Title

Matrilysin/MMP-7 Cleavage of Perlecan/HSPG2 Complexed with Semaphorin 3A Supports FAK-Mediated Stromal Invasion by Prostate Cancer Cells

Authors

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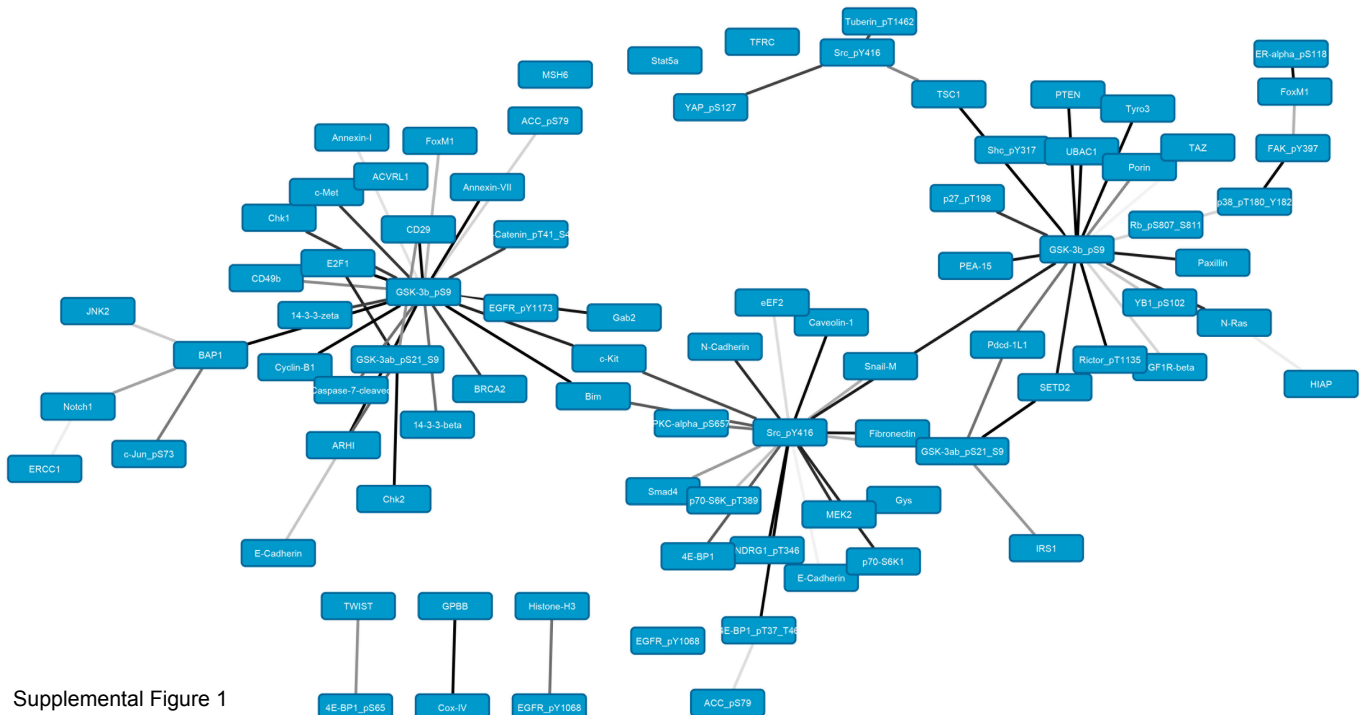
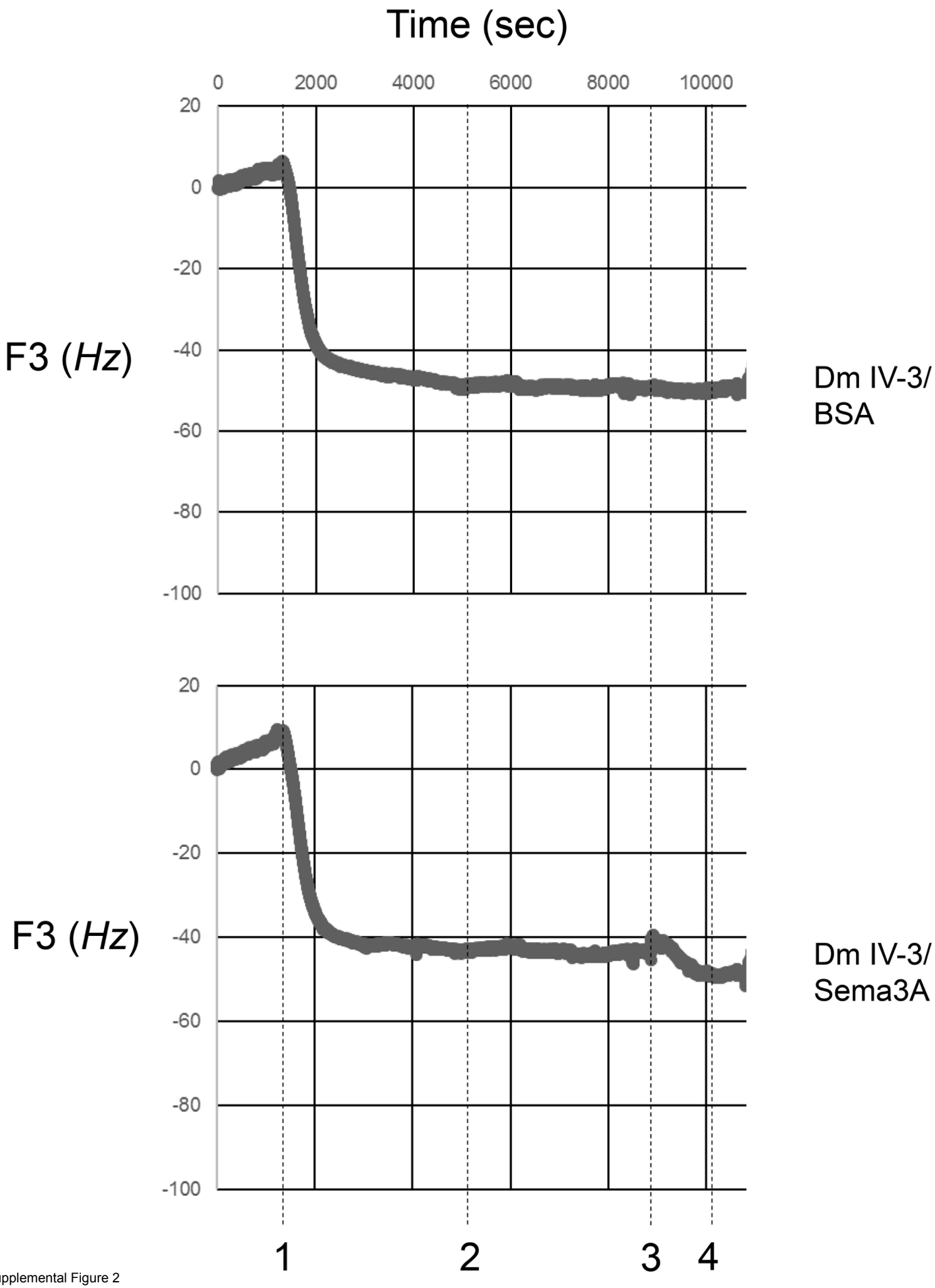
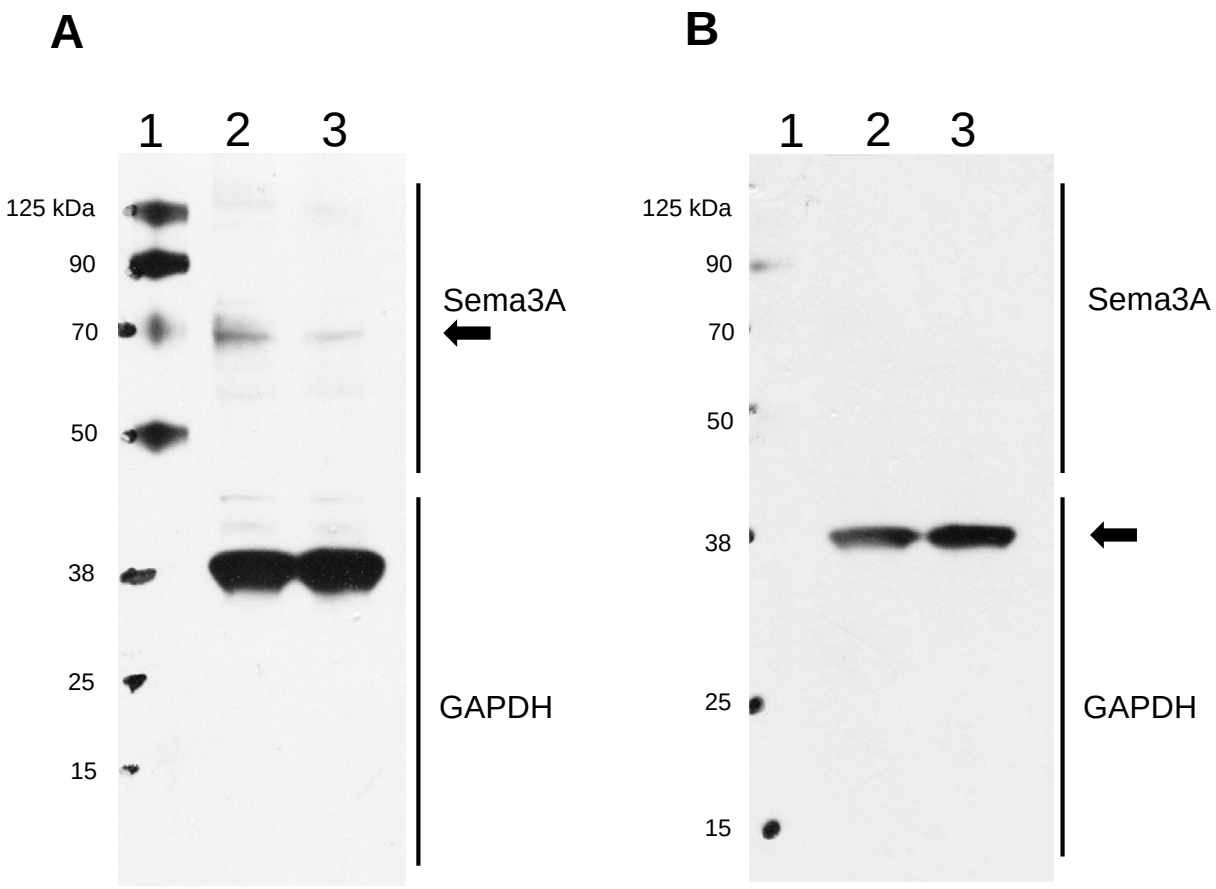


Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure S3

Supplemental Figure S1: Network informed by the transition of C4-2 cells in their clustered state on intact Domain IV-3 to a dispersed state on MMP-7 cleaved Domain IV-3. Edges are created by iterative comparison of each protein-protein interaction between the two states through a student's T-test. RPPA results presented as a clustered heat diagram are provided in supplemental data file.

Supplemental Figure S2: Raw quartz crystal microbalance (QCM) data demonstrates binding interaction between perlecan Domain IV-3 (Domain IV-3) and Sema3A. Domain IV-3 was adsorbed to a gold sensor (time point 1), washed with binding buffer and then blocked in BSA (time point 2) and flowed with either control BSA or Sema3A (time point 3) and then washed with additional binding buffer (time point 4). Flowing Sema3A over Domain IV-3 (lower graph) results in a frequency drop that is retained during washing with the binding buffer. No frequency drop is observed with flowing BSA (upper graph).

Supplemental Figure S3: Sema3A protein levels are reduced by shRNA. C4-2 cells were treated for 24 hrs with either shRNA control or SEMA3A targeting shRNA . Whole cell lysates were probed for A) Sema3A and B) GAPDH. Lanes 1-3 show the molecular weight markers, shRNA control treated cell lysate, or shRNA sema3A treated cell lysates, respectively. C) Sema3A levels from shRNA control and shRNA treated cells were quantified using standard densitometry. A knockdown of near 80% was achieved.

Supplemental Video: Perlecan Domain IV-3 as a substrate induces rapid clustering of C4-2 cells. Live cell imaging (5% CO_2 atmosphere, 37°C) of C4-2 cells after being seeded into a cell culture well that has been spotted with 1.5 µg of Domain IV-3. Domain IV-3 is spotted approximately within the black fiduciary markers. Cells caught within the Domain IV-3 coated area immediately cluster while cells outside are unaffected. Tile imaged under 10X Objective and stitched together. Time lapse 4.5 hours with 10-minute intervals.

Supplemental data to support:

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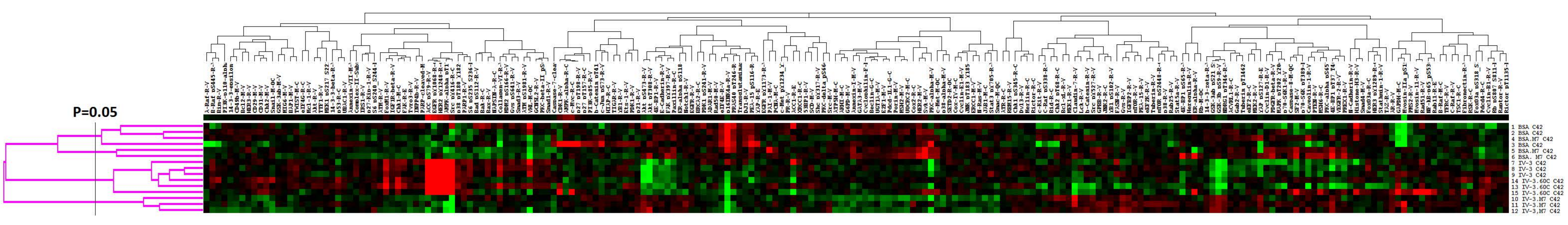
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4 BSA.M7 C42
3 BSA C42
5 BSA.M7 C42
6 BSA. M7 C42
7 IV-3 C42
8 IV-3 C42
9 IV-3 C42
14 IV-3.60C C42
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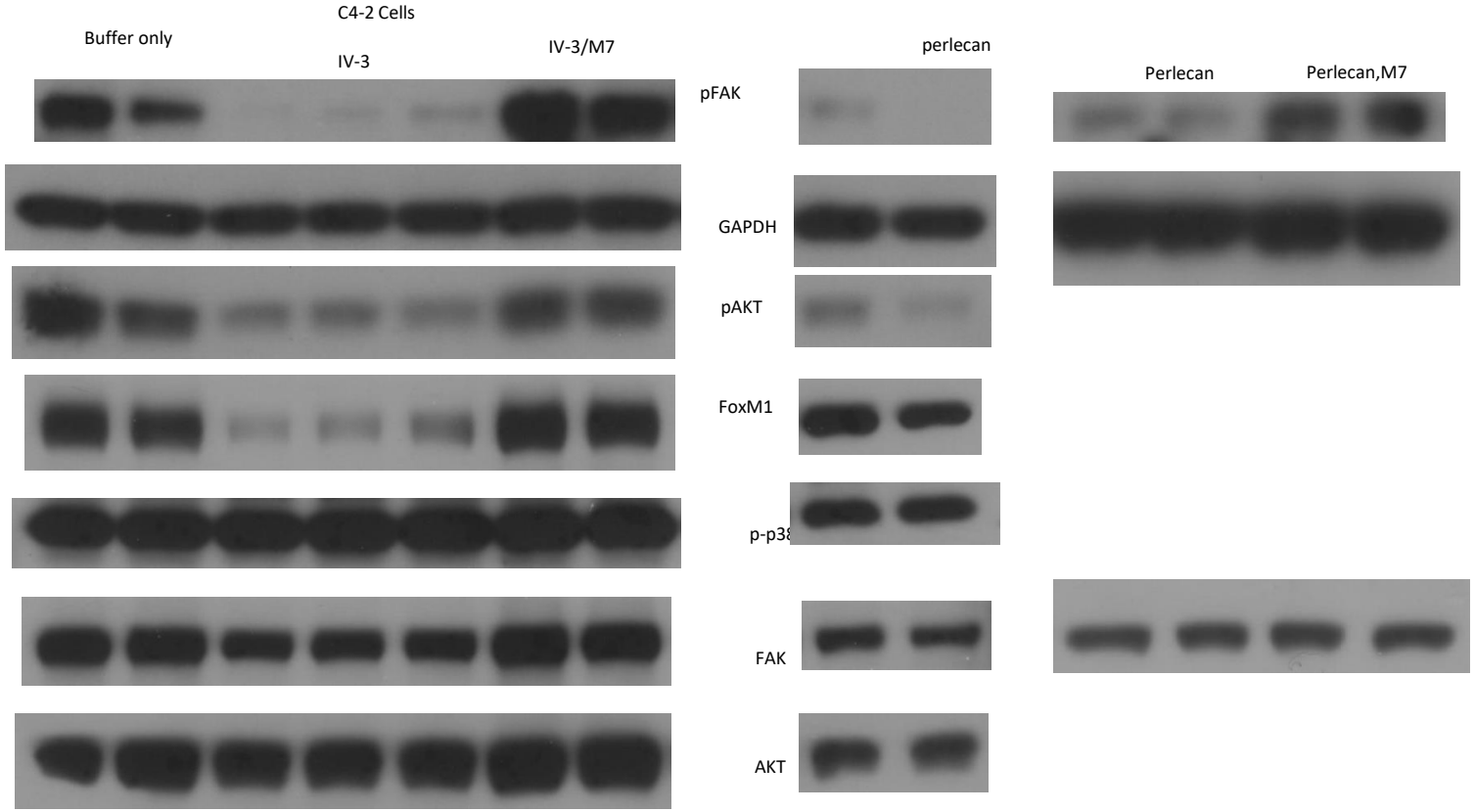
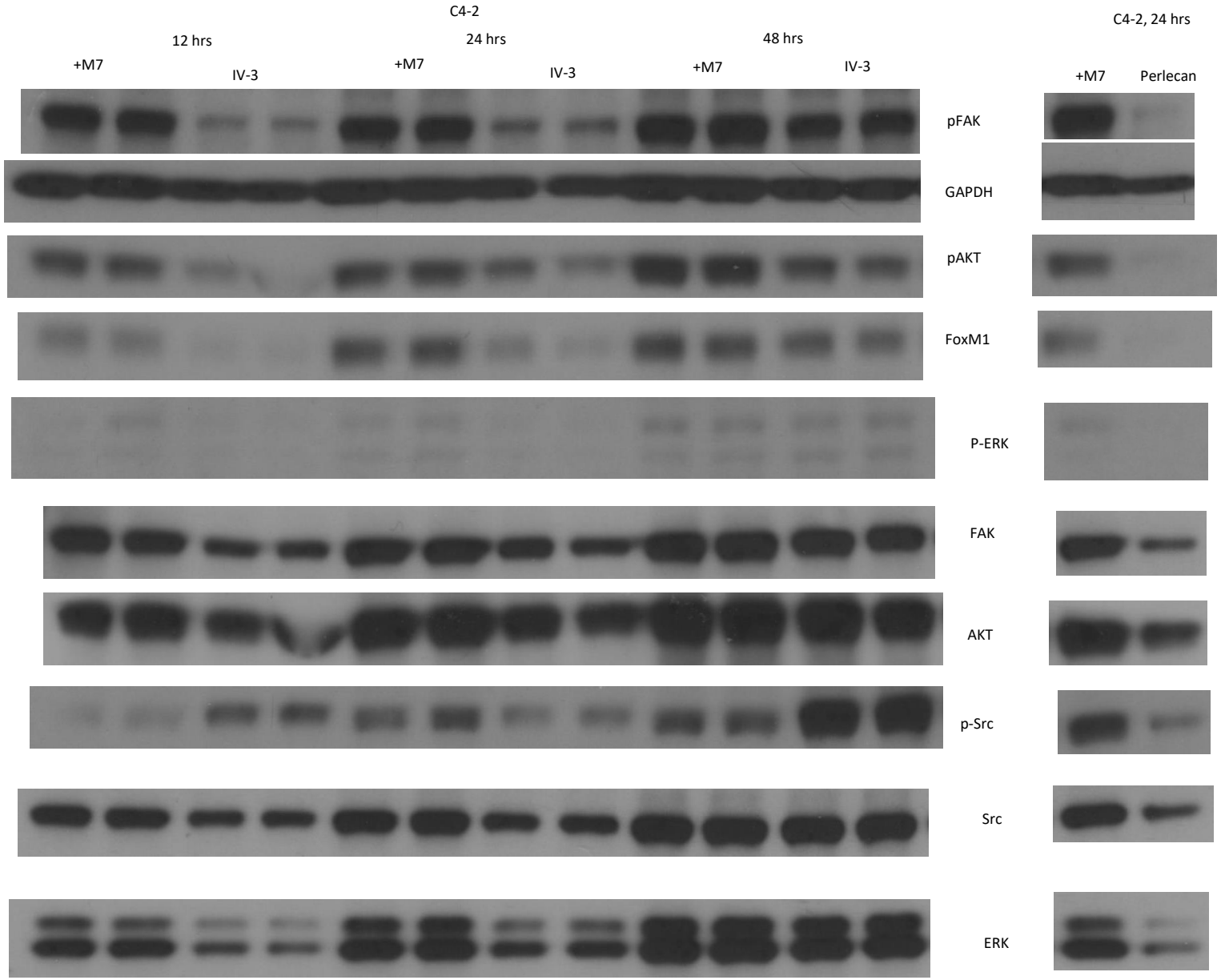
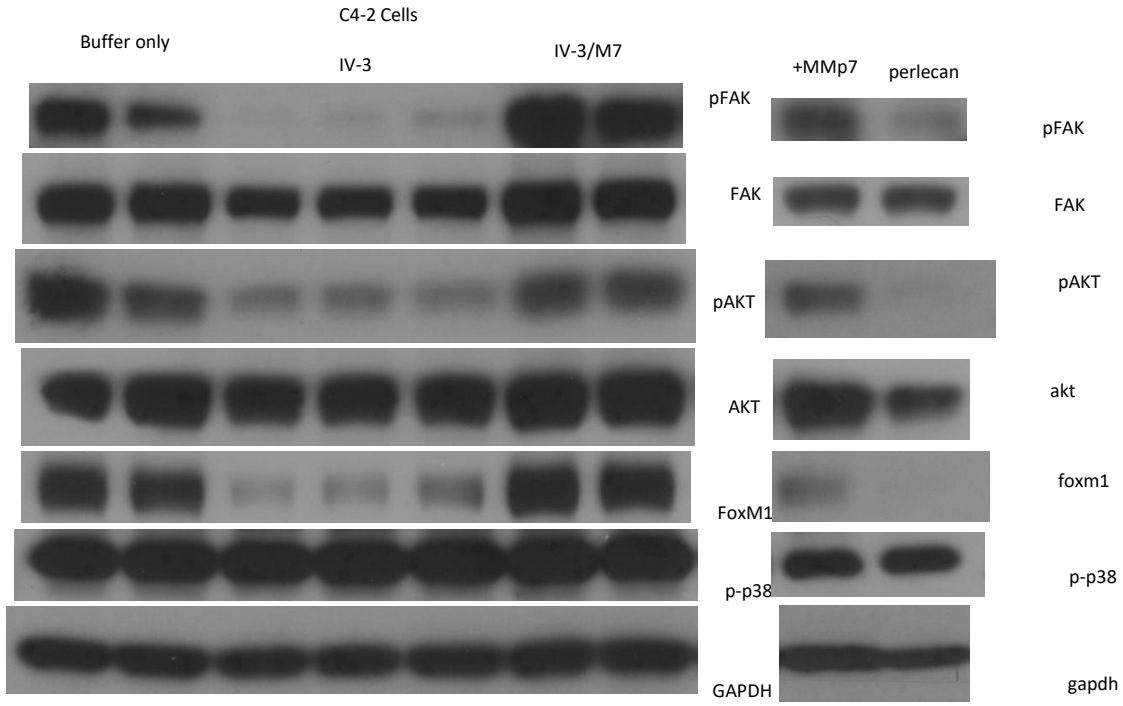
10 IV-3.M7 C42
11 IV-3.M7 C42
12 IV-3.M7 C42

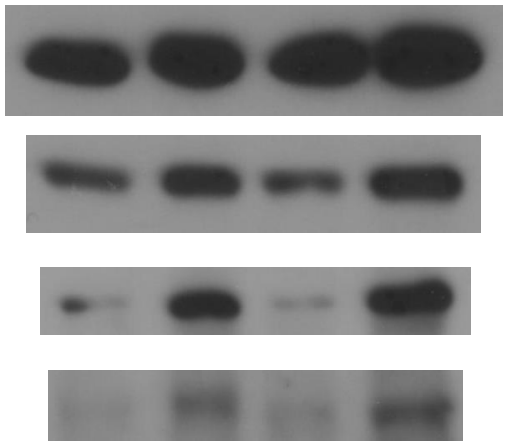

C4-2 Cells
Buffer only
IV-3
IV-3/M7
pFAK
GAPDH
pAKT
FoxM1
p-p38
FAK
AKT
perlecan
Perlecan
Perlecan,M7


C4-2
12 hrs
24 hrs
48 hrs
+M7
IV-3
+M7
IV-3
+M7
IV-3
C4-2, 24 hrs
+M7
Perlecan
pFAK
GAPDH
pAKT
FoxM1
P-ERK
FAK
AKT
p-Src
Src
ERK


C4-2 Cells
Buffer only
IV-3
IV-3/M7
pFAK
FAK
pAKT
AKT
FoxM1
p-p38
GAPDH
+MMp7
perlecan
pFAK
FAK
pAKT
akt
foxm1
p-p38
gapdh

C42, 24 hrs

IV-3 Rabbit IgG Sema3aAb BSA



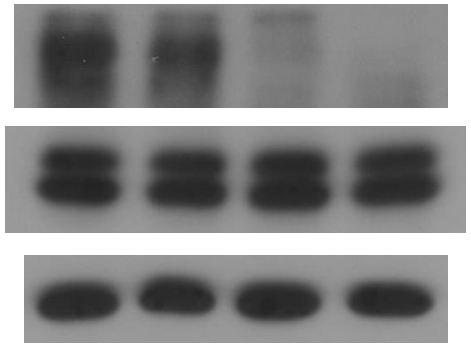
GAPDH

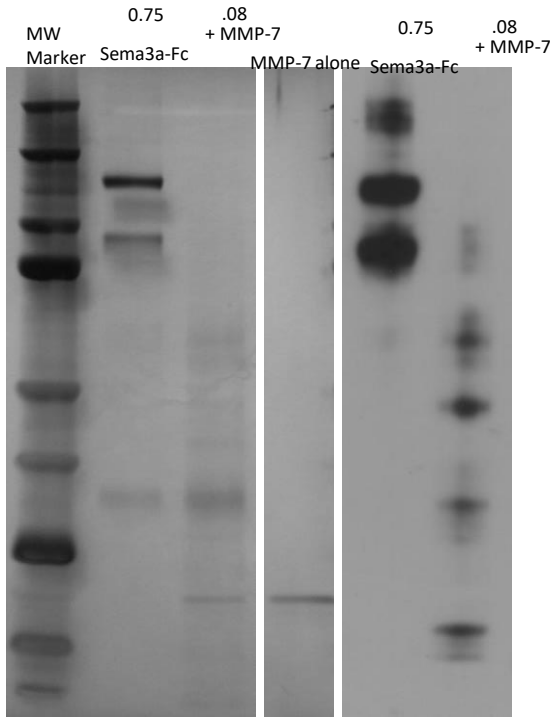
pAKT

FoxM1

pFAK

Rabbit IgG Sema3a Ab




MW
Marker
0.75
Sema3a-Fc
.08
+ MMP-7
MMP-7 alone
0.75
Sema3a-Fc
.08
+ MMP-7
Silver stain
WB sema3a