Supplemental information

Targetable Purinergic Receptors $P2Y_{12}$ And A2b Antagonistically Regulate Bladder Function

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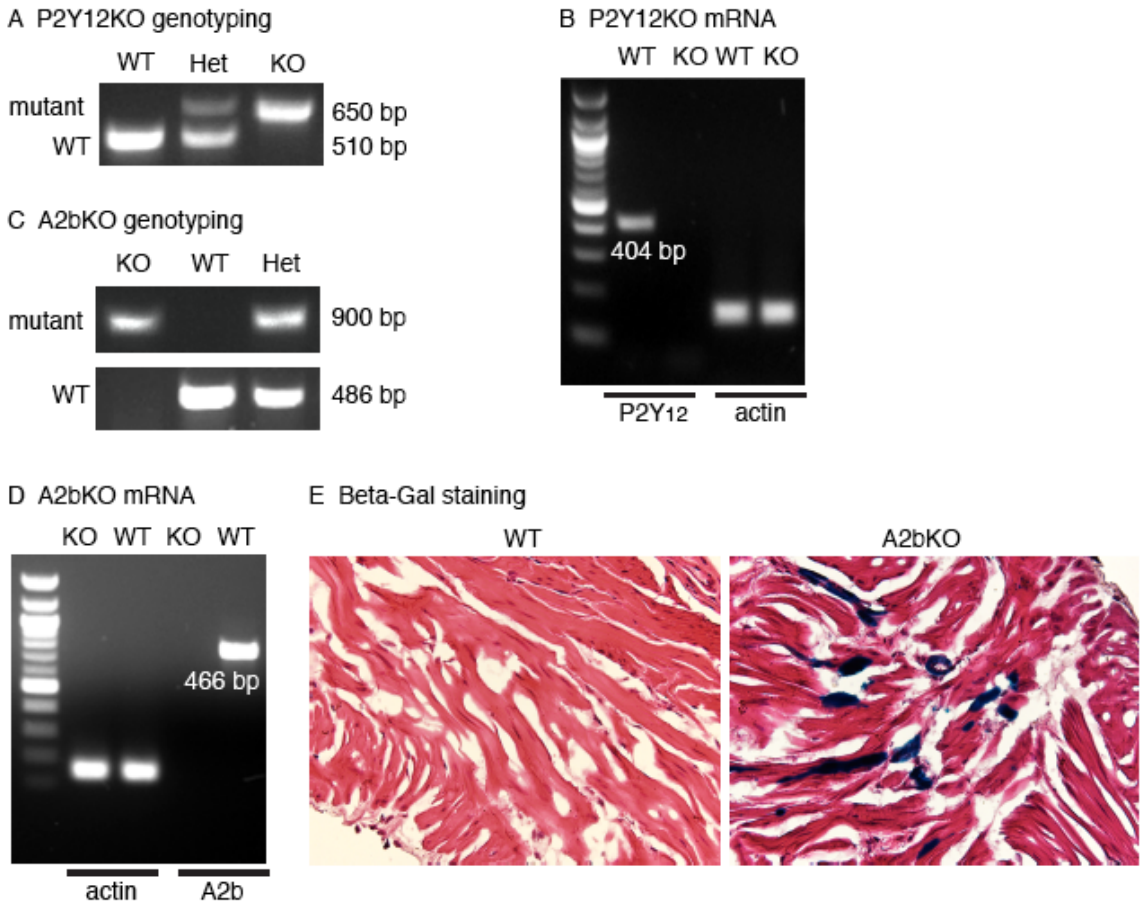
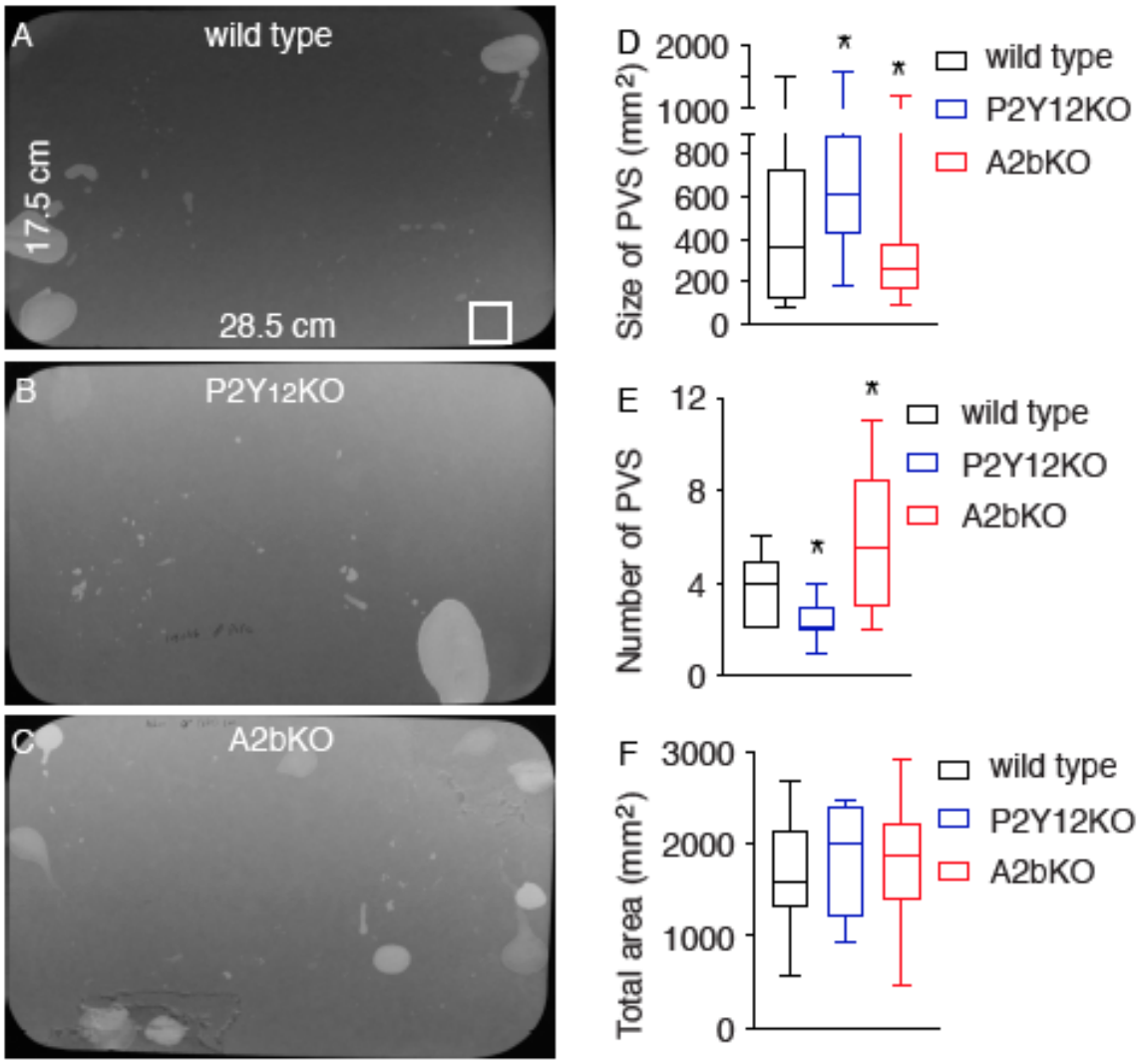
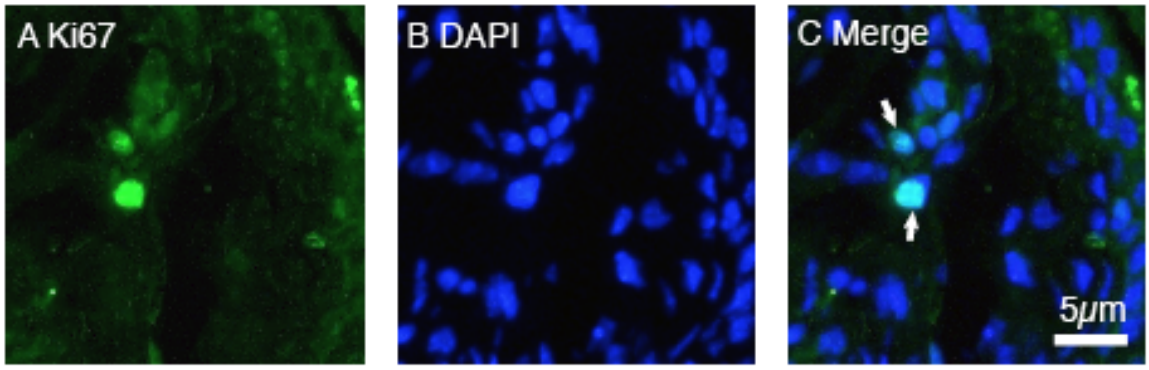
Supplemental figure 1. Genotyping of $P2Y_{12}KO$ (A) and A2bKO (C) mice. (B) and (D) mRNA expression by RT-PCR for $P2Y_{12}KO$ and A2bKO mice. (E) β-Gal staining of A2bKO BSM.



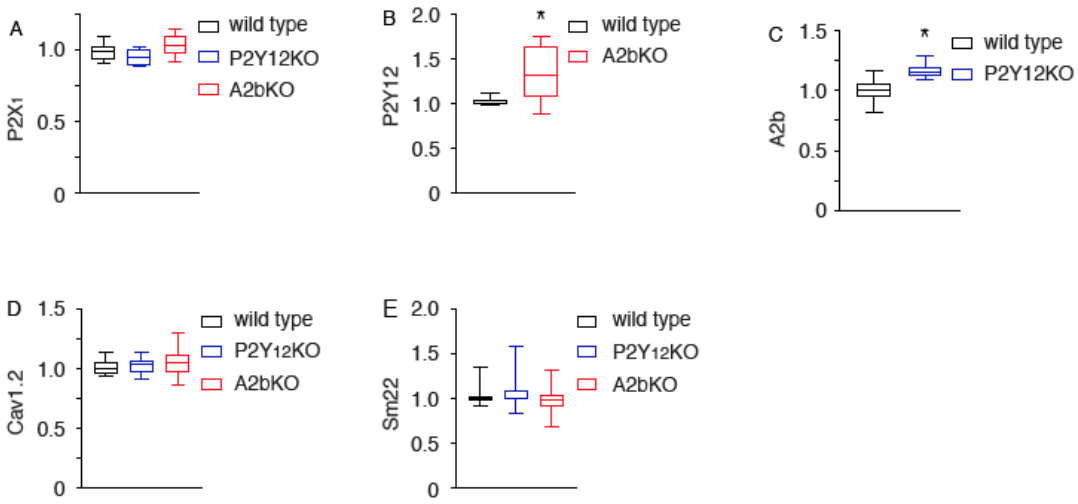
Supplemental figure 2. Male $P2Y_{12}KO$ and A2bKO mice exhibit altered bladder function. (A-C) are representative images of VSA filter papers from wild-type (n=11), $P2Y_{12}KO$ (n=7), and A2bKO (n=22) mice respectively, which are quantitated in (D-F). The square at bottom right corner of panel (A) (surface area of 400 mm^2) serves as a size standard. Primary void spots (PVS) are defined as spots >80 mm^2. Data are shown as Box and Whiskers, whiskers are from Min to Max, Student's t-test is used to compare between wild type and knock out animals, and * indicates P<0.05.



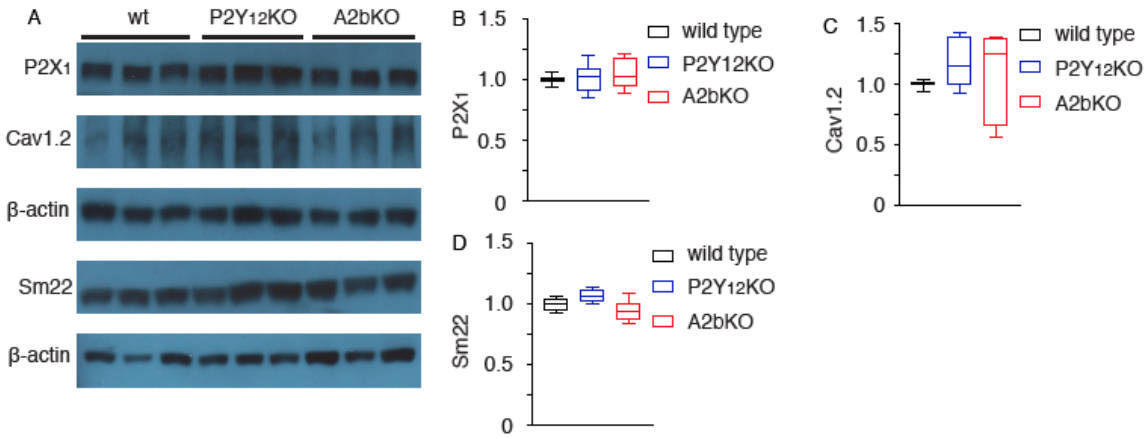
Supplemental figure 3. An enlarged view of Ki67 staining and nuclei co-localization. (A) Ki67 immuno-staining which is co-localized with nuclei (B). (C) merged image. White arrows indicate merged signaling.



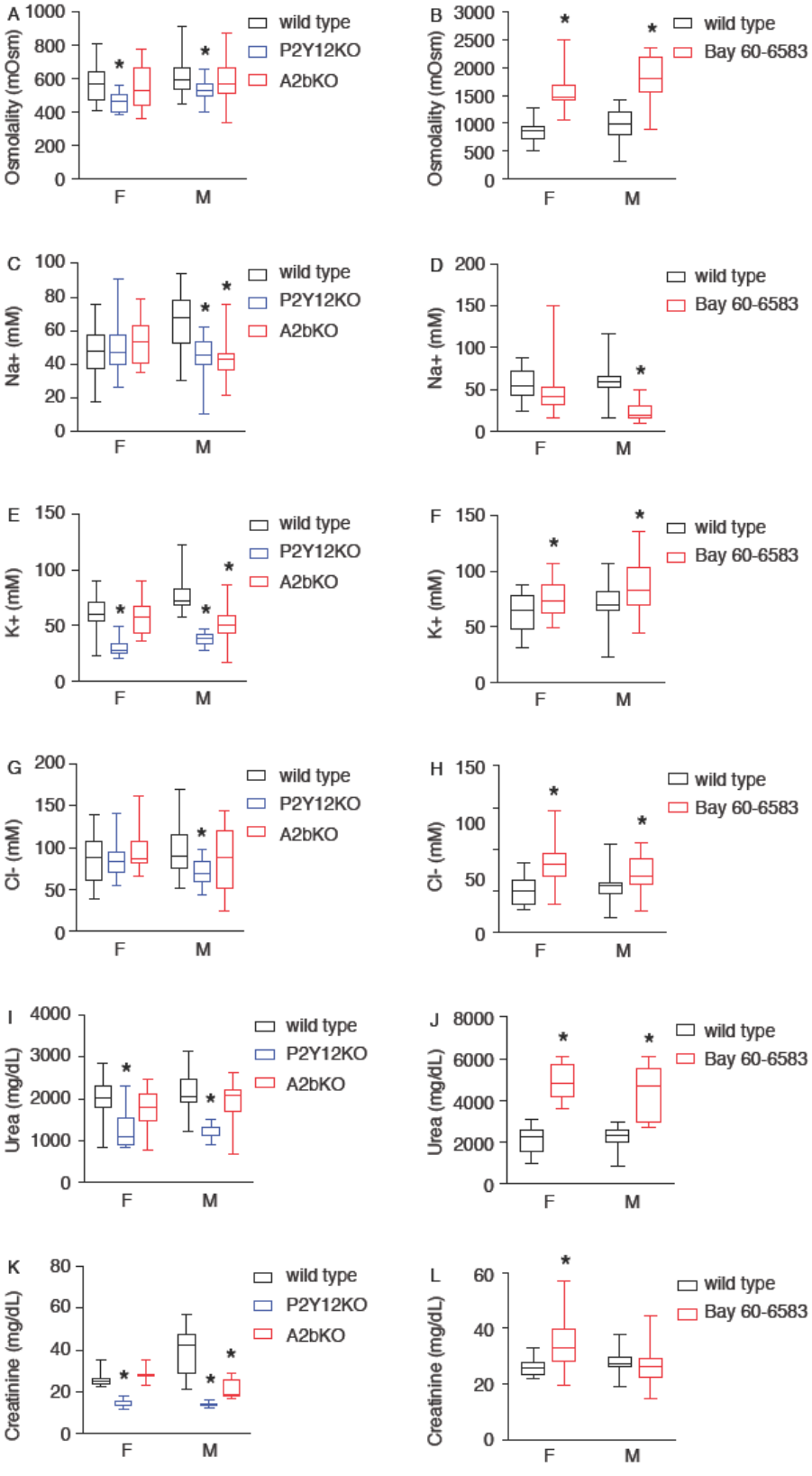
Supplemental figure 4. Quantitative RT-PCR of mRNA from wild type, $P2Y_{12}KO$, and A2bKO mice bladders. n=3. Data are shown as Box and Whiskers, whiskers are from Min to Max, Student's t-test is used to compare between wild type and knock out animals, and * indicates $P<0.05$.



Supplemental figure 5. (A) Western blots of proteins from wild type, $P2Y_{12}KO$, and A2bKO mice bladders. n=6. (B-D) Densitometric quantitation with normalization to β-actin. Data are shown as Box and Whiskers, whiskers are from Min to Max, Student's t-test is used to compare between wild type and knock out animals, and * indicates P<0.05.



Supplemental figure 6. Urine osmolality and chemistry was determined in both male (M below X-axis) and female (F below X-axis) mice (A, C, E, G, I, K), including wild type control: F=36, M=33; $P2Y_{12}KO$: F=17, M=19; and A2bKO: F=15, M=16). Effect of A2b agonist Bay 60-6583 (F=36, M=24) on urine osmolality and chemistry was also evaluated in comparison to wild type mice with vehicle (10 µl DMSO) treatment (B, D, F, H, J, L). Data are shown as Box and Whiskers, whiskers are from Min to Max, Student's t-test is used to compare between wild type and knock out animals, and * indicates P<0.05.


A
Osmolality (mOsm)
1000
800
600
400
200
0
F
M
wild type
P2Y12KO
A2bKO
B
Osmolality (mOsm)
3000
2500
2000
1500
1000
500
0
F
M
wild type
Bay 60-6583
C
Na+ (mM)
100
80
60
40
20
0
F
M
wild type
P2Y12KO
A2bKO
D
Na+ (mM)
200
150
100
50
0
F
M
wild type
Bay 60-6583
E
K+ (mM)
150
100
50
0
F
M
wild type
P2Y12KO
A2bKO
F
K+ (mM)
150
100
50
0
F
M
wild type
Bay 60-6583
G
Cl- (mM)
200
150
100
50
0
F
M
wild type
P2Y12KO
A2bKO
H
Cl- (mM)
150
100
50
0
F
M
wild type
Bay 60-6583
I
Urea (mg/dL)
4000
3000
2000
1000
0
F
M
wild type
P2Y12KO
A2bKO
J
Urea (mg/dL)
8000
6000
4000
2000
0
F
M
wild type
Bay 60-6583
K
Creatinine (mg/dL)
80
60
40
20
0
F
M
wild type
P2Y12KO
A2bKO
L
Creatinine (mg/dL)
60
40
20
0
F
M
wild type
Bay 60-6583

Supplemental table 1, Quantitative RT-PCR for Hippo pathway genes. Data shown is mean ± SD, Student's t-test is used to compare between wild type and knock out animals, and * indicates P<0.05.

Genes	Wild type (n=5)		$P2Y_{12}$KO (n=6)		A2bKO (n=5)	
	Mean	SD	Mean	SD	Mean	SD
MST1	1.00	0.03	1.14	0.31	1.03	0.16
MST2	1.00	0.02	1.29*	0.31	1.14*	0.14
YAP	1.00	0.03	1.05	0.17	1.02	0.10
TAZ	1.00	0.05	1.28*	0.23	0.98	0.13
LAST1	1.00	0.03	1.13	0.24	1.00	0.16
LAST2	1.00	0.04	1.16	0.36	1.03	0.36
TEAD1	1.00	0.06	1.07	0.35	1.05	0.19
TEAD2	1.00	0.05	0.98	0.15	0.92	0.12
TEAD3	1.00	0.02	0.94	0.25	1.02	0.05

Supplemental materials:

1. Primers used for genotyping:

A2b

CTCACACAGAGCTCCATCT	A2b wt F genotyping	
TCTGGCAGCAGCTTTGAT	A2b wt R genotyping	485bp
CAGCCTCTGTTCCACATACACT	A2b-/- F genotyping	
GGCACCTCTCCCTCCAAGACAC	A2b-/- R genotyping	900bp

$P2Y_{12}$

ACGCGTCACCTTAATATGCG	$P2Y_{12}$ genotyping
GGCACTCTAGTGATGCTTTGCCTA	$P2Y_{12}$ genotyping
GGCTGCCTTGAGAAATATCAAGT	$P2Y_{12}$ genotyping

WT 510 bp

Mut 650 bp

2. Primers used for regular RT-PCR of mRNA of $P2Y_{12}$, A2b, and positive control gene β-actin

$P2Y_{12}$ 404 bp

GAGACACTCATATCCTTCAGATTC

CCCAGGAACGATATACTGATATAC

A2b 466 bp

TCTATTCAGCTGCTCTTACTGTGT

ATTTTCACACTTTAGGTCCCAATA

Actin 147 bp
ACC TTC TAC AAT GAG CTG CG
CTG GAT GGC TAC GTA CAT GG

3. Primers used for quantitative RT-PCR

$P2X_1$
GGT GTT TGG GAT TCG CTT TG
GCA GGA TGT GGA GCA ATA AGA G

$P2Y_{12}$
GCT TTG TTC CCT TCC ACT TTG
GTG CTC TCC TTC ACG TAG AAC

A2b
GTCCCGCTCAGGTATAAAGGT
CAGTTCTGTGCAGTTGCTGG

c-Jun
GTT GAA AGC TGT ATG AAG TGG C
TCA AAG TTG GAA GGA GAC ACC

c-Fos
TCC AGT CCT CAC CTC TTC C
AAG AGA AAA GAG ACA CAG ACC AG

Cav1.2
CTT GAA ATC CAC CTA CCA GAC C
CCT CAC TGT TTC TCT GTC ATC TG

Mst1
GGATTTTGTGAAGCAGTGTCTG
CGCTTCAGTTTCACATCCATG

Mst2
CAGCACCATGTTAGAGTCGG
TGTACTTGAGGTGACGTTGC
Lats1
TGCGAACAGGATATACACAGC
GTGAAGAGAAGTTTGCCAGTTG

Lats2
GACACCAAATTACATCGCTCC
TCAAAGAGAATCACACCGACG

Taz
ATGAGTTCTGAGTTCCTGCG
GAAGTAGGGTGGGCTGTTAG

Yap
ACCAATAGTTCCGATCCCTTTC
TGTCTCCTGTATCCATTTCATCC

Tead1
GAATAAACCGCTCGCCAATG
GAGTTTCTTGTGTATCCCTGTTTG

Tead2
CCTGGTTAATTTCCTGCACAAG
GTGGAGACTTCAAAGACGTAGG

Tead3
TGGAGTATTCTGCCTTCATGG
GGAACTTGTCGTAGATCTGTCG

SM22α
ATGTTCCAGACTGTTGACCTC
GTGAAGTCCCTCTTATGCTCC

SDHA
CTT GAA TGA GGC TGA CTG TG
ATC ACA TAA GCT GGT CCT GT

4. Antibodies used for western blots.

Antibodies used for western blots: purified polyclonal rabbit anti-$P2X_1$ antibody (Catalogue #: APR-001, alomone Labs); purified monoclonal rabbit anti-c-Fos (Catalogue #: 2250), purified monoclonal rabbit anti-c-Jun (Catalogue #: 9165), purified monoclonal rabbit anti-α-Sm22 (Catalogue #: 19245), and purified polyclonal rabbit anti-β-actin (Catalogue #: 4967) are from Cell Signaling Technology; purified polyclonal rabbit anti-Cav1.2 antibody (Catalogue #: AB5156, Millipore); and Immun-Star goat anti-rabbit (GAR)-HRP conjugate

(Catalogue#: 1705046, Bio-Rad). Antibodies are chosen based on our or other reported references which were verified by knockouts or artificial expression systems.

5. Measurement of urine osmolality and chemistry.

Individual mice were placed in a clean standard AN75 mouse cage without bedding materials, and spontaneously voided urine was collected immediately using a 200 µl pipette for 2 h. Mice were given standard chow and water for the duration of the collection. Urine osmolality was measured by freezing point osmometry (Osmette A, Precision Systems, Natick, MA) according to the manufacturer's instructions. Urine chemistry was determined by an Electrolyte Analyzer 16+ (Nova biomedical, Waltham, MA) according to the manufacturer's instructions.