Valsecchi Supplementary Figure 1

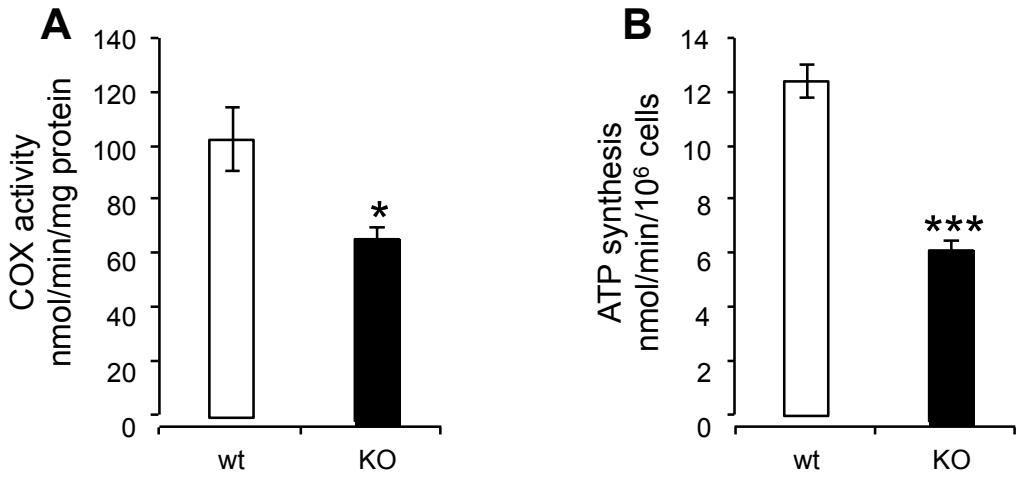

Figure S1: COX activity and ATP synthesis in wt2 and sAC KO2 MEFs

A) COX activity in independent lines of wt2 and sAC KO2 MEFs (n=3). B) ATP synthesis in permeabilized wt2 and sAC KO2 MEFs (n=3). Data is expressed as mean±s.e.m. in indicated number of different biological replicates (*p<0.05, ***p<0.001, Student's *t*-test).

Valsecchi Supplementary Figure 2

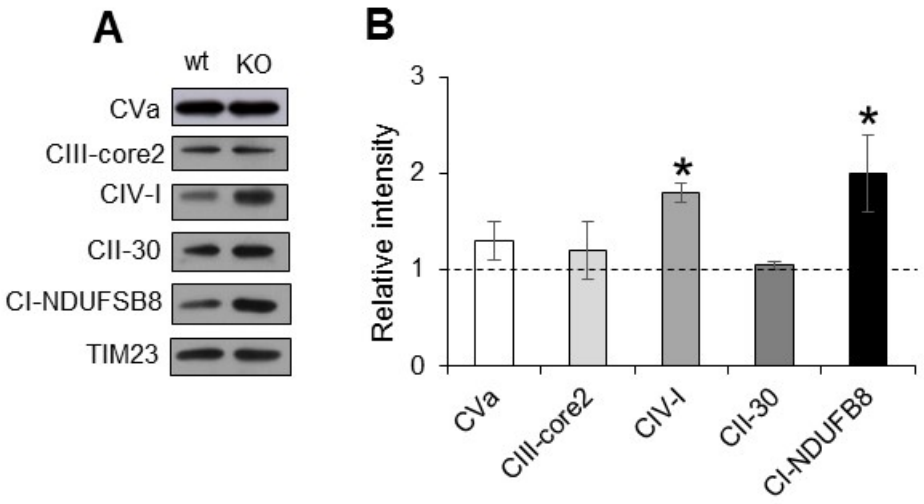

Figure S2: Quantification of OXPHOS proteins in isolated mitochondria from wt and sAC KO cells.

A) Representative western blot of OXPHOS subunits and TIM23. B) Quantification of band intensity normalized by TIM23 (n=5). Data is expressed as mean±s.e.m. in indicated number of different biological replicates (*p<0.05, Student's *t*-test).

Valsecchi Supplementary Figure 3

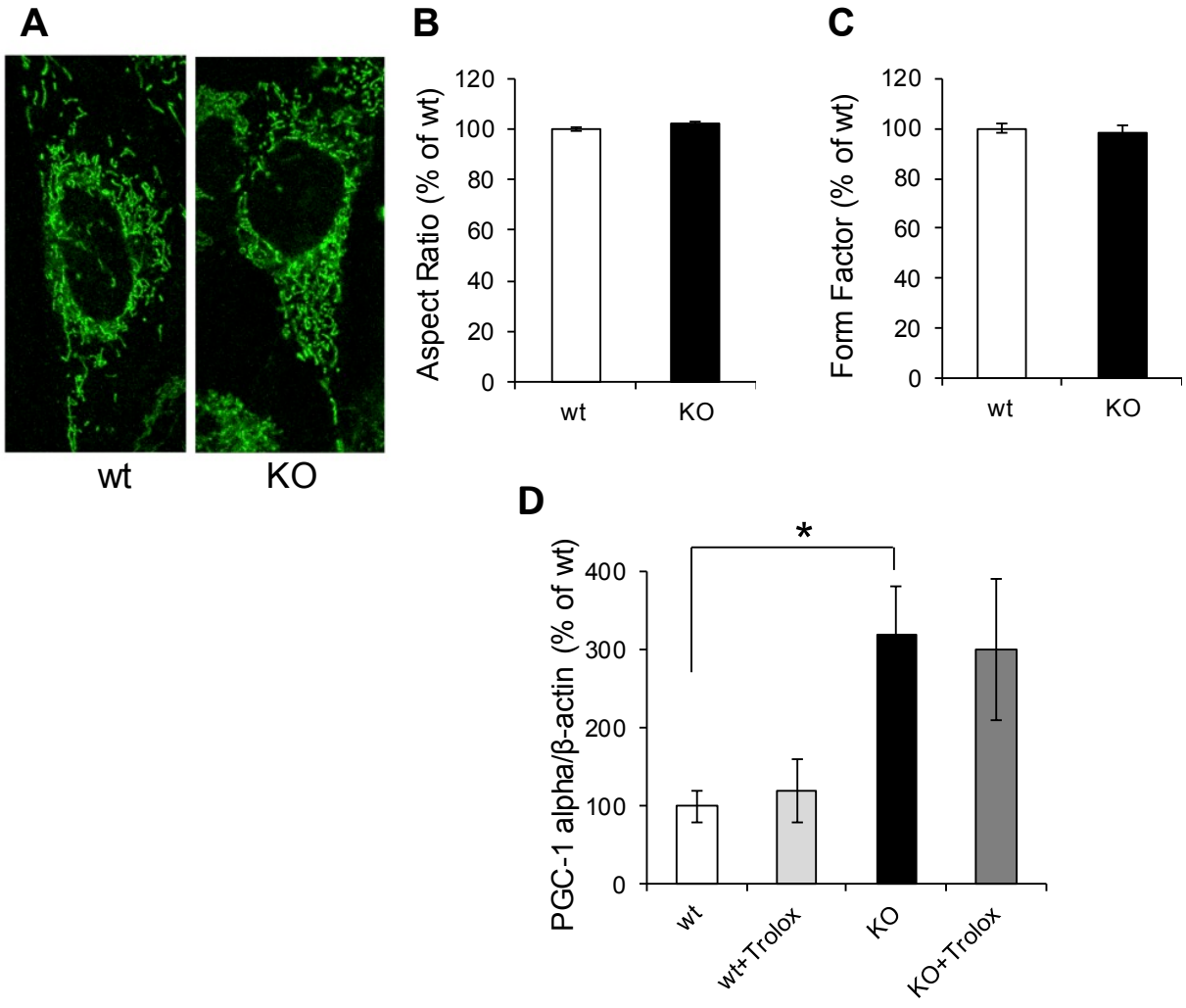

Figure S3: mitochondrial morphology and effect of antioxidants on PGC-1α expression. A) Representative image of wt and sAC KO cells loaded with MitoTracker Green 50nM. B) Mitochondria aspect ratio in wt and sAC KO MEFs expressed as % of wt. C) Mitochondrial form factor in wt and sAC KO MEFs expressed as a % of wt. In B and C, n=48-83 in 3 experiments. D) Effects of the antioxidant Trolox on PGC-1α mRNA content (n=3) in wt and sAc KO cells. Data is expressed as mean±s.e.m. in indicated number of different biological replicates (*$p<0.05$, ANOVA with Tukey's correction).

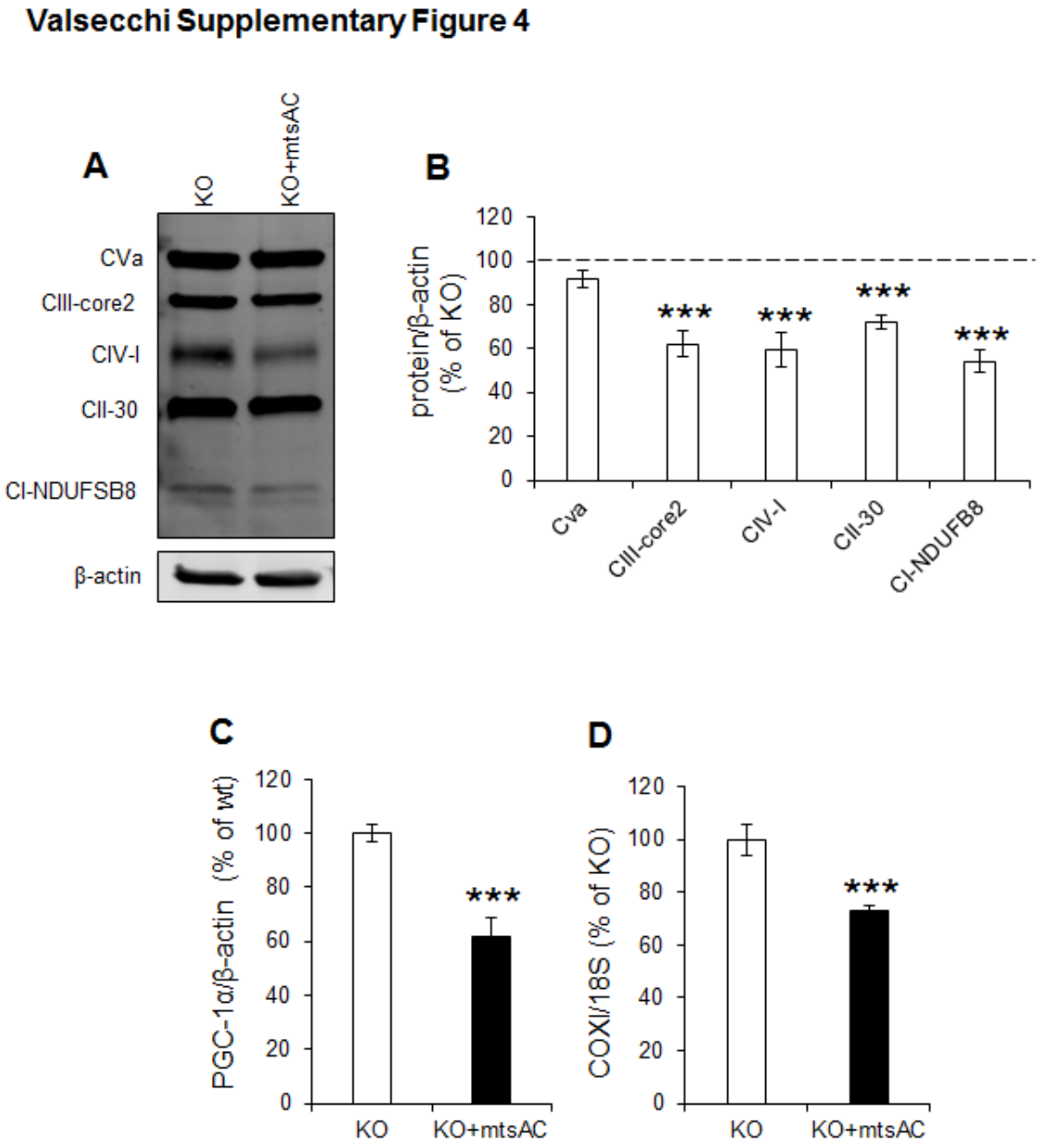

Figure S4: mtsAC expression decreases OXPHOS component content in KO cells. A) representative western blot of KO and KO+mtsAC cells probed with OXPHOS cocktails antibody. B) Quantification of the western blot. C) mRNA level of PGC1α expressed as % of KO. C) Mitochondrial DNA content expressed as % of KO (COXI gene normalized by 18S rRNA). Data is expressed as mean±s.e.m. in indicated number of different biological replicates (***p<0.001, Student's *t*-test).

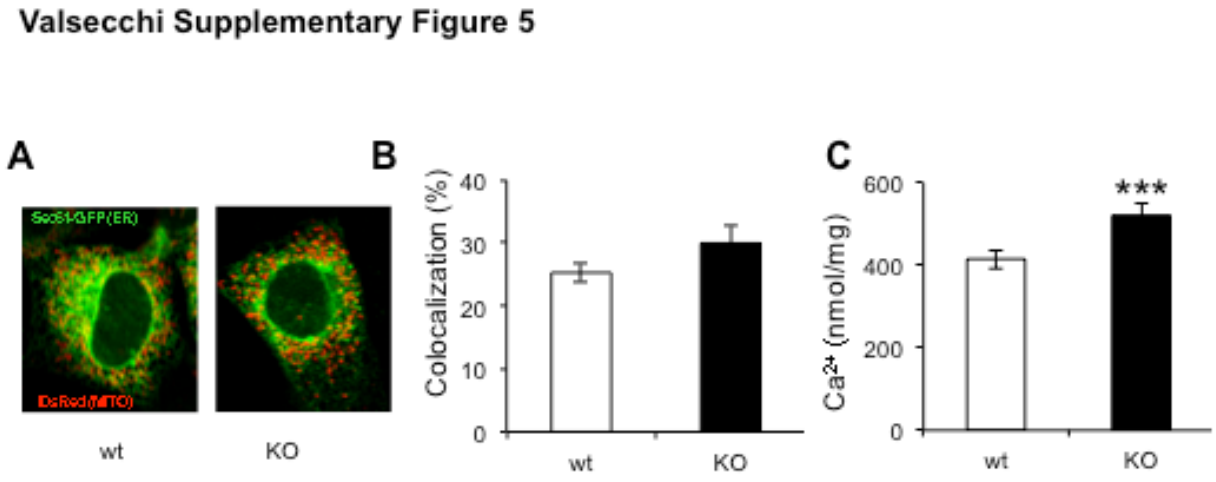

Figure S5: mitochondria Ca^{2+} uptake and ER-mitochondria colocalization

A) Representative images of wt and sAC KO cells co-transfected with the ER protein Sec61-GFP (green) and mitochondrial mitoDsRed (red). B) Quantification of ER-Sec61-GFP and mitoDsRed colocalization assessed by the co-localization function of MetaMorph expresses as % of red over green. C) Mitochondrial Ca^{2+} uptake in isolated mitochondria from wt and sAC KO MEFs (n=3). Data is expressed as mean±s.e.m. in indicated number of different biological replicates (***$p<0.001$, Student's *t*-test).

Valsecchi Supplementary Figure 6

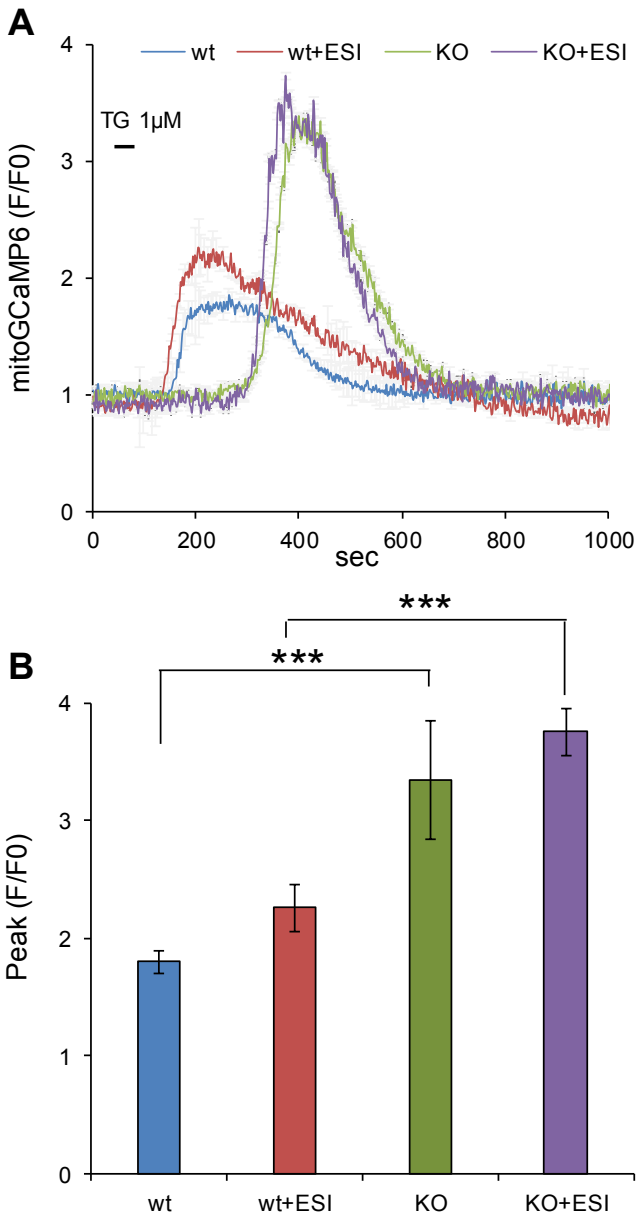

Figure S6: Effect of EPAC inhibitor ESI-09 on mitochondrial Ca^{2+} uptake A) Fluorescent traces of mitoGCaMP6 in wt and sAC KO (KO) MEFs treated with and without ESI-09 (ESI) and stimulated with TG. B) Quantification of mitoGCaMP6 fluorescence peaks (n=40, 32, 28, and 44 cells, for wt, wt + ESI, sAC KO, and sAC KO + ESI, respectively). Data is expressed as mean±s.e.m. in indicated number of different biological replicates (***p<0.001, ANOVA with Tukey's correction).

Valsecchi Supplementary Figure 7

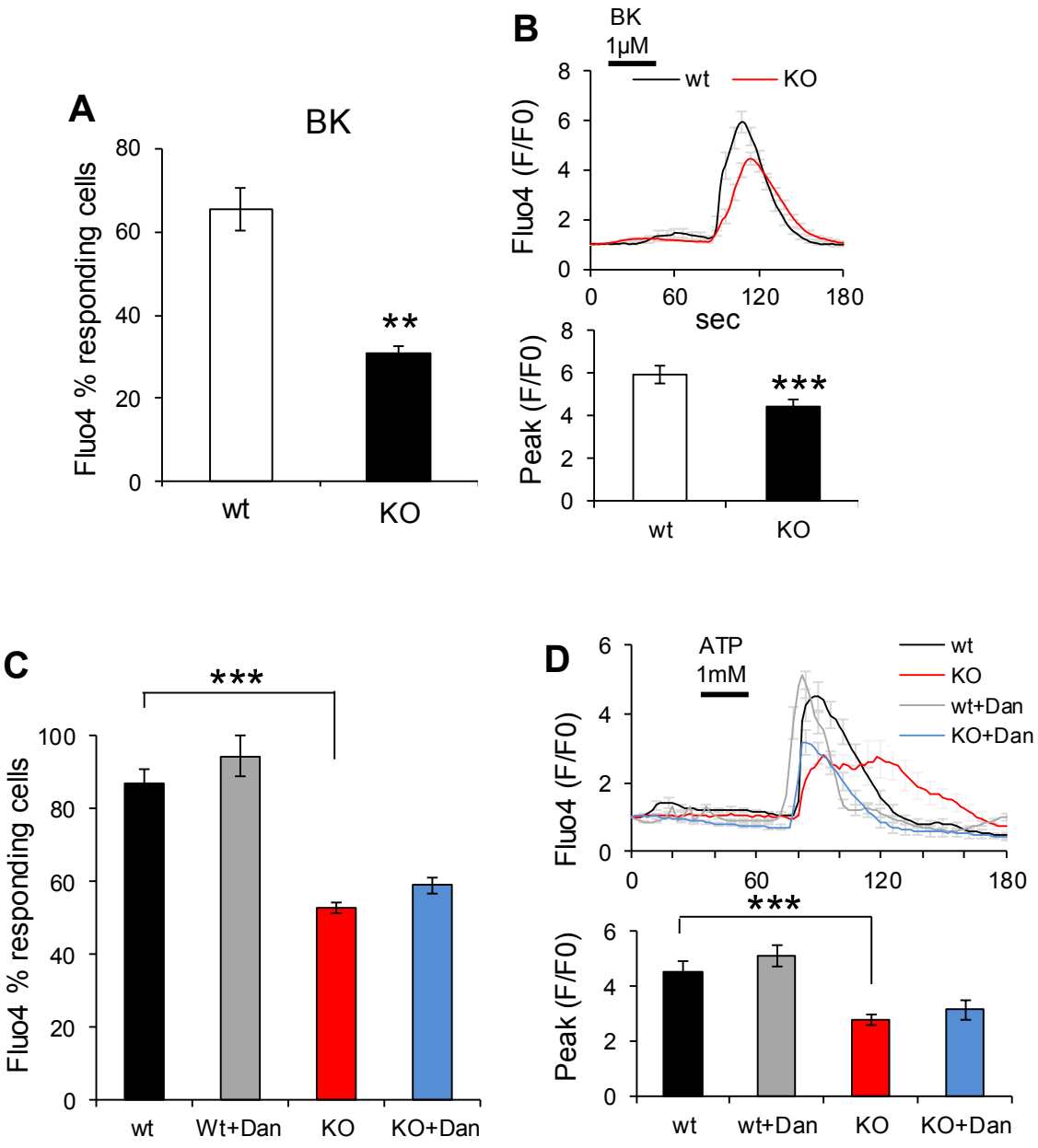

Figure S7: cytosolic ER Ca^{2+} release induced by Bradykinin stimulation, effects of dantrolene on ER Ca^{2+} release,

A) Percentage of responding wt and sAC KO MEFs upon Bradykinin (BK) stimulation. B) Top panel: Average fluo4 fluorescence curve in wt and sAC KO cells stimulated with 1µM bradykinin; Bottom panel: quantification of Ca^{2+} peak in responding wt and sAC KO cells

(n=33-66 cells, in 3 independent experiments). Data is expressed as mean±s.e.m. in indicated number of different biological replicates (**p<0.01, ***p<0.001, Student's *t*-test). C) Percentage of responding wt and sAC KO cells with and without dantrolene (10µM for 1 hour pre-incubation and in the perfusion buffer) and ATP (1mM). D) Top panel: fluo4 fluorescence in wt and sAC KO cells stimulated with ATP (1mM) in the presence or absence of dantrolene. Bottom Panel: quantification of the Fluo4 peak intensity in cells stimulated by ATP with and without dantrolene (n=15-27 cells, in 3 independent experiments). Data is expressed as mean±s.e.m. in indicated number of different biological replicates (***p<0.001, ANOVA with Tukey's correction).

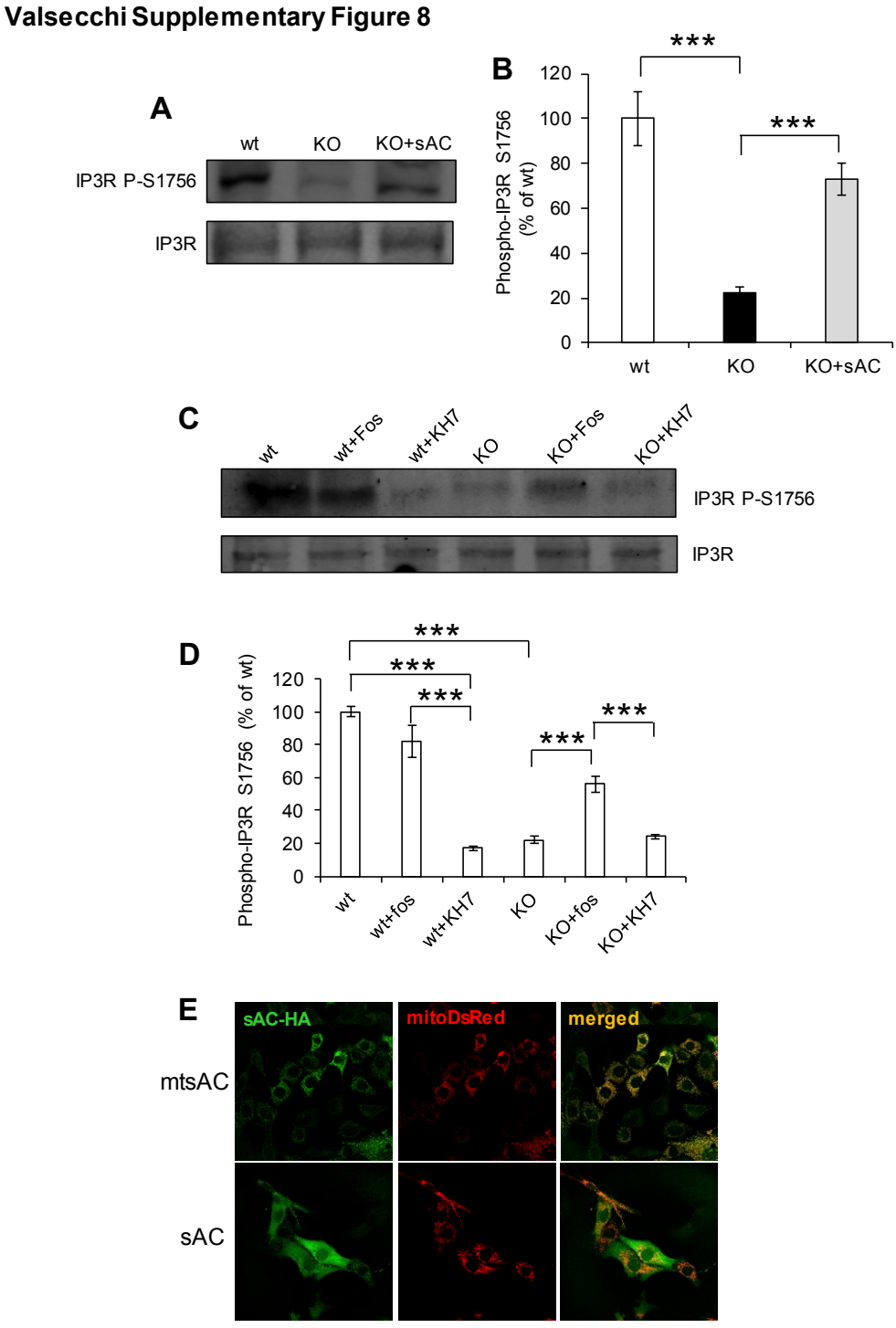

Figure S8: IP3R phosphorylation is regulated by sAC by forskolin or KH7.

A) Representative western blot of IP3R P-S1756 and total IP3R in wt, KO and KO+sAC cells. B) Quantification of P-S1756 normalized by total IP3R, in KO and KO+sAC cells expressed as % of wt (n=3). C) Representative western blot of IP3R P-S1756 and total IP3R in wt, and KO cells treated with forskolin and KH7. D) Quantification of P-S1756 normalized by total IP3R, in KO and KO+sAC cells expressed as % of wt (n=3). Data is expressed as mean±s.e.m. in

indicated number of different biological replicates (***$p<0.001$, ANOVA with Tukey's correction). E) Representative images of MEF cells co-transfected with mtsAC-HA or sAC-HA (green) and mitoDsRed (red) and merged images.