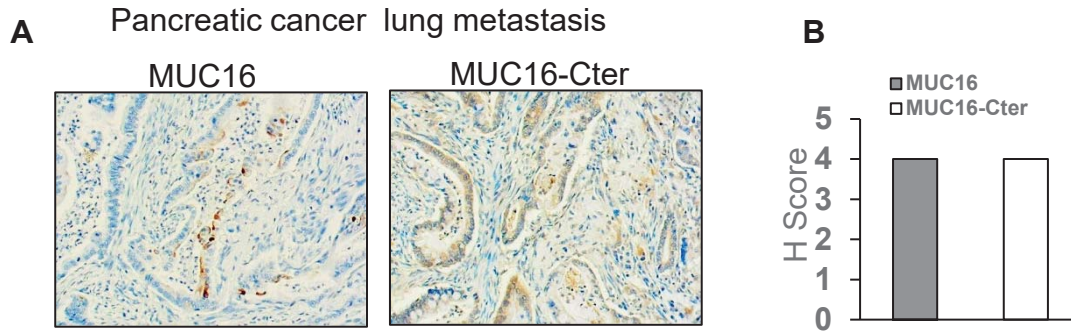


Supplementary Information

**MUC16 Promotes Liver Metastasis of Pancreatic
Ductal Adenocarcinoma by Upregulating NRP2
Associated Cell Adhesion**

Marimuthu et al.

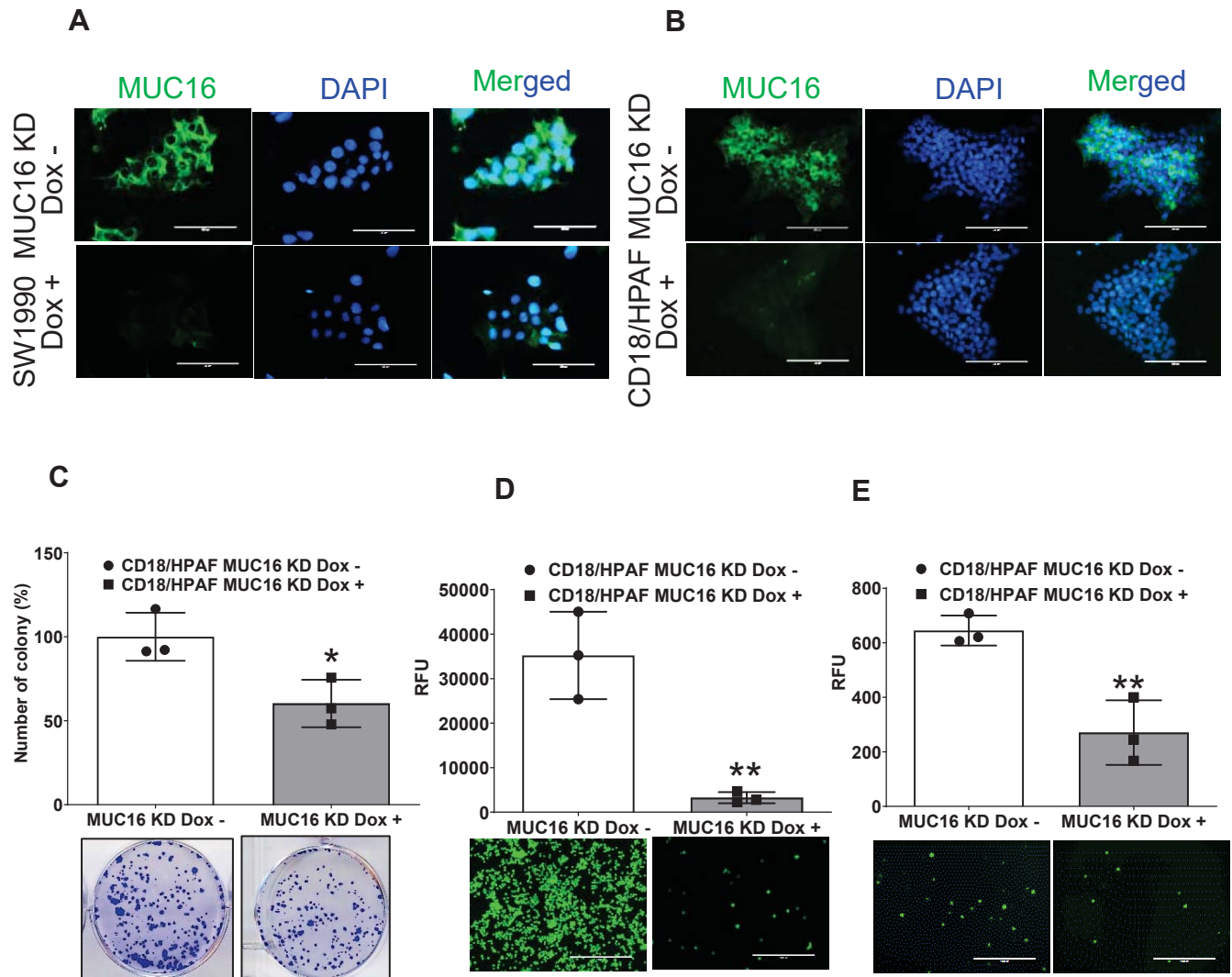
Supplementary Figure 1



Supplementary Figure 1 MUC16 expression in human PDAC lung metastasis and patient derived PDAC organoids

A) Histochemical staining of MUC16 and MUC16-Cter in pancreatic cancer lung-metastasis (TMAs) tissues. B) The bar graph shows the H-score expression of MUC16 and MUC16-Cter in matched tissues. Scale bar, 100 μ m.

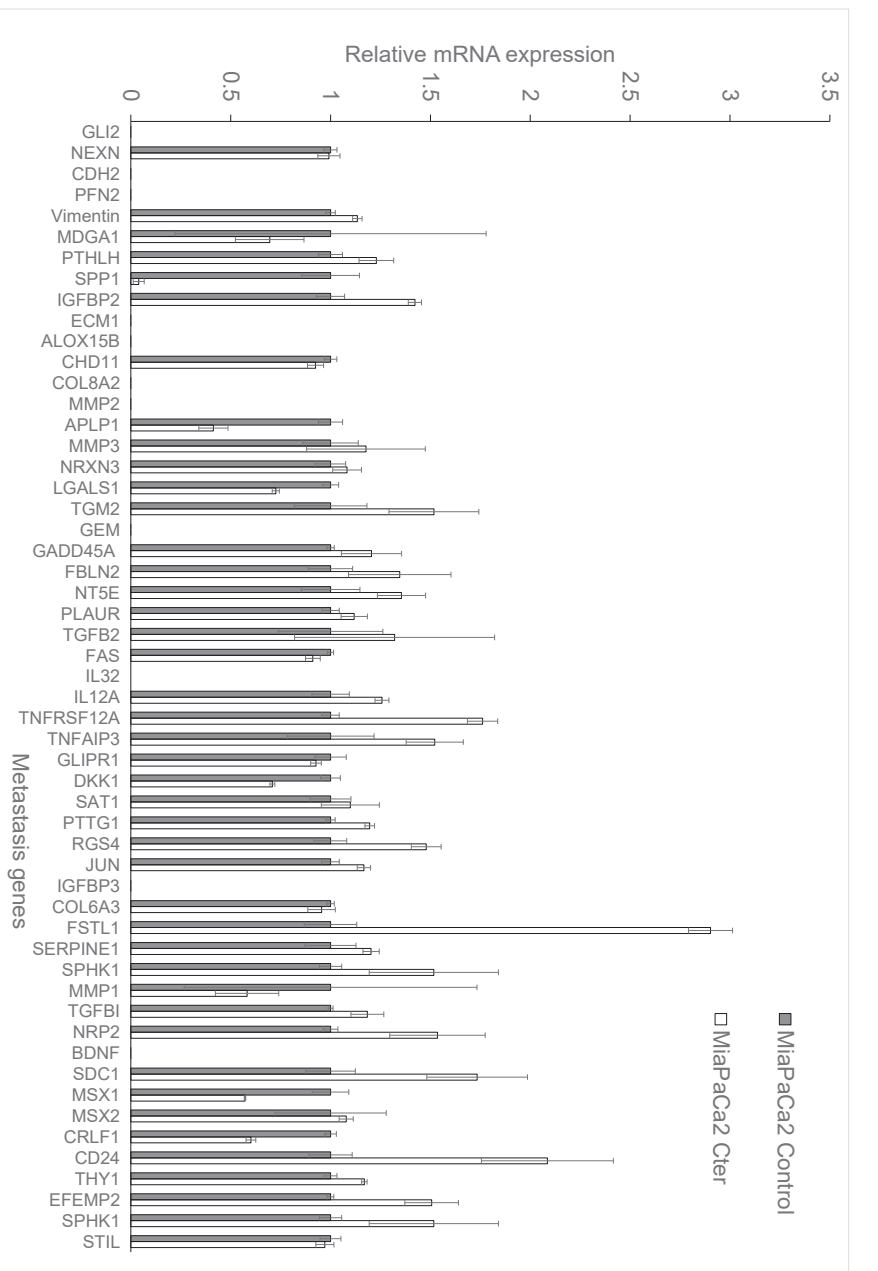
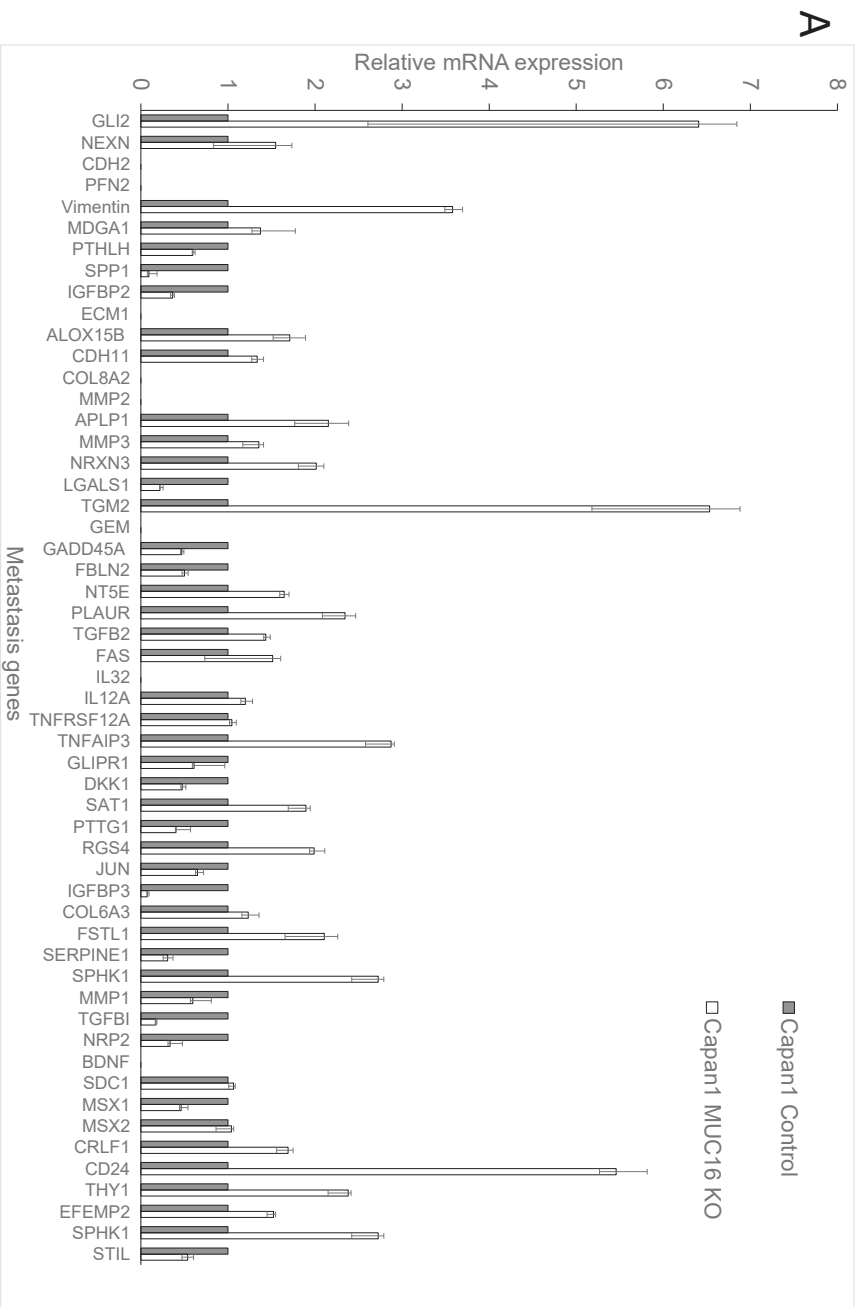
Supplementary Figure 2



Supplementary Figure 2 Loss of MUC16 decreases colony formation, endothelial binding, and p-selectin binding in PC

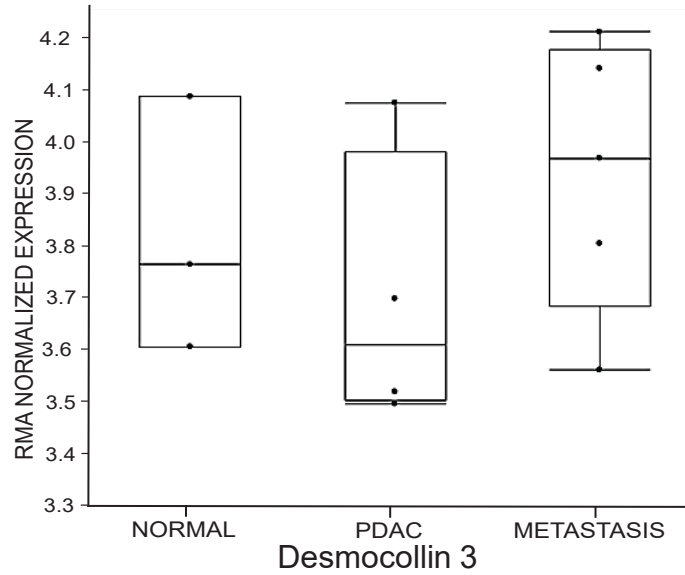
A, B). Immunofluorescence analysis to study MUC16 knockdown in PC cells. Scale bar, 200 μ m. C) CD18/HPAF MUC16 KD cells shows colony-forming ability. D, E) Tumor cell - endothelial/p-selectin binding assay, Scale bar, 1000 μ m. Student's t-test (** $p < 0.01$, * $p < 0.05$).

Supplementary Figure 3



Supplementary Figure 3 QRT-PCR analysis from our RNA-seq data.
A-B) Validation of mRNA expression in Capan1 MUC16 knockout and MiaPaCa2 MUC16-Cter overexpressed cells with respective control by using QRT-PCR analysis.

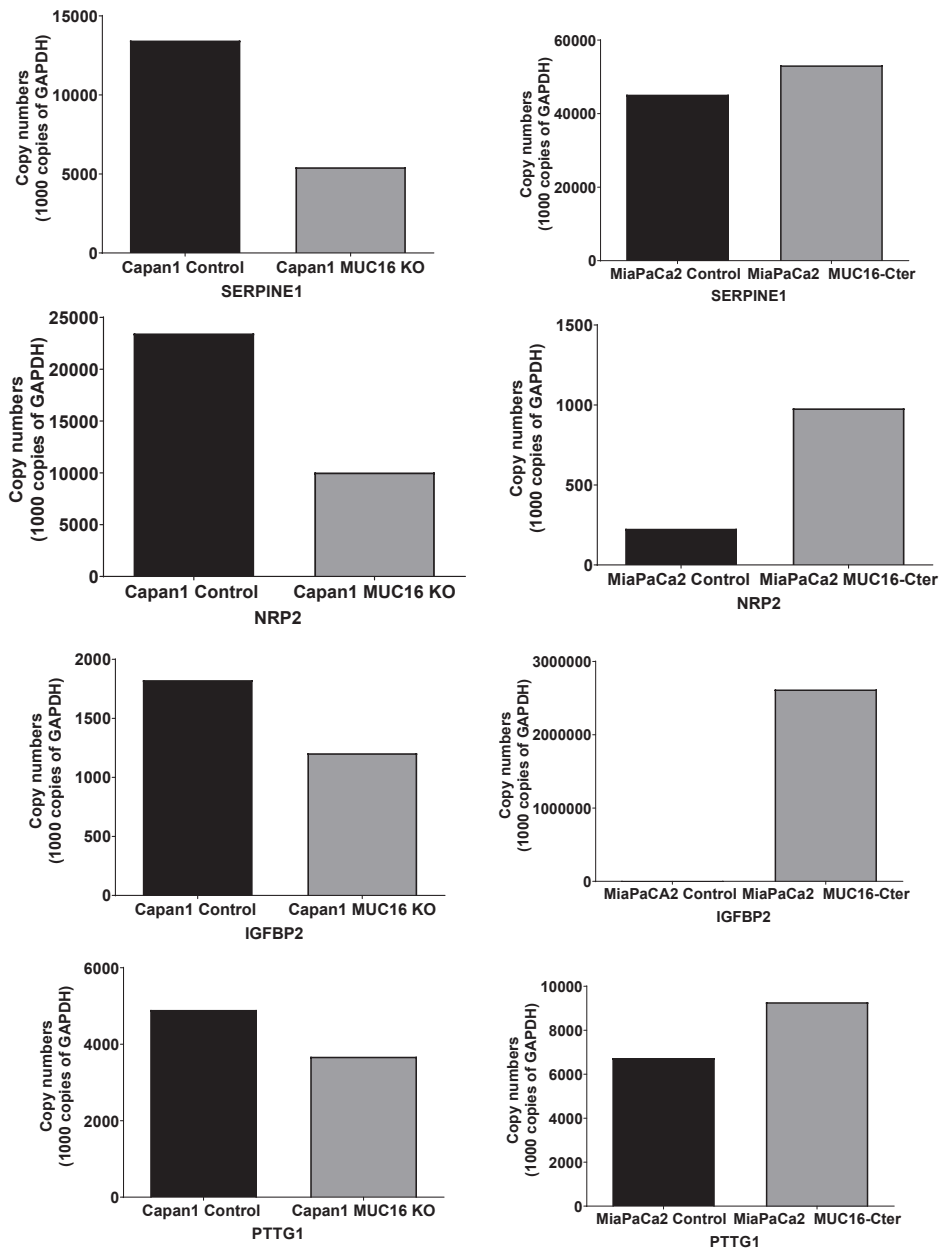
Supplementary Figure 4



Supplementary Figure 4 MUC16 associated cell adhesion Desmocollin3 expression from GSE19279 dataset

Microarray GSE19279 data analysis of Desmocollin3 gene expression in PDAC and metastasis samples compared to normal pancreas.

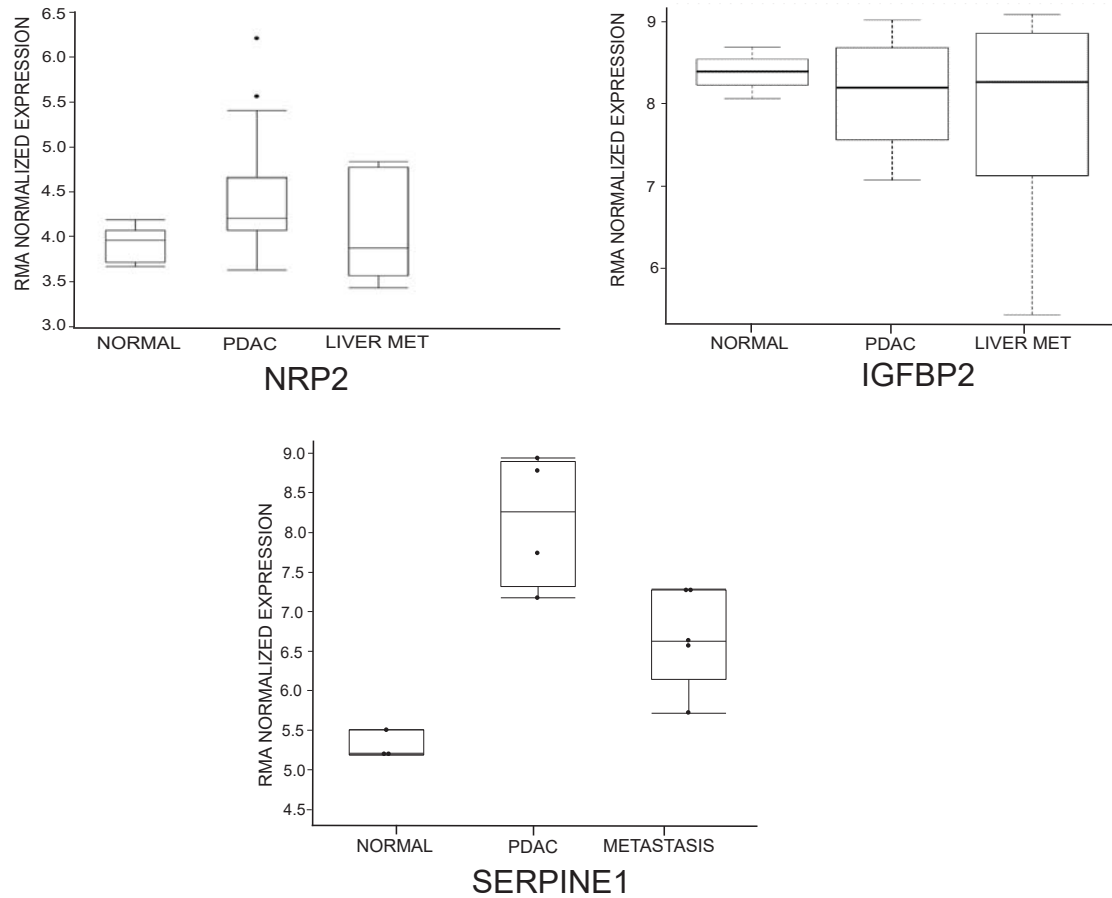
Supplementary Figure 5



Supplementary Figure 5 Droplet Digital PCR (ddPCR) absolute quantification analysis

The ddPCR analysis showed number transcripts of metastasis promoting genes, SERPINE1, NRP2, IGFBP2 and PTTG expression in MUC16 KO and overexpress PC cells. The number of transcripts/per 1000 number of GAPDH transcript were calculated. GAPDH is used as a housekeeping gene.

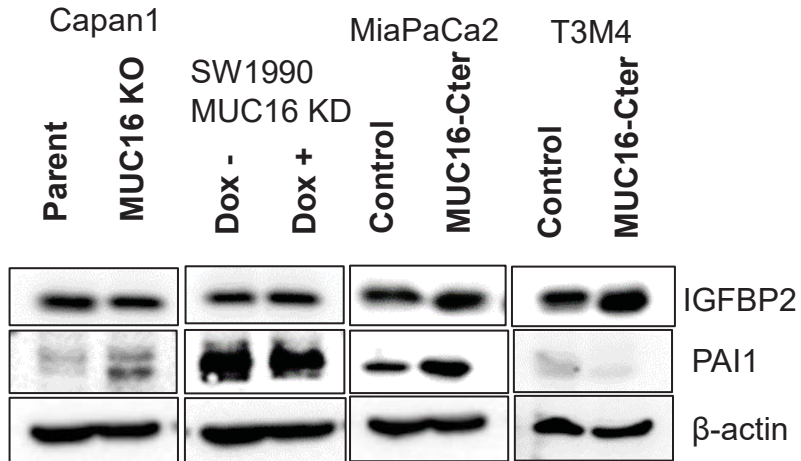
Supplementary Figure 6



Supplementary Figure 6 MUC16 associated metastatic gene expression from GSE19279 dataset

Microarray GSE19279 data analysis of NRP2, IGFBP3 and SERPINE1, gene expression in PDAC and metastasis samples compared to normal pancreas.

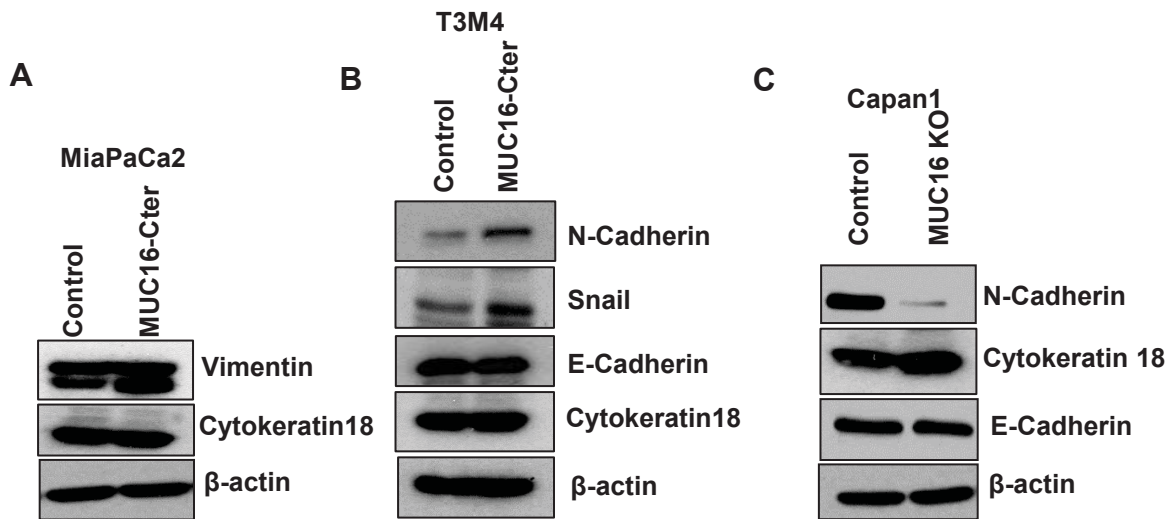
Supplementary Figure 7



Supplementary Figure 7 Validation of MUC16 associated metastatic gene expression in PDAC

Immunoblot analysis shows altered expression of IGFBP2, PAI1 in MUC16 depleted, and MUC16-Cter overexpressed in PC cells. β -actin was used as a loading control.

Supplementary Figure 8



Supplementary Figure 8 The role of MUC16 in epithelial to mesenchymal transition in PDAC

A, B, C) The expression of mesenchymal markers N-cadherin, Vimentin, Snail and epithelial cell-specific marker Cytokeratin-18, and E-cadherin in Capan1 MUC16 KO, and MUC16-Cter overexpressed MiaPaCa2, and T3M4 cells.