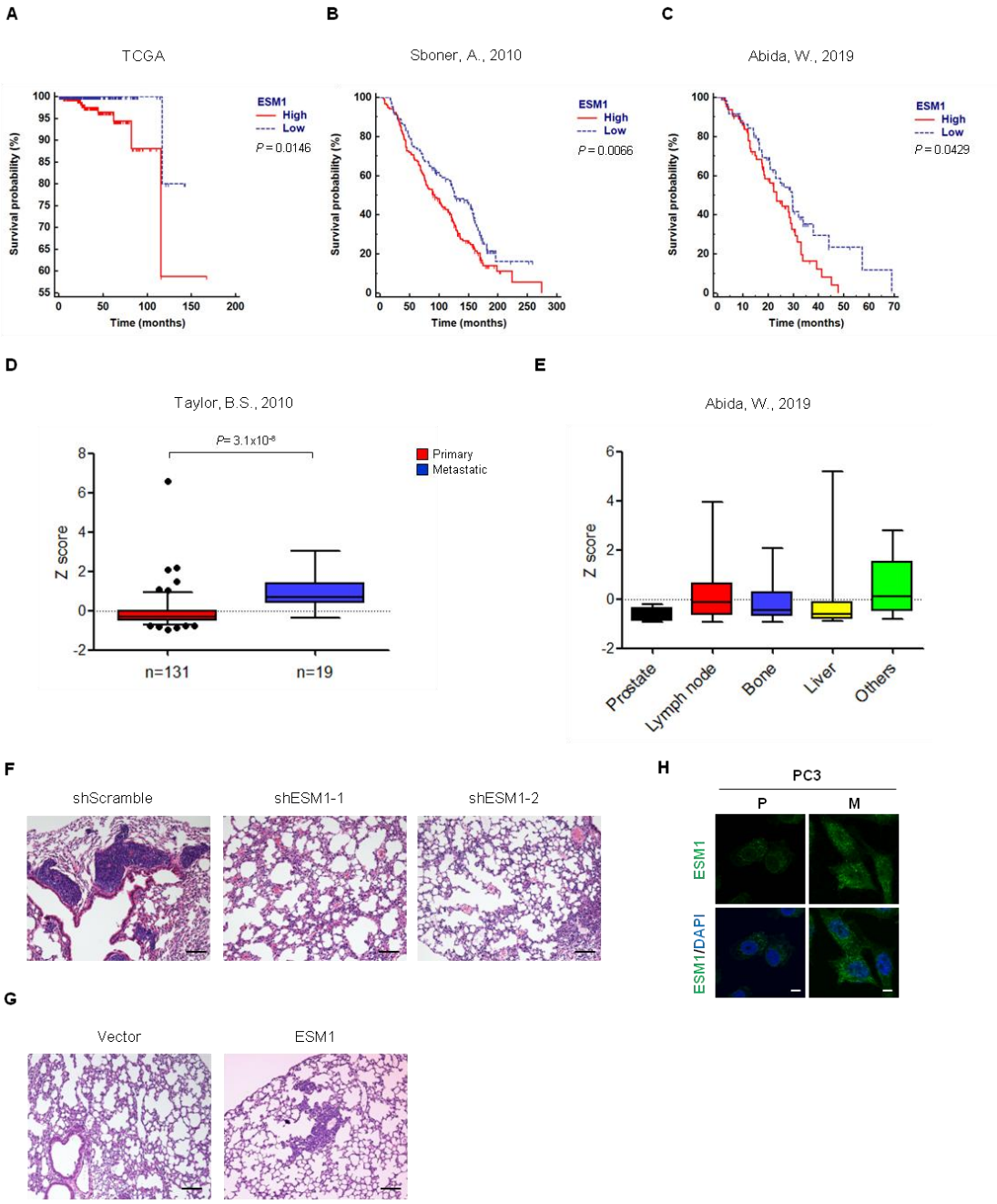
Appendix for

Direct interaction of β-catenin with nuclear ESM1 supports stemness of metastatic prostate cancer

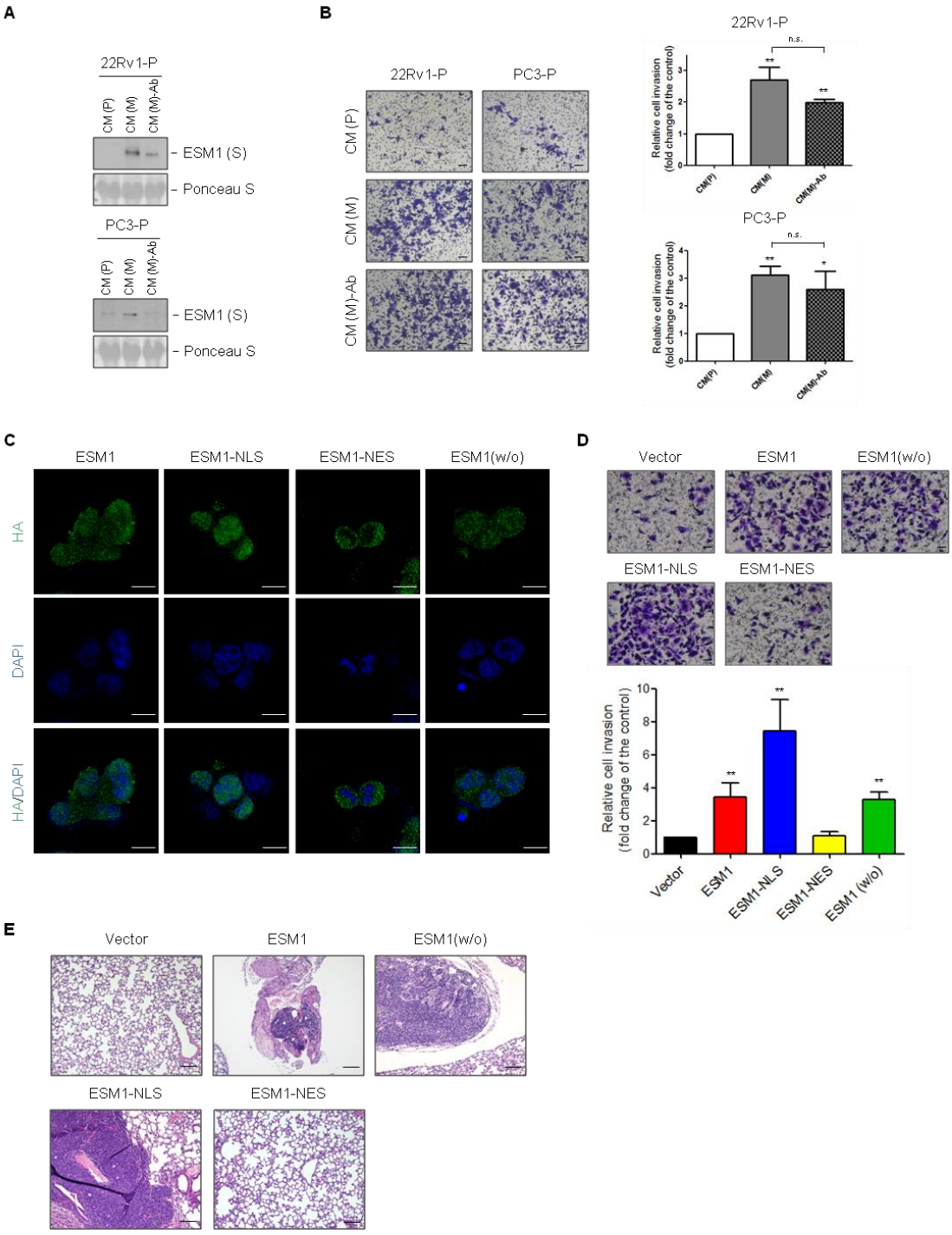
The PDF file includes:



Appendix Figure S1. High ESM1 expression in PCa is associated with shorter overall survival and metastasis.

(A to C) Graph derived from TCGA (A), published data available in the PubMed GEO database (GSE16560, B), and cBioPortal (C). For each respective analysis, 494 patients (A), 281 patients (B) or 429 patients (C) were segregated into two groups,

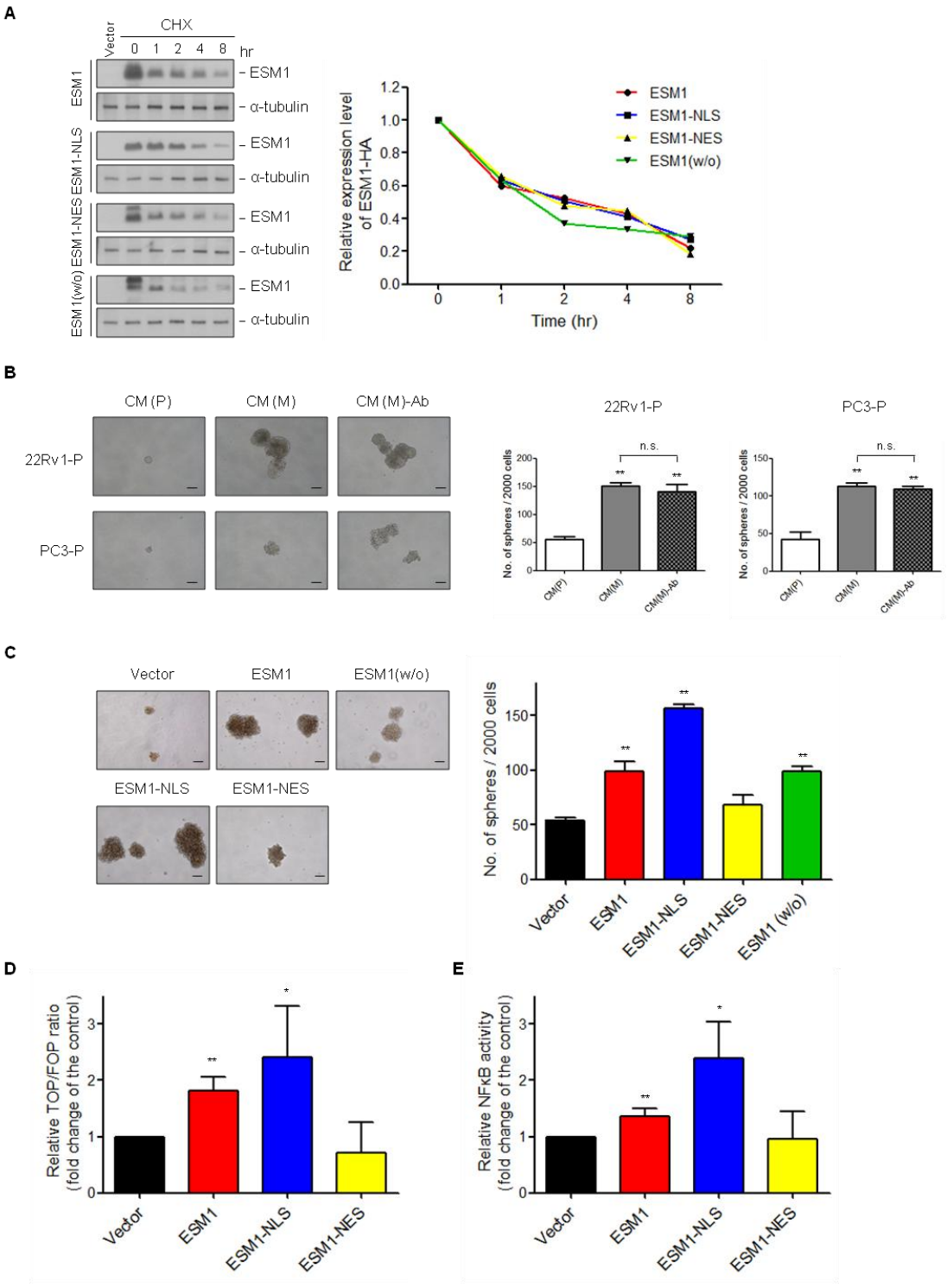
with the group designated “ESM1 high” representing the half who had tumors with higher levels of ESM1 mRNA and the group designated “ESM1 low” representing the half who had tumors with lower levels of ESM1 mRNA. Overall survival was determined by Kaplan-Meier analysis. **(D)** Graph derived from published data available in the PubMed GEO database (GSE21034). The box chart depicts the relative expression of ESM1 in primary and metastatic PCa patients. The centre line donates the median value while the box contains the 25^{th} to 75^{th} percentiles of dataset. The whiskers mark the 5^{th} and 95^{th} percentiles, and values beyond these upper and lower bounds are considered outliers. *P* value was obtained by two-tailed Student’s *t* test. **(E)** Graph derived from published data available in cBioPortal. The box chart depicts the relative expression of ESM1 in various metastatic sites among PCa patients. **(F, G)** H & E staining of lung metastatic tumors. Lungs from mice 8 weeks after orthotopical injection of 22Rv1-M cells transfected with either Scramble-shRNA or ESM1-shRNA (F). Lungs from mice 8 weeks after orthotopical injection of 22Rv1-P cells transfected with either vector control or ESM1 (G). Scale bar: 100 μm. **(H)** Representive photographs for immunofluorescence staining of endogenous ESM1 in PC3-P/M cells. Nuclei were counterstained with DAPI. Scale bar: 10 μm.



Appendix Figure S2. Nuclear ESM1, instead of secreted ESM1 is correlated with cell invasion and tumor metastasis.

(A) Immunoblotting analysis of ESM1 in supernatant (S) from parental cells, metastatic sublines with or without antibody-depletion were performed. **(B)** Left,

representative images of 22Rv1-P and PC3-P cells treated with supernatants from metastatic sublines along with ESM1-depletion in the transwell invasion assay. Scale bar: 100 μm. Right, statistic data of invasive ability of different treatment groups. **(C)** Representive photographs for immunofluorescence staining of HA in different ESM1 constucts overexpressing 22Rv1-P cells. Nuclei were counterstained with DAPI. Scale bar: 10 μm. **(D)** Upper, representative images of different subcellular localized ESM1-overexpressing PC3-P cells in the transwell invasion assay. Lower, statistic data of invasive ability of ESM1 expressing cells. **(E)** H & E staining of lung metastatic tumors in mice after tail vein injection of 22Rv1-Luc cells with different ESM1 expression patterns. Scale bar: 100 μm.



Appendix Figure S3. Nuclear ESM1 is functional in regulating the cancer stem cell population.

(A) Half-life analysis of ESM1. Different ESM1 expression plasmids were transfected

into 22Rv1-P cells, followed by cycloheximide (CHX) treatment for 0, 1, 2, 4, 8 hours. Whole-cell lysates were collected and immunoblotted with anti-ESM1 and α-tubulin antibody. **(B)** Left, representative images of 22Rv1-P and PC3-P cells treated with supernatants from metastatic sublines along with ESM1-depletion in the spheroid formation assay. Scale bar: 100 μm. Right, statistic data of the average number of spheres formed in different treatment groups. **(C)** Representative images of tumor spheres. Spheres from PC3-P cells were cultured for 7 days before counting. The histogram shows the mean numbers of spheres cultured. Scale bar: 100 μm. * $p < 0.05$, ** $p < 0.01$, and $n = 3$ biologically independent samples per group. **(D)** The transcriptional activity of β-catenin in PC3-P cells with different patterns of ESM1 overexpression. Bars are the mean ± SD of three independent experiments. **(E)** The transcriptional activity of NFκB in different patterns of ESM1-overexpressing PC3-P cells. β-catenin and NFκB promoter reporter activity was normalized by comparison with Renilla luciferase activity. (n=3 per group) * $p < 0.05$, ** $p < 0.01$ when compared to vector cells by two-tailed Student's *t* test.

Appendix Table S1. Sequences of qPCR primers

Gene	Forward primer (5’ to 3’)	Reverse primer (5’ to 3’)
ESM1	AGC AAT AAT TAT GCG GTG GAC T	ACC AAA CTC GTC ACC AAA AGG A
CD44	TGC CGC TTT GCA GGT GTA T	GGC CTC CGT CCG AGA GA
CD133	CAG TCT GAC CAG CGT GAA AA	GGC CAT CCA AAT CTG TCC TA
OCT4	CTC ACC CTG GGG GTT CTA TT	CTC CAG GTT GCC TCT CAC TC
NANOG	AAT ACC TCA GCC TCC AGC AGA TG	TGC GTC ACA CCA TTG CTA TTC TTC
ABCG2	TTA TCC GTG GTG TGT CTG GA	TTC CTG AGG CCA ATA AGG TG
SOX2	TAC AGC ATG TCC TAC TCG CAG	GAG GAA GAG GTA ACC ACA GGG
CTNNB1	CAC AAG CAG AGT GCT GAA GGT G	GAT TCC TGA GAG TCC AAA GAC AG
AXIN	CCC AAG CCC CAT AGT GCC CAA AG	CAG GGG AGG CAT CGC AGG GTC
LRP5	GCA GAG CCA CCA TCC ACA G	TCT TGC CCA TCC AAT CCA C
DKK1	CCT TGG ATG GGT ATT CCA GA	CCT GAG GCA CAG TCT GAT GA
RELA	TCA GTC AGC GCA TCC AGA CC	CAG AGC CGC ACA GCA TTC A
NFKBIA	CTC CGA GAC TTT CGA GGA AAT AC	GCC ATT GTA GTT GGT AGC CTT CA
IL6	CCT TCC AAA GAT GGC TGA AA	CAG GGG TGG TTA TTG CAT CT
IL8	ATG ACT TCC AAG CTG GCC GTG GCT	TCT CAG CCC TCT TCA AAA ACT TCT C
MyD88	GAC CCC TGG TGC AAG TAC C	AGT AGC TTA CAA CGC ATG ACA G
Actin	GGC GGC ACC ACC ATG TAC CCT	AGG GGC CGG ACT CGT CAT ACT

Appendix Table S2. Sequences of ChIP-qPCR primers

Gene promoter	Forward primer (5’ to 3’)	Reverse primer (5’ to 3’)
Ccnd1-reg. A	TAT GAA AAC CGG ACT ACA GG	CTG TTG TTA AGC AAA GAT CAA AG
Ccnd1-reg. B	GCCCATTCTGCCGGCTTGGA	GGGGTGAGGTGGAGGTGGCT
Ccnd1-reg. C	AGCGCATGCTAAGCTGAAAT	ACACGTGTAAATTGCAAGAA
Ccnd1-reg. D	TGAAGGGACGTCTACACCCC	CTGCCTTCCTACCTTGACCA
Ccnd1-reg. E	GAGGGCTTTTTCTATCAGTT	CAGGTTCTGTCTCTTTGGTG
c-myc-reg. A	TAGAGCTAGAGTGCTCGGCT	ACCCTCGCATTATAAAGGGC
c-myc-reg. B	ACTCTTGATCAAAGCGCGGC	CCTGTGAGTATAAATCATCG
c-myc-reg. C	GGTACAGACTGGCAGAGAGC	GCCAAAGCAGCAGATACCGC
c-myc-reg. D	GGAAAGAGGACCTGGAAAGG	GGGACCGGACTTCCTAAAAG
c-myc-reg. E	TGGGCAACTAGCTAAGTCGA	GGGGGAGTCTTGAGCTAATT