SUPPLEMENTARY METHODS

Isolation of tumor-associated exosomes: Ascites fluids (from ovarian tumor patients, or mice bearing DM6-Mut tumors), or medium in which DM6-Mut tumors were mechanically disrupted were first centrifuged at 300 x g to remove cells and large debris, followed by another round of centrifugation at 1,150 x g to remove smaller debris and membrane fragments. They were then diluted to 50% (with RPMI-1640 or PBS), passed through a 0.22 µm PVDF filter (Millipore), and ultracentrifuged at 200,000 x g for 90 minutes. The pellet was resuspended in RPMI-1640 + 1% HSA (for functional experiments) or PBS (for biophysical characterization).

Exosome antibody array: The identification of protein markers on isolated exosomes was done using a commercially available Exo-Check exosome antibody array (System Biosciences Inc.) kit as described by the manufacturer. The membrane was developed with SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific) and analyzed using ChemiDoc MP System (BioRad).

Nanotracking analysis (NTA): Particles were diluted in a final volume of 1 mL in sterile 1x PBS for NTA measurements. All NTA measurements were conducted with a ZetaView particle tracking analyzer equipped with a 488 nm laser (ParticleMetrix, Mebane, NC). Instrument performance was checked daily using 100 nm beads using the ZetaView acquisition software (version 8.05.04) quality control suite. The camera settings for the basic acquisition protocol for data acquisition included a sensitivity of 82, shutter speed of 150, and a frame rate of 30 with applied post-acquisition parameters of minimum brightness 20, minimum size 10, maximum size 200 and trace length 15. Eleven positions were recorded for each sample with 2 cycles at each position. Camera sensitivity settings were checked and adjusted where necessary for each sample but generally this would result in less than 2-3 percentage points from the saved protocols. For determining size distribution, the data (as fcs files) were analyzed using FlowJo.

Flow Exometry: Three hundred to five hundred micrograms of exosomes were attached to 100 µl of aldehyde/sulfate latex beads (4 µm; 4% w/v) and incubated overnight at 4°C on a rotator/ mixer. Glycine was then added to a final concentration of 100 mM to saturate remaining free binding sites on the beads. The beads were then washed in PBS with 0.5% BSA and used for immunofluorescence staining with an unconjugated anti-PS antibody (Clone 1H6, Upstate) and a secondary antibody goat anti-mouse IgG AlexaFluor 488 (Life Technologies).

T cell activation with antibodies to CD3 and CD28: Antibodies were immobilized on maxisorp 12 × 75 mm tubes (Nunc) by incubating 0.1 µg of purified anti-CD3 (Bio X Cell, catalog number BE001-2; clone OKT3) and 5 µg of purified anti-CD28 (Life Technologies, catalog number CD2800-4; clone 10F3) in 500 µl of PBS, at 4°C overnight. PBL from normal donors was thawed, resuspended in RPMI-1640 + 1% human serum albumin, and 5 x 10^5 total cells were incubated in anti-CD3/anti-CD28 in coated tubes at 37°C/5% CO_2 for the duration of activation.

Detection of NFκB translocation following T cell activation: Human NDPBL were activated for 2h at 37°C with immobilized anti-human CD3/CD28 with or without ovarian ascites fluid-derived exosomes. The percentage of activated T cells was determined by monitoring the translocation of NFκB from the cytosol into the nucleus using fluorescence microscopy as previously reported (7).

Detection of CD69 expression following T cell activation: Human NDPBL were activated for 2h at 37°C with immobilized anti-human CD3/CD28 with or without exosomes (derived from either ovarian ascites fluid or DM6-Mut xenograft ascites fluid). The cells were then incubated for 18h

in RPMI-1640 + 1% HSA at 37ºC/5% CO_2 in the absence of stimulation or exosomes. For flow cytometry, cells were labeled with the recommended amounts of fluorochrome-conjugated antibodies to CD3 (clone UCHT1, BD Biosciences) and CD69 (clone L78, BD Biosciences) for 30 mins at 4ºC. The cells were then washed with 2 mL of PBS and labeled with Sytox Red (Life Technologies) for 15 min, acquired on an LSR Fortessa (BD Biosciences) flow cytometer and analyzed using FlowJo software.

Detection of CD25 expression following T cell activation: Human NDPBL were activated for 72h at 37°C with immobilized anti-human CD3/CD28 with or without exosomes and ExoBlock. For flow cytometry, cells were labeled with the recommended amounts of fluorochrome-conjugated antibodies to CD3 (clone UCHT1, BD Biosciences) and CD25 (clone M-A251, BD Biosciences) for 30 mins at 4ºC. The cells were then washed with 2 mL of PBS and labeled with Sytox Red (Life Technologies) for 15 min, acquired on an LSR Fortessa (BD Biosciences) flow cytometer and analyzed using FlowJo software.

Detection of intracellular IL-2 expression following T cell activation: Human NDPBL were activated for 6h at 37ºC/5% CO_2 with immobilized anti-human CD3/CD28 in the presence of 1 µL/mL GolgiStop (BD Biosciences) with or without DM6-Mut ascites derived exosomes and ExoBlock. For flow cytometry, cells were labeled with fluorochrome-conjugated antibodies to CD3 (clone UCHT1, BD Biosciences) for 30 mins at 4ºC. The cells were then fixed and permeabilized with the fixation/permeabilization solution from the Cytofix/Cytoperm kit (BD Biosciences) as described by the manufacturer and labeled with fluorochrome-conjugated antibodies to IL-2 (clone 5344.111, BD Biosciences) at 4ºC for 30 min, washed, fluorescence emission acquired, and results analyzed as above.

Detection of intracellular IFN-γ expression following T cell activation: Human NDPBL were activated for 72h at 37ºC/5% CO_2 with immobilized anti-human CD3/CD28 with or without exosomes and ExoBlock. 1 µL/mL GolgiStop (BD Biosciences) was added and cells were cultured for 5 more hours. For flow cytometry, cells were labeled with fluorochrome-conjugated antibodies to CD3 (clone UCHT1, Tonbo Biosciences) for 30 min at 4ºC. The cells were then fixed and permeabilized with the fixation/permeabilization solution from the Cytofix/Cytoperm kit (BD Biosciences) as described by the manufacturer and labeled with fluorochrome-conjugated antibodies to IFN-γ (clone B27, BD Biosciences) at 4ºC for 30 min, washed, fluorescence emission acquired, and results analyzed as above.

Proliferation assay: Human NDPBL were labeled with CellTrace Violet Proliferation kit (Thermo Fisher Scientific) as recommended by the manufacturer. The labeled cells were incubated in tubes that were coated with immobilized antibodies to human CD3 and CD28 for 7 days with or without ascites fluid-derived exosomes and ExoBlock. On day 7, the cells were labeled with fluorochrome-conjugated anti-human CD3 (clone UCHT1, Tonbo Biosciences). Sytox Red was added 15 min before flow cytometry at a final concentration of 5 nM to label the dead cells. The fluorescence was acquired on an LSR Fortessa (BD Biosciences) flow cytometer. The data were analyzed using FlowJo software (Tree Star Inc.) to calculate the expansion index.

Calculation of % inhibition and % reversal: These were calculated using the formulae:
% Inhibition = [1- (% activation with exosomes / % activation without exosomes)] × 100
% Reversal = [1- (% inhibition in test group / % inhibition in control group)] × 100

Establishment of the X-mouse Model: NSG mice were implanted with 2.5 x 10^6 DM6-Mut cells intraperitoneally (i.p.) in a total volume of 0.5 mL. Cryopreserved neoantigen-specific TKT cells were thawed and incubated in complete medium for 6h at 37°C and 5% CO_2. 5 x 10^5 T cells were injected i.p. per mouse in a total volume of 0.5 mL, 5 days following the implantation of DM6-Mut cells.

Analysis of tumor burden in the X-mouse model: 25 days following implantation of the tumor, the mice were euthanized and the greater omentum from each mouse was surgically removed. A wet mount of the omentum in PBS was scanned using the Leica DM6 B upright fluorescence microscope. The entire omentum was scanned under the 5X objective using the GFP and DIC filters. Measurements were made in a random order for control and test groups to minimize confounders .The images were exported as TIF files and analyzed using ImageJ software to quantify the GFP signal. The polygon tool was used to draw a tight border around the omentum and the amount of signal was measured in the green channel. Background in the green channel was also measured by drawing a gate in a region of the omentum that was free of any tumor cells. Corrected total fluorescence was then calculated using the formula CTF = Intensity Density of Omentum- (Area of Omentum x Mean Gray Value of Background for that omentum).

Establishment of the OTX Model: NSG mice were implanted with 150 mg of ovarian tumor aggregates (obtained by mechanical disruption of the patient tumor) intraperitoneally (i.p.) in a total volume of 0.5 mL.

Recovery and analysis of T cells from OTX xenografts: T cells were recovered from the omenta of xenograft-bearing mice by the previously described walkout method[18]. Omenta were harvested under sterile conditions and placed in complete medium in wells of a 6-well tissue culture plate. The omenta were gently cut into small 4-5 mm pieces and incubated overnight at 37°C under 5% CO2. On the following day, the medium with the "walkout cells" was passed through a 70 µm cell strainer to exclude omental tissue and debris. For flow cytometry, the cells were labeled with CD3 (clone UCHT1, Tonbo Biosciences), CD4 (clone RPA-T4, BD Biosciences) and CD8 (clone RPA-78, BD Biosciences). Dead cells were excluded using Ghost Dye Red 710 (Tonbo Biosciences).

When blinding was not possible due to the nature of the experiment, we have minimized bias by randomization of animals assigned to control and test cohorts, using objective and reliable outcomes, duplicate assessment, and minimizing potential confounders.

Imaging Flow Cytometry (IFC): IFC analysis was performed on an ImageStreamX MK-II platform (ISX, Amnis®, Luminex Corp, Seattle, WA). An acquisition live gate was set based on the scatter signal (detected in Ch12) to include events with scatter from zero through to approximately 1-5 % of the Speedbead scatter signal. Speedbeads, which are an integral part of the platform, are 1-micron polystyrene beads that are intermixed with a sample during acquisition and are required to synchronize the sample velocity and time-delayed integration of the charge coupled device (CCD) camera in the system. All samples were acquired for a set time of 20 seconds. Single color controls were run (with the brightfield and scatter detectors off) to create a compensation matrix to correct the raw data for spectral overlap between the two dyes in the detection channels used. The 785nm laser (10mW) was used as the scatter and the 488nm laser (200 mW) was used to excite PsVue and Exoglow. For analyses in the IDEAS® software (version 6.2; Amnis, Luminex Corp) gates were applied to exclude any Speedbead events. Since the Imagestream platform is volumetric, the "objects/mL" parameter is a default statistic available for every experimental run. PsVue was detected in Ch2 and ExoGlow in Ch4. The particle concentrations were determined by the

objects/mL statistic and corrected for any pre-acquisition dilutions necessary to obtain an event rate that was within the camera processing speed capability of approximately 15,000 events/second. Final exosome concentrations reflect corrections for applied dilutions before acquisition and the subtraction of concentrations of non-detergent sensitive particle concentrations.