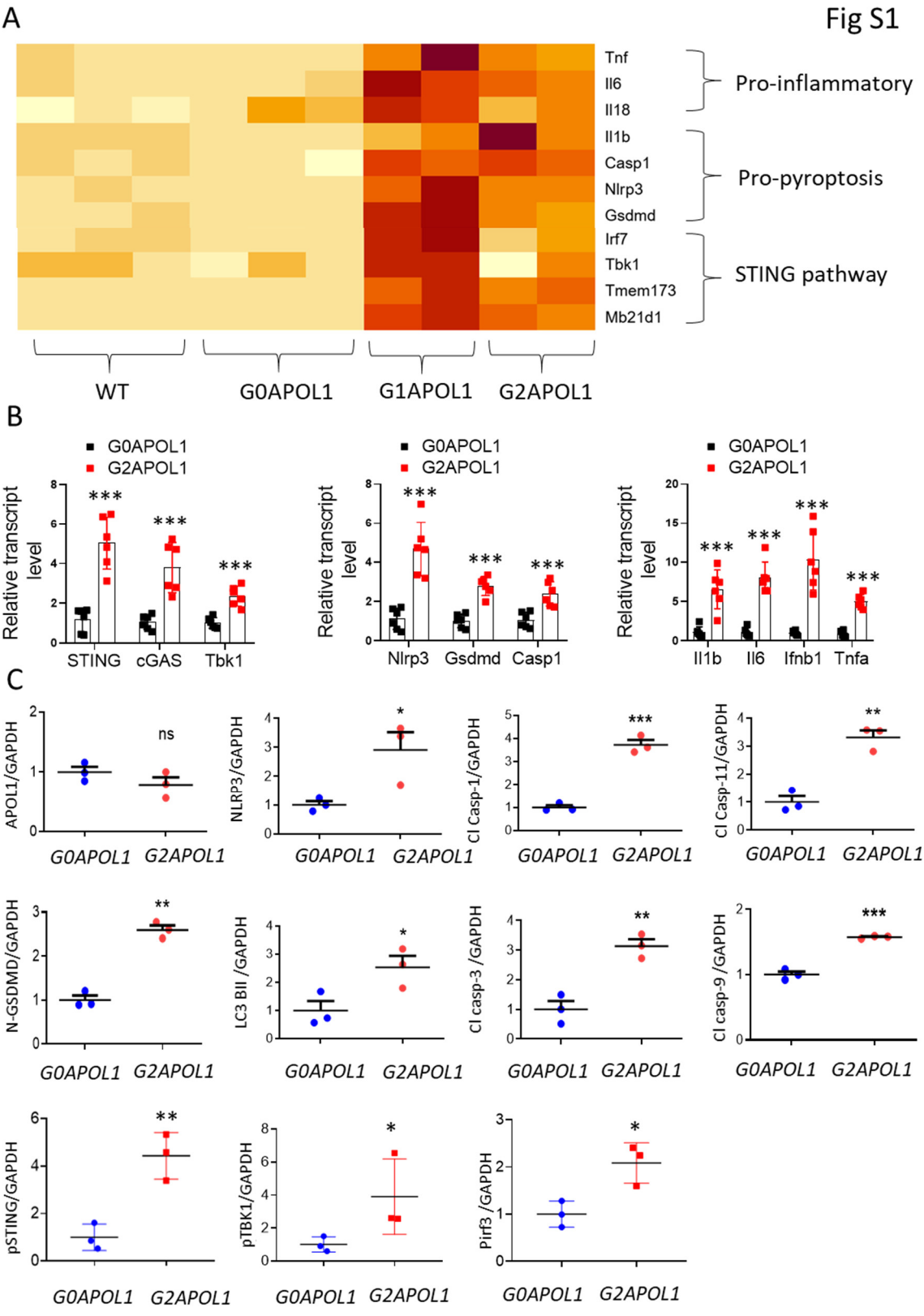
Fig S1


A
Tnf
Il6
Il18
Il1b
Casp1
Nlrp3
Gsdmd
Irf7
Tbk1
Tmem173
Mb21d1
Pro-inflammatory
Pro-pyroptosis
STING pathway
WT
G0APOL1
G1APOL1
G2APOL1
B
G0APOL1
G2APOL1
Relative transcript level
STING
cGAS
Tbk1
Nlrp3
Gsdmd
Casp1
Il1b
Il6
Ifnb1
Tnfa
C
APOL1/GAPDH
ns
NLRP3/GAPDH
Cl Casp-1/GAPDH
Cl Casp-11/GAPDH
N-GSDMD/GAPDH
LC3 BII /GAPDH
Cl casp-3 /GAPDH
Cl casp-9 /GAPDH
pSTING/GAPDH
pTBK1/GAPDH
Pirf3 /GAPDH

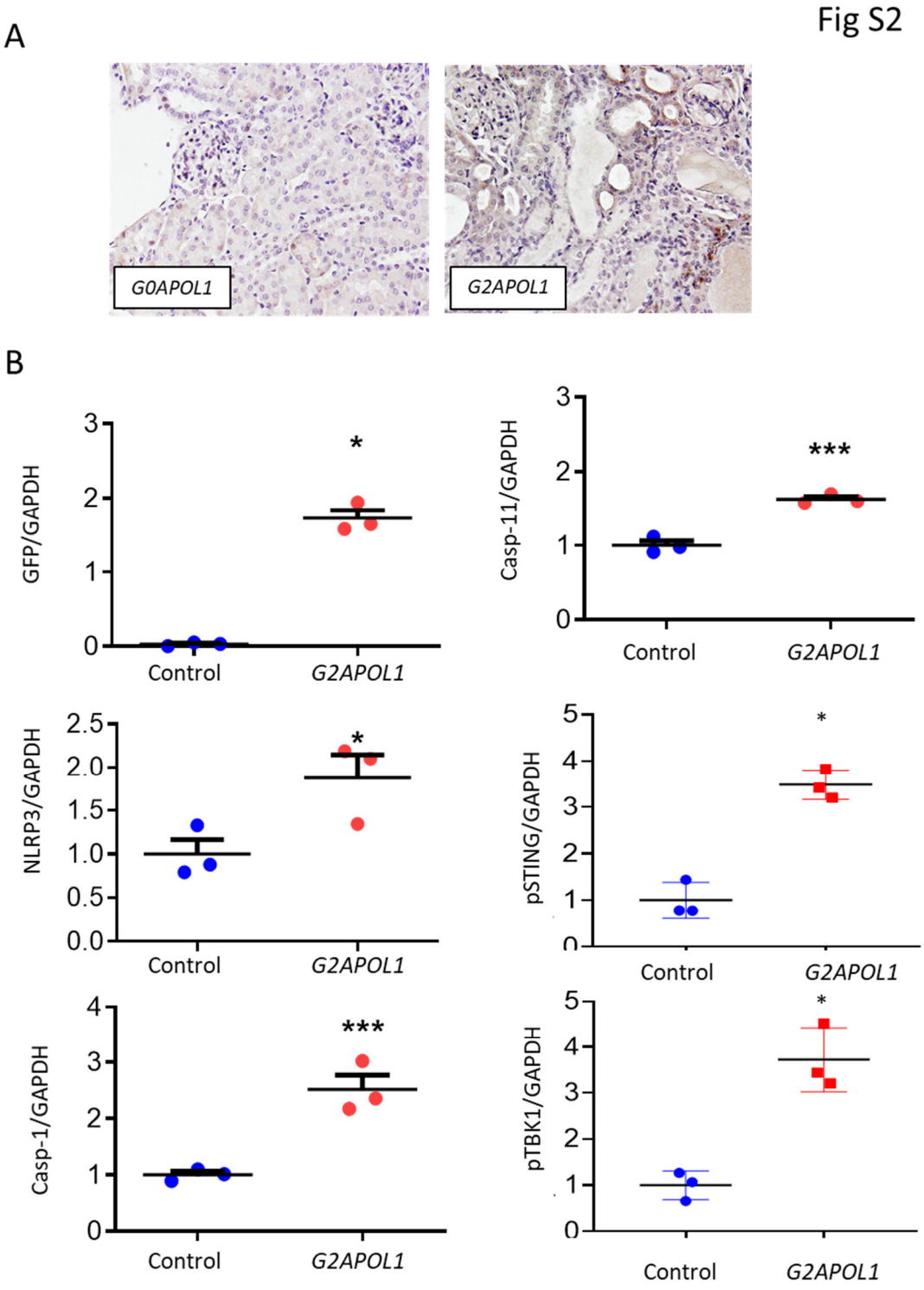
Supplemental Fig.1. Podocyte specific APOL1 risk allele expression activates cell death pathways in mice kidneys.

A. Heatmap of RNA-seq based gene expression in kidneys of wild type, *Nphs1*-rtTA/TRE-*APOL1*-G0/G1/G2 mice

B. Relative transcript level of *STING, cGAS, Tbk1, Nlrp3, Gsdmd, Casp1, Il1b, Il6, Ifnb1* and *Tnfa* in *G20POL1* and *G2APOL1* mice analyzed by QRT-PCR. ***p<0.001 vs *G0APOL1.*

C. Densitometric quantification of the expression of APOL1, NLRP3, Cleaved caspase-1 and LC3-II, Cleaved caspase-3 and Cleaved caspase-9, p-STING and p-TBK1, normalized to GAPDH between *Nphs1-rtTA/TREG0APOL1-GFP* (*G0APOL1*) and *Npsh1-rtTA/TREG2APOL1-GFP* (*G2APOL1*) mice. N = 3; *p <0.05 , **p<0.01, and ***p<0.001 vs *G0APOL1*.

Fig S2

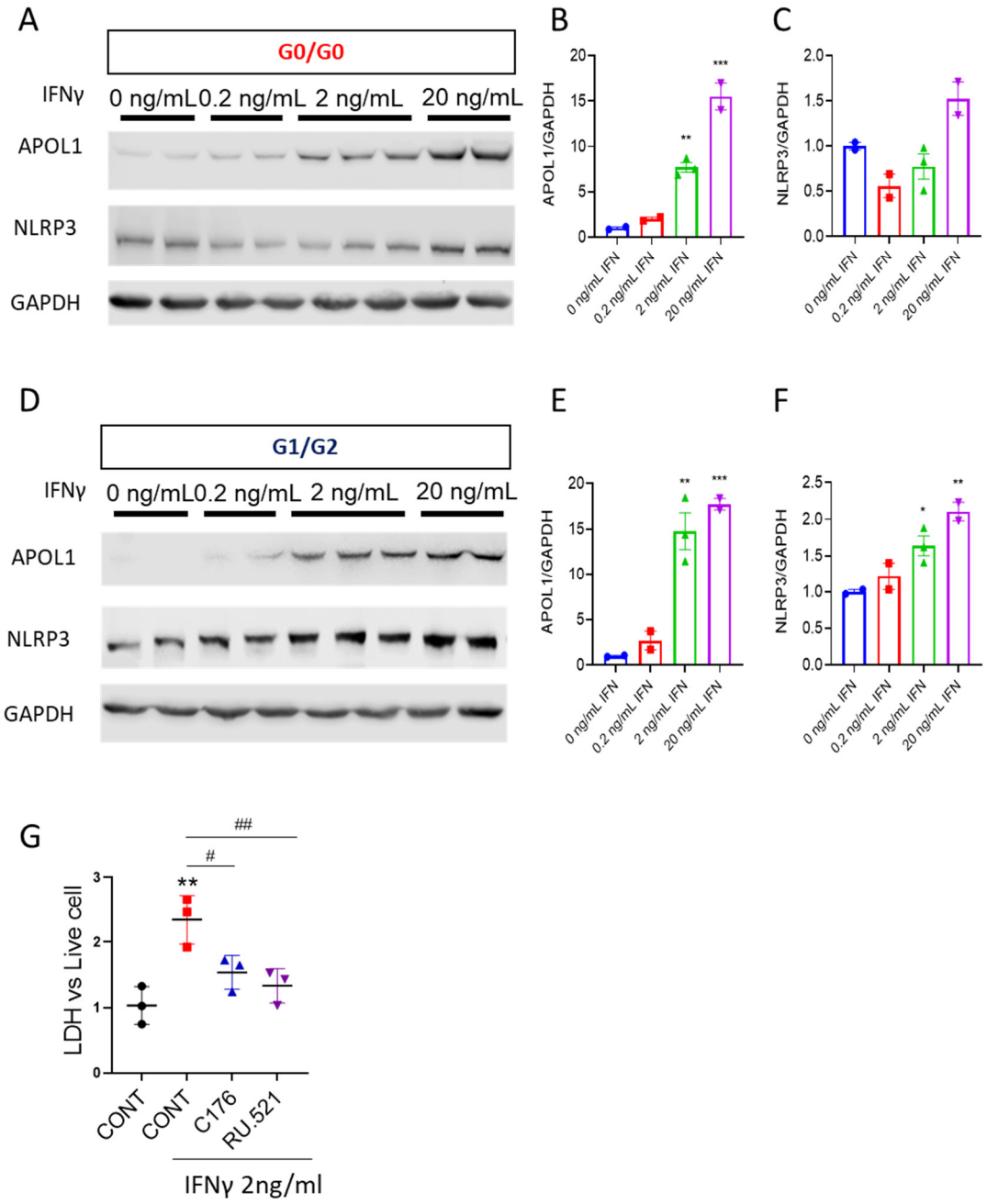


Supplemental Fig.2. Inflammasome activation in kidneys of podocyte specific APOL1 risk allele mice

A. Immunostaining of cleaved caspase-3 in *G0APOL1* and *G2APOL1* mice. Brown staining indicates cleaved caspase-3 expression. Scale bar =10µm.

B. Densitometric quantification of the expression of GFP (proxi for APOL1), NLRP3, Cleaved caspase-1, and Cleaved caspase-11, p-STING and p-TBK1, normalized to GAPDH in the glomeruli of Control and *G2APOL1* mice given doxycycline diet for 3 days to induce APOL1 expression. N = 3; *p <0.05, and ***p<0.001 vs Control.

Fig S3


A
G0/G0
IFNγ
0 ng/mL
0.2 ng/mL
2 ng/mL
20 ng/mL
APOL1
NLRP3
GAPDH
B
APOL1/GAPDH
C
NLRP3/GAPDH
D
G1/G2
E
F
G
LDH vs Live cell
CONT
CONT
C176
RU.521
IFNγ 2ng/ml

Supplemental.Fig.3. *APOL1*-induced cytotoxicity in high-risk human podocytes is inflammasome and STING dependent.

A. Representative western blots of APOL1, NLRP3, and GAPDH from G0/G0 and G1/G2 HUPEC cells treated with vehicle (0ng/ml IFN γ), or 0.2ng/ml, 2ng/ml, and 20 ng/ml IFN γ for 24h.

B. Densitometric quantification of APOL1 normalized to GAPDH in G0/G0 HUPEC cells. N = 2-3 independent experiments. *p<0.05, ***p< 0.001 vs G0/G0.

C. Densitometric quantification of NLRP3 normalized to GAPDH in G0/G0 HUPEC cells. N = 2-3 independent experiments.

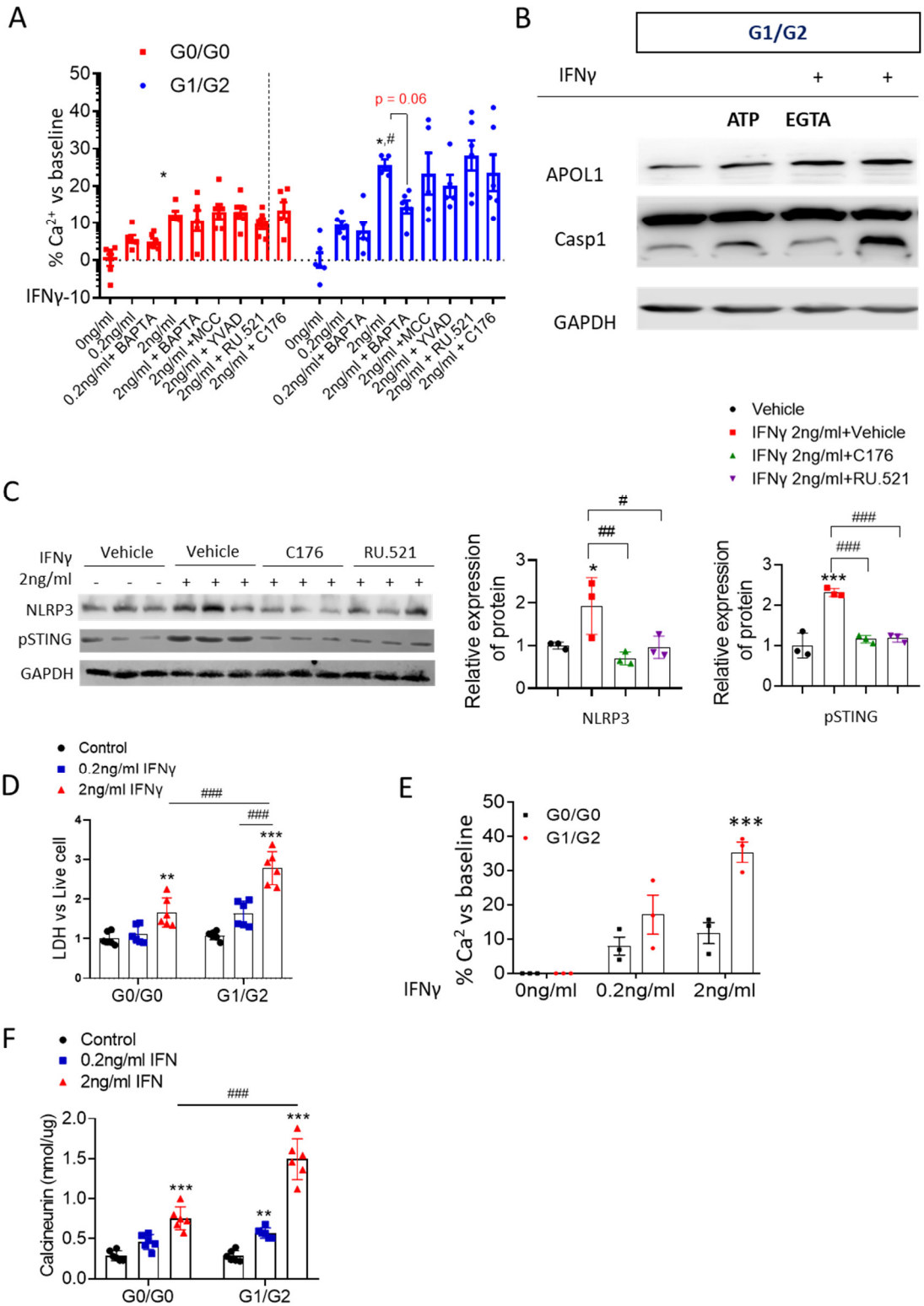
D. Representative western blots of APOL1, NLRP3, and GAPDH from high-risk G1/G2 HUPEC cells treated with 0ng/ml, 0.2ng/ml, 2ng/ml, and 20 ng/ml IFN γ for 24h.

E. Densitometric quantification of APOL1 normalized to GAPDH in G1/G2 HUPEC cells. N = 2-3 independent experiments. **p<0.01, ***p< 0.001 vs G0/G0.

F. Densitometric quantification of NLRP3 normalized to GAPDH in G1/G2 HUPEC cells. N = 2-3 independent experiments. *p<0.05, **p< 0.01 vs G1/G2.

G. Cell cytotoxicity in G1/G2 HUPEC cells. G1/G2 HUPEC cells were treated with 2ng/ml IFN γ for 24 hours in the presence inhibitors of STING: C176 or cGAS: RU.521. **p<0.01 vs Control. And $^{\#}$p<0.05, $^{\#\#}$p<0.01 vs indicated group.

Fig S4


A
G0/G0
G1/G2
% Ca2+ vs baseline
p = 0.06
IFNγ-10
0ng/ml
0.2ng/ml
0.2ng/ml+ BAPTA
2ng/ml
2ng/ml + BAPTA
2ng/ml +MCC
2ng/ml + YVAD
2ng/ml + RU.521
2ng/ml + C176
B
G1/G2
IFNγ
ATP
EGTA
APOL1
Casp1
GAPDH
C
Vehicle
IFNγ 2ng/ml+Vehicle
IFNγ 2ng/ml+C176
IFNγ 2ng/ml+RU.521
IFNγ
2ng/ml
Vehicle
C176
RU.521
NLRP3
pSTING
GAPDH
Relative expression of protein
NLRP3
pSTING
D
Control
0.2ng/ml IFNγ
2ng/ml IFNγ
LDH vs Live cell
G0/G0
G1/G2
E
G0/G0
G1/G2
% Ca2 vs baseline
IFNγ
0ng/ml
0.2ng/ml
2ng/ml
F
Control
0.2ng/ml IFN
2ng/ml IFN
Calcineurin (nmol/ug)
G0/G0
G1/G2

Supplemental.Fig.4. High risk APOL1-induced cytotoxicity is ameliorated by pyroptosis inhibition or calcium chelation.

A. Intracellular Ca^{2+} was measured in G0/G0 and G1/G2 cells using FURA-2 AM calcium indicator. Cells were treated with 0.2 ng/ml IFN γ ± BAPTA, 2ng/ml IFN γ ± BAPTA for 8h. Baseline Ca^{2+} release of G0/G0 and G1/G2 were normalized and set to zero. ***p<0.001 vs respective control treated cells; ##p<0.01 vs control treated G0/G0 cells. Red **p < 0.01 vs 2ng/ml ± BAPTA treatment. Representative experiment out of 3 independents. Each experiment was done in 6 replicates.

B. Immunoblot analysis of G1/G2 podocytes treated with ATP as a positive control for inflammasome activation and 2ng/ml IFN γ ± calcium chelator (EGTA). A representative experiment out of three experiments is presented here.

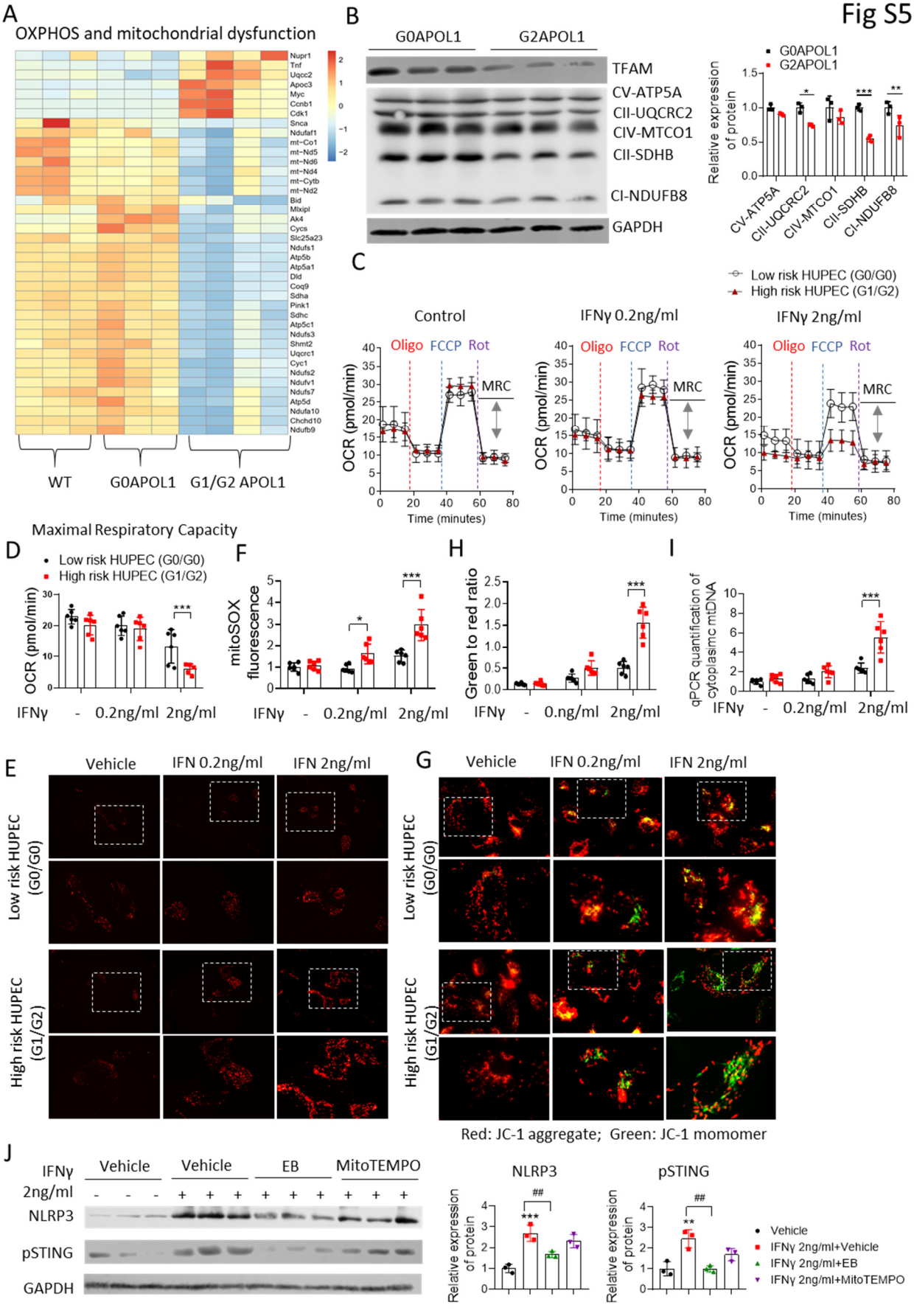
C. Immunoblot analysis of G1/G2 podocytes treated with Vehicle, C176 or RU.521. (Right panel) densitometric quantification of NLRP3 and phosphorylation STING normalized to GAPDH. N = 3 independent experiments; *p<0.05, ***p<0.001 vs G0/G0 and $^{\#}$p<0.05, $^{\#\#}$p<0.01， and $^{\#\#\#}$p<0.001 vs indicated group.

D. Cytotoxicity measured by LDH release was normalized to calcein absorbance as an indicator of live cell count of untreated G0/G0 and G1/G2 cells, respectively. G0/G0 and G1/G2 cells were treated for 24h with 0.2 ng/ml, 2ng/ml and 20ng/ml IFN γ. Representative experiment out of 3 experiments. *** p<0.001 vs 0ng/ml IFN γ treated G1/G2 cells.

E. Change in intracellular Ca^{2+} measured by FURA-2 AM (calcium indicator) as a percentage of baseline calcium (set to zero). Cells were treated with 0.2 ng/ml, 2ng/ml, and 20 ng/ml IFN γ for 8hours. N = 3; *p<0.05 vs control-treated cells.

F. G0/G0 and G1/G2 HUPEC were treated with the indicated concentrations of IFN γ or left untreated. Calcineurin activity was analysed using a calcineurin-activity assay. **p<0.05, ***p<0.001 vs. Control; ###p<0.001 vs. indicated group.

Fig S5


A
OXPHOS and mitochondrial dysfunction
WT
G0APOL1
G1/G2 APOL1
B
G0APOL1
G2APOL1
TFAM
CV-ATP5A
CII-UQCRC2
CIV-MTCO1
CII-SDHB
CI-NDUFB8
GAPDH
Relative expression of protein
C
Low risk HUPEC (G0/G0)
High risk HUPEC (G1/G2)
Control
IFNγ 0.2ng/ml
IFNγ 2ng/ml
Oligo FCCP Rot
MRC
OCR (pmol/min)
Time (minutes)
D
Maximal Respiratory Capacity
IFNγ - 0.2ng/ml 2ng/ml
F
mitoSOX fluorescence
H
Green to red ratio
IFNγ - 0.ng/ml 2ng/ml
I
qPCR quantification of cytoplasimc mtDNA
E
Vehicle
IFN 0.2ng/ml
IFN 2ng/ml
Low risk HUPEC (G0/G0)
High risk HUPEC (G1/G2)
G
Red: JC-1 aggregate; Green: JC-1 momomer
J
IFNγ
2ng/ml
Vehicle
EB
MitoTEMPO
NLRP3
pSTING
GAPDH
IFNγ 2ng/ml+Vehicle
IFNγ 2ng/ml+EB
IFNγ 2ng/ml+MitoTEMPO

Supplemental Figure.5. APOL1 risk allele induced defect in mitochondrial function.

A. Heatmap of expression OXPHOS genes analysed by RNA-seq in kidneys of wild type *Nphs1*-rtTA/TRE-*APOL1*-G0/G1/G2 mice

B. Western blots of whole kidney lysates from *G0APOL1* and *G2APOL1* mice, showing levels of TFAM, OXPHOS proteins (CV-ATP5A, CII-UQCRC2, CIV-MTCO1, CII-SDHB and CI-NDUFB8), and GAPDH. (Right panel) densitometric quantification of TFAM and OXPHOS proteins normalized to GAPDH. N = 3 treatments; *p<0.05, **p<0.01, and ***p<0.001 vs *G2APOL1* mice.

C. Real-time changes in the oxygen consumption ratio (OCR) of HUPEC after treatment with oligomycin (Oligo), FCCP, and rotenone (Rot). MRC, maximal respiratory capacity (double-headed arrow).

D. Maximal respiratory capacity of HUPEC measured by real-time changes in OCR. ***p<0.001 vs G1/G2 APOL1 HUPEC.

E. mtROS levels measured by MitoSOX staining in G0/G0 APOL1 and G1/G2 APOL1 HUPEC treated with vehicle, 0.2ng/ml IFN γ or 0.2ng/ml IFN γ . Representative images are shown. Scale bar=10 μ m.

F. Quantification of the mtROS in the experiment shown in panel E. ***p<0.001 vs *Vehicle*.

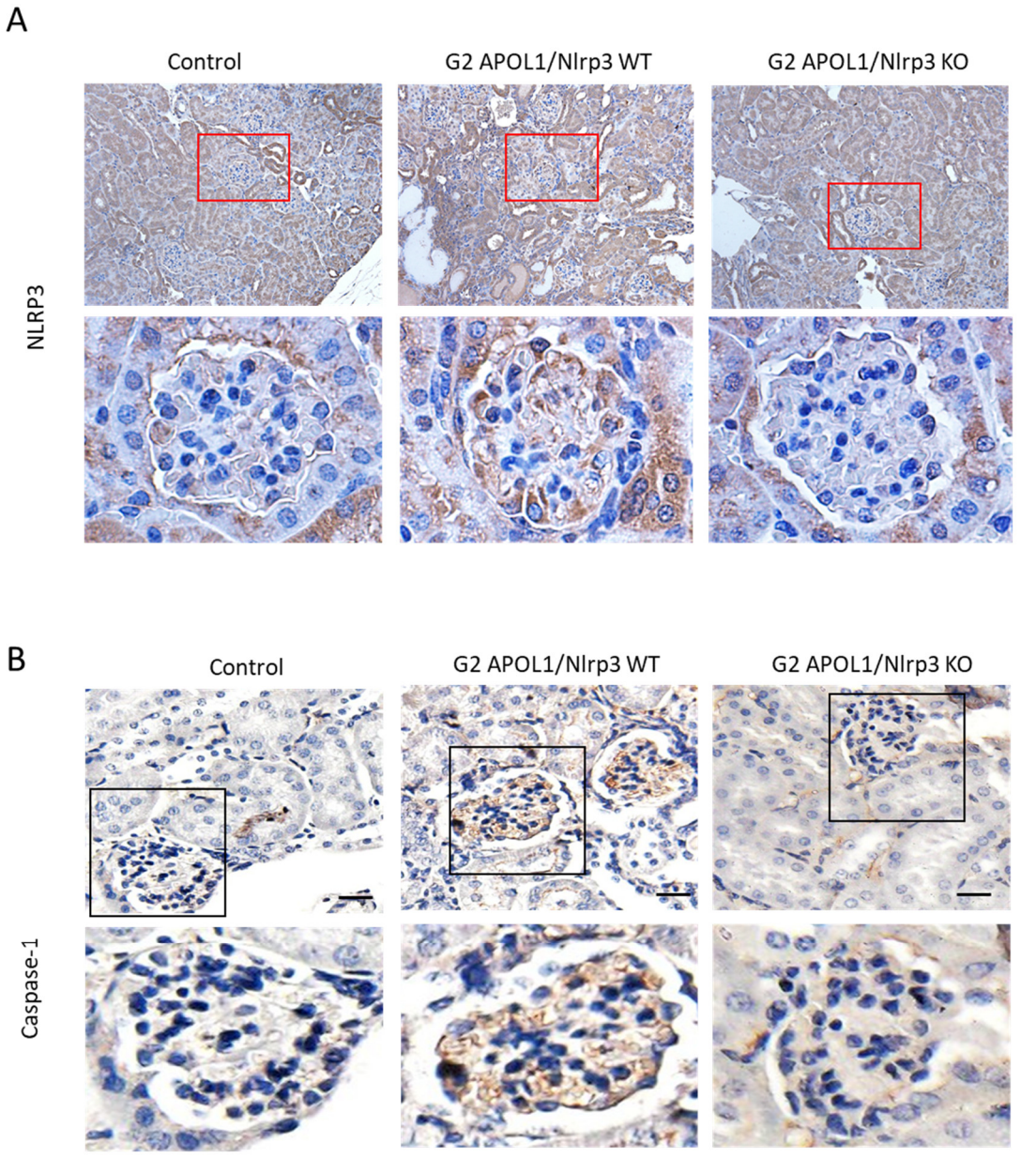
G. Mitochondrial membrane potential ($\Delta \psi$ m) was determined by the JC-1 assay in G0/G0 APOL1 and G1/G2 APOL1 HUPEC treated with vehicle, 0.2ng/ml IFN γ or 0.2ng/ml IFN γ . Scale bar = 10 μm.

H. Quantification of the JC-1 fluorescence ratio in the experiment shown in panel G. ***p<0.001 vs *Vehicle*.

I. Cytosolic mtDNA genes were normalized to respective nuclear RPL13A and presented as fold enrichment over media-treated controls. ***p<0.001 vs indicated group.

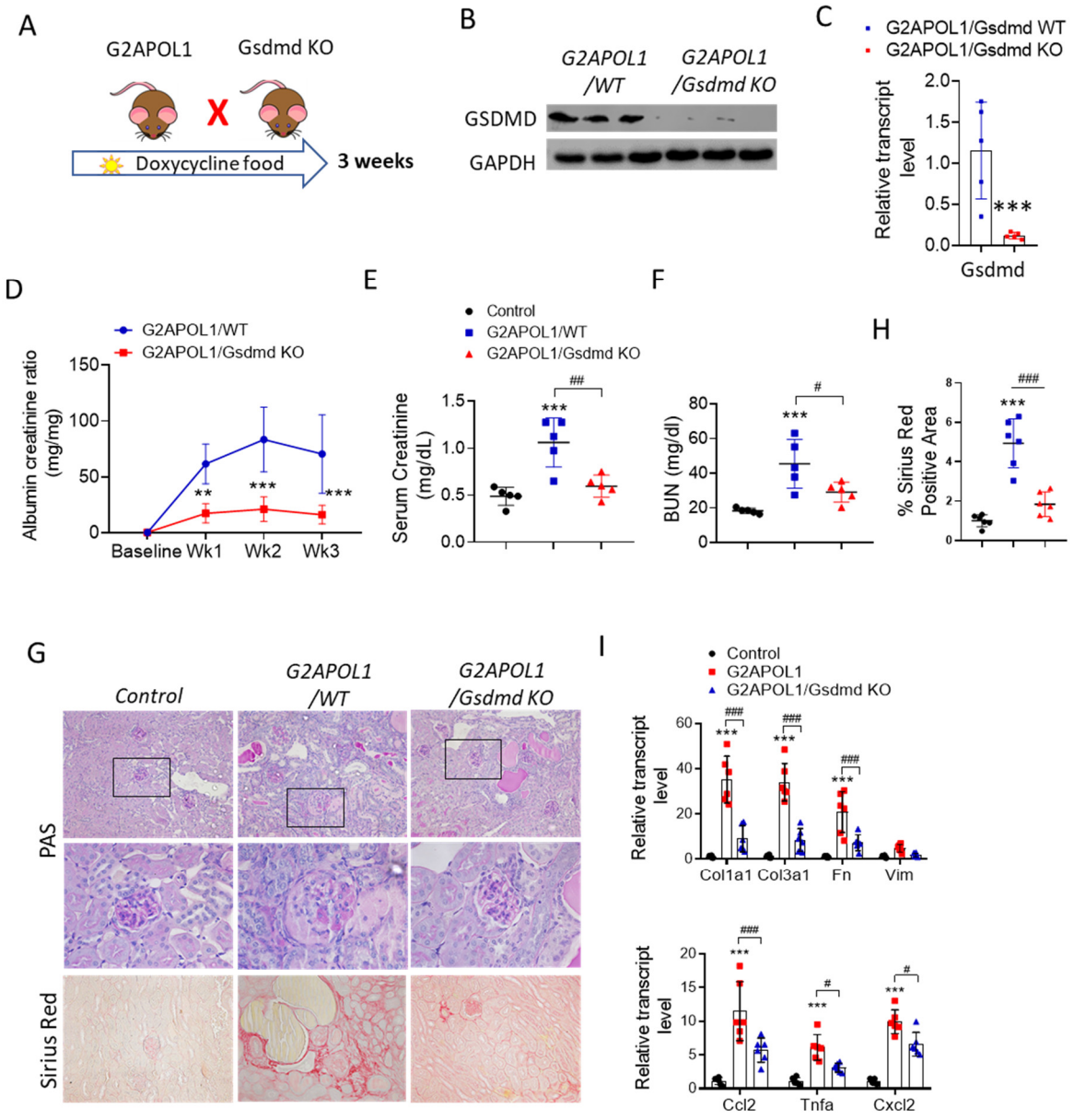
J. Immunoblot analysis of G1/G2 podocytes treated with Vehicle, ethidium bromide (EB) (72h, 150ng/ml), or MitoTEMPO (100nM, 4h). (Right panel) densitometric quantification of NLRP3 and phosphorylation STING normalized to GAPDH. N = 3 treatments; **p<0.01, ***p<0.001 vs G0/G0 and $^{\#\#}$p<0.01 vs indicated group.

Fig S6



Supplemental Fig.6. Expression of NLRP3 and Caspase1 in kidneys of podocyte specific APOL1 risk allele mice.

Immunohistochemical analysis of NLRP3 (A) and Cleaved Caspase-1(B) staining in Nphs1-rtTA (Control), Nphs1rtTA/TREG2APOL1 (G2APOL1/Nlrp3 WT), and Nphs1rtTA/TREG2APOL1/ $Nlpr3^{-/-}$ (G2APOL1/Nlrp3 KO) mice. Brown staining indicates cleaved NLRP3 expression. Scale bar =10 µM.



Supplemental Fig.7. Genetic deletion of Gsdmd in G2APOL1 transgenic mice markedly improves kidney function.

A. Experimental design for the generation of *Nphs1rtTA/TREG2APOL1/ Gsdmd KO* (*G2APOL1/Gsdmd KO*) mice.

B. Western blots of whole kidney lysates from *G2APOL1/Gsdmd WT* and *G2APOL1/Gsdmd KO* mice, showing levels *Gsdmd* and GAPDH.

C. Relative *Gsdmd* transcript level in kidney of *G2APOL1/Gsdmd WT* and *G2APOL1/Gsdmd KO* mice. ***p<0.01 vs *G2APOL1/Gsdmd WT*.

D. ACR of *G2APOL1/Gsdmd WT* (N = 5) and *G2APOL1/Gsdmd KO* mice (N = 5) at baseline, 1, 2 and 3 weeks on doxycycline diet. **p<0.01, ***p<0.001, compares *G2APOL1/ Gsdmd WT* mice at the same time points.

E. Serum creatinine levels in control, G2APOL1/ *Gsdmd* WT and G2APOL1/*Gsdmd* KO mice. ***p<0.001 vs. Control; ##p<0.01 vs. indicated group.

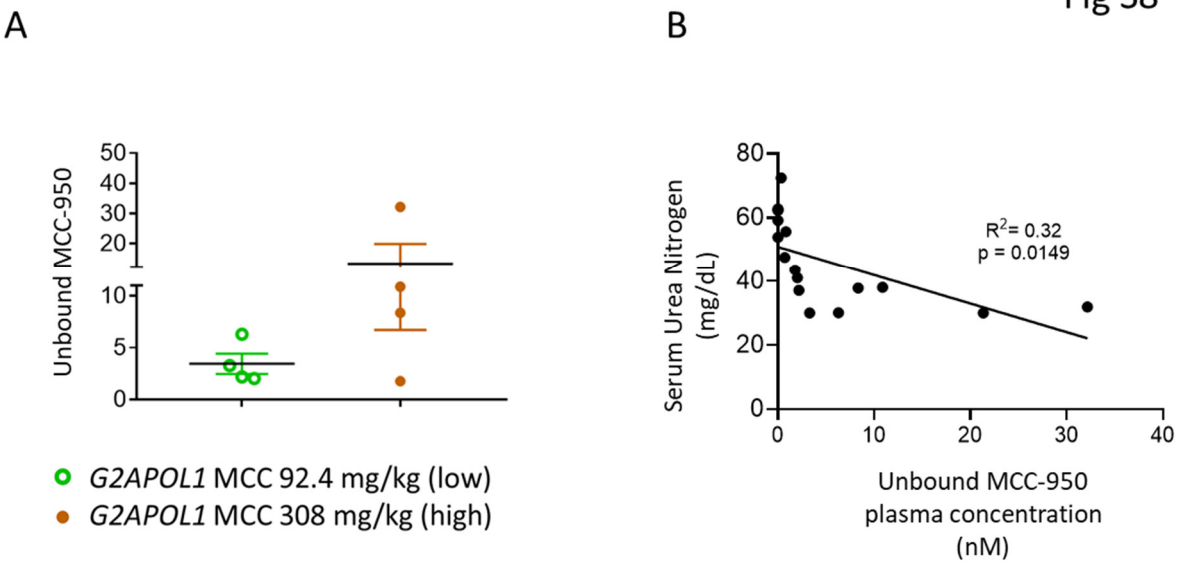
F. Serum urea nitrogen (BUN) levels in control, G2APOL1/ *Gsdmd* WT and G2APOL1/*Gsdmd* KO mice. ***p<0.001 vs. Control; #p<0.05 vs. indicated group.

G. PAS-stained and Sirius red-stained kidney sections of control, *G2APOL1/Gsdmd WT,* and *G2APOL1/Gsdmd KO* mice. Scale bar =10µm.

H. Quantification of Sirius red-positive area of control, *G2APOL1/Gsdmd WT* and *G2APOL1/Gsdmd KO* mice. N = 6 mice per group. ***p<0.001 vs. Control; ###p<0.001 vs. indicated group.

I. Relative mRNA levels markers of fibrosis; *Col1a1*. *Col3a1*, *Fn1*, and *Vim,* and markers of inflammation; *Ccl2*, *Tnfa*, *Cxcl2* were evaluated in the kidneys of control, *G2APOL1/Gsdmd WT* and *G2APOL1/Gsdmd KO* mice. N = 6 mice. ***p<0.001 vs. Control; #p<0.0 ###p<0.001 vs. indicated group.

Fig S8

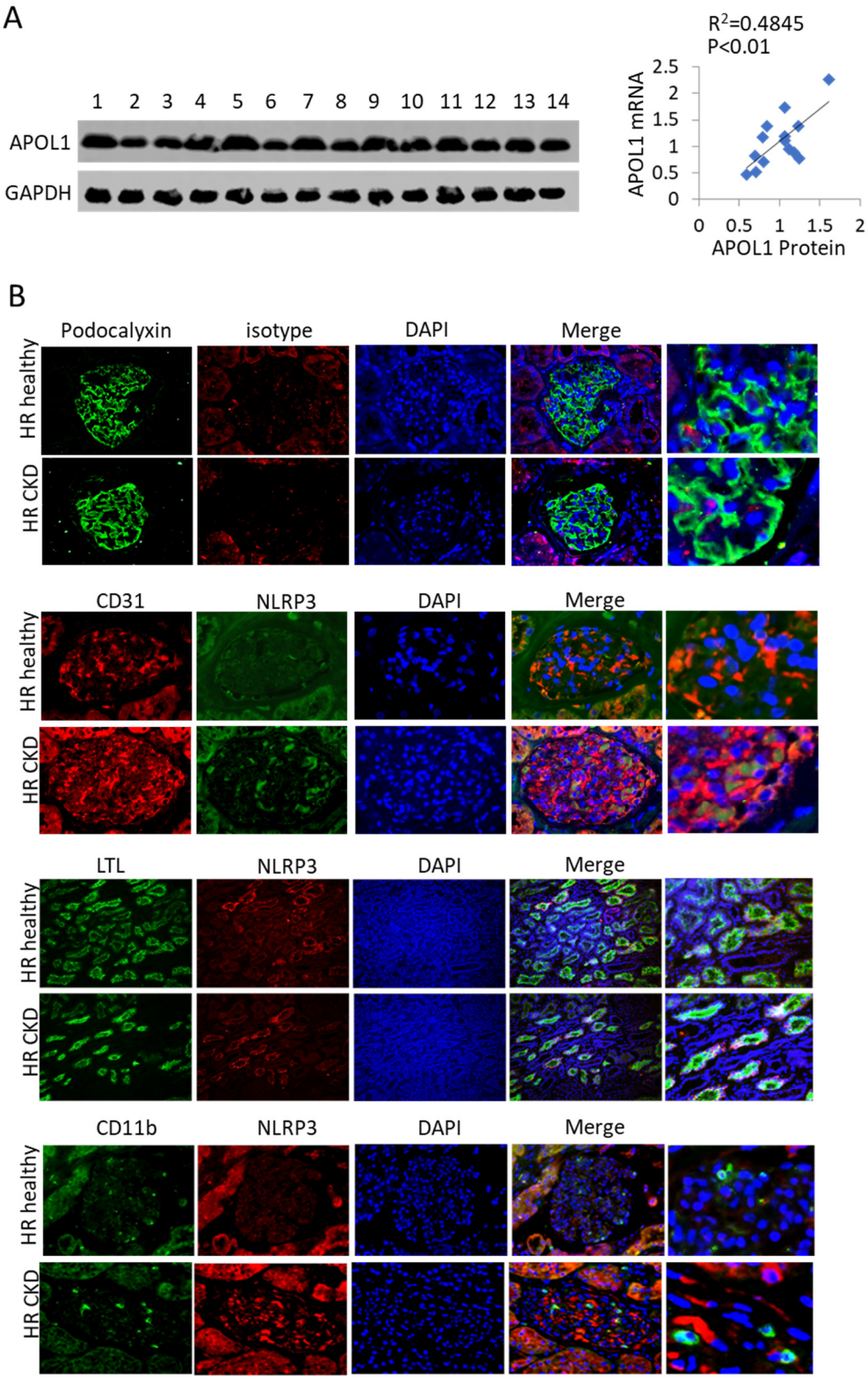


Supplemental Fig. 8. Plasma concentration of MCC-950 correlates with renal function of *G2APOL1* mice.

(A) Plasma concentration of unbound MCC-950 in was estimated by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) in *NPHS1-rtTA/TREG2APOL1* (*G2APOL1*) mice treated with *G2APOL1* MCC 92.4 mg/kg (N =4) , *G2APOL1* MCC 308 mg/kg (N= 4).

(B) Serum urea nitrogen plotted against unbound MCC-950 plasma concentration to determine if NLRP3 inhibitor concentration correlated with renal function.

Fig S9


A
1 2 3 4 5 6 7 8 9 10 11 12 13 14
APOL1
GAPDH
R2=0.4845
P<0.01
APOL1 mRNA
APOL1 Protein
0 0.5 1 1.5 2 2.5
B
Podocalyxin
isotype
DAPI
Merge
HR healthy
HR CKD
CD31
NLRP3
LTL
CD11b

Supplemental Fig. 9. Increase in podocyte NLRP3 and inflammasome expression in APOL1 high-risk patients

A. Western blots of whole kidney lysates from 14 individual kidney samples, showing levels APOL1 and GAPDH. (Right panel) The correlation of transcript level of APOL1 and protein level of APOL1 (Pearson correlation).

B. Representative immunofluorescence co-staining of NLRP3 isotype with Podocalyxin, or CD31, LTL, CD11B and DAPI in kidney samples of APOL1 high-risk control and CKD patients. Scale bar = 10μm.

Table 1:

Primary Cohort	
Subjects (n)	N=427
GFR (ml/min/1.73 m2 by CKD-EPI)	69.76 (24.92 %)
Gender (%Female)	155 (36.3 %)
Age	61.18 (12.56 %)
Race	
Asian (n)	6 (1.41 %)
Caucasian (n)	312 (73.07 %)
African American (n)	1 (0.23 %)
Hispanic (n)	64 (14.99 %)
Multi-racial (n)	10 (2.34 %)
Diabetes	166 (38.88 %)
Hypertension	301 (70.49 %)
Dipstick Protein (0=neg, 1=trace, 2=30, 3=100, 4=300, 5>300)	1.02 (1.51)
BMI (kg/m2)	31.09 (8.01)
HgbA1c	6.8 (1.52)
Serum glucose (mg/dl)	131.98 (62.62)
BP systolic (mmHg)	136.94 (20.84)
Serum-alb (gldl)	4.04 (1.06)
Glomeruli: Hypoperfused: 0-3	0.84 (0.68)
Glomeruli: Wall Thickening: 0-3	0.18 (0.5)
Glomeruli: Mesangial Matrix: 0-3	0.41 (0.76)
Glomeruli: Mesangial Cellularity: 0-3	0.33 (0.7)
Glomeruli: KW Nodules: 0-1	0.03 (0.17)
Glomeruli: Pericapsular Fibrosis: 0-2	0.71 (0.67)
Glomeruli: Globally Schlerotic %	11.81 (15.43)
Tubules: % Atrophy	10.72 (16.77)
Tubules: % Acute Tubular Injury	2.83 (6.32)
Tubules: Reabsorption: 0-3	0.37 (0.6)
Interstitium: % Fibrosis	11 (16.12)
Interstitium: Lymphocytic Infiltrate: 0-3	1.04 (0.85)
Interstitium: Plasmacytic Infiltrate: 0-3	0.38 (0.62)
Interstitium: Eosinophils: 0-3	0.26 (0.5)
Vessels: Medial Thickening: 0-3	0.11 (0.37)
Vessels: Intimal Fibrosis: 0-3	1.44 (0.81)
Vessels: Arteriolar Hyalinosis: 0-3	0.51 (0.74)

Table 2: Primers

Primers	Forward	Reverse
ASC	CAGAGTACAGCCAGAACAGGACAC	GTGGTCTCTGCACGAACTGCCTG
Casp1	CGTGGAGAGAAACAAGGAGTG	AATGAAAAGTGAGCCCCTGAC
Casp11	TCACTGAGGTATGGGGCTAA	TGACTTTGGGTTTGTCTCGTA
Col1a1	GCTCTTTTTAGATACTGTGGTGAGGAA	GTTTCCACGTCTCACCATTG
Col3a1	ACAGCTGGTGAACCTGGAAG	ACCAGGAGATCCATCTCGAC
Ccl2	CCCAATGAGTAGGCTGGAGA	AAAATGGATCCACACCTTGC
Cxcl2	GAGCTTGAGTGTGACGCCCCCAGG	GTTAGCCTTGCCTTTGTTCAGTATC
FN	ATGTGGACCCCTCCTGATAGT	GCCCAGTGATTTCAGCAAAGG
Gapdh	AGGTCATCCCAGAGCTGAACG	AGGTCATCCCAGAGCTGAACG
HPRT	CACAGGACTAGAACACCTGC	GCTGGTGAAAAGGACCTCT
IL1b	CCCTGCAGCTGGAGAGTGTGGA	TGTGCTCTGCTTGTGAGGTGCTG
IL6	CCTCTCTGCAAGAGACTTCCAT	AGTCTCCTCTCCGGACTTGT
Nlrp3	GGTCCTCTTTACCATGTGCTTC	AAGTCATGTGGCTGAAGCTGTA
Ubiquitin C	GCCCAGTGTTACCACCAAGAAG	GCTCTTTTTAGATACTGTGGTGAGGAA
Vim	GGAGGCCACGAACTTCACTCT	GGGATGCAACACCTATTGTCAGT
Ifit1	CTGAGATGTCACTTCACATGGAA	GTGCATCCCCAATGGGTTCT
Ifitm1	GACAGCCACCACAATCAACAT	CCCAGGCAGCAGAAGTTCAT
Mx2	CTTGCGCCTCTGATTGCTTTG	GTTGCCATCTTCGCGTTGC
Isg15	GGTGTCCGTGACTAACTCCAT	TGGAAAGGGTAAGACCGTCCT
Stat1	TCACAGTGGTTCGAGCTTCAG	GCAAACGAGACATCATAGGCA