



Supplemental Figure S3. Binding assessment of the anti-CD123 ADCs. MOLM-14 cells were incubated with a range of mAb and ADC concentrations from 0.1 nM to 300 nM, and binding activity was detected with a phycoerythrin-conjugated goat anti-mouse IgG Fc via flow cytometry. Data analysis was carried out using FlowJo software, and the calculated mean fluorescent intensity was fitted with a specific binding function with Hill slope in GraphPad Prism software. Each data point represents the mean of triplicate samples with standard deviation error bar. Both 7E7 and 11C3 ADCs demonstrated comparable binding affinity to their parent mAbs. (A) Estimated binding IC₅₀ of 7E7 mAb and ADC to MOLM-14 cells are 2.26 nM and 2.63 nM, respectively. (B) Estimated binding IC₅₀ of 11C3 mAb and ADC to MOLM-14 cells are 2.39 nM and 6.01 nM, respectively.