LIN28B promotes the development of neuroendocrine prostate cancer

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Supplementary Materials

I. Antibody Information

II. RNA and Plasmid Information

III. Primers for real-time qPCR

IV. Supplementary Figures

I. Antibodies used in this study

Antibody	Vendor	Catalogue Number	Application	Dilution
CD44 APC	eBioscience	17-0441-82	FC	1:10
CD133/1 APC	Miltenyi Biotec	130-098-829	FC	1:10
E-Cadherin	Santa Cruz	Sc-7870	WB	1:1000
N-Cadherin	Abcam	ab76011	WB	1:1000
Histone H3	Abcam	ab1791	WB	1:1000
HMGA2	Thermo Fisher	PA5-21320	WB	1:1000
Lin28B	Proteintech	16178-1-AP	IF, IHC	1:25
Lin28B	Abcam	ab71415	WB	1:500
Slug	Abcam	ab27568	WB	1:1000
Snail	Cell Signaling	3895	WB	1:1000
SOX2	Novus Biologicals	NB110-37235	IHC	1:25
SOX2	Cell Signaling	3579S	WB	1:1000
Tubulin	Abcam	ab18251	WB	1:1000
Vinculin	Sigma Aldrich	V9131-2ML	WB	1:2000
7-AAD	BD Pharmingen	51-68981E	FC	1:10

* FC = Flow Cytometry; * IF = Immunofluorescence; * IHC = Immunohistochemistry
* WB = Western blot

II. RNA and Plasmid Information

Reagent	Provider	Catalogue #
miRIDIAN microRNA human hsa-let-7d-5p mimic	Dharmacon	C-300478-07-0002
miRIDIAN microRNA mimic negative control	Dharmacon	CN-001000-01
TRC LIN28B shRNA	Dharmacon	RHS4533-EG389421
pcDNA3-FLAG-Lin28B	Addgene; pcDNA3-FLAG-Lin28B was a gift from Narry Kim	51373
pGL3-IRES-Lin28b-P3	Addgene; pGL3-IRES-Lin28b-P3 was a gift from Joshua Mendell	64794
MSCV puro spg-let-7	Addgene: MSCV puro spg-let-7 was a gift from Phil Sharp	29766
pMXS-hs-HMGA2	Addgene; pMXS-hs-HMGA2 was a gift from Shinya Yamanaka	52727
pCCLc-U6-shHMGA2.3-PGK-dTomato	Addgene; pCCLc-U6-shHMGA2.3-PGK-dTomato was a gift from Fernando Fierro	89606
pGL3-Sox2	Addgene; pGL3-Sox2 was a gift from Yuh-Shan Jou	101761

III. Primers for real-time qPCR

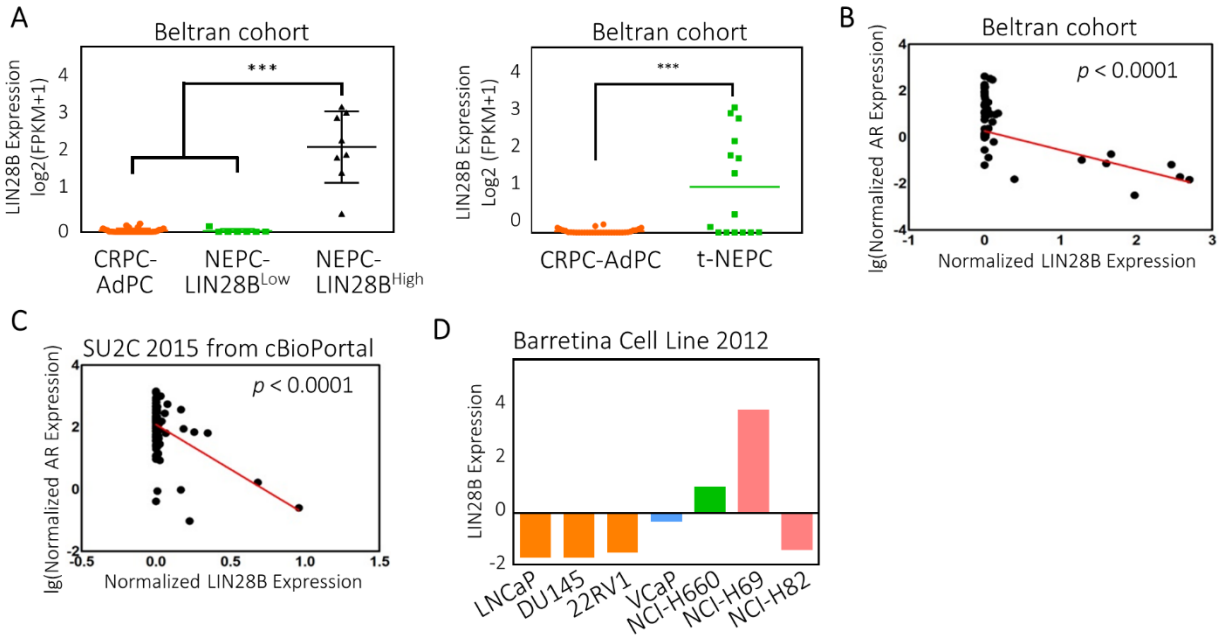
Primer Name	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')
ALDH1A2	TTGCAGGGCGTCATCAAAAC	ACACTCCAATGGGTTCATGTC
ASCL1	CCCAAGCAAGTCAAGCGACA	AAGCCGCTGAAGTTGAGCC
CCNF	AGGACAAGCGCTATGGAGAA	TCTGTCTTCCTGGAGGCTGT
CDH1	ATTTTTCCCTCGACACCCGAT	TCCCAGGCGTAGACCAAGA
CDH2	TGCGGTACAGTGTAACTGGG	GAAACCGGGCTATCTGCTCG
CDK6	CCAGATGGCTCTAACCTCAGT	AACTTCCACGAAAAAGAGGCTT
CHGA	TAAAGGGGATACCGAGGTGATG	TCGGAGTGTCTCAAAACATTCC
CHGB	CGAGGGGAAGATAGCAGTGAA	CAGCATGTGTTTCCGATCTGG
FOXC1	TGTTCGAGTCACAGAGGATCG	ACAGTCGTAGACGAAAGCTCC
FOXD3	TCACGCACCAATTCTAACGC	CACGGCTTGCTTACTGAAGG
GAPDH	GGACCTGACCTGCCGTCTAGAA	GGTGTCGCTGTTGAAGTCAGAG
HEY1	GTTCGGCTCTAGGTTCCATGT	CGTCGGCGCTTCTCAATTATTC
HMGA2	AGTCCCTCTAAAGCAGCTCAAAAG	GCCATTTCCTAGGTCTGCCTC
ID4	GGCCACTCAAGCAGCATTTG	TCTGGTTGCCTGGTTAGGAC
IGDCC3	TCATCGGCATCCACATCG	GAGGACCCTGCCCCTTTG
IGF2BP1	GGCCATCGAGAATTGTTGCAG	CCAGGGATCAGGTGAGACTG
INTS2	GTCTCTTGGTGGCCAATGTT	AGGGCCTGAGAAGGATTCAT
KRT8	TCCTCAGGCAGCTATATGAAGAG	GGTTGGCAATATCCTCGTACTGT
LIN28B	TGTAGTCTACCTCCTCAGCCAA	ATTCTGCTTCCTGTCTTCCCTG
miR-let-7a	CCAGCTGGGTGAGGTAGTAGGTTGT	CTGGTGTCGTGGAGTCGGCAATT
miR-let-7b	CCAGCTGGGTGAGGTAGTAGGTTGT	CTGGAGCTAGTTTCGTCGTAGGG
miR-let-7c	CCAGCTGGGTGAGGTAGTAGGTTGT	TCCAGTGCAGGGTCCGAGGTA
miR-let-7d	CCAGCTGGGAGAGGTAGTAGGTTGC	CTGGTGTCGTGGAGTCGGCAATT
miR-let-7e	CCAGCTGGGTGAGGTAGGAGGTTGT	CTGGTGTCGTGGAGTCGGCAATT
miR-let-7f1	CCAGCTGGGTGAGGTAGTAGATTGT	CTGGTGTCGTGGAGTCGGCAATT
miR-let-7g	CCAGCTGGGTGAGGTAGTAGTTTGT	CTGGTGTCGTGGAGTCGGCAATT
miR-let-7i	CCAGCTGGGTGAGGTAGTAGTTTGT	TCCAGTGCAGGGTCCGAGGTA
miR-98	CCAGCTGGGTGAGGTAGTAAGTTGT	CTGGTGTCGTGGAGTCGGCAATT
SCGN	GGCCATTTCTGAGGCTAAACT	GGGCTCCTGTTTTACTAACATCA
SIX2	AAGGCACACTACATCGAGGC	CACGCTGCGACTCTTTTCC
SOX2	GCCGAGTGGAAACTTTTGTCG	GGCAGCGTGTACTTATCCTTCT
SYP	TTAGTTGGGGACTACTCCTCG	GGCCCTTTGTTATTCTCTCGGTA
SYT4	ATGGGATACCCTACACCCAAAT	TCCCGAGAGAGGAATTAGAACTT
U6	GCTTCGGCAGCACATATACTAAAAT	CGCTTCACGAATTTGCGTGTCAT

Primer Name	Stem Loop Primer Sequence (5'-3')
miR-let-7a	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACTATAC
miR-let-7b	CTCAACTGGAGCTAGTTTCGTCGTAGGGCAGTTGAGAACCACAC
miR-let-7c	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAACCAT
miR-let-7d	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACTATGC
miR-let-7e	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACTATAC
miR-let-7f1	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACTATAC

miR-let-7g	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACTGTAC
miR-let-7i	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAACAGC
miR-98	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACAATAC
U6	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAAAAATATG

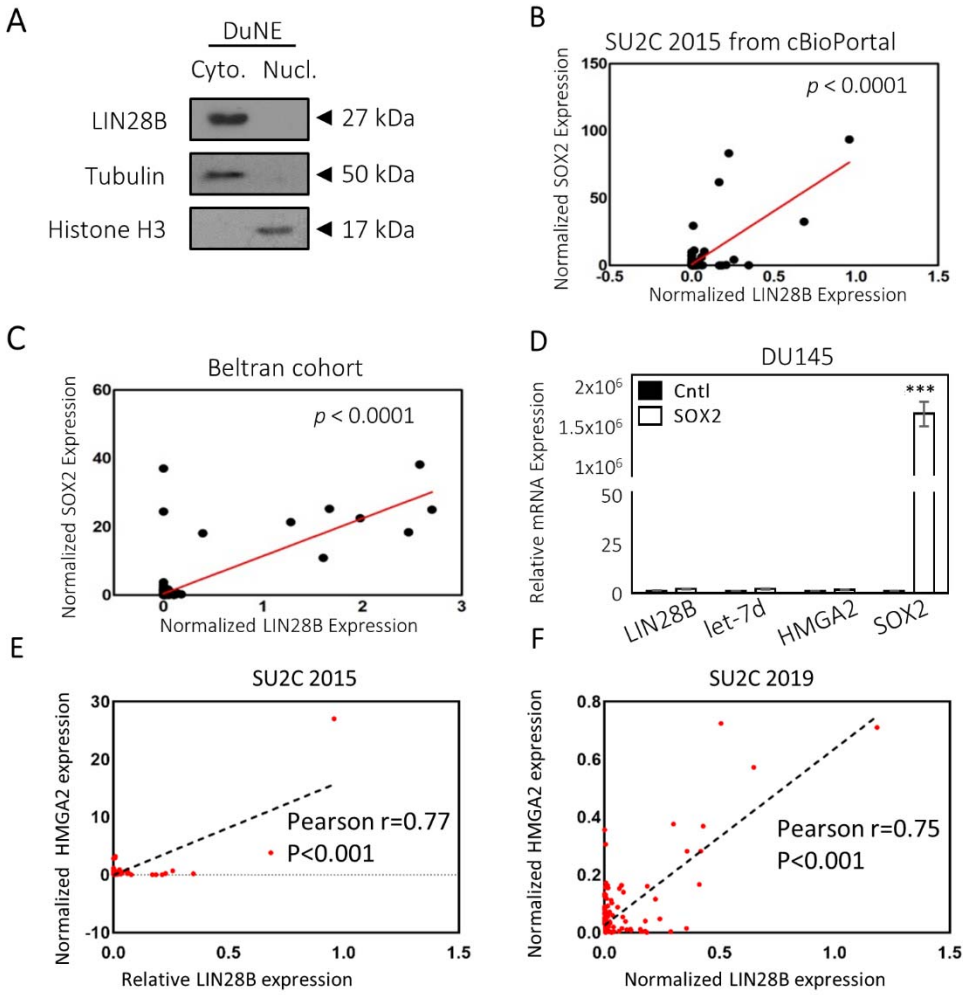
IV. Supplementary Figures

Figure S1



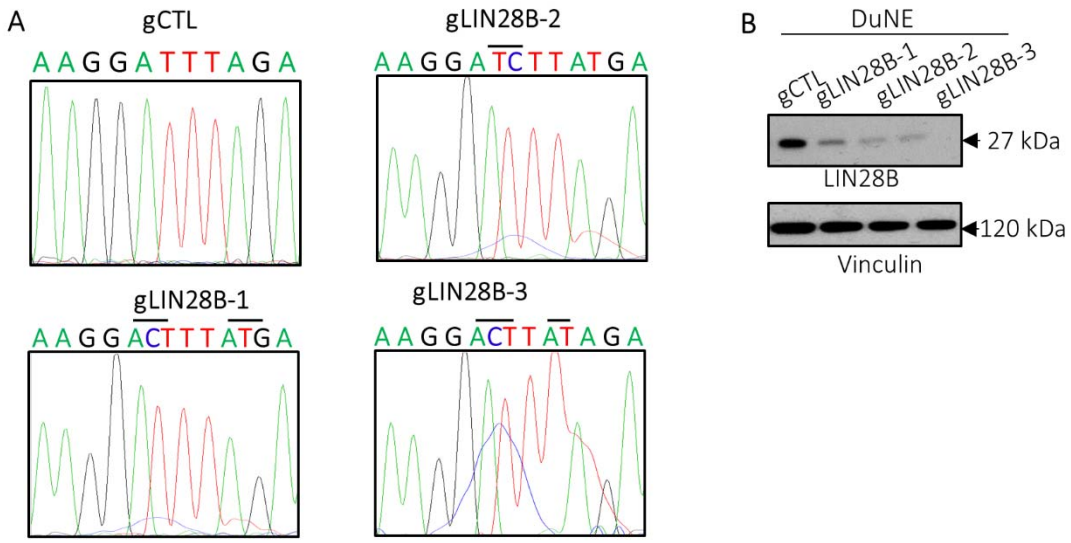
(A) LIN28B RNA expression from the Beltran 2016 patient cohort were plotted. **(B)** Pearson's correlation coefficient between *LIN28B* and *AR* expressions was r = -0.7 from the Beltran patient cohort. **(C)** *LIN28B* and *AR* are negatively correlated in SU2C 2015 cohort with Pearson's correlation coefficient of r = -0.46. **(D)** *LIN28B* expression in different cancer cell lines from Barretina et al. (2016). Results are presented as mean ± SD and statistical analyses were performed by one-way ANOVA or unpaired student's *t*-test with **, ***, denoting P<0.01, P<0.001, respectively.

Figure S2



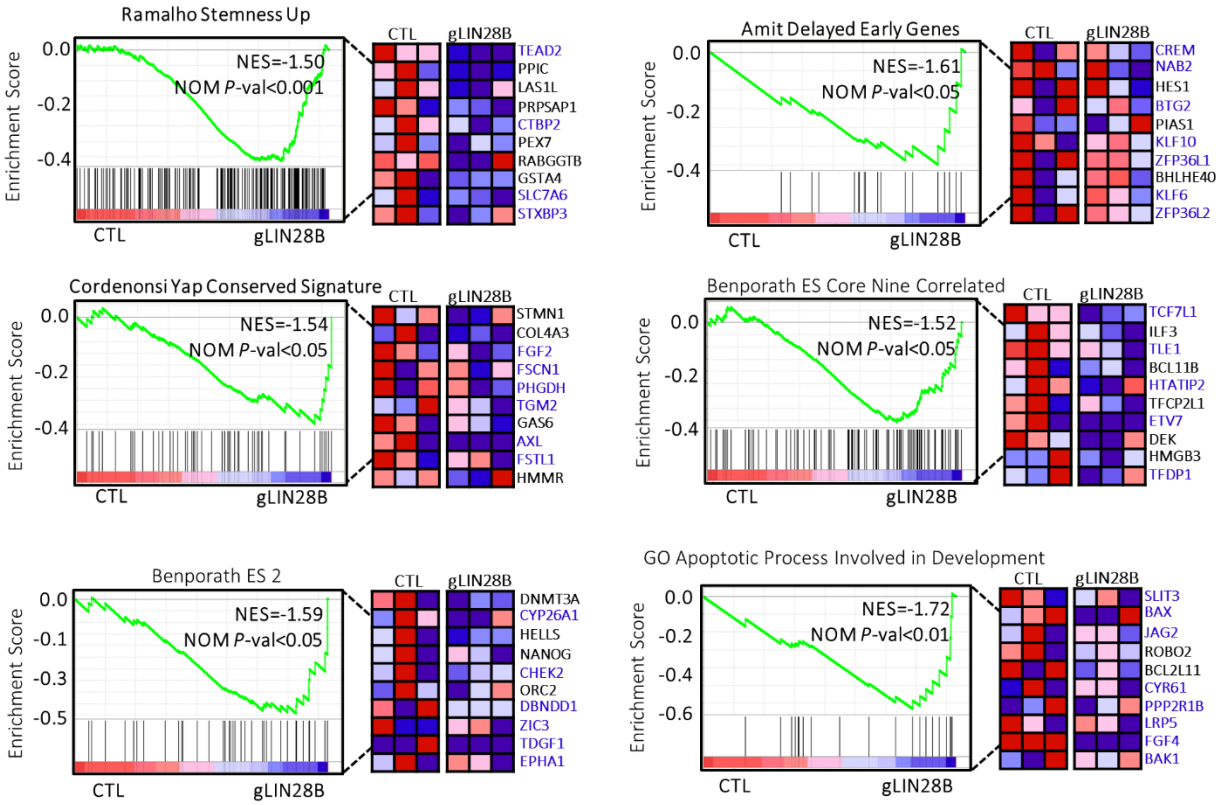
(A) DuNE cells were used to separate cytoplasmic and nuclear protein fractions. LIN28B protein was detected by immunoblotting. Histone H3 and tubulin were used as markers to confirm the efficacy of protein fractionation. **(B)** Pearson's correlation coefficient (r=0.73) between *LIN28B* and *SOX2* expressions from SU2C 2015 cohort. **(C)** Pearson's correlation coefficient (r =0.71) between *LIN28B* and *SOX2* expressions from the Beltran 2016 cohort. **(D)** DU145 cells were transiently transfected with the *SOX2* expression vector. Total RNA was extracted and used to measure LIN28B, let-7d, HMGA2, and SOX2 levels by real-time qPCR. Three independent biological replicates were performed for each experiment. All results are presented as mean ± SD. Statistical analyses were performed by student t-test, *** denoting p<0.001. **(E)** Pearson's correlation coefficient (r=0.77) between *LIN28B* and *HMGA2* expressions from SU2C (2015). **(F)** Pearson's correlation coefficient (r=0.75) between *LIN28B* and *HMGA2* expressions from SU2C (2019).

Figure S3



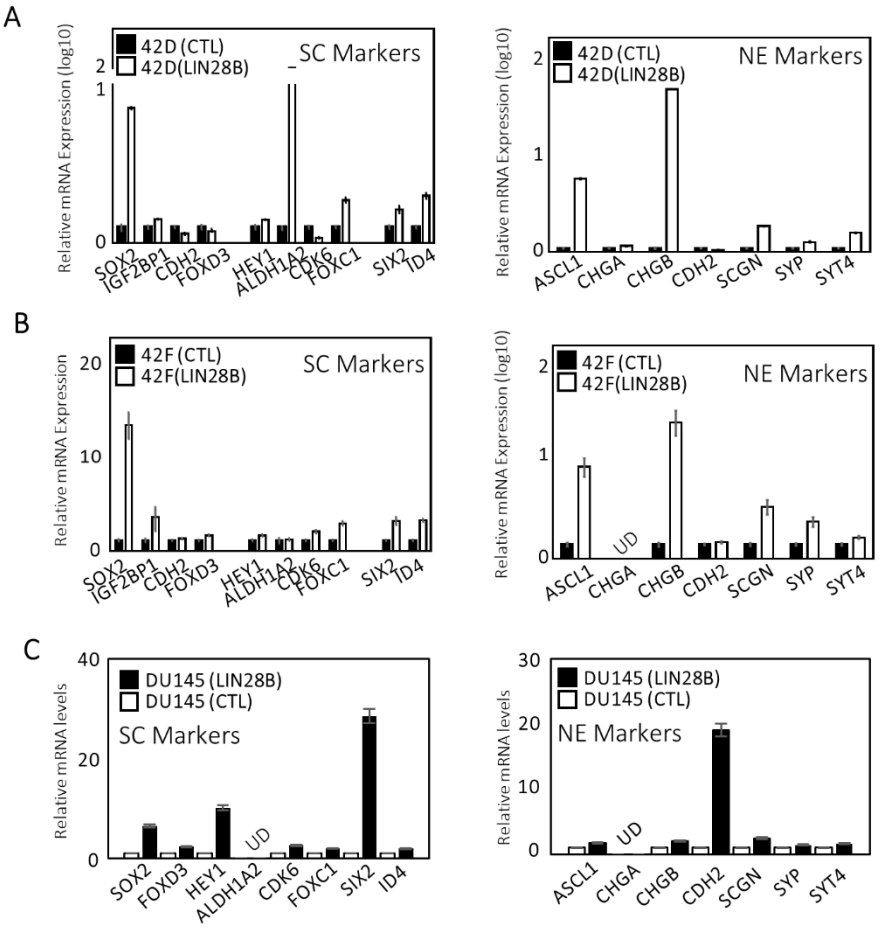
GeneArt CRISPR technology was used to knock out the *LIN28B* gene in the DuNE cell line. Three monoclones were used to validate LIN28B CRISPR by Sanger sequencing (A) and immunoblotting (B).

Figure S4



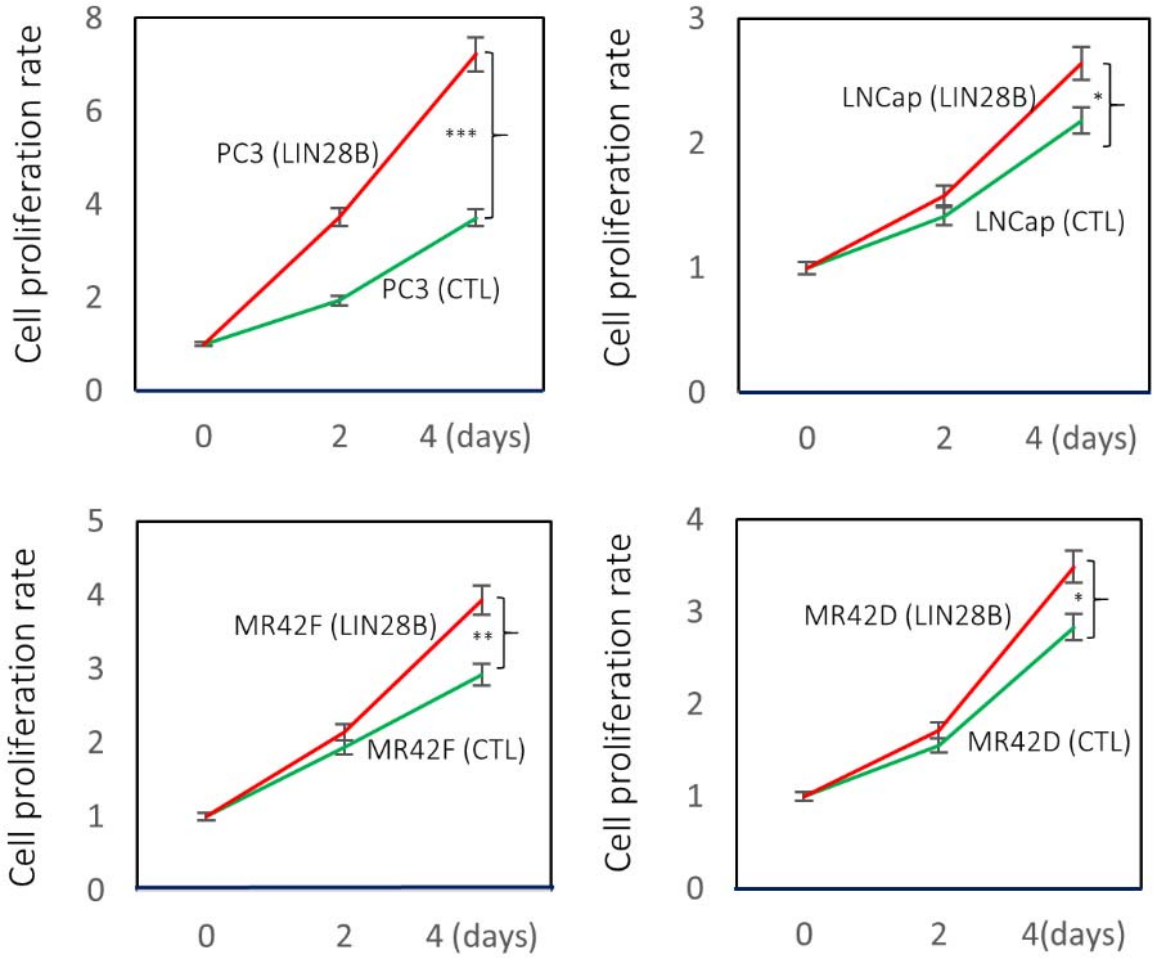
GSEA enrichment plots show the correlation of DuNE(gLIN28B) dataset (n=3302) with the GSEA gene sets in the lineage plasticity and embryogenesis subgroup from Figure 3A.

Figure S5



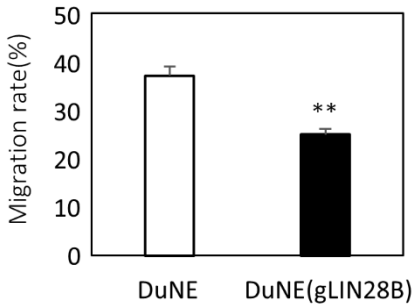
MR42D **(A)**, MR42F **(B)** and DU145 **(C)** cell lines were used to overexpress control or LIN28B expression vector. Real-time PCR was performed to measure stem cell (SC) and neuroendocrine (NE) biomarkers relatively to GAPDH. Three independent technical replicates were performed for each qPCR experiment. Note: *UD = undetermined*

Figure S6



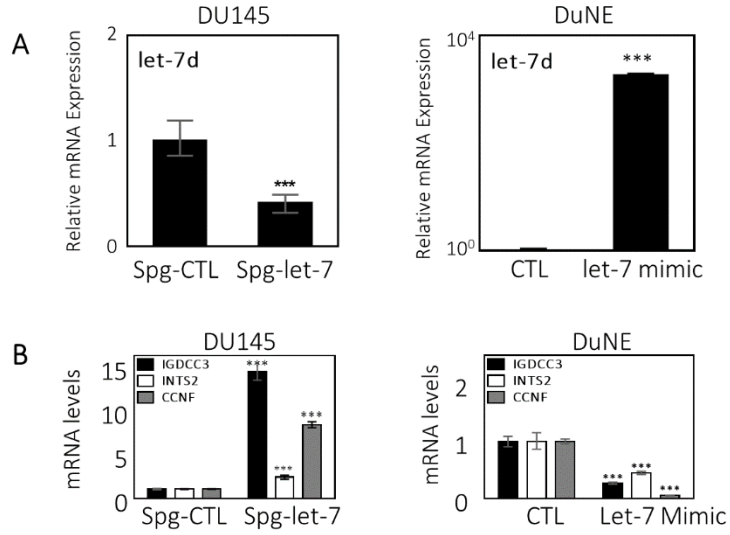
PC3, LNCaP, MR42F and MR42D cells overexpressing the control or LIN28B vector. Cell proliferation rates were measured by MTS assays. Results were normalized to the results from day 0. Statistical analyses were performed by student's *t*-test with **, ***, denoting $P<0.01$, $P<0.001$, respectively.

Figure S7



Wound healing assay measured DuNE and DuNE(gLIN28B) migration rates in 24 hours. The initiative unhealed area was used as a 100% control. Statistical analyses were performed by student's *t*-test with **, ***, denoting $P<0.01$, $P<0.001$, respectively.

Figure S8



DU145 cells were transfected with let-7 sponge vector (Addgene), while DuNE cells were transfected with let-7d mimic (Dharmacon). **(A)** Total RNA was extracted and used to measure let-7d expression levels by real-time qPCR. **(B)** The mRNA levels of IGDCC3, CCNF, and INTS2 were measured by real-time PCR. Three independent biological replicates were performed for each real-time PCR experiment. All results are presented as mean ± SD. Statistical analyses were performed by student's *t*-test with **, *** denoting $P<0.01$, $P<0.001$ respectively.