

**Figure. Focused ion beam milling of mammalian cells for in situ cryo-EM study of flotillin microdomains.**

(A) Top-down scanning electron microscope (SEM) view of a mammalian cell after trench milling by focused ion beam (FIB) to expose regions of interest for lamella preparation.

(B) Shallow-angled SEM view showing a thin lamella (~100–150 nm) generated from the milled region, suitable for high-resolution in situ imaging of native membrane structures.

(C) Low-magnification SEM overview of the cryo-FIB grid containing multiple prepared lamellae across a grid square array. These lamellae preserve the ultrastructure of the membrane and will be used for single-particle cryo-EM data collection to resolve flotillin assemblies in their native context.

