Expanded View Figures

Figure EV1. Aster-C deficiency increases cell size and proliferation.

A Schematic representation of Aster-C protein domains.

B, C Confocal imaging analysis depicting C2C12-vector control (Vector, B) and Aster-C KO (C) cells stained with CellMask™ Deep Red plasma membrane dye for cell size analysis. Cells were stained and analyzed 4 h after seeding. Scale bars, 50 μm.

D Quantification of cell size from B and C (n = 100 cells per group). Data are represented as mean ± SD. ***$P < 0.001$ by Student's t test.

E Cell proliferation rate analysis of vector and Aster-C KO C2C12 cells. Data are represented as mean ± SD (n = 3 biological replicates). *$P < 0.05$ and **$P < 0.01$ by Student's t test.

F Real-time RT-PCR analysis of *Aster-C* gene expression in vector control and Aster-C KO C2C12 cells. Data are represented as mean ± SD (n = 3 biological replicates). ***$P < 0.001$ by Student's t test.

G Western blot analysis of phosphorylation of Akt in Vector and Aster-C KO C2C12 cells.

H Western blot analysis of mTORC1 activity in HEK293T cells transiently over-expressing Myc tagged Aster-C in complete medium (CM), or in response to nutrient starvation and AA and/or insulin stimulation.

Data information: Data are representative of at least three independent experiments.

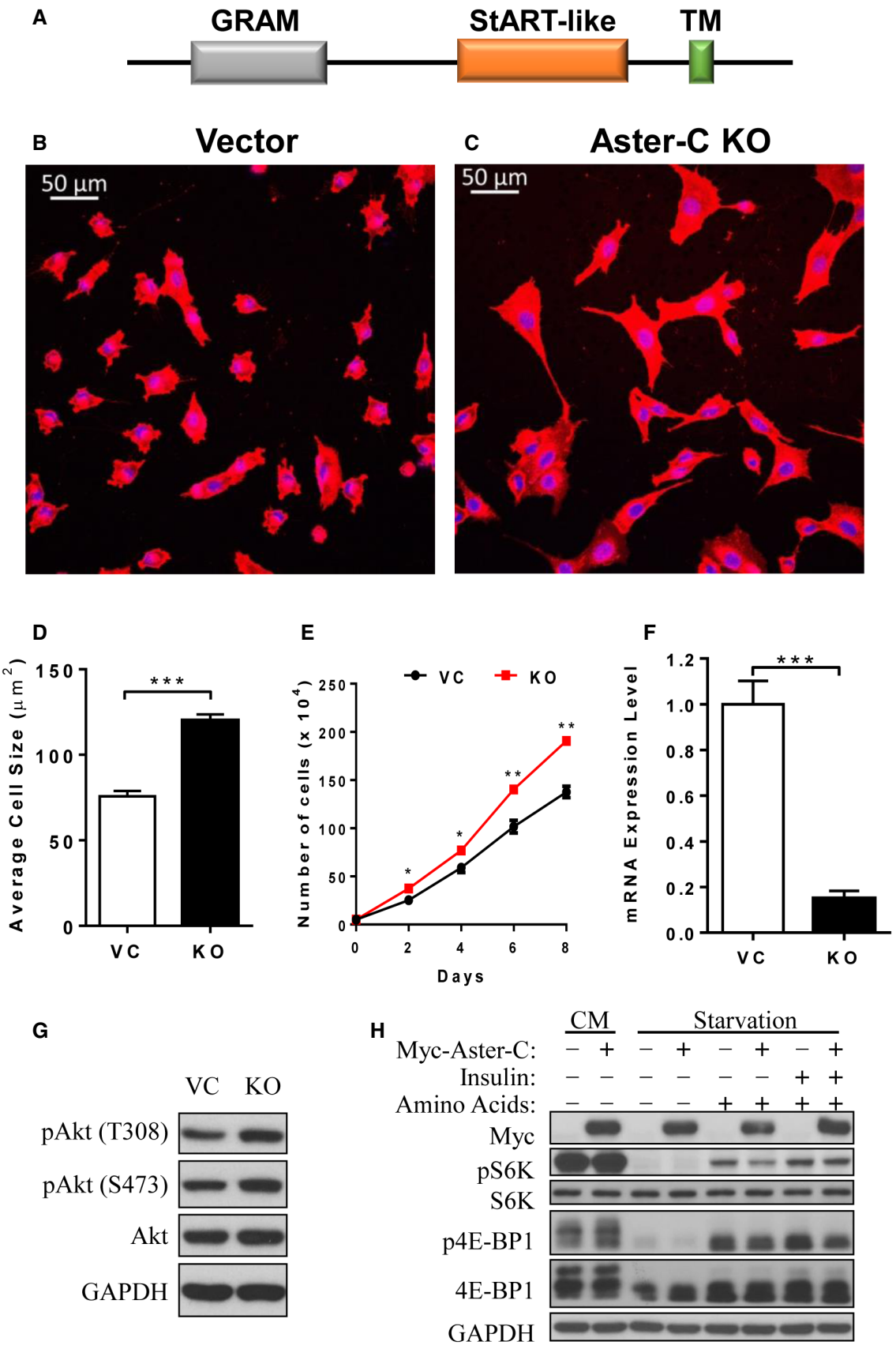

Figure EV1.

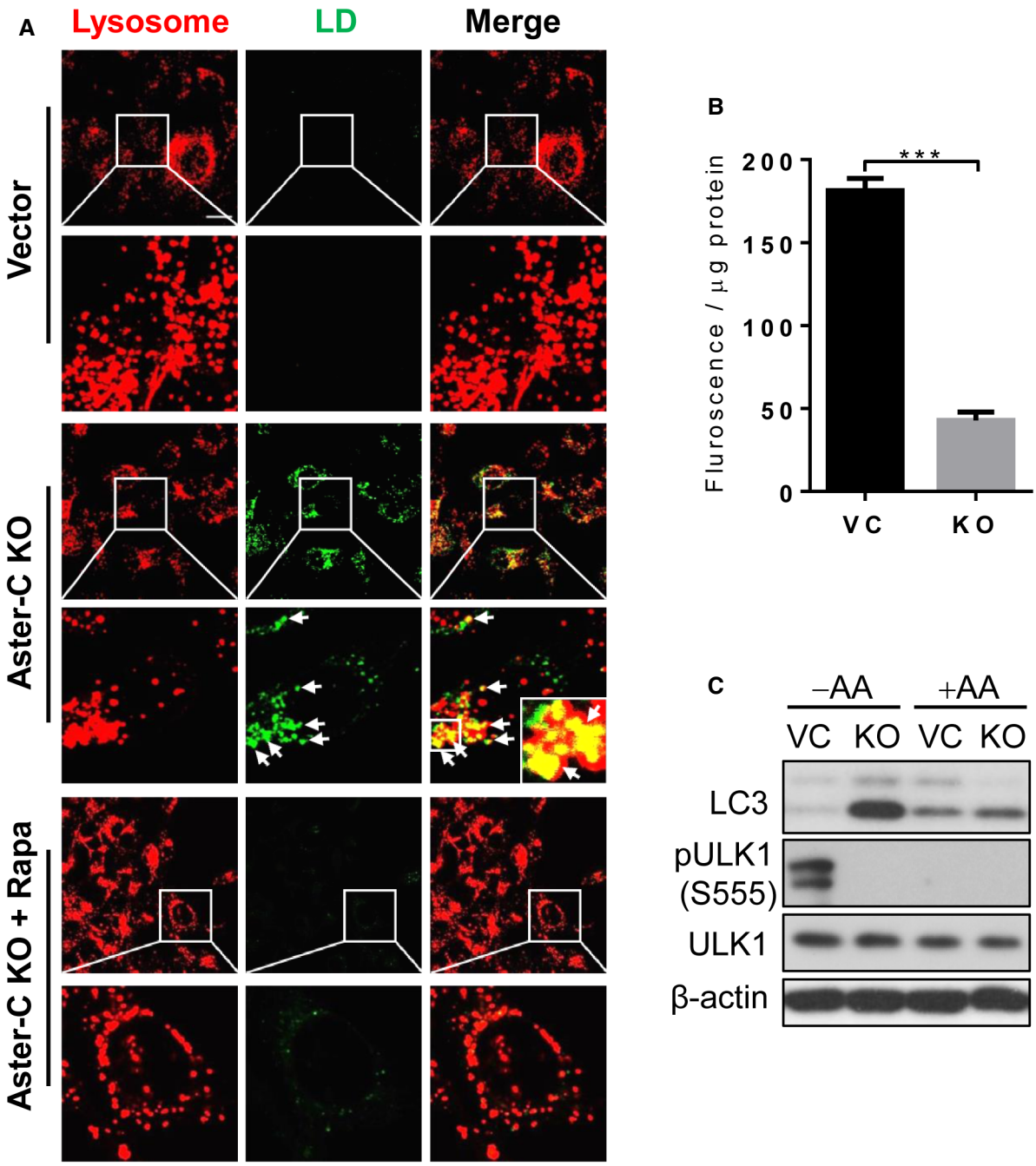

Figure EV2. Aster-C deficiency causes lipid droplets accumulation in lysosomes.

A Confocal image analysis of lipid droplets in C2C12-vector control (Vector) and Aster-C KO cells stained with LysoTracker Red under nutrient starvation in the presence or absence of rapamycin (Rapa) treatment. Lipid droplets were stained using BODIPY™ 650/665-X according to the manufacturer's instruction. Arrows highlight the accumulation of lipid droplets in the lysosomes. Scale bar, 20 μm.

B Lysosomal proteolytic degradation assay in C2C12-Vector and Aster-C KO cells under nutrient starvation using DQ-red-BSA as described in Materials and Methods. Data are represented as mean ± SD (n = 3 biological replicates). ***$P < 0.001$ by Student's t test.

C Western blot analysis of LC3 and phosphorylated ULK1 (S555) level in C2C12-Vector and Aster-C KO cells in response to nutrient starvation and AA stimulation.

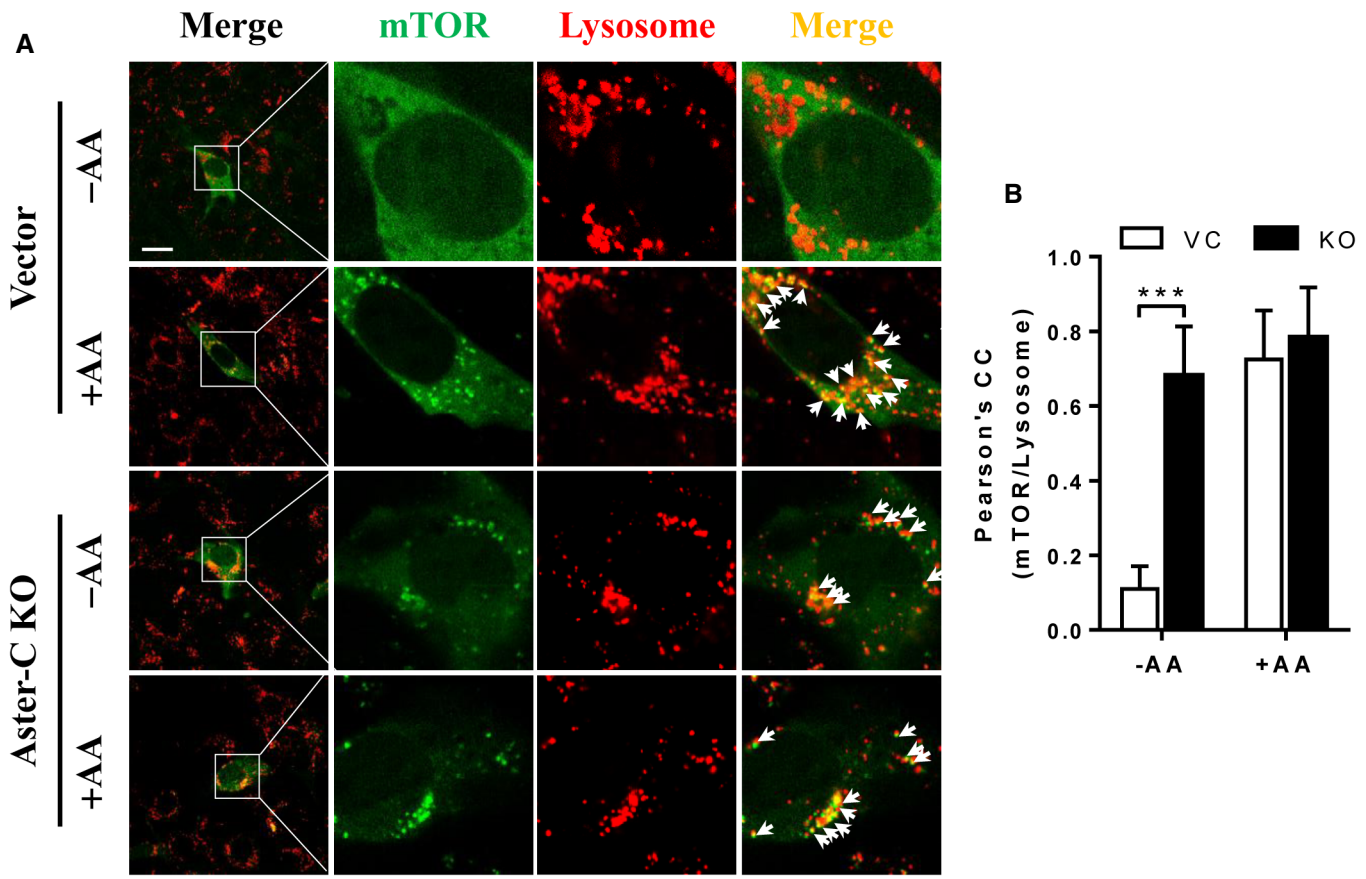

Figure EV3. Aster-C deficiency renders mTORC1 constitutively activated in live C2C12 cells.

A Confocal imaging analysis depicting YFP-mTOR (green) and lysosomes (red) in live C2C12 Aster-C KO and Vector cells transiently transfected with YFP-mTOR under nutrient starvation (−AA) and in response to AA stimulation (+AA). Arrows highlight co-localization of mTOR with lysosomes. Scale bar, 20 μm.

B Statistical analysis of the Pearson's correlation coefficient of mTOR and lysosomes in A (n = 10–15 cells per group). Data are represented as mean ± SD. ***$P < 0.001$ by one-way ANOVA.

Data information: Data are representative of at least two independent experiments.

Figure EV4. Aster-C prevents lysosomal association of TSC2 during nutrient starvation.

A Immunostaining of endogenous TSC2 and LAMP1 in C2C12-Vector and Aster-C KO cells under nutrient starvation (−AA) and in response to AA stimulation (+AA). Arrows highlight co-localization of TSC2 with LAMP1. Scale bar, 10 μm.

B Statistical analysis of the Pearson's correlation coefficient of TSC2 and LAMP1 in A (n = 10–15 cells per group). Data are represented as mean ± SD. ***$P < 0.001$ by one-way ANOVA.

C Western blot analysis of phosphorylation of TSC2 in C2C12-Vector and Aster-C KO cells under starvation and amino acid re-stimulation for 10 or 30 min.

D Co-IP analysis of the interaction of Aster-C with TSC2 in HEK293T cells transiently expressing FLAG-Aster-C in response to starvation and AA stimulation. FLAG-DEPDC5 was used as a negative control. Anti-FLAG antibody was used for immunoprecipitation. Black arrow indicates the FLAG-tagged Aster-C protein.

E Co-IP analysis of the interaction of Aster-C with TSC2 in HEK293T cells transiently expressing FLAG-Aster-C in response to starvation and AA stimulation. Anti-TSC2 antibody was used for immunoprecipitation.

Data information: Data are representative of at least two independent experiments.

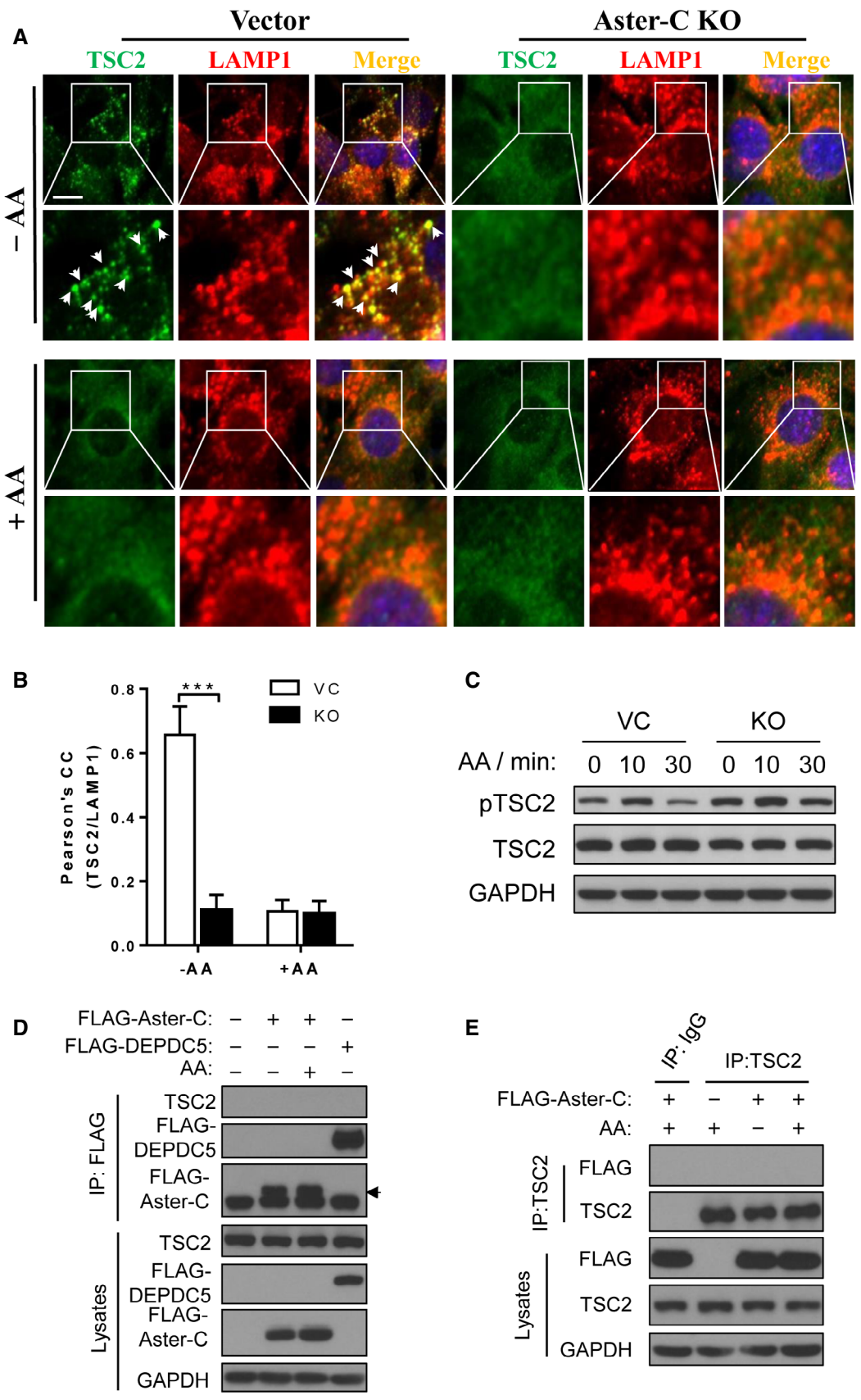
A
Vector
Aster-C KO
TSC2
LAMP1
Merge
TSC2
LAMP1
Merge
– AA
+ AA
B
Pearson's CC (TSC2/LAMP1)
0.8
0.6
0.4
0.2
0.0
***
VC
KO
-AA
+AA
C
VC
KO
AA / min:
0 10 30 0 10 30
pTSC2
TSC2
GAPDH
D
FLAG-Aster-C: – + + –
FLAG-DEPDC5: – – – +
AA: – – + –
IP: FLAG
TSC2
FLAG-DEPDC5
FLAG-Aster-C
Lysates
TSC2
FLAG-DEPDC5
FLAG-Aster-C
GAPDH
E
IP: IgG
IP:TSC2
FLAG-Aster-C: + – + +
AA: + + – +
IP:TSC2
FLAG
TSC2
Lysates
FLAG
TSC2
GAPDH

Figure EV4.

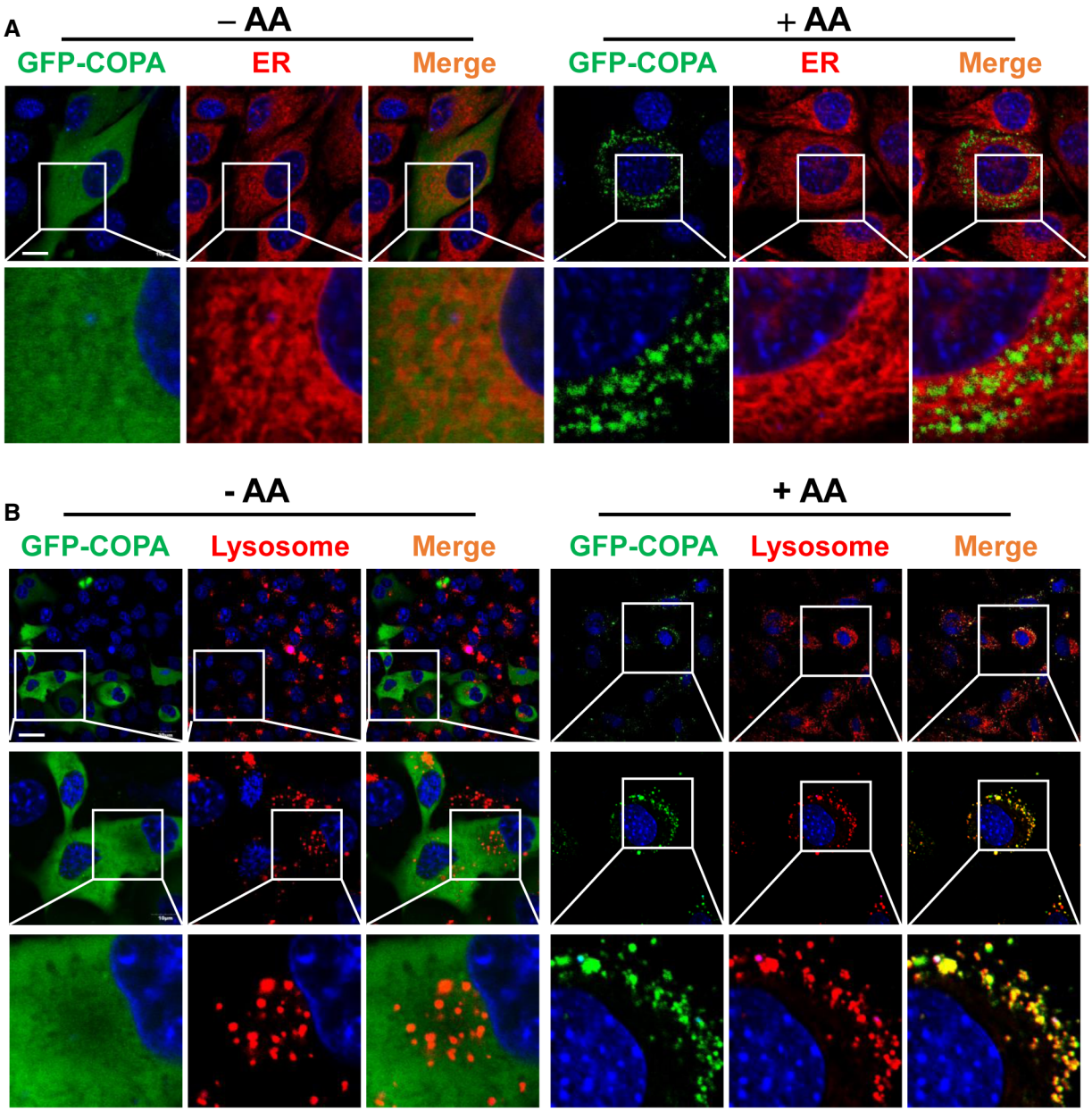

Figure EV5. Amino acids stimulate the biogenesis and lysosomal association of COP I vesicles.

A Confocal image analysis the co-localization of COPA with the ER in live C2C12 cells transiently expressing GFP-COPA and stained ER with ER-Tracker Red under starvation and in response to AA stimulation. Scale bar, 10 μm.

B Confocal image analysis co-localization of COPA with lysosomes in live C2C12 cells transiently expressing GFP-COPA and stained with LysoTracker Red under starvation and in response to AA stimulation. Scale bar, 30 μm.

Data information: Data are representative of at least two independent experiments.