Supplementary Tables

Table S1

PCR primers for genotyping			
Transgene	primer 1	primer 2	product size
Nphs1-hCD25	GTTTATTATCAGTGCGTCCAG	CTTGTCATCGTCGTCCTTGTA	409 bp
Nphs1-Cre	AGGTTCGTTCACTCATGGA	TCGACCAGTTTAGTTACCC	235 bp
Rpl22$^{tm1.1Psam}$	GGGAGGCTTGCTGGATATG	TTTCCAGACACAGGCTAAGTACAC	290 bp (243 bp in wild-type)

Table S2

	Company	Catalogue	dilution, concentration	pretreatment
for paraffin sections				
Nephrin	Progen	GP-N2	1/100	heat
HA-tag	Cell Signaling technology	#3724	1/8000	heat
hCD25	Thermo Scientific	MS-1088	(1/20)	heat
WT1	Abcam	ab89901	1/2000	heat
Podocalyxin	R&D systems	MAB1556	1/8000	trypsin
podocin	Sigma-Aldrich	P0372	1/5000	pepsin
Biotin-LTL	Vector	B-1325	10 μg/ml	
for fluorescent staining				
FITC-LTL	Vector	FL-1321	10 μg/ml	
WT1(f)	Abcam	ab89901	1/2000	
for immunoprecipitation				
HA.11 epitope	BioLegend	901501	12.5 μg/ml	

Table S3

Primers for quantitative PCR		
gene	Primer1	Primer2
Actb	ATGAGCTGCCTGACGGCCAGGTCATC	TGGTACCACCAGACAGCACTGTGTTG
Gadd45b	CTGCCTCCTGGTCACGAA	TTGCCTCTGCTCTCTTCACA
P2rx7	AGCAGAGGTGACGGAGAATG	CTGCACTTGGCCTTCTGACT
Cxcl1	GACTCCAGCCACACTCCAAC	TGACAGCGCAGCTCATTG
Egr1	CCTATGAGCACCTGACCACA	TCGTTTGGCTGGGATAACTC
Lrp2	AGGCTCCGACTTCGTAACTG	GCATGGTGATGGGGAGTT
Tnfaip2	GCCGAAAGCTACCACTACCA	GAAGCTCCTGTGCCAACTTC
Gja1	GTGCCGGCTTCACTTTCA	GGAGTAGGCTTGGACCTTGTC
Gja3	CCCCAGTGTCTGAGATCGAG	AGGCCGAACAAGAGCAAG
Gja5	CAGGGAGGAGGAAAGGAAGC	ACTGTGGAGTGCTTGTGGAC
Gjc1	ACAGGAGTTCTGGTGAACAGG	TCTCCTCTAGCAGGCGAGTC

Table S4

TaqMan Gene Expression Assays		
gene	Company	Assay ID
Nphs1	ThermoFisher	Mm00497828_m1
Nphs2	ThermoFisher	Mm00499929_m1
Wt1	ThermoFisher	Mm00460570_m1
hCD25	ThermoFisher	Hs00166229_m1

Supplementary Figure Legends

Figure S1. NEP25/Ribotag organoids treated with LMB2 for 3 days. The expression level of nephrin is reduced, whereas megalin and LTL staining is retained. Panels (a–d) represent the equivalent visual fields of the serial sections. Scale bar: 50 μm.

Figure S2. NEP25/Ribotag organoids with or without LMB2 treatment (2 days). In control organoids, podocytes strongly express WT1, HA, podocin, and nephrin. After LMB2 treatment, a few podocyte clusters remained and retained WT1 and HA expression, but the expression levels of podocin and nephrin are reduced. Panels (a–e) and (f–j) represent the equivalent visual fields of the serial sections. Scale bar: 20 μm.

Figure S3. Podocyte injury does not propagate between organoids. NEP25 and Ribotag organoids were cocultured on a single Transwell membrane. Without LMB2, both organoid types express podocin (a, b).After 3 days of LMB2 treatment, the expression of podocin is markedly suppressed in NEP25 organoids (arrows, c) but not in Ribotag organoids (d). Scale bar: 20 μm.

Figure S4. Effects of A83-01, SAR7334, and IKK-16 on indirect podocin suppression in chimeric organoids. LMB2 treatment for 2 days reduces podocin staining (b) despite the presence of HA(+) podocytes (a). Cotreatment with A83-01 (TGF-β inhibitor) (c and d), SAR7334 (TRPC6 inhibitor)(e and f), or

IKK-16 (NF-κB inhibitor)(g and h) does not restore podocin expression. Scale bar: 50 μm.

Figure S5. Connexin mRNA expression in mosaic mice and organoids. (a–d) hCD25(+) and hCD25(–) podocytes were isolated from mosaic mice either untreated (n = 3) or 7 days post-LMB2 injection (n = 5). Quantitative RT-PCR was performed using *Actb* as a control. Data are presented relative to heart RNA from a wild-type mouse. (e) Ratio of LMB2-treated to control polysomal RNA in NEP25/Ribotag organoids (n = 6) and chimeric organoids after 2 days (n = 6) or 4 days (n = 8) of LMB2 treatment. In NEP25/Ribotag organoids, IP RNA is derived from hCD25(+) podocytes. In chimeric organoids, IP RNA is derived from hCD25(−) podocytes. *Gja3* mRNA was undetectable in two IP samples of chimeric organoids treated with LMB2 for 4 days, which are not shown in the graph. LMB2 significantly reduces the mRNA expression level of *Gja3* in NEP25/Ribotag and chimeric organoids ($p < 0.01$).

Figure S6. *Tnfaip2* mRNA expression in mosaic mice and organoids. (a) hCD25(+) and hCD25(−) podocytes were isolated from mosaic mice without LMB2 (n = 3) or 7 days post-LMB2 injection (n = 5). *Tnfaip2* mRNA levels were measured via quantitative RT-PCR, normalized to *Actb*, and expressed relative to total kidney RNA from a wild-type mouse. (b) Ratio of LMB2-treated to control polysomal RNA in NEP25/Ribotag organoids (n = 6) and chimeric organoids after 2 days (n = 6) or 4 days (n = 8) of LMB2 treatment. In NEP25/Ribotag organoids, IP RNA is derived from hCD25(+) podocytes. In chimeric organoids, IP RNA is derived from hCD25(−) podocytes. LMB2

significantly reduces the mRNA expression level of *Tnfaip2* in NEP25/Ribotag organoids ($p < 0.01$) but not in chimeric organoids.

Figure S1

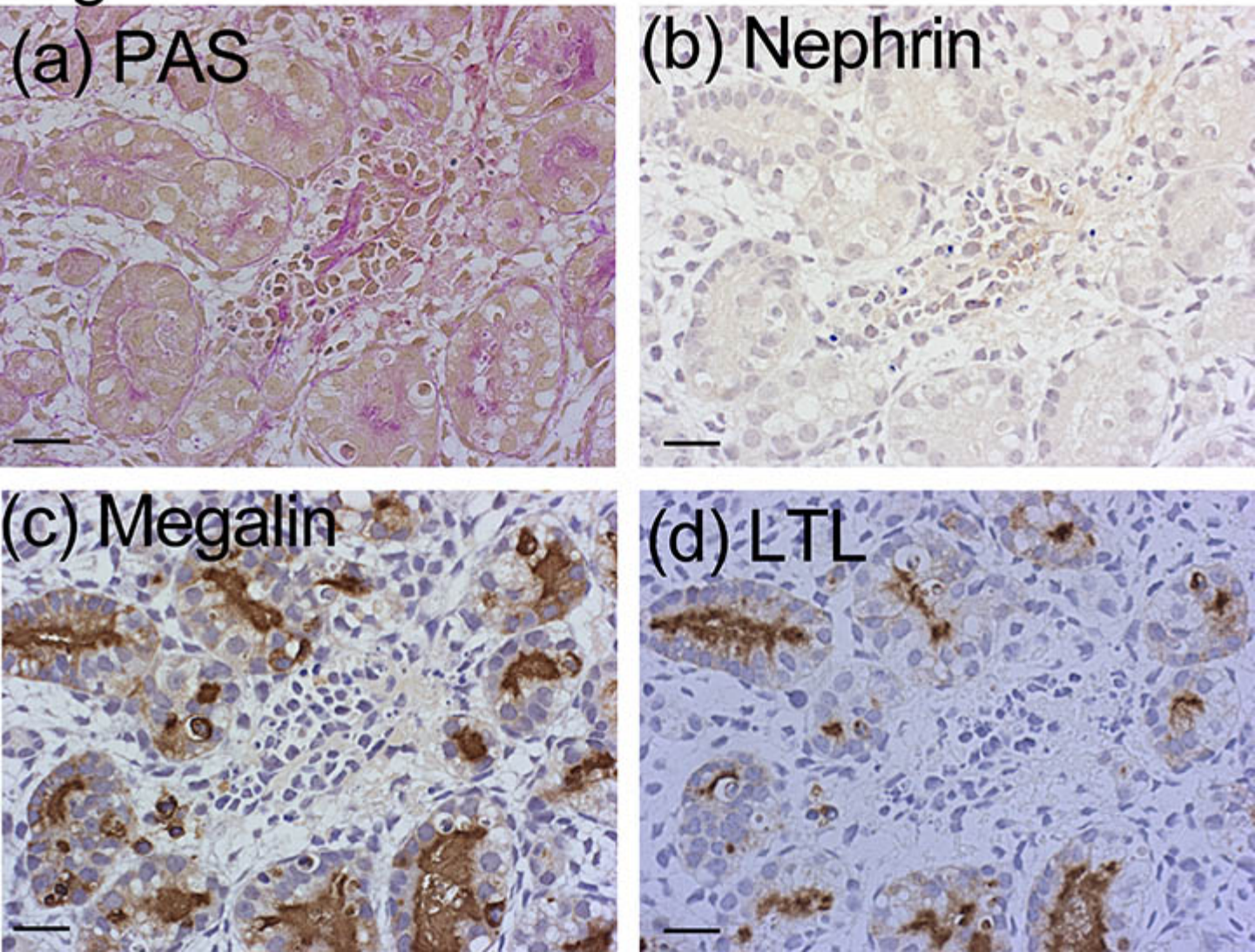


Figure S2

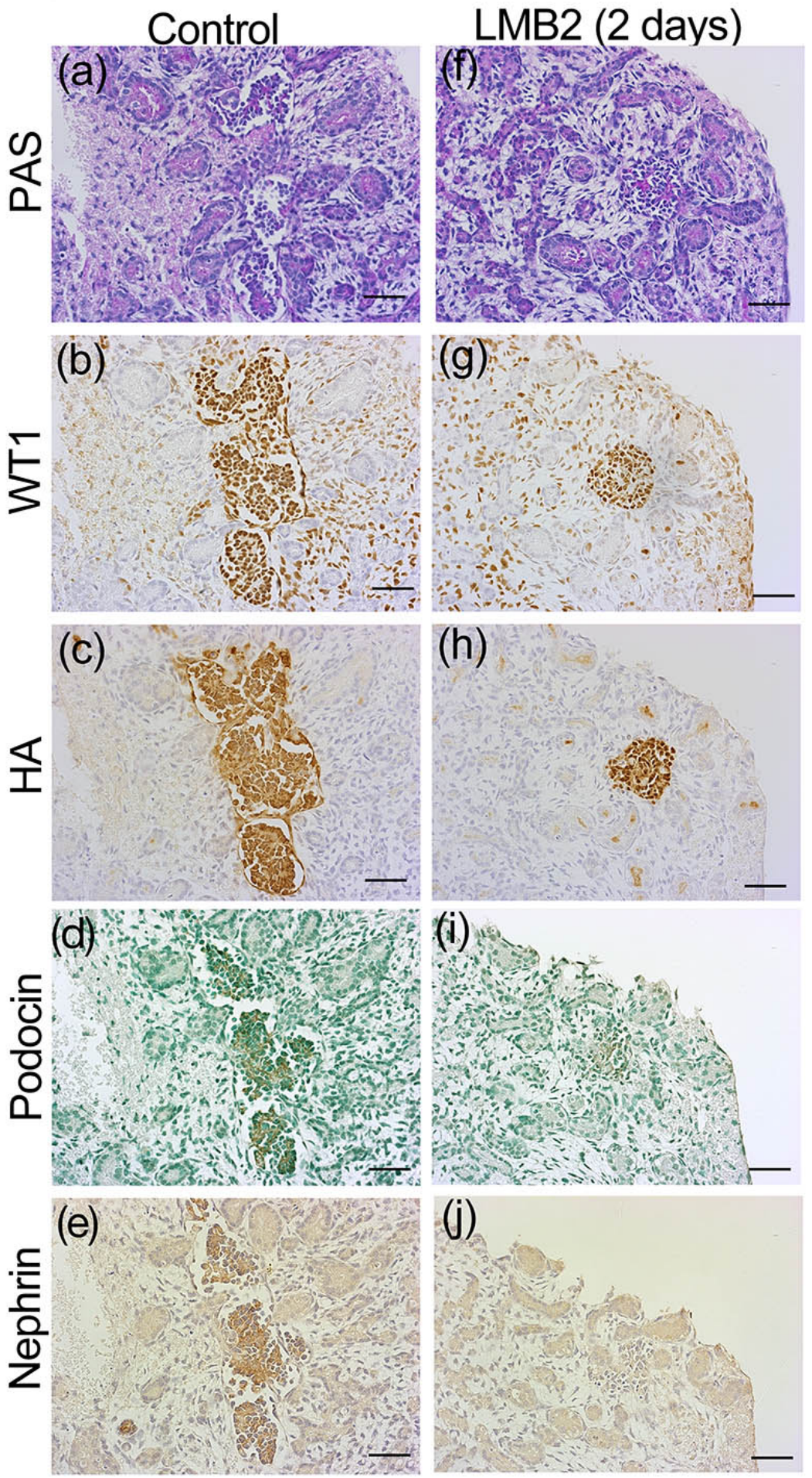


Figure S3

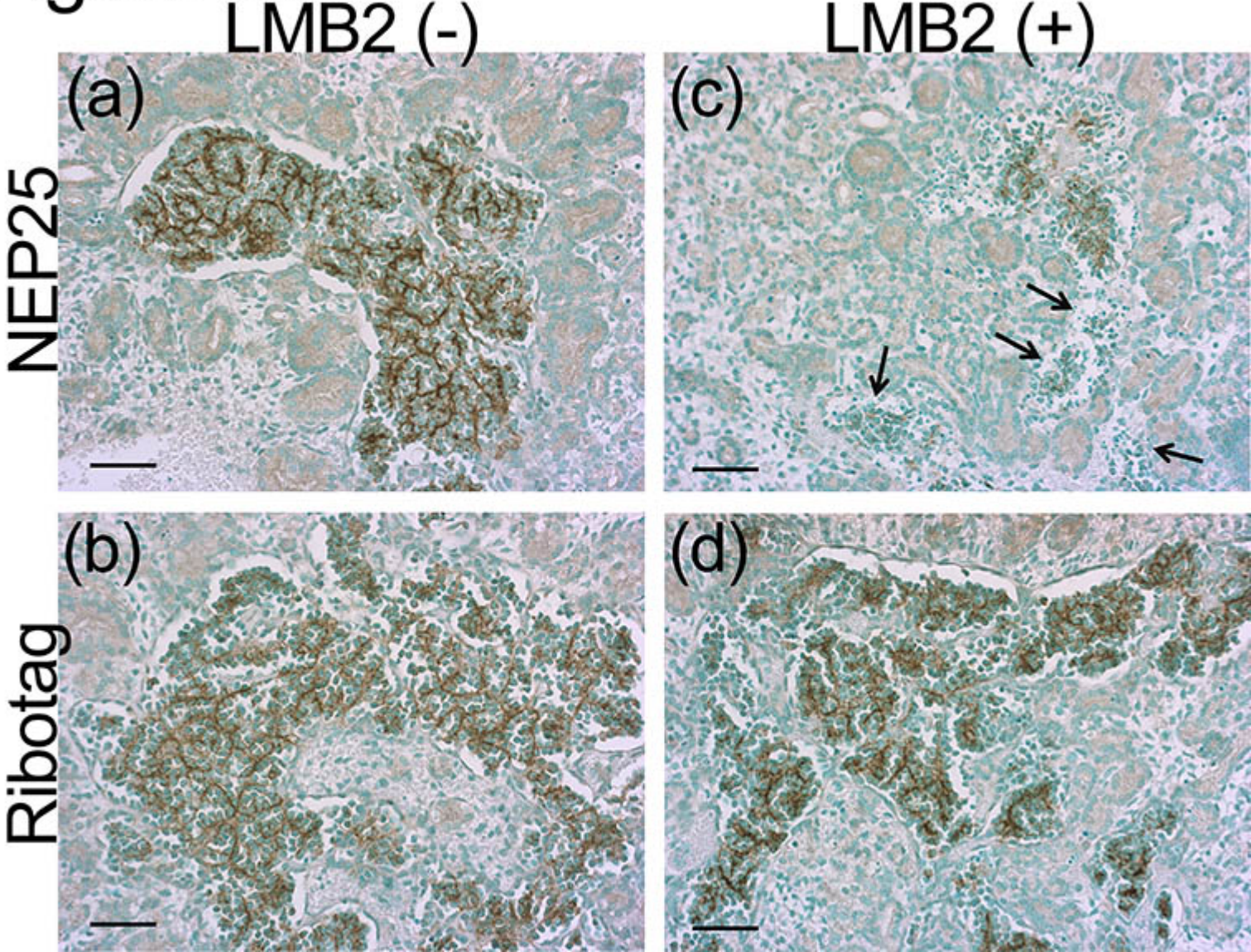


Figure S4

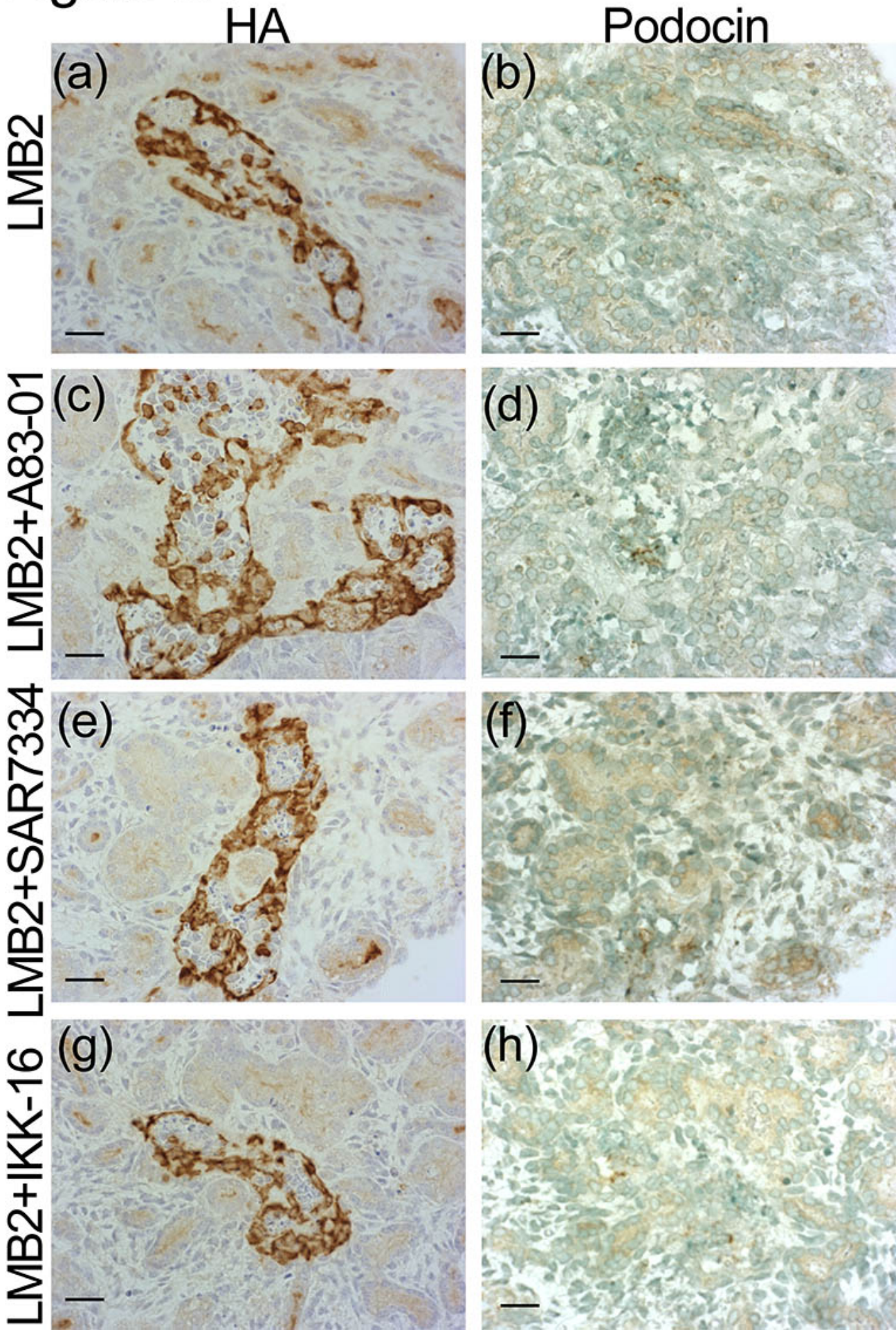

HA
Podocin
LMB2
LMB2+A83-01
LMB2+SAR7334
LMB2+IKK-16
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)

Figure S5

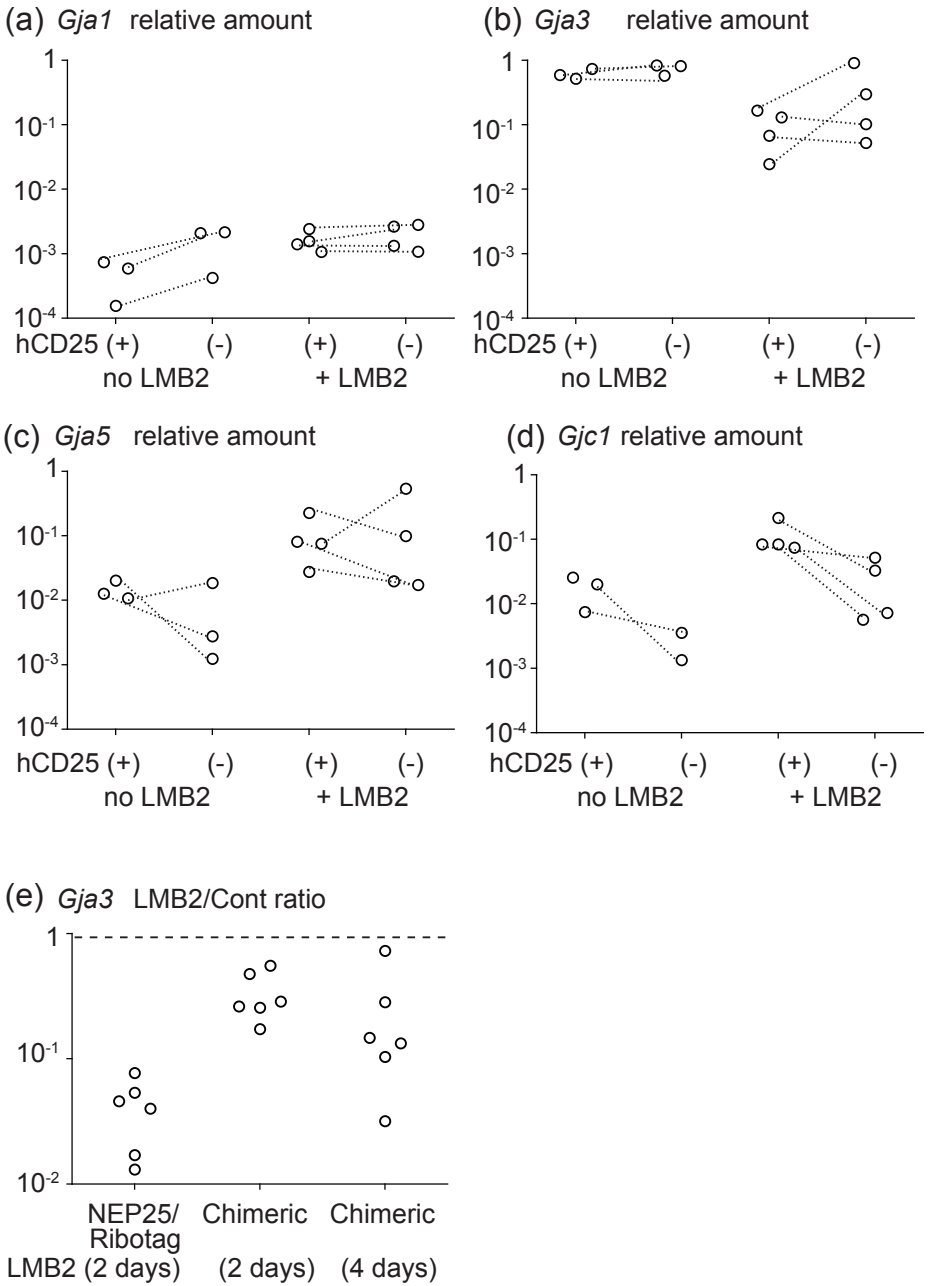
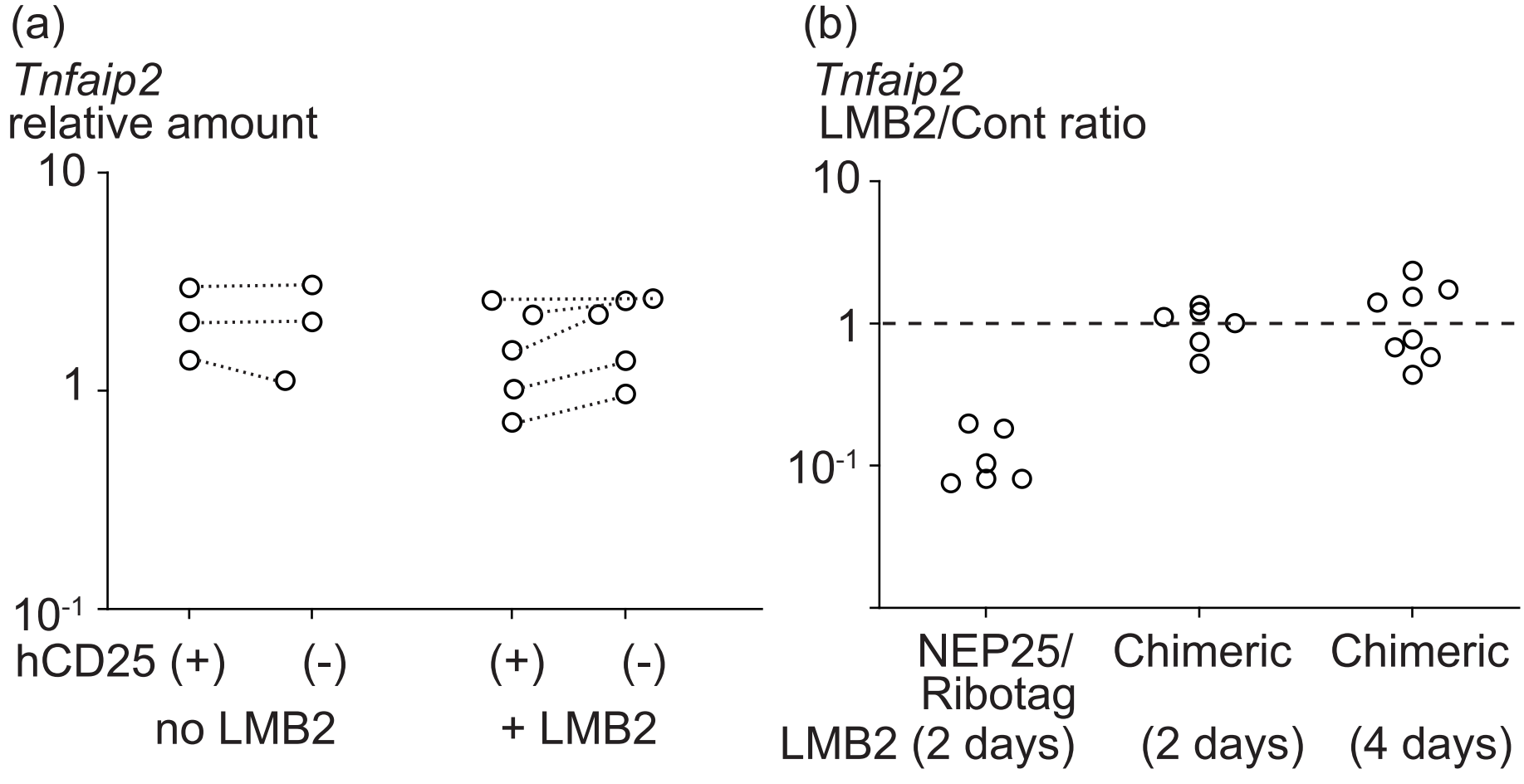

(a) Gja1 relative amount
1
10-1
10-2
10-3
10-4
hCD25 (+) (-) (+) (-)
no LMB2
+ LMB2
(b) Gja3 relative amount
1
10-1
10-2
10-3
10-4
hCD25 (+) (-) (+) (-)
no LMB2
+ LMB2
(c) Gja5 relative amount
1
10-1
10-2
10-3
10-4
hCD25 (+) (-) (+) (-)
no LMB2
+ LMB2
(d) Gjc1 relative amount
1
10-1
10-2
10-3
10-4
hCD25 (+) (-) (+) (-)
no LMB2
+ LMB2
(e) Gja3 LMB2/Cont ratio
1
10-1
10-2
NEP25/ Ribotag LMB2 (2 days)
Chimeric (2 days)
Chimeric (4 days)

Figure S6


(a)
Tnfaip2
relative amount
10
1
10-1
hCD25 (+)
(-)
(+)
(-)
no LMB2
+ LMB2
(b)
Tnfaip2
LMB2/Cont ratio
10
1
10-1
NEP25/
Ribotag
Chimeric
Chimeric
LMB2 (2 days)
(2 days)
(4 days)