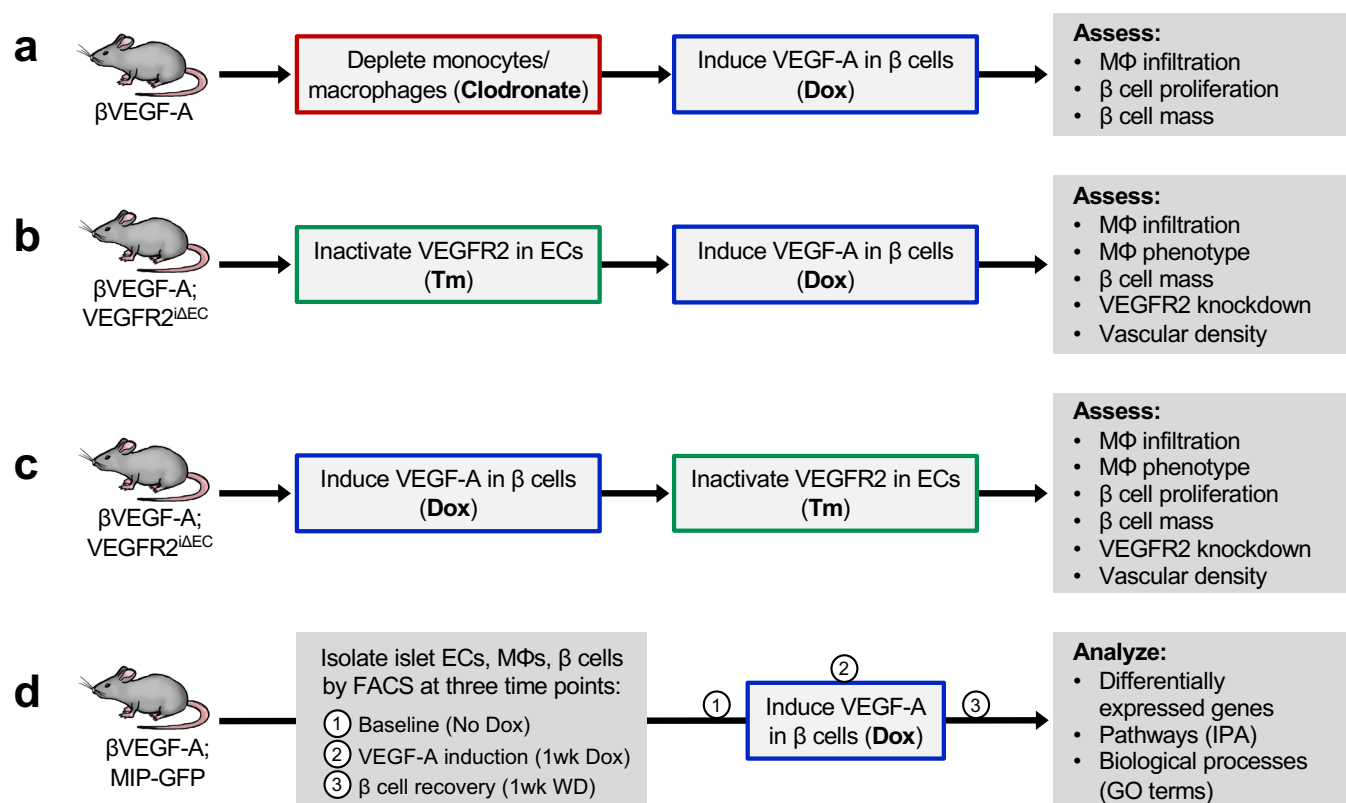
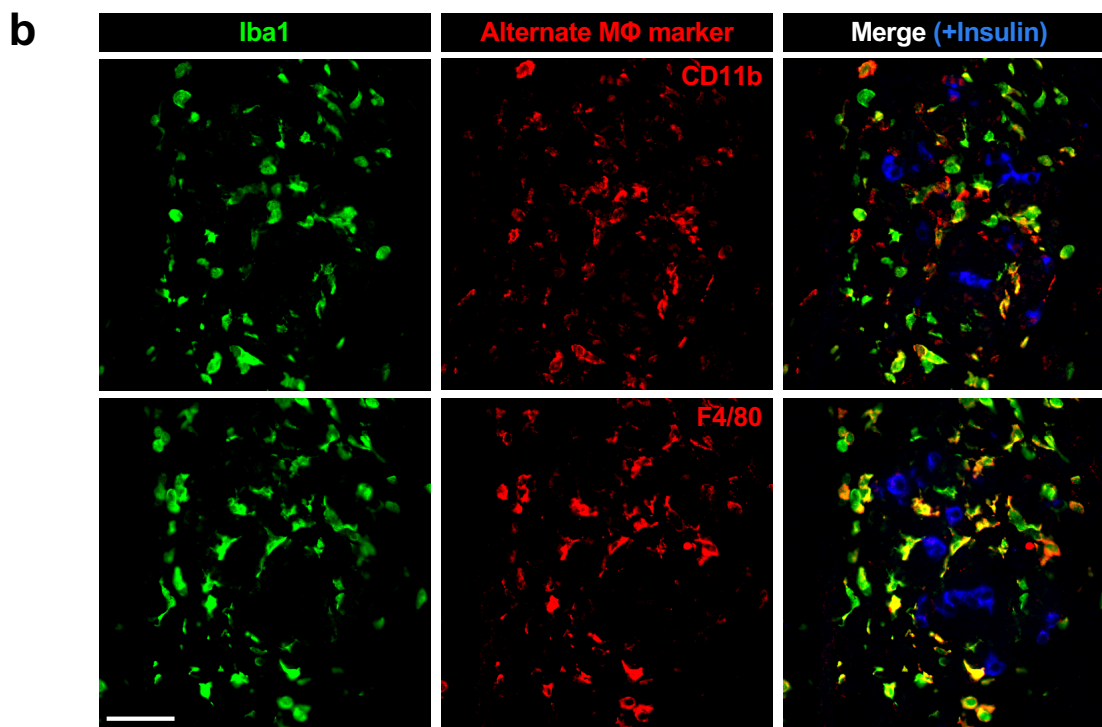
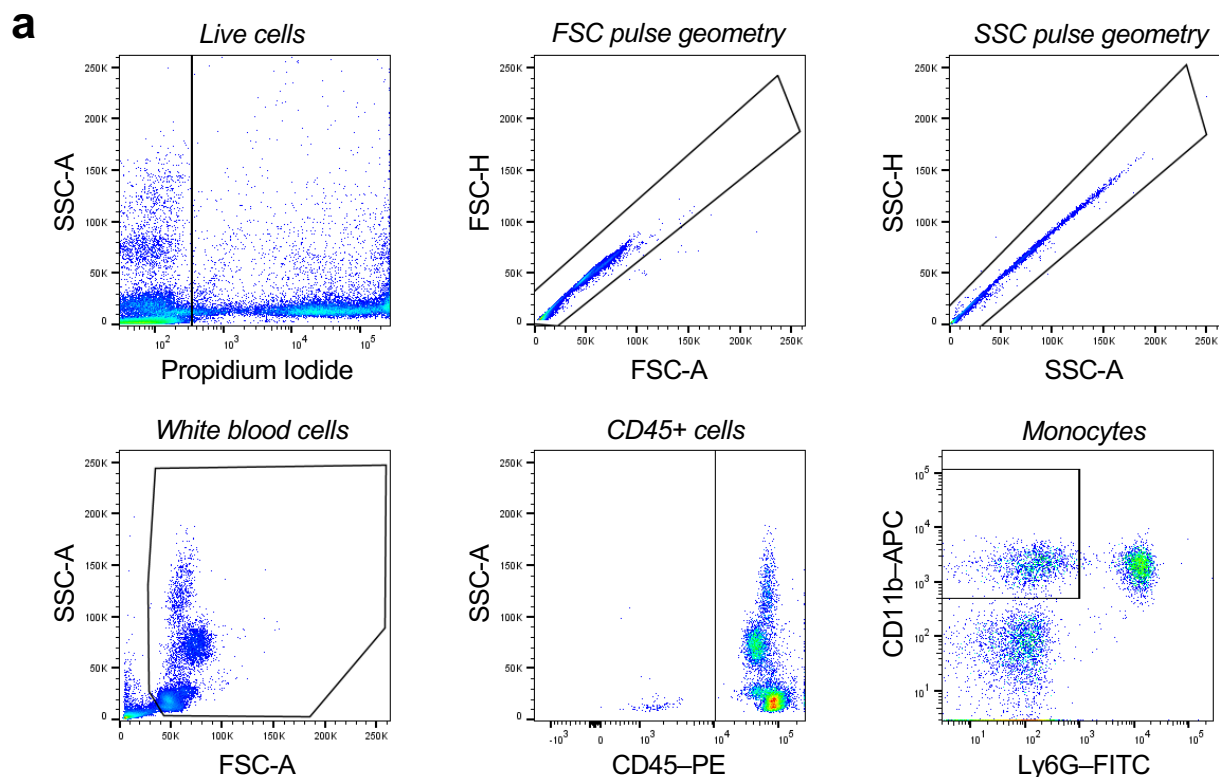


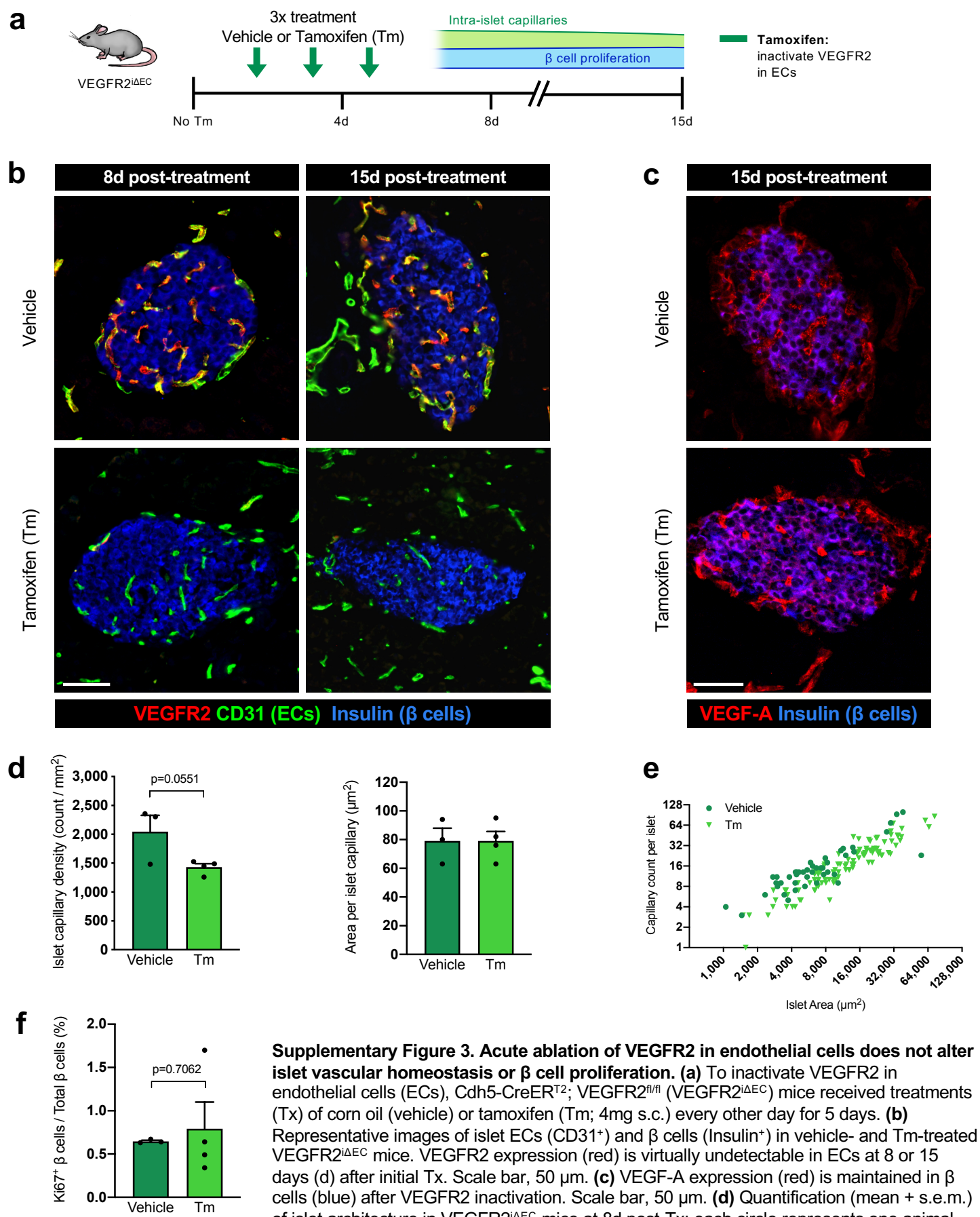
## SUPPLEMENTARY INFORMATION



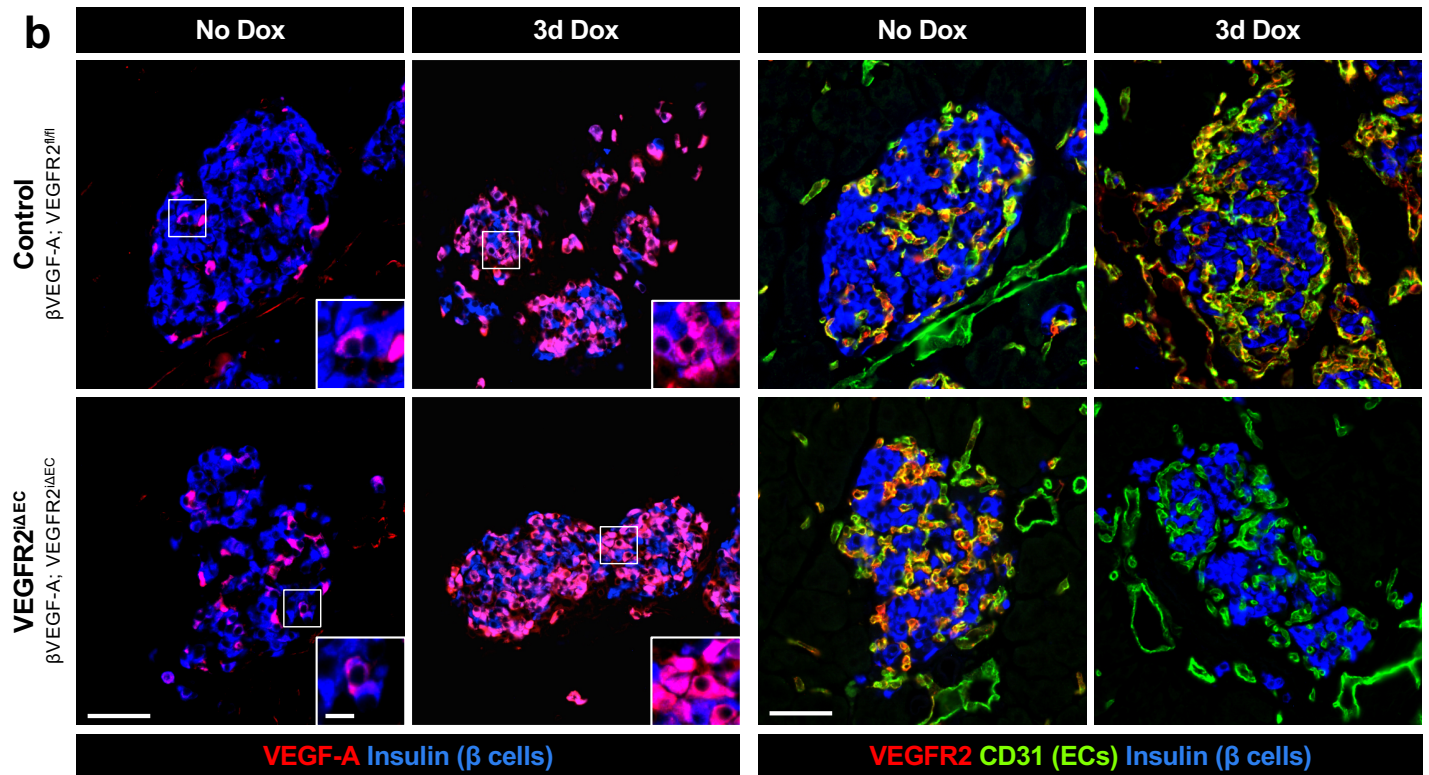
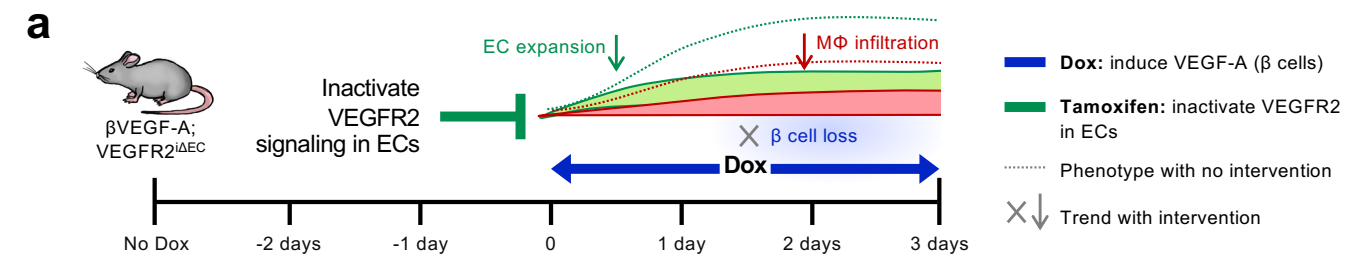
**Supplementary Figure 1. Schematic representation of experiments to determine the role of macrophages and endothelial cells in the  $\beta$ VEGF-A mouse model.** (a) Clodronate liposomes were used to suppress macrophage (M $\Phi$ ) infiltration, followed by administration of doxycycline (Dox) to overexpress VEGF-A in  $\beta$  cells. (b–c) To modulate endothelial cells (ECs), an inducible EC-specific VEGFR2 knockout mouse model Cd5-CreER; VEGFR2<sup>fl/fl</sup> (VEGFR2<sup>ΔEC</sup>)<sup>26</sup> was bred into the  $\beta$ VEGF-A line. Tamoxifen (Tm) was used to inactivate VEGFR2 in ECs either prior to (b) or after (c) VEGF-A induction to discern the role of proliferative and quiescent ECs, respectively in the  $\beta$  cell loss and recovery. (d) ECs, M $\Phi$ s, and  $\beta$  cells were isolated from  $\beta$ VEGF-A mice at three time points; introduction of the MIP-GFP transgene facilitated  $\beta$  cell sorting. For additional details, see **Methods**.



**Supplementary Figure 2. Experimental design for clodronate-mediated macrophage depletion. (a)** Gating strategy for flow cytometry analysis of circulating monocytes. Peripheral blood (50-100  $\mu$ l) was collected from the retro-orbital sinus of  $\beta$ VEGF-A mice to assess monocyte depletion following clodronate treatment. Cell debris were excluded by forward scatter (FSC) and side scatter (SSC), single cells were identified by voltage pulse geometry, and non-viable cells were excluded using propidium iodide. From remaining white blood cells, CD45 was used as a pan-leukocyte marker and monocytes were identified by a CD11b<sup>+</sup> Ly6G<sup>-</sup> profile. Approximately 10,000 white blood cells were analyzed and quantified per each animal. See also **Figure 2b**. **(b)** Representative images showing islet macrophage (M $\Phi$ ) infiltration during VEGF-A induction (1 wk Dox). These cells express Iba1 (green), which colocalizes with canonical M $\Phi$  markers CD11b and F4/80 (red). Scale bar, 50  $\mu$ m. See also **Figure 2c**.

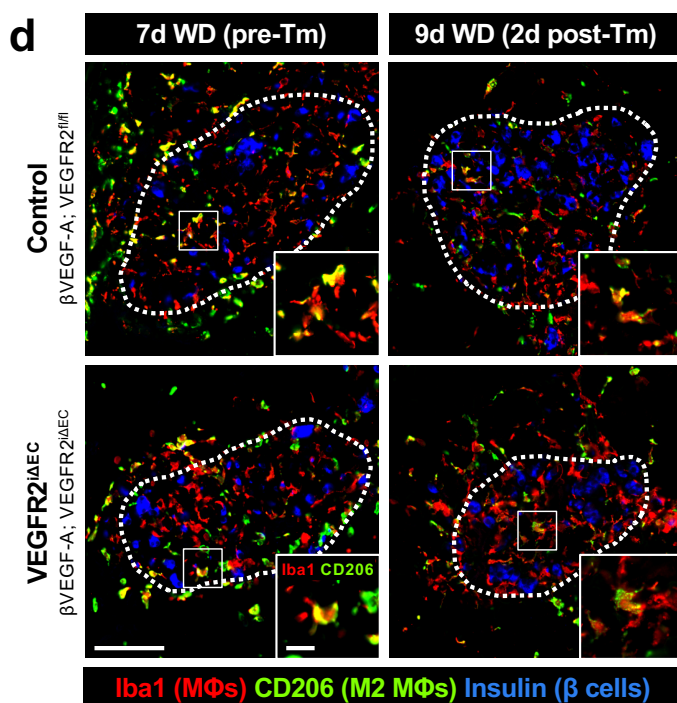
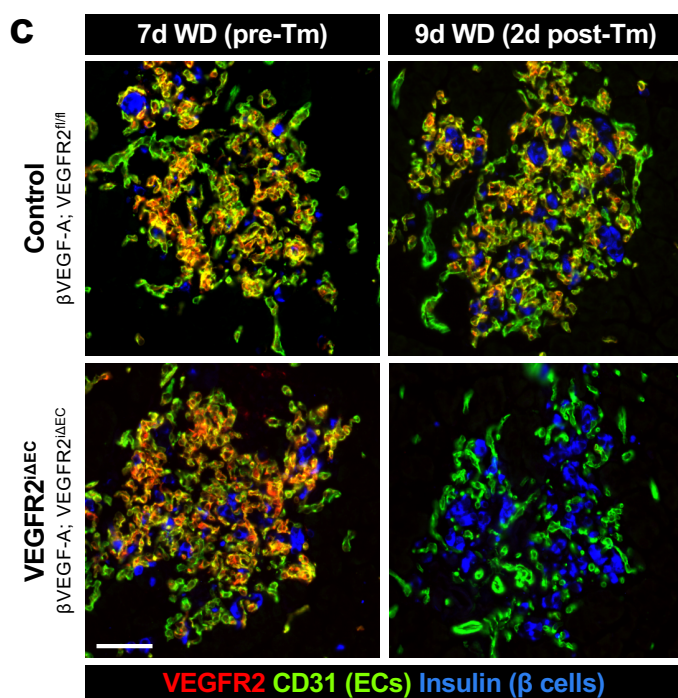
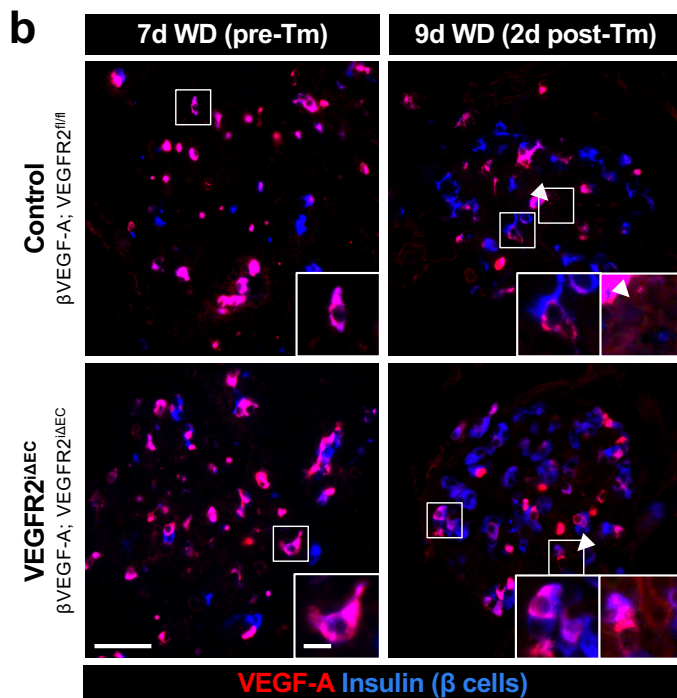
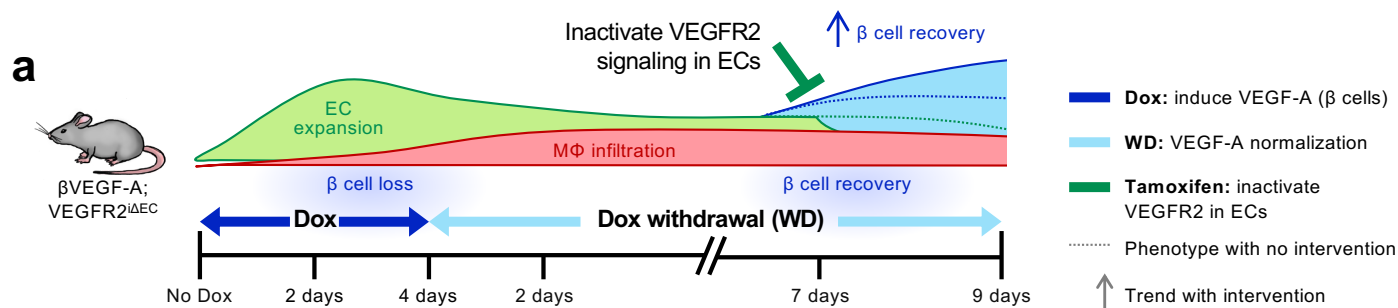


**Supplementary Figure 3. Acute ablation of VEGFR2 in endothelial cells does not alter islet vascular homeostasis or  $\beta$  cell proliferation.** (a) To inactivate VEGFR2 in endothelial cells (ECs),  $Cdh5-CreER^{T2}; VEGFR2^{fl/fl}$  ( $VEGFR2^{\Delta EC}$ ) mice received treatments (Tx) of corn oil (vehicle) or tamoxifen (Tm; 4mg s.c.) every other day for 5 days. (b) Representative images of islet ECs ( $CD31^{+}$ ) and  $\beta$  cells ( $Insulin^{+}$ ) in vehicle- and Tm-treated  $VEGFR2^{\Delta EC}$  mice. VEGFR2 expression (red) is virtually undetectable in ECs at 8 or 15 days (d) after initial Tx. Scale bar, 50  $\mu m$ . (c) VEGF-A expression (red) is maintained in  $\beta$  cells (blue) after VEGFR2 inactivation. Scale bar, 50  $\mu m$ . (d) Quantification (mean  $\pm$  s.e.m.) of islet architecture in  $VEGFR2^{\Delta EC}$  mice at 8d post-Tx; each circle represents one animal. Islet capillary density and area per islet capillary determined by  $CD31^{+}$  and  $Insulin^{+}$  stain;  $138 \pm 15 \times 10^5 \mu m^2$  total islet area analyzed per animal. (e) In both vehicle- and Tm-treated mice, the number of islet capillaries increases proportionately with islet size. Note both axes have a base 2 logarithmic scale. (f) Basal  $\beta$  cell proliferation rate;  $1,892 \pm 259$  cells counted per animal. Statistics shown in panels d and f reflect unpaired two-tailed t-tests.

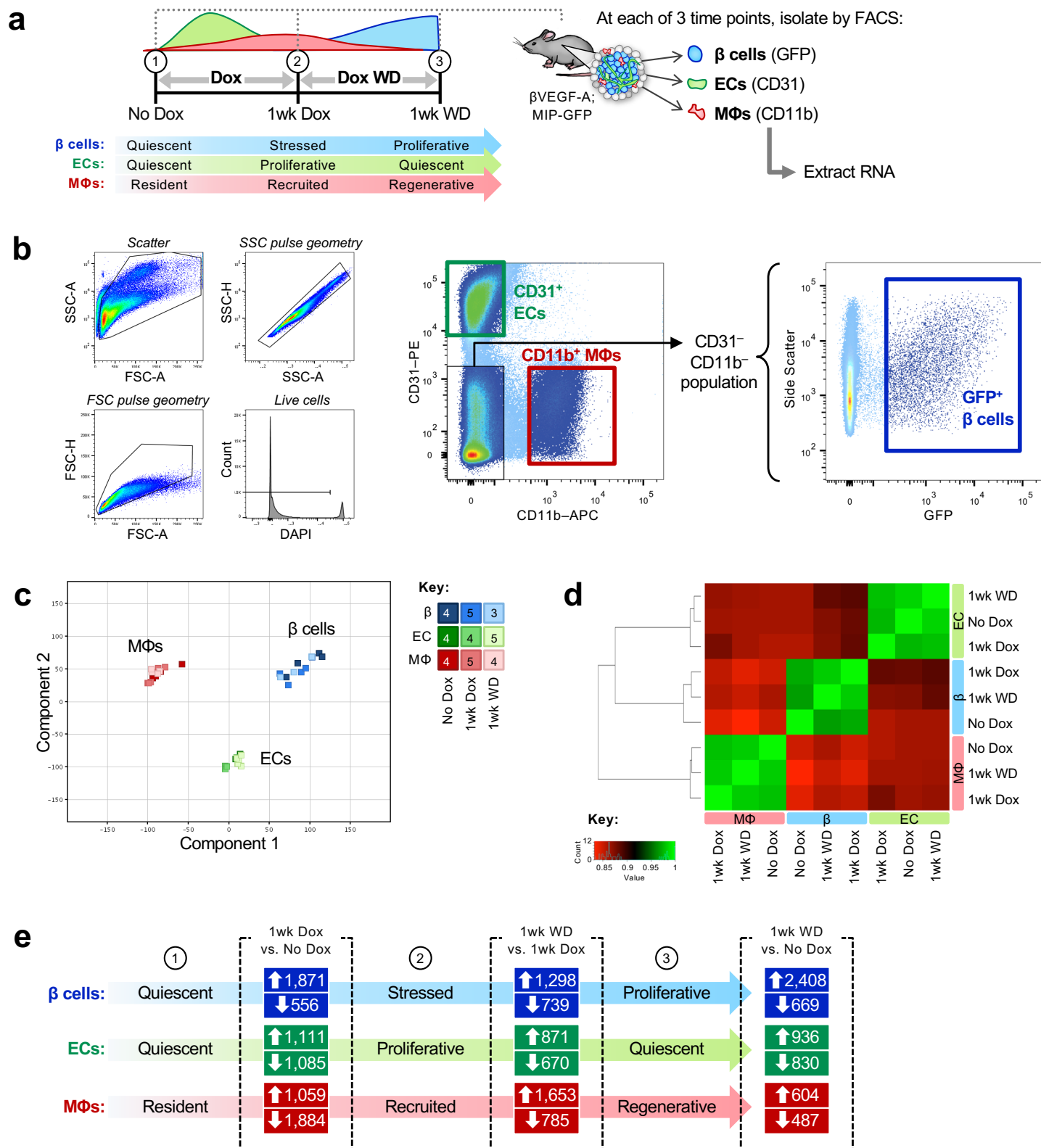


**Supplementary Figure 4. Effective VEGFR2 inactivation in endothelial cells prevents islet capillary expansion by acute elevation of VEGF-A in the islet microenvironment. (a)** To inactivate VEGFR2 in endothelial cells (ECs), βVEGF-A; R2<sup>ΔEC</sup> mice and βVEGF-A; R2<sup>fl/fl</sup> controls were treated with Tamoxifen (Tm; 4mg s.c.) prior to VEGF-A induction. **(b)** Left panel: induction of VEGFA expression at 3d Dox compared to baseline (No Dox) in βVEGF-A; R2<sup>ΔEC</sup> mice and controls; VEGF-A (red); β cells (Insulin<sup>+</sup>, blue). Boxes indicate regions enlarged in insets. Right panel: inactivation of VEGFR2 expression in ECs at baseline and 3d Dox; VEGFR2 (red), ECs (CD31<sup>+</sup>, green); β cells (Insulin<sup>+</sup>, blue). Scale bars, 50 μm; inset, 10 μm.

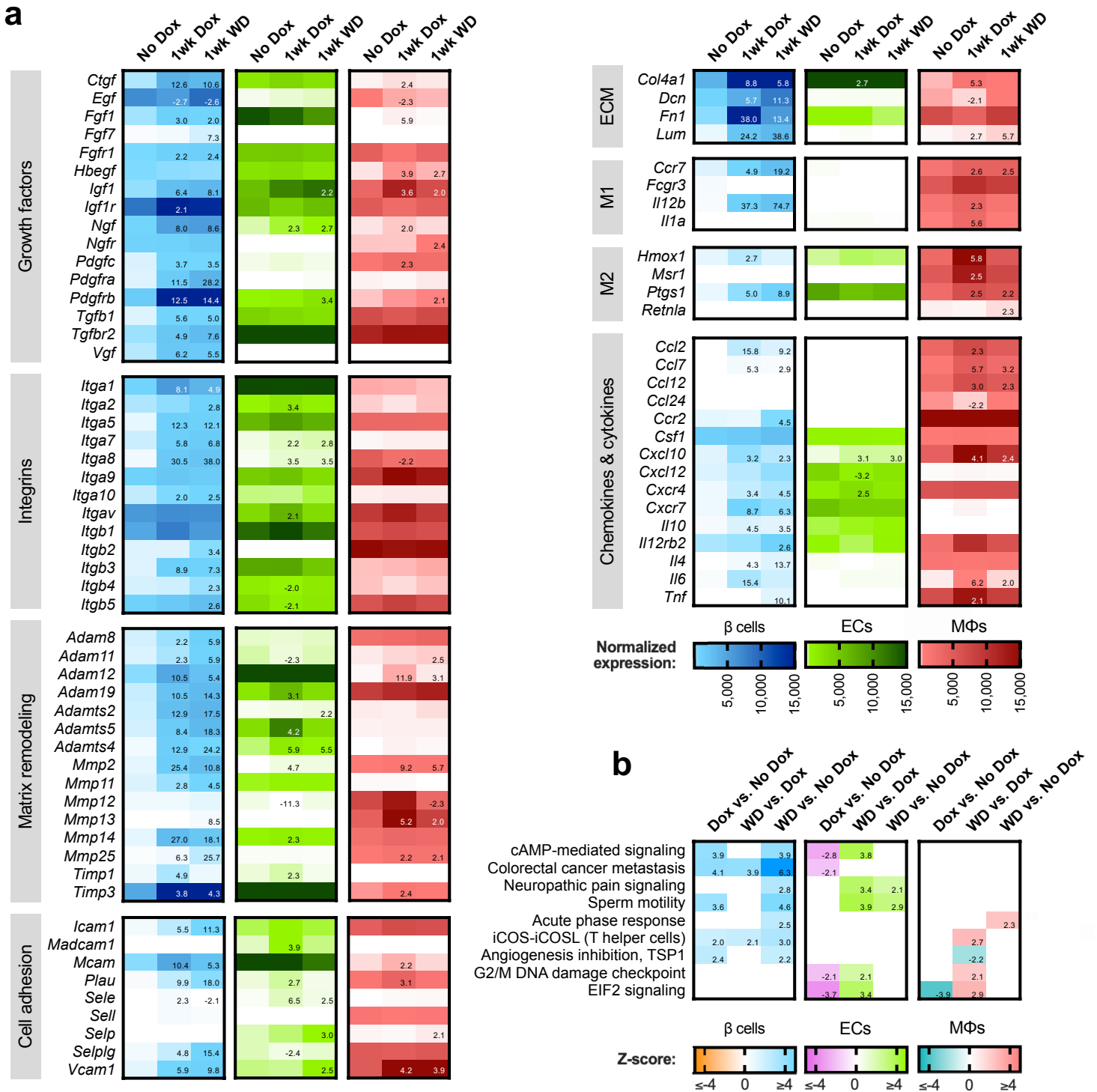




**Supplemental Figure 5. Effects of VEGF-A induction and VEGFR2 inactivation during β cell recovery.** (a) To inactivate VEGFR2 in endothelial cells (ECs) during β cell recovery, control (βVEGF-A; VEGFR2<sup>fl/fl</sup>) and VEGFR2<sup>ΔEC</sup> (βVEGF-A; VEGFR2<sup>ΔEC</sup>) mice received Tamoxifen (Tm; 4mg s.c.) after 7 days (d) of Dox withdrawal (WD). (b–c) Expression of VEGF-A in β cells (b) and VEGFR2 in ECs (c) at 7d WD and 9d WD. Boxes show regions enlarged in insets; arrowheads point to extracellular VEGF-A staining. (d) Intra-islet M2-like macrophages (MΦs, CD206<sup>+</sup> Iba1<sup>+</sup>) were present in both control and VEGFR2<sup>ΔEC</sup> mice before and after Tm treatment. Approximate islet area is outlined; boxes show regions enlarged in insets with insulin channel (blue) removed. Scale bars in b–d are 50 μm; insets, 10 μm.



**Supplemental Figure 6. Transcriptome analysis demonstrates unique gene expression profiles in β cells, endothelial cells and macrophages purified from islets during the period of β cell loss and recovery.** (a–b) Dispersed islet cells from βVEGF-A; MIP-GFP mice were immunolabeled with fluorophore-conjugated anti-CD31 and anti-CD11b antibodies to separate endothelial cell (EC) and macrophage (MΦ) populations, respectively. GFP<sup>+</sup> β cells were sorted from the CD31<sup>-</sup> CD11b<sup>-</sup> population. Initial gating is shown in first four plots. (c) Principal component analysis (PCA) plot shows the clustering of samples from sorted β cells (blue), ECs (green), and MΦs (red) at No Dox, 1wk Dox, and 1wk WD; n=3-5 biological replicates per time point as listed in Key. Islets from multiple mice were pooled to obtain adequate cells for each sample. (d) Pairwise correlation between all samples at all time points based on the Spearman correlation coefficient, which ranks and quantifies the degree of similarity between each pair of samples (perfect correlation=1; green). (e) Total number of genes for each cell type significantly up- or down-regulated (FC ≥2 or ≤-2) when comparing time points (dashed outlines): 1wk Dox vs. No Dox, 1wk WD vs. 1wk Dox, and 1wk WD vs. No Dox.





**Supplemental Figure 8. Biological processes enriched in islet macrophages, endothelial cells, and β cells of βVEGF-A mice during β cell loss and recovery.** Venn diagrams showing profiles of β cells (a), endothelial cells (ECs) (b), and macrophages (MΦs) (c) as determined by Gene Ontology (GO) term analysis; n=3-5 biological replicates per cell type per time point. Processes are organized by pairwise comparisons between time points (1wk Dox vs. No Dox, 1wk WD vs. 1wk Dox, and 1wk WD vs. No Dox), with location of text in Venn diagram accounting for processes that are represented in multiple time point comparisons. (d) Unique and shared processes of β cells, ECs, and MΦs during β cell recovery (1wk WD vs. 1wk Dox and/or 1wk WD vs. No Dox). All terms diagrammed in (a–d) appeared in top 20 hits (highest statistical significance) for the indicated population(s); those omitted due to space constraints are provided in **Supplementary Table 2**.



**Supplementary Table 1: GO Terms for sorted cell populations**

| Term       | Process                                                                                   | p value: | β cells  |          |          |          |       |       | ECs   |          |          |       |       |       | Macrophages |          |          |       |          |          |
|------------|-------------------------------------------------------------------------------------------|----------|----------|----------|----------|----------|-------|-------|-------|----------|----------|-------|-------|-------|-------------|----------|----------|-------|----------|----------|
|            |                                                                                           |          | G1vG0    | G2vG0    | G2vG1    | G1vG0    | G2vG0 | G2vG1 | G1vG0 | G2vG0    | G2vG1    | G1vG0 | G2vG0 | G2vG1 | G1vG0       | G2vG0    | G2vG1    | G1vG0 | G2vG0    | G2vG1    |
| GO:0000087 | M phase of mitotic cell cycle                                                             |          |          |          | 5.31E-09 | 1.13E-06 |       |       |       |          | 2.30E-10 |       |       |       |             |          |          |       |          |          |
| GO:0000278 | Mitotic cell cycle                                                                        |          |          |          |          | 3.29E-06 |       |       |       |          | 3.37E-10 |       |       |       |             |          |          |       |          |          |
| GO:0000279 | M phase                                                                                   |          |          |          | 8.80E-11 | 3.58E-07 |       |       |       |          | 2.60E-14 |       |       |       |             |          |          |       |          |          |
| GO:0000280 | Nuclear division                                                                          |          |          |          |          | 3.28E-06 |       |       |       |          | 1.05E-09 |       |       |       |             |          |          |       |          |          |
| GO:0000902 | Cell morphogenesis                                                                        |          |          |          |          | 6.64E-05 |       |       |       |          |          |       |       |       |             | 2.70E-09 | 6.23E-05 |       |          |          |
| GO:0000904 | Cell morphogenesis involved in differentiation                                            |          |          |          |          | 1.59E-06 |       |       |       |          |          |       |       |       |             | 2.25E-08 | 3.62E-04 |       |          |          |
| GO:0001501 | Skeletal system development                                                               |          | 2.97E-08 |          |          |          |       |       |       |          |          |       |       |       |             |          |          |       |          |          |
| GO:0001503 | Ossification                                                                              |          |          |          |          |          |       |       |       | 3.53E-04 |          |       |       |       | 0.002241    |          |          |       |          |          |
| GO:0001525 | Angiogenesis                                                                              |          | 7.92E-13 |          |          |          |       |       |       |          |          |       |       |       | 6.32E-05    |          |          |       |          |          |
| GO:0001568 | Blood vessel development                                                                  |          | 3.64E-21 | 9.44E-13 |          |          |       |       |       |          |          |       |       |       | 6.42E-06    |          |          |       |          |          |
| GO:0001775 | Cell activation                                                                           |          |          | 1.79E-15 | 1.55E-12 |          |       |       |       |          |          |       |       |       |             |          |          |       |          |          |
| GO:0001817 | Regulation of cytokine production                                                         |          |          |          | 1.57E-10 |          |       |       |       |          |          |       |       |       |             |          |          |       |          |          |
| GO:0001944 | Vasculature development                                                                   |          | 3.10E-22 | 1.52E-13 |          |          |       |       |       |          |          |       |       |       |             |          |          |       | 5.74E-06 |          |
| GO:0002504 | Antigen processing and presentation of peptide or polysaccharide antigen via MHC class II |          |          |          | 5.63E-10 |          |       |       |       |          |          |       |       |       |             |          |          |       |          |          |
| GO:0002526 | Acute inflammatory response                                                               |          |          |          |          |          |       |       |       | 6.27E-05 |          |       |       |       |             |          |          |       |          |          |
| GO:0002684 | Positive regulation of immune system process                                              |          |          | 1.63E-13 | 2.60E-13 |          |       |       |       |          |          |       |       |       |             |          |          |       |          |          |
| GO:0002694 | Regulation of leukocyte activation                                                        |          |          |          | 4.06E-09 |          |       |       |       |          |          |       |       |       |             |          |          |       |          |          |
| GO:0006323 | DNA packaging                                                                             |          |          |          |          |          |       |       |       |          | 2.18E-04 |       |       |       |             |          |          |       |          |          |
| GO:0006412 | Translation                                                                               |          |          |          |          |          |       |       |       |          | 1.06E-04 |       |       |       |             |          |          |       |          |          |
| GO:0006811 | Ion transport                                                                             |          |          |          |          |          |       |       |       |          |          |       |       |       | 5.92E-04    |          |          |       |          |          |
| GO:0006812 | Cation transport                                                                          |          |          |          |          |          |       |       |       | 2.18E-04 |          |       |       |       |             |          |          |       |          |          |
| GO:0006813 | Potassium ion transport                                                                   |          |          |          |          |          |       |       |       | 1.07E-04 |          |       |       |       | 4.04E-04    | 5.69E-07 | 9.98E-07 |       |          |          |
| GO:0006836 | Neurotransmitter transport                                                                |          |          |          |          |          |       |       |       | 2.24E-04 |          |       |       |       |             |          |          |       |          | 3.99E-04 |
| GO:0006928 | Cell motion                                                                               |          |          | 1.71E-10 |          |          |       |       |       |          |          |       |       |       |             |          |          |       |          | 5.17E-04 |
| GO:0006935 | Chemotaxis                                                                                |          |          | 1.78E-11 |          |          |       |       |       |          |          |       |       |       |             |          |          |       |          | 4.89E-04 |
| GO:0006952 | Defense response                                                                          |          |          | 1.51E-14 | 3.64E-11 |          |       |       |       |          |          |       |       |       | 0.001387    |          |          |       |          |          |
| GO:0006954 | Inflammatory response                                                                     |          |          | 2.18E-15 | 1.20E-09 |          |       |       |       |          | 2.59E-04 |       |       |       | 1.18E-04    |          |          |       |          |          |
|            |                                                                                           |          |          |          |          |          |       |       |       |          |          |       |       |       | 3.41E-06    | 4.13E-09 |          |       |          |          |

**Supplementary Table 1: GO Terms for sorted cell populations**

| Term       | Process                                                                           | p value: | β cells  |          |          |          |          |       | ECs   |          |          |          |          |          | Macrophages |          |          |       |       |          |
|------------|-----------------------------------------------------------------------------------|----------|----------|----------|----------|----------|----------|-------|-------|----------|----------|----------|----------|----------|-------------|----------|----------|-------|-------|----------|
|            |                                                                                   |          | G1vG0    | G2vG0    | G2vG1    | G1vG0    | G2vG0    | G2vG1 | G1vG0 | G2vG0    | G2vG1    | G1vG0    | G2vG0    | G2vG1    | G1vG0       | G2vG0    | G2vG1    | G1vG0 | G2vG0 | G2vG1    |
| GO:0006955 | Immune response                                                                   |          | 1.35E-10 | 4.67E-22 | 6.57E-12 |          |          |       |       |          |          | 1.12E-10 |          |          |             |          |          |       |       |          |
| GO:0007017 | Microtubule-based process                                                         |          |          |          |          |          |          |       |       |          | 4.58E-06 |          |          |          |             |          |          |       |       |          |
| GO:0007018 | Microtubule-based movement                                                        |          |          |          |          | 1.42E-05 |          |       |       |          | 8.85E-06 |          |          |          |             |          |          |       |       |          |
| GO:0007049 | Cell cycle                                                                        |          |          |          | 4.50E-09 | 8.81E-07 |          |       |       |          | 4.02E-13 |          |          |          |             |          |          |       |       |          |
| GO:0007059 | Chromosome segregation                                                            |          |          |          |          |          |          |       |       |          | 1.61E-05 |          |          |          |             |          |          |       |       |          |
| GO:0007067 | Mitosis                                                                           |          |          |          |          | 3.28E-06 |          |       |       |          | 1.05E-09 |          |          |          |             |          |          |       |       |          |
| GO:0007126 | Meiosis                                                                           |          |          |          |          |          |          |       |       |          | 2.02E-04 |          |          |          |             |          |          |       |       |          |
| GO:0007155 | Cell adhesion                                                                     |          | 1.06E-28 | 4.21E-36 |          | 2.29E-06 | 6.68E-10 |       |       |          |          | 4.43E-10 | 5.82E-14 | 9.81E-11 |             |          |          |       |       |          |
| GO:0007156 | Homophilic cell adhesion                                                          |          |          |          |          |          |          |       |       |          |          |          |          |          |             |          |          |       |       | 2.75E-08 |
| GO:0007167 | Enzyme linked receptor protein signaling                                          |          |          |          |          |          | 7.97E-05 |       |       |          |          |          |          |          |             |          |          |       |       |          |
| GO:0007169 | Transmembrane receptor protein tyrosine kinase signaling pathway                  |          |          |          |          |          | 5.49E-05 |       |       |          |          |          |          |          |             |          |          |       |       |          |
| GO:0007229 | Integrin-mediated signaling pathway                                               |          |          | 2.45E-11 |          |          |          |       |       |          |          |          |          |          |             |          |          |       |       |          |
| GO:0007267 | Cell-cell signaling                                                               |          |          |          |          |          |          |       |       |          |          |          |          |          |             | 2.88E-07 |          |       |       | 1.22E-04 |
| GO:0007409 | Axonogenesis                                                                      |          |          |          |          |          |          |       |       |          |          |          |          |          |             |          |          |       |       |          |
| GO:0007411 | Axon guidance                                                                     |          |          |          |          | 1.55E-04 |          |       |       |          |          |          |          |          |             |          |          |       |       |          |
| GO:0008284 | Positive regulation of cell proliferation                                         |          | 9.84E-11 |          |          |          |          |       |       |          |          |          |          |          | 1.03E-06    |          |          |       |       |          |
| GO:0009611 | Response to wounding                                                              |          | 6.84E-09 | 8.15E-18 |          |          |          |       |       | 5.42E-04 |          |          | 2.09E-07 | 3.20E-09 |             |          |          |       |       |          |
| GO:0015672 | Monovalent inorganic cation transport                                             |          |          |          |          |          |          |       |       | 4.95E-04 |          |          | 8.12E-04 |          |             |          | 3.65E-06 |       |       |          |
| GO:0016337 | Cell-cell adhesion                                                                |          |          |          |          |          |          |       |       |          |          |          |          |          | 1.17E-06    |          | 4.44E-06 |       |       |          |
| GO:0016477 | Cell migration                                                                    |          | 1.65E-08 |          |          |          |          |       |       |          |          |          |          |          |             |          | 7.82E-04 |       |       |          |
| GO:0016485 | Protein processing                                                                |          |          |          |          |          |          |       |       | 5.36E-05 |          |          |          |          |             |          |          |       |       |          |
| GO:0019886 | Antigen processing and presentation of exogenous peptide antigen via MHC class II |          |          |          | 1.18E-08 |          |          |       |       |          |          |          |          |          |             |          |          |       |       |          |
| GO:0022402 | Cell cycle process                                                                |          |          |          |          | 1.05E-06 |          |       |       |          | 2.12E-13 |          |          |          |             |          |          |       |       |          |
| GO:0022403 | Cell cycle phase                                                                  |          |          |          | 2.53E-09 | 1.90E-07 |          |       |       |          | 5.16E-15 |          |          |          |             |          |          |       |       |          |
| GO:0022610 | Biological adhesion                                                               |          | 1.32E-28 | 5.59E-36 |          | 2.52E-06 | 7.21E-10 |       |       |          |          | 4.92E-10 | 6.46E-14 | 1.07E-10 |             |          |          |       |       |          |
| GO:0030001 | Metal ion transport                                                               |          |          |          |          |          | 2.34E-05 |       |       |          |          |          |          |          | 1.93E-08    |          | 2.25E-06 |       |       |          |

**Supplementary Table 1: GO Terms for sorted cell populations**

| Term       | Process                                               | p value: | β cells  |          |          |          |          |       | ECs      |          |          | Macrophages |          |          |
|------------|-------------------------------------------------------|----------|----------|----------|----------|----------|----------|-------|----------|----------|----------|-------------|----------|----------|
|            |                                                       |          | G1vG0    | G2vG0    | G2vG1    | G1vG0    | G2vG0    | G2vG1 | G1vG0    | G2vG0    | G2vG1    | G1vG0       | G2vG0    | G2vG1    |
| GO:0030030 | Cell projection organization                          |          |          |          |          |          |          |       |          |          |          |             | 9.33E-07 |          |
| GO:0030155 | Regulation of cell adhesion                           |          | 5.67E-10 |          |          |          |          |       |          |          |          |             |          |          |
| GO:0030182 | Neuron differentiation                                |          |          |          |          |          |          |       |          |          |          |             | 3.32E-08 | 9.99E-04 |
| GO:0030198 | Extracellular matrix organization                     |          | 1.11E-13 | 1.62E-11 |          |          |          |       |          | 6.79E-04 |          |             |          |          |
| GO:0030218 | Erythrocyte differentiation                           |          |          |          |          |          |          |       |          |          | 9.14E-04 |             |          |          |
| GO:0031175 | Neuron projection development                         |          |          |          |          |          |          |       |          |          |          |             | 1.48E-07 |          |
| GO:0032940 | Secretion by cell                                     |          |          |          |          |          |          |       |          | 2.48E-04 |          |             |          | 8.57E-04 |
| GO:0032989 | Cellular component morphogenesis                      |          |          |          |          |          |          |       | 1.22E-04 |          |          |             | 3.34E-08 | 6.10E-04 |
| GO:0032990 | Cell part morphogenesis                               |          |          |          |          |          |          |       |          |          |          |             | 2.09E-07 |          |
| GO:0034101 | Erythrocyte homeostasis                               |          |          |          |          |          |          |       |          |          | 5.06E-04 |             |          |          |
| GO:0035295 | Tube development                                      |          | 7.39E-09 |          |          |          |          |       |          |          |          |             |          |          |
| GO:0042127 | Regulation of cell proliferation                      |          | 1.27E-14 | 7.62E-13 |          |          |          |       |          |          |          |             | 8.49E-07 |          |
| GO:0042330 | Taxis                                                 |          |          |          | 1.78E-11 |          |          |       |          |          |          | 0.001387    |          |          |
| GO:0043062 | Extracellular structure organization                  |          | 2.74E-10 | 5.05E-11 |          | 1.18E-04 |          |       |          |          |          | 0.001091    | 1.38E-06 |          |
| GO:0044057 | Regulation of system process                          |          |          |          |          |          | 7.29E-04 |       |          |          |          |             |          |          |
| GO:0045321 | Leukocyte activation                                  |          |          | 4.48E-15 | 3.83E-12 |          |          |       |          |          |          |             |          |          |
| GO:0045597 | Positive regulation of cell differentiation           |          | 2.62E-08 |          |          |          |          |       |          |          |          |             |          |          |
| GO:0045765 | Regulation of angiogenesis                            |          | 3.60E-08 |          |          |          |          |       |          |          |          |             |          |          |
| GO:0045785 | Positive regulation of cell adhesion                  |          | 1.37E-08 |          |          |          |          |       |          |          |          |             |          |          |
| GO:0046649 | Lymphocyte activation                                 |          |          | 3.34E-12 | 3.00E-10 |          |          |       |          |          |          |             |          |          |
| GO:0046903 | Secretion                                             |          |          |          |          |          |          |       |          | 7.69E-05 |          |             |          |          |
| GO:0048285 | Organelle fission                                     |          |          |          |          | 9.66E-06 |          |       |          |          | 3.91E-09 |             |          |          |
| GO:0048514 | Blood vessel morphogenesis                            |          | 7.41E-20 | 2.09E-12 |          |          |          |       |          |          |          | 4.47E-05    |          |          |
| GO:0048666 | Neuron development                                    |          |          |          |          |          |          |       |          |          |          |             | 8.58E-09 |          |
| GO:0048667 | Cell morphogenesis involved in neuron differentiation |          |          |          |          | 5.62E-05 |          |       |          |          |          |             | 1.10E-08 |          |
| GO:0048812 | Neuron projection morphogenesis                       |          |          |          |          | 2.34E-04 |          |       |          |          |          |             | 1.70E-07 |          |
| GO:0048858 | Cell projection morphogenesis                         |          |          |          |          |          |          |       |          |          |          |             | 5.79E-08 | 7.17E-04 |

**Supplementary Table 1: GO Terms for sorted cell populations**

| Term       | Process                                      | p value: | β cells  |       |          | ECs      |          |          | Macrophages |       |       |
|------------|----------------------------------------------|----------|----------|-------|----------|----------|----------|----------|-------------|-------|-------|
|            |                                              |          | G1vG0    | G2vG0 | G2vG1    | G1vG0    | G2vG0    | G2vG1    | G1vG0       | G2vG0 | G2vG1 |
| GO:0050863 | Regulation of T cell activation              |          |          |       | 6.50E-09 |          |          |          |             |       |       |
| GO:0050865 | Regulation of cell activation                |          |          |       | 1.74E-09 |          |          |          |             |       |       |
| GO:0050867 | Positive regulation of cell activation       |          |          |       | 4.69E-09 |          |          |          |             |       |       |
| GO:0050870 | Positive regulation of T cell activation     |          |          |       | 4.71E-11 |          |          |          |             |       |       |
| GO:0051094 | Positive regulation of developmental process |          | 3.48E-09 |       |          |          |          |          |             |       |       |
| GO:0051249 | Regulation of lymphocyte activation          |          |          |       | 6.63E-09 |          |          |          |             |       |       |
| GO:0051301 | Cell division                                |          |          |       |          | 5.30E-06 |          | 8.55E-10 |             |       |       |
| GO:0051321 | Meiotic cell cycle                           |          |          |       |          |          |          | 2.82E-04 |             |       |       |
| GO:0051327 | M phase of meiotic cell cycle                |          |          |       |          |          |          | 2.02E-04 |             |       |       |
| GO:0051604 | Protein maturation                           |          |          |       |          |          | 1.15E-04 |          |             |       |       |
| GO:0051605 | Protein maturation by peptide bond cleavage  |          |          |       |          |          | 4.52E-06 |          |             |       |       |
| GO:0060348 | Bone development                             |          |          |       |          |          |          |          | 0.001779    |       |       |



**Supplementary Table 2:** Mouse strains utilized in experimental models

| Abbreviation            | MGI Nomenclature or Strain Name | References                                                                                |
|-------------------------|---------------------------------|-------------------------------------------------------------------------------------------|
| Cd5-CreER               | Tg(Cdh5-cre/ERT2)#Ykub          | Okabe <i>et al.</i> , 2014 <sup>24</sup>                                                  |
| MIP-GFP                 | B6.Cg-Tg(Ins1-EGFP)1Hara/J      | Hara <i>et al.</i> , 2003 <sup>83</sup>                                                   |
| RIP-rtTA                | Tg(Ins2-rtTA)2Efr               | Milo-Landesman <i>et al.</i> , 2001 <sup>80</sup>                                         |
| TetO-VEGF               | n/a                             | Efrat <i>et al.</i> , 1995 <sup>79</sup> ; Ohno-Matsui <i>et al.</i> , 2002 <sup>81</sup> |
| VEGFR2 <sup>fl/fl</sup> | Kdr <sup>tm2Sato</sup>          | Hooper <i>et al.</i> , 2009 <sup>82</sup>                                                 |

**Supplementary Table 3:** Breeding scheme to generate  $\beta$ VEGF-A; VEGFR2 <sup>$\Delta$ EC</sup> mice

| Cross | Male breeder                                                                   | Female breeder                                        | Desired Offspring                                                                                           | Frequency |
|-------|--------------------------------------------------------------------------------|-------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|-----------|
| A1    | Cd5-CreER <sup>tg/+</sup>                                                      | VEGFR2 <sup>fl/fl</sup>                               | Cd5-CreER <sup>tg/+</sup> ; VEGFR2 <sup>fl/+</sup>                                                          | 50%       |
| A2    | Cd5-CreER <sup>tg/+</sup> ; VEGFR2 <sup>fl/+</sup>                             | VEGFR2 <sup>fl/fl</sup>                               | Cd5-CreER <sup>tg/+</sup> ; VEGFR2 <sup>fl/fl</sup>                                                         | 25%       |
| A3    | Cd5-CreER <sup>tg/+</sup> ; VEGFR2 <sup>fl/fl</sup>                            | RIP-rtTA <sup>tg/tg</sup>                             | Cd5-CreER <sup>tg/+</sup> ; VEGFR2 <sup>fl/+</sup> ; RIP-rtTA <sup>tg/+</sup>                               | 50%       |
| A4    | Cd5-CreER <sup>tg/+</sup> ; VEGFR2 <sup>fl/+</sup> ; RIP-rtTA <sup>tg/+</sup>  | VEGFR2 <sup>fl/fl</sup>                               | Cd5-CreER <sup>tg/+</sup> ; VEGFR2 <sup>fl/fl</sup> ; RIP-rtTA <sup>tg/+</sup>                              | 12.5%     |
| B1    | VEGFR2 <sup>fl/fl</sup>                                                        | TetO-VEGFA <sup>tg/tg</sup>                           | TetO-VEGFA <sup>tg/+</sup> ; VEGFR2 <sup>fl/+</sup>                                                         | 100%      |
| B2    | TetO-VEGFA <sup>tg/+</sup> ; VEGFR2 <sup>fl/+</sup>                            | TetO-VEGFA <sup>tg/tg</sup>                           | TetO-VEGFA <sup>tg/tg</sup> ; VEGFR2 <sup>fl/+</sup>                                                        | 50%       |
| B3    | TetO-VEGFA <sup>tg/+</sup> ; VEGFR2 <sup>fl/+</sup>                            | TetO-VEGFA <sup>tg/tg</sup> ; VEGFR2 <sup>fl/+</sup>  | TetO-VEGFA <sup>tg/tg</sup> ; VEGFR2 <sup>fl/fl</sup>                                                       | 25%       |
| C1    | Cd5-CreER <sup>tg/+</sup> ; VEGFR2 <sup>fl/fl</sup> ; RIP-rtTA <sup>tg/+</sup> | TetO-VEGFA <sup>tg/tg</sup> ; VEGFR2 <sup>fl/fl</sup> | TetO-VEGFA <sup>tg/+</sup> ; Cd5-CreER <sup>tg/+</sup> ; VEGFR2 <sup>fl/fl</sup> ; RIP-rtTA <sup>tg/+</sup> | 25%       |

**Supplementary Table 4:** PCR primers and conditions for genotyping

| Gentotype               | Primers                                                                                                                                                                                   | PCR Conditions                                                                                                                                        | Product(s)                                          |
|-------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------|
| Cd5-CreER               | 5'- GCG GTC TGG CAG TAA AAA CTA TC -3'<br>3'- TT CAC TGT CGT TAC GAC AAA GTG -5'                                                                                                          | 93°C 3'<br>-----<br>93°C 20"<br>60°C 20" 30 cycles<br>72°C 45"<br>-----<br>72°C 5'<br>4°C hold                                                        | 100 bp                                              |
| MIP-GFP                 | 5'- AAG TTC ATC TGC ACC ACC G -3' (GFP)<br>3'- GC GTG GTA GAA GAA GTT CCT -5' (GFP)<br><br>5'- CTA GGC CAC AGA ATT GAA AGA TCT -3' (IC)<br>3'- C CTA CTA CGA TCT TAA AGG TGG ATG -5' (IC) | 94°C 1.5'<br>-----<br>94°C 30"<br>60°C 1' 35 cycles<br>72°C 1'<br>-----<br>72°C 2'<br>4°C hold                                                        | 173 bp (GFP)<br>324 bp (IC)                         |
| RIP-rtTA                | 5'- GTG AAG TGG GTC CGC GTA CAG -3'<br>3'- CTA CGG GAA CCT TAA CTG CTC ATG -5'                                                                                                            | 92°C 2'<br>-----<br>94°C 30"<br>57°C 30" 30 cycles<br>72°C 30"<br>-----<br>72°C 10'<br>4°C hold                                                       | 400 bp                                              |
| TetO-VEGF               | 5'- TCG AGT AGG CGT GTA CGG -3'<br>3'- GCT ACG CCC CCG ACG ACG -5'                                                                                                                        | 95°C 4'<br>-----<br>95°C 1'<br>57°C 30" 29 cycles<br>72°C 1'<br>-----<br>72°C 10'<br>4°C hold                                                         | 420 bp                                              |
| VEGFR2 <sup>fl/fl</sup> | 5' - CCA CAG AAC AAC TCA GGG CTA - 3'<br>3'- AA AGG TCT CTG AAA CGA GGG -5'                                                                                                               | 94°C 2'<br>-----<br>94°C 20"<br>65°C 15" 10 cycles<br>68°C 10"<br>-----<br>94°C 15"<br>60°C 15" 28 cycles<br>72°C 10"<br>-----<br>72°C 1'<br>4°C hold | 179 bp (VEGFR2)<br>230 bp (VEGFR2 <sup>loxP</sup> ) |

*GFP, green fluorescent protein; IC, internal control*

**Supplementary Table 5:** Antibodies used for immunohistochemistry and flow cytometry

| Antigen/Conjugate    | Species    | Source                  | Catalog #   | Application | Dilution        |
|----------------------|------------|-------------------------|-------------|-------------|-----------------|
| Caveolin-1           | Rabbit     | Abcam                   | ab2910      | IHC (1°)    | 1:2000          |
| CD11b                | Rat        | Novus                   | NB600-1327  | IHC (1°)    | 1:400           |
| CD11b – APC          | Rat        | BD Pharmingen           | 561690      | FC          | 1:100,<br>1:500 |
| CD206 (Mrc1)         | Rat        | BioLegend               | 141701      | IHC (1°)    | 1:500           |
| CD31                 | Rat        | BD Pharmingen           | 553370      | IHC (1°)    | 1:100           |
| CD31 – PE            | Rat        | BD Pharmingen           | 561073      | FC          | 1:500           |
| CD45 – PE            | Rat        | BD Pharmingen           | 561087      | FC          | 1:500           |
| Col-IV               | Rabbit     | Abcam                   | ab6586      | IHC (1°)    | 1:1000          |
| F4/80                | Rat        | ThermoFisher Scientific | 14-4801-82  | IHC (1°)    | 1:200           |
| Goat IgG – Cy3       | Donkey     | Jackson Immunoresearch  | 705-165-147 | IHC (2°)    | 1:500           |
| Guinea pig IgG – Cy2 | Donkey     | Jackson Immunoresearch  | 706-225-148 | IHC (2°)    | 1:500           |
| Guinea pig IgG – Cy5 | Donkey     | Jackson Immunoresearch  | 706-175-148 | IHC (2°)    | 1:200           |
| Iba1                 | Rabbit     | Wako                    | 019-19741   | IHC (1°)    | 1:500           |
| Iba1                 | Rabbit     | Abcam                   | ab221790    | IHC (1°)    | 1:1000          |
| Insulin              | Guinea pig | Dako                    | A0564       | IHC (1°)    | 1:1000          |
| Insulin              | Guinea pig | Cell Marque             | 273A-15     | IHC (1°)    | 1:500           |
| Ki67                 | Rabbit     | Abcam                   | ab15580     | IHC (1°)    | 1:5000          |
| Ly6G-FITC            | Rat        | BD Pharmingen           | 551460      | FC          | 1:500           |
| pERK                 | Rabbit     | Cell Signaling          | 4370        | IHC (1°)    | 1:200           |
| Rabbit IgG – Cy2     | Donkey     | Jackson Immunoresearch  | 711-225-152 | IHC (2°)    | 1:500           |
| Rabbit IgG – Cy3     | Donkey     | Jackson Immunoresearch  | 711-165-152 | IHC (2°)    | 1:500           |
| Rat IgG – Cy2        | Donkey     | Jackson Immunoresearch  | 712-225-153 | IHC (2°)    | 1:500           |
| VEGF-A               | Goat       | R&D Systems             | AF564       | IHC (1°)    | 1:200           |
| VEGFR2               | Goat       | R&D Systems             | AF644       | IHC (1°)    | 1:2000          |

1°, primary antibody; 2°, secondary antibody; FC, flow cytometry; IHC, immunohistochemistry