SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Association of Nr4a1 knockout mutation with proteinuria by linkage analysis. (**A**) Location of Nr4a1 mutation in exon 1 induced by ENU mutagenesis. The point mutation results in a stop codon [TAC (Tyrosine) to TAA (stop)]. (**B**) AseI restriction site polymorphism was used to perform linkage analysis in an F_2 (FHH x Nr4a1$^{-/-}$) population (n=175). (**C**) Association of Nr4a1 mutation (A/A) with increased proteinuria (week 8 and 12), and reduced body weight (BW) and increased kidney weight (KW) at week 12 compared to wild-type (C/C). * $p<0.05$ (week 8); † $p<0.05$ (week 12); mean values + SE are presented.

Figure S2: Autoregulation of renal blood flow (RBF) in FHH and Nr4a1$^{-/-}$ rats. (**A**) RBF was measured as renal perfusion pressure (RPP) was varied from 150 to 90 mmHg in steps of 10 mmHg. The dashed line represents an autoregulation curve that demonstrates perfect autoregulation. (**B**) Autoregulatory index (AI) was calculated over the range of pressures from 100 to 140 mmHg. AI of 0 indicates perfect autoregulation of RBF and AI of 1 is characteristic of a circulation with a fixed vascular resistance and impaired RBF autoregulation. FHH (as expected) and Nr4a1$^{-/-}$ rats demonstrate impaired regulation of RBF.

Figure S3: Serum lipid levels and hepatic steatosis in FHH and Nr4a1$^{-/-}$ rats at week 24. (**A**) Serum cholesterol and triglyceride levels. (**B**) H&E and Oil Red O straining in liver sections. *$p<0.05$ vs. FHH; mean values + SE are presented.

Figure S4: Representative electron microscopy images of FHH and Nr4a1$^{-/-}$kidney at week 24. (**A**) Image of glomerular capillary and podocyte food processes. Thin arrows denote podocyte

foot process effacement. (**B**) Proximal tubule images. (**C**) Semi-quantitative assessment of cell degeneration (e.g., evidence of cells that are falling apart, loss of organelles, etc.) and lipid accumulation from electron microscopy images [n=15-20 tubule images per group (n=4 each strain]. Each parameter was scored in blinded fashion on semi-quantitative scale of 0 (no evidence) to 3 (severe). Thick arrows denote lipid droplet in proximal tubule. *$p<0.05$ vs. FHH; mean values + SE are presented.

Figure S5: Measurement of tubular injury in FHH and Nr4a1-/- rats at week 8, 16, and 24. (**A**) Semi-quantitative analysis of tubular injury. Tubular injury was analyzed for degree of tubular atrophy, vacuolization, dilation, and proteinaceous casts on a scale from 0 (normal) to 4 (severe with > 75% tubules demonstrating injury). (**B**) Quantitative real-time PCR of macrophage markers (Cd-68 and Socs3) and tubular injury genes [kidney injury molecule (Kim-1), and Neutrophil gelatinase-associated lipocalin (Ngal)]. n=20 images per group (n=6); * $p<0.05$ compared to FHH; mean values + SE are presented.

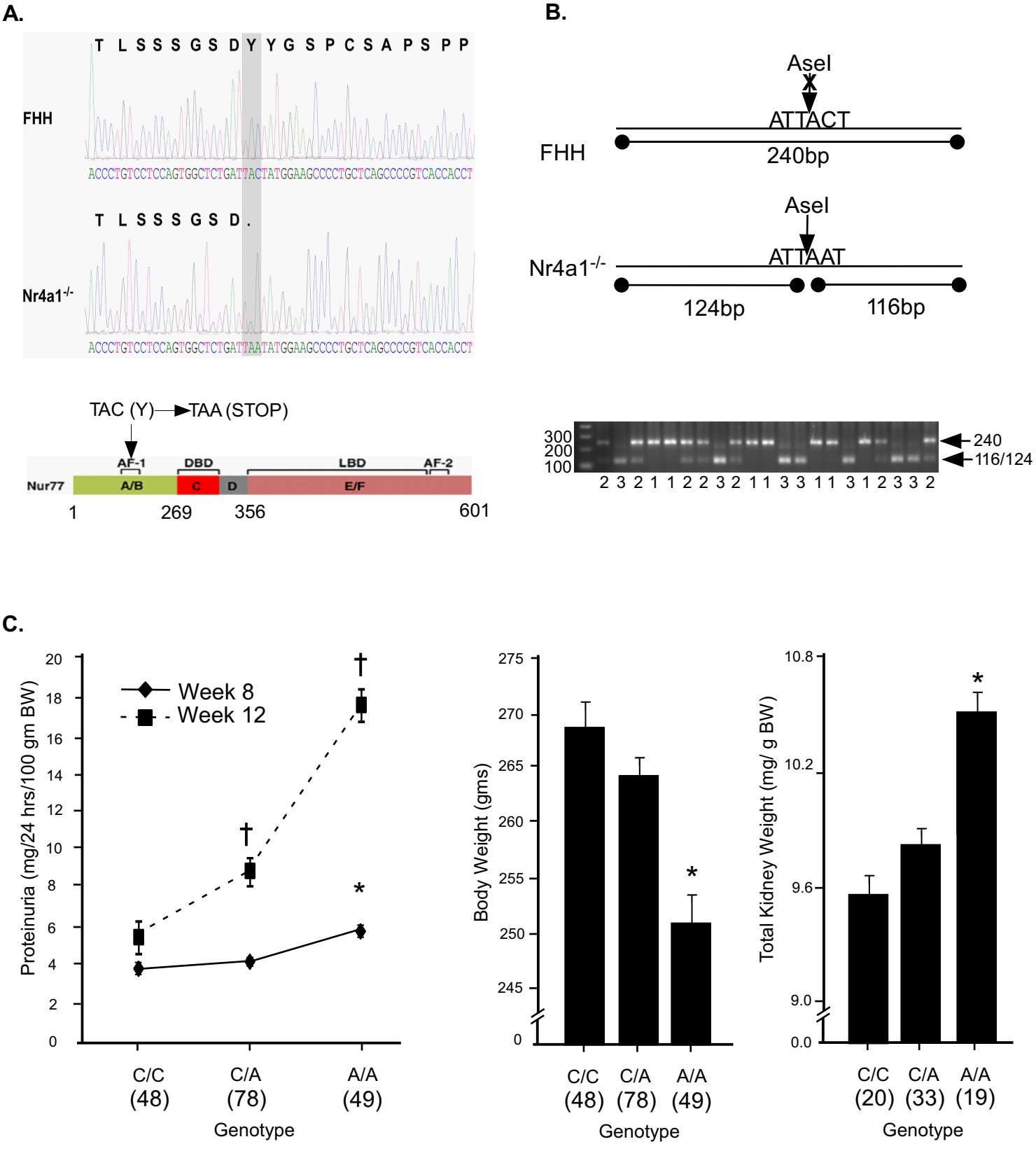
Figure S6: Microarray analysis of kidney cortex from FHH and Nr4a1$^{-/-}$ at week 8, 16, and 24. (**A**) Hierarchical clustering of immune related genes depicting gene expression changes with time and between the FHH and Nr4a1$^{-/-}$ strains. (**B**) Principal component analysis (PCA) illustrating the relationship between sample groups and gene expression changes over time. At an early time point (week 8) gene expression patterns are similar in kidney from Nr4a1$^{-/-}$ and FHH. From week 8 to week 16-24, gene expression differences of Nr4a1$^{-/-}$ are distinct from FHH and each other. By week 16, FHH gene expression differences are distinct from week 8, but similar to week 24. (**C**) Top biological functions (Ingenuity Pathway Analysis) and upstream regulators

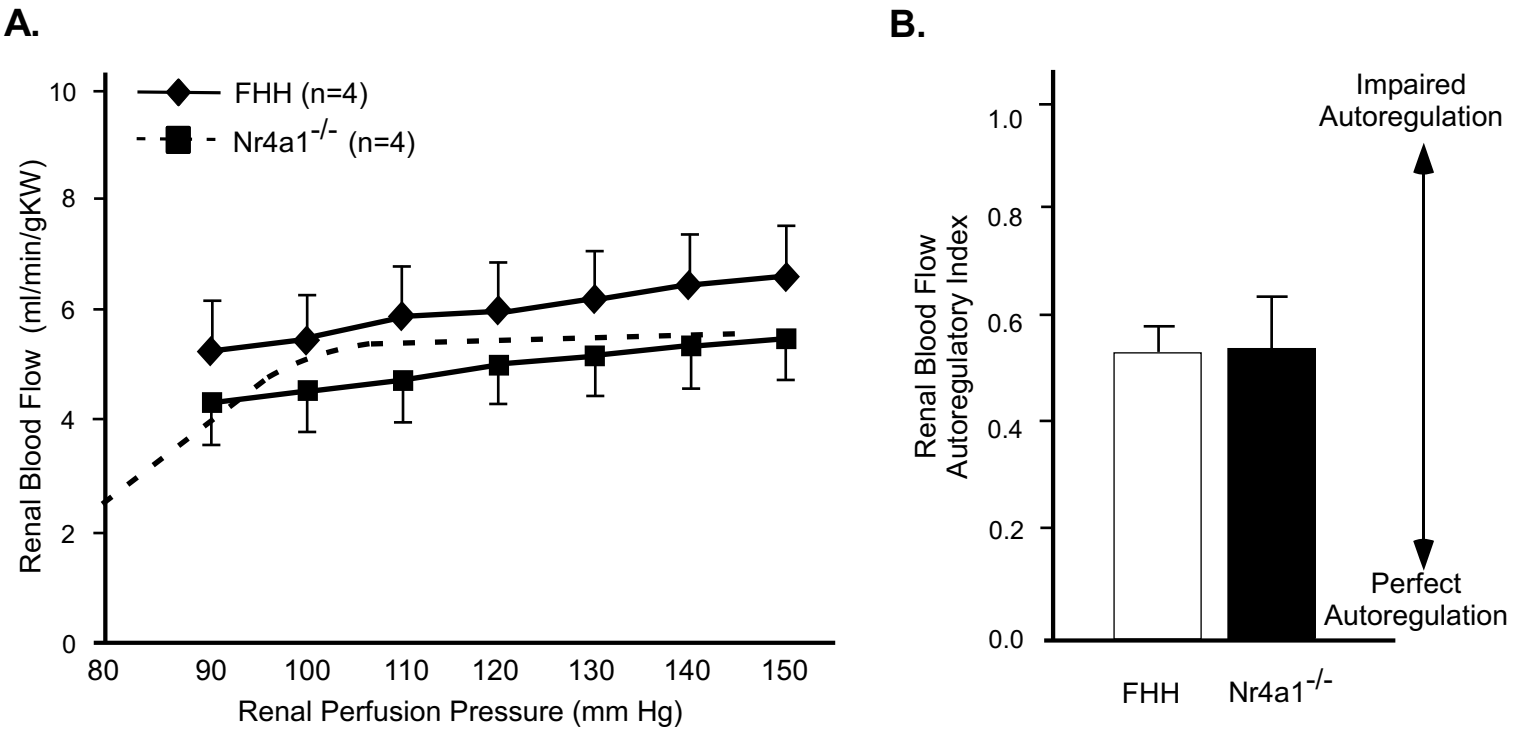
associated with gene expression difference (between samples and with time). (**D**) Quantitative real-time PCR validation of important chemokines/cytokines identified as differentially expressed between Nr4a1$^{-/-}$ and FHH.

Figure S7: Genotype, metabolic and renal injury measurements for bone marrow cross-transplantation studies using FHH and Nr4a1$^{-/-}$. (**A**) Validation that bone marrow transplanted from FHH was successfully transplanted into Nr4a1$^{-/-}$ (FHH$_{bm}$ into Nr4a1$^{-/-}{}_{irr)}$ by PCR. All FHH$_{bm}$ into Nr4a1$^{-/-}{}_{irr}$ animals exhibited a heterozygous genotype (i.e., FHH and Nr4a1), whereas each parental control demonstrated homozygous genotype. (**B**) Serum cholesterol and triglyceride levels. (**C**) Semi-quantitative analysis of tubular injury [n=20 images per group (n=6-8)]. * $p<0.05$ compared to FHH; mean values + SE are presented. † $p<0.05$ vs. other groups.

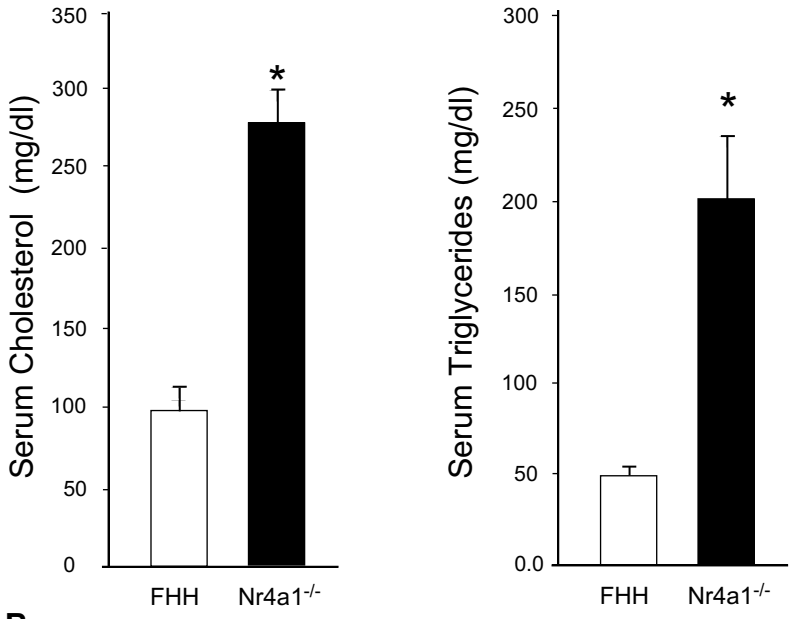
Figure S8: Quantitative real-time PCR of important inflammatory genes in thioglycolate elicited macrophages (**Fig. 4**) and bone-marrow derived macrophages. (**A**) Thioglycolate elicited macrophages isolated from Nr4a1$^{-/-}$ at week 4, 8, 12, and 20 under baseline conditions (unstimulated) demonstrated down-regulation (compared to FHH macrophages) of Il10, Il12b, and Tnfa and up-regulation of Nos2 from week 4-8, but by week 12 or 20 only Nos2 was significantly changed (increased in Nr4a1$^{-/-}$). Kruppel-like factor 4 (Klf4) was significantly down-regulated and suppressor of cytokine signaling 3 (Socs3) was up-regulated, along with increased Nos2 expression suggest that macrophage isolated from Nr4a1$^{-/-}$ are primed toward M1 polarized pro-inflammatory phenotype. (**B**) Quantitative real-time PCR of inflammatory genes using bone marrow derived macrophage (n=6 independent cell cultures/treatment derived from

n=2 rats/group) from untreated and LPS treated macrophages from FHH and Nr4a1$^{-/-}$. Bone marrow Nr4a1$^{-/-}$ macrophages demonstrated a significant up-regulation of Il-6, Il-12b, Tnfa, and Nos2 compared to FHH macrophages after stimulation with LPS. ND; not detected. * $p<0.05$ compared to FHH; mean values + SE are presented.

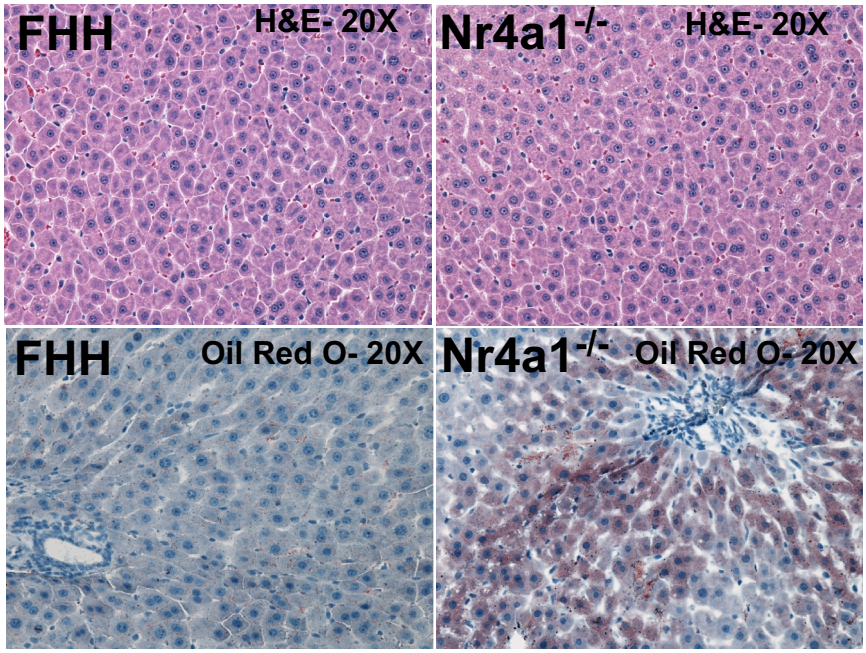

A.
T L S S S G S D Y Y G S P C S A P S P P
FHH
ACCCTGTCCTCCAGTGGCTCTGATTACTATGGAAGCCCCTGCTCAGCCCCGTCACCACCT
T L S S S G S D .
Nr4a1-/-
ACCCTGTCCTCCAGTGGCTCTGATTAATATGGAAGCCCCTGCTCAGCCCCGTCACCACCT
TAC (Y)→TAA (STOP)
AF-1
DBD
LBD
AF-2
Nur77
A/B
C
D
E/F
1
269
356
601
B.
AseI
ATTACT
FHH
240bp
AseI
Nr4a1-/-
ATTAAT
124bp
116bp
300
200
100
240
116/124
2 3 2 1 1 2 2 3 2 1 1 3 3 1 1 3 1 2 3 3 2
C.
Proteinuria (mg/24 hrs/100 gm BW)
Week 8
Week 12
†
†
*
C/C
(48)
C/A
(78)
A/A
(49)
Genotype
Body Weight (gms)
*
C/C
(48)
C/A
(78)
A/A
(49)
Genotype
Total Kidney Weight (mg/ g BW)
*
C/C
(20)
C/A
(33)
A/A
(19)
Genotype


A.
FHH (n=4)
Nr4a1-/- (n=4)
Renal Blood Flow (ml/min/gKW)
Renal Perfusion Pressure (mm Hg)
0
2
4
6
8
10
80
90
100
110
120
130
140
150
B.
Renal Blood Flow Autoregulatory Index
0.0
0.2
0.4
0.6
0.8
1.0
Impaired Autoregulation
Perfect Autoregulation
FHH
Nr4a1-/-

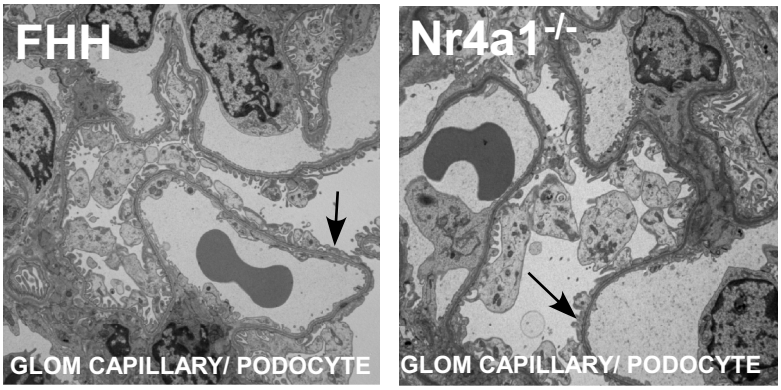
A.


Serum Cholesterol (mg/dl)
350
300
250
200
150
100
50
0
*
FHH
Nr4a1-/-
Serum Triglycerides (mg/dl)
300
250
200
150
100
50
0.0
*
FHH
Nr4a1-/-

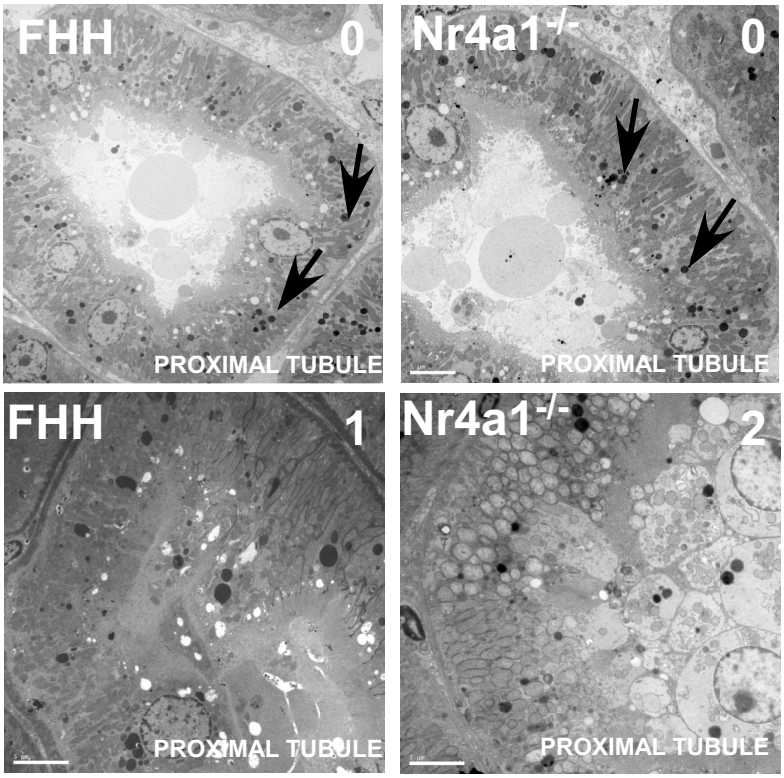
B.


FHH
H&E- 20X
Nr4a1-/-
H&E- 20X
FHH
Oil Red O- 20X
Nr4a1-/-
Oil Red O- 20X

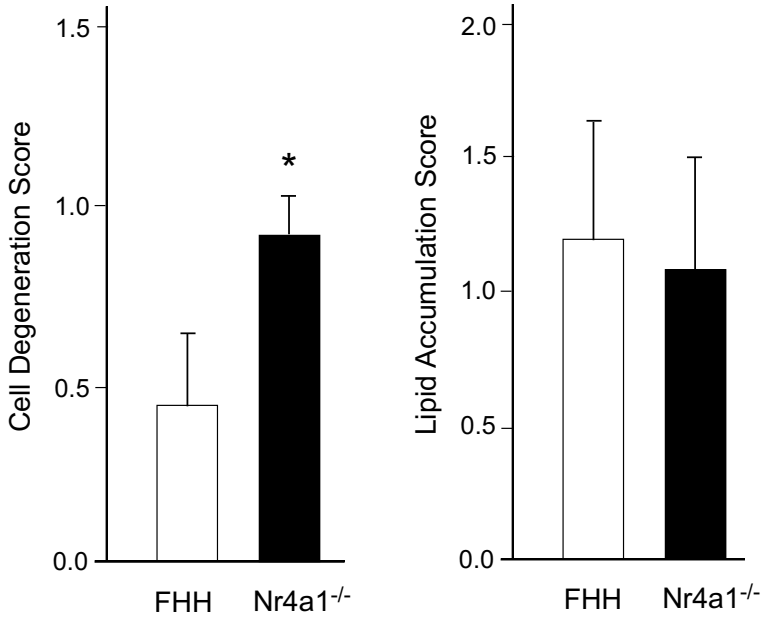
A.

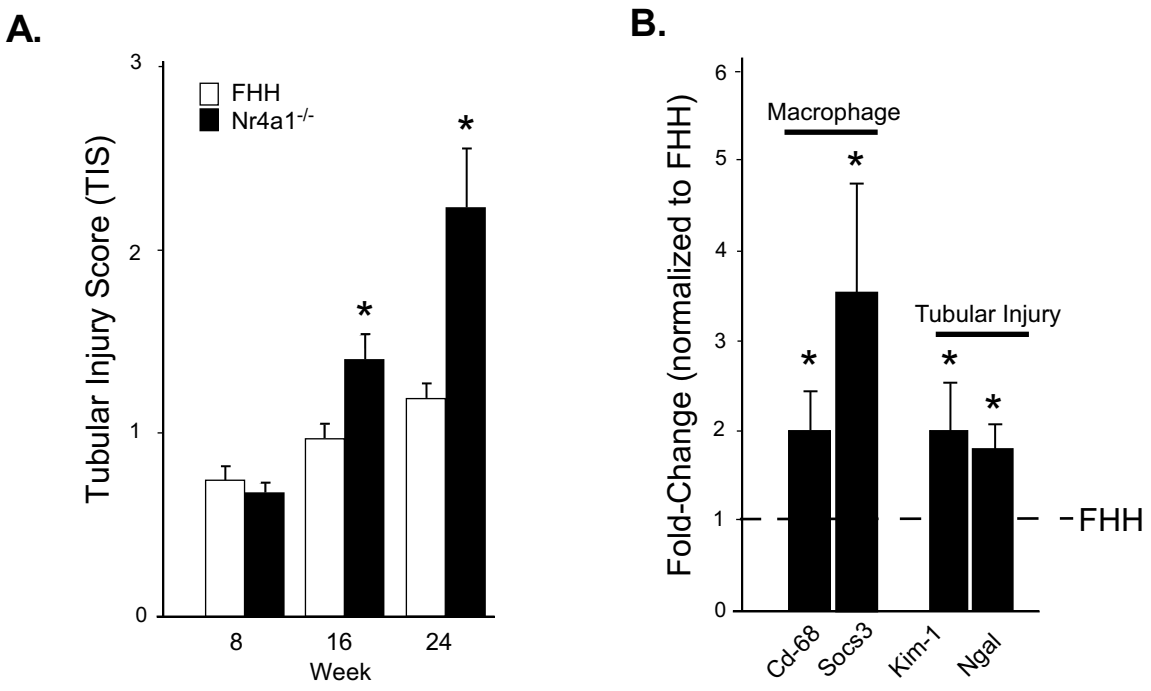

FHH
GLOM CAPILLARY/ PODOCYTE
Nr4a1-/-
GLOM CAPILLARY/ PODOCYTE

B.

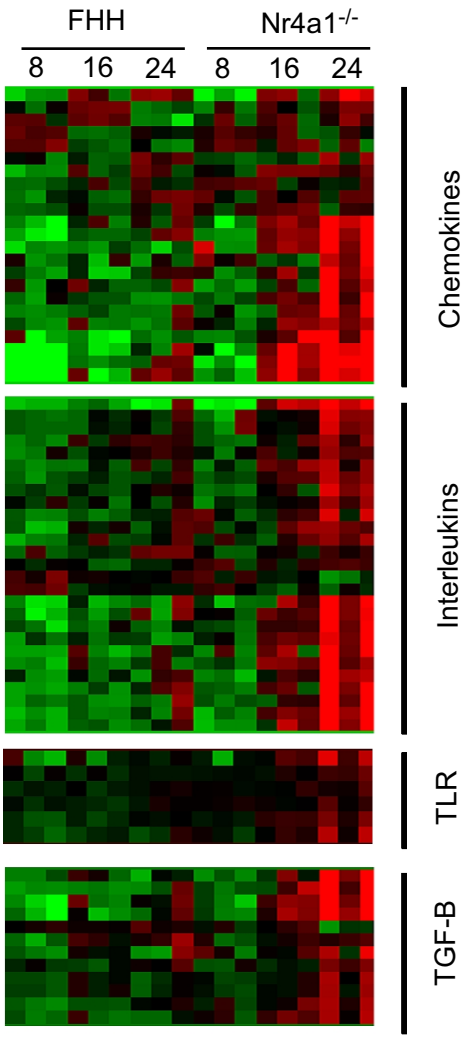

FHH
0
PROXIMAL TUBULE
Nr4a1-/-
0
PROXIMAL TUBULE
FHH
1
PROXIMAL TUBULE
Nr4a1-/-
2
PROXIMAL TUBULE

C.

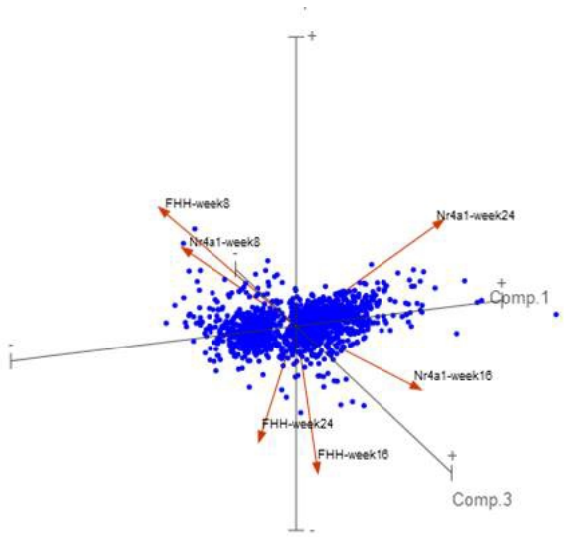

Cell Degeneration Score
1.5
1.0
0.5
0.0
*
FHH
Nr4a1-/-
Lipid Accumulation Score
2.0
1.5
1.0
0.5
0.0
FHH
Nr4a1-/-


A.
Tubular Injury Score (TIS)
FHH
Nr4a1-/-
0
1
2
3
8
16
24
Week
*
*
B.
Fold-Change (normalized to FHH)
Macrophage
Tubular Injury
FHH
0
1
2
3
4
5
6
Cd-68
Socs3
Kim-1
Ngal
*
*
*
*

A.



B.



C.

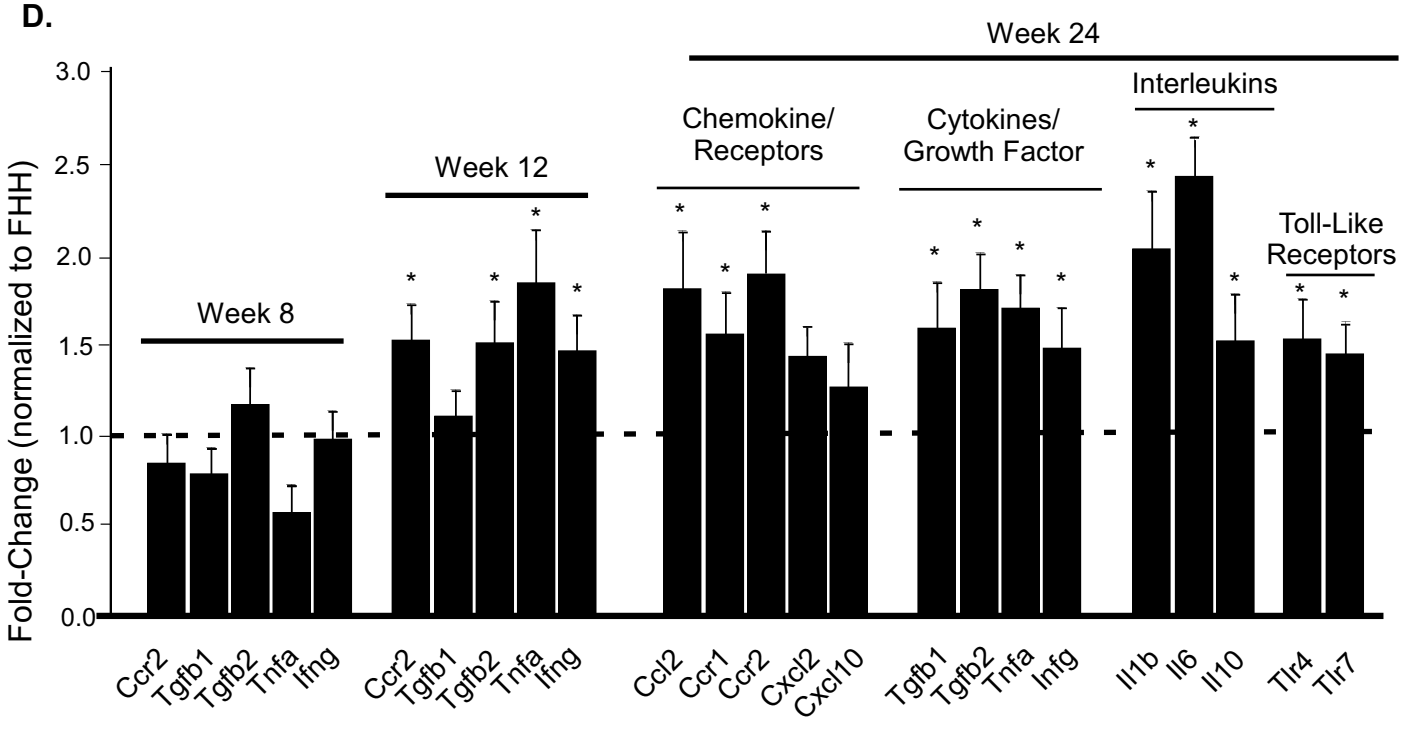
Top Bio Functions

Diseases and Disorders

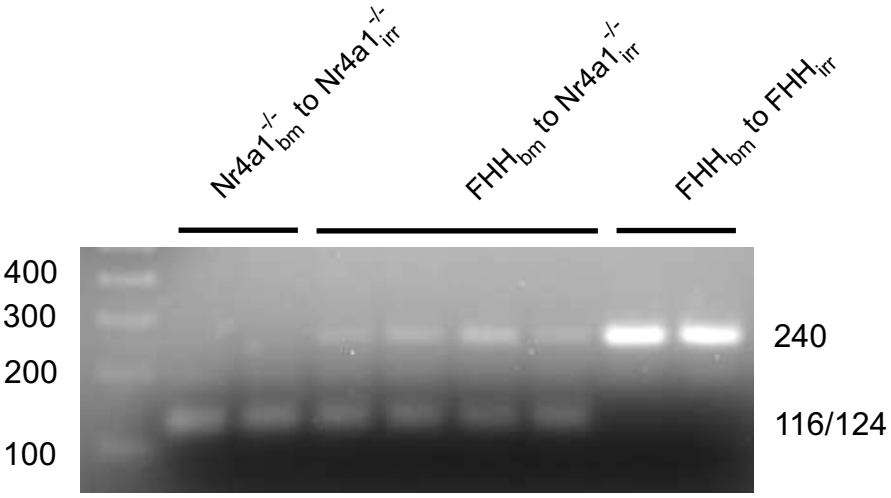
Name	p-value	# Molecules
Inflammatory Response	2.45E-14 - 3.15E-04	65
Connective Tissue Disorders	5.23E-12 - 1.47E-04	48
Inflammatory Disease	5.23E-12 - 2.50E-04	67
Skeletal and Muscular Disorders	5.23E-12 - 1.47E-04	47
Immunological Disease	8.85E-12 - 2.74E-04	54

Upstream Regulator	p-value of overlap
lipopolysaccharide	3.11E-17
IFNG (includes EG:15978)	6.42E-16
IL1B	4.92E-12
IL6	9.14E-12
TNF	1.03E-11

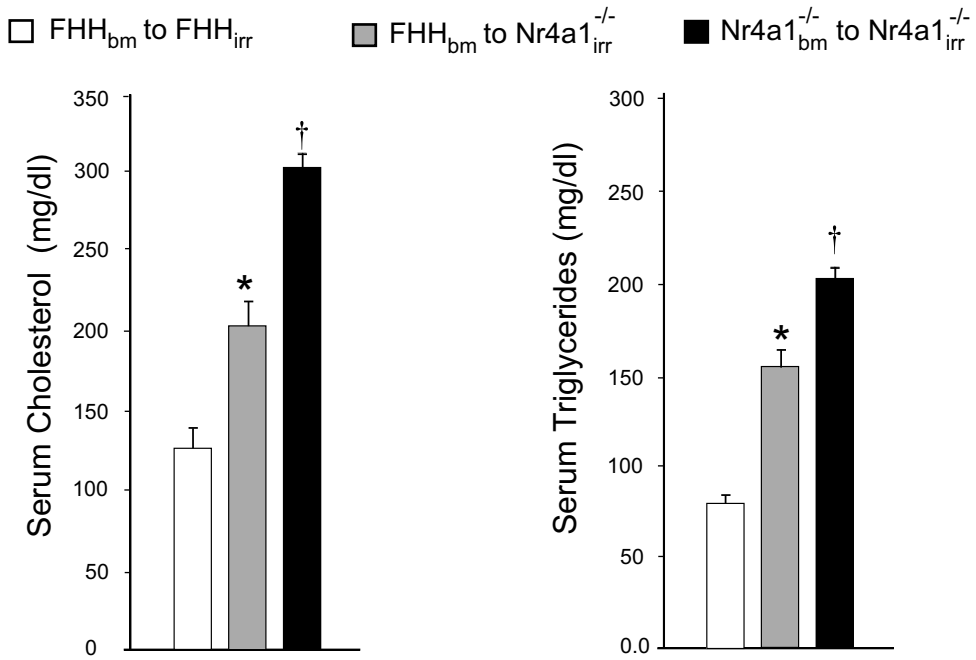
D.



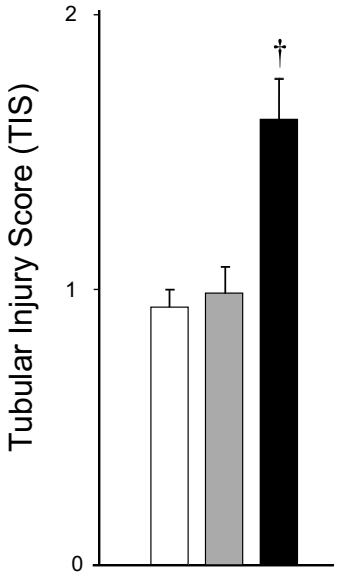
A.


Nr4a1-/- bm to Nr4a1-/- irr
FHH bm to Nr4a1-/- irr
FHH bm to FHH irr
400
300
200
100
240
116/124

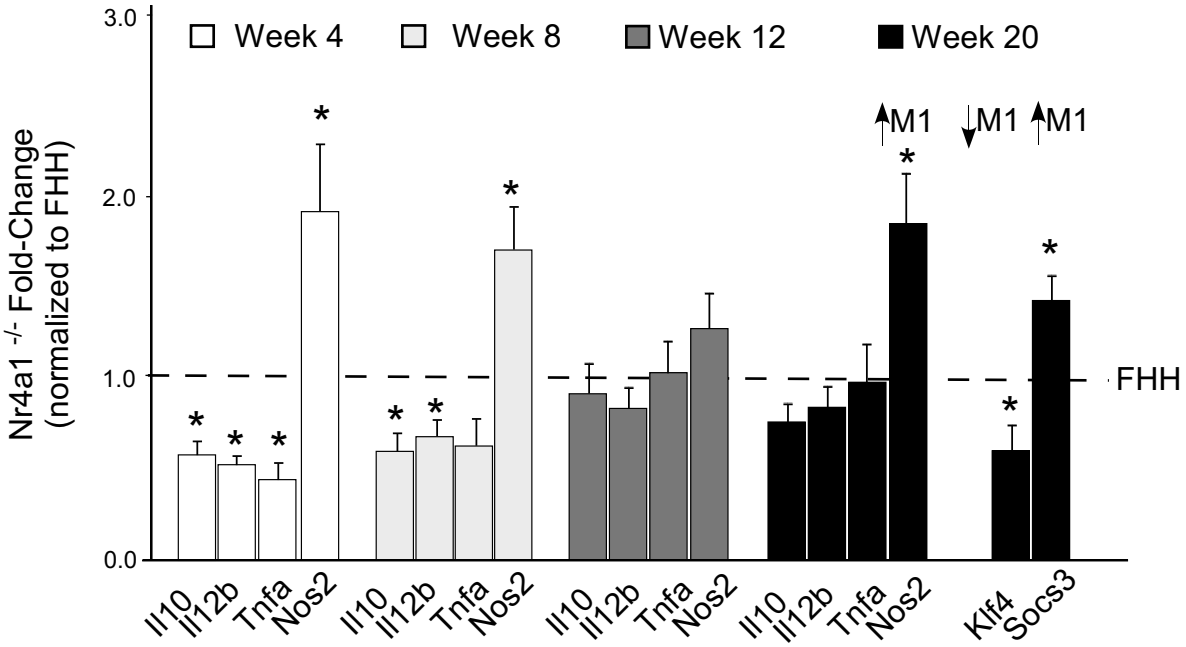
B.


FHH bm to FHH irr
FHH bm to Nr4a1-/- irr
Nr4a1-/- bm to Nr4a1-/- irr
Serum Cholesterol (mg/dl)
350
300
250
200
150
100
50
0
*
†
Serum Triglycerides (mg/dl)
300
250
200
150
100
50
0.0
*
†

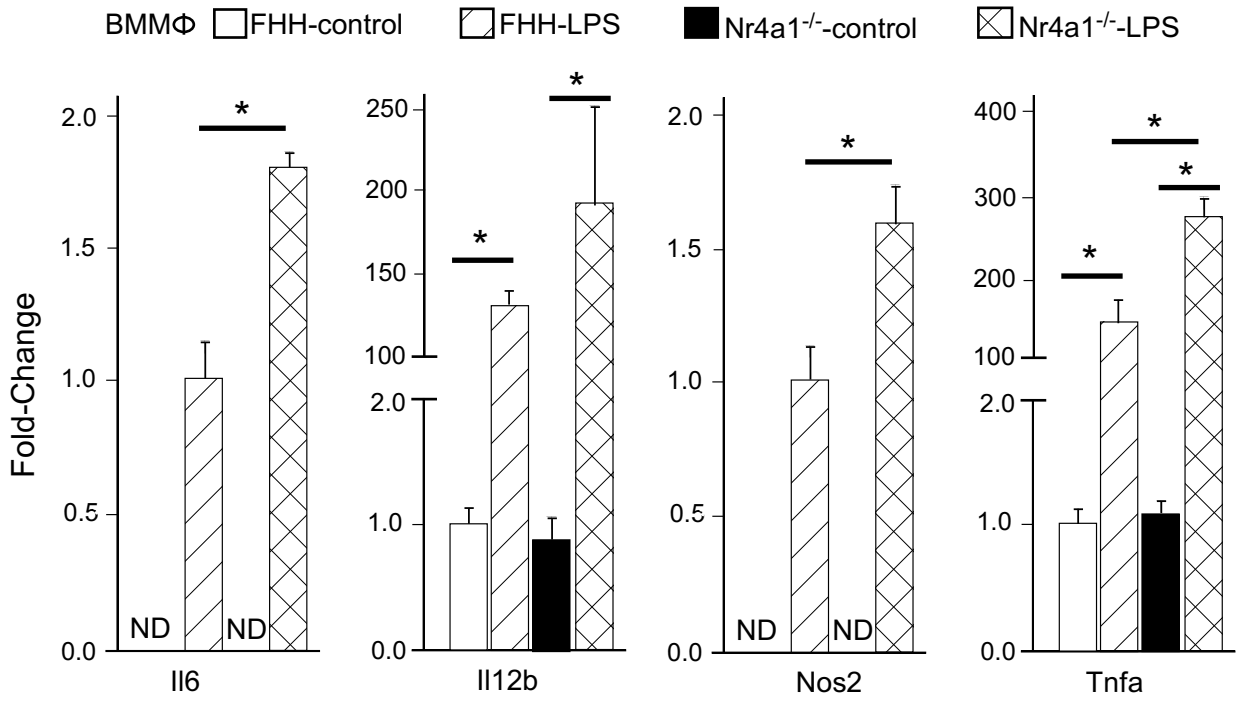
C.


Tubular Injury Score (TIS)
2
1
0
†

A.


Week 4
Week 8
Week 12
Week 20
Nr4a1 -/- Fold-Change (normalized to FHH)
3.0
2.0
1.0
0.0
FHH
M1
M1
M1
Il10
Il12b
Tnfa
Nos2
Il10
Il12b
Tnfa
Nos2
Il10
Il12b
Tnfa
Nos2
Il10
Il12b
Tnfa
Nos2
Klf4
Socs3

B.


BMMΦ
FHH-control
FHH-LPS
Nr4a1-/--control
Nr4a1-/--LPS
Fold-Change
ND
ND
Il6
Il12b
Nos2
Tnfa