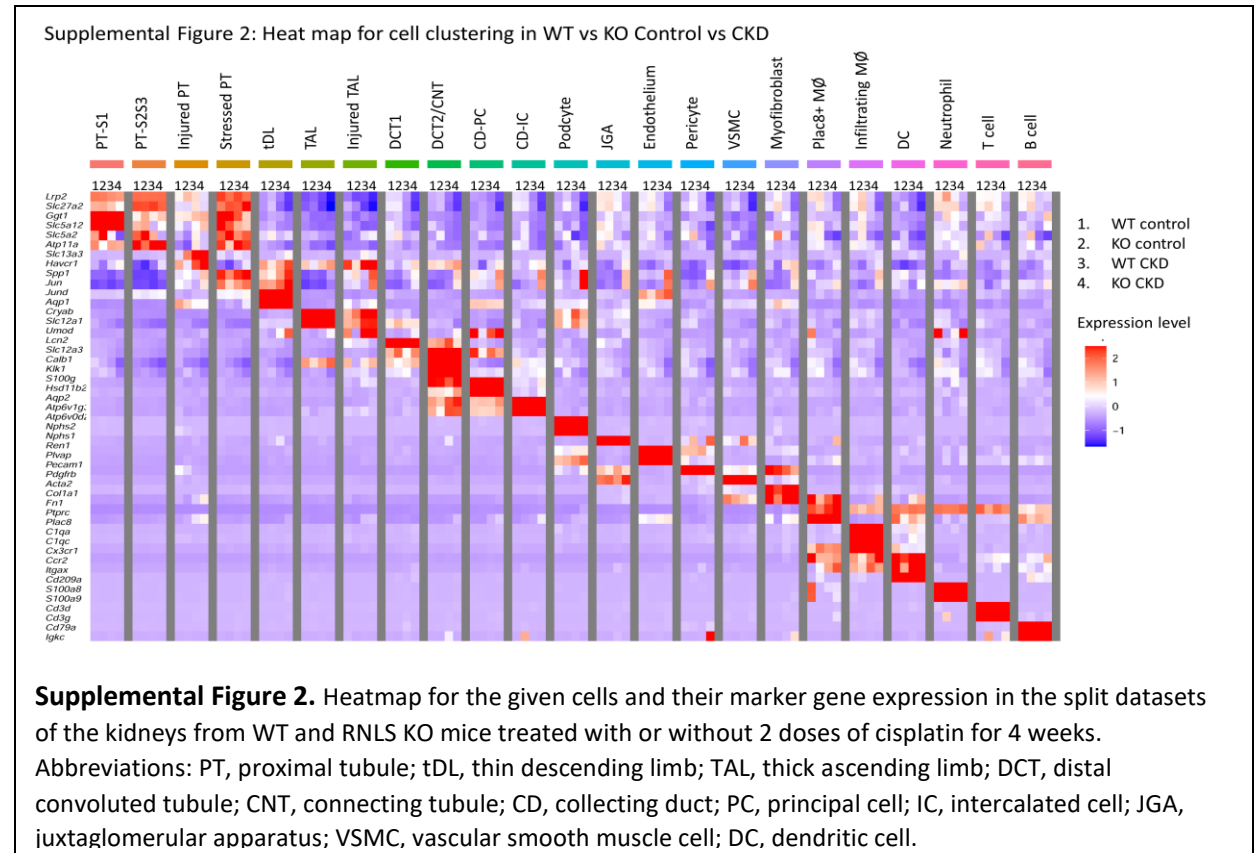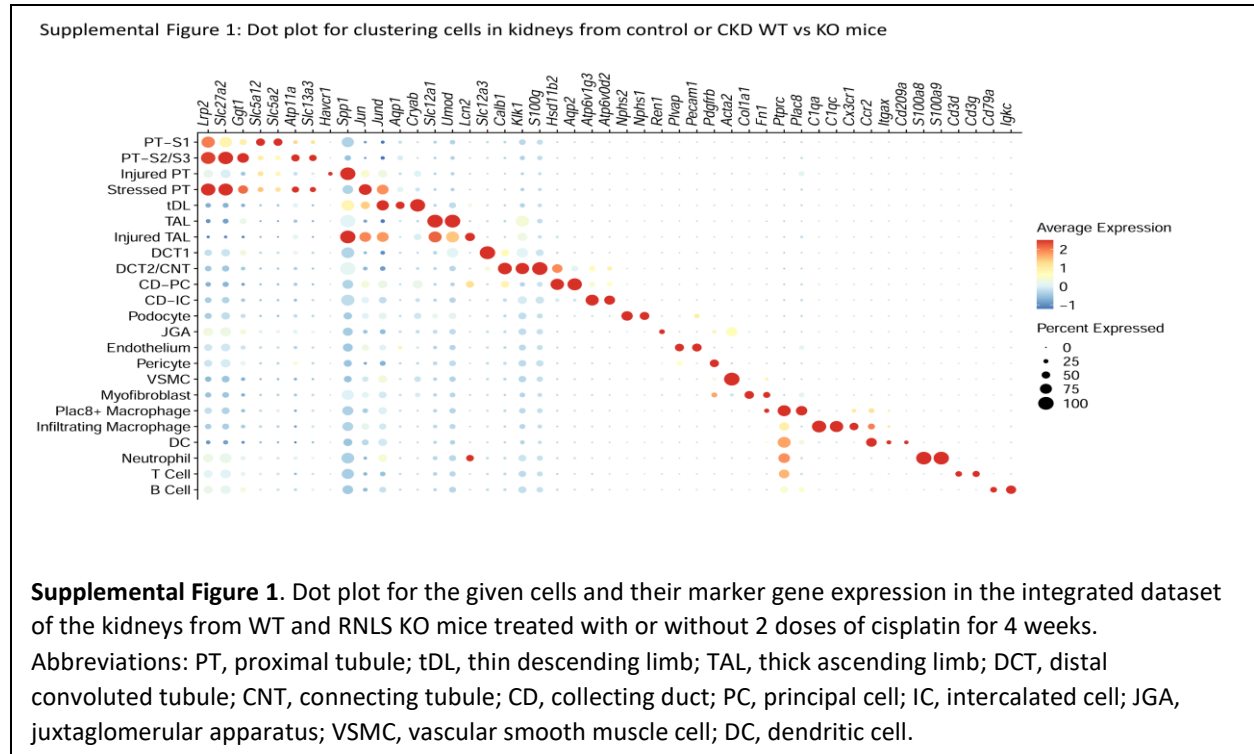Supplemental data.

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7 Figures and 4 Tables.

Supplemental Data


Supplemental Figure 1: Dot plot for clustering cells in kidneys from control or CKD WT vs KO mice

Supplemental Figure 1. Dot plot for the given cells and their marker gene expression in the integrated dataset of the kidneys from WT and RNLS KO mice treated with or without 2 doses of cisplatin for 4 weeks. Abbreviations: PT, proximal tubule; tDL, thin descending limb; TAL, thick ascending limb; DCT, distal convoluted tubule; CNT, connecting tubule; CD, collecting duct; PC, principal cell; IC, intercalated cell; JGA, juxtaglomerular apparatus; VSMC, vascular smooth muscle cell; DC, dendritic cell.

Supplemental Figure 2: Heat map for cell clustering in WT vs KO Control vs CKD

Supplemental Figure 2. Heatmap for the given cells and their marker gene expression in the split datasets of the kidneys from WT and RNLS KO mice treated with or without 2 doses of cisplatin for 4 weeks. Abbreviations: PT, proximal tubule; tDL, thin descending limb; TAL, thick ascending limb; DCT, distal convoluted tubule; CNT, connecting tubule; CD, collecting duct; PC, principal cell; IC, intercalated cell; JGA, juxtaglomerular apparatus; VSMC, vascular smooth muscle cell; DC, dendritic cell.

Supplemental Figure 3: IF for PEG in TKPTS cells
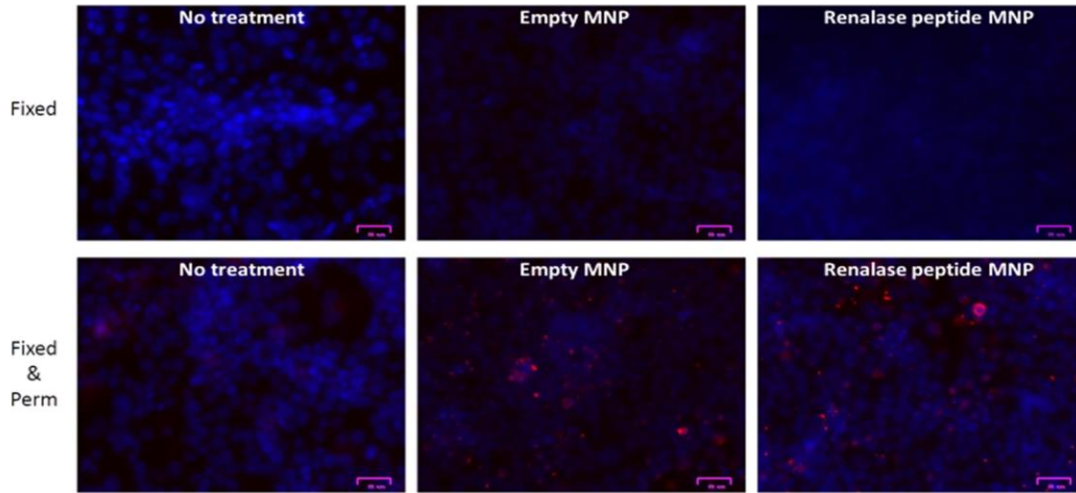
Supplemental Figure 3. Immunofluorescence staining for PEG in TKPTS cells treated with RP81MNP. Mouse proximal tubule cells TKPTS were cultured on coverslips and incubated without or with empty MNP or RP81MNP at 100ug/ml in cell growth medium for 24h. Cells were fixed in 4% paraformaldehyde for 20 min on ice. Some samples were permeabilized with ice-cold methanol for 5 min. Cells were stained with anti-PEG for MNP (red) and DAPI for nuclei (blue).

Supplemental Figure 4: RP81 protects WT mice from CP-induced CKD
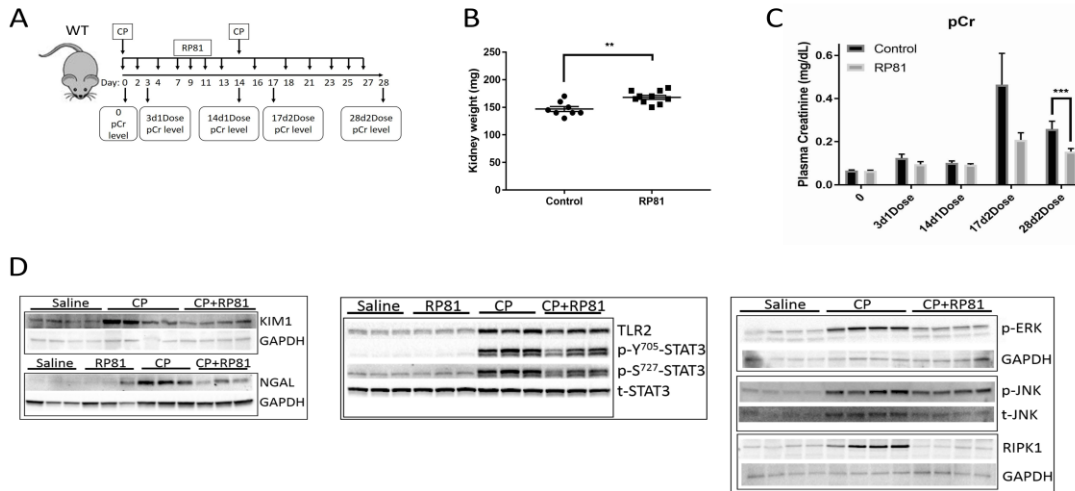
Supplemental Figure 4. RP81 protects WT mice from CP-induced CKD. A: schematic of treatment protocol: C57BL/6J mice were injected with 15mg/Kg on day 0 and day 14. Renalase peptide RP81 (KIDVPWAGQYITSNPCIRFVSIDNKKRNIESSEIGP) was administrated to a separate group of mice at 4 mg/kg on days indicated; B: mouse kidney weight was measured at the end of the experiment; C: plasma creatinine levels of mice treated with CP (n=8) or CP plus RP81 (n=10) were measured at the time points indicated; D: Immunoblotting analysis of protein isolated from kidneys of mice treated with saline or 2 doses of cisplatin for 4 weeks without (CP) or with renalase peptide RP81 (CP+RP81) for KIM-1, NGAL, TLR2, STT3, ERK, JNK, and RIPK1.

Supplemental Figure 5: Dot plot for clustering cells in kidneys from RNLS KO CKD mice treated with control or RP81MNP
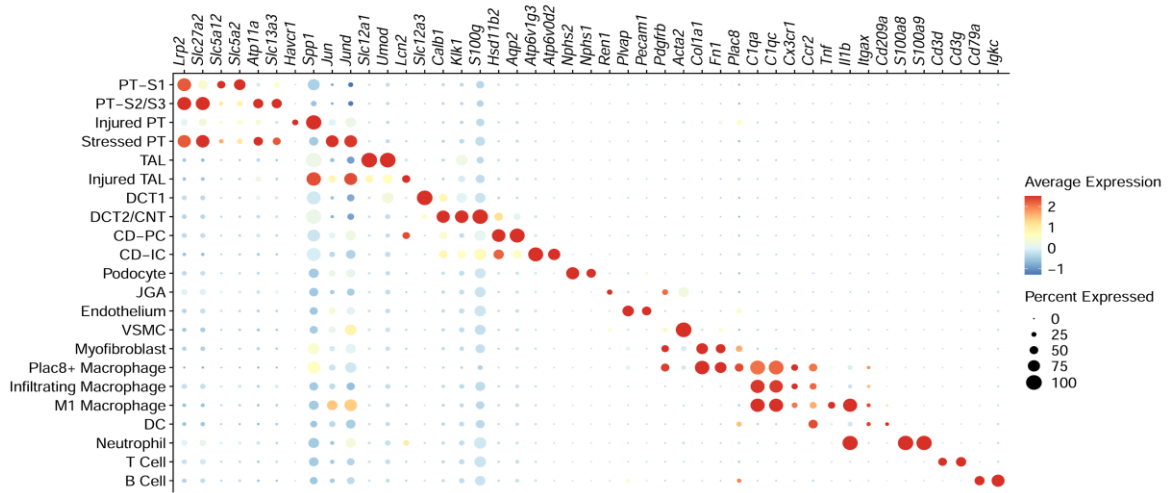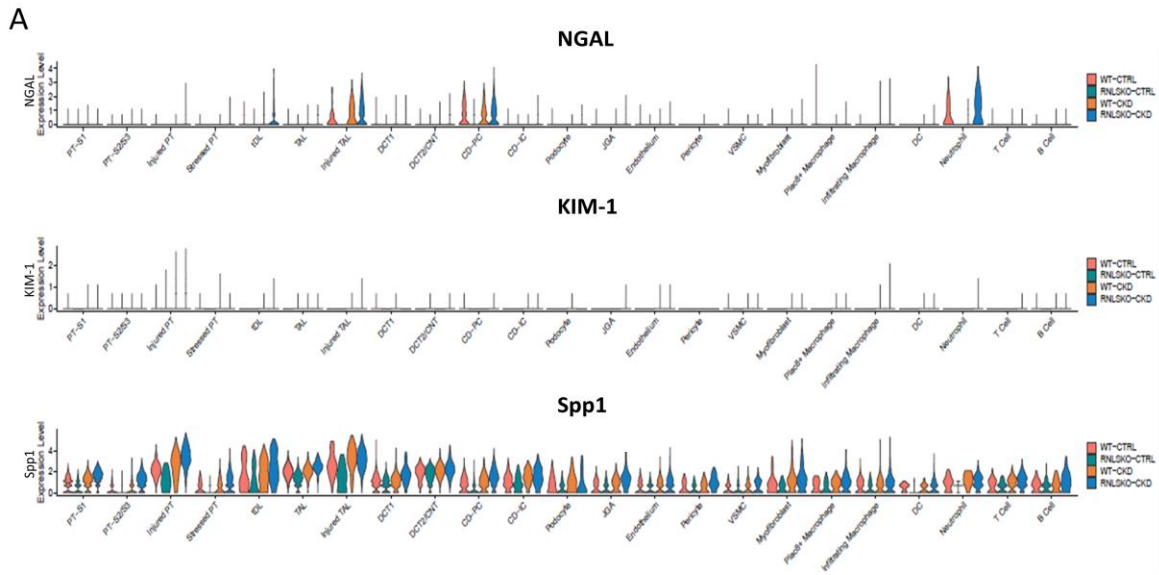
Supplemental Figure 5. Dot plot for the given cells and their marker gene expression in the integrated dataset of the kidneys from RNLS KO CKD mice treated with control or RP81MNP. Abbreviations: PT, proximal tubule; TAL, thick ascending limb; DCT, distal convoluted tubule; CNT, connecting tubule; CD, collecting duct; PC, principal cell; IC,

Supplemental Figure 6: Heat map for clustering cells in kidneys from RNLS KO CKD mice treated with control or RP81MNP
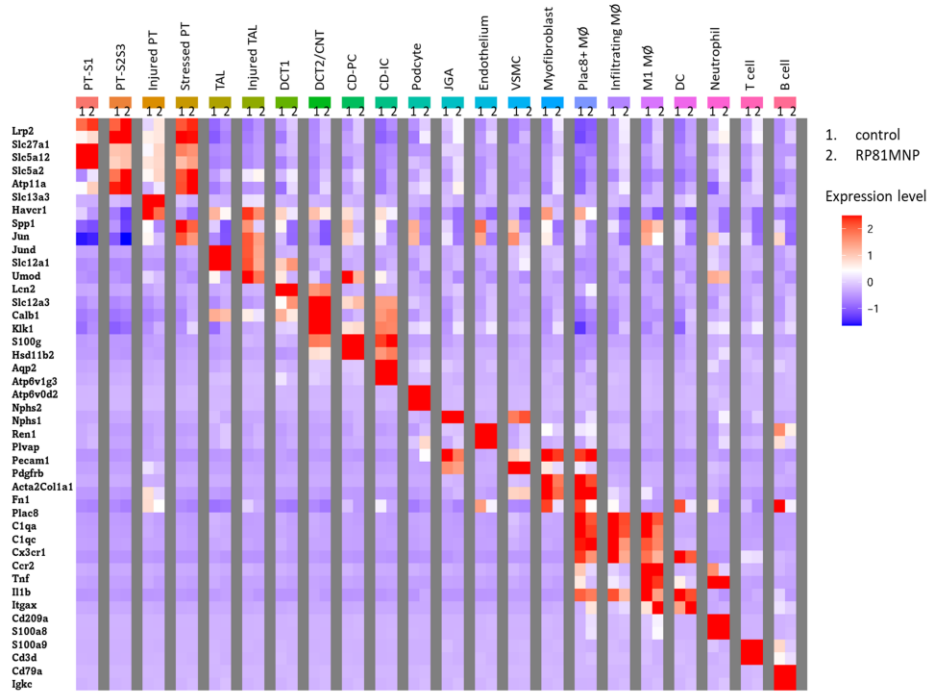
Supplemental Figure 6. Heatmap for the given cells and their marker gene expression in the split datasets of the kidneys from RNLS KO CKD mice treated with control or RP81MNP. Abbreviations: PT, proximal tubule; tDL, thin descending limb; TAL, thick ascending limb; DCT, distal convoluted tubule; CNT, connecting tubule; CD, collecting duct; PC, principal cell; IC, intercalated cell; JGA, juxtaglomerular apparatus; VSMC, vascular smooth muscle cell; DC, dendritic cell.

Supplemental Figure 7: Gene expression of KIM-1, NGAL, and Spp1 in kidneys from WT and KO mice

Supplemental Figure 7A. Violin plots of NGAL, KIM-1, and Spp1 gene expression in the split datasets of the kidneys from WT and RNLS KO mice treated with or without 2 doses of cisplatin for 4 weeks (A) and in the split datasets of the kidneys from RNLS KO CKD mice treated with control or RP81MNP

Supplemental Figure 7: Gene expression of KIM-1, NGAL, and Spp1 in kidneys from KO with and without RP81-MNP models of this study
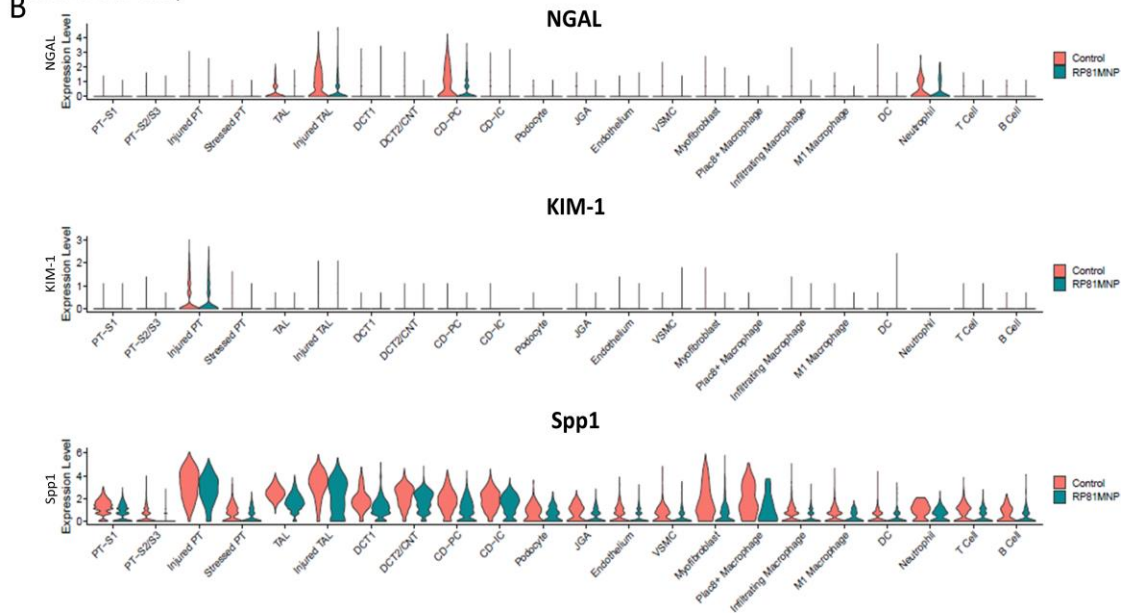
Supplemental Figure 7B. Abbreviations: PT, proximal tubule; tDL, thin descending limb; TAL, thick ascending limb; DCT, distal convoluted tubule; CNT, connecting tubule; CD, collecting duct; PC, principal cell; IC, intercalated cell; JGA, juxtaglomerular apparatus; VSMC, vascular smooth muscle cell; DC, dendritic cell.

Supplemental Table 1: Antibodies used for this study

Antigen Name	Abbreviations	Resource	Clone # / Catalog #	Applicatio dilution
Renalase	RNLS	in house	m28	Immunoblotting: 0.25ug/ml
glyceraldehyde 3 phosphate dehydrogenase	GAPDH	Cell Signaling Technologies (Danvers, MA)	2118	Immunoblotting: 1:2,000
Toll-like receptor 2	TLR2	Cell Signaling Technologies (Danvers, MA)	13744	Immunoblotting: 1:1,000
phospho-p44/42 Early response kinase	p-Erk	Cell Signaling Technologies (Danvers, MA)	4370	Immunoblotting: 1:2,000
total Early response kinase	t-Erk	Cell Signaling Technologies (Danvers, MA)	4695	Immunoblotting: 1:2,000
phospho-Tyr705-STAT3	p-Y-STAT3	Cell Signaling Technologies (Danvers, MA)	9145	Immunoblotting: 1:1,000
phospho-Ser727-STAT3	p-S-STAT3	Cell Signaling Technologies (Danvers, MA)	9134	Immunoblotting: 1:1,000
total STAT3	t-STAT3	Cell Signaling Technologies (Danvers, MA)	4904	Immunoblotting: 1:1,000
phospho-Thr183/Tyr185-SAPK/JNK	p-JNK	Cell Signaling Technologies (Danvers, MA)	4668	Immunoblotting: 1:1,000
total JNK	t-JNK	Cell Signaling Technologies (Danvers, MA)	9252	Immunoblotting: 1:1,000
receptor-interacting protein	RIPK-1	Cell Signaling Technologies (Danvers, MA)	3493	Immunoblotting: 1:1,000
kidney injury molecule-1	KIM-1	R&D System (Minneapolis, MN)	AF1817	Immunoblotting: 1:500
Lipocalin-2 / NGAL	NGAL	R&D System (Minneapolis, MN)	AF1857	Immunoblotting: 1:500
Polyethylene glycol	PEG	Abcam (Cambridge, MA)	ab51257	Immunohistochemistry: 1:200
Alexa Fluor 647 labeled goat anti-rabbit		Abcam (Cambridge, MA)	ab150079	Immunohistochemistry: 1:200
Anti-rabbit IgG, HRP-linked Antibody		Cell Signaling Technologies (Danvers, MA)	7074	Immunoblotting: 1:2,000
Anti-mouse IgG, HRP-linked Antibody		Cell Signaling Technologies (Danvers, MA)	7076	Immunoblotting: 1:2,000
Anti-rat IgG, HRP-linked Antibody		Cell Signaling Technologies (Danvers, MA)	7077	Immunoblotting: 1:2,000
Anti-Goat IgG, HRP conjugate		Sigma-Aldrich (St. Louis, MO)	AP180P	Immunoblotting: 1:5,000

Supplemental Table 1. List of Antibodies used in this study.

Supplemental Table 2

Supplemental Table 2. Human subject characteristics, eGFR, serum creatinine, KIM-1, and RNLS levels

Subject #	Gender	Age	Cisplatin Tx (days)	BW (kg)	eGFR (mL/min/1.73m²)	sCr (mg/dL)	KIM-1 (pg/ml)	RNLS (µg/ml)
1	M	68	0	69.9	>60	0.93	14.86	6.26
			1	71.5			23.75	5.74
			2	73.1			40.14	5.58
			14	66.9	57	1.25	420.42	4.71
2	M	51	0	110.9	>60	0.90	50.97	4.96
			1	114.8			56.81	5.66
			2	114.3			56.25	5.26
			14	110.2		0.90	350.42	6.51
3	M	57	0	67.22	>60	0.85	22.64	4.34
			1				40.69	5.40
			2	70.4			55.14	7.82
			14	64.7	>60	0.89	194.03	8.31
4	M	50	0	76.9	>60	0.90	44.58	11.21
			1				44.03	12.47
			2				56.53	5.50
			14	74.9	>60	0.91	230.69	12.58
5	F	60	0	87.6	>60	0.76	44.31	2.52
			1				62.64	6.98
			2	86.2			85.69	6.44
			14	85.2	>60	0.89	225.14	6.21
6	M	64	0	105.2	>60	0.92	87.92	12.99
			1				174.58	11.39
			2	106.3			152.36	11.05
			14	107.2	>60	0.91	209.86	8.88
7	M	48	0	108	>60	0.59	52.92	11.96
			1				54.58	14.60
			2	106.7			67.92	12.80
			14	105.2	>60	0.97	880.97	9.81
8	F	59	0	49.6	>60	0.59	127.36	11.21
			2				128.75	8.52
			14	48.9	>60	0.65	1029.58	5.80
9	M	45	0	89.1	>60	0.65	42.36	10.17
			1				44.58	8.83
			2	52	>60	0.68	62.08	7.75
			14	87.2	>60	0.84	549.03	9.53
10	M	57	0	99.1	>60	0.74	44.58	20.51
			1				52.64	18.59
			2	89.3			67.64	15.96
			14	95.7	>60	1.20	698.75	12.69
11	M	61	0	94.3	>60	0.75	134.31	10.62
			1		>60	0.69	192.64	10.85
			2	100.5	>60	0.71	230.42	8.89
			14	89.1	>60	0.89	424.86	5.19

Supplemental Table 2. Human subject characteristics and serum levels of eGFR, KIM-1, creatinine, and RNLS

Supplemental Table 3: Cell numbers in each cluster and % of cells in kidneys of WT vs. RNLS KO mice treated with or without 2 doses of cisplatin for 4 weeks

	WT-CTRL		RNLSKO-CTRL		WT-CKD		RNLSKO-CKD	
PT-S1	2838	30.8%	3742	26.6%	2473	26.1%	3071	20.6%
PT-S2/S3	1370	14.9%	3800	27.1%	1787	18.8%	1201	8.0%
Injured PT	89	1.0%	45	0.3%	223	2.3%	1486	10.0%
Stressed PT	125	1.4%	230	1.6%	286	3.0%	1542	10.3%
tDL	282	3.1%	334	2.4%	249	2.6%	560	3.8%
TAL	429	4.7%	880	6.3%	489	5.2%	346	2.3%
Injured TAL	30	0.3%	29	0.2%	96	1.0%	1190	8.0%
DCT1	492	5.3%	680	4.8%	354	3.7%	90	0.6%
DCT2/CNT	596	6.5%	958	6.8%	401	4.2%	263	1.8%
CD-PC	448	4.9%	537	3.8%	366	3.9%	548	3.7%
CD-IC	204	2.2%	155	1.1%	182	1.9%	180	1.2%
Podocyte	64	0.7%	162	1.2%	43	0.5%	42	0.3%
JGA	215	2.3%	342	2.4%	213	2.2%	166	1.1%
Endothelium	916	10.0%	1016	7.2%	930	9.8%	1191	8.0%
Pericyte	63	0.7%	206	1.5%	63	0.7%	31	0.2%
VSMC	287	3.1%	256	1.8%	221	2.3%	294	2.0%
Myofibroblast	92	1.0%	39	0.3%	146	1.5%	464	3.1%
Plac8+ Macrophage	62	0.7%	52	0.4%	90	0.9%	178	1.2%
Infiltrating Macrophage	279	3.0%	182	1.3%	405	4.3%	1141	7.6%
DC	27	0.3%	72	0.5%	96	1.0%	460	3.1%
Neutrophil	74	0.8%	14	0.1%	25	0.3%	75	0.5%
T Cell	118	1.3%	175	1.2%	228	2.4%	326	2.2%
B Cell	103	1.1%	142	1.0%	124	1.3%	82	0.5%
Sum	9203		14048		9490		14927	

Supplemental Table 3. Single cell RNA sequencing of kidneys from WT and RNLS KO mice treated with or without 2 doses of cisplatin for 4 weeks: cell numbers in each cluster and % of cells in the kidneys.

Supplemental Table 4: Cell numbers in each cluster and % of cells in kidneys of RNLS KO mice treated with control or RP81

	Control		RP81MNP	
PT-S1	1425	8.5%	4188	18.6%
PT-S2/S3	2480	14.8%	4148	18.4%
Injured PT	1113	6.7%	497	2.2%
Stressed PT	952	5.7%	722	3.2%
TAL	555	3.3%	960	4.3%
Injured TAL	814	4.9%	704	3.1%
DCT1	108	0.6%	607	2.7%
DCT2/CNT	604	3.6%	1348	6.0%
CD-PC	523	3.1%	1274	5.7%
CD-IC	351	2.1%	863	3.8%
Podocyte	110	0.7%	154	0.7%
JGA	229	1.4%	391	1.7%
Endothelium	1250	7.5%	2540	11.3%
VSMC	375	2.2%	378	1.7%
Myofibroblast	974	5.8%	662	2.9%
Plac8+ Macrophage	177	1.1%	36	0.2%
Infiltrating Macrophage	2082	12.4%	1618	7.2%
M1 Macrophage	1131	6.8%	86	0.4%
DC	442	2.6%	359	1.6%
Neutrophil	76	0.5%	146	0.6%
T Cell	827	4.9%	604	2.7%
B Cell	135	0.8%	258	1.1%
Sum	16733		22543	

Supplemental Table 4. Single cell RNA sequencing of kidneys from RNLS KO mice treated with control or RP81MNP and 2 doses of cisplatin for 4 weeks: cell numbers in each cluster and % of cells in the kidneys.