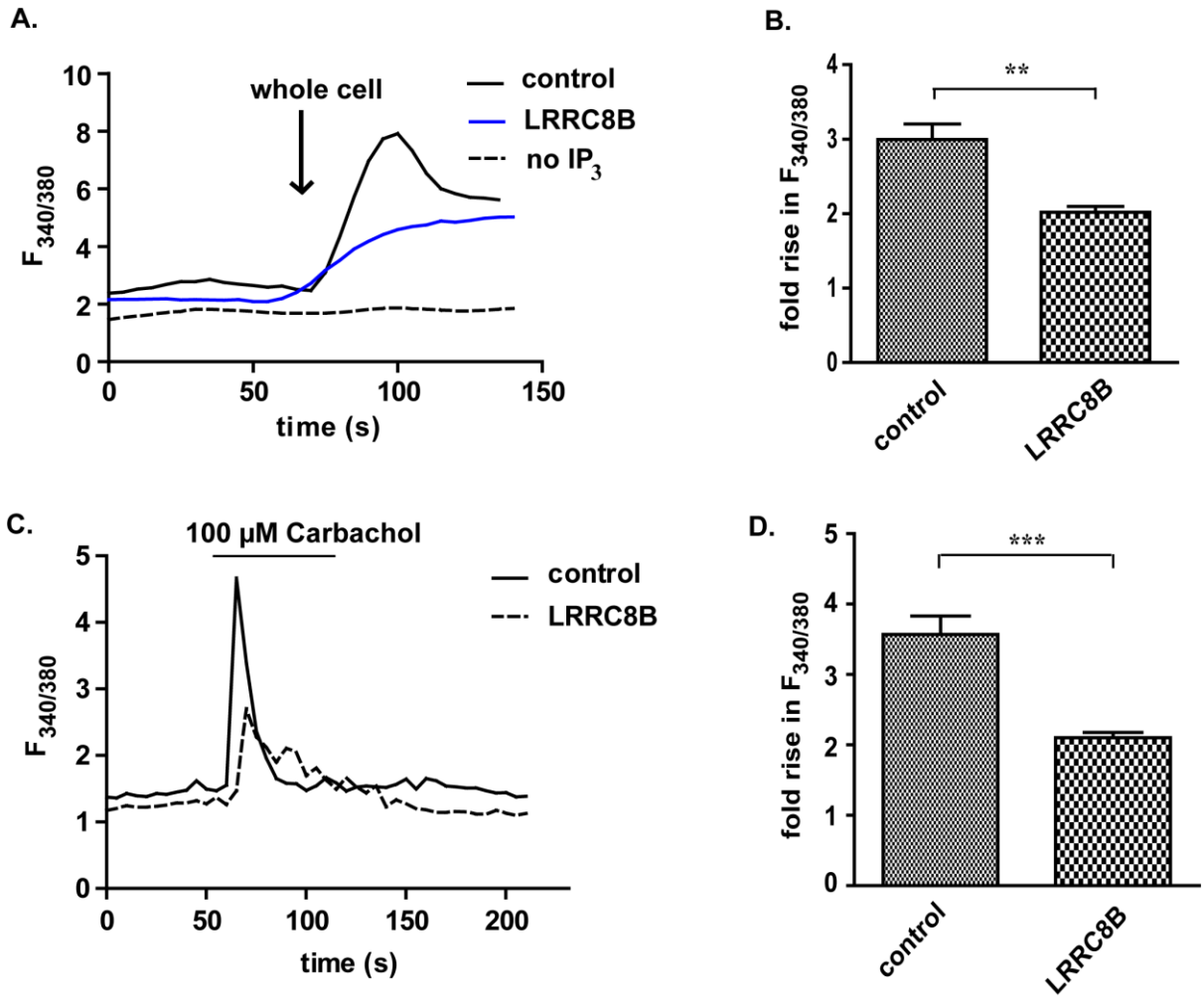
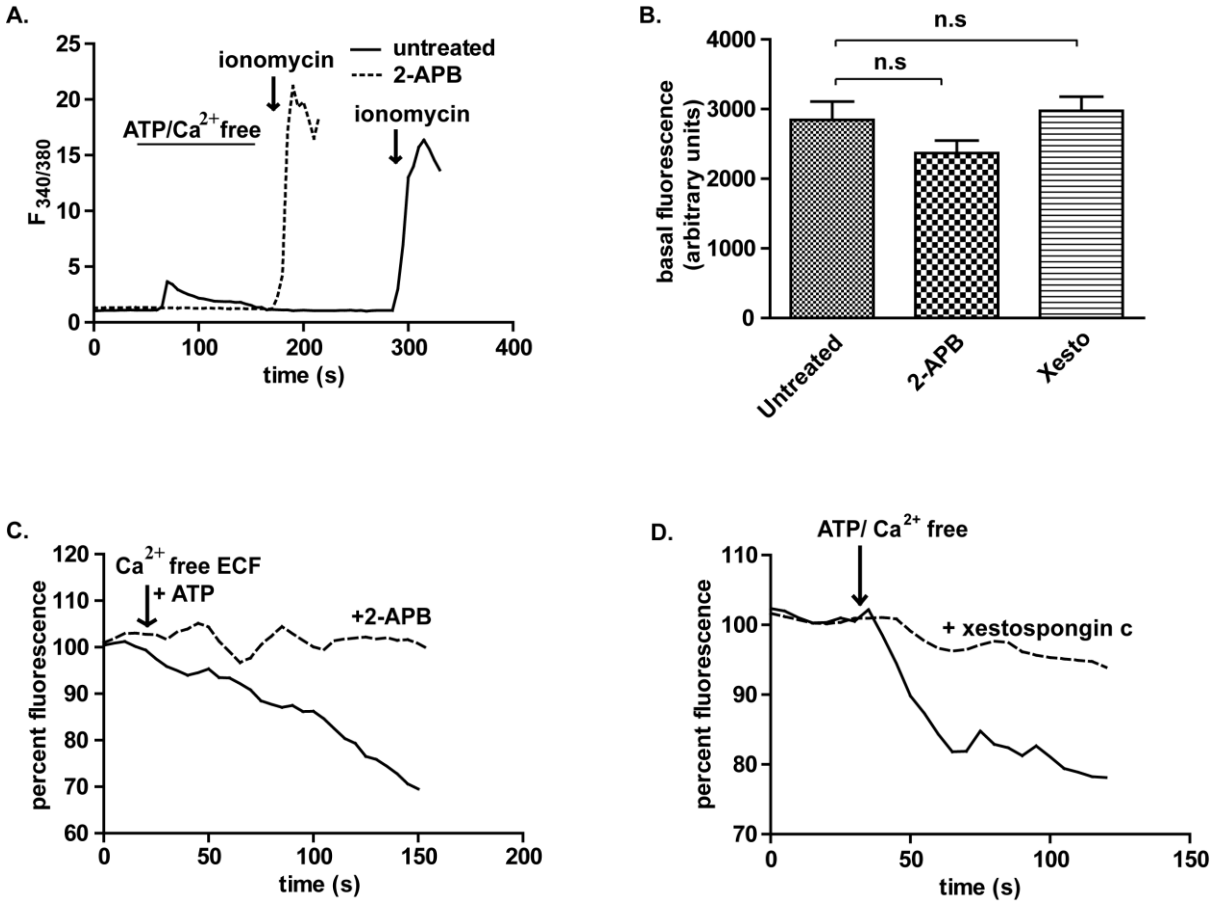
Supplementary figures



S1. Overexpression of LRRC8B supresses IP3R-activated intracellular Ca^{2+} ($[Ca^{2+}]_c$) rise

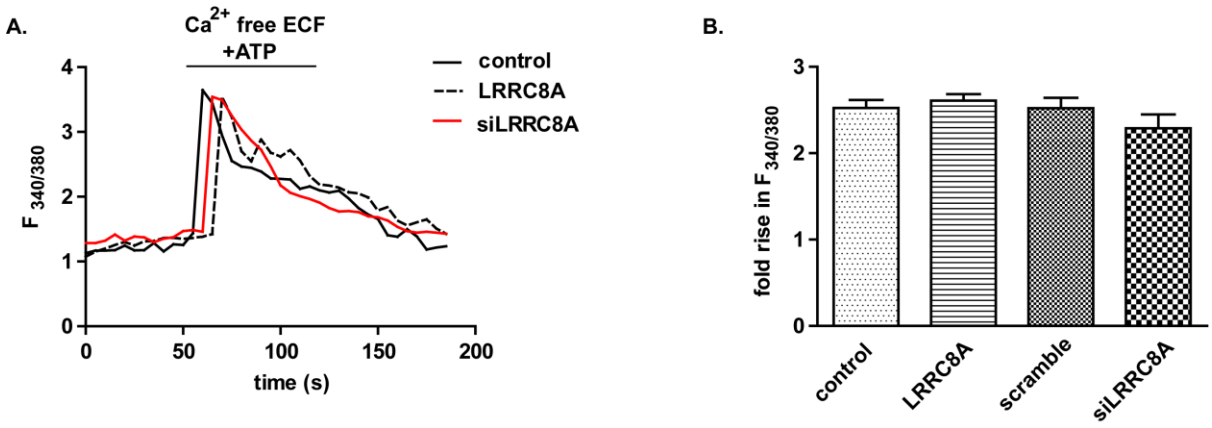
A: $[Ca^{2+}]_c$ transients evoked by injecting IP_3 (50µM) in HEK293 cells. IP_3 was administered through a patch pipette by making 'whole cell'. Both pipette solution and ECF had no Ca^{2+}. *B:* Cumulative data (mean ± SEM, n = 7-10) showing significantly lesser IP_3-induced $[Ca^{2+}]_c$ rise in LRRC8B overexpressed cells . *C:* Representative traces showing $[Ca^{2+}]_c$ rise, elicited by the application of 100 µM carbachol, in nominally Ca^{2+}-free external buffer. *D:* Cumulative data (mean ± SEM, n = 36-42) showing significantly lesser rise of $[Ca^{2+}]_c$ by carbachol in LRRC8B overexpressed cells .



S 2. 2-APB and xestospongin c do not alter ER-Ca^{2+} stores

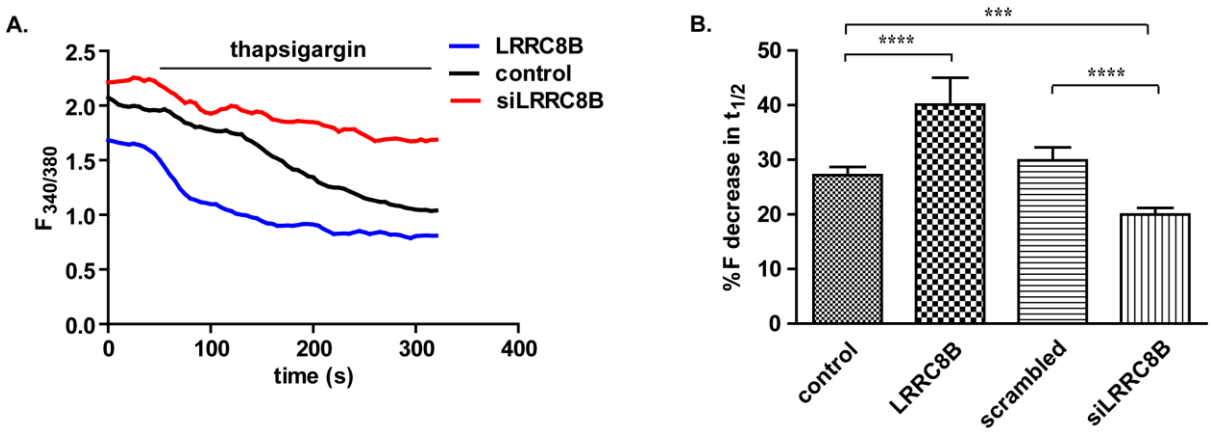
A: ER-Ca^{2+}stores were accessed with high concentration (5µM) of ionomycin in Ca^{2+}-free ECF. Robust rise of $[Ca^{2+}]_c$ following ionomycin treatment reflects the amount of Ca^{2+} present in stores. Both control and 2-APB treated cells exhibited ionomycin-mediated $[Ca^{2+}]_c$ rise to the similar extent. Cells were first incubated with or without 2-APB for 10 mins, after which ATP was applied in Ca^{2+}-free ECF. Then ionomycin (in Ca^{2+}-free ECF) was applied to release ER-Ca^{2+}. ATP (100µM) caused a rise in $[Ca^{2+}]_c$ in control cells but not in 2-APB

treated cells. *B:* ER-Ca^{2+} imaging with ER-targeted Ca^{2+} indicator RCEPIA1er reveals no significant difference in basal $[Ca^{2+}]_{ER}$ between untreated HEK293 cells and cells subjected to 2-APB (50µM) and xestospongin c (5µM) treatment. *C:*and *D:* Ca^{2+} imaging in ER reveals no notable decay of luminal Ca^{2+} following ATP treatment (in Ca^{+2}-free ECF) in 2-APB (50µM) and xestospongin c (3 µM)-treated cells. Untreated control cells (no blocker) showed quick release of ($[Ca^{2+}]_{ER}$ by ATP.



S 3. LRRC8A does not affect ATP-induced release of Ca^{2+} from stores.

A: Characteristic $[Ca^{2+}]_c$ traces in response to 100 µM ATP in nominally Ca^{2+}-free external buffer in HEK-293 Cells. Overexpression or knockdown of LRRC8A did not affect ATP-induced $[Ca^{2+}]_c$ rise. *B:* Cumulative data (mean ± SEM, n = 41-50) showing the level of LRRC8A does not influence $[Ca^{2+}]_c$ rise elicited by ATP in Ca^{2+}-free conditions.



S 4. Modulation of $[Ca^{2+}]_{ER}$ leak rate by LRRC8B.

A: Typical traces showing the decay of luminal Ca^{2+} ($[Ca^{2+}]_{ER}$), by blocking SERCA pump with 1µM TG. $[Ca^{2+}]_{ER}$ was measured with Mag-fura, as described in methods section. LRRC8B overexpressed and knocked down increased and decreased the decay rate respectively. *B:* Cumulative data for decrease in % fluorescence in $t_{1/2}$ (mean ± SEM, n = 16-27) are plotted.

Table S1. Antibody Validation

Protein	Antibody catalogue no.	Company	References	Dilution
LRRC8A	# HPA016811	Sigma Aldrich	Voss FK et. al Science, 344(6184), 634-638 (2014)	1:500
LRRC8B	# HPA017950	Sigma Aldrich	Voss FK et. al Science, 344(6184), 634-638 (2014)	1:500
β-actin	# A5441	Sigma Aldrich	Hu W et. al PNAS, 107(16), 7455-7460 (2010)	1:1000
ERp72	# D70D12	Cell SignallingTechnology	Kebede MA et al. *J Clin Invest.* 2014 Oct;124(10):4240-56	1:1000
Connexin 43	#3512	Cell Signalling Technology	Nimlamool W et al. *Mol Biol Cell.* 2015 Aug1;26(15):2755-	1:1000

			68.	
VDAC	# D73D12	Cell Signalling Technology	Tewari D et al. Biochim Biophys Acta 1848 (1 Pt A), 151-158. 2014 Oct 23	1:1000
PARP	# 46D11	Cell Signalling Technology	De Waal L et al. *Nat Chem Biol.* 2016 Feb;12(2):102-8.	1:1000
Bcl2	# sc-7382	Santa Cruz Biotechnology	Pilchova I et al. *Cell Mol Neurobiol.* 2015 Jan;35(1):23-31	1:750