The Notch-driven long non-coding RNA repertoire in T-cell acute lymphoblastic leukemia

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Supplementary Methods

Cell lines and compound treatment

HPB-ALL, TALL-1 and ALL-SIL cells were obtained from the DSMZ cell line repository, CUTLL1 cells were a kind gift of H.G. Wendel (Memorial Sloan Kettering Cancer Center, New York, USA). Cells were maintained in RPMI-1640 medium (Life Technologies, 52400-025), supplemented with 10 % or 20 % (ALL-SIL and CUTLL1) fetal bovine serum (Biochrom AG, S 0615), 1 % L-glutamin (Life Technologies, 15140-148) and 1 % penicillin/streptomycin (Life Technologies, 15160-047). CUTLL1, HPB-ALL, TALL-1 and ALL-SIL cells were seeded at a density of $1*10^6$ cells/ml and treated with either DMSO or 1 µM of Compound E (Enzo Life Sciences). Cells were harvested 12 and 48 hours after treatment.

RNA-isolation, cDNA synthesis and RT-qPCR

Total RNA was harvested with the miRNeasy mini kit (Qiagen) with DNase treatment on-column. RNA-concentrations were measured by means of spectrophotometry (Nanodrop). cDNA was synthesized using the iScript cDNA synthesis kit (Biorad, 170-8891) according to the manufacturers' protocol, starting with 500 ng of RNA, followed by RT-qPCR using the Light Cycler 480 (Roche). Finally, qPCR data was analyzed using the qBasePLUS software (Biogazelle) according to the ΔΔCt-method.

Target	**Forward primer**	**Reverse primer**
c-MYC	GCCACGTCTCCACACATCAG	TGGTGCATTTTCGGTTGTTG
HES1	TGTCAACACGACACCGGATAAA	CCATAATAGGCTTTGATGACTTTCTG
DTX1	ACGAGAAAGGCCGGAAGGT	GGTGTTGGACGTGCCGATAG
HPRT	TGACACTGGCAAAACAATGCA	GGTCCTTTTCACCAGCAAGCT
HMBS	GGCAATGCGGCTGCAA	GGGTACCCACGCGAATCAC
TBP	CACGAACCACGGCACTGATT	TTTTCTTGCTGCCAGTCTGGAC
B2M	TGCTGTCTCCATGTTTGATGTATCT	TCTCTGCTCCCCACCTCTAAGT

Reaction conditions for RT-qPCR

Components	**Amount**
sSo Advanced 2x mastermix	2,5 µl
Forward Primer (5 µM)	0,25 µl
Reverse Primer (5 µM)	0,25 µl
cDNA (2,5 ng/µl)	2 µl

Thermocycling parameters

Step	Temperature	Time	Cycles
Enzyme activation	95 °C	2 min	1
Amplification	95 °C	5 sec	44
	60 °C	30 sec	
	72 °C	1 sec	
Melting cyclus	95 °C	5 sec	1
	60 °C	1 min	
	95 °C	continuous	
Cooling	37 °C	3 min	1

Western blotting

Total protein isolation was performed with RIPA-lysis buffer, supplemented with protease inhibitors and SDS-PAGE was performed according to standard protocols. For immunoblotting, the rabbit polyclonal antibody to cleaved NOTCH1 (Val 1744) (Cell Signaling, 2421S) was used in a 1:500 dilution in BSA.

Protein coding potential calculation

We used PhyloCSF to identify putative protein coding transcripts in the unannotated, novel putative lncRNA loci obtained by RNA-seq. This algorithm employs codon substitution frequencies in whole-genome multi-species alignments to distinguish between coding and non-coding loci. Whole-genome alignments of 46 species are obtained from the UCSC website and processed using the PHAST package (version 1.3) to obtain the required input format for PhyloCSF.

To validate our workflow, we benchmarked PhyloCSF with transcripts annotated in Ensembl (version 75). Transcripts with biotype ‘lincRNA’ or ‘antisense’ (20,320 transcripts) serve as a negative set while transcripts with biotype ‘protein coding’ and an annotated coding sequence (36,959 transcripts) serve as a positive set. Using these sets, we have determined 41.2019 as an optimal treshold for the PhyloCSF score (precision of 95% and sensitivity of 91%).

Micro-array based gene expression profiling

RNA samples from T-ALL cells treated with GSI, CD34$^+$ thymocytes cultured on the OP9-GFP/DLL1 system, sorted T-cell subsets (CD34$^+$ and CD4$^+$CD8$^+$) and two primary T-ALL patient cohorts of which one cohort including samples with known *NOTCH1* mutation status (n=15) (all *FBXW7* wild type) and a larger cohort (n=64) were profiled on a custom designed Agilent micro-array covering all protein coding genes and 12,000 lncRNAs (23,042 unique lncRNA probes) as described by Volders et al.[46]

Profiling was performed according to the manufacturers protocol (One-color Microarray-Based Gene Expression Analysis, Low Input Quick Amp Labeling, Agilent Technologies), with 100 ng RNA as input. Normalization of the expression data was performed with the VSN-package (BioConductor release 2.12) in R. Expression values were further subjected to background subtraction by selecting those probes detecting a 10 % higher expression than the negative control probes of the array design in at least one treatment. Differential expression analysis was performed in R using Limma. A multifactorial design was used to control for batch effects.

Correlation analysis for functional annotation of selected lncRNAs

Normalized micro-array based gene expression profiles were generated for all samples of the primary T-ALL patient cohort (n=64). Spearman's rho values were calculated between 15 out of the set of 27 overlapping annotated lncRNAs (Figure 2E) (and also for *LUNAR1* (*lnc-IGF1R-1*)) for which a probe on the custom micro-array[46] was available. This output was used to generate a ranked (.rnk) file and used as an input for a GSEA pre-ranked analysis using the c2v3.1 MsigDB collection as geneset database. The output files were subsequently loaded into Cytoscape. By means of the Cytoscape plug-in 'enrichment mapping' (Isserling et al., F1000Research, 2014), enrichment maps were built representing functional gene set clusters that were significantly correlated (red nodes) or anti-correlated (blue nodes) with the lncRNA-of-interest.

Supplementary Tables

Supplementary Table 1: RNA-seq alignment summary for CUTLL1 samples

Sample name	Paired-end reads	Aligned Pairs	Aligned Pairs (%)	Multimapping pairs	Multimapping pairs (%)	Concordant aligned pairs (%)
DMSO 12h repl1	146323593	131275766	89.7	3216065	2.4	88.8
DMSO 48h repl1	131215273	117807914	89.8	2895717	2.5	88.9
GSI 12h repl1	159416630	143627829	90.1	3462073	2.4	89.3
GSI 48h repl1	95100288	85514736	89.9	2019348	2.4	89.1
DMSO 12h repl2	135554412	122299013	90.2	2828382	2.3	89.5
DMSO 48h repl2	100687539	90796028	90.2	2110770	2.3	89.4
GSI 12h repl2	199650982	180374457	90.3	4257865	2.4	89.6
GSI 48h repl2	169001713	152387296	90.2	3637367	2.4	89.3
DMSO 12h repl3	153193248	138567335	90.5	3389716	2.4	89.6
DMSO 48h repl3	98349936	88513219	90.0	2165418	2.5	89.1
GSI 12h repl3	117967005	106283685	90.1	2499311	2.4	89.2
GSI 48h repl3	95918114	86593969	90.3	2058472	2.4	89.4

Supplementary Table 2: RNA-seq alignment summary for OP9 samples

Sample Name	Paired-end reads	Aligned Pairs	Aligned Pairs (%)	Multimapping pairs	Multimapping pairs (%)	Concordant aligned pairs (%)
OP9-GFP donor 1	175557925	157523775	89.7	3998129	2.5	88.9
OP9-DLL1 donor 1	121468805	110981627	91.4	2682569	2.4	90.4
OP9-GFP donor 2	133723750	119466289	89.3	3172892	2.5	88.4
OP9-DLL1 donor 2	194441409	177560274	91.3	4350749	2.5	90.5

Supplementary Table 3: Overview of 27 differentially expressed (baseMean > 100) and annotated Notch positively regulated lncRNAs shared between CUTLL1 T-ALL cells and CD34+ T-cells on OP9

Chr	start	stop	Ensembl ID	lncipedia
chr1	113499037	113544813	*ENSG00000226419*	*lnc-LRIG2-4*
chr1	173833038	173838020	*ENSG00000234741*	*lnc-SERPINC1-1*
chr1	230394440	230404229	*ENSG00000227006*	*lnc-PGBD5-2*
chr1	239866684	239893765	*ENSG00000233355*	*lnc-GREM2-6*
chr2	87754887	87906324	*ENSG00000222041*	*lnc-PLGLB2-1*
chr2	99378401	99388543	*ENSG00000226791*	*lnc-c2orf55-1*
chr2	111965353	112252677	*ENSG00000172965*	*lnc-AC108463.1-3*
chr2	132160474	132166622	*ENSG00000223631*	*lnc-PLEKHB2-1*
chr2	136577761	136580657	*ENSG00000226806*	*lnc-UBXN4-1*
chr5	987295	997423	*ENSG00000215246*	*lnc-SLC12A7-4*
chr5	111496223	111499973	*ENSG00000224032*	*lnc-APC-6*
chr6	86370710	86388451	*ENSG00000203875*	*lnc-SYNCRIP-2*
chr7	22893875	22901021	*ENSG00000228649*	*lnc-IL6-2*
chr8	130228728	130253496	*ENSG00000250400*	*lnc-GSDMC-2*
chr9	96197614	96199403	*ENSG00000227603*	*lnc-FAM120AOS-1*
chr9	139440664	139444345	*ENSG00000237886*	*lnc-c9orf163-2*
chr16	2204798	2205359	*ENSG00000260260*	*lnc-PKD1-1*
chr16	66921918	66922834	*ENSG00000261705*	*lnc-CA7-1*
chr16	66923072	66924996	*ENSG00000261088*	*lnc-CA7-2*
chr16	81416874	81424489	*ENSG00000261609*	*lnc-GAN-1*
chr17	13932609	13972775	*ENSG00000223385*	*lnc-CDRT15-2*
chr17	14113805	14121239	*ENSG00000231595*	*lnc-COX10-1*
chr17	14207171	14208822	*ENSG00000266709*	*lnc-COX10-3*
chr17	16342136	16381992	*ENSG00000175061*	*lnc-TRPV2-1*
chr21	47013568	47017005	*ENSG00000233922*	*lnc-PCBP3-1*
chr22	23804273	23829167	*ENSG00000178248*	*lnc-IGLL1-2*
chrX	130836678	130964671	*ENSG00000213468*	*lnc-FRMD7-1*

Supplementary Table 4: Overview of 13 differentially expressed (baseMean > 100) and unannotated Notch positively regulated lncRNAs shared between CUTLL1 T-ALL cells and CD34+ T-cells on OP9

Chr	CUTLL1_Start	CUTLL1_End	CUTLL1_Name	OP9_Start	OP9_End	OP9_Name
chr3	72762617	72774075	*XLOC_032451*	72767224	72773948	*XLOC_032318*
chr3	73027441	73028329	*XLOC_032454*	73027514	73028331	*XLOC_032320*
chr3	138253033	138254792	*XLOC_032825*	138252949	138255180	*XLOC_032820*
chr5	81616893	81636923	*XLOC_036711*	81616441	81629071	*XLOC_036960*
chr5	81667707	81678632	*XLOC_036719*	81672215	81678702	*XLOC_036966*
chr12	113652353	113654532	*XLOC_010851*	113649939	113654123	*XLOC_010919*
chr14	72878457	72906694	*XLOC_012963*	72875211	72906687	*XLOC_012750*
chr15	99557714	99589675	*XLOC_014517*	99557739	99586083	*XLOC_014390*
chr17	3577525	3578999	*XLOC_019018*	3578539	3578908	*XLOC_018585*
chr17	3599783	3616722	*XLOC_018313*	3600043	3602985	*XLOC_018591*
chr20	13350996	13368579	*XLOC_028304*	13350988	13366426	*XLOC_027976*
chr21	46975354	46985114	*XLOC_029412*	46975468	46978095	*XLOC_029330*
chr22	23874749	23889951	*XLOC_029562*	23878831	23890363	*XLOC_029452*

Supplementary Table 5: Overview of 18 differentially expressed (baseMean > 100) and annotated Notch negatively regulated lncRNAs shared between CUTLL1 T-ALL cells and CD34+ T-cells on OP9

Chr	start	stop	Ensembl ID	lncipedia
chr1	111196182	111216076	*ENSG00000259834*	*lnc-KCNA3-1*
chr4	153021906	153025872	*ENSG00000245954*	*lnc-FAM160A1-6*
chr4	174243357	174250845	*ENSG00000248774*	*lnc-HMGB2-1*
chr6	114290865	114792869	*ENSG00000228624*	*lnc-MARCKS-5*
chr6	71961061	72037787	*ENSG00000232295*	*lnc-B3GAT2-2*
chr7	141404138	141438146	*ENSG00000228775*	*lnc-KIAA1147-2*
chr8	60031599	60034363	*ENSG00000167912*	*lnc-SDCBP-2*
chr12	47599681	47610239	*ENSG00000247774*	*lnc-AMIGO2-1*
chr13	30914407	30951327	*ENSG00000238121*	*lnc-KATNAL1-3*
chr14	98602411	98628990	*ENSG00000258511*	*lnc-C14orf177-3*
chr15	58879706	58883875	*ENSG00000259250*	*lnc-LIPC-1*
chr15	38794807	38797182	*ENSG00000259326*	*lnc-FAM98B-1*
chr16	66441932	66447150	*ENSG00000246898*	*lnc-CDH5-1*
chr16	89179583	89181687	*ENSG00000261546*	*lnc-CBFA2T3-2*
chr17	67547499	67550002	*ENSG00000267194*	*lnc-MAP2K6-5*
chr19	35302493	35305249	*ENSG00000261754*	*lnc-ZNF599-1*
chr19	35279486	35323773	*ENSG00000267767*	*lnc-ZNF599-2*
chr19	54862991	54864894	*ENSG00000268802*	*lnc-LAIR1-1*

Supplementary Table 6: Overview of 7 differentially expressed (baseMean>100) and unannotated Notch positively regulated lncRNAs shared between CUTLL1 T-ALL cells and CD34+ T-cells on OP9

Chr	CUTLL1_Start	CUTLL1_End	CUTLL1_Name	OP9_Start	OP9_End	OP9_Name
chr2	191439671	191455565	*XLOC_026974*	191439699	191456830	*XLOC_027082*
chr3	141515902	141519763	*XLOC_032844*	141515791	141519759	*XLOC_030962*
chr5	80698801	80702028	*XLOC_036686*	80699345	80701960	*XLOC_036927*
chr14	53020359	53032577	*XLOC_013366*	53020411	53032938	*XLOC_013224*
chr15	58731929	58747956	*XLOC_015245*	58737362	58747955	*XLOC_015119*
chr15	60824624	60829179	*XLOC_015269*	60824529	60829869	*XLOC_015183*
chr22	22609022	22620320	*XLOC_030158*	22615017	22620331	*XLOC_029961*

Supplementary Table 7: Unannotated putative lncRNA loci identified in CUTLL1 by RNA-seq and predicted as ‘coding’ by PhyloCSF analysis (precision: 95%; sensitivity: 90%)

ID	Score	Chr	Start	End
TCONS_00056766	52.15	chr1	2246753	2251281
TCONS_00086798	43.68	chr1	51628045	51630685
TCONS_00081942	44.76	chr1	111333342	111342403
TCONS_00056764	52.15	chr1	117354279	117370293
TCONS_00051781	54.1	chr2	102007909	102010517
TCONS_00048597	60.08	chr2	235384613	235389131
TCONS_00056543	49.11	chr2	240516862	240526394
TCONS_00059899	41.56	chr2	241126710	241128149
TCONS_00080921	72.2	chr4	187952855	187953853
TCONS_00086799	43.68	chr5	39099174	39103319
TCONS_00086796	43.68	chr5	82223281	82226577
TCONS_00056765	52.15	chr6	14444424	14450217
TCONS_00081943	44.76	chr6	14451309	14514496
TCONS_00080012	48.79	chr6	33548126	33551228
TCONS_00048598	60.08	chr6	85587066	85588203
TCONS_00056767	52.15	chr7	44122439	44135578
TCONS_00076833	65.01	chr7	64940991	64949641
TCONS_00088333	466.21	chr8	53673358	53694399
TCONS_00086792	43.68	chr8	53675310	53676444
TCONS_00081938	44.76	chr8	144272996	144280254
TCONS_00040364	48.99	chr9	46278172	46282364
TCONS_00055762	113.61	chr9	139472437	139475056
TCONS_00090830	60.61	chr10	8393180	8395114
TCONS_00055763	112.45	chr10	89871096	89872775
TCONS_00077100	103.42	chr10	125757085	125759130
TCONS_00081941	44.76	chr11	134570645	134576828
TCONS_00048600	60.08	chr12	10472892	10473695
TCONS_00086793	43.68	chr13	87409766	87431966
TCONS_00086794	43.68	chr13	87441562	87444206
TCONS_00051777	86.28	chr13	105817944	105838505
TCONS_00086795	43.68	chr13	110359240	110360897
TCONS_00088327	117.61	chr14	97646784	97665870
TCONS_00077099	103.42	chr14	106837417	106838015
TCONS_00022415	43.96	chr14	106856726	106857157
TCONS_00056483	65.43	chr14	107161628	107161912
TCONS_00087227	86.57	chr14	107164554	107166077
TCONS_00062955	46.92	chr15	46675349	46676557
TCONS_00083990	45.3	chr16	13337081	13337749
TCONS_00040365	48.35	chr17	3599782	3616722
TCONS_00088325	117.61	chr17	63491521	63501505

TCONS_00088330	117.61	chr17	72486556	72487602
TCONS_00081939	44.76	chr18	10298402	10305837
TCONS_00056542	61.16	chr18	10319095	10322466
TCONS_00086797	43.68	chr18	76390627	76397128
TCONS_00091468	158.24	chr18	76393073	76397128
TCONS_00086800	43.68	chr18	76393688	76397128
TCONS_00038764	42.54	chr18	76423717	76444074
TCONS_00080917	46.34	chr18	76525660	76529323
TCONS_00091467	158.49	chr20	24688672	24707491
TCONS_00088326	466.21	chr20	39373455	39385194
TCONS_00088328	50	chr20	39373455	39383151
TCONS_00088332	117.61	chr20	56293787	56304682
TCONS_00081940	44.76	chr20	56293787	56304682
TCONS_00058284	85.71	chr20	56414001	56415852
TCONS_00021977	58.74	chr21	47037435	47038182
TCONS_00059898	41.56	chr21	47045984	47059169
TCONS_00022416	43.96	chr21	47372575	47376597
TCONS_00021976	85.3	chr22	23874748	23889951
TCONS_00088331	117.61	chr22	46544029	46545743
TCONS_00088329	466.21	chrX	18902412	18903831
TCONS_00087753	186.24	chrX	63985367	64002448
TCONS_00056486	95.4	chrX	78606175	7861790
TCONS_00087636	70.65	chrX	130828109	130829871

Supplementary Table 8: Unannotated putative lncRNA loci identified in CD34+ thymocytes cultured on an OP9 stromal feeder layer by RNA-seq and predicted as 'coding' by PhyloCSF analysis (precision: 95%; sensitivity: 90%)

ID	Score	Chr	Start	End
TCONS_00072393	44.47	chr1	59763517	59766075
TCONS_00000024	71.81	chr1	94482764	94483785
TCONS_00049576	119.44	chr1	184071088	184080918
TCONS_00018092	49.19	chr1	201492686	201496026
TCONS_00013282	57.67	chr1	204579092	204583098
TCONS_00005447	74.64	chr1	206266987	206269412
TCONS_00028199	47.43	chr2	8578376	8582043
TCONS_00056874	57.99	chr2	47765920	47769139
TCONS_00052880	192.83	chr2	75488665	75490774
TCONS_00008149	71.82	chr2	75944631	75947096
TCONS_00006489	53.11	chr2	89074227	89074933
TCONS_00059446	73.91	chr2	102979438	102980995
TCONS_00036912	58.06	chr2	128352279	128353933
TCONS_00037996	44.64	chr2	129448171	129449912
TCONS_00007201	51.59	chr2	173364320	173364905
TCONS_00025943	65.72	chr2	197044762	197046167
TCONS_00061061	99.23	chr3	16005716	16014366
TCONS_00006917	203.31	chr3	18572889	18574386
TCONS_00046453	72.11	chr3	45844195	45846999
TCONS_00009952	43.38	chr3	45854323	45856659
TCONS_00029725	42.29	chr3	72767223	72773948
TCONS_00050097	130.58	chr3	107844588	107860198
TCONS_00041198	54.4	chr3	129325763	129327842
TCONS_00009908	98.04	chr3	167462678	167466894
TCONS_00071913	41.51	chr3	185543397	185547711
TCONS_00024054	385.19	chr3	194355573	194360483
TCONS_00050052	113.61	chr3	194356984	194360483
TCONS_00023792	46.54	chr4	25171054	25176183
TCONS_00062252	43.52	chr4	25213770	25219660
TCONS_00019745	42.84	chr4	40187190	40191932
TCONS_00071014	138.77	chr4	123200409	123200928
TCONS_00064666	45.23	chr5	345191	345528
TCONS_00029210	73.7	chr5	5315687	5317724
TCONS_00062251	43.52	chr5	65386257	65389744
TCONS_00024695	55.93	chr5	86362230	86371165
TCONS_00007271	57.61	chr5	89774583	89776807
TCONS_00011164	49.19	chr5	133376357	133393098
TCONS_00062270	49.32	chr5	150606606	150631743
TCONS_00057599	66.44	chr5	179890063	179891602
TCONS_00052572	57.74	chr6	2403148	2406370

TCONS_00050701	47.74	chr6	3304830	3310274
TCONS_00026406	90.5	chr6	5072922	5073683
TCONS_00064968	68.85	chr6	6702961	6704251
TCONS_00047535	48.93	chr6	14398440	14401713
TCONS_00064969	68.85	chr6	14452366	14452809
TCONS_00064967	68.85	chr6	14511669	14512993
TCONS_00013283	57.67	chr6	14661252	14662655
TCONS_00013983	87.82	chr6	15744959	15749318
TCONS_00045206	66.38	chr6	29850640	29852333
TCONS_00006709	61.13	chr6	37009596	37012118
TCONS_00029415	50.56	chr6	106277928	106340820
TCONS_00052342	50.46	chr6	130516350	130518701
TCONS_00066073	79.81	chr6	130519728	130521019
TCONS_00045196	49.01	chr6	130538723	130540284
TCONS_00068680	78.18	chr6	149453055	149454182
TCONS_00028198	52.78	chr6	150206104	150207553
TCONS_00066074	79.81	chr6	156149206	156204567
TCONS_00007518	47.02	chr6	157795798	157802109
TCONS_00068988	94.15	chr6	161585933	161586608
TCONS_00049704	82.1	chr7	1549453	1560923
TCONS_00057036	51.44	chr7	23562289	23569431
TCONS_00052257	66.33	chr7	99822288	99824735
TCONS_00023996	48.35	chr10	88159462	88162084
TCONS_00013280	57.67	chr10	98774668	98775476
TCONS_00039798	49.01	chr11	36618167	36618905
TCONS_00029745	61.14	chr11	64098670	64099428
TCONS_00023407	51.88	chr11	127920833	128057011
TCONS_00029266	125.21	chr11	127921365	127931679
TCONS_00019744	42.84	chr12	2853698	2858699
TCONS_00029267	125.21	chr12	2862395	2865432
TCONS_00050031	69.4	chr12	9756644	9758808
TCONS_00029209	73.7	chr12	12881631	12883860
TCONS_00018582	172.63	chr12	92032126	92039218
TCONS_00056635	46.92	chr12	113649938	113654123
TCONS_00064971	43.71	chr12	113671963	113673219
TCONS_00050079	72.43	chr12	117254966	117257189
TCONS_00059445	73.91	chr13	49105331	49107037
TCONS_00028236	57.24	chr13	100065629	100070089
TCONS_00064970	49.99	chr14	23437727	23438969
TCONS_00013979	180.78	chr14	53068696	53069630
TCONS_00007335	42.33	chr14	65420816	65423040
TCONS_00067832	284.96	chr14	72893256	72906687
TCONS_00062629	47.14	chr14	72893256	72906552
TCONS_00036913	58.06	chr14	72900554	72900967
TCONS_00049502	51.09	chr14	72991218	72992231
TCONS_00034745	42.14	chr14	73032380	73033337

TCONS_00029813	69.45	chr14	100528647	100529255
TCONS_00009953	43.98	chr14	106969092	106969523
TCONS_00024694	55.93	chr15	40338297	40342680
TCONS_00036185	49.23	chr15	56939054	56939702
TCONS_00063288	47.57	chr15	56942051	56942536
TCONS_00013977	46.88	chr15	66761087	66770639
TCONS_00050927	52.15	chr15	69117878	69120611
TCONS_00057832	48.94	chr15	70551056	70553387
TCONS_00050925	52.15	chr15	90597897	90599313
TCONS_00059609	215.5	chr15	99557738	99574337
TCONS_00034744	42.54	chr15	99569810	99574337
TCONS_00063770	88.29	chr16	2469884	2470738
TCONS_00063304	49.76	chr16	49672974	49674445
TCONS_00048613	42.52	chr16	67602034	67603724
TCONS_00059447	73.91	chr16	68428413	68430973
TCONS_00052256	41.29	chr16	72260597	72265505
TCONS_00047337	74.83	chr16	83976427	83979066
TCONS_00029774	63.28	chr16	85495679	85496270
TCONS_00071013	138.77	chr16	87931351	87934262
TCONS_00033907	63.44	chr17	26218043	26218928
TCONS_00067628	142.98	chr17	29042829	29051874
TCONS_00024062	248.12	chr17	37314134	37315360
TCONS_00072724	53.63	chr17	43277314	43281145
TCONS_00029705	64.89	chr17	45023736	45030864
TCONS_00018205	59.56	chr17	62396691	62407826
TCONS_00050080	53.87	chr17	66361368	66363633
TCONS_00059448	73.91	chr17	75842425	75847375
TCONS_00008150	71.82	chr18	3696263	3697848
TCONS_00014925	41.49	chr19	16259690	16259996
TCONS_00067730	47.41	chr19	21714994	21717673
TCONS_00063275	119.25	chr19	54878348	54879779
TCONS_00057833	45.18	chr20	4185348	4187974
TCONS_00026403	42.58	chr20	13770520	13773490
TCONS_00063302	44.62	chr21	45614432	45619053

Supplementary Table 9: Correlation of selected annotated lncRNAs with *DTX1* expression in T-cell subsets of 4 healthy donors

Spearman correlation lncRNA with *DTX1*	**r**	**p (two-tailed)**	**p-value summary**
LUNAR1	0.9386	0,0165	*
lnc-SYNCRIP-2	0.2912	0.3344	ns
lnc-SLC12A7-4	0.7308	0,0045	**
lnc-PLEKHB2-1	0.8022	0,0010	***
lnc-c2orf55-1	0.7033	0,0073	**
lnc-UBXN4-1	0.6154	0,0252	*
lnc-PGBD5-2	0.3681	0,2159	ns
lnc-FAM120AOS-1	-0.06593	0,8305	ns
lnc-COX10-1	-0.2692	0,3737	ns
lnc-SERPINC1-1	0.7857	0,0015	**
lnc-c9orf163-2	0.01099	0,9716	ns
lnc-GSDMC-2	0.7582	0,0027	**
lnc-PKD1-1	0.7527	0,0030	**
lnc-CA7-2	0.7967	0,0011	**
lnc-GAN-1	0.3242	0,2799	ns
lnc-CA7-1	0.7198	0,0055	**

Supplementary Table 10: *NOTCH1* mutation status of primary T-ALL samples

	***NOTCH1* WT**	***NOTCH1* MUT PEST**	***NOTCH1* MUT HD**	***NOTCH1* MUT PEST+HD**
n	8	3	2	2

Supplementary Table 11: Differential expression and significance level of selected annotated lncRNAs in primary T-ALL samples of 8 *NOTCH1* WT and 7 *NOTCH1* MUT cases

lncRNA	**logFC**	**P**
LUNAR1	2.376	0.0290
lnc-SYNCRIP-2	0.3544	0.4765
lnc-SLC12A7-4	-0.0466	0.9400
lnc-PLEKHB2-1	0.3689	0.5840
lnc-c2orf55-1	0.3275	0.6443
lnc-UBXN4-1	1.3274	0.0600
lnc-PGBD5-2	0.2793	0.3982
lnc-FAM120AOS-1	0.9387	0.0072
lnc-COX10-1	0.3860	0.5047
lnc-SERPINC1-1	0.2643	0.4333
lnc-c9orf163-2	0.5812	0.0982
lnc-GSDMC-2	2.2868	0.1052
lnc-PKD1-1	0.2334	0.6461
lnc-CA7-2	0.1776	0.6448
lnc-GAN-1	0.3355	0.4639
lnc-CA7-1	0.0539	0.8577

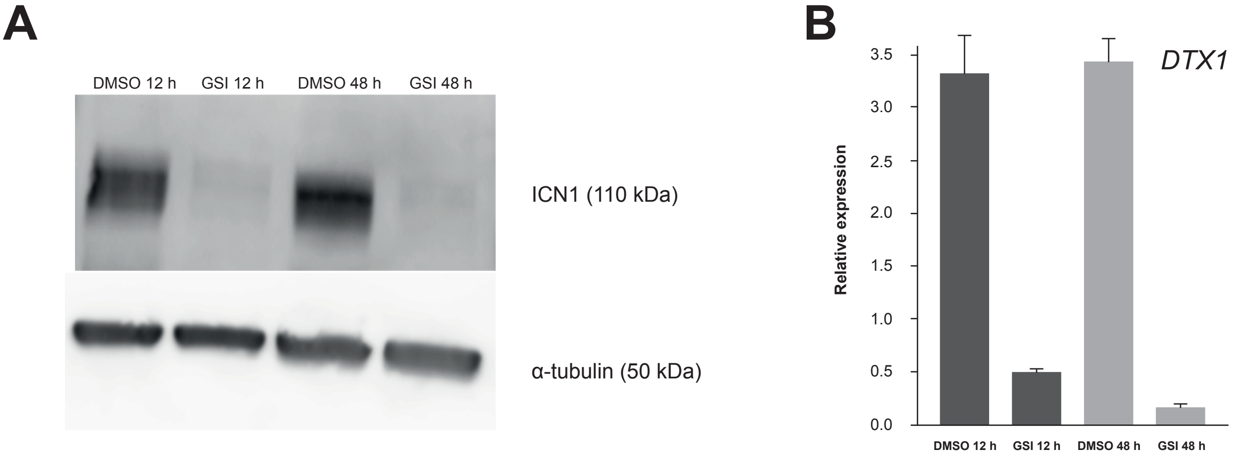
Supplementary Table 12: Notch1, Brd4, Med1 and H3K27ac ChIP-sequencing peaks for the selected annotated lncRNAs

lncRNA	Notch1	Brd4	Med1	H3K27ac
lnc-SYNCRIP-2	X	X	X	X
lnc-SLC12A7-4	-	X	X	X
lnc-PLEKHB2-1	X	X	-	X
lnc-c2orf55-1	X	X	X	X
lnc-UBXN4-1	X	X	X	X
lnc-PGBD5-2	-	X	-	X
lnc-FAM120AOS-1	-	-	-	-
lnc-COX10-1	-	-	-	-
lnc-SERPINC1-1	X	X	X	X
lnc-c9orf163-2	X	X	X	X
lnc-GSDMC-2	X	X	X	X
lnc-PKD1-1	X	X	X	X
lnc-CA7-2	-	-	-	-
lnc-GAN-1	-	-	-	-
lnc-CA7-1	-	-	-	-
lnc-AC108463.1-3	-	-	-	-
lnc-TRPV2-1	X	X	X	X
lnc-IGLL1-2	-	X	X	X
lnc-FRMD7-1	-	-	-	-
lnc-PLGLB2-1	-	-	-	-
lnc-CDRT15-2	-	X	X	X
lnc-APC-6	X	X	X	X
lnc-LRIG2-4	X	X	X	X
lnc-IL6-2	X	X	X	X
lnc-GREM2-6	X	X	X	X
lnc-PCBP3-1	-	X	-	X
lnc-COX10-3	-	-	-	-

X: presence of a ChIP-sequencing peak, -: absence of a ChIP-sequencing peak

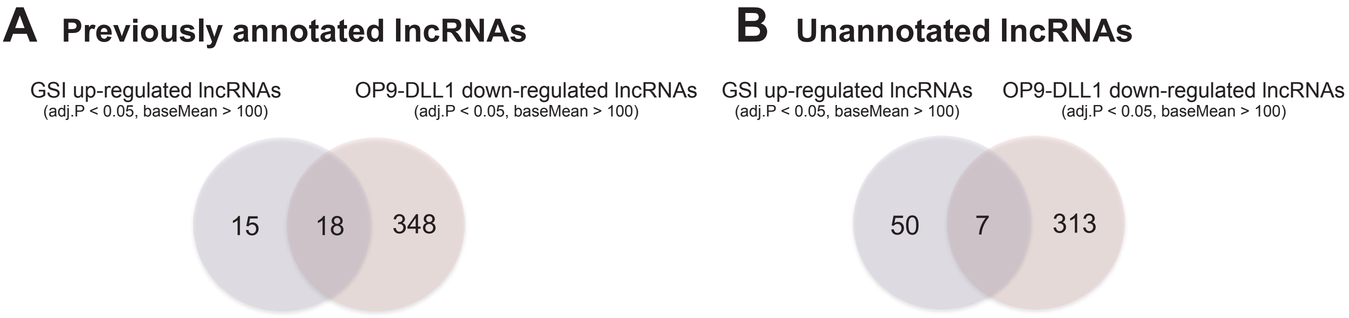
Supplementary Figures and Methods

Supplementary Figure 1



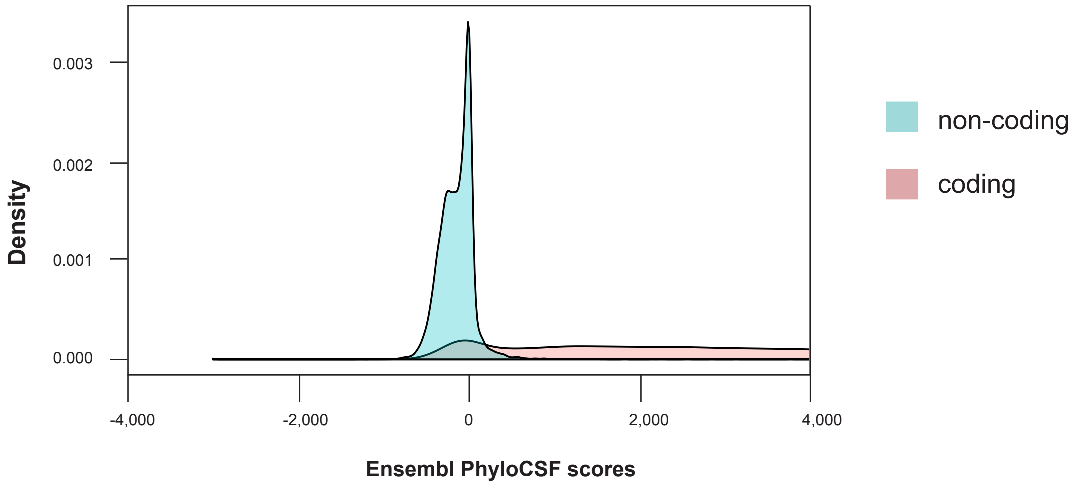
Supplementary Figure 1: Validation of the pharmacological Notch inhibition model in CUTLL1.
(A) Down-regulation of ICN1 protein levels upon GSI treatment for 12 h and 48 h in CUTLL1 was validated by Western blot analysis. **(B)** RT-qPCR could confirm down-regulation of *DTX1* expression upon GSI-treatment of CUTLL1 T-ALL cells.

Supplementary Figure 2



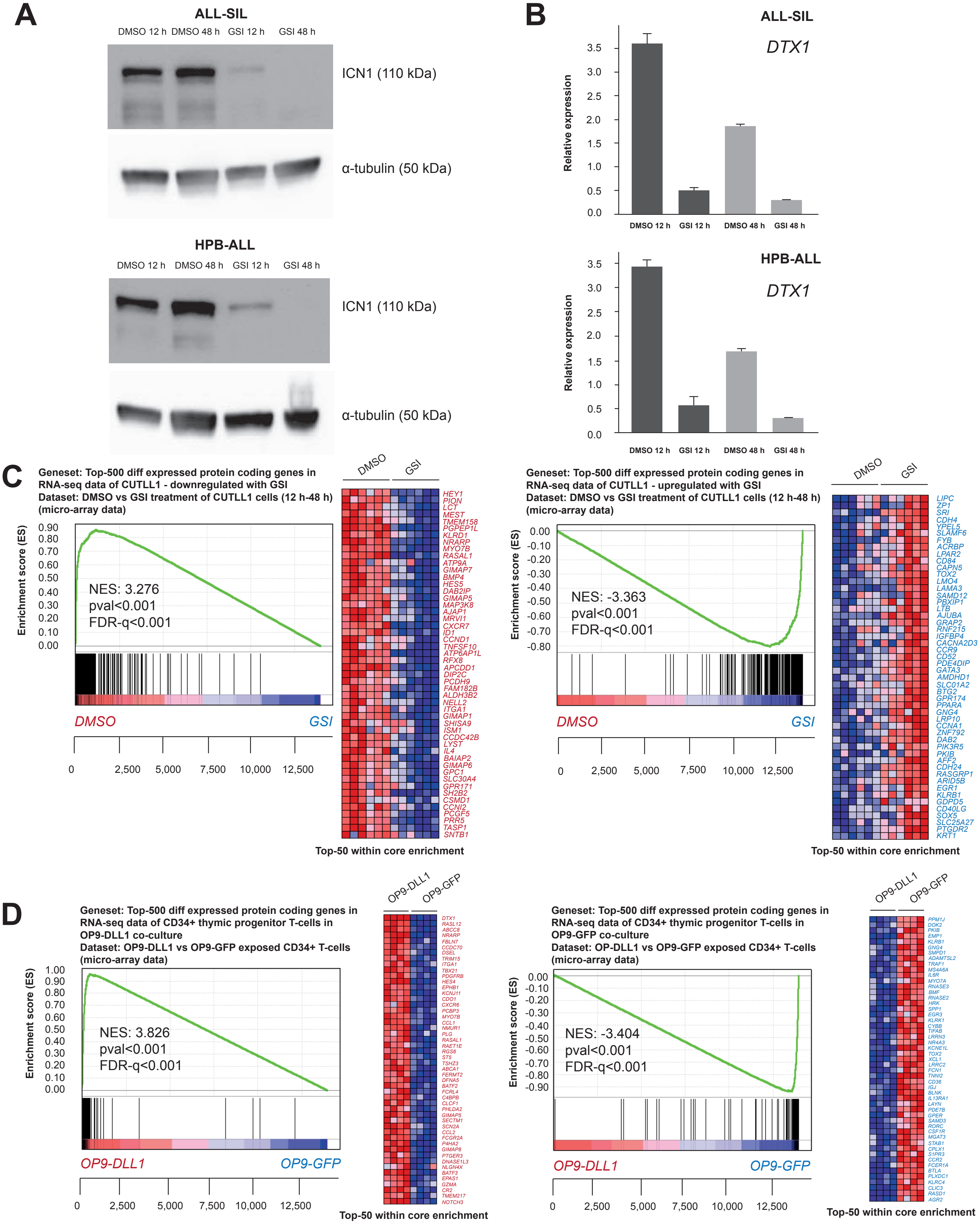
Supplementary Figure 2: Overlap between lncRNAs that are negatively correlated with Notch signaling in GSI treatment of CUTLL1 cells and co-culturing of CD34+ thymocytes on the OP9-DLL1 feeder layer.
(A) Venn diagram depicting the overlap between previously annotated lncRNAs that are up-regulated upon GSI treatment of the CUTLL1 cell line and down-regulated upon co-culturing of CD34+ thymocytes on the OP9-DLL1 feeder layer. **(B)** Venn diagram depicting the overlap between previously unannotated lncRNAs that are up-regulated upon GSI treatment of the CUTLL1 cell line and down-regulated upon co-culturing of CD34+ thymocytes on the OP9-DLL1 feeder layer.

Supplementary Figure 3



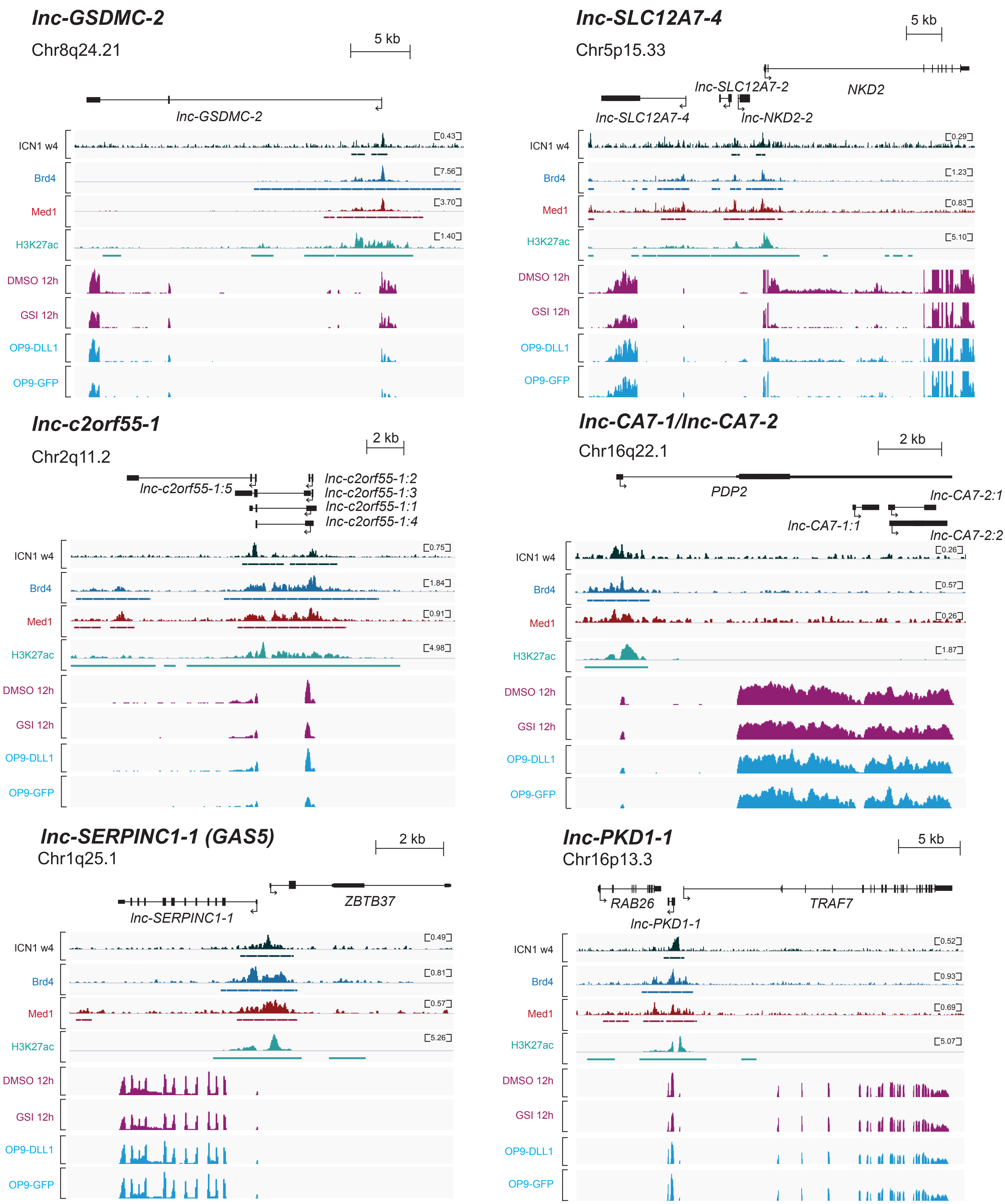
Supplementary Figure 3: PhyloCSF density plot to evaluate the protein coding potential of novel, unannotated lncRNA loci by RNA-seq.
The putative protein coding potential of unannotated lncRNA loci in CUTLL1 T-ALL cells and CD34+ T-cell progenitors cultured on an OP9 stromal feeder layer was calculated using the PhyloCSF algorithm.
The optimal treshold for the PhyloCSF score was determined as 41.2019 to obtain a precision of 95% and sensitivity of 90%.

Supplementary Figure 4



Supplementary Figure 4: Validation of Notch regulated lncRNAs in other model systems. **(A)** Western blot analysis confirms down-regulation of ICN1 in HPB-ALL and ALL-SIL cells upon GSI treatment for 12 h and 48 h. **(B)** RT-qPCR shows *DTX1* down-regulation upon GSI treatment of HPB-ALL and ALL-SIL T-ALL cells. GSEA shows significant overlap for differentially expressed protein coding genes found by RNA-seq and micro-array data of **(C)** GSI-treated CUTLL1 cells and **(D)** CD34+ T-cell progenitor on OP9-DLL1/GFP co-cultures.

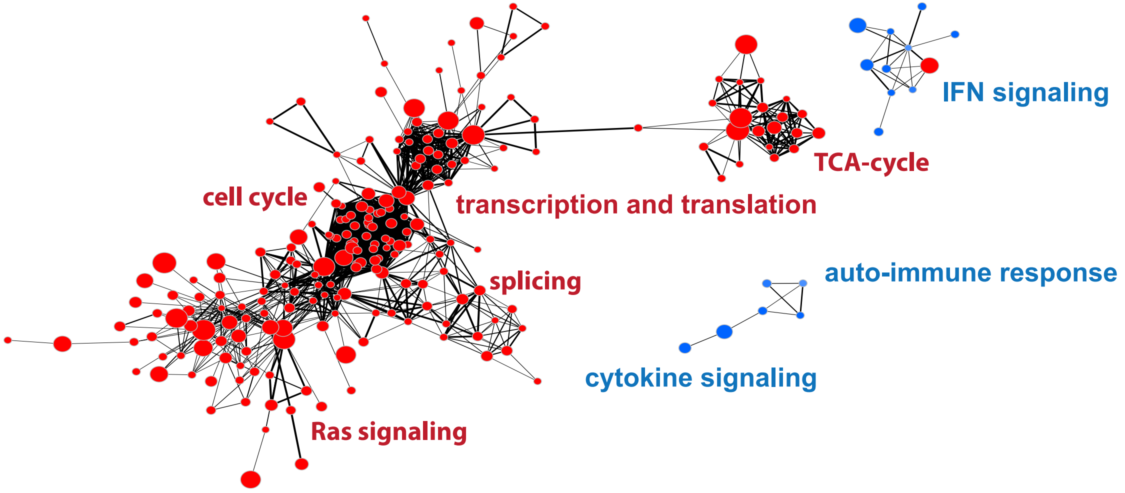
Supplementary Figure 5



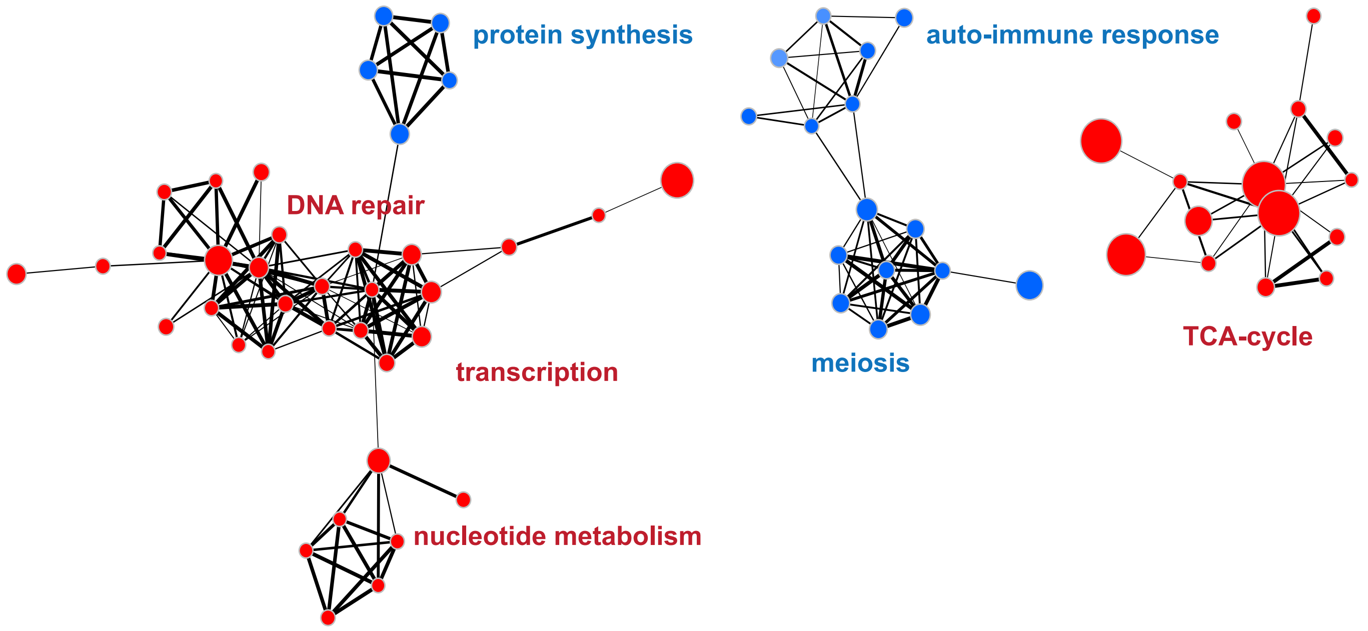
Supplementary Figure 5: Validation of direct regulation of selected lncRNAs by Notch1.
ChIP-seq tracks in CUTLL1 cells of Notch1, Brd4, Med1 and H3K27ac are depicted for the selection of annotated lncRNAs that were identified as overlapping Notch1 driven lncRNAs in CUTLL1 cells and CD34+ progenitor cells cultured on OP9 stromal cells. Representative RNA-seq tracks are shown for both in vitro model systems.

Supplementary Figure 6

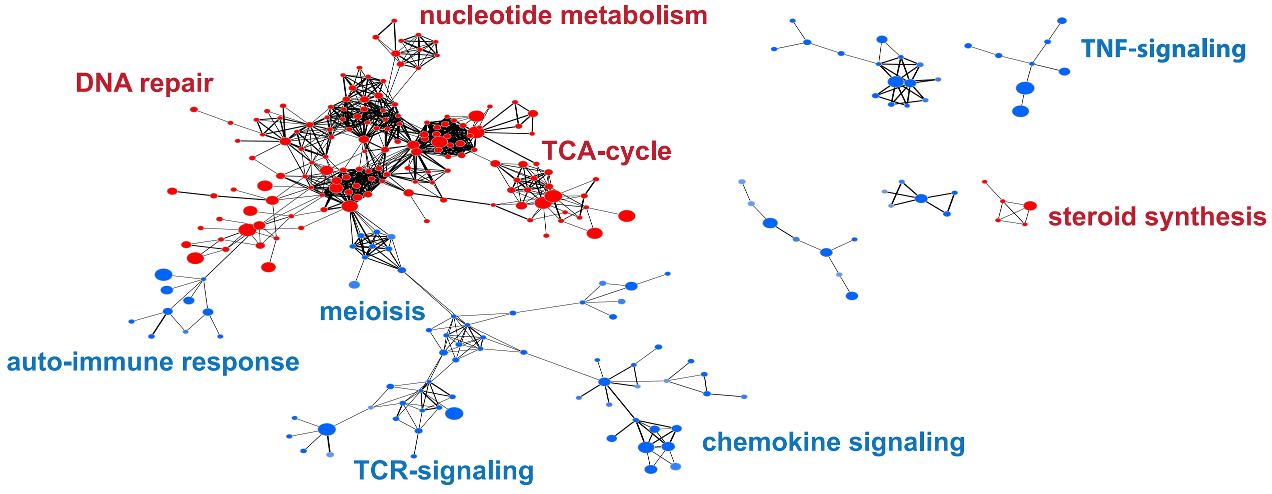
A *lnc-SYNCRIP-2*



B *lnc-SLC12A7-4*

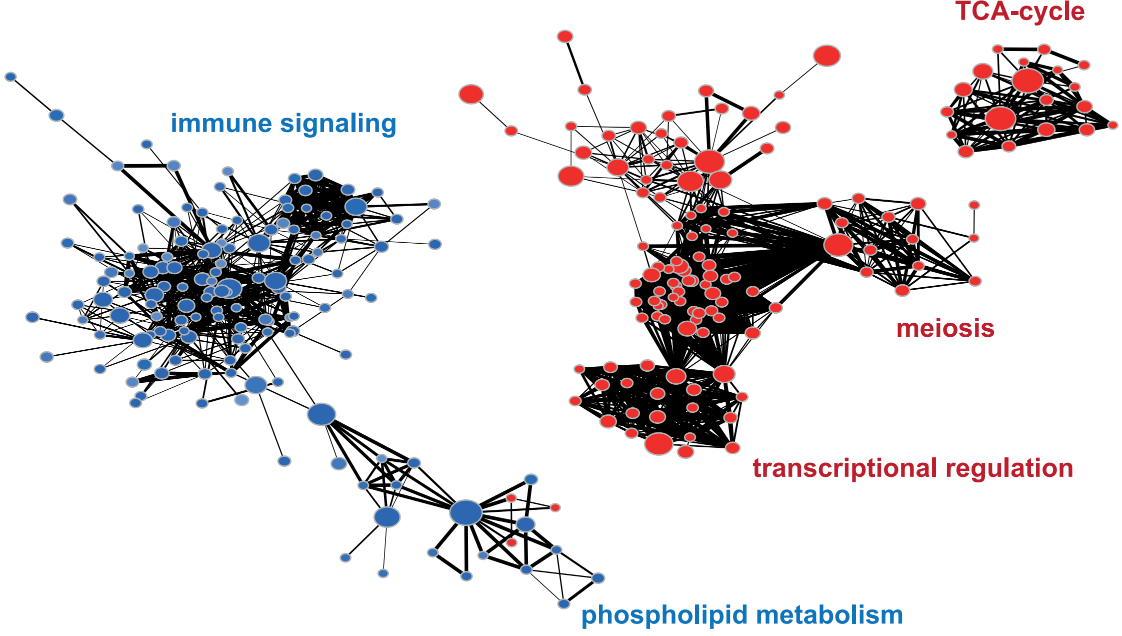


C *lnc-CA7-2*

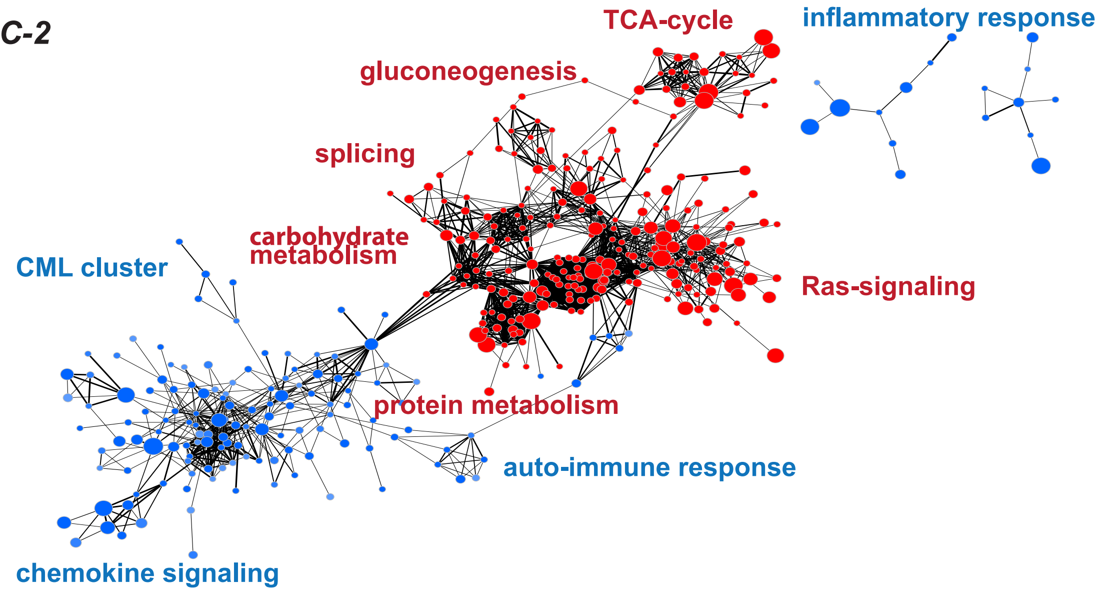


Supplementary Figure 6 (continued)

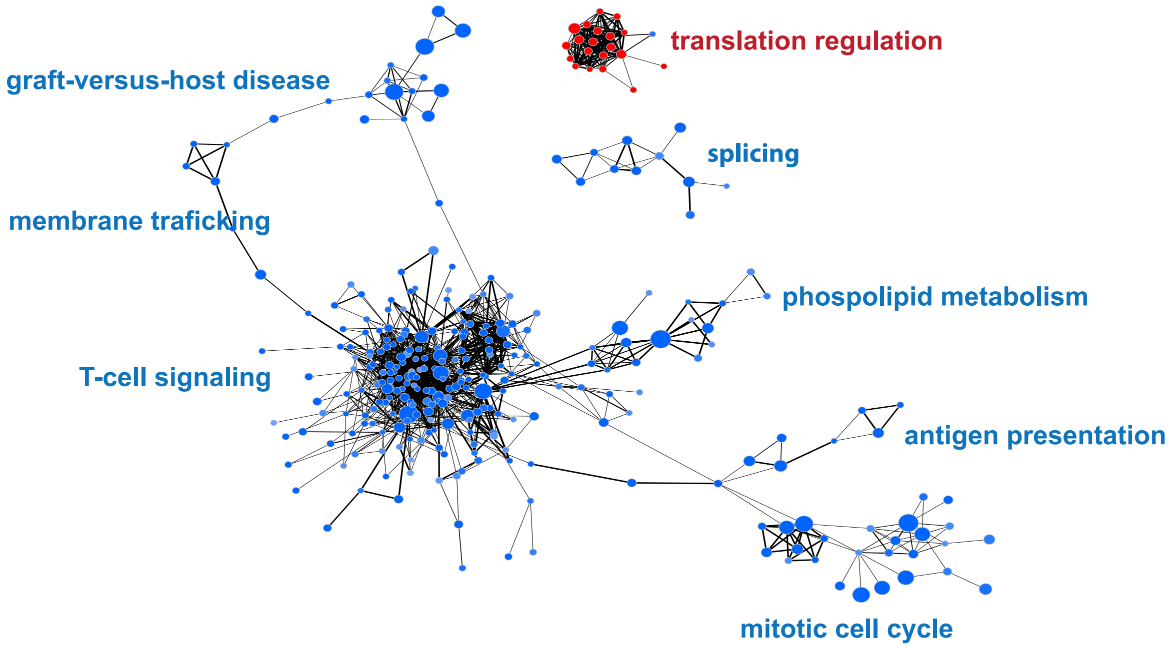
D *lnc-c2orf55-1*



E *lnc-GSDMC-2*

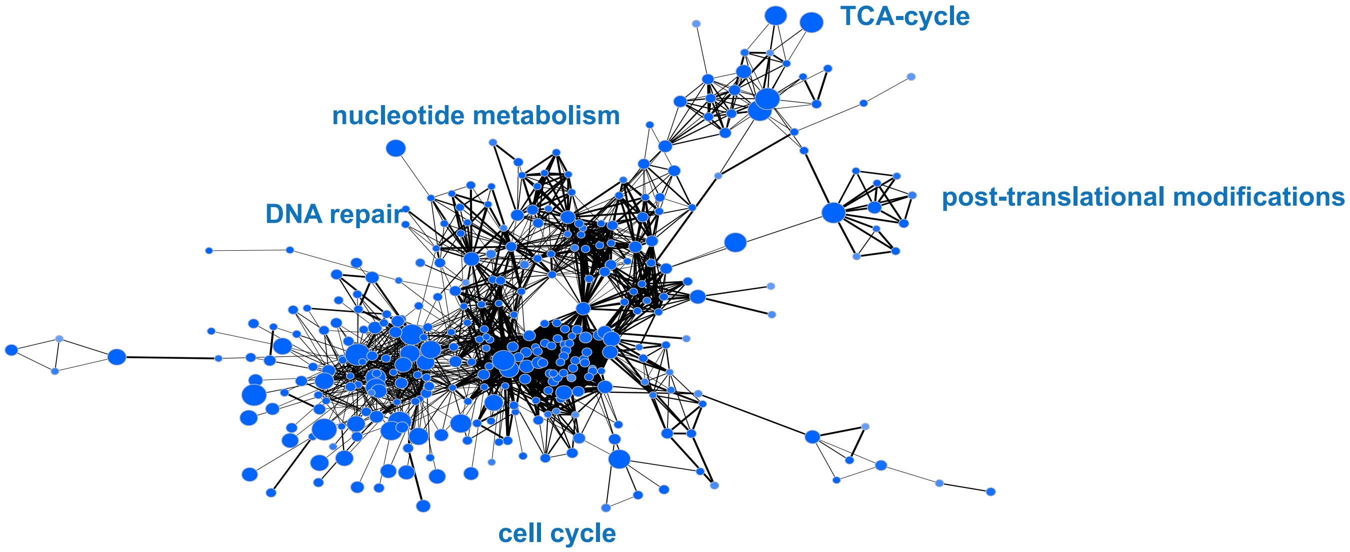


F *lnc-PGBD5-2*

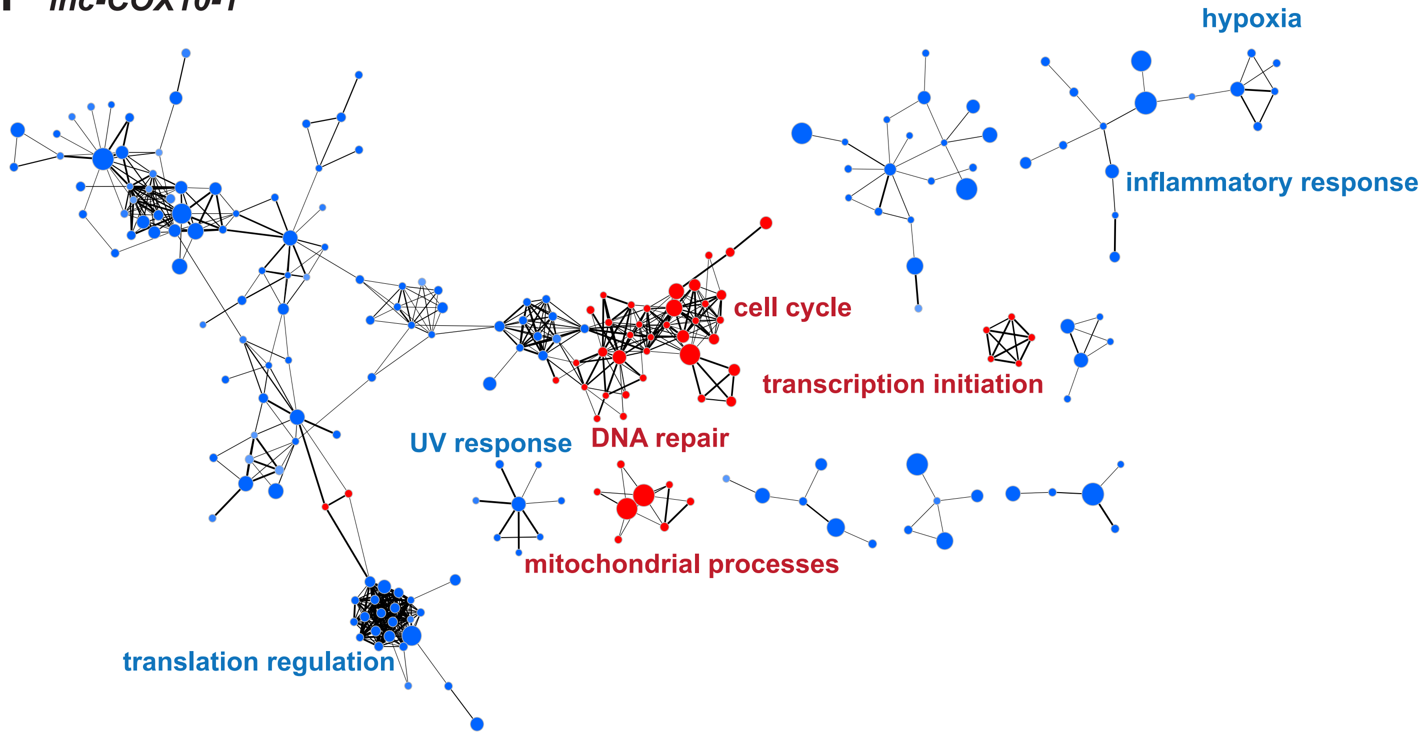


Supplementary Figure 6 (continued)

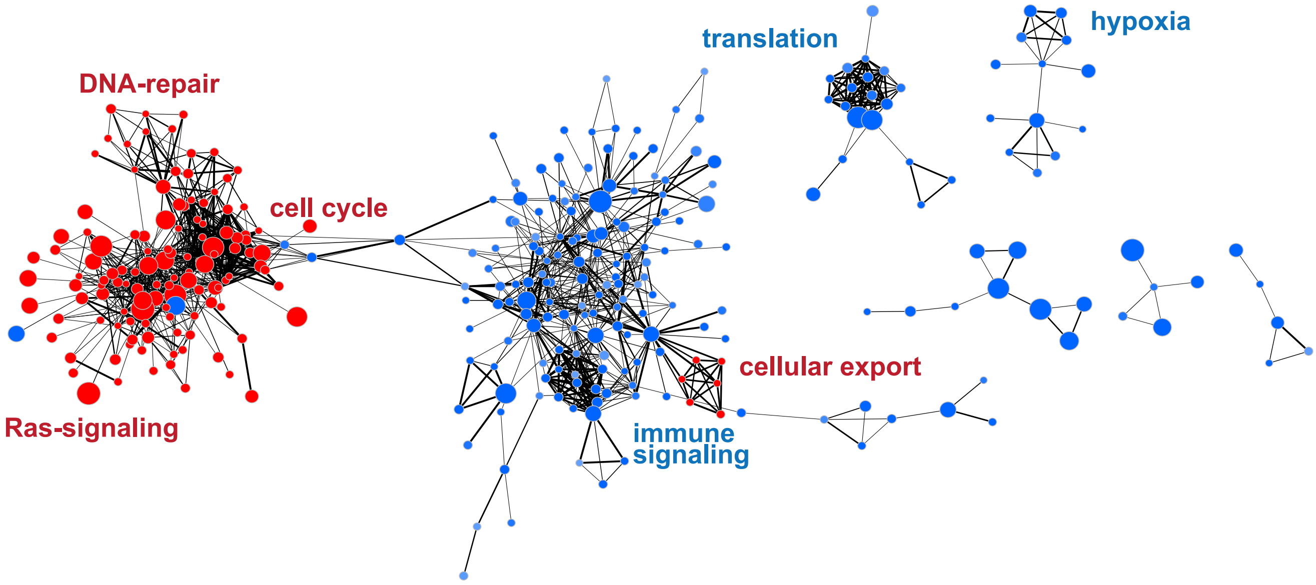
G *lnc-FAM120AOS-1*



H *lnc-COX10-1*

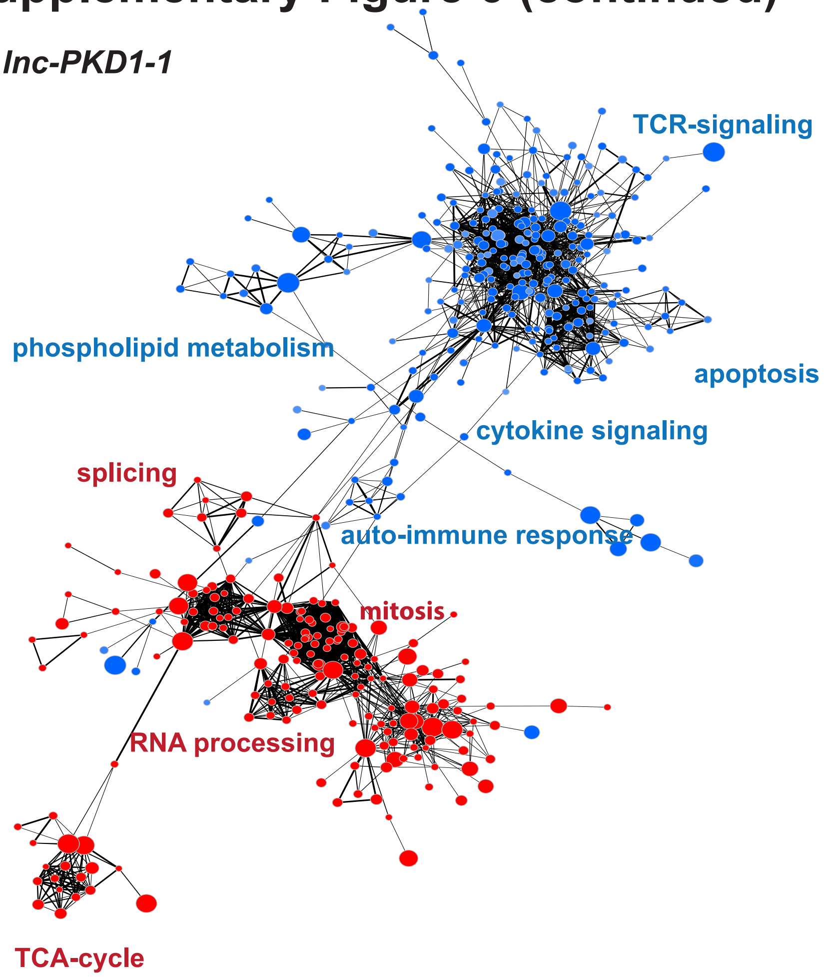


I *lnc-c9orf163-2*

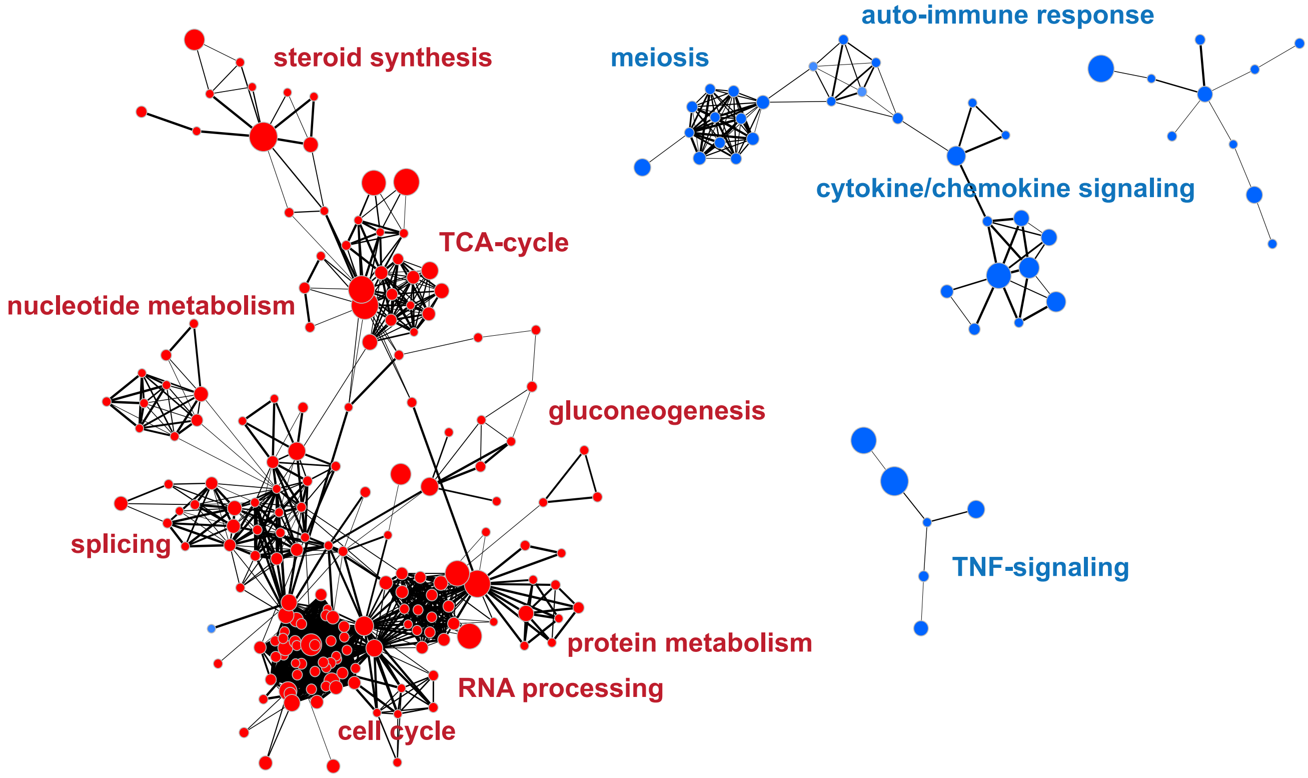


Supplementary Figure 6 (continued)

J *lnc-PKD1-1*

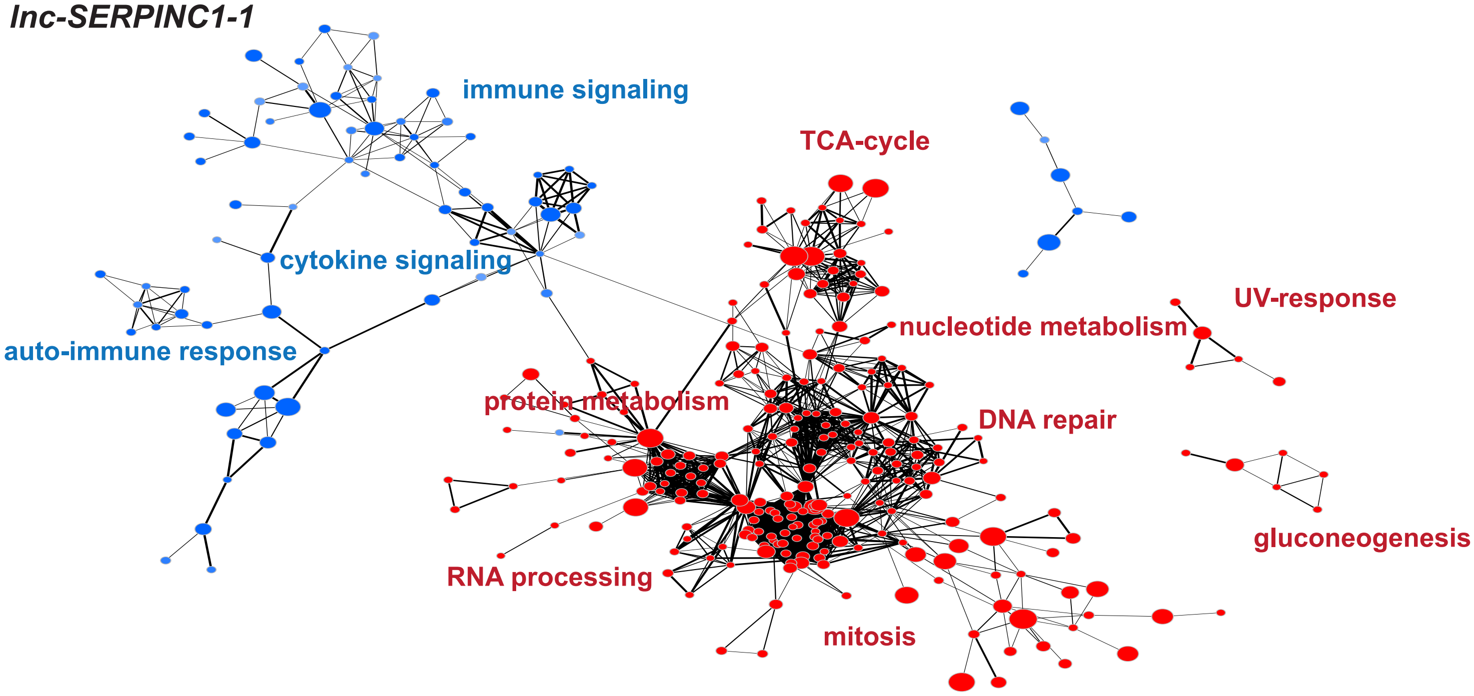


K *lnc-GAN-1*

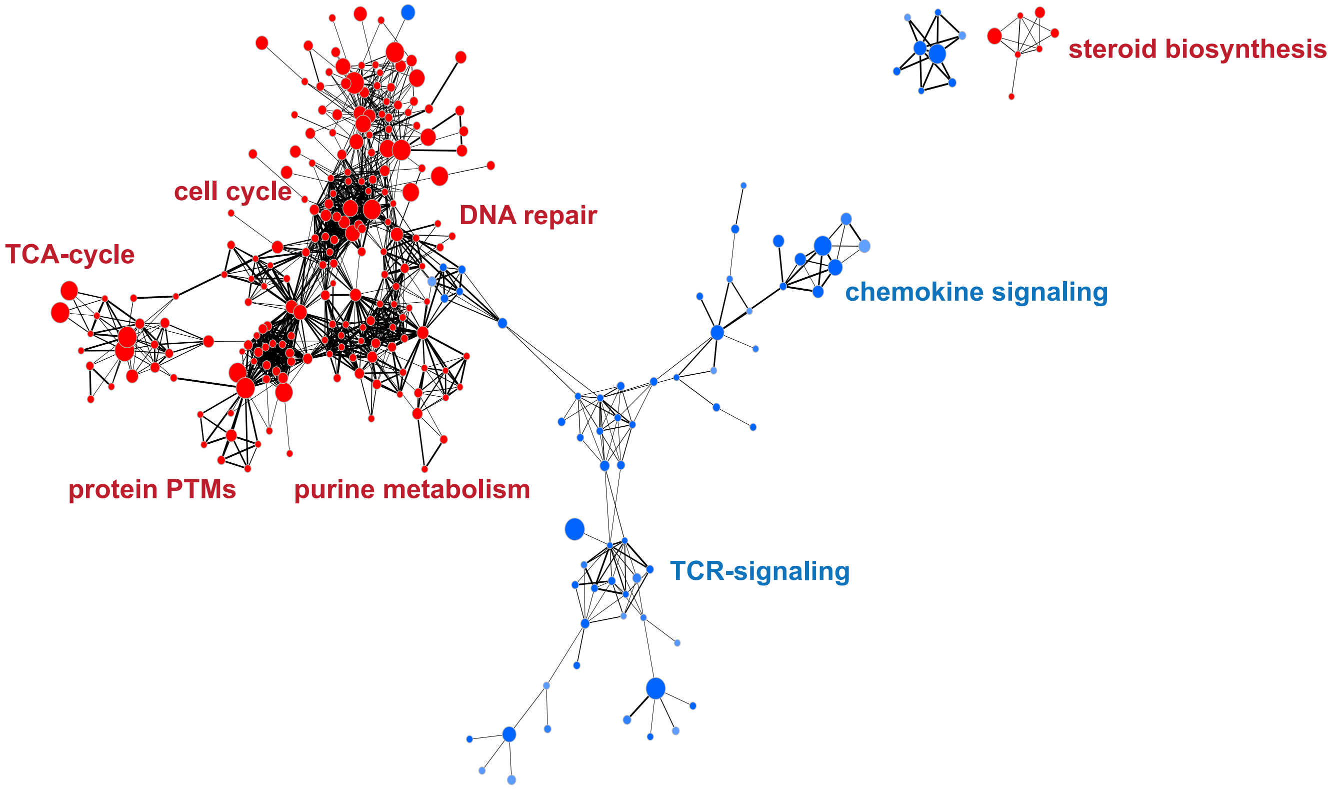


Supplementary Figure 6 (continued)

L *lnc-SERPINC1-1*



M *lnc-CA7-1*



Supplementary Figure 6: Functional annotation of candidate Notch driven lncRNAs in CUTLL1 T-ALL cells and CD34+ thymic progenitor T-cells through enrichment mapping.
(A-M) Pairwise Spearman's rho correlations were calculated between the selected Notch driven lncRNAs (with probes on the custom array) and all protein coding genes and used for functional annotation of each of the candidate lncRNAs by GSEA. Followingly, enrichment maps were generated in Cytoscape for all selected Notch driven lncRNAs and indicates potential clusters of functionalities linked to each of the candidate lncRNAs such as involvement in the TCA-cycle, meiosis, TCR-signaling, ...