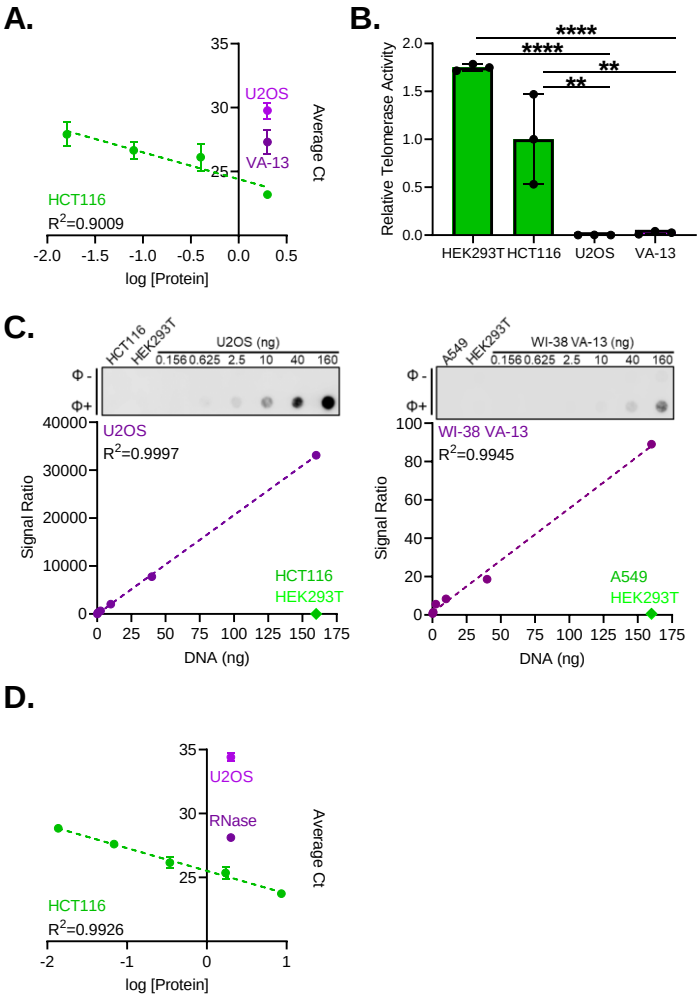
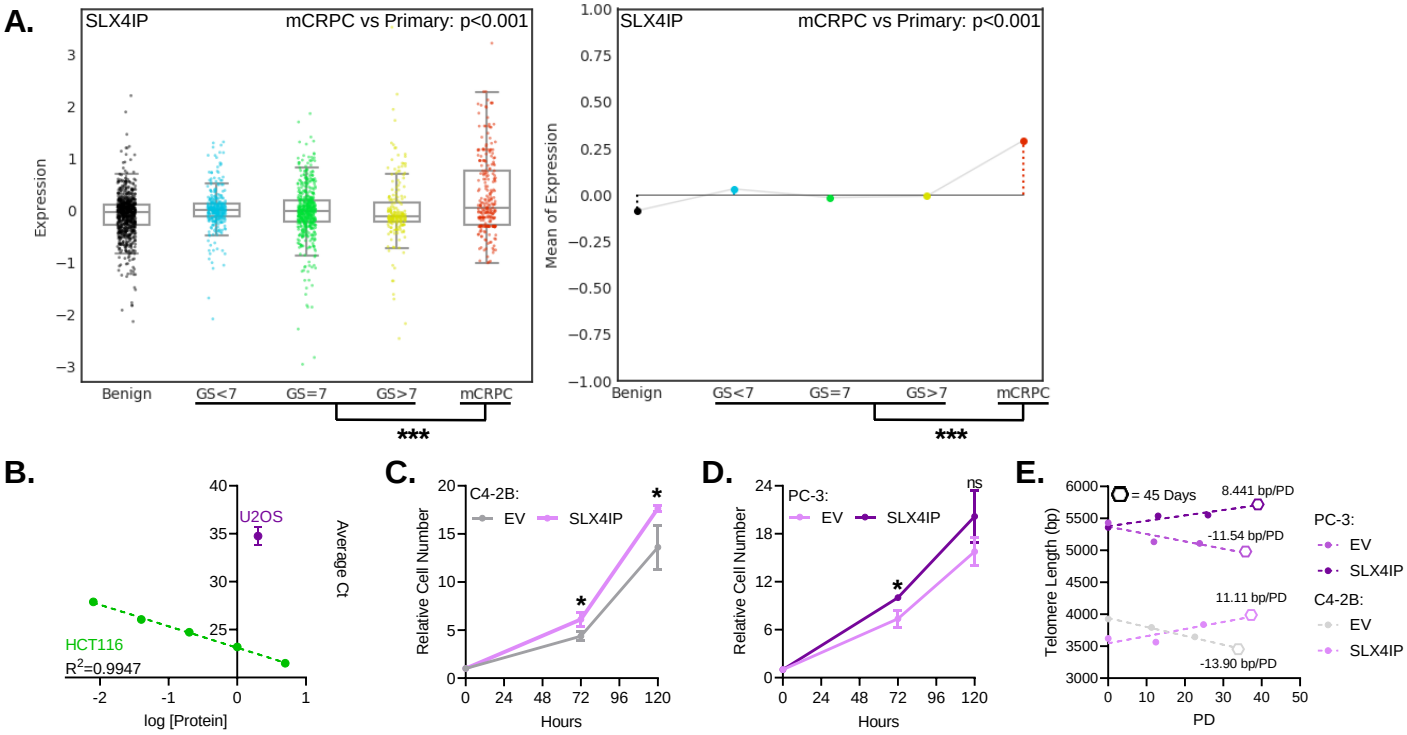
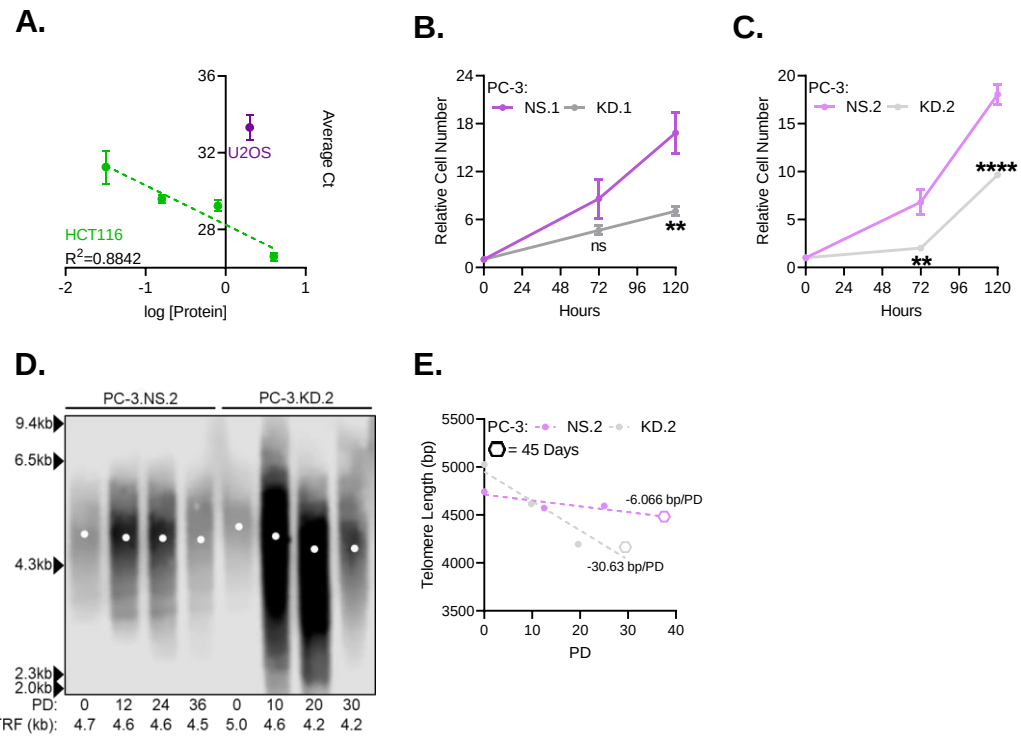


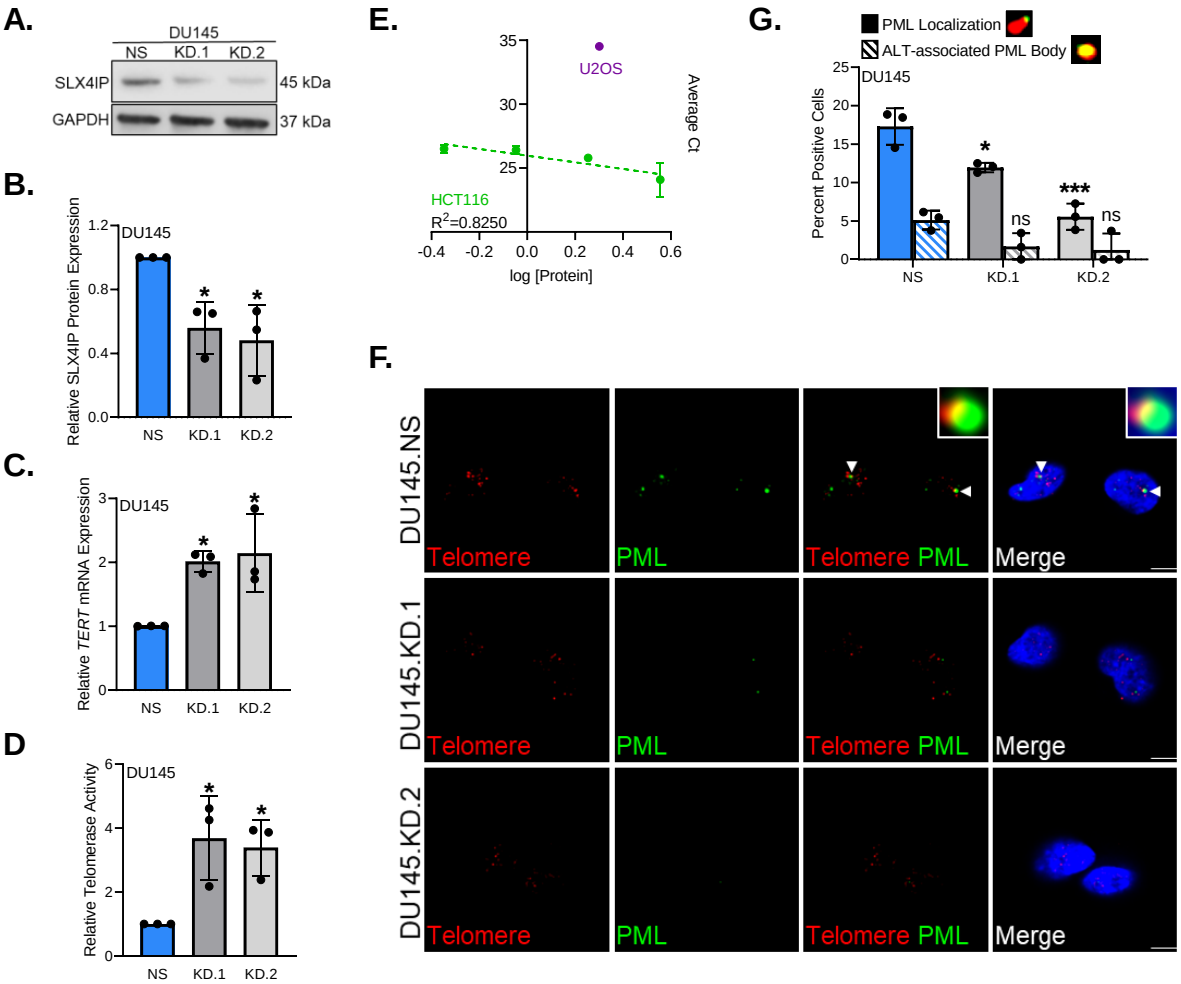
Supplemental Figure 1: Validation of TMM characterization techniques. (A) Standard curve of telomerase-positive HCT116 cells using Q-TRAP protocol. ALT-positive U2OS and WI-38 VA-13 cells included as negative controls. **(B)** Relative telomerase activity of telomerase-positive HEK293T and HCT116 cells and ALT-positive U2OS and WI-38 VA-13 cells calculated from (A). **(C)** Representative dot blots demonstrating the presence of telomeric C-circles. Φ+ lane indicates reactions with Φ29 polymerase and Φ- lane indicates control reaction. Standard curve of ALT-positive U2OS and WI-38 VA-13 DNA versus signal ratio of Φ+ to Φ- reaction is shown below. Telomerase-positive HEK293T, HCT116, and A549 cells included as negative controls (DNA at 160ng). **(D)** Representative standard curve of telomerase-positive HCT116 cells using Q-TRAP protocol for Figure 1B. ALT-positive U2OS and RNase-treated HCT116 sample included as negative controls. Data represented as mean±SD; n=3; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.



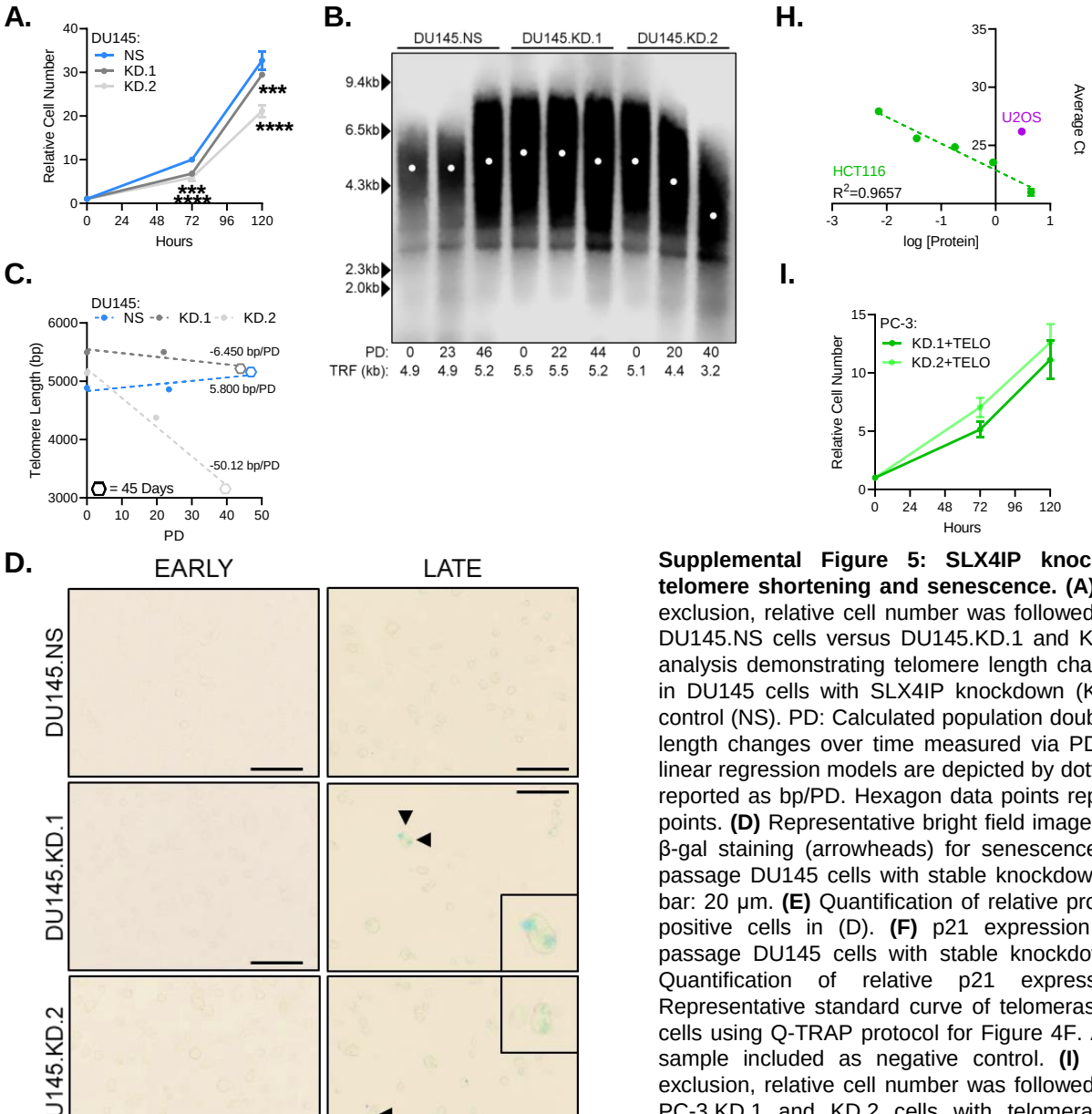
Supplemental Figure 2: Expression of SLX4IP in mCRPC versus primary disease. (A) SLX4IP expression data from the PCTA arranged based upon patient diagnosis of benign disease, primary disease and Gleason score, or mCRPC. Ranksums-test was used to compare primary (GS<7,=7,>7) versus mCRPC cohorts. **(B)** Representative standard curve of telomerase-positive HCT116 cells using Q-TRAP protocol for Figure 2E. ALT-positive U2OS sample included as negative control**. (C)** Using trypan blue exclusion, relative cell number was followed over 120 hours for C4-2B and **(D)** PC-3 cells with SLX4IP overexpression. **(E)** Telomere length changes over time measured via PDs from Figure 2I and J. Simple linear regression models are depicted by dotted lines and slopes reported as bp/PD. Hexagon data points represent 45 day time points. Data represented as mean±SD; n=3; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.



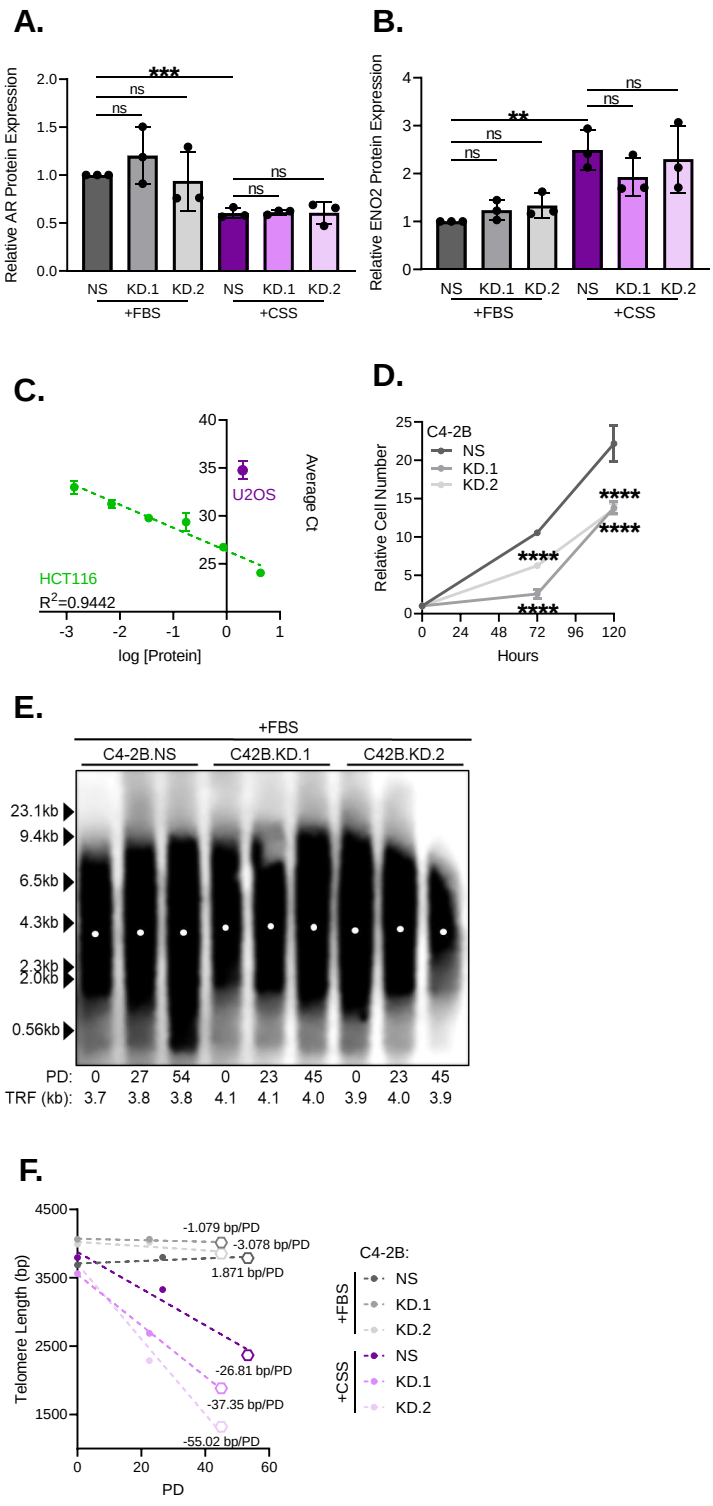
Supplemental Figure 3: SLX4IP knockdown correlates with accelerated telomere shortening. (A) Representative standard curve of telomerase-positive HCT116 cells using Q-TRAP protocol for Figure 3G. ALT-positive U2OS sample included as negative control**. (B)** Using trypan blue exclusion, relative cell number was followed over 120 hours in PC-3.NS.1 versus KD.1 or **(C)** NS.2 versus KD.2 cells. **(D)** Telomere restriction fragment analysis demonstrating telomere length changes over 45 days in PC-3 cells with SLX4IP knockdown (KD.2) versus control (NS.2). PD: Calculated population doublings. **(E)** Telomere length changes over time measured via PDs from (D). Simple linear regression models are depicted by dotted lines and slopes reported as bp/PD. Hexagon data points represent 45 day time points. Data represented as mean+SD; n=3; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.



Supplemental Figure 4: SLX4IP knockdown is accompanied by a blunted atypical ALT phenotype. (A) Confirmation of stable SLX4IP knockdown using two shRNAs (KD.1, KD.2) in DU145 cells with scrambled shRNA control (NS) at the protein level. **(B)** Quantification of (A). **(C)** Relative *TERT* mRNA expression and **(D)** telomerase activity following SLX4IP knockdown in DU145 cells. **(E)** Representative standard curve of telomerase-positive HCT116 cells using Q-TRAP protocol for (D). ALT-positive U2OS sample included as negative control**. (F)** Representative IF-FISH images demonstrating the presence (arrowheads) or absence of PML (green) at telomeres (red) with zoom insets provided. Scale bar: 5 μm. **(G)** Quantification of (F) for percent positive cells with at least one APB. Cells lacking typical APBs were quantified for ALT-like PML localization events. Representative foci are included defining these events. Data represented as mean+SD; n=3; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.



Supplemental Figure 5: SLX4IP knockdown promotes telomere shortening and senescence. (A) Using trypan blue exclusion, relative cell number was followed over 120 hours in DU145.NS cells versus DU145.KD.1 and KD.2 cells. **(B)** TRF analysis demonstrating telomere length changes over 45 days in DU145 cells with SLX4IP knockdown (KD.1, KD.2) versus control (NS). PD: Calculated population doublings. **(C)** Telomere length changes over time measured via PDs from (B). Simple linear regression models are depicted by dotted lines and slopes reported as bp/PD. Hexagon data points represent 45 day time points. **(D)** Representative bright field images demonstrating SA β-gal staining (arrowheads) for senescence in early and late-passage DU145 cells with stable knockdown of SLX4IP. Scale bar: 20 μm. **(E)** Quantification of relative proportion of SA β-gal positive cells in (D). **(F)** p21 expression in early and late passage DU145 cells with stable knockdown of SLX4IP. (**G)** Quantification of relative p21 expression in (F). **(H)** Representative standard curve of telomerase-positive HCT116 cells using Q-TRAP protocol for Figure 4F. ALT-positive U2OS sample included as negative control. **(I)** Using trypan blue exclusion, relative cell number was followed over 120 hours in PC-3.KD.1 and KD.2 cells with telomerase overexpression (+TELO). Data represented as mean±SD; n=3; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.



Supplemental Figure 6: Androgen-independent marker expression and telomere length changes following androgen deprivation. **(A)** Quantification of AR and **(B)** ENO2 expression in Figure 6A. **(C)** Representative standard curve of telomerase-positive HCT116 cells using Q-TRAP protocol for Figure 6D. ALT-positive U2OS sample included as negative control**. (D)** Using trypan blue exclusion, relative cell number was followed over 120 hours in C4-2B.NS cells versus C4-2B.KD.1 and KD.2 cells. **(E)** TRF analysis demonstrating telomere length changes in C42B cells with SLX4IP knockdown (KD.1, KD.2) versus C4-2B.NS grown in +FBS media. PD: Calculated population doublings. **(F)** Telomere length changes over time measured via PDs from (E) and Figure 6G. Simple linear regression models are depicted by dotted lines and slopes reported as bp/PD. Hexagon data points represent 45 day time points. Data represented as mean+SD; n=3; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.