

Expanded View Figures

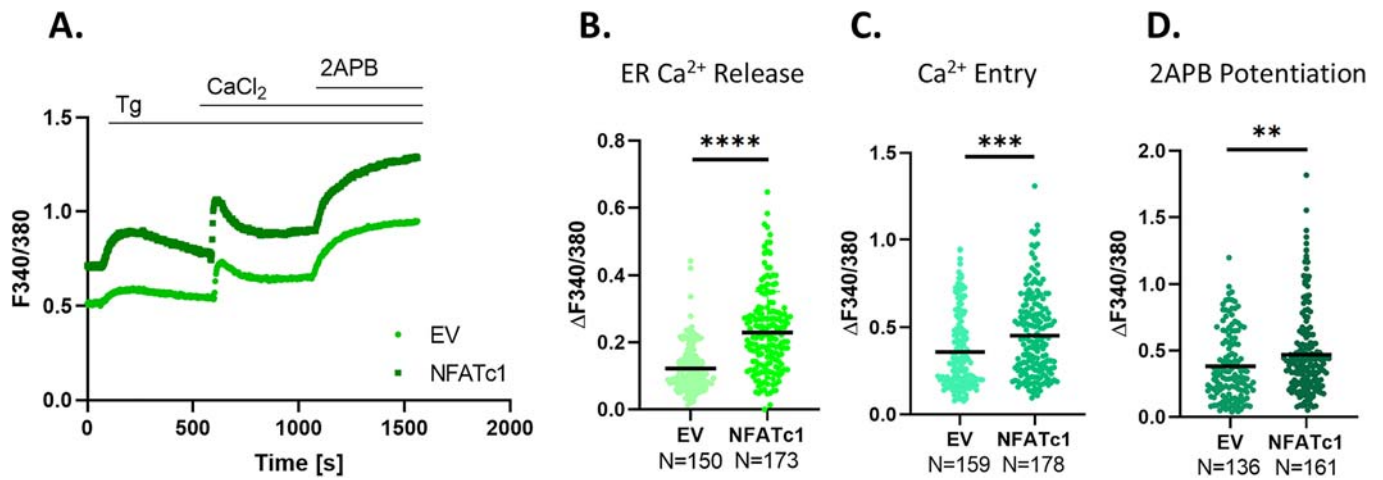


Figure EV1. Calcium imaging in MiaPaCa-2 using standard thapsigargin activated SOCE protocol.

(A) Representative Ca^{2+} imaging traces of empty vector control and NFATc1 overexpression in MiaPaCa-2. (B) Quantitation of ER Ca^{2+} release upon NFATc1 overexpression in MiaPaCa-2 compared to empty vector control where "N" denotes the number of ROIs. $****P < 0.0001$. (C) Change in Ca^{2+} entry upon overexpression of NFATc1 compared to empty vector control in MiaPaCa-2 where "N" denotes the number of ROIs. $***P = 0.0002$. (D) 2-APB potentiation of Orai3 in NFATc1 overexpressed and empty vector control MiaPaCa-2 where "N" denotes the number of ROIs. $**P = 0.0097$. Data presented are mean $\pm$ SEM. For statistical analysis, unpaired Student's *t* test was performed for (B–D) using GraphPad Prism software. Here, $**P < 0.01$; $***P < 0.001$ and $****P < 0.0001$. Source data are available online for this figure.

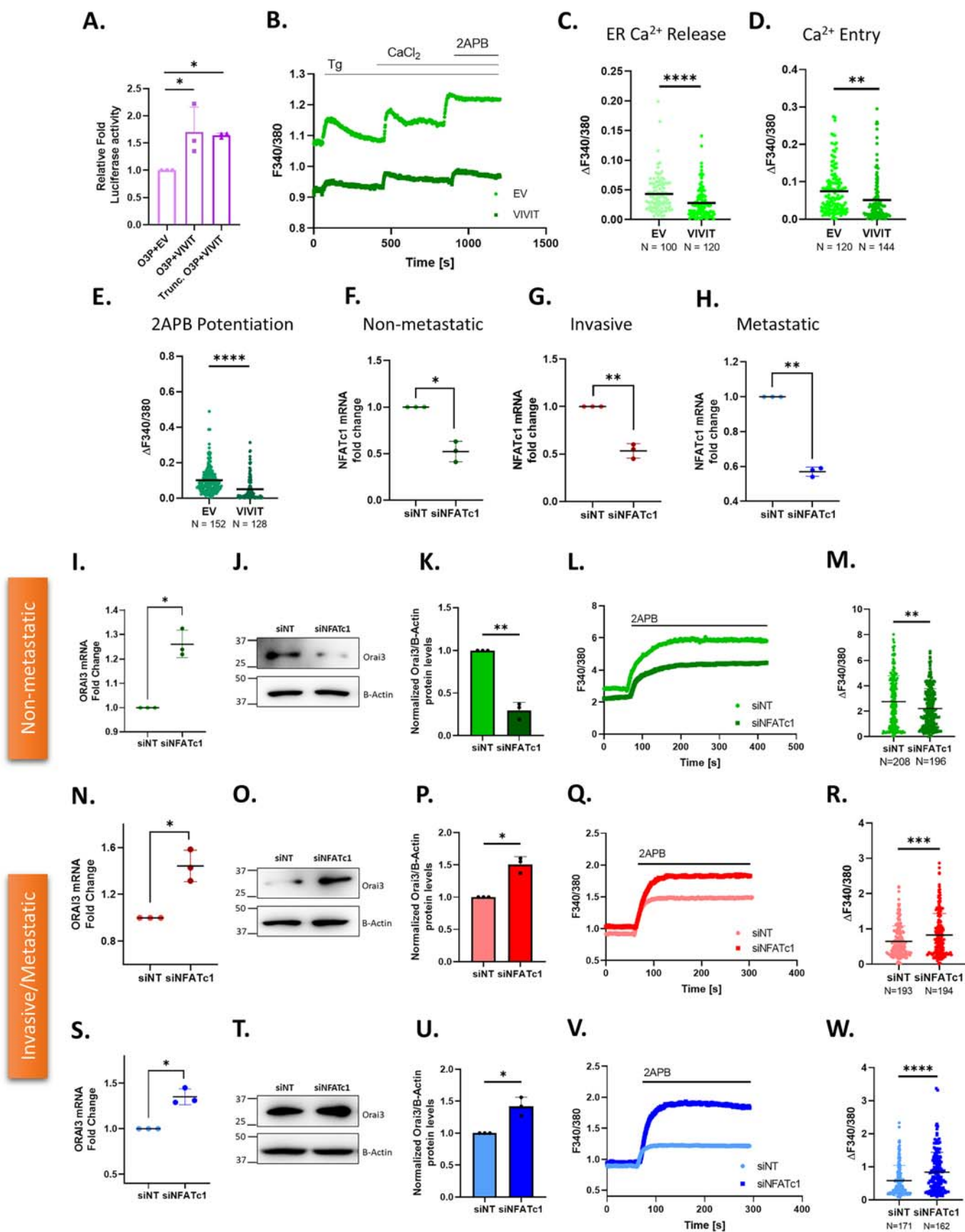


Figure EV2. siRNA mediated NFAT knockdown validates dichotomous regulation of Orai3 in non-metastatic v/s metastatic PC cells.

(A) Normalized luciferase activity of wild-type Orai3 promoter and truncated Orai3 promoter in PANC-1 cells upon VIVIT transfection for 48 h ($N = 3$). $*P = 0.0307$; $*P = 0.0438$. (B) Representative Ca^{2+} imaging trace of cells transfected with control vector pEGFP-N1 plasmid and VIVIT transfection in MiaPaCa-2. (C) Quantitation of ER Ca^{2+} release after VIVIT transfection in MiaPaCa-2 compared to empty vector control where "N" denotes the number of ROIs. $****P < 0.0001$. (D) Change in Ca^{2+} entry upon VIVIT Transfection in MiaPaCa-2 where "N" denotes the number of ROIs. $**P = 0.0018$. (E) 2-APB potentiation of Orai3 in VIVIT-transfected and empty vector control MiaPaCa-2 where "N" denotes the number of ROIs. $****P < 0.0001$. (F) qRT-PCR analysis showing NFATc1 knockdown validation in MiaPaCa-2 ($N = 3$). $*P = 0.0173$. (G) qRT-PCR analysis showing NFATc1 knockdown validation in PANC-1 ($N = 3$). $**P = 0.0088$. (H) qRT-PCR analysis showing NFATc1 knockdown validation in CFPAC-1 ($N = 3$). $**P = 0.0013$. (I) qRT-PCR analysis showing increase in Orai3 mRNA levels upon NFATc1 knockdown in MiaPaCa-2 compared to control ($N = 3$). $*P = 0.0148$. (J) Representative western blots showing decrease in Orai3 protein levels upon NFATc1 knockdown in MiaPaCa-2 cells compared to control. (K) Densitometric quantitation of Orai3 protein levels in NFATc1 knockdown MiaPaCa-2 cells compared to control ($N = 3$). $**P = 0.0060$. (L) Representative Ca^{2+} imaging trace of MiaPaCa-2 cells transfected with either control siNT or siNFATc1. (M). 2-APB potentiation of Orai3 in siNT and siNFATc1-transfected MiaPaCa-2 where "N" denotes the number of ROIs. $**P = 0.0024$. (N) qRT-PCR analysis showing increase in Orai3 mRNA expression upon NFATc1 knockdown in PANC-1 compared to control ($N = 3$). $*P = 0.0295$. (O) Representative western blots showing increase in Orai3 protein levels due to NFATc1 knockdown in PANC-1 compared to control. (P) Western blot densitometry of Orai3 protein levels after NFATc1 knockdown in PANC-1 cells compared to control ($N = 3$). $*P = 0.0185$. (Q) Representative Ca^{2+} imaging trace of PANC-1 cells transfected with either control siNT or siNFATc1. (R) Potentiation of Orai3 by 2-APB in control siNT and siNFATc1-transfected PANC-1 where "N" denotes the number of ROIs. $***P = 0.0009$. (S) qRT-PCR analysis showing increase in Orai3 mRNA expression upon NFATc1 knockdown in CFPAC-1 compared to control ($N = 3$). $*P = 0.0201$. (T) Representative western blots showing increase in Orai3 protein levels due to NFATc1 knockdown in CFPAC-1 compared to control. (U) Western blot densitometry of Orai3 protein in NFATc1 knockdown CFPAC-1 cells compared to control ($N = 3$). $*P = 0.0381$. (V) Representative Ca^{2+} imaging trace of CFPAC-1 cells transfected with either control siNT or siNFATc1. (W). Potentiation of Orai3 by 2-APB in siNT and siNFATc1-transfected CFPAC-1 where "N" denotes the number of ROIs. $****P < 0.0001$. Data presented are mean $\pm$ SEM. For statistical analysis, unpaired Student's t test was performed for (C-E, M, R, W) while one-sample t test was performed for (F, G, H, I, K, N, P, S, U) using GraphPad Prism software. Here, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ and $****P < 0.0001$. Source data are available online for this figure.

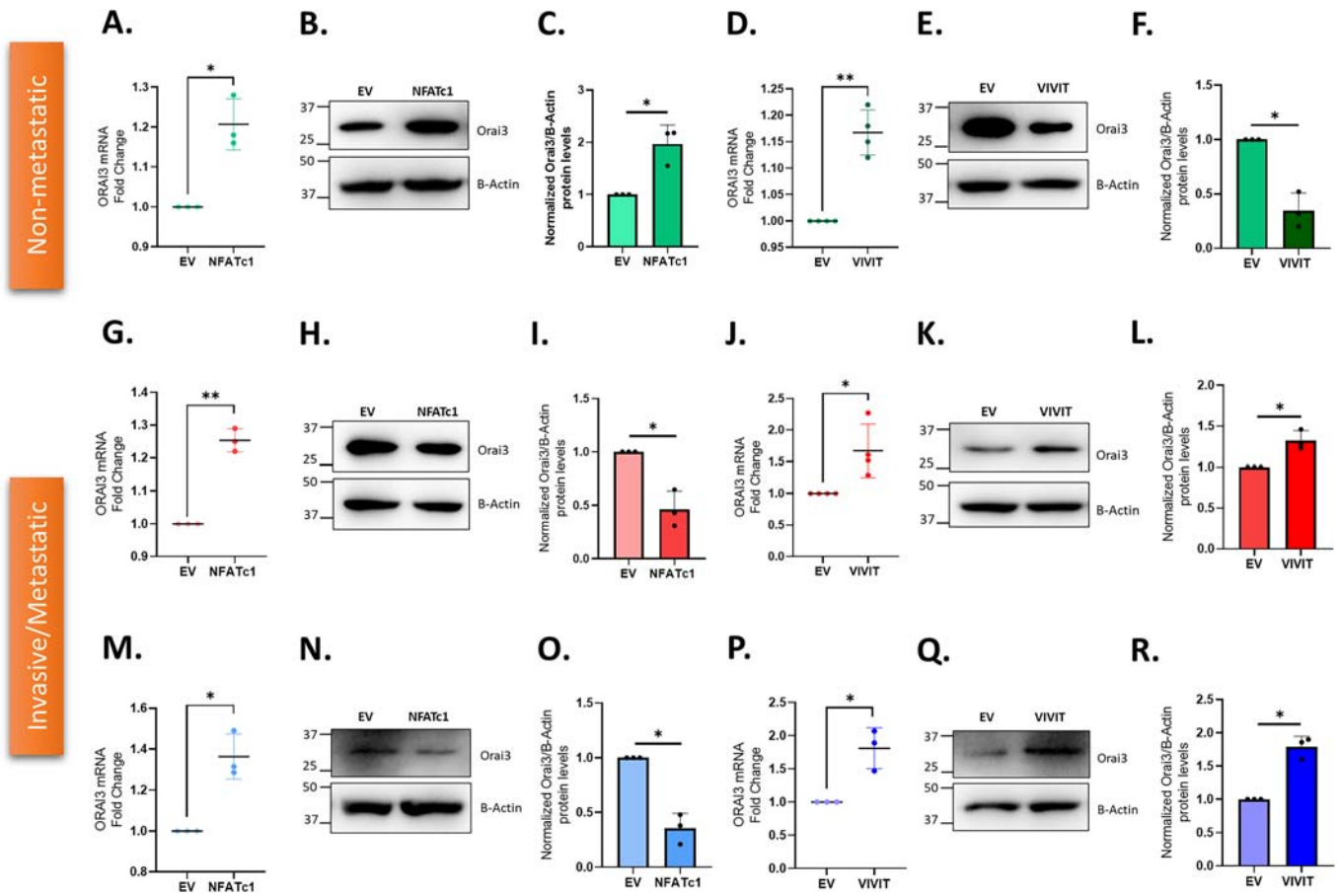


Figure EV3. NFATc1 differentially regulates Orai3 expression in BxPC-3 (non-metastatic), ASPC-1 (invasive) and SW1990 (metastatic) PC cells.

(A) qRT-PCR analysis showing increase in Orai3 mRNA levels upon NFATc1 overexpression in BxPC-3 compared to control ($N = 3$). $*P = 0.0308$. (B) Representative western blots showing increase in Orai3 protein levels due to NFATc1 overexpression in BxPC-3 cells compared to control. (C) Densitometric quantitation of Orai3 protein levels in NFATc1 overexpressed BxPC-3 compared to control ($N = 3$). $*P = 0.0435$. (D) qRT-PCR analysis showing increase in Orai3 mRNA levels upon VIVIT transfection in BxPC-3 compared to control ($N = 3$). $**P = 0.0045$. (E) Representative western blots showing decrease in Orai3 protein levels due to VIVIT transfection in BxPC-3 cells compared to control. (F) Densitometric quantitation of Orai3 protein levels in VIVIT-transfected BxPC-3 compared to control ($N = 3$). $*P = 0.0198$. (G) qRT-PCR analysis showing increase in Orai3 mRNA levels upon NFATc1 overexpression in ASPC-1 compared to control ($N = 3$). $**P = 0.0063$. (H) Representative western blots showing decrease in Orai3 protein levels due to NFATc1 overexpression in ASPC-1 cells compared to control. (I) Densitometric quantitation of Orai3 protein levels in NFATc1 overexpressed ASPC-1 compared to control ($N = 3$). $*P = 0.0327$. (J) qRT-PCR analysis showing increase in Orai3 mRNA levels upon VIVIT transfection in ASPC-1 compared to control ($N = 3$). $*P = 0.0494$. (K) Representative western blots showing increase in Orai3 protein levels due to VIVIT transfection in ASPC-1 cells compared to control. (L) Densitometric quantitation of Orai3 protein levels in VIVIT-transfected ASPC-1 compared to control ($N = 3$). $*P = 0.0436$. (M) qRT-PCR analysis showing increase in Orai3 mRNA levels upon NFATc1 overexpression in SW1990 compared to control ($N = 3$). $*P = 0.0296$. (N) Representative western blots showing decrease in Orai3 protein levels due to NFATc1 overexpression in SW1990 cells compared to control. (O) Densitometric quantitation of Orai3 protein levels in NFATc1 overexpressed SW1990 compared to control ($N = 3$). $*P = 0.0147$. (P) qRT-PCR analysis showing increase in Orai3 mRNA levels upon VIVIT transfection in SW1990 compared to control ($N = 3$). $*P = 0.0449$. (Q) Representative western blots showing increase in Orai3 protein levels due to VIVIT transfection in SW1990 cells compared to control. (R) Densitometric quantitation of Orai3 protein levels in VIVIT-transfected SW1990 compared to control ($N = 3$). $*P = 0.0140$. Data presented are mean $\pm$ SEM. For statistical analysis, one-sample t test was performed for (A, C, D, F, G, I, J, L, M, O, P, R) using GraphPad Prism software. Here, $*P < 0.05$ and $**P < 0.01$. Source data are available online for this figure.

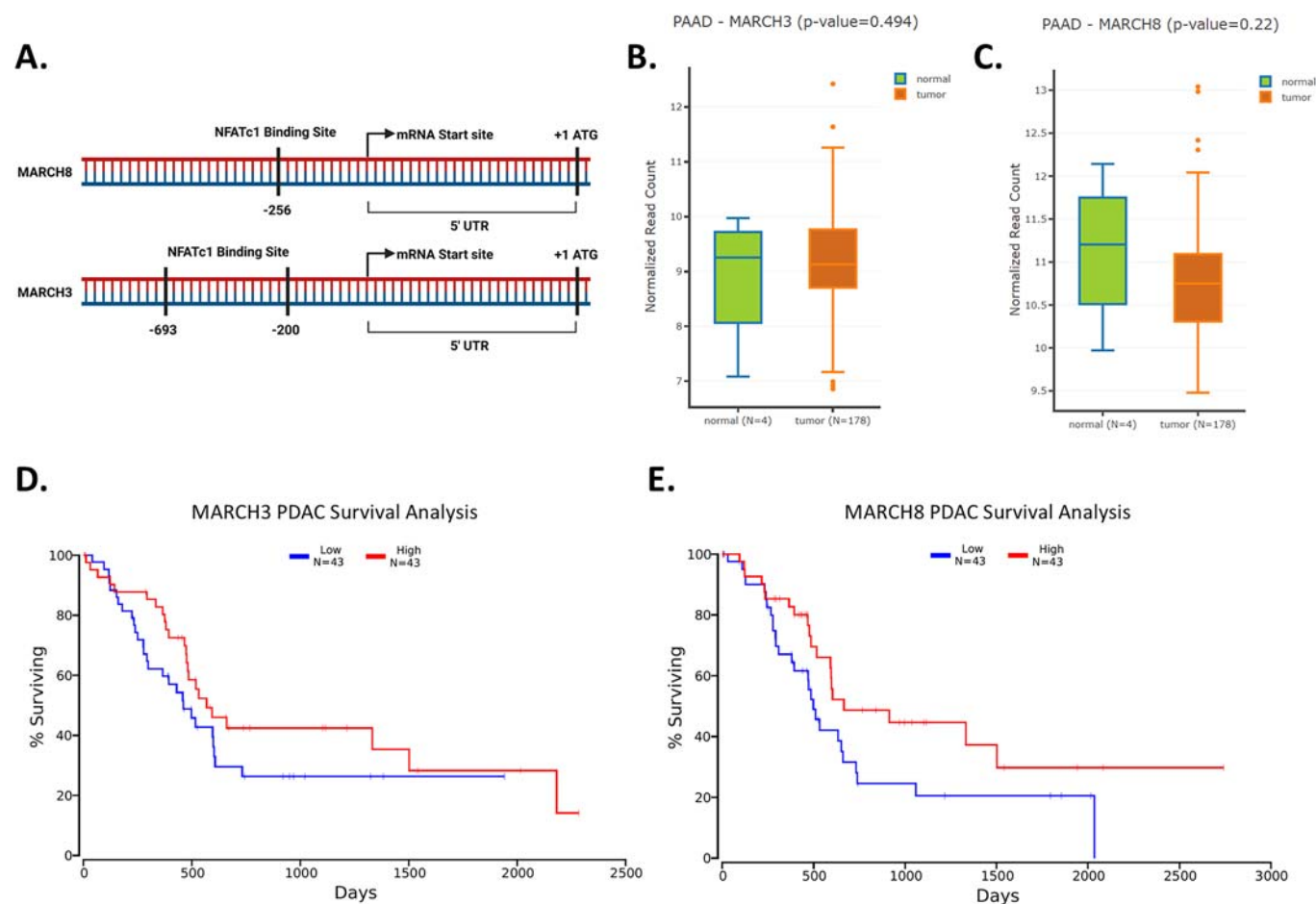


Figure EV4. Bioinformatic analysis of MARCH3 and MARCH8 E3 ubiquitin ligase.

(A) Identification of putative NFATc1 binding sites on the human MARCH3 and MARCH8 promoter using the EPD-Search Motif Tool at P value cut-off of 0.01. (B) MARCH3 expression levels in normal pancreatic tissues and pancreatic adenocarcinoma (PAAD) tissues analyzed by the DNMIIVD database. (Normal: maxima-9.971; minima-7.082; median-9.252; Q1-8.061; Q3-9.718. Tumor: maxima-12.418; minima-6.856; median-9.129; Q1-8.704; Q3-9.762). (C) MARCH8 expression levels in normal pancreatic tissues and pancreatic adenocarcinoma (PAAD) tissues analyzed by the DNMIIVD database. (Normal: maxima-12.14; minima-9.97; median-11.20; Q1-10.51; Q3-11.74. Tumor: maxima-13.04; minima-9.47; median-10.74; Q1-10.30; Q3-12.04). (D) Survival analysis of pancreatic cancer patients wherein blue trace corresponds to low MARCH3 expression ($n = 43$) and red trace corresponds to high MARCH3 expression ($n = 43$). (E) Survival analysis of pancreatic cancer patients wherein blue trace corresponds to low MARCH8 expression ($n = 43$) and red trace corresponds to high MARCH8 expression ($n = 43$). For statistical analysis, Mann-Whitney U test was performed for (B, C). Source data are available online for this figure.

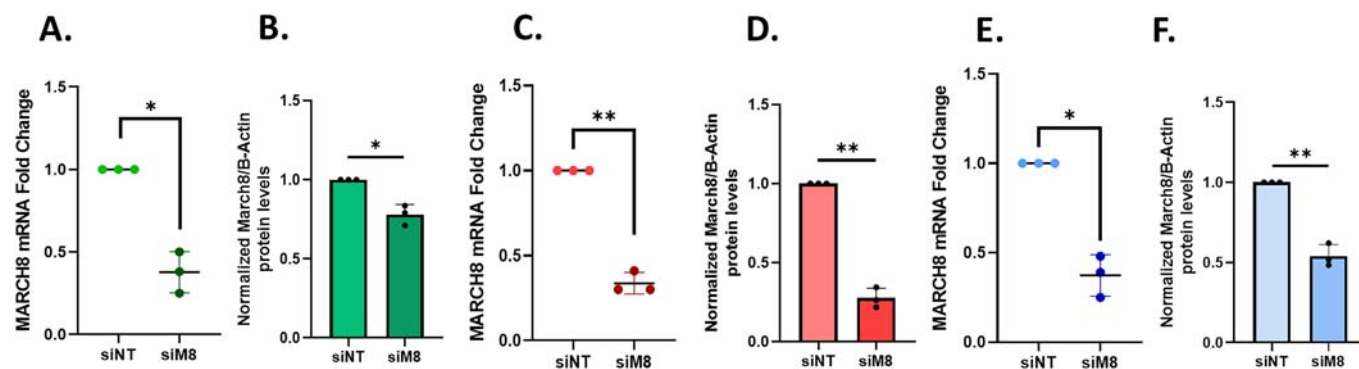


Figure EV5. siRNA mediated MARCH8 knockdown validation at RNA and protein levels in PC cells.

(A) qRT-PCR analysis showing decrease in MARCH8 mRNA levels upon siMARCH8 transfection in MiaPaCa-2 compared to control siINT ($N = 3$). $^*P = 0.0131$. (B) Densitometric quantitation of MARCH8 protein levels in siMARCH8 transfection MiaPaCa-2 cells compared to siINT control ($N = 3$). $^*P = 0.0266$. (C) qRT-PCR analysis showing decrease in MARCH8 mRNA levels upon siMARCH8 transfection in PANC-1 compared to control siINT ($N = 3$). $^{**}P = 0.0030$. (D) Densitometric quantitation of MARCH8 protein levels in siMARCH8 transfection in PANC-1 cells compared to siINT control ($N = 3$). $^{**}P = 0.0025$. (E) qRT-PCR analysis showing decrease in MARCH8 mRNA levels upon siMARCH8 transfection in CFPAC-1 compared to control siINT ($N = 3$). $^*P = 0.0112$. (F) Densitometric quantitation of MARCH8 protein levels in siMARCH8 transfection in CFPAC-1 cells compared to siINT control ($N = 3$). $^{**}P = 0.0080$. Data presented are mean $\pm$ SEM. For statistical analysis, one-sample t test was performed for (A-F) using GraphPad Prism software. Here, $^*P < 0.05$ and $^{**}P < 0.01$. Source data are available online for this figure.