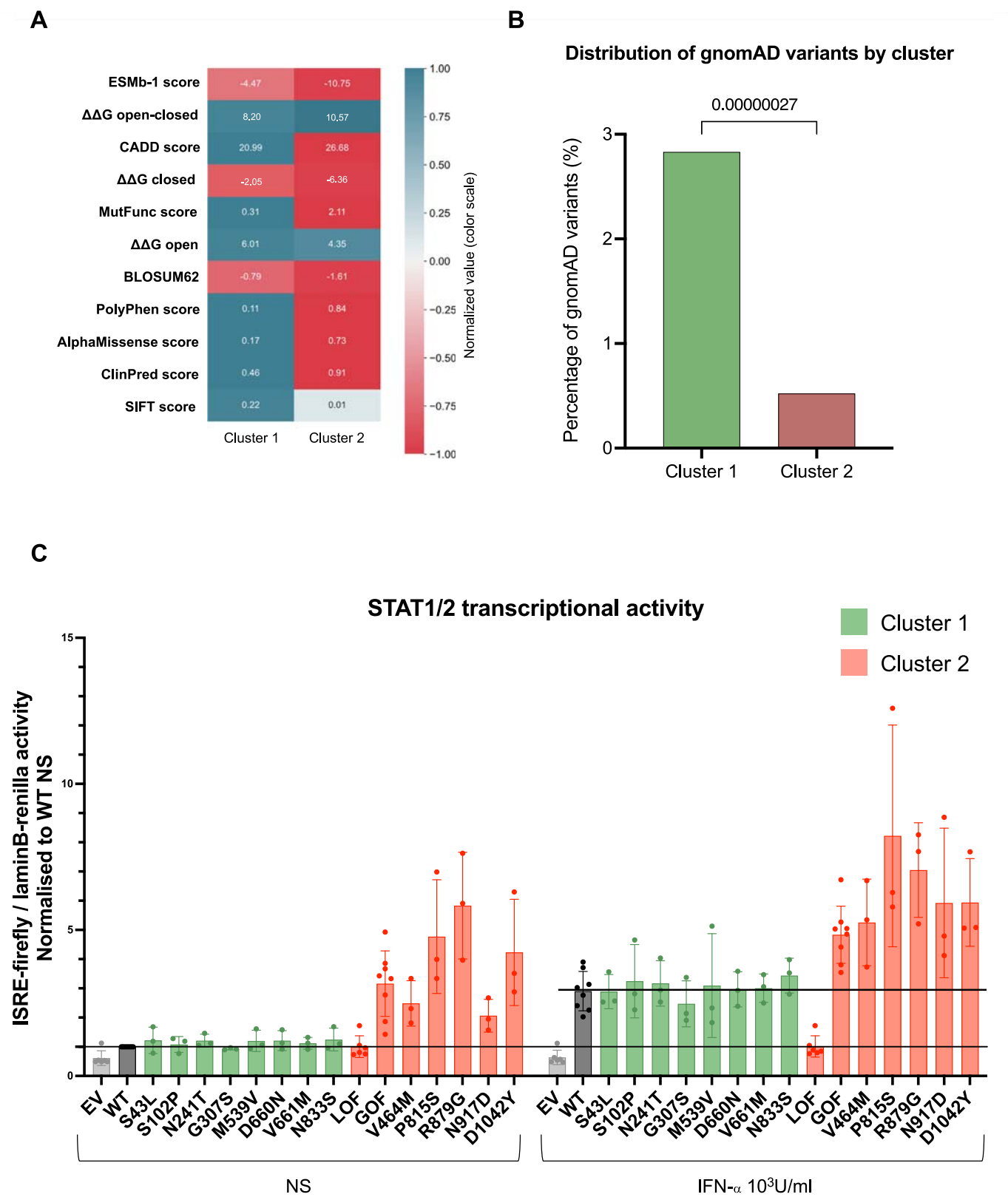
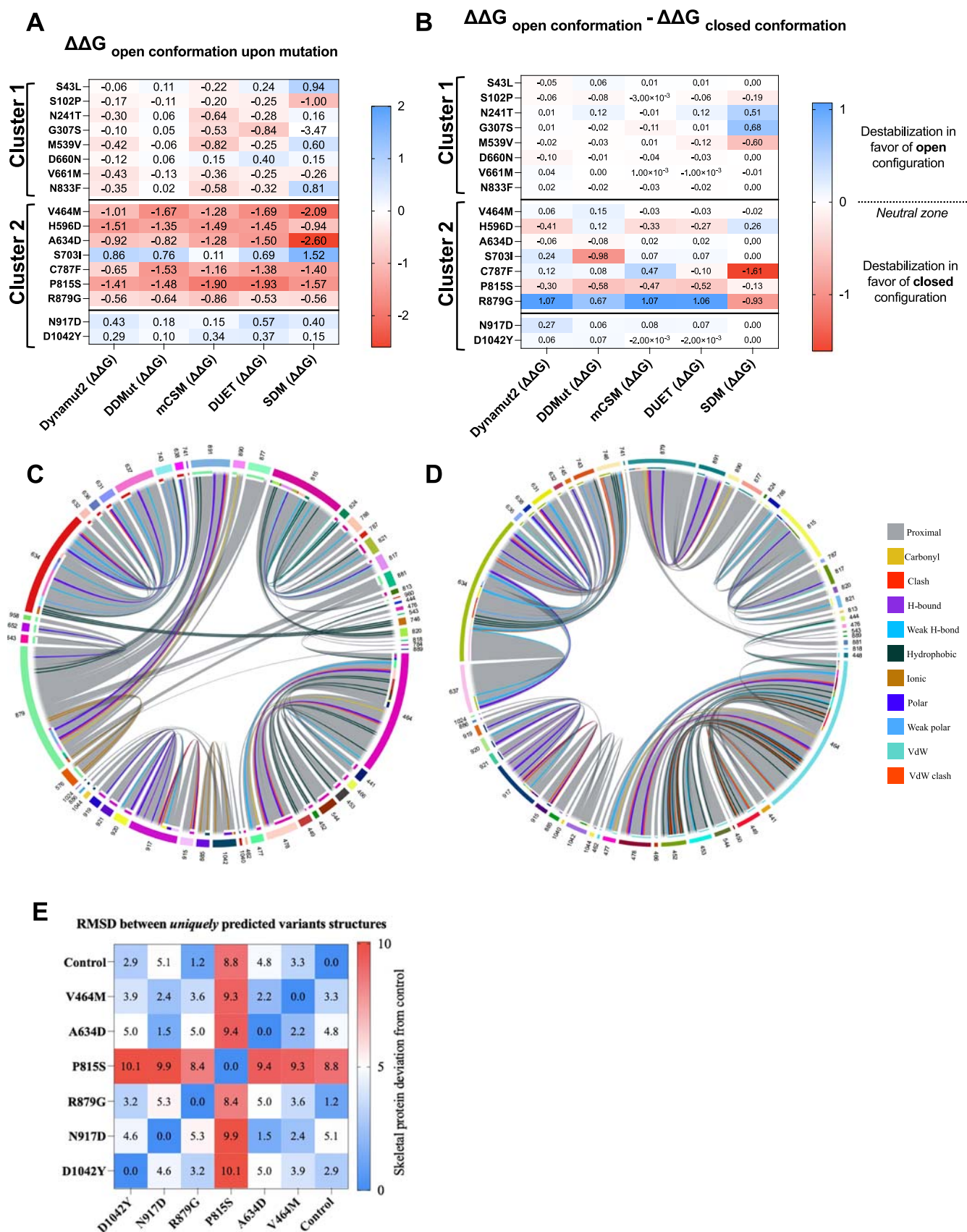


Expanded View Figures

Figure EV1. Validation of in silico pathogenicity prediction for JAK1 variants.

(A) Heatmap showing mean cluster values of in silico pathogenicity scores for JAK1 missense variants. Red indicates higher pathogenic values or negative $\Delta\Delta G$ (protein destabilization), blue indicates lower pathogenic values or positive $\Delta\Delta G$ (protein stabilization). Actual mean values are displayed in each cell. Color scale represents normalized values for visualization (red to blue). (B) Histogram showing distribution of gnomAD variants ($n = 287$) across the two clusters. P value was computed with Bonferroni-corrected Chi-square test and shown in the figure. (C) ISGF3 complex (STAT1, STAT2 and IRF9) luciferase reporter activity in U4C cells co-transfected with a plasmid containing the Interferon-Stimulated Response Element (ISRE) and WT or JAK1 variants at baseline or after stimulation with IFN- α (10^3 U/ml for 6 h); $n = 3$. JAK1 variants are grouped on the basis of their cluster affiliation. Error bars represent standard error of the mean.





**Figure EV2. Structural insights into uniquely predicted variants.**

(A) Predicted stability perturbation ($\Delta\Delta G$) of JAK1 variants in the open conformation, computed using five different methods (see Appendix Supplementary Methods). (B) Comparative analysis of $\Delta\Delta G$ values between open and closed JAK1 conformations, revealing the intrinsic tendency of mutated JAK1 to favor either conformation by minimizing the associated Gibbs free energy costs. Negative values indicate the tendency to adopt the closed conformation, while positive values point to a preference for the open conformation. (C, D) Chord plots illustrating residue-residue interactions in the JAK1 closed conformation. (C) Interactions involving wild-type residues. (D) Interactions altered by the variant. Each thread represents an atomic interaction, color-coded by interaction type. (E) Heatmap of root-mean-square deviation (RMSD) computed between pair of structures, quantifying the similarity between two superimposed atomic coordinates (see Appendix Supplementary Methods).

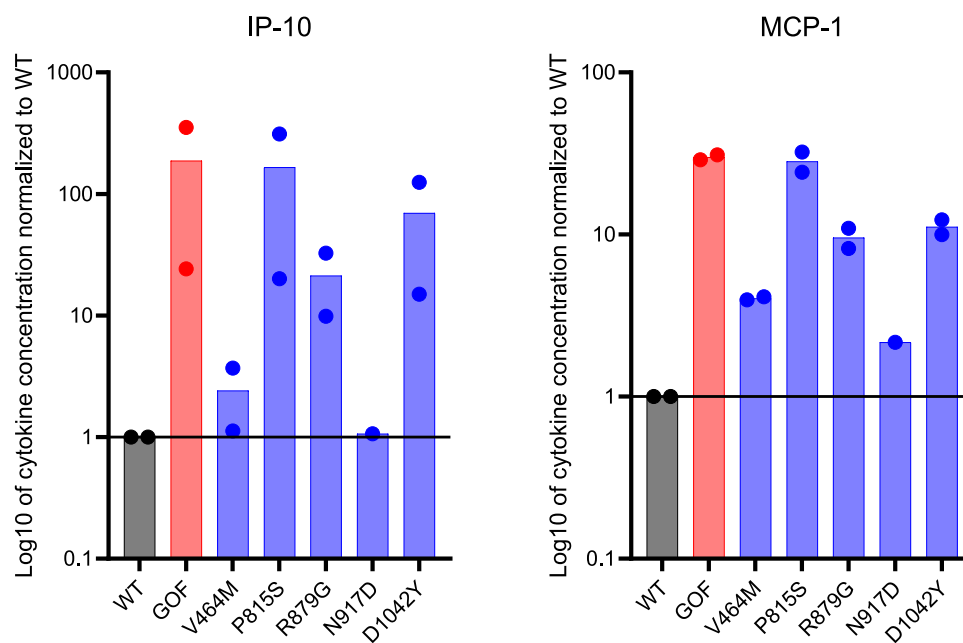


Figure EV3. Inflammatory cytokines secreted by U4C cells expressing *JAK1* variants.

MCP-1 or IP-10 levels measured in the supernatant of transfected U4C cells 24 h after transfection with WT, or JAK1 GOF (p.A634D), or patient's variants. $n = 2$ independent experiments.

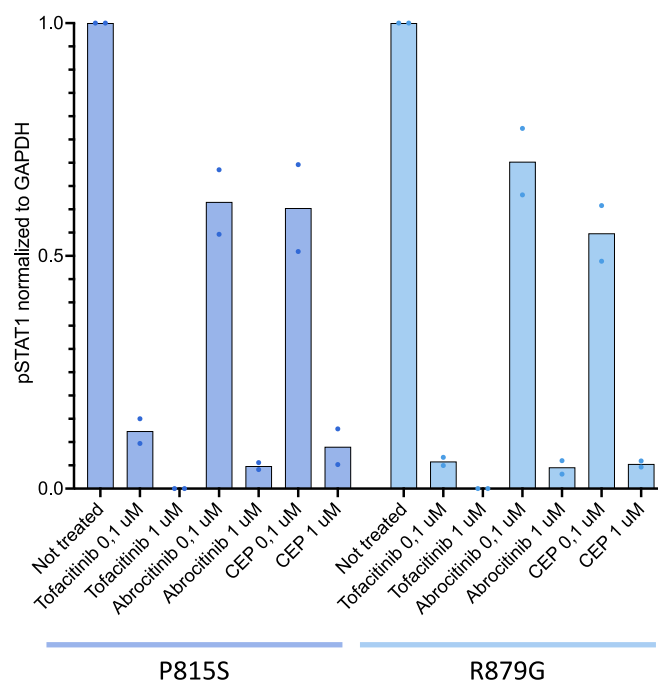


Figure EV4. Differential effect of JAKinibs on transduced U4C cells.

Western blot quantification of STAT1 phosphorylation in U4C cells transduced with different JAK1 variants at baseline and after different JAK inhibitors treatment. pSTAT1 band quantification is normalized to total GAPDH and baseline condition. $n = 2$ independent experiments. Histogram represents the mean.