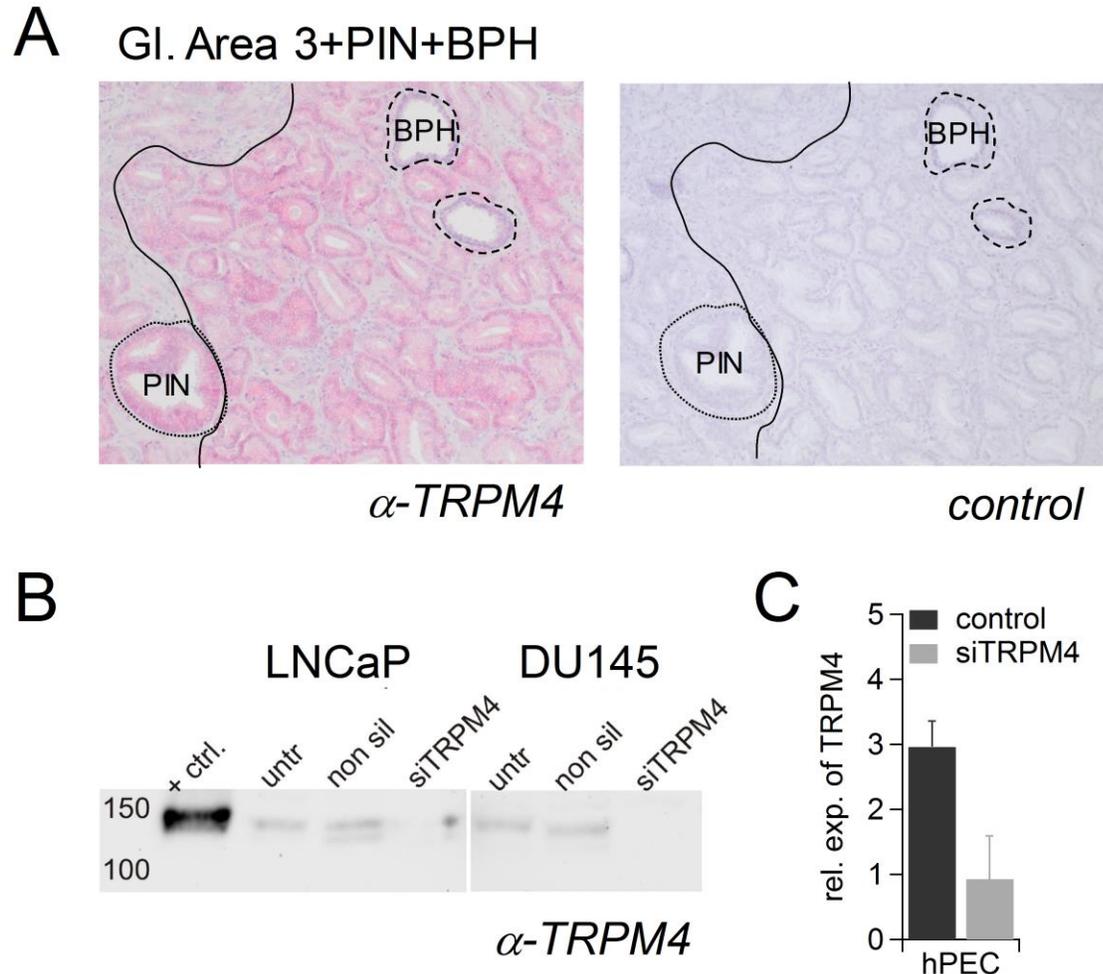
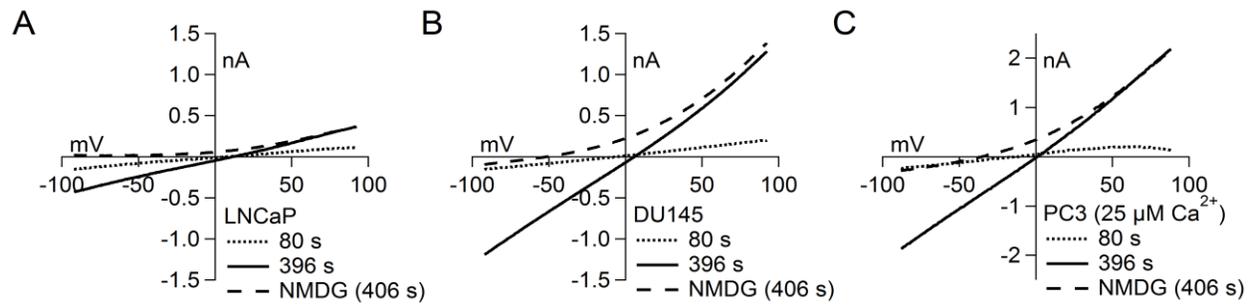


# Transient receptor potential melastatin 4 channel contributes to migration of androgen-insensitive prostate cancer cells

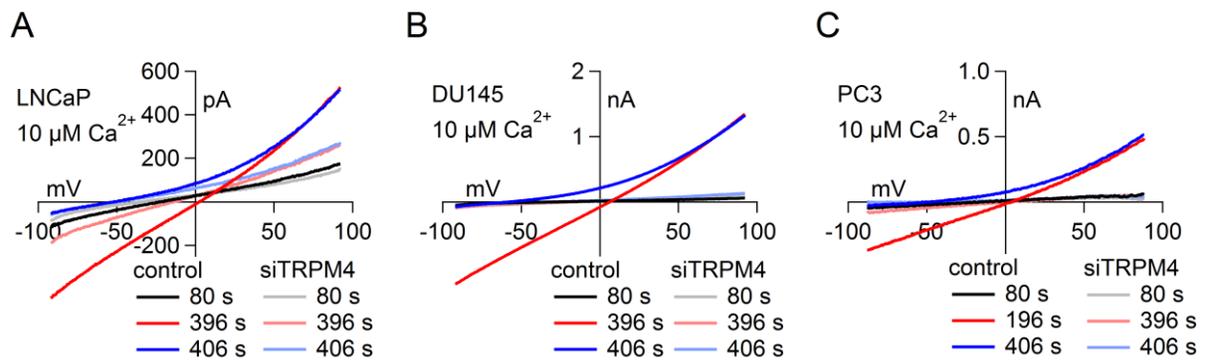
## Supplementary Material



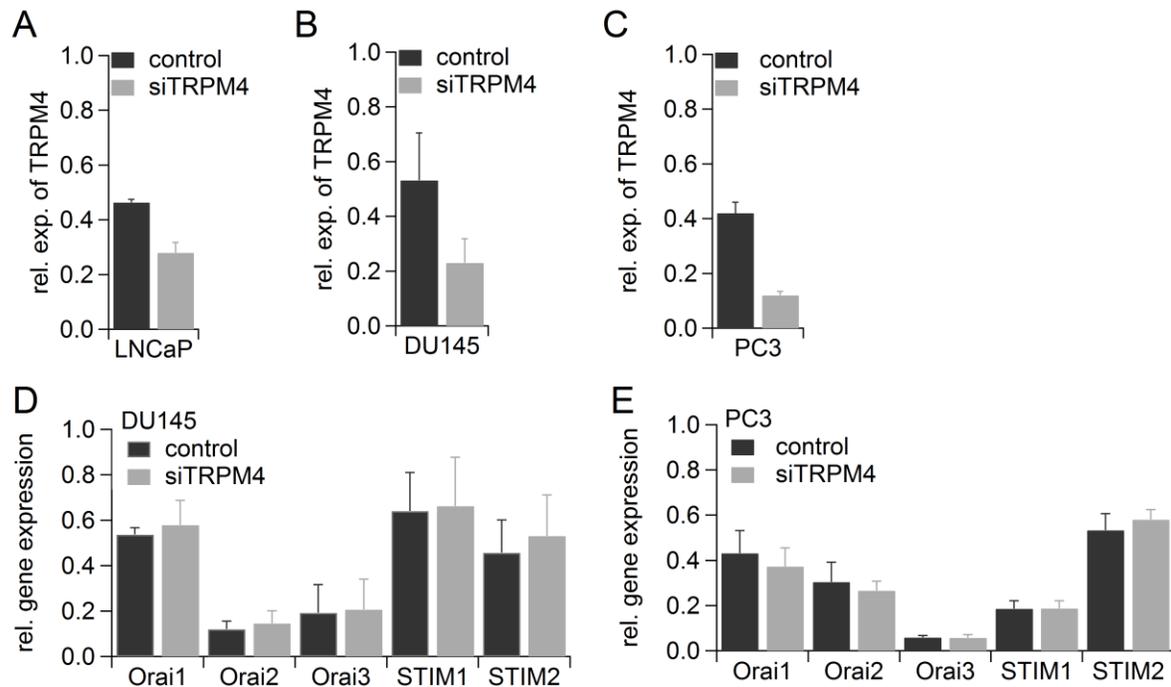
**Supplementary Fig. 1: Specificity of  $\alpha$ -TRPM4 antibody and knockdown efficiency of TRPM4 siRNA.** (A) Immunofluorescence staining for TRPM4 in prostate cancer tissue or control in two consecutive tissue slices. (B) Western blot analysis of TRPM4 protein in LNCaP or DU145 that were non-transfected or transfected with control RNA or siRNA targeting TRPM4. (C) Efficiency of TRPM4 down-regulation by siRNA 72 h after transfection detected by qRT-PCR and normalized to TBP in hPEC ( $n = 3$ ).



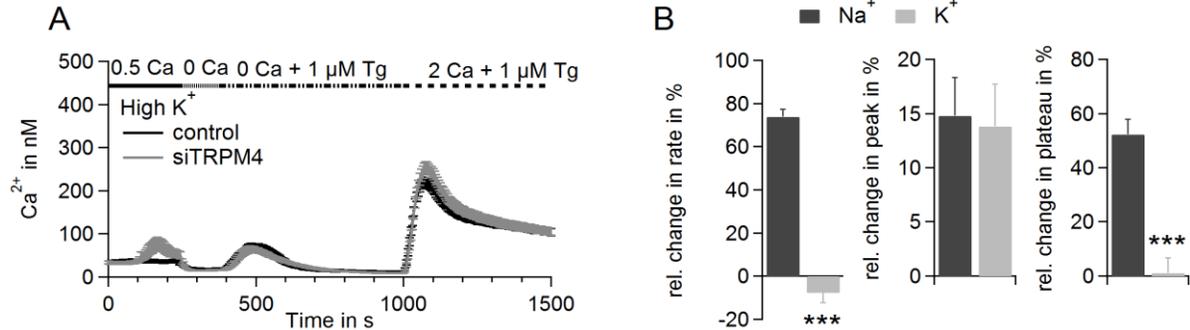
**Supplementary Fig. 2: IV of TRPM4 in non-transfected cells.** (A-C) IV from measurements in LNCaP, DU145 and PC3 cells shown in **Fig. 4B** extracted at indicated time points.



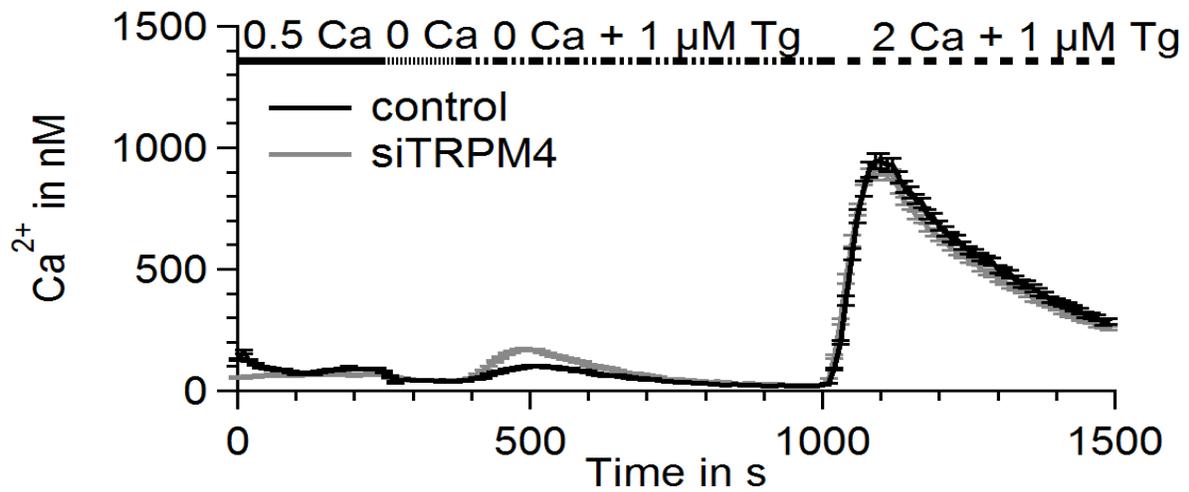
**Supplementary Fig. 3: IV of TRPM4 in transfected cells.** (A-C) IV from measurements in LNCaP, DU145 and PC3 cells shown in **Fig. 4D-F** extracted at indicated time points.



**Supplementary Fig. 4: Knockdown efficiency of TRPM4 on mRNA levels and off-target effects upon knockdown.** (A-C) Efficiency of TRPM4 down-regulation by siRNA 72 h after transfection when normalized to TBP in LNCaP ( $n = 3$ ), DU145 ( $n = 3$ ) and PC3 cells ( $n = 3$ ) detected by qRT-PCR. (D-E) mRNA levels of Orai1, Orai2, Orai3, STIM1 and STIM2 upon knockdown of TRPM4 ( $n = 3$ ).



**Supplementary Fig. 5: SOCE in DU145 upon knockdown of TRPM4 when external Na<sup>+</sup> was replaced with K<sup>+</sup>.** (A) Tg-induced SOCE in DU145 transfected with control RNA (black,  $n = 126$ ) or TRPM4 siRNA (grey,  $n = 137$ ) 72 h after transfection measured in a Fura-2-based Ca<sup>2+</sup> readdition protocol when extracellular Na<sup>+</sup> was replaced by K<sup>+</sup>. (B) Relative changes in Ca<sup>2+</sup> entry rate, peak and plateau of SOCE upon knockdown of TRPM4 under standard conditions (Na<sup>+</sup>, black bars, from cells in **Fig. 5A**) or when Na<sup>+</sup> was replaced by K<sup>+</sup> (grey bars, from cells in **SFig. 5A**).



**Supplementary Fig. 6: SOCE in PC3 upon knockdown of TRPM4.** (A) Tg-induced SOCE in PC3 transfected with control RNA (black,  $n = 292$ ) or TRPM4 siRNA (grey,  $n = 358$ ) 72 h after transfection measured in a Fura-2-based  $\text{Ca}^{2+}$  readdition protocol.

**Supplementary Table 1**

Clinical characteristics and signal intensity of TRPM4 immune reactivity in prostate carcinoma tissue

<b>case</b>	<b>Gleason Score</b>	<b>T stage</b>	<b>N</b>	<b>M</b>	<b>Tumor</b>	<b>PIN</b>	<b>BPH</b>
PCA001	3+2=5	pT4	N0	M0	++	++	+-
PCA002	4+3=7	pT3b	N0	M0	+++	+++	n.e.
PCA003	4+3=7	pT3a	N0	M0	+++	+++	+
PCA004	4+3=7	pT3a	N0	M0	+++	+++	+
PCA005	4+3=7	pT3a	N0	M0	++	++	-
PCA006	4+3=7	pT2c	N0	M0	++	+++	-
PCA007	3+4=7	pT2c	N0	M0	+++	+++	++
PCA008	4+5=9	pT3b	N0	M0	++	+++	+
PCA009	4+3=7	pT3a(est)	N0	M0	++	++	-
PCA010	4+3=7	pT3a(est)	N0	M0	++	++	-
PCA011	3+4=7	pT2c	N0	M0	++	++	n.e.
PCA012	3+4=7	pT3a(est)	N0	M0	+++	++	n.e.
PCA013	5+4=9	pT2c	N0	M0	+++	++	-
PCA014	3+4=7	pT2c	N0	M0	+++	++	-
PCA015	3+4=7	pT2c	N0	M0	+++	++	-
PCA016	3+4=7	pT3b	N0	M0	+++	++	-
PCA017	4+4=8	pT2a	N0	M0	+++	++	+
PCA018	3+4=7	pT2c	N0	M0	+++	++	-
PCA019	3+4=7	pT2c	N0	M0	+++	+	-
PCA020	3+3=6	pT2c	N0	M0	+++	+	-