

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

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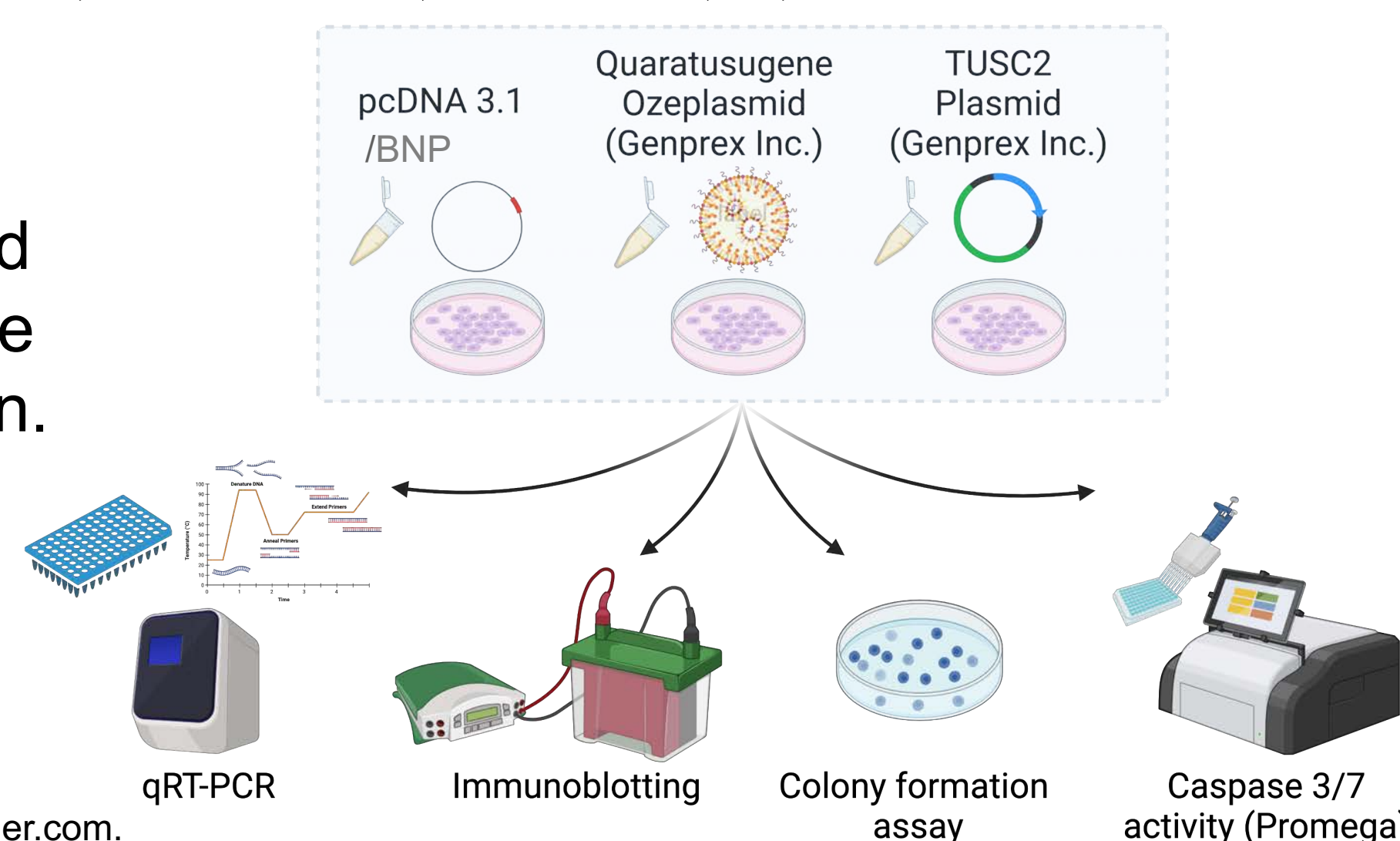
ABSTRACT

Non-Small Cell Lung Carcinoma (NSCLC) with the EML4-ALK fusion gene (Echinoderm microtubule-associated protein-like 4-Anaplastic Lymphoma Kinase) accounts for about 5% of NSCLC cases. Patients with these tumors initially respond well to ALK Tyrosine Kinase Inhibitors (TKIs), which serve as the primary treatments. However, ALK+ lung cancers eventually develop resistance to these inhibitors, highlighting the critical need for alternative or companion therapies. Tumor Suppressor Candidate 2 (TUSC2) is a tumor suppressor gene whose endogenous expression is typically low in NSCLC. Quaratusugene ozeplasmid (QO), developed by Genprex, Inc. is a novel gene therapy that delivers the functional TUSC2 gene via non-viral lipid nanoparticles, upregulating TUSC2 expression in cancer cells. We evaluated the effect of QO in several ALK+ NSCLC cell lines and patient-derived organoids (PDOs) both before and after exposure to treatment. QO-mediated upregulation of TUSC2 was found to significantly induce apoptosis, as demonstrated by increased caspase 3/7 activity, higher levels of pro-apoptotic markers, greater DNA fragmentation, and decreased colony formation ability in these models. Notably, this apoptotic response remained robust in ALK+ NSCLC cell lines with acquired resistance to the ALK inhibitor, alectinib. When QO was combined with alectinib in these resistant models, cell viability was further reduced compared to either agent alone. To explore these effects in vivo, we established xenograft models by subcutaneously implanting NCI-H2228 ALK+ cells into mice. Tumor-bearing mice were randomized to receive vehicle control, QO alone, alectinib alone, or a combination of QO and alectinib. Tumor reduction was most pronounced in the QO alone and QO plus alectinib groups (both 79%) compared to alectinib alone (60%). Collectively, our in vitro and in vivo findings suggest that QO-mediated TUSC2 restoration is effective in suppressing growth and inducing apoptosis in ALK+ NSCLC, including drug-resistant models. These results support further clinical evaluation of QO, alone or with TKIs, as a novel strategy for treating ALK+ NSCLC.

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 plural effusion.



*This figure was made using BioRender.com.

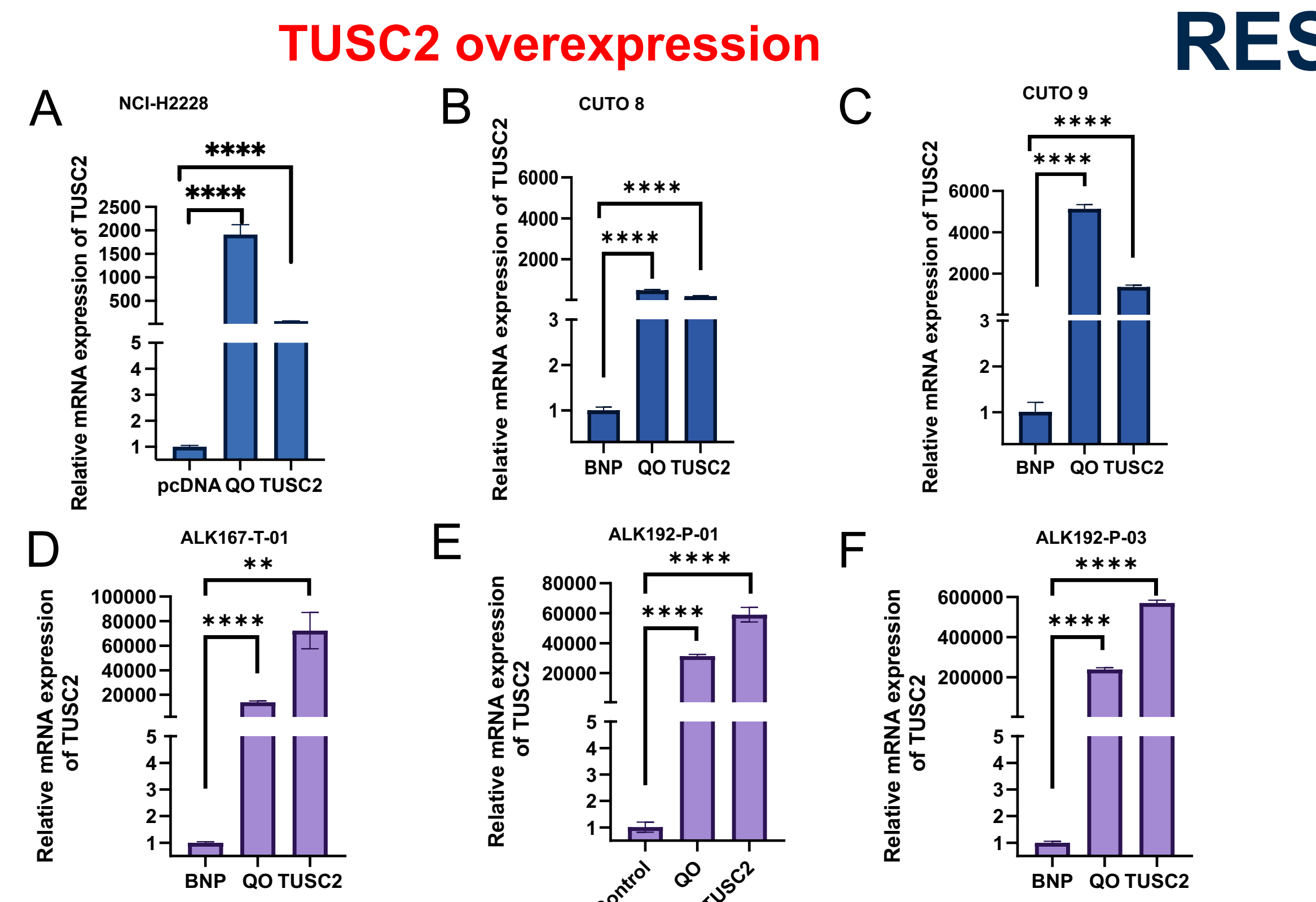


Fig 1: QO can overexpress TUSC2 in EML4-ALK+ NSCLC cell lines and patient derived organoids (PDOs). A-C, TUSC2 is significantly overexpressed in EML4-ALK+ NSCLC cell lines and D-F, organoids derived from patient tissue (T) and pleural effusion (P) on transfection with QO for 48 hours. Data analyzed using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

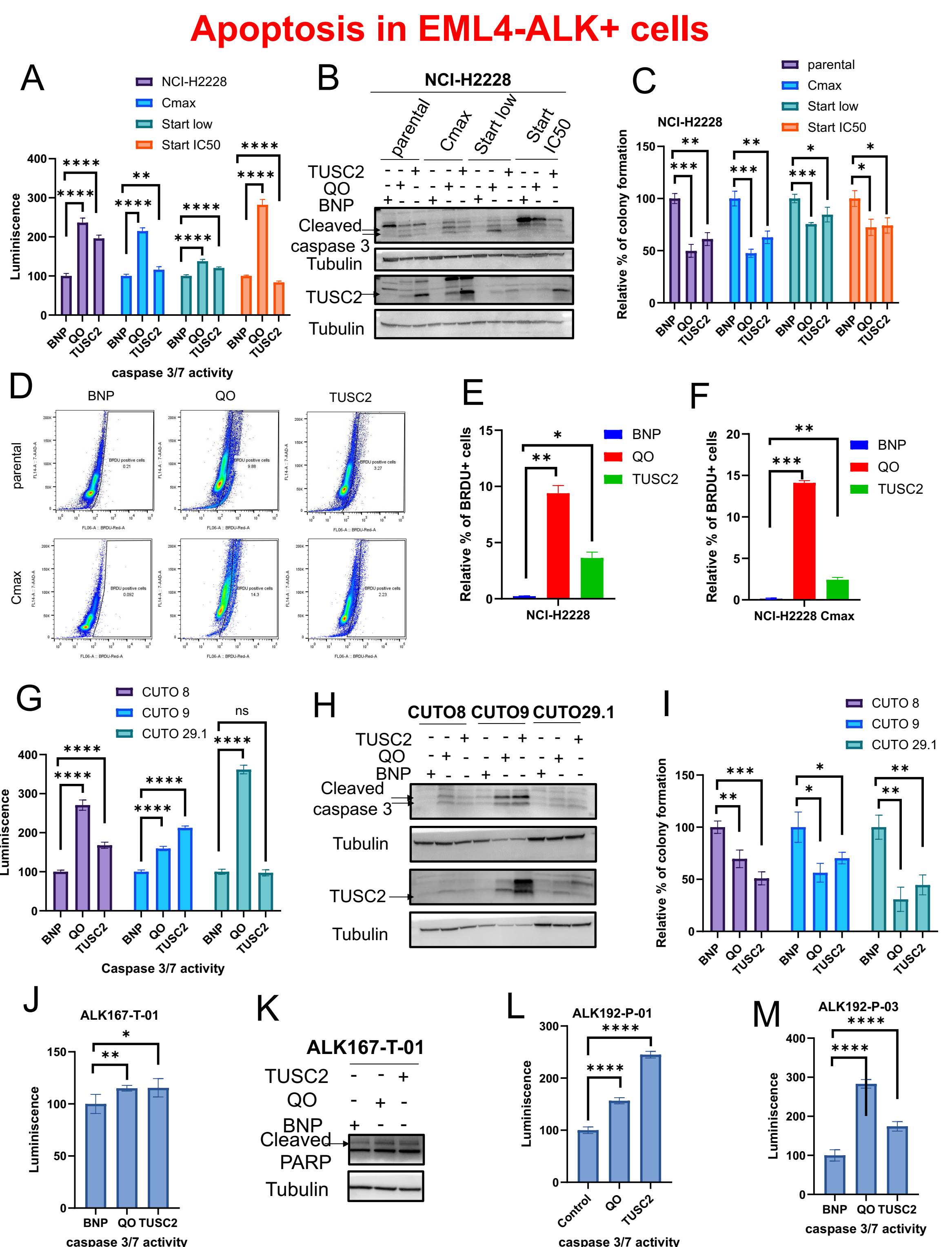


Fig 2: QO mediated TUSC2 overexpression can induce apoptosis in EML4-ALK+ NSCLC cell lines. QO mediated overexpression of TUSC2 A,G, increases the caspase 3/7 activity in ALK+ NSCLC cell lines J,L-M, and in PDOs. B,H,K, increases the expression of cleaved caspase 3 and cleaved PARP. C,I, reduces colony formation ability and D-F, increases DNA fragmentation. Cells were transfected with QO for 48 hours. Data analyzed using t-test. ****:p<0.0001, ***:0.0001≤p<0.001, **:0.001≤p<0.01, *:0.01≤p<0.05, ns: p ≥ 0.05.

RESULTS

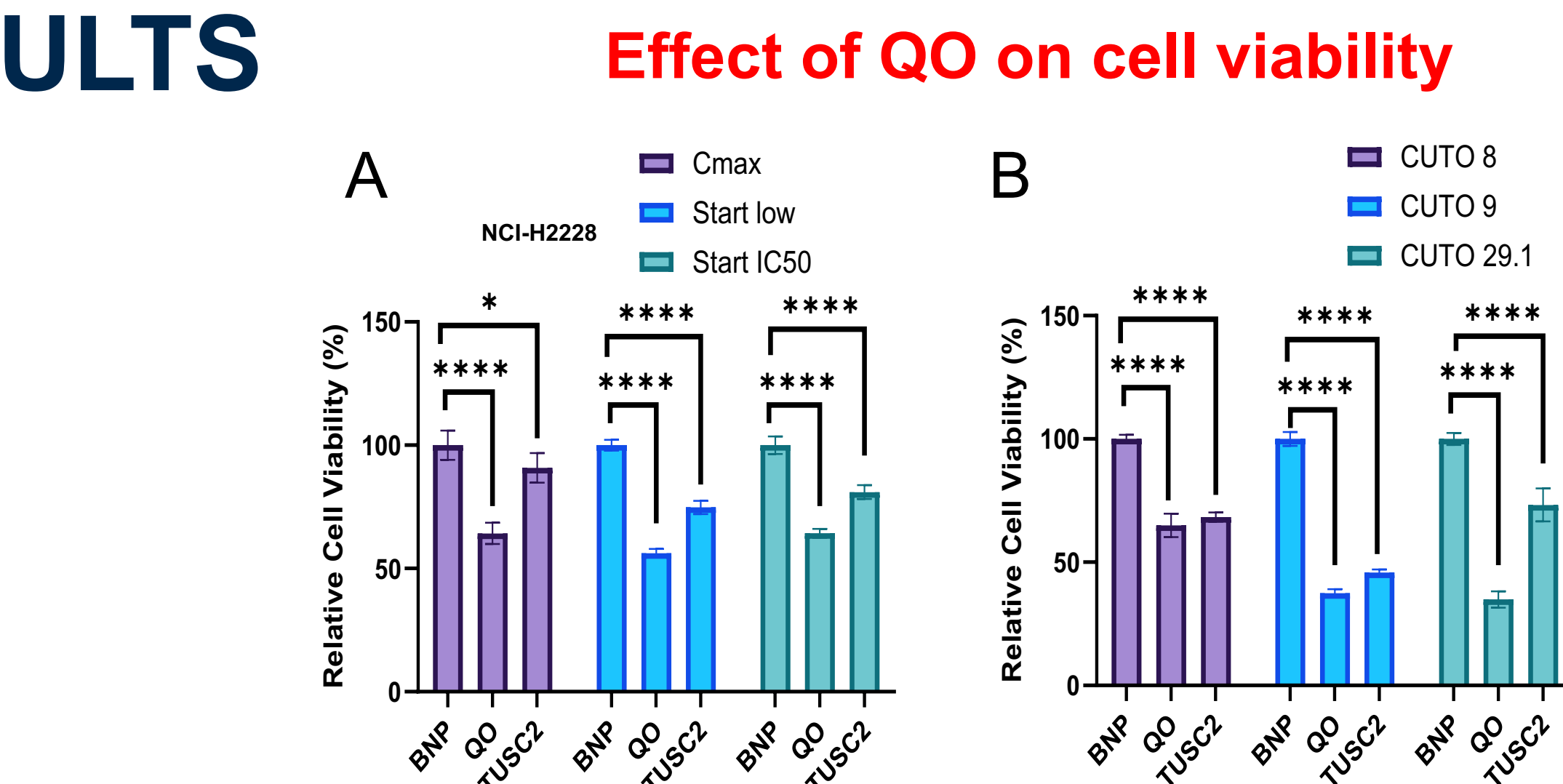


Fig 3: QO mediated TUSC2 overexpression can reduce cell viability in ALK+ cells resistant to ALK inhibitor, Alectinib. QO mediated overexpression of TUSC2 A,B, significantly decreases cell viability (120 hours) in ALK+ cell lines that are resistant to Alectinib. All data have been analyzed using t-test.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

QO in combination with ALK inhibitor, Alectinib

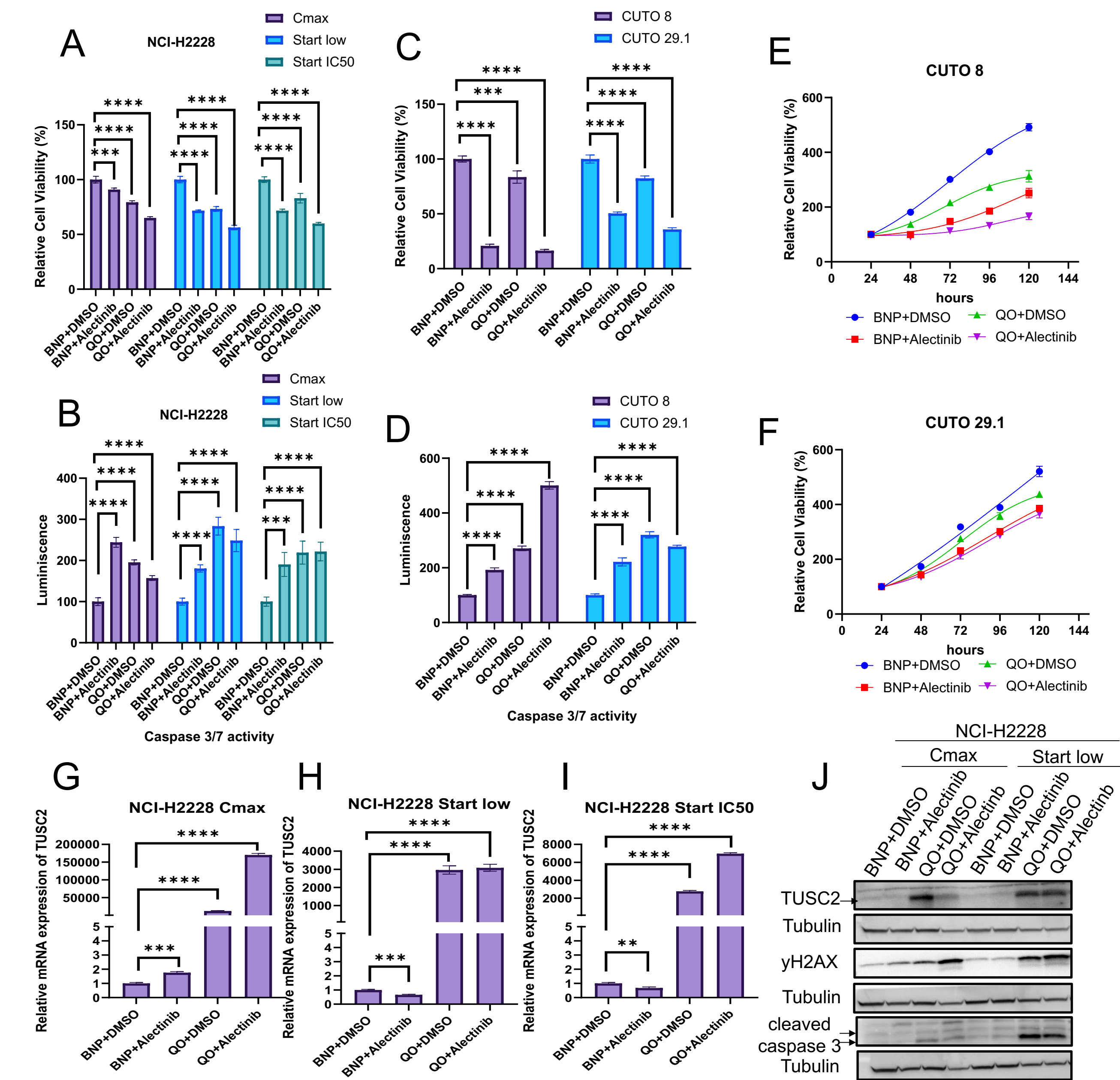
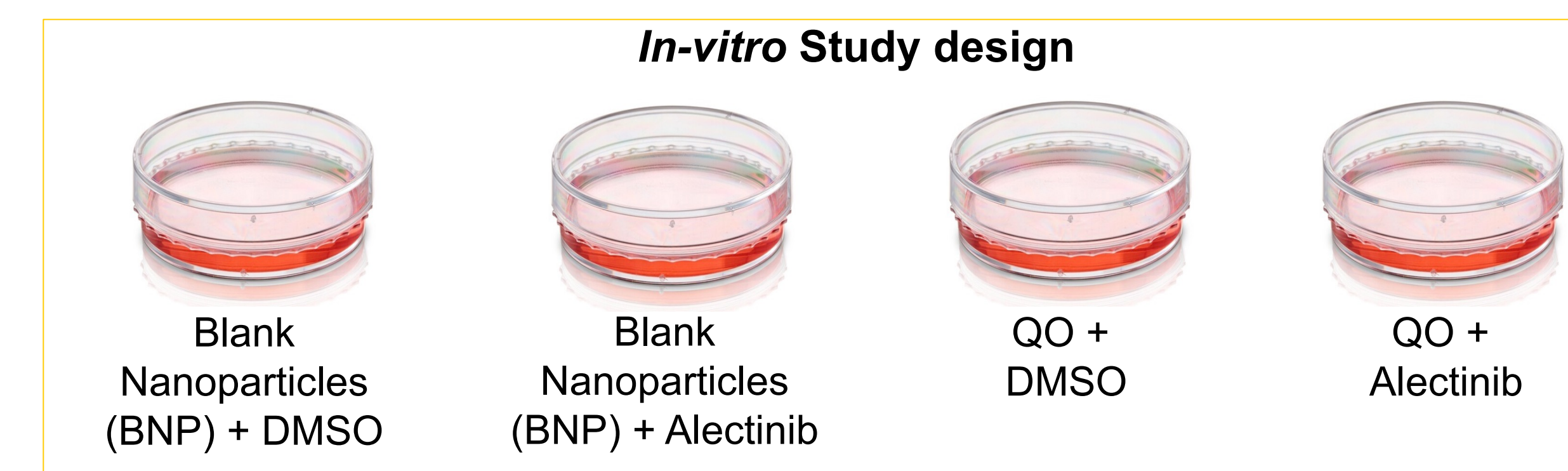
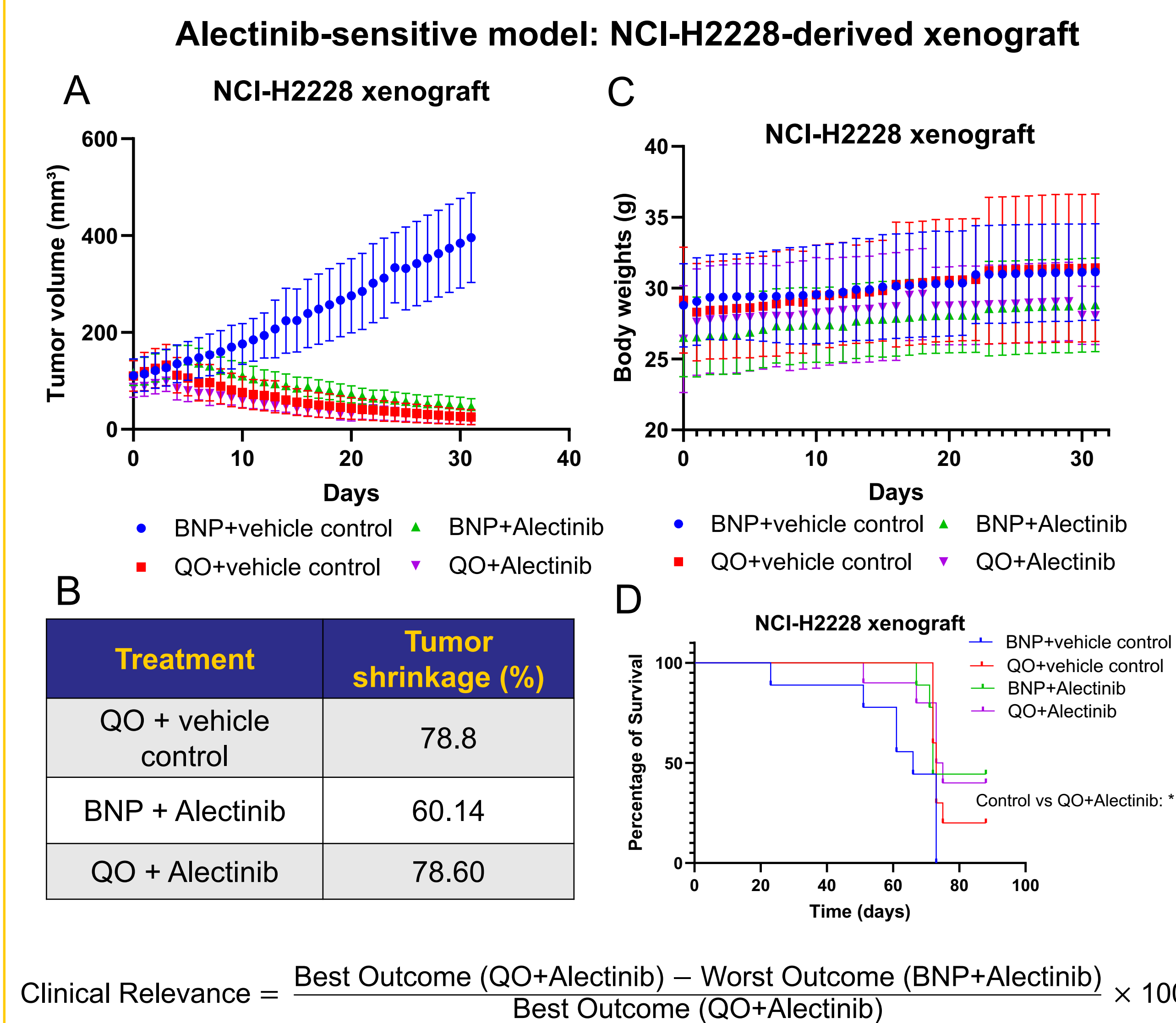
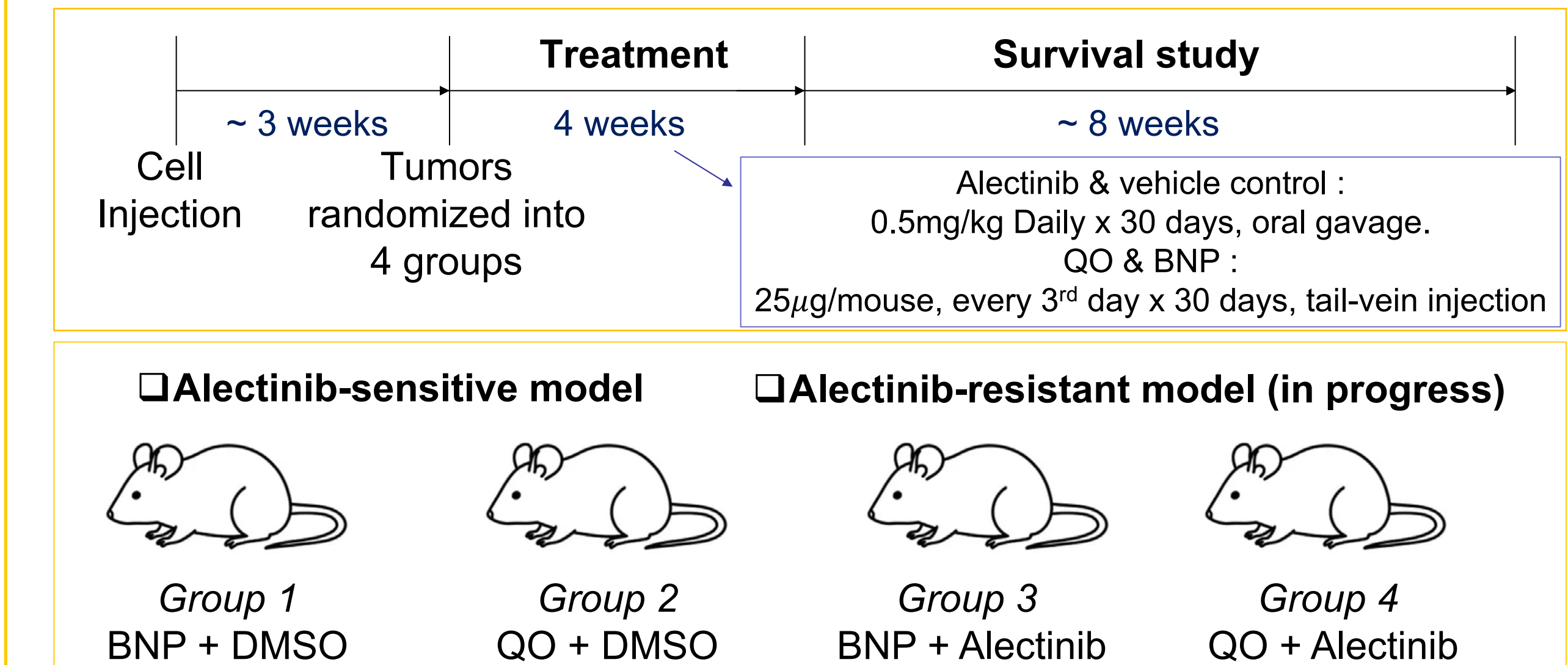


Fig 4: QO in combination with ALK inhibitor, Alectinib, can induce cell death. QO in combination with Alectinib A,C, can reduce cell viability, B,D, increase caspase 3/7 activity E,F, inhibit cell proliferation in ALK+ cells that are resistant to Alectinib. QO with Alectinib can also further increase G-J, the expression of TUSC2 and cleaved caspase 3 indicating increased apoptosis and the expression of γH2AX, indicating increased DNA damage. Cells were transfected with QO followed by treatment with Alectinib at 10μM for 24 hours. Data analyzed using t-test. ****:p<0.0001, ***:0.0001≤p<0.001, **:0.001≤p<0.01.

In-vivo Study design & Timeline



Clinical Relevance = $\frac{\text{Best Outcome (QO+Alectinib)} - \text{Worst Outcome (BNP+Alectinib)}}{\text{Best Outcome (QO+Alectinib)}} \times 100$

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

Fig 5: QO in combination with Alectinib has a better efficacy and overall improved outcome than individual treatments. A,B, Mice were treated for a duration of 30 days, and tumor measurements were recorded daily. C, Mice were regularly monitored for the entire duration of the study, and their weights were measured using vernier calipers. D, Post-treatment, mice were monitored during a drug free period to determine their survival. Data analyzed using t-test. *:0.01≤p<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

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