

RESEARCH ARTICLE

Coordinated activities of Myosin Vb isoforms and mTOR signaling regulate epithelial cell morphology during development

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ABSTRACT

The maintenance of epithelial architecture necessitates tight regulation of cell size and shape. However, mechanisms underlying epithelial cell size regulation remain poorly understood. We show that the interaction of Myosin Vb with Rab11 prevents the accumulation of apically derived endosomes to maintain cell-size, whereas that with Rab10 regulates vesicular transport from the trans-Golgi. These interactions are required for the fine-tuning of the epithelial cell morphology during zebrafish development. Furthermore, the compensatory cell growth upon cell-proliferation inhibition involves a preferential expansion of the apical domain, leading to flatter epithelial cells, an efficient strategy to cover the surface with fewer cells. This apical domain growth requires post-trans-Golgi transport mediated by the Rab10-interacting Myosin Vb isoform, downstream of the mTOR-Fatty Acid Synthase (FASN) axis. Changes in trans-Golgi morphology indicate that the Golgi synchronizes mTOR-FASN-regulated biosynthetic input and Myosin Vb-Rab10 dependent output. Our study unravels the mechanism of polarized growth in epithelial cells and delineates functions of Myosin Vb isoforms in cell size regulation during development.

KEY WORDS: Cell size regulation, Compensatory cell growth, Tissue homeostasis, Myosin Vb, mTOR signaling, Epidermis, Zebrafish

INTRODUCTION

Epidermis is a stratified epithelium, which acts as a physical and biological barrier to protect the interior milieu in metazoans. To acquire and maintain the architecture of such a tissue, tight regulation of cellular attributes such as cell size, cell number and cell morphology is crucial (Gómez-Gálvez et al., 2018; Hannezo et al., 2014; Park et al., 2015). Epithelial cells display a high degree of homogeneity in terms of size and shape (Ginzberg et al., 2015; Puliafito et al., 2017; Sugimura and Ishihara, 2013), and irregularity in cell size may indicate a tissue anomaly (Ginzberg et al., 2015; Lloyd, 2013). In the epidermis, squamous cell morphology possibly allows efficient coverage of large surface area. Furthermore, under conditions like wounding, mechanical stretch and inhibition of cell proliferation, epidermal cells grow in size (Razzell et al., 2014; Sonal et al., 2014; Tamori and Deng, 2014). Whether such growth involves the acquisition of specific cell morphology and how such cell growth is regulated remains unclear.

Myosin Vb, an actin based motor, functions in the maintenance of epidermal cell size, by regulating plasma membrane homeostasis (Sonal et al., 2014). It consists of a head domain having ATPase activity and three well-characterized tail domains which bind to Rab8a, Rab10 and Rab11a to mediate exocytosis, membrane biogenesis and recycling, respectively (Lapierre et al., 2001; Liu et al., 2013; Roland et al., 2011). In intestinal epithelial cells, loss of Myosin Vb promotes bulk endocytosis from the apical surface (Engevik et al., 2019), contributing to the pathophysiology of Microvillus inclusion disease (MVID) (Müller et al., 2008; Ruumelle et al., 2010). The Myosin Vb-Rab8 interaction is required for proper microvilli formation, whereas disruption of its interaction with Rab11 results in apical membrane internalization and formation of microvillus inclusions (Knowles et al., 2014). Two functional isoforms have been identified for Myosin Vb; one containing Rab10-, Rab8- and Rab11-binding domains and the other having only Rab8- and Rab11-binding domains (Roland et al., 2009). In rat hippocampal and cortical neurons and zebrafish retinal ganglion cells, interaction of Myosin Vb with Rab10 promotes copious post-mitotic growth in axons (Liu et al., 2013). The role of Myosin Vb in membrane biogenesis in epithelial cells has not been explored so far.

Acquisition of cell size and its maintenance requires synthesis of plasma membrane components. In mammalian cells, *de novo* fatty acid synthesis contributes to membrane biogenesis during mitotic expansion and formation of cellular protrusions (Scaglia et al., 2015; Zhang et al., 2017). Key genes controlling membrane biogenesis, such as *fatty acid synthase (fasn)*, are regulated by mTOR signaling (Yan et al., 2014; Yoon et al., 2007). mTORC1, along with other metabolic cues, governs the expression of FASN and other lipid biogenic enzymes via sterol regulatory element binding proteins (SREBPs) (Eberlé et al., 2004; Laplante et al., 2009; Madison, 2016; Peterson et al., 2011; Porstmann et al., 2008; Wen et al., 2012). Because of its central role in lipid as well as protein biosynthesis, mTOR signaling regulates cell size and tissue growth in various systems (Boehlke et al., 2010; Jones et al., 2009; Kuo et al., 2015; Tumaneng et al., 2012). So far, the role of mTOR-FASN-mediated membrane biogenesis in the acquisition or maintenance of cell size in the epidermal cells has remained unclear. Besides, it is poorly understood whether membrane biosynthesis coordinates with the cellular transport machinery in targeting the newly synthesized components to the plasma membrane during cell growth.

Using developing zebrafish epidermis as a model, we show that the interactions of Myosin Vb with Rab11 and Rab10 regulate different transport modes to maintain the epithelial cell morphology during development. Our analyses further reveal that the compensatory growth in the epidermal cells is characterized by a preferential increase in the apical domain – an efficient strategy to cover a large surface – and is regulated by mTOR and FASN. Interestingly, Myosin Vb-Rab10 interaction is essential for this

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polarized growth of the epidermal cells. Our investigation further establishes a hierarchical connection between mTOR-FASN-mediated membrane biogenesis and directional vesicle transport via Myosin Vb during cell growth, with the trans-Golgi acting as an intermediate reservoir.

RESULTS

Importance of Myosin Vb-Rab11 and Myosin Vb-Rab10 interactions in the developing epidermis

The *gsp/myosin Vb* mutants and morphants expressing truncated versions of Myosin Vb – devoid of the complete tail domain – exhibit increased endocytosis and accumulation of early, recycling and late endosomes and lysosomes. This leads to increased membrane influx, perturbed membrane homeostasis and subsequent reduction in total cell surface area in zebrafish peridermal or outer epidermal cells. As a consequence, some of the peridermal cells round up and form clusters on the head and flank region (Phatak et al., 2021; Sonal et al., 2014).

To unravel the importance of Myosin Vb-Rab10 and Myosin Vb-Rab11 interactions in the developing zebrafish epidermis, we aimed at rescuing the Myosin Vb deficiency by injecting mRNAs for: (1) full-length Myosin Vb having both Rab10- as well as Rab11-binding abilities; (2) an isoform of Myosin Vb without Rab10-binding domain; (3) Myosin Vb mutant lacking Rab11-binding ability; and (4) Myosin Vb incapable of binding to Rab10 and Rab11 (see Materials and Methods). Hereafter, these four conditions are referred to as MyoVb-Rab10⁺/Rab11⁺, MyoVb-Rab10⁻/Rab11⁺, MyoVb-Rab10⁺/Rab11⁻ and MyoVb-Rab10⁻/Rab11⁻. To generate functional deficiency of Myosin Vb, we injected a previously validated splice site morpholino (Sonal et al., 2014) into a transgenic line *Tg(cldnb:lynEGFP)* in which peridermal cell membranes are marked with lyn-EGFP. Supplementing *myosin Vb* morphants by mRNAs for MyoVb-Rab10⁺/Rab11⁺ and MyoVb-Rab10⁻/Rab11⁺ rescued the morphological cell rounding phenotype (Fig. 1A,B) as well as previously reported (Sonal et al., 2014) cellular phenotypes, such as loss of microridges, reduction in cell size and accumulation of vesicles (Fig. 1C). In *myosin Vb* morphants, peridermal cells exhibit accumulation of apically derived endosomes arising from fluid-phase endocytosis as revealed by dextran uptake assay. This phenotype is rescued when embryos are supplemented with mRNA for MyoVb-Rab10⁺/Rab11⁺ and MyoVb-Rab10⁻/Rab11⁺ (Fig. S1B). Interestingly, injections of mRNAs for MyoVb-Rab10⁺/Rab11⁻ and MyoVb-Rab10⁻/Rab11⁻ rescued neither the morphological nor the cellular phenotypes (Fig. 1A,C; Fig. S1B), suggesting that whereas Myosin Vb and Rab11 interaction is essential for maintenance of cell size and shape, the interaction of Myosin Vb with Rab10 is apparently dispensable for this process. However, staining with wheat germ agglutinin (WGA), which marks the trans-Golgi network (TGN) and post-TGN vesicles (Kanazawa et al., 2008), revealed that the injection of mRNA for MyoVb-Rab10⁻/Rab11⁺ in Myosin Vb-deficient embryos – but not for MyoVb-Rab10⁺/Rab11⁺ – results in a Golgi phenotype characterized by the increased branching of the trans-Golgi and large WGA-positive vesicles closely apposed to the trans-Golgi (Fig. 1C). The radiating long-branched trans-Golgi was more obviously present. Embryos showing the Golgi phenotype in a larger proportion of epidermal cells (more than ~40-50%) were counted as embryos showing Rab10-binding deficiency phenotype. Although the penetrance varied across experimental sets, the number of embryos showing the Golgi phenotype could reach up to 70% in the *myosin Vb* morphants rescued with Rab10⁻/Rab11⁺ mRNA (Fig. 1D).

To confirm that the cellular phenotype is because of the predicted perturbation in either Rab10 or Rab11 binding, we selectively rescued the *myosin Vb* morphants with either MyoVb-Rab10⁺/Rab11⁻ or MyoVb-Rab10⁻/Rab11⁺ mRNA and analyzed the accumulation of Rab10 and Rab11 labeled vesicles (Fig. S2). We observed the partial rescue of Rab11 vesicles in MyoVb-Rab10⁻/Rab11⁺-injected morphants (Fig. S2C,D; Kruskal–Wallis test with Dunn’s post hoc test, $P < 0.05$), whereas the Golgi phenotype with Rab10 vesicle accumulation was present mostly in MyoVb-Rab10⁻/Rab11⁺-injected morphants (Fig. S2A,B). It is important to note that MyoVb-deficient embryos and the MyoVb-deficient embryos injected with MyoVb-Rab10⁺/Rab11⁻ mRNA did not exhibit a higher number of Rab10 vesicles. In both these conditions, trans-Golgi showed highly perturbed organization (Fig. S2A) presumably due to massive accumulation of late endosomes and lysosomes (Sonal et al., 2014). This may either affect the biogenic function of the Golgi or result in a faster clearance of post-TGN vesicles.

To conclude, the phenotypic features seen in Myosin Vb-deficient embryos, such as increased endosome accumulation, reduction in cell surface area, cell rounding up and loss of apical microridges, are attributed to the loss of Myosin Vb-Rab11 interaction, whereas the Golgi phenotype may be attributed to the loss of Myosin Vb-Rab10 interaction.

Knockdown of Rab10-binding isoform of Myosin Vb results in a bigger and branched trans-Golgi and an accumulation of post-trans-Golgi vesicles

The Golgi phenotype in Myosin Vb-deficient embryos rescued with mRNA for MyoVb-Rab10⁻/Rab11⁺ was intriguing and suggested the role of Myosin Vb-Rab10 interaction in the post-TGN traffic. We reasoned that investigating this phenotype would lead towards an understanding of the functional importance of the Myosin Vb-Rab10 interaction in the epithelial cells. As the penetrance of the Golgi phenotype in the rescued embryos varied, we used a previously published splice site morpholino for this purpose. This morpholino prevents the inclusion of Exon D – which encodes the Rab10-binding domain – into the transcript, selectively knocking down the Myosin Vb isoform consisting of the Rab10-binding domain (Liu et al., 2013). Our RT-PCR analysis confirmed that the morpholino indeed knocked down Exon D-containing *myosin Vb* transcripts (Fig. S3A) and is henceforth referred to as Exon D MO. The previously reported optic nerve thinning phenotype (Liu et al., 2013) in the Exon D morphant was also present in the *gsp*^{NS042} (*myosin Vb*) mutant embryos as well as in the *myosin Vb* morphants (Fig. S3B).

To delineate the function of the Rab10-binding isoform of Myosin Vb in the epidermis, we characterized the Exon D morphant phenotype. The morphological phenotype was characterized by a slight reduction in the head size and reduced tail fin expansion. No epidermal defects were observed at the morphological level (Fig. 2A). To characterize the trans-Golgi morphology in morphant embryos, we marked the trans-Golgi compartment with a plasmid-encoded EGFP-tagged trans-Golgi-resident enzyme galactose-1-phosphate uridylyltransferase (EGFP-GalT) (Gupta and Sonawane, 2020; Nilsson et al., 1993; Rabouille et al., 1995; Sepich and Solnica-Krezel, 2016) and WGA, which marks both TGN and vesicles derived from TGN (Kanazawa et al., 2008). In Exon D-deficient embryos, the trans-Golgi appeared to be larger and highly branched. In addition, large post trans-Golgi vesicles – labeled only by WGA, but not with GalT – were closely apposed to trans-Golgi, and this phenotype appeared to be the most severe at 36 h post-fertilization (hpf) (Fig. 2B). This analysis suggested that the differentiated post trans-Golgi vesicles (WGA labeled but GalT

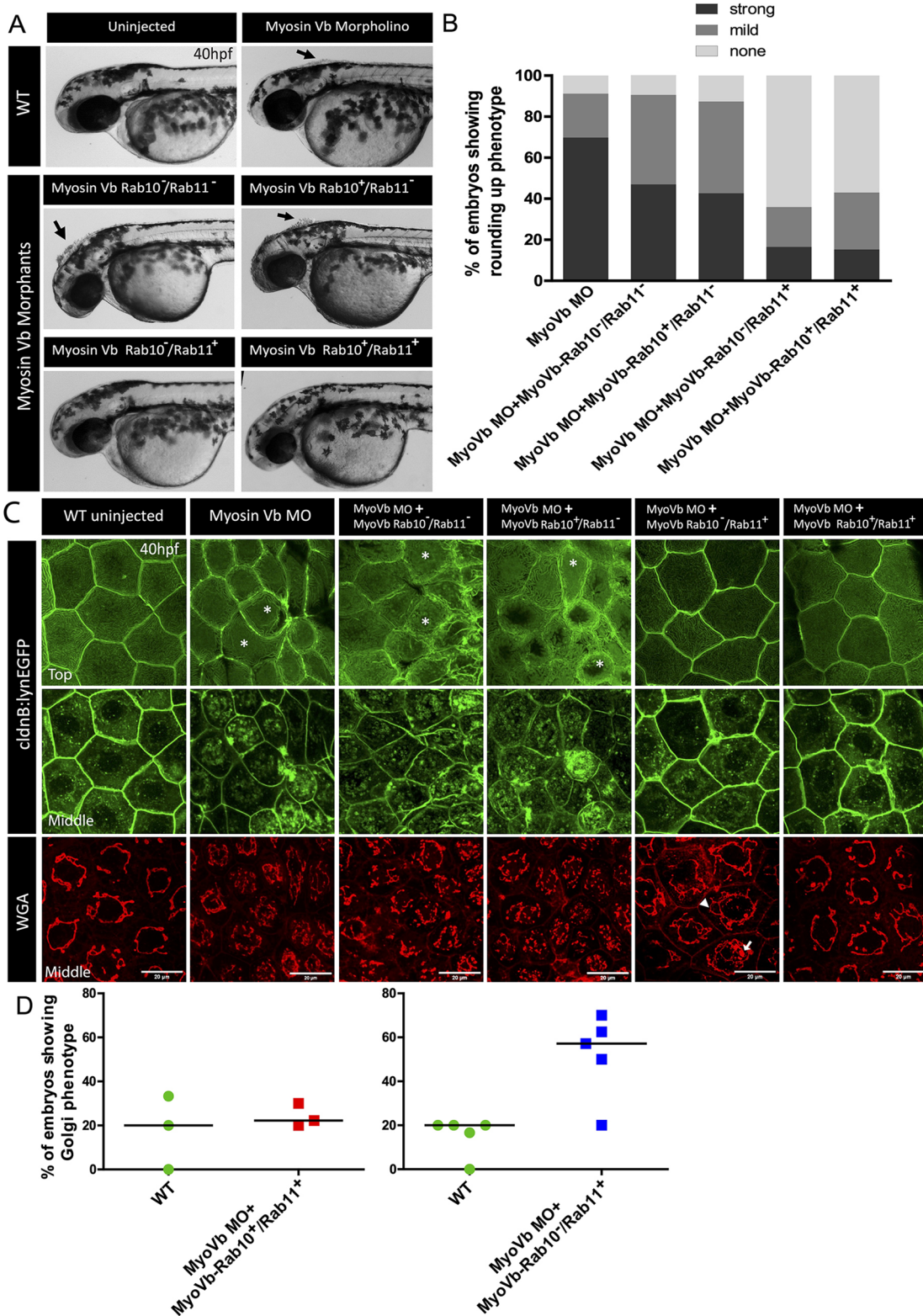


Fig. 1. Myosin Vb-Rab11 interaction is essential to maintain cell size and shape, whereas Myosin Vb-Rab10 interaction controls trans-Golgi morphology. (A) Representative brightfield images of Myosin Vb morphants at 40 hpf, with and without compensation with mRNA encoding different Myosin Vb isoforms mentioned in the figure. (B) Graphical representation of proportion of Myosin Vb morphants (MyoVb MO) showing either strong, mild or no cell rounding phenotype upon injections of mRNA for the mentioned Myosin Vb isoforms. (C) Confocal images of the 40 hpf morphant peridermal cells in *Tg(cldnB::lynEGFP)* transgenic line with the membrane and the vesicles marked with Lyn-GFP and trans-Golgi marked with WGA (top panel confocal z-stack at 0 μ m; middle panel z-stack at 1.9-2.2 μ m). (D) Percentage of the morphant embryos showing trans-Golgi phenotype characterized by branching of trans-Golgi and the presence of large vesicles in the morphant embryos injected with mRNA for stated Myosin Vb isoforms. Black arrows in A show bunches of rounded-up cells. White arrow and arrowhead in C indicate a trans-Golgi vesicle and branch, respectively. Asterisks in C point to the absence of microridges in the apical domain of the peridermal cells. Horizontal bar shows mean. Scale bars: 20 μ m (C).

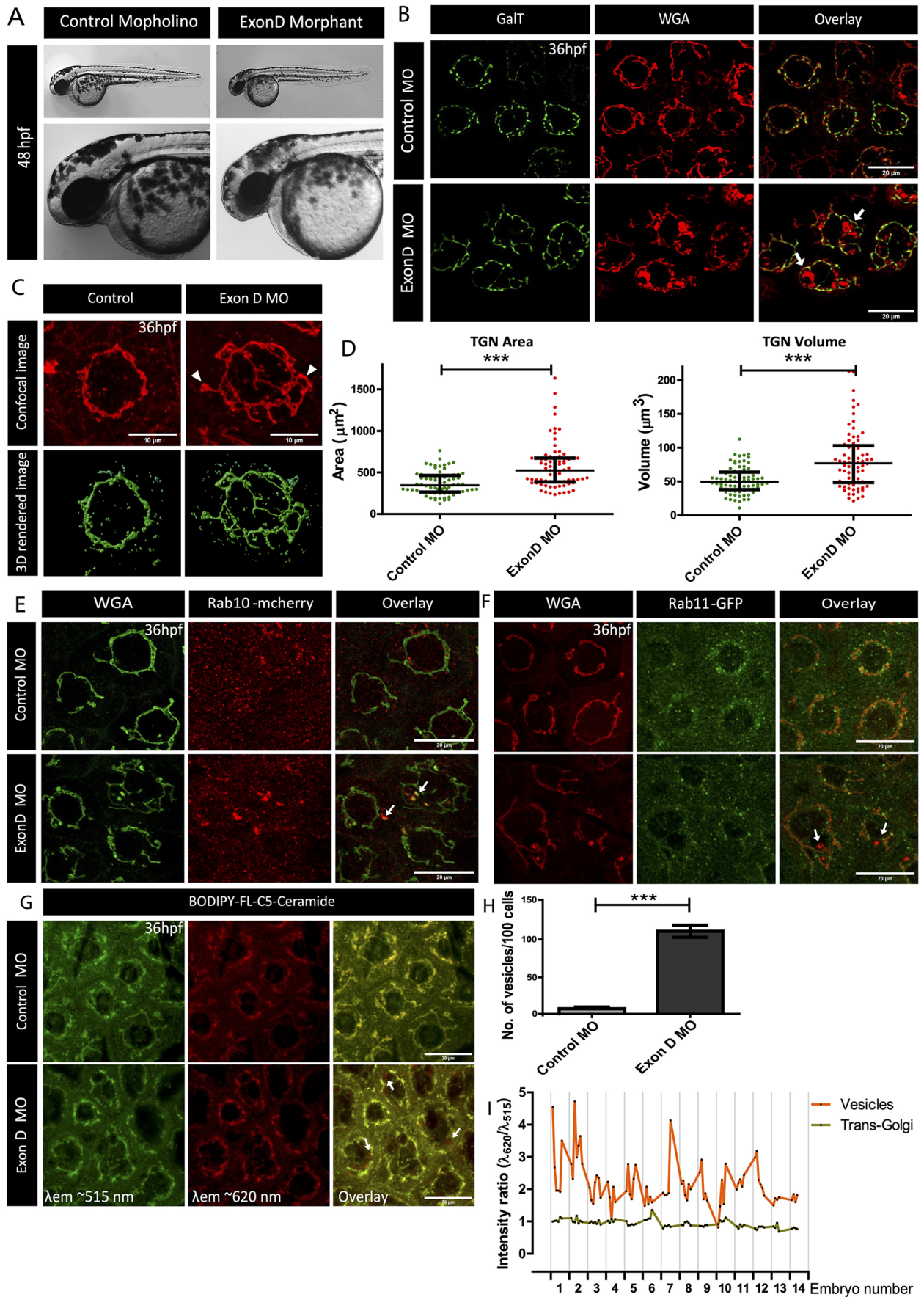


Fig. 2. See next page for legend.

Fig. 2. Myosin Vb Exon D knockdown results in increased trans-Golgi branching and accumulation of post-TGN vesicles in the peridermal cells. (A) Brightfield images of control and Exon D morphants (Exon D MO) at 48 hpf show that knockdown of Exon D isoform (Rab10 binding) does not result in rounded up cells in the head region. (B) Confocal images of peridermal cells of the control (Control MO) and Exon D morphants (Exon D MO) at 36 hpf immunostained for GalT-GFP and trans-Golgi using WGA. (C,D) 3D rendering of TGN by Huygen's software (C) followed by quantification (D) revealed an increase in both surface area and volume of the TGN. (E,F) Confocal stacks of control and Exon D morphants stained for trans-Golgi (WGA) and Rab10 (E) and trans-Golgi (WGA) and Rab11 (F). (G-I) Confocal micrographs of BODIPY-FL-C₅-Ceramide-labeled peridermal cells from control and Exon D morphants at 36 hpf, imaged using $\lambda_{ex}=488$ nm and λ_{em} at ~ 515 nm (green) and ~ 620 nm (red) (G) followed by quantification of number of vesicles (H), and intensity ratio of red:green fluorescence of large Ceramide-rich compartments [red' appearing vesicles in overlays (G)] as well as the TGN (I). White arrows in B, E-G point to trans-Golgi vesicles in overlay panels. White arrowheads in C point to trans-Golgi branches. Data are median \pm interquartile range. *** $P<0.001$ (Mann-Whitney U test; Table S1). Scale bars: 20 μ m (B,E-G); 10 μ m (C).

negative) accumulate at the trans-Golgi, possibly owing to the absence of further transport. Quantifications using 3D deconvoluted images of WGA-stained TGN (Fig. 2C; see Materials and Methods for details) revealed a statistically significant increase in the surface area and volume of TGN in the peridermal cells of Exon D morphants (Fig. 2D; Mann-Whitney U -test, $P<0.05$). To verify the specificity of the morpholino, we rescued the Golgi phenotype in Exon D morphants by injecting mRNA for MyoVb-Rab10⁺/Rab11⁺ in a clonal manner. We observed qualitative as well as quantitative rescue of the TGN phenotype specifically in the mRNA clones compared with the neighboring non-clonal cells that were deficient for the Exon-D isoform and did not receive mRNA for MyoVb-Rab10⁺/Rab11⁺. Notably, we did not observe the rescue in TGN phenotype when mRNA for MyoVb-Rab10⁻/Rab11⁺ was used for the clonal analysis (Fig. S3C,D; Kruskal-Wallis test with Dunn's post hoc test, $P<0.05$). We also confirmed the TGN phenotype using the second splice morpholino to knock down the Exon D isoform (Fig. S3E).

To test whether transport of Rab10 vesicles is specifically disrupted, we injected Rab10-mCherry mRNA into the control and Exon D morphants, and visualized the Rab10 vesicles both in live and in fixed samples co-stained with WGA. Live imaging of the periderm revealed that larger Rab10 vesicles were exclusively present in Exon D morphants (Fig. S3F). In fixed preparations, most of these large Rab10-mcherry labeled vesicles showed co-staining with WGA and were located in the peri-Golgi region (Fig. 2E). Further analysis using the *Tg(h2afx:EGFP-Rab11a)^{mw6}* transgenic line (Clark et al., 2011) revealed that Rab11-labeled endosomes did not co-localize with WGA in Exon D morphants (Fig. 2F). These analyses suggest that most of the large post-TGN vesicles are Rab10 compartments that accumulate owing to absence of the Rab10-interacting Myosin Vb isoform. To ascertain the nature of these post-TGN vesicles, we pulse labeled embryos with BODIPY-FL-C₅-Ceramide followed by imaging of the periderm. Enrichment of this BODIPY-FL-C₅-Ceramide dye in sphingolipid-rich biogenic compartments results in a shift in its λ_{em} from ~ 515 nm (green) to ~ 620 nm (Pagano et al., 1991). In the Exon D morphant embryos, we observed large vesicles juxtaposed to the trans-Golgi showing increased emission at 620 nm (Fig. 2G,H; Mann-Whitney U -test, $P<0.05$), suggesting their enrichment with sphingolipids. This BODIPY-FL-C₅-Ceramide enrichment in the vesicles was clearly discernible when an intensity ratio of λ_{620} nm/ λ_{515} nm was compared between the vesicles and TGN (Fig. 2I).

We further investigated the fate of the large vesicles that accumulate at the trans-Golgi surface. We reasoned that these vesicles would acquire late endo-lysosomal fate for the cells to reuse the accumulated vesicular contents. To test this idea, we knocked down the Exon D isoform in a transgenic line *Tg(h2afx:EGFP-Rab7)^{mw7}* that expresses EGFP-tagged Rab7 (Clark et al., 2011). Strikingly, large Rab7-labeled compartments were observed in Exon D morphants, compared with smaller Rab7-labeled vesicles seen in controls (Fig. S4A). Further immunostaining analysis revealed that the accumulated post-TGN vesicles labeled by WGA were co-stained for Rab7 and LAMP-1 – a lysosomal marker – in the Exon D morphants at 36 hpf (Fig. S4B). This indicates endo-lysosomal processing of the vesicular load accumulated around Golgi in the absence of the Exon D isoform. Consistent with this observation, the temporal analysis revealed that the size and number of the vesicles increase from 22 hpf to 36 hpf and decrease thereafter by 72 hpf (Fig. S4C,D; Kruskal-Wallis test with Dunn's post hoc test, $P<0.05$).

To conclude, in the absence of the Myosin Vb-Rab10 interaction, afferent transport from the trans-Golgi is inhibited, resulting in increased TGN size as well as accumulation of Rab10-labeled and sphingolipid-rich, presumably biogenic, vesicles that remain apposed to the trans-Golgi surface. In the absence of their further transport, the accumulated vesicles acquire late endosomal-lysosomal fate, gradually degrading the contents.

Compensatory growth of peridermal cells involves preferential expansion of the apical domain and requires the Rab10-binding isoform of Myosin Vb

Membrane biogenesis involves *de novo* synthesis of membrane lipids and their transport from trans-Golgi to plasma membrane. As we observed accumulation of sphingolipid-rich vesicles in the vicinity of trans-Golgi, we asked whether epidermal cells display reduction in cell size in the absence of this Exon D-containing isoform. To test this, we injected the Exon D morpholino in the embryos from *Tg(cldnB:lynEGFP)* transgenic line and quantified the total surface area of the peridermal cells. To our surprise, the Exon D morphants showed a slight increase in the basolateral surface area as well as cell height, whereas the apical surface remained unchanged, yielding a taller morphology (Fig. 3A,B; Mann-Whitney U -test, $P<0.05$). The marginal increase observed in the total surface can plausibly be attributed to the increase in the basolateral surface area. These data suggest that the Rab10-binding isoform of Myosin Vb is essential to maintain the cell morphology but does not have a major developmental function in cell size determination in the epidermis.

We further asked whether the function of the Rab10-binding Myosin Vb isoform becomes essential when the epidermal cells undergo hypertrophy. To test this, we used previously established paradigms (Sonal et al., 2014) to achieve compensatory cell growth by inhibiting cell proliferation by downregulation of EGFR signaling using PD 168393 and knockdown of Δ Np63 transcription factor (Bakkers et al., 2002; Lee and Kimelman, 2002; Sonal et al., 2014). Interestingly, the reduction in cell proliferation results in an anisometric growth, wherein ~ 30 - 50% increase in the apical surface contributes towards cell expansion (Fig. 3C,D; Kruskal-Wallis test with Dunn's post hoc test, $P<0.05$). The basolateral membrane increased by $\sim 10\%$ in this experiment (Fig. 3C,D), but the increase appears to vary between 4- 25% in the other experiments reported in this study and was not always statistically significant (e.g. Fig. 3F). As the apical growth is much larger than the basolateral growth, the height of the cells does not increase. This results in flatter peridermal

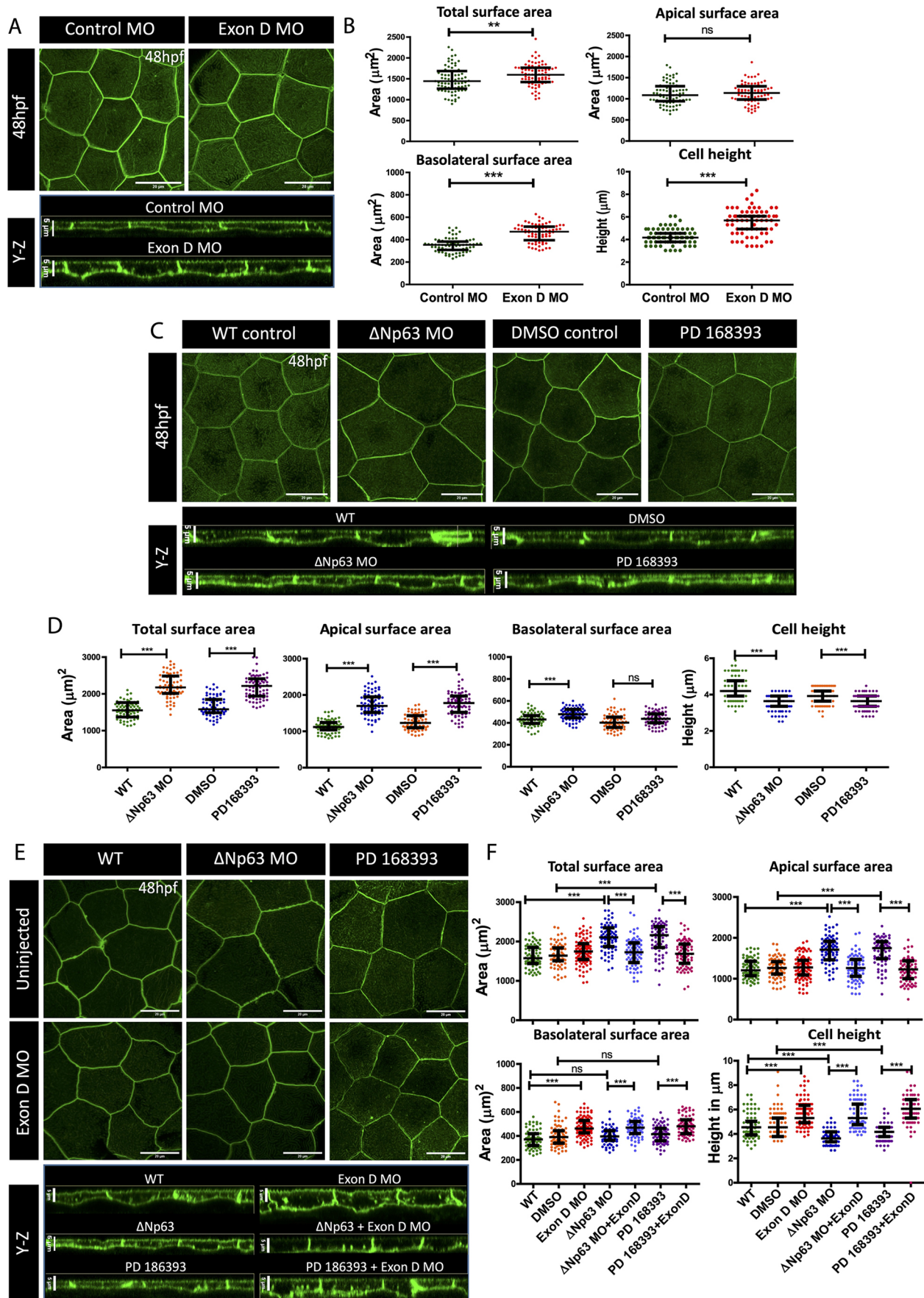


Fig. 3. See next page for legend.

Fig. 3. Rab10-interacting isoform of Myosin Vb regulates cell morphology during development and is essential for the apical domain expansion during compensatory cell growth. (A,C,E) Confocal scans and orthogonal (Y-Z) sections of the peridermal cells at 48 hpf of immunostained embryos of *Tg(cldnb:lynEGFP)* line injected with control (Control MO) and Exon D morphants (Exon D MO) (A), upon knockdown of Δ Np63 (Δ Np63 MO) and inhibition of EGF signaling by treatment with PD168393 (C) or uninjected or Exon-D morphants injected with Δ Np63 morpholino or treated with PD168393 (E). (B,D,F) Graphical representation of quantification of total, apical and basolateral surface area, and the cell height, under given genetic conditions or treatments in A,C,E, respectively. Data are median \pm interquartile range. $**P<0.01$, $***P<0.001$ [Mann–Whitney U test (B); Kruskal–Wallis test with Dunn's post hoc test (D,F)]. ns, not significant. Note that only essential paired comparisons are shown in D and F using horizontal line segments. For the details of all the comparisons please refer to Table S2. Scale bars: 20 μ m (X-Z plane of A,C,E, top panels); 5 μ m (Y-Z plane of A,C,E, bottom panels).

cells having a large cross-sectional area (Fig. 3C). Notably, the Exon D morphants were unable to display compensatory hypertrophy and showed inability to grow the apical domain, but the basolateral membrane and cell height were able to grow (Fig. 3E,F; Kruskal–Wallis test with Dunn's post hoc test, $P<0.05$).

To conclude, compensatory cell growth involves selective increase in the apical surface, which results in peridermal cells acquiring flatter morphologies. The Exon D isoform of Myosin Vb, although dispensable for cell size regulation in the developing epidermis, becomes essential for the apical membrane growth during compensatory hypertrophy.

Cellular hypertrophy in the periderm is regulated cooperatively by mTOR signaling and Rab10-dependent Myosin Vb transport

The cell surface area increase under the proliferation inhibition condition presents a compensatory hypertrophy paradigm with 30–40% increase in the total surface area. We asked how membrane biogenesis is regulated during this cell-growth and whether the Rab10-binding isoform of Myosin Vb cooperates with the membrane biogenic pathway. We checked the status of lipid anabolism by looking at levels of one of the essential and key enzymes of the *de novo* lipid synthesis pathway, FASN, which is required for the growth of the plasma membrane (Knobloch et al., 2013; Paoletti et al., 2007; Schuller et al., 1992; Vadia et al., 2017; Yao et al., 2012; Zhang et al., 2017). Under both the paradigms of compensatory cell size increase, FASN levels showed an upregulation (Fig. 4A,B; Mann–Whitney *U*-test, $P<0.05$). To confirm the role of FASN in cell size increase, we inhibited the function of FASN using Cerulenin (Kawaguchi et al., 1982; Omura, 1976; Vance et al., 1972). We observed a qualitative decrease in FASN levels in the peridermal cells (Fig. S5A). In addition, Cerulenin treatment led to an overall reduction in the peridermal cell size, with a prominent decrease in the apical domain in control embryos as well as in the embryos treated with EGFR inhibitor or injected with Δ Np63 morpholino, whereas the decrease in the size of the basolateral domain was marginal (Fig. 4C,E; Kruskal–Wallis test with Dunn's post hoc test, $P<0.05$).

mTOR signaling is a master regulator of metabolism, and controls lipid synthesis by regulating SREBP-dependent fatty acid synthesis (Peterson et al., 2011; Porstmann et al., 2008; Soliman, 2011; Yoon et al., 2007). We found that treatment with Rapamycin – an established mTOR inhibitor – resulted in downregulation of phospho-ribosomal protein S6 (pS6; a known target of mTORC1) along with FASN in the zebrafish periderm (Fig. S5B,C). Although the Rapamycin treatment did not have an effect on the peridermal cell size in developing embryos, in proliferation-inhibition paradigms the Rapamycin-treated peridermal cells did not attain

compensatory cell-hypertrophy and remained similar to those in the control embryos (Fig. 4D,F; Kruskal–Wallis test with Dunn's post hoc test, $P<0.05$).

The fact that both mTOR signaling and the Exon D isoform are required for compensatory growth indicates that these two components may have a cooperative relationship during cellular hypertrophy. To probe this notion, we established mTOR signaling activation paradigms using compound C – an AMPK inhibitor. Compound C reverses the effect of AMPK-induced reduction in FASN levels and increases the phosphorylation of acetyl-CoA carboxylase (ACC), thereby favoring lipogenesis (Fediuc et al., 2006; Fernández-Galilea et al., 2014; Zhou et al., 2001).

Compound C treatment resulted in an increase in the levels of pS6 as well as FASN (Fig. S6A,B). Quantification revealed a significant increase in total cell surface area in Compound C-treated embryos. In contrast to the proliferation-inhibition paradigms (Fig. 3F), here the membrane growth was proportional in both apical (25–35%) as well as basolateral surface area (30–40%) (Fig. S6C,D). Importantly, the Exon D morphants treated with compound C were unable to expand their apical surface. The basolateral area in these embryos showed a mild increase, which was statistically not significant, possibly because the increase due to the loss of the Exon D isoform is marginal over the increase resulting from compound C treatment. Nevertheless, we observed a significant increase in cell height in the Exon D morphants treated with compound C compared with the treated control embryos, likely a combined consequence of marginal increase in the basolateral area and reduced apical area (Fig. 5A,B; Kruskal–Wallis test with Dunn's post hoc test, $P<0.05$).

To conclude, our analyses indicate that compensatory hypertrophy requires mTOR-FASN-dependent membrane biogenesis. The Rab10-interacting isoform of Myosin Vb functions in mTOR-dependent polarized growth of peridermal cells. Our data indicate that vesicular transport by Myosin Vb-Rab10 at the trans-Golgi acts as a permissive step downstream of mTOR signaling during the regulation of epidermal cell growth.

Coordination between mTOR-FASN-mediated biosynthesis and Myosin Vb-mediated transport takes place at the TGN

Our analyses showed that the epidermal cell growth is regulated by coordinated activities of the membrane biosynthetic pathway and Myosin Vb. Given that newly synthesized membrane lipids such as phospholipids and sphingolipids are transported to the plasma membrane via trans-Golgi (Blom et al., 2011; Griffiths and Simons, 1986; Ikonen and Simons, 1998), and the fact that Myosin Vb function is required at the trans-Golgi surface, we hypothesized that the coordination would take place at the level of Golgi. If true, TGN morphology would alter depending upon the extent of input (synthesis) and output (transport from the TGN).

To test whether decreased biosynthesis would alter trans-Golgi morphology, we analyzed embryos treated with Rapamycin. This treatment resulted in a stark reduction in the TGN surface area, volume and trans-Golgi branching in the peridermal cells compared with control (Fig. 6A,C; Mann–Whitney *U*-test, $P<0.05$). Although the cell size did not decrease upon Rapamycin treatment (Fig. 6A,B; Mann–Whitney *U*-test, $P<0.05$), a stronger inhibition of mTOR signaling by Torin 1 (Liu et al., 2010; Thoreen et al., 2009) did result in a decrease in trans-Golgi branching, TGN size and cell size (Fig. S7A–C).

We further analyzed TGN morphologies linked with the cell size phenotypes under proliferation-inhibition paradigms, mTOR activation and proliferation-inhibition paradigms in combination with Exon D knockdown. In all the three conditions – EGFR

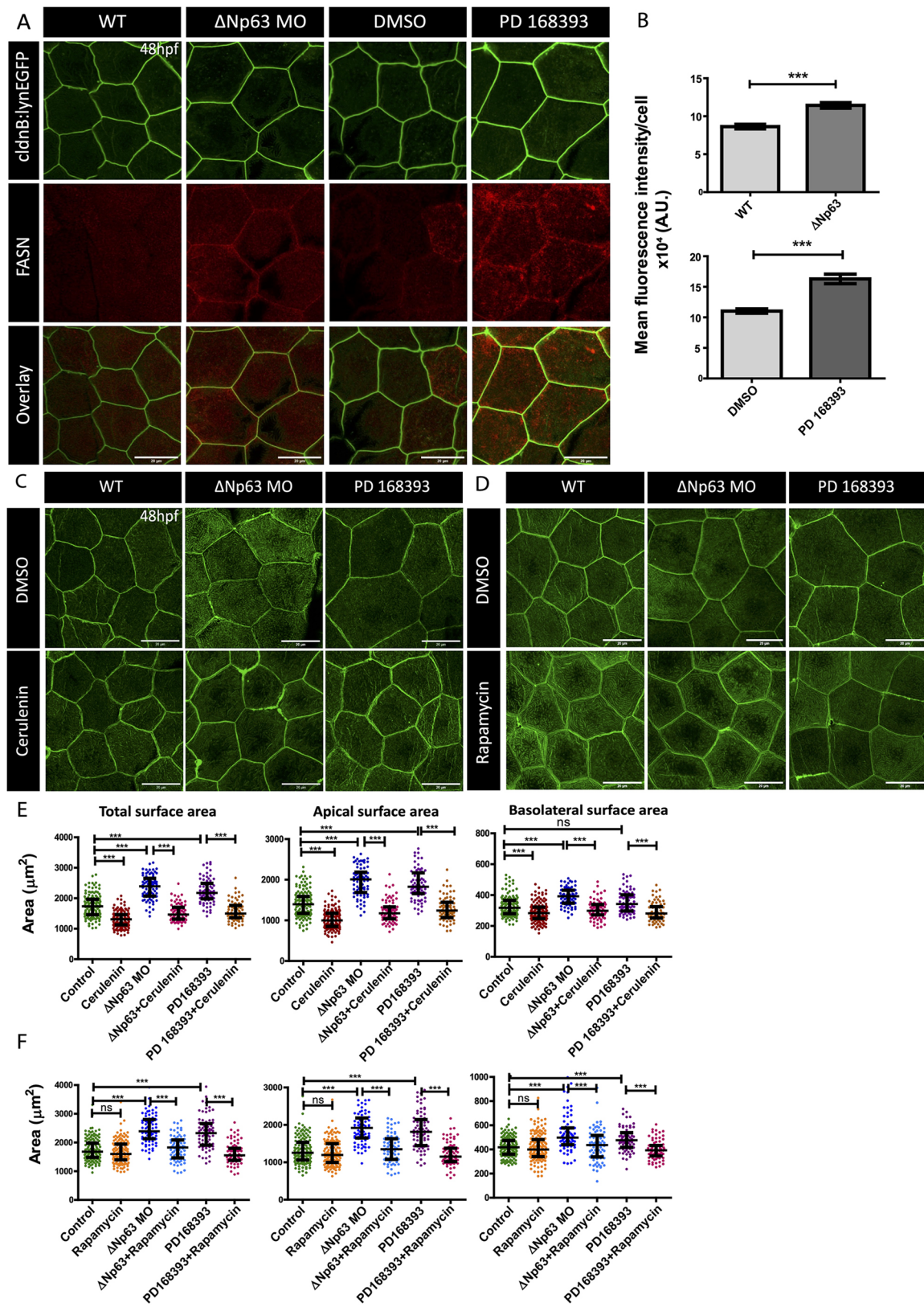


Fig. 4. Activity of FASN and mTOR is essential for compensatory cell growth in the developing periderm. (A,B) Immunostainings using anti-FASN antibody followed by confocal imaging (A) and fluorescence intensity quantification (B) in the embryos obtained from the *Tg(cldnB:lynEGFP)* line that are morphants for Δ Np63 (Δ Np63 MO) or treated with PD168393, and fixed at 48 hpf. (C-F) Confocal scans (C,D) and graphical representation of the total, apical and basolateral area quantifications (E,F) in Δ Np63 morphants (Δ Np63 MO) and PD168393-treated embryos upon FASN inhibition by Cerulenin (C,E) or mTOR inhibition by Rapamycin (D,F). Data are mean \pm s.e.m. (B) and median \pm interquartile range (E,F). *** $P < 0.001$ [Mann–Whitney U test (B); Kruskal–Wallis test with Dunn’s post hoc test (E,F)]. ns, not significant. Please note that only important paired comparisons are shown. For the entire pair-wise analyses please refer to Table S3. Scale bars: 20 μm .

inhibition, Δ Np63 knockdown and mTOR activation – the trans-Golgi morphology showed increased branching, surface area and volume (Fig. 6D,E; Fig. S7D,E; Kruskal–Wallis test with Dunn’s post hoc test, $P<0.05$), suggesting a tight correlation between increased lipid synthesis, trans-Golgi size and increased cell size. Embryos subjected to proliferation-inhibition paradigms when treated with Rapamycin or Cerulenin did not show an increase in TGN surface area and volume in the peridermal cells (Fig. 6F,G; Fig. S7F,G; Kruskal–Wallis test with Dunn’s post hoc test, $P<0.05$), and showed inability to undergo compensatory growth (Fig. 4C–F). This analysis suggested that the size and branching of the trans-Golgi compartment in developing wild-type embryos, as well as those subjected to proliferation-inhibition paradigms, depends upon mTOR-FASN-dependent inputs, which are also essential for cell size maintenance and cell growth.

Results presented above indicate that the trans-Golgi shows an increase in size and branching due to increased lipid synthesis, which is essential to acquire and maintain cellular hypertrophy. However, in the Exon D morphants, though the accumulation of TGN vesicles was understandable, the increase observed in trans-Golgi size and branching (Fig. 2C,D) was puzzling. We asked whether this increase in TGN size is also a consequence of the increased membrane biosynthesis in Exon D morphants. Indeed, we found significant increase in FASN levels in Exon D morphant peridermal cells, suggesting a feedback upregulation of lipid biosynthesis under reduced anterograde trafficking from the Golgi (Fig. S8A,B; Mann–Whitney U -test, $P<0.05$). To understand the significance of such an upregulation in the lipid synthesis and TGN expansion, we treated Exon D morphants with Rapamycin. Upon

treatment, Exon D morphants exhibited diminished branching and reduction in TGN size, while the large post trans-Golgi vesicles were still present (Fig. 7A,B; Kruskal–Wallis test with Dunn’s post hoc test, $P<0.05$). Importantly, we found a significant reduction in the basolateral surface area and in cell height in Rapamycin-treated Exon D morphants, suggesting that the basolateral membrane growth in Exon D morphants is a consequence of the compensatory synthesis of membrane components and their delivery independent of Myosin Vb-mediated transport. Intriguingly, we also observed a slight reduction in the apical area in Rapamycin-treated Exon D morphant peridermal cells compared with the control embryos and Exon D morphants but not with the Rapamycin-treated embryos. It is possible that in wild-type embryos the apical domain is regulated cooperatively by both mTOR and the Exon D isoform, and that in Exon D morphants mTOR signaling-mediated but Myosin Vb-independent membrane biogenesis is essential for the maintenance of the apical domain (Fig. 7C,D; Kruskal–Wallis test with Dunn’s post hoc test, $P<0.05$).

We further investigated the Golgi phenotypes in proliferation-inhibition and mTOR-activation paradigms combined with Exon D knockdown, wherein peridermal cells do not show cellular hypertrophy. We observed an increase in FASN level upon knockdown of the Exon D isoform under proliferation-inhibition conditions (Fig. S8C). Upon EGFR inhibition in Exon D morphants, the TGN surface area and volume also remained higher than uninjected controls (Fig. 7E,F; Kruskal–Wallis test with Dunn’s post hoc test, $P<0.05$). We also observed the same trend upon mTOR activation in Exon D morphants (Fig. S8D,E). Intriguingly, this trend was not conserved for Δ Np63-Exon D

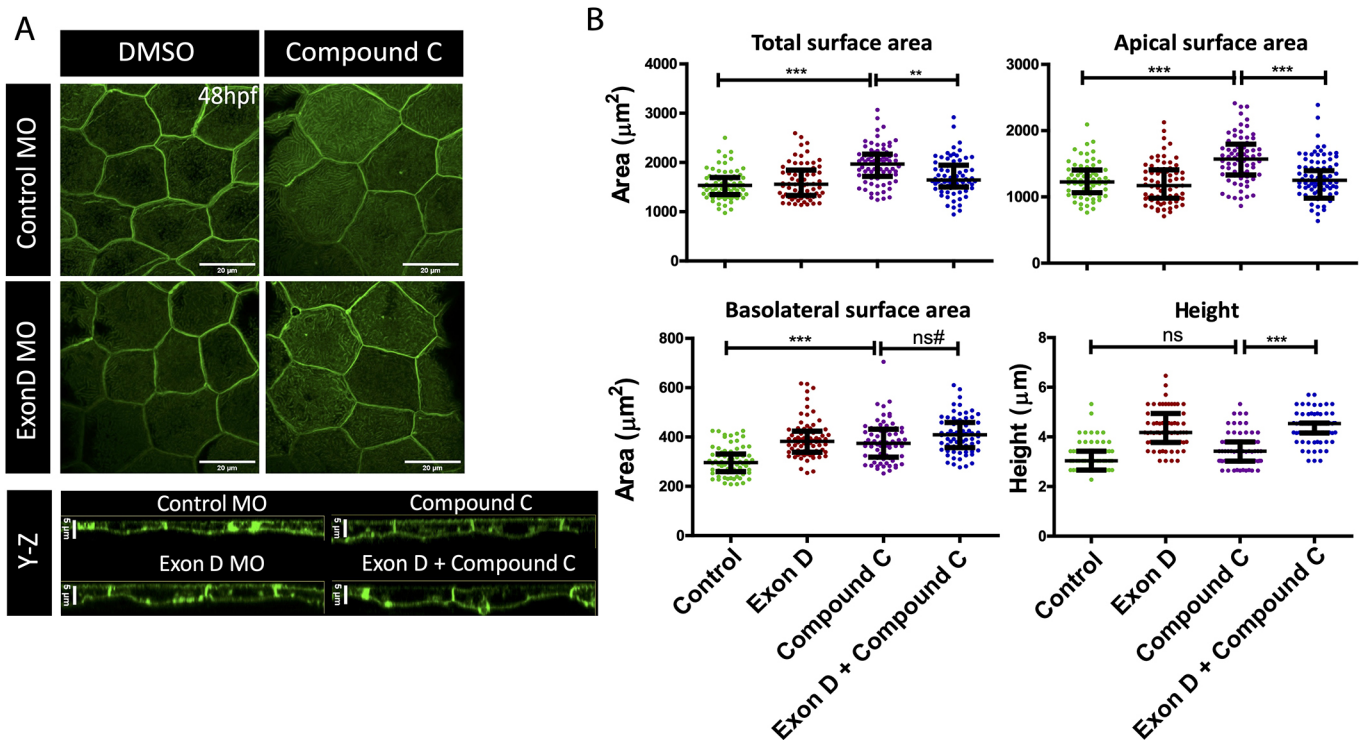


Fig. 5. Rab10-binding MyoVb isoform functions downstream of mTOR signaling during peridermal cell growth. (A) Confocal scans of IynEGFP-labeled peridermal cells of wild-type and Exon D morphant (Exon D MO) embryos treated with Compound C and DMSO at 48 hpf. (B) Graphical representation of the quantification of the total, apical and basolateral surface area, and the cell height in given genetic conditions and treatment. Data are median \pm interquartile range. ** $P<0.01$, *** $P<0.001$ (Kruskal–Wallis test with Dunn’s post hoc test). ns, not significant. ns#, although the difference is statistically not significant ($P=0.097$) the increasing trend might be biologically relevant (see the main text for details). Only key pairwise comparisons are shown in B; for the rest of the comparisons please refer to Table S4. Scale bars: 20 μ m.

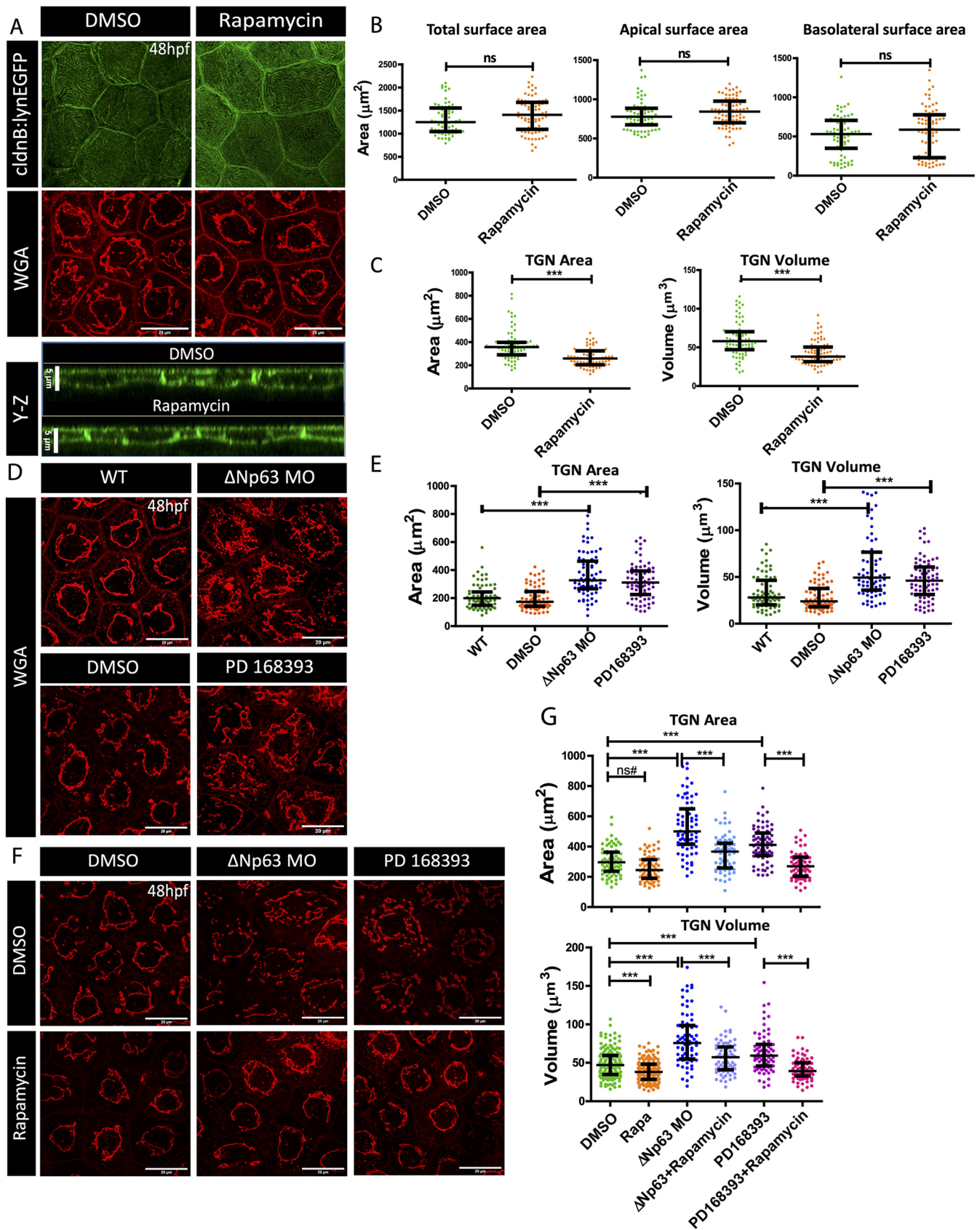


Fig. 6. See next page for legend.

Fig. 6. mTOR-mediated increase in the cell size corresponds to enlarged TGN size. (A) Confocal scans (X-Y and Y-Z planes) of the peridermal cells from 48 hpf embryos treated with DMSO (vehicle control) and Rapamycin and stained for lyn-EGFP and trans-Golgi (WGA). (B,C) Quantifications of total, apical and basolateral surface area (B), and TGN area and volume (C) upon Rapamycin treatment. (D,F) Confocal micrographs of TGN, labeled using WGA, from the peridermal cells from Δ Np63 morphants (Δ Np63 MO) and PD16393-treated embryos (D), and DMSO- or Rapamycin-treated Δ Np63 morphants and PD16393-treated embryos (F). (E,G) Graphical representation of TGN area and volume quantification in Δ Np63 morphants and PD16393-treated embryos (E), and DMSO or Rapamycin treated Δ Np63 morphants and PD16393-treated embryos (G). Data are median \pm interquartile range. *** P <0.001 [Mann–Whitney U test (B,C); Kruskal–Wallis test with Dunn's post hoc test (E,G)]. ns, not significant. ns#, although the comparison is not significant, the trend is similar to the other comparisons that have been seen to be statistically significant in other experiments (for example, in Figs 6C and 7B) performed in this study. Note that only essential pairwise comparisons are shown in panels E and G. A complete list of pairwise comparisons for B,C,E and G is given in Table S5. Scale bars: 20 μ m (X-Y plane of A,D,F); 5 μ m (Y-Z plane of A).

double morphants, in which we observed TGN surface area as well as volume to be comparable with that of the wild type (Fig. 7E,F). The absence of an increase in TGN surface area and volume in Δ Np63-Exon D double morphants is likely because of lysosomal processing of the accumulated cargo upon loss of Δ Np63 function and needs to be further investigated.

To conclude, the trans-Golgi compartment shows enlargement during compensatory cell growth and in the Exon D morphants. This enlargement is a consequence of the increased biosynthetic activity of mTOR-FASN axis. During the growth of the apical domain, the Exon D isoform acts downstream of the mTOR-FASN axis at the TGN. The increase in FASN levels in the absence of a functional Exon D isoform is a feedback response to increase membrane biogenesis. However, in the absence of Myosin Vb-Exon D, this compensatory growth is basolaterally directed.

DISCUSSION

Maintenance of the epithelial integrity is crucial for survival in metazoans. Under homeostatic conditions, epithelial cells maintain their morphology and number to conserve the tissue architecture. Whether and how epithelial cell morphologies are modulated under perturbed homeostatic conditions has remained unclear. In this study, we have unraveled the importance of interactions of Myosin Vb with Rab11 and Rab10 and its cooperativity with mTOR signaling in regulation of cell size in the developing zebrafish epidermis under normal and perturbed homeostatic conditions.

Our study shows that the interaction of Myosin Vb with Rab11 is of vital importance for the maintenance of epidermal cell size and shape during development. A complete rescue of the epidermal phenotype characterized by the restoration of cell size, cell shape and microridges was seen in Myosin Vb-deficient embryos supplemented with Myosin Vb that has the ability to interact with Rab11 but not with Rab10. Very interestingly, this also rescued the increased endosomal accumulation. It appears that MyosinVb-Rab11 interaction is essential to prevent either the excessive apical endocytosis or for clearing off the increased endocytic load by recycling it back to the plasma membrane (Fig. 7G). Recently, it has been shown that the apical endocytic flux in the absence of Myosin Vb is a cause of microvillus inclusions in enterocytes leading to MVID (Engevik et al., 2019; Sidhaye et al., 2016; Weis et al., 2016). Our data reveal that the disruption of Myosin Vb-Rab11 interaction results in apical inclusion in other epithelial systems such as the epidermis, and is not just restricted to the gut epithelium.

In many tissues such as liver, kidney, heart and cornea, the post-mitotic growth of the cells is observed upon reduction in the cell number. Such post-mitotic growth is known as compensatory cellular hypertrophy and is reported to be important for the restoration of the organ size and functionality (Tamori and Deng, 2014). In the absence of timely growth, the epithelial cells become stretched and flattened until the tissue breaks (Tamori and Deng, 2014). So far, cellular hypertrophy has been shown to be achieved via polyploidization, cell fusion and activation of metabolic signaling pathways (Al-Awqati, 2015; Haga et al., 2005; Honda et al., 1982; Losick et al., 2013; Tamori and Deng, 2013). Our results indicate that in an epithelial system, cells show preferential increase in the apical domain during hypertrophic growth (Fig. 7G). As a consequence of this directional growth, cells acquire flatter morphology and each cell covers a larger area. This is an effective strategy to cover a large surface with relatively fewer cells and by minimizing the energy expenditure on membrane synthesis.

How is the preferential apical growth regulated during cellular hypertrophy? Our data indicate that Myosin Vb and the mTOR pathway cooperate to achieve the preferential apical growth. Our analyses reveal that, in the absence of cell proliferation, cellular hypertrophy is driven by mTOR-dependent activity of FASN. The activation of mTOR by AMPK inhibition (compound C treatment) results in FASN-dependent cellular hypertrophy that is non-directional. The prominent effect of loss of the Exon D isoform on the apical domain in all these hypertrophy conditions indicates that Myosin Vb directs the transport of apical membrane components after their mTOR-dependent synthesis. Although the importance of Myosin Vb-Rab11 is well established in apically directed transport (Hobby-Henderson et al., 2003; Khandelwal et al., 2013; Lapierre et al., 2001; Roland et al., 2011; Swiatecka-Urban et al., 2007; Vogel et al., 2015; Weisz and Rodriguez-Boulan, 2009), our report establishes for the first time that the interaction of Myosin Vb-Rab10 at the Golgi contributes to the growth of the apical domain (Fig. 7G). Our analyses reveal that biogenic vesicles generated at the Golgi depend on Myosin Vb-Rab10 interactions to get transported away from the Golgi to the plasma membrane to achieve compensatory cell growth. In the absence of these interactions, the biogenic vesicles accumulate at the Golgi, acquire endo-lysosomal fate and get cleared up from the cytoplasm. Such accumulation of Golgi-derived endo-lysosomal compartment, in the absence of appropriated transport of lipids from the Golgi, has been previously described (D'Souza et al., 2019; Robenek and Schmitz, 1991). Myosin Vb is an actin-based motor. We did observe short actin tracks near Golgi and actin punctae proximal to the trans-Golgi (K.G. and M.S., unpublished). It is likely that the transient actin polymerization around trans-Golgi vesicles allows the motor to pull these vesicles away from the Golgi and load them onto the microtubular cytoskeleton for further transport to the plasma membrane.

In the absence of the Rab10 interacting isoform of Myosin Vb, we observed accumulation of vesicles near the Golgi; however, there was no reduction in the size of the apical domain in the developing peridermal cells. Given that plasma membrane components are recycled and replenished as part of membrane surveillance mechanisms (Stefan et al., 2017), it is likely that, in the absence of Rab10-Myosin Vb interaction, such mechanisms are perturbed, leading to altered plasma membrane composition. In proliferation-inhibition conditions, cell expansion is essential to maintain the integrity. Our data indicate that the mTOR-FASN axis gets activated by either sensing the increased demand of plasma membrane components during cell growth or due to the altered plasma membrane attributes as a consequence of the disruption of transport

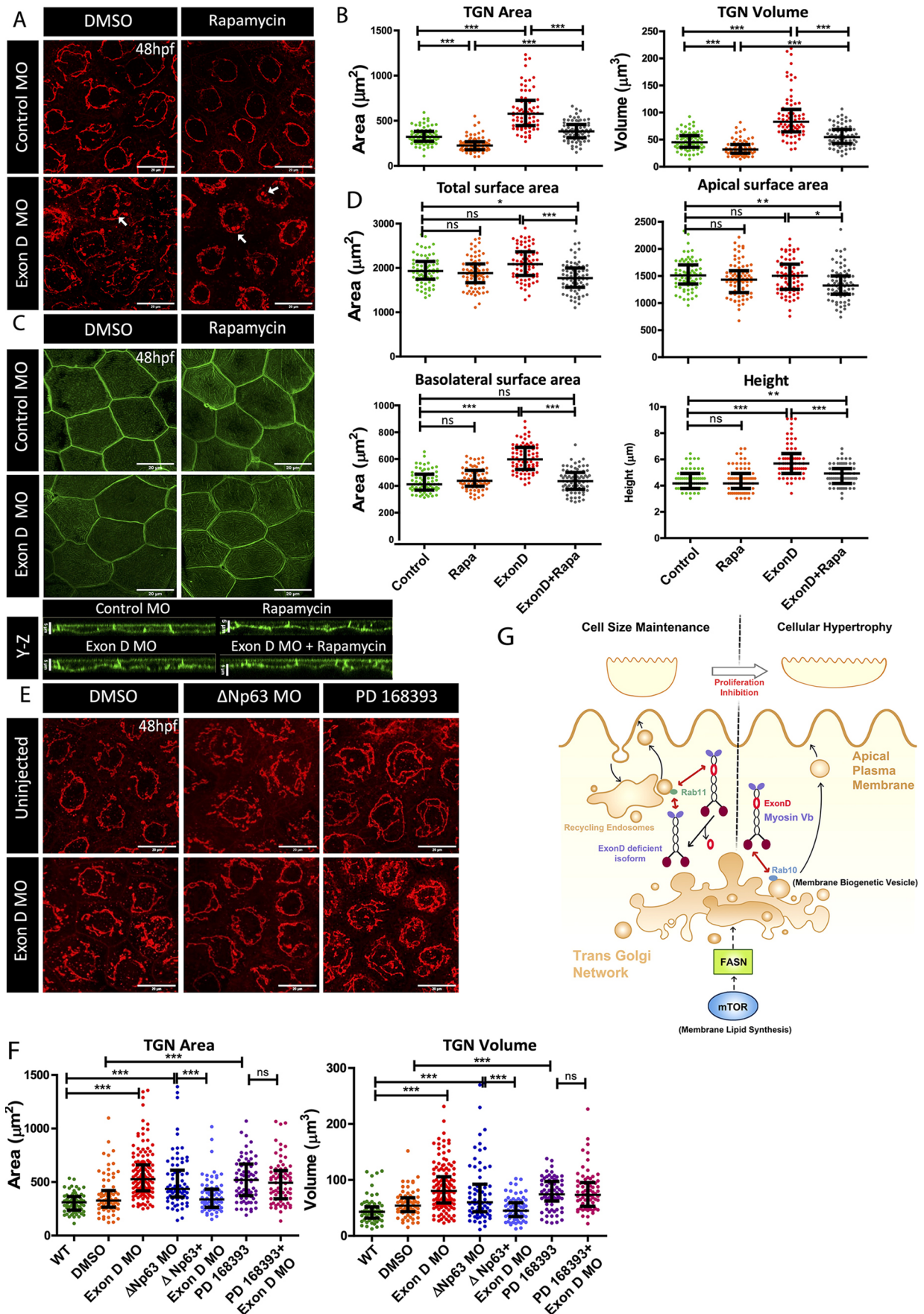


Fig. 7. See next page for legend.

Fig. 7. Myosin Vb and mTOR co-ordination at the TGN. (A,C) Confocal scans of the peridermal cells showing TGN (A) and cell membranes (C) of 48 hpf *Tg(cldnB:lynEGFP)* embryos injected with Exon D morpholino (Exon D MO) and the control embryos treated with DMSO or Rapamycin. (B,D) Quantification of the TGN surface area and volume (B) and total, apical and basolateral cell surface area and cell height (D) under different genetic conditions mentioned along the x-axes. (E,F) TGN morphologies (E) and quantifications of TGN surface area and volume (F) from the uninjected and Exon D morphant embryos either co-injected with Δ Np63 morpholino (Δ Np63 MO) or treated with PD16393 drug. (G) A schematic of Myosin Vb isoforms showing different outcomes depending upon their interaction with either Rab11 or Rab10. Although Myosin Vb-Rab11 interaction is essential to maintain the cell size during development, presumably by regulating recycling of the apical endosomes, MyosinVb-Rab 10 interaction regulates the apically directed transport from TGN downstream of the mTOR-FASN axis, during compensatory cell growth. Data are median \pm interquartile range. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Kruskal–Wallis test with Dunn's post hoc test). ns, not significant. Note that only essential pairwise comparisons are shown in the figure panels B,D and F. For the complete list of pairwise comparisons please refer to Table S6. Scale bars: 20 μ m (X-Y plane of A,C,E); 5 μ m (Y-Z plane of C).

from the Golgi in Exon D morphants. However, in the absence of the Exon D isoform, the newly synthesized membrane lipids under the control of mTOR do not get transported to the apical domain but accumulate at the Golgi and presumably get rerouted to the basolateral domain, making the cells more columnar.

The mTOR activation and FASN upregulation is strongly linked with an increase in the trans-Golgi size as well as cell size. In addition, inhibition of mTOR, as well as FASN synthesis, results in a decrease in Golgi size and prevents cellular hypertrophy. Besides, though not revealed by the Rapamycin treatment, presumably due to weaker inhibition, Torin 1 treatment reveals that mTOR signaling is also essential for peridermal cell growth during development. These data suggest a linear relationship between mTOR-directed lipid synthesis and trans-Golgi morphology, and show that such trans-Golgi scaling is ancillary for cells to grow big, probably by allowing storage of additional membrane and facilitating regulated membrane transport towards increased requirement. Our data also reveal that Myosin Vb functions as a permissive step at the trans-Golgi surface, downstream to the membrane biogenesis step. Thus, the coordination between lipid synthesis and transport takes place at the trans-Golgi, which is a known sorting and trafficking hub of the cell.

Overall, our study reveals that interactions of Myosin Vb with Rab11 and Rab10 fine tune epithelial cell morphology during both development and compensatory cell growth.

MATERIALS AND METHODS

Ethics statement

Zebrafish rearing, handling and experimentation were carried out as per the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and approved by the institutional animal ethics committee (TIFR/IAEC/2017-11).

Fish strains

We used *Tübingen* (Tü) strain and *gsp^{NS042}* allele of *goosepimples/myosin Vb* in this study (Sonal et al., 2014). Transgenic lines *Tg(cldnB:lynEGFP)* (Haas and Gilmour, 2006), *Tg(h2afx:EGFP-Rab11a)^{mw6}* and *Tg(h2afx:EGFP-Rab7)^{mw7}* (Clark et al., 2011) were used for experiments requiring visualization of plasma membrane, recycling endosomes and late endosomes, respectively.

Morpholino and plasmid injections

Myosin Vb splice site morpholino (250–300 pl of 200 μ M) (Sonal et al., 2014), Δ Np63 morpholino (250–300 pl of 50 μ M) (Bakkers et al., 2002), Myosin Vb Exon D splice site morpholino (5'-GCTTTACTGCCATCCGAGTGCAAGA-3'; 300–500 pl of 500 μ M)

(Liu et al., 2013) and its 5-base mismatch control (5'-GCTTTAGTCC-CATGCCAGTCCAAGA-3') were microinjected in zebrafish embryos at the one-cell stage using PV830 Pneumatic PicoPump. We also used the second Exon D slice-site morpholino (5'-ACTGCCATCCGAGTGCAAGA-GAGCC-3') for validation of the phenotype.

EGFP-GalT plasmid (500–600 pl of 50 ng/ μ l) was injected at the one-cell stage. This plasmid was generated by the Jennifer Lippincott-Schwartz lab (Addgene plasmid #11929) and previously used by the Lila Solnica-Krezel lab in zebrafish.

RNA synthesis and injections

For RNA rescue of *myosin Vb* morphants, both Exon D (+) and Exon D (–) isoforms of zebrafish *Myosin Vb* coding DNA sequences (CDS) were synthesized. Using Clustal Omega software (McWilliam et al., 2013), the zebrafish *myosin Vb* sequence was aligned with the human CDS (Roland et al., 2011) to determine the sites for introduction of mutations to abolish Rab11 binding (Fig. S1A). By introducing these mutations in both Exon D (+) and Exon D (–) isoforms, two additional Rab11^{−/−} versions for D1705E and Q1739R in Exon D (+), and D1678E and Q1712R in Exon D (–) were synthesized (GeneArt, Thermo Fisher Scientific). All Myosin Vb constructs were synthesized in pMK vectors with additional restriction sites and a linker at 3' terminal (GGAGGAAGCGGAGGAAGCGGAGGAGGAAGC) and sub-cloned into pCS2+8CmCherry and pCS2+8CeGFP vectors.

Following linearization of the plasmid, mRNA was synthesized using the SP6 mMessageMachine *In-vitro* Transcription Kit (Thermo Fisher Scientific, AM1340) followed by mRNA purification using BioRad MicroBio-Spin columns P-30, Tris (732–6250) and eluted in nuclease-free water. For the rescue experiments, the desired RNA (1.2 nl of 65–75 ng/ μ l in nuclease-free distilled water) was injected in the cytoplasm at the one-cell stage before the Myosin Vb splice site morpholino injection. For clonal rescue in Exon D morphants, the Exon D morpholino was injected at the one-cell stage, followed by injecting 0.5–0.8 nl of mRNA for the desired Myosin Vb isoform (75 ng/ μ l) and Citrine mRNA (100 ng/ μ l) as a tracer in one of the cells at the 16-cell stage. The phenotypic rescues in embryos and clones were assessed at 40 hpf.

Rab10 CDS was synthesized (GeneArt, Thermo Fisher Scientific) and subcloned in pCS2+8NmCherry and pCS2+8NeGFP vectors with the same linker at the N-terminal of the gene as stated above. This plasmid was linearized and, post *in vitro* transcription, \sim 1.2 nl of 120 ng/ μ l RNA was microinjected. pCS2+8NmCherry (Addgene plasmid #34936) and pCS2+8NeGFP (Addgene plasmid #34953) were gifts from Amro Hamdoun (Gökirmak et al., 2012).

RT-PCR for the validation of knockdown of the Exon D isoform

To confirm specific knockdown of the Exon D isoform, both control as well as Exon D morpholino-injected embryos (30–40 each) were fixed in TRIzol Reagent (Thermo Fisher Scientific, 15596026) at 48 hpf. Embryos were homogenized, stored at -80°C for 3–4 h and processed for RNA isolation. The RNA was dissolved in nuclease-free water and used to prepare cDNA using SuperScript™ IV First-Strand Synthesis System (Invitrogen, 18091050). PCR amplification of Myosin Vb along with Actin as a control from cDNA was performed using the following primers: Exon D FP 5'-CTCAACGTGACAGGACGATATAAACG-3', Exon D RP 5'-GTGAAGGGCTTCCAACATCC-3' (sequence provided by Liu et al., 2013), Actin FP 5'-ATCACACCTTCTACAACGAGC-3', Actin RP 5'-CATCACCAGAGTCCATCAGC-3'.

Drug treatments

Embryos at 20 hpf were treated with 10 μ M concentration of EGFR inhibitor PD168393 (Calbiochem, 513033) and 30 μ g/ml Cerulenin (Sigma-Aldrich, C2389). Treatments with 2 μ M Rapamycin (Santa Cruz Biotechnology, SC-3504), 5 μ M Torin 1 (Abcam, ab218606) and 20 μ M Compound C (Sigma-Aldrich, P5499) were started from 24 hpf. Rapamycin was changed once at 36 hpf. Before fixation at 48 hpf, embryos were given three washes of E3 (embryonic media) of 5 min each (without methylene blue). All the stocks were prepared in DMSO (Sigma-Aldrich, D8418). During treatments, DMSO

concentration was adjusted to 1% in the test and control samples. DMSO-treated wild-type embryos are used as controls in all drug experiments.

BODIPY C5-Ceramide, dextran and lysotracker assays

To mark the biogenic vesicles in the live periderm, embryos were pulsed with 3 μ M BODIPYTM FL C5-Ceramide complexed to bovine serum albumin (Invitrogen, B22650) for 2.5 h at 29°C followed by three 5 min washes with E3, and chased in E3 for another 3 h before fixing at 36 hpf in 4% paraformaldehyde (PFA) in PEMTT (0.1 M PIPES, 5 mM EGTA, 2 mM MgCl₂ · 6H₂O, 0.1% Triton X-100, 0.1% Tween 20, pH 6.8) (Song et al., 2013) overnight followed by serial glycerol upgradation (30%, 50% and 70%) preceding confocal imaging.

For endocytosis assays, the larvae were incubated in 50 μ g/ml solution of 10 kDa Alexa 546-conjugated Dextran (Invitrogen, D-22911) in E3 for 3 h followed by washing and mounting for live imaging, or for 12 h followed by washing, PFA fixation and immunostaining for fixed imaging. For visualizing acidic compartments, embryos were incubated in 5 μ M Lysotracker Red DND-99 (Invitrogen; L7528) in E3 for 3 h followed by washes with E3 and live imaging.

Immunostaining and WGA staining

Immunostainings were performed according to previously published protocol (Gupta and Sonawane, 2020). Briefly, embryos were fixed in 4% PFA in PBS followed by methanol post-fixation. For Rab7 immunostaining, fixation was carried out in 4% PFA in PEMTT. Embryos were permeabilized by washing in PBT, followed by blocking in 10% normal goat serum (NGS) in PBT and primary antibody treatment. The following primary antibodies were used in this study: rabbit anti-GFP (1:200, Torrey Pines Biolabs, TP401), 12A6 mouse anti-GFP (1:100, Developmental Studies Hybridoma Bank, 12A6), rabbit anti-RFP (1:200, Abcam, ab62341), rabbit anti-pS6 ribosomal protein (S240/244) (1:100, Cell Signaling Technology, 2214S), mouse anti-E cadherin (1:200, BD Biosciences, 610182), rabbit anti-FASN (1:100, Novus Biologicals, NB400-114), anti-acetylated tubulin (1:100, Sigma-Aldrich, T7451), rabbit anti-Rab10 (1:100, Sigma prestige, HPA045611). Primary antibodies were washed off, and embryos were incubated with secondary antibodies or fluorescent conjugates. The following secondary antibodies and fluorescent conjugates were used in this study: conjugated WGA Alexa Fluor 594 and 633 (1:200, Invitrogen, W11262 and W21404); Alexa 488 conjugated goat anti-rabbit IgG and goat anti-mouse IgG antibodies (1:250, Invitrogen, A-11034 and A-11029); Cy3- and Cy5-conjugated goat anti-rabbit IgG antibody (1:750, Jackson ImmunoResearch, 111-165-144 and 111-175-144); Cy3- and Cy5-conjugated goat anti-mouse IgG antibody (1:750, Jackson ImmunoResearch, 115-165-146 and 115-175-146) and Cy3-conjugated goat anti-rat IgG antibody (1:750, Jackson ImmunoResearch, 112-165-167). Post incubation, secondary antibodies were washed off and embryos were post fixed in 4% PFA followed by serial upgradation in glycerol.

Image acquisition

Brightfield images were taken on stereomicroscope (SteREO Discovery, Zeiss) mounted with AxioCam (Zeiss), after anesthetizing the embryos with 0.04% MESAB (Sigma-Aldrich, E10521) and mounting in low melting agarose (Sigma-Aldrich, A9414) on a glass slide.

For live imaging, embryos were anesthetized in 0.04% Tricaine solution in E3 buffer and mounted in a 35 mm glass bottom Petri dish in low melting agarose with the widest part of the dorsal head against the glass bottom. For fixed samples, embryo heads were dissected out and mounted on a glass slide in 70% glycerol, with the widest part of the dorsal head against the glass coverslip (Gupta and Sonawane, 2020). Imaging was performed on the dorsal head epidermis using Zeiss 710 and 880 confocal microscopes. For cell surface area quantifications, confocal stacks were obtained using EC neo-fluor 63 \times 1.3 oil objective with digital zoom 1.5, pinhole 1 AU, resolution 1024 \times 1024, averaging 4, bit depth 16 and optimal slice interval of 0.378 μ m. High sensitivity detectors on Zeiss 880 were used for visualizing Rab7-GFP and Rab10 mCherry localization. For imaging BODIPYTM FL C5-Ceramide, samples were subjected to λ_{exc} =488 nm on

Zeiss 710. The signal was collected simultaneously at λ_{em} ~515 nm (green) and ~620 nm (red) on two different tracks. Some of the images were optimized post acquisition for better representation by adjusting brightness/contrast equally for both control and test images.

Image analysis

Cell surface area quantification

To measure the total cell surface area of the peridermal cells, experiments were conducted using the *Tg(cldnB:lynEGFP)* line (Haas and Gilmour, 2006) and embryos were immunostained using anti-GFP and E-cadherin antibodies. To quantify surface area, cell outlines in each z-slice were manually traced, and area and perimeter were measured using the ImageJ measure function. The area of the top-first slice was estimated as the cross-section area and added to the ridge area to calculate the apical surface area. To quantify the ridge surface area, maximum intensity projection for the two apical slices spanning the approximate height of the microridges was smoothed and thresholded using an auto local thresholding function (Otsu method with radius 8 represented the microridges faithfully). Microridges for each cell were cropped out, converted to binary, and the perimeter was measured using the analyze particle function. The sum of the perimeter was then multiplied by two times the slice interval to obtain the total ridge area. To find out basolateral surface area, the perimeter of each slice starting with the first E-cadherin (basolateral marker) marked slice was measured by tracing GFP-marked membrane and multiplied by 0.378 (height of an optical z-slice in μ m), and added to the surface area of the last slice. The apical and basolateral surface areas were summed to estimate the total cell surface area.

TGN volume and surface area quantification

We used *Tg(cldnB:lynEGFP)* line to mark the membrane for visualizing the cell boundaries of individual cells along with WGA as a TGN marker for these experiments. All images were deconvolved with Huygens Professional version 18.10 (Scientific Volume Imaging, <http://svi.nl>), and volume and surface area of the TGN were calculated using the following parameters: average background value=computed from the image, number of iterations=30, signal to noise ratio=20 and quality change threshold=0.01. These settings were kept constant across all the samples. GFP channel was used to crop the individual cells from the deconvolved images and cropped cells were then surface rendered for WGA-marked TGN to quantify the volume and surface area of the TGN of each cell. The surface rendered images were compared with the original deconvolved images and visually checked for accurate representation. The deconvolved images of the TGN were then analyzed using the advanced object analyzer plugin (garbage volume=5 and threshold=10-12% were used for all experiments). The individual values of volume and surface area per object in the WGA channel were added to obtain total volume and surface area of the TGN, which were then plotted using GraphPad PRISM software.

Fluorescence intensity quantification

For estimation of the fluorescence intensity ratio for BODIPY C5-Ceramide staining, selected post-TGN vesicle boundaries were marked using the freehand selection tool and total intensity readings in each of the channels [λ_{em} ~515 nm (green) and ~620 nm (red)] were measured using the measure tool of the ImageJ software. The same selection mark was then placed on the adjoining TGN region chosen randomly from the same cell to calculate the intensity ratio. Equal number of vesicles and TGN regions were quantified per embryo.

For estimating mean fluorescent intensity for FASN, GFP-marked membrane was used to trace the cell boundaries in each z-slice of the confocal z-stack by using the freehand selection tool in the ImageJ software, and mean intensity measurements were recorded and summed for each cell.

Statistical analysis and graphs

Sigma Plot software was used for statistical analysis for all the quantitative tests. We used the Mann-Whitney test to compare the medians of two test groups, and the Kruskal-Wallis test with Dunn's post hoc test to compare medians of multiple test groups. The datasets represented by dot-plots

show medians with interquartile range, and those represented with bar graph show error bars denoting \pm s.e.m. Graphs were plotted using GraphPad PRISM software. Detailed statistical comparisons are presented in Tables S1-S12).

The sample sizes were empirically determined based on our previous studies. The sample size (n) was sufficiently large to correctly and reproducibly parse out the difference and/or reveal the trend. No data were excluded during the statistical analysis. Only those experiments that failed owing to mRNA or morpholino quality, injection volumes or quality of embryos were not considered for further analysis. For the knockdown studies, rescue experiments, drug treatments and imaging, embryos were selected randomly. Absolutely no attempts were made to pre-select embryos based on any criterion. For quantification of cellular features, randomization was achieved by analyzing every alternate cell starting from the upper left corner. Most of the experiments were quantified with appropriate quantification methods eliminating any bias and requirement for the blind analysis. During statistical analysis, an *a priori* assessment of data parameters (e.g. normality and variance) was carried out to ensure that the data met the assumptions of the test used.

Details of number of experimental repeats, number of embryos imaged and number of cells analyzed for quantifications are listed in Table S13.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: K.G., M.S.; Methodology: K.G., S.M., S.S.; Formal analysis: K.G., S.M., S.S.; Investigation: K.G., S.M., S.S.; Writing - original draft: K.G.; Writing - review & editing: M.S., K.G.; Supervision: M.S.; Project administration: M.S.; Funding acquisition: M.S.

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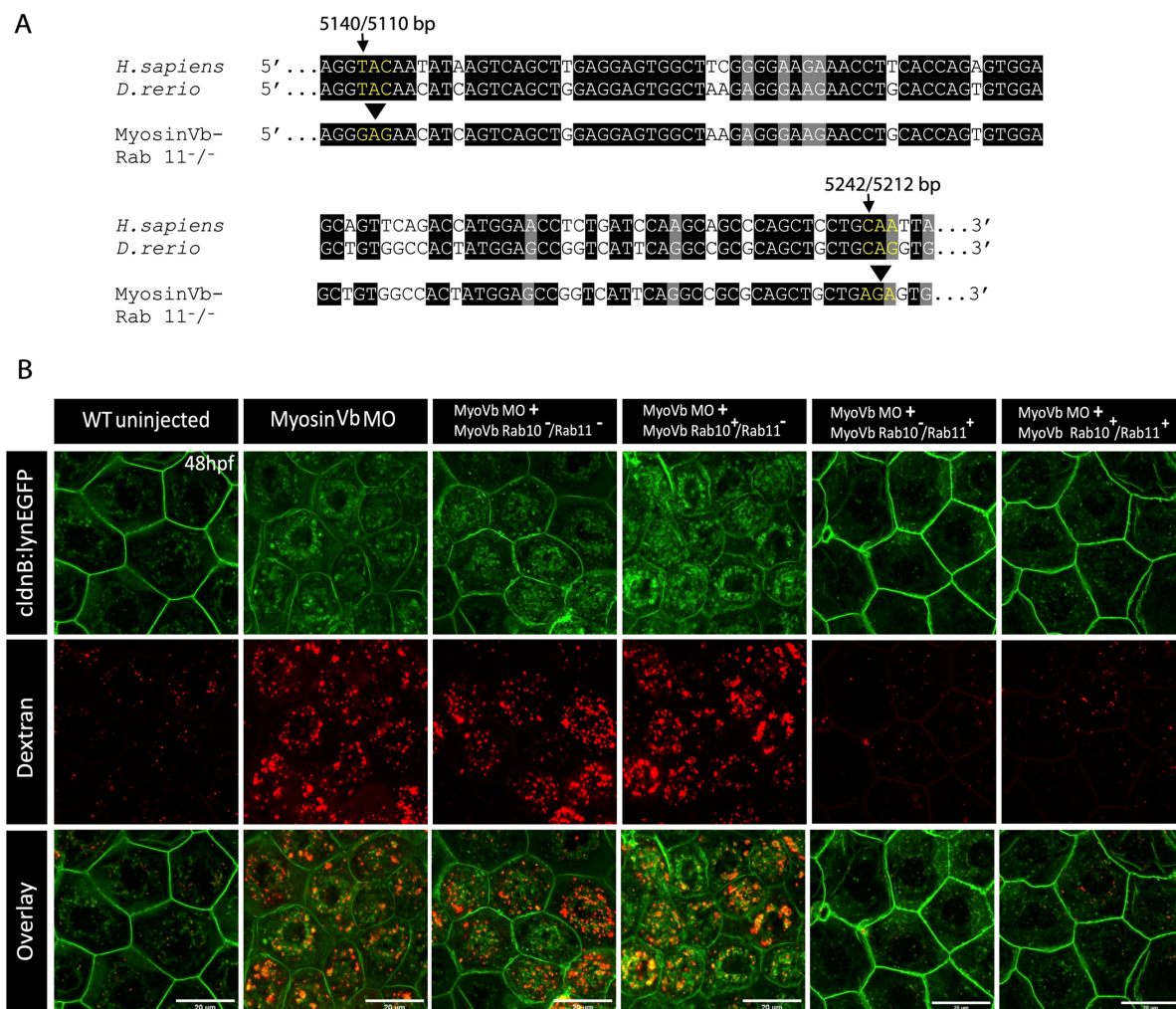


Fig. S1. Myosin Vb-Rab11 interaction is essential to prevent the accumulation of apically derived endosomes arising from fluid phase endocytosis

(A) Homology analysis for Human vs Zebrafish Myosin Vb ExonD CDS from 5137/5107

(Human/Zebrafish) to 5247/5217 bases using Clustal omega multiple sequence alignment

tool. Mutations introduced to abolish Rab11 binding of Myosin Vb (MyoVb-Rab11^{-/-}) are

shown in yellow. (B) Representative confocal images of the Lyn-EGFP marked peridermal

cells in 48hpf embryos of the given genetic combinations incubated in Rhodamine-Dextran

(10 kDa).

Scale Bars in B = 20μ.

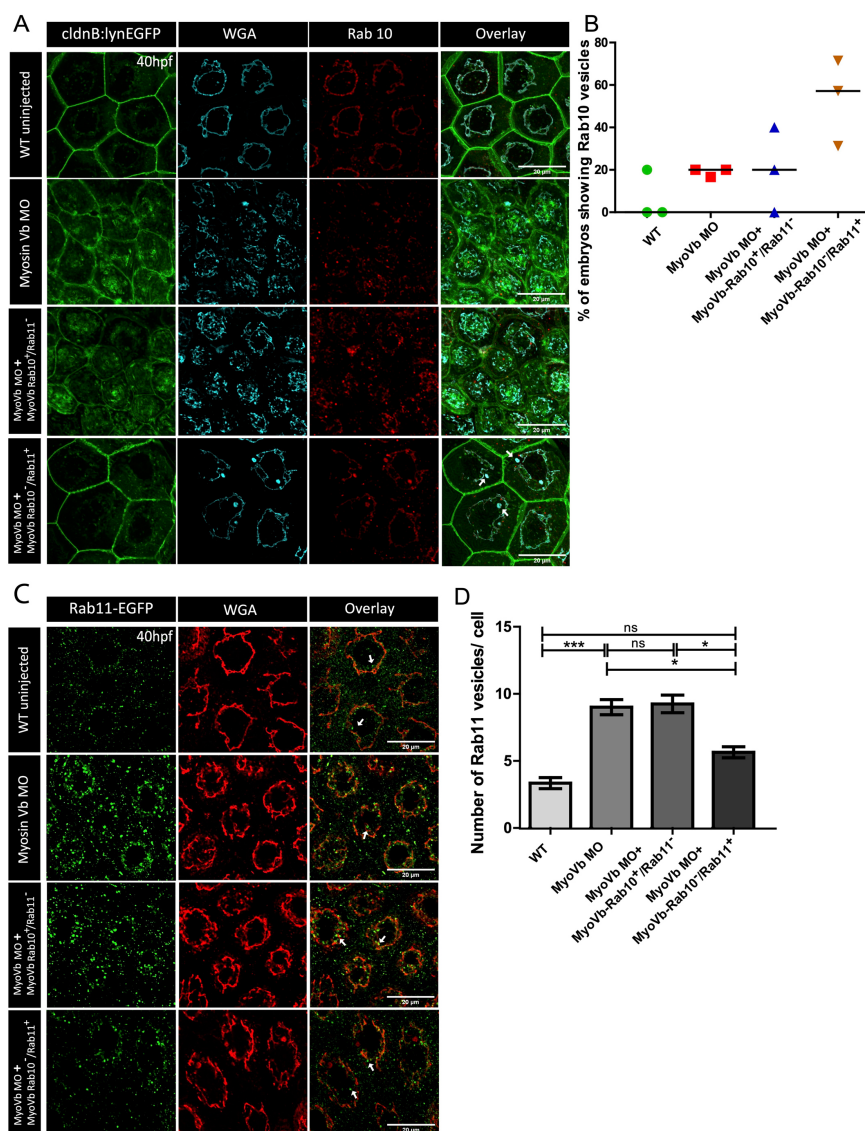


Fig. S2. The effect of Myosin Vb isoforms on Rab10 and Rab11 compartments

(A) Confocal images of the peridermal cells of the 40 hpf *Tg(cldnB:lynEGFP)* Myosin Vb morphants rescued with mRNA encoding for MyoVb-Rab10⁺/Rab11⁻ or MyoVb-Rab10⁻/Rab11⁺ and stained using anti-GFP antibody, WGA and anti-Rab10 antibody. (B) Quantification of percentage embryos showing Rab10 associated TGN phenotype in all the different genetic conditions mentioned. (C) Confocal images of the peridermal cells of the 40hpf *Tg(h2afx:EGFP Rab11a)^{mw6}* MyoVb morphants rescued with MyoVb-Rab10⁺/Rab11⁻ or MyoVb-Rab10⁻/Rab11⁺ mRNA and labeled using anti-GFP antibody and WGA. (D) Quantification of the number of Rab11 vesicles in all the different genetic conditions mentioned.

Scale bars in A and C correspond to 20µm. White arrows in A and C point to Rab10 and Rab11 vesicles, respectively. Asterisks in D denote the statistical significance at p<0.05. Key pairwise comparisons are shown in D; for the rest of the comparisons please refer to Table S7.

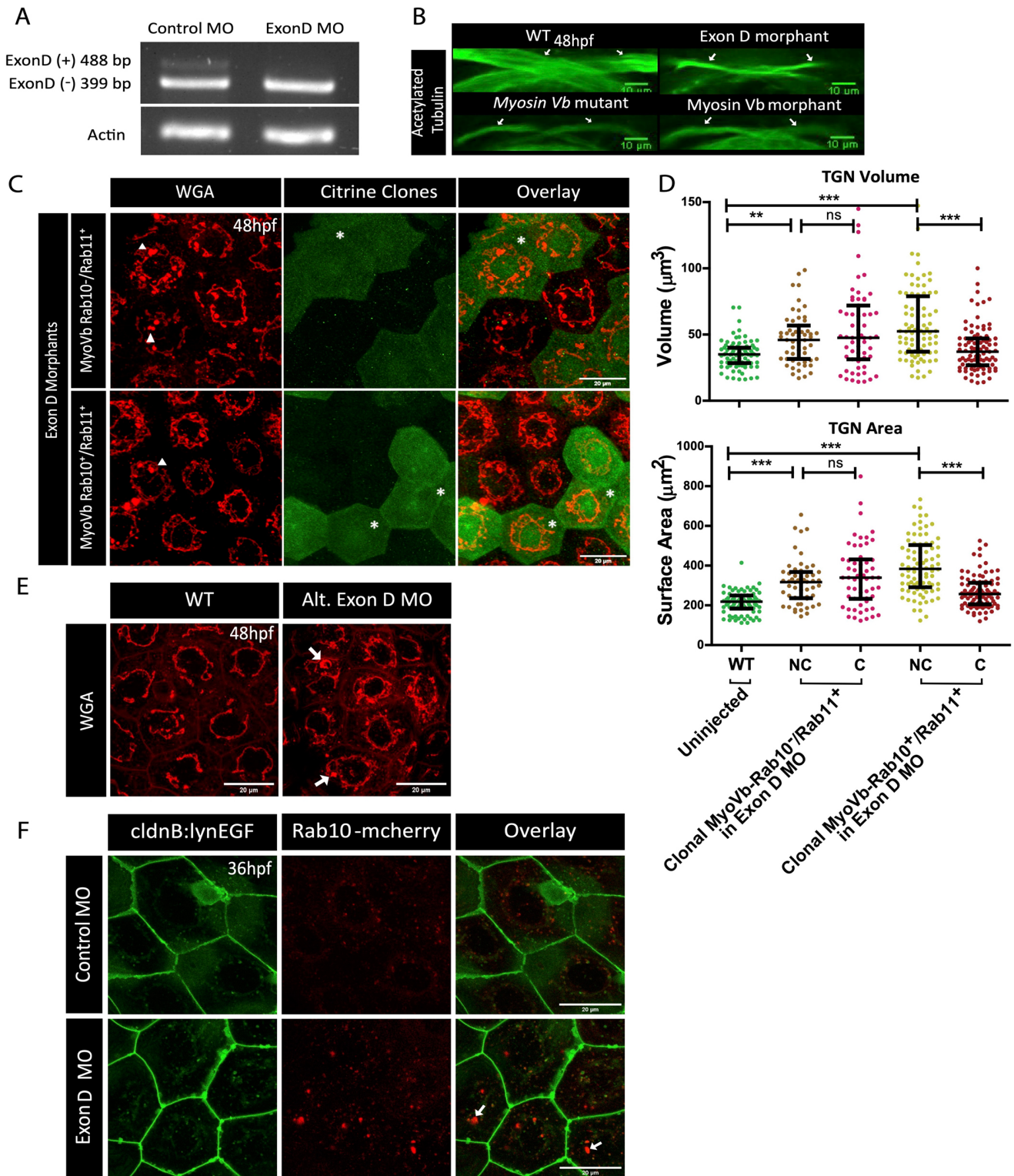


Fig. S3. Validation of Exon D morpholino.

(A) An RT-PCR analysis of control (control MO) and Exon D morphants (Exon D MO) at 48hpf reveals specific loss of 488 bp band representing isoform of Myosin Vb containing Exon D domain, upon morpholino injection, while the 399 bp band corresponding to the isoform without Exon D does not show any reduction. Actin is used as an RT-PCR control. (B) Confocal images of optic nerve of 48hpf wild type, Exon D morphants, *gsp* mutant and Myosin Vb morphants embryos stained using acetylated tubulin antibody. Note that Exon D morphants display thinning of optic nerve, which is also observed in the *gsp* mutants and Myosin Vb morphants. (C) Confocal images of 48 hpf *Tübingen* (*Tü*) embryos showing TGN and post-TGN marked with WGA in Exon D morphants clonally injected with either MyoVb-Rab10⁺/Rab11⁺ or MyoVb-Rab10⁻/Rab11⁺ mRNA along with Citrine mRNA (tracer) to mark the clones. (D) Quantification of TGN volume and surface area in clonal (=C) and non-clonal cells (=NC) in different genetic conditions mentioned in the figure. (E) WGA staining reveals that the alternative morpholino for Exon D splice site (Alt. Exon D MO) shows the similar branching and vesicle accumulation trans-Golgi phenotype. (F) Live confocal scan of the control and Exon D morphant peridermal cells at 36hpf show large Rab10-mCherry labeled vesicles in the morphants.

Scale bars in B correspond to 10µm and those in C, E and F to 20µm. White arrows in B indicate the optic nerve and those in E and F point to accumulated vesicles; white arrowheads and asterisks in C point to post-TGN vesicles and clones, respectively. Asterisks in D denote the statistical significance at $p < 0.05$. Only key pairwise comparisons are shown in D; for the rest of the comparisons please refer to Table S8.

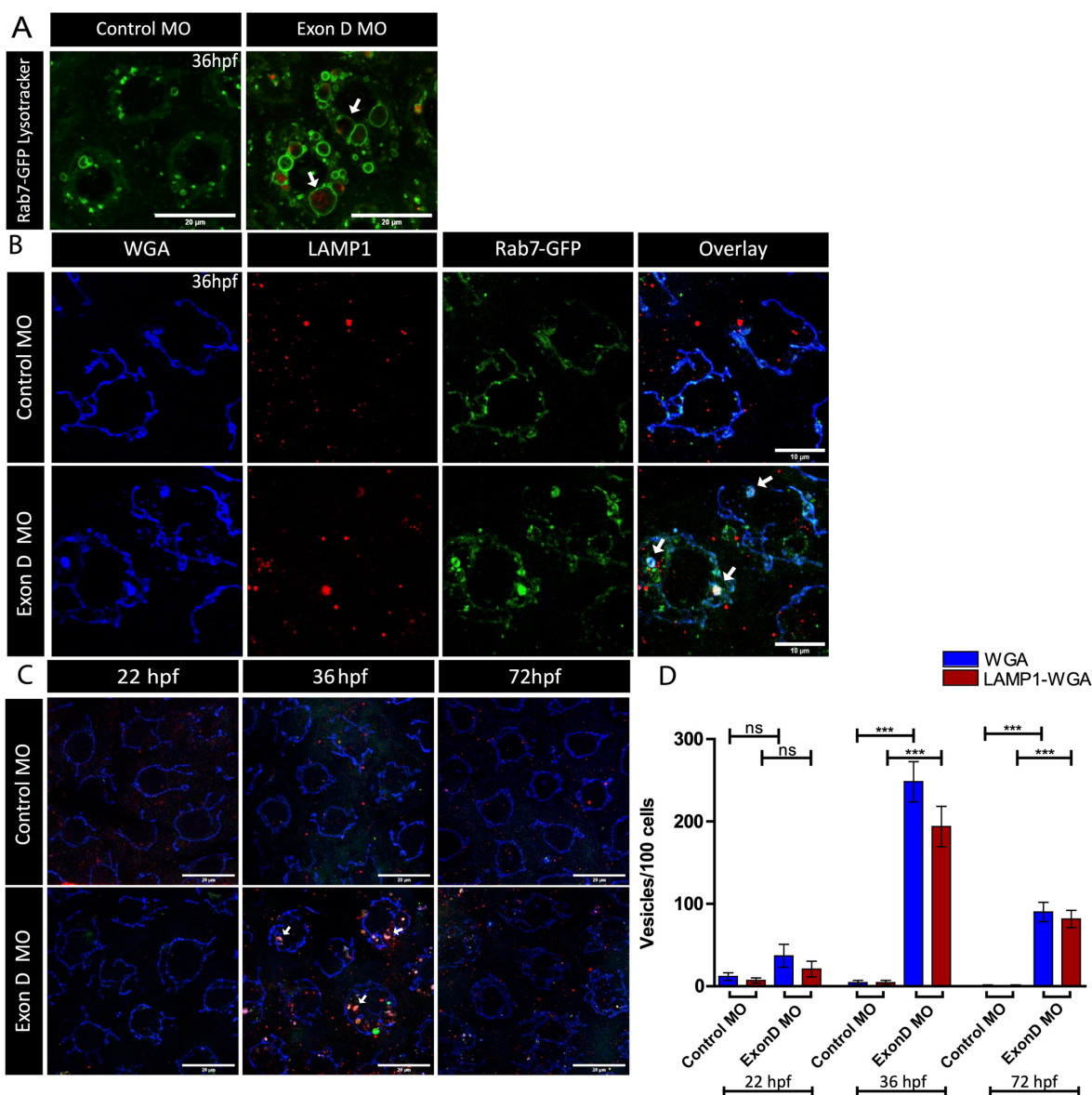


Fig. S4. Trans-Golgi vesicles accumulated upon the knockdown of Exon D isoform acquire late-endolysosomal fate in the peridermal cells.

Confocal images of the peridermal cells (A, B, C) of the embryos from *Tg(h2afx: EGFP-Rab7)^{mw7}* line injected with Exon D morpholino and (A) labeled with lysotracker dye and imaged live at 36hpf or (B) immunolabeled with WGA and LAMP-1 upon fixation at 36-hpf or (C) during development at 22, 36 and 72hpf. Note the presence of large Rab7 marked vesicles (white arrows in A). Many of the large post trans-Golgi vesicles show co-labeling with Rab7 and LAMP-1 (white arrows in B and C), and their size decreases by 72hpf. (D) Quantification of vesicles (presented in C) stained with WGA and their fraction showing co-localization with LAMP1 at 22, 36 and 72 hpf.

Asterisks in D denote the statistical significance at $p < 0.05$. For detailed statistical analysis table please refer to Table S9. Scale bars in A and C corresponds to 20μ and those in B to 10μ .

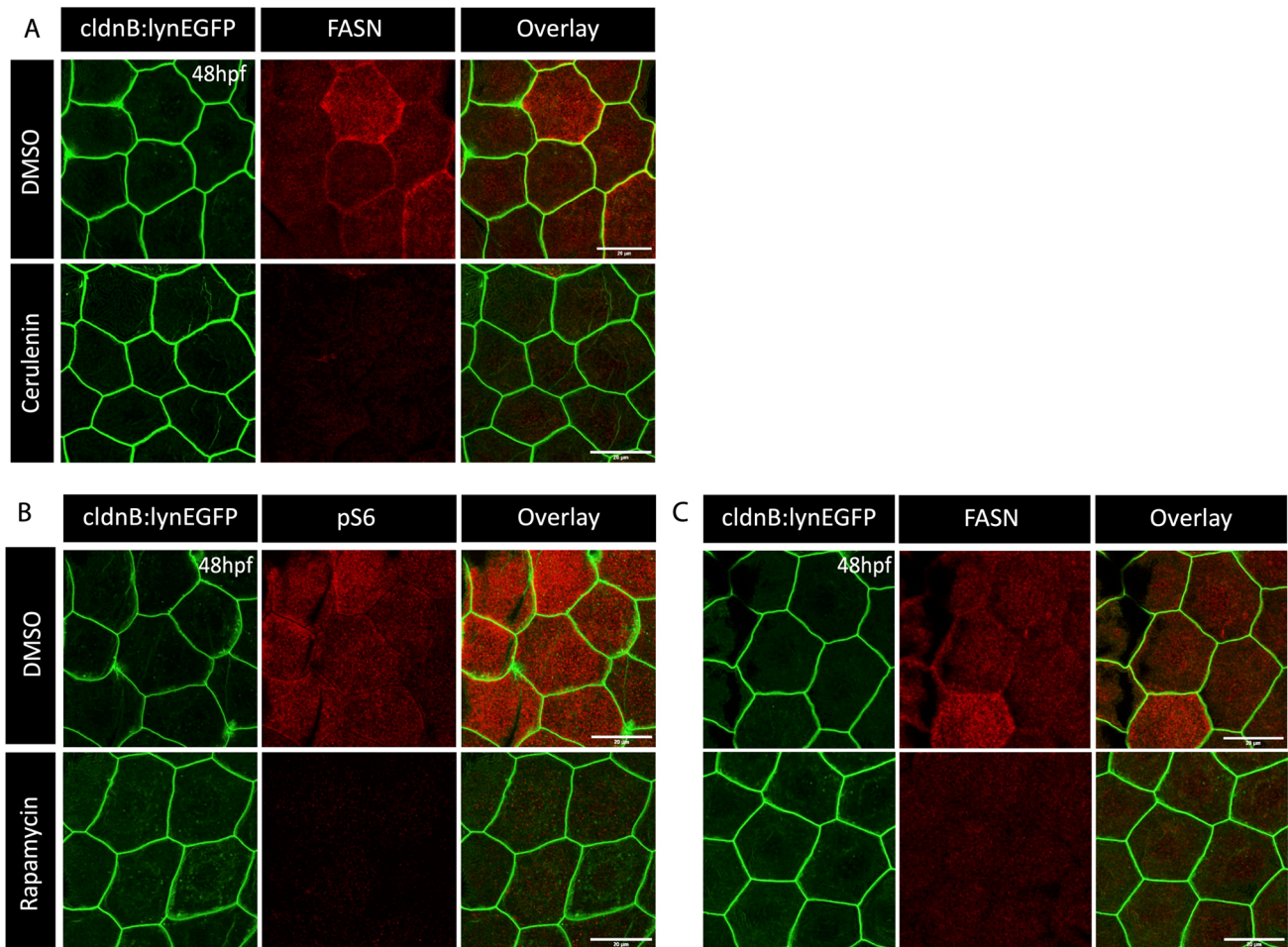


Fig. S5. Cerulenin treatment leads to a decrease in FASN levels while Rapamycin treatment results in a reduction in both pS6 and FASN levels in the peridermal cells. Immunostainings using anti-GFP (A, B, C), anti-pS6 (B) and anti-FASN (A, C) antibodies followed by confocal microscopy of 48h old embryos from the *Tg(cldnB:lynEGFP)* line treated with (A) Cerulenin and (B, C) Rapamycin. Scale bars in A, B and C equals to 20μ.

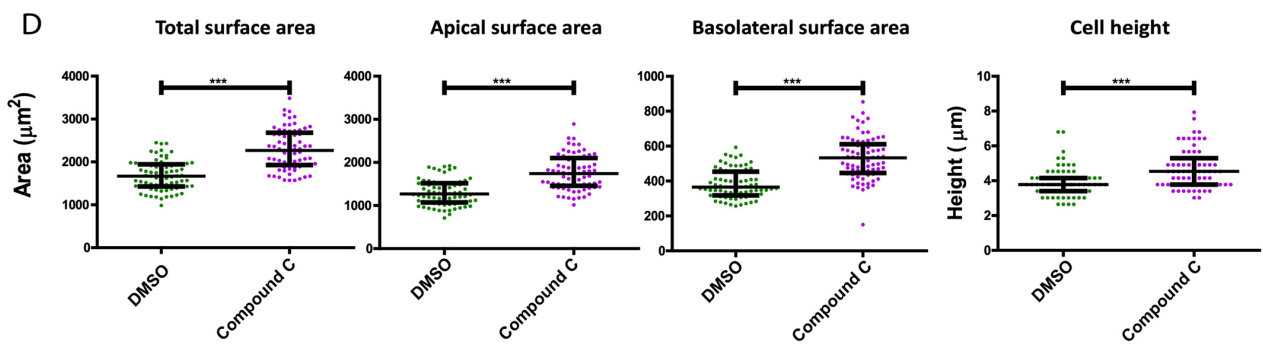
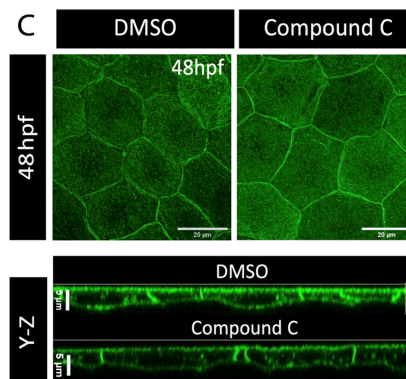
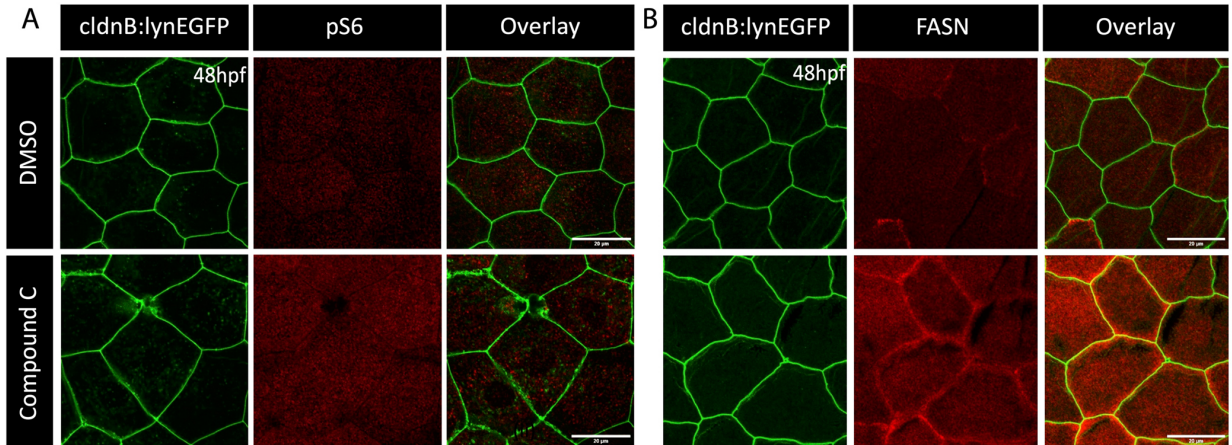


Fig. S6. Compound C treatment activates mTOR signaling and results in increased cell size in the zebrafish embryonic periderm.

Immunostaining using anti-GFP (A, B, C), anti-pS6 (A) and anti-FASN (B) antibodies at 48 hpf followed by confocal microscopy on embryos obtained from *Tg(cldnb:lynEGFP)* line and treated with compound C and DMSO as a vehicle control (A-C). Note that Compound C treated embryos display increased levels of mTOR downstream target - (A) pS6, and (B) FASN, and (C) increased size of peridermal cells. (D) Quantifications of the total, apical and basolateral surface area, and cell height in Compound C treated embryos.

Scale Bars in A-C (X-Y plane) correspond to 20 μ and in C (Y-Z plane) to 5 μ . Asterisks in D indicate statistically significant difference at $p < 0.05$ (refer to Table S10).

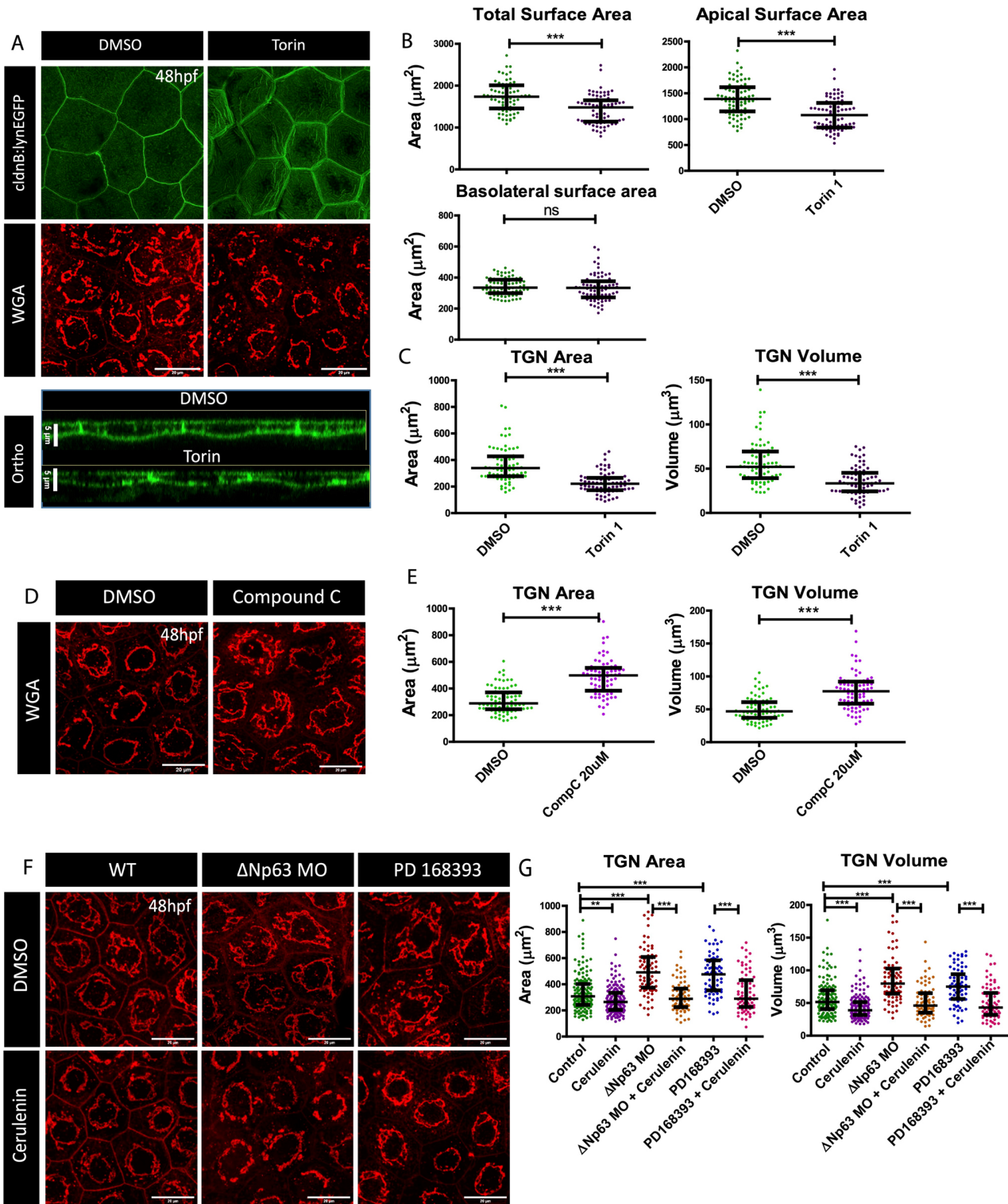


Fig. S7. mTOR signaling and FASN regulates cell size and TGN morphology in the peridermal cells.

(A) Confocal scans along X-Y and Y-Z planes of the peridermal cells from the embryos at 48hpf treated with DMSO or Torin 1, and stained using anti-GFP antibody and WGA (TGN).

(B) Graphical representation of quantification of total, apical, and basolateral surface area,

and (C) TGN area and volume upon Torin 1 treatment. Confocal micrographs showing WGA

marked peridermal TGN of (D) Compound C treated embryos or (F) Cerulenin treated Δ Np63

morphants and PD16393 delivered embryos. (E, G) Quantifications of TGN surface area and

volume in given genetic conditions and/or under given treatments. Scale bars: X-Y plane =

20 μ ; Y-Z plane = 5 μ . Asterisks in B, C, E and G denote the statistically significant difference at

$p < 0.05$. ns = not significant. Note that only the essential comparisons are shown in G. For the

complete list of comparisons please refer to Table S11.

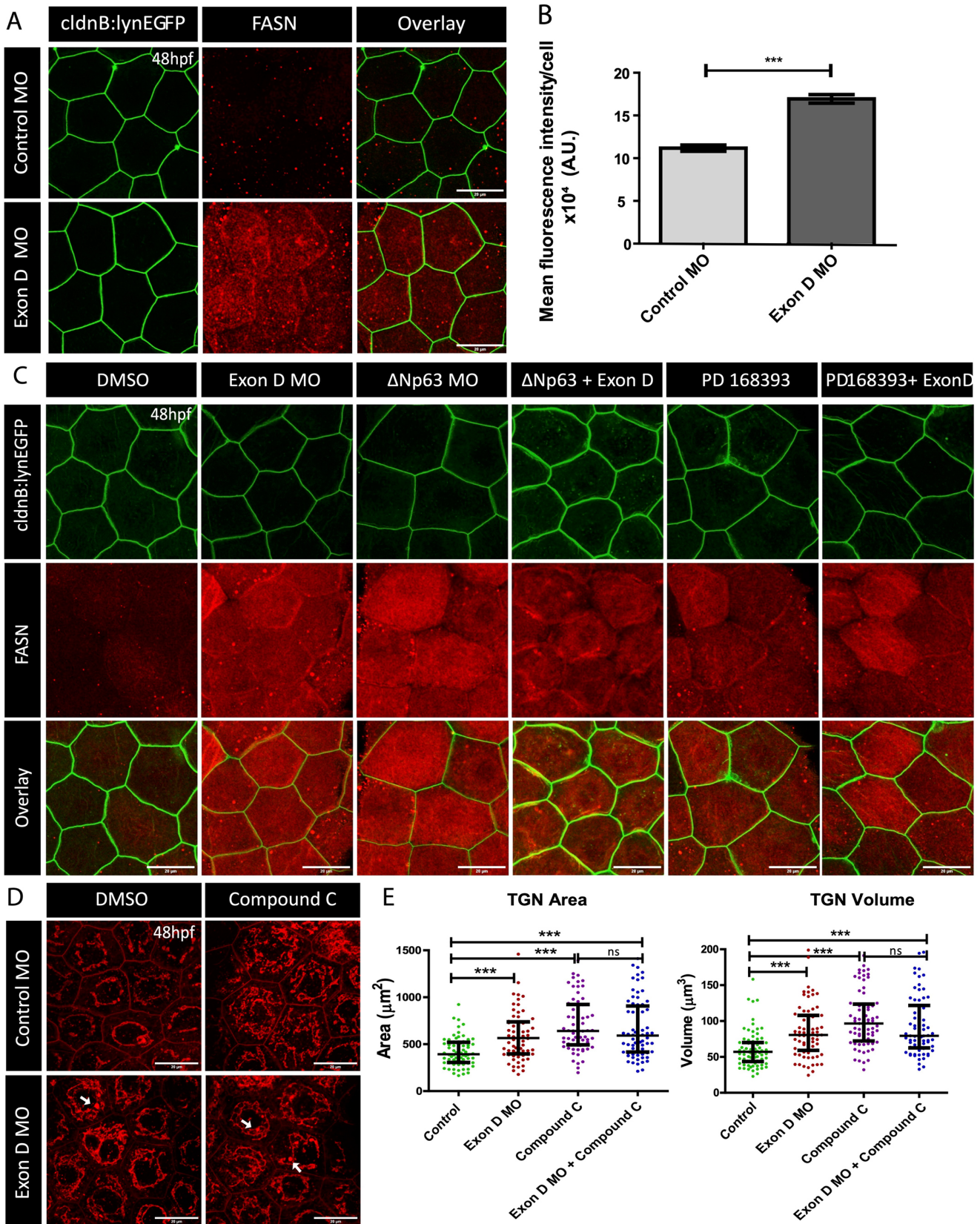


Fig. S8. FASN is upregulated upon cell proliferation inhibition in Exon D morphant; TGN expansion phenotype in Compound C treated embryos is not rescued upon Exon D knockdown.

Confocal images of the peridermal cells of 48hpf control and Exon D morphant *Tg(cldnB:lynEGFP)* embryos showing (A) FASN immunostaining and (B) intensity quantification. (C) Confocal images of the peridermal cells of 48hpf control and Exon D morphant embryos in *Tg(cldnB:lynEGFP)* background under cell proliferation inhibition (Δ Np63 MO and PD16393 treatment) paradigms showing FASN immunostaining. (D) Confocal micrographs of WGA marked TGN in Exon D morphant *Tg(cldnB:lynEGFP)* embryos treated with Compound C or DMSO and (E) quantification of TGN surface and volume.

Scale bars in A, C and D = 20 μ . White arrows in D point to post trans-Golgi vesicles.

Asterisks in B and E denote the statistically significant difference at $p < 0.05$. Only essential pairwise comparisons are shown in E. For the complete list of pairwise comparisons please refer to Table S12.

Table S1. Statistical analyses for quantitative data in Figure 2.

(A) Statistical analysis for comparing TGN surface area and volume between control embryos and Exon D morphants as shown in Figure 2D:

Table Analyzed	TGN Surface Area
Column A	WT control
vs	vs
Column B	ExonD MO
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	4160 , 7015

Table Analyzed	TGN Volume
Column A	WT Control
vs	vs
Column B	ExonD MO
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	4327 848

(B) Statistical analysis of comparing BODIPY-ceramide vesicle count between control embryos and Exon D morphants as shown in Figure 2H:

Table Analyzed	Vesicle count
Column A	Control
vs	vs
Column B	Exon D MO
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A, B	154, 342
Mann-Whitney U	18.00

Table S2. Statistical comparison for cell surface area as shown in Figure 3. (A)

Statistical analysis for cell surface area quantification in Figure 3B.

Table Analyzed	Total surface area
Column A	Control MO
vs	vs
Column B	Exon D MO
Mann Whitney test	
P value	0.0081
Exact or approximate P value?	Gaussian Approximation
P value summary	**
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	4958 , 6367
Mann-Whitney U	2108

Table Analyzed	Apical surface area
Column A	Control MO
vs	vs
Column B	Exon D MO
Mann Whitney test	
P value	0.518
Exact or approximate P value?	Gaussian Approximation
P value summary	ns
Are medians signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	5490 , 5835
Mann-Whitney U	2640

Table Analyzed	basolateral surface area
Column A	Control MO
vs	vs
Column B	Exon D MO
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	3729 , 7596
Mann-Whitney U	879

Table Analyzed	Cell Height
Column A	Control MO
vs	vs
Column B	Exon D MO
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	3796 , 7530
Mann-Whitney U	945.5

(B) Statistical analysis for cell surface area quantification in Figure 3D.

Table Analyzed	Total surface area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	171.8		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
WT vs Δ Np63 MO	-143.6	Yes	***
WT vs DMSO	-22.45	No	ns
WT vs PD168393	-139.4	Yes	***
Δ Np63 MO vs DMSO	121.1	Yes	***
Δ Np63 MO vs PD168393	4.187	No	ns
DMSO vs PD168393	-117	Yes	***

Table Analyzed	Apical surface area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	174.9		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
WT vs Δ Np63 MO	-144.9	Yes	***
WT vs DMSO	-31.52	No	ns
WT vs PD168393	-147.8	Yes	***
Δ Np63 MO vs DMSO	113.3	Yes	***
Δ Np63 MO vs PD168393	-2.907	No	ns
DMSO vs PD168393	-116.2	Yes	***

Table Analyzed	Basolateral surface area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	46.65		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
WT vs Δ Np63 MO	-63.8	Yes	***
WT vs DMSO	30.55	No	ns
WT vs PD168393	-6.56	No	ns
Δ Np63 MO vs DMSO	94.35	Yes	***
Δ Np63 MO vs PD168393	57.24	Yes	***
DMSO vs PD168393	-37.11	No	ns

Table Analyzed	Cell Height		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	81.61		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
WT vs Δ Np63 MO	110.4	Yes	***
WT vs DMSO	44.27	Yes	**
WT vs PD168393	100.4	Yes	***
Δ Np63 MO vs DMSO	-66.11	Yes	***
Δ Np63 MO vs PD168393	-10.03	No	ns
DMSO vs PD168393	56.08	Yes	***

(C) Statistical analysis for cell surface area quantification in Figure 3F.

Table Analyzed	Total surface area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	7		
Kruskal-Wallis statistic	123.4		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
WT vs DMSO	-15.99	No	ns
WT vs ExonD MO	-60.57	No	ns
WT vs PD	-192.4	Yes	***
WT vs PD+ExonD	-38.23	No	ns
WT vs Δ Np63 MO	-204	Yes	***
WT vs Δ Np63 MO+ExonD	-51.47	No	ns
DMSO vs ExonD MO	-44.58	No	ns
DMSO vs PD	-176.4	Yes	***
DMSO vs PD+ExonD	-22.23	No	ns
DMSO vs Δ Np63 MO	-188	Yes	***
DMSO vs Δ Np63 MO+ExonD	-35.48	No	ns
ExonD MO vs PD	-131.8	Yes	***
ExonD MO vs PD+ExonD	22.35	No	ns
ExonD MO vs Δ Np63 MO	-143.4	Yes	***
ExonD MO vs Δ Np63 MO+ExonD	9.1	No	ns
PD vs PD+ExonD	154.2	Yes	***
PD vs Δ Np63 MO	-11.57	No	ns
PD vs Δ Np63 MO+ExonD	140.9	Yes	***
PD+ExonD vs Δ Np63 MO	-165.7	Yes	***
PD+ExonD vs Δ Np63 MO+ExonD	-13.25	No	ns
Δ Np63 MO vs Δ Np63 MO+ExonD	152.5	Yes	***

Table Analyzed	Apical surface area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	7		
Kruskal-Wallis statistic	153.4		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
WT vs DMSO	-6.707	No	ns
WT vs exonD	-18.87	No	ns
WT vs PD	-187.2	Yes	***
WT vs PD+exonD	9.633	No	ns
WT vs ΔNp63	-200.4	Yes	***
WT vs ΔNp63+exonD	-12.45	No	ns
DMSO vs exonD	-12.17	No	ns
DMSO vs PD	-180.5	Yes	***
DMSO vs PD+exonD	16.34	No	ns
DMSO vs ΔNp63	-193.7	Yes	***
DMSO vs ΔNp63+exonD	-5.74	No	ns
exonD vs PD	-168.4	Yes	***
exonD vs PD+exonD	28.51	No	ns
exonD vs ΔNp63	-181.5	Yes	***
exonD vs ΔNp63+exonD	6.425	No	ns
PD vs PD+exonD	196.9	Yes	***
PD vs ΔNp63	-13.14	No	ns
PD vs ΔNp63+exonD	174.8	Yes	***
PD+exonD vs ΔNp63	-210	Yes	***
PD+exonD vs ΔNp63+exonD	-22.08	No	ns
ΔNp63 vs ΔNp63+exonD	187.9	Yes	***

Table Analyzed	Basolateral surface area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	7		
Kruskal-Wallis statistic	127.6		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
WT vs DMSO	-41.49	No	ns
WT vs exonD	-183.3	Yes	***
WT vs PD	-77.72	No	ns
WT vs PD+exonD	-203.5	Yes	***
WT vs Δ Np63 MO	-45.07	No	ns
WT vs Δ Np63 MO+ExonD	-180.9	Yes	***
DMSO vs exonD	-141.8	Yes	***
DMSO vs PD	-36.23	No	ns
DMSO vs PD+exonD	-162	Yes	***
DMSO vs Δ Np63 MO	-3.587	No	ns
DMSO vs Δ Np63 MO+ExonD	-139.4	Yes	***
exonD vs PD	105.6	Yes	***
exonD vs PD+exonD	-20.15	No	ns
exonD vs Δ Np63 MO	138.3	Yes	***
exonD vs Δ Np63 MO+ExonD	2.458	No	ns
PD vs PD+exonD	-125.8	Yes	***
PD vs Δ Np63 MO	32.65	No	ns
PD vs Δ Np63 MO+ExonD	-103.2	Yes	**
PD+exonD vs Δ Np63 MO	158.4	Yes	***
PD+exonD vs Δ Np63 MO+ExonD	22.61	No	ns
Δ Np63 MO vs Δ Np63 MO+ExonD	-135.8	Yes	***

Table Analyzed	Cell height		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	7		
Kruskal-Wallis statistic	256.3		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
WT vs DMSO	-29.53	No	ns
WT vs Exon D MO	-134.8	Yes	***
WT vs PD	82.86	Yes	*
WT vs PD+Exon D	-176.9	Yes	***
WT vs ΔNp63 MO	136.7	Yes	***
WT vs ΔNp63+ ExonD	-124.4	Yes	***
DMSO vs Exon D MO	-105.2	Yes	***
DMSO vs PD	112.4	Yes	***
DMSO vs PD+Exon D	-147.4	Yes	***
DMSO vs ΔNp63 MO	166.2	Yes	***
DMSO vs ΔNp63+ ExonD	-94.91	Yes	**
Exon D MO vs PD	217.6	Yes	***
Exon D MO vs PD+Exon D	-42.17	No	ns
Exon D MO vs ΔNp63 MO	271.5	Yes	***
Exon D MO vs ΔNp63+ ExonD	10.33	No	ns
PD vs PD+Exon D	-259.8	Yes	***
PD vs ΔNp63 MO	53.86	No	ns
PD vs ΔNp63+ ExonD	-207.3	Yes	***
PD+Exon D vs ΔNp63 MO	313.6	Yes	***
PD+Exon D vs ΔNp63+ ExonD	52.49	No	ns
ΔNp63 MO vs ΔNp63+ ExonD	-261.2	Yes	***

Table S3. Statistical comparison for cell surface area as shown in Figure 4. (A)

Statistical analysis for FASN quantification in Figure 4B.

Table Analyzed	Mean intensity
Column A	DMSO
vs	vs
Column B	PD 168393
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	3655 , 6215
Mann-Whitney U	1170

Table Analyzed	Mean Intensity
Column A	WT
vs	vs
Column B	Δ Np63
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	4250 , 7075
Mann-Whitney U	1400

(B) Statistical analysis for cell surface area quantification in Figure 4E.

Table Analyzed	Total Surface Area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	6		
Kruskal-Wallis statistic	324.7		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO vs PD168393	-152.3	Yes	***
DMSO vs Cerulenin	182.6	Yes	***
DMSO vs PD 168393 +Cerulenin	67.07	No	ns
DMSO vs Δ Np63	-180	Yes	***
DMSO vs Δ Np63 Cerulenin	88.13	Yes	**
PD168393 vs Cerulenin	334.9	Yes	***
PD168393 vs PD 168393 +Cerulenin	219.4	Yes	***
PD168393 vs Δ Np63	-27.63	No	ns
PD168393 vs Δ Np63 Cerulenin	240.5	Yes	***
Cerulenin vs PD 168393 +Cerulenin	-115.5	Yes	***
Cerulenin vs Δ Np63	-362.5	Yes	***
Cerulenin vs Δ Np63 Cerulenin	-94.44	Yes	**
PD 168393 +Cerulenin vs Δ Np63	-247	Yes	***
PD 168393 +Cerulenin vs Δ Np63 Cerulenin	21.05	No	ns
Δ Np63 vs Δ Np63 Cerulenin	268.1	Yes	***

Table Analyzed	Apical Surface Area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	6		
Kruskal-Wallis statistic	323.7		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO vs PD168393	-155.8	Yes	***
DMSO vs Cerulenin	184.5	Yes	***
DMSO vs PD 168393 + Cerulenin	58.69	No	ns
DMSO vs Δ Np63	-174.6	Yes	***
DMSO vs Δ Np63 Cerulenin	87.54	Yes	**
PD168393 vs Cerulenin	340.4	Yes	***
PD168393 vs PD 168393 + Cerulenin	214.5	Yes	***
PD168393 vs Δ Np63	-18.71	No	ns
PD168393 vs Δ Np63 Cerulenin	243.4	Yes	***
Cerulenin vs PD 168393 + Cerulenin	-125.8	Yes	***
Cerulenin vs Δ Np63	-359.1	Yes	***
Cerulenin vs Δ Np63 Cerulenin	-96.97	Yes	**
PD 168393 + Cerulenin vs Δ Np63	-233.2	Yes	***
PD 168393 + Cerulenin vs Δ Np63 Cerulenin	28.85	No	ns
Δ Np63 vs Δ Np63 Cerulenin	262.1	Yes	***

Table Analyzed	Basolateral Surface Area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	6		
Kruskal-Wallis statistic	133.8		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO vs PD168393	-57.35	No	ns
DMSO vs Cerulenin	105	Yes	***
DMSO vs PD 168393 + Cerulenin	91.97	Yes	**
DMSO vs ΔNp63	-140.7	Yes	***
DMSO vs ΔNp63 Cerulenin	57.15	No	ns
PD168393 vs Cerulenin	162.3	Yes	***
PD168393 vs PD 168393 + Cerulenin	149.3	Yes	***
PD168393 vs ΔNp63	-83.32	Yes	*
PD168393 vs ΔNp63 Cerulenin	114.5	Yes	***
Cerulenin vs PD 168393 + Cerulenin	-12.99	No	ns
Cerulenin vs ΔNp63	-245.6	Yes	***
Cerulenin vs ΔNp63 Cerulenin	-47.81	No	ns
PD 168393 + Cerulenin vs ΔNp63	-232.6	Yes	***
PD 168393 + Cerulenin vs ΔNp63 Cerulenin	-34.82	No	ns
ΔNp63 vs ΔNp63 Cerulenin	197.8	Yes	***

(C) Statistical analysis for cell surface area quantification in Figure 4F.

Table Analyzed	Total Cell Surface Area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	6		
Kruskal-Wallis statistic	183.2		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO vs Rapamycin	25.75	No	ns
DMSO vs Δ Np63 MO	-222.9	Yes	***
DMSO vs Δ Np63+Rapamycin	-30.37	No	ns
DMSO vs PD168393	-184.2	Yes	***
DMSO vs PD168393+Rapamycin	45.17	No	ns
Rapamycin vs Δ Np63 MO	-248.7	Yes	***
Rapamycin vs Δ Np63+Rapamycin	-56.12	No	ns
Rapamycin vs PD168393	-210	Yes	***
Rapamycin vs PD168393+Rapamycin	19.42	No	ns
Δ Np63 MO vs Δ Np63+Rapamycin	192.5	Yes	***
Δ Np63 MO vs PD168393	38.69	No	ns
Δ Np63 MO vs PD168393+Rapamycin	268.1	Yes	***
Δ Np63+Rapamycin vs PD168393	-153.8	Yes	***
Δ Np63+Rapamycin vs PD168393+Rapamycin	75.54	No	ns
PD168393 vs PD168393+Rapamycin	229.4	Yes	***

Table Analyzed	Apical Surface Area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	6		
Kruskal-Wallis statistic	179.7		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO vs Rapamycin	34.78	No	ns
DMSO vs Δ Np63 MO	-214.6	Yes	***
DMSO vs Δ Np63+Rapamycin	-24.31	No	ns
DMSO vs PD168393	-182.3	Yes	***
DMSO vs PD168393+Rapamycin	41.5	No	ns
Rapamycin vs Δ Np63 MO	-249.3	Yes	***
Rapamycin vs Δ Np63+Rapamycin	-59.09	No	ns
Rapamycin vs PD168393	-217.1	Yes	***
Rapamycin vs PD168393+Rapamycin	6.717	No	ns
Δ Np63 MO vs Δ Np63+Rapamycin	190.3	Yes	***
Δ Np63 MO vs PD168393	32.23	No	ns
Δ Np63 MO vs PD168393+Rapamycin	256.1	Yes	***
Δ Np63+Rapamycin vs PD168393	-158	Yes	***
Δ Np63+Rapamycin vs PD168393+Rapamycin	65.81	No	ns
PD168393 vs PD168393+Rapamycin	223.8	Yes	***

Table Analyzed	Basolateral Surface Area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	6		
Kruskal-Wallis statistic	74.63		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO vs Rapamycin	9.973	No	ns
DMSO vs Δ Np63 MO	-146.5	Yes	***
DMSO vs Δ Np63+Rapamycin	-20.27	No	ns
DMSO vs PD168393	-107.2	Yes	***
DMSO vs PD168393+Rapamycin	43.34	No	ns
Rapamycin vs Δ Np63 MO	-156.5	Yes	***
Rapamycin vs Δ Np63+Rapamycin	-30.24	No	ns
Rapamycin vs PD168393	-117.2	Yes	***
Rapamycin vs PD168393+Rapamycin	33.37	No	ns
Δ Np63 MO vs Δ Np63+Rapamycin	126.3	Yes	***
Δ Np63 MO vs PD168393	39.34	No	ns
Δ Np63 MO vs PD168393+Rapamycin	189.9	Yes	***
Δ Np63+Rapamycin vs PD168393	-86.93	Yes	*
Δ Np63+Rapamycin vs PD168393+Rapamycin	63.61	No	ns
PD168393 vs PD168393+Rapamycin	150.5	Yes	***

Table S4. Statistical comparison for cell surface area as shown in Figure 5B.

Table Analyzed	Total surface area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	48.36		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO (Control) vs CompC	-91.6	Yes	***
DMSO (Control) vs ExonD	-15	No	ns
DMSO (Control) vs ExonD + CompC	-41.27	Yes	*
CompC vs ExonD	76.6	Yes	***
CompC vs ExonD + CompC	50.33	Yes	**
ExonD vs ExonD + CompC	-26.27	No	ns

Table Analyzed	Apical Surface Area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	49.52		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO (Control) vs CompC	-79.28	Yes	***
DMSO (Control) vs ExonD	11.99	No	ns
DMSO (Control) vs ExonD + CompC	-14.39	No	ns
CompC vs ExonD	91.27	Yes	***
CompC vs ExonD + CompC	64.89	Yes	***
ExonD vs ExonD + CompC	-26.37	No	ns

Table Analyzed	Basolateral Surface Area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	82.88		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO (Control) vs CompC	-85.61	Yes	***
DMSO (Control) vs ExonD + DMSO	-98.37	Yes	***
DMSO (Control) vs ExonD + CompC	-119.6	Yes	***
CompC vs ExonD + DMSO	-12.75	No	ns
CompC vs ExonD + CompC	-33.96	No	ns
ExonD + DMSO vs ExonD + CompC	-21.21	No	ns

Table Analyzed	Height		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	119.5		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO (Control) vs Compound C	-23.24	No	ns
DMSO (Control) vs Exon D	-112.5	Yes	***
DMSO (Control) vs Exon D + Compound C	-125.5	Yes	***
Compound C vs Exon D	-89.25	Yes	***
Compound C vs Exon D + Compound C	-102.3	Yes	***
Exon D vs Exon D + Compound C	-13.03	No	ns

Table S5. Statistical comparison for cell surface area and TGN surface area and volume as shown in Figure 6.

(A) Statistical analysis for cell surface area quantification in Figure 6B.

Table Analyzed	Total cell surface area
Column A	DMSO
vs	vs
Column B	Rapamycin
Mann Whitney test	
P value	0.1302
Exact or approximate P value?	Gaussian Approximation
P value summary	ns
Are medians signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	4727 , 5858
Mann-Whitney U	2242

Table Analyzed	Apical Surface Area
Column A	WT
vs	vs
Column B	Rapamycin
Mann Whitney test	
P value	0.1078
Exact or approximate P value?	Gaussian Approximation
P value summary	ns
Are medians signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	4703 , 5882
Mann-Whitney U	2218

Table Analyzed	Basolateral Surface Area
Column A	WT
vs	vs
Column B	Rapa
Mann Whitney test	
P value	0.3597
Exact or approximate P value?	Gaussian Approximation
P value summary	ns
Are medians signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	4878 , 5707
Mann-Whitney U	2393

(B) Statistical analysis for TGN surface area and volume quantification in Figure 6C.

Table Analyzed	TGN Surface Area
Column A	DMSO
vs	vs
Column B	Rapamycin
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	7207 , 4118
Mann-Whitney U	1268

Table Analyzed	TGN Volume
Column A	DMSO
vs	vs
Column B	Rapamycin
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	7144 , 4181
Mann-Whitney U	1331

(C) Statistical analysis for TGN surface area and volume quantification in Figure 6E.

Table Analyzed	TGN Surface area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	84.49		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO vs PD168393	-87.6	Yes	***
DMSO vs WT	-12.65	No	ns
DMSO vs Δ Np63 MO	-106.4	Yes	***
PD168393 vs WT	74.95	Yes	***
PD168393 vs Δ Np63 MO	-18.79	No	ns
WT vs Δ Np63 MO	-93.73	Yes	***

Table Analyzed	TGN Volume		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	67.44		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO vs PD168393	-78.89	Yes	***
DMSO vs WT	-24.88	No	ns
DMSO vs Δ Np63 MO	-103	Yes	***
PD168393 vs WT	54.01	Yes	***
PD168393 vs Δ Np63 MO	-24.13	No	ns
WT vs Δ Np63 MO	-78.15	Yes	***

(D) Statistical analysis for TGN surface area and volume quantification in Figure 6G

Table Analyzed	TGN Surface Area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	6		
Kruskal-Wallis statistic	170.7		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO vs Rapa	55.64	No	ns
DMSO vs Δ Np63 MO	-170.4	Yes	***
DMSO vs Δ Np63+Rapamycin	-52.68	No	ns
DMSO vs PD168393	-108.6	Yes	***
DMSO vs PD168393+Rapamycin	36	No	ns
Rapa vs Δ Np63 MO	-226.1	Yes	***
Rapa vs Δ Np63+Rapamycin	-108.3	Yes	***
Rapa vs PD168393	-164.3	Yes	***
Rapa vs PD168393+Rapamycin	-19.64	No	ns
Δ Np63 MO vs Δ Np63+Rapamycin	117.7	Yes	***
Δ Np63 MO vs PD168393	61.79	No	ns
Δ Np63 MO vs PD168393+Rapamycin	206.4	Yes	***
Δ Np63+Rapamycin vs PD168393	-55.96	No	ns
Δ Np63+Rapamycin vs PD168393+Rapamycin	88.68	Yes	***
PD168393 vs PD168393+Rapamycin	144.6	Yes	***

Table Analyzed	TGN Volume		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	6		
Kruskal-Wallis statistic	154.6		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO vs Rapa	82.75	Yes	***
DMSO vs Δ Np63 MO	-176.6	Yes	***
DMSO vs Δ Np63+Rapamycin	-63.29	No	ns
DMSO vs PD168393	-102.4	Yes	***
DMSO vs PD168393+Rapamycin	63.69	No	ns
Rapa vs Δ Np63 MO	-259.3	Yes	***
Rapa vs Δ Np63+Rapamycin	-146	Yes	***
Rapa vs PD168393	-185.2	Yes	***
Rapa vs PD168393+Rapamycin	-19.05	No	ns
Δ Np63 MO vs Δ Np63+Rapamycin	113.3	Yes	***
Δ Np63 MO vs PD168393	74.16	No	ns
Δ Np63 MO vs PD168393+Rapamycin	240.3	Yes	***
Δ Np63+Rapamycin vs PD168393	-39.12	No	ns
Δ Np63+Rapamycin vs PD168393+Rapamycin	127	Yes	***
PD168393 vs PD168393+Rapamycin	166.1	Yes	***

Table S6. Statistical comparison for cell surface area and TGN surface area as well as volume as shown in Figure 7.

(A) Statistical analysis for TGN surface area and volume quantification in Figure 7B.

Table Analyzed	TGN Surface Area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	166		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO (Control) vs Rapa	67.73	Yes	***
DMSO (Control) vs ExonD	-111.3	Yes	***
DMSO (Control) vs ExonD+Rapa	-34.79	No	ns
Rapa vs ExonD	-179.1	Yes	***
Rapa vs ExonD+Rapa	-102.5	Yes	***
ExonD vs ExonD+Rapa	76.53	Yes	***

Table Analyzed	TGN Volume		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	144.2		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO (Control) vs Rapa	58.32	Yes	***
DMSO (Control) vs Exon D	-108.3	Yes	***
DMSO (Control) vs Exon D +Rapa	-31.95	No	ns
Rapa vs Exon D	-166.6	Yes	***
Rapa vs Exon D +Rapa	-90.27	Yes	***
Exon D vs Exon D +Rapa	76.32	Yes	***

(B) Statistical analysis for cell surface area quantification in Figure 7D.

Table Analyzed	Total surface area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	29.37		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO (Control) vs ExonD	-33.15	No	ns
DMSO (Control) vs Rapa	14.15	No	ns
DMSO (Control) vs ExonD+Rapa	42.23	Yes	*
ExonD vs Rapa	47.29	Yes	**
ExonD vs ExonD+Rapa	75.37	Yes	***
Rapa vs ExonD+Rapa	28.08	No	ns

Table Analyzed	Apical surface area		
Kruskal-Wallis test			
P value	0.0016		
Exact or approximate P value?	Gaussian Approximation		
P value summary	**		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	15.32		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO (Control) vs ExonD	9.587	No	ns
DMSO (Control) vs Rapa	23.93	No	ns
DMSO (Control) vs ExonD+Rapa	51.95	Yes	**
ExonD vs Rapa	14.35	No	ns
ExonD vs ExonD+Rapa	42.36	Yes	*
Rapa vs ExonD+Rapa	28.01	No	ns

Table Analyzed	Basolateral surface area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	110		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO (Control) vs ExonD	-130.6	Yes	***
DMSO (Control) vs Rapa	-21.29	No	ns
DMSO (Control) vs ExonD+Rapa	-10.44	No	ns
ExonD vs Rapa	109.3	Yes	***
ExonD vs ExonD+Rapa	120.2	Yes	***
Rapa vs ExonD+Rapa	10.85	No	ns

Table Analyzed	Height		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	90.92		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO (Control) vs Exon D MO	-118.4	Yes	***
DMSO (Control) vs Rapa	-6.173	No	ns
DMSO (Control) vs Exon D MO+Rapa	-51.53	Yes	**
Exon D MO vs Rapa	112.2	Yes	***
Exon D MO vs Exon D MO+Rapa	66.88	Yes	***
Rapa vs Exon D MO+Rapa	-45.35	Yes	**

(C) Statistical analysis for TGN surface area and volume quantification in Figure 7F.

Table Analyzed	TGN Surface Area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	7		
Kruskal-Wallis statistic	151.8		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO (Control) vs PD 168393	-143.5	Yes	***
DMSO (Control) vs Exon D MO	-180.3	Yes	***
DMSO (Control) vs PD 168393+Exon D MO	-125.1	Yes	***
DMSO (Control) vs WT	64.56	No	ns
DMSO (Control) vs Δ Np63 MO	-120	Yes	***
DMSO (Control) vs Δ Np63+Exon D MO	-2.333	No	ns
PD 168393 vs Exon D MO	-36.8	No	ns
PD 168393 vs PD 168393+Exon D MO	18.37	No	ns
PD 168393 vs WT	208.1	Yes	***
PD 168393 vs Δ Np63 MO	23.48	No	ns
PD 168393 vs Δ Np63+Exon D MO	141.2	Yes	***
Exon D MO vs PD 168393+Exon D MO	55.17	No	ns
Exon D MO vs WT	244.9	Yes	***
Exon D MO vs Δ Np63 MO	60.28	No	ns
Exon D MO vs Δ Np63+Exon D MO	178	Yes	***
PD 168393+Exon D MO vs WT	189.7	Yes	***
PD 168393+Exon D MO vs Δ Np63 MO	5.107	No	ns
PD 168393+Exon D MO vs Δ Np63+Exon D MO	122.8	Yes	***
WT vs Δ Np63 MO	-184.6	Yes	***
WT vs Δ Np63+Exon D MO	-66.89	No	ns
Δ Np63 MO vs Δ Np63+Exon D MO	117.7	Yes	***

Table Analyzed	TGN Volume		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	7		
Kruskal-Wallis statistic	136.1		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO (Control) vs PD 168393	-113.3	Yes	**
DMSO (Control) vs Exon D MO	-137.5	Yes	***
DMSO (Control) vs PD 168393+Exon D MO	-114.2	Yes	***
DMSO (Control) vs WT	78.72	No	ns
DMSO (Control) vs Δ Np63 MO	-63.03	No	ns
DMSO (Control) vs Δ Np63+Exon D MO	57.28	No	ns
PD 168393 vs Exon D MO	-24.22	No	ns
PD 168393 vs PD 168393+Exon D MO	-0.9333	No	ns
PD 168393 vs WT	192	Yes	***
PD 168393 vs Δ Np63 MO	50.28	No	ns
PD 168393 vs Δ Np63+Exon D MO	170.6	Yes	***
Exon D MO vs PD 168393+Exon D MO	23.29	No	ns
Exon D MO vs WT	216.3	Yes	***
Exon D MO vs Δ Np63 MO	74.5	Yes	*
Exon D MO vs Δ Np63+Exon D MO	194.8	Yes	***
PD 168393+Exon D MO vs WT	193	Yes	***
PD 168393+Exon D MO vs Δ Np63 MO	51.21	No	ns
PD 168393+Exon D MO vs Δ Np63+Exon D MO	171.5	Yes	***
WT vs Δ Np63 MO	-141.7	Yes	***
WT vs Δ Np63+Exon D MO	-21.44	No	ns
Δ Np63 MO vs Δ Np63+Exon D MO	120.3	Yes	***

Table S7. Statistical analysis for the quantification of number of Rab11 vesicles as shown in Supplementary Figure 2D.

Table Analyzed	Number of Rab11 vesicles		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	28.75		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
WT vs Myosin Vb MO	-22.90	Yes	***
WT vs Myosin Vb-Rab10+/11-	-23.65	Yes	***
WT vs Myosin Vb-Rab10-/11+	-9.050	No	ns
Myosin Vb MO vs Myosin Vb-Rab10+/11-	-0.7500	No	ns
Myosin Vb MO vs Myosin Vb-Rab10-/11+	13.85	Yes	*
Myosin Vb-Rab10+/11- vs Myosin Vb-Rab10-/11+	14.60	Yes	*

Table S8. Statistical analysis for the TGN surface area and volume between Myosin Vb isoform injected clones and non-clones in the Exon D morphants as shown in Supplementary Figure 3D.

Table Analyzed	TGN Volume		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	5		
Kruskal-Wallis statistic	52.55		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
Uninjected Wildtype vs MyoVbRab10 ^{+/11+} Non-clones	-107.0	Yes	***
Uninjected Wildtype vs MyoVbRab10 ^{+/11+} Clones	-21.21	No	ns
Uninjected Wildtype vs MyoVbRab10 ^{-/11+} Non-clones	-66.77	Yes	**
Uninjected Wildtype vs MyoVbRab10 ^{-/11+} Clones	-76.45	Yes	***
MyoVbRab10 ^{+/11+} Non-clones vs MyoVbRab10 ^{+/11+}	85.81	Yes	***
MyoVbRab10 ^{+/11+} Non-clones vs MyoVbRab10 ^{/11}	40.25	No	ns
MyoVbRab10 ^{+/11+} Non-clones vs MyoVbRab10 ^{/11+}	30.57	No	ns
MyoVbRab10 ^{+/11+} Clones vs MyoVbRab10 ^{/11+}	-45.56	No	ns
MyoVbRab10 ^{+/11+} Clones vs MyoVbRab10 ^{/11+} Clones	-55.24	Yes	*
MyoVbRab10 ^{/11+} Non-clones vs MyoVbRab10 ^{/11+}	-9.683	No	ns

Table Analyzed	TGN Surface area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	5		
Kruskal-Wallis statistic	104.6		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
Uninjected Wildtype vs MyoVbRab10 ^{+/11+} Non-clones	-161.1	Yes	***
Uninjected Wildtype vs MyoVbRab10 ^{+/11+} Clones	-59.99	Yes	**
Uninjected Wildtype vs MyoVbRab10 ^{-/11+} Non-clones	-102.8	Yes	***
Uninjected Wildtype vs MyoVbRab10 ^{-/11+} Clones	-117.1	Yes	***
MyoVbRab10 ^{+/11+} Non-clones vs MyoVbRab10 ^{+/11+}	101.1	Yes	***
MyoVbRab10 ^{+/11+} Non-clones vs MyoVbRab10 ^{-/11+}	58.33	Yes	*
MyoVbRab10 ^{+/11+} Non-clones vs MyoVbRab10 ^{-/11+}	43.98	No	ns
MyoVbRab10 ^{+/11+} Clones vs MyoVbRab10 ^{-/11+}	-42.79	No	ns
MyoVbRab10 ^{+/11+} Clones vs MyoVbRab10 ^{-/11+} Clones	-57.14	Yes	*
MyoVbRab10 ^{-/11+} Non-clones vs MyoVbRab10 ^{-/11+}	-14.35	No	ns

Table S9. Statistical analysis for the number of WGA positive vesicles and their co-localization with LAMP1 at different developmental time points in the Exon D morphants vs controls as shown in Supplementary Figure 4D.

Table Analyzed	Number of vesicles at 22hpf		
Kruskal-Wallis test			
P value	0.1556		
Exact or approximate P value?	Gaussian Approximation		
P value summary	ns		
Do the medians vary signif. (P < 0.05)	No		
Number of groups	4		
Kruskal-Wallis statistic	5.231		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
Control MO WGA vs Control MO LAMP1-WGA	3.214	No	ns
Control MO WGA vs ExonD WGA	-8.692	No	ns
Control MO WGA vs ExonD LAMP1-WGA	-4.328	No	ns
Control MO LAMP1-WGA vs ExonD WGA	-11.91	No	ns
Control MO LAMP1-WGA vs ExonD LAMP1-WGA	-7.542	No	ns
ExonD WGA vs ExonD LAMP1-WGA	4.364	No	ns

Table Analyzed	Number of vesicles at 36hpf		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	37.13		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
Control MO WGA vs Control LAMP1-WGA	0.0000	No	ns
Control MO WGA vs Exon D WGA	-26.35	Yes	***
Control MO WGA vs Exon D LAMP1-WGA	-21.65	Yes	***
Control LAMP1-WGA vs Exon D WGA	-26.35	Yes	***
Control LAMP1-WGA vs Exon D LAMP1	-21.65	Yes	***
ExonD WGA vs Exon D LAMP1-WGA	4.692	No	ns

Table Analyzed	Number of vesicles at 72hpf		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	34.79		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
Control WGA vs Control LAMP1-WGA	-0.07143	No	ns
Control WGA vs Exon D WGA	-25.92	Yes	***
Control WGA vs Exon D LAMP1-WGA	-23.32	Yes	***
Control LAMP1-WGA vs Exon D WGA	-25.85	Yes	***
Control LAMP1-WGA vs Exon D LAMP1-WGA	-23.25	Yes	***
ExonD WGA vs ExonD LAMP1-WGA	2.600	No	ns

Table S10. Statistical comparison for cell surface area as shown in Supplementary Figure 6D.

Table Analyzed	Total Surface Area
Column A	DMSO
vs	vs
Column B	Compound C
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	3695 , 7630
Mann-Whitney U	845

Table Analyzed	Apical Surface Area
Column A	DMSO
vs	vs
Column B	Compound C
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	3895 , 7430
Mann-Whitney U	1045

Table Analyzed	Basolateral Surface Area
Column A	DMSO
vs	vs
Column B	Compound C
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	3654 , 7671
Mann-Whitney U	804

Table Analyzed	Cell height
Column A	DMSO
vs	vs
Column B	Compound C
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	4459 , 6866
Mann-Whitney U	1609

Table S11. Statistical comparison for cell surface area, and TGN surface area and volume as shown in Supplementary Figure 7.

(A) Statistical analysis for cell surface area quantification in Supplementary Figure 7B.

Table Analyzed	Total Surface Area
Column A	DMSO
vs	vs
Column B	Torin 1
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	7015 , 4310
Mann-Whitney U	1460

Table Analyzed	Apical Surface Area
Column A	DMSO
vs	vs
Column B	Torin 1
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	7101 , 4224
Mann-Whitney U	1374

Table Analyzed	Basolateral Surface Area
Column A	DMSO
vs	vs
Column B	Torin 1
Mann Whitney test	
P value	0.2757
Exact or approximate P value?	Gaussian Approximation
P value summary	ns
Are medians signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	5953 , 5372
Mann-Whitney U	2522

(B) Statistical analysis for TGN surface area and volume quantification in Supplementary Fig 7C

Table Analyzed	TGN Surface Area
Column A	DMSO
vs	vs
Column B	Torin 1
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	7570 , 3755
Mann-Whitney U	905

Table Analyzed	TGN Volume
Column A	DMSO
vs	vs
Column B	Torin 1
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	7184 , 4141
Mann-Whitney U	1291

(C) Statistical analysis for TGN surface area and volume quantification in Supplementary Figure 7E

Table Analyzed	TGN Surface Area
Column A	DMSO
vs	vs
Column B	Compound C
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	3626 , 7699
Mann-Whitney U	776

Table Analyzed	TGN Volume
Column A	DMSO
vs	vs
Column B	Compound C
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	3943 , 7382
Mann-Whitney U	1093

(D) Statistical analysis for TGN surface area and volume quantification in Supplementary Figure 7G

Table Analyzed	TGN Surface Area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	6		
Kruskal-Wallis statistic	142.8		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO vs Cerulenin	73.93	Yes	**
DMSO vs Δ Np63 MO	-161.4	Yes	***
DMSO vs Δ Np63 MO + Cerulenin	33.53	No	ns
DMSO vs PD168393	-143.5	Yes	***
DMSO vs PD168393 + Cerulenin	10.23	No	ns
Cerulenin vs Δ Np63 MO	-235.3	Yes	***
Cerulenin vs Δ Np63 MO + Cerulenin	-40.41	No	ns
Cerulenin vs PD168393	-217.4	Yes	***
Cerulenin vs PD168393 + Cerulenin	-63.7	No	ns
Δ Np63 MO vs Δ Np63 MO + Cerulenin	194.9	Yes	***
Δ Np63 MO vs PD168393	17.88	No	ns
Δ Np63 MO vs PD168393 + Cerulenin	171.6	Yes	***
Δ Np63 MO + Cerulenin vs PD168393	-177	Yes	***
Δ Np63 MO + Cerulenin vs PD168393 + Cerulenin	-23.29	No	ns
PD168393 vs PD168393 + Cerulenin	153.7	Yes	***

Table Analyzed	TGN Volume		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	6		
Kruskal-Wallis statistic	141.4		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO vs Cerulenin	97.23	Yes	***
DMSO vs Δ Np63 MO	-146.3	Yes	***
DMSO vs Δ Np63 MO + Cerulenin	35.28	No	ns
DMSO vs PD168393	-111.6	Yes	***
DMSO vs PD168393 + Cerulenin	52.33	No	ns
Cerulenin vs Δ Np63 MO	-243.5	Yes	***
Cerulenin vs Δ Np63 MO + Cerulenin	-61.95	No	ns
Cerulenin vs PD168393	-208.9	Yes	***
Cerulenin vs PD168393 + Cerulenin	-44.89	No	ns
Δ Np63 MO vs Δ Np63 MO + Cerulenin	181.5	Yes	***
Δ Np63 MO vs PD168393	34.63	No	ns
Δ Np63 MO vs PD168393 + Cerulenin	198.6	Yes	***
Δ Np63 MO + Cerulenin vs PD168393	-146.9	Yes	***
Δ Np63 MO + Cerulenin vs PD168393 + Cerulenin	17.05	No	ns
PD168393 vs PD168393 + Cerulenin	164	Yes	***

Table S12. Statistical comparison for FASN intensity quantification, TGN volume and surface area, and cell surface area as shown in Supplementary Figure 8.

(A) Statistical analysis for FASN intensity quantification in Supplementary Figure 8B.

Table Analyzed	FASN Mean Intensity
Column A	Control MO
vs	vs
Column B	Exon D MO
Mann Whitney test	
P value	< 0.0001
Exact or approximate P value?	Gaussian Approximation
P value summary	***
Are medians signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	1535 , 3515
Mann-Whitney U	260.0

(B) Statistical comparison for TGN surface area and volume as shown in Supplementary Figure 8E.

Table Analyzed	TGN Surface Area		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	50.83		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO vs CompC	-93.28	Yes	***
DMSO vs Exon D MO	-59.85	Yes	***
DMSO vs Exon D MO + Comp C	-74.99	Yes	***
Comp C vs Exon D MO	33.43	No	ns
Comp C vs Exon D MO + Comp C	18.29	No	ns
Exon D MO vs Exon D MO + Comp C	-15.14	No	ns

Table Analyzed	TGN Volume		
Kruskal-Wallis test			
P value	< 0.0001		
Exact or approximate P value?	Gaussian Approximation		
P value summary	***		
Do the medians vary signif. (P < 0.05)	Yes		
Number of groups	4		
Kruskal-Wallis statistic	57.53		
Dunn's Multiple Comparison Test	Difference in rank sum	Significant? P < 0.05?	Summary
DMSO vs Comp C	-102.7	Yes	***
DMSO vs Exon D	-63.84	Yes	***
DMSO vs Exon D + Comp C	-78.55	Yes	***
CompC vs Exon D	38.81	Yes	*
Comp C vs Exon D + Comp C	24.11	No	ns
Exon D vs Exon D + Comp C	-14.71	No	ns

Table S13. The compilation of the number of experimental sets, embryos and cells used for the phenotypic assessment and statistical evaluation in various experiments

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