

Supplementary Figure legends

Supplementary Figure S1. Tumor growth curves for representative top hits in the screen. A, Validation of gene overexpression for representative ORFs. Primers only detecting endogenous transcripts were designed to flank introns, where those detecting both endogenous and exogenous transcript were inside exons. **B,** Growth curves for subcutaneous tumors with earliest onset day after injection for representative genes. **C,** Validation of gene overexpression for 26 randomly selected ORFs from the 238 negative genes of the screen using qRT-PCR. Red and blue dotted lines represent 1X and 2X fold change, respectively.

Supplementary Figure S2. PYGO2 overexpression promotes LHMK and LNCaP tumor progression. A, Copy number-correlated *PYGO2* mRNA expression in three datasets including primary and metastatic PCa. **B,** H&E stain for representative LHMK-RFP and LHMK-PYGO2 subcutaneous tumors. Scale bar, 2mm. **C,** H&E stain for representative subcutaneous tumor and associated draining lymph node filled with metastasis, formed by LNCaP-PYGO2 cells. Scale bar, 100 μ m.

Supplementary Figure S3. Effects of PYGO2 knockdown *in vitro* and *in vivo*. A, Modestly reduced 2D colony formation by PYGO2 knockdown in PC3. **B,** Significantly reduced invasion by PYGO2 knockdown in PC3 cells, detected by invasion assay. **C,** H&E stain of spontaneous lung metastasis formed from subcutaneous tumors by either PC3-shControl or PC3-shPYGO2 #860. Scale bar 5mm. **D,** PYGO2 IHC staining of spontaneous lung metastasis formed from subcutaneous tumors by PC3-shControl. Scale bar 100 μ m. **E,** Expression of PYGO2 in indicated human PCa cell lines, detected by western blot. **F,** PYGO2-targeting siRNA (1:1 ratio of SASI_Hs01_00059021 and SASI_Hs02_00363399 from Sigma, delivered by MaxSuppressor In Vivo RNA-LANCER II kit) impaired tumor growth of the PDX model MDA-PCa-180. ***P < 0.001, Student's t-test.

Supplementary Tables

Supplementary Table S1. List of primers for quantitative RT-PCR and shRNA sequences. Primers were designed to detect either endogenous transcripts only (endo), or both endogenous and ORF-expressed transcripts (endo + ORF).

Supplementary Table S2. List of genes generated from bioinformatics analyses and used to select ORFs for *in vivo* screen.

Supplementary Table S3. List of ORFs used for *in vivo* screen.

Supplementary Table S4. List of positive hits from the *in vivo* screen, shown by gene name, tumor incidence and first tumor onset day (post-injection).