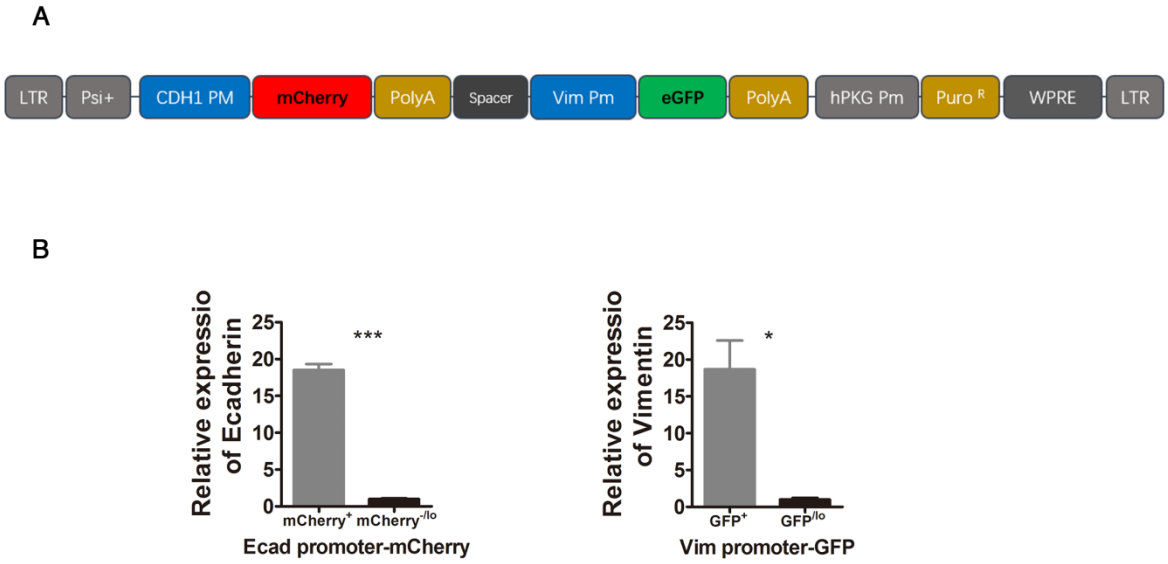
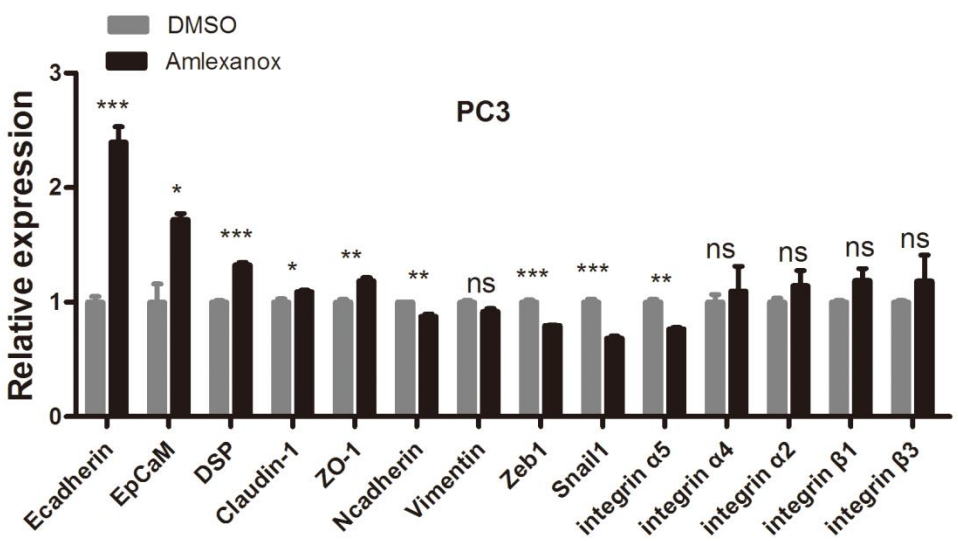
Supplementary Figures



Cheng Supplementary Fig. S1.

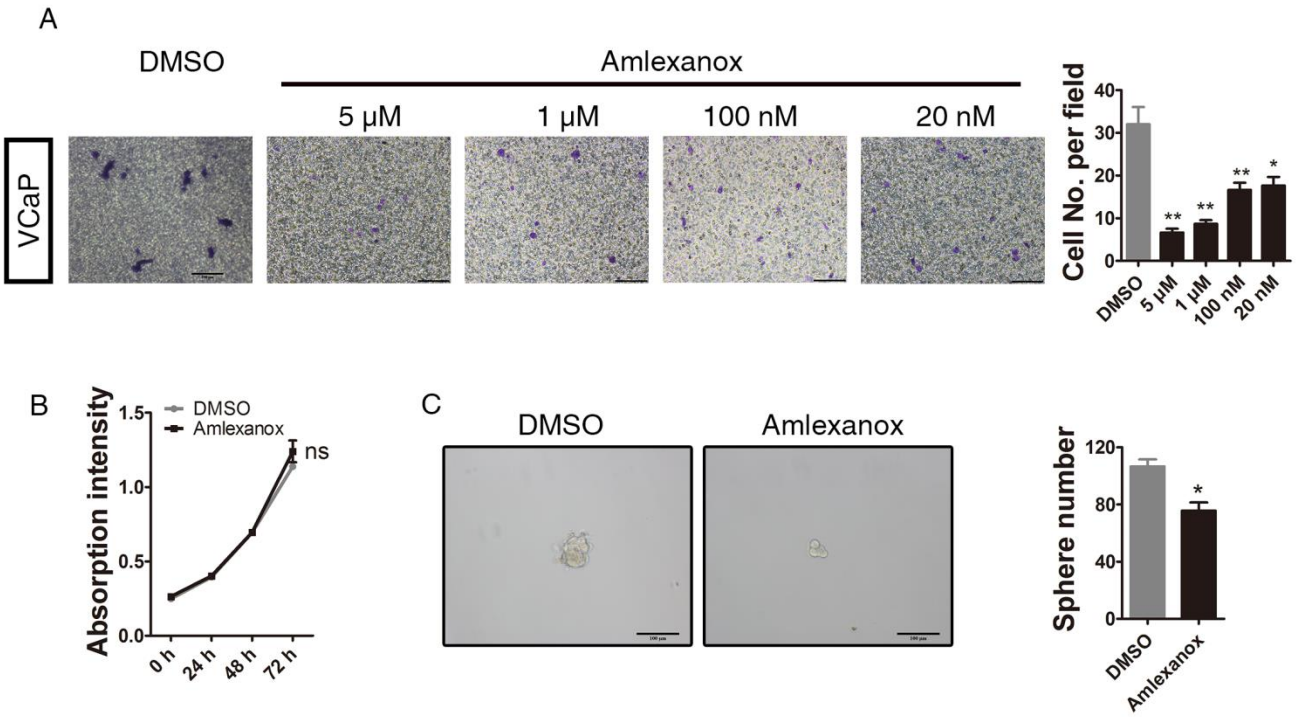
Figure S1. Validation of CDH1 and VIM gene promoters. (A) Map of the lentiviral dual-fluorescence EMT reporter plasmid in which the mCherry expression is driven by the CDH1 gene promoter, while the eGFP is driven by the VIM promoter. **(B)** qRT-PCR experiments confirm that the mCherry or eGFP fluorescent intensities are significantly correlated with endogenous expression levels of E-cadherin or Vimentin (n=3). (Unpaired t test was used for the statistical analysis. *, $P<0.05$; ***, $P<0.001$. Data are presented as means ± SEM.)

A



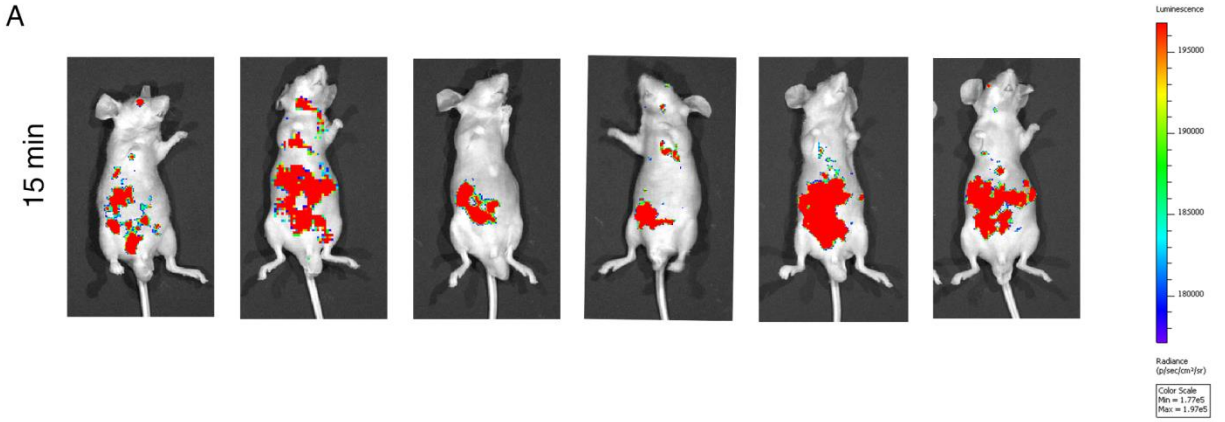
Cheng Supplementary Fig.S2

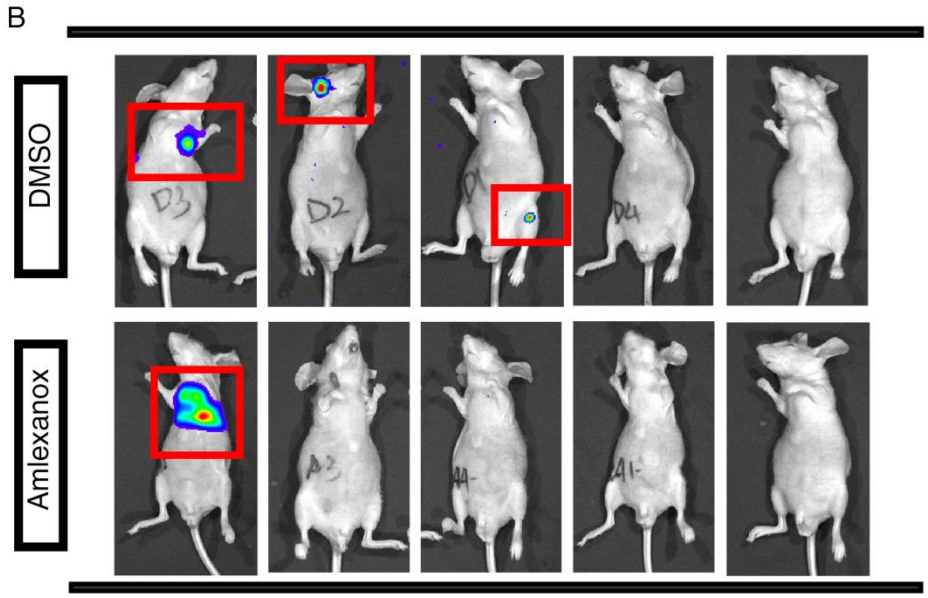
Figure S2. Amlexanox treatment leads to upregulated expression of adhesion molecules and suppression of mesenchymal genes and integrin α5 on PC3 cells. **(A)** Amlexanox treatment on PC3 cells leads to upregulated expression of adhesion molecules (EpCAM, DSP, Claudin1, ZO1 and E-cadherin) and suppression of mesenchymal genes and integrin α5. (Unpaired t test was used for the statistical analysis. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. Data are presented as means ± SEM.)

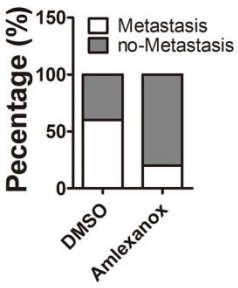


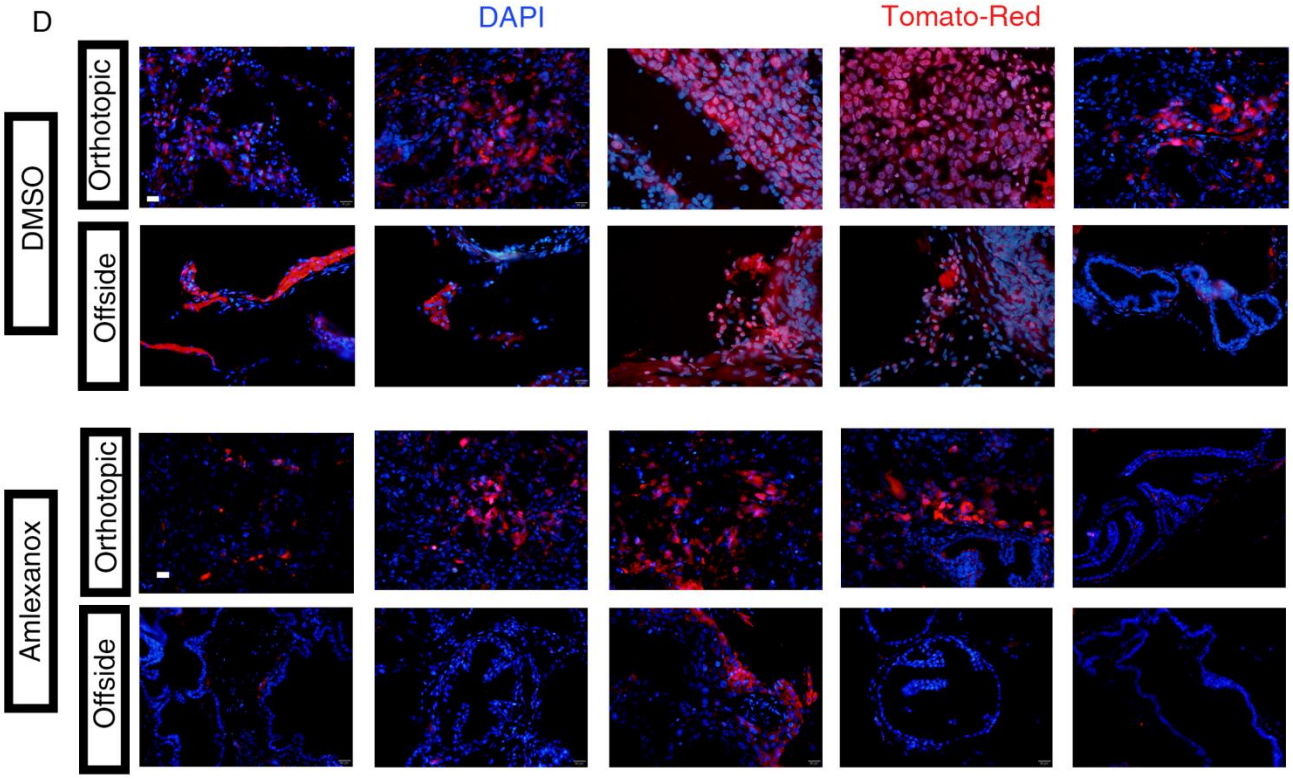
Cheng Supplementary Fig.S3.

Figure S3. Amlexanox treatment suppresses migration and sphere forming of VCaP cells *in vitro*. (A) Amlexanox treatment results in a dose dependent suppression of VCaP cell transwell migration in the Boyden chamber assay (n=3). Scale bar=100 μm. **(B)** Amlexanox does not cause a significant change of the proliferation rate of VCaP cells (n=6). **(C)** The sphere-forming capacity of VCaP cells is inhibited by Amlexanox treatment (n=3). Scale bar=100 μm. (Unpaired t test was used for the statistical analysis. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. Data are presented as means ± SEM.)



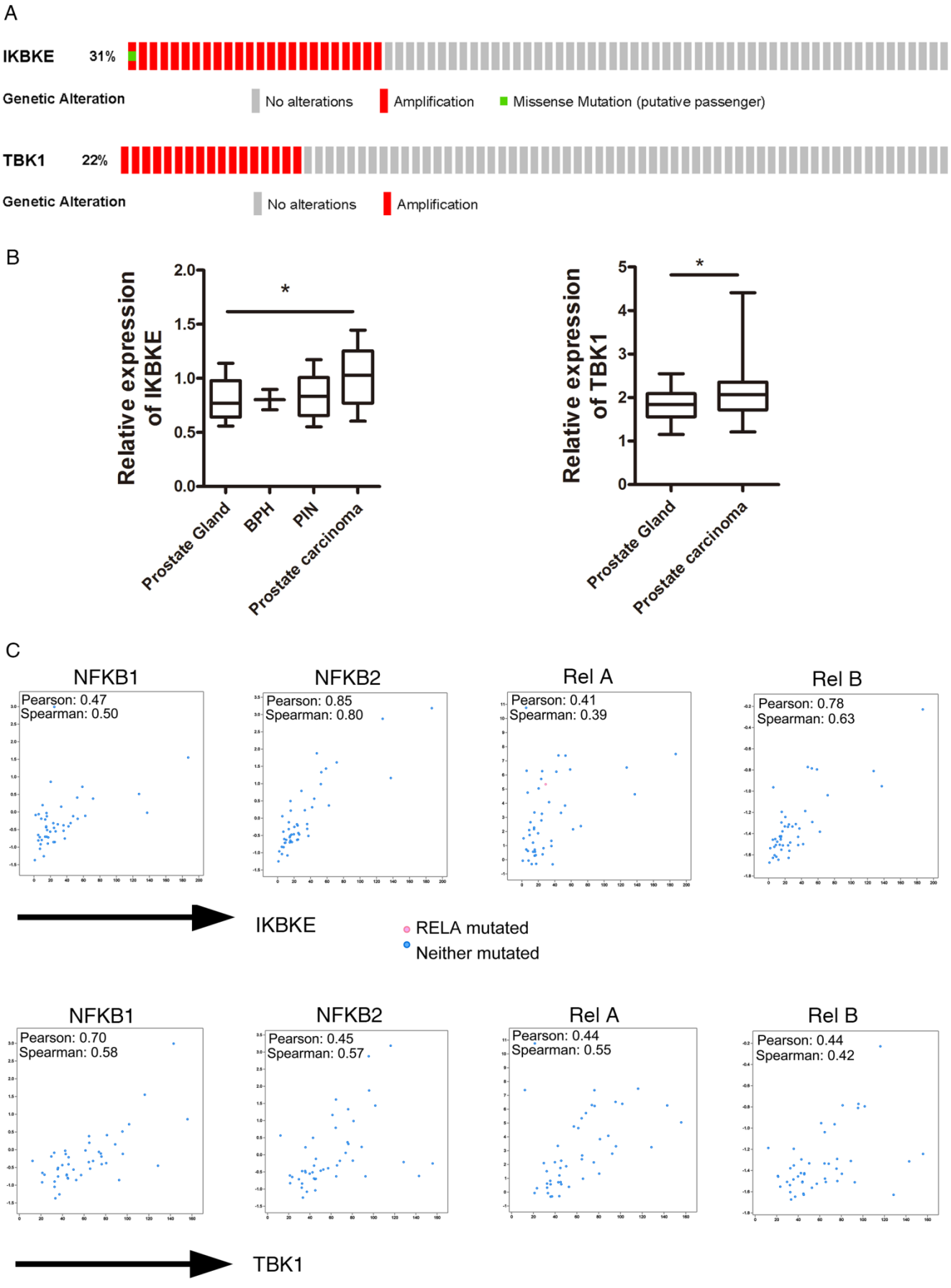






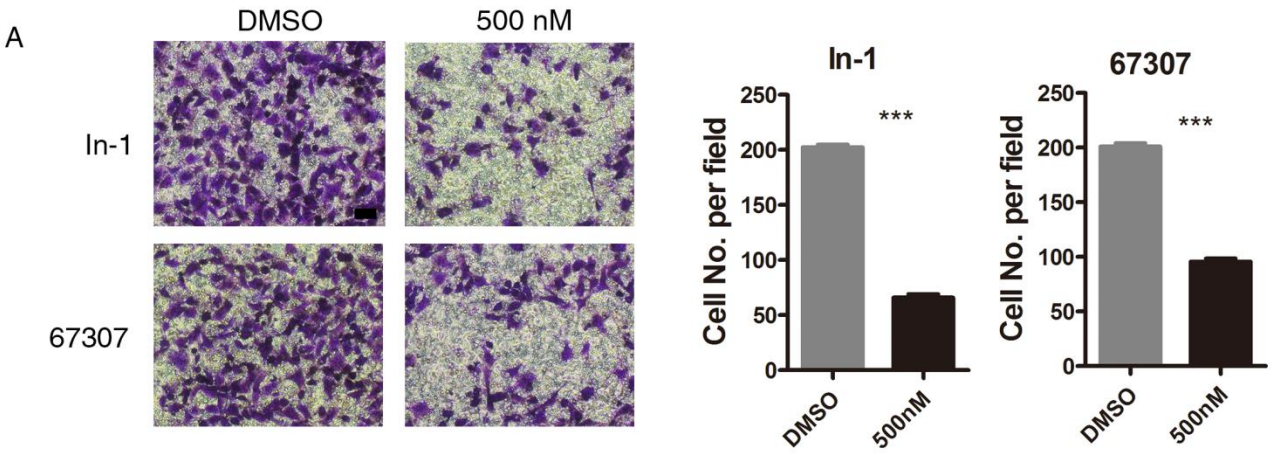
Cheng Supplementary Fig. S4

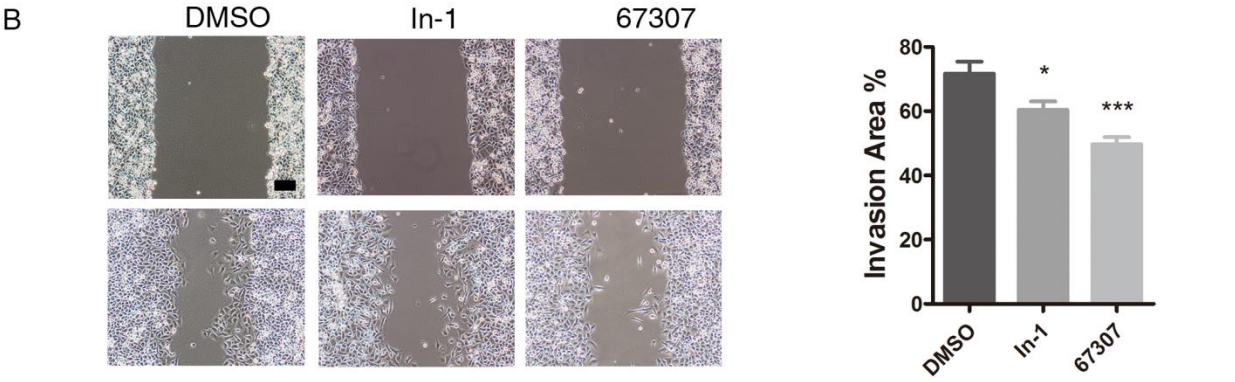
Figure S4. Amlexanox inhibits the in vivo metastatic ability of PC3-M cells without pre-treatment in vitro. (A) The recipient mice were administrated with 150mg/kg D-luciferin immediately after the intracardiac injection of PC3 cells. Bioluminescence imaging were taken 15 minutes later. Similar distribution pattern and bioluminescence intensity of PC3 cells are detected in day 1 among injected mice (n=6). **(B-C)** Untreated PC3-M cells were implanted into nude mice via intracardiac injection. Systemic Amlexanox administration suppresses the forming of tumor tumor metastasis (n=5). **(D)** Untreated tomato-Red reporter expressing PC3-M cells were injected into the left anterior lobe of nude mouse prostates. Systemic Amlexanox administration inhibits the forming of tumor metastasisthe in the offside of mouse prostates (n=6).

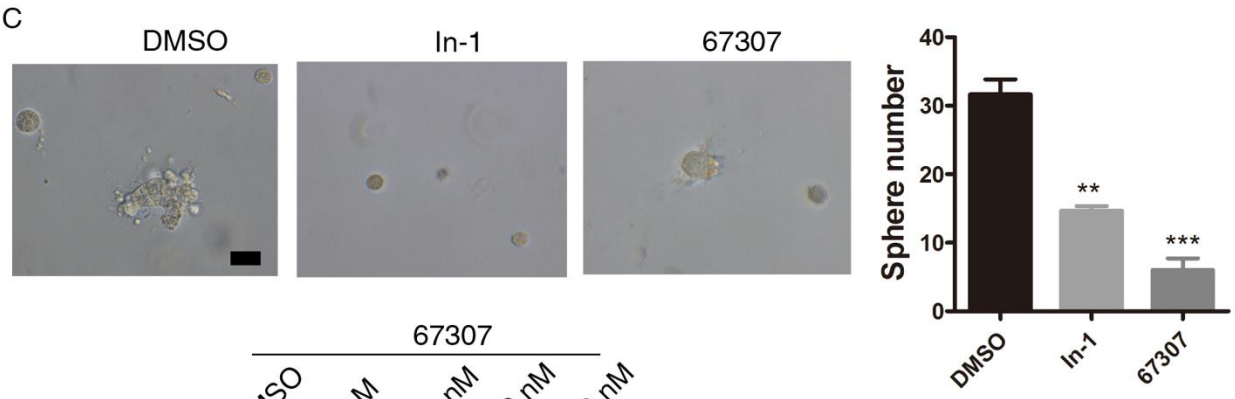


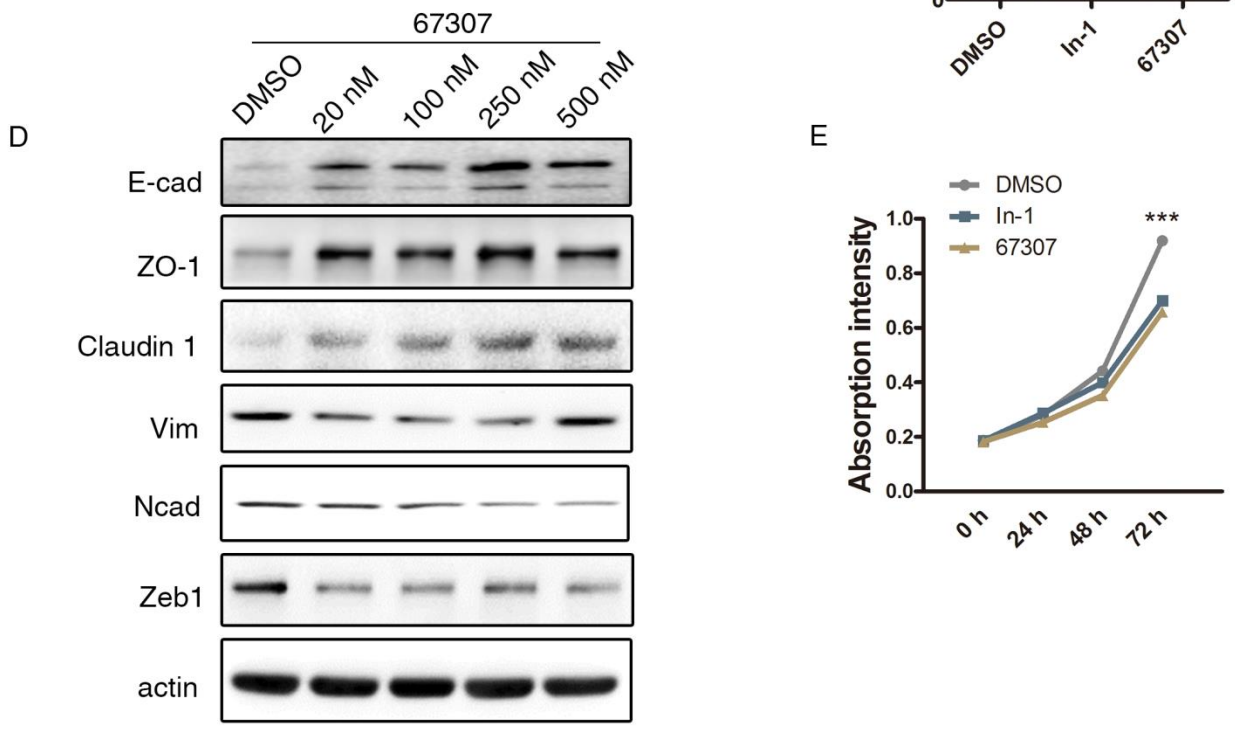
Cheng Supplementary Fig. S5.

Figure S5. Upregulation of IKKε/TBK1/NF-κB signaling axis in prostate cancers. **(A)** Amplification of IKKε and TBK1 is detected in human prostate cancer samples. Data are collected from the Trento/Cornell/Broad 2016 dataset at cbioportal (http://www.cbioportal.org/). **(B)** Transcription of IKKε and TBK1 are up-regulated in in human prostate cancer samples (Data are obtained from the Grasso Prostate Statistics at Oncomine). **(C)** Expressional correlation of IKKε, TBK1 and molecules in the NF-κB signaling pathway in human prostate cancer samples (0.3<pearson<0.5, moderate correlated; 0.5<pearson<1.0, significant correlated; Data are collected from the Trento/Cornell/Broad 2016 dataset at cbioportal (http://www.cbioportal.org/)









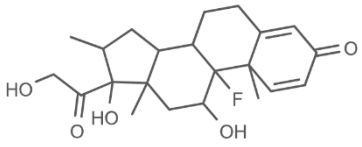
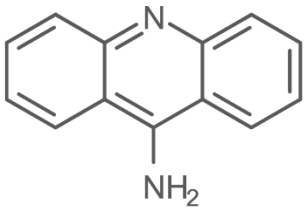
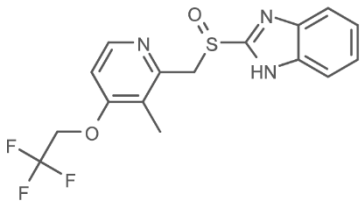
Cheng Supplementary Fig. S6.

Figure S6. IKKε/TBK1 inhibitor In-1 or 67307 treatment suppresses mobility and migration of PCa cells *in vitro*. (A) In-1 or 67307 causes a suppression of PC3 cells transwell migration (n=3). Scale bar=50 μm. **(B)** In-1 or 67307 treatment represses the mobility of PC3 cells (n=18). Scale bar=100 μm. **(C)** The sphere generation capacity of PC3 is inhibited by In-1 or 67307 treatment (n=3). Scale bar=50 μm. **(D)** IKKε/TBK1 inhibitor 67307 treatment upregulates the expression of epithelial marker E-cadherin, Claudin1 and ZO-1 and downregulate expression of mesenchymal marker Vimentin, N-cadherin and EMT transcriptional factor Zeb1. **(E)** Moderate suppressing effect by In-1 or 67307 on PC3 cell proliferation determined by the cell counting kit-8 assay (n=6). (Unpaired t test was used for the statistical analysis. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. Data are presented as means ± SEM.)

Table S1. Antibodies used for immunoblotting or immunofluorescence staining in this study.

Antibodies	Catalog
EMT Sampler Kit	CST #9782
NF-κB Pathway Sampler Kit	CST #9936
anti-IRF3	CST #4302
anti-pIRF3	CST #29047
anti-IKKε	CST #2690
anti-pIKKε	CST #8766
anti-TBK1	CST #3013
anti-pTBK1	CST #5483
Anti-Rabbit HRP	CST #7074
Anti-Mouse IgG, HRP-linked Antibody	CST #7076
Anti-Nuclei Antibody, clone 235-1	Merck MAB1281
DAB Staining kit	Gene Tech GK347010

Table S2. The structure and names of the top 4 lead compounds

	Name	Structure
Compound1	Betamethasone	
Compound2	Aminacrine	
Compound3	Lansoprazole	
Compound4	Amlexanox