

Figure 1 Nagasawa

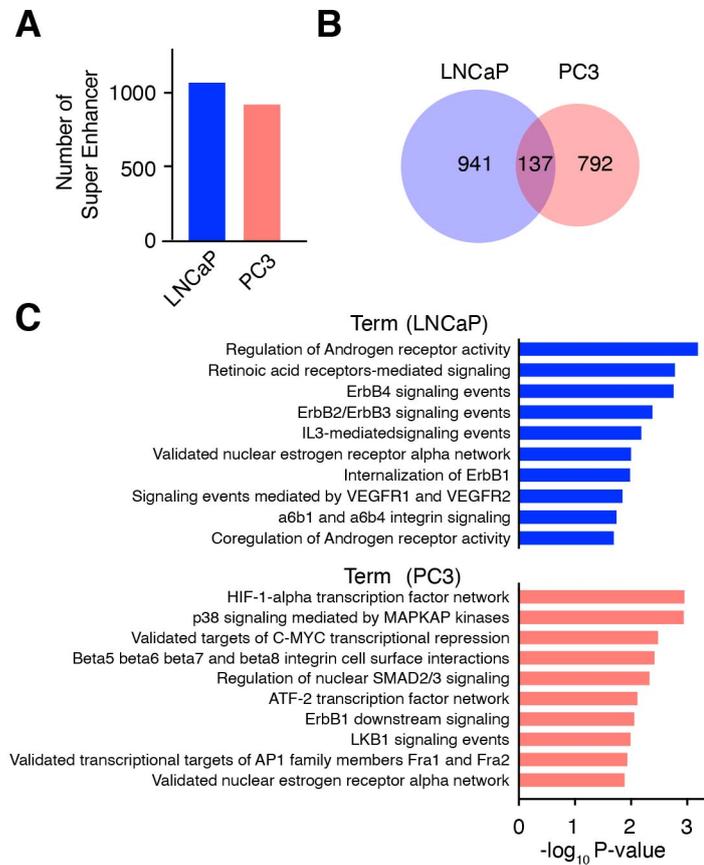


Figure 1. PC3-specific SE-associated genes include genes involved in cell migration and invasion.

(A) Numbers of SE-associated genes in the indicated cell lines. **(B)** Venn diagram showing the numbers of SE-associated genes in LNCaP and PC3 cells. **(C)** Pathway analysis was performed using the LNCaP- and PC3-specific SE-associated gene sets; the top 10 terms are indicated.

Figure 2 Nagasawa

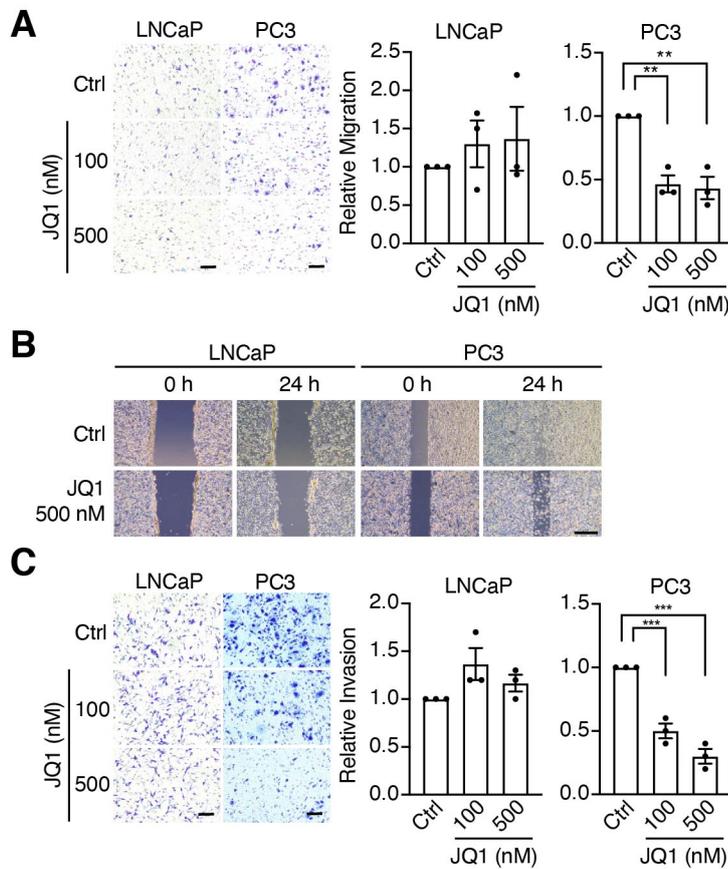


Figure 2. A BETi, JQ1, decreases migration and invasion abilities of PC3 cells.

(A) Migration and (C) invasion assays using LNCaP and PC3 cells. Cells were treated with DMSO (Ctrl) or 100 nM or 500 nM JQ1 and incubated in the chamber for 8 h for migration assays and 16 h for invasion assays. Error bars indicate mean \pm S.E.M. (n=3 biological replicates). *** P <0.001, ** P <0.01, one-way ANOVA. Scale bar, 200 μ m. (B) Wound healing assay using LNCaP and PC3 cells treated with 500 nM JQ1. Scale bar, 500 μ m.

Figure 3 Nagasawa

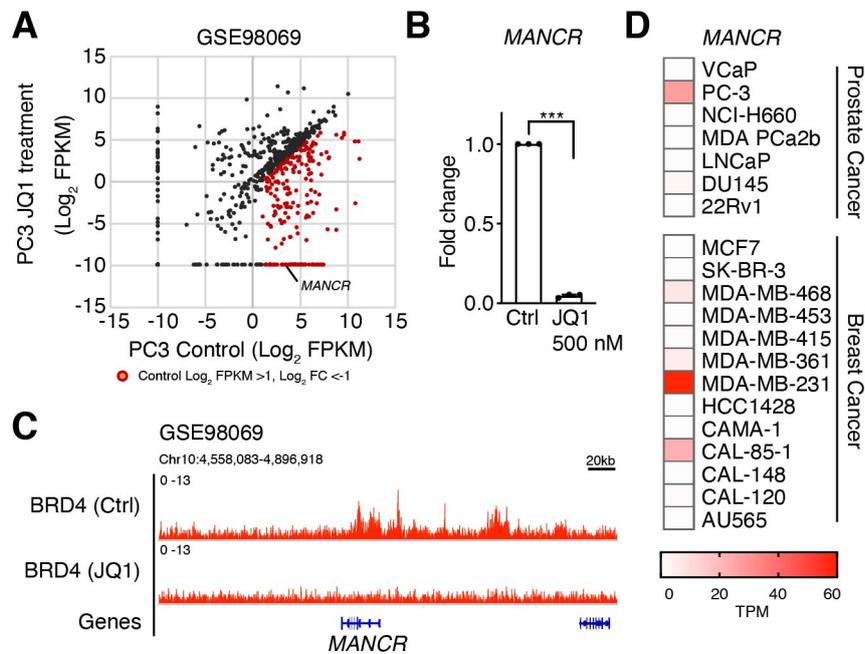


Figure 3. PC3-specific SE-associated lncRNA *MANCR* is down-regulated by JQ1.

(A) Scatter plot comparing Log₂ FPKM expression values for PC3-specific SE-associated genes as identified in Figure 1B. An RNA-seq dataset for untreated (Ctrl) and JQ1-treated PC3 cells were used (GSE98069). (B) RT-qPCR analysis of *MANCR* expression in PC3 cells treated with JQ1. Error bars indicate mean ± S.E.M. (n=3 biological replicates). ****P*<0.001; unpaired t-test. (C) Genome browser images of BRD4 ChIP-seq in PC3 cells. ChIP-seq dataset for untreated (Ctrl) and JQ1-treated PC3 cells were used (GSE98069). (D) Comparative analysis of *MANCR* expression in several cancer cell lines. Expression data were downloaded from Expression Atlas (<http://www.ebi.ac.uk/gxa>).

Figure 4 Nagasawa

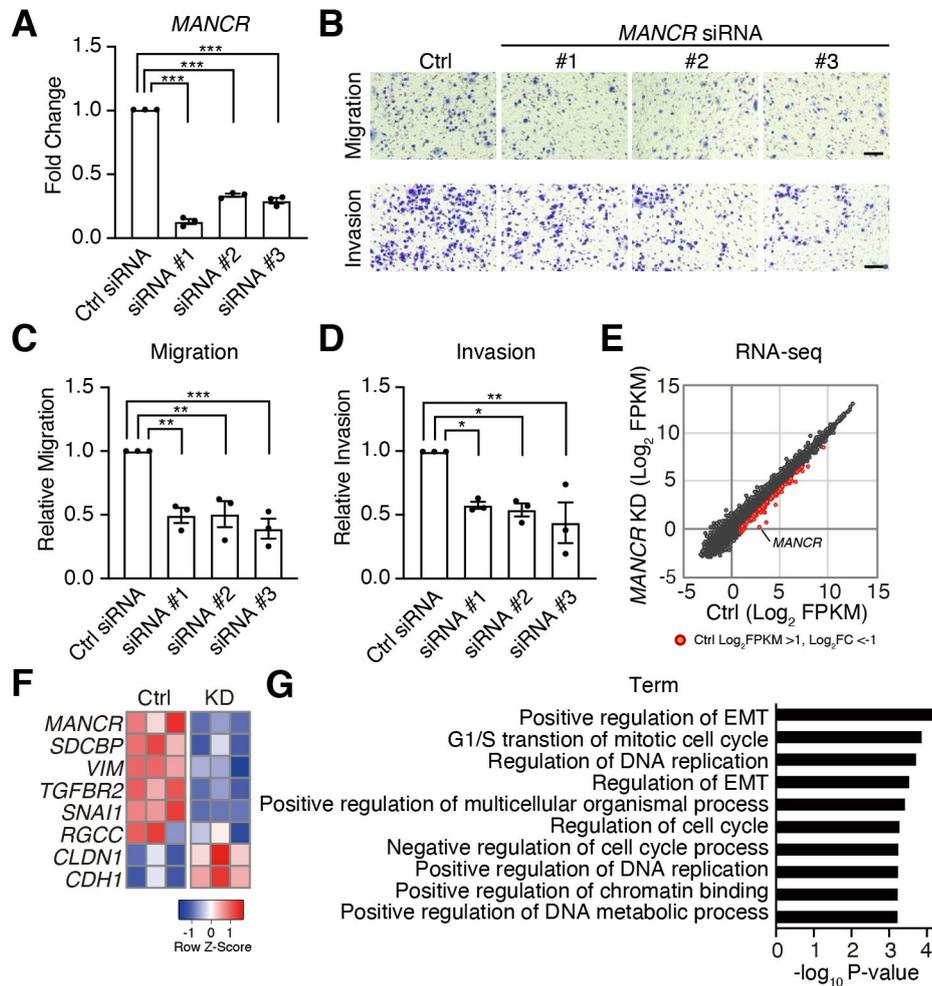


Figure 4. *MANCR* plays a critical role in migration and invasion of PC3 cells.

(A) RT-qPCR analysis of *MANCR* expression in PC3 cells transfected with control or *MANCR*-targeted siRNA. (B) Representative images of migration and invasion assays using PC3 cells transfected with the indicated siRNA. Scale bars, 100 μ m. (C, D) Quantification of the migration (C) and invasion assays (D). (A, C, D) Error bars indicate mean \pm S.E.M. (n=3 biological replicates). ***P<0.001, **P<0.01, *P<0.05; one-way ANOVA. (E) Scatter plot displaying mRNA expression levels in *MANCR*-knocked down PC3 cells compared with the control. Red dots indicate down-regulated genes (Log_2 FPKM > 1 in Ctrl, Log_2 FC < -1 by *MANCR* knockdown). (F) Heat map showing expression levels of genes involved in EMT. (G) Gene Ontology analysis using the gene set indicated by red dots in (E). Top 10 terms are indicated.