

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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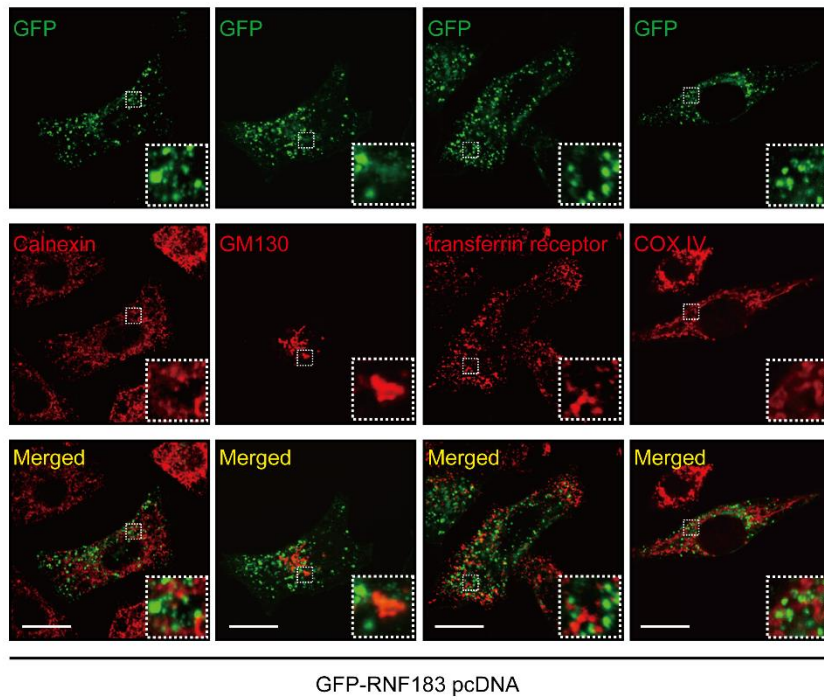
### **Supporting Information**

1. Table S1
2. Figures S1–S6

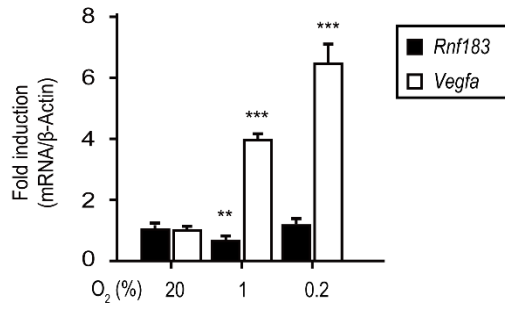
**Table S1. Putative transcription factor binding sites in the *RNF183* enhancer region.**

<i>Mus musculus</i>							<i>Rattus norvegicus</i>						
Transcription factor	JASPAR Score	Relative Score	Start	End	Strand	Predicted site sequence	Transcription factor	JASPAR Score	Relative Score	Start	End	Strand	Predicted site sequence
STAT5	15.281	98.1%	-3466	-3456	1	CTTTCCAGAA	STAT5	14.146	96.7%	-1978	-1968	1	CCTTCCAGAA
STAT1	17.040	99.3%	-3465	-3455	-1	TTTCTGGGAAA	Atoh1	12.065	97.0%	-1972	-1965	1	CAGAAGGC
STAT3	16.483	99.7%	-3465	-3455	-1	TTTCTGGGAAA	NFIC <sup>a</sup>	8.520	96.1%	-1935	-1930	1	TTGGCT
SP1	13.428	95.0%	-3438	-3428	1	CCCCGCCCG	ZNF354C <sup>a</sup>	8.723	99.2%	-1926	-1921	1	CTCCAC
KLF5	13.577	97.6%	-3438	-3429	1	CCCCGCCCG	NFE2L1-MafG <sup>a</sup>	8.812	100.0%	-1913	-1908	1	CATGAC
MafB	9.499	100.0%	-3430	-3423	-1	GCTGACGG	NFAT <sup>a</sup>	11.360	100.0%	-1866	-1860	1	TTTTCCA
SP1	13.428	95.0%	-3423	-3413	1	CCCCGCCCG	Atoh1 <sup>a</sup>	13.740	99.6%	-1861	-1854	1	CAGCTGGC
KLF5	13.577	97.6%	-3423	-3414	1	CCCCGCCCG	MZF1_1-4	9.085	100.0%	-1845	-1840	-1	TGGGGA
NFIC <sup>a</sup>	8.520	96.1%	-3407	-3402	1	TTGGCT	Prrx2 <sup>a</sup>	9.124	100.0%	-1826	-1822	1	AATTA
ZNF354C <sup>a</sup>	8.723	99.2%	-3398	-3393	1	CTCCAC	Prrx2	9.124	100.0%	-1823	-1819	-1	AATTA
NFE2L1-MafG <sup>a</sup>	8.072	96.8%	-3385	-3380	1	TATGAC	HOXA5	8.661	95.8%	-1813	-1806	1	CTCAAAAT
NFAT <sup>a</sup>	11.360	100.0%	-3338	-3332	1	TTTTCCA	MZF1_1-4	9.085	100.0%	-1797	-1792	-1	TGGGGA
Atoh1 <sup>a</sup>	13.740	99.6%	-3333	-3326	1	CAGCTGGC	GATA3	13.321	97.9%	-1769	-1762	1	AGATAAAA
MZF1_1-4	8.252	96.2%	-3317	-3312	-1	GGGGGA	FOXO1	11.362	96.1%	-1751	-1744	-1	GTAAACAG
Lhx3	16.202	96.3%	-3303	-3291	-1	AAATTAATTAGTT	FOXO3	12.142	100.0%	-1750	-1743	-1	TGTAACACA
Nobox	10.854	98.3%	-3302	-3295	-1	TAATTAGT	SOX10	8.910	100.0%	-1745	-1740	-1	CTTTGT
Pdx1	9.570	100.0%	-3301	-3296	1	CTAATT	NFE2L1-MafG	8.692	99.5%	-1687	-1682	1	GATGAC
Prrx2	9.124	100.0%	-3300	-3296	-1	AATTA	<i>Homo sapiens</i>						
Prrx2 <sup>a</sup>	9.124	100.0%	-3299	-3295	1	AATTA	Transcription factor	JASPAR Score	Relative Score	Start	End	Strand	Predicted site sequence
Prrx2	9.124	100.0%	-3296	-3292	-1	AATTA	NFIC <sup>a</sup>	8.520	96.1%	-4322	-4317	1	TTGGCT
Stat6	15.738	95.5%	-3283	-3269	1	AAGTTCTGAGAAGC	ZNF354C <sup>a</sup>	8.723	99.2%	-4313	-4308	1	CTCCAC
Klf1	14.517	95.7%	-3271	-3261	1	AGCCCCACCT	NFE2L1-MafG <sup>a</sup>	8.812	100.0%	-4300	-4295	1	CATGAC
KLF5	14.984	99.4%	-3270	-3261	1	GCCCCACCT	Hltf	7.794	95.6%	-4279	-4270	-1	TACCTTAGAC
Klf4	15.246	99.6%	-3270	-3261	-1	AGGGTGGGC	NFAT <sup>a</sup>	11.360	100.0%	-4252	-4246	1	TTTTCCA
GATA3	13.321	97.9%	-3242	-3235	1	AGATAAAA	Atoh1 <sup>a</sup>	14.033	100.0%	-4247	-4240	1	CAGATGGC
FOXO1	11.362	96.1%	-3223	-3216	-1	GTAAACAG	Pdx1	9.570	100.0%	-4208	-4203	-1	CTAATT
FOXO3	11.549	98.0%	-3222	-3215	-1	GGTAACACA	Prrx2 <sup>a</sup>	9.124	100.0%	-4208	-4204	1	AATTA
MafB	8.818	97.0%	-3171	-3164	1	GCTGACTC	NFAT	11.360	100.0%	-4151	-4145	-1	TTTTCCA
NFE2L1-MafG	8.692	99.5%	-3159	-3154	1	GATGAC	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	CACAAATT
							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

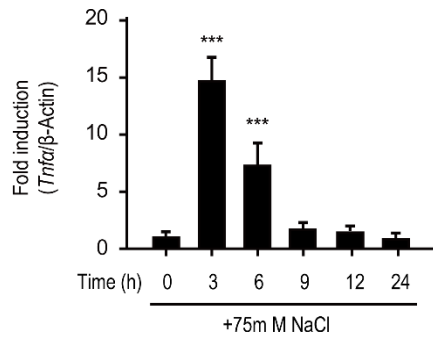
Transcription factor-binding sites predicted using the JASPAR software are shown in the mammalian conserved region of *RNF183* genes (mouse, -3,466 to -3,136; rat, -1,978 to -1,664; human, -4,367 to -4,049). The potential binding sites with a >95% chance (relative score) of binding to any of the listed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



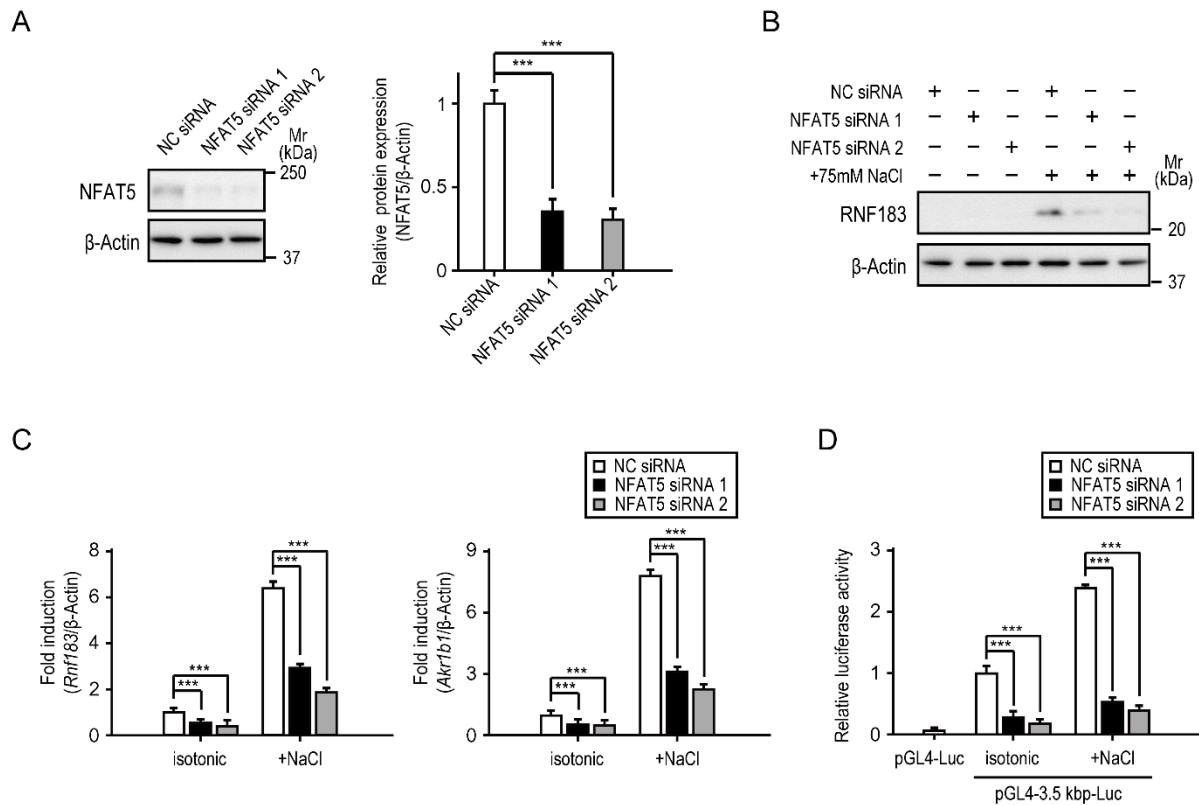
**Figure S1. Subcellular localization of RNF183.** The confocal images of immunofluorescence with GFP signals (*upper panels, green*) and various antibodies for organelle markers (Calnexin, GM130, transferrin receptor, and COX IV; *middle panels, red*) in HeLa cells transfected with GFP-RNF183. Bars,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



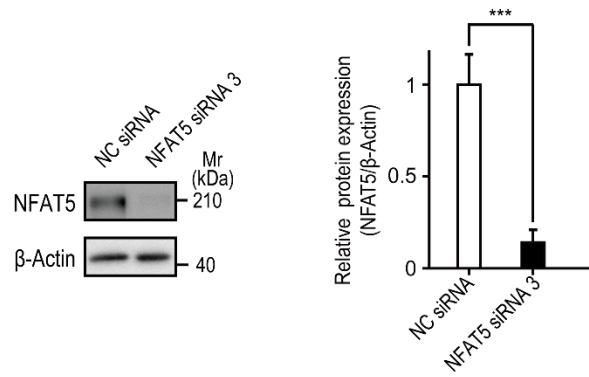
**Figure S2. RNF183 expression is not up-regulated under hypoxia.** Effect of hypoxia on *Rnf183* expression. Mouse IMCD-3 cells were maintained in hypoxia (0.1% or 0.3% O<sub>2</sub>) or normoxia (20% O<sub>2</sub>) chambers for 12 h at 37°C and analyzed by qRT-PCR analysis. *Vegfa* was used as a hypoxia-induced 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  $\pm$  SD. \*\* $P < 0.01$ , \*\*\* $P < 0.001$  (versus normoxic control).



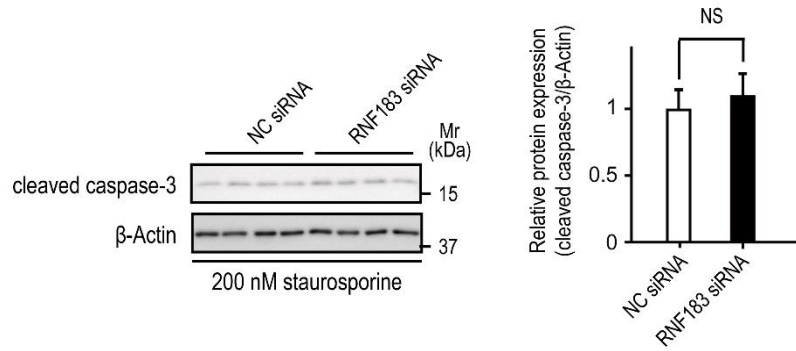
**Figure S3. *Tnfa* mRNA peaked after 3h of hypertonic stimulation.** Time-course analysis of *Tnfa* mRNA in response to hypertonic stress. Mouse IMCD-3 cells were treated with 75 mM NaCl-supplementation for indicated time and analyzed by qRT-PCR analysis. Expression levels were compared with those in 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  $\pm$  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  $\pm$  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  $\pm$  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  $\pm$  SD. N.S.  $P > 0.05$  (versus NC).