

# Supplementary Figures and Tables for NPEPPS is a novel and druggable driver of platinum resistance

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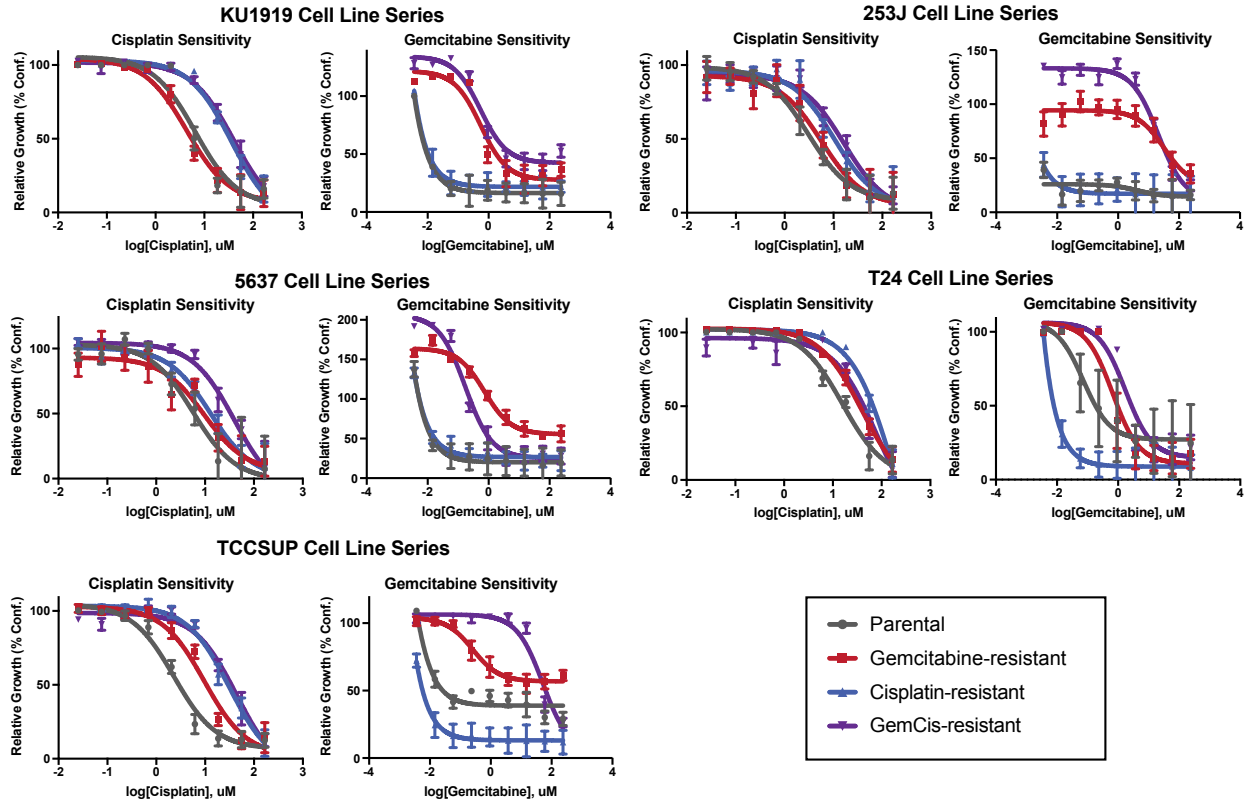
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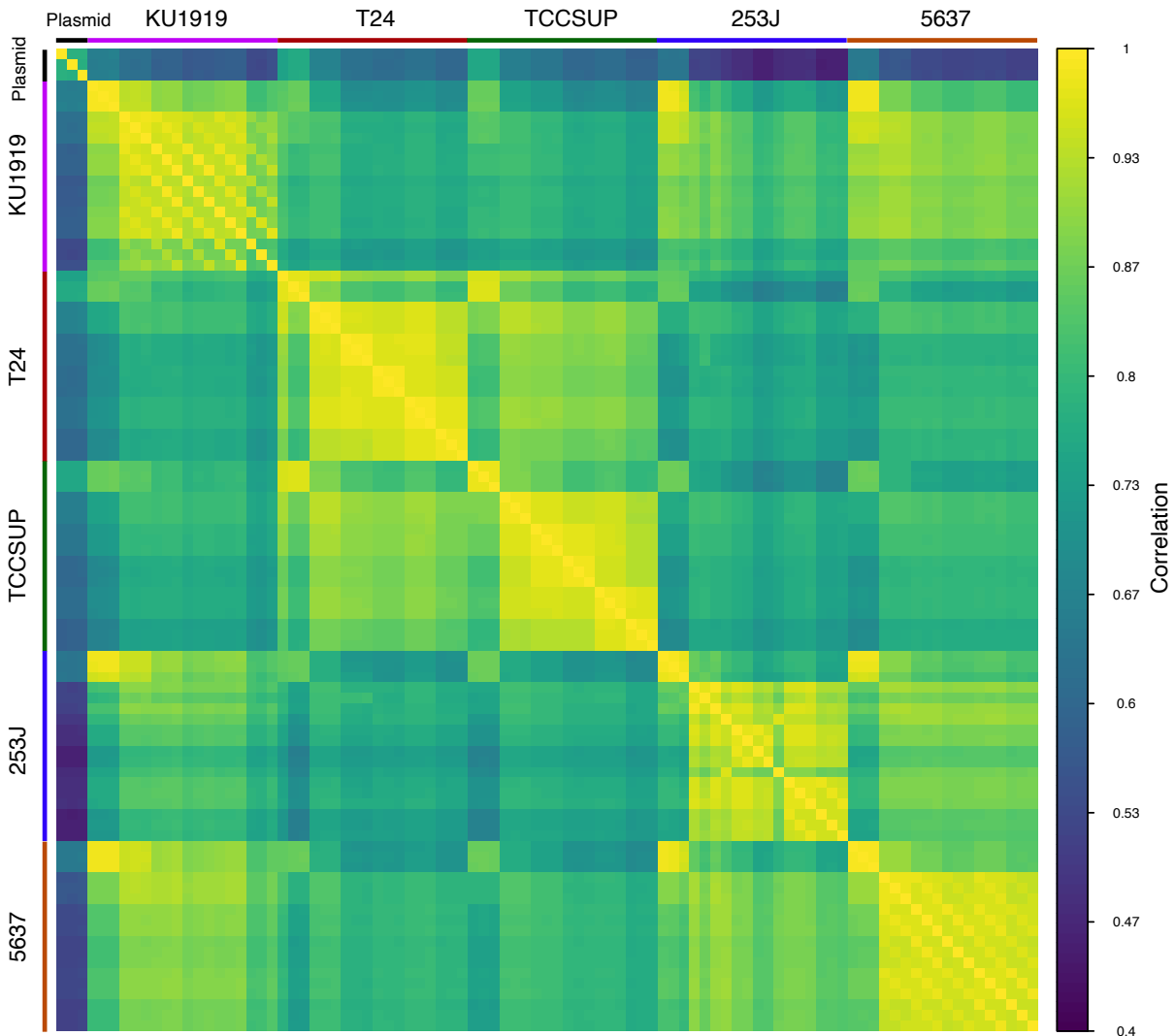
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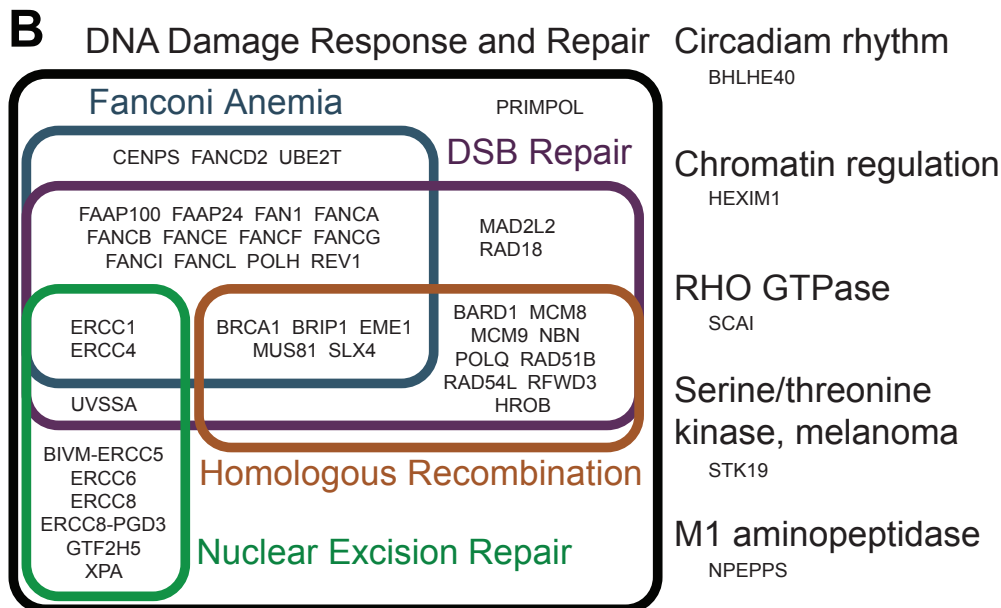
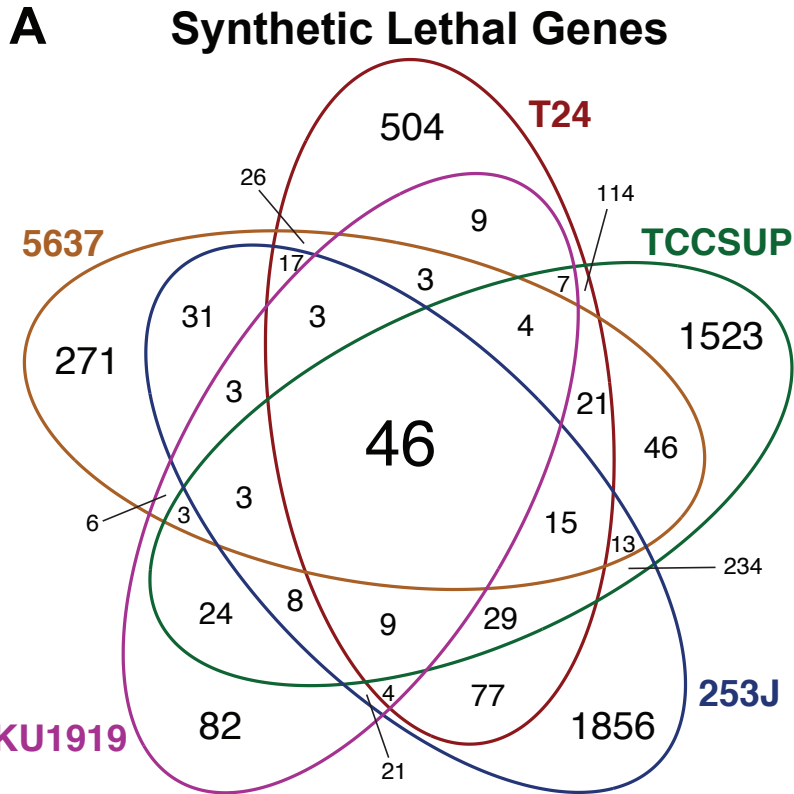


**Figure S1. Dose response for all cell line derivatives.** Parental, cisplatin-resistant, gemcitabine-resistant, and gemcitabine plus cisplatin (GemCis)-resistant cells for each of the five bladder cancer cell lines were treated with increasing doses of cisplatin or gemcitabine. Dose response curves were calculated. The resistant derivative lines were more resistant to the associated drug. Data represent a single experiment with each condition measured in technical triplicate wells (mean  $\pm$  SEM).

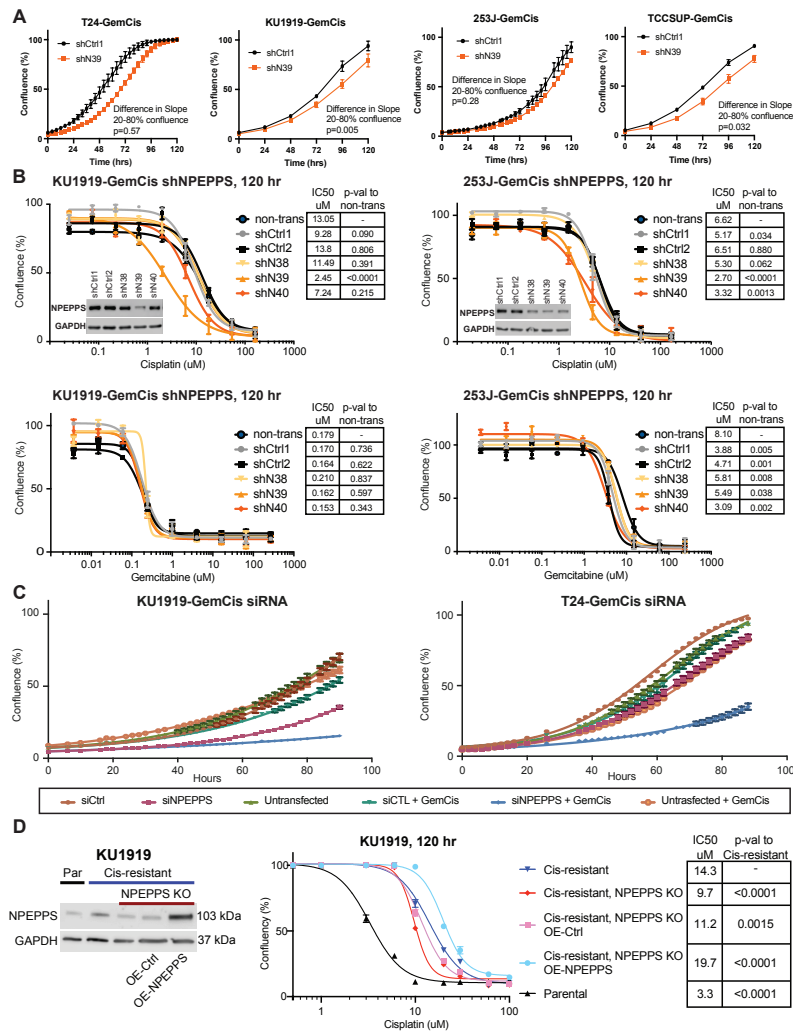


**Figure S2. Correlation of genes across conditions and cell lines from the CRISPR screen.** Pearson correlation was calculated between normalized gene counts across all tested conditions and replicates, from the original plasmid pool in triplicate to all cell lines. The ordering for each cell line includes: Day 0 (replicate 1, replicate 2, replicate 3), Day 10 (all replicates), Day 19 with PBS treatment (all replicates), Day 25 with PBS treatment (all replicates), Day 19 with gemcitabine plus cisplatin treatment (all replicates), and Day 25 with gemcitabine plus cisplatin treatment (all replicates).

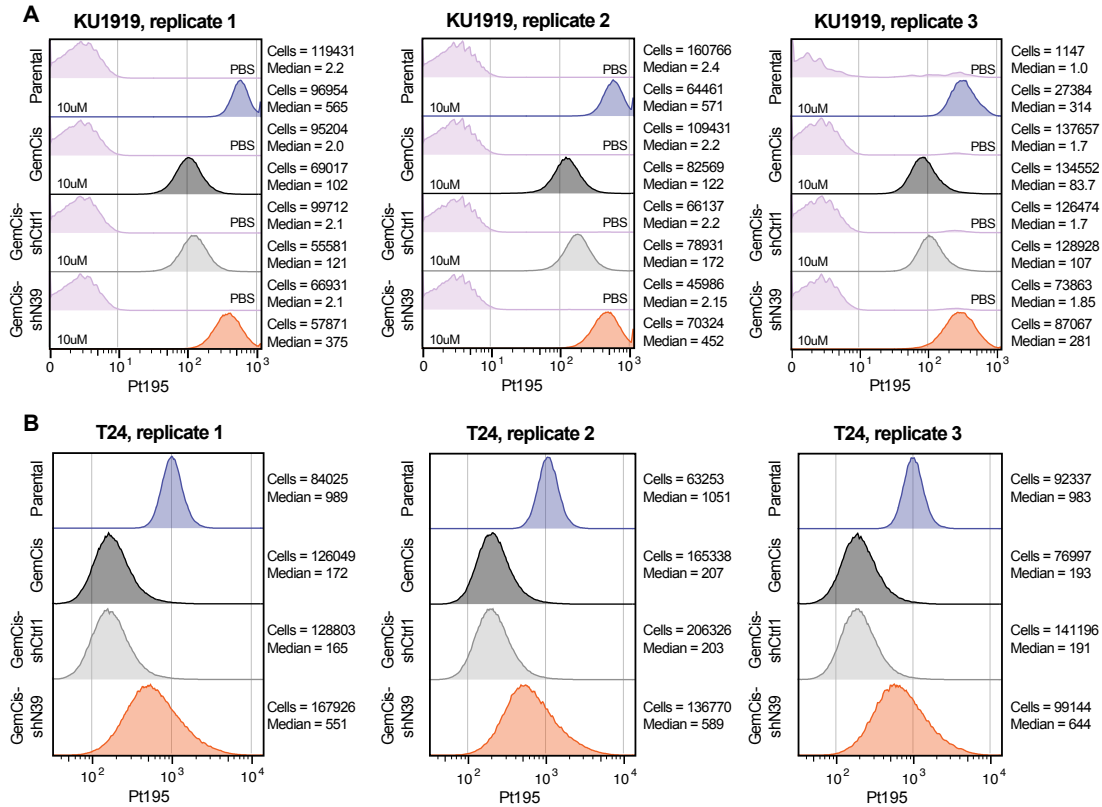




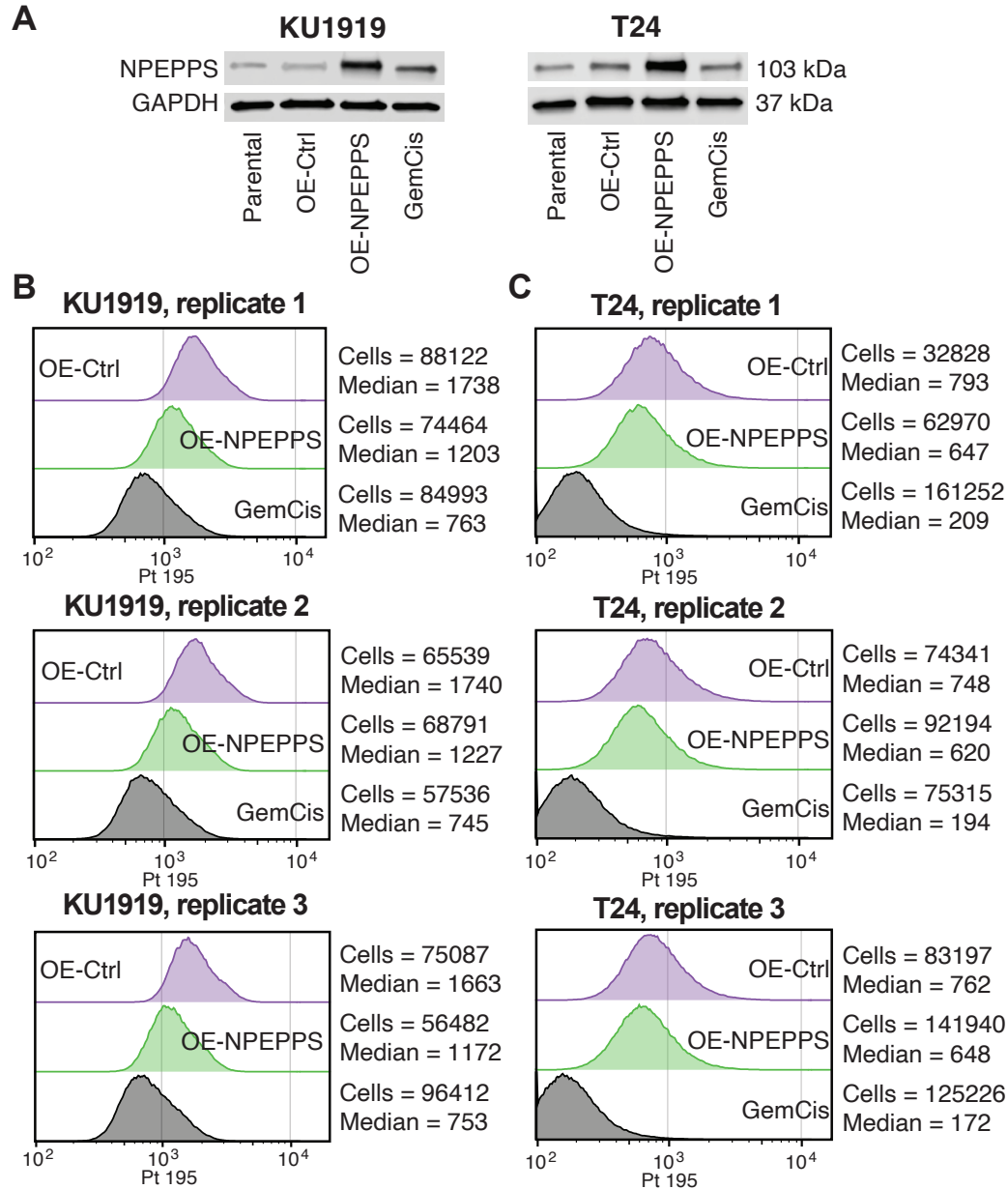
**Figure S3. Summary of synthetic lethal genes.** (A) Venn diagram of all gene counts across all 5 synthetic lethal screen results. The number of genes that were statistically significantly synthetic lethal (FDR < 0.05) are reported. (B) The putative annotations for the 46 commonly synthetic lethal genes. Detailed annotation information can be found in Supplementary Table S14.



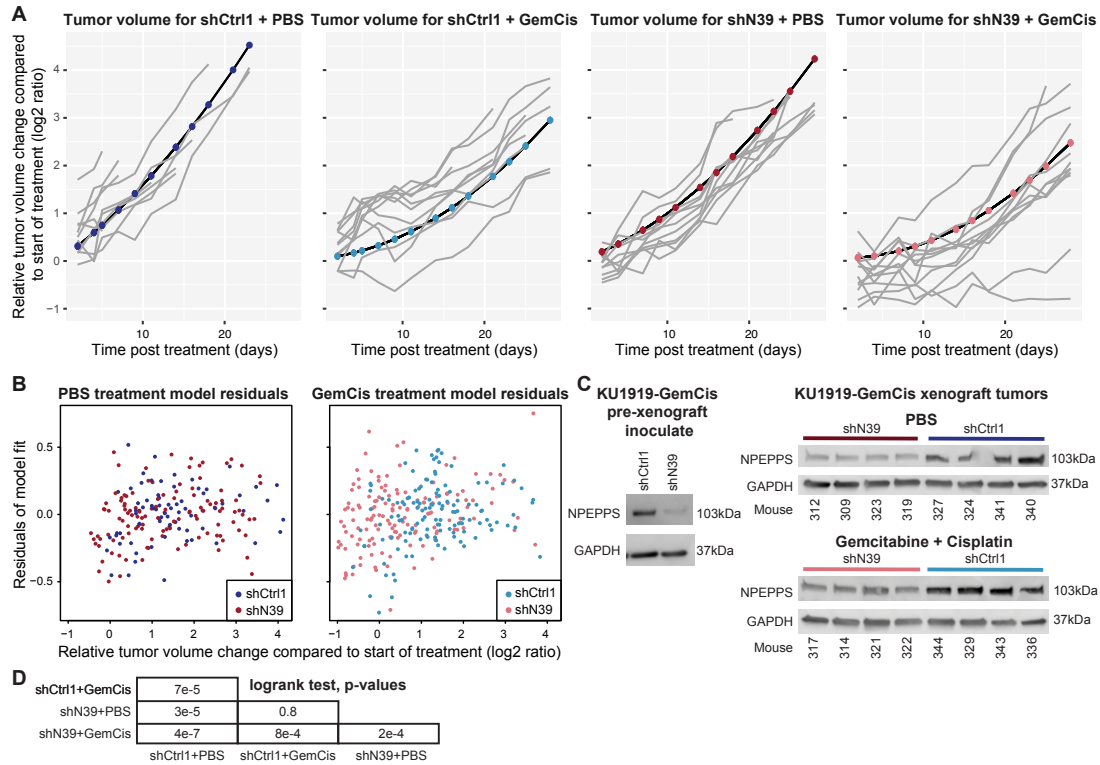
**Figure S4. shRNA and siRNA targeting NPEPPS resensitizes to cisplatin and gemcitabine plus cisplatin.** (A) Growth of GemCis-resistant cells with control knockdown (shCtrl1) or knockdown of NPEPPS (shN39) were measured over 120 hours in control (PBS) treatment conditions. Growth rates were calculated as the slope of the curve between 20%-80% confluency. (B) Dose response for shRNA targeting NPEPPS (shN38 = TRCN0000073838; shN39 = TRCN0000073839; shN40 = TRCN0000073840) or non-targeting controls (shCtrl1 = MISSION pLKO.1-puro Non-Mammalian shRNA Control; shCtrl2 = MISSION pLKO.1-puro Non-Target shRNA Control). Cells were treated with cisplatin or gemcitabine separately. Data for KU1919-GemCis shown from two independent experiments with 3 technical replicates per dose (mean  $\pm$  SEM). Data from 253J-GemCis represent a single experiment with 3 technical replicate wells per dose (mean  $\pm$  SEM). Immunoblot analysis of NPEPPS protein across the different shRNAs and cell lines are inset in the cisplatin treatment graph. (C) Cell confluency was measured using Incucyte Zoom across untransfected cells, siRNA controls (siCtrl), and siRNA transfected cells targeting NPEPPS. Cells were treated with PBS or gemcitabine plus cisplatin (GemCis) at the resistant doses for each cell line (Supplementary Table S1). Data shown represent a single experiment with 3 technical replicates (mean  $\pm$  SEM) per timepoint represented. (D) Cisplatin-resistant KU1919 cells were CRISPR edited using a gRNA from the Brunello library, GCAAAGGCTGTAGTTGATGG. Overexpression of NPEPPS, along with the empty vector control (OE-Ctrl), was performed in the NPEPPS knockout cells. Immunoblot for NPEPPS protein expression is shown.



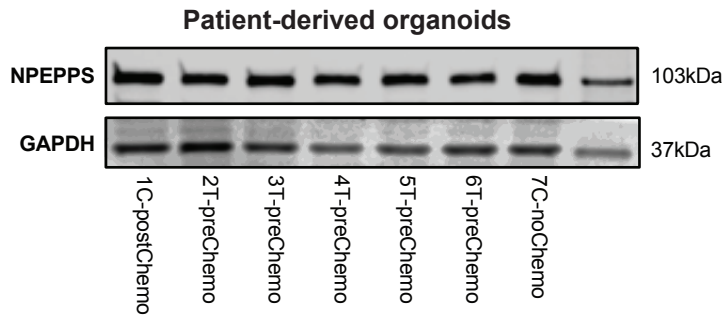
**Figure S5. Intracellular cisplatin measured using CyTOF. (A)** KU1919-Parental, KU1919-GemCis, KU1919-GemCis-shCtrl1, and KU1919-GemCis-shN39 cells were treated with PBS vehicle ( $0\mu\text{M}$ ) or  $10\mu\text{M}$  cisplatin for 4 hours and then intracellular cisplatin was measured using cytometry by time of flight (CyTOF). Results from replicates are shown. **(B)** The same experiment with  $10\mu\text{M}$  cisplatin treated for 4 hours was performed with T24 cell lines and results from replicates are shown.



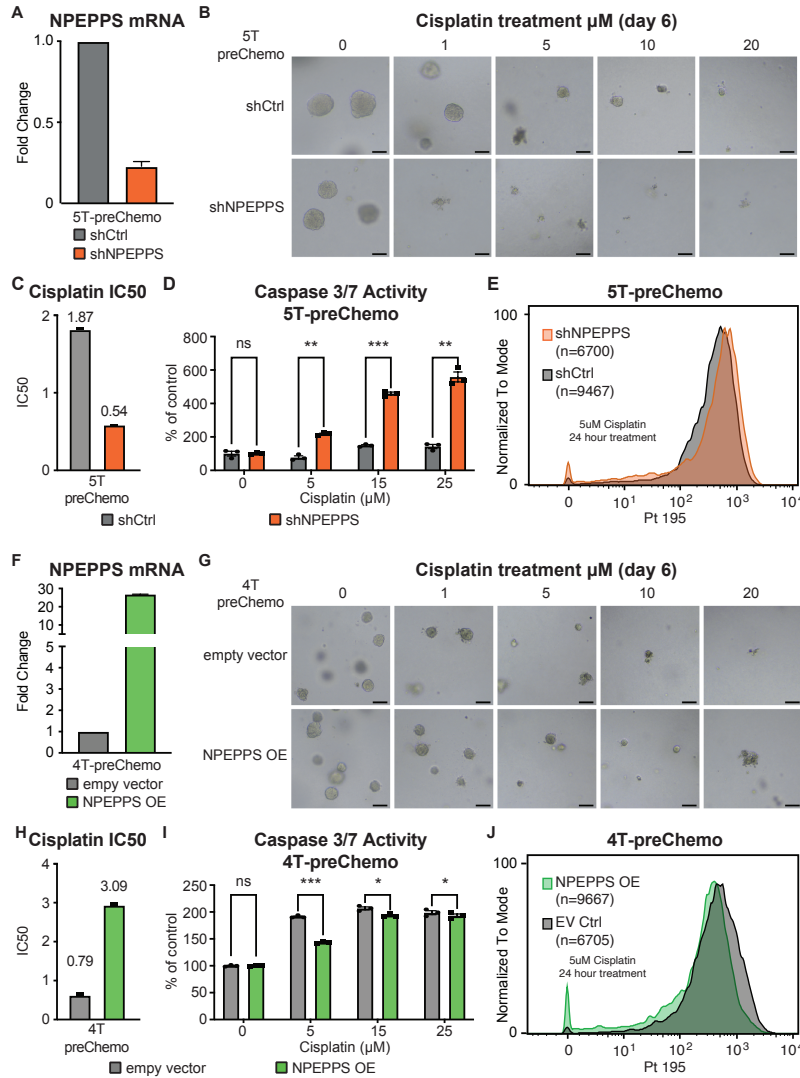
**Figure S6. Intracellular cisplatin in response to overexpression of NPEPPS. (A)** Immunoblot of NPEPPS to confirm the overexpression of NPEPPS. CyTOF on **(B)** KU1919 and **(C)** T24 parental, overexpression control (OE-Ctrl), NPEPPS overexpression (OE-NPEPPS), or GemCis cells was used to measure intracellular cisplatin. The number of cells measured and the median value for each group are reported.



**Figure S7. KU1919-GemCis xenograft tumor growth modeling and validation. (A)** Fixed effects population-level model fit (thick lines) overlaid on top of the observations (grey lines). Longitudinal tumor volumes were divided by the baseline tumor volume and then  $\log_2$ -transformed before modelling. **(B)** Residuals for the final mixed-effects model (y-axis) coupled with the original observations (x-axis). No systematic trends were detected in model diagnostics, suggesting that the single fitted model successfully captured variation over the treatment arms and individuals. **(C)** Immunoblot on the left is from KU1919-GemCis cells that were injected into mice to establish tumors. Immunoblot on the right are from tumor samples after mice met the endpoint of the experiment of  $> 2\text{cm}^3$ . **(D)** Logrank test comparing all pairwise groups from **Fig. 3G**.

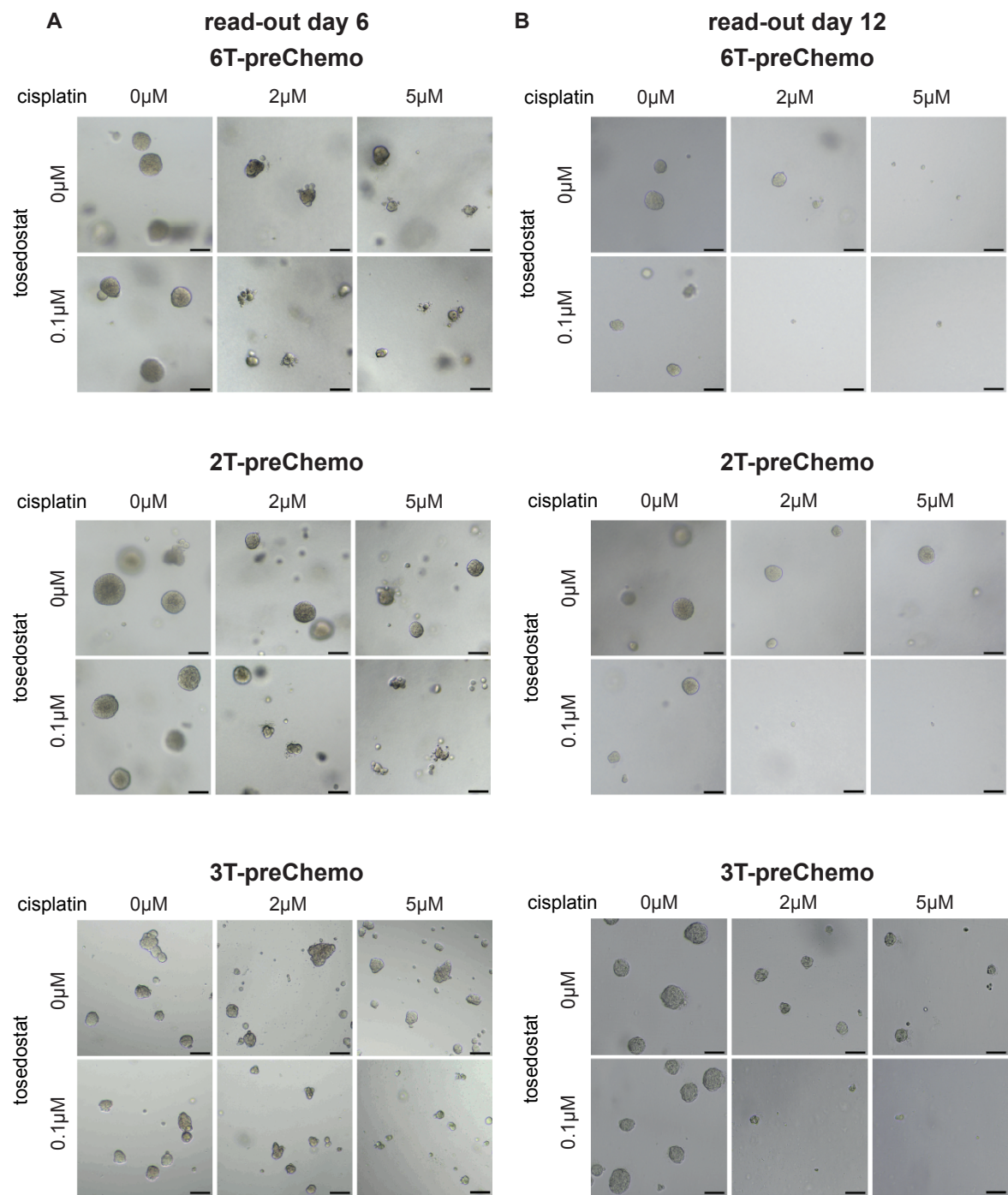


**Figure S8. Immunoblot analysis of NPEPPS in the seven tumor-derived organoids.** Information on individual organoids are reported in **Fig. 4A-C**.



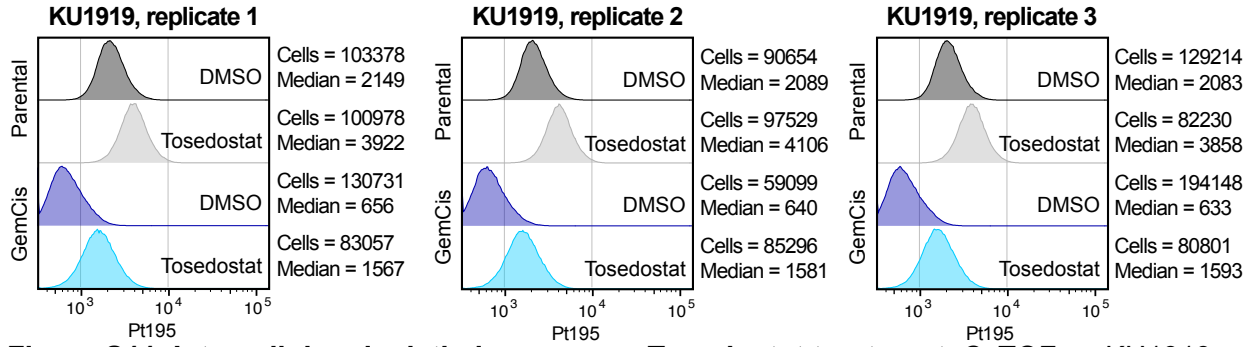
**Figure S9. Ex vivo NPEPPS knockdown and overexpression regulates cisplatin resistance.** (A) NPEPPS expression was evaluated by RT-PCR in shNPEPPS and shCtrl PDO lines normalized to cyclophilin. Error bars represent mean  $\pm$  SD. (B) Representative bright-field images of control and NPEPPS-depleted 5T-preChemo organoid lines. (C) IC50 values estimated from dose curves for cell viability measured through CellTiter-Glo (biological triplicates; mean  $\pm$  SEM). (D) Relative caspase-3 and -7 activity in cisplatin-treated shCtrl and shNPEPPS. Caspase activity was measured by Caspase-Glo and normalized to untreated PDOs. (biological triplicates; mean  $\pm$  SEM). (E) Intracellular cisplatin levels were measured after 24 hours of 5 $\mu$ M cisplatin treatment using CyTOF, with the number of live cells analyzed as indicated. (F) NPEPPS expression was evaluated by RT-PCR in empty vector and NPEPPS overexpression normalized to cyclophilin in the 4T-preChemo organoid lines. Error bars represent mean  $\pm$  SD. (G) Representative bright-field images of empty vector control and NPEPPS overexpression. Scale bar = 400 $\mu$ m. (H) IC50 values estimated from dose curves for cell viability measured through CellTiter-Glo (biological triplicates; mean  $\pm$  SEM). (I) Relative caspase-3 and -7 activity in cisplatin-treated empty vector control and NPEPPS overexpression. Caspase activity was measured by Caspase-Glo and normalized to untreated PDOs. (biological triplicates; mean  $\pm$  SEM). (J) Intracellular cisplatin levels were measured after 24 hours of 5 $\mu$ M cisplatin treatment using CyTOF, with the number of live cells analyzed as indicated.





**Figure S10.** (A) Representative bright-field images of pre-POC organoids treated for six days at the indicated concentrations of drug. (B) Organoids originally treated at indicated concentrations of drug were dissociated to single cell and reseeded, allowed to grow new organoids for six days. Images were taken six days after reseeded, allowing organoids to re-grow. Scale bar = 200 $\mu$ m.





**Figure S11. Intracellular cisplatin in response Tosedostat treatment.** CyTOF on KU1919 parental and GemCis cells treated with DMSO or 1  $\mu$ M Tosedostat. Cells were pre-treated for 72 hours in drug or vehicle, then treated with 10  $\mu$ M Cisplatin for 4 hours. The number of cells measured and the median value for each group are reported.

## SUPPLEMENTAL TABLES

**Supplemental Table 1.** Established gemcitabine and cisplatin concentrations used for resistance cell line maintenance as reported by the RCCL.  
(<https://research.kent.ac.uk/industrial-biotechnology-centre/the-resistant-cancer-cell-line-rccl-collection/>)

**Supplemental Table 2.** Differential expression for RNAseq results for all derivatives compared to parental lines and for the KU1919, T24, TCCSUP, 253J, 5637, and all cell lines combined.

**Supplemental Table 3.** Alignment statistics from whole exome sequencing.

**Supplemental Table 4.** Comparison of mutation profiles reported through the CCLE.

**Supplemental Table 5.** Quality control measures for the CRISPR screening samples.

**Supplemental Table 6.** CRISPR screening parameters used for each cell line.

**Supplemental Table 7.** Broad Institute GPP LentiCRIPRv2 Library Prep Primers used in this study (P7 Index primer sequences A01-E09; P5 Staggered Pool).

**Supplemental Table 8.** Raw CRISPR screen counts.

**Supplemental Table 9.** Synthetic lethality as determined by gemcitabine plus cisplatin treated GemCis-resistant cells compared to saline treated cells with samples from day 19 and day 25 treated as covariates.

**Supplemental Table 10.** Gene essentiality as determined by Day 25 saline treated samples compared to Day 0 samples in the GemCis-resistant cell lines.

**Supplemental Table 11.** Mutations filtered for quality and location in exonic gene regions.

**Supplemental Table 12.** Copy number alterations with the reported mean log<sub>2</sub> ratio of the associated segment and the call of amplification (+), loss (-), or diploid (0).

**Supplemental Table 13.** Gene Set Enrichment (fgSEA) results for synthetic lethal screen.

**Supplemental Table 14.** Gene annotations and references for top 46 hits from synthetic lethal screen.

**Supplemental Table 15.** Differential expression from mass spec proteomics results for all derivatives compared to parental lines and for the KU1919, T24, TCCSUP, 253J, 5637, and all cell lines combined.

**Supplemental Table 16.** Summary of patient-derived bladder tumor organoid lines and corresponding clinical data