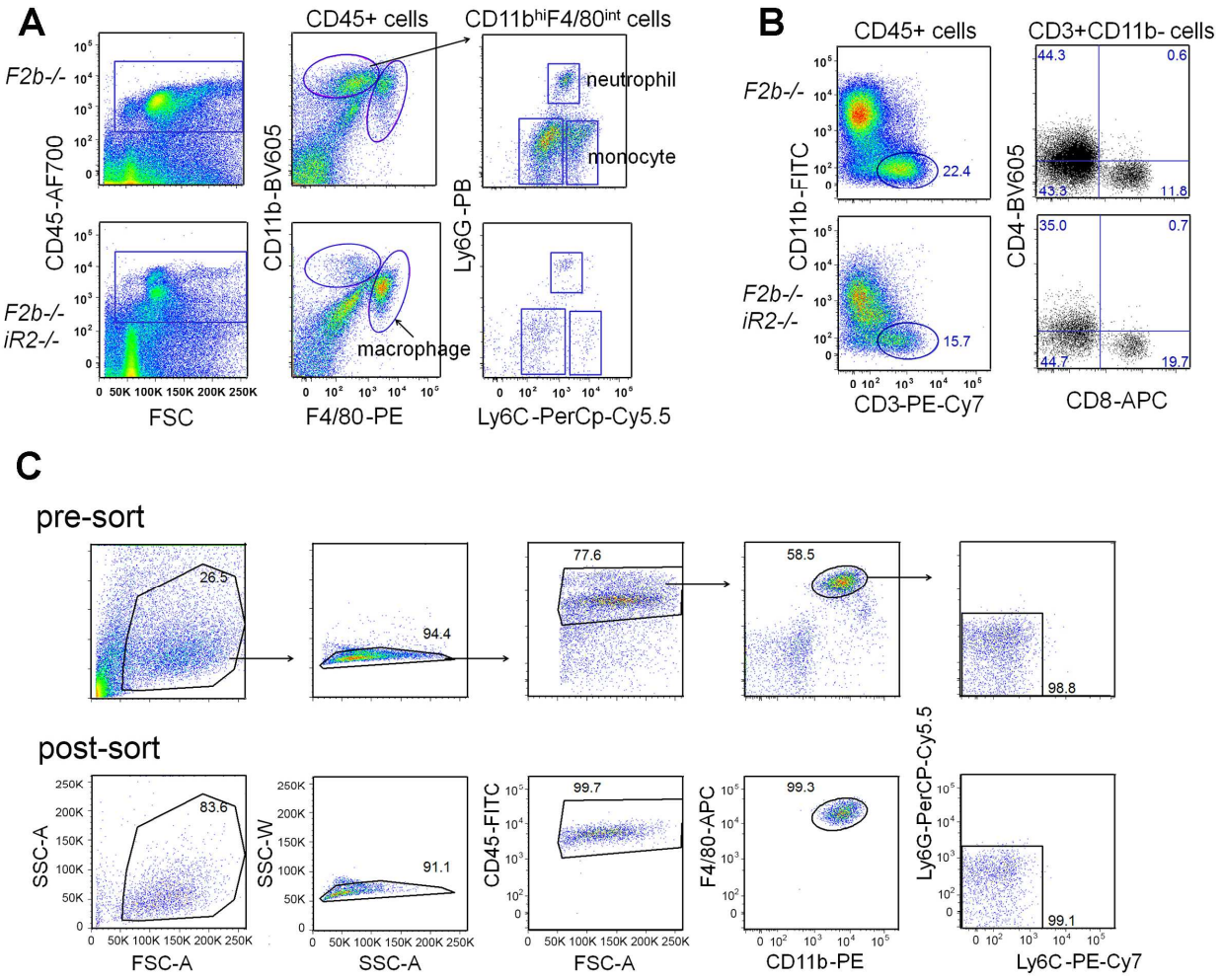
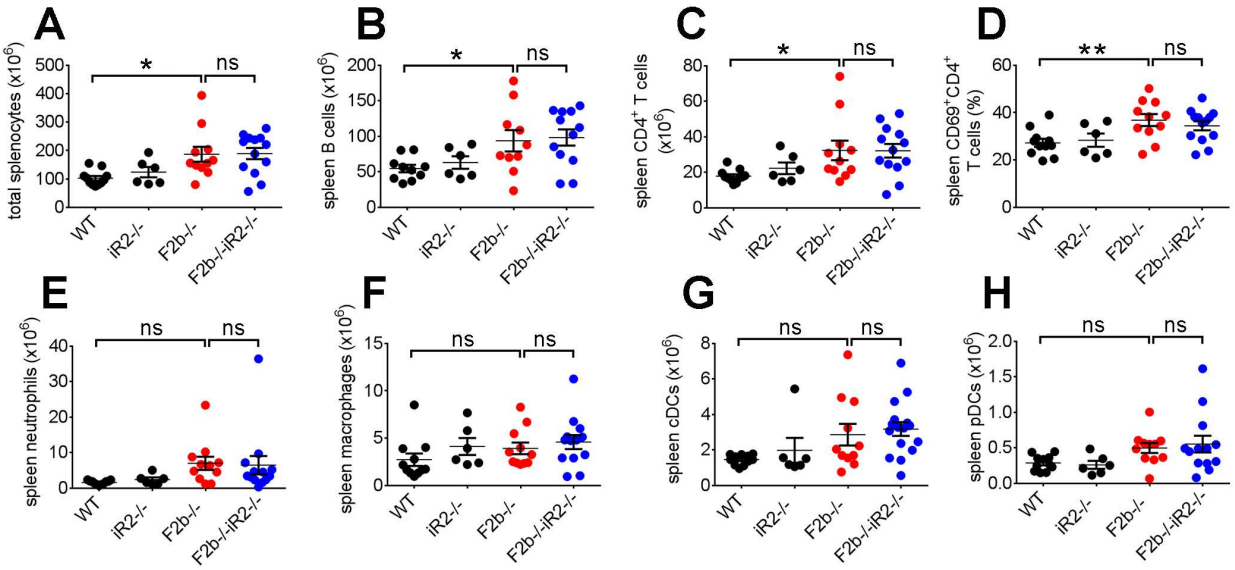
SUPPLEMENTAL FIGURES

Sup. Figure 1



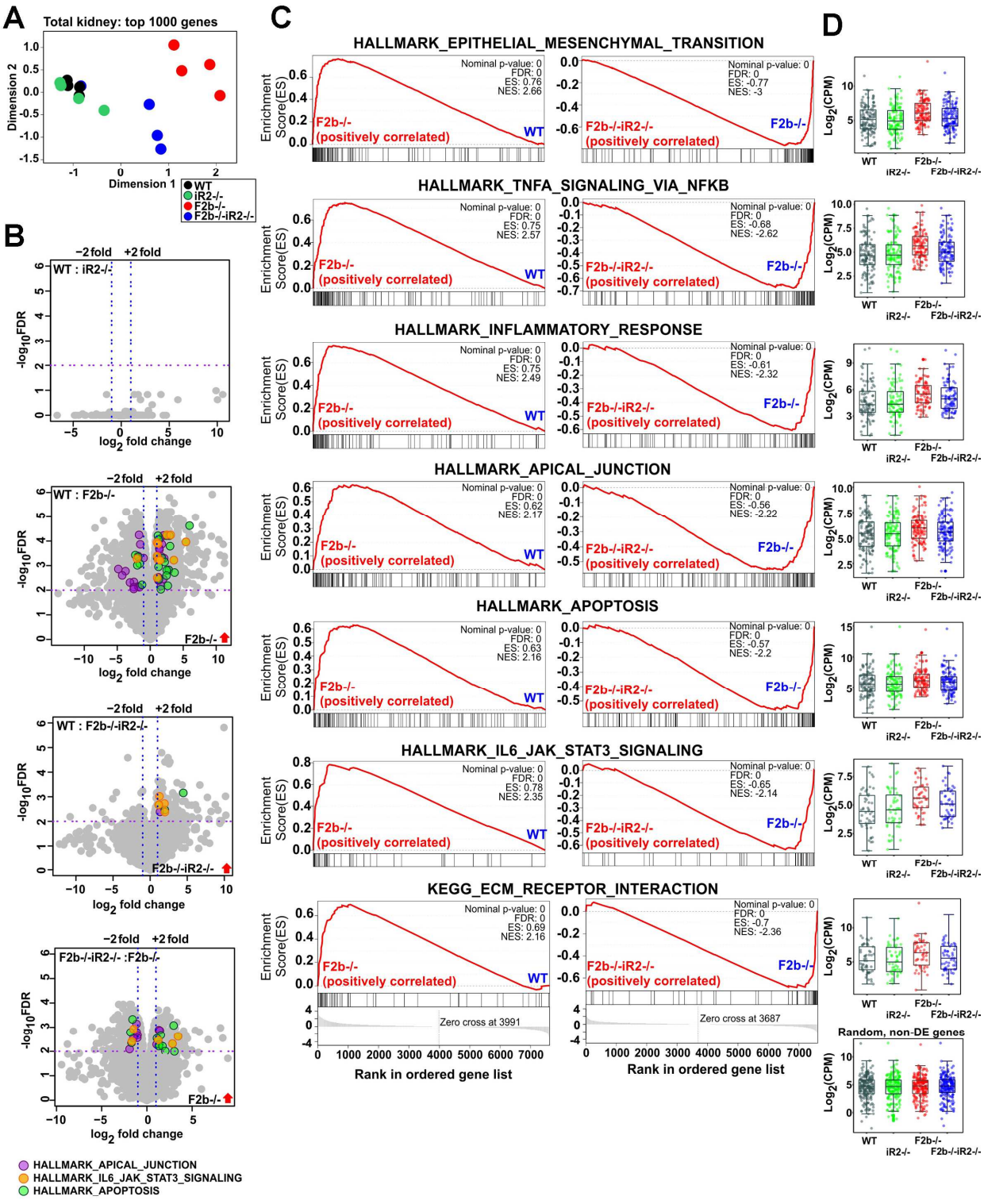
Supplemental figure 1. Flow cytometry gating and sorting strategies. (**A** and **B**) Gating strategy for flow cytometry analysis of inflammatory cell infiltrates in the kidneys. (**A**) $CD45^+$ leukocytes, $CD45^+F4/80^{hi}CD11b^+$ macrophages, $CD45^+F4/80^{int}CD11b^{hi}Ly6C^+Ly6G^+$ neutrophils, $CD45^+F4/80^{int}CD11b^{hi}Ly6C^{hi}Ly6G^-$ $Ly6C^{hi}$ monocytes. (**B**) T cell subsets ($CD45^+CD11b^-CD3^+$ total T cells, $CD45^+CD11b^-CD3^+$ $CD4^+CD8^-$ T cells, $CD45^+CD11b^-CD3^+CD4^-CD8^+$ T cells, and $CD45^+CD11b^-CD3^+CD4^-CD8^-$ double negative T cells). (**C**) Sorting macrophages from kidneys for RNA-seq. Macrophages were sorted as $CD45^+F4/80^{hi}CD11b^+Ly6G^-Ly6C^-$ population in perfused kidneys for RNA-seq.

Sup. Figure 2



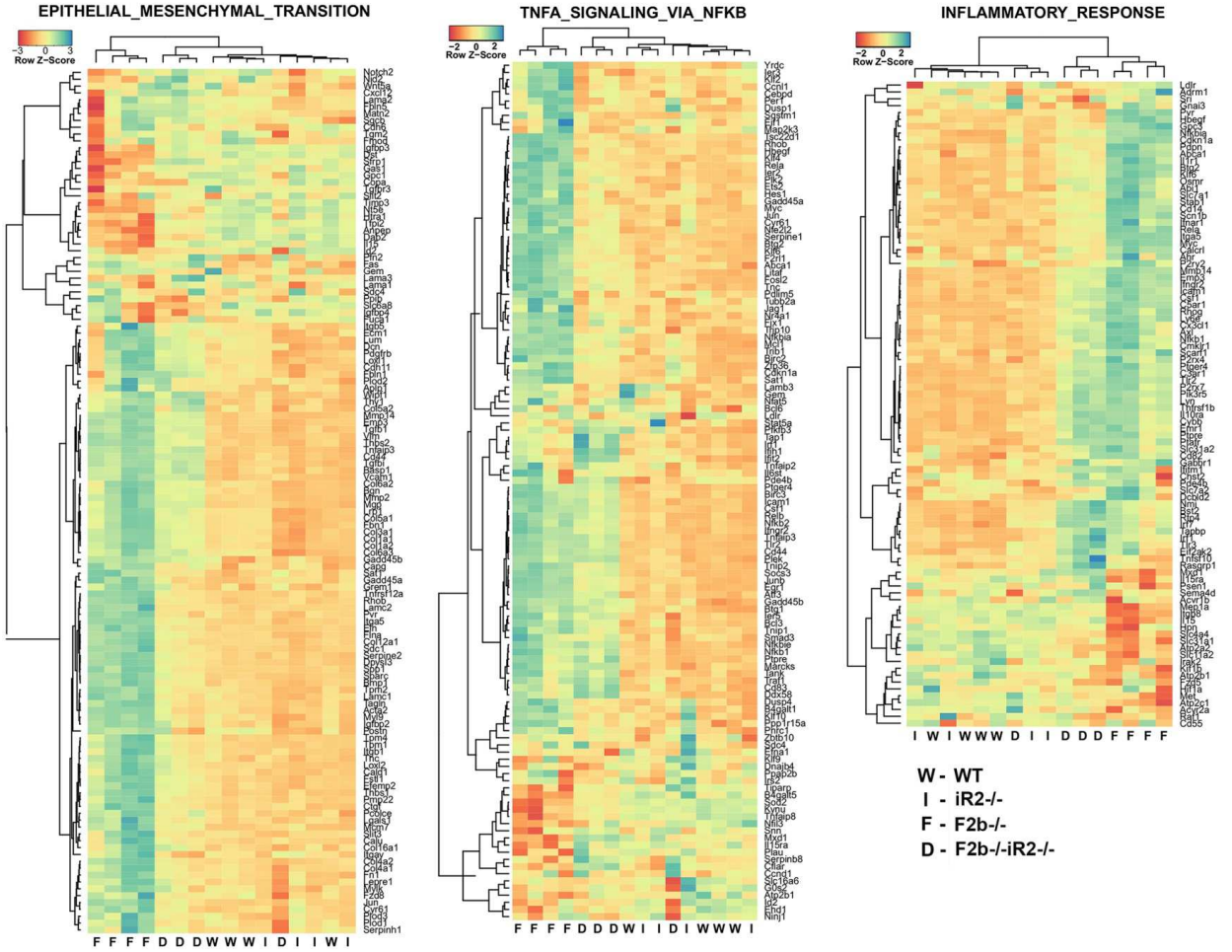
Supplemental Figure 2. Expansion of immune cells in the spleen of *Fcgr2b-/-* mice was not affected by *iRhom2* deficiency. Spleen cells from 7-9 month old mice were assessed by flow cytometry. (**A**) Numbers of total splenocytes. (**B**) Numbers of $CD11b^{-}CD3^{-}B220^{+}CD4^{-}$ B cells. (**C**) $CD11b^{-}CD3^{+}B220^{-}CD4^{+}$ T cells. (**D**) Percentage of $CD69^{+}$ activated $CD4^{+}$ T cells. (E) Numbers of $CD11b^{hi}Ly6G^{+}$ neutrophils. (**F**) $F4/80^{hi}CD11b^{+}$ macrophages. (**G**) $CD11c^{hi}MHCclassII^{+}PDCA^{-}$ conventional dendritic cells (cDCs). (**H**) $CD11c^{lo}PDCA^{+}$ plasmacytoid dendritic cells (pDCs). A-H, 11 *WT*, 6 *Rhbdf2-/-*, 11 *Fcgr2b-/-*, and 13 *Fcgr2b-/-Rhbdf2-/-* mice. All are mean ± s.e.m, One-way ANOVA with Dunnett's multiple comparisons test. * $P<0.05$, ** $P<0.01$, ns, not significant.

Sup. Figure 3


A
Total kidney: top 1000 genes
Dimension 2
Dimension 1
WT
iR2-/-
F2b-/-
F2b-/-iR2-/-
B
WT : iR2-/-
WT : F2b-/-
WT : F2b-/-iR2-/-
F2b-/-iR2-/- :F2b-/-
-2 fold
+2 fold
-log10FDR
log2 fold change
F2b-/-
F2b-/-iR2-/-
HALLMARK_APICAL_JUNCTION
HALLMARK_IL6_JAK_STAT3_SIGNALING
HALLMARK_APOPTOSIS
C
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_APICAL_JUNCTION
HALLMARK_APOPTOSIS
HALLMARK_IL6_JAK_STAT3_SIGNALING
KEGG_ECM_RECEPTOR_INTERACTION
Enrichment Score(ES)
Nominal p-value: 0
FDR: 0
ES: 0.76
NES: 2.66
ES: -0.77
NES: -3
ES: 0.75
NES: 2.57
ES: -0.68
NES: -2.62
ES: 0.75
NES: 2.49
ES: -0.61
NES: -2.32
ES: 0.62
NES: 2.17
ES: -0.56
NES: -2.22
ES: 0.63
NES: 2.16
ES: -0.57
NES: -2.2
ES: 0.78
NES: 2.35
ES: -0.65
NES: -2.14
ES: 0.69
NES: 2.16
ES: -0.7
NES: -2.36
F2b-/-
(positively correlated)
F2b-/-iR2-/-
WT
Zero cross at 3991
Zero cross at 3687
Rank in ordered gene list
D
Log2(CPM)
WT
iR2-/-
F2b-/-
F2b-/-iR2-/-
Random, non-DE genes

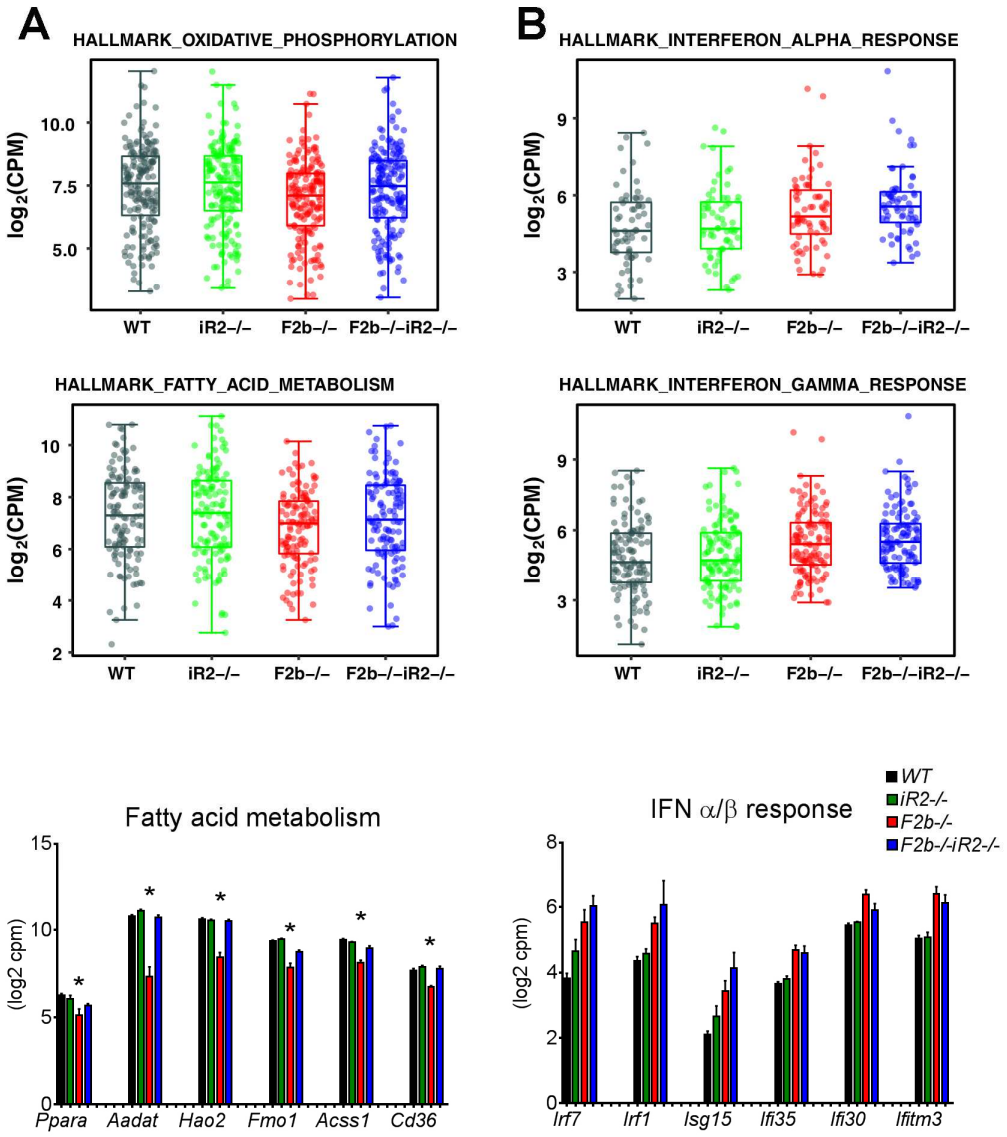
Supplemental figure 3. **RNA-seq profiling of mouse kidneys.** (**A**) Multidimensional scaling plot for the top 1000 genes with the largest expression variance identified by RNA-seq. (**B-D**) GSEA of RNA-seq data. (**B**) Differentially expressed genes that belonged to the hallmark gene sets in *Fcrg2b*-/- vs. WT mice were highlighted in the volcano plots. (**C**) GSEA profiles for the top hallmark gene sets are shown for *Fcrg2b*-/- vs. WT (left panels) and *Fcrg2b*-/- vs. *Fcrg2b*-/- *Rhbdf2-/-* kidneys (right panels). FDR, False discovery rate. ES, Enrichment score. NES, Normalized enrichment score. (**D**) Expression of genes from hallmark gene sets is illustrated for all 4 groups of mice. A set of random non-differentially expressed genes (*n*=200) were shown as control on the bottom. (*n*=4 mice per group)

Sup. Figure 4



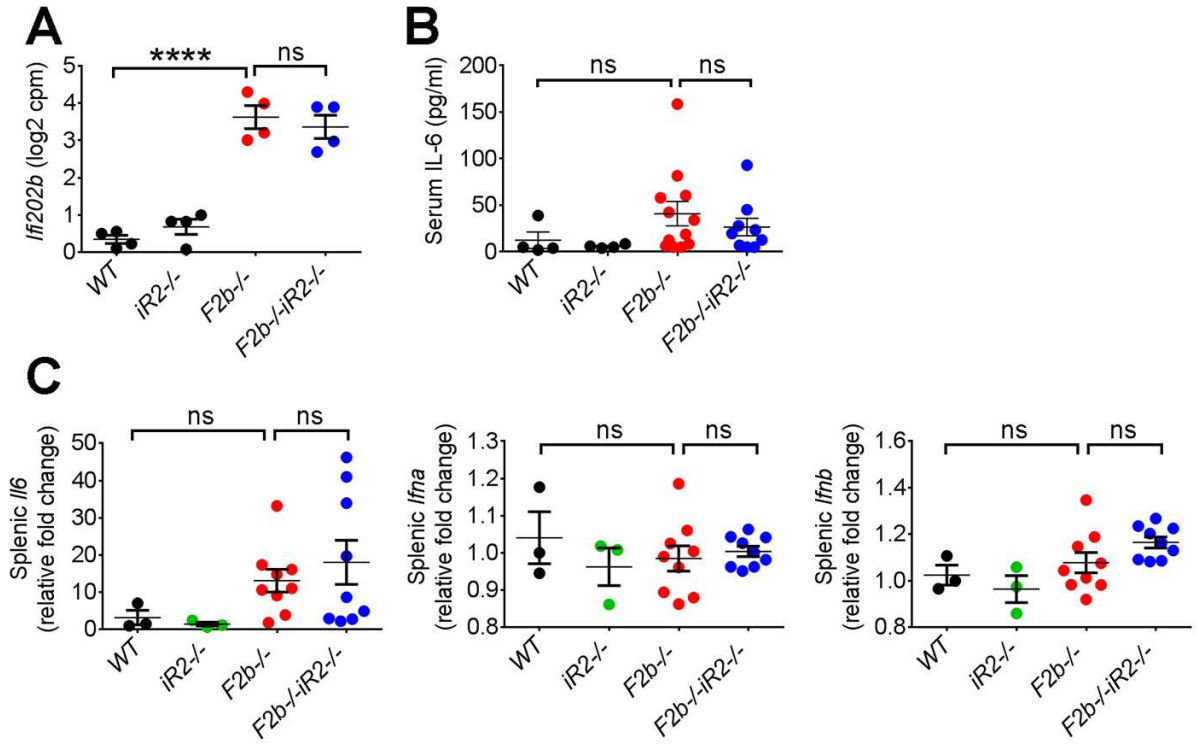
Supplemental figure 4. **Hierarchical clustering of genes from the EMT, TNF signaling and inflammatory response gene sets in mouse kidneys**. GSEA of RNA-seq data from lupus kidneys identified the EMT, TNF signaling and inflammatory response as the top 3 hallmark gene sets differentially expressed between *Fcrg2b*-/- vs. WT mice. Unsupervised hierarchical clustering indicates substantial upregulation of genes from these gene sets in *Fcrg2b*-/- compared to other genotypes (*n*=4 mice per group).

Sup. Figure 5



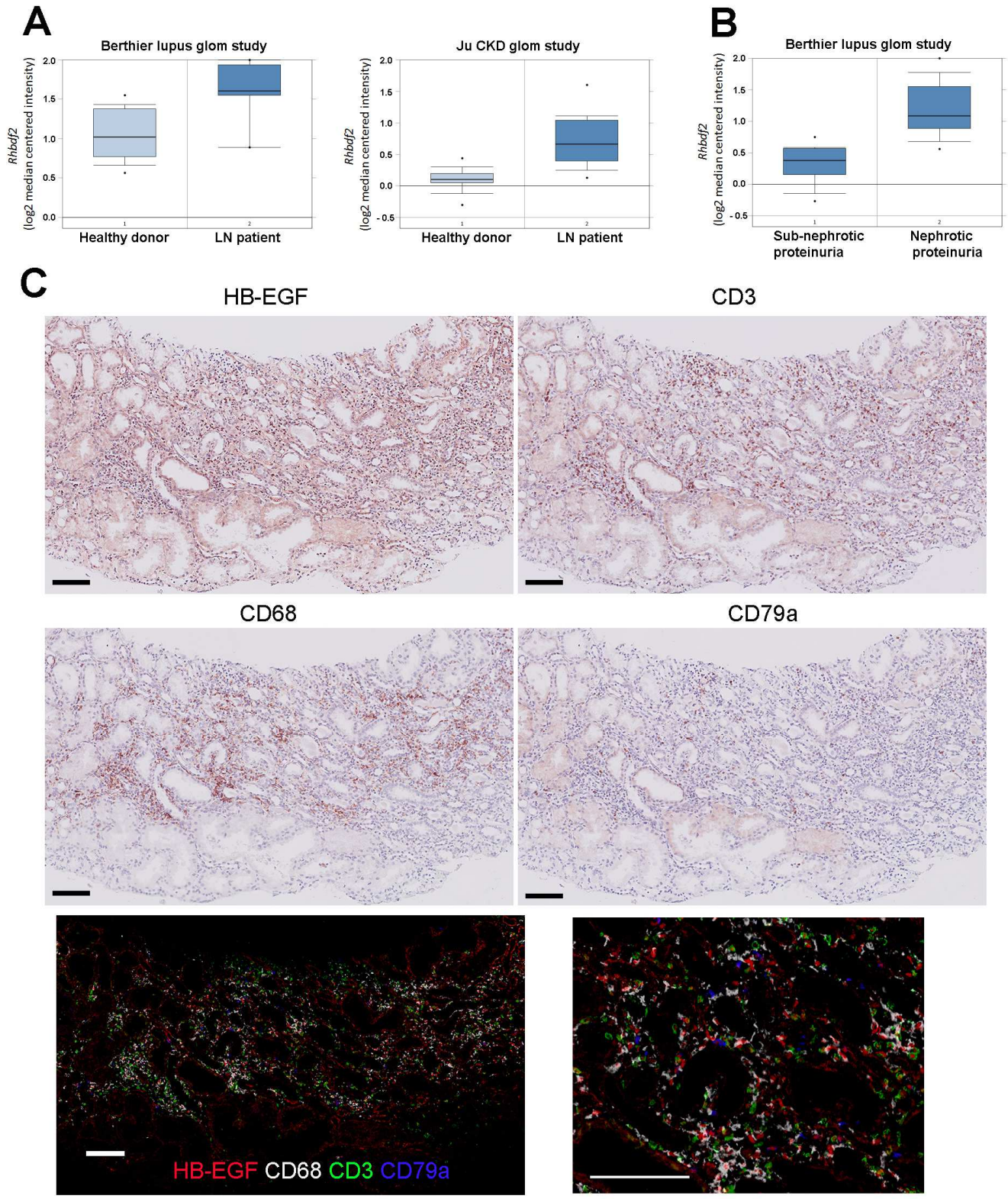
Supplemental figure 5. **Fatty acid metabolism and IFN responses in the kidneys of *Fcrg2b-/-* mice.** GSEA was performed on RNA-seq results from total kidneys. Expression of gene sets encoding oxidative phosphorylation, fatty acid metabolism (**A**) and IFNα, IFNγ responses (**B**) was illustrated. Representative genes shown: *Ppara,* peroxisome proliferative activated receptor, alpha; *Aadat*, aminoadipate aminotransferase; *Hao2*, hydroxyacid oxidase 2 (long chain); *Fmo1*, flavin containing monooxygenase 1; *Acss1*, acyl-CoA synthetase short-chain family member 1; *Cd36*, thrombospondin receptor; *Irf7*, interferon regulatory factor 7; *Irf1*, interferon regulatory factor 1; *Isg15*, ISG15 ubiquitin-like modifier; *Ifi35*, interferon-induced protein 35; *Ifi30*, interferon-induced protein 30; *Ifitm3*, interferon induced transmembrane protein 3. *n*= 4 mice per group. * *P*<0.05

Sup. Figure 6



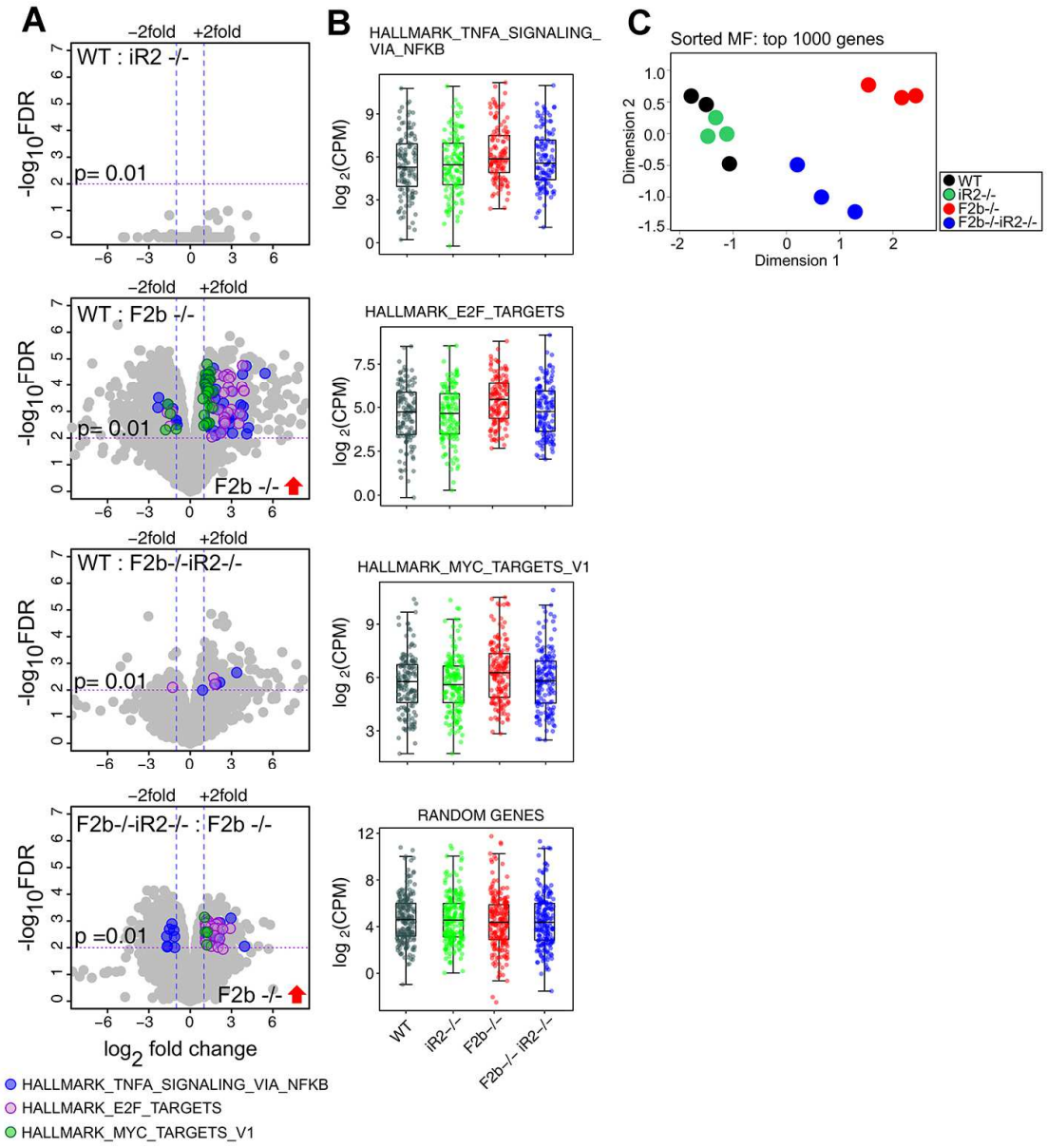
Supplemental Figure 6. Type I IFN induced genes and serum IL-6 are not altered by *iRhom2* deficiency in *Fcgr2b-/-* mice. (**A**) Expression of *Ifi202b* in the kidneys on RNA-seq. *n*=4 mice per group, mean ± s.e.m. (**B**) IL-6 was measured by ELISA in the serum of WT (*n*=4), *Rhbdf2*-/- (*n*=4), *Fcrg2b*-/- (*n*=12) and *Fcrg2b*-/-*Rhbdf2*-/- mice (*n*=10) at the age of 7-9 months upon euthanasia. (**C**) Expression of *Il6*, *Ifna*, and *Ifnb* mRNA in the spleen measured by qPCR. 3 WT, 3 *Rhbdf2*-/-, 9 *Fcrg2b*-/- and 9 *Fcrg2b*-/-*Rhbdf2*-/- mice. Mean ± s.e.m. (B and C) One-way ANOVA with Dunnett's multiple comparisons test. **** $P<0.0001$, ns, not significant.

Sup. Figure 7


A
Berthier lupus glom study
Rhbdf2
(log2 median centered intensity)
2.0
1.5
1.0
0.5
0.0
Healthy donor
LN patient
Ju CKD glom study
2.0
1.5
1.0
0.5
0.0
-0.5
Healthy donor
LN patient
B
Berthier lupus glom study
Rhbdf2
(log2 median centered intensity)
2.0
1.5
1.0
0.5
0.0
-0.5
Sub-nephrotic proteinuria
Nephrotic proteinuria
C
HB-EGF
CD3
CD68
CD79a
HB-EGF CD68 CD3 CD79a

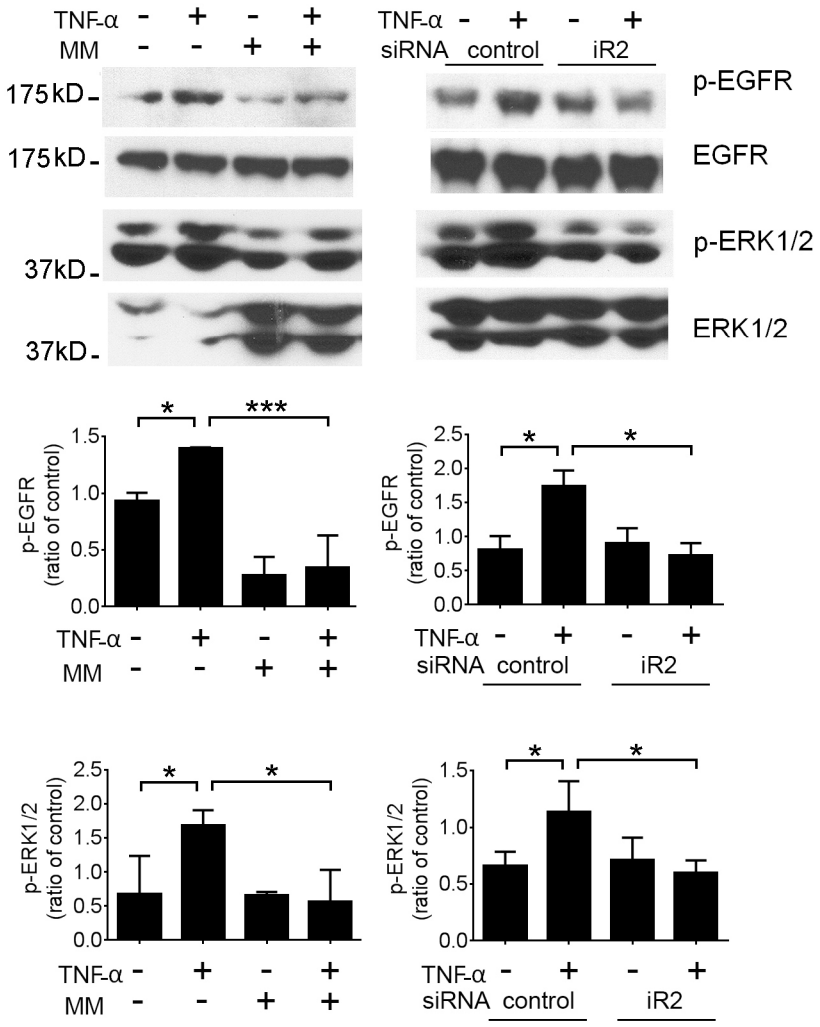
Supplemental figure 7. ***Rhbdf2*** **and HB-EGF are over-expressed in the kidneys of LN patients**. (**A** and **B**) Expression of *Rhbdf2* in kidneys of LN patients measured by cDNA microarray. (**A**) Healthy donors vs. LN patients. Berthier lupus glom study, 14 healthy donor and 32 LN patients, mean ± s.d., fold change=1.813, *P*=6.00E-10 (35) (GSE32591); Ju CKD glom study, 21 healthy donor and 32 LN patients, mean ± s.d., fold change=1.532, *P*=2.99E-10 (34). (**B**) Patients with sub-nephrotic proteinuria vs. patients with nephrotic proteinuria. Berthier lupus glom study, 18 patients with sub-nephrotic proteinuria and 9 patients with nephrotic proteinuria, fold change=1.518, *P*=2.69E-10 (35) (GSE32591). (**C**) Sequential staining of HB-EGF (red signal) and leukocytes in the kidney interstitium of LN patients. CD68 (white signal) for macrophages, CD3 (green signal) for T cells and CD79a (blues signal) for B cells. Scale bar, 100 μm. Representative image from 24 LN patients (18 class IV and 6 class V). This data is also shown in Figure 10B.

Sup. Figure 8



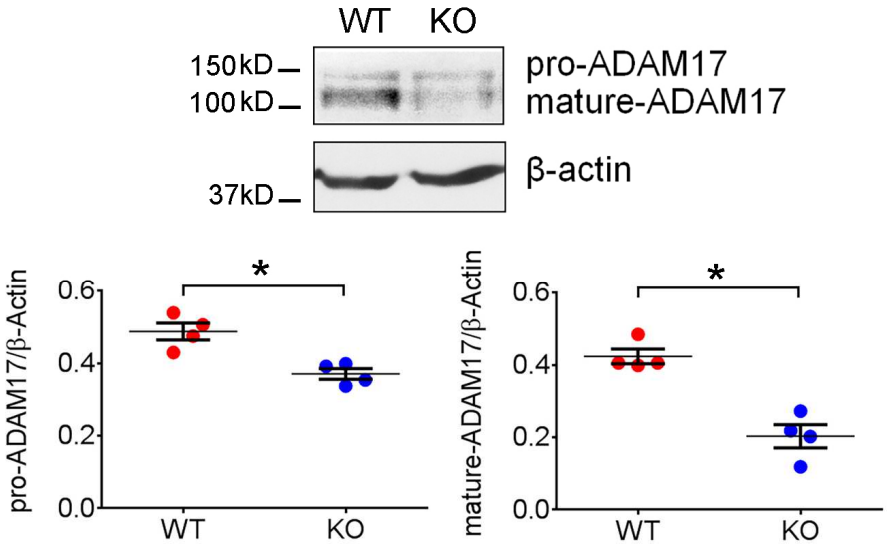
Supplemental figure 8. **RNA-seq analysis of kidney macrophages from *Fcgr2b-/-* mice.** (**A**) Volcano plots of genes differentially expressed between each group (*P*=0.01, FC=2). Differentially-expressed genes that belong to the enriched hallmark gene sets identified by GSEA are shown in indicated colors. (**B**) Expression of genes from TNF signaling, E2F targets and Myc targets gene sets. A set of random non-differentially expressed genes (*n*=200) is shown as control. (**C**) Multidimensional scaling plot for the top 1000 genes with the largest expression variance identified by RNA-seq.

Sup. Figure 9



Supplemental figure 9. TNF-α transactivates EGFR via the iRhom2/ADAM17 pathway in kidney tubular epithelial cells. C1 cells pretreated with MM or transfected with siRNA were stimulated with mouse recombinant TNF-α. Expression of p-EGFR, EGFR, p-ERK1/2 and ERK1/2 was assessed in cell lysates. The p-EGFR/EGFR and p-ERK1/2/ERK bands in the siRNA experiments shown here are replicate samples run on parallel gels. **P**-EGFR and p-ERK1/2 expression was calculated as ratios against total EGFR or ERK1/2 respectively. n=3 independent experiments, mean ± s.e.m. One-way ANOVA with Dunnett's multiple comparisons test. * $P<0.05$, *** $P<0.001$.

Sup. Figure 10



Supplemental figure 10. Decreased ADAM17 expression in the nephrons of *iRhom2* deficient mice. Nephrons (glomeruli and tubules) isolated from *Rhbdf2*-/- and WT mice were examined for ADAM17 expression (pro- and mature forms). n=4 mice per group, β-actin ratio as loading control, mean ± s.e.m. two-tailed Mann-Whitney test. * $P<0.05$.

Supplemental Table 1. Demographics of patients with kidney biopsies

Diagnosis	Kidney biopsies (n)	Age (Years, Mean ± SD)	Female (n)	Male (n)
LN class IV with crescents	9	39 ± 16	7	2
LN class IV without crescents	9	35 ± 17	5	4
LN class V	6	44 ± 15	4	2
ANCA associated vasculitis	10	56 ± 18	4	6