

Supplementary Figure 1: *Nanobody 7D12 is able to block stimulation by different EGFR-ligands.* Wt EGFR expressing Ba/F3 cells were seeded in triplicates at equal densities, and the average number of viable cells were measured after trypan blue staining using Vi-CELL. Cells were stimulated for 72 h with 10 ng/ml of indicated EGFR ligand under treatment with 60 µM 7D12-hcAb (grey) or without as control (white). One experiment out of two experiments is shown. Results are presented as mean ± SD.

Statistical significance was calculated using unpaired student’s t-test (\*\*\* = p < 0.001).



Supplementary Figure 2: **(A)***Expression of EGFR wt or mutated EGFR.*EGFR wt or mutant-transduced Ba/F3 cells were fluorescently labelled with polyclonal EGFR antibody and specific secondary antibodies. Gating was done on EGFR high cells in comparison to unstained gate. Experiments were performed at least two times in triplicates. Results are presented as mean ± SD.

**(B)** *EGFR variants I491K, K489E, K467T and V441F are not limited in EGF-dependent growth.* EGFR-wt, I491K, K489E, K467T, I462A and V441F-transduced Ba/F3 cells were seeded in triplicates at equal densities, and the average number of viable cells were measured after trypan blue staining using Vi-CELL. After 72 h under EGF stimulation, increase of viable cells from indicated EGFR variant was calculated relative to EGFR wt Ba/F3 cells. One experiment out of two is shown. Results are presented as mean ± SD

Statistical significance was calculated using a one sample t-test against value 1 (B) (\*\* = p < 0.01).

Supplementary Figure 3

Rituximab 7mer peptide enrichment top 50 sequences using GLAM2:

Score: 403.918 Columns: 8 Sequences: 25

\*\*\*\*\*\*\*\*

1 1 QFSNPS.L 7 + 20.9

8 1 QYANPS.M 7 + 24.8

12 1 PYANPS.M 7 + 23.1

13 1 MYSNPS.M 7 + 22.7

14 1 VFANPS.M 7 + 20.9

16 1 IHANPS.M 7 + 20.1

17 1 NYANPS.L 7 + 18.2

18 1 PFANPS.M 7 + 22.5

20 1 EYANPS.M 7 + 22.1

21 1 QFSNPS.F 7 + 19.5

24 1 LWANPS.M 7 + 17.9

25 1 MFANPS.M 7 + 22.9

26 1 LYAN.SPF 7 + 2.80

29 1 PHANPS.M 7 + 20.4

31 1 QRSLPS.L 7 + 1.53

34 1 IFSNPS.L 7 + 18.9

35 1 QLSNPS.L 7 + 14.0

36 1 RYANPS.M 7 + 23.2

38 1 QFSTPS.L 7 + 9.63

39 1 QFSNPS.M 7 + 23.4

40 1 RYANPS.V 7 + 18.7

44 1 WFANPSQV 8 + 12.5

45 1 RYSNPS.L 7 + 20.0

48 1 EYANPS.L 7 + 19.7

50 1 DYANPS.M 7 + 20.1

QYANPSPM

FS QL

A C D E F G H I K L M N P Q R S T V W Y Del Ins Score

0 0 1 2 0 0 0 2 0 2 2 1 3 7 3 0 0 1 1 0 0 3.64

0 -0.110

0 0 0 0 9 0 2 0 0 1 0 0 0 0 1 0 0 0 1 11 0 55.7

0 -0.110

16 0 0 0 0 0 0 0 0 0 0 0 0 0 0 9 0 0 0 0 0 56.6

0 -0.110

0 0 0 0 0 0 0 0 0 1 0 23 0 0 0 0 1 0 0 0 0 78.0

0 -0.110

0 0 0 0 0 0 0 0 0 0 0 0 24 0 0 0 0 0 0 0 1 83.5

0 -0.110

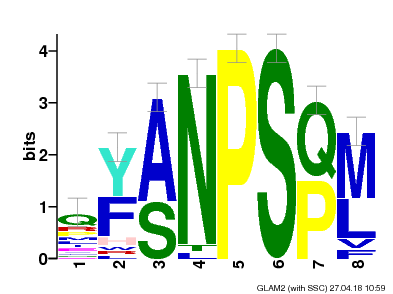
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 25 0 0 0 0 0 86.1

0 -0.110

0 0 0 0 0 0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 23 -19.9

0 -0.110

0 0 0 0 2 0 0 0 0 8 13 0 0 0 0 0 0 2 0 0 0 61.1



Nanobody 7mer peptide enrichment top 50 sequences using GLAM2:

Score: -23.9131 Columns: 12 Sequences: 2

\*\*\*\*\*\*\*\*\*\*\*\*

35 1 .DVWHSA..Y.Q 8 + -4.96

52 1 .SMWPGA.QN.. 8 + -5.73

DMWHGA QN Q

SV PS Y

A C D E F G H I K L M N P Q R S T V W Y Del Ins Score

0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 -5.89

0 -0.0429

0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 -0.247

0 -0.0429

0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 1 0 0 0 0.257

0 -0.0429

0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 0 4.92

0 -0.0429

0 0 0 0 0 0 1 0 0 0 0 0 1 0 0 0 0 0 0 0 0 -0.739

0 -0.0429

0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 -0.529

0 -0.0429

2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1.80

0 -0.0429

0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 -5.89

0 -0.0429

0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 1 -5.23

0 -0.0429

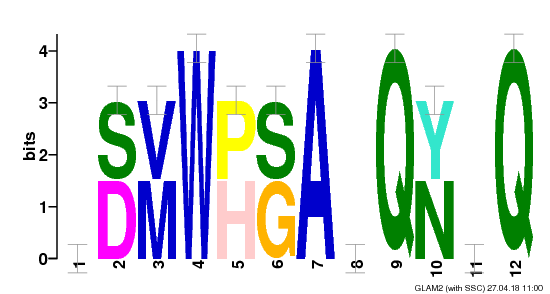
0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 1 0 -0.779

0 -0.0429

0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 -5.89

0 -0.0429

0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 1 -5.23



Supplementary Figure 3:*7mer peptide phage display results.* After three times of antibody selection with 7D12-hcAb nanobody or rituximab as control, 50 most prevalent 7mer peptide sequences were analysed as described in methods. To be eligible for GLAM2 analysis a random 8th position was added. Best found motif is shown as representative result with key positions marked (\*) and automatically calculated score. Result of one experiment is shown.

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| --- | --- | --- |
| **Supplementary Table 1: Primers for side-directed mutagenesis and In-Fusion cloning of**  **hEGFR ectodomain variants** | | |
| **Template** | **Primer name** | **Primer sequence 5'-3'** |
| hEGFR wt | **hEGFR\_hS492R-mutagen\_fw** | GGTCAGAAAACCAAAATTATA AGA AACAGAGGTGAAAACAGC |
| hEGFR wt | **hEGFR\_hS492R-mutagen\_rv** | GCTGTTTTCACCTCTGTTTCTTATAATTTTGGTTTTCTGACC |
| hEGFR wt | **hEGFR\_hI491K-mutagen\_fw** | CCTCCGGTCAGAAAACCAAAATTAAAAGCAACAGAGGT |
| hEGFR wt | **hEGFR\_hI491K-mutagen\_rv** | ACCTCTGTTGCTTTTAATTTTGGTTTTCTGACCGGAGG |
| hEGFR wt | **hEGFR\_hK489E-mutagen\_fw** | GGGACCTCCGGTCAGAAAACCGAAATTATAAGCAACAGAGG |
| hEGFR wt | **hEGFR\_hK489E-mutagen\_rv** | CCTCTGTTGCTTATAATTTCGGTTTTCTGACCGGAGGTCCC |
| hEGFR wt | **hEGFR\_hK467T-mutagen\_fw** | GGAGATGTGATAATTTCAGGAAACACAAATTTGTGCTATGC |
| hEGFR wt | **hEGFR\_hK467T-mutagen\_rv** | GCATAGCACAAATTTGTGTTTCCTGAAATTATCACATCTCC |
| hEGFR wt | **hEGFR\_hG465R-mutagen\_fw** | GTGATGGAGATGTGATAATTTCAAGAAACAAAAATTTGTGCTATGC |
| hEGFR wt | **hEGFR\_hG465R-mutagen\_rv** | GCATAGCACAAATTTTTGTTTCTTGAAATTATCACATCTCCATCAC |
| hEGFR wt | **hEGFR\_hS464L-mutagen\_fw** | GGAGATAAGTGATGGAGATGTGATAATTTTAGGAAAC |
| hEGFR wt | **hEGFR\_hS464L-mutagen\_rv** | GTTTCCTAAAATTATCACATCTCCATCACTTATCTCC |
| hEGFR wt | **hEGFR\_hI462A-mutagen\_fw** | GGAGATAAGTGATGGAGATGTGGCAATTTCAGGAAAC |
| hEGFR wt | **hEGFR\_hI462A-mutagen\_rv** | GGAGATAAGTGATGGAGATGTGGCAATTTCAGGAAAC |
| hEGFR wt | **hEGFR\_hR451C-mutagen\_fw** | CATCCTTGGGATTA TGC TCCCTCAAGGAG |
| hEGFR wt | **hEGFR\_hR451C-mutagen\_rv** | CTCCTTGAGGGAGCATAATCCCAAGGATG |
| hEGFR wt | **hEGFR\_hV441F-mutagen\_fw** | TCAGTTTTCTCTTGCAGTCTTCAGCCTGAACATAACATC |
| hEGFR wt | **hEGFR\_hV441F-mutagen\_rv** | GATGTTATGTTCAGGCTGAAGACTGCAAGAGAAAACTGA |
| hEGFR wt | **hEGFR\_hD379A-mutagen\_fw** | GTGGCATTTAGGGGTGCCTCCTTCACACATAC |
| hEGFR wt | **hEGFR\_hD379A-mutagen\_rv** | GTATGTGTGAAGGAGGCACCCCTAAATGCCAC |
| hEGFR wt | **hEGFR\_hR377A-mutagen\_fw** | CCGGTGGCATTTGCGGGTGACTCCTTCACAC |
| hEGFR wt | **hEGFR\_hR377A-mutagen\_rv** | GTGTGAAGGAGTCACCCGCAAATGCCACCGG |
| hEGFR start | **InFusion\_hEGFR-fw** | GGATCCAAGGCCTGCGGCCGCATGCGACCCT |
| hEGFR end | **InFusion\_hEGFR-rv** | CGTTAACACCGGTTCTAGATCATGCTCCAATAAATTCACTGCTTTG |
| wt: wildtype; fw: forward primer; rv: reverse primer; | |  |