Supplemental Table 1. Clinical characteristics of patients with and without diabetic nephropathy, from whom archival nephrectomy tissue was examined.

	Control Nx	Diabetes Nx
Sex (male/female)	8/4	8/4
Age (years)	65.6±3.1	67.6±2.5
Type of diabetes (Type 1/Type 2)	--	1/11
Serum creatinine (μmol/L)	80±4	101±10
eGFR (ml/min/1.73m^2)	81±4	66±7
Hemoglobin A_{1c} (%)	--	8.0±0.5 (unavailable for 4)
Hypertension (%)	6 (50)	10 (83)
RAS blockade (%)	4 (33)	7 (58)
Stage 3 CKD or worse (%)	0	5 (42)
Duration of diabetes (years)	--	7, 12, 13, 15, 20, 22, 30 (unavailable for 5)
Proteinuria	--	By 24h urine collection [1.64 g/day]; by urine ACR [22.8 mg/mmol, 8.1 mg/mmol, 3.4 mg/mmol, 1.4 mg/mmol]; by urine reagent [>3.0 g/L, 1.0g/L, 0.3 g/L, "trace"] (unavailable for 3)

Nx = nephrectomy, eGFR = estimated glomerular filtration rate, RAS = renin angiotensin system, CKD = chronic kidney disease, ACR = albumin:creatinine ratio.

Supplemental Table 2. Clinical characteristics of patients with and without FSGS, from whom biopsy tissue was examined.

	Control Bx	FSGS Bx
Sex (male/female)	6/3	7/3
Age (years)	47.4±4.1	52.7±5.1
Type of FSGS (primary/secondary)	--	6/4
Serum creatinine (μmol/L)	76±4	173±28**
eGFR (ml/min/1.73m^2)	101±4	45±6****
Hypertension (%)	0	10 (100)
RAS blockade (%)	0	6 (60)
Stage 3 CKD or worse (%)	0	9 (90)
Race (white/black/other)	9/0/0	9/1/0
Body mass index (kg/m^2)	29.3±1.3	34.2±2.6
24 hour urine protein excretion (g/day)	0.11±0.02	5.26±0.64 (unavailable for 2; however, in those patients, urine ACR was 436 mg/mmol and 104 mg/mmol)
Serum albumin (g/L)	42±1	31±3** (unavailable in 1)
Hematuria (yes/no)	--	6/4
Edema (yes/no)	--	5/5

Of the 4 patients with secondary FSGS, causes were: ischemia/non-steroidal anti-inflammatory drugs (NSAIDs); anabolic steroids/NSAIDS; obesity/NSAIDS; and obesity.
Bx = biopsy, eGFR = estimated glomerular filtration rate, RAS = renin angiotensin system, CKD = chronic kidney disease, ACR = albumin:creatinine ratio. **p<0.01, ****p<0.0001 by 2-tailed Student *t* test.

Supplemental Table 3. Expression of 84 Notch pathway-related genes in human podocytes under control conditions or treated with TGF-ß1 (10ng/ml for 48h) in the presence or absence of GSK-J4 (5µM) (n=4/condition).

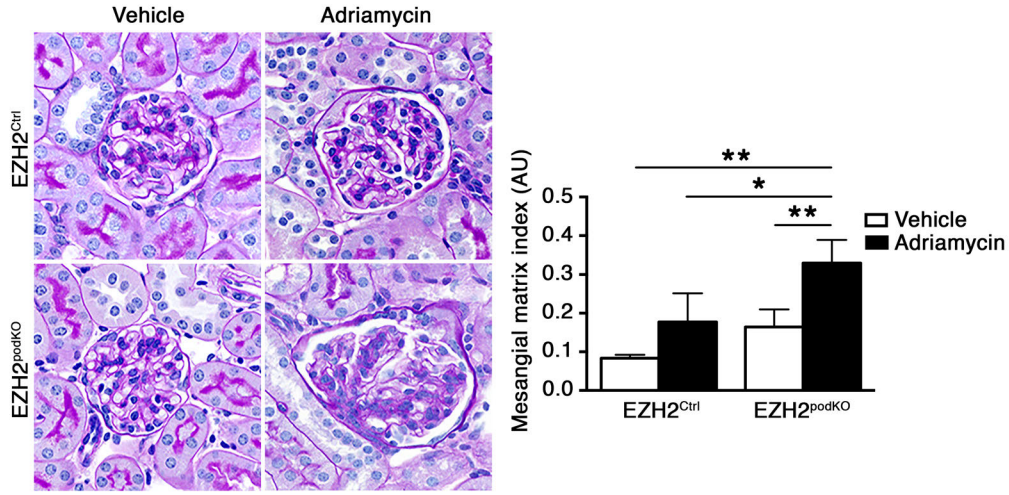
	2^(-Avg.(Delta(Ct))			
Symbol	**Control**	**TGF-ß1**	**GSK-J4**	**TGF-ß1 + GSK-J4**
ADAM10	0.000325	0.000341	0.00026	0.000342
ADAM17	0.000325	0.000341	0.000346	0.000342
AES	0.00065	0.000966	0.0006	0.002517
AXIN1	0.000402	0.001175	0.000466	0.001601
CBL	0.655274	0.724876	0.335862	0.334609
CCND1	0.017605	0.030707	0.017665	0.013576
CCNE1	0.000325	0.000439	0.000345	0.000427
CD44	0.002171	0.001832	0.001771	0.004271
CDKN1A	0.009664	0.007088	0.009596	0.009449
CFLAR	0.000488	0.000532	0.00051	0.000579
CHUK	0.721118	0.984654	0.276128	0.426669
CTNNB1	1.182867	1.964607	0.438588	0.451775
DLL1	0.000445	0.000449	0.00026	0.001312
DLL3	0.000752	0.002209	0.0003	0.002404
DLL4	0.002383	0.005712	0.000893	0.007847
DTX1	0.001039	0.00203	0.000707	0.000767
EP300	0.501223	0.636963	0.156656	0.217764
ERBB2	0.000325	0.00037	0.00026	0.000498
FIGF	0.001356	0.001366	0.000989	0.001445
FOS	6.233018	3.403967	0.675441	0.403894
FOSL1	0.000805	0.001083	0.002174	0.002377
FZD2	0.000442	0.000529	0.000558	0.000567
FZD3	0.000325	0.000341	0.00026	0.000342
FZD4	0.001671	0.001851	0.000411	0.001102
FZD7	0.710302	0.813511	0.189286	0.165989
GLI1	0.002514	0.003968	0.001133	0.002777
GSK3B	0.349666	0.650798	0.068119	0.184391
HDAC1	0.078758	0.099272	0.029799	0.032082
HES1	0.035784	0.137512	0.004687	0.007526
HES5	0.001176	0.002351	0.000583	0.001791
HEY1	0.000325	0.000341	0.00026	0.000408
HEY2	0.000325	0.000558	0.000298	0.000342
HEYL	0.00033	0.000366	0.00026	0.000768
HOXB4	0.006302	0.006038	0.00113	0.00565
HR	0.003392	0.006622	0.001146	0.006918
ID1	0.000325	0.000341	0.00026	0.000342
IFNG	0.000325	0.000341	0.00026	0.000342
IL17B	0.000325	0.000341	0.00026	0.000342
IL2RA	0.000325	0.000341	0.00026	0.000342
JAG1	0.010232	0.03645	0.002262	0.001874
JAG2	0.002065	0.002135	0.001759	0.004052
KRT1	0.000325	0.000341	0.00026	0.000342

LFNG	0.000325	0.000341	0.000527	0.000529
LMO2	0.000678	0.001127	0.000408	0.012586
LOR	0.000978	0.001859	0.000313	0.000536
LRP5	0.000325	0.000341	0.00026	0.000368
MAML1	0.000325	0.000341	0.00026	0.000342
MAML2	0.000325	0.000498	0.00026	0.000421
MFNG	0.000325	0.000341	0.00026	0.000342
MMP7	0.000388	0.000359	0.000311	0.000847
NCOR2	0.001842	0.002925	0.000748	0.003702
NCSTN	0.001713	0.001973	0.001163	0.002208
NEURL1	0.003295	0.00796	0.001611	0.010342
NFKB1	0.000482	0.000902	0.000273	0.001788
NFKB2	0.057706	0.107845	0.039001	0.037713
NOTCH1	0.000327	0.000365	0.00026	0.000351
NOTCH2	0.000614	0.00049	0.00026	0.000591
NOTCH2NL	0.000415	0.000341	0.000342	0.000444
NOTCH3	0.000371	0.000451	0.000607	0.00178
NOTCH4	0.000325	0.000341	0.00026	0.000342
NR4A2	0.014443	0.01501	0.000989	0.006611
NUMB	0.000686	0.000373	0.000393	0.000404
PAX5	0.000402	0.000669	0.000279	0.000983
POFUT1	0.000325	0.000341	0.00026	0.000397
PPARG	0.008181	0.006412	0.002942	0.013771
PSEN1	0.000616	0.000992	0.000459	0.001213
PSEN2	0.017063	0.009353	0.01421	0.019848
PSENEN	0.005277	0.010894	0.002792	0.013682
PTCRA	0.001315	0.002854	0.000405	0.003483
RBPJL	0.000325	0.000346	0.00026	0.000471
RFNG	0.000325	0.000341	0.000267	0.000513
RUNX1	0.037214	0.068557	0.018829	0.019872
SEL1L	0.001158	0.000881	0.001116	0.000866
SH2D1A	0.000325	0.000341	0.00026	0.000342
SHH	0.000325	0.000409	0.00026	0.000342
SMO	0.036648	0.055387	0.013013	0.014461
SNW1	0.022926	0.041206	0.006201	0.020977
STAT6	0.000325	0.000377	0.000301	0.000836
STIL	0.000325	0.000341	0.000341	0.000534
SUFU	0.000485	0.000992	0.00037	0.002721
TLE1	0.000353	0.000459	0.000298	0.00095
WISP1	0.002249	0.00416	0.000677	0.002525
WNT11	0.051931	0.136607	0.014908	0.058912
ZIC2	0.000325	0.000443	0.00026	0.000867

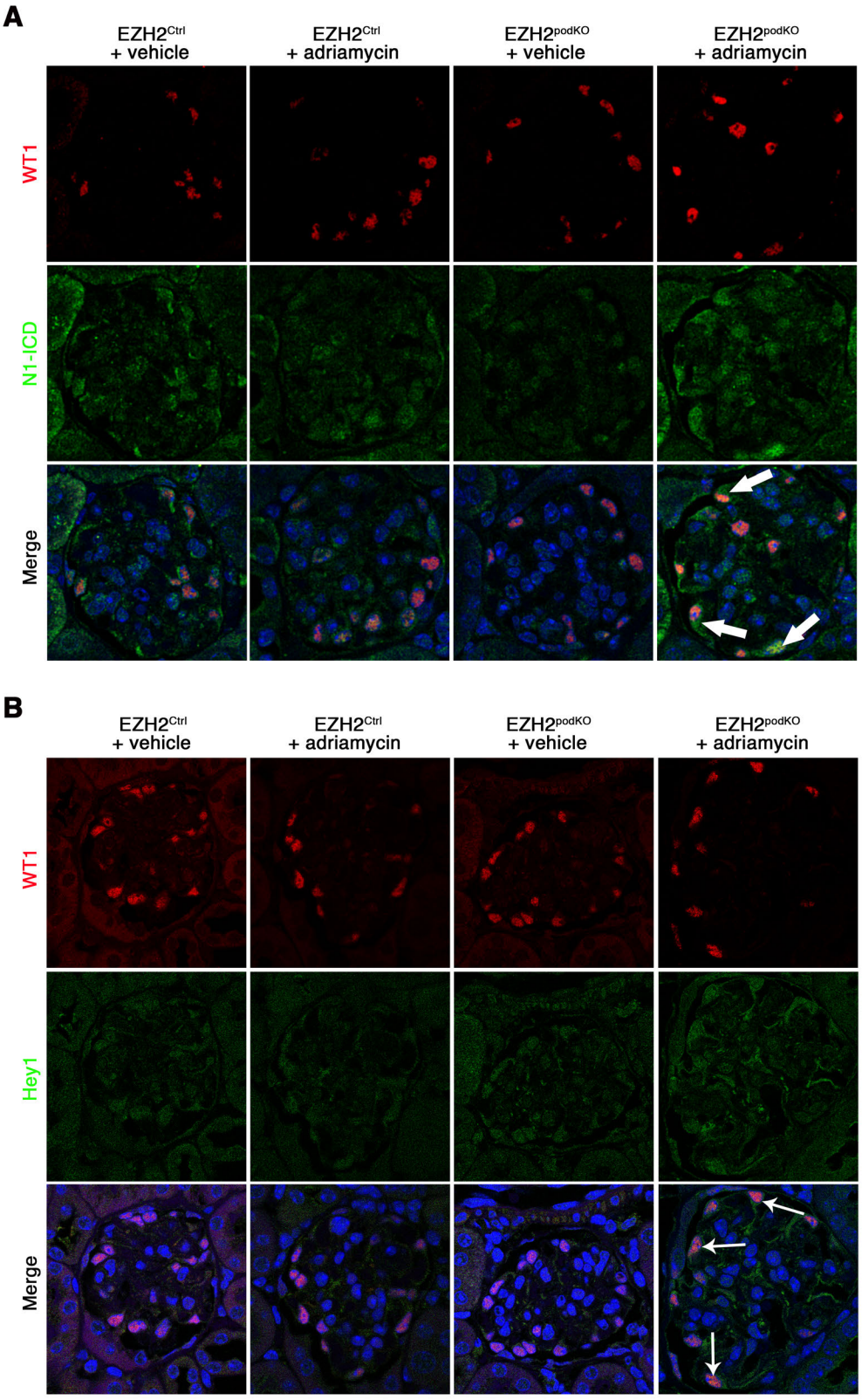
The genes highlighted in red are significantly upregulated in TGF-ß1 treated human podocytes compared to control ($P < 0.05$ by 2-tailed Student t test) and are shown in Figure 6F. The two genes highlighted in blue are significantly downregulated with TGF-ß1.

Supplemental Table 4. Primer sequences.

Target gene	Primer sequence (5' – 3')
Mouse Hey1 forward	GCTCACCCAGACTACAGCTC
Mouse Hey1 reverse	CGCTTCTCGATGATGCCTCT
Mouse α-SMA forward	CAGGGAGTAATGGTTGGAAT
Mouse α-SMA reverse	TCTCAAACATAATCTGGGTCA
Mouse podocin forward	TTGAGAATGGACAGCAGGGC
Mouse podocin reverse	GGGACTCTGAAGCAGCCTTT
Mouse Jagged-1 forward	GAAAGACCACTGCCGTACCA
Mouse Jagged-1 reverse	CACACAGGTCCCGCTATTGT
Mouse Notch1 forward	CTTGCCAGGTTTTGCTGGAC
Mouse Notch1 reverse	CTTTGCCGTTGACAGGGTTG
Mouse Notch2 forward	CCGTGGGGCTGAAAAATCTC
Mouse Notch2 reverse	GGGTCATCTTCCGACAGCAA
Mouse RPL13a forward	GCTCTCAAGGTTGTTCGGCTGA
Mouse RPL13a reverse	AGATCTGCTTCTTCTTCCGATA
Jag1 promoter forward	GAGTGTCGCTGCTAATTGCG
Jag1 promoter reverse	AGAACCTAACCGCTGTCGTG
Human Jagged-1 forward	ATGCGTTCCCCACGGAC
Human Jagged-1 reverse	CCCCACACACCTTGGCTC
Human Hes1 forward	AAAAATTCCTCGTCCCCGGT
Human Hes1 reverse	ATGCCGCGAGCTATCTTTCT
Human Wnt11 forward	TAACCCGCCGCCTCCG
Human Wnt11 reverse	GACAGCGCCAGCCACTT
Human GAPDH forward	AAGACCTTGGGCTGGGACTG
Human GAPDH reverse	TGGCTCGGCTGGCGAC

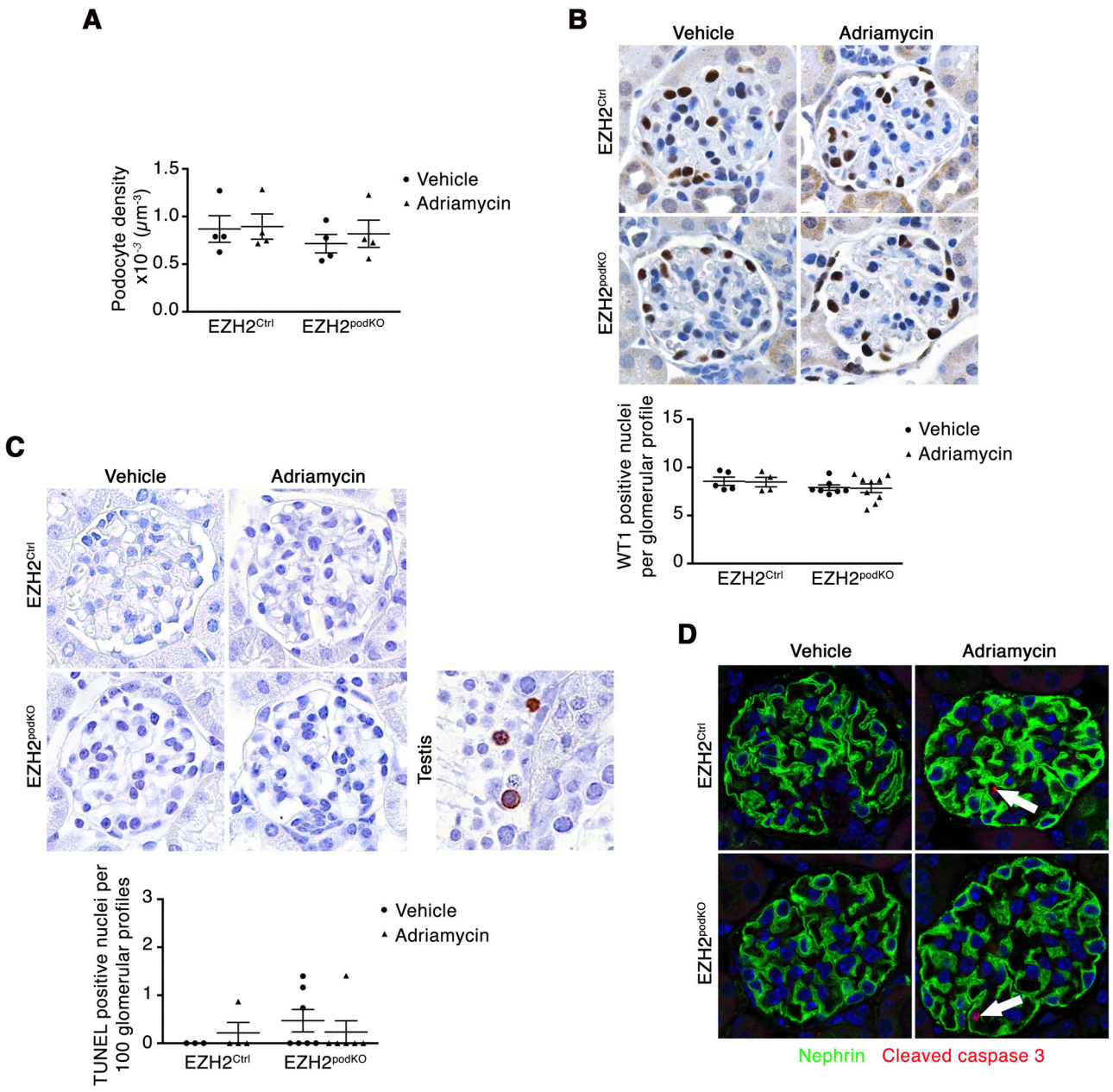


Supplemental Figure 1. Mesangial matrix accumulation in EZH2^{podKO} mice injected with adriamycin. Periodic acid-Schiff stained kidney sections and quantification of mesangial matrix in EZH2^{Ctrl} (vehicle, n=3; adriamycin, n=4) and EZH2^{podKO} (vehicle, n=7; adriamycin, n=8) mice 10 days after adriamycin (25mg/kg) injection. Original magnification x 400. AU = arbitrary units. Values are mean ± s.e.m.. *P < 0.05, **P < 0.01 by 1-way ANOVA followed by Fisher least significant difference post hoc test.



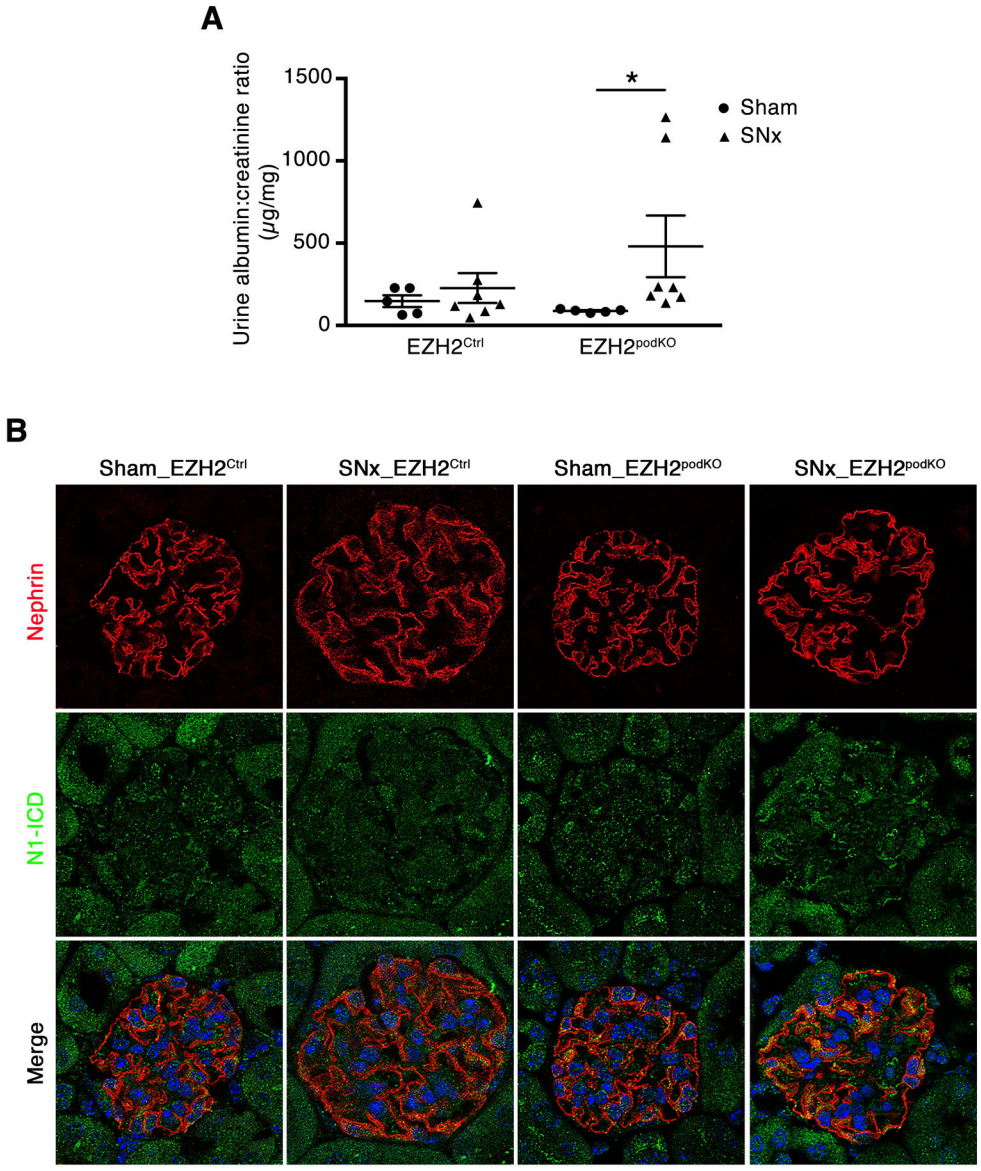
Supplemental Figure 2. Upregulation of the Notch pathway in podocytes of EZH2^{podKO} mice injected with adriamycin. Immunofluorescence staining for (**A**) the podocyte marker WT1 and the truncated Notch receptor, N1-ICD in EZH2^{Ctrl} and EZH2^{podKO} mice or (**B**) WT1 and the Notch target

gene, Hey1 in $EZH2^{Ctrl}$ and $EZH2^{podKO}$ mice 10 days after adriamycin (25mg/kg) injection (n=3/group). Blue = DAPI. Original magnification x 630. The thick white arrows mark N1-ICD in WT1 positive glomerular cells (podocytes) in the $EZH2^{podKO}$ mouse injected with adriamycin and the thin white arrows mark Hey1 expression in WT1 positive glomerular cells in the $EZH2^{podKO}$ mouse injected with adriamycin.

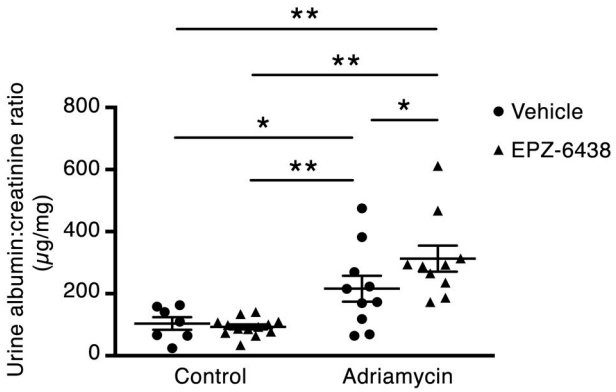


Supplemental Figure 3. Podocyte number and apoptosis are unaffected by adriamycin in EZH2^{podKO} mice. (**A**) Glomerular podocyte density determined by transmission electron microscopy in EZH2^{Ctrl} (vehicle, n=4; adriamycin, n=4) and EZH2^{podKO} (vehicle, n=4; adriamycin, n=4) mice 10 days after adriamycin (25mg/kg) injection. (**B**) Glomerular WT1 positive nuclei in EZH2^{Ctrl} (vehicle, n=5; adriamycin, n=4) and EZH2^{podKO} (vehicle, n=7; adriamycin, n=9) mice. Original magnification x 400. (**C**) TUNEL staining of kidney sections from EZH2^{Ctrl} (vehicle, n=3; adriamycin, n=4) and EZH2^{podKO} (vehicle, n=7; adriamycin, n=6) mice. TUNEL positive nuclei were present in less than 1 in 20 glomeruli in all cases. A photomicrograph of a TUNEL stained mouse testis is shown as a positive control. Original magnification x 400. (**D**) Immunofluorescence for the podocyte marker nephrin and cleaved caspase 3 in kidney sections from EZH2^{Ctrl} and EZH2^{podKO} mice injected with vehicle or adriamycin. Original magnification x 630. Twenty glomerular profiles were examined in four mice per group. Cells positively immunostaining for cleaved caspase 3 (labeled by thick white arrows in the photomicrographs) were found in 3/80 glomeruli in EZH2^{Ctrl} mice injected with

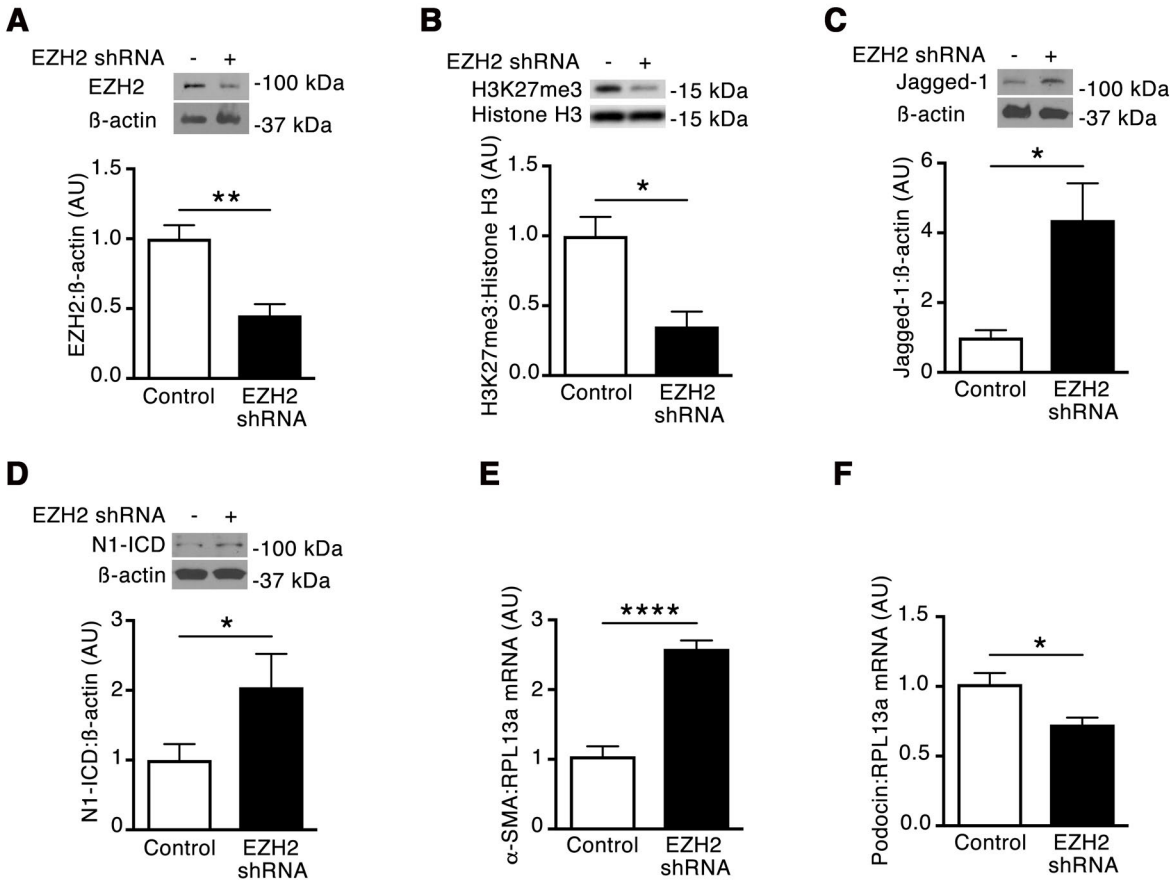
adriamycin; 1/80 glomeruli in EZH2^{podKO} mice injected with adriamycin and 0/80 glomeruli in EZH2^{Ctrl} and EZH2^{podKO} mice injected with vehicle. None of the cleaved caspase 3 positive cells co-stained with nephrin. The presence of apoptosis (crescent-like condensation of chromatin) was also sought in an average of 10 podocyte nuclei from three glomeruli in four mice per group by transmission electron microscopy and was not observed in any of the mice from any of the treatment groups. Values are mean ± s.e.m.



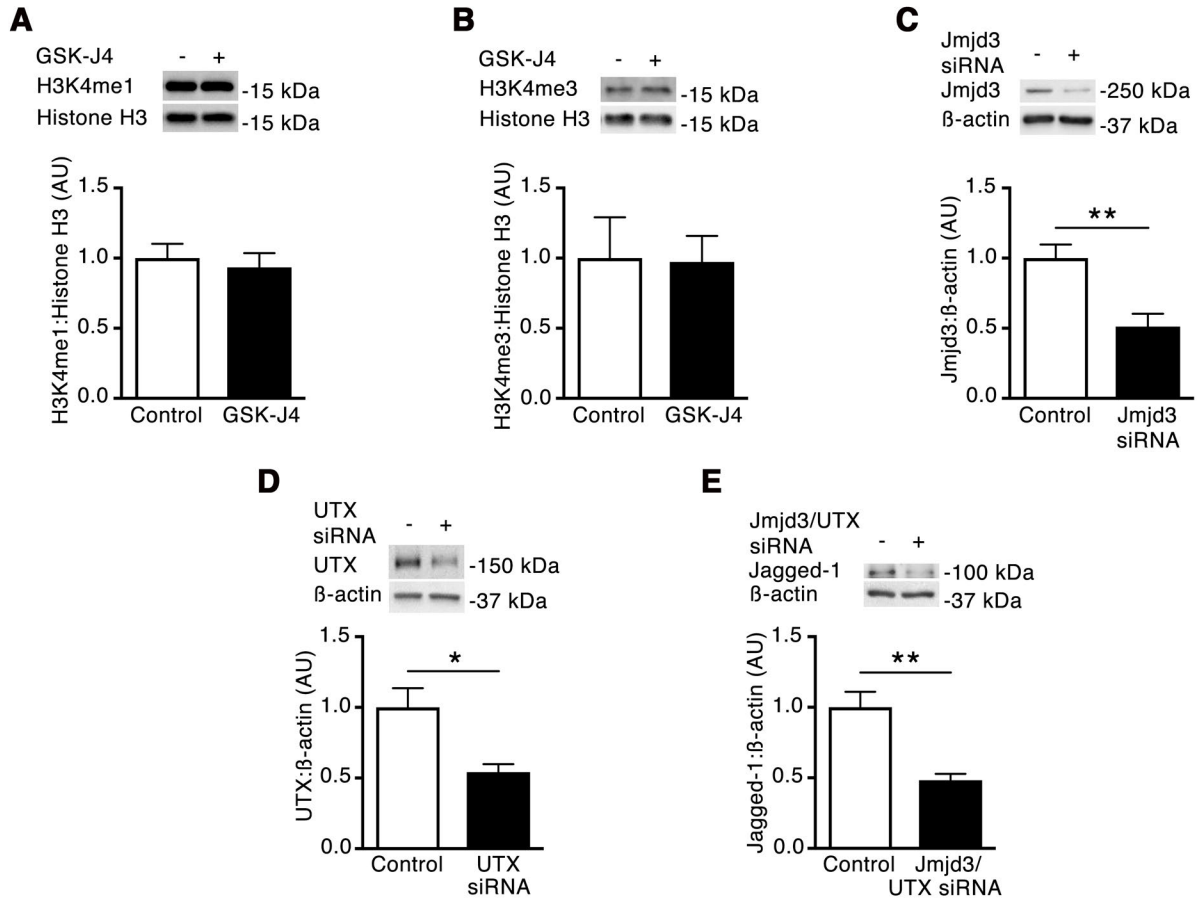
Supplemental Figure 4. EZH2 deletion from podocytes augments albuminuria and increases podocyte N1-ICD levels in mice subjected to subtotal nephrectomy. (**A**) Urine albumin:creatinine ratio and (**B**) immunofluorescence for the podocyte protein, nephrin and the truncated Notch receptor, N1-ICD in sham-operated and subtotally nephrectomized (SNx) $EZH2^{Ctrl}$ (sham, n=5; SNx, n=7) and $EZH2^{podKO}$ (sham, n=5; SNx n=7) mice after six weeks. Blue = DAPI. Original magnification x 630. Values are mean ± s.e.m.. *P < 0.05 by Kruskal-Wallis test followed by Dunn's post hoc comparison.



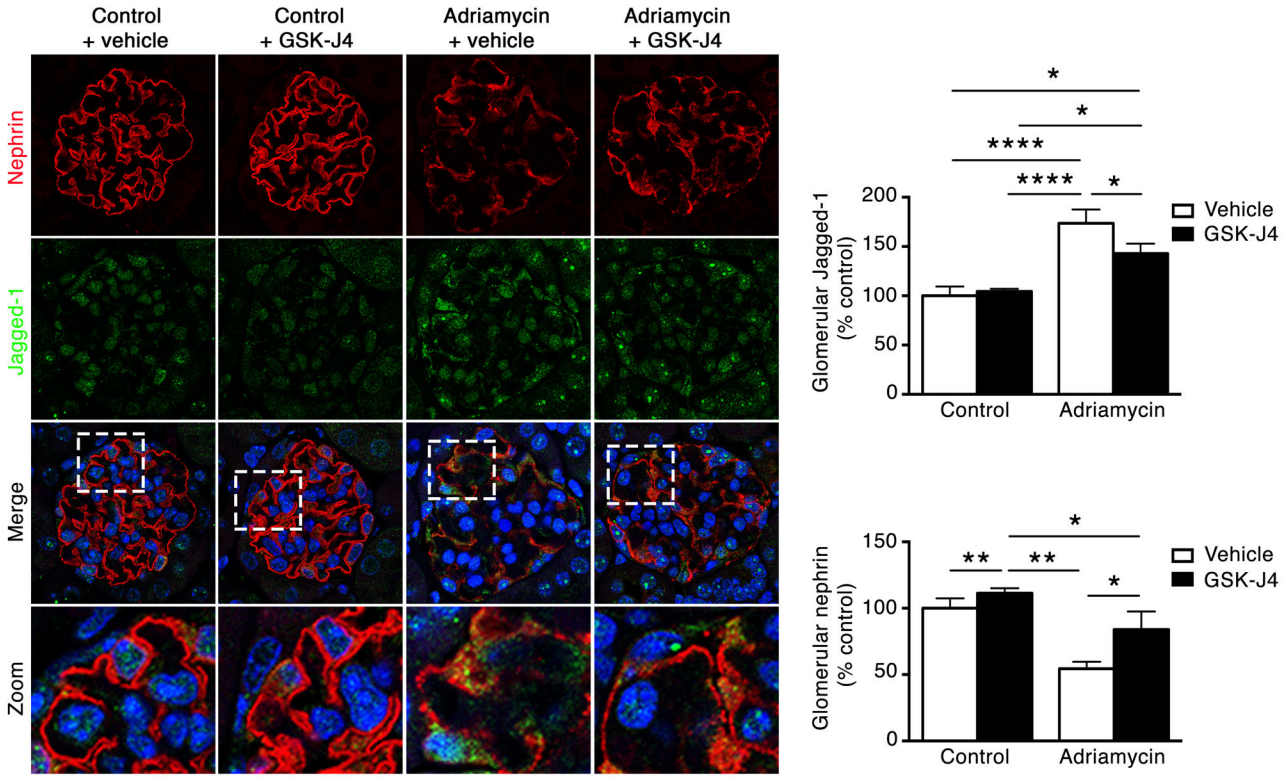
Supplemental Figure 5. The EZH2 inhibitor EPZ-6438 augments albuminuria in adriamycin-injected mice. Urine albumin:creatinine ratio in BALB/c mice after injection with adriamycin (10mg/kg) (or vehicle) and treated with vehicle or EPZ-6438 (100mg/kg by daily gavage for 10 days) (control + vehicle, n=7; control + EPZ-6438, n=13; adriamycin + vehicle, n=10; adriamycin + EPZ-6438, n=10). Values are mean ± s.e.m.. *P < 0.05, **P < 0.01 by 1-way ANOVA followed by Fisher least significant difference post hoc test.



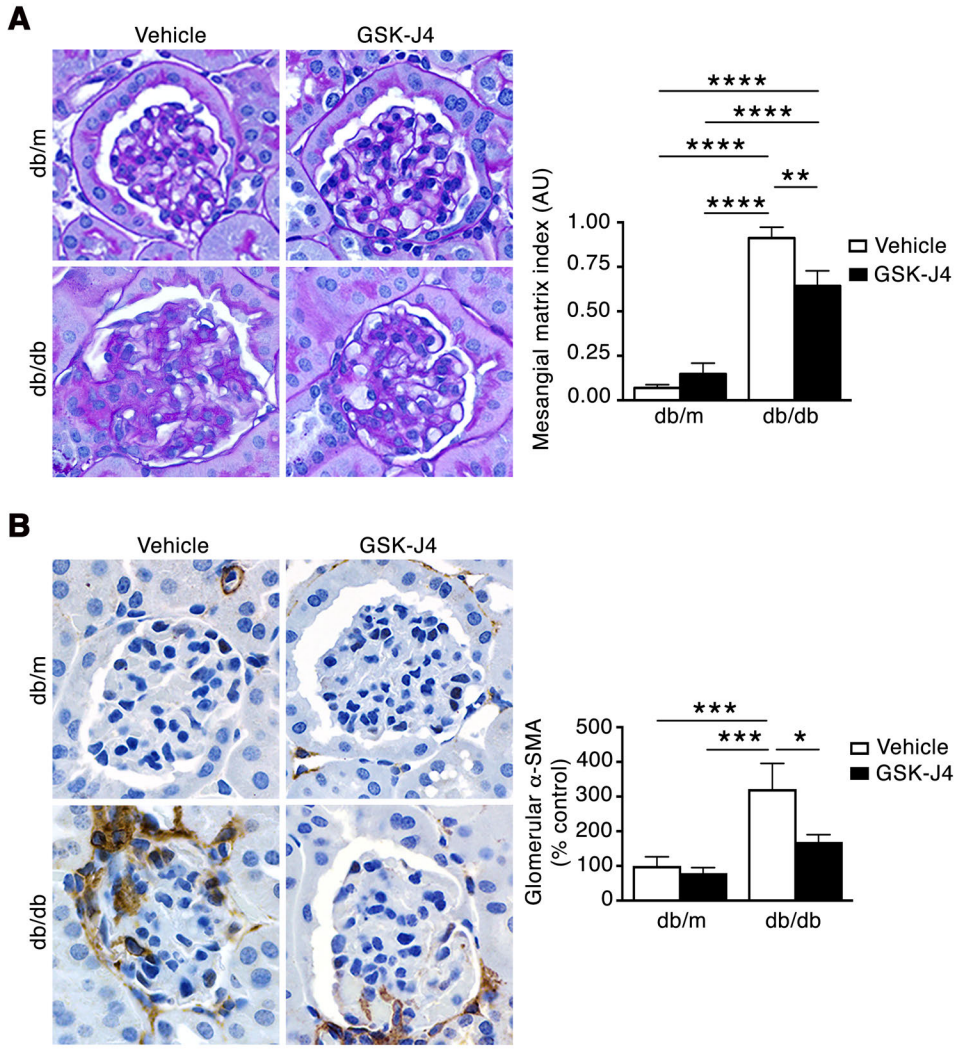
Supplemental Figure 6. Effect of EZH2 knockdown with shRNA on podocyte Notch pathway activation in cultured mouse podocytes. (**A**) Immunoblotting for EZH2 in mouse podocytes transfected with empty vector (control) or EZH2 shRNA (n=6). (**B**) Immunoblotting for H3K27me3 (n=3). (**C**) Immunoblotting for Jagged-1 (n=3). (**D**) Immunoblotting for N1-ICD (n=8). (**E** and **F**) RTQ-PCR for α-smooth muscle actin (α-SMA; **E**) and podocin (**F**) (n=6). AU = arbitrary units. Values are mean ± s.e.m.. **P* < 0.05, ***P* < 0.01, *****P* < 0.0001 by 2-tailed Student *t* test (A, B, C, E, F) and 2-tailed Mann-Whitney test (D).



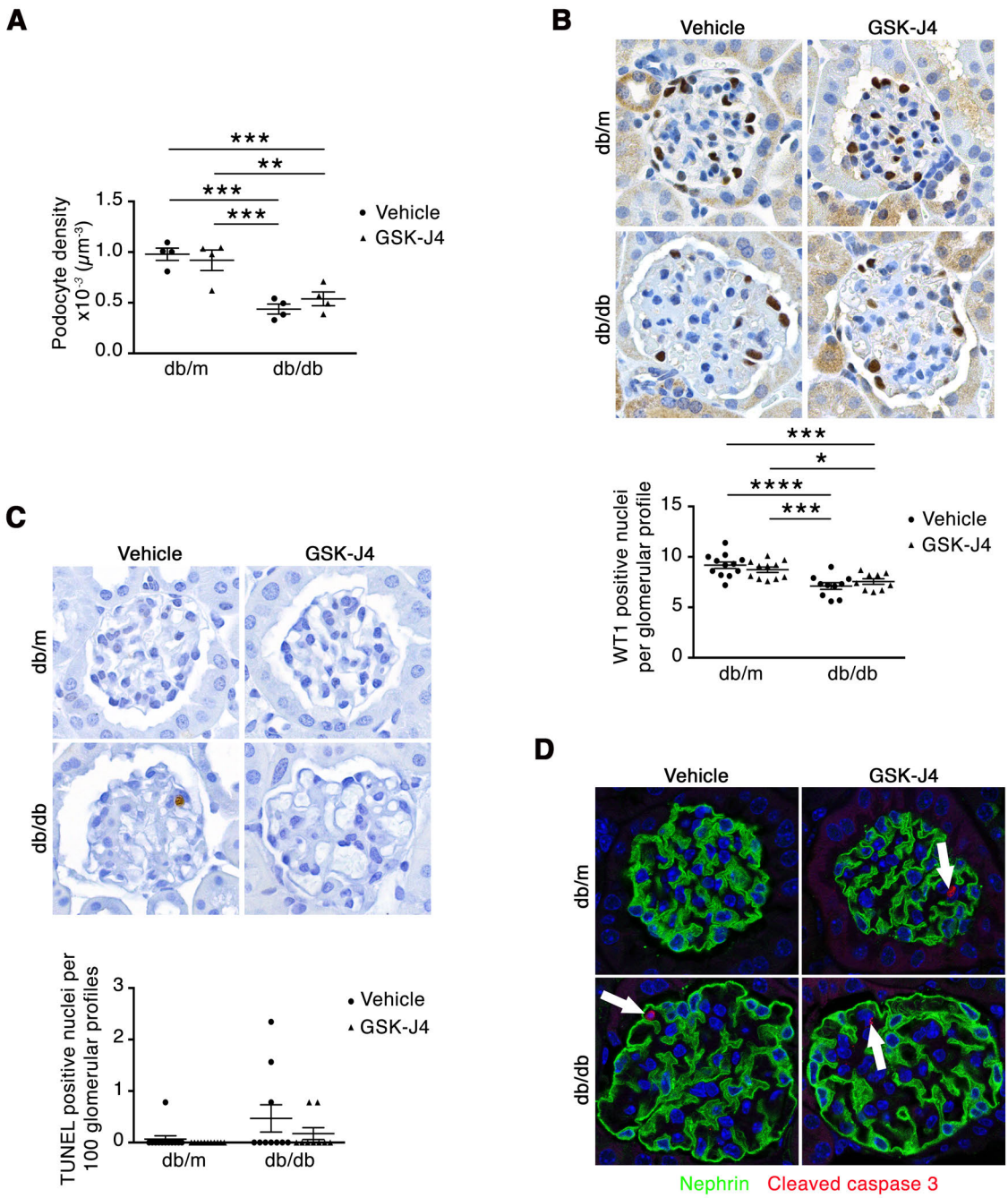
Supplemental Figure 7. The Jmjd3/UTX inhibitor GSK-J4 does not affect H3K4me1 or H3K4me3 content in mouse podocytes and Jmjd3/UTX siRNA decreases Jagged-1 expression. (A and B) Immunoblotting for H3K4me1 (A, n=6) and H3K4me3 (B, n=6) in mouse podocytes treated with or without exposure to GSK-J4 (5μM for 48h). (C and D) Effect of siRNA (or scramble control) on Jmjd3 (C, n=5) and UTX (D, n=4) expression in mouse podocytes. (E) Immunoblotting for Jagged-1 following transfection with scramble or Jmjd3/UTX siRNA (n=6). AU = arbitrary units. Values are mean ± s.e.m.. **P* < 0.05, ***P* < 0.01 by 2-tailed Student *t* test.



Supplemental Figure 8. Jmjd3/UTX inhibition attenuates Jagged-1 upregulation and nephrin loss in mice injected with adriamycin. Immunofluorescence staining for nephrin and Jagged-1 in glomeruli from BALB/c mice injected with adriamycin (10mg/kg) or vehicle and treated with GSK-J4 (10mg/kg thrice weekly i.p. 10 days) or vehicle (control + vehicle, n=5; control + GSK-J4, n=6; adriamycin + vehicle, n=6; adriamycin + GSK-J4, n=6). Blue = DAPI. Original magnification x 630. The zoom is an enlargement of the dashed areas. AU = arbitrary units. Values are mean ± s.e.m.. **P* < 0.05, ***P* < 0.01, *****P* < 0.0001 by 1-way ANOVA followed by Fisher least significant difference post hoc test.

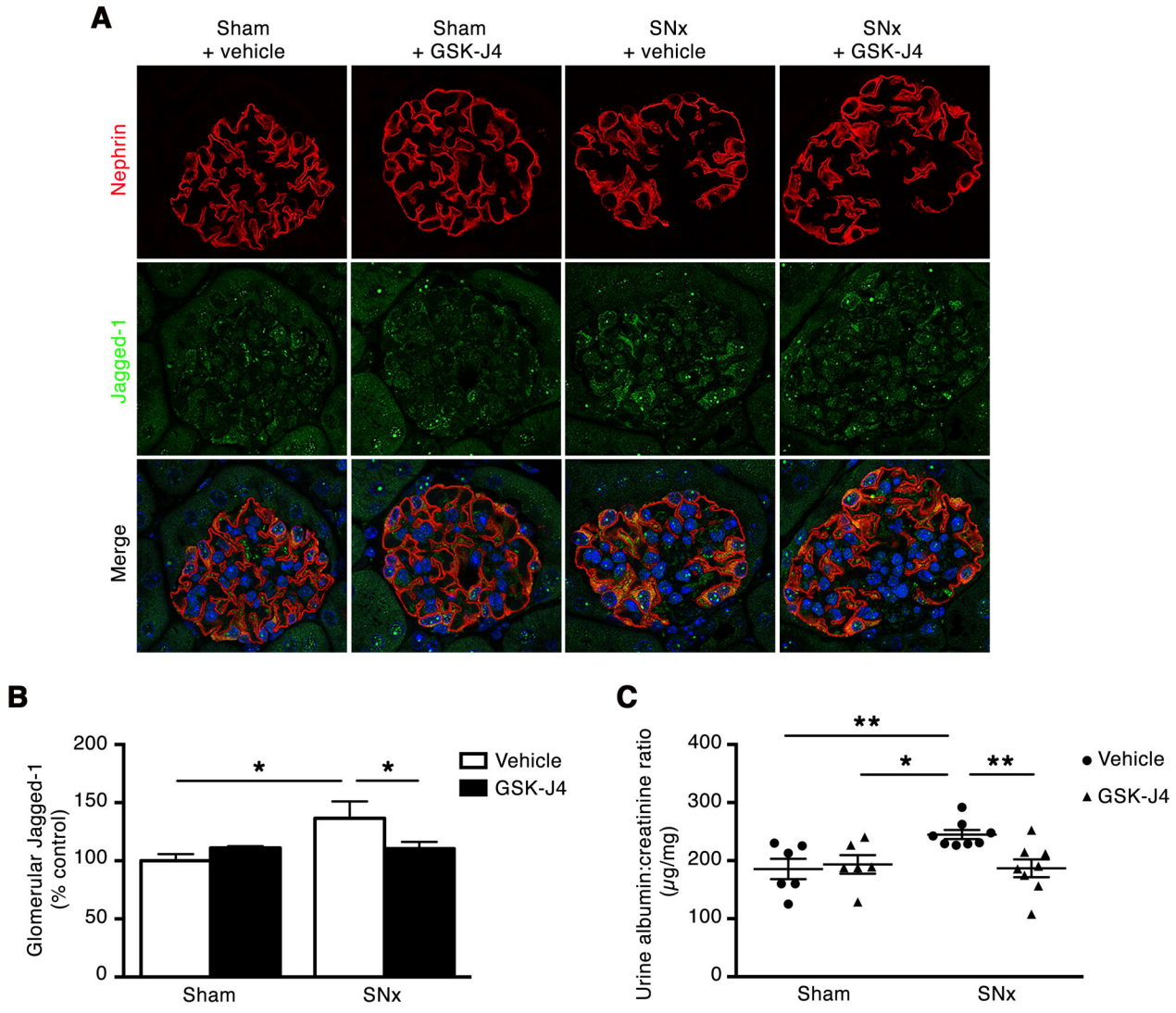


Supplemental Figure 9. Jmjd3/UTX inhibition attenuates mesangial matrix accumulation and glomerular α-smooth muscle actin (α-SMA) expression in diabetic mice. (**A**) Periodic acid-Schiff (PAS)-stained kidney sections and quantification of mesangial matrix and (**B**) immunostaining for α-SMA and quantification of glomerular α-SMA in non-diabetic db/m and diabetic db/db mice treated with vehicle or GSK-J4 (10mg/kg) thrice weekly i.p. for 10 weeks (db/m + vehicle, n=12; db/m + GSK-J4, n=11; db/db + vehicle, n=10; db/db + GSK-J4, n=9). Original magnification x 400. AU = arbitrary units. Values are mean ± s.e.m.. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 by 1-way ANOVA followed by Fisher least significant difference post hoc test.



Supplemental Figure 10. Jmjd3/UTX inhibition does not affect podocyte number or apoptosis in diabetic mice. (**A**) Glomerular podocyte density determined by transmission electron microscopy in non-diabetic db/m (vehicle, n=4; GSK-J4, n=4) and diabetic db/db (vehicle, n=4; GSK-J4, n=4) mice treated with GSK-J4 (10mg/kg thrice weekly i.p.) or vehicle for 10 weeks. (**B**) Glomerular WT1 positive nuclei in db/m (vehicle, n=12; GSK-J4, n=11) and db/db (vehicle, n=10; GSK-J4, n=9) mice. Original magnification x 400. (**C**) TUNEL staining in kidney sections from db/m (vehicle, n=12; GSK-J4, n=11) and db/db (vehicle, n=10; GSK-J4, n=9) mice. Original magnification x 400. (**D**) Immunofluorescence staining for the podocyte marker nephrin and cleaved caspase 3 in kidney sections from db/m and db/db mice injected with vehicle or GSK-J4. Original magnification x 630. Twenty glomerular profiles were examined in 3-4 mice/group. Cells positively immunostaining for cleaved caspase 3 (labeled by thick white arrows in the photomicrographs) were

found in 0/60 glomeruli from db/m mice treated with vehicle, 1/60 glomeruli from db/m mice treated with GSK-J4, 1/80 glomeruli from db/db mice treated with vehicle and 1/60 glomeruli from db/db mice treated with GSK-J4. None of the cleaved caspase 3 positive cells co-stained with nephrin. The presence of apoptosis (crescent-like condensation of chromatin) was also sought in an average of 10 podocyte nuclei from three glomeruli in four mice per group by transmission electron microscopy and was not observed in any of the mice from any of the treatment groups. Values are mean ± s.e.m. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 by 1-way ANOVA followed by Fisher least significant difference post hoc test.



Supplemental Figure 11. Jmjd3/UTX inhibition decreases glomerular Jagged-1 expression and attenuates albuminuria in sham and subtotally nephrectomized (SNx) C57BL/6 mice. Mice underwent sham or subtotal nephrectomy surgery and were followed for six weeks before randomization to receive GSK-J4 (10mg/kg thrice weekly i.p.) or vehicle for a further four weeks (sham + vehicle, n=6; sham + GSK-J4, n=6; SNx + vehicle, n=8; SNx + GSK-J4, n=8). (**A**) Immunofluorescence staining for the podocyte protein, nephrin and Jagged-1. Blue = DAPI. Original magnification x 630. (**B**) Quantification of glomerular Jagged-1 (sham + vehicle, n=5; sham + GSK-J4, n=5; SNx + vehicle, n=6; SNx + GSK-J4, n=6). (**C**) Urine albumin:creatinine ratio. AU = arbitrary units. Values are mean ± s.e.m.. *P < 0.05, **P < 0.01 by 1-way ANOVA followed by Fisher least significant difference post hoc test.