# TARGETING β-PIX SH3 DOMAIN-MEDIATED PROTEIN-PROTEIN INTERACTIONS IN BREAST CANCER USING PEPTIDE INHIBITORS

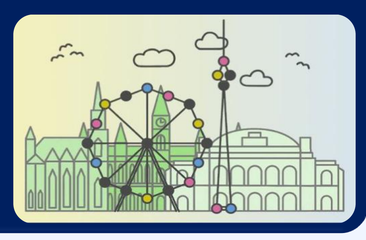

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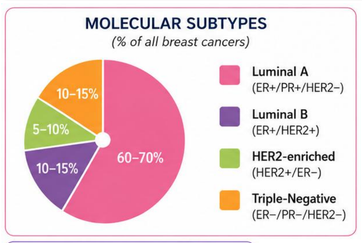


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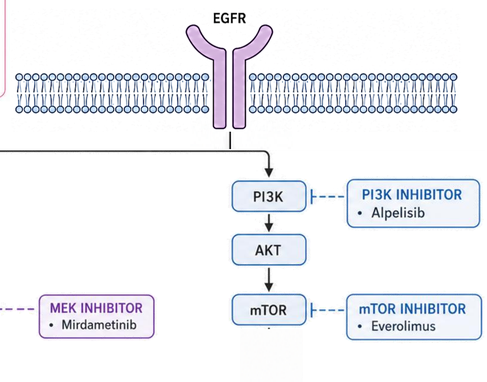


## BREAST CANCER STATISTICS AND CURRENT TREATMENT STRATEGIES

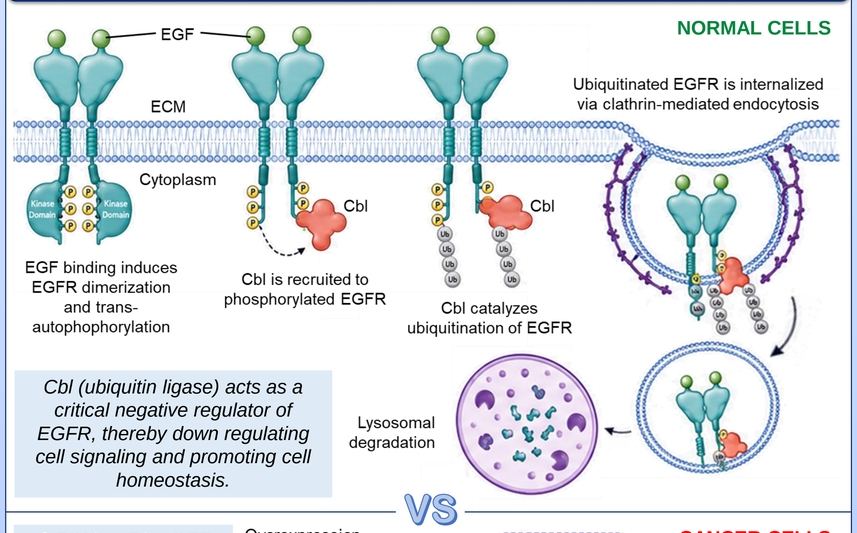



*Breast cancer is the largest cancer burden women face worldwide. Conventional therapies target the EGFR kinase directly – drugs like the tyrosine kinase inhibitors. This approach fails due to mutations, and reactivation of the kinase domain.*




*Where EGFR kinase inhibitors have failed, can disrupting the protein-protein interactions that sustain oncogenic signaling provide a novel therapeutic strategy in breast cancer?*

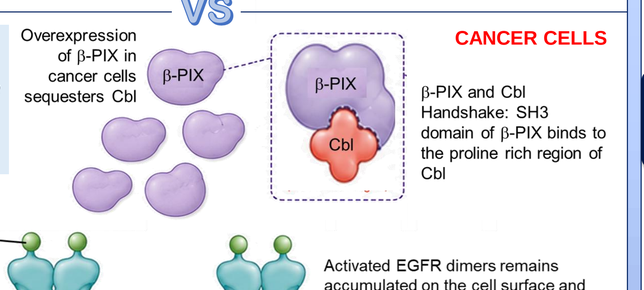
## EGFR SIGNALING:FROM NORMAL REGULATION TO ONCOGENIC DYSREGULATION

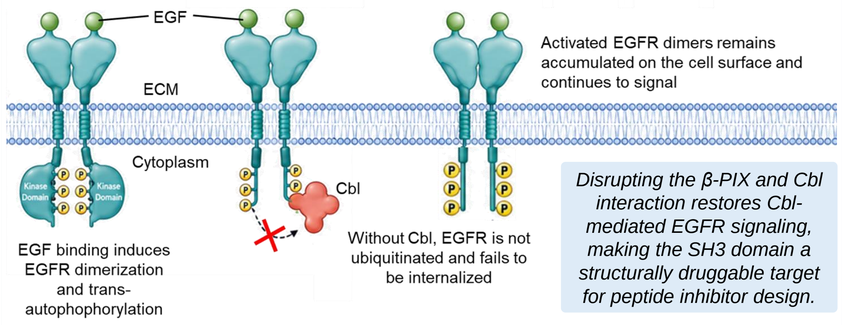



*Cbl (ubiquitin ligase) acts as a critical negative regulator of EGFR, thereby down regulating cell signaling and promoting cell homeostasis.*

VS

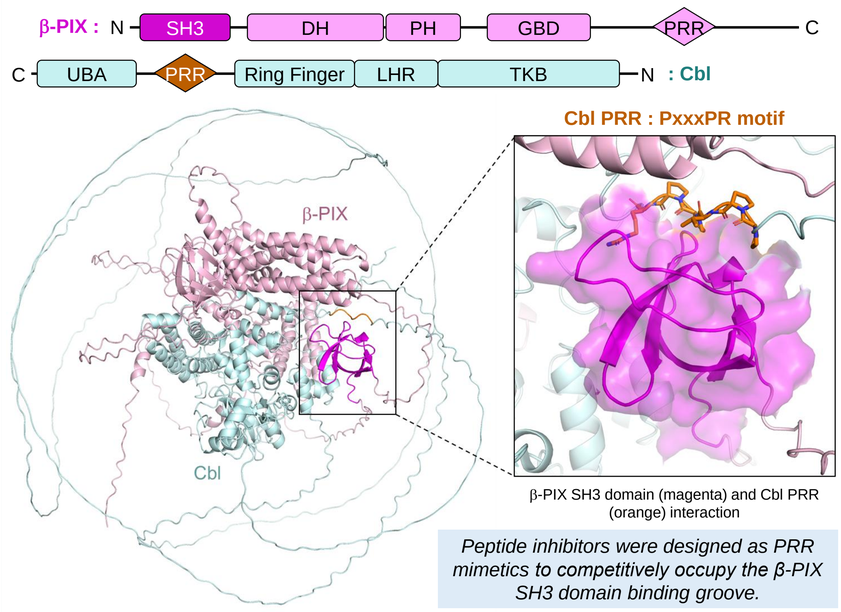
*β-PIX is a Rho-GEF that activates Rac1/Cdc42 to mediate enhanced migration/ invasion and drives metastasis.*



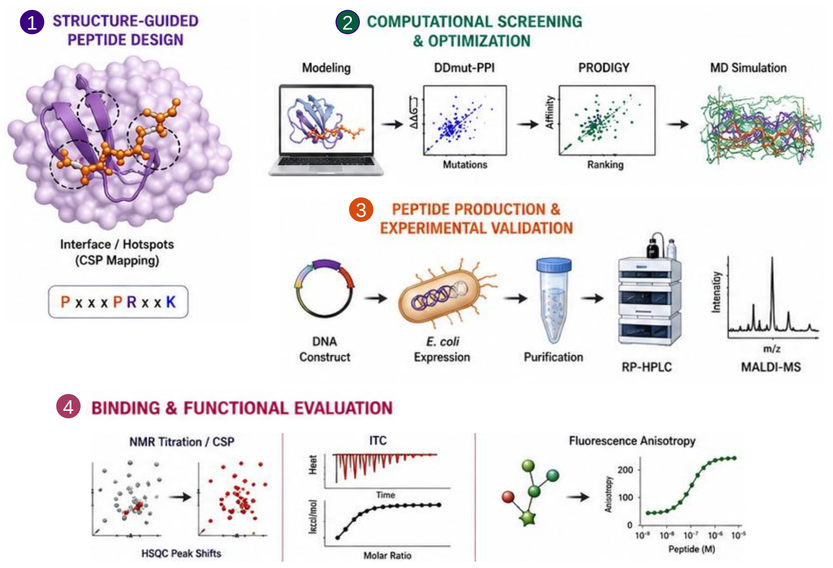

*Disrupting the β-PIX and Cbl interaction restores Cbl-mediated EGFR signaling, making the SH3 domain a structurally druggable target for peptide inhibitor design.*

## INTERACTION BETWEEN β-PIX SH3 DOMAIN AND PROLINE RICH REGION (PRR) OF CBL

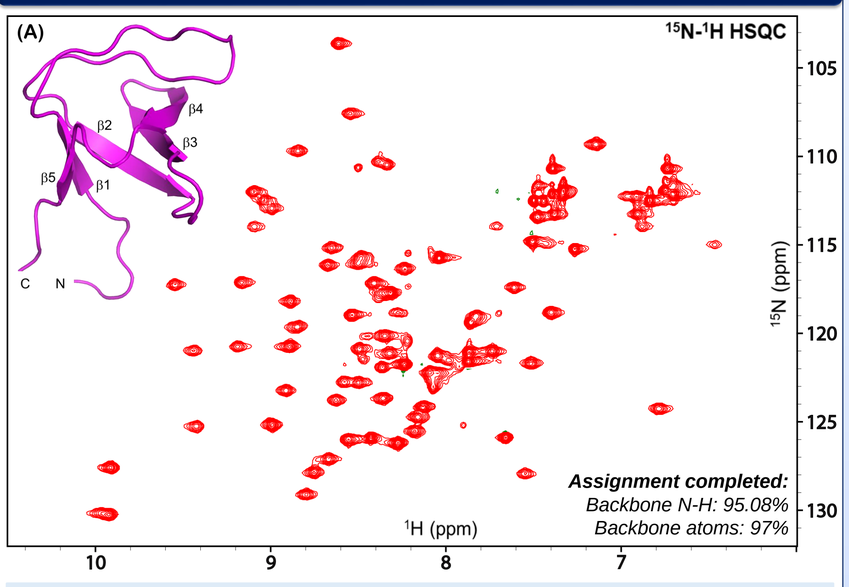



*Peptide inhibitors were designed as PRR mimetics to competitively occupy the β-PIX SH3 domain binding groove.*

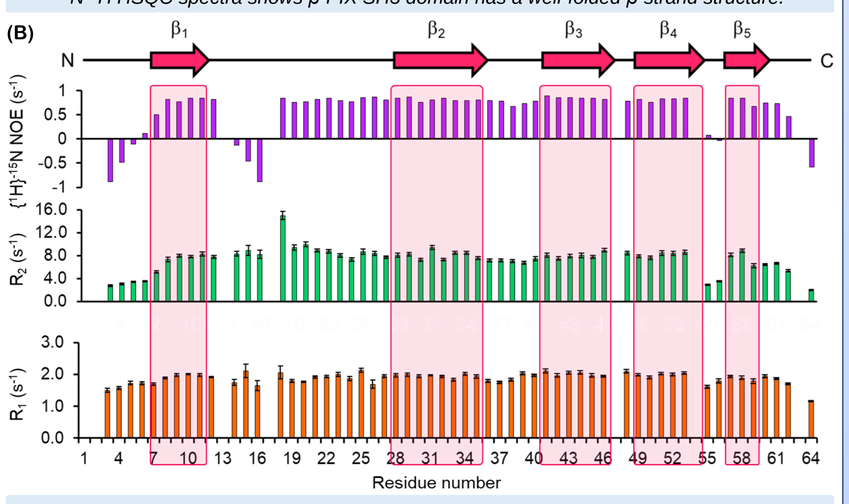
## METHODOLOGICAL WORKFLOW




## CHARACTERIZATION OF β-PIX SH3 DOMAIN FOR STRUCTURE-GUIDED PEPTIDE DESIGN




*$^{15}$N-$^{1}$H HSQC spectra shows β-PIX SH3 domain has a well-folded β-strand structure.*



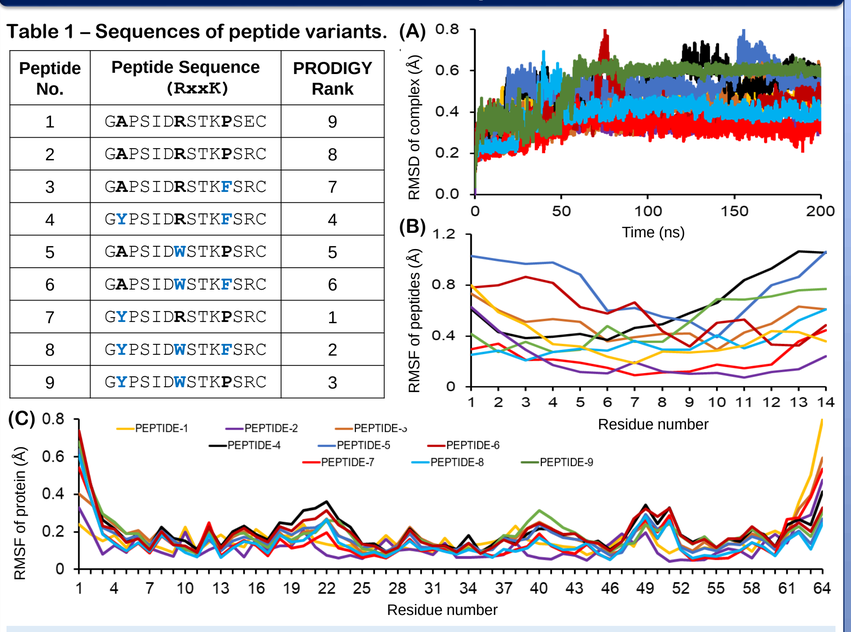

*$^{15}$N relaxation analysis of ps-ns timescale motions show uniform NOE values for each residue, indicating the domain having rigid folds. Decreased NOE values between β1 and β2 suggest local flexibility. T18 has significantly higher transverse relaxation ($R_2$) value, which suggests μs-ms conformation exchange.*

**Figure 1 – (A) $^{15}$N-$^{1}$H HSQC spectra and solution NMR structure of β-PIX SH3 domain. (B) Fast-timescale (ps–ns) dynamics of backbone amides in the β-PIX SH3 domain.**

## DESIGN AND SCREENING OF PEPTIDE INHIBITORS TARGETING β-PIX SH3 DOMAIN

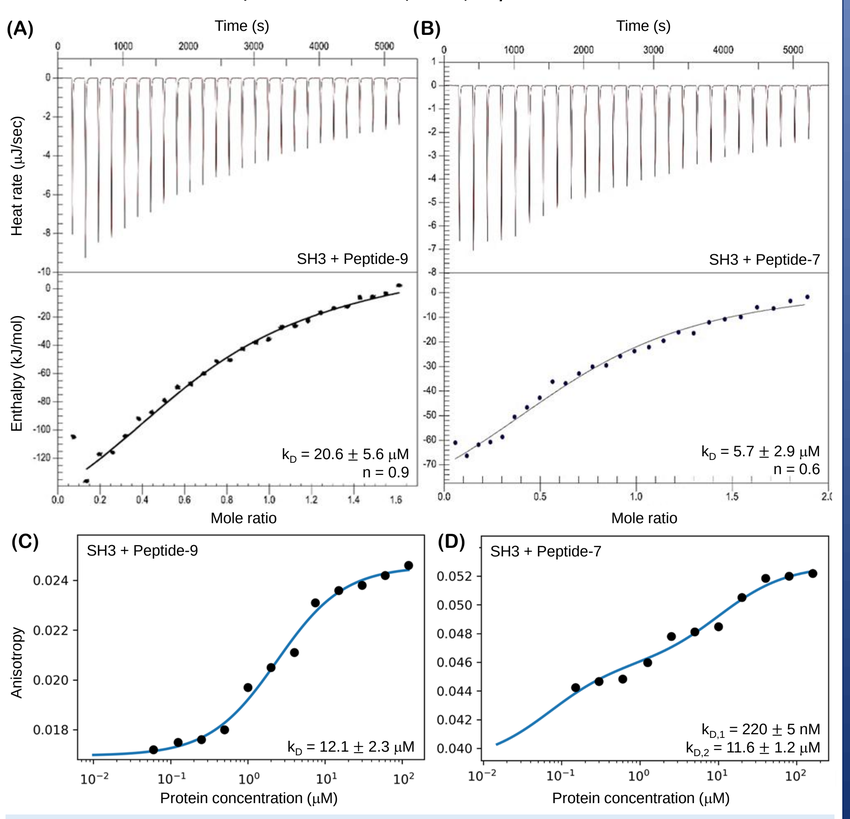
**Table 1 – Sequences of peptide variants.**

| Peptide No. | Peptide Sequence (RxxK) | PRODIGY Rank |
|---|---|---|
| 1 | GAPSIDRSTKPSEC | 9 |
| 2 | GAPSIDRSTKPSRC | 8 |
| 3 | GAPSIDRSTKFSRC | 7 |
| 4 | GYPSIDRSTKFSRC | 4 |
| 5 | GAPSIDWSTKPSRC | 5 |
| 6 | GAPSIDWSTKFSRC | 6 |
| 7 | GYPSIDRSTKPSRC | 1 |
| 8 | GYPSIDWSTKFSRC | 2 |
| 9 | GYPSIDWSTKPSRC | 3 |




*9 peptide variants were generated using DDmut-PPI and ranked by predicted binding affinity using PRODIGY. Following MD simulations, 5 variants were selected, purified, characterized, binding affinity was evaluated by ITC, fluorescence anisotropy, and NMR.*

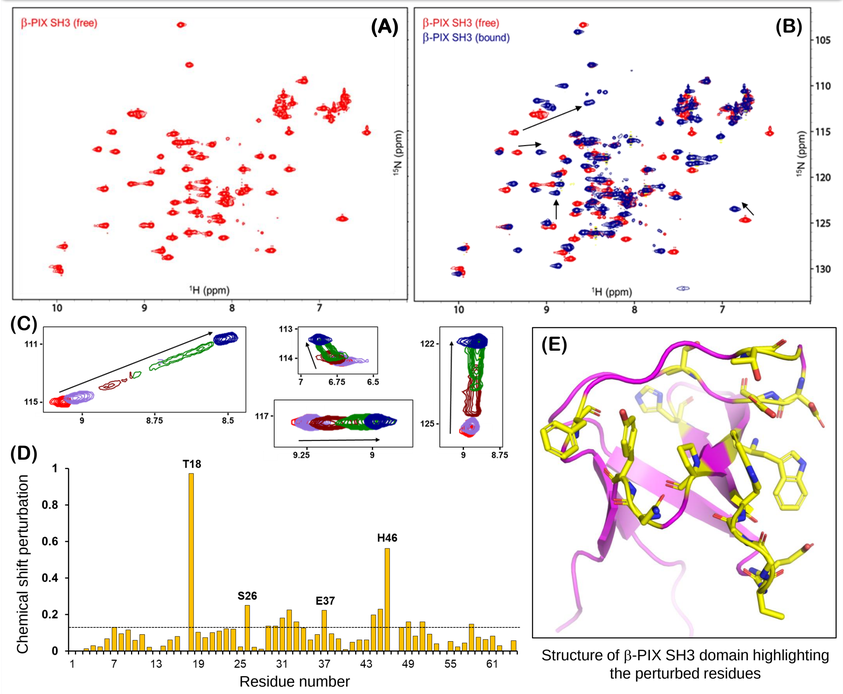
**Figure 2 – (A) Root mean square deviation (RMSD) of peptide bound β-PIX SH3 domain from 200 ns simulation. (B) Root mean square fluctuation (RMSF) of peptides. (C) Root mean square fluctuation (RMSF) of β-PIX SH3 domain.**




*Peptide-7 demonstrated enhanced binding to the β-PIX SH3 domain with 2:1 SH3:peptide binding stoichiometry. R→W mutation in Peptide-9 weakened SH3 binding and shifted the stoichiometry to 1:1, highlighting the role of Arg in the RxxK motif.*

**Figure 3 – ITC heat responses from titrating (A) Peptide-9 and (B) Peptide-7 into β-PIX SH3 domain. Anisotropy changes upon titrating β-PIX SH3 into fluorescently labelled (C) Peptide-9 and (D) Peptide-7**

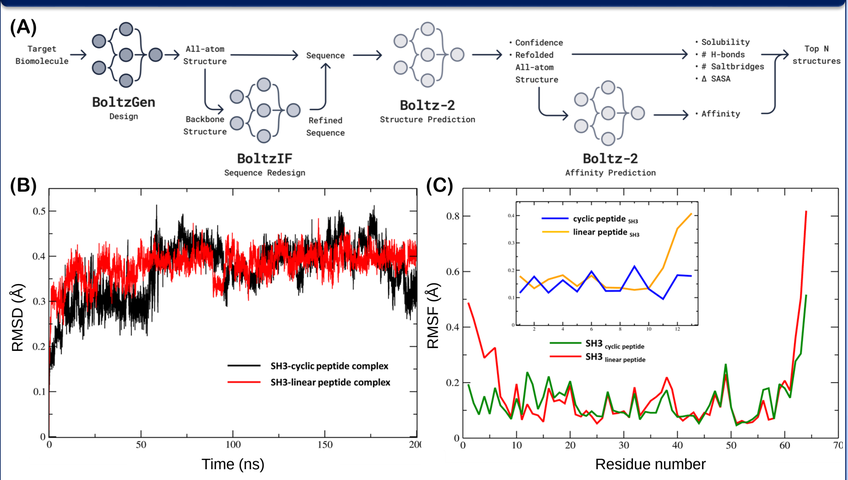
## STRUCTURAL MAPPING OF β-PIX SH3 DOMAIN AND PEPTIDE-7 INTERACTION

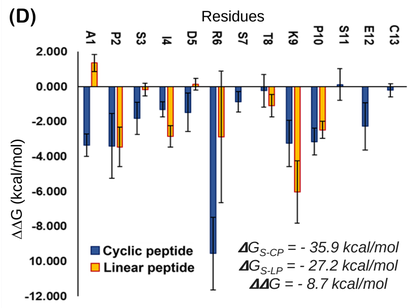



*NMR-based titration experiments demonstrate that Peptide-7 directly binds the β-PIX SH3 domain, inducing distinct residue-specific chemical shift perturbations that map the molecular recognition interface.*

**Figure 4 – $^{15}$N-$^{1}$H HSQC spectra of (A) apo and (B) overlay of free peptide-bound β-PIX SH3 domain. (C) Representative perturbed residues. (D) Chemical shift perturbations quantification and analysis. (E) Mapping of perturbed residues onto β-PIX SH3 domain.**

## AI-GUIDED DESIGN OF CYCLIC PEPTIDES




*Experimental validation of cyclic peptide binding via Spl DnaX split intein mediated cyclization is ongoing!*

*Cyclic peptide achieves lower average RMSD and RMSF values compared to the linear peptide. Lower RMSD values suggests a more favorable and consistent binding interaction of cyclic peptide with SH3. Cyclic peptide shows tighter binding from Molecular Mechanics/Generalized Born Surface Area (MMGBSA) calculations.*

**Figure 5 - (A) Cyclic peptide design using BoltzGen for structure prediction. (B) MD simulations showing the RMSD profiles of β-PIX SH3 complexes with cyclic (red) and linear (black) peptides. (C) Per-residue RMSF analysis and comparative residue fluctuations, with the inset showing peptide backbone RMSF. (D) Per-residue binding free-energy contributions (MMGBSA) comparing cyclic and linear peptides.**

## EVALUATION OF PEPTIDES USING CELL STUDY

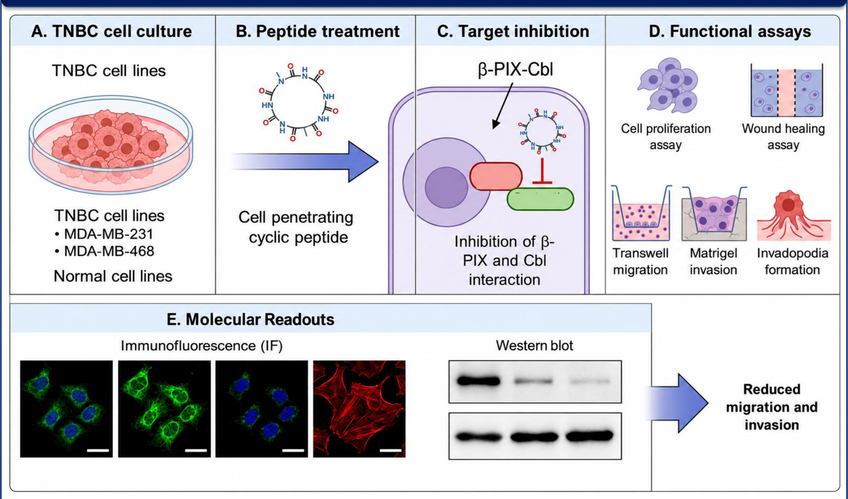



**Figure 6 - (A) Cell culture using TNBC cell lines (MDA-MB-231 and MDA-MB-468) and normal cell lines. (B) Treatment with a cell-penetrating peptides. (C) Inhibition of the β-PIX & Cbl interaction (D) Functional assays assessing proliferation, migration, invasion, and invadopodia formation. (E) Readouts by immunofluorescence and western blot.**

## CONCLUSION

- Disrupting the β-PIX-Cbl PPI offers a structurally tractable alternative to EGFR kinase inhibition, which has failed clinically due to resistance mutations and pathway reactivation.
- Solution NMR structural characterization of the apo β-PIX SH3 domain provided the structure-guided template for rational peptide inhibitor design against this target.
- An integrated computational-to-experimental pipeline identified Peptide-7 as a novel lead binder, with NMR chemical shift perturbation mapping confirming its interaction interface.
- The Arg of the RxxK motif in peptides is functionally essential for high affinity with 2:1 SH3:peptide binding – Mutating it to Trp weakens binding and shifts the stoichiometry to 1:1.
- Synthesis of the optimized cyclic peptide using split *Spl* DnaX intein is underway, with TNBC cell-based assays planned to assess functional inhibition of β-PIX-Cbl signaling.

## REFERENCES


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