

Targeted Antagonism of the Activator Protein 1 Transcription Factor Complex Results in Potent Anti-Tumor Activity in HNSCC Models

Abstract

The AP-1 transcription factor complex, comprised of Fos and Jun family heterodimers, plays a pivotal role in tumor pathogenesis, progression and metastasis. Bioinformatics analysis implicates dysfunctional AP-1 activity as a critical factor in head and neck squamous cell carcinoma (HNSCC) and non-small cell lung cancer (NSCLC), where expression of Fos family member Fra1 inversely correlates with prognosis. As dimerization is required for AP-1 complex DNA binding and transcriptional activity, we designed Fra1 antagonizing peptide (FraAP) to disrupt complex formation and prevent associated activity. FraAP displays low nanomolar binding affinity to Fra1 and Jun and selectivity for AP-1 family members. FraAP-mediated antagonism of the Fra1 and cJun protein-protein interaction attenuated AP-1 transcriptional activity in reporter assays, and transcriptomics analysis identified a significant impact on invasion, apoptosis and proliferation pathways that were confirmed by corresponding functional in vitro assays. Following exposure to FraAP, a phenotypic mesenchymal-to-epithelial transition was observed that inhibited in vitro invasion. Dose-dependent induction of apoptosis and G1 arrest were observed following FraAP exposure, while a negative control peptide had no impact on either event. Consistent with the impact on cell cycle, combination of FraAP with the CDK4/6 inhibitor abemaciclib resulted in synergistic anti-tumor activity in vitro. While FraAP results in significant anti-tumor activity as a monotherapy in HNSCC and NSCLC subcutaneous xenograft models, combination of sub-pharmacologic FraAP and abemaciclib results in significantly enhanced tumor growth inhibition. These data support FraAP as a potent peptide antagonist of the AP-1 transcription complex that warrants further investigation as a novel therapeutic for AP-1 driven tumors such as HNSCC and NSCLC.

Targeting Activator Protein 1 (AP-1)

The cancer dependency map (DepMap), a genome-scale CRISPR-Cas9 essentiality screen across 1170 cancer cell lines, implicates the *JUN* and *FOSL1* (gene encoding for Fra1) interaction as crucial in cancer cell survival. FraAP is a peptide designed to target the basic leucine zipper motif of Fra1 to antagonize the interaction between cJun and Fra1 and disrupt AP-1 activity.

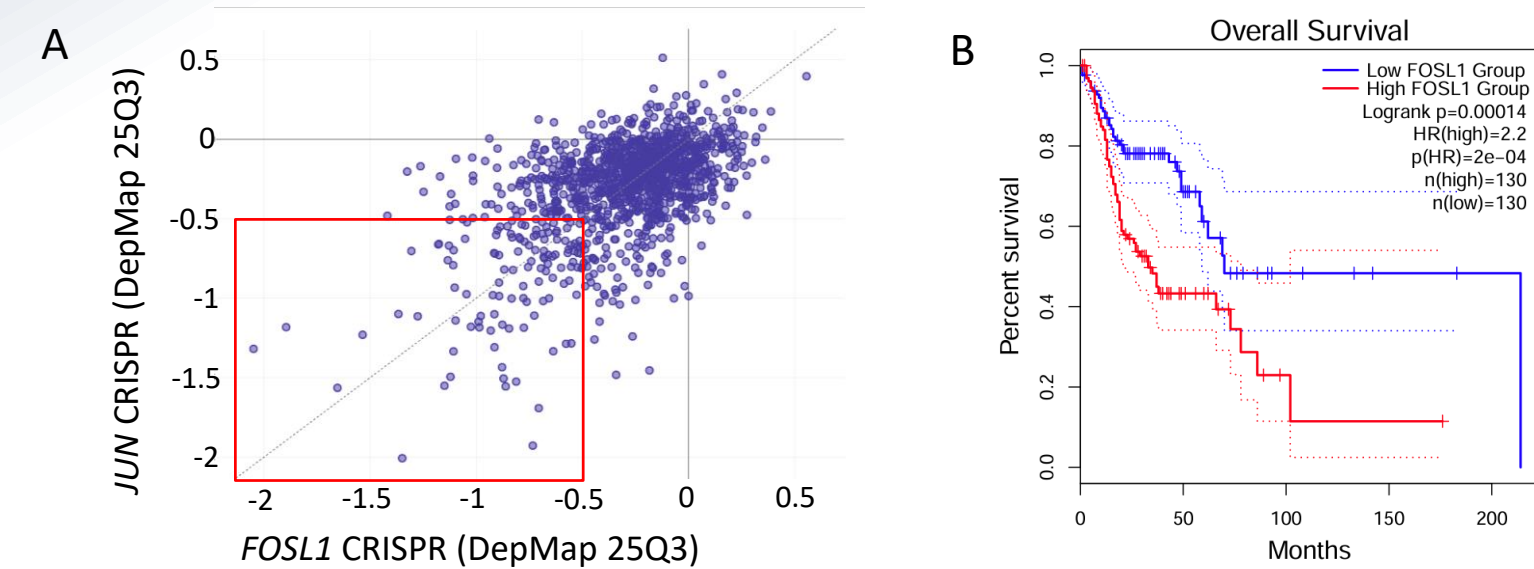


Figure 1. JUN and FOSL1 interaction is necessary for survival of a subset of cancer cells and elevated FOSL1 expression is predictive of poor prognosis in HNSCC. (A) DepMap portal CRISPR screen depicting *FOSL1* gene effect (x-axis) and *JUN* gene effect (y-axis). cJun/Fra1 dual-dependent cells are enriched in head and neck squamous cell carcinoma (HNSCC), non-small cell lung cancer (NSCLC), pleural mesothelioma, ovarian cancer, bladder cancer and glioma. Red box highlights cell lines with dependency scores ≤ -0.5 for that interaction. (B) Kaplan-Meier plots of overall survival for HNSCC were generated using quartile group cutoff (25% low; 75% high), 95% confidence interval (dotted line), high expression (red), low expression (blue) (TCGA dataset; KM curves generated with Gepia2).

FraAP Mechanism of Action

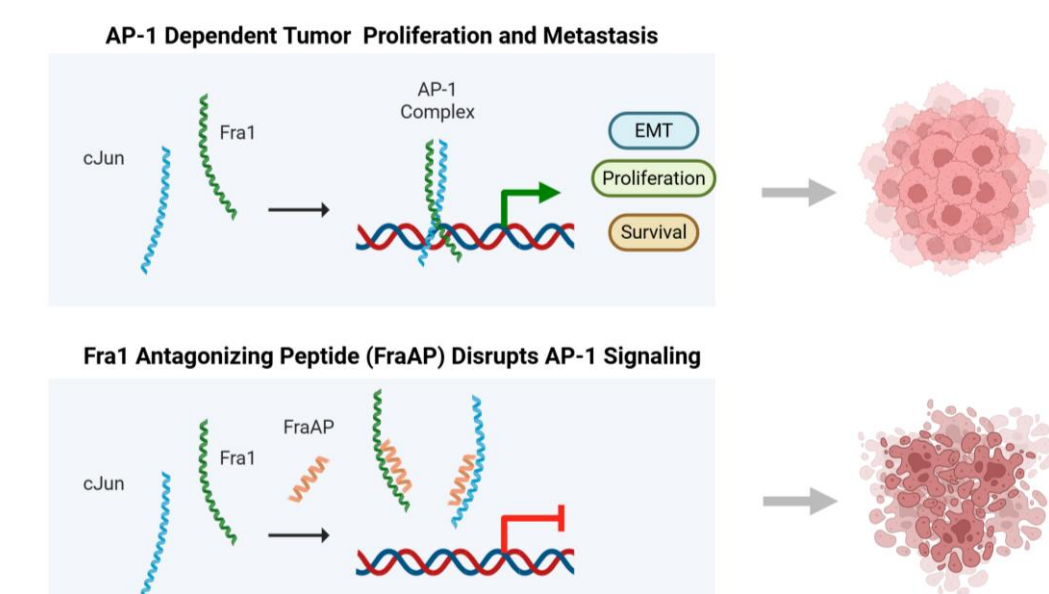


Figure 2. The AP-1 complex is a master regulator of proliferation, survival and EMT. Jun and Fra1 (*FOSL1* gene product) heterodimerize to form the AP-1 complex. FraAP is a stabilized peptide designed to disrupt AP-1 dimerization, preventing AP-1 mediated transcription. The result is antagonism of oncogenic gene transcription leading to selective tumor cell death and reduced tumor cell invasion.

Results

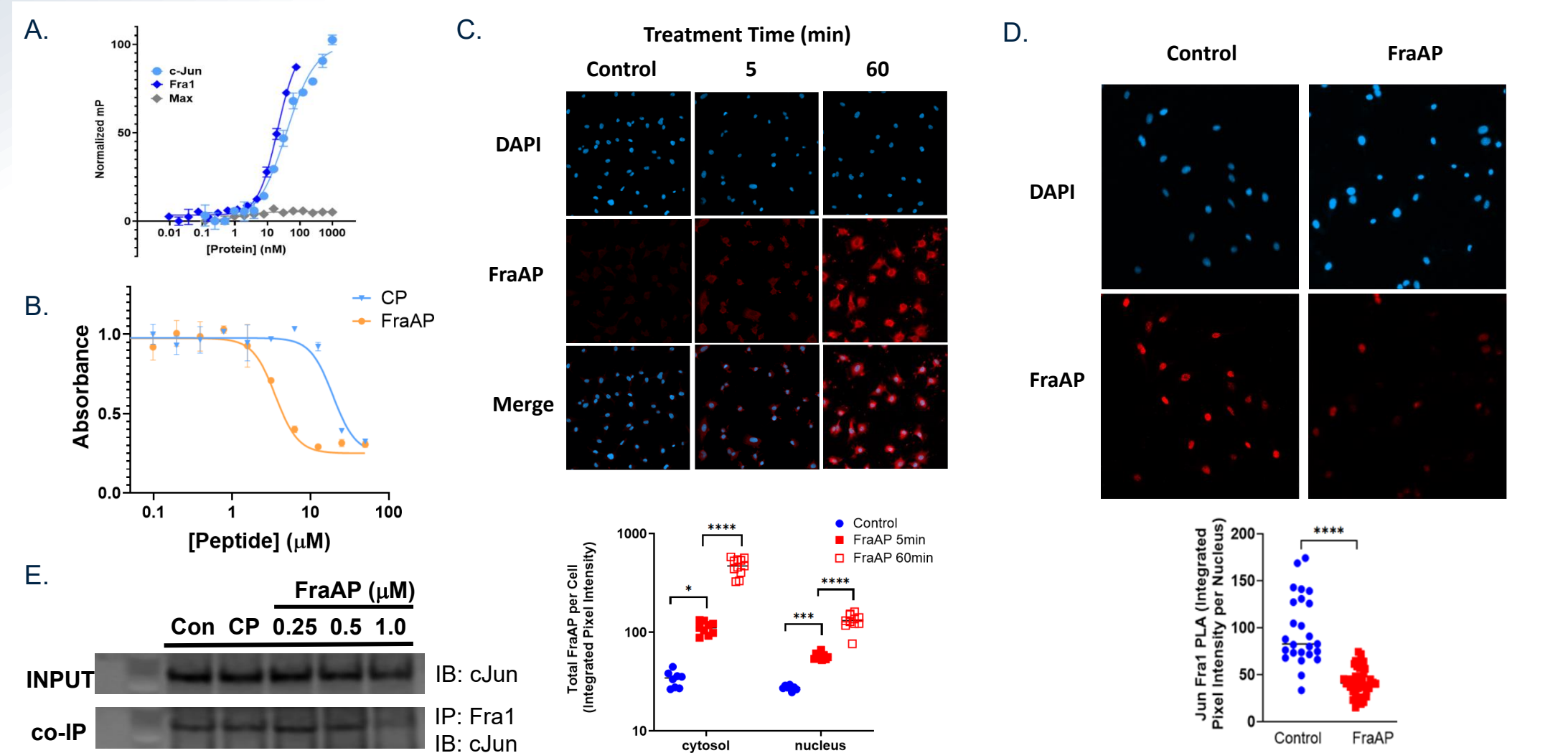


Figure 3. FraAP target engagement and disruption of Jun-Fra1 protein-protein interaction (PPI). A: Binding affinity of FraAP to leucine zipper proteins, as determined by fluorescence polarization, indicates K_d to Fra1 and cJun of 20.61 nM and 38.53 nM, respectively. K_d to Max (off-target leucine zipper) was undetermined. B: FraAP disrupts the binding of AP-1 complex to immobilized consensus-binding site oligo (5'-TGA(C/G)TCA-3') by ELISA. Control peptide (CP) used as negative control. C: IF staining of FraAP (250nm) penetration into SW579 cells at indicated times. Integrated pixel intensity per cell shown. (* $p=0.01$; *** $p=0.0003$; **** $p<0.0001$). D: FraAP (1 μ M for 6hrs) disrupts formation of AP-1 complex (Fra1 and Jun) in SW579 cells by proximity ligation assay (PLA). Integrated pixel intensity was calculated per nucleus for each condition (* $p=0.04$; **** $p<0.0001$). E: FraAP (1 μ M for 6 hrs) disrupted AP-1 complex (Fra1 and Jun) in SW579 cells by IP assay. CP (1 μ M) had no impact. Statistical significance determined by one-way ANOVA analysis, uncorrected Fisher's LSD.

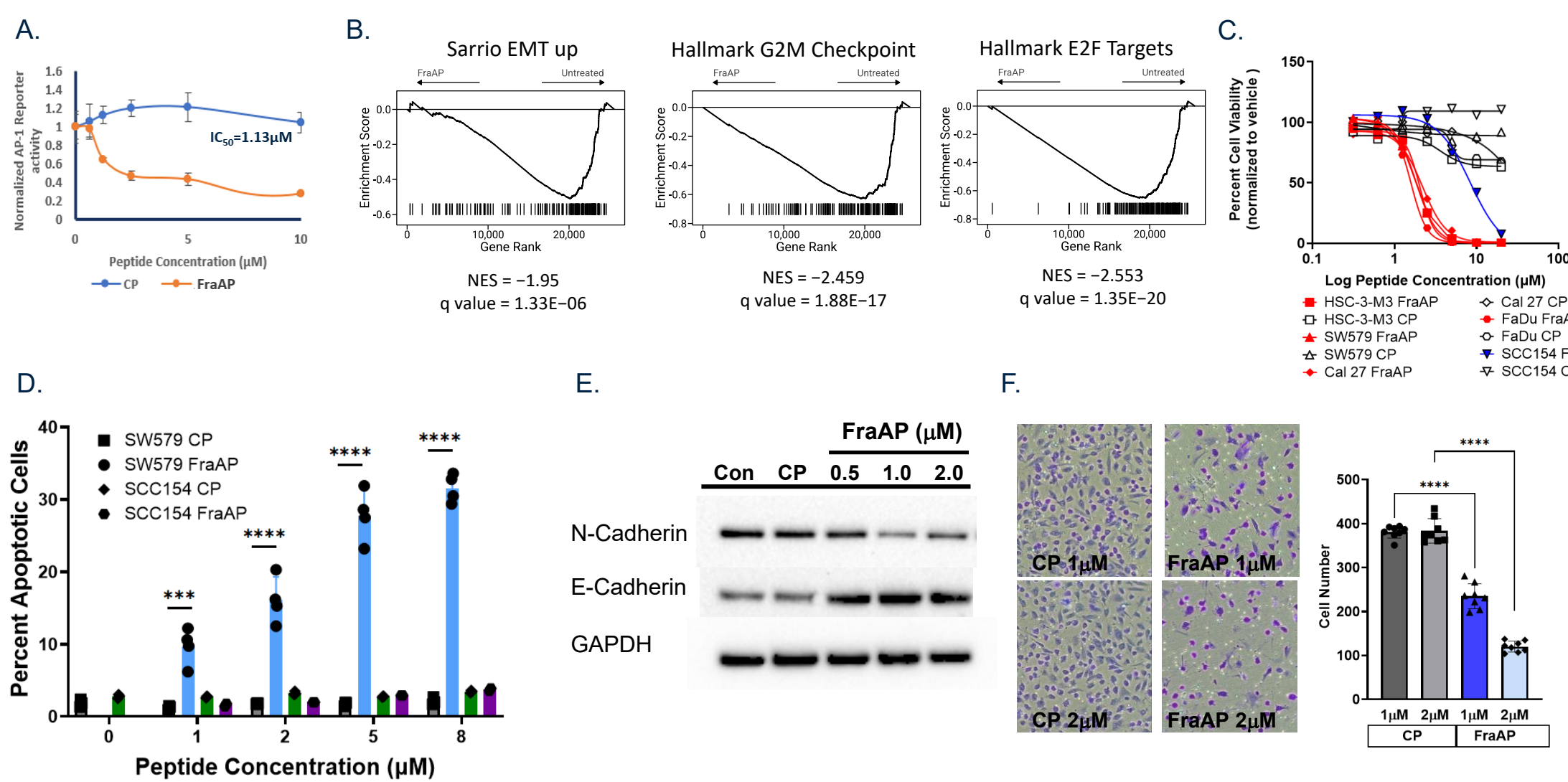


Figure 4. FraAP disrupts oncogenic signaling in AP-1-dependent tumor cells. A: FraAP inhibits AP-1 transcriptional activity in HEK293 AP-1 firefly luciferase reporter assay (IC_{50} of 1.13 μ M). B: GSEA pathway analysis of RNAseq data indicates FraAP inhibition of EMT and cell cycle/proliferation (G2M checkpoint, E2F target) pathways. C: FraAP demonstrates potent cytotoxicity in AP-1 dependent HNSCC cells (mean $IC_{50}=1.90\pm 0.13$ μ M). AP-1 independent HNSCC cells display reduced sensitivity (SCC154 $IC_{50}=8.392\mu$ M). Viability was measured by CellTiter Glo 48-hrs post peptide treatment. D: Dose-dependent increase in apoptosis in SW579 cells, but no impact in AP-1 independent SCC154 cells. Annexin PI staining was detected by flow cytometry at 24-hrs post peptide treatment (** $p=0.0001$; **** $p<0.0001$). E: FraAP treatment of SW579 induces a reduction in the mesenchymal marker N-Cadherin and an increase in the epithelial marker E-Cadherin by immunoblot. F: FraAP treated SW579 cells exhibit reduced invasion in an in vitro Matrigel-coated Boyden chamber assay (**** $p<0.0001$). One-way ANOVA analysis, uncorrected Fisher's LSD, was used to calculate significance.

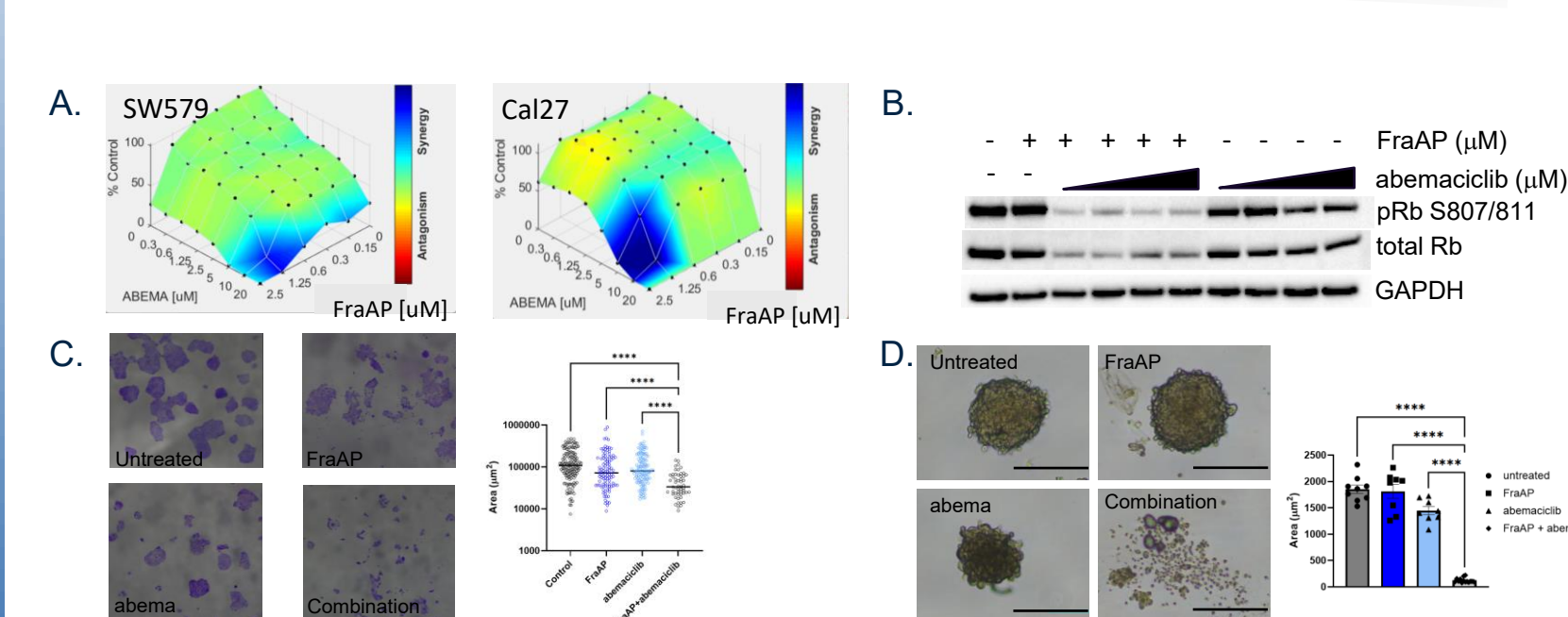
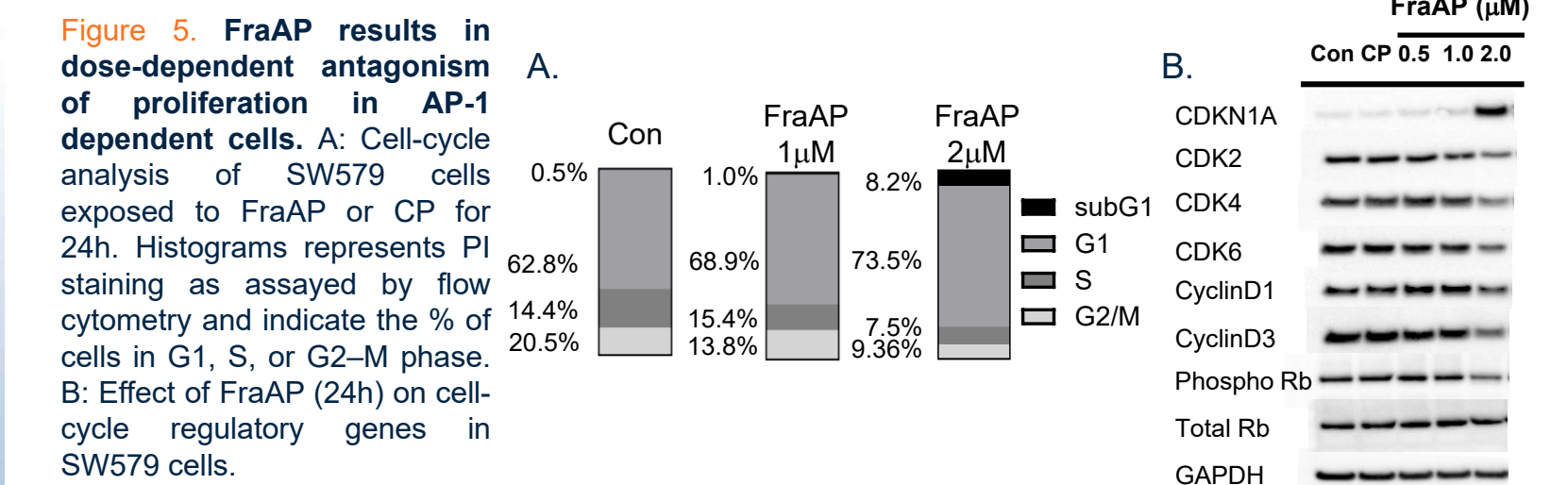


Figure 6. FraAP synergizes with CDK4/6 inhibitors in vitro. A: Bliss surface model for SW579 and Cal27 HNSCC cell lines treated with FraAP and abemaciclib. Synergy indicated by blue. Viability at 48 hours by CellTiter Glo. B: Immunoblot for phospho-Rb, total Rb, and GAPDH in SW579 cells treated with abemaciclib (0.075 μ M, 0.15 μ M, 0.3 μ M, 0.6 μ M), FraAP (1.25 μ M), or combination for 24hrs. C: Colony formation assay for Cal 27 cells treated with abemaciclib (0.075 μ M), FraAP (1.5 μ M), or combination for 7 days. D: Orosphere assay for Cal 27 cells treated with abemaciclib (1.25 μ M), FraAP (2.5 μ M), or combination for 7 days.

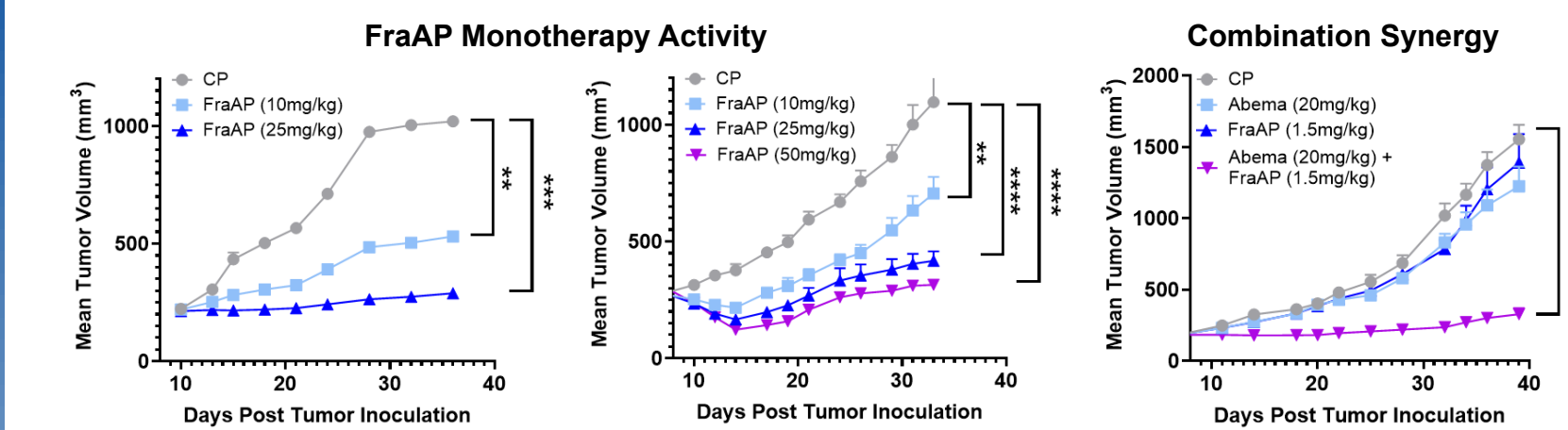


Figure 7. FraAP displays potent anti-tumor activity in HNSCC xenograft models. Tumor volumes of A: SW579 subcutaneous xenografts treated with FraAP or CP (n=6/group; ** $p=0.006$, *** $p=0.0002$); B: Cal 27 subcutaneous xenografts treated with FraAP or CP (n=5/group; ** $p=0.003$, **** $p<0.0001$); C: Cal 27 subcutaneous xenografts treated with subpharmacologic FraAP (1.5mg/kg) or CP, abemaciclib (20mg/kg) administered 5x/week SC, or combination (n=6/group; ** $p=0.002$). FraAP administered SC 3x/week in all studies. Error bars represent SEM. One-way ANOVA, uncorrected Fisher's LSD, used to calculate significance.

Conclusions

- FraAP disrupts the interaction between Fra1 and cJun, preventing AP-1 complex formation.
- FraAP inhibits AP-1 dependent cell survival, proliferation and invasion, and demonstrates potent in vitro cytotoxicity in AP-1-dependent HNSCC cells.
- FraAP and CDK4/6i demonstrate synergistic cytotoxicity in vitro.
- FraAP demonstrates potent anti-tumor activity in vivo in AP-1-dependent subcutaneous xenograft models as a single agent; synergistic anti-tumor activity in HNSCC is observed in vivo in combination with CDK4/6i.
- Antagonism of AP-1 complex formation with FraAP represents a novel approach to target AP-1 driven tumors.
- These data support potential development of FraAP as a therapy for AP-1 driven tumors including HNSCC.

References
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