

Electronic supplementary material

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Kidney Injury is Independent of Endothelial HIF-1 α

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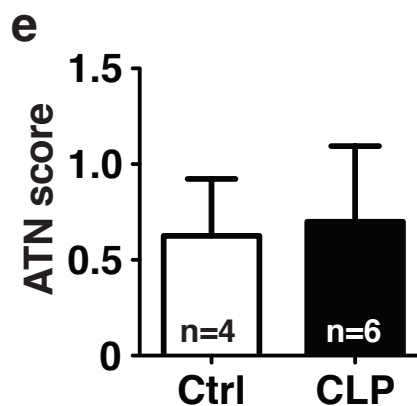
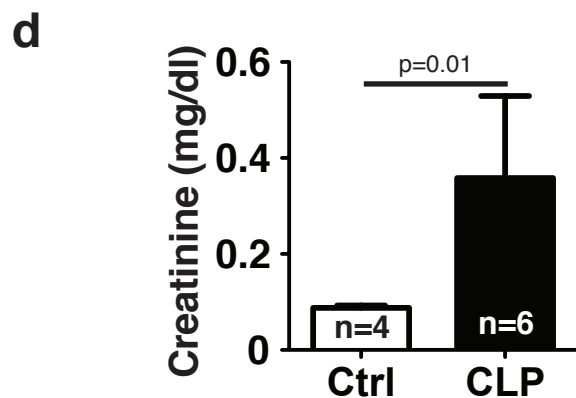
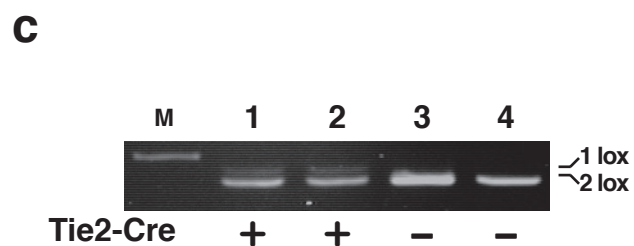
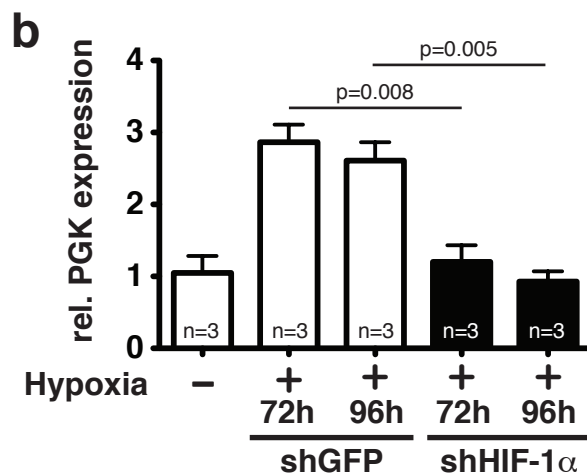
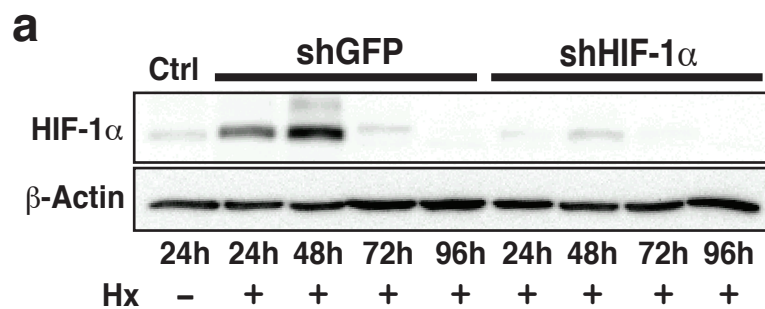
Supplemental Table 1

Primer Name	Sequence
HIF1 F1 (Deletion)	TTGGGGATGAAAACATCTGC
HIF1 F2 (Deletion)	GCAGTTAAGAGCACTAGTTG
HIF1 R (Deletion)	GGAGCTATCTCTCTAGACC
HIF-1alpha F (Genotyping)	GCAGTTAAGAGCACTAGTTG
HIF-1alpha R (Genotyping)	GGAGCTATCTCTCTAGACC
Tie2-Cre F (Genotyping)	CCC TGT GCT CAG ACA GAA ATG AGA
Tie2-Cre R (Genotyping)	CGC ATA ACC AGT GAA ACA GCA TTG C

Supplemental Table 2

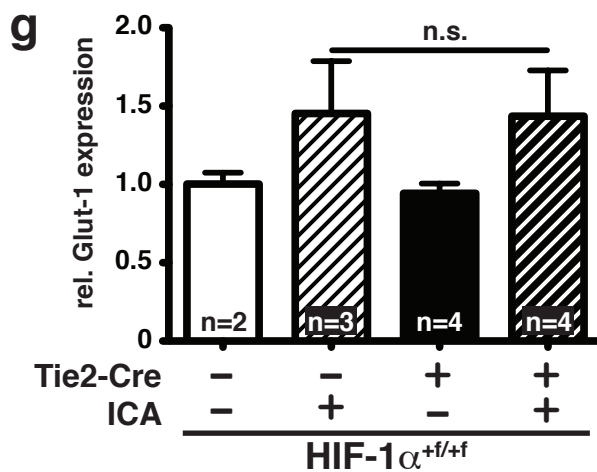
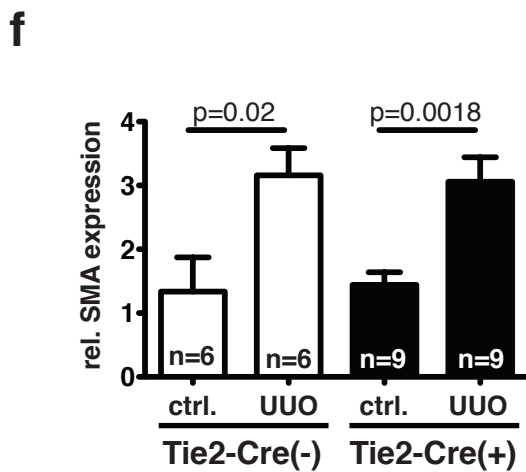
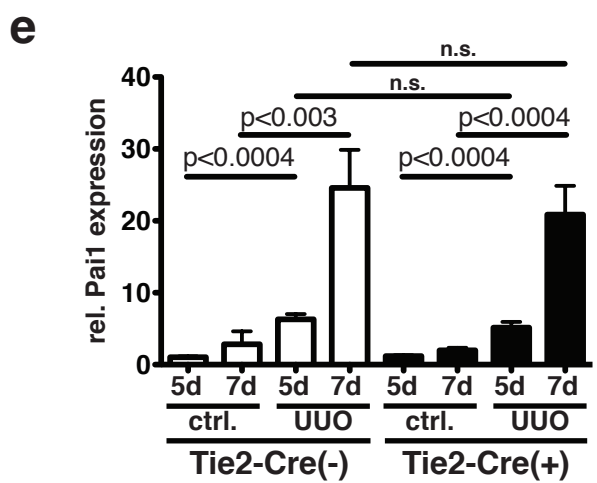
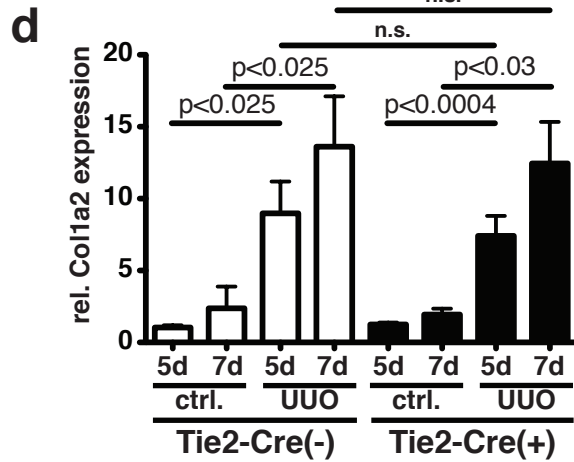
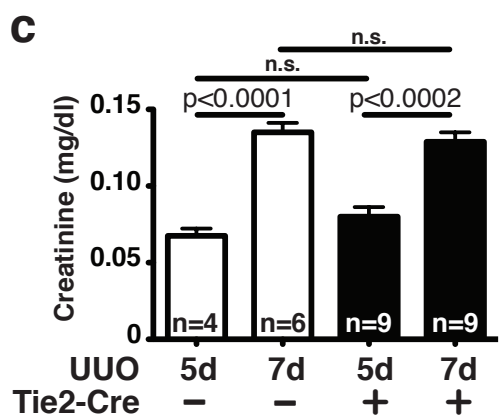
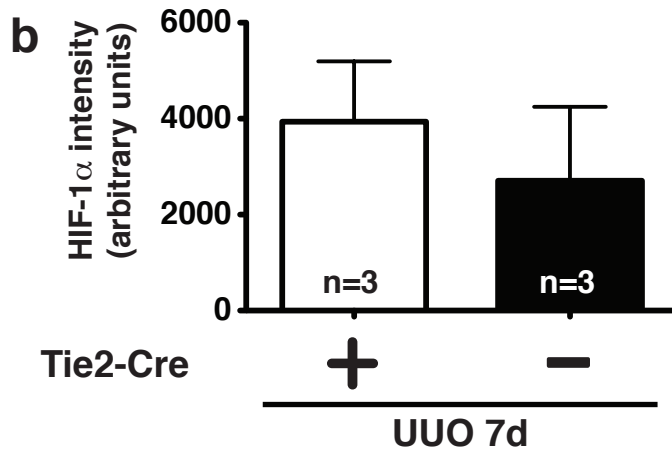
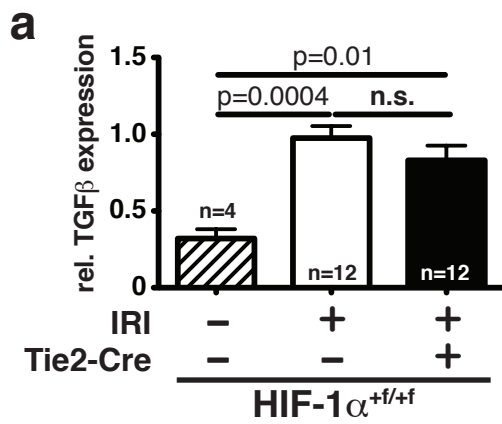
Primer Name	Sequence
Glut-1 F	GGGCATGTGCTTCCAGTATGT
Glut-1 R	ACGAGGAGCACCGTGAAGAT
ICAM F	CCGCAGGTCCAATTCACACT
ICAM R	CAGAGCGGCAGAGCAAAAG
VCAM F	CTCCCCTGAATACAAAACGATTG
VCAM R	GCCCGTAGTGCTGCAAGTG
Col1a2 F	GTAACACCCCAGCGAAGAACT
Col1a2 R	TCAAACCTGGCTGCCACCAT
18s F	CGGACAGGATTGACAGATTG
18s R	CAAATCGCTCCACCAACTAA
TGF β F	GAAACGGAAGCGCATCGA
TGF β R	GGGACTGGCGAGCCTTAGTT
TNF α F	CTAGTGGTGCCAGCCGATG
TNF α R	TAGTCGGGGCAGCCTTGTC
PGK F	CAGGACCATTCCAAACAATCTG
PGK R	CTGTGGTACTGAGAGCAGCAAGA
Ngal F	GGCCTCAAGGACGACAACA
Ngal R	TCACCACCCATTTCAGTTGTCA
SMA F	ATGGCATCAATCACTTCAACAGA
SMA R	ACGCTCTCAAATACCCCGTTT

Supplemental Fig. 1



Supplemental Figure 1 Successful deletion of HIF-1 α in a glomerular endothelial cell line (gIEND.2) and *in vivo* **(a)** Western blot of HIF-1 α in control (shGFP) and shHIF-1 α cells shows successful deletion of HIF-1 α protein. Note the negative feedback loop reducing HIF-1 α protein in control cells after prolonged hypoxia. **(b)** Expression of the HIF-1 α target gene PGK is significantly reduced shHIF-1 α gIENDs compared to control cells (n=3). **(c)** DNA deletion of the Hif1a gene in kidneys: PCR-products of kidney DNA of Tie2-cre(+) animals (lane 1&2) show both the recombined 1-lox band and the 2-lox band whereas DNA of cre-negative mice (lane 3&4) exhibit only the 2-lox bands. **(d)** CLP induced sepsis induces acute kidney injury as determined by increased plasma creatinine levels. **(e)** No renal specific pathology such as increased acute tubular necrosis is observed in animals with CLP-induced sepsis (for both CLP experiments: WT: n=4; KO: n=6).

Suppl. Fig. 2



Supplemental Figure 2: (a) 72 h after renal IRI TGF β mRNA expression in whole kidney lysates is significantly upregulated compared to sham. Loss of HIF-1 α in EC does not affect TGF β expression (sham: n=4; IRI n=12). **(b)** A non-significant trend to reduced HIF-1 α protein expression both in tubular cells and EC in HIF-1 α knockout kidneys 7d after obstruction is observed (n=3; 10 random images of each 200x section). **(c)** 7d after UUO renal function is significantly reduced compared to 5d obstruction. Deletion of HIF-1 α in EC does not influence renal function in UUO (WT: n=4-6, KO: n=9). **(d-e)** Fibrosis associated genes Col1a2 and Pai1 are significantly upregulated in obstructed kidneys compared to contralateral (unobstructed) controls. Deletion of endothelial HIF-1 α does not affect expression levels of both genes (all mRNA analyses: WT: n=4-6; KO: n=9). **(f)** mRNA expression of the fibrotic marker α -smooth muscle actin (α SMA) is significantly induced 7d after UUO irrespective of HIF-1 α in EC (WT: n=6; KO: n=9). **(g)** Deletion of HIF-1 α in EC does not influence Glut-1 mRNA expression in kidneys after systemic application of the PHD-inhibitor ICA (ICA treated: n=3-4).