**Supplementary figure legends**

**Supplementary Figure 1. Knockdown of ESE3/EHF and let-7 miRNAs in immortalized human prostate epithelial cells.** A.ESE3/EHF mRNA (left panels) and protein levels (right panels) evaluated by qRT-PCR and WB in control and ESE3KD-PrECs and RWPE-1 cells. β-actin (left) and tubulin (right) were used as loading control. Data are presented as fold change relative to control PrECs and RWPE-1 cells. ESE3/tubulin ratios determine by band intensity are reported at the bottom. B. Let-7 miRNAs in control and ESE3KD prostate epithelial cellsevaluated by qRT-PCR in control, ESE3KD-PrECs and ESE3KD-RWPE-1 cells. P values were determined using t-test. \*P<0.05; \*\*, P<0.01.

**Supplementary Figure 2. Lin28 protein expression in xenografts of human prostate cancer cell lines.** Protein level of Lin28 was evaluated by IHC in the indicated tumor xenografts. The relative expression of ESE3/EHF and Lin28 is reported at the bottom.

**Supplementary Figure 3. Ablation and forced expression of Lin28A and Lin28B affect the phenotype of normal and prostate cancer cells.** A-B.Knockdown of Lin28A and Lin28B evaluated by qRT-PCR (A) and WB (B) following transfection with siRNAs targeting Lin28A (siLin28A) and Lin28B (siLin28B) in ESE3KD-PrECs and RWPE-1 cells. Quantitative densitometric values for ESE3/tubulin ratio are relative to three separate western blots C. Level of Let-7b in cells transfected with Lin28 targeting siRNAs. D-F. Lin28A and Lin28B levels (D), colony formation (E) and sphere forming efficiency (F) in PrECs following transfection of Lin28A and B expression vectors. G-I. Lin28A and Lin28B levels (G), colony formation (H) and sphere forming efficiency (I) in LNCaP cells following transfection of Lin28A and B expression vectors. P values were determined using t-test. \*P<0.05; \*\*, P<0.01. Data are representative of three independent experiments.

**Supplementary Figure 4. Consequences of miR-let7b expression in prostate cancer cells.** A. Level of mature let-7b miRNA in DU145 cells following transient transfection of pre-miR-let7-b (pre-let-7b) or negative control (Neg Ctr). B. *In vitro* sphere forming efficiency (SFE) of DU145 cells following transfection with pre-let-7b or negative control evaluated at first (G1) and second (G2) generation. P values were determined using t-test. \*P<0.05; \*\*, P<0.01. Data are representative of three independent experiments.

**Supplementary Figure 5. Predicted ETS binding sites (EBS) in the promoters of five let-7 family members.** Sequence, score and position relative to the gene transcription start site (TSS) are indicated for each predicted EBS.

**Supplementary Figure 6.** **ESE3/EHF rescues the effects of forced expression of Lin28A on the CSC compartment in normal prostate epithelial cells.** A. Lin28 and ESE3/EHF protein expression following transfection of Lin28A and ESE3/EHF expression vectors in RWPE-1 cells. Tubulin was used as loading control. B. Colony formation in soft agar (left) and i*n vitro* sphere forming efficiency (right) of RWPE-1 cells after transient transfection with Lin28A and ESE3/EHF expression vectors. C. Level of mature let-7b miRNA in RWPE-1 cells determined by qRT-PCR after transient transfection with Lin28A and ESE3/EHF expression vectors*.* P values were determined using t-test. \*P<0.05; \*\*, P<0.01. Data are representative of three independent experiments.

**Supplementary Figure 7. Lin28A and Lin28B downregulation affect the phenotype of human prostate cancer cells.** A. Lin28A and Lin28B mRNA evaluated by qRT-PCR after transfection of DU145 (left) and PC3 (right) cells with control (siGL3) and Lin28 targeting siRNA (siLin28A and siLin28B). B. Level of mature let-7b evaluated by qRT-PCR after transfection as indicated in A. C. Flow cytometry analysis of cell senescence by fluorescein di-beta-D-galactopyranoside(FDG) staining following transfection of DU145 cells as indicated in A. P values were determined using t-test. \*P<0.05; \*\*, P<0.01. Data are representative of three independent experiments.

**Supplementary Figure 8. Efficiency of Lin28B downregulation by the Lin28B-2 siRNA in DU145 cells.** A.Lin28B mRNA evaluated in DU145 cells after transfection with siGL3, siLin28B and siLin28B-2 siRNA. B. Level of mature let-7b in DU145 cells transfected with the indicated siRNA as in panel A. C. Sphere forming efficiency (SFE) of DU145 cells transfected as in panel A. P values were determined using t-test. \*P<0.05; \*\*, P<0.01. Data are representative of three independent experiments.