SUPPLEMENTARY INFORMATION

Nanomolar potency and selectivity of a CRAC channel blocker against store-operated Ca^{2+}-entry and migration of vascular smooth muscle cells

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Supplementary Table

Supplementary Table I: PCR primer pairs (F, forward direction; R, reverse direction), PCR amplicon sizes, and siRNA probes sequences. The "Orai1 si.negative" was generated for the purpose of suppressing Orai1 expression but it failed to have any effect on the expression and so was used as a negative control.

Gene	Primer 5'-3'	Predicted amplicon (bp)	siRNA sequence (5'-3')
Orai1	F GCACAATCTCAACTCGG R GCGAAGACGATAAAGATCAG	300	Probe 1 (Orai1 si.1) GGGAAGAGGAUUUUUAUAAtt UUAUAAAAAUCCUCUUCCCtc
Orai1	-	-	Probe 2 (Orai1 si.2) CCUGUUUGCGCUCAUGAUCtt GAUCAUGAGCGCAAACAGGtg
Orai1	-	-	Negative (Orai1 si.negative) GCAACGUGCACAAUCUCAAtt UUGAGAUUGUGCACGUUGCtc
Orai2	F GCATCTGGTAGACCCG R ACCTCAAGTGATCCGC	232	Orai2 si GACCAAAGUUUUCCUCUUGtt CAAGAGGAAAACUUUGGUCtt
Orai3	F CAAGGCATTGGTCTAGC R AATTCAGTGTCAGAAGAGC	298	Orai3 si GCUCUUCUGACACUGAAUUtt AAUUCAGUGUCAGAAGAGCtt
STIM1	F CTCTCTTGACTCGCCA R GCTTAGCAAGGTTGATCT	276	
STIM2	F TGGACCTCTAACACGC R GCATACTGACGTCTACTCAA	351	

TRPC1	F TTAGCGCATGTGGCAA R CCACTTACTGAGGCTACTAAT	303	
TRPC4	F ATTAGCTTCACGGGGT R CTTCGTGGGTGACTGT	241	
TRPC5	F ACATTTTAAGTTCGTTGCG R ACATCGGATCCCCTTG	218	

Supplementary Figure Legends

Supplementary Figure I. Relevant gene expressions in human saphenous vein VSMCs. (**a**) Typical gel showing PCR products for Orai1, 2 and 3 mRNAs with (+) but not without (-) reverse transcriptase (RT) reaction. A DNA marker ladder is on the left. (**b**) Using real-time RT-PCR quantification, relative abundances of the mRNAs encoding the indicated proteins (n=3-4).

Supplementary Figure II. Example Ca^{2+} add-back signals in human saphenous vein VSMCs with and without store-depletion and showing comparison of effects of two chemically-different store-depletion agents, thapsigargin (TG) and cyclopiazonic acid (CPA). Intracellular Ca^{2+} was measured in cells pre-treated (as indicated below) in Ca^{2+}-free bath solution for 30 min and then exposed to 0.2 mM extracellular Ca^{2+} as indicated by the horizontal bar labeled 'add-back', except for one group of cells where the Ca^{2+} was not added back (0 Ca^{2+}). The pre-treatments in addition to the Ca^{2+}-free solution were: 1 μM TG; 10 μM CPA; 1 μM TG plus 10 μM CPA (TG+CPA); 0.2 % dimethylsulphoxide (DMSO) as the vehicle control for TG and CPA; or no TG, CPA or DMSO (none).

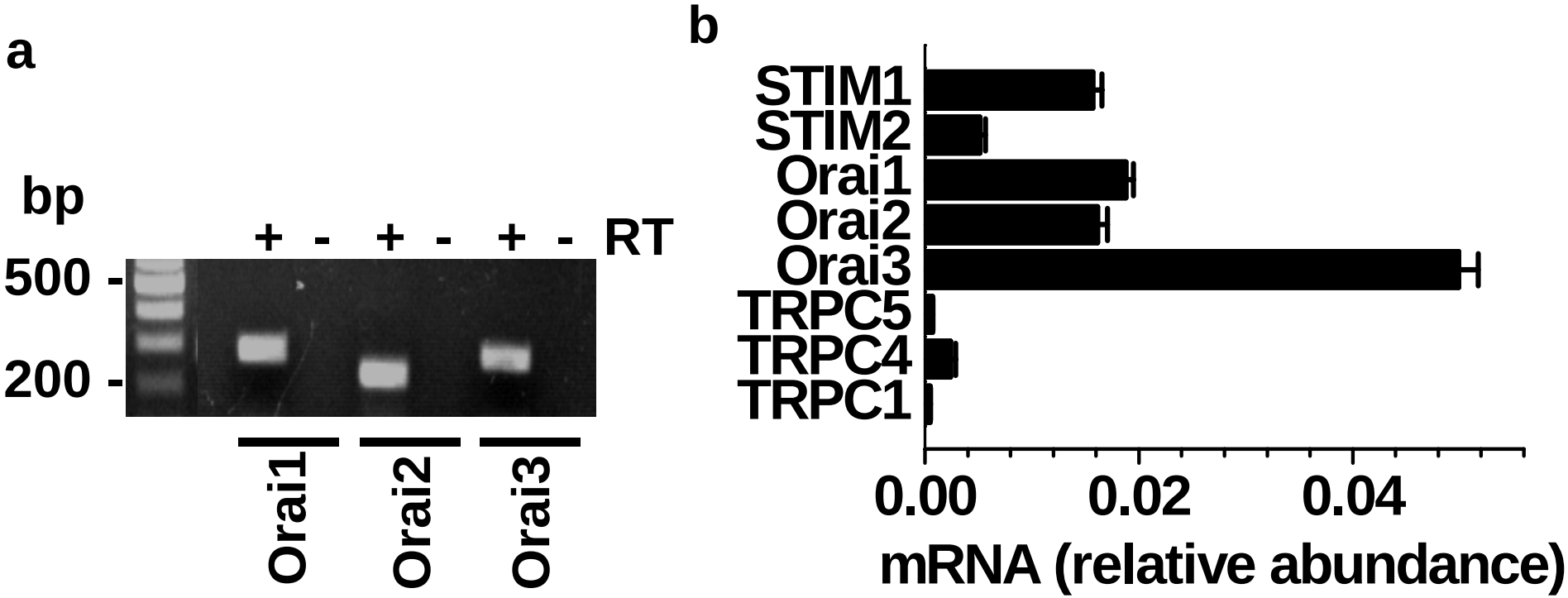
Supplementary Figure III. Validation and specificity of siRNA knock-down of *Orai* expression. See Supplementary Table I for annotations. (**a**) Validation of knock-down, showing relative abundances of Orai1, 2 and 3 mRNAs after transfection of vascular smooth muscle cells with Orai1, 2 and 3 siRNAs as indicated (n/N=3/6). The control was scrambled siRNA. (**b**) Validation of specificity, showing lack of effect on the non-target mRNAs (as indicated) after transfection of vascular smooth muscle cells with the indicated siRNAs (n/N=2-3/4-6).

Supplementary Figure IV. Endogenous Orai1 protein detection and knock-down in human saphenous vein VSMCs. Western blot for lysates from cells transfected with control (scrambled) siRNA (siRNA ctrl) or Orai1 siRNA 1 (Orai1 si.1). The blot was probed with anti-Orai1 antibody (upper panel) and anti-β-actin antibody (lower panel). The predicted protein mass of Orai1 is 33 kDa (arrow).

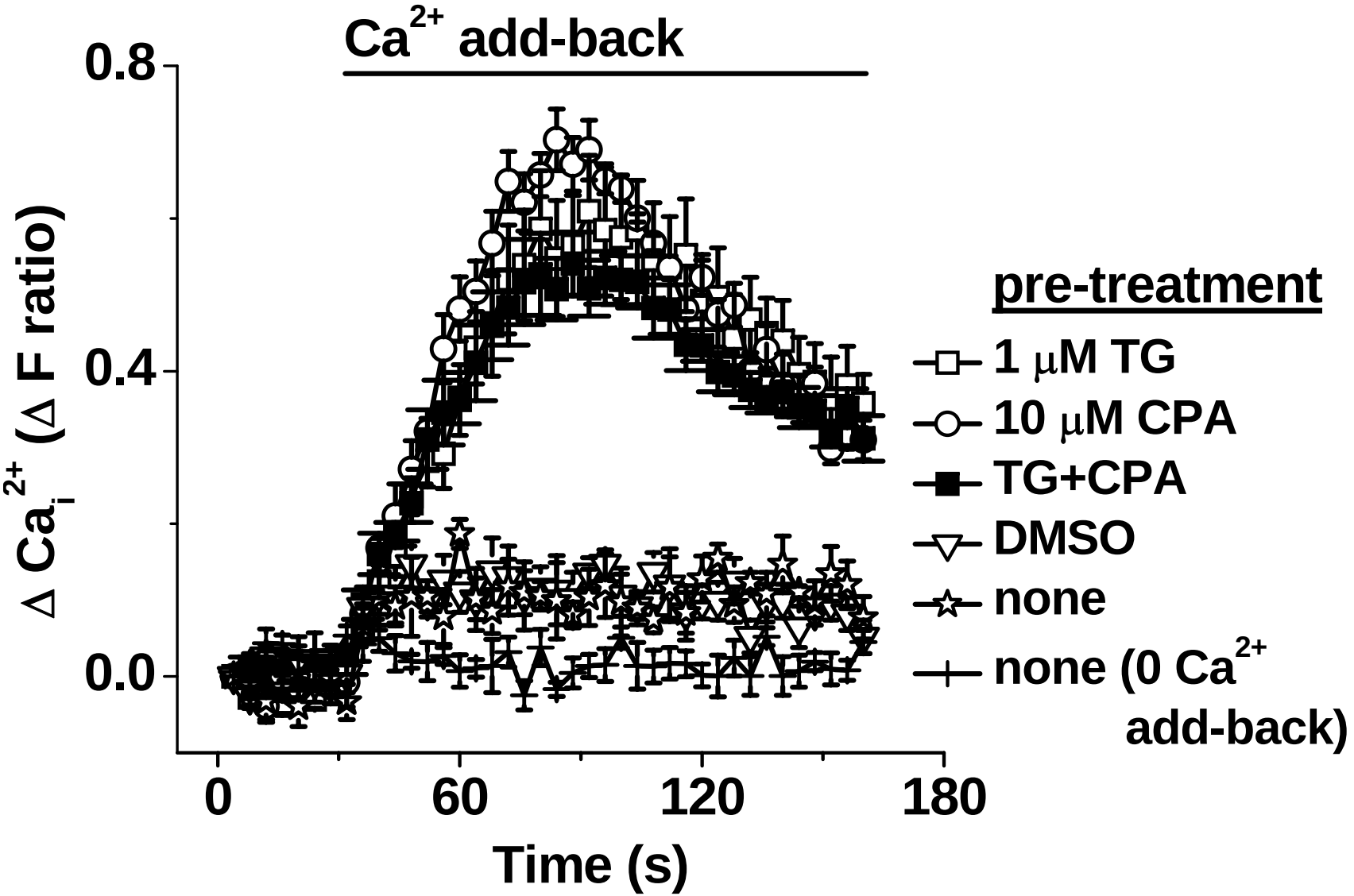
Supplementary Figure V. Typical original traces for the mean data shown in Figure 1a. For N=16 per individual experiment, intracellular Ca^{2+} measurements from VSMCs pre-treated with 1 μM thapsigargin in Ca^{2+}-free bath solution for 30 min and then exposed to 0.2 mM extracellular Ca^{2+} as indicated by the horizontal bars: comparing control (scrambled) siRNA with Orai1 siRNA 1 (**a**), Orai2 siRNA (**b**), Orai3 siRNA (**c**), Orai2 and Orai3 siRNAs (**d**) and Orai1 siRNA 2 (**e**); and comparing DNA vector with Orai1 R91W mutant (dominant negative) (**f**).

Supplementary Figure VI. In support of the dotted curve in Fig 2b, the graph shows the mean rate of rise data points without normalization and with the fitted Hill equation. The y-axis is the linear slope of the initial rate of rise of the fura-2 fluorescence after extracellular Ca^{2+} was added to the medium.

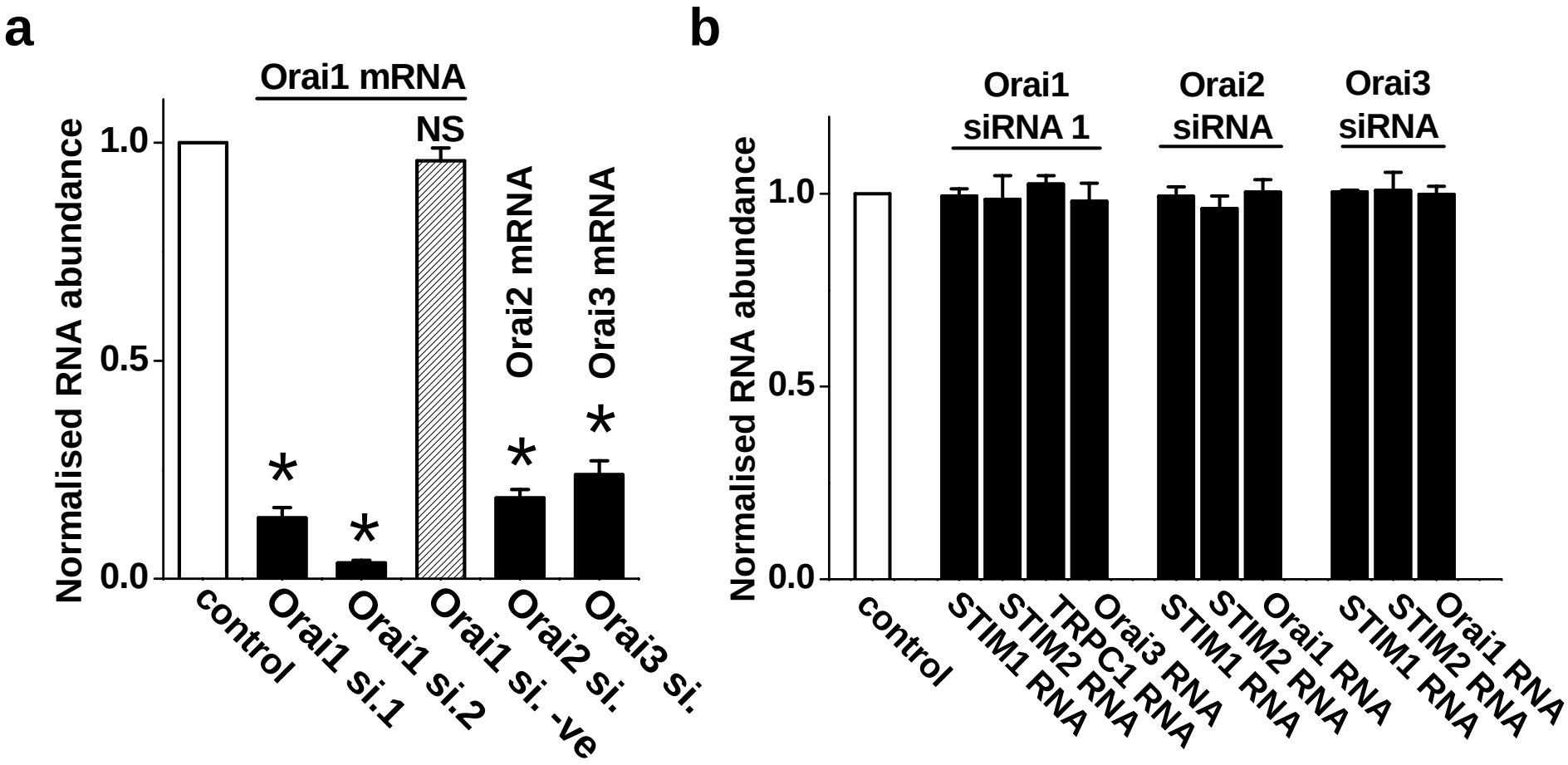
Supplementary Figure VII. Original data for VSMC migration assays. Typical bright-field images of human saphenous vein VSMCs that had moved through pores in the polycarbonate membrane for vehicle control (ctrl) and 1 μM S66. A cell is indicated by a white arrow and a pore by a black arrow.



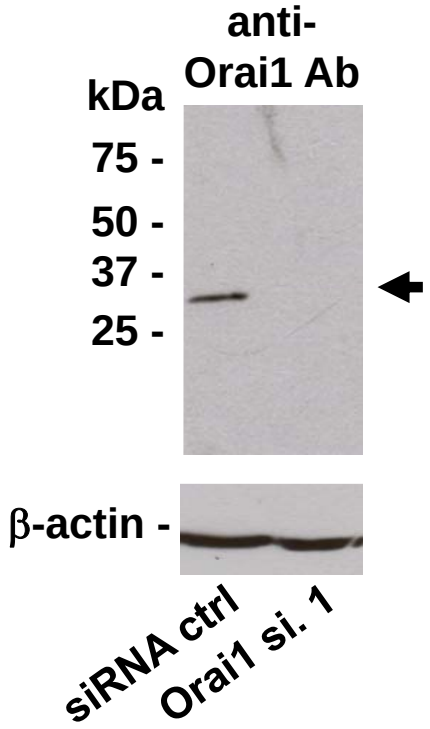
Supplemental Figure I



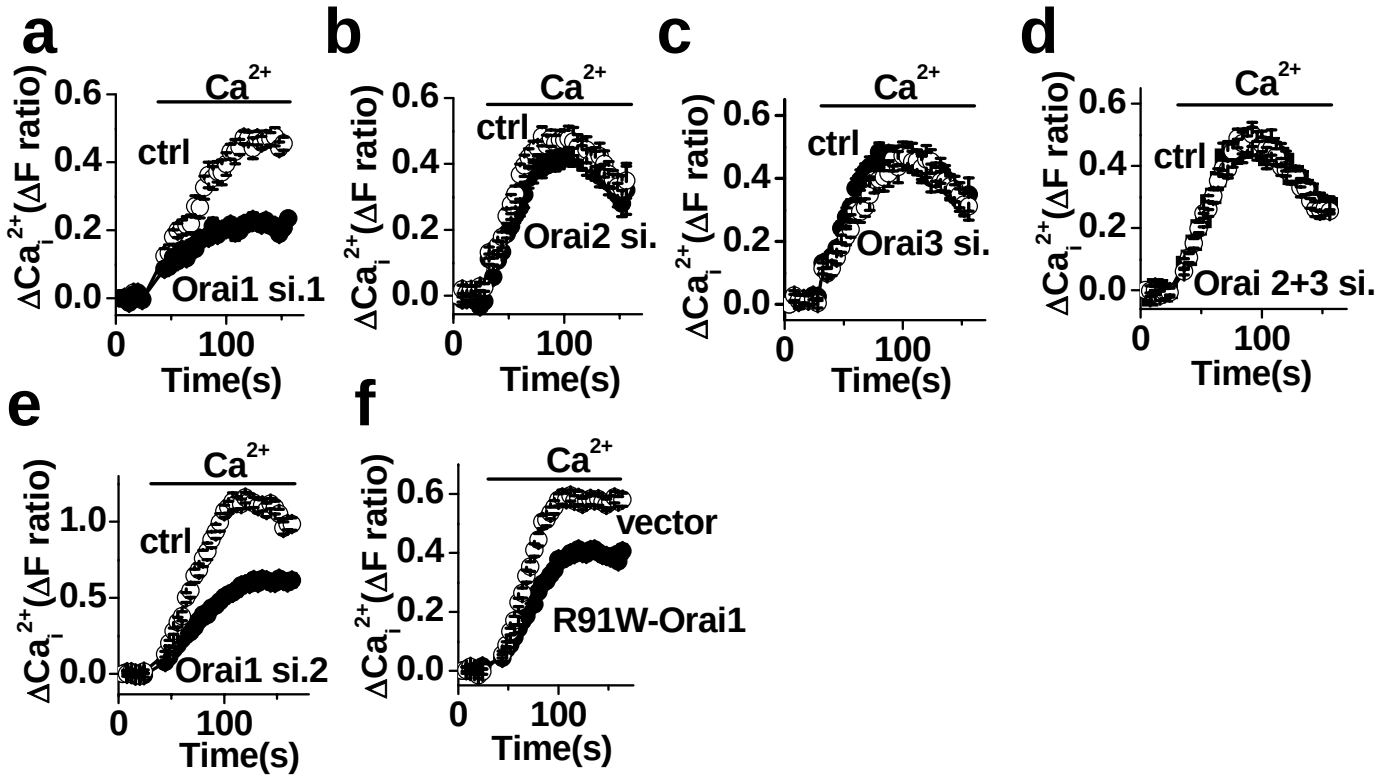
Supplemental Figure II



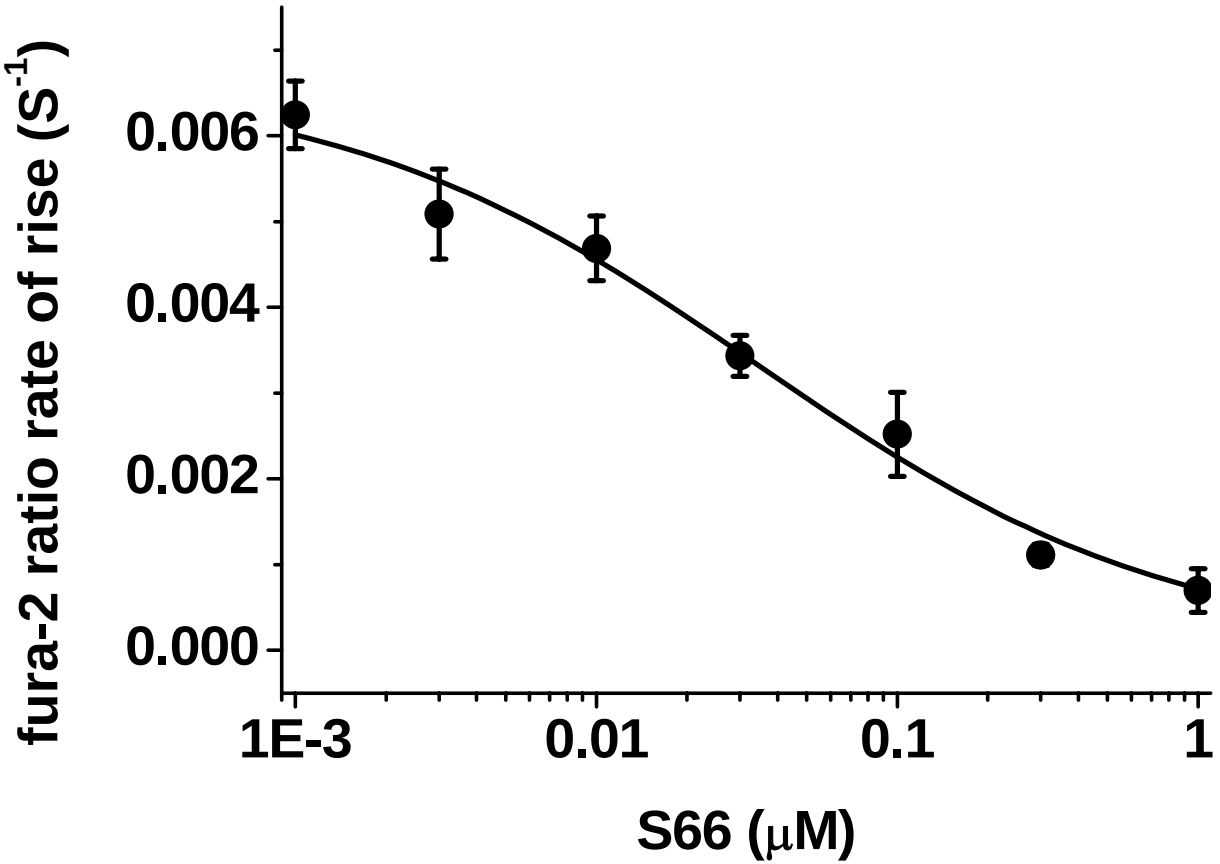
Supplemental Figure III



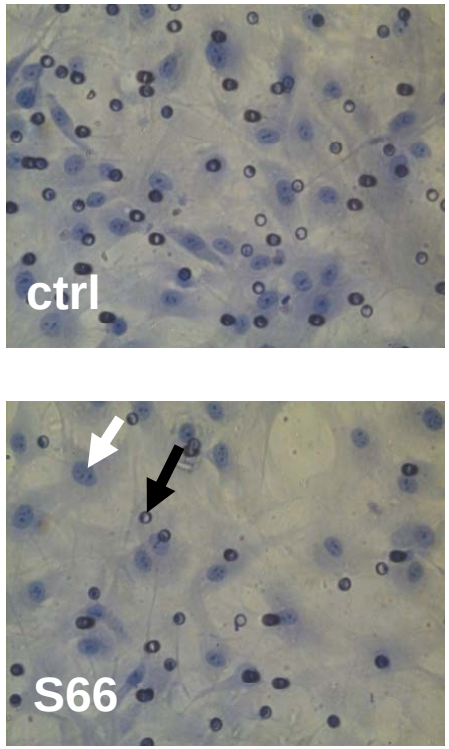
Supplemental Figure IV



Supplemental Figure V



Supplemental Figure VI



Supplemental Figure VII