

Supplementary Material

Supplementary Figure Legends

Supplementary Figure 1 Gating strategy for leukemic blast cells

Flow cytometry gating strategy in the identification of acute myeloid leukemia (AML) blasts. The blast gating was primarily based on CD45/SSC (Left); if blasts were uniformly positive for CD34, the CD34 gate would be used for better delineation of the blast population (Middle). For all cases, a comprehensive panel for AML work-up was performed, and blast population was further confirmed for the expression of other myeloid or stem cell markers. In this case, the blasts were also CD117+ and HLADR+.

Supplementary Figure 2 Examples of cytotoxicity of SGN-CD123A on primary AML samples

Cytotoxicity of primary AML samples after the treatment of SGN-CD123A or control ADC for 96 hours. Cell death was determined using flow cytometry analyses of live/dead cells in duplicate. **A.** Patient SG018 are MDR positive. **B.** Patient SG026 carries a FLT-3 mutation and are MDR positive.

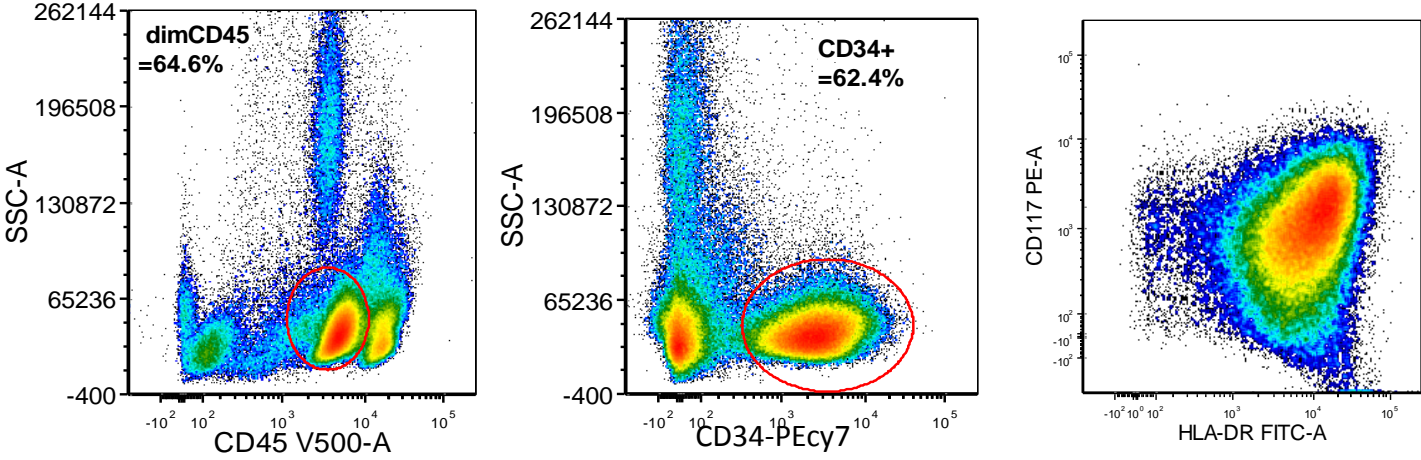
Supplementary Figure 3 Mechanisms of SGN-CD123A induced cell apoptosis

A. Flow cytometry measurement of phosphorylated H2AX in HNT-34 cells. Cells were treated with SGN-CD123A (10 ng/ml) or control ADC at 10 ng/mL. Cisplatin(5 μ M) was used as positive control Assays were run in quadruplicates. MFI ratio was set to compare pH2AX staining with isotype control staining. **B.** pH2AX measurement of KG-1 cells after the treatment of SGN-CD123A, control ADC (10 ng/mL), or cisplatin (5 μ M). **C.** pH2AX measurement of THP-1 cells after the treatment of SGN-CD123A (10 ng/mL), control ADC (10 ng/mL), or cisplatin (5 μ M). **D.**

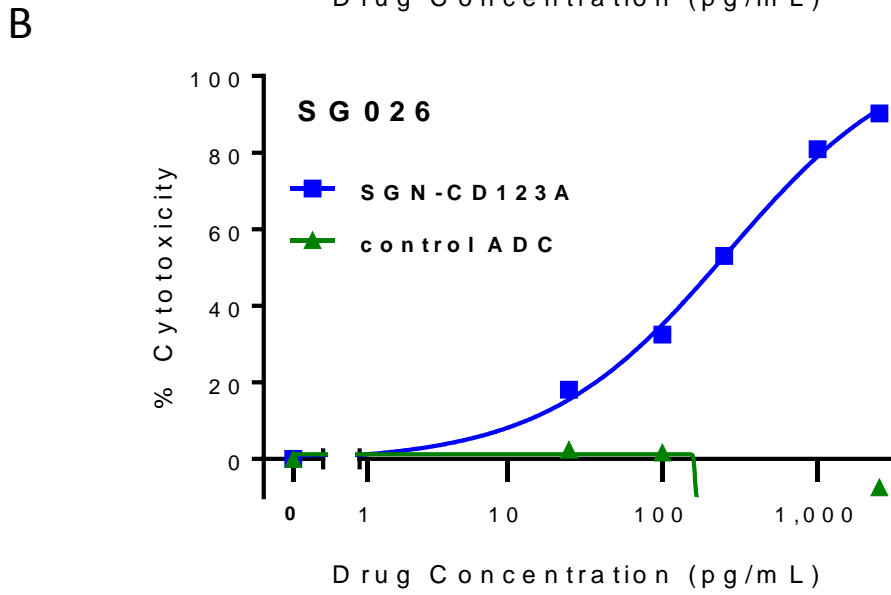
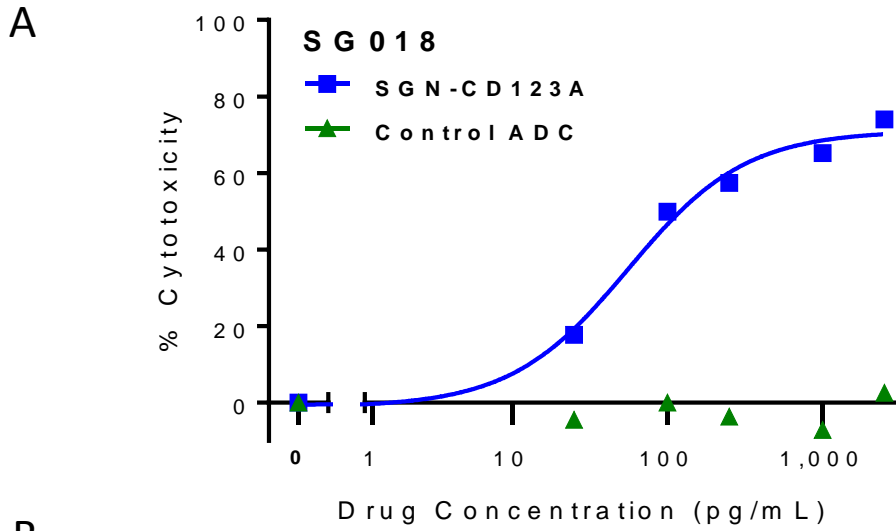
Caspase activity of GDM-1 cells post treatment of SGN-CD123A or control ADC. Cells were treated with ADC for 72 hours and the measurement was performed in duplicate.

Supplementary Figure 4: Sequence of the heavy chain and light chain of h7G3ec antibody

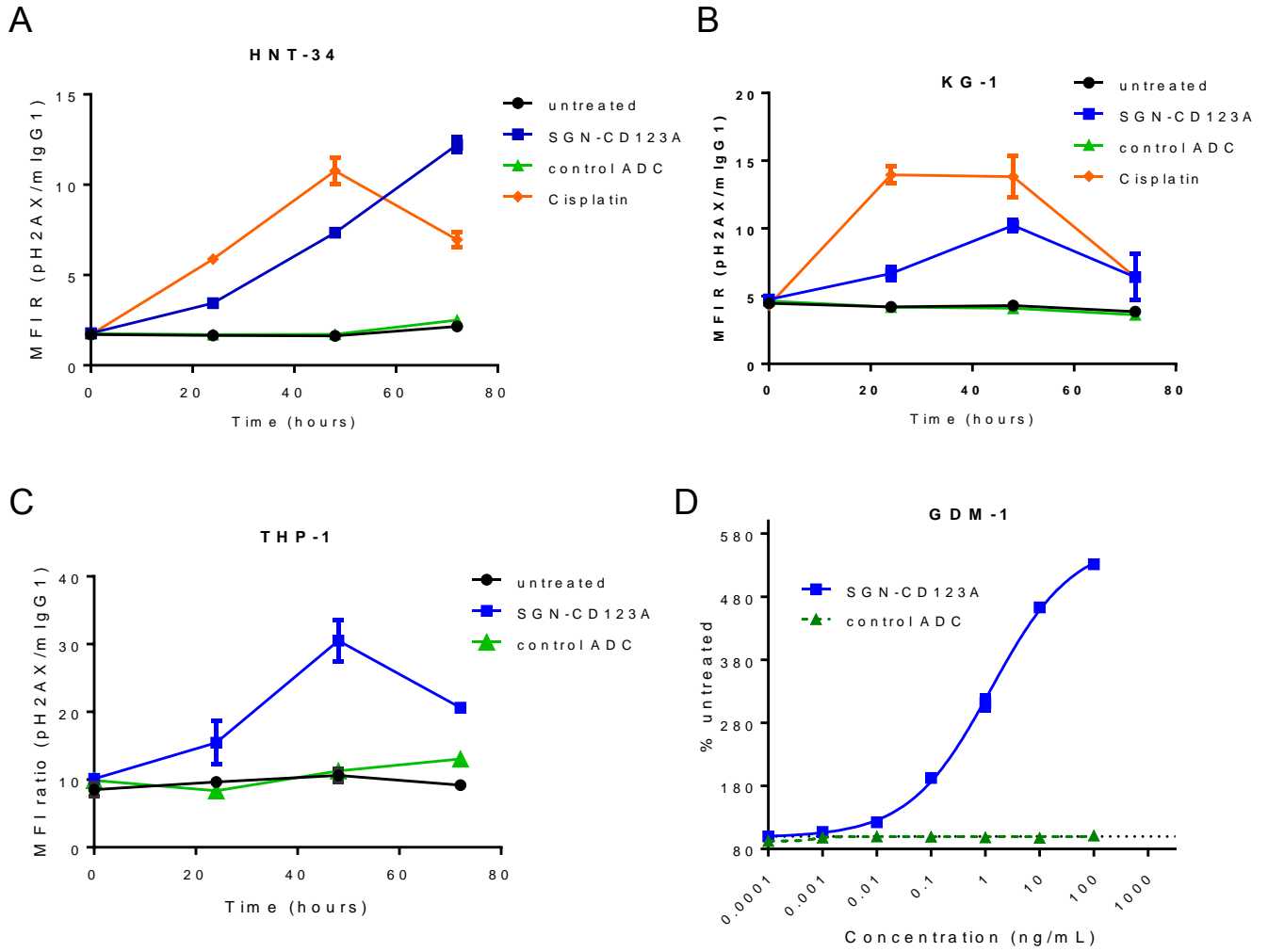
Supplementary Figure 1: Gating strategy for leukemic blast cells



Supplementary Figure 2: Examples of cytotoxicity of SGN-CD123A on primary AML samples



Supplementary Figure 3: SGN-CD123A induced DNA damage responses and apoptosis in AML cell lines



Supplementary Figure 4: Amino acid sequence of h7G3ec

h7G3ec heavy chain

QVQLVQSGAEVKKPGASVKMSCASGYTFTDYYMKWVKQAPGQGLEWIGDIIPSNG
ATFYNQKFKGKATLTVDRSISTAYMHLNRLRSDDTAVYYCTRSHELLRASWFAYWGQG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTC
PPCPAPELLGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLD
SDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

h7G3ec light chain

DFVMTQSPDSLAVSLGERATINCKSSQSLLSNGNQKNYLTWYLQKPGQPPKLLIYWA
STRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDYSYPYTFGQGTKLEIKRT
VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYLSLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC