

Fig S1. Conformational analysis of U-STAT1-U-STAT2 dimer.

- a. (i) Size-exclusion chromatography profile for STAT1-STAT1 homodimer and STAT1-STAT2 heterodimer. The fraction used for EM analysis is highlighted in red rectangle box. (ii) Two-dimension class averages of particles from negative stain EM micrographs. (iii) Fourier shell correlation (FSC) curve of the final EM map and the angular distribution of particles.
- b. Western analysis of STAT2 from a GST pull-down assay, using HEK293T cells transfected with GST-tagged truncations of STAT1.
- c. Western analysis of STAT1 from a GST pull-down assay, using HEK293T cells transfected with truncations of GST-tagged STAT2.
- d. EM density of STAT1-STAT2 dimers contain WT-STAT2 (contour level of 1.81) shown in blue, and T404A-STAT2 (contour level of 1.17) shown in orange. Correlation=0.9835, Overlap = 1.85e+05.
- e. U3A cells expressing WT, F77A, F172A, Q340W, or Y701F STAT1 were treated with IFN- β (100 IU/ml) for 30 min or were untreated. Whole-cell lysates were analyzed by the Western method.

