Supplemental Figure Legends

Supplemental Figure 1. MT1-MMP gelatinolytic activity was observed in the soluble phase of detergent lysates and present in a number of MT1-MMP-expressing cell lines. (A) HT1080 cell lysates (1% Brij 99) was centrifuged at 100,000 g where indicated and immunoprecipitated with anti-CD81 or anti-MHC I antibodies. Eluates were analyzed by gelatin zymography. (B) MDA-MB-231, HeLa, BT549, 293, U87, LN827, MCF-7, NT2, A549 and K562 cells were lysed in 1% Brij 99 lysis buffer and immunoprecipitated using an anti-tetraspanin cocktail (TSPAN: CD9, CD63, CD81, CD82, CD151 and TSPAN4), anti-CD81, or anti-MHC I and the eluates analyzed by gelatin zymography. MMP-2 standard (std) was loaded on the gel as a gelatinolytic control enzyme. pMMP-2: pro-MMP-2, aMMP-2: active MMP-2.

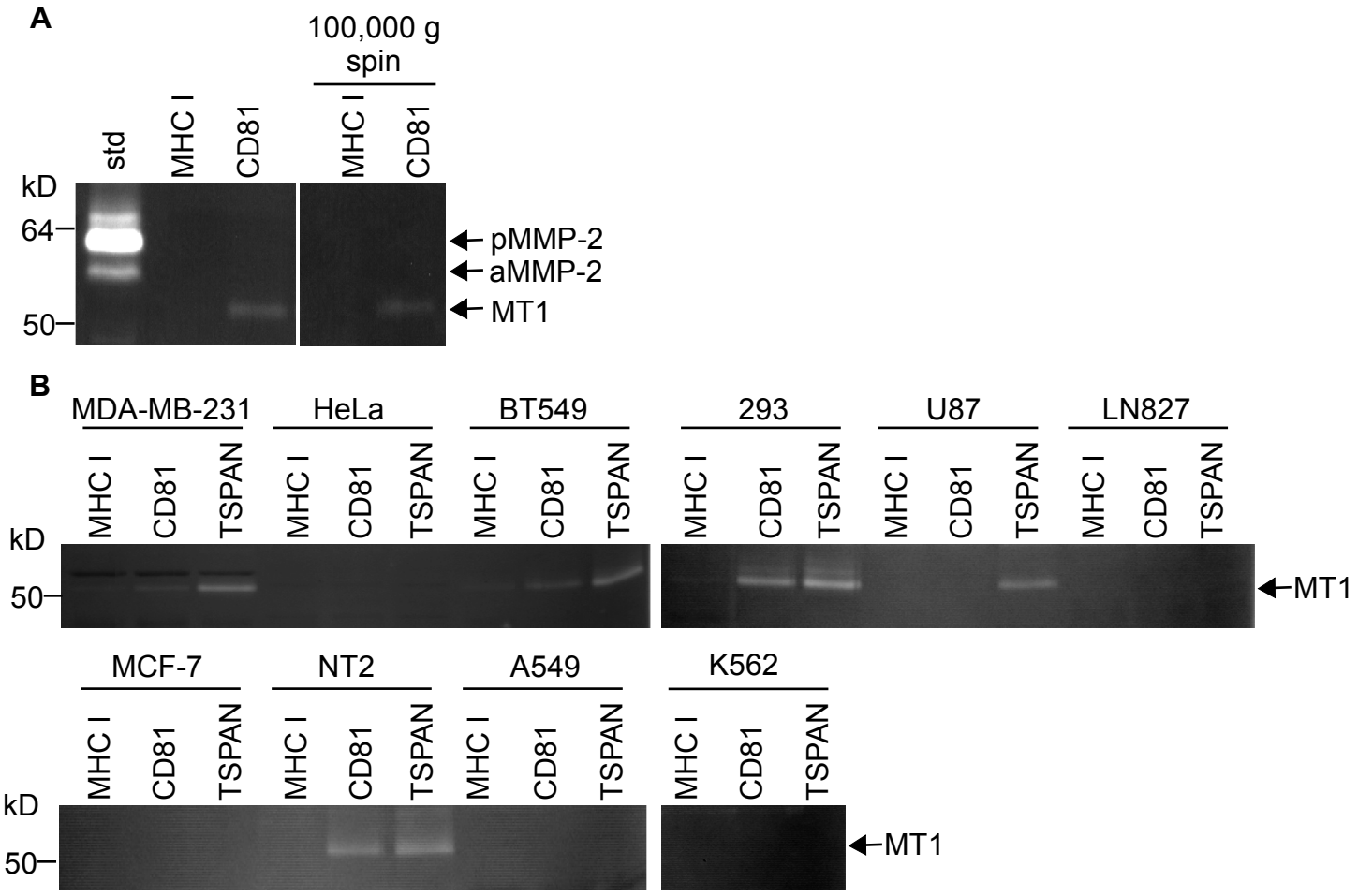
Supplemental Figure 2. Detergent specificity analysis of the MT1-MMP-tetraspanin associations. (A) MDA-MB-231 cells were lysed with the indicated detergent lysis buffers and immunoprecipitated with anti-CD9 or anti-MHC I antibodies. Eluates were analyzed by gelatin zymography and western blotting for MT1-MMP (AB815 antibody). (B) HT1080 cells transfected with MT1-MMP-GFP (MT1-GFP) or TSPAN12-FLAG were lysed in 1% Brij 96 lysis buffer and immunoprecipitated with anti-FLAG or anti-GFP antibodies. In addition, equal amounts of both HT1080-MT1-GFP and HT1080-TSPAN12-FLAG lysates were mixed in a 1:1 ratio after lysis and immunoprecipitated as above. Eluates were analyzed by gelatin zymography or western blotting probed for FLAG, GFP or MT1-MMP (AB815 antibody). pMMP-2: pro-MMP-2, aMMP-2: active MMP-2, n.s.: non-specific, std: MMP-2 standard.

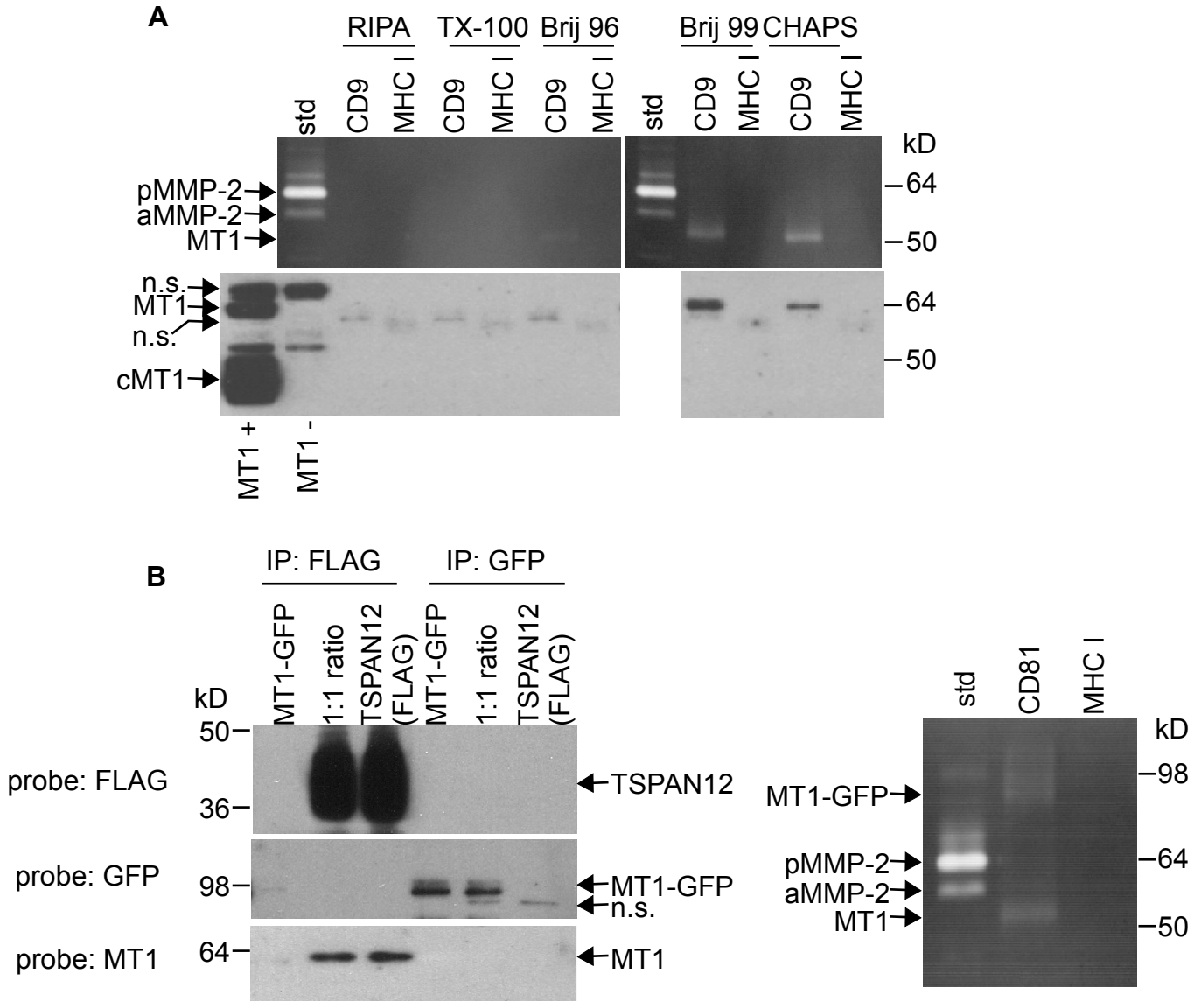
Supplemental Figure 3. Confirmation of tetraspanin knockdown. (A) MCF-7-VC or MCF-7-MT1 cells were transfected with the indicated siRNAs. Lysates were collected 4 days after siRNA transfection and western blot performed for CD9, CD81 and actin. (B) RNA was also collected 2 days after siRNA transfection and RT-PCR performed for TSPAN12 and GAPDH. Results are expressed as ratio of TSPAN12/GAPDH. (C) HT1080 cells expressing TSPAN12-FLAG were transfected with control or TSPAN12

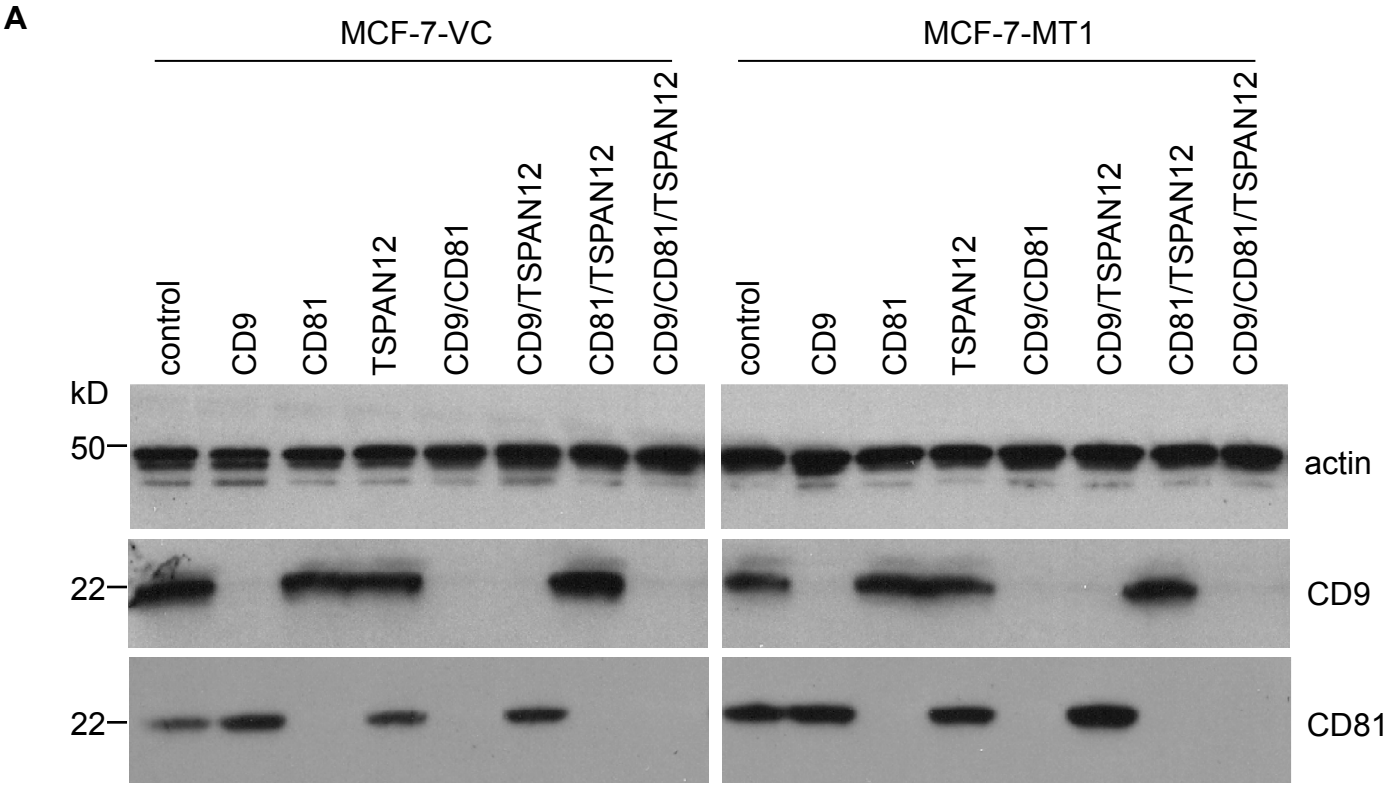
siRNAs. Lysates were collected 4 days post transfection and western blotting performed for FLAG (TSPAN12 antibodies are currently unavailable) and actin to confirm protein knockdown. (D) RNA was also collected 2 days after siRNA transfection and RT-PCR performed to confirm TSPAN12 knockdown in HT1080 cells relative to GAPDH.

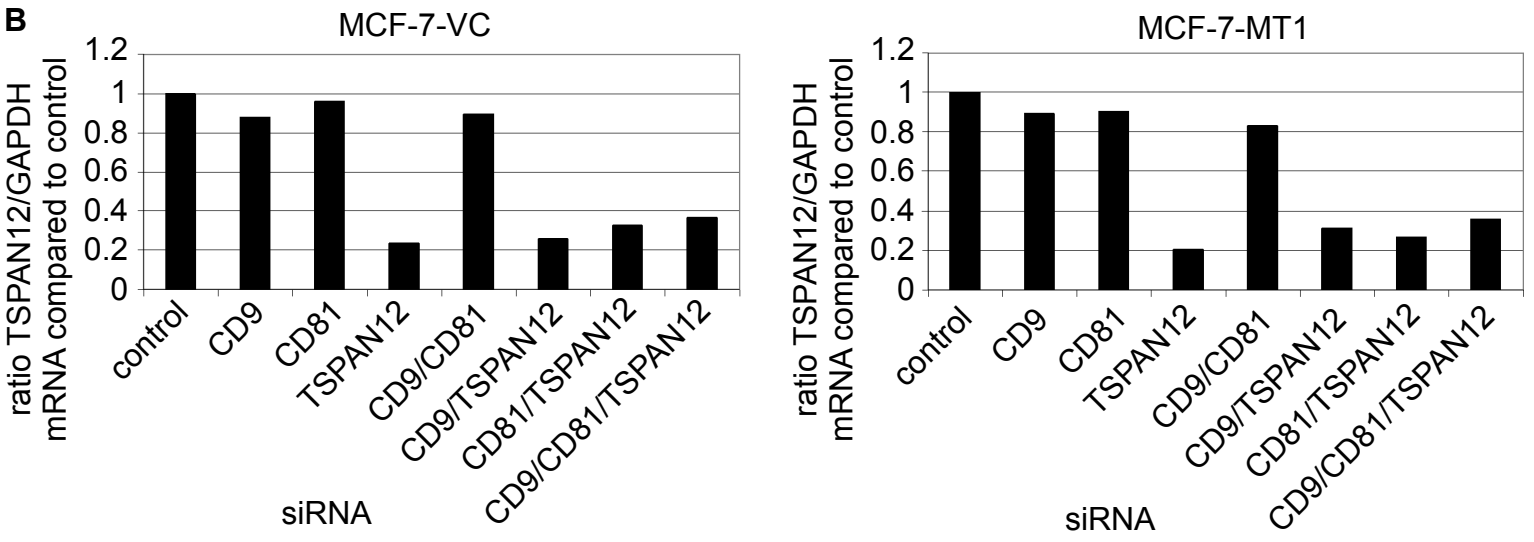
Supplemental Figure 4. Tetraspanin effects on MT1-MMP activity in cells grown in collagen gels. MCF-7-MT1 and MCF-7-VC cells were treated for 2 days with siRNAs, and then cells were embedded within collagen gels for the indicated times. The % area of cell coverage was determined to yield mean +/- S.D. (N=3, *, $P<0.05$; **, $P<0.005$ compared to control at each time point).

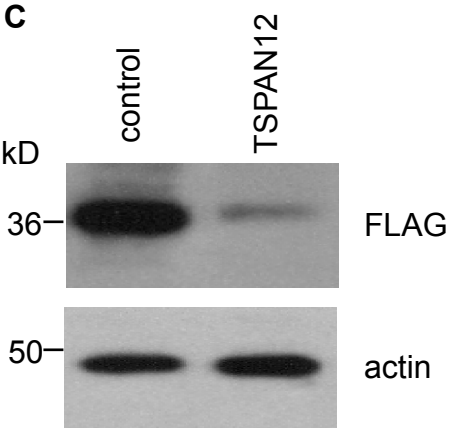
Supplemental Figure 5. Tetraspanin knockdown does not increase MT1-MMP internalization. An MT1-MMP cell surface internalization assay was performed on MCF-7-MT1-GFP cells transfected with control or CD9/CD81/TSPAN12 (3xTSPAN) siRNAs. Due to the lower total MT1-MMP levels in CD9/CD81/TSPAN12 siRNA treated cells, the internalized MT1-MMP was expressed as a % of internalized from the total initial amount on the cell surface. The slope indicating the internalization rate is similar in both cases. Similar results were also obtained for MCF-7-MT1 cells.

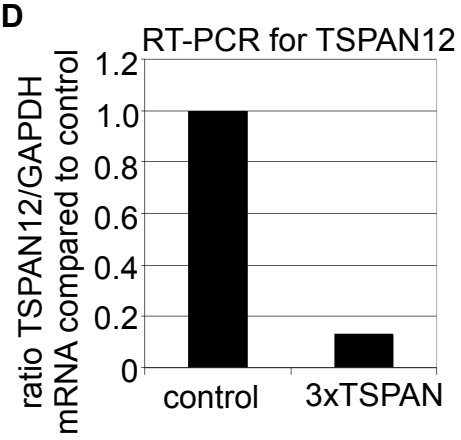

A
100,000 g spin
std
MHC I
CD81
MHC I
CD81
kD
64
50
pMMP-2
aMMP-2
MT1
B
MDA-MB-231
HeLa
BT549
293
U87
LN827
MHC I
CD81
TSPAN
kD
50
MT1
MCF-7
NT2
A549
K562
MHC I
CD81
TSPAN
kD
50
MT1

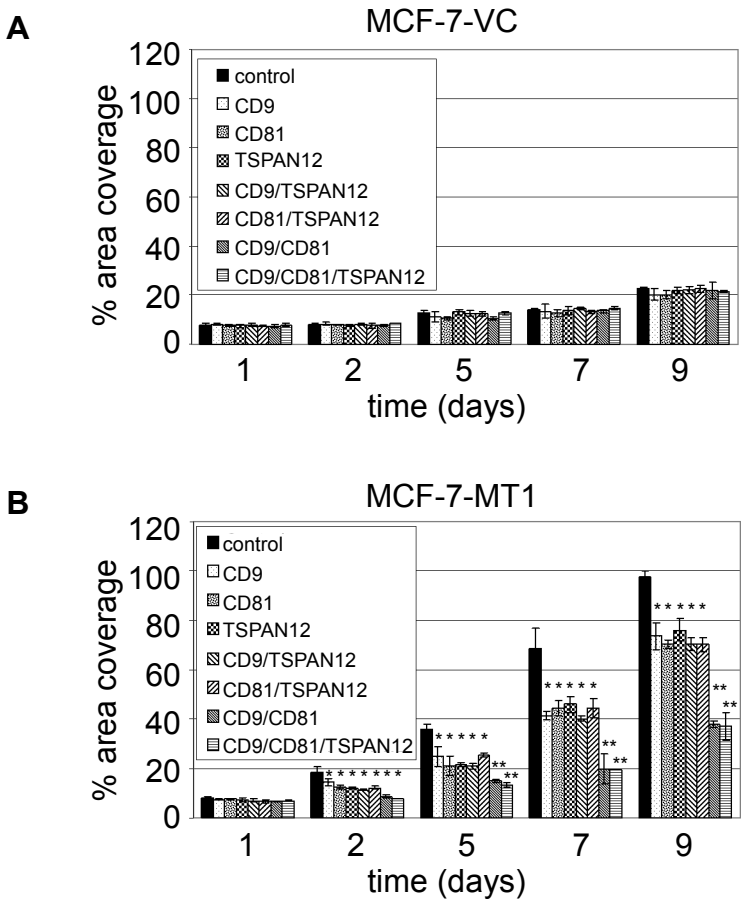

A
RIPA
TX-100
Brij 96
Brij 99
CHAPS
std
CD9
MHC I
kD
64
50
pMMP-2
aMMP-2
MT1
n.s.
cMT1
MT1 +
MT1 -
B
IP: FLAG
IP: GFP
MT1-GFP
1:1 ratio
TSPAN12 (FLAG)
probe: FLAG
probe: GFP
probe: MT1
98
36
TSPAN12
MT1-GFP
n.s.
CD81
MHC I

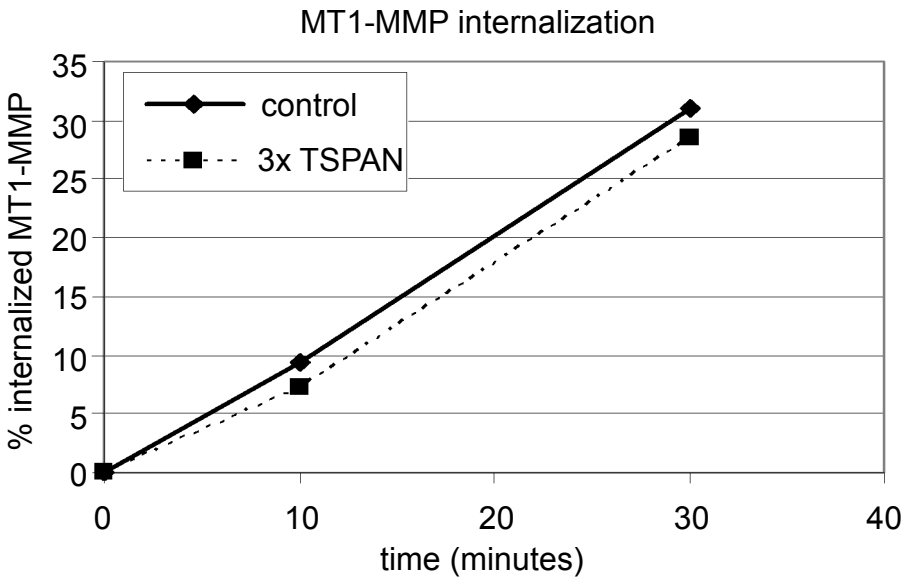

A
MCF-7-VC
MCF-7-MT1
control
CD9
CD81
TSPAN12
CD9/CD81
CD9/TSPAN12
CD81/TSPAN12
CD9/CD81/TSPAN12
kD
50
22
22
actin
CD9
CD81


B
MCF-7-VC
MCF-7-MT1
ratio TSPAN12/GAPDH mRNA compared to control
1.2
1
0.8
0.6
0.4
0.2
0
control
CD9
CD81
TSPAN12
CD9/CD81
CD9/TSPAN12
CD81/TSPAN12
CD9/CD81/TSPAN12
siRNA


C
control
TSPAN12
kD
36
50
FLAG
actin


D
RT-PCR for TSPAN12
ratio TSPAN12/GAPDH mRNA compared to control
1.2
1.0
0.8
0.6
0.4
0.2
0
control
3xTSPAN


A
MCF-7-VC
% area coverage
120
100
80
60
40
20
0
control
CD9
CD81
TSPAN12
CD9/TSPAN12
CD81/TSPAN12
CD9/CD81
CD9/CD81/TSPAN12
1
2
5
7
9
time (days)
B
MCF-7-MT1
% area coverage
120
100
80
60
40
20
0
control
CD9
CD81
TSPAN12
CD9/TSPAN12
CD81/TSPAN12
CD9/CD81
CD9/CD81/TSPAN12
1
2
5
7
9
time (days)


MT1-MMP internalization
% internalized MT1-MMP
35
30
25
20
15
10
5
0
control
3x TSPAN
0
10
20
30
40
time (minutes)

Cell surface expression of tetraspanins and other proteins by flow cytometry on MCF-7-MT1, HT1080 and MDA-MB-231 cells

MCF-7-MT1	MFI *	HT1080	MFI	MDA-MB-231	MFI
neg. control	6	neg. control	3	neg. control	4
CD9	935	CD9	3	CD9	526
CD63	603	CD63	274	CD63	406
CD81	704	CD81	1414	CD81	536
CD82	140	CD82	69	CD82	218
CD151	378	CD151	842	CD151	616
TSPAN4	68	TSPAN4	145	TSPAN4	222
MHC I	167	MHC I	492	MHC I	916
CD147	345	CD147	1857	CD147	954

* indicates mean fluorescence intensity (MFI) of each protein

Relative MT1-MMP recovery following CD9, CD81 or TSPAN12 immunoprecipitation (IP) in the indicated cell lines and indicated lysis buffers. A representative zymogram/western blot for MT1-MMP is shown in supplemental Fig. 2A.

Cell type and IP	RIPA	TX-100	Brij 96	Brij 99	CHAPS
MCF-7-MT1 IP: CD81	-	-	-	+++	+++
HT1080 IP: CD81	-	-	-	+++	+++
MDA-MB-231 IP: CD9	-	-	+	+++	+++
HT1080-TSPAN12 IP: FLAG (TSPAN12)	-	+	+++	+++	+++

Two distinct sets of tetraspanin siRNAs similarly reduced MT1-MMP protein expression analyzed by flow cytometry. Mean Fluorescent Intensity (MFI) values are indicated for MT1-MMP, CD9 and CD81. Confirmation of TSPAN12 knockdown was performed by RT-PCR.

	MFI			ratio TSPAN12/actin compared to control
	MT1	**CD9**	**CD81**	**TSPAN12**
neg. control	7.8	2.3	8.4	-
control siRNA	49.0	1016	1741	1
CD9/CD81/TSPAN12 siRNA (set 1)	24.6	575	480	0.06
CD9/CD81/TSPAN12 siRNA (set 2)	26.4	569	1120	0.16