**Supplementary Information**

# The survival of B cells is compromised in kidney disease

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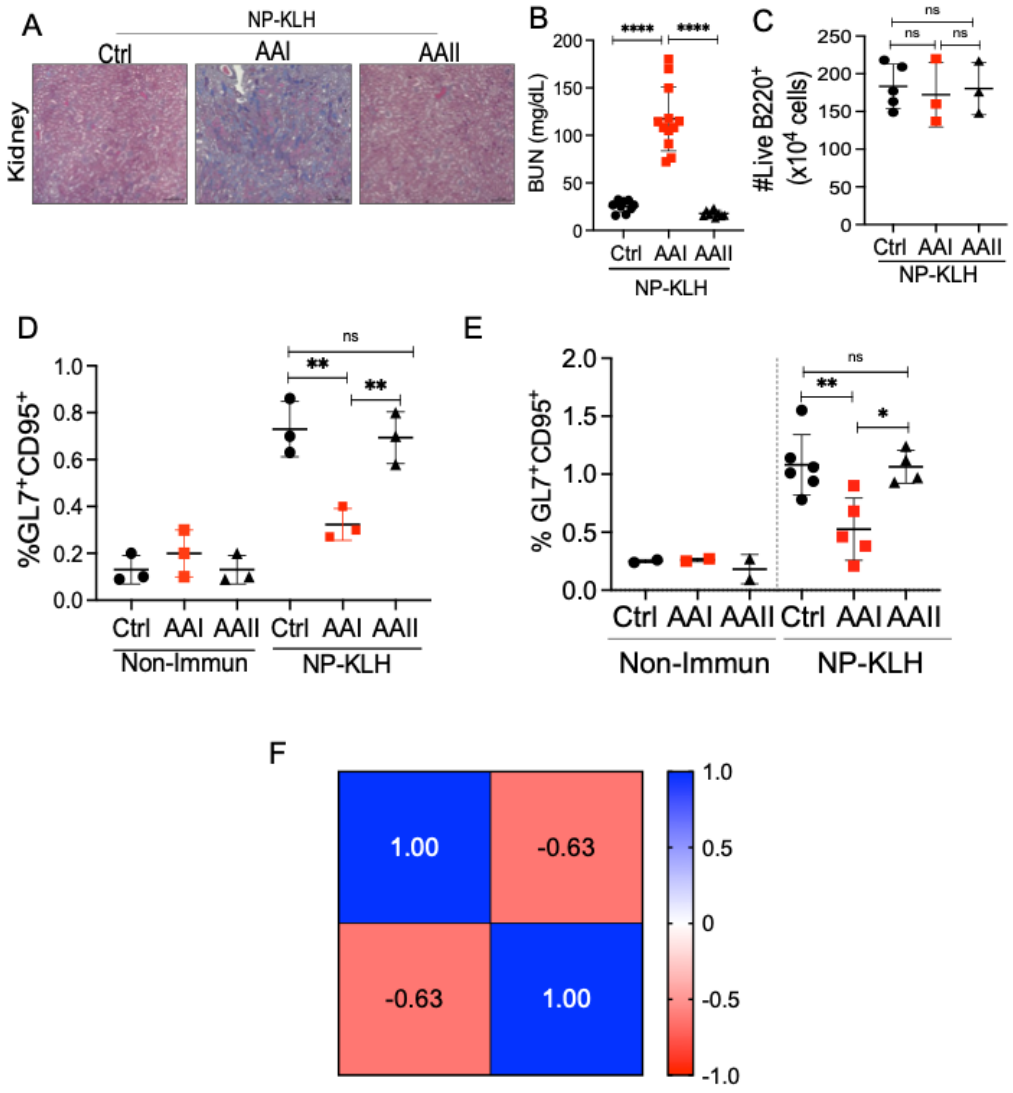
**Supplementary Figures and Figure Legends:**




| | Correlation Variable | Spearman r | Confidence interval (r2) = 95% | P value |
|---|---|---|---|---|
| 1 | BUN (mg/dL) | -0.630689207 | -0.8391 to -0.2608 | 0.002175 |
| 2 | B220+GL7+CD95+ (%) | | | |

***Fig S1: B cell response in NP-KLH immunized mice with kidney disease.*** WT mice were i.p. injected with a single dose of AAI, AAII (7.5 mg/kg b.wt) or PBS (Ctrl). Mice were either immunized with NP-KLH in alum or left non-immunized (non-immun) 4 days post-AAI injection.

At day 12 post-immunization, (**A**) Renal fibrosis (n=6) was evaluated following staining with Masson's trichrome stain of kidney sections. Image representative of 2 independent experiments. Scale bar = 50 μm. (**B**) Serum BUN was assessed by enzymatic assay [Ctrl (9), AAI (12), AAII (9)], (**C**) Total B cells (liveB220$^{+}$) in the bonemarrow were quantified by flow cytometry [Ctrl (5), AAI (3), AAII (3)]. (**D**) Renal lymph nodes were evaluated for total GC B cells. (**E**) At 21 days post-immunization, spleens were assessed for total GC B cells (liveB220$^{+}$GL7$^{+}$CD95$^{+}$) by flow cytometry [Non-immun: Ctrl (2), AAI (2), AAII (2); NP-KLH: Ctrl (6), AAI (5), AAII (4)]. (**F**) Nonparametric Spearman r correlation analysis for BUN (mg/dL) and percentage of GC B cells in NP-KLH immunized mice (n=21). Each dot represents individual mice and data are pooled from at least 2-3 independent experiments (B-E). Data expressed as Mean ± SD. Statistical analyses by One-way ANOVA (B-E) and nonparametric Spearman r correlation analysis (F). (B) **** $P<0.0001$. (D) Ctrl vs. AAI ** $P=0.0043$, AAI vs. AAII ** $P= 0.0065$. (E) Ctrl vs. AAI ** $P=0.0064$, AAI vs. AAII * $P=0.0147$. ns: statistically not significant. Source data are provided as a Source Data file.

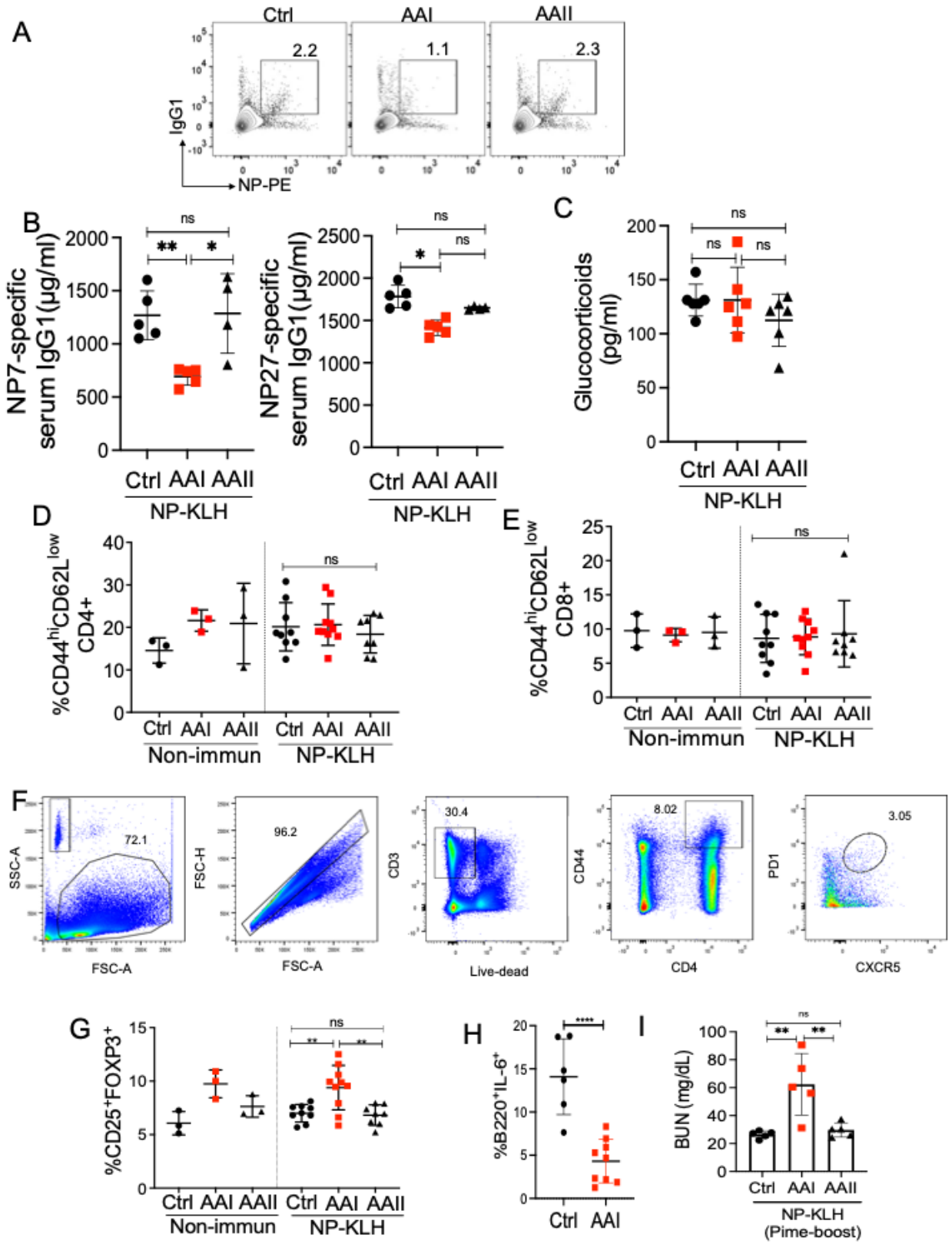

A
Ctrl
AAI
AAII
2.2
1.1
2.3
IgG1
NP-PE
B
NP7-specific serum IgG1(μg/ml)
NP27-specific serum IgG1(μg/ml)
ns
**
*
Ctrl AAI AAII
NP-KLH
C
Glucocorticoids (pg/ml)
D
%CD44hiCD62Llow CD4+
Non-immun
NP-KLH
E
%CD44hiCD62Llow CD8+
F
72.1
SSC-A
FSC-A
96.2
FSC-H
30.4
CD3
Live-dead
8.02
CD44
CD4
3.05
PD1
CXCR5
G
%CD25+FOXP3+
H
%B220+IL-6+
****
I
BUN (mg/dL)
NP-KLH (Pime-boost)

***Fig S2: T cell response in NP-KLH immunized mice with kidney disease.*** WT mice were i.p. injected with a single dose of AAI, AAII (7.5 mg/kg b.wt) or PBS (Ctrl). Mice were either immunized with NP-KLH in alum or left non-immunized (non-immun) 4 days post-AAI injection. At 12 days post-immunization, spleens were assessed for (**A**) NP-specific switched $IgG1^+$ B cells ($liveIgD^-IgM^-CD138^-Gr1^-B220^+NP^+IgG1^+$) by flow cytometry (representative flow plot). (**B**) Serum NP7 and NP27-specific IgG1 by ELISA at day 21 post-immunization [NP7-specific serum IgG1: Ctrl (5), AAI (5), AAII (4) and NP27-specific serum IgG1: Ctrl (5), AAI (5), AAII (4) and NP7/NP27: Ctrl (5), AAI (5), AAII (4)], (**C**) Serum glucocorticoids level by ELISA [Ctrl (6), AAI (6), AAII (6)], (**D**) activated $CD4^+$ T (live $CD4^+CD44^{hi}CD62L^{lo}$) cells [Non-immun: Ctrl (3), AAI (3), AAII (3); NP-KLH Ctrl (9), AAI (10), AAII (8)], (**E**) activated $CD8^+$ T (live $CD8^+CD44^{hi}CD62L^{lo}$) cells by flow cytometry at day 12 post-immunization [Non-immun: Ctrl (3), AAI (3), AAII (3); NP-KLH Ctrl (9), AAI (10), AAII (8)]. (**F**) Representative flow plot showing gating strategy for TFh cells in the spleen of immunized or non-immunized AAN and control mice, and (**G**) percentage of T regulatory ($liveCD4^+CD25^+Foxp3^+$) cells in the spleen was assessed by flow cytometry [Non-immun: Ctrl (3), AAI (3), AAII (3), NP-KLH Ctrl (9), AAI (10), AAII (8)]. (**H**) Intracellular IL-6 staining of purified splenic B cells from NP-KLH immunized AAN and control mice following LPS stimulation at day 12 post-immunization [Ctrl (6), AAI (9)]. (**I**) AAN and control mice were primed with NP-KLH in alum followed by NP-KLH boost 36 days later. The boosted (n=5) mice were evaluated for serum BUN six days later [Ctrl (5), AAI (5), AAII (5)]. Each dot represents individual mice and data pooled from 2-3 experiments (B-E and G-I). Data expressed as Mean ± SD. Statistical analyses by One-way ANOVA (B-E, G and I) and two-sided t-test (H). (B) Ctrl vs. AAI ** P = 0.0088, AAI vs. AAII * P = 0.0105 and Ctrl vs. AAI * P=0.0402. (G) Ctrl vs. AAI ** P=0.0040, AAI vs. AAII ** P=0.0028. (H) Ctrl vs. AAI ****

P<0.0001. (I) Ctrl vs. AAI ** P=0.0026, AAI vs. AAII ** P= 0.0052. ns: statistically not significant. Source data are provided as a Source Data file.

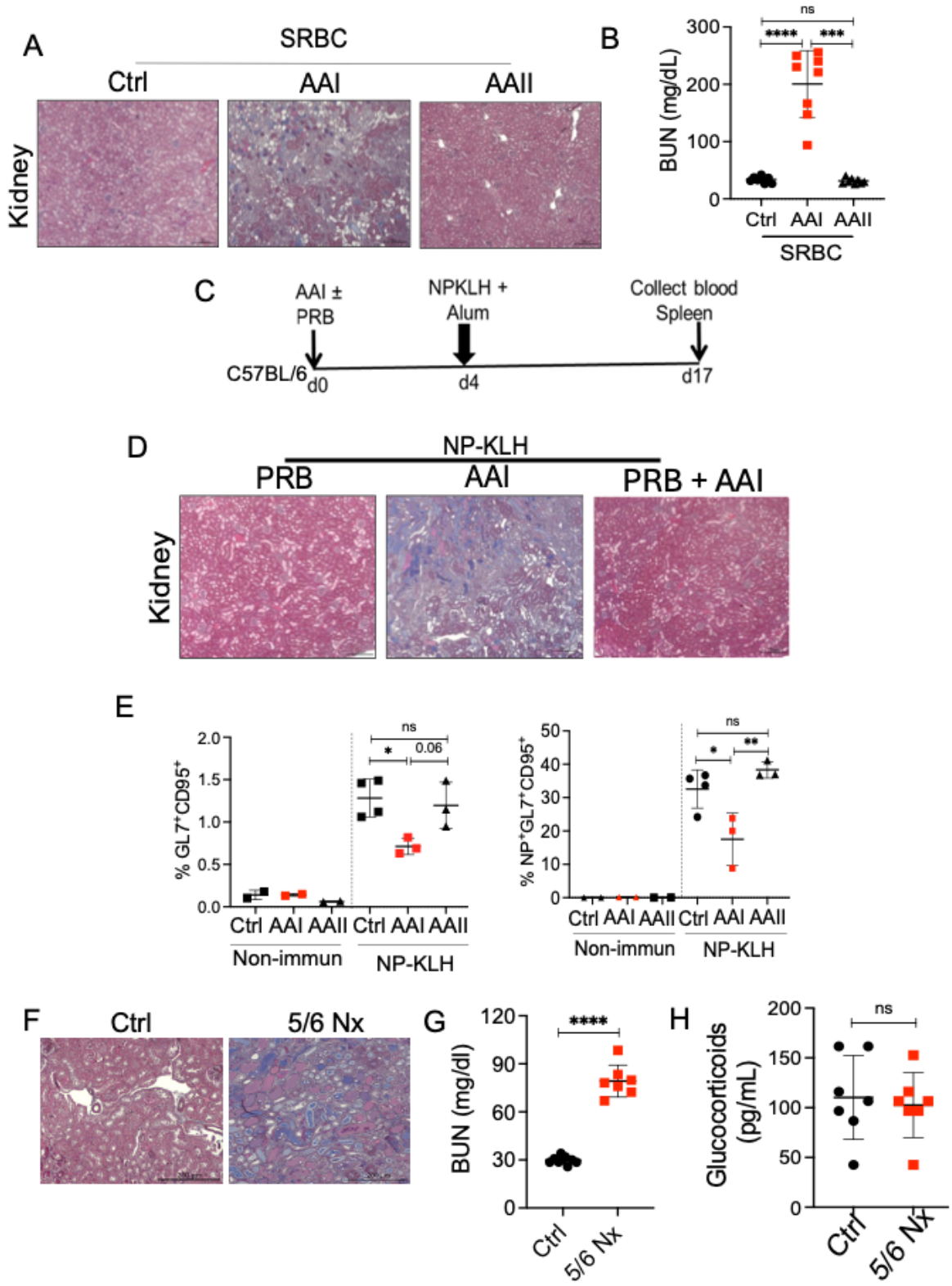

A
SRBC
Ctrl
AAI
AAII
Kidney
B
BUN (mg/dL)
ns
****
***
Ctrl AAI AAII
SRBC
C
AAI ± PRB
NPKLH + Alum
Collect blood Spleen
C57BL/6
d0
d4
d17
D
NP-KLH
PRB
AAI
PRB + AAI
Kidney
E
% GL7+CD95+
ns
*
0.06
Ctrl AAI AAII Ctrl AAI AAII
Non-immun
NP-KLH
% NP+GL7+CD95+
ns
*
**
Ctrl AAI AAII Ctrl AAI AAII
Non-immun
NP-KLH
F
Ctrl
5/6 Nx
G
BUN (mg/dl)
****
Ctrl
5/6 Nx
H
Glucocorticoids (pg/mL)
ns
Ctrl
5/6 Nx

***Fig S3: B cell response following SRBC immunization and 5/6 nephrectomy.*** AAN and control mice were i.p. immunized with 2.5% SRBC (n=8) 4 days post-AAI injection. (**A**) Renal fibrosis was evaluated following staining with Masson's trichrome stain of kidney sections at day 7 post-immunization (n=4). Image representative of 2 independent experiments. Scale bar = 50 μm. (**B**) Serum BUN was assessed at day 7 post-immunization [Ctrl (8), AAI (8), AAII (8)]. (**C**) Schematic representation of the probenecid treatment experiment. AAN mice were either treated with probenecid (PRB+AAI) once on day 0 (relative to AAI injection) or left untreated (n=8-10). Control mice received probenecid only (PRB). Four days later, mice received NP-KLH immunization and (**D**) renal fibrosis was assessed following Masson's trichome staining of kidney sections (Scale bar = 50 μm) (n=4). (**E**) C57BL/6 (WT) mice were i.p. injected with a single dose of AAI, AAII (7.5 mg/kg b.wt) or PBS (Ctrl). Mice were either immunized with NP-KLH in alum or left non-immunized (non-immun) 10 days post-AAI injection. At 12 days post-immunization, spleens were assessed for total GC B cells (liveB220$^{+}$GL7$^{+}$CD95$^{+}$) and NP-specific GC B cells (liveB220$^{+}$ NP$^{+}$GL7$^{+}$CD95$^{+}$) by flow cytometry [Non-immun: Ctrl (2), AAI (2), AAII (2); NP-KLH: Ctrl (4), AAI (3), AAII (3) and Non-immun: Ctrl (2), AAI (2), AAII (2); NP-KLH: Ctrl (4), AAI (3), AAII (3)]. WT mice were either subjected to 5/6 nephrectomy (5/6 Nx) or sham-operated (Ctrl). (**F**) Renal fibrosis was evaluated following staining with Masson's trichrome stain of 5/6 nephrectomized or control kidney sections at two weeks post-surgery (n=4). Image representative of 2 independent experiments. Scale bar = 50 μm. (**G**) Two weeks after surgery, 5/6 nephrectomized mice were evaluated for kidney function by measuring serum BUN by enzymatic analysis [Ctrl (9), 5/6 Nx (7)]. (**H**) Serum glucocorticoids level was measured in 5/6 nephrectomized mice at day 12 post-immunization [Ctrl (7), 5/6 Nx (7)]. Each dot represents individual mice and data pooled from 2 experiments (B, E, G, H). Data expressed as Mean ± SD.

Statistical analysis by one-way ANOVA (B, E) and two-sided t-test (G, H). (B) Ctrl vs AAI **** P<0.0001, AAI vs. AAII *** P=0.0002. (E) Ctrl vs. AAI * P=0.0240 and Ctrl vs. AAI * P=0.0265, AAI vs. AAII ** P= 0.0075. (G) Ctrl vs. 5/6 Nx **** P<0.0001. ns: statistically not significant. Source data are provided as a Source Data file.

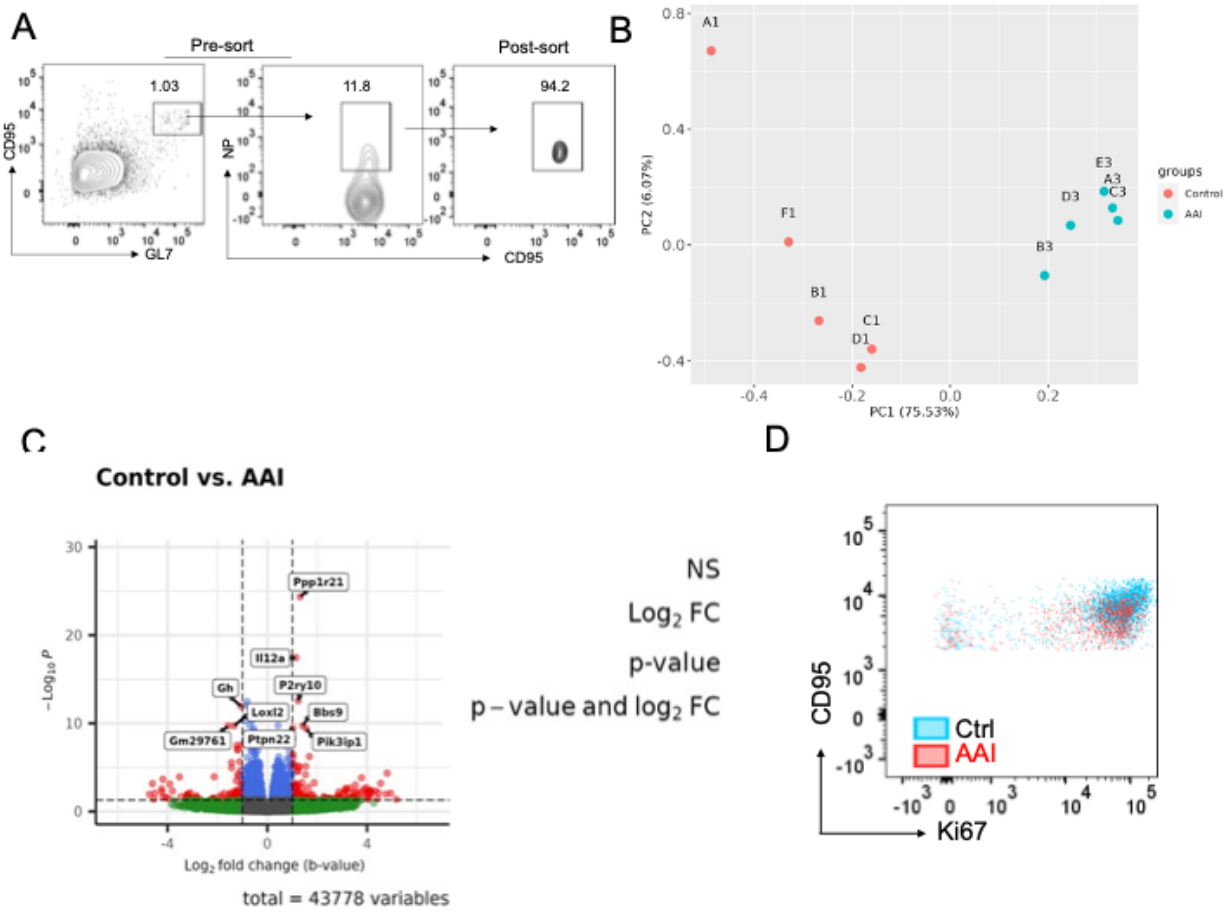



***Fig S4: Flow sorting of NP-specific GC B cells and RNA-Seq analysis.*** **(A)** NP-specific GC (live $NP^{+}B220^{+}$ $GL7^{+}CD95^{+}$) B cells from control and AAN mice (n=5) were flow sorted from spleen on day 12 post-immunization and subjected to RNA-seq analysis. Representative contour plot showing pre- and post-sorting gating strategy and post sort-purity. (**B**) Principal component analysis (PC1/PC2) of AAN and control NP-specific GC B cells. (**C**) The volcano plot showing differentially expressed genes between NP-specific GC B cells from mice with kidney disease and control after NP-KLH immunization. (**D**) $Ki67^{+}$ GC B cells (n=5-7) was analyzed at day 12 post-immunization by flow cytometry. Flow cytometry plot representative of two independent experiments. Source data are provided as a Source Data file.

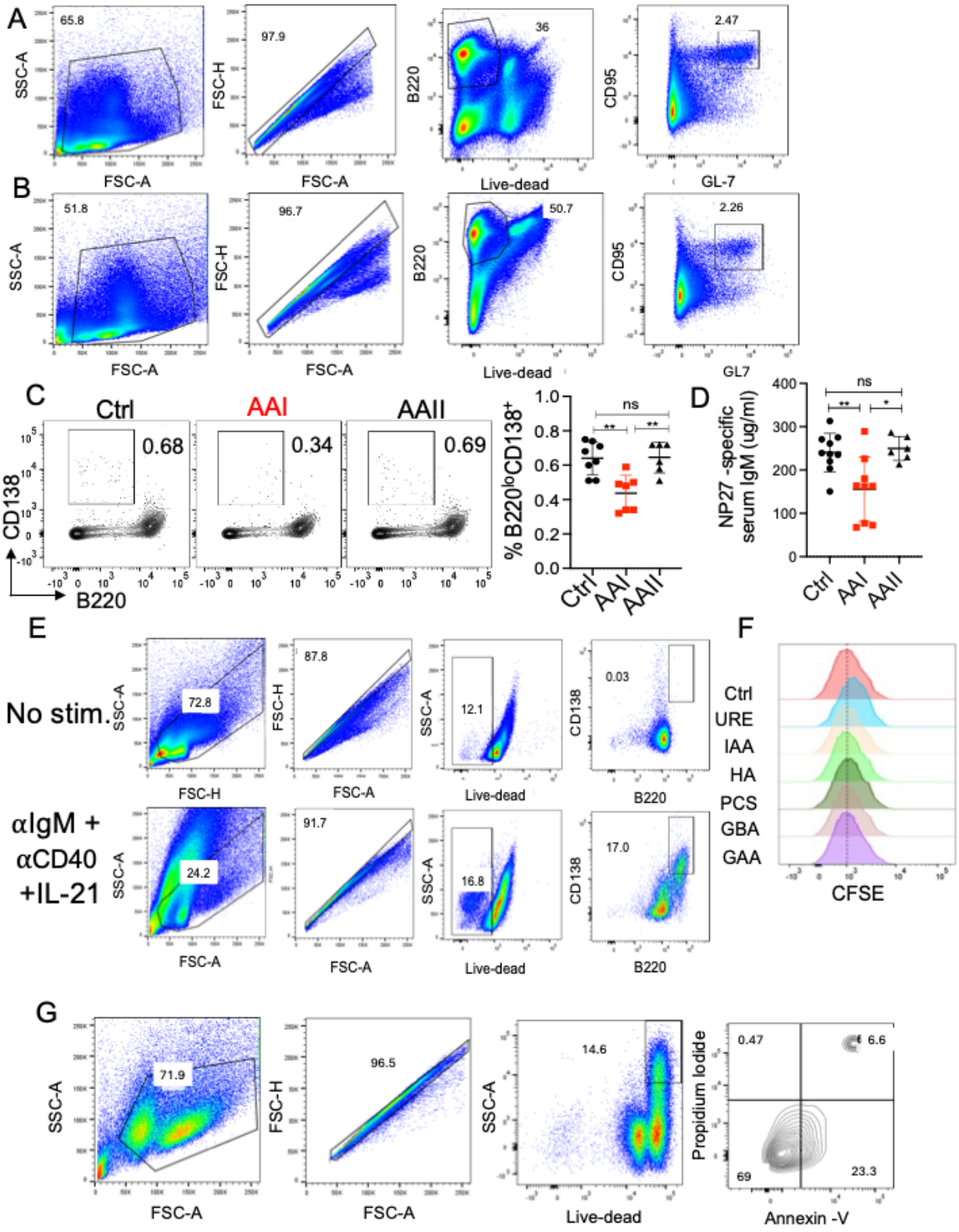

A
65.8
SSC-A
FSC-A
97.9
FSC-H
FSC-A
36
B220
Live-dead
2.47
CD95
GL-7
B
51.8
SSC-A
FSC-A
96.7
FSC-H
FSC-A
50.7
B220
Live-dead
2.26
CD95
GL7
C
Ctrl
AAI
AAII
0.68
0.34
0.69
CD138
B220
% B220loCD138+
ns
**
**
Ctrl
AAI
AAII
D
NP27 -specific serum IgM (ug/ml)
ns
**
*
Ctrl
AAI
AAII
E
No stim.
72.8
SSC-A
FSC-H
87.8
FSC-H
FSC-A
12.1
SSC-A
Live-dead
0.03
CD138
B220
αIgM +
αCD40
+IL-21
24.2
SSC-A
FSC-A
91.7
FSC-A
FSC-A
16.8
SSC-A
Live-dead
17.0
CD138
B220
F
Ctrl
URE
IAA
HA
PCS
GBA
GAA
CFSE
G
71.9
SSC-A
FSC-A
96.5
FSC-H
FSC-A
14.6
SSC-A
Live-dead
Propidium Iodide
0.47
6.6
69
23.3
Annexin -V

***Fig S5: Uremic toxins have negative impact on in vitro B cell differentiation.*** WT mice were i.p. injected with a single dose of AAI, AAII (7.5 mg/kg b.wt) or PBS (control). Mice were either immunized with NP-KLH in alum or left non-immunized (non-immun) 4 days post-AAI injection. At 12 days post-immunization, spleens were assessed for the loss of mitochondrial membrane potential (TMRE staining) and mitoROS (MitoSox Red) production in GC B cells. Representative flow plots showing gating strategy employed for (**A**) TMRE and (**B**) MitoSox Red staining. AAN and control mice were immunized with NP-KLH in alum 4 days post-AAI injection. Four days later mice were evaluated for (**C**) plasmablasts (live$B220^{lo}CD138^{+}$) by flow cytometry [Ctrl (8), AAI (7), AAII (6)] and (**D**) serum NP27-specific IgM antibody titer by ELISA [Ctrl (10), AAI (9), AAII (6)]. **(E)** WT resting splenic B cells ± uremic toxins were stimulated with αIgM/αCD40/IL-21 and the number of $B220^{+}CD138^{+}$ plasmablasts was determined by flow cytometry at 96 h. Representative flow plots showing gating strategy for determining the plasmablast frequency. (**F**) CFSE-labeled splenic B cells were stimulated with αIgM/αCD40/IL-21 ± uremic toxins (as indicated in the figure) and proliferation of $B220^{+}CD138^{+}$ plasmablasts was assessed at 48 h post-stimulation. **(G)** Representative flow plots showing gating strategy for assessing the frequency of apoptotic cells within the plasmablast population. Each dot represents individual mice and data are pooled from at least 2-3 independent experiments (C and D). (C) Ctrl vs. AAI ** P= 0.0021, AAI vs. AAII ** P= 0.0032. (D) Ctrl vs. AAI ** P= 0.0074, AAI vs. AAII * P= 0.016. Data expressed as Mean ± SD. Statistical analyses by One-way ANOVA (C and D). ns: statistically not significant. Source data are provided as a Source Data file.

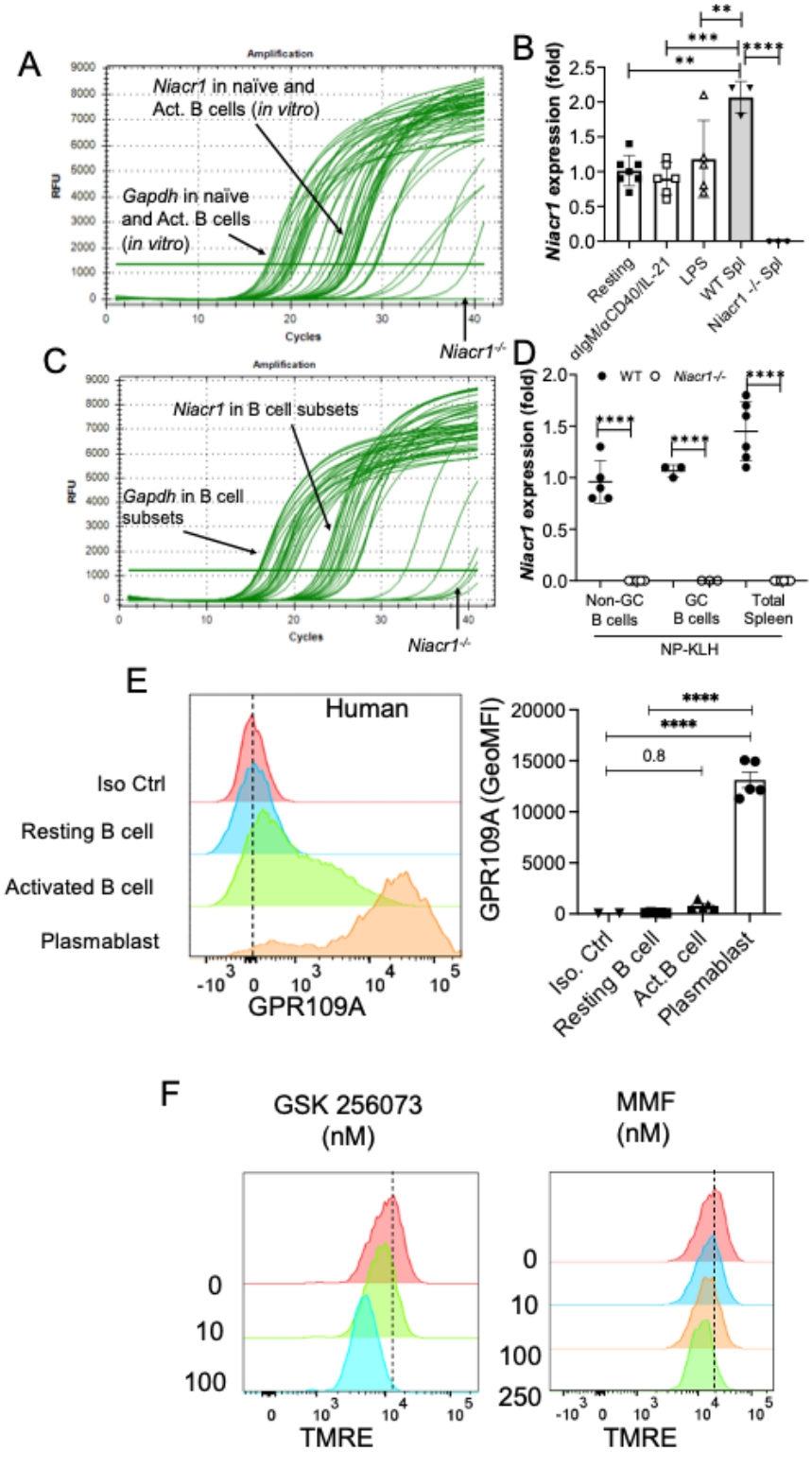

A
Amplification
Niacr1 in naïve and Act. B cells (in vitro)
Gapdh in naïve and Act. B cells (in vitro)
RFU
Cycles
Niacr1-/-
B
Niacr1 expression (fold)
Resting
αIgM/αCD40/IL-21
LPS
WT Spl
Niacr1 -/- Spl
C
Niacr1 in B cell subsets
Gapdh in B cell subsets
D
WT
Niacr1-/-
Non-GC B cells
GC B cells
Total Spleen
NP-KLH
E
Human
Iso Ctrl
Resting B cell
Activated B cell
Plasmablast
GPR109A
GPR109A (GeoMFI)
0.8
Iso. Ctrl
Resting B cell
Act.B cell
Plasmablast
F
GSK 256073 (nM)
MMF (nM)
TMRE

***Fig S6: Niacr1 gene expression in mouse and human B cells.*** Resting splenic B cells ± αIgM/αCD40/IL-21 or LPS were assessed for *Niacr1* mRNA expression by RT-qPCR at 24 h. Total splenocytes from WT and *Niacr1*$^{-/-}$ mice were used as positive and negative controls, respectively. **(A)** Representative real time qPCR amplification plot and (**B**) data expressed as fold change [Resting (7), αIgM/αCD40/IL-21 (6), LPS (5), WT spleen (3), *Niacr1*$^{-/-}$ spleen (3)]. *Niacr1* transcript expression was assessed by RT-qPCR on NP-KLH immunized and FACS-sorted non-GC (liveB220$^{+}$GL7$^{-}$CD95$^{-}$), GC (liveB220$^{+}$GL7$^{+}$CD95$^{+}$) (2 mice pooled together) and total spleen cells from WT and *Niacr1*$^{-/-}$ mice) at day 12 post-immunization. **(C)** Representative real time qPCR amplification plot and (**D**) data expressed as fold change [Non-GC B cells WT (5), *Niacr1*$^{-/-}$ (5), GC B cells WT (3), *Niacr1*$^{-/-}$ (3), Total spleen WT (6), *Niacr1*$^{-/-}$ (6)]. **(E)** Healthy donor B cells (n=5) ± αIgM/sCD40L/IL-21 and GPR109A expression was evaluated at 24 h by flow cytometry [Iso Ctrl (2), Resting B cell (5), Act.B cell (5), Plasmablast (5)]. Splenic B cells were stimulated with αIgM/αCD40/IL-21 ± **(F)** GSK256073 or ± MMF (GPR109A agonists) at indicated concentrations and the loss of mitochondrial membrane potential was evaluated (TMRE staining) at 24 h by flow cytometry. Each dot represents individual mice (B and D), or donor (E) and data are pooled from at least 2-3 independent experiments (B, D). (B) Resting vs WT spleen ** P= 0.008, αIgM/αCD40/IL-21 vs. WT spleen *** P= 0.0004, LPS vs. WT spleen ** P= 0.0042, WT vs. *Niacr1*$^{-/-}$ spleen **** P <0.0001. (D) **** P <0.0001. (E) Iso Ctrl vs. Act. B cell ** P=0.0049, Iso Ctrl vs. Plasmablast **** P<0.0001, Resting B cell vs. Plasmablast **** P<0.0001. Data expressed as Mean ± SD. Statistical analyses by One-way ANOVA (B, D and E). Source data are provided as a Source Data file.

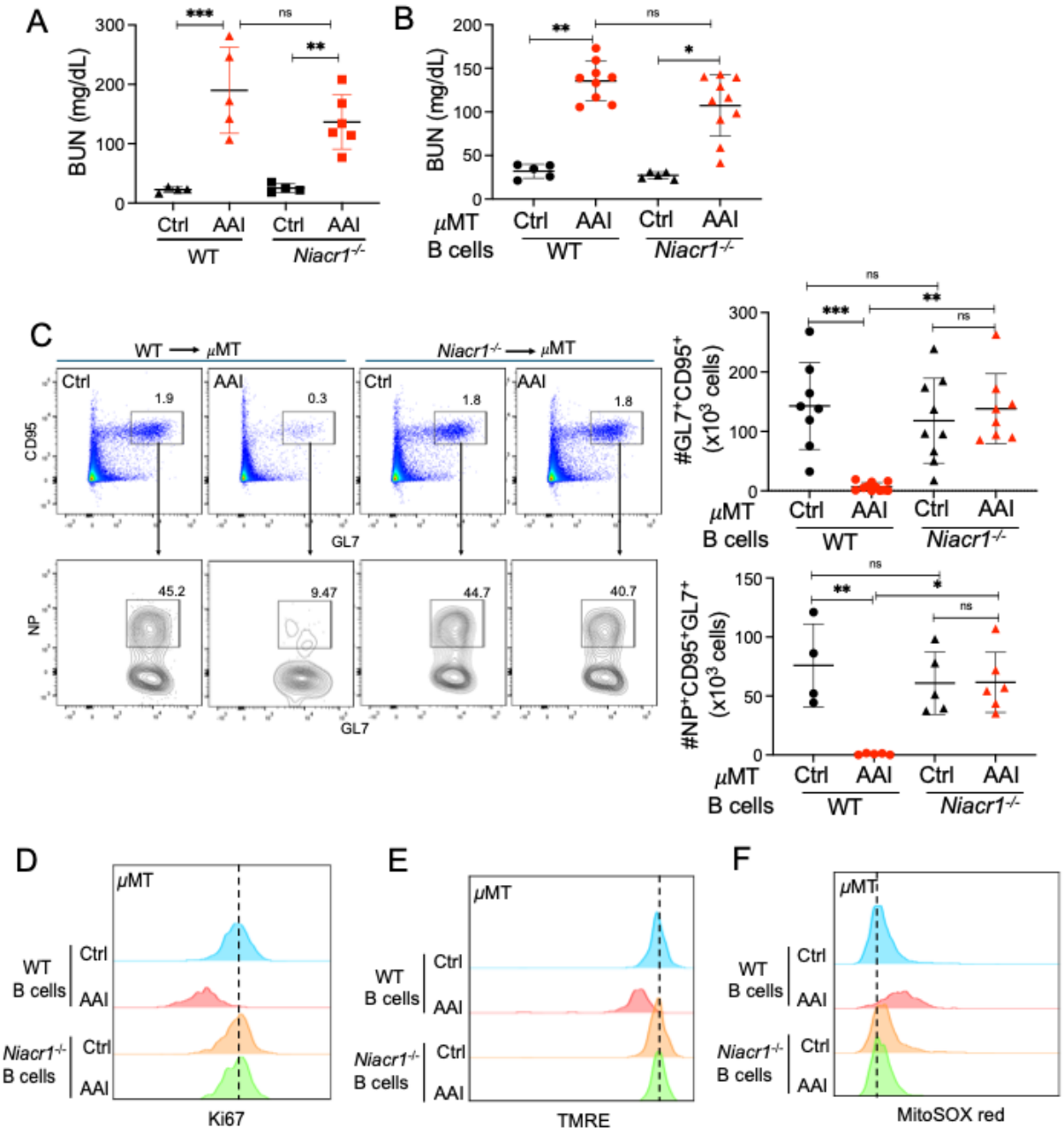



***Fig S7: HA-GPR109A-axis in B cells regulates apoptosis in kidney disease.*** (**A**) WT and *Niacr1*$^{-/-}$ mice were subjected to AAN followed by NP-KLH immunization. Serum BUN was measured in WT and *Niacr1*$^{-/-}$ mice at day 12 post-immunization by enzymatic assay [WT Ctrl (4), AAI (5), *Niacr1*$^{-/-}$ Ctrl (4), AAI (6)]. Control and AAI-injected $\mu$MT mice receiving either WT or *Niacr1*$^{-/-}$

resting splenic B cells were evaluated for (**B**) serum BUN [WT Ctrl (5), AAI (9), *Niacr1*$^{-/-}$ Ctrl (5), AAI (10)] and (**C**) absolute number of total and NP-specific GC B cells [WT Ctrl (8), AAI (11), *Niacr1*$^{-/-}$ Ctrl (9), AAI (8) and WT Ctrl (4), AAI (5), *Niacr1*$^{-/-}$ Ctrl (5), AAI (7)], (**D**) proliferation, (**E**) loss of mitochondrial membrane potential, and (**F**) mitochondrial ROS generation by total GC B cells at day 12 post-immunization (representative histogram). Each dot represents individual mice and data are pooled from at least 2-3 independent experiments (A-F). Data expressed as Mean ± SD (A-C). Statistical analyses by One-way ANOVA (A-C). (A) WT Ctrl vs. AAI *** P=0.0004, *Niacr1*$^{-/-}$ Ctrl vs. AAI ** P=0.0091. (B) WT Ctrl vs. AAI ** P=0.0050, *Niacr1*$^{-/-}$ Ctrl vs. AAI * P=0.0252. (C) WT Ctrl vs. AAI *** P=0.0003, *Niacr1*$^{-/-}$ Ctrl vs. AAI ** P=0.0025 and WT Ctrl vs. AAI ** P=0.0031, *Niacr1*$^{-/-}$ Ctrl vs. AAI * P=0.033. ns: statistically not significant. Source data are provided as a Source Data file.

**Supplementary Table:**

**Table S1: Demographic information of healthy participants (n=8)**

| ***Total number of healthy human participants*** | ***Race*** | ***Gender*** | ***Age*** |
|---|---|---|---|
| 8 | 3 Caucasians, 3 Asians, 1 African American and 1 Hispanic | 3 Female and 5 Male | 40.25 ± 8.3 |