Haroun-Izquierdo et al, Supplemental Material
Supplemental Figure 1. Extended phenotyping by flow- and mass cytometry of ADAPT-NK cells. A) Gating scheme for mass cytometry analysis of NKG2C⁺ and NKG2C⁻ CD56⁺ NK cells. B) Gating scheme for flow cytometry analysis of NKG2C⁺ and NKG2C⁻ CD56⁺ NK cells, gated upstream on fsc-h/a and ssc-h/a single cells. C-D) Summary data that are the basis of heatmaps in Fig. 2B, frequencies of marker expression or mean metal intensity (MMI). Data for Fcεr1γ, HLA-DR, CD98 and CD25 derive from flow cytometry data. p-value <0.05 * <0.01 ** <0.001 *** <0.0001 **** in one-way ANOVA with Tukey’s correction (C-D). For flow- and mass cytometry n=4-7.
Supplemental Figure 2. ADAPT-NK cells show a retained adaptive transcriptional signature with activation of effector programs. **A)** CIT-Seq workflow. **B)** Sort gates for day 0 and day 11 NK cells. **C-D)** Heatmaps showing the expression of the upregulated (C) and downregulated (D) genes in ADAPT-NK in the CIT-Seq data sorted by the identified clusters and showing the GO (BP) processes associated with these genes. **E)** Venn diagram showing the overlap between the up- and down-regulated genes in the KLRC2⁺ day 11 cluster and up- and down-regulated genes in exhausted CAR T cells (Good et al., 1). **F)** Exhaustion score computed using AUCCell showing the activity of this signature in the clusters. CIT-Seq n=1.
Supplemental Figure 3. ADAPT-NK cells are highly functional with predictable alloreactivity. Functional analysis of NK cells by flow cytometry in terms of degranulation (CD107a) and IFN-γ production. A) Responses of NKG2C+ KIR2DL1+ KIR2DL3+ KIR2DL2+ NKG2A+ or NKG2C+ KIR2DL1+ KIR2DL3+ KIR2DL2+ NKG2A+ ADAPT-NK cells to K562 with single chain β2m HLA-C1 or HLA-C2 dimers, in the HLA-C/KIR matched and mismatched setting. n=5-11, in 3 independent experiments with KIR2DL1+ and KIR2DL3+ subsets analyzed in all donors. B) Determination of HLA-E expression on tumor target lines. C-F) Determination of killing ability of ADAPT-NK cells over 48 h against tumor cell lines of various HLA-C genotypes and at different effector to target ratios (1:1 and 5:1) in the IncuCyte platform. In bold the expected KIR-Fe binding, n=4-9 donors, in at least two independent experiments for each target cell line. Data is displayed as mean (± SD) and significance is given between HLA-C/KIR mismatched and targets only. p-value <0.05 * <0.01 ** <0.001 *** <0.0001 **** in one-way ANOVA followed by Sidak’s multiple comparison correction (C-F).
Supplemental Figure 4. Cytotoxicity of ADAPT-NK cells against primary AML blasts. A) AML patient cytogenetics and HLA-C haplotypes. B) Cytotoxicity against 8 primary AML blasts by 9 different HLA-C/KIR-mismatched ADAPT-NK products in 3 independent experiments. Some AML samples were co-cultured with multiple NK products (up to 3) in separate experiments. A and B notation on ADAPT-NK#4 denotes a repeat combination in 2 independent experiments. C) Surface marker expression of indicated proteins evaluated by flow cytometry on 7 CD45^dim AML blasts and ordered based on decreased sensitivity to ADAPT-NK cell cytotoxicity, FMO = fluorescence minus one where indicated.
## Supplemental table 1. mAbs used for Flow cytometry

| Fluorochrome | Antigen | Clone | Vendor |
|--------------|---------|-------|--------|
| APC          | Anti-Biotin     | REA746 | Miltenyi |
| BV785        | CD3      | UCHT1 | BD Biosciences |
| PE-Cy5       | CD3      | UHCT1 | Beckman Coulter |
| ECD          | CD3      | UCHT1 | Beckman Coulter |
| BV421        | CD3      | SK7   | Biolegend |
| APC          | CD3      | HIT3a | BD Biosciences |
| V500         | CD14     | MoP9  | BD Biosciences |
| BV570        | CD14     | M5E2  | Biolegend |
| ECD          | CD14     | RMO52 | Beckman Coulter |
| FITC         | CD14     | M5E2  | BD Biosciences |
| BVU395       | CD16     | 3G8   | BD Biosciences |
| ECD          | CD16     | 3G8   | Beckman Coulter |
| BV785        | CD16     | 3G8   | Biolegend |
| V500         | CD19     | HIB19 | BD Biosciences |
| BV570        | CD19     | HIB19 | Biolegend |
| ECD          | CD19     | J3-119| Beckman Coulter |
| BV510        | CD19     | SJ25C1| BD Biosciences |
| BV421        | CD25     | 2A3   | BD Biosciences |
| PE-Vio770    | CD33     | REA775| Miltenyi |
| BUV395       | CD34     | 581   | BD Biosciences |
| BV711        | CD38     | HIT2  | Biolegend |
| BV650        | CD45     | H130  | Biolegend |
| PerCP-Cy5.5  | CD45     | H130  | BD Biosciences |
| BV421        | CD47     | B6H12 | BD Biosciences |
| BUV737       | CD56     | NCAM16.2 | BD Biosciences |
| ECD          | CD56     | NHK-1 | Beckman Coulter |
| PE-Cy7       | CD56     | NCAM16.2 | BD Biosciences |
| Pacific Blue | CD57     | HCD57 | Biolegend |
| BV650        | CD98     | UM7F8 | BD Biosciences |
| BUV395       | CD107a   | H4A3  | BD Biosciences |
| APC          | CD112    | TX31  | Biolegend |
| PE-Cy5.5     | CD117    | 104D2D1| Beckman Coulter |
| PE           | CD155    | SKII.4| Biolegend |
| PE           | EPCAM    | 9C4 (#271) | Biolegend |
| FITC         | FceRlg   | polyclonal | Merck-Millipore |
| BV785        | HLA-DR   | L243  | Biolegend |
| APC          | HLA-E    | 3D12  | Biolegend |
| Antibody/Kit                                      | Color | Name                     | Supplier                  |
|--------------------------------------------------|-------|--------------------------|---------------------------|
| PE HLA-E                                         |       | 3D12                     | Biolegend                 |
| PE-Cy7 HLA-E                                     |       | 3D12                     | Biolegend                 |
| AF700 IFN-γ                                       |       | B27                      | BD Biosciences            |
| BV785 IFN-γ                                      |       | 4S.B3                    | Biolegend                 |
| APC KIR2DL1                                      |       | REA284                   | Miltenyi                  |
| PE-Cy7 KIR2DL1/S1                                 |       | EB6B                     | Beckman Coulter           |
| Biotin KIR2DL3                                    |       | REA147                   | Miltenyi                  |
| PE-Cy5.5 KIR2DL3/L2/S2                            |       | GL183                    | Beckman Coulter           |
| AF700 KIR3DL1                                    |       | DX9                      | Biolegend                 |
| APC-Fire750 KIR3DL1                               |       | DX9                      | Biolegend                 |
| Vio-Bright-FITC NKG2A                              |       | REA110                   | Miltenyi                  |
| PE NKG2C                                         |       | FAB138P                  | R&D Systems               |
| APC NKG2C                                        |       | REA205                   | Miltenyi                  |
| PE NKG2C                                         |       | REA205                   | Miltenyi                  |
| BV711 Streptavidin                               |       |                         | BD Biosciences            |
| PE TIGIT                                         |       | MBSA43                   | eBiosciences – Thermo Fisher Scientific |
| BV650 TNF                                        |       | MAb11                    | Biolegend                 |
| Aqua LIVE/DEAD Fixable Aqua Dead Cell Stain Kit   |       |                         | Thermo Fisher Scientific  |
| Near-IR LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit |   |                         | Thermo Fisher Scientific  |
| eFluor780                                         |       |                         | Thermo Fisher Scientific  |
| FITC Cleaved Caspase-3 Staining Kit (FITC)        |       |                         | Abcam                     |
| Recombinant Human KIR2DL1/CD158a Fc Chimera Protein | #1844-KR-050 | R&D Systems               |
| Recombinant Human KIR2DL3/CD158b2 Fc Chimera Protein | #2014-KR-050 | R&D Systems               |
| Recombinant Human NKG2D Fc Chimera Protein        |       |                         | R&D Systems               |
| PE Goat anti-Human IgG Fc Secondary Antibody      |       | polyclonal                | Thermo Fisher Scientific  |
Supplemental table 2. mAbs used for Mass cytometry

| Mass  | Antigen | Clone     | Vendor   |
|-------|---------|-----------|----------|
| 89Y   | CD45    | HI30      | Fluidigm |
| 141Pr | CX3CR1  | REA385    | Miltenyi |
| 142Nd | CD57    | HCD57     | Fluidigm |
| 143Nd | CD2     | TSI/8     | Biolegend|
| 144Nd | CD38    | REA572    | Miltenyi |
| 145Nd | CXCR3   | REA232    | Miltenyi |
| 146Nd | CCR2    | REA538    | Miltenyi |
| 147Sm | CD96    | NK92.39   | Biolegend|
| 148Nd | CD3     | OKT3      | Biolegend|
| 148Nd | CD14    | RM052     | Fluidigm |
| 148Nd | CD19    | HIB19     | Biolegend|
| 149Sm | FasL    | NOK-1     | Biolegend|
| 150Nd | LAG3    | 11C3C65   | Fluidigm |
| 151Eu | CXCR4   | REA649    | Miltenyi |
| 152Sm | Siglec-7| 194211    | Fluidigm |
| 153Eu | TIM-3   | F38-2E2   | Fluidigm |
| 154Sm | NKG2C   | REA205    | Miltenyi |
| 155Gd | CXCR1   | REA958    | Miltenyi |
| 156Gd | KIR2DL1/S1 | 11PB6 | Miltenyi |
| 158Gd | KIR2DL1 | REA284    | Miltenyi |
| 159Tb | GITR    | 108-17    | Biolegend|
| 160Gd | GPR56   | CG4       | Biolegend|
| 161Dy | PLZF    | 17.10,17  | Biolegend|
| 162Dy | CD69    | FN50      | Fluidigm |
| 163Dy | KIR2DL2/S2/L3 | GL183 | Beckman Coulter |
| 164Dy | TIGIT   | MBSA43    | Thermo Fisher Scientific |
| 165Ho | CCR5    | REA245    | Miltenyi |
| 166Er | NKG2D   | ON72      | Fluidigm |
| 167Er | CCR7    | REA108    | Miltenyi |
| 168Er | Nkp30   | P30-15    | Miltenyi |
| 169Fm | NKG2A   | Z199      | Fluidigm |
| 170Er | CXCR2   | REA208    | Miltenyi |
| 171Yb | DNAM-1  | DX11      | Fluidigm |
| 172Yb | KIR3DL1 | DX9       | R&D      |
| 173Yb | Granzyme B | GB11 | Fluidigm |
| 174Yb | PD-1    | EH12.2H7  | Fluidigm |
| 175Lu | Perforin| B-D48     | Fluidigm |
| 176Yb | CD56    | NCAM16.2  | Fluidigm |
| 209Bi | CD16    | 3G8       | Fluidigm |
| Pathway                                                                 | Total | Expected | Hits  | FDR     |
|-----------------------------------------------------------------------|-------|----------|-------|---------|
| Cell activation                                                       | 960   | 12.6     | 54    | 9.25e-15|
| Lysocyte activation                                                  | 707   | 9.3      | 39    | 3.9e-12  |
| Lymphocyte activation                                                | 601   | 7.91     | 36    | 3.9e-12  |
| Regulation of immune response                                        | 727   | 9.57     | 39    | 7.11e-12 |
| Immune response                                                      | 1430  | 18.8     | 55    | 1.48e-11 |
| Lysocyte differentiation                                             | 404   | 5.32     | 28    | 7.03e-11 |
| Regulation of immune system process                                  | 1190  | 15.7     | 48    | 1.37e-10 |
| Positive regulation of lymphocyte activation                         | 250   | 3.29     | 22    | 1.83e-10 |
| Hemopoiesis                                                          | 640   | 8.82     | 34    | 2.15e-10 |
| T cell activation                                                    | 432   | 5.68     | 28    | 2.15e-10 |
| Lymphocyte differentiation                                            | 260   | 3.42     | 22    | 2.92e-10 |
| B cell activation                                                    | 194   | 2.55     | 19    | 6.62e-10 |
| Hematopoietic or lymphoid organ development                          | 679   | 8.93     | 34    | 8.41e-10 |
| Immune system development                                            | 722   | 9.5      | 35    | 9.24e-10 |
| Immune system process                                                | 2720  | 35.8     | 72    | 1.99e-09 |
| Cytokine-mediated signaling pathway                                  | 374   | 4.92     | 23    | 1.13e-09 |
| Positive regulation of immune system process                         | 739   | 9.72     | 34    | 6.35e-09 |
| Positive regulation of T cell activation                             | 202   | 2.66     | 18    | 7.87e-09 |
| Regulation of lymphocyte activation                                  | 360   | 4.74     | 23    | 1.59e-08 |
| B cell differentiation                                               | 82    | 1.08     | 12    | 2.88e-08 |
| Regulation of T cell activation                                      | 279   | 3.67     | 20    | 2.98e-08 |
| T cell differentiation                                               | 186   | 2.45     | 16    | 1.14e-07 |
| Regulation of response to stimulus                                   | 3360  | 44.2     | 80    | 1.78e-07 |
| Positive regulation of response to stimulus                          | 1550  | 20.4     | 48    | 3.52e-07 |
| Response to stress                                                   | 4150  | 54.6     | 91    | 4.62e-07 |
| Positive regulation of immune response                               | 487   | 6.41     | 24    | 8.01e-07 |
| Response to wounding                                                 | 1310  | 17.3     | 42    | 1.29e-06 |
| Regulation of body fluid levels                                      | 680   | 8.95     | 28    | 2.19e-06 |
| Blood coagulation                                                    | 564   | 7.42     | 24    | 1.1e-05  |
| Coagulation                                                          | 568   | 7.47     | 24    | 1.21e-05 |
| Hemostasis                                                           | 570   | 7.5      | 24    | 1.24e-05 |
| Response to radiation                                                | 345   | 4.54     | 18    | 1.79e-05 |
| Defense response                                                     | 1510  | 19.9     | 43    | 1.78e-05 |
| Interaction with host                                                | 426   | 5.61     | 20    | 2.08e-05 |
| Regulation of immune effector process                                | 249   | 3.28     | 15    | 2.44e-05 |
| Production of molecular mediator of immune response                  | 1330  | 17.1     | 11    | 2.53e-05 |
| Regulation of apoptotic process                                      | 1540  | 20.2     | 43    | 2.53e-05 |
| Activation of immune response                                        | 399   | 5.25     | 19    | 2.88e-05 |
| Regulation of programmed cell death                                  | 1350  | 20.4     | 43    | 3.27e-05 |
| Wound healing                                                        | 780   | 9.21     | 26    | 3.33e-05 |
| Immune effector process                                              | 576   | 7.58     | 23    | 4.12e-05 |
| Response to organic substance                                        | 2500  | 32.9     | 59    | 4.18e-05 |
| Innate immune response                                               | 638   | 8.39     | 24    | 6.52e-05 |
| Regulation of cell differentiation                                   | 1290  | 17       | 37    | 7.99e-05 |
| Positive regulation of cell proliferation                            | 786   | 10.3     | 23    | 7.99e-05 |
| Cell migration                                                       | 1050  | 13.9     | 32    | 0.000125 |
| Positive regulation of developmental process                         | 817   | 10.8     | 27    | 0.000156 |
| Peptidyl tyrosine phosphorylation                                    | 228   | 3        | 13    | 0.000173 |
| Positive regulation of metabolic process                             | 2690  | 35.4     | 60    | 0.000185 |
| Peptidyl tyrosine modification                                       | 230   | 3.03     | 13    | 0.000183 |
| Viral reproductive process                                            | 597   | 7.86     | 22    | 0.000201 |
| Regulation of cellular component organization                        | 1520  | 20.1     | 40    | 0.000226 |
| Response to ionizing radiation                                       | 112   | 1.47     | 9     | 0.00256 |
| Regulation of developmental process                                  | 1880  | 24.7     | 46    | 0.000256 |
| Regulation of protein metabolic process                              | 1820  | 24       | 45    | 0.000268 |
| Adaptive immune response                                             | 241   | 3.17     | 13    | 0.000268 |
| Inflammatory response                                                | 569   | 7.49     | 21    | 0.00028 |
| Regulation of myeloid cell differentiation                           | 145   | 1.91     | 10    | 0.000307 |
| Cell proliferation                                                   | 1900  | 25       | 46    | 0.000329 |
| Positive regulation of biological process                            | 5500  | 72.3     | 100   | 0.000361 |
| Positive regulation of cellular metabolic process                    | 2530  | 33.2     | 56    | 0.000389 |
| Negative regulation of apoptotic process                             | 679   | 8.93     | 23    | 0.000389 |
| Response to abortive stimulus                                       | 876   | 11.5     | 27    | 0.000595 |
| Adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains | 218   | 2.87     | 12    | 4.00E-04 |
| Regulation of protein modification process                            | 1250  | 16.4     | 34    | 0.000422 |
| Negative regulation of programmed cell death                        | 691   | 9.09     | 23    | 0.000481 |
| Negative regulation of signal transduction                           | 790   | 10.4     | 25    | 0.000491 |
| Pathway                                                                 | Significance Score | p Value          | Fold Change |
|------------------------------------------------------------------------|--------------------|-----------------|-------------|
| Positive regulation of cell migration                                  | 263                | 0.000542        |
| Cell surface receptor signaling pathway                                | 3340               | 0.000542        |
| Positive regulation of cellular process                                | 4780               | 0.000571        |
| Response to light stimulus                                             | 229                | 0.000584        |
| Homeostasis of number of cells                                         | 196                | 0.000641        |
| Exocytosis                                                             | 309                | 0.000656        |
| Interphase of mitotic cell cycle                                       | 309                | 0.000656        |
| Positive regulation of cell differentiation                             | 571                | 0.000688        |
| Regulation of multicellular organismal process                         | 2480               | 0.000748        |
| Epidermal growth factor receptor signaling pathway                     | 167                | 0.000752        |
| Intrinsic apoptotic signaling pathway                                   | 135                | 0.000752        |
| Interphase                                                             | 443                | 0.000783        |
| Positive regulation of protein metabolic process                       | 1080               | 0.000783        |
| Apoptotic process                                                      | 230                | 0.000877        |
| Positive regulation of transcription, DNA-dependent                    | 1260               | 0.000901        |
| Regulation of cell proliferation                                       | 1430               | 0.000917        |
| Peptidyl amino acid modification                                       | 739                | 0.00101         |
| Response to UV                                                         | 112                | 0.00106         |
| Regulation of cellular protein metabolic process                       | 1560               | 0.0011          |
| Programmed cell death                                                  | 2160               | 0.00111         |
| Negative regulation of transcription from RNA polymerase II promoter   | 552                | 0.00111         |
| Positive regulation of RNA metabolic process                           | 1330               | 0.00111         |
| T cell proliferation                                                   | 147                | 0.00123         |
| Myeloid cell differentiation                                           | 296                | 0.0013          |
| Negative regulation of metabolic process                               | 1820               | 0.00148         |
| Regulation of transferase activity                                     | 768                | 0.00161         |
| Nucleocytoplasmic transport                                           | 388                | 0.00161         |
| Nuclear transport                                                      | 392                | 0.00178         |
| Positive regulation of cytokine biosynthetic process                   | 66                 | 0.00184         |
| Positive regulation of nucleobase-containing compound metabolic process| 1490               | 0.00184         |
| Regulation of nucleobase-containing compound metabolic process         | 351                | 0.00184         |
| Regulation of cell cycle                                               | 886                | 0.00198         |
| Regulation of kinase activity                                          | 743                | 0.00246         |
| Regulation of phosphorylation                                          | 1070               | 0.00266         |
| Regulation of protein kinase activity                                   | 698                | 0.00299         |
| Viral reproduction                                                     | 803                | 0.00278         |
| Interleukin 2 production                                               | 47                 | 0.00278         |
| Negative regulation of cellular component organization                 | 370                | 0.00292         |
| Negative regulation of response to stimulus                            | 967                | 0.00292         |
| Protein complex assembly                                               | 861                | 0.00295         |
| Regulation of peptidyl tyrosine phosphorylation                        | 171                | 0.00319         |
| Regulation of cytokine production                                      | 513                | 0.00321         |
| Positive regulation of T cell proliferation                            | 75                 | 0.00326         |
| Negative regulation of cellular metabolic process                      | 1660               | 0.00326         |
| Regulation of cell-cell adhesion                                       | 77                 | 0.00368         |
| Regulation of protein phosphorylation                                  | 987                | 0.00368         |
| Negative regulation of transcription, DNA-dependent                    | 987                | 0.00368         |
| Cellular response to stress                                            | 1620               | 0.00398         |
## Supplemental table 4. GO terms for downregulated genes in Steiner Forest PPI network

| Pathway                                                                 | Total | Expected | Hits | FDR  |
|------------------------------------------------------------------------|-------|----------|------|------|
| Negative regulation of metabolic process                               | 1830  | 19.9     | 55   | 3.87e-10 |
| Negative regulation of cellular metabolic process                      | 1660  | 18.2     | 52   | 2.37e-10 |
| Negative regulation of signal transduction                             | 790   | 8.6      | 34   | 9.87e-10 |
| Negative regulation of cellular process                                | 4110  | 44.8     | 85   | 2.4e-09  |
| Regulation of signal transduction                                       | 2440  | 26.6     | 61   | 6.57e-09 |
| Negative regulation of response to stimulus                            | 967   | 10.6     | 36   | 6.57e-09 |
| Regulation of I-kappaB kinase/NF-kappaB cascade                         | 210   | 2.29     | 17   | 1.55e08  |
| Cytokine mediated signaling pathway                                     | 374   | 4.08     | 21   | 7.53e-08 |
| Negative regulation of biological process                               | 4590  | 50.1     | 87   | 7.56e-08 |
| I-kappaB kinase/NF-kappaB cascade                                       | 246   | 2.69     | 17   | 1.26e-07 |
| Regulation of apoptotic process                                         | 1540  | 16.8     | 43   | 2.67e07  |
| Regulation of programmed cell death                                     | 1550  | 17       | 43   | 2.75e-07 |
| Negative regulation of transcription, DNA-dependent                    | 987   | 10.8     | 33   | 2.75e-07 |
| Apoptotic process                                                       | 2130  | 23.2     | 52   | 2.75e-07 |
| Innate immune response                                                  | 638   | 6.9      | 26   | 2.75e-07 |
| Negative regulation of cellular biosynthetic process                    | 1220  | 13.3     | 37   | 3.58e-07 |
| Programmed cell death                                                   | 2160  | 23.5     | 52   | 3.79e-07 |
| Negative regulation of biosynthetic process                            | 1240  | 13.6     | 37   | 4.96e-07 |
| Negative regulation of nucleobase-containing compound metabolic process | 1130  | 12.3     | 35   | 4.96e-07 |
| Negative regulation of RNA metabolic process                           | 1020  | 11.2     | 33   | 5.1e-07  |
| Response to organic substance                                           | 2500  | 27.3     | 56   | 9.13e-07 |
| Protein import into nucleus                                             | 228   | 2.49     | 15   | 9.82e-07 |
| Nuclear import                                                         | 232   | 2.53     | 15   | 1.19e-06 |
| Interaction with host                                                   | 426   | 4.65     | 19   | 6.26e-06 |
| Regulation of defense response                                          | 519   | 5.6      | 21   | 6.95e-06 |
| Nucleocytoplasmic transport                                             | 388   | 4.24     | 18   | 6.99e-06 |
| Intracellular receptor mediated signaling pathway                        | 270   | 2.95     | 13   | 8.7e-06  |
| Nuclear transport                                                       | 392   | 4.28     | 18   | 7.59e-06 |
| Protein import                                                         | 272   | 2.97     | 15   | 7.68e-06 |
| Regulation of response to stimulus                                      | 3360  | 36.7     | 65   | 8.69e-06 |
| Regulation of nucleocytoplasmic transport                              | 172   | 1.88     | 12   | 9.82e-06 |
| Regulation of intracellular transport                                   | 290   | 3.17     | 15   | 1.59e-05 |
| Regulation of molecular function                                        | 2256  | 24.5     | 49   | 1.61e-05 |
| Regulation of cytokine production                                       | 513   | 5.6      | 20   | 1.79e-05 |
| Myeloid cell differentiation                                            | 296   | 3.23     | 15   | 1.89e-05 |
| Organ development                                                       | 3290  | 35.9     | 63   | 1.9e-05  |
| Regulation of developmental process                                     | 1880  | 20.5     | 43   | 2.38e-05 |
| Central nervous system development                                      | 784   | 8.56     | 25   | 2.56e-05 |
| Cell surface receptor signaling pathway                                 | 3340  | 36.4     | 63   | 2.6e-05  |
| Response to chemical stimulus                                           | 3830  | 41.8     | 69   | 3.49e-05 |
| Negative regulation of transcription from RNA polymerase II promoter    | 552   | 6.03     | 20   | 4.68e-05 |
| Regulation of transforming growth factor beta receptor signaling pathway | 107   | 1.17     | 9    | 4.7e-05  |
| Regulation of cell proliferation                                        | 1430  | 15.6     | 35   | 6.37e-05 |
| Defense response                                                        | 1510  | 16.5     | 36   | 8.16e-05 |
| Intracellular protein kinase cascade                                     | 1140  | 12.4     | 30   | 9.22e-05 |
| Negative regulation of cell proliferation                              | 585   | 6.39     | 20   | 9.97e-05 |
| Negative regulation of transferase activity                            | 189   | 2.06     | 11   | 0.00019  |
| Regulation of transcription from RNA polymerase II promoter             | 1610  | 17.6     | 33   | 0.00012  |
| Protein targeting                                                      | 545   | 5.95     | 19   | 0.000123 |
| Regulation of cell differentiation                                      | 1290  | 14.1     | 32   | 0.000123 |
| Positive regulation of developmental process                            | 817   | 8.92     | 24   | 0.000132 |
| Response to stress                                                      | 4150  | 45.3     | 71   | 0.000132 |
| Positive regulation of defense response                                 | 273   | 2.98     | 13   | 0.000136 |
| Regulation of protein metabolic process                                 | 1820  | 19.9     | 40   | 0.000136 |
| Positive regulation of signal transduction                              | 998   | 10.9     | 27   | 0.000142 |
| Regulation of multicellular organ simplistic process                    | 2480  | 27.1     | 49   | 0.000167 |
| Regulation of immune system                                              | 1190  | 13       | 30   | 0.000172 |
| Positive regulation of transcription, DNA-dependent                     | 1260  | 13.7     | 31   | 0.000173 |
| Regulation of sequence-specific DNA binding transcription factor activity| 372   | 4.06     | 15   | 0.000184 |
| G1 phase                                                                | 49    | 0.55     | 6    | 0.00019  |
| Negative regulation of apoptotic process                               | 679   | 7.41     | 23   | 0.000195 |
| Negative regulation of phosphorylation                                 | 246   | 2.69     | 12   | 2.0e-04  |
| Regulation of catalytic activity                                        | 1370  | 18.9     | 38   | 2.0e-04  |
| Cytokine production                                                     | 576   | 6.29     | 19   | 0.000203 |
| Positive regulation of transcription from RNA polymerase II promoter    | 800   | 8.73     | 23   | 0.000223 |
| Regulation of protein import into nucleus                               | 137   | 1.5      | 9    | 0.000223 |
| Negative regulation of phosphate metabolic process                     | 293   | 3.2      | 13   | 0.000228 |
| Negative regulation of programmed cell death                            | 691   | 7.54     | 21   | 0.000259 |
| Category                                               | q-value | FDR   | p-value |
|--------------------------------------------------------|---------|-------|---------|
| Negative regulation of binding                         | 0.000251| 7     | 0.852   |
| Immune response                                        | 0.000262| 11    | 1.58    |
| Defense response to virus                              | 0.000262| 11    | 1.58    |
| Cell proliferation                                     | 0.000271| 21    | 7.88    |
| Multi-organism process                                 | 0.000281| 27    | 11.17   |
| Regulation of cytokine production                      | 0.000281| 27    | 11.17   |
| Regulation of cell cycle                               | 0.000336| 24    | 9.67    |
| Positive regulation of catalytic activity              | 0.000345| 27    | 11.17   |
| Regulation of gene expression                          | 0.000373| 73    | 48.9    |
| Positive regulation of RNA metabolic process           | 0.000389| 31    | 14.6    |
| Intracellular signal transduction                      | 0.000404| 47    | 4.00E-04|
| Regulation of immune response                          | 0.000427| 21    | 7.94    |
| Regulation of transcription, DNA-dependent             | 0.000427| 64    | 4.12    |
| Regulation of nucleobase-containing compound metabolic process| 0.000519| 73    | 49.5    |
| Immune effector process                                | 0.000519| 18    | 6.29    |
| Positive regulation of sequence-specific DNA binding transcription factor activity| 0.000563| 10    | 2.16    |
| Immune system process                                  | 0.000578| 50    | 29.7    |
| Cell cycle                                             | 0.000604| 38    | 20.3    |
| Interphase of mitotic cell cycle                       | 0.000723| 15    | 4.75    |
| Negative regulation of protein metabolic process       | 0.000732| 17    | 5.9     |
| Viral reproductive process                             | 0.000778| 18    | 6.52    |
| Regulation of transferase activity                     | 0.000885| 21    | 8.39    |
| Interphase                                             | 0.000885| 15    | 4.84    |
| Reproductive process                                   | 0.000885| 36    | 19      |
| Positive regulation of metabolic process               | 0.000885| 49    | 29.4    |
| Embryo development                                     | 0.000907| 26    | 11.8    |
| Cell cycle process                                     | 0.000997| 31    | 15.4    |
| Positive regulation of response to stimulus            | 0.000997| 33    | 17      |
| Cellular response to stress                            | 0.01013 | 34    | 17.7    |
| Positive regulation of nucleobase-containing metabolic process| 0.01013| 32    | 16.3    |
| Regulation of cellular protein metabolic process       | 0.01013 | 33    | 17      |
| Regulation of RNA metabolic process                    | 0.01016 | 64    | 42.6    |
| Intracellular protein transport                        | 0.01019 | 21    | 8.66    |
| Transcription initiation from RNA polymerase II promoter| 0.01019 | 19    | 2.39    |
| Cell development                                       | 0.01019 | 37    | 20.1    |
| Hematopoietic or lymphoid organ development            | 0.01112 | 19    | 7.41    |
| G1 phase of mitotic cell cycle                         | 0.01112 | 5     | 0.513   |
| Reproduction                                           | 0.01118 | 37    | 20.3    |
| Enzyme linked receptor protein signaling pathway       | 0.01118 | 22    | 12.8    |
| Negative regulation of cellular protein metabolic process| 0.01118| 15    | 5.06    |
| Positive regulation of cell differentiation            | 0.01118 | 17    | 6.23    |
| Multicellular organismal development                   | 0.01118 | 85    | 62.4    |
| Cellular protein catalytic process                     | 0.0112  | 16    | 5.66    |
| Regulation of protein modification process             | 0.0112  | 28    | 13.6    |
Extended Methods

**Flow- and mass cytometry**

Details of all antibodies used for flow cytometry are found in sTable1 and for mass cytometry in sTable2. Recombinant Human NKG2D Fc Chimera Protein (#1299-NK-050, R&D Systems) was biotinylated using the One-Step Antibody Biotinylation Kit (Miltenyi Biotec) as per manufacturer’s recommendation. For all surface antibodies, cells were stained in PBS containing 2% FBS and 2 mM EDTA (Thermo Fisher, USA) for 15 min at room temperature followed by two washes. A second extracellular stain and incubation was performed with streptavidin and the pan-KIR binding antibodies KIR2DL2/S2/L3 and KIR2DL1/S1. After washing, cells were fixed for 15-20 min at 4 °C using BD Cytofix/Cytoperm™ Fixation and Permeabilization Solution (BD Biosciences). After two washes with BD Perm/Wash™ Buffer (BD Biosciences), intracellular markers were stained in this buffer for 30 min at room temperature. For HLA-C1/C2 expression, cells were stained with recombinant human KIR2DL1/CD158a or KIR2DL3/CD158b2-Fc Chimera Proteins (R&D systems) at 30 µg/ml in PBS 60 mins on ice, washed twice and incubated 15 mins at room temperature with Goat anti-Human IgG Fc Secondary Antibody, conjugated to PE. Samples were incubated with the secondary antibody only or isotype IgG1-PE as controls. Cells were fixed and acquired as described above. Flow cytometry samples were acquired on a BD LSRII or LSR Fortessa flow cytometer (BD Biosciences). Acquired data were analyzed in FlowJo v.10.6 (BD Biosciences) gated on live CD19−CD3−CD56+ single cells or CTV+CD56+ target cells for cytotoxicity assays. AML blasts were defined by low CD45 expression. For visualization of flow cytometry data, t-Distributed Stochastic Neighbor Embedding (t-SNE), an unsupervised nonlinear dimensionality reduction algorithm, was employed in FlowJo software with default settings and included all fluorescent markers stained for. For mass cytometry experiments, cells were stained with Cell-ID Intercalator-103Rh (Fluidigm) for viability assessment followed by an Fc
blocking reagent and a surface antibody cocktail. Antibodies that were not available pre-labeled were conjugated to metal isotopes using Maxpar X8 Antibody Labeling Kits (Fluidigm). Cells were fixed with 2% formaldehyde in Maxpar PBS (Fluidigm). Samples were washed, permeabilized and barcoded using the Cell-ID 20-Plex Pd Barcoding Kit (Fluidigm) before being pooled. Samples were then resuspended in cold methanol and stored overnight at -20 °C. Upon acquisition, cells were stained with an intracellular antibody cocktail, labeled with Cell-ID Intercalator-Ir (Fluidigm) and resuspended in Maxpar Cell Acquisition Solution (Fluidigm). EQ Four Element Calibration Beads (Fluidigm) were added to the cells before analysis on a Helios CyTOF instrument (Fluidigm). FCS files were concatenated, normalized and debarcoded using Helios software (Fluidigm), and gated using FlowJo v.10 (BD Biosciences). For subsequent analysis, data was imported into R (R Core Team, 2019) using the flowCore package, and transformed using arcsinh(x/5). For mass-cytometry data, t-SNE was performed using the Rtsne R package with default settings and results were visualized using the ggplot2 R package.

**Cellular indexing of transcriptomes and epitopes by sequencing - CITE-Seq.**

CITE-seq was performed as outlined in Biolegend ‘TotalSeqTM-A Antibodies and Cell Hashing with 10x Single Cell 3’ Reagent Kit v3 3.1 Protocol’ with minor modifications. Briefly, cells were stained with CD56-biotin (REA 196), followed by TotalSeq antibodies and TotalSeq streptavidin-PE and Live/Dead Aqua (Invitrogen) and subsequently sorted for viable CD56+ cells by flow cytometry. Day 0 cells from the same donor were thawed and recovered in IL-15 (3 ng/ml, Miltenyi) overnight prior to staining and sorting. To distinguish day 0 and day 11 cells, streptavidin-PE conjugated to different oligos were used, and equal number of sorted viable CD56+ cells from the two cell preparations were pooled as one sample. This was followed by standard 10X Genomics library preparation and sequencing workflow (Genomics
Core Facility UiO, Oslo, Norway). Sequencing was performed with the recommended read
lengths on the NextSeq500 sequencer (Illumina). Data were demultiplexed using bcl2fastq2
(Illumina) and further processed with the Cell Ranger version 6.0.1 software package to
generate raw count-matrix files. The antibody-derived tags (ADTs) were counted using the
package CITE-Seq-Count\(^2\). CITE-Seq data was analyzed using Scanpy\(^3\) as follows: During pre-
processing cells were filtered out based on number of genes and reads in each cell (minimum
300 genes, maximum 30 000 total counts) and genes were filtered based on number of cells in
which the genes were expressed (minimum 3 cells). Cells with more than 10% mitochondrial
reads were also filtered out. Cells with expression of CD3 were filtered out together with cells
expressing both day 0 and day 11 ADTs. The remaining cells (n=8013) were analyzed using
the probabilistic model totalVI\(^4\). This model was used to get a representation of the cells that
were used for clustering and for embedding using UMAP, get denoised expression values for
both the ADTs and mRNA, and to perform the statistical tests to identify differentially
expressed (DE) genes. Heatmaps, UMAP plots, violin plots and dot plots were generated using
Scanpy\(^3\). The p-values and fold change computed from the DE framework in totalVI were
plotted as volcano plots using bioinfokit\(^5\). The genes down- and upregulated in CAR T cells
after continuous antigen exposure were extracted from Good et al.\(^1\) and for both gene sets the
overlap with the genes upregulated in the day 11 CITE-Seq cluster was shown in Venn
diagrams. AUCell\(^6\) was used to identify the activity of a T cell-derived exhaustion signature
from Feucht et al.\(^7\) in the CITE-Seq data and the AUC scores were plotted as violin plots
showing the activity of this signature in the various clusters.

**Tumor cell lines**

The K562 cell line (chronic myeloid leukemia) and 721.221 (EBV-transformed B cell line)
were obtained from ATCC. HL-60 (acute myeloid leukemia) was obtained from the Miller lab.
PANC-1 (Pancreatic ductal adenocarcinoma) and A549 (lung adenocarcinoma) were obtained from Fate Therapeutics. K562, A549 and PANC-1 along with NALM-6 (B cell acute lymphoblastic leukemia) and BJAB (Burkitt’s Lymphoma) were re-authenticated using STR fingerprinting (ATCC). The K562, 721.221, BJAB and NALM-6 cell lines were cultured in RPMI-1640 (Thermo Fisher) with 10% FBS and 1 mM L-glutamine (Thermo Fisher) (‘complete RPMI’) and with or without 100 mg/ml of penicillin/streptomycin (Invitrogen). HL-60 was grown in IMDM, A549 and PANC-1 in DMEM, all with 10% FBS and with or without 100 mg/ml of penicillin/streptomycin (Invitrogen). Cell lines were KIR-ligand genotyped with PCR-based KIR-HLA Ligand kit (Olerup SSP). All cell lines were mycoplasma-tested using one of the following reagents: MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza), the PCR-based LookOut Mycoplasma (Sigma Aldrich), or Mycoplasma check (Eurofins Genomics).

**Flow cytometry-based NK cell functional assays**

NK cell degranulation and cytokine production was evaluated by mixing ADAPT-NK products at different E:T ratios with tumor targets and incubating in complete RPMI in 96-well U-bottom plates for 6 h at 37 °C and 5% CO₂. Monensin (GolgiStop, 1:1500, BD Biosciences), Brefeldin A (GolgiPlug, 1:1000, BD Biosciences) and anti-CD107a were added 1 h into the 6 h co-culture. To measure ADCC, MabThera (1 μg/ml) was added to co-culture experiments with 721.221 cells. Phorbol-12-myristate-13-acetate (PMA) (50 ng/ml) + ionomycin (1 μg/ml) (Sigma) was used as positive control. NK cell cytotoxicity was evaluated by mixing ADAPT-NK products at different E:T ratios with single target cell lines pre-stained with 2 μM of CellTrace Violet (Thermo Fisher) and incubating in 96-well V-bottomed plates for 6 h at 37 °C and 5% CO₂. FITC-DEVD-FMK (Abcam), a caspase-3 inhibitor used to detect the active/cleaved form of Caspase-3 was added at the start of the incubation and cells were
subsequently stained with eFluor780 Fixable viability dye (Thermo Fisher). All samples were fixed and acquired on a flow cytometer as described above. Dead cells were defined as CellTrace\(^+\) Caspase-3\(^+\) and/or dead cell marker (DCM)\(^+\) and specific cytotoxicity calculated as:

\[
\frac{\% \text{ dead experimental} - \% \text{ dead target only}}{(100\% - \% \text{ dead target only})} \times 100\%.
\]

For competitive cytotoxicity assays, HLA-C1- and HLA-C2-dimer expressing K562 were stained with either high (2 µM) or low (0.5 µM) concentrations of CellTrace Violet respectively, prior to being mixed at a 1:1 ratio and subsequently seeded at multiple E:T ratios with ADAPT-NK cells. The two different target cells were then identified based on CellTrace\(^+\) high or low, and in these populations the frequency of live (Caspase-3\(^-\) and/or DCM\(^-\)) remaining in the culture out of the total live CTV\(^+\) cells was determined. Cytokine stimulations with IL-12 (10 ng/ml) and IL-18 (10 ng/ml) (both Biotechne) were performed for 25 h at 37 °C and 5% CO\(_2\), and Monensin and Brefeldin A were added after 20 h.

**Genetic cell engineering**

In brief, K562 cells (ATCC Cat: CCL-243) were engineered by Fate Therapeutics using an in-house third-generation lentiviral transfer plasmid designed to contain a chimeric protein with the HLA-G leader peptide (1-24) and the mature HLA-E*0103 (22-358) driven by an EF1α promoter. Lentivirus was produced in Lenti-X™ 293T cells (Clontech 632180) using the second-generation packaging system that includes a transfer plasmid, a packaging plasmid, for expression of gag, pol, tat and rev, and an envelope plasmid, for expression of envelope protein VSV-G. The transduced population was sorted to enrich for cells expressing high levels of HLA-E. For HLA-C and -E variants, K562 were engineered in-house using VSV-G-pseudotyped lentiviral particles with a mammalian LeGO-G2 expression vector, to express the following synthetic proteins: β2m–HLA-C1 single chain dimer (HLA-C1-dimer): B2M signal peptide sequence and mature B2M (without STOP-codon) fused covalently to mature HLA-
C*07:01 (without STOP-codon) through a flexible (G4S)4 linker sequence, followed by a T2A peptide sequence and murine Thy1.1 (CD90.1); β2m–HLA-C2 single chain dimer (HLA-C2-dimer): B2M signal peptide sequence and mature B2M (without STOP-codon) fused covalently to mature HLA-C*04:01 (without STOP-codon) through a flexible a (G4S)4 linker sequence, followed by a T2A peptide sequence and murine Thy1.2 (CD90.2); HLA-G3,11–β2m–HLA-E single chain trimer (HLA-E-trimer): B2M signal peptide sequence and the HLA-G3,11 signal peptide sequence (VMAPRTLFL) fused covalently by a flexible (G4S)3 linker sequence to mature B2M (without STOP-codon), which is further covalently linked to mature HLA-E*01:01 by a flexible (G4S)4 linker sequence. Expression cassettes were sub-cloned into the mammalian expression vector LeGO-G2 (kind gift from Boris Fehse, Addgene plasmid #251917; http://n2t.net/addgene:25917; RRID: Addgene_25917) after removing EGFP. LeGO-HLA-C1-dimer, LeGO-HLA-C2-dimer, and LeGO-HLA-E-trimer were used to generate VSV-G-pseudotyped lentiviral particles. For production of VSV-G-pseudotyped lentiviral particles, 22 x 10⁶ LentiX 293T cells (Takara) were plated into T125 flask one day prior to transfection with 15 µg of LeGO expression vector, 10 µg pRSV-Rev (Addgene plasmid #12253), 15 µg pMDLg/pRRE (Addgene plasmid #12251) and 5 µg pCMV-VSV-G (Addgene plasmid #8454) using Lipofectamine 3000 (Invitrogen). Supernatant containing lentiviral particles was harvested at 24 and 48 h post transfection and concentrated using LentiX concentrator (Takara). Viral titers were determined using Lenti-X GoStix (Takara). Concentrated virus was stored as aliquots at -80 °C until use. Wildtype K562 cells were then transduced with 20 MOI of lentiviral particles at 2,5 x10⁵ / 24-well in a total volume of 1 ml. The plates were centrifuged at 900 g for 1 h at 32 °C followed by incubation at 37 °C overnight. Media was exchanged the next day and cells were cultivated for >5 passages. After >5 passages, genetically engineered K562 lines were sorted on a MA900 cell sorter (Sony) for high HLA-C-dimer expression using recombinant human KIR2DL3 Fc chimera proteins and
KIR2DL3 Fc chimera proteins, respectively or sorted for high HLA-E-trimer expression using an anti-HLA-E antibody (3D12, BioLegend). Sorted cells were routinely monitored for HLA-C-dimer and HLA-E-trimer expression and tested for presence of mycoplasma (Eurofins Mycoplasma check). Further, NALM-6 were engineered to knock-out HLA-E using Cas9 and a pool of synthetic guide RNAs (sgRNA) (5’- AUUUCCACACUUCGUGUCC-3’, 5’-ACAACGACGCGCCGAGUCC-3’ and 5’-GGGGUCAGAGUAUUGGGACC-3’) (CRISPRRevolution sgRNA EZ Kit, Synthego). 180 pmol sgRNA and 20 pmol Cas9 (Synthego) were used to form ribonucleoprotein (RNP) complexes. 1.5 x 10^5 cells in 3P buffer (5 mM KCl, 15 mM MgCl2, 90 mM NaCl, 10 mM Glucose, 0.4 mM Ca(NO3)2, 40 mM Na2HPO4/NaH2PO4 pH7.2) were mixed with RNP and electroporated using Lonza 4D-Nucleofector system with pulse code CV-104. Cells were grown for 7 days before single cells were sorted on a FACS Aria II (BD Biosciences) to establish multiple clones in 96-well round bottom plates. DNA sequencing and flow cytometry confirmed HLA-E depletion in the selected clones used in the study. HLA-E sgRNAs further targeted the HLA-C locus resulting in a combined KO for this line.

**Serial killing assays**

The cytotoxic potential of individual NK cells was evaluated using a previously described microwell chip screening assay. Briefly, NK cells labelled with 5 µg/ml CellTrace Yellow and target cells labelled with 5 µg/ml CellTrace Far Red (Thermo Fisher) were seeded in a microwell chip containing complete RPMI supplemented with 100 ng/ml Sytox Green (Thermo Fisher). Cells stochastically distributed in the 8064 60-µm-wide wells at an average effector to target ratio of 1:5. HLA-C matched and -mismatched co-cultures with the same ADAPT-NK product were investigated simultaneously in different compartments of the chip. The co-cultures were imaged every 3 h for 15 h using an inverted confocal microscope, LSM 880 (Carl
Zeiss AG) equipped with a 10x/0.5 Plan-Achromat objective and an environmental control unit (37°C, CO₂ 5%). The images were processed using a custom-built MATLAB script whereby live and dead cells were quantified at all time points. For each condition, a minimum of 500 wells containing a single NK cell at start along with at least 4 live target cells were included for analysis. Target cell death was adjusted for spontaneous death using wells containing only target cells.

**IncuCyte measurement of tumor killing**

Tumor killing was measured in real time using the IncuCyte® S3 platform. Target cells stably expressing NucLight Red (Essen Biosciences) were selected for NucLight Red expression after thawing with 2 µg/mL puromycin for 72 h, screened by flow cytometry for uniform NucLight Red expression and allowed to rest without puromycin for at least 48 h (1 passage) before assay. Target cells were seeded at 5000 cells/well in 200 µL of complete media and allowed to attach overnight in clear, 96-well flat-bottom plates (Thermo Fisher, cat. #167008). Wells used for target cells which habitually grow in suspension (HL60, K562, Bjab, Nalm6) were pre-coated with Poly-L-Ornithine solution (Sigma/Merck, cat #A-004-M). At assay start half of the media was removed and ADAPT-NK cells were serially diluted and added to the wells in SCGM (CellGenix) with 50 IU/mL IL-2 at different E:T ratios. Images (3/well) from at least two technical replicates for each condition were acquired every 90 min for 48 h, using a 10x objective lens and analyzed by IncuCyte Controller v2020A (Essen Biosciences). Graphed readouts represent percentage live target cells (NucLight Red⁺). To obtain percentage of live cells, live target cell count in each well was normalized by division to timepoint 0 h (T0), and then to the number of live cells in the “target cell only” control group, as calculated in the following equation: % Live cells = [(Tn/T0) test / (Tn/T0) ctrl] x 100. The means of the technical replicates for each condition were compiled for n=4-9 donors, tested in at least two
independent experiments for each target cell, and are displayed as mean (± SD). Area Under Curve for each condition was graphed and analyzed.

**In vivo AML tumor model**

Fifteen male 9-11 weeks old NOD.Cg-PrkdscidIl2rgtm1Wjl/SzJ mice (NSG, Jackson Laboratories) mice were injected intravenously with HL-60 cells stably expressing firefly luciferase (1.5x10^6/mouse). Four control mice on the same background received no injections. After allowing tumors to engraft for 4 days, bioluminescence imaging was performed, and mice were randomized into 3 groups. Mice in the first group received no treatment (tumor alone). Mice in the second group received intravenous injections of HLA-C/KIR matched ADAPT-NK cells (flat-dose 5 x10^6/mouse) expanded as above. Mice in the third group received intravenous injections of HLA-C/KIR mismatched ADAPT-NK cells (flat-dose 5x10^6/mouse). Mice receiving NK cell injections were also injected intraperitoneally with IL-15 (6ug/mouse, National Cancer Institute) twice weekly for three weeks. Bioluminescence imaging was performed weekly to track tumor burden using the IVIS Spectrum (Perkin-Elmer). Images were analyzed using Living Image software (Perkin-Elmer). D-Luciferin Sodium Salt (GoldBio) was dissolved into DPBS at a concentration of 30 mg/ml and injected intraperitoneally at a dose of 3 mg/20 g body weight.

**References extended methods**

1. Good, C.R. *et al.* An NK-like CAR T cell transition in CAR T cell dysfunction. *Cell* **184**, 6081-6100 e6026 (2021).
2. Gui, P.R.b.B.F.s.G. Hoohm/CITE-seq-Count: 1.4.2. Zenodo; 2019.
3. Wolf, F.A., Angerer, P. & Theis, F.J. SCANPY: large-scale single-cell gene expression data analysis. *Genome Biol* **19**, 15 (2018).
4. Gayoso, A. *et al.* Joint probabilistic modeling of single-cell multi-omic data with totalVI. *Nat Methods* **18**, 272-282 (2021).
5. Bedre, R. reneshbedre/bioinfokit: Bioinformatics data analysis and visualization toolkit. Zenodo; 2021.

6. Aibar, S. et al. SCENIC: single-cell regulatory network inference and clustering. *Nat Methods* **14**, 1083-1086 (2017).

7. Feucht, J. et al. Calibration of CAR activation potential directs alternative T cell fates and therapeutic potency. *Nat Med* **25**, 82-88 (2019).

8. Guldevall, K. et al. Microchip Screening Platform for Single Cell Assessment of NK Cell Cytotoxicity. *Front Immunol* **7**, 119 (2016).