

Supplementary Information for **Mechanism underlying Liquid-to-Solid Phase Transition in Fused in** **Sarcoma Liquid Droplets**

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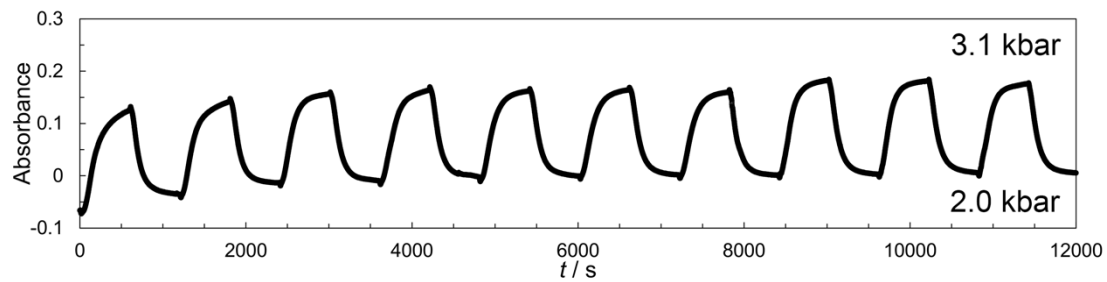


Figure S1. Changes in absorbance with 10 pressure-jump cycles between 2.0 kbar and 3.1 kbar at 9.3 °C. The time spent in 1-phase (2.0 kbar) or HP-LLPS (3.1 kbar) was approximately 600 s. This required ~5 s for pressurization up to 3.1 kbar but ~2 s for depressurization down to 2.0 kbar.

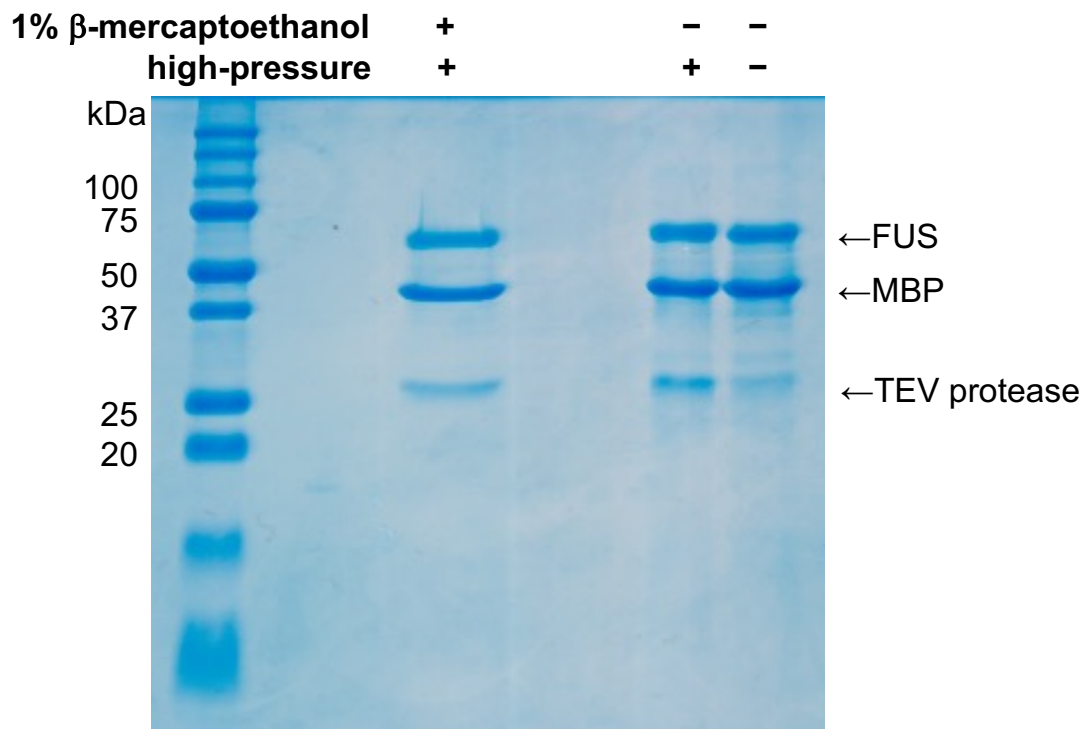


Figure S2. SDS-PAGE analysis of FUS before and after 3.1 kbar pressure treatment.

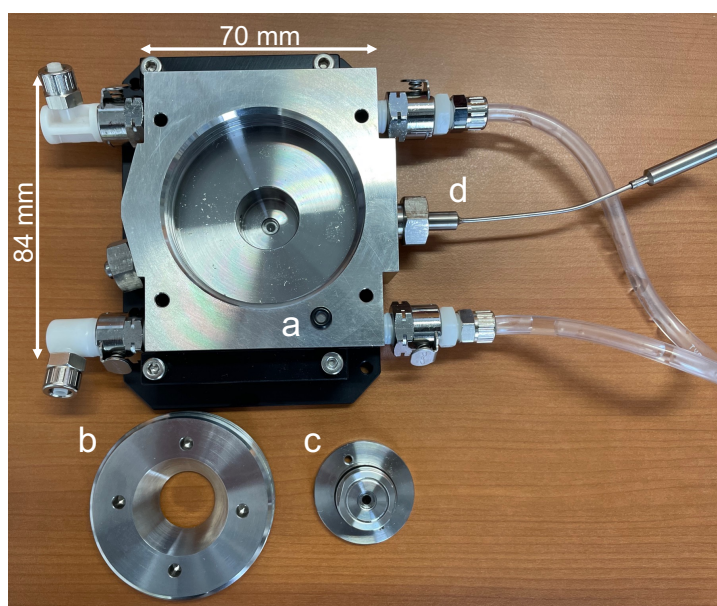


Figure S3. Pressure-resistant optical cell for microscopy (PMC-400; Syn-Corporation; a: i.d.=3 mm O-ring; b: grand nut; c: sapphire window unit; d: temperature detector).

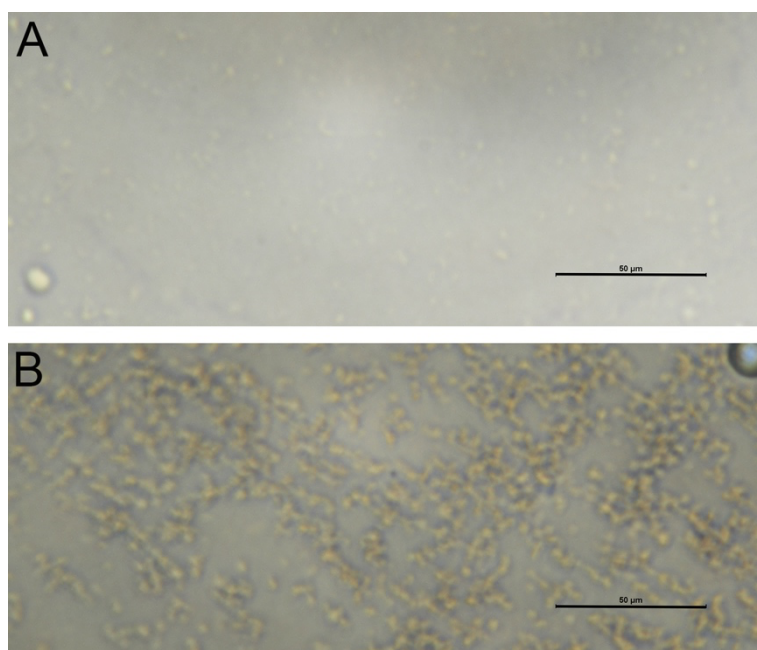


Figure S4. Reversibility and irreversibility of FUS-LLPS at 9.3 °C. (A) Microscopic image at 1.2 kbar after a 120 min incubation at 1 bar. (B) Microscopic image at 1 bar after a 120 min incubation at 3.1 bar. Black scale bar=50 μm .

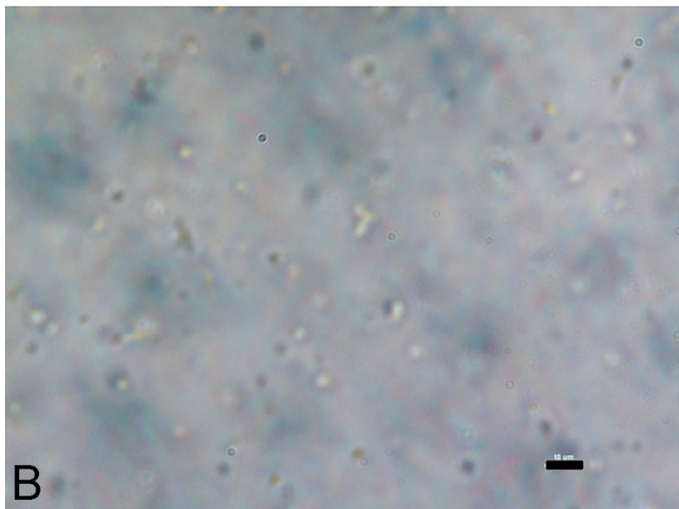
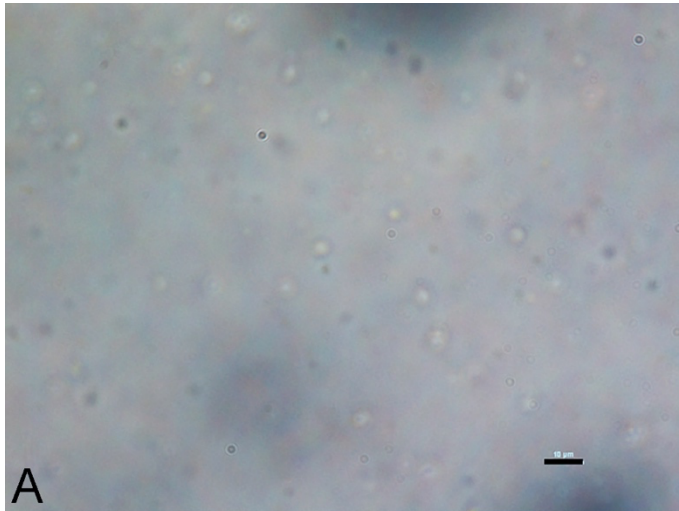


Figure S5. Microscopic images of FUS-LLPS. Images after 20 min (A) or 100 min (B) pressurization to 3.1 kbar at 9.3 °C, based on the middle layer of the sample chamber. Black scale bar=10 μm .

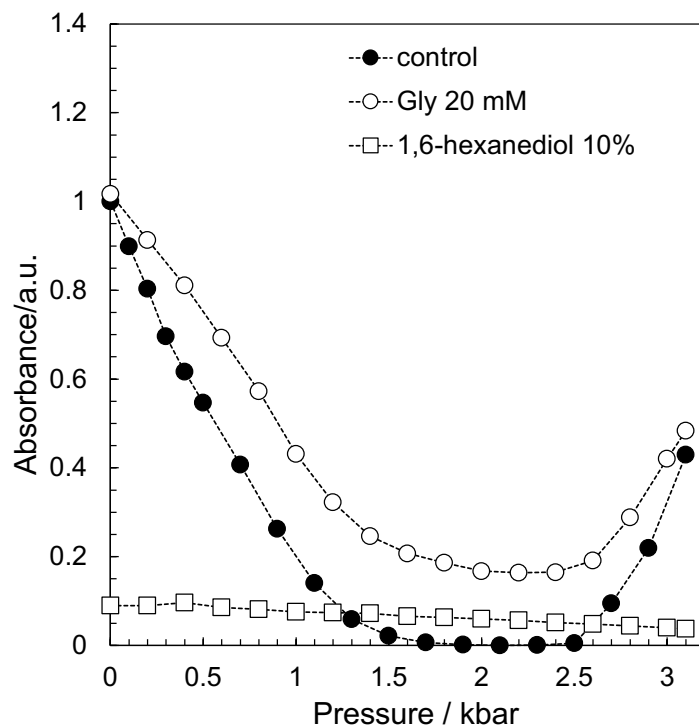


Figure S6. Effect of additives on LLPS formation with 5 μ M FUS. Pressure-dependent changes in the absorbance of FUS in 20 mM glycine and 10% 1,6-hexanediol at 9.3 $^{\circ}$ C. The absorbance was normalized to that without additives (i.e., control).

Table S1. Fluorescence recovery ratio after photobleaching of fused in sarcoma, FUS, less than 7 h after droplet formation.

Time/s	drop1	drop2	drop3	drop4	drop5	drop6	drop7
0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
1.638	0.994	0.999	0.997	0.999	0.996	0.971	1.001
3.292	0.994	0.991	0.998	1.002	0.981	1.003	1.001
5.086	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6.724	0.075	0.231	0.162	0.278	0.123	0.174	0.216
8.378	0.132	0.372	0.259	0.466	0.186	0.293	0.368
10.016	0.185	0.469	0.342	0.609	0.221	0.380	0.465
11.654	0.232	0.538	0.399	0.740	0.236	0.456	0.540
13.323	0.275	0.583	0.449	0.782	0.260	0.500	0.604
14.961	0.323	0.617	0.496	0.863	0.282	0.541	0.641
16.583	0.359	0.643	0.529	0.903	0.271	0.563	0.700
18.221	0.398	0.681	0.557	0.900	0.291	0.601	0.729
19.891	0.440	0.696	0.580	0.923	0.307	0.632	0.792
21.529	0.480	0.722	0.603	0.943	0.332	0.608	0.852
23.151	0.523	0.727	0.633	0.971	0.318	0.626	0.887
24.820	0.550	0.741	0.666	0.943	0.321	0.659	0.915
26.458	0.578	0.759	0.688	0.953	0.333	0.666	0.924
28.096	0.600	0.746	0.696	0.972	0.346	0.672	0.935
29.765	0.640	0.761	0.697	0.947	0.414	0.697	0.932
31.419	0.663	0.777	0.718	0.950	0.455	0.705	0.922
33.057	0.673	0.779	0.739	0.951	0.409	0.715	0.922
34.695	0.700	0.777	0.763	0.967	0.429	0.725	0.908
36.349	0.723	0.793	0.772	0.984	0.489	0.749	0.900
37.987	0.739	0.788	0.796	0.985	0.523	0.737	0.823
39.625	0.762	0.786	0.797	0.991	0.531	0.736	0.801
41.278	0.782	0.785	0.811	0.995	0.548	0.735	0.808
42.932	0.807	0.798	0.830	1.005	0.621	0.753	0.835
44.570	0.822	0.784	0.840	0.997	0.649	0.758	0.871
46.208	0.842	0.782	0.835	1.000	0.647	0.738	0.895
47.862	0.851	0.780	0.847	0.989	0.639	0.719	0.899
49.500	0.854	0.788	0.843	0.993	0.655	0.716	0.893
51.138	0.869	0.785	0.854	1.005	0.686	0.727	0.901
52.791	0.877	0.791	0.857	1.009	0.680	0.735	0.912
54.445	0.892	0.784	0.858	1.005	0.682	0.720	0.900
56.083	0.896	0.789	0.870	1.007	0.686	0.712	0.900
57.721	0.902	0.799	0.879	1.002	0.693	0.701	0.893
59.374	0.918	0.797	0.880	1.000	0.706	0.699	0.875
61.012	0.913	0.779	0.898	1.006	0.704	0.676	0.877
62.650	0.918	0.781	0.892	1.010	0.719	0.657	0.892
64.304	0.925	0.776	0.903	1.011	0.721	0.636	0.889

Table S2. Fluorescence recovery ratio after photobleaching of fused in sarcoma, FUS, more than 36 h after droplet formation.

Time/s	drop1	drop2	drop3	drop4	drop5	drop6	drop7	drop8	drop9
0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
1.638	0.998	1.025	0.996	1.000	0.974	1.000	1.004	0.993	1.034
3.276	1.020	1.007	1.000	1.000	0.970	1.000	0.998	0.981	1.046
5.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6.662	0.039	0.107	0.104	0.023	0.014	-0.035	0.013	0.027	0.009
8.300	0.065	0.094	0.129	0.028	0.010	-0.015	0.019	0.072	-0.026
9.984	0.108	0.102	0.126	0.032	-0.006	-0.014	0.011	0.087	-0.079
11.622	0.100	0.122	0.150	0.024	-0.011	-0.024	0.014	0.065	-0.050
13.260	0.118	0.159	0.161	0.019	-0.007	-0.027	0.002	0.076	-0.079
14.914	0.127	0.175	0.181	0.015	-0.014	-0.017	0.012	0.053	-0.058
16.568	0.120	0.180	0.194	0.021	-0.001	-0.015	0.001	0.044	-0.107
18.206	0.132	0.206	0.194	0.018	-0.013	0.008	0.010	0.056	-0.172
19.844	0.160	0.242	0.197	0.022	-0.006	-0.017	0.012	0.081	-0.188
21.466	0.157	0.247	0.167	0.016	-0.012	-0.020	-0.002	0.036	-0.228
23.104	0.148	0.206	0.224	0.026	-0.013	-0.034	-0.011	0.028	-0.219
24.742	0.187	0.229	0.212	0.017	-0.016	-0.053	-0.020	0.071	-0.233
26.489	0.181	0.206	0.210	0.027	-0.014	-0.035	-0.012	0.040	-0.262
28.221	0.186	0.203	0.207	0.030	0.006	-0.018	-0.002	0.063	-0.283
29.859	0.170	0.229	0.217	0.031	-0.010	0.001	-0.035	0.046	-0.316
31.513	0.167	0.271	0.210	0.033	-0.004	0.003	-0.014	0.049	-0.298
33.151	0.163	0.262	0.218	0.028	0.005	0.014	0.007	0.058	-0.259
34.789	0.218	0.288	0.227	0.025	0.025	0.024	-0.008	0.044	-0.224
36.442	0.220	0.296	0.247	0.027	0.034	0.016	-0.010	0.052	-0.198
38.096	0.212	0.255	0.208	0.019	0.030	0.007	-0.016	0.063	-0.212
39.734	0.202	0.266	0.227	0.012	0.013	-0.039	-0.018	0.057	-0.203
41.403	0.228	0.243	0.209	0.010	0.000	0.013	-0.021	0.060	-0.235
43.025	0.246	0.271	0.221	0.011	0.000	-0.006	-0.027	0.063	-0.248
44.663	0.237	0.273	0.216	0.007	-0.005	-0.008	-0.024	0.035	-0.295
46.301	0.255	0.279	0.198	0.009	-0.006	0.027	-0.034	0.059	-0.312
47.986	0.253	0.271	0.182	0.006	0.000	-0.011	-0.033	0.044	-0.299
49.624	0.281	0.270	0.201	0.008	-0.001	-0.001	-0.049	0.047	-0.313
51.262	0.243	0.293	0.207	0.012	-0.011	-0.024	-0.036	0.029	-0.263
52.916	0.247	0.295	0.197	0.004	-0.015	-0.031	-0.038	0.022	-0.209
54.570	0.241	0.318	0.200	0.003	-0.022	0.013	-0.025	0.047	-0.091
56.208	0.223	0.317	0.199	0.003	-0.025	0.032	-0.048	0.020	-0.106
57.830	0.215	0.310	0.183	0.000	-0.025	-0.019	-0.035	0.029	-0.121
59.499	0.252	0.365	0.186	-0.006	-0.018	-0.050	-0.035	0.035	-0.162
61.137	0.317	0.341	0.190	-0.006	-0.015	-0.037	-0.035	0.012	-0.171
62.775	0.312	0.350	0.182	-0.013	-0.014	0.012	-0.036	0.022	-0.228
64.413	0.330	0.351	0.196	-0.012	-0.015	-0.002	-0.048	0.020	-0.269

Table S3. Fluorescence recovery ratio after photobleaching of fused in sarcoma, FUS, for pressure-treated droplets (<7 h).

Time/s	drop1	drop2	drop3	drop4	drop5	drop6	drop7	drop8	drop9	drop10	drop11
0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
1.654	0.946	0.953	0.992	1.032	0.970	0.995	1.057	0.998	0.930	0.977	1.059
3.292	0.992	0.945	0.989	1.050	0.949	0.999	1.543	0.987	0.967	0.950	1.052
5.195	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6.817	0.050	0.067	0.082	0.067	0.092	0.130	0.206	0.065	0.271	0.383	0.267
8.502	0.056	0.116	0.139	0.121	0.155	0.184	0.354	0.137	0.358	0.574	0.303
10.156	0.060	0.146	0.183	0.127	0.185	0.257	0.331	0.197	0.383	0.596	0.399
11.794	0.091	0.174	0.207	0.161	0.215	0.262	0.217	0.265	0.352	0.673	0.410
13.432	0.110	0.182	0.250	0.236	0.230	0.266	0.172	0.282	0.322	0.677	0.338
15.085	0.131	0.204	0.290	0.274	0.250	0.284	0.455	0.312	0.344	0.697	0.434
16.723	0.166	0.210	0.317	0.289	0.274	0.293	0.194	0.335	0.387	0.729	0.458
18.361	0.181	0.233	0.355	0.273	0.275	0.275	0.020	0.370	0.354	0.714	0.552
20.015	0.213	0.234	0.375	0.231	0.279	0.256	0.146	0.343	0.364	0.719	0.655
21.653	0.217	0.253	0.400	0.217	0.294	0.220	0.095	0.385	0.349	0.741	0.763
23.291	0.218	0.261	0.418	0.222	0.301	0.186	0.013	0.412	0.378	0.770	0.702
24.929	0.231	0.260	0.419	0.201	0.298	0.176	0.152	0.418	0.468	0.715	0.647
26.551	0.239	0.270	0.438	0.127	0.311	0.135	-0.014	0.408	0.505	0.719	0.679
28.189	0.241	0.282	0.470	0.138	0.316	0.171	0.222	0.381	0.483	0.728	0.615
29.827	0.246	0.286	0.494	0.121	0.317	0.196	0.299	0.394	0.384	0.754	0.590
31.512	0.268	0.285	0.497	0.134	0.279	0.189	0.266	0.439	0.284	0.756	0.537
33.166	0.275	0.294	0.505	0.145	0.261	0.173	0.120	0.366	0.246	0.731	0.549
34.804	0.276	0.295	0.538	0.113	0.275	0.169	0.395	0.401	0.270	0.706	0.613
36.442	0.271	0.292	0.517	0.148	0.288	0.138	0.258	0.427	0.307	0.731	0.716
38.064	0.291	0.297	0.511	0.176	0.295	0.131	0.457	0.423	0.291	0.724	0.767
39.702	0.309	0.309	0.526	0.177	0.309	0.123	0.418	0.401	0.289	0.723	0.749
41.340	0.330	0.307	0.546	0.171	0.320	0.048	0.337	0.419	0.299	0.751	0.861
42.994	0.341	0.307	0.573	0.197	0.294	0.051	0.502	0.407	0.311	0.740	0.878
44.647	0.339	0.313	0.563	0.264	0.265	0.154	0.291	0.410	0.279	0.774	0.835
46.286	0.350	0.311	0.536	0.232	0.273	0.318	0.554	0.411	0.221	0.771	0.894
47.924	0.372	0.320	0.495	0.240	0.283	0.389	0.235	0.404	0.362	0.791	0.896
49.577	0.381	0.312	0.463	0.224	0.292	0.469	0.216	0.388	0.532	0.739	0.811
51.293	0.358	0.306	0.437	0.198	0.335	0.519	0.280	0.417	0.551	0.722	0.877
52.978	0.388	0.316	0.445	0.187	0.336	0.535	0.269	0.438	0.447	0.714	0.818
54.632	0.372	0.306	0.437	0.208	0.311	0.532	0.202	0.434	0.524	0.754	0.820
56.270	0.423	0.308	0.425	0.219	0.312	0.559	0.064	0.425	0.423	0.754	0.856
57.908	0.421	0.291	0.430	0.209	0.305	0.530	0.024	0.466	0.403	0.744	0.872
59.561	0.444	0.289	0.423	0.179	0.281	0.553	-0.004	0.450	0.401	0.781	0.819
61.199	0.443	0.301	0.407	0.174	0.294	0.551	-0.021	0.467	0.343	0.783	0.847
62.837	0.440	0.316	0.404	0.143	0.309	0.538	-0.004	0.486	0.370	0.749	0.796
64.491	0.455	0.327	0.430	0.140	0.298	0.528	-0.084	0.463	0.416	0.790	0.827
										(LP-LLPS)	(LP-LLPS)

Table S4. Fluorescence recovery ratio after photobleaching of fused in sarcoma, FUS, for droplets incubated in buffer containing 20 mM arginine for more than 36 h.

Time/s	drop1	drop2	drop3	drop4
0.000	1.000	1.000	1.000	1.000
1.653	0.977	1.000	1.007	0.902
3.291	1.025	1.000	1.020	0.934
5.054	0.000	0.000	0.000	0.000
6.708	0.199	0.576	0.113	0.209
8.346	0.291	0.752	0.201	0.316
9.984	0.398	0.800	0.339	0.362
11.637	0.507	0.939	0.419	0.438
13.291	0.509	0.892	0.450	0.345
14.929	0.541	0.939	0.517	0.463
16.567	0.580	0.965	0.569	0.482
18.221	0.614	0.976	0.666	0.509
19.859	0.708	0.926	0.699	0.481
21.497	0.709	0.954	0.736	0.414
23.150	0.709	0.967	0.826	0.473
24.788	0.687	0.994	0.872	0.500
26.426	0.718	0.992	0.898	0.468
28.064	0.763	0.993	0.920	0.471
29.702	0.817	0.959	0.941	0.499
31.340	0.853	0.938	0.990	0.603
32.978	0.843	0.900	1.006	0.480
34.647	0.900	0.943	0.995	0.526
36.301	0.881	0.935	1.003	0.552
37.939	0.930	0.941	1.034	0.530
39.577	0.930	0.878	0.998	0.482
41.231	0.898	0.890	1.026	0.503
42.869	0.911	0.871	1.037	0.474
44.507	0.904	0.845	1.029	0.491
46.160	0.940	0.787	1.042	0.503
47.798	0.939	0.792	1.028	0.531
49.436	0.979	0.826	1.046	0.536
51.074	1.005	0.802	1.023	0.579
52.744	1.025	0.857	1.008	0.577
54.382	1.008	0.891	1.011	0.569
56.004	1.018	0.899	1.012	0.519
57.658	1.033	0.823	0.986	0.570
59.311	1.033	0.818	0.985	0.647
60.949	1.014	0.843	0.959	0.666
62.587	1.025	0.836	0.968	0.740
64.241	1.058	0.792	0.953	0.725