SUPPLEMENTARY MATERIAL

Figure S1. Na^+-dependence and cation specificity of hCNT1-mediated uridine transport. Oocytes were injected with 10 nl of water without (control) or with 10 ng of RNA transcripts encoding hCNT1. The expression vector was pGEM-T. **A.** Inward current generated by perfusing an RNA-injected oocyte with 100 μM uridine in Na^+-containing transport medium (100 mM NaCl; pH 7.5). **B.** The same oocyte perfused with 100 μM uridine in transport medium in which Na^+ was replaced by choline (100 mM ChCl; pH 7.5). **C** and **D** show the same experiment described in **A** and **B**, but with a control water-injected oocyte. No inward currents were generated. **E.** Current was measured in an RNA-injected oocyte in medium in which Na^+ was replaced by choline (100 mM ChCl) at pH 6.5. **F.** The same oocyte perfused with 100 μM uridine in transport medium in which Na^+ was replaced by choline (100 mM ChCl) at pH 5.5.

Figure S2. hCNT1 steady-state current/voltage relationship. A. Time courses of transmembrane currents measured in the presence of 100 μM external uridine in Na^+-containing transport medium recorded in 10 mV increments from a holding potential (V_h) of -50 mV to potentials ranging between -90 and +60 mV (*left trace*). Current responses are shown only at V_t= -90, -50, +10 and +60 mV and the capacitive transients have been truncated to more clearly demonstrate the steady-state currents. Currents from the same oocyte were also recorded in the absence of uridine (*right trace*). **B.** Differences in membrane currents with and without uridine are shown at V_t= -90, -50, +10 and +60 mV. **C.** The current/voltage (I/V) curve was generated by subtraction of steady-state currents in the presence and absence of uridine. The current produced by uridine at each potential (± SEM) was averaged from 4 - 5 different oocytes. The expression vector was pGEM-T.

Figure S3. hCNT1 competition studies. A. The current produced by perfusing an hCNT1-producing oocyte with 100 μM uridine (*left trace*) in Na^+-containing transport medium was compared to the current generated when the same oocyte was simultaneously perfused with 100 μM uridine plus 100 μM thymidine (*right trace*). **B.** The same experiment was performed, but with 100 μM uridine plus 100 μM adenosine added to the transport medium. **C.** The nucleosides guanosine and uridine (each at a concentration of 100 μM) were added simultaneously to the oocyte. The expression vector was pGEM-T.

Figure S4. Transport of zidovudine by hCNT1. Oocytes were injected with 10 nl of water without (control) or with 10 ng of RNA transcripts encoding hCNT1. The expression vector was pGEM-HE. Current responses generated by 100 μM or 1 mM zidovudine in Na^+-containing transport medium or 1 mM zidovudine in choline-containing medium are shown in **B**, **C** and **D**, respectively. The current produced by 100 μM uridine in Na^+-containing medium is given for comparison in **A**. Zidovudine (1 mM) was also added to a control water-injected oocyte (**E** and **F**).

Figure S5. Inhibition of hCNT1-mediated uridine influx by β-DFP-5M. The uptake of 10 μM ^{14}C-uridine in oocytes producing hCNT1 was measured either in the absence or presence of various concentrations of β-DFP-5M (0 - 10 mM) in Na^+-containing transport medium under standard initial rate conditions (1 min flux, 20 °C). Mediated transport was calculated as uptake in RNA-injected oocytes *minus* uptake in water-injected oocytes. Each value is the mean ± SEM of 10 - 12 oocytes and is expressed as a percentage of influx in the absence of β-DFP-5M. The expression vector was pGEM-HE.

Figure S6. Inhibition of hCNT1-mediated Na^+ currents by phloridzin. A. Inward current induced in an hCNT1-producing oocyte by 100 μM uridine in Na^+-containing transport medium (*left current trace*) or after a 10 min incubation with 10 mM phloridzin (*right current trace*). **B.** Inward current induced in hCNT1-producing oocytes in response to 100 μM uridine was measured in Na^+-containing transport medium before and after incubation with various concentrations of phloridzin (0 - 5 mM). The current produced after incubation with phloridzin was expressed as a percentage of the current produced in the same oocyte before incubation with inhibitor. Values are means (± SEM) of 5 - 7 different oocytes. **C.** Oocytes producing hCNT1 were incubated either in the absence or presence of various concentrations of phloridzin (0 - 5 mM) in Na^+-containing medium, and influx of 10 μM ^{14}C-uridine was then measured under standard initial rate conditions (1 min flux, 20 °C). Mediated transport was calculated as uptake in RNA-injected oocytes *minus* uptake in water-injected oocytes. Each value is the mean ± SEM of 10 - 12 oocytes and is expressed as a percentage of uptake in the absence of phloridzin. The expression vector was pGEM-HE.

Figure S7. Nucleoside analog hCNT1 steady-state kinetics. hCNT1-mediated currents for 2'-deoxyuridine (**A**), 5-fluoro-2'-deoxyuridine (**B**), 5'-fluorouridine (**C**) and zidovudine (**D**) were measured in Na^+-containing transport medium. Values are mean ± SEM of 5 - 6 different oocytes. The expression vector was pGEM-HE.

Figure S8. hCNT1 presteady-state currents elicited by voltage pulses. A. Voltage pulse protocol: the oocyte membrane was held at a holding potential (V_h) of -50 mV and stepped to a range of test potentials (V_t). Shown are V_t from -170 and +70 mV (20 mV increments). **B.** An hCNT1-producing oocyte in Na^+-containing transport medium. **C.** An hCNT1-producing oocyte in choline-containing medium. **D.** A control water-injected oocyte in Na^+-containing medium. The expression vector was pGEM-HE.

Figure S9. Relationship between hCNT1 ON and OFF charge movements. Correlation between charge movements in an hCNT1-producing oocyte in Na^+-containing transport medium obtained from the time integral of transient currents following command pulses to a range of V_t between -170 and +130 mV (Q_{ON}) and charge movements following return to V_h (-50 mV) (Q_{OFF}). Linear regression analysis of the data gave a slope (± SE) of 0.90 ± 0.03 (*solid line*) compared to a reference slope of unity (*broken line*). The expression vector was pGEM-HE.

Figure S10. Effect of uridine on hCNT1 presteady-state currents. A. Representative presteady-state current recording of an hCNT1-producing oocyte in Na^+-containing transport medium in the presence of 100 μM uridine. **B.** Maximal charge moved (Q_T) as a function of uridine concentration. Charge movements with uridine present are shown as percentages of the charge movement in the absence of permeant (control). Each bar represents the average charge (± SEM) of 5 - 6 different oocytes. The expression vector was pGEM-HE.

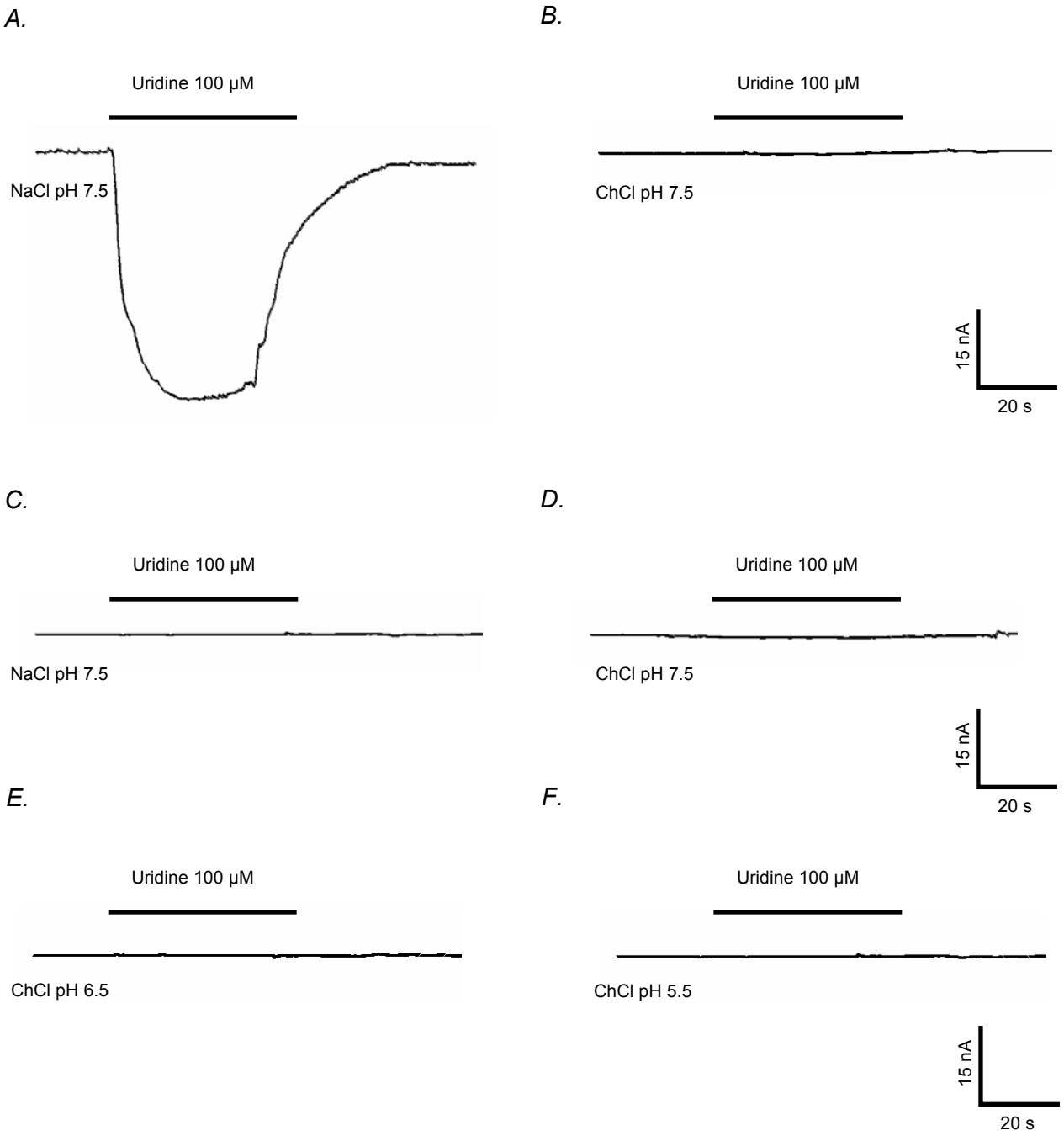
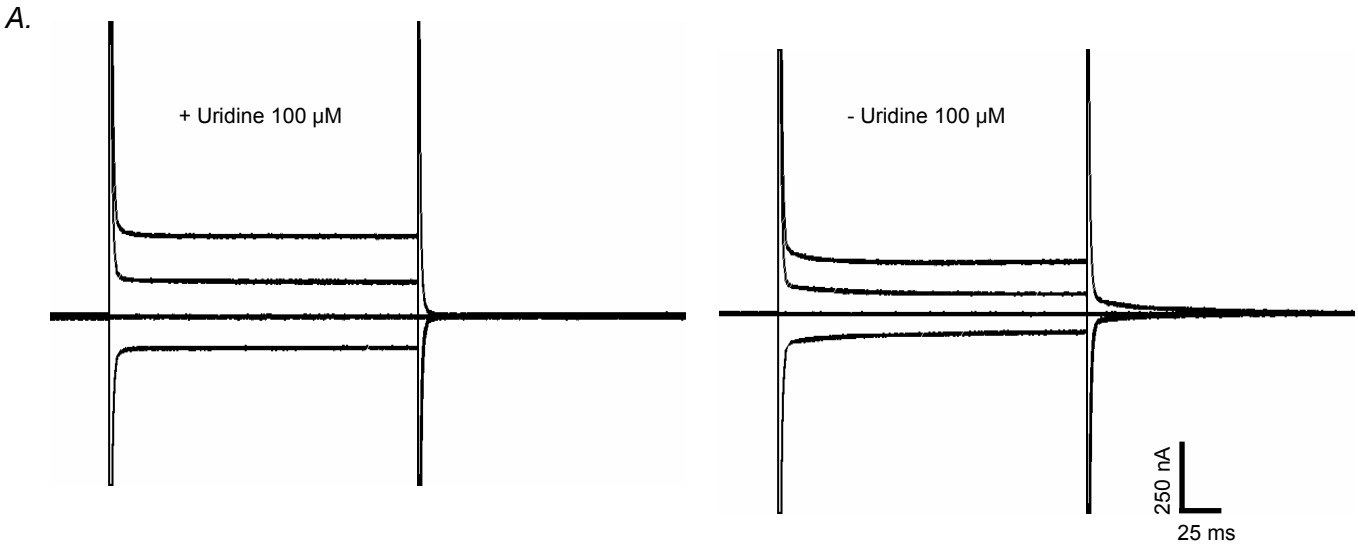
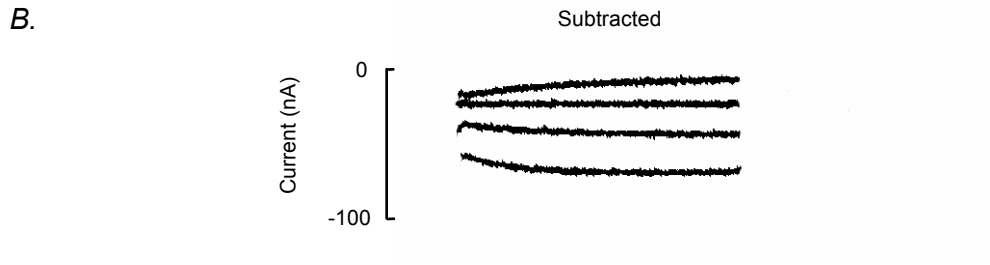

A.
Uridine 100 µM
NaCl pH 7.5
B.
Uridine 100 µM
ChCl pH 7.5
15 nA
20 s
C.
Uridine 100 µM
NaCl pH 7.5
D.
Uridine 100 µM
ChCl pH 7.5
15 nA
20 s
E.
Uridine 100 µM
ChCl pH 6.5
F.
Uridine 100 µM
ChCl pH 5.5
15 nA
20 s

Figure S1


A.
+ Uridine 100 µM
- Uridine 100 µM
250 nA
25 ms


B.
Subtracted
Current (nA)
0
-100

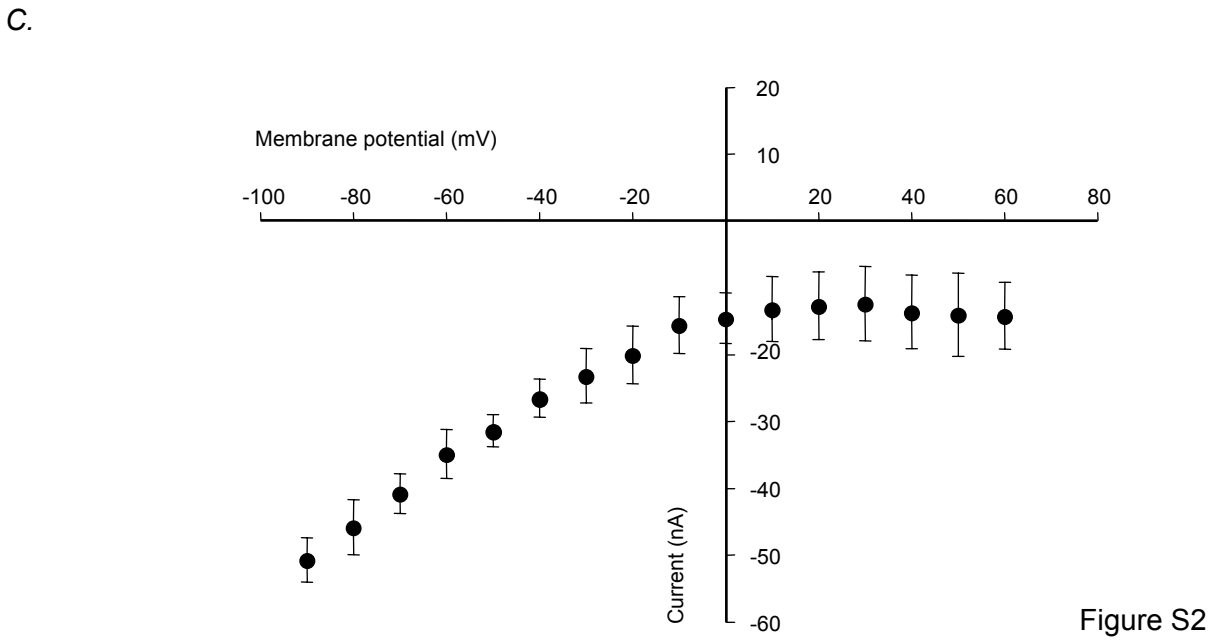
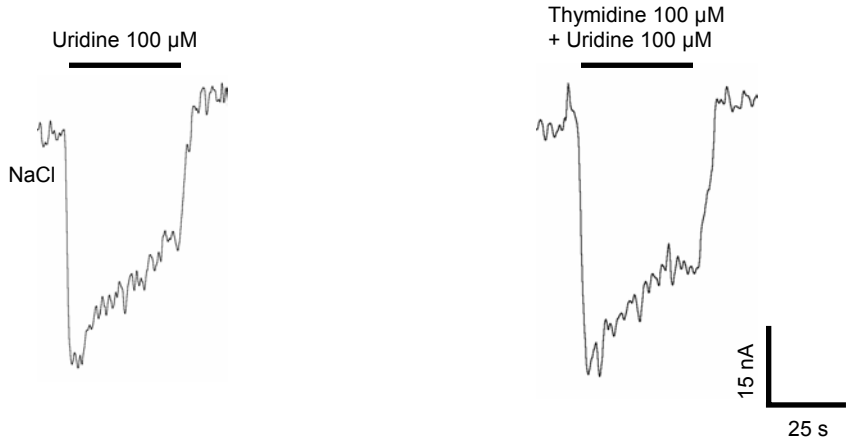
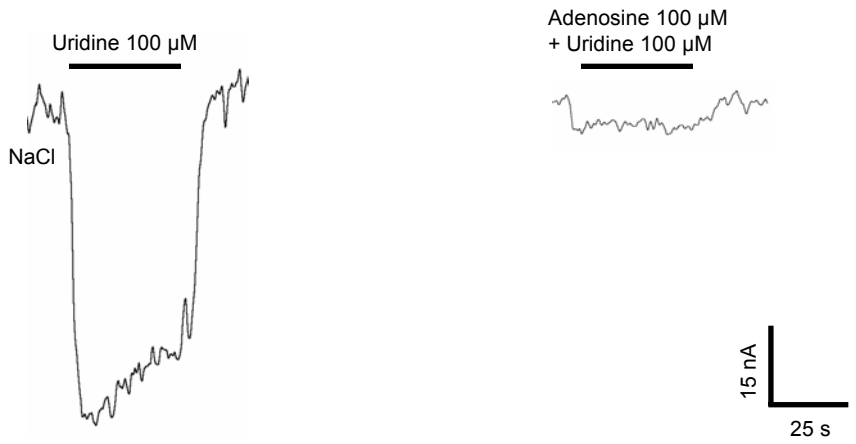

C.
Membrane potential (mV)
20
10
-100
-80
-60
-40
-20
20
40
60
80
-20
-30
-40
-50
-60
Current (nA)

Figure S2

A.



B.



C.

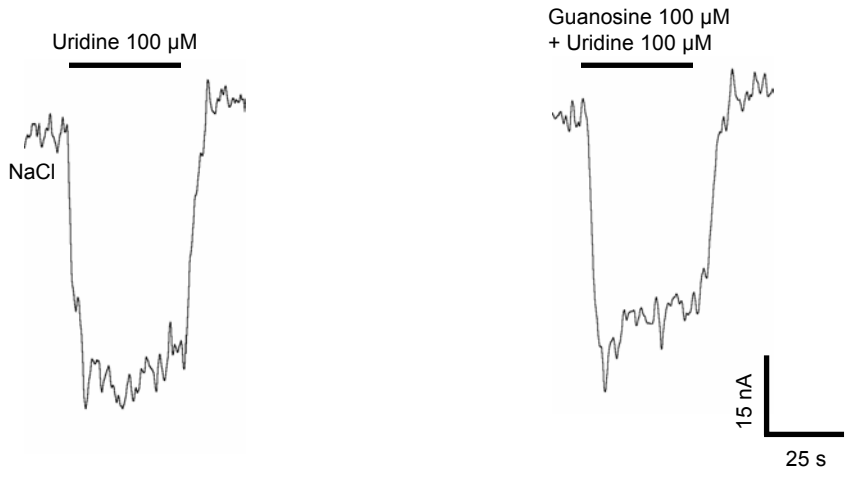


Figure S3

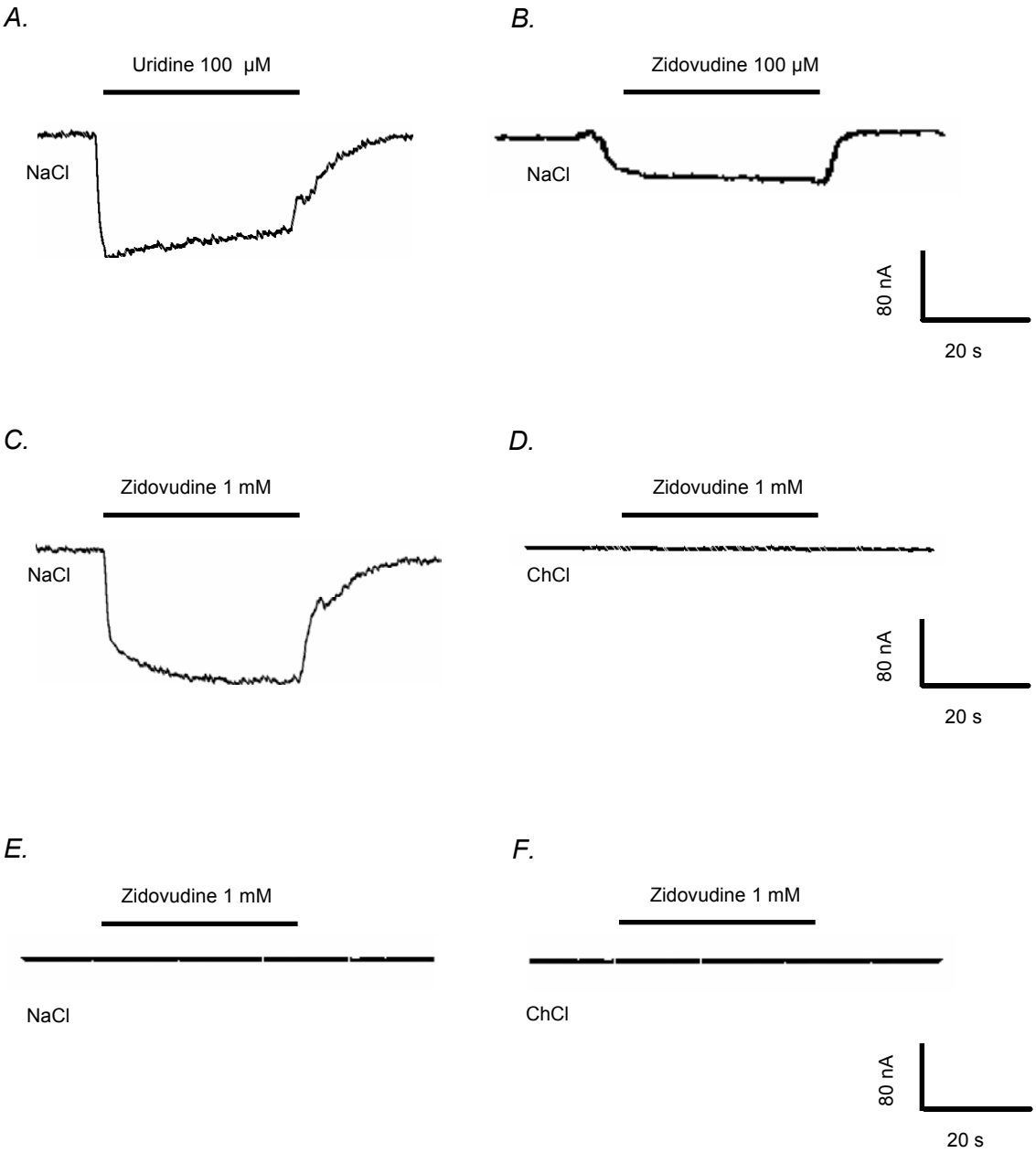

A.
Uridine 100 µM
NaCl
B.
Zidovudine 100 µM
NaCl
80 nA
20 s
C.
Zidovudine 1 mM
NaCl
D.
Zidovudine 1 mM
ChCl
80 nA
20 s
E.
Zidovudine 1 mM
NaCl
F.
Zidovudine 1 mM
ChCl
80 nA
20 s

Figure S4

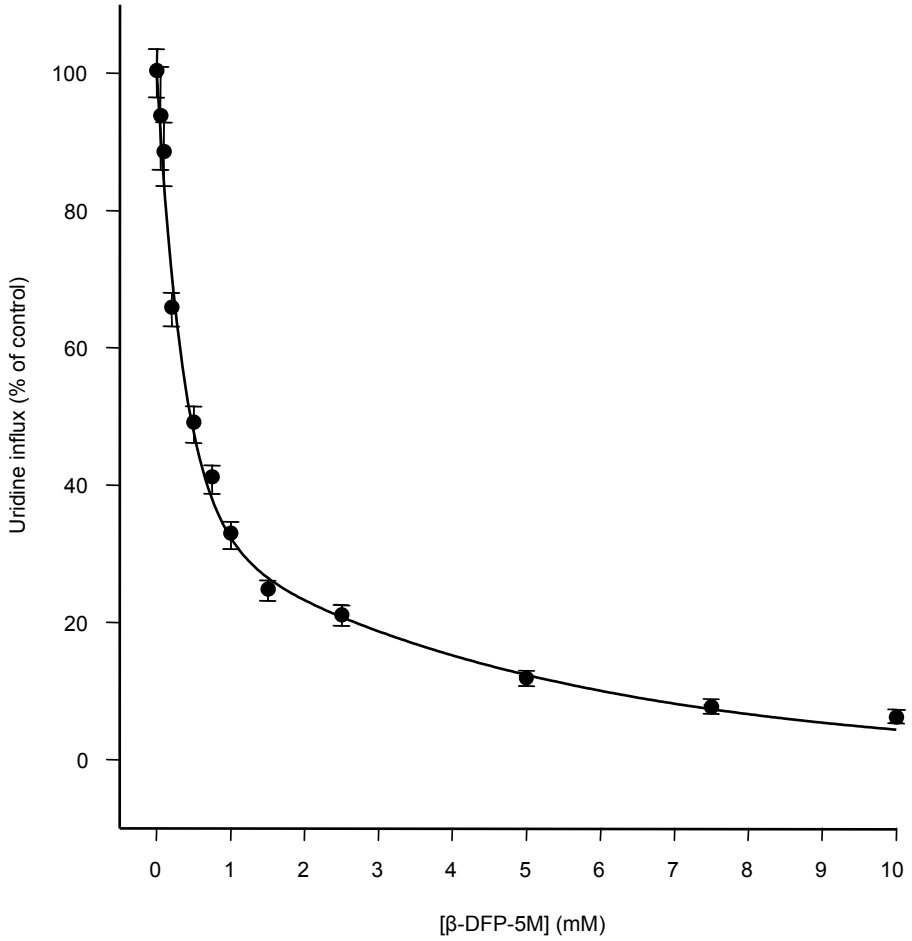
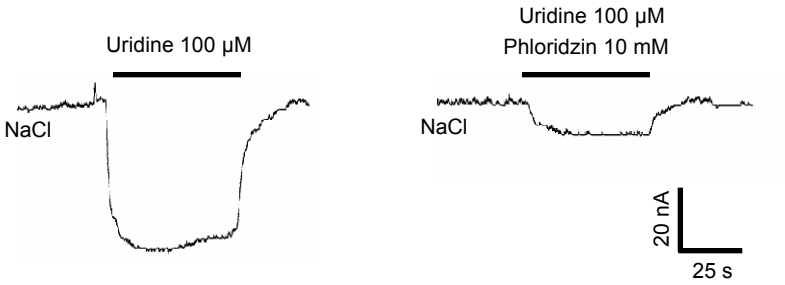
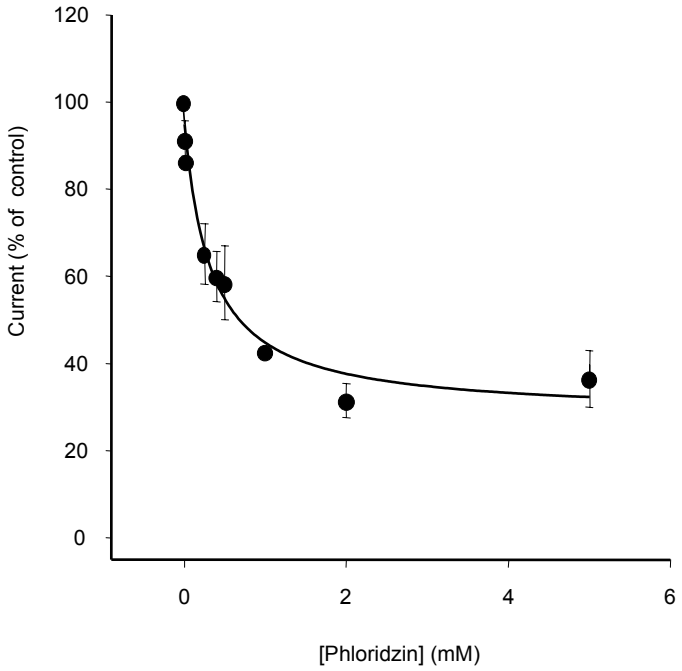

Uridine influx (% of control)
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
9
10
[β-DFP-5M] (mM)

Figure S5

A.



B.



C.

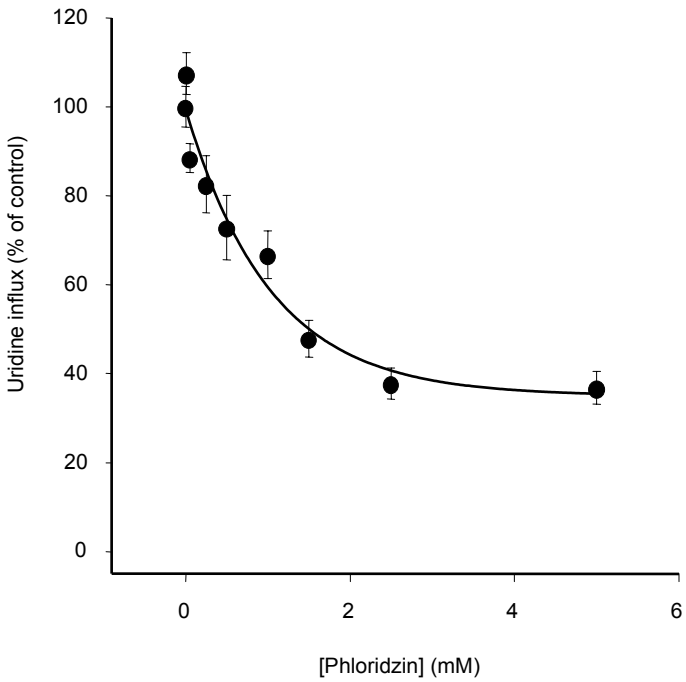


Figure S6

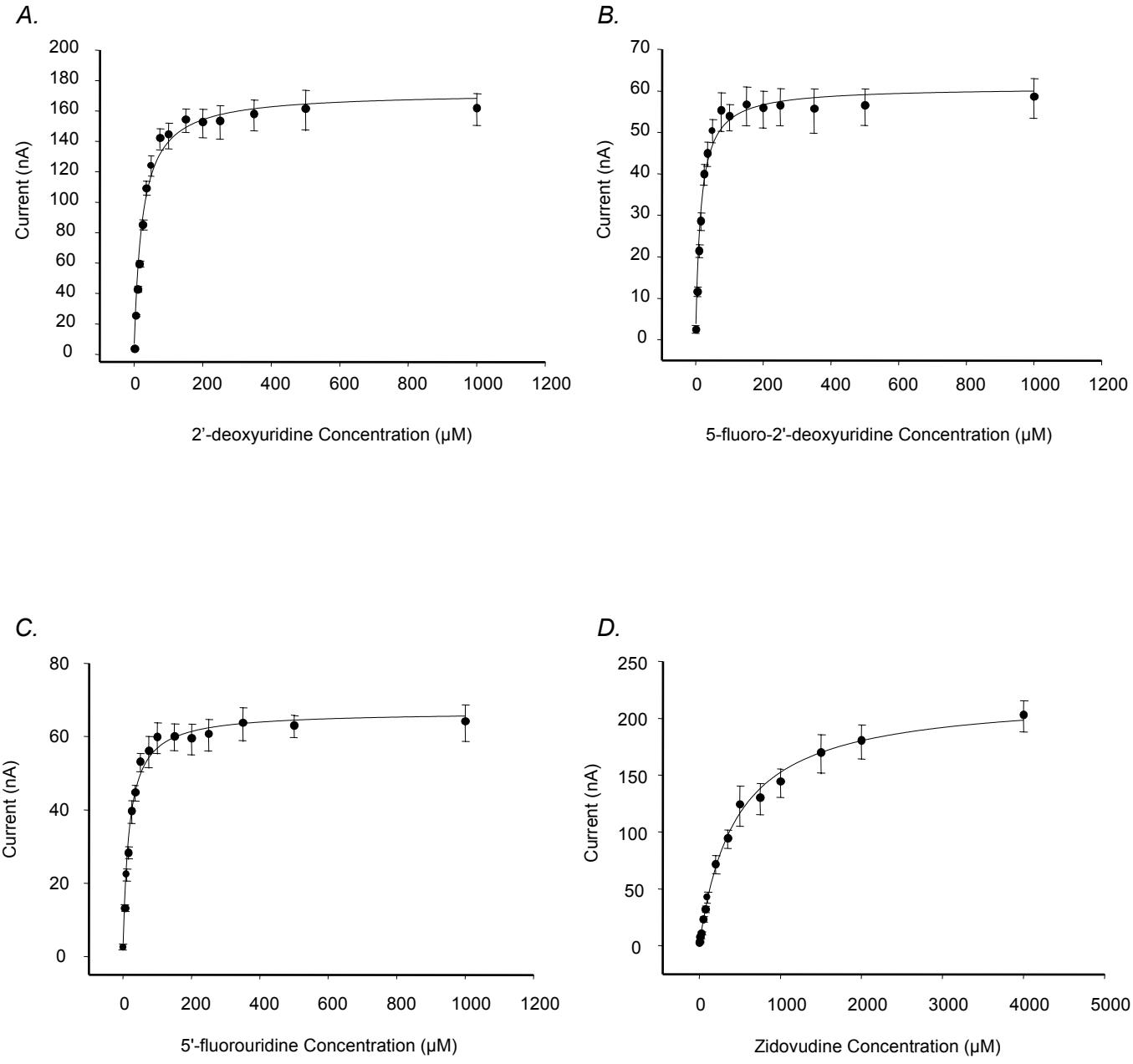

A.
Current (nA)
2'-deoxyuridine Concentration (μM)
B.
Current (nA)
5-fluoro-2'-deoxyuridine Concentration (μM)
C.
Current (nA)
5'-fluorouridine Concentration (μM)
D.
Current (nA)
Zidovudine Concentration (μM)

Figure S7

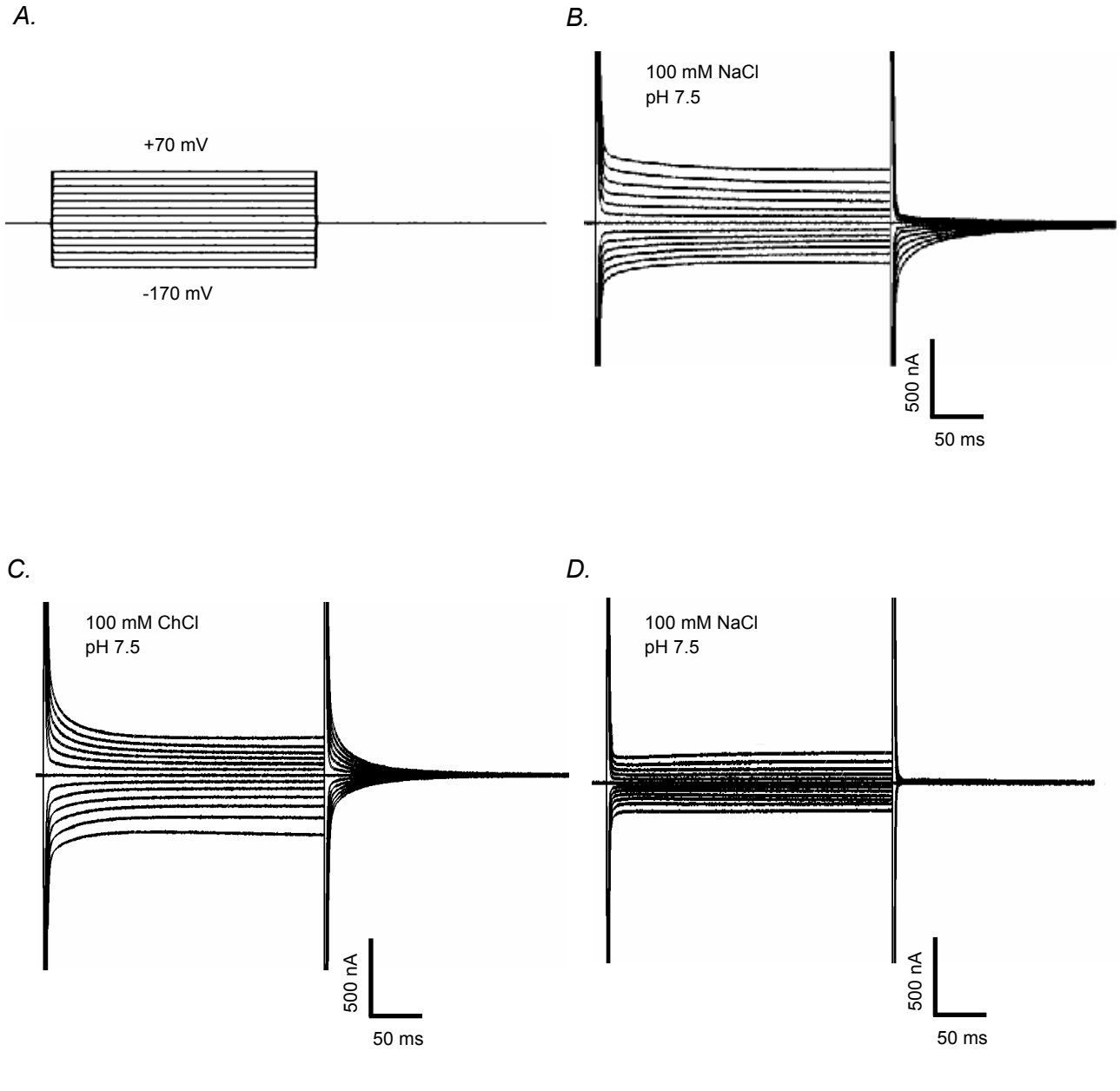

A.
+70 mV
-170 mV
B.
100 mM NaCl
pH 7.5
500 nA
50 ms
C.
100 mM ChCl
pH 7.5
500 nA
50 ms
D.
100 mM NaCl
pH 7.5
500 nA
50 ms

Figure S8

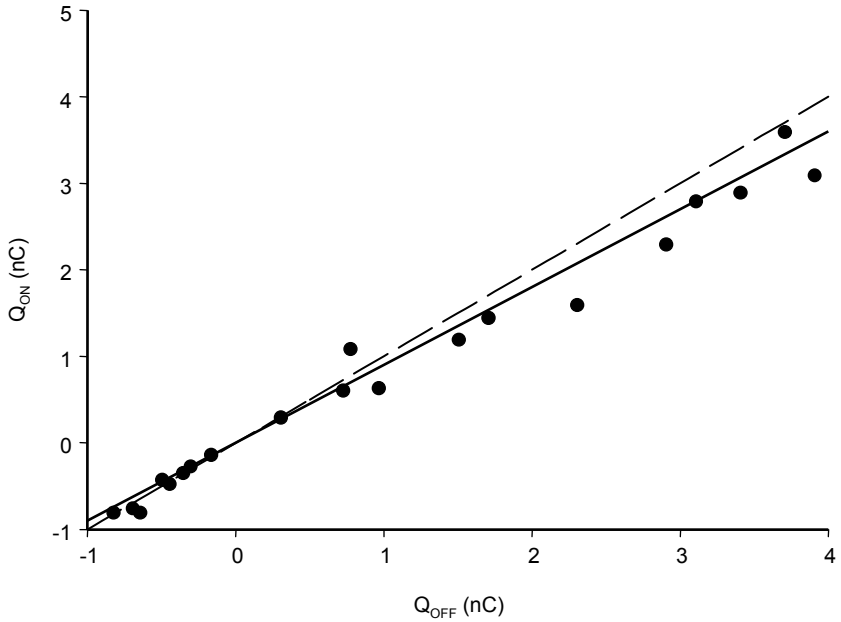
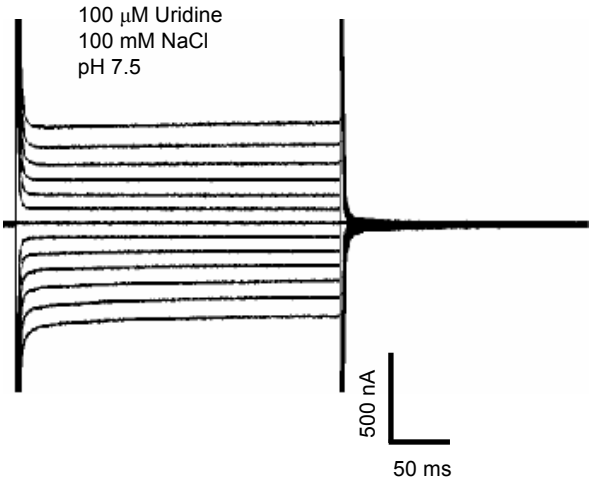


Figure S9

A.



B.

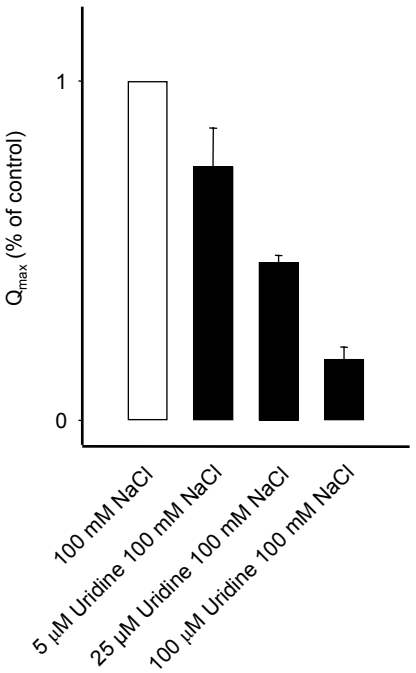


Figure S10