Supplemental Figure legends

Figure S1. Schematic of the PDO generation and analysis pipeline.

Figure S2. A. CNA by chromosome using whole exome (top panel) and whole genome sequencing (bottom panel, hT117). **B.** Indels per megabase (MB) in the human organoids. Line represents the mean and error bars the standard deviation. hF43 is highlighted in orange. **C**. Genomic alterations evaluated by whole genome sequencing following germline correction of the PDO library. **D.** CNA by gene evaluated by whole genome sequencing following germline correction.

Figure S3. Circos plots from germline corrected WGS of matched primary and hT organoid specimens as well as biopsy derived hF PDO cultures. TvN: Primary tumor specimen following germline correction to normal tissue. OvN: PDO following germline correction to normal tissue.

Figure S4. A. NMF cluster ranking of clustering of the 2,000 most variably expressed genes. **B.** NMF cluster performance.

Figure S5. Chemotherapy dose response curves of representative PDO cultures evaluated at different passage numbers.

Figure S6. A. Progression free survival of advanced patients following diagnosis and isolation of a PDO. Historical median progression free survival is indicated by a dotted line (180 days). The chemotherapeutic regimen received by the patient is indicated in parenthesis and color coded for PDO pharmacotyping AUC results: blue denotes a sensitive drug, grey denotes an intermediate drug and red denotes a resistant drug. **B.** The response of hF2 to chemotherapy among the distribution of all PDO AUC responses. **C**. Patient MR scans at the time of organoid isolation and following two different regiments of chemotherapy. The chemotherapeutic regimen received by the patient is indicated and color coded for PDO cohort normalized AUC results: blue is top 33% sensitive organoids, red is the top 34% of resistant organoids.

Figure S7. A. Normalized AUC values from targeted therapy pharmacotyping. **B.** Mutation and CNA in the homologous repair deficiency pathway following germline correction of PDO organoid whole genome sequencing data.

Figure S8. Schematic representation of PDO-pharmacotranscriptomic signature workflow on a per drug basis.

Figure S9. The Spearman's correlation value for each gene within one drug-specific signature are shown as violin plots for Gemcitabine (**A**), Paclitaxel (**B**), SN-38 (**C**), 5-FU (**D**) and Oxaliplatin (**E**).

Figure S10. A. The Spearman's correlation between gene expression and individual chemotherapy response signatures for Gemcitabine, Paclitaxel, SN-38, 5-FU, and Oxaliplatin. Only genes whose expression increased with decreased AUC are shown (negative rho). Low AUC is indicative of increased sensitivity. **B.** Clustering of PDO cultures using the individual Paclitaxel, SN-38, 5-FU, and Oxaliplatin chemotherapy sensitivity signatures with the associated PDO pharmacotyping results (log_2 transformed z-scores).

Figure S11. A. The Gemcitabine sensitivity signature was applied to RNA-seq data from patients receiving Gemcitabine as a single agent or in combination with other chemotherapy. Additional data regarding the C1/C2 subtype, Basal/Classical subtype, and stage are shown. **B.** The OS of patients receiving only gemcitabine as a single agent and identified by the Gemcitabine sensitivity signature. **C-D**. The PFS (**C**) and OS (**D**) of patients identified as Gemcitabine Sensitive or Non-Sensitive as described in **A**. **E.** The Gemcitabine sensitivity signature was applied to RNA-seq data from untreated patients. Additional data regarding the C1/C2 subtype, Basal/Classical subtype, and stage are shown. **F**. The OS of patients identified as described in **E**. Log-rank (Mantel-Cox) test P values are shown.

Figure S12. The PDO-derived Gemcitabine (**A**), Paclitaxel (**B**), 5-FU (**C**) and SN-38 (**D**) signatures were used to cluster the COMPASS RNA-seq data (n=73).

Figure S13. Waterfall plot of the percent tumor response in the target lesion at 8 weeks for the patients receiving Gemcitabine and nab-Paclitaxel. Sensitivity to Gemcitabine and Paclitaxel is indicated. Additional data regarding the mean chemotherapeutic signature scores, C1/C2 subtype, and Basal/Classical subtype are shown.