Expanded View Figures

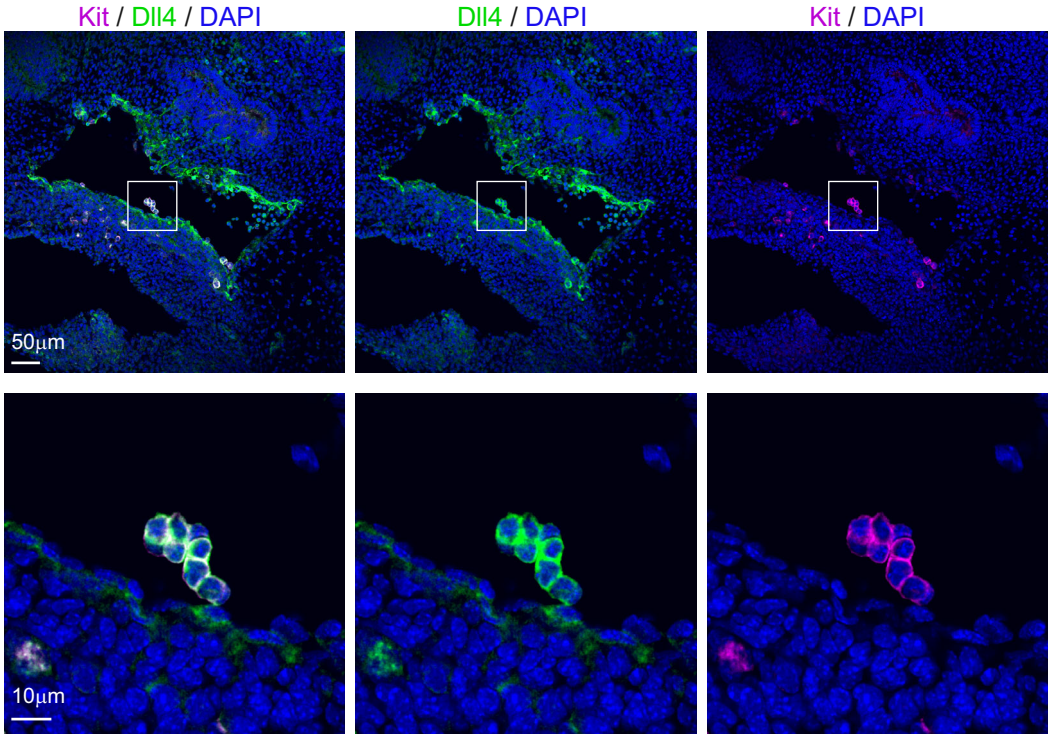

Figure EV1. The αDll4 blocking antibody recognizes both endothelial and cluster cells.
Upon intracardiac injection, the αDll4 antibody diffuses along the aorta and is detected in both endothelial cells (upper panels, scale bar: 50 μm) and IAHC (upper and lower panels, scale bar: 10 μm).

Figure EV2. Analysis of Kit^{+}CD45^{-} single cells and decrease in Notch activity after blocking Dll4.

A Heatmap from single-cell RNA-seq data to define groups using ICGS. Columns represent cells, and rows represent genes. ICGS cell clusters are indicated at the top (ColCluster), and treatment conditions are stated (Condition, αDll4 treated in cyan, IgG controls in orange). Seven cell clusters (colors used also in Fig 5G) and 10 gene guide clusters (RowCluster, left and Appendix Table S1) were identified. Selected ICGS guide genes are indicated (right).

B Integration of our dataset with scRNA-seq dataset from Zhou *et al* (2016). Differentially expressed genes for endothelial, pre-HSC, and HSC cells (type of genes, left) were used to perform hierarchical clustering of our dataset (top), and their expression is depicted in the heatmap. ICGS cluster and treatment of each cell are also shown at the top.

C Functional enrichment analysis of differentially expressed genes in the comparison of IgG- versus Dll4-treated cells.

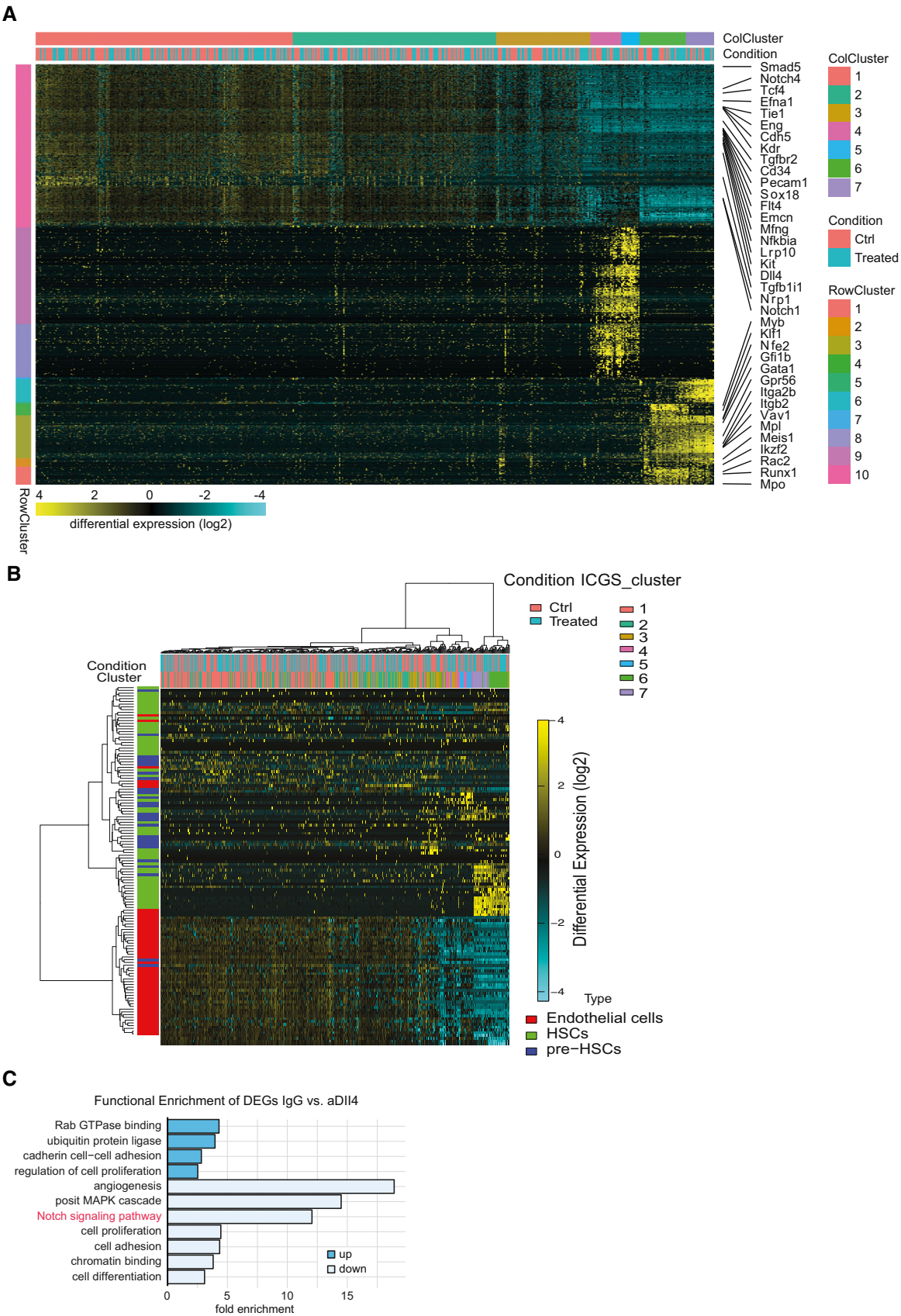

Figure EV2.

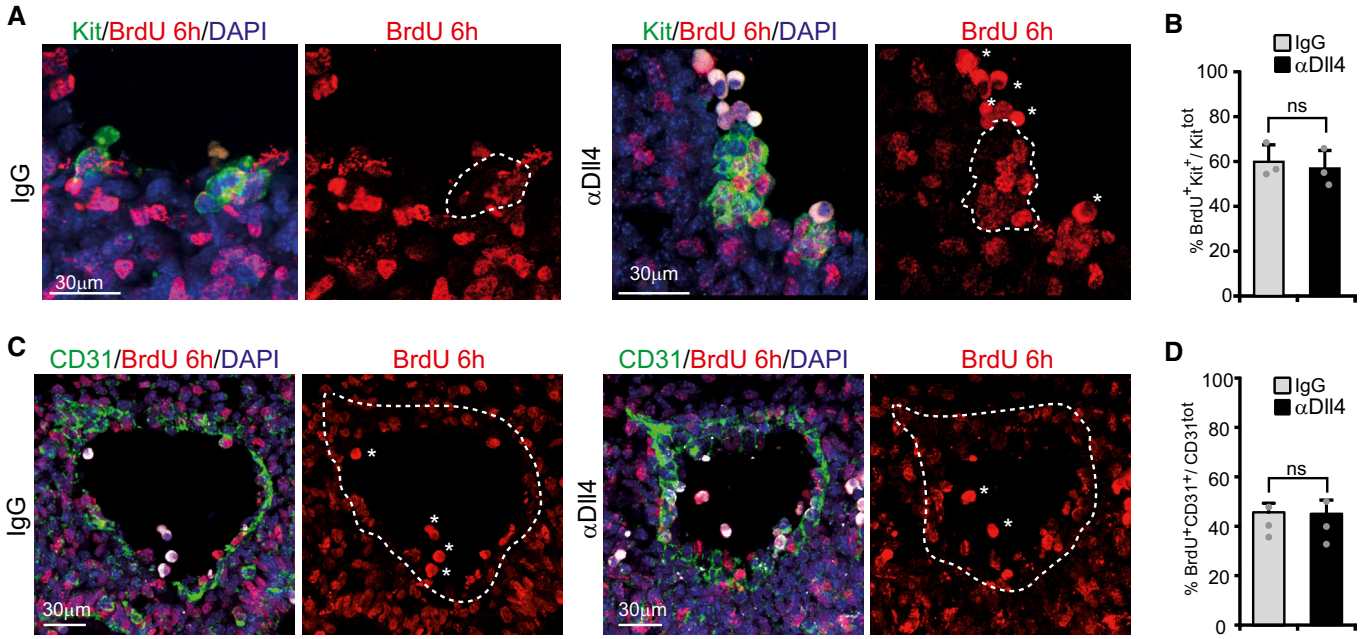

Figure EV3. Proliferation of IAHCs and endothelial cells is not changed upon αDll4 treatment.

BrdU incorporation assay upon IgG or αDll4 treatment in Kit^+ (A, B) and $CD31^+$ endothelial cells (C, D).

A, C Representative confocal images of transversal sections in the AGM region. Autofluorescent circulating cells are signed by asterisks. Scale bar: 30 μm.

B, D Bars represent the percentage of Kit^+ or $CD31^+$ cells that incorporated BrdU. Mean ± SE (n = 3).

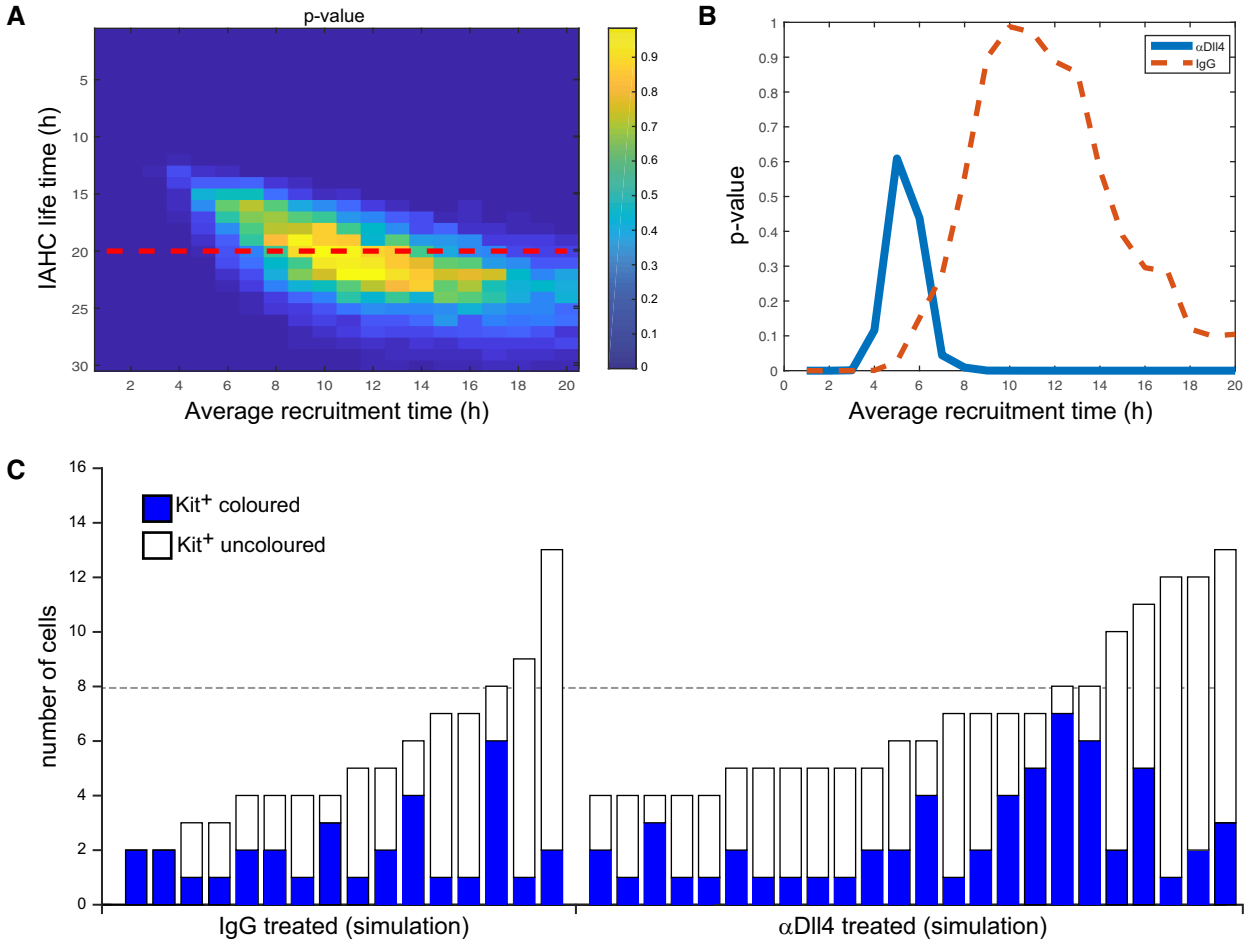

Figure EV4. Analysis using the mathematical model of the data with the two big clusters split in two.

A–C Similar analysis as done in Fig 7 to the data with the two extremely large clusters divided equally into two smaller clusters each. The colored cells were also divided equally between the new clusters.

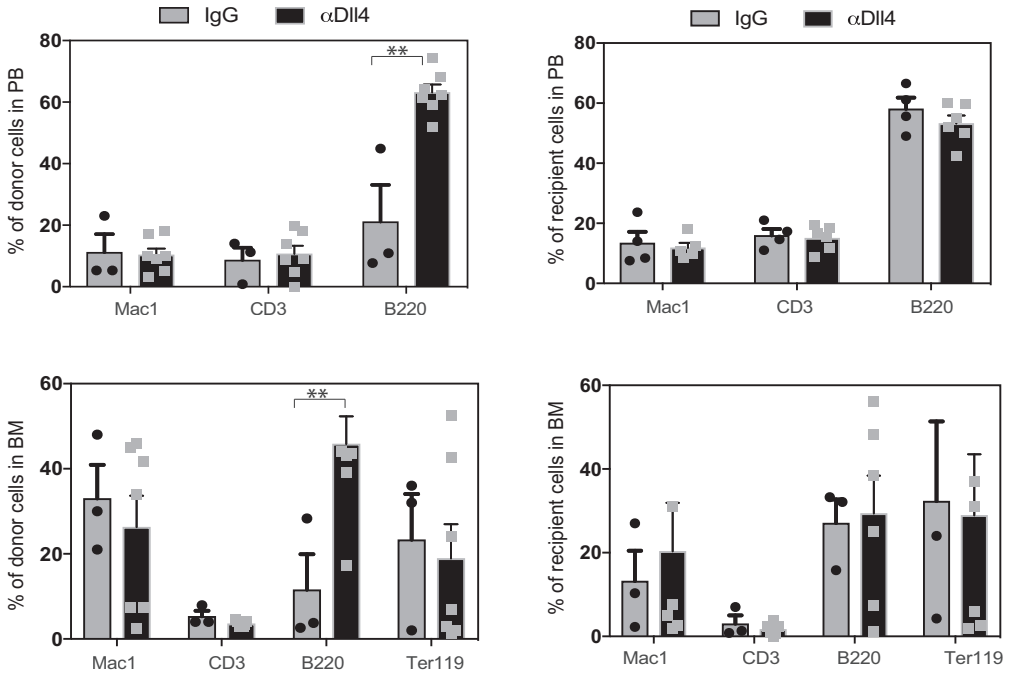

Figure EV5. Multilineage reconstitution from cells engrafted in transplantations from Fig 8F and G.
We analyzed the peripheral blood and bone marrow from transplanted irradiated mice after 4 months. CD45.1 or CD45.2 donor or recipient cells were analyzed for the contribution to the main hematopoietic lineages in the mice where engraftment was detected. Mac1 (myeloid lineage), CD3 (T lymphoid lineage), B220 (B lymphoid lineage), and Ter119 (erythroid lineage). Graphs represent the percentage of lineage cells derived from IgG-treated (n = 4) and aDll4 (n = 7)-treated AGM.