Supplemental Material and Methods

Ethic statement and patients’ information. Leukocyte concentrates and heparinized blood from healthy adult blood donors was provided by the Department of Transfusion Medicine, the Institute of Immunology or the Clinic of Gynaecology and Obstetrics, all in Kiel, Germany, whereas blood from PDAC-patients was obtained from the Department of General and Thoracic Surgery of the University-Hospital Schleswig-Holstein or the municipal hospital in Kiel.

Flow cytometry. mAb used for surface staining: anti-CD3 clone SK7, anti-TCRγδ clone 11F2 (BD Biosciences) anti-TCRαβ clone IP26 (Biolegend), anti-TCRVD2 clone Immu389 (Beckman Coulter), anti-TCRVδ1 clone TS8.2 (Thermo Fisher Scientific), and corresponding isotype controls (BD Biosciences or Biolegend). To analyze Her2/neu expression, we used 20 µg/ml trastuzumab (Roche Pharma AG, Grenzach Wyhlen), and the corresponding humanized IgG control (20 µg/ml) followed by a second step staining with goat-anti-human F(ab)2 (Medac, Hamburg). All samples were analyzed on a FACS-Calibur flow cytometer (BD Biosciences) using CellQuestPro software.

Culturing of pure lymphocyte populations. γδ T-cells with a purity < 90% were labeled with anti-TCRαβ mAb clone IP26 (Biolegend, San Diego, CA, USA) and subjected to magnetic separation in order to deplete remaining αβ T-cells. Magnetically depleted γδ T-cells as well as γδ T-cells of donors of whom we initially received only 1 ml blood with a low percentage of γδ T-cells were re-stimulated to ensure large-scale expansion of pure γδ T-cell lines as described (15).
ELISA and CD107a-degranulation assay. 20,000 PDAC-cells in 96-well microtiter plates were cultured overnight. Medium, 300 nM BrHPP, 1 µg/ml [(Her2)2xVγ9] were added for 1 h before γδ T-cell lines supplemented with 12.5 U/ml rIL-2 were co-cultured with target cells in a 12.5:1 ratio. Human perforin and granzyme B were measured by a sensitive sandwich ELISA following the procedures outlined by the manufacturer after 4h of co-culture in duplicates (Abcam, Cambridge, UK; Bender MedSystems, Vienna, Austria).

For CD107a-assay, 10 µl anti-human CD107a mAb clone H4A3 (50 µg/ml, Biolegend), 3 µM monensin (1 h after co-culture) and anti-TCRγδ mAb (3 h after culture) were added, and cells were analyzed by flow cytometry.

SDS-PAGE, coomassie staining of proteins and capillary electrophoresis. Antibody derivatives were analysed by SDS-PAGE under reducing and non-reducing conditions, according to standard procedures. Purity and concentrations of the purified proteins were determined by Coomassie staining (colloidal Coomassie brilliant blue G250 solution, Carl Roth GmbH, Karlsruhe) or by capillary electrophoresis using an Experion™ Automated Electrophoresis System (Bio-Rad), according to the manufacturer’s instructions.

Gelfiltration chromatography. Gelfiltration chromatography was performed on an ÄKTA purifier (GE Healthcare) using PBS as running buffer at a constant flow rate of 1 ml/min. 200 µg protein were loaded in a volume of 1 ml on a Superdex 200 10/300 GL column (GE Healthcare). Ferritin (440 kDa), human IgG1 (150 kDa), Conalbumin (75 kDa) and Ribonuclease A (13.7 kDa) were used for calibration. Data were analyzed with Unicorn 5.1 software (GE Healthcare).

Binding analysis of bispecific antibodies. Briefly, 3x10^5 cells were incubated with purified antibody derivatives in PBS supplemented with 1% bovine serum albumin (BSA) (Carl Roth
GmbH) and 0.1% sodium-azide (FACS-buffer) for 30 minutes at 4°C. Cells were washed two times with FACS-buffer. After incubation with 10 µg/ml Penta-His™ Alexa Fluor® 488 conjugated IgG (Qiagen) for 30 min. at 4°C and an additional washing step, cells were analysed on a flow cytometer (Epics XL, Beckman Coulter).