

Targeted epigenomic control of MYC as a strategy to treat EGFR inhibitor-resistant NSCLC

Eugene Lee, Padraic Flahardy, Cameron Vergato, Stephen Siecinski, Justin Chen, Charles O'Donnell, Graeme Hodgson, Defne Yarar, Thomas McCauley

Omega Therapeutics, Cambridge, MA, USA

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Background

EGFR tyrosine kinase inhibitors (EGFRi) have improved outcomes for non-small cell lung cancer (NSCLC) patients harboring activating *EGFR* mutations. However, patients receiving EGFRi therapy frequently relapse with an EGFR T790M mutation. While osimertinib, a third generation EGFRi, blocks EGFR T790M activity, resistance eventually develops through various EGFR-dependent and EGFR-independent mechanisms, such as acquiring additional mutations in EGFR (e.g. C797S), activating bypass signaling pathways, or undergoing an epithelial to mesenchymal transition (EMT).



MYC is a master transcription factor critical for mediating oncogenic signal transduction and has been implicated in EGFRi resistance, suggesting that MYC targeting may overcome multiple EGFRi resistance mechanisms. To evaluate this, we have developed a NSCLC-specific programmable epigenomic mRNA therapy, termed a MYC epigenomic controller (NSCLC MYC-EC), designed to selectively target regulatory elements in MYC's insulated genomic domain and downregulate MYC expression. We have previously shown that NSCLC MYC-EC effectively decreases MYC expression pre-transcriptionally and have demonstrated that MYC-EC combination with osimertinib synergistically reduces viability of EGFR mutant NSCLC cells. Here, we demonstrate NSCLC MYC-EC activity in models that have developed EGFRi resistance through EGFR-dependent and -independent mechanisms.

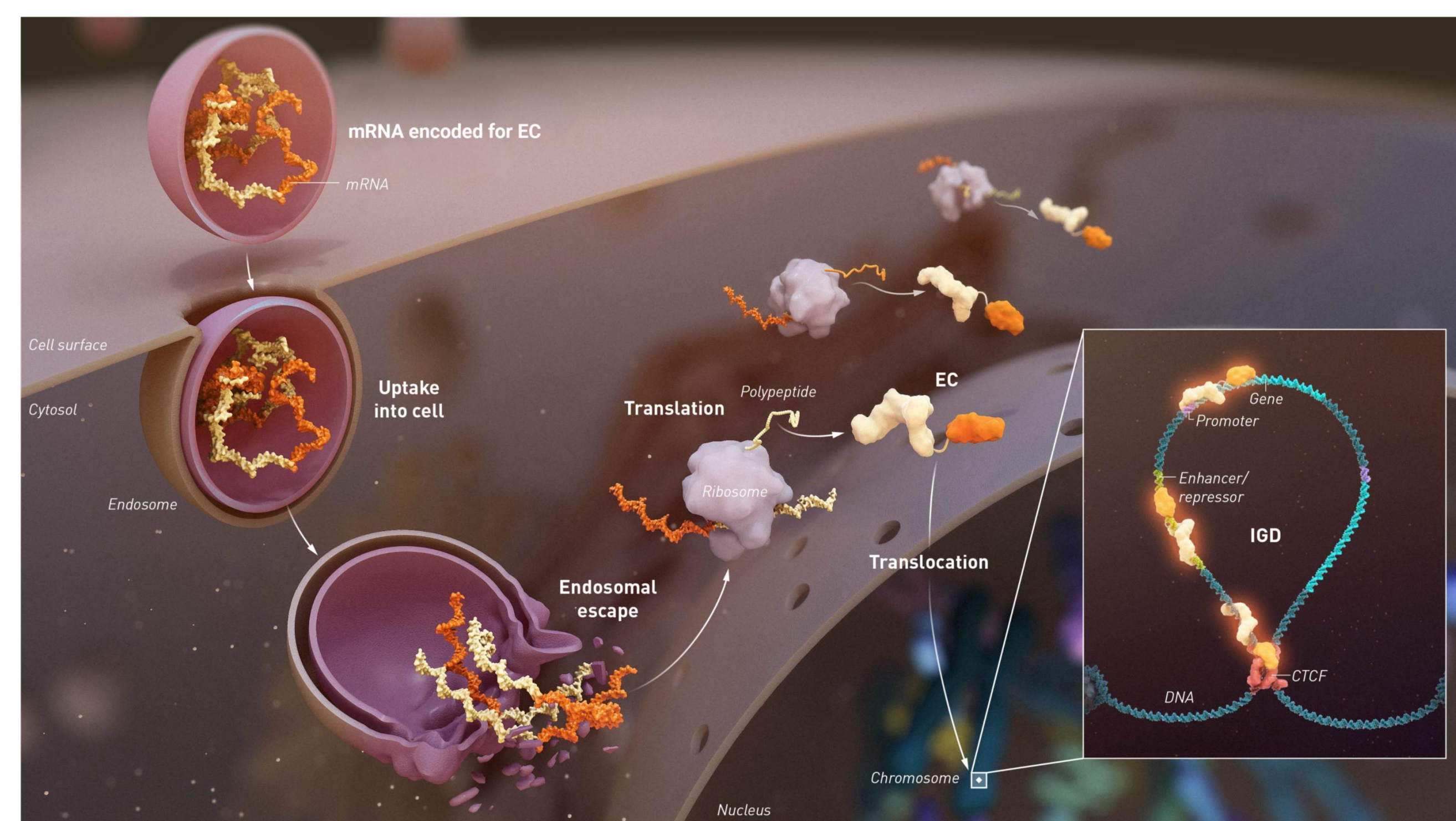
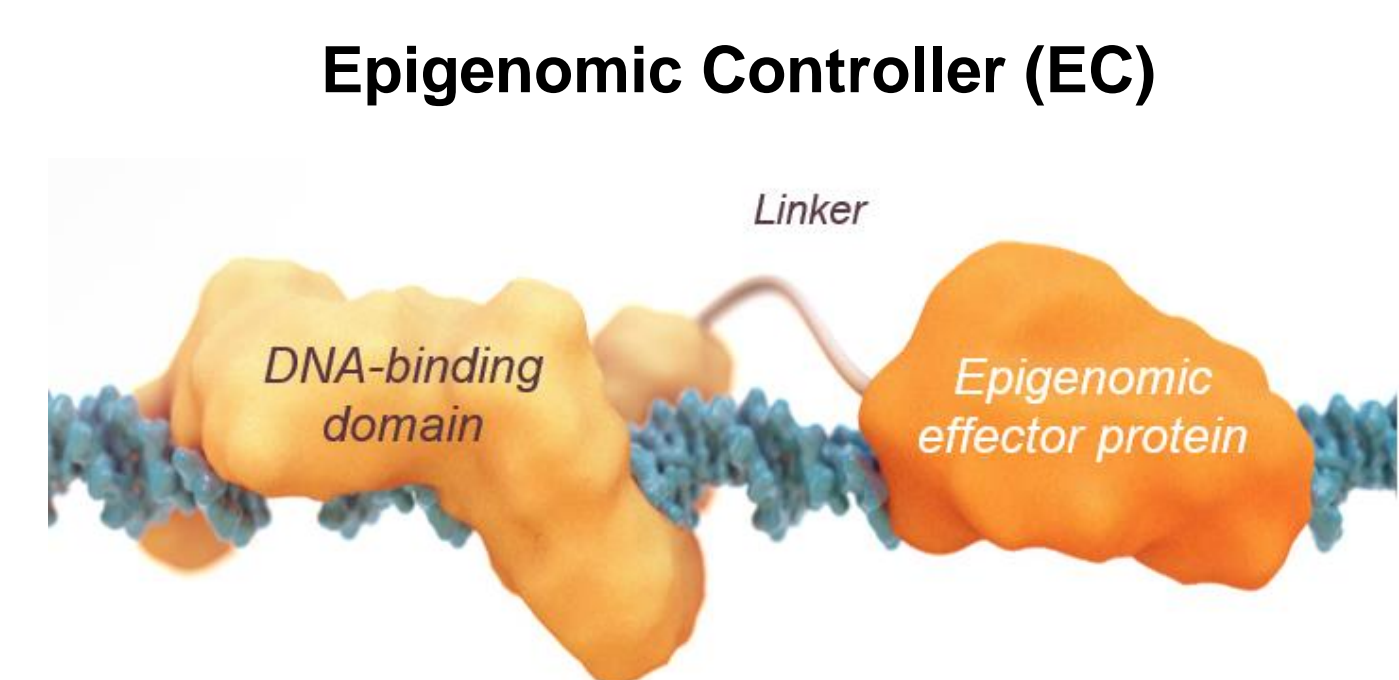


Figure 1. Structure and Mechanism of Action of ECs

NSCLC MYC-EC combination with osimertinib enhanced MYC protein downregulation and synergistically reduced cell viability in PC9 cells engineered to have EGFR T790M mutation

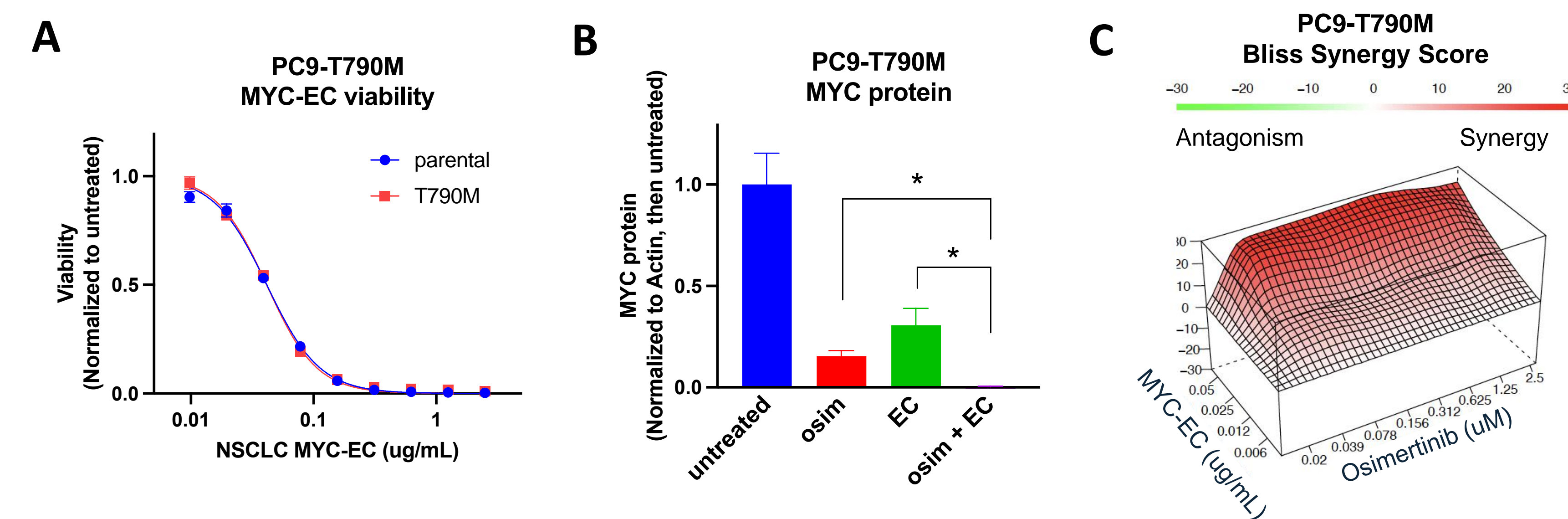


Figure 2: (A) PC9 cells were engineered to have EGFR T790M mutation via CRISPR/Cas9 knock-in (PC9-T790M). PC9-T790M cells retain sensitivity to NSCLC MYC-EC treatment. (B) NSCLC MYC-EC combination therapy with osimertinib enhances MYC protein downregulation compared to monotherapy in PC9-T790M cells. Cells were treated with PBS (untreated), 0.1 uM osimertinib (osim), 0.5 ug/ml MYC-EC (EC) or a combination of both for 48hr. ** denotes $p < 0.05$. (C) Bliss synergy analysis of varying NSCLC MYC-EC concentrations (0.006-0.05 ug/mL) and osimertinib concentrations (0.02-2.5 uM) in PC9-T790M cells. Bliss scores from the most synergistic area is 20. Score ≥ 10 demonstrates synergy.

H1975 and PC9 cells with acquired resistance to osimertinib through EGFR C797S mutation retain sensitivity to NSCLC MYC-EC

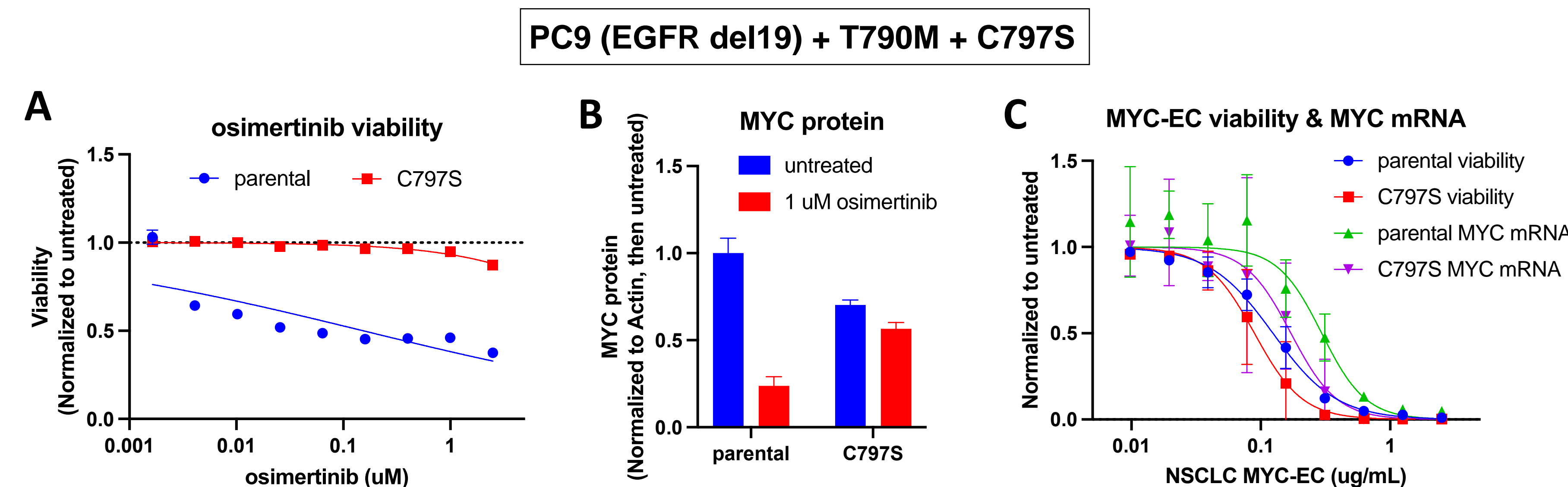


Figure 3: (A) PC9-T790M cells engineered to have EGFR C797S mutation via CRISPR/Cas9 knock-in (PC9-C797S) are resistant to osimertinib treatment compared to parental PC9 cells. (B) MYC protein expression in PC9-C797S cells are minimally impacted by osimertinib treatment while the same treatment highly reduces MYC protein level in parental PC9 cells. (C) NSCLC MYC-EC treatment reduces MYC mRNA levels and cell viability in PC9-C797S cells to a similar degree as in parental PC9 cells.

H1975 (EGFR L858R, T790M) + C797S

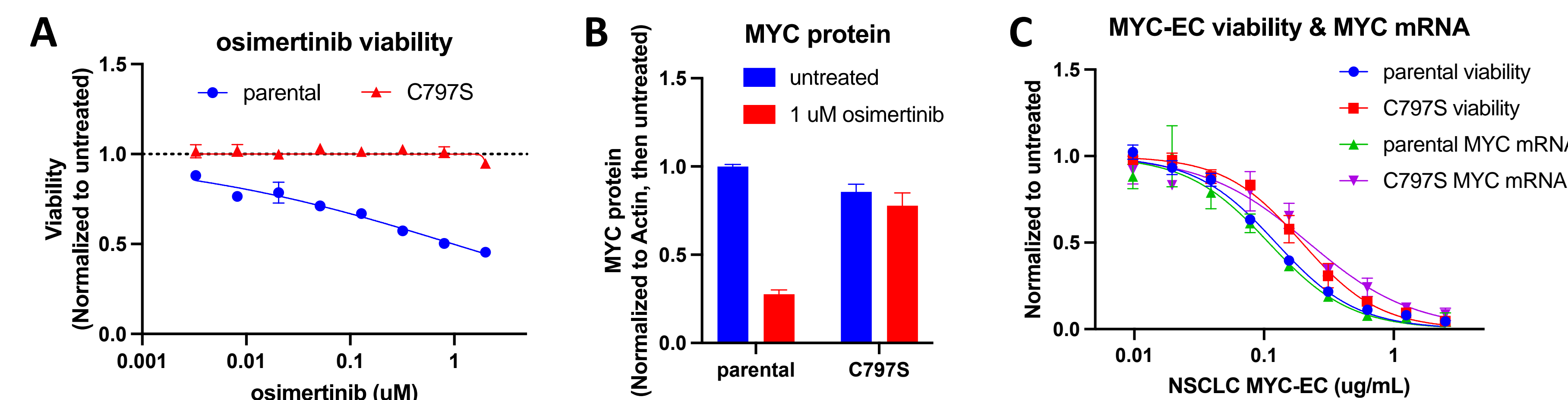


Figure 4: (A) H1975 cells engineered to have EGFR C797S mutation via CRISPR/Cas9 knock-in (H1975-C797S) are resistant to osimertinib treatment compared to parental H1975 cells. (B) MYC protein expression in H1975-C797S cells are minimally impacted by osimertinib treatment while the same treatment highly reduces MYC protein level in parental H1975 cells. (C) NSCLC MYC-EC treatment reduces MYC mRNA levels and cell viability in H1975-C797S cells to a similar degree as in parental H1975 cells.

H1975 cells with acquired resistance to osimertinib through epithelial to mesenchymal transition (EMT) retain sensitivity to NSCLC MYC-EC

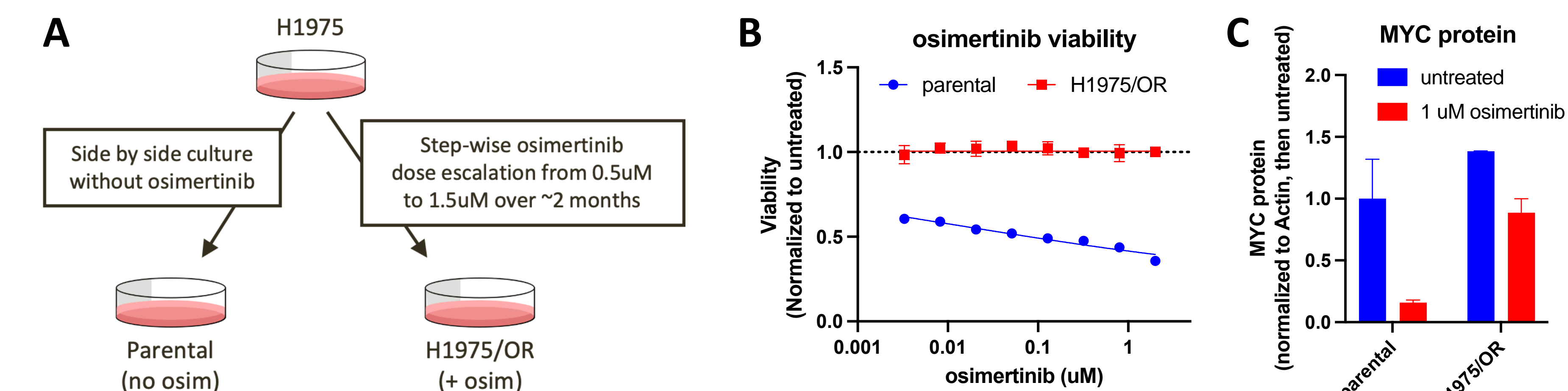


Figure 5: (A) Osimertinib-resistant H1975 cells (H1975/OR) were generated by progressive exposure to escalating doses of osimertinib (osim) over ~2 months. (B) H1975/OR cells are resistant to osimertinib treatment compared to parental H1975 cells. (C) Osimertinib treatment shows modest effect on MYC protein expression in H1975/OR cells while MYC protein level is highly reduced by the same treatment in parental H1975 cells.

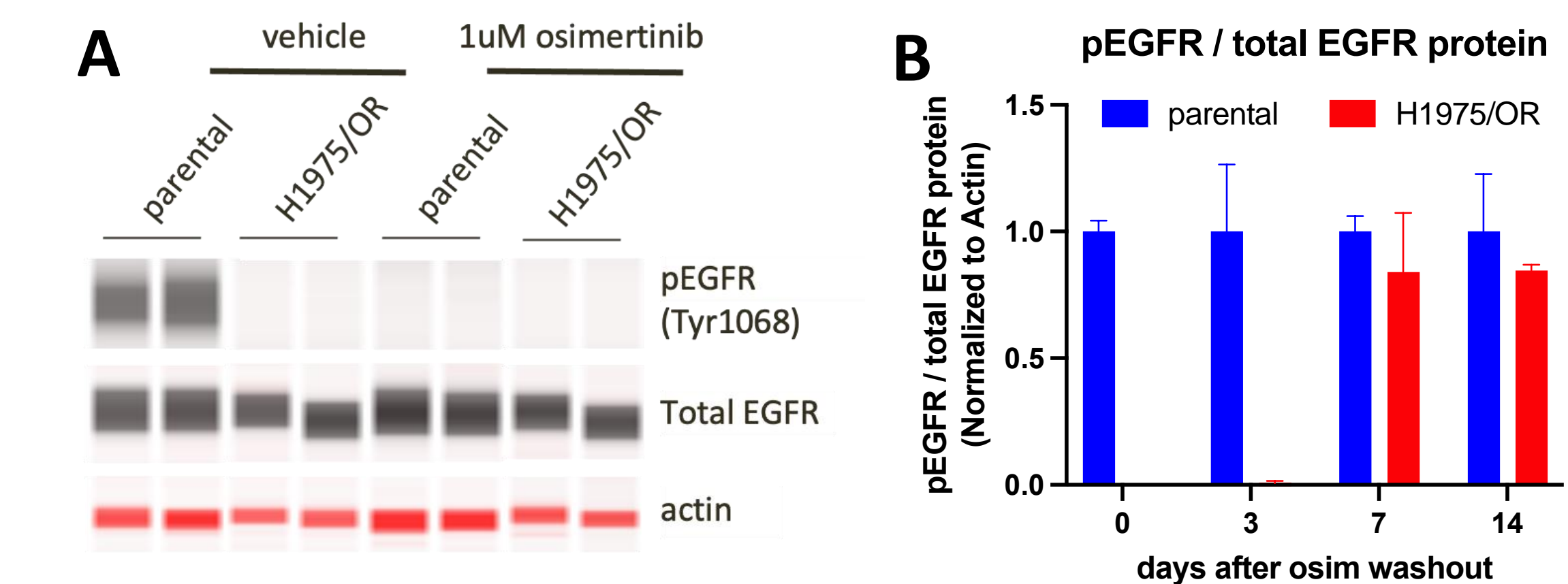


Figure 6: (A) Protein analysis shows that phospho-EGFR levels are highly reduced in H1975/OR cells in the absence of osimertinib treatment, suggesting an EGFR-independent resistance mechanism to osimertinib. (B) Seven days of osimertinib washout period restored phospho-EGFR levels in H1975/OR cells, indicating that H1975/OR cells are resistant to osimertinib via a non-genetic mechanism.

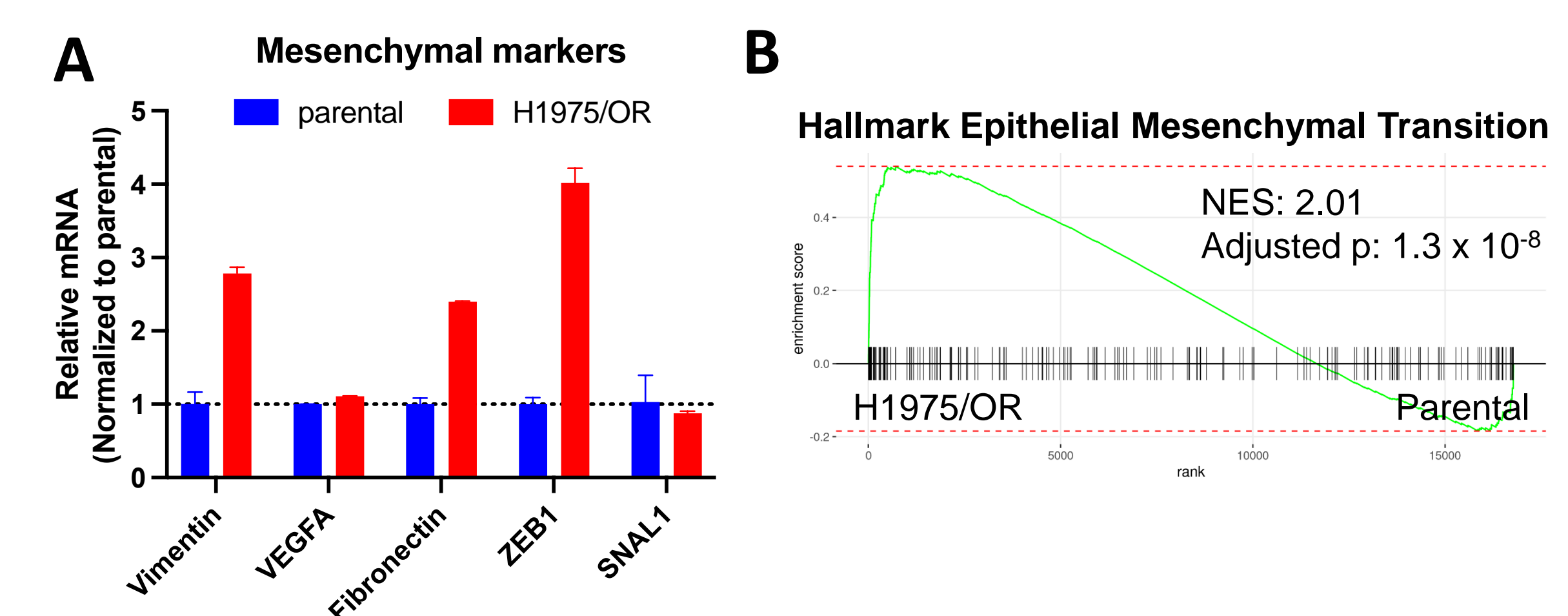


Figure 7: (A) qPCR analysis shows increased expression of subset of mesenchymal markers in H1975/OR cells. (B) RNA-sequencing followed by Gene Set Enrichment Analysis demonstrates that an epithelial to mesenchymal transition (EMT) pathway is highly activated in H1975/OR cells.

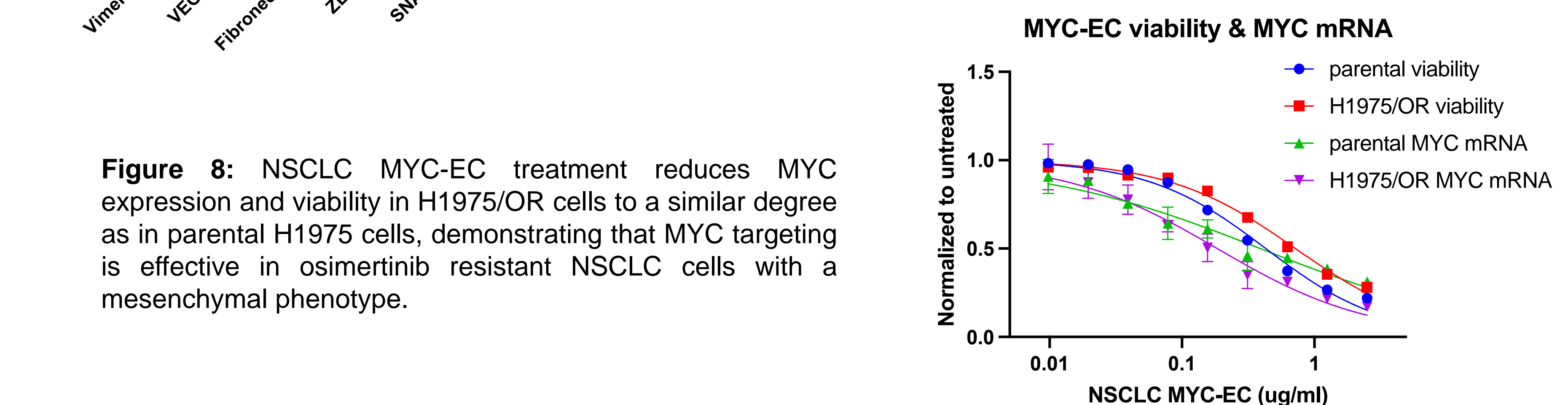


Figure 8: NSCLC MYC-EC treatment reduces MYC expression and viability in H1975/OR cells to a similar degree as in parental H1975 cells, demonstrating that MYC targeting is effective in osimertinib resistant NSCLC cells with a mesenchymal phenotype.

Conclusions

- Epigenomic downregulation of MYC expression synergistically inhibits viability of EGFR-T790M mutant NSCLC cells in combination with osimertinib
- NSCLC cells resistant to osimertinib through EGFR-C797S mutation or epithelial to mesenchymal transition (EMT) retain sensitivity to epigenomic downregulation of MYC expression with NSCLC MYC-EC
- These results support development of NSCLC MYC-EC in EGFR-mutant NSCLC as a combination therapy with osimertinib, and as a monotherapy in osimertinib-resistant NSCLC