

## Supplemental file

### Preclinical evaluation of CD8<sup>+</sup> anti-BCMA mRNA CAR T-cells for treatment of multiple myeloma

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**Running title:** CD8<sup>+</sup> anti-BCMA mRNA CAR T-cells to control myeloma

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## **Supplemental Methods:**

### **Cell lines and primary MM patient cells**

All MM cell lines were obtained from American Type Culture Collection (ATCC), and all express BCMA on the cell membrane. Lenalidomide- and pomalidomide-resistant MM cell lines H929(R) and MM1S(R) were derived from H929 and MM1S cell lines, respectively. They were generated by culturing these cells in media containing gradually increasing concentrations of lenalidomide and pomalidomide [1]. All cell lines were grown in RPMI-1640 (Invitrogen, Carlsbad, CA) with 10% FBS (Hyclone, Logan, UT), 100 U/ml penicillin, and 100 µg/ml streptomycin (Invitrogen). All cell lines were used between passage 4-10. They are routinely checked by Human short tandem repeat (STR) DNA Profiling Cell Authentication for their authenticity (Molecular Diagnostic Laboratory, DFCI) and for mycoplasma contamination by PCR and the MycoSEQ Mycoplasma detection assay kit (ThermoFisher Scientific).

MM patient samples were obtained with IRB approval. Written informed consent was obtained in all cases according to the Declaration of Helsinki. Peripheral blood (PB) and bone marrow (BM) aspirate samples from patients were collected in heparinized tubes. Patients met the International Myeloma Working Group (IMWG) Criteria for the diagnosis of MM. PB and BM mononuclear cells (PBMC and BMMCs) were isolated from PB and BM, respectively, via density gradient centrifugation using Ficoll-Hypaque (GE Healthcare). Primary CD138+ cells were purified via positive selection from BM mononuclear cells (BMMCs) with anti-CD138 microbeads (Miltenyi Biotec). Residual CD138-negative cells were cultured in RPMI-1640 with 10% FBS to generate BM stromal cells (BMSC) [1-4].

Cells (BM mononuclear cells (BMMCs) or CD138+ cells from MM patients, MM cell lines were co-incubated in 96-well plates (100,000 cells/well) with Descartes-08 or control CD8+ cells. Co-cultures were maintained in a final volume of 100  $\mu$ l culture media (5% Human Serum in LGM-3 with 2ng/ml IL-2) at 37°C and 5% CO<sub>2</sub>. For extended incubation, 100  $\mu$ l of prewarmed whole culture media was added on day 4.

### **Flow cytometric (FCM) analysis**

The analysis of CAR status, degranulation of effector cells, cytotoxic effect, and cytokine expression were performed by flow cytometry. The majority of patient samples were evaluated using real-time multicolor flow cytometry, within 8 hours after arrived at our lab. Human Fc block reagent (BD Biosciences) was used to prevent nonspecific antibody binding. Human Fc block reagent (BD Biosciences) was used to prevent nonspecific antibody binding. The LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Invitrogen) was routinely used to determine the viability of cells and in conjunction with PE-Annexin V (BioLegend) to determine if cells were apoptotic. All FCM analyses were performed using BD FACSCanto II flow cell analyzer with FACSDiva Version 5.0 acquisition/analysis software (BD Biosciences). All data were analyzed using FlowJo Version 8.6.6 (TreeStar Inc).

Monoclonal antibodies (MoAbs) were obtained from Biolegend (San Diego, CA): anti-CD38 (PB), anti-CD138 (FITC, MI15), PE Streptavidin, Anti-CD107a (FITC, H4A3), anti-CD8 (APC, SK1), anti-IFN $\gamma$  (FITC, 4S.B3), anti-IL-2 (APC, MQ1-17H12) and anti-TNF- $\alpha$  (PE, MAb11). Anti-CD8 (APC/Cy7, SK1) and anti-CD3 (APC/Cy7) MoAbs were obtained from BD Biosciences.

### **Degranulation assay (CD107a mobilization)**

Descartes-08 or the paired control CD8<sup>+</sup> T cells were incubated in 96-well plates (100,000 cells/well), together with MM cell lines (H929, U266, MM1S, MM1R), bone marrow mononuclear cells (BMMC) or purified CD138<sup>+</sup> cells from MM patients. Co-cultures were maintained in a final volume of 100µl culture media (5% Human Serum in LGM-3 with 2 ng/ml IL-2) for 6h at 37°C and 5% CO<sub>2</sub> with Protein Transport Inhibitors (BFA and Monensin). CD107a staining was done during cell stimulation by the addition of a fluorescent anti-CD107a antibody during the last hour of the co-culture, as described previously.[4, 5] The degranulation activity was evaluated by flow cytometry analysis and determined as the percentage of CD107a<sup>+</sup> CD8<sup>+</sup> cells.

### **Cytokine expression analysis**

For intracellular cytokine staining, Protein Transport Inhibitors (BFA and Monensin) were first added immediately after effector cells and MM cells were co-cultured. After 6h of incubation, the cells were permeabilized and fixed by Cytofix/Cytoperm kit (BD), according to manufacturer's instruction. Then the cells were stained with anti- IFN- $\gamma$ , anti-IL-2, and anti-TNF- $\alpha$  antibodies. The analysis was performed by flow cytometry.

### **References**

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Supplemental Figures

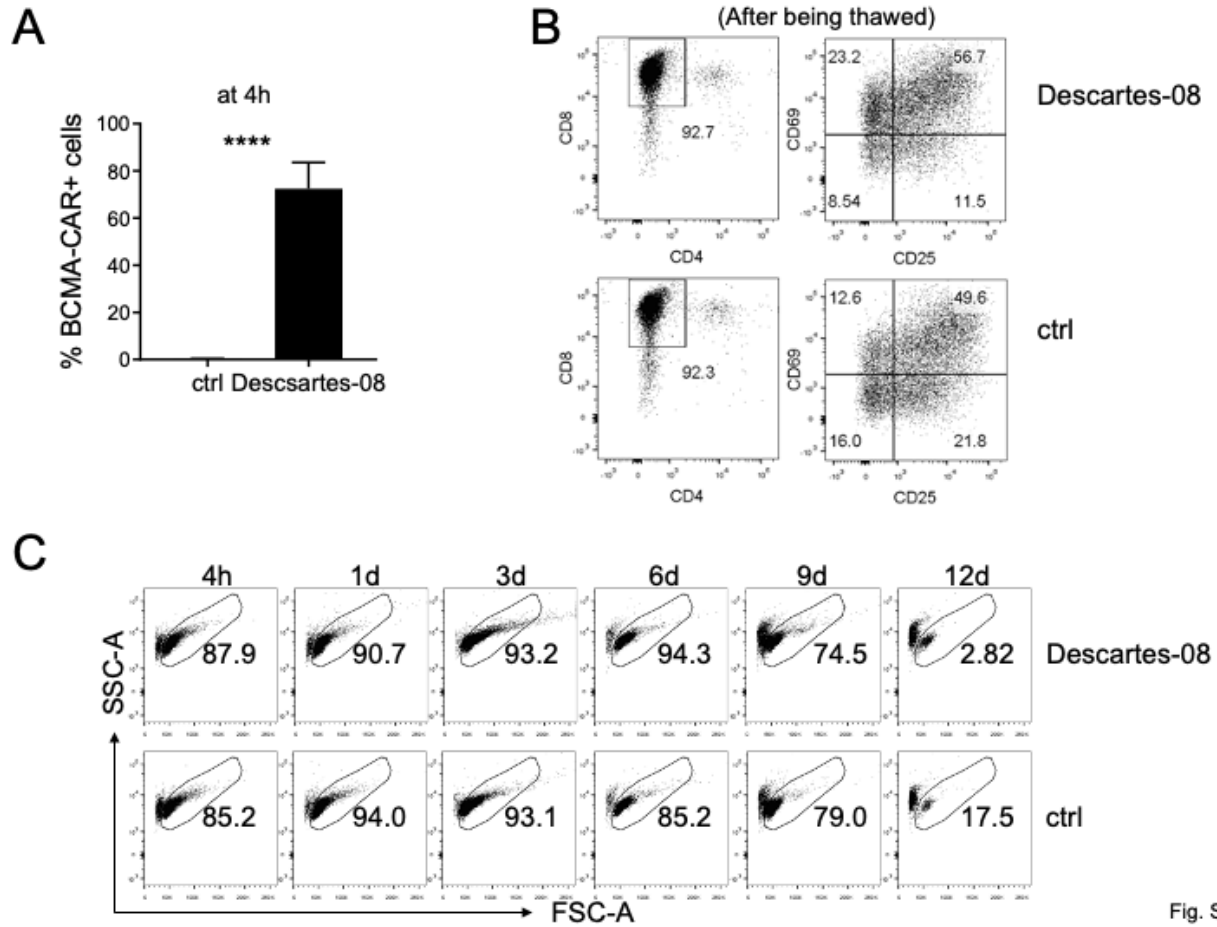
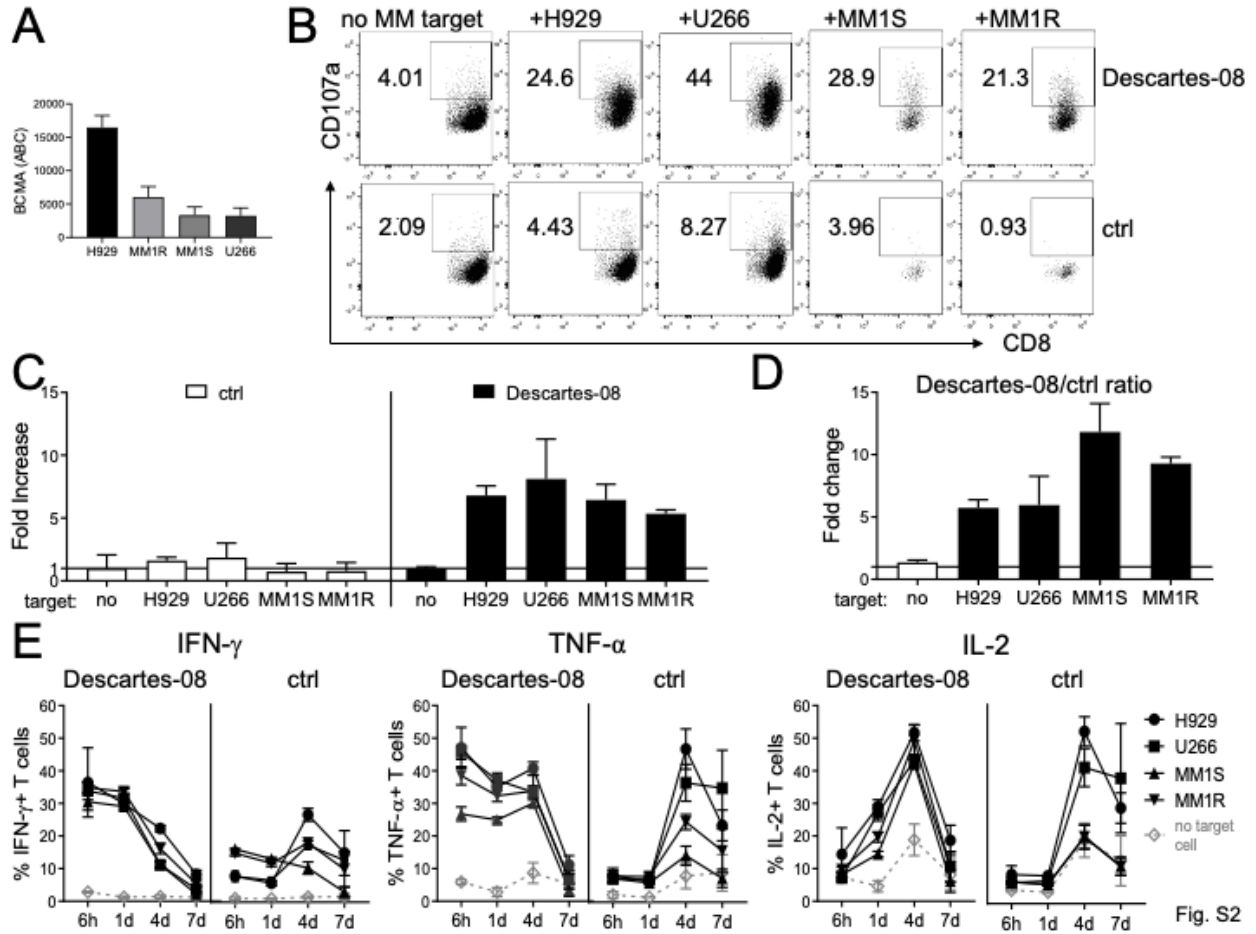


Fig. S1

**Fig. S1 Time course studies of Descartes-08 cell viability *in vitro* after being thawed.**

(A) Summarized BCMA CAR expression data (n = 3) as means  $\pm$  standard deviations (SDs) (n = 3 donor CD8<sup>+</sup> T) at 4h after transfection with mRNA encoding BCMA CAR (**Fig. 1b**). \*\*\*\* $P < .0001$  (B) FCM analysis were done to confirm the viable human CD8 positive percentage (>90%) and activation status (CD25/CD69) of Descartes-08 and the paired control (ctrl) CD8<sup>+</sup> T cells after being thawed. (C) Using FCM analysis, cell viability after being thawed was evaluated by FSC-A and SSC-A at indicated time periods (4 hours to 12 days, 4h to 12d).



**Fig. S2 Descartes-08 cells induced potent and selective cytolytic activity against MM cell lines in a time-dependent manner.**

(A-C) Descartes-08 and the paired control (ctrl) CD8<sup>+</sup> T cells (n = 3) were co-cultured for 6h with indicated target MM cell lines with a range of BCMA expression [1] (A, ABC, antibody binding capacity) (E:T = 1:1). Shown are representative dot plot data of % CD107a<sup>+</sup> CD8<sup>+</sup> T cells (B, Descartes-08 (upper panel) and the paired ctrl T (lower panel)) and fold increases in % CD107a<sup>+</sup> CD8 T cells (n = 3) co-cultured with indicated MM cells vs no target cells (C, Descartes-08 (right) and the paired ctrl T (left)). (D) Fold changes (increases) of % CD107a<sup>+</sup> cells in Descartes-08 vs ctrl T cells. (E) Changes in expression of indicated cytokines in Descartes-08 vs the paired ctrl



CD8<sup>+</sup> T cells (n = 4) in the co-cultures (E:T = 1:10) at indicated time periods (**Fig. 2b**). All data are presented as means  $\pm$  SDs (error bars) from > 3 independent experiments with triplicates for each condition.

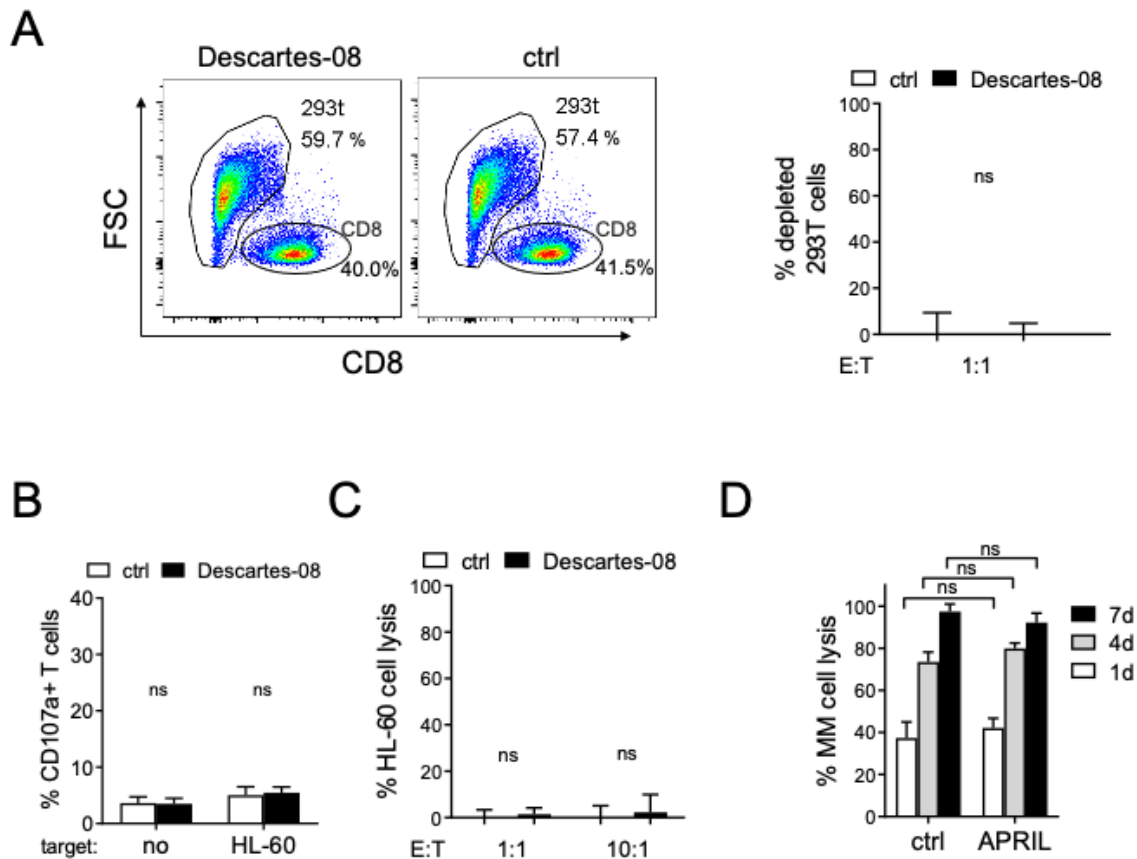


Fig. S3

**Fig. S3 No significant degranulation and cytolytic function were seen in Descartes-08 vs control T cells co-cultured with BCMA-negative cells.**

(A) The cytolytic ability of Descartes-08 vs the paired control (ctrl) CD8 T cells ( $n = 3$ ) was evaluated 1d after co-culture with BCMA-negative 293t cells (E:T = 1:1). Shown are the representative FCM dot plots (left) and summarized results of % depleted 293t cells (means  $\pm$  SDs) (right). (B-C) Descartes-08 or the paired ctrl CD8+ T cells ( $n = 4$ ) were co-cultured with or without BCMA-negative HL-60 cells (B, E:T = 1:1). The percentages of CD107a+ (B) and the cytolytic activity (C) of T cells were determined at 6h (B) and 1d (C), respectively. (D) Descartes-08 cells ( $n = 4$ ) were co-cultured with H929 target cells (E:T = 1:1) in the presence or absence of APRIL

(100 ng/ml). The percentages of H929 MM cell lysis in the co-cultures was determined at indicated time points. All data are presented as means  $\pm$  SDs. ns, not significant

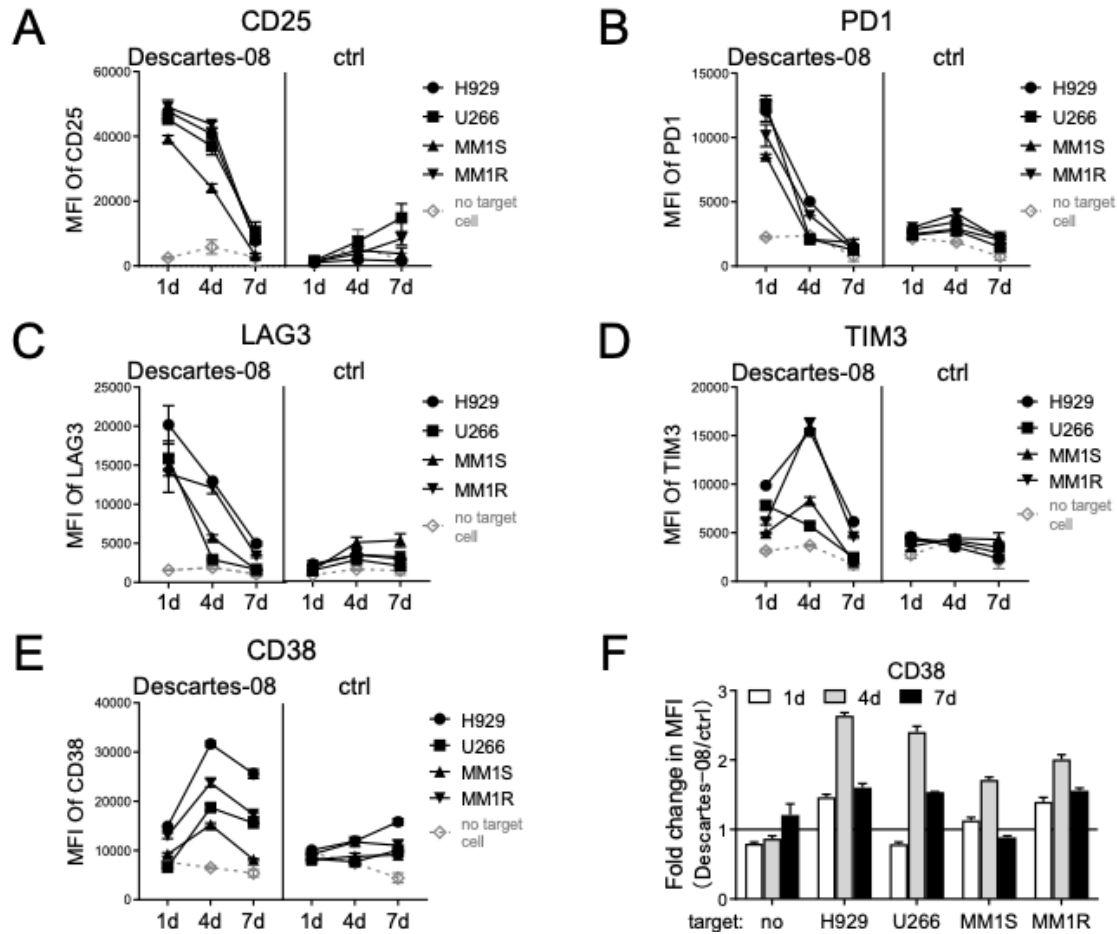


Fig. S4

**Fig. S4 Time course studies on expression of activation and checkpoint proteins in Descartes-08 vs control T cells in co-cultures**

(A-E) Descartes-08 and the paired control (ctrl) CD8+ T cells (n = 3) were co-cultured with various target MM cells (solid black symbols) or no target cells (open gray diamond) (E:T = 1:10) (Fig. 3). Shown are values of median fluorescence intensity (MFI) of CD25 (A), PD1 (B), LAG3 (C), TIM3 (D) and CD38 (E) on Descartes-08 (left) vs the paired ctrl T (right) with or without target MM cells at indicated time periods. (F) Fold changes of MFI values for CD38 in Descartes-08 vs the paired ctrl CD8+ T cells at the same indicated time periods. Three independent experiments

were done in triplicates at each condition using T cells from 3 individuals. All data were expressed as means  $\pm$  SDs.

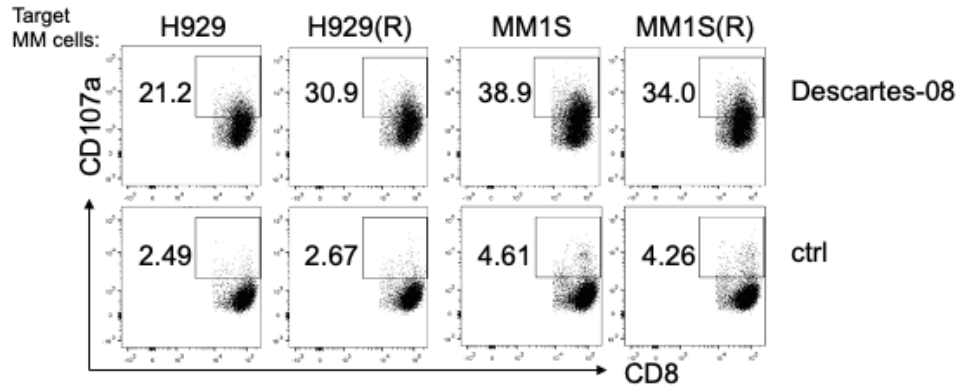


Fig. S5

**Fig. S5. Robust induction of degranulation in Descartes-08 cells following co-culture with IMiDs-resistant MM cells.**

Descartes-08 (upper panel) or the paired control CD8+ (ctrl) (lower panel) T cells were co-cultured for 6h with paired IMiDs (lenalidomide and pomalidomide)-sensitive (H929 and MM1S) and -resistant (H929(R) and MM1S(R)) MM cell lines (E:T = 1:1). Shown are representative FCM dot plot data of % CD107a+ T cells for each group.

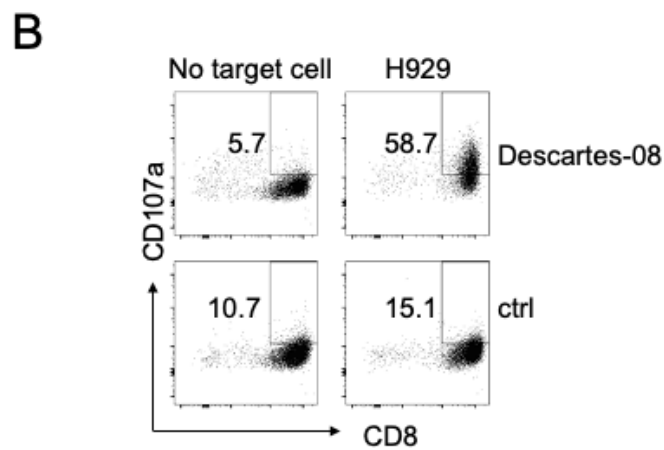
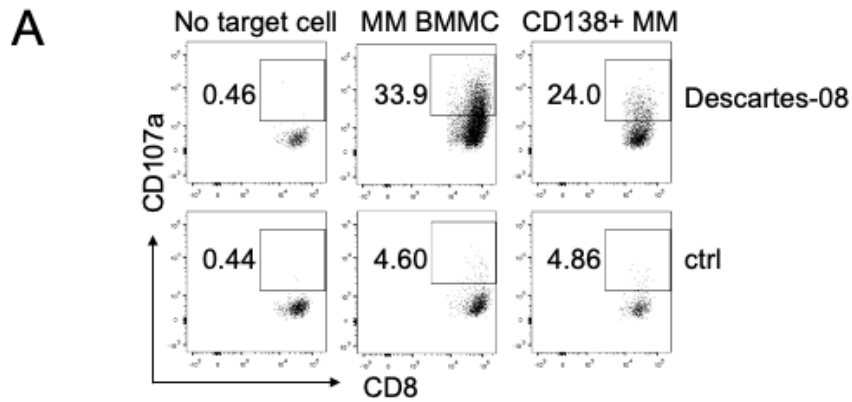


Fig. S6

**Fig. S6 Degranulation of Descartes-08 cells were significantly induced following co-culture with patient MM cells.**

(A) Descartes-08 (upper panel) or paired control (ctrl) (lower panel) CD8<sup>+</sup> T cells were co-cultured for 6h with BMMC or purified CD138<sup>+</sup> cells (E:T = 1:1) from a representative MM patient sample. No target cells served as baseline controls. (B) CD8<sup>+</sup> T cells were purified from peripheral blood of MM patients (n = 6). Patient-derived Descartes-08 cells vs paired ctrl T cells were co-cultured with target H929 MM cells for 6h followed by FCM analysis to determine % CD107a<sup>+</sup> in Descartes-08 vs ctrl T cells. No target cells served as baseline controls. Shown are representative FCM dot plot data of T cells from one patient sample.





\*\*\*\* $P < .0001$  (**B**) Weights (means + SDs, error bars) of animals in indicated groups were followed through timeframe of study in **Fig. 6**.