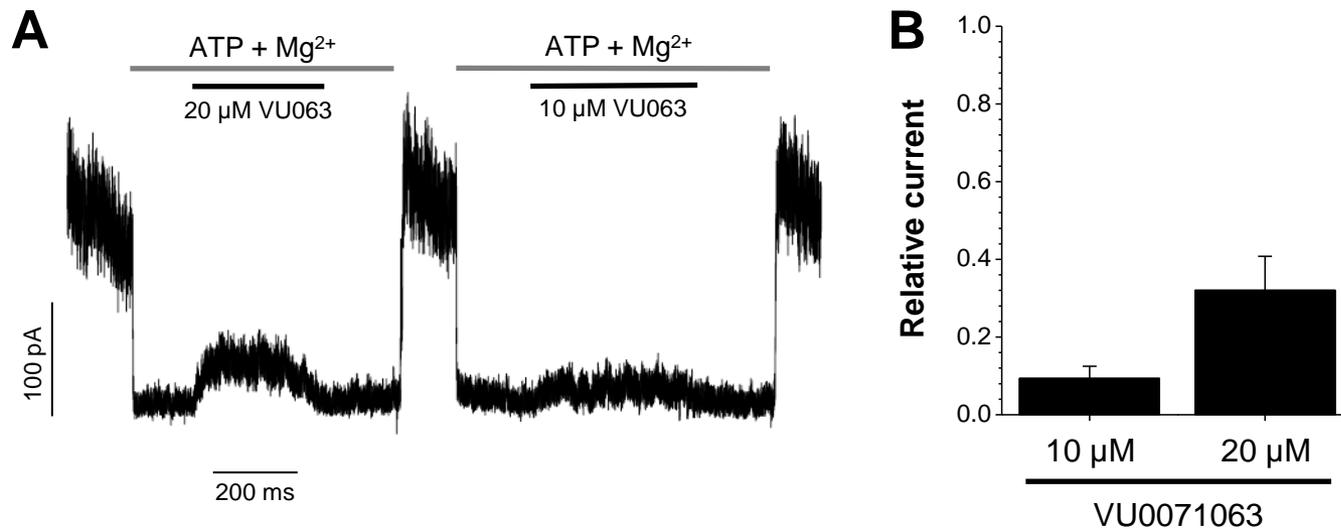
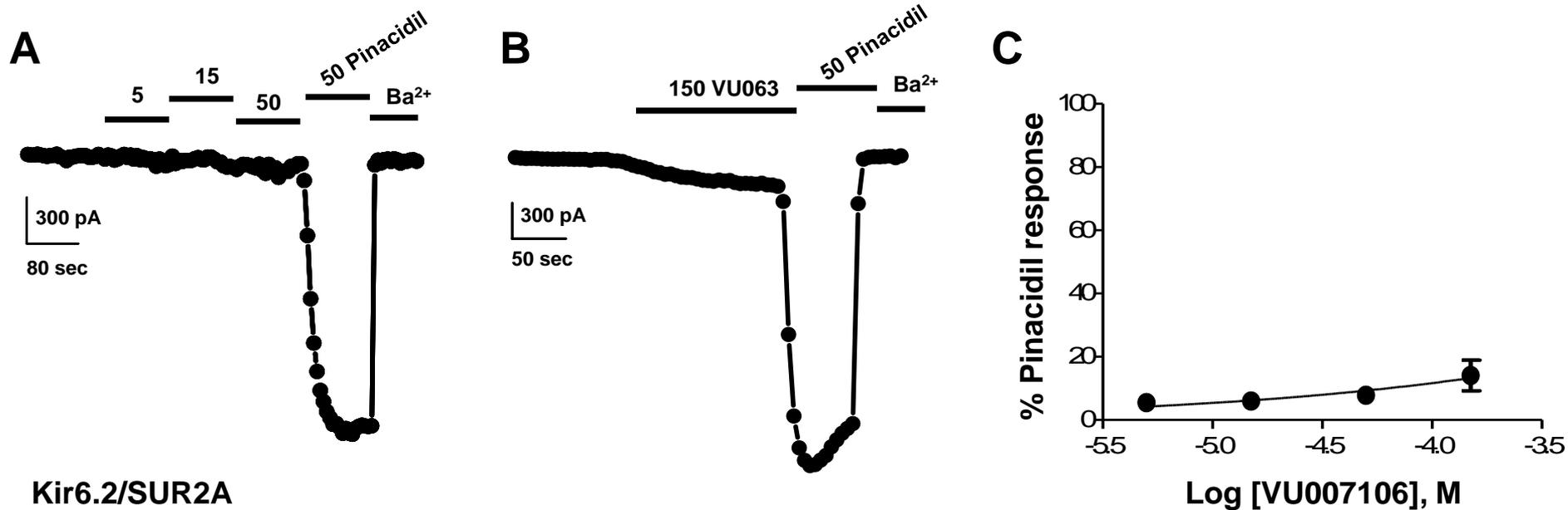


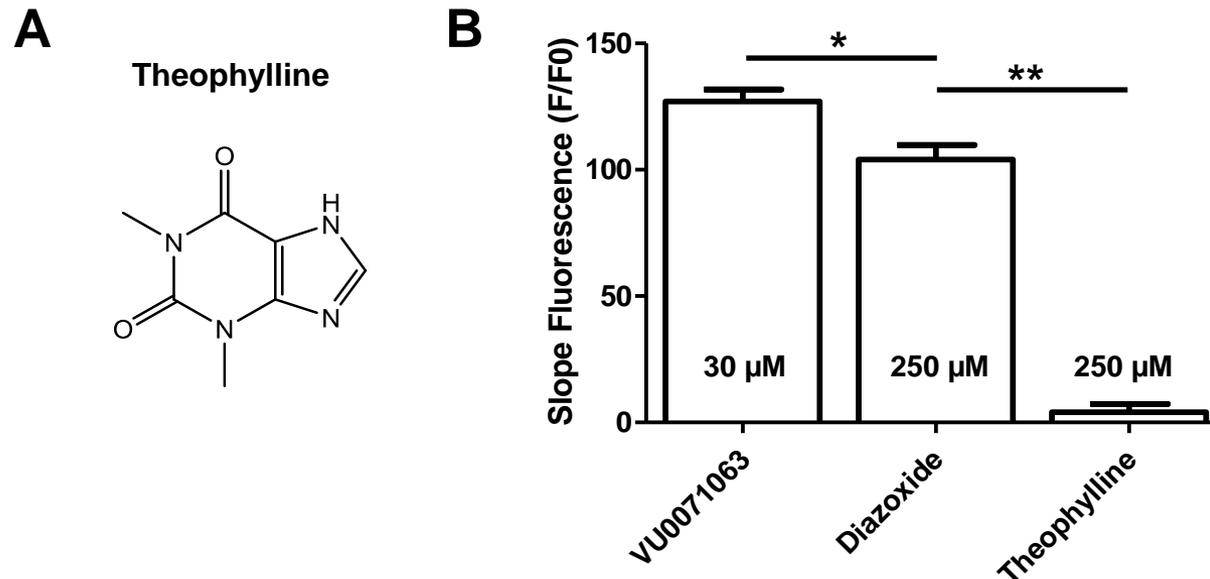
Direct activation of β -cell K_{ATP} channels with a novel xanthine derivative. Raphemot, R., Swale, D.R., Dadi, P.K., Jacobson, D.A., Cooper, P., Wojtovich, A.P., Banerjee, S., Nichols, C., Denton, J.S., *Molecular Pharmacology*. T-REx-HEK293-Kir6.2/SUR1 cell line characterization. A, Cells were cultured overnight with the indicated concentration of tetracycline and subjected to Western blot analysis of Kir6.2 expression in whole-cell lysates. Membranes were stripped and re-probed for b-actin as a loading control. B, Representative Tl^+ flux experiment in cells cultured overnight with (red) or without (black) tetracycline and then pre-treated with 250 μ M diazoxide for 20 min before Tl^+ (12 mM Tl_2SO_4) addition.



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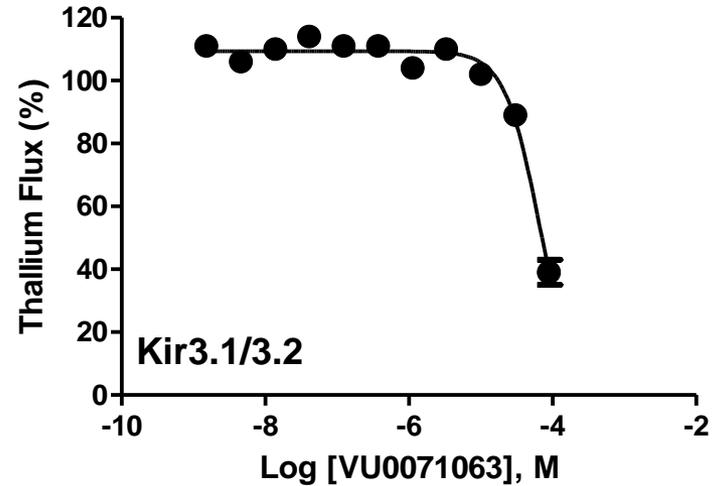
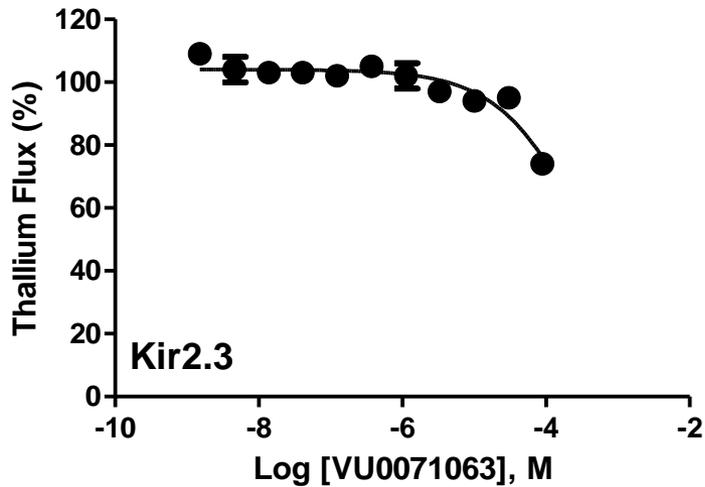
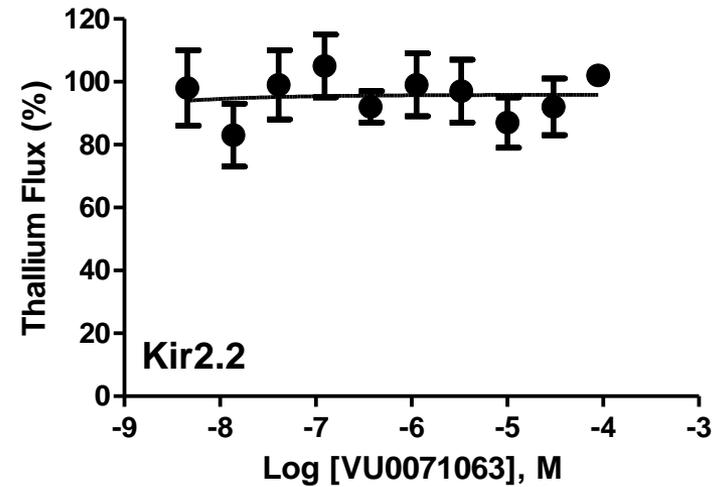
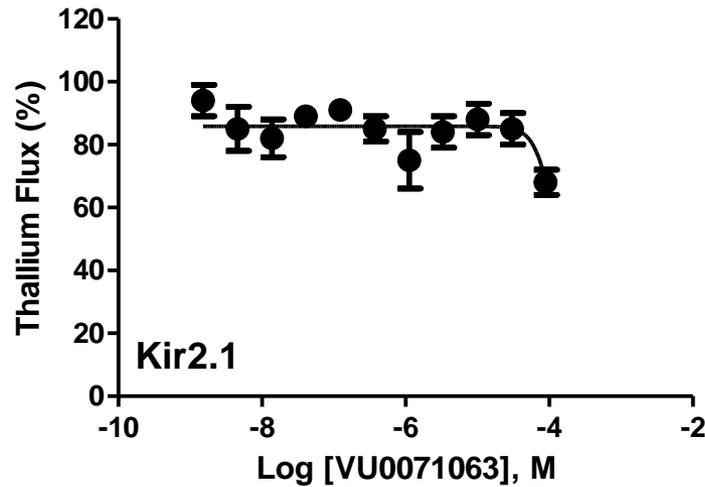


Direct activation of b-cell K_{ATP} channels with a novel xanthine derivative. Raphemot, R., Swale, D.R., Dadi, P.K., Jacobson, D.A., Cooper, P., Wojtovich, A.P., Banerjee, S., Nichols, C., Denton, J.S., *Molecular Pharmacology*. Lack of effect of VU0071063 on Kir6.2/SUR2A channels. A, Whole-cell Kir6.2/SUR2A currents were measured every 5 sec at -120 mV before and after addition of 5, 15, or 50 μM VU007106 or 50 μM Pinacidil (positive control), followed by application of 2 mM Ba²⁺. B, Single-dose exposure to 150 μM VU0071063 has only a modest effect on Kir6.2/SUR2A channel activity. C, VU0071063 CRC data normalized to 50 μM pinacidil response. Data are means ± SEM (n = 4 at each dose).

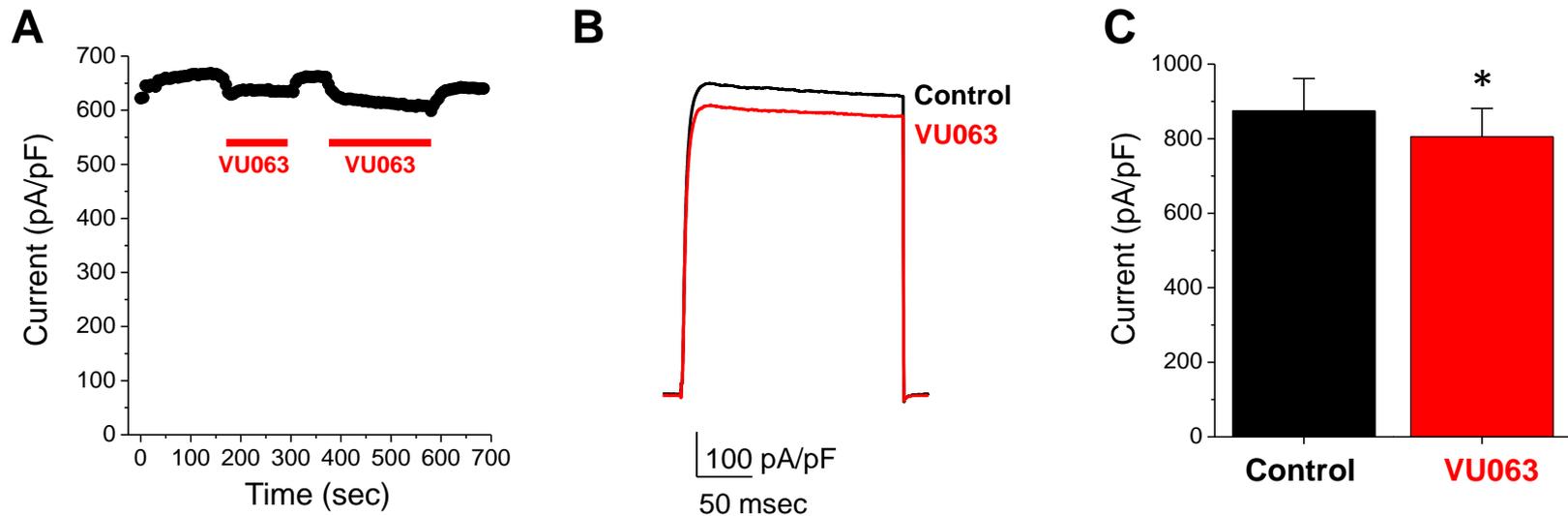


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Supplementary Figure 5



Direct activation of b-cell K_{ATP} channels with a novel xanthine derivative. Raphemot, R., Swale, D.R., Dadi, P.K., Jacobson, D.A., Cooper, P., Wojtovich, A.P., Banerjee, S., Nichols, C., Denton, J.S., *Molecular Pharmacology*. Selectivity of VU0071063 among members of the Kir channel family. 11-point CRC of VU0071063 were established for Kir2.1, Kir2.2, Kir2.3, and Kir3.1/3.2 expressing cell lines (n = 2 independent experiments performed in triplicate per cell line).



Direct activation of b-cell K_{ATP} channels with a novel xanthine derivative. Raphemot, R., Swale, D.R., Dadi, P.K., Jacobson, D.A., Cooper, P., Wojtovich, A.P., Banerjee, S., Nichols, C., Denton, J.S., *Molecular Pharmacology*. Effects of VU0071063 on heterologously expressed Kv2.1. A, Time versus current amplitude plot illustrating the rapid and reversible inhibition of Kv2.1 current at 40 mV by 10 μ M VU0071063. B, Representative current traces recorded at 40 mV before and during bath perfusion with 10 μ M VU0071063. C, Mean SEM current amplitude at 40 mV in the absence (control) or presence of 10 μ M VU0071063. * $P = 0.01$, paired Students t-test.

Supplemental Table 1

[Compound] μM	Complex II activity (% inhibition)	
	VU0071063	Diazoxide
1	1.5 \pm 3.5	-
3	-2.2 \pm 4.5	-
10	2.4 \pm 2.7	7.6 \pm 2.5
30	0.0 \pm 4.3	14.9 \pm 6.0
100	4.9 \pm 2.9	24.2 \pm 4.3
300	-	36.0 \pm 3.5
600	-	41.6 \pm 0.7

Direct activation of b-cell K_{ATP} channels with a novel xanthine derivative. Raphemot, R., Swale, D.R., Dadi, P.K., Jacobson, D.A., Cooper, P., Wojtovich, A.P., Banerjee, S., Nichols, C., Denton, J.S., *Molecular Pharmacology*. Percent inhibition of Complex II activity in response to VU0071063 and diazoxide. underwent three cycles of freeze-thaw and subject to the complex II enzymatic activity assay. Complex II activity was measured mitochondria isolated from mouse hearts using an enzymatic activity. Data are mean \pm S.E.M. percent inhibition (n=4 independent mitochondria preparations). Control complex II activity was 88.0 \pm 3.1 nmol/min/mg protein.