**Sex-Specific Angiogenic Responses in Endothelial Cells – Role of the Pluripotency Factor OCT4**

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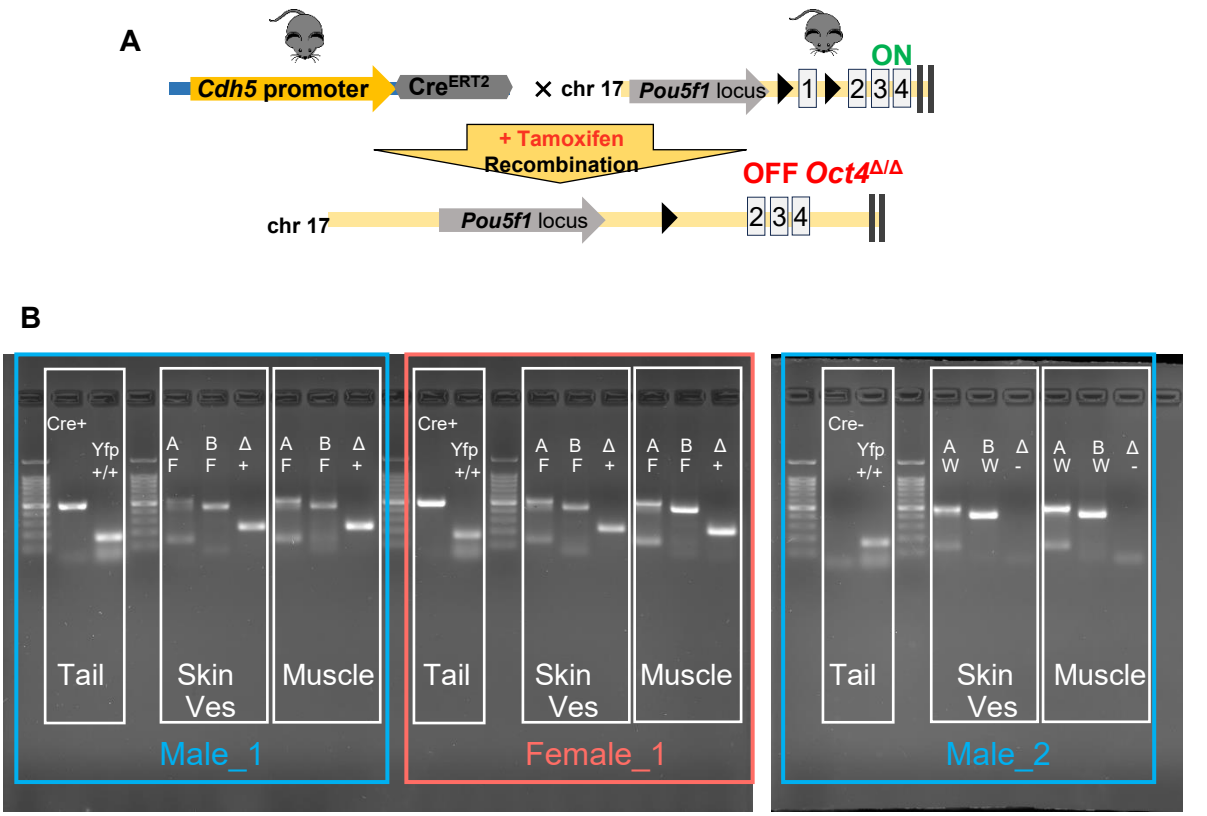
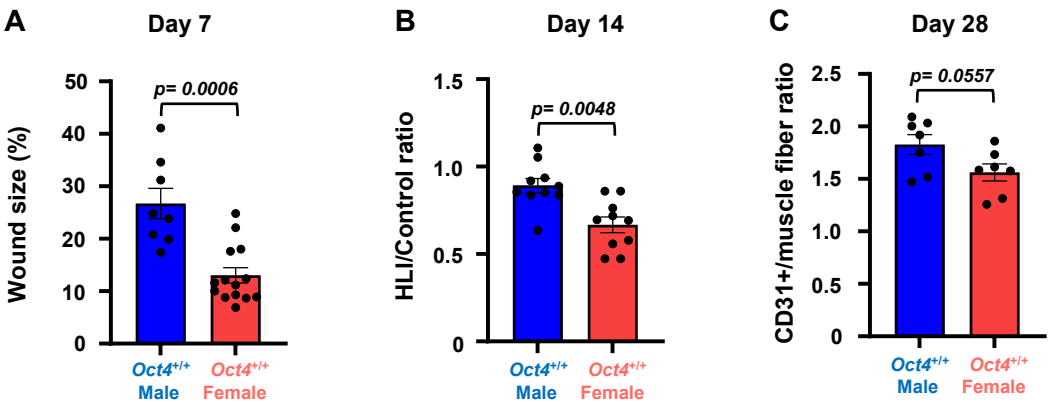
# Supplementary Figures 1-7

*Supplementary Figure 1*




**Supplemental Figure 1. Mouse model.** (**A**) Schematic illustrating an EC-specific tamoxifen-inducible OCT4 knockout mouse model (*Oct4*$^{\Delta/\Delta}$) and littermate wild-type controls (*Oct4*$^{+/+}$). (**B**) Male and female *Oct4*$^{Flox/Flox}$ PAC-*Cdh5*-CreERT2$^{+}$ Rosa-Stop-*Yfp*$^{+/+}$ mice (Male_1 and Female_1) and male *Oct4*$^{WT/WT}$ PAC-*Cdh5*-CreERT2$^{-}$ Rosa-Stop-*Yfp*$^{+/+}$ mouse (Male_2) received ten intraperitoneal tamoxifen injections between 6 and 8 weeks of age. At age 10 weeks of age, mice were euthanized and tail, dorsal skin blood vessels, and hind limb tissues were subjected to genotyping using primers specific for Cre, Yfp, and *Oct4* Flox/WT/Δ. Oct4 Flox (F) and wild type (W) loci were identified using two different primers sets, as described by *Kehler et al., 2004* (see Material and Method section).

*Supplementary Figure 2*

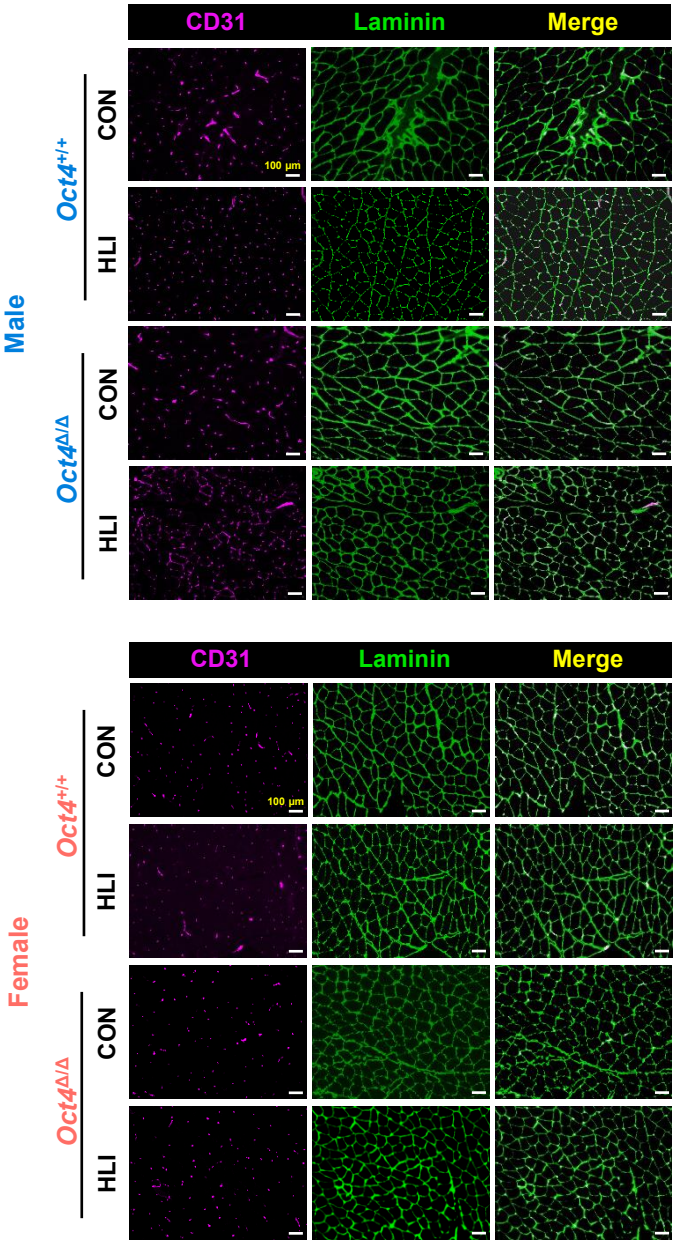



**D**

| Baseline<br>M WT vs F WT | Phenotype | Vascularization |
|---|---|---|
| Inflammatory physiological model (Skin injury) | Female WT: Faster wound healing | ♂ >♀ |
| Inflammatory pathological model (Tumor injection) | Male WT: higher tumor growth | ♂ >♀ |
| Low-inflammatory ischemia model (Hindlimb ischemic model) | Male WT: faster blood flow recovery | ♂ >♀ |

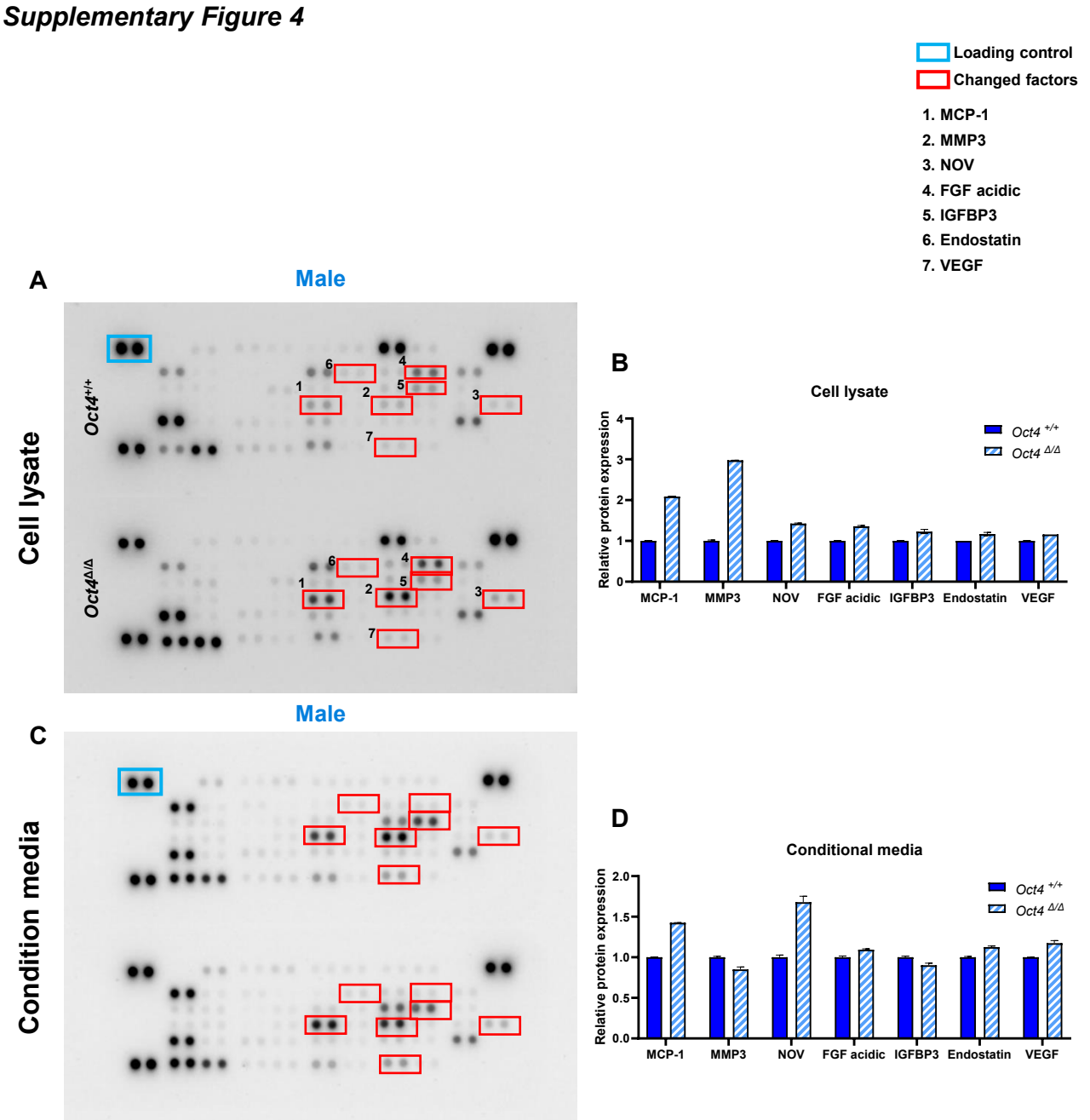
**Supplementary Figure 2. Female wild type mice show a faster skin wound healing but slower blood flow recovery in hind limb ischemia (HLI) model compared to male mice.** (**A**) Comparison of skin wound healing between male *Oct4*$^{+/+}$ and female *Oct4*$^{+/+}$ mice at day 7 post-injury (*n*=8-14). Data represent the wound size as % of the original wound size on day 0. (**B**) Blood flow was compared between male *Oct4*$^{+/+}$ and female *Oct4*$^{+/+}$ mice at day 14 post-HLI surgery (*n*=10). **C.** Skeletal muscle capillarization was compared between male *Oct4*$^{+/+}$ and female *Oct4*$^{+/+}$ mice at day 28 post-HLI surgery (*n*=6-7). Data were analyzed via an unpaired nonparametric Mann-Whitney test. Values are presented as mean ± s.e.m. (**D**) Summary of the baseline sex differences in wild-type male vs. female mice in three used models.

*Supplementary Figure 3*



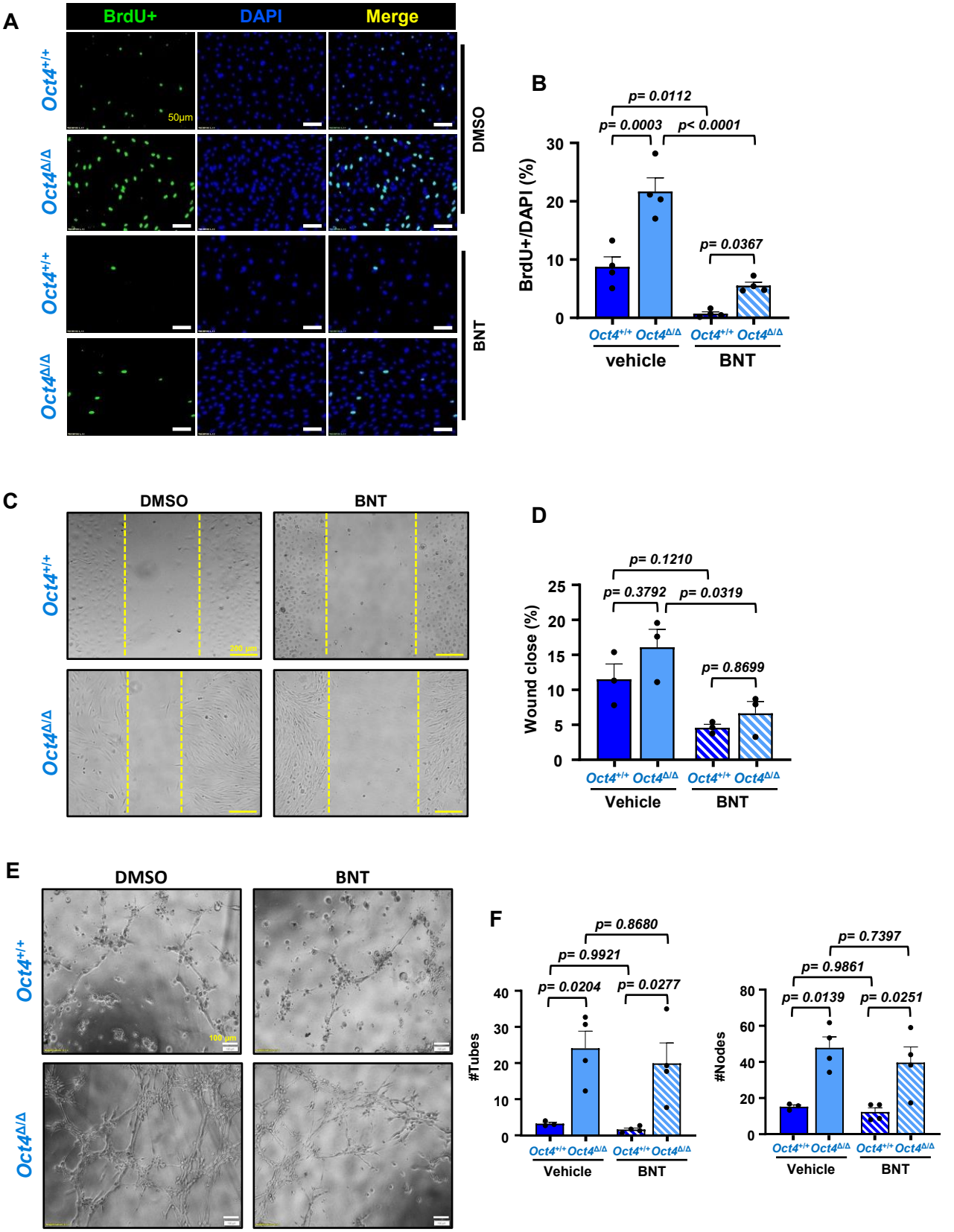

**Supplementary Figure 3. OCT4 deficiency in EC increased capillarization in male mice, not in females.** Representative images of capillarization in gastrocnemius/soleus muscles stained for CD31 (to visualize endothelial cells) and laminin (to visualize muscle fibers) in *Oct4*$^{+/+}$ versus *Oct4*$^{\Delta/\Delta}$ male and female mice at days 28 post-hind-limb ischemia surgery. Scale bar = 100 μm.

*Supplementary Figure 4*



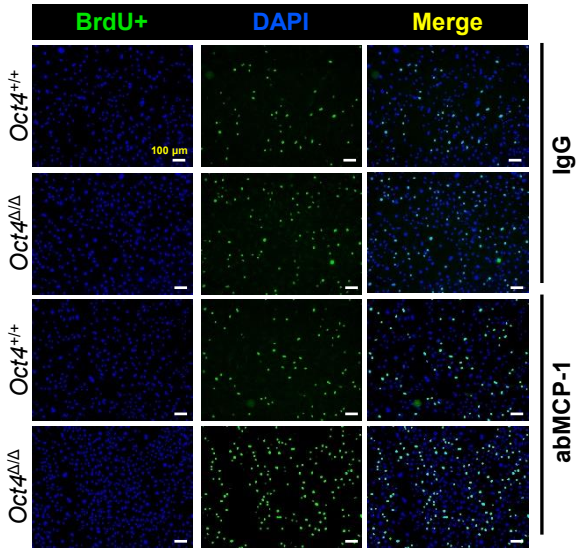

**Supplementary Figure 4.** (**A** and **C**) Representative images of angiogenesis proteome profiling array (**C**) and the quantification of array dots intensity (**B and D**) in total cell lysate and condition media from *Oct4*$^{+/+}$ versus *Oct4*$^{\Delta/\Delta}$ cultured male MAECs. MCP-1; monocyte chemoattractant protein-1, MMP3; matrix metalloproteinase-3, NOV; nephroblastoma overexpressed, FGF1; fibroblast growth factor 1, IGFBP3; insulin-like growth factor-binding protein 3, VEGF; vascular endothelial growth factor.

*Supplementary Figure 5*



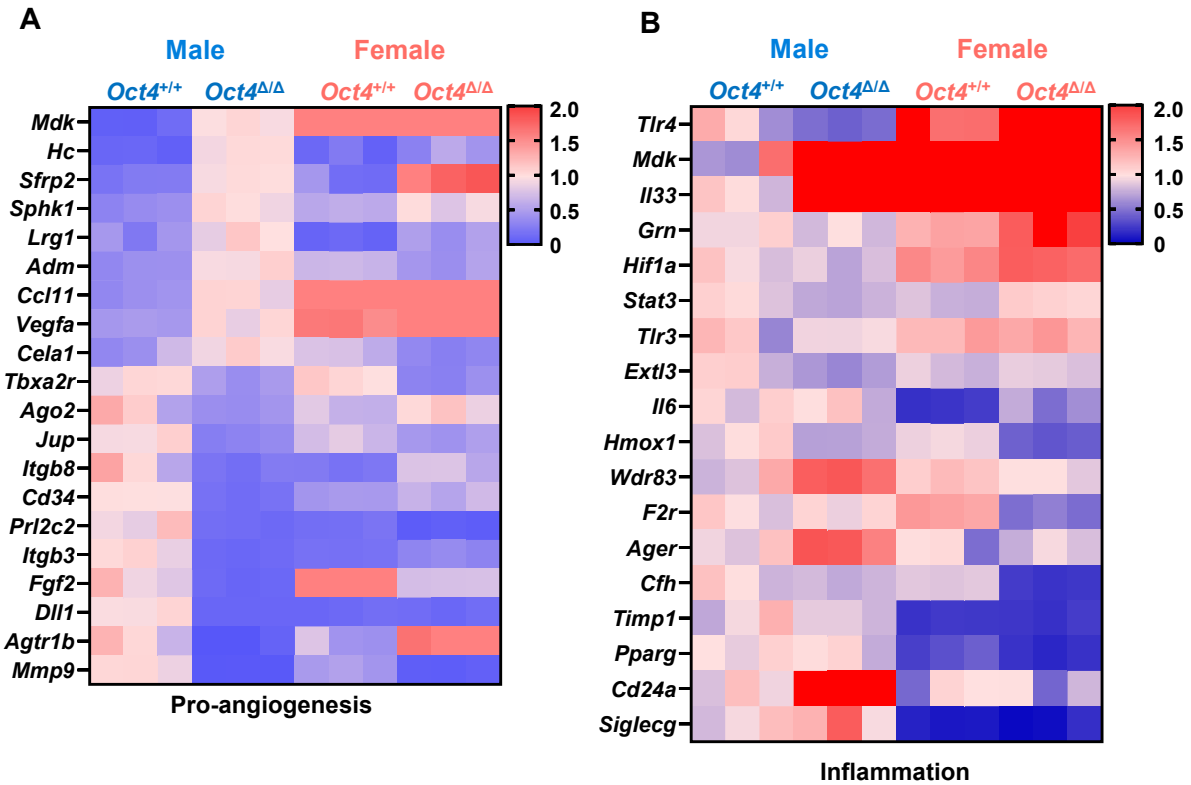
**Supplementary Figure 5. MCP1 inhibitor Bindarit partially rescues angiogenesis in OCT4 deficient male mouse aortic ECs (MAECs).** (**A** and **B**) Representative images for BrdU proliferation assay (**A**) and the quantification of $BrdU^{+}DAPI^{+}$ cells (**B**) in $Oct4^{+/+}$ and $Oct4^{\Delta/\Delta}$ male MAECs treated with Bindarit or vehicle control. (**C** and **D**) Representative images for *in vitro* scratch assay (**C**) and the quantification of the scratch area closure (**D**) in $Oct4^{+/+}$ and $Oct4^{\Delta/\Delta}$ male MAECs treated with Bindarit or vehicle control. (**E** and **F**) Representative images of Matrigel-based tube formation (**E**) and the quantification of tubes and nodes (**F**) in male $Oct4^{+/+}$ versus $Oct4^{\Delta/\Delta}$ MAECs treated with Bindarit or vehicle control. Data were analyzed via Two-way ANOVA with Tukey's post hoc test. Values are presented as mean ± s.e.m.

*Supplementary Figure 6*




**Supplementary Figure 6. BrdU proliferation assay in *Oct4*$^{+/+}$ versus *Oct4*$^{\Delta/\Delta}$ female MAECs.** Representative images for BrdU proliferation assay in *Oct4*$^{+/+}$ versus *Oct4*$^{\Delta/\Delta}$ cultured female MAECs treated with abMCP-1 or IgG control. Scale bar = 100 μm.

*Supplementary Figure 7*




**Supplementary Figure 7. Bulk-RNA sequencing and gene set enrichment analysis (GSEA) of *Oct4*$^{+/+}$ versus *Oct4*$^{\Delta/\Delta}$ male and female MAECs.** Bulk RNAseq has been performed on mRNA from *Oct4*$^{+/+}$ and *Oct4*$^{\Delta/\Delta}$ male and female MAECs. (**A** and **B**) The heat map showing the differential gene expression related to pro-angiogenesis (**A**) and inflammation (**B**).