Supporting Information

Multi-Functional Transmembrane Protein Ligands for Cell-Specific Targeting of Plasma Membrane-Derived Vesicles

Chi Zhao, David J. Busch, Connor P. Vershel, Jeanne C. Stachowiak*

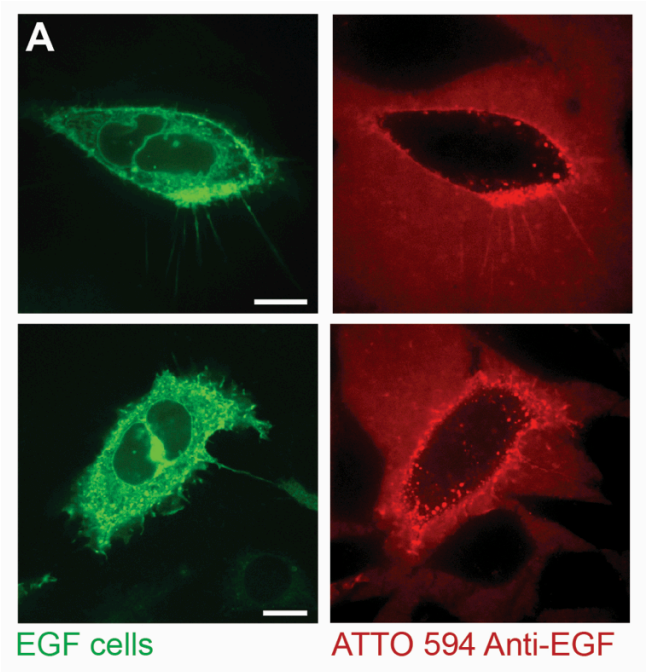

Figure S1. Additional confocal images of donor cells expressing the EGF targeting protein, recruiting the soluble antibody (ATTO 594 Anti-EGF) from solution. Notably, the EGF targeting protein contains an eGFP domain.

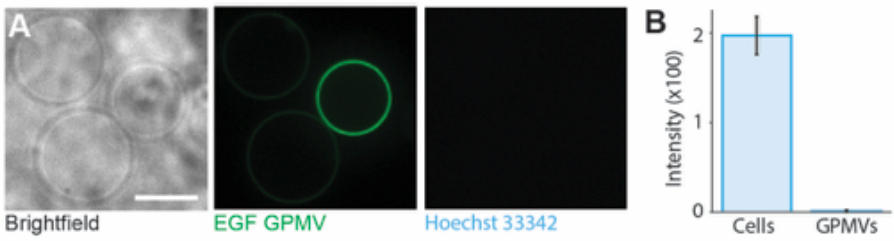

Figure S2. A) GPMVs harvested from donor cells with stable expression of the EGF targeting proteins were stained using Hoechst 33342. No detectable signal was seen in the lumen of the GPMVs. The brightness and contrast of the Hoechst image was kept the same as Figure 1D. **B)** A quantitative comparison of blue (Hoechst) fluorescent intensity between donor cells after

plasma membrane extraction and the harvested GPMVs suggested that GPMVs have 200-fold lower Hoechst 33342 signals than cellular nuclei. Collectively, these results suggest that GPMVs are free of contamination from the nuclei. Error bars represent the standard deviations of 33 cells and 90 GPMVs analyzed.

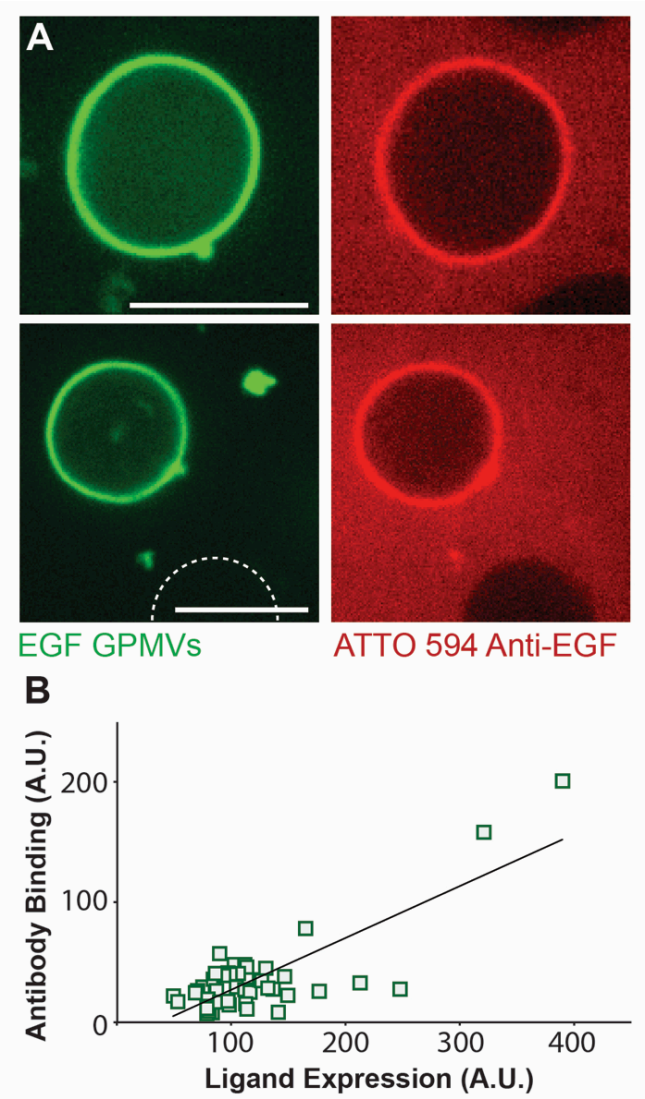

Figure S3. A) Additional confocal images of GPMVs derived from donor cells displaying the EGF targeting protein (EGF-GPMVs), recruiting the soluble labeled antibody (ATTO 594 Anti-EGF) from solution. **B)** Display of the eGFP-labeled EGF targeting protein and the binding of soluble ATTO 594 Anti-EGF were correlated based on fluorescence intensity analysis of two-color fluorescence images of GPMVs, similar to those in Figure S3A. A total of 23 GPMVs were analyzed. All scale bars, 10 μm.

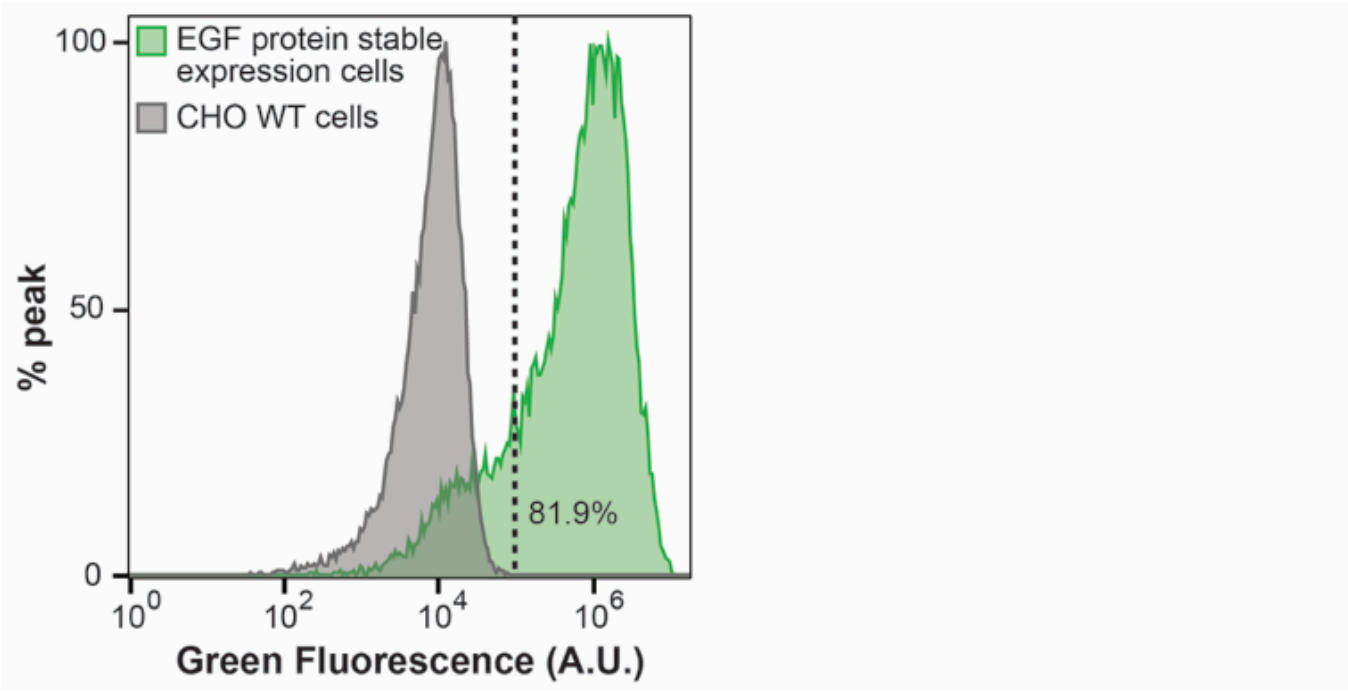

Figure S4. The GFP-channel fluorescence distributions of CHO cells with stable expression of the EGF targeting protein (GFP) and CHO wildtype control cells (gray) were evaluated using flow cytometry. More than 80% of cells in the stable line have higher fluorescence than all control cells.

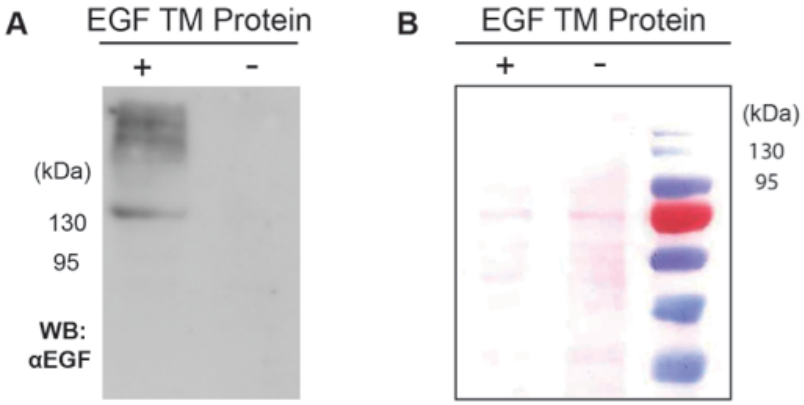

Figure S5. A) Western blot analysis of GPMVs from CHO cells stably expressing the EGF targeting protein (EGF TM) (+) and CHO wild type cells (-). **B)** The ponceau stain of the nitrocellulose membrane indicates that at least as much protein was transferred to the blot membrane for the (-) condition in comparison to the (+) condition.

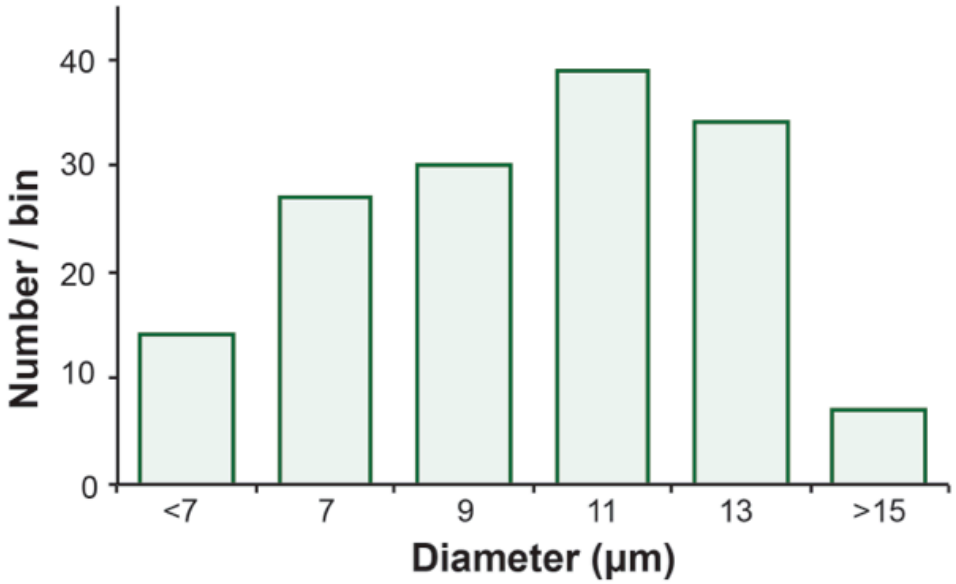

Figure S6. Size distribution of GPMVs harvested from cells expressing the EGF targeting protein. 150 GPMVs from 3 independent sample preparations were analyzed.

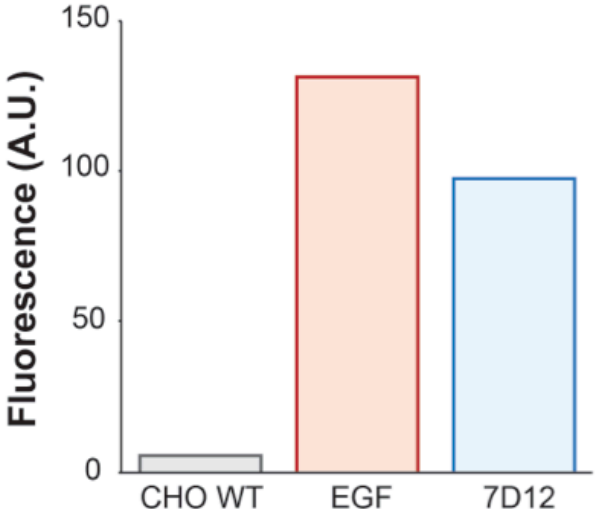

Figure S7. Background subtracted eGFP fluorescence intensity normalized to $2x10^7$ of GPMVs derived from CHO WT cells (grey bar), CHO cells stably expressing the EGF targeting protein (red bar) and the 7D12 targeting protein (blue bar) respectively. These results indicate that the auto-fluorescence of the GPMVs accounts for only a few percent of the fluorescence signal from the eGFP-containing targeting proteins.

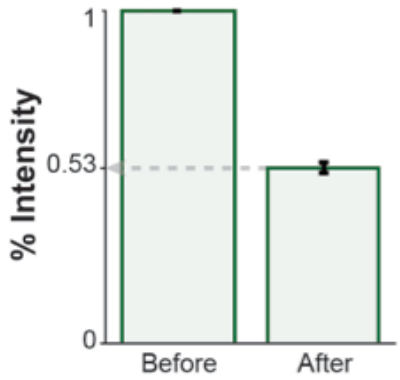

Figure S8. Extrusion efficiency was estimated by measuring fluorescence intensity before and after the extrusion process. Data were normalized to the starting concentration. The error bar represents the standard deviation of three independent trials. Approximately 50% of the membrane material is lost during the extrusion process, likely coating the surfaces of the extruder device. It is likely that preparing larger batches of PMVs or using a smaller volume extrusion device would reduce the loss of membrane material.

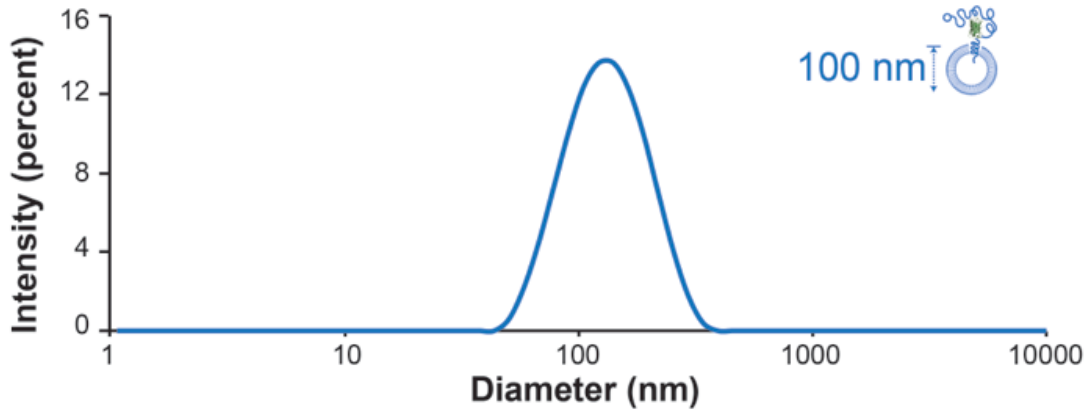

Figure S9. The size distribution of PMVs extruded through 100 nm membranes, as determined by dynamic light scattering.

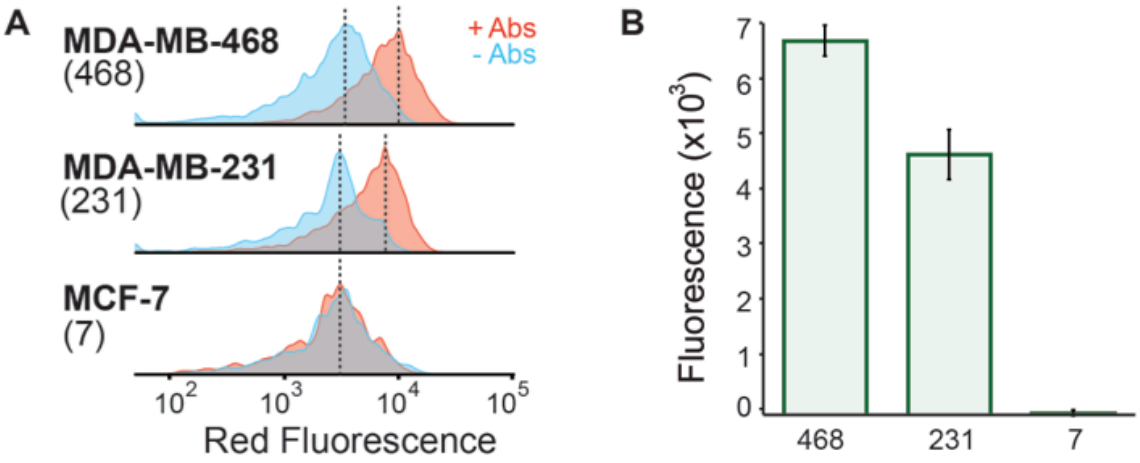

Figure S10. A) Example histograms of the fluorescence intensity of cells with (red curve) or without (blue curve) binding of ATTO 594 labeled anti-EGFR with a dye to protein ratio of 1.8:1. **B)** The mean fluorescence peak shift between anti-EGFR bound and anti-EGFR free cells increases with increasing EGFR expression level. Results from this study were consistent with previous studies.[47] Error bars represent the standard deviations of 3 independent trials.

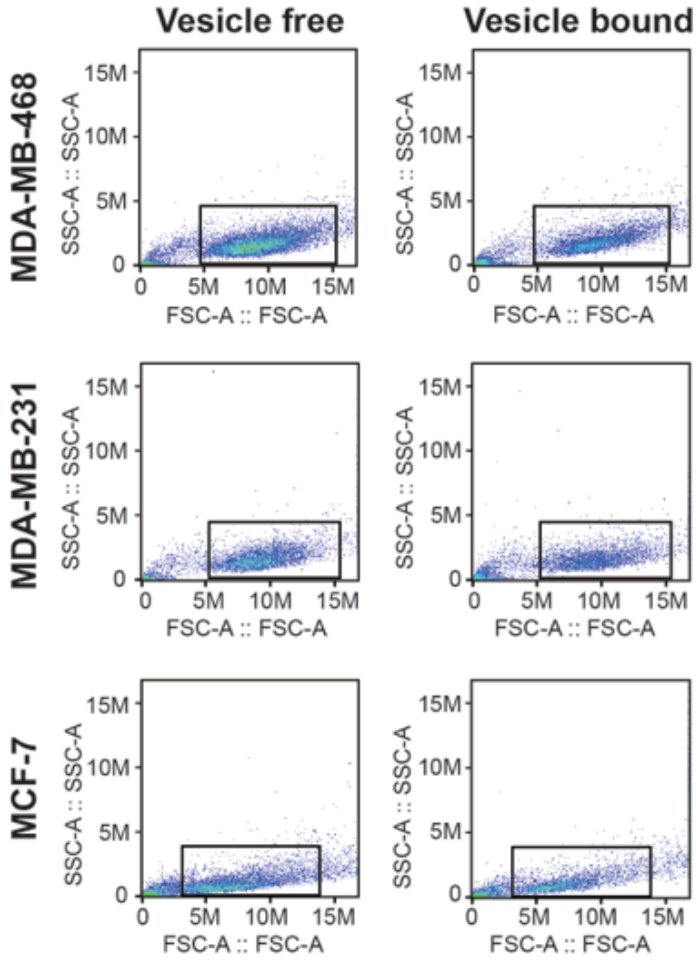

Figure S11. Example flow cytometry scatterplots from populations of cells with and without addition of targeted EGF-PMVs. The black rectangle represents the gate, which varied slightly depending on the cell line but was used uniformly across all experiments with a given cell line.

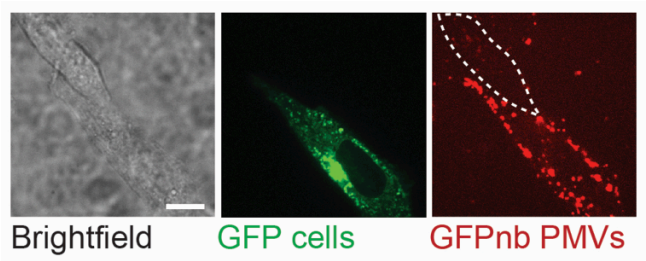

Figure S12. The competitive binding experiment, illustrated in Figure 6A and 6B, was repeated with purified GFPnb PMVs. The cells were incubated with vesicles at a 1300 vesicles per cell concentration. Similar to Figure 6B, these images show that purified GFPnb PMVs bind selectively to cells that express extracellular GFP domains. Detailed purification information can be found in the experimental section.