***Supplementary Figure 1 Related to Figure 1. T-ALL associated lncRNAs have low coding potential and are enriched the nucleus.***

(A) Cumulative frequency plot of PhyloCSF scores for lncRNAs (blue) and RefSeqNM entries (red). (B) Heat map representation of lncRNA expression in whole cell RNA extracts compared to nuclear RNA extract. (C) Bar plots showing the proportion of T-ALL lncRNAs that are included in GENCODE. (D) Bar plots showing the proportion of divergent (yellow) versus intergenic (blue) lncRNAs that are active (FPKM >1) in primary T-ALL (left) compared to thymus (right).

***Supplementary Figure 2 Related to Figure 2. LncRNAs in the T-ALL transcriptional circuitry.***

(A) Immunoblot for NOTCH1 and qPCR for *HES1, TUG1* and a panel of T-ALL associated lncRNAs following γSI treatment. (B) Gene track of three Notch-dependent lncRNA loci, which show occupancy by both NOTCH1 and RBPJκ at their regulatory elements (highlighted yellow). (C) Venn-diagram depicting lncRNAs that showed occupancy by NOTCH1 (red), ZNF143 (blue) and were down-regulated upon Notch inhibition (yellow).(D) Heat map representation of T-ALL associated lncRNAs that are differentially expressed between T-ALL subtypes.

***Supplementary Figure 3 Related to Figure 3. LUNAR1 chromatin state across diverse tissues.***

(A) Gene track of *LUNAR1* locus depicting H3K36me3 (green), H3K79me2 (blue), H3K4me1 (gray) and H3K4me3 (black) in DND41 cells. (B) Gene track image of ChromHMM states at the *LUNAR1* locus in all tissues available from the Roadmap Epigenomics project (http://www. roadmapepigenomics.org/).

***Supplementary Figure 4 Related to Figure 4. Notch1 occupies an enhancer in the IGF1R gene.***

(A) ChIP assay for NOTCH1, MED1, BRD4, and H3K27ac followed by qPCR for a region in the last intron of *IGF1R*. (B) Schematic of reporter constructs

generated. (C) Relative reporter activity of constructs containing the *IGF1R* enhancer (blue) or promoter only (yellow) upon co-transfection of ICN1. Error bars represent SEM of at least 3 experiments. * indicates p-value < 0.05.

***Supplementary Figure 5 Related to Figure 5. LUNAR1 is required for efficient T-ALL growth.***

(A) qPCR for *LUNAR1* (blue) and *IGF1R* (yellow) in CUTLL1 cells transduced with empty vector or *LUNAR1.* (B) Line graph showing a similar competition assay as in Figure 5B but with AML cells. (C) qPCR for *LUNAR1* (blue) and *IGF1R* (yellow) in CUTLL1 cells treated with non-targeting (Scr) or *LUNAR1* ASOs. (D) Growth curve for CUTLL1 cells treated with non-targeting (red) or *LUNAR1* (blue) ASOs. (E) Representative FACS histogram of DNA content stained with 7AAD. Quantification percentage of CUTLL1 cells in S/G2/M phase following depletion of *LUNAR1* are shown in the bar graph. (F) GSEA analysis with genes down-regulated upon pharmacological inhibition of IGF1R (left) or targets of IGF1/2 from mSig database (right). Expression datasets used are from *LUNAR1* knockdown experiments with two different shRNAs (top and bottom respectively). * indicates p-value < 0.05. ** indicates p-value < 0.01.

***Supplementary Table 1 Related to Figure 1.***

Excel spreadsheet which documents the filtration process for annotating T-ALL lncRNAs. Each tab indicates a processing step as indicated.

***Supplementary Table 2 Related to Figure 3-7.***

Excel spreadsheet listing primer sequences, shRNA sequences and ASO sequences used throughout the manuscript.

***Supplementary Table 3 Related to Figure 3.***

Sequence of the *LUNAR1* transcript isolated by 5’ and 3’ RACE in FASTA format.

***Supplementary Table 4 Related to Figure 1.***

A final annotation file of T-ALL lncRNAs in GTF format.

## SUPPLEMENTARY METHODS

### *LncRNA discovery*

We sequenced two samples from T-ALL cell lines (CUTLL1 and HPBALL), two primary human thymus samples to ultra high depth (>200 million million mate pairs each), ten primary pediatric T-ALL samples (60-80 million mate pairs each) and data generated by the Roadmap Epigenomics project for Naïve CD4+ and CD8+ T-cells to be used for *ab initio* transcirptome assembly with Cufflinks v1.3. Briefly, Cufflinks was run with the following options: -u, -N, -g (RefSeq GTF file provided as guide), -M (rRNA and 7SK RNA mask file provided). We generated transcriptome assemblies for each of these samples separately and then used Cuffmerge to combine all annotations. We then removed any transcript that overlapped a known coding region (RefSeq NM entries) or T-cell receptor and B-cell receptor gene loci in order to remove products of antigen receptor recombination. We next removed any transcript that did not overlap a region of enrichment for either H3K27ac, H3K4me1 or H3K4me3 in T-ALL or T-cells respectively and then merged the T-ALL and T-cell annotations using Cuffmerge. We removed any gene locus that did not have at least one multi-exonic isoform or at least one isoform with > 3X nucleotide coverage in one of our samples. Any isoform with a length < 200nt was removed. Next, we used the PhyloCSF algorithm to predict protein coding potential of the remaining transcripts. We extracted multiple alignments for the 29 mammals supported by PhyloCSF using the Galaxy tools Extract multiple alignments and Stitch gene blocks. PhyloCSF was then run using –orf ATGStop to identify putative open reading frames with a minimum ORF length of 50 amino acids. We then removed any transcript with a

PhyloCSF score greater than 100 as was previously used by Alvarez-Dominguez and colleagues (Alvarez-Dominguez et al., 2014). Finally all miRNA and snRNA host genes were removed. The resulting T-ALL lncRNA annotation was then compared to the GENCODE v18 lncRNA annotation in order to determine the number of novel genes discovered here. Transcripts were considered divergent if their TSS was less than 2.5kb from a RefSeq_NM TSS on the opposite strand. All other lncRNAs were considered to be intergenic. This T-ALL lncRNA annotation was merged with the RefSeqNM annotation using Cuffmerge and used for all subsequent RNA-Seq expression analyses. For a copy of this annotation in GTF format see supplementary data.

***RT-qPCR analysis***

For all RT-qPCR analyses, whole RNA was isolated using the Qiagen RNeasy mini plus kit (Life Technologies, Carlsbad, CA). cDNA was generated using the High Capacity cDNA Reverse Transcription kit (Life Technologies, Carlsbad, CA). All qPCR primers were verified to produce specific primers and to perform efficiently. qPCR reactions were performed with technical triplicates on a Roche Lightcycler 480i with Roche 2X SYBR mastermix. Relative quantification of target genes was performed using the $\Delta\Delta$Ct method with *GAPDH* as a reference gene.

***Rapid amplification of cDNA ends (RACE)***

5' and 3' RACE were performed using the FirstChoice-RLM RACE kit according to the manufacturers instructions (Life Technologies, Carlsbad CA).

***shRNA design and cloning***

shRNAs were generated using the DSIR algorithm (http://biodev.extra.cea.fr/DSIR/DSIR.html). Briefly, FASTA sequences for each gene were provided to DSIR using the 21nt siRNA setting. We took the top 10 ranking siRNAs and used the reverse complement from the "AS sequence" output field as the basis for designing 97mer template oligos at:

http://katahdin.cshl.org/siRNA/RNAi.cgi?type=shRNA. 97mers were synthesized, diluted to 0.1ng/uL and mixed into a pool. Pools were then used as template for a PCR reaction which added XhoI and EcoRI sites for cloning into LMP vectors. PCR products were purified using Qiagen PCR cleanup kit and digested with EcoRI and XhoI and subsequently ligated into EcoRI/XhoI-digested LMP vector. Ligation products were transformed into E. coli and clones were screened for correct inserts.

***Chromatin immunoprecipitation (ChIP)***

The following antibodies were used for chromatin immunoprecipitation experiments: Notch1 C-20 (Santa Cruz sc-6014), Med1 (Bethyl, A300-793A), Med12 (Bethyl, A300-774A), RNA PolII N-20 (Santa Cruz, sc-899), H3K4me1 (Abcam, ab8895), H3K4me3 (Active Motif, 39159), H3K27ac (Abcam, ab4729). ChIP assays were performed essentially as described previously(Whyte et al., 2013). Briefly cells were crosslinked in 1% formaldehyde for 10 minutes at room temperature and the reaction was stopped by the addition of .125M glycine and incubated for an additional 5 minutes. Cells were washed twice with ice cold PBS. Cells were then lysed in LB1 (50mM HEPES pH7.5, 140mM NaCl, 1mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton-X) for 10 minutes at 4C and crude nuclei were pelleted by centrifugation. Nuclei were then resuspended in sonication buffer (20mM Tris pH8, 150mM NaCl, 0.1% SDS, 1% Triton-X, 2mM EDTA) and chromatin was sheared using a QSonica Q500 with microtip (40% amplitude, 15 seconds on 60 seconds off, total time 7.5 minutes). Following sonication chromatin was cleared by high-speed centrifugation and supernatant was kept for immunoprecipitations. Antibodies (2.5ug/IP for histones and 5ug/IP for others) coupled to Protein-G Dynabeads (Life Technologies) were added to chromatin and incubated at 4C overnight. The following day immune complexes were washed once with sonication buffer, once with high salt wash buffer (20mM Tris pH8, 500mM NaCl, 0.1% SDS, 1% Triton-X, 2mM EDTA), once with lithium chloride wash buffer (10mM Tris pH8, 250mM LiCl, 1% NP-40) and once with TE supplemented with 50mM NaCl. Immunoprecipitates were then eluted by the

addition of 1% SDS and 25mM sodium bicarbonate and incubated with shaking at 65C for 1 hour. Crosslinks were reversed by incubation at 65C for 6-18 hours and then treated with RNase A followed by Proteinase K. DNA was purified using Qiagen PCR purification columns and used as template for qPCR.

***ChIP-Seq data analysis***

ChIP-Seq for Notch1, Rbpjk, H3K4me3, H3K27ac and p300 in CUTLL1 cells were performed previously (Wang et al., 2014; Wang et al., 2011). RNAP2 ChIP-Seq was preformed previously by us(King et al., 2013). For all data sets, FASTQ files were aligned to hg19 using Bowtie with the following options: -n 2, -m 1. Resulting SAM files were then converted to BAM format and sorted by chromosomal location using Picard. Enriched regions were determined using MACS (Zhang et al., 2008) v1.4 with a p-value cutoff of 1e-9. ChIP-Seq density heat maps and histograms were generated using Genomic-Tools (Tsirigos et al., 2012). LncRNAs were considered bound by Notch1 if they had an enriched region either within the gene or 5kb up or downstream.

***Chromosome conformation capture (3C)***

3C experiments were performed essentially as described previously(Hagege et al., 2007). Briefly, 10^7 CUTLL1 cells per experiment were cross linked with 1% formaldehyde in PBS for 10 minutes at room temperature and quenched by adding 0.125M glycine for 5 minutes. Cells were then washed twice in ice cold PBS. Cells were then lysed in 3C lysis buffer (10mM Tris-HCl, pH 7.5; 10mM NaCl; 5mM $MgCl_2$; 0.1mM EGTA; 1X Sigma protease inhibitor cocktail) for 10 minutes on ice and nuclei were pelleted by centrifugation at 600 x g for 5 minutes. Pellets were then resuspended in 1X NEB buffer 2 with 1X BSA, 0.3% SDS and incubated with rotation at 37C for 1 hour. Triton X was then added and to a final concentration of 2% incubated again for 1 hour at 37C with rotation. 400U of highly concentrated HindIII (NEB, Ipswich, MA), was added and incubated overnight at 37C with rotation. The following day SDS was added to a final concentration of 1.6% and samples were incubated at 65C for 20 minutes.

Sample were then brought up to a final volume of 7mL in 1X NEB T4 ligase buffer with 1X BSA and 1% Triton X. Samples were rotated at 37C for 1 hour. Sample were then chilled on ice for 5 minutes and 4000U T4 DNA ligase was added (NEB, Ipswich, MA) and samples were incubated at 16C for 6 hours followed by 30 minutes at room temperature. Next, 300ug of proteinase K was added and cross links were reversed at 65C overnight. The following day an additional 300ug of proteinase K was added and incubated at 50C for 1 hr. Finally genomic DNA was purified by phenol chloroform extraction followed by ethanol precipitation. Ligation events were detected using specific primers and Zen double quenched probes with 5' 6-FAM and 3' Zen/Iowa black quenchers (IDT DNA, Coralville, IA). qPCRs were performed on a Roche Lightcycler 480i using 2X Roche Probes mastermix. Specificity and efficiency of all 3C primes was verified by performing digestion and ligation of BACs containing the regions of interest. Ligation products were then serially diluted in sheared genomic DNA and the efficiency of each PCR reaction was verified. Amplicons from BAC qPCRs and actual 3C template were run on agarose gel to verify the production of a single band the expected size.

***Luciferase reporter assays***

293T or 293-UAS-TK-Luc cells were grown until 60% confluent and transfected with indicated plasmids plus pTK-Ren using polyethylenimine (PEI). For all experiments as the amount of BoxB-lncRNA plasmid was decreased, an equal amount of BoxB-Empty-vector was added in its place. 24 hours after transfection cells were harvested and assayed for reporter activity using the Dual-Glo Luciferase Assay System and a GloMax-Multi Jr. luminometer according to manufacturers instructions (Promega, Madison WI). Each data point was taken as the average Luc/Ren ratio of 3 triplicate wells.

***Xenograft in vivo competition assay***

CUTLL1 cells were transduced with retroviruses expressing either LMP-shRenilla-GFP or LMP-sh*LUNAR*-mCherry. 48 hours following the infection,

puromycin was added to culture media until cultures were >95% GFP or mCherry positive and >90% alive. GFP and mCherry cells were then mixed 1:1 and the relative contribution of cells containing each shRNA was verified by FACS. This mixture was then xenografted into sublethally irradiated (350 cGy) $Rag2^{-/-}IL2R\gamma^{-/-}$ animals at 1 million cells per animal via tail vein injection. After 4 weeks, animals were sacrificed and spleens were harvested and stained with anti-human CD45 to mark human leukocytes. We then measured the relative contribution of cells harboring each shRNA by FACS analysis.

***Hi-C data analysis***

Hi-C sequencing data were pre-processed (iterative alignment and outlier removal) using the pipeline described by Imakaev and colleagues(Imakaev et al., 2012). The heat map in the *IGF1R/LUNAR1* locus shows the Hi-C interactions as paired-read counts between pairs of sliding windows of 50kb in length.

***Antisense oligonucleotide (ASO) design and delivery***

ASOs were designed using the IDT Antisense Design Tool (www.idtdna.com) using the chimeric 25mer setting. The top 3 ASOs generated by the design tool were ordered and tested for knockdown efficiency. The ASO with the highest potency was used for experiments. Sequence for the scrambled ASO was taken from Li et al.(Li et al., 2013). For ASO experiments in T-ALL cells oligonucleotides were delivered simply by adding them to the growth media at 2μM. For ASO knockdown in BoxB tethering experiments, ASOs were co-transfected with plasmid DNA at 50nM.

***Primary T-ALL samples***

Primary T-ALL samples were provided by the Childrens Oncology Group with informed consent and analyzed under the supervision of the New York University Langone Medical Center Institutional Review Board.

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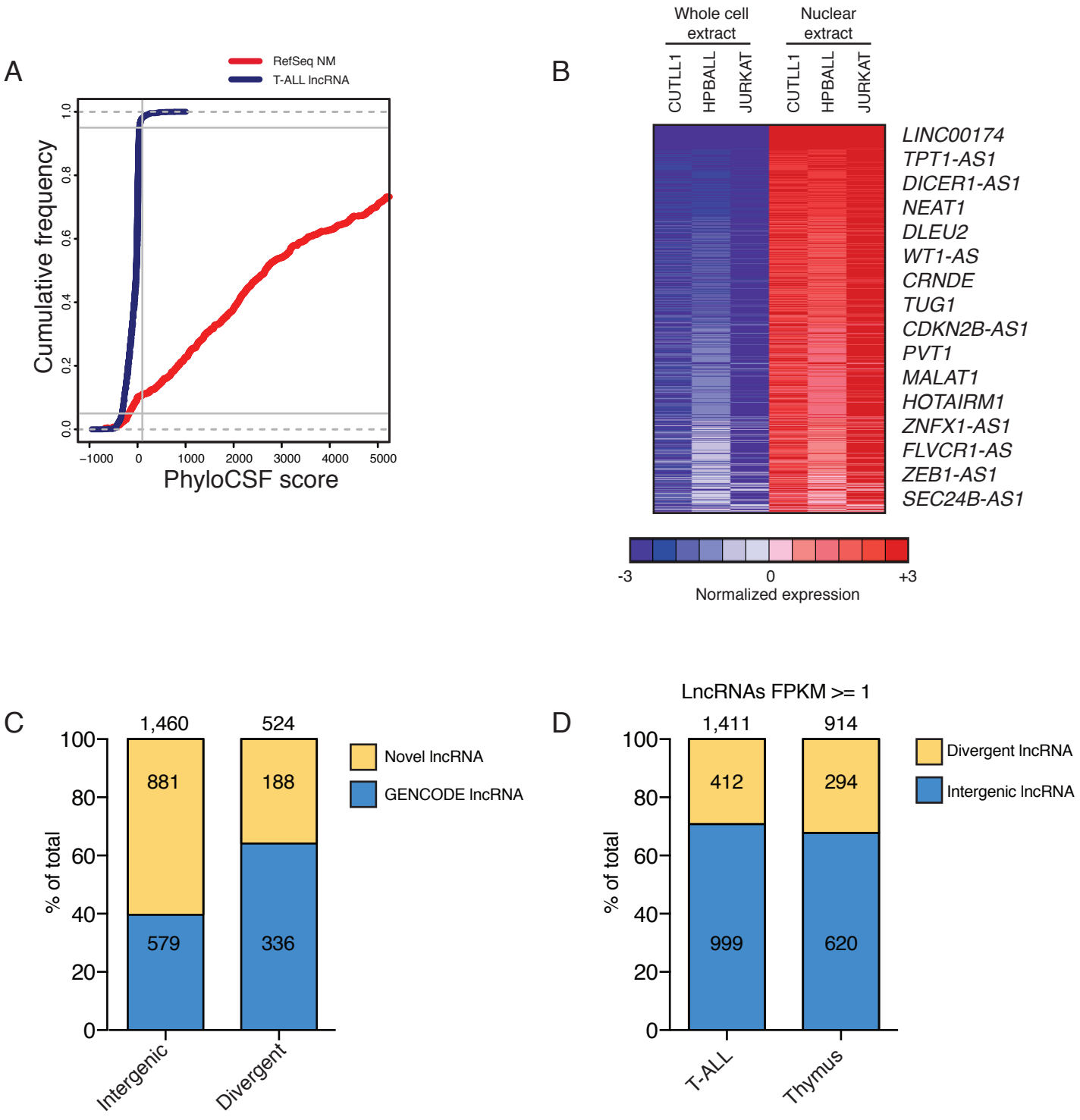
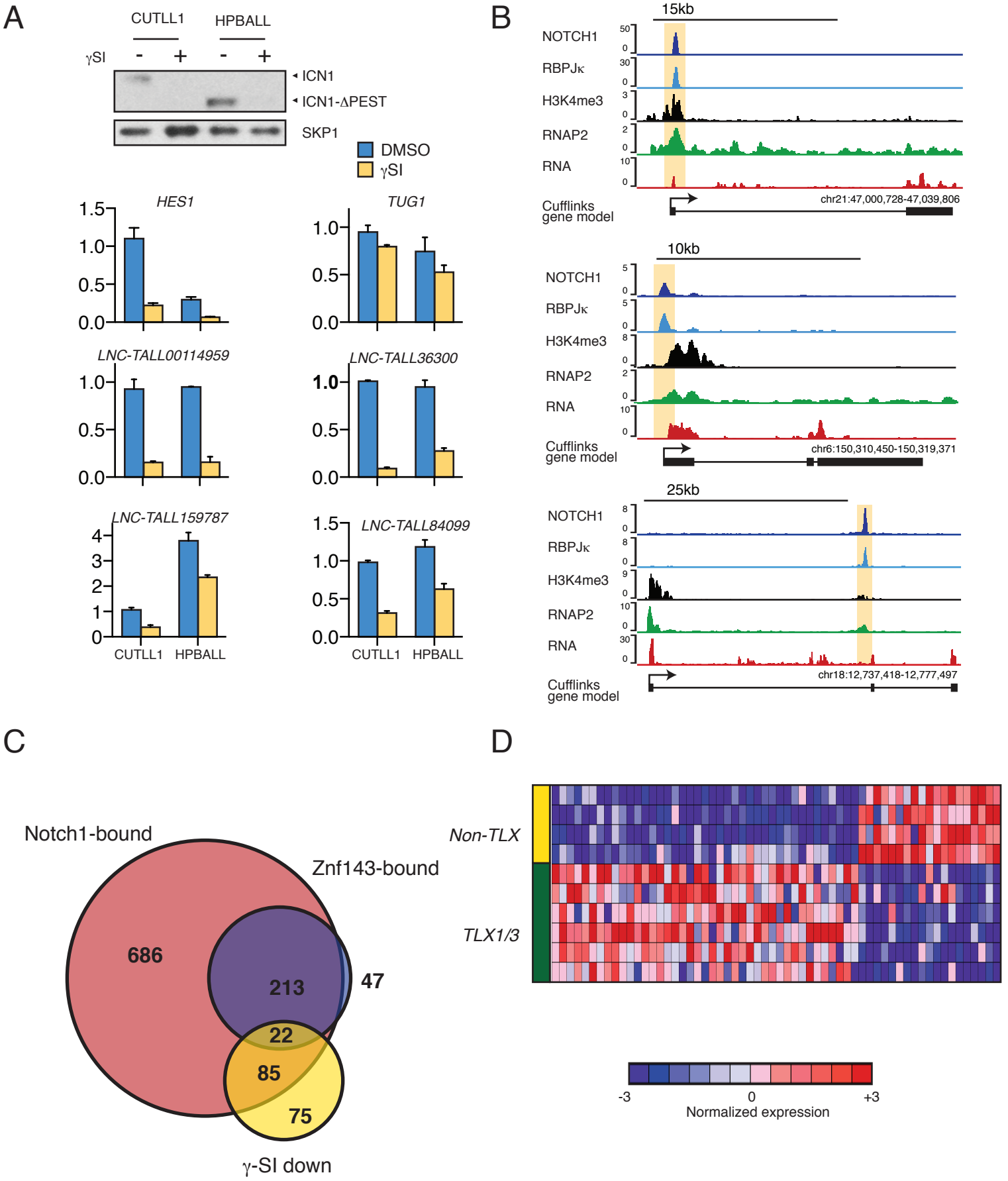
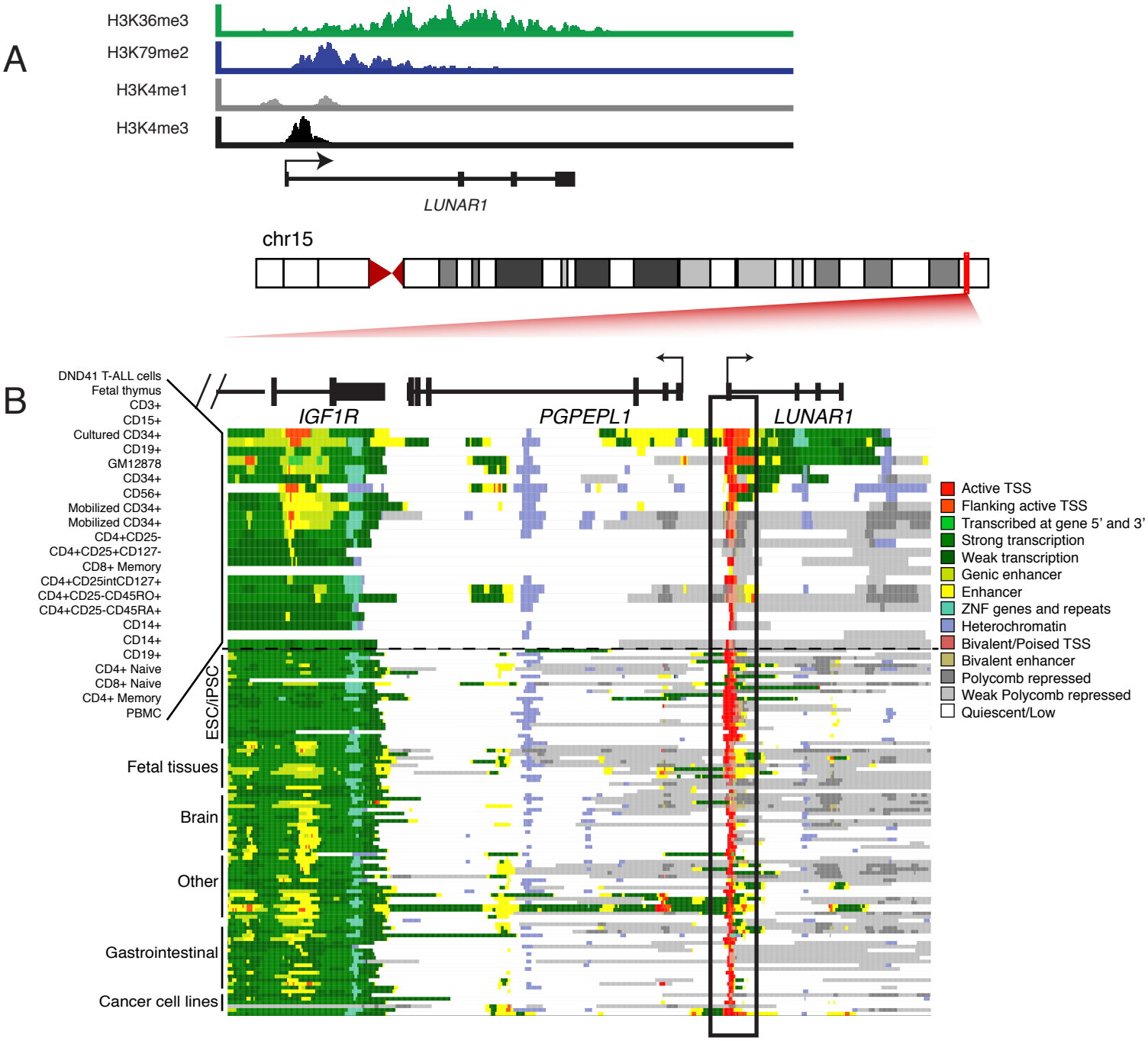
Supplemental Figure1




Supplementary figure 1, Related to Figure 1




Supplementary figure 2, Related to Figure 2

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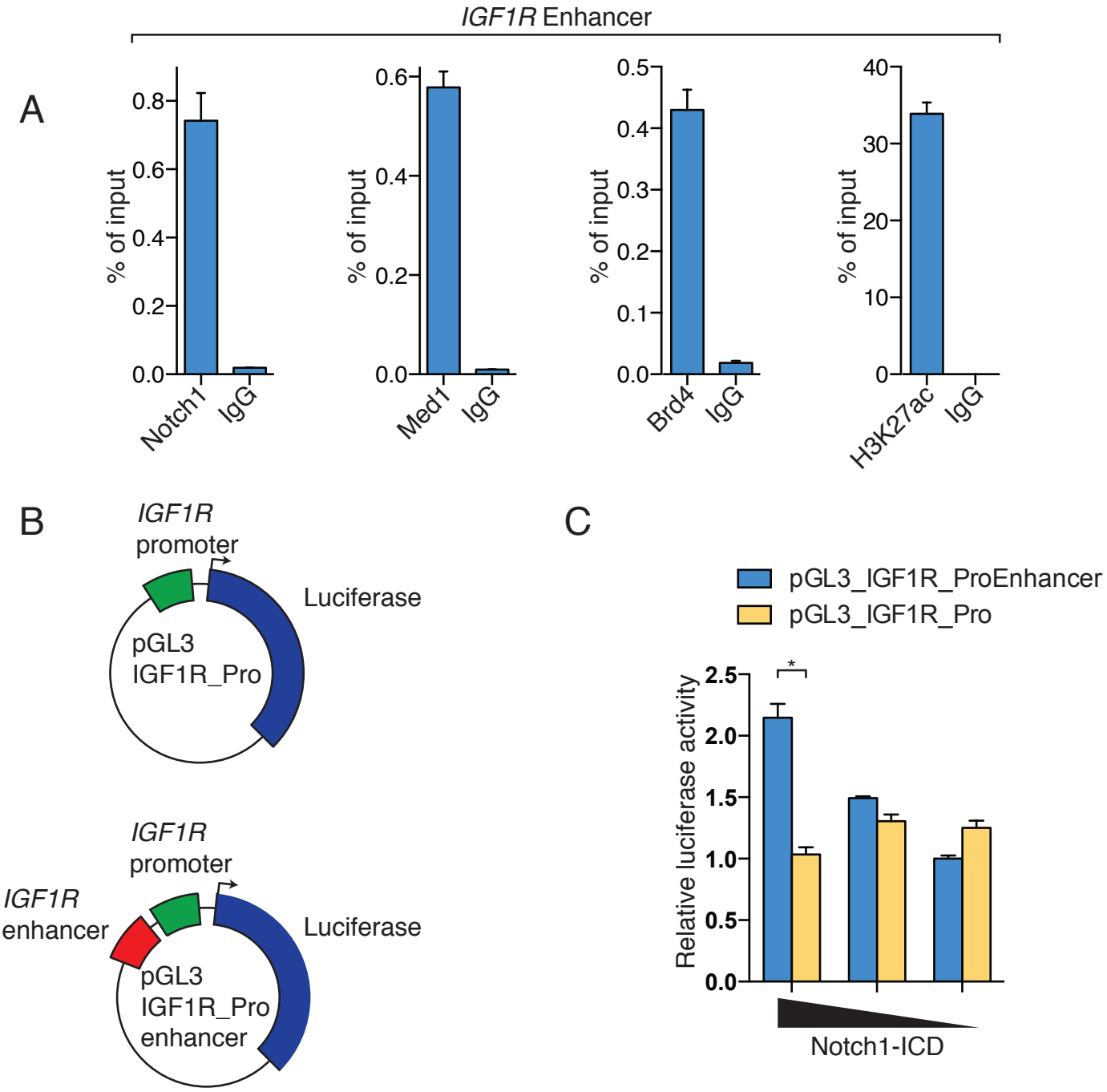
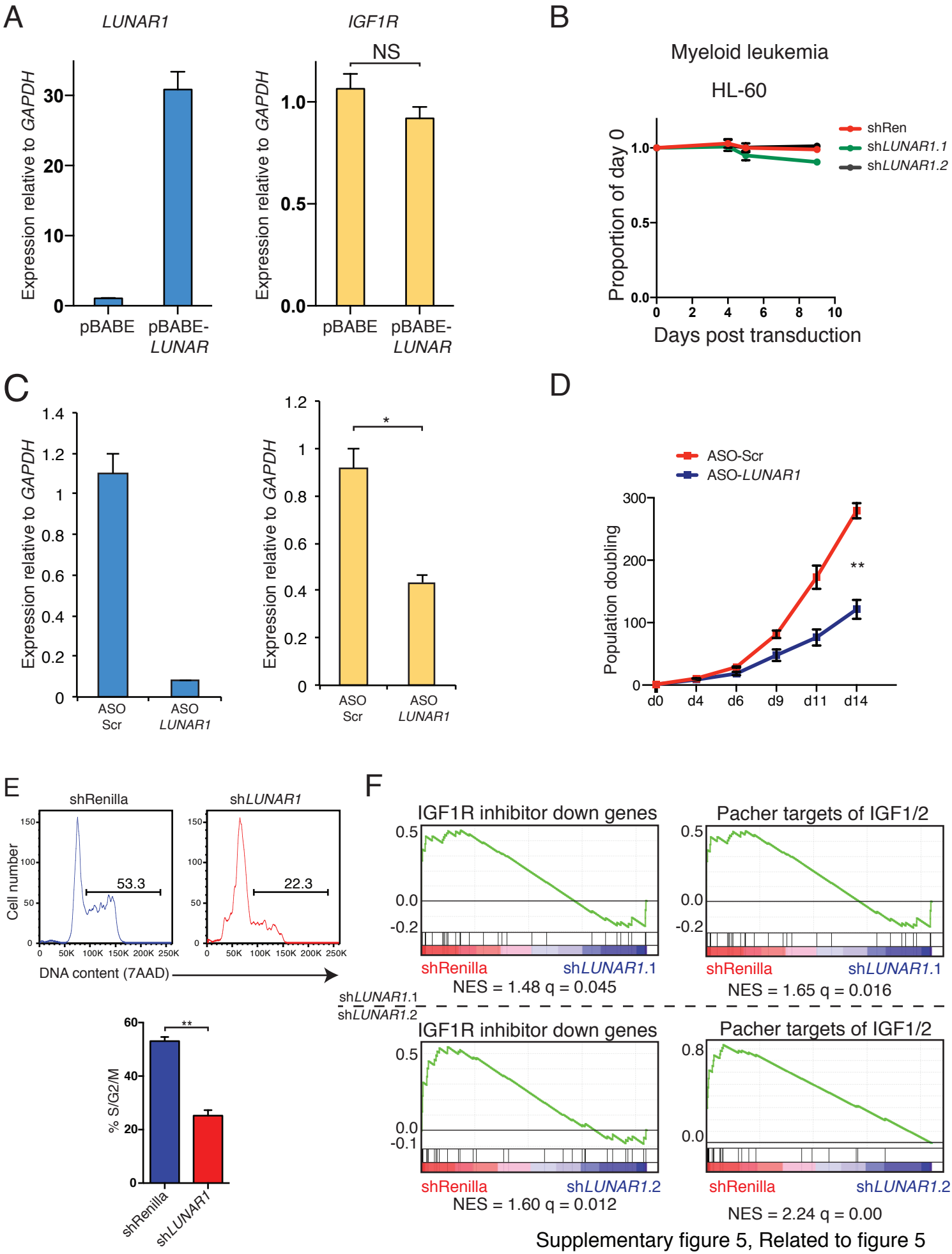



Supplementary figure 4, Related to Figure 4

Supplemental Figure5




Supplementary figure 5, Related to figure 5