Supporting information - Supplementary Figures and Tables

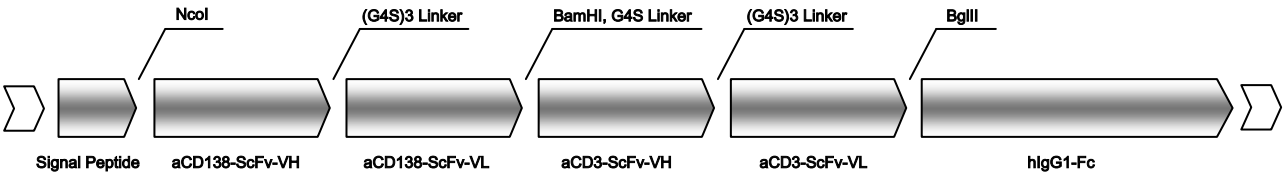

Fig. S1. Design of BiTE-hIgFc molecule construction.

From the 5' end to the 3' end (left to right), the structure of pFUSE-BiTE-hIgFc (STL001) includes, in order, a signal peptide that leads to cellular extra secretion of the expressed protein, a NcoI cloning site, aCD138-ScFv-VH, a (G4S)3 linker for easy assembly of the variable regions of heavy and light chains (VH and VL), aCD138-ScFv-VL, a BamHI cloning site, a G4S Linker, aCD3-ScFv-VH, a (G4S)3 Linker, aCD3-ScFv-VL, a BglII cloning site and hIgG1-Fc.

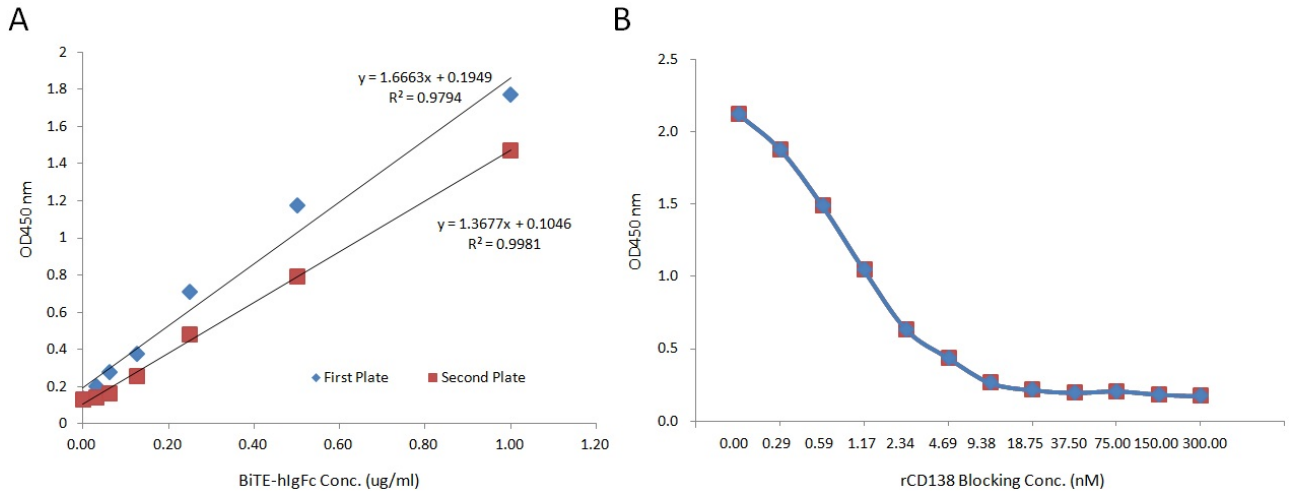

Fig. S2. The competitive ELISA analysis of rCD138 and BiTE-hIgFc (STL001). In both ELISA plates, the rCD138 coating protein concentration was 0.1 μg/mL. (**A**) After incubation with 0.00, 0.03, 0.06, 0.13, 0.25, 0.50 and 1.00 μg/mL antibody, the primary antibody solution in the first 96-well ELISA plate (blue line) was pipetted into the second ELISA plate (red line). R2 shows the fit of linear regression for both plates. (**B**) A gradual blocking concentration of rCD138 solution (0.00-300.00 nM) and 1.00 μg/mL primary BiTE-hIgFc (STL001) was used to determine the blocking ability of rCD138 against BiTE-hIgFc (STL001). Three repeated measures per group were performed in the competitive ELISA.

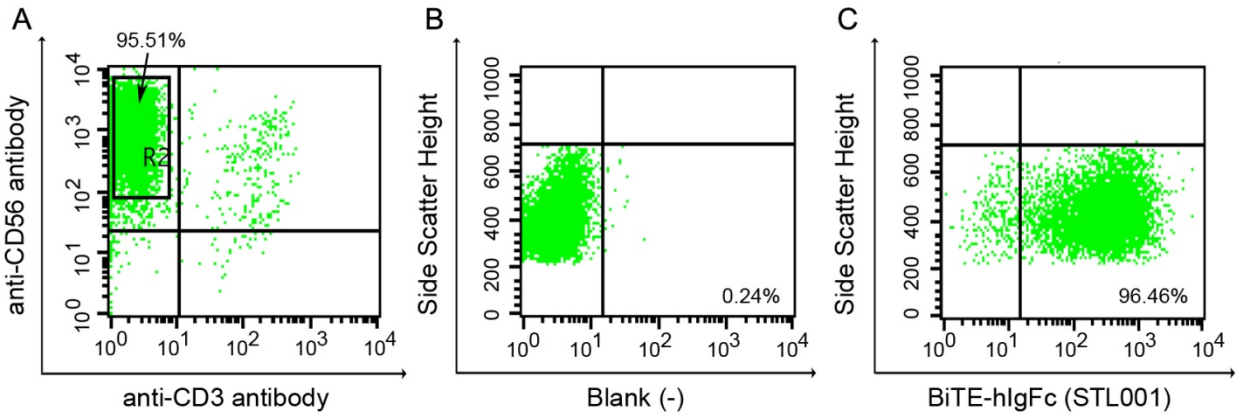

Fig. S3. Specific binding of NK cells via Fc gamma receptor. After 2 weeks of PBMC stimulation by irradiated K562 cell lines transfected with membrane-bound IL-21, IL-15, CD137L and CD86 molecules, and cell culture with additional IL-2, enriched NK cells accounted for 95.51% (**A**) of the total cell populations. Compared with the negative control (**B**), the binding rate of NK cells by BiTE-hIgFc via Fc was more than 96% (**C**) of the total NK cells gated by CD3(-) and CD56(+) (R2, **A**). Staining with PBS solutions was set as the negative control (**B**).

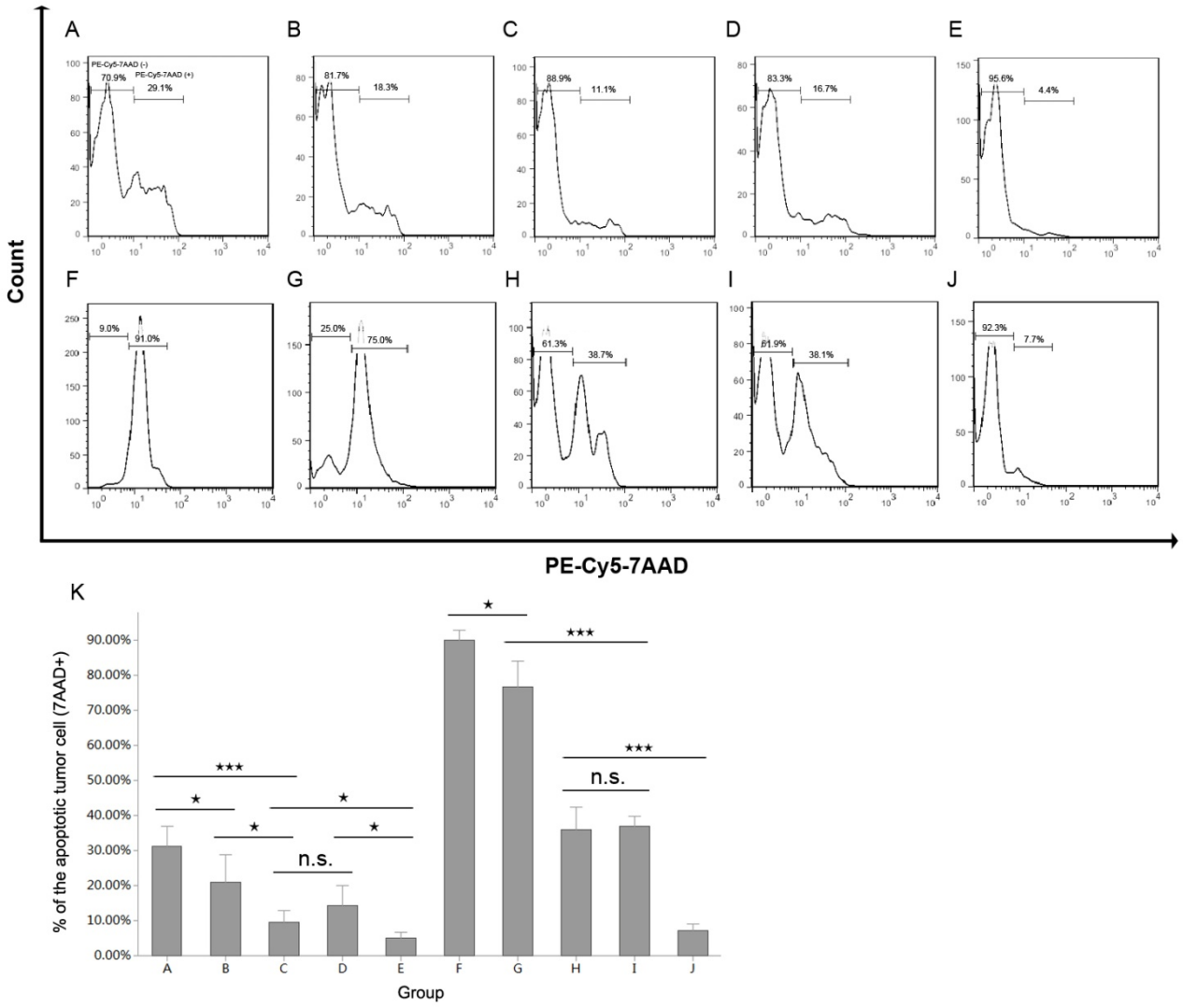

Fig. S4. Anti-tumor effect analysis of anti-CD138-ScFv-hIgFc and BiTE-hIgFc (STL001). RPMI-8226 cells were stained with 10 μM CFSE solution at 37°C for 15 min. 1.4×10^6 PBMC effector cells and 2.0×10^5 tumor cells (E:T ratio = 7:1) were mixed in a 96-well microtitre plate in a total volume of 200 μL. Anti-CD28 mAb (100 ng/mL) and IL-2 (10 U/mL) were also added to each group. After 24 h (A–E) or 48 h (F–J) incubation of PBMCs and MM cells with the different antibodies, BiTE-hIgFc (STL001) (A, 29.1%; F, 91.0%), or the mixture of aCD138-ScFv-hIgFc and aCD3-ScFv-hIgFc (B, 18.3%; G, 75.0%) showed stronger cell lysis of RPMI-8226 than that of single aCD138-ScFv-hIgFc (C, 11.1%; H, 38.7%) or isotype control groups (D, 16.7%; I, 38.1%). Moreover, the groups without PBMCs (E, 4.4%; J, 7.7%) showed much lower cytotoxicity (E; J) though the presence of BiTE-hIgFc (STL001). Before cytotoxicity analysis by flow cytometry, the RPMI-8226 cells were stained with PE-Cy5-labeled-7-AAD reagent. Data shown in K are means ± standard error of the mean (SEM). *P < 0.05, **P < 0.01, ***P < 0.005 by Student's t-test. And n.s. means non-significant.

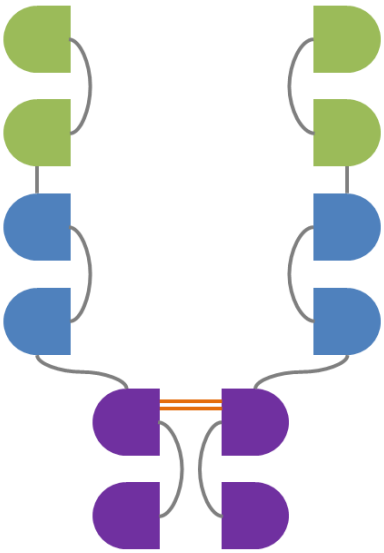

Fig. S5. Backbone of the BiTE-hIgFc (STL001) molecule. The molecule presents a 'Y' shape, similar to the traditional full IgG antibody. In this study, green, blue, and purple fragments represent aCD138-ScFv, aCD3-ScFv, and hIgG1 Fc, which target MM tumor cells, T cells, and NK cells, respectively. The orange lines indicate two disulfide-bonds between the hinge regions of the Fc homodimer.

Table S1. Staining percentages of different antibodies targeting RPMI-8226, U266 and Jurkat cells and PBMCs.

Group	Antibody used for cell staining	The staining percentage of different cell lines (%)			
		RPMI-8226	U266	Jurkat	PBMCs
1	BiTE-hIgFc (STL001)	A, 97.91 ★★★★★	B, 94.55 ★★★★★	C, 75.65 ★★★★	D, 72.02 ★★★★
2	aCD138-ScFv-hIgFc	E, 97.95 ★★★★★	F, 96.08 ★★★★★	G, 0.31 ☆	H, 15.95 ★
3	aCD3-ScFv-hIgFc	I, 1.13 ☆	J, 3.83 ☆	K, 73.00 ★★★★	L, 69.34 ★★★★

The BiTE-hIgFc (STL001) and aCD138-ScFv-hIgFc antibodies showed similar cell staining patterns (five stars) on MM cell lines RPMI-8226 and U266. On the other hand, BiTE-hIgFc (STL001) and aCD3-ScFv-hIgFc antibodies achieved the same pattern (four stars) on Jurkat cells and PBMCs. Due to the bispecific characteristic of BiTE-hIgFc (STL001), targeting hCD138 and hCD3, the antibody stained all four cell lines strongly. However, aCD138-ScFv-hIgFc specifically stained the MM cell lines RPMI-8226 and U266 cells only, and aCD3-ScFv-hIgFc specifically stained CD3+ Jurkat cells and PBMCs only. Five black stars or one hollow star indicates a strong or negative cell staining, respectively.