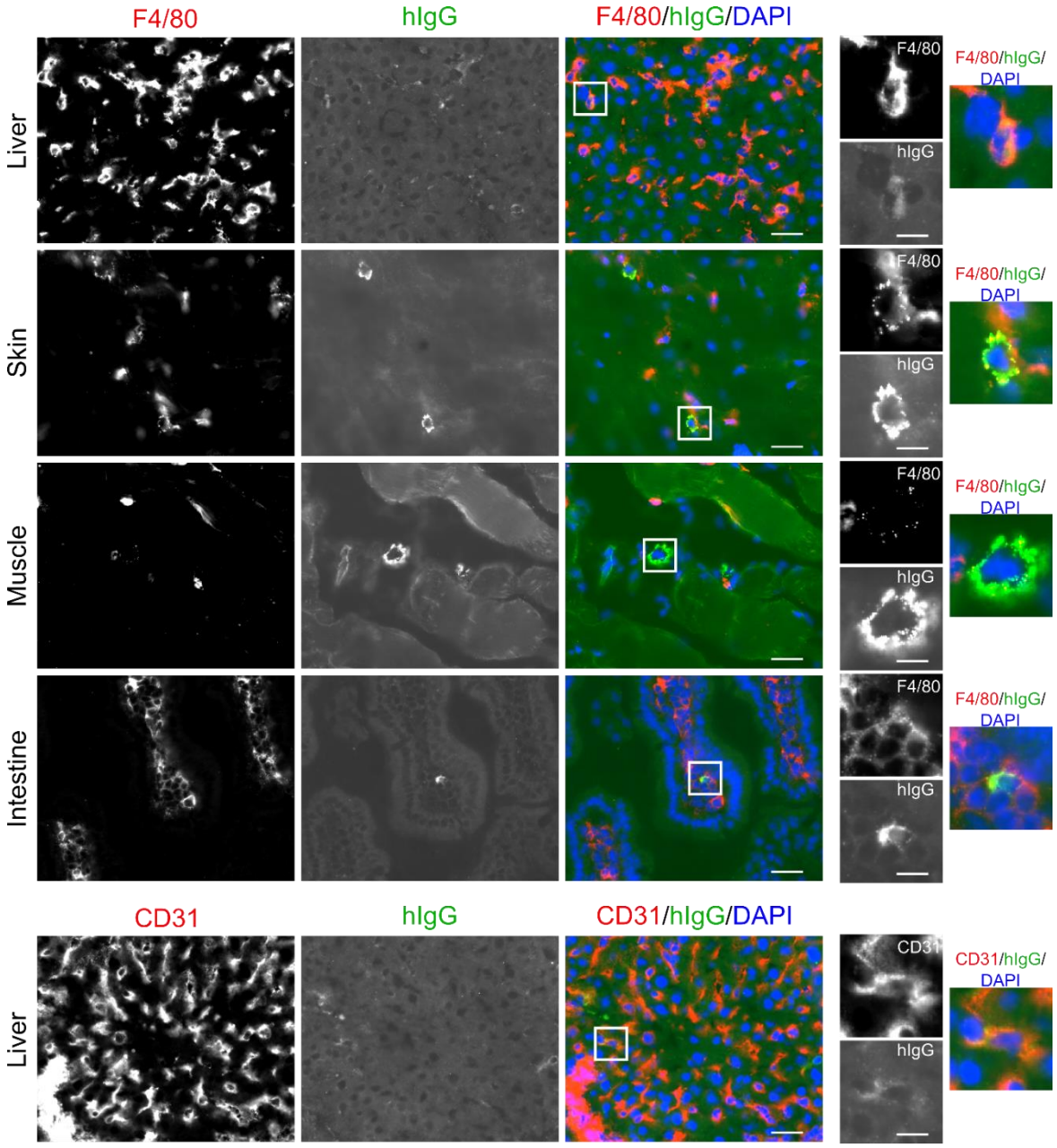
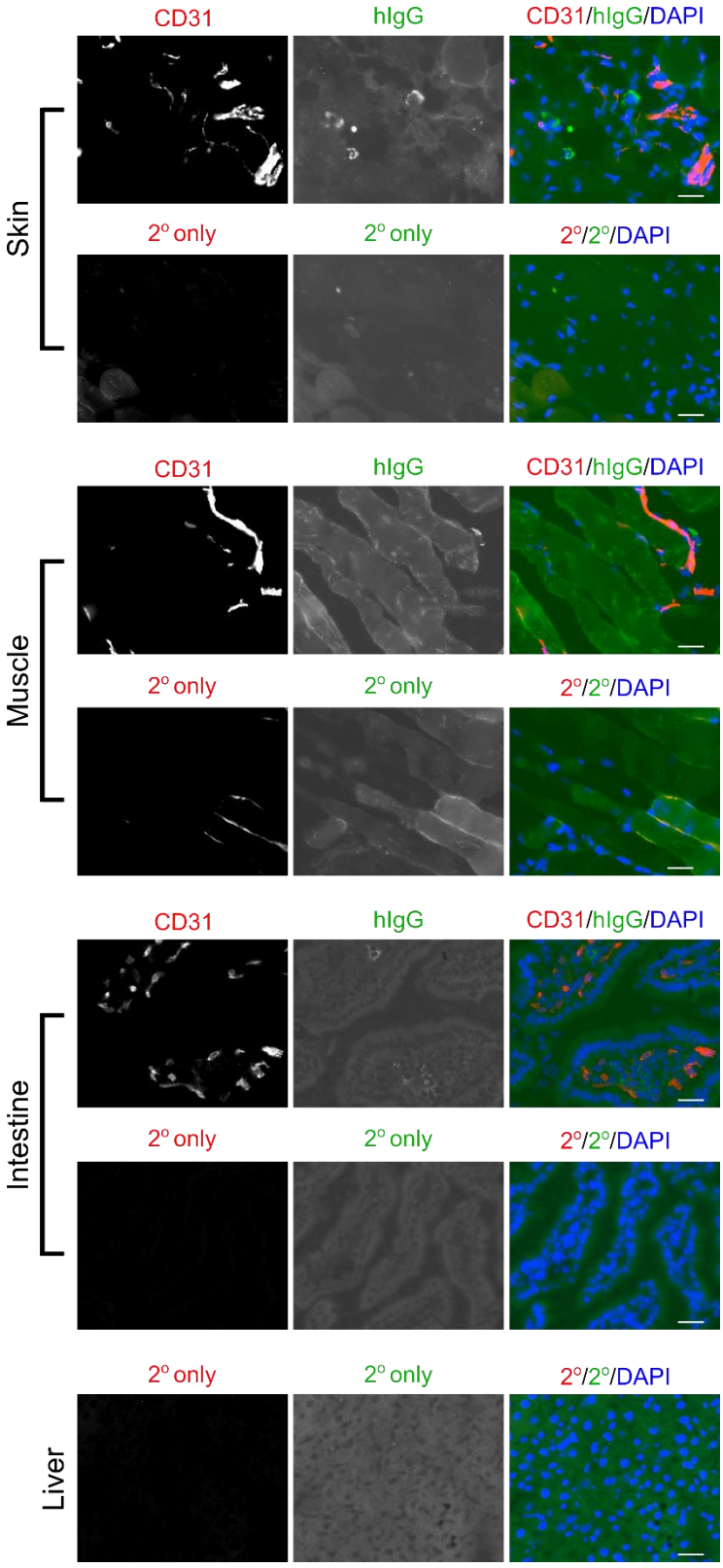
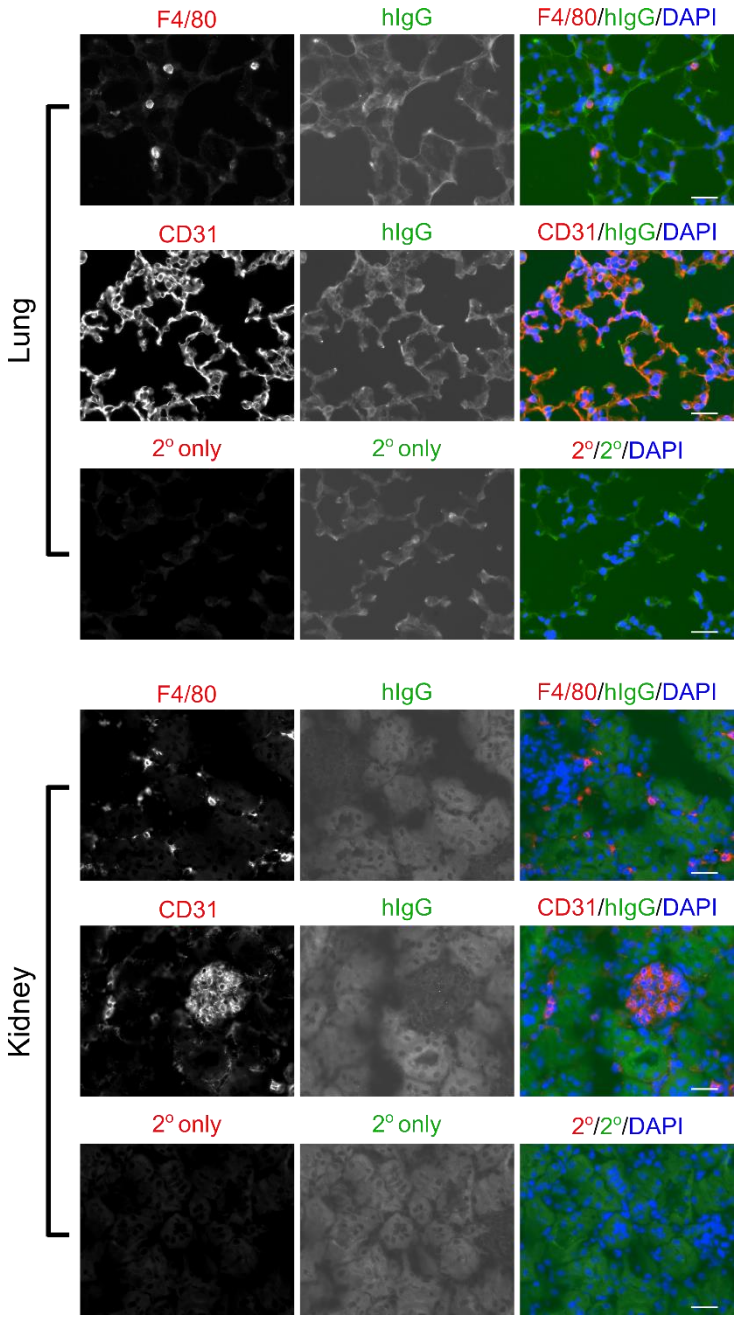
Supplementary Figures



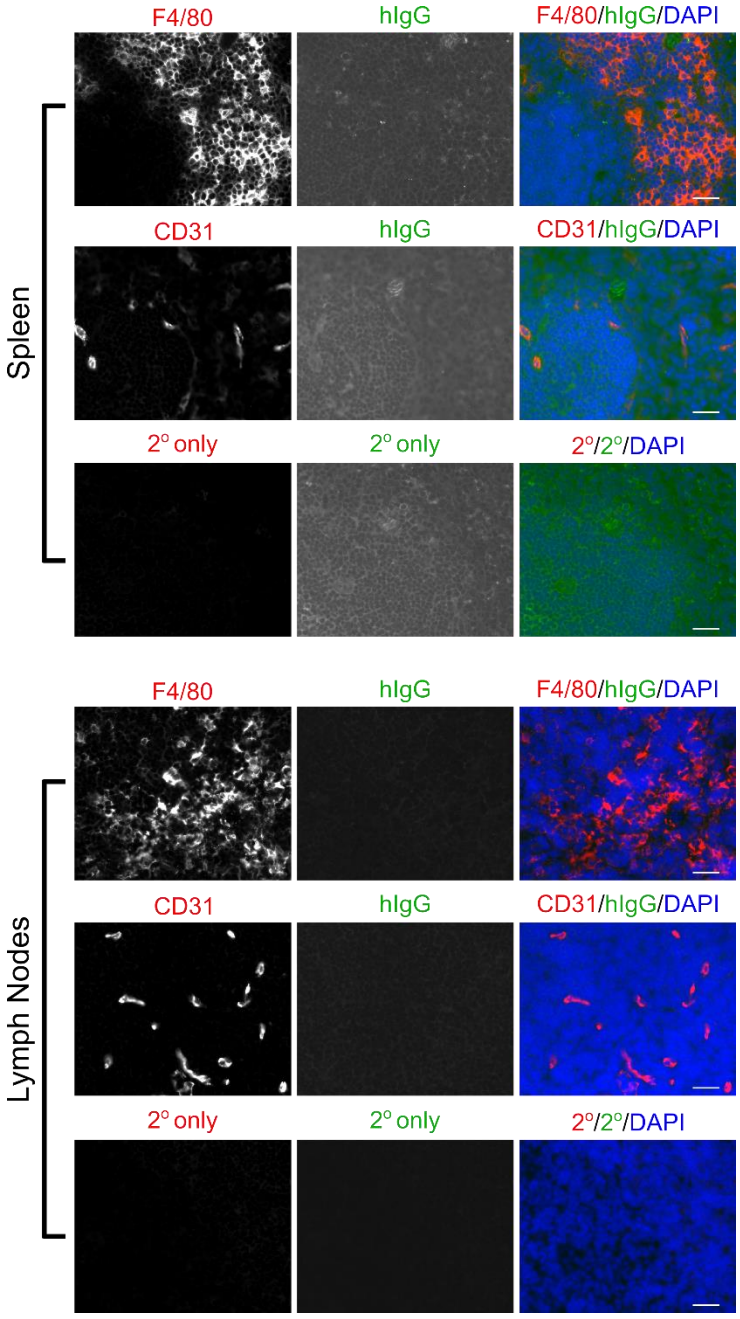
Supplementary Figure 1. Macrophages are highly pinocytic *in vivo*. G-KO mice were injected (i.v.) with 1.5 mg hIgG1^{D265A}, followed by perfusion and organ collection 10 hours later. Colocalization of the injected antibody (pseudocolored green) with F4/80^{+} macrophages or CD31^{+} endothelial cells (pseudocolored red) is shown. Representative cells (boxed) are cropped and expanded in the panels on the right hand side. Scale bars = 30 μm, and for expanded images, scale bars = 10 μm. Data shown is derived from immunohistochemical analyses of two mice from two independent experiments (≥ 51 images were acquired per tissue).


CD31
hIgG
CD31/hIgG/DAPI
Skin
2° only
2° only
2°/2°/DAPI
CD31
hIgG
CD31/hIgG/DAPI
Muscle
2° only
2° only
2°/2°/DAPI
CD31
hIgG
CD31/hIgG/DAPI
Intestine
2° only
2° only
2°/2°/DAPI
2° only
2° only
2°/2°/DAPI
Liver

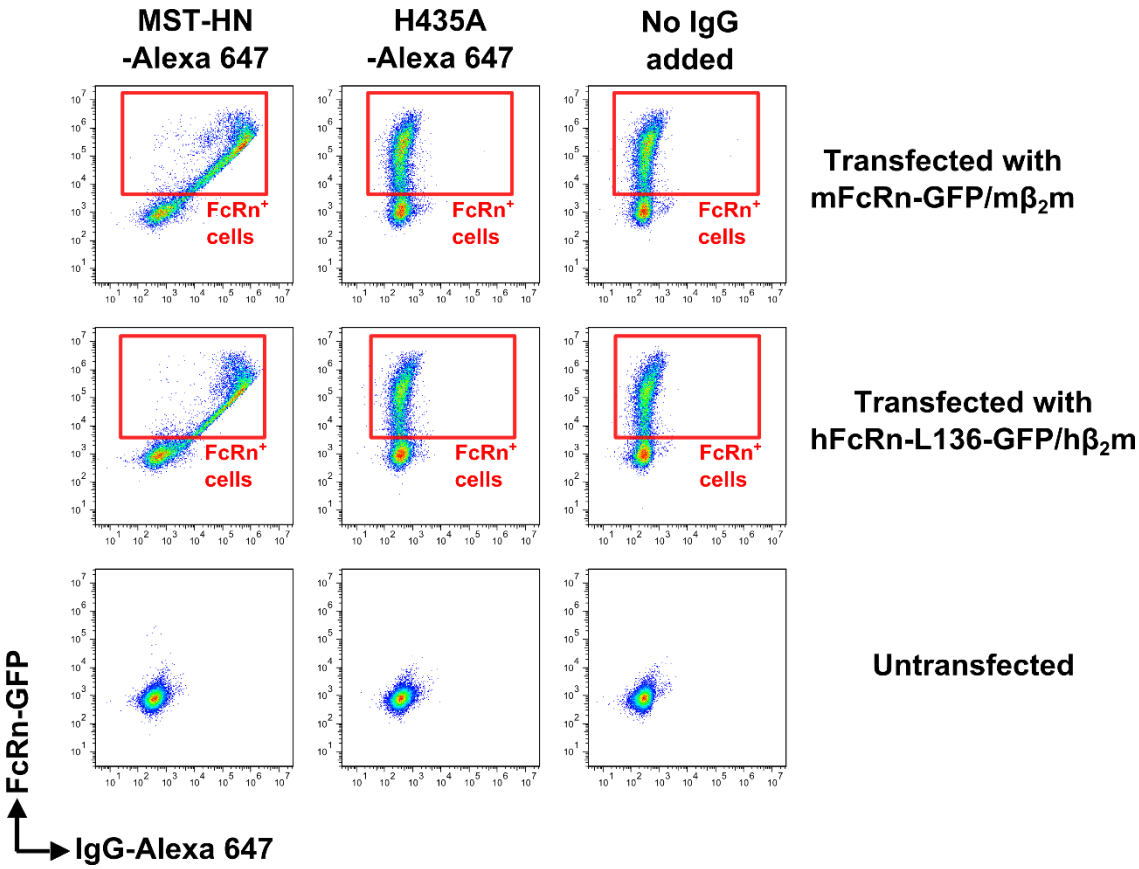
Supplementary Figure 2. Analysis of accumulation of IgG and secondary antibody staining/background autofluorescence levels in skin, muscle, intestine and liver. G-KO mice were treated as in Supplementary Figure 1. Endothelial cells ($CD31^+$; pseudocolored red), injected antibody (pseudocolored green) and secondary antibody staining/background autofluorescence levels (2^o; pseudocolored red/green) are shown. Scale bars = 30 μm. Data shown is derived from immunohistochemical analyses of two mice from two independent experiments (≥ 51 images were acquired per tissue).



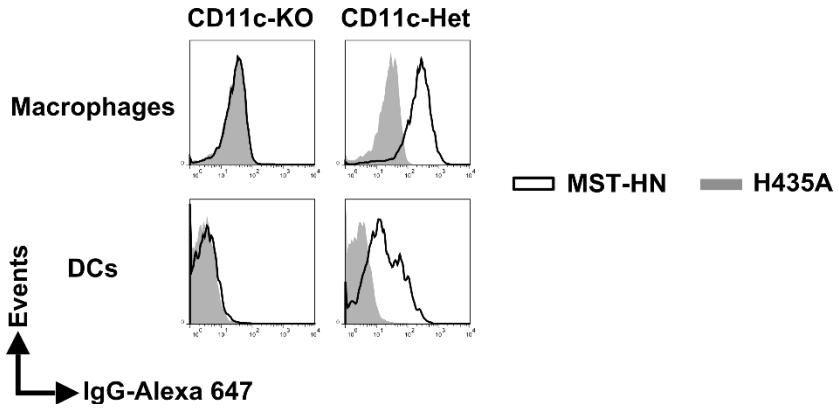
Supplementary Figure 3. Analysis of accumulation of IgG and secondary antibody staining/background autofluorescence levels in lung and kidney. G-KO mice were treated as in Supplementary Figure 1. Injected antibody (pseudocolored green), macrophages ($F4/80^+$, pseudocolored red), endothelial cells ($CD31^+$; pseudocolored red) and secondary antibody staining/background autofluorescence levels (2^o; pseudocolored red/green) are shown. Scale bars = 30 μm. Data shown is derived from immunohistochemical analyses of two mice from two independent experiments (≥ 46 images were acquired per tissue).



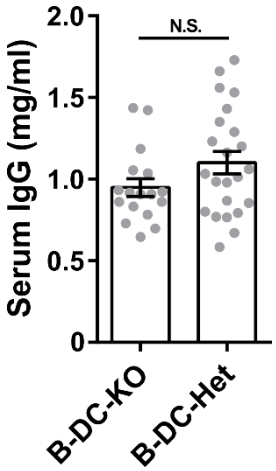
Supplementary Figure 4. Analysis of accumulation of IgG and secondary antibody staining/background autofluorescence levels in spleen and lymph nodes. G-KO mice were treated as in Supplementary Figure 1. Injected antibody (pseudocolored green), macrophages (F4/80^{+}, pseudocolored red), endothelial cells (CD31^{+}; pseudocolored red) and secondary antibody staining/background autofluorescence levels (2°; pseudocolored red/green) are shown. Scale bars = 30 μm. Data shown is derived from immunohistochemical analyses of two mice from two independent experiments (≥ 78 images were acquired per tissue).



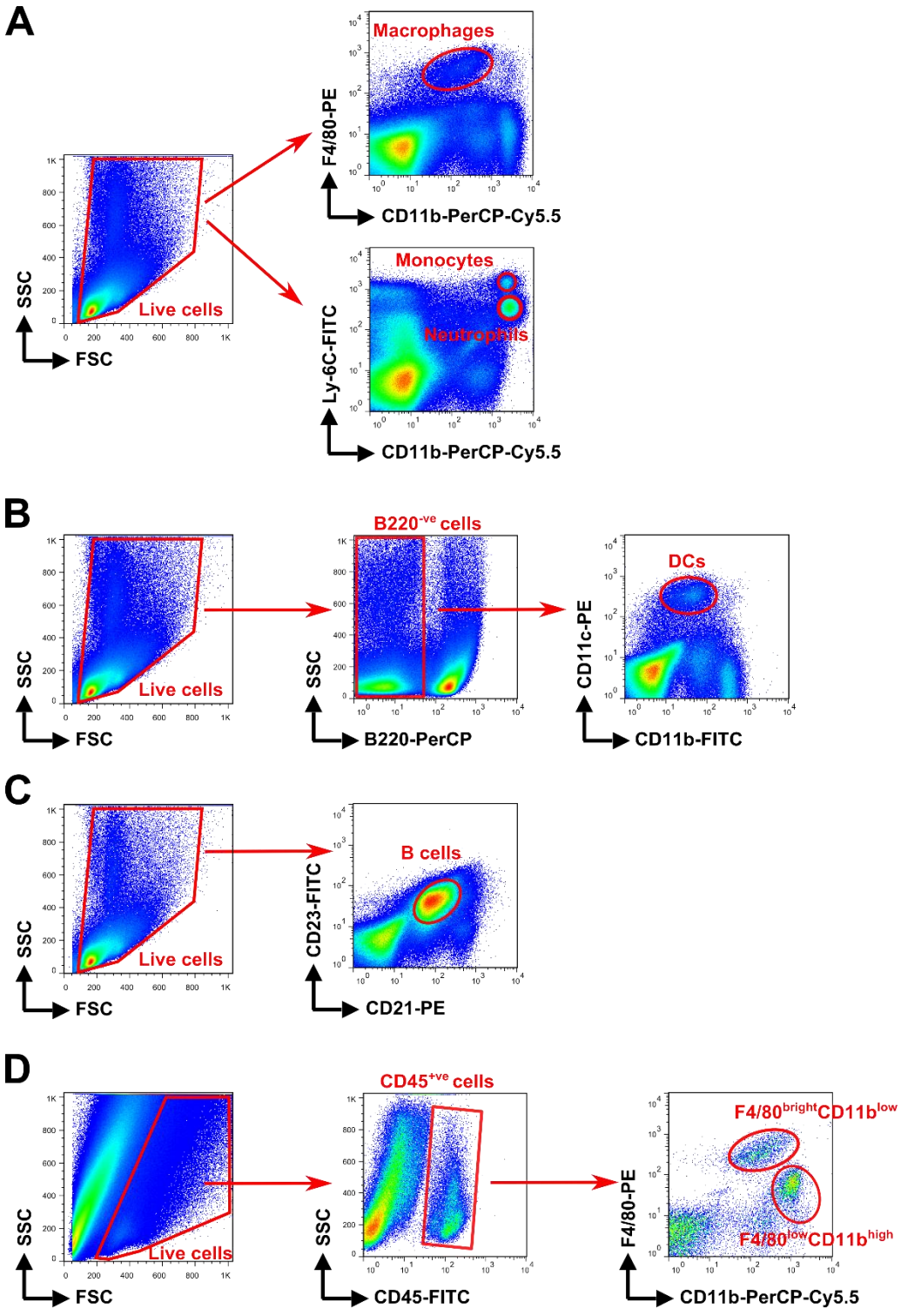
Supplementary Figure 5. MST-HN accumulation in cells is dependent on FcRn expression levels. Human endothelial cells (HMEC-1) were co-transfected with expression plasmids encoding either mFcRn-GFP and mβ$_2$m or hFcRn-L136-GFP and hβ$_2$m. Untransfected HMEC-1 cells were used as controls. The cells were incubated with Alexa 647-labeled MST-HN, H435A or vehicle at 37 °C to assess FcRn-mediated uptake of fluorescently-labeled antibodies. Levels of cell-associated fluorescence were determined using flow cytometry. Each experiment was carried out using triplicate samples and one representative flow cytometry plot for each transfection or treatment condition is shown. mFcRn-GFP, mouse FcRn tagged with enhanced green fluorescent protein; mβ$_2$m, mouse β2-microglobulin; hFcRn-L136-GFP, mutated human FcRn tagged with enhanced green fluorescent protein; hβ$_2$m, human β2-microglobulin; MST-HN, mutated human IgG1 with increased affinity for FcRn;[1] H435A, mutated, control human IgG1 with negligible binding for FcRn.[2] Data shown is representative of two independent experiments.



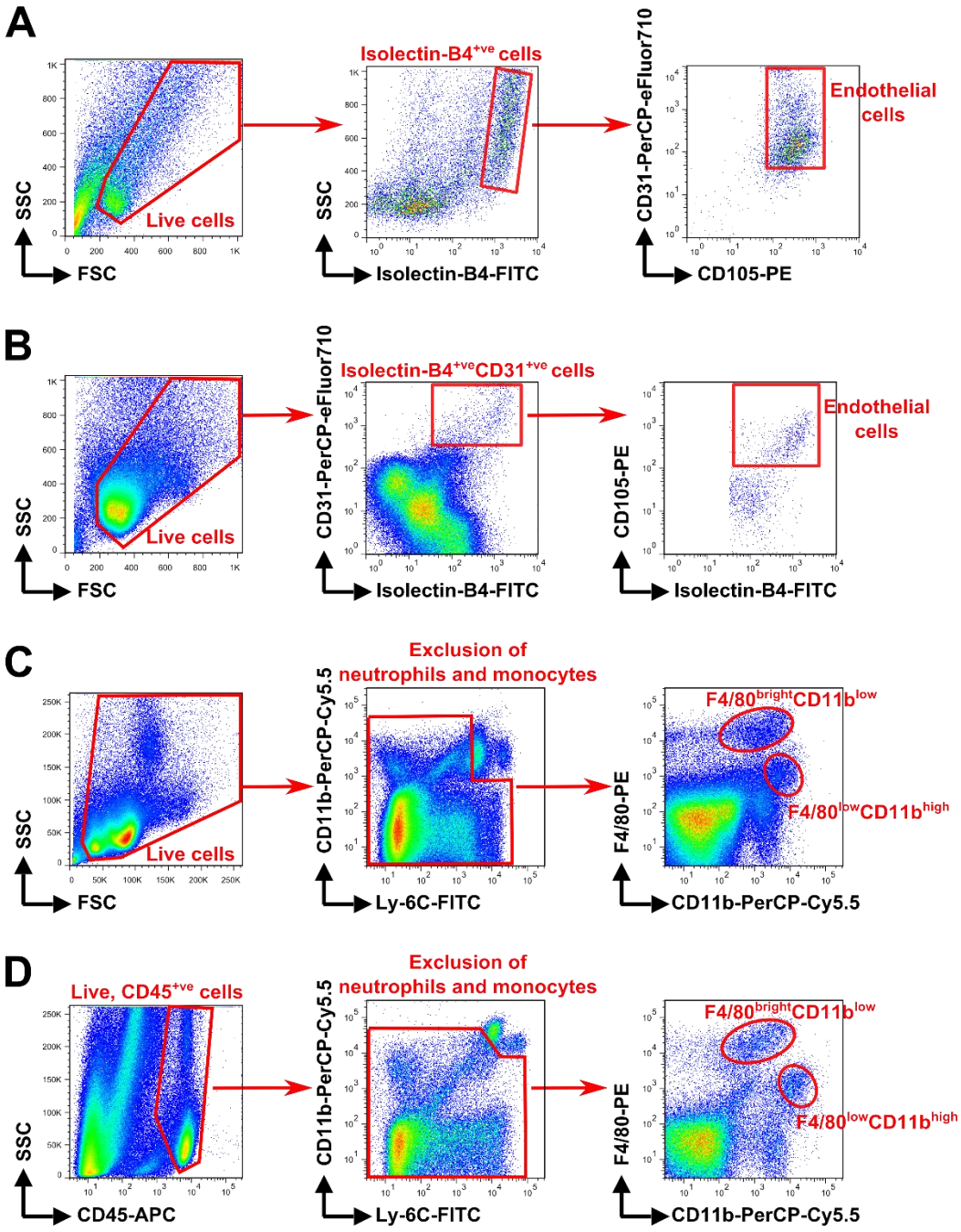
Supplementary Figure 6. CD11c-Cre-mediated deletion of FcRn in CD11c-Cre-FcRn$^{flox/flox}$ mice is not restricted to DCs. Splenocytes were isolated, pooled (from 2-3 mice/genotype) and incubated with anti-FcγRIIB/III (2.4G2) antibody at 4 °C followed by Alexa 647-labeled MST-HN or H435A mutant at 37 °C to assess FcRn-mediated uptake. Fluorescence levels associated with each of the indicated cell types were determined using flow cytometry. Macrophages and DCs were identified as F4/80^{bright}CD11b^{low} and CD11c^{+}CD11b^{+}, respectively. The gating strategies employed for the identification of these cell types are shown in Supplementary Fig. 8A and B. CD11c-KO, CD11c-Cre-FcRn$^{flox/flox}$; CD11c-Het, CD11c-Cre-FcRn$^{flox/+}$; MST-HN, mutated human IgG1 with increased affinity for FcRn;[1] H435A, mutated (control) human IgG1 with negligible binding towards FcRn.[2] Data shown is representative of at least two independent experiments.



Supplementary Figure 7. Loss of FcRn function in B cells and dendritic cells does not affect serum IgG levels. Serum IgG levels in B-DC-KO and B-DC-Het mice are shown. Error bars indicate SEM. N.S., no significant difference ($p > 0.05$; two-tailed Student's *t*-test). B-DC-KO, CD19-Cre-FcRn$^{flox/flox}$ (B cell- and DC-specific FcRn KO); B-DC-Het, CD19-Cre-FcRn$^{flox/+}$ (control). Data shown is derived from 17-23 mice/genotype.



Supplementary Figure 8. Gating strategy used for analyzing functional FcRn levels in different immune cell types. Gating strategy used to identify splenic macrophages (**A**), monocytes (**A**), neutrophils (**A**), classical DCs (**B**), follicular B cells (**C**) and macrophage subtypes (**D**) in kidney, lung and liver is shown.



Supplementary Figure 9. Gating strategy employed for analyzing functional FcRn levels in endothelial cells and percentage of macrophages. Gating strategy employed to identify endothelial cells in the heart (**A**) and lung (**B**), and macrophage subtypes in the spleen (**C**) and liver (**D**) of liposome-treated mice is shown.

REFERENCES

1. Vaccaro C, Zhou J, Ober RJ, Ward ES. Engineering the Fc region of immunoglobulin G to modulate *in vivo* antibody levels. Nat Biotechnol. 2005;23(10):1283-1288.
2. Firan M, Bawdon R, Radu C, Ober RJ, Eaken D, Antohe F, Ghetie V, Ward ES. The MHC class I related receptor, FcRn, plays an essential role in the maternofetal transfer of gammaglobulin in humans. Int Immunol. 2001;13:993-1002.