Supplemental Information

Multiplex epitope mapping using bacterial surface display reveals both linear and conformational epitopes

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Supplemental methods

Construction of VEGF fragments

Oligonucleotides corresponding to top and bottom strands of the Avastin epitopes (F1, F2, and F3) were ordered (MWG Eurofins) with *NotI* and *AscI* sites (lower case) for cloning into the pSCEM2 vector. To construct the F3 fragment we annealed and ligated four oligos simultaneously into the pSCEM2 vector.

AVA F1 F: ggccgcaCCTCACCAAGGCCAGCACATAGGAGAGATGgg
AVA F1 R: cgcgccCATCTCTCCTATGTGCTGGCCTTGGTGAGGtgc

AVA F2 F: ggccgcaACCATGCAGATTATGCGGATCAAACCTgg
AVA F2 R: cgcgccAGGTTTGATCCGCATAATCTGCATGGTtgc

AVA F3 Fi: ggccgcaATCACCATGCAGATTATGCGGATCAAACCTCACC
AVA F3 Fii: AAGGCCAGCACATAGGAGAGATGAGCgg
AVA F3 Ri: gcgccGCTCATCTCTCCTATGTGCTGGCCTT
AVA F3 Rii: GGTGAGGTTTGATCCGCATAATCTGCATGGTGATtgc

Successful insertion of the F1, F2, or F3 fragment was confirmed with DNA sequencing.

Supplemental File 1. SI TargetList.xls A Microsoft Excel file containing details on all targets in the MTF library, including primers used for PCR amplification of ECDs.

Table SI 1. Epitopes determined using single-gene and MTF libraries.

PSMA epitopes (PSMA Uniprot ID Q04609) PSMA pAb HPA HPA010593		
PSMA mAb E1	473-491	LVHNLTKELKSPDEGFEGK
PSMA pAb antigen (PrEST)	232-337	ADYFAPGVKSYPDGWNLPGGGVQRGNILNLNGAGDPLTPGYPANEYAYRRGIAEAVGLPSIPVHPIGYYDAQKLLEKMGGSAPPDSSWRGSLKVPYNVGPGFTGNF
PSMA pAb E1	231-245	PADYFAPGVKSYPDG
PSMA pAb E2	253-264	VQRGNILNLNGA
PSMA pAb E3	287-301	VGLPSIPVHPIGYYD
PSMA pAb E4	317-331	SSWRGSLKVPYNVGP
EGFR epitopes (EGFR Uniprot ID Q504U8) EGFR pAb HPA001200		
EGFR pAb antigen (PrEST)	299-447	EFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIIS
EGFR pAb E1	344-352	PQELDILKT
EGFR pAb E2	381-398	IRGRTKQHGQFSLAVVSL
EGFR pAb E3	425-442	CYANTINWKKLFGTSGQK
VEGF epitopes (VEGF Uniprot ID P15692)		
VEGF mAb E1	45-50	YIFKPS
VEGF pAb E1	3-8	AEGGGQ
VEGF pAb E2	11-16	HEVVKF
Avastin epitopes and constructs		
Avastin E1 (Enriched fragment)	34-121	DIFQEYPDEIEYIFKPSCVPLMRCGGCCNDEGLECVPTEESNITMQIMRIKPHQGQHIGEMSFLQHNKCECRPKKDRARQEKCDKPRR
F1 construct	84-94	KPHQGQHIGEM
F2 construct	77-85	TMQIMRIKP
F3 construct	75-96	NITMQIMRIKPHQGQHIGEMSF
EGFR pAb Off-target binding		
ITGAL2b enriched (Uniprot ID **P08514)**	877-953	PSPIHPAHHKRDRRQIFLPEPEQPSRLQDPVLVSCDSAPCTVVQCDLQEMARGQRAMVTVLAFLWLPSLYQRPLDQF
IL-17 not enriched (Uniprot ID Q16552)	79-100	NEDPERYPSVIWEAKCRHLGCI

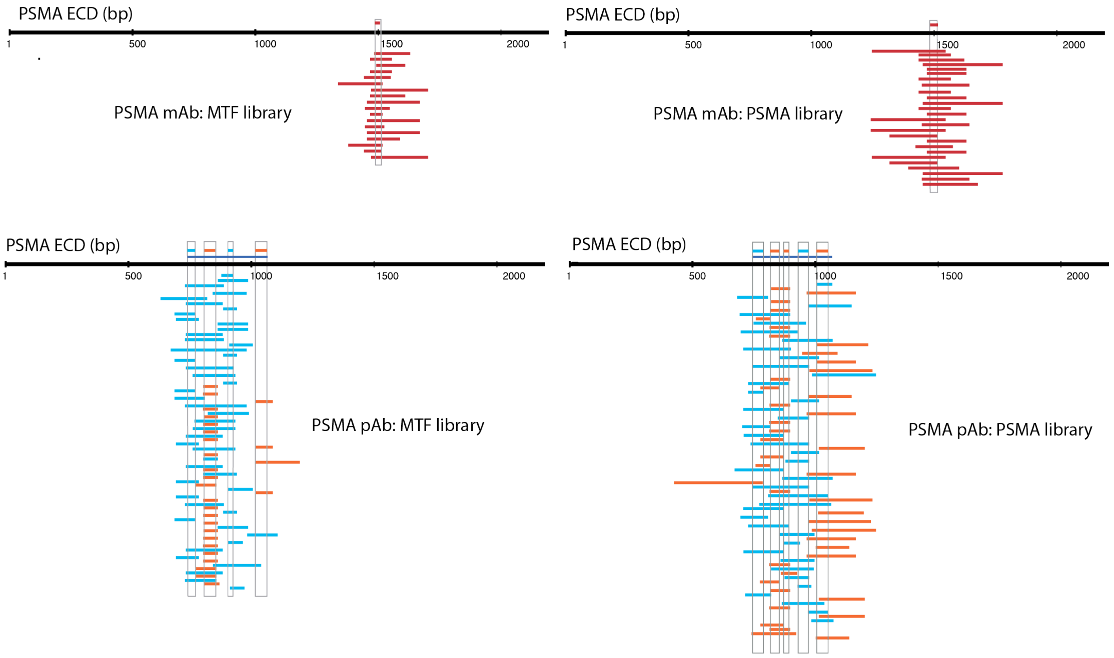

Figure SI 1. Details of anti PSMA mAb and anti PSMA pAb HPA010593 epitope mapping using dedicated and MTF libraries. Epitope mapping as described in Methods. Top: Epitope mapping of mAb. Bottom: Eptiope mapping of pAb. Blue bars: High gate alignments, Orange bars: Low gate alignments. The blue bar over the gene is the antigen PrEST (described in Table SI 1).

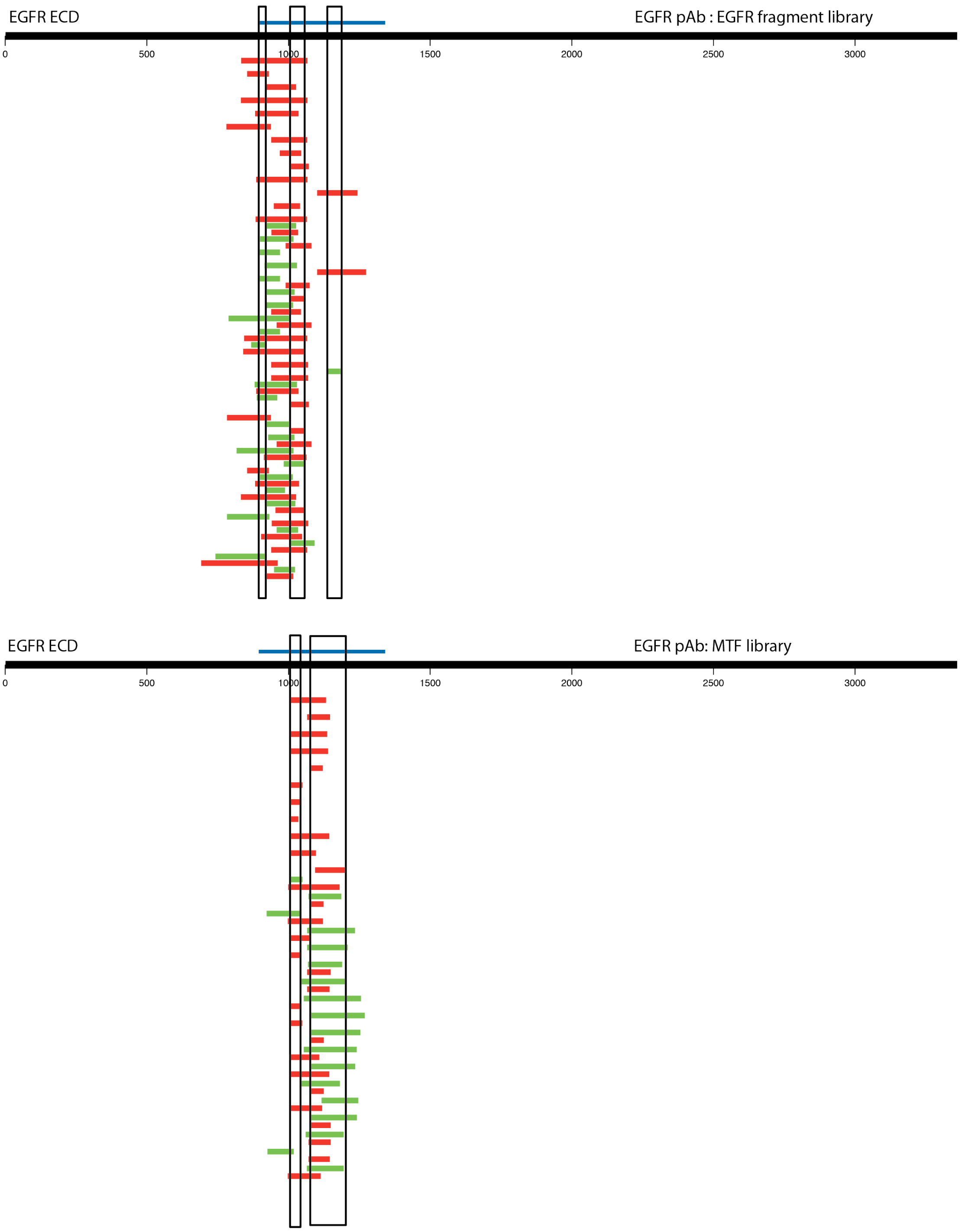

Figure SI 2. Details of anti EGFR pAb HPA001200 epitope mapping using dedicated and MTF libraries. Epitope mapping as described in Methods. Red bars: High gate alignments, Green bars: Low gate alignments. The blue bar over the gene is the antigen PrEST (described in Table SI 1).

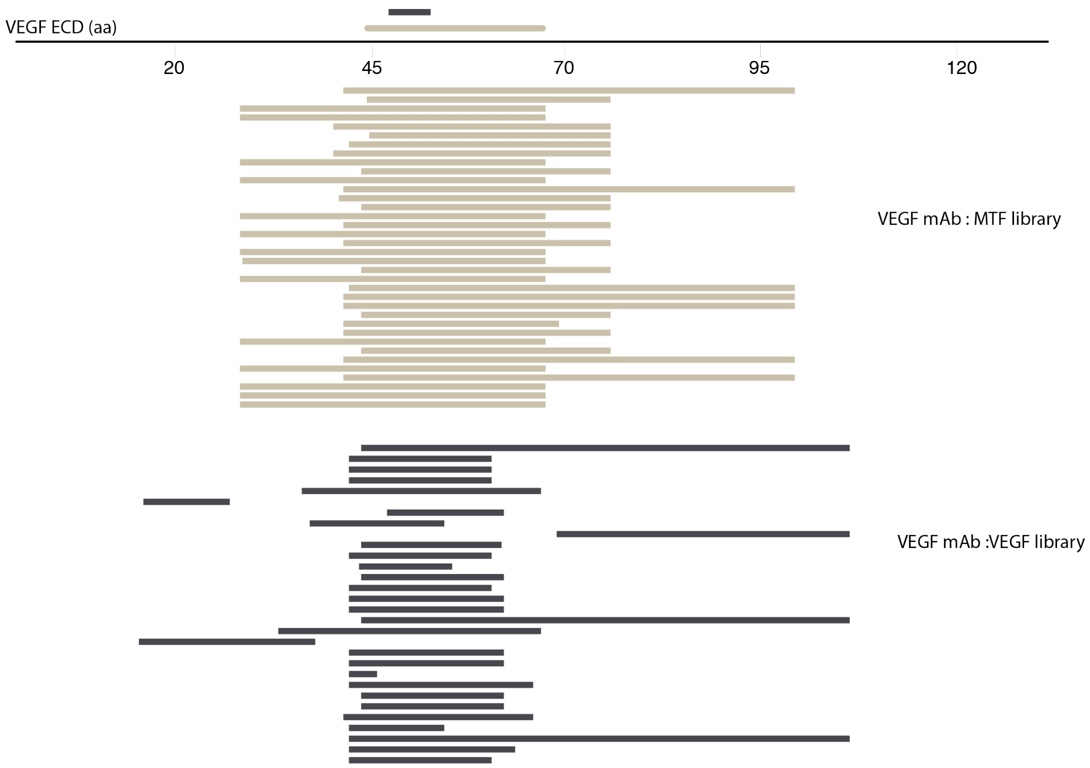

Figure SI 3. Details of anti VEGF mAb epitope mapping using dedicated and MTF libraries. Epitope mapping as described in Methods. Consensus epitopes are shown over the gene. Details of eptiopes are shown in Table SI 1.

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27.8% identity in 36 residues overlap; Score: 42.0; Gap frequency: 11.1%

EGFR_PrEST    19 CTSISGDLHILPVAFRG----DSFTHTPPLDPQELD
ITGA2b_enr    40 CTVVQCDLQEMARGQRAMVTVLAFLWLPSLYQRPLD
                 **    **       *       *   * *    **
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26.3% identity in 19 residues overlap; Score: 36.0; Gap frequency: 0.0%

EGFR_PrEST    27 HILPVAFRGDSFTHTPPLD
ITGA2b_enr    57 MVTVLAFLWLPSLYQRPLD
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Figure SI 4. Homology alignments of EGFR PrEST and ITGA2b$_{877\text{-}956}$ fragment enriched by FACS. Alignments were performed with the ExPASy SIM alignment tool (http://web.expasy.org/sim/) with the BLOSUM30 algorithm and gap and extension penalties at -12 and -4, respectively.