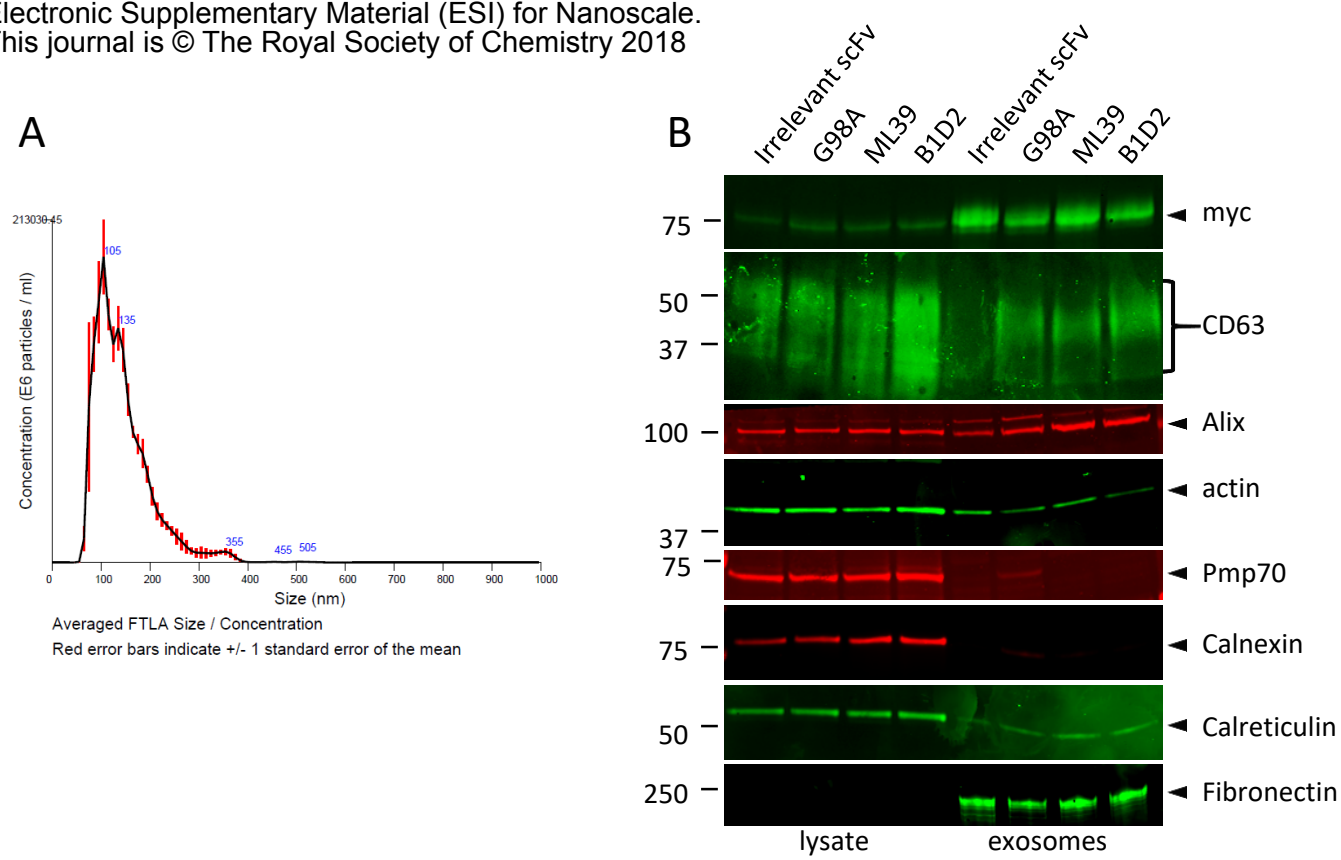
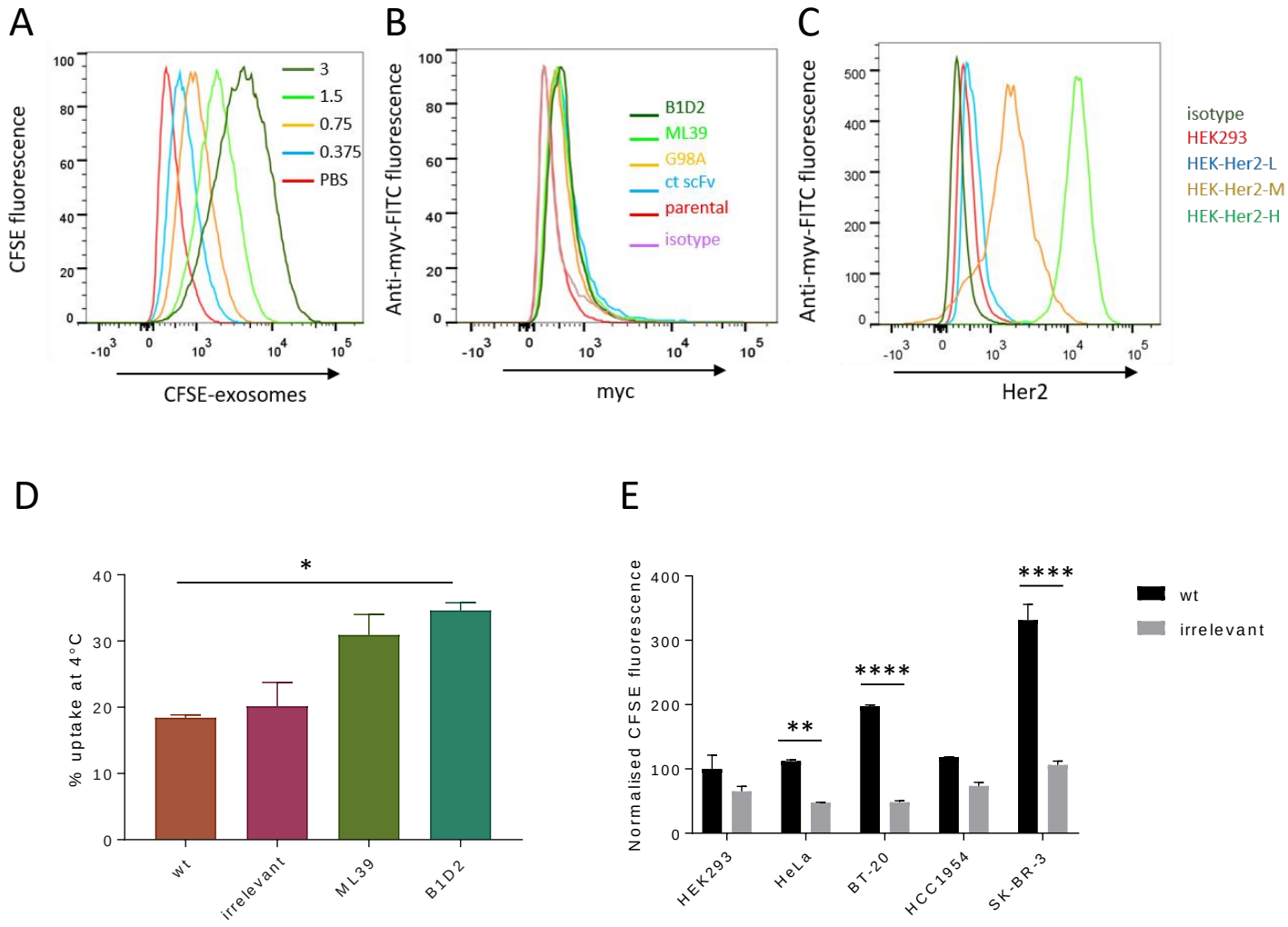
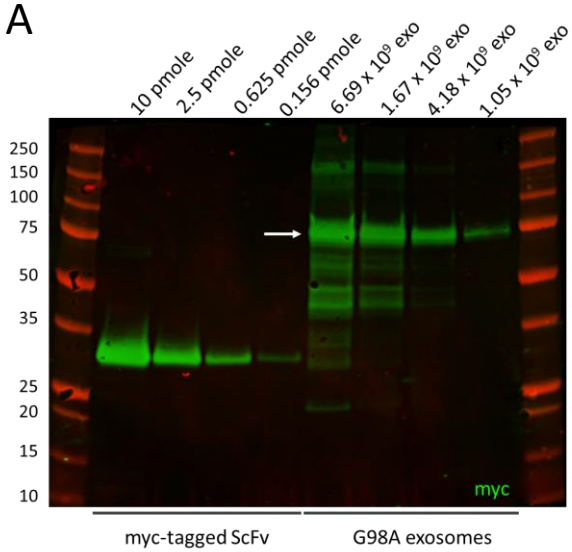


Supplementary figure 1 A) The concentration of exosomes, and their mean size and size distribution was determined by nanoparticle tracking analysis using a NS300 system (Malvern) and the NTA3.0 software; the same instrument and software settings used for all samples. Graph illustrates an averaged representative trace. B) Immunoblot analysis of cell lysates and exosome lysates from cells expressing different scFv-C1C2 fusion proteins. Myc represents the C1C2-scFv fusion protein. CD63 and Alix are exosomal markers. Calnexin and Calreticulin are endoplasmic reticulum proteins, whereas Pmp70 is a mitochondrial protein and fibronectin is a component of the extracellular matrix, all of which are used to assess exosome purity. Molecular mass is indicated on the left.

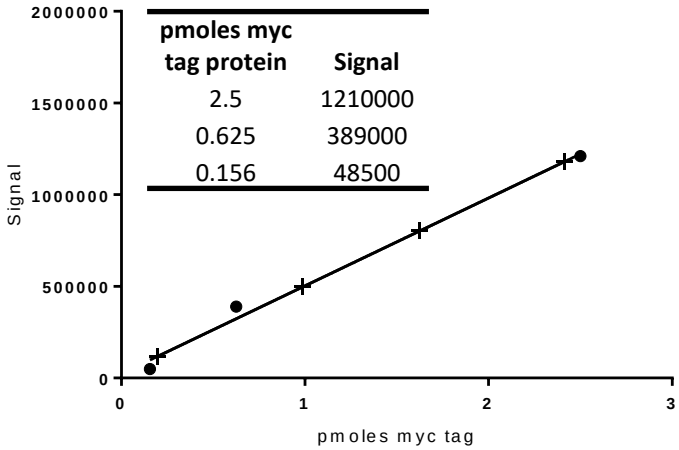


Supplementary figure 2 A) HEK293 cells were incubated with increasing concentrations of purified CFSE-exosomes (x 10^{11}/ml), or with a PBS control, for 4 h and recipient cell fluorescence was assessed by flow cytometry. Histogram plots are shown. B) Parental HEK cells or HEK cells expressing different scFv-C1C2 fusion proteins were stained with a FITC-labelled anti-myc antibody and the fluorescence was measured by flow cytometry. Histogram plots are shown. C) Parental HEK cells or clonal HEK cells expressing different Her2 were stained with a FITC-labelled anti-Her2 antibody and the fluorescence was measured by flow cytometry. Histogram plots are shown. D) HEK-Her2-H cells were incubated with CFSE-labelled non-modified (wt) or scFv-bearing exosomes in duplicate for 4 h at 37˚C or at 4˚C and cell fluorescence was assessed by flow cytometry. MFI values for the 4˚C exosome samples were normalised to the 37 ˚C values, to calculate a % uptake at 4˚C value. The values plotted represent the average of two independent experiments. Statistical analysis was performed using a one-way ANOVA, comparing all conditions to wt exosome values. Only statistically significant values are shown; * = p≤0.05. E) Cell lines were incubated with non-modified (wt) or irrelevant scFv CFSE-exosomes in duplicate for 4 h, and cells were analysed for CFSE-exosome uptake by flow cytometry. The MFI values were calculated and normalised to the MFI of the HEK cells incubated with the wt exosomes. Statistical analysis to compare uptake between the groups was performed using two-way ANOVA with multiple comparisons; **** = p<0.0001, *** = p ≤ 0.001, ** = p≤ 0.01, * = p≤ 0.05; error bars indicate SEM.

A



B



C

Exosome number	Signal	Extrapolated pmoles	Extrapolated moles	Total myc molecules	myc tag molecules/exosome
6.69E+09	1180000	2.41343996	2.41344E-12	1.45E+12	217.25
1.67E+09	803000	1.626717654	1.62672E-12	9.80E+11	586.61
4.18E+08	497000	0.988157904	9.88158E-13	5.95E+11	1423.64
1.05E+08	119000	0.199348801	1.99349E-13	1.20E+11	1143.34

Supplementary figure 3 A) Immunoblot analysis of serial dilutions of a purified myc-tagged scFv of known concentration and G98A-scFv exosomes, detected via anti-myc antibody. White arrow indicates the molecular weight of the relevant band for quantification. B) Linear regression plot for the purified myc protein (circles) with interpolated values for the G98A exosome preparation dilutions (crosses). C) Calculations performed to obtain the number of myc tag molecules per exosome.