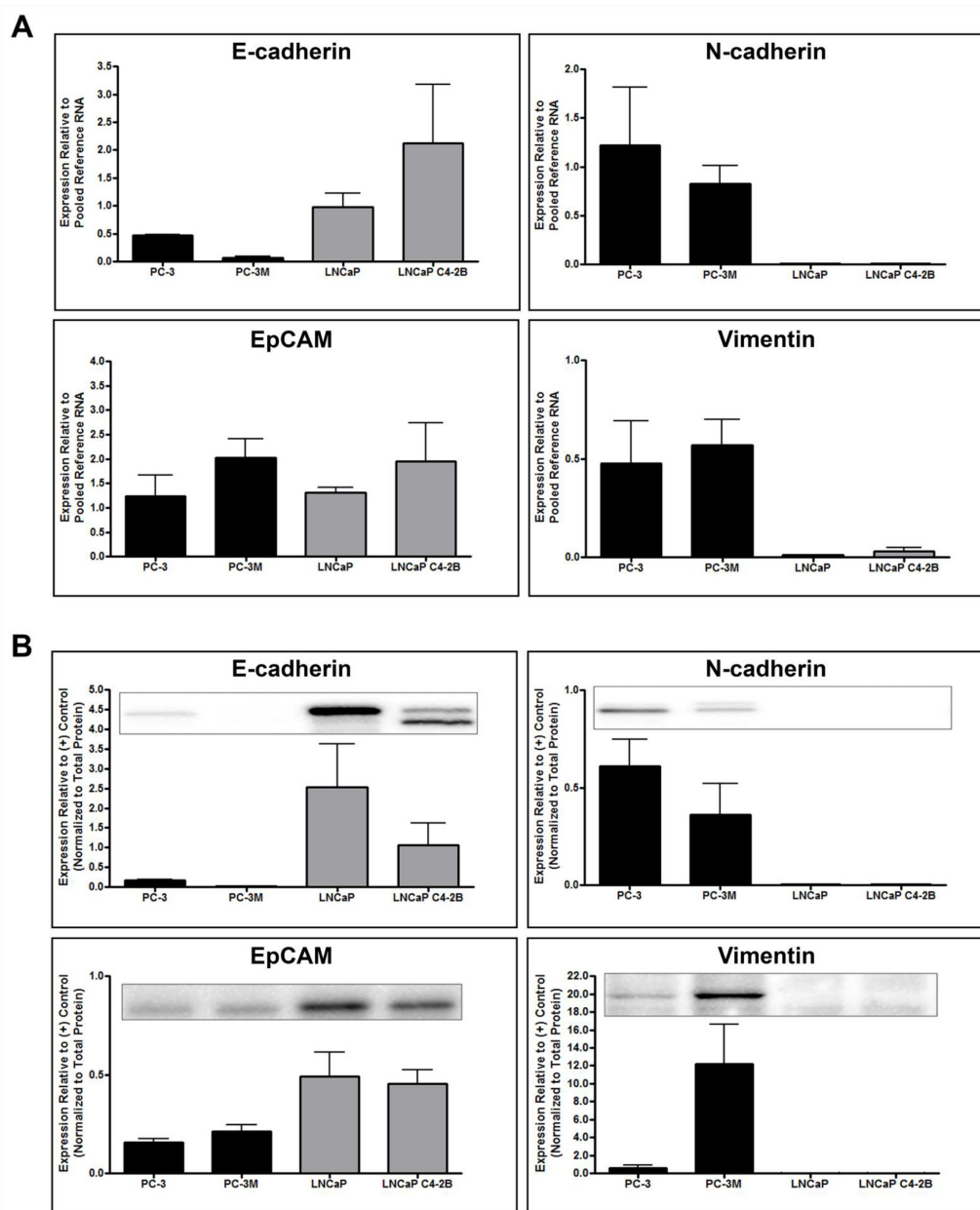
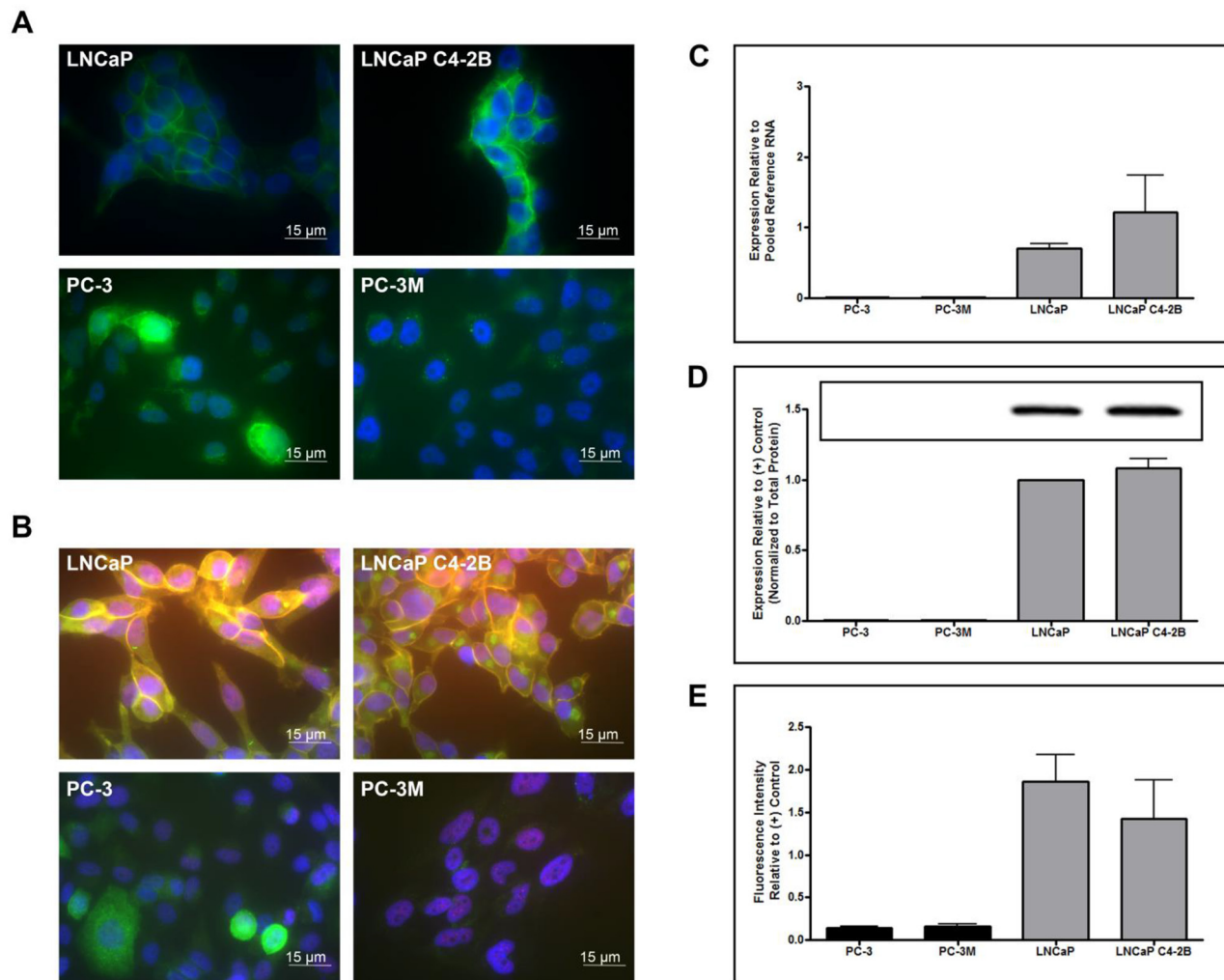


Epithelial-to-mesenchymal transition leads to disease-stage differences in circulating tumor cell detection and metastasis in pre-clinical models of prostate cancer

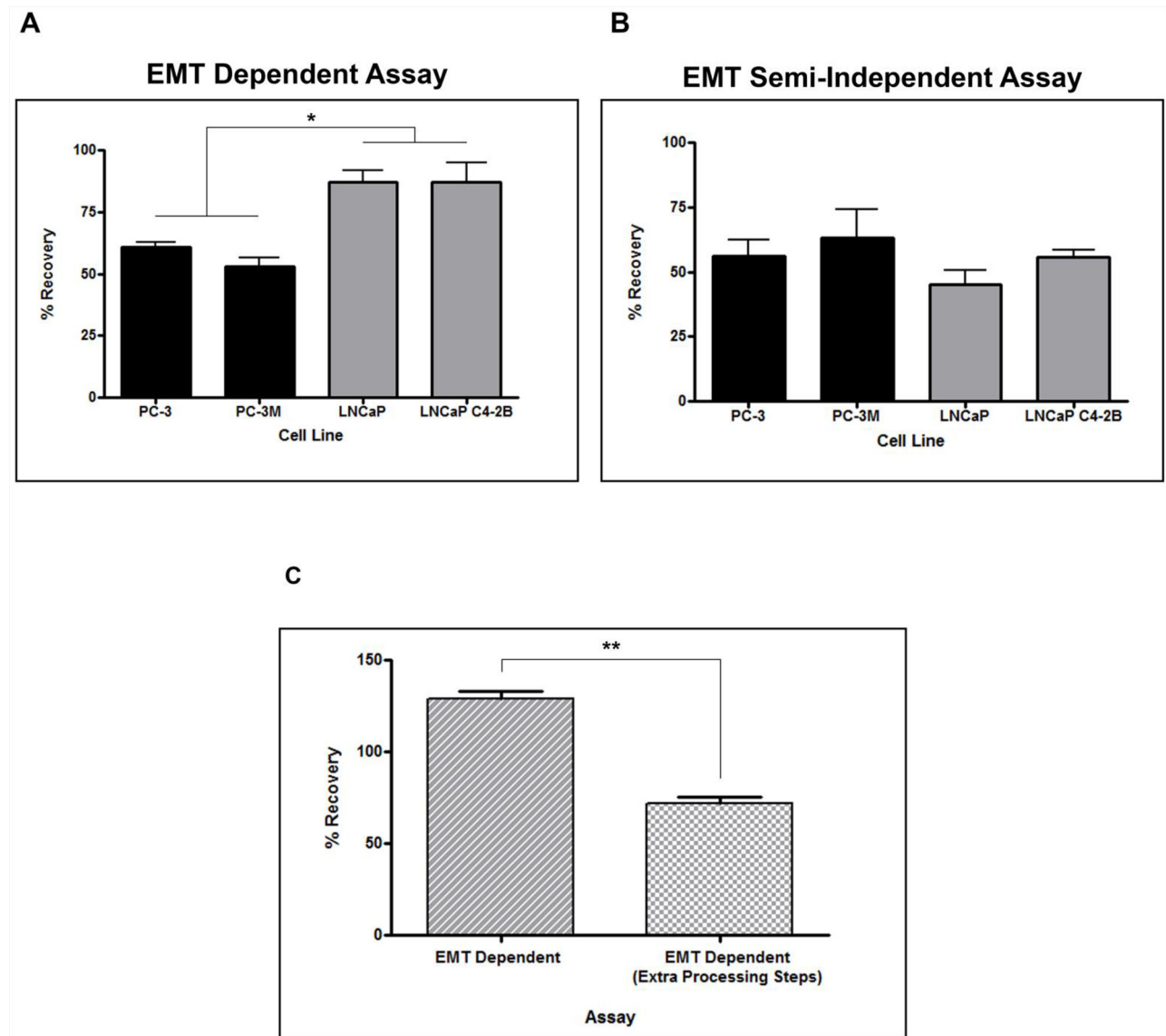
SUPPLEMENTARY FIGURES AND TABLES



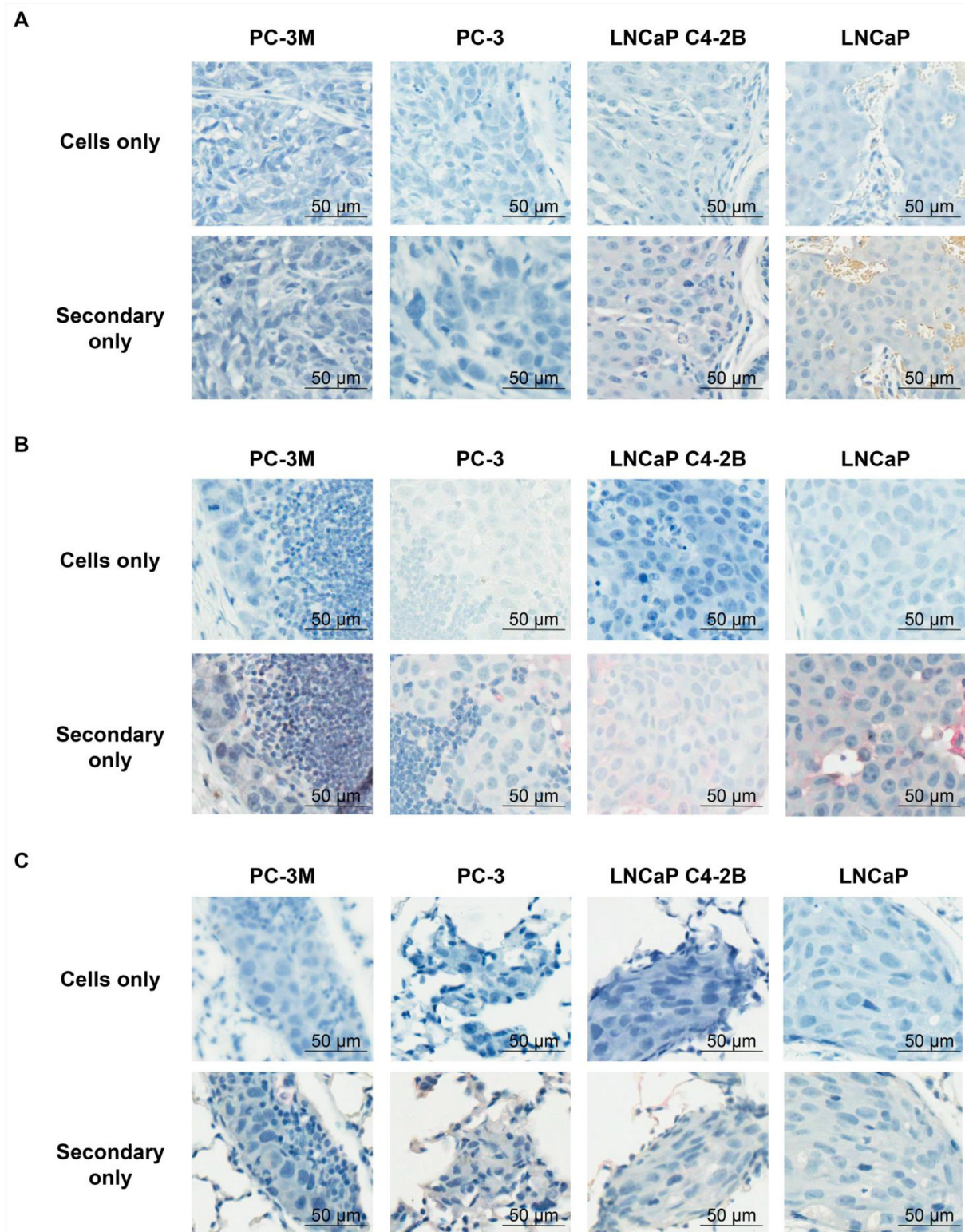
Supplementary Figure S1: Human prostate cancer cell lines display differences in epithelial-to-mesenchymal transition (EMT) phenotype at the RNA and protein level. A-B. The expression of epithelial-associated markers E-cadherin and EpCAM and mesenchymal-associated markers N-cadherin and vimentin is associated with previously reported cell aggressiveness and *in vivo* metastatic capacity of the human prostate cancer cell lines PC-3, PC-3M (black bars), LNCaP and LNCaP C4-2B (grey bars). (A) RT-qPCR analysis of mRNA expression; data presented as relative expression (mean \pm SEM) compared to pooled reference RNA (n=3). (B) Immunoblot analysis of protein expression; presented as quantitative densitometric data (mean \pm SEM) relative to appropriate positive control cell lines and normalized to total protein loaded as assessed by amido black staining, and as representative immunoblots shown as cropped gel images (n=3).



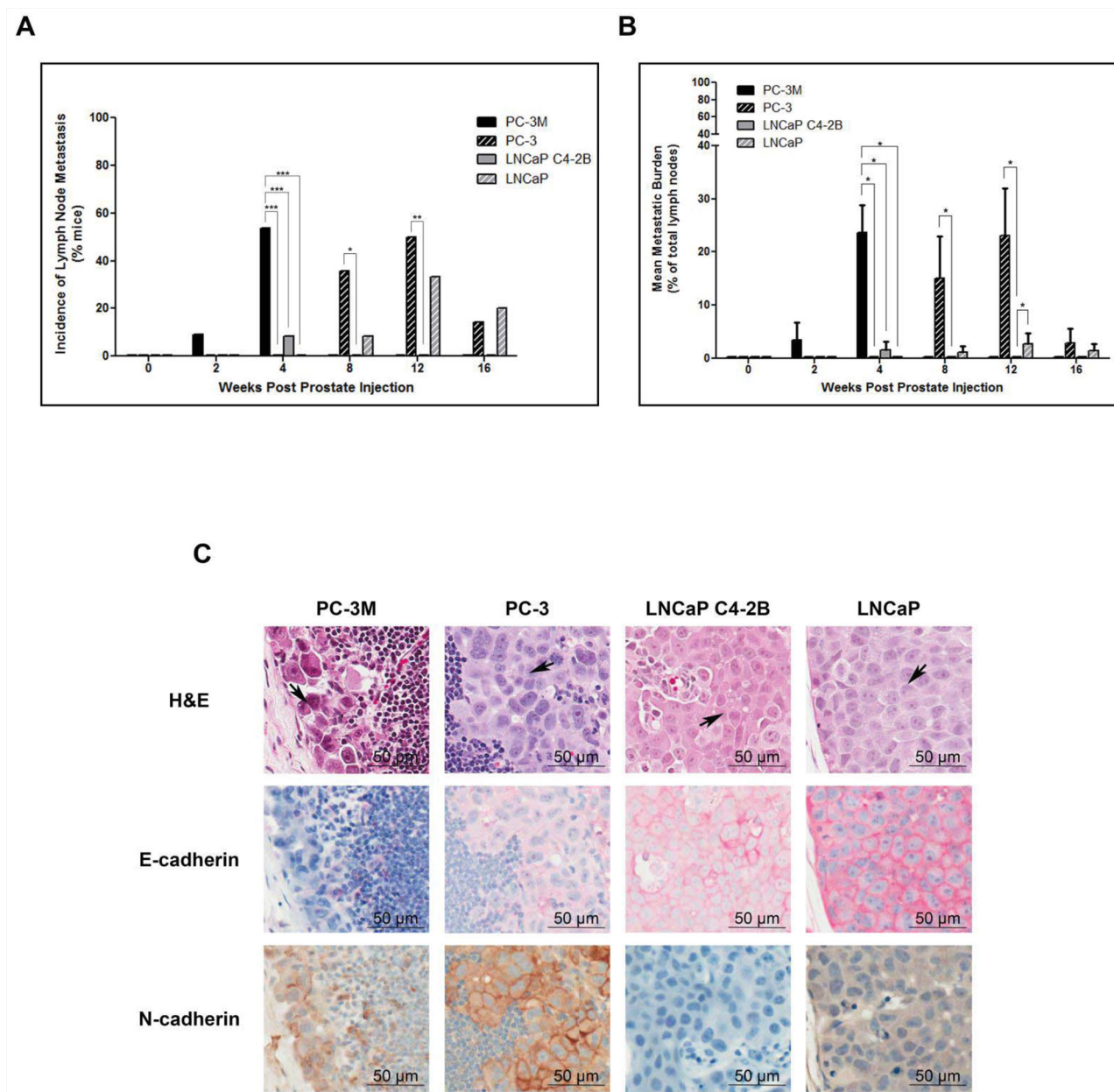
Supplementary Figure S2: E-cadherin cell membrane localization is aberrant in human prostate cancer cell lines that do not express α -catenin. **A.** Representative immunofluorescent images of E-cadherin (*green*) and DAPI (*blue*) stained PC-3, PC-3M, LNCaP, and LNCaP C4-2B cell lines cultured on glass chamber slides. **B.** Representative immunofluorescent images of co-localization (*yellow*) of E-cadherin (*green*) and α -catenin (*red*) in PC-3, PC-3M, LNCaP, and LNCaP C4-2B cell lines cultured on glass chamber slides (DAPI [blue]). Images were obtained at 60x magnification, scale bars = 15 μ m (n=3). **C.** RT-qPCR analysis of mRNA; data presented as relative expression (mean \pm SEM) compared to pooled reference RNA (n=3). **D.** Immunoblot analysis of protein expression; presented as quantitative densitometric data (mean \pm SEM) relative to an appropriate positive control cell line and as a representative immunoblot, shown as a cropped gel image (n=3). **E.** Flow cytometry analysis; presented as relative fluorescence intensity (expression; mean \pm SEM) compared to an appropriate positive control cell line (n=3).



Supplementary Figure S3: CTC recovery using the CellSearch® system is reduced in human prostate cancer cells with a mesenchymal phenotype. Prostate cancer cells were counted by hemocytometer and spiked at a concentration of 1,000 tumor cells/50 μ l of whole mouse blood. 50 μ l of mouse blood was subsequently processed using 1 of 2 mouse-adapted protocols and CTC recovery was measured as a percentage recovery of the number of spiked cells. **A.** CTC recovery using the EMT-dependent assay is significantly lower in cells with a more mesenchymal and metastatic phenotype. **B.** CTC recovery using the EMT semi-independent assay is lower relative to the EMT dependent assay. However, equivalent recovery is observed across all 4 cell lines regardless of EMT phenotype. **C.** Reduced recovery observed when using the EMT semi-independent assay was further investigated and determined to be as a result of extra processing steps required in this protocol. Data are presented as the mean \pm SEM (n=3). Analysis of differences in CTC recovery using the 2 assays was performed using 1-way ANOVA with Tukey's post-test for multiple comparisons. * = significantly different ($p \leq 0.05$).



Supplementary Figure S4: Cells only and secondary antibody controls for IHC. Cells only and secondary only controls for IHC performed on **A.** primary tumors, **B.** lymph node metastases, and **C.** lung metastases. Histological sections are presented at 40x magnification. Scale bars=50 μ m.



Supplementary Figure S5: The mesenchymal PC-3M and PC-3 cell lines exhibit a greater incidence of lymph node metastases and mean lymph node metastatic burden. **A.** Incidence of lymph node metastasis in mice injected with PC-3M, PC-3, LNCaP C4-2B, and LNCaP prostate cancer cell lines based on microscopic histological examination of formalin fixed, H&E stained tissue following orthotopic injection. Data are presented as the percentage of mice per cell line per timepoint with detectable lymph node metastases (n=7-39 mice/group). **B.** Quantitative analysis of tumor burden (mean % of lymph nodes occupied by tumor) following orthotopic injection of prostate cancer cell lines. Data are presented as the mean ± SEM (n=7-39 mice/group). Differences in the incidence of lymph node metastasis were assessed using Fisher’s Exact Test. Differences in metastatic burden to the lymph nodes were assessed by Wilcoxon Score followed by a Kruskal-Wallis test. * = significantly different (p≤0.05). **C.** Representative H&E and IHC (E-cadherin and N-cadherin) images of lymph node metastases for each investigated cell line. Histological sections are presented at 40x magnification. Arrowheads on H&E images indicate regions of tumor within the given tissue. Scale bars=50 μm.

Supplementary Table S1: Forward and reverse primers used for qPCR analysis

Target Gene	Forward Primer (5' → 3')	Reverse Primer (5' → 3')
E-cadherin	TGCTGATGCCCCCAATACCCCA	GTGATTTCCTGGCCCACGCCAA
N-cadherin	TGACTCCAACGGGGACTGCACA	AGCTCAAGGACCCAGCAGTGGGA
EpCAM	CGACTTTTGCCCGCAGCTCAGGA	GGGCCCCCTTCAGGTTTTGCTCT
Vimentin	AACCAACGACAAAGCCCGCGTC	TTCCGGTTGGCAGCCTCAGAGA
α -catenin	CCACGTTTTACTGAGCAAGT	AGTCAGAGTCATCCAACCTCC
GAPDH	TCCATGGCACCGTCAAGGCTGA	GCCAGCATCGCCCCACTTGATT

Sequence specific primers were designed based on gene sequence information from the National Center for Biotechnology Information (NCBI; Bethesda, MD).

Supplementary Table S2: Anti-human antibodies used for immunoblot analysis

Target Protein	Clone	Commercial Source	1° Ab Host	kDa	1° Ab Conditions
N-Cadherin	EPR1791-4	Abcam	Rabbit	100	1:1,000 (1 hr @ RT)
E-Cadherin	36/E-cadherin	BD Biosciences	Mouse	120	1:20,000 (1 hr @ RT)
Vimentin	V9	Millipore	Mouse	60	1:1,000 (1 hr @ RT)
EpCAM	E144	Abcam	Rabbit	39	1:1,000 (1 hr @ RT)
α -catenin	EP1793Y	Abcam	Rabbit	100	1:50,000 (1 hr @ RT)
β -Actin	Polyclonal	Sigma	Rabbit	42	1:5,000 (1 hr @ RT)

Supplementary Table S3: Anti-human antibodies used for flow cytometry analysis

Target Protein	Clone	Commercial Source	1° Ab Amount per 5×10^5 cells
N-Cadherin	EPR1791-4	Abcam	0.40 μ g
E-Cadherin	36/E-cadherin	BD Biosciences	0.80 μ g
Vimentin	V9	Millipore	0.80 μ g
EpCAM	E144	Abcam	0.15 μ g
α -catenin	EP1793Y	Abcam	0.35 μ g
CK 8/18/19	Polyclonal	Janssen Diagnostics (CellSearch)	100 μ l