A Bioinformatics Pipeline Reveals a Shared IκBα Interface for NF-κB and Histone Binding

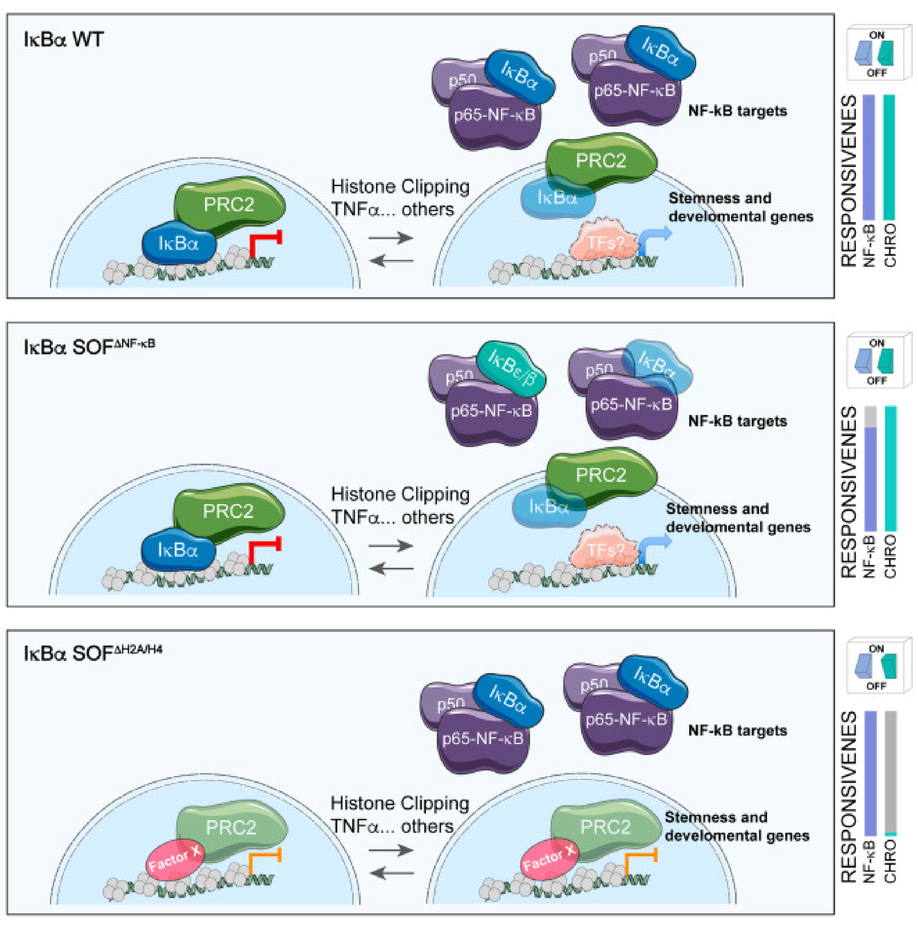
UNIVERSITAT DE VIC
UNIVERSITAT CENTRAL
DE CATALUNYA

Iris CC

Martin Floor[1,3], Jordi Villà-Freixa[1,2], Joan Bertran[1,2] and Lluís Espinosa[4]
[1]Computational Biochemistry and Biophysics Lab, Faculty of Sciences, Engineering and Technology, Universitat de Vic - Universitat Central de Catalunya
[2]Institut de Recerca i Innovació en Ciències de la Vida i de la Salut a la Catalunya Central
[3]Barcelona Supercomputing Center, Life Sciences Department
[4]Hospital del Mar Research Center

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 binding energies:

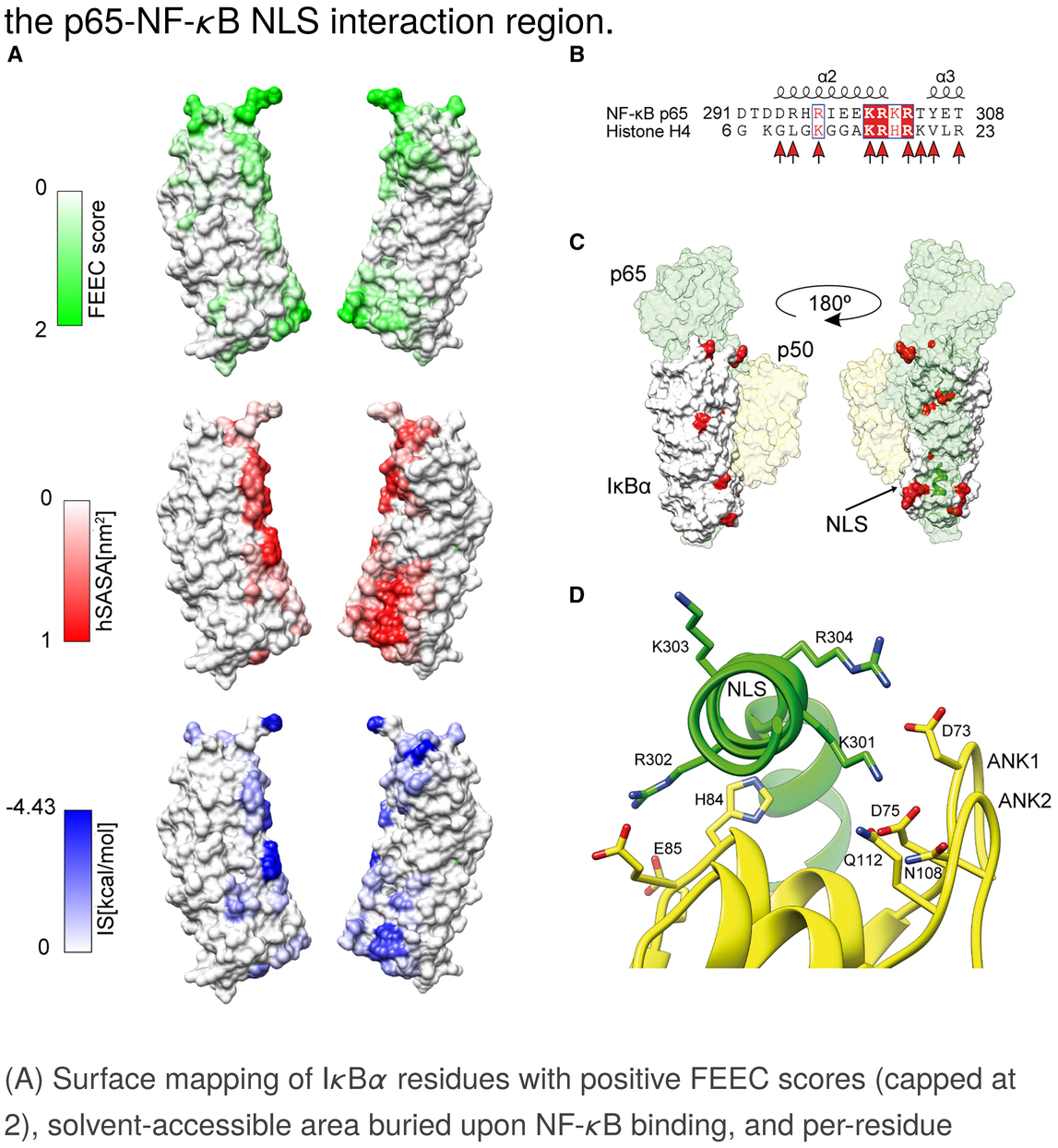
$$P_i = \frac{e^{-E_i/kT}}{\sum_{i=1}^{N} e^{-E_i/kT}},$$

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

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. (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. (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. (PDB: 1NFI, 3UW9; UniProt Q04206/p65 and P25963/IκBα).

REFERENCES

Álvarez-Villanueva, Daniel et al. (2025). "Separation-of-function mutants reveal the NF-κB-independent involvement of IκBα in the regulation of intestinal stemness". 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.

Read the full article - Cell Reports (2025)