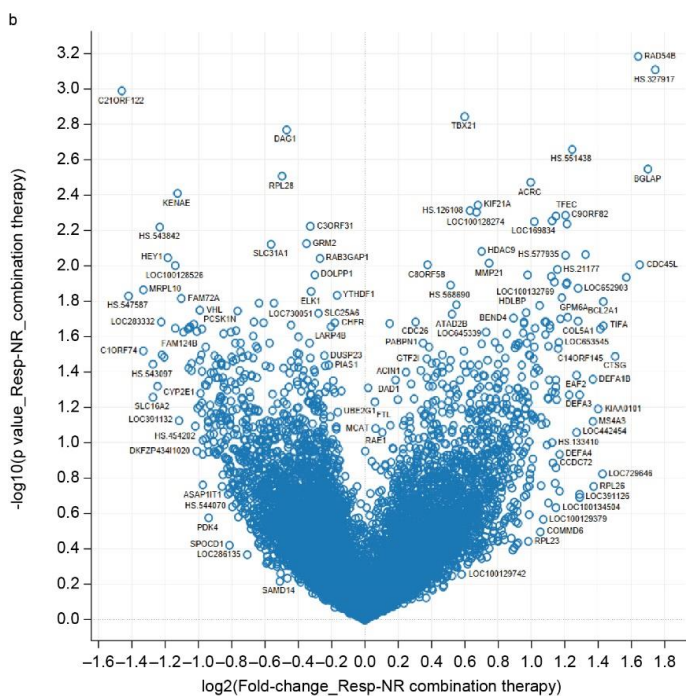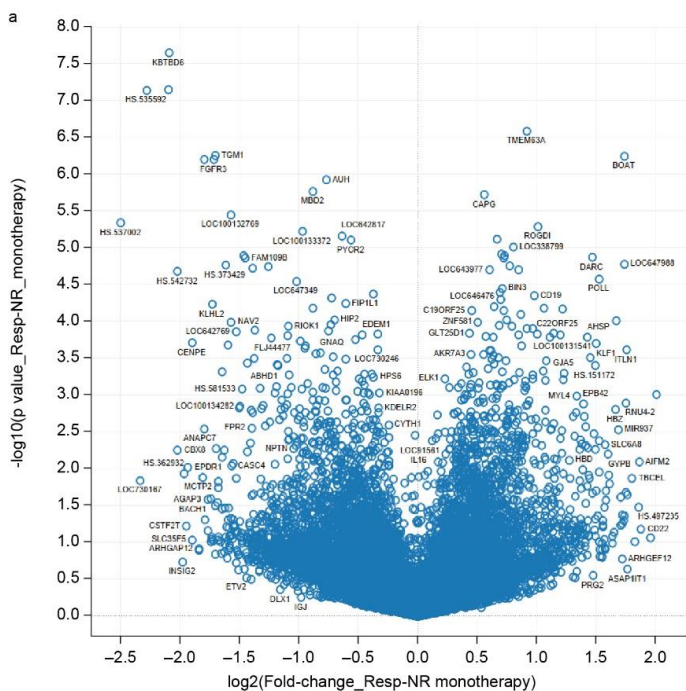**Overman et al.** **A randomized phase 2 study of the Bruton tyrosine kinase inhibitor**

**acalabrutinib, alone or with pembrolizumab in patients with advanced pancreatic cancer**

**Additional material**

Additional file 1: pdf file.

1

a



b

**Figure S1.** Gene expression profiles in peripheral blood of patients with response or stable

disease and in patients with progressive disease.

(a) Volcano plot comparing subtracted PB GEP data for three patients with response or stable

disease vs 15 patients with progressive disease treated with acalabrutinib monotherapy and (b)

seven patients with response or stable disease vs 13 patients with progressive disease treated

with combination therapy.

*PB* peripheral blood, *GEP* gene expression profiling, *Resp-NR* responders/stable disease vs non-responders (progressive disease)
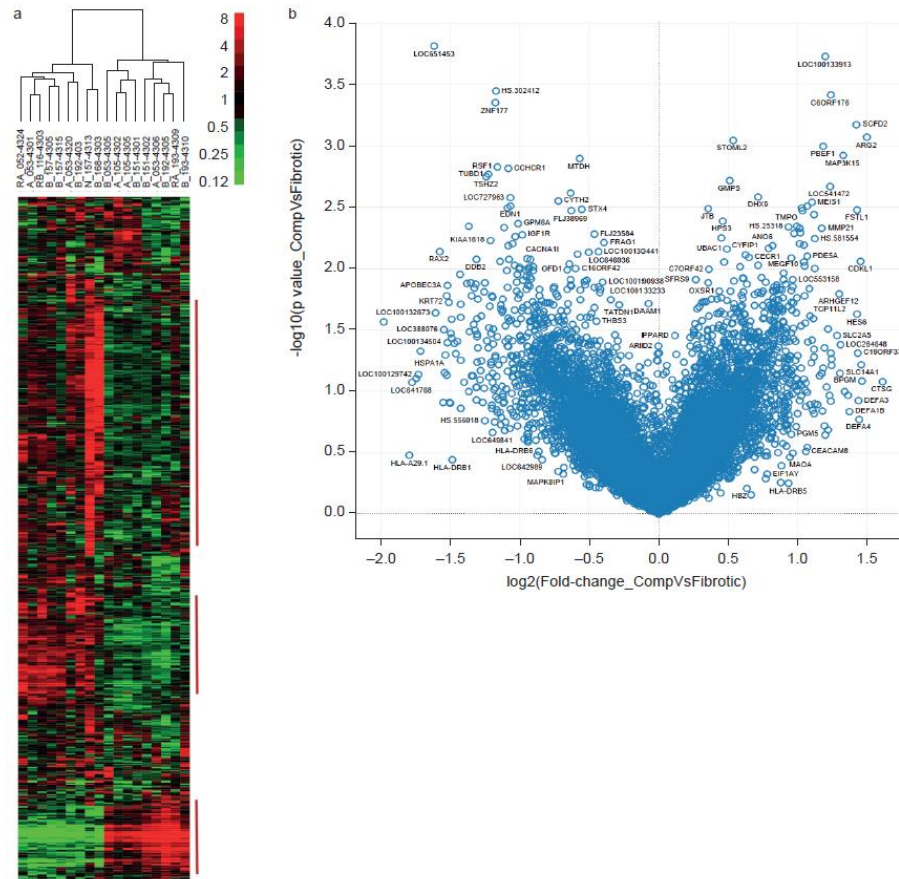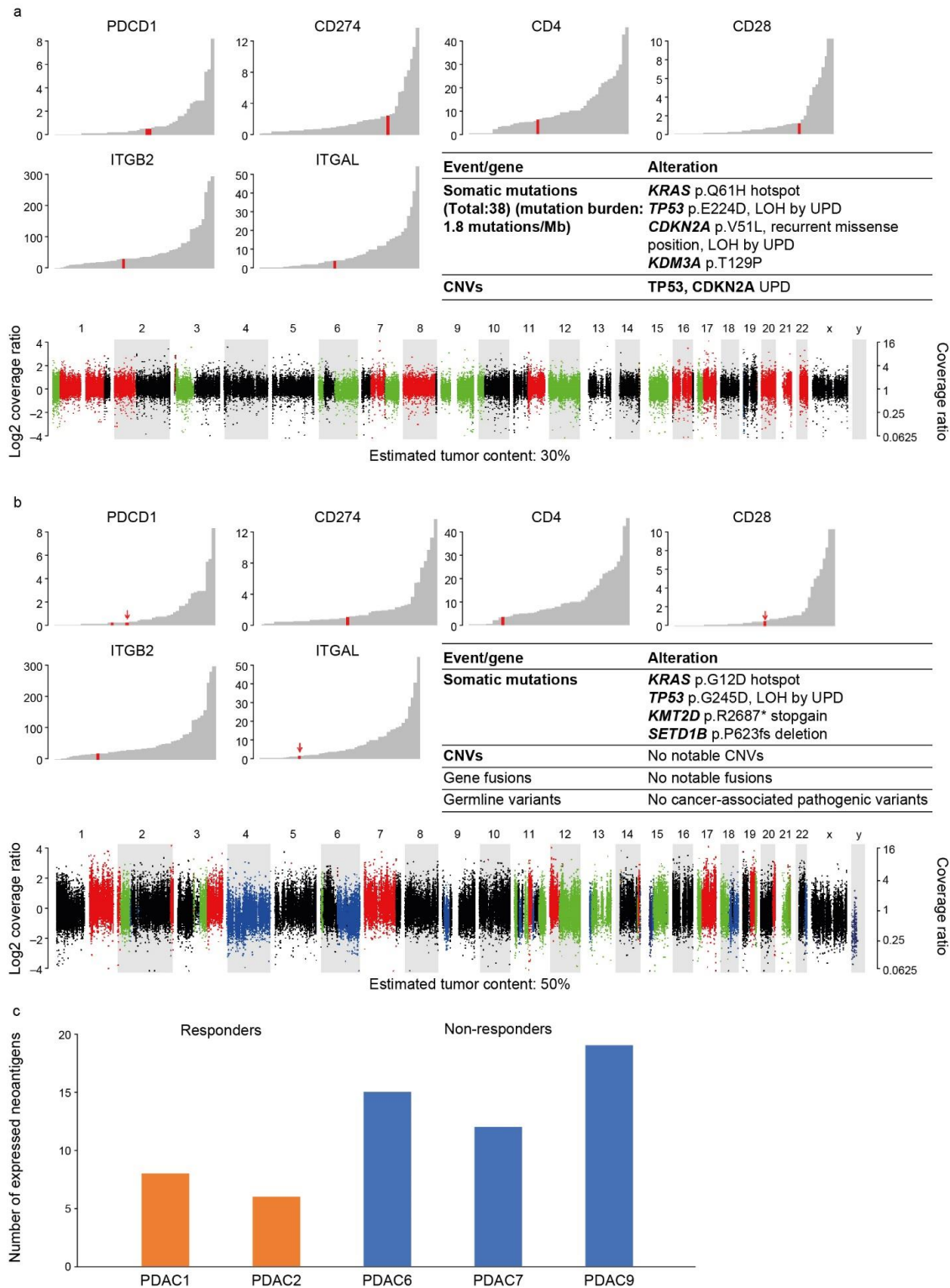
**Figure S2.** Gene expression profiles in tumor biopsies.

(a) Heat map of GEP data from pre-treatment metastatic tumor biopsies from 18 patients,

measured for an immune-focused panel of 1458 genes with a NanoString nCounter. Log2

expression values are shown for 694 genes that displayed a standard deviation of at least 1,

after median-centering and coloring by fold-difference according to the color bar shown. Genes

(rows) and samples (columns) were hierarchically clustered for similarity. "R" precedes sample

labels for patients showing clinical responses; "A" indicates treatment with acalabrutinib

monotherapy; "B" indicates treatment with combination therapy; "N" indicates no treatment.

(b) Volcano plot comparing pre-treatment PB GEP data for nine patients with complement-type

tumors vs eight patients with fibrotic-type tumors.

*PB* peripheral blood, *GEP* gene expression profiling

a



| Event/gene | Alteration |
|---|---|
| **Somatic mutations (Total:38) (mutation burden: 1.8 mutations/Mb)** | ***KRAS*** p.Q61H hotspot<br>***TP53*** p.E224D, LOH by UPD<br>***CDKN2A*** p.V51L, recurrent missense position, LOH by UPD<br>***KDM3A*** p.T129P |
| **CNVs** | **TP53, CDKN2A** UPD |



Estimated tumor content: 30%

b



| Event/gene | Alteration |
|---|---|
| **Somatic mutations** | ***KRAS*** p.G12D hotspot<br>***TP53*** p.G245D, LOH by UPD<br>***KMT2D*** p.R2687* stopgain<br>***SETD1B*** p.P623fs deletion |
| **CNVs** | No notable CNVs |
| **Gene fusions** | No notable fusions |
| **Germline variants** | No cancer-associated pathogenic variants |



Estimated tumor content: 50%

c

**Figure S3.** Summary of the integrative molecular analysis of two responder patient samples (in

a and b). RNA expression of select immune-related marker genes in the "responder" samples

(in red) is shown relative to the cohort of pancreatic cancer samples sequenced under the

Michigan Oncology Sequencing Program (in gray); y-axis shows normalized expression values as

FPKM. Inset includes summary of salient mutations, and the panel below represents copy

number profile of the tumor samples.

 (c) Predicted number of HLA class I strong binding neoantigens in the two responders (in

orange) and three patients with no response or progressive disease (in blue).

*CNV* copy number variation, *FPKM* fragments per kilobase of transcript per million mapped

reads, *HLA* human leukocyte antigen, *LOH* loss of heterozygosity, *UPD* uniparental disomy.