Figure S1: **Scheme describing the masked selection procedure**. Non relevant (normal) antigens are depicted as empty black shapes, immobilized onto a surface. Relevant (i.e. cancer specific) antigen is depicted as a full black star. A phage-sdAb library is used to select binders against the non-relevant sample. Corresponding sdAbs are produced, purified and added in a large excess during a second selection performed with the same library on the relevant sample. Only relevant epitopes are available for binding, leading to the preferential selection of binders against proteins or epitopes absent in the non relevant sample.

Figure S2: Transfection level. Expression HER2, mGluR4 or CXCR4 (fused to SNAP and Flag tag) on the surface of HEK cells was followed by flow cytometry. HER2-transfected HEK cells (HEK-HER2), mGluR4-transfected HEK cells (HEK-mGLuR4) and CXCR4-transfected HEK cells (HEK-CXCR4) were incubated with anti-Flag mAb (black line) or control isotype (gray line). Captured antibodies were detected using PE-conjugated anti-mouse antibodies. Cells were analyzed by flow cytometry assay on a MACSQuant flow cytometer (Miltenyi). Histograms were overlaid and normalized for peak height to facilitate comparison.

Figure S3: **Specificity analysis of anti-HER2 sdAbs by ELISA**. HER2-Fc recombinant fusion or human Fc portion were covalently immobilized on epoxy magnetic beads. Soluble sdAbs were incubated. After washing, bound sdAbs were detected using a HRP-labeled anti-6his mAb. anti-Fc: anti human Fc sdAb used as positive control.

Figure S4: **Characterization of anti-HER2 binders by flow cytometry**. **A)** All selected phage-sdAbs were incubated with $HER2^+$ SKOv3 cells. After washing, bound phage were detected using PE-conjugated anti-M13 mAb. Cells were analyzed by flow cytometry assay on a MACSQuant flow cytometer (Miltenyi). anti-Fc: anti-human Fc sdAb used as negative control. **B**) Competition flow cytometry assay. Cells were incubated with phage-sdAbs in the presence of an excess of soluble anti-HER2 sdAbs C.E4, A.E4 or A.H10. Bound phage were detected as in A). **C)** Phage-sdAbs and sdAbs able to compete (sharing overlapping epitopes) were classified into 3 groups.

Fig S5: Affinity analysis of sdAbs selected on cell surface antigens by HTRF. Various concentrations of sdAbs were incubated on transfected and labeled cells and binding was followed by FRET as for Fig 2. Dissociation constants were calculated using a non-linear curve fitting software (Prism, GraphPad). **A)** Dose response curves obtained by anti-HER2 sdAbs incubated on cells transfected with SNAP-HER2 fusions. **B)** Calculated K_D of anti-HER2 sdAbs. **C)** Dose response curves obtained with anti-mGluR4 sdAbs incubated on cells transfected with SNAP-mGluR4 fusions . **D)** Calculated K_D of anti-HER2 sdAbs. **E)** Dose response curves obtained with anti-CXCR4 sdAbs incubated on cells transfected with SNAP-CXCR4 fusions. **F)** Calculated K_D of anti-CXCR4 sdAbs.

Fig S6: Sequences and epitope analysis of sdAbs targeting overexpressed targets. A) Amino acid sequences and frequency of biopsy specific binders. Only complementarity determining regions CDR1, CDR2 and CDR3 are represented. **B)** Phage ELISA competition assays (in the presence of an excess of soluble sdAbs) were performed with all different biopsy lysate binders to determine competitive sdAbs sharing overlapping epitopes. **C)** Aminoacid sequences and frequency of cell surface marker-specific sdAbs. Only CDR1, CDR2 and CDR3 are represented. **D)** A flow cytometry competition assay using phage-sdAbs in the presence of soluble sdAbs was performed to determine competitive clones.

Figure S1

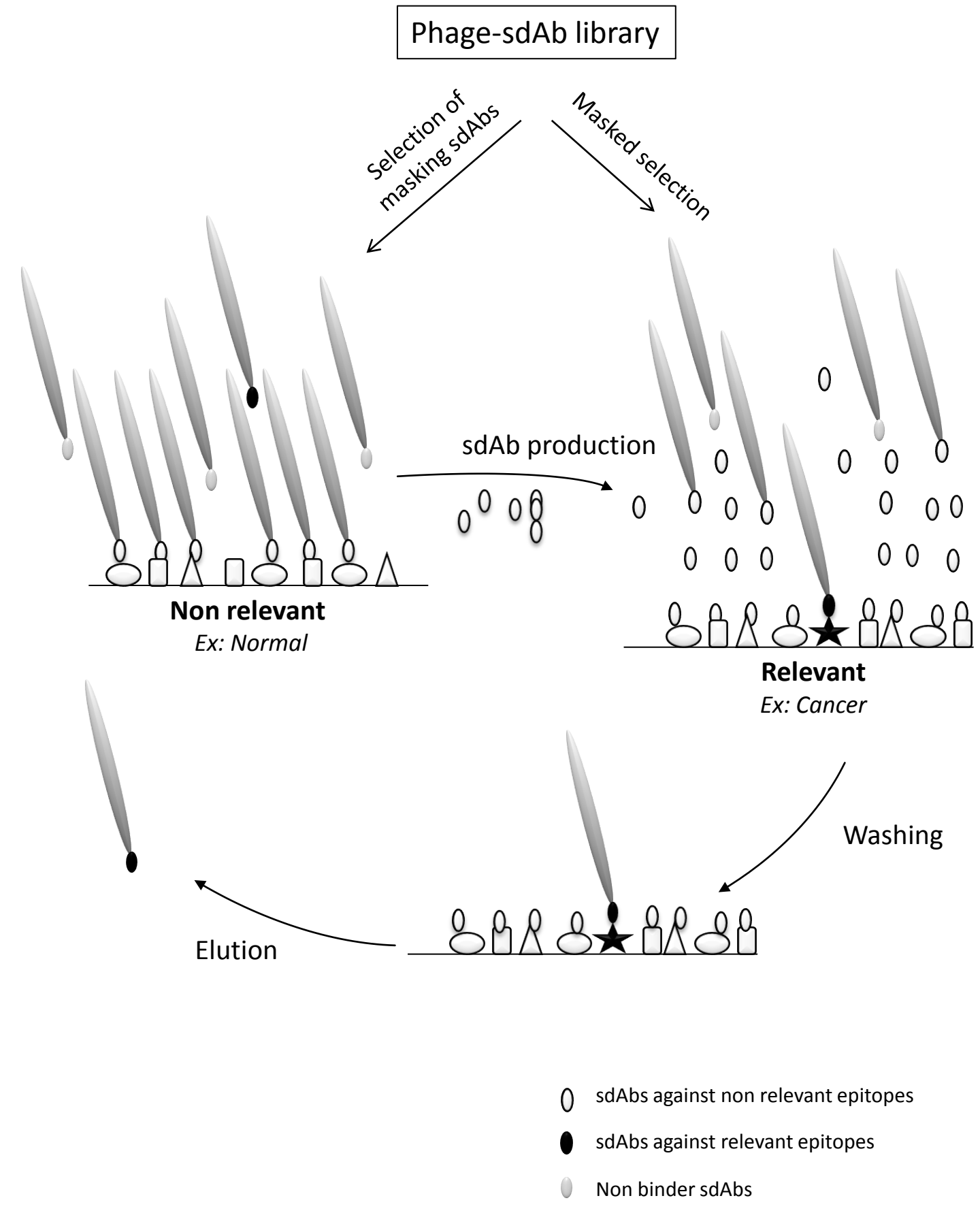
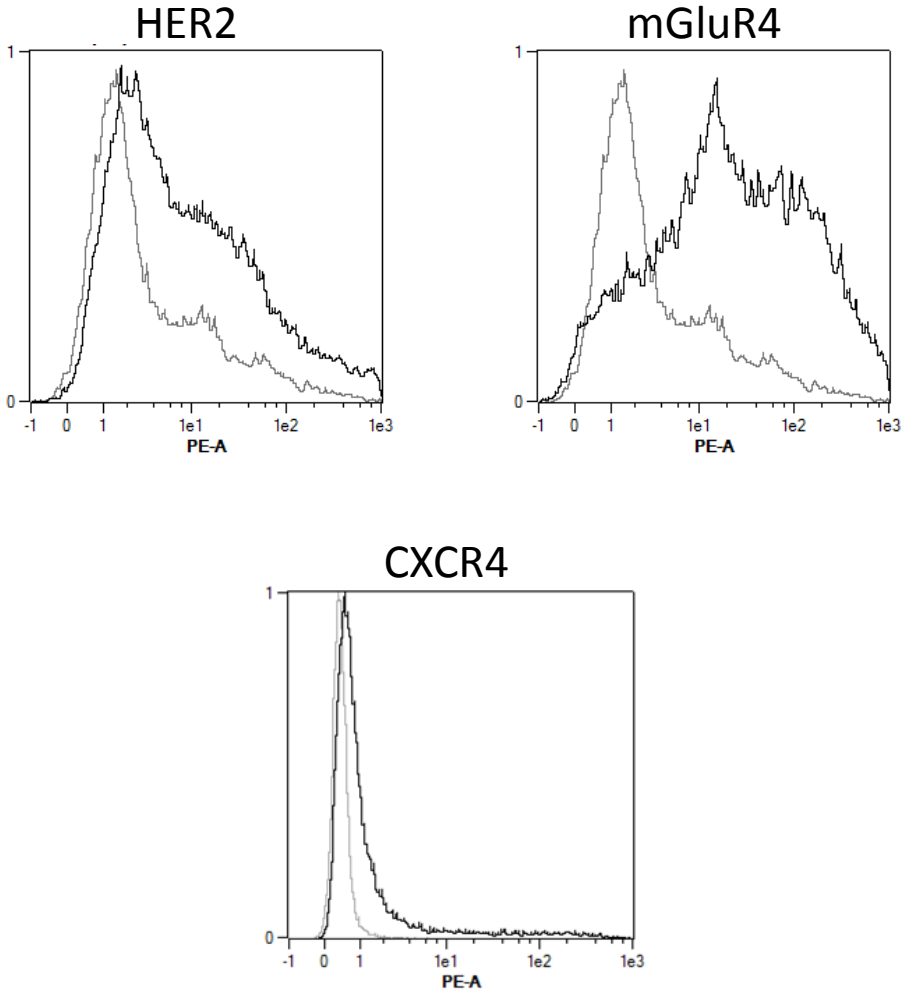

Phage-sdAb library
Selection of masking sdAbs
Masked selection
sdAb production
Non relevant
Ex: Normal
Relevant
Ex: Cancer
Washing
Elution
sdAbs against non relevant epitopes
sdAbs against relevant epitopes
Non binder sdAbs

Figure S2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1e2
1e3
PE-A
mGluR4
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0
-1
0
1
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1e2
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PE-A
CXCR4
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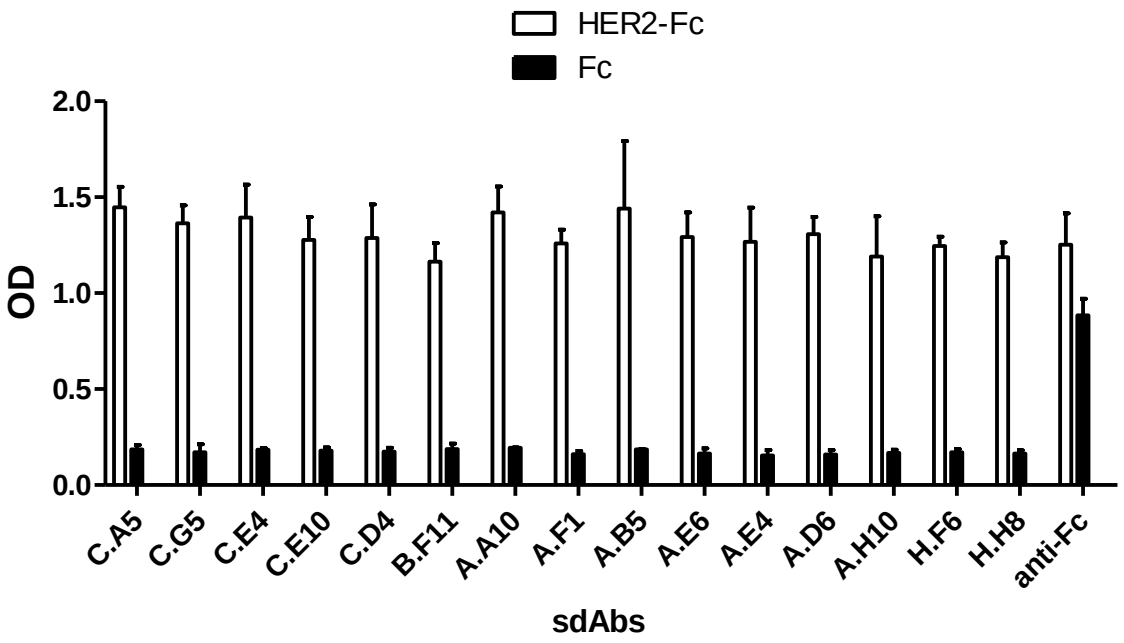
0
-1
0
1
1e1
1e2
1e3
PE-A

Figure S3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Fc
OD
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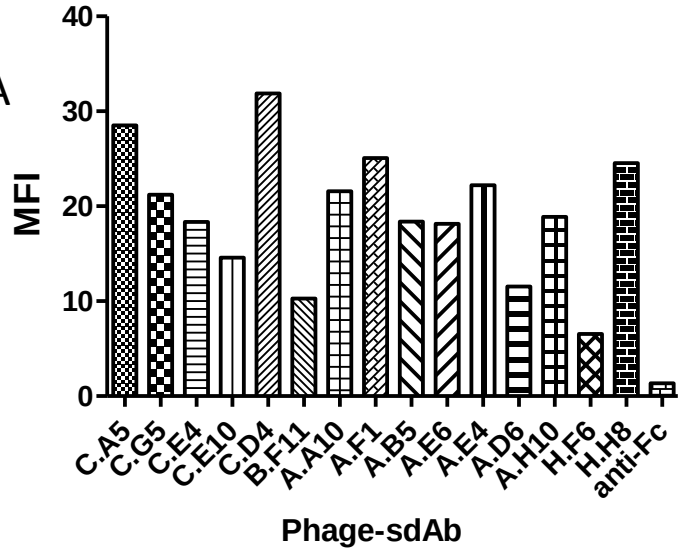
1.5
1.0
0.5
0.0
C.A5
C.G5
C.E4
C.E10
C.D4
B.F11
A.A10
A.F1
A.B5
A.E6
A.E4
A.D6
A.H10
H.F6
H.H8
anti-Fc
sdAbs

Figure S4

A



C

Competitive cytometry assay results

Group 1	Group 2	Group 3
C.A5 C.G5 C.E4 H.H8	C.E10 A.E6 A.E4	A.B5 A.D6 A.H10

B

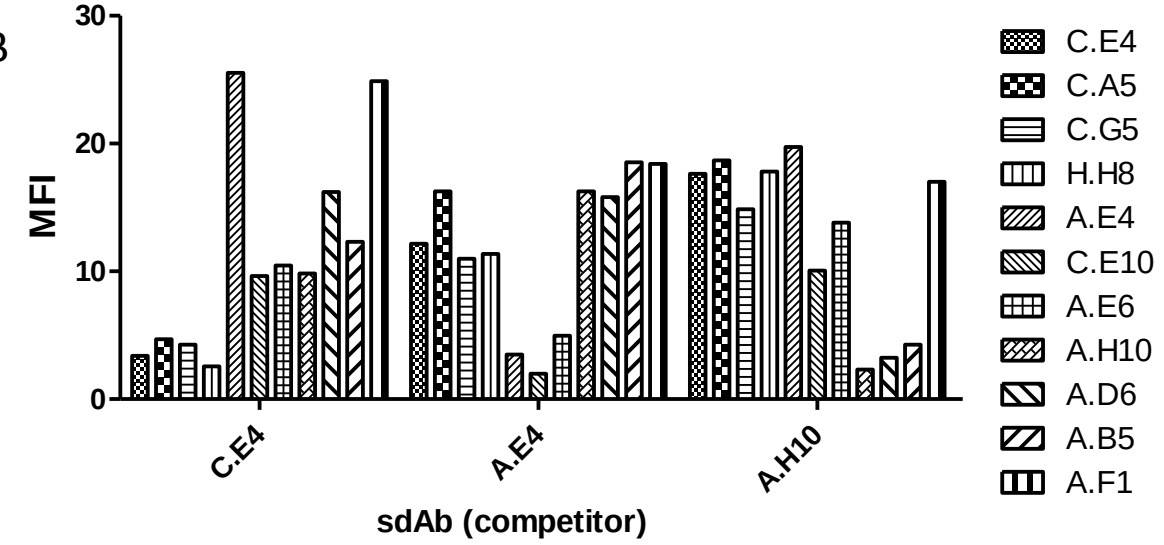
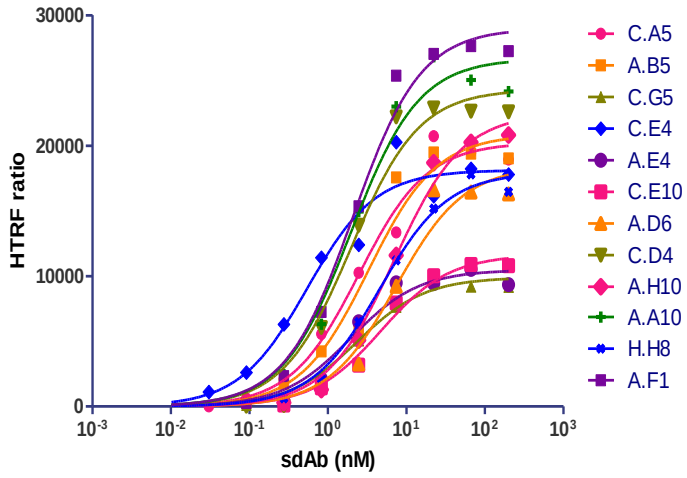


Figure S5

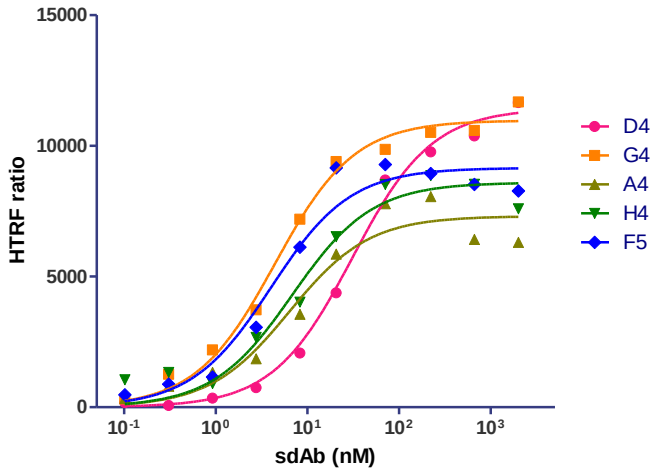
A



B

sdAb	K_D (nM)
A.A10	2.7
A.F1	2.2
A.B5	3.2
A.E4	1.9
A.D6	7.3
A.H10	7
C.A5	2.4
C.E4	0.5
C.D4	2
C.G5	2.3
C.E10	4.7
H.H8	5.3

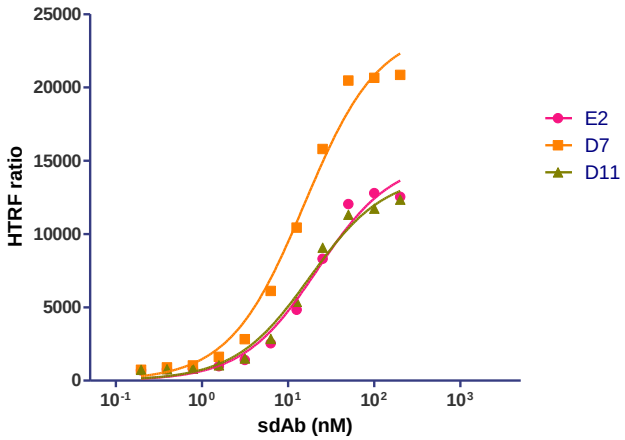
C



D

sdAb	K_D (nM)
H4	6.8
A4	6.3
D4	30
F5	3.9
G4	4.4

E



F

sdAb	K_D (nM)
E2	22
D7	15
D11	17

Figure S6

A

Name	CDR1	CDR2	CDR3	Frequency
J.A4	GDTFSRYR	VTIDGAT	NALNREGPFEY	x1
J.C1	GSIFSINF	IDSRGSLN	RSFGTGGDY	x45
J.D9	GSIKSIGT	ITSGGST	TADVLYMRKTYYARDTF	x1
J.F3	GRTFSNYA	INGSGSSI	AAVRWGGSRHNGKYDS	x1
J.F8	GLTFANYH	ISRSSDTT	AAAPYWYGSAWSSAAVDY	x1
J.G9	GSIKSIGA	ITSGGST	TADVLYYSGGYYARDAY	x1
J.H6	GRTFST	ITWSGITT	AVKKRSPAGWTTSTADYDP	x1
J.H4	GRTFDNYV	IIWSSGST	AAHPYGLIRRLHQPDEYRY	x1
J.G12	GFTFSIYY	IDSSGGST	ARGPGTSWYWPWGY	x1

B

Competitive ELISA results	
Group 1	Non competitive clones
J.C1 J.D9 J.G12 J.F3	J.A4 J.F8 J.G9 J.H6 J.H4

C

Name	CDR1	CDR2	CDR3	Frequency
M.C9	GATFSRNI	ITWVRETT	AASVGSRLYGAYHKEGGYDY	x1
M.B9	GPSFLLYA	ITYIGGST	AASGHSYSDSANQYDD	x1
M.E10	TSVFSIDT	ITSGGST	KAITTRWDRTSDSY	x1
M.D5	GSISSRNT	QASGSYI	YLSQYSGSY	x4
M.D7	GMIFSNYG	ITRGGST	YANTN	x1
M.H3	GIIVSIRS	ITGRGST	NTRRQPLP	x1
M.H12	GSSISSIA	ISSGGTI	NTGRRLQTGS	x1
M.A9	GSIFEIND	ITRGRST	KADHSTYDNWNENY	x1
M.A4	EISVSIKS	VISGRSP	NLHTWSGYDY	x1
M.F5	ESTFSSNA	ISPGGSA	YSRFR	x2
M.H11	GRTLSAYT	LVGSADNT	AAKWRTDYYRDPENYAY	x1

D

Competitive cytometry assay results		
Group 1	Group 2	Non competitive clones
M.H11 M.A4	M.E10 M.A9 M.D7 M.H12 M.F5	M.C9 M.B9 M.D5 M.H3