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A BIOINFORMATICS PIPELINE REVEALS A SHARED IκBα INTERFACE FOR NF-κB AND HISTONE BINDING

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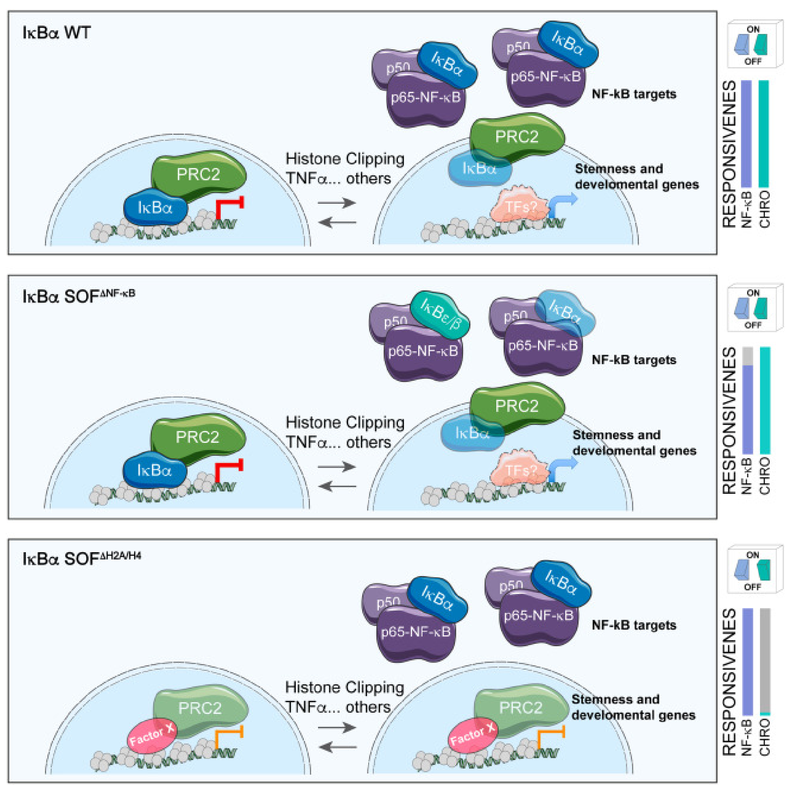
SUMMARY

IκBα, traditionally a negative regulator of NF–κB, has recently been linked to chromatin-dependent transcriptional control(Marruecos et al., 2021). We developed a bioinformatics pipeline to identify conserved, surface-exposed residues mediating protein interactions. Our Fold-Excluded Evolutionary Conservation (FEEC) metric integrates structural packing (WCN) and evolutionary conservation (ConSurf) to prioritize IκBα binding residues. FEEC highlighted an ANK-repeat surface engaging both the *p*65 NLS and histone H4 tail. Guided by FEEC, we engineered separation-of-function (SOF) mutants disrupting either NF–κB binding ($SOF^{\Delta NF\text{-}\kappa B}$) or histone association ($SOF^{\Delta H2A/H4}$). Structural modeling, mutagenesis, and functional assays confirmed their specificity. Transcriptomic analysis of $SOF^{\Delta NF\text{-}\kappa B}$ cells revealed repression of intestinal stem-cell genes independent of NF–κB, underscoring a chromatin-related role for IκBα. FEEC thus provides a generalizable framework to resolve multifunctional protein interfaces.(Álvarez-Villanueva et al., 2025)



Regulation of NF-κB and PRC2 target genes by IκBα WT and SOF mutants

METHODOLOGY

The **Weighted Contact Number (WCN)** quantifies atomic contact density from interatomic deistances as

$$\mathrm{WCN}_i = \sum_{j \neq i} \frac{1}{r_{ij}^2}, \quad \mathrm{iWCN}_i = \frac{1}{\mathrm{WCN}_i}.$$

Sequence conservation scores ($S_{C,i}$) were obtained from the ConSurf server and expressed as Z-scores. The **Fold-Excluded Evolutionary Conservation (FEEC)** score identifies residues with conservation beyond structural constraints:

$$\mathrm{FEEC}_i = \mathrm{iWCN}_i - S_{C,i}.$$

Interface energies were computed from Rosetta-generated conformations weighted by Boltzmann probabilities and expected bingin energies:

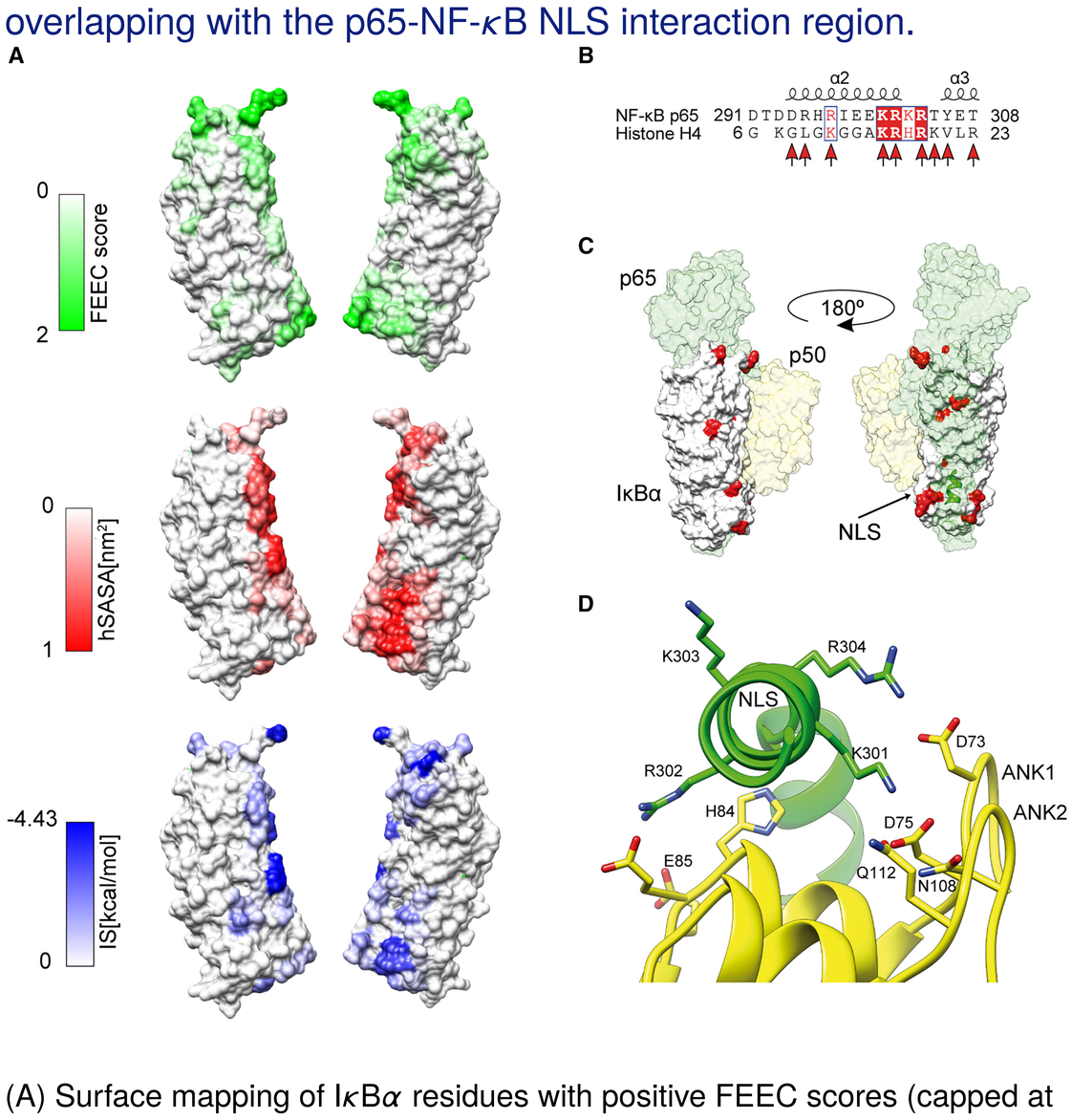
$$P_i = \frac{e^{-E_i/kT}}{\sum_{i=1}^{N} e^{-E_i/kT}},$$

$$\langle E_{\mathrm{binding}} \rangle = \sum_i P_i E_{b,i}, \quad E_{b,i} = E_{\mathrm{complex},i} - (E_{A,i} + E_{B,i}).$$

Mutational effects were estimated after repacking and minimizing residues within 8 Å of each mutation site.

COMMON DOMAIN OF IκBα BINDING TO P65-NF-κB AND H2A/H4

FEEC scores accurately predicted most IκBα/NF-κB interface residues (83% for p65, 73% for p50), validating FEEC as a reliable predictor of binding sites. Mapping positive FEEC residues revealed strong overlap with known interface regions from structural data. Negatively charged FEEC-positive residues in the ANK1-ANK2 domains (e.g., D73, D75, E85, E86) likely form the binding site for the histone H4 N-terminal tail, overlapping with the p65-NF-κB NLS interaction region.



(A) Surface mapping of IκBα residues with positive FEEC scores (capped at 2), solvent-accessible area buried upon NF–κB binding, and per-residue interface scores (PDB: 1NFI). (B) Alignment of the p65-NF–κB NLS region with the H4 N-terminal tail; conserved positions (blue), similar/identical residues (red/white), and IκBα-contacting residues (red arrows) are indicated (PDB: 3UW9). (C) IκBα (white)-NF–κB (p65, green; p50, yellow) complex showing negatively charged IκBα residues with FEEC>0 (red). (D) Interaction of the p65 NLS motif (KRKR, green) with IκBα ANK1-2 repeats; polar interacting residues in yellow. UniProt Q04206 (p65) and P25963 (IκBα).

REFERENCES

Álvarez-Villanueva, Daniel et al. (2025). "Separation-of-function mutants reveal the NF-κB (independent) role of chromatin-bound IκBα in intestinal stemness and differentiation". In: *Cell Reports* 44.7, p. 115949. DOI: 10.1016/j.celrep.2025.115949.

Marruecos, L. et al. (2021). "Dynamic chromatin association of IκBα is regulated by acetylation and cleavage of histone H4". In: *EMBO Reports* 22.8, e52649. DOI: 10.15252/embr.202152649.