

**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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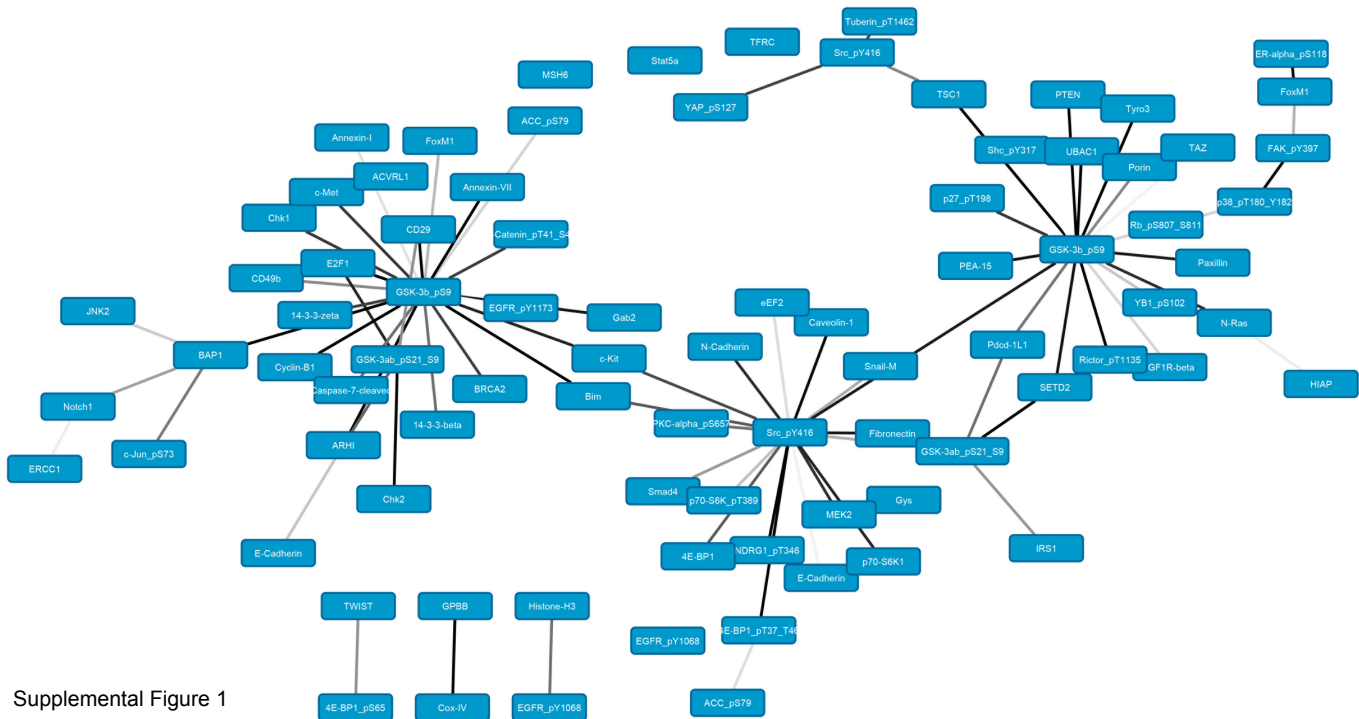
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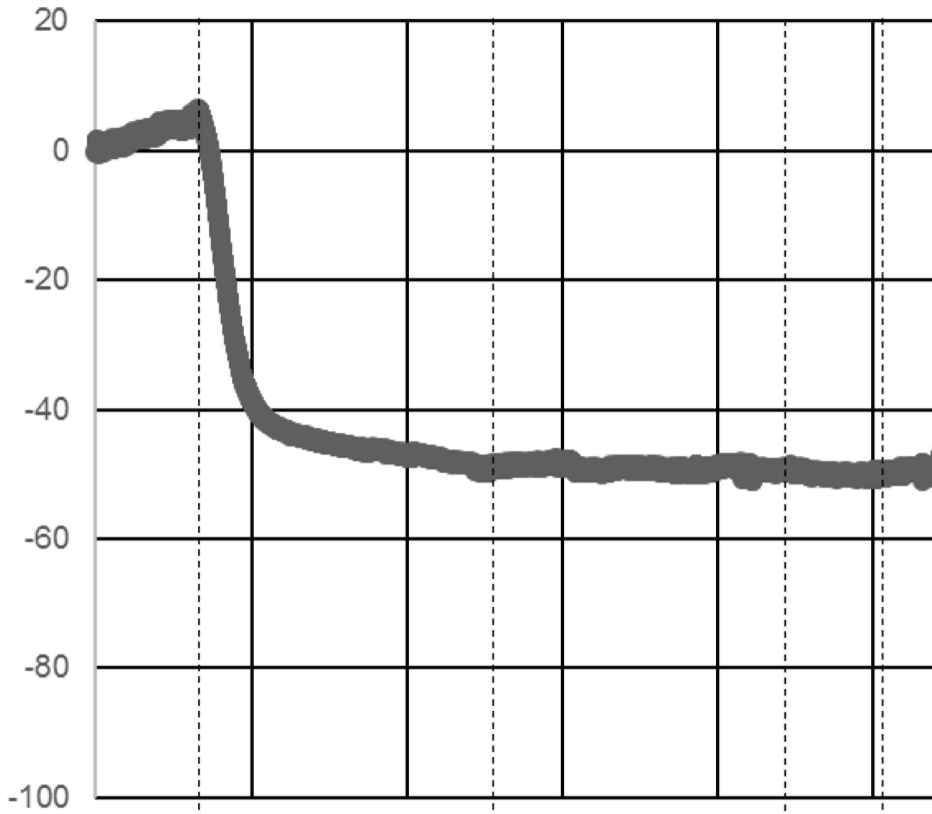


Supplemental Figure 1

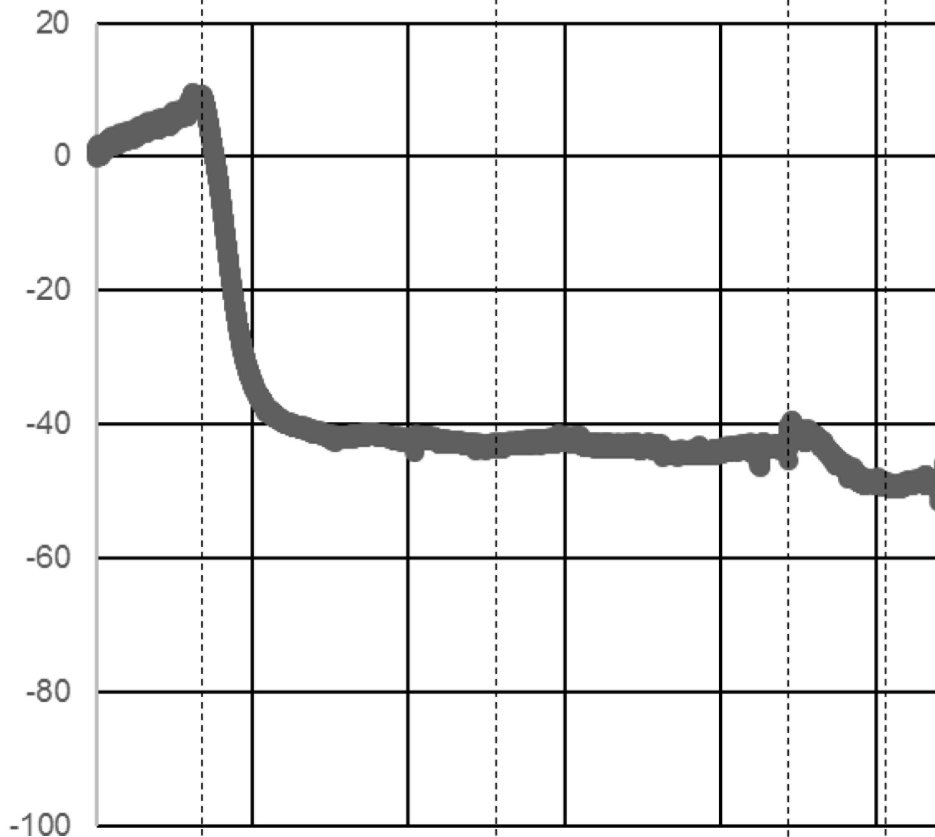
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F3 (Hz)

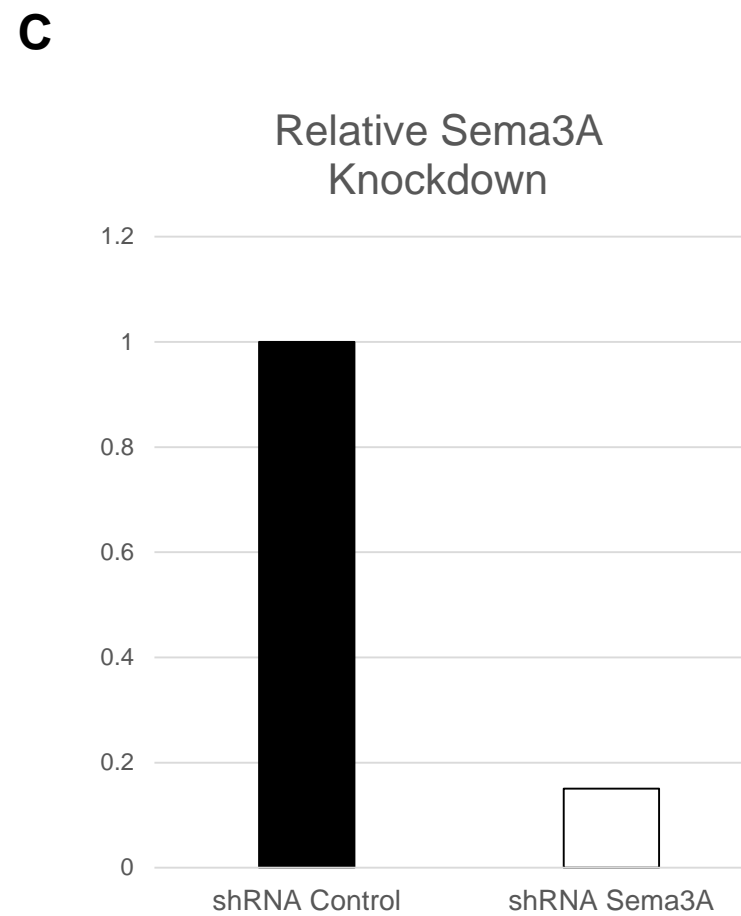
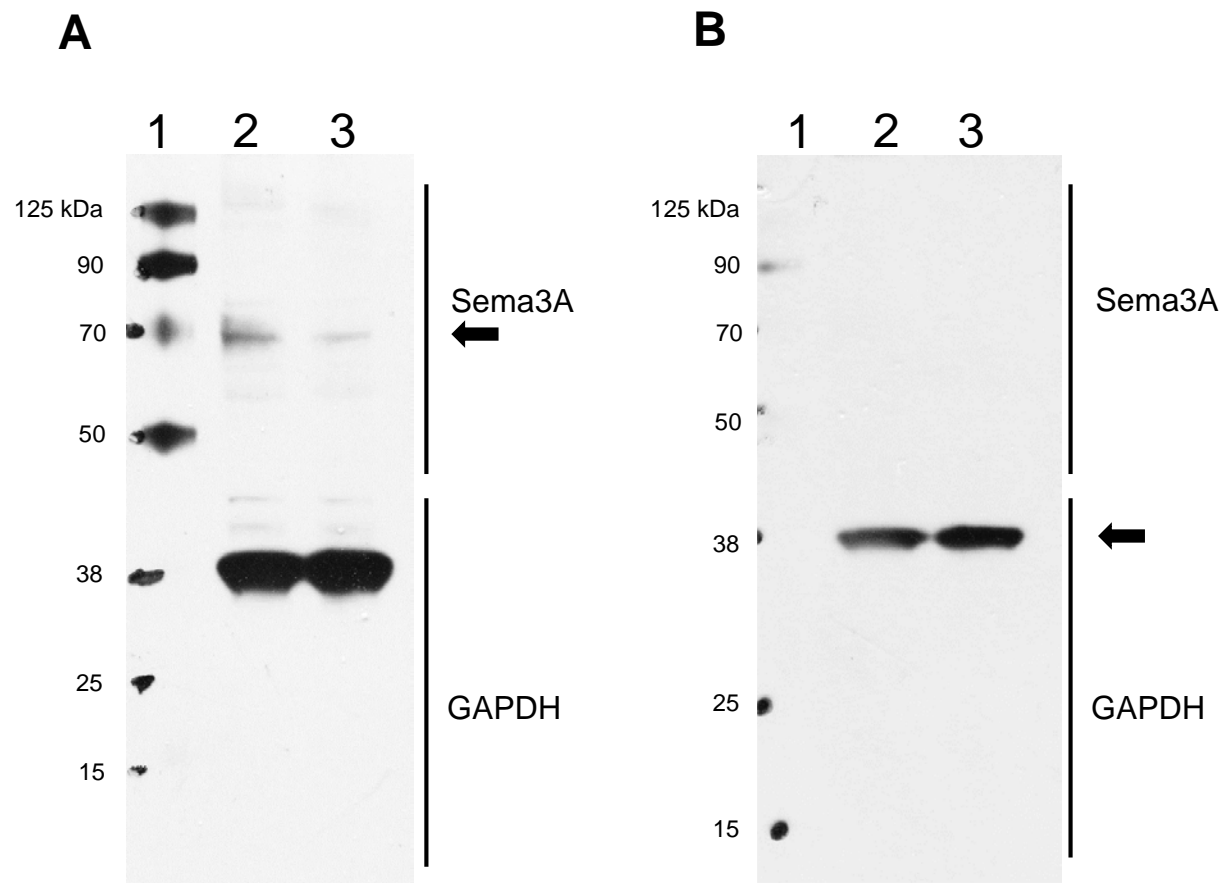


1

2

3

4



**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<sub>2</sub> 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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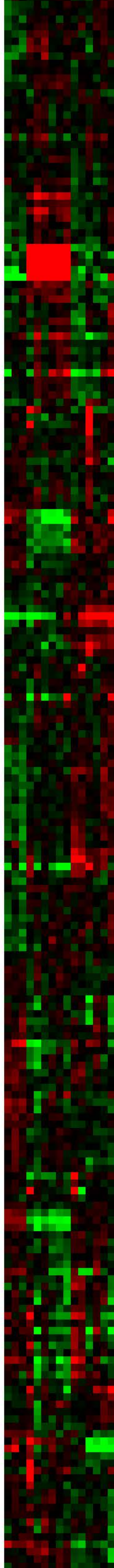
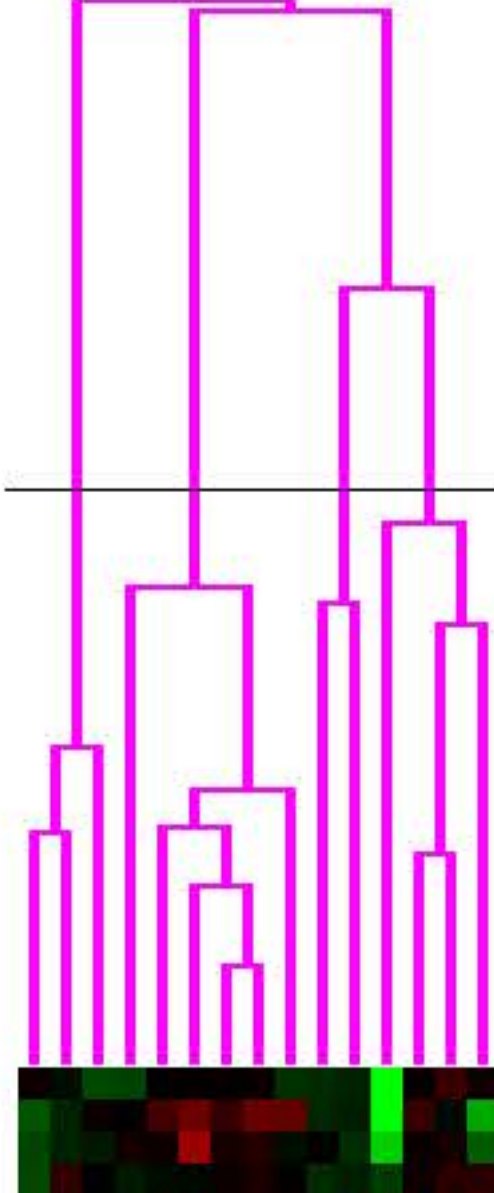
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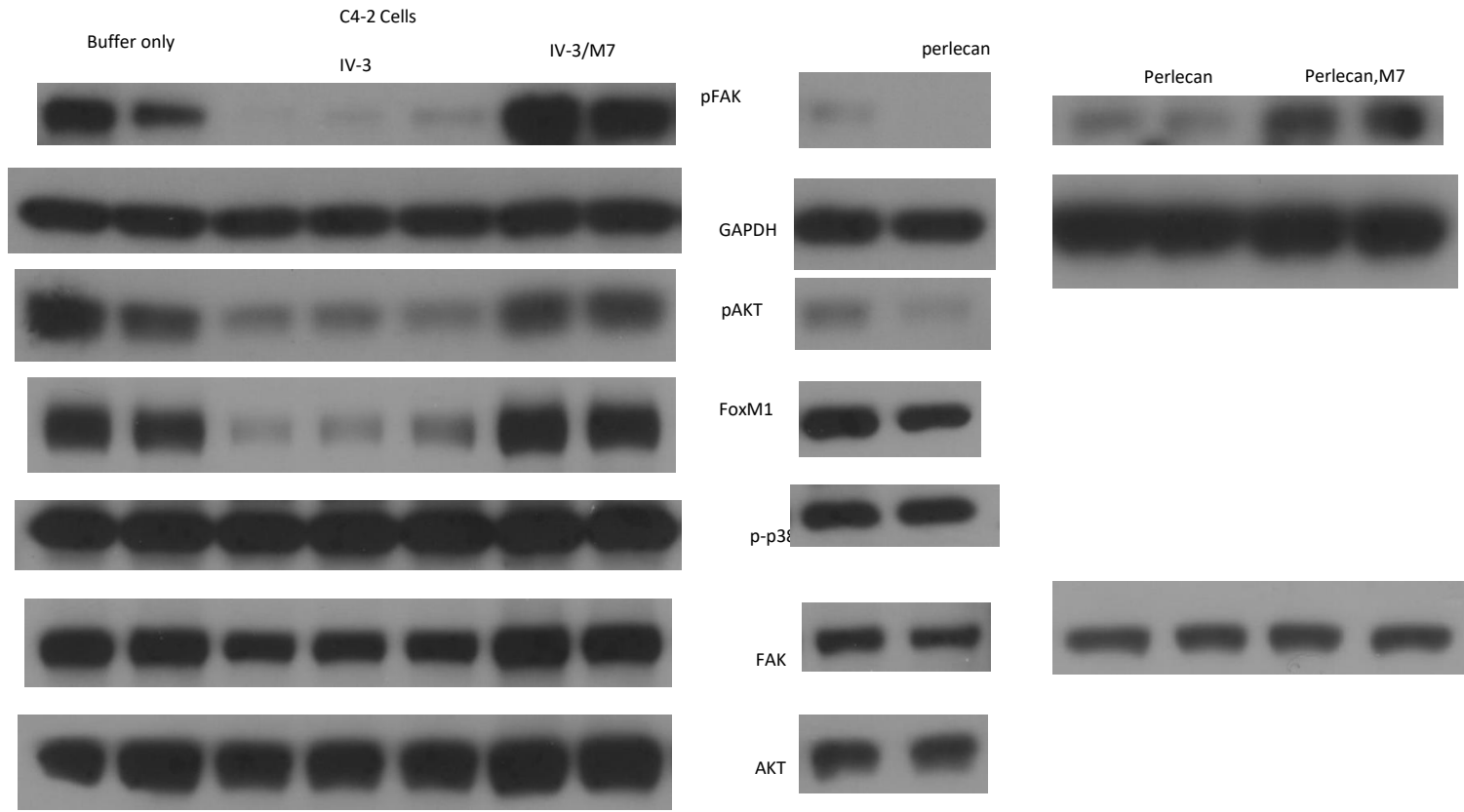
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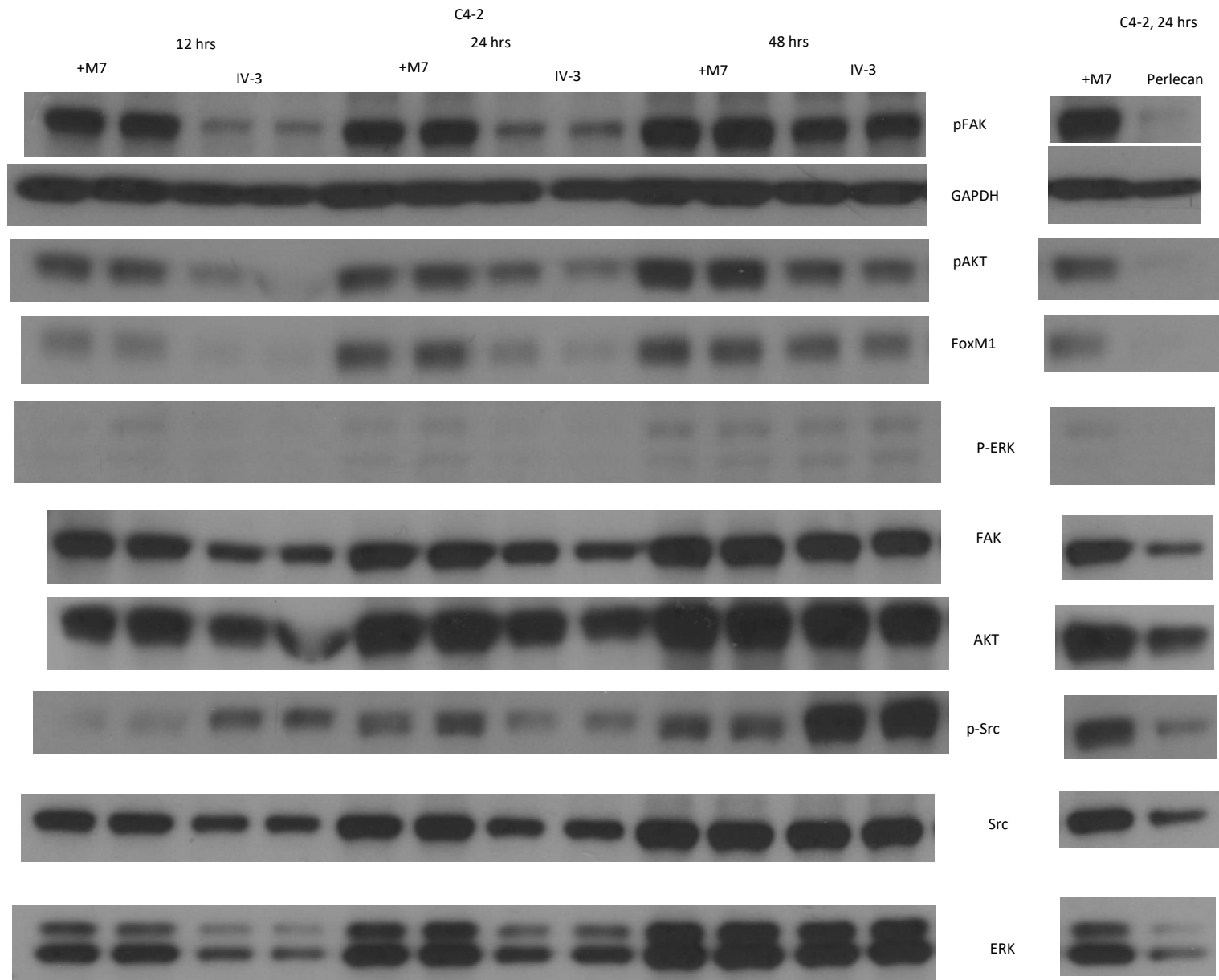


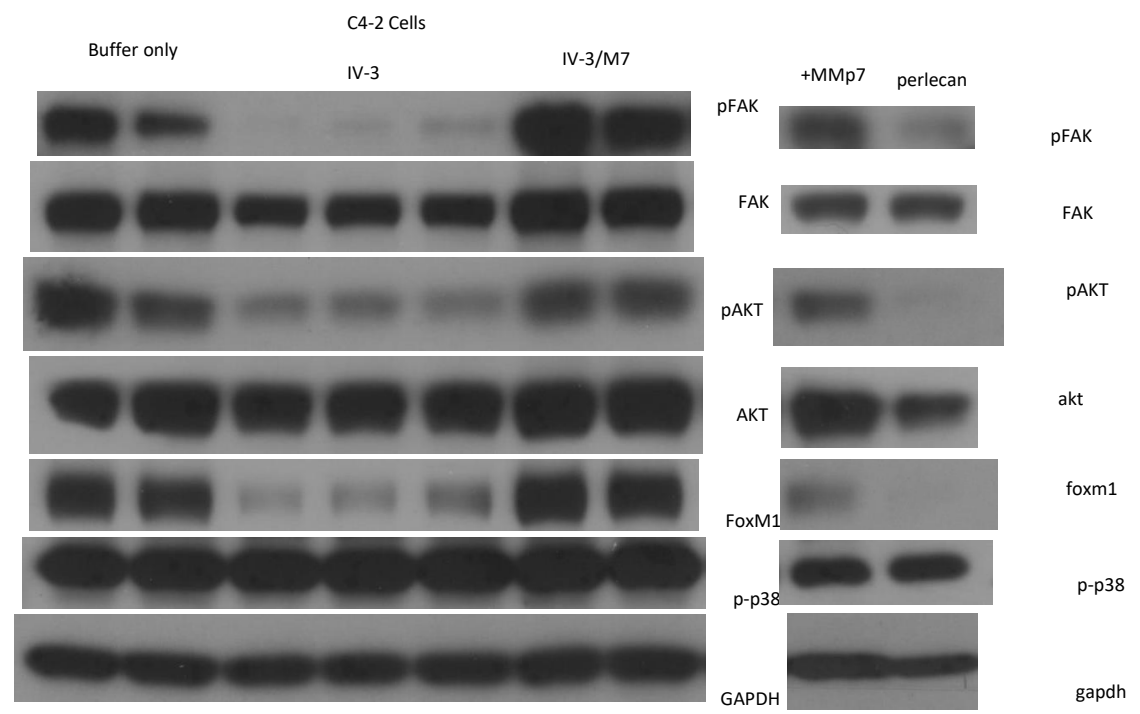
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 2 BSA C42  
 3 BSA, M7 C42  
 4 BSA, M7 C42  
 5 BSA, M7 C42  
 6 BSA, M7 C42  
 7 IV-3 C42  
 8 IV-3 C42  
 9 IV-3 C42  
 10 IV-3, 60C C42  
 11 IV-3, 60C C42  
 12 IV-3, M7 C42

- A-Raf-R-V
- B-Raf pS445-R-V
- Bim-R-V
- PI3K-p110-alpha
- 14-3-3-epsilon
- CD49b-M-V
- Bcl2-M-V
- HER3-R-V
- CD29-M-V
- CD31-M-V
- Chk2-M-V
- Snail-M-OC
- GSK-3ab-M-V
- MIG6-M-V
- BAP1-M-V
- TWIST-M-C
- eIF4G-R-C
- Mcl-1-R-V
- Akt-R-V
- TTF1-R-V
- MEK1 pS217 S22
- 14-3-3-beta-R-V
- p53-R-C
- UBAC1-R-V
- Annexin-VII-M-V
- Complex-II-Sub
- IRS1-R-V
- p27-Kip-1-R-V
- S6 pS240 S244-I
- ATM-R-V
- FoxM1-R-V
- IGF1R-beta-R-V
- Chk1-M-C
- FAK-R-E
- Tvra3-R-V
- INPP4b-R-V
- PARP-cleaved-M
- ACC pS79-R-V
- EGFR pY1068-R-V
- HER2 pY1248-R-V
- AMPK-alpha pT1
- Src pY416-R-C
- p38 pT180 Y182
- PARP1-R-V
- S6 pS235 S236-I
- Bad pS112-R-V
- Bak-R-C
- Bax-R-V
- NAPSIN-A-R-C
- Collagen-VI-R-V
- MDM2 pS166-R-V
- Gvs pS641-R-V
- Smad3-R-V
- ATM pS1981-R-V
- VHL-M-OC
- GCM5L2-R-V
- PKC-beta-II\_pS
- Smad1-R-V
- Caspase-7-clea
- CDK1-R-V
- AMPK-alpha-R-C
- c-Myc-R-C
- p27 pT198-R-V
- p27 pT157-R-C
- PI3K-p85-R-V
- b-Catenin pT41
- c-Jun pS73-R-V
- HIAP-R-C
- TIGAR-R-V
- YB1-R-V
- Ets-1-R-V
- PDK1-R-V
- p21-R-V
- Akt pS473-R-V
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- E-Cadherin-R-V
- FAK pY397-R-V
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- Translucina
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- PEA-15 pS116-R
- YAP-R-E
- EGFR pY1173-R-V
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- c-Met pY1234\_Y
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- ACC1-R-E
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- SCD-M-V
- Shc pY317-R-V
- PKC-delta\_pS66
- Src-M-V
- ATP5H-M-C
- ARHI-M-C
- G6PD-M-V
- Annexin-I-M-V
- GATA3-M-V
- Cyclophilin-F-I
- Beclin-G-C
- UGT1A-M-V
- E2F1-M-V
- Pdcd-1L1-G-C
- Porin-M-V
- MSH2-M-V
- U0CRC2-M-C
- Cox2-R-C
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- Dvl3-R-V
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- Rictor-R-C
- c-Kit-R-V
- C-Raf pS338-R-V
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- YB1 pS102-R-V
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- IGFBP2-R-V
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- Rab25-R-V
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- 4E-BP1 pS65-R-V
- Heregulin-R-V
- ER-alpha-R-V
- Rb-M-OC
- 14-3-3-zeta-R-V
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- PREX1-R-V
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- Histone-H3-R-V
- Smad4-M-V
- FoxO3a-R-C
- HER3 pY1289-R-V
- Stathmin-1-R-V
- TAZ-R-V
- AR-R-V
- GAPDH-M-C
- Mvosin-IIa\_pS1
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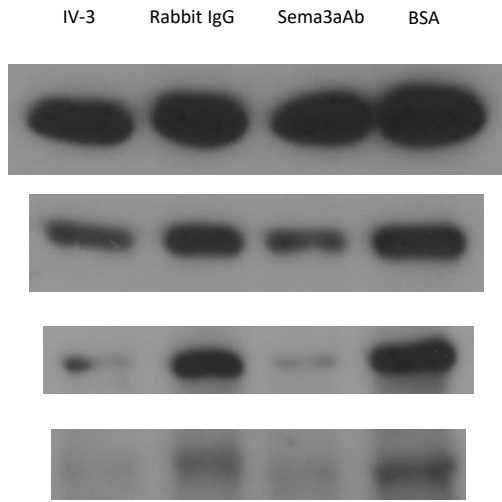








C42, 24 hrs

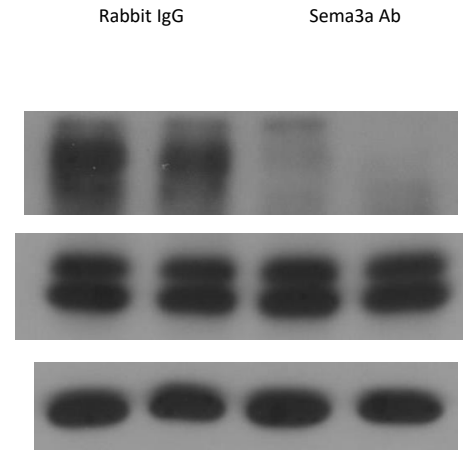


GAPDH

pAKT

FoxM1

pFAK



Rabbit IgG

Sema3a Ab

