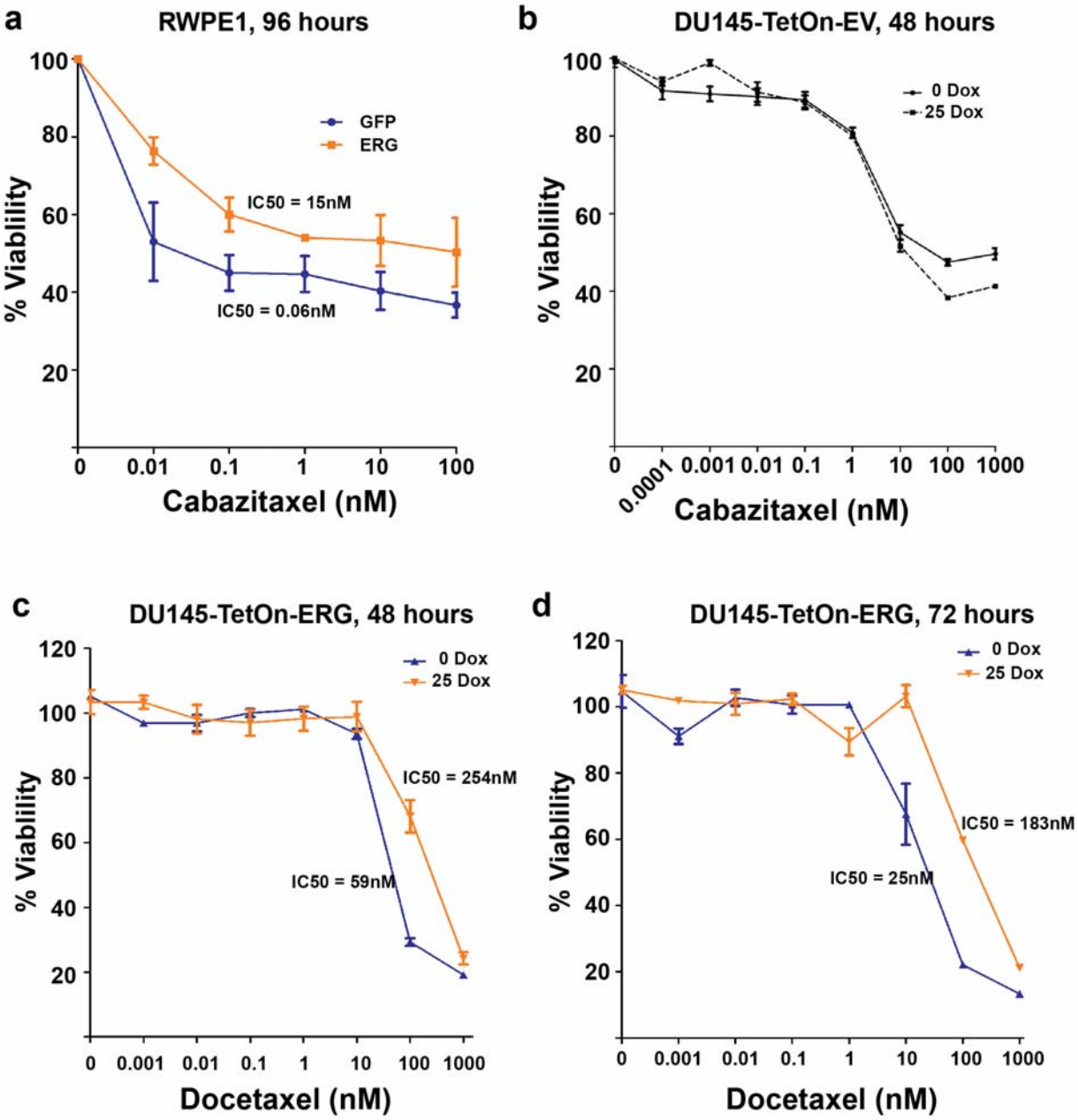
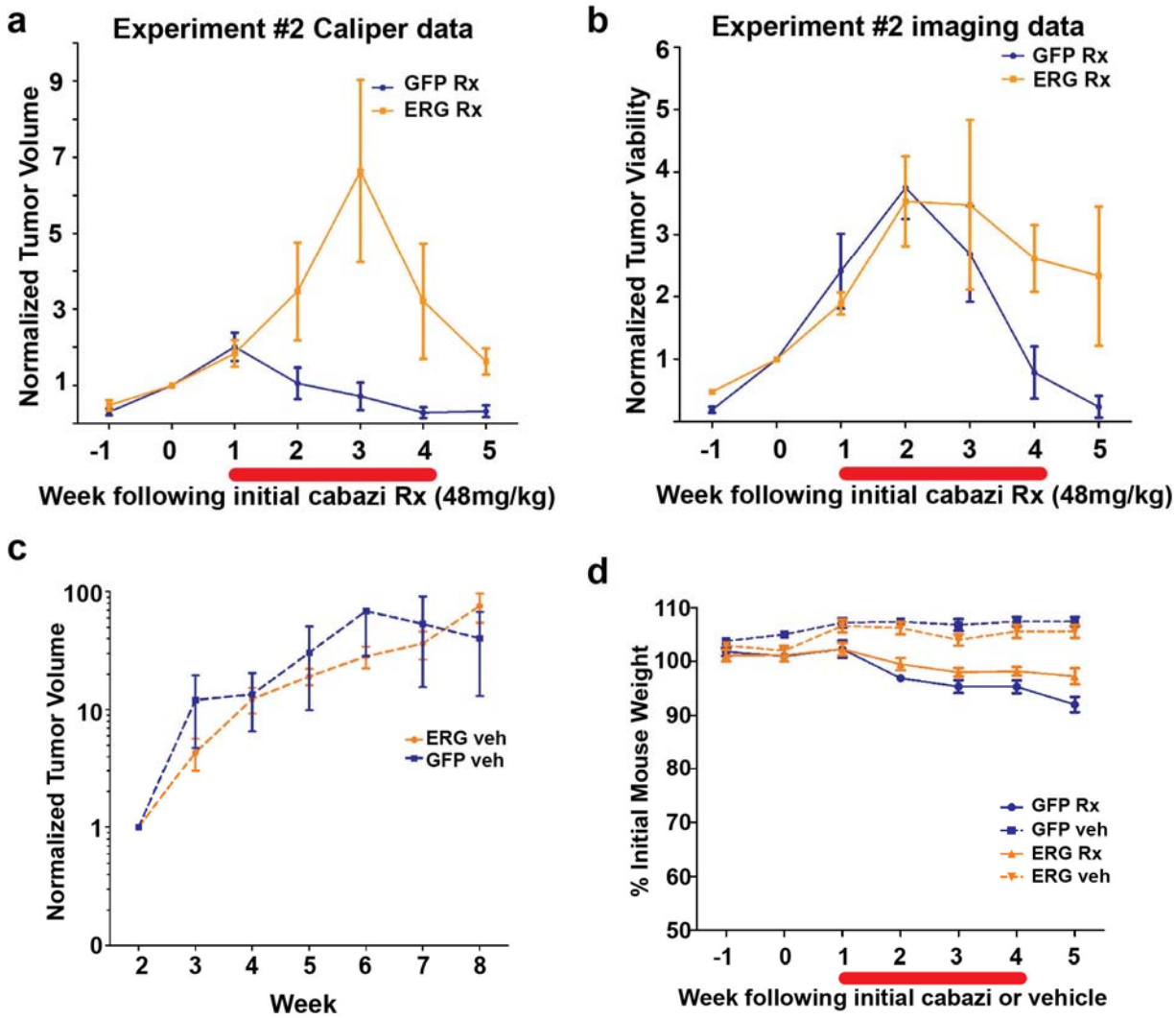
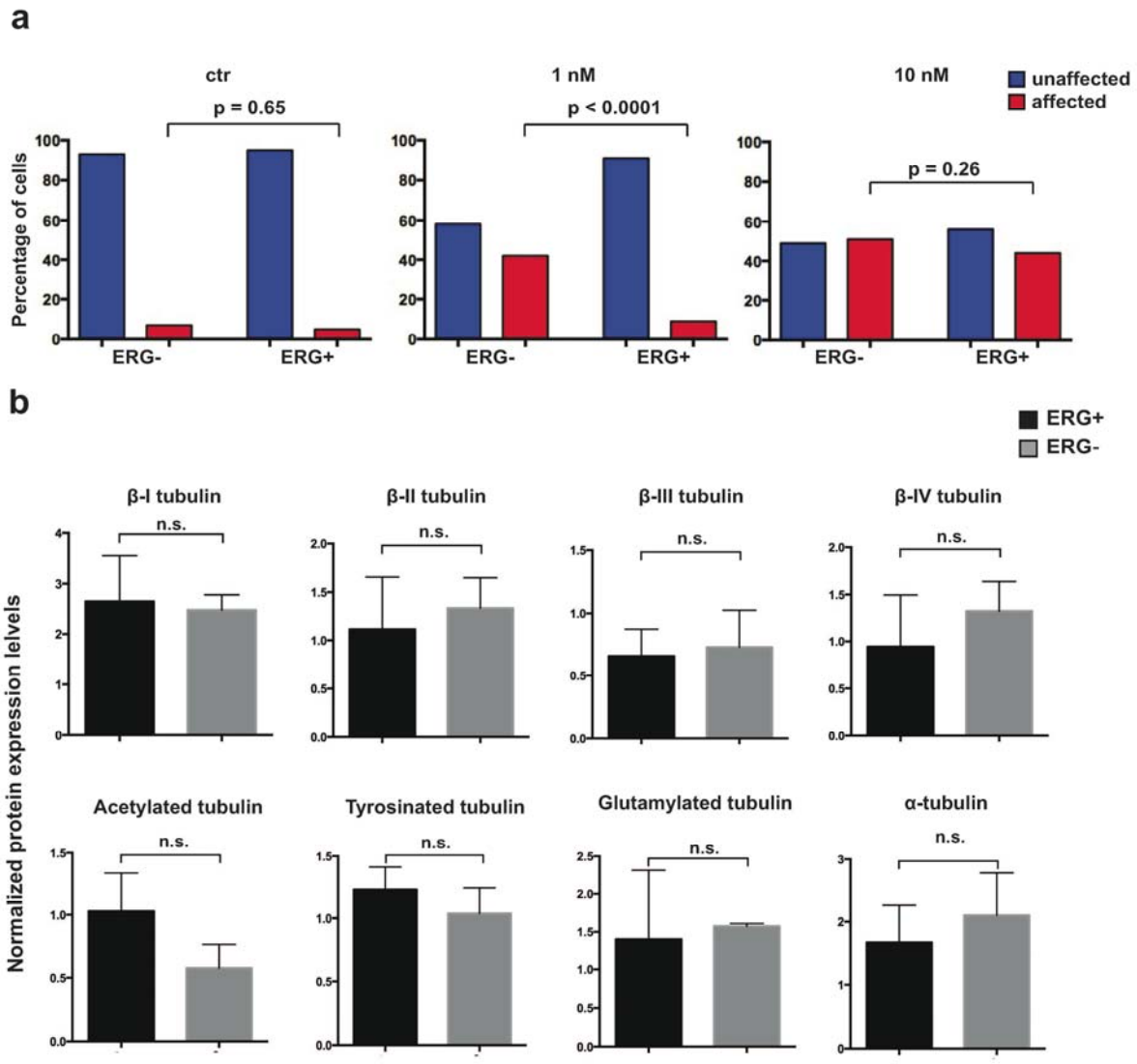


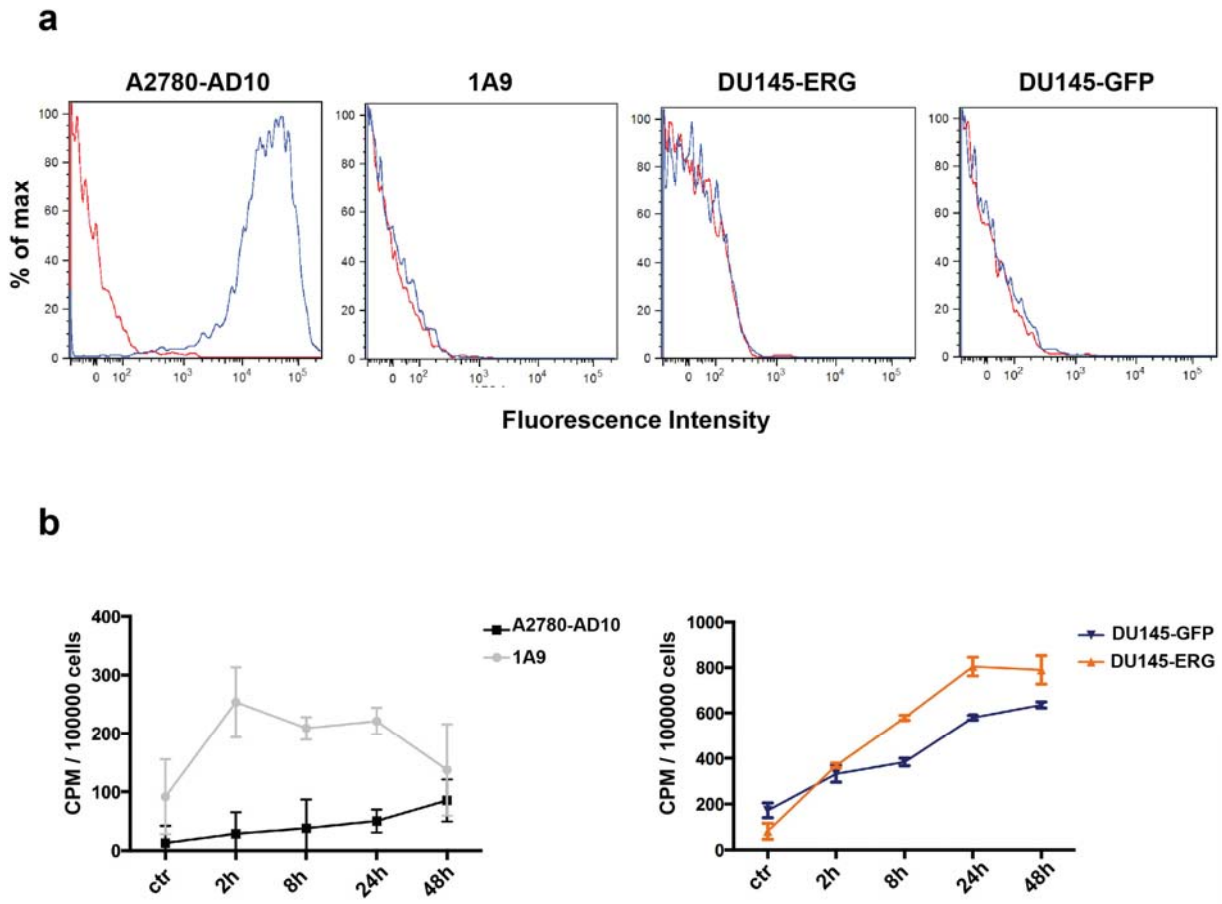
Supplementary Figure 1. Cytotoxicity assays of RWPE1 clones stably over-expressing ERG (orange) or GFP (blue) (**A**[1]) or DU145-TetOn-empty vector (EV) following 96 or 48 hr of cabazitaxel treatment, respectively. Inset, ERG immunoblot in Du145-ERG clones stably expressing ERG-targeting shRNA. Tubulin was used as loading control (**B**, there is no inset for RWPE1) (**D)** Apoptosis induction (caspase 3/7 activity) 24 hours following cabazitaxel treatment of DU145 cells stably expressing GFP (blue) or ERG (orange). Values are normalized to vehicle-treated cells. **(E)** Cabazitaxel anti-proliferative activity in DU145 with inducible ERG expression (Tet-ON-ERG). Cell viability results were obtained from a 48 hr treatment with cabazitaxel following a 2 day-exposure to the indicated doses of doxycycline (Dox) Inset: immunoblot showing ERG protein expression in response to increasing doses of Dox. Cells with endogenous (VCaP) or stable ERG overexpression (Du-ERG) are shown as controls. **(F)**. Docetaxel antiproliferative activity following 72 hr of drug treatment in DU145-TetOn-ERG cells versus control DU145-TetOn-EV cells.



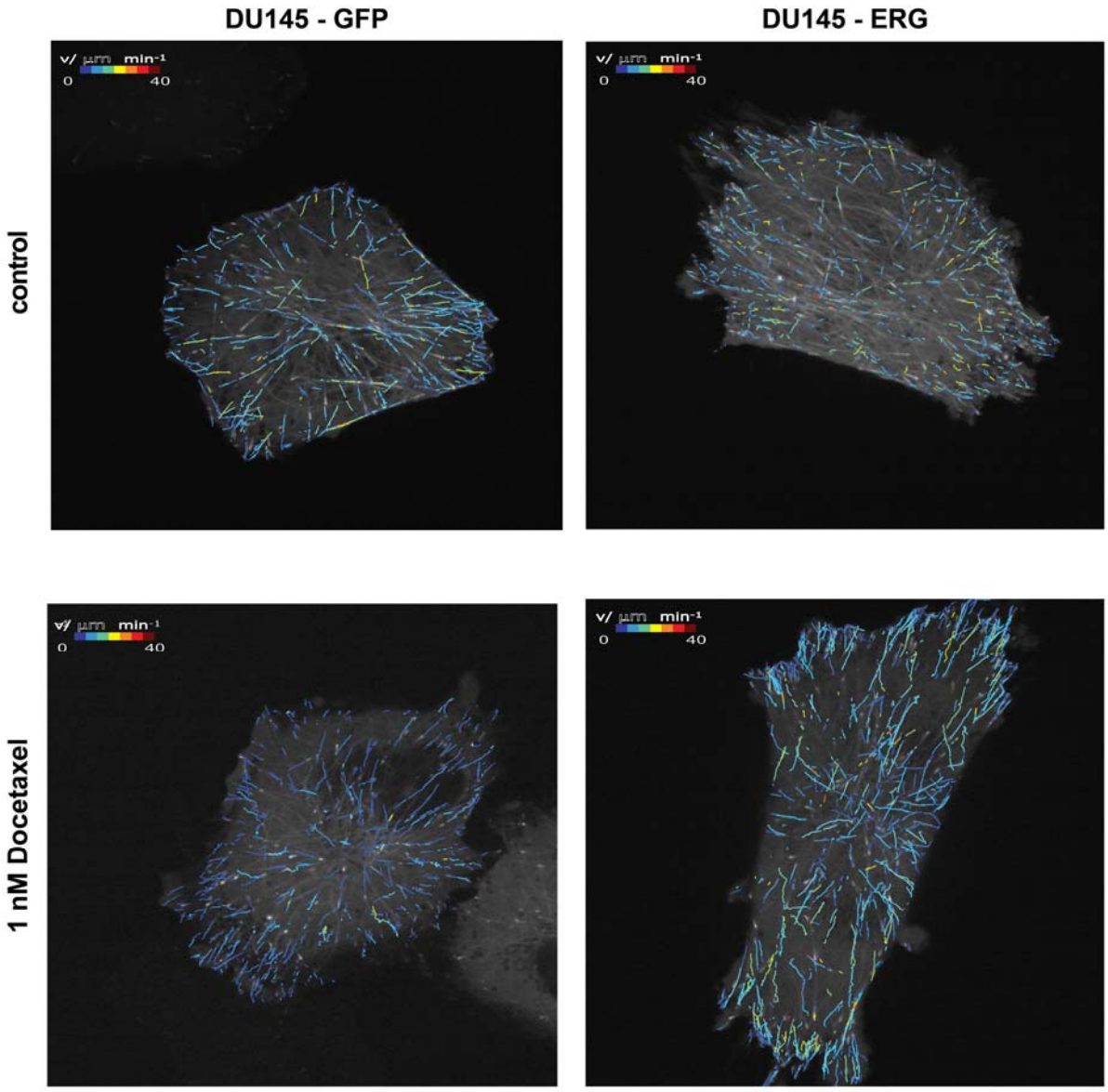
Supplementary Figure 2. Mouse weight (**B)** or tumor assessment **(C-E)** from DU145-ERG (orange) or DU145-GFP (blue) tumor xenograft volume assessment (average +/- error) before, during and after treatment with 48mg/kg cabazitaxel (solid lines) or vehicle (dashed lines) at the indicated week following cell subcutaneous injection (n = 5 mice/group). **C, left.**



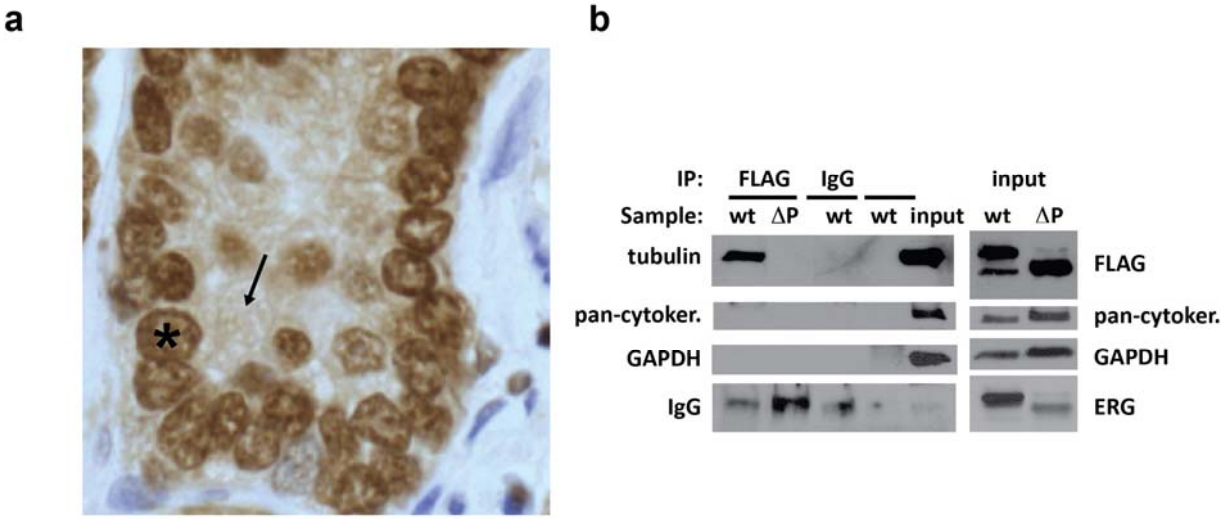
Supplementary Figure 3. (a) Quantification of immunofluorescence drug target engagement in ERG-positive and ERG-negative DU145 cells. Cell percentage and p values calculated from Fischer exact tests are shown graphically for each condition. Cells arrested in mitosis or with visible MT bundling are considered cabazitaxel affected and thus grouped together (red bars), unaffected cells are depicted in blue bars. **(b)** Quantification of tubulin isotypes and post-translational modification expression levels in ERG-positive (black bars) and ERG-negative (grey bars) DU145 cells. Densitometry was performed with Image J software (National Institute of Health, Bethesda, MD, USA) and data were analyzed using Graphpad software. SD showed as error bars. Statistical significance was determined using t-test provided by GraphPad software.



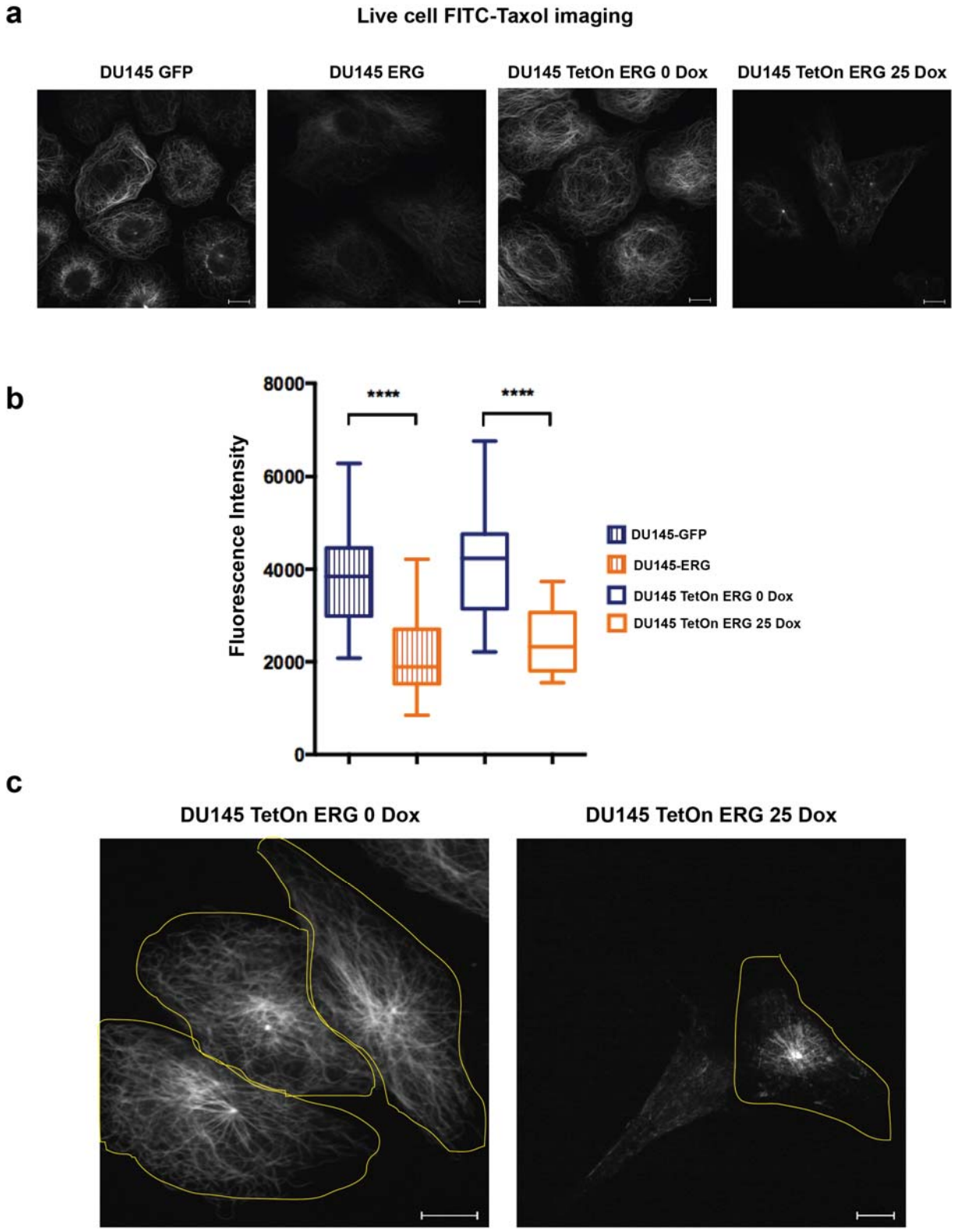
Supplementary Figure 4. Evaluation of the MDR phenotype in ERG-positive and ERG-negative DU145 cells. **(a)** Assessment of protein expression levels of P-glycoprotein (P-gp) in DU145-ERG and DU145-GFP cells by flow cytometry. Ovarian cancer cell lines 1A9 and the isogenic P-gp over-expressing cell line A2780-AD10 were used as negative and positive control for P-gp protein expression, respectively. P-gp protein expression levels in ERG-positive cells are similar to background levels and to the levels detected for the isogenic ERG-negative cell line. Blue: P-gp; red: IgG isotype control. **(b)** Intracellular drug accumulation assay using C-14 radiolabeled cabazitaxel in DU145-ERG and DU145-GFP cells. Intracellular levels of the radiolabeled drug in DU145-ERG are equivalent (2h time point) or even superior (8-48h time points) to the values documented for the DU145-GFP cells.



Supplementary Figure 5. (a) Representative images of DU145 ERG- and ERG+ cells infected with EB1-EGFP. The bright comets are clusters of EB1 dimers that bind the tips of MTs during growth. MT growth tracks are for DU145-ERG or DU145-GFP cells in the presence of 1nM docetaxel or vehicle are shown. Color-coded computer-vision tracking of MT dynamics shows speed of MT growth with warmer colors indicating high speed rates and colder colors indicating low speed rates. Inset shows corresponding EB1 comet velocity histograms of the number of tracks per micron/minute speed.



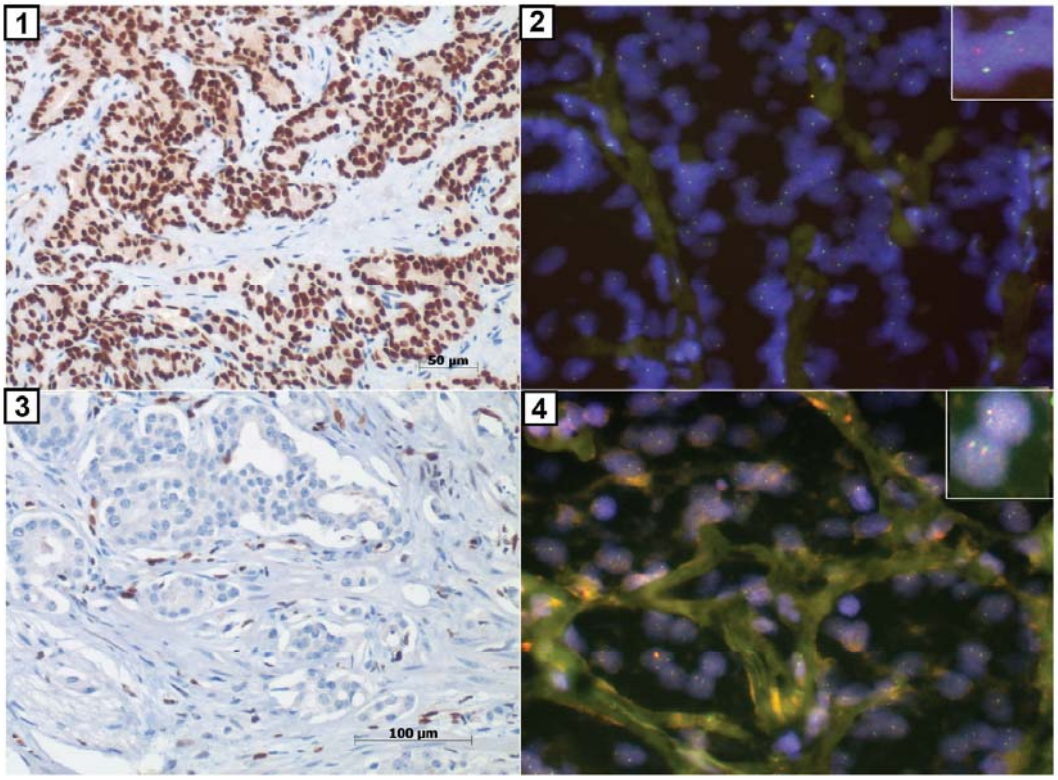
Supplementary Figure 6. (a) ERG immunohistochemistry images from of a tissue microarray with locally advance prostate cancer specimens. The top 2 images correspond to tissue from 2 different patients that were characterized with ERG rearrangements and that are positive for high levels of ERG protein levels. Boxes indicate the area that is shown at higher magnification below. To the right is an example of ERG presence in the nucleus (*****) and in the cytoplasm (arrow). To the left is an example of weak to little ERG cytoplasmic localization with strong nuclear localization. **(b)** Co-immunoprecipitation (IP) of whole cell extracts from DU145-GFP 72 hours following transient transfection with the indicated Flag-tagged wild type or pointed domain deletion (ΔP) ERG constructs anti-Flag or anti α-tubulin antibody followed by immunoblot for the indicated proteins. Isotype-matched IgGs were used to control for non-specific binding to the beads used for immunoprecipitation. Equal amounts of input lysate were also immunoblotted in the same fashion and shown to the left of the IP blot. Western blot images have been cropped for presentation. Full size images are presented in supplementary **Fig. S11**.



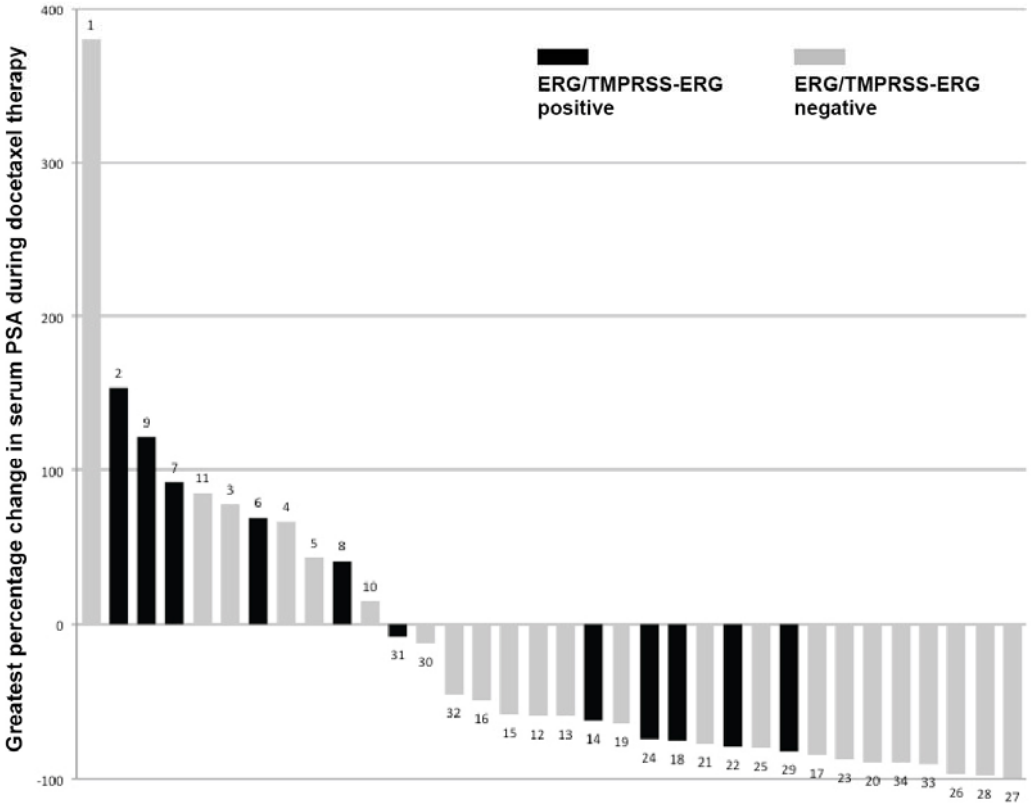
Supplementary Figure 7. Live cell FITC-Taxol imaging. **(a**) Microtubule network decoration using fluorescently labeled paclitaxel-induced decoration in ERG-negative (DU145 TetOn ERG doxycycline 0 and DU145-GFP) and ERG-positive (DU145 TetOn ERG doxycycline 25 and DU145-ERG) cells. Live cells were permeabilized with 0.5% Triton X-100 and treated with 1

μM FITC-labeled paclitaxel for 5 minutes. Images of live cells were subsequently acquired with confocal microscope (63x/1.4NA objective, Zeiss, Germany). Representative images are shown for each condition. Scale bar: 10 μM. **(b)** Quantification of fluorescence intensity of S7a; fluorescence intensity of an average of 30 cells per condition was done using MetaMorph image analysis software (Molecular Device, CA, USA). Data were analyzed using Graphpad software. Statistical significance was determined using t-test provided by GraphPad software. (c) Schematic representation of cell edges is presented to show the difference in microtubule network extension in ERG-positive and ERG negative DU145 cell. Representative images of both cell lines are shown. Scale bar: 10 μm.

a


1
50 µm
2
3
100 µm
4

b


Greatest percentage change in serum PSA during docetaxel therapy
400
300
200
100
0
-100
ERG/TMPRSS-ERG positive
ERG/TMPRSS-ERG negative
1
2
9
7
11
3
6
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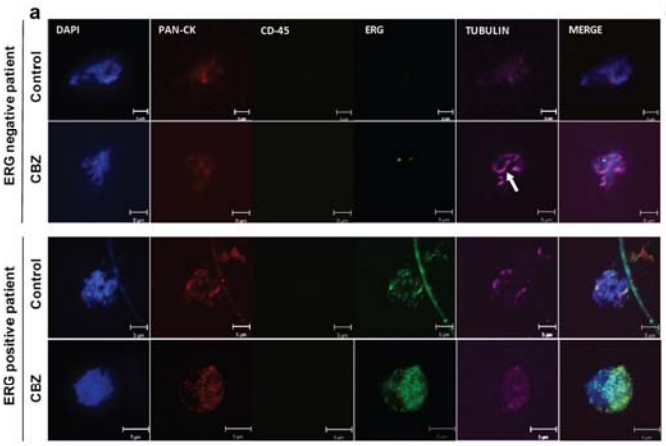
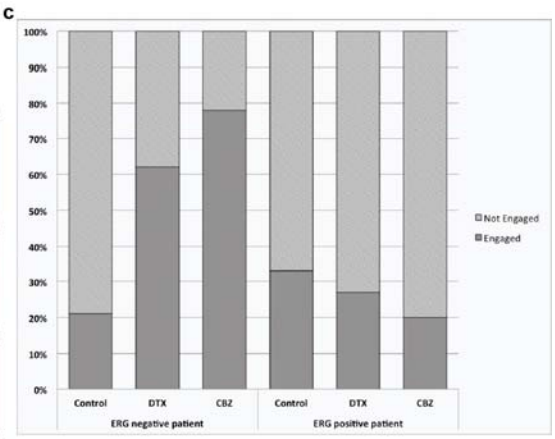
Supplementary Figure 8. Expression of TMPRSS-ERG translocation and ERG protein in primary prostate cancer tissues from men later treated with docetaxel for metastatic castrate-resistant disease. Case 9, transrectal biopsy: **(a)** Positive staining (>95% of cancer cells) for ERG by immunohistochemistry (x200) and **(b)** positive FISH for TMPRSS-ERG translocation (x400), inset higher magnification of break apart probe. Case 5, radical prostatectomy: **(c)** Negative staining for ERG by immunohistochemistry (x200) and **(d)** negative FISH for TMPRSS-ERG translocation (x400), inset higher magnification of break apart probe. Waterfall plot of the greatest percentage change in serum PSA from baseline to end of docetaxel chemotherapy in men with metastatic castrate resistant prostate cancer. The cases in black represent men whose primary prostate cancer tissue was ERG/TMPRSS-ERG positive.

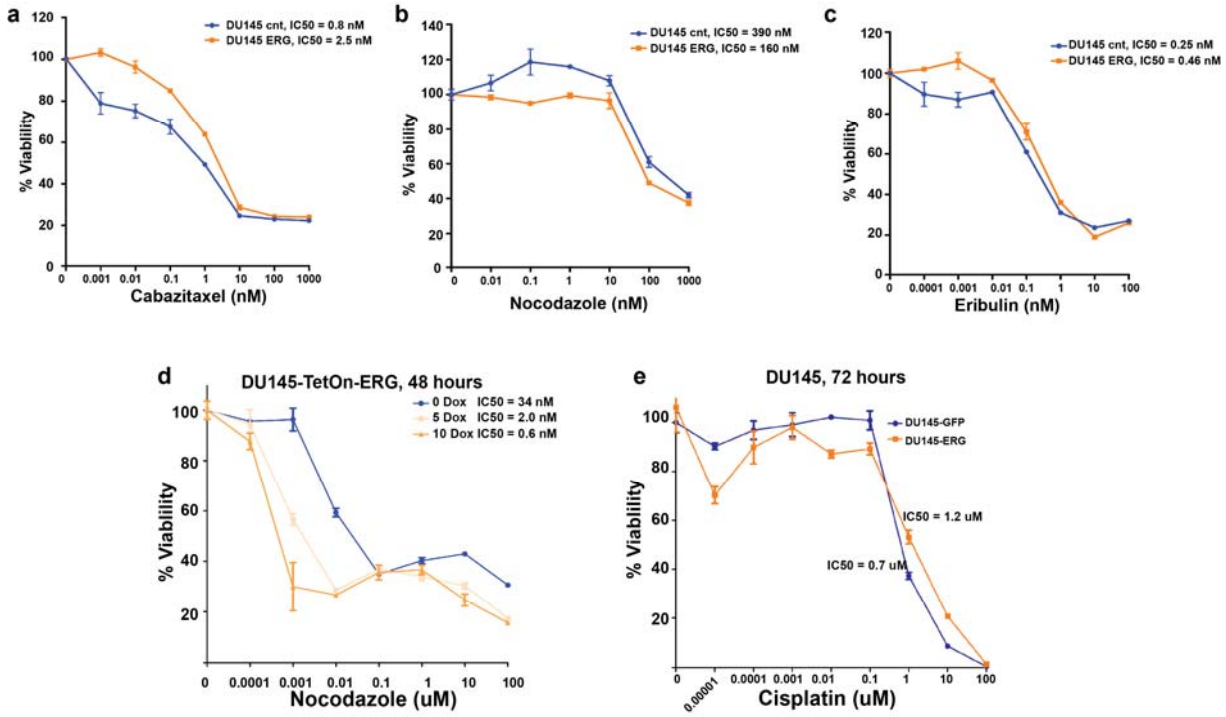




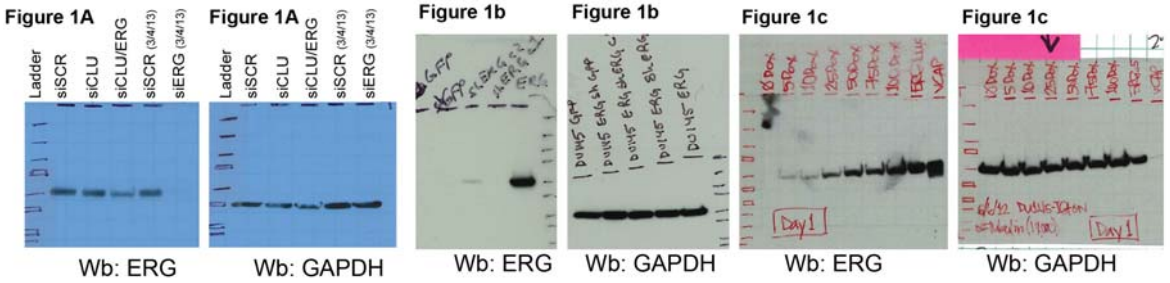
b

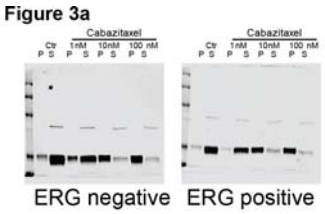
	ERG negative patient's CTCs		ERG positive patient's CTCs	
	Not engaged	Engaged	Not engaged	Engaged
Control	34 (79%)	9 (21%)	24 (67%)	12 (33%)
Docetaxel	25 (38%)	41 (62%)	35 (73%)	13 (27%)
Cabazitaxel	23 (22%)	82 (78%)	24 (80%)	6 (20%)

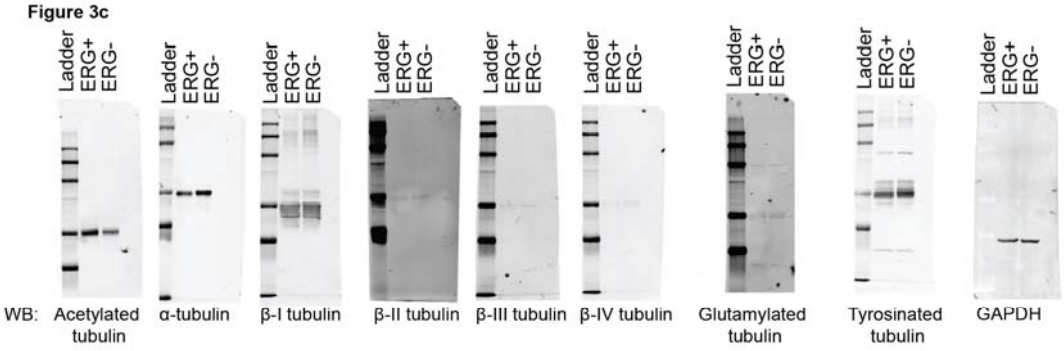
Supplementary Figure 9. (a) Analysis of CTCs isolated from the peripheral blood of ERG negative and positive CRPC patients. One ml of peripheral blood from each patient was flown through each GEDI microfluidic device, and the captured CTCs were treated on the device with either DMSO (Control), Docetaxel 100 nM (DTX) or Cabazitaxel 10 nM (CBZ) overnight. GEDI-captured cells were fixed, immunostained with the indicated antigens and imaged by confocal microscopy. CTCs were identified as nucleated, CD45-/pan-CK+ cells, enumerated manually and the microtubule network pattern was assessed for the presence of drug-target engagement. ERG immunostaining is used to confirm ERG expression or lack of expression on the captured CTCs. Representative images of isolated CTCs are shown in untreated or CBZ-treated devices. Microtubule bundles (arrow) are evident in the ERG negative CRPC patient CTCs after CBZ exposure. Scale bar: 5 μm. (**b**) Percentage of CTCs with effective DTE is identified as "engaged" CTC calculated according to the following formula: (engaged CTCs/ not engaged CTCs + engaged CTCs) x 100. (**c**) Graphic representation of data shown in **b**.

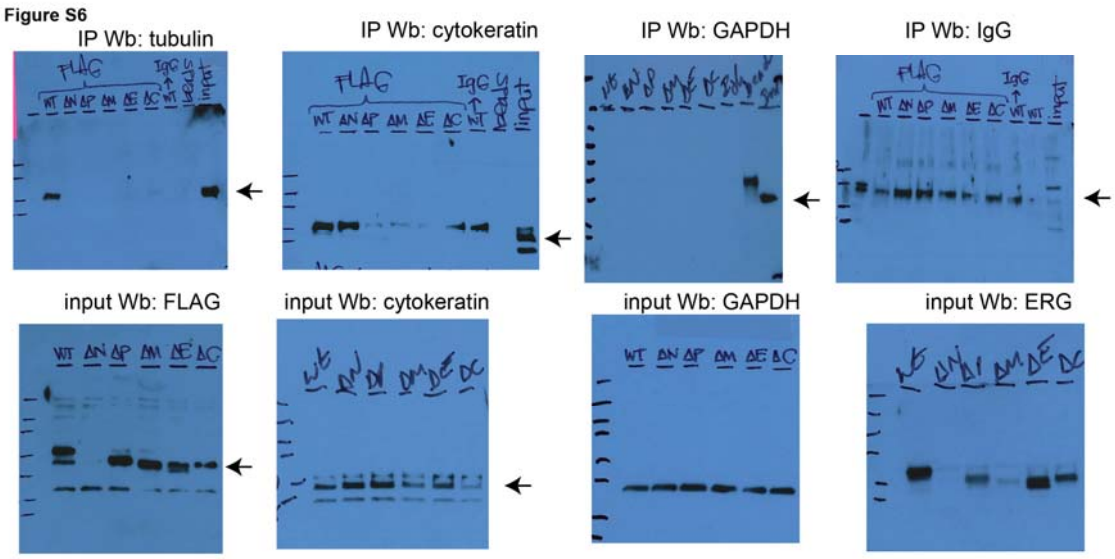


Supplementary Figure 10. Cytotoxicity assays of DU145 clones stably over-expressing ERG (orange) or GFP (blue), following treatment with cabazitaxel **(a)**, Nocodazole **(b)**, Eribulin **(c)** or Cisplatin **(e)**. Cytotoxicity assay of DU145-TetOn-ERG cells with the indicated dose of doxycycline following Nocodazole treatment **(d)**.

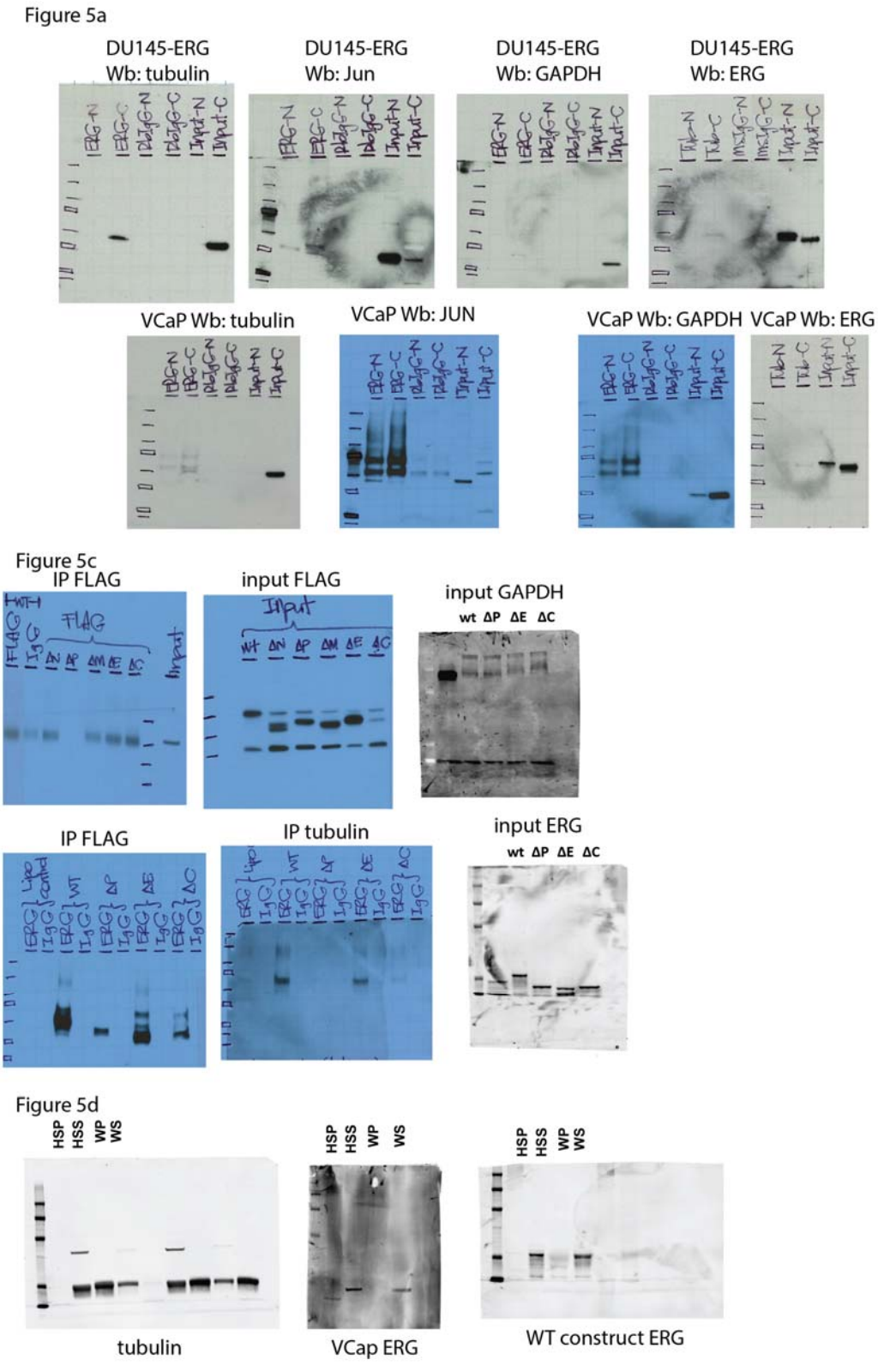








Supplementary Figure 11. Western blots of the cropped images throughout the text for the indicated figure. Arrow indicates the protein in question.



Supplementary Figure 12. Western blots of the cropped images throughout the text for the indicated figure.

Parameter\conditions	DU145-GFP	DU145-GFP 1nM DTX	Statistical significance	DU145-ERG	DU145-ERG 1nM DTX	Statistical significance
movies (#)	12	10		15	14	
growth track speed (μm min^{-1})	11.46 ± 1.88	8.14 ± 1.02	*	9.58 ± 1.04	10.26 ± 1.53	n.s.
gap/pause speed (μm min^{-1})	5.07 ± 0.97	3.22 ± 0.41	*	3.85 ± 0.69	4.45 ± 0.81	n.s.
Growth Times (s)	8.03 ± 0.74	7.87 ± 0.93	n.s.	6.83 ± 0.94	8.41 ± 0.67	*
Gap/pause Times (s)	10.84 ± 0.79	11.12 ± 0.93	n.s.	10.93 ± 1.04	10.88 ± 0.57	n.s.
growth segment speed (μm min^{-1})	7.64 ± 1.35	5.32 ± 0.71	*	6.32 ± 0.99	6.84 ± 0.96	n.s.
gaps per segment	1.32 ± 0.11	1.35 ± 0.04	n.s.	1.33 ± 0.008	1.37 ± 0.085	n.s.
time ratio (Tgap / Tsegment)	0.39 ± 0.038	0.42 ± 0.042	n.s.	0.43 ± 0.04	0.40 ± 0.02	*
Δ*Tsegment* (s)	32.31 ± 2.50	32.42 ± 1.36	n.s.	30.29 ± 1.98	34.17 ± 2.64	*
shrinkage speed (μm min^{-1})	8.79 ± 1.86	4.81± 1.01	*	7.02 ± 1.00	7.86 ± 1.58	n.s.
Δtshrink (s)	15.69 ± 0.70	15.71 ± 1.00	n.s.	16.18 ± 0.98	16.74 ± 1.08	n.s.
P*shrink* (%)	0.29 ± 0.043	0.25 ± 0.044	n.s.	0.19 ± 0.004	0.26 ± 0.06	n.s.
T*shrink* (%)	0.21 ± 0.036	0.18 ± 0.035	*	0.16 ± 0.005	0.21 ± 0.05	n.s.

Supplementary Table 1. Microtubule dynamics parameters. Results of grouping of visible growth tracks of DU145 ERG+ or ERG- cells in comparing vehicle versus taxane per cell condition. Per condition: number of movies, growth track speeds ± std, gap/pause speeds ± std, growth track time, gap/pause time growth segment speeds ± std, number of gaps/pauses per growth segment, percentage of time spent in gap/pause within a growth segment, growth segment time, shortening speeds ± std, shortening time, probability of catastrophe at the end of grouped growth track, percentage of time spent in shortening within a multi-track trajectory.

Parameter\conditions	DU145-GFP veh	DU145-ERG veh	Stat. sig.
movies (#)	12	15	
growth track speed (μm min-1)	11.46 ± 1.88	9.58 ± 1.04	n.s.
gap/pause speed (μm min-1)	5.07 ± 0.97	3.85 ± 0.69	*
Growth Times (s)	8.03 ± 0.74	6.83 ± 0.94	*
Gap/pause Times (s)	10.84 ± 0.79	10.93 ± 1.04	n.s.
growth segment speed (μm min-1)	7.64 ± 1.35	6.32 ± 0.99	n.s.
gaps per segment	1.32 ± 0.11	1.33 ± 0.008	n.s.
time ratio Tgap / Tsegment	0.39 ± 0.038	0.43 ± 0.04	n.s.
dT segment (s)	32.31 ± 2.50	30.29 ± 1.98	*
shrinkage speed (mm min-1)	8.79 ± 1.86	7.02 ± 1.00	n.s.
Dt shrink (s)	15.69 ± 0.70	16.18 ± 0.98	n.s.
P*shrink (%)*	0.29 ± 0.043	0.19 ± 0.004	n.s.
T*shrink (%)*	0.21 ± 0.036	0.16 ± 0.005	n.s.

Supplementary Table. 2 Results of grouping of visible growth tracks in vehicle treated DU145 ERG+ or ERG- cells.

Patient Number	ERG IHC staining	% nuclei ERG-positive	Intensity nuclei positive	Intensity Cytoplasmic positive	TMPRSS-ERG FISH	Type of tissue	PCWG2 PSA progression[2,4]	Baseline PSA	PSA at 12 weeks	PSA at 15 weeks	PSA at >15 weeks	Greatest percentage change in PSA over course of
1	Negative				Negative	TRB	Yes	4	16.2	19.2		380
2	Positive	60%	3+	1+	N/A	TRB	Yes	260	640	661		154
3	Negative				Negative	TRB	Yes	10.5	18.7	17.7		78
4	Negative				Negative	TRB	Yes	7.65	12.7	Died		66
5	Negative				Negative	RP	Yes	58.1	83.4	79.2		43
6	Positive	95%	3+	1+	Positive	TRB	Yes	65	88.2	91.3		69
7	Negative	N/A	N/A	N/A	Positive	CELL BLOCK	Yes	67.6	88	N/A	130	92
8	Positive	95%	3+	1+	Positive	TRB	Yes	951	1193	1338		41
9	Positive	95%	3+	1+	Positive	TRB	Yes	416.3	489.5	926		122
10	Negative				Negative	TRB	No	175	202	184		15
11	Negative				Negative	TURP	No	216	189	232		85
12	Negative				Negative	TRB	No	76.7	44.5	31.7		-59
13	Negative				N/A	Bone	No	342	194	282		-59
14	Positive	95%	2+	1+	Positive	TRB	No	4984	1918	2295		-62
15	Negative				Negative	TURP	No	914.9	474.7	475.9		-58
16	Negative				Negative	TRB	No	376	193	226		-49
17	Negative				Negative	TRB	No	223.2	92.54	63.46		-84
18	Positive	95%	3+	1+	Positive	TRB	No	95.7	30.2	39		-75
19	Negative				N/A	TRB	No	10054	4264	3676		-64
20	Negative				Negative	TRB	No	38.14	12.86	7.21		-89
21	Negative				Negative	RP	No	94.6	27.6	22.35		-77
22	Positive	95%	3+	1+	Positive	TRB	No	1376	375	330		-79
23	Negative				Negative	RP	No	154	41.7	29.5		-87
24	Positive	95%	3+	1+	Positive	TRB	No	105	27.8	N/A	37.9	-74
25	Negative				Negative	TRB	No	35.69	9.02	7.92		-80
26	Negative				Negative	TRB	No	2286	93.36	77.69		-97
27	Negative				N/A	RP	No	141	6.87	2.26		-99
28	Negative				Negative	CELL BLOCK	No	202.8	4.38	4.13		-98
29	Positive	90%	2+	0+		RP	No	4.33	1.55		0.78	-82
30	Negative				N/A	TRB	Yes	49	56.69	53.18	43.14	-12
31	Positive	100%	3+	0+		TRB	Yes	126	145.6	149.21	115.4	-8
32	Negative						No	98.98	65.3		54.13	-45
22	Negative						No	170.55	32.41		17.5	-90
34	Negative						No	188.2	20.6		20.6	-89

Supplementary Table 3: ERG/TMPRSS-ERG expression in men with metastatic castrate resistant prostate cancer treated with docetaxel.
Abbreviations: IHC = immunohistochemistry, FISH = fluorescent in situ hybridization, PCWG = Prostate Cancer Working Group, PR = partial response, SD = stable disease, PD = progressive disease, TRB = transrectal biopsy, RP = radical prostatectomy, TURP = trans-urethral resection of prostate, N/A = not available

References

1 Rickman, D. S. *et al.* Oncogene-mediated alterations in chromatin conformation. *Proc Natl Acad Sci U S A* **109**, 9083-9088, doi:1112570109 [pii] 10.1073/pnas.1112570109 (2012).