Supplementary information, Figure S3

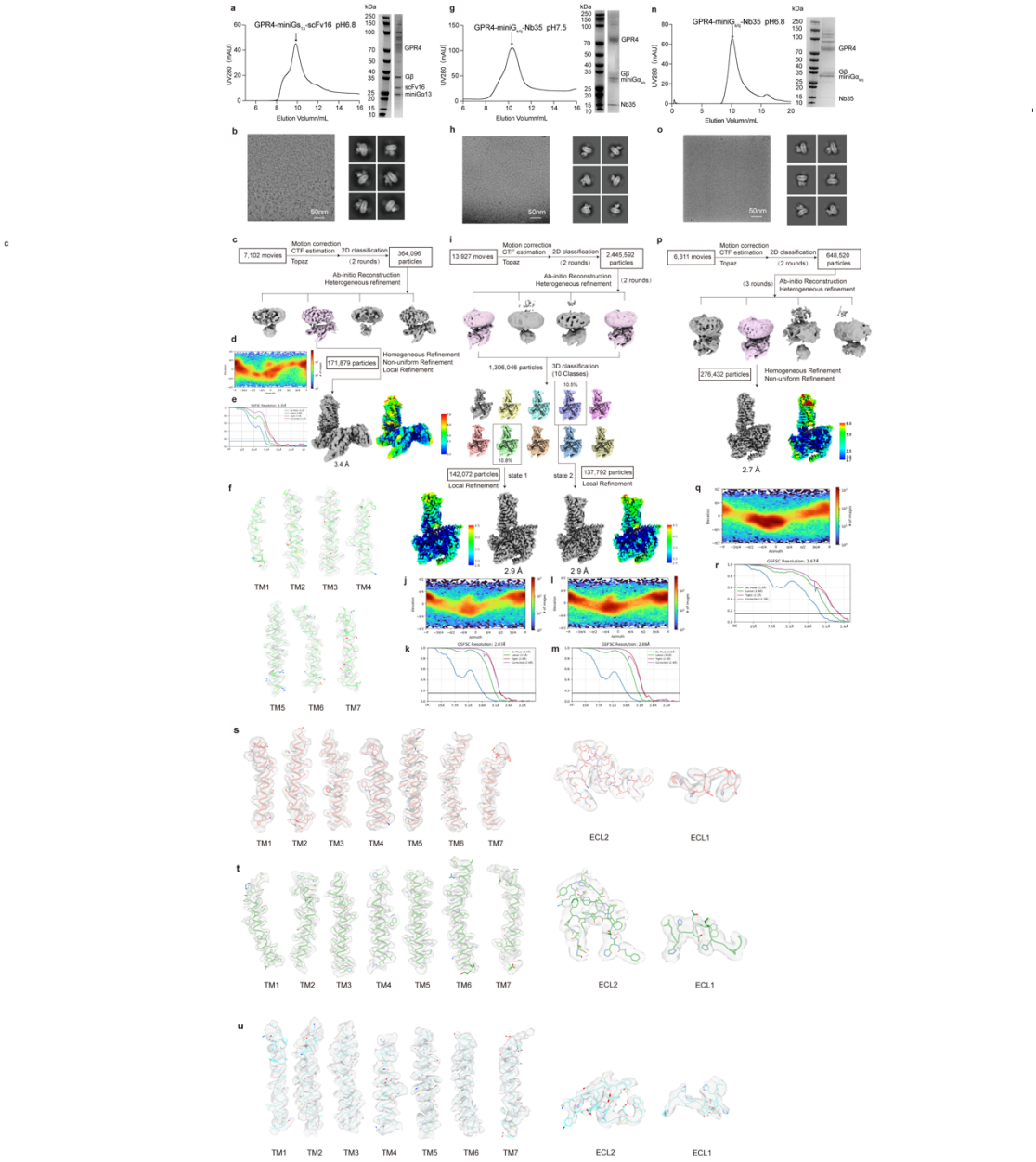

Fig. S3 Sample preparation and cryo-EM data processing of $_{pH6.8}$GPR4-miniG$_{13}$-scFv16, $_{pH7.5}$GPR4-miniG$_{s/q}$-Nb35 and $_{pH6.8}$GPR4-miniG$_{s/q}$-Nb35 complexes. **a, g, n,** Superdex200 size-exclusion chromatography elution profiles of the purified complex samples and SDS-PAGE analysis. **b, h, o,** Representative cryo-EM micrography and selected 2D classification. **c, i, p,** Schematic representation of cryo-EM data processing workflow. **d-e, j-m, q-r,** Angular distribution of the particles used for final reconstruction**,** cryo-EM maps are colored by local resolution (Å). The Fourier shell correlation (FSC) curves of $_{pH6.8}$GPR4-G$_{13}$, $_{pH7.5}$GPR4-G$_q$ and $_{pH6.8}$GPR4-G$_q$. The global resolution of the final processed density map estimated at the FSC = 0.143. **f,**

Cryo-EM maps and models of TMs for $_{pH6.8}$GPR4-miniG$_{13}$-scFv16. **s-u,** Cryo-EM maps and models of TMs and ECLs for $_{pH7.5}$GPR4-miniG$_{s/q}$-Nb35-state-1 (**s**), $_{pH7.5}$GPR4-miniG$_{s/q}$-Nb35-state-1 (**t**) and $_{pH6.8}$GPR4-miniG$_{s/q}$-Nb35 (**u**) complexes, respectively.