

Inducing synthetic lethality for selective targeting of acute myeloid leukemia cells harboring *STAG2* mutations

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Inducing synthetic lethality as a targeted therapy for acute myeloid leukemia harboring STAG2 mutations

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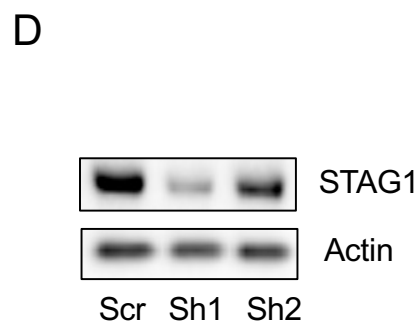
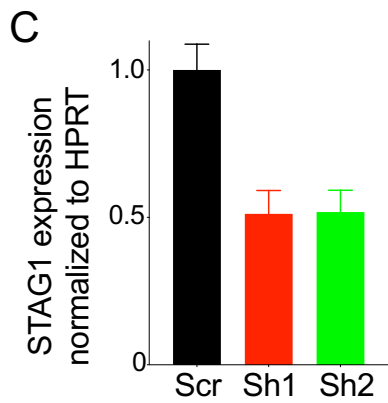
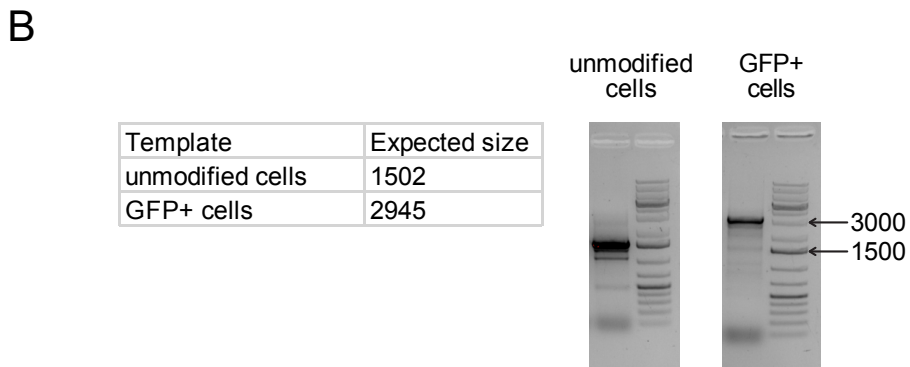
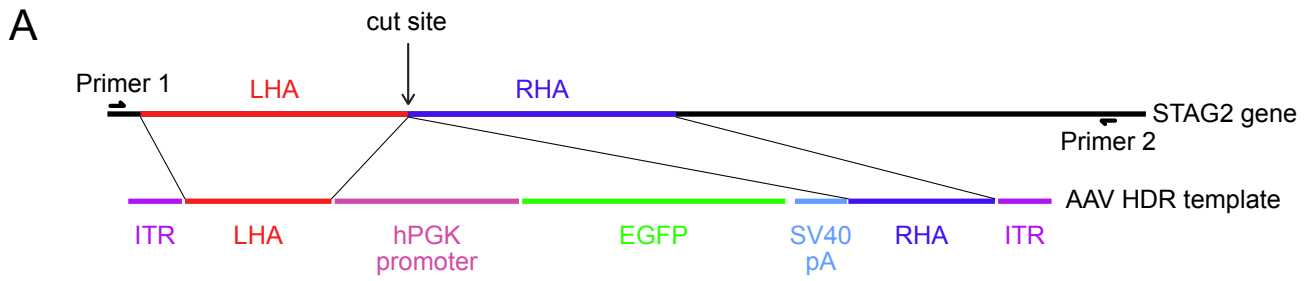
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Supplementary figure legends

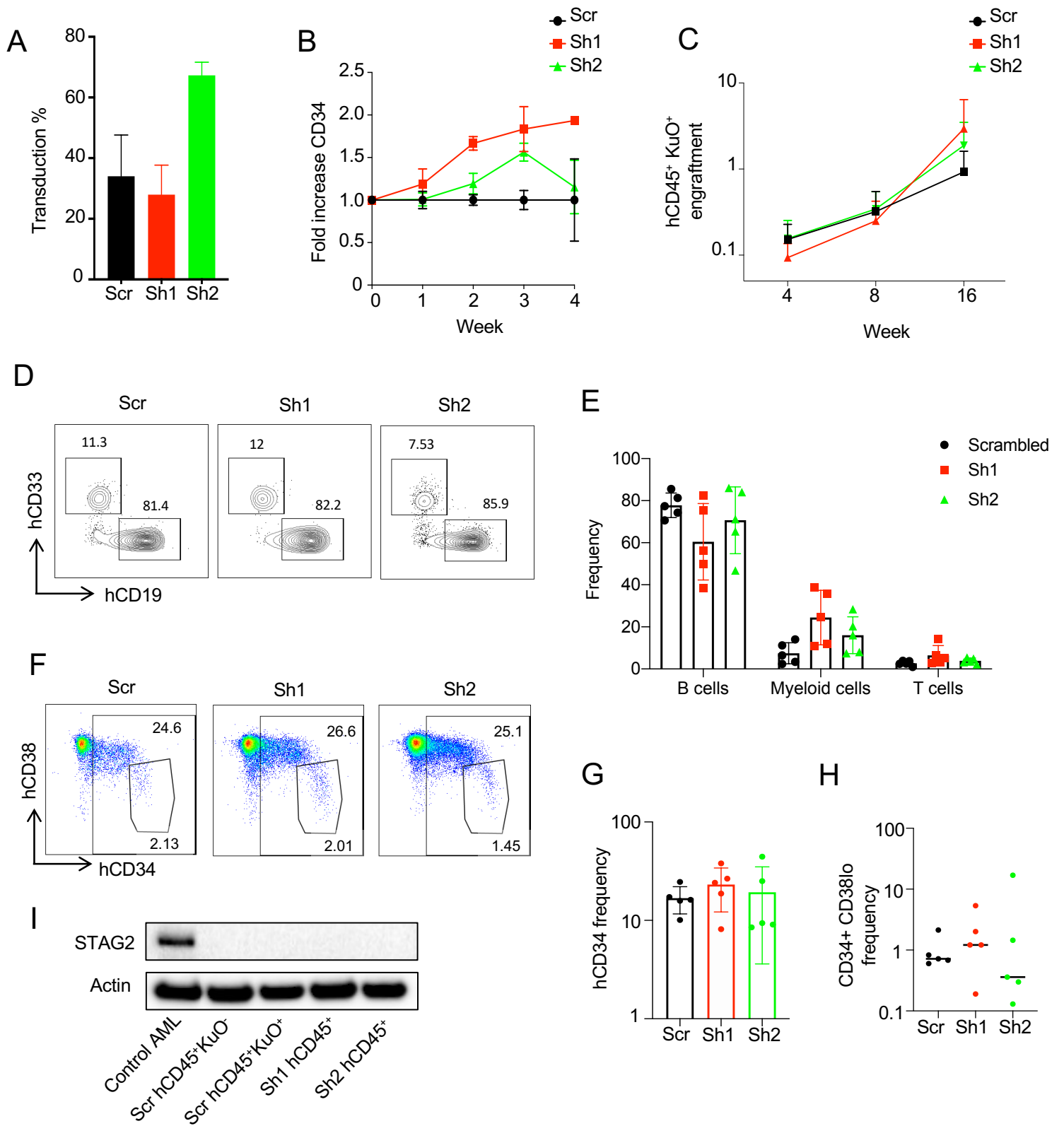
Supplementary Figure 1. **A.** Construction of the eGFP knock in construct and validation of the integration in the STAG2 locus with PCR. **B.** Integration of eGFP construct was analyzed with PCR with the following primers, Primer 1; TGGGGTAGGCACAGTTTTATCCT and Primer 2; TGGTTTACATCAGCATATTTTTGACCA. **C.** UCB CD34⁺ cells were transduced with STAG1 shRNAs for 3 days and the knockdown efficiency was quantified with qPCR and **D.** with Western blotting.

Supplementary Figure 2. **A.** Transduction frequencies of scrambled and two independent STAG1 shRNAs (Sh1 and Sh2) in AML PDX, two days post transduction, quantified from 3 independent replicates. **B.** UCB CD34⁺ cells were transduced with STAG1 shRNAs and fold expansion of CD34 cell numbers were quantified over four weeks of *in vitro* culture. **C.** UCB CD34⁺ cells were transduced with STAG1 shRNAs and transplanted in NSG mice. Engraftment of transduced Kusabira orange positive cells in the peripheral blood at week 4, 8 and 16. **D.** CD19 B-cell and CD33 myeloid lineage contribution of shRNA transduced cells in bone marrow at week 16. **E.** B-, Myeloid and T- cell lineage frequencies were quantified for each shRNA. **F.** Frequency of CD34⁺ and CD34⁺CD38^{low} cells in observed in the bone marrow of NSG mice transplanted with shRNA transduced UCB CD34⁺ cells. Quantification of the frequency of **G.** CD34⁺ and **H.** CD34⁺CD38^{low} cells for each shRNA. **I.** STAG2 protein expression in the hCD45⁺ PDX materials obtained from the Scrambled (KuO⁺ and KuO⁻), Sh1 and Sh2 conditions.

Supplementary table 1. Next generation sequencing analysis-based frequencies of the somatic mutations in AML samples.



Supplementary Figure 1



Supplementary Figure 2

Sample	Gene	Chromosome	Start position	Reference sequence	Alternative sequence	Variant Allele Frequency
AML16	STAG2	chrX	123205077	TTAGTGTATACCCCT	TTTT	0.926
	<i>FLT3</i>	chr13	28592642	C	A	0.486
	<i>MYT1L</i>	chr2	1926741	AG	GG	0.471
	<i>EVPL</i>	chr17	74005949	C	T	0.439
	<i>ABCA4</i>	chr1	94506929	T	C	0.431
	<i>TET2</i>	chr4	106197210	CA	AA	0.431
	<i>ZFYVE9</i>	chr1	52798497	AA	AG	0.414
	<i>TRIM51</i>	chr11	55653082	C	T	0.394
	<i>NPM1</i>	chr5	170837544	TCTG	TCTGTCTG	0.328
<i>HMCN2</i>	chr9	133305778	GG	AG	0.286	
AML21	STAG2	chrX	123220481	TTCTTT	TGAAAGCT	0.89
	<i>GRIA4</i>	chr11	105758236	T	C	0.64
	<i>MPP4</i>	chr2	202547667	A	C	0.64
	<i>EDAR</i>	chr2	109546594	C	T	0.63
	<i>HNF1A</i>	chr12	121432080	C	T	0.55
	<i>TUBB3</i>	chr16	89999064	G	A	0.48
	<i>NRAS</i>	chr1	115258747	C	T	0.47
	<i>SERPINB4</i>	chr18	61310786	G	A	0.46
	<i>PROX1</i>	chr1	214170676	G	A	0.46
	<i>DRD5</i>	chr4	9784632	G	A	0.45
	<i>IDH2</i>	chr15	90631934	C	T	0.45
	<i>TTN</i>	chr2	179436904	A	G	0.45
	<i>ANXA2P2</i>	chr9	33625306	T	C	0.44
	<i>SRSF2</i>	chr17	74732959	G	T	0.44
	<i>SH2B3</i>	chr12	111885251	T	C	0.43
	<i>SARS</i>	chr1	109779189	G	C	0.41
	<i>TGFBR3</i>	chr1	92149368	C	T	0.40
<i>TGM4</i>	chr3	44952468	C	T	0.39	
<i>MMP13</i>	chr11	102820988	G	C	0.39	
AML94	<i>BCORL1</i>	chrX	129150099	GAAGAT	CAAGATAGGGTC CCCT	0.99
	<i>FLT3</i>	chr13	28608240	G	<DUP>	0.981
	STAG2	chrX	123184086	GGA	GAA	0.979
	<i>BCOR</i>	chrX	39921391	CC	TC	0.94
	<i>IGFBP3</i>	chr7	45956889	G	A	0.505
	<i>PRKAB2</i>	chr1	146634130	CCC	CCA	0.481
	<i>PLEKHA1</i>	chr10	124189419	TT	TC	0.473
	<i>DNMT3A</i>	chr2	25457242	C	T	0.467
	<i>ANKH</i>	chr5	14749340	GA	AA	0.444
	<i>IDH1</i>	chr2	209113112	CG	CC	0.429
	<i>TSHR</i>	chr14	81606046	CT	TT	0.405
	<i>TIAM1</i>	chr21	32526645	TC	CC	0.404
	<i>RUNX1</i>	chr21	36164462	GCGCGCG	GCGCGCGCG	0.396
<i>DMRTA1</i>	chr9	22451795	GG	AG	0.092	
Control AML	<i>PER1</i>	chr17	8048009	C	CA	0.671053
	<i>BCL2</i>	chr18	60985779	CCG	C	0.5625
	<i>FANCA</i>	chr16	89836872	T	C	0.492754
	<i>PER1</i>	chr17	8048283	G	A	0.470588
	<i>JAK1</i>	chr1	65339110	C	CT	0.323077
	<i>MYC</i>	chr8	128750540	A	G	0.473896
	<i>FLT3</i>	chr13	28624294	G	A	0.443243
<i>TIMP3</i>	chr22	33253290	TCC	T	0.366864	

Supplementary table 1