Reduced *Zeb1* Expression in Prostate Cancer Cells Leads to an Aggressive Partial-EMT Phenotype Associated with Altered Global Methylation Patterns

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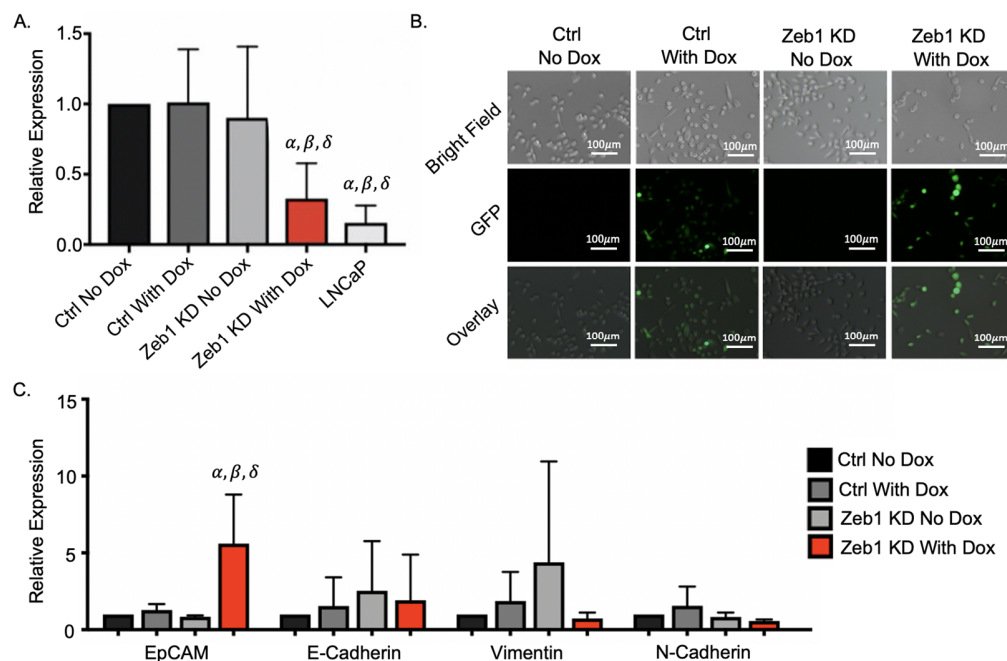
Supplemental Figure S1. *Zeb1* RNA can be inducibly knocked down in PC-3 human prostate cancer cells. Mesenchymal human PC-3 prostate cancer cells were engineered to knockdown expression of the master EMT regulator Zeb1 using the SMARTvector inducible lentiviral shRNA system (Dharmacon), which features Tet-on® induction of the target shRNA in the presence of Doxycycline (Dox). **(A)** qRT- PCR analysis of *Zeb1* mRNA expression in the presence of absence of Dox in Zeb1[KD] or Ctrl cells, or LNCaP cells. **(B)** Immunofluorescence of Zeb1[KD] and Ctrl cells ± Dox. Scale bars = 100 μm. **(C)** qRT- PCR analysis of *EpCAM*, *E-Cadherin*, *Vimentin*, and *N-Cadherin* mRNA expression in Zeb1[KD] or Ctrl cells ± Dox. Data is presented as the mean ± SEM (n=3). α= significantly different than PC-3 Ctrl no Dox. β= significantly different than Ctrl with Dox. δ= significantly different than Zeb1[KD] no Dox ($p \le 0.05$).
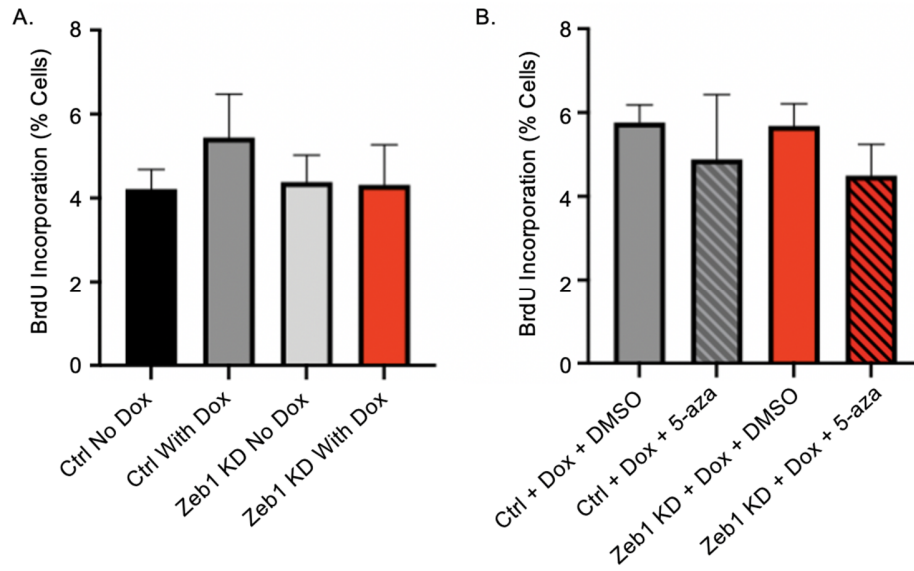
Supplemental Figure S2. Knockdown of *Zeb1* in PC-3 prostate cancer cells does not alter cell proliferation. Cells (1.6x10^4/well) were seeded on 8-well chamber slides with or without Dox, DMSO, and/or 5uM 5-azacytidine (5-aza), a global demethylating agent. Cells were serum starved for 3 days and then treated with media containing fetal bovine serum albumin for 24h. Cells were then treated with BrdU for 30 min and formalin fixed. Cells were incubated with a BrdU antibody overnight and visualized using 5 high-powered fields of view (FOV) using DAPI mounting media. **(A)** BrdU incorporation of Ctrl and Zeb1^{KD} cells ± Dox. **(B)** BrdU incorporation of Ctrl and Zeb1^{KD} cells + Dox ± DMSO or 5-aza (5uM). Data is presented as the mean ± SEM (n=3).
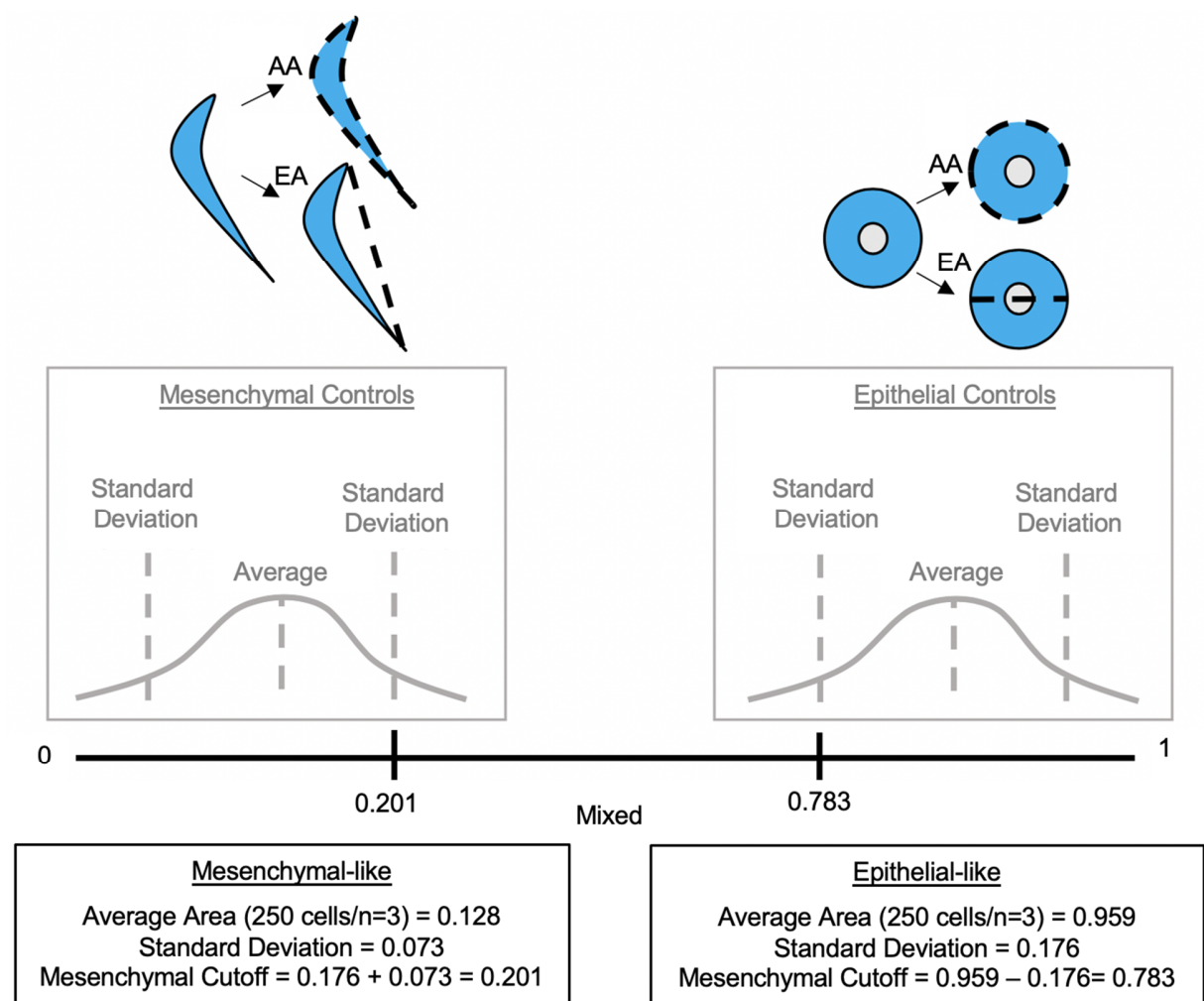
Supplemental Figure S3: Cell morphology assay calculations. Epithelial (MDA-MB-468 breast cancer cells) and mesenchymal (primary lung fibroblasts) control cells were assessed for cell shape (250 cells, n=3). The average was calculated and the "cut-off" points for a round (epithelial) cell and an elongated (mesenchymal) cell were calculated by subtracting/adding the standard deviation from/to the average. Zeb1[KD] and Ctrl cells were then analyzed for cell shape (250 cells, n=3). Any value within the mesenchymal "cut-off" (0-0.201) was considered mesenchymal-like, any value within the epithelial "cut-off" (0.783-1) was considered epithelial-like, and any value which fell in between (0.202-0.782) was considered mixed.

Supplemental Table S1. Primary antibodies for immunoblotting.

Target Protein	1° Host	kDa	1° Conditions (Overnight @ 4℃)	2° Conditions (1 hour @ room temperature)	Reduced?	Poly/Mono Clonal
Zeb1 (Cell Sig.- 3396)	Rabbit	200	1:500	1:1000	Yes	Mono (D80D3)
EpCAM (Abcam- ab32392)	Rabbit	39	1:1000	1:2000	Yes	Mono (E144)
E-Cadherin (BD Biosciences- 610181)	Mouse	120	1:2000	1:2000	Yes	Mono (36)
N-Cadherin (Abcam- ab76011)	Rabbit	100	1:1000	1:2000	Yes	Mono (EPR1791-4)
Vimentin (Millipore-MAB3400)	Mouse	60	1:1000	1:2000	Yes	Mono (V9)
P-Cadherin (Abcam- ab137729)	Rabbit	91	1:1000	1:2000	Yes	Poly
Integrin $\beta4$ (Abcam- ab29042)	Mouse	202	1:1000	1:1000	Yes	Mono (M126)
Actin (Sigma- A2006)	Rabbit	42	1:5000	1:5000	Yes	Poly

Supplemental Table S2. Forward and reverse primers used for qRT-PCR

Target Gene	Forward Primer (5′ → 3′)	Reverse Primer (5′ → 3′)
Zeb1	AGCACTTGTCTTCTGTGTGATG	CAGGCTTCTCAGCTTCTGCT
EpCAM	CGACTTTTGCCGCAGCTCAGGA	GGGCCCCTTCAGGTTTTGCTCT
E-Cadherin	TGCTGATGCCCCCAATACCCCA	GTGATTTCCTGGCCCACGCCAA
N-Cadherin	TGACTCCAACGGGGACTGCACA	AGCTCAAGGACCCAGCAGTGGA
Vimentin	AACCAACGACAAAGCCCGCGTC	TTCCGGTTGGCAGCCTCAGAGA
P-Cadherin	AAGTGCTGCAGCCAAAGACAGA	AGGTAGACCCACCTCAATCATCCTC
Integrin $\beta4$	GCTTCACACCTATTTCCCTGTC	GACCCAGTCCTCGTCTTCTG
GAPDH	TCCATGGCACCGTCAAGGCTGA	GCCAGCATCGCCCCACTTGATT