Combinatorial Targeting of Multiple Myeloma by complementing T cell Engaging Antibody Fragments

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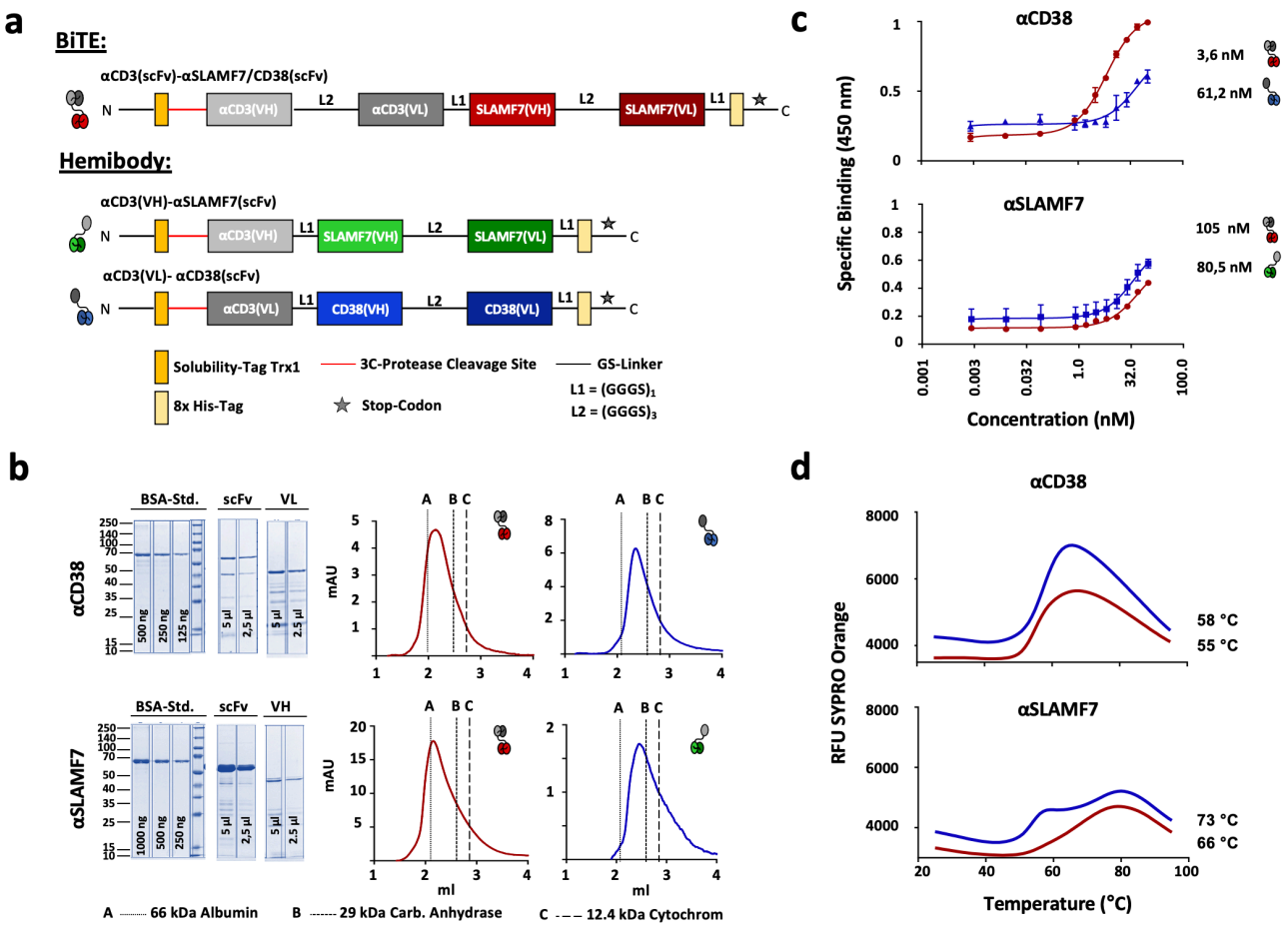
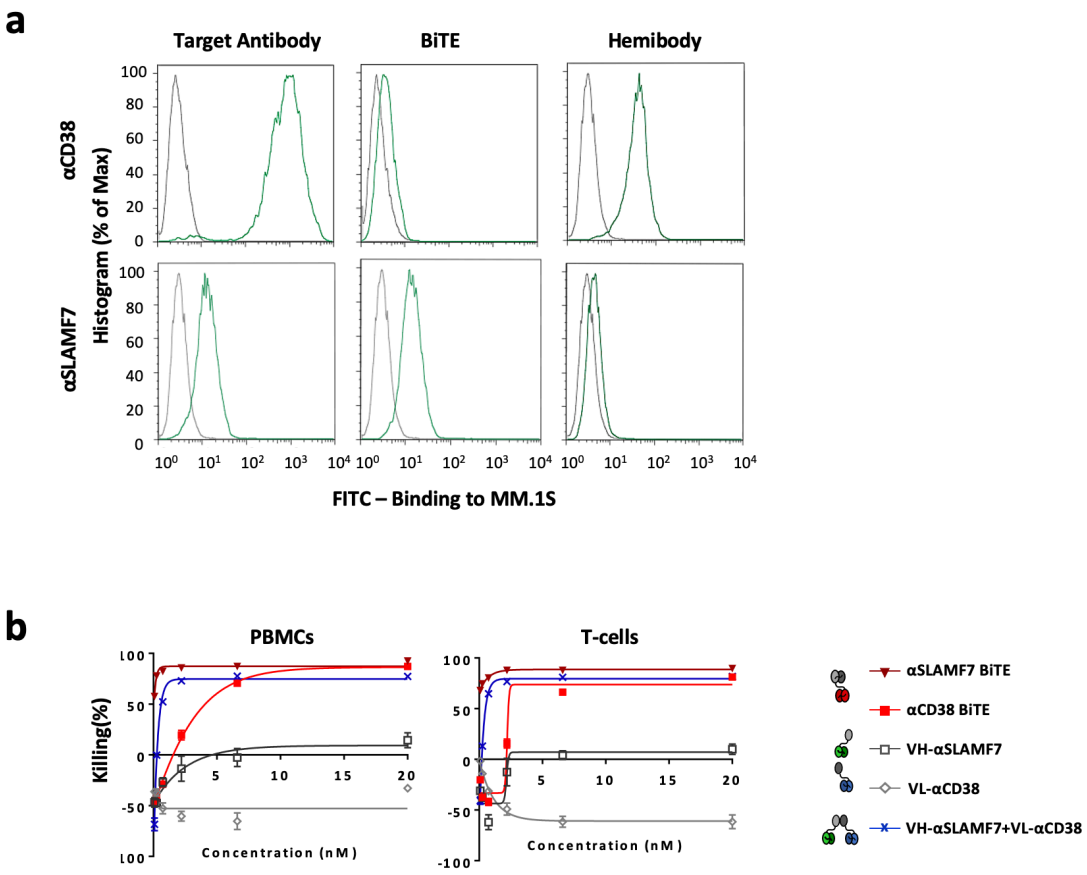
Supplementary Information:

Supplement Figure 1: Construction and biochemical characteristics of hemibodies.



(a) Schematic construction of BiTEs and hemibodies targeting CD38 and SLAMF7. **(b)** Antibodies were isolated by immobilized metal ion affinity chromatography and size-exclusion chromatography. Production yield, purity and monomeric content were analysed by FPLC using 50 ul of size-exclusion eluate and by SDS-PAGE under reducing conditions followed by Coomassie staining in concentrations as indicated (n>3, single experiment is shown). **(c)** Hemibodies were incubated with 10 000 CD38- or SLAMF7-positive CHO cells for 2 h as indicated and the specific antibody binding was detected using ELISA techniques (n=3, single experiment is shown). **(d)** SYPRO Orange based heat stability curve of used antibody constructs from 25-95 °C with a stepwise increase of the temperature of 1 °C/min (n=2-3, single experiment is shown).

Supplement Figure 2: Functional analysis of hemibodies.



(a) Binding profile of 500 ng hemibody and BiTE constructs to their specific target on MM.1S cells compared to conventional IgG antibodies by flow cytometry (n=3, single experiment is shown). **(b)** Luciferase-positive MM.1S cells were incubated with PBMCs or purified T-cells and different antibody concentrations for 24 h. The T-cell mediated lysis was detected by a luciferase-based cytotoxicity assay (n=3, single experiment is shown).