Supplemental Data

STIM2 Is a Feedback Regulator that

Stabilizes Basal Cytosolic and

Endoplasmic Reticulum Ca^{2+} Levels

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Table S1.

Units are fold standard deviations from median Fura-2 ratio. Gene names are based on the NCBI nomenclature.

gene	low Ca$^{2+}_{ext}$ condition	high Ca$^{2+}_{ext}$ condition
ATP2A1	-0.39289	-0.9418
ATP2A2	11.3003	0.142401
ATP2A3	-0.35855	1.1669
ATP2B1	1.641	33.0733
ATP2B2	-0.57369	-2.2089
ATP2B3	-0.12276	-0.035867
ATP2B4	0.33795	-1.5009
CACNA1A	-0.58811	0.79601
CACNA1B	0.17067	0.36025
CACNA1C	-0.65638	0.73057
CACNA1D	-0.17218	0.2902
CACNA1E	-1.8773	1.03
CACNA1F	0.088143	2.1226
CACNA1G	0.086275	-0.19972
CACNA1H	1.3862	1.4276
CACNA1I	-0.9814	-0.79492
CACNA1S	-2.4325	-1.098
ITPKA	1.7505	-0.64677
ITPKB	1.5912	1.0806
ITPR1	1.6406	-0.12037
ITPR2	1.2437	0.9335
ITPR3	0.51593	-2.2621
PSEN1	-2.2494	0.18205

PSEN2	-0.11029	-1.7258
SLC8A1	-2.6531	0.15688
SLC8A2	0.063456	0.049516
SLC8A3	-2.4966	-0.81094
TRPA1	-2.0646	-0.63463
TRPC1	0.31108	0.63618
TRPC3	-1.3776	2.274
TRPC4	0.32254	0.58842
TRPC5	-0.21547	-0.79262
TRPC6	-0.15239	-0.66884
TRPC7	-1.2503	1.7674
TRPM1	-0.55947	-2.1096
TRPM2	-1.6407	0.45256
TRPM3	-0.31696	1.0616
TRPM4	-1.0307	-0.45337
TRPM5	-0.89363	0.5181
TRPM6	-0.42829	1.6638
TRPM7	-3.5	0.67418
TRPM8	-0.19636	-0.49052
TRPV1	0.43127	-0.058567
TRPV2	-0.019	1.6494
TRPV3	-0.72963	-0.76843
TRPV4	-0.36298	0.85333
TRPV5	0.23015	2.4792
TRPV6	-0.19674	2.9569

Table S2.

Units are fold standard deviations from median Fura-2 ratio.

gene	low Ca$^{2+}_{ext}$ condition	high Ca$^{2+}_{ext}$ condition
ACTR3	-2.6427	1.032
AKAP7	3.0534	-0.91885
CALM1	2.0908	9.6558
CLMN	-0.47456	-2.4542
FLJ45651	1.1813	-2.1474
FNBP2	-0.55684	-2.683
HAPIP	-2.9538	0.29199
KIAA0007	-1.3395	2.0522
LOC124685	0.031095	2.4768
LOC389246	2.5197	1.12
LOC55971	3.2384	0.34667
PIP5K1A	-2.0378	1.1826
PPEF1	0.87681	-2.7225
PPP1R12A	2.3008	0.33042
PRKAR1A	1.9521	-3.0284
RAC1	-3.4616	-2.6044
SEC23IP	1.3801	-2.3268
SSH1	-0.18364	-2.0778
STIM2	-6.5051	-1.8468

Figure S1
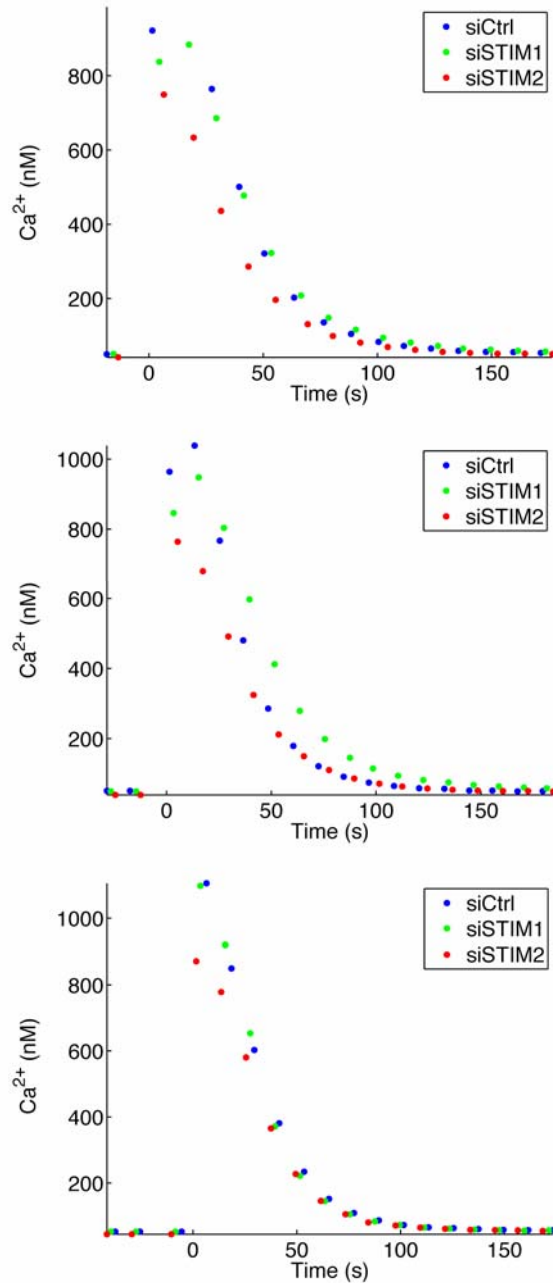

Single-well traces to indirectly assay ER Ca2+ concentration in Figure 2C. Addition of ionomycin and EGTA was used to rapidly release ER Ca2+ and the resulting cytosolic Ca2+ signal was measured using Fura-2. Cells were transfected with siRNA for STIM1, STIM2 or GL3 (control).

Figure S2
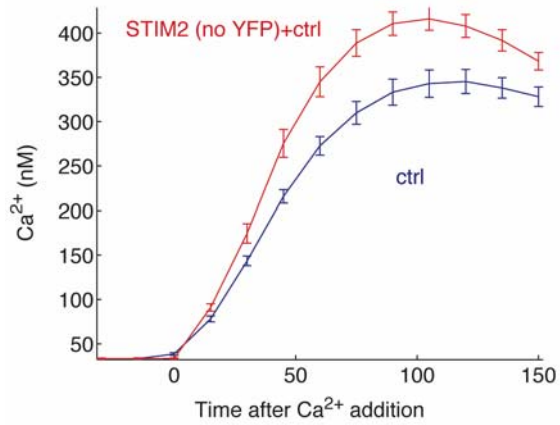
A wildtype STIM2 construct (without YFP tag) also increases R-SOC Ca^{2+} influx. Cells were transfected with YFP or co-transfected with YFP and YFP-free STIM2 for 5 hours. Cells were selected expressing 7.5 to 15 times background. N=6 sites from 3 wells. Error bars represent standard error.

Figure S3
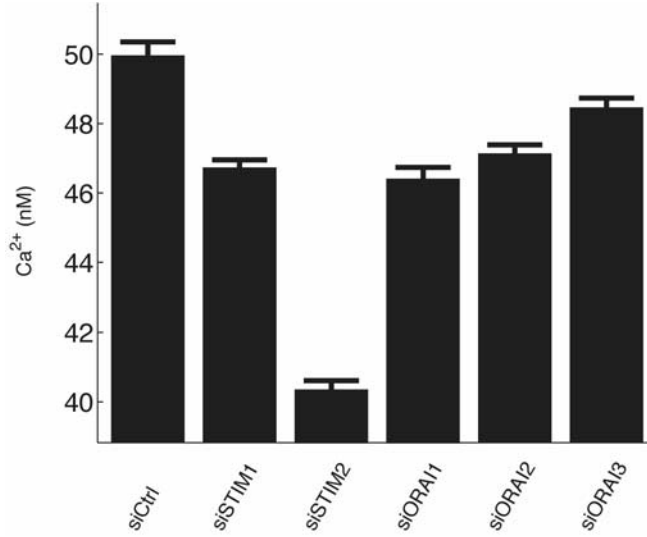
Basal cytosolic Ca^{2+} measurements after 3 day transfection with siSTIM1-2 and, for comparison, siOrai1-3 and GL3 (control). N=10 sites. Error bars represent standard error.

Figure S4
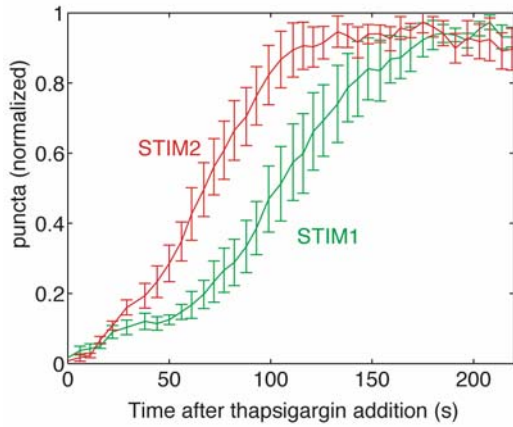
Time-course of STIM1 and STIM2 puncta formation upon thapsigargin addition. 1 μM thapsigargin was added to HeLa cells and imaged for 220 seconds. Images were then analyzed for puncta content as in described in Materials and Methods section. N=4 cells each. Error bars represent standard error.

Figure S5
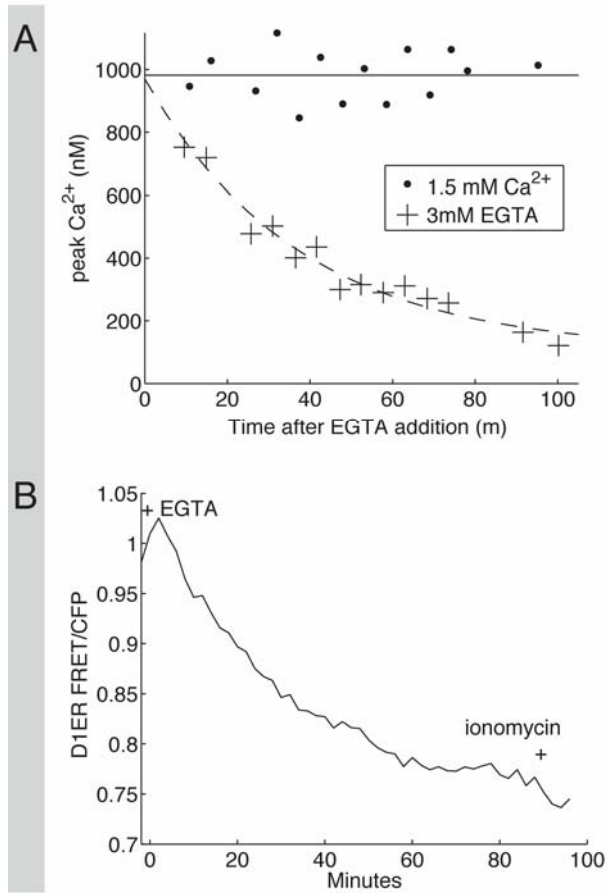
Calibration of the ER Ca^{2+} content at different time-points following external addition of EGTA. (A) 3 mM EGTA was added to wells at time = 0 min. Ionomycin was added to different wells at the indicated time points. The measured ΔCa^{2+} peak heights were fit to an exponential decay. (B) FRET measured using the D1ER cameleon probe. Average relative FRET signal for 6 cells imaged using a 40x confocal microscope. 1 μM ionomycin was added near the end of the timecourse.

Figure S6
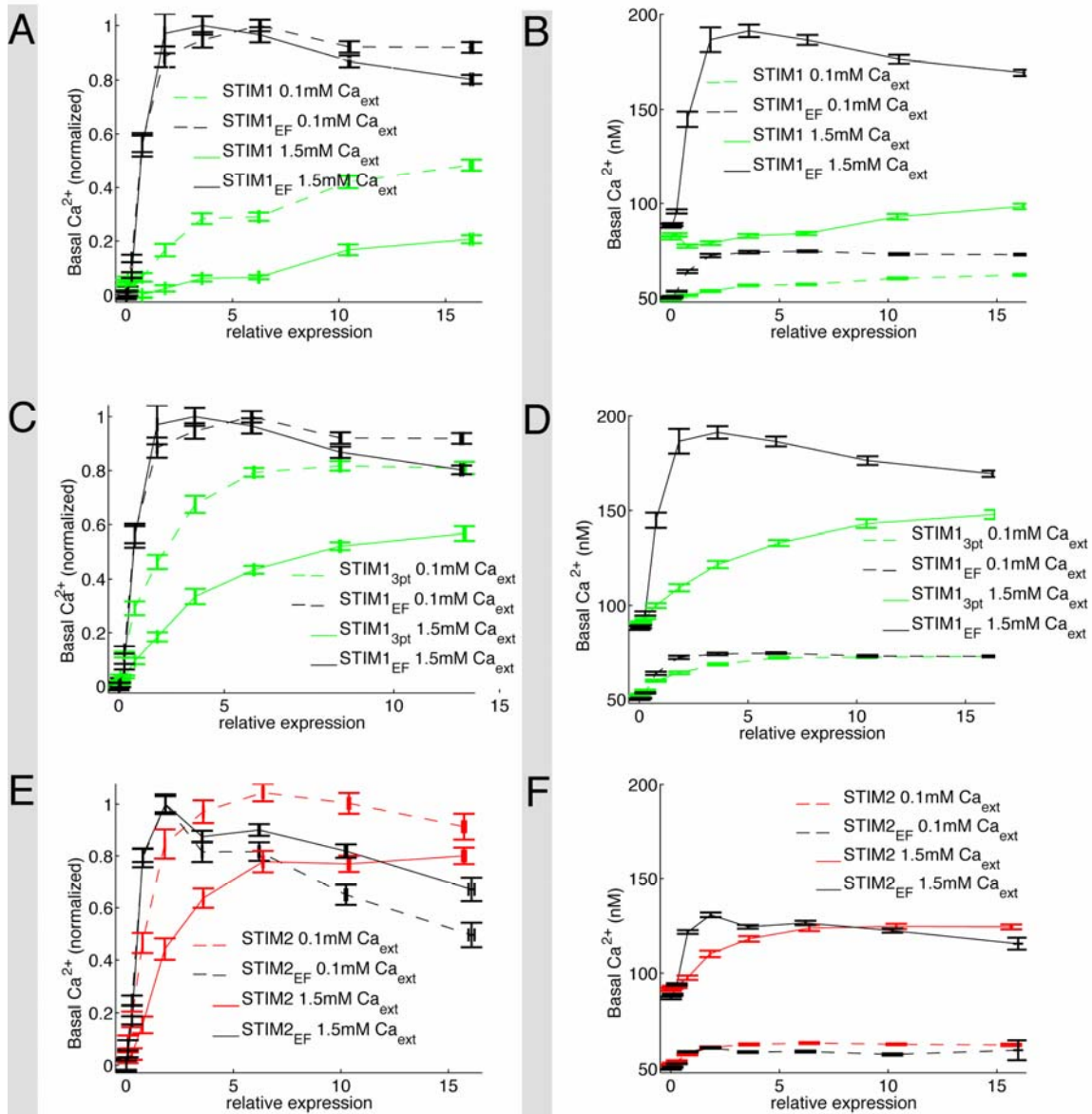
Ca^{2+} levels in cells expressing different concentrations of STIM1 and STIM2 constructs. Basal Ca^{2+} was measured for the reduced and normal ER conditions as described in the main text (reduced conditions are the low Ca2+ conditions from the siRNA screen). Both raw traces and traces normalized to constitutively active mutants are shown. EF hand switch mutant ($STIM1_{EF->STIM2}$) is labeled with the subscript "3pt". Error bars represent standard error.

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Figure S7
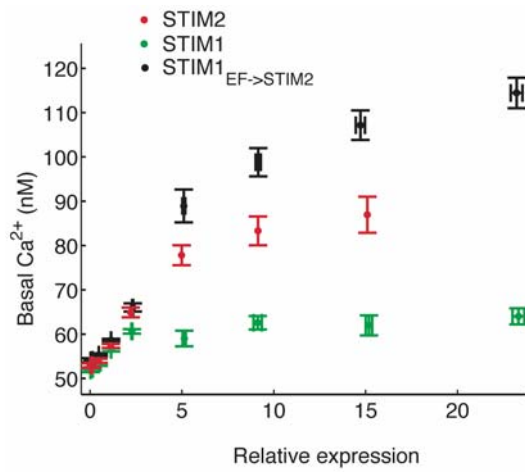
Basal Ca^{2+} levels in cells expressing a STIM1 construct with its EF hand mutated to be similar to STIM2 (STIM1$_{EF->STIM2}$). Basal Ca^{2+} concentration is shown as a function of the expression level of YFP-STIM1$_{EF->STIM2}$ and compared to that of YFP-STIM1, YFP-STIM2 and YFP control. Error bars represent standard error.

Figure S8

STIM2 Primary accession number: Q9P246

Construct name	Description	Differences from Swiss-Prot
STIM2	CMV_SP_YFP_STIM2(15-746AA)	P38L, V222I, E575V
STIM2 (no tag)	CMV_SP_STIM2(15-746AA)	P38L, V222I, E575V
STIM2$_{EF}$	CMV_SP_YFP_STIM2(15-746AA)	P38L, V222I, E575V, D80A
STIM2$_{EF->STIM1}$	CMV_SP_YFP_STIM2(15-746AA)	P38L, V222I, E575V, K83A,D84N, G86D
STIM1	CMV_SP_YFP_STIM1(23-685AA)	
STIM1$_{EF}$	CMV_SP_YFP_STIM1(23-685AA)	D76A
STIM1$_{EF->STIM2}$	CMV_SP_YFP_STIM1(23-685AA)	A79K, N80D, D82G

Cloned STIM2 DNA sequence (15-746AA):

TGCGAGCTTGTGCCCCGGCACCTCCGCGGGCGGCGGGCGACTGGCTCTGCCGCAACTGCCGCCTCCTCTCTCGCCGCGGC
GGCCGGCGATAGCCCGGCGCTCATGACAGATCCCTGCATGTCACTGAGTCCACCATGCTTTACAGAAGAAGCACAGATTTA
GTCTGGAAGCTCTTCAAACAATACATAAACAAATGGATGATGACAAAGATGGTGGAATTGAAGTAGAGGAAAGTGATGAA
TTCATCAGAGAAGATATGAAATATAAAGATGCTACTAATAAACACAGCCATCTGCACAGAGAAGATAAACATATAACGAT
TGAGGATTTATGGAAACGATGGAAAACATCAGAAGTTCATAATTGGACCCTTGAAGACACTCTTCAGTGGTTGATAGAGT
TTGTTGAACTACCCCAATATGAGAAGAATTTTAGAGACAACAATGTCAAAGGAACGACACTTCCCAGGATAGCAGTGCAC
GAACCTTCATTTATGATCTCCCAGTTGAAAATCAGTGACCGGAGTCACAGACAAAAACTTCAGCTCAAGGCATTGGATGT
GGTTTTGTTTGGACCTCTAACACGCCCACCTCATAACTGGATGAAAGATTTTATCCTCACAATTTCTATAGTAATTGGTG
TTGGAGGCTGCTGGTTTGCTTATACGCAGAATAAGACATCAAAAGAACATGTTGCAAAAATGATGAAAGATTTAGAGAGC
TTACAAACTGCAGAGCAAAGTCTAATGGACTTACAAGAGAGGCTTGAAAAGGCACAGGAAGAAAACAGAAATGTTGCTGT
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GGGAGGGAGCTGAATGTGAATTGAGTAGACGTCAGTATGCAGAACAGGAATTGGAACAGGTTCGCATGGCTCTGAAAAAG
GCCGAAAAAGAATTTGAACTGAGAAGCAGTTGGTCTGTTCCAGATGCACTTCAGAAATGGCTTCAGTTAACACATGAAGT
AGAAGTGCAATACTACAATATTAAAAGACAAAACGCTGAAATGCAGCTAGCTATTGCTAAAGATGAGGCAGAAAAAATTA
AAAAGAAGAGAAGCACAGTCTTTGGGACTCTGCACGTTGCACACAGCTCCTCCCTAGATGAGGTAGACCACAAAATTCTG
GAAGCAAAGAAAGCTCTCTCTGAGTTGACAACTTGTTTACGAGAACGACTTTTTCGCTGGCAACAAATTGAGAAGATCTG
TGGCTTTCAGATAGCCCATAACTCAGGACTCCCCAGCCTGACCTCTTCCCTTTATTCTGATCACAGCTGGGTGGTGATGC
CCAGAGTCTCCATTCCACCCTATCCAATTGCTGGAGGAGTTGATGACTTAGATGAAGACACACCCCCAATAGTGTCACAA
TTTCCCGGGACCATGGCTAAACCTCCTGGATCATTAGCCAGAAGCAGCAGCCTGTGCCGTTCACGCCGCAGCATTGTGCC
GTCCTCGCCTCAGCCTCAGCGAGCTCAGCTTGCTCCACACGCCCCCCCACCCGTCACACCCTCGGCACCCTCACCACCCGC
AACACACACCACACTCCTTGCCTTCCCCTGATCCAGATATCCTCTCAGTGTCAAGTTGCCCTGCGCTTTATCGAAATGAA
GTGGAGGAAGAGGCCATTTACTTCTCTGCTGAAAAGCAATGGGAAGTGCCAGACACAGCTTCAGAATGTGACTCCTTAAA
TTCTTCCATTGGAAGGAAACAGTCTCCTCCTTTAAGCCTCGAGATATACCAAACATTATCTCCGCGAAAGATATCAAGAG
ATGAGGTGTCCCTAGAGGATTCCTCCCGAGGGGATTCGCCTGTAACTGTGGATGTGTCTTGGGGTTCTCCCGACTGTGTA
GGTCTGACAGAGACTAAGAGTATGATCTTCAGTCCTGCAAGCAAAGTGTACAATGGCATTTTGGAGAAATCCTGTAGCAT
GAACCAGCTTTCCAGTGGCATCCCGGTGCCTAAACCTCGCCACACATCATGTTCCTCAGCTGGCAACGACAGTAAACCAG
TTCAGGAAGCCCCAAGTGTTGCCAGAATAAGCAGCATCCCACATGACCTTTGTCATAATGGAGAGAAAAGCAAAAAGCCA
TCAAAAATCAAAAGCCTTTTTAAGAAGAAATCTAAGTGA

STIM1 DNA sequence (23-685AA):

CTCAGCCATAGTCACAGTGAGAAGGCGACAGGAACCAGCTCGGGGGCCAACTCTGAGGAGTCCACTGCAGCAGAGTTTTG
CCGAATTGACAAGCCCCTGTGTCACAGTGAGGATGAGAAACTCAGCTTCGAGGCAGTCCGTAACATCCACAAACTGATGG
ACGATGATGCCAATGGTGATGTGGATGTGGAAGAAAGTGATGAGTTCCTGAGGGAAGACCTCAATTACCATGACCCAACA
GTGAAACACAGCACCTTCCATGGTGAGGATAAGCTCATCAGCGTGGAGGACCTGTGGAAGGCATGGAAGTCATCAGAAGT
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AGCTGCAGCTCAGTGGCCATGCCATGCCAAGGCTGGCTGTCACCAACACCACCATGACAGGGACTGTGCTGAAGATGACA
GACCGGAGTCATCGGCAGAAGCTGCAGCTGAAGGCTCTGGATACAGTGCTCTTTGGGCCTCCTCTCTTGACTCGCCATAA

TCACCTCAAGGACTTCATGCTGGTGGTGTCTATCGTTATTGGTGTGGGCGGCTGCTGGTTTGCCTATATCCAGAACCGTT
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GAAAGGCTGCACAAGGCCCAGGAGGAGCACCGCACAGTGGAGGTGGAGAAGGTCCATCTGGAAAAGAAGCTGCGCGATGA
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CCGCCAGCGTGTGGCCCCCAAACCTCCTCAGATGAGCCGTGCTGCAGACGAGGCTCTCAATGCCATGACTTCCAATGGCA
GCCACCGGCTGATCGAGGGGGTCCACCCAGGGTCTCTGGTGGAGAAACTGCCTGACAGCCCTGCCCTGGCCAAGAAGGCA
TTACTGGCGCTGAACCATGGGCTGGACAAGGCCCACAGCCTGATGGAGCTGAGCCCCTCAGCCCCACCTGGTGGCTCTCC
ACATTTGGATTCTTCCCGTTCTCACAGCCCCAGCTCCCCAGACCCAGACACACCATCTCCAGTTGGGGACAGCCGAGCCC
TGCAAGCCAGCCGAAACACACGCATTCCCCACCTGGCTGGCAAGAAGGCTGTGGCTGAGGAGGATAATGGCTCTATTGGC
GAGGAAACAGACTCCAGCCCAGGCCGGAAGAAGTTTCCTCTCAAAATCTTTAAGAAGCCTCTTAAGAAGTAG

Signal Peptide DNA sequence from STIM1(1-22AA):

ATGGATGTATGCGTCCGTCTTGCCCTGTGGCTCCTCTGGGGACTCCTCCTGCACCAGGGCCAGAGC

Cloned STIM2 Protein sequence (15-746AA):

CELVPRHLRGRRATGSAATAASSLAAAAGDSPALMTDPCMSLSPPCFTEEDRFSLEALQT
IHKQMDDDKDGGIEVEESDEFIREDMKYKDATNKHSHLHREDKHITIEDLWKRWKTSEVH
NWTLEDTLQWLIEFVELPQYEKNFRDNNVKGTTLPRIAVHEPSFMISQLKISDRSHRQKL
QLKALDVVLFGPLTRPPHNWMKDFILTISIVIGVGGCWFAYTQNKTSKEHVAKMMKDLES
LQTAEQSLMDLQERLEKAQEENRNVAVEKQNLERKMMDEINYAKEEACRLRELREGAECE
LSRRQYAEQELEQVRMALKKAEKEFELRSSWSVPDALQKWLQLTHEVEVQYYNIKRQNAE
MQLAIAKDEAEKIKKKRSTVFGTLHVAHSSSLDEVDHKILEAKKALSELTTCLRERLFRW
QQIEKICGFQIAHNSGLPSLTSSLYSDHSWVVMPRVSIPPYPIAGGVDDLDEDTPPIVSQ
FPGTMAKPPGSLARSSSLCRSRRSIVPSSPQPQRAQLAPHAPHPSHPRHPHHPQHTPHSL
PSPDPDILSVSSCPALYRNEVEEEAIYFSAEKQWEVPDTASECDSLNSSIGRKQSPPLSL
EIYQTLSPRKISRDEVSLEDSSRGDSPVTVDVSWGSPDCVGLTETKSMIFSPASKVYNGI
LEKSCSMNQLSSGIPVPKPRHTSCSSAGNDSKPVQEAPSVARISSIPHDLCHNGEKSKKP
SKIKSLFKKKSKstop

STIM1 Protein sequence (23-685AA):

LSHSHSEKATGTSSGANSEESTAAEFCRIDKPLCHSEDEKLSFEAVRNIHKLMDDDANGD
VDVEESDEFLREDLNYHDPTVKHSTFHGEDKLISVEDLWKAWKSSEVYNWTVDEVVQWLI
TYVELPQYEETFRKLQLSGHAMPRLAVTNTTMTGTVLKMTDRSHRQKLQLKALDTVLFGP
PLLTRHNHLKDFMLVVSIVIGVGGCWFAYIQNRYSKEHMKKMMKDLEGLHRAEQSLHDLQ
ERLHKAQEEHRTVEVEKVHLEKKLRDEINLAKQEAQRLKELREGTENERSRQKYAEEEELE
QVREALRKAEKELESHSSWYAPEALQKWLQLTHEVEVQYYNIKKQNAEKQLLVAKEGAEK
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NNPGIHSLVAALNIDPSWMGSTRPNPAHFIMTDDVDDMDEEIVSPLSMQSPSLQSSVRQR
LTEPQHGLGSQRDLTHSDSESSLHMSDRQRVAPKPPQMSRAADEALNAMTSNGSHRLIEG
VHPGSLVEKLPDSPALAKKALLALNHGLDKAHSLMELSPSAPPGGSPHLDSSRSHSPSSP
DPDTPSPVGDSRALQASRNTRIPHLAGKKAVAEEDNGSIGEETDSSPGRKKFPLKIFKKP
LKKstop

Signal Peptide from STIM1(1-22AA):
MDVCVRLALWLLWGLLLHQGQS

Figure S9

7Dharmacon SMART STIM2 siRNA pool

1)

sense sequence

AGACACAGCUUCAGAAUGUUU

antisense sequence

5'-PACAUUCUGAAGCUGUGUCUUU

2)

sense sequence

GCUUUCAGAUAGCCCAUAAUU

antisense sequence

5'-PUUAUGGGCUAUCUGAAAGCUU

3)

sense sequence

CUUUAAGCCUCGAGAUAUAUU

antisense sequence

5'-PUAUAUCUCGAGGCUUAAAGUU

4)

sense sequence

AGAAGCAGUUGGUCUGUUCUU

antisense sequence

5'-PGAACAGACCAACUGCUUCUUU

Ambion STIM1 siRNA pool

1)

sense sequence

GGAUGCUGUCAUUUUUUGAtt

antisense sequence

UCAAAAAAUGACAGCAUCCtt

2)

sense sequence

GGAUGAGAAACUCAGCUUCtt

antisense sequence

GAAGCUGAGUUUCUCAUCCtc

3)

sense sequence

GGGAAGACCUCAAUUACCAtt

antisense sequence

UGGUAAUUGAGGUCUUCCCtc

Dharmacon SMART ORAI1 siRNA pool

1)

sense sequence

GCUCACUGGUUAGCCAUAAUU

antisense sequence

5'-PUUAUGGCUAACCAGUGAGCUU

2)

sense sequence

GGCCUGAUCUUUAUCGUCUUU

antisense sequence

5'-PAGACGAUAAAGAUCAGGCCUU

3)

sense sequence

UGAGCAACGUGCACAAUCUUU

antisense sequence

5'-PAGAUUGUGCACGUUGCUCAUU

4)

sense sequence

GCACCUGUUUGCGCUCAUGUU

antisense sequence

5'-PCAUGAGCGCAAACAGGUGCUU

Dharmacon SMART ORAI2 siRNA pool

1)

sense sequence

GAGCUUAACGUGCCUAUCGUU

antisense sequence

5'-PCGAUAGGCACGUUAAGCUCUU

2)

sense sequence

UGGAACUGGUCACCUCUAAUU

antisense sequence

5'-PUUAGAGGUGACCAGUUCCAUU

3)

sense sequence

GGGCAUGGAUUACCGGGACUU

antisense sequence

5'-PGUCCCGGUAAUCCAUGCCCUU

4)

sense sequence

CACCGUGCUUGGCAUCCUAUU

antisense sequence

5'-PUAGGAUGCCAAGCACGGUGUU

Dharmacon SMART ORAI3 siRNA pool

1)

sense sequence

GGAACUAGAGGAACUGAAUUU

antisense sequence

5'-PAUUCAGUUCCUCUAGUUCCUU

2)

sense sequence

GCACCUCUUUGCACUCAUGUU

antisense sequence

5'-PCAUGAGUGCAAAGAGGUGCUU

3)

sense sequence

CAAGACAGACCGCUACAAGUU

antisense sequence

5'-PCUUGUAGCGGUCUGUCUUGUU

4)

sense sequence

GGGUCAAGUUUGUGCCCAUUU

antisense sequence

5'-PAUGGGCACAAACUUGACCCUU