

## **Supplemental Information**

### **Renal COP9 signalosome deficiency alters CUL3-KLHL3-WNK signaling pathway**

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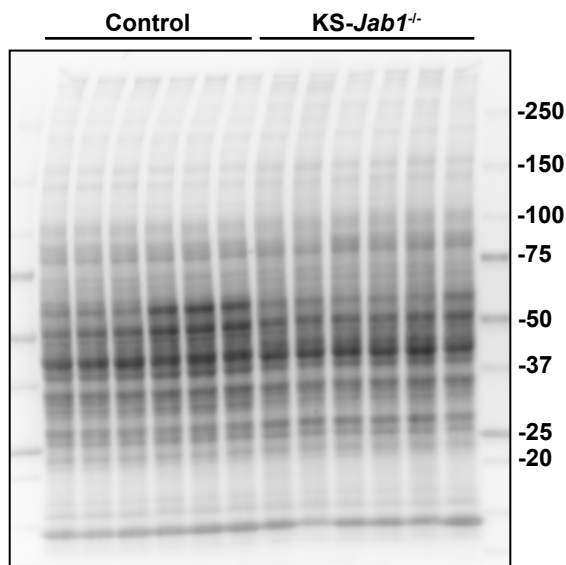
**Table S1.** Antibodies used for Western blot and immunofluorescent staining

<i>Target</i>	<i>Figure</i>	<i>Source</i>	<i>ID</i>	<i>App</i>	<i>Dilution</i>
JAB1	1	Santa Cruz	FL-334, SC-9074	IF, WB	1:50, 1h 1:1,000, o/n
Calbindin D-28	1, S1, S2	Swant	CB300	IF	1:50-1:200 1h
NHE3	1	Chemicon	MAB3134	IF	1:50, 1h
CUL3	2	Cell Signalling	2759	WB	1:1,000, o/n
NEDD8	2	Cell Signaling	19E3, 2754	WB	1:1,000, o/n
KLHL3*	3	Proteintech	16951-1-AP	WB, IF	1:500, o/n 1:50, o/n
Parvalbumin	3	Swant	GP72	IF	1:50, 1h
WNK4	4, S1	Ellison Lab		WB, IF	1:1,000, 1h, 1:500, 1h
pWNK4 <sup>S1196</sup>	4	Ellision Lab		WB	1:500, o/n
WNK1	4	Ellison Lab		WB	1:1,000, 1h
SPAK	4, S2	Delpire Lab		WB, IF	1:5,000, o/n 1:200 1h
OSR1	4, S2	Delpire Lab		WB, IF	1:5,000, o/n 1:200 1h
pSPAK/pOSR1	4	Millipore	07-2273	WB	1:1,000, o/n
NCC	5	Ellison Lab		WB	1:6,000, 1h
pNCC <sup>T53</sup>	5	Ellison Lab		WB	1:2,000, o/n
Keap1	3	Millipore	MABS514, c144	WB	1:1,000, o/n
NKCC2	6	Bachmann Lab		WB	1:1,000, o/n
pNKCC2	6	Bachmann Lab		WB	1:1,000, o/n
AQP2	6	Santa Cruz	SC-9882	WB	1:1,000, 1h
Cyclin E	6	Roberts Lab		WB	1:500, 1h
HA	5C	Covance	HA1.1, MMS-101P	WB	1:1,000, 1h

Abbreviations: App, application; WB, Western blot; IF, immunofluorescence; o/n, overnight.

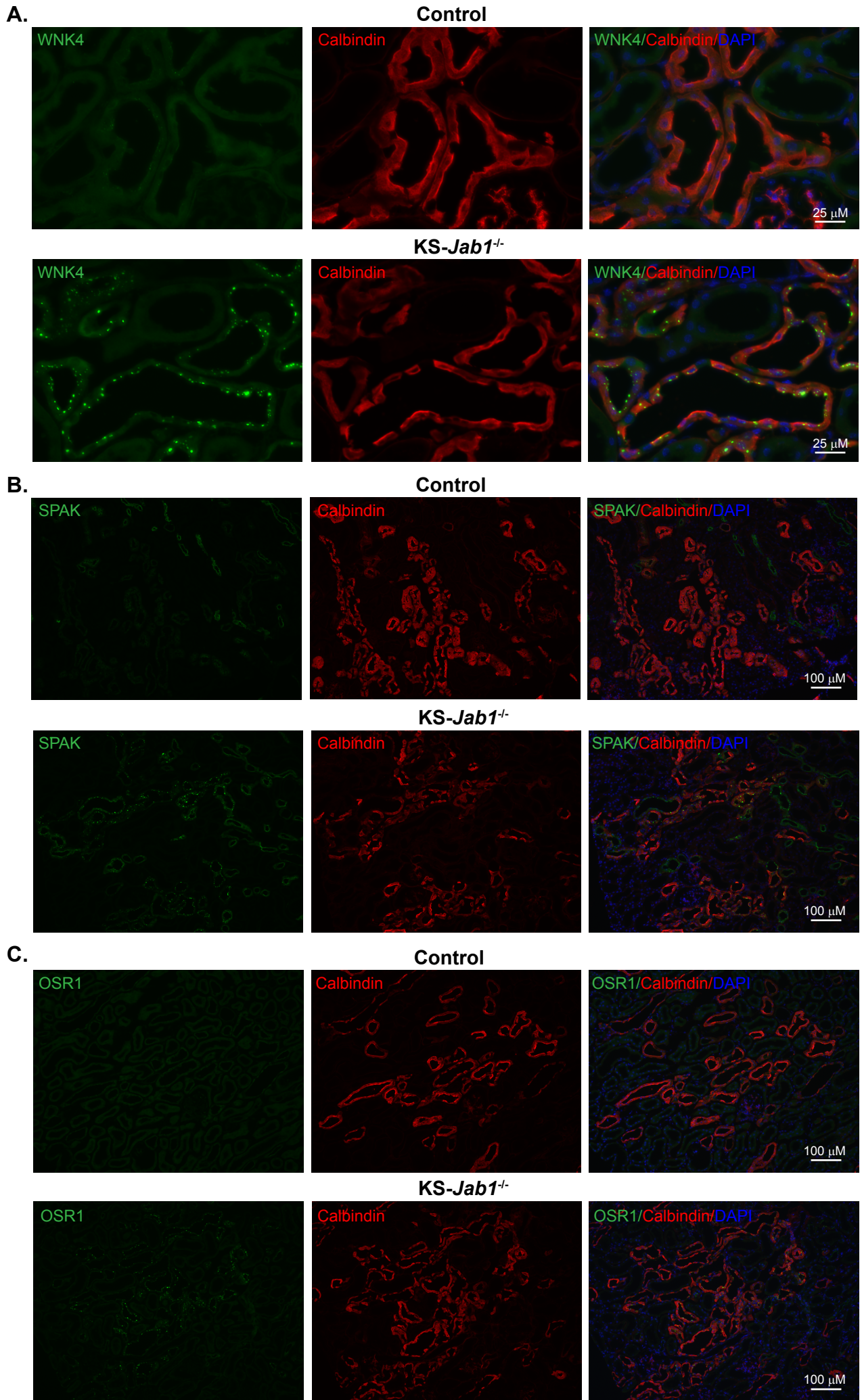
\* KLHL3 antibody was incubated in Can Get Signal for WB as stated in methods section.

**Figure S1**



**Figure S1. Stain-free gel imaging.** Image of a stain-free gel membrane, shown here as an example of stain-free imaging used for loading control.

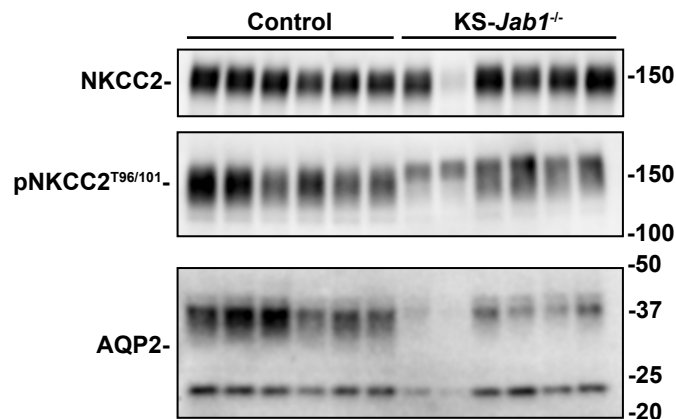
Figure S2



**Figure S2. Co-immunofluorescent staining of WNK4, SPAK, and OSR1 with calbindin.**

Immunofluorescent staining of kidney cortex sections from control and *KS-Jab1<sup>-/-</sup>* mice. WNK4 (A) SPAK (B) and OSR1 (C) localization was examined in kidney cortex by co-staining with the distal nephron marker calbindin. There was low abundance of WNK4, SPAK and OSR1 staining in control mice relative to *KS-Jab1<sup>-/-</sup>* mice. Staining of *KS-Jab1<sup>-/-</sup>* mice kidney sections showed protein translocated into puncta in calbindin-positive cells.

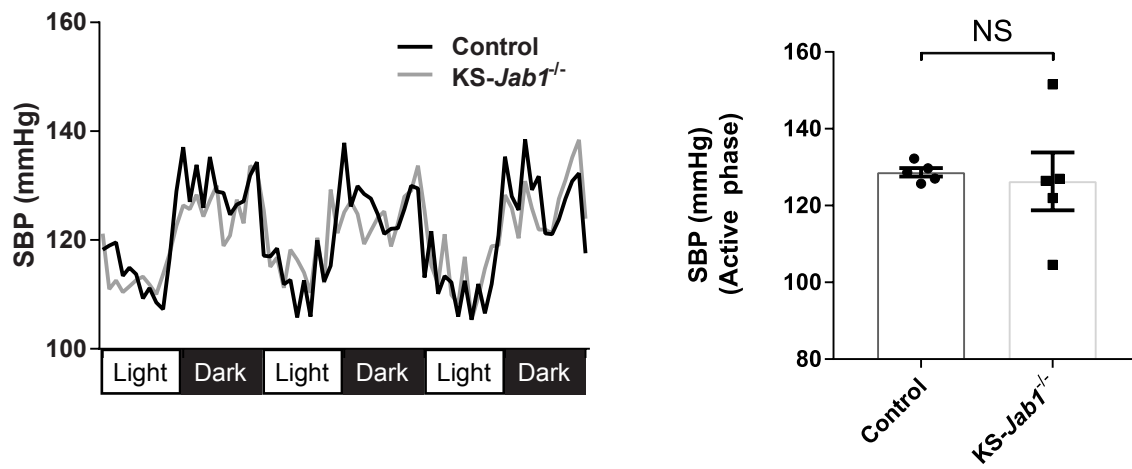
**Figure S3**



**Figure S3. Effects of nephron-specific *Jab1* deletion on NKCC2, pNKCC2, and AQP2 protein abundance.**

Western blot of whole-kidney lysates from control and *KS-Jab1<sup>-/-</sup>* mice. Immunoblotting was performed with antibodies against the Na-K-2Cl cotransporter (NKCC2), phosphorylated NKCC2 at threonine 96/101 (pNKCC2<sup>T96/101</sup>), or aquaporin 2 (AQP2). There was no difference in NKCC2 or pNKCC2<sup>T96/101</sup> protein abundance between *KS-Jab1<sup>-/-</sup>* and control mice. AQP2 abundance was lower in *KS-Jab1<sup>-/-</sup>* mice.

**Figure S4**



**Figure S4. Effects of nephron-specific *Jab1* deletion on blood pressure.** A) Radiotelemetry was used to measure systolic blood pressure in control and *KS-Jab1*<sup>-/-</sup> mice. The tracing (left), using 1 h average values, and the analysis of the mean of the 1 h averages of three dark periods (right) showed that there was no difference between *KS-Jab1*<sup>-/-</sup> mice and control mice. Data represent individual values as well as mean  $\pm$  SEM (control,  $n = 5$ ; *KS-Jab1*<sup>-/-</sup>,  $n = 5$ ).