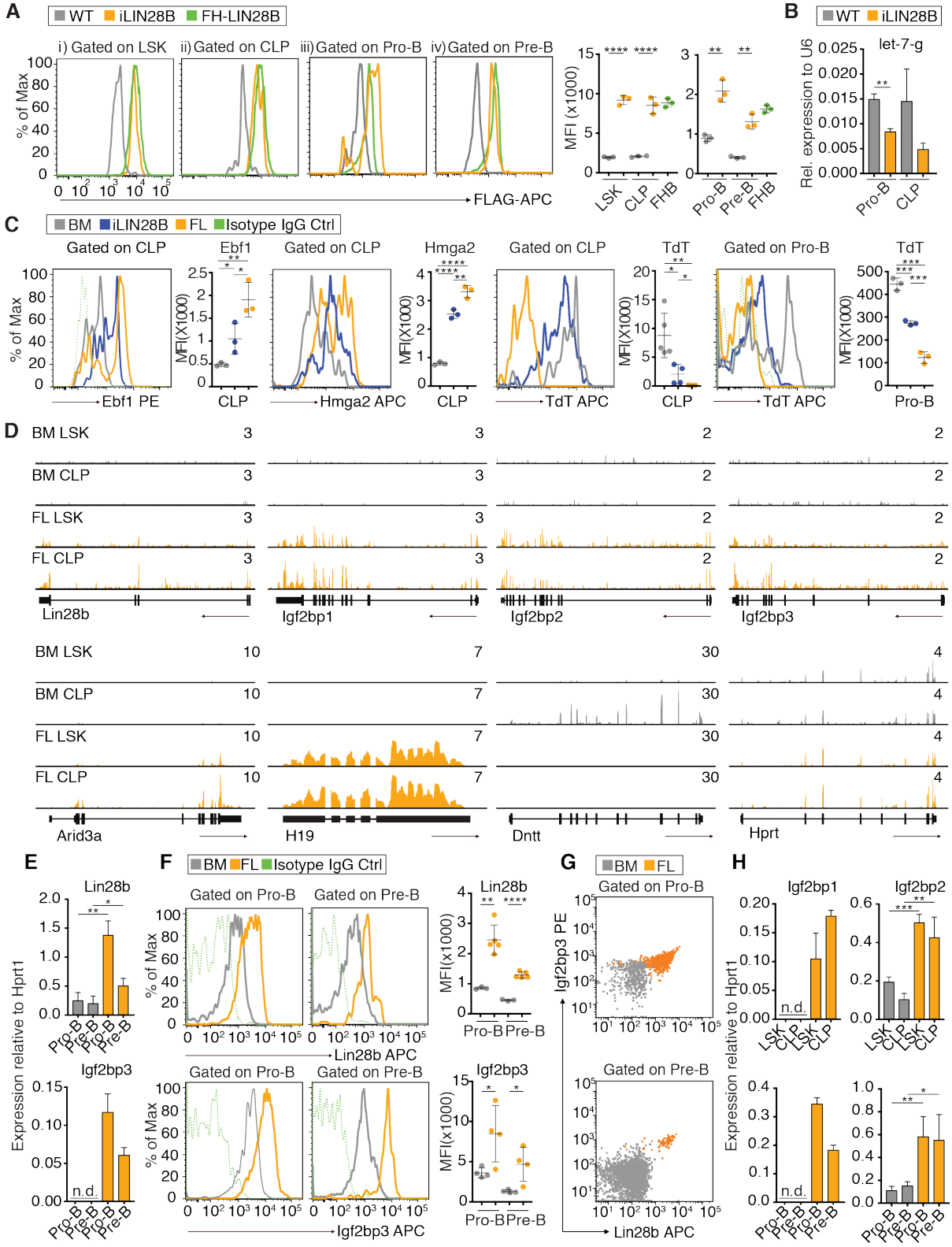
Supplemental Figures and Legends

Supplemental Figure S1



Supplemental Figure S1. Validation of scRNA-seq and low input RNA-seq.

(A) The flow cytometric histograms show FLAG epitope expression in (i) LSK, (ii) CLP, (iii) pro-B and (iv) pre-B cells from iLIN28B mouse and WT littermate control. FH-LIN28B-220-8 (FHB) cell line (green) included for comparison. The geometric mean of fluorescence intensities (MFI) from biological replicates are plotted. ** $P \leq 0.01$ and **** $P \leq 0.0001$ represent results of two-tailed *t*-test. Error bars represent standard deviation of three biological replicates.

(B) Levels of mature let-7g in CLP and pro-B cells from iLIN28B mouse and WT littermate control were measured using TaqMan assays and normalized to *Rnu6* (U6) snRNA. ** $P \leq 0.01$ represents result of two-tailed *t*-test. Error bars represent standard deviation of three biological replicates.

(C) The flow cytometric histograms show Ebf1, Hmga2 and TdT protein expression in CLP or pro-B (*right panel,* for TdT) cells from BM of wildtype (gray), iLIN28B mouse (blue) and E16.5 fetal liver (orange). Isotype IgG controls are also shown (green, dashed). The MFI from biological replicates are plotted. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and **** $P \leq 0.0001$ represent results of two-tailed *t*-test. Error bars represent standard deviation of three to five biological replicates.

(D) The genome browser tracks of indicated genes show coverage tracks for low-input RNA-seq described in Fig. 1C. *Dntt* is included as a known example of a transcript expressed in adult BM CLPs but not in FL.

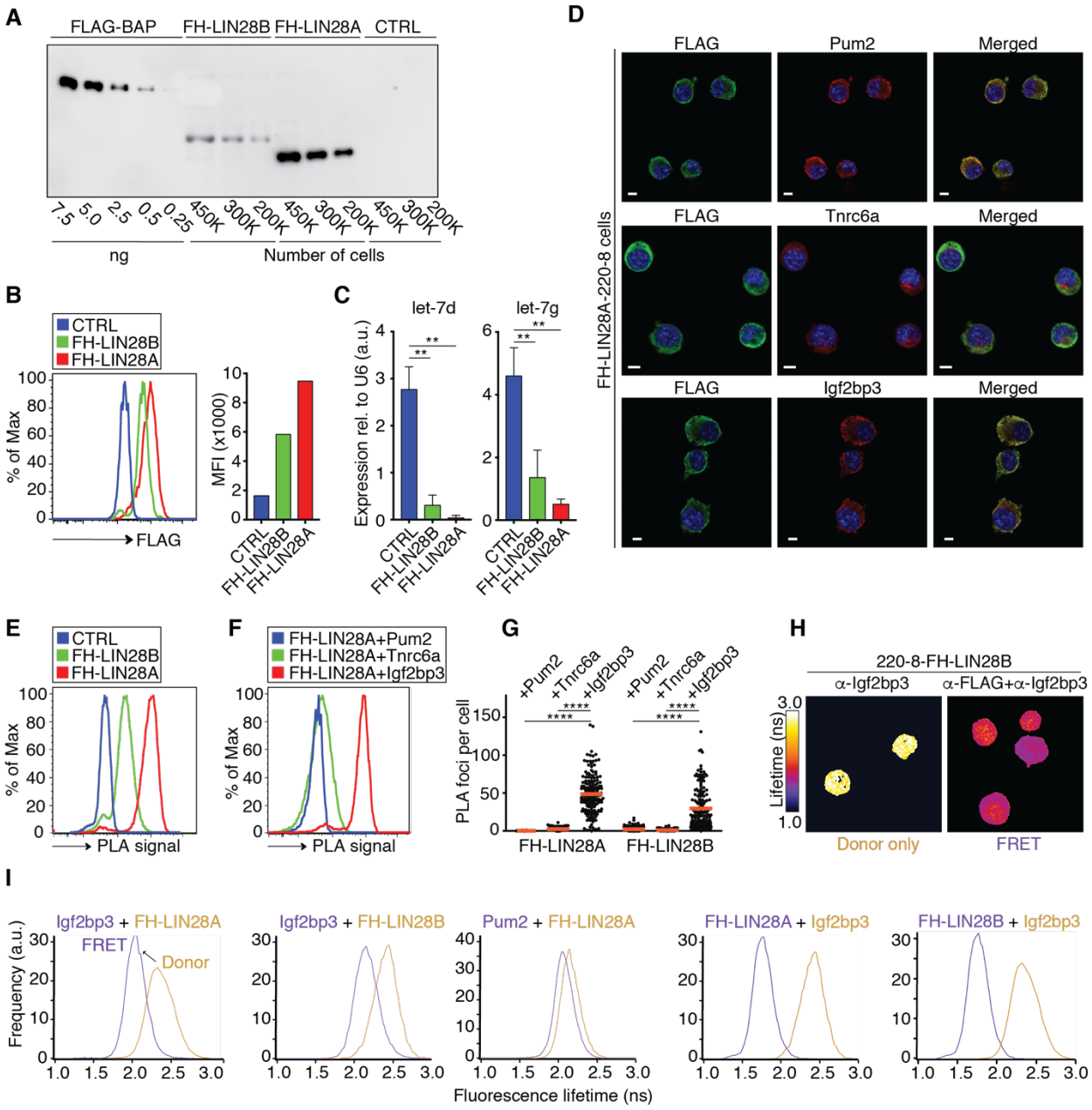
(E) The RT-qPCR analyses quantify *Lin28b* and *Igf2bp3* mRNA expression normalized to *Hprt1* in pro-B and pre-B cells sorted from FL (orange) or BM (gray). n.d. indicates not detectable. * $P \leq 0.05$, and ** $P \leq 0.01$ represent results of two-tailed *t*-test. Error bars represent standard deviation of three biological replicates.

(F) The flow cytometric histograms show Lin28b and Igf2bp3 expression in pro-B and pre-B cells from BM (gray) and FL (orange). Isotype IgG controls are also shown (green, dashed). The geometric mean of fluorescence intensities (MFI) from biological replicates are plotted. * $P \leq 0.05$, ** $P \leq 0.01$, and **** $P \leq 0.0001$ represent results of two-tailed *t*-test. Error bars represent standard deviation of three to five biological replicates.

(G) The dot plots quantify Lin28b (x-axis) and Igf2bp3 (y-axis) protein co-expression in pro-B and pre-B cells from BM and FL based on FACS. Dots represent individual cells color coded by sample.

(H) The RT-qPCR analyses quantify *Igf2bp1* and *Igf2bp2* mRNA expression normalized to *Hprt1* in LSK, CLP, pro-B and pre-B cells sorted from FL (orange) or BM (gray). n.d. indicates not detectable. * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$ represent results of two-tailed *t*-test. Error bars represent standard deviation of three biological replicates.

Supplemental Figure S2



Supplemental Figure S2. LIN28A and LIN28B are in close proximity to Igf2bp3 *in situ*.

(A) Lysates from different cell numbers were loaded for semi-quantitative Western analysis to estimate the copy number of FH-LIN28A and FH-LIN28B molecules per cell. Serial dilutions of recombinant FLAG-BAP protein (0.25 – 7.5 ng) were used as a standard for quantitation of signal from anti-FLAG detection.

(B) The flow cytometric histograms show FLAG epitope expression in wild-type 220-8 cells and transgenic 220-8 cells stably expressing FH-LIN28A or B (*left panel*). Mean fluorescence intensities are plotted (*right panel*).

(C) Levels of mature let-7d and let-7g in absence or presence FH-LIN28A or -B were measured using TaqMan assays and normalized to *Rnu6* (U6) snRNA. ** $P \leq 0.01$ represent results of two-tailed *t*-test. Error bars represent standard deviation of three biological replicates.

(D) Representative confocal images show immunofluorescence staining in FH-LIN28A-220-8 cells using indicated primary antibodies: anti-FLAG, anti-Igf2bp3, anti-Pum2, and anti-Tnrc6a. Secondary antibodies were anti-mouse Alexa488 and anti-rabbit Alexa555. Nuclei were stained with DAPI. Scale Bar = 5μm.

(E) Histograms show flow cytometric quantification of PLA signal in wild-type 220-8 cells and transgenic 220-8 cells expressing FH-LIN28A or B, indicating proximity between Igf2bp3 and FLAG epitopes.

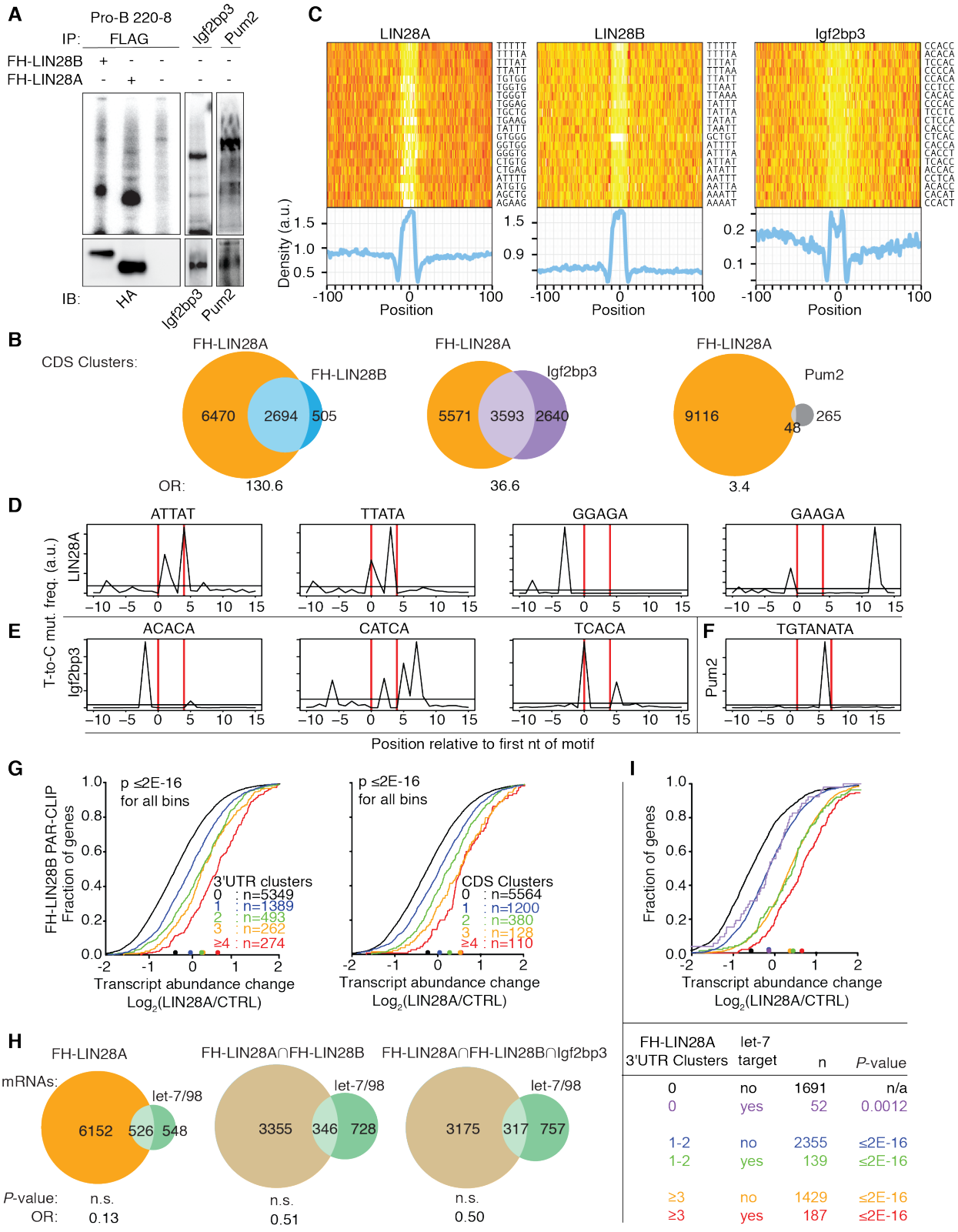
(F) Histograms show flow cytometric quantification of PLA signal in wild-type 220-8 cells and transgenic 220-8 cells expressing FH-LIN28A, comparing proximity between FLAG epitopes and Igf2bp3, Pum2, or Tnrc6a, respectively. Pum2 and Tnrc6a serve as specificity controls.

(G) Single cell PLA foci count in 220-8 cells transduced with either FH-LIN28A or FH-LIN28B are plotted to compare as in Supplemental Fig. S2F. Each dot in the graph represents a single cell with the number of PLA foci in that cell. The red bar indicates median foci number within each population. **** $P \leq 0.0001$ by Mann-Whitney Test.

(H) The representative microscopy images show fluorescence lifetime for FH-LIN28A and Igf2bp3 in FH-LIN28A-220-8 cells as determined by FLIM-FRET assay. Fluorescence lifetime of immunostaining with anti-Igf2bp3 conjugated to Alexa 488 dye only (*left panel*, donor only), or co-stained with anti-FLAG conjugated to Alexa 555 (*right panel*, FRET). The color scale indicates fluorescence lifetime (ns).

(I) The histograms depict reciprocal FLIM-FRET of FH-LIN28/Igf2bp3 pair in FH-LIN28A and FH-LIN28B-220-8 cells. In each sample, the donor antibody is indicated in orange. The FLAG/Pum2 pair represents a non-interacting protein pair in FH-LIN28A-220-8 cells and was used as negative control.

Supplemental Figure S3



FH-LIN28A 3'UTR Clusters	let-7 target	n	*P*-value
0	no	1691	n/a
0	yes	52	0.0012
1-2	no	2355	≤2E-16
1-2	yes	139	≤2E-16
≥3	no	1429	≤2E-16
≥3	yes	187	≤2E-16

Supplemental Figure S3. FH-LIN28A, FH-LIN28B, Igf2bp3 and Pum2 PAR-CLIPs.

(A) *Top panel*, The autoradiograph shows UV-crosslinked, immunoprecipitated, and radiolabeled FH-LIN28A, FH-LIN28B, Igf2bp3, and Pum2 RNPs that were fractionated on an SDS-PAGE. *Bottom panel*, Western blots of immunoprecipitates were probed with anti-HA for FH-LIN28A or -B, anti-Igf2bp3, and anti-Pum2.

(B) The Venn diagrams show CDS binding sites (clusters) identified in FH-LIN28A PAR-CLIP that overlap by at least one nt with FH-LIN28B, Igf2bp3, and Pum2, respectively.

(C) *Top panels*, Heatmaps indicate the relative enrichment (see Methods) of the 5-mer motifs within ±100 bp around FH-LIN28A (*left panel*), FH-LIN28B (*middle panel*), and Igf2bp3 binding sites (*right panel*). Here we compared k-mer enrichment in FH-LIN28A/B vs. Igf2bp3. *Bottom panels,* Lineplots show the relative density of occurrence of the 20 top 5-mer sequence motifs within ±100 bp of the PAR-CLIP binding sites.

(D) Lineplots show the relative frequency of crosslinking induced mutations (T-to-C) mutations within ±10 bp of the indicated motif for FH-LIN28A (*top panels*).

(E) Same as in **(D)**, only for the Igf2bp3 PAR-CLIP.

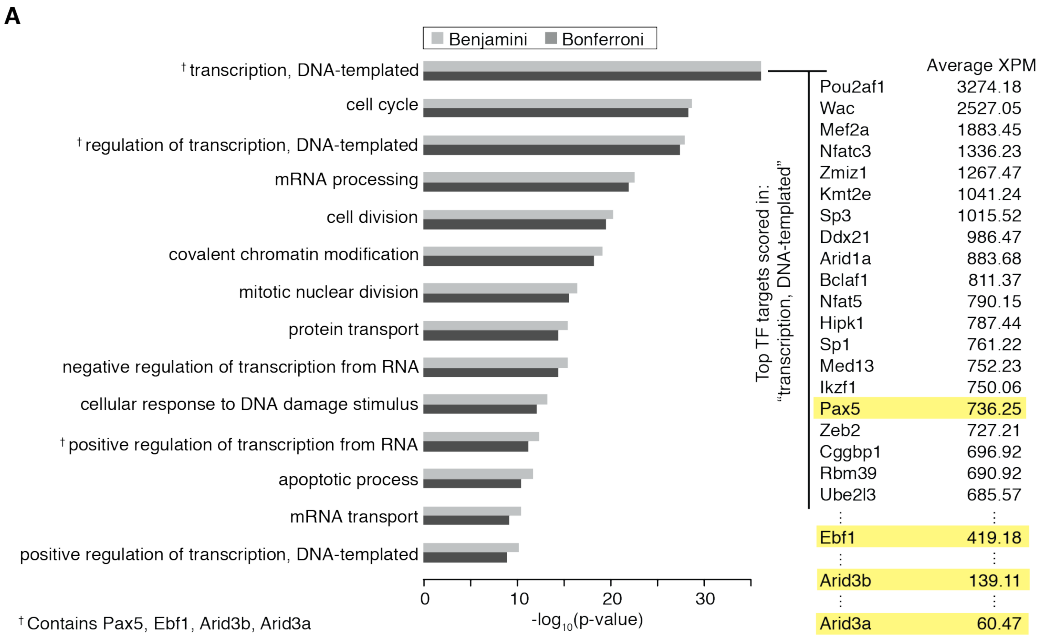
(F) Same as in **(D)**, only for the Pum2 PAR-CLIP.

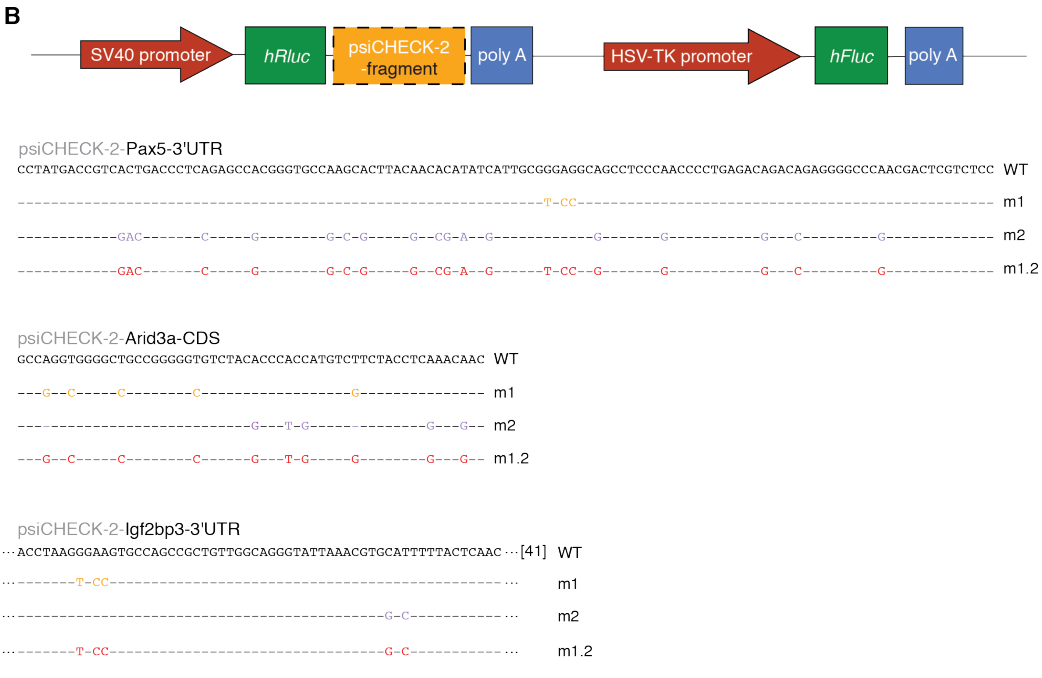
(G) Cumulative distribution of log-transformed fold changes of mRNA expression was plotted, comparing 220-8 cells tranduced with LIN28 or empty vector (CTRL). FH-LIN28B-target mRNAs are binned based on the number of PAR-CLIP binding sites in the 3'UTR (*left)* or the CDS (*right*). Significance was determined using a two-sided Kolmogorov-Smirnov (KS) test. Bin sizes are indicated.

(H) Venn diagrams show numbers of mRNAs co-targeted by let-7 (based on TargetScan predictions) and FH-LIN28A; or both FH-LIN28A/B; or both Igf2bp3 and FH-LIN28A/B. Not significant is denoted by n.s. The odds ratio (OR) of each overlap is indicated.

(I) Cumulative distribution analysis of change in mRNA expression comparing 220-8 cells tranduced with LIN28A or empty vector (CTRL). FH-LIN28A-target mRNAs are binned based on the number of PAR-CLIP binding sites in the 3'UTR (either 0, 1-2 or ≥3) and whether they were predicted to be a let-7 target or not. Significance was determined using a two-sided Kolmogorov-Smirnov (KS) test. Bin sizes are indicated.

Supplemental Figure S4



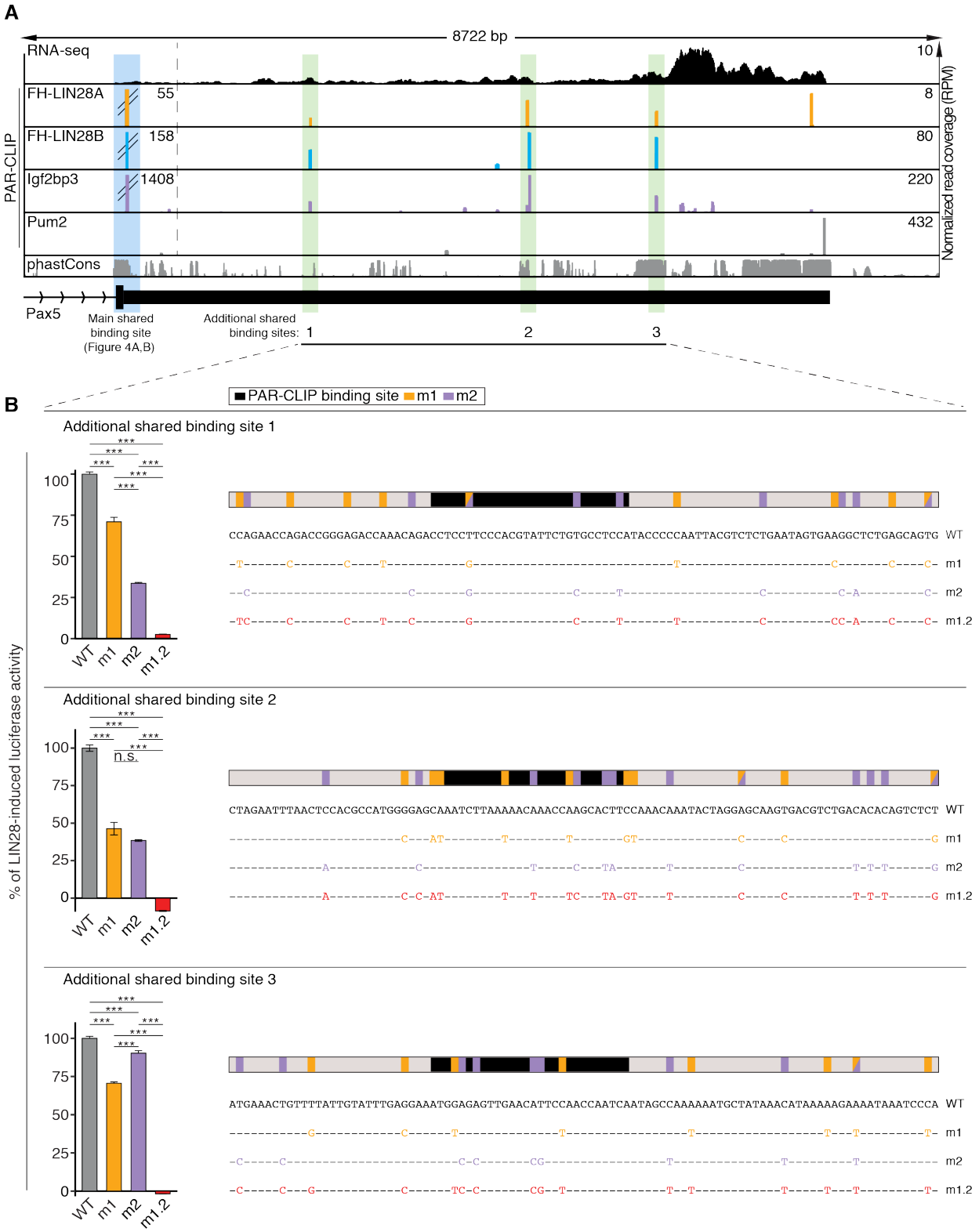


Supplemental Figure S4. Gene ontology analysis of shared PAR-CLIP targets and luciferase reporter constructs.

(A) Benjamini (light gray) and Bonferroni (dark gray) corrected *P*-values are plotted for the top enriched Biological Process GO categories, as calculated by DAVID. The top 2,500 shared targets ([FH-LIN28A ∪ FH-LIN28B] ∩ Igf2bp3) based on average PAR-CLIP XPM were used as input. The top transcription factors from the category "transcription, DNA templated" are listed, ordered by average XPM (*right*).

(B) The schematics depict the dual luciferase reporter constructs used in the assays for Fig. 4B, 4G, and 5B. Sequence of the unmutated (WT) 117 bp fragment from 3'UTR of *Pax5*, 56 bp fragment from *Arid3a* CDS and 131 bp fragment from *Igf2bp3* 3'UTR are shown. The first set of mutations (m.1) alter the putative Lin28 ZnF RRE. The second set of mutations (m.2) alter all the potential Igf2bp3 RREs. Mutated bases are shown in orange (m.1) or purple (m.2). The third combines both sets of mutations (m1.2, red).

Supplemental Figure S5

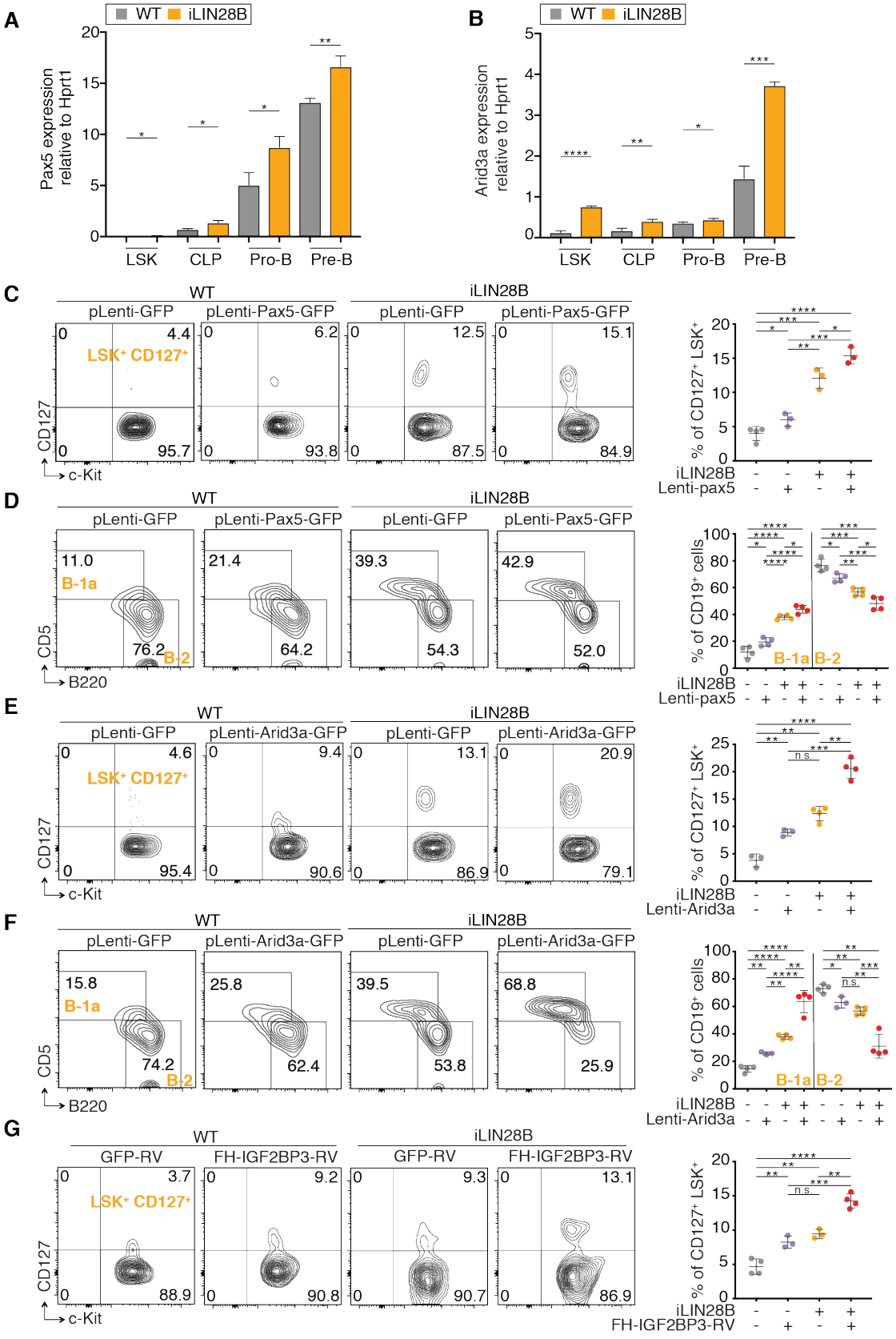


Supplemental Figure S5. Luciferase reporter constructs and results for additional shared binding sites in the *Pax5* 3'UTR

(A) Genome browser track of the last exon of *Pax5*, including the entire 3'UTR and other shared binding sites. The strongest shared binding site depicted in Fig. 4A is highlighted in blue, with its PAR-CLIP coverage truncated to increase visibility of other sites in the 3'UTR. Additional putative binding sites in the 3'UTR are highlighted in green.

(B) Luciferase reporter assays were performed for these additional fragments with or without mutations to the LIN28 motifs (m1), the Igf2bp3 motifs (m2) or both (m1.2). The results and mutations are visualized as described for Fig. 4B and Supplemental Fig. S4B.

Supplemental Figure S6



Supplemental Figure S6. *In vivo* validation of *Pax5*, *Arid3a* and *Igf2bp3* as shared targets of LIN28 and Igf2bp3.

(A,B) RT-qPCR analyses quantify *Pax5* and *Arid3a* mRNA expression normalized to *Hprt1* in LSK, CLP, pro-B and pre-B cells sorted from WT and iLIN28B mice from same litter. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, and **** $P \leq 0.0001$ represent results of two-tailed *t*-test. Error bars represent standard deviation of three biological replicates.

(C-F) Adult BM HSPCs from WT and iLIN28B mice were transduced with empty vector (pLenti-GFP), **(C,D)** pLenti-Pax5-GFP or **(E,F)** pLenti-Arid3a-GFP and transplanted in *Rag1*$^{-/-}$ recipients. **(C,E)** Representative contour plots depict flow cytometric analyses of CD127^{+} LSK^{+} HSPCs in BM of the indicated chimeric mice 4-6 weeks post-transplantation. *Right panel*s, Percentages of LSK^{+} CD127^{+} cells in multiple independent BM chimeras are plotted. **(D,F)** Representative contour plots depict flow cytometric analyses of B-1a (CD19^{+}B220$^{lo/-}$ CD5^{+}) and B-2 (CD19^{+} B220^{hi} CD5^{-}) cells among CD19^{+} GFP^{+} cells in peritoneal cavity of the indicated chimeric mice 4-6 weeks post-transplantation. *Right panel*s, Percentages of B-1a and B-2 cells in multiple independent BM chimeras are plotted. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$ represent results of two-tailed *t*-test. Error bars represent standard deviation of three to four biological replicates.

(G) Adult BM HSPCs from WT and iLIN28B mice were transduced with empty vector (GFP-RV) or FH-IGF2BP3-RV, and transplanted in *Rag1*$^{-/-}$ recipients. Representative contour plots depict flow cytometric analyses of B-1a and B-2 cells among CD19^{+} GFP^{+} cells in peritoneal cavity of indicated BM chimeric mice 4-6 weeks post-transplantation. *Right panel*, Percentages of B-1a and B-2 cells in multiple independent BM chimeras are plotted. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$ represent results of two-tailed *t*-test. Error bars represent standard deviation of three to four biological replicates.

Supplementary Tables and Legends

Supplemental Table 1. Summary statistics for single-cell RNA-seq of CLPs

Sample name	Starting cell #	Detected cell #	Post-filter cell #	Total reads	Reads in cells	Mean reads per cell	Median genes per cell	Total genes detected	Median UMI counts per cell	Sequencing saturation
WT BM	4000	1087	858	37,393,092	91.8%	34400	2086	14129	5356	65.9%
WT BM +Dox	4000	1653	1083	36,526,664	92.0%	22097	1871	14464	4500	60.5%
iLIN28B +Dox	4000	1638	1238	40,948,667	93.3%	24999	1930	14155	4800	61.3%
FL	4000	1127	653	35,125,028	86.3%	31166	1668	14158	3962	67.9%

Supplemental Table 2. Summary statistics for low-input RNA-seq of LSKs and CLPs

Sample name	Total reads	Uniquely mapped reads	Uniquely mapped reads %	Multi-mapped	Unmapped too short	Unmapped other	Total mapped	Total unmapped
BM_LSK	18,870,288	11,786,522	62.5%	832,343	32.4%	0.24%	12,618,865	7,916,109
BM_CLP	26,824,642	16,120,702	60.1%	1,124,129	35.2%	0.11%	17,244,831	11,828,069
FL_LSK	19,912,754	12,498,331	62.8%	913,033	32.2%	0.13%	13,411,364	8,327,456
FL_CLP	19,806,013	12,865,772	65.0%	964,631	29.8%	0.09%	13,830,403	7,904,872

Supplemental Table 3. Proteins interacting with FH-LIN28A in 220-8 pro-B cells determined by affinity purification and mass spectrometry.

Protein name	NCBI link	Mass (kDa)	Spectrum counts (no RNase)	Spectrum counts (+RNase)	%Sequence coverage
Protein lin-28 homolog A	81914711	22.7	414	398	73%
Polyadenylate-binding protein 1	341941223	70.6	185	66	61%
Insulin-like growth factor 2 mRNA-binding protein 3	81916748	63.5	69	12	53%
Polypyrimidine tract-binding protein-associated-splicing factor	68566057	75.4	76	1	29%
Fragile X mental retardation syndrome-related protein 1	25090336	76.2	60	1	48%
Lupus La protein homolog	417240	47.7	264	0	62%
Ras GTPase-activating protein-binding protein 1	14916571	51.8	86	0	50%
A-kinase anchor protein 9	300681022	435.9	82	0	22%
Putative helicase MOV-10	50403726	113.5	75	0	45%
60 kDa SS-A/Ro ribonucleoprotein	12643534	60.1	71	0	44%
Ataxin-2-like protein	52000711	110.6	65	0	32%
Non-POU domain-containing octamer-binding protein	67460966	54.5	56	0	48%
Nuclear fragile X mental retardation-interacting protein 2	81862045	75.6	53	0	35%

Supplemental Table 4. Summary statistics for PAR-CLIP in 220-8 pro-B cells

PAR-CLIP	Total reads	Uniquely aligned	%Uniquely aligned	3'UTR clusters	CDS clusters	Transcripts targeted
FH-LIN28A_1	34,027,694	8,167,808	25.3%	25640	19360	9294
FH-LIN28A_2	58,260,755	14,068,227	24.2%	33397	25406	10167
FH-LIN28A_3	50,535,146	11,354,555	22.5%	25923	19153	9542
FH-LIN28A_4	42,963,267	1,758,861	4.2%	9338	4734	5664
FH-LIN28B_1	61,660,584	1,874,937	3.1%	4996	3805	4580
FH-LIN28B_2	84,198,466	4,020,255	4.8%	15799	9198	7887
FH-LIN28B_3	53,483,484	3,369,146	6.4%	7470	4114	5296
FH-LIN28B_4	47,212,819	3,852,105	8.3%	8630	4461	5825
Igf2bp3_1	27,543,980	6,632,891	24.2%	21287	14844	8457
Igf2bp3_2	46,551,091	6,231,498	13.4%	22093	13185	8376
Igf2bp3_3	20,594,088	1,446,708	7.1%	8058	5401	5201
Igf2bp3_4	124,649,193	13,319,556	10.9%	14030	7251	6219
Pum2	19,220,265	1,483,886	7.8%	6685	568	3691

Supplemental Table 5. The coordinates, number of sequence reads, and number of crosslinked sequence reads for binding sites of FH-LIN28A, FH-LIN28B, Igf2bp3, and Pum2 are shown. Data for each RBP are found in separate sheets. FH-LIN28A, FH-LIN28B, and Igf2bp3 binding sites reported that were found in at least three out of four biological replicates.
(please see Excel file online)

Supplemental Table 6. Z-scores of 5-mers highlighted in Fig. 3E grouped according to the same colors.

	5-mer	LIN28	Igf2bp3	Pum2
	GGAGA	3.70	0.82	0.59
	GAAGA	4.09	0.70	0.09
	AGAGA	2.96	0.82	0.36
	AAAAA	2.97	0.21	2.66
	UUUUU	4.67	0.78	1.95
	AGAAG	4.37	0.90	0.07
	UGGAG	3.84	0.95	1.57
	GAGAA	3.26	0.87	0.33
	AUUUU	4.70	0.84	0.45
	UGGAA	3.23	1.32	0.62
	UUUCC	2.87	1.16	1.29
	UCAUC	0.95	2.84	-0.33
	CACAG	0.92	3.31	0.71
	CACAC	0.37	3.49	1.27
	UCACA	0.49	3.91	0.12
	CAUCA	1.46	4.39	-0.06
	CUUCA	2.52	3.80	0.31
	CAGCC	1.51	3.62	0.58
	CAGCA	0.96	3.65	0.76
	CUCUG	1.43	2.87	1.08
	CCACA	1.27	2.71	0.53
	ACACA	0.31	3.92	1.82
	CCAUC	0.76	2.91	-0.01
	CACCA	0.78	3.63	0.77
	CAUCU	1.54	3.43	0.20
	CUCCA	2.39	2.87	0.38
	UCUUC	3.59	3.34	0.31
	CUUCU	2.35	2.08	0.48
	CUUCC	3.11	2.53	1.00
	CAUUU	2.56	2.65	0.96
	UUCCA	2.82	2.77	0.47
	UGCUG	2.84	1.71	0.71
	UCUCU	2.48	2.27	0.78
	UUUCU	3.84	2.13	1.3
	CUCCU	2.29	2.28	0.58
	UAUAU	-1.64	-1.47	4.26
	GUAAA	-2.03	-0.52	3.62
	AUGUA	-2.16	-0.64	4.24
	UGUAC	-1.19	-0.71	3.47
	UGUAA	-1.84	-0.71	5.01
	UGUAU	-1.67	-0.33	4.94
	UUGUA	-1.79	-0.39	4.80
	AUAUA	-2.86	-1.53	3.31
	UUUGU	0.13	0.11	3.36
	CUGUA	-0.76	-0.18	3.38

Supplemental Table 7. The table summarizes the PAR-CLIP results across the transcriptome. The number of binding sites and crosslinked reads for different annotation categories (5'UTR, 3'UTR, CDS, and intron) are shown. The gene expression levels in WT 220-8 and LIN28A-220-8 are also shown. Predicted let-7 target genes are indicated. (please see Excel file online)

Supplemental Table 8. The table summarizes data for luciferase reporter assays presented in Fig. 4B (Pax5), 4G (Arid3a), 5B (Igf2bp3), and Supplemental Fig. S5B (Pax5, additional sites). The schematics of reporter constructs are described in Supplemental Fig. S4B. Raw data are available upon request.

Supplemental Table 9. List of antibodies and staining reagents used in this study

Antigen/description	Antibody (clone)	Conjugate	Applications and dilutions	Source
mouse/human β-actin	mouse (AC-15)	HRP	WB (1:25,000)	Sigma #A3854
mouse/human α-tubulin	B-5-1-2		WB (1:2000)	Sigma #T5168
FLAG® epitope	mouse (M2)		IF (1:100), WB (1:2000)	Sigma #F1804
FLAG® epitope	mouse (M2)	Cy3	IF (1:100)	Sigma #A9594
Anti-FLAG Magnetic Beads	mouse (M2)		PAR-CLIP, IP	Sigma #M8823
mouse/human PUM2	rabbit		IF (1:200)	Sigma #HPA030316
mouse/human PUM2	rabbit		PAR-CLIP (1:120), WB (1:10000)	Bethyl #A300-202A
HA epitope	rabbit (C29F4)		WB (1:1000)	CST #3724
mouse/human Igf2bp3	rabbit		PAR-CLIP (1:50), IF (1:800), IP (1:50), WB (1:1000), FACS (1:100)	Millipore #03-198
mouse/human LIN28A	rabbit		WB (1:1000)	Millipore #03-105
mouse LIN28B	rabbit		WB (1:1000), FACS (1:100)	CST #5422
human LIN28B	rabbit		WB (1:1000)	CST #4196
Alexa Fluor™ 647 Antibody Labeling Kit	n/a	Alexa647	FACS (primary antibody labeling)	Thermo Fisher #A20186
Alexa Fluor™ 555 Antibody Labeling Kit	n/a	Alexa555	FACS (primary antibody labeling)	Thermo Fisher #A20187
DYKDDDDK (aka FLAG epitope)	rabbit	Alexa647	FACS (1:50)	CST #3196
mouse/human TNRC6A (GW182)	rabbit		IF (1:200)	Abcam #ab156173
mouse IgG (H+L chains)	goat (highly cross-adsorbed)	Alexa555	IF (1:500)	Life Tech #A-21422
mouse IgG (H+L chains)	goat (highly cross-adsorbed)	Alexa488	IF (1:500)	Life Tech #A-11029
rabbit IgG (H+L chains)	goat (highly cross-adsorbed)	Alexa555	IF (1:500)	Life Tech #A-21428
rabbit IgG (H+L chains)	goat (highly cross-adsorbed)	Alexa488	IF (1:1000)	Life Tech #A-11008
rabbit IgG (H+L chains)	goat (highly cross-adsorbed)	Alexa647	FACS (1:1000)	Thermo Fisher #A-21242
mouse IgG (L chain)	goat (highly cross-adsorbed)	HRP	WB (1:3000)	Jackson Immuno #115-035-174

rabbit IgG (H+L chains)	goat (affinity purified)	HRP	WB (1:3000)	CST #7074
mouse Ebf1	mouse (T26-818)	PE	FACS (1:100)	BD #T26-818
human/mouse/rat Hmga2	rabbit		FACS (1:100)	CST #5269
mouse/human TdT	mouse (19-3)	APC	FACS (1:100)	Miltenyi 130-100-749
mouse/human Pax5	rat (1H9)	APC	FACS (1:100)	eBioscience #17-9918-80
mouse/human Arid3a	rabbit		FACS (1:100)	Sigma #AV32869
rabbit IgG isotype CTRL	rabbit (PP64)		FACS (1:100)	Millipore #637709
IgG isotype CTRL	rat (eBR2a)	PE	FACS (1:100)	eBioscience #12-4321-80
IgG isotype CTRL	rat (RTK4530)	APC	FACS (1:100)	BioLegend #400611
IgG isotype CTRL	mouse (MOPC-21)	PE	FACS (1:100)	BD #554680
mouse Sca-1 (Ly-6A/E)	rat (D7)	PerCP-Cy5.5	FACS (1:100)	eBioscience #45-5981-82
mouse CD117 (c-Kit)	rat (2B8)	APC	FACS (1:100)	BioLegend #105812
mouse CD117 (c-Kit)	rat (ACK2)	APC-eFluor 780	FACS (1:100)	eBioscience #47-1171-82
mouse CD117 (c-Kit)	rat (2B8)	eFluor 450	FACS (1:100)	eBioscience #48-1171-82
mouse CD127 (IL7Rα)	rat (A7R34)	PE	FACS (1:100)	BioLegend #135010
mouse/human CD45R (B220)	rat (RA3-6B2)	eFluor 450	FACS (1:100)	eBioscience #48-0452-82
mouse/human CD45R (B220)	rat (RA3-6B2)	PerCP-Cy5.5	FACS (1:100)	BioLegend #103236
mouse IgM	rat (RMM-1)	FITC	FACS (1:100)	BioLegend #406505
mouse IgM	rat (RMM-1)	PerCP-Cy5.5	FACS (1:100)	BioLegend #406512
mouse CD25 (IL2Rα)	rat (3C7)	PE	FACS (1:100)	BioLegend #101904
mouse CD5	rat (53-7.3)	eFluor 450	FACS (1:100)	eBioscience #48-0051-82
mouse CD19	rat (eBio1D3)	PE	FACS (1:100)	eBioscience #12-0193-82
mouse CD23	rat (B3B4)	PE	FACS (1:100)	BioLegend #101608
mouse CD1d	rat (1B1)	APC	FACS (1:100)	BioLegend #123522
mouse hematopoietic Lineage (Lin) cocktail	rat (145-2C11, RB6-8C5, M1/70, RA3-6B2, Ter-119)	FITC	FACS (1:100)	BioLegend #133302
100nm, DOPC/CHOL Liposomes	n/a	PE	FACS (1:100)	FormuMax #F60103F-NBD

Supplemental Table 10. List of oligonucleotides used in plasmid construction

Name of construct	Forward Oligo (5'→3')	Reverse Oligo (5'→3')
pMSCVpuro-FH-LIN28A	GGAGATCTACCATGGACTACAAGGACGACGATG	GGCTCGAGTCAATTCTGTGCCTCCGGGAG
pMSCVpuro-FH-LIN28B	GCCGGAATTAGATCTCACCATGGACTACAAGGAC	TCCCCTACCCGGTAGTTATGTCTTTTTCCTTTTTTGAAC
pGFP-RV-LIN28A	CTAGGCGCCGGAATTAACCATGGGCTCCGTGTCC	TCGATACCGTCGACCTAATTCTGTGCCTCCGGG
pGFP-RV-LIN28B	CTAGGCGCCGGAATTAACCATGGCCGAAGGCGGG	TCGATACCGTCGACCTTATGTCTTTTTCCTTTTTTGAACTGAAGGCCC
pMSCVneo-FH-IGF2BP3	GGGAATTCACCATGGACTACAAGGACGACGATG	GGCTCGAGTTACTTCCGTCTTGACTGAGG
pGFP-RV-FH-IGF2BP3	CTAGGCGCCGGAATTAATGGACTACAAGGACGAC	TCGATACCGTCGACCTTACTTCCGTCTTGACTG
psiCHECK-2-Igf2bp3-3'UTR WT	TGTCTCCTGTTTCTCTAACACTAACATGGATAACCTAAGGGAAGTGCCAGCCGCTGTTGGCAGGGTATTAAACGTGCATTTTTACTCAACTACCTCAGGTATTCAGTAATACAGTTAAAAGCAAAATTATT	AATAATTTTGCTTTTAACTGTATTACTGAATACCTGAGGTAGTTGAGTAAAAATGCACGTTTAATACCCTGCCAACAGCGGCTGGCACTTCCCTTAGGTTATCCATGTTAGTGTTAGAGAAACAGGAGACA
psiCHECK-2-Igf2bp3-3'UTR m1	TGTCTCCTGTTTCTCTAACACTAACATGGATAACCTAAGTGCCGTGCCAGCCGCTGTTGGCAGGGTATTAAACGTGCATTTTTACTCAACTACCTCAGGTATTCAGTAATACAGTTAAAAGCAAAATTATT	AATAATTTTGCTTTTAACTGTATTACTGAATACCTGAGGTAGTTGAGTAAAAATGCACGTTTAATACCCTGCCAACAGCGGCTGGCACGGCACTTAGGTTATCCATGTTAGTGTTAGAGAAACAGGAGACA
psiCHECK-2-Igf2bp3-3'UTR m2	TGTCTCCTGTTTCTCTAACACTAACATGGATAACCTAAGGGAAGTGCCAGCCGCTGTTGGCAGGGTATTAAACGTGGACTTTTACTCAACTACCTCAGGTATTCAGTAATACAGTTAAAAGCAAAATTATT	AATAATTTTGCTTTTAACTGTATTACTGAATACCTGAGGTAGTTGAGTAAAAGTCCACGTTTAATACCCTGCCAACAGCGGCTGGCACTTCCCTTAGGTTATCCATGTTAGTGTTAGAGAAACAGGAGACA
psiCHECK-2-Igf2bp3-3'UTR m1.2	TGTCTCCTGTTTCTCTAACACTAACATGGATAACCTAAGTGCCGTGCCAGCCGCTGTTGGCAGGGTATTAAACGTGGACTTTTACTCAACTACCTCAGGTATTCAGTAATACAGTTAAAAGCAAAATTATT	AATAATTTTGCTTTTAACTGTATTACTGAATACCTGAGGTAGTTGAGTAAAAGTCCACGTTTAATACCCTGCCAACAGCGGCTGGCACGGCACTTAGGTTATCCATGTTAGTGTTAGAGAAACAGGAGACA
psiCHECK-2-Pax5-3'UTR WT	CCTATGACCGTCACTGACCCTCAGAGCCACGGGTGCCAAGCACTTACAACACATATCATTGCGGGAGGCAGCCTCCCAACCCCTGAGACAGACAGAGGGGCCCAACGACTCGTCTCC	GGAGACGAGTCGTTGGGCCCCTCTGTCTGTCTCAGGGGTTGGGAGGCTGCCTCCCGCAATGATATGTGTTGTAAGTGCTTGGCACCCGTGGCTCTGAGGGTCAGTGACGGTCATAGG
psiCHECK-2-Pax5-3'UTR m1	CCTATGACCGTCACTGACCCTCAGAGCCACGGGTGCCAAGCACTTACAACACATATCATTGCGTGCCGCAGCCTCCCAACCCCTGAGACAGACAGAGGGGCCCAACGACTCGTCTCC	GGAGACGAGTCGTTGGGCCCCTCTGTCTGTCTCAGGGGTTGGGAGGCTGCGGCACGCAATGATATGTGTTGTAAGTGCTTGGCACCCGTGGCTCTGAGGGTCAGTGACGGTCATAGG
psiCHECK-2-Pax5-3'UTR m2	CCTATGACCGTCGACGACCCTCCGAGCCGCGGGTGCCGACCGCTTACGACCGAAATGATTGCGGGAGGCGGCCTCCCGACCCCTGAGACGGACCGAGGGGCCCGACGACTCGTCTCC	GGAGACGAGTCGTCGGGCCCCTCGGTCCGTCTCAGGGGTCGGGAGGCCGCCTCCCGCAATCATTTCGGTCGTAAGCGGTCGGCACCCGCGGCTCGGAGGGTCGTCGACGGTCATAGG
psiCHECK-2-Pax5-3'UTR m1.2	CCTATGACCGTCGACGACCCTCCGAGCCGCGGGTGCCGACCGCTTACGACCGAAATGATTGCGTGCCGCGGCCTCCCGACCCCTGAGACGGACCGAGGGGCCCGACGACTCGTCTCC	GGAGACGAGTCGTCGGGCCCCTCGGTCCGTCTCAGGGGTCGGGAGGCCGCGGCACGCAATCATTTCGGTCGTAAGCGGTCGGCACCCGCGGCTCGGAGGGTCGTCGACGGTCATAGG

psiCHECK-2-Arid3a-CDS WT	GCCAGGTGGGGCTGCCGGGGGTGTCTAC ACCCACCATGTCTTCTACCTCAAACAAC	GTTGTTTGAGGTAGAAGACATGGTGGGTG TAGACACCCCCGGCAGCCCCACCTGGC
psiCHECK-2-Arid3a-CDS m1	GCCGGGCGGGGCCGCCGGGGGCGTCT ACACCCACCATGTCGTCTACCTCAAAC AAC	GTTGTTTGAGGTAGACGACATGGTGGGTG TAGACGCCCCCGGCGGCCCCG CCCGGC
psiCHECK-2-Arid3a-CDS m2	GCCAGGTGGGGCTGCCGGGGGTGTCT ACGCCCTCGATGTCTTCTACCTCGAAC GAC	GTCGTTCGAGGTAGAAGACATCGAGG GCG TAGACACCCCCGGCAGCCCCACCTGGC
psiCHECK-2-Arid3a-CDS m1.2	GCCGGGCGGGGCCGCCGGGGGCGTCT ACGCCCTCGATGTCGTCTACCTCGAAC GAC	GTCGTTCGAGGTAGACGACATCGAGGGCG TAGACGCCCCCGGCGGCCCCGCCCGGC
psiCHECK-2-Pax5 additional shared binding site 1 WT	CCAGAACCAGACCGGGAGACCAAACAG ACCTCCTTCCCACGTATTCTGTGCCTCCA TACCCCCAATTACGTCTCTGAATAGTGA AGGCTCTGAGCAGTG	CACTGCTCAGAGCCTTCACTATTCAGAGAC GTAATTGGGGGTATGGAGGCACAGAATACG TGGGAAGGAGGTCTGTTTGGTCTCCCGGTC TGGTTCTGG
psiCHECK-2-Pax5 additional shared binding site 1 m1	CTAGAACCCGACCGGGCGACCTAACAG ACCTCCGTCCCACGTATTCTGTGCCTCC ATACCCCTAATTACGTCTCTGAATAGTG ACGGCTCTGCGCAGCG	CGCTGCGCAGAGCCGTCACTATTCAGAGAC GTAATTAGGGGTATGGAGGCACAGAATACG TGGGACGGAGGTCTGTTAGGTCGCCCGGTC GGGTTCTAG
psiCHECK-2-Pax5 additional shared binding site 1 m2	CCCGAACCAGACCGGGAGACCAAACCG ACCTCCGTCCCACGTATTCTGCGCCTCT ATACCCCCAATTACGTCTCCGAATAGTG AACGATCTGAGCAGCG	CGCTGCTCAGATCGTTCACTATTCGGAGAC GTAATTGGGGGTATAGAGGCGCAGAATACG TGGGACGGAGGTCGGTTTGGTCTCCCGGTC TGGTTCGGG
psiCHECK-2-Pax5 additional shared binding site 1 m1.2	CTCGAACCCGACCGGGCGACCTAACCG ACCTCCGTCCCACGTATTCTGCGCCTCT ATACCCCTAATTACGTCTCCGAATAGTG ACCGATCTGCGCAGCG	CGCTGCGCAGATCGGTCACTATTCGGAGAC GTAATTAGGGGTATAGAGGCGCAGAATACG TGGGACGGAGGTCGGTTAGGTCGCCCGGTC GGGTTCGAG
psiCHECK-2-Pax5 additional shared binding site 2 WT	CTAGAATTTAACTCCACGCCATGGGGAG CAAATCTTAAAAACAAACCAAGCACTTC CAAACAAATACTAGGAGCAAGTGACGT CTGACACACAGTCTCT	AGAGACTGTGTGTCAGACGTCACTTGCTCC TAGTATTTGTTTGGAAGTGCTTGGTTTGTTT TTAAGATTTGCTCCCCATGGCGTGGAGTTA AATTCTAG
psiCHECK-2-Pax5 additional shared binding site 2 m1	CTAGAATTTAACTCCACGCCATGGCGAG ATAATCTTAATAACAAACCTAGCACTTG TAAACAAATACTAGGCGCAAGCGACGT CTGACACACAGTCTCG	CGAGACTGTGTGTCAGACGTCGCTTGCGCC TAGTATTTGTTTACAAGTGCTAGGTTTGTTA TTAAGATTATCTCGCCATGGCGTGGAGTTA AATTCTAG
psiCHECK-2-Pax5 additional shared binding site 2 m2	CTAGAATTTAACTACACGCCATGGGGCG CAAATCTTAAAAACTAACCACGCATATC CAAACTAATACTAGGCGCAAGTGACGTC TGATATATAGTCTCG	CGAGACTATATATCAGACGTCACTTGCGCC TAGTATTAGTTTGGATATGCGTGGTTAGTTT TTAAGATTTGCGCCCCATGGCGTGTAGTTA AATTCTAG
psiCHECK-2-Pax5 additional shared binding site 2 m1.2	CTAGAATTTAACTACACGCCATGGCGCG ATAATCTTAATAACTAACCTCGCATATG TAAACTAATACTAGGCGCAAGCGACGTC TGATATATAGTCTCG	CGAGACTATATATCAGACGTCGCTTGCGCC TAGTATTAGTTTACATATGCGAGGTTAGTTA TTAAGATTATCGCGCCATGGCGTGTAGTTA AATTCTAG

psiCHECK-2-Pax5 additional shared binding site 3 WT	ATGAAACTGTTTTATTGTATTTGAGGAA ATGGAGAGTTGAACATTCCAACCAATCA ATAGCCAAAAAATGCTATAAACATAAA AAGAAAATAAATCCCA	TGGGATTTATTTTCTTTTTATGTTTATAGCAT TTTTTGGCTATTGATTGGTTGGAATGTTCAA CTCTCCATTTCCTCAAATACAATAAAACAGT TTCAT
psiCHECK-2-Pax5 additional shared binding site 3 m1	ATGAAACTGTTGTATTGTATTTGACGAA ATGTAGAGTTGAACATTCTAACCAATCA ATAGCCAATAAATGCTATAAACATAAAT AGATAATAAATCCTA	TAGGATTTATTATCTATTTATGTTTATAGCA TTTATTGGCTATTGATTGGTTAGAATGTTCA ACTCTACATTTCGTCAAATACAATACAACA GTTTCAT
psiCHECK-2-Pax5 additional shared binding site 3 m2	ACGAAACCGTTTTATTGTATTTGAGGAA ATGGCGCGTTGAACCGTCCAACCAATCA ATAGCTAAAAAATGCTATAAATATAAA AAGATAATAAATCCCA	TGGGATTTATTATCTTTTTATATTTATAGCA TTTTTTAGCTATTGATTGGTTGGACGGTTCA ACGCGCCATTTCCTCAAATACAATAAAACG GTTTCGT
psiCHECK-2-Pax5 additional shared binding site 3 m1.2	ACGAAACCGTTGTATTGTATTTGACGAA ATGTCGCGTTGAACCGTCTAACCAATCA ATAGCTAATAAATGCTATAAATATAAAT AGATAATAAATCCTA	TAGGATTTATTATCTATTTATATTTATAGCA TTTATTAGCTATTGATTGGTTAGACGGTTCA ACGCGACATTTCGTCAAATACAATACAACG GTTTCGT

Supplemental Table 11. List of qPCR primers and probes used in this study

Gene name	Forward primer (5'→3')	Reverse primer (5'→3')	Probe	Exons
Lin28b	CCAGTGGATGTATTTG TACACCA	GCCTGTTACCCGTAT TGACTC	/6-FAM/AGAAGCTTG /ZEN/AAAGAAGGAGAGC CAGTG/3IABkFQ/	2-3
Igf2bp1	CTTGCTCACAGTTCTC CACT	CGTCTAGAGATTGAA CACTCAGT	/6-FAM/AGGCTATCT /ZEN/AGCACTTCCCAT CGGA/3IABkFQ/	2-4
Igf2bp2	ACCATCCTCTCACTGA CATCT	ACACATCAAACAGC TCGCT	/6-FAM/TGCCTCCAT /ZEN/CAAGATTGCTCC AGC/3IABkFQ/	12-13
Igf2bp3	CCAGCACCTCCCATTG TAAG	CGCTTTCAGGTAAAA TGGAAC TAC	/6-FAM/CACTCGGTC /ZEN/CCTAAACGGCAG A/3IABkFQ/	1-4
Pax5	GCCTGTAGACACTAT GCTGTG	AGAGCGAGTCTGTG ACAATG	/6-FAM/TCCGAATGA /ZEN/TCCTGTTGATGG AGCTG/3IABkFQ/	3-5
Arid3a	CAACATGAACAGGTC GAGGAC	GACCCCAAGAGGAA AGAGTTC	/6-FAM/ACTGGAGTG /ZEN/CCCCGCTTCTG/3IABkFQ/	4-5
Hprt1	CCCCAAAATGGTTAA GGTTGC	AACAAAGTCTGGCCT GTATCC	/6-JOEN/CTTGCTGGT /ZEN/GAAAAGGACCTC TCGAA/3IABkFQ/	5-6

Abbreviations or trademark names used:

6-FAM	6-carboxyfluorescein amidite
ZEN	internal ZEN™ quencher
JOEN	6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein NHS ester
3IABkFQ	3' Iowa Black® FQ

Supplemental Movie 1

Physical co-localization between FH-LIN28A and endogenous Igf2bp3 in 220-8 pro-B cells was detected using proximity ligation assay (PLA) as described for Figure 2E. Confocal microscopy was used to visualize in situ the fluorescent foci from PLA (green) and DAPI staining (blue). 3D animation was generated using Imaris software (Bitplane) to demonstrate electronic rendering of nuclei (blue) and PLA signal (green dots), and their spatial arrangement in cytoplasm of representative cells.