Supplementary Material

Supplementary Table S1. Clinicopathological characteristics of 30 DLBCL patients.

Characteristic	n	GINS1		P value
		High expression* (number of cases)	Low expression* (number of cases)	
Gender				
Male	20	18	2	0.3
Female	10	7	3	
Age (median age: 56)				
<60	19	16	3	0.999
≥ 60	11	9	2	
Subgroups				
GCB	14	10	4	0.1571
ABC	16	15	1	
PS*				
0-1	12	10	2	0.999
2-4	18	15	3	
Stage				
I-II	9	5	4	**0.0195**
III-IV	21	20	1	
LDH*				
Normal	16	12	4	0.3359
High	14	13	1	
IPI scores*				
0-2	18	15	3	0.999
3-5	12	10	2	
PAX5*				
Positive	26	24	2	**0.0093**
Negative	4	1	3	
Extranodal involvement				
<2	17	13	4	0.3549
≥2	13	12	1	

Note:

*PS: Performance status

*LDH: Lactate dehydrogenase

*IPI scores: international prognostic index

*PAX5: The expression of PAX5 was performed by Immunohistochemical staining. PAX5 positive DLBCL was defined as 30% or more of the lymphoma cells showing distinct PAX5 nuclear staining. Among PAX-negative DLBCL cases, PAX5 staining cells was less 30% of the lymphoma cells.

*GINS1: Western blotting was used to measure the GINS1 protein levels. ImageJ was used to compare the density of bands on western blot. Compared with the GINS1 average value of control people, when relative density values were above 3.5, then it was defined as high expression. While relative density values were below 3.5, then it was defined as low expression.

Supplementary Table S2. Primers sequences and shRNA target sequences

Name	Sequences
H/m PAX5-Forward	GCATAGTGTCTACAGGCTCCG
H/m PAX5-Reverse	GATGGGTTCCGTGGTGGT
Human GINS1-Forward	CTTCAACGAGGATGGACT
Human GINS1-Reverse	CATTTGGCAAGACGCTAC
Mouse GINS1-Forward	CTCAGGTGGGAATATGGGAGT
Mouse GINS1-Reverse	CACCGAGGCAAAAAGTGCTG
Human β-actin-Forward	CCATCGTCCACCGCAAAT
Human β-actin -Reverse	CCTGTAACAACGCATCTCATA
Mouse GAPDH-Forward	TGTGTCCGTCGTGGATCTGA
Mouse GAPDH-Reverse	TTGCTGTTGAAGTCGCAGGAG
Divergent circ1857-Forward	TCCTCGGTGAGCACGGATTC
Divergent circ1857-Reverse	ACAACCATGGCTGACCCGAA
Convergent circ1857-Forward	CTTGGCAGGTATTATGAGACA
Convergent circ1857-Reverse	ACTGGTTGGTTGGGTGGC
Human PRDM1-Forward	GGGATTCTGGTGCTGATGGC
Human PRDM1-Reverse	GGTTGGCAGGGATGGGCTTA
Mouse PRDM1-Forward	GAACCTGCTTTTCAAGTATGCTG
Mouse PRDM1-Reverse	AGTGTAGACTTCACCGATGAGG
Human U6-Forward	GCTTCGGCAGCACATATACTAAAAT
Human U6-Reverse	CGCTTCACGAATTTGCGTGTCAT
shcontrol	CCTAAGGTTAAGTCGCCCTCG
shPAX5-1	GACTATCCATCCATCATAA
shPAX5-2	CGGCCACTCGCTTCCGGGC
shGINS1-1	TCAGGTGGACGAAGTGATT
shGINS1-2	CCTGTATGACCGCTTGCTT
shcirc1857-1	AGAGAGACGAAGGACATGGAG
shcirc1857-2	GAGACGAAGGACATGGAGGAG
P-1500/+50-LUC sense	CCTGAGCTCGCTAGCCTCGAGAAAAAGGCAATGTTTGCT
P-1500/+50-LUC antisense	CAGTACCGGATTGCCAAGCTTACAGCGCCAGGCTCTCGC
P-550/+50-LUC sense	CCTGAGCTCGCTAGCCTCGAGGAAAGCCTAAATAACAGT
P-550/+50-LUC antisense	CAGTACCGGATTGCCAAGCTTACAGCGCCAGGCTCTCGC
P-400/+50-LUC sense	CCTGAGCTCGCTAGCCTCGAGTACCCAATAAAAGAGAGT
P-400/+50-LUC antisense	CAGTACCGGATTGCCAAGCTTACAGCGCCAGGCTCTCGC
P-1500mut1/+50-LUC sense	GGTGAAAAAAAATGTAATTCCAGCACTTTGG
P-1500mut1/+50-LUC antisense	TACATTTTTTTTCACCGCACCCAGCAAACAT
P-550mut2/+50-LUC sense	AGCTAAAAAAAATTGGGCAAGTTACCTAACT
P-550mut2/+50-LUC antisense	CCAATTTTTTTTAGCTCTCCAGAGTGATTAG
pmirGLO GINS1-F	CCTCGAGTTGGGAGAATTGACATCTTAA
pmirGLO GINS1-R	GTCTAGATTAGAAAGGAAGGAGTAAAAC
pmirGLO GINS1Mut-F	AACATACCACCAACAAATGGTATTTTAAAATT
pmirGLO GINS1Mut-R	AAATGCTGACATGGTTTTGTTGGTGGTATGTT

Supplementary Table S3. Plasmids

Reagent or Resource	Source	Identifier
pCMV-N-Flag Control Vector	Beyotime	Cat#D2722
pCDH-CMV-MCS-EF1-CopGFP-T2A-puro	Addgene	Cat#72263
pLP1	Miaolingbio	P0264
pLP2	Miaolingbio	P0265
pLP/VSVG	Miaolingbio	P0266
pLKO.1 puro	Addgene	Cat#8453
psPAX2	Addgene	Cat#12260
pMD2.G	Addgene	Cat#12259
pCMV-N-Flag-PAX5	This paper	N/A
pCMV-N-Flag-PAX5 P80R	This paper	N/A
pET-32a-Trx	Miaolingbio	P0033
pET-32a-Trx-PAX5	This paper	N/A
pET-32a-Trx-PAX5 P80R	This paper	N/A
pLKO.1-shcontrol	This paper	N/A
pLKO.1-shGINS1 -1	This paper	N/A
pLKO.1-shGINS1 -2	This paper	N/A
pLKO.1-shPAX5 -1	This paper	N/A
pLKO.1-shPAX5 -2	This paper	N/A
pLKO.1-shcirc1857 -1	This paper	N/A
pLKO.1- shcirc1857 -2	This paper	N/A
pCDH-CMV-MCS-EF1-GINS1-CopGFP-T2A-puro	This paper	N/A
pCDH-CMV-MCS-EF1-PAX5-CopGFP-T2A-puro	This paper	N/A
pLCDH-ciR-circ1857	This paper	N/A
pLCDH-ciR	Geneseed	GS0103
pGL-4.17 Basic	Promega	#9PIE672
P-1500/+50-LUC	This paper	N/A
P-1500mut1/+50-LUC	This paper	N/A
P-550/+50-LUC	This paper	N/A
P-550mut2/+50-LUC	This paper	N/A
P-400/+50-LUC	This paper	N/A
pmirGLO GINS1	This paper	N/A
pmirGLO GINS1 Mut	This paper	N/A

Supplementary Table S4. Probes and primers sequences

name	Sequences
Probe-1	GGTGCGGTGGCTCACGCTGTAATTCC
Probe-2	TCTGGAGCTGTGTGACCTTGGGCAAG
Probe-1-mut	GCTGGGTGCGGTGTGTAATTCCAGCA
Probe-2-mut	TCACTCTGGAGCTTTGGGCAAGTTAC
ChIP-GINS1-1F	GGAAATGGAAAGCCTAAAT
ChIP-GINS1-1R	CAGAGCTGGAAATAAATGC
ChIP-GINS1-2F	CAAAGGGTGGTACTGTGGTG
ChIP-GINS1-2R	CATTGGTAGGACGCAAGGGT
ChIP-GAPDH-F	GGCTGGATGGAATGAAAGG
ChIP-GAPDH-R	GCACGGAAGGTCACGATGT

Supplementary Table S5. Stable cell lines

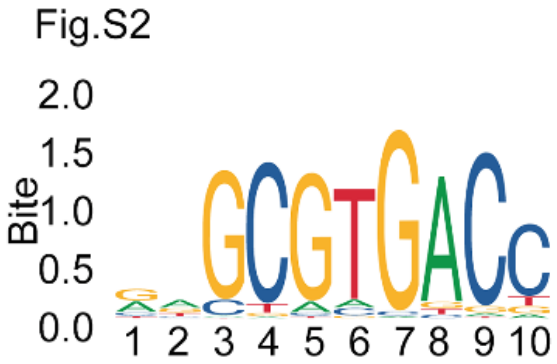
Cell lines name	Source
Farage-Control	This paper
Farage-OE-GINS1	This paper
Farage-OE-PAX5	This paper
Farage-OE-circ1857	This paper
SU-DHL-2- shcontrol	This paper
SU-DHL-2- shGINS1-1	This paper
SU-DHL-2- shGINS1-2	This paper
SU-DHL-2- shPAX5-1	This paper
SU-DHL-2- shPAX5-2	This paper
SU-DHL-2- shcirc1857-1	This paper
SU-DHL-2- shcirc1857-2	This paper
SU-DHL-2- shPAX5-1+OE-GINS1	This paper
SU-DHL-2- shPAX5-2+OE-GINS1	This paper
IM-9-Control	This paper
IM-9-OE-PAX5	This paper
IM-9-OE-circ1857	This paper
DB- shcontrol	This paper
DB- shPAX5-1	This paper
DB- shPAX5-2	This paper
DB- shPAX5-1+OE-GINS1	This paper
DB- shPAX5-2+OE-GINS1	This paper

Supplementary Table S6. Chemicals and antibodies

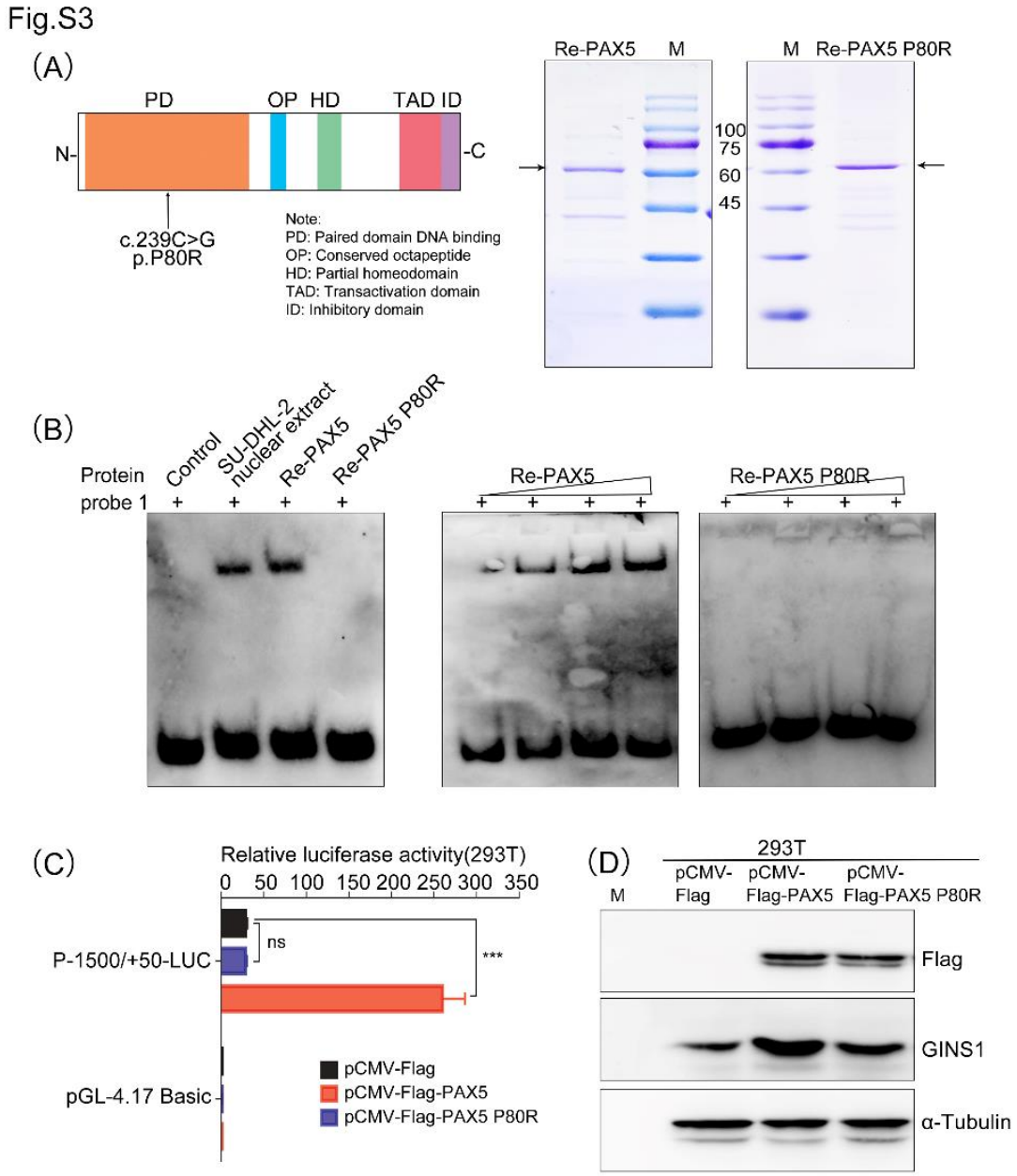
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals		
Fetal Bovine Serum	Gibco	Cat# 10099-141
GenEscortII	Nanjing Wisegen Biotechnology Company	Cat# WIS 2100
ClonExpress II One Step Cloning Kit	Vazyme	Cat# C112-01
Mut Express™ II Fast Mutagenesis Kit	Vazyme	Cat# C214-01
TRIzol	Beyotime	Cat# R0016
BeyoRT™ II cDNA first chain synthesis kit	Beyotime	Cat# D7168L
IPTG	ThermoFisher	Cat# 34060
Firefly Luciferase Reporter Gene Assay Kit	Beyotime	Cat# RG005
β-galactosidase Assay Kit	Beyotime	Cat# RG0036
Chromatin Immunoprecipitation (ChIP)Assay Kit	Beyotime	Cat# P2078
Chemiluminescent EMSA Kit	Beyotime	Cat# GS009
Lipopolysaccharides	Beyotime	Cat# ST1470
Super ECL Detection Reagent ECL	YEASEN	Cat# 36208ES60
Cell Counting Kit-8	Apexbio	Cat# K1018
SYBR® Premix Ex Taq™ II	Takara	Cat# DRR820A
RIPA Lysis buffer	Beyotime	Cat# P0013B
BSA	Beyotime	Cat# ST025
Paraformaldehyde	Sangon Biotech	Cat# A500684
Ficoll 400	MULTI Sciences	Cat# 70-MLSM1092
Actinomycin D	Xiyashiji	A14832
Recombinant human IL-21 Protein	R&D systems	8879-IL
BCA Protein Assay Kit	Thermo Fisher Scientific	Cat# 23227
Antibodies		
Anti-PAX5	Abcam	ab109443
Anti-GINS1	Abcam	ab181112
Anti-PRDM1	Thermos fisher scientific	MA1-16874
Anti-a-Tubulin	Proteintech company	66031-1-Ig
Anti-Flag	Proteintech company	66008-4-Ig
CD45R (B220) MicroBeads,mouse	Miltenyi Biotec	130-049-501
CD138 MicroBeads, mouse	Miltenyi Biotec	130-098-257
Streptavidin MicroBeads	Miltenyi Biotec	130-048-101
IgD Antibody, anti-mouse, Biotin	Miltenyi Biotec	130-101-899
Anti-Mouse IgM MicroBeads	Miltenyi Biotec	130-047-301
Anti-human CD40 antibodies	Thermofisher	14-0409-82

Supplementary Figure S1. A sketch map for plasmid construction of circ1857 overexpression.

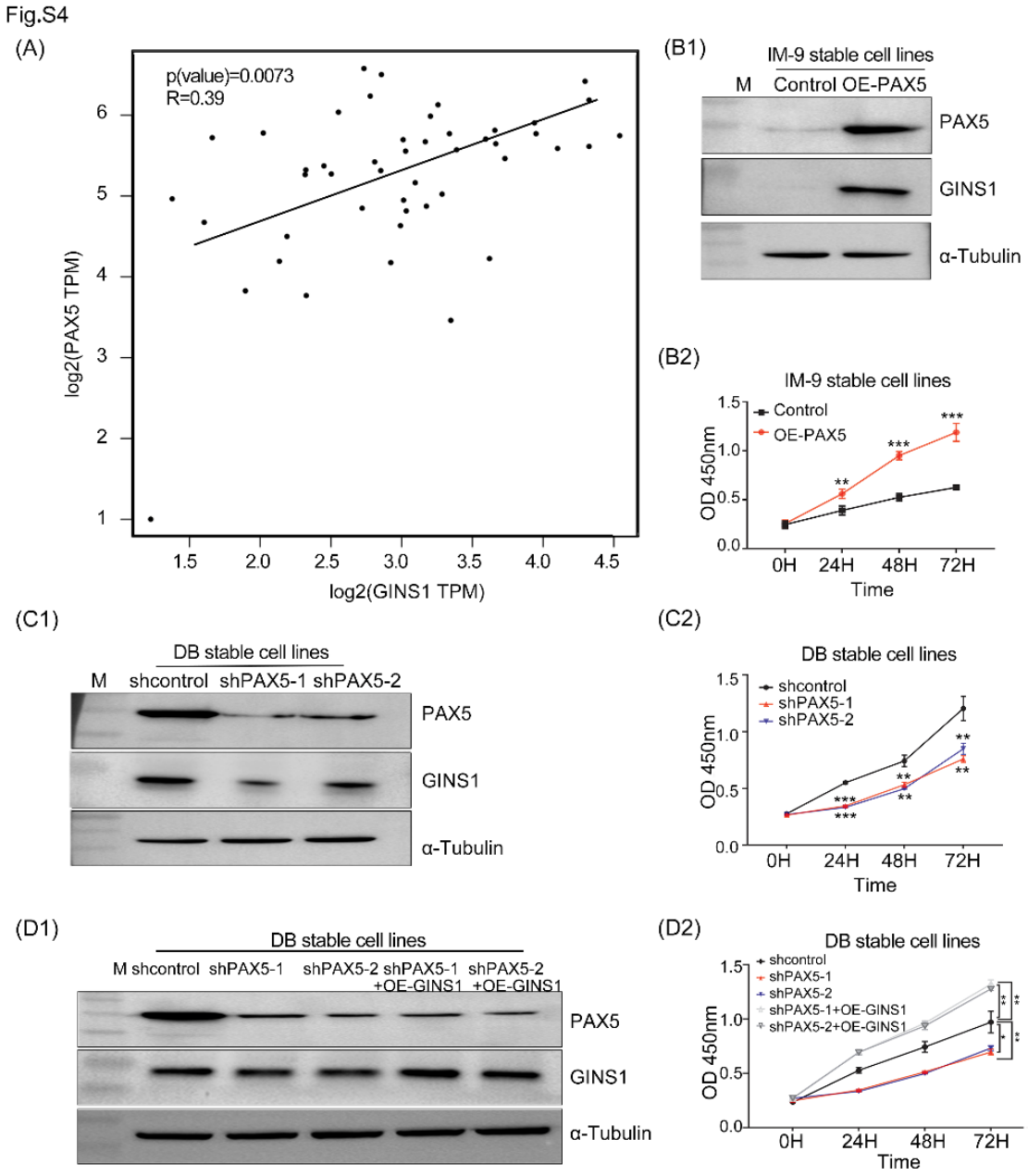
Supplementary Figure S2. The DNA-binding consensus sequence of the PAX5 according to the JASPAR database.



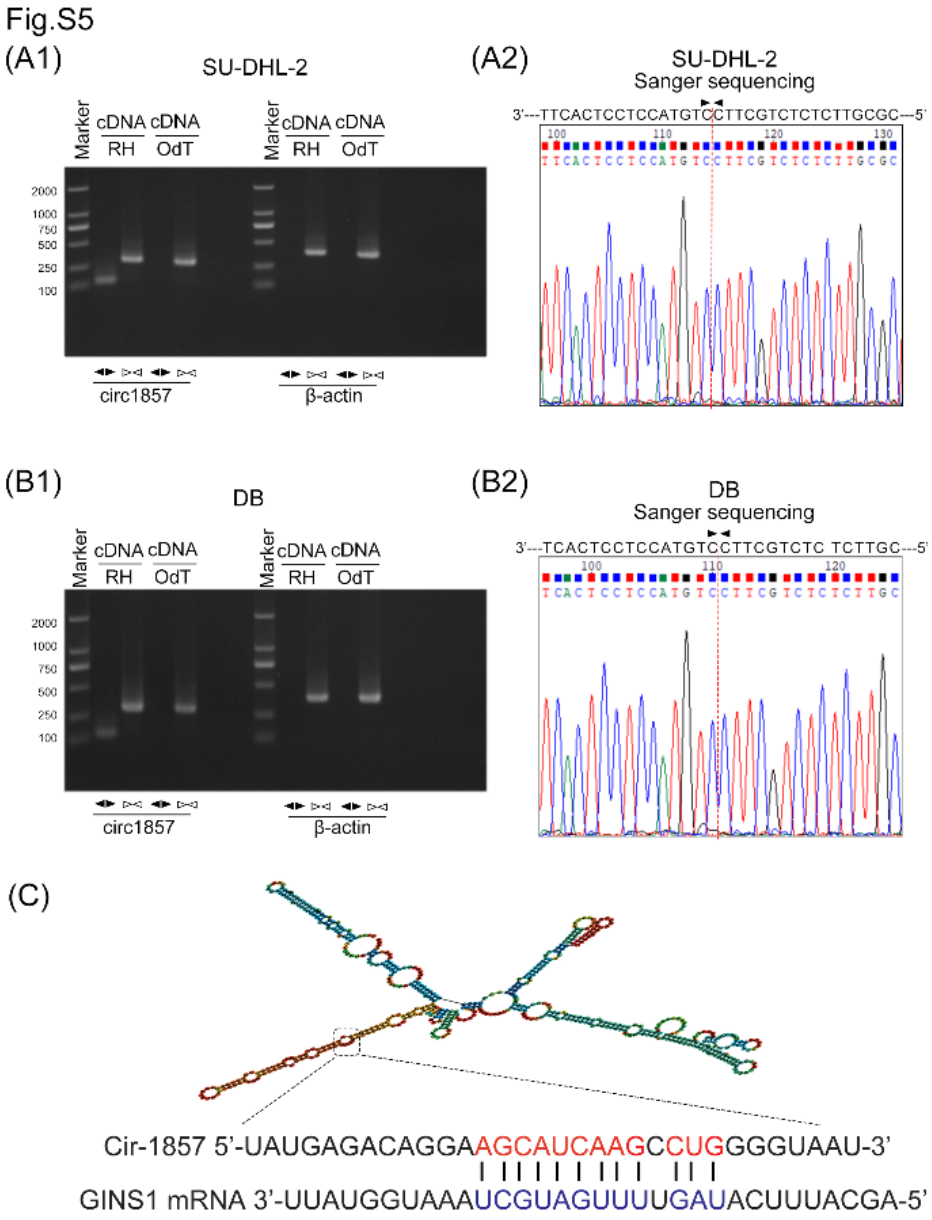
Supplementary Figure S3. PAX5 P80R mutation led to loss of function of binding with GINS1 promoter. (A) Recombinant PAX5 and P80R mutation protein production. Left: schematic representation of PAX5 domain structure. P80R mutation was in DNA binding region. Right: The recombinant PAX5 WT and PAX5 P80R were expressed and purified, then analyzed by SDS-PAGE. (B) EMSA. The left panel showed that SU-DHL-2 nuclear extract and recombinant PAX5 protein (Re-PAX5) can bind with probes and form the complex band. The other two panel showed that increased amount of recombinant PAX5 protein (Re-PAX5) can bind with probes and form the complex band, while recombinant PAX5 P80R protein (Re-PAX5 P80R) could not bind with probes and no complex band was observed. (C) Luciferase reporter assay(***P<0.001). (D) The influence of ectopic expression of Flag-PAX5 WT or Flag-PAX5 P80R on GINS1.



Supplementary Figure S4. The correlation between GINS1 and PAX5 expression level in DLBCL tissues in GEPIA database and the DLBCL cell lines. (A)The correlation between GINS1 and PAX5 expression level in DLBCL by GEPIA database. (B-C) Overexpressing PAX5 in IM-9 cell enhanced GINS1 level and facilitated cell proliferation, while knockdown PAX5 in DB cell decreased GINS1 level and inhibited cell proliferation. (B) Overexpression of PAX5 in IM-9. (B1) western blot. (B2) CCK8 assay. (C)Knockdown of PAX5 in DB cells. (C1) western blot. (C2) CCK8 assay. (D) GINS1 overexpression reversed GINS1 expression level decreased by PAX5 knock down in DB stable cell lines. (D1) western blot. (D2) CCK8 assay.



Supplementary Figure S5. Identification of circ1857 in SU-DHL-2 and DB cells. A-B. Circ1857 in SU-DHL-2 and DB cells was validated by RT-PCR with divergent primers and confirmed by sanger sequencing. PCR analysis for circ1857 and β-actin in cDNA in SU-DHL-2 (A1) and DB (B1) cells. RH: random hexamers, OdT: oligo(dT) 18 primers. (C) Predicted secondary stem-loop structure of circ1857. circ1857 was predicted to bind with 3-UTR of GINS1 mRNA.



Supplementary Figure S6. Correlation between GINS1 and circ1857 in B cells. (A)The RNA level of GINS1 and circ1857 stimulated by LPS in IM-9 cells. (B)The RNA level of GINS1 and circ1857 stimulated by IL-21+anti-CD40 in SU-DHL-2 cells.

Fig.S6