# SUPPLEMENTARY MATERIAL

## Intracellular delivery of therapeutic antibodies into specific cells using antibody-peptide fusions

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## Supplementary Tables

Supplementary Table S1: Affinity of CPP-Abs

| Name | ka [1/(M·s)] | kd [1/s] | KD [M] | KD ratio (/WT) |
|---|---|---|---|---|
| Pep-1-AH | 1.82E+05 | 3.03E-03 | 1.66E-08 | 0.9 |
| Pep-1-BH | 1.24E+05 | 3.54E-03 | 2.85E-08 | 1.5 |
| Pep-1-LCC | 1.32E+05 | 3.09E-03 | 2.34E-08 | 1.3 |
| Ab-ctrl1 | 2.38E+05 | 2.89E-03 | 1.22E-08 | 1.0 |

ka: association rate; kd: dissociation rate; KD: equilibrium constant.

Supplementary Table S2: Affinity of CPP-Abs with cytosolic tag

| Name | ka [1/(M·s)] | kd [1/s] | KD [M] | KD ratio (/WT) |
|---|---|---|---|---|
| Ab-ctrl2 | 3,95E+05 | 4,19E-03 | 1,05E-08 | 0,8 |
| PEPth-LCC | 2,95E+05 | 6,24E-03 | 2,12E-08 | 1,5 |
| PEPth-BH | 2,93E+05 | 6,39E-03 | 2,18E-08 | 1,6 |
| PEPth-AH | 2,97E+05 | 5,76E-03 | 1,94E-08 | 1,4 |
| Pep-1-LCC | 2,91E+05 | 5,48E-03 | 1,88E-08 | 1,4 |
| Pep-1-BH | 3,23E+05 | 7,43E-03 | 2,30E-08 | 1,7 |
| Pep-1-AH | 3,68E+05 | 6,62E-03 | 1,80E-08 | 1,3 |
| aurein-LCC | 3,02E+05 | 3,48E-03 | 1,15E-08 | 0,9 |
| aurein-BH | 2,00E+05 | 3,91E-03 | 1,96E-08 | 1,6 |
| aurein-AH | 2,27E+05 | 3,43E-03 | 1,51E-08 | 1,2 |
| MTS-LCC | 3,15E+05 | 3,29E-03 | 1,05E-08 | 0,9 |
| MTS-BH | 3,47E+05 | 3,52E-03 | 1,01E-08 | 0,8 |
| MTS-AH | 4,28E+05 | 3,00E-03 | 7,00E-09 | 0,6 |
| GFWFG-LCC | nd | nd | nd | nd |
| GFWFG-BH | 3,61E+05 | 2,97E-03 | 8,23E-09 | 0,7 |
| GFWFG-AH | 3,31E+05 | 3,10E-03 | 9,39E-09 | 0,8 |

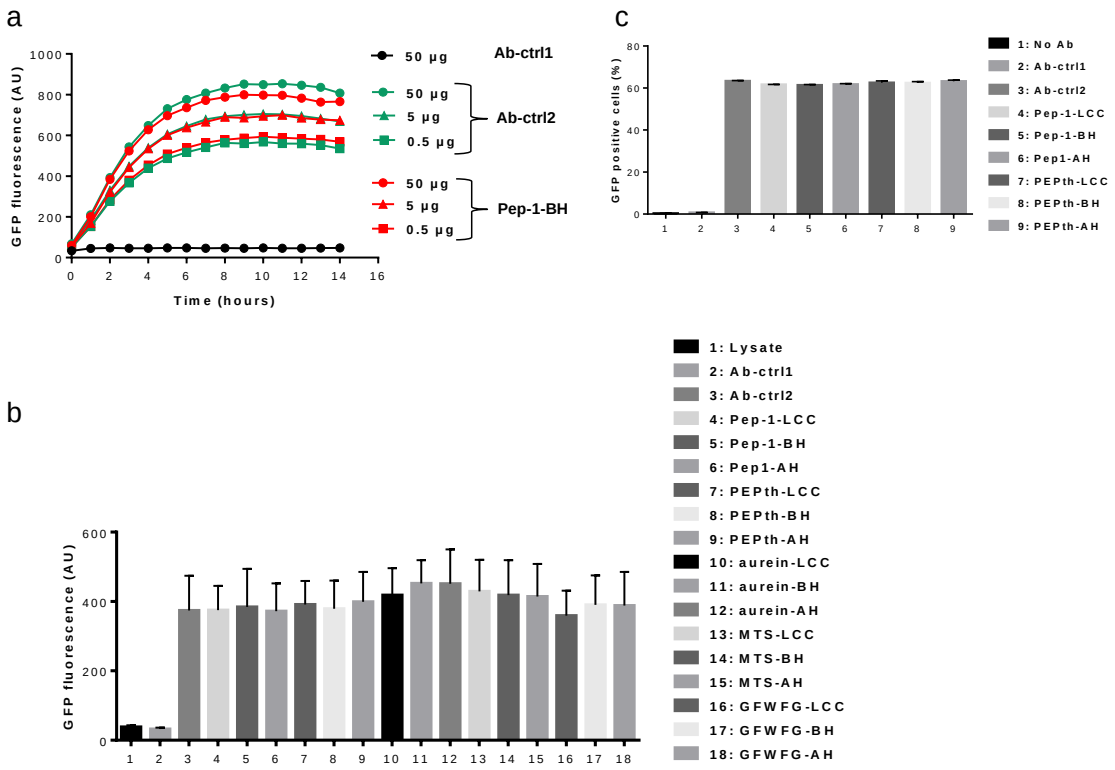
ka: association rate; kd: dissociation rate; KD: equilibrium constant. nd: not determined.
GFWFG-LCC was not efficiently captured on surface and the binding to CEACAM5 could not be determined.

Supplementary Table S3: MFI values corresponding to the number of GFP molecules coated on beads (using calibration beads)

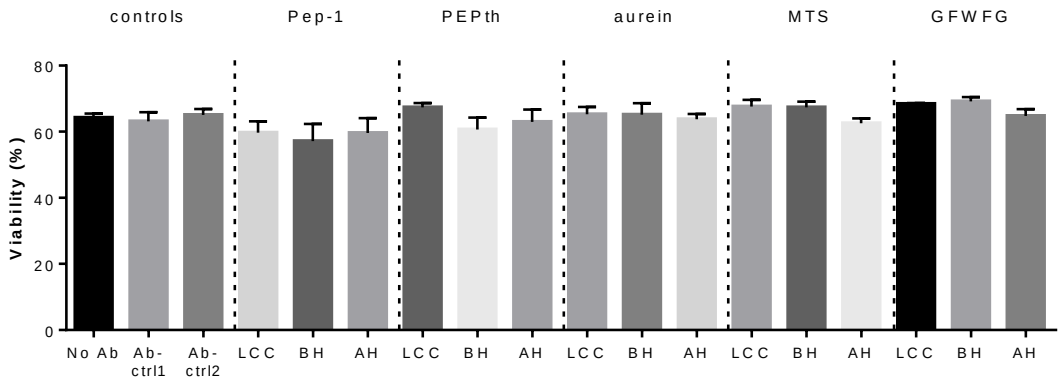
| peaks | B | C | D | E | F | G |
|---|---|---|---|---|---|---|
| **MESF** | 74471 | 164770 | 410977 | 1291440 | 7372326 | 18463961 |
| **MFI (x100)** | 0,52 | 1,45 | 5,52 | 20,56 | 119,07 | 353,61 |

MESF: Molecular Equivalent of Soluble Fluorophore

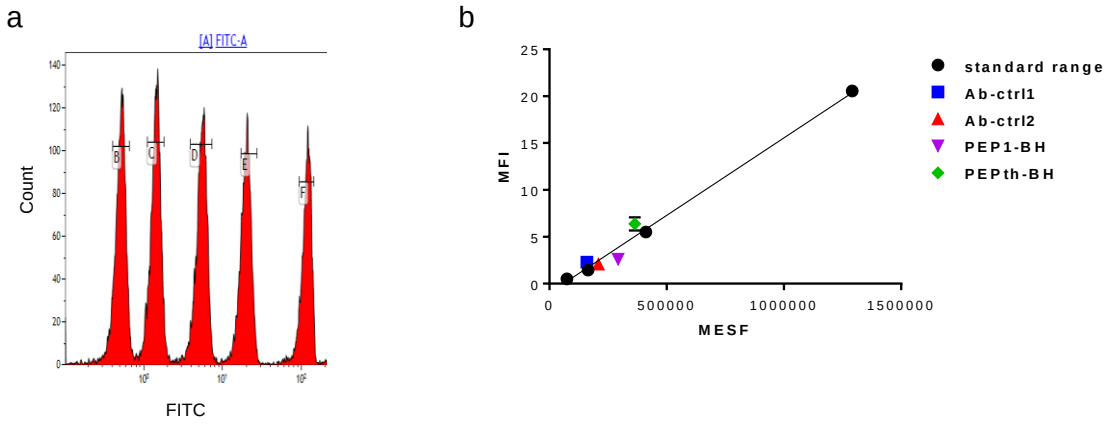
## Supplementary Figures




Supplementary Figure S1. Validation of split GFP complementation assay. Fixed amount of HEK293FS cell lysate expressing GFP1-10SA was incubated with different amounts of antibodies (0.5, 5 or 50 µg). Ab-ctrl1 without the cyto-Tag, Ab-ctrl2, which carries the GFP11-SBP2 cyto-Tag at C-terminus of the HC, and Pep-1-BH were evaluated for their capacity to complement with GFP1-10SA. Fluorescence is analyzed by spectrofluorimetry (**a**). All the CPP-Abs were incubated at 0.5 µg in HEK293FS cell lysate expressing GFP1-10SA for 8 hours and fluorescence intensity was analyzed by spectrofluorimetry (**b**). HEK293FS cells were transiently transfected with GFP1-10SA (transfection efficiency ~90%) and after 48 hours were electroporated to force antibodies delivery into the cytosol. Percentage of GFP-positive cells was analyzed by FACS (**c**).




Supplementary Figure S2. Cell viability analysis. Cell viability after transfection and incubation with cpAbs is calculated by FACS using cell viability marker on all cells.




Supplementary Figure S3. Histogram showing the fluorescence of GFP-coated beads. Five different intensities (from B to F) which represent 5 different known amounts of coated GFP (**a**). Standard plot for GFP fluorescence showing linear regression of the amount of GFP coated on beads versus the GFP fluorescence intensities R2= 0.999. Results with 5 µM samples were added (**b**).