

Supplementary Data

Tumour exosomes inhibit binding of tumour-reactive antibodies to tumour cells and reduce ADCC

Cancer Immunology, Immunotherapy

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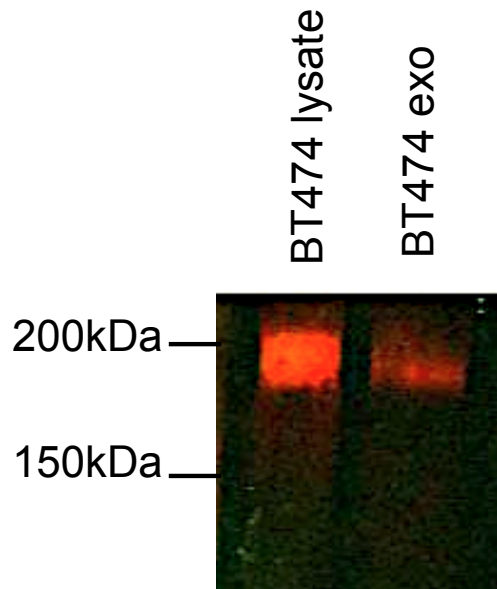


Figure S1: Exosomes from BT474 cells contain full-length HER2. Exosomes were isolated from BT474 supernatants, homogenized in lysis buffer, separated on 9% SDS-PAGE and transferred to PVDF membrane. After blotting, the 185kD full-length HER2 could be readily detected using an antibody directed against the extracellular domain of HER2 and a secondary Cy3-labeled antibody and visualized with an Odyssey imaging system (LI-COR Biosciences, Bad Homburg, Germany). In contrast, we were not able to identify the 95kD truncated C-terminal fragment (CTF) {Anido et al., 2006, #84238}, using an antibody directed against the intracellular domain of HER2.

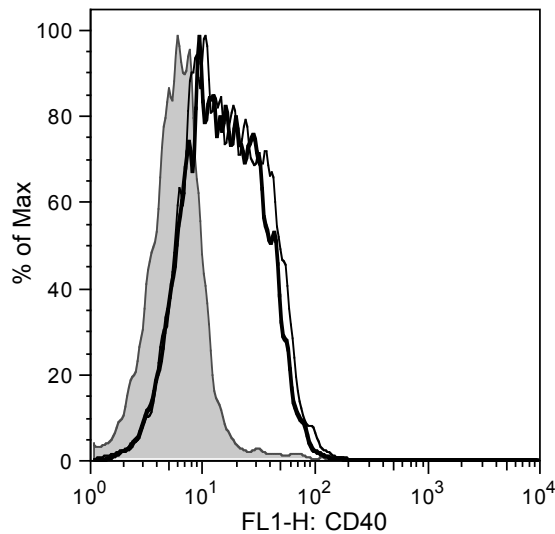


Figure S2: BT474-exosomes do not inhibit binding of an anti-CD40 antibody to a human B-cell line. In order to demonstrate the specificity of antibody sequestration by BT474 exosomes (Figure 2 of the manuscript), we investigated the sequestration of an antibody that binds to a protein not contained in BT474 exosomes. For that, we pre-incubated BT474 exosomes with an anti-CD40 antibody and then measured its binding to the human B cell line RZ-LCL. As shown, no inhibition of binding was observed, demonstrating the specificity of antibody sequestration.

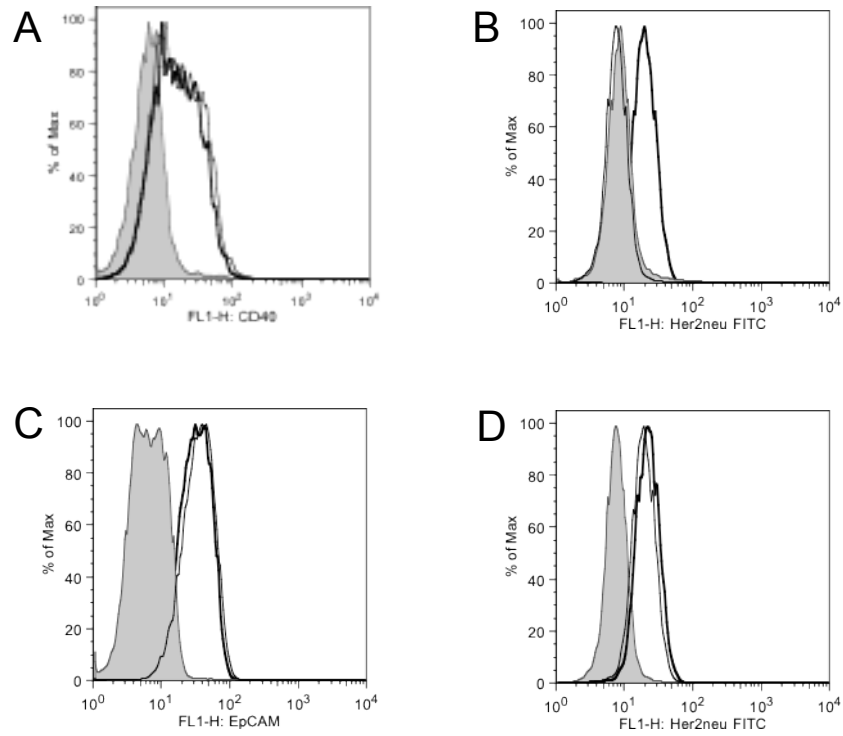


Figure S3: HER+EpCAM+ TEX from SkBr3 cells but not HER2–EpCAM– TEX from A375 melanoma cells prevent binding of trastuzumab and C215 to SkBr3 cells. 50 ng/ml trastuzumab or C215 were pre-incubated with 100 μ g/ml exosomes from SkBr3 cells and A375 cells for 30 min and then used to stain SkBr3 cells. SkBr3 exosomes prevented binding of C215 (A) and trastuzumab (B) whereas A375 exosomes did not (C and D). Grey histogram: unstained cells. Bold line: Maximal staining of trastuzumab and C215 on SkBr3 cells. Thin line: staining of SkBr3 cells with pre-incubated trastuzumab and C215.

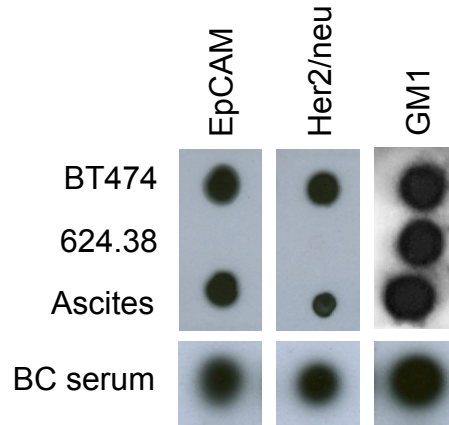


Figure S4: Exosomes from malignant ascites and sera from breast cancer (BC) patients carry the tumour-associated antigens EpCAM and Her2/neu. Exosomes from the ascites of a patient with ovarian cancer, from serum of a patient with breast cancer and from the supernatants of BT474 and Mel624.38 cells were isolated by ultracentrifugation and further purified by floatation into a sucrose gradient. Fractions 3 and 4, corresponding to densities of 1.13 and 1.17, were pooled and tested for EpCAM, Her2/neu and the exosomal marker GM1 by an immunoblot.