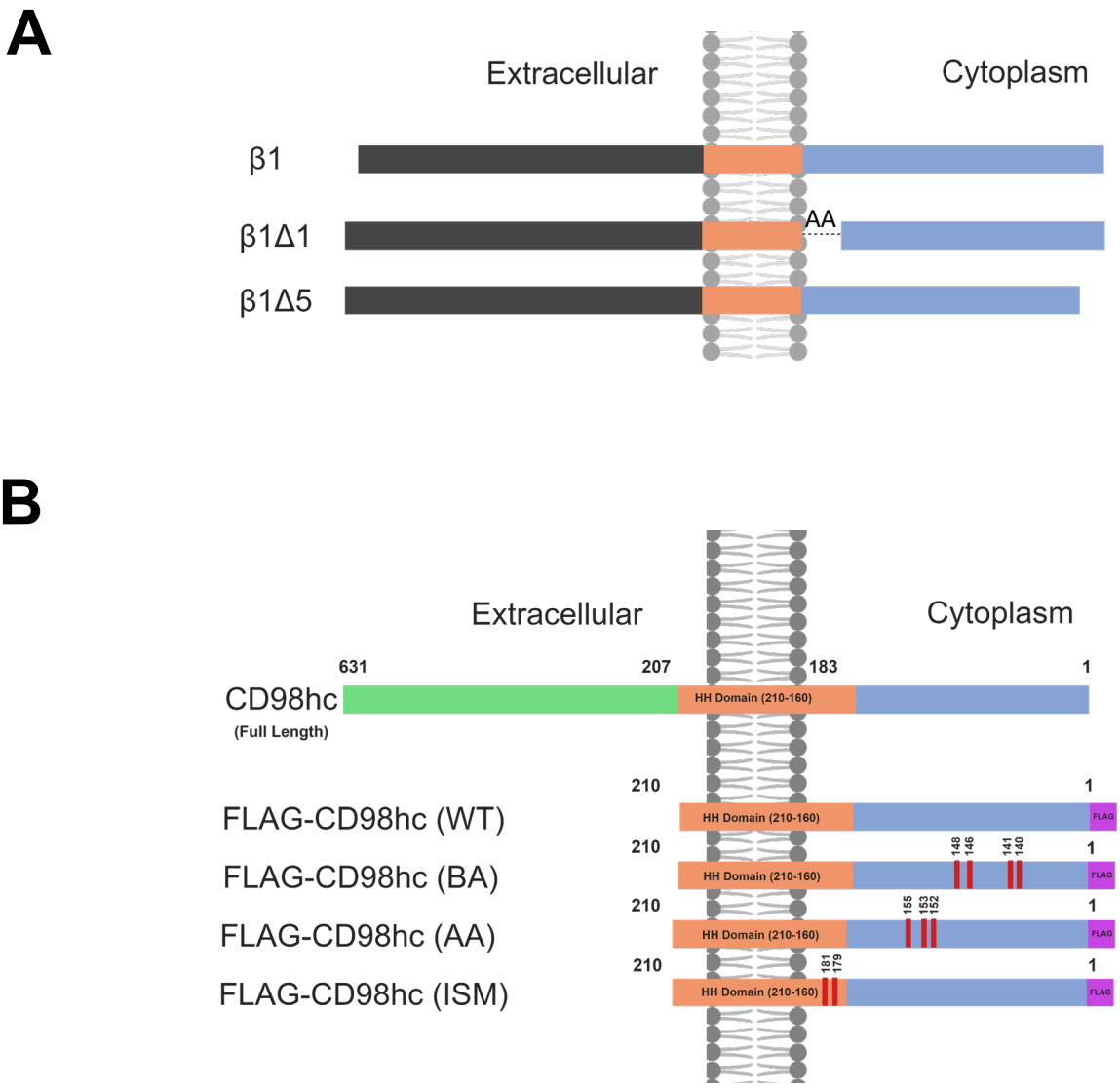

Supplementary Figure S1

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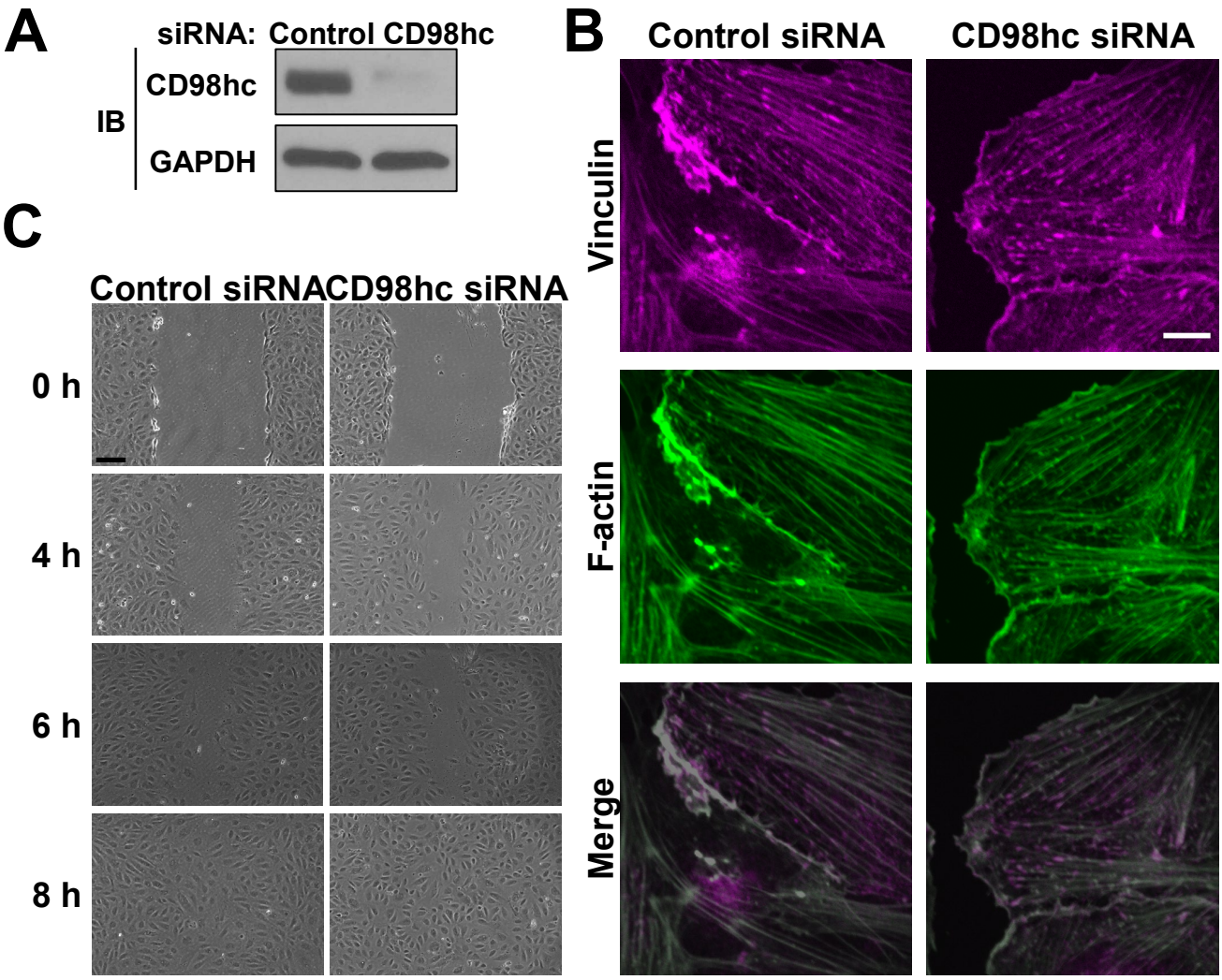
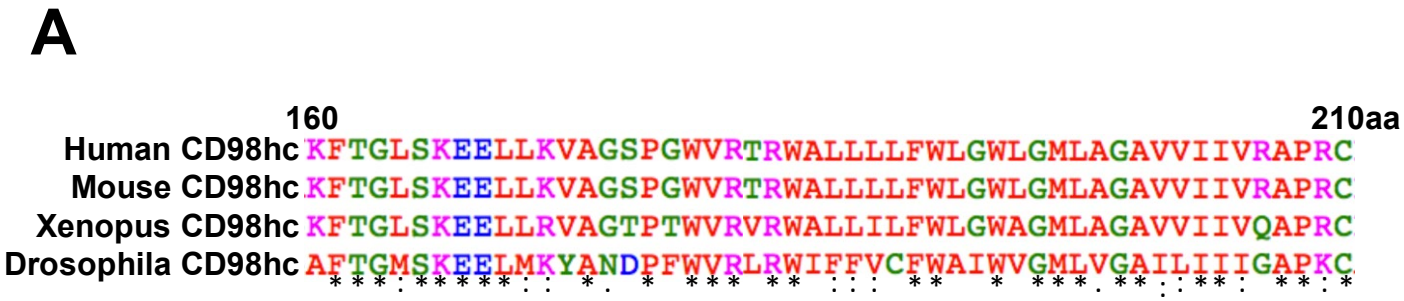
Figure S1 - Diagram of mutant β1-integrin and CD98hc constructs.

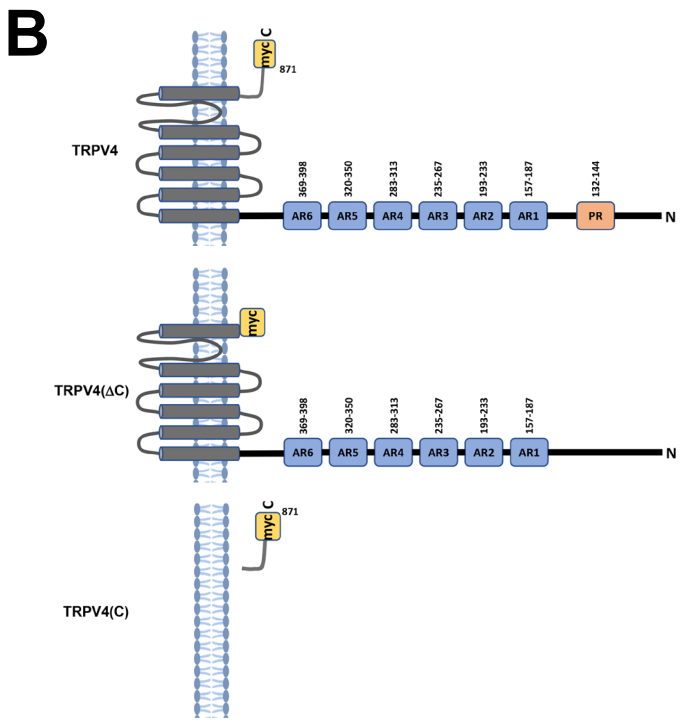
(A) Each mutant construct contains the carbonic anhydrase IV (CAIV) enzyme extracellular (EC, black) domain connected to the transmembrane (orange) domain of LDL, and the whole or partial deletions (Δ1 and Δ5) of the β1-integrin intracellular (blue) domain; AA represents replacement of deleted segment with alanine. β1Δ5 represents a construct that lacks last six AA β1-integrin intracellular domain. (B) Schematic showing full length CD98hc with its high homology domain (HH domain). Schematic depicting truncated, FLAG tagged CD98hc mutants with wild type (FLAG-CD98hc (WT)), mutated acidic residues (FLAG-CD98hc (AA)), mutated basic residues (FLAG-CD98hc (BA)) and mutated β1-integrin interacting residues (FLAG-CD98hc (ISM)).(C) Aminoacid composition of wild type (FLAG CD98), acidic and basic residue mutants (FLAG-CD98ΔTSM) and mutant integrin interacting residues (FLAG-CD98ΔISM).

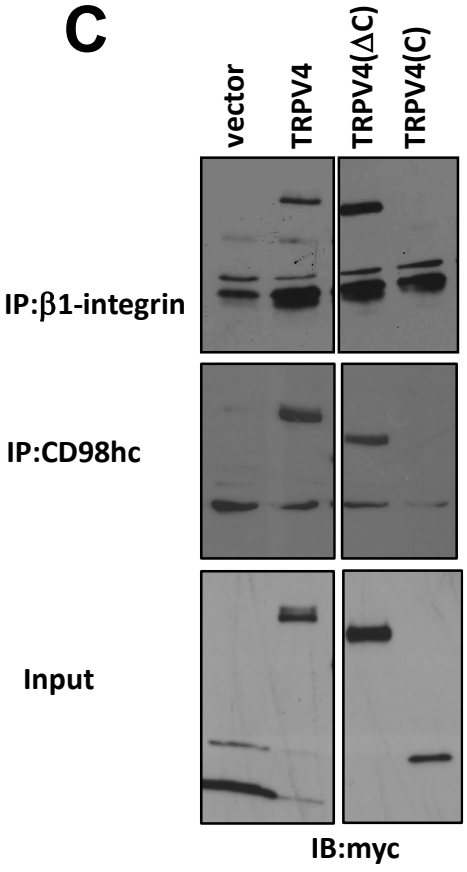


Supplementary Figure S2

Figure S2 - CD98hc is not required for cell migration. (A) Immunoblots showing CD98hc and GAPDH protein levels in HUVE cells transfected with control or CD98hc siRNA. (B) Fluorescence micrographs of HUVE cells transfected with control or CD98hc siRNA showing formation of focal adhesions containing vinculin (magenta) and F-actin (green). (C) Phase contrast photomicrographs showing cell migration following scratch wound of monolayer of HUVE cells transfected with control or CD98hc siRNA (bar, 200 μm).(n=3 replicates)

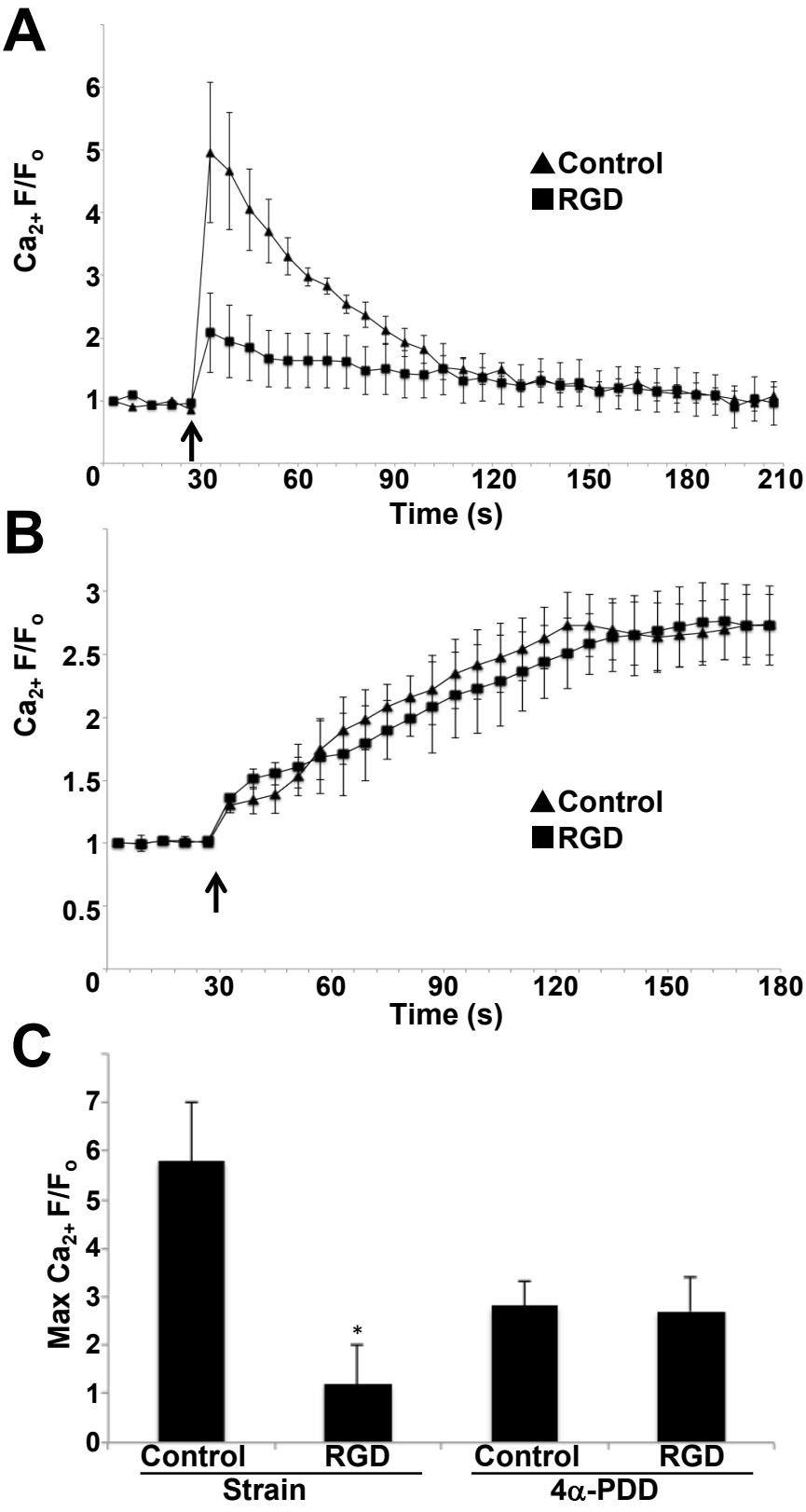






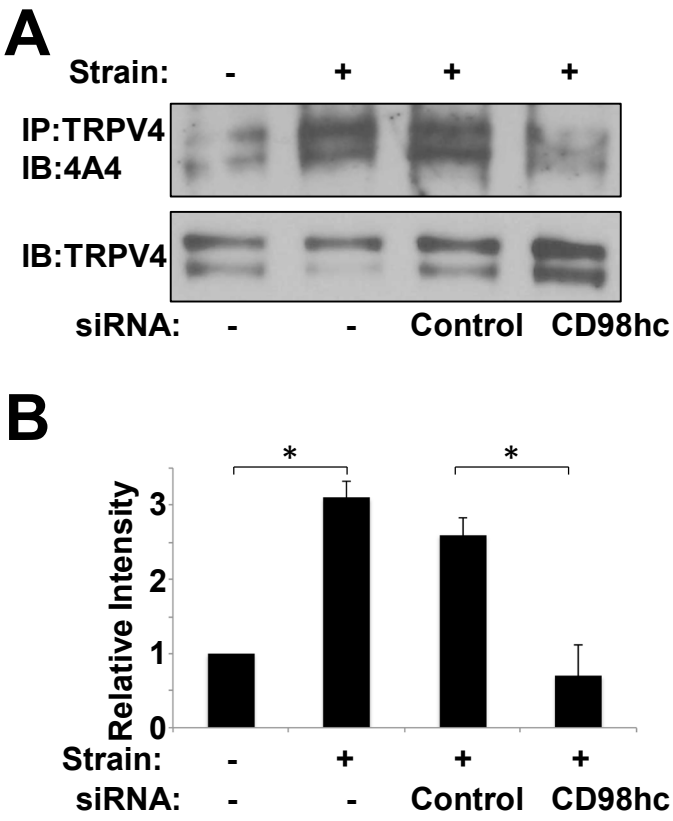
Supplementary Figure S3

Figure S3 - CD98hc HH domain is highly conserved from drosophila to mammals. (A) Schematic diagram of CD98hc, CD98hc(HH), and CD98hc(ΔHH) constructs containing high homology (HH, orange) within amino acid (aa) sequences 160-210. Amino acid sequences 160-210 in human, mouse, *Xenopus*, and *Drosophila* CD98hc ("*" indicates identity, ":" indicates high similarity, and "." indicates low similarity in amino acids between the different species). (B) Schematic diagram of various full length and deletion TRPV4 constructs from top to bottom: full length TRPV4 (TRPV4), , TRPV4 lacking carboxy terminal (TRPV4(ΔC)), and TRPV4 carboxy terminal only (TRPV4(C)). (C) Myc-tagged TRPV4 constructs immunoprecipitated with β1-integrin (Top) or CD98hc (middle) antibody in lysates from HEK293T cells transfected with indicated DNA constructs; inputs from corresponding IPs are shown in the bottom panel.



Supplementary Figure S4

Figure S4 - Mechanisms of mechanical and chemical activation of TRPV4 are distinct. Relative changes in cytosolic calcium (Ca_{2+} F/F_0) in Fluo-4-loaded HUVE cells in response to 15% static strain for 4 s (**A**) or the TRPV4 chemical activator, 10μM 4α-PDD (**B**) in the presence or absence of RGD peptide (10 μM). (**C**) Average maximum relative changes in cytosolic calcium (Max Ca_{2+} F/F_0) in cells described in panels **A** and **B** (n=3, * $p<0.05$ vs strain control).



Supplementary Figure S5

Figure S5 - CD98hc knockdown prevents TRPV4 phosphorylation induced by mechanical strain. (A) Immunoblots showing phosphorylated TRPV4 from lysates of control HUVE cells or HUVE cells treated with control or CD98hc siRNA and exposed to cyclic substrate strain (15%, 1 Hz, 2 h) detected using the 4A4 anti-phosphoserine antibody. (B) Histogram showing the densitometric quantification of immunoblots in A (n=3,*p<0.05).

Table S1 Interaction residues between hTRPV4 and CD98 (P121-S166). Predicted interacting residues in hTRPV4 and CD98 (P121-S166), and corresponding hydrogen (H) – bonds between the residues**. Notice:** The human TRPV4 (PDB ID: 4DX2) forms a dimer in crystal. R224a and R224b are from different monomers from hTRPV4.

hTRPV4	CD98 (P121-S166)	H-bonds (Å)
K311	K148	1.7
R224[a]	D152	1.9
K310	D152	1.9
R224[b]	D152	2.4
R316	E153	1.9 and 2.3
R224[b]	E155	1.8 and 2.0

Notice: The human TRPV4 (PDB ID: 4DX2) forms a dimer in crystal. R224[a] and R224[b] are from different monomers from hTRPV4.

Table S2. Interaction residues between the cytoplasmic tail of β1 integrin (N792-K798) to the HH domain of CD98hc (K161-R210).

β1 integrin (N792-K798)	CD98hc (K161-R210)	H-bonds (Å)
K794	**E168**	2.5 and 2.7
Y795	**W179**	2.8
E796	**R181**	2.8 and 3.2

Table S3. Key reagents.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit Anti-Mouse TRPV4 Polyclonal Antibody, Unconjugated	Abcam	Abcam Cat# ab39260, RRID:AB_1143677
Rabbit Anti-TRPV4 Polyclonal Antibody, Unconjugated	Alomone Labs	Alomone Labs Cat# ACC-034, RRID:AB_2040264
Mouse Anti-CD29 Monoclonal Antibody, Unconjugated, Clone 18	BD Biosciences	BD Biosciences Cat# 610467, RRID:AB_2128060
Rabbit Anti-human CD98 Monoclonal Antibody, Unconjugated	Abcam	Abcam Cat# ab108300, RRID:AB_10863340
Mouse Anti-Vinculin Monoclonal Antibody, Unconjugated	Sigma-Aldrich	Sigma-Aldrich Cat# V9131, RRID:AB_477629
Rabbit Anti-HA tag Polyclonal Antibody, Unconjugated	Sigma-Aldrich	Sigma-Aldrich Cat# SAB4300603, RRID:AB_10620829
Mouse Anti-Human Carbonic anhydrase iv Monoclonal antibody, Unconjugated, Clone 310413	R and D Systems	R and D Systems Cat# MAB2186, RRID:AB_2259244
Rabbit Anti-Myc tag Polyclonal Antibody, Unconjugated	Millipore	Millipore Cat# 06-549, RRID:AB_11212682
Mouse Anti-Phosphoserine Monoclonal antibody, Unconjugated, Clone 4A4	Millipore	Millipore Cat# 05-1000, RRID:AB_11210897

Mouse Anti-Glyceraldehyde-3-PDH (GAPDH) Monoclonal antibody, Unconjugated	Millipore	Millipore Cat# MAB374, RRID:AB_2107445
Rabbit Anti-FLAG tag Polyclonal Antibody, Unconjugated	Sigma-Aldrich	Sigma-Aldrich Cat# F7425, RRID:AB_439687
Mouse Anti-FLAG tag Monoclonal Antibody, Unconjugated	Sigma-Aldrich	Sigma-Aldrich Cat# F3165, RRID:AB_259529
Mouse Anti-FLAG(R) M2 Affinity Gel antibody	Sigma-Aldrich	Sigma-Aldrich Cat# A2220, RRID:AB_10063035
Mouse Anti-human CD98 (E-5) Monoclonal Antibody, Unconjugated	Santa Cruz Biotechnology	Santa Cruz Biotechnology Cat# sc-376815
Goat Anti-Human CD98 (C-20) Polyclonal, Unconjugated antibody	Santa Cruz Biotechnology	Santa Cruz Biotechnology Cat# sc-7095, RRID:AB_638284
Alexa Fluor 488 Phalloidin antibody	Thermo Fisher Scientific	Thermo Fisher Scientific Cat# A12379, RRID:AB_2315147
Talin-1 (C45F1) Rabbit mAb antibody	Cell Signaling Technology	Cell Signaling Technology Cat# 4021, RRID:AB_2204018
Chemicals, Peptides, and Recombinant Proteins		
4α-Phorbol 12,13-didecanoate	Sigma-Aldrich	Sigma-Aldrich Cat# P8014
Phalloidin-iFluor 405 Reagent	Abcam	Abcam Cat# ab176752
Fluo-4, AM, cell permeant	Thermo Fisher Scientific	Thermo Fisher Scientific Cat# F14201
Linear RGD peptide	Bachem	Bachem Cat# H-7356

Targefect-HUVEC	Targeting systems	Targeting systems Cat# HUVEC-01
Deposited Data		
Crystal structure of the human TRPV4 ankyrin repeat domain	10.1021/bi300279b	10.2210/pdb4DX2/pdb
Experimental Models: Cell Lines		
Primary Umbilical Vein Endothelial Cells; Normal, Human, Pooled (HUVEC)	ATCC	ATCC Cat# PCS-100-013
293T/17 [HEK 293T/17]	ATCC	ATCC Cat# CRL-11268
Oligonucleotides		
siRNA targeting sequence human CD98hc #1: 5'-GAAUGGUCUGGUGAAGAUC-3'	Qiagen	N/A
siRNA targeting sequence human CD98hc #2: 5'-GAUCUUCACCAGACCAUUC-3'	Qiagen	N/A
Recombinant DNA		
pCMV-HA vector	Takara Bio	Takara Bio Cat# 635690
pCMV-CD98hc-HA	This paper	Based on NM_001012662

pCMV-CD98hc(ΔHH)-HA	This paper	N/A
pCMV-CD98hc(HH)-HA	This paper	N/A
pCMV-myc vector	Takara Bio	Takara Bio Cat# 635689
pCMV-TRPV4-myc	This paper	N/A
pCMV-TRPV4(DPR)-myc	This paper	N/A
pCMV-TRPV4(DAR1-3)-myc	This paper	N/A
pcDNA™3.1/Zeo(-) Mammalian Expression Vector	Thermo Fisher Scientific	Thermo Fisher Scientific Cat#V86520
pcDNA3.1(-)-FLAG-CD98hc(WT)	This paper	N/A
pcDNA3.1(-)-FLAG-CD98hc(ISM)	This paper	N/A
pcDNA3.1(-)-FLAG-CD98hc(AA)	This paper	N/A
pcDNA3.1(-)-FLAG-CD98hc(BA)	This paper	N/A
Software and Algorithms		
PEP-FOLD 3.0	10.1093/nar/gkw329	http://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py#forms::PEP-FOLD3
ClusPro 2.0	10.1038/nprot.2016.169	https://cluspro.org
GraphPad Prism	Graphpad	https://www.graphpad.com/scientific-software/prism/
Leica Application Suite	Leica Microsystems	https://www.leica-microsystems.com/products/microsc

		ope-software/details/product/leica-application-suite/
ZEN imaging software	Zeiss	https://www.zeiss.com/microscopy/us/products/microscope-software/zen.html
Cell profiler	https://doi.org/10.1186/gb-2006-7-10-r100	https://cellprofiler.org/releases/