SUPPLEMENTAL MATERIAL

Inhibition of urea transporter ameliorates uremic cardiomyopathy in mice with chronic kidney disease

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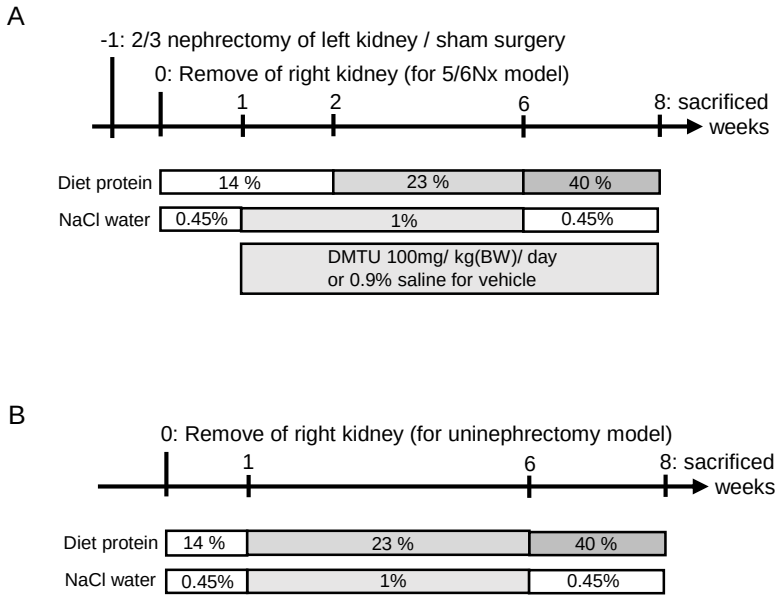
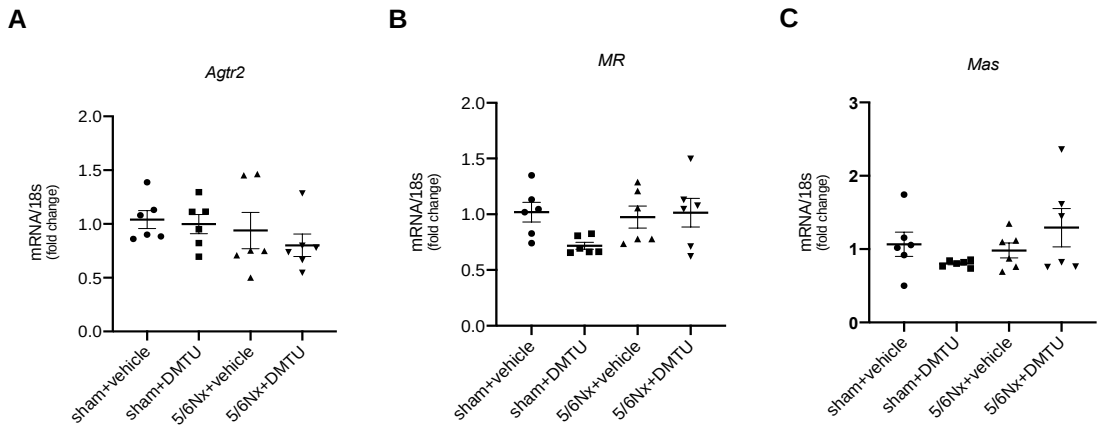
Supplementary Table 1. The primers for quantitative PCR

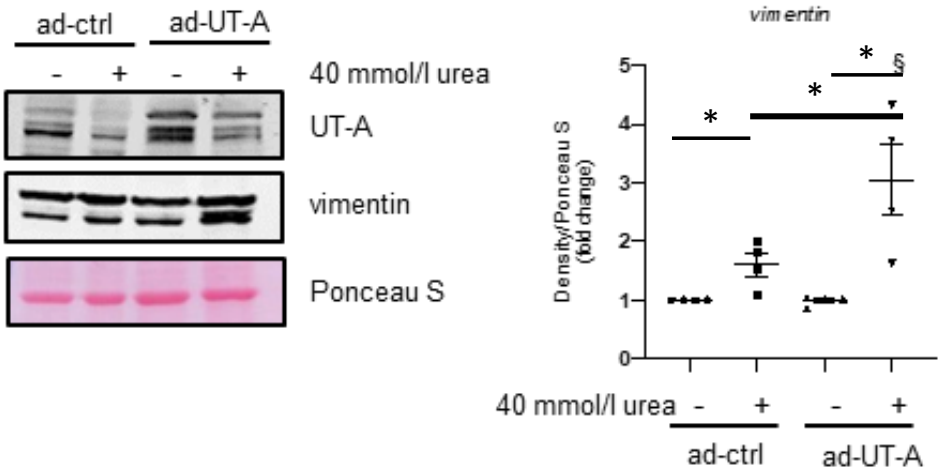
Name	Sequence	Amplicon (bp)	Code
Collagen 1a1			
Forward	GAGCGGAGAGTACTGGATCG	204	NM_007742.4
Reverse	TACTCGAACGGGAATCCATC		
Collagen 4a1			
Forward	ATTCCTTTGTGATGCACACCAG	151	NM_009931
Reverse	AAGCTGTAAGCATTCGCGTAGTA		
Fibronectin			
Forward	CGAGGTGACAGAGACCACAA	149	NM_010233.2
Reverse	CTGGAGTCAAGCCAGACACA		
α-SMA			
Forward	ACTGGGACGACATGGAAAAG	240	NM_007392.3
Reverse	CATCTCCAGAGTCCAGCACA		
Agt			
Forward	TCTCCTTTACCACAACAAGAGCA	121	NM_007428.4
Reverse	CTTCTCATTCACAGGGGAGGT		
Renin			
Forward	CTCTCTGGGCACTCTTGTTGC	198	NM_031192.3
Reverse	GGGAGGTAAGATTGGTCAAGGA		
Ace			
Forward	GGAGTACTTCCAACCGGTCA	147	NM_207624
Reverse	GCCTTGGCTTCATCAGTCTC		
Agtr1			
Forward	CTGTGGCCAGTGTCTTTCT	161	NM_177322
Reverse	GGCGTAGAGGTTGAAACTGA		
Agtr2			
Forward	AACTGGCACCAATGAGTCCG	209	NM_007429.5
Reverse	CCAAAAGGAGTAAGTCAGCCAAG		
MR			
Forward	GAAAGGCGCTGGAGTCAAGT	127	NM_001083906.2
Reverse	TGTTCGGAGTAGCACCGGAA		
Mas			
Forward	AGAAATCCCTTCACGGTCTACA	146	NM_008552.5
Reverse	GTCACCGATAATGTCACGATTGT		
UT-B			
Forward	CCAACATCACGTGGTCTGA	61	AF448798
Reverse	ACTCCCACCGGAAGAGACTT		
18s			
Forward	CCAGAGCGAAAGCATTTGCCAAGA	101	X00686
Reverse	TCGGCATCGTTTATGGTCGGAACT		



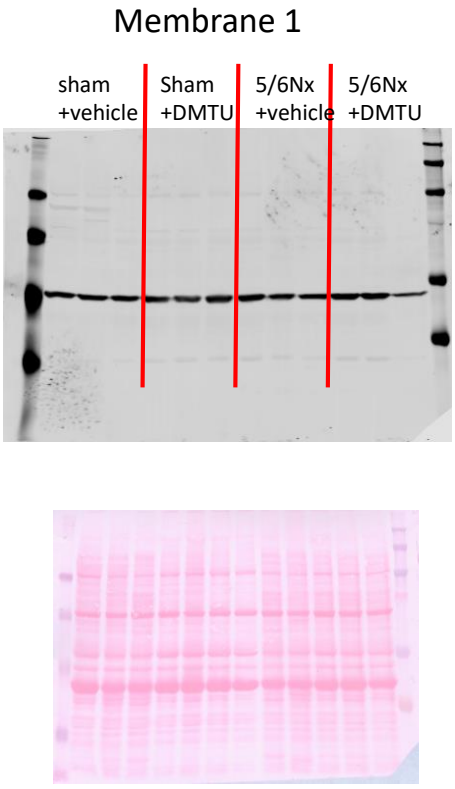
Supplementary Figure 1. Animal experimental protocol:
(A) Experiment of C57BL/6J wild type mice. Mice underwent 5/6 nephrectomy (5/6Nx) or sham surgery. Mice were fed 14% protein diet (0 weeks to 2 weeks), 23% protein diet (2 weeks to 6 weeks), and 40% protein diet (6 weeks to 8 weeks), and 0.9% saline for vehicle or dimethylthiourea (DMTU; UT-A inhibitor) was administered. (B) Experiment of C57BL/6J wild type and *UT-A1/A3*$^{-/-}$ mice. Mice underwent uninephrectomy (Nx) or sham surgery. Mice were fed 14% protein diet (0 weeks to 1 week), 23% protein diet (1 weeks to 6 weeks), and 40% protein diet (6 weeks to 8 weeks). For both experiments, mice drank 1% NaCl water one week to six weeks after second surgery.

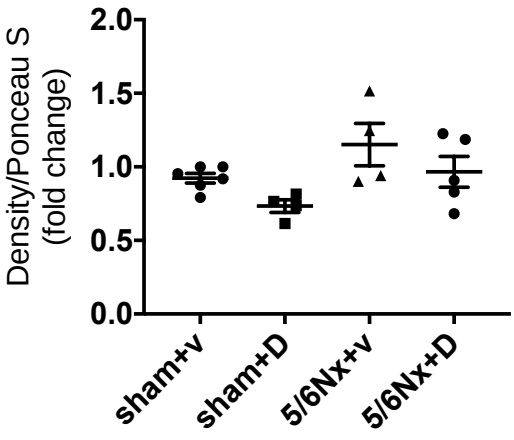


Supplementary Figure 2. Renin-angiotensin system related genes in mouse hearts :
Quantitative mRNA expression in mouse hearts performed by real-time PCR. All gene expressions were calculated by ΔΔcq and standarized by housekeeping gene 18s. (A) Agtr2: angiotensin II receptor type2, (B) MR: mineralocorticoid receptor, (C) Mas: Mas receptor. All data; mean±SEM (*N*=6).



Supplementary Figure 3. The effect of urea on vimentin protein amount with or withour upregulation of UT.
H9c2 cardiac myoblasts transduced with control adenovirus (ad)-GFP and ad-UT-A/GFP in normal medium, respectively. Protein was isolated after 48 hours after virus transduction. Western blot of lysates from H9c2 cells was performed to analyse UT and vimentin protein abandance. Data; mean±SEM (*N*=4). **P*<0.005, by two-way ANOVA anslysis.





Supplementary Figure 4:
Western blot of lysates from sham/vehicle, sham/DMTU, 5/6Nx/vehicle or 5/6Nx/DMTU mouse hearts probed for urea transport proteins. UT protein amount has a tendency to be decreased in 5/6Nx/DMTU treated mice compared with 5/6Nx/vehicle treated mice, but is not statistically different.