

Brailoiu et al., <http://www.jcb.org/cgi/content/full/jcb.200904073/DC1>

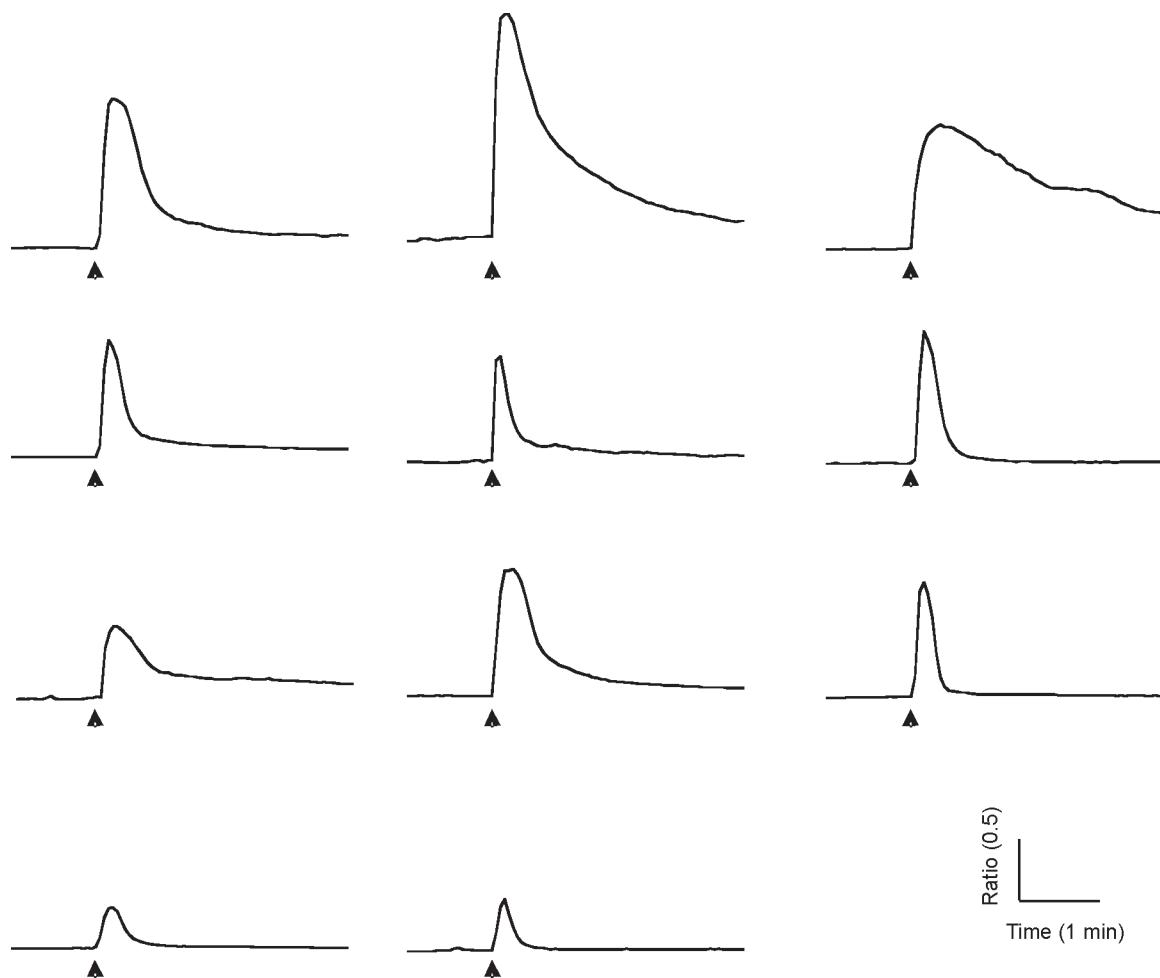
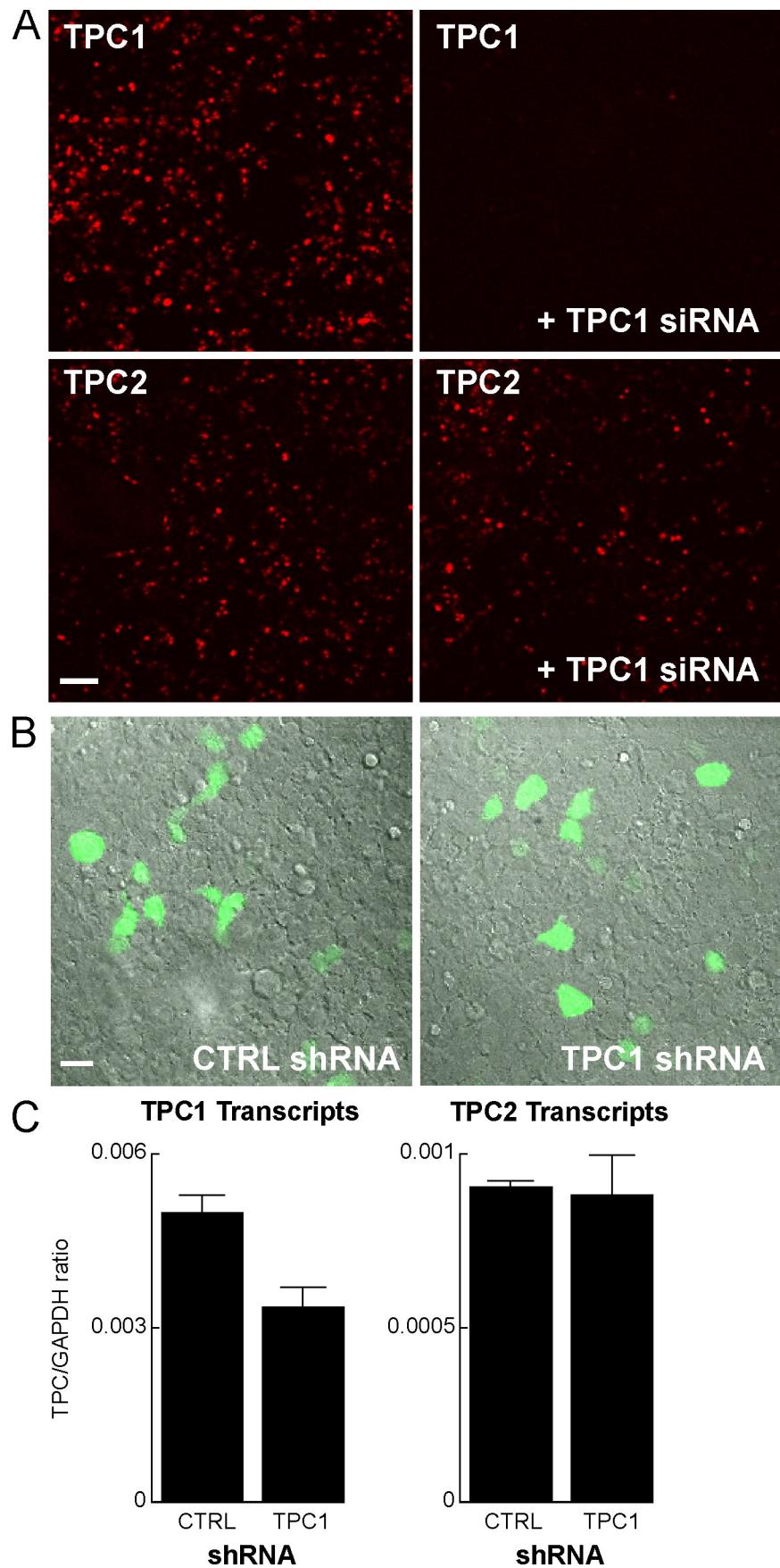
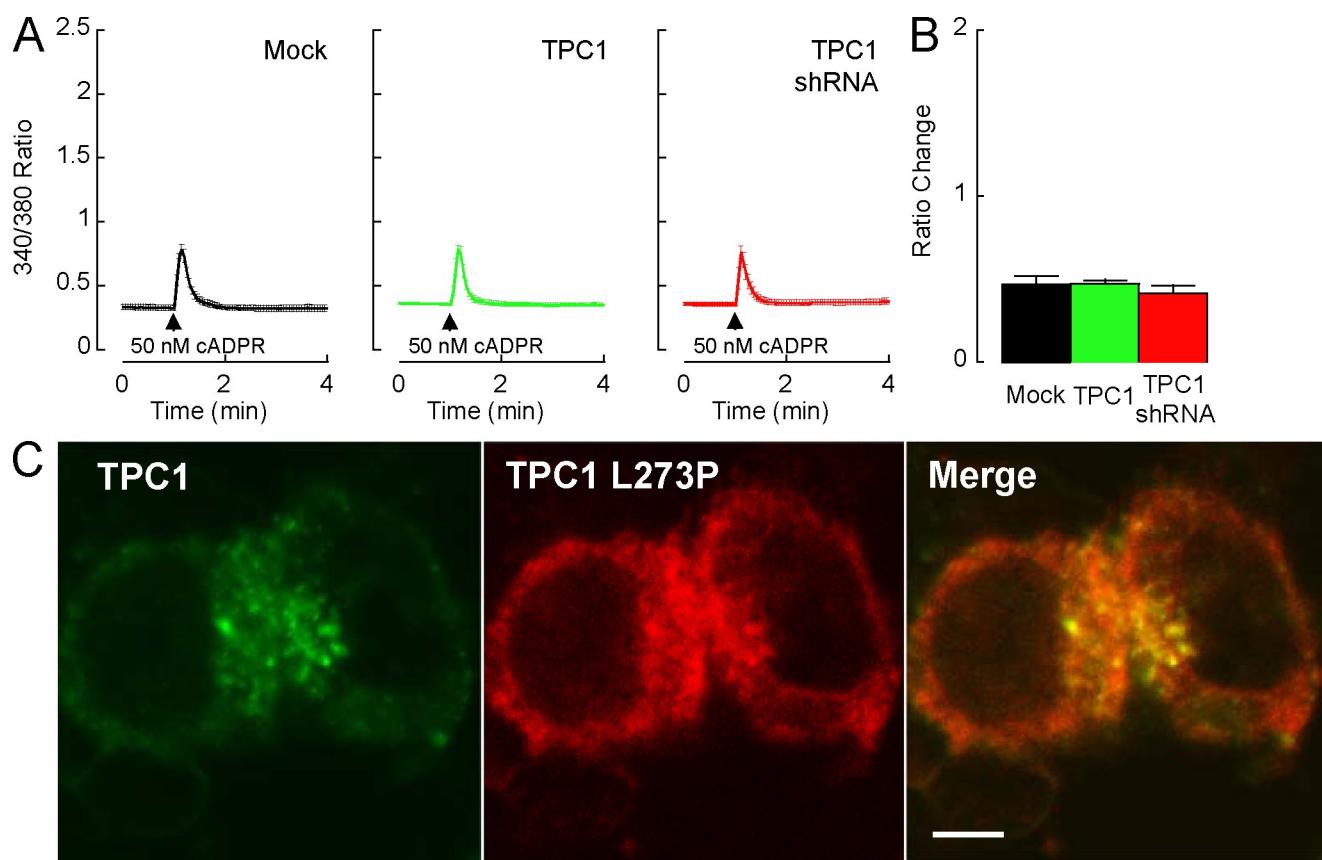


Figure S1. Variation in single-cell responses to NAADP injection. Cytosolic calcium responses of 11 individual fura-2-loaded SKBR3 cells microinjected with 10 nM NAADP (arrowheads). Cells were from cultures expressing TPC1 mRFP.

**Figure S2. Validation of TPC1 siRNA.** (A) Wide-field confocal images of HEK cells transfected with either TPC1-mRFP (top) or TPC2-mRFP (bottom) in the absence (left) or presence (right) of siRNA duplexes targeting TPC1 (2 nM). Note the selective suppression of TPC1 mRFP expression. Bar, 100  $\mu$ m. (B) Confocal images of HEK cells transfected with the plasmids encoding for both GFP and the indicated shRNA. GFP fluorescence (green) is overlayed on the transmitted light images (gray). Bar, 20  $\mu$ m. (C) Quantitative RT-PCR analysis of transcript levels for TPC1 (left) and TPC2 (right) in HEK cells transfected for 3 d with the indicated shRNA construct. Data were normalized to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Note the selective reduction in TPC1 transcripts, which represents a major underestimate of the level of knockdown at the individual cell given the modest transfection efficiency. CTRL, control. Error bars indicate SEM.





**Figure S3. Overexpression or knockdown of TPC1 does not affect cyclic ADP-ribose-mediated calcium signals.** (A) Cytosolic calcium responses of individual fura-2-loaded SKBR3 cells microinjected with 50 nM cyclic ADP-ribose (cADPR). Cells were from mock-transfected cultures (black) or cultures expressing either TPC1 (green) or an shRNA-targeting TPC1 (red). (B) Pooled data quantifying the magnitude of the ratio changes under the various experimental conditions. Error bars indicate SEM. (C) TPC1 L273P colocalizes with wild-type TPC1. Confocal images of an SKBR3 cell cotransfected with TPC1 GFP (left) and TPC1 L273P mRFP (middle). (right) An overlay of the two images is shown. Bar, 5  $\mu$ m.

Table S1. Protein sequences used for phylogenetic analyses

Name	Organism	Common name	Identifier	Database
<b>Choanoflagellida</b>				
MbrTPC1a	<i>M. brevicollis</i>	Marine choanoflagellate	EDQ93044.1	NCBI Protein
MbrTPC1b <sup>a</sup>	<i>M. brevicollis</i>	Marine choanoflagellate	EDQ84309.1	NCBI Protein
MbrTPC3	<i>M. brevicollis</i>	Marine choanoflagellate	XP_001744080.1	NCBI Protein
<b>Invertebrates - Cnidarians</b>				
NvetPC1	<i>N. vectensis</i>	Starlet sea anemone	XP_001639580.1	NCBI Protein
NvetPC2	<i>N. vectensis</i>	Starlet sea anemone	XP_001626812.1	NCBI Protein
NvetPC3	<i>N. vectensis</i>	Starlet sea anemone	XP_001625114.1	NCBI Protein
<b>Invertebrates - Arthropods</b>				
NviTPC1	<i>Nasonia vitripennis</i>	Jewel wasp	XP_001608064.1	NCBI Protein
AmeTPC1	<i>Apis mellifera</i>	Honey bee	XR_015086.1 (nt)	GenBank
<b>Invertebrates - Echinoderms</b>				
SputPC1	<i>S. purpuratus</i>	Purple sea urchin	XP_784701.2	NCBI Protein
SputPC2	<i>S. purpuratus</i>	Purple sea urchin	XP_796820.2	NCBI Protein
SputPC3	<i>S. purpuratus</i>	Purple sea urchin	XP_001181199.1	NCBI Protein
<b>Invertebrates - Cephalochordates (<i>Amphioxus</i>)</b>				
BflTPC1	<i>Branchiostoma floridae</i>	Florida lancelet	estExt_fgenesh2_pg.C_1470040	JGI
BflTPC2	<i>Branchiostoma floridae</i>	Florida lancelet	e_gw.502.22.1	JGI
BflTPC3a	<i>Branchiostoma floridae</i>	Florida lancelet	fgenesh2_pg.scaffold_579000007	JGI
BflTPC3b <sup>a</sup>	<i>Branchiostoma floridae</i>	Florida lancelet	fgenesh2_pg.scaffold_8000137	JGI
<b>Vertebrates - Nonmammalian</b>				
XtrTPC1	<i>X. tropicalis</i>	Western clawed frog	e_gw1.91.312.1	JGI
XtrTPC2	<i>X. tropicalis</i>	Western clawed frog	e_gw1.82.433.1	JGI
XtrTPC3	<i>X. tropicalis</i>	Western clawed frog	e_gw1.604.9.1	JGI
AcATPC1	<i>Anolis carolinensis</i>	Anole lizard	AAWZ01026922.1 (nt)	GenBank
AcATPC2	<i>Anolis carolinensis</i>	Anole lizard	AAWZ01009654.1 (nt)	GenBank
AcATPC3	<i>Anolis carolinensis</i>	Anole lizard	AAWZ01034767.1 (nt)	GenBank
<b>Vertebrates - Mammals</b>				
RnoTPC1	<i>Rattus norvegicus</i>	Norway rat	NP_647548.2	NCBI Protein
RnoTPC2	<i>Rattus norvegicus</i>	Norway rat	NP_001101036.1	NCBI Protein
CfaTPC1	<i>Canis familiaris</i>	Dog	XP_534690.2	NCBI Protein
CfaTPC2	<i>Canis familiaris</i>	Dog	XP_540804.2	NCBI Protein
CfaTPC3	<i>Canis familiaris</i>	Dog	XP_540172.2	NCBI Protein
HsaTPC1	<i>Homo sapiens</i>	Human	NP_060371.2	NCBI Protein
HsaTPC2	<i>Homo sapiens</i>	Human	EAW74735.1	NCBI Protein
<b>Protists</b>				
TpsTPC	<i>T. pseudonana</i>	Marine phytoplankton	AAFD01000009.1 (nt)	GenBank
PrdTPC	<i>P. ramorum</i>	Sudden oak death agent	AAQX01001438.1 (nt)	GenBank
<b>Plants</b>				
AthTPC	<i>A. thaliana</i>	Thale cress	NP_567258.1	NCBI Protein
OsaTPC	<i>Oryza sativa</i>	Rice	NP_001043858.1	NCBI Protein

JGI, Joint Genome Institute; NCBI, National Center for Biotechnology Information; nt, nucleotide. Sequences (retrieved in 2008) are abbreviated according to the first letter of the genus followed by the first two letters of the species. For multiple isoforms, the sequences are numbered according to their phylogenetic grouping.

<sup>a</sup>Sequences that failed in the  $\chi^2$  test in Tree Puzzle or contained >15% gaps in the refined alignments.

Table S2. Amino acid sequence identity and similarity between members of the TPC family

Isoform	HsaTPC1	HsaTPC2	RnoTPC1	RnoTPC2	SpuTPC1	SpuTPC2	SpuTPC3
HsaTPC1	<b>100</b>	37	93	35	60	36	39
HsaTPC2	21	<b>100</b>	37	81	33	57	34
RnoTPC1	89	21	<b>100</b>	35	59	36	39
RnoTPC2	19	71	19	<b>100</b>	31	55	34
SpuTPC1	42	19	41	17	<b>100</b>	33	37
SpuTPC2	20	40	20	38	18	<b>100</b>	35
SpuTPC3	21	18	21	18	19	21	<b>100</b>

Tabulated values were calculated from pairwise alignments. Percentage identities of the sequences are listed below the diagonal, and percentage similarities are listed above.

Table S3. Oligonucleotide primer sequences

Transcript	Forward primer	Reverse primer
End point		
SpuTPC1	5'-TAATAGTGATGTATAGTGAAGCATGGTAG-3'	5'-AGGCAGTGACTGGATGATTGAC-3'
SpuTPC2	5'-TGGTCACTATCAACTTCGTCCTGC-3'	5'-TCTTCATCAACTGGGAGTTCTGG-3'
SpuTPC3	5'-ACATCTCGGTGTCAATTGTCATTATC-3'	5'-CTCTAAGGCACTCTCATACTCTGTC-3'
RnoTPC1	5'-CTGGAGAACAGCATCGCAA-3'	5'-AAGCTCGTCAAACCCAGTGCT-3'
RnoTPC2	5'-TGCTGCTGACTATCCACCTG-3'	5'-AAAGTAGCGGTGGCTGAAGA-3'
HsaTPC1	5'-ATTTCCTGGTGGACTGTGCG-3'	5'-AGGAGGGCATCATCACATCT-3'
HsaTPC2	5'-TGTGCCCTTCACCATGTC-3'	5'-ATGGCTGTCAAGCAGGTTCAT-3'
Quantitative		
SpuTPC1	5'-TAATAGTGATGTATAGTGAAGCATGGTAG-3'	5'-AGGCAGTGACTGGATGATTGAC-3'
SpuTPC2	5'-TGGTCACTATCAACTTCGTCCTGC-3'	5'-TCTTCATCAACTGGGAGTTCTGG-3'
SpuTPC3	5'-ACATCTCGGTGTCAATTGTCATTATC-3'	5'-CTCTAAGGCACTCTCATACTCTGTC-3'
SpuUQ	5'-AAGGCCAAGATCCAGGGACAAAG-3'	5'-TATGGGAAGAAGTGGGTTCATCTG-3'
RnoTPC1	5'-TCCAAGGTTCTCATAAGCTAGGTG-3'	5'-GCCTGCCTCTGCCACTTC-3'
RnoTPC2	5'-GTCTTTACCCACAAGCTCATCTG-3'	5'-CTGAAACAAAGAGGTGACATTCCC-3'
Rno-GAPDH	5'-GTATGACTCTACCCACGGCAAG-3'	5'-TTCTCCATGGTGGTGAAGACG-3'
HsaTPC1	5'-TTCTGTGTTGCTTAGGG-3'	5'-ATTCCGCTCCATTAGATCC-3'
HsaTPC2	5'-GTTGACATGGAGAGAACCTTGAC-3'	5'-GATGAAAATAACTGGCAATCAGAAC-3'
Hsa-GAPDH	5'-TGTGAACCATGAGAAGTATGAC-3'	5'-ATGAGTCCTCCACGGATACC-3'

GAPDH, glyceraldehyde 3-phosphate dehydrogenase. Sequences of the oligonucleotide primers used for end point and quantitative PCR are shown.