

## Supplementary data

### PI3KC2 $\beta$ depletion rescues endosomal trafficking defects in *Mtm1* knockout skeletal muscle cells

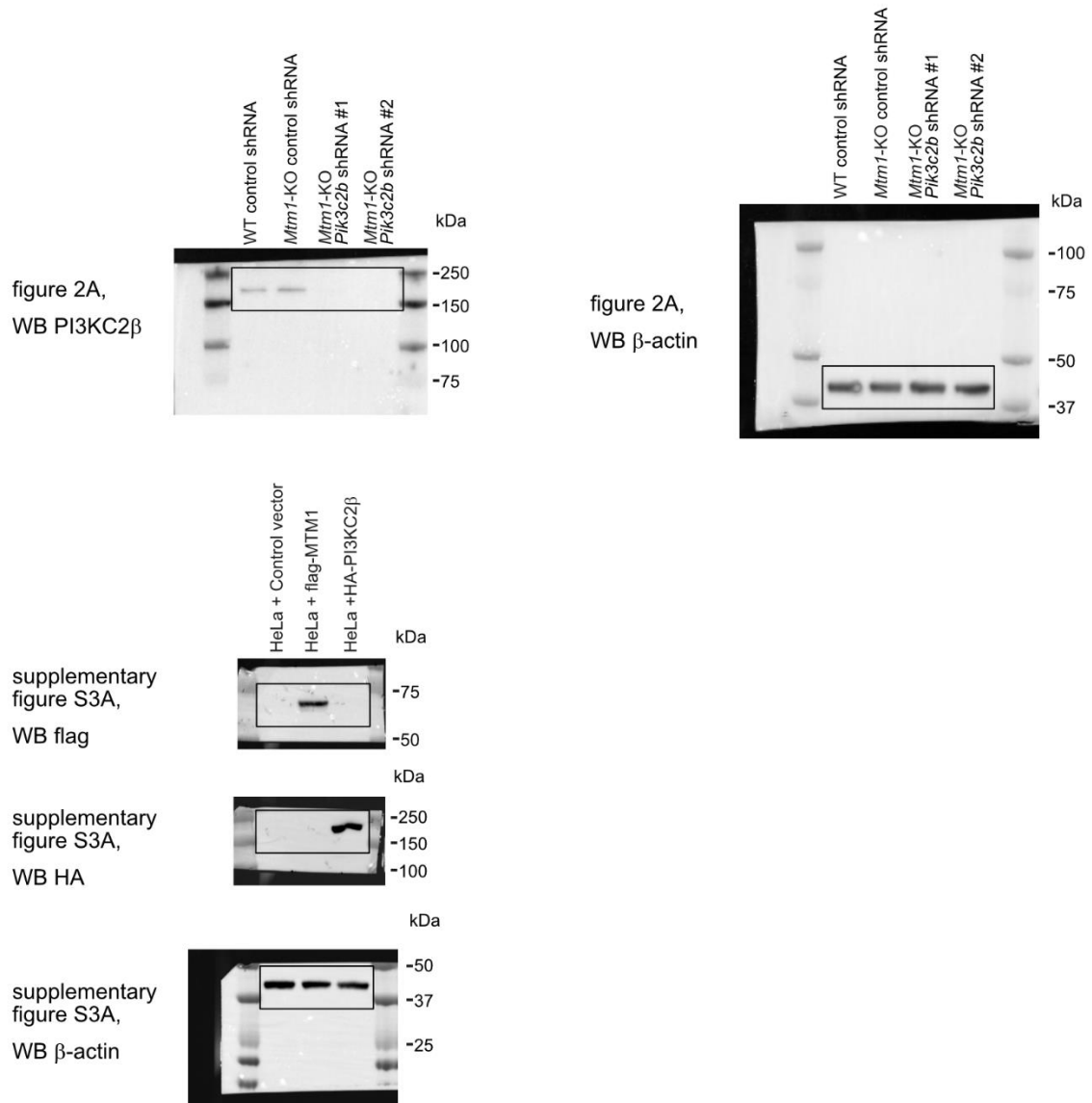
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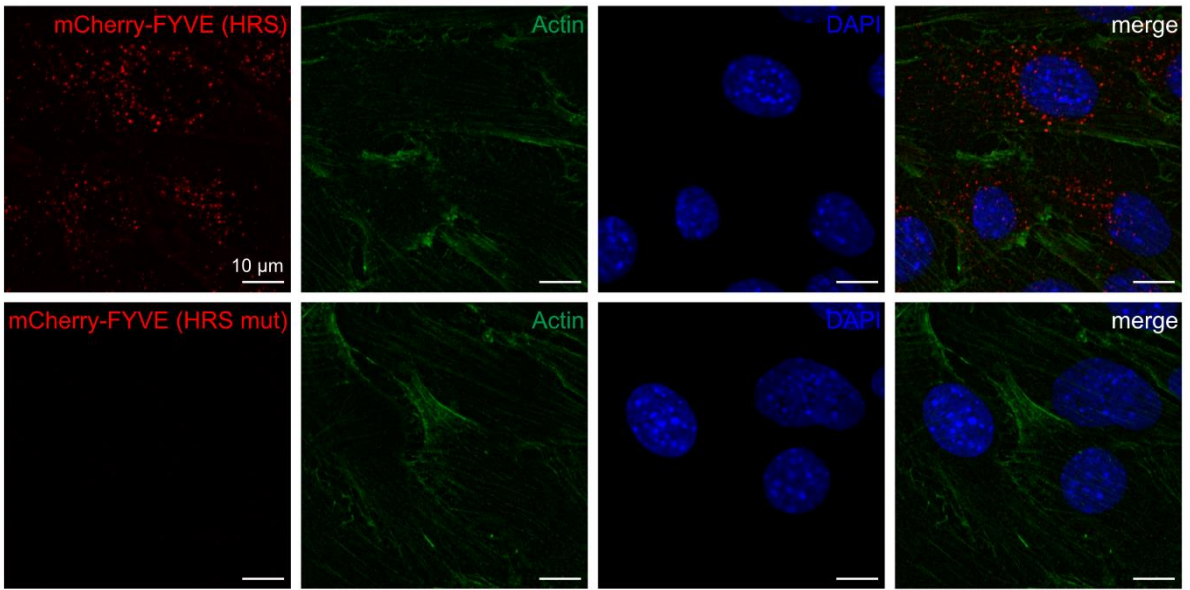
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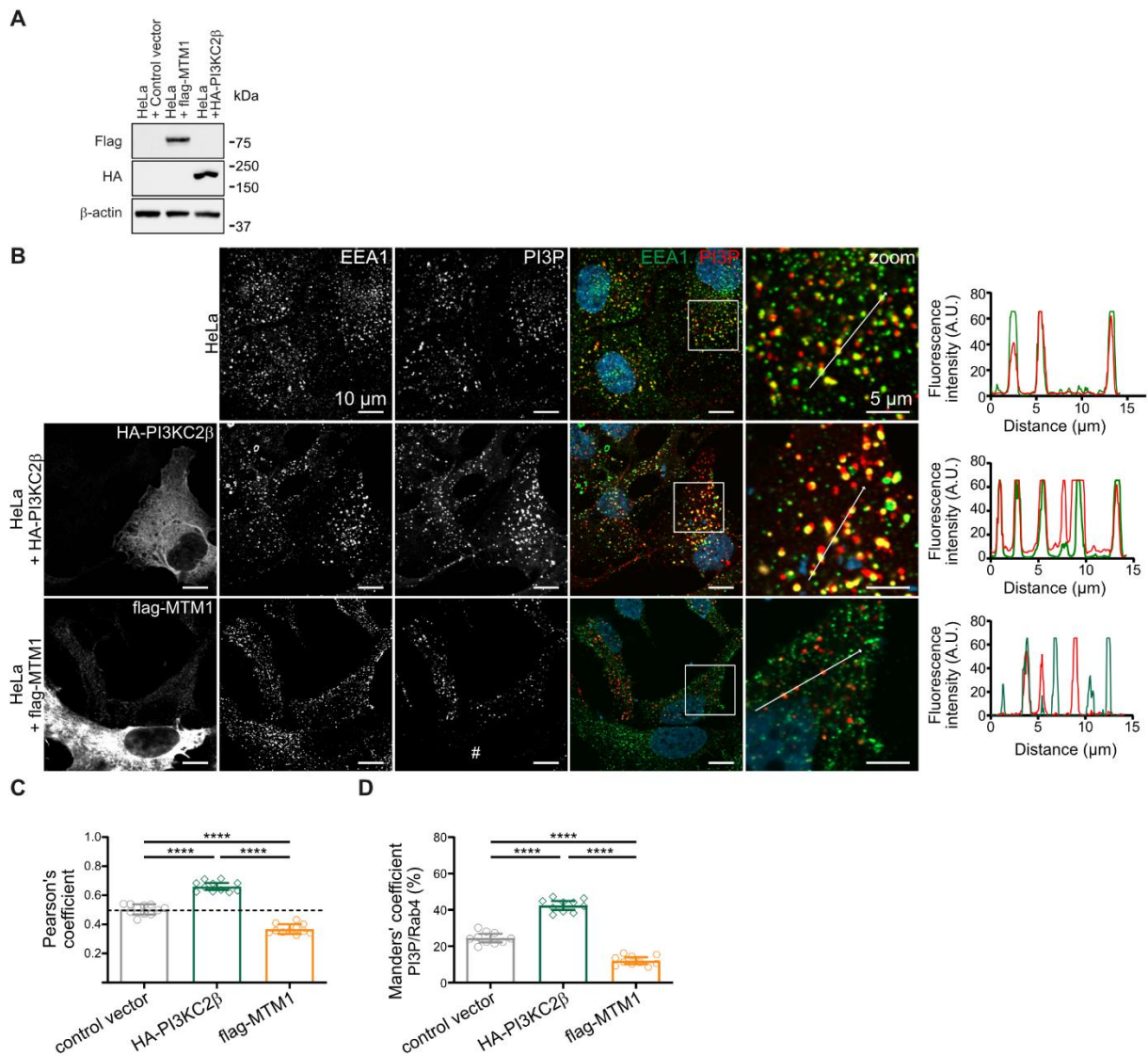
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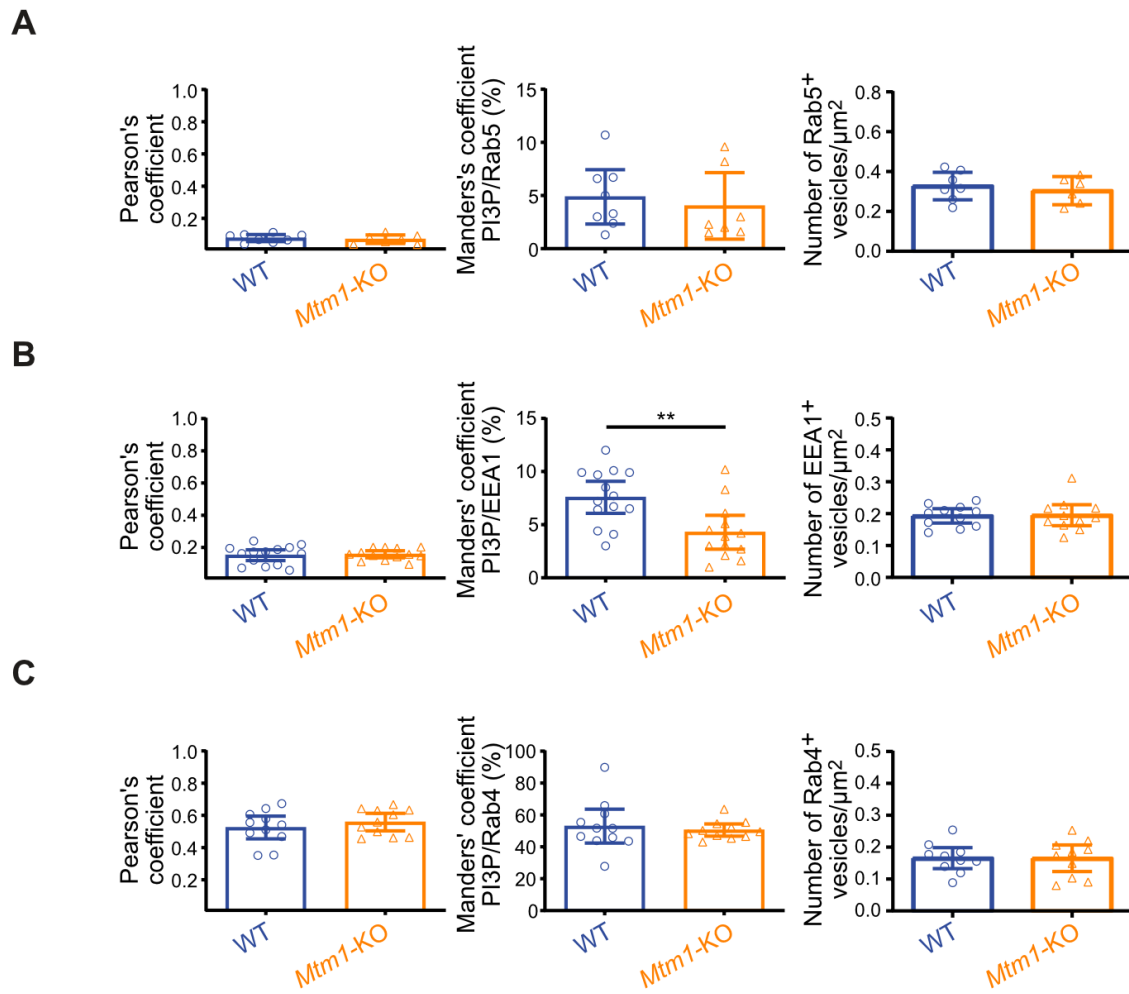
**Fig. S1.** Uncropped western blot images from figure 2 and supplementary Fig. S3.



**Fig. S2.** Top: Representative images of PI3P labeling with the mCherry-FYVE (HRS) probe in C2C12 WT myoblast. Bottom: Representative images of PI3P labeling with the mCherry-FYVE (HRS mut) probe in C2C12 WT myoblast. The mutations consist of R24A/K25A/R29A. Scale bar, 10 μm.



**Fig. S3.** PI3P accumulates on EEA1-positive vesicles in HeLa cells expressing HA-PI3KC2β, while flag-MTM1 overexpression leads to a marked reduction. **A:** Expression of HA-PI3KC2β and flag-MTM1 in HeLa cells transfected with plasmids encoding these constructs, analyzed by Western blot. Images are representative of three independent experiments. **B:** Representative confocal images of HeLa cells expressing or not expressing HA-PI3KC2β or flag-MTM1, labeled with a probe for PI3P (FYVE domain of Hrs protein), EEA1, and DAPI (blue). Scale bar: 10 μm. # indicates a cell highly expressing flag-MTM1, in which PI3P is not detected. Magnification of the boxed area shown on the right. Respective line scans are shown on the right of the zoom panel. Scale bar: 5 μm. **C:** Quantification of Pearson's coefficient from the experiment described in B. For flag-MTM1-expressing cells, only those with low expression levels were considered. Results are shown as mean ± 95% confidence interval, n=3, each point represents one field of view. \*\*\*\*p<0.0001 according to one-way ANOVA followed by Šidák's multiple comparison test. **D:** Quantification of the Manders' coefficient from the experiment described in B. Results are represented as mean ± 95% confidence interval, n=3, each point represents one field of view. \*\*\*\*p<0.0001 according to one-way ANOVA followed by Šidák's multiple comparison test.



**Fig. S4.** Quantification of Pearson's coefficient, Manders' coefficient, and the number of indicated positive vesicles in WT and *Mtm1*-KO myoblasts labeled with a probe for PI3P (FYVE domain of Hrs protein) and the indicated endosomal marker. A: Rab5. B: EEA1. C: Rab4. Results are shown as mean  $\pm$  95% confidence interval,  $n=3$ , each point represents one field of view. \*\* $p < 0.01$  according to Student's t-test.