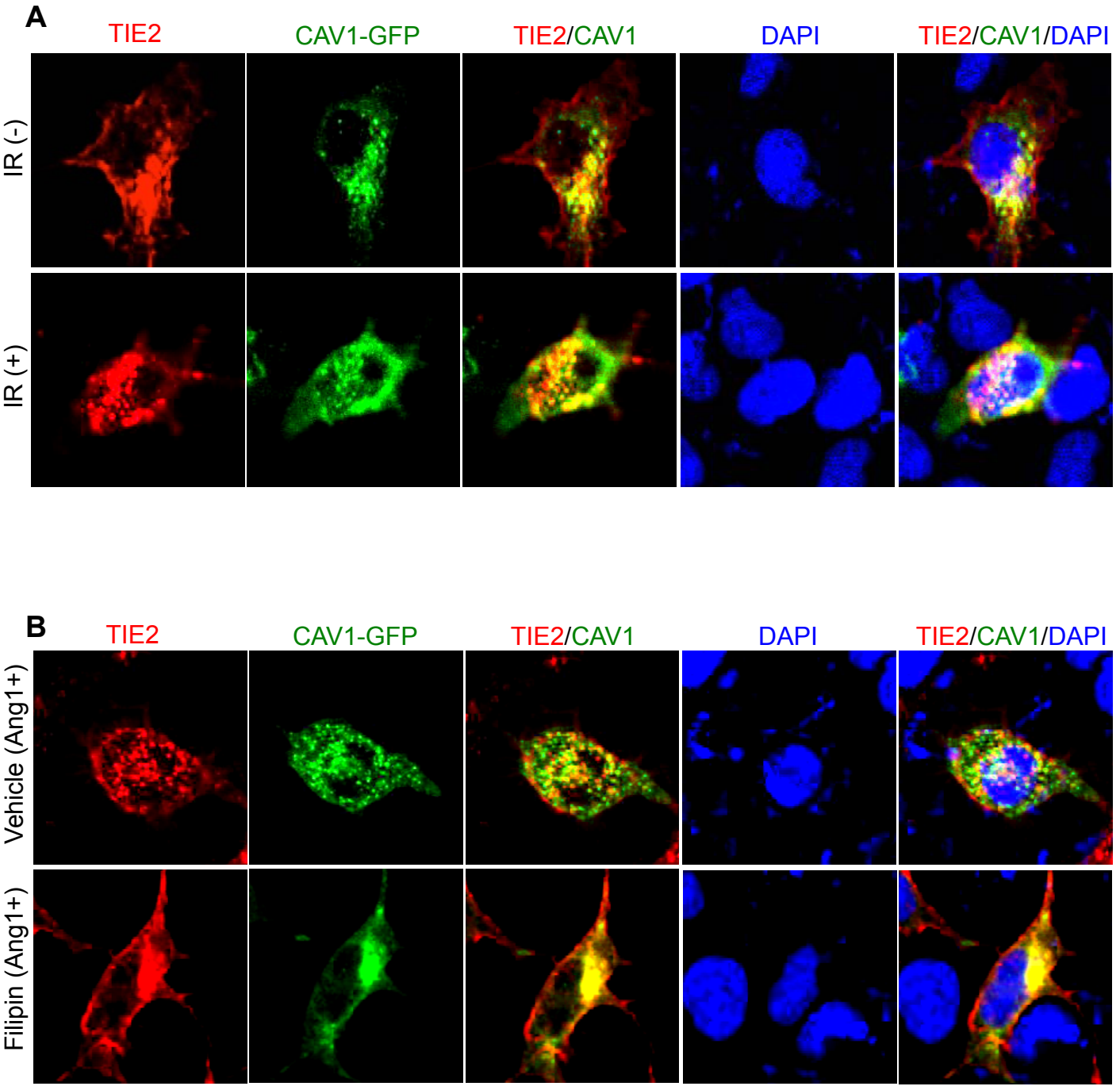
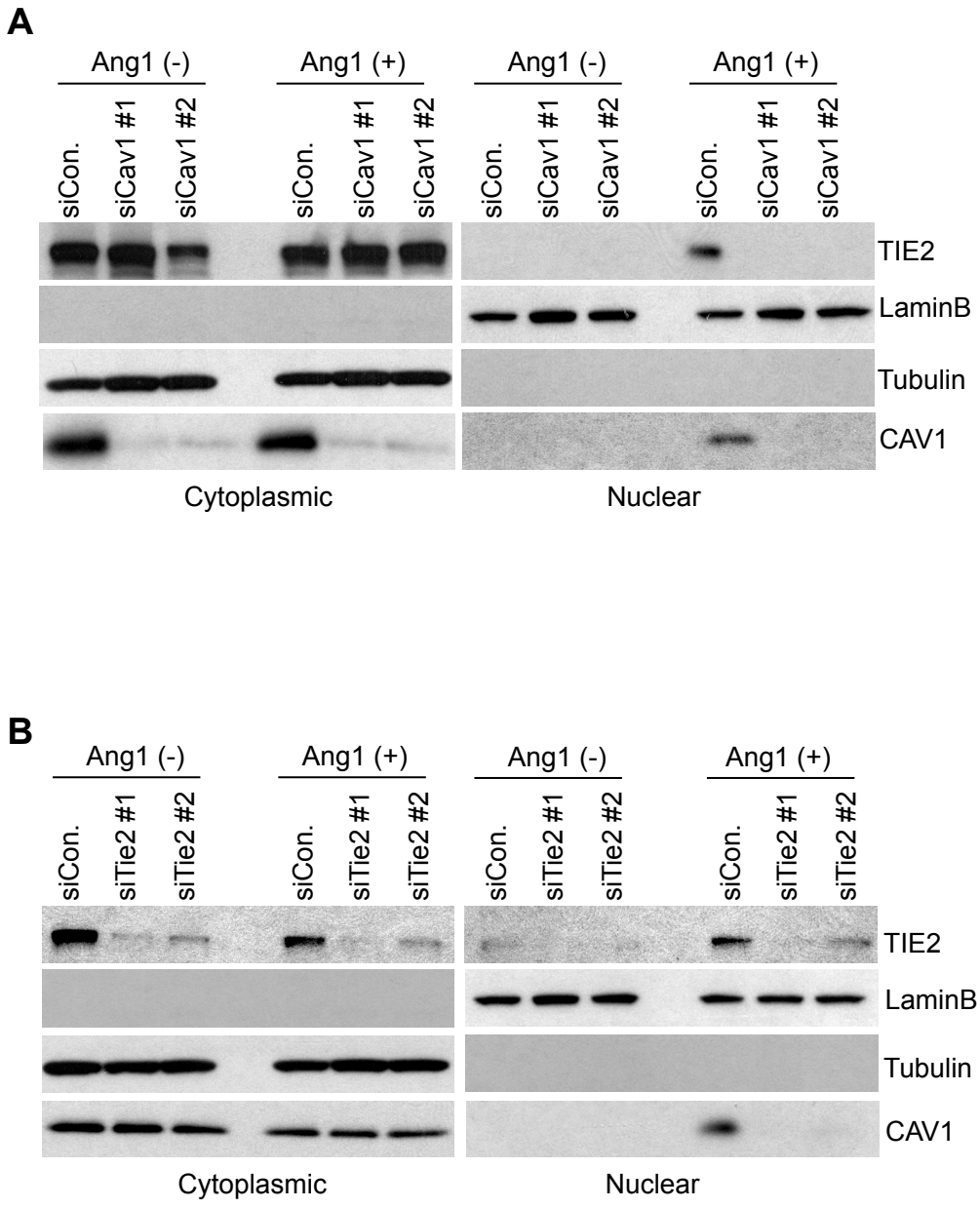
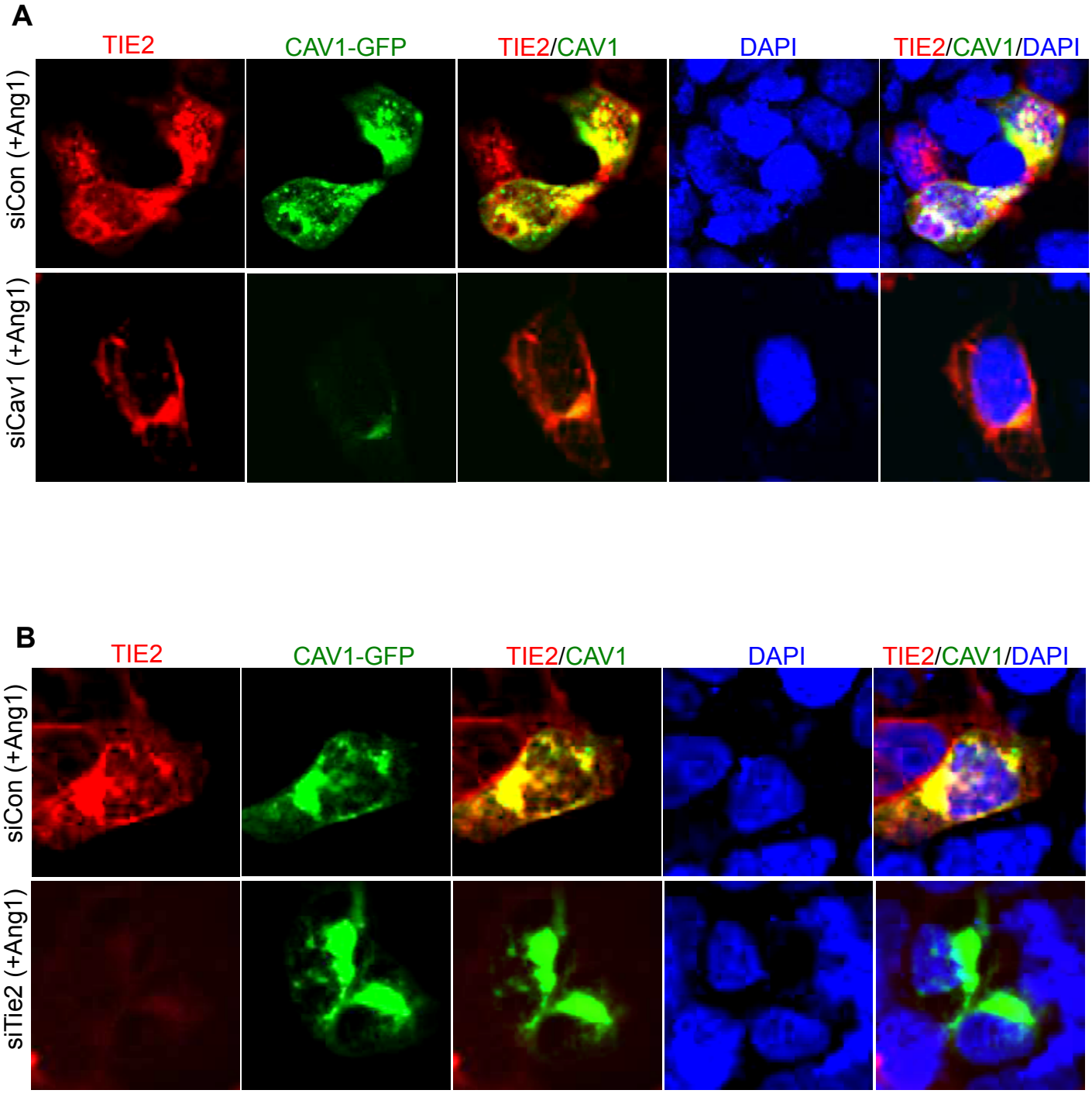


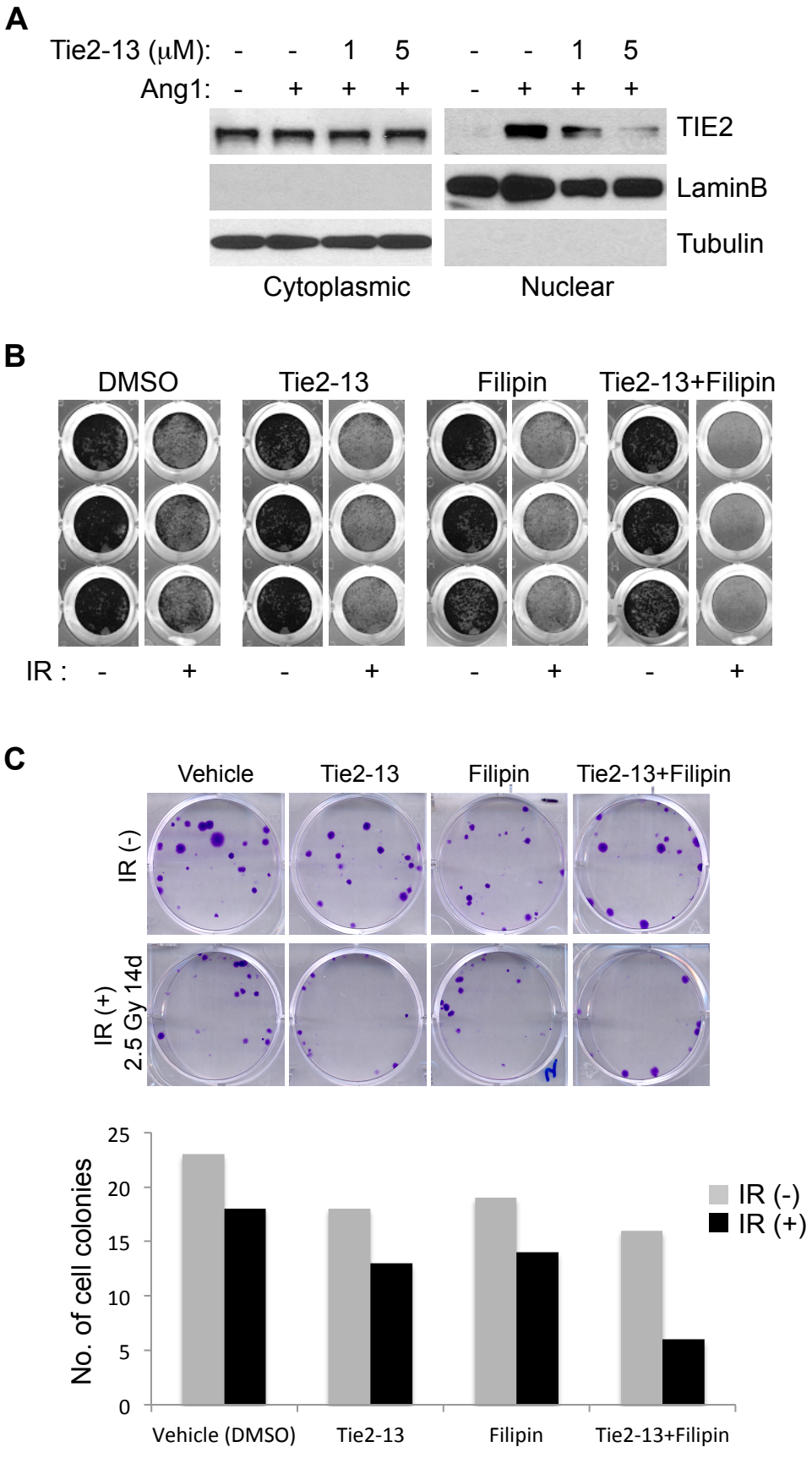
Supplemental Figure S1. Additional data on caveolin-1-dependent TIE2 nuclear translocation using confocal microscopy .



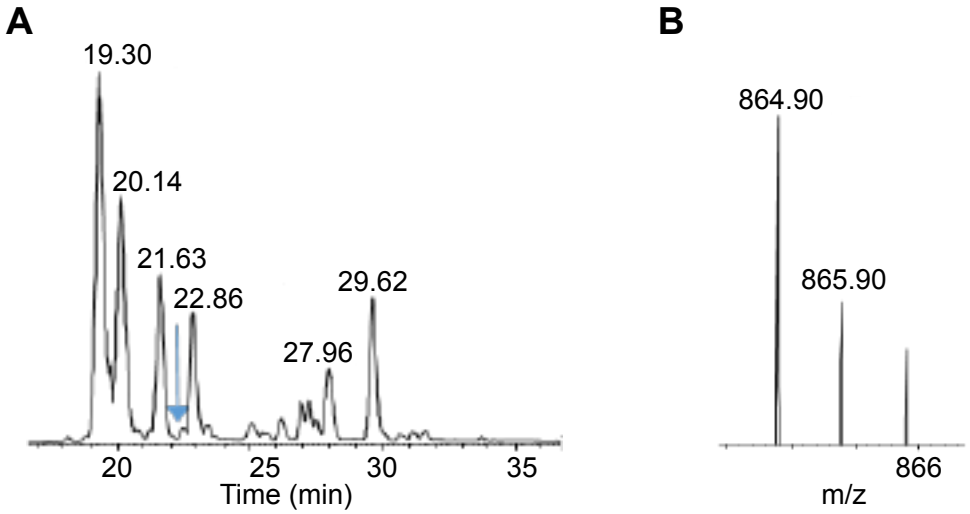
Supplemental Figure S2. Additional data on caveolin-1-dependent TIE2 nuclear translocation.



Supplemental Figure S3. Additional data on caveolin-1-dependent TIE2 nuclear translocation using confocal microscope.



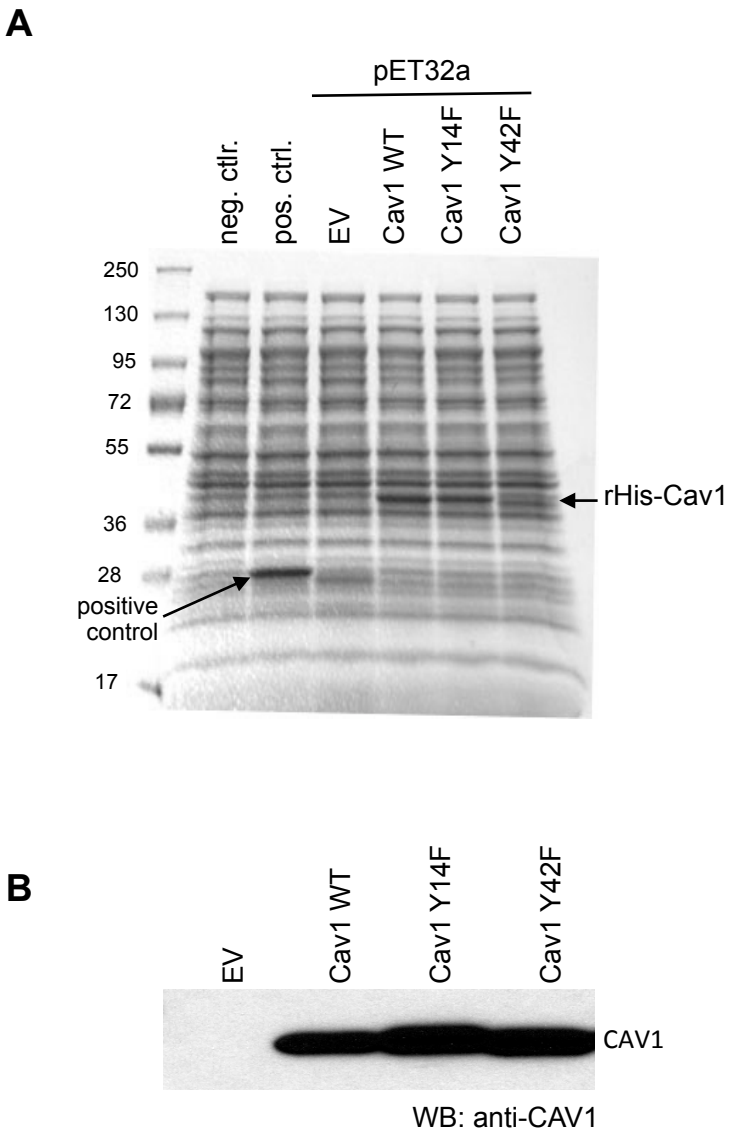
Supplemental Fig. S4. Additional data on the role of TIE2/caveolin-1 in radioresistance.



C

Sequence	phosphoRS Site Probabilities (%)
ASFTTFTVTK	
YVDSEGHL**y**TVPIR	Y(1): 0.0; **Y(9): 100.0**
HLNDDVVK	
IDFEDVIAEPEGTHSFDGIWK	
EQGNI**Y**KPnnKAmADELSEK	Y(6): 0.0
EIDLVNRDPK	
EIDLVnRDPK	
YWF**Y**R	Y(1): 0.0; Y(4): 0.0
EIDLVNR	

Supplemental Fig. S5. Additional analyses of mass spectrometry of TIE2-mediated caveolin-1 phosphorylation.



Supplemental Fig. S6: *In vitro* caveolin-1 expression, as assessed using a cell free protein expression system.

SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. S1. Additional data on caveolin-1-dependent TIE2 nuclear translocation using confocal microscopy. (A) HEK293.Tie2-myc cultures were serum starved for 4 hours before IR (10Gy; 30 min). Cell fixation and immunofluorescence studies were performed as previously described (1), using anti-myc-tag antibody (red fluorescence). Green fluorescence from the GFP signal was used as a reporter for caveolin-1 expression. DAPI was used for nuclear staining. Merged images are provided for visualization of TIE2/caveolin-1 nuclear co-localization. (B) HEK293.Tie2-myc cultures were serum starved for 4 hours and treated with Filipin (1µg/ml); 60 min later, Ang1 was added (400 ng/ml; 30 min). Images were captured using a confocal microscope (Olympus FluoView FV1000).

Supplemental Fig. S2. Additional data on caveolin-1-dependent TIE2 nuclear translocation. (A) U251.Tie2 cultures were transfected with two different siRNAs against caveolin-1 (10 nM); 48 hours later, they were exposed to Ang1 for 30 min. BSA (0.1% in PBS) was used as the vehicle/control in Ang1(-) samples. siCon, non-targeted siRNA. Cytoplasmic and nuclear proteins were obtained after subcellular fractionation and analyzed by Western blot analysis. LaminB and tubulin expression levels were used as nuclear and cytoplasmic protein loading controls, respectively. (B) U251.Tie2 cells were transfected with two different siRNAs against Tie2 (10 nM) and exposed to Ang1; proteins were analyzed as described in (A). siCon, non-targeted siRNA. The siRNA sequences used are listed in Supplemental Table 1. The antibodies and working dilutions are listed in Supplemental Table 3.

Supplemental Fig. S3. Additional data on caveolin-1-dependent TIE2 nuclear translocation using confocal microscope. siRNA against caveolin-1 (A) or siRNA against Tie2 (B) was transfected into HEK293.Tie2-myc cells, and 24 hours later, Cav1-GFP plasmid was transfected, and after 36 hours cells were serum starved for additional 4 hours before treatment with Ang1(400 ng/ml; 30 min). Confocal microscopy was performed as Fig S1.

Supplemental Fig. S4. Additional analyses on the role of TIE2/caveolin-1 in radioresistance. **(**A) U251.Tie2 cells treated with the TIE2 inhibitor Tie2-13 (1 and 5 μM; Enzo Life Sciences; ALX-270-434) (2) and exposed to Ang1 (400 ng/ml; 30 min). Nuclear and cytoplasmic proteins were obtained by subcellular fractionation and subjected to Western blot analysis. LaminB and tubulin were used for nuclear and cytoplasmic protein loading controls, respectively. (B) U251.Tie2 cells were plated in a 96-well plate at subconfluent density and treated with Tie2-13 (5 μM) and Filipin (2 μg/ml) (3), alone or in combination; 24 h later, they were irradiated (15 Gy) and kept in an incubator for an additional 48 h at 37°C. Cell viability was visualized with crystal violet solution (0.1% crystal violet and 20% methanol) for 15 min at room temperature (RT); a representative experiment is presented. (C) U251.Tie2 cells (25 cells) were plated in a 6-well plate and treated with Tie2-13 (5 μM) and Filipin (1 μg/ml), alone or in combination; 24 h later, they were irradiated (2.5 Gy) and kept in an incubator for 2 weeks at 37°C. Cells were stained with crystal violet solution (0.1% crystal violet and 20% methanol) for 15 min at room temperature (RT); a representative experiment is presented.

Supplemental Fig. S5. Additional analyses of mass spectrometry of TIE2-mediated caveolin-1 phosphorylation. As shown in Figure 4. Nano HPLC chromatographs of the

caveolin-1 protein tryptic peptide mixture; the target ion was eluted as indicated (arrow) in 22.86 min (A) and a zoom scan shows the target ion at 864.9Da (B). (C) Shown are the probabilities of phosphorylation of different Tyr residues of caveolin-1 after *in vitro* exposure to active TIE2, analyzed by mass spectrometry.

Supplemental Fig. S6. *In vitro* caveolin-1 expression, as assessed using a cell-free protein expression system. **(**A) We constructed *pET32a-Cav1WT* by cloning human caveolin-1 cDNA into the pET-32a(+) vector (Novagen, no. 69015-3) between the *BamH*I and *Xho*I sites. *pET32a-Cav1Y14F* and *pET32a-Cav1Y42F* were generated using the QuikChange II site-directed mutagenesis kit (Agilent Technologies), following the manufacturer's recommended protocol. The primers used are listed in Supplementary Table 2. Colloidal blue staining (Invitrogen) of 4-20 Tris-Glicine gel showing *in vitro* wild-type (WT) and mutant (Y14F; Y42F) caveolin-1 protein expression using a cell-free protein expression system (AccuRapid cell-free protein expression kit, Bioneer, no. K7250) according to the manufacturer's protocol. No template was used in the negative control sample, and the manufacturer provided the positive control template. *EV*, pET-32a empty vector. (B) Western blot analysis of the *in vitro* cell-free system, which generated wild-type and mutant caveolin-1 proteins. The antibodies and working dilutions are listed in Supplemental Table 3.

Supplemental Table 1. List of siRNA sequences used in this study

Target protein	Company	5´ to 3´ sequence
TIE2	Life Technology	S: GCUUCUAUACAAACCCGUUTT
(TIE2 #1)	ID: s13984; no. 4390824	As: AACGGGUUUGUAUAGAAGCTT
TIE2	Life Technology	S: CAAACCCGUUAAUCACUAUTT
(TIE2 #2)	ID: s13982; no. 4392420	As: AUAGUGAUUAACGGGUUUGTA
CAV1	Life Technology	S: GCUUCCUGAUUGAGAUUCATT
(CAV #1)	ID: s2446; no. 4390824	As: UGAAUCUCAAUCAGGAAGCTC
CAV1	Life Technology	S: CCUUCACUGUGACGAAAUATT
(CAV #2)	ID: s2448; no. 4457298	As: UAUUUCGUCACAGUGAAGGTG

Supplemental Table 2. List of primer sequences

Primer name	5´ to 3´ sequence
Cav1 Y14F sense	GGATGGGAACGGTGAAGAGATGTCCCTCC
Cav1Y14F antisense	GGAGGGACATCTCTTCACCGTTCCCATCC
Cav1Y14D Sense	CGGAGGGACATCTCGACACCGTTCCCATC
Cav1Y14D Antisense	GATGGGAACGGTGTCGAGATGTCCCTCCG
Cav1 Y42F sense	GTGCGCGTCGAACACTTGCTTCTCGCTCAG
Cav1 Y42F antisense	CTGAGCGAGAAGCAAGTGTTCGACGCGCAC
Tie2 F (for RT-PCR)	TTACGGGCCAGATTGTAAGC
Tie2 R (for RT-PCR)	CATCCCCAAAGTAAGGCTCA
Cav1 F (for RT-PCR)	GGGCAAATACGTAGACTCGGAGGGAC
Cav1 R (for RT-PCR)	GCGAAGTAAATGCCCCAGATGAGTGC
GAPDH sense	ATGGGGAAGGTGAAGGTCGG
GAPDH antisense	GACGGTGCCATGGAATTTGC

Supplemental Table 3. List of antibodies used in this study

Antibody	Company	Experimental use
TIE2 (C-20)	Santa Cruz Biotechnology, Inc. (no. sc-324)	WB (1:2000), IF (1:500), IP
CAV1	Abcam (no. ab32577)	WB (1:2000)
CAV1 (N-20)	Santa Cruz Biotechnology, Inc. (no. sc-894)	WB (1:2000)
CAV2 (H-96)	Santa Cruz Biotechnology, Inc. (no. sc-7942)	WB (1:1000)
CAV3 (I-16)	Santa Cruz Biotechnology, Inc. (no. sc-5437)	WB (1:1000)
pCAV1 (Y14)	Abcam (no. ab75876)	WB (1:2000)
CD36	Abcam (no. ab133625)	WB (1:1000)
GFP	Novus Biologicals (no. NB600-308)	WB (1:5000)
LaminB	Santa Cruz Biotechnology, Inc. (no. sc-6216)	WB (1:4000)
Alpha-tubulin	Santa Cruz Biotechnology, Inc. (no. sc-23948)	WB (1:5000)
Myc-tag	Cell Signaling Technology (no. 2276)	WB (1:2000), IF (1:500), IP
γH2AX (mouse)	Abcam (no. ab26350)	WB (1:2000), IF (1:1000)
PTRF	Novus USA (no. H00284119-B01P)	IB (1:500)
Alexa Fluor 555 goat anti-mouse IgG (H+L)	Life Technologies (no. A-21422)	IF (1:1000)

WB, Western blotting; IF, immunofluorescence; IP, immunoprecipitation.

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