

Vault Nanoparticles: Chemical Modifications for Imaging and Enhanced Delivery

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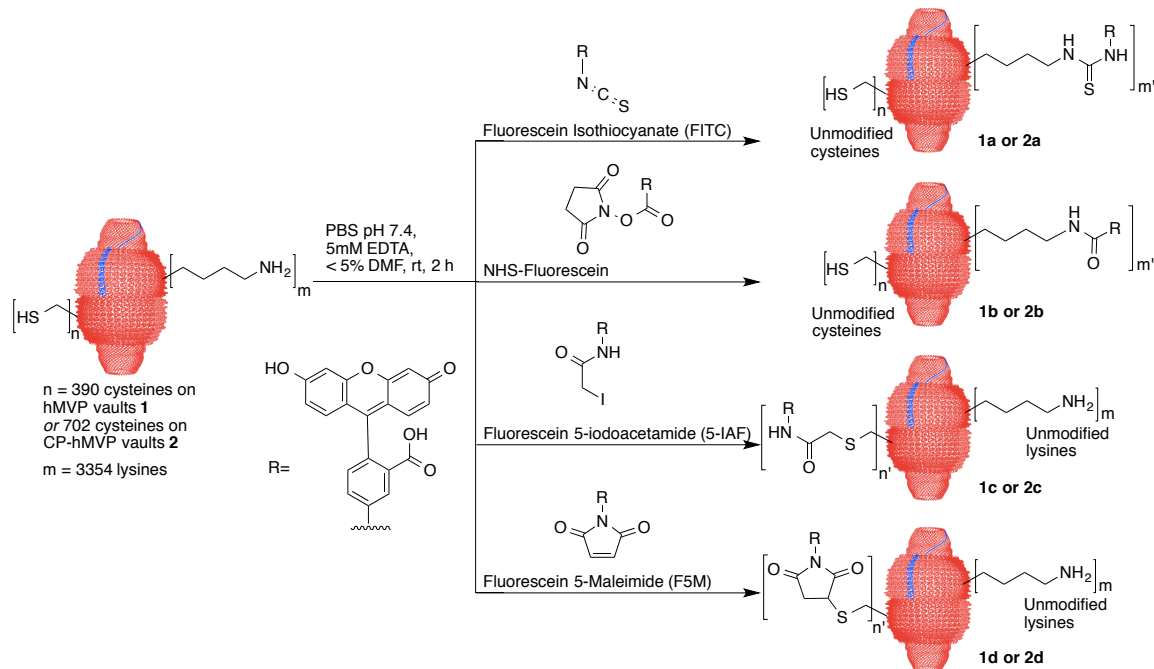
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SUPPORTING INFORMATION

Figure S1. Fluorescein Labeling of Vault Nanoparticles.



Protein recovery after fluorescein labeling and purification with Ultra-0.5 30K Centrifugal

Filter Devices (Millipore):

1a FITC labeled hMVP vaults: 58%

1b NHS-Fluorescein labeled hMVP vaults: 46%

1c 5-IAF labeled hMVP vaults: 53%

1d F5M labeled hMVP vaults: 55%

2a FITC labeled CP-hMVP vaults: 43%

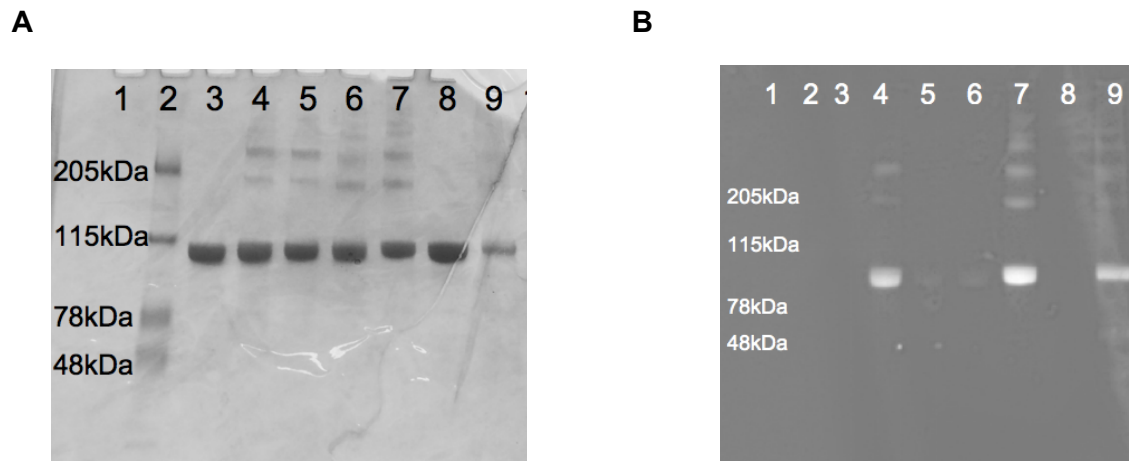
2b NHS-Fluorescein labeled CP-hMVP vaults: 39%

2c 5-IAF labeled CP-hMVP vaults: 58%

2d F5M labeled CP-hMVP vaults: 49%

Protein loss is presumably due to vaults sticking to the membrane of the filter devices.

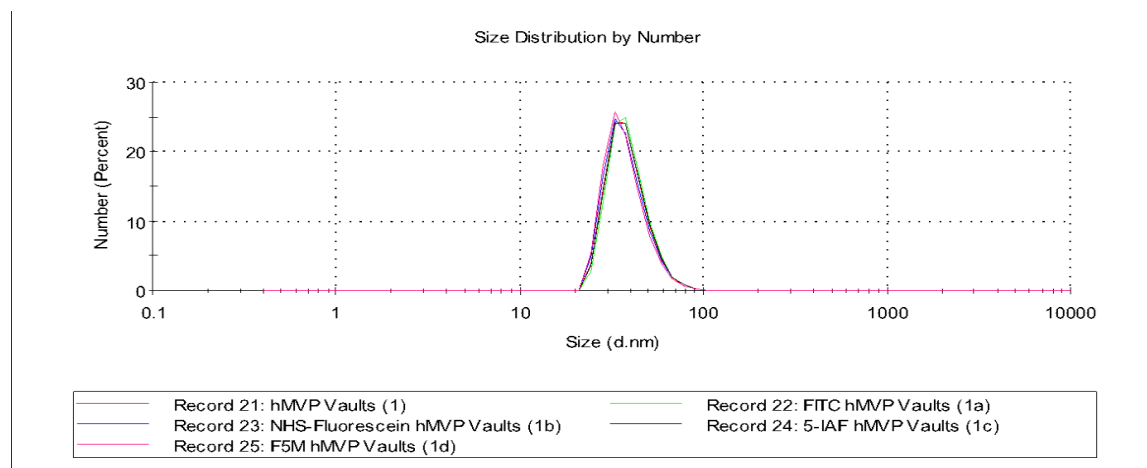
Figure S2. SDS-PAGE of unmodified and modified hMVP vaults visualized by (A) Coomassie blue staining and (B) under UV light (lane 1, blank; lane 2, prestained SDS-PAGE protein standard (broad range); lane 3, hMVP vault; lane 4, F5M-hMVP vault; lane 5, 5-IAF-hMVP vault; lane 6, FITC-hMVP vault; lane 7, NHS-Fluorescein-hMVP vault; lane 8, Traut's modified hMVP vault; lane 9, F5M-Traut's modified hMVP vault). The major protein band ~100 kDa indicates the hMVP or modified hMVP. Higher molecular weight band in fluorescein-modified vaults is likely due to the formation of vault dimers and trimers of fluorescent hMVP. Greater fluorescence was observed in lanes 4, 7, and 9 corresponding to F5M-hMVP vaults, NHS-Fluorescein-hMVP vaults, and F5M labeled Traut's modified hMVP vaults, respectively.



Qualitative Analysis of Protein by SDS-PAGE: The vault proteins (20 μ L of 0.1-0.2 mg/mL solutions in PBS) and 4 \times SDS-PAGE sample loading buffer (2 μ L) were mixed and incubated at 100 $^{\circ}$ C for 10 min. A 7 μ L sample was loaded on a 4-15% Bis-Tris SDS-PAGE gradient gel, and then separated by electrophoresis for 25 min at 200 V. Proteins bands were visualized under UV light or stained with Coomassie blue.

Figure S3. DLS traces of unmodified and fluorescein labeled (A) hMVP and (B) CP-hMVP vaults.

A)



B)

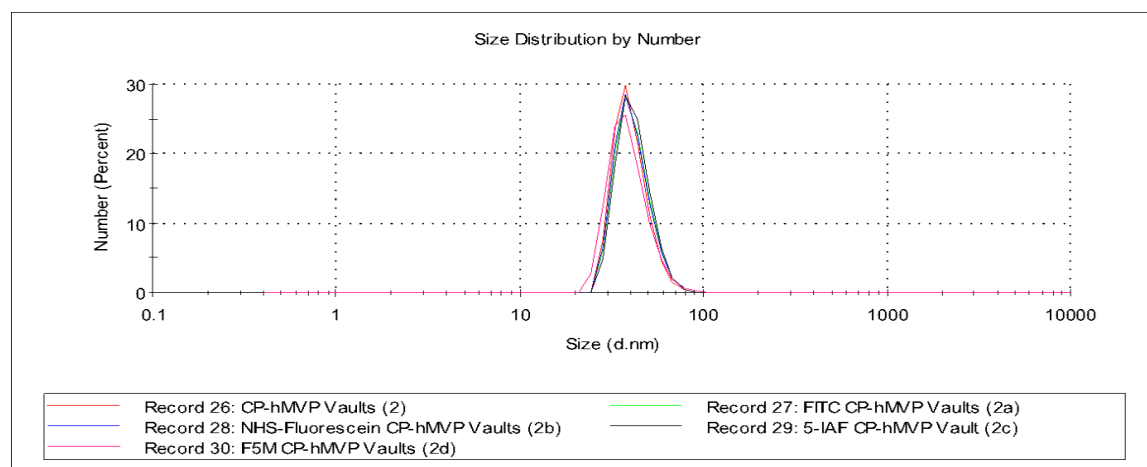
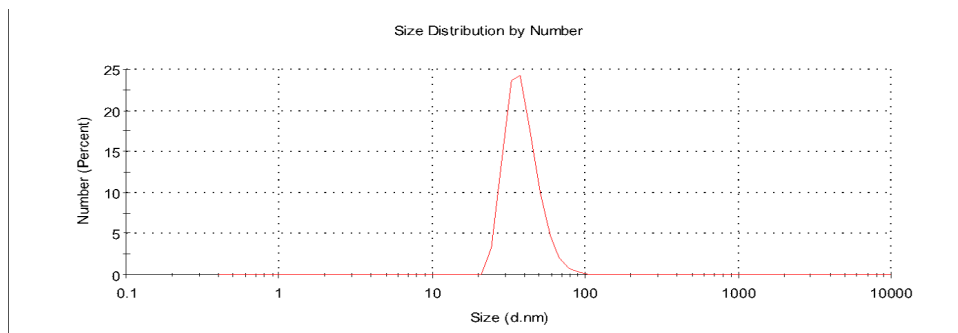
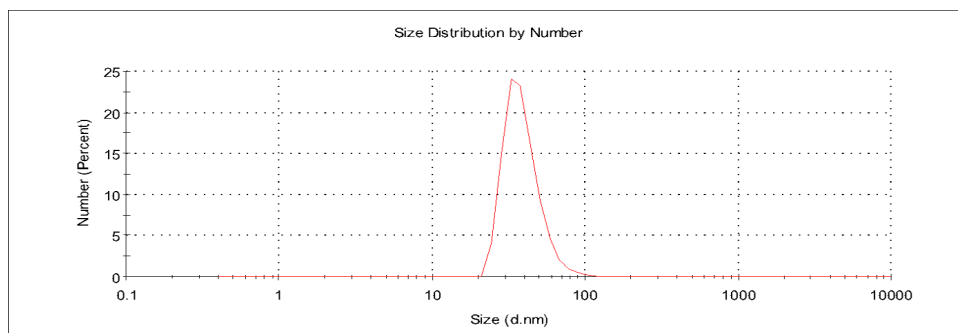


Figure S4. DLS traces of Traut's modified vaults and F5M labeled Traut's modified hMVP vaults.

A) Traut's modified hMVP vaults. Size: 39.3 ± 10.4 d.nm



B) F5M labeled Traut's modified hMVP vaults. Size: 39.2 ± 11.4 d.nm



C) Traut's modified CP-hMVP vaults. Size: 45.1 ± 11.4 d.nm

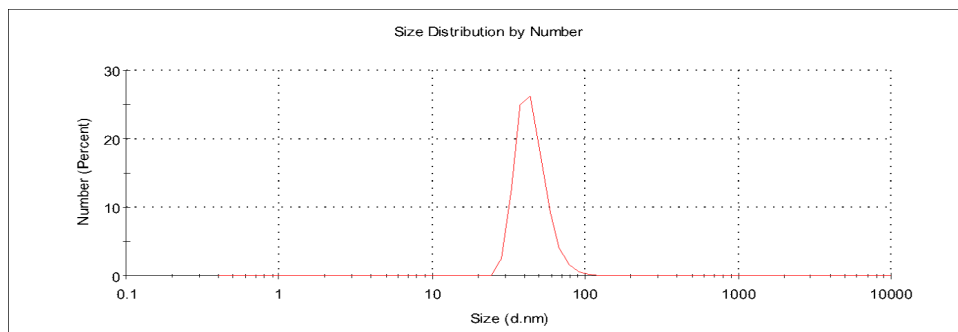


Figure S5. Electron microscopy of CP-hMVP vaults and Traut's modified CP-hMVP vaults. Negative staining with 1% uranyl acetate and imaged with a JEOL 1200EX microscope.

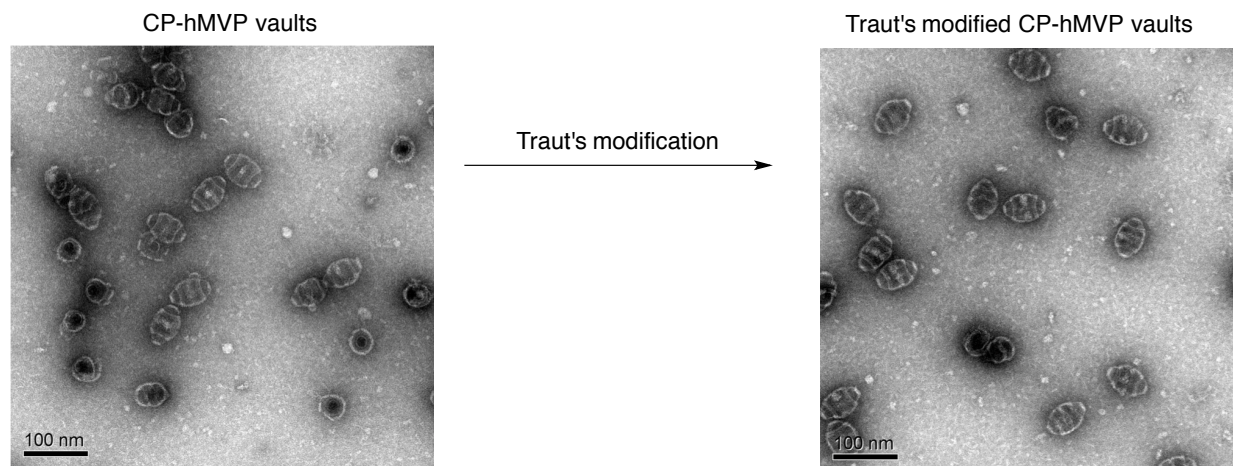


Figure S6. Circular dichroism spectra of hMVP vaults **1**, FITC hMVP vaults **1a**, FITC-Vault-r8_{Cleavable} **8**, and FITC-Vault-r8_{Non-cleavable} **12**. CD spectra were taken on a Jasco J-815 CD Spectrometer with a 1 mm quartz cuvette using 200 μ L of sample at 50-100 μ g/mL. These spectra show that the hMVP vaults maintain their secondary structure after covalent modifications.

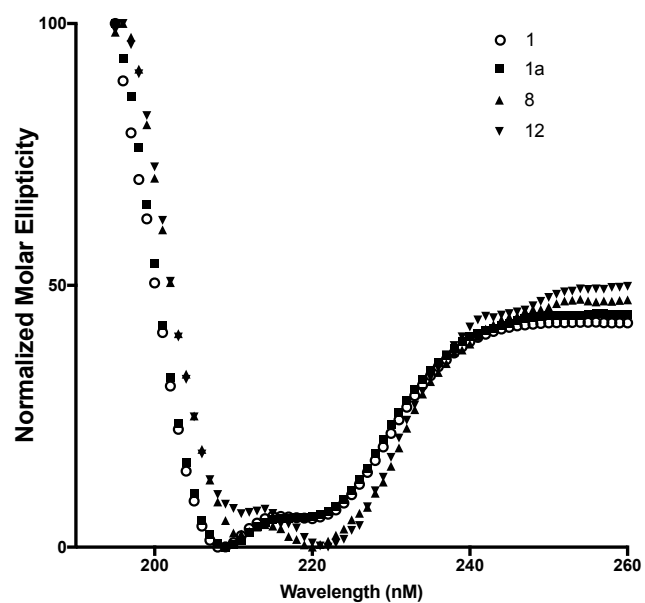
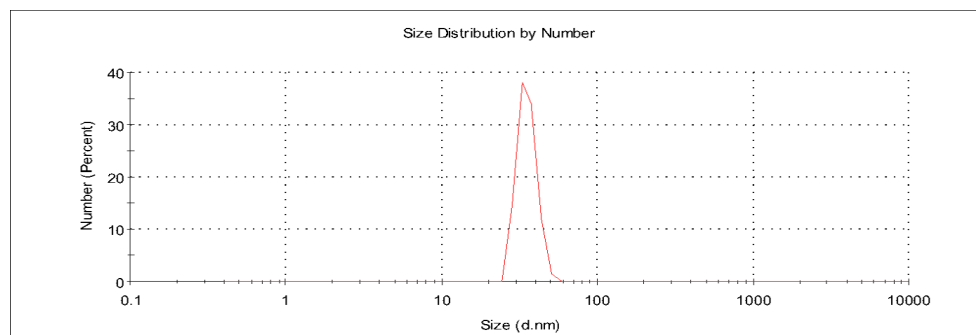


Figure S7. DLS traces of (A) FITC-Vault-r8_{Cleavable} **8** and (B) FITC-Vault-r8_{Non-cleavable} **12**. The increase in size is likely due to octaarginines on the vault surface.

(A)



(B)

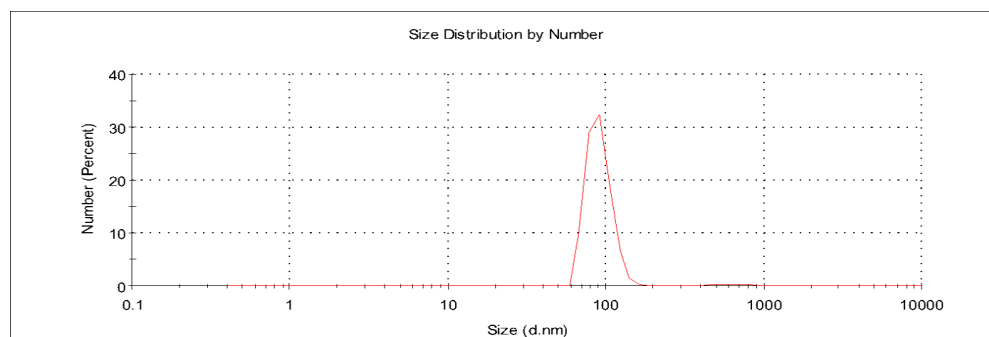


Figure S8. SDS-PAGE of unmodified and modified hMVP vaults visualized by Coomassie blue staining (lane 1, prestained SDS-PAGE protein standard; lane 2, hMVP vault; lane 3, FITC hMVP vault; lane 4, FITC-Vault-r8_{Cleavable}; lane 5, FITC-Vault-r8_{Non-cleavable}). This gel indicates that vaults remain mainly monomeric after the modifications.

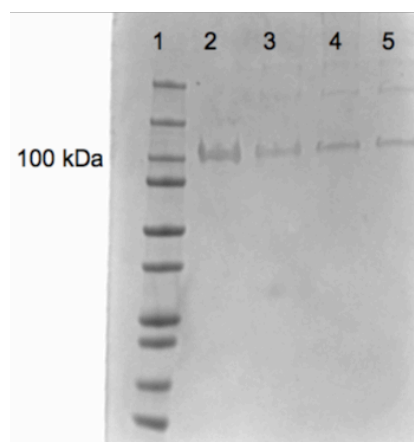


Figure S9. A. Overlay confocal images of live RAW264.7 cells (top), HeLa cells (middle), and CHO-K1 cells (bottom) incubated with 30 μg of modified vaults/500,000 cells.

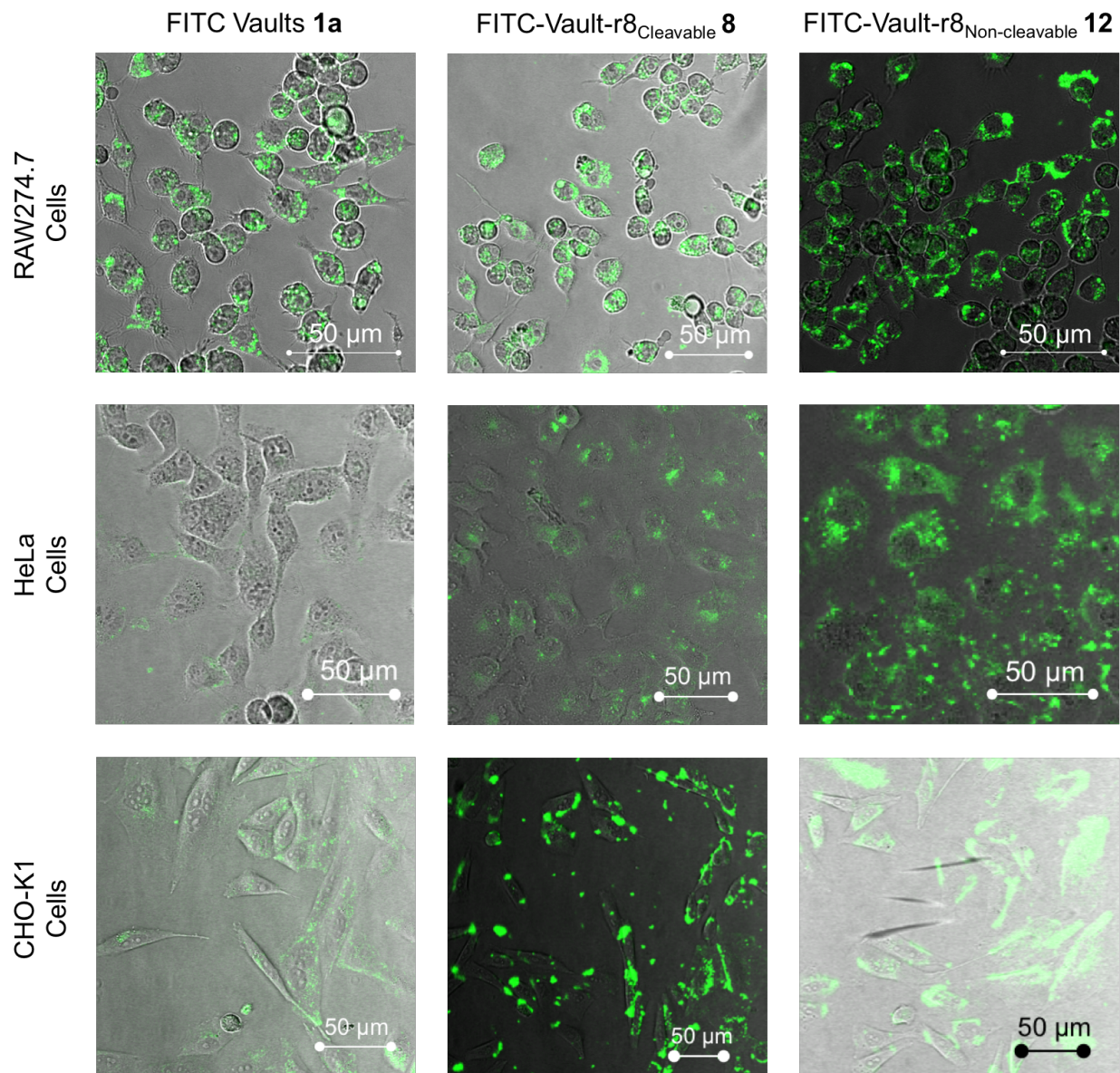


Figure S10. Normalized viability of cells treated with modified vaults, as determined by MTT assay over 16 hours. Modified vaults were tested at indicated concentrations in (A) RAW264.7 cells, (B) HeLa cells, (C) CHO-K1 cells. Data represents mean \pm SD, $N \geq 3$ for all measurements.

