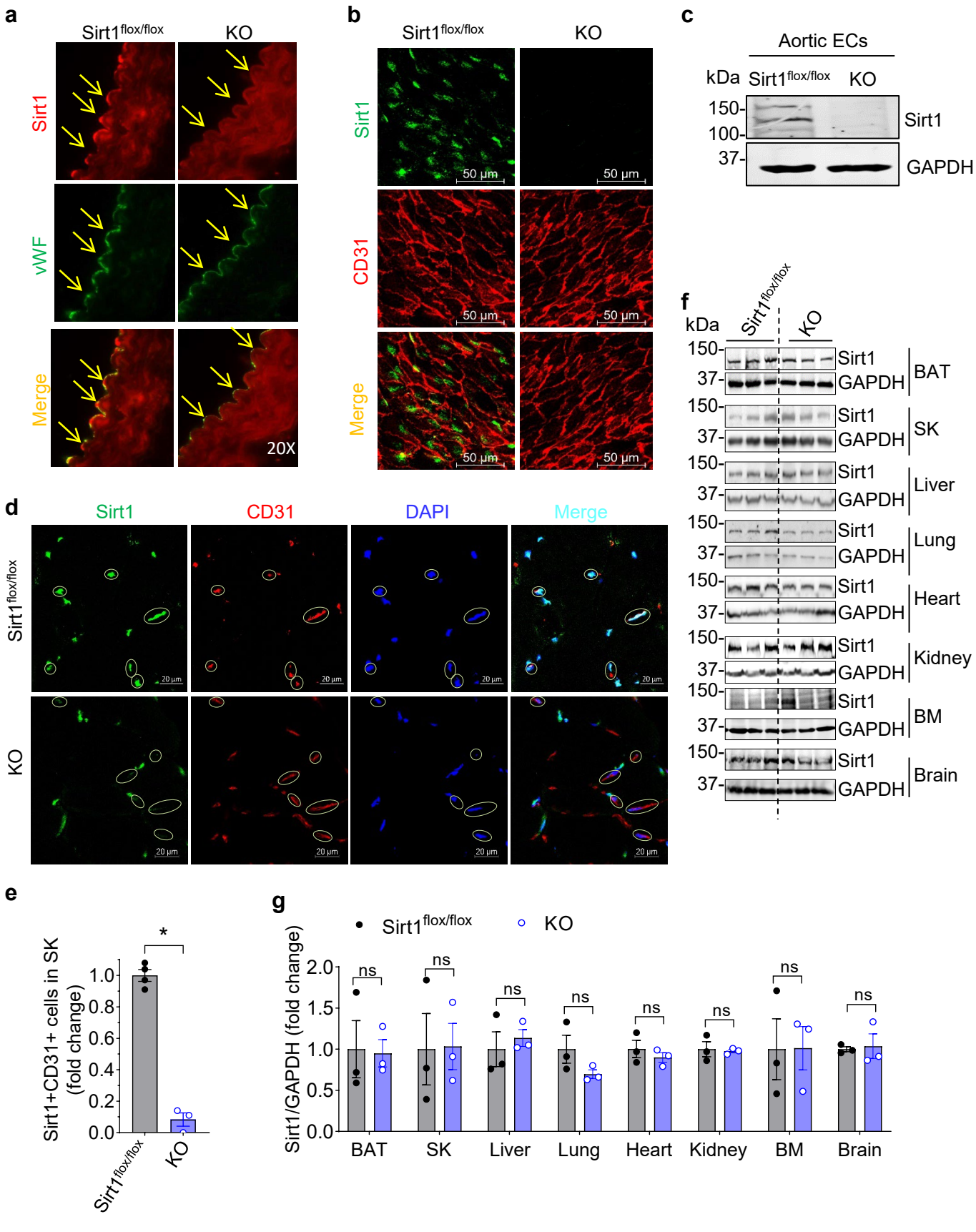
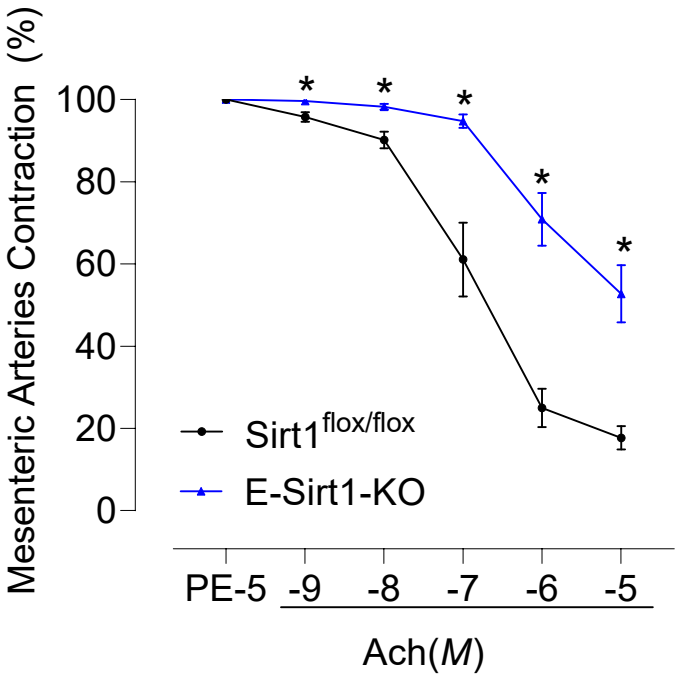
Supplementary Fig. 1



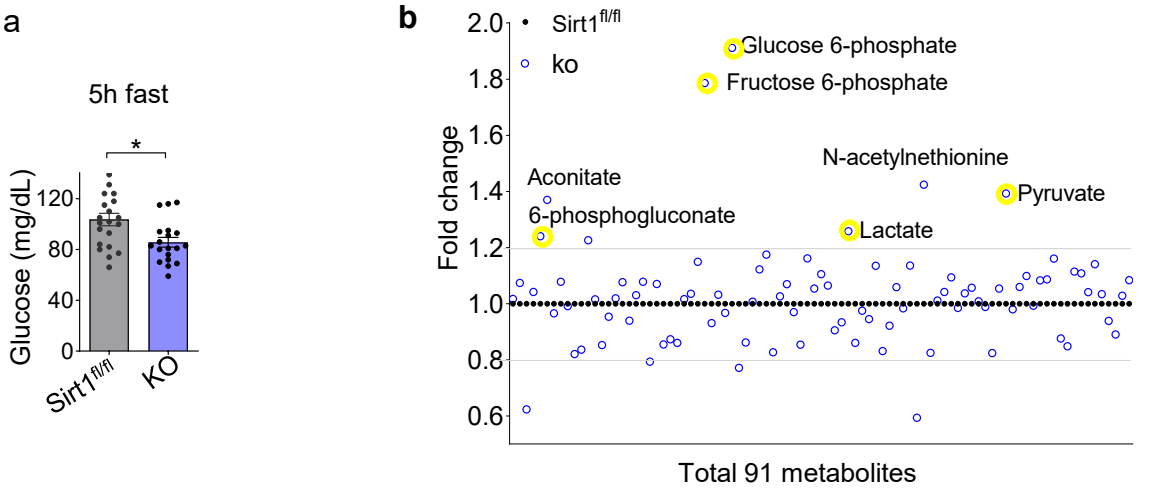
Supplementary Figure 1. Endothelium-specific deletion of Sirt1 in E-Sirt1-KO mice. a Immunostaining for Sirt1 and endothelial vWF in whole aortas of male Sirt1$^{flox/flox}$ and E-Sirt1-KO mice. Yellow arrows indicate endothelium. Green is vWF, an endothelium marker, and red is sirt1; **b** en face staining for endothelial CD31 (red) and Sirt1 (green) in aortas of male Sirt1$^{flox/flox}$ and E-Sirt1-KO mice; **c** Immunoblotting for Sirt1in aortic endothelial cells enzymatically isolated from aortas of Sirt1$^{flox/flox}$ (n = 4) and E-Sirt1-KO (n = 4) mice; **d** Immunostaining for Sirt1 (green), CD31 (red), and DAPI (blue) in gastrocnemius of Sirt1$^{flox/flox}$ and E-Sirt1-KO mice. CD31+ endothelial cells were circled; **e** Fold change of Sirt1+/CD31+ cells in gastrocnemius of Sirt1$^{flox/flox}$ mice (n = 4 areas from 3 mice) vs. E-Sirt1-KO mice (n = 3 areas from 3 mice), *p=0.00002. **f, g** Sirt1 expression in whole tissues of male Sirt1$^{flox/flox}$ (n = 3) and E-Sirt1-KO (n = 3) mice. **f** Immunoblotting and **g** relative quantification are shown. ns: p>0.05. The gray bar represents Sirt1$^{flox/flox}$ mice. The blue bar represents E-Sirt1-KO mice. Each back dot (Sirt1$^{flox/flox}$) or blue circle (KO) represents one sample. Data are shown as mean±SEM. Two-tailed Student's t-test is used to compare the differences between groups. BAT: Brown adipose tissue; SK: Skeletal muscle; BM: bone marrow; vWF: von Willebrand factor. Source data are provided as a Source Data file.

Supplementary Fig. 2



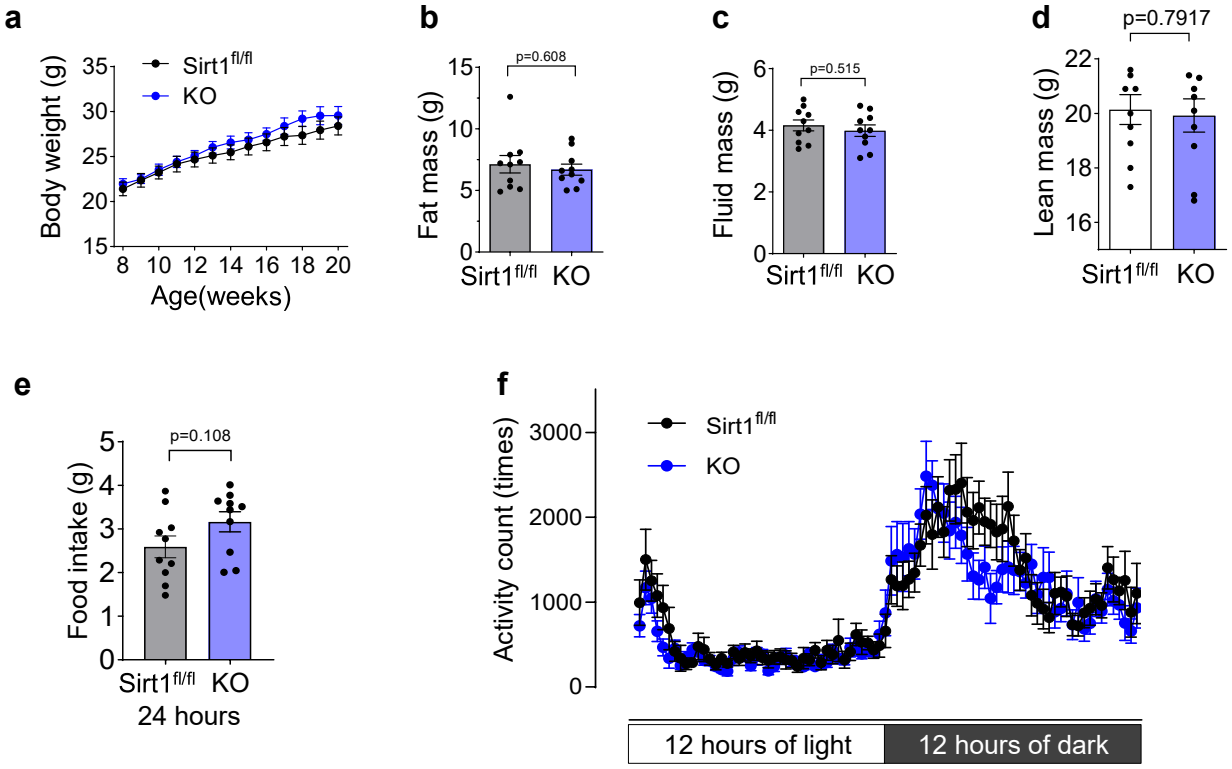
Supplementary Figure 2. Female E-Sirt1-KO mice exhibit vascular dysfunction.
Acetylcholine (Ach)-induced endothelium-dependent relaxation of mesenteric artery rings from female Sirt1$^{flox/flox}$ mice (n = 6 rings from 3 mice) and E-Sirt1-KO (n = 11 rings from 4 mice) mice. Data are shown as mean ± SEM. *p<0.05 Sirt1$^{flox/flox}$ vs. E-Sirt1-KO. Two-tailed unpaired Student's t-test was used at each indicated point. The black line represents Sirt1$^{flox/flox}$ mice. The blue line represents E-Sirt1-KO mice. Source data are provided as a Source Data file.

Supplementary Figure 3



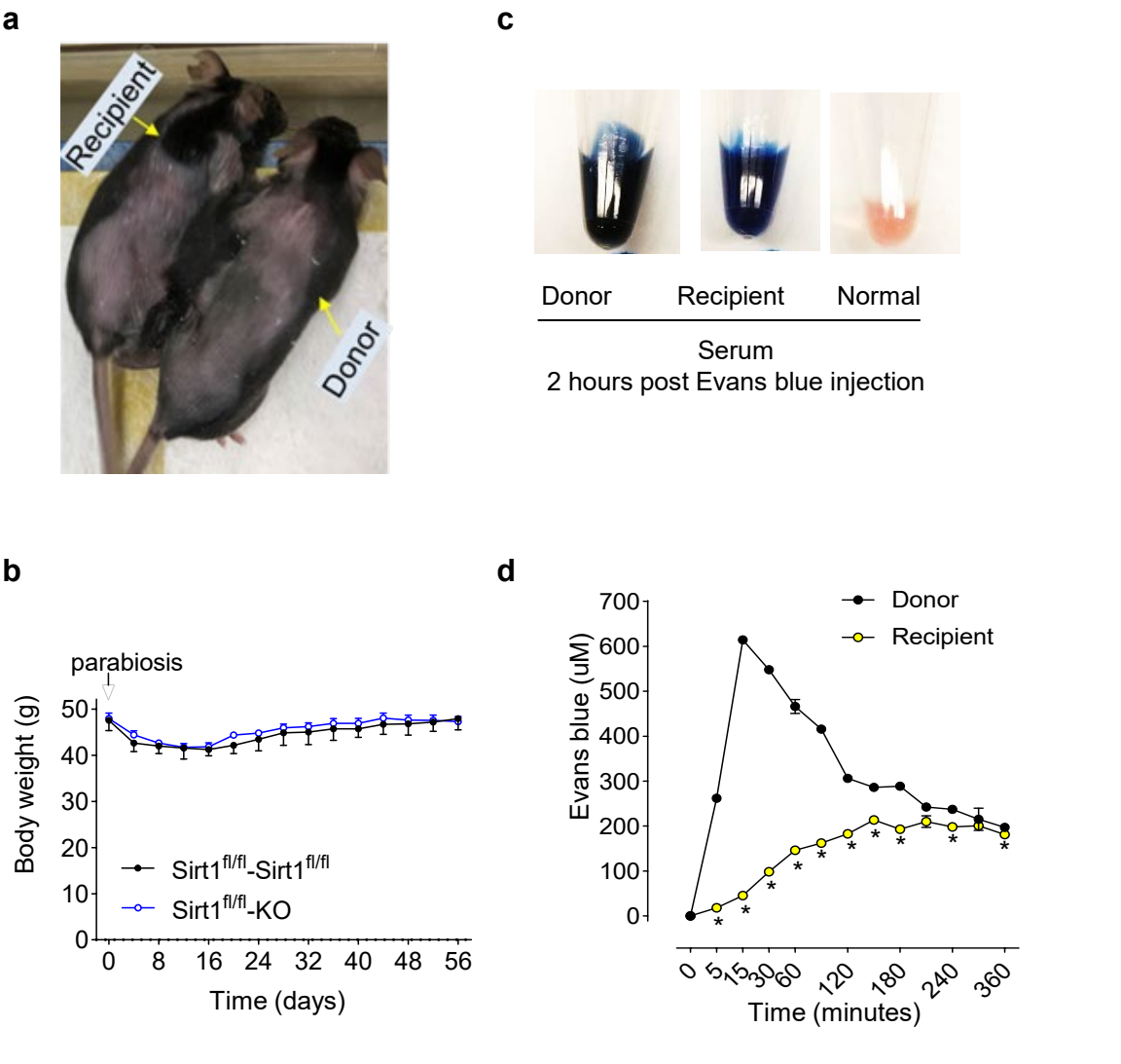
Supplementary Figure 3. Fasting blood glucose, tissue glucose uptake, and skeletal muscle metabolites in Sirt1$^{flox/flox}$ and E-Sirt1-KO mice. a Blood glucose after 5−6 h fasting in male Sirt1$^{fl/fl}$ mice (n=21) vs. KO mice (*n* = 19), **p*= 0.007. Data are shown as mean±SEM. Two-tailed unpaired Student's T-test was used to compare the differences between groups. The gray bar represents Sirt1$^{fl/fl}$ mice. The blue bar represents KO mice. Each back dot represents an individual sample. **b** Metabolomics in tibialis anterior of male KO mice (*n* = 6) relative to Sirt1$^{flox/flox}$ mice (*n* = 3). Each black dot (Sirt1$^{fl/fl}$) or blue circle (KO) represents an individual metabolite. Note the increase in metabolites of glycolysis (yellow circles). Sirt1$^{fl/fl}$: Sirt1$^{flox/flox}$; KO: E-Sirt1 KO. Source data are provided as a Source Data file.

Supplementary Fig. 4



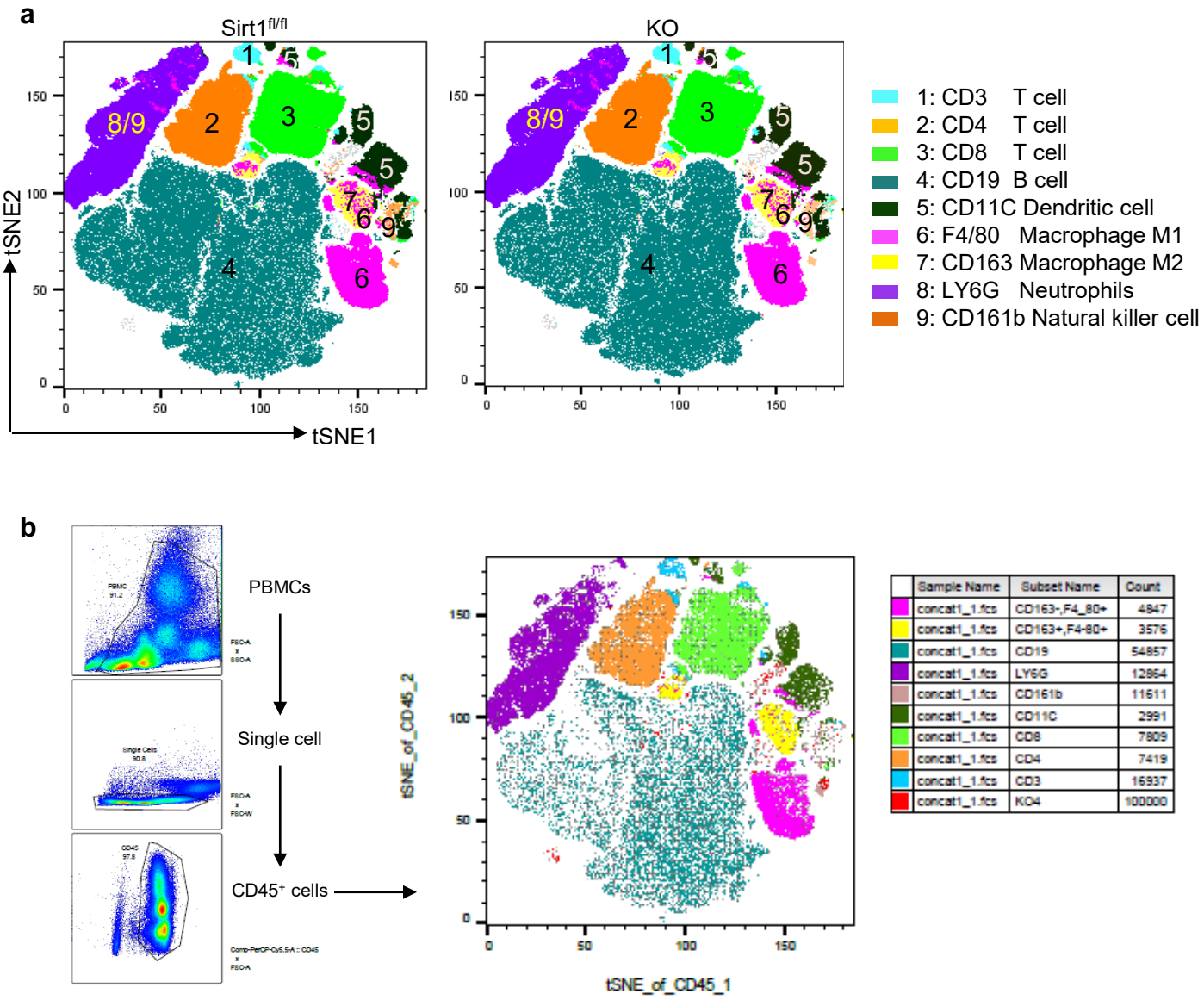
Supplementary Figure 4. Endothelium-specific deletion of Sirt1 does not affect body weight, body composition, activity, and food intake. a Gain in bodyweight over time of male Sirt1$^{fl/fl}$ (*n* = 8) and KO (*n* = 8) mice, *p*>0.05 at each indicated point; **b** Fat mass of male Sirt1$^{fl/fl}$ (*n* = 10) and KO (n=10) mice, p=0.608; **c** fluid mass of male Sirt1$^{fl/fl}$ (*n* = 10) and KO (*n* = 10) mice, p=0.515; and **d** lean mass of male Sirt1$^{fl/fl}$ (*n* = 10) and KO (*n* = 10) mice, *p*=0.7917; **e** food intake of male Sirt1$^{fl/fl}$ (*n* = 10) and KO (*n* = 10) mice, *p*=0.108; and **f** activity in 20-week-old male Sirt1$^{fl/fl}$ (*n* = 11) and KO (*n* = 13) mice. *p*>0.05 at each indicated point. Data are shown as mean±SEM. Two-tailed unpaired Student's T-test was used to compare the differences between groups. The black lines in **a** and **f** represent Sirt1$^{fl/fl}$ mice, and the blue lines in **a** and **f** represent KO mice. In **b**, **c**, **d**, and **e**, the gray bar represents Sirt1$^{fl/fl}$ mice, and the blue bar represents KO mice. Each back dot represents one sample. Sirt1$^{fl/fl}$: Sirt1$^{flox/flox}$ mice; and KO: E-Sirt1-KO mice. Source data are provided as a Source Data file.

Supplementary Figure 5



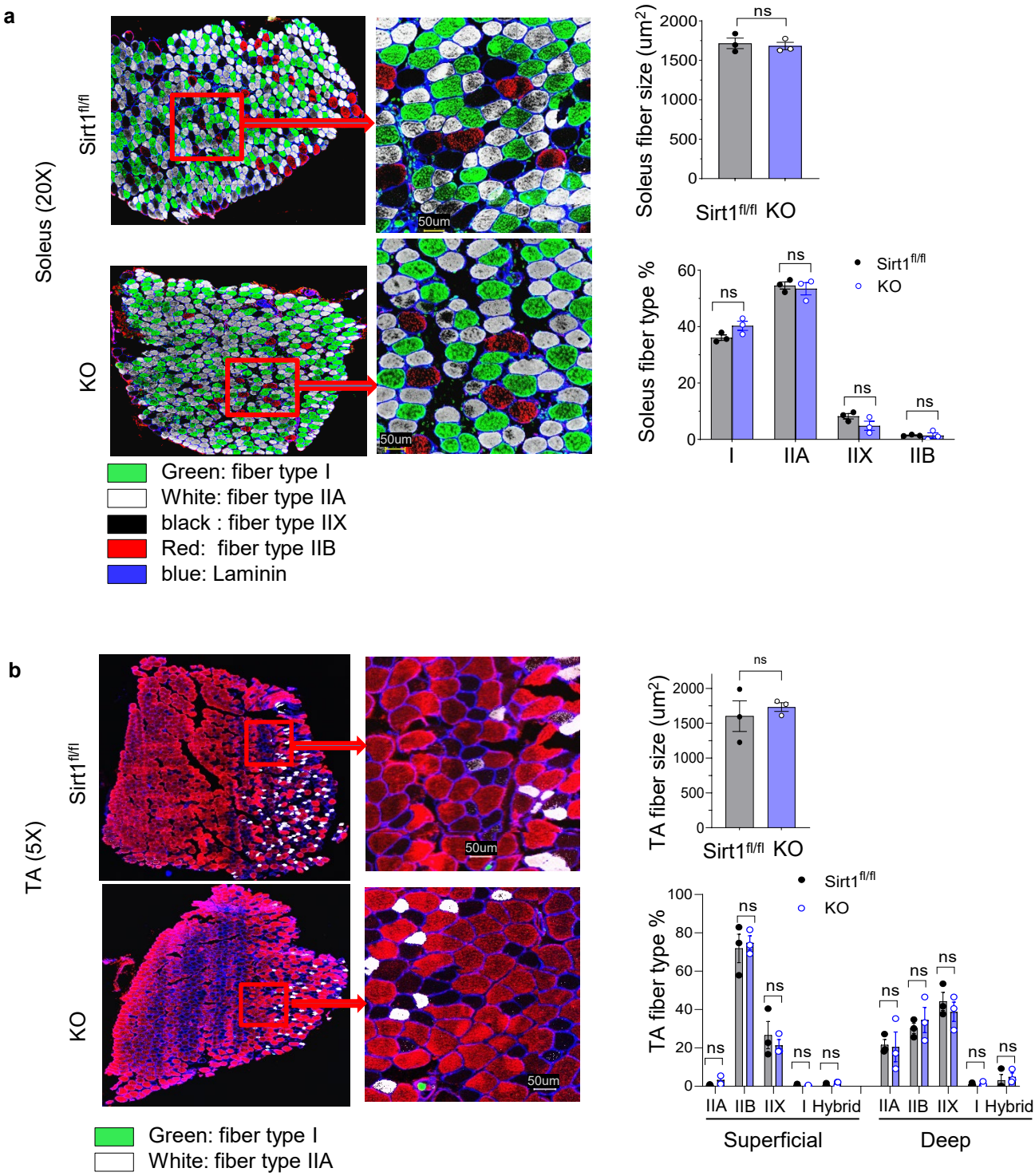
Supplementary Figure 5. Parabiosis of mice results in shared circulation. **a** Weight-matched male parabiosed mouse pair; **b** Body weights of male Sirt1$^{fl/fl}$ - Sirt1$^{fl/fl}$ mice (n = 6 pairs) and Sirt1$^{fl/fl}$-KO mice (n = 8 pairs) were monitored after parabiosis surgery. p>0.05 at each indicated point. The black line represents Sirt1$^{fl/fl}$-Sirt1$^{fl/fl}$ paired mice, and the blue line represents Sirt1$^{fl/fl}$-KO paired mice; **c** Appearance of Evans blue dye in serum of donor (injected) and recipient parabiosed male mice; **d** Venous concentration of Evans blue dye over time in donor (injected) male mice (n = 2 blood samples from 1 mouse, black line with the black dots) and recipient mice (n = 2 blood samples from 1 mouse, black line with the yellow dot) of parabiosed mice,*p<0.05. Data are shown as mean±SEM. Two-tails Student's t-test was used at each indicated point. Sirt1$^{fl/fl}$: Sirt1$^{flox/flox}$ mice; and KO: E-Sirt1-KO mice. Source data are provided as a Source Data file.

Supplementary Figure 6

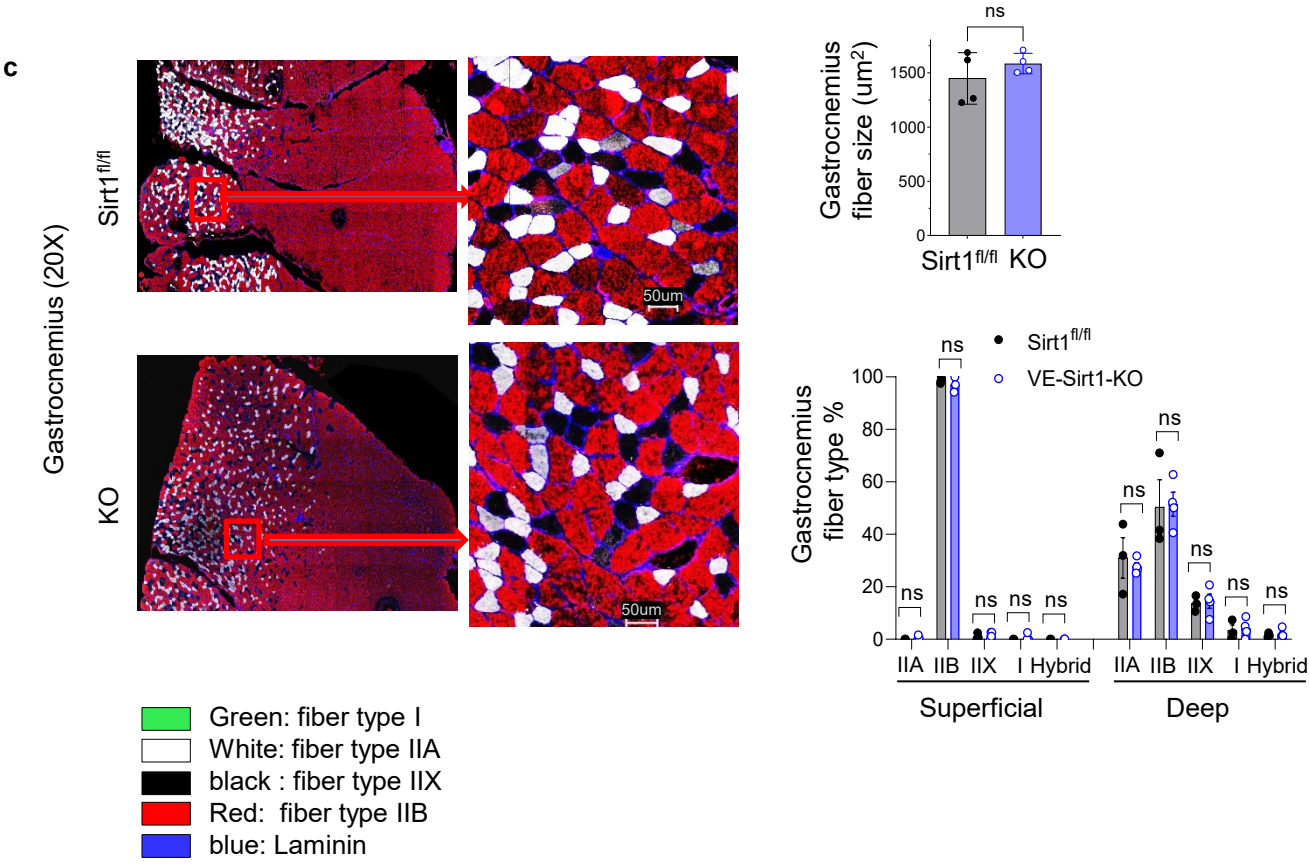


Supplementary Figure 6. Endothelium-specific deletion of Sirt1 does not alter immune cell populations. **a** t-SNE analysis followed by clustering of mass cytometry data for peripheral blood cells in male Sirt1$^{fl/fl}$ (*n* = 6) and KO (*n* = 6) mice. Merged t-SNE plots for all Sirt1$^{flox/flox}$ and KO, with each cluster indicated by number and color, are shown. **b** Gate strategy. All alive PBMCs are first gated on the basis of FSC-A (forward scatter) and SSC-A (side scatter), and then single cell is gated on the basis of FSC-S (forward scatter) and FSC-W (forward scatter width). Then, CD45-positive cells are gated on the basis of the surface expression of CD45, a white blood cell marker. The t-distributed stochastic neighbor embedding (t-SNE) tool is used to analyze the sub-populations of CD45 positive cells on the basis of surface expression markers, and the t-SNE map of 100,000 CD45+ cells was created. PBMCs: peripheral blood mononuclear cells; Sirt1$^{fl/fl}$: Sirt1$^{flox/flox}$ mice; KO: E-Sirt1-KO mice. Each color represents one subpopulation of the PBMCs as indicated. Source data are provided as a Source Data file.

Supplementary Figure 7

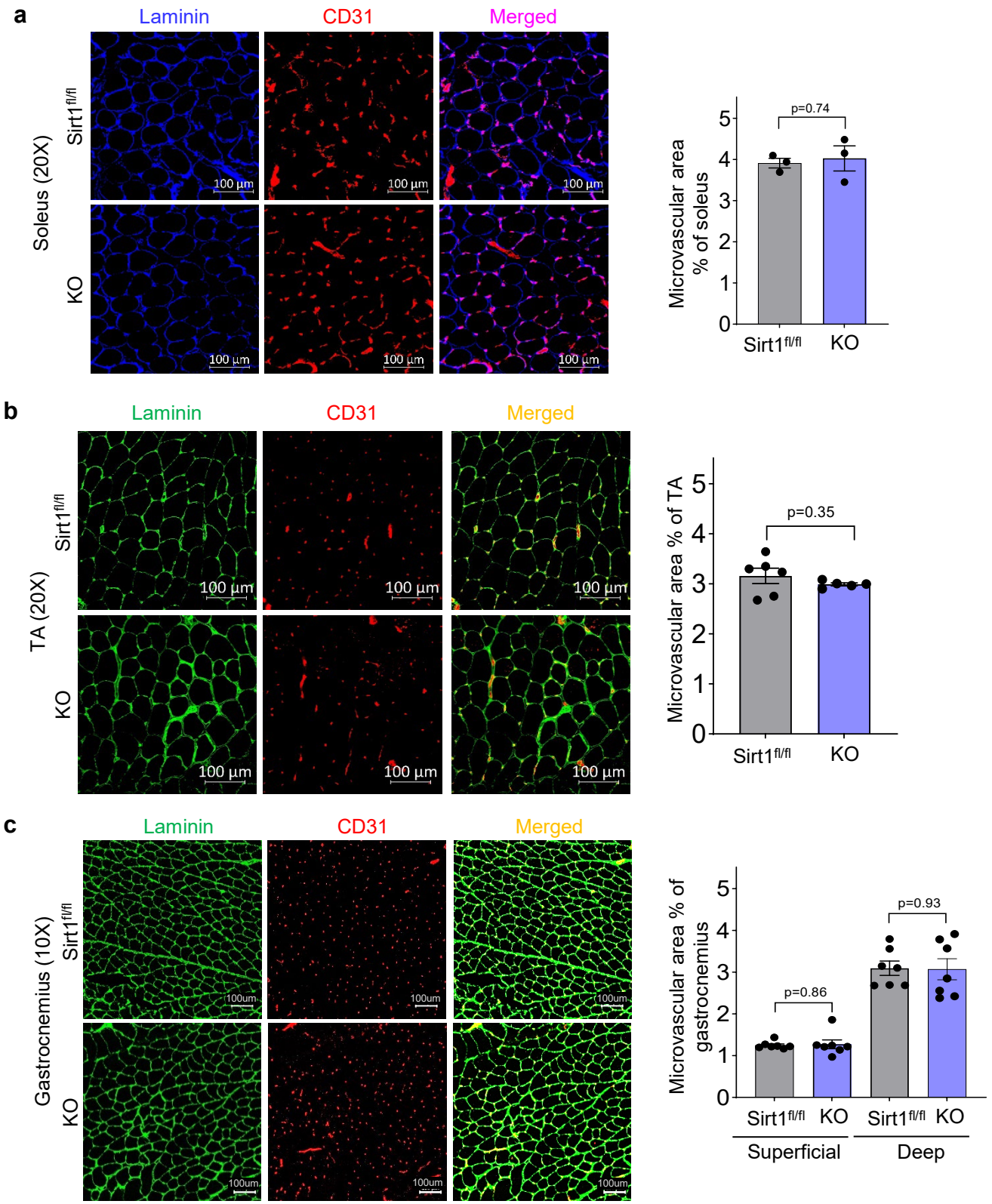


Supplementary Figure 7 (continued)



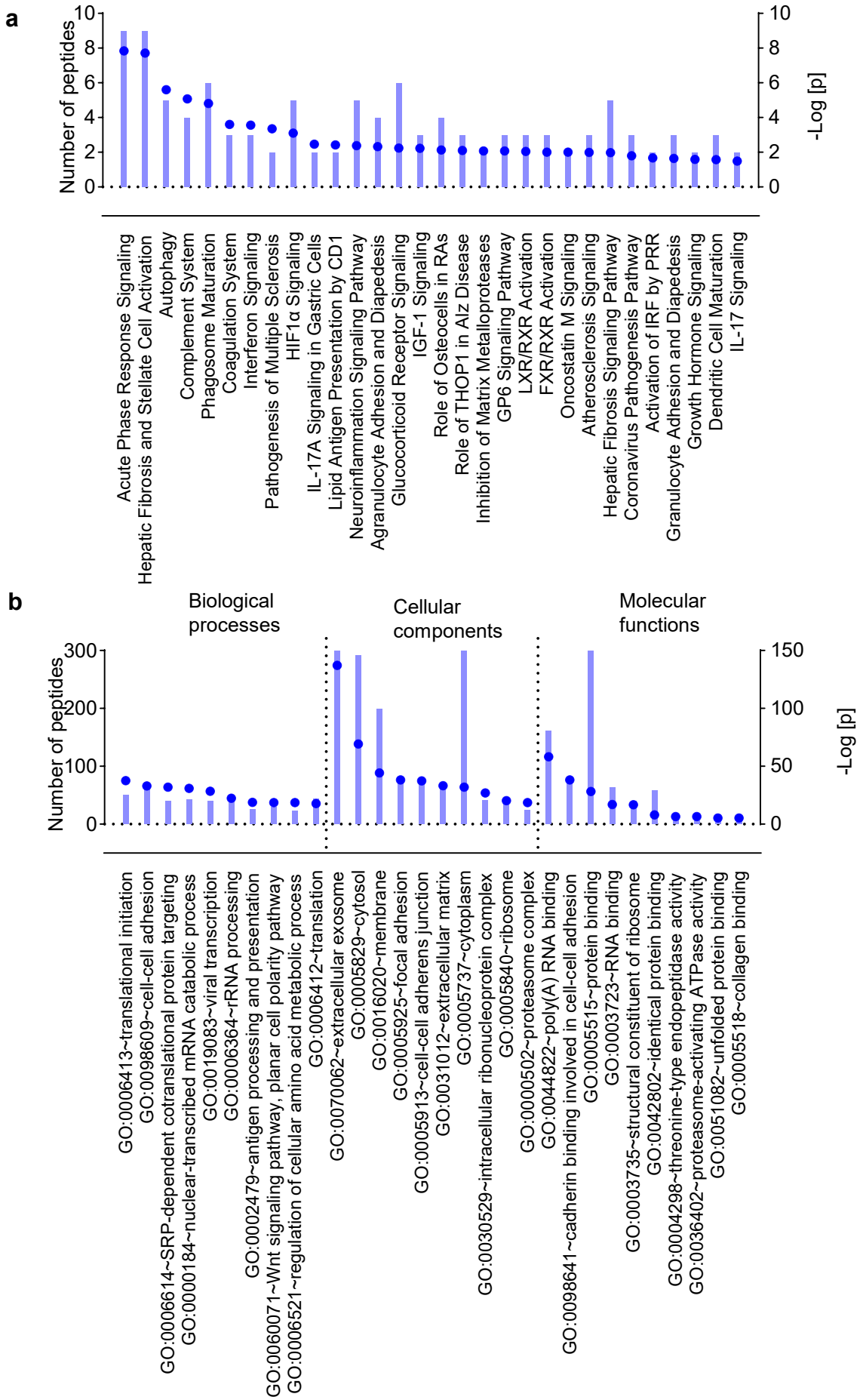
Supplementary Figure 7. Deletion of endothelial Sirt1 does not alter skeletal muscle fiber size and type. Representative fluorescence images (left), fiber size (right upper), and percentage of fiber type of **a** soleus muscle from male Sirt1$^{fl/fl}$ (*n* = 3) and KO (*n* = 3) mice, **b** tibialis anterior muscle from male Sirt1$^{fl/fl}$ (*n* = 3) and KO (*n* = 3) mice, and **c** gastrocnemius from male Sirt1$^{fl/fl}$ (*n* = 4) and KO (*n* = 4) mice. ns P>0.05. Data are shown as mean±SEM. Two-tailed unpaired Student's t-test was used to compare the differences between groups. Green: fiber type i, White: fiber type iiA, black: fiber type iiX, red: fiber type iiB, blue: laminin. The gray bar represents Sirt1$^{fl/fl}$ mice and the blue bar represents KO mice. Each back dot (Sirt1$^{fl/fl}$) and blue circle (KO) represents one sample. Sirt1$^{fl/fl}$: Sirt1$^{flox/flox}$ mice; KO: E-Sirt1-KO mice. TA: Tibialis anterior muscle. Source data are provided as a Source Data file.

Supplementary Figure 8



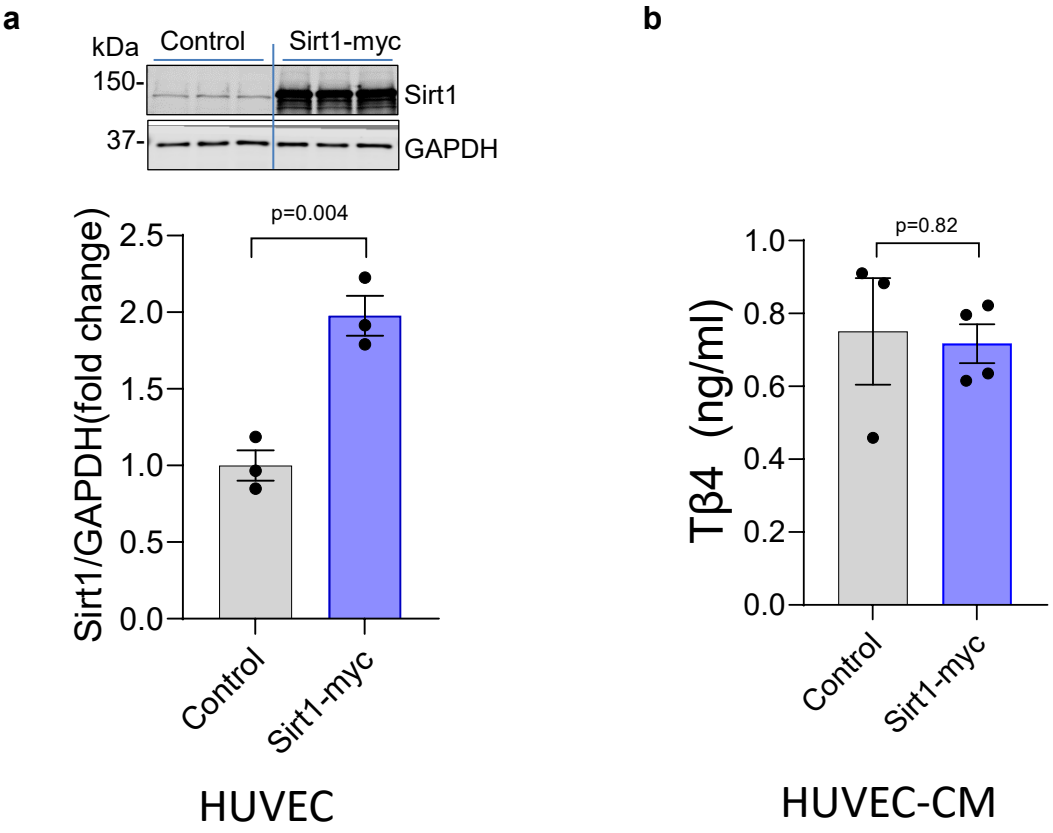
Supplementary Figure 8. Deletion of endothelial Sirt1 does not alter microvascular density in skeletal muscle. Representative images with quantification of microvascular density percentage in skeletal muscle are shown. Immunostaining for Laminin (blue in **a** and green in **b** and **c)** and endothelial marker CD31 (red) in **a** soleus of male $Sirt1^{fl/fl}$ (*n* = 3 areas of 3 mice) and KO (*n* = 3 areas of 3 mice) mice, *p*=0.74; **b** tibialis anterior of male $Sirt1^{fl/fl}$ (*n* = 6 areas of 3 mice) and KO (*n* = 5 areas of 3 mice) mice, *p*=0.35; and **c** gastrocnemius of male $Sirt1^{flox/flox}$ (*n* = 7 areas of 3 mice) and KO mice (n=7 areas of 3 mice), *p*=0.86 in superficial area group and *p*=0.93 in the deep area group. The gray bar represents $Sirt1^{fl/fl}$ mice. The blue bar represents KO mice. Each back dot represents one sample. $Sirt1^{fl/fl}$: $Sirt1^{flox/flox}$ mice; KO: E-Sirt1-KO mice. TA: Tibialis anterior muscle. Data are shown as mean ± SEM. Two-tailed unpaired Student's T-test was used to compare the differences between groups. Source data are provided as a Source Data file.

Supplementary Figure 9



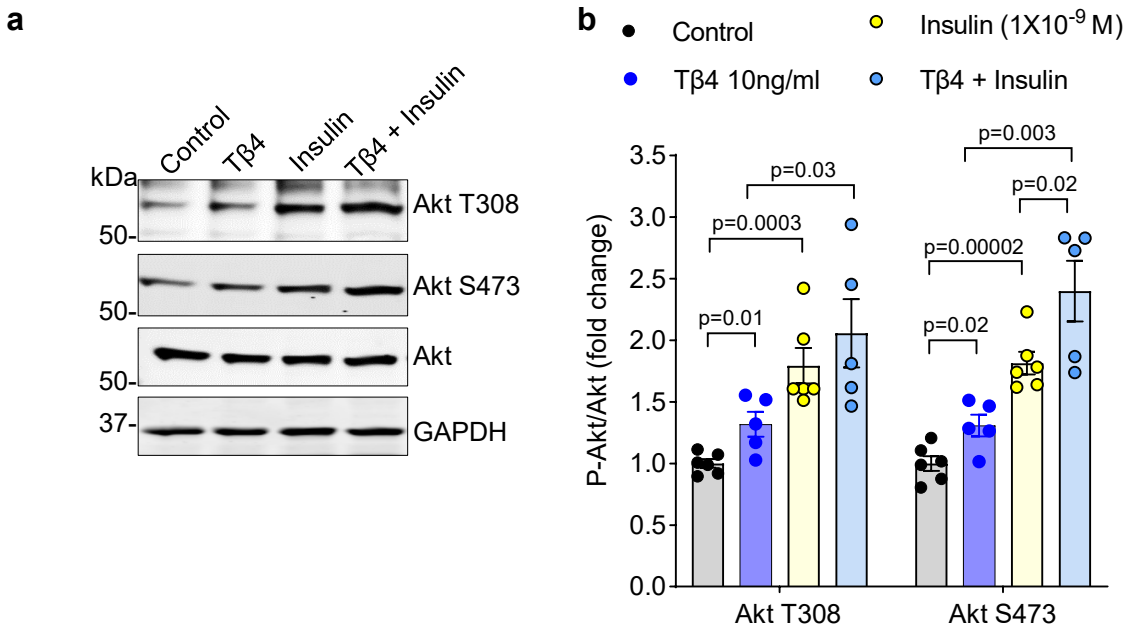
Supplementary Figure 9. Pathway and Gene-ontology analysis of proteins enriched in conditioned media of endothelial cells deficient for Sirt1. **a** Top 30 canonical pathways based on ingenuity pathway analysis of differentially regulated peptides in the conditioned media of Sirt1-silenced HUVECs. **b** Gene-ontology enrichment analysis using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) v6.8 showing the top 10 biological processes, cellular components, and molecular function of differentially regulated peptides in the conditioned media of Sirt1-silenced HUVECs. Blue bars indicate numbers of peptides and blue dots show statistical significance. Source data are provided as a Source Data file**.**

Supplementary Figure 10



Supplementary Figure 10. Overexpression of endothelial Sirt1 does not increase Tβ4 secretion. a Sirt1 protein expression in the control HUVECs (*n* = 3 cell culture) vs. Sirt1-myc HUVECs (*n* = 3 cell culture), *p*=0.004; and **b** Tβ4 concentration in conditioned medium (CM) of HUVECs transfected with Sirt1 plasmid (*n* = 4 cell culture) vs. control plasmid (*n* = 3 cell culture), *p*=0.82. Representative immunoblots are shown. Data are shown as mean ± SEM. Two-tail Student's T-test was used to compare the differences between groups. The blue bar represents the Sirt1-myc group, and the gray bar represents the scramble control group. Each dot represents one sample. HUVECs: human umbilical endothelial cells. Source data are provided as a Source Data file.

Supplementary Figure 11



Supplementary Figure 11. Tβ4 activates Akt in skeletal myotubes independently and synergistically with insulin. a Representative immunoblots and **b** fold change of phosphorylation and total Akt in C2C12 myotubes treated with recombinant human Tβ4 (10 ng/mL for 20 min), insulin (10^{-9} M for 20 min), or a combination of both. Data are shown as mean ± SEM. The gray bar is the control group (*n* = 6 cell culture), the blue bar represebt the recombinant human Tβ4 treated group (*n* = 6 cell culture), the yellow bar is the insulin treated group (*n* = 6 cell culture), and the light blue bar represents the group treated with Tβ4 and insulin (*n* = 5 cell culture). Each dot represents one sample. Two-tail Student's T-test was used to compare the differences between groups. The uncropped Western blots and Source data are provided as a Source Data file.