

### Supplementary Data:

**Supplementary Figure S1.** Targeting *Vhl* and *Bap1* in *Pax8* lineage. **A**, PCR genotyping evaluating deletion of *Vhl* and *Bap1* floxed sequences 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 assays. CAIX with strong membranous expression in normal proximal tubules and parietal epithelial cells in *Pax8-Cre;Vhl<sup>F/F</sup>* mice, but not *Pax8-Cre;Vhl<sup>F/+</sup>;Bap1<sup>F/F</sup>*. Phospho-S6 is negative in *Pax8-Cre;Vhl<sup>F/F</sup>* kidneys, but patchy positive in normal proximal tubules and parietal epithelial cells of *Pax8-Cre;Vhl<sup>F/+</sup>;Bap1<sup>F/F</sup>* mice.

**Supplementary Figure S2.** Targeting *Vhl* and *Pbrm1* in *Pax8* lineage. **A**, PCR genotyping evaluating deletion of *Vhl* and *Pbrm1* floxed sequences 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 expression in normal proximal tubules and parietal epithelial cells in the *Pax8-Cre;Vhl<sup>F/F</sup>;Pbrm1<sup>F/+</sup>* mice. CAIX was negative in *Pax8-Cre;Pbrm1<sup>F/F</sup>* and *Pax8-Cre;Vhl<sup>F/+</sup>;Pbrm1<sup>F/F</sup>* kidneys. Negative phospho-S6 in all genotypes. **C**, Microphotograph of cell line in culture 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 HIF1 $\alpha$  and HIF2 $\alpha$  were overexpressed in HEK293 cells (empty vector, EV). **G**, Analysis of the COSMIC database identified ccRCCs with mutations in *VHL*, *PBRM1* and *TSC1*.

**Supplementary Figure S3.** Targeting *Vhl* and either *Bap1* or *Pbrm1* with *Villin-Cre* and *Sglt2-Cre*. **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 (arrows) 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 sequences or (**C**) *Vhl* and *Pbrm1* floxed alleles by *Pax8-Cre*, *Villin-Cre*, or *Sglt2-Cre*. **D**, Analysis of a combined cohort of human RCC for the expression of Villin (VIL1) and SGLT2 (SLC5A2) showing levels in ccRCC similar or reduced compared to normal kidney.