Supplementary Information

Supercharged Protein Nanosheets for Cell Expansion on Bioemulsions

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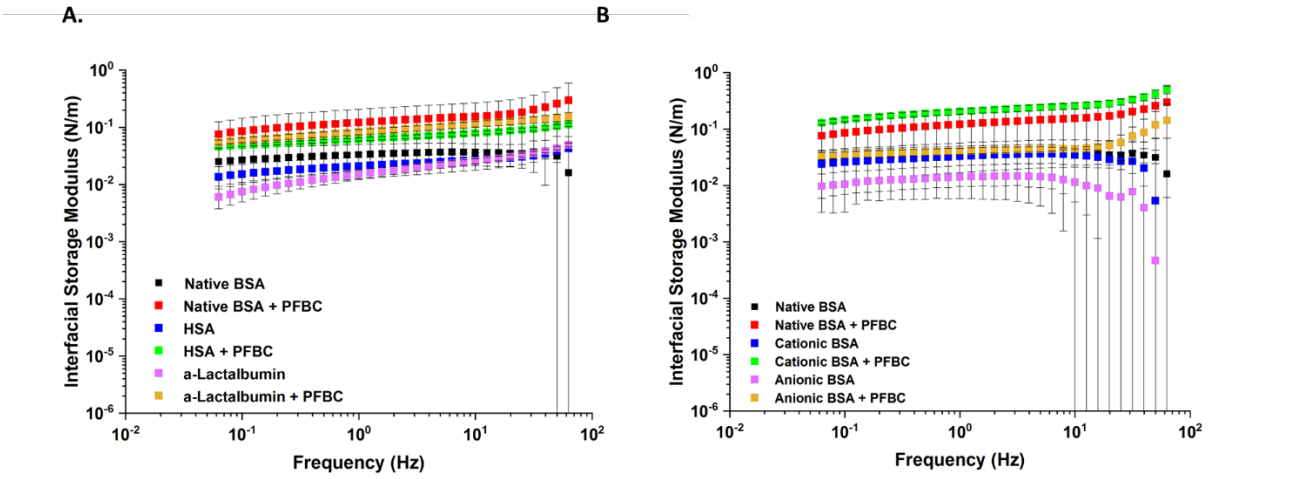

Figure S1. Frequency sweep at oscillating amplitude of 10^{-4} rad carried out for the characterization of (A) BSA, HSA and α-Lactalbumin (all at 1 mg/mL) with and without pro-surfactant PFBC and (B) native, cationic (cBSA) and anionic BSA (aBSA) with and without pro-surfactant PFBC (10 μg/mL). All experiments were carried out at interfaces between PBS and Novec 7500 (fluorinated) oil.

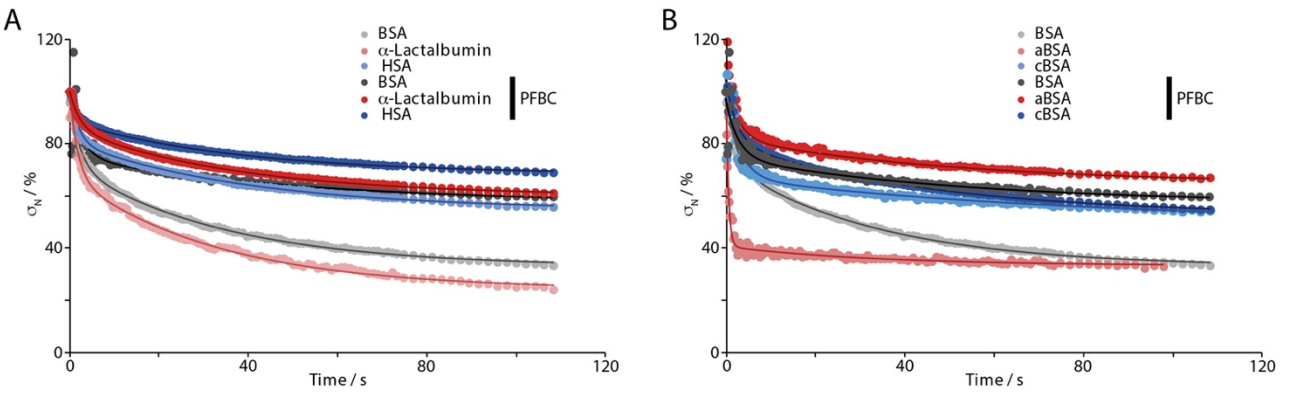

Figure S2. Normalised stress relaxation experiments carried out on protein nanosheets formed at liquid-liquid interfaces with and without PFBC (10 μg/mL). Data is shown as normalised stress (σ_N) extracted from stress relaxation experiments at a strain 0.5%. (A) Comparison of BSA, HSA, α-Lactalbumin and (B) native BSA, cBSA and aBSA.

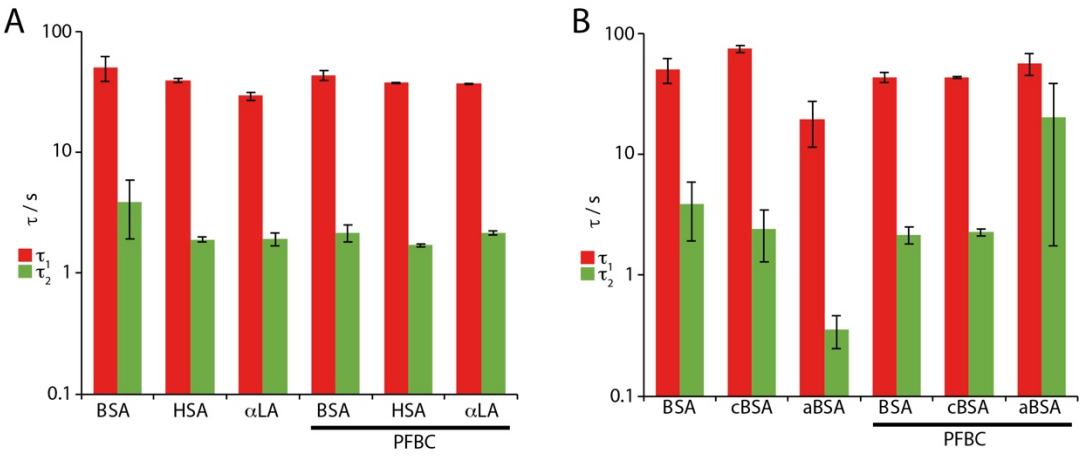

Figure S3. Characterisation of the stress relaxation profiles associated with protein nanosheets studied. Data were extracted from stress relaxation at a strain of 0.5%. (A) Comparing BSA, HSA, α-Lactalbumin (all at 1 mg/mL) and (B) native BSA, cBSA and aBSA. Error bars are s.e.m; n=3.

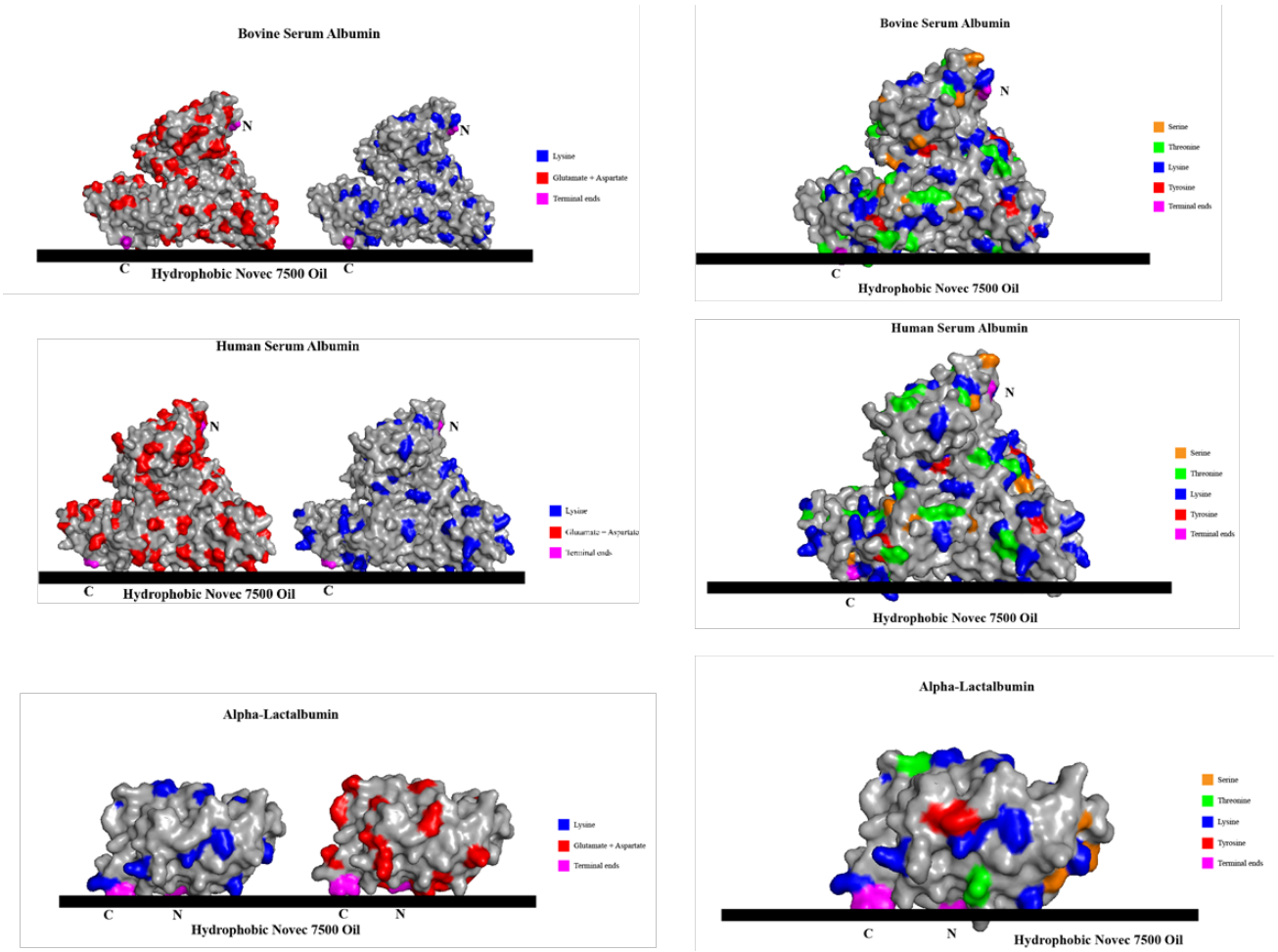

Figure S4. Proposed idealised model (not taking into account protein denaturation) for the adsorption of different albumin proteins at hydrophobic Novec 7500 oil interfaces. Orientations proposed are based on the hydrophobicity of corresponding protein surfaces and are not further optimised via molecular dynamics. Note that it is likely that significant denaturation and remodelling takes place following on from such initial interaction configuation. Left images. Surface accessible residues potentially reacting during the supercharging of corresponding proteins; Red – Glutamate and Aspartate residues, Blue – lysine residues. Pink - Terminal ends. Right images. Surface accessible hydroxyl-based amino acids, and in blue lysine residues. Images were orientated and generated using Pymol software; the draw "Connolly surfaces" command was used to trace the surface of the proteins that are proposed to be in direct contact with the buffer.

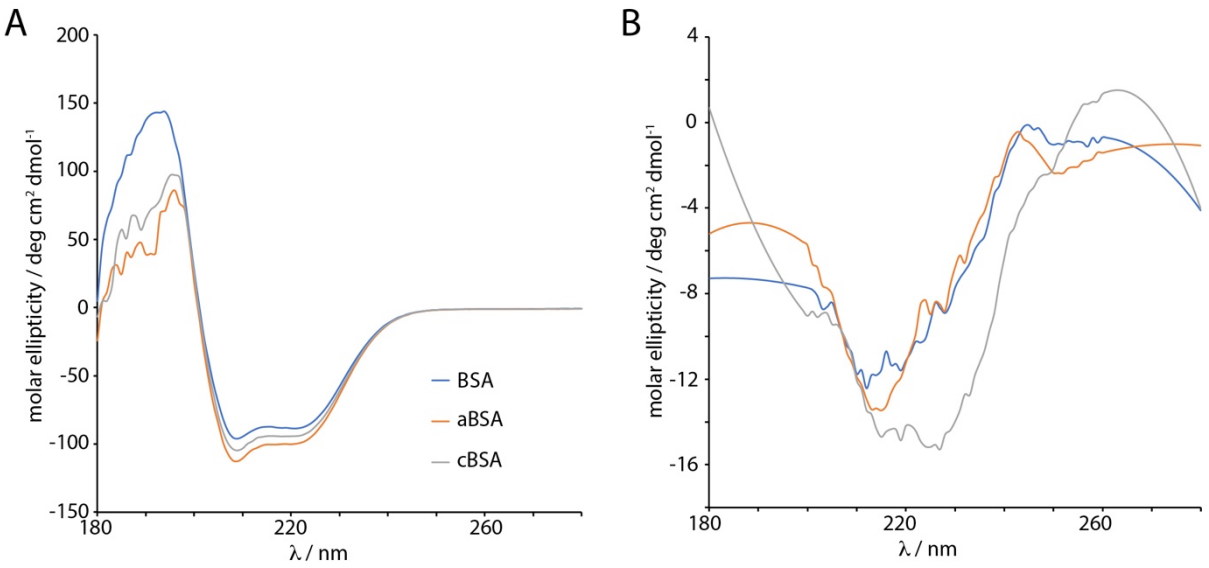

Figure S5. Circular dichroism spectra of BSA, aBSA and cBSA in solution (A) and after adsorption at liquid-liquid interfaces (Novec 7500/α-α-α-trifluorotoluene emulsions; excess free proteins removed).

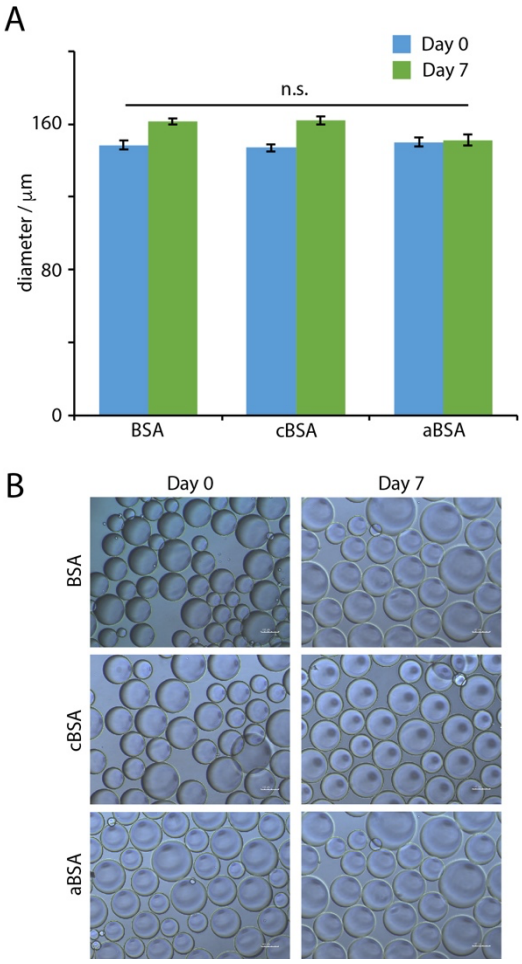

Figure S6. A. Quantification of droplet dimensions shortly after formation and after 7 days of incubation. B. Images of corresponding droplets.

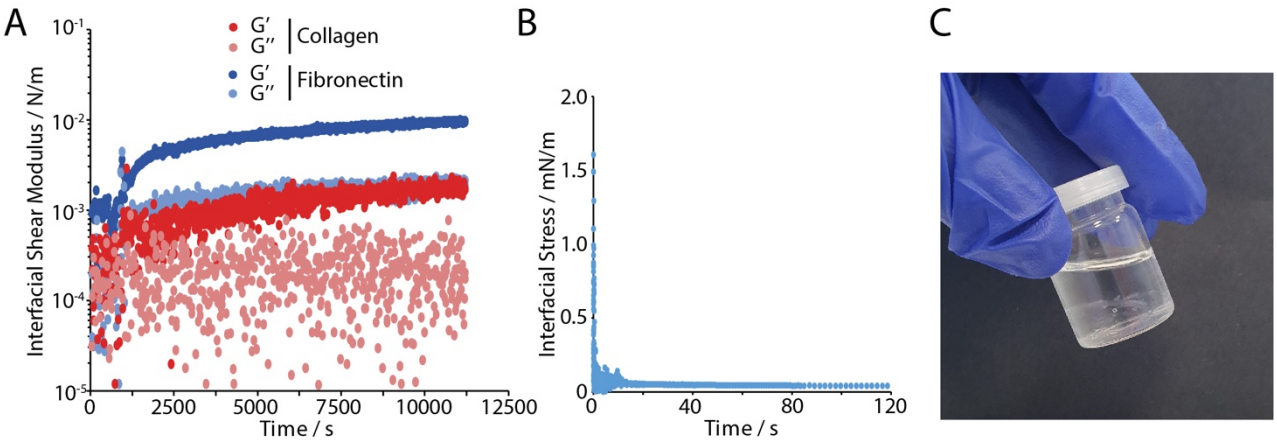

Figure S7. A. Time sweeps of the evolution of interfacial storage moduli of Novec 7500/PBS interfaces during the assembly of fibronectin and collagen (10 and 100 μg/mL, respectively; direct assembly to the oil interface; 1.0 10^{-3} rad, strain of 1 %). B. Stress relaxation experiment carried out on the corresponding interface, with adsorbed fibronectin (strain 0.5%). C. Images of a vial in which an attempt at stabilising an emulsion using fibronectin (10 μg/mL) was made, but failed (apparent phase separation).

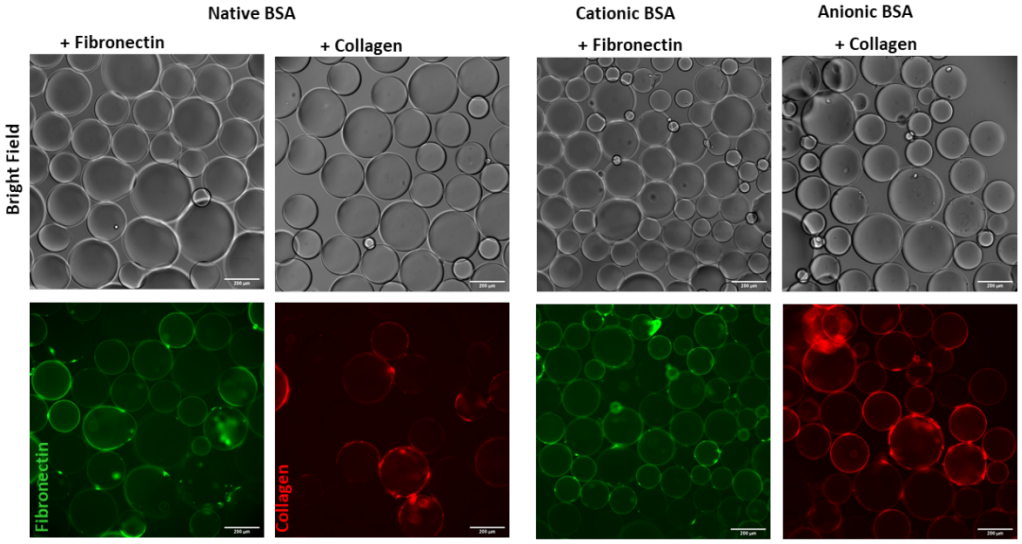

Figure S8. Epifluorescence microscopy images were with rabbit (green, AB F3648) and mouse (red, AB ab90395) antibodies and species specific secondary antibodies conjugated. Scale bars, 200 μm.

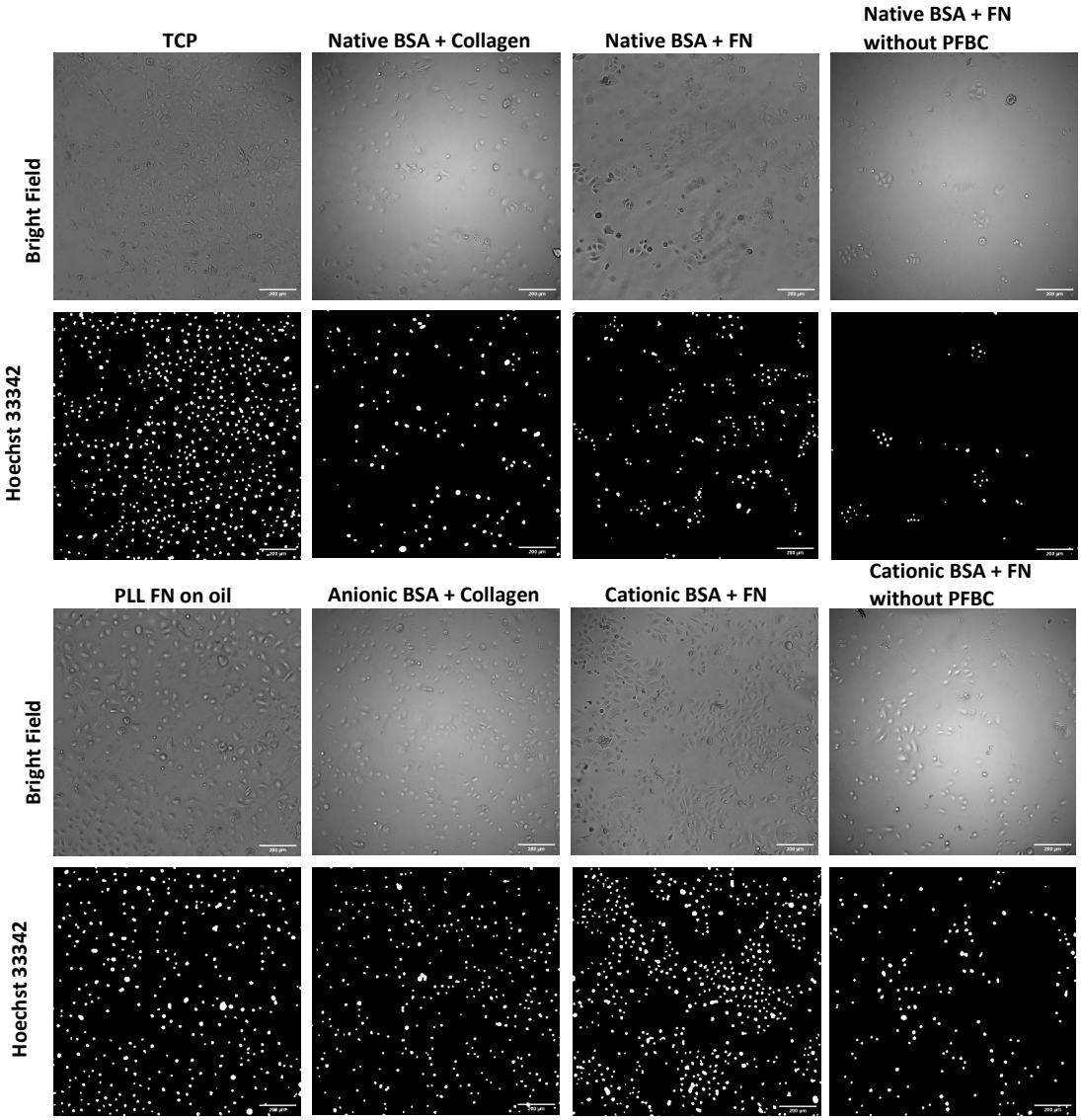

Figure S9. Bright field and epifluorescence images of HPKs adhered on pinned droplets after three days in culture. Images are corresponding nuclear stainings (Hoechst 33342). Scale bar, 200 µm.

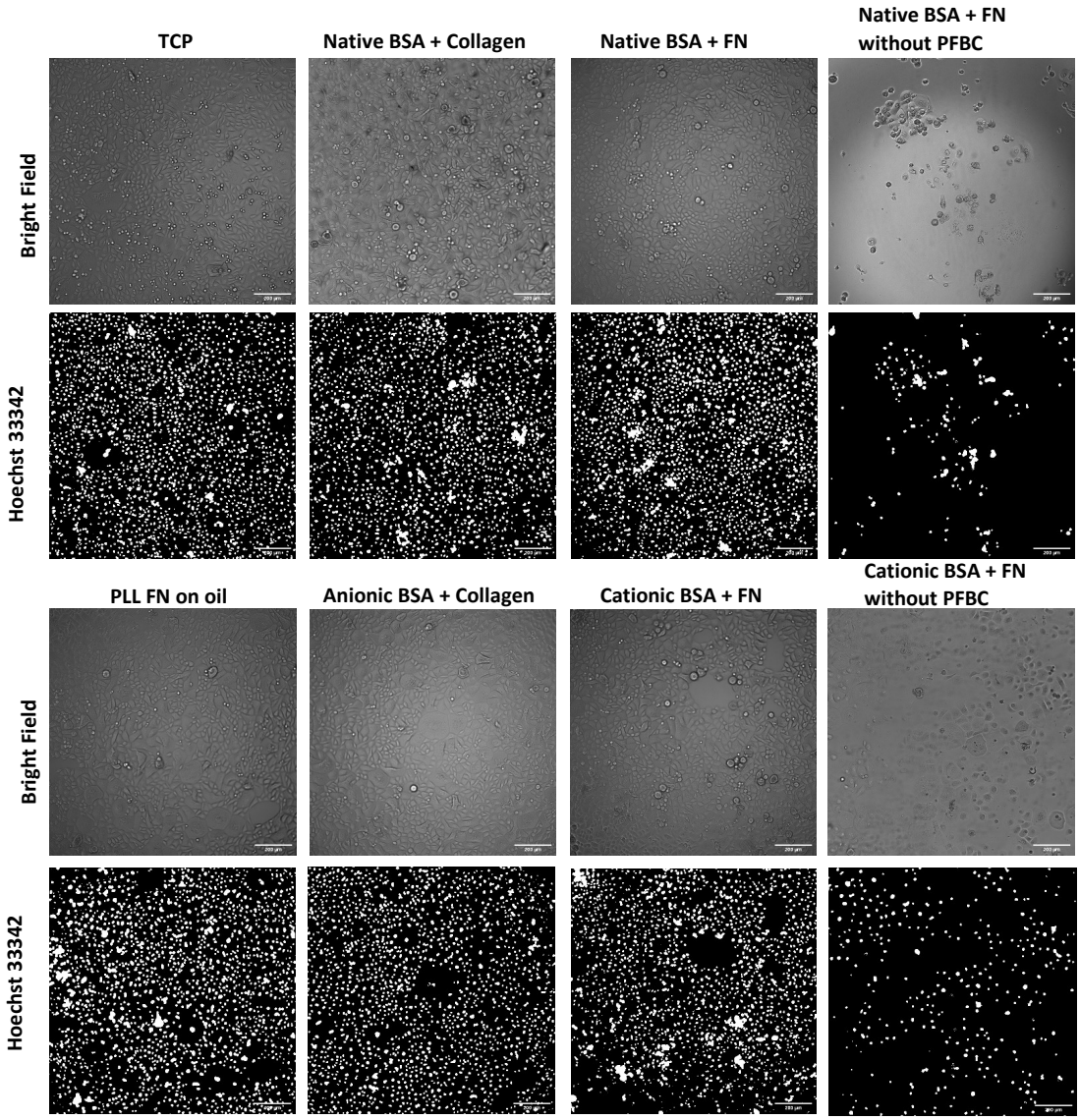

Figure S10. Bright field and epifluorescence images of HPKs adhered on pinned droplets after seven days in culture. Images are corresponding nuclear stainings (Hoechst 33342). Scale bars, 200 µm.

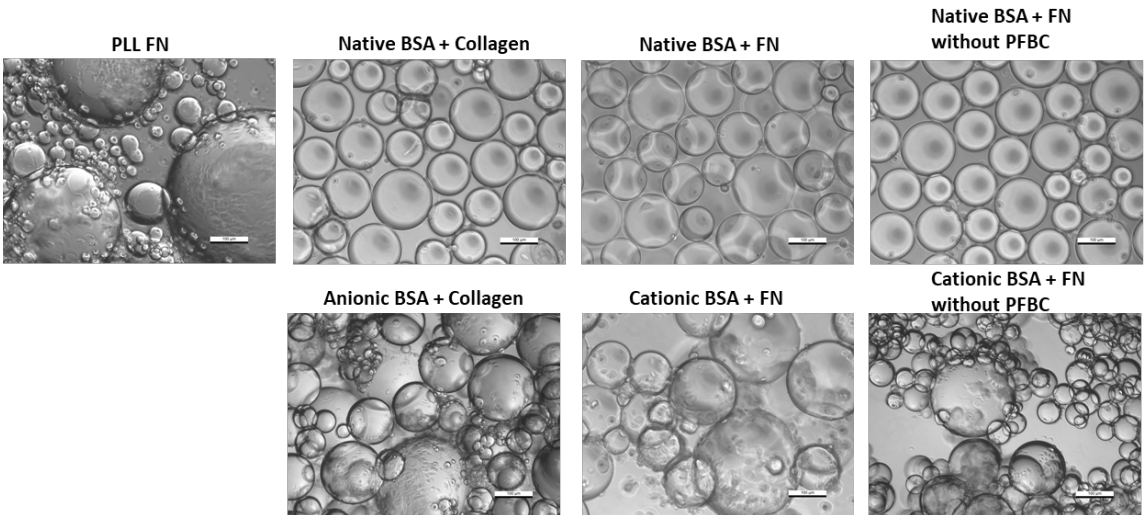

Figure S11. Bright field images showing the HPKs adhesion and growing on the oil droplets after three days of culture. Scale bars, 100 µm.

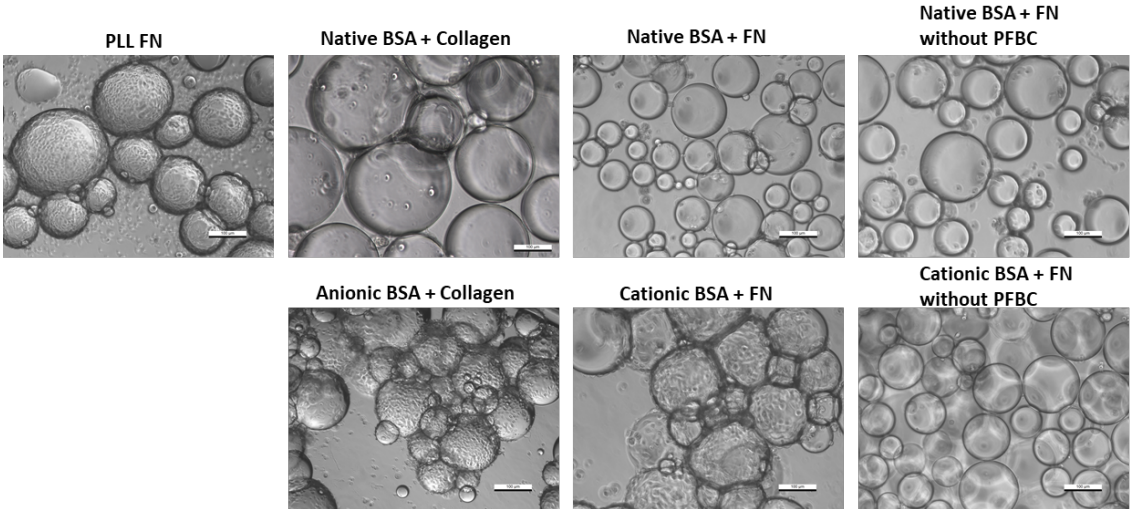

Figure S12. Bright field images showing the HPKs adhesion and growing on the oil droplets after seven days of culture. Scale bars, 100 µm.

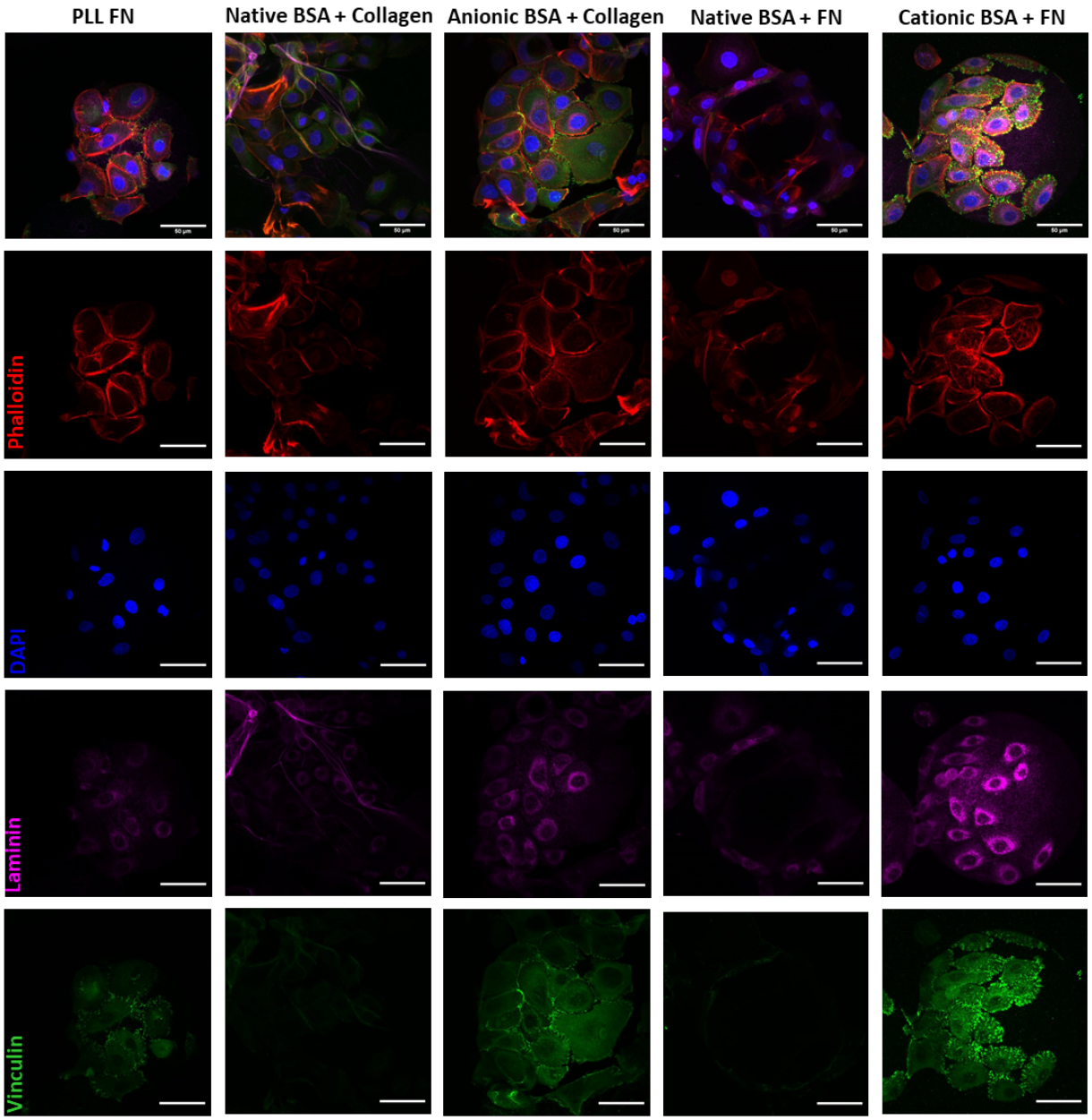

Figure S13. Confocal fluorescence microscopy images of HPKs spreading after seven days on emulsion (blue, DAPI; red, phalloidin; green, vinculin; purple, laminin). Error bars are s.e.m.; n = 3. Scale bars, 50 µm.

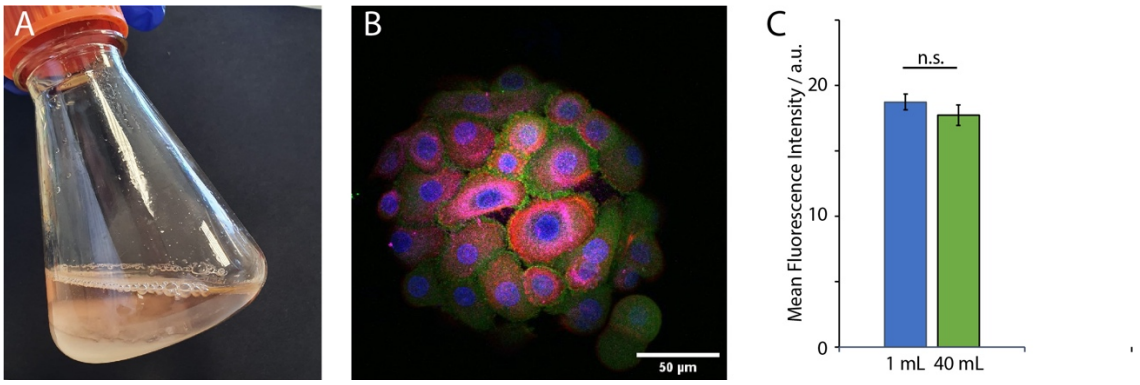

Figure S14. A. Image of a conical flask used for the culture of HPKs on cBSA/FN stabilised bioemulsions. B. Confocal fluorescence microscopy images of HPKs spreading after seven days on cBSA/FN-stabilised emulsion, in a conical flask (blue, DAPI; red, phalloidin; green, vinculin; purple, laminin). C. Quantification of laminin expression by HPKS grown on cBSA/FN-stabilised emulsions, in multi-well plate (1 mL volume) or conical flasks (40 mL). Error bars are s.e.m.; n = 3. Scale bars, 50 µm.

Supplementary Tables

Table S1. Summary of statistical analysis of data obtained by frequency sweep after the proteins were adsorbed at fluorinated-PBS interfaces with and without co-surfactant (PFBC) at 10 μg/ mL (Figures 1E and 2E).

	MeanDiff	**Prob**	
Cationic BSA Native BSA	-0.02877	1.58E-04	***
Anionic BSA Native BSA	-0.04202	5.37E-06	***
Anionic BSA Cationic BSA	-0.01325	0.03919	*
α-Lactalbumin Native BSA	-0.01816	0.00563	**
α-Lactalbumin Cationic BSA	0.01061	0.11291	n.s
α-Lactalbumin Anionic BSA	0.02385	7.38E-04	***
HSA Native BSA	-0.01692	0.00907	**
HSA Cationic BSA	0.01185	0.06883	n.s
HSA Anionic BSA	0.0251	4.91E-04	***
HSA α-Lactalbumin	0.00124	0.99721	n.s
Cationic BSA with PFBC Native BSA with PFBC	0.21018	3.49E-07	***
Anionic BSA with PFBC Native BSA with PFBC	-4.25E-04	1	n.s
Anionic BSA with PFBC Cationic BSA with PFBC	-0.21061	3.43E-07	***
α-Lactalbumin with PFBC Native BSA with PFBC	0.06796	0.0055	**
α-Lactalbumin with PFBC Cationic BSA with PFBC	-0.14222	1.31E-05	***
α-Lactalbumin with PFBC Anionic BSA with PFBC	0.06839	0.00527	**
HSA with PFBC Native BSA with PFBC	0.03371	0.20575	n.s
HSA with PFBC Cationic BSA with PFBC	-0.17648	2.23E-06	***
HSA with PFBC Anionic BSA with PFBC	0.03413	0.19724	n.s
HSA with PFBC α-Lactalbumin with PFBC	-0.03425	0.19491	n.s

Table S2. Summary of statistical analysis of data obtained by frequency sweep after the proteins were adsorbed at the fluorinated-PBS interface with and without the surfactant at 10 μg/ mL. Pairwise comparison to determine the significance of the effect of prosurfactant (Figures 1E and 2E).

	MeanDiff	**Prob**	
Native BSA with PFBC Native BSA	0.00165	0.57633	n.s
Cationic BSA with PFBC Cationic BSA	0.24061	3.42E-04	***
Anionic BSA with PFBC Anionic BSA	0.04324	2.39E-07	***
α-Lactalbumin with PFBC α-Lactalbumin	0.08778	3.15E-04	***
HSA with PFBC HSA	0.05228	9.98E-04	***

Table S3. Summary of statistical analysis of data obtained from stress relaxation experiments at a strain of 0.5 % (Figures 1F and 2F).

	MeanDiff	Prob	
BSA PFBC BSA	24.309	0.03934	*
cBSA PFBC cBSA	4.34233	0.63335	n.s
aBSA PFBC aBSA	36.47233	0.02059	*
HSA PFBC HSA	16.679	0.05404	n.s
α-Lactalbumin PFBC α-Lactalbumin	41.525	0.0022	**

Table S4. Summary of statistical analysis of data obtained from the SPR data for the protein binding (1 mg/mL) at the perfluorodecanethiol pre-treated chips (Figure 3B).

	MeanDiff	Prob	
Anionic BSA Native BSA	296.9	0.07194	n.s
Cationic BSA Native BSA	1381.267	3.27E-05	***
Cationic BSA Anionic BSA	1084.367	1.32E-04	***

Table S5. Summary of statistical analysis of data obtained from the SPR data for the fibronectin or collagen binding at the surface of supercharged protein layers (native, cationic and anionic BSA) (Figure 3D).

	MeanDiff	Prob	
BSA/Col BSA/FN	-37.3333	0.98415	n.s
cBSA/FN BSA/FN	-30	0.99159	n.s
cBSA/FN BSA/Col	7.33333	0.99987	n.s
aBSA/Col BSA/FN	679	9.71E-04	***
aBSA/Col BSA/Col	716.3333	6.76E-04	***
aBSA/Col cBSA/FN	709	7.25E-04	***

Table S6. Summary of statistical analysis of data obtained from the epifluorescence images for the fibronectin or collagen binding on native, anionic and cationic BSA emulsion droplets (Figure 3F).

	MeanDiff	Prob	
Cationic BSA + FN Native BSA + FN	4425.399	0.00282	**
Anionic BSA + Collagen Native BSA + Collagen	6989.911	0.00253	**
Native BSA + Collagen Native BSA + FN	-2893.23	0.02859	*
Anionic BSA + Collagen Cationic BSA + FN	-328.722	0.72984	n.s

Table S7. Summary of statistical analysis of data obtained from cell (HPK) density on pinned droplets after three days in culture (Figure 4A).

	MeanDiff	Prob	
PLL FN TPS	-104.667	0.90088	n.s
Native BSA Collagen TPS	-432.444	0.00173	*
Native BSA Collagen PLL FN	-327.778	0.0199	*
Anionic BSA Collagen TPS	-351.667	0.01138	*
Anionic BSA Collagen PLL FN	-247	0.12135	n.s
Anionic BSA Collagen Native BSA Collagen	80.77778	0.97227	n.s
Native BSA FN TPS	-354.556	0.01064	*
Native BSA FN PLL FN	-249.889	0.11422	n.s
Native BSA FN Native BSA Collagen	77.88889	0.9772	n.s
Native BSA FN Anionic BSA Collagen	-2.88889	1	n.s
Cationic BSA FN TPS	-201.111	0.29522	n.s
Cationic BSA FN PLL FN	-96.4444	0.93205	n.s
Cationic BSA FN Native BSA Collagen	231.3333	0.16716	n.s
Cationic BSA FN Anionic BSA Collagen	150.5556	0.62167	n.s
Cationic BSA FN Native BSA FN	153.4444	0.601	n.s
Native BSA FN no PFBC TPS	-534.111	1.78E-04	***
Native BSA FN no PFBC PLL FN	-429.444	0.00185	**
Native BSA FN no PFBC Native BSA Collagen	-101.667	0.91308	n.s
Native BSA FN no PFBC Anionic BSA Collagen	-182.444	0.40249	n.s
Native BSA FN no PFBC Native BSA FN	-179.556	0.42085	n.s
Native BSA FN no PFBC Cationic BSA FN	-333	0.01762	*
Cationic BSA FN no PFBC TPS	-460	9.18E-04	***
Cationic BSA FN no PFBC PLL FN	-355.333	0.01044	*
Cationic BSA FN no PFBC Native BSA Collagen	-27.5554	0.99997	n.s
Cationic BSA FN no PFBC Anionic BSA Collagen	-108.333	0.88469	n.s
Cationic BSA FN no PFBC Native BSA FN	-105.444	0.89756	n.s
Cationic BSA FN no PFBC Cationic BSA FN	-258.889	0.09432	n.s
Cationic BSA FN no PFBC Native BSA FN no PFBC	74.11122	0.98265	n.s

Table S8. Summary of statistical analysis of data obtained from cell (HPK) density on pinned droplets after seven days in culture (Figure 4A).

	MeanDiff	Prob	
PLL FN TPS	-129.333	0.99923	n.s
Native BSA Collagen TPS	-461.778	0.56631	n.s
Native BSA Collagen PLL FN	-332.444	0.85717	n.s
Anionic BSA Collagen TPS	-543.667	0.37824	n.s
Anionic BSA Collagen PLL FN	-414.333	0.68207	n.s
Anionic BSA Collagen Native BSA Collagen	-81.8889	0.99996	n.s
Native BSA FN TPS	-592.889	0.28432	n.s
Native BSA FN PLL FN	-463.556	0.56197	n.s
Native BSA FN Native BSA Collagen	-131.111	0.99916	n.s
Native BSA FN Anionic BSA Collagen	-49.2222	1	n.s
Cationic BSA FN TPS	-473.111	0.53874	n.s
Cationic BSA FN PLL FN	-343.778	0.83641	n.s
Cationic BSA FN Native BSA Collagen	-11.3333	1	n.s
Cationic BSA FN Anionic BSA Collagen	70.55556	0.99999	n.s
Cationic BSA FN Native BSA FN	119.7778	0.99953	n.s
Native BSA FN no PFBC TPS	-2030.89	6.72E-06	***
Native BSA FN no PFBC PLL FN	-1901.56	1.58E-05	***
Native BSA FN no PFBC Native BSA Collagen	-1569.11	1.64E-04	***
Native BSA FN no PFBC Anionic BSA Collagen	-1487.22	3.01E-04	***
Native BSA FN no PFBC Native BSA FN	-1438	4.38E-04	***
Native BSA FN no PFBC Cationic BSA FN	-1557.78	1.78E-04	***
Cationic BSA FN no PFBC TPS	-1660.33	8.43E-05	***
Cationic BSA FN no PFBC PLL FN	-1531	2.17E-04	***
Cationic BSA FN no PFBC Native BSA Collagen	-1198.56	0.00284	**
Cationic BSA FN no PFBC Anionic BSA Collagen	-1116.67	0.00548	**
Cationic BSA FN no PFBC Native BSA FN	-1067.44	0.00815	**
Cationic BSA FN no PFBC Cationic BSA FN	-1187.22	0.00311	**
Cationic BSA FN no PFBC Native BSA FN no PFBC	370.5556	0.78223	n.s

Table S9. Summary of statistical analysis of data obtained from cell adhesion area on pinned droplets after 48h in culture (Figure 4C).

	MeanDiff	Prob	
PLL FN TCP	238.244	0.51286	n.s
Anionic BSA Collagen Native BSA Collagen	463.9954	0.51532	n.s
Cationic BSA FN Native BSA FN	449.8663	0.34017	n.s
Native BSA Collagen PLL FN	-1006.69	0.03179	*
Anionic BSA Collagen PLL FN	-542.691	0.44682	n.s
Native BSA FN PLL FN	-683.948	0.0558	n.s
Cationic BSA FN PLL FN	-234.082	0.62417	n.s

Table S10. Summary of statistical analysis of data obtained by mean fluorescence intensity for laminin deposition from cells cultured on emulsion droplets (Figure 4D).

	MeanDiff	Prob	
Native BSA + Collagen PLL + FN	0.89844	0.91187	n.s
Anionic BSA + Collagen PLL + FN	7.97922	1.63E-04	***
Anionic BSA + Collagen Native BSA + Collagen	7.08078	4.40E-04	***
Native BSA + FN PLL + FN	-1.66067	0.55494	n.s
Native BSA + FN Native BSA + Collagen	-2.55911	0.19424	n.s
Native BSA + FN Anionic BSA + Collagen	-9.63989	3.12E-05	***
Cationic BSA + FN PLL + FN	12.44644	2.99E-06	***
Cationic BSA + FN Native BSA + Collagen	11.548	6.04E-06	***
Cationic BSA + FN Anionic BSA + Collagen	4.46722	0.01276	*
Cationic BSA + FN Native BSA + FN	14.10711	1.42E-06	***