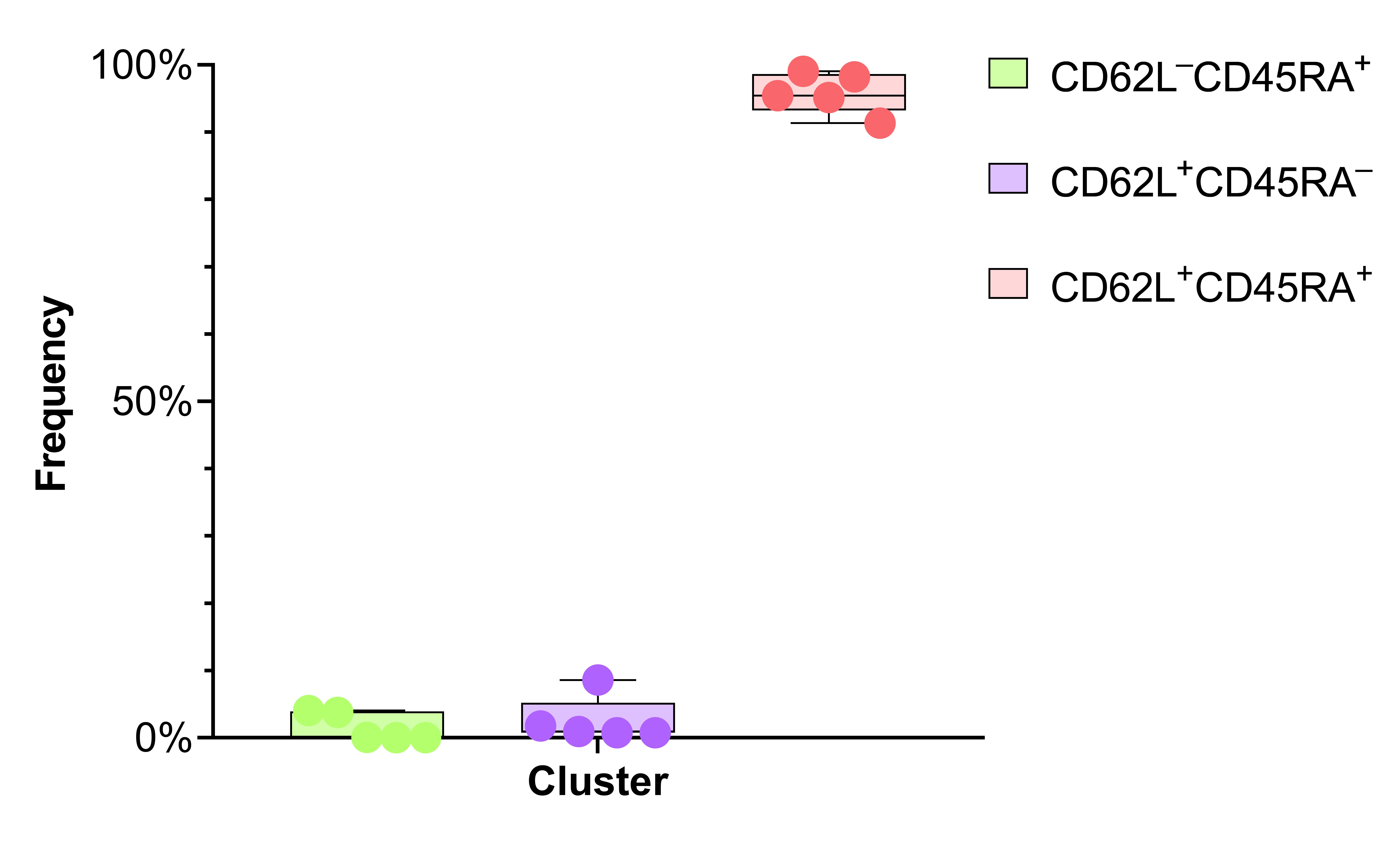
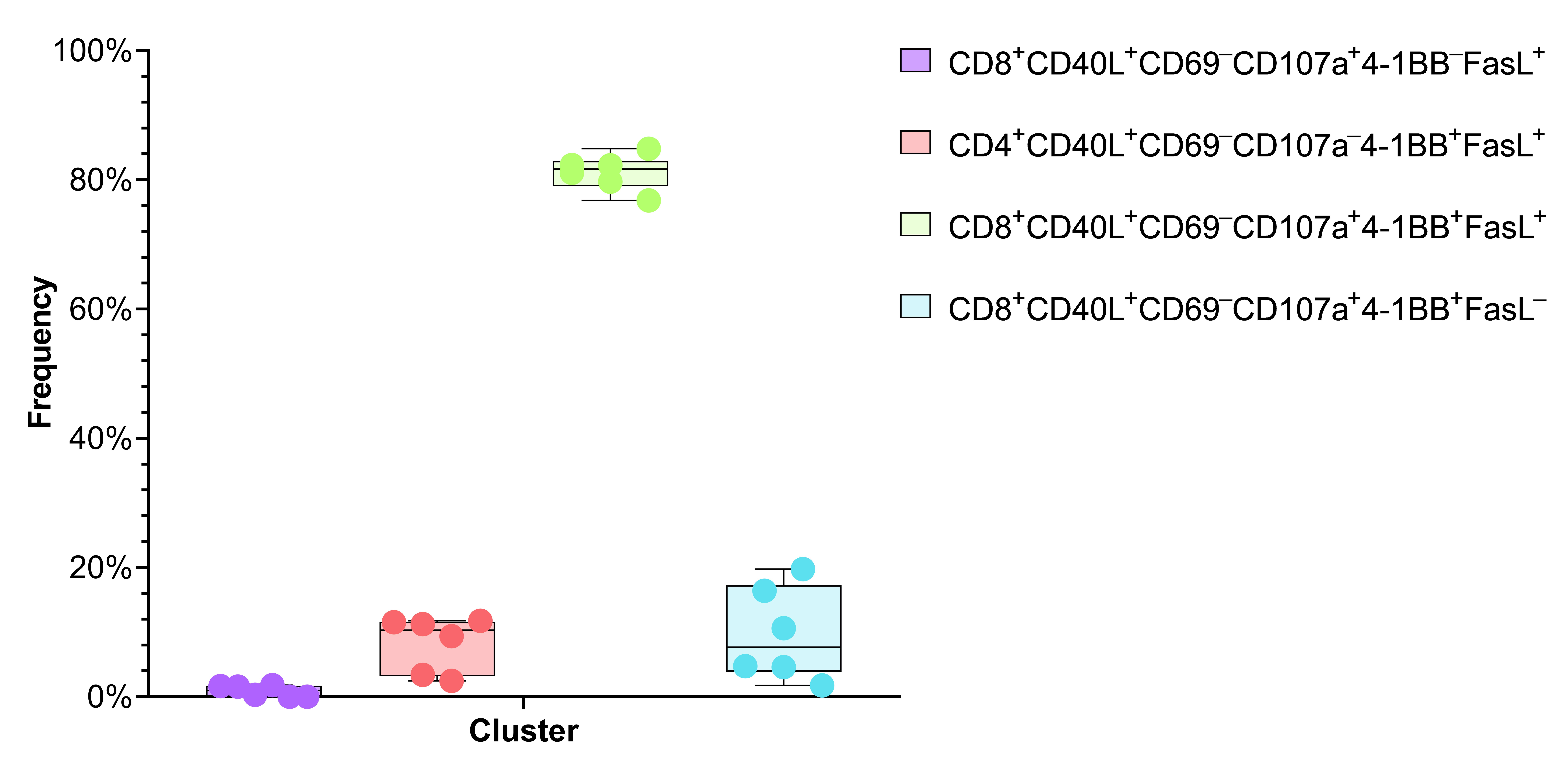


**Supplementary Fig. 1. Flow cytometric analysis of retroviral transduction in anti-CD3 primed PBMCs.** Lymphocytes (**A**) were gated from the debris, singlets cells (**B**) from the lymphocytes, live cells (**C**) from the singlets cells, CD3+ cells (**D**) from the live cells, and transduced cells (**E**) from the CD3+ cells.



**Supplementary Fig. 2. HSNE-based analysis of the CD8+ GD2.CAR T cell memory subpopulations.** Box plots show the percentage composition of each cluster, identified by CD62L and CD45RA expression: Temra (green), Tcm (violet), and Tn/scm (red).



**Supplementary Fig. 3. HSNE-based analysis of the GD2.CAR T cells activation and cytotoxicity.** Box plots show the percentage composition of CD8+ GD2.CAR T cells within each cluster expressing activation and cytotoxicity markers (CD69, 4-1BB, CD107a, FasL, and CD40L) after 4–6 hours co-culture with the SK-MEL-37 cells.

**Supplementary Table 1. Significantly up- and down-regulated genes in the GD2.CAR T cells after 2 hours of co-culture with SK-MEL-37 cells.**

|  |  |
| --- | --- |
| Up-regulated genes | *AHR, APP, BCL6, BST1, C1QBP, C1R, C1S, C3, CCL2, CCL20, CCL3, CCL4, CD22, CD276, CD59, CD83, CD9, CEBPB, CFI, CIITA, CSF1, CSF2, CTNNB1, CXCL1, CXCL10, CXCL11, CXCL2, EDNRB, EGR2, FCGR2A, FN1, GBP1, HLA-DMA, HLA-DMB, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DRA, HLA-DRB1, HLA-DRB3, ICAM1, IFNG, IL13, IL13RA1, IL1A, IL1B, IL1RAP, IL6, IL6ST, IL8, IRAK2, IRF1, LIF, LTBR, NCAM1, NT5E, PLAUR, PSMB5, PSMB7, PTK2, SELE, SMAD5, SPP1, TCF4, TFRC, THY1, TLR2, TNFRSF9, TRAF1* |
| Down-regulated genes | *ADA, ARHGDIB, BAX, BCL2, CASP1, CASP8, CCND3, CCR1, CCR2, CCR5, CCR7, CCRL2, CD244, CD247, CD27, CD28, CD45RA, CD45RB, CD48, CD53, CD6, CD79B, CD96, CISH, CX3CR1, CXCR3, CXCR4, CXCR6, DPP4, FCGRT, GBP5, GZMA, GZMK, HAVCR2, HLA-B, ICAM2, ICAM3, IFITM1, IKBKE, IKZF1, IKZF3, IL10RA, IL12RB1, IL21R, IL23A, IL2RG, IL4R, IL7R, IRAK4, ITGA4, ITGAL, ITGAM, ITGAX, ITGB2, JAK1, JAK2, JAK3, KLRB1, LAIR1, LCK, LEF1, LTB4R, MAF, MAP4K1, MAP4K2, MBP, MUC1, MYD88, NCF4, NFATC3, PDGFRB, PRF1, PSMB10, PYCARD, S1PR1, SELPLG, SIGIRR, SLAMF7, STAT4, STAT5B, STAT6, TAGAP, TAPBP, TCF7, TGFB1, TGFBR1, TLR1, TMEM173, TNFRSF14, TNFRSF1B, TNFSF12, TNFSF8, XBP1, ZAP70, ZBTB16* |

**Supplementary Table 2. Gene Set Enrichment Analysis (GSEA) of the up-regulated genes in the GD2.CAR T cells.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Term** | **Overlap** | **Combined Score** | **Genes** |
| Cytokine-mediated signaling pathway | 38/621 | 3094.915 | *CIITA, CSF2, CSF1, CXCL1, IL1RAP, CXCL2, ICAM1, PSMB7, PSMB5, IRAK2, CCL4, CCL3, CCL2, NCAM1, GBP1, HLA-DQA1, HLA-DPA1, IL13RA1, CCL20, TNFRSF9, IL13, FN1, LIF, TRAF1, IL1A, CXCL10, CXCL11, IL6, BCL6, IFNG, IL1B, IRF1, HLA-DPB1, HLA-DRA, LTBR, IL6ST, HLA-DRB3, HLA-DRB1* |
| Cellular response to interferon-gamma | 17/121 | 2943.425 | *CIITA, CCL20, ICAM1, IFNG, IRF1, CCL4, HLA-DPB1, CCL3, HLA-DRA, CCL2, NCAM1, HLA-DRB3, GBP1, HLA-DQA1, HLA-DRB1, TLR2, HLA-DPA1* |
| Cellular response to cytokine stimulus | 25/482 | 1114.687 | *CSF2, CSF1, CXCL1, IL1RAP, CXCL2, ICAM1, IRAK2, CCL4, CCL3, CCL2, GBP1, HLA-DPA1, IL13RA1, CCL20, IL13, FN1, LIF, IL1A, CXCL10, IL6, BCL6, IL1B, IRF1, IL6ST, TLR2* |
| Interferon-gamma-mediated signaling pathway | 12/68 | 2711.983 | *CIITA, IFNG, IRF1, HLA-DPB1, HLA-DRA, NCAM1, HLA-DRB3, GBP1, HLA-DQA1, HLA-DRB1, ICAM1, HLA-DPA1* |
| Inflammatory response | 16/230 | 939.9529 | *CIITA, CEBPB, CCL20, CXCL1, CXCL2, SELE, IL1A, CXCL10, CXCL11, IL6, IFNG, IL1B, CCL4, CCL3, CCL2, TLR2* |
| Regulation of T cell proliferation | 10/76 | 1807.625 | *IL6, CEBPB, HLA-DMB, TFRC, IL1B, HLA-DPB1, IL6ST, HLA-DRB1, CD276, HLA-DPA1* |
| Positive regulation of cytokine production | 16/335 | 525.3742 | *APP, CEBPB, CSF2, IL13, C3, IL1A, IL6, IFNG, IL1B, IRF1, HLA-DPB1, CTNNB1, IL6ST, TLR2, CD276, HLA-DPA1* |
| Positive regulation of T cell activation | 10/75 | 1431.652 | *IL6, HLA-DMB, TFRC, IL1B, HLA-DPB1, CCL2, THY1, IL6ST, CD276, HLA-DPA1* |
| Regulation of cell population proliferation | 21/764 | 304.2711 | *APP, CSF2, CSF1, TFRC, TNFRSF9, FN1, LIF, CXCL1, PTK2, IL1A, CXCL10, BST1, CXCL11, IL6, BCL6, IFNG, IL1B, IRF1, CTNNB1, IL6ST, IL13RA1* |
| Positive regulation of lymphocyte proliferation | 9/75 | 1086.013 | *BST1, IL6, HLA-DMB, TFRC, IL1B, HLA-DPB1, IL6ST, CD276, HLA-DPA1* |

Results from GSEA using Gene Ontology Biological Process terms; *q*-value < 0.000001.