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# Supplemental Material to:

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# Bupivacaine inhibits large conductance, voltage- and Ca2+- activated K+ channels in human umbilical artery smooth muscle cells

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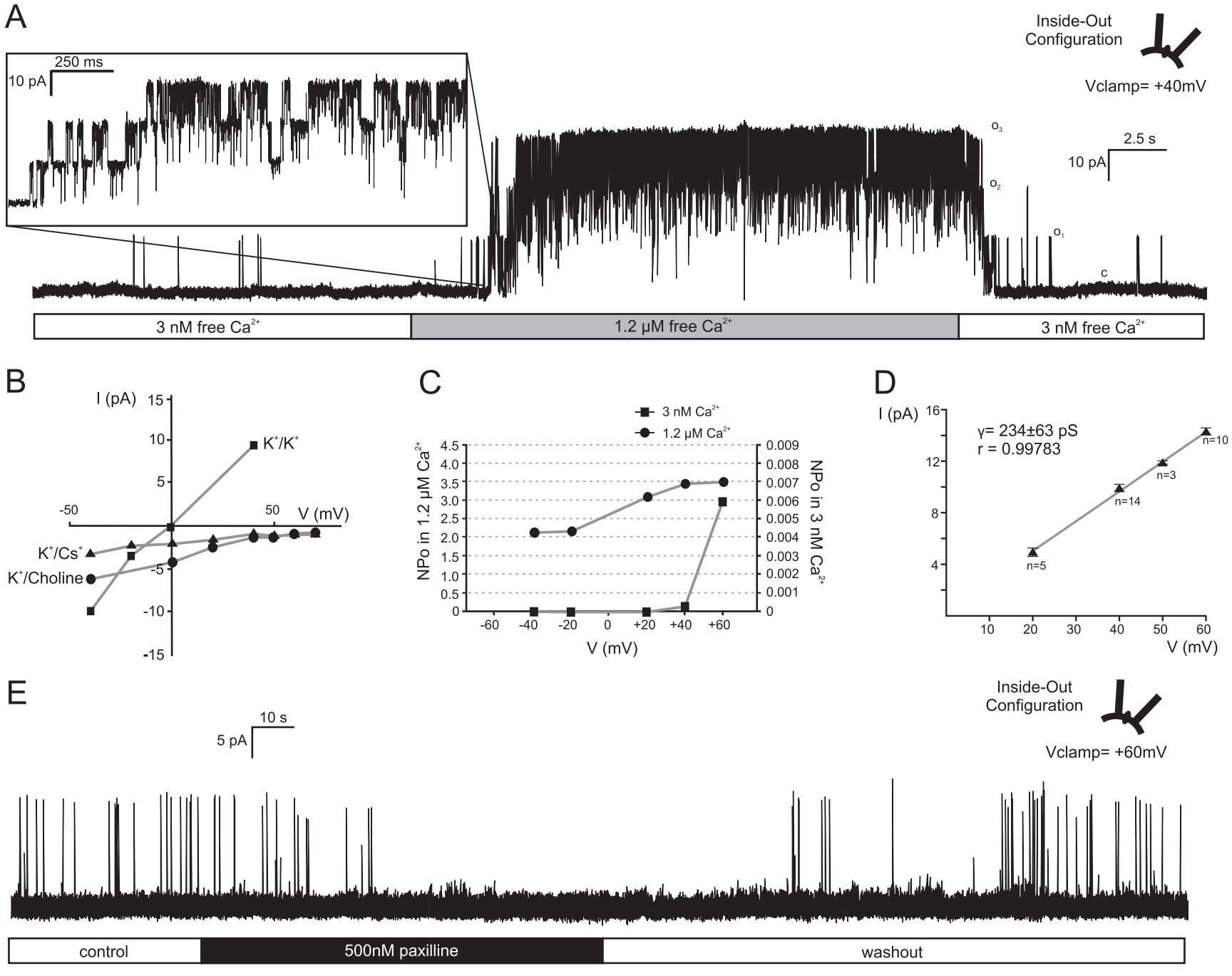
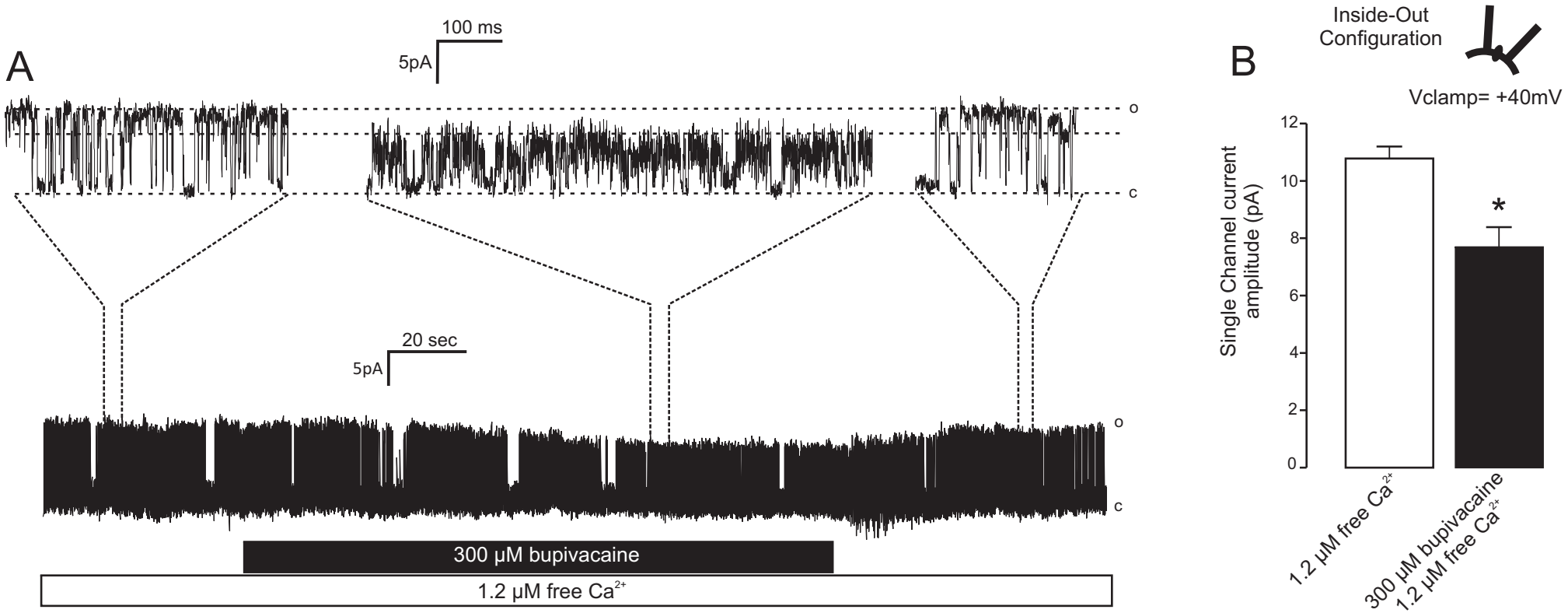
**Supplementary Figure 1:** *Characterization of high-conductance voltage- and $Ca^{2+}$-activated $K^+$ channels ($BK_{Ca}$) in human umbilical artery smooth muscle cells in the inside-out (IO) single-channel configuration.* **A:** Typical recording of $BK_{Ca}$ channel activity in an IO patch clamped at +40 mV at a low (3 nM) and a high (1.2 µM) $Ca^{2+}$ concentration in contact with the cytosolic face of the membrane. The closed and open levels are indicated as C and O, respectively. **B:**Typical IV curves of $BK_{Ca}$ channel in IO configuration obtained with a control pipette solution (containing 140 mM KCl) and three bath solutions in contact with the cytosolic face of the membrane: control bath solution (with 140 mM KCl), bath solution in which KCl was replaced equimolarly by CsCl, and bath solution in which KCl was replaced equimolarly by choline chloride. **C:** Typical plots of open probability (NPo) versus voltage for $BK_{Ca}$ channel in IO configuration obtained at a low (3 nM) and a high (1.2 µM) $Ca^{2+}$ concentration in contact with the cytosolic face of the membrane. **D:** Mean IV curve for $BK_{Ca}$ channel in IO configuration under control conditions. Data represent the mean ± SEM of 3-14 membrane patches. E: Typical recording of the block by 500 nM paxilline of the $BK_{Ca}$ channel in an IO patch clamped at +60 mV.

# Figure S1


A
Inside-Out Configuration
Vclamp= +40mV
250 ms
10 pA
2.5 s
10 pA
O3
O2
O1
C
3 nM free Ca2+
1.2 µM free Ca2+
3 nM free Ca2+
B
I (pA)
K+/K+
K+/Cs+
K+/Choline
V (mV)
C
3 nM Ca2+
1.2 µM Ca2+
NPo in 1.2 µM Ca2+
NPo in 3 nM Ca2+
V (mV)
D
I (pA)
γ= 234±63 pS
r = 0.99783
n=5
n=14
n=3
n=10
V (mV)
E
10 s
5 pA
Inside-Out Configuration
Vclamp= +60mV
control
500nM paxilline
washout

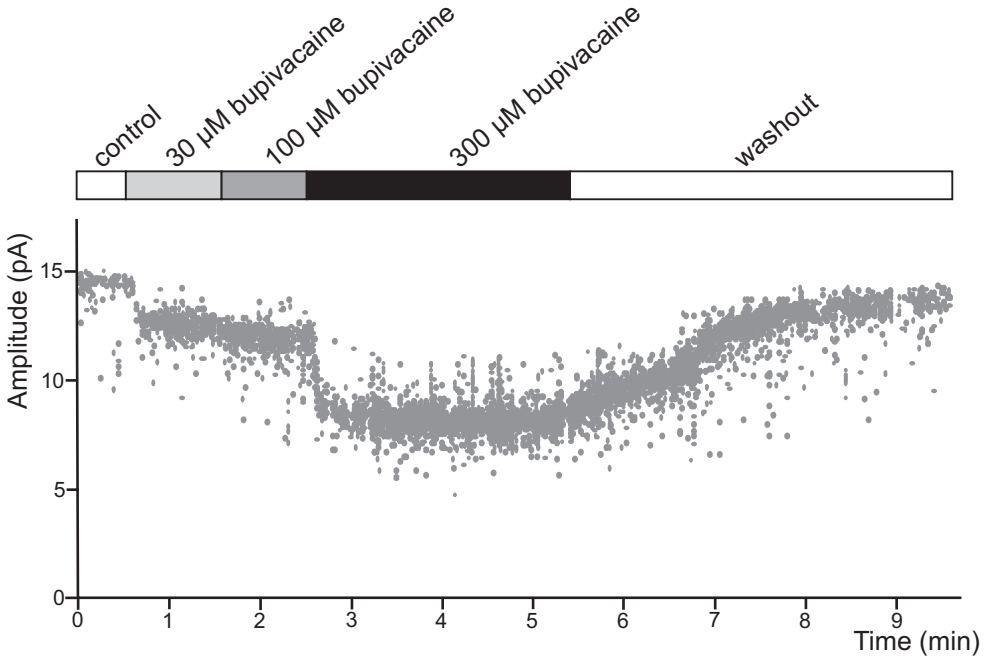
**Supplementary Figure 2:** *Bupivacaine induces a decrease in the amplitude of single-channel current and a flickery mode of the open channel state.* **A:** Typical recording of bupivacaine (300 µM) effects on $BK_{Ca}$ channel activity in an IO patch clamped at +40 mV with 1.2 µM $Ca^{2+}$ in contact with the cytosolic face of the membrane. The closed and open levels are indicated as C and O, respectively. **B:** Mean current amplitude of $BK_{Ca}$ channels recorded as shown in A in control (1.2 µM $Ca^{2+}$) and with 300 µM bupivacaine (n=6). The symbol * indicates a statistically significant difference from controls (Student´s t test, $P<0.05$).

# Figure S2


A
100 ms
5pA
o
c
20 sec
5pA
o
c
300 µM bupivacaine
1.2 µM free Ca2+
B
Inside-Out
Configuration
Vclamp= +40mV
Single Channel current
amplitude (pA)
12
10
8
6
4
2
0
*
1.2 µM free Ca2+
300 µM bupivacaine
1.2 µM free Ca2+

**Supplementary Figure 3:** Temporal course of reduction in open channel current amplitude produced by increasing concentrations of bupivacaine obtained in a typical IO patch clamped at +60 mV.

# Figure S3


control
30 μM bupivacaine
100 μM bupivacaine
300 μM bupivacaine
washout
Amplitude (pA)
15
10
5
0
0
1
2
3
4
5
6
7
8
9
Time (min)