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CD56 targeted in vivo genetic engineering of Natural Killer cells mediates immunotherapy for acute myeloid leukemia

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Supplementary Figures

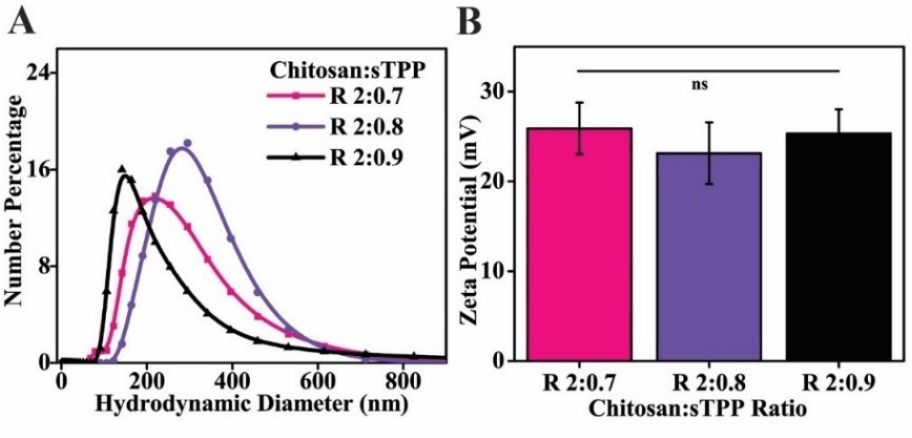

Fig. S1: Optimization of synthesis of Chitosan nanoparticles and characterization with (A) Dynamic Light Scattering and (B) Zeta Potential analysis.

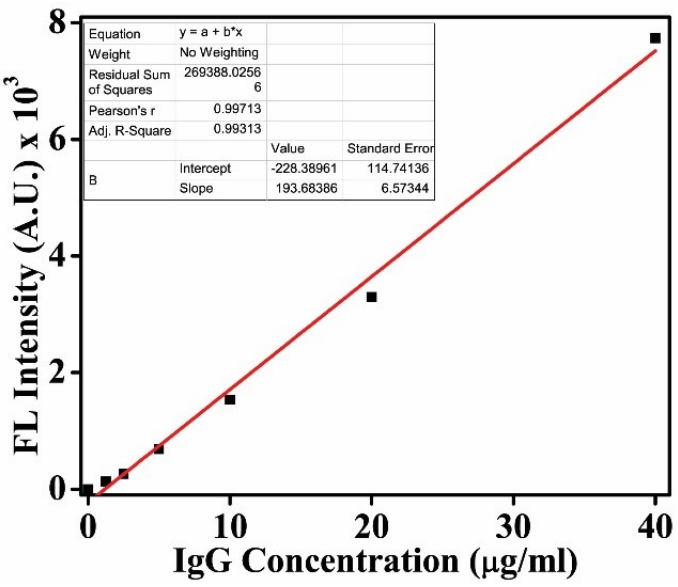

Fig. S2: Standard curve of intrinsic fluorescence of Tryptophan of IgG. All experiments were performed with n=3.

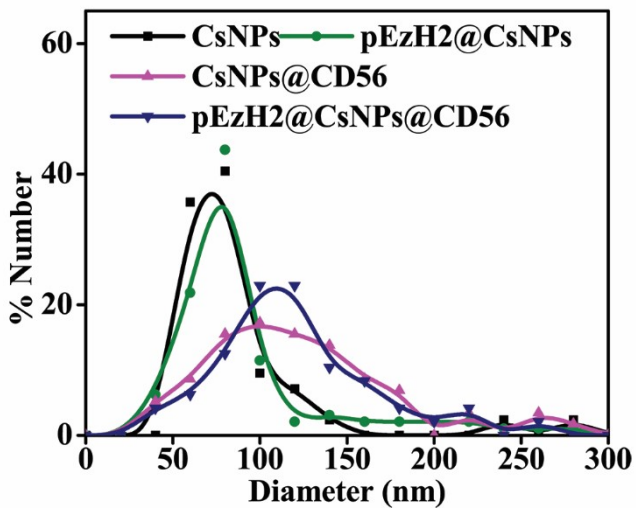

Fig. S3: Size distribution analysis based on TEM imaging of CsNPs, pEzH2@CsNPs, CsNPs@CD56, and pEzH2@CsNPs@CD56.

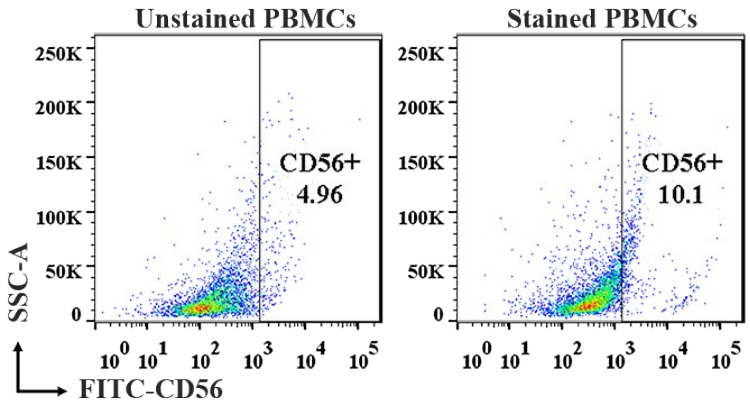

Fig. S4: Flow cytometric Analysis of CD56+NK cells in Peripheral Blood Mononuclear (PBMCs). All experiments were performed with n=3.

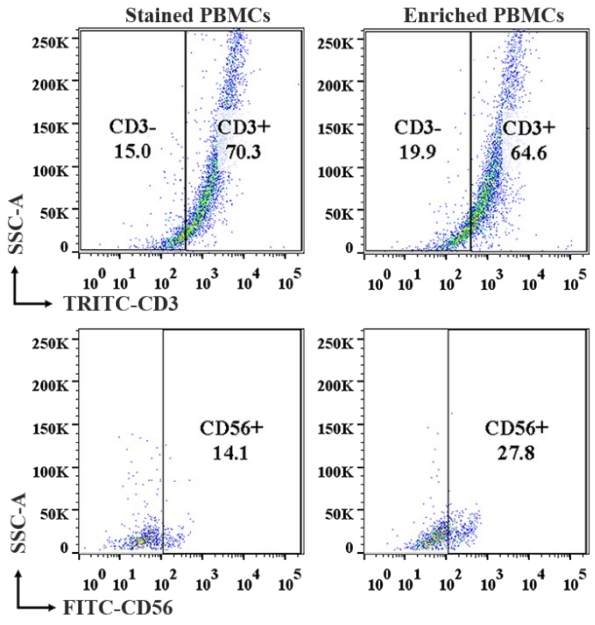

Fig. S5: Flow cytometric analysis of CD56+CD3-NK cells in enriched Peripheral Blood Mononuclear (PBMCs). All experiments were performed with n=3.

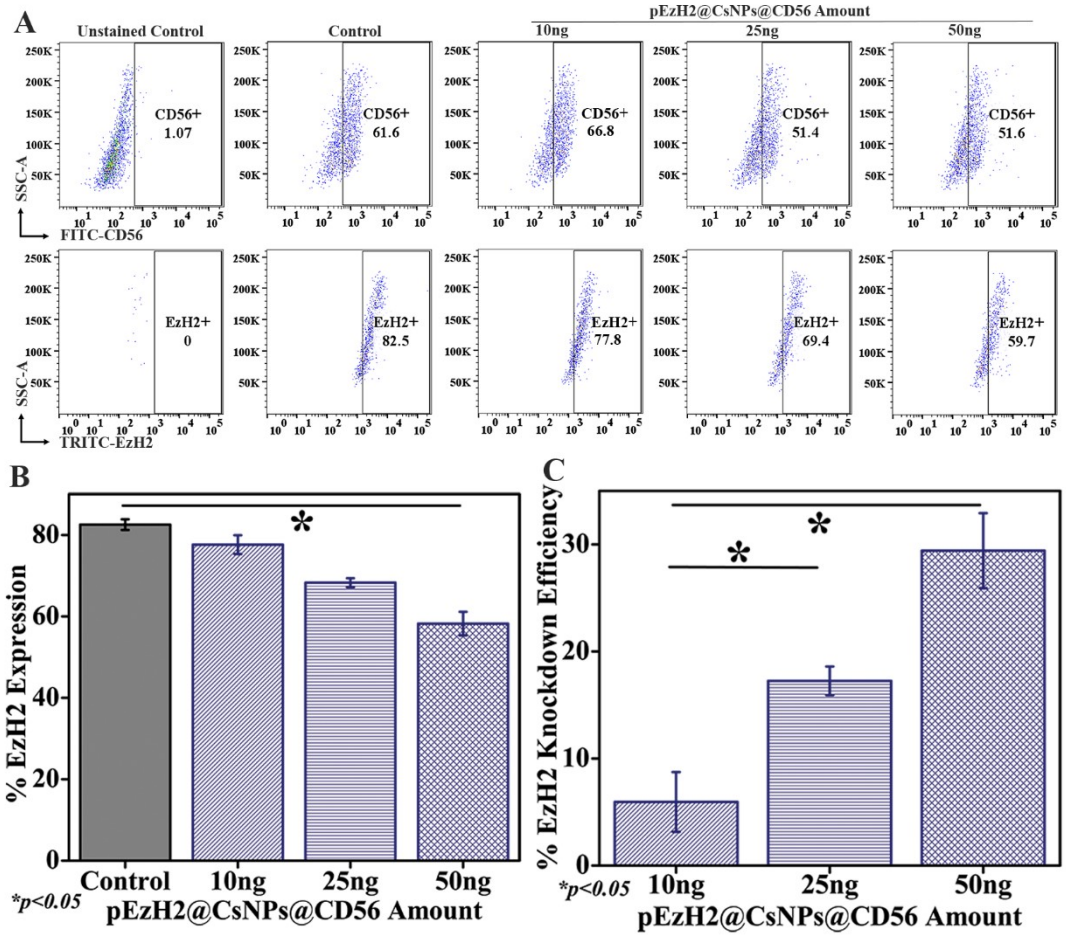

Fig S6: (A) Flow cytometric analysis EzH2 expression in sorted CD56+CD3-NK cells; (B) its quantification; and (C) calculation of percentage EzH2 knockdown efficiency after the treatment of different amounts of pEzH2@CsNPs@CD56 infection in the presence of Puromycin. All experiments were performed with n=3.

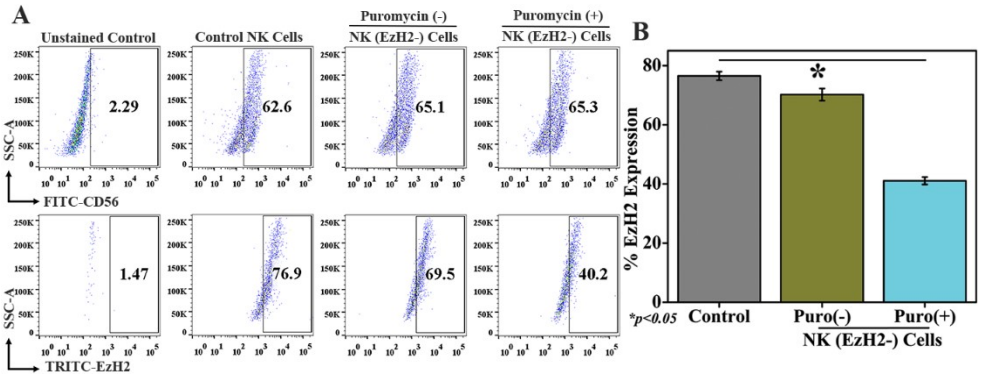

Fig. S7: Preparation of NK (EzH2-) cells; (A) Flow cytometric analysis of EzH2 expression in sorted CD56+CD3-NK cells and (B) its quantification after infection with pSMP-EzH2- viral particle in the absence and presence of Puromycin. All experiments were performed with n=3.

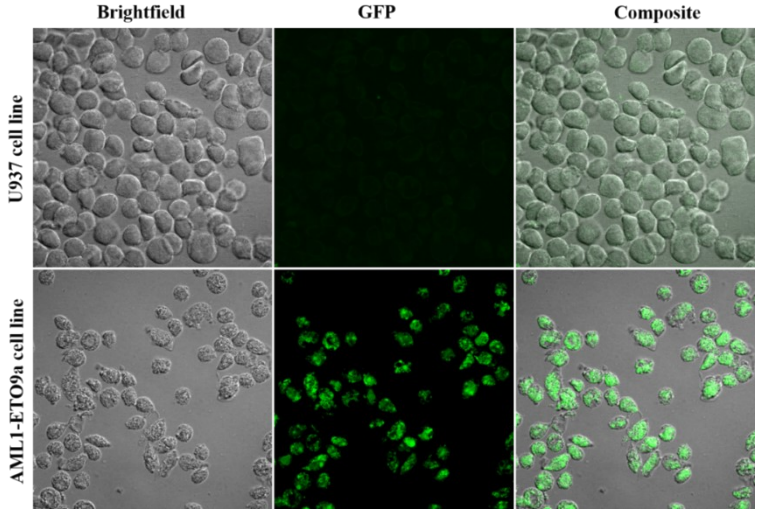

Fig. S8: Green Fluorescent Protein (GFP) expression analysis after infection with AML1-ETO9a viral particles. All experiments were performed with n=3.

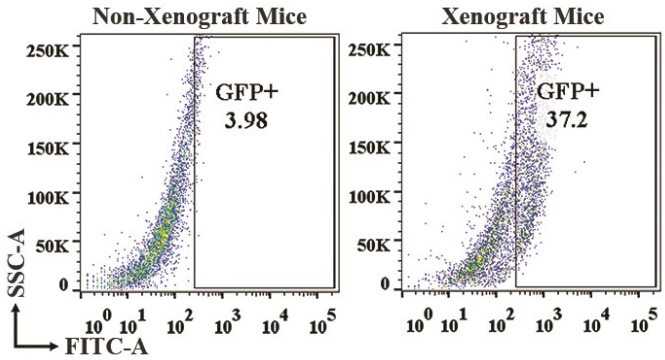

Fig. S9: Validation of Xenograft development; GFP Expression analysis in athymic mice PBMCs after 21 days of AML1-ETO9a cells administration. All experiments were performed with n=3.

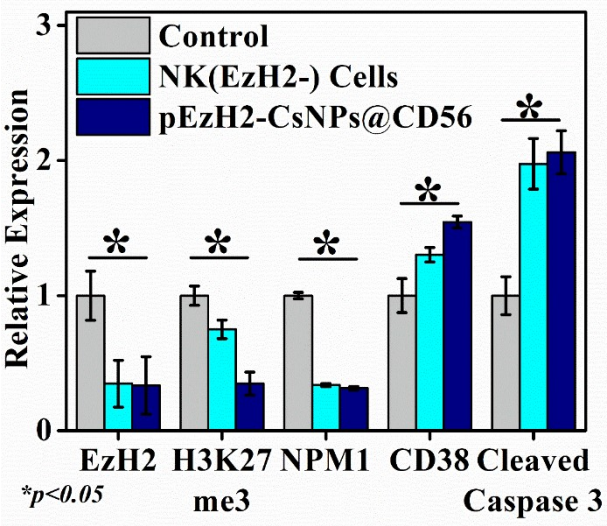

Fig. S10: Quantification of western blot analysis of spleen tissues from NK (EzH2-) cells-treated and pEzH2-CsNPs@CD56-treated xenograft mice.

Supplementary tables

S. No.	Antibody:CsNPs ratio	Percentage antibody conjugation
1	1:100	51.8%
2	1:500	70.9%
3	1:1000	45.1%
4	pEzH2@CsNPs@CD56	70.1%

Supplementary table S1: Percentage of Antibody conjugation with CsNPs as calculated based on intrinsic tryptophan fluorescence of IgG.

S. No.	Primers Name	Sequence (5`-3`)
1	Fragment A_**(-742/-376)**_Forward	TCTTAGGGCGATGTCCTTGC
2	Fragment A_**(-742/-376)**_Reverse	GCTGTGTAAGATATGGCGGG
3	Fragment B_**(-273/+48)**_Forward	TGCGCGTTACGACTGGAAAG
4	Fragment B_**(-273/+48)**_Reverse	AGAACGCTGCTCCAGAGAAC
5	Fragment C_**(+561/+913)**_Forward	AAGCATGGGCCTGCTTGTTG
6	Fragment C_**(+561/+913)**_Reverse	GAGAGCTGCCATCACAGTAC

Supplementary table S2: List of primer for NPM1 promoter region for ChIP-qPCR analysis.