

### Supplementary Data:

**Supplementary Figure S1.** Validation of *Pax8-Cre;Vhl;Bap1* mice. **A**, PCR genotyping for deletion of *Vhl* and *Bap1* floxed alleles by *Pax8-Cre*. **B**, Representative sections of *Pax8-Cre;Vhl<sup>F/F</sup>* and *Pax8-Cre;Vhl<sup>F/+</sup>;Bap1<sup>F/F</sup>* kidneys showing normal renal parenchyma with preserved normal architecture on H&E, and IHC for CAIX highlighting strong membranous expression in normal proximal tubules and parietal epithelial cells, and negative phospho-S6 in *Pax8-Cre;Vhl<sup>F/F</sup>* mice. *Pax8-Cre;Vhl<sup>F/+</sup>;Bap1<sup>F/F</sup>* kidneys on the other hand are negative for CAIX and show patchy positive expression for phospho-S6 in normal proximal tubules and parietal epithelial cells.

**Supplementary Figure S2.** Validation of *Pax8-Cre;Vhl;Pbrm1* mice. **A**, PCR genotyping for deletion of *Vhl* and *Pbrm1* floxed alleles by *Pax8-Cre*. **B**, Representative H&E sections showing normal renal parenchyma in *Pax8-Cre;Vhl<sup>F/F</sup>;Pbrm1<sup>F/+</sup>* (n=11), *Pax8-Cre;Pbrm1<sup>F/F</sup>* (n=5), and *Pax8-Cre;Vhl<sup>F/+</sup>;Pbrm1<sup>F/F</sup>* (n=23) mice. IHC studies reveal strong membranous CAIX and negative phospho-S6 expression in normal proximal tubules and parietal epithelial cells in the *Pax8-Cre;Vhl<sup>F/F</sup>;Pbrm1<sup>F/+</sup>* mice. CAIX and phospho-S6 was negative in *Pax8-Cre;Pbrm1<sup>F/F</sup>* and *Pax8-Cre;Vhl<sup>F/+</sup>;Pbrm1<sup>F/F</sup>* kidneys. **C**, Cells in culture generated from a 16-month-old *Pax8-Cre;Vhl<sup>F/F</sup>;Pbrm1<sup>F/F</sup>* kidney tumor after 10 passages (100X magnification). **D**, Representative H&E staining of the tumor cell line. **E**, PCR genotyping of *Vhl* and *Pbrm1* in tumor cell line. **F**, Western blot analysis of the indicated proteins in the tumor cell line (TC) or normal kidney (WT). As a control, mouse Hif-1 $\alpha$  and Hif-2 $\alpha$  were transfected/expressed in HEK293 cells (empty vector, EV). **G**, Analysis of the COSMIC database identified ccRCCs with mutations in *VHL*, *PBRM1* and *TSC1*.

**Supplementary Figure S3.** Validation of *Villin-Cre;Vhl;Bap1/Pbrm1* and *Sglt2-Cre;Vhl;Bap1/Pbrm1* mice. **A**, Representative H&E microphotographs of kidney sections from the indicated mice showing normal renal parenchyma with preserved normal architecture. IHC for CAIX highlighting focal strong membranous expression in some normal proximal tubules as well as focal loss of *Bap1* and *Pbrm1* (arrow) in *Villin-Cre;Vhl<sup>F/F</sup>;Bap1<sup>F/F</sup>* and *Sglt2-Cre;Vhl<sup>F/F</sup>;Pbrm1<sup>F/F</sup>* mice. **B and C**, PCR genotyping for deletion of **(B)** *Vhl* and *Bap1* floxed alleles or **(C)** *Vhl* and *Pbrm1* floxed alleles by *Pax8-Cre*, *Villin-Cre*, or *Sglt2-Cre*. **D**, Analysis of the combined cohort of human RCC for the expression of Villin (VIL1) and SGLT2 (SLC5A2) showed similar or lower levels than normal kidney.