

SUPPLEMENTARY MATERIALS

Supplementary Figure 1. Robo2 is required for epithelial cell development in kidney, and Robo2 deficiency induce cellular senescence.

Supplementary Figure 2. Effect of Robo2 on ciliogenesis in mIMCD3 cells.

Supplementary Figure 3. Direct binding assay for Robo2 and Baiap2.

Supplementary Figure 4. Robo2 colocalizes with Baiap2, ZO-1 and Claudin-2 in epithelial cell.

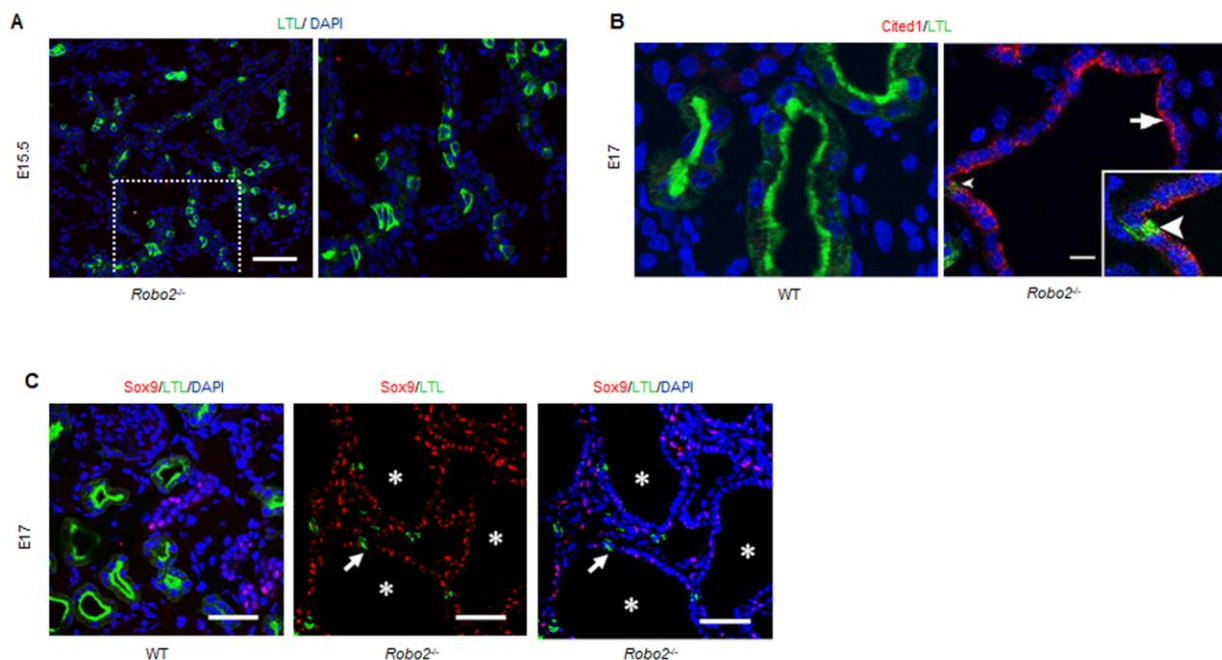
Supplementary Figure 5. Senescent progenitor cells in *Robo2*^{-/-} kidneys

Supplementary Figure 6. Changes in signaling pathways of Robo2.

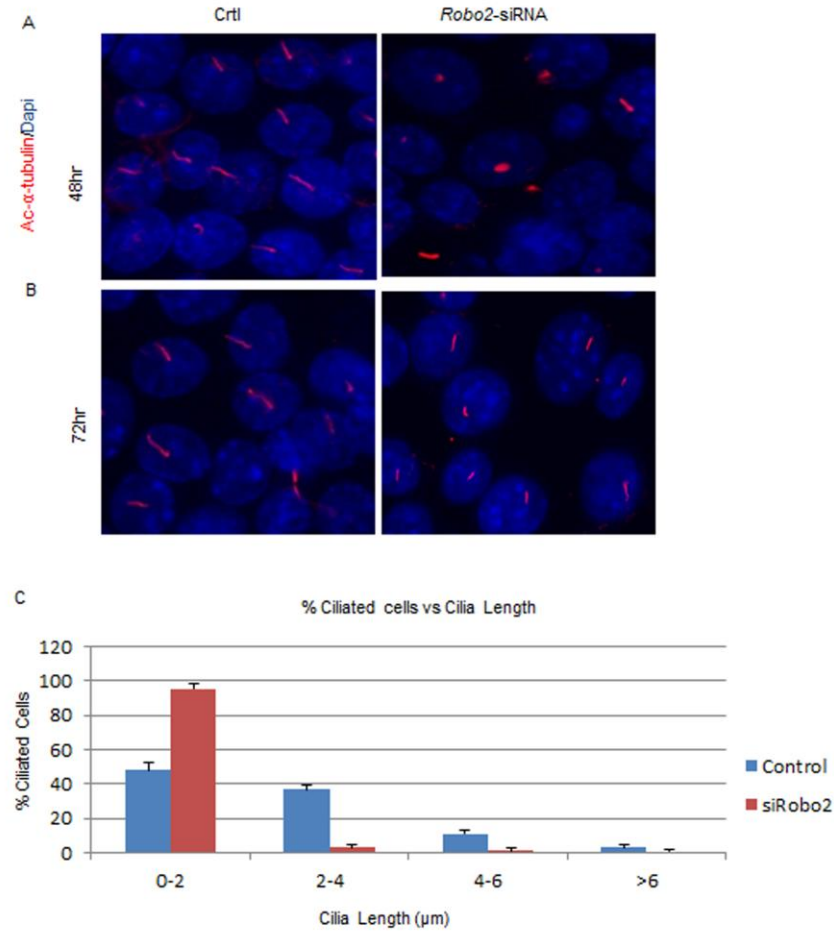
Supplementary Table1. Mascot search results of proteins from Robo2 IP purified excised protein bands

Supplementary Table 2. The top 60 most differentially expressed genes between the kidneys from wild-type and *Robo2*^{-/-} mice

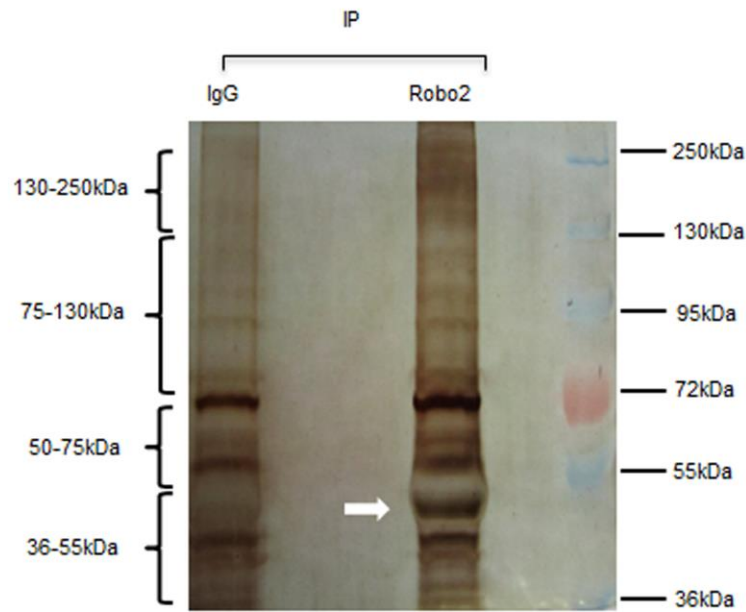
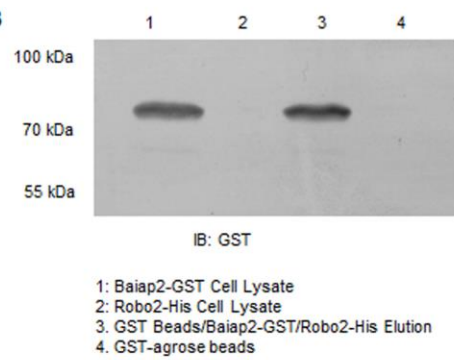
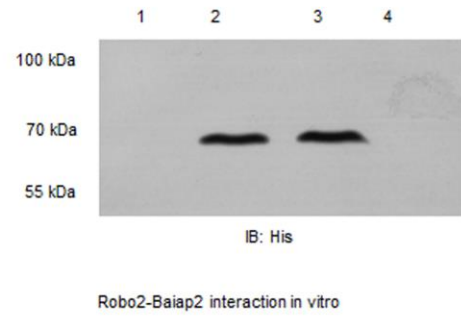
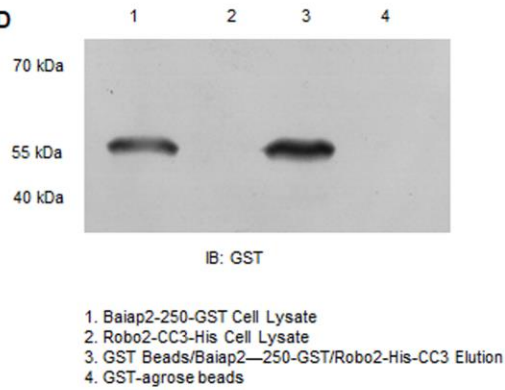
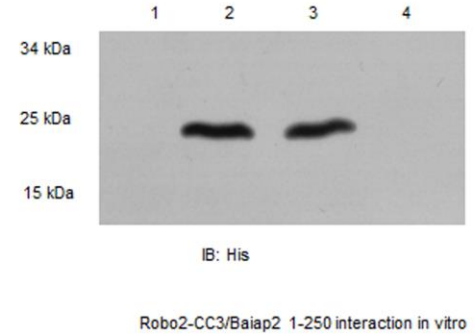
Supplementary Figures and Figure legends



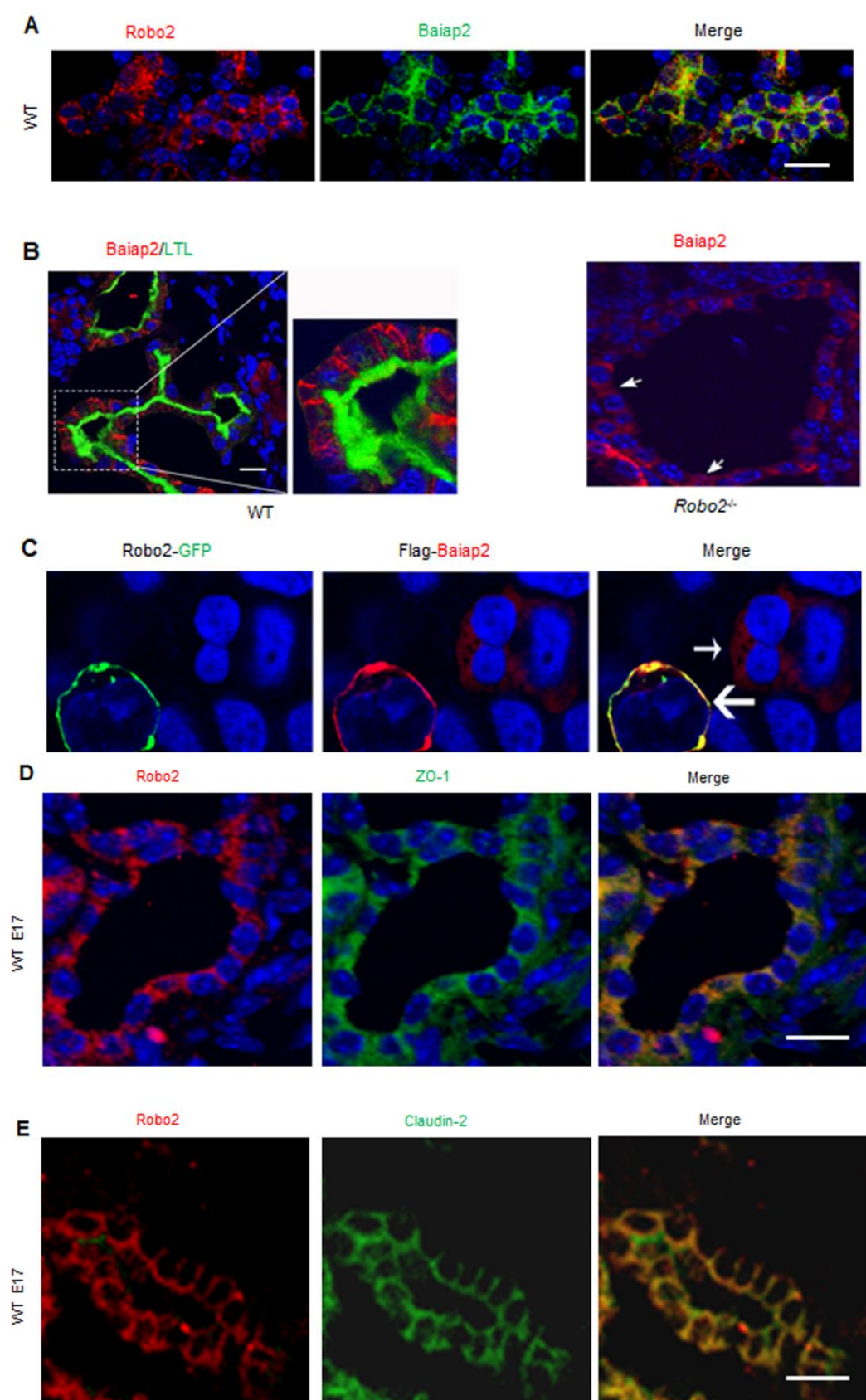
Supplementary Figure 1. Robo2 is required for epithelial cell development in kidney and *Robo2* deficiency induce cellular senescence. **(A)** IF staining of LTL in the cryosections of E15.5 *Robo2*^{-/-} kidney. The misexpression of LTL was found in the *Robo2*^{-/-} kidneys. The right panel shows the magnification of the renal tubules in the *Robo2*^{-/-} kidney. Scale bar, 50 μ m. **(B)** IF staining of Cited1 and LTL in renal tubular epithelial cells in E17 wildtype and *Robo2* knockout kidneys. **(C)** IF staining of Sox9 and LTL in renal tubular epithelial cells in E17 wildtype and *Robo2* knockout kidneys. Sox9 was expressed ectopically in cystic segments with reduced or absent LTL staining (white arrow). The representative image showing cysts (*) in the kidney. Scale bars, 50 μ m.



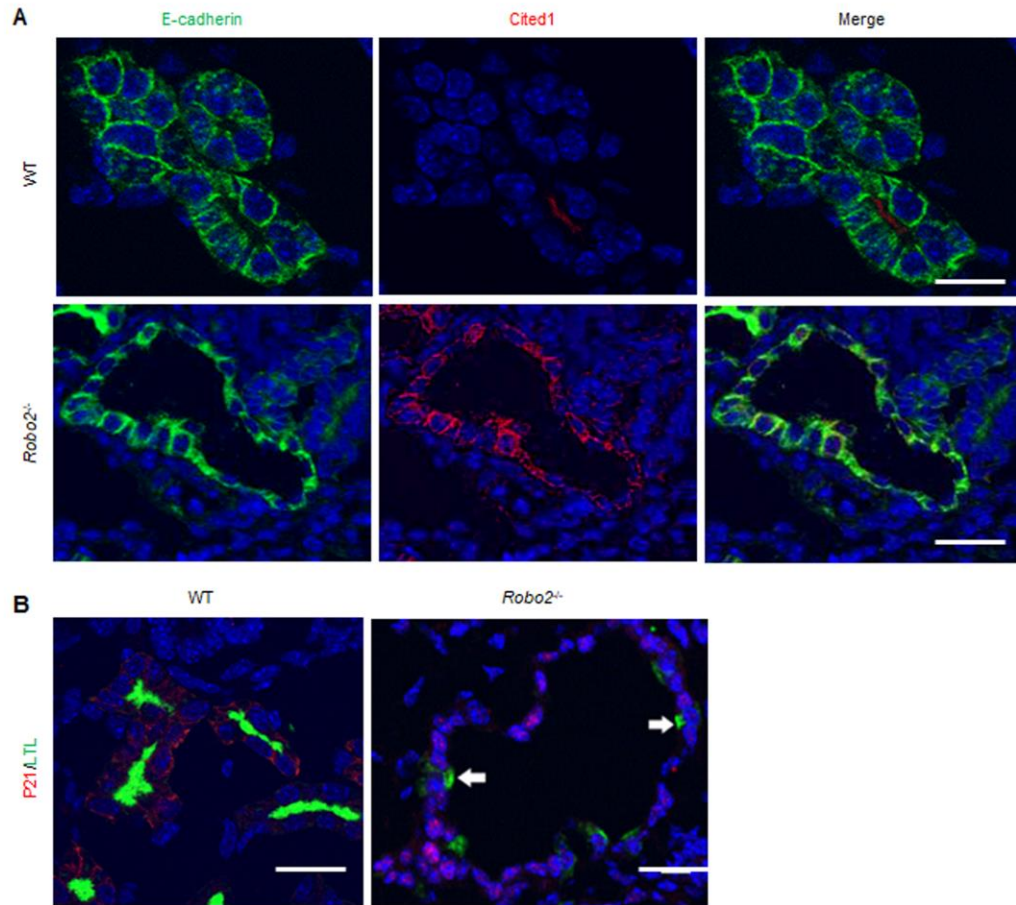
Supplementary Figure 2. Effect of Robo2 on ciliogenesis in mIMCD3 cells. **(A-b)** IF staining of cilia with α -actetaled tubulin in mIMCD3 cells, cultured 48hr, 72hr, respectively. **(C)** Knockout of *Robo2* resulted in the short and bar-like cilia in mouse IMCD3 cells.

A**B****C****D****E**

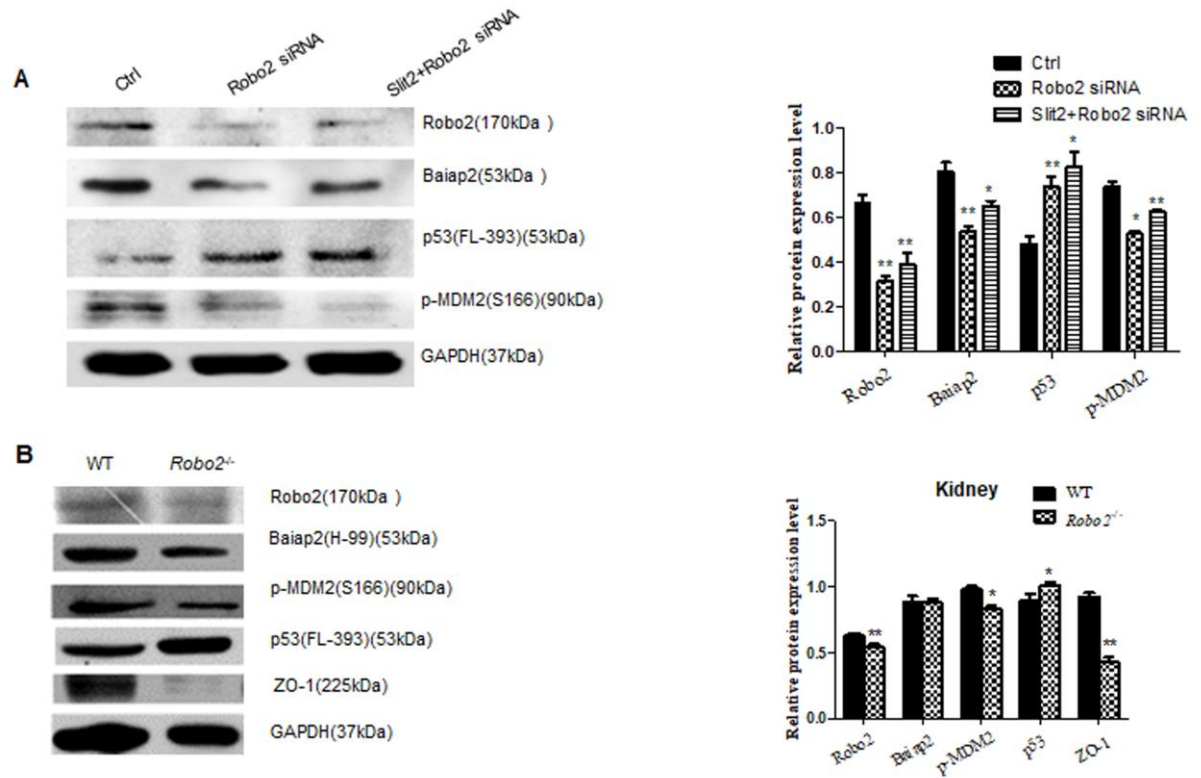
Supplementary Figure 3. Direct binding assay for Robo2 and Baiap2. **(A)** SDS-PAGE of the membrane proteins precipitated with IgG (control) and Robo2 antibody. The PAGE gel was divided into four groups (*left*) based on the protein markers on the right. **(B-E)** GST pull -down assays showed that Robo2 interacted directly with Baiap2.



Supplementary Figure 4. Robo2 colocalizes with Baiap2, Zo-1 and Claudin-2 in epithelial cell. **(A)** Robo2 is co-localized with Baiap2 in the renal tubular epithelial cells in wildtype kidneys. Scale bars, 100 μm . **(B)** Baiap2 is co-localized with LTL in wildtype kidneys but not in *Robo2*^{-/-} kidneys. The enlarged image (*middle panel*) shows the basolateral localization of Baiap2 in renal tubule cells. *Robo2* deficiency also reduced the recruitment of Baiap2 to the tight junctions in *Robo2*^{-/-} kidneys (n=7). Scale bars, 10 μm . **(C)** Robo2 mediates the recruitment of Baiap2 to the junctional areas of the cell surface in MDCK cells. Scale bars, 20 μm . **(D)** Robo2 is co-localized with Zo-1 in wildtype kidneys. Scale bars, 10 μm . **e** Robo2 is co-localized with Claudin-2 in wildtype kidneys. Scale bars, 10 μm .



Supplementary Figure 5. Senescent progenitor cells in *Robo2*^{-/-} kidneys. **(A)** IF staining of Cited1 and E-cadherin in renal tubular epithelial cells in wildtype and Robo2 knockout kidneys. **(B)** p21 staining showing senescent cells in *Robo2*^{-/-} kidneys, except for the LTL-positive cells. Scale bars, 10μm.



Supplementary Figure 6. Changes in signaling pathways of Robo2. **(A)** Slit2 has no significant direct effect on the Robo2-Baiap2 signaling pathway (n=3). The cells were treated with Robo2 siRNAs and then with SLIT2 N-terminus (SLIT2-N) (Sigma-Aldrich, SRP3155) as indicated. Compared to control group, * $p < 0.05$, ** $p < 0.01$. **(B)** The expression of the Robo2-related proteins, including Baiap2, p-MDM2, p53 and ZO-1, were decreased in *Robo2*^{-/-} kidneys compared to those in wild-type kidneys (n=3). Compared to wild-type group, * $p < 0.05$, ** $p < 0.01$.

Supplementary Table

Supplementary Table 1

Mascot search results of proteins from Robo2 IP purified excised protein bands

Excised fragment	Protein name	Gene name	Mass	Score	Peptide match
130-250kDa	Kif20b	KI20B	204969	239	13(10)
	Robo1	ROBO1	177552	76	5(4)
	Cps1	CPSM	165711	182	14(7)
	Robo2	ROBO2	162002	1303	60(45)
	Nid1	NID1	139387	83	1(1)
75-130kDa	Luzp1	LUZP1	119809	271	13(9)
	Agap1	AGAP1	95322	106	2(2)
	Tgm1	TGM1	90682	61	4(4)
	Jup	PLAK	82490	316	12(10)
	Pkp1	PKP1	82270	72	4(1)
	Mpo	PERM	81931	69	3(2)
	Ltf	TRFL	79698	60	11(2)
	Ddx4	DDX4	77278	85	4(2)
	Ddx3y	DDX3Y	73838	95	3(2)
50-75kDa	Ddx5	DDX5	69790	681	28(21)
	Hnf1b	HNF1B	61778	63	1(1)
	Tcp1	TCPA1	60930	91	6(5)
	Baiap2	BAIP2	59428	64	3(2)
	Hmgcs1	HMCS1	58160	78	5(1)
36-55kDa	Idh2	IDHP	51330	60	2(1)
	Gfap	GFAP	49927	117	4(3)
	Exosc9	EXOS9	49533	77	2(2)
	Hnrnph1	HNRH1	49454	96	3(2)
	Eif4a1	IF4A1	46353	363	10(9)
	Ppp2ca	PP2AA	36156	90	3(3)

Supplementary Table 2

The top 60 most differentially expressed genes between the kidneys from wild-type and *Robo2*^{-/-} mice

Gene ID	Gene Name	Genbank Accession	Fold change
Mylpf	myosin light chain, phosphorylatable, fast skeletal muscle	Mm.14526	31.44
Actc1	actin, alpha, cardiac muscle 1	Mm.686	25.23
Scd2	stearoyl-Coenzyme A desaturase 2	Mm.487021	24.83
Npy	neuropeptide Y	Mm.154796	9.32
Slc15a2	solute carrier family 15 (H+/peptide transporter), member 2	Mm.281804	7.69
Angptl4	angiopoietin-like 4	Mm.196189	6.31
Fubp1	far upstream element (FUSE) binding protein 1	Mm.278922	4.11
Eno3	enolase 3, beta muscle	Mm.251322	4.02
Tet2	Tet methylcytosine dioxygenase 2	Mm.347816	3.85
Vwf	Von Willebrand factor homolog	Mm.22339	3.83
Pabpn1	poly(A) binding protein, nuclear 1	Mm.7723	3.74
Ube2i	ubiquitin-conjugating enzyme E2I	Mm.240044	3.53
Ube2b	ubiquitin-conjugating enzyme E2B	Mm.384918	3.43
Nfat5	nuclear factor of activated T cells 5	Mm.390057	3.32
Ranbp3l	RAN binding protein 3-like	Mm.418007	3.30
Cblb	Casitas B-lineage lymphoma b	Mm.328206	3.28
Kcnq1ot1	KCNO1 overlapping transcript 1	Mm.356096	3.20
Taf1d	TATA box binding protein (Tbp)-associated factor, RNA polymerase I, D	Mm.354115	3.13
Cox6a2	cytochrome c oxidase, subunit VI a, polypeptide 2	Mm.43824	3.01
Dnm3os	dynamamin 3, opposite strand	Mm.485366	3.00
Ap2b1	adaptor-related protein complex 2, beta 1 subunit	Mm.39053	2.94
Hnrpd1	heterogeneous nuclear ribonucleoprotein D-like	Mm.389579	2.92
Sdc2	syndecan 2	Mm.234266	2.70
Zfp101	zinc finger protein 101	Mm.4417	2.38
Mylik	myosin, light polypeptide kinase	Mm.33360	2.13
Tnnc1	troponin C, cardiac/slow skeletal	Mm.439921	2.09
Rhox4b	Reproductive homeobox 4B	Mm.327419	2.07
Ccl9	chemokine (C-C motif) ligand 9	Mm.416125	2.06
Kng1	kininogen 1	Mm.2160	2.04
Csnk2a1	casein kinase 2, alpha 1 polypeptide	Mm.23692	2.03
Nedd4l	neural precursor cell expressed, developmentally down-regulated gene 4-like	Mm.98668	2.02
Cd36	CD36 antigen	Mm.18628	2.00
Trp53	transformation related protein 53	Mm.222	2.00
Prp40a	PRP40 pre-mRNA processing factor 40 homolog A (yeast)	Mm.257474	-1.99
Prp40a	PRP40 pre-mRNA processing factor 40 homolog A (yeast)	Mm.257474	-1.99
Rdh11	retinol dehydrogenase 11	Mm.291799	-1.99
Magi3	membrane associated guanylate kinase, WW and PDZ domain containing 3	Mm.264849	-1.98
Polr3k	polymerase (RNA) III (DNA directed) polypeptide K	---	-1.98
Gtpbp4	GTP binding protein 4	Mm.41800	-1.91
Chek1	checkpoint kinase 1	Mm.16753	-1.90
Stf1	suppression of tumorigenicity 7-like	Mm.206379	-1.89
Sqle	squalene epoxidase	Mm.296169	-1.89
Pvr13	poliovirus receptor-related 3	Mm.328072	-1.89
Eif4e	eukaryotic translation initiation factor 4E	Mm.3941	-1.88
Idi1	predicted gene 13502 /// isopentenyl-diphosphate delta isomerase	Mm.29847	-1.88
Slu7	SLU7 splicing factor homolog (S. cerevisiae)	Mm.28200	-1.87
Dtx3l	deltex 3-like (Drosophila)	Mm.390852	-1.86
Endod1	endonuclease domain containing 1	Mm.41423	-1.86
Ppih	predicted gene, 17748 /// peptidyl prolyl isomerase H	Mm.304080	-1.86
Soat1	sterol O-acyltransferase 1	Mm.28099	-1.86
Rrm2	ribonucleotide reductase M2	Mm.99	-1.86
Serbp1	serpine1 mRNA binding protein 1	Mm.240490	-1.85
Dcun1d1	DCN1, defective in cullin neddylation 1, domain containing 1 (S. cerevisiae)	Mm.488396	-1.85
Srsf10	serine/arginine-rich splicing factor 10	Mm.10229	-1.83
Pola1	polymerase (DNA directed), alpha 1	Mm.1923	-1.82
Hsp90aa1	heat shock protein 90, alpha (cytosolic), class A member 1	Mm.398379	-1.81
Dapp1	dual adaptor for phosphotyrosine and 3-phosphoinositides 1	Mm.254835	-1.80
Lss	lanosterol synthase	Mm.55075	-1.80
Rpa2	replication protein A2	Mm.2870	-1.80
Creb3l2	cAMP responsive element binding protein 3-like 2	Mm.169929	-1.78