Supplementary Materials (List)

Supplementary Materials and Methods List

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Antibodies used for FACS surface staining: IA/E-PE (Biolegend , M5/114.15.2), Bst-2-biotin (in-house conjugated, eBio927-bi), CD11c-PE/Cy7 (Biolegend, N418), CD45R-APC/Cy7 (BD Pharmigen, RA3-6B2), SiglecH-Al647 (eBioscience, eBio440c), CD19-Pacblue (in-house conjugated 1D3.2), CD40-PE (BD Pharmigen, 3/23), Ly6G-Al488 (in-house conjugated 1A8), Gr1-PE/Cy7 (Biolegend, RB6-8C5), CD11b-APC/Cy7 (Biolegend, M1/70), F4/80-Al647 (in-house conjugated, BM8), CD44-Al488 (in-house conjugated, 1M7), TcRβ-PE/Cy7 (Biolegend, H57-597), CD62L-APC/Cy7 (Biolegend, Mel-14), CD8-Al647 (in-house conjugated, TIB 105), CD4-Pacblue (in-house conjugated, GK1.5), CD4-PE (Biolegend, GK1.5), CD25-PE/Cy7 (BD Bioscience, PC61), TcRβ-APC/Cy7 (Biolegend, H57-597), FoxP3-APC (eBioscience, FJK-16s), CD8-Pacblue(in-house conjugated, TIB 105).

Details of Nephritis Evaluation: Glomerulonephritis was scored in the following manner: A score of 0 indicated that capillary loops were evident and the mesangium was unexpanded. A score of 1 indicated evident capillary loops, with an expanded mesangium. A score of 2 indicated that capillary loops were evident, the mesangium was expanded with increased cellularity. A score of 3 indicated diminished capillary loops with swollen glomeruli. A score of 4 indicated no capillary loops, basement membrane thickening, and significant mesangial proliferation. A score of 5 indicated the presence of crescentic glomerular disease. A score of 6 indicated crescents, necrosis, obliteration of glomerular architecture, and global sclerosis.

Neutrophil Isolation and Assessment of DNA release: Neutrophils were isolated from the bone marrow using a layered Percoll (Sigma) gradient of 52%, 64%, and 78%. Neutrophils were collected at the 64%-78% interface. Cells were plated in a Gibco polypropylene plate at a

density of $1x10^6$ cells per mL in RPMI without serum. Cells were either left untreated or stimulated with 100nM PMA (VWR Scientific) or 10mM hydrogen peroxide (Sigma) in the presence of Pico Green (Invitrogen). Fluorescence was measured with a Wallac Victor^2 1420 plate reader at thirty minute intervals for six hours. This protocol was adapted from Ermert et al. *(27)*.

Supplementary Figure Legends

Fig. S1. Neutrophils from Nox2-deficient mice fail to release DNA in response to NET-inducing stimuli. **(A)** Bone marrow derived neutrophils from 8-week old Nox2-sufficient (left panel) or Nox2-deficient (right panel) mice were stimulated with PMA, H_2O_2, or left unstimulated. DNA release was measured with the DNA dye PicoGreen over six hours. Data points represent mean fluorescence of four mice. Error bars indicate standard deviation. Statistical significance was assessed with a two-way repeated measures ANOVA. **(B)** DNA release from 8-week old Balb/c (left) Balb/c.*Fas*^{lpr} (middle) and MRL.*Fas*^{lpr} (right) as determined by PicoGreen fluorescence. Cells from two mice were pooled and plated in duplicate. Error bars indicate standard deviation of replicates. **(C)** Number (left panel) and percentage (right panel) of neutrophils in the spleens of Nox2-sufficient and deficient mice as determined by FACS at 14 weeks of age.

Fig. S2. Increased spleen weight and expanded myeloid compartment in Nox2-deficient F2 mice. (**A**) Spleen weight in grams as a function of Nox2 genotype. (**B**) FACS analysis of percentage of CD11b^{+} F4/80^{+} Gr1$^{int\text{-}lo}$ macrophages. Each group represents the mean of seven or more mice. Statistical analysis was performed using a two-tailed Mann-Whitney test.

Fig. S3. Altered ANA profile in Nox2-deficient F2 mice. (**A**) Representative ANA patterns seen in plasma from Nox2-sufficient (left) and Nox2-deficient F2 animals (right). (**B**) Dominant ANA pattern quantitated for each genotype in a blinded manner. (**C**) Intensity of cytoplasmic HEp-2 staining quantitated for each genotype on an arbitrary scale of 0-4 by a blinded observer. (**D**) ELISA assessment of Anti-Sm antibodies in the plasma of F2 mice of the indicated

genotype. A two-tailed Chi-Squared test was used to determine statistical significance. The threshold for positivity (indicated by dashed line) was set at 25,000 AU for Anti-Sm.

Fig. S4. Nox2 deficiency has no impact on anti-nucleosome Abs, rheumatoid factor, or IFNα in plasma. ELISA assessment of anti-nucleosome **(A)** and rheumatoid factor Abs **(B)** in plasma. Plasma IFNα was quantified by ELISA **(C)**. All samples were collected at 14 weeks from fully backcrossed animals.

Fig. S5. Increased renal disease in Nox2-deficient F2 mice. (**A**) Proteinuria was assessed using Siemens Albustix$^{(R)}$. (**B**) Glomerulonephritis was scored blindly by M.K. on a scale of 1 to 4, and is represented here as a function of Nox2 genotype in fully backcrossed mice. (**C-D**) Example of a Nox2-sufficient kidney (**C**) and deficient kidney (**D**) at 10X magnification.

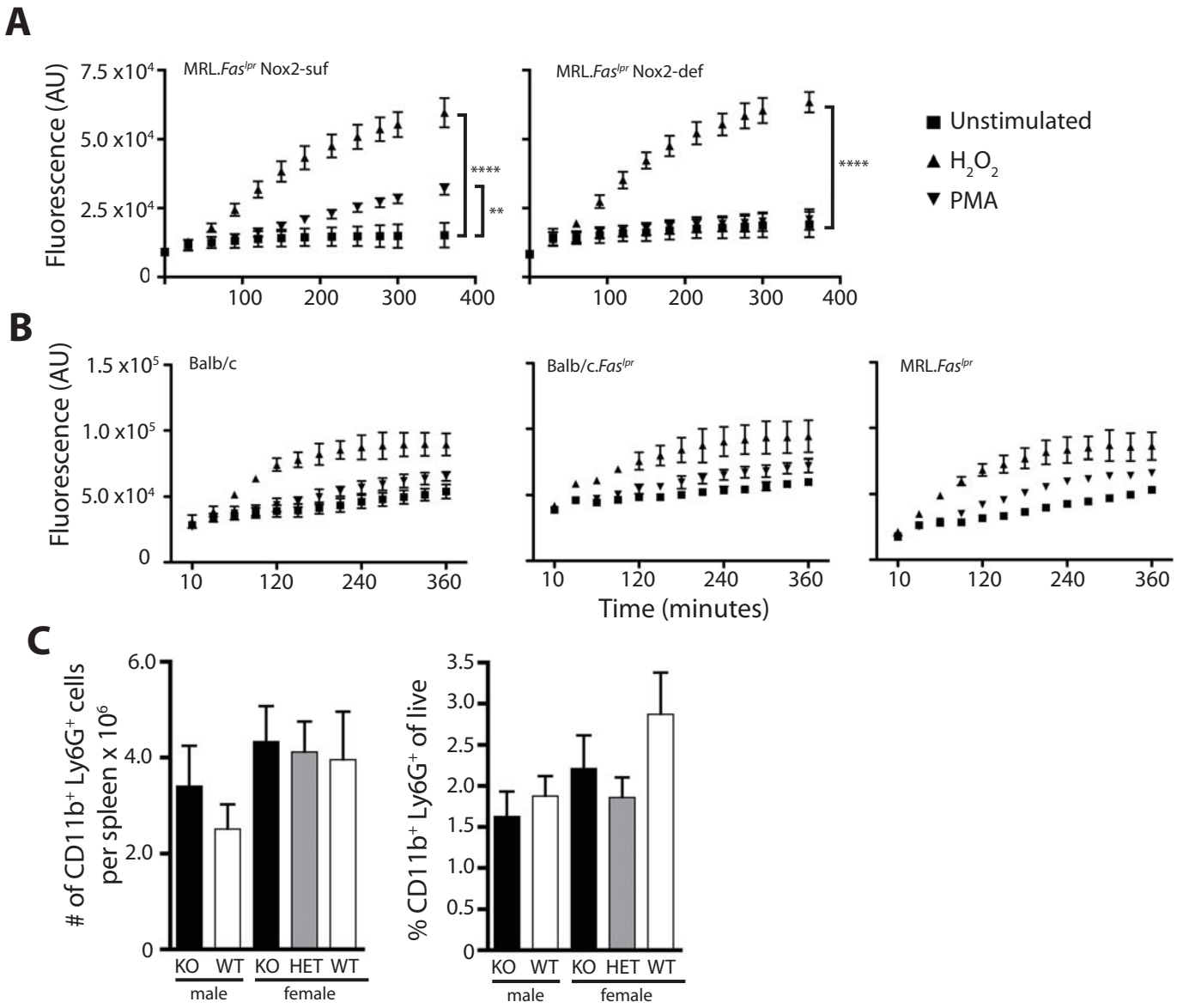
A
MRL.Fas^lpr Nox2-suf
MRL.Fas^lpr Nox2-def
Fluorescence (AU)
7.5 x10^4
5.0 x10^4
2.5 x10^4
0
100
200
300
400
****
**
Unstimulated
H2O2
PMA
B
Balb/c
Balb/c.Fas^lpr
MRL.Fas^lpr
1.5 x10^5
1.0 x10^5
5.0 x10^4
10
120
240
360
Time (minutes)
C
# of CD11b+ Ly6G+ cells per spleen x 10^6
% CD11b+ Ly6G+ of live
KO WT KO HET WT
male
female

Figure S1

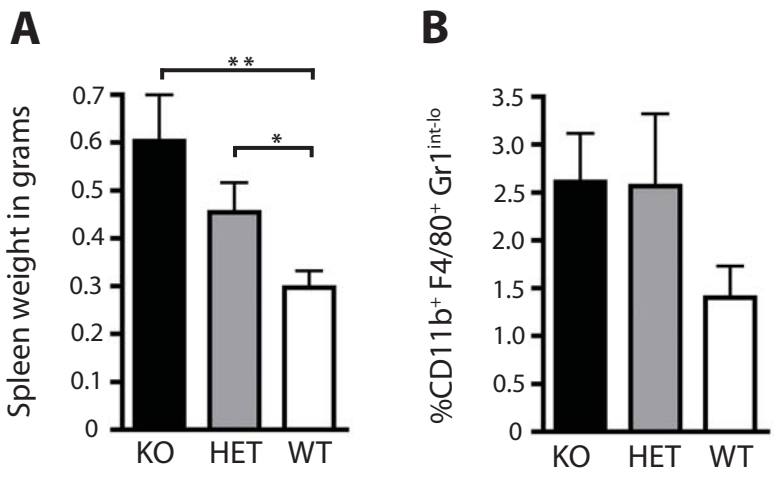
A
B
Spleen weight in grams
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
**
*
KO
HET
WT
%CD11b+ F4/80+ Gr1int-lo
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
KO
HET
WT

Figure S2

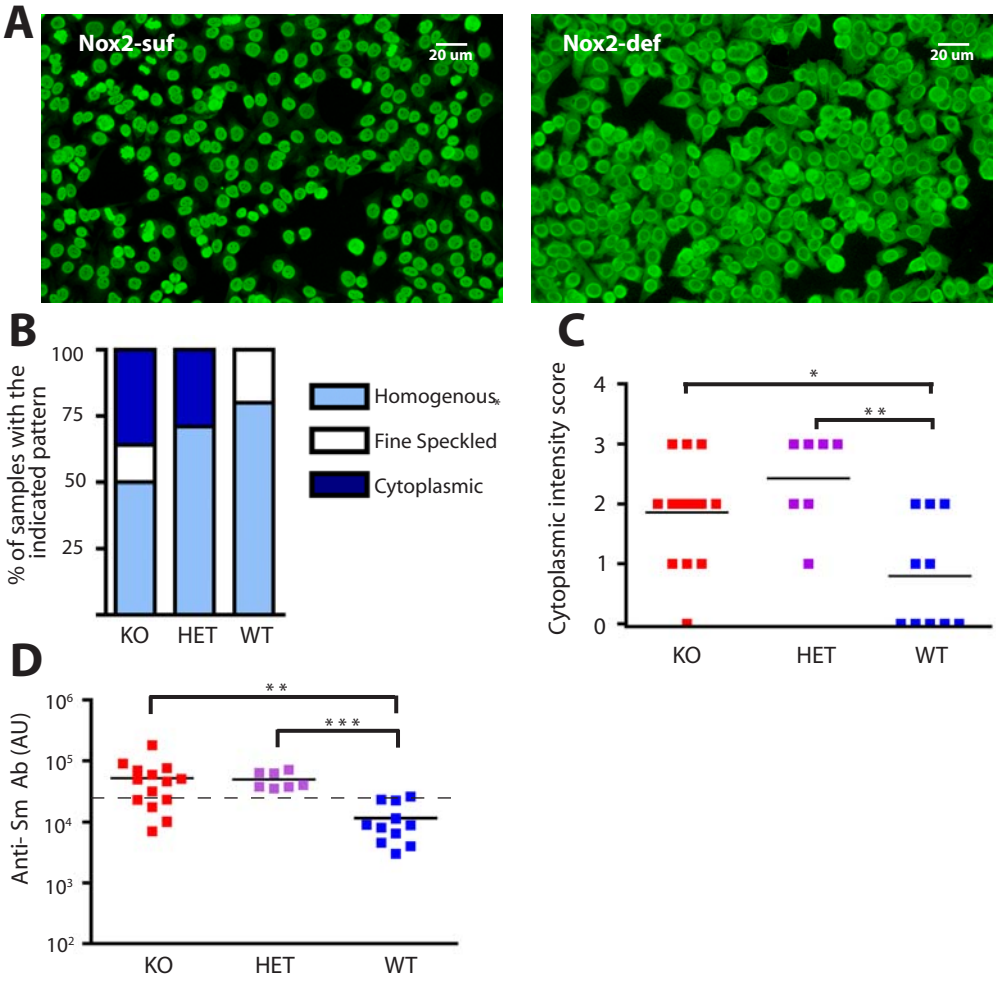
A
Nox2-suf
20 um
Nox2-def
20 um
B
% of samples with the indicated pattern
100
75
50
25
KO
HET
WT
Homogenous
Fine Speckled
Cytoplasmic
C
Cytoplasmic intensity score
4
3
2
1
0
*
**
KO
HET
WT
D
Anti- Sm Ab (AU)
10⁶
10⁵
10⁴
10³
10²
**
***
KO
HET
WT

Figure S3

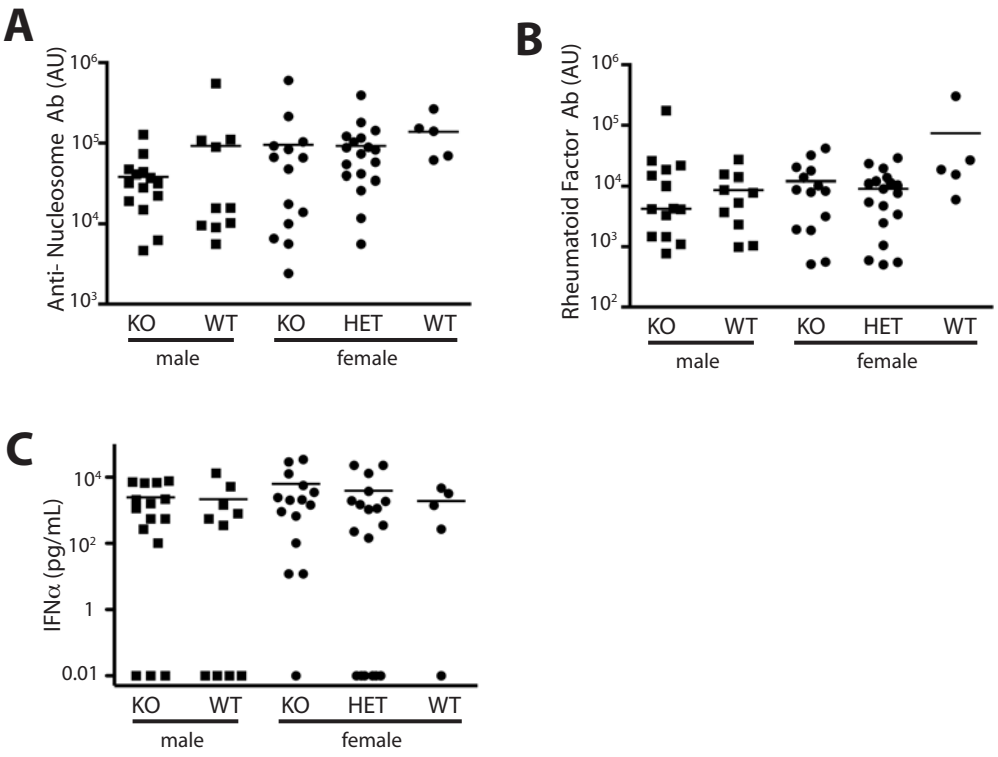
A
Anti- Nucleosome Ab (AU)
10^6
10^5
10^4
10^3
KO
WT
KO
HET
WT
male
female
B
Rheumatoid Factor Ab (AU)
10^6
10^5
10^4
10^3
10^2
KO
WT
KO
HET
WT
male
female
C
IFNα (pg/mL)
10^4
10^2
1
0.01
KO
WT
KO
HET
WT
male
female

Figure S4

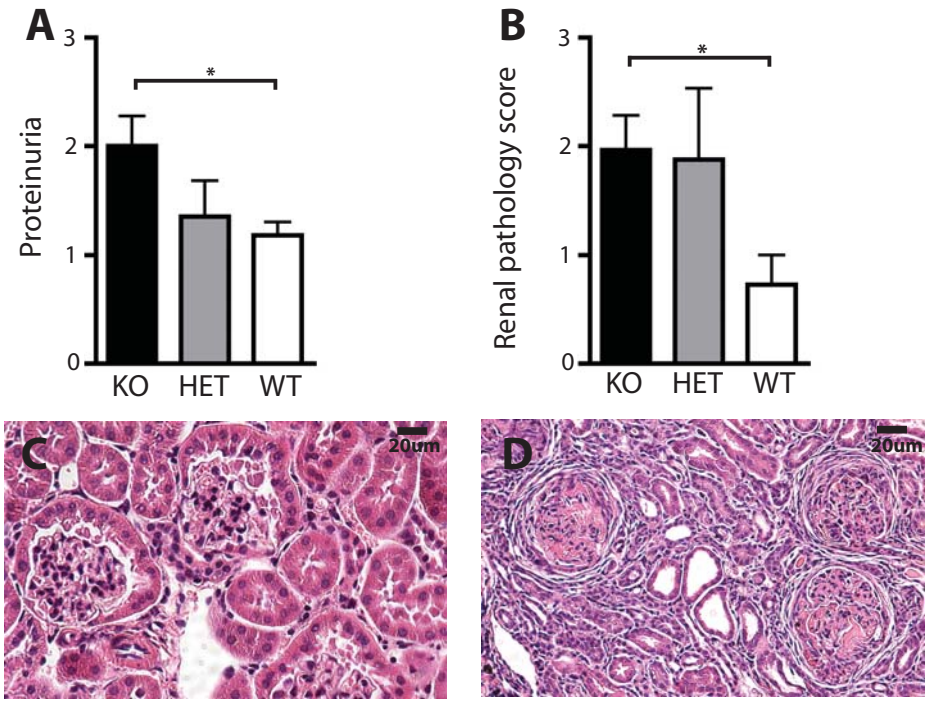
A
Proteinuria
3
2
1
0
*
KO
HET
WT
B
Renal pathology score
3
2
1
0
*
KO
HET
WT
C
20um
D
20um

Figure S5