

Insights into the Molecular Association of Aqueous Deep Eutectic Solvents using Cell Permeability – Supplementary Information

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Two Parameter Formalism

The two parameter formalism is described in the literature¹⁻³ and is based on two primary equations, one for water flux (1):

Equation 1:

$$\frac{dV_w}{dt} = -L_p A R T (M^e - M^i)$$

and the other for solute flux (2):

Equation 2:

$$\frac{dN_s}{dt} = P_s A (M_s^e - M_s^i)$$

Where L_p is the water permeability (hydraulic conductivity), A is the cell area, R is the universal gas constant, T is absolute temperature, P_s is the solute permeability, subscript w refers to water, subscript s refers to permeating solute, and M^i and M^e are respectively the intracellular and extracellular osmolality.

Equation 3:

$$V_c = V_w + V_s + V_b$$

25 Where V_c is the total cell volume, V_w is the partial molar volume of water, V_s is the solute volume
26 determined by Equation 4 (below), and V_b is the osmotically inactive volume (including cell
27 solids). The value of V_b was determined by the Boyle van't Hoff relationship⁴ (below).

28 Equation 4:

$$29 \quad V_s = N_s \bar{V}_s$$

30 Where N_s is the osmoles of solute inside the cell and \bar{V}_s is the partial molar fraction.

31 The validity of this method and of the fixed parameters was confirmed by using DMSO as a
32 control. As shown in Figure S1 and S2, a good model fit was achieved not only for all of the PBS
33 data, but also for 10 wt% DMSO which had a $P_s = 1.9 \mu\text{m}/\text{min}$ with THP-1 and $7.0 \mu\text{m}/\text{min}$ for
34 HaCat. These values are well within the expected range based on studies of similar cell types in
35 the literature.⁵⁻⁹

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37 **Boyle van't Hoff Relationship**

38 The osmotically inactive volume (V_b) can be determined using the Boyle van't Hoff Relationship⁴:

39 Equation 5:

$$40 \quad \frac{V}{V_o} = \frac{V_w^o \pi_o}{V_o \pi} + \frac{V_b}{V_o} = (1 - b) \frac{\pi_o}{\pi} + b$$

41 where V is the cell volume, V_o is the total cell isotonic volume, V_w^o is the isotonic volume of water
42 in the cell, and π and π_o are the osmolality and isotonic osmolality respectively. Shrink-swell
43 experiments were performed on THP-1 and HaCat cells using various concentrations of
44 phosphate buffered saline (a non-penetrating solute), the results of which are shown in Figures
45 S1 and S2. The final/minimum volume for each solute concentration was determined and plotted
46 against the normalised inverse osmolality (Figure S3). The intercept of the trendline for this data
47 is equal to the normalised osmotically inactive volume, i.e. b and was determined to be
48 0.279 ± 0.008 for THP-1 and 0.4023 ± 0.101 for HaCat.

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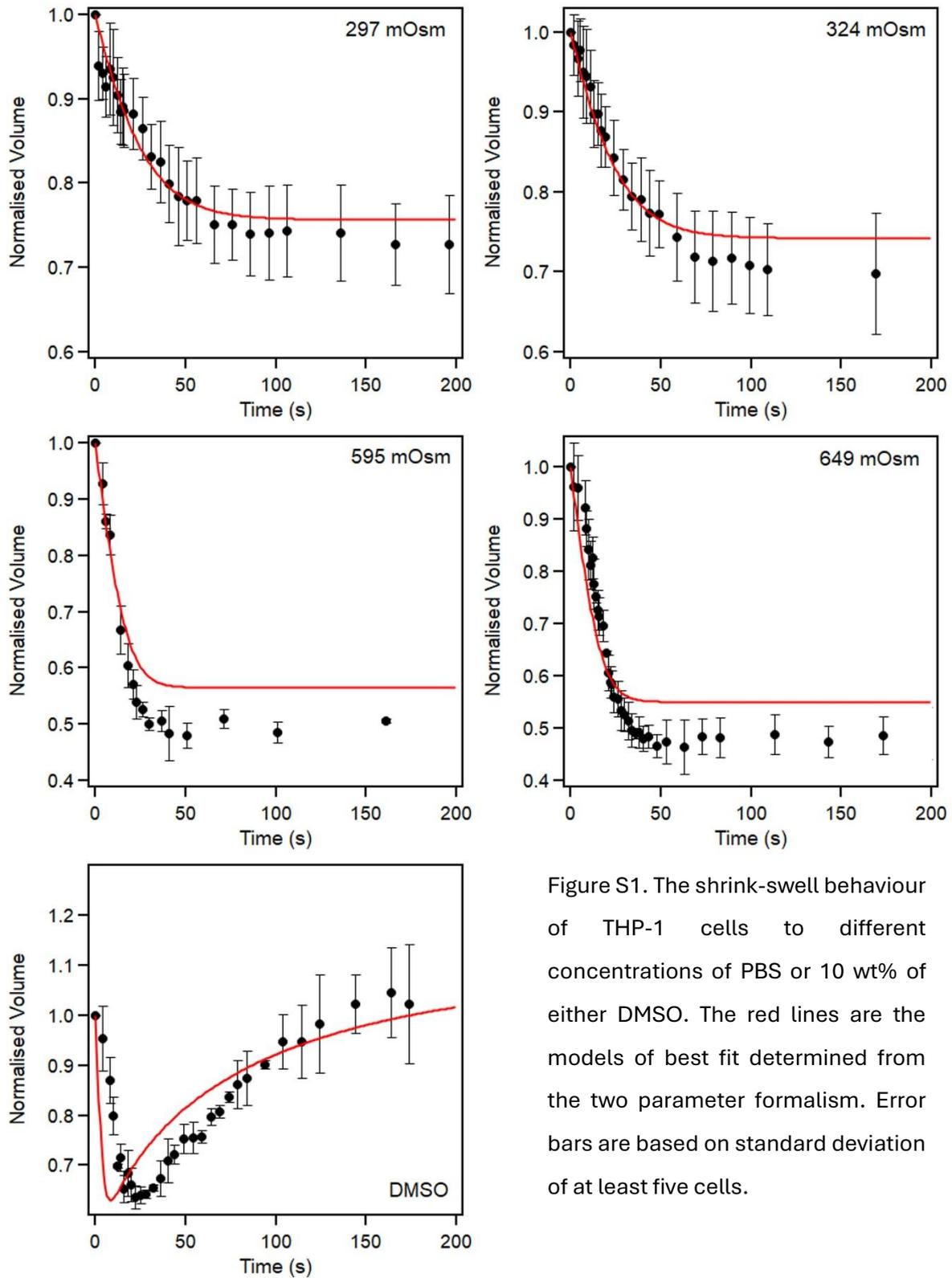


Figure S1. The shrink-swell behaviour of THP-1 cells to different concentrations of PBS or 10 wt% of either DMSO. The red lines are the models of best fit determined from the two parameter formalism. Error bars are based on standard deviation of at least five cells.

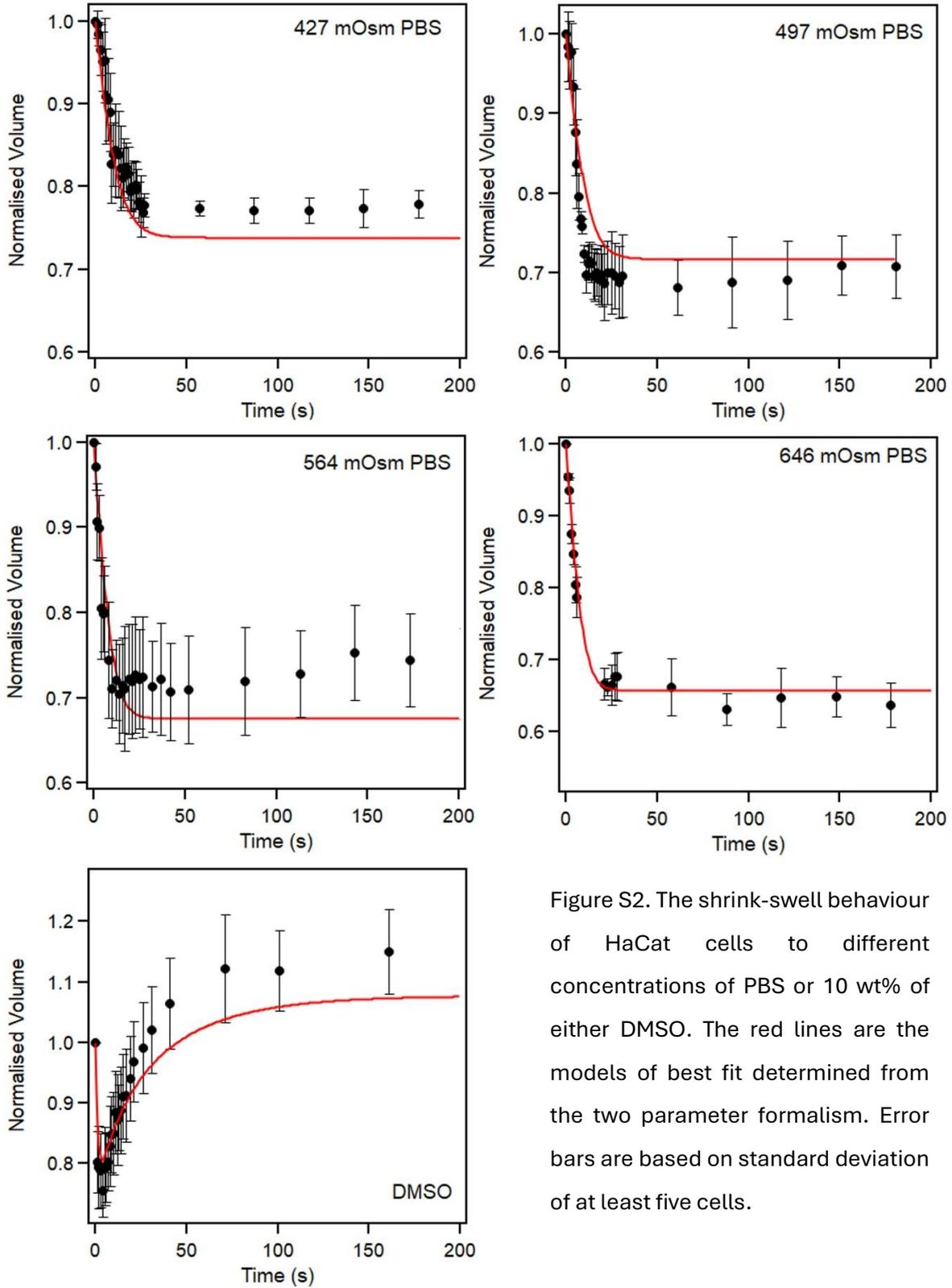
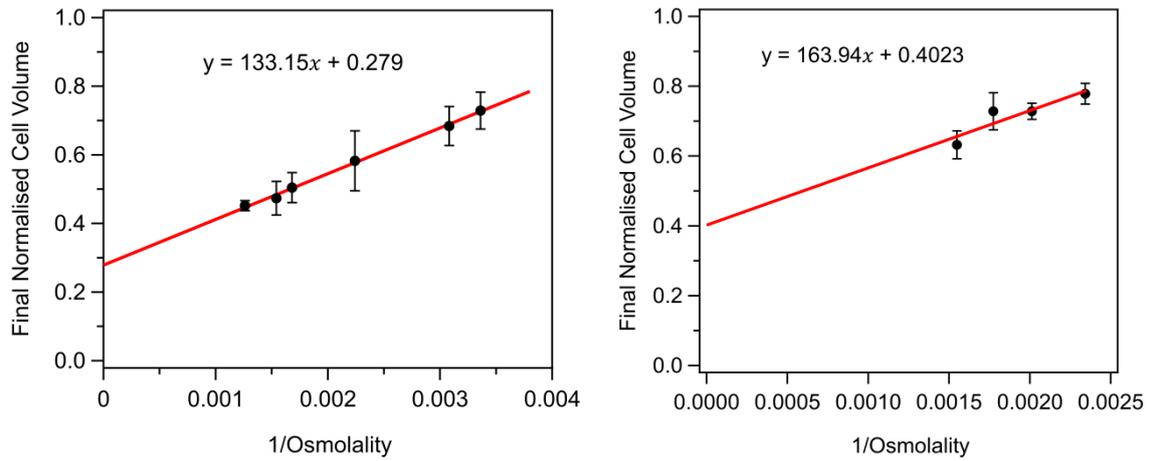


Figure S2. The shrink-swell behaviour of HaCat cells to different concentrations of PBS or 10 wt% of either DMSO. The red lines are the models of best fit determined from the two parameter formalism. Error bars are based on standard deviation of at least five cells.

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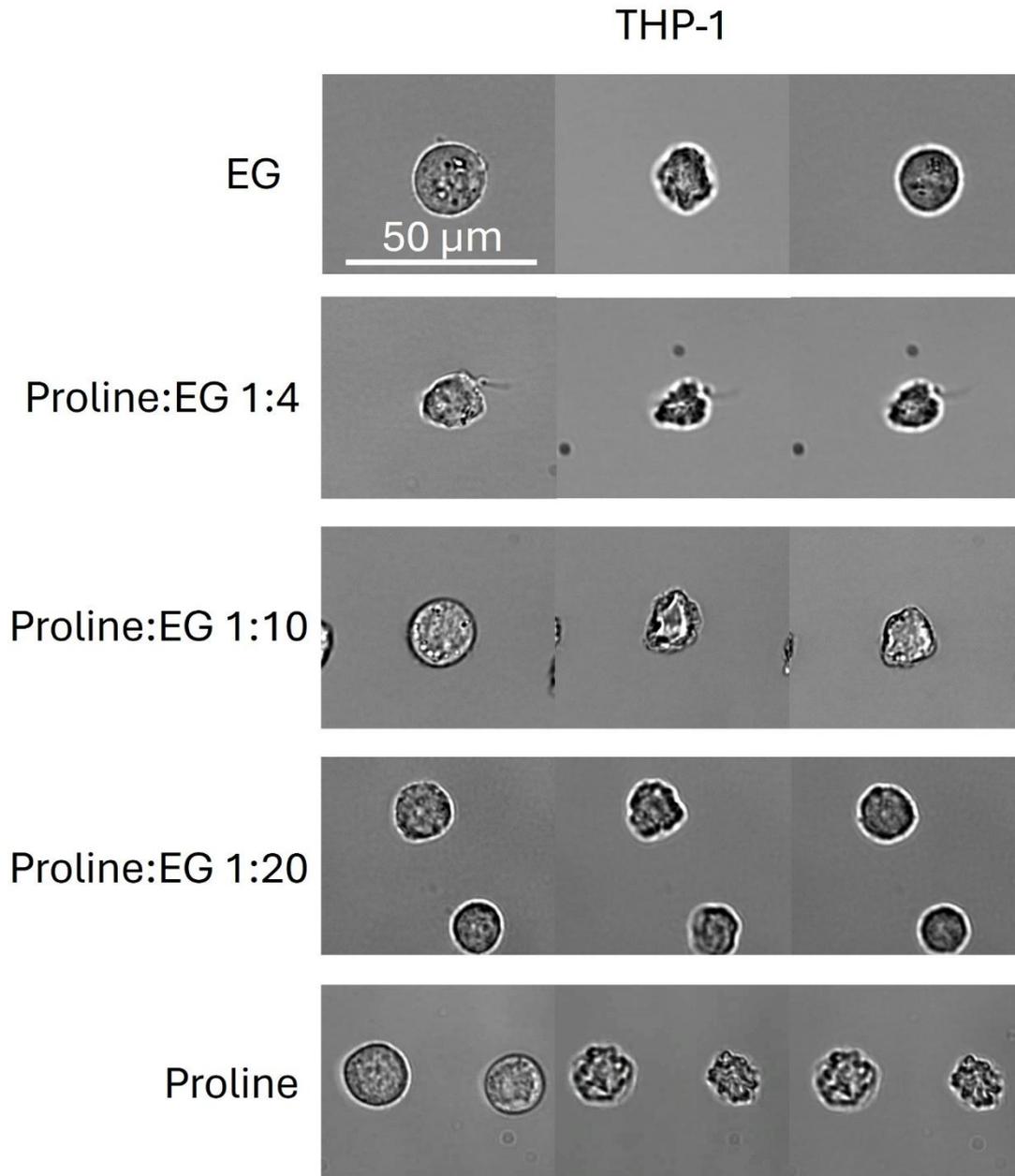
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57 Figure S3. The final normalised cell volume of THP-1 (left) or HaCat (right) cells exposed to
 58 different concentrations of phosphate buffered saline. The intercept point (0.279 ± 0.008 for THP-
 59 1 and 0.4023 ± 0.101 for HaCat) is equal to the normalised osmotically inactive volume (b),

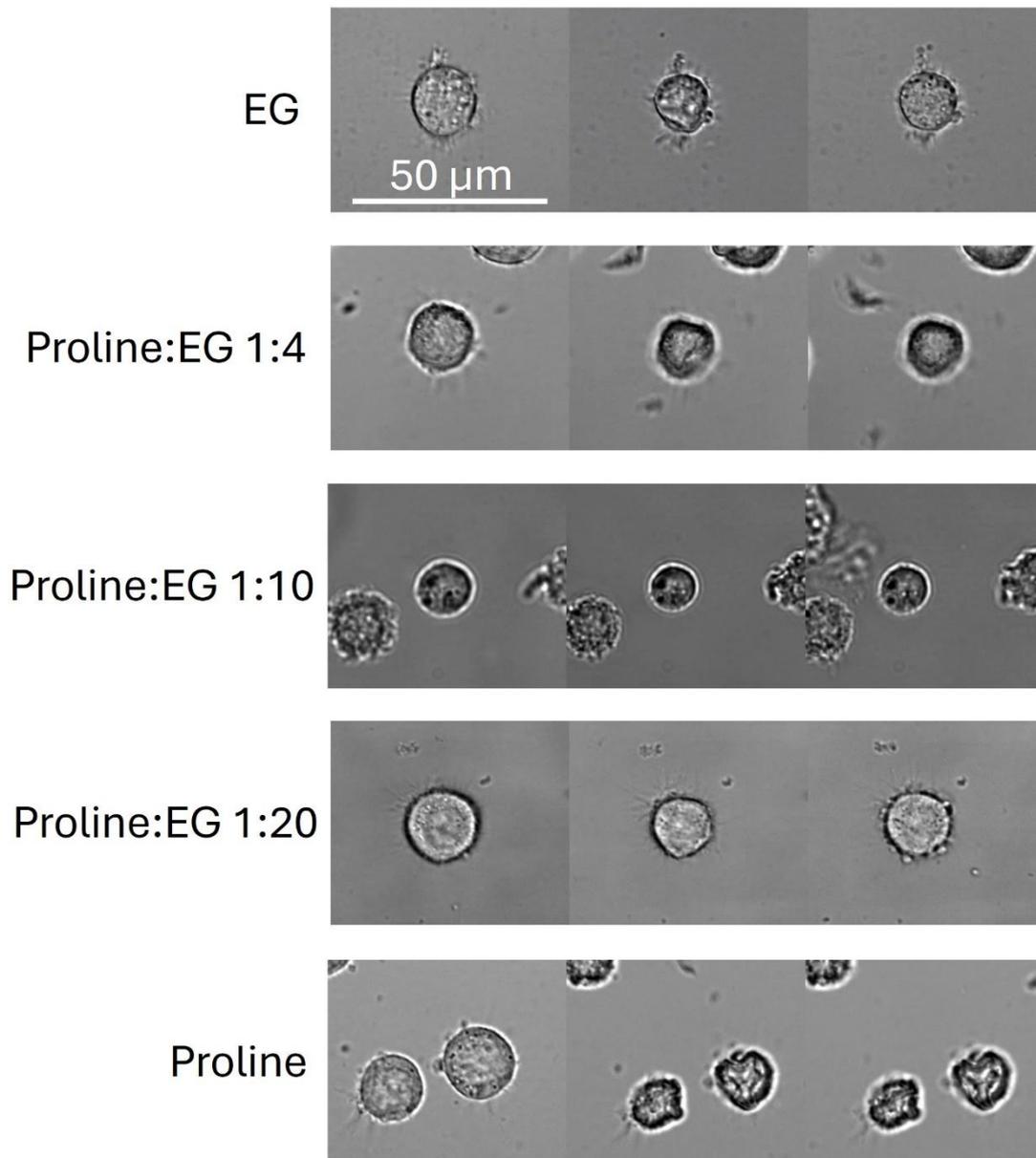
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62 Figure S4. Micrographs from shrink-swell experiments showing THP-1 cells before
 63 addition of the 'treatment' (list to the left of each row), immediately after (middle), and
 64 after thirty seconds (right).

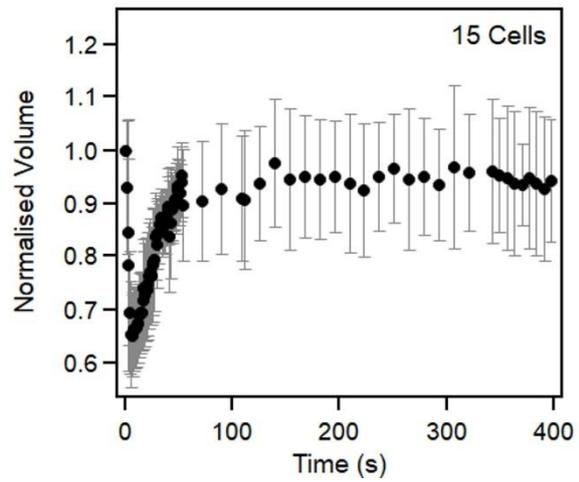
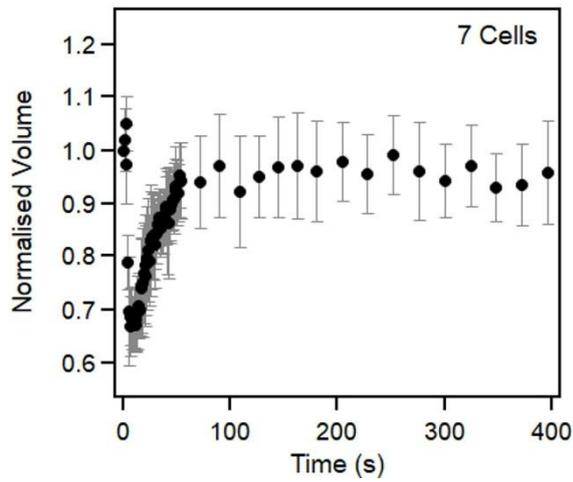
HaCat



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66 Figure S5. Micrographs from shrink-swell experiments showing THP-1 cells before addition of
67 the 'treatment' (list to the left of each row), immediately after (middle), and after thirty
68 seconds (right).

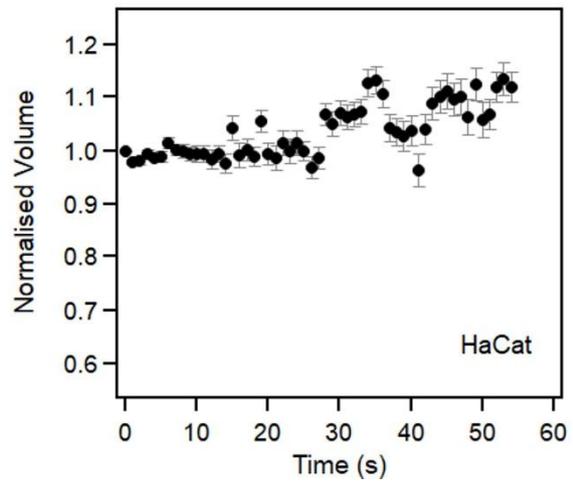
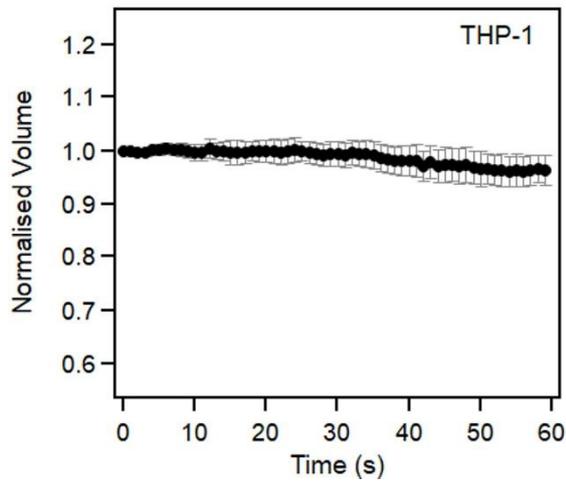
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71 Figure S6. Shrink-swell behaviour of HaCat cells exposed to 10 wt% of Proline:EG (1:20
 72 ratio). The graphs show the average and standard deviation of 7 cells (left) or 15 cells
 73 (right).

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76 Figure S7. Changes to normalised volume of THP-1 and HaCat cells exposed to just cell
 77 media over 1 minute.

78 Table S1. Raw shrink-swell data for THP-1 cells

EG_Time	EG_Volume	EG_Error	Proline_Time	Proline_Volume	Proline_Error	1to4_Time	1to4_Volume	1to4_Error	1to10_Time	1to10_Volume	1to10_Error	1to20_Time	1to20_Volume	1to20_Error
0	1.00	0.00	0	1.00	0.00	0	1.00	0.00	0	1.00	0.00	0	1.00	0.00
1	1.18	0.25	1	1.00	0.01	3	0.84	0.09	1	0.86	0.04	1	0.75	0.04
2	0.84	0.16	2	1.07	0.21	4	0.77	0.07	2	0.81	0.05	2	0.73	0.05
3	1.08	0.35	3	0.69	0.31	5	0.73	0.08	3	0.76	0.05	3	0.69	0.06
4	0.73	0.06	4	0.57	0.20	6	0.75	0.07	4	0.75	0.07	4	0.67	0.06
5	0.70	0.05	5	0.57	0.11	7	0.75	0.08	5	0.75	0.07	5	0.67	0.06
6	0.69	0.11	6	0.57	0.14	8	0.75	0.08	6	0.76	0.06	6	0.68	0.05
7	0.67	0.18	7	0.57	0.14	9	0.76	0.07	7	0.78	0.08	7	0.68	0.05
8	0.69	0.13	8	0.54	0.12	10	0.75	0.08	8	0.78	0.06	8	0.68	0.05
9	0.77	0.07	9	0.55	0.13	11	0.75	0.08	9	0.78	0.08	9	0.69	0.05
10	0.77	0.08	10	0.55	0.13	12	0.74	0.09	10	0.78	0.08	10	0.70	0.04
11	0.79	0.11	11	0.54	0.12	13	0.74	0.09	11	0.79	0.08	11	0.71	0.04
12	0.76	0.08	12	0.53	0.11	14	0.76	0.08	12	0.79	0.07	12	0.70	0.04
13	0.76	0.07	13	0.52	0.10	15	0.74	0.08	13	0.77	0.08	13	0.71	0.04
14	0.82	0.06	14	0.54	0.12	16	0.73	0.09	14	0.78	0.08	14	0.72	0.04
15	0.76	0.11	15	0.54	0.13	17	0.75	0.09	15	0.78	0.08	15	0.71	0.04
16	0.75	0.10	16	0.52	0.11	18	0.75	0.09	16	0.77	0.09	16	0.72	0.04
17	0.78	0.07	17	0.53	0.10	19	0.76	0.08	17	0.77	0.10	17	0.72	0.03
18	0.79	0.10	18	0.51	0.09	20	0.77	0.08	18	0.76	0.10	18	0.72	0.03
19	0.83	0.07	19	0.50	0.07	21	0.76	0.08	19	0.76	0.10	19	0.72	0.03
20	0.84	0.09	20	0.53	0.10	22	0.76	0.08	20	0.77	0.10	20	0.73	0.04
21	0.82	0.07	21	0.52	0.09	23	0.77	0.08	21	0.76	0.10	21	0.73	0.04
22	0.81	0.08	22	0.52	0.09	24	0.77	0.08	22	0.76	0.10	22	0.73	0.03
23	0.81	0.06	23	0.54	0.10	25	0.77	0.08	23	0.75	0.12	23	0.73	0.04
24	0.81	0.15	24	0.53	0.10	26	0.77	0.07	24	0.76	0.11	24	0.74	0.03
25	0.86	0.06	25	0.55	0.11	27	0.77	0.07	25	0.76	0.11	25	0.70	0.10
26	0.85	0.06	26	0.55	0.11	28	0.77	0.08	26	0.75	0.10	26	0.74	0.03
27	0.86	0.06	27	0.53	0.09	29	0.77	0.07	27	0.76	0.10	27	0.74	0.03
28	0.90	0.08	28	0.54	0.10	30	0.76	0.08	28	0.76	0.09	28	0.70	0.10
29	0.84	0.09	29	0.54	0.10	31	0.79	0.07	29	0.75	0.10	29	0.70	0.10
30	0.84	0.08	30	0.53	0.09	32	0.78	0.06	30	0.75	0.08	30	0.69	0.11
31	0.86	0.08	31	0.54	0.09	33	0.79	0.07	31	0.74	0.07	31	0.70	0.10
32	0.88	0.09	32	0.54	0.11	34	0.80	0.07	32	0.75	0.07	32	0.74	0.04
33	0.86	0.09	33	0.54	0.09	35	0.80	0.08	33	0.75	0.06	33	0.74	0.04
34	0.82	0.16	34	0.54	0.10	36	0.78	0.06	34	0.76	0.08	34	0.70	0.10
35	0.92	0.08	35	0.54	0.09	37	0.79	0.08	35	0.74	0.06	35	0.73	0.03
36	0.92	0.08	36	0.55	0.10	38	0.78	0.07	36	0.73	0.06	36	0.74	0.04
37	0.93	0.08	37	0.54	0.08	39	0.78	0.07	37	0.74	0.06	37	0.73	0.04
38	0.94	0.08	38	0.55	0.09	40	0.76	0.08	38	0.74	0.06	38	0.74	0.03
39	0.95	0.08	39	0.52	0.11	41	0.76	0.06	39	0.74	0.06	39	0.73	0.03
40	0.94	0.08	40	0.56	0.11	42	0.76	0.06	40	0.75	0.06	40	0.74	0.03
41	0.92	0.10	41	0.54	0.09	43	0.77	0.06	41	0.75	0.05	41	0.71	0.06

42	0.92	0.09	42	0.54	0.10	44	0.74	0.08	42	0.76	0.06	42	0.74	0.02
43	0.94	0.07	43	0.55	0.10	45	0.75	0.07	43	0.76	0.07	43	0.71	0.07
44	0.94	0.08	44	0.56	0.11	46	0.75	0.06	44	0.75	0.06	44	0.71	0.09
45	0.92	0.08	45	0.56	0.11	47	0.75	0.07	45	0.75	0.06	45	0.72	0.06
46	0.88	0.18	46	0.55	0.10	48	0.75	0.06	46	0.75	0.06	46	0.72	0.05
47	0.93	0.08	47	0.54	0.09	49	0.75	0.06	47	0.75	0.06	47	0.73	0.06
48	0.95	0.08	48	0.55	0.10	50	0.74	0.06	48	0.76	0.06	48	0.71	0.11
49	0.96	0.08	49	0.55	0.10	51	0.73	0.07	49	0.76	0.06	49	0.71	0.09
50	0.96	0.08	50	0.56	0.10	52	0.73	0.09	50	0.75	0.06	50	0.73	0.07
51	0.96	0.08	51	0.56	0.10	53	0.74	0.06	51	0.76	0.07	51	0.73	0.07
52	0.95	0.07	52	0.56	0.10	54	0.72	0.07	52	0.77	0.06	52	0.75	0.05
53	0.97	0.07	53	0.55	0.09	55	0.75	0.06	53	0.78	0.07	53	0.75	0.01
54	0.97	0.07	54	0.55	0.08	56	0.74	0.09	54	0.77	0.07	54	0.75	0.07
55	0.97	0.07	55	0.53	0.09	57	0.74	0.10	55	0.78	0.07	55	0.75	0.02
56	0.96	0.10	56	0.56	0.11	58	0.73	0.08	56	0.78	0.09	56	0.78	0.03
57	0.97	0.07	57	0.55	0.10	59	0.74	0.08	57	0.78	0.07	57	0.73	0.08
58	0.99	0.06	58	0.56	0.11	60	0.75	0.08	58	0.79	0.09	58	0.77	0.03
59	0.99	0.06	59	0.55	0.10	61	0.74	0.09	59	0.78	0.09	59	0.71	0.12
60	0.98	0.06	60	0.56	0.11	62	0.73	0.07	60	0.79	0.09	60	0.74	0.05
61	0.97	0.08	61	0.57	0.11	63	0.73	0.05	61	0.77	0.12	61	0.74	0.10
62	0.99	0.06	62	0.56	0.11	64	0.75	0.09	62	0.80	0.09	62	0.72	0.11
63	1.01	0.04	63	0.56	0.11	65	0.72	0.08	63	0.81	0.10	63	0.77	0.12
64	1.01	0.05	64	0.56	0.10	75	0.71	0.04	64	0.81	0.10	64	0.79	0.03
74	1.06	0.05	65	0.56	0.10	85	0.69	0.06	65	0.79	0.10	65	0.75	0.10
84	1.07	0.09	66	0.56	0.09	95	0.70	0.08	75	0.84	0.12	66	0.77	0.10
94	1.08	0.11	67	0.55	0.09	105	0.68	0.09	85	0.83	0.13	67	0.77	0.10
104	1.12	0.12	68	0.55	0.09	115	0.71	0.06	95	0.87	0.13	68	0.83	0.04
114	1.10	0.09	69	0.56	0.09	125	0.71	0.06	105	0.88	0.15	74	0.83	0.04
124	1.10	0.16	70	0.56	0.10	141	0.71	0.06	115	0.86	0.15	80	0.84	0.03
134	1.15	0.07	71	0.55	0.09	157	0.69	0.06	125	0.91	0.13	86	0.87	0.04
144	1.15	0.08	72	0.56	0.10	173	0.67	0.13	135	0.91	0.09	92	0.89	0.04
154	1.19	0.10	73	0.55	0.10	189	0.68	0.10	145	0.94	0.13	98	0.89	0.04
184	1.20	0.05	74	0.55	0.09	205	0.70	0.07	155	0.91	0.11	104	0.91	0.04
214	1.20	0.11	75	0.57	0.11	235	0.69	0.08	165	0.89	0.09	110	0.91	0.04
244	1.20	0.07	76	0.57	0.12	265	0.69	0.07	175	0.90	0.11	116	0.87	0.07
274	1.20	0.08	77	0.54	0.10	295	0.67	0.08	185	0.92	0.12	122	0.87	0.07
304	1.20	0.12	78	0.54	0.09	325	0.70	0.07	205	0.93	0.11	128	0.90	0.03
334	1.19	0.10	79	0.56	0.10	355	0.72	0.07	235	0.89	0.09	134	0.90	0.03
364	1.14	0.06	80	0.56	0.11	385	0.71	0.07	265	0.91	0.07	140	0.89	0.04
394	1.16	0.05	81	0.56	0.11				295	0.92	0.06	146	0.90	0.02
			82	0.55	0.09				325	0.89	0.11	152	0.89	0.01
			83	0.56	0.09				355	0.91	0.10	158	0.89	0.02
			84	0.55	0.09				385	0.98	0.09	164	0.87	0.03
			85	0.56	0.10							170	0.88	0.02

			86	0.55	0.09							182	0.89	0.03
			87	0.56	0.10							194	0.88	0.04
			88	0.55	0.10							206	0.89	0.05
			89	0.56	0.11							218	0.90	0.04
			90	0.57	0.11							230	0.91	0.04
			91	0.55	0.09							242	0.89	0.02
			92	0.56	0.10							254	0.88	0.03
			93	0.56	0.10							266	0.88	0.03
			94	0.55	0.10							290	0.86	0.05
			95	0.56	0.11							326	0.93	0.08
			96	0.55	0.10							362	0.93	0.07
			97	0.55	0.09									
			98	0.55	0.09									
			99	0.55	0.10									
			105	0.57	0.12									
			111	0.56	0.13									
			117	0.56	0.10									
			123	0.57	0.11									
			129	0.57	0.11									
			135	0.56	0.11									
			141	0.56	0.12									
			147	0.56	0.10									
			153	0.57	0.11									
			159	0.57	0.12									
			171	0.57	0.11									
			183	0.55	0.10									
			195	0.57	0.11									
			207	0.55	0.10									
			219	0.57	0.12									
			231	0.57	0.12									
			243	0.57	0.11									
			255	0.57	0.12									
			267	0.56	0.11									
			279	0.56	0.10									
			291	0.55	0.10									
			303	0.56	0.11									
			315	0.57	0.11									
			327	0.56	0.11									
			336	0.57	0.12									
			337	0.72	0.11									
			338	0.64	0.30									
			339	0.57	0.26									
			340	0.59	0.11									
			341	0.58	0.10									

			342	0.54	0.08									
			343	0.56	0.10									
			344	0.57	0.10									

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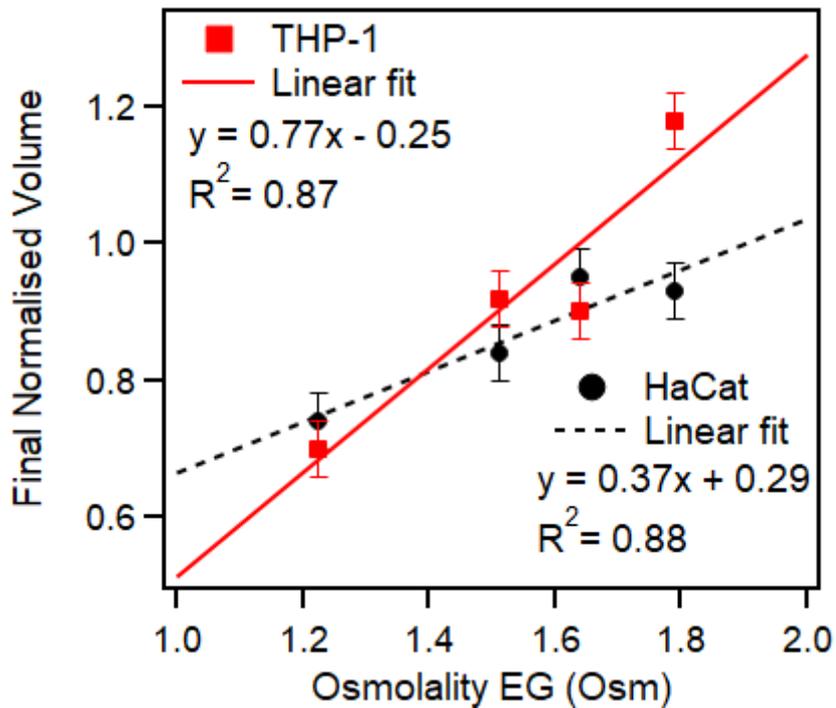
80 Table S2. Raw shrink-swell data for HaCat cells

EG_Time	EG_Volume	EG_Error	Proline_Time	Proline_Volume	Proline_Error	1to4_Time	1to4_Volume	1to4_Error	1to10_Time	1to10_Volume	1to10_Error	1to20_Time	1to20_Volume	1to20_Error
0	1.00	0.00	0	1.00	0.00	0	1.00	0.00	0	1.00	0.00	0	1.00	0.00
5	0.87	0.06	1	1.09	0.07	1	0.99	0.05	1	0.91	0.30	1	0.93	0.13
6	0.77	0.02	2	1.00	0.02	2	0.98	0.02	2	0.80	0.05	2	0.84	0.21
7	0.72	0.02	3	0.92	0.05	3	1.03	0.06	3	0.81	0.04	3	0.78	0.20
8	0.68	0.05	4	0.74	0.08	4	0.91	0.03	4	0.81	0.08	4	0.69	0.12
9	0.67	0.06	5	0.66	0.07	5	0.78	0.14	5	0.66	0.14	5	0.65	0.10
10	0.60	0.14	6	0.58	0.05	6	0.73	0.08	6	0.62	0.15	6	0.65	0.08
11	0.59	0.16	7	0.54	0.06	7	0.66	0.07	7	0.63	0.17	7	0.65	0.08
12	0.61	0.16	8	0.56	0.04	8	0.75	0.07	8	0.65	0.16	8	0.66	0.08
13	0.62	0.13	9	0.55	0.06	9	0.75	0.02	9	0.66	0.16	9	0.66	0.07
14	0.69	0.07	10	0.56	0.04	10	0.75	0.12	10	0.66	0.14	10	0.66	0.08
15	0.71	0.07	11	0.54	0.06	11	0.75	0.09	11	0.69	0.13	11	0.67	0.08
16	0.70	0.06	12	0.54	0.06	12	0.75	0.14	12	0.69	0.12	12	0.67	0.07
17	0.71	0.06	13	0.54	0.06	13	0.75	0.13	13	0.72	0.11	13	0.69	0.08
18	0.72	0.06	14	0.53	0.05	14	0.75	0.13	14	0.71	0.12	14	0.69	0.08
19	0.72	0.06	15	0.53	0.05	15	0.75	0.14	15	0.73	0.10	15	0.69	0.07
20	0.73	0.06	16	0.54	0.07	16	0.78	0.09	16	0.70	0.13	16	0.74	0.09
21	0.72	0.07	17	0.55	0.06	17	0.75	0.12	17	0.76	0.08	17	0.72	0.09
22	0.72	0.07	18	0.55	0.06	18	0.78	0.12	18	0.76	0.08	18	0.73	0.10
23	0.75	0.07	19	0.52	0.06	19	0.76	0.09	19	0.77	0.08	19	0.74	0.10
24	0.77	0.07	20	0.54	0.05	20	0.77	0.07	20	0.78	0.08	20	0.74	0.09
25	0.78	0.07	21	0.54	0.06	21	0.77	0.09	21	0.79	0.09	21	0.75	0.10
26	0.77	0.06	22	0.54	0.07	22	0.77	0.16	22	0.79	0.08	22	0.76	0.10
27	0.77	0.07	23	0.54	0.07	23	0.77	0.09	23	0.79	0.08	23	0.77	0.10
28	0.79	0.06	24	0.55	0.07	24	0.75	0.10	24	0.79	0.09	24	0.76	0.09
29	0.78	0.07	25	0.53	0.06	25	0.79	0.08	25	0.79	0.09	25	0.78	0.11
30	0.83	0.07	26	0.53	0.07	26	0.79	0.07	26	0.80	0.09	26	0.79	0.10
31	0.84	0.09	27	0.55	0.06	27	0.80	0.10	27	0.80	0.10	27	0.79	0.10
32	0.83	0.11	28	0.53	0.07	28	0.79	0.08	28	0.80	0.10	28	0.84	0.08
33	0.83	0.08	29	0.54	0.07	29	0.79	0.11	29	0.82	0.09	29	0.82	0.08
34	0.85	0.09	30	0.53	0.08	30	0.76	0.15	30	0.82	0.10	30	0.84	0.07
35	0.87	0.10	31	0.53	0.07	31	0.75	0.14	42	0.83	0.10	31	0.84	0.06
36	0.89	0.12	32	0.51	0.09	32	0.72	0.09	54	0.85	0.14	32	0.86	0.05
37	0.91	0.13	33	0.53	0.06	33	0.79	0.08	64	0.85	0.14	33	0.87	0.05
38	0.91	0.13	34	0.54	0.06	34	0.72	0.10	84	0.86	0.15	34	0.87	0.05
39	0.89	0.12	35	0.53	0.07	35	0.77	0.10	104	0.87	0.16	35	0.85	0.05
40	0.92	0.13	36	0.51	0.05	36	0.77	0.09	119	0.85	0.15	36	0.86	0.05

56	0.99	0.17	37	0.51	0.05	48	0.76	0.13	139	0.86	0.16	37	0.86	0.06
72	1.08	0.18	38	0.50	0.06	60	0.74	0.09	154	0.87	0.18	38	0.87	0.05
88	1.01	0.17	39	0.53	0.07	72	0.73	0.08	169	0.90	0.17	39	0.89	0.06
104	0.99	0.16	40	0.52	0.07	83	0.73	0.07	199	0.88	0.15	40	0.89	0.07
120	0.92	0.19	41	0.52	0.07	95	0.74	0.08	229	0.84	0.16	41	0.84	0.10
136	0.90	0.19	42	0.54	0.07	107	0.76	0.03	259	0.84	0.16	42	0.86	0.11
152	0.95	0.19	43	0.54	0.06	119	0.74	0.05	289	0.84	0.16	43	0.89	0.06
176	0.91	0.14	44	0.53	0.06	143	0.75	0.03	299	0.82	0.13	44	0.89	0.06
200	0.93	0.12	45	0.54	0.06	167	0.75	0.02	304	0.82	0.10	45	0.90	0.05
215	0.91	0.18	46	0.54	0.07	191	0.76	0.03	324	0.85	0.15	46	0.91	0.06
245	0.97	0.13	47	0.54	0.05	215	0.76	0.05				47	0.91	0.07
275	0.95	0.17	48	0.52	0.07	239	0.76	0.07				48	0.93	0.07
305	0.96	0.17	60	0.53	0.08	263	0.74	0.04				49	0.93	0.06
335	0.89	0.18	72	0.54	0.06	287	0.74	0.03				50	0.92	0.06
365	0.93	0.17	84	0.53	0.05	311	0.73	0.03				51	0.92	0.05
			96	0.54	0.06	335	0.72	0.06				52	0.94	0.07
			120	0.54	0.06	359	0.75	0.06				53	0.95	0.06
			132	0.55	0.06	383	0.74	0.07				54	0.90	0.11
			150	0.53	0.07							72	0.90	0.11
			162	0.54	0.04							90	0.93	0.12
			186	0.55	0.05							108	0.91	0.12
			204	0.55	0.03							111	0.91	0.13
			247	0.54	0.05							125	0.94	0.11
			272	0.54	0.04							139	0.98	0.12
			293	0.54	0.04							153	0.95	0.13
			310	0.55	0.03							167	0.95	0.12
			327	0.55	0.04							181	0.94	0.11
			339	0.55	0.05							195	0.95	0.11
			351	0.54	0.03							209	0.94	0.13
			363	0.55	0.04							223	0.92	0.13
			375	0.56	0.04							237	0.95	0.10
			387	0.55	0.04							251	0.97	0.10
												265	0.95	0.13
												279	0.95	0.11
												293	0.94	0.11
												307	0.97	0.15
												321	0.96	0.11
												342	0.96	0.14
												349	0.95	0.11
												356	0.95	0.13
												363	0.94	0.14
												370	0.93	0.08
												377	0.95	0.13
												384	0.94	0.14

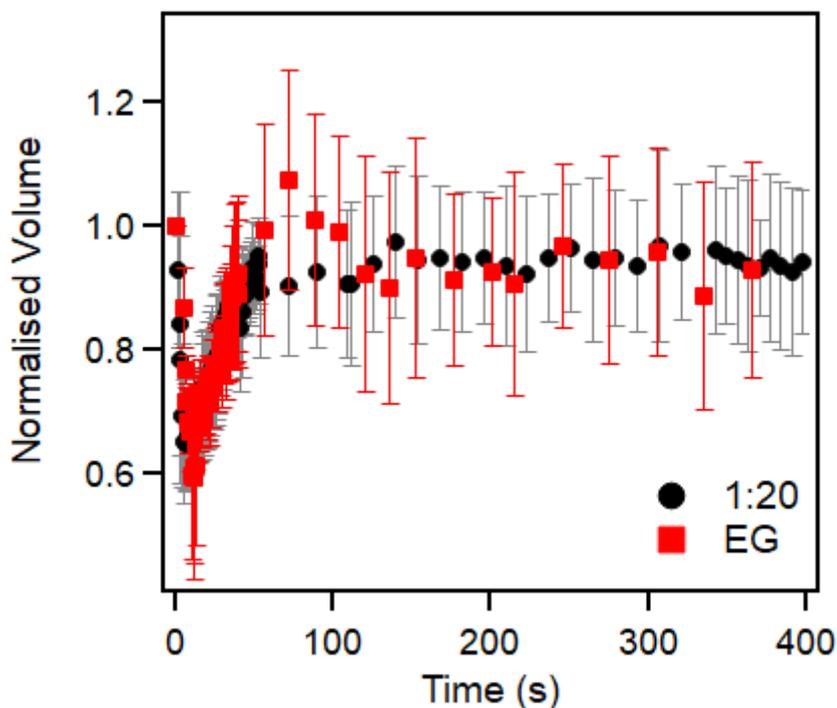
													391	0.93	0.13
													398	0.94	0.12

81



82

83 Figure S8. Final normalised equilibrium volume of THP-1 and HaCat cells following
 84 exposure to proline/ethylene glycol mixtures at different ratios. The lines are linear fits to
 85 the data with the equations and R^2 values provided on the graph. Error bars are based on
 86 the maximum standard deviation of the average value from 200-400 seconds
 87 (experiment time), and do not represent the actual variation between cells.



88

89 Figure S9. Changes to normalised volume HaCat cells exposed to 10 wt% of either EG or
 90 a 1:20 mix of proline and EG. Error bars are based on the standard deviation of at least 5
 91 cells.

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