## Supplementary Information

### Table. S1:

| NK Source     | Method of selection           | Feeder cells                                    | Stimulation                                      | Expansion                                                | References         |
|---------------|-------------------------------|------------------------------------------------|------------------------------------------------|----------------------------------------------------------|--------------------|
| PBMC          | Negative selection for NK     | Autologous and allogeneic osteoclasts          | IL-2, anti-CD16 mAb, sAJ2                        | 21,000-132,000 fold expansion at 20 days, 0.3-5.1 million at 31 days; 17-21 population doublings with an average of 19 population doublings within 4 weeks | Current paper |
| PBMC          | Whole PBMCs                   | None                                            | IL-2, anti-CD16Ab-immobilized flask, OK432      | 637–5712 fold at 21 days                                 | [6]                |
| PBMC (negative selection) | Negative selection for NK | Irradiated autologous PBMC’s (pretreated for 3-5 days with IL2, IL-15, and concanavalin A) | IL-2 and IL-15, (phytohemagglutinin (PHA) and ionomycin first 24 hours) | 100 fold at 16 days | [7]                |
| PBMC          | CD3 depletion of PBMCs        | Autologous irradiated PBMCs stimulated with OKT3 | IL-2                                            | 79-322 fold at 21-26 days                               | [8]                |
| PBMC          | Whole PBMCs                   | Wilms tumor cell line (HFWT)                    | IL-2                                            | 58-401-fold at 10-21 days                                | [9]                |
| PBMC | Whole PBMCs | K562-mb15-41BBL | IL-2 | 165 (4-567) fold at 14 days | [10] |
|------|-------------|-----------------|------|-----------------------------|-----|
| PBMC | Whole PBMCs | K562-mb15-41BBL | IL-2 | 21.6 (5.1-86.6) fold at 7 days | [11] |
| PBMC | Whole PBMCs | K562-GM (transmembrane expression of IL-15, 4-1BBL, and IL-18) | IL-2 | 500 fold at 21 days | [12] |
| PBMC | CD3 depletion followed by CD56+ selection | Irradiated EBV-TM-LCL | IL-2 | 490 (+/- 260) at 21 days | [13] |
| Cord Blood (CB) | CD34+ positive selection | None | SCF, TPO, IL-7, Fit3L, IL-15, IL-2, G-CSF, GM-CSF, IL-6, LIF, MIP-1α | >15,000 fold at 5 weeks | [14] |
| Cord Blood (CB) | CD34+ positive selection | None | SCF, Fit3L, TPO, IL-7, GM-CSF, G-CSF, IL-6, IL-15, LMWH, and IL-2 | >2,000 fold at 6 weeks (using GMP) | [15] |
| NK-92 | N/A (cell line) | None | IL-2 | 218- 250 fold at 15-17 days | [16] |
| PBMC (genetically modified with TERT for immortalization) | Whole PBMCs used initially, residual T-cells removed after 7 days using anti-CD3 Dynabeads | K562-mb15-41BBL (continued stimulation) | IL-2 | TERT transformed (130-227 population doublings over 1000 days) Non-transformed (11-20 population doublings at 8-15 weeks) | [17] |
PBMCs (after 7 days of culture, retrovirally transduced with NKG2D-DAP10-CD3ζ) | Whole PBMCs used initially, residual T-cells removed after 7 days using anti-CD3 Dynabeads | K562-mb15-41BBL | IL-2 | N/A | [18]

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Fig. S1A

| CD3<sup>+</sup>-FITC | Day 6   | Day 9   | Day 12  | Day 15  | Day 19  | Day 24  | Day 29  | Day 34  |
|----------------------|---------|---------|---------|---------|---------|---------|---------|---------|
| +sAJ2                | 7.24    | 0.33    | 10.9    | 1.74    | 0.67    | 1.77    | 1.62    | 0.82    |
|                      | 65.6    | 26.8    | 15.0    | 73.3    | 7.02    | 78.4    | 6.61    | 70.0    |
| +OCs                 | 29.8    | 1.77    | 29.4    | 1.22    | 19.6    | 1.65    | 18.2    | 1.16    |
|                      | 27.1    | 41.3    | 4.58    | 64.8    | 0.90    | 77.8    | 1.80    | 78.9    |
| +sAJ2+OCs            | 35.1    | 0.52    | 26.1    | 2.17    | 7.63    | 0.8    | 2.86    | 0.39    |
|                      | 4.98    | 59.4    | 7.31    | 64.5    | 3.19    | 88.4    | 2.01    | 94.7    |

CD16<sup>+</sup>CD56<sup>-</sup>-PE
Fig. S1D
**Fig. S1:** Higher expression of NK activating ligands by Osteoclasts.

To generate osteoclasts (OCs), monocytes were cultured in medium containing M-CSF (25 ng/ml) and RANKL (25 ng/ml) for 21 days. Highly purified NK cells (1x10^6 cells/ml) were treated with the combination of IL-2 (1000 U/ml) and anti-CD16mAb (3ug/ml) for 18 hours before they were co-cultured with autologous OCs in the presence or absence of sAJ2 bacteria at 1:2:4 ratios (OCs:NK:sAJ2), respectively. Surface expression of CD3, CD16, and CD56 was analyzed in 1x10^4 lymphocyte samples from co-cultures at days 6, 9, 12, 15, 19, 24, 29, and 34 using flow cytometry, and culture medium was refreshed and supplemented with rh-IL-2 (1000 U/ml) **(A)**. Cells were co-cultured as described in Fig. S1A and expanded lymphocytes were counted manually using microscope **(B)**. Monocytes were isolated from the PBMCs of a healthy donor. To generate dendritic cells, monocytes were cultured in medium containing GM-CSF (150 ng/ml) and IL-4 (50 ng/ml) for 8 days. Osteoclasts were generated and NK cells were purified as described in Fig. S1A, before they were co-cultured with autologous cells in the presence of sAJ2 bacteria at 1:2:4 ratios (target cells:NK:sAJ2), respectively. On day 6 of the culture, the culture media was removed and the NK cells were treated with rh-IL-2 for 5 hours before the supernatants were harvested, and IFN-γ secretions were determined using single ELISA **(C)**. Monocytes were isolated, dendritic cells and osteoclasts were generated as described in Figs. S1A and D, OSCSCs and K562 tumor cell lines were cultured as described in materials and methods, 1x10^4 cells were used to analyze MHC-1, CD54, KIR2, KIR3, KLRG1 and MICA/B surface expressions, employing PE-conjugated antibodies and flow cytometry. IgG2 isotype was used as a control **(D)**.
Fig. S2A

| CD16^+CD56^+PE | CD3-FITC |
|-----------------|----------|
| 1.34            | 86.2     |
| 7.54            | 4.95     |
| 11.8            | 0.04     |

| CD8-PE          | CD3-FITC |
|-----------------|----------|
| 30.3            | 58.1     |
| 11.4            | 0.01     |

| CD4-PE          | CD3-FITC |
|-----------------|----------|
| 11.7            | 0.08     |

Fig. S2B

**NK cells**

| IgG2a-PE | IgG2a-FITC |
|----------|------------|
| 99.8     | 0.17       |

| CD16^+CD56^+PE |
|----------------|
| 4.75           |
| 95.2           |

**T cells**

| IgG2a-PE | IgG2a-FITC |
|----------|------------|
| 99.8     | 0.17       |

| CD16^+CD56^+PE |
|----------------|
| 92.4           |
| 3.2            |
Fig. S2. Unlike NK cells, T cells purified from osteoclast-expanded NK cells do not mediate cytotoxicity against OSCSCs and secrete IFN-γ moderately.
Freshly purified NK cells were treated and co-cultured with monocyte-derived autologous osteoclasts as described in Materials and Methods. Surface expression of CD3, CD16, CD56, GL3 (TCR γ/δ), CD4 and CD8 was analyzed in lymphocyte samples from co-cultures at day 9 using FITC- and PE-conjugated antibodies and flow cytometry (A). NK cells were treated and co-cultured with autologous osteoclasts as described in Fig. S1A and on day 9, CD3T-positive cells were sorted out using CD3T positive selection kit, purity of CD3T-negative (NK) cells was assessed using CD3, CD16, CD56 FITC and PE-conjugated antibodies and flow cytometry (B). CD3T-positive cells and CD3T-negative cells (CD16 positive cells) were treated with rh-IL-2 (1000 U/ml) for 18-20 hours before they were tested for cytotoxicity using a standard 4-hour $^{51}$Cr release assay against the OSCSCs (C) and K562 (D) cell lines. The lytic units 30/10$^6$ cells were determined using the method described in Materials and Methods, for OSCSCs and K562 respectively. The supernatant was harvested from the culture and IFN-$\gamma$ secretion was determined using single ELISA (E). NK cells, CD3T, CD4T, CD8T, and $\gamma\delta$T cells were purified from PBMC as described in materials and methods, and were activated with rh-IL-2 for 18-20 hours, before they were tested for cytotoxicity using a standard 4-hour $^{51}$Cr release assay against the OSCSCs (F). The lytic units 30/10$^6$ cells were determined using the inverse number of lymphocytes required to lyse 30% of OSCSCs x 100 (F). NK and T cells were purified from PBMCs as described in materials and methods, NK cells were treated as described in Materials and Methods. T cells were activated with anti-CD3 (1 ug/ml) and anti-CD28 (3 ug/ml) 18-20 hours before they were cultured with autologous OCs, and expanded lymphocytes were counted manually using microscope day 4 after the culture (G). NK and T cells were purified and cultured with OCs, and counted on day 4 as described in Fig. S2G, fold expansion of
lymphocytes expanded by the OCs were divided by fold expansion of lymphocytes without the OCs (H).

Fig. S3A

Fig. S3B
To generate osteoclasts, monocytes were cultured in medium containing M-CSF (25 ng/ml) and RANKL (25 ng/ml) for 21 days, K562 tumor cell lines were cultured as described in materials and methods. Highly purified NK cells (1x10^6 cells/ml) were treated with the combination of IL-2 (1000 U/ml) and anti-CD16mAb (3ug/ml) for 18 hours before they were co-cultured with K562 and autologous OCs in the presence of sAJ2 bacteria at 1:2:4 ratios (OCs:NK:sAJ2), respectively. Cells from the cultured were counted manually using microscope on day 6, 10 and 13 (A). The osteoclasts generated as described in Fig. S1A and K562 tumor cells lines were irradiated at 40 grays (Gy) as described in the materials and methods. NK cells were purified and treated as described in Materials and Methods, before they were co-cultured with irradiated K562 and irradiated autologous OCs in the presence of sAJ2 bacteria at 1:2:4 ratios (OCs:NK:sAJ2), respectively. Cells from the cultured were counted manually using microscope on day 6, 10 and 13 (B). NK cells were purified and cultured with OCs and K562 as described in Fig. S1A, cytotoxicity of lymphocytes co-cultured for 6 days was determined using a standard 4-hour $^{51}$Cr release assay against OSCSCs. The lytic units 30/10^6 cells were determined using method described in Fig. S2F (C). NK cells were purified and cultured with OCs and K562 as described in Fig. S3B, cytotoxicity of lymphocytes co-cultured for 6 days was determined using a standard 4-hour $^{51}$Cr release assay against OSCSCs. The lytic units 30/10^6 cells were determined using method described in Fig. S2F (D). NK cells were purified and cultured with OCs and OSCSCs as described in Fig. S3A, cytotoxicity of lymphocytes co-cultured for 6 days was determined using a standard 4-hour $^{51}$Cr release assay against OSCSCs. The lytic units 30/10^6 cells were determined using method described in Fig. S2F (E). NK cells were purified and cultured with OCs and K562 as described in Fig. S2A,
supernatant was harvested on day 3, 6, 7, 10 and 13, and IFN-γ secretion was determined using single ELISA (F). NK cells were purified and cultured with irradiated OCs and irradiated K562, as described in Fig. S3B, supernatant was harvested on day 3, 6, 7, 10 and 13, and IFN-γ secretion was determined using single ELISA (G). NK cells were purified and cultured with OCs and OSCSCs as described in Fig. S3A, supernatant was harvested on day 1, 3, 6 and 8, and IFN-γ secretion was determined using single ELISA (H).
Fig. S4C

Number of Lymphocytes x 10^6

Day 36  Day 32  Day 28  Day 24  Day 21  Day 17  Day 13  Day 10  Day 6  Day 0

Healthy Donor  Cancer Patient

Fig. S4D

Number of NK cells x 10^6

Day 36  Day 32  Day 28  Day 24  Day 21  Day 17  Day 13  Day 10  Day 6  Day 0

Healthy Donor  Cancer Patient

Fig. S4E

Number of T cells x 10^6

Day 36  Day 32  Day 28  Day 24  Day 21  Day 17  Day 13  Day 10  Day 6  Day 0

Healthy Donor  Cancer Patient
Fig. S4F

Number of NK cells x 10^6

Healthy Donor
Cancer Patient

Day 0  Day 6  Day 10  Day 13  Day 17  Day 21  Day 24  Day 28  Day 32  Day 36

Number of NK cells x 10^6

Healthy Donor
Cancer Patient

Day 0  Day 6  Day 9  Day 12  Day 15  Day 18  Day 21  Day 24  Day 27  Day 31
Fig. S4. Small fraction of contaminating T cells within purified NK cells from cancer patient expand faster and crowd out NK cells likely due to decreased NK cell function.

Freshly purified NK cells from a healthy donor and a pancreatic cancer patient were treated and co-cultured with monocyte-derived allogeneic (from different healthy donor) osteoclasts as described in Fig. S1A. Surface expression of CD3, CD16 and CD56 was analyzed in 1x10^4 lymphocyte samples from co-cultures at days 6, 10, 13, 17, 21, 24, 28, 32 and 36 of cancer patient (A) and healthy donor (B) using FITC- and PE-conjugated antibodies and flow cytometry. After 6, 10, 13, 17, 21, 24, 28 and 32 days of co-culture, expanded lymphocytes were counted manually using microscope (C). Cells counted as mentioned in Fig. S4C and were adjusted based on the surface expression analyzed in Fig. S4A and S4B to determine the number T/NKT cells (D) and NK cells at each day (E and F). Cytotoxicity of lymphocytes co-cultured for 18-20 hours, 13, 20 and 32 days was determined using a standard 4-hour ^51Cr release assay against OSCSCs. The lytic units 30/10^6 cells were determined using method described in Fig. S2F (F). Lytic units from Fig. S4F was adjusted based on surface expression analyzed is Fig. S4A and S4B to determine the cytotoxicity mediated by 1 NK cell against OSCSCs (G). The supernatant was harvested from the overnight, 6, 10, 13, 17, 21, 24, 28 and 32 days of co-culture and IFN-γ (H), IL-10 (I), and IL-6 (J) secretion was determined using single ELISA.
Fig. S5. Osteoclast-expanded NK cells retain their cytokine secretion and cytotoxic function after freezing.

Freshly purified NK cells were treated and co-cultured with monocyte-derived autologous osteoclasts as described in Fig. S1A, day 9 after the cultures, expanded NK cells were frozen. NK cells were thawed and treated with rh-IL-2 (1000 U/ml), day 6 and day 9 after the culture, the supernatant was harvested and IFN-γ secretion was determined using single ELISAs (A). NK cells were cultured as described in Fig. S1A, and the cytotoxicity of lymphocytes day 6 and 9 after the culture was determined using a standard 4-hour $^{51}$Cr release assay against OSCSCs. The lytic units $30/10^6$ cells were determined using method described in Fig. S2F (B)
Fig. S6. Phenotype of CD3 T cell depleted lymphocytes from the splenocytes of hu-BLT mice.

Humanized-BLT (hu-BLT; human bone marrow/liver/thymus) mice were generated by surgical implantation of human fetal liver and thymus tissue under the renal capsule of 6-8 weeks old immunocompromised NOD.CB17-Prkdcscid/J and NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice. 4-6 weeks post tissue transplant, mice were sub-lethally irradiated and intravenously injected with CD34+ cells isolated from fetal liver to support full reconstitution of human bone marrow. 8-12 weeks after injection with CD34+ cells, reconstitution of human immune system was analyzed using blood specimens. At the end of this experiment engraftment of human immune cells was confirmed by staining splenocytes and bone marrow cells with anti-human CD45, CD3, CD4 and CD8 antibodies and analyzed by flow cytometry (data not shown). Successfully reconstituted hu-BLT mice (levels and lineages of T cells comparable to healthy donors) were orthotopically injected with 1x10^6 of human OSCSCs into the floor of the mouth. Disease
progression and weight loss was monitored for another 3-4 weeks. Animals were sacrificed, spleens were harvested from the sacrificed animals, and single cell suspensions were obtained as described in materials and methods. CD3T cells were sorted out using human CD3T positive selection kit. Flow through cells (CD3-negative cells) were analyzed for surface expression of human CD3, CD16, CD56, CD45, CD19, CD14, after staining with the respective PE-conjugated, PE-Cy5-conjugated and FITC-conjugated antibodies. Isotype control antibodies were used as a control (Fig. S6).
Fig. S7A

[Graph showing various cytokine levels in primary and expanded cells]

- G-CSF (pg/mL)
- GM-CSF (pg/mL)
- IFN-γ (pg/mL)
- IFN-α (pg/mL)
- TNF-α (pg/mL)
- TNF-β (pg/mL)
- IL-3 (pg/mL)
- IL-4 (pg/mL)
- IL-5 (pg/mL)
- IL-6 (pg/mL)
- IL-7 (pg/mL)
- IL-8 (pg/mL)
- IL-10 (pg/mL)
- IL-12p70 (pg/mL)
- IL-12p9 (pg/mL)
- IL-15 (pg/mL)
- IL-17A (pg/mL)
- IL-23 (pg/mL)
- IL-40 (pg/mL)

Primary vs. Expanded
Fig. S7B

- Eotaxin (pg/mL)
- Fractalkine (pg/mL)
- MCP-3 (pg/mL)
- IP-10 (pg/mL)
- MCP-1 (pg/mL)
- MIP-1α (pg/mL)
- MIP-1β (pg/mL)
- RANTES (pg/mL)

Comparison between Primary and Expanded conditions.
Fig. S7C

Fig. S7. Cytokines, chemokines and growth factors and ligands secreted by primary and osteoclast-expanded NK cells

Highly purified NK cells and monocytes were obtained from peripheral blood mononuclear cells (PBMCs) of healthy donors and NK cells were treated (1x10^6 cells/ml) with IL-2 (1000 U/ml) for 18 hours before the supernatant was harvested. To generate osteoclasts, monocytes were cultured in alpha-MEM media containing M-CSF (25 ng/ml) and RANKL (25 ng/ml) for 21 days. For expansion,
purified NK cells (1x10^6 cells/ml) were treated with the combination of IL-2 (1000 U/ml) and anti-CD16mAb (3ug/ml) for 18 hours before they were co-cultured with autologous osteoclasts in the presence of sAJ2 bacteria at 1:2:4 ratios (OC:NK:sAJ2), respectively. The supernatant was harvested after 6 days of co-culture and multiplex assay was used to determine cytokines (A), chemokines (B) and growth factors (C) levels secreted by the primary and expanded NK cells.

Fig. S8

[Image showing flow cytometry results with CD3^+ and CD16^+CD56^+ cells and control compared to +anti-CD3 conditions with percentages and fluorescence values]
**Fig. S8.** Purified T cells treated with anti-CD3mAb in the absence of NK cells did not lose forward and side scatter.

Highly purified T cells and monocytes were obtained from peripheral blood mononuclear cells (PBMCs) of healthy donors and T cells were treated (1x10^6 cells/ml) with IL-2 (100 U/ml) and anti-CD3 (1ug/ml) for 18 hours before they were co-cultured with autologous osteoclasts in the presence of sAJ2 bacteria at 1:2:4 ratios (OC:T cells:sAJ2), respectively. The cells were analyzed for CD3, CD16 and CD56 on day 9 after the culture. Anti-CD3 treated T cells did not lose Forward and side scatter in the absence of NK cells (**Fig. S8**).