Expanded View Figures

Figure EV1. Targeting of A2 precursors in the yolk sac labels F4/80^{hi} MΦ (related to Fig 1).

A Flow cytometric quantification of YFP$^+$ A1 and A2 yolk sac cells at E 10.5 after TAM application at E 9.0 in pregnant *Cx3cr1^{CreER}:R26yfp* mice. Each symbol represents one mouse. Data represent mean ± s.e.m. Two independent experiments are depicted.

B Efficacies of microglia targeting (YFP$^+$CD11b$^+$CD45^{lo}) at E 16.0 after TAM application either at E 7.0, E 8.0, or E 9.0 in plug-positive *Cx3cr1^{CreER}:R26-yfp* mice. One representative blot is shown. Gray area depicts fluorescence signal in MΦ of non-transgenic littermates. At least three mice with similar results were investigated.

C Representative images of the fetal liver in E 10.5 *Cx3cr1$^{GFP/WT}$* embryos. CX_3CR1-GFP expression (green), F4/80 (red), CD31 (blue), c-kit, or CD41 (white). Arrow points to a c-KIT- and CD31-positive cell. No co-localization of CD31, CD41, or c-KIT with CX_3CR1-GFP$^+$ cells was detected. Scale bars represent 50 μm. Representative pictures out of two embryos are shown.

D Representative images of the AGM in E 10.0 *Cx3cr1$^{GFP/WT}$* embryos. CX_3CR1-GFP expression (green), CD31 (white or red), F4/80 (red). No CX_3CR1-GFP signal is observed close to native HSC (* marks the HSC cluster in the aorta). CX_3CR1-GFP$^+$ cells are close to vessels; some are also F4/80$^+$ (arrows). Scale bars represent 100 μm. Representative pictures out of two embryos are shown.

E Immunofluorescence of YFP (green) and Iba-1 (red) from brain, liver, and kidney of adult (P42) *Cx3cr1^{CreER}:R26-yfp* mice that received TAM at E 9.0. Scale bars represent 25 μm.

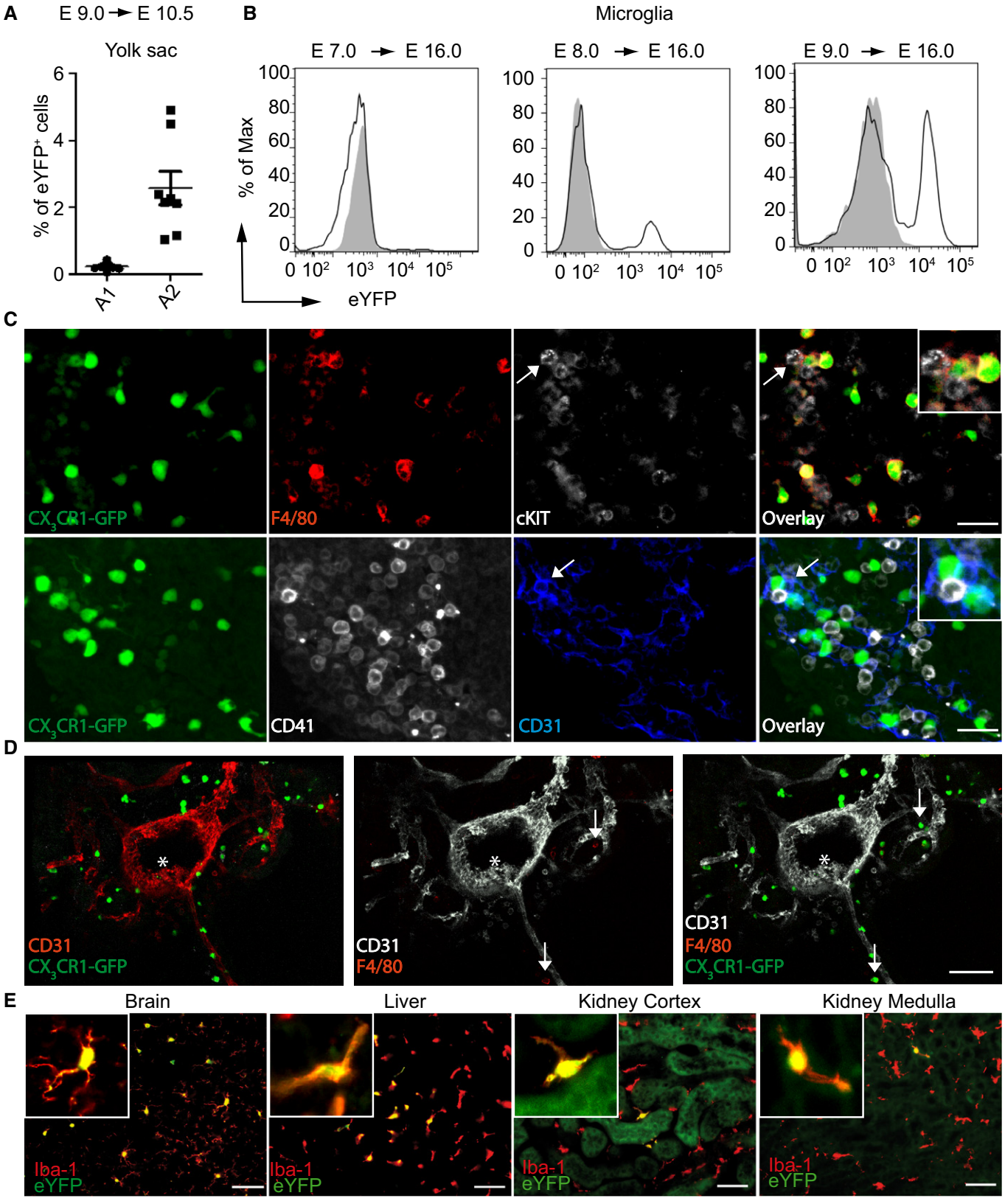

Figure EV1.

Figure EV2. Gating strategy used for flow cytometry of MΦ (related to Figs 1–6).
Single-cell suspensions were prepared using enzymatic digestion, density gradient centrifugation, or mechanical dissociation. Side scatter (SSC) and forward scatter (FSC) discrimination of cells of interest was followed by the gating for the respective MΦ population.

A Microglia were discriminated by CD45 and CD11b.
B Liver cells were gated for CD45-positive cells; CD146, lineage- (CD3, CD19, NK1.1) and Gr1-positive cells were excluded to select $CD11b^{hi}$ and $F4/80^{hi}$ MΦ as described recently (Huang *et al*, 2013).
C Kidney cells were gated on $CD45^+$ cells followed by the exclusion of lineage markers (CD3, CD19, NK1.1) and $Gr1^+$ cells to select the $CD11b^{hi}$ and $F4/80^{hi}$ MΦ.
D Epidermal cells were first selected by a living dye stain, followed by selection of $CD45^+$, $EpCAM^+$, $Ly6C^-$, $MHCII^+$, and $CD11b^+$ characteristics.

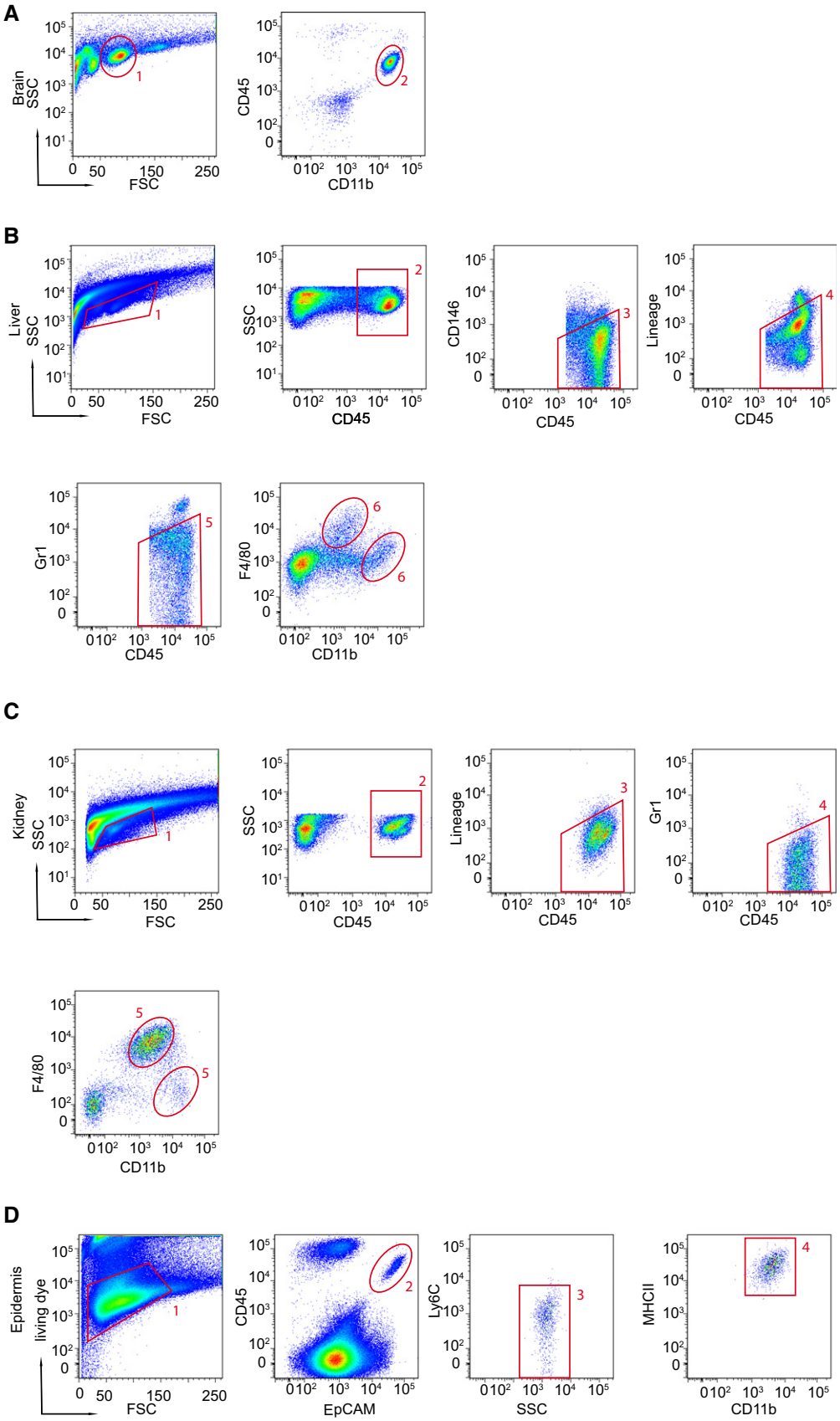
A
Brain
SSC
FSC
CD45
CD11b
B
Liver
SSC
FSC
SSC
CD45
CD146
CD45
Lineage
CD45
Gr1
CD45
F4/80
CD11b
C
Kidney
SSC
FSC
SSC
CD45
Lineage
CD45
Gr1
CD45
F4/80
CD11b
D
Epidermis
living dye
FSC
CD45
EpCAM
Ly6C
SSC
MHCII
CD11b

Figure EV2.

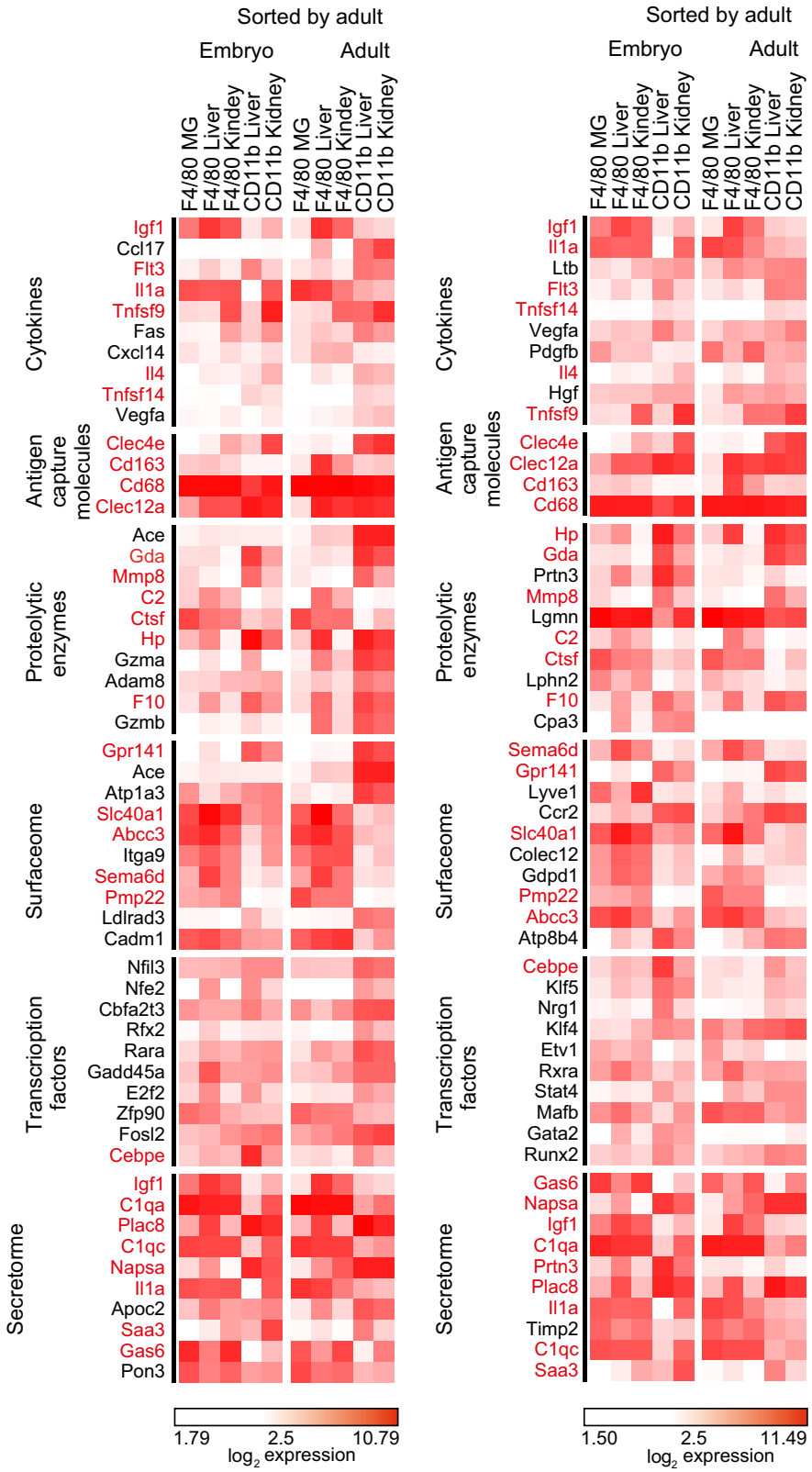

Figure EV3. Visualization of genes differentially expressed between F4/80^{hi} and CD11b^{hi} MΦ during embryogenesis and adulthood (related to Fig 3).

Heat map visualization of differentially expressed genes between F4/80^{hi} and CD11b^{hi} MΦ in different tissues during embryogenesis and adulthood. The top 10 genes based on fold change (FC) (left panel: FC F4/80^{hi} vs. CD11b^{hi} in adult; right panel: FC F4/80^{hi} vs. CD11b^{hi} in embryo) among the differentially expressed genes related to macrophage function such as cytokines, antigen capturing molecules, proteolytic enzymes, cell surface markers, and transcription factors are shown. Values are represented in log2 expression. Genes being common in both heat maps are highlighted by red color.

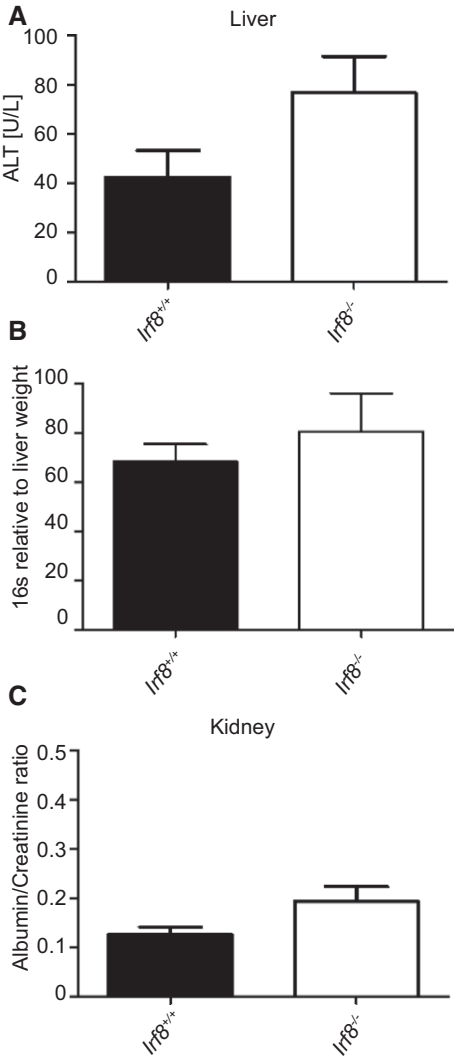

Figure EV4. Normal liver and kidney parameters in *Irf8*-deficient mice under homeostatic conditions (related to Fig 5).

A Alanine aminotransferase (ALT) activity in the serum of adult $Irf8^{-/-}$ and $Irf8^{+/+}$ mice.

B qPCR analysis for bacterial 16S rRNA in the livers of adult $Irf8^{-/-}$ and $Irf8^{+/+}$ animals

C Albumin:creatinine ratio in the urine of adult $Irf8^{-/-}$ and $Irf8^{+/+}$ mice. Bars represent mean ± s.e.m. of at least three mice per group. Significant differences were not detectable by using an unpaired *t*-test.

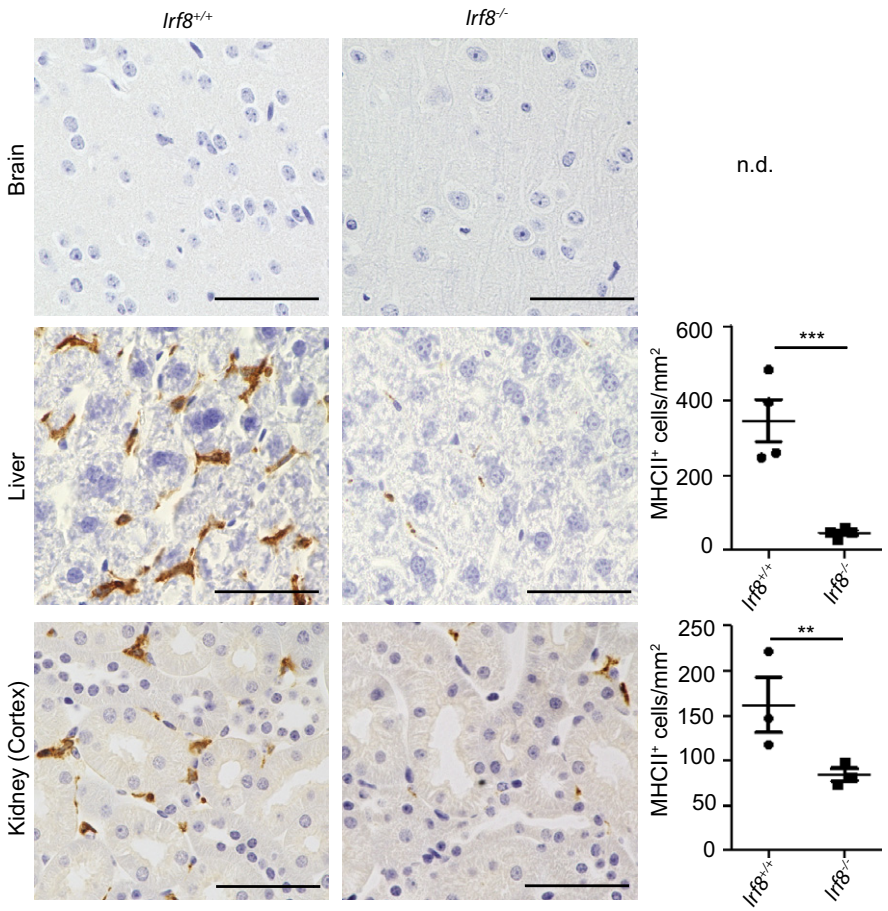

Figure EV5. Down-regulation of MHC class II in *Irf8*-deficient mice (related to Fig 6).
MHCII immunohistochemistry of brain, liver, and kidney MΦ in adult *Irf8*$^{-/-}$ or *Irf-8*$^{+/+}$ mice (left) and quantification thereof (right). Scale bar represents 50 μm. Each symbol represents one mouse with at least four examined tissue section per mouse. Significant differences were examined by an unpaired *t*-test and marked with asterisks (**$P < 0.01$, ***$P < 0.001$); n.d.: not detectable.