SUPPLEMENTAL MATERIAL

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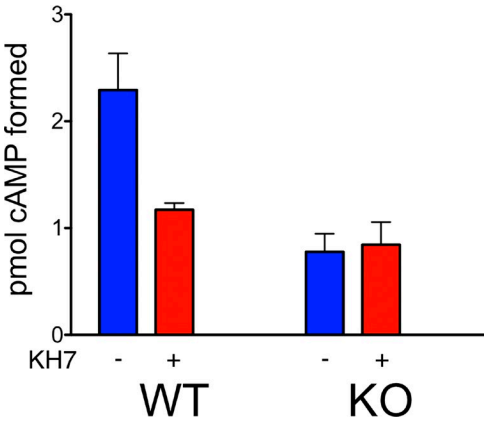

Figure S1. **Functional characterization of sAC KO MEFs.** cAMP accumulation in WT and sAC KO 3T3 MEFs in the absence (−, blue bars) or presence (+, red bars) of the sAC-specific inhibitor KH7 (30 µM). Data represent triplicate determinations of picomoles of cAMP formed over 5 min in 2.5×10^6 cells in the presence of 500 µM IBMX and 50 µM dipyridamole of an experiment performed at least three times; error bars indicate standard deviation.

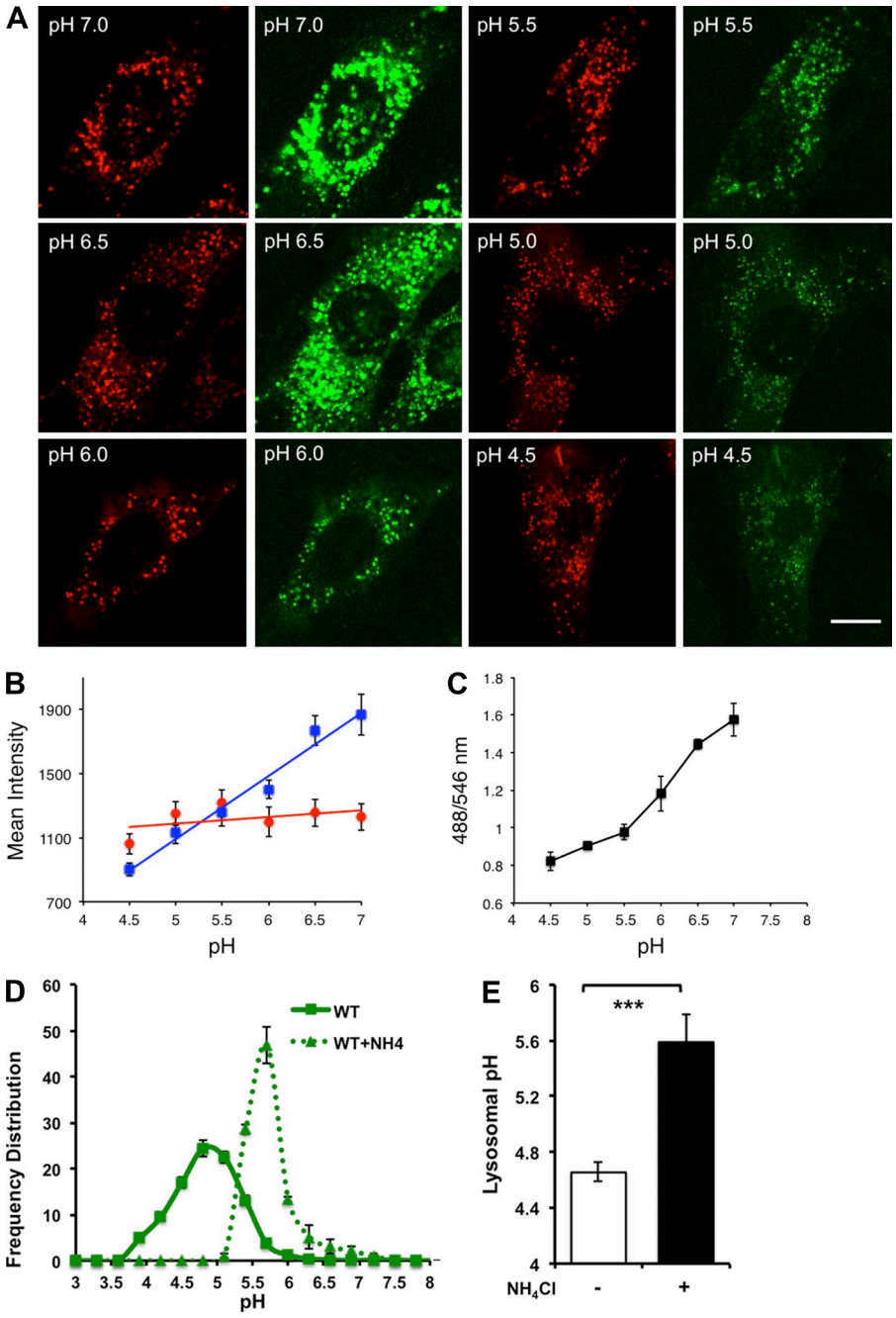

Figure S2. **Controls for the ratiometric measurement of lysosomal pH by confocal microscopy using pH-sensitive fluorescein fluorescence and pH-insensitive rhodamine fluorescence.** (A) Representative images of WT MEFs used for ratiometric analysis using the pH-sensitive fluorescein and pH-insensitive Rhodamine dextran. Images were collected while permeabilized WT cells were incubated in buffer calibrated at different pH values (pH 7.0, 6.5, 6.0, 5.5, 5.0, and 4.5). Bar, 10 µm. (B) Graph showing intensities of fluorophore of the dual-emission probe. pH-sensitive fluorescein is shown in blue, and pH-insensitive Rhodamine is shown in red. (C) Graph showing the standard curve from one independent experiment (n = 100 lysosomes/pH). (D) Chemical acidification of lysosomes caused by addition of 20 mM NH_4Cl for 30 min. WT MEFs were incubated with fluorescein-rhodamine dextran for 16 h, followed by a 4-h chase. WT cells were incubated with NH_4Cl, and the lysosomal pH frequency distribution curve was plotted. WT lysosomes = 3,141, n = 18 (from six independent experiment days). WT+NH_4Cl = 395, n = 3. (E) Mean lysosomal pH in WT cells treated with 20 mM NH_4Cl for 30 min. ***, $P < 0.001$. All values are given as mean ± SEM.

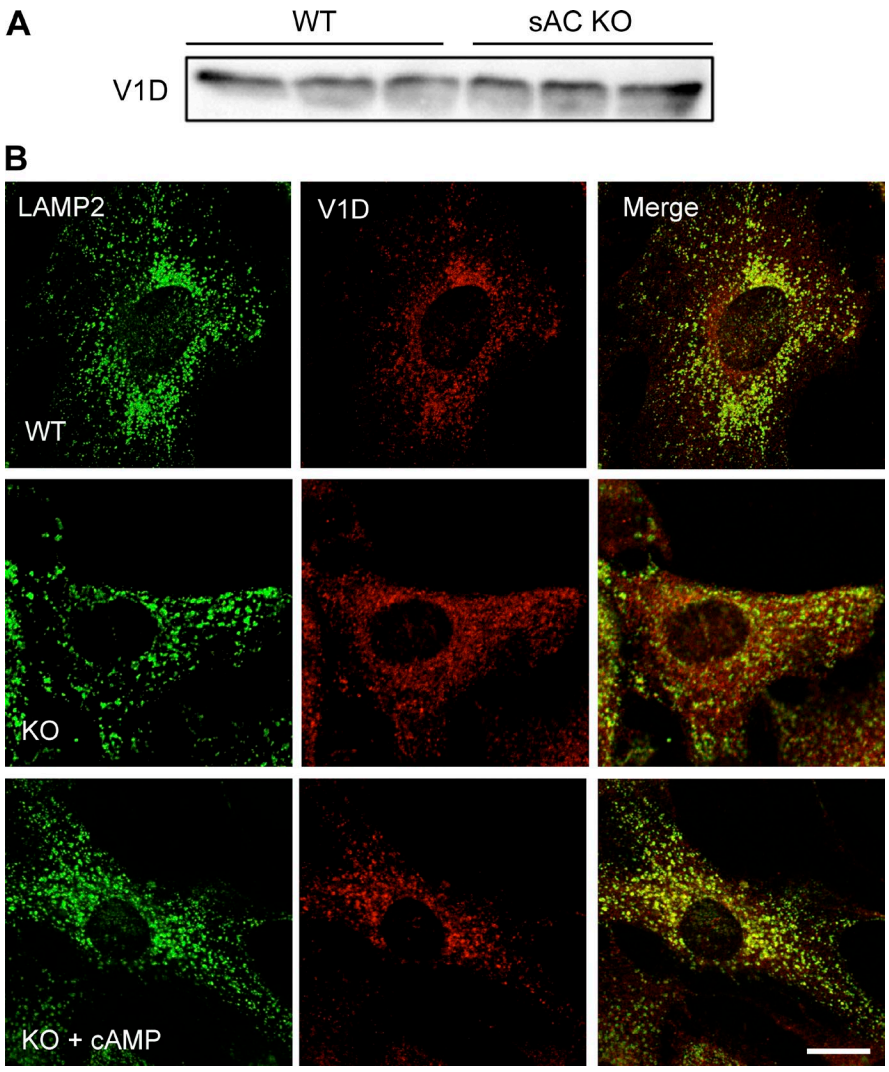

Figure S3. **sAC regulates V-ATPase localization to lysosomes.** (A) Representative immunoblot of the V-ATPase subunit V1D. Shown are whole cell extracts from three independently grown cultures of WT and sAC KO MEFs. *n* = 3. This immunoblot is a reprobing of the blot shown in Fig. 2 A; therefore, the GAPDH control showed in Fig. 2 A is also relevant for this immunoblot. (B) Double-immunofluorescence labeling of V-ATPase V1D subunit (red) and LAMP2 (green) in WT MEFs, sAC KO MEFs, and sAC KO MEFs treated with 500 µM sp-8-cpt-cAMP for 1 h. Bar, 10 µm.

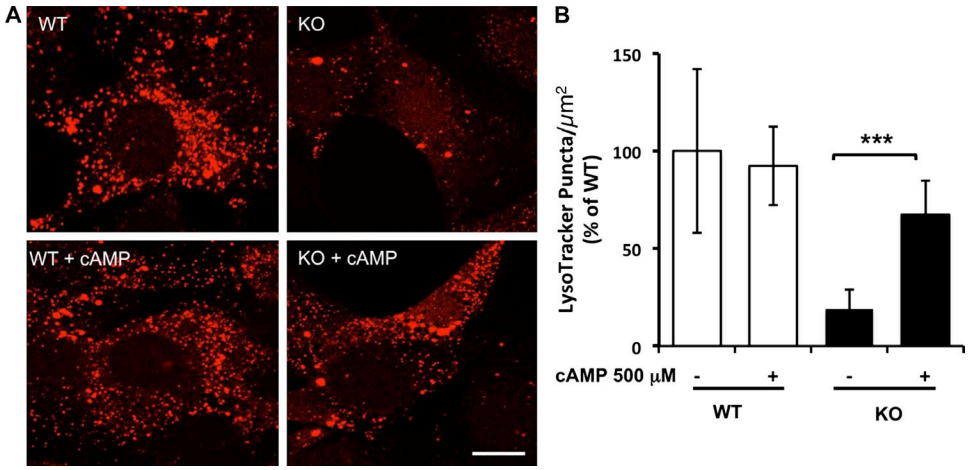

Figure S4. **LysoTracker reflects organellar acidification in MEFs.** (A) Representative images of staining with LysoTracker (red) in WT or sAC KO MEFs grown in the absence or presence of 500 µM Sp-8-cpt-cAMP (1 h), with the addition of 100 nM LysoTracker for the final 30 min. Bar, 10 µm. (B) LysoTracker puncta per square micrometer were quantified using MetaMorph in multiple cells from three independent experiments. ***, P < 0.001, compared with control untreated cells. Values are given as mean ± SEM.

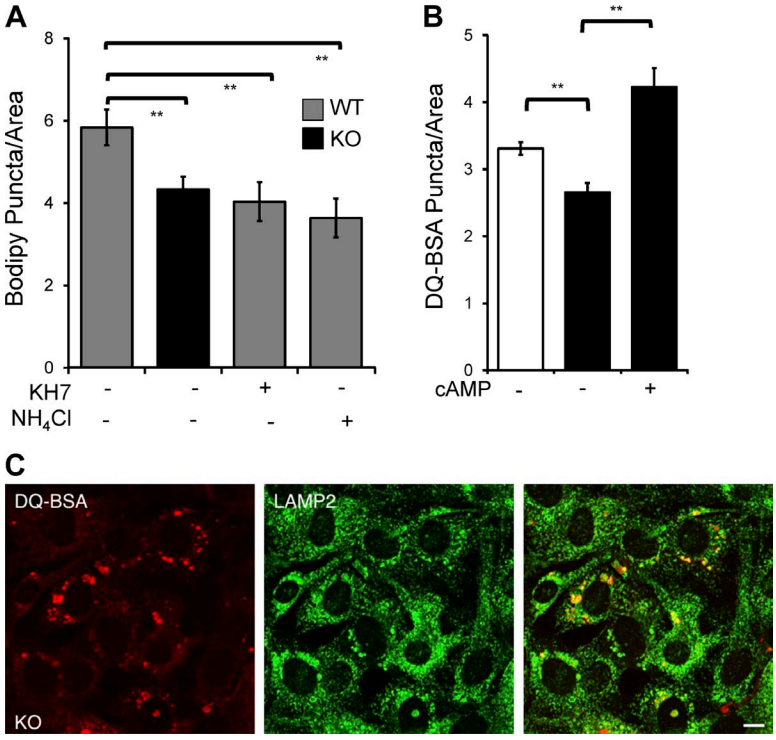

Figure S5. **Impaired lysosomal degradation in sAC KO MEFs.** (A) BODIPY-FL–Pepstatin A–stained puncta were quantified using MetaMorph in multiple cells from three independent experiments. **, P < 0.01. (B) WT and KO cells were preincubated with 10 µg/ml DQ-BSA and chased for 4 h. DQ-BSA puncta were quantified using MetaMorph in multiple cells from three independent experiments. **, P < 0.02. All values are given as mean ± SEM. (C) Subset of sAC KO cells depict enlarged DQ-BSA fluorescence, which colocalized with LAMP2-positive lysosomes. Bar, 10 µm.

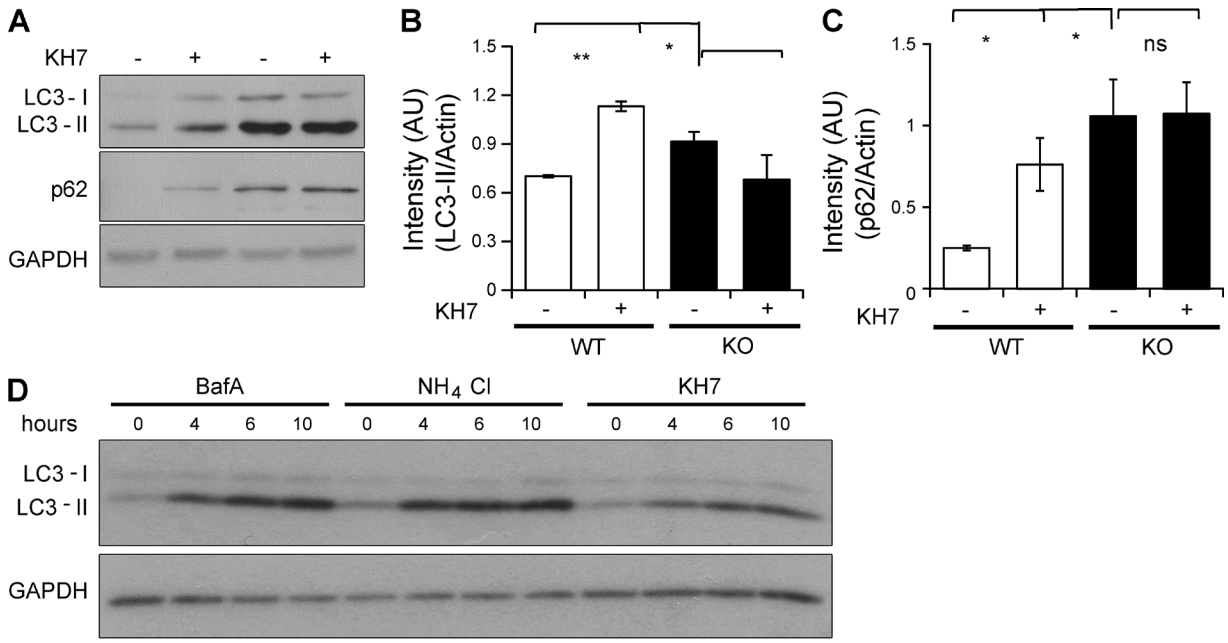

Figure S6. **In the absence of sAC activity, APs accumulate in neurons and Huh7 cells.** (A) Representative immunoblot of LC3 and p62 in 12 DIV primary WT and sAC KO neurons treated with and without 30 μM KH7 for 3 h. Protein levels of each protein were normalized to Actin as an internal control. (B) Densitometric analysis of LC3-II from A. Error bars represent the SEM. $n = 6$. *, $P < 0.05$; **, $P < 0.001$, compared with KO cells. (C) Densitometric analysis of p62 from A. Error bars represent the SEM. $n = 6$. *, $P < 0.05$, compared with KO cells. (D) Representative immunoblot of LC3 in Huh7 human liver cells treated with 30 μM KH7 for 0, 4, 6, and 10 h. 200 nM Bafilomycin A1 (BafA; an inhibitor of V-ATPase proton pump) and 20 mM NH_4Cl (which alkalinizes lysosomes) were used as positive controls. GAPDH was used as loading control. $n = 3$.