Supplemental materials

Suppl. experimental procedures

Solutions for Ca^{2+} Imaging. The standard solutions with (Ca2) or without (Ca0) calcium were used for cell imaging studies. The chemical composition of the solutions is summarized in Table S1. The osmolality of imaging solution was 319 ± 7 mOsm. The pH of imaging solutions was 7.35, adjusted by sodium hydroxide. Gd^{3+} was added as $GdCl_3$.

Supplemental figure legends

Suppl. Fig. S1. Validation of effective mRNA silencing in HUVECs. RT-PCRs and quantitative-PCRs were performed using gene-specific primers. (*A*) Representative RT-PCR results confirm siRNA-mediated gene silencing of STIM1 in HUVECs. The mRNA levels of GAPDH from individual samples were examined to verify the application of equal amount of total RNAs for RT-PCR assay. M: DNA markers; Ctl: control; NT: non-targeting. (*B*) Quantitative-PCR analysis of STIM1 expression at the mRNA level indicates effective silencing of STIM1 by STIM1-siRNA. KD: knocking-down. (*C*) Representative RT-PCR data show specific silencing of the Orai genes in cells treated with targeting siRNAs. GAPDH mRNA levels were examined to verify the equal loading of RT-PCR assay. (*D* - *F*) Bar graphs indicate relative mRNA levels of Orai1 (*D*), Orai2 (*E*), and Orai3 (*F*) from four groups of samples: control HUVECs, HUVECs treated with Orai1-siRNA (O1-KD), Orai2-siRNA (O2-KD), or Orai3-siRNA (O3-KD).

Suppl. Fig. S2. Endogenous expression of Orai2 or Orai3 proteins is not detectable in HUVECs. Representative Western blot results with three different anti-Orai2 antibodies and two different anti-Orai3 antibodies are shown. Cells transfected with human Orai2 (left) or Orai3 plasmids (right) were used as positive controls and the corresponding cell lysates were loaded with (1:10, lane 2 and 1:100, lane 3) or without dilutions (lane 1). Cell lysates from untransfected HUVECs were loaded into lane 4. All the antibodies could effectively detect the corresponding, exogenous Orai proteins. MW: molecular weight.

Supplemental tables

Table S1. Standard Solutions (all concentrations in mM)

Solution	Ca2	Ca0
NaCl	155	153
KCl	4.5	4.5
$CaCl_2$	2	0
$MgCl_2$	1	3
EGTA	0	1
HEPES	5	5
D-glucose	10	10

Table S2. Mean Values, Standard Error of the Mean (SEM) and Sample Size (*n*) of Experiments

Figure 1*G*, Peak $[Ca^{2+}]_i$ (nM)			
	Mean	SEM	*n* (cell numbers)
1 μM histamine in Ca2	97	7	179
1 μM histamine in Ca0	97	13	93
10 μM histamine in Ca2	223	6	278
10 μM histamine in Ca0	191	11	135
100 μM histamine in Ca2	278	4	813
100 μM histamine in Ca0	256	12	98
Figure 1*H*, Sustained $[Ca^{2+}]_i$ (nM), 4 min after His application			
	Mean	SEM	*n* (cell numbers)
1 μM histamine in Ca2	88	6	179
1 μM histamine in Ca0	21	1	93
10 μM histamine in Ca2	126	4	278
10 μM histamine in Ca0	19	1	135
100 μM histamine in Ca2	115	2	813
100 μM histamine in Ca0	24	1	98
Figure 2*J*, Peak $[Ca^{2+}]_i$ (nM)			
	Mean	SEM	*n* (cell numbers)
10 μM histamine in Ca0	191	11	135
10 μM His in Ca0, Dip	63	2	102
10 μM His in Ca0, FEX	60	2	138
Figure 2*J*, Sustained $[Ca^{2+}]_i$ (nM), 4 min after His application			
	Mean	SEM	*n* (cell numbers)
10 μM histamine in Ca0	19	1	135
10 μM His in Ca0, Dip	38	1	102
10 μM His in Ca0, FEX	28	1	138
Figure 2*K*, Peak $[Ca^{2+}]_i$ (nM)			
	Mean	SEM	*n* (cell numbers)
10 μM histamine in Ca2	223	6	278
10 μM His in Ca2, Dip	45	1	131
10 μM His in Ca2, FEX	62	3	264

Figure 2*K*, Sustained $[Ca^{2+}]_i$ (nM), 4 min after His application			
	Mean	SEM	*n* (cell numbers)
10 μM histamine in Ca2	126	4	278
10 μM His in Ca2, Dip	39	1	131
10 μM His in Ca2, FEX	57	3	264
Figure 2*L*, Peak $[Ca^{2+}]_i$ (nM)			
	Mean	SEM	*n* (cell numbers)
2 μM TG in Ca2	149	6	134
2 μM TG in Ca2, Dip	183	8	132
2 μM TG in Ca2, FEX	165	8	130
Figure 2*L*, Sustained $[Ca^{2+}]_i$ (nM), 4 min after TG application			
	Mean	SEM	*n* (cell numbers)
2 μM TG in Ca2	137	5	134
2 μM TG in Ca2, Dip	174	8	132
2 μM TG in Ca2, FEX	154	8	130
Figure 3*F*, Sustained $[Ca^{2+}]_i$ (nM) with TG, 5 min after Ca2 application			
	Mean	SEM	*n* (cell numbers)
Ca2	155	5	303
Ca2 + Gd^{3+}	74	5	92
Ca2 + 2-APB	61	4	69
Ca2 + SKF96365	48	3	68
Ca2 + BTP2	58	3	64
Figure 3*L*, Sustained $[Ca^{2+}]_i$ (nM), 4 min after His application			
	Mean	SEM	*n* (cell numbers)
100 μM histamine in Ca2	115	2	813
100 μM His in Ca2, Gd^{3+}	74	5	147
100 μM His in Ca2, 2-APB	33	2	115
100 μM His in Ca2, SKF96365	78	3	252
100 μM His in Ca2, BTP2	65	2	218
Figure 4*D*, Sustained $[Ca^{2+}]_i$ (nM), 4 min after His application			
	Mean	SEM	*n* (cell numbers)

100 μM histamine in Ca2	115	2	813
100 μM His in Ca2, Gd^{3+}	54	3	65
100 μM His in Ca2, BTP2	48	2	96
Figure 5*G*, Peak $[Ca^{2+}]_i$ (nM) with TG			
	Mean	SEM	*n* (cell numbers)
Control	223	7	337
Non-targeting siRNA	190	11	163
STIM1 siRNA	59	8	132
Figure 5*H*, Sustained $[Ca^{2+}]_i$ (nM), 4 min after His application			
	Mean	SEM	*n* (cell numbers)
Control	115	2	813
Non-targeting siRNA	93	5	107
STIM1 siRNA	40	4	135
Figure 7*D*, Peak $[Ca^{2+}]_i$ (nM) with TG			
	Mean	SEM	*n* (cell numbers)
eGFP	205	16	44
eGFP-Orai1-E106A	34	3	34
eGFP-Orai1-R91W	83	8	24
Figure 7*H*, Sustained $[Ca^{2+}]_i$ (nM), 4 min after His application			
	Mean	SEM	*n* (cell numbers)
eGFP	130	7	65
eGFP-Orai1-E106A	27	5	31
eGFP-Orai1-R91W	63	6	32
Figure 8*C*, Peak $[Ca^{2+}]_i$ (nM) with TG			
	Mean	SEM	*n* (cell numbers)
eGFP	205	16	44
STIM1 + eGFP-Orai1	948	64	31
Figure 8*F*, Sustained $[Ca^{2+}]_i$ (nM), 4 min after His application			
	Mean	SEM	*n* (cell numbers)
eGFP	130	7	65
STIM1 + eGFP-Orai1	806	44	16
Figure 9*L*, Nuclear translocation of NFAT (% of total cells)			

	Mean	SEM	*n* (cell numbers)
Control	7	4	85
100 μM histamine in Ca2	95	2	139
100 μM His in Ca2, FEX	14	10	54
100 μM His in Ca0	10	10	53
100 μM His in Ca2, CsA	2	2	50
100 μM His in Ca2, Gd^{3+}	16	9	46
100 μM His in Ca2, STIM1 siRNA	30	4	38
100 μM His in Ca2, Orai1 siRNA	23	9	51

Figure 11*B*, IL-8 (ng/ml)

	Mean	SEM	*n* (exp. numbers)
Control	0.55	0.03	3
100 μM histamine	1.84	0.09	3
100 μM His, CsA	0.57	0.05	3
100 μM His, Orai1 siRNA	0.89	0.05	3
100 μM His, STIM1 siRNA	0.73	0.04	3

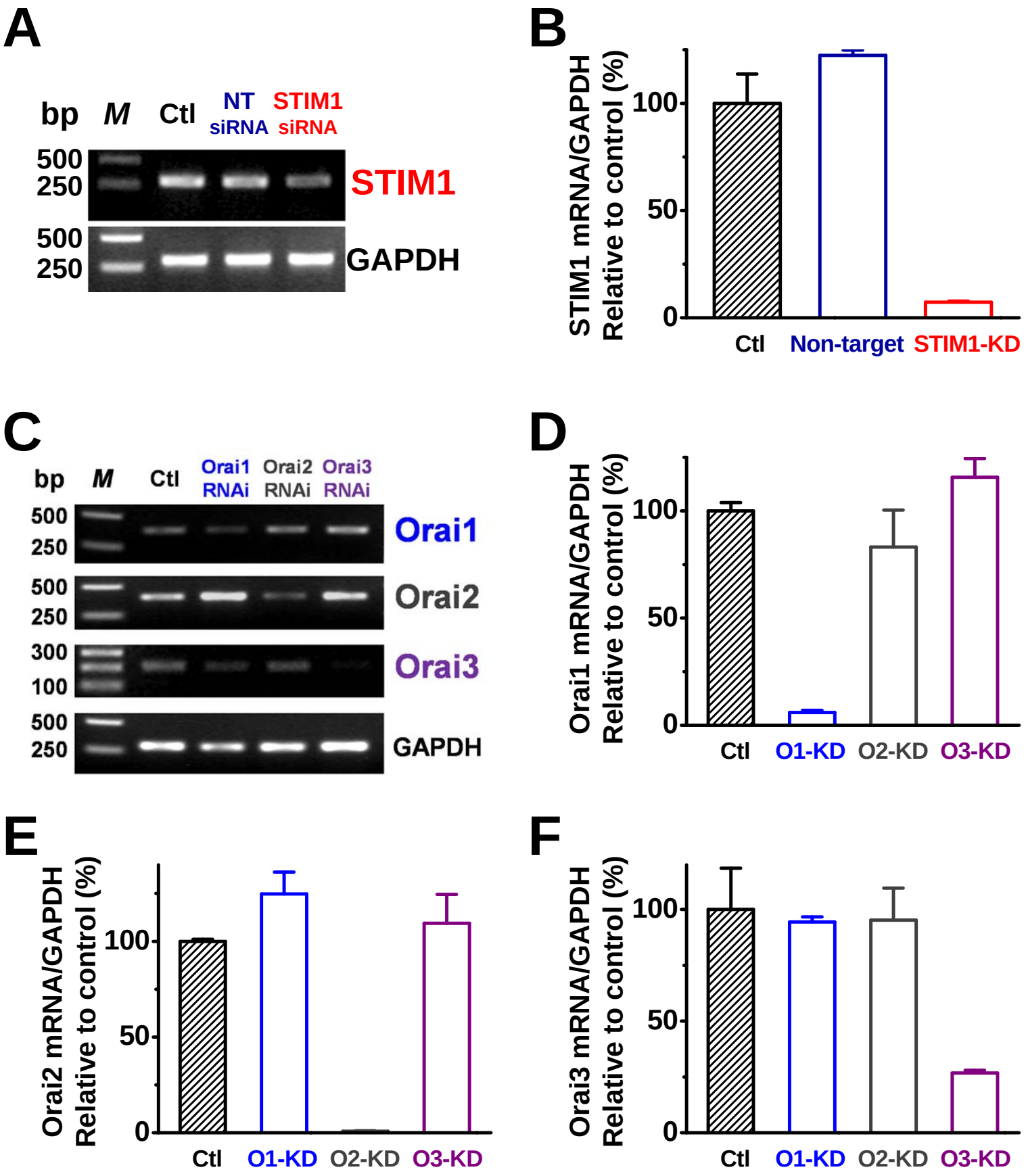
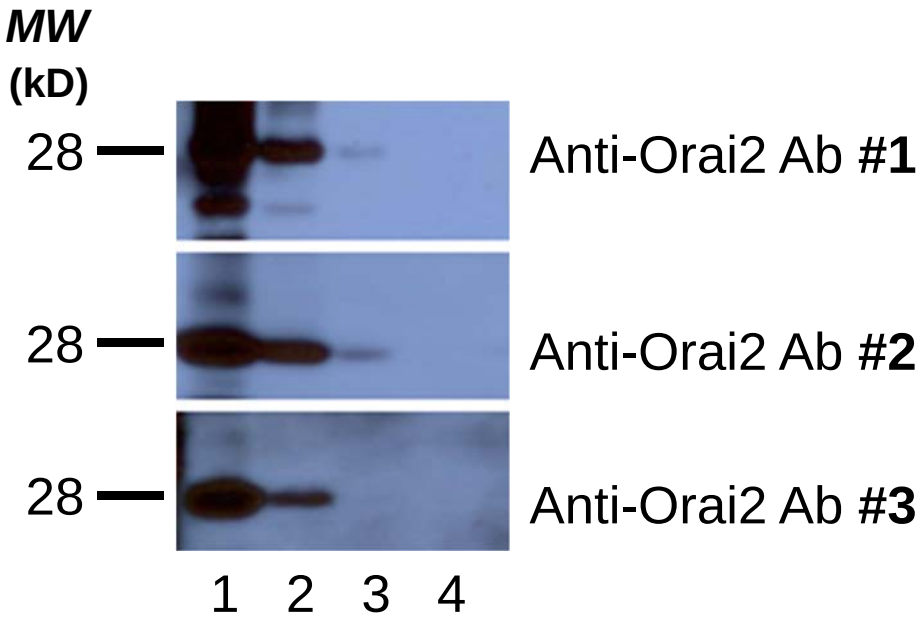

A
bp
M
Ctl
NT siRNA
STIM1 siRNA
500
250
STIM1
500
250
GAPDH
B
STIM1 mRNA/GAPDH Relative to control (%)
100
50
0
Ctl
Non-target
STIM1-KD
C
bp
M
Ctl
Orai1 RNAi
Orai2 RNAi
Orai3 RNAi
500
250
Orai1
500
250
Orai2
300
100
Orai3
500
250
GAPDH
D
Orai1 mRNA/GAPDH Relative to control (%)
100
50
0
Ctl
O1-KD
O2-KD
O3-KD
E
Orai2 mRNA/GAPDH Relative to control (%)
100
50
0
Ctl
O1-KD
O2-KD
O3-KD
F
Orai3 mRNA/GAPDH Relative to control (%)
100
50
0
Ctl
O1-KD
O2-KD
O3-KD

Figure S1


MW
(kD)
28
Anti-Orai2 Ab #1
28
Anti-Orai2 Ab #2
28
Anti-Orai2 Ab #3
1 2 3 4

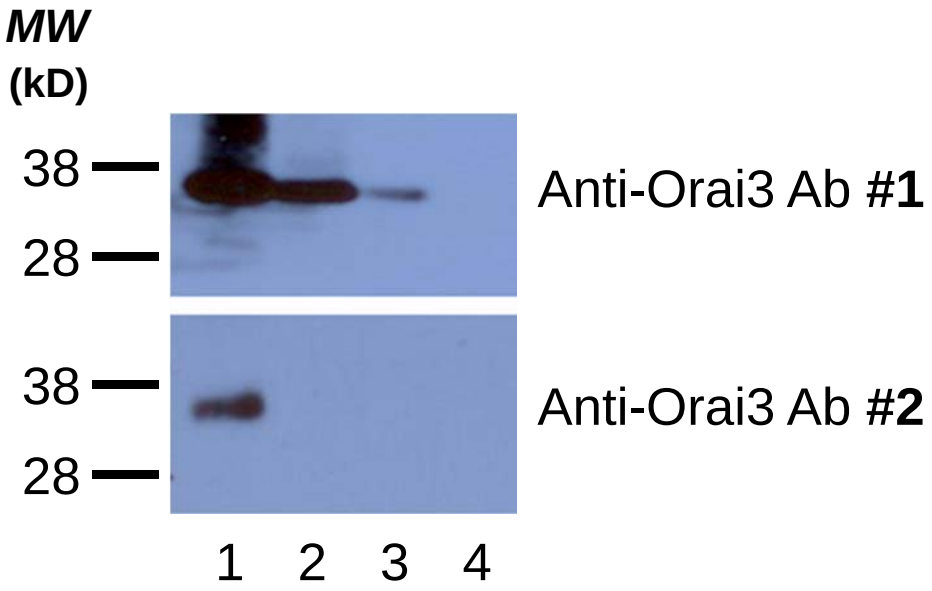

MW
(kD)
38
28
Anti-Orai3 Ab #1
38
28
Anti-Orai3 Ab #2
1 2 3 4

Figure S2