# Supplementary materials

## CREB Regulates Cisplatin Resistance by Targeting TNKS and KDM6A in NSCLC cell-Derived Tumor Spheroids

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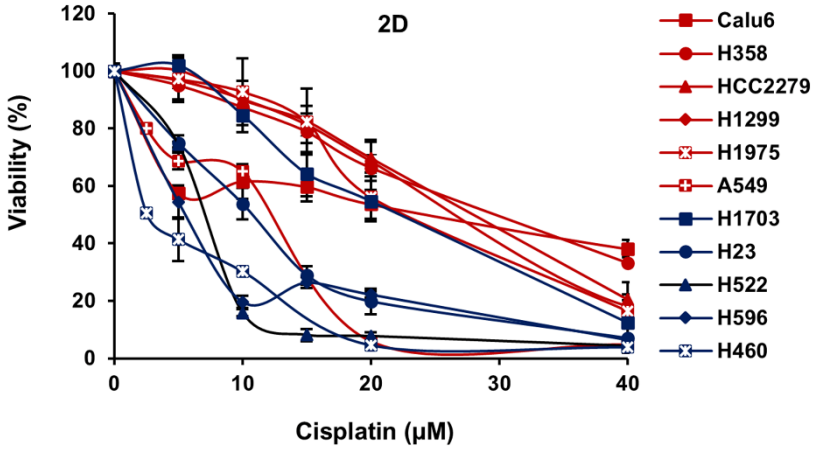
**Supplementary Table 1. Characteristics of the 11 NSCLC cell lines used in this study**

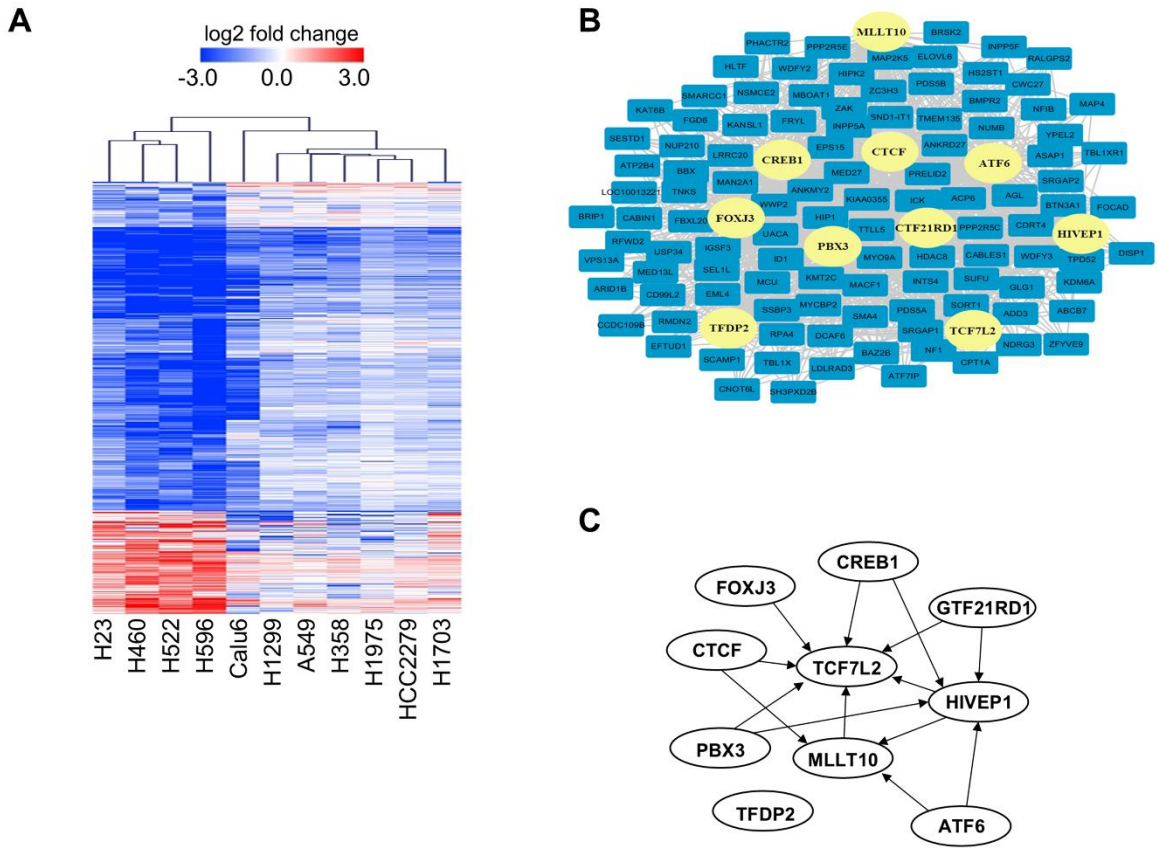
| Cell line | Histology | EGFR | RAS | TP53 | Growth property |
|---|---|---|---|---|---|
| A549 | Adenocarcinoma | WT | K-RAS G12S | WT | non |
| NCI-H1299 | Large cell carcinoma | WT | N-RAS Q61K | Null | growing |
| NCI-H358 | Adenocarcinoma | WT | K-RAS G12C | Null | growing |
| NCI-H1975 | Adenocarcinoma | L858R, T790M | WT | R273H | growing |
| HCC2279 | Adenosquamous Carcinoma | del(E746-A750) | WT | Y234C | non |
| Calu-6 | Adenocarcinoma | WT | K-RAS Q61K | R196Ter | growing |
| NCI-H1703 | Squamous Cell Carcinoma | WT | WT | c.919+1G>T | growing |
| NCI-H460 | Large cell carcinoma | WT | K-RAS Q61H | WT | growing |
| NCI-H522 | Adenocarcinoma | WT | WT | P191fsTer56 | growing |
| NCI-H596 | Adenosquamouse carcinoma | WT | WT | G245C | non |
| NCI-H23 | Adenocarcinoma | WT | K-RAS G12C | M246I | growing |

Abbreviations: WT, wild-type; Ter, termination; fs, frameshift

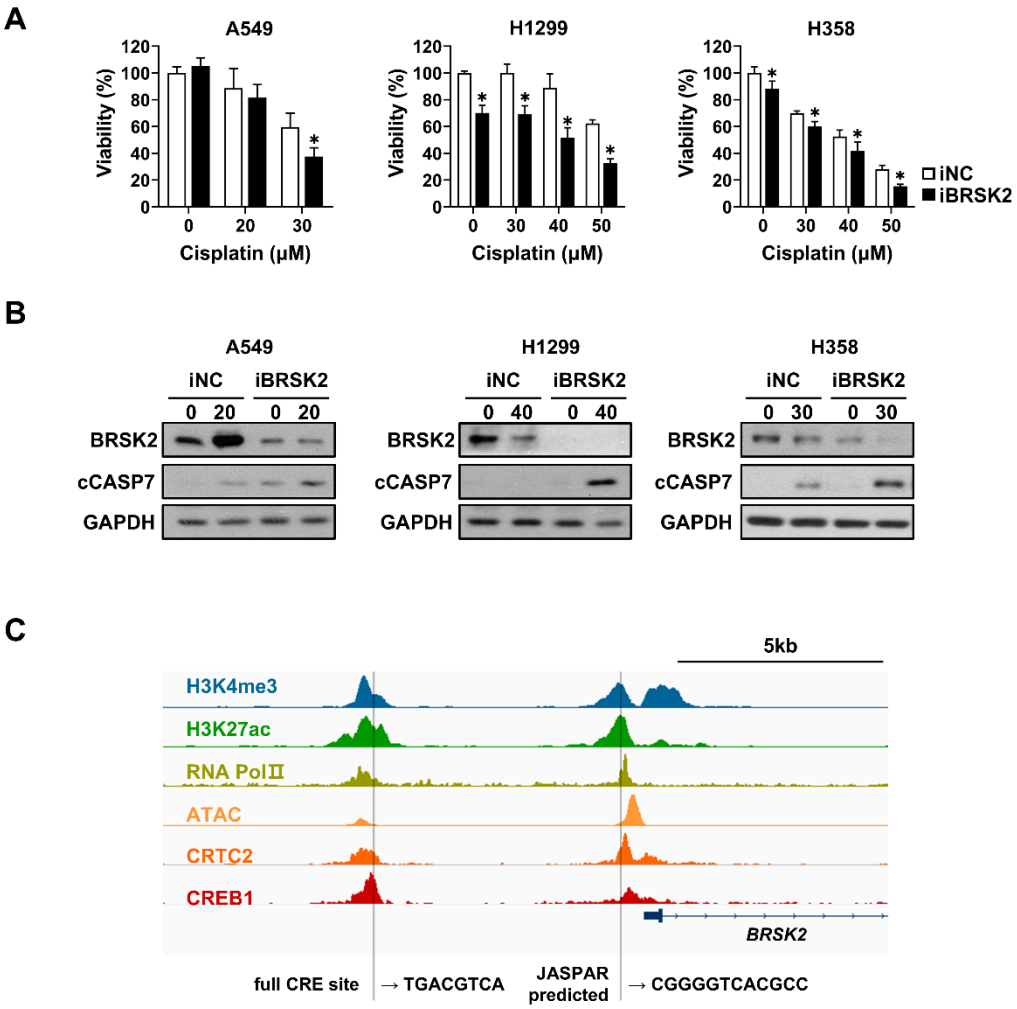
Cell line information accessed from DepMap portal website: https://depmap.org/portal



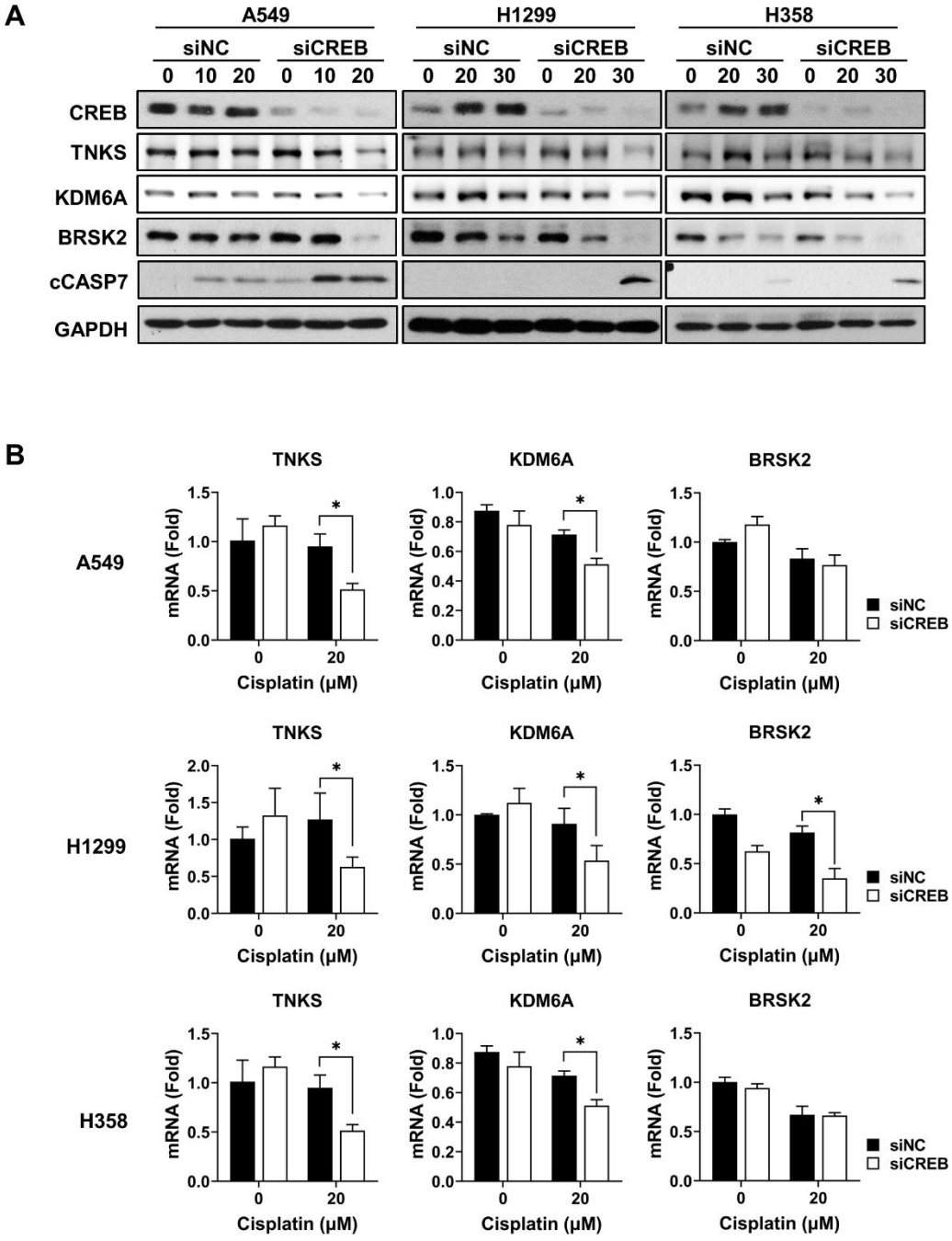

**Supplementary Figure 1. Dose-response curves of cisplatin in 2D cultured NSCLC cells.** Cells were treated with various concentrations of cisplatin for 48 h. Cell viability was assessed by the ATP content (CellTiter-Glo 2.0). Data represent the mean ± SEM of three independent experiments.



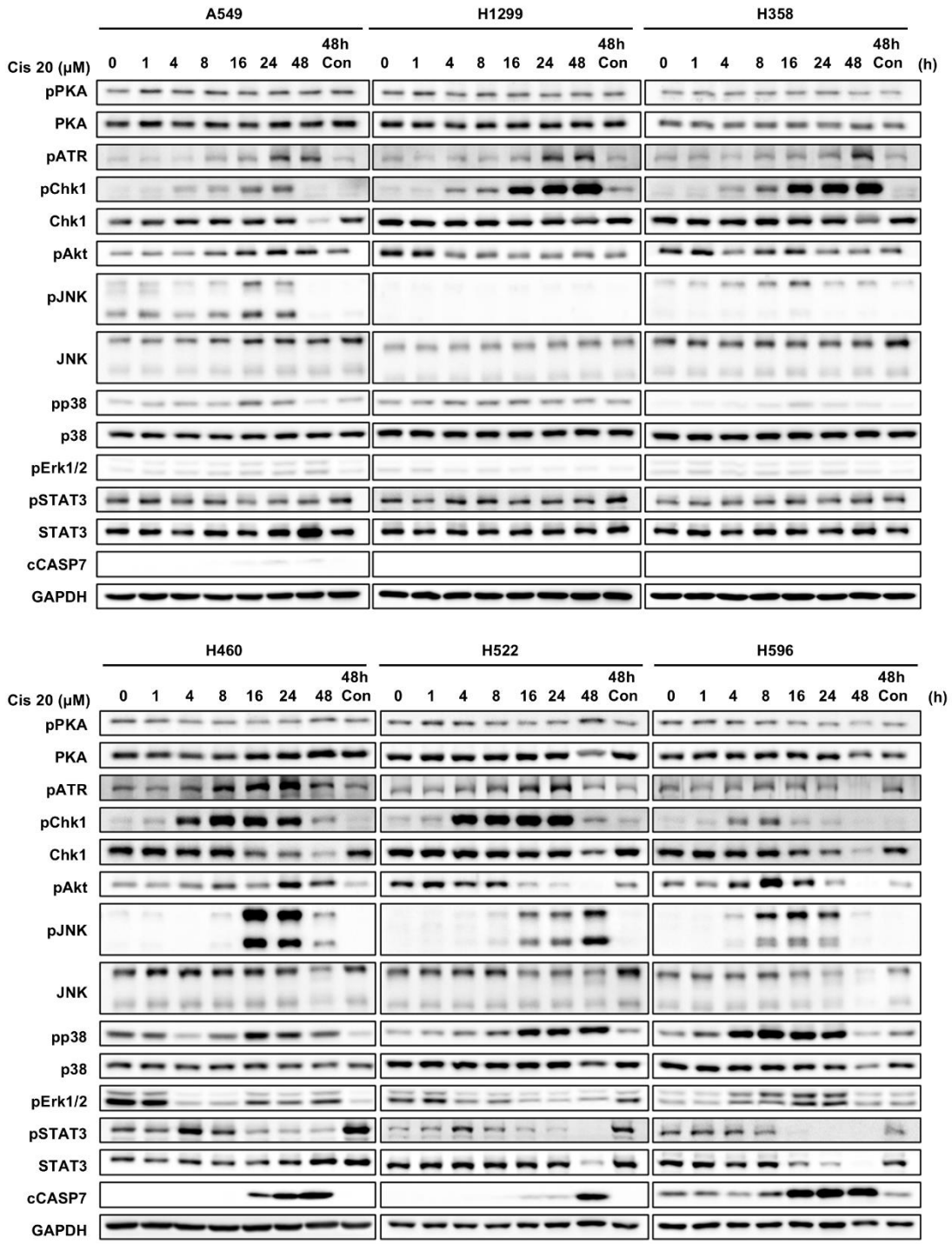

**Supplementary Figure 2. Transcriptional regulatory networks associated with cisplatin resistance.** (A) A heatmap showing the hierarchical clustering of the cisplatin-sensitive and -resistant cell lines and the fold-change values of 668 genes. (B) The network consists of the top 10 master regulators and their target genes. Yellow nodes represent the master regulators. (C) A schematic representation of transcriptional network between top 10 master regulators.



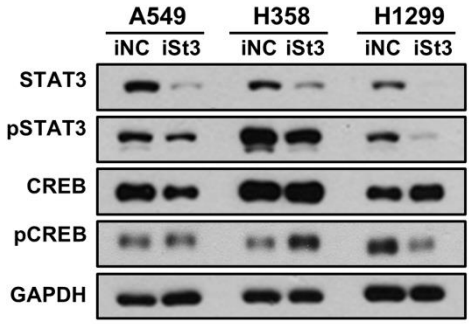

**Supplementary Figure 3. Inhibition of BRSK2 enhances the cisplatin sensitivity.** (A, B) The effect of BRSK2 silencing on cisplatin sensitivity was analyzed in three cisplatin-resistant cell lines (A549, H1299, and H358). Tumor spheroids with BRSK2 silencing were treated with the indicated dose of cisplatin for 48 h, and changes in cisplatin sensitivity were assessed via viability assay (A) and western blot analysis (B). (C) Analysis of CREB binding sites in the regulatory regions of *BRSK2*. The Integrative Genomics Viewer browser displays ChIP-seq signals in the regulatory regions of *BRSK2*. CREB binding sites predicted by JASPAR are indicated by grey bars.




**Supplementary Figure 4. CREB knockdown combined with cisplatin treatment reduces the expression of TNKS, KDM6A, and BRSK2.** Tumor spheroids with CREB knockdown were treated with the indicated dose of cisplatin for 48 h, and changes in the protein (A) and mRNA (B) expression levels of CREB target genes were evaluated by western blotting and qPCR, respectively.




**Supplementary Figure 5. Comparison of signaling pathways between cisplatin-resistant and cisplatin-sensitive tumor spheroids.** Tumor spheroids were treated with 20 μM cisplatin for the indicated time. Protein expression associated with cell survival, cell death, and DNA damage response was analyzed by western blotting.




**Supplementary Figure 6. STAT3 is not involved in CREB expression.** Cells were transfected with siRNA targeting STAT3 for 48 h, and the expression levels of CREB and pCREB were analyzed by western blotting.