

Quaratusugene Ozeplasmid Mediated TUSC2 Upregulation In EML4-ALK Bearing Non-small Cell Lung Carcinoma Induces Apoptosis And Is Highly Effective In Preclinical Studies

Ananya Banerjee¹, Neeke Busette¹, Xu Cheng¹, Kerslee Kohagen¹, Liwei Bao¹, Lluís Lopez-Barcons¹, Rachel Sexton², Ross Camidge³, Sharon Pine³, Mark S. Berger⁴, Matthew B. Soellner², Angel Qin¹, Sofia D. Merajver^{1*}, Nathan M. Merrill^{1*}

¹ Department of Internal Medicine, University of Michigan, Ann Arbor, MI, ² Departments of Medicinal Chemistry and Chemistry, University of Michigan, Ann Arbor, MI, ³ Department of Medicine - Medical Oncology, University of Colorado Anschutz, Aurora, CO, ⁴ Genprex, Inc. Austin, TX, * Equal contribution

ABSTRACT

Background: Non-Small Cell Lung Carcinoma (NSCLC) with the EML4-ALK fusion gene represents about 5% of all NSCLC cases. While these tumors initially respond to ALK Tyrosine Kinase Inhibitors (TKIs), which are standard first- and second-line treatments, resistance develops in nearly all patients, creating an urgent need for new therapeutic strategies. This study investigates the potential of quaratusugene ozeplasmid (QO), a novel gene therapy developed by Genprex that encapsulates the Tumor Suppressor Candidate 2 (*TUSC2*) gene in non-viral lipid nanoparticles. *TUSC2* is typically expressed at low levels in NSCLC, and its upregulation by QO was evaluated in a variety of ALK+ cell lines and patient-derived organoids (PDOs) before and after QO treatment.

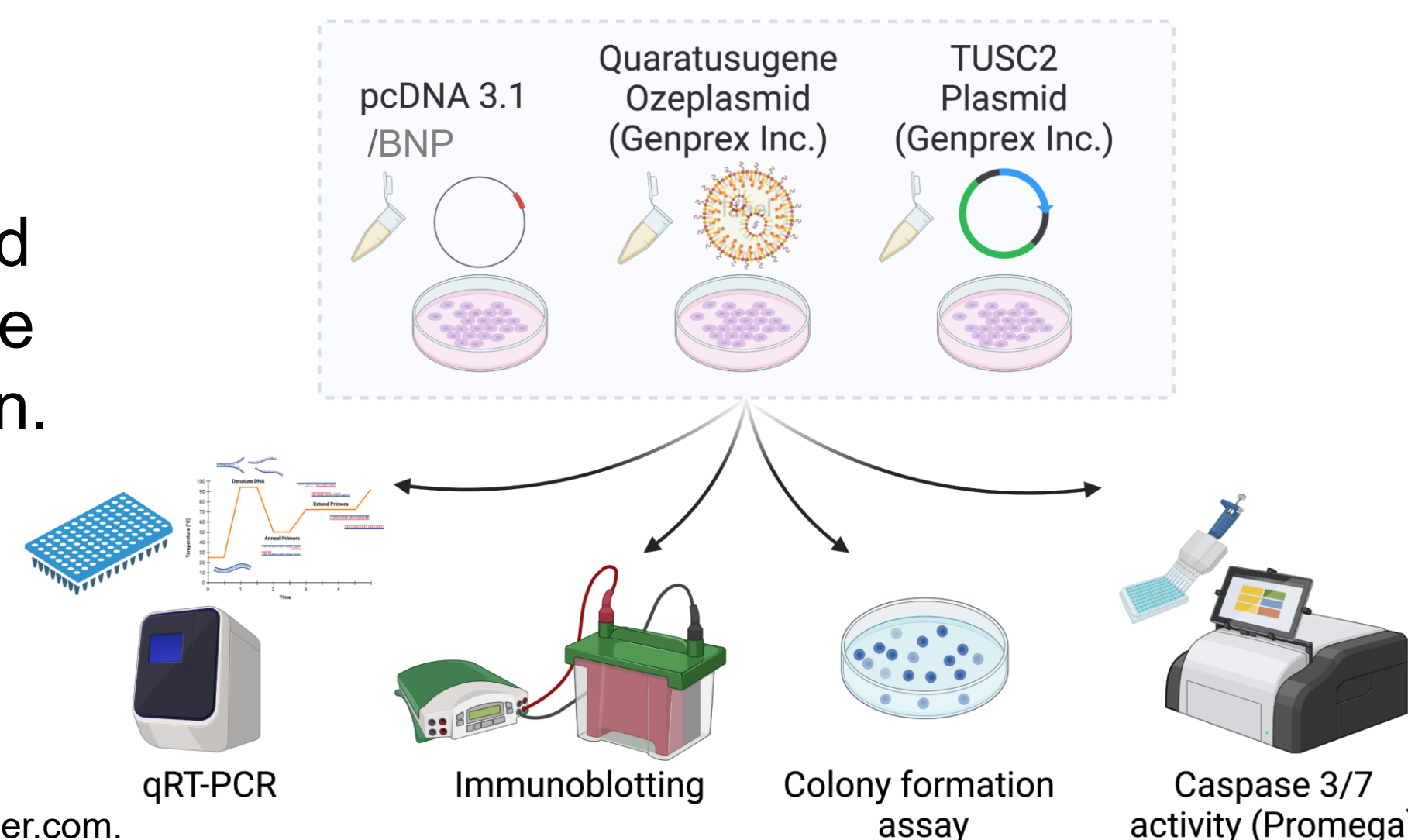
Methods and Results: The results demonstrated that QO-induced *TUSC2* overexpression triggered strong pro-apoptotic responses in both ALK+ cells sensitive to and resistant (lab-generated) to the ALK inhibitor alectinib, as shown by increased apoptotic markers and decreased cell viability, especially when QO was combined with alectinib. To further explore this combination, two in vivo mouse models were used: one with alectinib-sensitive NCI-H2228 ALK+ cells and another with alectinib-resistant ALK167 PDX implants. Mice were divided into four groups: vehicle control, QO alone, alectinib alone (0.5 mg/kg for sensitive or 15 mg/kg for resistant, oral, daily), and QO plus alectinib. In the sensitive model, alectinib alone caused 60% tumor shrinkage, but QO alone and in combination with alectinib achieved 79% tumor reduction ($p = 0.0135$ vs. control), showing a 23% improvement over alectinib alone.

Major new unpublished results: In the resistant model, the QO and alectinib combination showed a synergistic effect, leading to the greatest tumor reduction and improved overall survival ($p = 0.0001$ vs. control), underscoring the clinical promise of this approach. Collectively, these in vitro and in vivo findings support the advancement of QO-mediated *TUSC2* upregulation as a strategy to limit tumor growth and overcome drug resistance in ALK+ NSCLC, warranting further development toward clinical trials.

MATERIALS AND METHODS

- Cell lines: EML4-ALK+ NSCLC cell lines, NCI-H2228 parental & its corresponding Alectinib resistant cell lines generated in the lab, Cmax, Start low, Start IC50, CUTO 8, 9, 29.1.

- Patient-derived material (PDM): Organoids derived from human tissue and pleural effusion.



*This figure was made using BioRender.com.

RESULTS

QO can overexpress TUSC2 in EML4-ALK+ NSCLC cell lines and patient derived organoids (PDOs)

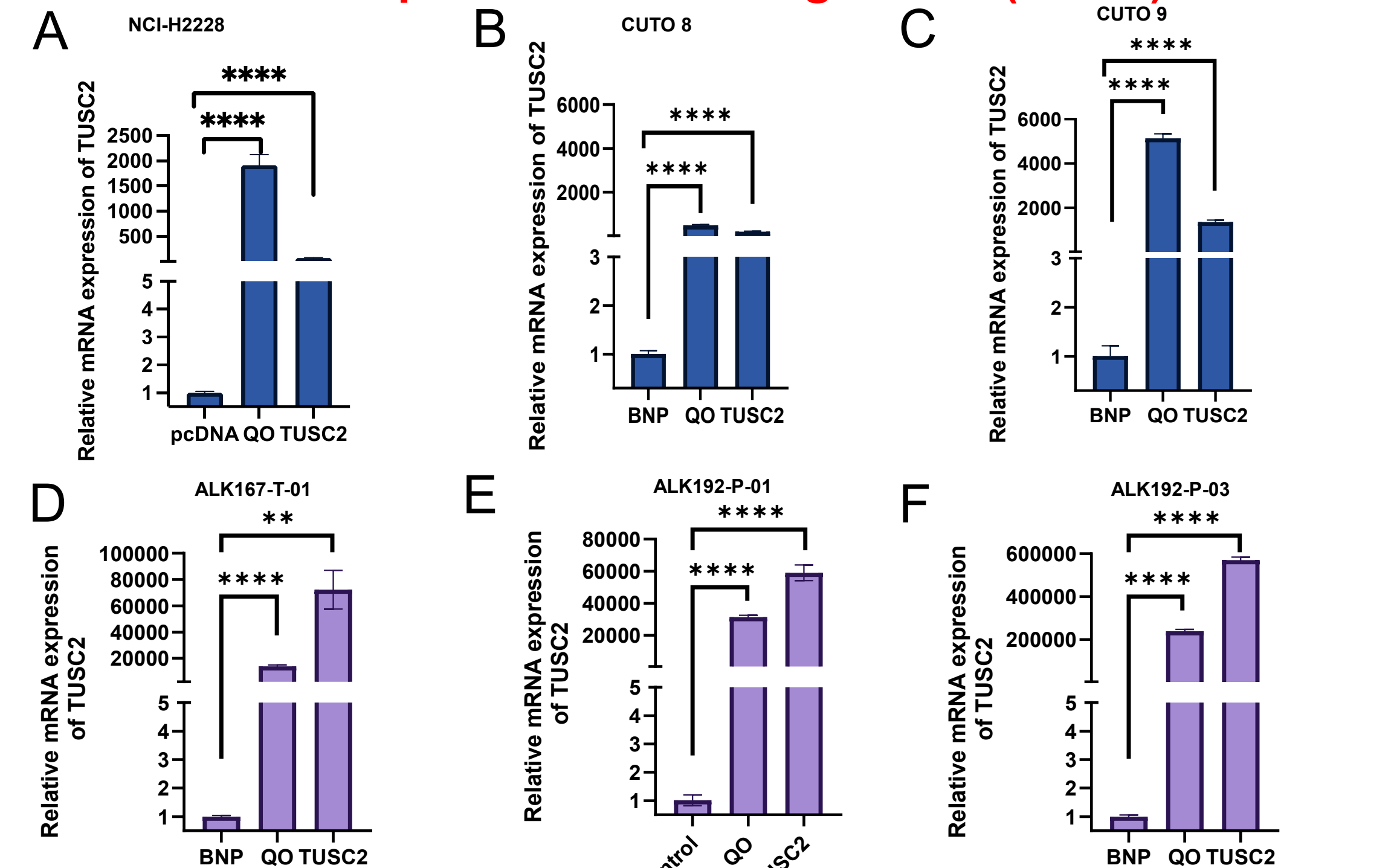


Fig 1: A-C, *TUSC2* is significantly overexpressed in EML4-ALK+ NSCLC cell lines and D-F, organoids derived from patient tissue (T) and pleural effusion (P) on transfection with QO for 48 hours. Data analyzed using t-test. **: $0.001 \leq p \leq 0.01$, ****: $p < 0.0001$.

QO mediated TUSC2 overexpression can induce apoptosis in EML4-ALK+ NSCLC cell lines

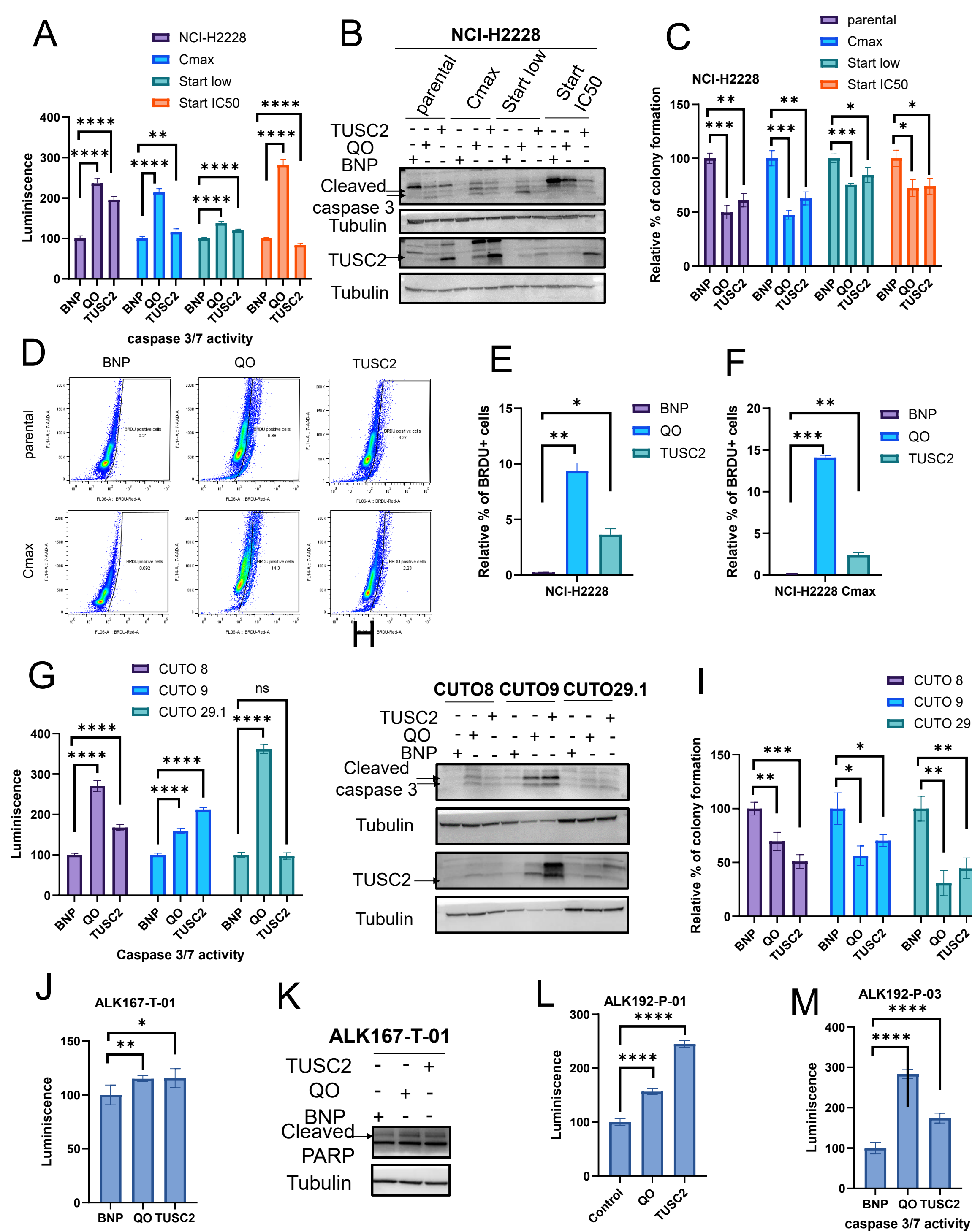


Fig 2: QO mediated overexpression of *TUSC2* A,G, increases the caspase 3/7 activity in ALK+ NSCLC cell lines J,L-M, and in PDOs. B,H,K, increases the expression of cleaved caspase 3 and cleaved PARP. C,I, reduces colony formation ability and D-F, increases DNA fragmentation. Cells were transfected with QO for 48 hrs. Analyzed using t-test. ****: $p < 0.0001$, ***: $0.0001 \leq p \leq 0.001$, **: $0.001 \leq p \leq 0.01$, *: $0.01 \leq p \leq 0.05$, ns: $p \geq 0.05$.

QO mediated TUSC2 overexpression can reduce cell viability in ALK+ cells resistant to ALK inhibitor, Alectinib

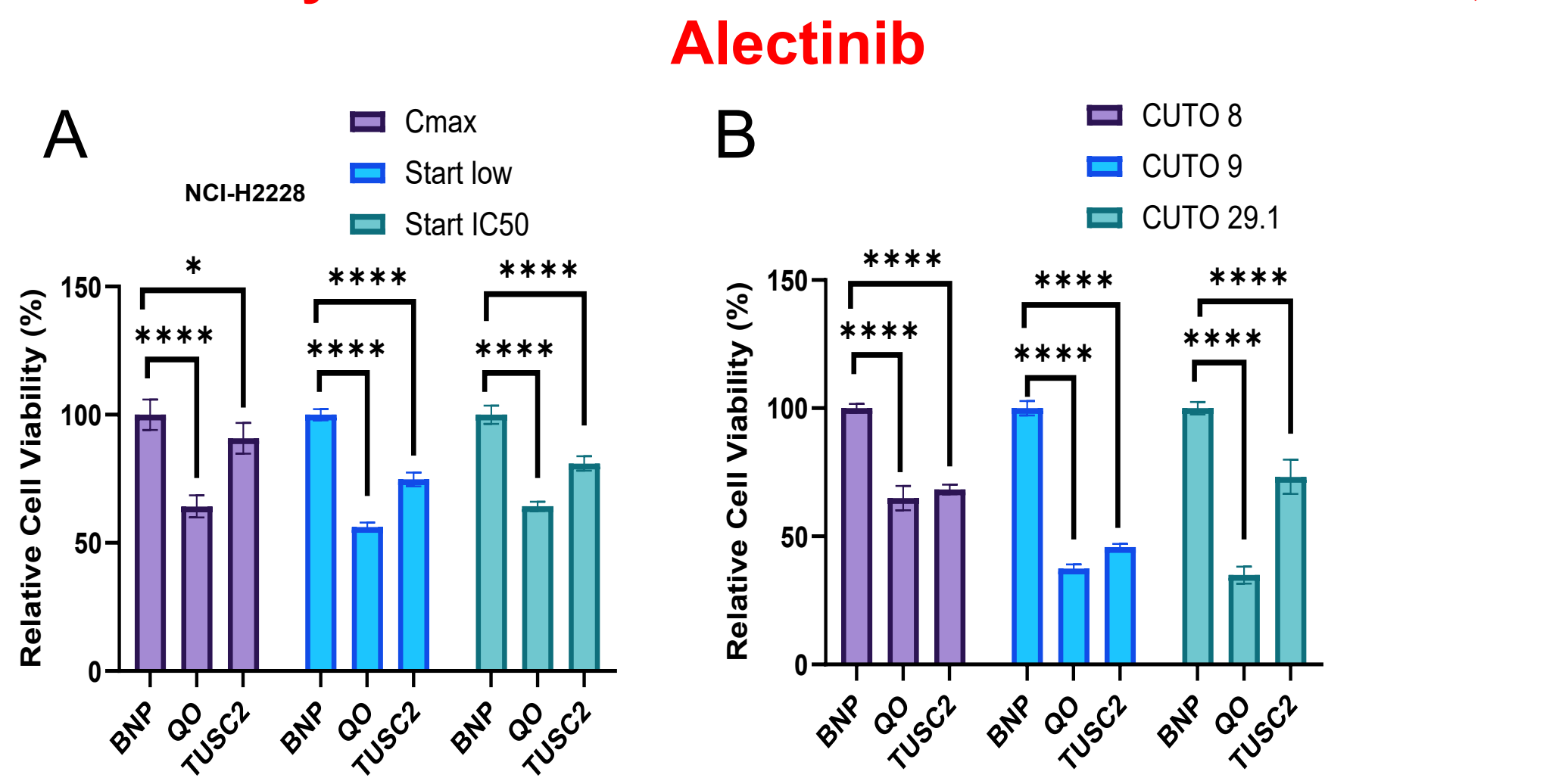


Fig 3: QO mediated overexpression of *TUSC2* A,B, significantly decreases cell viability (120 hours) in ALK+ cell lines that are resistant to Alectinib. All data have been analyzed using t-test. ****: $p < 0.0001$, *: $0.01 \leq p \leq 0.05$.

QO in combination with ALK inhibitor, Alectinib, can induce cell death

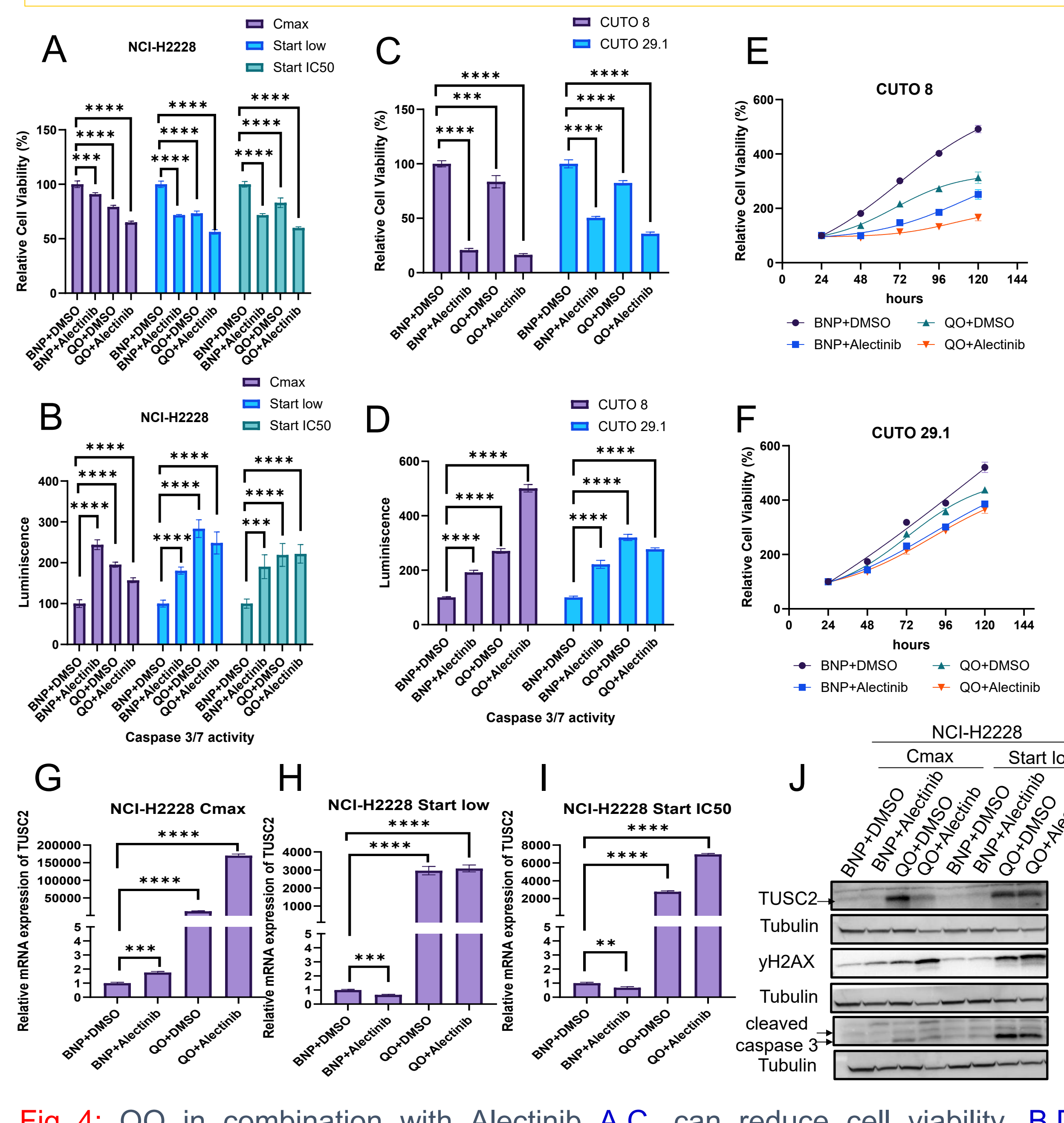
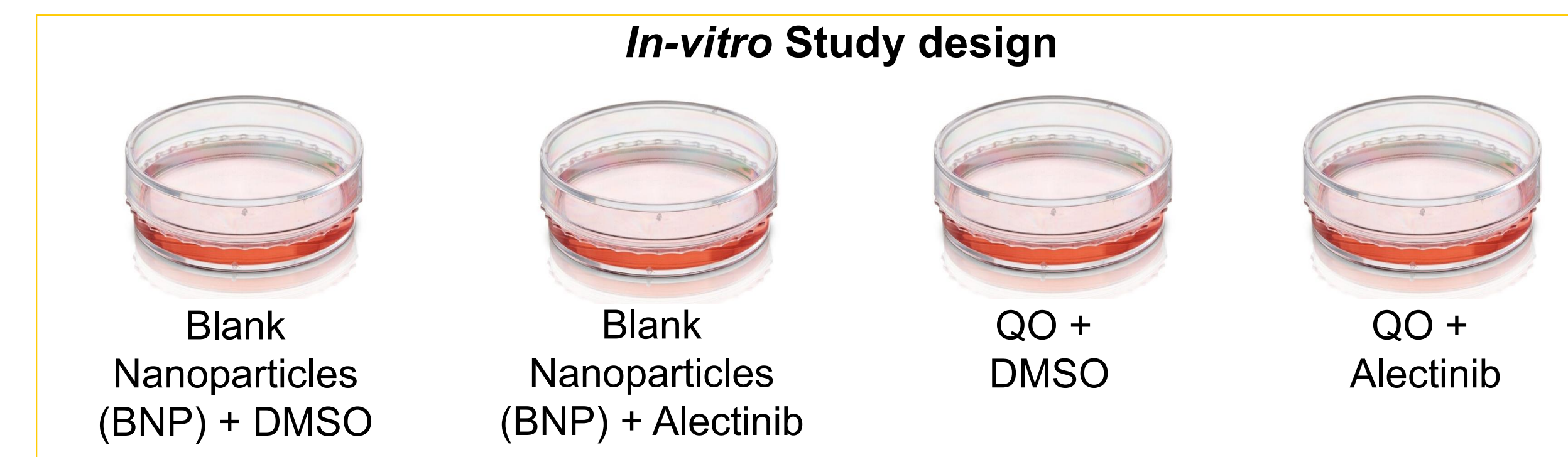
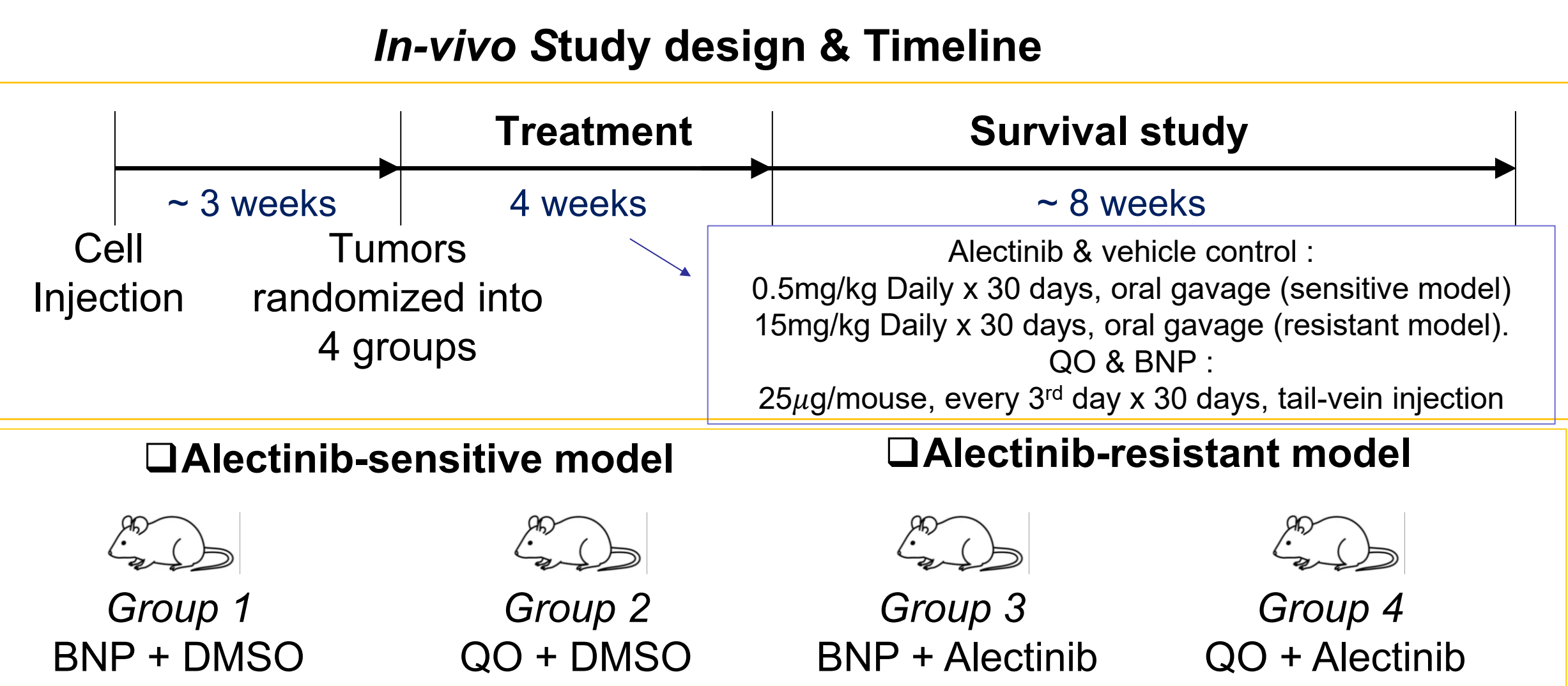
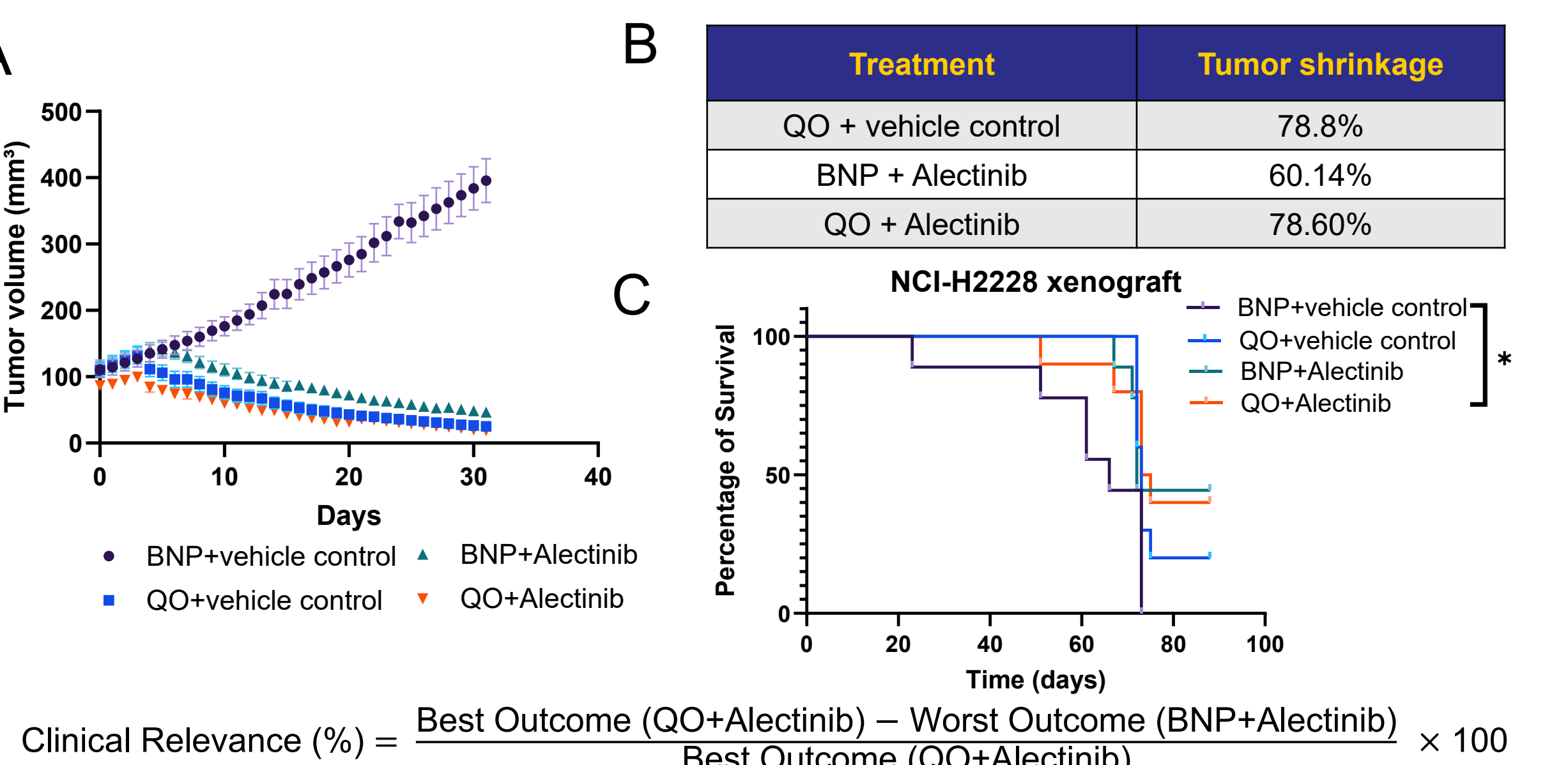


Fig 4: QO in combination with Alectinib A,C, can reduce cell viability, B,D, increase caspase 3/7 activity E,F, inhibit cell proliferation in ALK+ cells that are resistant to Alectinib. QO with Alectinib can also further increase G-J, the expression of *TUSC2* and cleaved caspase 3 indicating increased apoptosis and the expression of yH2AX, indicating increased DNA damage. Cells were transfected with QO followed by treatment with Alectinib at $10 \mu\text{M}$ for 24 hours. Data analyzed using t-test. ****: $p < 0.0001$, ***: $0.0001 \leq p \leq 0.001$, **: $0.001 \leq p \leq 0.01$.

QO in combination with Alectinib has contrasting effects on Alectinib-sensitive and Alectinib-resistant models

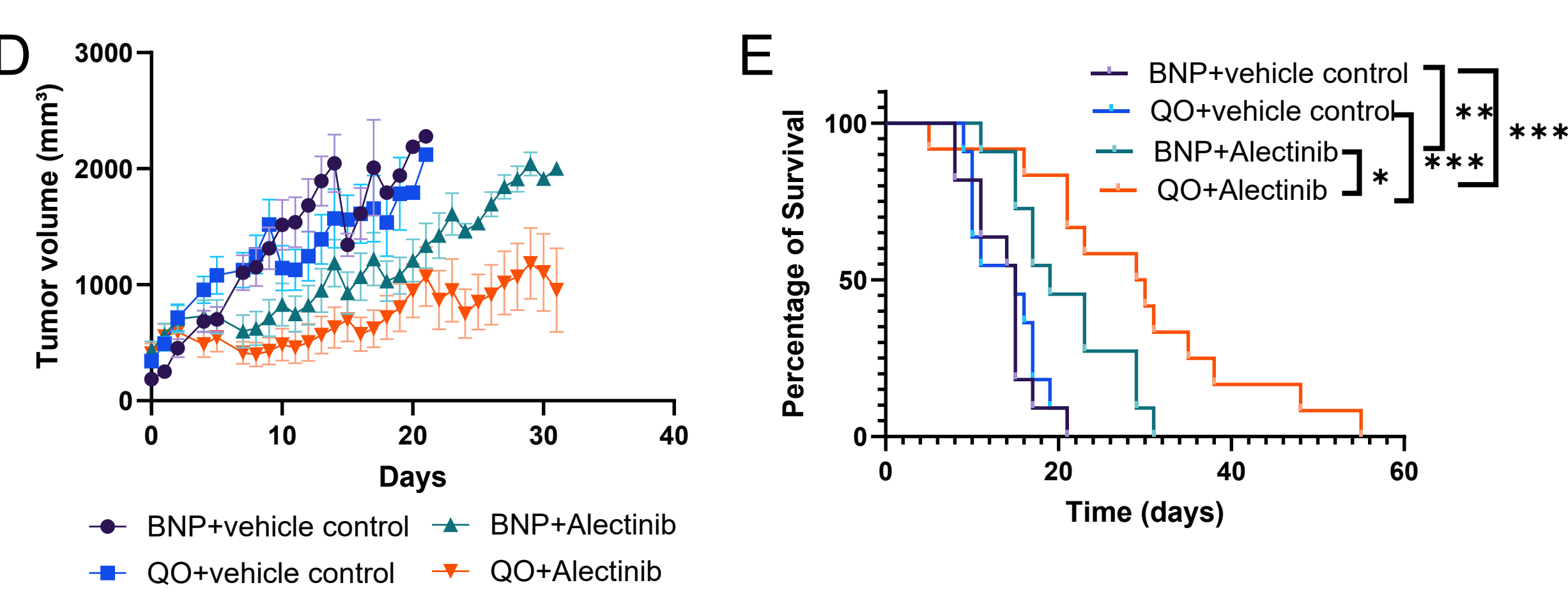


Alectinib-sensitive model: NCI-H2228-derived xenograft



QO with Alectinib has a 23.5% improved outcome than Alectinib alone.

Alectinib-resistant model: ALK167 patient derived xenograft



Combination of QO with Alectinib exhibit significant synergy.

Fig 5: A, B, D, Mice were treated for a duration of 30 days, and tumor measurements were recorded daily. C,E, Post-treatment, mice were monitored during a drug free period to determine their survival. Data analyzed using t-test. ****: $0.0001 \leq p \leq 0.001$, **: $0.001 \leq p \leq 0.01$, *: $0.01 \leq p \leq 0.05$.

CONCLUSION

- The upregulation of *TUSC2* by QO induces apoptosis in ALK+ NSCLC cells, including those resistant to Alectinib.
- Combining QO with Alectinib further increases apoptosis and improves treatment outcomes, but the precise mechanism through which *TUSC2* regulates cell signaling requires additional research.

ACKNOWLEDGEMENT

- This work was supported by the Judith Tam ALK Lung Cancer Initiative.
- We thank Genprex, Inc. for providing the Quaratusugene Ozeplasmid (QO) and the *TUSC2* plasmid, and UC Anschutz for providing the CUTO cell lines.
- We thank ALK Positive and Genprex, Inc. for support with in-vivo studies.
- Travel to San Diego, CA for AACR 2026 was supported in-part by Rogel Cancer Center Postdoctoral Travel Award.