Supplementary material

Identification of TENM4 as a novel Cancer Stem Cell-associated molecule and potential target in Triple Negative Breast Cancer

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In this section the raw colorimetric image of each PVDF membrane/blot with the transferred protein ladder, the ECL image and the superimposition between the colorimetric (containing the marker bands) and the ECL image to see the protein of interest and the ladder at the same time are reported. The lanes in the black rectangles are those of the samples shown in the figures of the manuscript.

For the current study the ThermoScientific (Rodano; Milan, Italy) PageRuler™ Prestained Protein Ladder ranging from 10 kDa to 170 kDa (cat#26616) and that ranging from 10 to 250 KDa (cat#26619) were used.

Densitometric analyses have been carried out by using Image Lab software (BioRad; Segrate; Milan, Italy). Densitometric values are shown at the end of each lane in the image showing the protein of interest and the ladder. For each blot we chose a reference lane that we marked as "100". All the other bands have been normalized accordingly.

A) W. Blot Fig. 4b; left panel (4T1), and central panel (HCC1806), for **TENM4** and **Vinculin**. Samples in black rectangles are shown.

Colorimetric blot with ladder

250 kDa

130 kDa
100 kDa

ECL image of the blot

4T1   HCC1806

TENM4 >

4T1   HCC1806

Vinculin >

Merge showing **TENM4**

250 kDa

13:100
Ep.:T

0:100
Ep.:T

Merge showing **Vinculin**

130 kDa
100 kDa

80:100
Ep.:T

94:100
Ep.:T
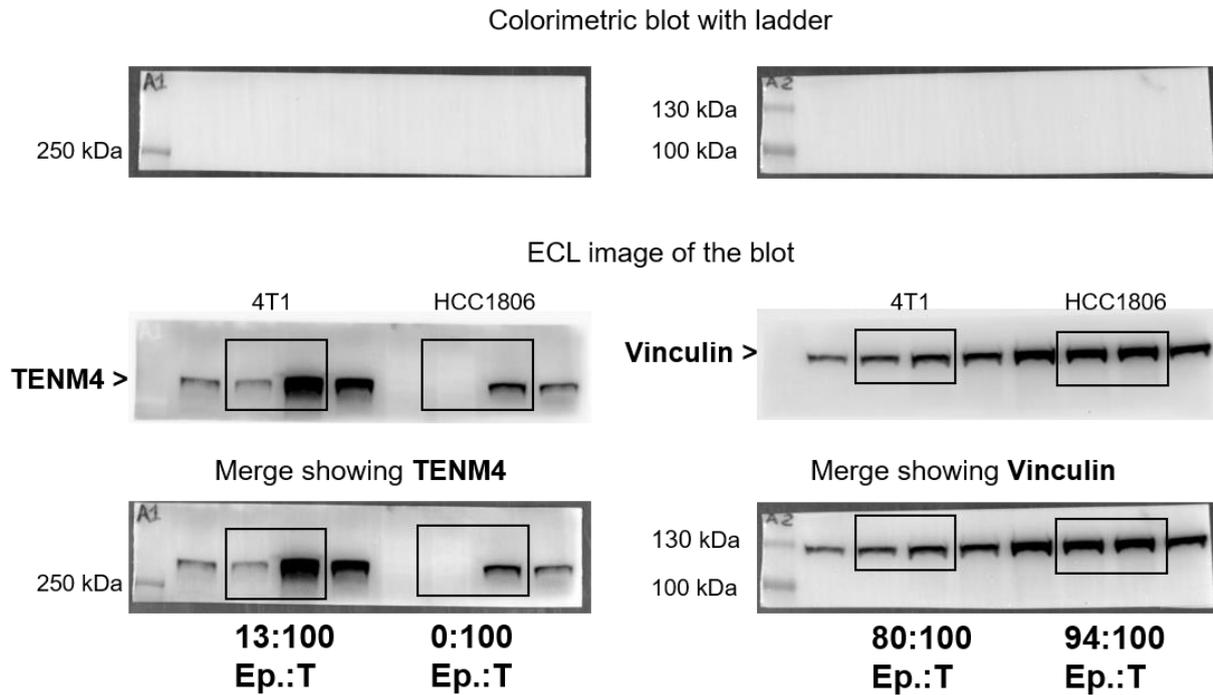
**B)** W. Blot Fig. 4b; left panel (4T1), and central panel (HCC1806), for **FAK**. Samples in black rectangles are shown.

Colorimetric blot with ladder

130 kDa
100 kDa

ECL image of the blot

FAK >

Merge showing **FAK**

130 kDa
100 kDa

41:100
Ep.:T

88:100
Ep.:T
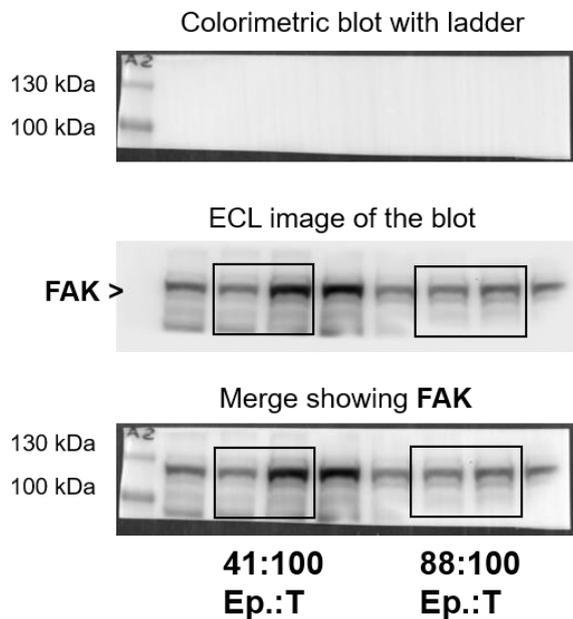
A) W. Blot Fig. 4b; right panel (MDA-MB-231); for **TENM4** and **β-actin**. Samples in black rectangles are shown in the fugure.

Colorimetric blot with ladder

55 KDa
35 KDa

250 KDa

ECL image of the blot

β-actin >

TENM4 >

Merge showing **TENM4**

Merge showing **β-actin**

55 KDa

35 KDa

250 KDa

100:873
Ep.:T

100:74
Ep.:T
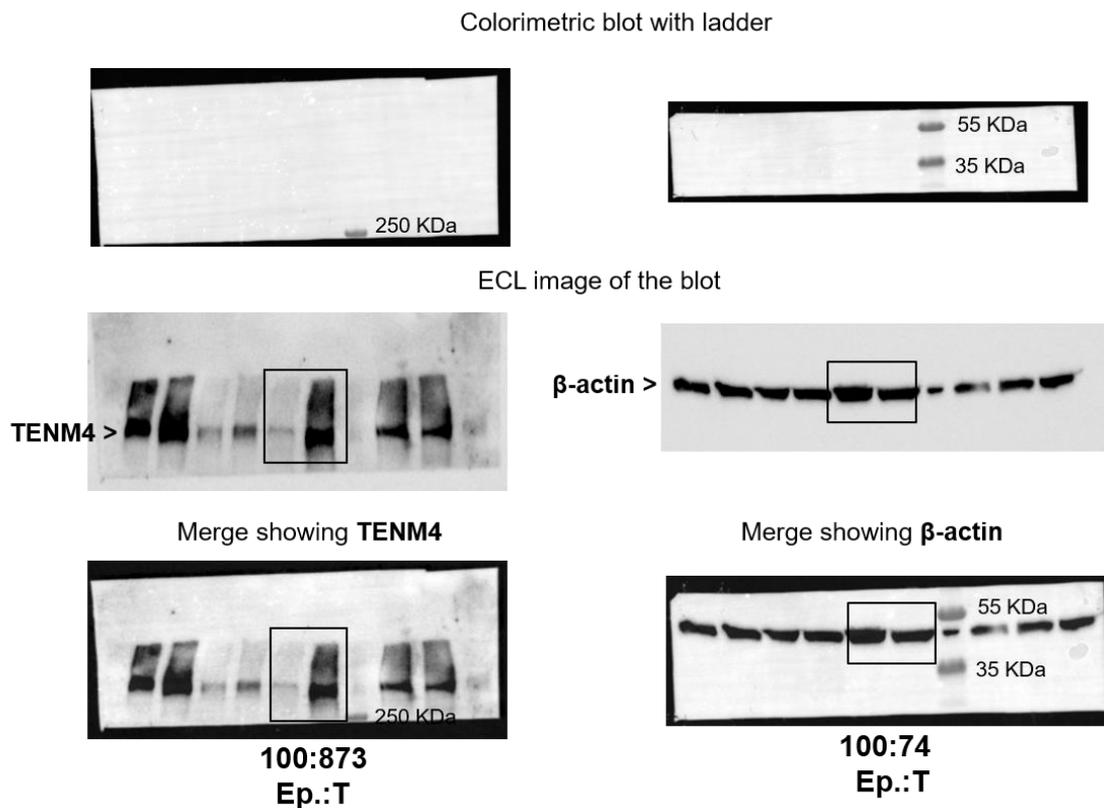
B) W. Blot Fig. 4b; right panel (MDA-MB-231); for **FAK**. Samples in black rectangles are shown in the fugure.

ECL image of the blot

130 KDa
100 KDa
70 KDa

< FAK

Colorimetric blot with ladder

Merge showing **FAK**

130 KDa
100 KDa
70 KDa

130 KDa
100 KDa
70 KDa

100:159
Ep.:T
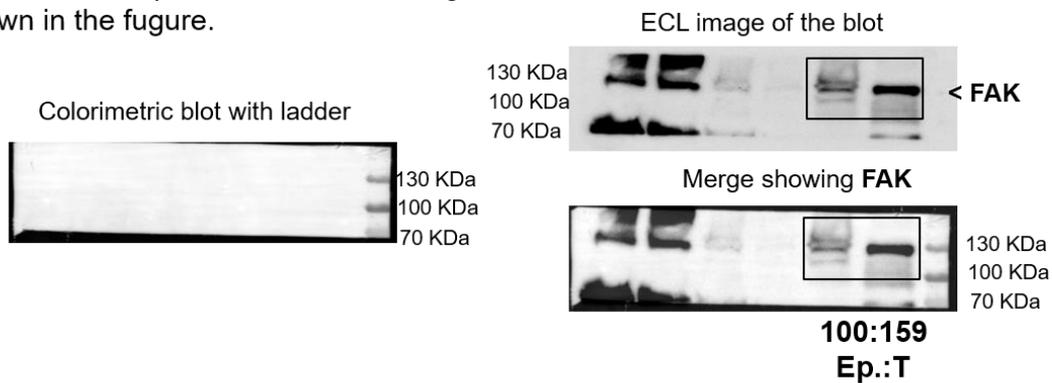
A) W. Blot Fig. 4c for **TENM4** and **Vinculin**. Samples in black rectangles are shown in the fugure.

Colorimetric blot with ladder

ECL image of the blot

TENM4 >   Vinculin >

Merge showing **TENM4**   Merge showing **Vinculin**

TENM4 >
250 kDa >

130 kDa >
100 kDa >

100:39
Mock:siRNA

100:94
Mock:siRNA
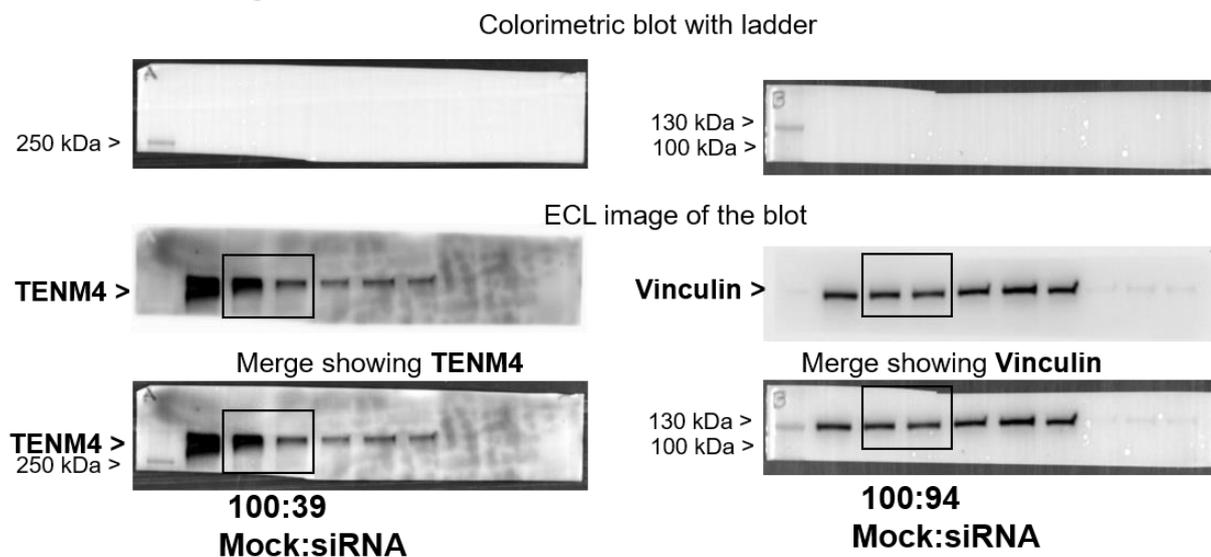
B) W. Blot Fig. 4c for **FAK** and **pFAK^{Y925}**. Samples in black rectangles are shown in the fugure.

Colorimetric blot with ladder

130 kDa >
100 kDa >

130 kDa >
100 kDa >

ECL image of the blot

FAK >   pFAK^{Y925}>

Merge showing **FAK**   Merge showing **pFAK^{Y925}**

130 kDa >
100 kDa >

130 kDa >
100 kDa >

100:95
Mock:siRNA

100:76
Mock:siRNA
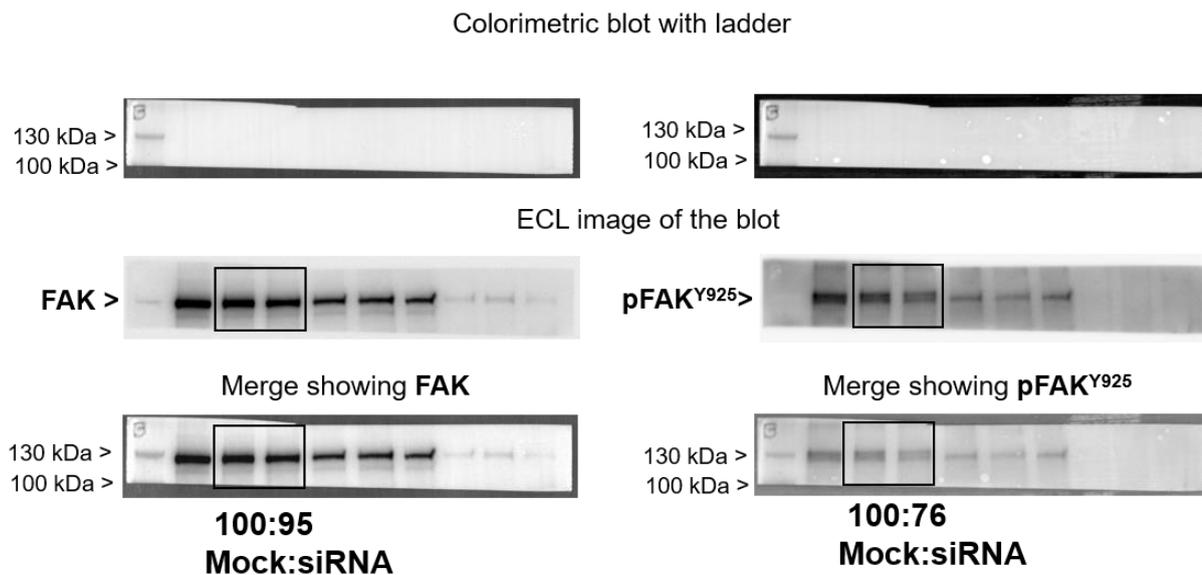
A) W. Blot Fig. 4f for **TENM4** and **β-actin**. Samples in black rectangles are shown in the fugure.

Colorimetric blot with ladder

250
130
100
70
KDa

55
35
KDa

ECL image of the blot

TENM4 >

β-actin >

Merge showing **TENM4**

Merge showing **β-actin**

250
130
100
70
KDa

55
35
KDa

100:36,1
Mock:siRNA

100:112
Mock:siRNA
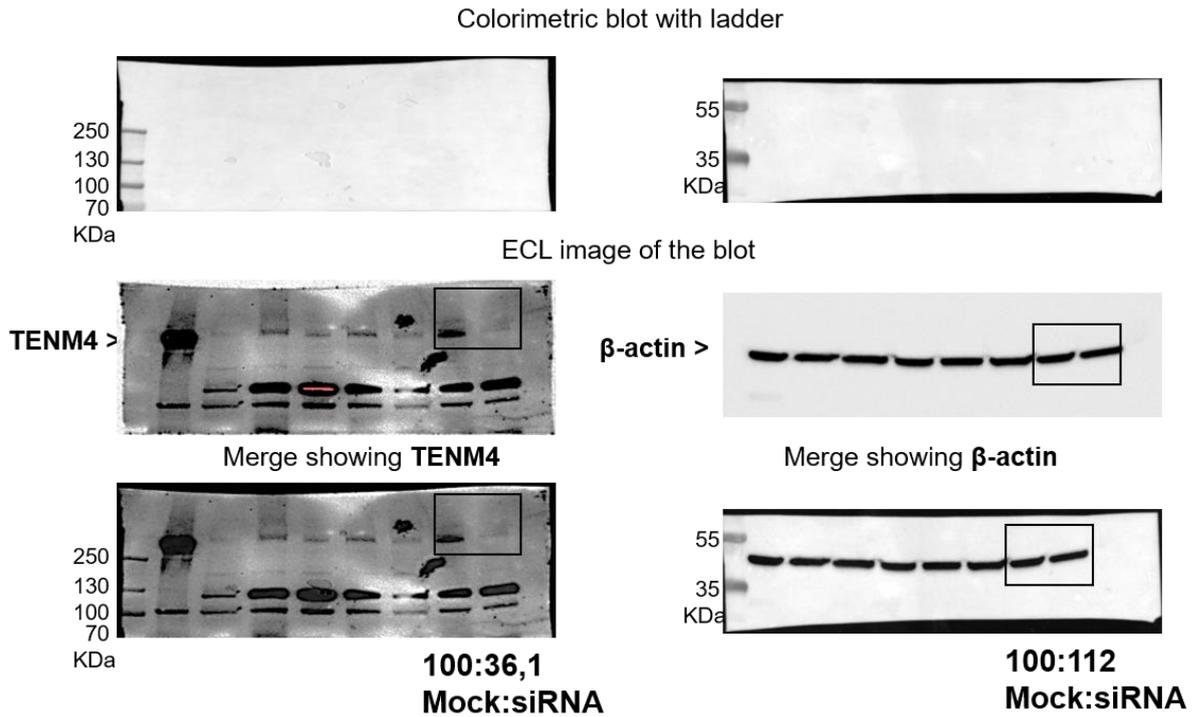
B) W. Blot Fig. 4f for **FAK** and **pFAK^{Y925}**. Samples in black rectangles are shown in the fugure.

Colorimetric blot with ladder

130
100
KDa

130
100
KDa

ECL image of the blot

FAK >

pFAK^{Y925}>

Merge showing **FAK**

Merge showing **pFAK^{Y925}**

130
100
KDa

130
100
KDa

100:112
Mock:siRNA

100:75
Mock:siRNA
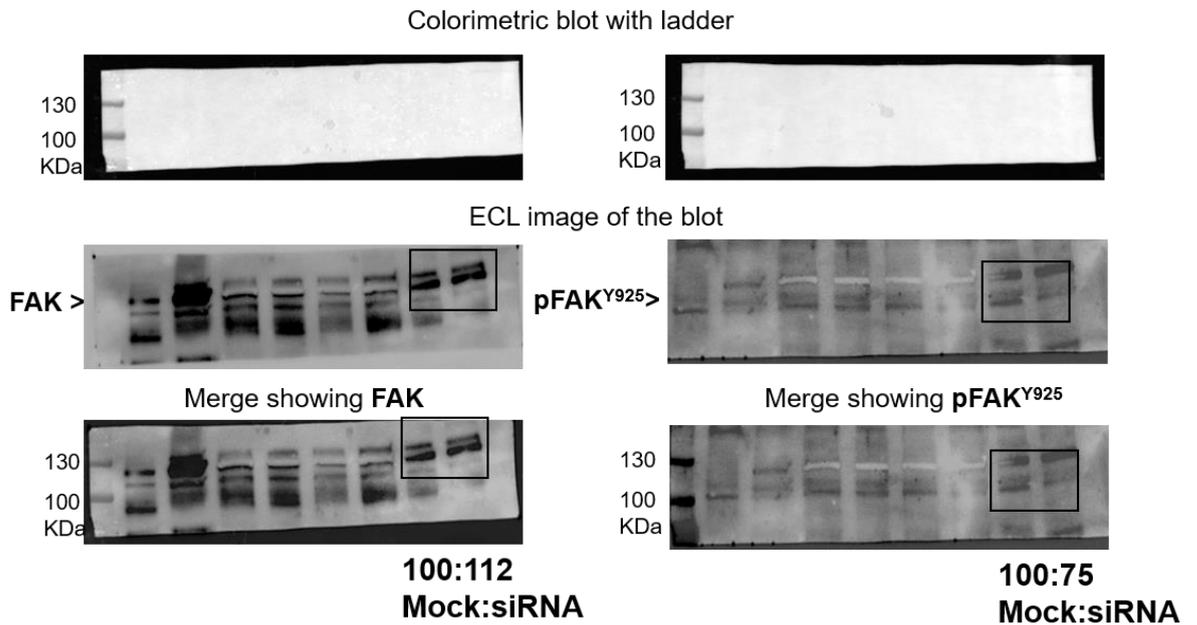
W. Blot Figure 5a (4T1; upper panel) for **TENM4** and **β-actin**. Samples in black rectangles are shown)

Colorimetric blot with ladder

ECL image of the blot

TENM4 >

β-actin >


33:65:100

86:88:100

Merge showing
TENM4

or
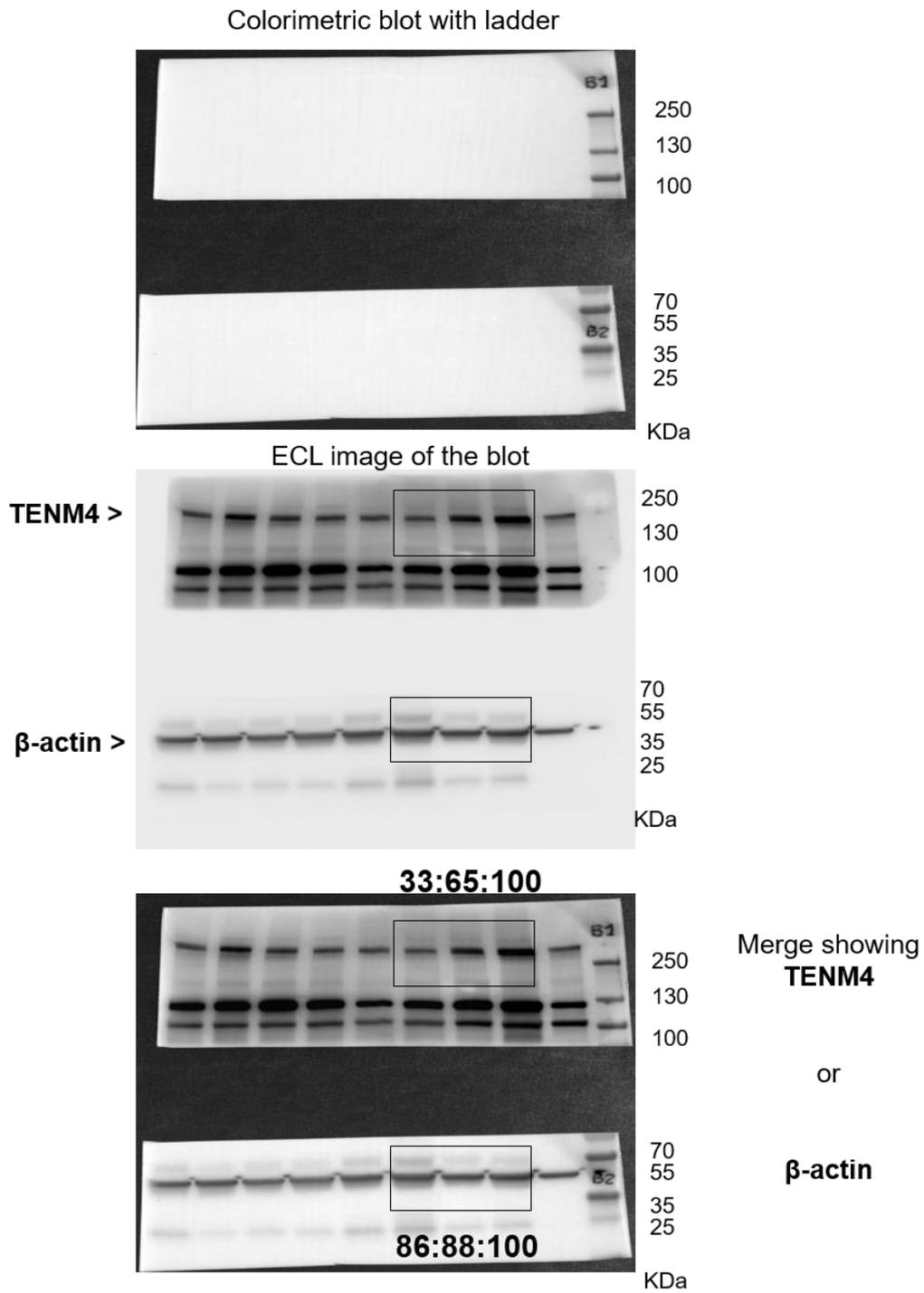
β-actin

W. Blot Figure 5a (MDA-MB-231; lower panel) for **TENM4** and **β-actin**. Samples in black rectangles are shown).

Colorimetric blot with ladder


ECL image of the blot

TENM4 >

β-actin >

250
130
100
70
55
35
25
KDa

100:125:76

100:131:114

Merge showing
TENM4

or
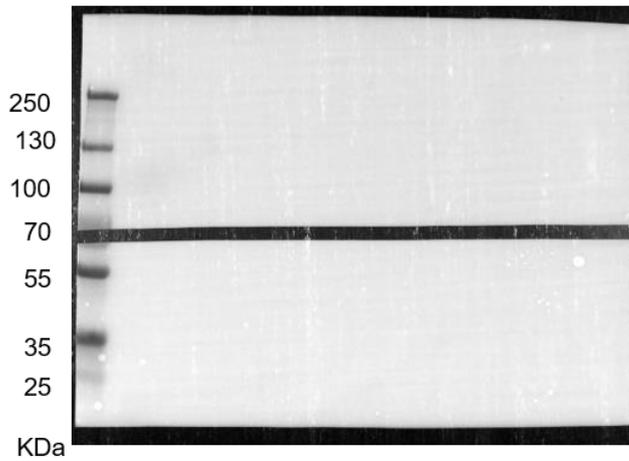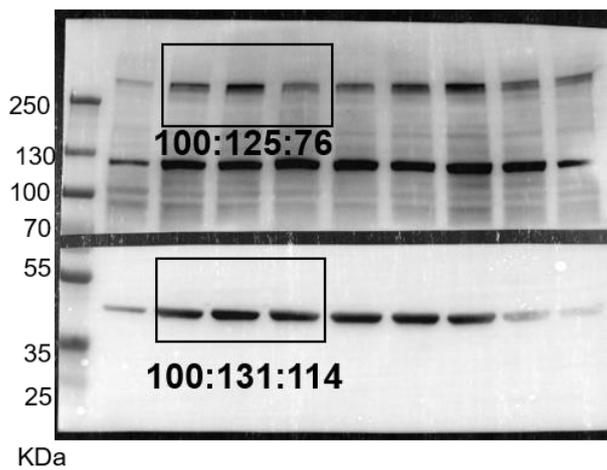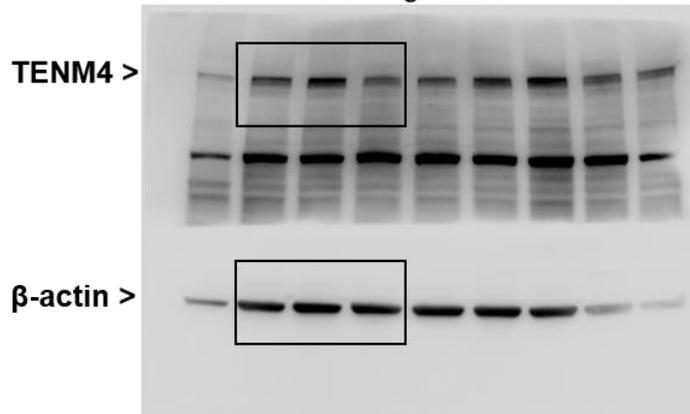
β-actin

A) W. Blot Figure 5f, for **TENM4**. Samples in black rectangles are shown.

Colorimetric blot with ladder	ECL image of the blot	Merge showing **TENM4**

< TENM4

100:91
4T1:MDA-MB-231
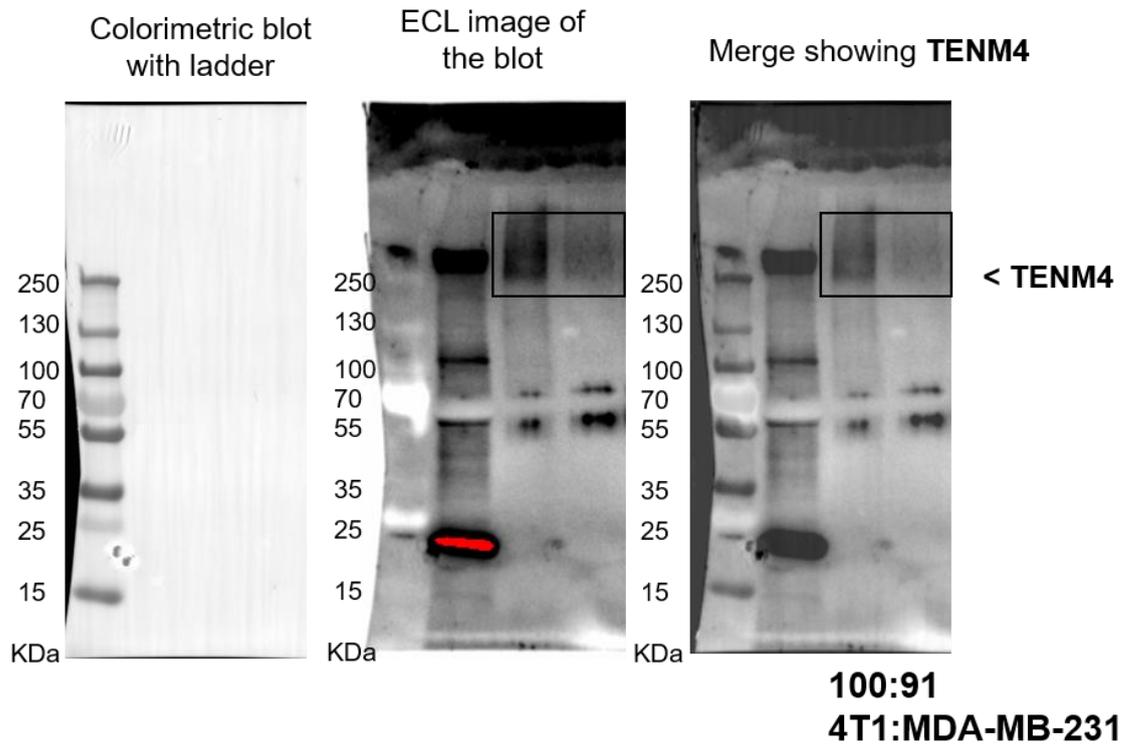
B) W. Blot Figure 5f, for **CD9**. Samples in black rectangles are shown.

Colorimetric blot with ladder	ECL image of the blot	Merge showing **CD9**

<CD9

100:46
4T1:MDA-MB-231
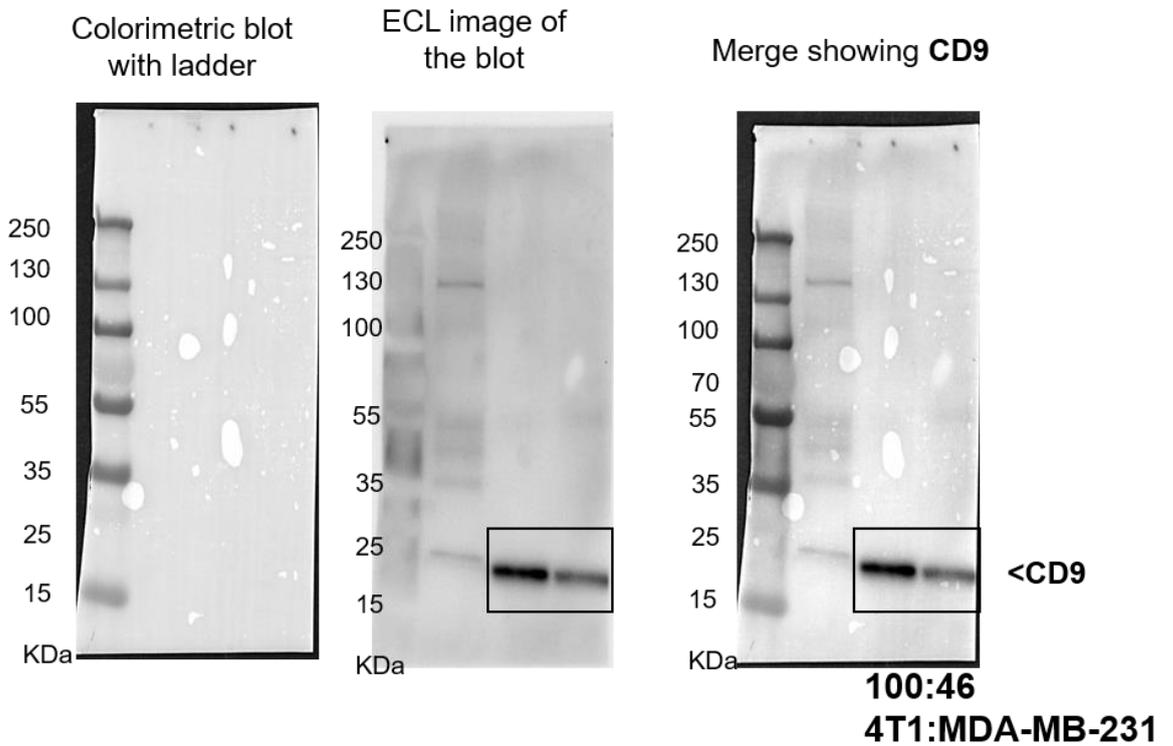
A) W. Blot Figure 5g; left panel (4T1), and central panel (MDA-MB-231) for **TENM4**. Samples in black rectangles are shown.

Colorimetric blot with ladder

250
130
100
KDa

ECL image of the blot

250
130
100
KDa

< **TENM4**

Merge showing **TENM4**

250
130
100
KDa

**100:369**   **100:307**
4T1   **MDA-MB-231**
NT:TB   **NT:TB**
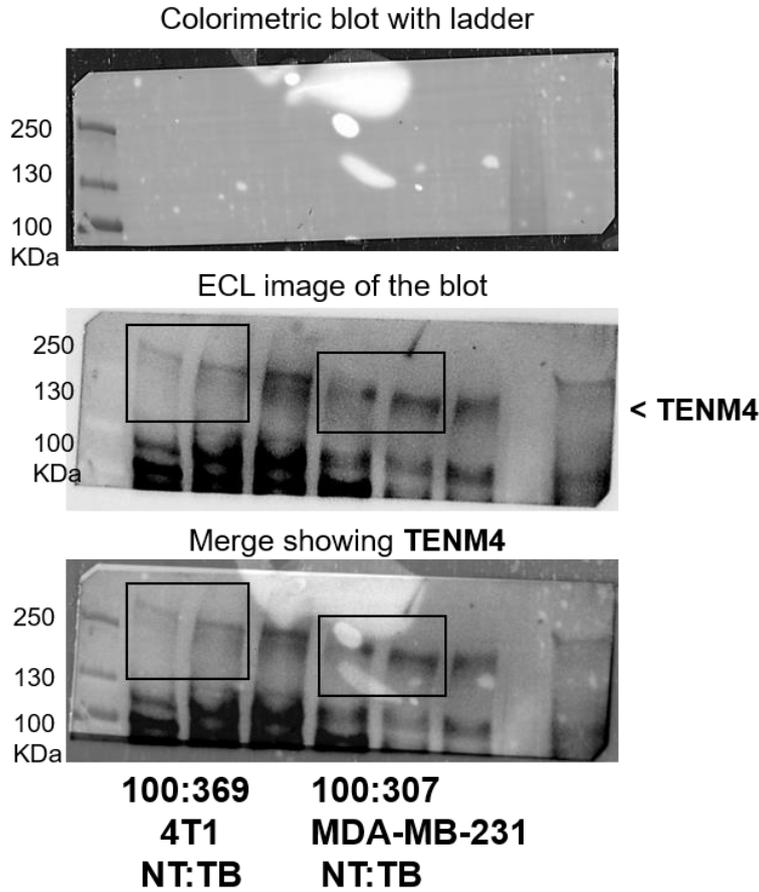
B) W. Blot Figure 5g; left panel (4T1), and central panel (MDA-MB-231) for **CD9**. Samples in black rectangles are shown.

Colorimetric blot with ladder

55
35
25
KDa

Merge showing **CD9**

55
35
25
KDa

100:112   **100:80**
4T1   **MDA-MB-231**
NT:TB   **NT:TB**

ECL image of the blot

CD9 >
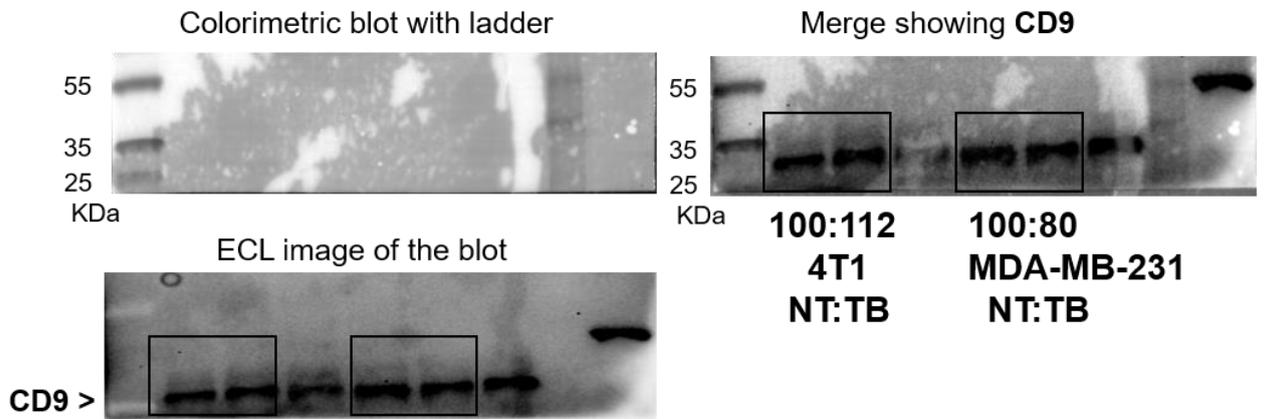
A) W. Blot Figure 5g; right panel (patients), for **TENM4**. Samples in black rectangles are shown.

Colorimetric blot with ladder

ECL image of the blot

< **TENM4**

Merge showing **TENM4**

100:85:142:114
HD:HD:TNBC:TNBC
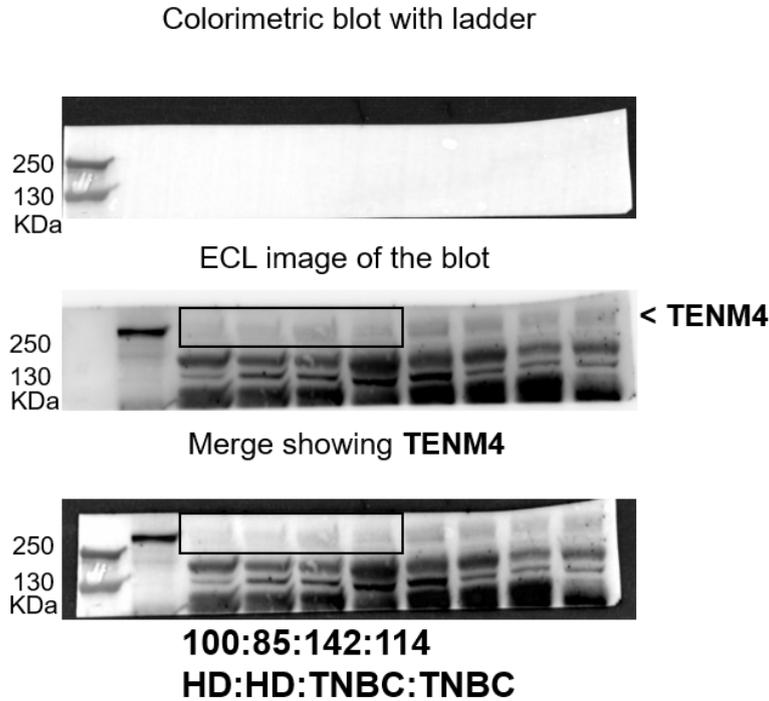
B) W. Blot Figure 5g; right panel (patients), for **CD9**. Samples in black rectangles are shown.

< **CD9**

100:97:53:59
HD:HD:TNBC:TNBC
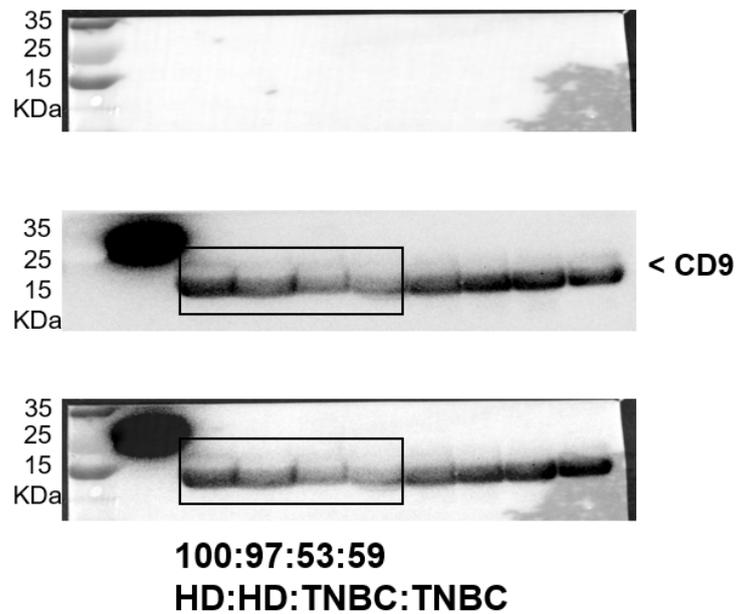
A) W. Blot Figure S3b, for **TENM4**. Samples in black rectangles are shown.

Colorimetric blot with ladder

ECL image of the blot

< TENM4

Merge showing **TENM4**

142:100:41:35
LA:LB:LB:LB
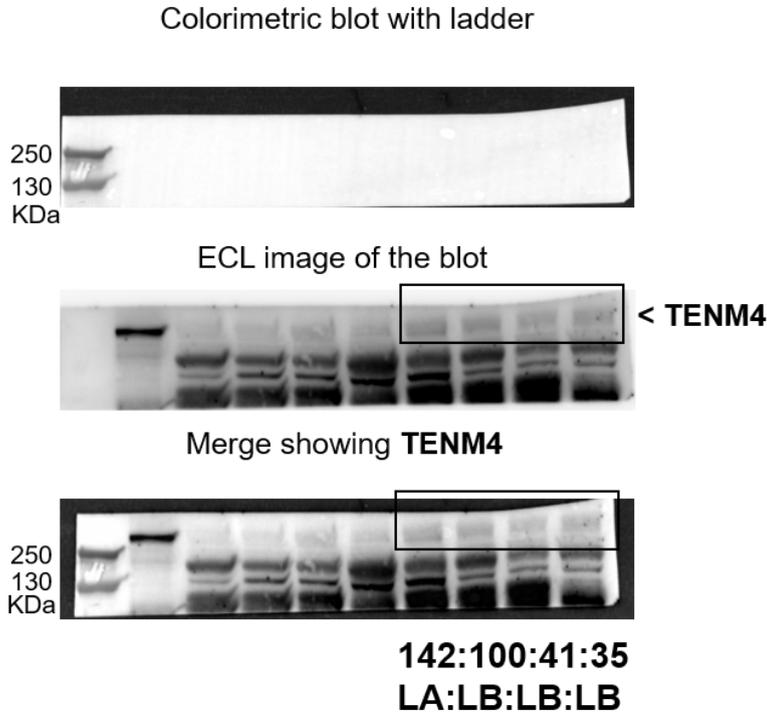
B) W. Blot Figure S3b, for **CD9**. Samples in black rectangles are shown.

Colorimetric blot with ladder

ECL image of the blot

< CD9

Merge showing **CD9**

98:100:94:98
LA:LB:LB:LB