Supplementary Information

Multiplex profiling of serum proteins in solution using barcoded antibody fragments and next generation sequencing

Mattias Brofelth, Anna Isinger Ekstrand, Shashank Gour, Ronnie Jansson, My Hedhammar, Björn Elleby, Anders Kvist, Christer Wingren, Ulrika Axelsson, Carl A.K. Borrebaeck*

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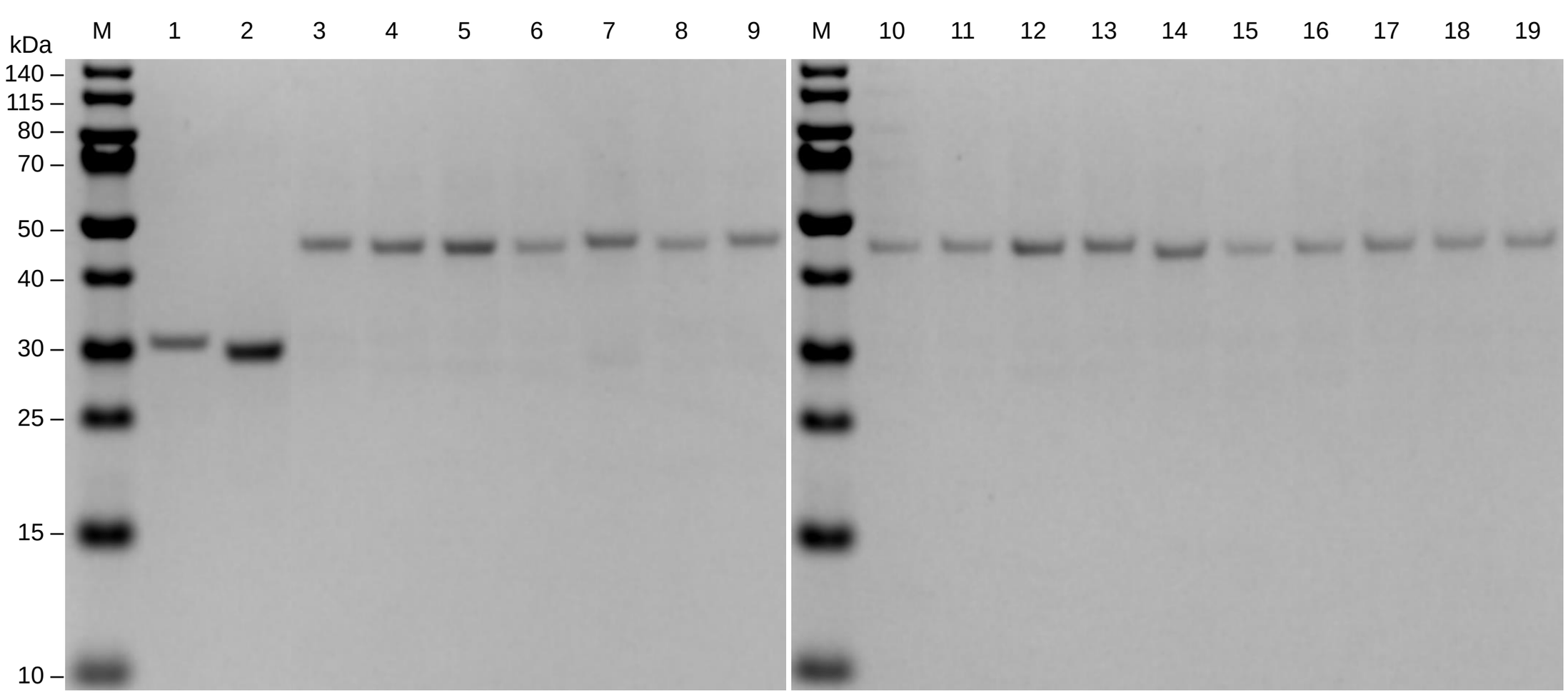
Supplementary Figures



Supplementary Figure 1.

SDS-PAGE of conjugated scFv antibody fragments and oligonucleotides.

Lane 1: Sortase A enzyme.

Lane 2: Non-conjugated scFv-LPETG (scFv(1))

Lane 3-19: scFv(1)-scFv(17) conjugated to oligo barcodes, after purification with 30 kDa filter.

M = Molecular Weight Marker

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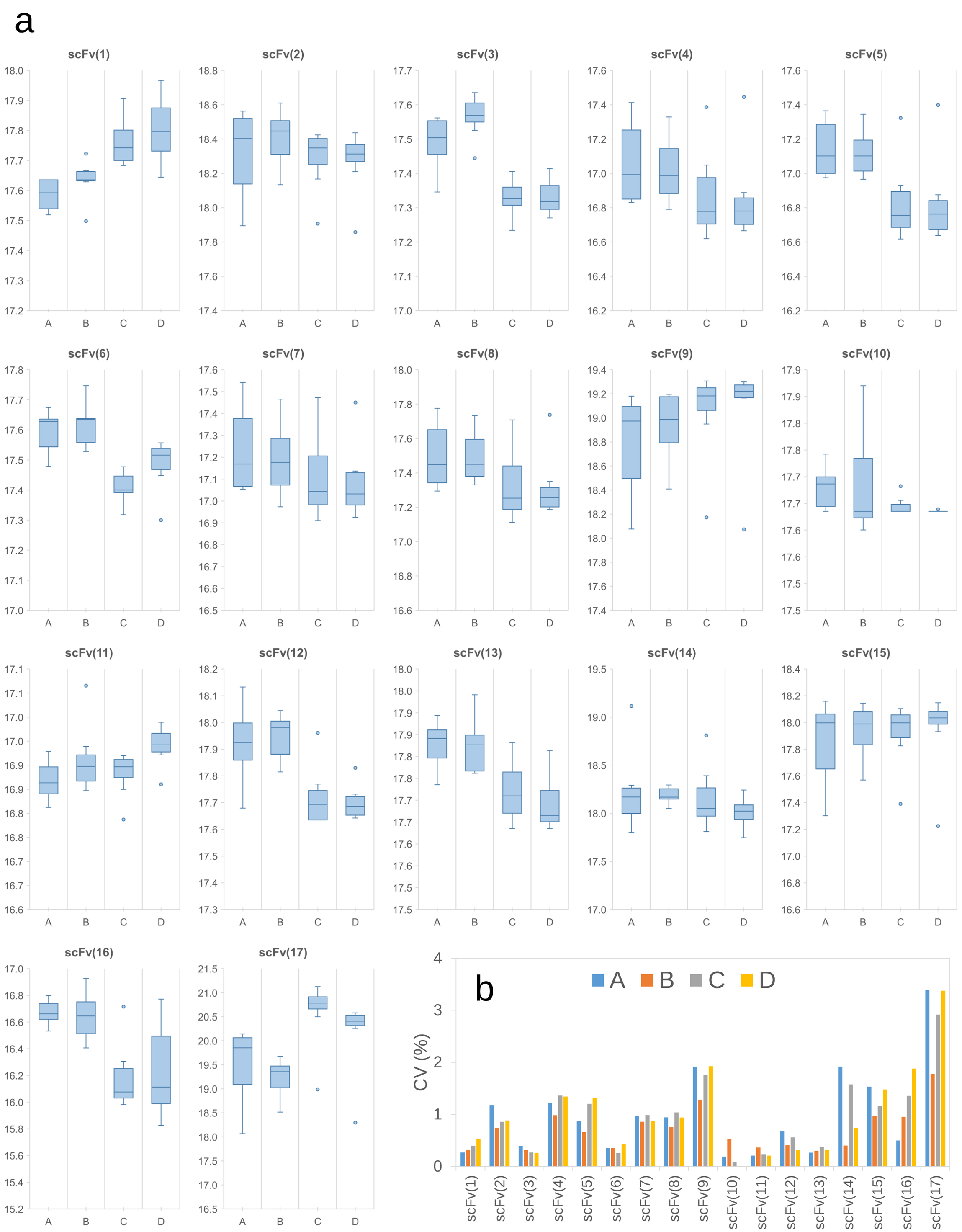

Supplementary Figure 2. Intra-assay precision study. Variability for each scFvs antibody was tested using n=4 biologically independent serum samples (A: Normal, B: Normal, C: PDAC stage III, D: PDAC stage IV) with 10 technical replicates each. Each of the in total 40 parallel samples were incubated with an equally sized part of a single scFv cocktail containing all 17 scFv-oligos **a)** Box plots with median, quartiles and range. Outliers are indicated by filled circles. **b)** Coefficient of variation (CV).

Supplementary Tables

Supplementary Table 1. Barcode oligonucleotide sequences. 55555555 represents a sequence of 8 random bases intended to be used as an UMI (Unique Molecular Identifier), however, the UMI was not used at this stage in the development.

	Barcode oligonucleotide sequences
1	TTCCCTACACGACGCTCTTCCGATCT55555555TGGCCTATAGATCGGAAGAGCACACGTCTGAACT
2	TTCCCTACACGACGCTCTTCCGATCT55555555ACAGTATCAGATCGGAAGAGCACACGTCTGAACT
3	TTCCCTACACGACGCTCTTCCGATCT55555555GTTAGGCAAGATCGGAAGAGCACACGTCTGAACT
4	TTCCCTACACGACGCTCTTCCGATCT55555555CACTACGGAGATCGGAAGAGCACACGTCTGAACT
5	TTCCCTACACGACGCTCTTCCGATCT55555555TGGCTAGAAGATCGGAAGAGCACACGTCTGAACT
6	TTCCCTACACGACGCTCTTCCGATCT55555555GTAGGTACAGATCGGAAGAGCACACGTCTGAACT
7	TTCCCTACACGACGCTCTTCCGATCT55555555GTTAACTGAGATCGGAAGAGCACACGTCTGAACT
8	TTCCCTACACGACGCTCTTCCGATCT55555555CACTGTACAGATCGGAAGAGCACACGTCTGAACT
9	TTCCCTACACGACGCTCTTCCGATCT55555555TGCTTACGAGATCGGAAGAGCACACGTCTGAACT
10	TTCCCTACACGACGCTCTTCCGATCT55555555ACTCAGTAAGATCGGAAGAGCACACGTCTGAACT
11	TTCCCTACACGACGCTCTTCCGATCT55555555CAGACCGTAGATCGGAAGAGCACACGTCTGAACT
12	TTCCCTACACGACGCTCTTCCGATCT55555555TGCGGAGAAGATCGGAAGAGCACACGTCTGAACT
13	TTCCCTACACGACGCTCTTCCGATCT55555555ACTACTCGAGATCGGAAGAGCACACGTCTGAACT
14	TTCCCTACACGACGCTCTTCCGATCT55555555GTATAGTCAGATCGGAAGAGCACACGTCTGAACT
15	TTCCCTACACGACGCTCTTCCGATCT55555555CAGCTCATAGATCGGAAGAGCACACGTCTGAACT
16	TTCCCTACACGACGCTCTTCCGATCT55555555ACTTAGGTAGATCGGAAGAGCACACGTCTGAACT
17	TTCCCTACACGACGCTCTTCCGATCT55555555GTAACTCCAGATCGGAAGAGCACACGTCTGAACT