Endoplasmic reticulum-resident protein 57 (ERp57) oxidatively inactivates human transglutaminase 2

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SUPPORTING INFORMATION

Supplemental Figures:

Supplemental Table:

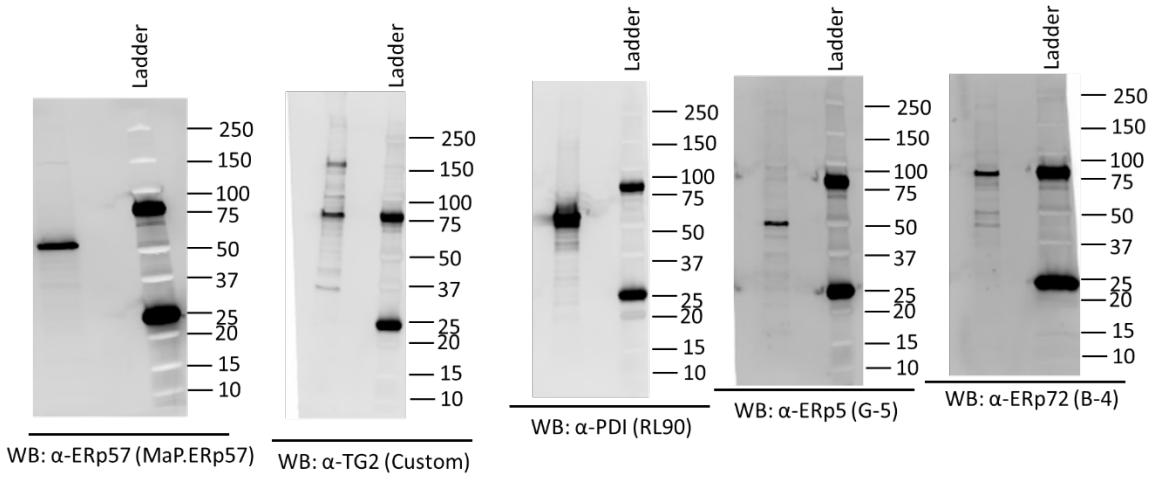

Figure S1. Specificity of antibodies used in this study. HUVEC cultures were lysed with RIPA buffer and resolved by reducing SDS-PAGE. Antibody specificity was assessed by Western immunoblotting with the listed antibodies. Ladder represents molecular mass in kDa.

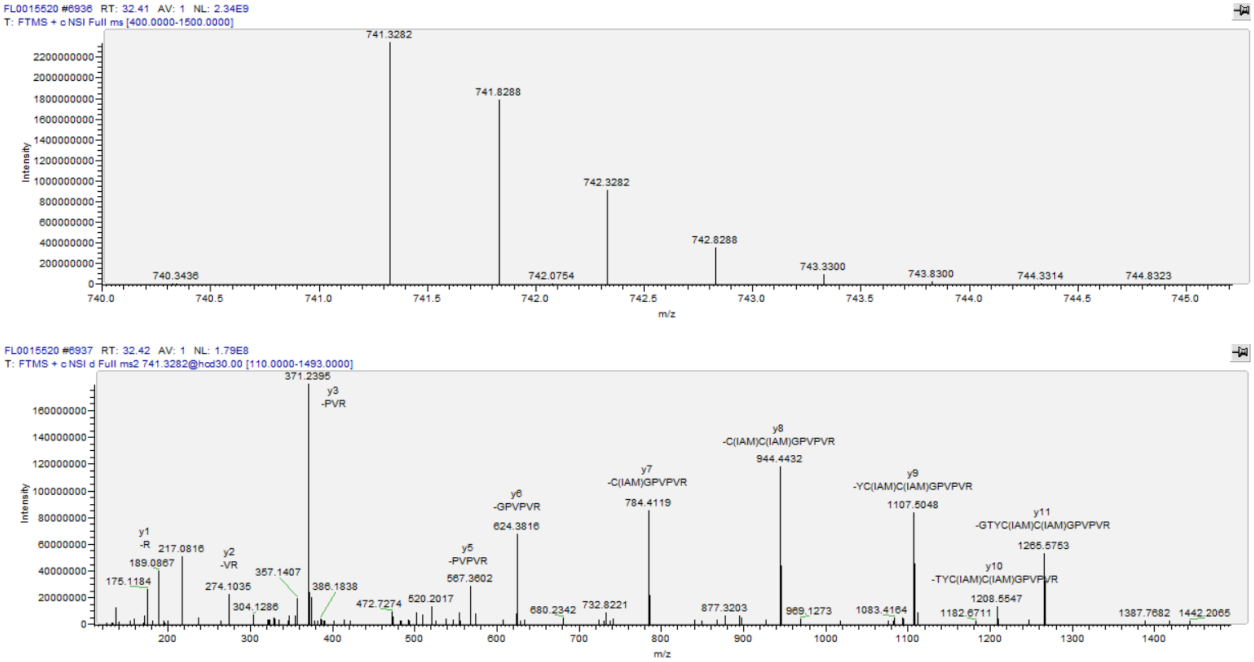

Figure S2. Identification of reduced Cys370 and Cys371 in TG2. *Top,* Isotopic distribution of ^{365}SEGTYC(IAM)C(IAM)GPVPVR^{377}. *Bottom,* Identity of the peptide confirmed by tandem mass spectrum (*MS/MS*) with each *y* fragment ion labeled.

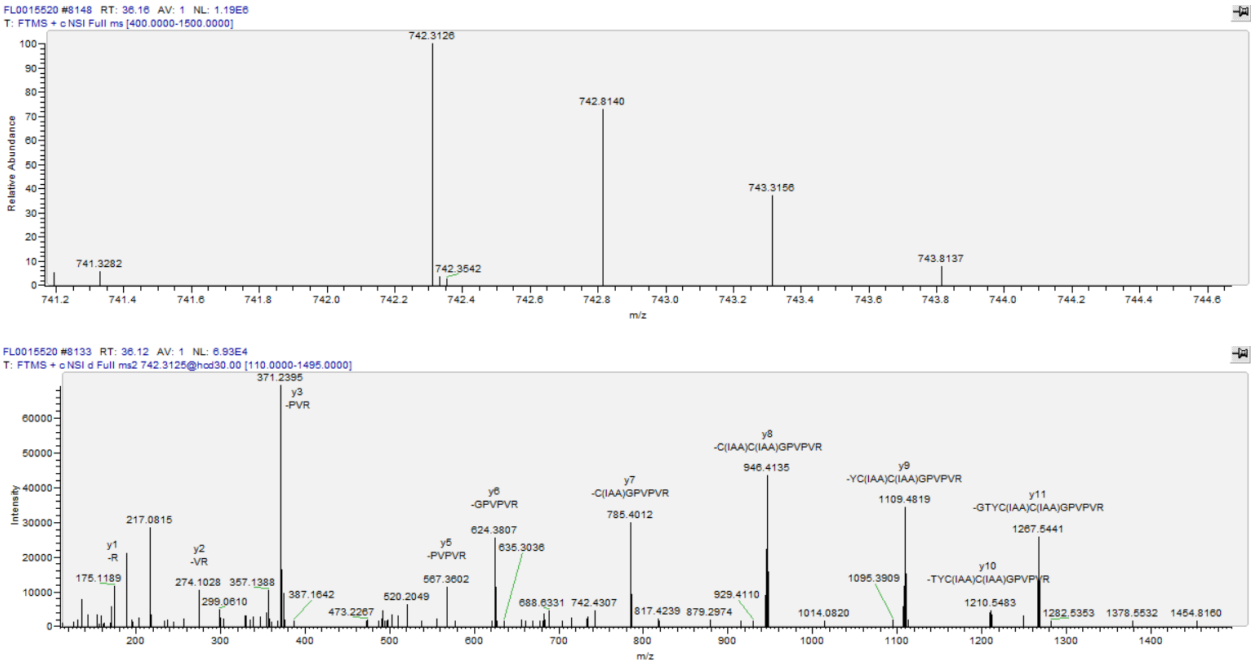

Figure S3. Identification of the Cys370-Cys371 disulfide bond in TG2. *Top,* Isotopic distribution of ^{365}SEGTYC(IAA)C(IAA)GPVPVR^{377}. *Bottom,* Identity of the peptide confirmed by tandem mass spectrum (*MS/MS*) with each *y* fragment ion labeled.

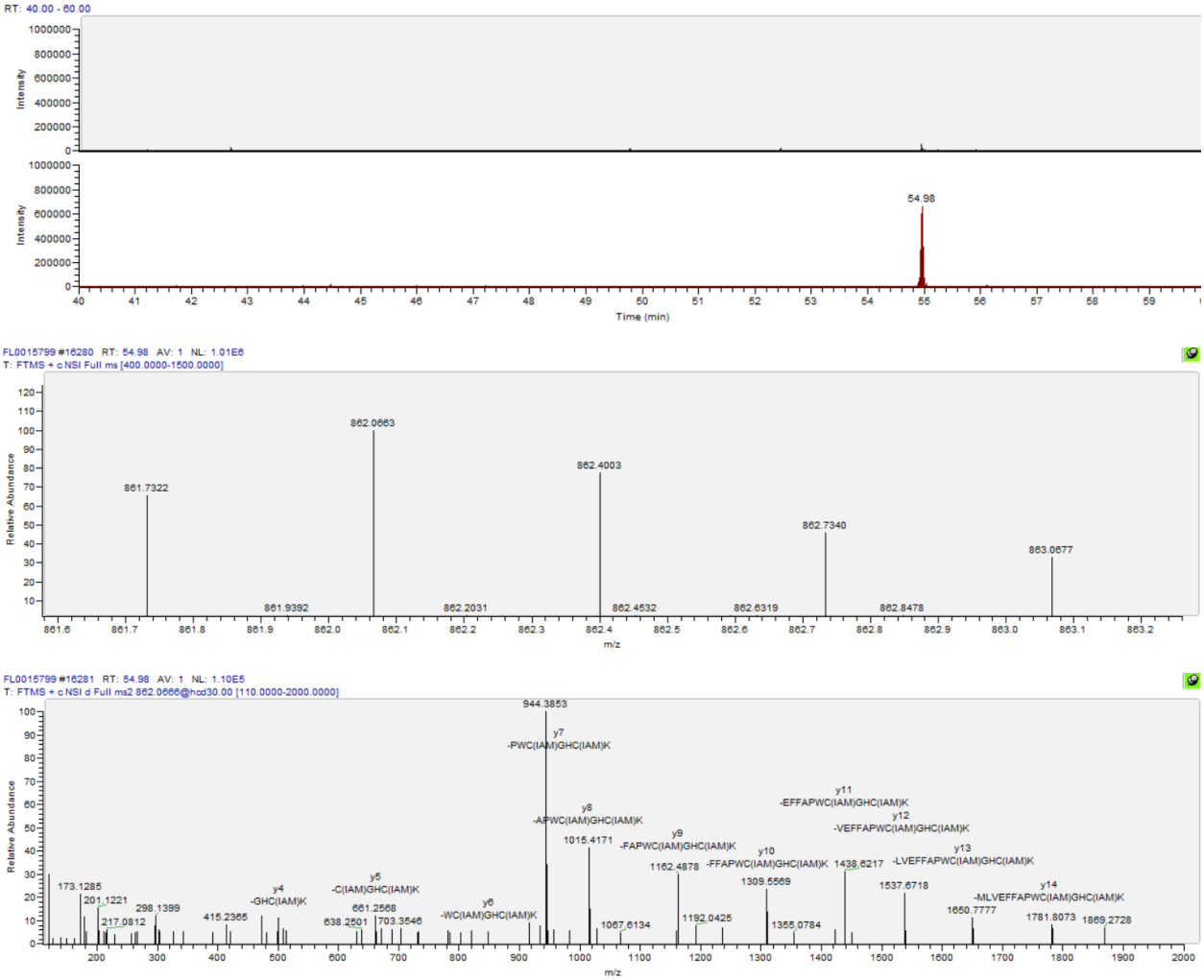

Figure S4. Identification of reduced domain a CXXC in ERp57. *Top,* Isotopic distribution of ^{39}ISDTGSAGLMLVEFFAPWC(IAM)GHC(IAM)K^{61}. *Bottom,* Identity of the peptide confirmed by tandem mass spectrum (*MS/MS*) with each *y* fragment ion labeled.

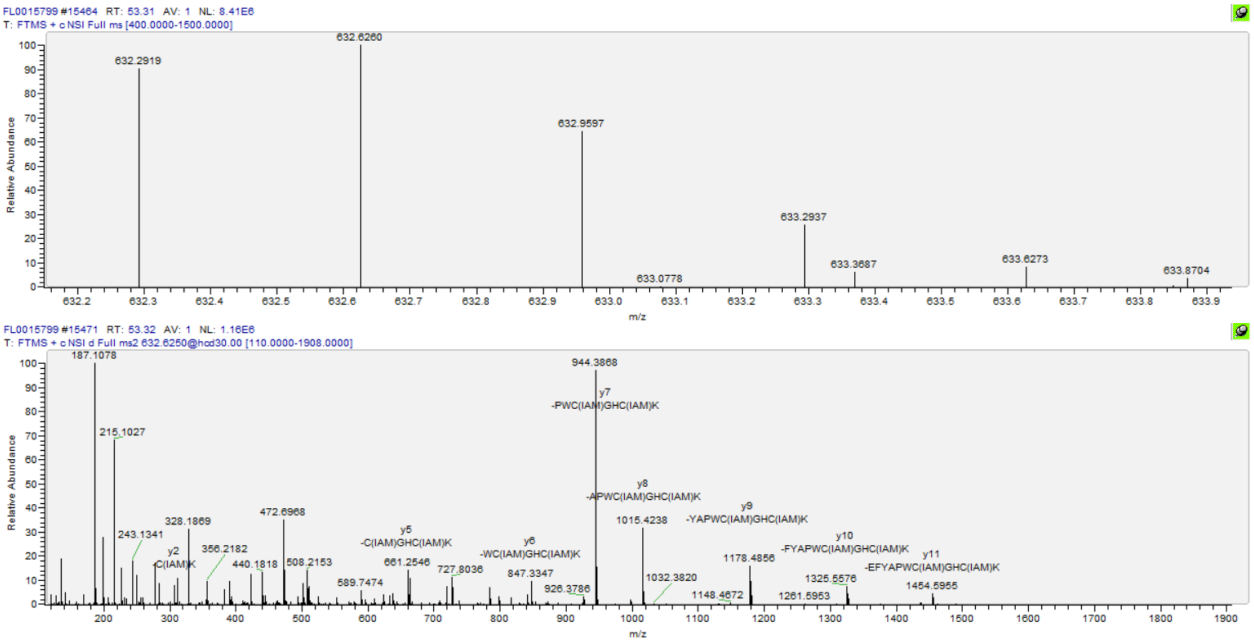

Figure S5. Identification of reduced domain a' CXXC in ERp57. *Top,* Isotopic distribution of ^{396}DVLIEFYAPWC(IAM)GHC(IAM)K^{410}. *Bottom,* Identity of the peptide confirmed by tandem mass spectrum (MS/MS) with each *y* fragment ions labeled.

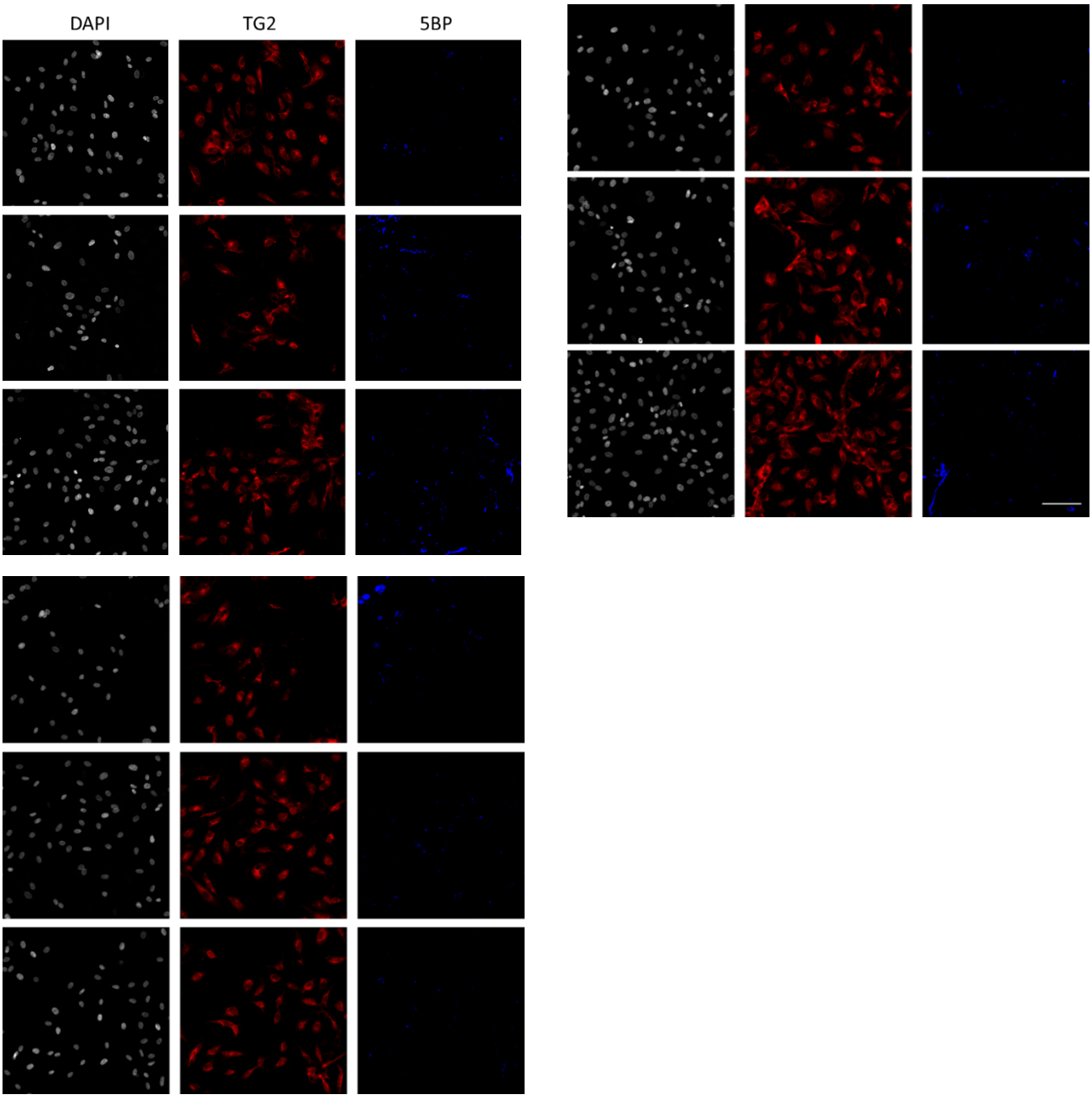

Figure S6. Basal extracellular TG2 activity in HUVEC. 200 µM 5BP was added for 3 h to control siRNA-transfected cells to assay for TG2 activity. 5BP incorporation (blue) is proportional to TG2 activity. Experimental conditions were performed in triplicate. During confocal microscopy analysis, three images were taken per replicate using a 20x objective and all images are presented. Images were post-processed and analyzed in FIJI. Images in Row 8 were used in Figure 8E. *Scale bar = 100 µm.*

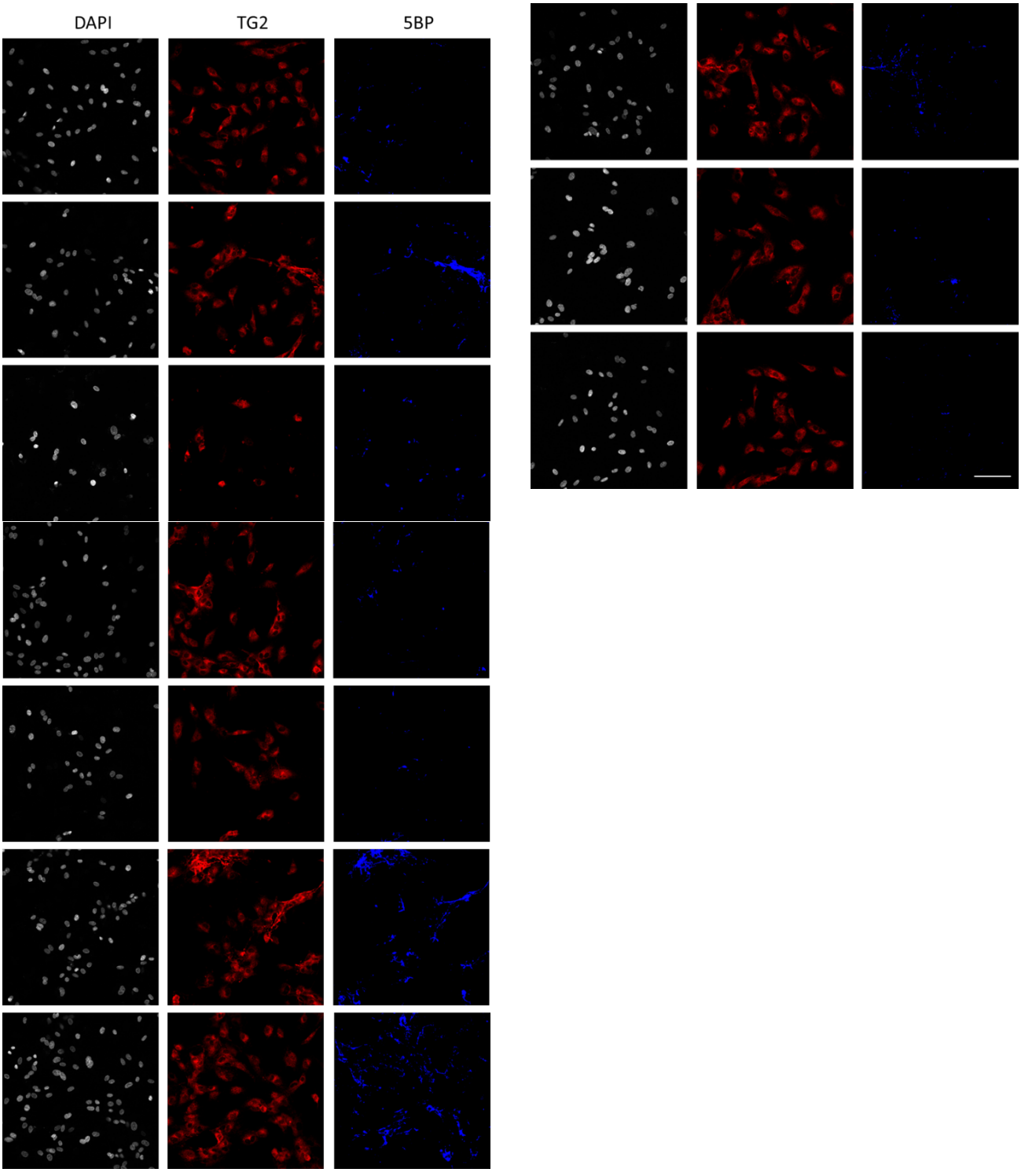

Figure S7. ERp57-targeted siRNA knockdown in HUVEC. 200 µM 5BP was added for 3 h to ERp57 siRNA-transfected cells to assay for TG2 activity. 5BP incorporation (blue) is proportional to TG2 activity. Experimental conditions were performed in triplicate. During confocal microscopy analysis, at least three images were taken per replicate using a 20x objective, and all images are presented. Images were post-processed and analyzed in FIJI. Images in Row 6 were used in Figure 8E. *Scale bar = 100 µm.*

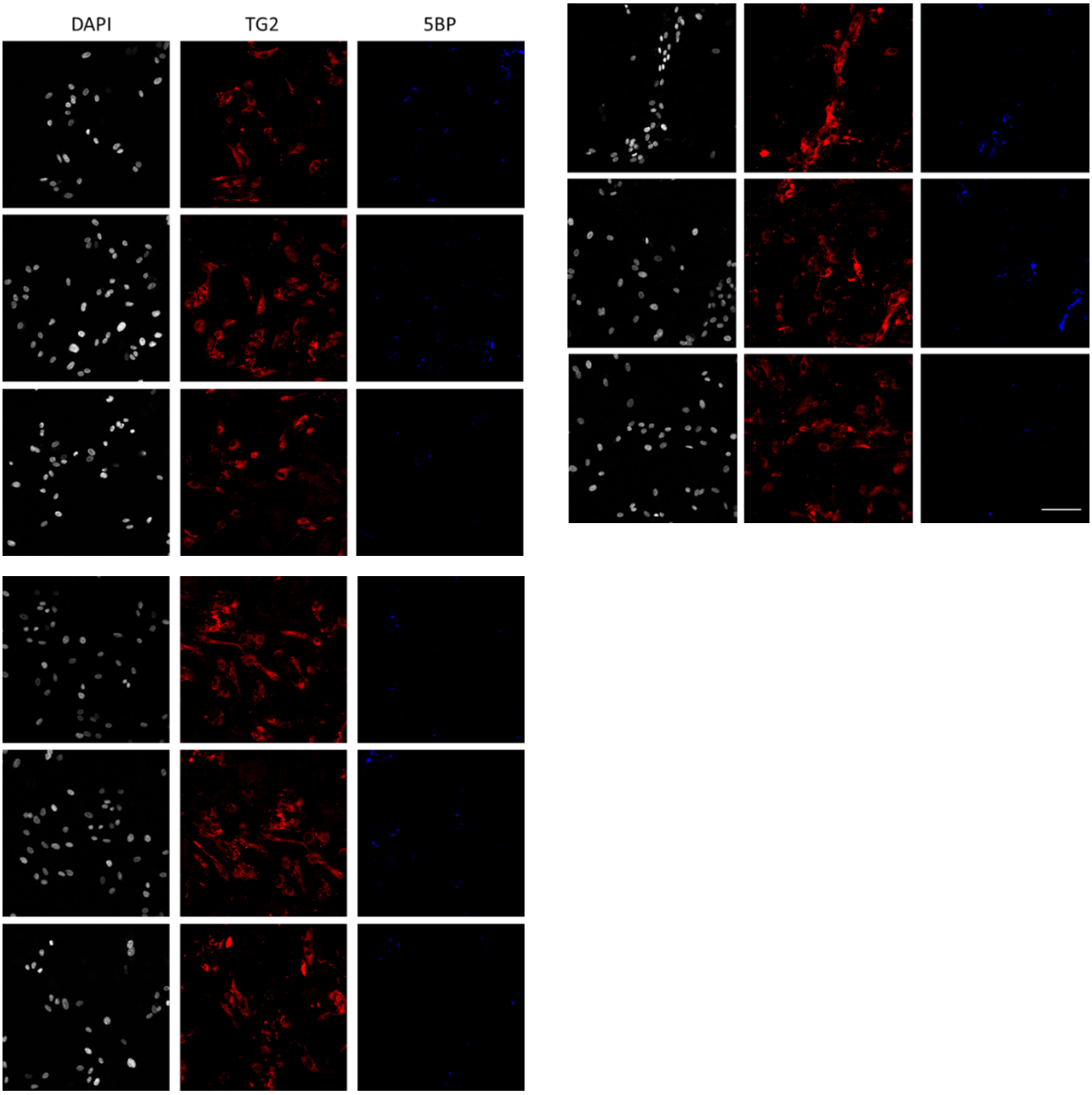

Figure S8. ERp57-targeted siRNA knockdown in HUVEC supplemented with exogenous 1 µM oxidized ERp57. 1 µM oxidized ERp57 was pre-incubated with ERp57 siRNA-treated HUVEC cultures for 30 min. After, 200 µM 5BP was added for 3 h to assay for TG2 activity. 5BP incorporation (blue) is proportional to TG2 activity. Experimental conditions were performed in triplicate. During confocal microscopy analysis, three images were taken per replicate using a 20x objective, and all images are presented. Images were post-processed and analyzed in FIJI. Images in Row 2 were used in Figure 8E. *Scale bar = 100 µm.*

Protein	Peptide Sequence	Theoretical m/z	Observed m/z	Charge
TG2 Reduced Cys370, Cys371	SEGTYC(**IAM**)C(**IAM**)GPVPVR	741.3078	741.3282	2
TG2 Oxidized Cys370, Cys371	SEGTYC(**IAA**)C(**IAA**)GPVPVR	742.3136	742.3078	2
ERp57 Reduced Domain **a**	ISDTGSAGLMLVEFFAPWC(**IAM**)GHC(**IAM**)K	861.7182	861.7324	3
ERp57 Reduced Domain **a'**	DVLIEFYAPWC(**IAM**)GHC(**IAM**)K	632.2777	632.2917	3

Table S1: Peptides derived from reduced/oxidized TG2 and reduced ERp57 that were detected by mass spectrometry.