Paradoxical Role of Glypican-1 in Prostate Cancer Cell and Tumor Growth

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Supplemental Figure 1: Effect of GPC-1 Inhibition on GPC-1 Expression and Cell Growth and Morphology in DU-145 Cells. A. DU-145 cells were transfected with GPC-1 shRNA (Genecopoeia, Rockville, MD) prior to analysis of GPC-1 expression using immunoblot analysis. Effect of GPC-1 inhibition on DU-145 cells morphology (**B**), proliferation (**C**) and migration (**D**) were assessed using phase contrast microscopy, crystal violet staining (11 days) and scratch assays, respectively. Data in **C** and **D** are presented as the mean ± the S.E.M. of at least 3 (n = 3) separate passages. *Indicates a significant difference ($p < 0.05$) as compared to control as determined using a Student t-test.

Supplemental Figure 2: Effect of GPC-1 Inhibition on the Expression of GPC Isoforms. qRT-PCR was used to measure the mRNA expression level of GPC isoforms in PC-3 (**A**) and DU-145 (**B**) cells transfected with GPC-1 shRNA (Sigma, St. Louis, MO). Data are presented as the mean ± the S.E.M. of at least 3 (n = 3) separate passages. *Indicates a significant difference ($p < 0.05$) as compared to control as determined using a Student t-test.

Supplemental Figure 3: Effect of GPC-1 Inhibition Using Different shRNA Plasmid Sequences on Prostate Cancer Cell Growth and Morphology. A. Effect of GPC-1 shRNAs (Sigma, St. Louis, MO) on expression of GPC-1 in PC-3 cells. **B.** Effect of GPC-1 inhibition on cell morphology visualized by crystal violet staining at low density. **C.** Changes in cell growth in GPC-1 knockdown PC-3 cells visualized by crystal violet staining after 11 days of culture. **D.** Inhibition of GPC-1 results in decrease in cell migration in the scratch wound assay as visualized by crystal violet staining. Data in **C** and **D** are representative of at least 3 separate experiments with at least 3 independent cell passages. Data in **C** and **D** are presented as the mean ± the S.E.M. of at least 3 (n = 3) separate passages. *Indicates a significant difference ($p < 0.05$) as compared to control as determined using a Student t-test.

Supplemental Figure 4: H&E Staining of Tumor Section from Scrambled (A) and GPC-1 shRNA (B) Mouse xenografts. Data in **A** and **B** are representative of at least three individual mice. The scale bar indicates 50 μm.

Supplemental Figure 5: Ki-67 Expression in Tumor Sections from Scrambled (A) and GPC-1 shRNA (B) Mouse Xenografts. Data in **A** and **B** are representative of at least 3 separate mice. Data in **C** are presented as the mean ± S.E.M. of at least 5 separate images taken from at 5 different section of each tumor slice. The scale bar in **A** and **B** indicates 50 μm.

Supplemental Figure 6: E-Cad and N-Cad Staining in Tumor Sections from Scrambled (A) and GPC-1 shRNA (B) Mouse Xenografts. Data in **A** and **B** are representative of at least 3 individual mice. E-Cad staining is indicated by red fluorescence while N-Cad is indicated by green. The scale bar indicates 50 μm.

Supplemental Figure 7: Effect of GPC-1 Inhibition on MMP9/2 Activity. TCM from control PC-3 cells (scrambled shRNA) and from GPC-1 shRNA cells were collected and applied to confluent Hs27 and hMSCs at confluence for 24 hours. Media from these stromal cells were collected for MMP-9 and 2 activity analysis using gelatin zymography.

Supplemental Figure 8: GPC-1 mRNA Expression Profile in Human Prostate Cancer Patients and Correlation to Prostate Cancer Patient Survival. A TCGA Prostate Cancer (PRAD) cohort of 550 patients was analyzed using the UCSC Xena Cancer Database (http://xena.ucsc.edu/). Gene expression RNAseq (ployA+ IlluminaHiSeq pancan normalized) was selected to analyze GPC-1 expression in solid normal and primary prostate tissue samples (**A**). Kaplan-Meier Survival Curves were generated for these same patients (**B**) using the default Xena database algorithm. Even though the statistical log-ranked test (p= 0.9387) does not indicate significant correlation between patient with high and low GPC-1 expression, patients with low expression of GPC-1 (blue lines) had a lower survival rate compared to high GPC-1 expressing patients (highlighted region).

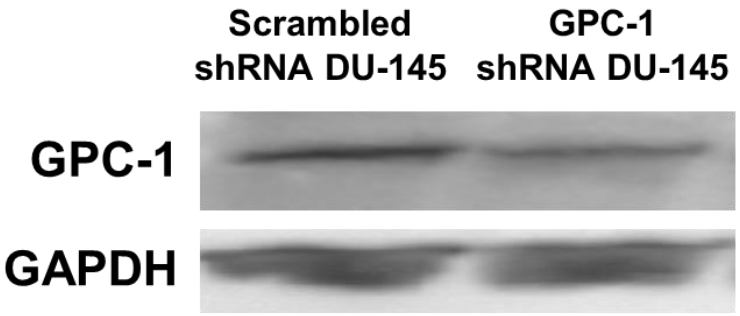
Supplemental Figure 9: Full western blot images used in Figure 1 and 2

Supplemental Figure 10: Full western blot images used in Figure 5

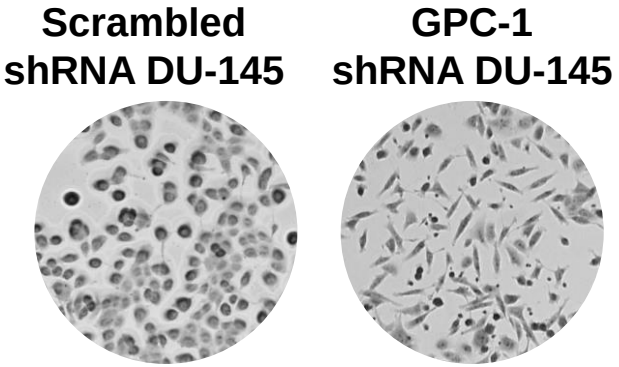
Supplemental Figure 11: Full western blot images used in Supplementary Figure 1 and 2.

Supplemental Figure 1

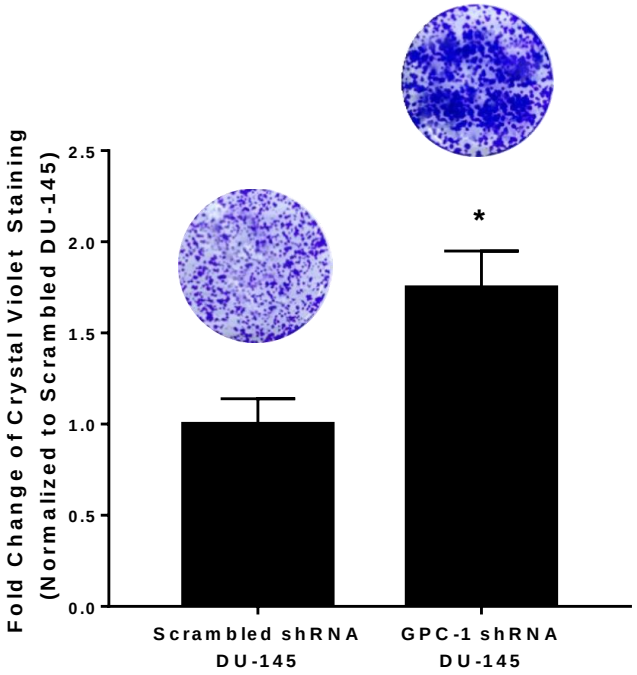
A. Immunoblot Analysis



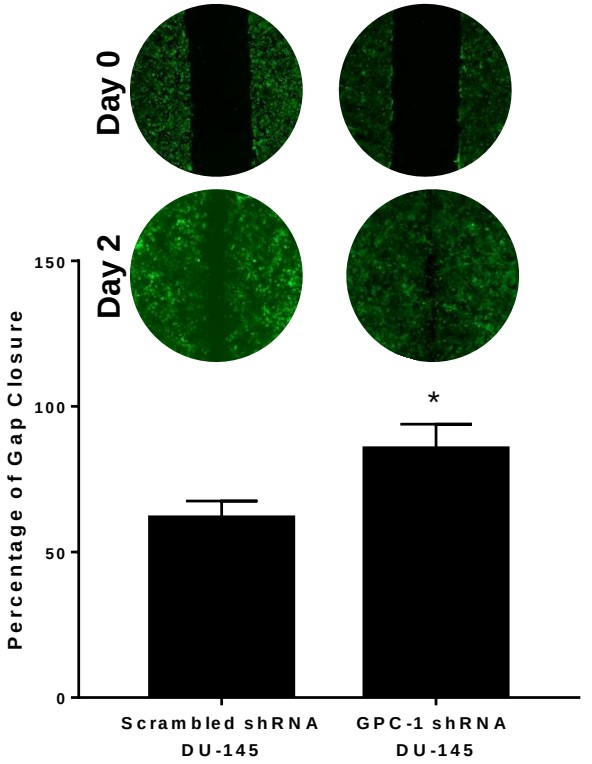
B. Cell Morphology



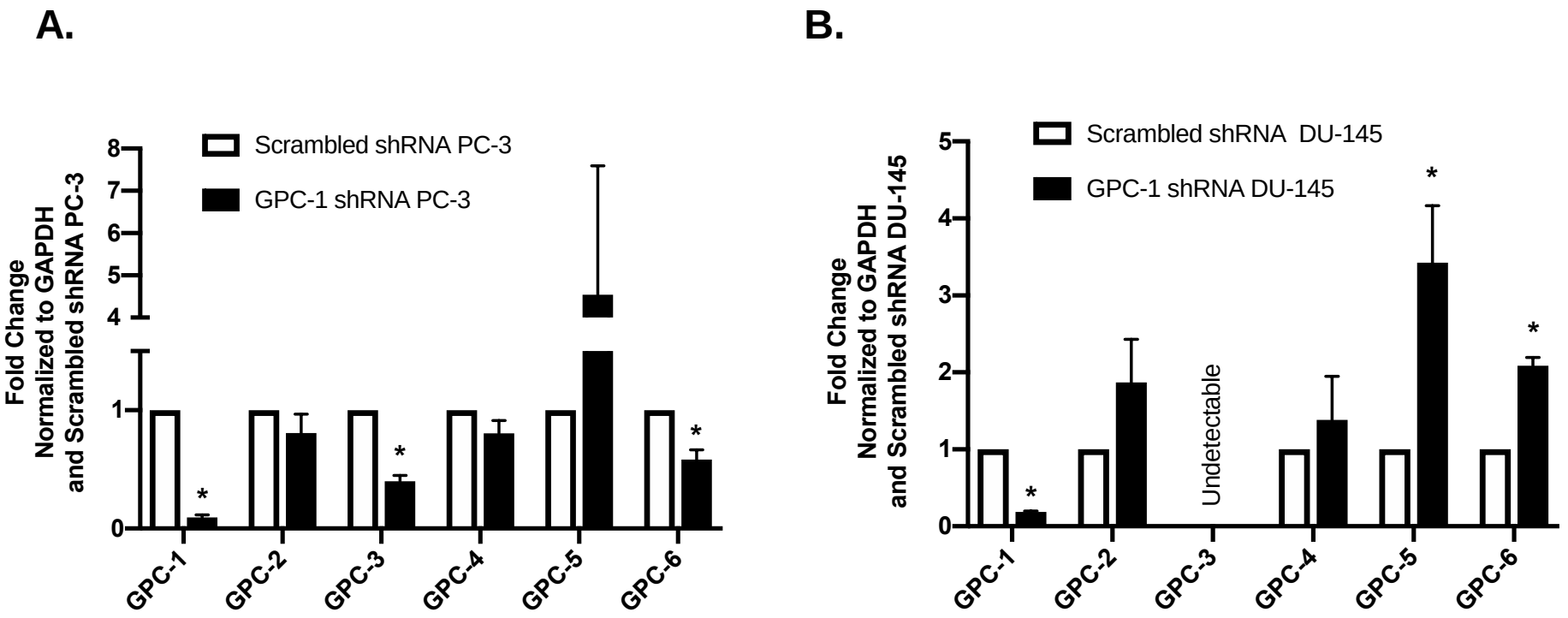
C. Cell Proliferation



D. Cell Migration

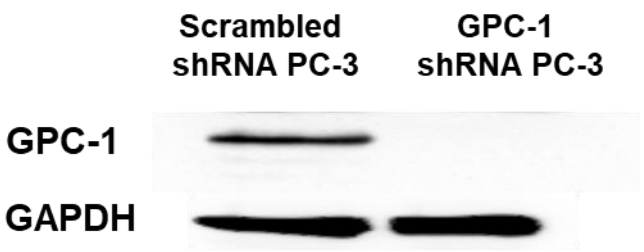


Supplemental Figure 2

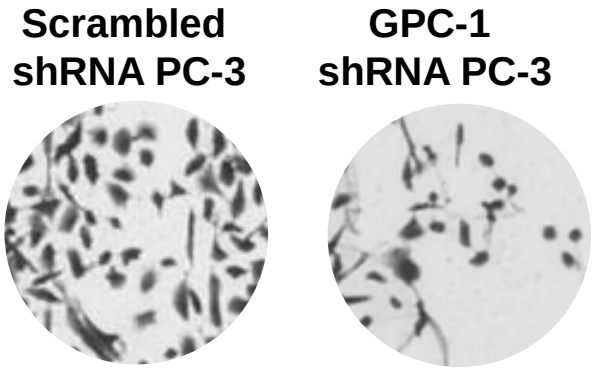


Supplemental Figure 3

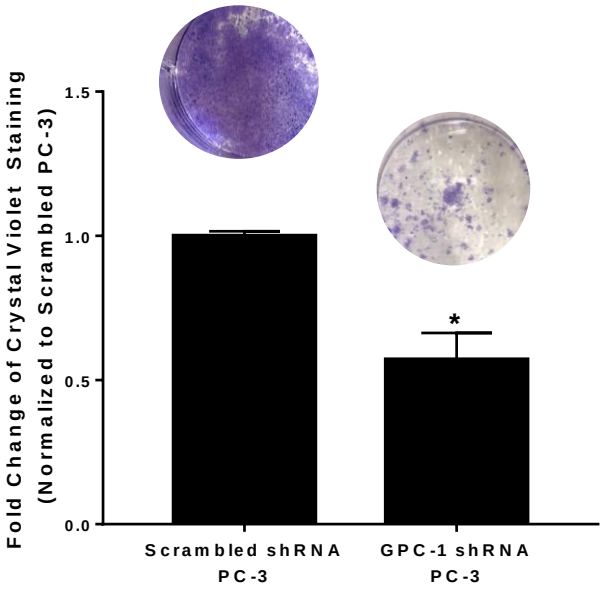
A. Immunoblot Analysis



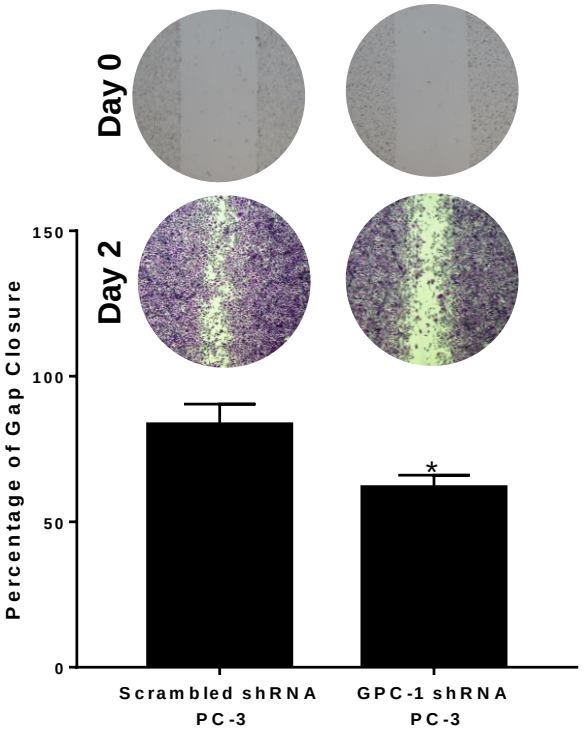
B. Cell Morphology



C. Cell Proliferation

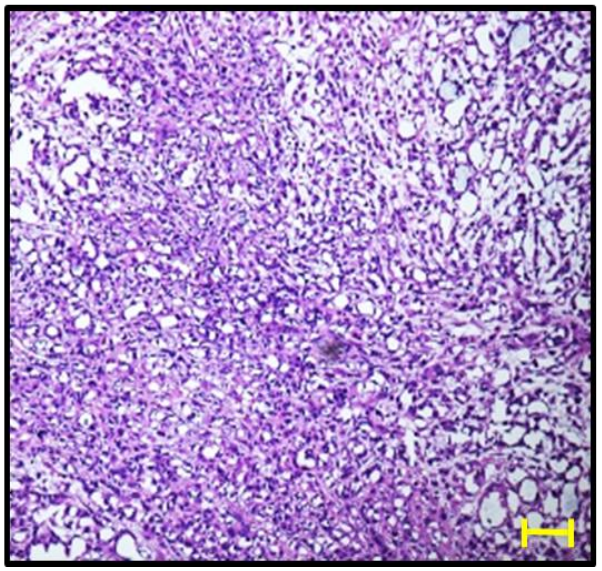


D. Cell Migration

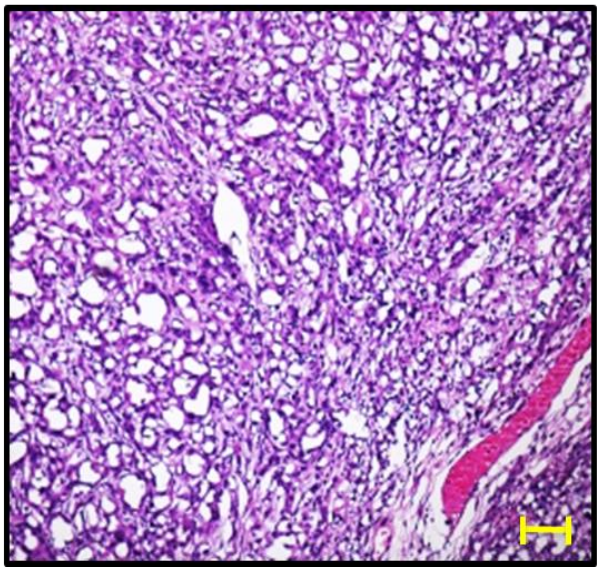


Supplemental Figure 4

A. Scrambled shRNA PC-3

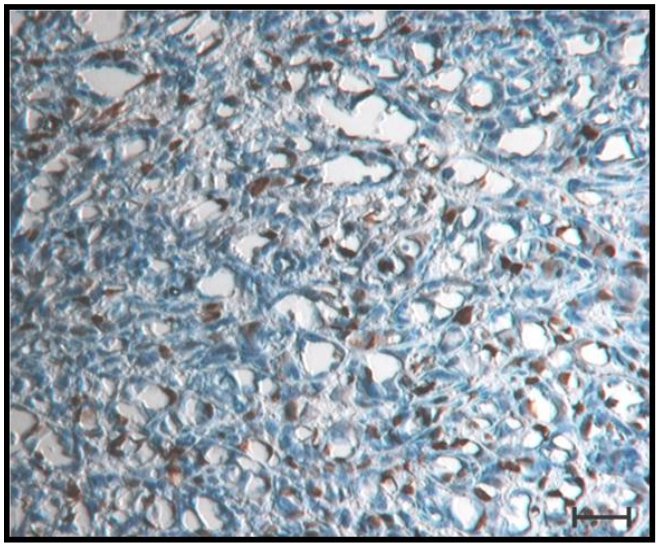


B. GPC-1 shRNA PC-3

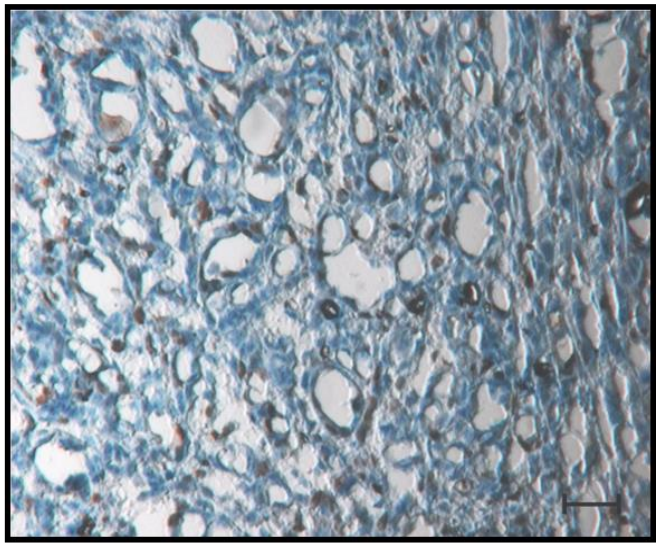


Supplemental Figure 5

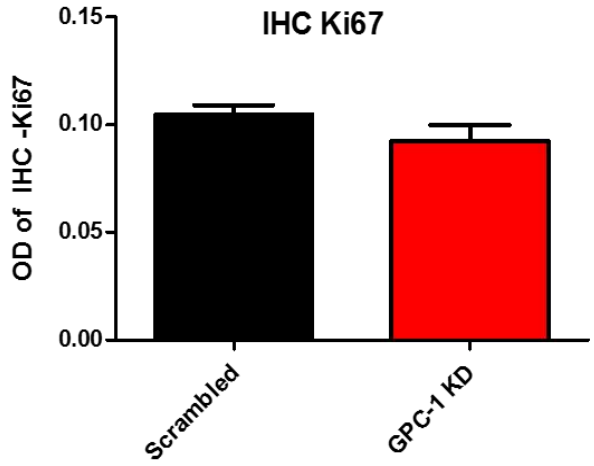
A. Scrambled shRNA PC-3



B. GPC-1 shRNA PC-3

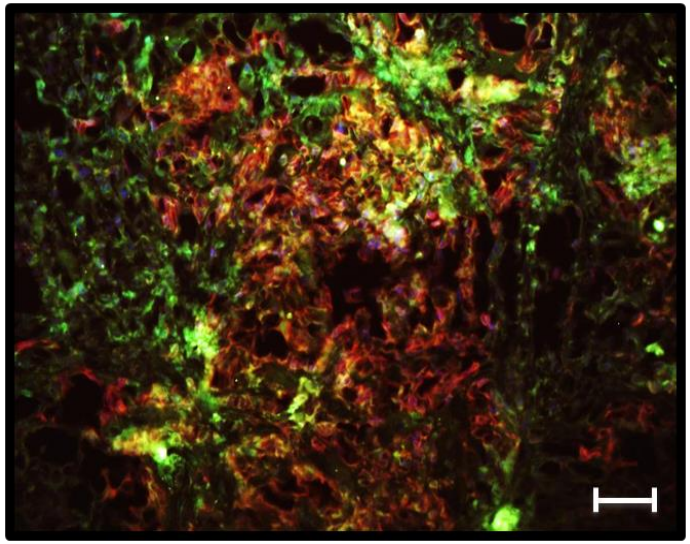


C.

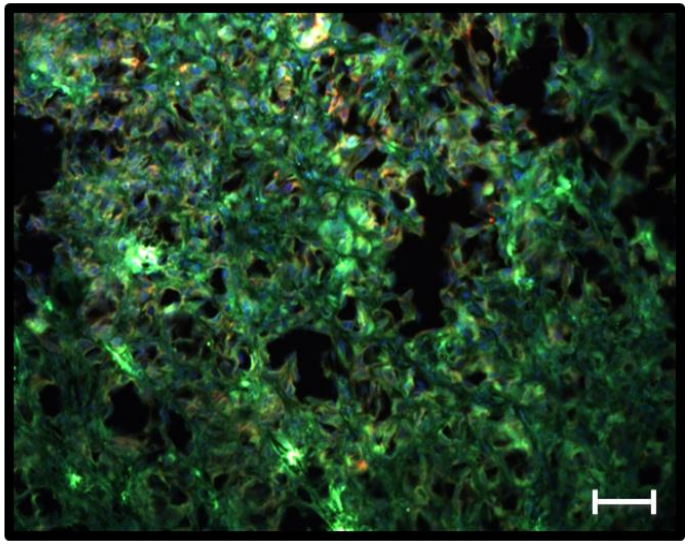


Supplemental Figure 6

A. Scrambled shRNA PC-3

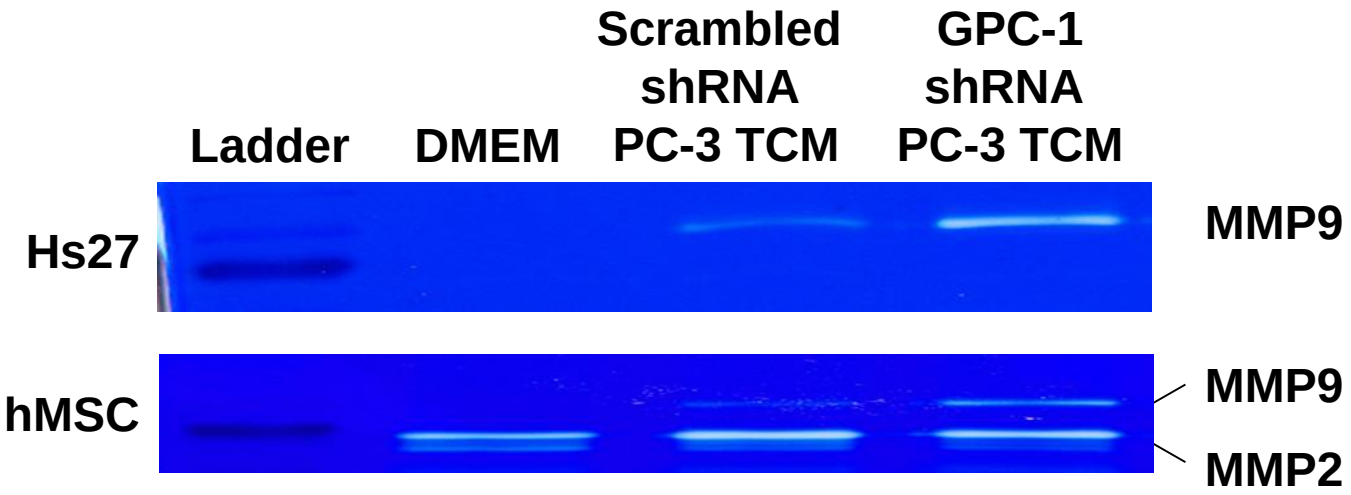


B. GPC-1 shRNA PC-3



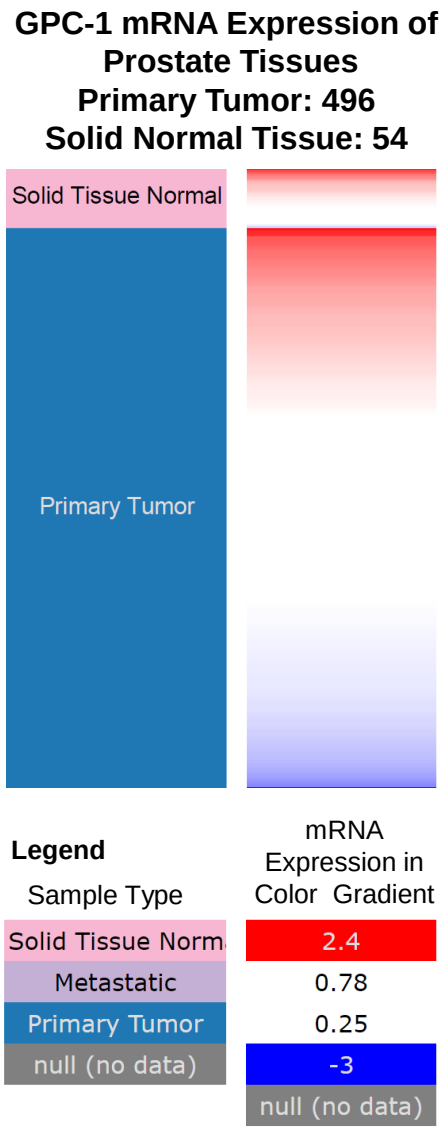
ECad/ NCad

Supplemental Figure 7

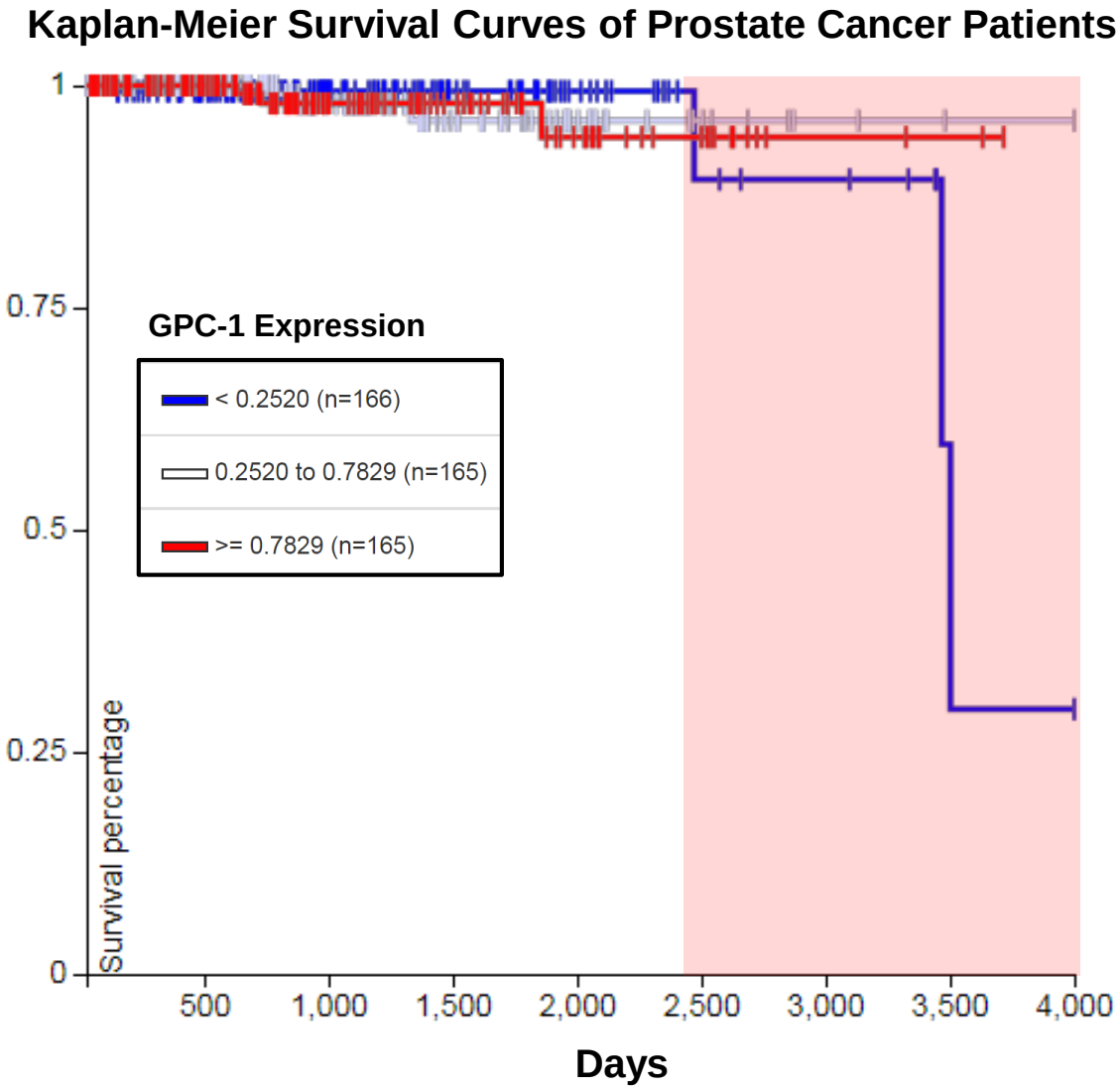


Supplemental Figure 8

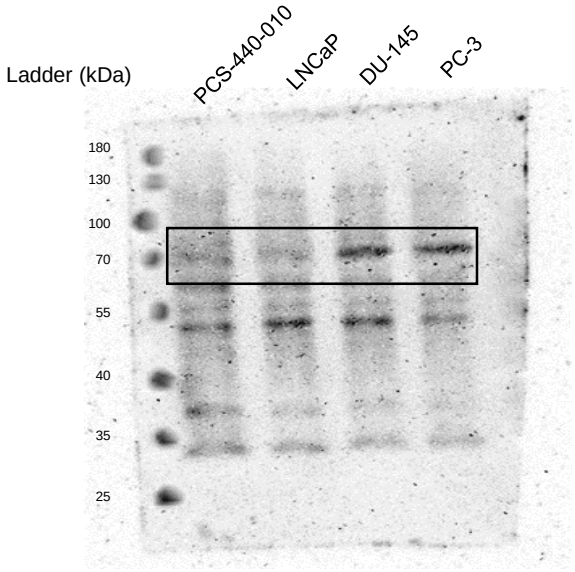
A.

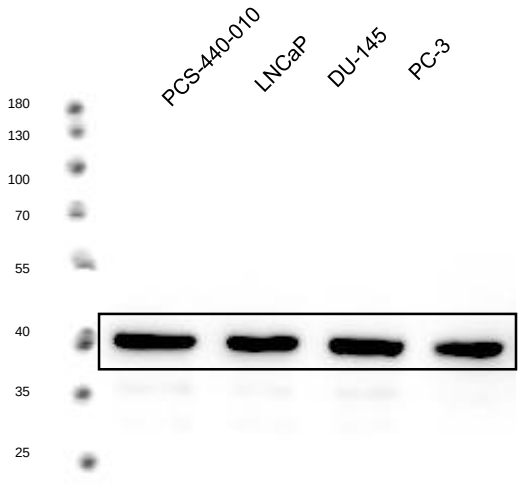


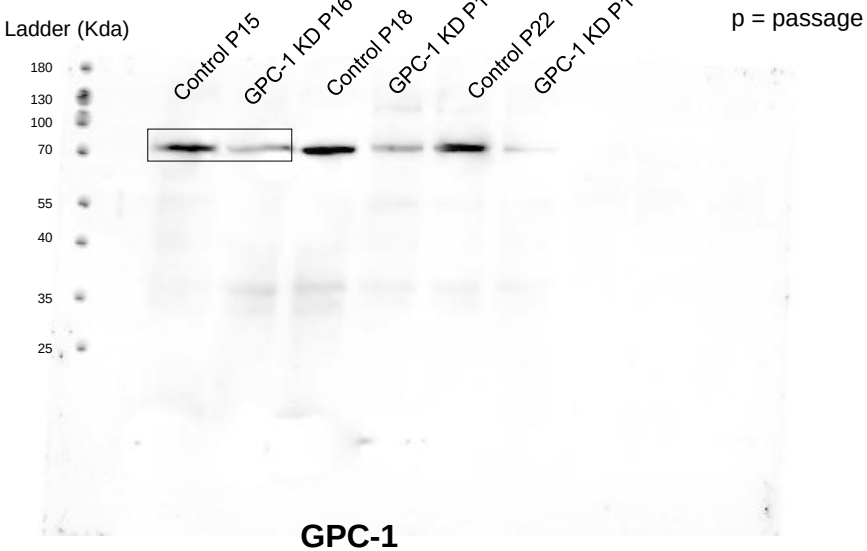
B.



Supplemental Figure 9

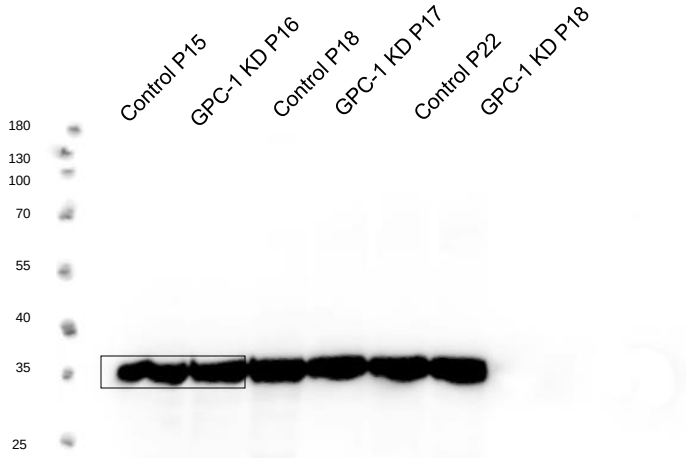






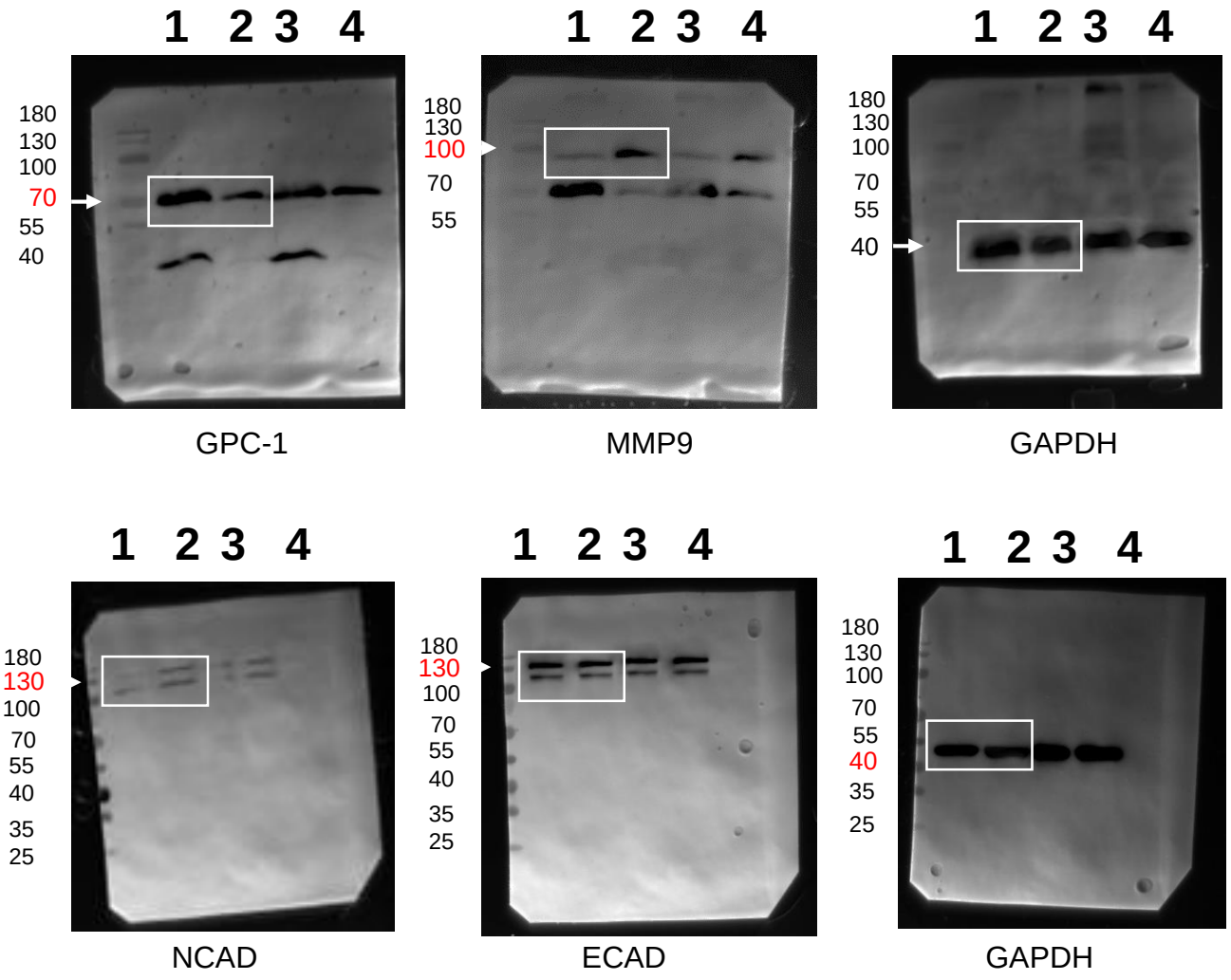
p = passage

GPC-1



GAPDH

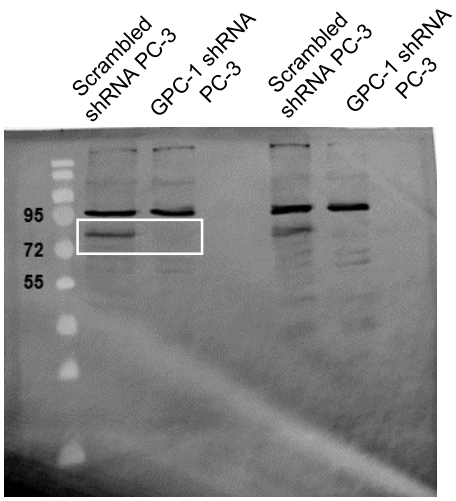
Supplemental Figure 10



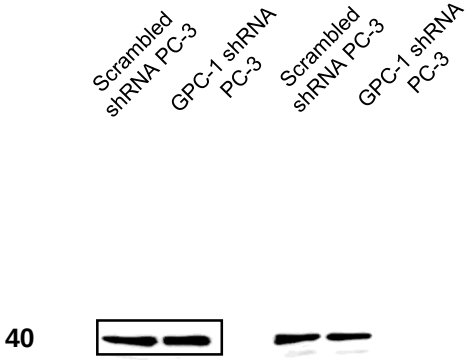
Legend

1 – Control tumor M1
2 – GPC-1 KD tumor M3
3 – Control Tumor M2
4 – GPC-1 KD tumor M4

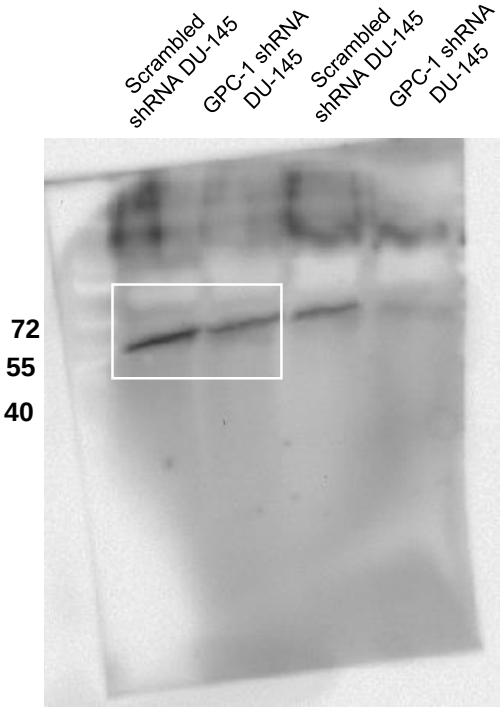
Supplemental Figure 11



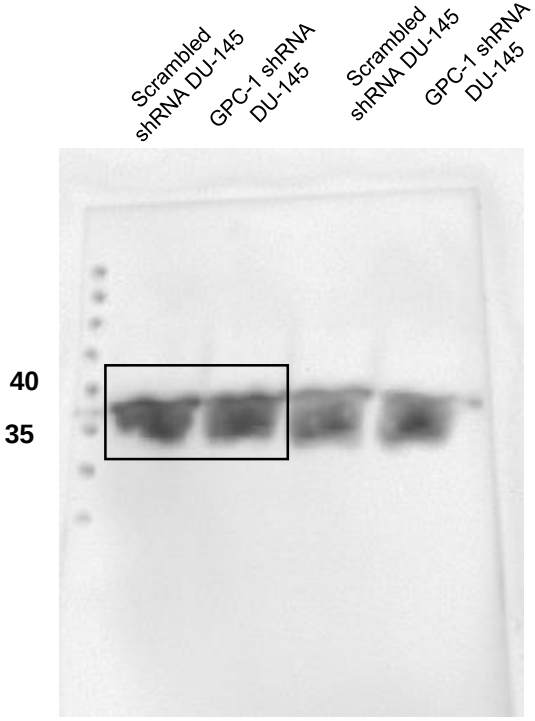
GPC-1



GAPDH



GPC-1



GAPDH