SUPPLEMENTAL MATERIAL

Figure S1

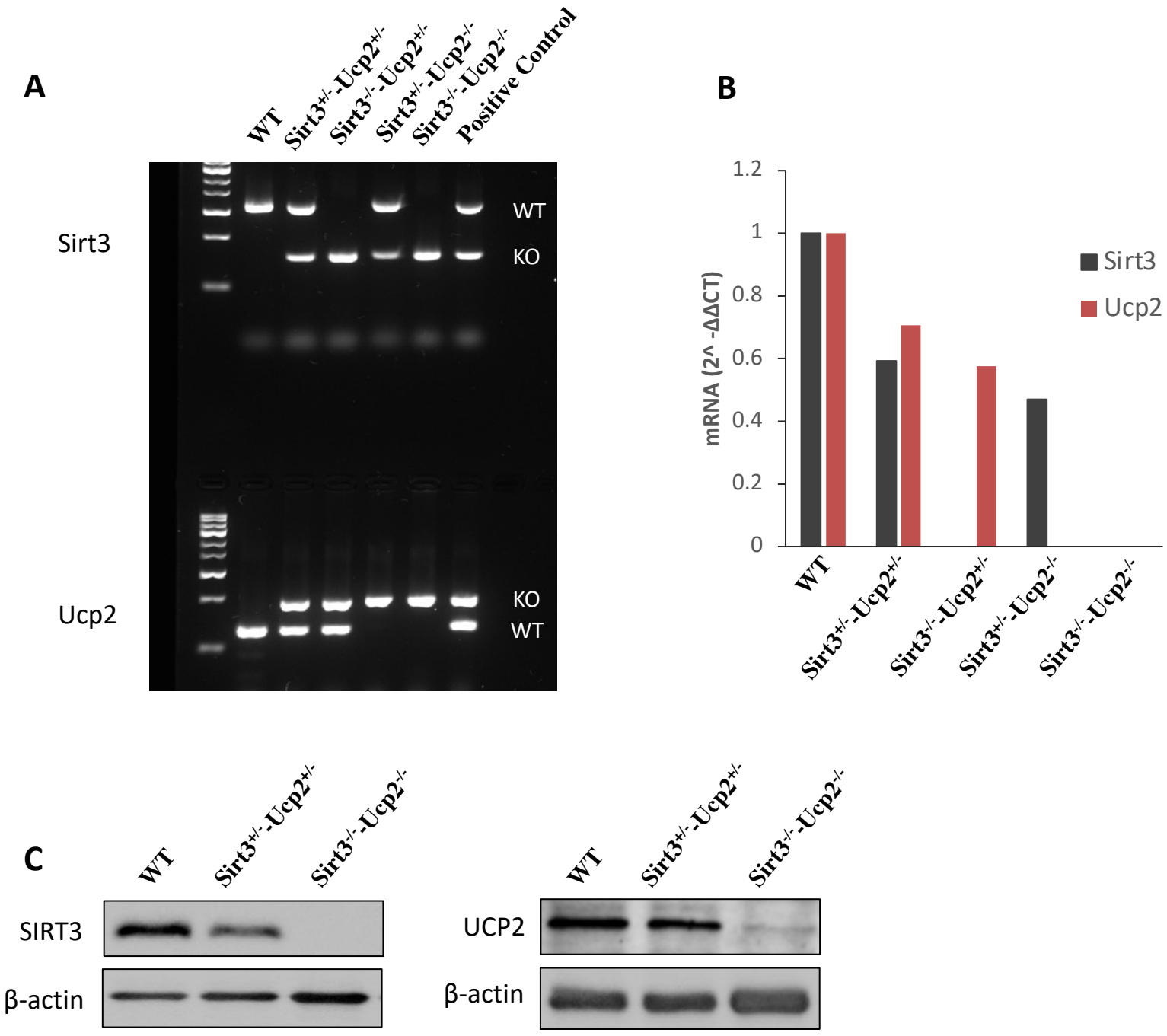


Figure S1. A. Representative PCR blot confirming the genotype for the Sirt3 and Ucp2 mutant mice. Sirt3 mutant mice have deletion of exons 2-3. Based on the primer sequences used, the wild type allele shows at 562bp and the mutant allele shows at 200bp. The Sirt3 heterozygous mice are positive for both alleles (Top blot). The Ucp2 mutant mice have an insertion of a PGK-NEO cassette replacing exons 3-7 to create the larger mutant allele. The Ucp2 mutant allele shows at 280bp, while the wild type allele shows at 156bp. The Ucp2 heterozygous mice are positive for both alleles (bottom). **B.** qRT-PCR (in lung tissues) shows that the double knockout mice have no detectable levels of Sirt3 or Ucp2 mRNA, compared to wild type controls, while the double heterozygous mice express ~50% mRNA levels. **C.** Lung tissue from mice heterozygous or homozygous to the lack of both genes shows no detectable levels of either Sirt3 or Ucp2, and decreased expression of either SIRT3 or UCP2 compared to WT mice, using immunoblots. The remaining "smear" in the Ucp2 blot of Sirt3$^{-/-}$-Ucp2$^{-/-}$ mice, is likely due to the well-known cross-reactivity of the Ucp2 antibody with Ucp3, in all known commercially available antibodies (Ucp2 shares a 72% homology with Ucp3).

Figure S2

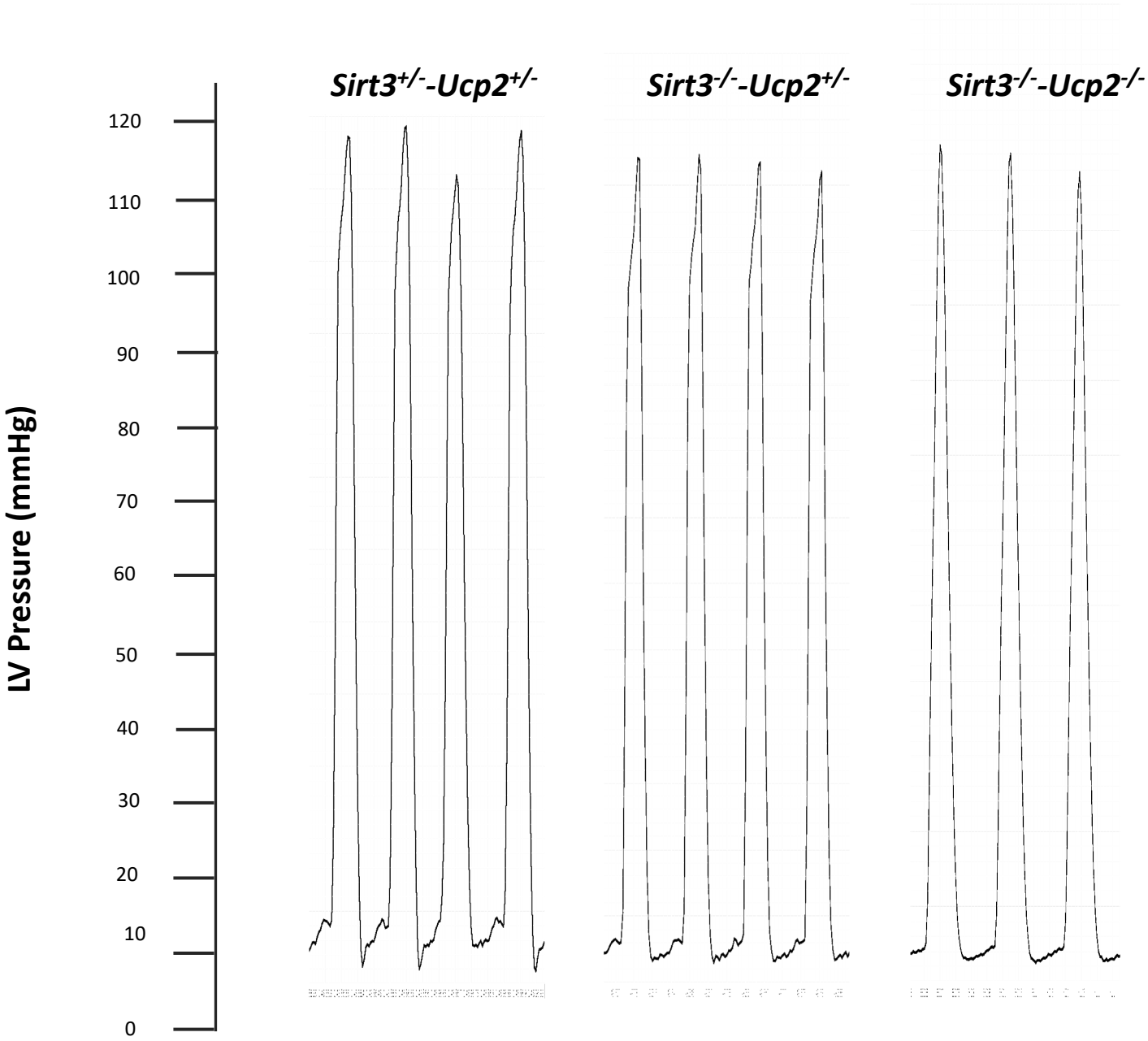


Figure S2. Representative LVEDP traces from anesthetized mice lacking Sirt3 and Ucp2 shows that they all have normal values.

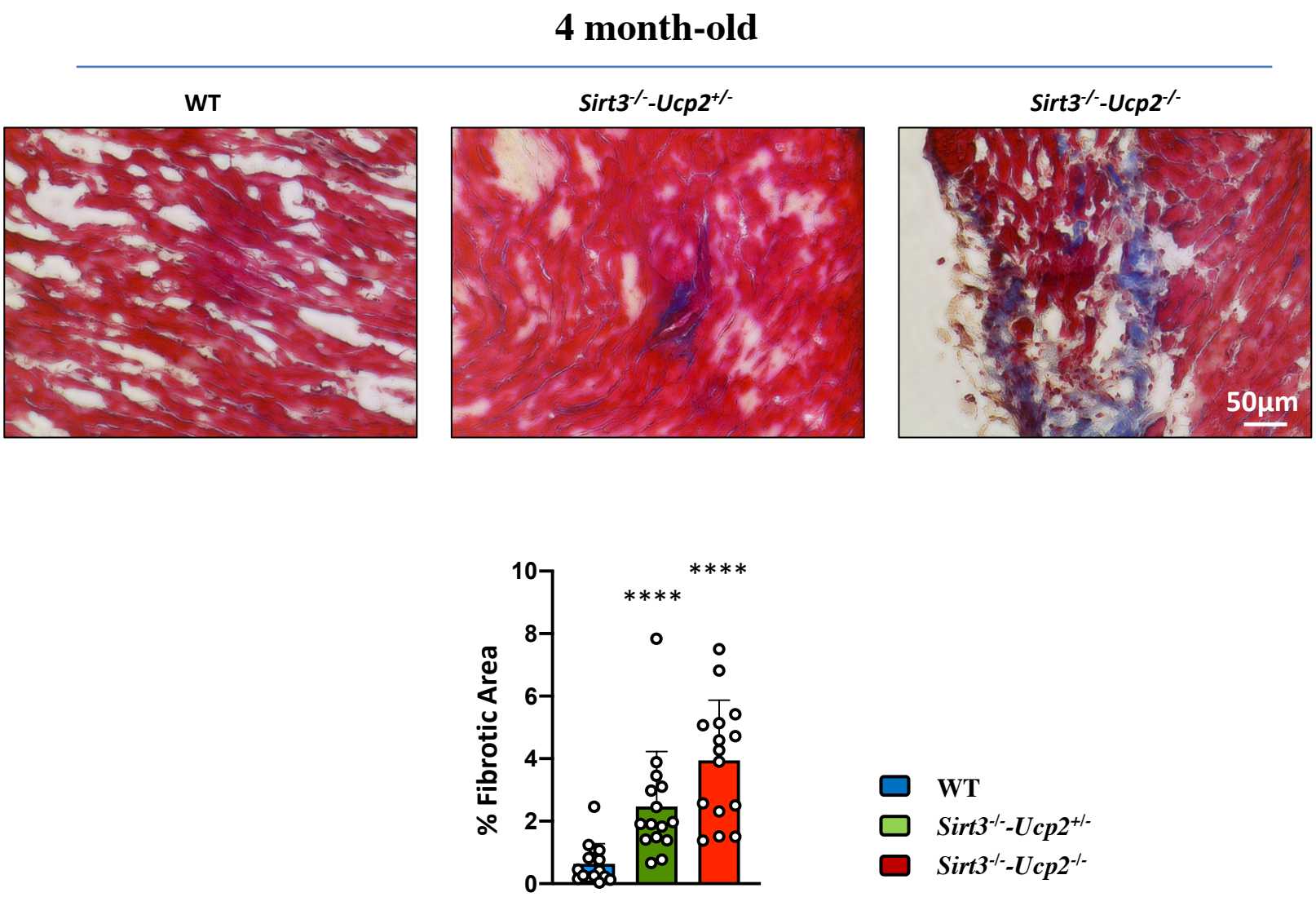


Figure S3. Representative Masson's trichrome pictures of right ventricles from Sirt3$^{-/-}$-Ucp2$^{+/-}$ and Sirt3$^{-/-}$-Ucp2$^{-/-}$ mice shows that they have a significant increase of fibrosis compared to WT mice. ****p<0.0001 (Kruskal-Wallis test) compared to WT mice, n=15 images from 3 mice / group.

Figure S4

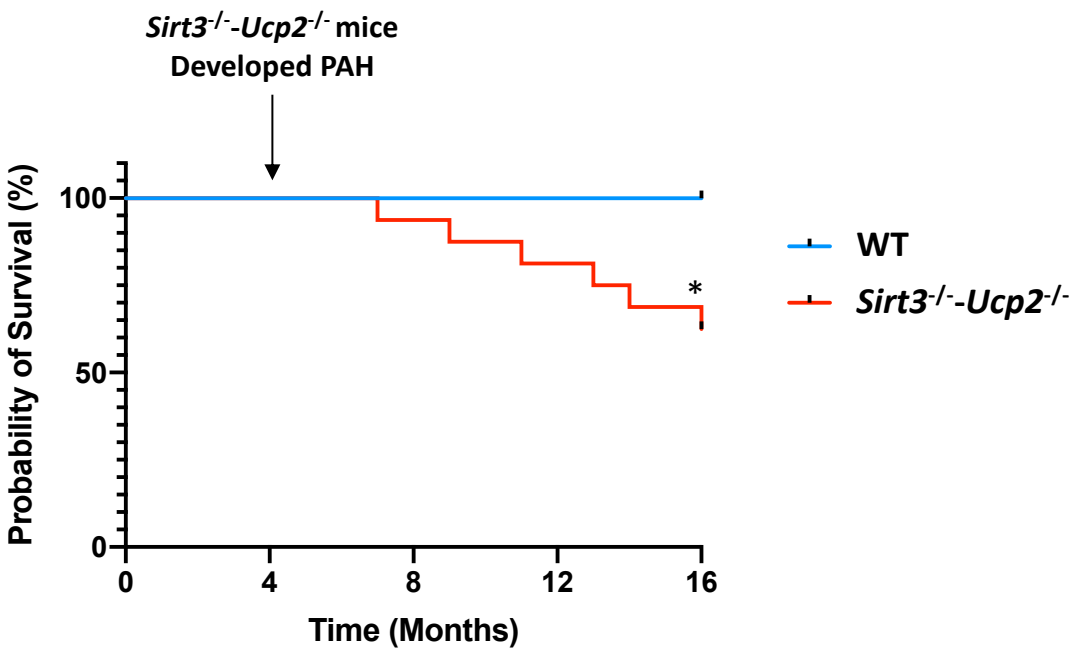


Figure S4. Sixteen-month (12 months after developing PAH) Kaplan-Meier survival plot shows the Sirt3$^{-/-}$-Ucp2$^{-/-}$ mice have decreased survival compared to WT mice. *$p<0.05$ compared to WT mice, n=16 mice / group.

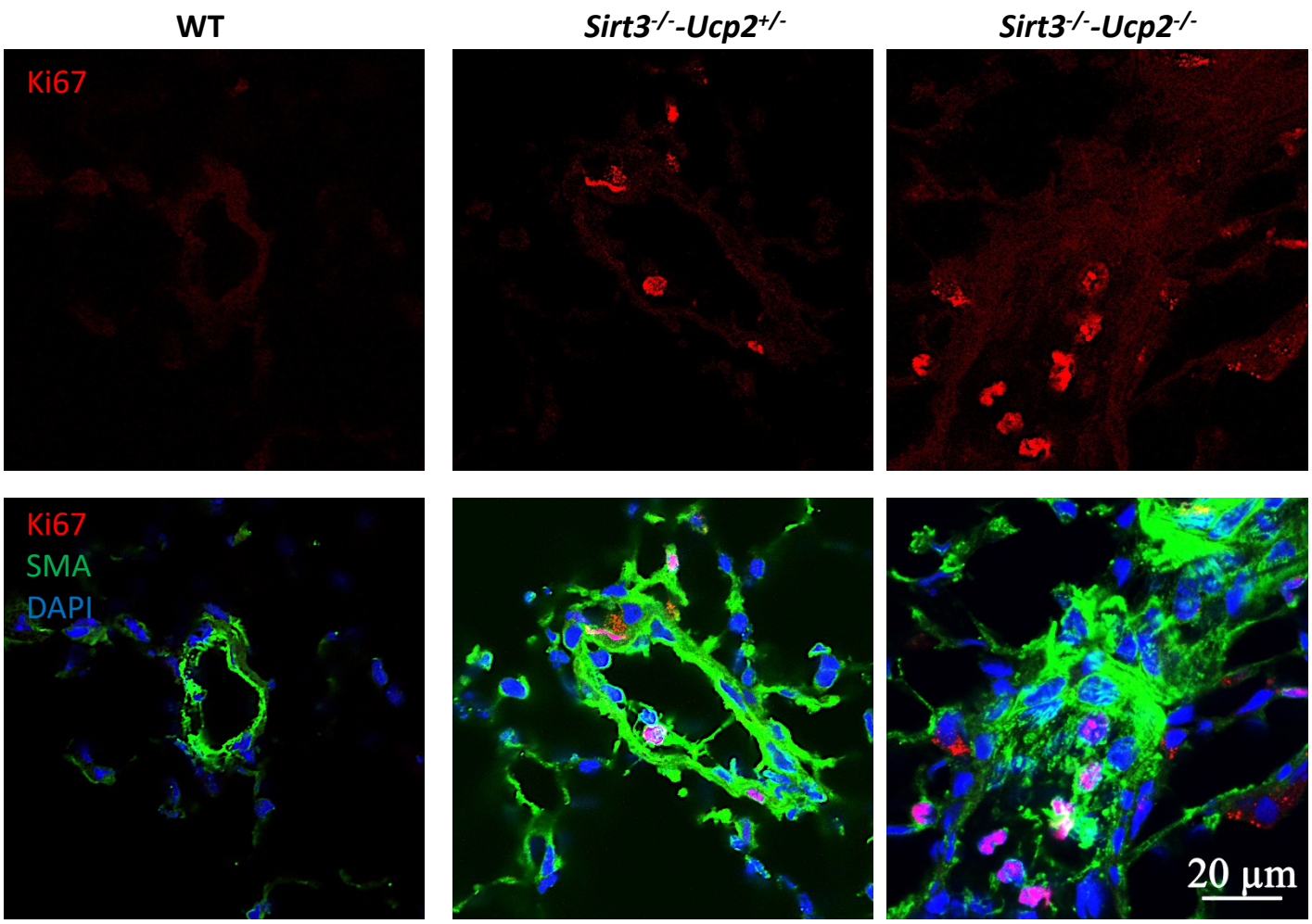


Figure S5. Representative confocal immunohistochemistry images of pulmonary arteries from mice lacking *Sirt3* and *Ucp2* shows that they express higher levels of the proliferation marker Ki67 in their vascular wall.

Figure S6

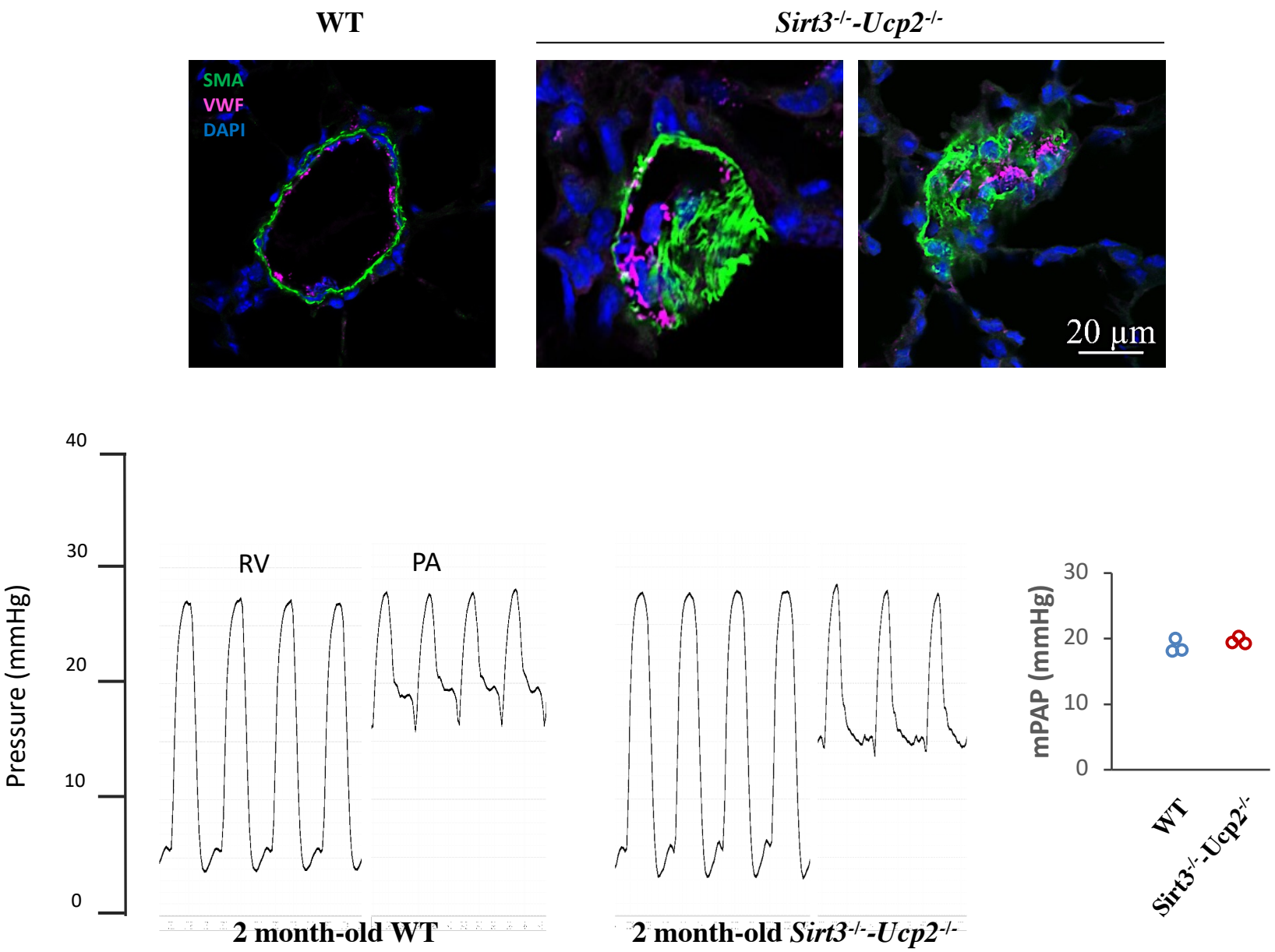


Figure S6. Confocal immunohistochemistry of small pulmonary arteries (showing plexogenic lesions) as well as right heart catheterization data (pulmonary artery tracings and mean PA pressure data) shows normal pressures in 3 WT and 3 Sirt3$^{-/-}$-Ucp2$^{-/-}$ 2 month-old mice, suggesting that the plexogenic arteriopathy is a very early event in the disease process.

Figure S7

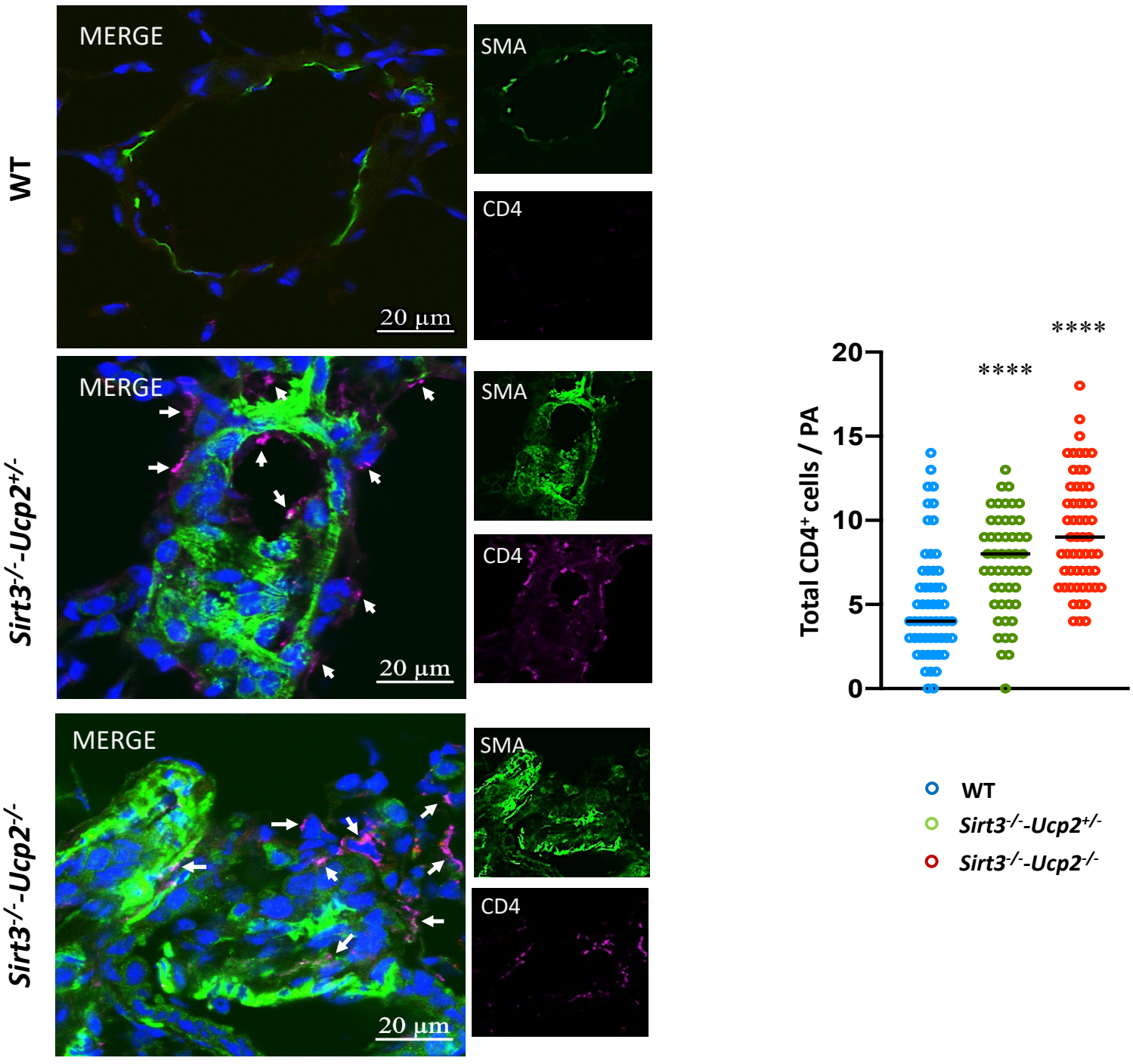
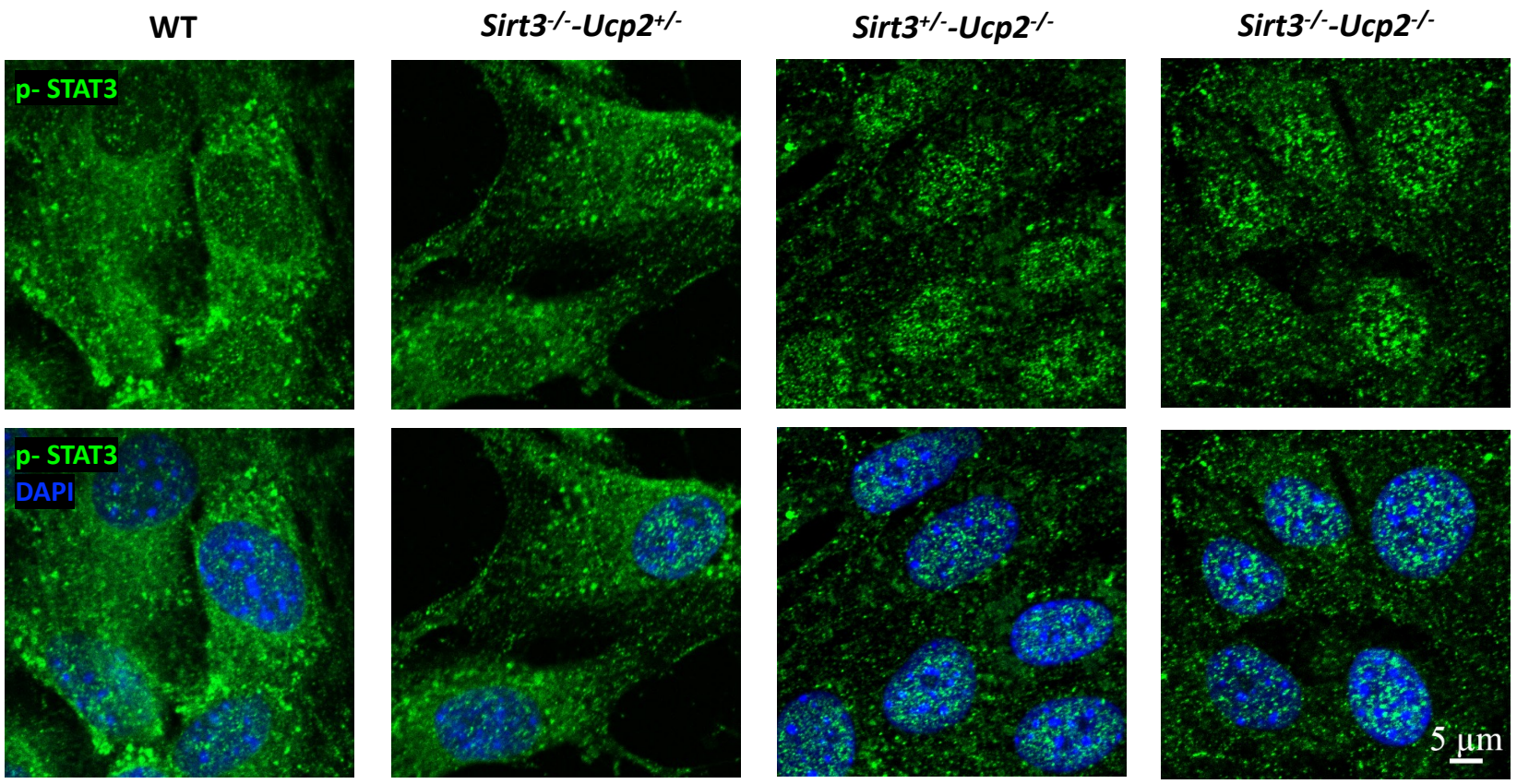


Figure S7. Representative confocal immunohistochemistry images of small pulmonary arteries and mean data shows increased $CD4^+$ T cells within and around the vascular wall of $Sirt3^{-/-}$-$Ucp2^{+/-}$, and $Sirt3^{-/-}$-$Ucp2^{-/-}$ mice compared to WT mice. ****$p<0.0001$ (Bonferroni) compared to WT mice, n = 50 arteries / group.

Figure S8



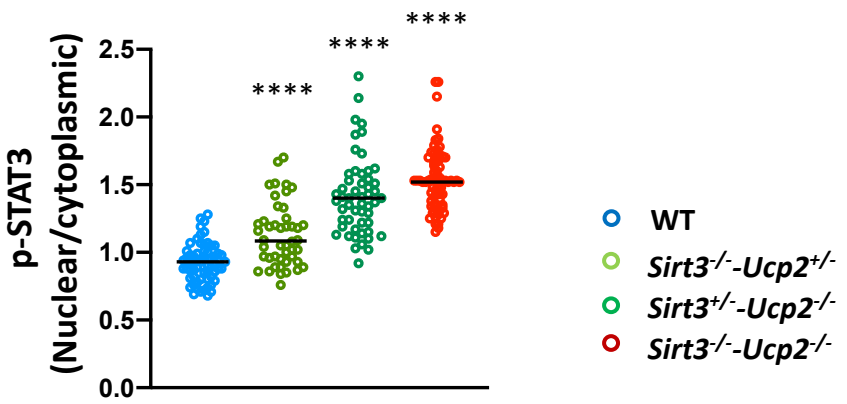


Figure S8. Representative confocal immunohistochemistry images of PASMCs shows increased ratio of nuclear/cytoplasmic of ^{Y705}p-STAT3 in PASMCs from mice lacking Sirt3 and Ucp2 compared to PASMCs from WT mice. ****p<0.0001 (Bonferroni) compared to WT mice, n = 60 cells / group (3 experiments).

Figure S9

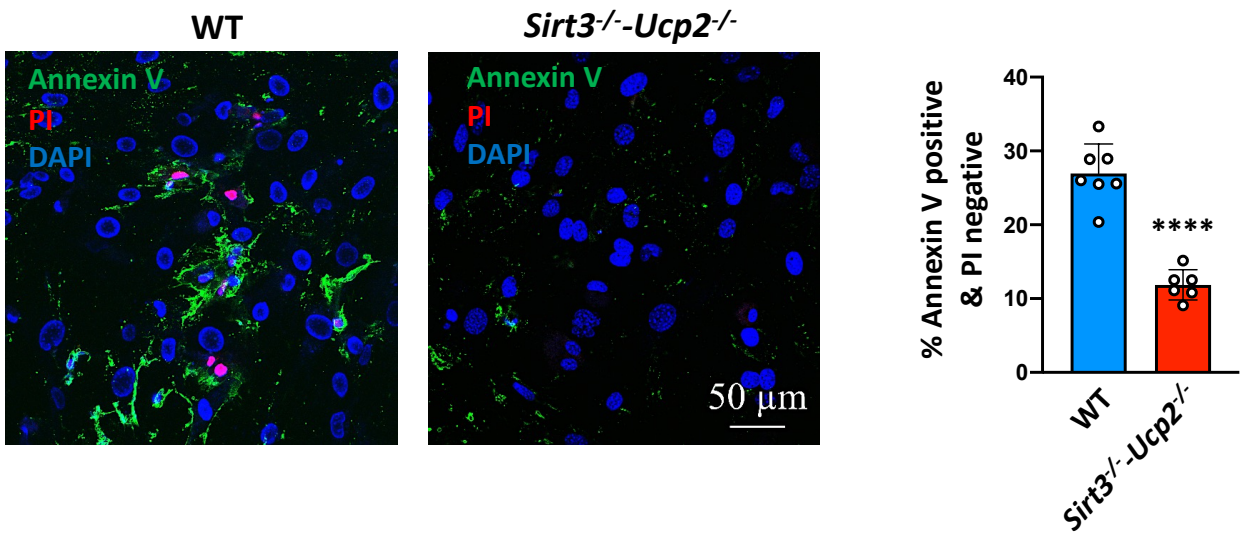


Figure S9. Annexin V / Propidium Iodide (PI) staining of PASMCs exposed to serum starvation for 48 hours shows decreased apoptosis in the $Sirt3^{-/-}$-$Ucp2^{-/-}$ compared to the WT PASMCs. The cells stained only for Annexin V (marking apoptosis) and not PI (which marks death) are counted to measure apoptosis (and not necrosis). ****$p<0.0001$ (Mann-Whitney U test) compared to the WT PASMCs, n = ~240 cells / group (3 experiments).