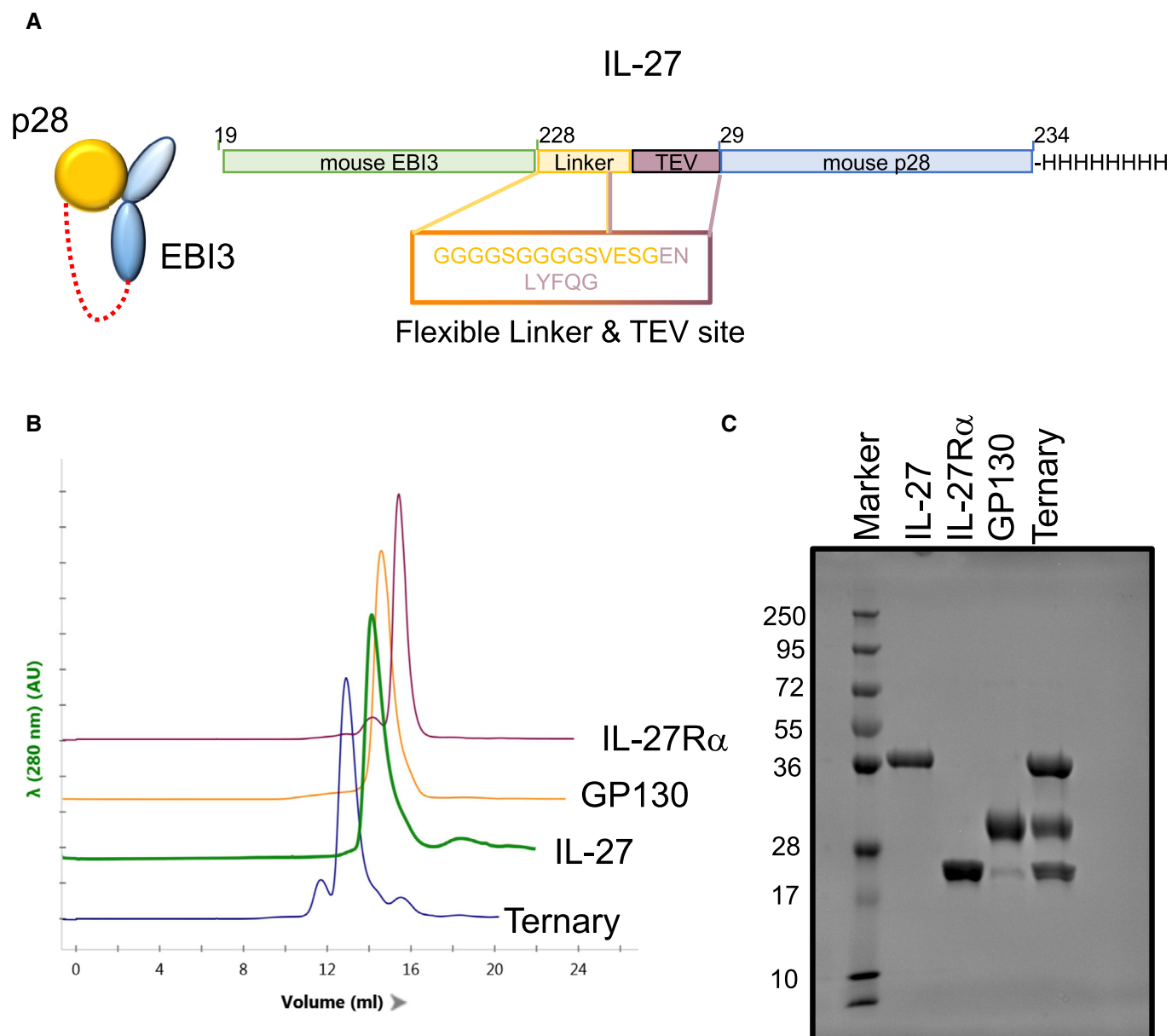
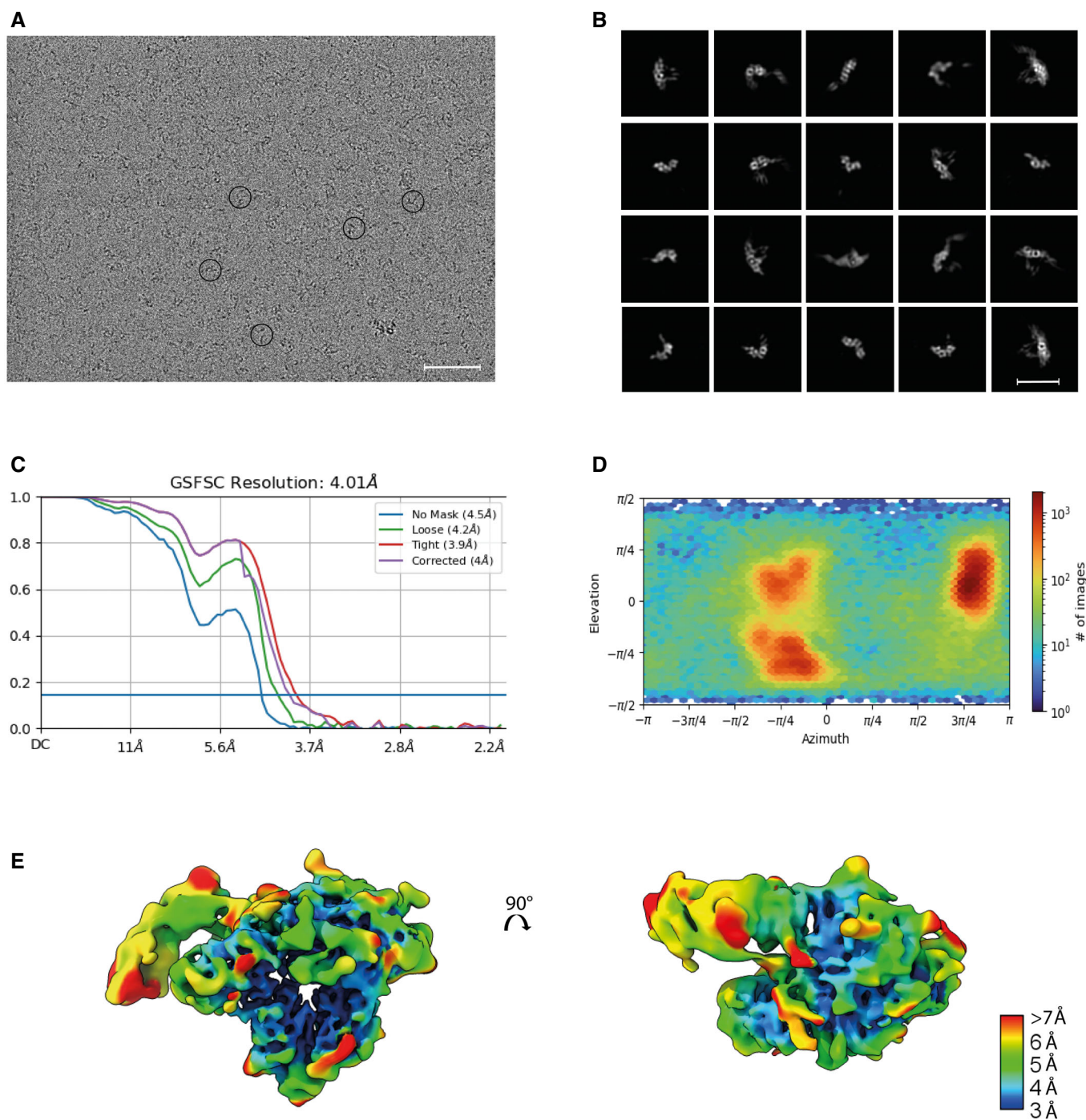


## Expanded View Figures



**Figure EV1. Assembly and characterization of the IL-27-receptor complex.**

- A Schematic representation of the single chain IL-27 heterodimer.  
 B Chromatogram overlay from size exclusion chromatography of the different IL-27 complex components.  
 C Coomassie-stained SDS-PAGE analysis under reducing conditions of the individual components plus the IL-27:IL-27R $\alpha$ :GP130 complex used for cryoEM studies.



**Figure EV2. Supplementary cryoEM data.**

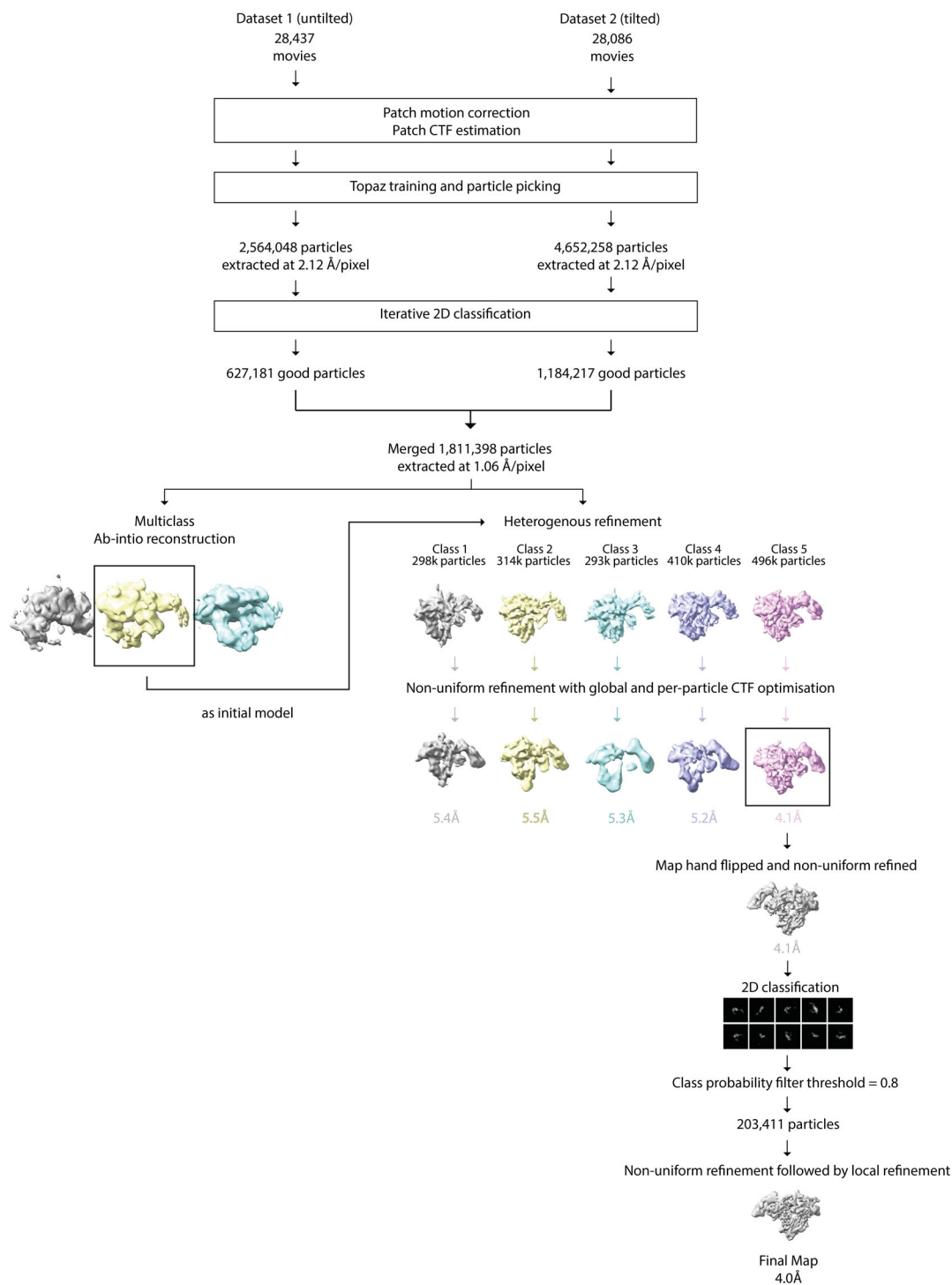
A Raw cryoEM micrograph with examples of the IL-27 receptor complex circled. Scale bar 50 nm.

B A subset of representative 2D class averages. Scale bar 110 Å.

C Gold standard Fourier shell correlation (GSFSC) for the final reconstruction. The resolution at the 0.143 cutoff is reported.

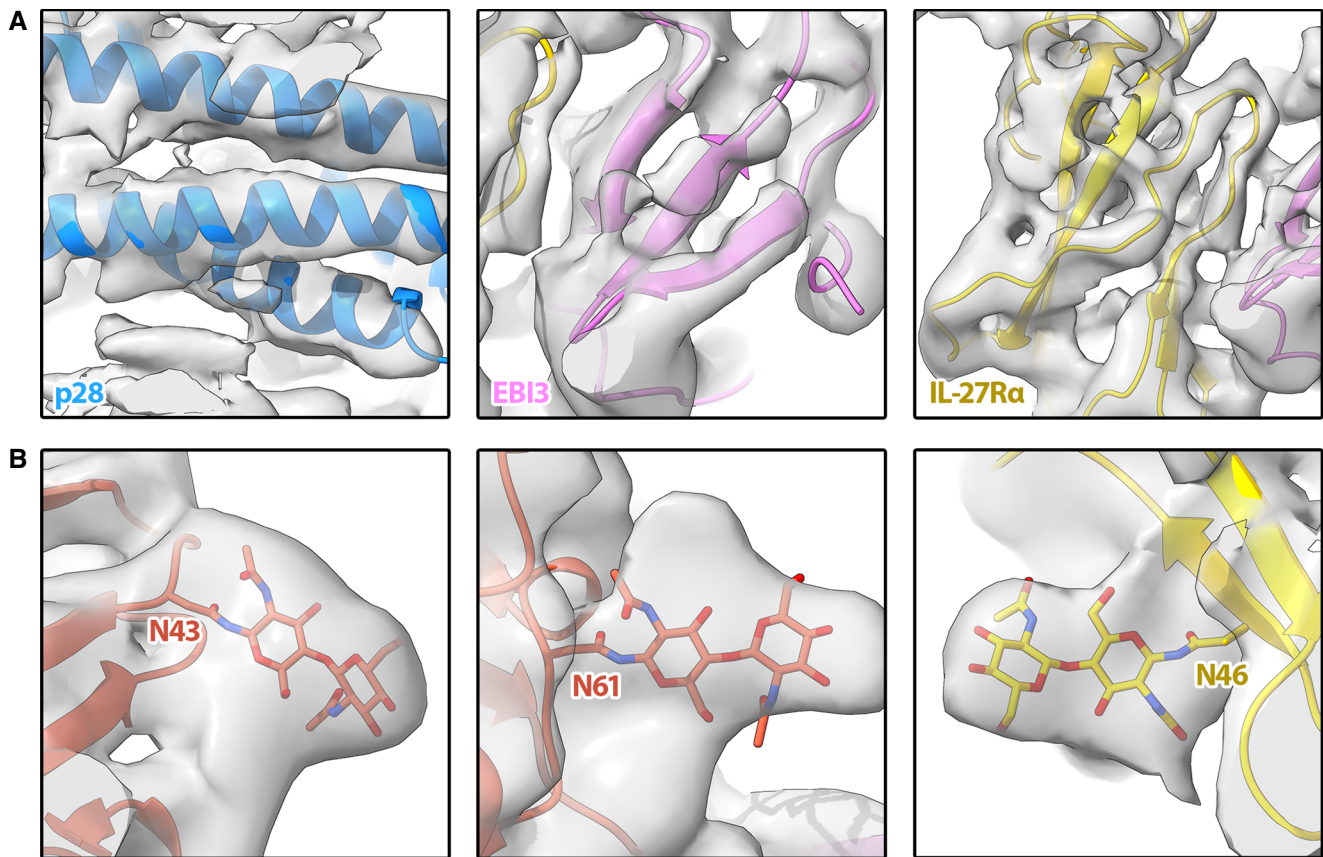
D Angular distribution plot.

E IL-27 receptor recognition reconstruction filtered according to local resolution ranging from 3 Å (blue) to 7–15 Å (red).



**Figure EV3. CryoEM image processing workflow outlining steps performed to obtain the structure of IL-27 heterodimer complex.**

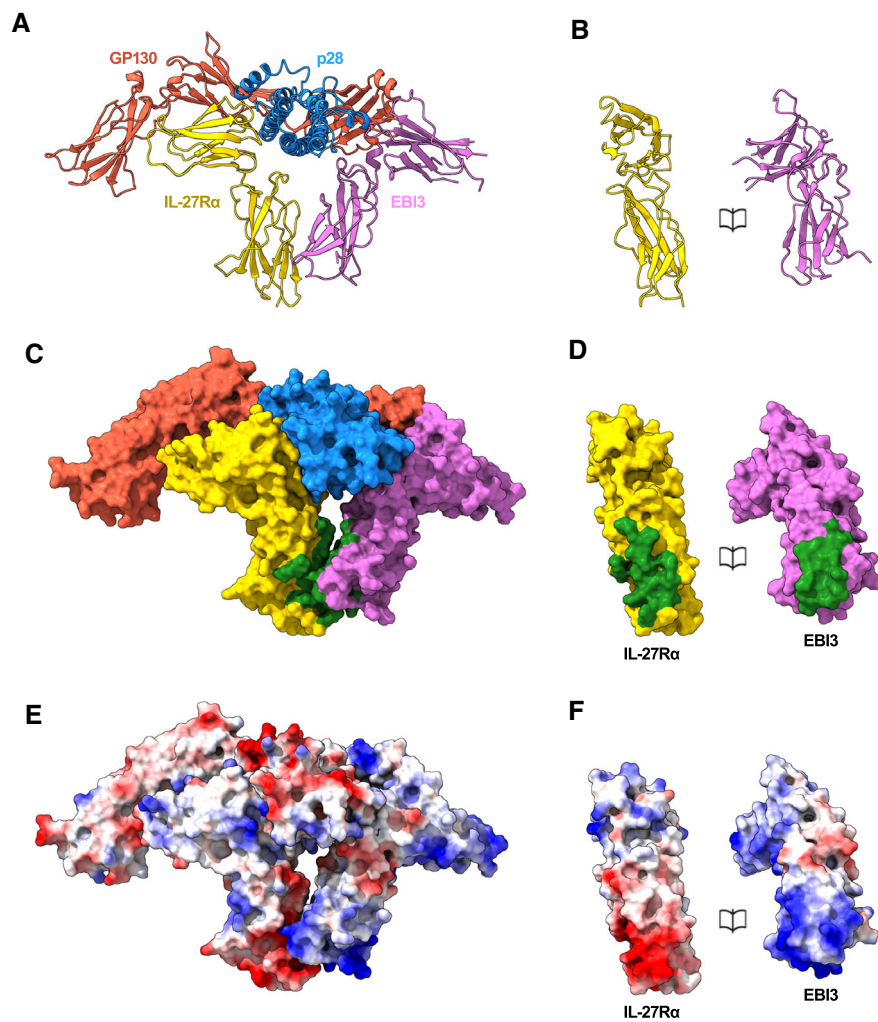
All processing was performed using CryoSPARC v.3.3.1 (see Materials and Methods for details).



**Figure EV4. Model for the IL-27 receptor signaling complex overlaid with the cryoEM map.**

A Map/model overlays for density corresponding to p28 (left panel), EB13 (middle panel), and IL-27R $\alpha$  (right panel).

B Representative glycan density in the map corresponding to known glycosylation sites on GP130 (left and middle panels) and IL-27R $\alpha$  (right panel).

**Figure EV5. EB13:IL-27R $\alpha$  interface.**

A–F Ribbon representation of the IL-27 receptor recognition complex (A) and book representation of the EB13:IL-27R $\alpha$  interface (B). Individual proteins colored: EB13 (purple), IL-27R $\alpha$  (yellow), p28 (blue), GP130 (red). Surface representation of the complex (C) and book representation of the interface (D). Proteins colored as in (A) with EB13: IL-27R $\alpha$  interface residues in green. Coulombic electrostatic potential ranging from  $-10$  (red) to  $10$  (blue) kcal/(mole) calculated from the models in A (E) and corresponding book representation of the interface (F).