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Supplemental Information

NOTCH1 Nuclear Interactome Reveals Key Regulators of Its Transcriptional Activity and Oncogenic Function

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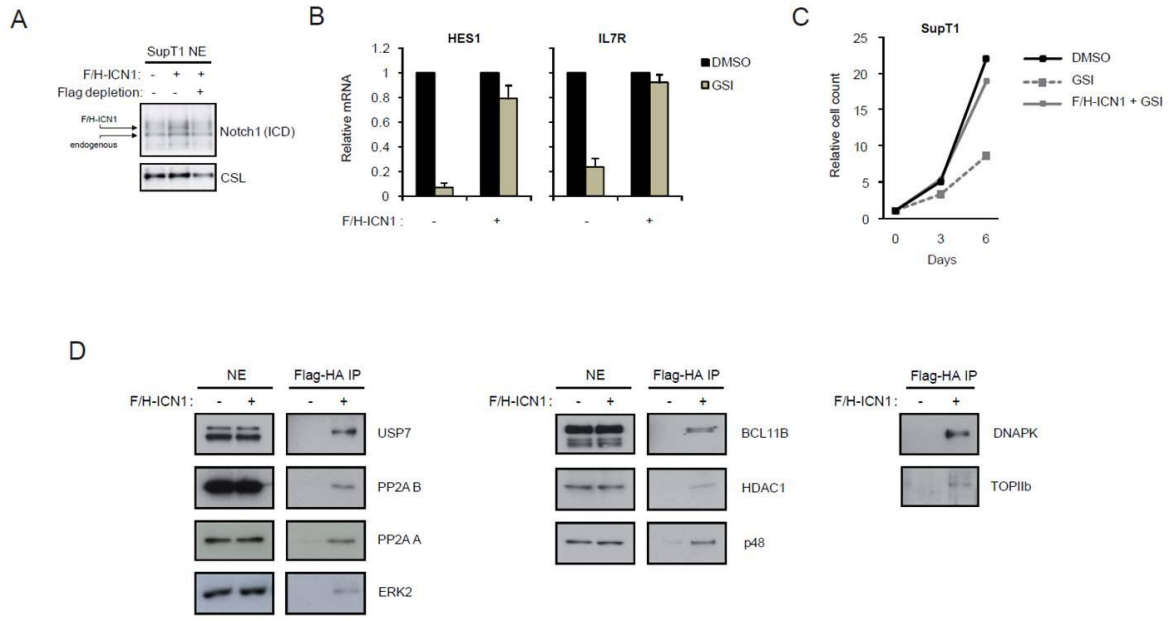

Figure S1, related to Figure 1:

(A) Expression level of Flag-HA tagged ICN1 (F/H-ICN1) in SupT1 nuclear extracts (NEs) was measured by Western blot (WB). The antibody against NOTCH1 intra-cellular domain (anti-ICD) recognizes both endogenous and tagged ICN1. F/H-ICN1 was depleted from SupT1 NEs using anti-Flag beads.

(B) F/H-ICN1 restores Notch-responsive genes expression. Non-transduced and F/H-ICN1 expressing SupT1 cells were treated for 8 hours with DMSO or GSI. The expression of two Notch-responsive genes (*HES1* and *IL7R*) was measured by quantitative RT-PCR. The graphs represent mean +/-SD (n=2).

(C) SupT1 cells expressing F/H-ICN1 are insensitive to GSI-mediated growth arrest. Cell count proliferation assays were performed using non-transduced and F/H-ICN1 expressing SupT1 cells treated with DMSO or GSI.

(D) Nuclear extracts (NEs) derived from non-transduced and F/H-ICN1 expressing SupT1 cells were subjected to immunoprecipitation using anti-Flag and anti-HA beads. ICN1-associated proteins were analyzed by Western blot using the indicated antibodies.

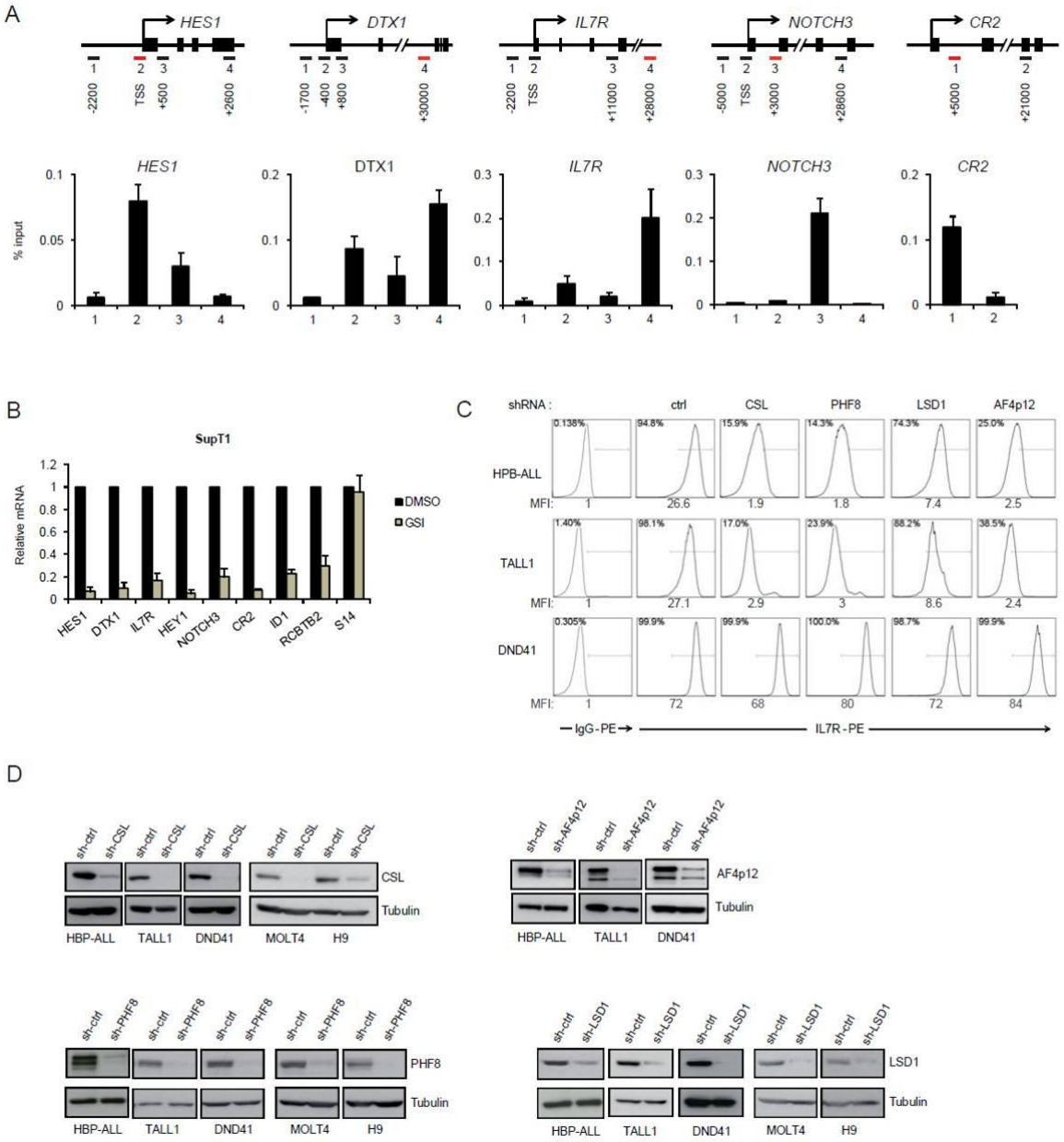

Figure S2, related to Figure 3:

(A) The upper panel depicts the basic gene structure of the five Notch-target genes (shown in Figure 3A) *HES1*, *DTX1*, *IL7R*, *NOTCH3* and *CR2* and positions of amplicons used in ChIP experiments. Amplicons used in Figure 3A (corresponding to ICN1-binding sites) are represented in red. Position relative to the transcription start site (TSS) is indicated for each amplicons. ChIP assay with anti-Notch1 antibody was performed in SupT1 cells. Results are expressed as percentage relative to input DNA. Shown is the mean +/- SD of three independent experiments. Numbers on the x-axes represent the different amplicons.

(B) mRNA expression of 8 Notch-target genes (shown in Figure 3C) in SupT1 cells treated with DMSO or GSI was assessed by quantitative RT-PCR (Q-RT-PCR). mRNA levels were normalized to those of GAPDH. *S14* mRNAs expression was used as a control.

(C) Cell surface expression of IL7R in HPB-ALL, TALL1 and DND41 (expressing the indicated shRNA) was analyzed by flow cytometry using anti-IL7R-PE antibody. The gate and percentage in the histograms indicate IL7R-positive cells as determined relative to irrelevant isotype-matched antibody (IgG-PE). The means of fluorescence intensity (MFI) relative to the isotype-control staining are indicated under the histograms.

(D) Efficiency of shRNA-mediated knockdown in T-ALL cells determined by western blot.

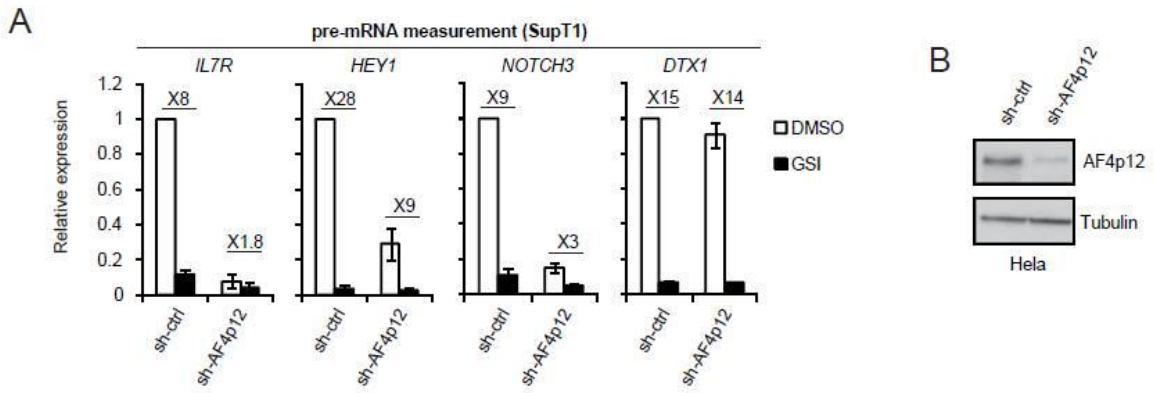

Figure S3, related to Figure 4:

(A) Quantitative analysis of nascent primary transcripts (pre-mRNA) in AF4p12 knockdown cells. SupT1 cells expressing control (ctrl) or AF4p12 specific shRNA were treated with DMSO or GSI. Pre-mRNA measurements were performed by Q-RT-PCR using specific intronic primers and normalized to GAPDH. Numbers in the graph represent the fold activation by Notch (DMSO-treated /GSI-treated).

(B) The efficiency of AF4p12 knockdown in HeLa cells was determined by western blot (related to Figure 4C).

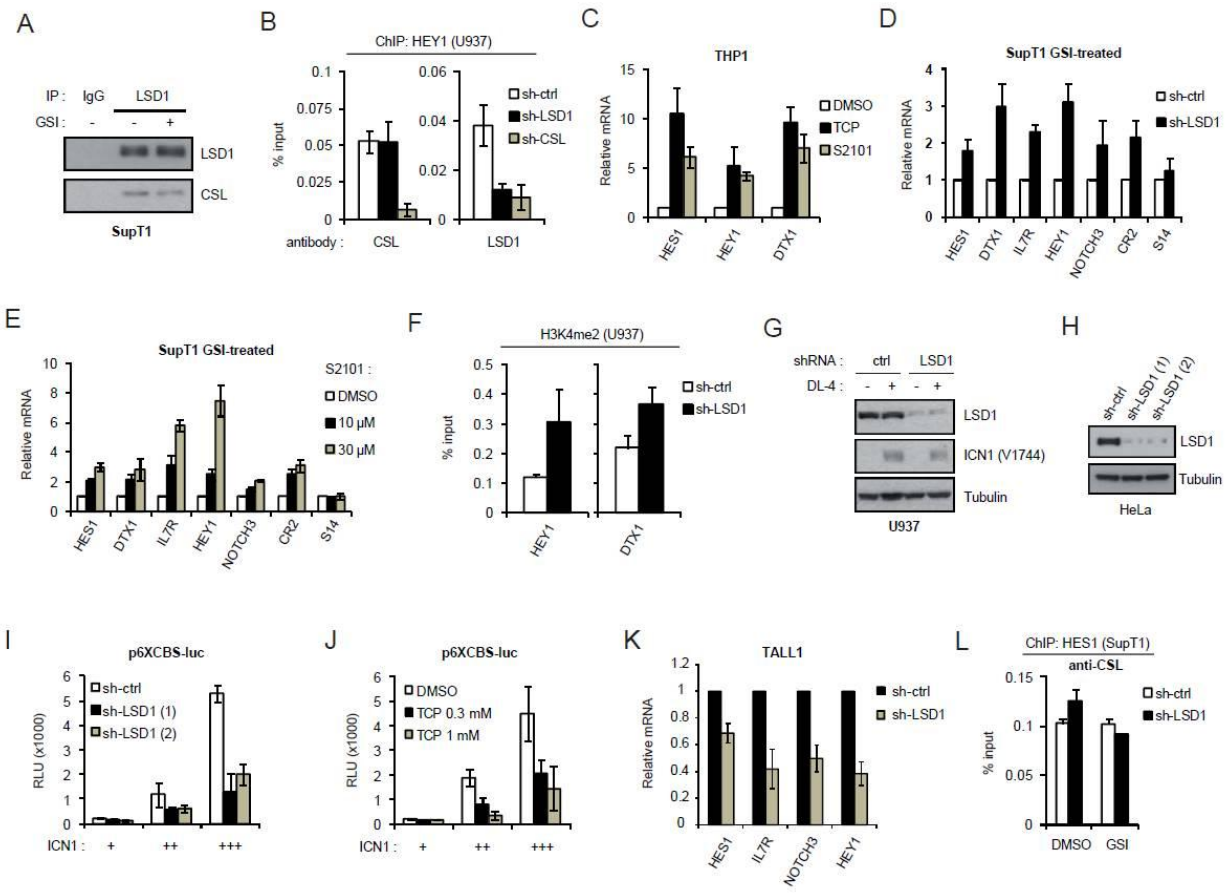

Figure S4, related to Figure 5:

(A) Endogenous LSD1 was immunoprecipited from DMSO or GSI-treated SupT1 cells. The presence of CSL in the precipitate was revealed by WB.

(B) U937 cells expressing control, LSD1 or CSL specific shRNAs were subjected to ChIP using antibodies against CSL and LSD1. CSL and LSD1 occupancy of the *HEY1* enhancer is shown as percentage relative to input DNA (n=2).

(C) THP1 cells were treated for 8 hours with LSD1 inhibitors tranylcypromine (TCP, 1mM) and S2101 (30µM). *HES1*, *HEY1* and *DTX1* expression was measured by Q-RT-PCR as described in Figure 5G.

(D) SupT1 cells expressing control or LSD1 specific shRNA were treated with 1µM GSI for 48 hours. The expression levels of the indicated Notch-target genes was monitored by Q-RT-PCR and normalized to GAPDH levels. The expression of *S14* served as a control.

(E) SupT1 cells were treated with 1μM GSI for 48 hours and further treated with DMSO or S2101 (10μM or 30μM) for 8 hours. Q-RT-PCR were performed as described in (D).

(F) H3K4me2 levels at the *HEY1* and *DTX1* enhancers were assessed after LSD1 knockdown in U937 cells by ChIP (related to Figure 5H).

(G) WB assays were performed on U937 cells used in Figure 5I to assess the knockdown efficiency of LSD1, and ICN1 production after 1 hour culture on DL-4.

(H) Efficiency of LSD1 knockdown in Hela cells was analyzed by western blot.

(I) LSD1 is required for Notch transcriptional activity in transient report assay. HeLa cells expressing control or two LSD1 specific shRNAs (from S4H) were transfected with the Notch-responsive reporter p6XCBS-luc and various amounts of active ICN1. Luciferase levels were measured 24 hours post-transfection and represented as fold induction by ICN1 (relative to basal luciferase activity in the absence of ICN1).

(J) Hela cells were transfected as described in (I) and treated 12 hours post-transfection with DMSO or TCP at the indicated concentration. Luciferase activity was measured 24 hours post-transfection. Show in (I and J) is the mean +/- SD of the luciferase activity from three independent experiments.

(K) TALL1 were transduced with control or LSD1 shRNA. Expression of *HES1*, IL7R, *NOTCH3* and *HEY1* was measured by Q-RT-PCR (n=2).

(L) SupT1 cells expressing control or LSD1 shRNA were treated with DMSO or GSI and subjected to ChIP assays. Results for CSL occupancy at *HES1* promoter are shown as percentage of input DNA (n=2). Related to Figure 5M.

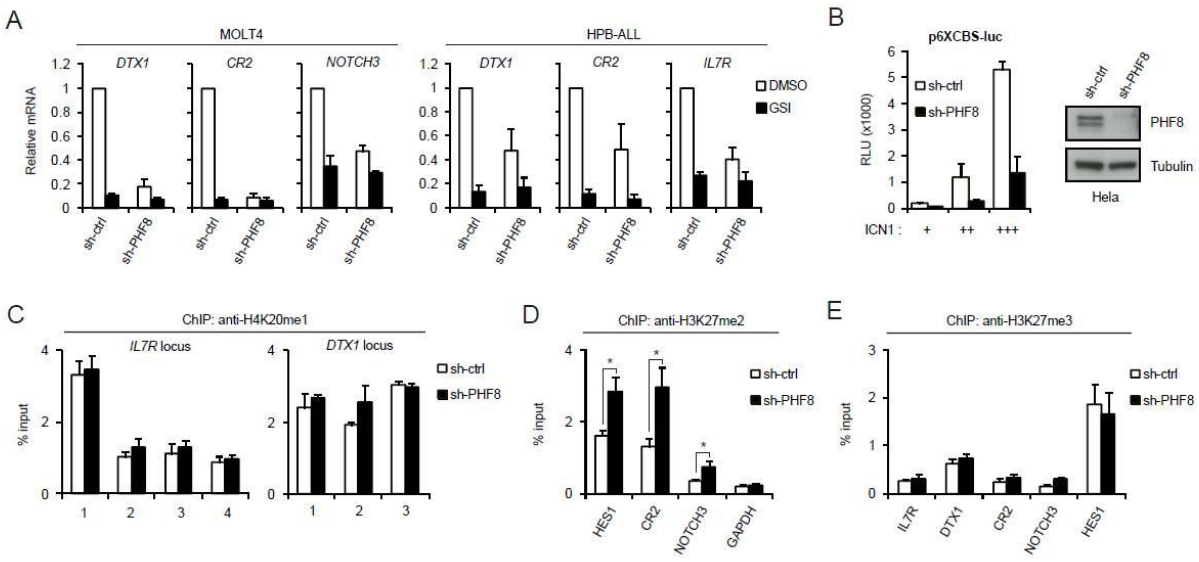

Figure S5, related to Figure 6:

(A) MOLT4 and HPB-ALL cells expressing control or PHF8 shRNA were treated with DMSO or GSI. The expression of the indicated genes was analyzed by Q-RT-PCR and normalized to *GAPDH* levels. Shown is the mean +/- SD (n=2 for MOLT4, n=3 for HPB-ALL).

(B) HeLa expressing control or PHF8 shRNA were transfected with the Notch-responsive reporter (p6XCBS-luc) and a vector encoding the active form of Notch1 (ICN1). Luciferase levels were measured 24 hours post-transfection and represented as fold induction by ICN1 (mean +/- SD, n>3). Efficiency of PHF8 knockdown is shown in the right panel.

(C) ChIP assay, related to Figure 6D and 6E. Results for H4K20me1 at *IL7R* and *DTX1* loci in SupT1 cells expressing control or PHF8 shRNA are represented as percentage of input DNA. The amplicons indicated by the numbers on the x axis are positioned at each locus in Figure 6D and 6E. Error bars represent SD from at least three experiments.

(D) H3K27me2 levels at *HES1*, *CR2* and *NOTCH3* were analyzed by ChIP in SupT1 cells expressing control or PHF8 shRNA. The ICN1-binding region (as determined in Figure S2A) was PCR amplified from the precipitated and input DNA. Primers specific to the *GAPDH* promoter were used as a control (n=3, *p<0.05).

(E) ChIP assay for H3K27me3 at the indicated Notch-target genes were performed in SupT1 cells expressing control or PHF8 shRNA.

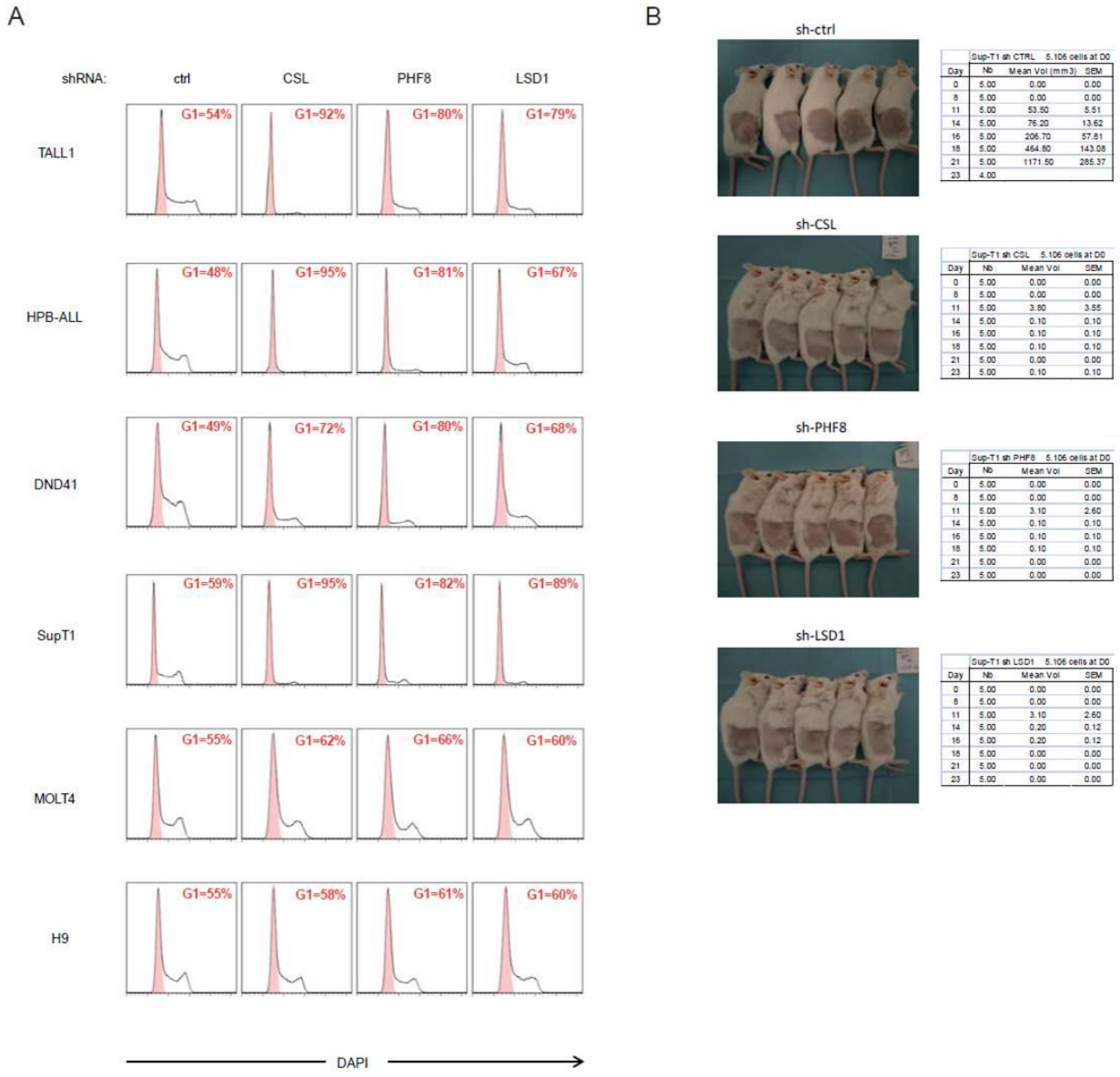

Figure S6, related to Figure 7:

(A) Effects of LSD1 and PHF8 depletion on cell cycle progression in a panel of human T-ALL cell lines. The DNA content of DAPI-stained cells was measured by flow cytometry. The G0/G1 phase of cell cycle is indicated in red.

(B) SCID mice were xenografted subcutaneously with $5x10^6$ SupT1 cells expressing control (ctrl), CSL, PHF8 and LSD1 shRNAs (n=5 per group). Tumor volume (mm3) +/-SEM over a period of 23 days post-injection is indicated in the tables. Pictures of the mice at the end of the follow-up period are shown.

Table S2. Identified Notch partners previously linked to Notch signaling.

Protein	Function	Link to Notch signaling	references
CSL	Notch signaling	DNA-binding subunit of the Notch-activation complex	(Fortini and Artavanis-Tsakonas, 1994)
MAML1	Notch signaling	Orthologues of the drosophila Mastermind. Transcriptional coactivator and component of the Notch-activation complex.	(Wu et al., 2000; Xu et al., 1990)
MAML3	Notch signaling		
NOTCH2	Notch signaling	Heterodimerization between Notch paralogues may play a role in the regulation of Notch1 activity.	(Nam et al., 2007)
NOTCH3	Notch signaling		
Trafficking			
NBEA	Trafficking	The drosophila orthologue of NBEA (rugose) was described as a positive regulator of Notch signaling.	(Shamloula et al., 2002; Wech and Nagel, 2005)
LRBA	Trafficking	C. Elegans LRBA is a negative regulator of lin12/Notch activity	(de Souza et al., 2007)
Transcriptional Regulators			
TBLR1	Corepressor for coactivator exchange factor	Depletion of TBLR1 or its drosophila orthologue EBI abrogates Notch-dependent transcription.	(Marygold et al., 2011; Perissi et al., 2008)
HCF1	Transcriptional activator	Genome-wide RNAi screen in Drosophila revealed a role of HCF in promoting Notch-dependent transcription.	(Mourikis et al., 2010)
SRRT	RNAi pathway component and transcriptional activator	Knockdown of SRRT (ARS2) in neuronal stem cells reduced the expression of Notch-target genes (HES1 and HES5).	(Andreu-Agullo et al., 2012)
ERH	Might be involved in transcriptional regulation	The drosophila ERH orthologue was recently described as a positive regulator of the Notch signaling	(Tsubota et al., 2011)
BRG1	Components of the PBAF chromatin remodeling complex	PBAF catalytic subunit (BRG1) is required for Notch signaling in mouse embryos and neural progenitors. Moreover, RNAi screen identified drosophila PB1 (Polybromo) as a positive regulator of Notch-dependent transcription.	(Lamba et al., 2008; Mourikis et al., 2010; Takeuchi et al., 2007)
PB1			
BAF170			
BAF155			
RNF40	Subunit of BRE1, which monoubiquitinates H2B	Drosophila BRE1 is required for Notch-mediated activation of its target genes	(Bray et al., 2005; Tenney et al., 2006)
LSD1	Histone demethylase	Repressor of Notch-target genes expression in the absence of Notch activation. Genetic interaction with the Notch signaling pathway has been reported in drosophila.	(Di Stefano et al., 2011; Mulligan et al., 2011; Wang et al., 2007)
Protein-Modifiers			
FBW7	E3 ubiquitin ligase	Mammalian FBW7 and its C Elegans orthologue SEL-10 negatively regulates Notch signaling by targeting ICN1 for ubiquitination and degradation. Inactivating mutations of FBW7 in human T-ALL promote oncogenic ICN1 stabilization.	(Gupta-Rossi et al., 2001; O'Neil et al., 2007; Oberg et al., 2001; Thompson et al., 2007; Wu et al., 2001a)
HDAC1	Histone and non-histone proteins deacetylase	Component of the CSL-repressor complex. HDAC1 deacetylates active Notch3 promoting its stabilization.	(Hsieh et al., 1999; Kao et al., 1998; Palermo et al., 2011)
GPS1	Subunit of COP9 signalosome deneddylation complex	Genome-wide RNAi screen in Drosophila identified the COP9 deneddylation complex as a Notch signaling regulator.	(Mummery-Widmer et al., 2009)
Signaling cross-talk			
VAV1	Guanine exchange factor. Activator of Rho/Rac GTPases	VAV1 negatively regulates Notch signaling during early T-cell development. C. Elegans VAV1 suppresses Notch activity.	(Dumont et al., 2009; Yoo and Greenwald, 2005)
SMAD9	TGF/BMP signaling effector	A physical interaction between Smad proteins and the Notch-activation complex refines Notch transcriptional responses during Notch-TGF/BMP crosstalk.	(Blokzijl et al., 2003; Li et al., 2011; Samon et al., 2008)
Lineage-specific transcription factors			
HEB	Transcription factor. Regulator of T-cell development	Notch1 signaling is defective in HEB-deficient T-cell precursors	(Braunstein and Anderson, 2011)
BCL11B	Transcription factor. Regulator of T-cell development	BCL11B is required for T-cell commitment and is a downstream target of Notch. Enforced expression of Bcl11b during early T-cell development abrogates Notch/IL7-dependent self-renewal of uncommitted progenitors.	(Ikawa et al., 2010; Li et al., 2010a; Li et al., 2010b; Wakabayashi et al., 2003)
RUNX1	Transcription factor. Regulator of T-cell development	RUNX1 has been reported to cooperate with Notch during drosophila and zebrafish development. RUNX1 DNA motifs are enriched near ICN1 binding sites in T-ALL cells.	(Burns et al., 2005; Flores et al., 2000; Wang et al., 2011)
IKAROS	Transcription factor. Regulator of T-cell development	IKAROS suppresses Notch transcriptional responses and proliferation in developing and leukemic T-cells. Ikaros deficient mice develop Notch-dependent T-cell leukemia.	(Beverly and Capobianco, 2003; Dumortier et al., 2006; Winandy et al., 1995)
HELIOS	Transcription factor. Mainly expressed in T cells	In T cells, Helios is a major partner of the Notch suppressor Ikaros. Its overexpression blocks early T-cell development.	(Cai et al., 2009; Sridharan and Smale, 2007; Zhang et al., 2007)

Table S3. Identified Notch partners involved in transcription

Protein	Primary function	Role in transcriptional activation	references
Transcriptional activators			
BRG1	Nucleosome remodeling	Components of the PBAF chromatin remodeling complex. PBAF regulates transcription by altering the chromatin structure.	(Ho and Crabtree, 2010)
PB1			
BAF170			
BAF155			
RNF40	Histone H2B ubiquitin ligase	Monoubiquitination of H2B-K120 is a prerequisite for the methylation of H3K4 (initiation) and H3K79 (elongation).	(Osley, 2006; Zhu et al., 2005)
LSD1	Histone demethylase	Activates transcription by demethylating the repressive mark H3K9me1/2 and non-histone proteins (such as the HIV-1 transactivator Tat).	(Garcia-Bassets et al., 2007; Metzger et al., 2005; Perillo et al., 2008; Sakane et al., 2011)
PHF8	Histone demethylase	Activates transcription by removing multiple repressive marks including H3K9me1/2, H3K27me2 and H4K20me1.	(Feng et al., 2010; Fortschegger et al., 2010; Horton et al., 2010; Kleine-Kohlbrecher et al., 2010; Liu et al., 2010; Loenarz et al., 2010; Qi et al., 2010; Qiu et al., 2010; Zhu et al., 2010)
TBLR1	Corepressor/coactivator exchange factor	Mediates the exchange of corepressor for coactivator during activation by signal-dependent transcription factors	(Perissi et al., 2004; Perissi et al., 2008)
MED23	Transcriptional initiation	Components of the mediator complex, which promotes the assembly of RNA polymerase II and general transcription factors.	(Malik and Roeder, 2005)
MED25			
C14ORF166	RNA PolII regulation	Interacts with RNA polymerase II and positively regulates its activity.	(Perez-Gonzalez et al., 2006)
TATSF1	Transcriptional elongation	Couple transcription elongation to RNA processing.	(Li and Green, 1998; Zhou and Sharp, 1996)
HCF1	Transcriptional coactivator	Transcriptional coactivator for multiple cellular and viral transcription factors.	(Kristie and Sharp, 1993; Vogel and Kristie, 2000)
TAZ	Transcriptional coactivator	Transcriptional coactivator in the Hippo signaling pathway	(Liu et al., 2011)
ASCC3	Transcriptional activator	Helicase that unwind duplex DNA. Play an essential role in transcriptional activation by various transcription factors.	(Dango et al., 2011; Jung et al., 2002)
AF4P12	Uncharacterized	Exhibits transcriptional activation properties	(Hayette et al., 2005)
Factors involved in transcription			
SMC1A	Cohesin complex. Involved in chromosome cohesion during cell cycle	Cohesin facilitates transcriptional activation by promoting enhancer-promoter communication.	(Fay et al., 2011; Kagey et al., 2010; Pauli et al., 2010; Seitan et al., 2011)
SMC3			
PDS5A			
MAU2			
AMPK	Metabolic pathway kinase	Stimulates transcriptional elongation by directly phosphorylating histone H2B at serine 36.	(Bungard et al., 2010)
ERK2	Signaling pathway kinase	Upon MAPK pathway activation, ERK2 phosphorylates and activates transcription factors. Component of hormone receptors activation complex.	(Agoulnik et al., 2008; Chen et al., 2007; Madak-Erdogan et al., 2011; Vicent et al., 2006)
DNAPK	DNA repair	DNA-PK complex induces DNA double-strand breaks required for transcription activation by various transcription factors.	(Abramson et al., 2010; Haffner et al., 2010; Ju et al., 2006; Nock et al., 2009; Tyagi et al., 2011; Wong et al., 2009)
TOP2B			
PARP1			
RANBP9	Ran-GTPase binding partners	Essential for transcriptional activation by nuclear hormone receptors.	(Harada et al., 2008; Poirier et al., 2006; Rao et al., 2002)
RANBP10			
MMS19	DNA repair	Interacts with the estrogen receptor and stimulates its transcriptional activity	(Wu et al., 2001b)
MCM5	DNA replication	Directly interacts with STAT1 and regulates interferon-induced gene expression.	(Snyder et al., 2005; Zhang et al., 1998)
SRRT	RNAi pathway component	Recently reported to directly activate transcription.	(Andreu-Agullo et al., 2012)
DDX17	RNA helicase	DDX17 acts as transcriptional coactivators for several transcription factors (such as estrogen receptor)	(Watanabe et al., 2001; Wortham et al., 2009)
PRP19	mRNA splicing	In Saccharomyces cerevisiae, Prp19 acts as a transcription elongation factor.	(Chanarat et al., 2011)

Supplemental Experimental Procedures

Cell culture and treatment

Human T-ALL cell lines SupT1, HPB-ALL, TALL1, DND41, MOLT4 and H9 were used in this study. NOTCH1 signaling in SupT1, HPB-ALL, TALL1, DND41 and MOLT4 is constitutively active and requires γ-secretase cleavage for activation. Notch signaling was inhibited by treating cells with the γ-secretase inhibitor (GSI) compound E (santa cruz) at a final concentration of 0.5-1μM. For ligand-mediated Notch signaling activation, the monocytic cell line U937 was cultured for 1 hour with precoated recombinant Notch ligand Delta-like 4 (5 μg/mL). LSD1 demethylase activity was inhibited by addition of cell-permeable LSD1 inhibitors: tranylcypromine (TCP) and compound S2101. The general monoamine oxidase inhibitor TCP (Sigma P8511) was used at a final concentration of 1mM. The recently designed compound S2101 (LSD1 Inhibitor II, Calbiochem), which exhibits stronger LSD1 inhibition (IC50=0.99μM vs. 184μM) and much weaker effect on monoamine oxidases (Mimasu et al., 2010), was used at 30μM.

shRNA vectors

shRNA-mediated knockdown experiments were performed using the pSUPER.retro.puro vector (Oligoengine). DNA oligos containing specific shRNA sequence were synthesized with a forwad sequence: GATCCCC(shRNA-sens-sequence)TTCAAGAGA(shRNA-reverse-complementary-sequence)TTTTTC; and reverse sequence: TCGAGAAAAA(shRNA-sens-sequence)TCTCTTGAA(shRNA-reverse-complementary-sequence)GGG. After annealing, the synthesized DNA insert was cloned into XhoI/BglII digested pSUPER vector.

Target	shRNA sequence
Luciferase (control)	CGTACGCGGAATACTTCGA
SCR (control)	TCTCGCTTGGGCGAGAGTAAG
CSL (1)	GCATGGCACTCCCAAGATTGA

CSL (2)	GAGTCTCAACCGTGTGCAT
LSD1 (1)	GAAGGCTCTTCTAGCAATA
LSD1 (2)	GCACCTTATAACAGTGATA
PHF8	GCTTCATGATCGAGTGTGACA
BRG1 (1)	CGACGTACGAGTACATCAT
BRG1 (2)	GGGTACCCTCAGGACAACA
AF4p12 (1)	GCAGGAATGTGCTCAGTAT
AF4p12 (2)	GGCTGTTTCAGACAATTCA

Expression vectors

Retroviral pOZ constructs containing a single tag (FLAG or HA) were made by modifying the pOZ-Flag/HA (F/H) vector (Nakatani and Ogryzko, 2003) and the pOZ.puro-F/H vector (Kumar et al., 2009). Human NOTCH1 intracellular domain (ICN1) was PCR amplified from the MigRI-ICN1 vector (a kind gift from J. Aster) and inserted into the XhoI/NotI sites of pOZ vectors. pOZ-MAML1 and pOZ-LSD1 constructs were generated by PCR amplification of human MAML1 and LSD1 coding region from pFLAG-CMV2-MAML1 vector (kind gift from L. Wu) and pcDNA3-LSD1 vector (kind gift from T. Kristie). pOZ-F/H vectors encoding human wide-type PHF8 and the catalytic mutant F278S were obtained from H. Qi and Y. Shi (Qi et al., 2010). These constructs contain silent mutations that confer shRNA resistance (R). PHF8 was subcloned into pOZ.puro vectors. pBABE-BRG1-Flag vector was obtained from Addgene (1959, Robert Kingston). All constructs were verified by sequencing.

Vector	Backbone	encoded gene	Tag	Selection
MigRI-ICN1	MigRI	ICN1	none	GFP
pOZ-F/H-ICN1	pOZ	ICN1	FLAG-HA (C)	IL2R
pOZ.puro-F/H-ICN1	pOZ	ICN1	FLAG-HA (C)	Puromycin
pOZ-HA-ICN1	pOZ	ICN1	HA (C)	IL2R
pOZ.puro-HA-ICN1	pOZ	ICN1	HA (C)	Puromycin
pBABE-BRG1-Flag	pBABE	BRG1	FLAG (C)	Puromycin
pOZ.puro-Flag-LSD1	pOZ	LSD1	FLAG (N)	Puromycin
pOZ-F/H-PHF8 (R)	pOZ	PHF8 (R)	FLAG-HA (N)	IL2R
pOZ -F/H-PHF8 FS (R)	pOZ	PHF8 F279S (R)	HA (N)	IL2R

pOZ.puro-Flag-PHF8 (R)	pOZ	PHF8 (R)	FLAG (N)	Puromycin
pOZ-F/H-MAML1	pOZ	MAML1	FLAG-HA (N)	IL2R
pOZ-Flag-MAML1	pOZ	MAML1	FLAG (N)	IL2R

Virus production and cell line transduction

293T cells were transfected with a packaging mixture and the retroviral vector (pOZ, pSUPER, pBABE) using the calcium phosphate precipitation method. For transfection, 5µg of the retroviral vector, 2.5µg of the packaging plasmid (gag/pol) and 2.5µg of the envelope plasmid were mixed with 100µL of CaCl2 (1.25M) and 500µL of HBS2X (sigma) in a final volume of 1mL. The mixture was incubated 1min at room temperature then added dropwise to the cells. The medium was changed the following day and the viral-containing supernatant was collected 48 hours after transfection, filtered through a 0.45 µm filter and subsequently used to infect cells.

To establish stable SupT1 cell lines expressing tagged ICN1, MAML1, LSD1, PHF8 or BRG1, we transduced SupT1 with recombinant retroviruses expressing a bicistronic mRNA that encodes the tagged protein and a selection marker (either IL-2 receptor subunit alpha or puromycin resistance gene). Transduced cells were purified by affinity cell sorting (for IL2R) or selected by puromycin treatment (2µg/mL).

For shRNA-mediated knockdown experiments, cells were transduced with pSUPER retroviral vectors. After an overnight incubation, a second round of infection was performed using the same vector (for PHF8 and control shRNAs) or a second shRNA targeting the same mRNA (for CSL, LSD1, AF4p12 and BRG1). The medium was refreshed the following day and puromycin was added 72 hours post-infection at a final concentration of 2µg/mL. Protein expression was analyzed by western blot after 3 days of selection. All the experiments were performed between day 6 and day 14 post-transduction.

Purification of Proteins complexes

Nuclear extracts were prepared using the Dignam protocol with slight modifications (Dignam et al., 1983). For the purification of ICN1-associated complexes, 12 x 10^9 SupT1 cells stably

expressing Flag-HA tagged ICN1 and control SupT1 were harvested by centrifugation, washed in cold PBS and resuspended in 4 packed cell pellet volumes of hypotonic buffer (20 mM Tris-HCl pH 7.4, 10 mM NaCl, and 1.5 mM MgCl2). The suspension was incubated on ice for 10 min and then cells were lysed by 12 strokes using a Dounce homogenizer fitted with a B pestle. The nuclei were pelleted by centrifugation and resuspended in one packed nuclear pellet volume of a buffer containing 20 mM Tris-HCl pH 7.4, 300 mM NaCl, 25% glycerol, 0.2 mM EDTA, 1.5 mM MgCl2 and PMSF. One packed nuclear pellet volume of a high salt buffer (containing 20 mM Tris-HCl pH 7.4, 720 mM NaCl, 25% glycerol, 0.2 mM EDTA, 1.5 mM MgCl2 and PMSF) was added dropwise to the suspension gently stirring with a magnetic bar. After stirring for 30 min to allow extraction of transcription factors, the suspension was centrifuged at 13.000g for 30 min at 4°C and the supernatant was dialyzed against 100 volumes of buffer BC100 (20 mM Tris-HCl pH 7.4, 100 mM NaCl, 10% glycerol, 0.2 mM EDTA, 1.5 mM MgCl2 and PMSF) for 6 hours. The dialysate (nuclear extract) was cleared by centrifugation at 13.000g for 30 min. Nuclear extracts were incubated for 4 hr (at 4°C with rotation) with anti-FLAG M2 agarose beads (Sigma) (1% v/v) equilibrated in BC100. Beads were washed 3 times with 10 mL buffer B015 (20 mM Tris-HCl pH 7.4, 150 mM NaCl, 10% glycerol, 0.5 mM EDTA, 5 mM MgCl2, 0.05% Triton X-100, 0.1% Tween, and PMSF) and bound proteins were eluted with 4 bead volumes of B015 containing 0.2 mg/mL of FLAG peptide (Sigma) for 1 hr. The FLAG affinity purified complexes were further immunopurified by affinity chromatography using 10 µL of anti-HA conjugated agarose beads (Santa Cruz). After incubation for 4 hr, HA beads were washed 4 times with 800µL of buffer B015 in spin columns (Pierce, 69702) and eluted under native conditions using HA peptide (Roche). Ten percent of the eluate was resolved on SDS-PAGE and Sylver stained using the silverquest kit (from invitrogen). The remaining material was stained with Coomassie-R250 and subsequently analysed by mass spectrometry at the Taplin Biological Mass Spectrometry facility (Harvard Medical School, Boston, MA).

In order to isolate MAML1-associated proteins in the presence or absence of activated Notch1, two-step affinity purification was performed on nuclear extracts from 4 x 10^9 SupT1 cells stably expressing FLAG-HA tagged MAML1 treated for 8 hr with DMSO or GSI, followed by western blot analysis. Reciprocal immunoprecipitations of tagged-proteins were performed on Dignam nuclear extracts derived from SupT1 stably expressing: HA tagged-ICN1/FLAG-tagged MAML1 (4 x 10^9 cells), HA tagged-ICN1/FLAG-tagged LSD1 (2 x 10^9 cells), HA tagged-ICN1/FLAG-tagged PHF8 (2 x 10^9 cells), HA tagged-ICN1/FLAG-tagged BRG1 (2 x 10^9 cells) and control SupT1. After two step affinity chromatography, protein complexes containing both tagged-proteins were peptide eluted and analyzed by western blot. For endogenous protein immunoprecipitations, nuclear extracts (500 µg-1 mg) were incubated with antibodies (1-2 µg) for 4 hr, followed by addition of 10 µL protein G Sepharose beads (Fast flow, Sigma) for 45 min before washing five times with 800µL of buffer B015 in spin columns (Pierce).

Chromatin Immunoprecipitation assays (ChIP)

For ChIP experiments, 6 x 10^7 cells were cross-linked for 10 min with 1% formaldehyde (sigma) at room temperature. The cross-linking reaction was stopped by adding glycine to a final concentration of 0.125 M for 10 min at room temperature. Cells were washed twice with cold PBS and incubated on ice for 7 min in 2 mL of buffer containing 15 mM Tris-HCl (pH 7.4), 0.3 M sucrose (sigma), 60 mM KCl, 15 mM NaCl, 5 mM MgCl2, 0.1 mM EGTA and 0.1 % NP-40. Each cell suspension was then layered over 8 mL sucrose cushion (15 mM Tris-HCl, 1.2 M sucrose, 60 mM KCl, 15 mM NaCl, 5 mM MgCl2 and 0.1 mM EGTA) and centrifuged at 10.000 g for 20 min at 4°C. Nuclear pellet was lysed with 1 mL lysis buffer (50 mM Tris-HCl pH 8, 10 mM EDTA, 1% SDS) complemented with Protease Inhibitor Cocktail (Roche). Chromatin was sonicated to generate DNA-fragments of approximately 300 to 500 bp in an ultra sonicator water bath (Bioruptor, Diagenode) using ten cycles of 30s/on and 30s/off. After centrifugation at 13.000g for 20 min, an aliquot of sonicated DNA was reverse-crosslinked by addition of 250 mM NaCl and incubation at 65 °C for 6 h. DNA

was extracted by phenol-chloroform, quantified using nanodrop and run on a 1% agarose gel to confirm DNA fragment size. The antibodies were pre-bound to Invitogen Dynal magnetic beads (Protein A or G beads) in PBS containing BSA (5 mg/mL) and chromatin was pre-cleared with beads for 4 h at 4°C. Immunoprecipitation was performed using 20 µg of chromatin and 2-3 µg of antibody coupled to 15 µL of beads in ChIP buffer (20 mM Tri-HCl pH 8, 150 mM NaCl, 2 mM EDTA, and 1% Triton X-100) complemented with Protease Inhibitor Cocktail. After overnight incubation at 4°C, beads were washed 4 times with wash buffer 1 (20 mM Tri-HCl pH 8, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100 and 0.1% SDS) and 4 times with wash buffer 2 (20 mM Tri-HCl pH 8, 500 mM NaCl, 2 mM EDTA, 1% Triton X-100 and 0.1% SDS) using the DynaMag-2 magnet (Invitogen). Elution of immunoprecipitated DNA was performed in buffer containing 1% SDS and 100 mM NaHCO3. Crosslinking was reversed by incubation at 65 °C and proteins were degraded by addition of proteinase K (Sigma). Eluted DNA and 10% of input DNA were purified using phenol-chloroform extraction followed by isopropanol precipitation or using QIAquick PCR purification (Qiagen), according to the manufacturer instructions. Resultant DNA was dissolved in 60 µL of water containing 10 mM Tris-HCl pH 8. ChIP DNA was analysed by SYBR Green quantitative PCR (Qiagen) using specific primers. qPCR was carried out in the LightCycler480 (Roche) with a 15 min DNA denaturation step at 95°C, followed by 40 cycles of 15 s at 95°C, 30 s at 58°C and 30 s at 72°C. PCR measurements were performed in duplicate. The average of the technical replicates was normalized to input DNA per set of primer using the comparative CT method ($2^{-\Delta\Delta CT}$). Averages and standard deviations of the biological replicate values are shown in the figures. The number of biological replicates is indicated in the figure legends.

Antibodies used for ChIP experiments:

Antibody	Reference
NOTCH1	Cell Signaling Technology - 2421
NOTCH1	Bethyl – A301-895A

MAML1	Bethyl – A300-672A
BRG1	Santa Cruz – sc10768
LSD1	Cell Signaling Technology – 4218
PHF8	Abcam – ab36068
PB1	Bethyl - A301-591A
AF4P12 (FRYL)	Bethyl - A302-644A
CSL	cosmobio – 2ZRBP1
RNA Polymerase II	Millipore – 041569
H3K4me1	Abcam – ab8895
H3K4me2	Abcam – ab32356
H3K4me3	Abcam - ab8580
H3K9me1	Millipore – 07450
H3K9me2	Abcam – ab1220
H3K27me2	Millipore – 07452
H3K27me3	Millipore – 17622
H4K20me1	Abcam – ab9051
Histone 3	Abcam – ab1791
Control IgG	Sigma – C2288

ChIP primers:

Locus	Primer position	Forward	Reverse
NOTCH3	-5kb	TAGCCCCTGGTCAGTCATTC	GGTGCATCGTATCAGGAGGT
NOTCH3	TSS	TGGCCTCAGTTTCCAGAGTT	CACACCCAACCTCGTGAAC
NOTCH3	+3000 (CBS)	GTCTCAGCACACCCCATTCT	AACCACAAAGCAGGGGAAG
NOTCH3	+28600	GGGGGCTAAAGACACAAACA	GTTCCTTCTCTCCCCACTCC
IL7R	-2200	CCCAGTTACTCACCCATGAAG	AGCCTGATTCACAAAAATTGG
IL7R	TSS	CCTCCCTCCCTTCCTCTTAC	TTGAGCATAGCCACTTTCTCC
IL7R	+11000	CATCACGGAAGGCAATCTAC	GTTGAAGGGGGAGCGAATAG
IL7R	+28000 (CBS)	CAAGCCAGGTTGTTCAGACA	CACTTCACCCCACCCTATTG
DTX1	-1700	TGTGAATGACATGGCAGAGG	TGAATCTCCTGCCAGTACCC
DTX1	-400	TTCGGTTCCCCATTGTTCT	GACCTGCCAGGAGACAAGAG
DTX1	+800	AATCTGCTGATGCCAAATCC	CAACTTGCTCAAGGCCCTAC
DTX1	+30000 (CBS)	ACATGCCAGACAGCAGAACA	AACCTTCCAGACCCTGTGTG
HES1	-2200	AGGTCACCCAGAGTCAGGAA	CCAGCGTCTTGTTTGATGTG
HES1	TSS (CBS)	CGTGTCTCCTCCTCCCATT	GAGAGGTAGACGGGGGATTC

HES1	+450	TCAACACGACACCGGATAAA	TCAGCTGGCTCAGACTTTCA
HES1	+2600	GGCTTTTGGTGGAATTTGAA	TCATGGAGGATTGGTGAAAAG
CR2	+5000 (CBS)	GCCGGAAGGATGTTCTTGTA	CAGGGAAGGCCATGAAAATA
CR2	+21000	CCCCACAGTGCTTACGATCT	AAGCCAGGATTGCAGTCAAC

The position of each amplicon is given relative to the Transcription Start Site (TSS). The amplicons corresponding to the CSL-ICN1 binding site (CBS) are indicated.

Western blots

Cells were lysed in lysis buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM EDTA, 0.5% NP-40 and PMSF) and briefly sonicated. Cell lysates and immunoprecipitates were boiled in SDS sample buffer and resolved on a 7% SDS-PAGE gel (Biorad). Proteins were liquid-transferred (Biorad) to nitrocellulose membrane in transfer buffer (20% methanol, 25 mM Tris, 192 mM Glycine, 0.037% SDS) during 90 min at 100V. Western blotting was performed using the following antibodies and conditions:

- Primary antibodies (incubated 2 hr at room temperature in PBS 0.1% tween):

Antibody	Reference	Dilution	Source
HA	Roche – Clone 3F10	1/1000	Rat
FLAG	Sigma – Clone M2	1/10.000	Mouse
ICN1 (V1744)	Cell Signaling Technology - 2421	1/1000	Rabbit
NOTCH1 (ICD)	Bethyl – A301-895A	1/1000	Rabbit
MAML1	Bethyl – A300-672A	1/1000	Rabbit
CSL	Cosmo Bio – SIM-2ZRBP2	1/500	Rat
BRG1	Santa Cruz – sc10768	1/1000	Rabbit
LSD1	Cell Signaling Technology – 4218	1/500	Mouse
LSD1	Bethyl – A300-215A	1/1000	Rabbit
PHF8	Abcam – ab36068	1/2000	Rabbit
PB1	Bethyl - A301-591A	1/2000	Rabbit
AF4P12 (FRYL)	Bethyl - A302-644A	1/2000	Rabbit
USP7	Cell Signaling Technology – 4833	1/1000	Rabbit
PP2A A	Cell Signaling Technology – 2041	1/1000	Rabbit
PP2A B	Cell Signaling Technology – 2290	1/1000	Rabbit

PP2A C	Cell Signaling Technology – 2259	1/500	Rabbit
ERK2	Cell Signaling Technology – 9108	1/1000	Rabbit
HDAC1	Abcam – ab7028	1/1000	Rabbit
RBBP4	Cell Signaling Technology - 4633	1/500	Rabbit
BCL11B	Santa Cruz – sc56014	1/1000	Rat
TOP2B	Abcam – ab72334	1/1000	Rabbit
Tubulin	Sigma	1/10.000	Mouse

The anti-ICN1(V1744) antibody detects the γ-secretase cleaved form of NOTCH1. The anti-NOTCH1 (ICD) antibody recognizes the intracellular domain of endogenous NOTCH1 (when bound to membrane or cleaved) and ectopically expressed Flag-HA-ICN1.

- Secondary antibodies (incubated 1 hr at room temperature in PBS-5% milk-0.1% tween):

Antibody	Reference	Dilution
anti-rabbit-HRP	Cell Signaling Technology - 7074	1/2000
anti-mouse-HRP	GE Healthcare – NA931V	1/2500
anti-rat-HRP	GE Healthcare – NA935V	1/2500

Quantitative RT–PCR

Total RNA was isolated using Trizol reagent (Invitrogen) and reverse transcription was performed with 500 ng of RNA using SuperScript II (Invitrogen) and oligo-dT, according to the manufacturer's instructions. PCR measurements were performed in duplicate using SYBR Green (Qiagen). Amplification was carried out in the LightCycler480 (Roche) with a 15 min DNA denaturation step at 95°C, followed by 40 cycles of : 15 s at 95°C, 30 s at 60°C and 30 s at 72°C. The average of the technical replicates was normalized to GAPDH levels using the comparative CT method ($2^{-\Delta\Delta CT}$). Averages and standard deviations of at least 3 experiments are shown in the figures.

Q-RT-PCR were performed using the following primers:

Gene	Forward	Forward
HES1	CCTCTCTTCCCTCCGGACTCT	CAAATATAGTGCATGGTCAGTCACT
DTX1	CGGCTTCCCTGATACCCAGACC	GCAGCACCTTCCGGCCTTTCTC
IL7R	TCGCTCTGTTGGTCATCTTG	GGAGACTGGGCCATACGATA
NOTCH3	TTCCCAGTGAGCACCCTTAC	ACTTGCCTCTTGGGGGTAAC
HEY1	TCGGCTCCTTCCACTTACTG	TTCCCCTCCCTCATTCTACA
CR2	CCCCACAGTGCTTACGATCT	AAGCCAGGATTGCAGTCAAC
ID1	CGGATCTGAGGGAGAACAAG	TCCCACCCCCTAAAGTCTCT
RCBTB2	AGAAGGATGGTGGTGAGTGG	TGAGACCAGGGTACCAAAGG
GAPDH	CGGAGTCAACGGATTTGGTCGTAT	AGCCTTCTCCATGGTGGTGAAGAC
S14 (RPS14)	GGCAGACCGAGATGAATCCTCA	CAGGTCCAGGGGTCTTGGTCC

Quantification of nascent transcripts

RNAs were isolated using the Trizol reagent (Invitrogen) and treated with DNase (M610A promega) for 30min at 37°C. The reaction was stopped according to the manufacturer's instructions and reverse transcription was performed with 1µg of RNA using SuperScript II (Invitrogen) and random primers. PCR measurements were performed as described above (Q-RT-PCR) using intronic primers.

Notch-responsive reporter assay

HeLa cells were co-transfected with 1µg of a Notch-responsive firefly luciferase reporter containing 6 CSL-binding sites (p6XCBS-luc), 100ng of TK-Renilla-luciferase vector (transfection control) and various amount (0.1 - 0.3 - 1 µg) of the MigR1-ICN1 expression vector. Transfections were performed in 6-wells plates using JetPEI reagent (Polyplus) according to manufacturer's instruction. Firefly luciferase activity was measured 24 hours post-transfection and normalized to Renilla luciferase expression. The values in the figures are Relative Luciferase Units (RLU) represented as fold induction over the luciferase activity measured in the absence of ICN1 (cells transfected with p6XCBS-luc and an empty vector). The mean and standard deviations from several experiments are shown in the figures. The

number of experiments is indicated in the figure legends. The p6XCBS-luc reporter was a kind gift from Alain Israel (Pasteur Institute, Paris).

Subcutaneous xenograft tumor model

Female SCID mice (C.B.-17/IcrHan™Hsd-Prkdc^{scid}) were obtained from *Harlan Laboratories* (Gannat, France). Animals were maintained in specific pathogen-free animal housing at the Center for Exploration and Experimental Functional Research (CERFE, Evry, France) animal facility. The human T-ALL cell line SupT1 was infected with retroviral vectors encoding shRNA directed against human PHF8, CSL, and LSD1, or a control shRNA. 72 hours post-infection, cells were selected with 2 µg/mL puromycin for 72 hours. At this point, the cells were maintained in fresh media for 2 days prior to injection into animals. Prior injection, cells were washed and resuspended in DMEM : 50% Matrigel (BD Pharmingen). 5X10^6 cells (in 100µL) were injected to each mouse by subcutaneous route in the right flank (n=5 per group). Tumor volume was evaluated by measuring tumor diameters, with a calliper, three times a week during the follow-up period (23 days). The formula TV (mm^3) = [length (mm) x width (mm)^2]/2 was used, where the length and the width are the longest and the shortest diameters of the tumor, respectively.

Flow cytometry, cell proliferation and cell cycle analysis

The following antibodies were used for flow cytometry: CD127-PE clone R34.34 and the IgG1-PE (from Beckman Coulter). Flow cytometry was performed on a BD FACSCalibur or MACSQUANT cytometer (Miltenyi). For cell proliferation assays, cells were plated at 2X10^5/mL in triplicate. Proliferation of shRNA-transduced T-ALL cells was followed by cell counting using the MACSQUANT cytometer (gated on live cells). Flow-cytometric cell cycle analysis was performed by staining DNA content of T-ALL cell lines using DAPI. SupT1 cells were also analyzed with EdU-DAPI staining to precisely define the percentage of cells at the G0/G1 phase. Briefly, SupT1 expressing control, CSL, PHF8 or LSD1 shRNAs were treated

with 10 µM EdU for 2 hrs. Cells were washed with PBS and fixed with PBS-4% PFA for 10 minutes at room temperature. After permeabilization, EdU incorporation was detected following the manufacturer's instructions (Click-iT, invitrogen) and total DNA content was measured using DAPI.

Nuclear Run-On

SupT1 cells expressing control or AF4p12 shRNA were harvest, washed twice with cold PBS and incubated on ice for 7 min in 2 mL of buffer containing 15 mM Tris-HCl (pH 7.4), 0.3 M sucrose (sigma), 60 mM KCl, 15 mM NaCl, 5 mM MgCl2, 0.1 mM EGTA and 0.1 % NP-40. Cell suspension was layered over 8 mL sucrose cushion (15 mM Tris-HCl, 1.2 M sucrose, 60 mM KCl, 15 mM NaCl, 5 mM MgCl2 and 0.1 mM EGTA) and centrifuged at 10.000 g for 20 min at 4°C. Nuclei were resuspended in freezing buffer (50 mM Tris-HCl pH=8, 40% glycerol, 5 mM MgCl2 and 0.1 mM EDTA) at a concentration of $5X10^6$/mL and freezed (at -80°C). Nuclear Run-on assays were performed as described previously (Core et al., 2008), except that we used $5X10^5$ nuclei per reaction containing 500µM ATP, CTP, GTP and br-UTP and 0,5% sarkosyl. The reaction was performed at 30°C for 5 minutes. RNAs transcribed during the assay were purified using anti-BrdU beads (santa cruz) and reverse transcription was performed using SuperScript II (Invitrogen) and random primers. PCR measurements were performed as described above (Q-RT-PCR) using intronic primers.

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