

Expanded View Figures

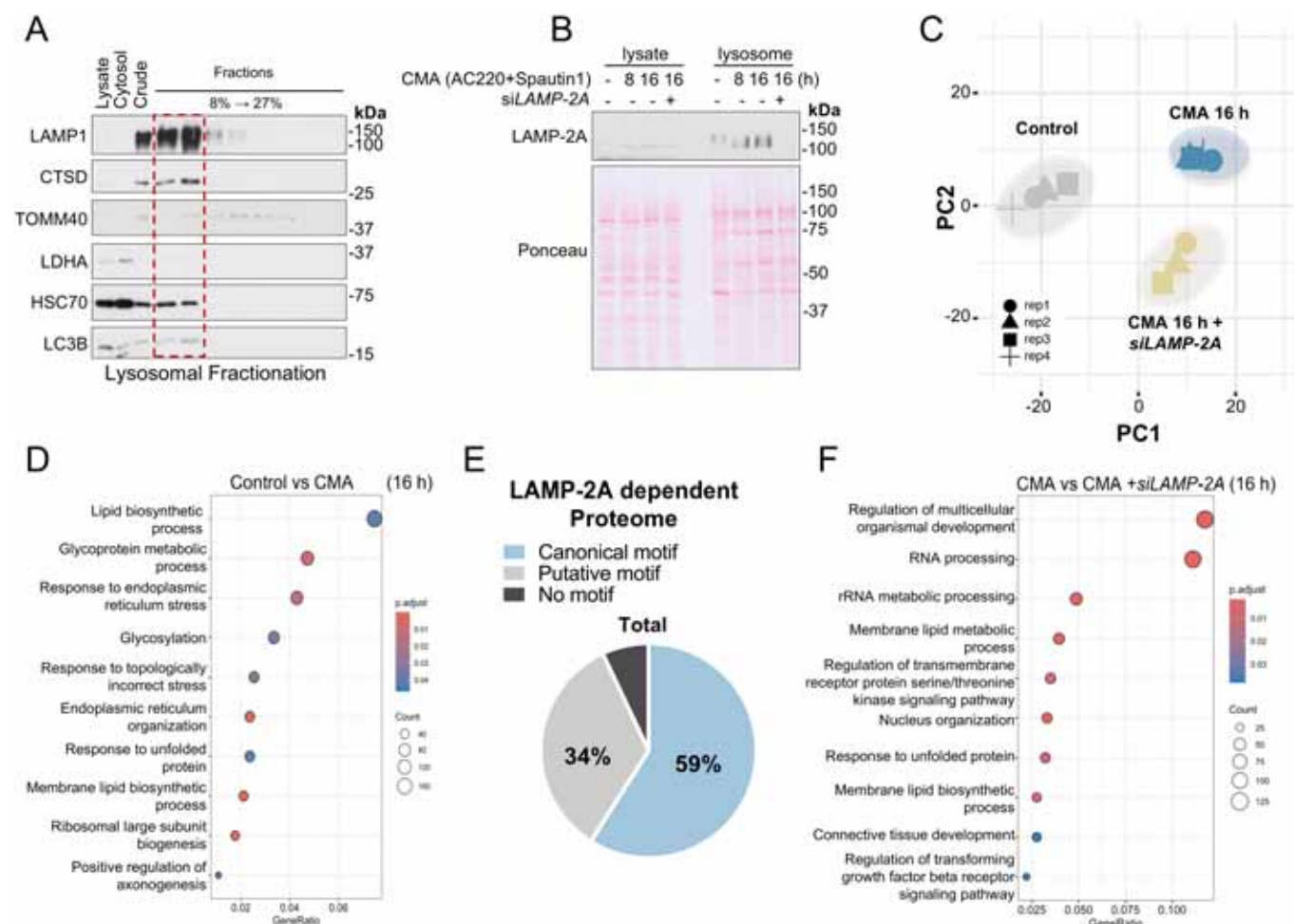


Figure EV1. Lysosomal fractionation and quantitative proteomics of ES2 cells.

(A) Cellular fractions isolated from ES2 cells by differential large-scale multi-layered density gradient centrifugations and analyzed by immunoblotting using antibodies against lysosomal membrane protein LAMP1, lysosomal matrix protein cathepsin D (CTSD), mitochondrial protein translocase of outer mitochondrial membrane 40 (TOMM40), cytosolic protein Lactate dehydrogenase A (LDHA), the Hsc70 chaperone protein and macroautophagy marker LC3B. The red square indicates the low percentage gradient fraction, enriched in lysosomes, and chosen for further analysis. (B) Cellular lysate and lysosomal fractions from control (DMSO), 8 and 16 h CMA activated (AC220+Spautin1) and 16 h CMA+siLAMP-2A conditions analyzed by immunoblotting using LAMP-2A antibody. Total protein loading is visualized by Ponceau S Red staining. (C) Score plot of principle component analysis (PCA) in 2D for control, 16 h CMA, and 16 h CMA+siLAMP-2A treated sample sets ($n_{exp} = 4$). (D) Pathway enrichment analysis by Gene ontology (GO) of biological processes comparing control vs 16 h CMA treated sample sets (Over Representation Analysis). (E) Pie graphs of the experimentally validated LAMP-2A-dependent lysosome proteome showing the percentage of proteins with indicated types of KFERQ-like motifs. (F) GO analysis comparing 16 h CMA vs CMA+siLAMP-2A treated sample sets. Dot plots of top 10 enriched pathways are indicated as the ratio of the differentially expressed gene number to the total gene number for a certain annotation. The size and color of the dots represent the gene ratio and the range of adjusted P values, respectively (Over Representation Analysis). Source data are available online for this figure.

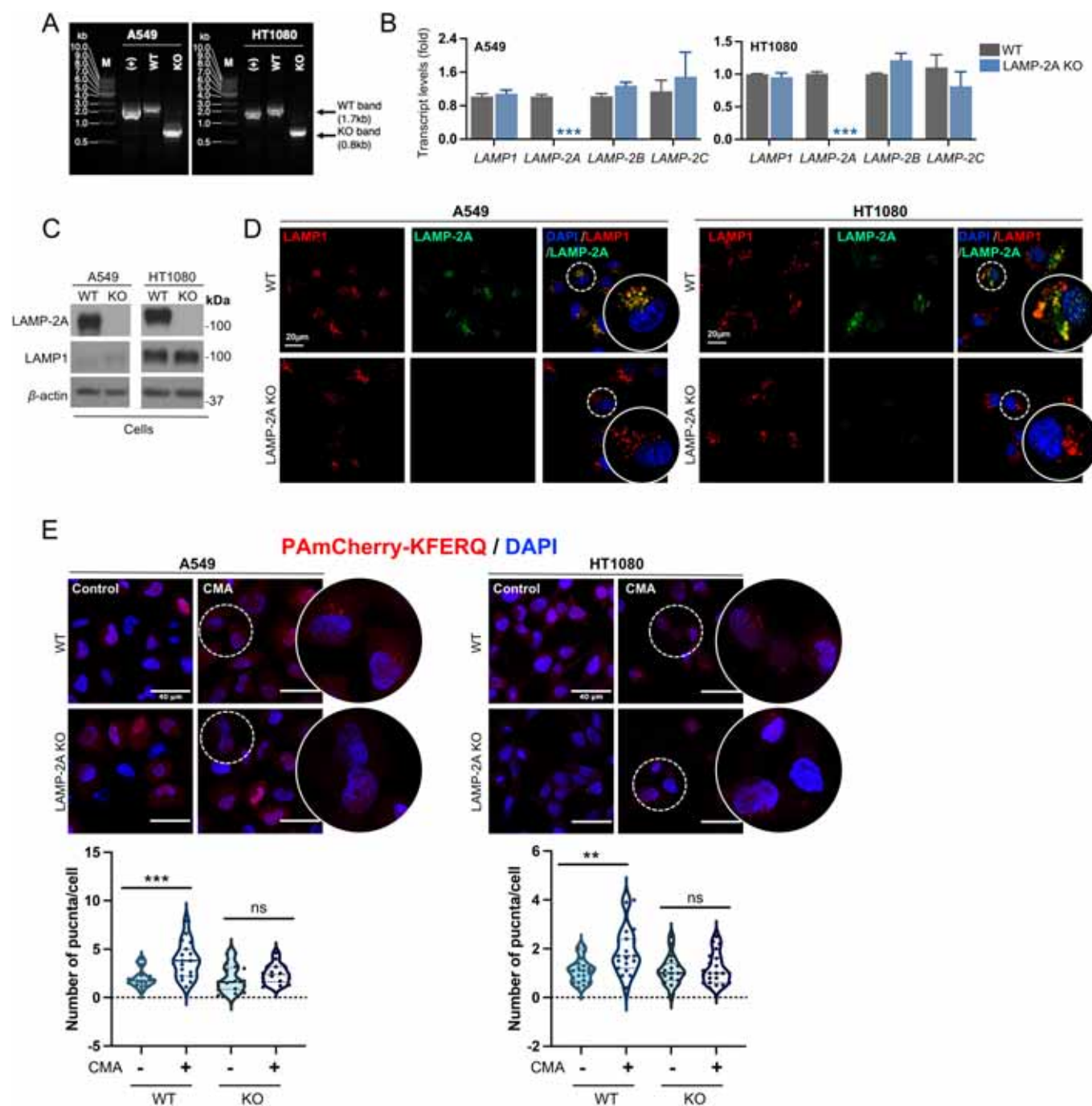


Figure EV2. Generation and validation of isoform-specific knockout of *LAMP-2A*.

(A) CRISPR/Cas9 genome editing and genotyping by agarose gel electrophoresis/PCR in regular (+), wild-type (WT), and *LAMP-2A* knockout (KO) A549 and HT1080 cells. Target amplicon in human *LAMP2* gene is detected by the fragment of ~800 bp. M: marker. (B) RT-qPCR detection of *LAMP1*, *LAMP-2A*, -2B, and -2C expression in WT and *LAMP-2A* KO A549 and HT1080 cell lines. Bars represent mean $\pm$ sd ($n_{\text{exp}} = 3$). P values refer to *** $P < 0.001$ (A549: $P_{\text{LAMP-2A}} = 0.0001$; HT1080: $P_{\text{LAMP-2A}} < 0.0001$ Student's t -test). (C) Immunoblot detection of *LAMP-2A* and *LAMP1* in WT and *LAMP-2A* KO A549 and HT1080 cells. β -Actin: loading control. (D) Representative immunofluorescence confocal images of WT and *LAMP-2A* KO A549 and HT1080 cell lines stained with anti-*LAMP-2A* (green), anti-*LAMP1* (red), and DAPI (blue) for nuclei highlighted with (circular insets) magnification. Scale bars: 20 μm . (E) CMA activity measured in WT and *LAMP-2A* KO A549 and HT1080 cells expressing PAmCherry-KFERQ CMA reporter. Cells were treated with AC220+Spautin1 to activate CMA for 16 h. Confocal images (upper panel) of merged red (PAmCherry-KFERQ) and blue (DAPI) for nuclei highlighted with (circular insets) magnification. Scale bars: 40 μm . Quantification (lower panel) of CMA activity as number of fluorescent puncta per cell. For each replicate ($n_{\text{exp}} = 3$), at least four images were analyzed. Each dot in the graph represents the average number of puncta per cell. P values refer to *** $P < 0.001$, ns: non-significant (A549: $P_{\text{WT Ctrl vs CMA}} = 0.0008$, $P_{\text{KO Ctrl vs CMA}} = 0.28$; HT1080: $P_{\text{WT Ctrl vs CMA}} = 0.0013$, $P_{\text{KO Ctrl vs CMA}} = 0.75$; Student's t -test). Source data are available online for this figure.

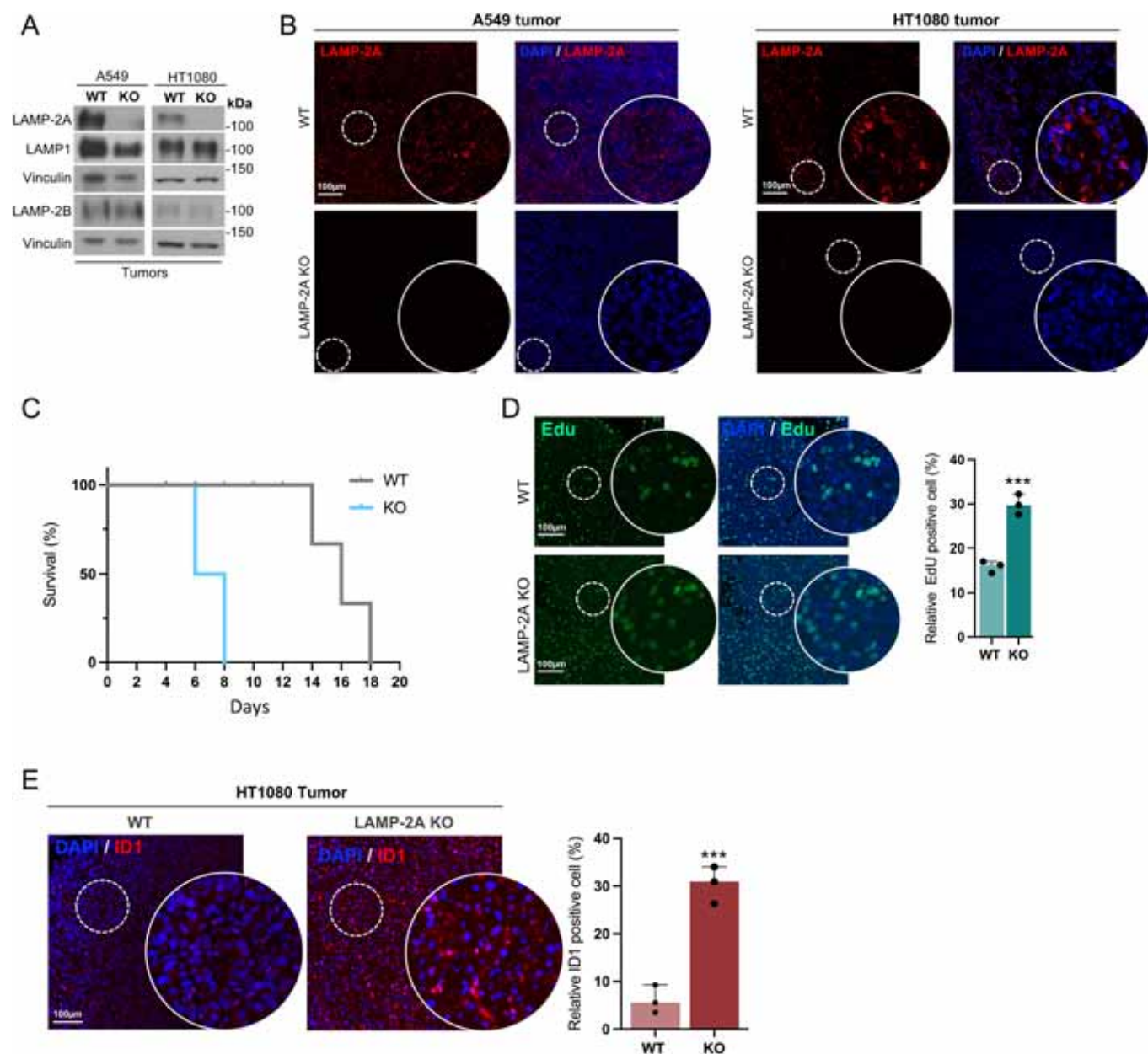


Figure EV3. The impact of *LAMP-2A* knockout in cancer cells and tumors.

(A) Immunoblot detection of LAMP-2A, LAMP-2B, and LAMP1 in WT and *LAMP-2A* KO A549 and HT1080 tumor lysates. Vinculin: loading control. (B) Immunofluorescence confocal images of WT and *LAMP-2A* KO A549 and HT1080 tumor sections stained with anti-LAMP-2A (red) and DAPI- (blue) for nuclei highlighted with (circular insets) magnification. Scale bars: 100 µm. (C) Kaplan-Meier survival curves for mice bearing WT or *LAMP-2A* KO HT1080 tumors. (D) Immunofluorescence confocal images (left panel) of Edu positive (green) and DAPI (nuclei, blue) labeled WT and *LAMP-2A* KO HT1080 tumor sections ($n_{\text{exp}} = 3$). Scale bars: 100 µm. Quantification (right panel) of the percentage Edu+ cell ratio per 20× field (bar graph). Bars present mean $\pm$ sd. P values refer to *** $P < 0.001$ ($P = 0.0008$; Student's t -test). (E) Representative immunofluorescence confocal images (left panel) and quantification (right panel) of HT1080 WT and *LAMP-2A* KO tumor section stained with anti-ID1 (red) and DAPI (blue) for nuclei highlighted with (circular insets) magnification. Scale bar: 100 µm. Quantification of the ID1+ cell ratio per 20× field (bar graph) ($n = 3$). Error bars, $\pm$ SD. *** $P < 0.001$ ($P = 0.0010$; Student's t -test). Source data are available online for this figure.

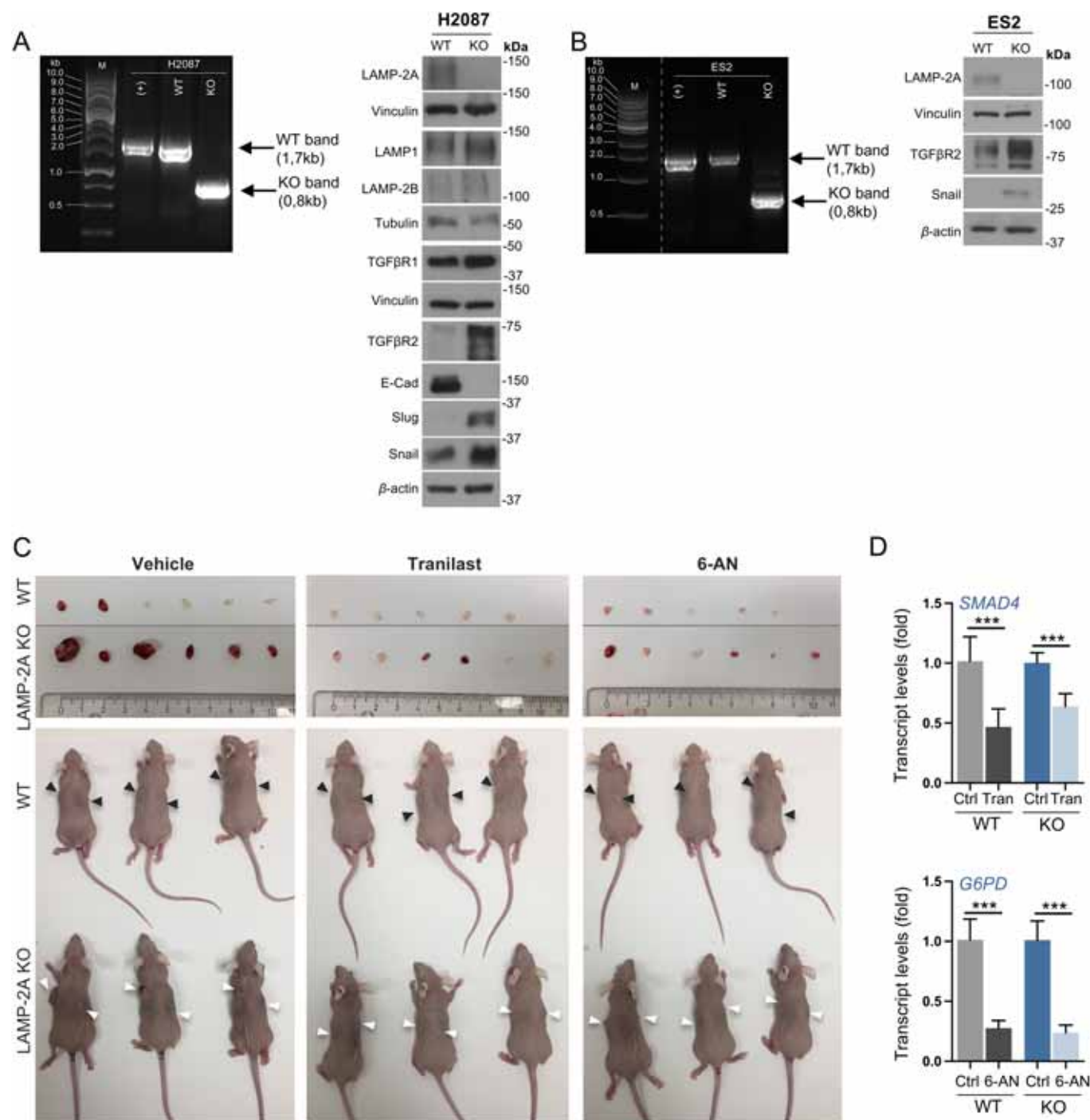


Figure EV4. LAMP-2A-KO tumor regression in vivo.

(A) (Left panel) CRISPR/Cas9 genome editing and genotyping by agarose gel electrophoresis/PCR in regular (+), WT, and LAMP-2A KO NCI-H2087 cells. Target amplicon in human LAMP2 gene is detected by the fragment of ~800 bp. M: marker. (Right panel) Immunoblot detection of indicated LAMP proteins, TGFβ receptors, and EMT proteins in WT and LAMP-2A KO H2087 cells. Vinculin and tubulin: loading controls. (B) (Left panel) CRISPR/Cas9 genome editing and genotyping by agarose gel electrophoresis/PCR in regular (+), WT and LAMP-2A KO ES2 cells. Target amplicon in human LAMP2 gene is detected by the fragment of ~800 bp. M: marker. (Right panel) Immunoblot detection of LAMP-2A, TGFβR2 and Snail in WT and LAMP-2A KO ES2 cells. β-Actin: loading control. (C) Representative images of WT and LAMP-2A KO HT1080 xenograft nude mice (two transplants per mouse ($n_{\text{mice}} = 3$)) and harvested tumors ($n = 6$) after administration with Tranilast or 6-AN for nine days compared to vehicle (corn oil) treated mice/tumors. (D) RT-qPCR detection of SMAD4 or G6PD in WT and LAMP-2A KO HT1080 tumors after administration with Tranilast (Tran) or 6-AN, respectively, compared to control (Ctrl) vehicle (corn oil) treatment. ($n_{\text{mice}} = 3$; Tran: $P_{\text{WT}} = 0.0003$, $P_{\text{KO}} < 0.0001$; 6-AN: $P_{\text{WT}} < 0.0001$, $P_{\text{KO}} < 0.0001$, Student's *t*-test). Bars present mean $\pm$ sd. *P* values refer to ****P* < 0.001. Source data are available online for this figure.