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Supporting Information

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Reversible Shielding and Immobilization of Liposomes and Viral Vectors by Tailored Antibody-Ligand Interactions

*Oliver S. Thomas, Balder Rebmann, Matthias Tonn, Ivo C. Schirmeister, Sarah Wehrle, Jan Becker, Gabriel J. Zea Jimenez, Sebastian Hook, Sarah Jäger, Melissa Klenzendorf, Mateo Laskowski, Alexander Kaier, Gerhard Pütz, Matias D. Zurbriggen, Wilfried Weber, Maximilian Hörner, and Hanna J. Wagner**

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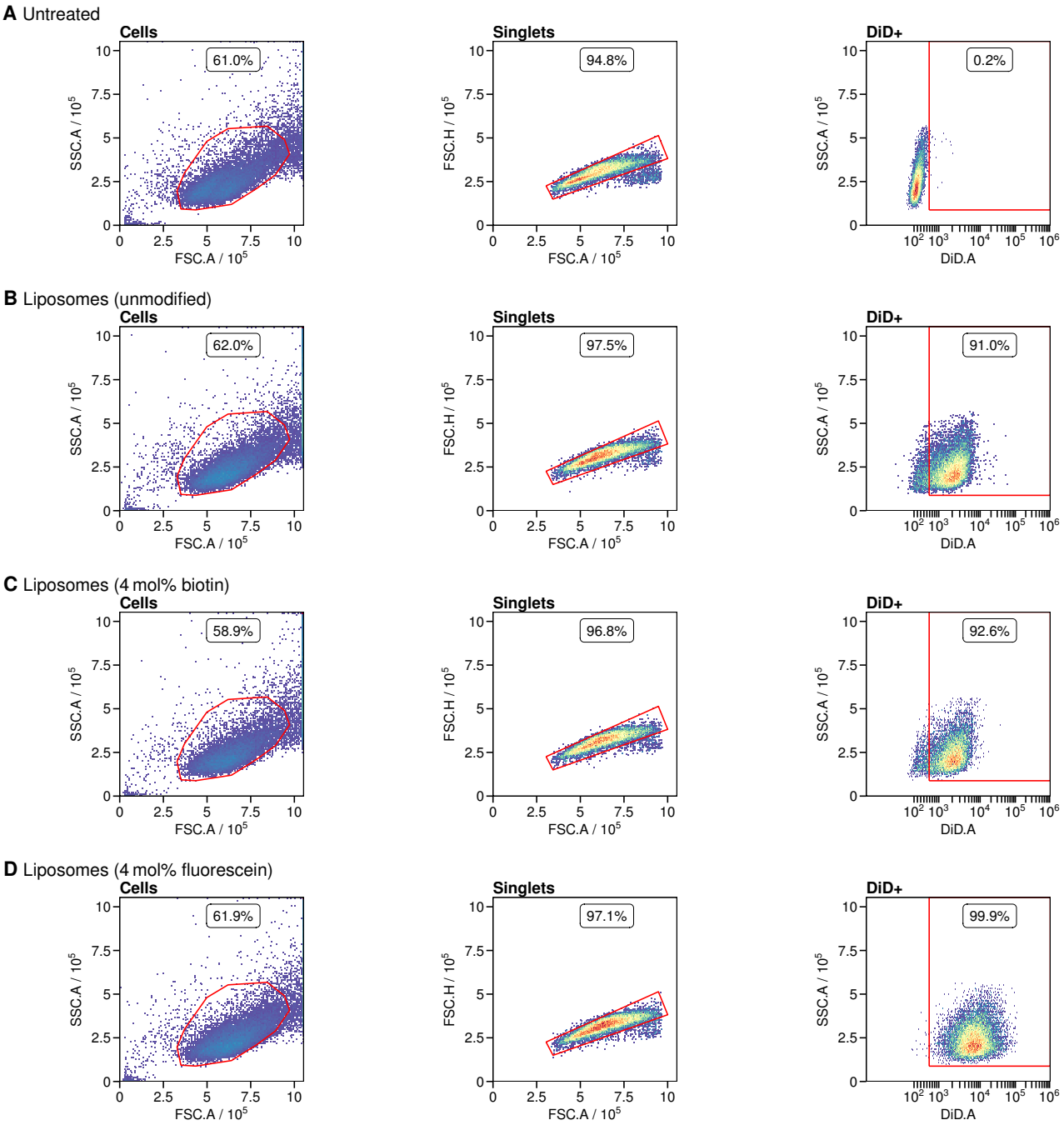


Figure S1: Gating strategy for cell association kinetics.
Representative FACS plots for cell association kinetics presented in Figure 2A. The data shown corresponds to 8 h of incubation with **A** no liposomes (untreated), **B** unmodified liposomes, **C** liposomes with 4 mol% biotin, or **D** liposomes with 4 mol% fluorescein.

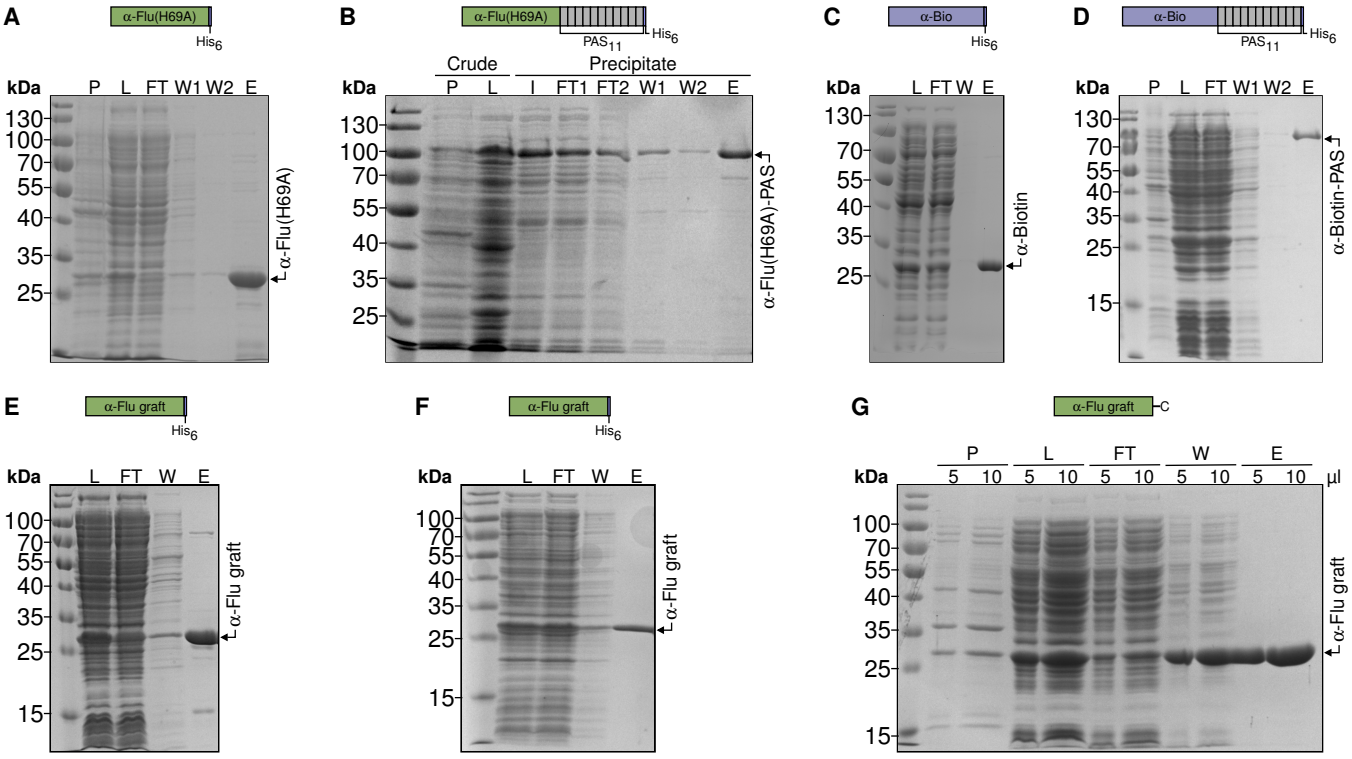


Figure S2: Purification of scFvs.
scFvs binding to fluorescein or biotin were produced in *E. coli* SHuffle T7 Express cells and samples were analyzed at different steps of the purification procedure via SDS PAGE and subsequent staining with Coomassie Brilliant Blue to stain proteins indiscriminately. Fractions: P, pellet (insoluble fraction); L, cleared lysate (soluble fraction); FT, flow-through; W, wash; E, eluate.
A Purification of α-Flu(H69A) by metal affinity chromatography over a TALON Co^{2+} resin.
B Purification of α-Flu(H69A)-PAS by metal affinity chromatography over a Ni^{2+}-NTA resin after precipitation with a 40 % saturated solution of ammonium sulfate. Crude fractions were loaded before precipitation. I, input (resuspended precipitate); FT1/FT2, I was applied to the column twice and separate flowthrough fractions were collected.
C Purification of α-Biotin by affinity chromatography over a Protein L resin.
D Purification of α-Biotin-PAS by affinity chromatography over a Protein L resin.
E, F Purification of α-Flu graft by affinity chromatography over a **E** Ni^{2+}-NTA resin or **F** a Protein A resin.
G Purification of α-Flu graft with a terminal cysteine by affinity chromatography over a Protein A resin.

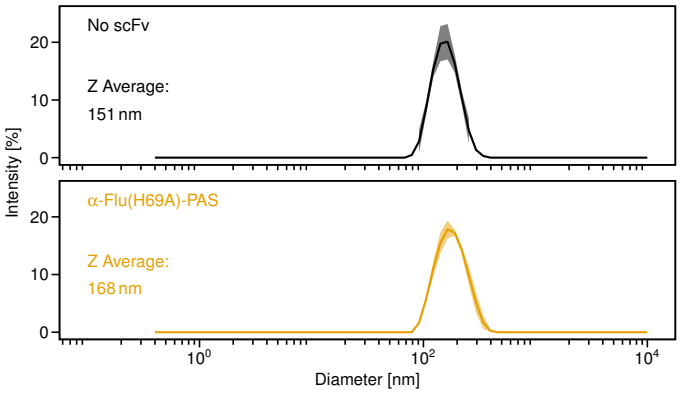


Figure S3: Liposome size increase upon binding of PASylated scFv.
Fluoresceinated liposomes were incubated for 1 h with or without α-Flu(H69A)-PAS and particle size was determined by DLS. $n = 3$ measurements for each protein. Lines mark the mean and ribbons show standard deviation.

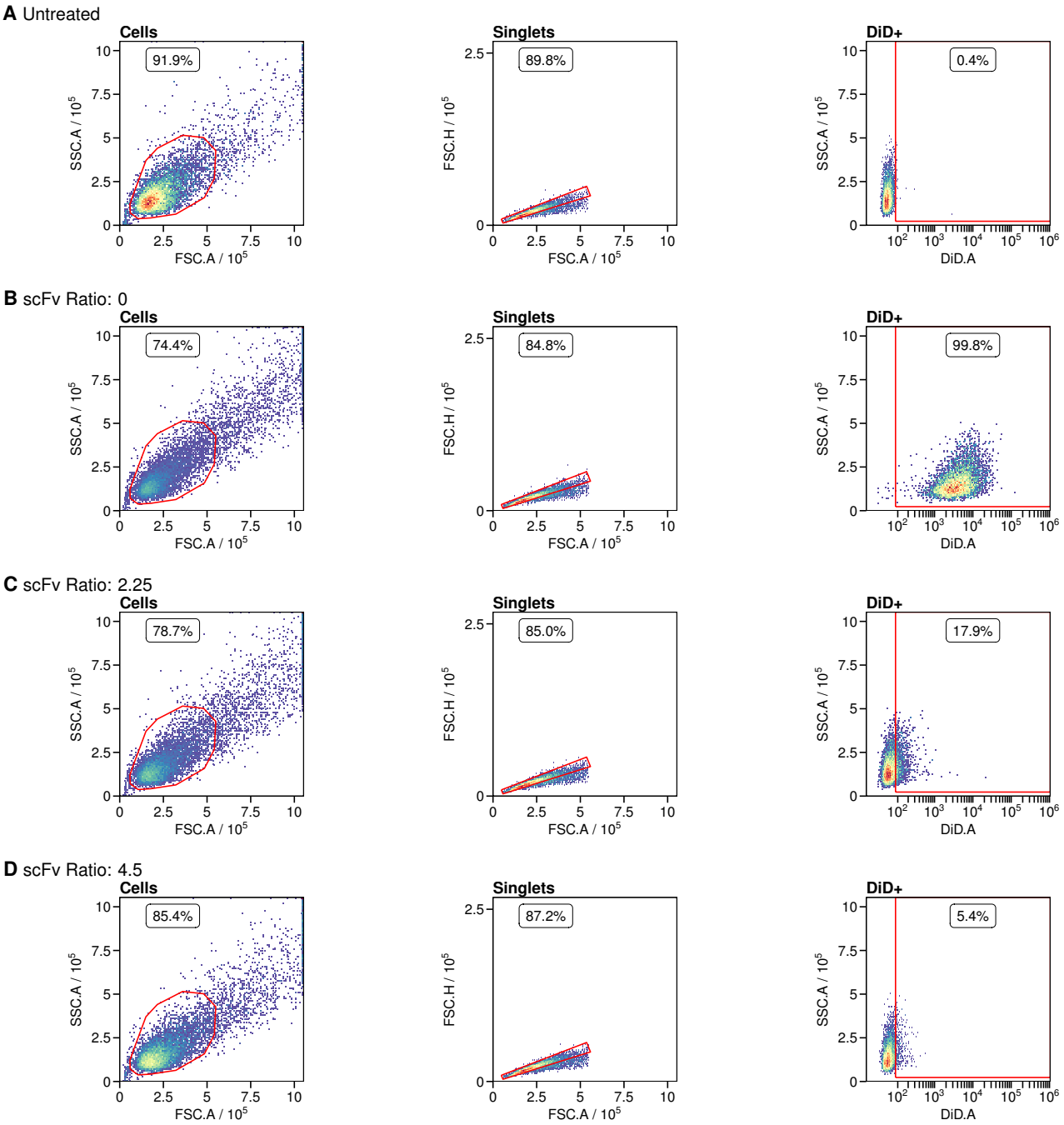


Figure S4: Gating strategy for cell association after shielding of fluoresceinated liposomes.
Representative FACS plots for shielding with α-Flu(H69A)-PAS presented in Figure 2B. **A** No liposomes (untreated). **B, C, D** Liposomes with fluorescein, with an scFv:DSPE-Flu (mol:mol) ratio of **B** 0, **C** 2.25, or **D** 4.5.

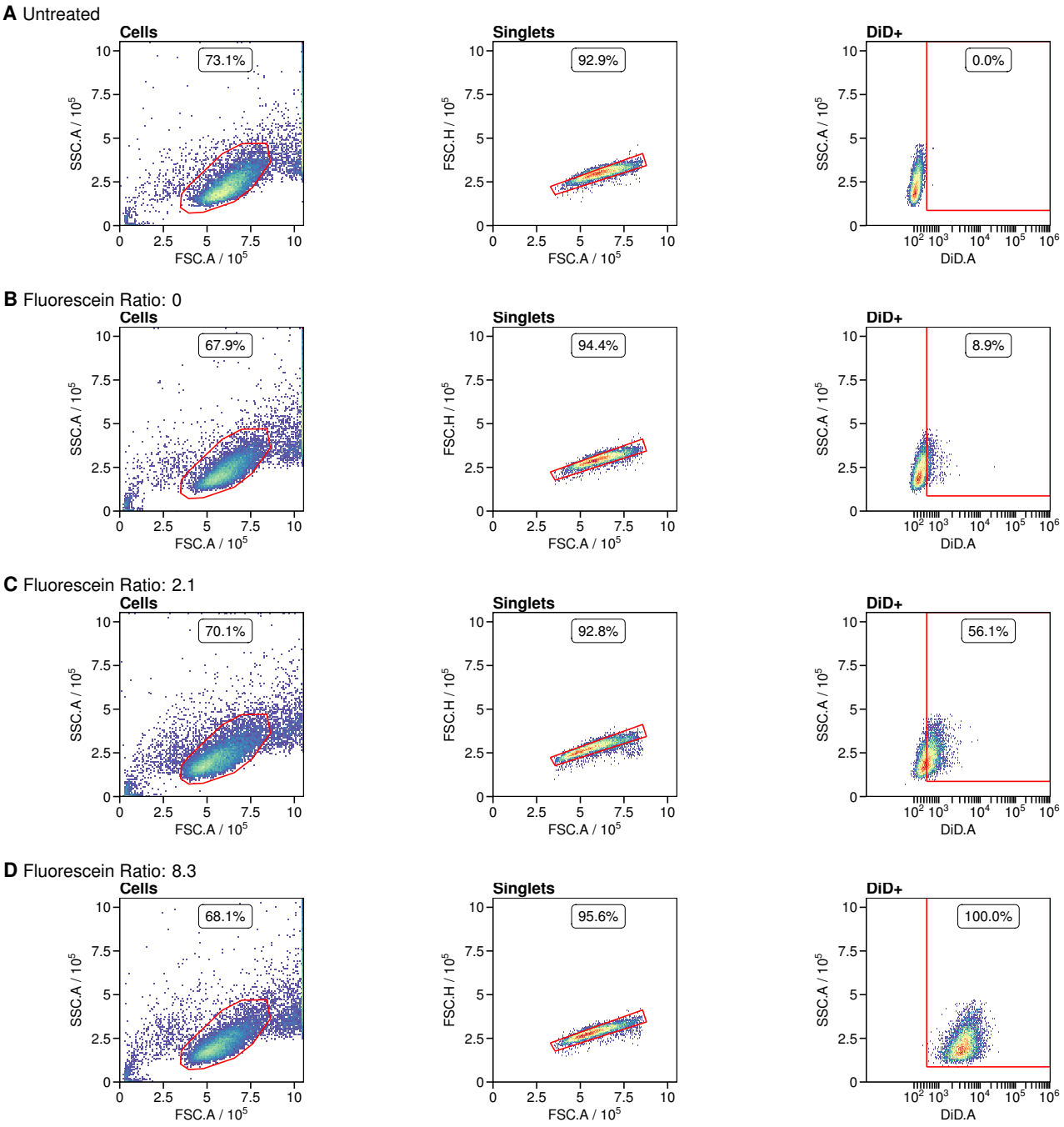


Figure S5: Gating strategy for cell association after deshielding of fluoresceinated liposomes.
Representative FACS plots for deshielding after shielding with α-Flu(H69A)-PAS presented in Figure 2C. **A** No liposomes (untreated). **B, C, D** Liposomes with fluorescein, with an scFv:DSPE-Flu (mol:mol) ratio of 4.5. The ratio of fluorescein:scFv (mol:mol) was **B** 0, **C** 2.1, or **D** 8.3.

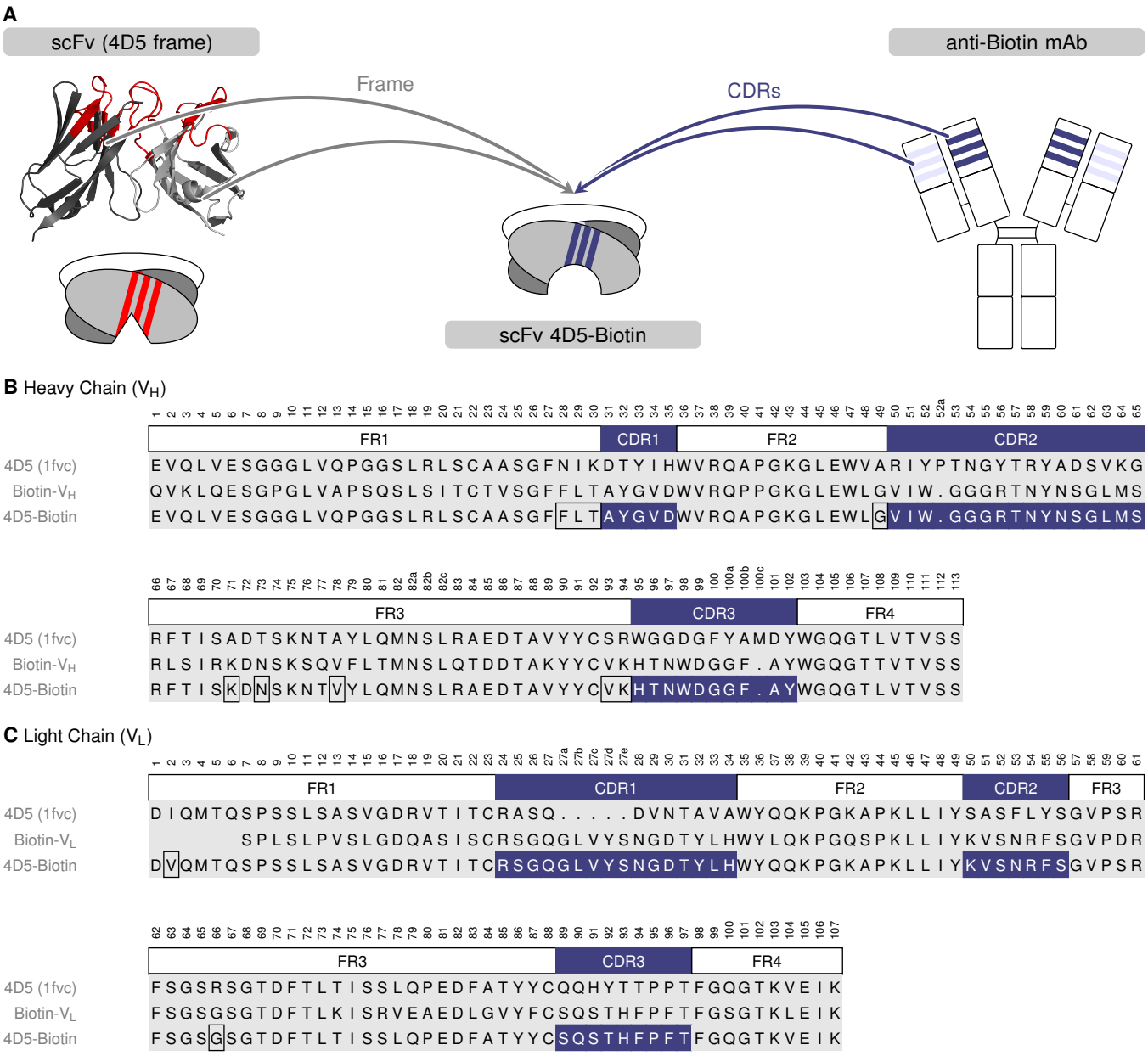
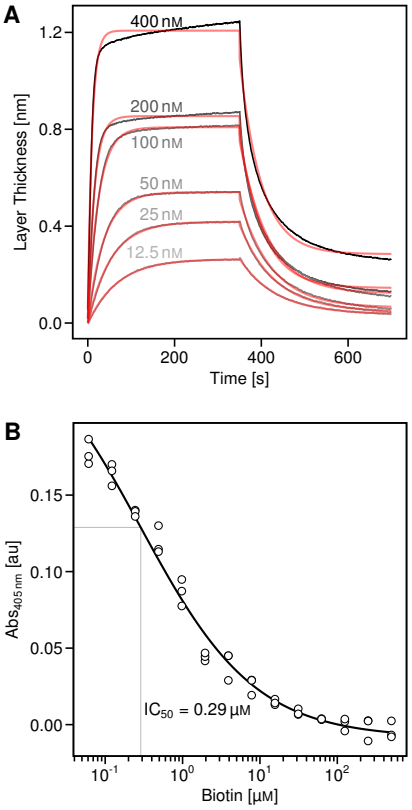


Figure S6: CDR grafting of a biotin-binding scFv.
A Design of the CDR grafting. Framework regions were taken from the 4D5 V_H and V_L consensus sequences and combined with the CDR regions of a biotin-binding murine mAb.[1]
B, C Sequence alignments of the **B** V_H and **C** V_L domains of the CDR acceptor framework (4D5, PDB: 1fvc), the CDR donor construct (Biotin-V_H/Biotin-V_L), and the grafted biotin-binding scFv (4D5-Biotin). The numbering scheme is according to Kabat.[2] CDR regions are highlighted in blue. Residues in the framework regions (FR) that correspond to the Vernier zone[3] (enframed) contribute to the conformation of the CDR loops and were thus matched according to the donor mAb.



c [nM]	K_D [M]	K_{on} ($M^{-1}\,s^{-1}$)	K_{off} (s^{-1})
400.0	1.03×10^{-7}	1.96×10^{5}	$2.01(3) \times 10^{-2}$
200.0	7.97×10^{-8}	2.31×10^{5}	$1.84(2) \times 10^{-2}$
100.0	6.22×10^{-8}	2.46×10^{5}	$1.53(1) \times 10^{-2}$
50.0	5.08×10^{-8}	2.68×10^{5}	$1.36(1) \times 10^{-2}$
25.0	4.90×10^{-8}	2.53×10^{5}	$1.24(1) \times 10^{-2}$
12.5	5.02×10^{-8}	2.34×10^{5}	$1.18(1) \times 10^{-2}$

Figure S7: Characterization of the grafted, biotin-binding scFv.
A Biolayer interferometry sensorgram traces of the association and dissociation of different concentrations of the grafted α-Bio scFv to/from biotin-conjugated BSA. The red curves represent the local fitting according to a 1:1 bimolecular interaction. Fitted parameters are reported in the table.
B Competitive ELISA to determine dissociation curve of α-Bio scFv. 0.47 nM scFv (corresponds to the EC_{50} value of the binding curve in Figure 3B) was mixed with free biotin 30 min prior to the addition to BSA-biotin coated ELISA wells. $n = 3$ for each biotin concentration.

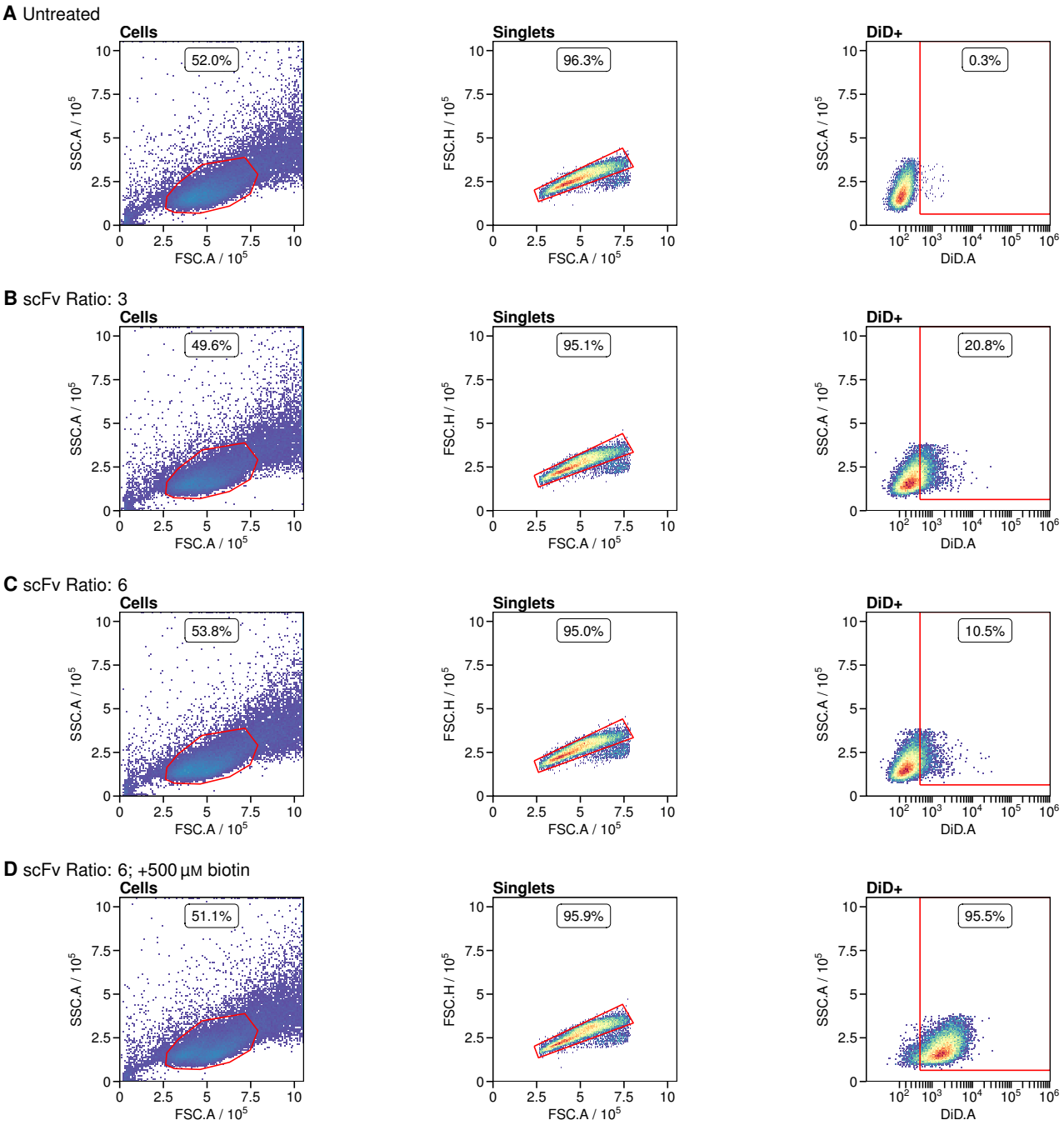


Figure S8: Gating strategy for cell association after shielding and deshielding of biotinylated liposomes. Representative FACS plots for shielding and deshielding with α-Biotin-PAS presented in Figure 4A. **A** No liposomes (untreated). **B, C, D** Liposomes with biotin, with an scFv:DPPE-Bio (mol:mol) ratio of **B** 3, **C** 6, or **D** 6, with addition of 500 µM biotin.

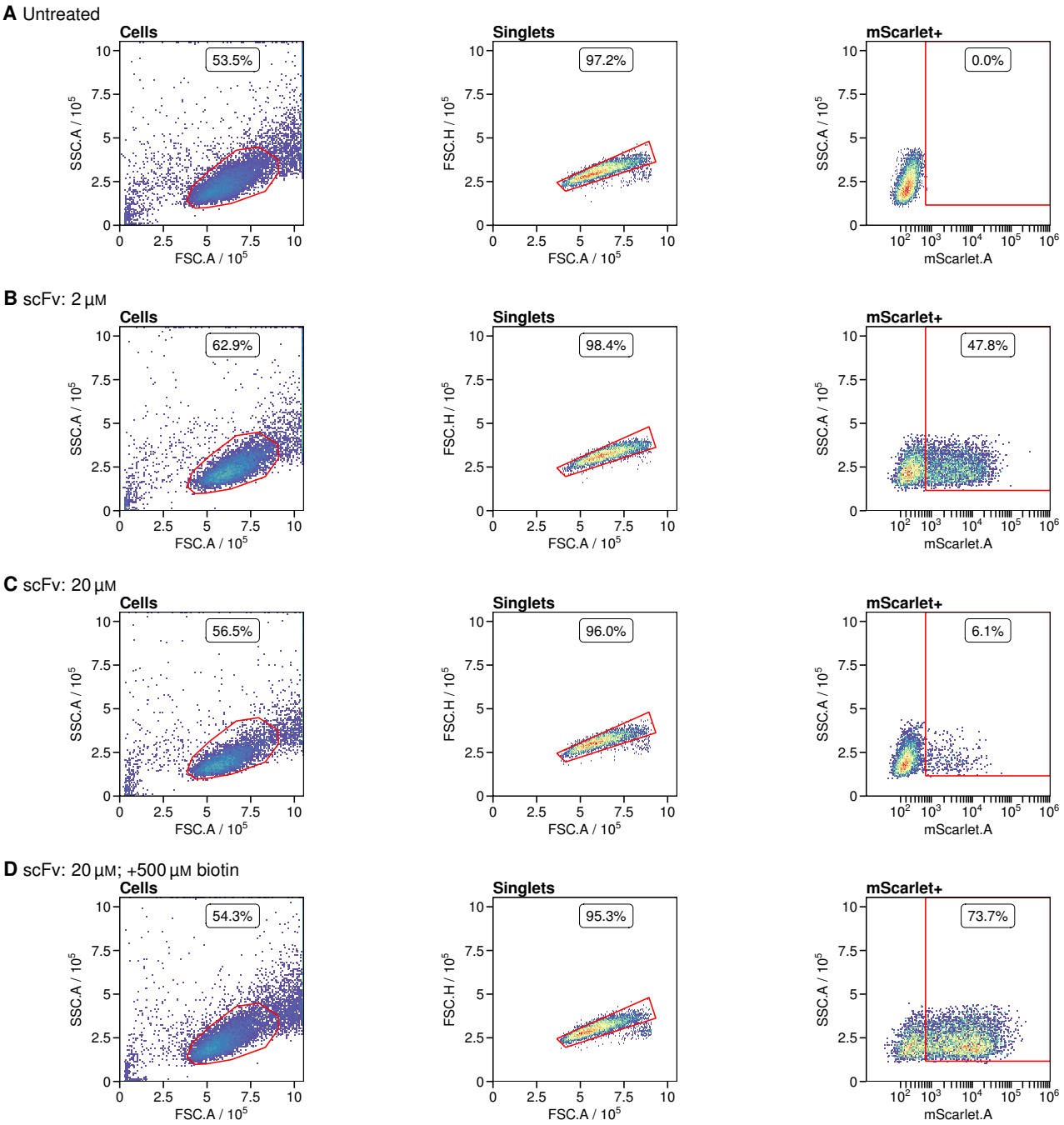


Figure S9: Gating strategy for transduction after shielding and deshielding of biotinylated AAVs.
Representative FACS plots for shielding and deshielding with α-Biotin-PAS presented in Figure 4C. **A** No AAVs (untreated). **B, C, D** AAVs with biotin, with an scFv concentration of **B** 2 µM, **C** 20 µM, or **D** 20 µM, with addition of 500 µM biotin.

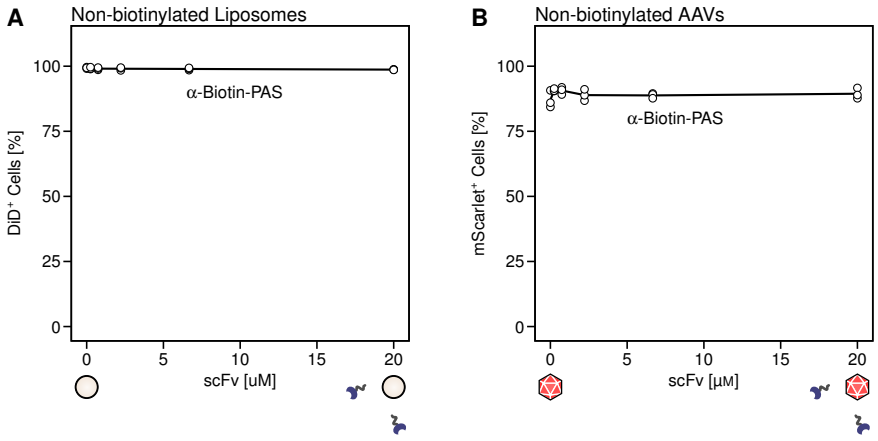


Figure S10: The PASylated biotin-binding scFv does not unspecifically prevent cellular association.
Non-biotinylated liposomes or AAVs were incubated with α-Biotin-PAS and incubated with HeLa cells for 24 h before measurement of cell association (liposomes) or transduction (AAVs) by flow cytometry, as described in Figure 4. **A, B** $n = 3$ for each scFv concentration. **A** An scFv concentration of 20 µM corresponds to the highest scFv:DPPE-Bio (mol:mol) ratio in Figure 4A.

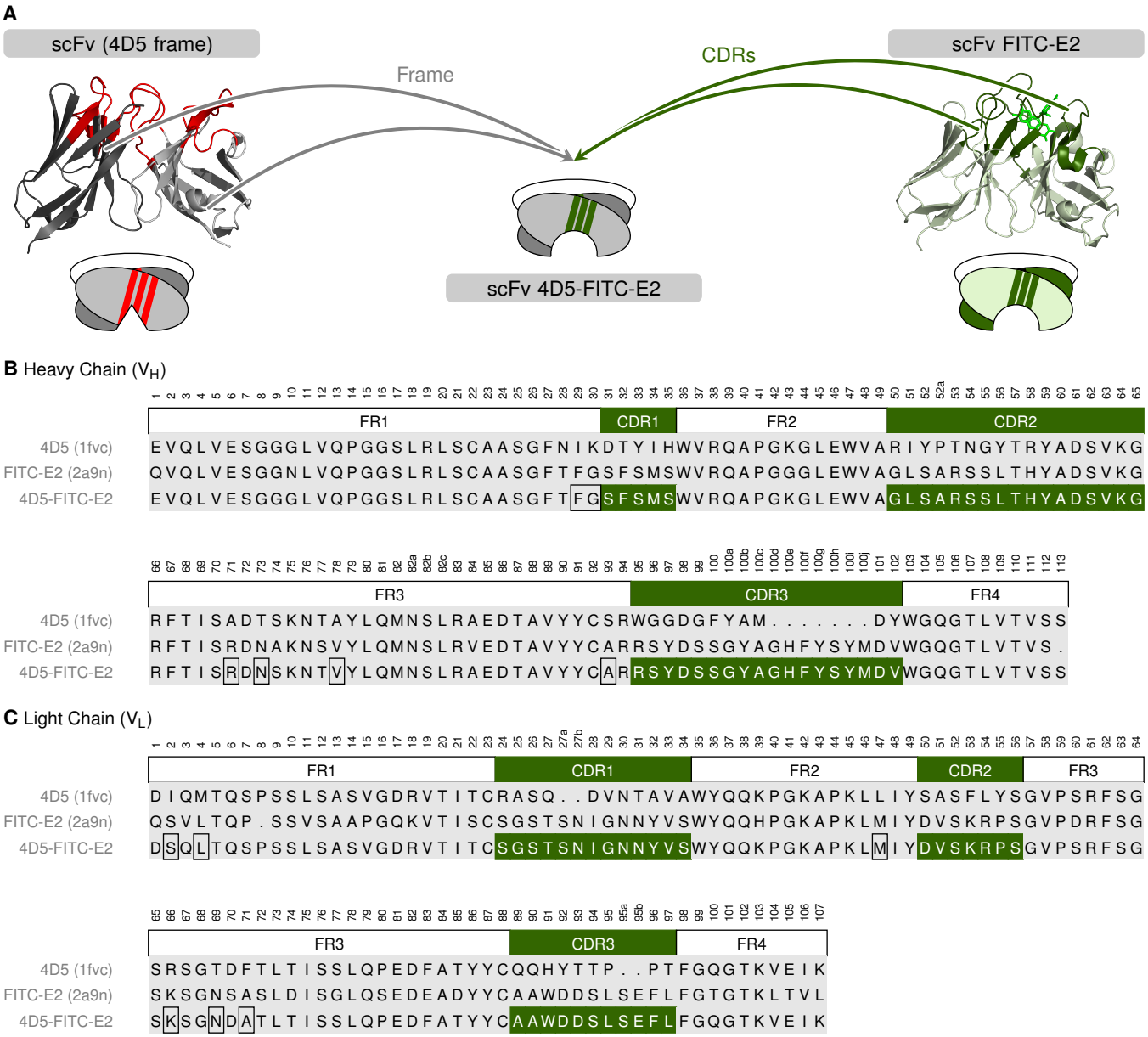


Figure S11: CDR grafting of a fluorescein-binding scFv.
A Design of the CDR grafting. Framework regions were taken from the 4D5 V_H and V_L consensus sequences and combined with the CDR regions of the fluorescein-binding FITC-E2 scFv.
B, C Sequence alignments of the **B** V_H and **C** V_L domains of the CDR acceptor framework (4D5, PDB: 1fvc), the CDR donor construct (FITC-E2, PDB: 2a9n), and the grafted fluorescein-binding scFv (4D5-FITC-E2). The numbering scheme is according to Kabat.[2] CDR regions are highlighted in green. Residues in the framework regions (FR) that correspond to the Vernier zone[3] (enframed) contribute to the conformation of the CDR loops and were thus matched according to the donor scFv.

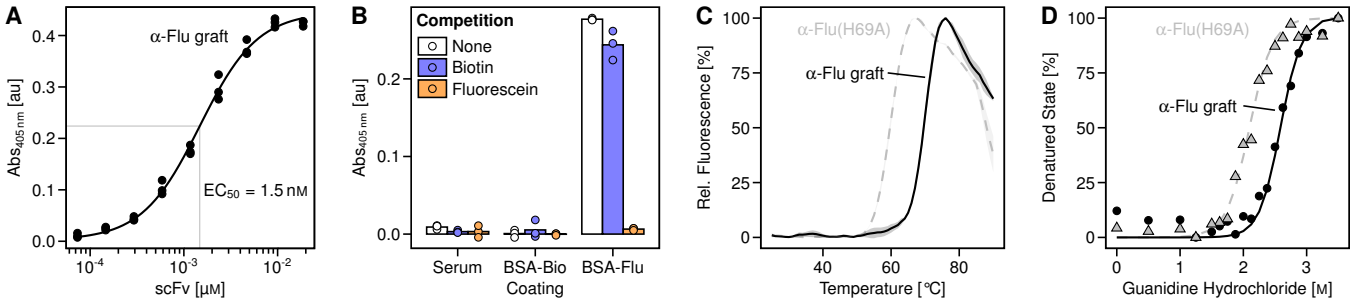
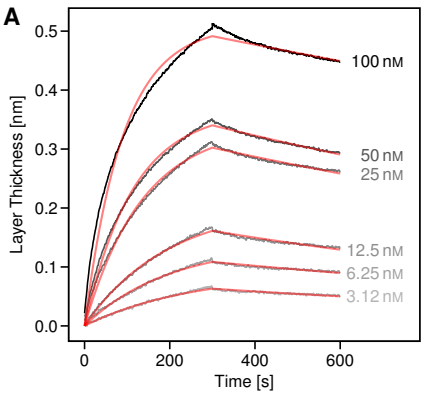


Figure S12: Characterization of a CDR-grafted, fluorescein-binding scFv.
A Binding curve of the grafted α-Flu scFv. The binding capability of the grafted α-Flu scFv to fluorescein-BSA was determined by ELISA. $n = 3$ for each scFv concentration.
B Specificity of the grafted α-Flu scFv. Binding of the scFv to immobilized bovine serum, biotin-BSA or fluorescein-BSA was measured by ELISA. During the binding step, either no free ligand, 500 µM free biotin or 500 µM free fluorescein was added for competition with the immobilized ligand. $n = 3$ for each combination of coating and competition.
C Thermal stability. Melting curves of the grafted scFv and the CDR donor scFv (H69A variant) were measured using the fluorescent dye SYPRO Orange, which fluoresces upon binding to hydrophobic regions of proteins. $n = 2$ for each protein. Lines mark the mean, and ribbons show standard deviation.
D Guanidine hydrochloride denaturation transitions of the grafted and CDR donor (H69A) scFv. The denatured state was determined by measuring the shift in tryptophan fluorescence emission maxima. $n = 1$ for each protein and concentration of guanidine hydrochloride.



c [nM]	K_D [M]	K_{on} ($M^{-1}\,s^{-1}$)	K_{off} (s^{-1})
100.00	$2.77(14) \times 10^{-9}$	$1.08(1) \times 10^{5}$	$2.99(15) \times 10^{-4}$
50.00	$3.16(6) \times 10^{-9}$	$1.66(1) \times 10^{5}$	$5.24(9) \times 10^{-4}$
25.00	$1.91(4) \times 10^{-9}$	$2.74(3) \times 10^{5}$	$5.23(10) \times 10^{-4}$
12.50	$2.07(4) \times 10^{-9}$	$3.55(4) \times 10^{5}$	$7.36(10) \times 10^{-4}$
6.25	$8.96(22) \times 10^{-10}$	$7.10(11) \times 10^{5}$	$6.36(12) \times 10^{-4}$
3.13	$7.29(23) \times 10^{-10}$	$1.06(3) \times 10^{6}$	$7.70(15) \times 10^{-4}$

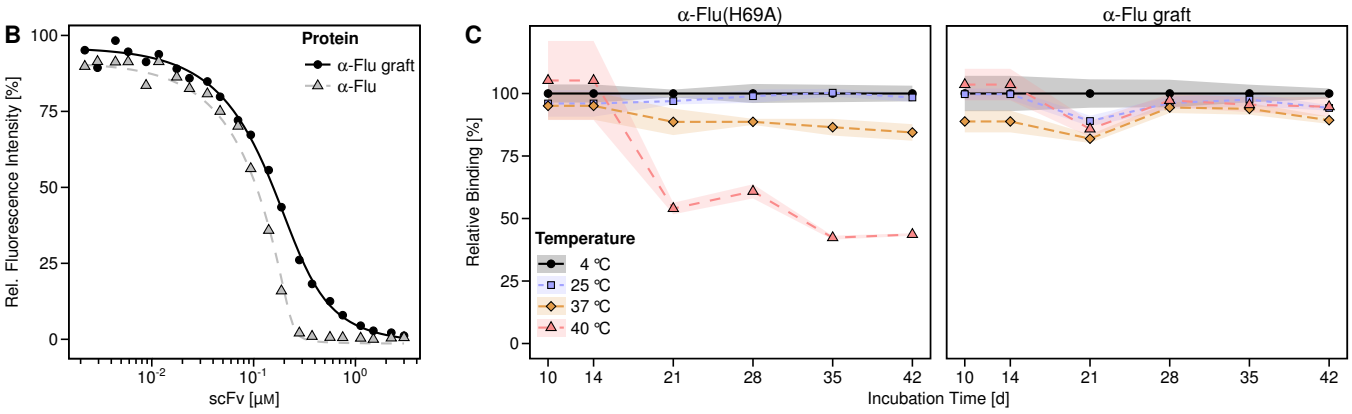


Figure S13: Affinity and stability of the grafted fluorescein-binding scFv.
A Biolayer interferometry sensorgram traces of the association and dissociation of different concentrations of the grafted fluorescein-scFv to/from fluorescein-conjugated BSA. The red curves represent the local fitting according to a 1:1 bimolecular interaction. Fitted parameters are reported in the table.
B Affinity determination by fluorescence quenching of fluorescein. A constant concentration of fluorescein (0.1 µM) was incubated with the indicated concentrations of grafted scFv or CDR donor scFv. Fluorescence intensity was normalized to the condition without added scFv. The fits were calculated as described by Pedrazzi et al.[4] $n = 1$ for each scFv and scFv concentration.
C Long term stability of the grafted scFv. The ungrafted and grafted scFv species were incubated in SBF at different temperatures for up to 6 weeks before measuring their capacity to bind to BSA-Flu by ELISA. For each time point and each protein, the measured signal was normalized to the corresponding signal at 4 °C. $n = 3$ for each protein. The same samples were subjected to ELISA analysis on different days. Symbols represent mean, and ribbons represent standard deviation.

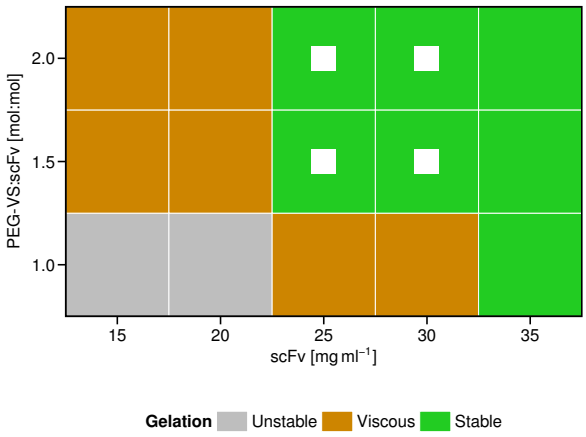


Figure S14: Determination of hydrogel synthesis conditions.
Hydrogels were synthesized with the indicated final protein concentrations and PEG-VS:scFv molar ratios. After 20 h, their polymerization state was qualitatively assessed. If polymerization occurred, gels were placed in PBS with 500 µM fluorescein to determine their dissolution capability by visual inspection. White squares in tiles indicate complete dissolution.

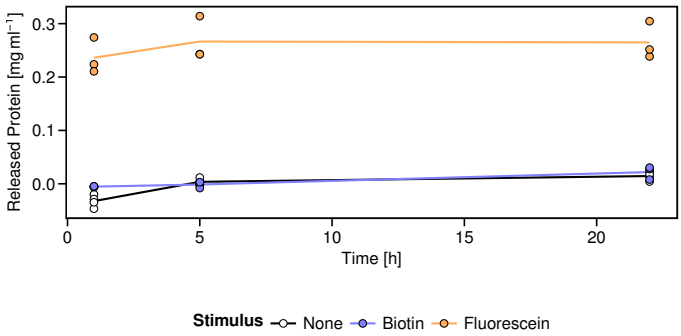


Figure S15: Specific response of fluorescein-triggered hydrogels.
Hydrogels were incubated in PBS, either without an additional stimulus, with 500 µM fluorescein, or with 500 µM biotin. Protein released into the supernatant was quantified with the BCA assay. For gels without stimulus (None), $n = 4$. For gels treated with biotin, $n = 4$ for time points 1–5 h, $n = 3$ for time point 22 h. For gels treated with fluorescein, $n = 3$.

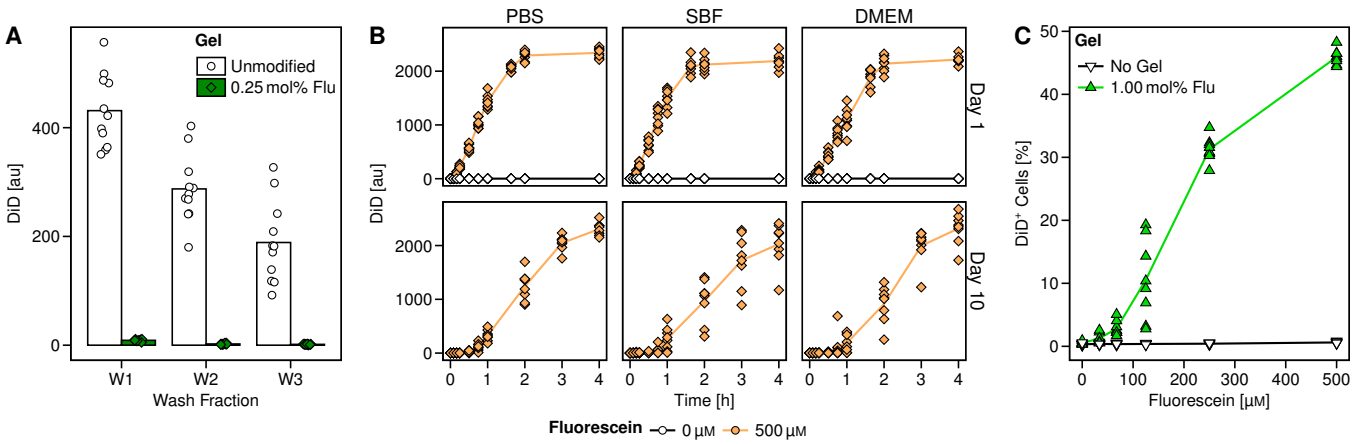


Figure S16: Stability and functionality of liposome-loaded hydrogels.
A Surface-exposed fluorescein on liposomes is required for stable entrapment within the hydrogel framework. After gelation, gels were washed 3 times for 1 h each. Passively escaping liposomes were quantified by measuring DiD fluorescence in the wash fractions. $n = 11$ gels with unmodified liposomes. $n = 12$ gels with liposomes with 0.25 mol% fluorescein.
B Liposome-loaded hydrogels are stable and functional in different buffers. On day 1 after gelation, liposome-loaded hydrogels were placed in PBS, synthetic body fluid (SBF) or DMEM (+FCS, +P/S), with or without fluorescein, and liposome release kinetics were determined by measuring DiD fluorescence in the supernatant. On day 10 after gelation, the previously undissolved hydrogels from day 1 were then placed in buffer containing fluorescein. $n = 8$ gels per dissolution time course.
C Fluorescein dose-dependent uptake of liposomal gel cargo. Gels were placed on HeLa cells in medium with the indicated concentrations of fluorescein. After 24 h, association of liposomes with cells was quantified by flow cytometry. $n = 8$ for each combination of gel/no gel and fluorescein concentration.

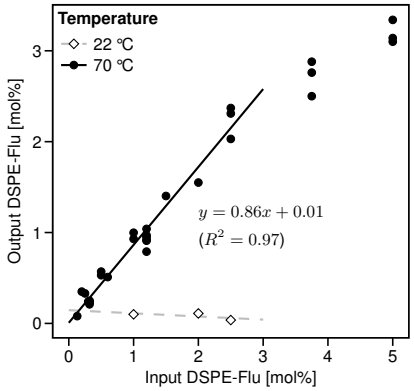


Figure S17: Post-insertion of DSPE-Flu into liposomes.
Post-insertion of DSPE-Flu micelles into preformed liposomes was performed with the amounts required to achieve the saturations indicated on the x axis, assuming 100 % yield. After separation of unincorporated micelles, the fluorescein concentration in the liposome fraction was measured to determine the actual yield of the reaction. Only reactions with input values below 3 mol% were considered for the fit.

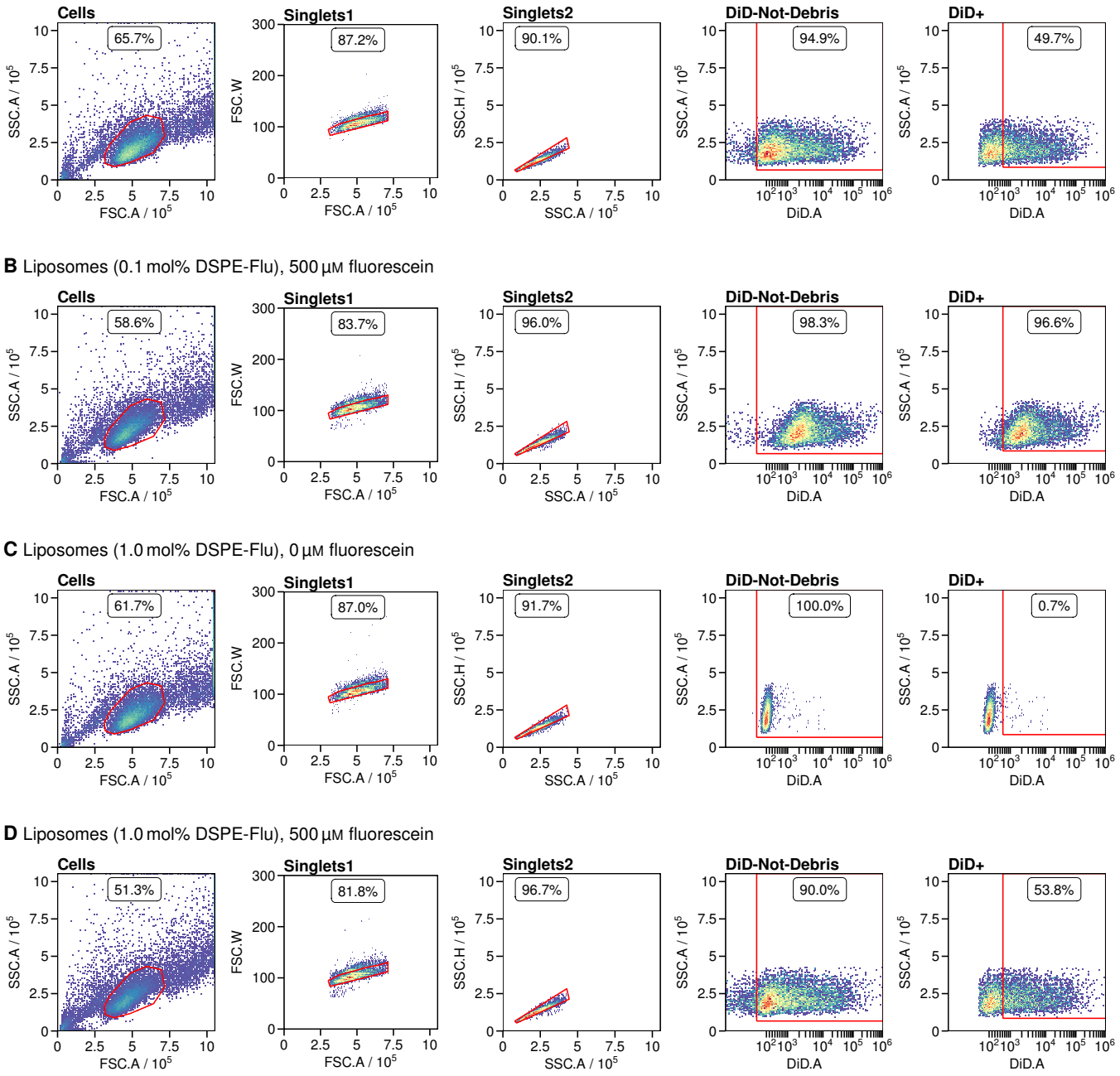


Figure S18: Gating strategy for cell association of liposomes released from gels.
Representative FACS plots for cell association of liposomes with undissolved or dissolved hydrogels presented in Figure 7B. **A** Undissolved hydrogels with liposomes with 0.1 mol% DSPE-Flu. **B** Hydrogels with liposomes with 0.1 mol% DSPE-Flu, dissolved with 500 µM fluorescein. **C** Undissolved hydrogels with liposomes with 1.0 mol% DSPE-Flu. **D** Hydrogels with liposomes 1.0 mol% DSPE-Flu, dissolved with 500 µM fluorescein.

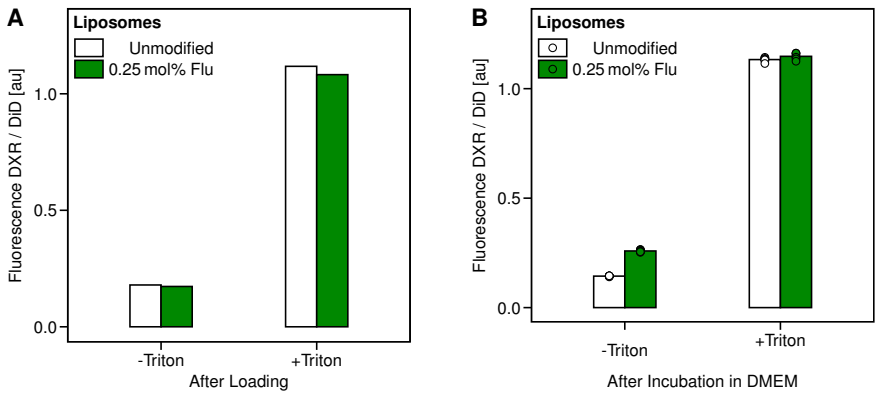


Figure S19: Doxorubicin leakage from fluorescein-modified liposomes.
At high concentrations of doxorubicin (DXR) – ie., when loaded in liposomes – its fluorescence is quenched. Addition of Triton X-100 lyses the liposomes and restores fluorescence.[5] The doxorubicin signal was normalized to the sample's DiD signal. Spillover correction was applied to remove bleeding of the fluorescein signal into the doxorubicin channel. **A** Liposomes were hydrated in ammonium sulfate and a gradient was established. Doxorubicin (DXR) was added to liposomes, and loading was allowed to proceed for 45 min at 70 °C. $n = 1$ loading reaction per liposome type. **B** Subsequently, buffer or DSPE-Flu micelles were added to the reaction and incubation at 70 °C proceeded for a further 45 min. Unencapsulated doxorubicin was removed. $n = 8$ aliquots from the loading reactions shown in **A** were incubated in DMEM without or with Triton X-100 (for lysis of liposomes and complete release of doxorubicin) for 24 h at 37 °C.

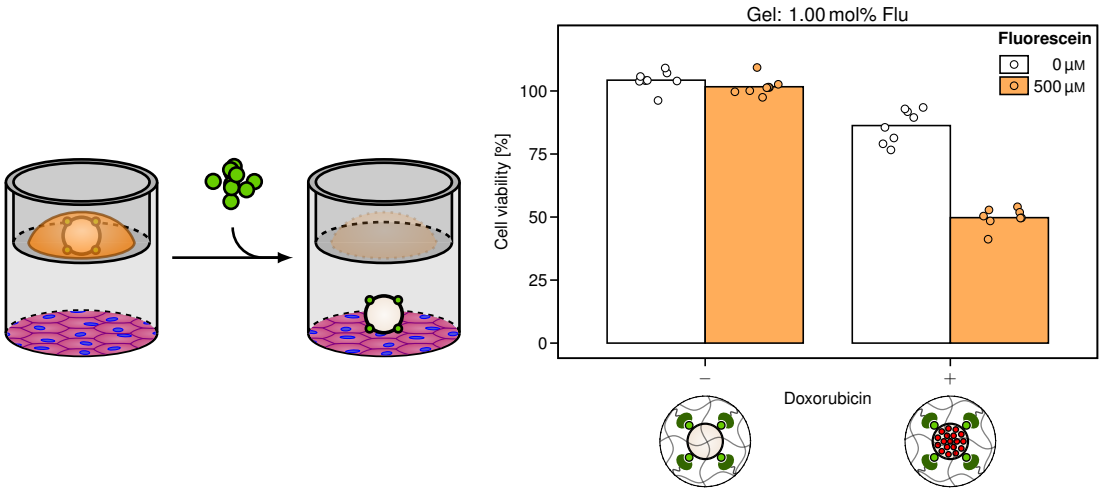


Figure S20: Entrapment of doxorubicin-loaded liposomes in fluorescein-responsive hydrogels.
Liposomes with 1 mol% surface-exposed fluorescein were loaded with doxorubicin and incorporated in hydrogels. Hydrogels were placed in the upper compartment of a transwell plate, with HeLa cells seeded in the lower compartment. The transwell mesh prevented direct contact of the cells with the hydrogels, but was permeable to released liposomes. After 24 h, toxicity was quantified by determining cell viability via WST-1 assay, normalized to the untreated control. $n = 8$ gels for each condition.

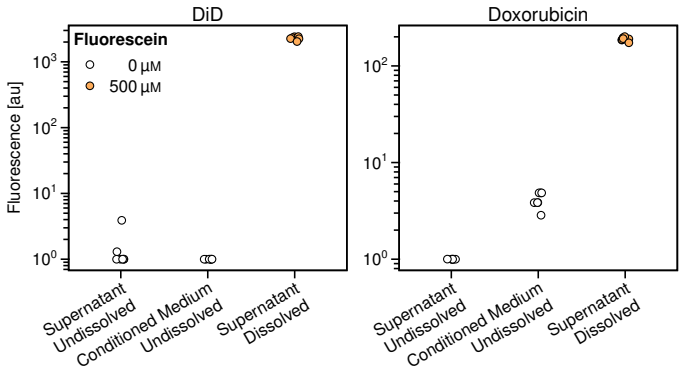


Figure S21: Comparison of liposome and doxorubicin leakage from gels.
Hydrogels were prepared with liposomes with 1 mol% DSPE-Flu and charged with doxorubicin. Samples were taken after synthesis and washing (Supernatant Undissolved) and after incubation on HeLa cells for 24 h (Conditioned Medium Undissolved). Fluorescence of DiD and doxorubicin was measured and background was subtracted. Values for complete release were determined by dissolving gels with 500 µM fluorescein (Supernatant Dissolved). $n = 7$ gels.

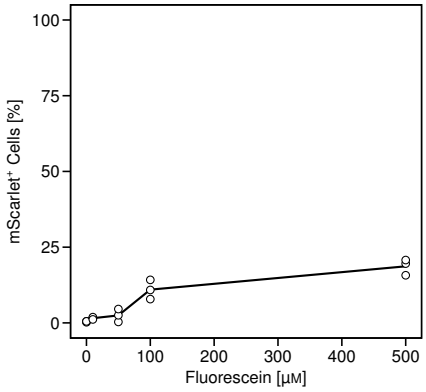


Figure S22: Release of AAVs from fluorescein-responsive hydrogels.
AAVs were labeled with 0.18 mM NHS-fluorescein, concentrated by ultrafiltration and added to the synthesis reaction for fluorescein-responsive hydrogels. The gels were placed on HeLa cells and dissolved by addition of different concentrations of free fluorescein. After 24 h, transduction was quantified by flow cytometry. $n = 3$ gels for each fluorescein concentration.

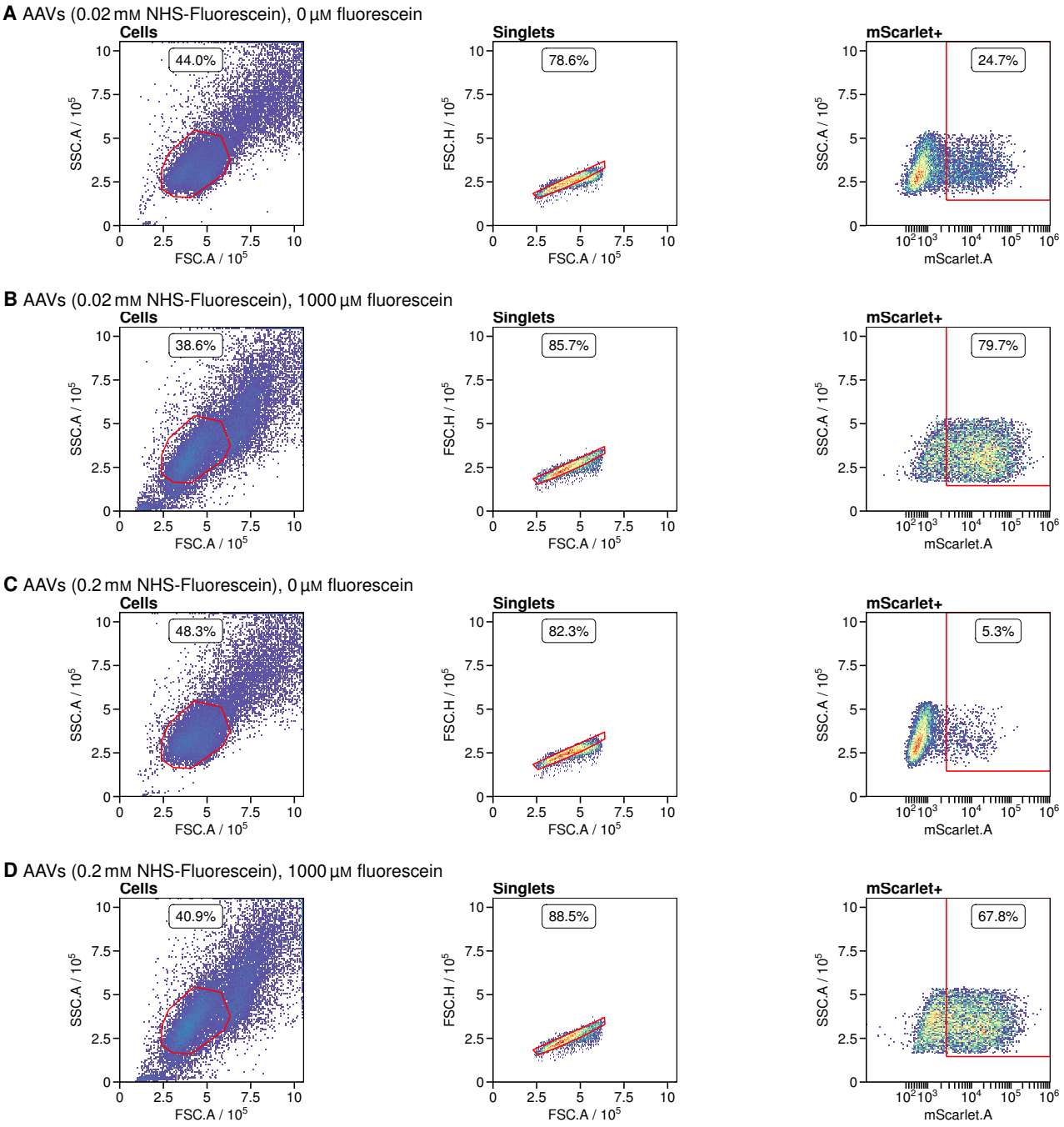
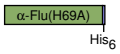
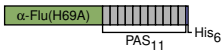
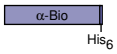
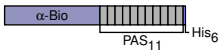


Figure S23: Gating strategy for transduction by AAVs released from gels.
Representative FACS plots for transduction by AAVs with undissolved or dissolved hydrogels presented in Figure 7D. **A** Undissolved hydrogels with AAVs conjugated with 0.02 mM NHS-Fluorescein. **B** Hydrogels with 0.02 mM NHS-Fluorescein, dissolved with 1000 µM fluorescein. **C** Undissolved hydrogels with 0.2 mM NHS-Fluorescein. **D** Hydrogels with 0.2 mM NHS-Fluorescein, dissolved with 1000 µM fluorescein.

Constructs and Sequences

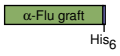
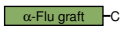
Table S1: Constructs, expression conditions and sequences.

Plasmid / Construct	DNA Sequence
pBR100 α-Flu(H69A)  *Host*: *E. coli* T7 SHuffle Express[6] *Promoter*: T7 *Expression*: 20 h at 25 °C in TB medium *Purification*: IMAC (Co^{2+}) *or* Protein A	ATGCAGGTGCAGCTGGTGGAGTCTGGGGGAAACTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGC AGCCTCTGGATTCACCTTTGGCAGCTTTGCCATGAGCTGGGTCCGCCAGGCTCCAGGGGGGGGGCTGGAGT GGGTCGCAGGTCTGAGTGCTCGTAGTAGTCTCACAGCATATGCAGACTCCGTGAAGGGCCGGTTCACCATC TCCAGAGACAACGCCAAGAACTCAGTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCTGTGTA TTACTGTGCGAGAAGATCGTATGATAGTAGTGGTTATTGGGGCCACTTCTACTCCTACATGGACGTCTGGG GCCAAGGCACCCTGGTCACCGTCTCGAGTGGTGGAGGCGGTTCAGGCGGAGGTGGCAGCGGCGGTGGCGGA TCGCAGTCTGTGTTGACGCAGCCGTCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTCACCATTTCCTGCTC TGGAAGCACCTCCAACATTGGGAATAATTATGTCTCCTGGTACCAACAGCACCCAGGCAAAGCCCCCAAAC TCATGATTTATGATGTCAGTAAGCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCAAC TCAGCCTCCCTGGACATCAGTGGGCTCCAGTCTGAGGATGAGGCTGATTATTACTGTGCAGCATGGGATGA CAGCCTGAGTGAATTTCTCTTCGGAACTGGGACCAAGCTGACCGTCCTAGGGGCCTCGGGGGCCGATCACC ATCATCACCATCATTAG
pOT009 α-Flu(H69A)-PAS  *Host*: *E. coli* T7 SHuffle Express[6] *Promoter*: T7 *Expression*: 20 h at 25 °C in TB medium *Purification*: IMAC (Ni^{2+}) after precipitation in 40 % ammonium sulfate *or* Protein A	ATGCAGGTGCAGCTGGTGGAGTCTGGGGGAAACTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGC AGCCTCTGGATTCACCTTTGGCAGCTTTGCCATGAGCTGGGTCCGCCAGGCTCCAGGGGGGGGGCTGGAGT GGGTCGCAGGTCTGAGTGCTCGTAGTAGTCTCACAGCATATGCAGACTCCGTGAAGGGCCGGTTCACCATC TCCAGAGACAACGCCAAGAACTCAGTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCTGTGTA TTACTGTGCGAGAAGATCGTATGATAGTAGTGGTTATTGGGGCCACTTCTACTCCTACATGGACGTCTGGG GCCAAGGCACCCTGGTCACCGTCTCGAGTGGTGGAGGCGGTTCAGGCGGAGGTGGCAGCGGCGGTGGCGGA TCGCAGTCTGTGTTGACGCAGCCGTCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTCACCATTTCCTGCTC TGGAAGCACCTCCAACATTGGGAATAATTATGTCTCCTGGTACCAACAGCACCCAGGCAAAGCCCCCAAAC TCATGATTTATGATGTCAGTAAGCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCAAC TCAGCCTCCCTGGACATCAGTGGGCTCCAGTCTGAGGATGAGGCTGATTATTACTGTGCAGCATGGGATGA CAGCCTGAGTGAATTTCTCTTCGGAACTGGGACCAAGCTGACCGTCCTAGGGGCCTCGGGGGCCGATGGCT CTTCTGCCTCTCCAGCTGCACCTGCTCCAGCAAGCCCTGCTGCACCAGCTCCGTCTGCTCCTGCTGCCTCT CCAGCTGCACCTGCTCCAGCAAGCCCTGCTGCACCAGCTCCGTCTGCTCCTGCTGCCTCTCCAGCTGCACC TGCTCCAGCAAGCCCTGCTGCACCAGCTCCGTCTGCTCCTGCTGCCTCTCCAGCTGCACCTGCTCCAGCAA GCCCTGCTGCACCAGCTCCGTCTGCTCCTGCTGCCTCTCCAGCTGCACCTGCTCCAGCAAGCCCTGCTGCA CCAGCTCCGTCTGCTCCTGCTGCCTCTCCAGCTGCACCTGCTCCAGCAAGCCCTGCTGCACCAGCTCCGTC TGCTCCTGCTGCCTCTCCAGCTGCACCTGCTCCAGCAAGCCCTGCTGCACCAGCTCCGTCTGCTCCTGCTG CCTCTCCAGCTGCACCTGCTCCAGCAAGCCCTGCTGCACCAGCTCCGTCTGCTCCTGCTGCCTCTCCAGCT GCACCTGCTCCAGCAAGCCCTGCTGCACCAGCTCCGTCTGCTCCTGCTGCCTCTCCAGCTGCACCTGCTCC AGCAAGCCCTGCTGCACCAGCTCCGTCTGCTCCTGCTGCCTCTCCAGCTGCACCTGCTCCAGCAAGCCCTG CTGCACCAGCTCCGTCTGCTCCTGCTGCCCACCATCATCACCATCATTAG
Highlighted: His Tag PAS Repeat	*continued on next page*

Plasmid / Construct	DNA Sequence
pHJW275 α-Biotin  *Host*: *E. coli* T7 SHuffle Express[6] *Promoter*: T7 *Expression*: 20 h at 18 °C in LB medium *Purification*: Protein L	ATGGAAGTTCAGCTGGTTGAATCTGGTGGTGGTCTGGTTCAGCCGGGTGGTTCTCTGCGTCTGTCTTGCGC TGCTTCTGGTTTCTTCCTGACCGCTTACGGTGTTGACTGGGTTCGTCAGGCTCCGGGTAAAGGTCTGGAAT GGCTGGGTGTTATCTGGGGTGGTGGTCGTACCAACTACAACTCTGGTCTGATGTCTCGTTTCACCATCTCT AAAGACAACTCTAAAAACACCGTTTACCTGCAGATGAACTCTCTGCGTGCTGAAGACACCGCTGTTTACTA CTGCGTTAAACACACCAACTGGGACGGTGGTTTCGCTTACTGGGGTCAGGGTACCCTGGTTACCGTTTCTT CTGGTGGAGGCGGTTCAGGCGGAGGTGGCAGCGGCGGTGGCGGATCGGGTGGAGGCGGTTCAGACGTTCAG ATGACCCAGTCTCCGTCTTCTCTGTCTGCTTCTGTTGGTGACCGTGTTACCATCACCTGCCGTTCTGGTCA GGGTCTGGTTTACTCTAACGGTGACACCTACCTGCACTGGTACCAGCAGAAACCGGGTAAAGCTCCGAAAC TGCTGATCTACAAAGTTTCTAACCGTTTCTCTGGTGTTCCGTCTCGTTTCTCTGGTTCTGGTTCTGGTACC GACTTCACCCTGACCATCTCTTCTCTGCAGCCGGAAGACTTCGCTACCTACTACTGCTCTCAGTCTACCCA CTTCCCGTTCACCTTCGGTCAGGGTACCAAAGTTGAAATCAAACGTGGTAGCGGCAGCGGTAGCCATCATC ACCATCATCATTGA
pOT200 α-Biotin-PAS  *Host*: *E. coli* T7 SHuffle Express[6] *Promoter*: T7 *Expression*: 20 h at 18 °C in LB medium *Purification*: Protein L	ATGGAAGTTCAGCTGGTTGAATCTGGTGGTGGTCTGGTTCAGCCGGGTGGTTCTCTGCGTCTGTCTTGCGC TGCTTCTGGTTTCTTCCTGACCGCTTACGGTGTTGACTGGGTTCGTCAGGCTCCGGGTAAAGGTCTGGAAT GGCTGGGTGTTATCTGGGGTGGTGGTCGTACCAACTACAACTCTGGTCTGATGTCTCGTTTCACCATCTCT AAAGACAACTCTAAAAACACCGTTTACCTGCAGATGAACTCTCTGCGTGCTGAAGACACCGCTGTTTACTA CTGCGTTAAACACACCAACTGGGACGGTGGTTTCGCTTACTGGGGTCAGGGTACCCTGGTTACCGTTTCTT CTGGTGGAGGCGGTTCAGGCGGAGGTGGCAGCGGCGGTGGCGGATCGGGTGGAGGCGGTTCAGACGTTCAG ATGACCCAGTCTCCGTCTTCTCTGTCTGCTTCTGTTGGTGACCGTGTTACCATCACCTGCCGTTCTGGTCA GGGTCTGGTTTACTCTAACGGTGACACCTACCTGCACTGGTACCAGCAGAAACCGGGTAAAGCTCCGAAAC TGCTGATCTACAAAGTTTCTAACCGTTTCTCTGGTGTTCCGTCTCGTTTCTCTGGTTCTGGTTCTGGTACC GACTTCACCCTGACCATCTCTTCTCTGCAGCCGGAAGACTTCGCTACCTACTACTGCTCTCAGTCTACCCA CTTCCCGTTCACCTTCGGTCAGGGTACCAAAGTTGAAATCAAACGTGGCTCTTCTGCCTCTCCAGCTGCAC CTGCTCCAGCAAGCCCTGCTGCACCAGCTCCGTCTGCTCCTGCTGCCTCTCCAGCTGCACCTGCTCCAGCA AGCCCTGCTGCACCAGCTCCGTCTGCTCCTGCTGCCTCTCCAGCTGCACCTGCTCCAGCAAGCCCTGCTGC ACCAGCTCCGTCTGCTCCTGCTGCCTCTCCAGCTGCACCTGCTCCAGCAAGCCCTGCTGCACCAGCTCCGT CTGCTCCTGCTGCCTCTCCAGCTGCACCTGCTCCAGCAAGCCCTGCTGCACCAGCTCCGTCTGCTCCTGCT GCCTCTCCAGCTGCACCTGCTCCAGCAAGCCCTGCTGCACCAGCTCCGTCTGCTCCTGCTGCCTCTCCAGC TGCACCTGCTCCAGCAAGCCCTGCTGCACCAGCTCCGTCTGCTCCTGCTGCCTCTCCAGCTGCACCTGCTC CAGCAAGCCCTGCTGCACCAGCTCCGTCTGCTCCTGCTGCCTCTCCAGCTGCACCTGCTCCAGCAAGCCCT GCTGCACCAGCTCCGTCTGCTCCTGCTGCCTCTCCAGCTGCACCTGCTCCAGCAAGCCCTGCTGCACCAGC TCCGTCTGCTCCTGCTGCCTCTCCAGCTGCACCTGCTCCAGCAAGCCCTGCTGCACCAGCTCCGTCTGCTC CTGCTGCCCACCATCATCACCATCATTAG

Highlighted: His Tag PAS Repeat

continued on next page

Plasmid / Construct	DNA Sequence
pHJW17 α-Flu graft  *Host*: *E. coli* T7 SHuffle Express[6] *Promoter*: T7 *Expression*: 20 h at 18 °C in TB medium *Purification*: Protein A	ATGGAAGTTCAGCTGGTTGAATCTGGTGGTGGTCTGGTTCAGCCGGGTGGTTCTCTGCGTCTGTCTTGCGC TGCTTCTGGTTTCACCTTCGGTTCTTTCTCTATGTCTTGGGTTCGTCAGGCTCCGGGTAAAGGTCTGGAAT GGGTTGCTGGTCTGTCTGCTCGTTCTTCTCTGACCCACTACGCTGACTCTGTTAAAGGTCGTTTCACCATC TCTCGTGACAACTCTAAAAACACCGTTTACCTGCAGATGAACTCTCTGCGTGCTGAAGACACCGCTGTTTA CTACTGCGCTCGTCGTTCTTACGACTCTTCTGGTTACGCTGGTCACTTCTACTCTTACATGGACGTTTGGG GTCAGGGTACCCTGGTTACCGTTTCTTCTGGTGGAGGCGGTTCAGGCGGAGGTGGCAGCGGCGGTGGCGGA TCGGGTGGAGGCGGTTCAGACTCTCAGCTGACCCAGTCTCCGTCTTCTCTGTCTGCTTCTGTTGGTGACCG TGTTACCATCACCTGCTCTGGTTCTACCTCTAACATCGGTAACAACTACGTTTCTTGGTACCAGCAGAAAC CGGGTAAAGCTCCGAAACTGATGATCTACGACGTTTCTAAACGTCCGTCTGGTGTTCCGTCTCGTTTCTCT GGTTCTAAATCTGGTAACGACGCTACCCTGACCATCTCTTCTCTGCAGCCGGAAGACTTCGCTACCTACTA CTGCGCTGCTTGGGACGACTCTCTGTCTGAATTCCTGTTCGGTCAGGGTACCAAAGTTGAAATCAAACGTC ATCATCACCATCATCATTAA
pHJW121 α-Flu graft (terminal Cys)  *Host*: *E. coli* T7 SHuffle Express[6] *Promoter*: T7 *Expression*: 20 h at 18 °C in TB medium *Purification*: Protein A	ATGGAAGTTCAGCTGGTTGAATCTGGTGGTGGTCTGGTTCAGCCGGGTGGTTCTCTGCGTCTGTCTTGCGC TGCTTCTGGTTTCACCTTCGGTTCTTTCTCTATGTCTTGGGTTCGTCAGGCTCCGGGTAAAGGTCTGGAAT GGGTTGCTGGTCTGTCTGCTCGTTCTTCTCTGACCCACTACGCTGACTCTGTTAAAGGTCGTTTCACCATC TCTCGTGACAACTCTAAAAACACCGTTTACCTGCAGATGAACTCTCTGCGTGCTGAAGACACCGCTGTTTA CTACTGCGCTCGTCGTTCTTACGACTCTTCTGGTTACGCTGGTCACTTCTACTCTTACATGGACGTTTGGG GTCAGGGTACCCTGGTTACCGTTTCTTCTGGTGGAGGCGGTTCAGGCGGAGGTGGCAGCGGCGGTGGCGGA TCGGGTGGAGGCGGTTCAGACTCTCAGCTGACCCAGTCTCCGTCTTCTCTGTCTGCTTCTGTTGGTGACCG TGTTACCATCACCTGCTCTGGTTCTACCTCTAACATCGGTAACAACTACGTTTCTTGGTACCAGCAGAAAC CGGGTAAAGCTCCGAAACTGATGATCTACGACGTTTCTAAACGTCCGTCTGGTGTTCCGTCTCGTTTCTCT GGTTCTAAATCTGGTAACGACGCTACCCTGACCATCTCTTCTCTGCAGCCGGAAGACTTCGCTACCTACTA CTGCGCTGCTTGGGACGACTCTCTGTCTGAATTCCTGTTCGGTCAGGGTACCAAAGTTGAAATCAAACGTG GTGGAGGCGGTTCAGGCTGTTAA

Highlighted: His Tag PAS Repeat

Supplementary References

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6. Lobstein, J. *et al.* SHuffle, a Novel Escherichia Coli Protein Expression Strain Capable of Correctly Folding Disulfide Bonded Proteins in Its Cytoplasm. *Microb. Cell Factories* **11,** 56 (2012).