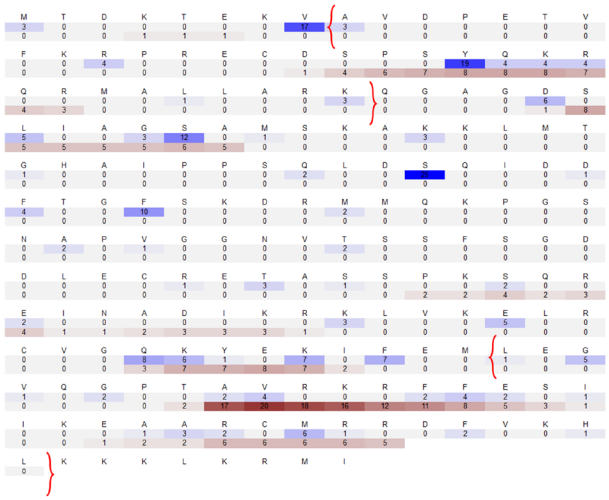
# Multipeptide stimulated PBMCs generate $T_{EM}/T_{CM}$ for adoptive cell therapy in multiple myeloma

## SUPPLEMENTARY MATERIALS

**A**



**B**

1. SEA1 (MUC1): SPQLSTGVSFFFLSFHISNLQFNSSLEDPSTD (32 MER)
2. SEA2 (MUC1): STDYYQELQRDISEMFLQIYKQGGFLGLSNIKFRPGSVV (39 MER)
3. SEA3 (MUC1): DVETQFNQYKTEAASRYNLTISDVSVSDVPFP (32 MER)
4. BCMA 2: FPLPAMEEGATILVTTKTNDYCKSLPAALSATEIEKS (37 MER)
5. CD38.1: YQSCPDWRKDCSNNPVSVFWKTVSRR (26 MER)
6. CD38.2: ACDVVHVMLNGSRSKIFDKNSTFGSV (26 MER)
7. FcRH5.1: CTADNGFGPQRSEVVSLFVTVPVSRPILTLRVPRAQAV (38 MER)
8. FcRH5.2: AQHSDTISLSVIVPVSRPILTFRAPRAQA (29 MER)
9. MCL1.1: GAEVPDVTATPARLLFFAPTRRAAPLEEM (29 MER)
10. RHAMM 2: RLNAALREKTSLSANNATLEKQLIELTRTNELLKSK (36 MER)
11. RHAMM 3: SENGNQKNLRILSLELMKLRNKRETKMRGMMAKQEGME (38 MER)
12. RHAMM 4: AHTQATLLLQEKYDSMVQSLEDVTAQFESYKA (32 MER)
13. SLAMF7.2: DVIYTWKALGQAANESHNGSILPISWRWGESDMTFICVAR (40 MER)
14. SLAMF7.5: LGLFLWFLKRERQEENNPKGRSSKYGLL (28 MER)
15. SOX2.1: NSPDRVKRPMNAFMVWSRGQRRKMAQENPKMHNSEISK (38 MER)
16. SOX2.2: EWKLLSETEKRPFIDEAKRLRALHMKEHPDYKYRPRRK (38 MER)
17. XBP(S)1.1: AAAPNPADGTPKVLLLSGQPASAAGAPAGQ (30 MER)
18. XBP(S)1.2: SDILLGILDNLDPVMFFKCPSPEPASLEELPEVYPEGP (38 MER)
19. CT45.1: AVDPETVFKRPRECDSPSYQKRQRMALLARK (31 MER)
20. CT45.4: LEGVQGPTAVRKRFFESIIKEAARCMRRDFVKHL (34 MER)
21. MAGEA3: TFPDLESEFQAALSRKVAELVHFLLLK (27 MER)
22. MAGEA4.1: PDAESLFREALSNKVDELAHFLLRKYRAKEL (31 MER)
23. MAGEA6.1: TFPDLESEFQAALSRKVAKLVHFLLLK (27 MER)
24. MAGEA6.3: FVQENYLEYRQVPGSDPACYEFLWGPRALIETSYVKVLHHM (41 MER)
25. MAGEC1.4: SPLQRPVSSFFSYTLASLLQSSHESPQS (28 MER)
26. MAGEC1.5: SSTSSSLSKSSPESPLQSPVISFSS (25 MER)
27. MAGEC1.8: NSSPPRYEFLWGPRAHSEVIKRKVVEFLAMLKNTVPI (37 MER)
28. NY-ESO-1.1: ARGPESRLLEFYLAMPFATPMEAELARRSLAQDAPPL (37 MER)
29. NY-ESO-1.2: VPGVLLKEFTVSGNILTIRLTAADHRQL (28 MER)
30. WT1.1: RSASETSEKRPFMCAYPGCNKRYFKLSHLQMH (32 MER)



**Supplementary Figure 1: Synthetic peptides were designed for different antigens based on predictive algorithms.** (**A**) An example depicting the methodology used to design peptides. Immunogenic heat map that recognizes regions with high binding affinity for MHC I (blue letters) and MHC II (area highlighted in red) grooves for antigen, CT45 (brackets indicate the designed peptide sequence). (**B**) The peptide sequences for different antigens (17–41 mers) were synthesized that consist of overlapping regions for MHC I and MHC II binding. The peptides: 1–18 are designed from antigens that are overexpressed in MM; peptides 19 through 30 were constructed from cancer testis antigens. The list consists of antigens (MUC1 (SEA1,2,3), CD38, FcRH5, RHAMM, SLAMF7, SOX2, XBP(S)1, CT45, MAGEA6, MAGEC1 and NY-ESO-1) that showed more than one region with overlapping MHC I & II hotspots that could be synthesized. To indicate that the peptides are synthesized from the same antigen, the peptides are labelled accordingly (e.g., CD38.1, CD38.2).

| MUC1 Cocktail | Cocktail 1 | Cocktail 3 | Cocktail 4 |
|---|---|---|---|
| | SEA 1 | SLAMF7.2 | RHAMM 2 |
| SEA1 | MAGEA6.1 | SLAMF7.5 | RHAMM 3 |
| SEA2 | MAGEA6.3 | FcRH5.1 | RHAMM 4 |
| SEA3 | CT45.4 | FcRH5.2 | NYESO 1.1 |
| | SEPT 9.1 | BCMA2 | NYESO 1.2 |

**Supplementary Figure 2: Four peptide cocktails were used for subsequent experiments.** Based on the data obtained following treatment of healthy donor PBMCs with single peptides, four different peptide cocktails were designed to assess the ability of different antigens to co-operatively induce T cell responses from PBMCs isolated from healthy donors or MM patients.

**A**

**Cocktail 1**

| HD | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 |
| $T_{EM}$ | 49 | 53 | 52 | 68 | 57 | 50 | 67 | 48 | 100 | 96 |
| $T_{CM}$ | 47 | 42 | 43 | 15 | 41 | 49 | 33 | 49 | 0 | 0 |

| MM | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 |
| $T_{EM}$ | 77 | 69 | 76 | 76 | 96 | 94 | 60 | 42 | 100 | 97 |
| $T_{CM}$ | 22 | 30 | 23 | 19 | 4 | 3 | 21 | 57 | 0 | 1 |

**B**

**Cocktail 3**

| HD | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 |
| $T_{EM}$ | 31 | 64 | 54 | 64 | 56 | 54 | 79 | 64 | Insufficient Cells | |
| $T_{CM}$ | 60 | 26 | 36 | 10 | 41 | 44 | 20 | 34 | Insufficient Cells | |

| MM | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 |
| $T_{EM}$ | 68 | 79 | 90 | 76 | 93 | 89 | Insufficient Cells | | 100 | 94 |
| $T_{CM}$ | 30 | 19 | 9 | 18 | 7 | 7 | Insufficient Cells | | 0 | 1 |

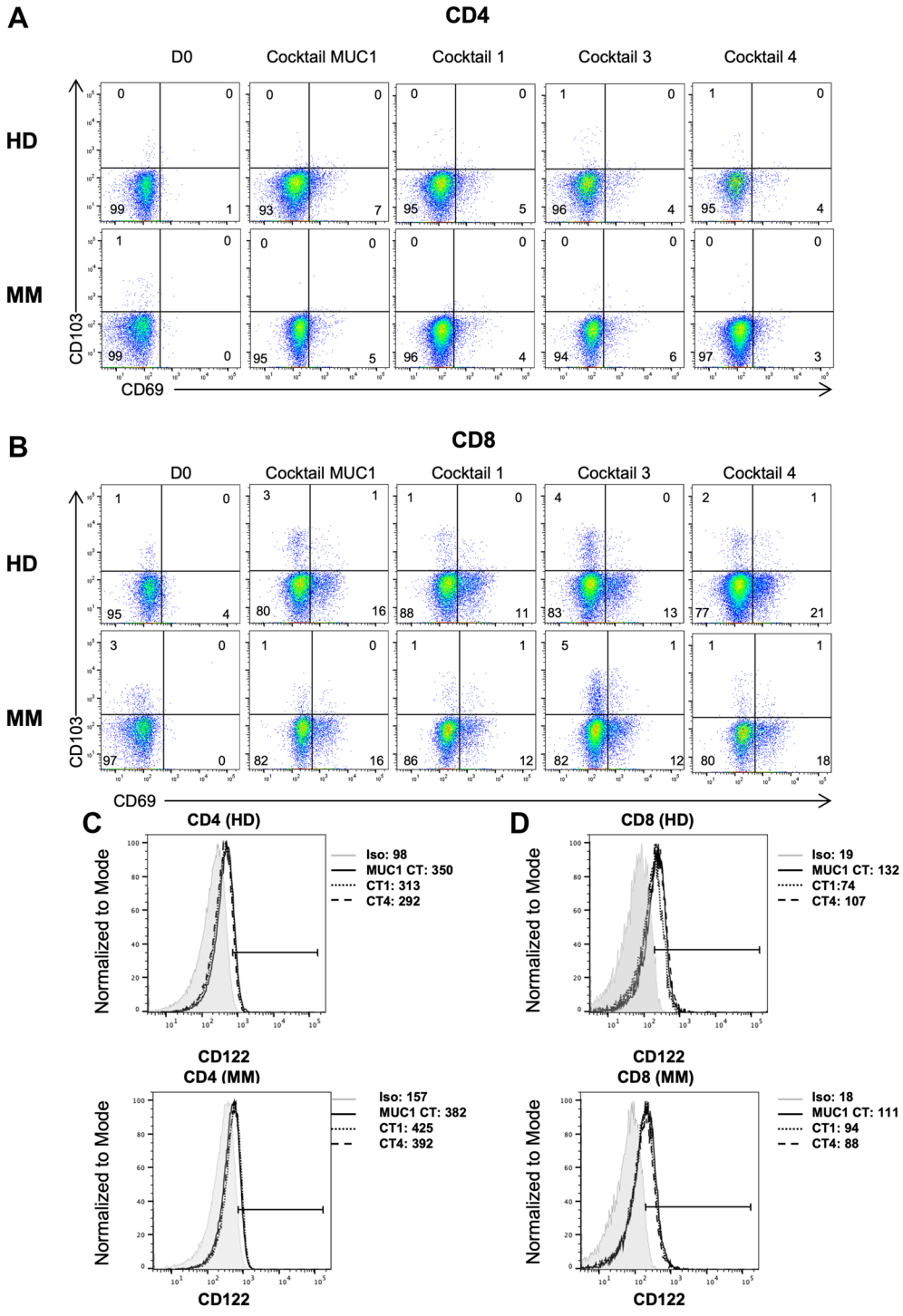
**C**

**Cocktail 4**

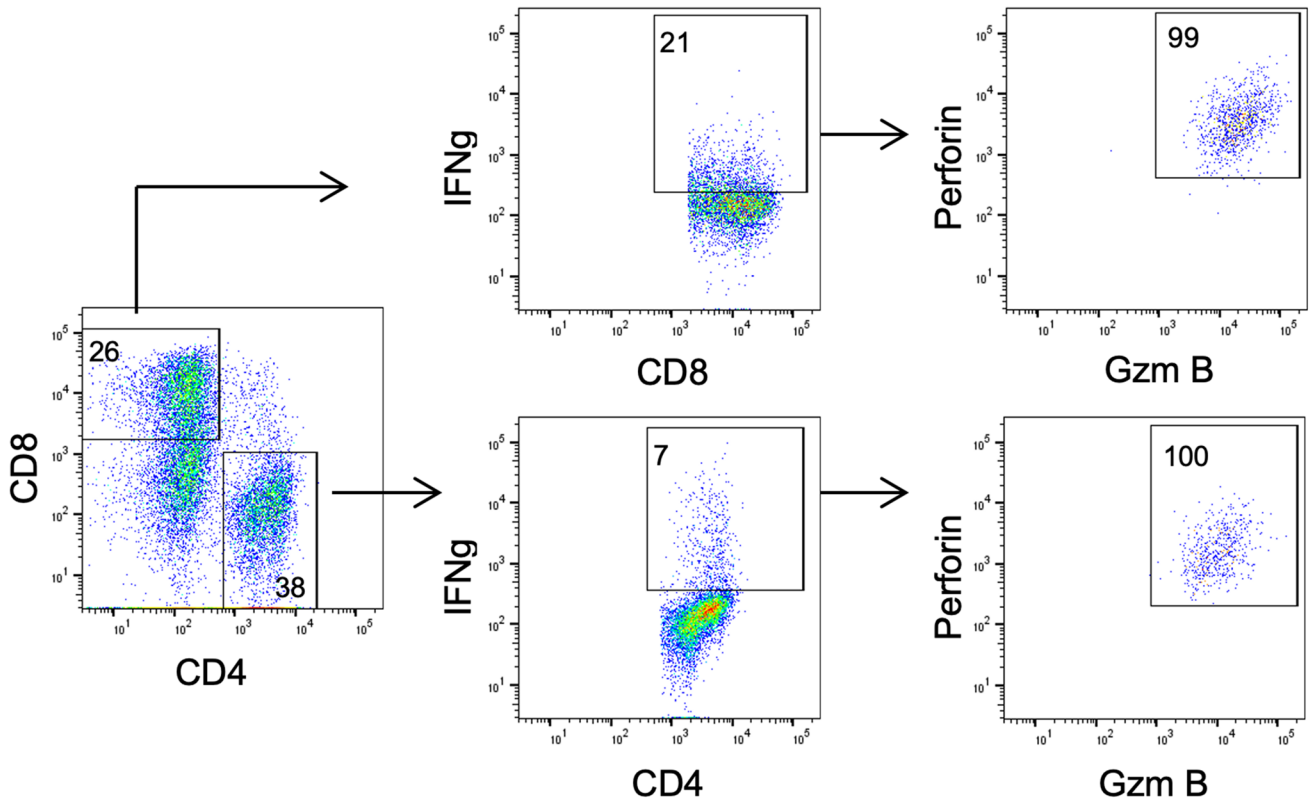
| HD | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 |
| $T_{EM}$ | 42 | 52 | 57 | 72 | 61 | 24 | 74 | 45 | 37 | 47 |
| $T_{CM}$ | 51 | 42 | 33 | 12 | 37 | 75 | 25 | 51 | 0 | 0 |

| MM | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 | CD4 | CD8 |
| $T_{EM}$ | 78 | 57 | 83 | 70 | 92 | 84 | Insufficient Cells | | 100 | 96 |
| $T_{CM}$ | 22 | 42 | 16 | 23 | 7 | 13 | Insufficient Cells | | 0 | 1 |

**Supplementary Figure 3: Culture activation generates $T_{EM}$ and $T_{CM}$ populations in both $CD4^+$ and $CD8^+$ T cells.** Table depicting the percentage of $T_{EM}$ and $T_{CM}$ for $CD4^+$ and $CD8^+$ T cells obtained at the end of the culture period following treatment with cocktails (**A**) CT1, (**B**) CT3 and (**C**) CT4.




**Supplementary Figure 4: Stimulation with peptide cocktail leads to enhanced expression of $T_{RM}$ markers, CD69 and CD103, on $CD4^+$ and $CD8^+$ T cells.** (**A**) Expression of CD69 and CD103 on $CD4^+$ T cells or (**B**) $CD8^+$ T cells on D0 or on D19 following stimulation of PBMCs isolated from healthy donor (HD) or MM patient (MM) with either MUC1 Cocktail, Cocktail 1, Cocktail 3 or Cocktail 4. Representative data are shown. CD122 expression on $CD4^+$ T cells (**C**) and $CD8^+$ T cells (**D**) were generated following exposure of PBMCs from HD (top panel) and MM patient (bottom panel) to MUC1 cocktail (solid line histogram), Cocktail 1 (dotted line histogram), and Cocktail 4 (dashed line histogram). The isotype control is depicted by grey histogram. Data representative of four individuals (2 HDs, 2 MM patients).




**Supplementary Figure 5: Effector memory ($T_{EM}$) and central memory ($T_{CM}$) $CD4^+$ and $CD8^+$ T cells possess anti-tumor profile.** Representative dot plot showing the gating hierarchy to define different functional subsets of $CD4^+$ and $CD8^+$ T cells. First, viable cells were gated based on the absence of UV Blue stain. These cells are then gated on CD3 and then on CD8, which was used to define $CD8^+IFN\text{-}\gamma^+$. The expression of perforin and granzyme B was examined on $CD8^+IFN\text{-}\gamma^+$. Similar strategy was used for $CD4^+$ T cells. Representative data are shown.