SUPPLEMENTAL MATERIAL

Pharmacological Npt2a inhibition causes phosphaturia and reduces plasma phosphate in mice with normal and reduced kidney function

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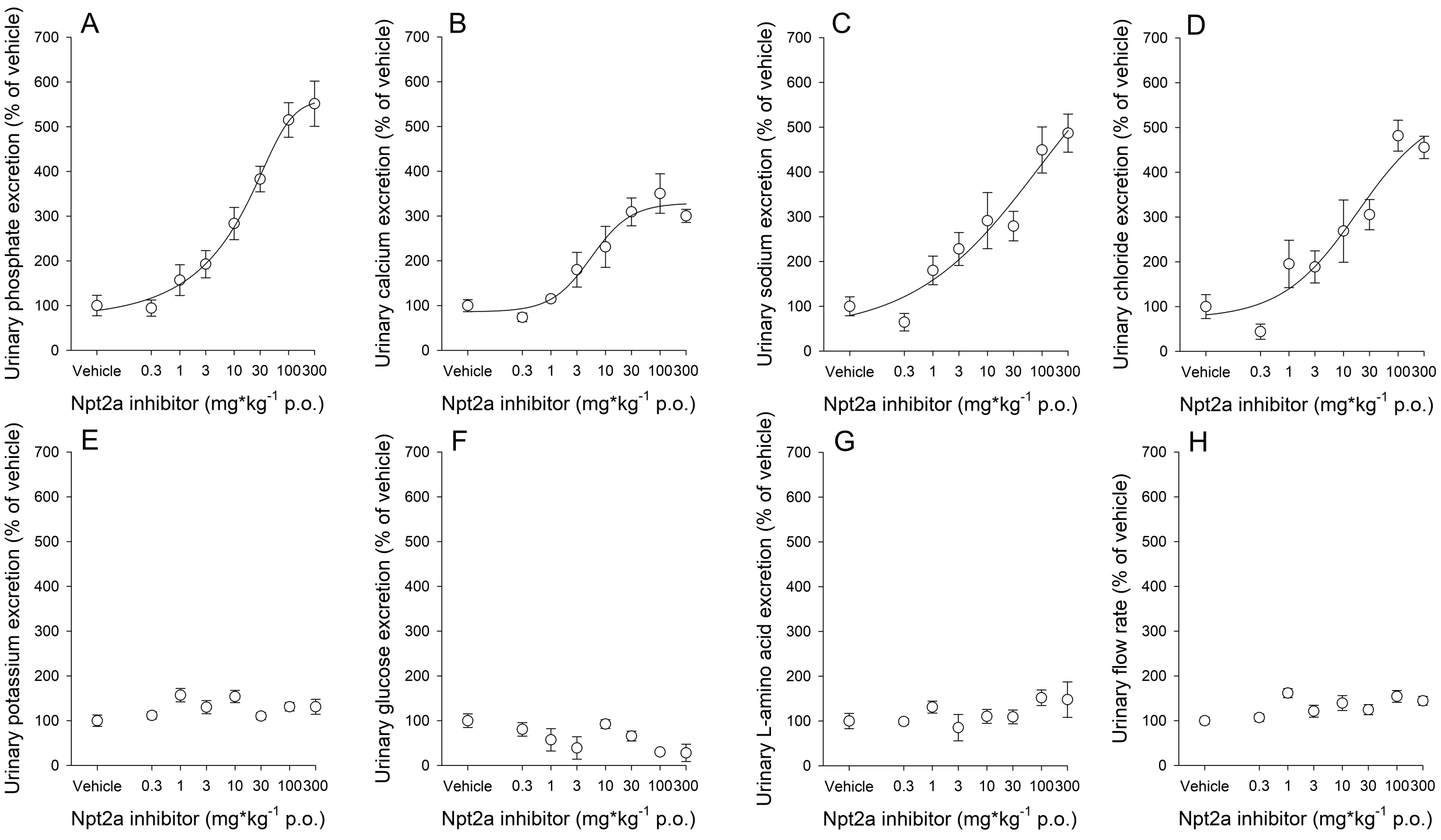
Supplemental Figure 1. Percent change in urinary parameters in response to Npt2a inhibition. Response to acute oral (p.o.) application of Npt2a-I (0.3-300 mg*kg^{-1} via oral gavage) or vehicle in short-term (3 hour) metabolic cage experiments. (A) Phosphate, (B) calcium, (C) sodium, (D) chloride, (E) potassium, (F) glucose, (G) amino acids and (H) urinary flow rate. Data are expressed as mean±S.E.M, *n*=6-14/0.3-100 mg*kg^{-1}, *n*=3 for 300 mg*kg^{-1}.

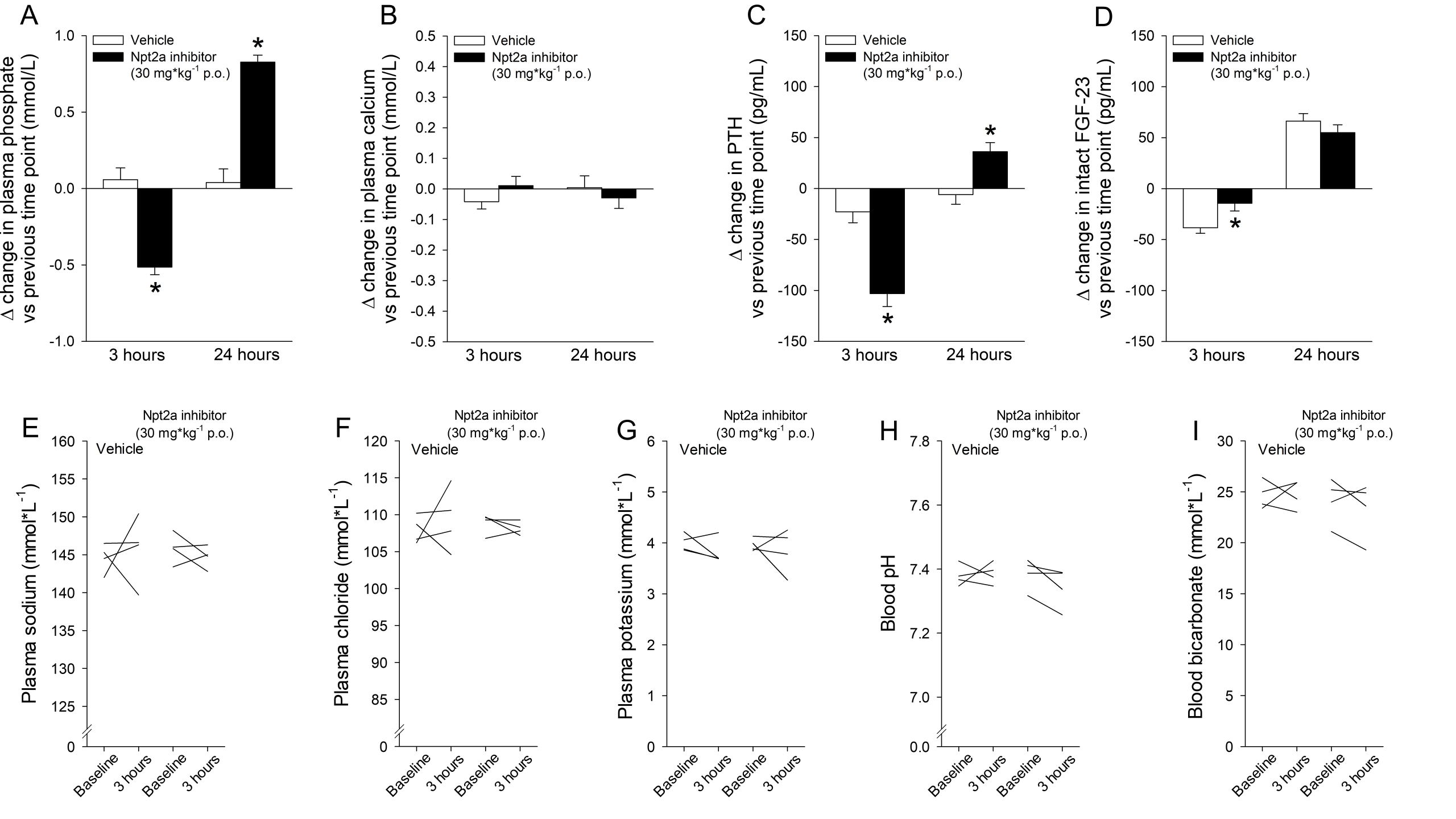
Supplemental Figure 2. Change in plasma parameters in response to Npt2a inhibition. Response to acute oral (p.o.) application of Npt2a-I (30 mg*kg^{-1} via oral gavage). (A) Phosphate, (B) calcium, (C) PTH, (D) FGF-23, (E) sodium, (F) chloride, (G) potassium, (H) blood pH and (i) blood bicarbonate. Data were analyzed by 2-way ANOVA followed by Tukey's multiple comparisons test and are expressed as mean±S.E.M. *n*=6-18 (A-D), n=4 (E-I). *P<0.05 versus vehicle.

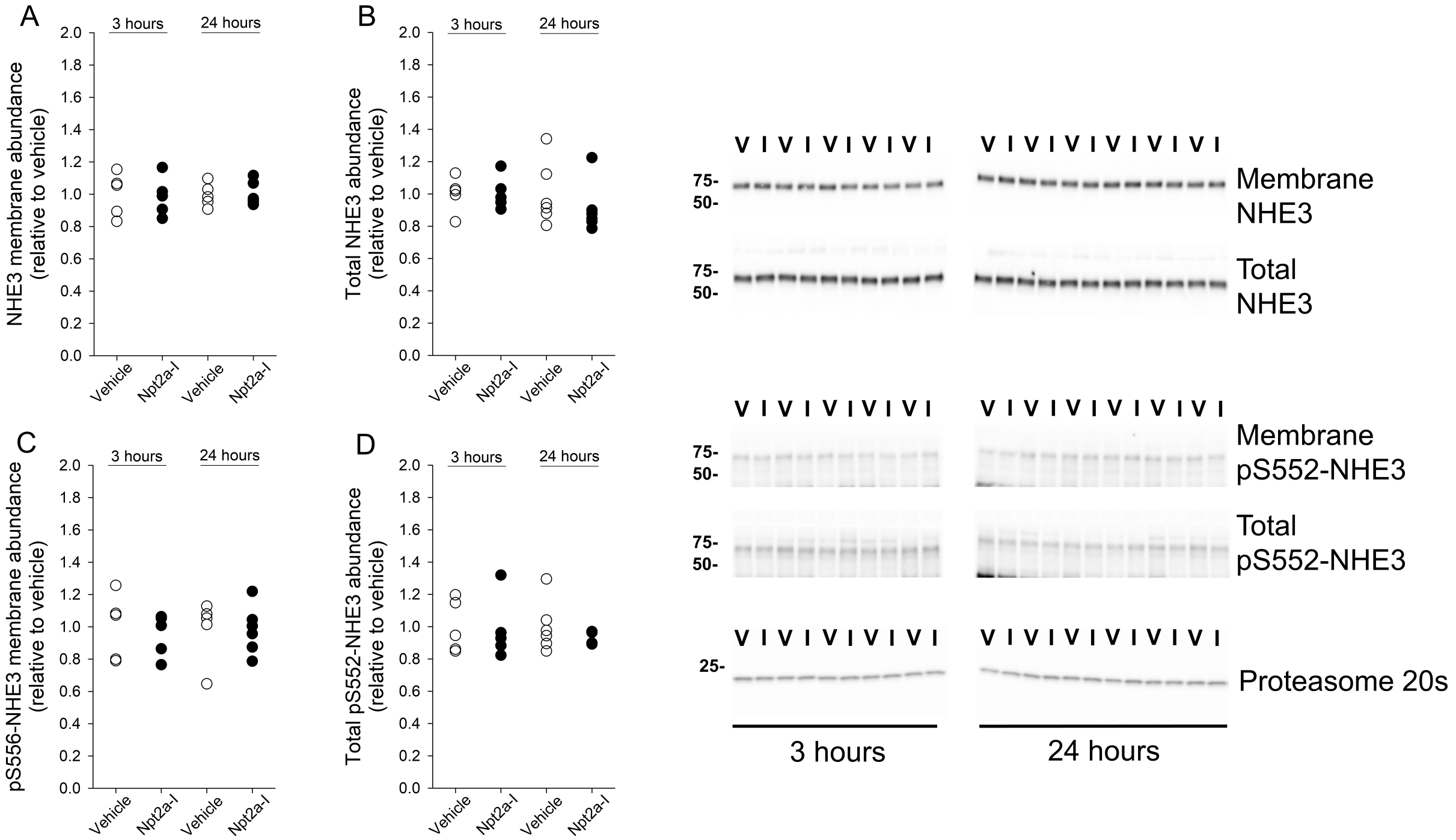
Supplemental Figure 3. Npt2a-inhibition does not affect NHE3 and pS552-NHE3. Western blot analysis of NHE3 and pS552-NHE3 abundances in membrane fractions and total homogenates of kidneys from vehicle (V) or Npt2a inhibitor (I) treated mice (30 mg*kg-1). NHE3 membrane and total abundances were not significantly affected 3 hours (A and B) or 24 hours (C and D) after Npt2a inhibitor treatment. Data were analyzed by unpaired Student t test and are expressed as mean±S.E.M, *n*=5-6. **P*<0.05 versus vehicle.

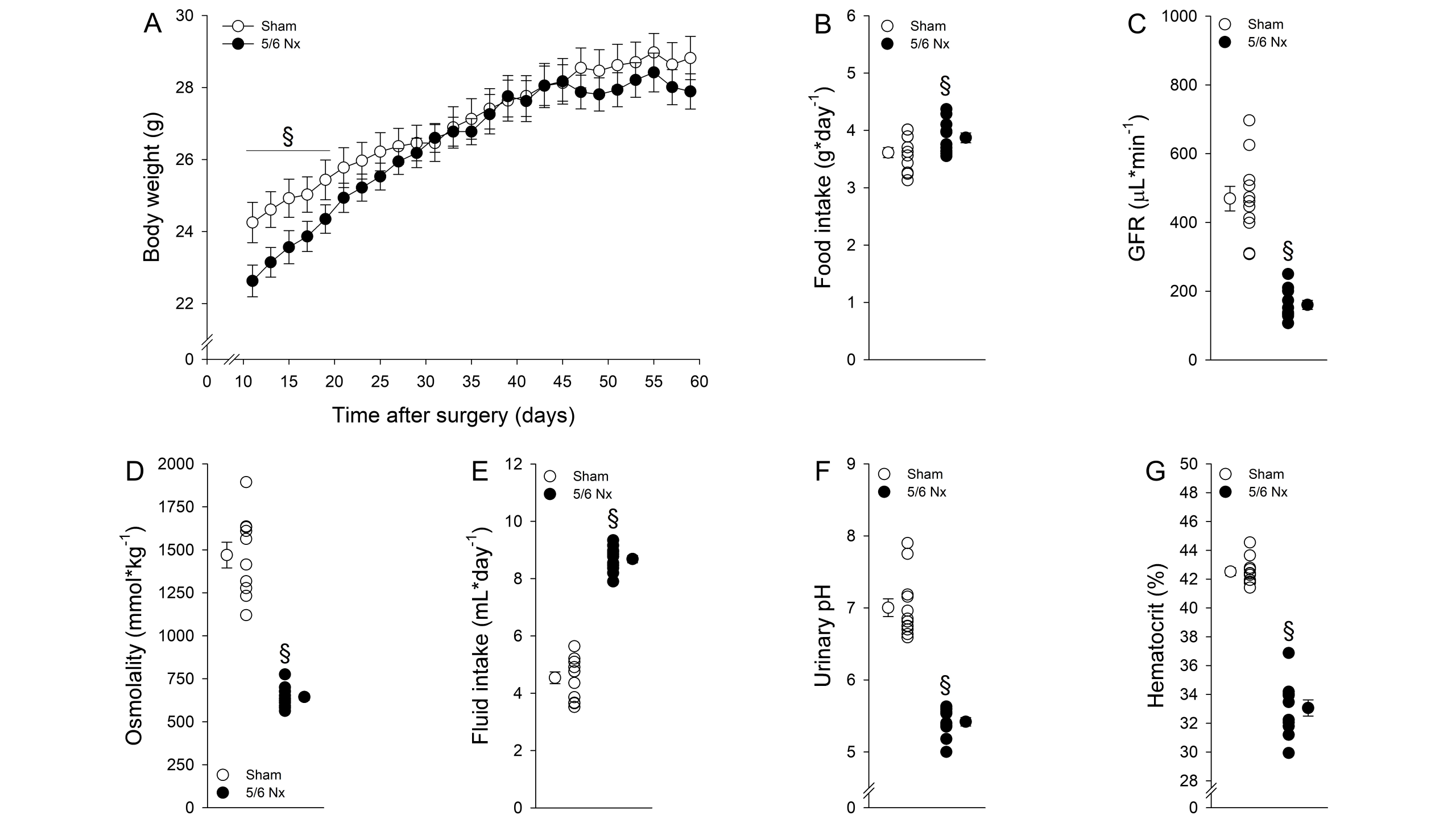
Supplemental Figure 4. Pathophysiological consequences of 5/6 nephrectomy (Nx). Effects on body weight (A), food intake (B), glomerular filtration rate (GFR, C), osmolality (D), fluid intake (E), urinary pH (F) and hematocrit (G). Data were analyzed by repeated measures 2-way ANOVA (A) and 2-way ANOVA (B-G) followed by Tukey's multiple comparisons test and are expressed as mean±S.E.M. *n*=11-12. §*P*<0.05 versus Sham.

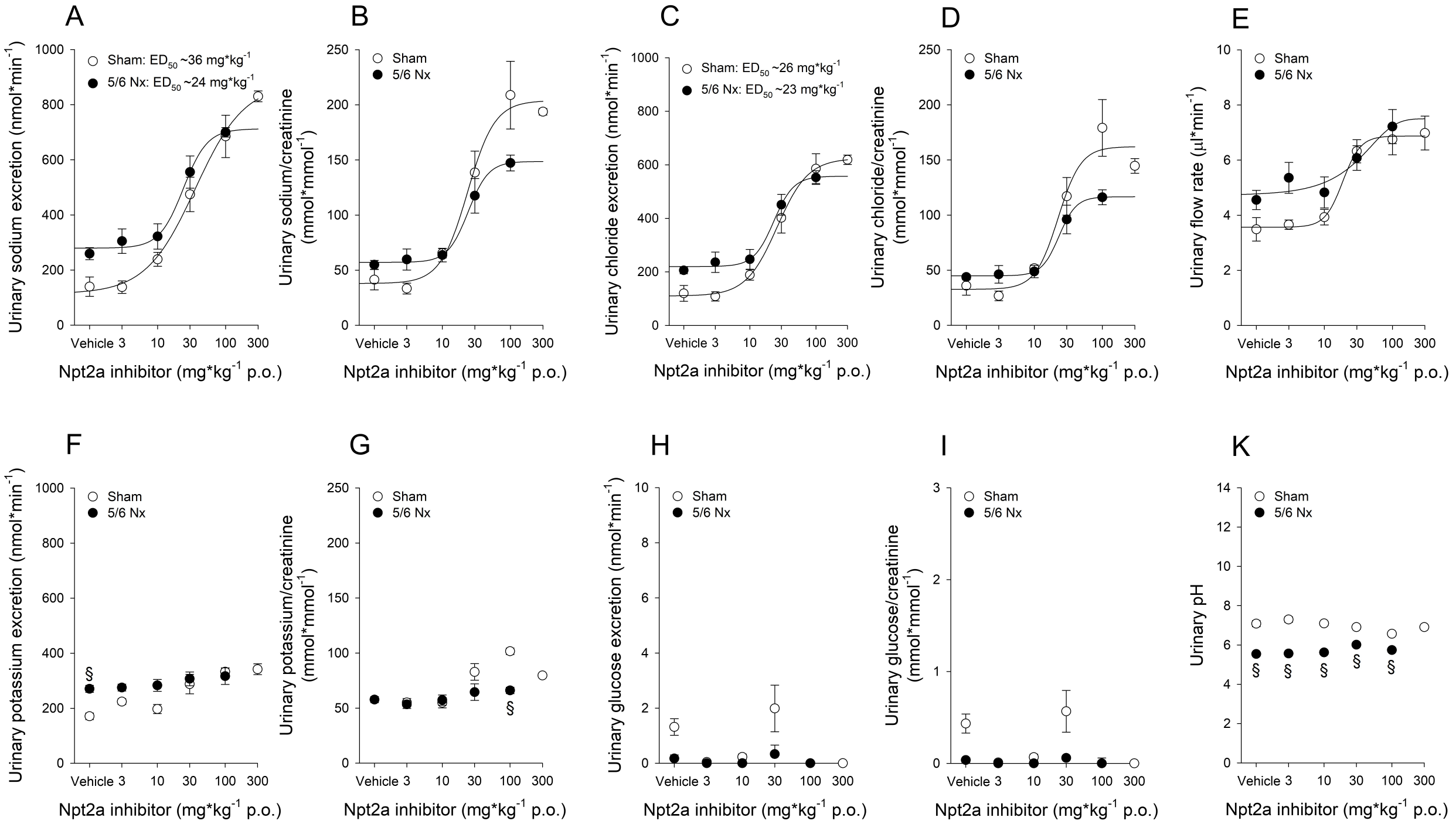
Supplemental Figure 5. Comparison of Npt2a inhibitor dose-response effects between Sham and 5/6 nephrectomy (Nx) mice. Response to acute oral (p.o.) application of Npt2a-I (0.3-300 mg*kg^{-1} via oral gavage) or vehicle in short-term (3 hour) metabolic cage experiments. (A) Sodium, (B) sodium/creatinine, (C) chloride, (D) chloride/creatinine, (E) urinary flow rate, (F) potassium, (G) potassium/creatinine, (H) urinary flow rate, (I) glucose, (J) glucose/creatinine and (K) urinary pH. The dose of 300 mg*kg^{-1} in 5/6 Nx mice was not included in the analysis because mice appeared lethargic after application. Data were analyzed by one-way ANOVA followed by Newman-Keuls multiple comparisons test and are expressed as mean±S.E.M. *n*=6-14/0.3-100 mg*kg^{-1}, *n*=4 for 300 mg*kg^{-1}. §*P*<0.05 versus Sham.


A
Urinary phosphate excretion (% of vehicle)
B
Urinary calcium excretion (% of vehicle)
C
Urinary sodium excretion (% of vehicle)
D
Urinary chloride excretion (% of vehicle)
E
Urinary potassium excretion (% of vehicle)
F
Urinary glucose excretion (% of vehicle)
G
Urinary L-amino acid excretion (% of vehicle)
H
Urinary flow rate (% of vehicle)
0
100
200
300
400
500
600
700
Vehicle
0.3
1
3
10
30
100
300
Npt2a inhibitor (mg*kg-1 p.o.)


A
Δ change in plasma phosphate vs previous time point (mmol/L)
Vehicle
Npt2a inhibitor (30 mg*kg-1 p.o.)
3 hours
24 hours
B
Δ change in plasma calcium vs previous time point (mmol/L)
C
Δ change in PTH vs previous time point (pg/mL)
D
Δ change in intact FGF-23 vs previous time point (pg/mL)
E
Plasma sodium (mmol*L-1)
F
Plasma chloride (mmol*L-1)
G
Plasma potassium (mmol*L-1)
H
Blood pH
I
Blood bicarbonate (mmol*L-1)
Baseline
3 hours


A
3 hours
24 hours
NHE3 membrane abundance (relative to vehicle)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Vehicle
Npt2a-I
Vehicle
Npt2a-I
B
3 hours
24 hours
Total NHE3 abundance (relative to vehicle)
Vehicle
Npt2a-I
Vehicle
Npt2a-I
C
3 hours
24 hours
pS556-NHE3 membrane abundance (relative to vehicle)
Vehicle
Npt2a-I
Vehicle
Npt2a-I
D
3 hours
24 hours
Total pS552-NHE3 abundance (relative to vehicle)
Vehicle
Npt2a-I
Vehicle
Npt2a-I
V I V I V I V I V I
V I V I V I V I V I
75-
50-
Membrane NHE3
75-
50-
Total NHE3
V I V I V I V I V I
V I V I V I V I V I
75-
50-
Membrane pS552-NHE3
75-
50-
Total pS552-NHE3
V I V I V I V I V I
V I V I V I V I V I
25-
Proteasome 20s
3 hours
24 hours


A
Sham
5/6 Nx
Body weight (g)
Time after surgery (days)
§
B
Food intake (g*day-1)
C
GFR (µL*min-1)
D
Osmolality (mmol*kg-1)
E
Fluid intake (mL*day-1)
F
Urinary pH
G
Hematocrit (%)


A
Sham: ED50 ~36 mg*kg-1
5/6 Nx: ED50 ~24 mg*kg-1
Urinary sodium excretion (nmol*min-1)
Npt2a inhibitor (mg*kg-1 p.o.)
B
Sham
5/6 Nx
Urinary sodium/creatinine (mmol*mmol-1)
C
Sham: ED50 ~26 mg*kg-1
5/6 Nx: ED50 ~23 mg*kg-1
Urinary chloride excretion (nmol*min-1)
D
Urinary chloride/creatinine (mmol*mmol-1)
E
Urinary flow rate (µl*min-1)
F
Urinary potassium excretion (nmol*min-1)
G
Urinary potassium/creatinine (mmol*mmol-1)
H
Urinary glucose excretion (nmol*min-1)
I
Urinary glucose/creatinine (mmol*mmol-1)
K
Urinary pH
Vehicle 3 10 30 100 300
§