

## **Supplemental Materials**

### **Supplementary Figures pp. 2-4**

Supplementary Figure 1. Plasmids used to generate CD19 CAR-T cells

Supplementary Figure 2. Comparison of integration profiles among three donors

Supplementary Figure 3. Local sequences near vector integration sites

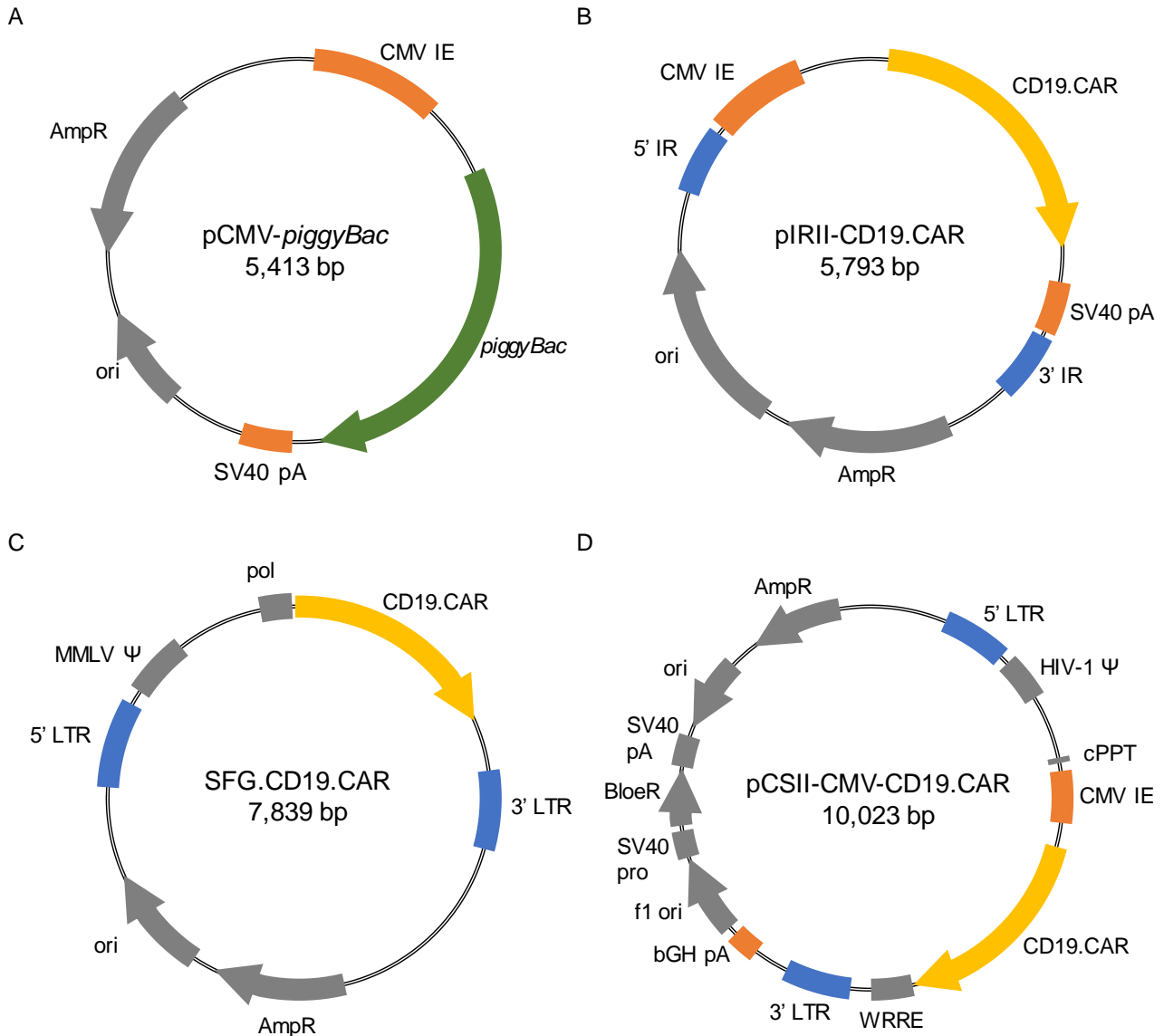
### **Supplementary Tables pp. 5-8**

Supplementary Table 1. List of sequences of primers

Supplementary Table 2. Pairing of primers for 1st PCR

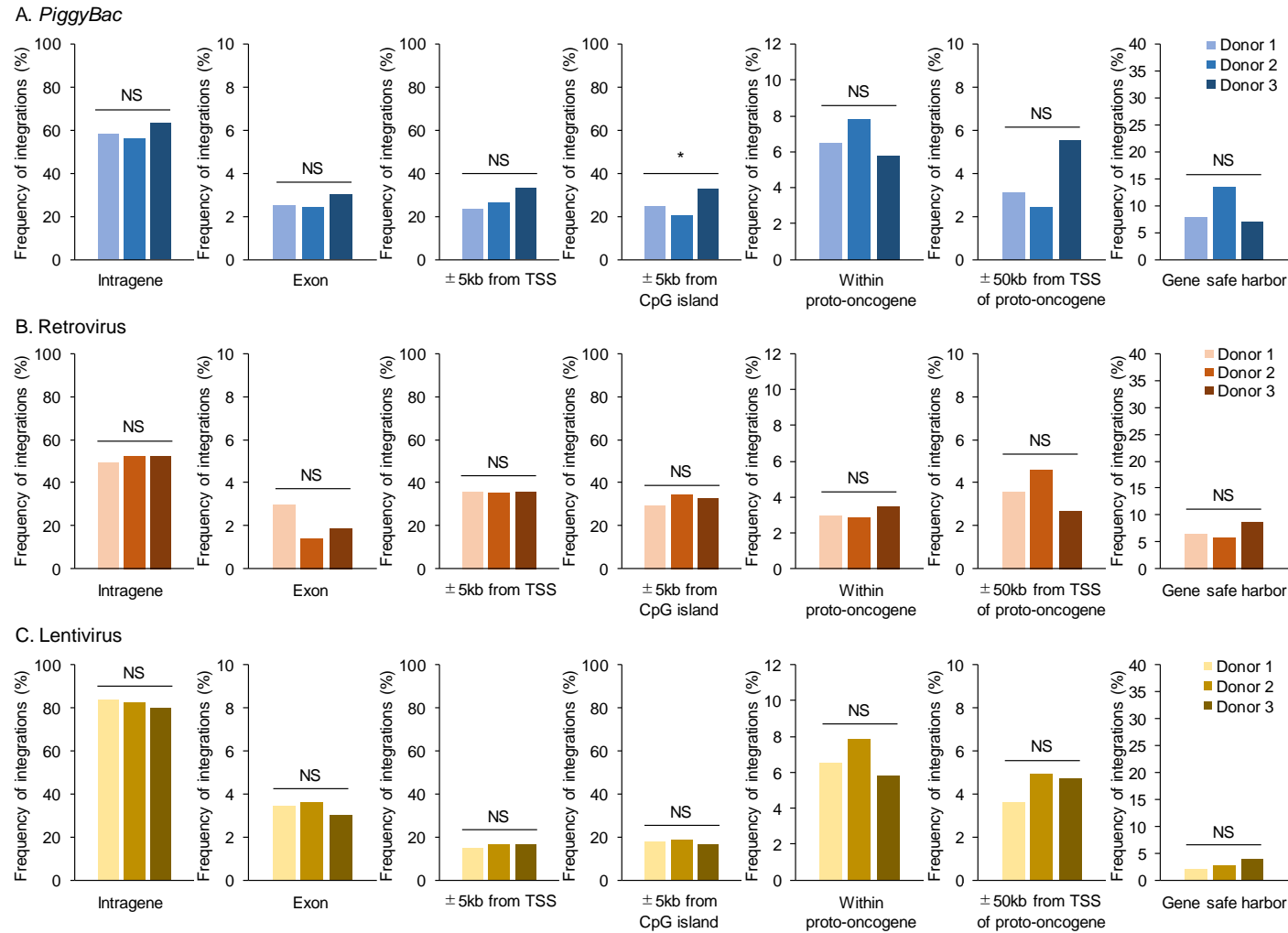
Supplementary Table 3. List of integration sites (a separate Excel file)

Supplementary Table 4. List of oncogenes with integrations into or within 50kb from TSSs



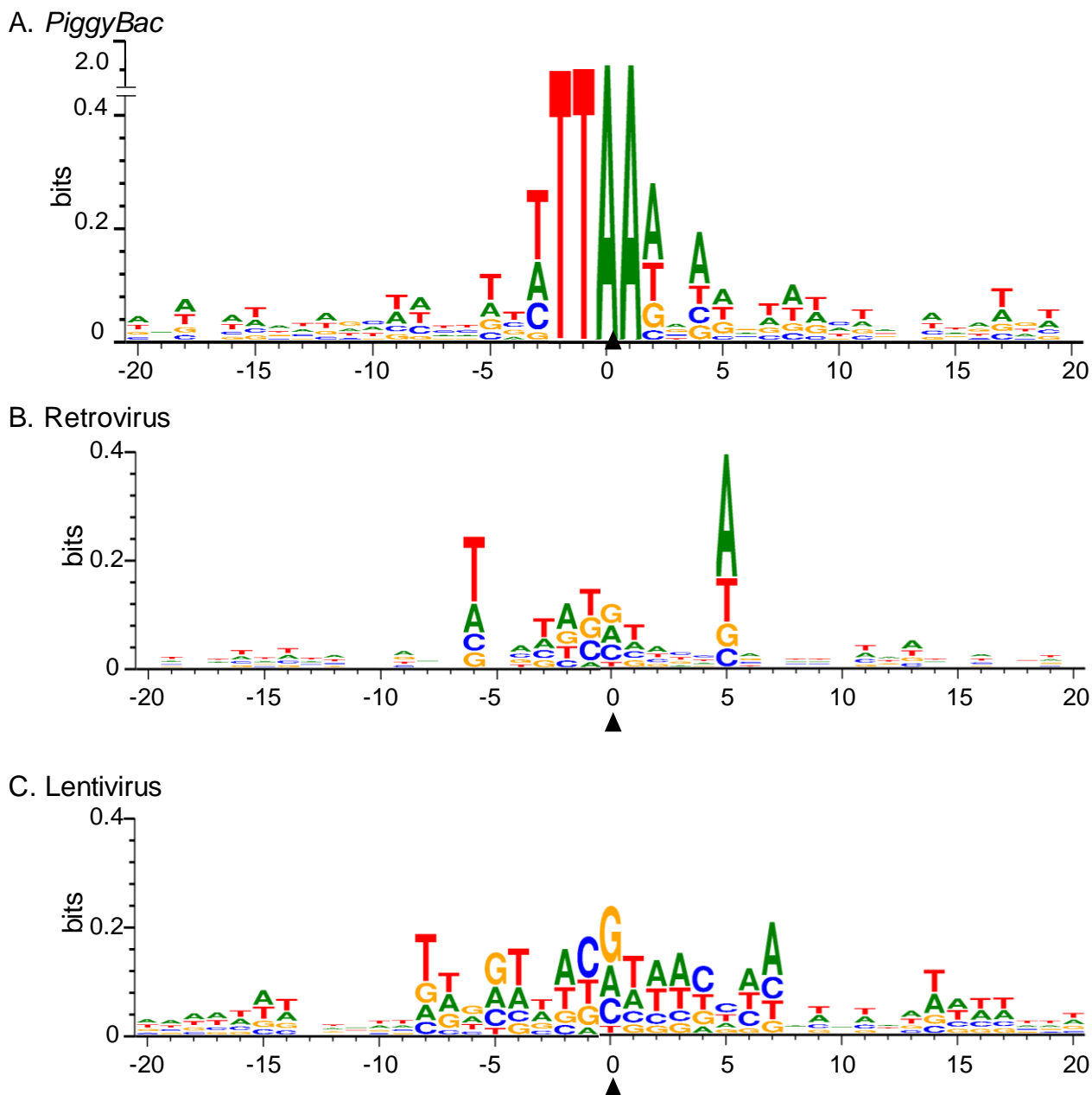
**Supplementary Fig. 1. Plasmids used to generate CD19 CAR-T cells**

Vector illustration of *PiggyBac* transposase plasmid (A), *piggyBac* transposon plasmid (B), retrovirus vector plasmid (C), and lentivirus vector plasmid (D). Genes of interest are shown in yellow (CD19.CAR) and green (*piggyBac* transposase). Specific sequences necessary for insertion of transgene are shown in blue. Promoters and enhancers for transgenes are shown in orange. Other components are shown in gray. AmpR, ampicillin resistance gene; bGH pA, bovine growth hormone polyadenylation signal; BleoR, bleomycin resistance gene; CD19.CAR, CD19-specific chimeric antigen receptor; CMV IE, cytomegalovirus immediate early promoter; cPPT, central polypurine tract; f1 ori, f1 bacteriophage origin of replication; HIV-1 Ψ, Human immunodeficiency virus type-1 packaging signal; IR, inverted terminal repeat; LTR, long terminal repeat; MMLV Ψ, Moloney murine leukemia virus packaging signal; ori, high-copy-number ColE1/pMB1/pBR322/pUC origin of replication; *piggyBac*, *piggyBac* transposase; pol, Moloney murine leukemia virus pol region containing the splice acceptor site; SV40 pA, simian virus 40 polyadenylation signal; SV40 pro, SV40 early promoter; WPRE, woodchuck hepatitis virus posttranscriptional regulatory element.



**Supplementary Fig. 2. Comparison of integration profiles among three donors**

Frequency of integration within or around genetic components of CD19 CAR-T cells generated by *piggyBac* transposon (A), retrovirus (B), and lentivirus (C), separately analyzed for each individual donor. Genetic components include genes, exons, 5kb windows around transcriptional start sites (TSSs), 5kb windows around CpG islands, proto-oncogenes, 50kb windows around TSSs of proto-oncogenes, and gene safe harbors. \* P < 0.05; NS, not significant.



**Supplementary Fig. 3. Local sequences near vector integration sites**

The WebLogo diagrams indicate consensus sequences near integration sites of CD19 CAR T cells generated by *piggyBac* transposon (A), retrovirus (B), and lentivirus (C). The X-axis denotes the distance from vector integration sites and arrowheads indicate vector integration sites. The overall height of each stack indicates the sequence conservation at each position (measured in bits), and the height of the letters within the stack reflects the relative frequency of the corresponding nucleic acids.

**Supplementary Table 1. List of sequences of primers**

Round	Primer name	Sequence (5'-3')
1 <sup>st</sup> PCR	NTSR1-F1	TCGTCGGCAGCGTCAGAT
	NTSR2-F1	GTCTCGTGGGCTCGGAGATG
	PB-NTSR1-R1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACGCAGACTATCTTTCTAGGGTT
	PB-NTSR1-R2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTCAAGAATGCATGCGTCAATTT
	PB-NTSR1-RCR1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACCGATAAAAACACATGCGTCA
	PB-NTSR2-R1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTACGCAGACTATCTTTCTAGGGTT
	PB-NTSR2-R2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCGACGGATTTCGCGCTATTT
	PB-NTSR2-RCR1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGACCGATAAAAACACATGCGTCA
	RV-NTSR1-R1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATGCCTTGCAAATGGCGTTA
	RV-NTSR1-RCR1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCAATAAACCTCTTGCAGTTG
	RV-NTSR1-RCR2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTCTCGCTGTTCCCTGGGAG
	RV-NTSR2-R1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGCCTTGCAAATGGCGTTA
	RV-NTSR2-RCR1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCCAATAAACCTCTTGCAGTTG
	RV-NTSR2-RCR2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTCTCGCTGTTCCCTGGGAG
	LV-NTSR1-R1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAGCAGTGGGTTCCCTAGTT
	LV-NTSR1-RCR1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTAGTGTGTGCCCGTCTGTTG
	LV-NTSR1-RCR2	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAACTAGAGATCCCTCAGACCCT
	LV-NTSR2-R1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAAGCAGTGGGTTCCCTAGTT
	LV-NTSR2-RCR1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTAGTGTGTGCCCGTCTGTTG
	LV-NTSR2-RCR2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAACTAGAGATCCCTCAGACCCT
2 <sup>nd</sup> PCR	Nextera-2nd-i5-N501	AATGATACGGCGACCACCGAGATCTACACTAGATCGCTCGTCGGCAGCGTC
	Nextera-2nd-i7-N716	CAAGCAGAAGACGGCATAACGAGATTAGCGAGTGTCTCGTGGGCTCGG
	Nextera-2nd-i7-N718	CAAGCAGAAGACGGCATAACGAGATGTAGCTCCGTCTCGTGGGCTCGG
	Nextera-2nd-i7-N719	CAAGCAGAAGACGGCATAACGAGATTACTACGCGTCTCGTGGGCTCGG
	Nextera-2nd-i7-N720	CAAGCAGAAGACGGCATAACGAGATAGGCTCCGTCTCGTGGGCTCGG
	Nextera-2nd-i7-N721	CAAGCAGAAGACGGCATAACGAGATGCAGCGTAGTCTCGTGGGCTCGG
	Nextera-2nd-i7-N722	CAAGCAGAAGACGGCATAACGAGATCTGCGCATGTCTCGTGGGCTCGG
	Nextera-2nd-i7-N723	CAAGCAGAAGACGGCATAACGAGATGAGCGCTAGTCTCGTGGGCTCGG
	Nextera-2nd-i7-N724	CAAGCAGAAGACGGCATAACGAGATCGCTCAGTGTCTCGTGGGCTCGG
	Nextera-2nd-i7-N726	CAAGCAGAAGACGGCATAACGAGATGTCTTAGGGTCTCGTGGGCTCGG

\*Blue and purple letters indicate incomplete adapter sequences for P5 and P7 adapters, respectively. Green letters indicate sequences for completing adapters.

**Supplementary Table 2. Pairing of primers for 1<sup>st</sup> PCR**

Vector	Forward primer	Reverse primer
<i>PiggyBac</i>	NTSR1-F1	PB-NTSR1-R1
		PB-NTSR1-R2
		PB-NTSR1-RCR1
	NTSR2-F1	PB-NTSR2-R1
		PB-NTSR2-R2
		PB-NTSR2-RCR1
Retrovirus	NTSR1-F1	RV-NTSR1-R1
		RV-NTSR1-RCR1
		RV-NTSR1-RCR2
	NTSR2-F1	RV-NTSR2-R1
		RV-NTSR2-RCR1
		RV-NTSR2-RCR2
Lentivirus	NTSR1-F1	LV-NTSR1-R1
		LV-NTSR1-RCR1
		LV-NTSR1-RCR2
	NTSR2-F1	LV-NTSR2-R1
		LV-NTSR2-RCR1
		LV-NTSR2-RCR2

**Supplementary Table 4. List of oncogenes with integrations into or within 50kb from TSSs**

<i>PiggyBac</i>	Retrovirus	Lentivirus
<i>ATM</i>	<i>ACKR3</i>	<i>ABI1</i>
<i>ATRX</i>	<i>ARHGAP26</i>	<i>AKAP9</i>
<i>BTG1</i>	<i>ARID1A</i>	<i>ARHGAP26</i>
<i>CALR</i>	<i>BCL11B</i>	<i>ARID1A</i>
<i>CBL</i>	<i>BRD4</i>	<i>ARNT</i>
<i>CBLB</i>	<i>CARD11</i>	<i>ASXL1</i>
<i>CNBP</i>	<i>CCND3</i>	<i>ATM</i>
<i>DDX6</i>	<i>CD274</i>	<i>BCL2</i>
<i>ELF4</i>	<i>CDK6</i>	<i>BRAF</i>
<i>EXT2</i>	<i>EWSR1</i>	<i>BRCA1</i>
<i>FBXO11</i>	<i>FGFR1OP</i>	<i>BRD4</i>
<i>FNBP1</i>	<i>GOLGA5</i>	<i>BRIP1</i>
<i>FOXP1</i>	<i>HLA-A</i>	<i>CASP8</i>
<i>ITK</i>	<i>IKZF1</i>	<i>CBL</i>
<i>LPP</i>	<i>IL7R</i>	<i>CCND3</i>
<i>MDS2</i>	<i>KAT6A</i>	<i>CCNE1</i>
<i>MLLT6</i>	<i>KAT6B</i>	<i>CD274</i>
<i>MYB</i>	<i>KDM6A</i>	<i>CNTRL</i>
<i>NCOA2</i>	<i>LCK</i>	<i>COL2A1</i>
<i>NF2</i>	<i>MALAT1</i>	<i>CRTC3</i>
<i>NIN</i>	<i>MYH9</i>	<i>CYLD</i>
<i>NTRK1</i>	<i>NCOA2</i>	<i>DDX6</i>
<i>PML</i>	<i>NF1</i>	<i>ERCC3</i>
<i>PMS1</i>	<i>NFATC2</i>	<i>FANCA</i>
<i>PPARG</i>	<i>PRF1</i>	<i>FBXW7</i>
<i>PTPRC</i>	<i>PTPRC</i>	<i>FOXP1</i>
<i>RAD51B</i>	<i>PTPRK</i>	<i>GOLGA5</i>
<i>RPL22</i>	<i>RARA</i>	<i>IL7R</i>
<i>RUNX1</i>	<i>RNF213</i>	<i>IRF4</i>
	<i>RUNX1</i>	<i>JAK3</i>
	<i>SUFU</i>	<i>KDM6A</i>
	<i>TNFAIP3</i>	<i>KIF5B</i>
	<i>TPM4</i>	<i>KTN1</i>
	<i>UBR5</i>	<i>LCK</i>
	<i>WHSC1L1</i>	<i>LPP</i>
		<i>LSM14A</i>

	<i>NCOA4</i>
	<i>NCOR1</i>
	<i>NSD1</i>
	<i>NUP214</i>
	<i>PBRM1</i>
	<i>PCM1</i>
	<i>PHF6</i>
	<i>PLAG1</i>
	<i>PWWP2A</i>
	<i>RABEP1</i>
	<i>RAC1</i>
	<i>RAP1GDS1</i>
	<i>RHOH</i>
	<i>RNF213</i>
	<i>RUNX1</i>
	<i>SETD2</i>
	<i>SH3GL1</i>
	<i>SMAD4</i>
	<i>SS18</i>
	<i>STAT3</i>
	<i>STAT5B</i>
	<i>TBL1XR1</i>
	<i>TCF3</i>
	<i>TFRC</i>
	<i>TOP1</i>
	<i>TSC2</i>
	<i>U2AF1</i>
	<i>WHSC1</i>
	<i>WHSC1L1</i>
	<i>ZNF331</i>