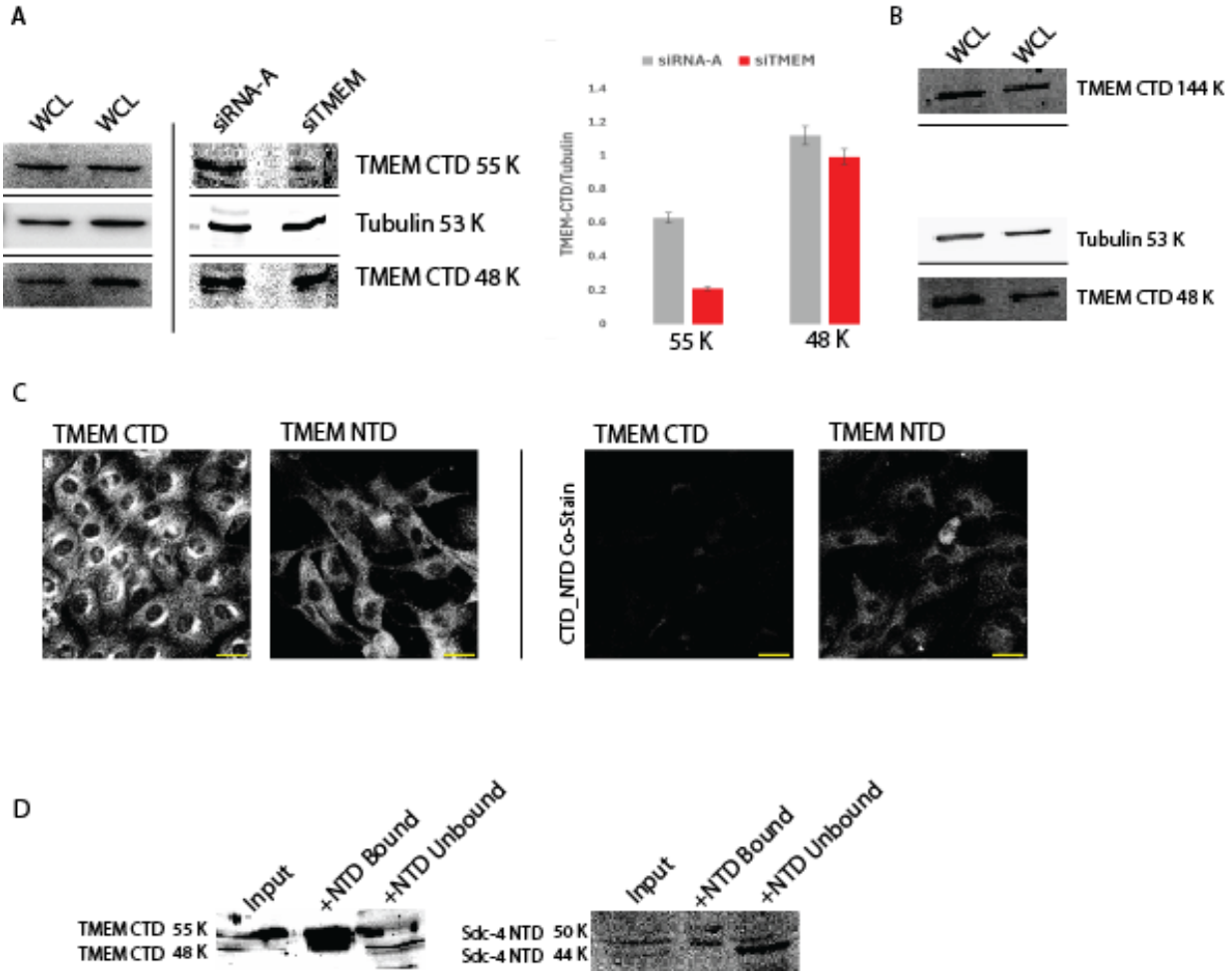
A
WCL
WCL
siRNA-A
siTMEM
TMEM CTD 55 K
Tubulin 53 K
TMEM CTD 48 K
siRNA-A
siTMEM
TMEM-CTD/Tubulin
55 K
48 K
B
WCL
WCL
TMEM CTD 144 K
Tubulin 53 K
TMEM CTD 48 K
C
TMEM CTD
TMEM NTD
CTD_NTD Co-Stain
TMEM CTD
TMEM NTD
D
Input
+NTD Bound
+NTD Unbound
TMEM CTD 55 K
TMEM CTD 48 K
Input
+NTD Bound
+NTD Unbound
Sdc-4 NTD 50 K
Sdc-4 NTD 44 K

Figure S1. TMEM184A CTD verification in BAOECs. A. WCL TMEM184A CTD rabbit polyclonal (1:500) antibody confirmation staining (55 kDa and 48 kDa) compared to Tubulin loading control (53 kDa). In siTMEM cells, band densities normalized to Tubulin are decreased. B. A higher molecular weight band (144 kDa) representing TMEM184A is also confirmed in a separate WCL harvest with a lower molecular weight band (48 kDa) compared to Tubulin loading control (53 kDa). C. IF staining of TMEM184A CTD and NTD antibodies, stained separately (left) at 1:100 dilutions and co-stained (right) at 1:100 dilutions. D. TMEM184A NTD pull down confirmation with TMEM184A CTD polyclonal staining shown in A. (left). The same blot stained with the Sdc4 mouse monoclonal (right) shows the reciprocal of the pull down in Figure 1. C.

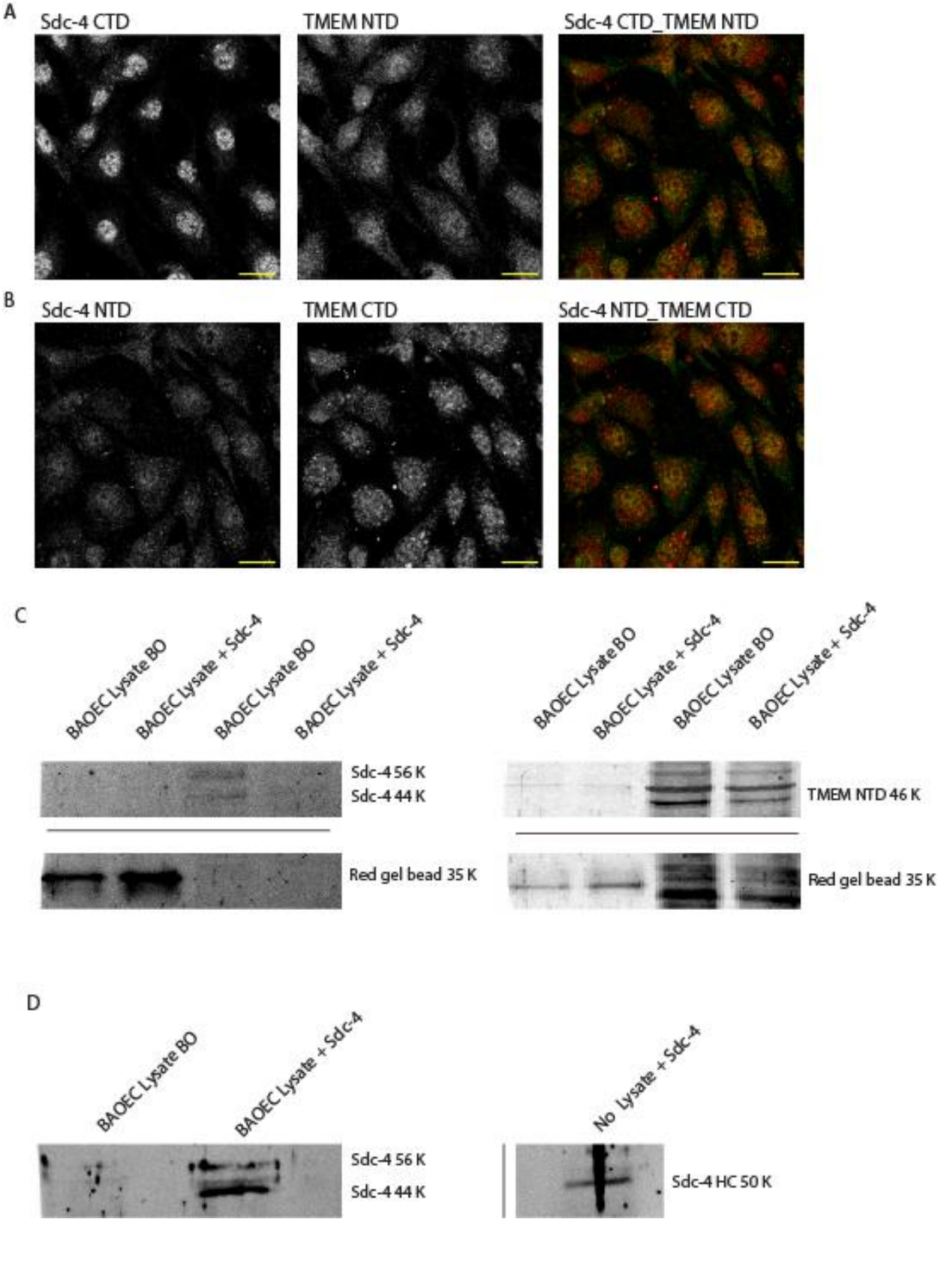
A
Sdc-4 CTD
TMEM NTD
Sdc-4 CTD_TMEM NTD
B
Sdc-4 NTD
TMEM CTD
Sdc-4 NTD_TMEM CTD
C
BAOEC Lysate BO
BAOEC Lysate + Sdc-4
BAOEC Lysate BO
BAOEC Lysate + Sdc-4
Sdc-4 56 K
Sdc-4 44 K
Red gel bead 35 K
BAOEC Lysate BO
BAOEC Lysate + Sdc-4
BAOEC Lysate BO
BAOEC Lysate + Sdc-4
TMEM NTD 46 K
Red gel bead 35 K
D
BAOEC Lysate BO
BAOEC Lysate + Sdc-4
Sdc-4 56 K
Sdc-4 44 K
No Lysate + Sdc-4
Sdc-4 HC 50 K

Figure S2. TMEM184A-Sdc4 interactions in BAOECs are abrogated with increased detergent and incubation times. IF staining of Sdc4 CTD (green) merged with TMEM184A NTD (red) in 0.3% Triton X-100 permeabilized conditions. Scale 20 μm. B. IF staining of Sdc4 NTD (green) and TMEM184A CTD (red) in 0.3% Triton X-100 permeabilized conditions from one independent experiment of each antibody combination. Scale 20 μm. Six images from each experiment were compared. C. Comparison of bound and unbound fractions in WB of a Sdc4 mouse monoclonal (+Sdc4) pull down in BAOEC cell lysate incubated overnight and stained with anti-Sdc4 rabbit polyclonal antibody (56 kDa and 44 kDa doublet) and TMEM184A NTD (46 kDa band) as in Figure 1 C. Red gel bead shed is shown at 35 kDa. Sdc4 IP and WB represents one experiment. D. Sdc4 rabbit polyclonal stain of beads only (BO) lysate, +Sdc4 lysate, and +Sdc4 no lysate control with Sdc4 (56 kDa and 44 kDa doublet) and Sdc4 heavy chain (HC) antibody (50 kDa). No lysate control was obtained within the second IP Sdc4 pull down (TMEM NTD stain is not shown).

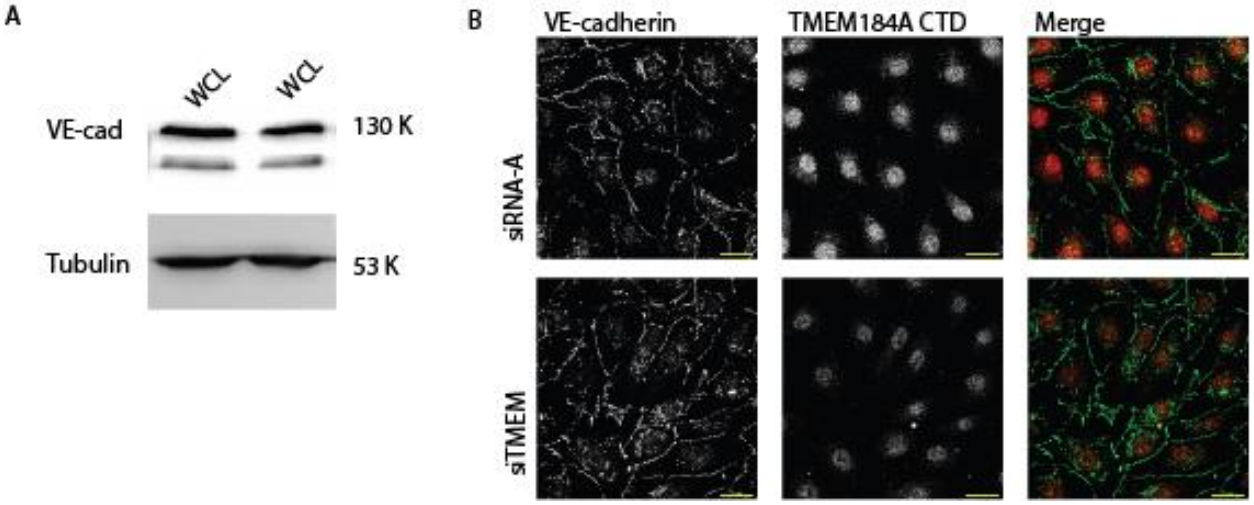
A
WCL
WCL
VE-cad
130 K
Tubulin
53 K
B
VE-cadherin
TMEM184A CTD
Merge
siRNA-A
siTMEM

Figure S3. VE-cad goat antibody verification with minimal decreases in VE-cad goat fluorescence in confluent siTMEM cells. A. WB of WCL stained with VE-cad goat polyclonal (130 kDa), Tubulin (53 kDa). B. Representative images of IF staining of VE-cad (green) and TMEM184A CTD (red) in confluent control and siTMEM cell groups quantified in Figure 3. B.