

Expanded View Figures

Figure EV1. Proteome profile of muscle cells during myogenesis.

(A) Representative WB of total cell lysate from C2C12 cells and myotubes. MF20: Myosin heavy chain; MyoG: Myogenin. Bar plots represent the densitometric quantification of WB bands. (B–D) Heatmap (Log_2 LFQ normalized intensity) of proteins related to the ER (B), lysosome (C) and autophagy-ubiquitin pathway (D) that are significantly deregulated in differentiated myotubes versus C2C12 myoblasts. (E, F) Representative WB of ER and lysosomal proteins from total cell lysate from myoblasts and myotubes. (G) Heatmap (Log_2 LFQ normalized intensity) of significantly regulated proteins in murine primary myoblasts and myotubes differentiated in growth medium (GM) or in differentiation medium (DM). The profile plot highlights significantly upregulated proteins. (H) Principal components analysis (PCA) of the full proteomes from primary murine myoblasts and myotubes differentiated in growth medium (GM) or in differentiation medium (DM). (I) GOCC enrichment analysis from MS data with ER elements in purple and lysosomal components in green. (J) GOCC terms and frequencies of the upregulated proteins identified in murine myotubes. (K) Heatmap (LFQ normalized intensity) of significantly deregulated proteins in human myoblasts and myotubes differentiated in growth medium (GM) or in differentiation medium (DM). The profile plot highlights significantly upregulated proteins. (L) Principal components analysis (PCA) of the full proteomes from primary human myoblasts and myotubes differentiated in growth medium (GM) or in differentiation medium (DM). (M) GOCC enrichment analysis from MS data with ER elements in purple and lysosomal components in green. (N) GOCC terms and frequencies of the upregulated proteins identified in human myotubes. (O) The bar graph represents the quantification of the ER area in myoblasts and myotubes (Fig. 1A). All data are represented as mean $\pm$ s.d.; *** $P < 0.001$ (Student t test). Mass spectrometry was performed in triplicate. Western blots were performed in three independent experiments. Source data are available online for this figure.



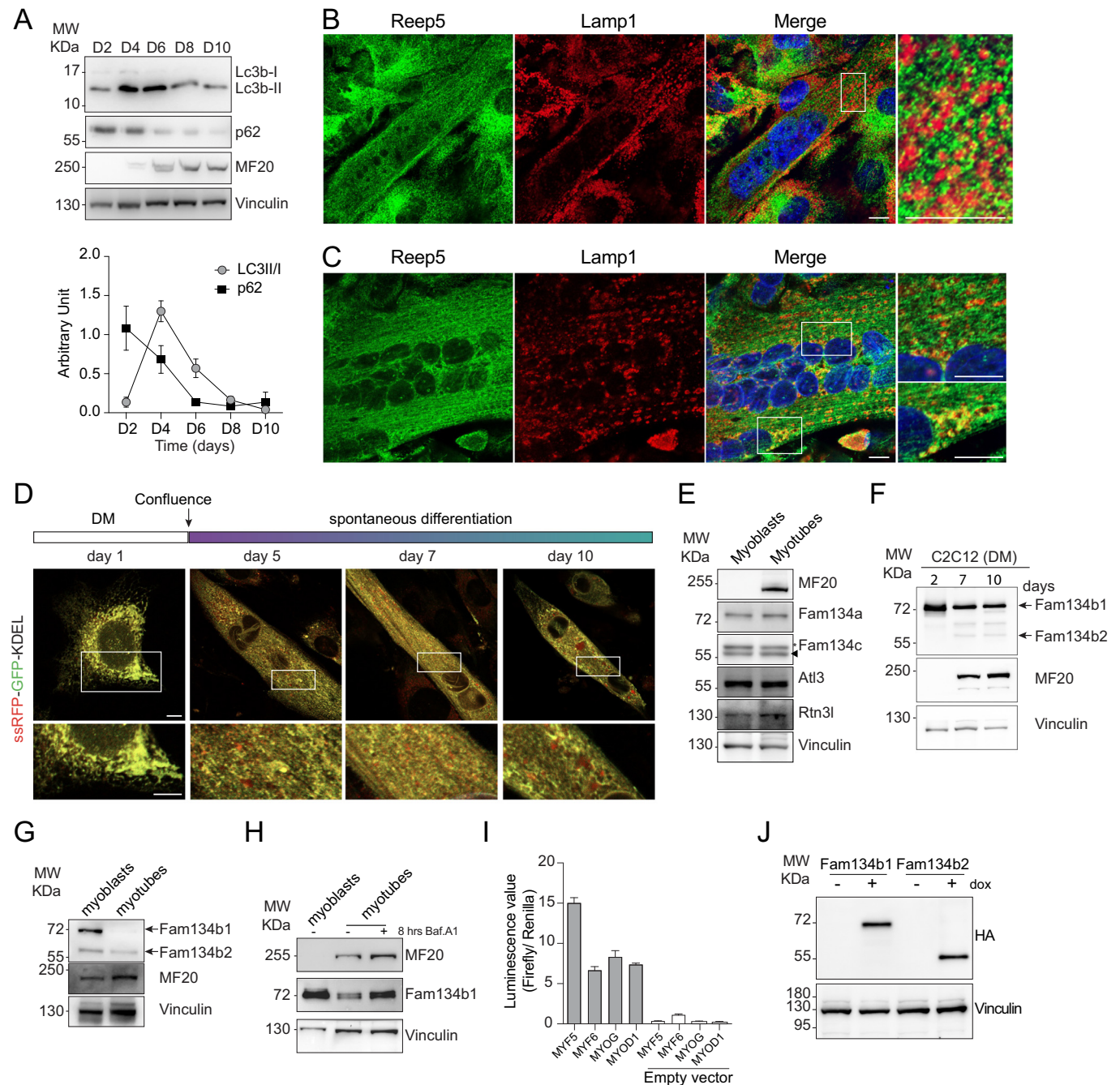


Figure EV2. Fam134b isoforms are differently modulated during myogenesis.

(A) WB analysis showing Lc3b, p62, and MF20 protein levels at various differentiation time points during C2C12 differentiation. Graphs represent the densitometric analysis of WB bands. (B) Confocal images of differentiated C2C12 cells stably expressing TMEM192-3xHA stained for HA and the ER protein REEP5. Scale bar: 10 μ m. (C) Confocal images of differentiated human myoblasts, stained for the ER protein REEP5 and the lysosomal protein LAMP1. Scale bar: 10 μ m. (D) Confocal images of C2C12 cells, stably overexpressing ssRFP-GFP-KDEL during their differentiation. Scale bar: 10 μ m. Inset Scale bar: 5 μ m. DM: differentiation medium (2% horse serum) (E). (E) WB analysis of the endogenous ER-phagy receptors in C2C12 myoblasts and myotubes. (F, G) WB analysis of endogenous FAM134B1 and FAM134B2 in C2C12 cells (F) and human myoblasts (G) differentiated into myotubes using differentiation medium (DM). (H) WB analysis of endogenous Fam134b1 in C2C12 myoblasts and myotubes, with and without Bafilomycin A1 (Baf.A1) treatment. MF20 was used as a muscle differentiation marker. (I) Graphical quantification of luciferase assays demonstrating the activity of the indicated transcription factors co-transfected, in HEK 293T, with a plasmid containing the firefly luciferase gene downstream of the Fam134b1 promoter. (J) WB representing the protein levels of HA-Fam134b1 and HA-Fam134b2 in C2C12 stable cell lines. Imaging, western blots and luciferase assay were performed in three independent experiments. Source data are available online for this figure.

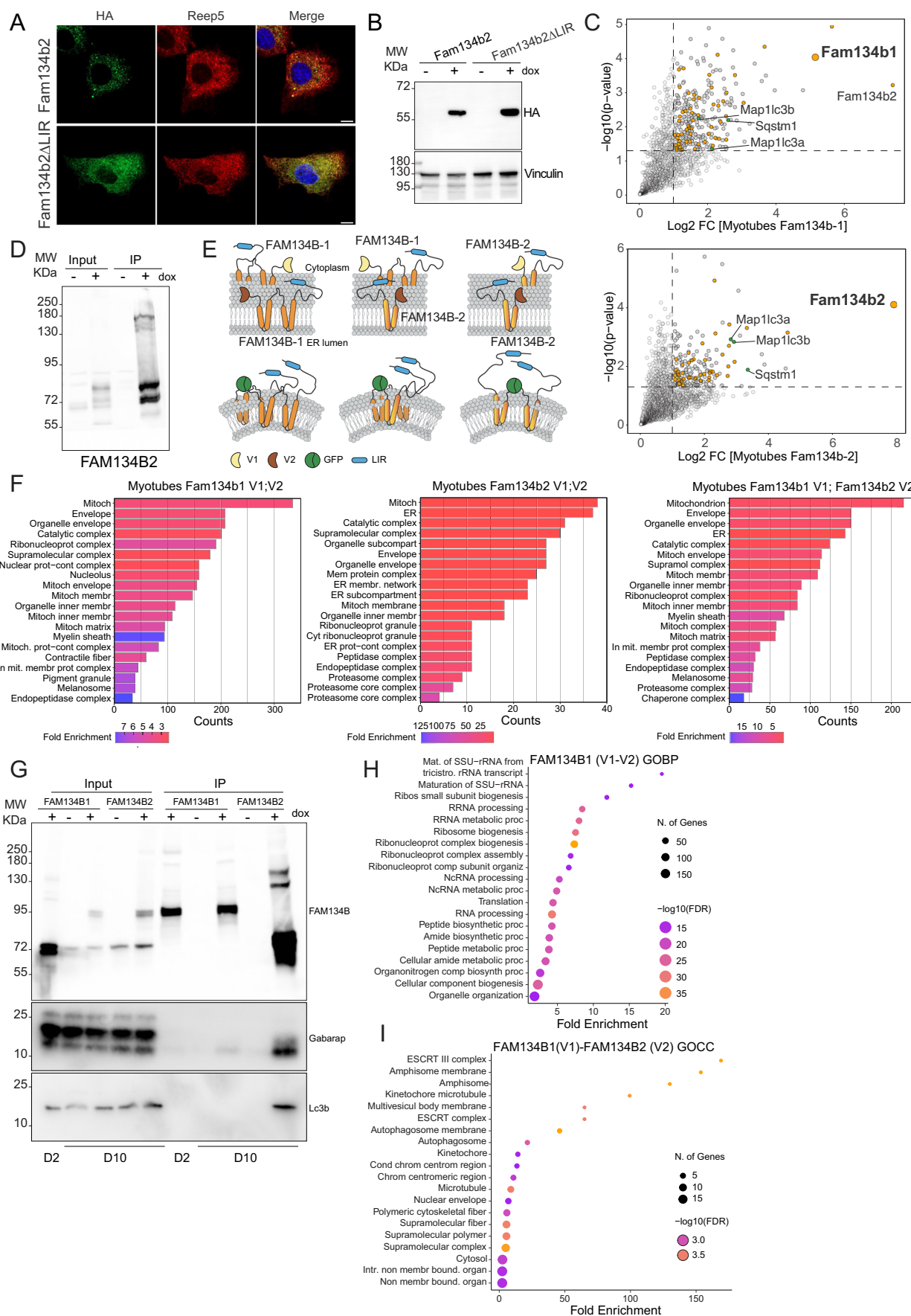


Figure EV3. FAM134B2 mostly interacts with ER and autophagy proteins.

(A) Confocal images of C2C12 cells expressing HA-Fam134b2 and HA-Fam134b2ΔLIR, stained for the ER protein REEP5 and HA. Scale bar: 10 μm. (B) WB analysis of HA tag in C2C12 cells expressing HA-Fam134b2 and HA-Fam134b2ΔLIR. (C) Volcano plot illustrating the interactome of Fam134b1 (left) and Fam134b2 (right) in differentiated myotubes, highlighting ER proteins (orange dots) and the autophagy proteins (green dots). (D) WB analysis following RFP-tagged Fam134b2 pull-down in differentiated myotubes. (E) Schematic representation of the bimolecular complementation affinity purification system employed to pulldown FAM134B complexes. (F) GOCC terms and frequencies of the different FAM134B complexes interactors in myotubes. (G) IP of GFP tag in C2C12 cells expressing V1/V2-FAM134B1 or V1/V2-FAM134B2 and WB for Lc3b and Gabarap. (H) GOBP enrichment analysis from FAM134B1 homodimers interactors. (I) GOCC enrichment analysis from FAM134B1-FAM134B2 heterodimers interactors. Image, western blots and mass spectrometry were performed in three independent experiments. Source data are available online for this figure.

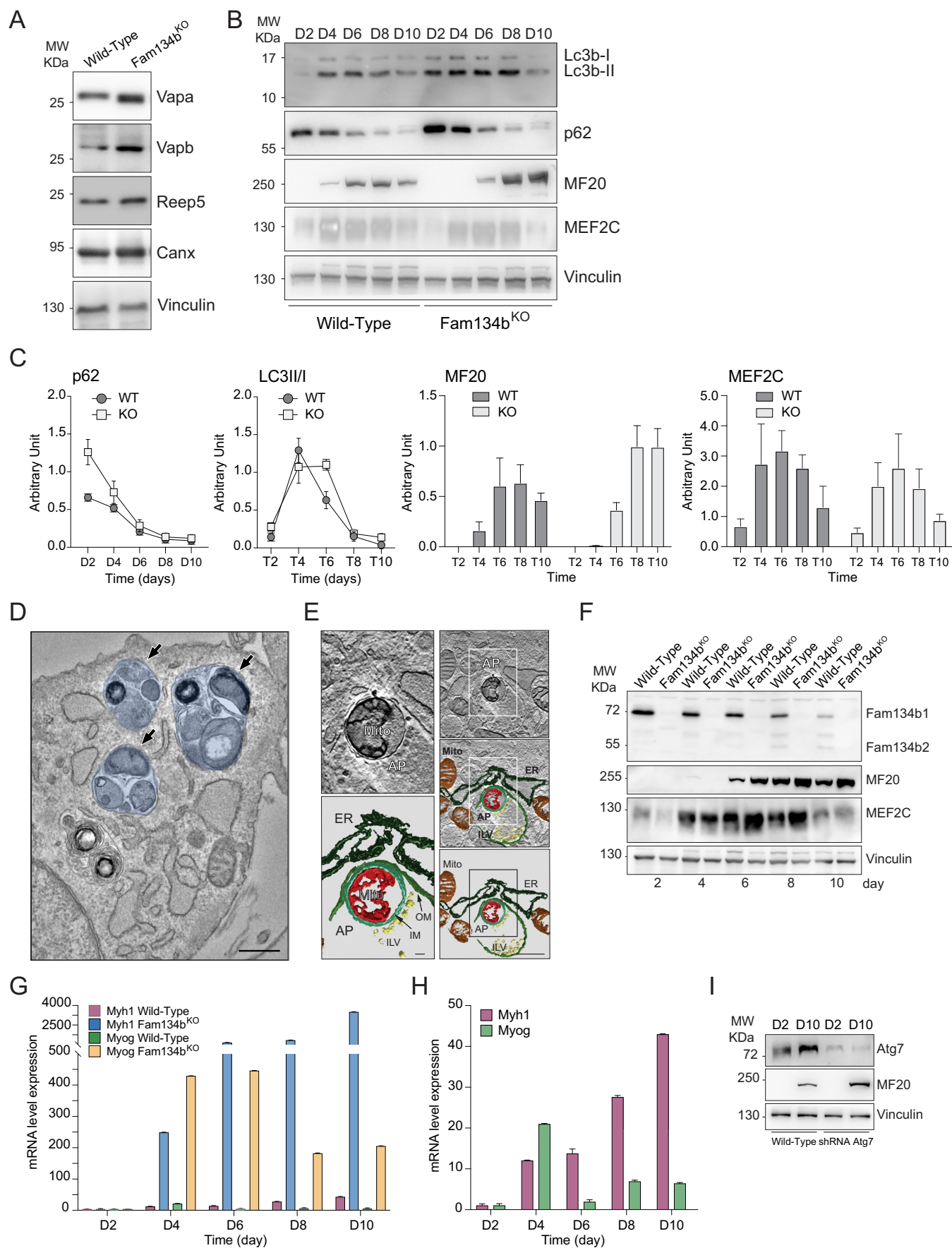
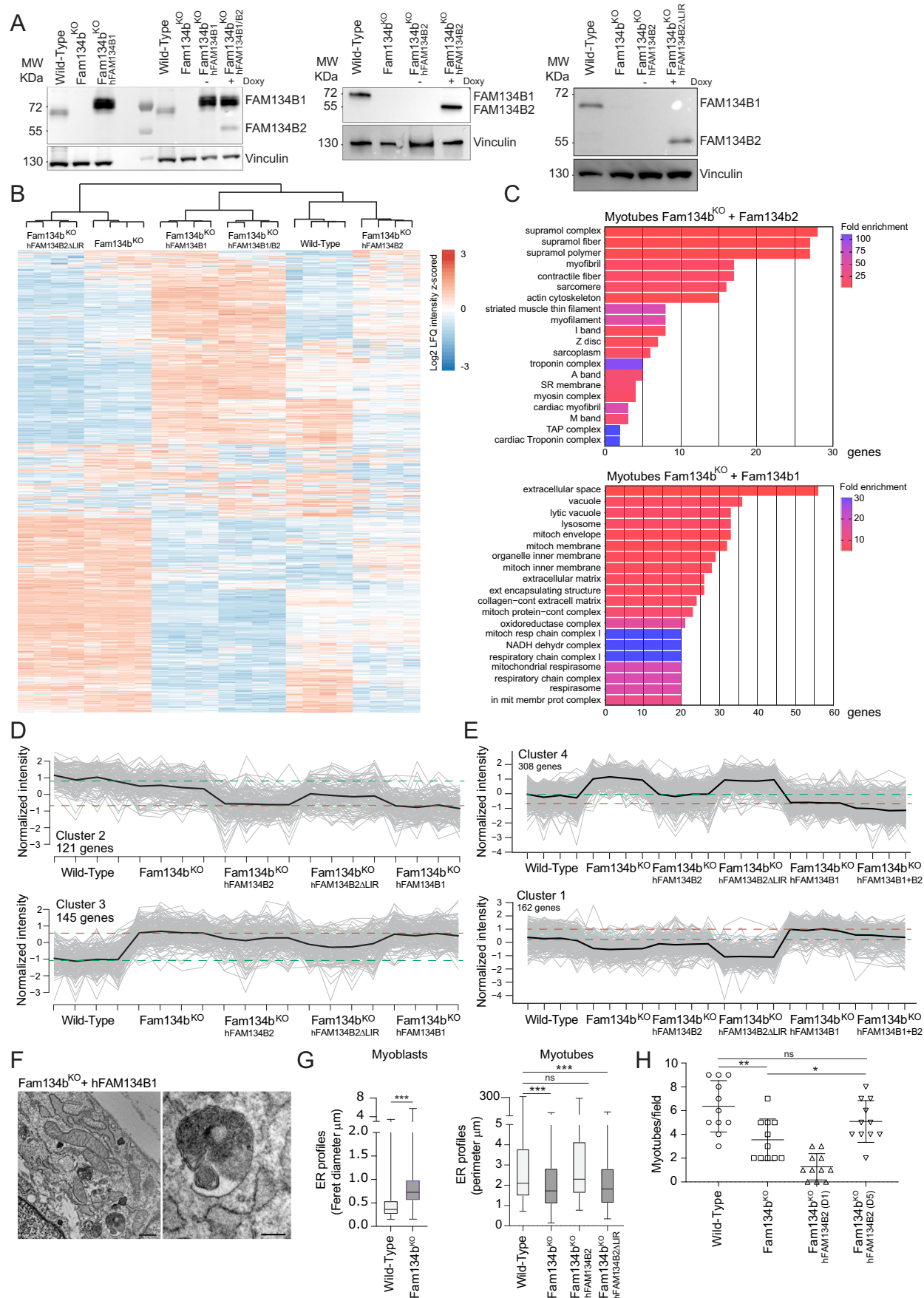




Figure EV4. A proper ER-phagy flux is necessary for myogenesis.

(A) Representative WB analysis of endogenous ER proteins in WT and Fam134b^{KO} myotubes. (B) Representative WB analysis of Lc3b, p62, MF20 and MEF2C in WT and Fam134b^{KO} C2C12 cells throughout differentiation. (C) Densitometric analysis of WB bands in panel (B). (D) Electron microscopy images of Fam134b^{KO} myotubes, with black arrows indicating autophagosome structures. Scale bar: 400 nm. (E) Tomography and image reconstruction depicting a mitochondrion inside an autophagosome in a Fam134b^{KO} myotube. AP autophagosome, ER endoplasmic reticulum, Mito mitochondria, OM outer autophagosomal membrane (dark green), IM inner autophagosomal membranes (light green), ILV intra luminal vesicles. Red: mitochondria inside the autophagosome. Scale bar: 200 nm. (F) Representative WB analysis of endogenous Fam134b isoforms and the muscle differentiation markers MF20 and MEF2C in WT and Fam134b^{KO} C2C12 cells throughout differentiation. (G) Real time PCR analysis illustrating the expression profiles of the indicated genes during the differentiation of WT and Fam134b^{KO} C2C12 cells. (H) Magnified view of the bar graphs presented in panel G, focusing on WT samples. (I) Representative WB images of endogenous Atg7 and MF20 protein levels in WT and Atg7^{KO} C2C12 cells during differentiation. Data are represented as mean $\pm$ s.d. Western blots, imaging and real time pcr were performed in three independent experiments. Source data are available online for this figure.



◀ **Figure EV5. Fam134b2 reconstitution is sufficient to rescue the proteomic abnormalities in Fam134b^{KO} myotubes.**

(A) WB analysis of FAM134B in Fam134b^{KO} C2C12 cells reconstituted with the indicated FAM134B isoforms. (B) Heatmap (Log₂ LFQ normalized intensity) of the full proteomes from WT, Fam134b^{KO} and reconstituted myotubes. (C) GOCC terms and frequencies of the significantly deregulated proteins in the proteomic analysis of Fam134b^{KO} myotubes reconstituted with hFAM134B2 or hFAM134B1. (D) Profile plot showing significantly regulated proteins according to ANOVA, specifically within cluster 2 and 3 of ER associated proteins. (E) Profile plots of significantly altered ER proteins belonging to clusters 4 and 1, as identified by ANOVA. (F) Electron microscopy images of the ER in Fam134b^{KO} myotubes reconstituted with hFAM134B1. Scale bar: 500 nm. Insets highlight autophagosome structures. Scale bar: 200 nm. (G) Left histograms represent the Feret's diameter measures from the ER of Wild-Type and Fam134b^{KO} myoblasts. Right histograms represent the perimeter of the ER from Wild-Type, Fam134b^{KO} and reconstituted myotubes. (H) Quantitative analysis of myotubes number in Wild-Type and Fam134b^{KO} C2C12 after 10 days of differentiation as well as in Fam134b^{KO} C2C12 overexpressing hFAM134B1 since D1 or hFAM134B2 from D5 of differentiation. Data are represented as mean ± s.d. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 (Mann-Witney EV5G; one-way ANOVA EV5H). Mass spectrometry was performed in quadruplicate. Western blots and imaging were performed in three independent experiments. Source data are available online for this figure.