Supporting Information

Wang et al. 10.1073/pnas.1109023108

SI Materials and Methods

γ-Secretase-Inhibitor Washout Studies. CUTLL1 cells (2×10^5/mL) were treated with compound E (1 μM, EMD cat. #565790) for 3 d, washed twice with warm complete medium, and incubated for 2 to 4 h in the presence or absence of 20 μM cycloheximide (Sigma). To control for "off-Notch" effects of γ-secretase-inhibitor (GSI), CUTLL1 cells were transduced with empty MigRI retrovirus or MigRI-dominant negative MAML1 (1) and sorted before GSI treatment. To identify direct target genes, cycloheximide (20 μg/mL) was added before GSI washout. To control for cycloheximide effects, "mock" GSI washout was perform in the presence of cycloheximide.

Antibodies. Antibodies used in ChIP-seq and ChIP analyses were as follows: Notch1, rabbit polyclonal raised against amino acids 2278 to 2470 of human Notch1; RBPJ, rabbit polyclonal raised against amino acids 1 to 48 of human RBPJ; H3K4me1, rabbit polyclonal (Abcam, cat. #ab8895); H3K4me3, rabbit monoclonal (Millipore, cat. #04–745); H3K27me3, rabbit polyclonal (Millipore, cat. #07–449); ZNF143, mouse monoclonal (Novus Biologicals, cat. #H00007702-M01); GABPA, rabbit polyclonal (Santa Cruz Biotechnology, cat. #sc-22810); RUNX1, rabbit polyclonal (Abcam, cat. #ab23980); ETS1, rabbit polyclonal (Santa Cruz Biotechnology, cat. #sc-350); CREB, rabbit monoclonal (Cell Signaling Technology, cat. #9197); REST, rabbit polyclonal (Santa Cruz Biotechnology, cat. #sc-25398); mouse nonimmune IgG (Millipore, cat. #12–371). Western blot analysis was performed with the antibodies above or rabbit antiactivated Notch1 (Cell Signaling Technology, cat. #2412), and mouse anti–β-actin (Sigma).

1. Weng AP, et al. (2004) Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science* 306:269–271.

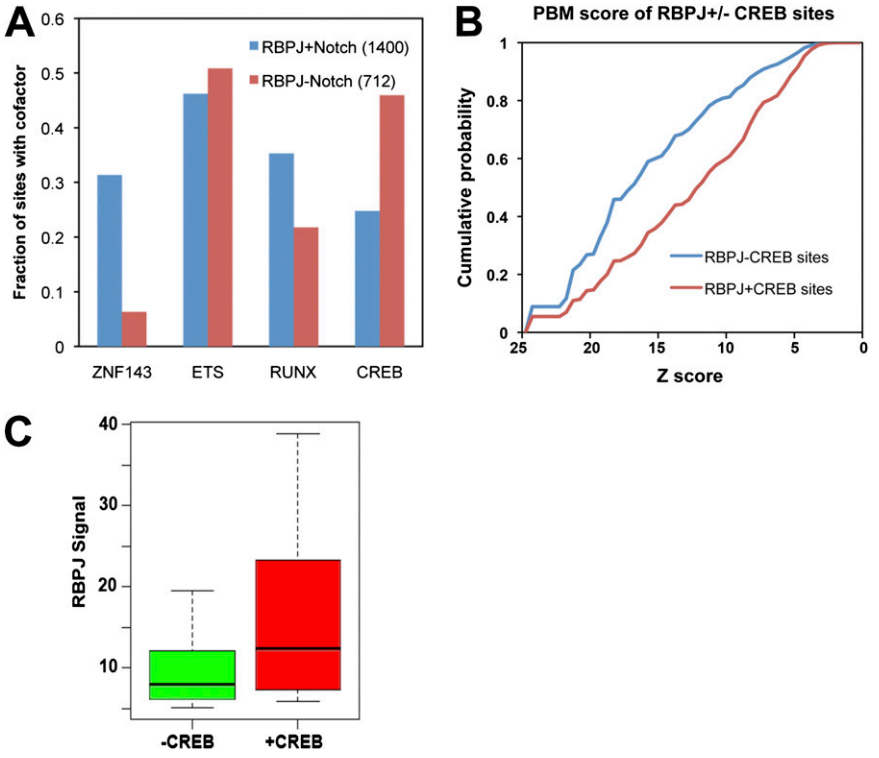

Fig. S1. Transcription factor motifs enriched at RBPJ-only and RBPJ/Notch sites cosites. (*A*) Fraction of RBPJ binding sites associated with the indicated motifs that do or do not bind Notch1. (*B*) Cumulative probability distributions of RBPJ binding affinities at RBPJ sites with or without CREB motifs. Relative RBPJ binding affinity for all possible 8-bp sequences measured by protein binding microarray is expressed as a *z*-score (1). (*C*) Comparison of RBPJ ChIP-Seq signals at RBPJ-only sites with and without CREB binding. Signals are expressed in reads per kilobase per million aligned reads (RPKM).

1. Del Bianco C, et al. (2010) Notch and MAML-1 complexation do not detectably alter the DNA binding specificity of the transcription factor CSL. *PLoS ONE* 5:e15034.

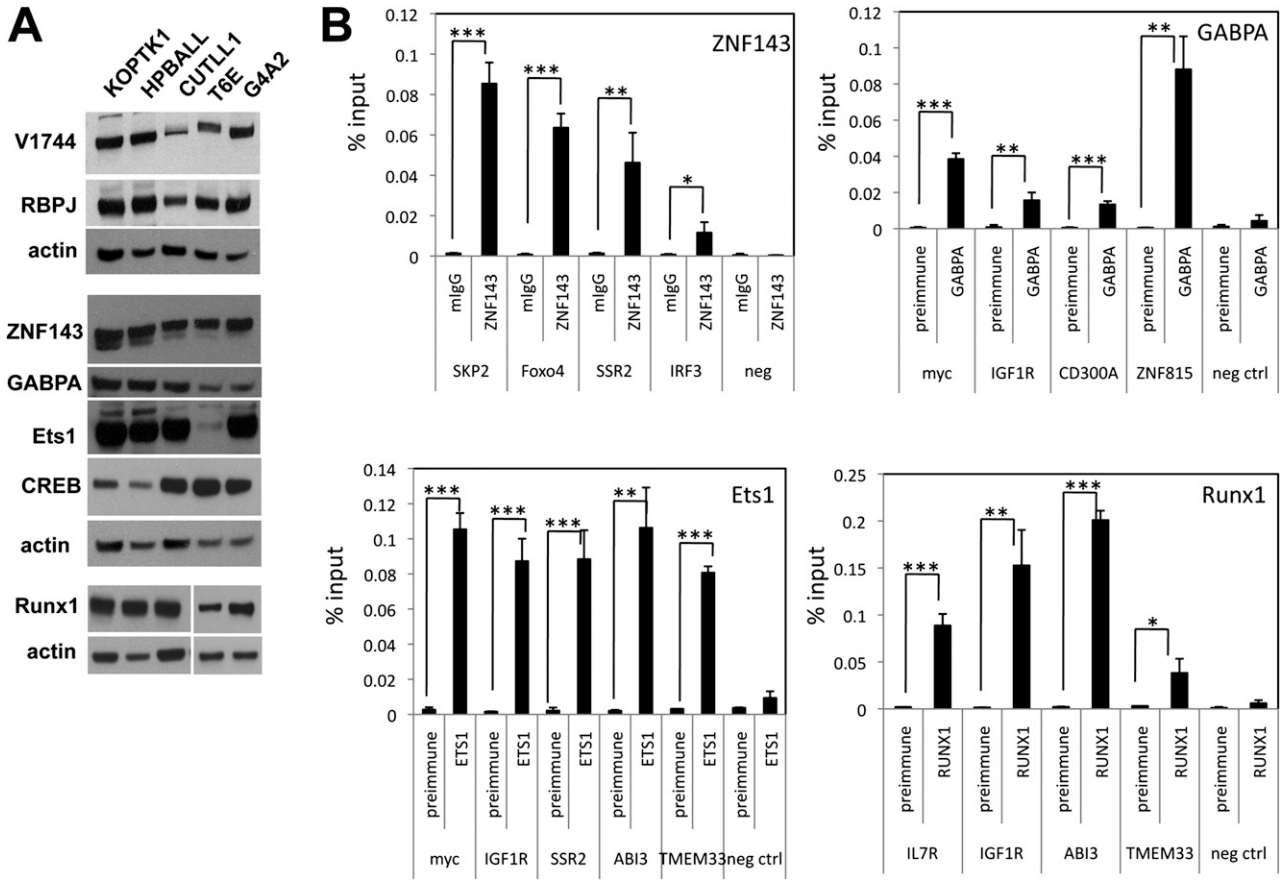

Fig. S2. ZNF143, GABPA, ETS1, and RUNX1 association with RBPJ/Notch1 binding elements in CUTLL1 cells. (*A*) Western blot detection of various transcription factors in human and murine T-lymphoblastic leukemia (TLL) whole-cell lysates. (*B*) Detection of ZNF143, GABPA, ETS1, and RUNX1 binding to imputed sites near Notch1 binding elements. Binding elements in transcription factor genes (*FOXO4*, *IRF3*), known (*MYC*, *IL7R*, *SKP2*), and novel (*IGF1R*, CD300A) Notch1 target genes, as well as elements associated with the genes *SSR2*, *ZNF815*, *ABI3*, and *TMEM133* that bind RBPJ in both CUTLL1 cells and Epstein-Barr virus-transformed B cells (1), were chosen to test the predicted binding of ZNF143, GABPA, ETS1, and RUNX1 by local ChIP. In each experiment, an extraneous genomic sequence lacking the indicated motifs was used as a negative control. $*P < 0.05$, $**P < 0.01$, $***P < 0.005$ (*t* test). All ChIPs were performed in triplicate.

1. Zhao B, et al. (2011) Epstein-Barr virus exploits intrinsic B-lymphocyte transcription programs to achieve immortal cell growth. *Proc Natl Acad Sci USA*, in press.

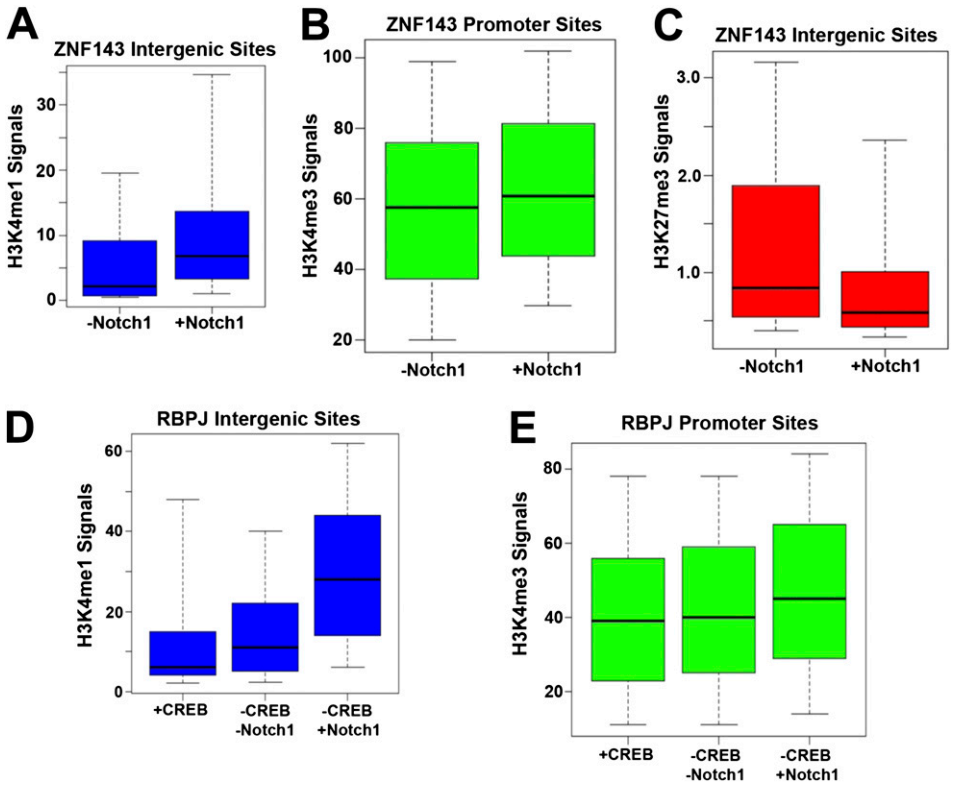

Fig. S3. Comparison of chromatin mark signals associated with RBPJ/Notch1, RBPJ, and RBPJ/CREB binding sites. (*A*) H3K4me1 signals at intergenic ZNF143 binding sites with and without Notch1. (*B*) H3K4me3 signals at promoter ZNF143 binding sites with and without Notch1. (*C*) H3K27me3 signals at intergenic ZNF143 binding sites with and without Notch1. (*D*) H3K4me1 signals at intergenic RBPJ binding sites with and without CREB motifs and Notch1 binding. (*E*) H3K4me3 signals at promoter RBPJ binding sites with and without CREB motifs and Notch1 binding. Signals are expressed in RPKM.

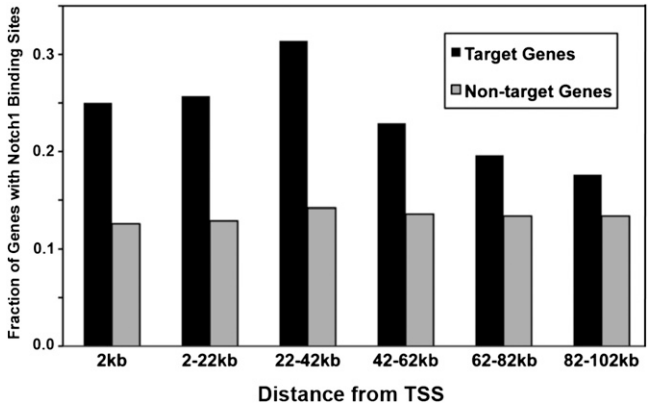

Fig. S4. Genomic Notch1 binding sites are more frequent near the transcriptional start sites (TSS) of Notch1 target genes. The frequency and distribution of Notch1 binding sites near the TSS of robust Notch1 target genes (defined in the main text) is compared with the frequency and distribution of Notch1 binding sites near nontarget genes.

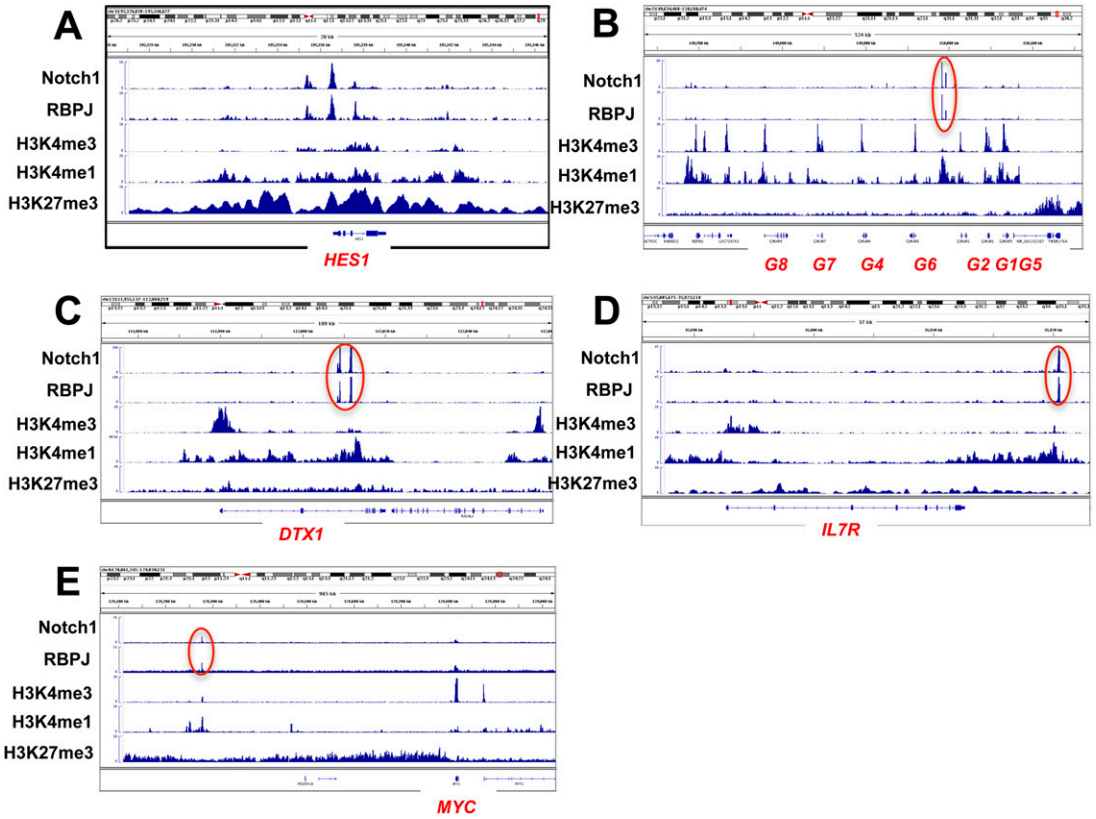

Fig. S5. RBPJ/Notch1 aligned reads and associated chromatin marks near (*A*) *HES1*, (*B*) the GIMAP gene cluster, (*C*) *DTX1*, (*D*) *IL7R*, and (*E*) *MYC*. G1-G8 in *B* corresponds to the positions of the *GIMAP1* through *GIMAP8* genes.

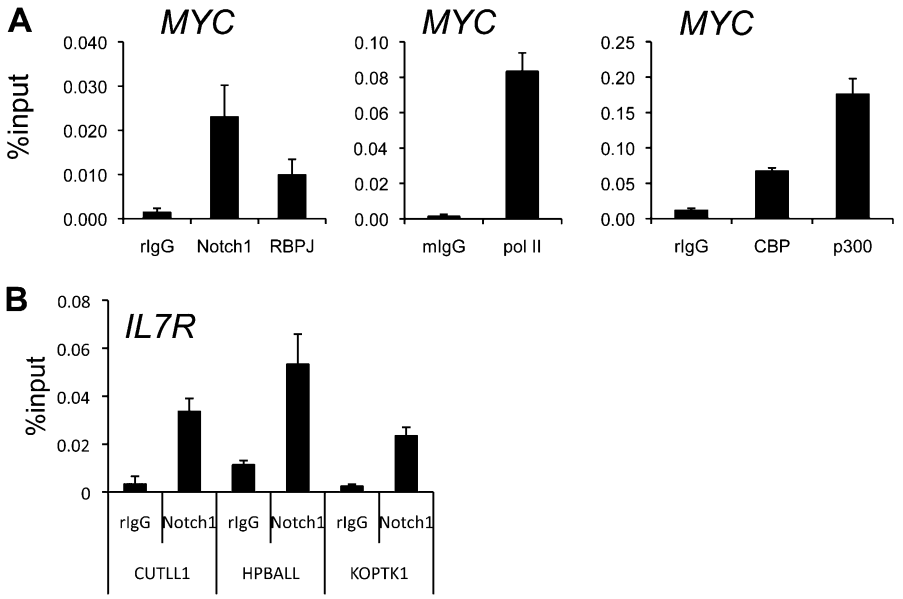

Fig. S6. Local ChIP analyses of the putative *MYC* (*A*) and IL7R (*B*) enhancers. (*A*) Chromatin immunoprecipitated with antibodies against Notch1, RBPJ, RNA polymerase II, CREB binding protein (CBP), or p300, or with control preimmune rabbit IgG (rIg) or nonimmune mouse Ig, was amplified with primers specific for the candidate 5′ enhancer near *MYC*. (*B*) Chromatin immunoprecipitated with anti-Notch1 antibody or preimmune rabbit IgG (rIgG) from the cell lines CUTLL1, HPB-ALL, and KOP-TK1 was amplified with primers specific for Notch1 binding elements flanking the *IL7R* gene. All ChIPs were performed in triplicate.

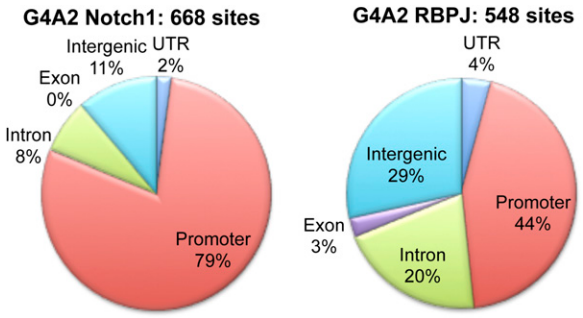

Fig. S7. Distribution of RBPJ and Notch1 binding sites in the genome of the murine TLL cell line G4A2.

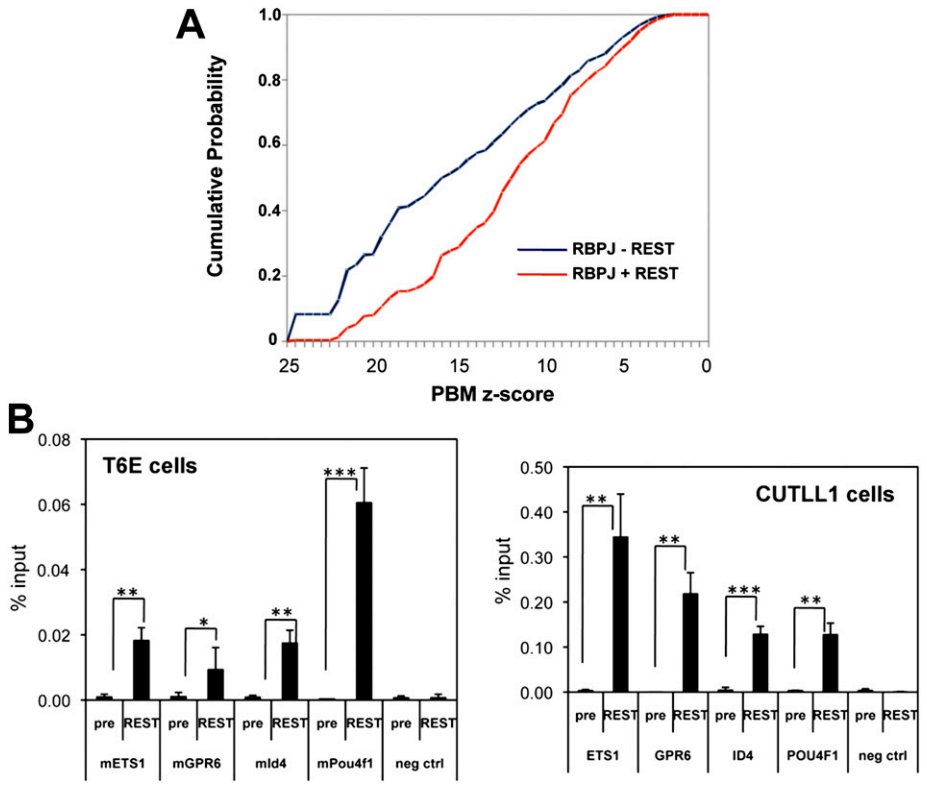

Fig. S8. RBPJ/REST binding in murine T6E and human CUTLL1 genomes. (*A*) Cumulative RBPJ binding affinities for RBPJ binding sites associated or unassociated with REST motifs identified by ChIP-Seq in murine T6E cells. Relative RBPJ binding affinity for all possible 8-bp sequences measured by protein binding microarray is expressed as a *z*-score (1). (*B*) REST binding assessed by ChIP analysis of four conserved imputed REST sites near the genes *Ets1*, *Gpr6*, *Id4*, and *Pou4F1*. In each experiment, an extraneous genomic sequence lacking the indicated motifs was used as a negative control. $*P < 0.05$, $**P < 0.01$, $***P < 0.005$ (*t* test). All ChIPs were performed in triplicate.

1. Del Bianco C, et al. (2010) Notch and MAML-1 complexation do not detectably alter the DNA binding specificity of the transcription factor CSL. *PLoS ONE* 5:e15034.

Table S1. Factors/motifs correlated with conserved Notch1 binding to orthologous elements in murine and human T-lymphoblastic leukemia cells

Factor or motif	Probability ratio of conserved Notch1 binding	*P* value
RBPJ*	5.19	$<10^{-16}$
ZNF143	1.91	10^{-7}
ETS	1.33	10^{-3}
CREB	1.3	0.01
RUNX	0.87	ns

ns, not significant.
*Based on conserved binding sites identified by ChIP-Seq.

Table S2. Factors/motifs correlated with conserved RBPJ binding to orthologous elements in murine and human T-lymphoblastic leukemia cells

Motif	Probability ratio of conserved RBPJ binding	*P* value
CREB	2.92	$<10^{-16}$
ETS	1.88	10^{-5}
RUNX	1.08	ns

ns, not significant.

Other Supporting Information Files

Dataset S1 (XLS)