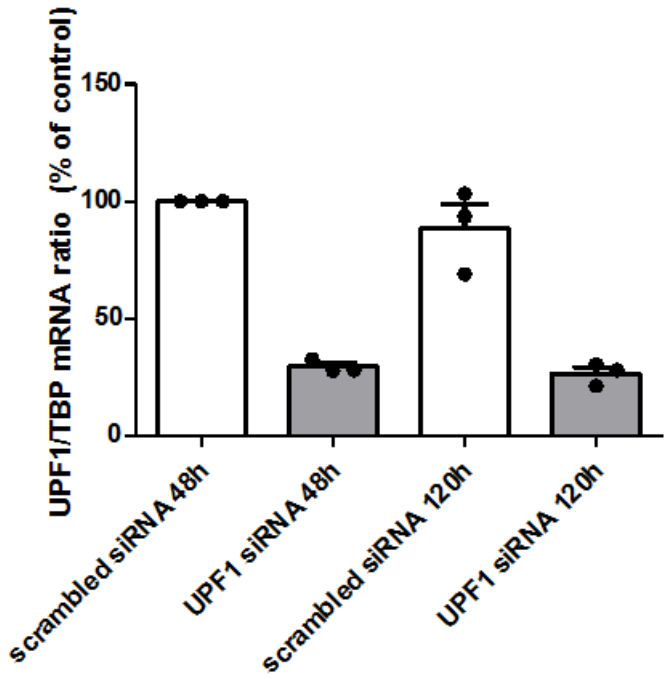
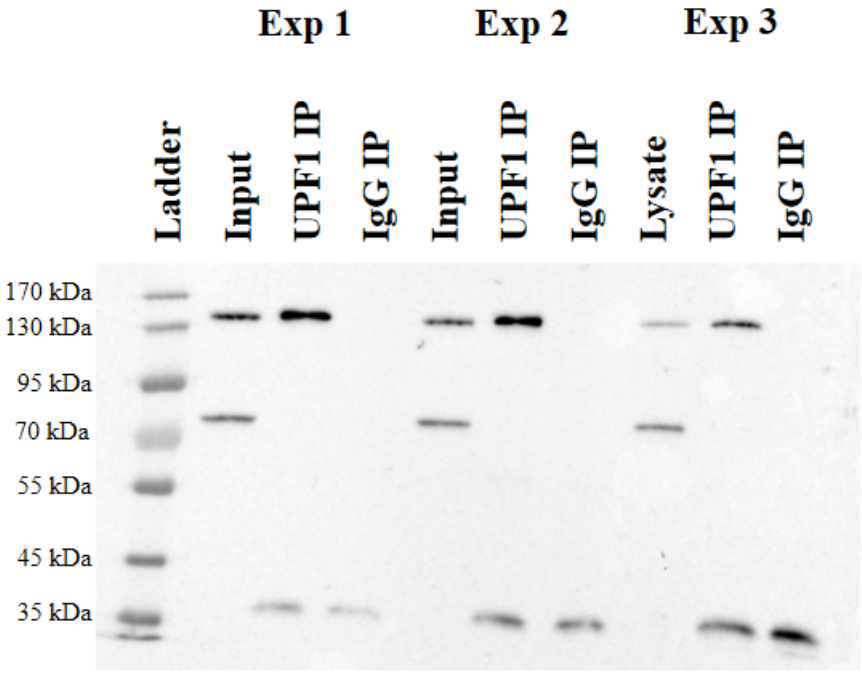
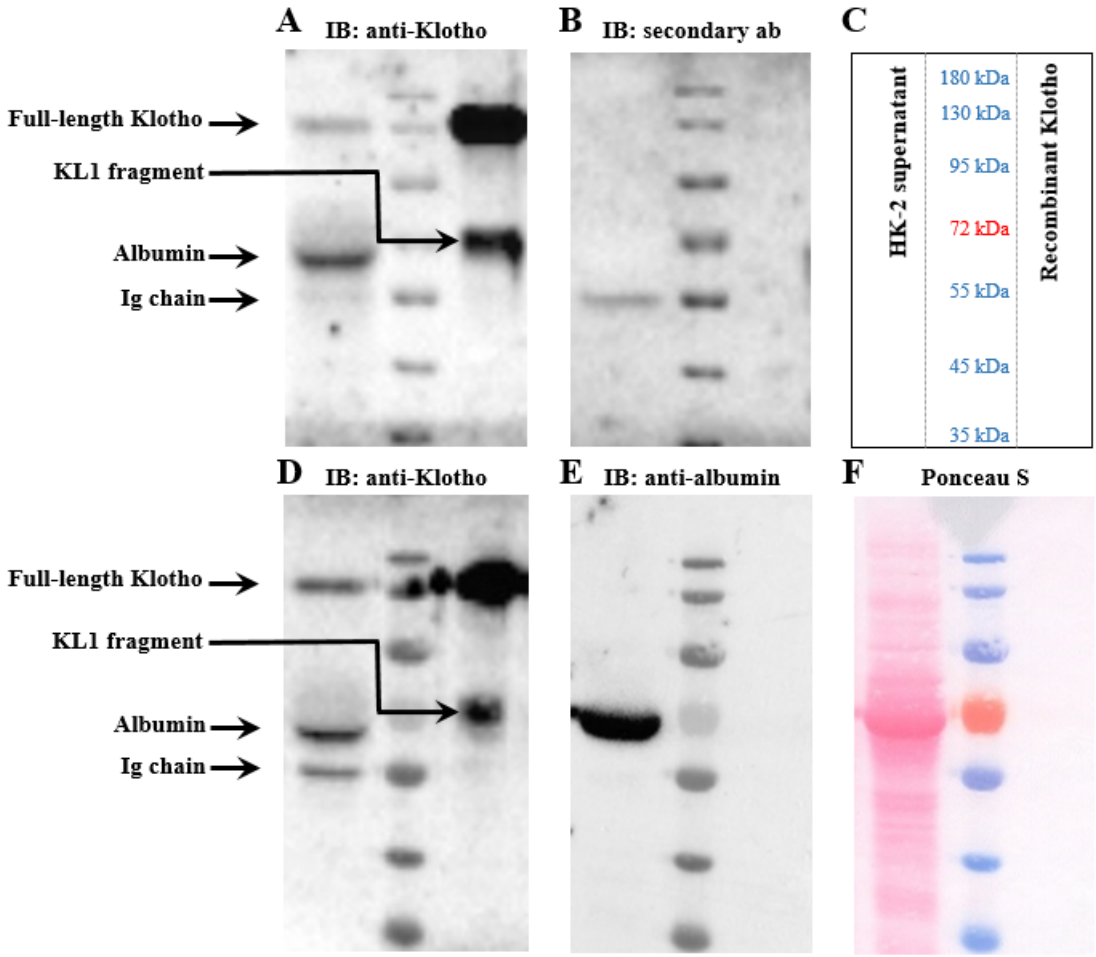


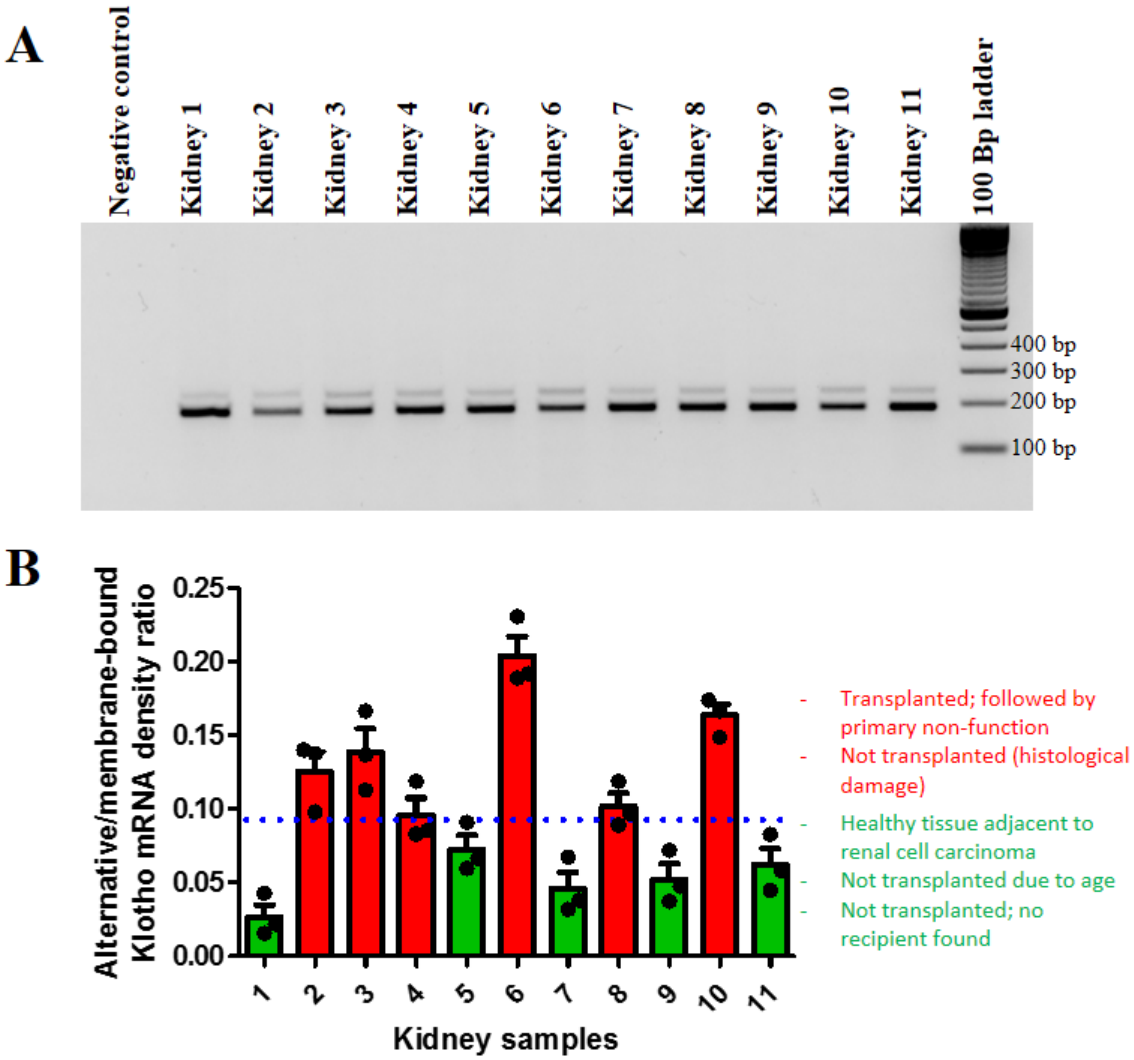
Supplemental figure 1. qRT-PCR control of RNA interference efficacy of *Upf1* in HK-2 cells 48 h and 120 h after transfection. Individual data points represent means from independent experiments, plotted with means ± SD.



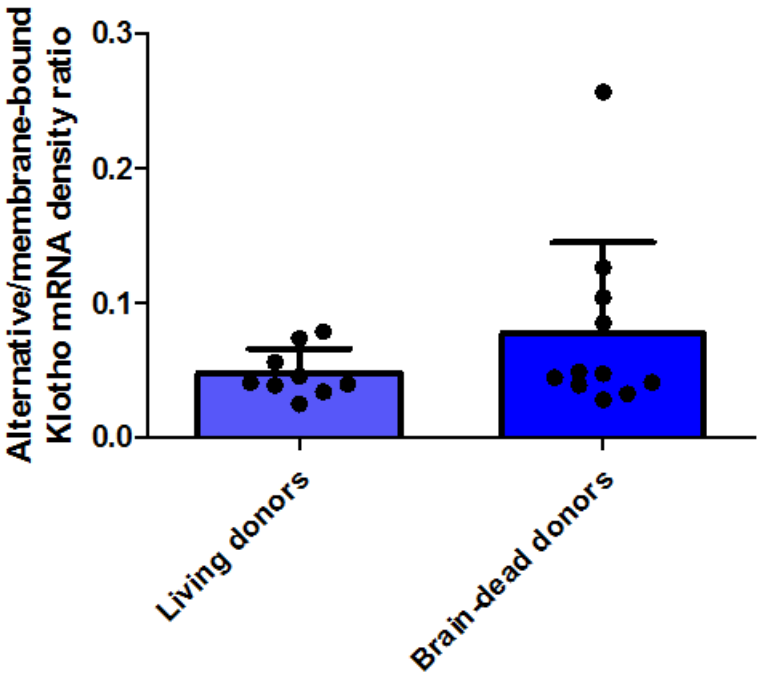
Supplemental figure 2. Full Western blots for UPF1 of three independent UPF1 RIP experiments.



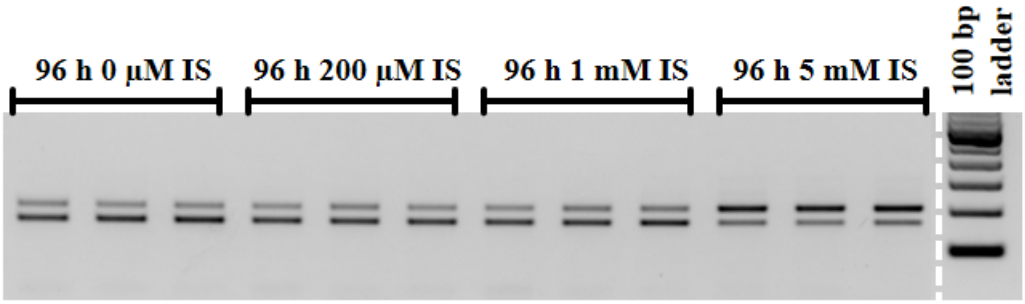
Supplemental figure 3. Characterization of bands detected on Western blot in HK-2 cell supernatant. (A) Western blot for Klotho (using KM2076) on HK-2 cell supernatant (48 h conditioning; left lane) and recombinant human Klotho (right lane), showing a pattern similar to Figure 6A. **(B)** Secondary antibody control for (A), showing that the ~50 kDa band is reactive with polyclonal rabbit anti-rat antibodies, making it likely an immunoglobulin heavy chain. **(C)** Clarification of samples and ladder protein sizes for all panels. **(D)** Western blot for Klotho (using KM2076) on HK-2 cell supernatant (72 h conditioning) and recombinant human Klotho, showing a pattern similar to Figure 6A. **(E)** Re-probing of (D) for albumin, identifying the 65 kDa band in (D) as albumin. **(F)** Ponceau S protein staining for (D), depicting a prominent band at 65 kDa, identified as residual albumin in (E).



Supplemental figure 4. Klotho gene splicing is highly variable between different human kidneys and there may be a link between renal damage and dysregulation of Klotho gene splicing. **(A)** RT-PCR of the membrane-bound and alternative Klotho mRNA transcripts in random samples of healthy and acute kidney injury human kidney samples. **(B)** Densitometric quantification of three independent gene expression analyses, plotted as individual data points and depicted with mean ± SD. The healthiest kidneys (green bars) appear to be the kidneys with the lowest relative alternative Klotho mRNA expression.



Supplemental figure 5. Densitometric quantification of RT-PCR analysis for both *Klotho* transcripts on biopsies from living kidney donors (N = 9) and brain-dead kidney donors (N = 10), showing no difference between these groups. Individual data points are plotted with mean ± SD.



Supplemental figure 6. Proof of principle that the relative abundance of the *Klotho* gene transcripts can be highly dysregulated *in vitro*. RT-PCR analysis for both *Klotho* transcripts after stimulation of HK-2 cells with different concentrations of indoxyl sulfate (IS) for 96 h, shows a marked shift towards the alternative *Klotho* mRNA using 5 mM.

Supplemental Table 1. Densitometric values and qPCR data of the example experiments depicted in Figure 2A-C.

		T = 0 h			T = 2 h			T = 4 h			T = 6 h		
Measurement	Sample	1	2	3	1	2	3	1	2	3	1	2	3
Densitometry													
Alternative *Klotho* band		1.00	1.13	1.03	1.01	1.12	1.08	1.55	1.53	1.58	1.71	1.90	1.76
Membrane-bound *Klotho* band		5.94	6.08	6.87	5.50	5.48	5.00	4.46	4.74	5.29	4.22	3.81	3.63
Ratio		0.17	0.19	0.15	0.18	0.21	0.22	0.35	0.32	0.30	0.41	0.50	0.49
qPCR													
$\Delta Ct_{(Ct(alternative\ Klotho) - Ct(membrane\text{-}bound\ Klotho)}$		0.79	0.75	0.82	0.54	0.85	0.79	0.43	0.74	0.50	0.22	0.26	0.26
$2^{(-\Delta Ct)}$		0.58	0.59	0.57	0.69	0.56	0.58	0.74	0.60	0.71	0.86	0.84	0.84

Supplemental Table 2. Densitometric values and qPCR data of the example experiments depicted in Figure 2D-F.

		Scrambled 48 h			Scrambled 120 h			UPF1 48 h			UPF1 120 h		
Measurement	Sample	1	2	3	1	2	3	1	2	3	1	2	3
Densitometry													
Alternative *Klotho* band		1.00	1.06	1.03	1.12	1.18	1.15	1.15	1.19	1.13	1.59	2.20	1.65
Membrane-bound *Klotho* band		5.39	5.11	4.89	4.65	4.82	4.98	5.39	5.60	5.11	4.80	5.82	4.76
Ratio		0.19	0.21	0.21	0.24	0.25	0.23	0.21	0.21	0.22	0.33	0.37	0.35
qPCR													
$\Delta Ct_{(Ct(alternative\ Klotho) - Ct(membrane\text{-}bound\ Klotho)}$		0.45	1.15	0.75	0.53	0.48	0.84	0.80	1.09	0.96	0.78	-0.28	0.53
$2^{(-\Delta Ct)}$		0.73	0.45	0.59	0.69	0.72	0.56	0.58	0.47	0.51	0.58	1.21	0.69

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