Supporting Information

PKC inhibition results in a $K_v1.5$+$K_v\beta1.3$ pharmacology closer to $K_v1.5$ channels

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Running title: *$K_v1.5$+$K_v\beta1.3$ pharmacology and PKC*

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SUPPLEMENTAL TABLES

SUPPLEMENTAL TABLE 1: IC_{50} and n_H from the of the concentration-response curves to bupivacaine in $K_v1.5$+$K_v\beta1.3$ channels expressed in cells treated with hispidin and bisindolylmaleimide II in comparison with those obtained of bupivacaine on $K_v1.5$ and $K_v1.5$+$K_v\beta1.3$ channels.

	IC_{50} (μM)	n_H
$K_v1.5$	13.1 ± 0.8*	---
$K_v1.5$+$K_v\beta1.3$ Control	47.5 ± 5.1*	---
$K_v1.5$+$K_v\beta1.3$ Bis II	12.4 ± 1.8	0.88 ± 0.09
$K_v1.5$+$K_v\beta1.3$ Hispidin	27.3 ± 1.3	1.23 ± 0.10

*: Taken from (Gonzalez *et al.*, 2002)

REFERENCES

Gonzalez T, Navarro-Polanco R, Arias C, Caballero R, Moreno I, Delpon E, et al. (2002). Assembly with the Kvβ1.3 subunit modulates drug block of hKv1.5 channels. Mol Pharmacol 62: 1456-1463.

FIGURE LEGENDS

FIGURE S1: **Concentration dependence of bupivacaine-induced blockade of hispidin-treated $K_v1.5$+$K_v\beta1.3$ channels**. (A) The dashed and dotted lines represent the dose-response curves obtained for the bupivacaine-induced blockade of $K_v1.5$ and $K_v1.5$+$K_v\beta1.3$ channels, respectively (taken from González et al., 2002). Reduction in the current (relative to the control) at the end of depolarising steps from -80 to +60 mV was used as an index of blockade. (●): Concentration-response curves for bupivacaine in bisindolylmaleimide II-treated $K_v1.5$+$K_v\beta1.3$ channels and in hispidin-treated $K_v1.5$+$K_v\beta1.3$ channels (○). (B) Reduction in the current (relative to the control) at 50 ms (○) and at 250 ms (●) depolarising steps from -80 to +60 mV. Each point represents the mean±S.E.M. of three to four experiments. The lines represent the fit of the experimental data to a monophasic Hill equation.

FIGURE S2: **Concentration dependence of bupivacaine- (A) and quinidine-induced (B) blockade of calphostine C-treated $K_v1.5$+$K_v\beta1.3$ channels**. The dashed and continuous lines represent the dose-response curves obtained for the bupivacaine- or quinidine-induced blockade at 50 ms (○) and at 250 ms (●) depolarising steps from -80 to +60 mV, respectively. Each point represents the mean±S.E.M. of three to eight experiments. The continuous line represents the fit of the experimental data to a biphasic Hill equation.

FIGURE S3: **Concentration dependence of bupivacaine- (A) and quinidine-induced (B) blockade of bisindolylmaleimide II-treated $K_v1.5$+$K_v\beta1.3$ channels**. The dashed and continuous lines represent the dose-response curves obtained for the bupivacaine- or quinidine-induced blockade at 50 ms (○) and at 250 ms (●) depolarising steps from -80 to +60 mV, respectively. Each point represents the mean±S.E.M. of three to eight experiments. The continuous line represents the fit of the experimental data to a monophsic Hill equation.

FIGURE S4: **Absolute values for bupivacaine- and quinidine- blockade of calphostin C- and bisindolylmaleimide II-treated $K_v1.5$+$K_v\beta1.3$ channels**. Magnitude of calphostin C-$K_v1.5$+$K_v\beta1.3$ currents in the absence and in the presence of different bupivacaine (A) and quinidine (B) concentrations. Magnitude of bisindolylmaleimide II-$K_v1.5$+$K_v\beta1.3$ currents in the absence and in the presence of different bupivacaine (C) and quinidine (D) concentrations. Each point represents the mean±S.E.M. of three to eight experiments.

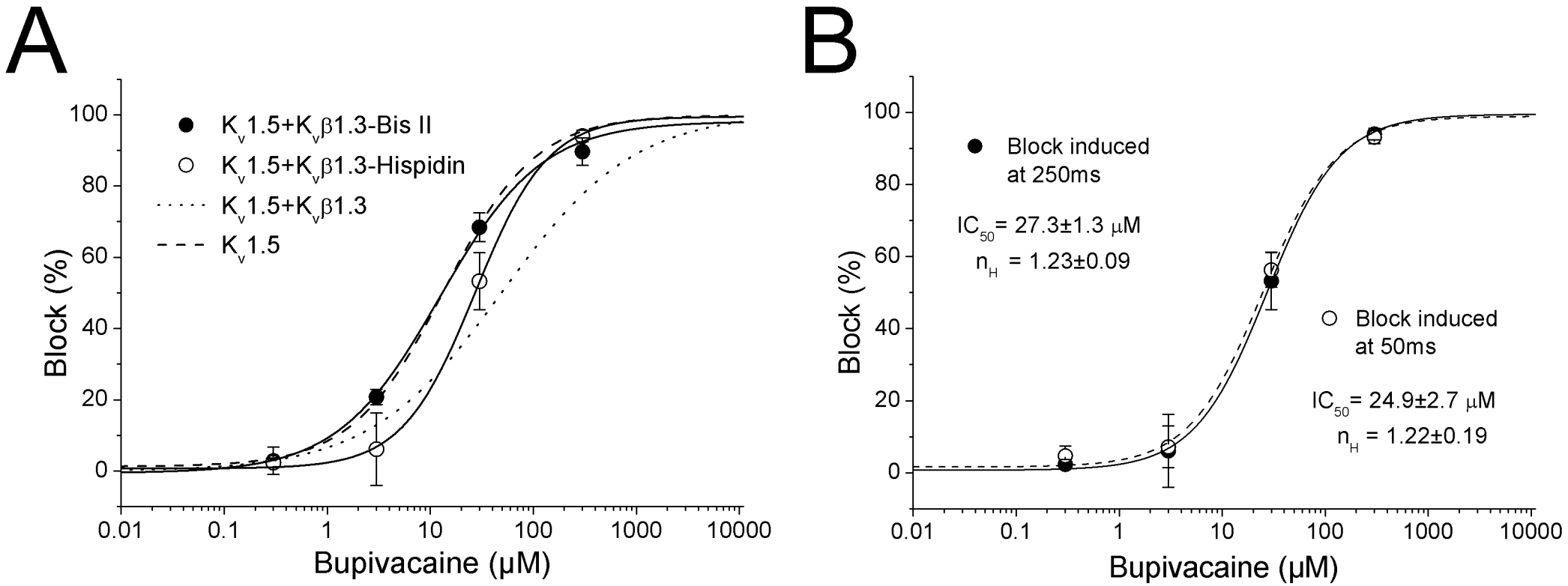
A
B
Block (%)
Bupivacaine (µM)
0.01
0.1
1
10
100
1000
10000
0
20
40
60
80
100
Kv1.5+Kvβ1.3-Bis II
Kv1.5+Kvβ1.3-Hispidin
Kv1.5+Kvβ1.3
Kv1.5
Block induced at 250ms
IC50= 27.3±1.3 µM
nH = 1.23±0.09
Block induced at 50ms
IC50= 24.9±2.7 µM
nH = 1.22±0.19

Figure S1

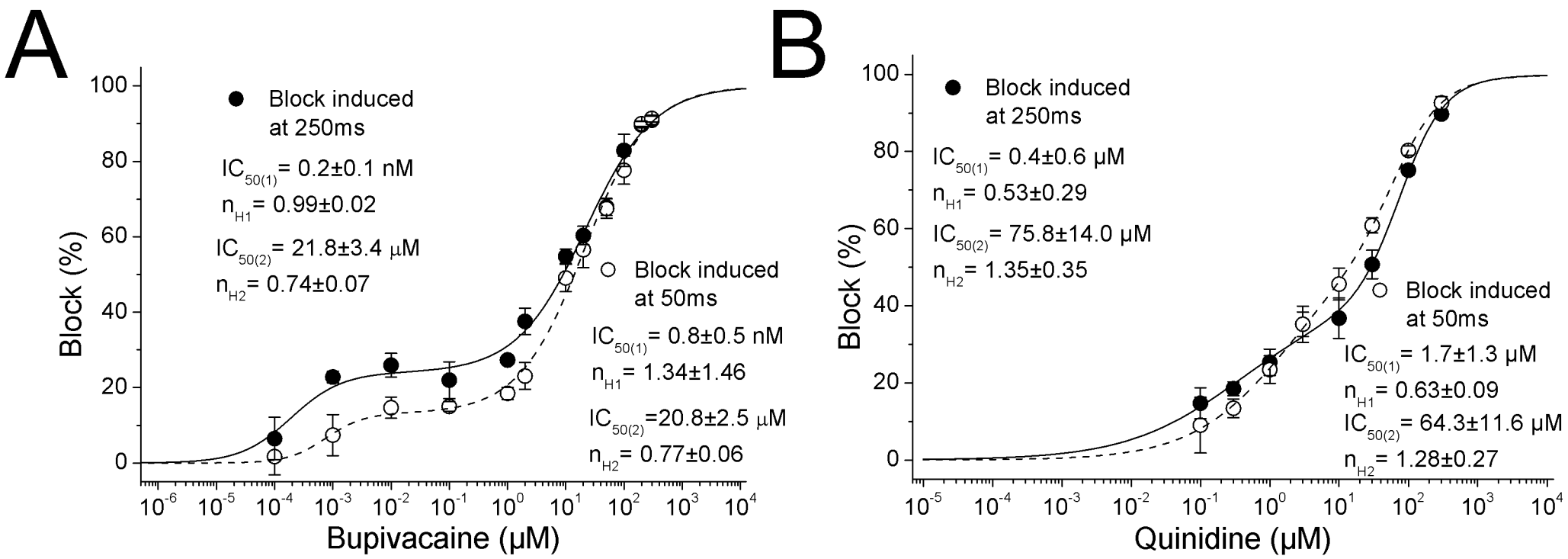
A
Block induced
at 250ms
IC50(1)= 0.2±0.1 nM
nH1= 0.99±0.02
IC50(2)= 21.8±3.4 μM
nH2= 0.74±0.07
Block induced
at 50ms
IC50(1)= 0.8±0.5 nM
nH1= 1.34±1.46
IC50(2)=20.8±2.5 μM
nH2= 0.77±0.06
Block (%)
Bupivacaine (μM)
B
Block induced
at 250ms
IC50(1)= 0.4±0.6 μM
nH1= 0.53±0.29
IC50(2)= 75.8±14.0 μM
nH2= 1.35±0.35
Block induced
at 50ms
IC50(1)= 1.7±1.3 μM
nH1= 0.63±0.09
IC50(2)= 64.3±11.6 μM
nH2= 1.28±0.27
Block (%)
Quinidine (μM)

Figure S2

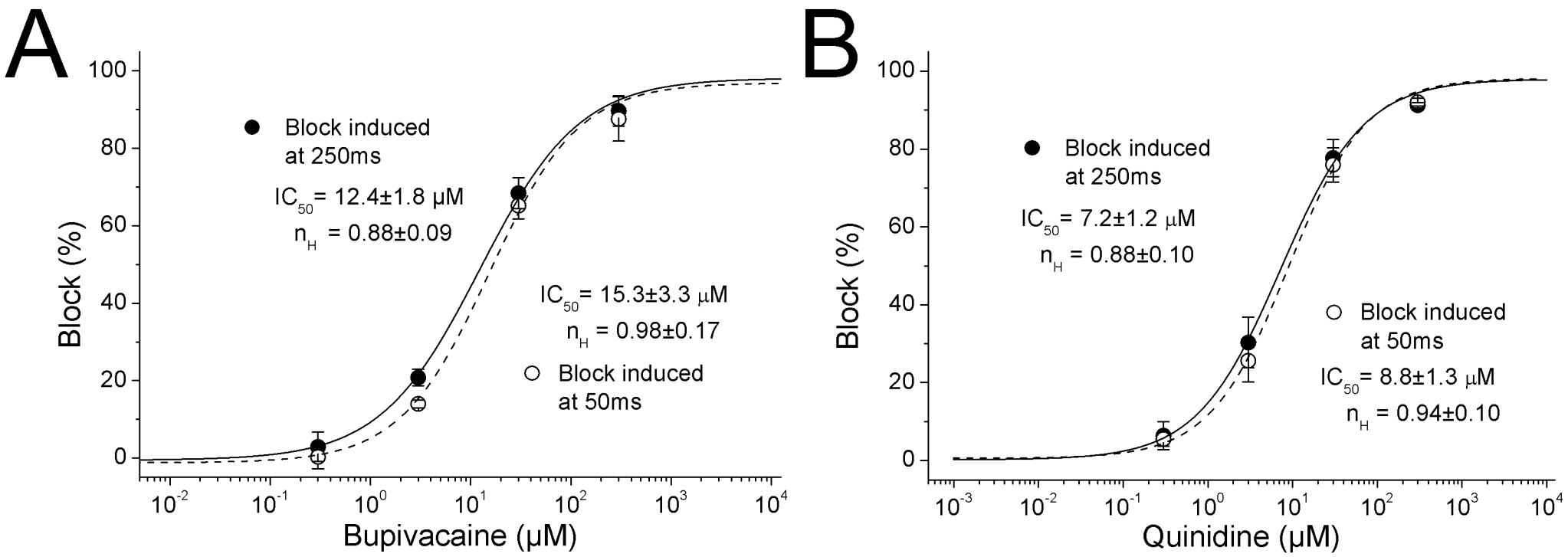
A
Block (%)
Bupivacaine (μM)
Block induced at 250ms
IC50= 12.4±1.8 μM
nH = 0.88±0.09
IC50= 15.3±3.3 μM
nH = 0.98±0.17
Block induced at 50ms
B
Block (%)
Quinidine (μM)
Block induced at 250ms
IC50= 7.2±1.2 μM
nH = 0.88±0.10
Block induced at 50ms
IC50= 8.8±1.3 μM
nH = 0.94±0.10

Figure S3

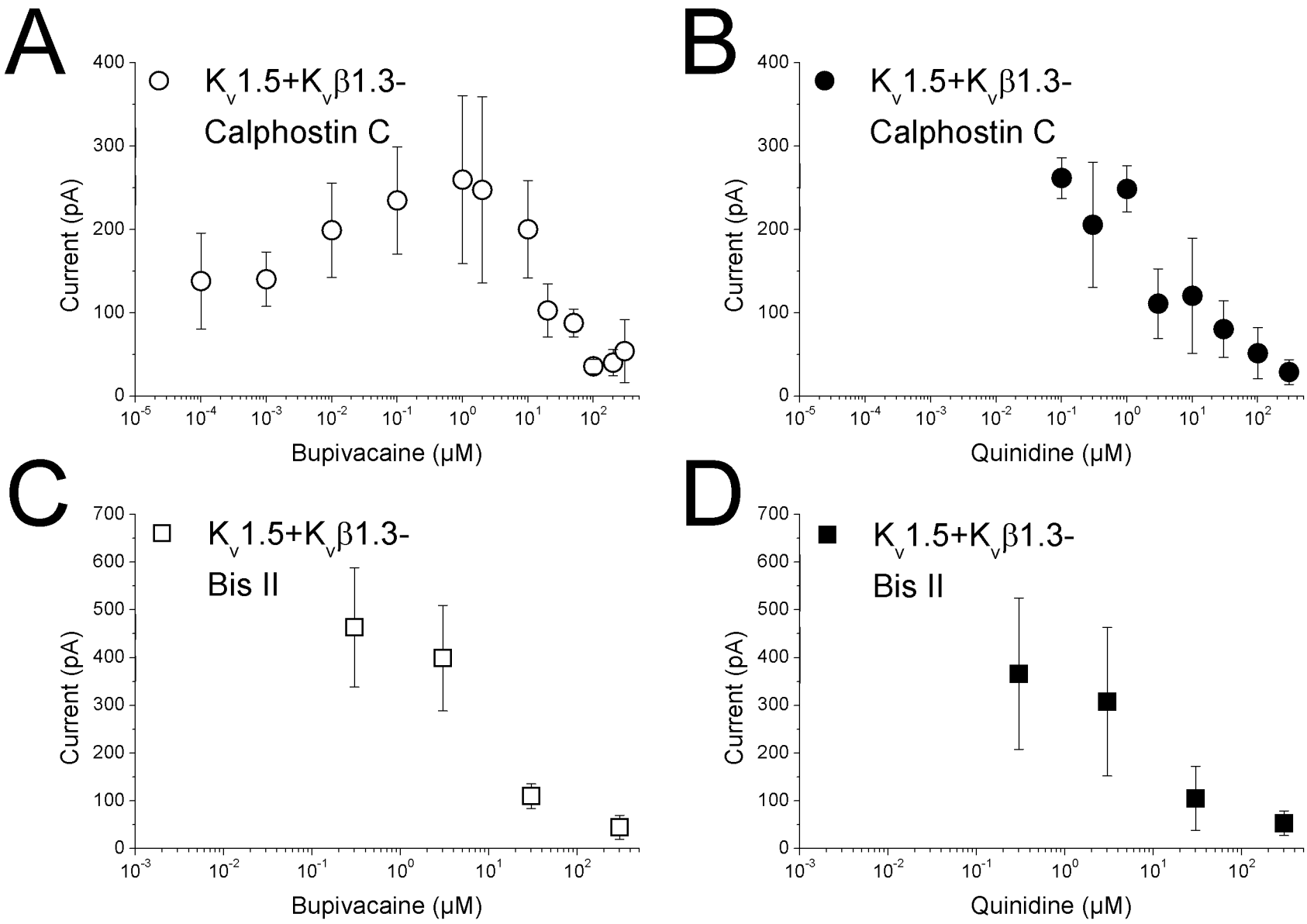
A
$K_v1.5+K_v\beta1.3$-
Calphostin C
Current (pA)
Bupivacaine (μM)
B
$K_v1.5+K_v\beta1.3$-
Calphostin C
Current (pA)
Quinidine (μM)
C
$K_v1.5+K_v\beta1.3$-
Bis II
Current (pA)
Bupivacaine (μM)
D
$K_v1.5+K_v\beta1.3$-
Bis II
Current (pA)
Quinidine (μM)

Figure S4