

**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

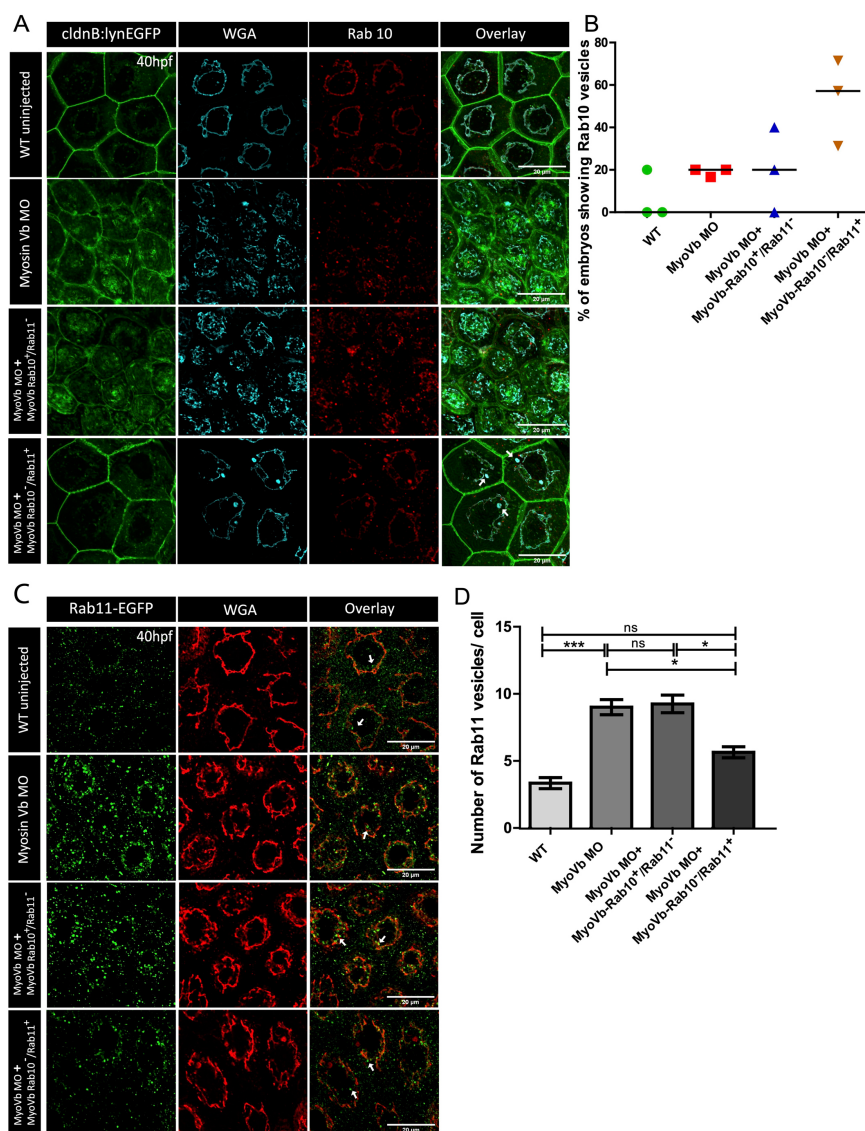
tool. Mutations introduced to abolish Rab11 binding of Myosin Vb (MyoVb-Rab11<sup>-/-</sup>) are

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).

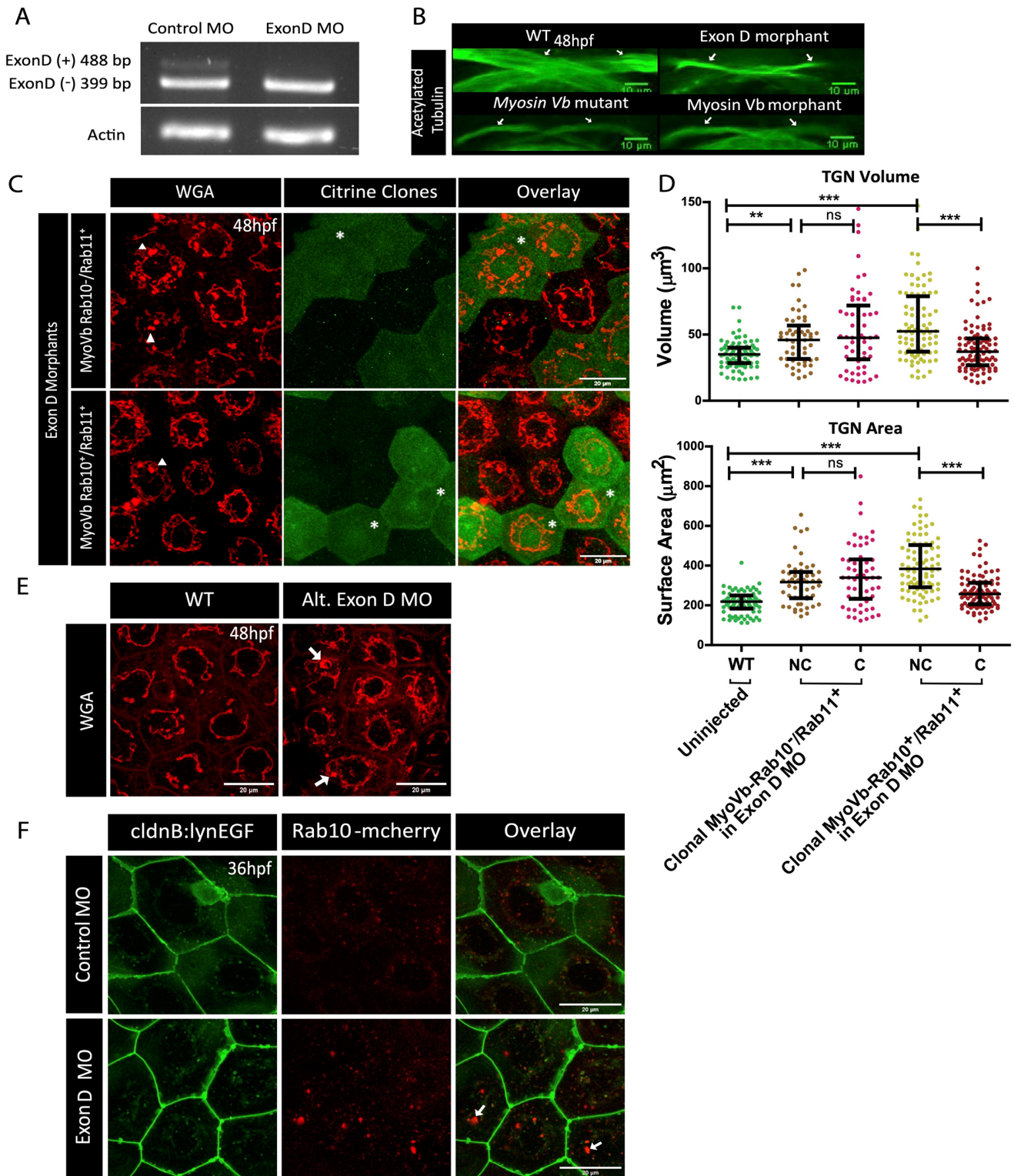
Scale Bars in B = 20μ.



**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<sup>+</sup>/Rab11<sup>-</sup> or MyoVb-Rab10<sup>-</sup>/Rab11<sup>+</sup> 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)<sup>mw6</sup>* MyoVb morphants rescued with MyoVb-Rab10<sup>+</sup>/Rab11<sup>-</sup> or MyoVb-Rab10<sup>-</sup>/Rab11<sup>+</sup> mRNA and labeled using anti-GFP antibody and WGA. (D) Quantification of the number of Rab11 vesicles in all the different genetic conditions mentioned.

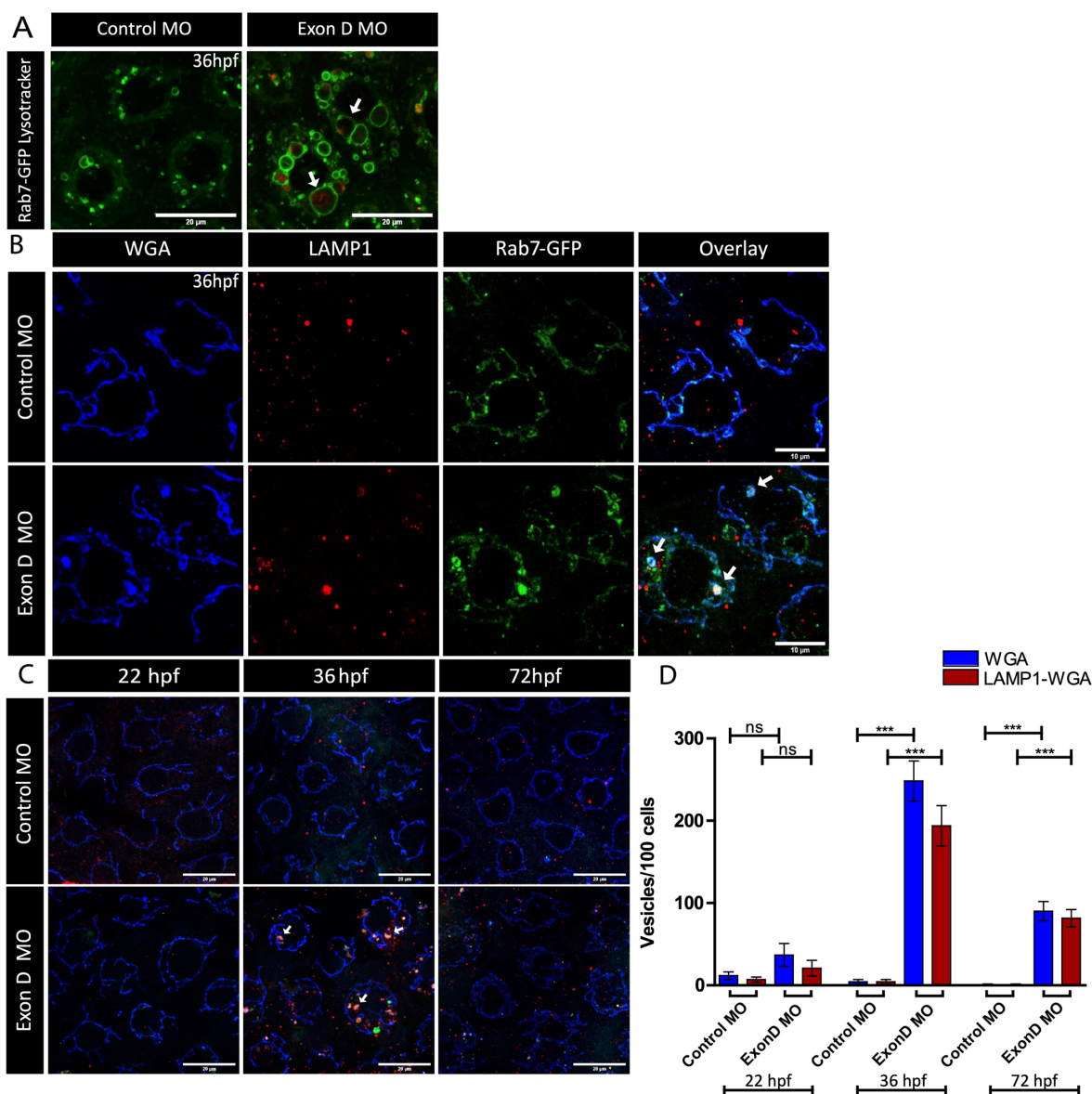
Scale bars in A and C correspond to 20µm. White arrows in A and C point to Rab10 and Rab11 vesicles, respectively. Asterisks in D denote the statistical significance at p<0.05. Key pairwise comparisons are shown in D; for the rest of the comparisons please refer to Table S7.



**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<sup>+</sup>/Rab11<sup>+</sup> or MyoVb-Rab10<sup>-</sup>/Rab11<sup>+</sup> 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.

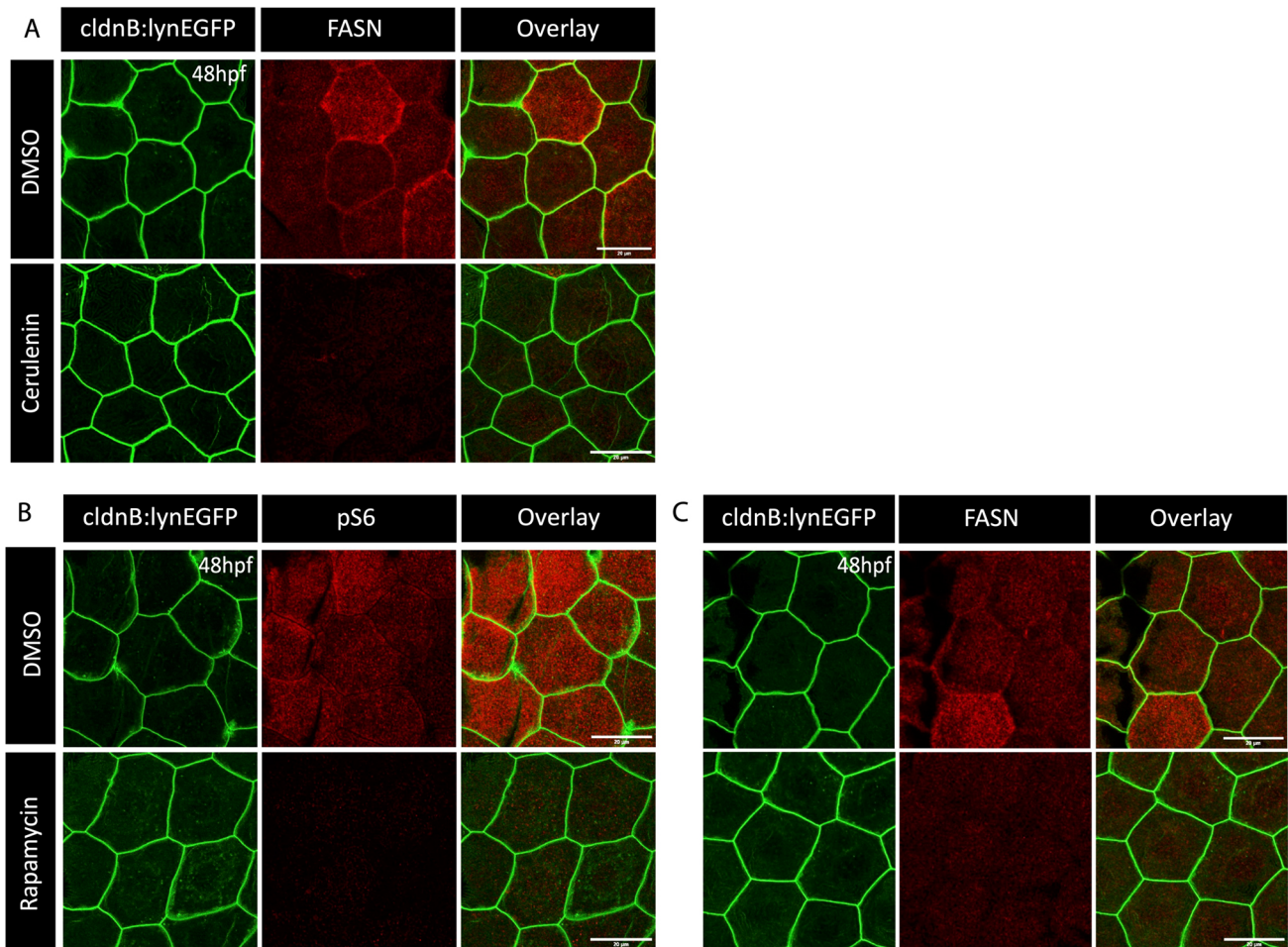
Scale bars in B correspond to 10µm and those in C, E and F to 20µm. White arrows in B indicate the optic nerve and those in E and F point to accumulated vesicles; white arrowheads and asterisks in C point to post-TGN vesicles and clones, respectively. Asterisks in D denote the statistical significance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



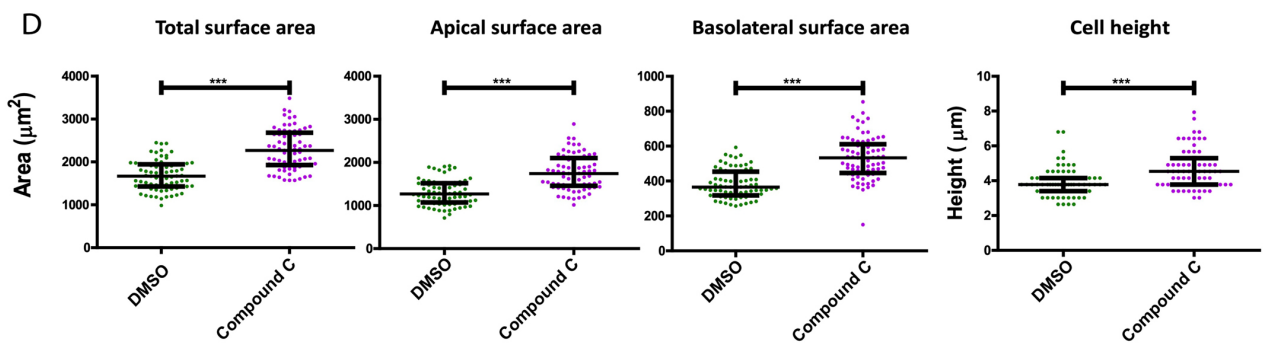
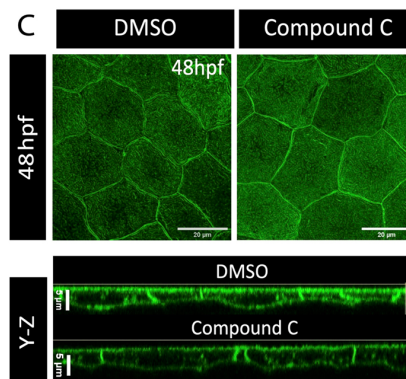
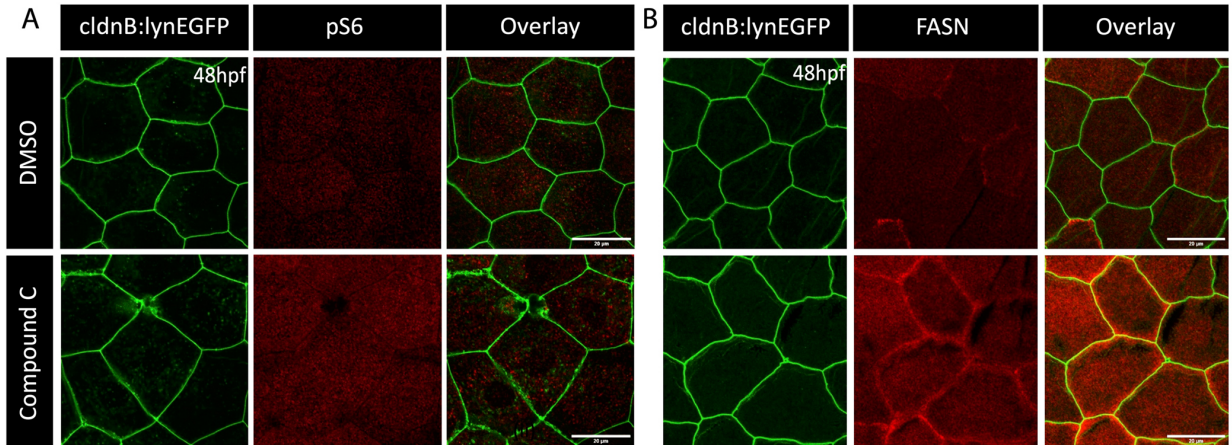
**Fig. S4.** Trans-Golgi vesicles accumulated upon the knockdown of Exon D isoform acquire late-endolysosomal fate in the peridermal cells.

Confocal images of the peridermal cells (A, B, C) of the embryos from *Tg(h2afx: EGFP-Rab7)<sup>mw7</sup>* 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  $20\mu$  and those in B to  $10\mu$ .



**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 20 $\mu$ .

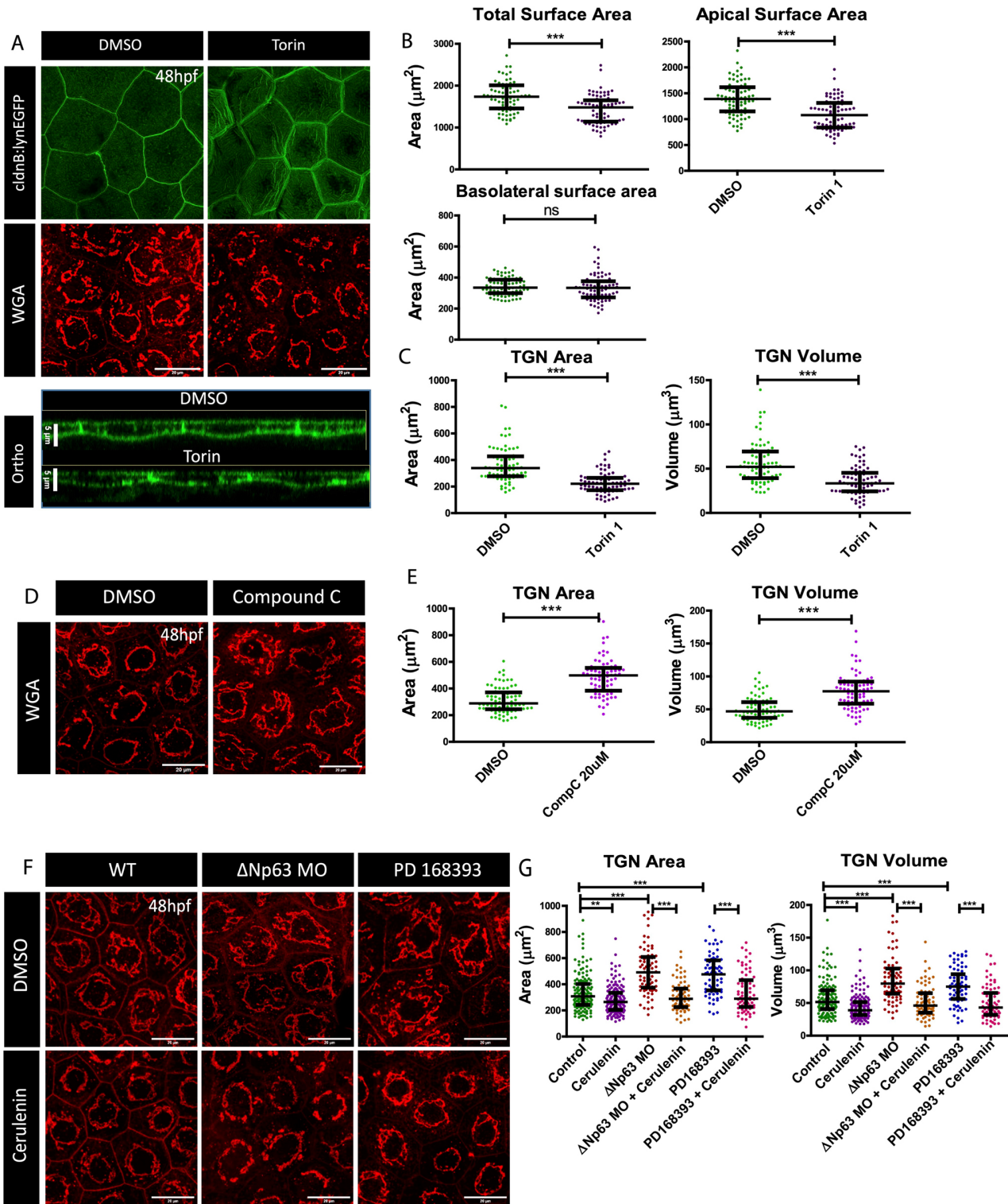


**Fig. S6.** Compound C treatment activates mTOR signaling and results in increased cell size in the zebrafish embryonic periderm.

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 $\mu$  and in C (Y-Z plane) to 5 $\mu$ . 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  $\Delta$ Np63

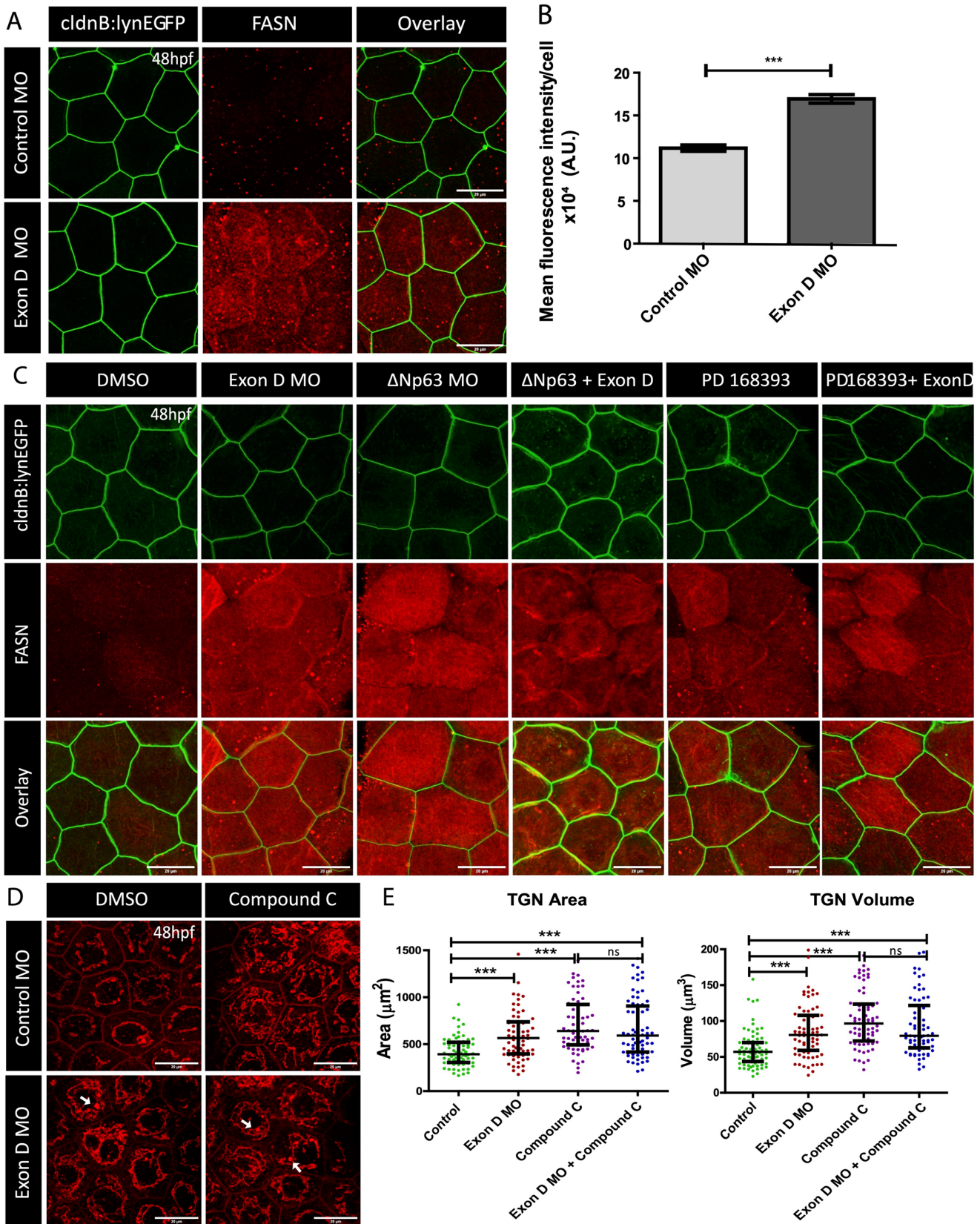
morphants and PD16393 delivered embryos. (E, G) Quantifications of TGN surface area and

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

20 $\mu$ ; Y-Z plane = 5 $\mu$ . 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 ( $\Delta$ 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  $\mu$ . 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 $\Delta$ Np63 MO	-143.6	Yes	***
WT vs DMSO	-22.45	No	ns
WT vs PD168393	-139.4	Yes	***
$\Delta$ Np63 MO vs DMSO	121.1	Yes	***
$\Delta$ 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 $\Delta$ Np63 MO	-144.9	Yes	***
WT vs DMSO	-31.52	No	ns
WT vs PD168393	-147.8	Yes	***
$\Delta$ Np63 MO vs DMSO	113.3	Yes	***
$\Delta$ 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 $\Delta$ Np63 MO	-63.8	Yes	***
WT vs DMSO	30.55	No	ns
WT vs PD168393	-6.56	No	ns
$\Delta$ Np63 MO vs DMSO	94.35	Yes	***
$\Delta$ 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 $\Delta$ Np63 MO	110.4	Yes	***
WT vs DMSO	44.27	Yes	**
WT vs PD168393	100.4	Yes	***
$\Delta$ Np63 MO vs DMSO	-66.11	Yes	***
$\Delta$ 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 $\Delta$ Np63 MO	-204	Yes	***
WT vs $\Delta$ 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 $\Delta$ Np63 MO	-188	Yes	***
DMSO vs $\Delta$ 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 $\Delta$ Np63 MO	-143.4	Yes	***
ExonD MO vs $\Delta$ Np63 MO+ExonD	9.1	No	ns
PD vs PD+ExonD	154.2	Yes	***
PD vs $\Delta$ Np63 MO	-11.57	No	ns
PD vs $\Delta$ Np63 MO+ExonD	140.9	Yes	***
PD+ExonD vs $\Delta$ Np63 MO	-165.7	Yes	***
PD+ExonD vs $\Delta$ Np63 MO+ExonD	-13.25	No	ns
$\Delta$ Np63 MO vs $\Delta$ 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 $\Delta$ Np63	-200.4	Yes	***
WT vs $\Delta$ Np63+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 $\Delta$ Np63	-193.7	Yes	***
DMSO vs $\Delta$ Np63+exonD	-5.74	No	ns
exonD vs PD	-168.4	Yes	***
exonD vs PD+exonD	28.51	No	ns
exonD vs $\Delta$ Np63	-181.5	Yes	***
exonD vs $\Delta$ Np63+exonD	6.425	No	ns
PD vs PD+exonD	196.9	Yes	***
PD vs $\Delta$ Np63	-13.14	No	ns
PD vs $\Delta$ Np63+exonD	174.8	Yes	***
PD+exonD vs $\Delta$ Np63	-210	Yes	***
PD+exonD vs $\Delta$ Np63+exonD	-22.08	No	ns
$\Delta$ Np63 vs $\Delta$ Np63+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 $\Delta$ Np63 MO	-45.07	No	ns
WT vs $\Delta$ 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 $\Delta$ Np63 MO	-3.587	No	ns
DMSO vs $\Delta$ Np63 MO+ExonD	-139.4	Yes	***
exonD vs PD	105.6	Yes	***
exonD vs PD+exonD	-20.15	No	ns
exonD vs $\Delta$ Np63 MO	138.3	Yes	***
exonD vs $\Delta$ Np63 MO+ExonD	2.458	No	ns
PD vs PD+exonD	-125.8	Yes	***
PD vs $\Delta$ Np63 MO	32.65	No	ns
PD vs $\Delta$ Np63 MO+ExonD	-103.2	Yes	**
PD+exonD vs $\Delta$ Np63 MO	158.4	Yes	***
PD+exonD vs $\Delta$ Np63 MO+ExonD	22.61	No	ns
$\Delta$ Np63 MO vs $\Delta$ 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 $\Delta$ Np63 MO	136.7	Yes	***
WT vs $\Delta$ Np63+ 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 $\Delta$ Np63 MO	166.2	Yes	***
DMSO vs $\Delta$ Np63+ 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 $\Delta$ Np63 MO	271.5	Yes	***
Exon D MO vs $\Delta$ Np63+ ExonD	10.33	No	ns
PD vs PD+Exon D	-259.8	Yes	***
PD vs $\Delta$ Np63 MO	53.86	No	ns
PD vs $\Delta$ Np63+ ExonD	-207.3	Yes	***
PD+Exon D vs $\Delta$ Np63 MO	313.6	Yes	***
PD+Exon D vs $\Delta$ Np63+ ExonD	52.49	No	ns
$\Delta$ Np63 MO vs $\Delta$ Np63+ 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	$\Delta$ 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 ΔNp63	-180	Yes	***
DMSO vs ΔNp63 Cerulenin	88.13	Yes	**
PD168393 vs Cerulenin	334.9	Yes	***
PD168393 vs PD 168393 +Cerulenin	219.4	Yes	***
PD168393 vs ΔNp63	-27.63	No	ns
PD168393 vs ΔNp63 Cerulenin	240.5	Yes	***
Cerulenin vs PD 168393 +Cerulenin	-115.5	Yes	***
Cerulenin vs ΔNp63	-362.5	Yes	***
Cerulenin vs ΔNp63 Cerulenin	-94.44	Yes	**
PD 168393 +Cerulenin vs ΔNp63	-247	Yes	***
PD 168393 +Cerulenin vs ΔNp63 Cerulenin	21.05	No	ns
ΔNp63 vs ΔNp63 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 ΔNp63	-174.6	Yes	***
DMSO vs ΔNp63 Cerulenin	87.54	Yes	**
PD168393 vs Cerulenin	340.4	Yes	***
PD168393 vs PD 168393 + Cerulenin	214.5	Yes	***
PD168393 vs ΔNp63	-18.71	No	ns
PD168393 vs ΔNp63 Cerulenin	243.4	Yes	***
Cerulenin vs PD 168393 + Cerulenin	-125.8	Yes	***
Cerulenin vs ΔNp63	-359.1	Yes	***
Cerulenin vs ΔNp63 Cerulenin	-96.97	Yes	**
PD 168393 + Cerulenin vs ΔNp63	-233.2	Yes	***
PD 168393 + Cerulenin vs ΔNp63 Cerulenin	28.85	No	ns
ΔNp63 vs ΔNp63 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 $\Delta$ Np63 MO	-222.9	Yes	***
DMSO vs $\Delta$ Np63+Rapamycin	-30.37	No	ns
DMSO vs PD168393	-184.2	Yes	***
DMSO vs PD168393+Rapamycin	45.17	No	ns
Rapamycin vs $\Delta$ Np63 MO	-248.7	Yes	***
Rapamycin vs $\Delta$ Np63+Rapamycin	-56.12	No	ns
Rapamycin vs PD168393	-210	Yes	***
Rapamycin vs PD168393+Rapamycin	19.42	No	ns
$\Delta$ Np63 MO vs $\Delta$ Np63+Rapamycin	192.5	Yes	***
$\Delta$ Np63 MO vs PD168393	38.69	No	ns
$\Delta$ Np63 MO vs PD168393+Rapamycin	268.1	Yes	***
$\Delta$ Np63+Rapamycin vs PD168393	-153.8	Yes	***
$\Delta$ 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 $\Delta$ Np63 MO	-214.6	Yes	***
DMSO vs $\Delta$ Np63+Rapamycin	-24.31	No	ns
DMSO vs PD168393	-182.3	Yes	***
DMSO vs PD168393+Rapamycin	41.5	No	ns
Rapamycin vs $\Delta$ Np63 MO	-249.3	Yes	***
Rapamycin vs $\Delta$ Np63+Rapamycin	-59.09	No	ns
Rapamycin vs PD168393	-217.1	Yes	***
Rapamycin vs PD168393+Rapamycin	6.717	No	ns
$\Delta$ Np63 MO vs $\Delta$ Np63+Rapamycin	190.3	Yes	***
$\Delta$ Np63 MO vs PD168393	32.23	No	ns
$\Delta$ Np63 MO vs PD168393+Rapamycin	256.1	Yes	***
$\Delta$ Np63+Rapamycin vs PD168393	-158	Yes	***
$\Delta$ 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 $\Delta$ Np63 MO	-146.5	Yes	***
DMSO vs $\Delta$ Np63+Rapamycin	-20.27	No	ns
DMSO vs PD168393	-107.2	Yes	***
DMSO vs PD168393+Rapamycin	43.34	No	ns
Rapamycin vs $\Delta$ Np63 MO	-156.5	Yes	***
Rapamycin vs $\Delta$ Np63+Rapamycin	-30.24	No	ns
Rapamycin vs PD168393	-117.2	Yes	***
Rapamycin vs PD168393+Rapamycin	33.37	No	ns
$\Delta$ Np63 MO vs $\Delta$ Np63+Rapamycin	126.3	Yes	***
$\Delta$ Np63 MO vs PD168393	39.34	No	ns
$\Delta$ Np63 MO vs PD168393+Rapamycin	189.9	Yes	***
$\Delta$ Np63+Rapamycin vs PD168393	-86.93	Yes	*
$\Delta$ 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 $\Delta$ Np63 MO	-106.4	Yes	***
PD168393 vs WT	74.95	Yes	***
PD168393 vs $\Delta$ Np63 MO	-18.79	No	ns
WT vs $\Delta$ 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 $\Delta$ Np63 MO	-103	Yes	***
PD168393 vs WT	54.01	Yes	***
PD168393 vs $\Delta$ Np63 MO	-24.13	No	ns
WT vs $\Delta$ 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 $\Delta$ Np63 MO	-170.4	Yes	***
DMSO vs $\Delta$ Np63+Rapamycin	-52.68	No	ns
DMSO vs PD168393	-108.6	Yes	***
DMSO vs PD168393+Rapamycin	36	No	ns
Rapa vs $\Delta$ Np63 MO	-226.1	Yes	***
Rapa vs $\Delta$ Np63+Rapamycin	-108.3	Yes	***
Rapa vs PD168393	-164.3	Yes	***
Rapa vs PD168393+Rapamycin	-19.64	No	ns
$\Delta$ Np63 MO vs $\Delta$ Np63+Rapamycin	117.7	Yes	***
$\Delta$ Np63 MO vs PD168393	61.79	No	ns
$\Delta$ Np63 MO vs PD168393+Rapamycin	206.4	Yes	***
$\Delta$ Np63+Rapamycin vs PD168393	-55.96	No	ns
$\Delta$ 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 $\Delta$ Np63 MO	-176.6	Yes	***
DMSO vs $\Delta$ Np63+Rapamycin	-63.29	No	ns
DMSO vs PD168393	-102.4	Yes	***
DMSO vs PD168393+Rapamycin	63.69	No	ns
Rapa vs $\Delta$ Np63 MO	-259.3	Yes	***
Rapa vs $\Delta$ Np63+Rapamycin	-146	Yes	***
Rapa vs PD168393	-185.2	Yes	***
Rapa vs PD168393+Rapamycin	-19.05	No	ns
$\Delta$ Np63 MO vs $\Delta$ Np63+Rapamycin	113.3	Yes	***
$\Delta$ Np63 MO vs PD168393	74.16	No	ns
$\Delta$ Np63 MO vs PD168393+Rapamycin	240.3	Yes	***
$\Delta$ Np63+Rapamycin vs PD168393	-39.12	No	ns
$\Delta$ 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 $\Delta$ Np63 MO	-120	Yes	***
DMSO (Control) vs $\Delta$ 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 $\Delta$ Np63 MO	23.48	No	ns
PD 168393 vs $\Delta$ 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 $\Delta$ Np63 MO	60.28	No	ns
Exon D MO vs $\Delta$ Np63+Exon D MO	178	Yes	***
PD 168393+Exon D MO vs WT	189.7	Yes	***
PD 168393+Exon D MO vs $\Delta$ Np63 MO	5.107	No	ns
PD 168393+Exon D MO vs $\Delta$ Np63+Exon D MO	122.8	Yes	***
WT vs $\Delta$ Np63 MO	-184.6	Yes	***
WT vs $\Delta$ Np63+Exon D MO	-66.89	No	ns
$\Delta$ Np63 MO vs $\Delta$ 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 $\Delta$ Np63 MO	-63.03	No	ns
DMSO (Control) vs $\Delta$ 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 $\Delta$ Np63 MO	50.28	No	ns
PD 168393 vs $\Delta$ 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 $\Delta$ Np63 MO	74.5	Yes	*
Exon D MO vs $\Delta$ Np63+Exon D MO	194.8	Yes	***
PD 168393+Exon D MO vs WT	193	Yes	***
PD 168393+Exon D MO vs $\Delta$ Np63 MO	51.21	No	ns
PD 168393+Exon D MO vs $\Delta$ Np63+Exon D MO	171.5	Yes	***
WT vs $\Delta$ Np63 MO	-141.7	Yes	***
WT vs $\Delta$ Np63+Exon D MO	-21.44	No	ns
$\Delta$ Np63 MO vs $\Delta$ 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 <sup>+/11+</sup> Non-clones	-107.0	Yes	***
Uninjected Wildtype vs MyoVbRab10 <sup>+/11+</sup> Clones	-21.21	No	ns
Uninjected Wildtype vs MyoVbRab10 <sup>-/11+</sup> Non-clones	-66.77	Yes	**
Uninjected Wildtype vs MyoVbRab10 <sup>-/11+</sup> Clones	-76.45	Yes	***
MyoVbRab10 <sup>+/11+</sup> Non-clones vs MyoVbRab10 <sup>+/11+</sup>	85.81	Yes	***
MyoVbRab10 <sup>+/11+</sup> Non-clones vs MyoVbRab10 <sup>/11</sup>	40.25	No	ns
MyoVbRab10 <sup>+/11+</sup> Non-clones vs MyoVbRab10 <sup>/11+</sup>	30.57	No	ns
MyoVbRab10 <sup>+/11+</sup> Clones vs MyoVbRab10 <sup>/11+</sup>	-45.56	No	ns
MyoVbRab10 <sup>+/11+</sup> Clones vs MyoVbRab10 <sup>/11+</sup> Clones	-55.24	Yes	*
MyoVbRab10 <sup>/11+</sup> Non-clones vs MyoVbRab10 <sup>/11+</sup>	-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 <sup>+/11+</sup> Non-clones	-161.1	Yes	***
Uninjected Wildtype vs MyoVbRab10 <sup>+/11+</sup> Clones	-59.99	Yes	**
Uninjected Wildtype vs MyoVbRab10 <sup>-/11+</sup> Non-clones	-102.8	Yes	***
Uninjected Wildtype vs MyoVbRab10 <sup>-/11+</sup> Clones	-117.1	Yes	***
MyoVbRab10 <sup>+/11+</sup> Non-clones vs MyoVbRab10 <sup>+/11+</sup>	101.1	Yes	***
MyoVbRab10 <sup>+/11+</sup> Non-clones vs MyoVbRab10 <sup>-/11+</sup>	58.33	Yes	*
MyoVbRab10 <sup>+/11+</sup> Non-clones vs MyoVbRab10 <sup>-/11+</sup>	43.98	No	ns
MyoVbRab10 <sup>+/11+</sup> Clones vs MyoVbRab10 <sup>-/11+</sup>	-42.79	No	ns
MyoVbRab10 <sup>+/11+</sup> Clones vs MyoVbRab10 <sup>-/11+</sup> Clones	-57.14	Yes	*
MyoVbRab10 <sup>-/11+</sup> Non-clones vs MyoVbRab10 <sup>-/11+</sup>	-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 $\Delta$ Np63 MO	-161.4	Yes	***
DMSO vs $\Delta$ Np63 MO + Cerulenin	33.53	No	ns
DMSO vs PD168393	-143.5	Yes	***
DMSO vs PD168393 + Cerulenin	10.23	No	ns
Cerulenin vs $\Delta$ Np63 MO	-235.3	Yes	***
Cerulenin vs $\Delta$ Np63 MO + Cerulenin	-40.41	No	ns
Cerulenin vs PD168393	-217.4	Yes	***
Cerulenin vs PD168393 + Cerulenin	-63.7	No	ns
$\Delta$ Np63 MO vs $\Delta$ Np63 MO + Cerulenin	194.9	Yes	***
$\Delta$ Np63 MO vs PD168393	17.88	No	ns
$\Delta$ Np63 MO vs PD168393 + Cerulenin	171.6	Yes	***
$\Delta$ Np63 MO + Cerulenin vs PD168393	-177	Yes	***
$\Delta$ 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 $\Delta$ Np63 MO	-146.3	Yes	***
DMSO vs $\Delta$ Np63 MO + Cerulenin	35.28	No	ns
DMSO vs PD168393	-111.6	Yes	***
DMSO vs PD168393 + Cerulenin	52.33	No	ns
Cerulenin vs $\Delta$ Np63 MO	-243.5	Yes	***
Cerulenin vs $\Delta$ Np63 MO + Cerulenin	-61.95	No	ns
Cerulenin vs PD168393	-208.9	Yes	***
Cerulenin vs PD168393 + Cerulenin	-44.89	No	ns
$\Delta$ Np63 MO vs $\Delta$ Np63 MO + Cerulenin	181.5	Yes	***
$\Delta$ Np63 MO vs PD168393	34.63	No	ns
$\Delta$ Np63 MO vs PD168393 + Cerulenin	198.6	Yes	***
$\Delta$ Np63 MO + Cerulenin vs PD168393	-146.9	Yes	***
$\Delta$ 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 Cvs Exon D +Comp C	24.11	No	ns
Exon D vs Exon D + CompC	-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**

[Click here to download Table S13](#)