

## Supplementary Information

**Supplementary Movie 1. Example of  $\gamma\delta$  T cell mediated death through granzyme B (a) or death receptors (b, start at 11 sec).** A498<sup>GBDR</sup> target cell stably expressing the dual fluorescent reporter NES-ELQTD-GFP-T2A-NES-VGPD-mCherry co-cultured with unlabelled  $\gamma\delta$  T cells. Under attack of a  $\gamma\delta$  T cell, the fluorescent reporter is cleaved and mCherry (granzyme B-mediated death (a) or GFP (death receptor-mediated death through caspase 8 cleavage (b) can diffuse in the nucleus. Frame rates are slowed down to highlight nuclear staining.

**Supplementary Movie 2. Examples for individual outcomes of  $\gamma\delta$  T cells co-cultured with A498<sup>GBDR</sup> target cells.** (a)  $\gamma\delta$  T cell migrating, dividing (timepoint 11 sec), and contacting A498 target cells during 16-hour assay, without being able to kill the target cell. (b, start at 21 sec) Consecutive kills by a  $\gamma\delta$  T cell. After three kills the  $\gamma\delta$  T cell divides (timepoint 42 sec) before continuing to kill the fourth target. (c, start at 53 sec)  $\gamma\delta$  T cell kills one target cell, keeps on migrating and dividing but is not able to mediate a second kill. Frame rates are slowed down to highlight nuclear staining and cell division.

**Supplementary Movie 3. Characterization of unidentified deaths.** (a) Both GFP and mCherry, are cleaved and diffuse into the nucleus, resulting in a double positive death. (b, start at 15 sec) The target cell starts shrinking before a signal in the nucleus is visible and then collapses over a long period. (c, start at 32 sec) The target cell shrinks and collapses quickly in 2 frames (or less) without a visible nuclei signal. Frame rates are slowed down to highlight nuclear staining and collapsing of the target cells.

**Supplementary Movie 4. Addition of 15  $\mu$ m recombinant granulysin to A498<sup>GBDR</sup>.** With the addition of granulysin, target cells are collapsing slowly. The fluorescent reporter can diffuse into the nucleus indicating nuclear envelope rupture.

**Supplementary Movie 5. Killing events by  $\gamma\delta$  T cells can be mediated through committed (a) or uncommitted (b, start at 11 sec) contacts.** (a)  $\gamma\delta$  T cell contacts the A498 target and converges toward the contact site forming a committed contact that lasts longer than 2 imaging frames. (b)  $\gamma\delta$  T cell is interacting with the target cell while continue to migrate. While no long contacts or commitment to the target are observed, the target is still killed. Frame rates are slowed down to highlight contact of  $\gamma\delta$  T cell and target cell.

**Supplementary Movie 6.  $\gamma\delta$  T cells without target cells in the 80  $\mu$ m well platform.** (a)  $\gamma\delta$  T cells are viable, migrating and proliferating inside the 80  $\mu$ m microwells over a 16-hour assay. (b, start at 18 sec) Treatment with HMBPP can lead to fratricide if  $\gamma\delta$  T cell are in close contact. Frame rate is slowed down to highlight fratricide.

**Supplementary Movie 7.  $\gamma\delta$  T cells co-cultured with A498<sup>GBDR</sup> target cells in 350  $\mu$ m wells show limited serial killing potential.**  $\gamma\delta$  T cell No. 1 (red) kills one target through death receptor-mediated death by cleaving caspase 8 (event E).  $\gamma\delta$  T cells No. 4 (cyan) and 10 (blue) are contacting targets together and kill two consecutive targets through granzyme B mediated death (event A and B).  $\gamma\delta$  T cell No. 8 (purple) kills two consecutive targets through granzyme B mediated death (event C and D). All remaining  $\gamma\delta$  T cells fail to induce target cell death. Frame rates are slowed down to highlight contact of  $\gamma\delta$  T cell, nuclear staining and target death.

**Supplementary Table 1:** List of the antibodies/staining used in this study.

| Antigen                | Clone       | Fluorophore     | Supplier         | Code        | RRID        |
|------------------------|-------------|-----------------|------------------|-------------|-------------|
| Actin/Phalloidin       |             | Alex Fluor 568  | Thermo Fisher    | A12380      |             |
| BTN2A1                 |             |                 | Sigma-Aldrich    | HPA019208   | AB_1845492  |
| CCR6                   | 11A9        | PE-Cy7          | BD Pharmingen    | 560620      | AB_1727440  |
| CCR7                   | 150503      | PE              | BD Pharmingen    | 560765      | AB_2033949  |
| CD107a                 | H4A3        | Biotin          | BD Pharmingen    | 555799      | AB_396133   |
| CD107a                 | H4A3        | BV421           | BD Horizon       | 562623      | AB_2737685  |
| CD107a                 | H4A3        | Alex Fluor 647  | BD Pharmingen    | 562622      | AB_2737684  |
| CD16                   | 3G8         | APC             | BD Pharmingen    | 561248      | AB_10612010 |
| CD16                   | 3G8         | BV605           | BD Pharmingen    | 563172      | AB_2744297  |
| CD160                  | BY55        | FITC            | Invitrogen       | MA5-16600   | AB_2538099  |
| CD19                   | SJ25C1      | PE-Cy7          | BD Pharmingen    | 557835      | AB_396893   |
| CD200R                 | OX108       | PE              | Invitrogen       | 12-9201-42  | AB_10717665 |
| CD223/Lag3             | 11C3C65     | PE              | BioLegend        | 369306      | AB_2629592  |
| CD244                  | eBioDM244   | APC             | Invitrogen       | 17-5837-42  | AB_10670227 |
| CD253/TRAIL            | RIK-2       | BV421           | BD Horizon       | 564243      | AB_2738696  |
| CD261/TRAIL-R1         | S35-934     | PE              | BD Pharmingen    | 564180      | AB_2738648  |
| CD262/TRAIL-R2         | YM366       | PE              | BD Pharmingen    | 565499      | AB_2732871  |
| CD27                   | M-T271      | BV421           | BD Horizon       | 562513      | AB_11153497 |
| CD277/BTN3A1           | 232-5       | BV605           | BD OptiBuild     | 742828      | AB_2741080  |
| CD277/BTN3A1           | 20.1, BT3.1 |                 | Invitrogen       | 14-2779-82  | AB_467550   |
| CD28                   | CD28.2      | BV510           | BioLegend        | 302936      | AB_2562030  |
| CD3                    | UCHT1       | Alexa Fluor 700 | BD Pharmingen    | 557943      | AB_396952   |
| CD45RA                 | HI100       | BV605           | BD Horizon       | 562886      | AB_2737865  |
| CD45RO                 | UCHL1       | PE-Cy7          | BD Pharmingen    | 560608      | AB_1727499  |
| CD56                   | B159        | PerCP-Cy5.5     | BD Pharmingen    | 560842      | AB_2033964  |
| CD62L                  | DREG-56     | PE              | BD Pharmingen    | 555544      | AB_395928   |
| CD63                   | H5C6-s      |                 | DSHB             |             | AB_528158   |
| CD8                    | SK1         | APC-H7          | BD Pharmingen    | 560179      | AB_1645481  |
| CD95/Fas               | DX2         | BV421           | BD Horizon       | 562616      | AB_2737679  |
| CX3CR1                 | 2A9-1       | BV605           | BD OptiBuild     | 744488      | AB_2742268  |
| CD178/FasL             | NOK-1       | APC             | BD Pharmingen    | 564262      | AB_2738714  |
| CD226/DNAM1            | DX11        | BV605           | BD OptiBuild     | 742495      | AB_2740828  |
| Chondroitin Sulphate 4 | 2B6         |                 | AMSBIO           | 270432      | AB_10891938 |
| CTLA4                  | BNI3        | BV421           | BD Horizon       | 562743      | AB_2737762  |
| Granulysin             | RB1         | Alexa Fluor 488 | BD Pharmingen    | 558254      | AB_2869123  |
| Granzyme A             | GzA-3G8.5   | PE              | Invitrogen       | 12-5831-82  | AB_2572631  |
| Granzyme B             | GB11        | Alexa Fluor 647 | BD Pharmingen    | 561999      | AB_10897997 |
| Granzyme B             | 496B        |                 | Invitrogen       | 14-8889-82  | AB_2572909  |
| Granzyme H             | P20718      | Alexa Fluor 488 | G Biosciences    | ITA2121     |             |
| Granzyme K             | G3H69       | Alexa Fluor 647 | BD Pharmingen    | 566655      | AB_2869812  |
| Granzyme M             | 4B2G4       | eFluor660       | Invitrogen       | 50-9774-42  | AB_2574374  |
| NKG2A                  | 131411      | BV605           | BD OptiBuild     | 747921      | AB_2872382  |
| NKG2C                  | REA205      | Vio Bright FITC | Miltenyi Biotech | 130-117-707 | AB_2728023  |
| NKG2D                  | 1D11        | PerCP-Cy5.5     | BD Pharmingen    | 562364      | AB_11154225 |
| Nkp30                  | p30-15      | BV510           | BD OptiBuild     | 743170      | AB_2741321  |

|                     |         |                 |                  |              |             |
|---------------------|---------|-----------------|------------------|--------------|-------------|
| Nkp44               | p44-8   | BV421           | BD Pharmingen    | 744299       | AB_2742129  |
| Nkp46               | 9E2     | PE-Cy7          | BD Pharmingen    | 562101       | AB_10894195 |
| PD1                 | EH12.1  | PE-Cy7          | BD Pharmingen    | 561272       | AB_10611585 |
| Perforin            | dG9     | Pacific Blue    | BioLegend        | 308118       | AB_10899565 |
| Perforin            | dG9     | Alexa Fluor 488 | BioLegend        | 308108       | AB_493252   |
| Perforin            | dG9     | PerCP-Cy5.5     | BD Pharmingen    | 563762       | AB_2738409  |
| Perforin            | Pf-344  | FITC            | Mabtech          | 3465-7       | AB_1925742  |
| Perforin            | Pf-344  | PF647P          | Mabtech          | 3465-72-100T | AB_2888642  |
| Streptavidin        |         | Alexa Fluor 568 | Invitrogen       | S11226       | AB_2315774  |
| TCR gd pan          | 11F2    | PE              | Miltenyi Biotech | 130-113-504  | AB_2733905  |
| TCR gd pan          | IMMU510 |                 | Beckman Coulter  | IM1349       | AB_131619   |
| TCR Vd1             | TS8.2   | FITC            | Invitrogen       | TCR2730      | AB_223624   |
| TCR Vd2             | B6      | PE              | BioLegend        | 331408       | AB_1089232  |
| TCR Vd2             | B6      | APC/Cy7         | BioLegend        | 331439       | AB_2860864  |
| TCR Vg9             | B3      | FITC            | BioLegend        | 331306       | AB_1236403  |
| TCR Vg9             | B3      | PE-Cy7          | BioLegend        | 331320       | AB_2814209  |
| Tigit               | A15153G | Alexa Fluor 647 | BioLegend        | 372724       | AB_2715972  |
| TIM3                | 7D3     | BV605           | BD OptiBuild     | 742856       | AB_2744030  |
| Viability stain 510 |         |                 | BD Horizon       | 564406       | AB_2869572  |
| Viability stain 700 |         |                 | BD Horizon       | 564997       | AB_2869637  |