Supplementary Figure S1

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┌──────────────────────────────┐          ┌────────────────────────────┐
│ Insufficient tissue or poor  │          │ 79 patients undergo biopsy │
│ RNA quantity/quality (n=16)  │          │    on PancSeq Protocol     │
│ • Insufficient tumor by H&E  │          └────────────────────────────┘
│   of biopsy (n=6)            │                                          ┌──────────────────────┐
│ • Insufficient tissue for RNA│◄──────┐              ┌──────────────────►│ Insufficient tumor by│
│   extraction (n=7)           │       │              │                   │ H&E of biopsy (n = 6)│
│ • Insufficient RNA after     │       │              │                   └──────────────────────┘
│   extraction (n=3)           │       ▼              ▼
└──────────────────────────────┘  ┌─────────────┐ ┌─────────────┐
                                  │ RNA-seq data│ │    WES      │
                                  │  (n = 63)   │ │  (n = 73)   │
                                  └─────────────┘ └─────────────┘
┌──────────────────────┐                                          ┌──────────────────────┐
│ Insufficient ABSOLUTE│◄──────┐              ┌──────────────────►│ Insufficient ABSOLUTE│
│   purity by WES      │       │              │                   │  purity for mutation │
│     (n = 3)          │       ▼              ▼                   │    calling (n = 2)   │
└──────────────────────┘  ┌─────────────┐ ┌──────────────────┐   └──────────────────────┘
                          │RNA-seq data │ │ WES Analysis of  │
                          │  analysis   │ │ mutations and CNAs│
                          │  (n = 60)   │ │    (n = 71)      │
                          └─────────────┘ └──────────────────┘
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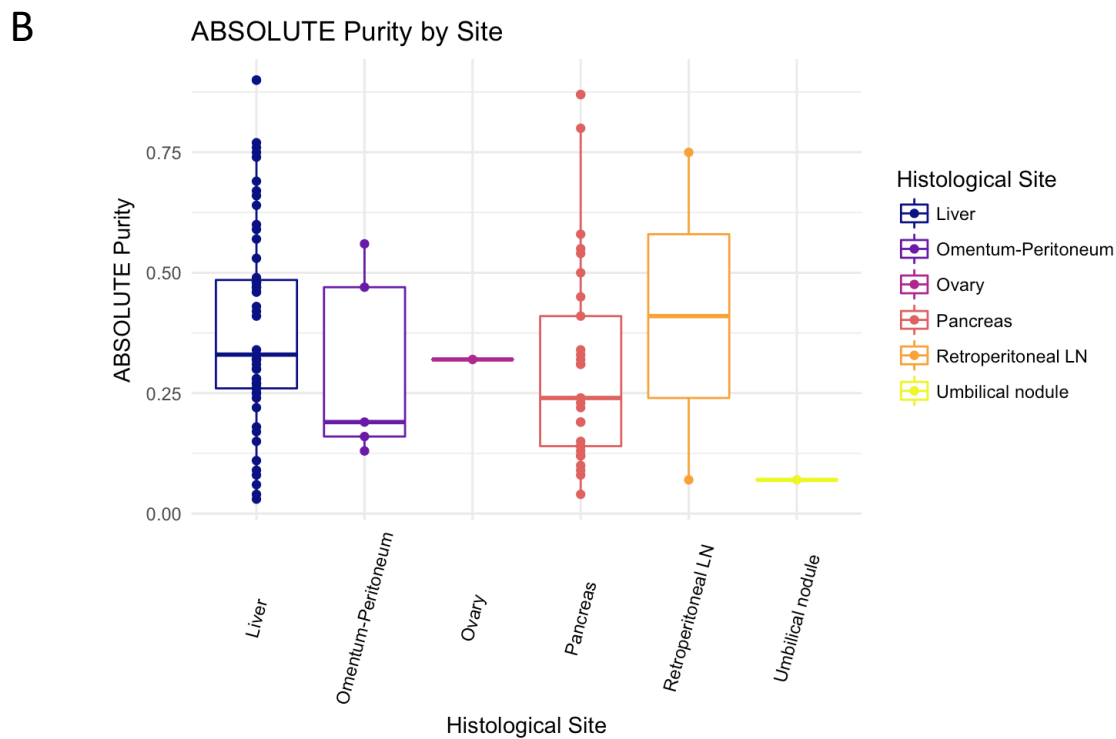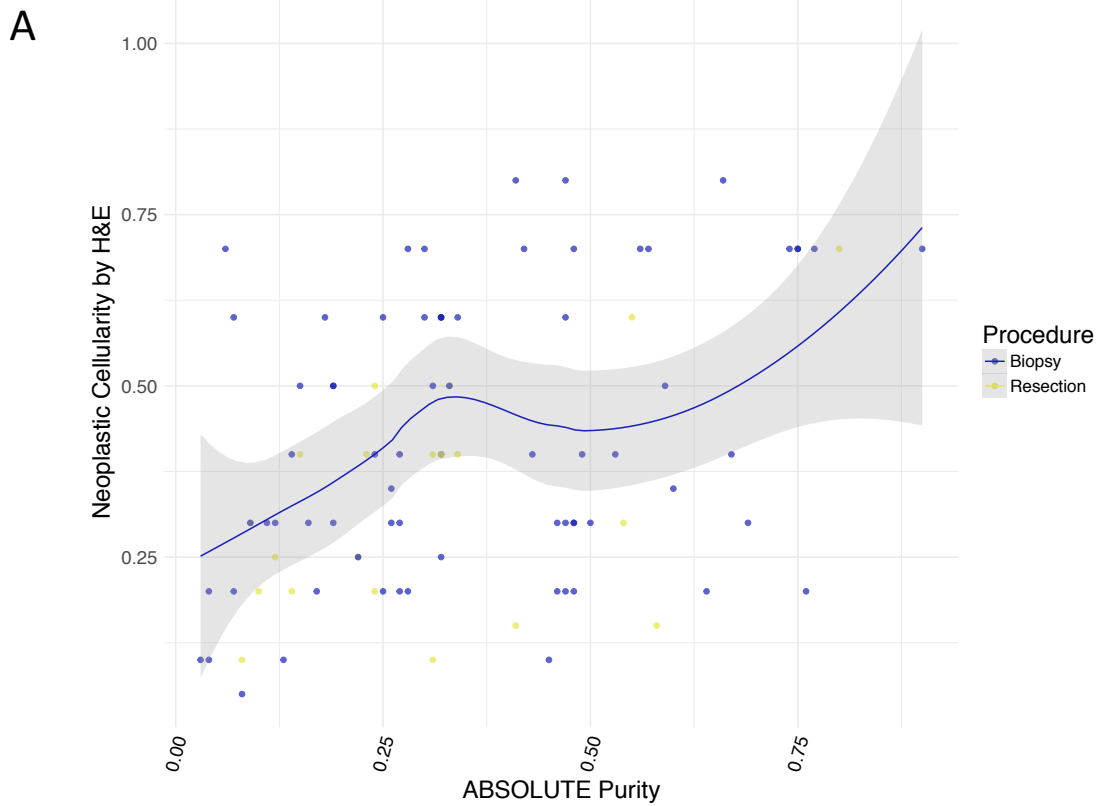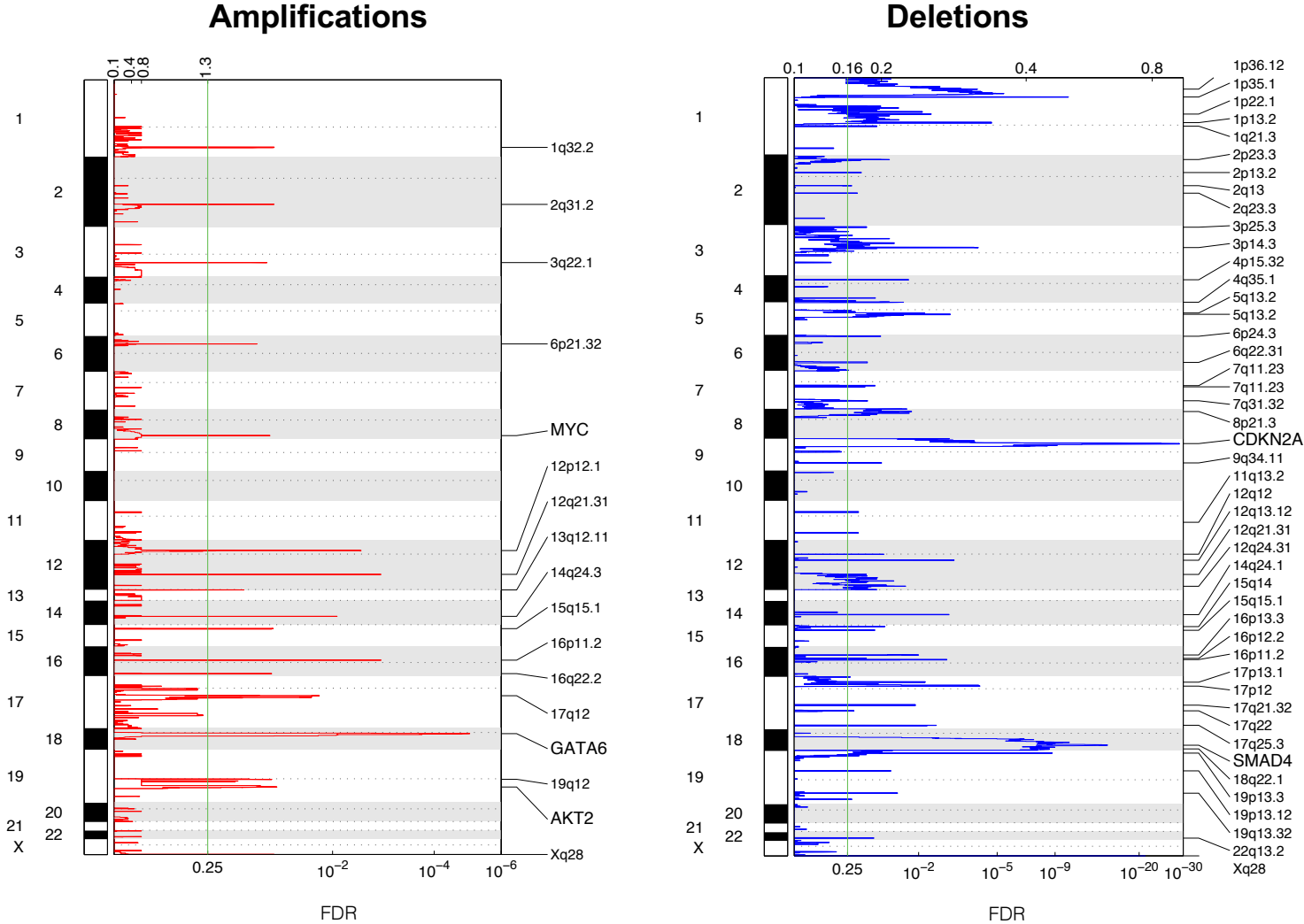
Supplementary Figure S1: Overview of workflow and data generation for patients enrolled on the PancSeq protocol.
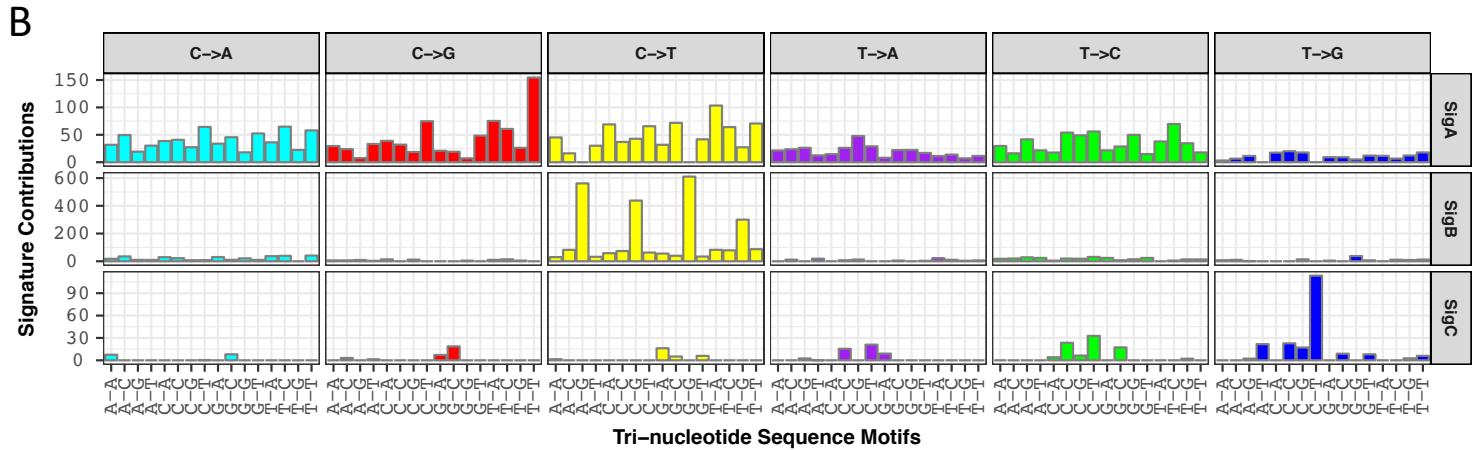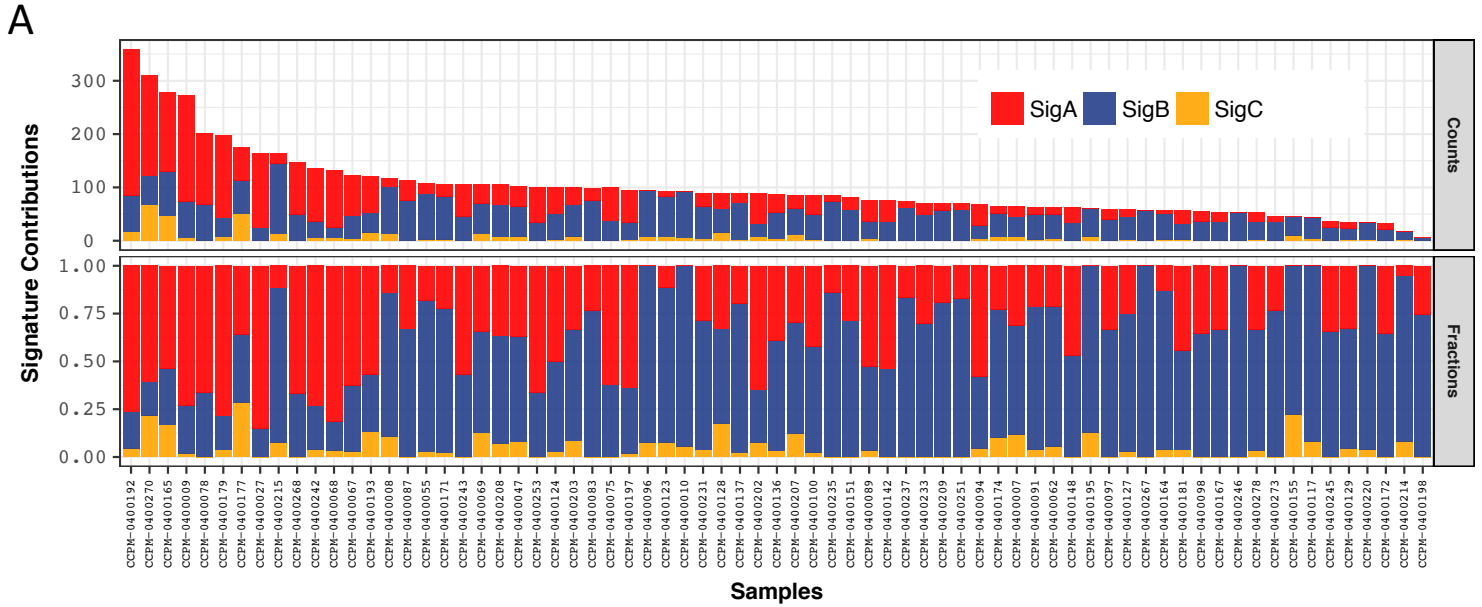
Supplementary Figure S2

A

B

Supplementary Figure S2: Analysis of neoplastic cellularity within the biopsy cohort of advanced PDAC patients. A) Correlation of neoplastic cellularity estimates by histology (H&E stain, Y-axis) and ABSOLUTE purity by WES analysis (X-axis). Overall, the Pearson correlation was 0.386, 95% CI (0.195 - 0.548); P = 0.00016. For metastatic biopsies, the correlation was 0.367, 95% CI (0.133 - 0.562), P = 0.0029. For primary tumor resections, the correlation was 0.308, 95% CI(-0.081, 0.616), P = 0.118. B) ABSOLUTE purity by biopsy site. Pancreatic primary tumors (0.30) showed a non-significant trend toward lower ABSOLUTE purity than liver metastatic lesions (0.39), Welch Two Sample t-test, p = 0.07.
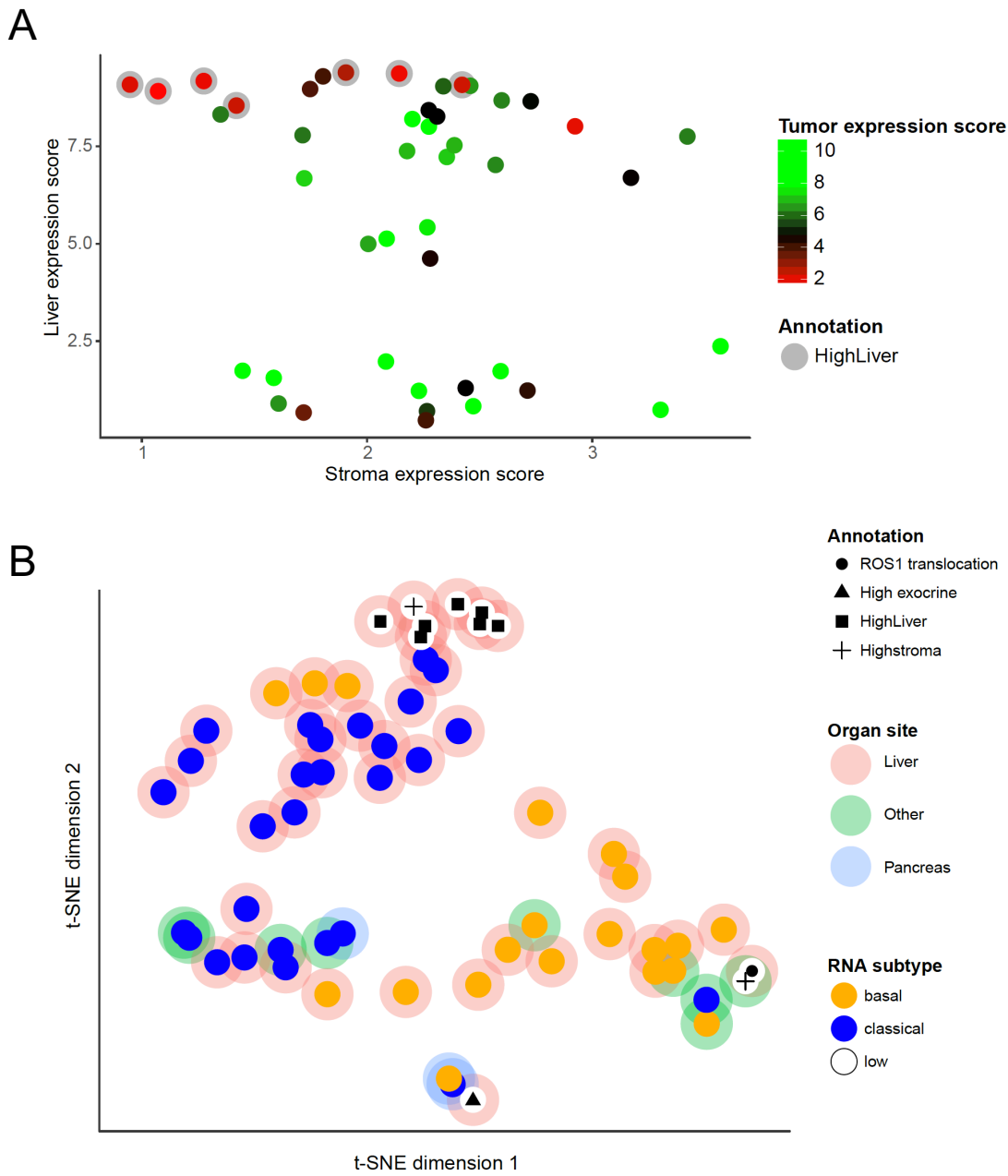
Supplementary Figure S3

Supplementary Figure S3: GISTIC 2.0 analysis of recurrent copy number alterations (CNAs). Chromosome position is shown on the Y-axis and statistical significance (q-value) is shown on the X-axis. Recurrent amplifications are depicted in red (left) and deletions in blue (right).

Supplementary Figure S4

Supplementary Figure S4: De novo mutational signature analysis from whole exome sequencing data for advanced PDAC patients. A) Signature activities are shown for all 71 samples in the cohort for three primary signatures (Sig A, B, C), representing 4 main mutational processes: C>T transitions at CpG dinucleotides, Aging (COSMIC1), APOBEC (COSMIC2 and 13), homologous recombination deficiency (HRD) or BRCA deficiency (COSMIC3), and a signature of unknown etiology (COSMIC17) B) Lego plot showing identification of three signatures.
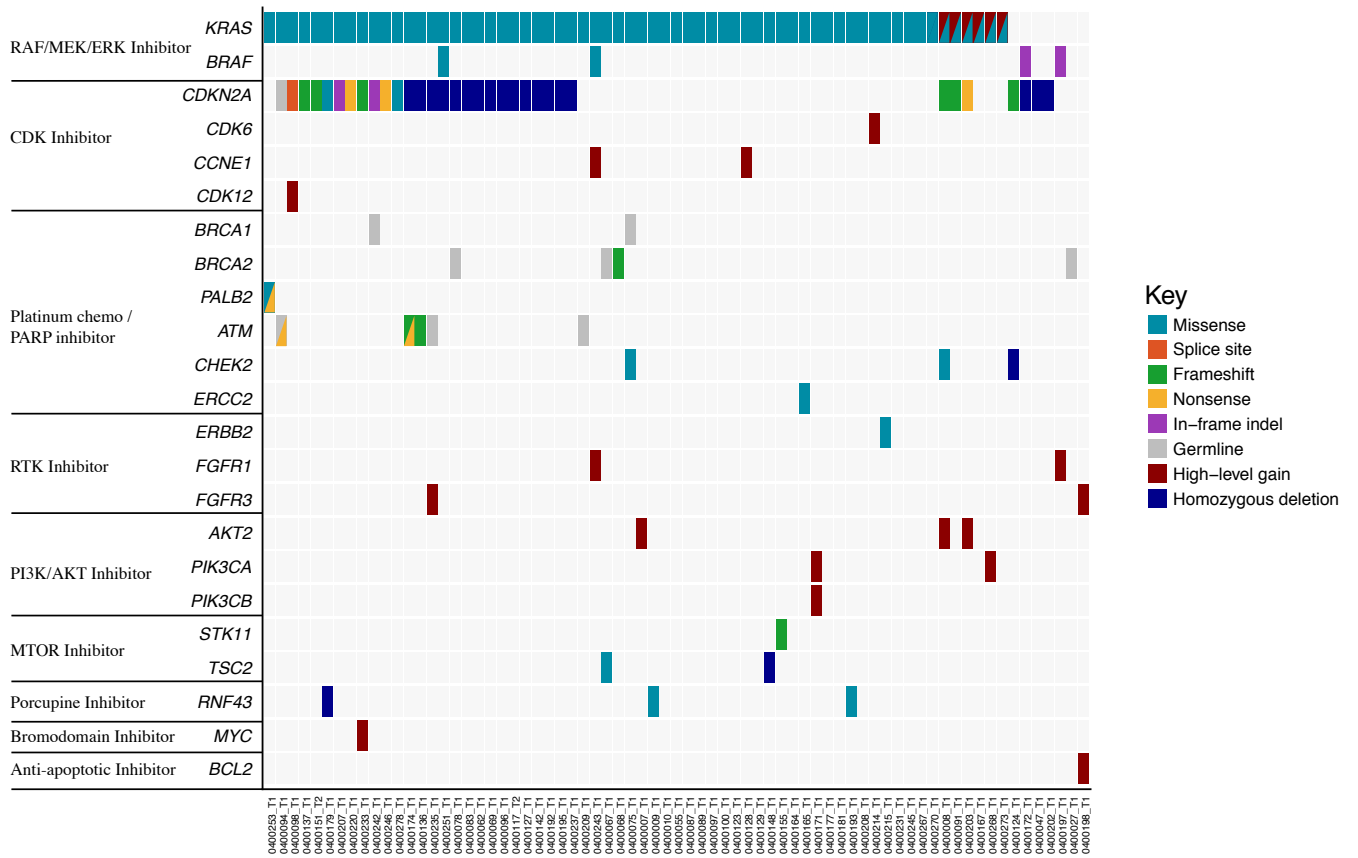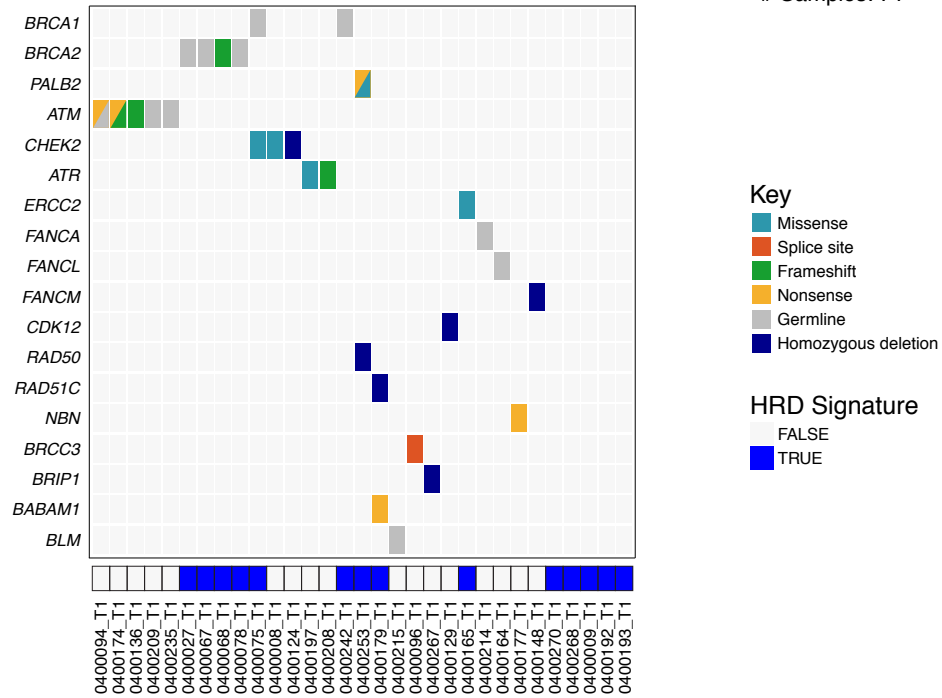
Supplementary Figure S5

Supplementary Figure S5: Analysis of PDAC gene expression signatures in the biopsy cohort. A) Liver gene expression scores (Y-axis) and composite stromal gene expression scores (X-axis) are displayed for each liver metastasis. Color indicates composite tumor expression score, with the lowest expressing samples from Figure 3A highlighted in grey. B) t-SNE plot showing clustering of samples based on gene expression with samples labeled by expression subtype, anatomic location and additional annotations highlighting atypical features of select samples.

Supplementary Figure S6

Supplementary Figure S6: Clinically relevant alterations in the cohort. (A) Clinically relevant alterations with potential therapeutic implications are shown, with genes grouped according to therapy class shown on the left. (B) Alterations in DNA damage repair genes. The presence of an HRD signature ("true" = blue) from whole exome sequencing data is shown in the track at the bottom, defined as a sample having an HRD/BRCA signature score greater than or equal to the *BRCA* or *PALB2* mutant sample with the lowest HRD/BRCA signature score.