NFAT5 up-regulates expression of the kidney-specific ubiquitin ligase gene *Rnf183* under hypertonic conditions in inner-medullary collecting duct cells

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Supporting Information

1. Table S1
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Table S1. Putative transcription factor binding sites in the *RNF183* enhancer region.

Mus musculus

Transcription factor	JASPAR Score	Relative Score	Start	End	Strand	Predicted site sequence
STAT5	15.281	98.1%	-3466	-3456	1	CTTTCCCAGAA
STAT1	17.040	99.3%	-3465	-3455	-1	TTTCTGGGAAA
STAT3	16.483	99.7%	-3465	-3455	-1	TTTCTGGGAAA
SP1	13.428	95.0%	-3438	-3428	1	CCCCCGCCCCG
KLF5	13.577	97.6%	-3438	-3429	1	CCCCCGCCCC
Mafb	9.499	100.0%	-3430	-3423	-1	GCTGACGG
SP1	13.428	95.0%	-3423	-3413	1	CCCCCGCCCCG
KLF5	13.577	97.6%	-3423	-3414	1	CCCCCGCCCC
NFIC [a]	8.520	96.1%	-3407	-3402	1	TTGGCT
ZNF354C [a]	8.723	99.2%	-3398	-3393	1	CTCCAC
NFE2L1-MafG [a]	8.072	96.8%	-3385	-3380	1	TATGAC
NFAT [a]	11.360	100.0%	-3338	-3332	1	TTTTCCA
Atoh1 [a]	13.740	99.6%	-3333	-3326	1	CAGCTGGC
MZF1_1-4	8.252	96.2%	-3317	-3312	-1	GGGGGA
Lhx3	16.202	96.3%	-3303	-3291	-1	AAATTAATTAGTT
Nobox	10.854	98.3%	-3302	-3295	-1	TAATTAGT
Pdx1	9.570	100.0%	-3301	-3296	1	CTAATT
Prrx2	9.124	100.0%	-3300	-3296	-1	AATTA
Prrx2 [a]	9.124	100.0%	-3299	-3295	1	AATTA
Prrx2	9.124	100.0%	-3296	-3292	-1	AATTA
Stat6	15.738	95.5%	-3283	-3269	1	AAGTTCCTGAGAAGC
Klf1	14.517	95.7%	-3271	-3261	1	AGCCCCACCCT
KLF5	14.984	99.4%	-3270	-3261	1	GCCCCACCCT
Klf4	15.246	99.6%	-3270	-3261	-1	AGGGTGGGGC
GATA3	13.321	97.9%	-3242	-3235	1	AGATAAAA
FOXD1	11.362	96.1%	-3223	-3216	-1	GTAAACAG
FOXO3	11.549	98.0%	-3222	-3215	-1	GGTAAACA
Mafb	8.818	97.0%	-3171	-3164	1	GCTGACTC
NFE2L1-MafG	8.692	99.5%	-3159	-3154	1	GATGAC

Rattus norvegicus

Transcription factor	JASPAR Score	Relative Score	Start	End	Strand	Predicted site sequence
STAT5	14.146	96.7%	-1978	-1968	1	CCTTCCCAGAA
Atoh1	12.065	97.0%	-1972	-1965	1	CAGAAGGC
NFIC [a]	8.520	96.1%	-1935	-1930	1	TTGGCT
ZNF354C [a]	8.723	99.2%	-1926	-1921	1	CTCCAC
NFE2L1-MafG [a]	8.812	100.0%	-1913	-1908	1	CATGAC
NFAT [a]	11.360	100.0%	-1866	-1860	1	TTTTCCA
Atoh1 [a]	13.740	99.6%	-1861	-1854	1	CAGCTGGC
MZF1_1-4	9.085	100.0%	-1845	-1840	-1	TGGGGA
Prrx2 [a]	9.124	100.0%	-1826	-1822	1	AATTA
Prrx2	9.124	100.0%	-1823	-1819	-1	AATTA
HOXA5	8.661	95.8%	-1813	-1806	1	CTCAAATT
MZF1_1-4	9.085	100.0%	-1797	-1792	-1	TGGGGA
GATA3	13.321	97.9%	-1769	-1762	1	AGATAAAA
FOXD1	11.362	96.1%	-1751	-1744	-1	GTAAACAG
FOXO3	12.142	100.0%	-1750	-1743	-1	TGTAAACA
SOX10	8.910	100.0%	-1745	-1740	-1	CTTTGT
NFE2L1-MafG	8.692	99.5%	-1687	-1682	1	GATGAC

Homo sapiens

Transcription factor	JASPAR Score	Relative Score	Start	End	Strand	Predicted site sequence
NFIC [a]	8.520	96.1%	-4322	-4317	1	TTGGCT
ZNF354C [a]	8.723	99.2%	-4313	-4308	1	CTCCAC
NFE2L1-MafG [a]	8.812	100.0%	-4300	-4295	1	CATGAC
Hltf	7.794	95.6%	-4279	-4270	-1	TACCTTAGAC
NFAT [a]	11.360	100.0%	-4252	-4246	1	TTTTCCA
Atoh1 [a]	14.033	100.0%	-4247	-4240	1	CAGATGGC
Pdx1	9.570	100.0%	-4208	-4203	-1	CTAATT
Prrx2 [a]	9.124	100.0%	-4208	-4204	1	AATTA
NFAT	11.360	100.0%	-4151	-4145	-1	TTTTCCA
BRCA1	7.447	95.7%	-4136	-4130	-1	CCAACAG
NFIC	9.697	100.0%	-4133	-4128	1	TTGGCA
HOXA5	9.854	100.0%	-4069	-4062	-1	CACTAATT
Pdx1	9.570	100.0%	-4069	-4064	-1	CTAATT
Prrx2	9.124	100.0%	-4069	-4065	1	AATTA
NFAT	11.360	100.0%	-4058	-4052	-1	TTTTCCA

Transcription factor-binding sites predicted using the JASPAR software are shown in the mammalian conserved region of *RNF183* genes (mouse, −3,466 to −3,136; rat, −1,978 to −1,664; human, −4,367 to −4,049). The potential binding sites with a >95% chance (relative score) of binding to any of the listed transcription factors. [a] Conserved binding sites among mouse, rat, and human. The conserved NFAT5 DNA binding site is indicated as *yellow background*.

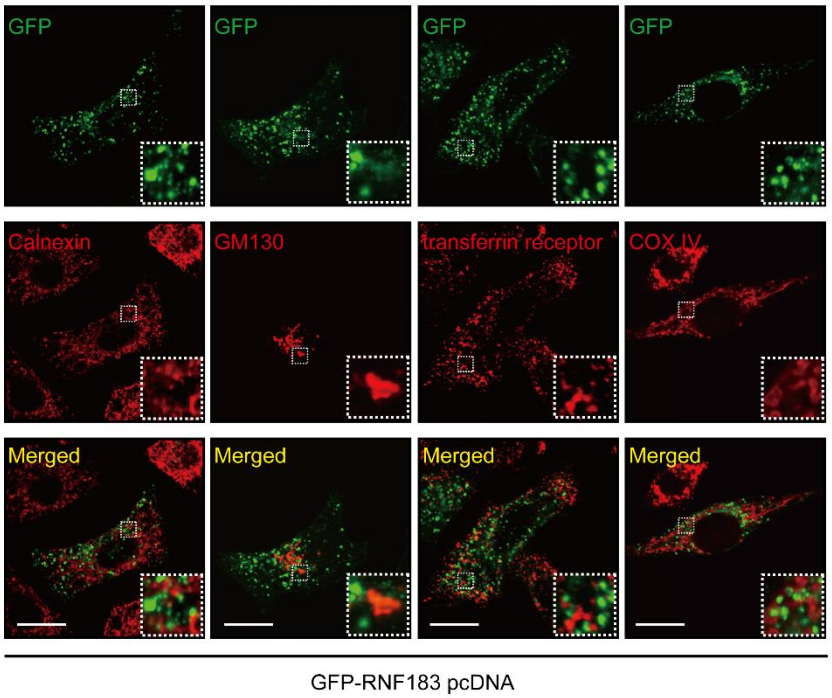

Figure S1. Subcellular localization of RNF183. The confocal images of immunofluorescence with GFP signals (*upper panels, green*) and various antibodies for organelle markers (Calnexin, GM130, transferrin receptor, and COX IV; *middle panels, red*) in HeLa cells transfected with GFP-RNF183. Bars, 10 μm. All images shown are representative of at least three replicates in independent observations.

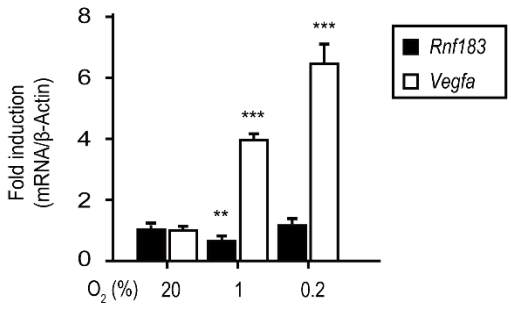

Figure S2. RNF183 expression is not up-regulated under hypoxia. Effect of hypoxia on *Rnf183* expression. Mouse IMCD-3 cells were maintained in hypoxia (0.1% or 0.3% O_2) or normoxia (20% O_2) chambers for 12 h at 37°C and analyzed by qRT-PCR analysis. *Vegfa* was used as a hypoxia-induced positive control. Expression levels were compared with those in normoxia (n = 6). Data were analyzed by one-way ANOVA, followed by the *post hoc* tests using *t* tests with Bonferroni correction. Values represent mean ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$ (versus normoxic control).

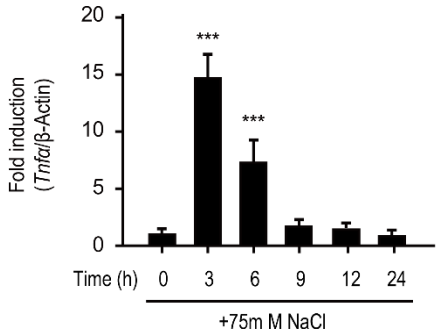

Figure S3. *Tnfa* mRNA peaked after 3h of hypertonic stimulation. Time-course analysis of *Tnfa* mRNA in response to hypertonic stress. Mouse IMCD-3 cells were treated with 75 mM NaCl-supplementation for indicated time and analyzed by qRT-PCR analysis. Expression levels were compared with those in isotonic control ($n = 6$). Data were analyzed by one-way ANOVA followed by the *post hoc* tests using *t* tests with Bonferroni correction. Values represent mean ± S.D. *** $P < 0.001$ (versus isotonic control).

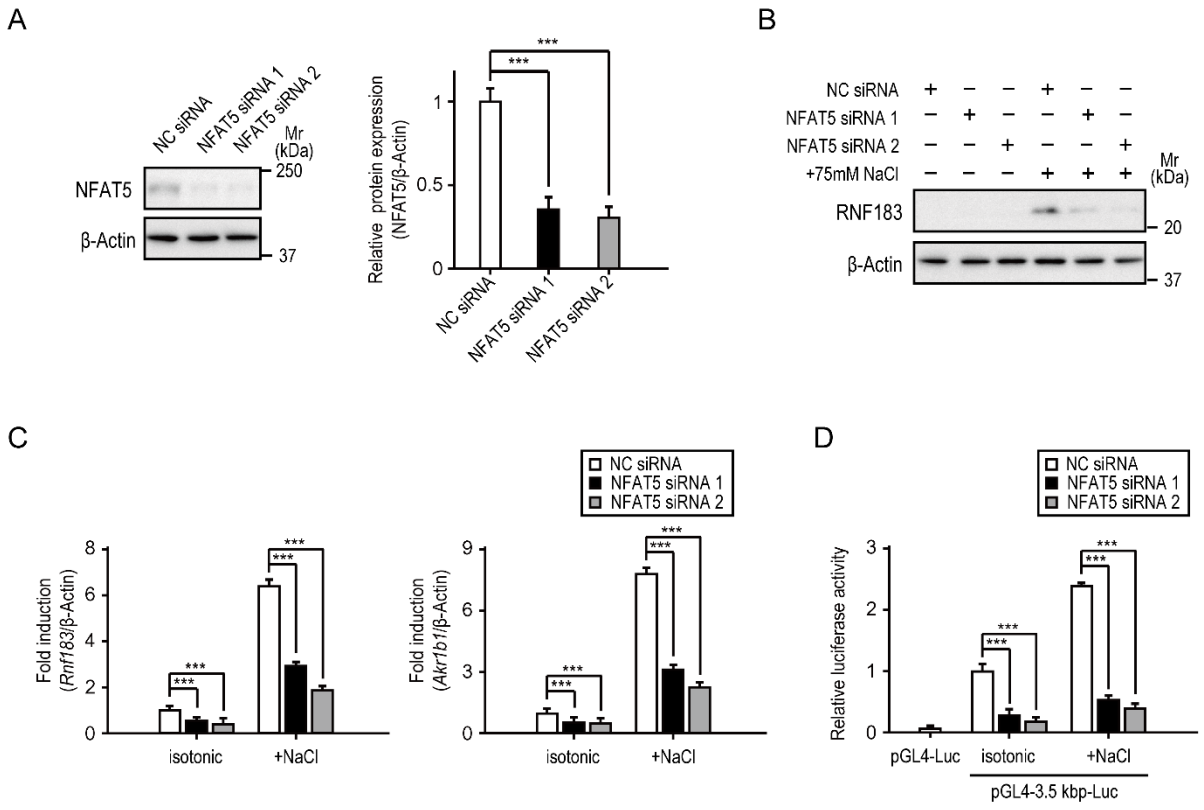

Figure S4. NFAT5 knockdown attenuates hypertonicity-induced RNF183 expression. A. Inhibition of NFAT5 expression using siRNA 2-mediated knockdown. Mouse IMCD-3 cells transfected with NFAT5 siRNA 1, NFAT5 siRNA 2, or negative control (NC) siRNA were analyzed by western blotting. The quantification of NFAT5 is summarized in the accompanying bar graph (n = 3). **B and C.** Effects of NFAT5 siRNA 2-mediated knockdown on **(B)** expression of RNF183 protein and **(C)** expression of *Rnf183* (*left*) and *Akr1b1* (*right*) mRNA (n = 4). mIMCD-3 cells transfected with NFAT5 siRNA 1, NFAT5 siRNA 2, or NC siRNA were treated with isotonic or 75 mM NaCl -supplemented medium for 12 h and analyzed using **(B)** western blot and **(C)** qRT-PCR. **D.** Effect of NFAT5 siRNA 2-mediated knockdown on hypertonicity-induced promoter activities. Cells co-transfected with 3.5 kbp-Luc and siRNA (NFAT5 1, NFAT5 2 or NC) were treated with isotonic or 75 mM NaCl- or sucrose-supplemented medium for 24 h (n = 4). Data were analyzed by one-way ANOVA, followed by the *post hoc* tests using t tests with Bonferroni correction. Values represent mean ± SD. ***P < 0.001 (versus NC).

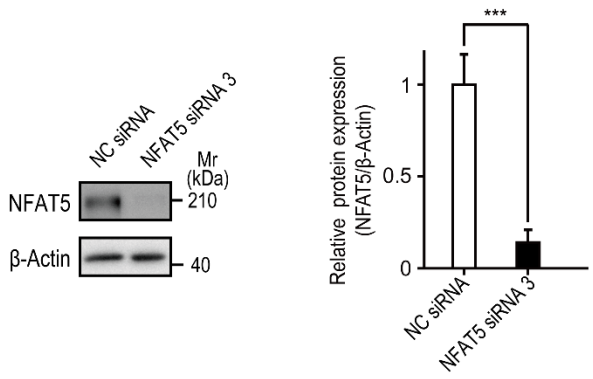

Figure S5. Inhibition of NFAT5 expression using NFAT5 siRNA 3-mediated knockdown. Mouse IMCD-3 cells transfected with NFAT5 siRNA 3 or negative control (NC) siRNA were analyzed by western blotting. The quantification of NFAT5 is summarized in the accompanying bar graph (n = 3). Data were analyzed by t test. Values represent mean ± SD. ***$P < 0.001$.

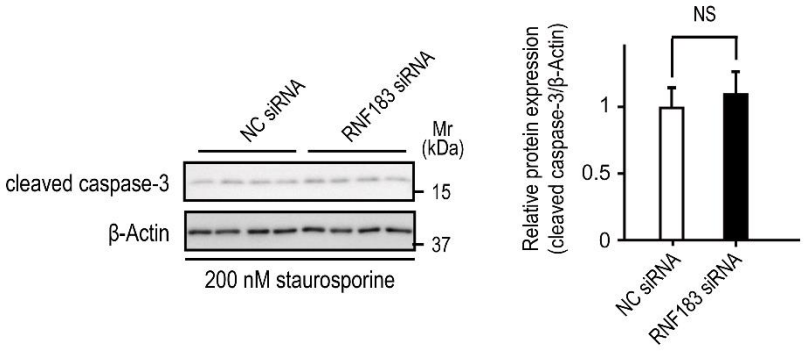

Figure S6. RNF183 expression did not protect against staurosporine-induced apoptosis

Cells transfected with the indicated siRNAs were treated with 200 nM staurosporine-supplemented medium for 12 h and analyzed using western blotting. The accompanying bar graph summarizes the quantification of relative amounts of cleaved caspase-3 (n = 4). Values represent mean ± SD. N.S. P > 0.05 (versus NC).