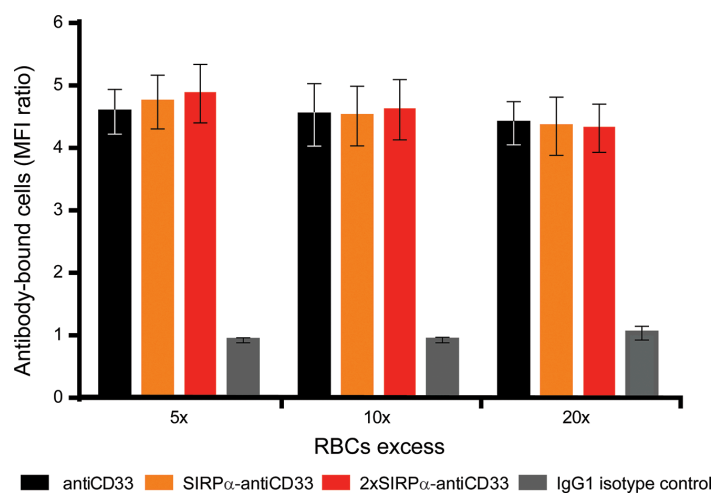
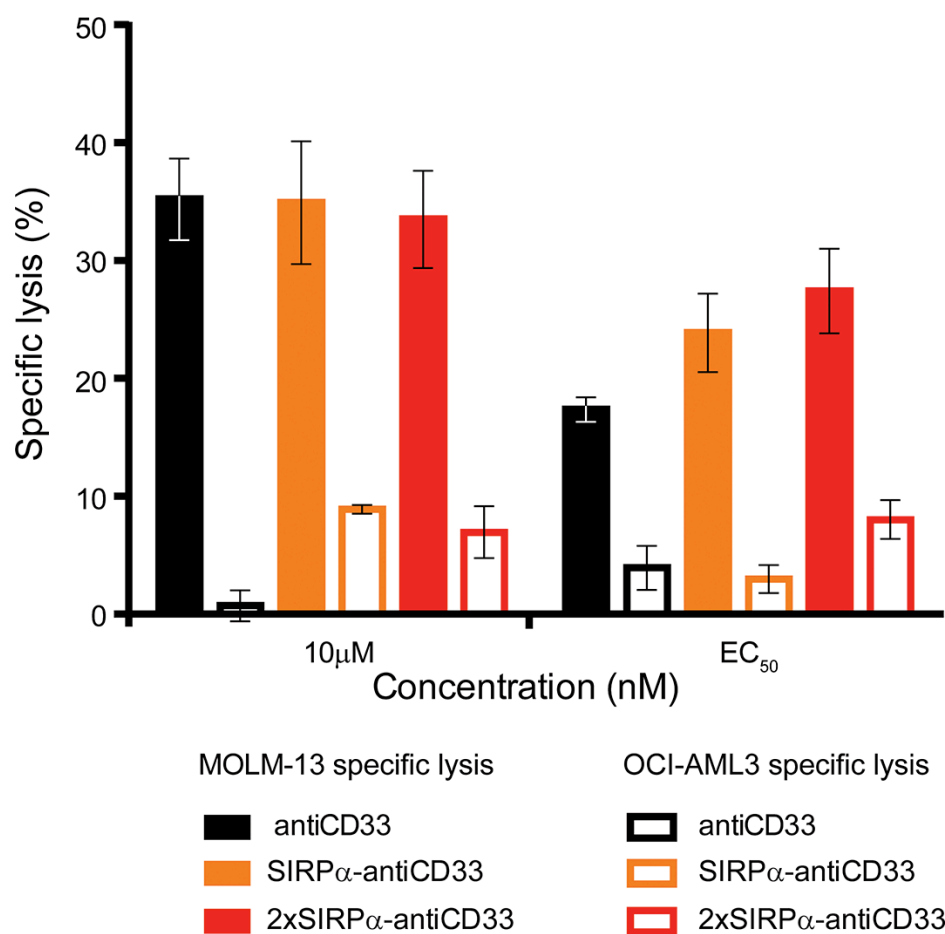


SIRP α -antibody fusion proteins stimulate phagocytosis and promote elimination of acute myeloid leukemia cells

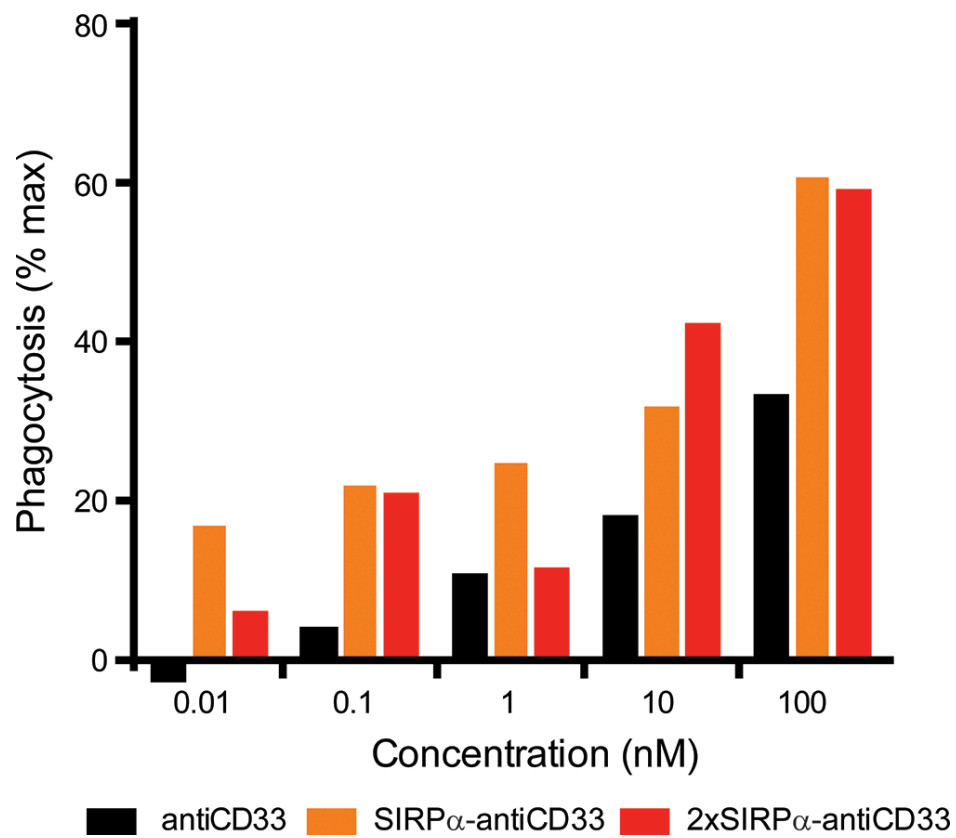
Supplementary Materials



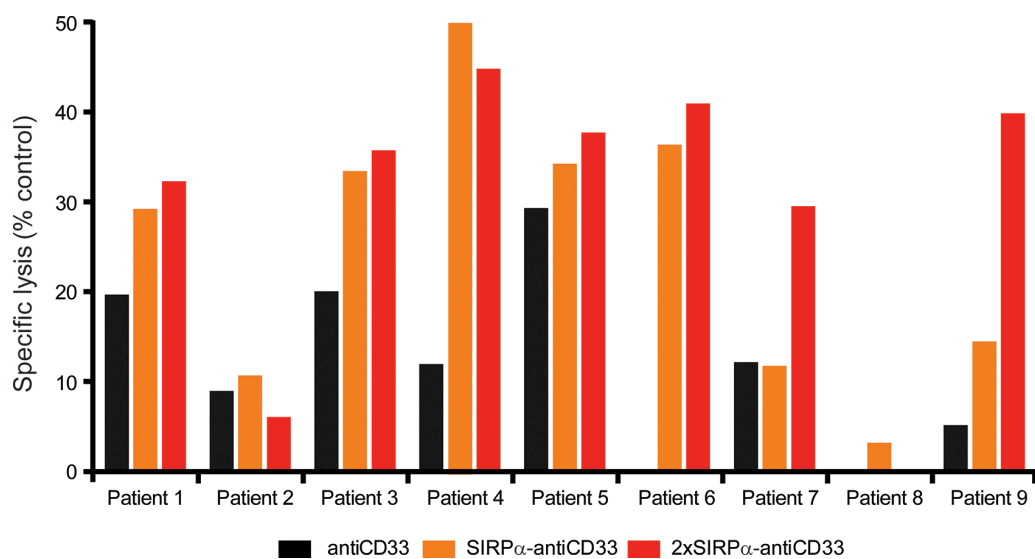
Supplementary Figure S1: LicMAB targeting is dictated by the high binding to CD33. MFI ratio of antibody-bound cells of the MOLM-13 and RBCs mixture was measured for antiCD33 mAb, SIRP α -antiCD33 licMAB, 2xSIRP α -antiCD33 licMAB and human IgG1 isotype control (sample / secondary staining). Error bars indicate SEM of three independent experiments with three different donors.



Supplementary Figure S2: LicMABs preferentially target and induce clearance of cells expressing high levels of CD33. Preferential lysis of MOLM-13 cells respect to OCI-AML3 cells is mediated by antiCD33 mAb, SIRP α -antiCD33 licMAB and 2xSIRP α -antiCD33 licMAB at concentrations of 10 nM and at the EC₅₀. Error bars indicate SEM of triplicates with three different donors.



Supplementary Figure S3: LicMAB molecules stimulate phagocytosis of OCI-AML3 cells. antiCD33 mAb, SIRP α -antiCD33 licMAB and 2xSIRP α -antiCD33 licMAB induced phagocytosis of CD33 and CD47 positive AML-derived OCI-AML3 cell line by donor-derived human macrophages.



Supplementary Figure S4: Cytotoxicity of primary AML patient cells is mediated by licMABs. Individual responses to licMAB-induced NK cell-dependent cytotoxicity (summarized in Figure 7) of the 9 analyzed AML patient samples are depicted.

Supplementary Table S1: AML patient characteristics

PT	Gender	Disease Phase	Material	Blasts (%)	<i>NPM1</i> mut.	<i>FLT3-ITD</i>	Karyotype	ELN genetic group	CD33 MFI ratio	CD47 MFI ratio
1	F	ID	BM	91	+	–	normal	favorable	111.8	47.1
2	F	ID	BM	88	+	+	normal	intermediate I	110.0	76.3
3	M	ID	PB	89	–	+	47, XY, + 8	intermediate II	73.0	95.2
4	M	Relapse	BM	93	–	–	complex	adverse	52.3	34.5
5	M	ID	BM	71	–	–	normal	intermediate I	54.2	58.2
6	M	ID	BM	94	–	–	normal	intermediate I	12.6	75.9
7	W	Relapse	BM	91	–	+	46,XX; t (5,11)	Intermediate II	90.0	27.1
8	M	ID	PB	71	–	–	46,XX; der (16) t (1;16) (q12;q21)	Intermediate II	37.0	31.7
9	W	ID	BM	n.a.	+	+	n.a.	n.a.	n.a.	n.a.

PT, patient; M, male; F, female; ID, initial diagnosis; PB, peripheral blood; BM, bone marrow; mut, mutation; ELN, European Leukemia Net; n.a., not available.