Supporting Information:

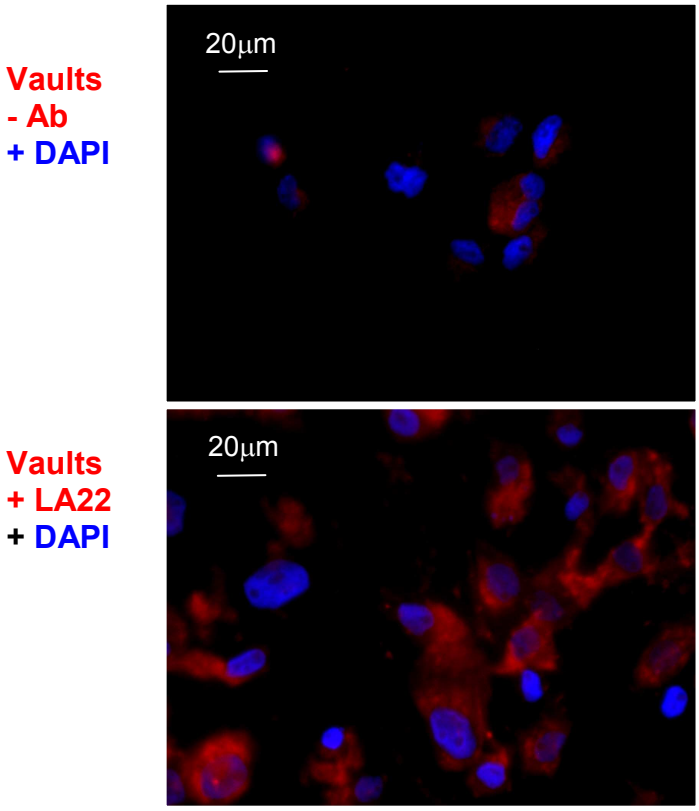

Figure 1. Antibody-mediated binding. Binding of CP-MVP-Z/mCherry-mINT vaults to A431 cells in the absence (-Ab) or presence of LA22 (+Ab). The red fluorescence is due to the intrinsic fluorescence from the mCherry protein packaged inside of the vaults, nuclei were stained with DAPI (blue), and images were merged. LA22 antibody is required to efficiently bind vaults to the cell surface EGFR receptor.

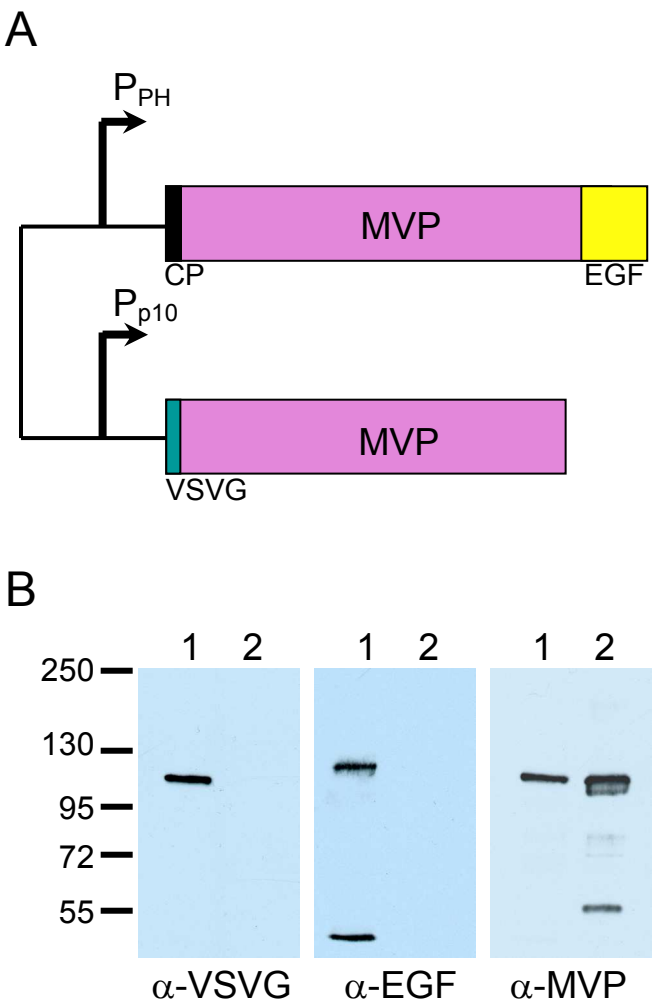

Figure 2. Combination recombinant vaults V-MVP/CP-MVP-EGF. A. Schematic diagram of VSVG-MVP (V-MVP) and CP-MVP-EGF inserted into the pFastBac Dual expression vector (P_{PH}, polyhedron promoter; P_{10}, P10 promoter). B. Western blot analyses of purified V-MVP/CP-MVP-EGF (lane 1) and CP-MVP (lane 2) recombinant vaults probed with the indicated antibodies. Vaults were purified from Sf9 cells infected with either the dual MVP (vMVP/CP-MVP-EGF) or CP-MVP only baculoviruses. The purified vaults vMVP/CP-MVP-EGF (lane 1) and CP-MVP (lane 2) were analyzed by immunoblotting with the indicated antibodies. The anti-VSVG and anti-EGF antibodies only reacted with vaults purified from the dual infected cells, whereas the anti-MVP antibody recognized both vault preparations. We estimated that the dual vaults vMVP/CP-MVP-EGF contain 6-8 copies of the CP-MVP-EGF incorporated into each particle using an ELISA.

C-terminal tag size threshold. There may be a threshold for the size or type of tag that can be added to the C-terminus of MVP as the crystal structure model showed that 48 copies of MVP meet in the cap and form a double iris, containing two C-terminal disks built upside down relative to each other.[1] However, exactly where the C-termini are located is beyond the resolution of the x-ray crystallographic model. Based on the cryoEM reconstruction of CP-MVP-VSVG we propose that many of the 48 copies of the C-terminal peptide tags are likely protruding from the vault cap. The vertical density slice through the CP-MVP-VSVG reconstruction shows only a little additional peptide tag density on the interior side of the caps. It is possible that in vaults without C-terminal tags half of the C-termini are on the exterior surface and half are on the interior surface of the vault. Addition of C-terminal peptide tags may perturb the vault cap slightly to allow most of the C-termini to extend outside of the cap. C-terminal extensions up to 33 aa seemed to have no deleterious effect on vault particle formation/solubility. In contrast, when the 55 aa encoding EGF were fused to the C-terminus of MVP, the resulting proteins expressed in *Sf9* insect cells were insoluble. This finding was somewhat surprising as the green fluorescence protein (a 238 aa protein) has been fused onto the C-terminus of MVP and transiently expressed in mammalian cells, resulting in an immunofluorescence pattern similar to endogenous vaults.[2] We reasoned that the endogenously expressed MVP in these cells likely interacted with the transfected GFP-MVP resulting in formation of composite vaults. To mimic this situation in *Sf9* cells (that do not contain any endogenous MVP) we utilized a dual expression vector so that we could simultaneously express two modified MVPs in the same infected cells (one with both an N- and C-terminal addition and the other with only an N-terminal addition). We co-expressed V-MVP with CP-MVP-EGF and found that this system allowed us to purify recombinant vaults that contain 6-8 copies of CP-MVP-EGF per vault. Here, we demonstrated that EGF vaults are functional in a cell proliferation assay and can stimulate the autophosphorylation of the EGFR receptor.

1. Anderson, D. H.; Kickhoefer, V. A.; Sievers, S. A.; Rome, L. H.; Eisenberg, D. Draft Crystal Structure of the Vault Shell at 9-Å Resolution. *PLoS Biol*. **2007**, *5*, 2661-2670.
2. van Zon, A.; Mossink, M. H.; Schoester, M.; Houtsmuller, A. B.; Scheffer, G. L.; Scheper, R. J.; Sonneveld, P.; Wiemer, E. A. The Formation of Vault-Tubes: A Dynamic Interaction between Vaults and Vault PARP. *J Cell Sci*. **2003**, *116*, 4391-4400.