Supplement

Table 1: *PCR primer sequences*

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
P2Y2R (canine)	ACAGGTGGGACAGAACTGACG	TGTCCGCCTAGAATCCTCACT
P2Y2R (mouse)	GGGACGAACTGGGATACAAGTG	ACACGGGCAACAGCACGTA
ANO6 (canine)	AAGCAGCCCTTGGACCTTATC	AGTGTAGTAGCCCAGCCAAGC
ANO6 (mouse)	TGGCAACCTCAACTGGTTCA	ACTCCTGCTCTGGCTTGATGA
KCa3.1 (canine)	GCTGCTGGCTGCCATTAAC	TTCACTTGTTCTCGGAGCTTTCT
KCa3.1 (mouse)	CGTGCACAACTTCATGATGGA	CGCCGCTGACTCCTTCA
ANO1 (canine)	CTGCACGACGGAGACTACGA	GTAGCTCGCCCACTCTTGGT
ANO1 (mouse)	TGAGGGTGACAACGTTGAGTTC	CGTAACTTGCCCATTCCTCATAC
NKCC1 (canine)	GATGATTTGAGAGAAGGTGCACAGT	GACAAGTGTGTTTGGCTTCATACG
NKCC1 (mouse)	GGTGGTGCAATTGGCTTGAT	CGAATCCGACAACATACATAGCA
CFTR (canine)	CGGAGACAACGAAGACAGTCTGT	CTTCGGTGAATGCTCTGACCTT
CFTR (mouse)	CAGCTCAAACAACTGGAATCTGA	GCTCGAAGTGTCCAGAGTCCTT
HPRT1 (canine)	AGCTTGCTGGTGAAAAGGAC	TTATAGTCAAGGGCATATCC
Rn18S (mouse)	TGATTAAGTCCCTGCCCTTTGTA	CGATCCGAGGGCCTCACTA

Figure legends

S1. PLC cysts within a collagen matrix are exposed to hypoxia. A Typical setting of the PLC cyst assays showing PLC cysts within a collagen matrix at the bottom of a well and medium on top of the collagen/cell suspension. **B** Prior to the ratiometric luminescence imaging (RLI) of oxygen by the use of the VisiSens^{TM} imaging technology, RLI-sensor foils (depicted by dotted red lines) were two-point calibrated. To this end, left sensor foil was incubated with $Na_2S_2O_5$ (which binds molecular oxygen) and the right sensor foil was

exposed to room air. **C** Calibration curve obtained from B. **D-G** Sensor foils were placed at different heights in the well as depicted by the red arrow and oxygen was measured on top of the collagen matrix (**D**), within the collagen matrix (**E,F**) and at the bottom of the well (**G**). **H** PLC cysts were incubated with pimonidazole before fixation and stained with FITC-conjugated hypoxyprobe antibody. **I** PLC cysts incubated with FITC-conjugated hypoxyprobe antibody without pimonidazole serving as control.

S2. THP is expressed early in embryonic kidney development. S2 shows a cross section of a metanephric kidney E13.0 stained for THP revealing expression of THP apically in branching tubules (*) and to a lesser extent also partly in comma shaped bodies (#). Scale bar whole kidney: 100µm; magnifications: 50µm.

S3. THP is expressed in the cyst epithelium of embryonic kidneys stimulated with forskolin. S3 shows a cross section of an embryonic kidney from a THP-Cre;$VHL^{lox/lox}$ mouse, that was dissected at E13.5 and cultured *ex vivo* for 5 days including forskolin stimulation for the last 4 days. Most of the cysts are stained positive for THP (*), a few cysts show a subtle or no staining (#) whereas proximal tubule segments stain negative for THP (arrow).

S4. THP-dependent deletion of VHL leads to a significant increase of tubular expression of HIF-1α in embryonic kidneys. Embryonic kidneys E13.5 with THP-dependent deletion of VHL (Cre+; n=4) were stained for HIF-1α and compared with VHL-competent kidneys (Cre-; n=4). The number of HIF-1α positive tubular cells was normalized to the total number of tubular cells. Right shows representative embryonic kidney sections. Scale bar: 200µm.

S5. HIF-1α leads to transcriptional activation of genes involved in calcium-activated chloride secretion. A,B Relative mRNA expression of the purinergic receptor P2Y2R, the

Ca^{2+}-activated Cl^- channels anoctamin 6 (ANO6), and anoctamin 1 (ANO1), the calcium-activated K^+ channel KCa3.1, the Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1), and the Cl^- channel CFTR in PLC in the presence or absence of ICA (**A**; n=3; control set to 1) or in PLC stably expressing scrambled shRNA (Scr) or shRNA directed against HIF-1α (shHIF-1α, clone 5.1) (**B**; n=3). **C** Relative mRNA expression of ANO6, P2Y2R, ANO1, KCa3.1, NKCC1, and CFTR in kidneys from 7 mice treated with ICA (40mg/kg) relative to 3 control kidneys (set to 1).

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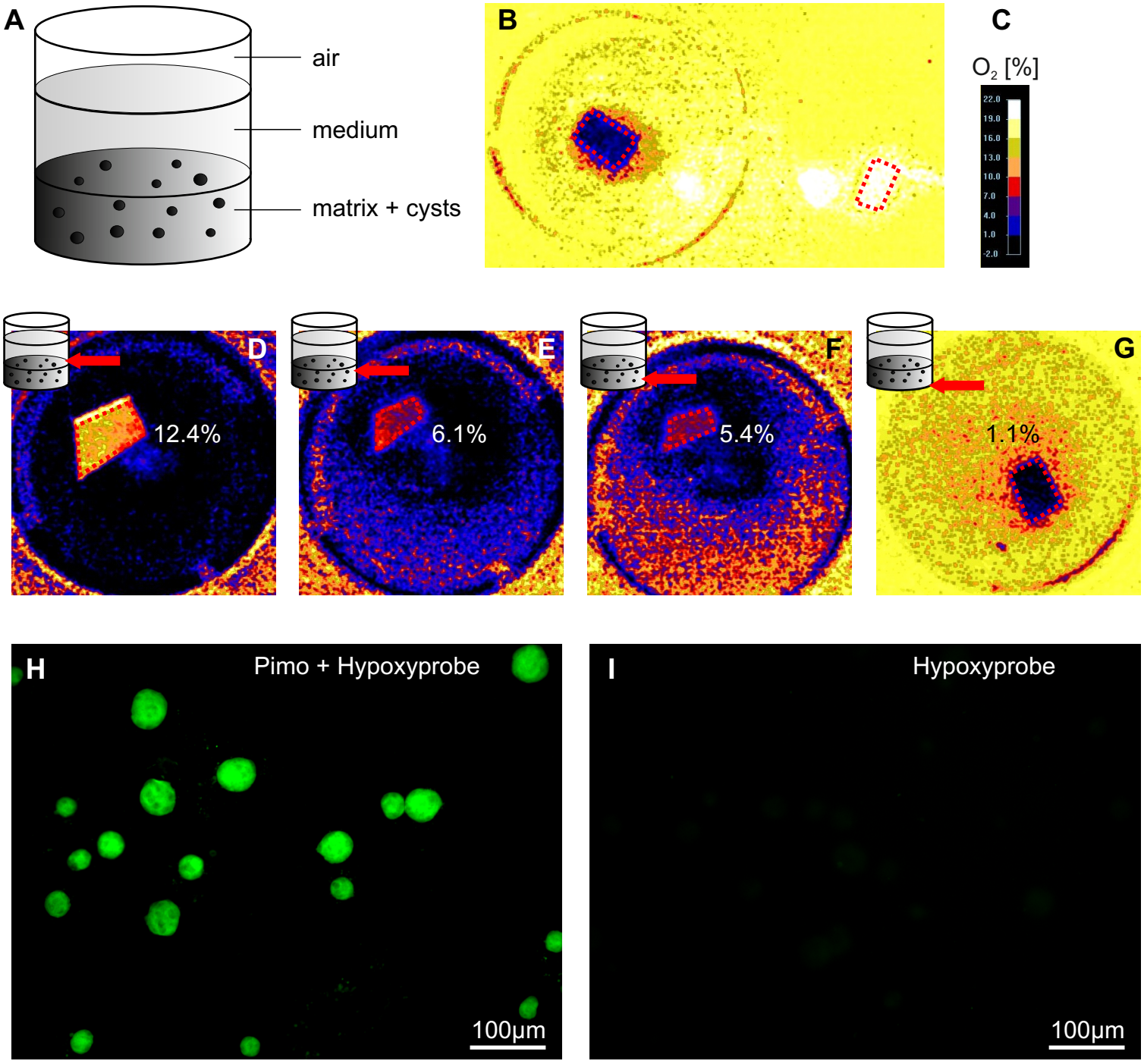
Fig. S1



Supplement

Fig. S2

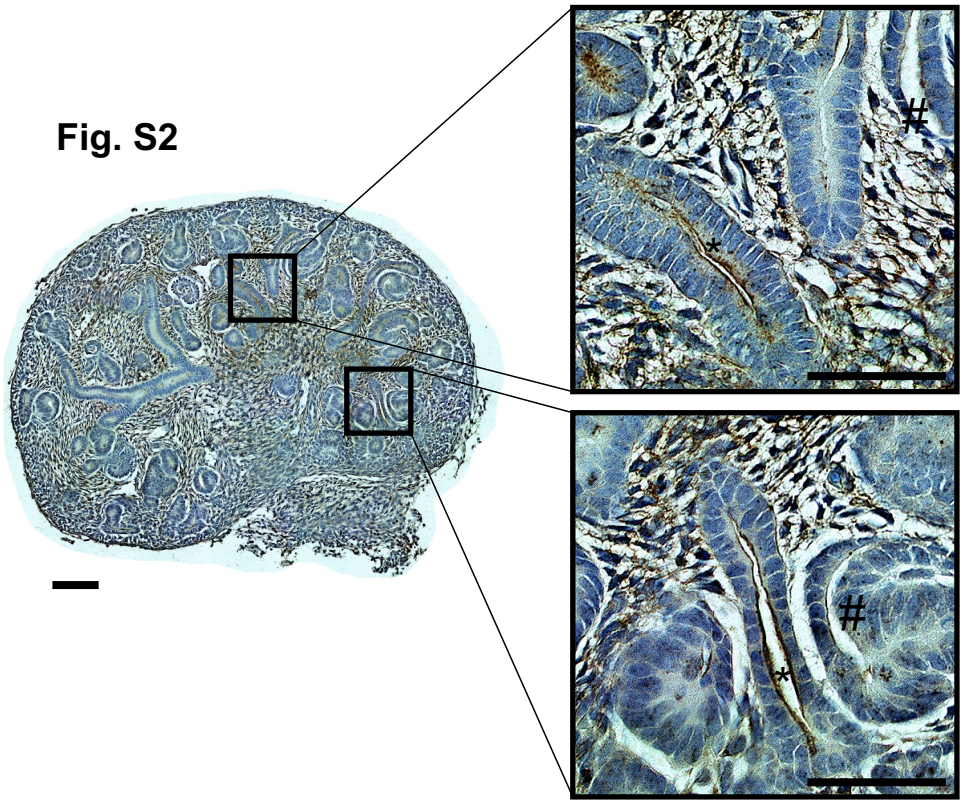


Fig.S3

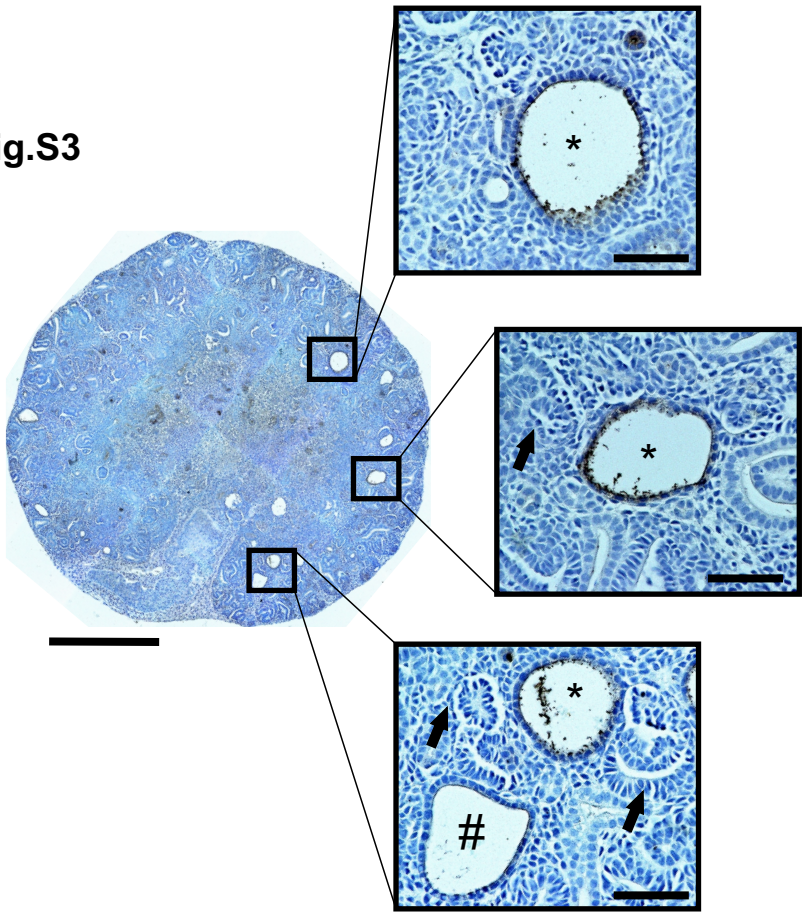


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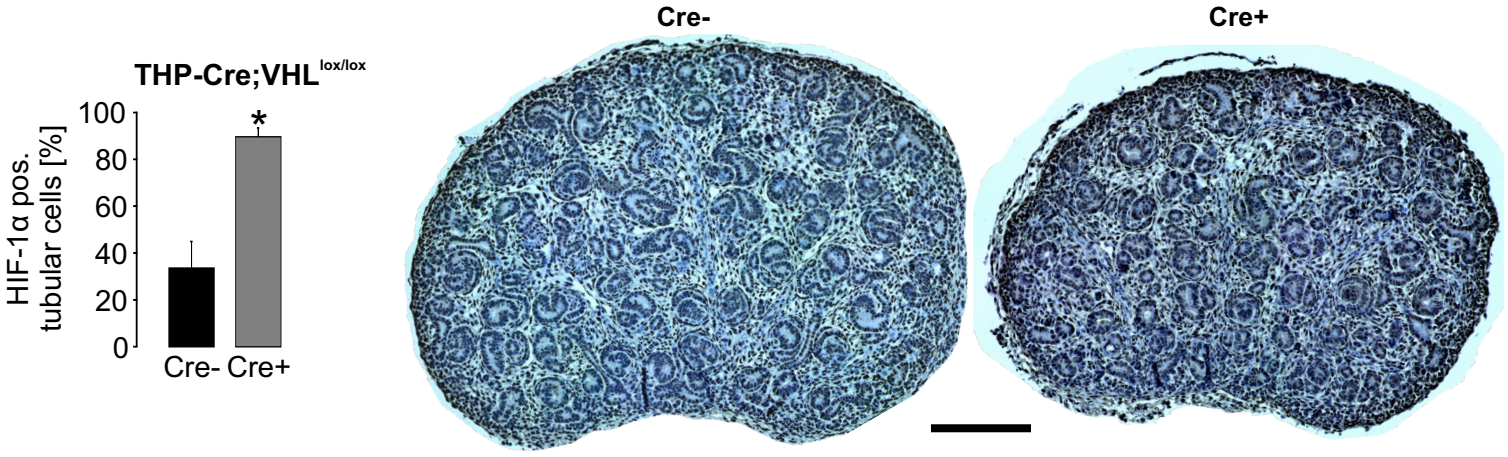
Fig. S4



Supplement 5

Fig. S5