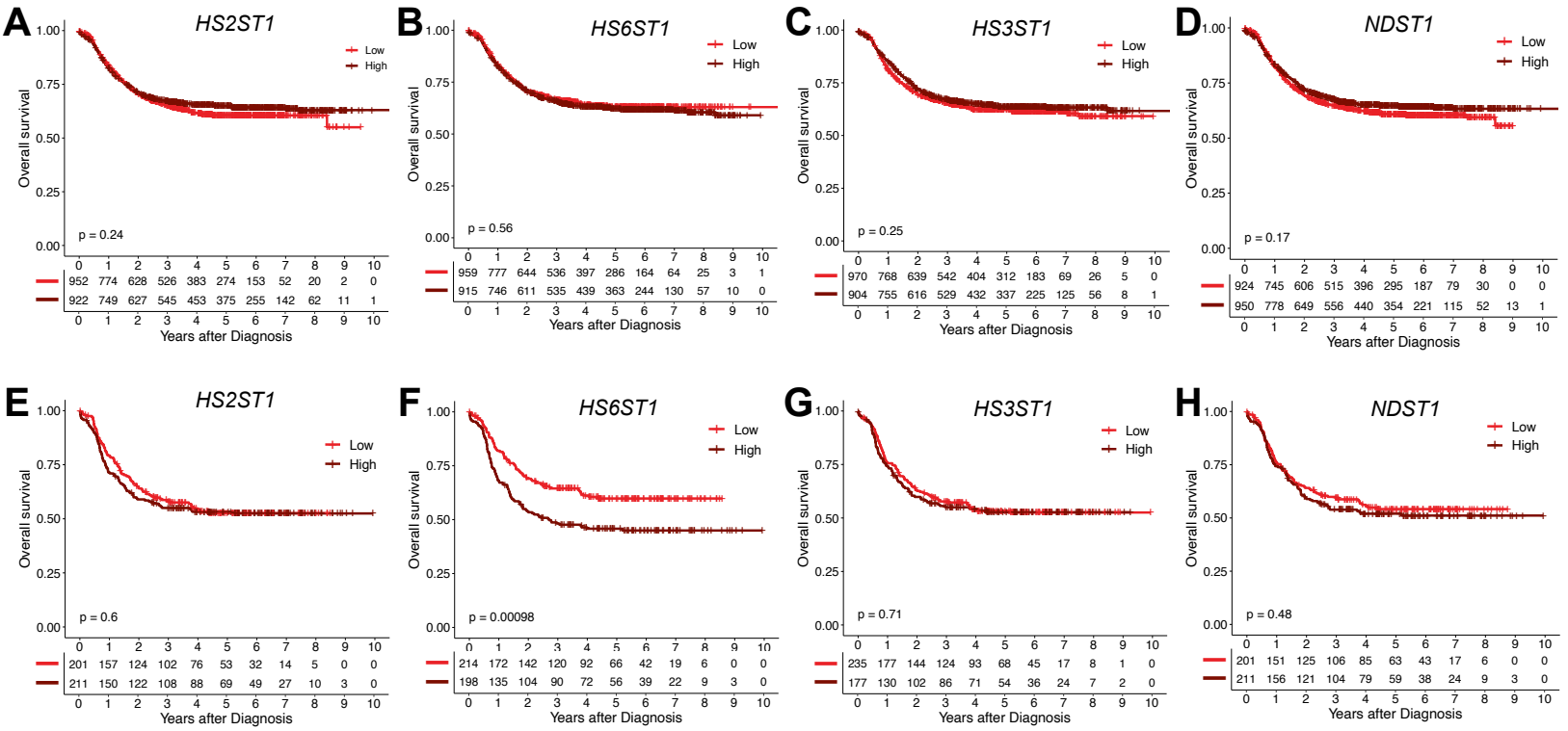
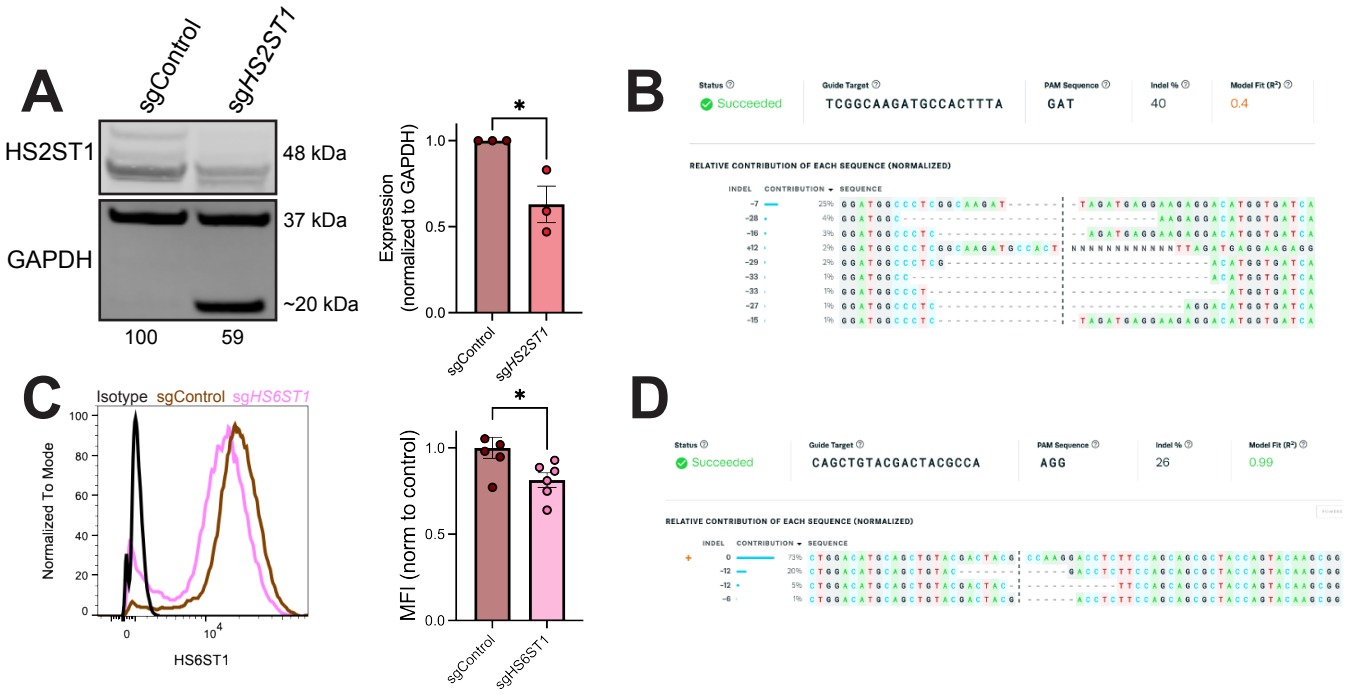
Supplemental Figure 1



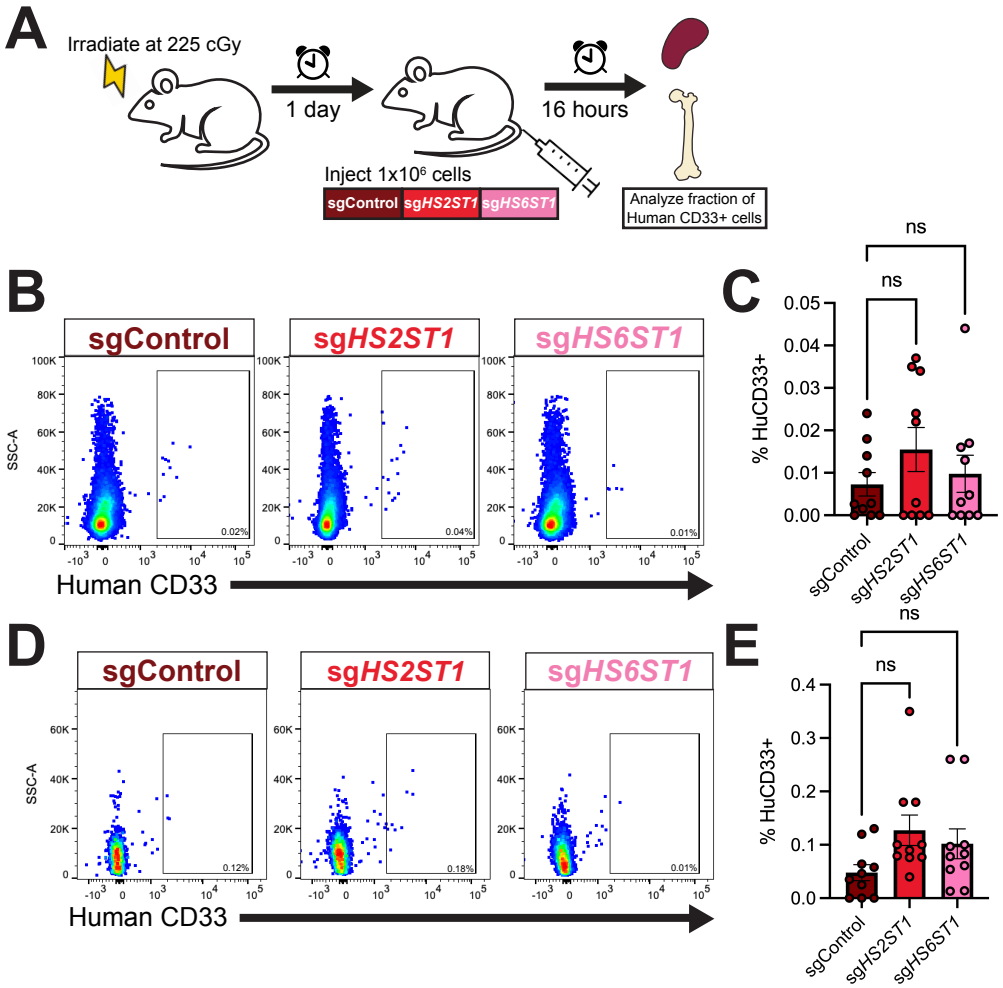
Supplemental Figure 1. High HS6ST1 expression predicts poor event-free survival in KMT2A-rearranged AML. Overall survival of AML patients from the TARGET AML database was analyzed based on above-median (high) or below-median (low) expression of (A) HS2ST1, (B) HS6ST1, (C) HS3ST1, (D) or NDST1 in patient bone marrow mononuclear cells (n=1874 AML patients). Overall survival of AML patients harboring KMT2A-rearrangements from the TARGET AML database was analyzed based on above-median (high) or below-median (low) expression of (E) HS2ST1, (F) HS6ST1, (G) HS3ST1, (H) or NDST1 in patient bone marrow mononuclear cells. Statistics denote the log-rank test (n=412 KMT2A-rearranged AML patients).

Supplemental Figure 2



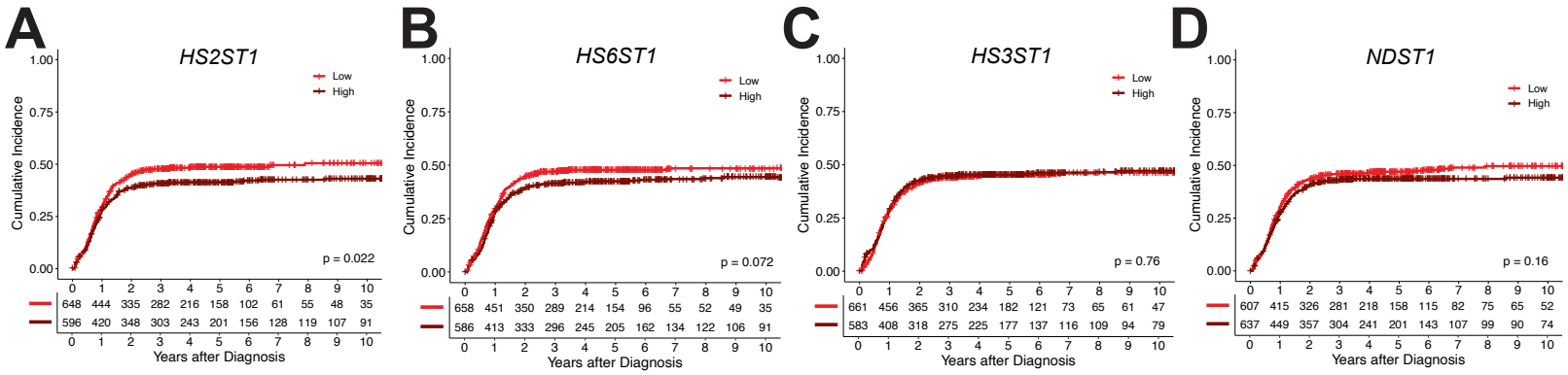
Supplemental Figure 2. HS2ST1 and HS6ST1 were targeted using CRISPR-Cas9 lentivirus. (A) Western blot analysis and quantification of HS2ST1 expression in sgControl and sgHS2ST1 MOLM-13 cells. (B) Sanger sequencing and Inference of CRISPR Edits analysis of the guide RNA target region in sgHS2ST1 MOLM-13 cells. (C) Intracellular flow cytometry and quantification of HS6ST1 expression in sgControl and sgHS6ST1 MOLM-13 cells. (D) Sanger sequencing and Inference of CRISPR Edits analysis of the guide RNA target region in sgHS6ST1 cells. (n=3 biological replicates; statistics denote unpaired t-tests; *p<0.05; error bars denote SEM).

Supplemental Figure 3



Supplemental Figure 3. HS2ST1 and HS6ST1 do not impact AML homing. (A) Experimental design for in vivo leukemia burden study. (B) Representative flow cytometry scatter plots and (C) quantification of bone marrow human CD33 burden from xenograft at 16 hours post-transplant. (D) Representative flow cytometry scatter plots and (E) quantification of spleen human CD33 burden from xenograft at 16 hours post-transplant. (n=10 mice from n=2 independent experiments; statistics denote one-way ANOVA with Holm-Sidak corrected t-tests; error bars denote SEM).

Supplemental Figure 4



Supplemental Figure 4. Increased HS2ST1 is associated with elevated relapse risk in AML. TARGET patient relapse risk for all AML subtypes based on above-median (high) or below-median (low) expression of (A) HS2ST1, (B) HS6ST1, (C) HS3ST1, or (D) NDST1. (n=1244 AML patients, statistics denote the log-rank test).

Supplemental Table 1: Manuscript Resources table. Vendor information for antibodies, assays, mouse models, and software used throughout manuscript.

ANTIBODIES				
Name	**Vendor**	**Catalog Number**	**Clone**	**Dilution**
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 555	Invitrogen	A21428	Polyclonal	2 µg/mL
HS6ST1 Polyclonal Antibody	Invitrogen	PA5-112578	Polyclonal	1:100
HS2ST1 polyclonal antibody	Invitrogen	PA5-89472	Polyclonal	1:1000
GAPDH Loading Control Monoclonal Antibody (GA1R)	Invitrogen	MA5-15738	GA1R	1:1000
PE anti-human CD33 Antibody	Biolegend	366608	P67.6	5 µL/test
BD Horizon™ V450 Mouse Anti-Human CD45	BD Biosciences	560367	HI30	5 µL/test
Brilliant Violet 605™ anti-mouse CD45 Antibody	Biolegend	103140	30-F11	5 µL/test
BD Horizon™ V450 Mouse IgG1, κ Isotype Control	BD Biosciences	560373	MOPC-21	5 µL/test
Brilliant Violet 605™ Rat IgG2b, κ Isotype Ctrl Antibody	Biolegend	400649	RTK4530	5 µL/test
Pacific Blue™ Annexin V	Biolegend	640918	N/A	5 µL/test
Alexa Fluor® 647 Annexin V	Biolegend	640943	N/A	5 µL/test
680RD Goat Anti-Mouse IgG Secondary Antibody	LICOR	926-68070	Polyclonal	1:10000
800 CW Goat Anti-Mouse IgG Secondary Antibody	LICOR	926-32210	Polyclonal	1:10000
Phospho-SMAD2 (S465/467) SMAD3 (S423/425) (D27F4)	Cell Signaling Technology	8828S	D27F4	1:100
MOUSE MODELS				
Name	**Vendor**	**Catalog Number**		
NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ				
REAGENTS				
Name	**Vendor**	**Catalog Number**		
RPMI 1640 (+)L-Glu (+)HEPES	Gibco	11875-093		
Iscove's Modified Dulbecco's Medium	Gibco	12440-053		
Penicillin-Streptomycin Solution	ThermoFisher	MT30002CI		
Puromycin Dihydrochloride	ThermoFisher	A1113803		
Dulbecco's Phosphate Buffered Saline	Gibco	14190-144		
Fetal bovine serum	Fisher Scientific	501527078		
Retronectin	Takara	T100A		
Compensation beads	Fisher Scientific	3031-5813-28		
7AAD cell viability stain	BD Biosciences	559925		
Human Methocult H4034	STEMCELL Technologies	4034		
OneTaq 2X Master Mix with Standard Buffer	New England Biolabs	M0482S		
Surfen	EMD Millipore Corp.	362330-100MG		

Cytarabine	MedChemExpress	MCEW250109126
Human Recombinant TGF-beta 1, 5 µg	Stem Cell Technologies	78067
COMMERCIAL KITS		
Name	**Vendor**	**Catalog Number**
RNeasy Micro Kit	Qiagen	74004
High-Capacity cDNA Reverse Transcription Kit	Fisher Scientific	4368814
Applied Biosystems TaqMan Universal PCR Master Mix, Kit	Fisher Scientific	4304437
Invitrogen™ Bolt™ Bis-Tris Plus Mini Protein Gels, 4-12%, 1.0 mm, WedgeWell™ format	Invitrogen	NW04125BOX
Invitrogen™ iBlot™ 2 Transfer Stacks, PVDF, mini	Invitrogen	IB24002
FlexiGene DNA Kit	Qiagen	51206
QIAquick Gel Extraction Kit	Qiagen	28704
Cell Proliferation Kit I (MTT)	Roche Diagnostics	11465007001
CellTrace Violet Cell Proliferation Kit	Invitrogen	C34571
QPCR PROBES		
Name	**Vendor**	**Catalog Number**
HS2ST1 TaqMan Probe Hs00202138_m1	ThermoFisher	4351368
HS3ST1 TaqMan Probe Hs01099196_m1	ThermoFisher	4351368
HS6ST1 TaqMan Probe Hs00757137_m1	ThermoFisher	4351368
NDST1 TaqMan Probe Hs00155454_m1	ThermoFisher	4351368
GAPDH TaqMan Probe Hs02786624_m1	ThermoFisher	4351368
CDKN1C TaqMan Probe Hs00175928_m1	ThermoFisher	4351368
LENTIVIRUS		
Control Lenti	Sigma-Aldrich	NEGATIVECONTROL1
HS2ST1 Lenti	Sigma-Aldrich	HSPD0000056420: HS2ST1
HS6ST1 Lenti	Sigma-Aldrich	HSPD0000054591: HS6ST1
PCR PRIMERS		
Name	**Vendor**	**Sequence**
HS2ST1 forward primer	IDT	5’ ACTCTGTGGTCTGTGCTATTCTGC 3’
HS2ST1 reverse primer	IDT	5’ GAGACACAAGACTAAGGTTGGC 3’
HS6ST1 forward primer	IDT	5’ CTACTACATCACCCTGCTACG 3’
HS6ST1 reverse primer	IDT	5’ CTGGCCTCCTTGGATTTATCG 3’

Supplemental Table 2

Analysis	Sample ID	Peripheral Blood Blasts	FLT3 Result	NPM1 Result	DNMT3A Result	Cytogenetics
Glycomics	RO50739	85	Positive	Positive	ND	Normal
	RO50772	91	Positive	Positive	ND	Normal
	RO50898	73	Positive	Positive	Positive	Normal
	RO51092	55	Negative	Negative	Negative	+8; t(9;11)
	RO50640	13	Negative	Negative		t(9;11)

Supplemental Table 2. AML patient characteristics from samples used for glycomics analysis.