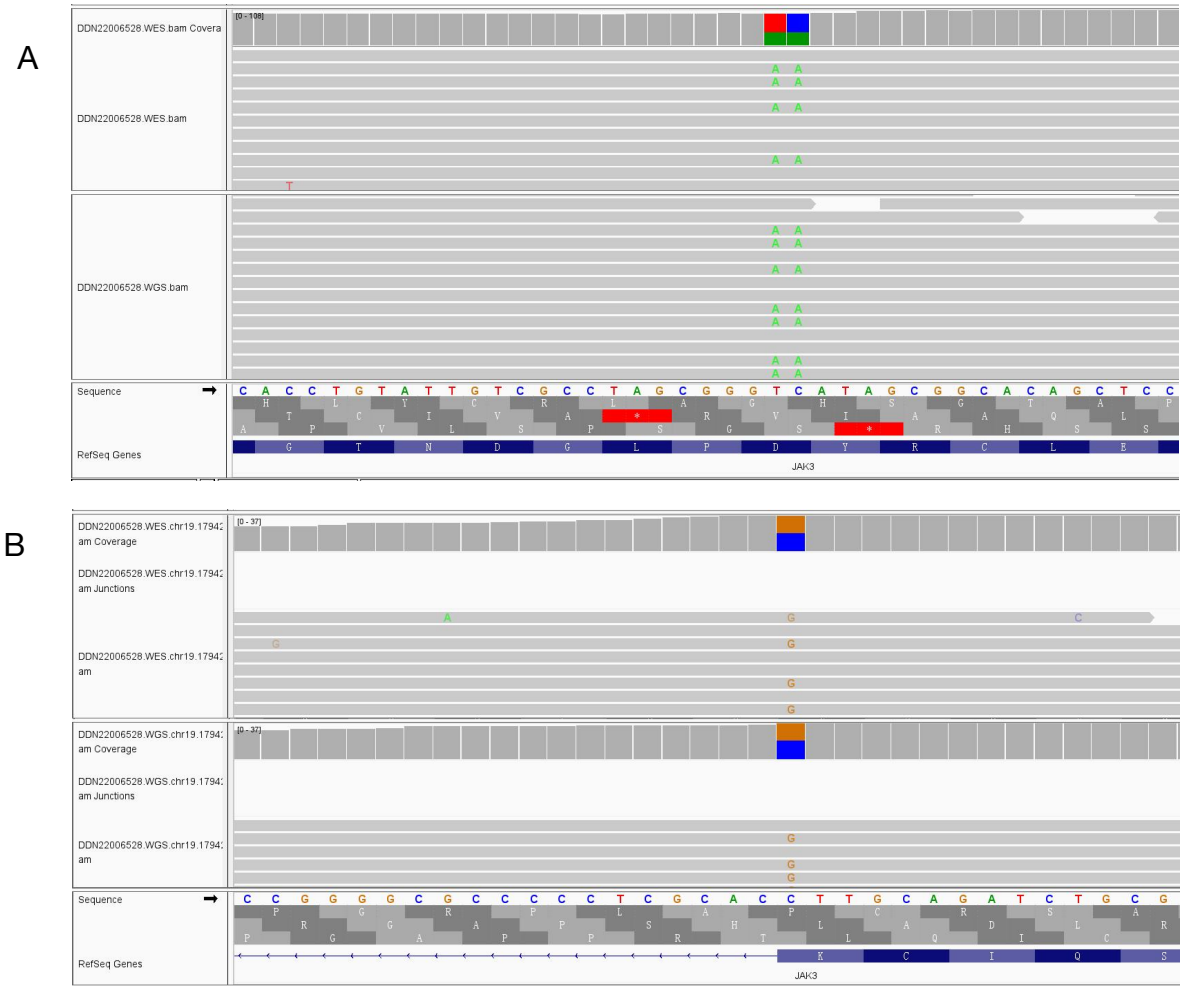
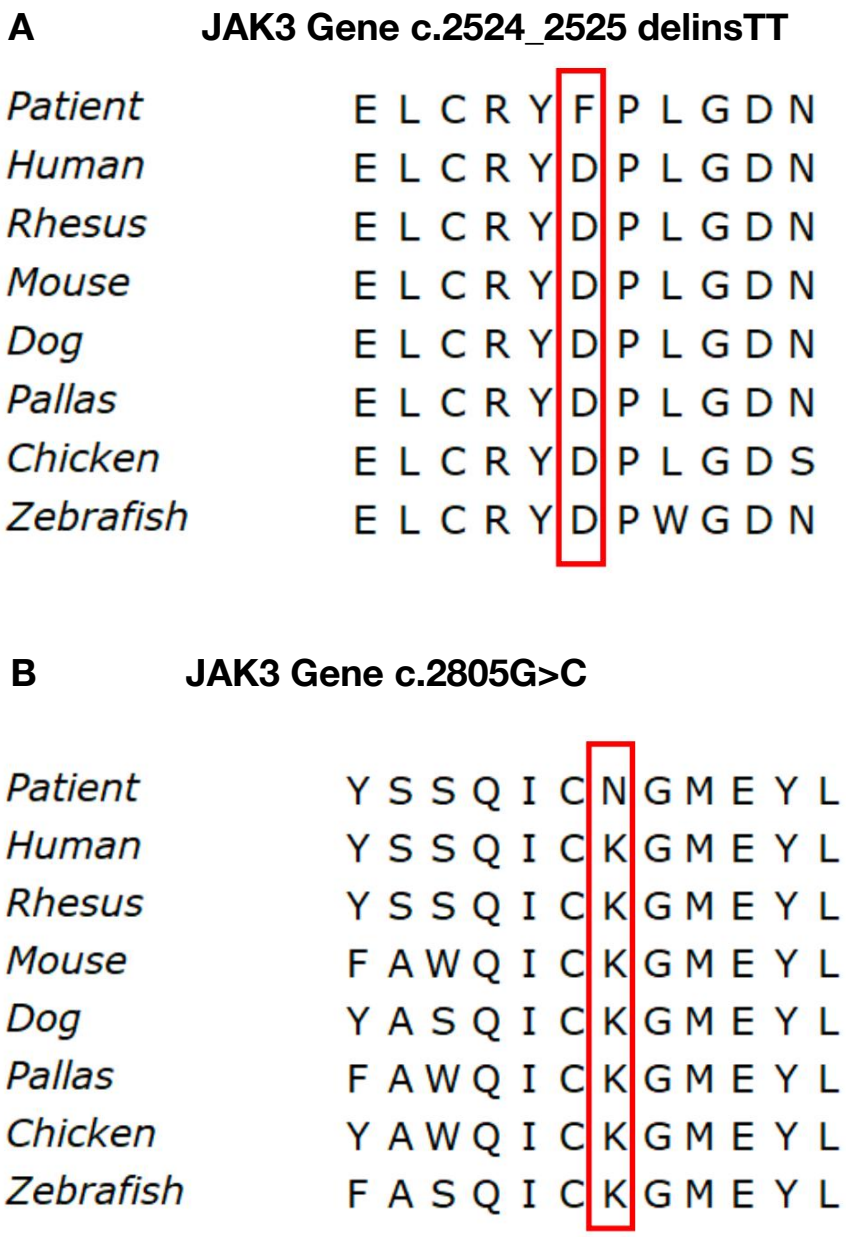
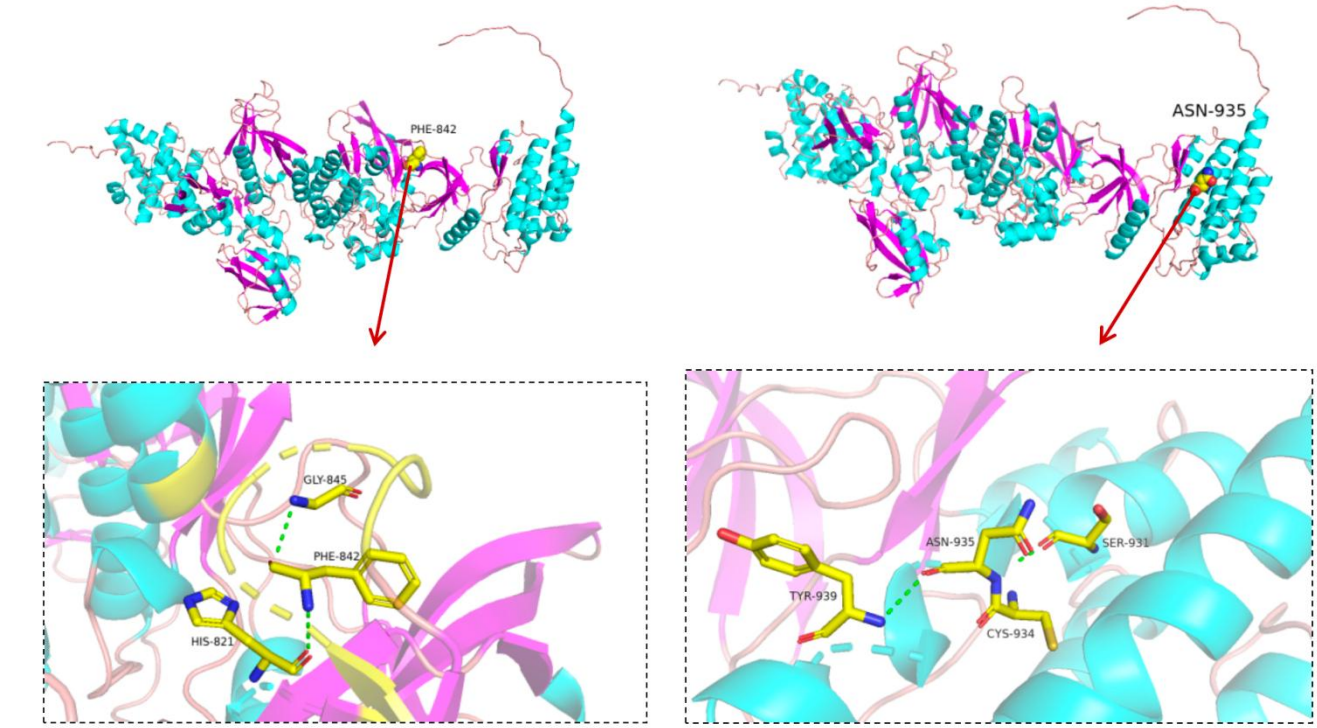


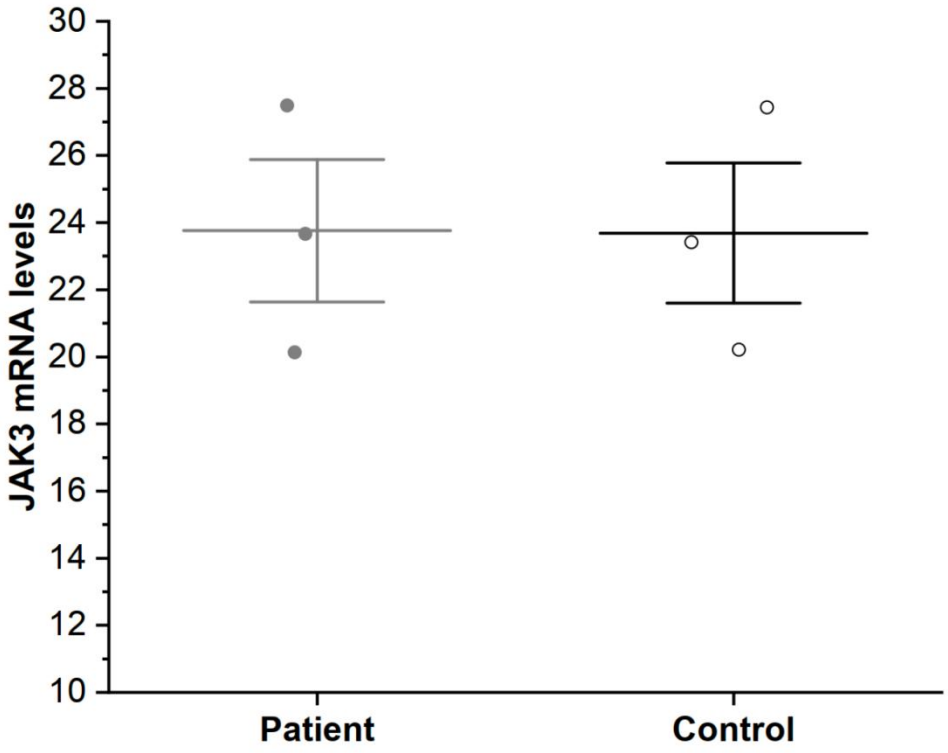
Supplementary Fig 1. Integrative Genomics Viewer Plot of JAK3 Gene Variant. (A) Whole-genome and whole-exome sequencing reads mapping to JAK3 locus c.2524_2525, with variant nucleotides displayed in green. The WES coverage depth is 95 reads, with the wild-type TC accounting for 58% and the variant TT accounting for 42%. The WGS coverage depth is 37 reads, with the wild-type TC accounting for 48% and the variant TT accounting for 62%. (B) Whole-genome and whole-exome sequencing reads mapping to JAK3 locus c.2805, with variant nucleotides displayed in brown. The WES coverage depth is 36 reads, with the wild-type G accounting for 50% and the variant C accounting for 50%. The WGS coverage depth is 36 reads, with the wild-type G accounting for 53% and the variant C accounting for 47%.



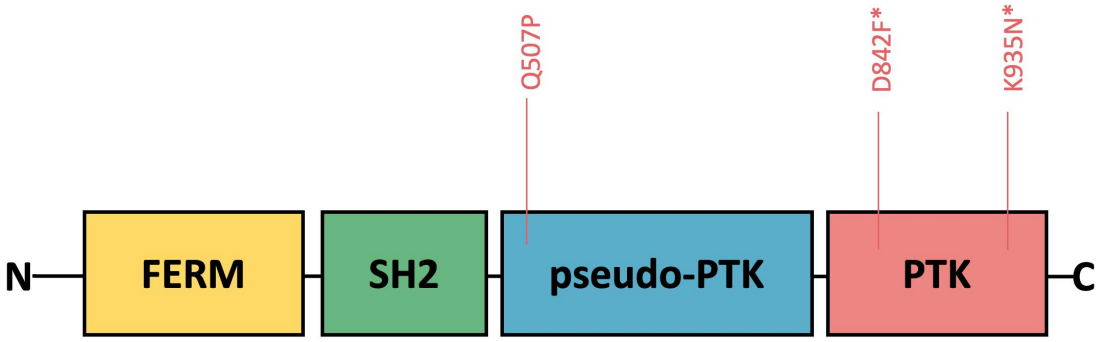
Supplementary Fig 2. Conservation Analysis of JAK3 Protein. Homologous protein sequences were downloaded from the HomoloGene database on the NCBI website, and sequence alignment and visualization were performed using UniproUGENE software. Comparative analysis across multiple species shows that both the 842nd and 935th amino acids of the JAK3 gene are highly conserved. The patient's c.2524_2525delinsTT variant results in an aspartic acid to phenylalanine substitution at position 842 (A), while the c.2805G>C variant leads to a lysine to asparagine substitution at position 935 (B).



Supplementary Fig 3.Visual Analysis of Structural Impacts of Variants at Positions 842 and 935 in the JAK3 Protein. The 3D protein structure analysis was conducted using protein sequences downloaded from the NCBI website (https://www.ncbi.nlm.nih.gov/). Homology modeling of the protein structure was performed using the online analysis tool Swiss-Model (https://swissmodel.expasy.org/interactive). The selected model's PDB file was downloaded and visualized using PyMOL viewer software, and images were saved. The c.2524_2525delinsTT variant results in an aspartic acid (D) to phenylalanine (F) substitution at position 842, altering the local hydrogen bonding network and potentially affecting protein conformation. Similarly, the c.2805G>C variant leads to a lysine (K) to asparagine (N) substitution at position 935, disrupting the hydrogen bond interactions with surrounding residues. Blue represents α -helices, purple represents β -sheets, and pink loops represent loop structures. The observed hydrogen bonds are displayed as stick models, with each color representing different atoms: yellow for carbon (C), gray for hydrogen (H), blue for nitrogen (N), red for oxygen (O), and orange for sulfur (S). Hydrogen bonds are shown in green.



Supplementary Fig 4. Comparison of JAK3 mRNA Levels in Peripheral Blood Between Patient and Healthy Control. The JAK3 mRNA levels in the patient with JAK3 mutation are comparable to those in control group.



Supplementary Figure 5: Germline gain-of-function mutations in JAK3. JAK3 mutations associated with immune cell diseases. JAK3 protein has four domains: four-point-one, ezrin, radixin and moesin (FERM), Src-homology 2 (SH2), pseudo-protein tyrosine kinase (pseudo-PTK), and protein tyrosine kinase (PTK) domains. Reported germline gain-of-function mutations.

Supplementary Table 1. JAK3 Gene Mutation Analysis and ACMG Evaluation

Gene	Exon Number	Nucleotide alteration	Amino acid change	Zygosity	From	SIFT Score	Mutation Taster Prediction	dbSNP	1000 Genomes Project	gnomAD	ACMG Evaluation
JAK3	exon 19	c.2524_2525delinsTT	p.Asp842Phe	Het	Father	<0.05	D	NC	NC	NC	PM1+PM2+PM3+PP3
	exon20	c.2805G>C	p.Lys935Asn	Het	Mother	<0.05	D	NC	NC	NC	PM1+PM2+PM3+PP3

Het: heterozygous; D: disease causing; NC: Not collected; ACMG: American College of Medical Genetics and Genomics.