Protein charge parameters that influence stability and cellular internalization of PEC micelles

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Supporting Information

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S.1 Materials and Methods

Unless otherwise noted, all competent cells were purchased from New England Biolabs and affinity resin was purchased from ThermoFisher. All media components and chemicals were purchased from Sigma Aldrich or Fisher Scientific and were used as received unless indicated otherwise.

S.2 Protein Preparation

The anionic proteins used in this study and their primary sequences have been reported previously.[1] The charge that is reported for each protein is the expected charge at pH 7.4 based on the primary amino acid sequence and the following isolated amino acid pK_a values: 12.48 (Arg), 3.65 (Asp), 4.25 (Glu), 6.00 (His), 10.53 (Lys), 10.07 (Tyr), 9.42 (N-terminus), 2.18 (C-terminus). The predicted charge (with and without histidine included) is summarized in Table S1. A summary of the mutations (relative to superfolder GFP) in each protein can be found in Table S1.

All GFP mutants were expressed in NiCo21(DE3) cells in 1 L cultures of LB media supplemented with 100 μg/mL ampicillin. Cultures were incubated at 37 °C with shaking at 250 rpm. Cultures were grown to an OD_{600} of ca. 0.8-1.0 and were subsequently induced by addition of 1 mL of 1 M isopropyl β-D-1-thiogalactopyranoside (IPTG). Cultures were incubated at 25 °C for 16-18 h after induction.

Cells were harvested by centrifugation (4000 rpm for 15 min) and cell pellets were resuspended in lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, pH 8.0; 15 mL buffer per L of culture). Protease inhibitor cocktail (Sigma P8849) was added to the resuspended cell pellet prior to lysis (100 μL per L of culture). The cells were lysed by sonication and cell debris was removed by centrifugation at 10,000 rpm for 30 min. The soluble protein was purified using Ni-NTA affinity chromatography according to the manufacturer's instructions with the following modifications: (i) 6 mL of resin was used per L of culture, (ii) imidazole was eliminated from the lysis buffer, (iii) the imidazole concentration in the wash buffer was increased to 35 mM, (iv) the volume of wash and elution buffer was optimized to maximize protein yield and purity. The flow through, wash, and elution fractions were analyzed by SDS-PAGE and pure fractions were combined and concentrated via centrifugal ultrafiltration with a 10 kDa molecular weight cutoff (MWCO) filter.

Protein solutions were prepared by dialyzing the purified protein against 10 mM tris buffer and 2 mM EDTA, pH 7.4 (at 4 °C, in the dark) using a cellulose dialysis membrane with a 3.5 kDa MWCO. This buffer was used for four buffer changes over a minimum of 12 h and was then switched to 10 mM tris buffer, pH 7.4 for an additional three buffer changes over a minimum of 9 h to ensure complete buffer exchange. The concentration of all GFP variants was determined by absorbance at 280 nm. This wavelength was used as the supercharged variants can have increased protonation of the GFP chromophore, resulting in decreased absorbance at 488 nm. An extinction coefficient for each variant at 280 nm was determined using the superfolder GFP as a reference. The protein solution volume was adjusted with additional buffer to create a stock solution of 1 mg mL^{-1} protein. Protein solutions at this concentration were stored at 4 °C until use.

S.3. Summary of mutations in protein library

Table S1. Mutations relative to superfolder GFP in each protein

sfGFP sequence*	MGHHHHHHGG ASKGE**E**LF**T**G VVPILVELDG DVNGH**K**FSVR GEGEGDAT**N**G **K**LTLKFICTT G**K**LPVPWPTL VTTLTYGVQC FS**R**YP**D**HM**K**Q HDFFKSAMPE GYVQERTISF K**D**DGTYKTRA EVKFEG**D**TLV NRIELKG**I**DF KEDGNILGHK LEYNFNSH**N**V YITADKQ**K**NG IKA**N**F**K**IRHN VEDGSVQLAD HYQQNTPIGD GPVLLP**D**N**H**Y LST**Q**SALSKD P**N**E**K**RDHMVL LEFVTAAGI**T** HGMDE**L**YK					
Protein	**GFP(-18)**	**GFP(-24)**	**GFP(-30)**	**tag-GFP(-18)**	**tag-GFP(-24)**	**GFP(+6)-6R**
Expected Charge	-18.02	-24.02	-30.02	-19.02	-25.02	11.97
Expected Charge (w/ His)	-17.41	-23.41	-29.40	-18.41	-24.41	12.58
Mutations	N49E K62E K89D N159D K168E Q214E K224D	K36E N49E K62E R83D K89D N159D K168E N174E Q214E K224D T240D	T19D K36E N49E K51E K62E R83D K89D N159D K168E N174E K176E N208D Q214E K224D T240D	C-terminal + DEEEDD DEEEDD	C-terminal + DEEEDD DEEEDD DEEEDD	E16R D86K D112K D127R I138R D207R N222K L246R C-terminal + RRRRRR

* bold **red** letters indicate amino acids that are mutated in the anionic GFP variants; bold **blue** letters indicate amino acids that are mutated in the cationic GFP variant

S.4 Polymer Preparation

RAFT polymerization was used to synthesize a block copolymer from 4-vinylpyridine (4VP, MW = 105.14 g mol^{-1}) and oligo(ethylene glycol) methyl ether methacrylate (OEGMA, M_n = 300 g mol^{-1}) with a narrow molecular weight distribution. OEGMA and 4VP were passed through basic alumina columns prior to polymerization to remove inhibitor.

4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid (52.4 mg) and azobisisobutyronitrile (AIBN) (6.1 mg, recrystallized twice from methanol and stored at -20 °C) were added to a solution of OEGMA (10.05 g) in 21.1 g of 1,4-dioxane in the ratio of 180:1:0.2 (OEGMA:CTA:AIBN). The solution was degassed by three freeze-pump-thaw cycles. The polymerization was carried out in a sealed 100 mL flask at 65 °C for 7 h. The reaction was terminated by removal of heat and exposure to air.

The POEGMA homopolymer was purified by precipitation into hexanes three times. After each precipitation the polymer was collected by filtration, dried, and then dissolved in a minimal volume of acetone. The molecular weight, dispersity, and purity (M_n = 23.7 kg mol^{-1}, $Đ$ = 1.14, DP = 79) were determined by gel permeation chromatography (Bruker 400SL). A Waters Alliance 2695 separation module was equipped with a PL-aqua gel-OH 8-micron Mixed-M column (300 x 7.5 mm) as the stationary phase. The instrument was calibrated to polystyrene standards using tetrahydrofuran (THF) as the mobile phase. The mobile phase used

was THF and the polymer was monitored by a Waters 2998 Photodiode Array Detector and a Waters 2414 Refractrometer detector (Supporting Figure S2).

The P4VP block was synthesized by adding a molar ratio of 350:1:0.2 4VP:POEGMA:AIBN. The components were dissolved in 6 g of a mixture of 1,4-dioxane and *N*,*N*-dimethylformamide (DMF). The solution was degassed by three freeze-pump-thaw cycles. The polymerization was carried out in a sealed 25 mL flask at 70 °C for 6 h. The reaction was terminated by removal of heat and exposure to air. POEGMA-*b*-P4VP was purified by precipitation into cold diethyl ether three times. The block ratio was determined to be 2.21 4VP monomers to 1 POEGMA monomer by ^{1}H NMR (Supporting Figure S4).

This diblock copolymer, $POEGMA_{79}$-*b*-$P4VP_{175}$, was quaternized by adding excess iodomethane (5 equiv) to a solution of the polymer in DMF. The reaction was stirred at room temperature for 24 h. The modified polymer was purified by precipitation into hexanes three times. The precipitated polymer was then dried under reduced pressure to remove residual solvent. The degree of quaternization was determined to be ~95% by ^{1}H NMR (Supporting Figure S4).

S.5 Dynamic Light Scattering (DLS) sample preparation

Stock solutions of $POEGMA_{79}$-*b*-$qP4VP_{175}$ were prepared at 1 mg mL^{-1} in 10 mM tris, pH 7.4. Protein solutions adjusted to 1 mg mL^{-1} and were then filtered through a 0.2 µm polyethersulfone (PES) syringe filter prior to use.

S.6 DLS measurements as a function of mixing ratio

DLS measurements were performed on a Malvern Zetasizer ZS. Pure protein solutions were measured at 1 mg mL^{-1} in 10 mM tris, pH 7.4. Micelle samples were prepared to a final macromolecule concentration of 0.2 mg mL^{-1} (0.14 to 0.06 mg mL^{-1} protein with 0.06 to 0.14 mg mL^{-1} polymer) by adding relevant volumes of protein stock solution, 10 mM tris, pH 7.4 and polymer stock, in that order, to a low volume disposable cuvette. Cuvettes were sealed with parafilm and vortexed for 3 s. Samples were measured by DLS within 15 min of mixing. Micelle samples were prepared in triplicate and each sample was measured once by DLS.

S.7 DLS salt and temporal stability experiments

Micelle samples were prepared to a final macromolecule concentration of 0.2 mg mL^{-1} by adding relevant protein stock solution, 10 mM tris with 5 M NaCl, 10 mM tris, pH 7.4, and polymer stock, in that order, to a low volume disposable cuvette. Cuvettes were sealed with parafilm and vortexed for 3 s. Samples were measured by DLS within 45 min of mixing. Samples were stored at 4 °C between measurements.

S.8 Fluorescence correlation spectroscopy

Samples were prepared as described above (S.5 and S.7), with the exception that the pure GFP (1 mg mL^{-1}) was diluted 10-fold with 10 mM tris, pH 7.4 prior to measurement. Measurements of micelle samples were performed within 60 min of sample preparation.

Fluorescence Correlation Spectroscopy (FCS) measurements were performed on an inverted Leica TCS-SP8 STED 3X equipped with a 63x 1.20 NA water immersion objective. Fluorophores were excited at rate of 40 MHz, using the 488 nm line from a white light laser. Fluorescence was collected through a size adjustable pinhole set to 70 µm, at 510 ± 10 nm and detected using a HyD detector, coupled to a TCSPC module. Data acquisition and calculation of the correlation curve $G(\tau)$ were performed using SymPhoTime software (PicoQuant, Germany) Ten, 30 s measurements were collected and averaged for each sample. Values represent the average of three measurements performed on three different days. Errors were calculated from the standard deviation of these results.

Averaged autocorrelation curves were fit as described by Nolles, et al.[2] Briefly, autocorrelation curves for **GFP** were fit to a single-component model (1), including triplet state:

$$G(\tau) = \left[1 - \tau + \tau e^{\frac{-\tau}{\tau_T}}\right] \frac{1}{\left[N\left(1+\frac{\tau}{\tau_D}\right)\left(1+\frac{\tau}{\kappa^2\tau_D}\right)^{0.5}\right]} \quad (1)$$

Where G(τ) is the autocorrelation function as a function of time, τ. N is the average number of molecules in the focal volume. τ_D is the diffusion time, the average amount of time a molecule spends diffusing through the observation volume, τ_T is the lifetime of the triplet state. $\kappa = \frac{z_0}{\omega_0}$ represents the ratio of the axial (z_0) to radial (ω_0) dimensions of the Gaussian excitation volume. This value was determined by calibration using Atto488-carboxylic acid (D = 4.0 x 10^{-6} cm^2 s^{-1} at 25 °C).

Autocorrelation curves for **PEC micelles** were fit to a two-component diffusion model. One component, diffusion time for GFP, was fixed at the value calculated for free GFP. The hydrodynamic radii of GFP and micelles were calculated from the Stokes-Einstein relation.

To estimate the number of GFPs per micelle as described by Nolles et al., an assumption that all GFPs are incorporated was used (the number of free GFPs found at 40 μM divided by the number of micelles). A second method, using counts per molecule of free GFP versus per micelle was also used to estimate the number of GFPs per micelle. This is only accurate if GFP has the same intensity in buffer as in the PEC micelles. Measurement of fluorescence lifetimes (Table S3) show similar lifetimes for GFP in micelles at 150 mM NaCl and GFP in buffer, but at lower NaCl concentration the lifetimes are shorter in micelles suggesting a lower CPM in the micelle compared to buffer. Consequently, CPM/GFP(CPM) is similar to GFP(N)/(N) at 150 mM but progressively underestimates the number of GFP per micelle at lower salt concentrations.

Table S2. Summary of FCS Data with GFP(-24)

	Radius (nm)	Counts per molecule (CPM)	Number of molecules (N)	% GFP in micelles	CPM/ GFP(CPM)	10 x GFP (N) / (N)
GFP(-24) (diluted 10x)	1.9 ± 0.1	2365 ± 130	74 ± 15	-	-	-
PEC – 0 mM NaCl	97 ± 5	547587 ± 69197	2.4 ± 0.05	99± 6	231 ± 19	303 ± 67
PEC – 25 mM NaCl	61 ± 3	452426 ± 31627	3.3 ±0.3	98± 2	192 ±19	226 ± 54
PEC – 125 mM NaCl	88 ± 5	337005 ± 50406	4.5 ±0.5	96± 1	142 ± 15	164 ± 29
PEC – 150 mM NaCl	93 ± 1	228502 ± 46507	8.8 ± 3	96± 2	98 ± 25	100 ± 65

Table S3. Lifetimes, corresponding A factors and average lifetimes

	Lifetime 1 (ns)	Lifetime 2 (ns)	A1	A2	Average lifetime (ns)
GFP(-24) (diluted 10x)	2.698	1.33	0.7966	0.2034	2.4
PEC – 0 mM NaCl	2.041	0.796	0.7049	0.2951	1.7
PEC – 25 mM NaCl	2.104	0.781	0.7651	0.2349	1.8
PEC – 125 mM NaCl	2.4	1.04	0.7333	0.2667	2.0
PEC – 150 mM NaCl	2.55	1.1	0.7856	0.2414	2.2

S.9 Transmission electron microscopy

Micelle samples were prepared at the indicated macromolecule mixing ratio by adding the relevant amounts of protein stock solution, 10 mM tris buffer with 5 M NaCl, 10 mM tris buffer, pH 7.4, and polymer solution, in that order, to a 1.7 mL tube. Samples were vortexed for 3 s. An equal volume of water was then added to the sample, resulting in a final macromolecule concentration of 0.1 mg mL^{-1}. 5 µL of this solution was pipetted onto a Formvar coated, 300 mesh, copper grid (Ted Pella 01701-F), the grid was covered, and then incubated at room temperature for 3 min. The sample was then wicked with filter paper. To reduce the salt concentration on the grid, the grid was washed by adding 5 µL of milliQ water to the grid and immediately wicking with filter paper. Samples were dried at room temperature for a minimum of 1 h at reduced pressure and stored at room temperature.

The micelle radius was determined using FIJI. 50 particles were measured, unless otherwise noted.

S.10 Flow Cytometry

Experiments were conducted in a 96 well round bottom plate. Jurkat cells were maintained in cell culture treated T-flasks in RPMI supplemented with 10% FBS and 1% penicillin/streptomycin. For the protein delivery assays, cells were collected by centrifugation and resuspended in complete media. 100 µL of cell solutions were plated in wells at a density of $5x10^6$ cells mL^{-1}.

100 µL micelle solutions were prepared with a macromolecule concentration of 0.2 mg mL^{-1} by adding relevant protein stock solution, DPBS, and $POEGMA_{79}$-b-$qP4VP_{175}$ dissolved in DPBS, in that order, to a 1.7 mL tube. Tubes were vortexed for 3 s. Micelle samples were prepared immediately prior to plating. The micelle solutions were diluted into cell solutions to a final macromolecule concentration of 0.1 mg mL^{-1} and 0.05 mg mL^{-1}. For the lower concentration, the samples were diluted two-fold with DPBS immediately prior to addition to cell solutions. The concentrations of free protein and polymer solutions were the same as their respective concentrations in the PEC micelle samples (e.g. polymer and GFP(+6)-6R had final concentrations of 0.05 mg mL^{-1} and 0.025 mg mL^{-1}).

Micelle solutions were added to the plated cells and incubated at 37 °C with 5% CO_2 for 1 h. Cells were washed 3 times by pelleting by centrifugation, discarding the supernatant, and resuspending in 100 µL of DPBS supplemented with 1% FBS. During the final wash step, cells were resuspended in 200 µL DPBS + 1% FBS. Cells were incubated at 37 °C with 5% CO_2 for a 15 min recovery period. Following this incubation, 1 drop of SYTOX AADvanced™ Ready Flow™ Reagent (Invitrogen #R37173) dead stain was added to each well. Cells were incubated at 37 °C with 5% CO_2 for an additional 15 min.

Flow cytometry was conducted on an Attune NxT Acoustic Focusing Cytometer using the blue laser with the 530/30 and 695/40 emission filters.

S.11 Confocal Imaging

Samples were prepared in a 96 well round bottom plate as they were for flow cytometry, through the final wash step. On the final wash, cells were resuspended in 200 µL of 150 nM LysoTracker Red DND-99 in DPBS supplemented with 1% FBS. Cells were incubated at 37 °C with 5% CO_2 for 30 min. Cells were pelleted and fixed in 200 µL of 4% formaldehyde in DPBS.

50 µL of the fixed cell solution was spotted onto 18x18 mm coverslips in a 6 well plate. The cells were collected on the coverslips by centrifuging for 15 min at 2000 rpm. Cell coated coverslips were washed 2 times with DPBS in the 6 well plate. Coverslips were stained with 2 drops of NucBlue stain in 3 mL of DPBS for 15 min. Coverslips were removed from the well plates and mounted onto slides with ProLong Diamond Antifade Mountant (Invitrogen #P36961). Coverslips were allowed to cure on the bench for 24 h, protected from light, before being stored at 4 °C prior to imaging.

Cells were imaged on a Nikon Ti Eclipse inverted confocal microscope with a 60x oil objective (NA = 1.49) at the Columbia University Herbert Irving confocal and specialized microscopy core (NIH #P30 CA013696).

S.12 Tables of PEC micelle data at varying salt concentrations

Table S4. Summary of PEC micelle data at 0 mM NaCl

Protein	*f*+	Protein Fraction	Intensity Peak 1 (r_H, nm)	PDI	Derived Count Rate (kcps)	TEM (r, nm)
sfGFP	0.91	0.5	67 ± 3	0.15 ±0.02	22200 ± 1900	37 ± 9
GFP(-18)	0.85	0.4	90 ± 4	0.08 ± 0.01	177000 ± 6300	-
	0.79	0.5	117 ± 12	0.17 ± 0.07	143000 ± 14000	57 ± 13
GFP(-24)	0.73	0.5	101 ± 12	0.21 ± 0.04	60000 ± 37000	36 ± 12
GFP(-30)	0.69	0.5	92 ± 14	0.15 ± 0.01	101000 ± 22000	40 ± 8
tag-GFP(-18)	0.79	0.5	119 ± 7	0.26 ± 0.04	5200 ± 180	-
tag-GFP(-24)	0.74	0.5	60 ± 1	0.07 ± 0.02	52000 ± 9400	59 ± 13

Table S5. Summary of PEC micelle data at 25 mM NaCl

Protein	*f*+	Protein Fraction	Intensity Peak 1 (r_H, nm)	PDI	Derived Count Rate (kcps)	TEM (r, nm)
sfGFP	0.91	0.5	98 ± 3	0.05 ± 0.02	72000 ± 5000	-
GFP(-18)	0.85	0.4	68 ± 2	0.07 ± 0.01	177000 ± 6300	59 ± 15
	0.79	0.5	83 ± 1	0.08 ± 0.02	177000 ± 6900	58 ± 12
GFP(-24)	0.73	0.5	72 ± 4	0.09 ± 0.02	65000 ± 42000	45 ± 11
GFP(-30)	0.69	0.5	65 ± 5	0.08 ± 0.01	83000 ± 9700	59 ± 13
tag-GFP(-18)	0.79	0.5	105 ± 6	0.18 ± 0.01	9000 ± 450	-
tag-GFP(-24)	0.74	0.5	70 ± 2	0.06 ± 0.02	89000 ± 3000	-

Table S6. Summary of PEC micelle data at 125 mM NaCl

Protein	*f*+	Protein Fraction	Intensity Peak 1 (r_H, nm)	PDI	Derived Count Rate (kcps)	TEM (r, nm)
sfGFP	0.91	0.5	127 ± 7	0.17 ±0.02	6300 ± 140	-
GFP(-18)	0.85	0.4	97 ± 4	0.08 ± 0.01	99000 ± 800	-
	0.79	0.5	95 ± 2	0.05 ± 0.04	93000 ± 2300	-
GFP(-24)	0.73	0.5	81 ± 3	0.08 ± 0.01	73000 ± 51000	48 ± 7
GFP(-30)	0.69	0.5	66 ± 4	0.06 ± 0.01	74000 ± 3100	51 ± 9
tag-GFP(-18)	0.79	0.5	154 ± 9	0.17 ± 0.1	7800 ±1400	-
tag-GFP(-24)	0.74	0.5	148 ± 10	0.16 ± 0.01	37000 ± 13000	-

Table S7. Summary of PEC micelle data at 150 mM NaCl

Protein	*f*+	Protein Fraction	Intensity Peak 1 (r_H, nm)	PDI	Derived Count Rate (kcps)	TEM (r, nm)
sfGFP	0.91	0.5	122 ± 6	0.19 ± 0.01	5500 ± 125	-

GFP(-18)	0.85	0.4	110 ± 2	0.09 ± 0.01	58000 ± 4700	-
	0.79	0.5	108 ± 4	0.11 ± 0.04	58000 ± 7300	-
GFP(-24)	0.73	0.5	84 ± 4	0.08 ± 0.02	62000 ± 42000	145 ± 39
GFP(-30)	0.69	0.5	68 ± 3	0.07 ± 0.02	69000 ± 8100	43 ± 8
tag-GFP(-18)	0.79	0.5	169 ± 5	0.19 ± 0.02	6800 ± 670	-
tag-GFP(-24)	0.74	0.5	251 ± 37	0.26 ± 0.06	9200 ± 3200	-

S.13 Table summarizing flow cytometry data

Table S8. Summary of Flow Cytometry Analysis

Sample	Protein Fraction	Polymer Fraction	[GFP] (μM)	[Polymer] (mg mL^{-1})	Ungated Count	T Cell Count	Fraction Viable T cells	MFI T cells
Untreated	0	1	0	0	50000 ± 0	39703 ± 123	0.98 ± 0.002	101 ± 6
GFP(-18)	1	0	1.8	0	50000 ± 0	38752 ± 299	0.98 ± 0.002	126 ± 2
GFP(-18)	0.5	0.5	1.8	0.05	50000 ± 0	27559 ± 837	0.78 ± 0.02	2050 ± 120
GFP(-18)	0.5	0.5	0.9	0.025	50000 ± 0	32536 ± 1336	0.85 ± 0.01	1080 ± 69
GFP(-18)	0.4	0.6	1.4	0.06	50000 ± 0	25083 ± 543	0.74 ± 0.05	3060 ± 465
GFP(-18)	0.4	0.6	0.7	0.03	50000 ± 0	29868 ± 316	0.8 ± 0.01	1590 ± 44
GFP(-24)	1	0	1.8	0	50000 ± 0	38663 ± 3150	0.97 ± 0.01	105 ± 8
GFP(-24)	0.5	0.5	1.8	0.05	38720 ± 3084	29726 ± 2179	0.94 ± 0.01	2110 ± 546
GFP(-24)	0.5	0.5	0.9	0.025	37662 ± 10802	28436 ± 7146	0.93 ± 0.01	1670 ± 381
GFP(-30)	1	0	2.2	0	50000 ± 0	39362 ± 645	0.98 ± 0.002	110 ± 3
GFP(-30)	0.6	0.4	2.2	0.04	50000 ± 0	38759 ± 156	0.97 ± 0.004	777 ± 165
GFP(-30)	0.6	0.4	1.1	0.02	50000 ± 0	38407 ± 471	0.97 ± 0.01	688 ± 134
GFP(-30)	0.5	0.5	1.8	0.05	50000 ± 0	37679 ± 255	0.94 ± 0.003	544 ± 33
GFP(-30)	0.5	0.5	0.9	0.025	50000 ± 0	38794 ± 263	0.96 ± 0.01	557 ± 25
tag-GFP(-24)	1	0	2.0	0	50000 ± 0	39401 ± 482	0.98 ± 0.002	115 ± 21
tag-GFP(-24)	0.6	0.4	2.0	0.04	50000 ± 0	35323 ± 969	0.9 ± 0.02	28800 ± 3680
tag-GFP(-24)	0.6	0.4	1.0	0.02	50000 ± 0	37549 ± 415	0.94 ± 0.01	5900 ± 1090
tag-GFP(-24)	0.5	0.5	1.7	0.05	50000 ± 0	31139 ± 452	0.79 ± 0.01	30030 ± 2350
tag-GFP(-24)	0.5	0.5	0.8	0.025	50000 ± 0	34237 ± 930	0.86 ± 0.02	12087 ± 1806
Polymer	0	1	0	0.05	23771 ± 3137	13967 ± 1751	0.77 ± 0.04	117 ± 1
Polymer	0	1	0	0.025	43484 ± 7277	25316 ± 4584	0.8 ± 0.09	101 ± 3
GFP(+6)-6R	1	0	1.8	0	50000 ± 0	40523 ± 217	0.97 ± 0.002	1330 ± 72
GFP(+6)-6R	1	0	0.9	0	50000 ± 0	40280 ± 278	0.97 ± 0.003	1323 ± 40

Ungated Count, T Cell Count, Fraction Viable T cells and MFI T cells are the mean and standard deviation of 3 replicates.

S.14 Supplementary References

1 R. A. Kapelner and A. C. Obermeyer, *Chem. Sci.*, 2019, **10**, 2700–2707.

2 A. Nolles, A. H. Westphal, J. A. de Hoop, R. G. Fokkink, J. M. Kleijn, W. J. H. van Berkel and J. W. Borst, *Biomacromolecules*, 2015, **16**, 1542–1549.

S.15 Figure S1. Protein characterization

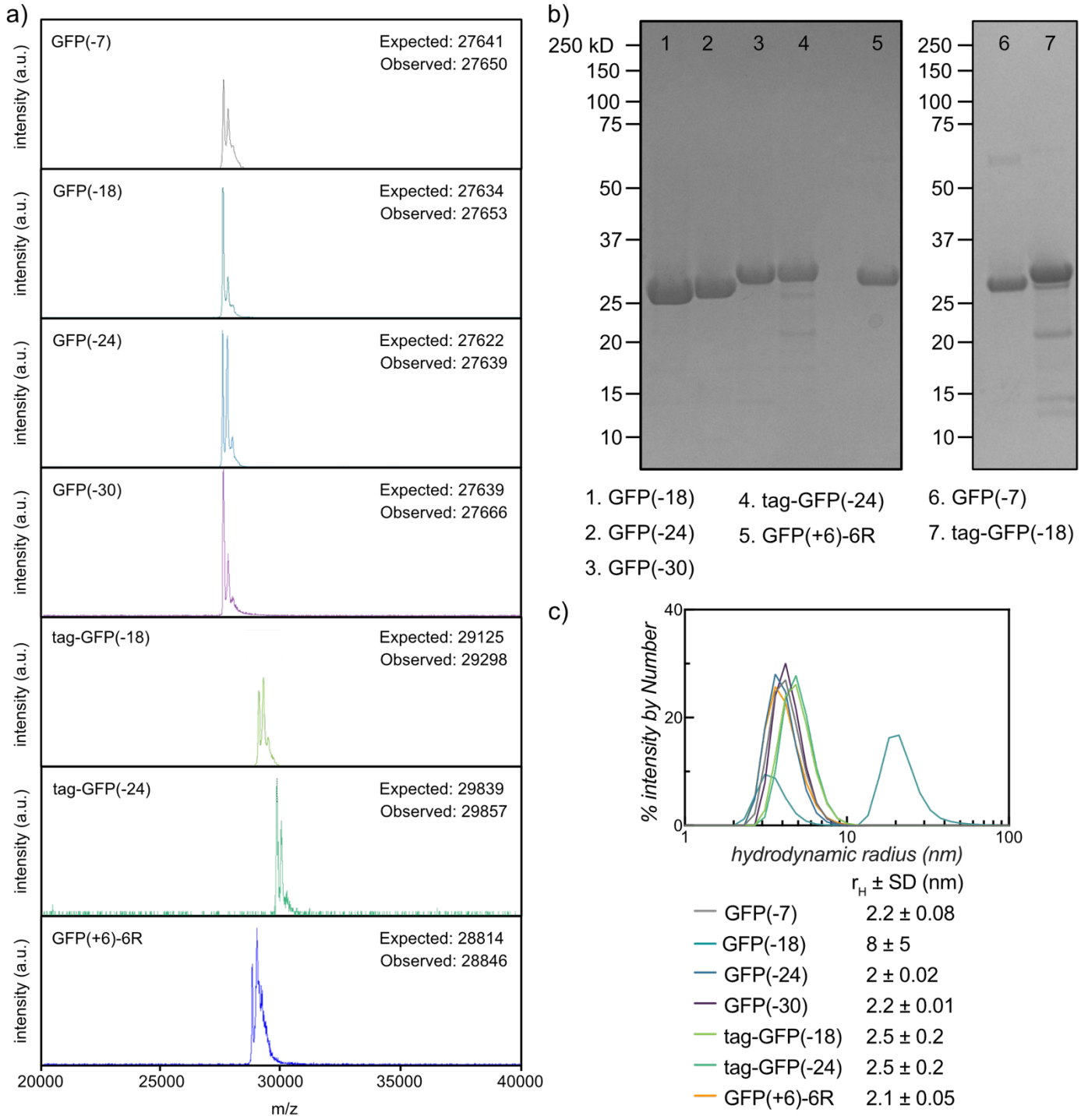

Figure S1. (a) MALDI-TOF MS analysis of purified proteins was performed using a Bruker ultrafleXtreme MALDI-TOF. The observed protein mass was consistent with the expected protein mass. (b) SDS-PAGE of proteins studied. Protein concentration was 0.25 mg mL^{-1}. (c) Number Intensity DLS traces of protein (1 mg mL^{-1}) in 10 mM Tris, pH = 7.4. Average of three replicates shown.

S.16 Figure S2. POEGMA GPC

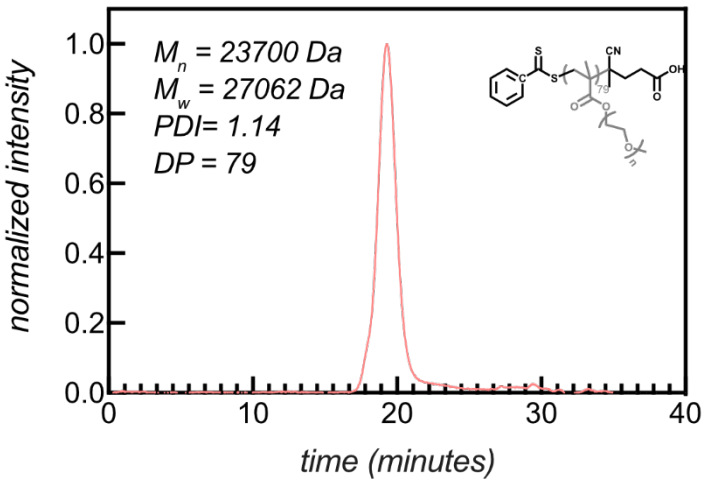

Figure S2. THF gel permeation chromatogram of the POEGMA homopolymer.

S.17 Figure S3. ^{1}H NMR of polymers

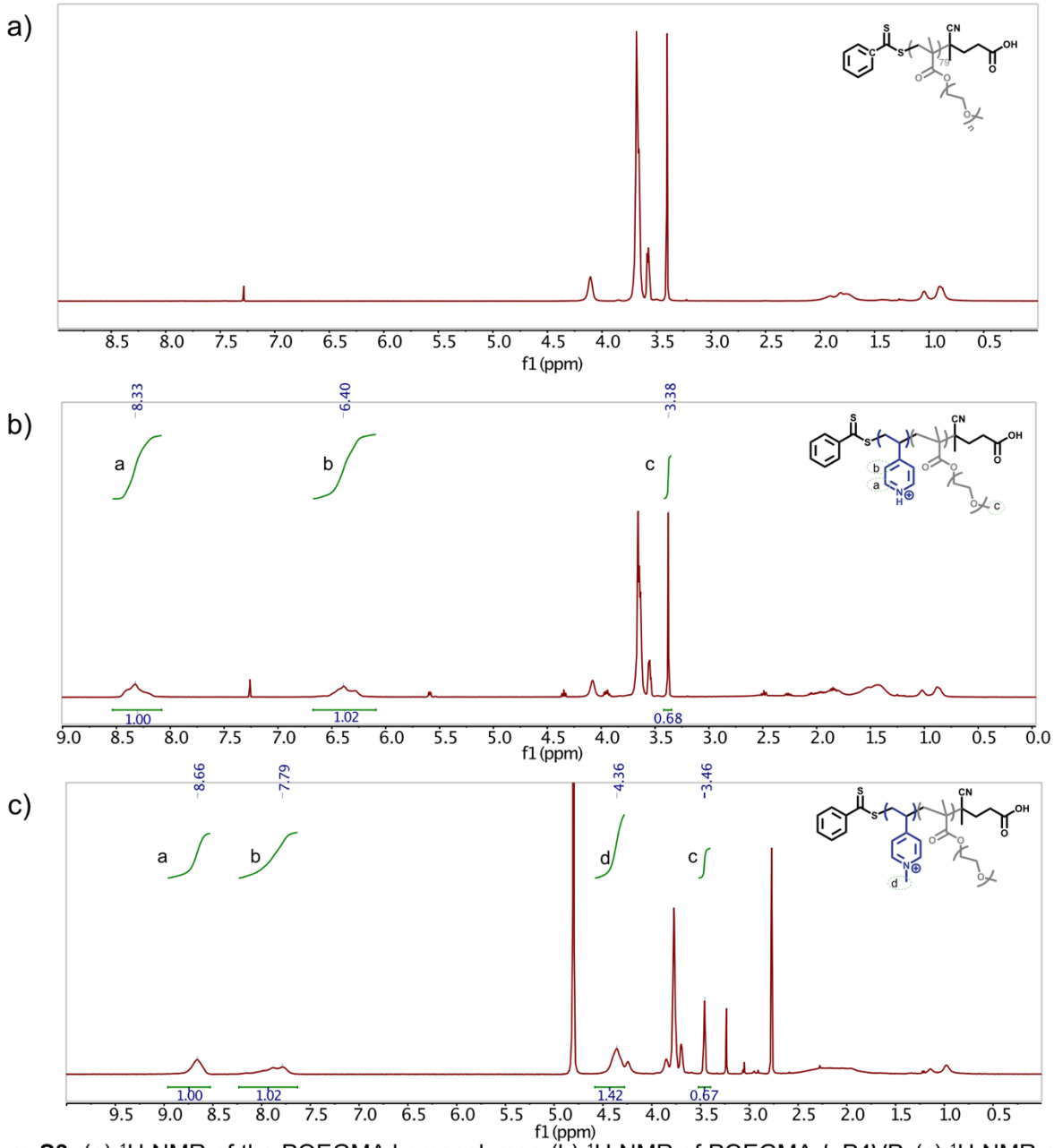

Figure S3. (a) ^{1}H NMR of the POEGMA homopolymer. (b) ^{1}H NMR of POEGMA-*b*-P4VP. (c) ^{1}H NMR of POEGMA-*b*-qP4VP.

S.18 Figure S4. DLS results for PEC micelles at different mixing ratios

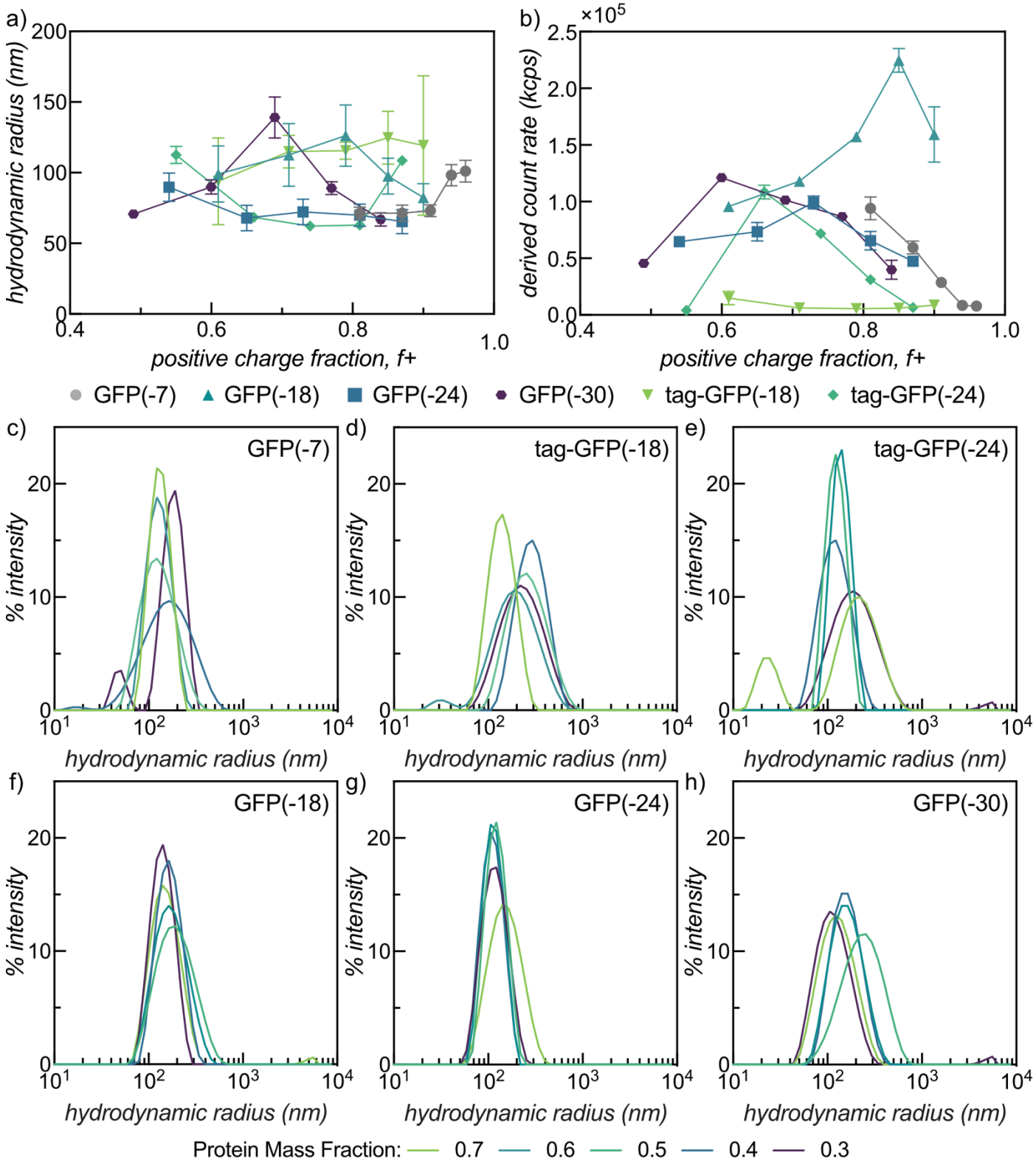

Figure S4. (a) Hydrodynamic radius as a function of mixing ratio. [macromolecule] = 0.2 mg mL^{-1}, 10 mM Tris pH 7.4. Mean and standard deviation of 3 measurements. (b) Derived count rate as a function of mixing ratio. [macromolecule] = 0.2 mg mL^{-1}, 10 mM Tris pH 7.4. Mean and standard deviation of 3 measurements. (c-h) DLS intensity traces of various protein and polymer mixing ratios for (c) GFP(-7) (d) tag-GFP(-18) (e) tag-GFP(-24) (f) GFP(-18) (g) GFP(-24) (h) GFP(-30). Buffer conditions: 10 mM Tris, pH = 7.4. Total macromolecule concentration is 0.2 mg mL^{-1}.

S.19 Figure S5. DLS correlation curves at different mixing ratios

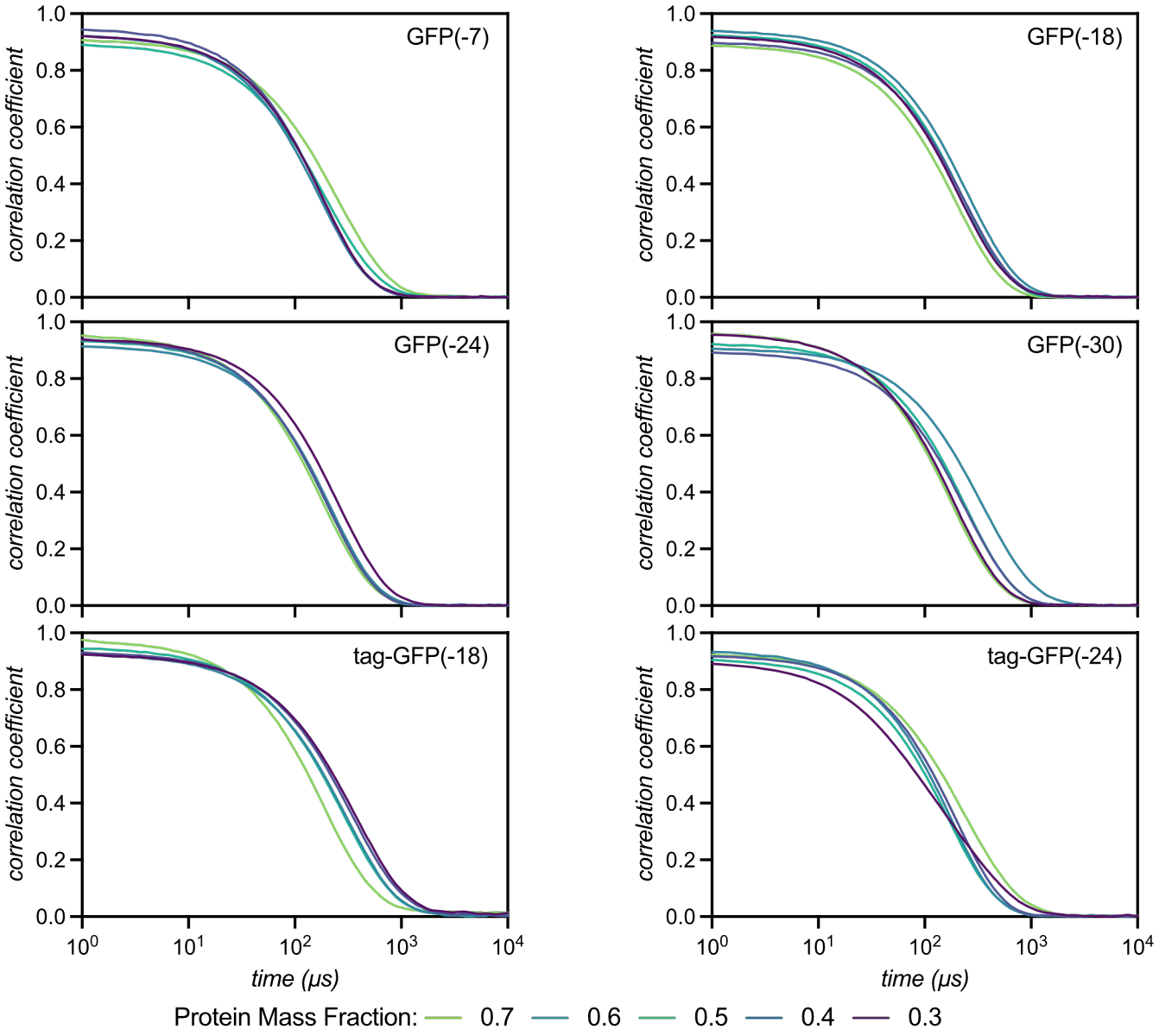

Figure S5. Representative dynamic light scattering autocorrelation curves at varying macromolecular mixing ratios, corresponding to data shown in Figure S4.

S.20 Figure S6. FCS results for GFP(-24) PEC micelles as a function of salt

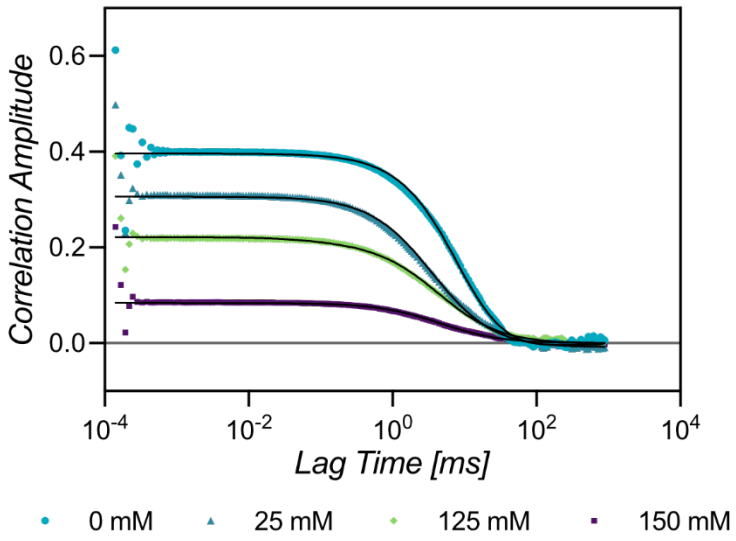

Figure S6. Representative fluorescence correlation spectroscopy autocorrelation curves at varying salt concentrations for PEC micelles with GFP(-24), corresponding to data shown in Figure 2 and Figure S8.

S.21 Figure S7. TEM and DLS salt titration of GFP(-18) at different mixing ratios

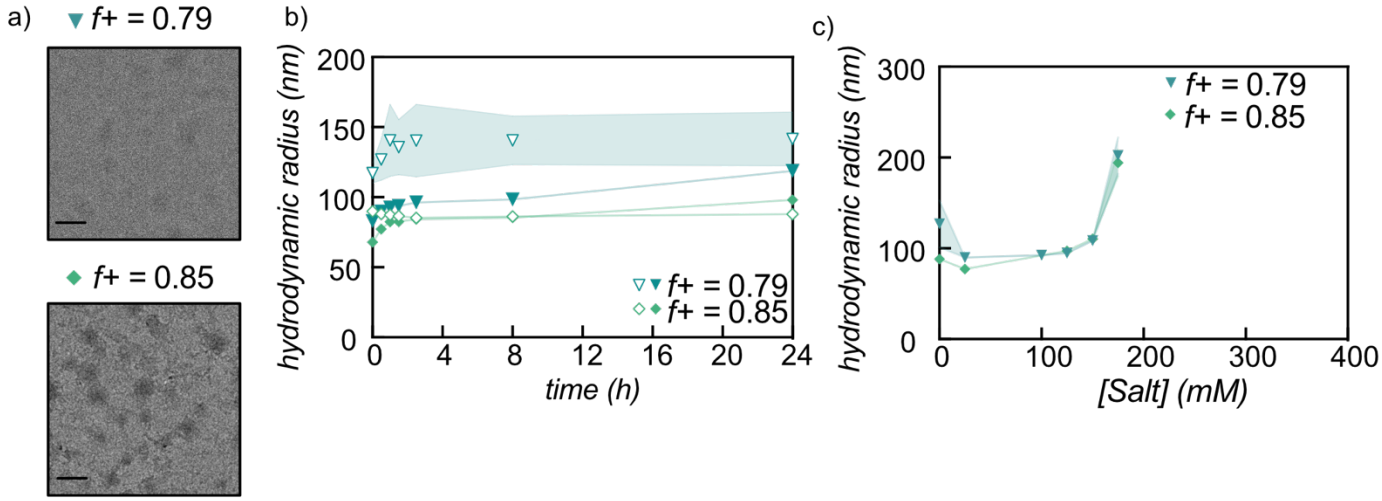

Figure S7. (a) TEM micrographs of GFP(-18) PEC micelles at different mixing ratios in 25mM NaCl. Scale bar = 200 nm. (b) Temporal stability of GFP(-18) PEC micelles in 0 M (open symbol) and 25 mM (closed symbol) NaCl evaluated by DLS. The hydrodynamic radius determined from the intensity distribution is plotted as a function of time. The shaded regions represent the standard deviation between 3 samples. (c) PEC micelle stability as a function of ionic strength.

S.22 Figure S8. Derived count rate at different salt concentrations over time

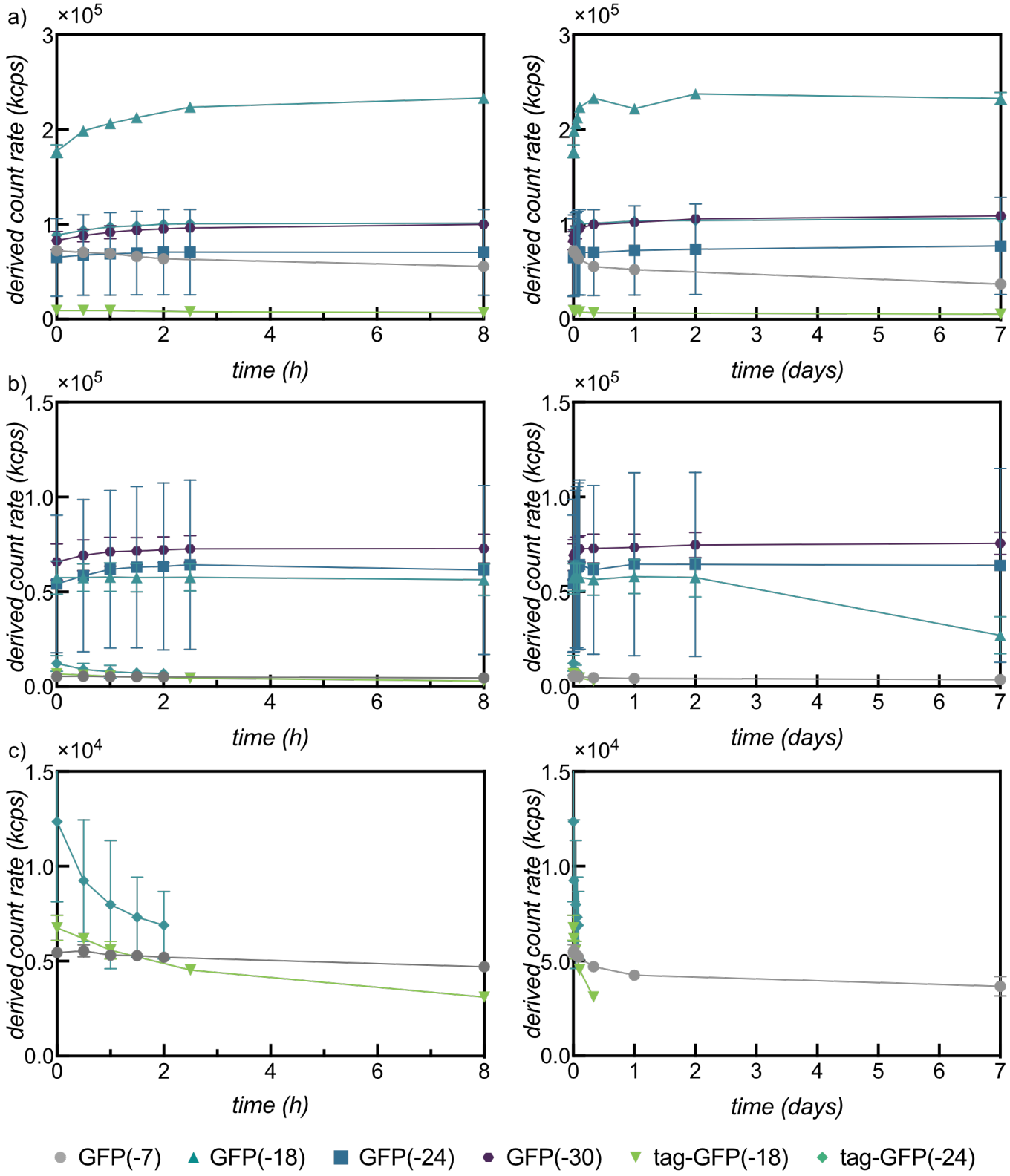

Figure S8. PEC micelle temporal stability as a function of protein charge. Derived count rate as a function of time for samples prepared with 0.1 mg mL^{-1} protein and 0.1 mg mL^{-1} polymer in 10 mM Tris pH 7.4 with (a) 25 mM NaCl, (b) and (c) 150 mM NaCl. Error bar represents the standard deviation between 3 samples.

S.23 Figure S9. DLS correlation curves as a function of time at 25 mM NaCl

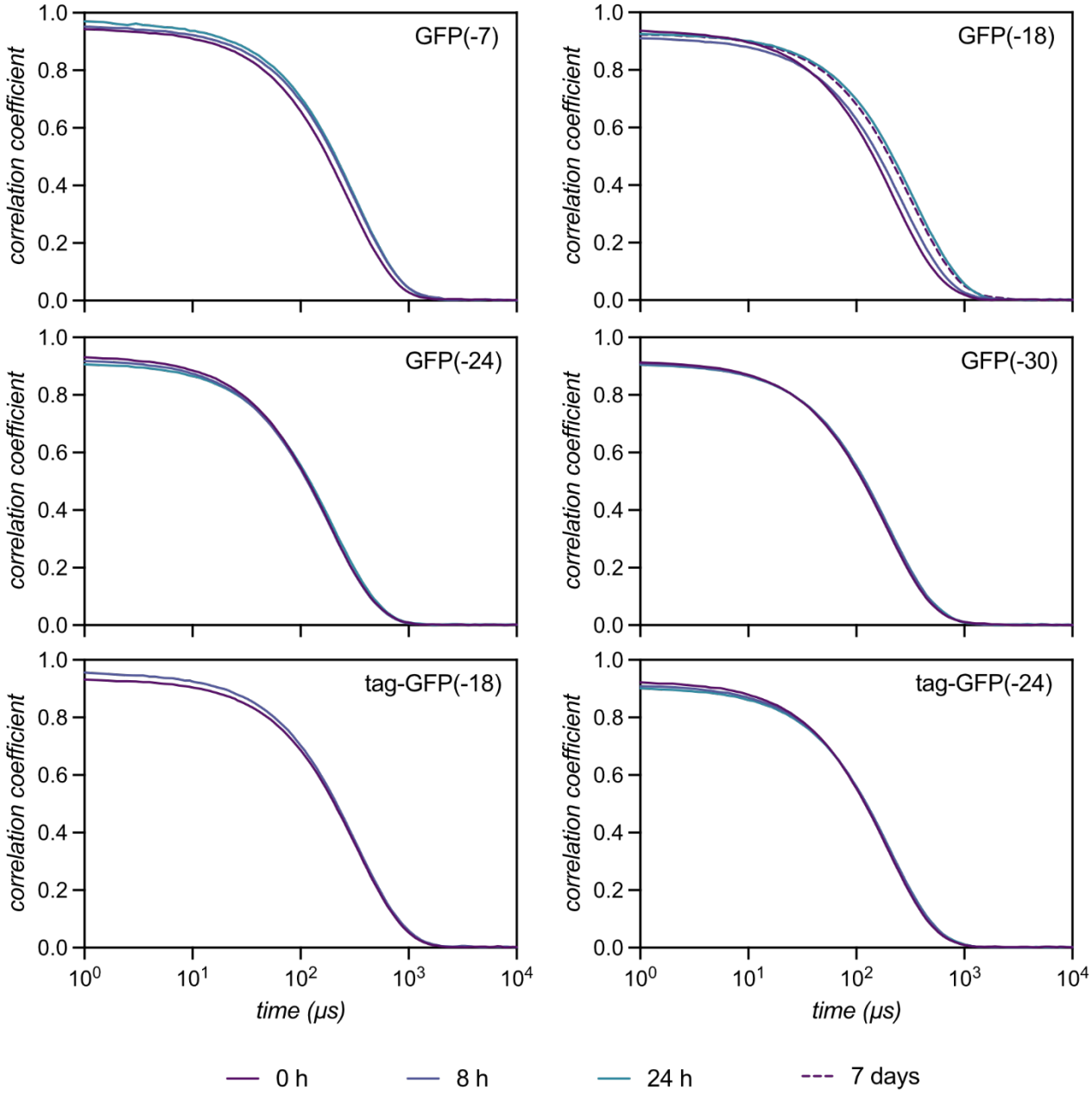

Figure S9. Representative dynamic light scattering autocorrelation curves at varying time points for samples in 10 mM tris buffer, pH 7.4 with 25 mM NaCl corresponding to data shown in Figure 2a and Figure S6a.

S.24 Figure S10. DLS correlation curves as a function of time at 150 mM NaCl

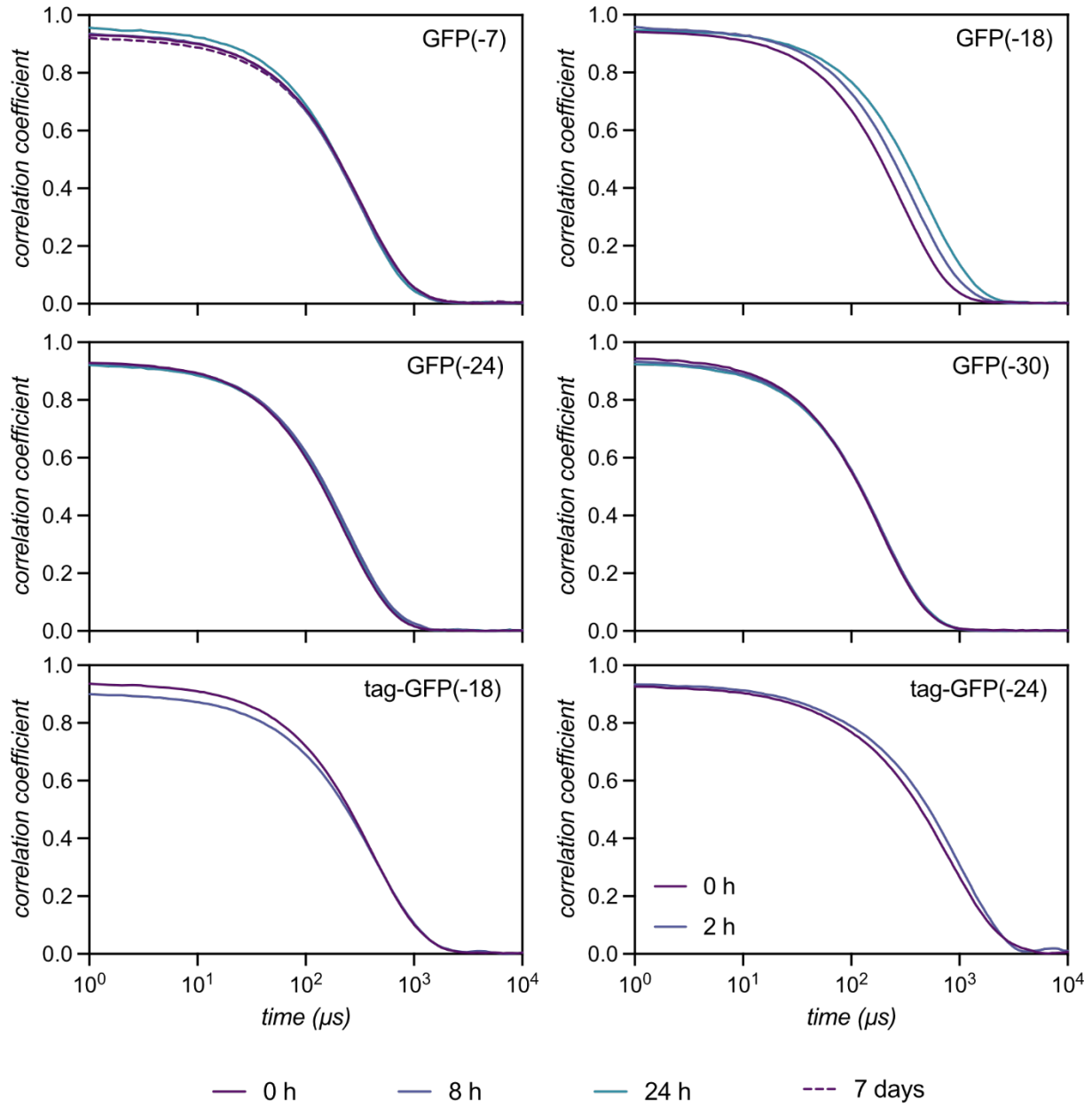

Figure S10. Representative dynamic light scattering autocorrelation curves at varying time points for samples in 10 mM tris buffer, pH 7.4 with 150 mM NaCl corresponding to data shown in Figure 2c and Figure S6b,c. Legend applies to all samples except tag-GFP(-24), which has a separate legend shown on the graph.

S.25 Figure S11. Derived count rate as a function of salt concentration

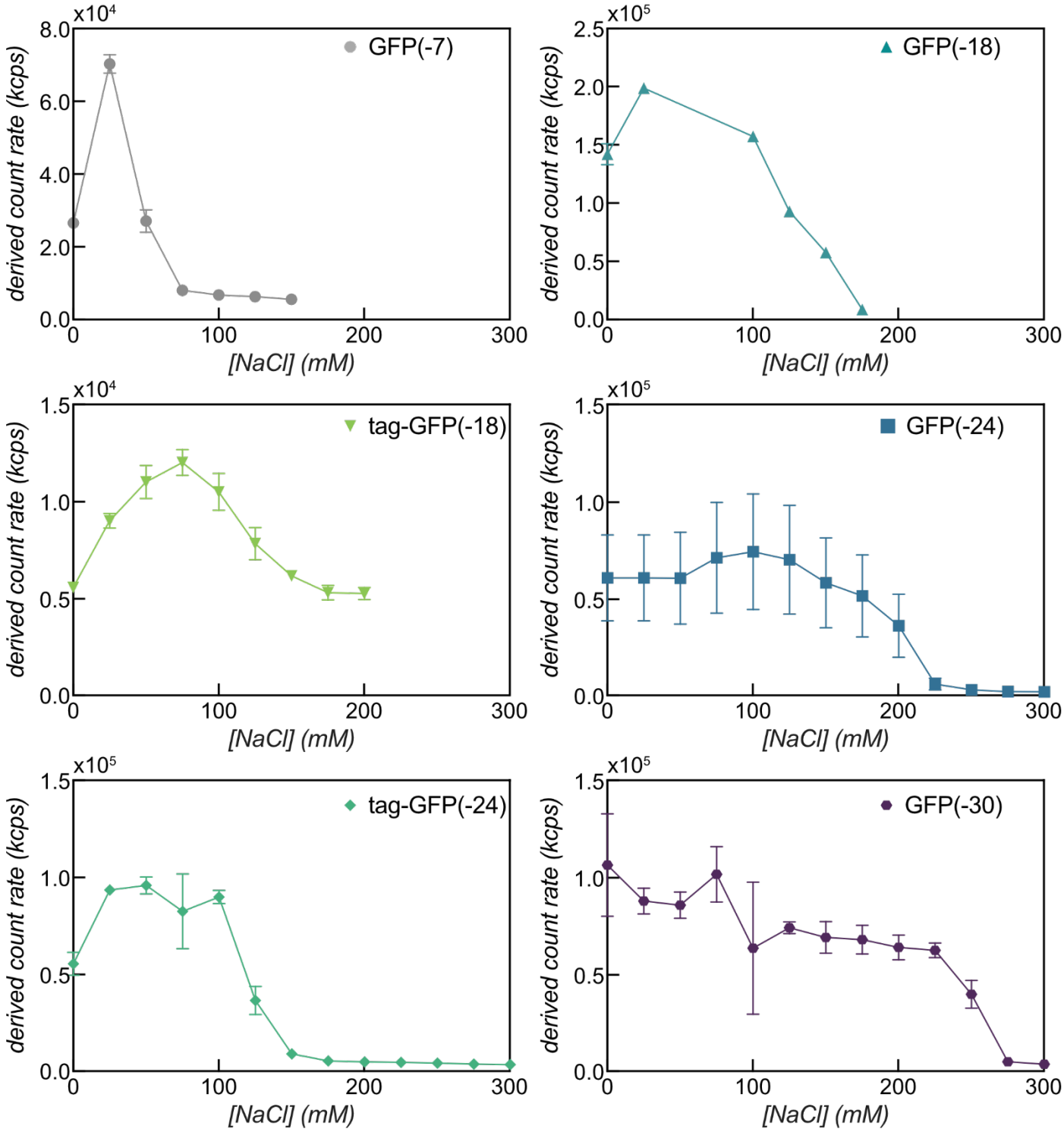

Figure S11. Derived count rate as a function of salt concentration for micelles prepared with 0.1 mg mL^{-1} protein and 0.1 mg mL^{-1} polymer in 10 mM Tris pH 7.4 + NaCl. The data points represent the mean and the error bars show the standard deviation of 3 samples.

S.26 Figure S12. DLS correlation curves as a function of salt

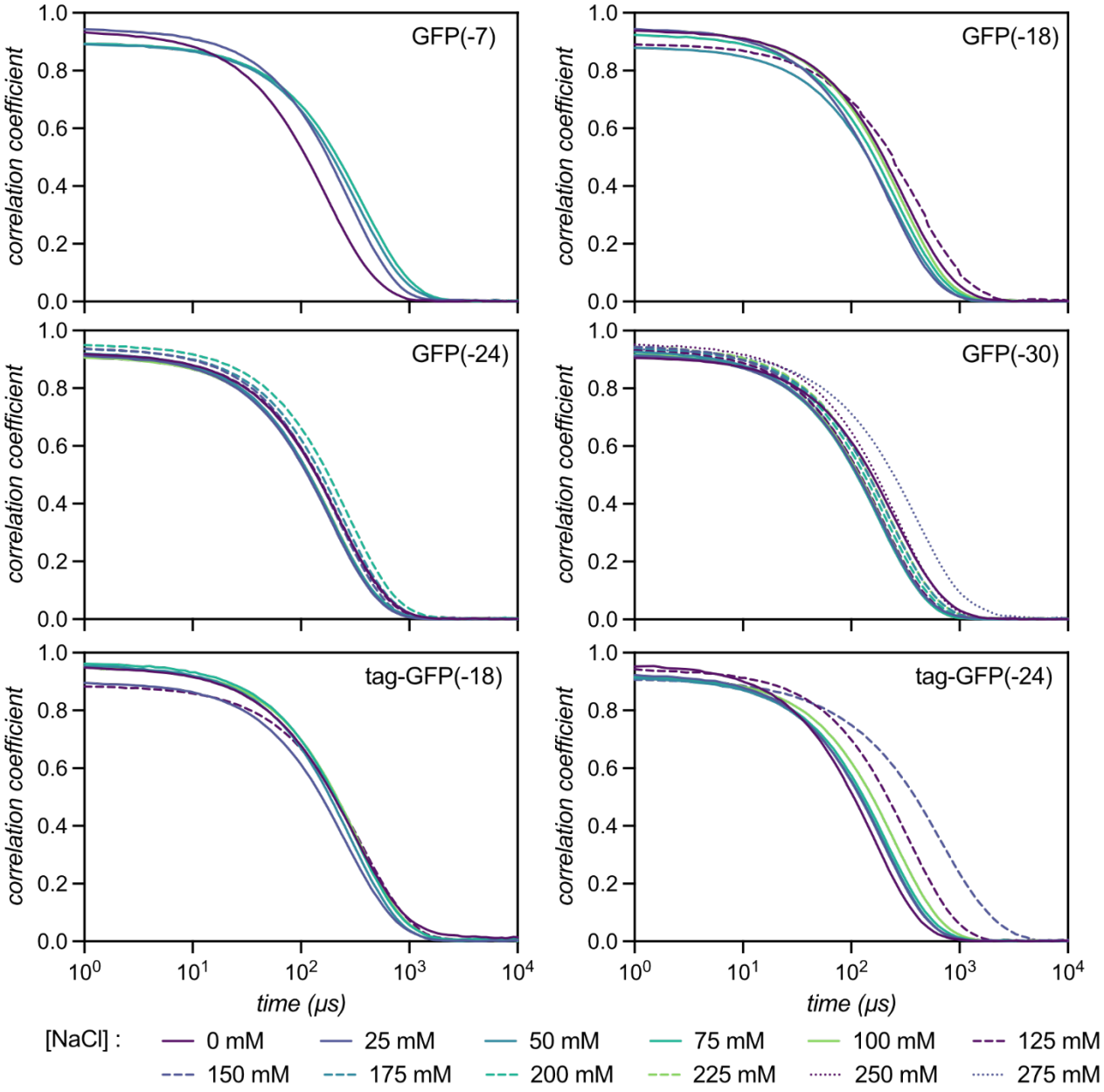

Figure S12. Representative dynamic light scattering autocorrelation curves at varying salt concentrations corresponding to data shown in Figure 2b and Figure S7.

S.27 Figure S13. DLS intensity by number of –24 variants as a function of salt

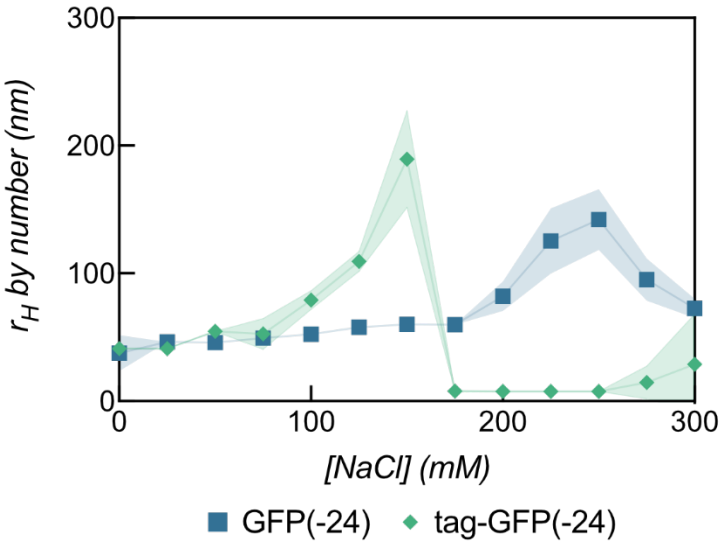

Figure S13. PEC micelle analysis using the number distribution. Hydrodynamic radius of PEC micelles as a function of ionic strength for GFP(-24) and tag-GFP(-24). Samples were prepared with 0.1 mg mL^{-1} protein and 0.1 mg mL^{-1} polymer in 10 mM Tris pH 7.4 + NaCl. The data points represent the mean and the shaded region shows the standard deviation of 3 samples.

S.28 Figure S14. Flow cytometry data for polymer controls

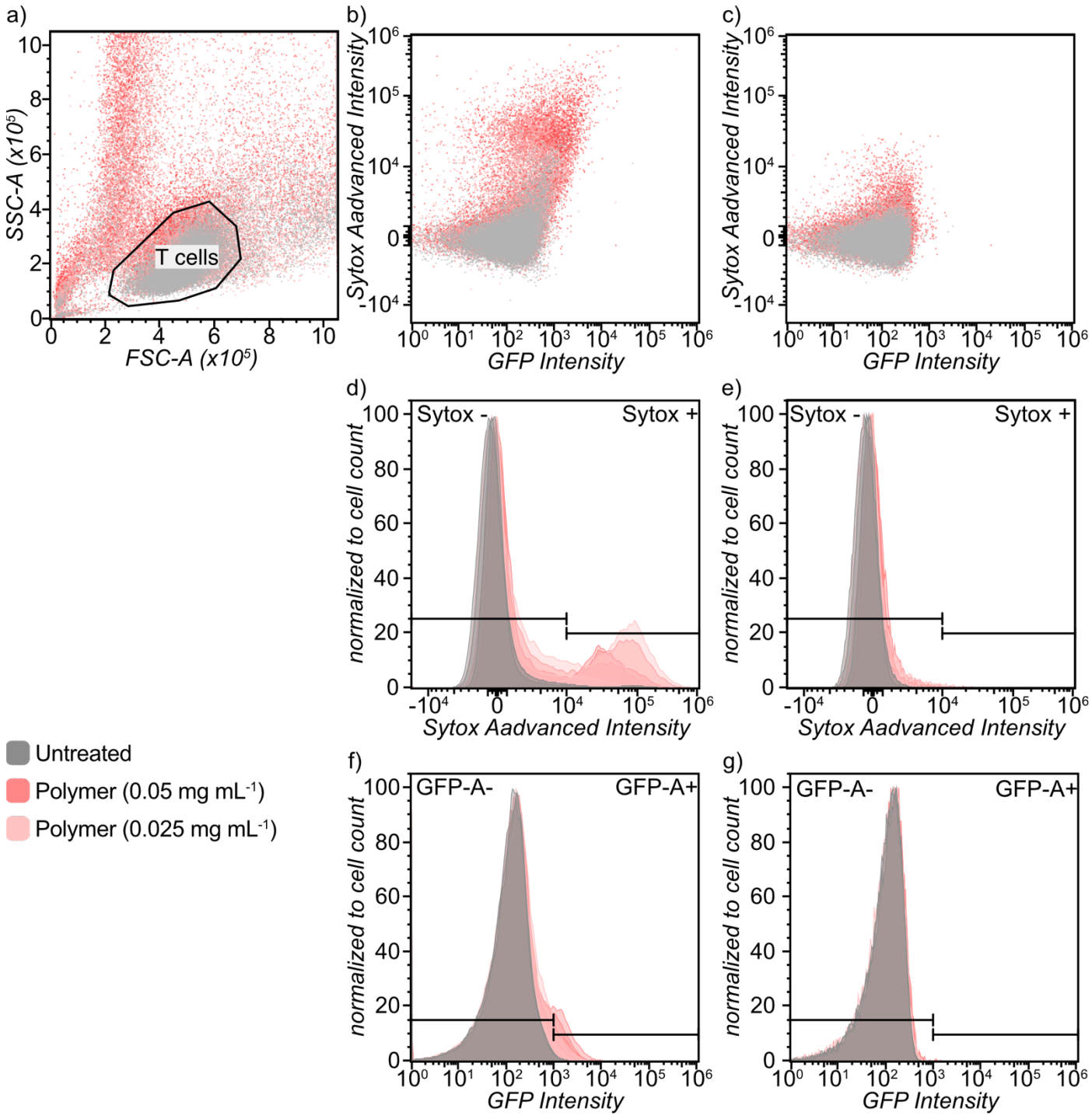

Figure S14. Summary of flow cytometry results for POEGMA-*b*-qP4VP. All samples were treated with Sytox Aadvanced. (a) Forward scatter area vs. side scatter area. T cells were gated as shown. (b) Sytox Aadvanced intensity vs. GFP intensity, ungated. (c) Sytox Aadvanced intensity vs. GFP intensity, gated for T cells. (d) Histogram of Sytox Aadvanced intensity, normalized to cell count, ungated. Sytox+ indicates dead cells. (e) Histogram of Sytox Aadvanced intensity, normalized to cell count and gated for T cells. Sytox+ indicates dead cells (f) Histogram of GFP intensity, normalized to cell count, ungated. (g) Histogram of GFP intensity, normalized to cell count and gated for T cells.

S.29 Figure S15. Flow cytometry data for GFP(+6)-6R

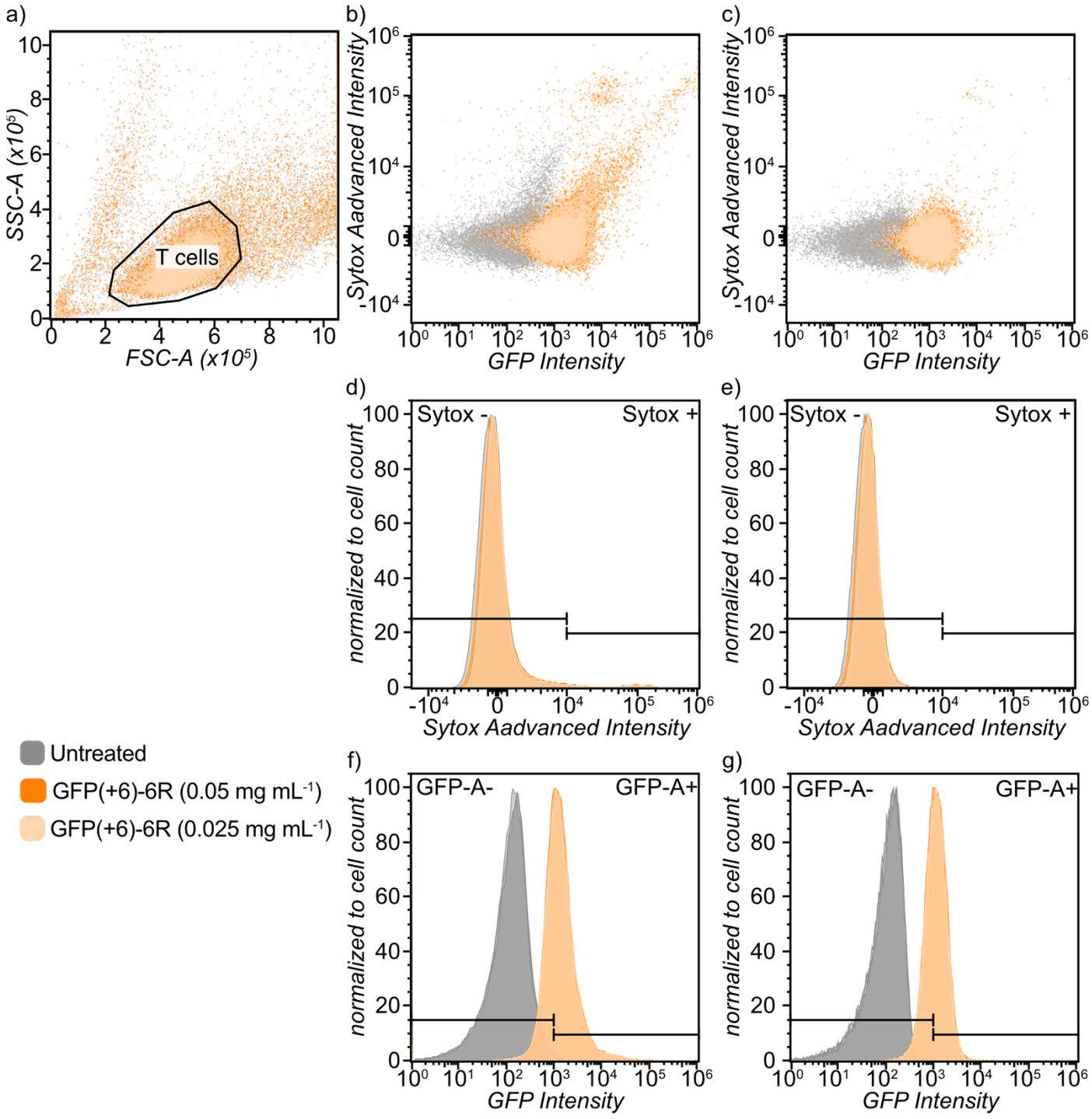

Figure S15. Summary of flow cytometry results for GFP(+6)-6R. All samples were treated with Sytox Aadvanced. (a) Forward scatter area vs. side scatter area. T cells were gated as shown. (b) Sytox Aadvanced intensity vs. GFP intensity, ungated. (c) Sytox Aadvanced intensity vs. GFP intensity, gated for T cells. (d) Histogram of Sytox Aadvanced intensity, normalized to cell count, ungated. Sytox+ indicates dead cells. (e) Histogram of Sytox Aadvanced intensity, normalized to cell count and gated for T cells. Sytox+ indicates dead cells (f) Histogram of GFP intensity, normalized to cell count, ungated. (g) Histogram of GFP intensity, normalized to cell count and gated for T cells.

S.30 Figure S16. Flow cytometry data for GFP(-18)

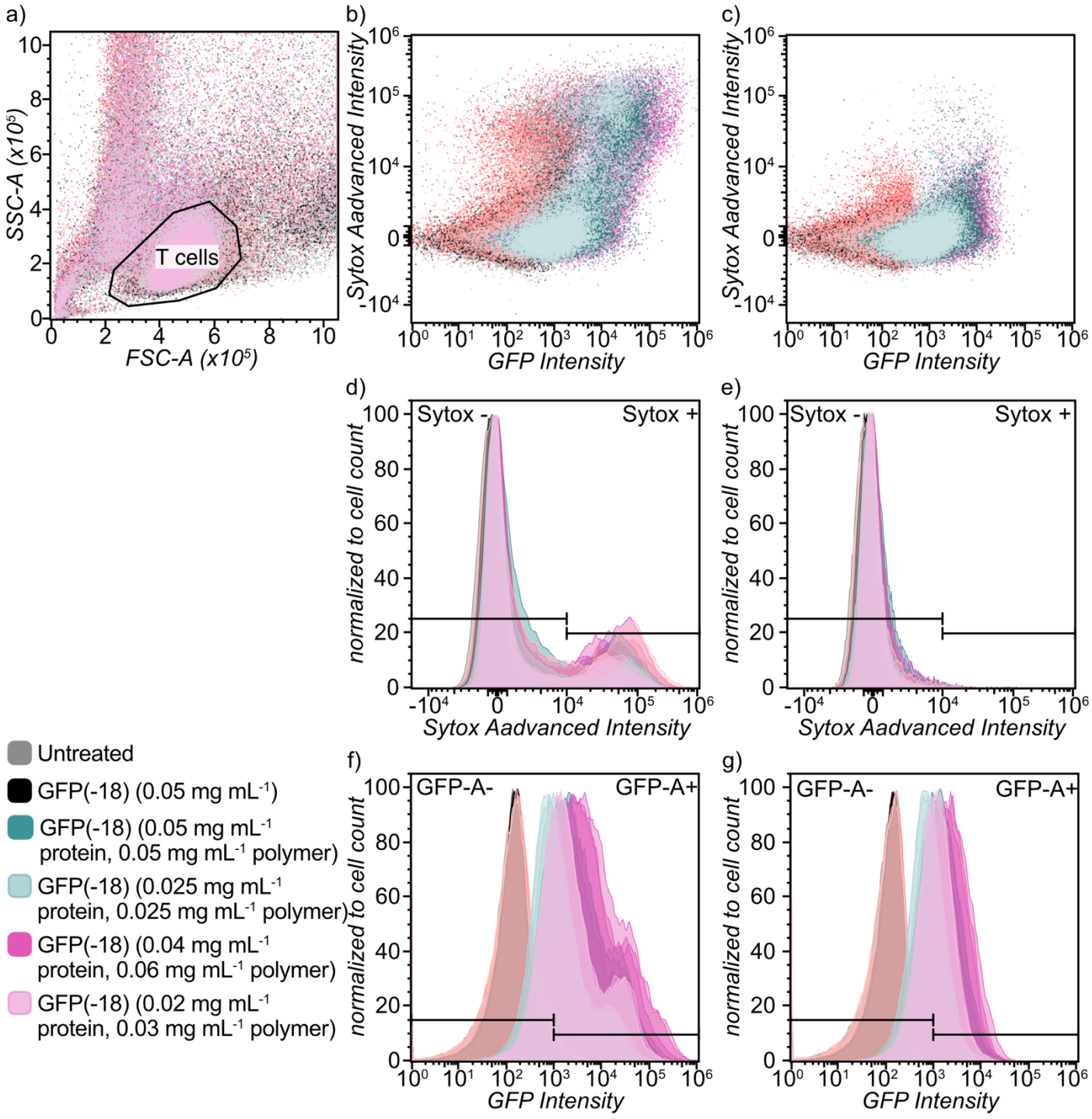

Figure S16. Summary of flow cytometry results for GFP(-18). All samples were treated with Sytox Aadvanced. (a) Forward scatter area vs. side scatter area. T cells were gated as shown. (b) Sytox Aadvanced intensity vs. GFP intensity, ungated. (c) Sytox Aadvanced intensity vs. GFP intensity, gated for T cells. (d) Histogram of Sytox Aadvanced intensity, normalized to cell count, ungated. Sytox+ indicates dead cells. (e) Histogram of Sytox Aadvanced intensity, normalized to cell count and gated for T cells. Sytox+ indicates dead cells (f) Histogram of GFP intensity, normalized to cell count, ungated. (g) Histogram of GFP intensity, normalized to cell count and gated for T cells.

S.31 Figure S17. Flow cytometry data for GFP(-24)

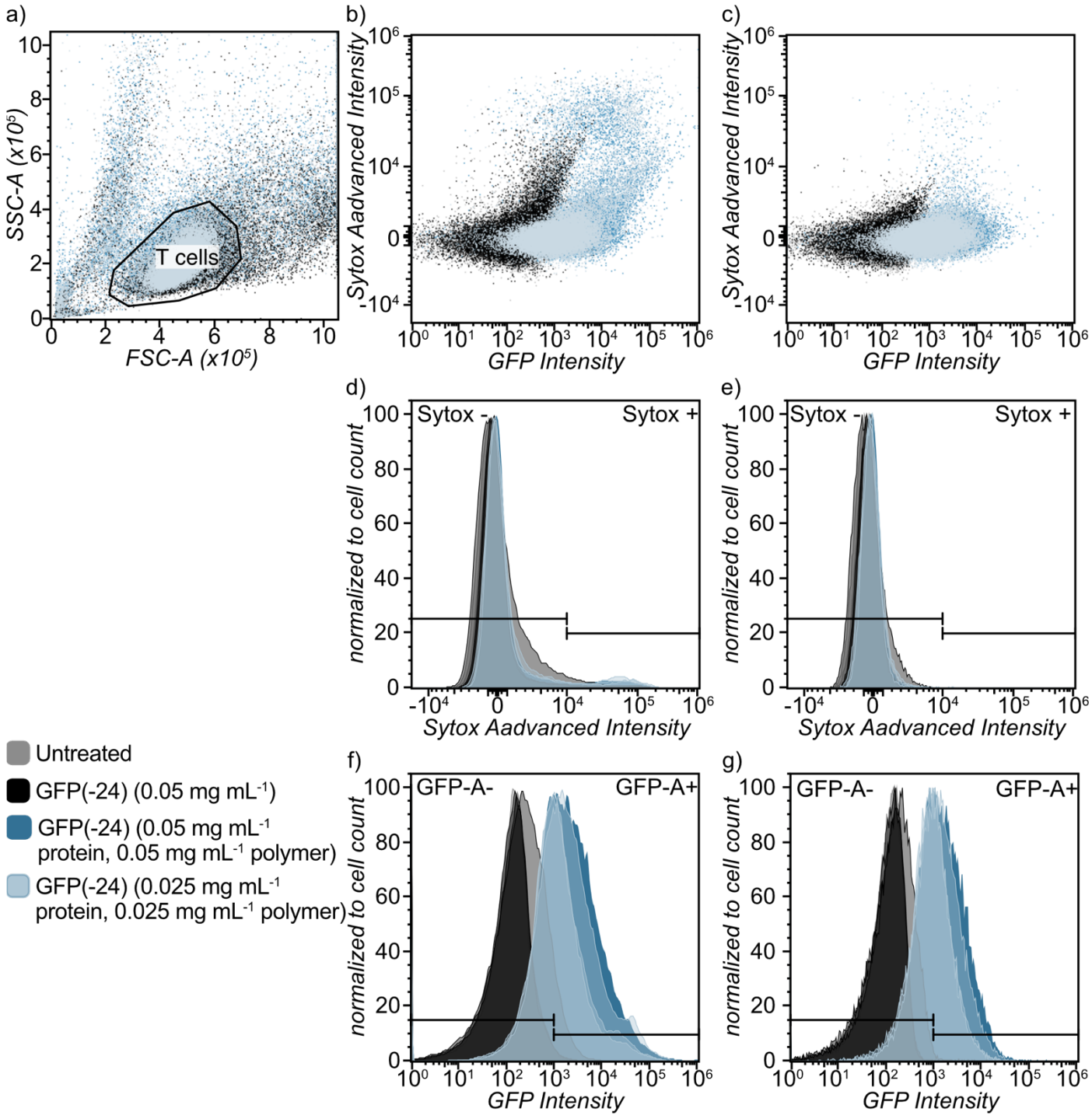

Figure S17. Summary of flow cytometry results for GFP(-24). All samples were treated with Sytox Aadvanced. (a) Forward scatter area vs. side scatter area. T cells were gated as shown. (b) Sytox Aadvanced intensity vs. GFP intensity, ungated. (c) Sytox Aadvanced intensity vs. GFP intensity, gated for T cells. (d) Histogram of Sytox Aadvanced intensity, normalized to cell count, ungated. Sytox+ indicates dead cells. (e) Histogram of Sytox Aadvanced intensity, normalized to cell count and gated for T cells. Sytox+ indicates dead cells (f) Histogram of GFP intensity, normalized to cell count, ungated. (g) Histogram of GFP intensity, normalized to cell count and gated for T cells.

S.32 Figure S18. Flow cytometry data for GFP(-30)

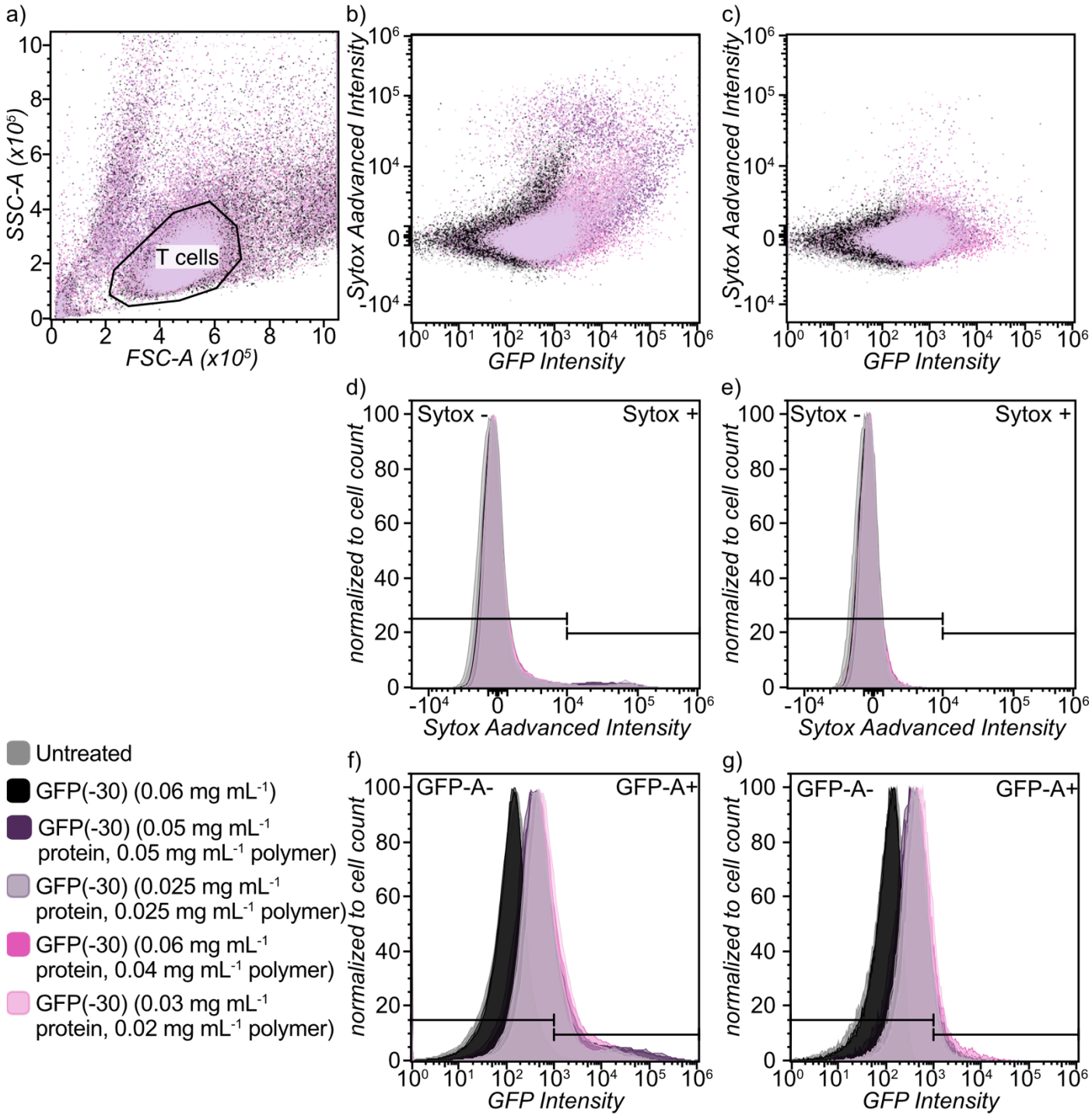

Figure S18. Summary of flow cytometry results for GFP(-30). All samples were treated with Sytox Aadvanced. (a) Forward scatter area vs. side scatter area. T cells were gated as shown. (b) Sytox Aadvanced intensity vs. GFP intensity, ungated. (c) Sytox Aadvanced intensity vs. GFP intensity, gated for T cells. (d) Histogram of Sytox Aadvanced intensity, normalized to cell count, ungated. Sytox+ indicates dead cells. (e) Histogram of Sytox Aadvanced intensity, normalized to cell count and gated for T cells. Sytox+ indicates dead cells (f) Histogram of GFP intensity, normalized to cell count, ungated. (g) Histogram of GFP intensity, normalized to cell count and gated for T-cells.

S.33 Figure S19. Flow cytometry data for tag-GFP(-24)

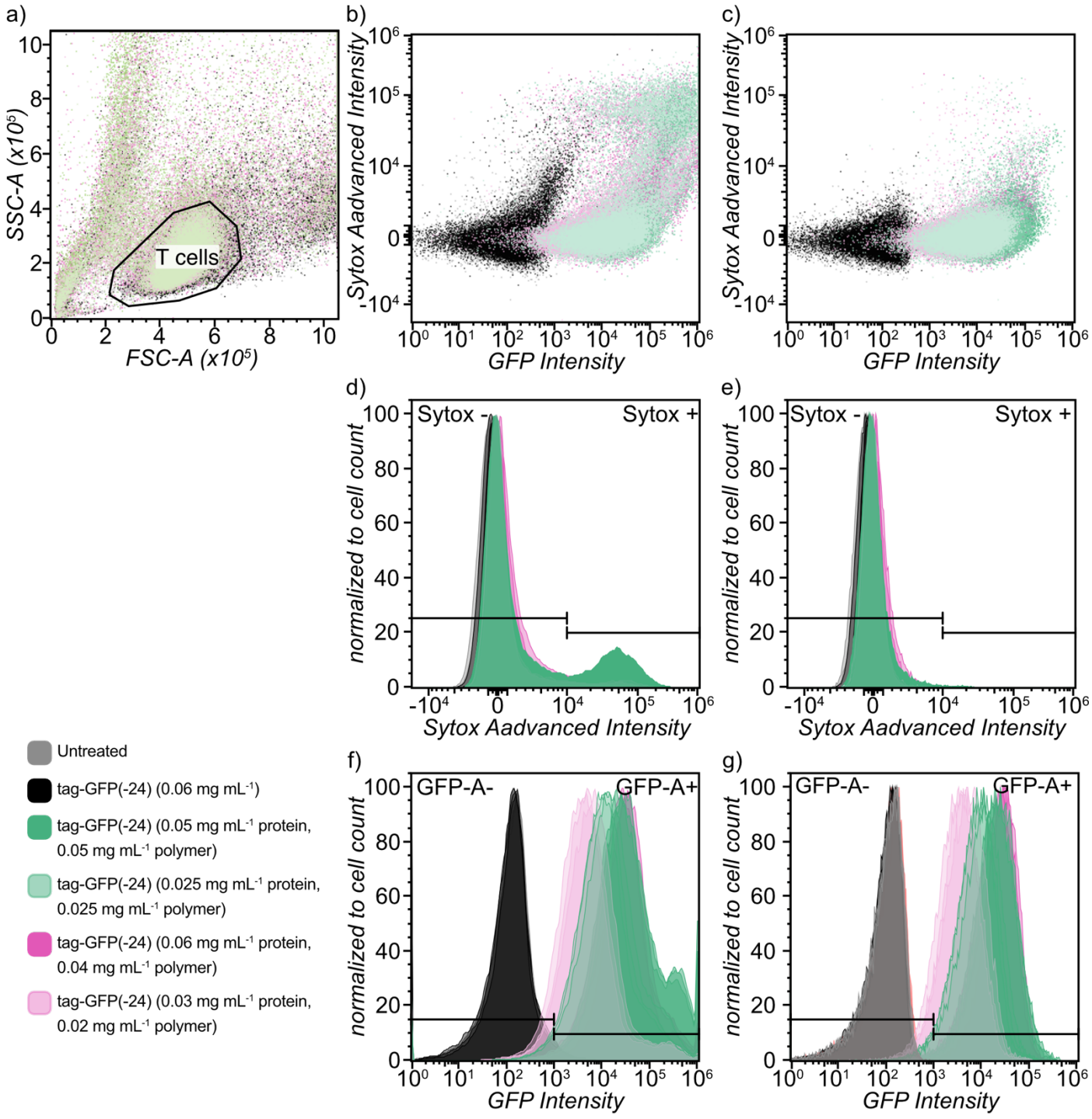

Figure S19. Summary of flow cytometry results for tag-GFP(-24). All samples were treated with Sytox Aadvanced. (a) Forward scatter area vs. side scatter area. T cells were gated as shown. (b) Sytox Aadvanced intensity vs. GFP intensity, ungated. (c) Sytox Aadvanced intensity vs. GFP intensity, gated for T cells. (d) Histogram of Sytox Aadvanced intensity, normalized to cell count, ungated. Sytox+ indicates dead cells. (e) Histogram of Sytox Aadvanced intensity, normalized to cell count and gated for T cells. Sytox+ indicates dead cells (f) Histogram of GFP intensity, normalized to cell count, ungated. (g) Histogram of GFP intensity, normalized to cell count and gated for T cells.

S.34 Figure S20. Overlayed flow cytometry histograms for PECMs

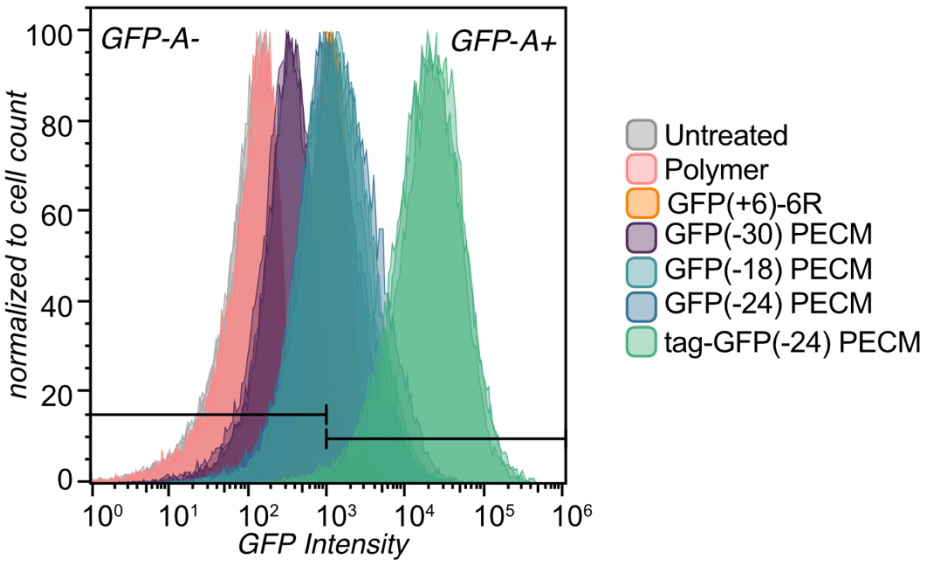

Figure S20. Histogram of GFP intensity, normalized to cell count and gated for T cells. 3 replicates were conducted and overlayed. All samples treated with Sytox Aadvanced. Polymer and GFP(+6)-6R contain 0.05 mg mL^{-1} of polymer and protein respectively. PEC micelle samples contain 0.05 mg mL^{-1} of polymer and protein each.

S.35 Figure S21. Mean fluorescence intensity from flow cytometry

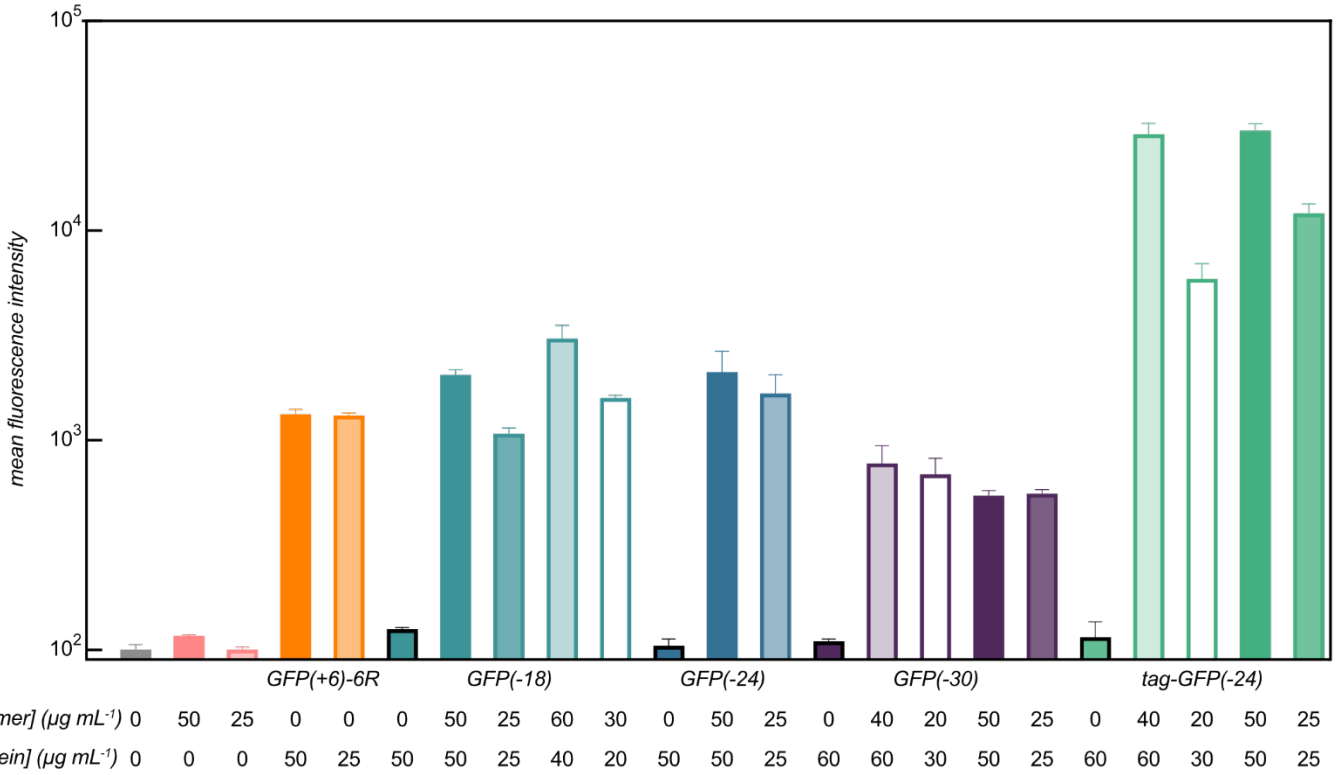

Figure S21. Mean fluorescence intensity of Jurkat T-cells. All samples treated with Sytox Aadvanced. Gated for T-cells. Shows mean and standard deviation of 3 replicates.

S.36 Figure S22. Widefield confocal microscopy images

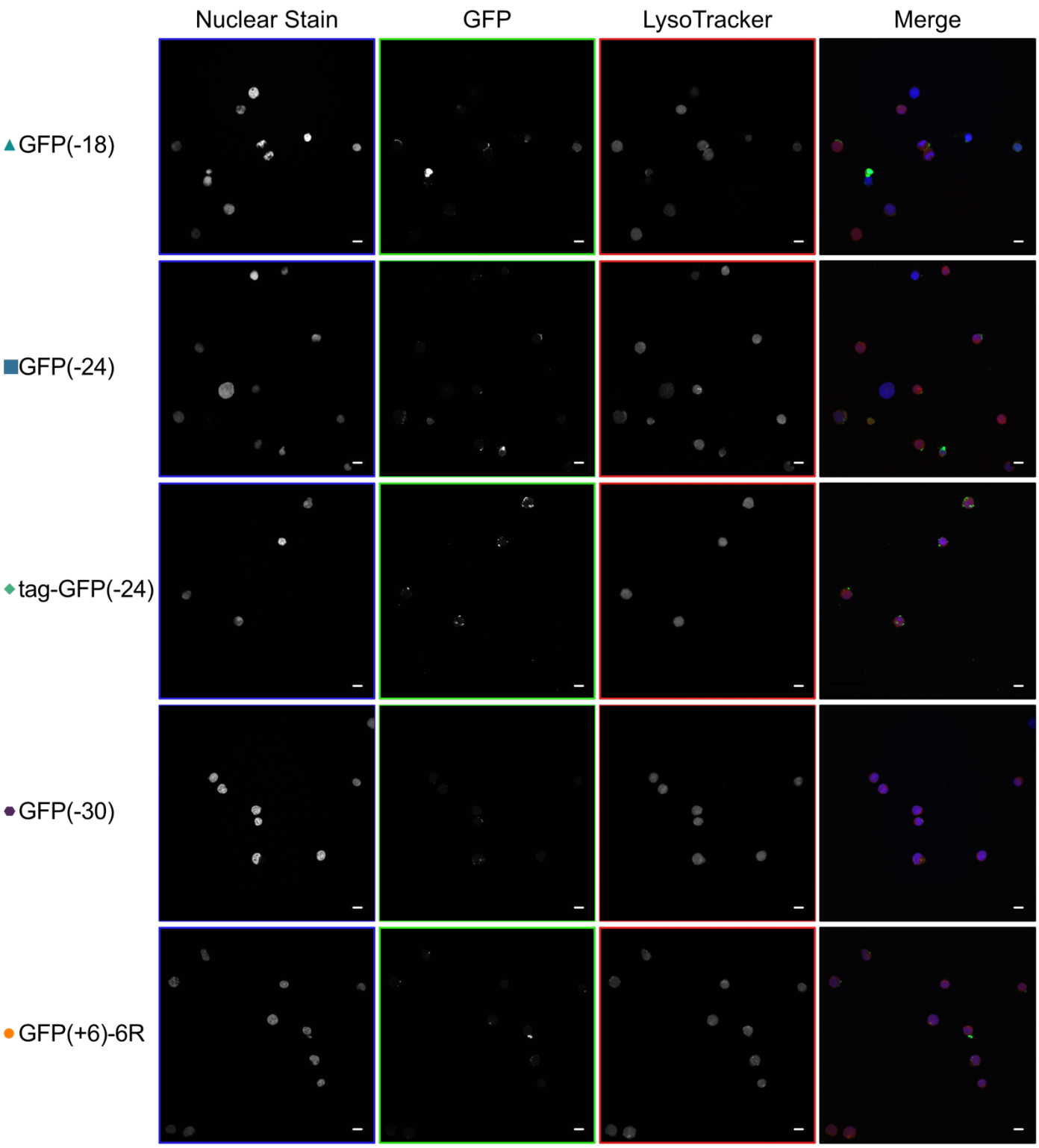

Figure S22. Confocal images of Jurkat T cells. Cells were incubated with GFP containing micelles or free protein (GFP(+6)-6R) for 1 h. After washing 3x with DPBS, they were incubated with 150 nM LysoTracker Red DND-99 at 37 °C for 30 minutes. Cells were fixed with 4% formaldehyde + DPBS and stained with NucBlue for 15 min. Scale bar = 10 µm.