Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Biomaterial screening of protein coatings and peptide additives: towards a simple synthetic mimic of a complex natural coating for a bio-artificial kidney

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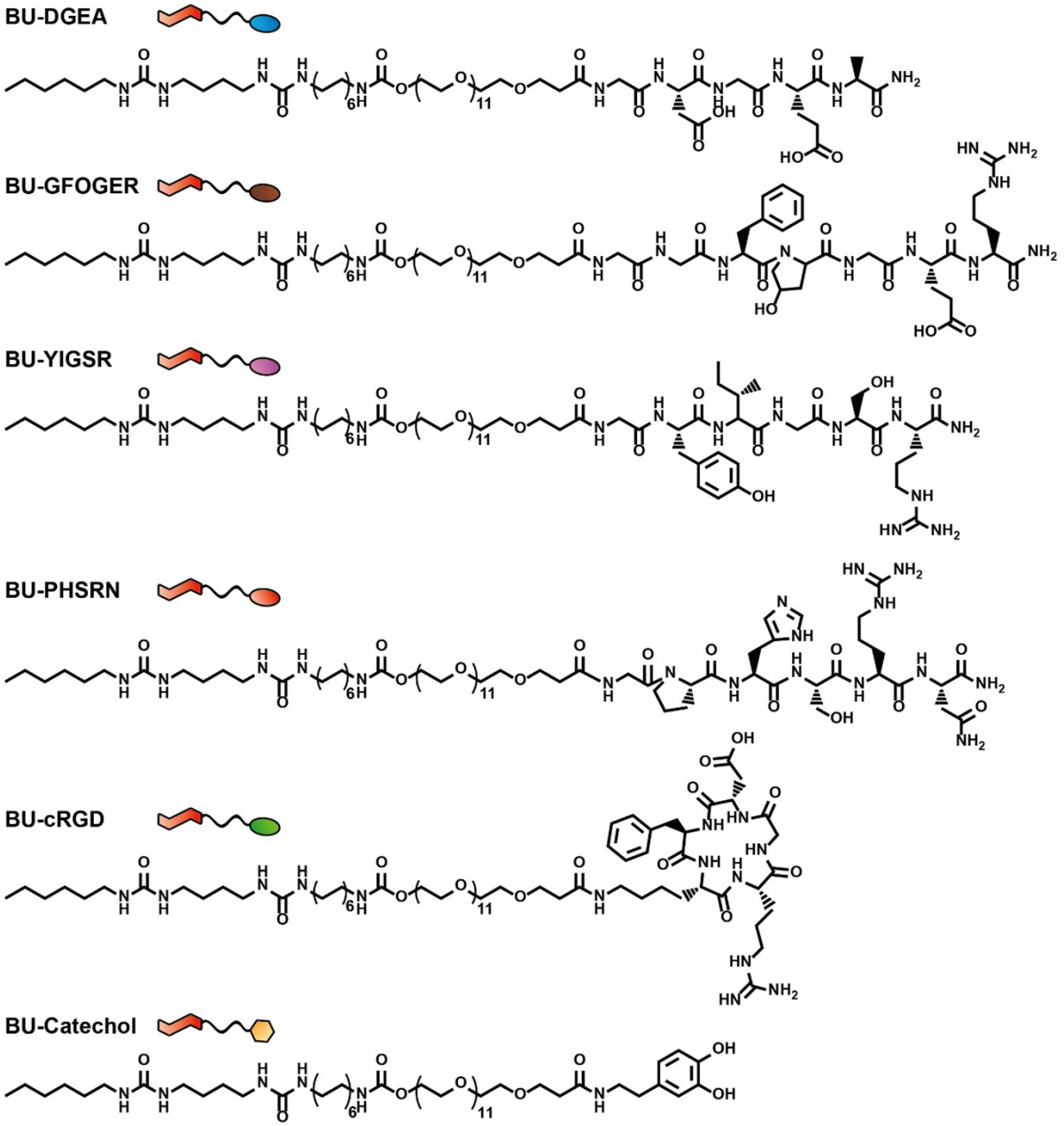


Scheme S1. Chemical structures and schematic representations of BU-additives.

Table S1. ECM Library composition. "+" present, "-" absent in coating combination, *Not required for DoE analysis, performed as control. Sole presence of PCL-BU is termed Pristine.

Run	Collagen type IV	Laminin	Fibronectin	Collagen type I	L-DOPA
1	-	-	-	-	+
2	+	-	-	-	-
3	-	+	-	-	-
4	+	+	-	-	+
5	-	-	+	-	-
6	+	-	+	-	+
7	-	+	+	-	+
8	+	+	+	-	-
9	-	-	-	+	-
10	+	-	-	+	+
11	-	+	-	+	+
12	+	+	-	+	-
13	-	-	+	+	+
14	+	-	+	+	-
15	-	+	+	+	-
16	+	+	+	+	+
17*	-	-	-	-	-

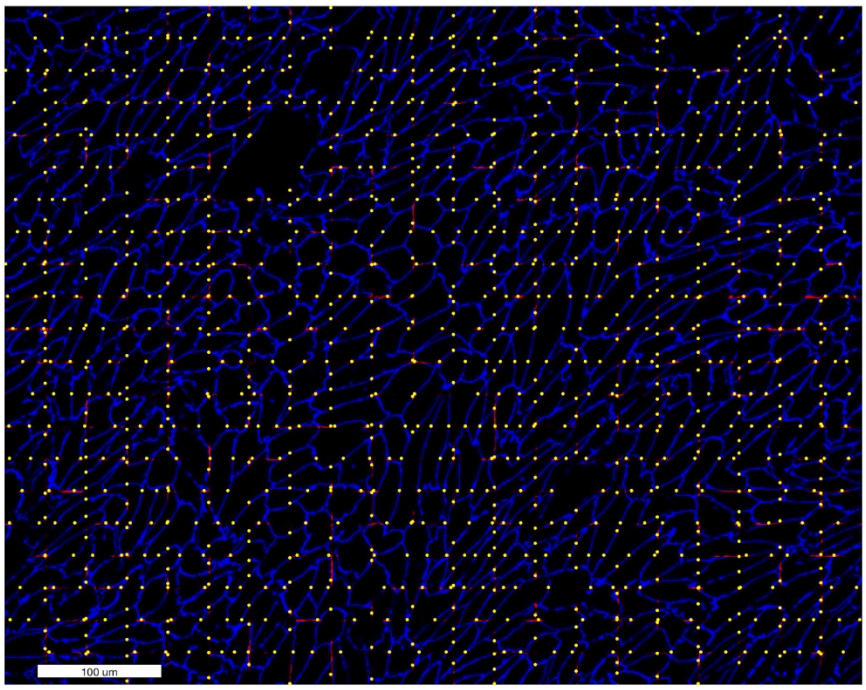


Fig. S1 Example of quantification method ZO-1 intersections.

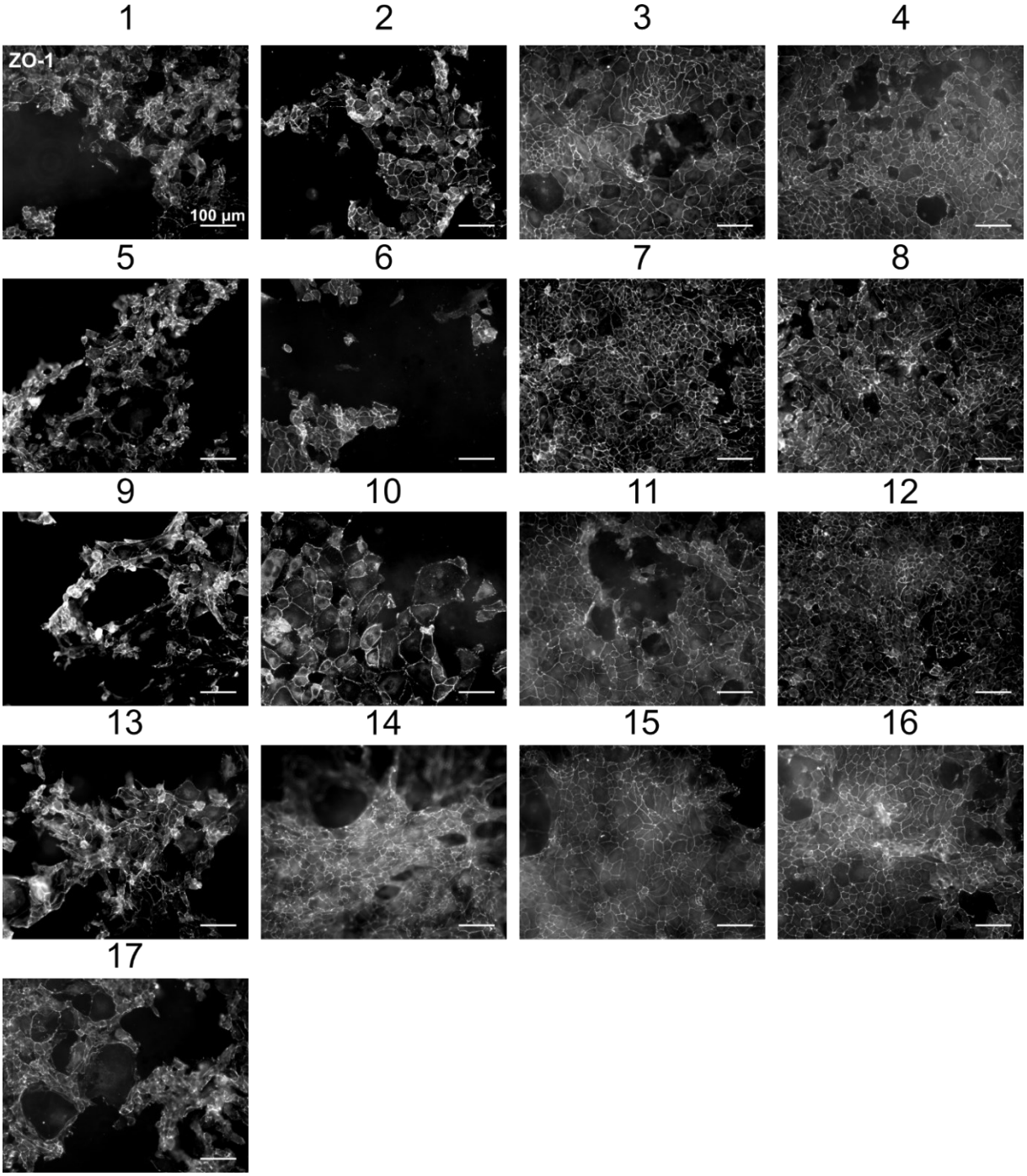


Fig. S2 Response of HK-2 to all ECM library conditions. HK-2 cells cultured for 3 weeks on biomaterial conditions listed in Table S1. Representative images of cells stained for Zona Occludens-1 (ZO-1), scale bar is 100 µm, n=9.

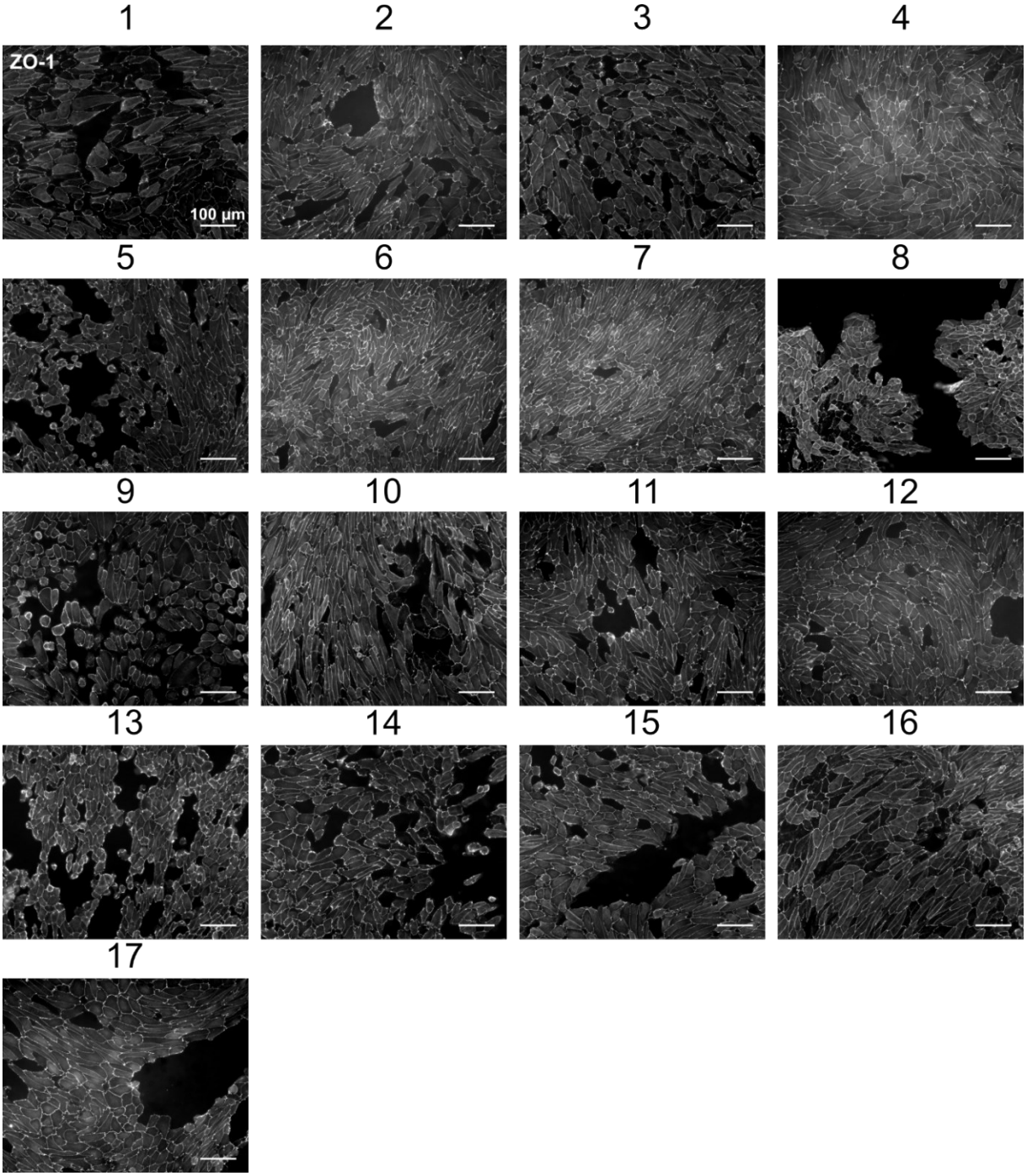


Fig. S3 Response of RPTEC to all ECM library conditions. RPTEC cells cultured for 3 weeks on biomaterial conditions listed in Table S1. Representative images of cells stained for Zona Occludens-1 (ZO-1), scale bar is 100 µm, n=9.

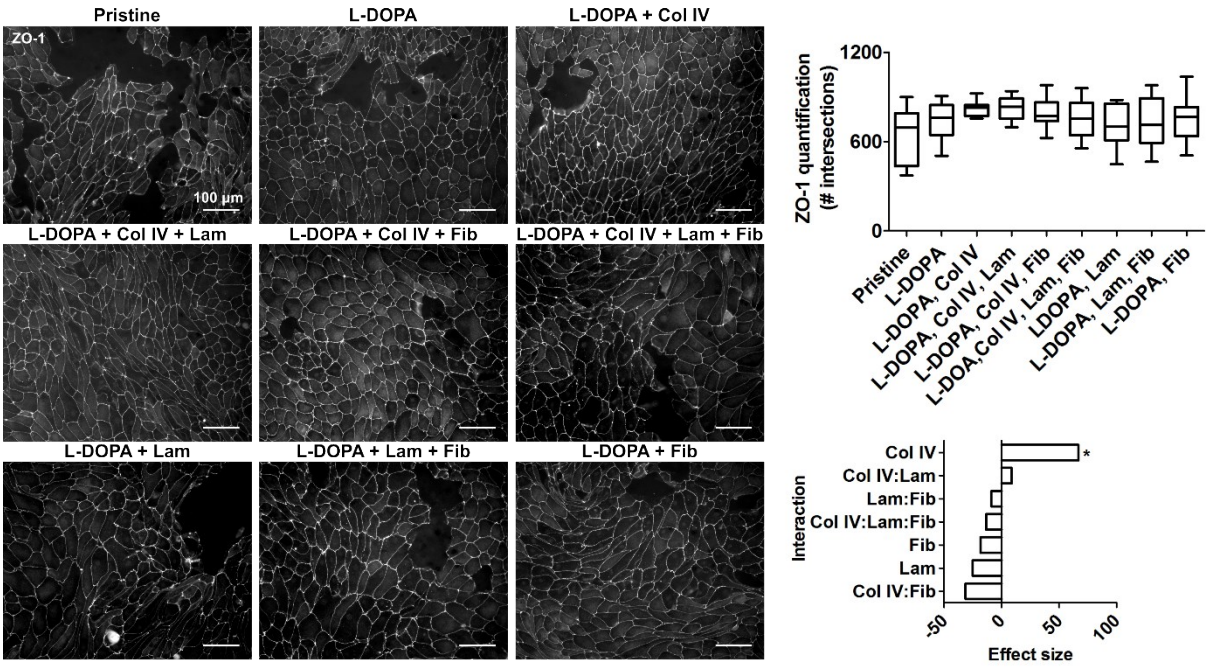
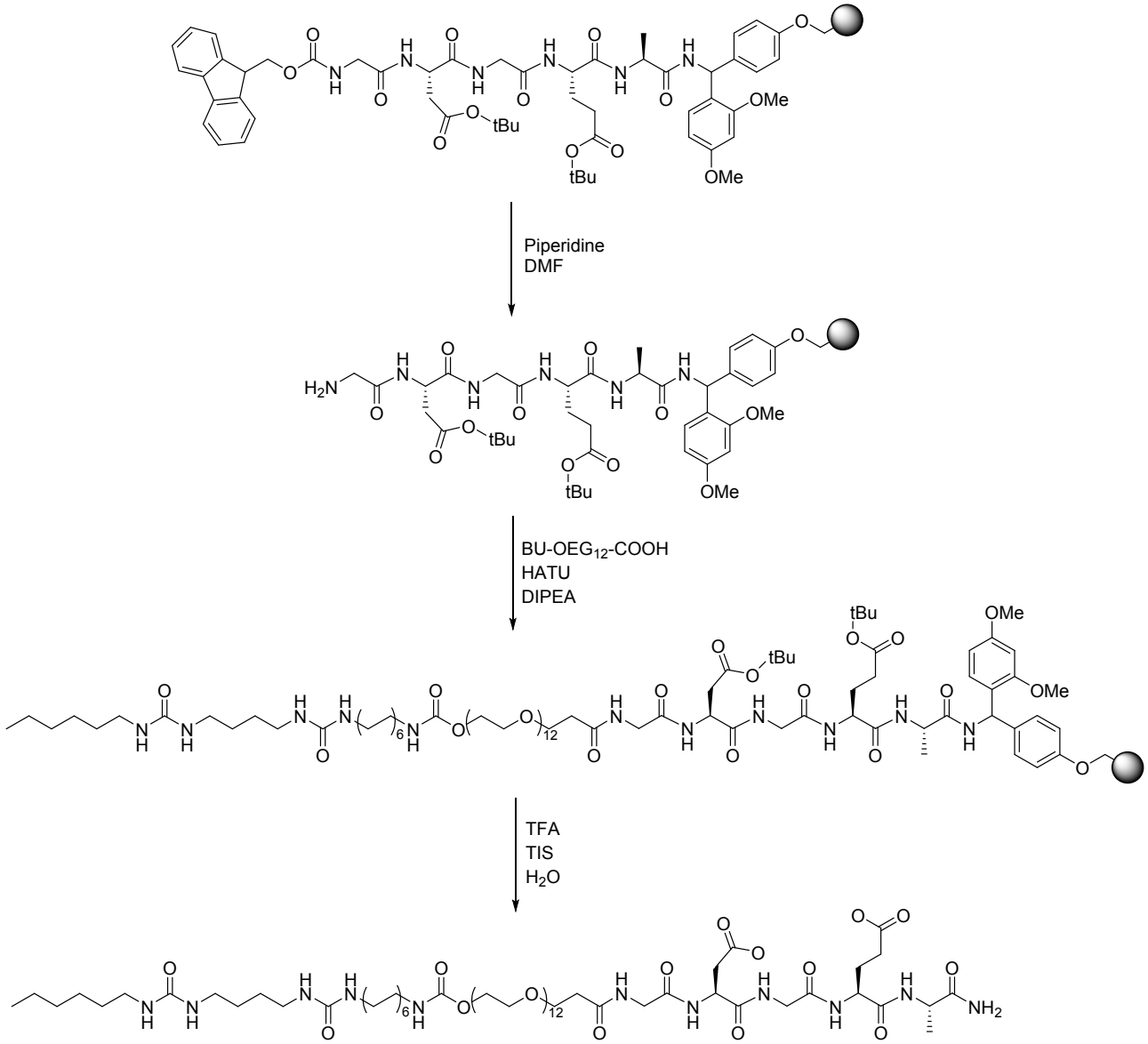


Fig. S4 Response of RPTEC to ECM proteins with L-DOPA constant. RPTEC cells cultured on biomaterial conditions coated with L-DOPA, Collagen type IV (Col IV), Laminin (Lam) or Fibronectin (Fib) or left pristine (no coating on PCL-BU) for 3 weeks. Representative images of cells stained for Zona Occludens-1 (ZO-1; Left), scale bar are 100 µm. Quantification of ZO-1 (Right top), effect size components (Right bottom), *n=3*, *p≤0.05.

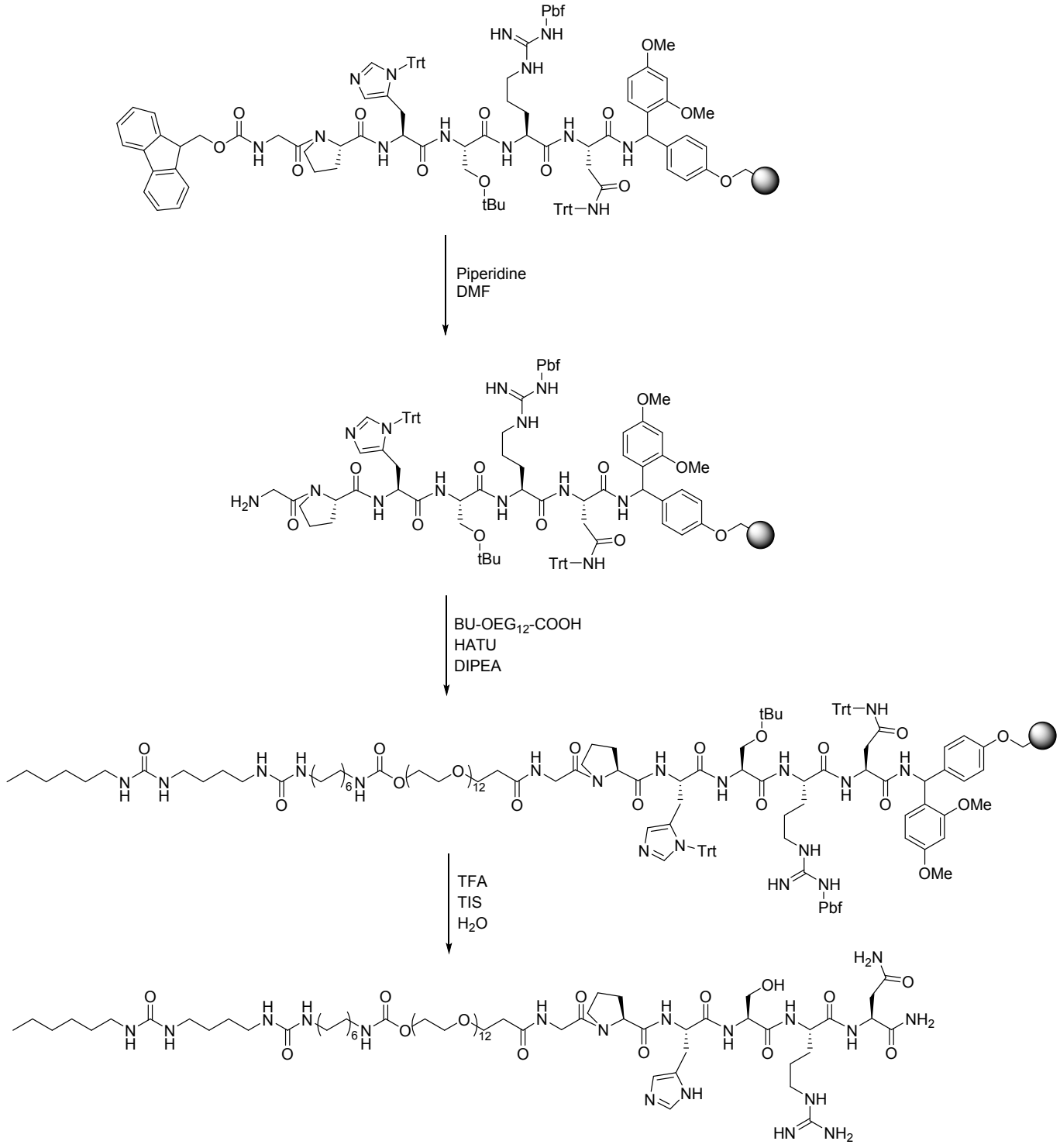
Synthesis bis-urea additives

BU-$(OEG)_{12}$-peptide

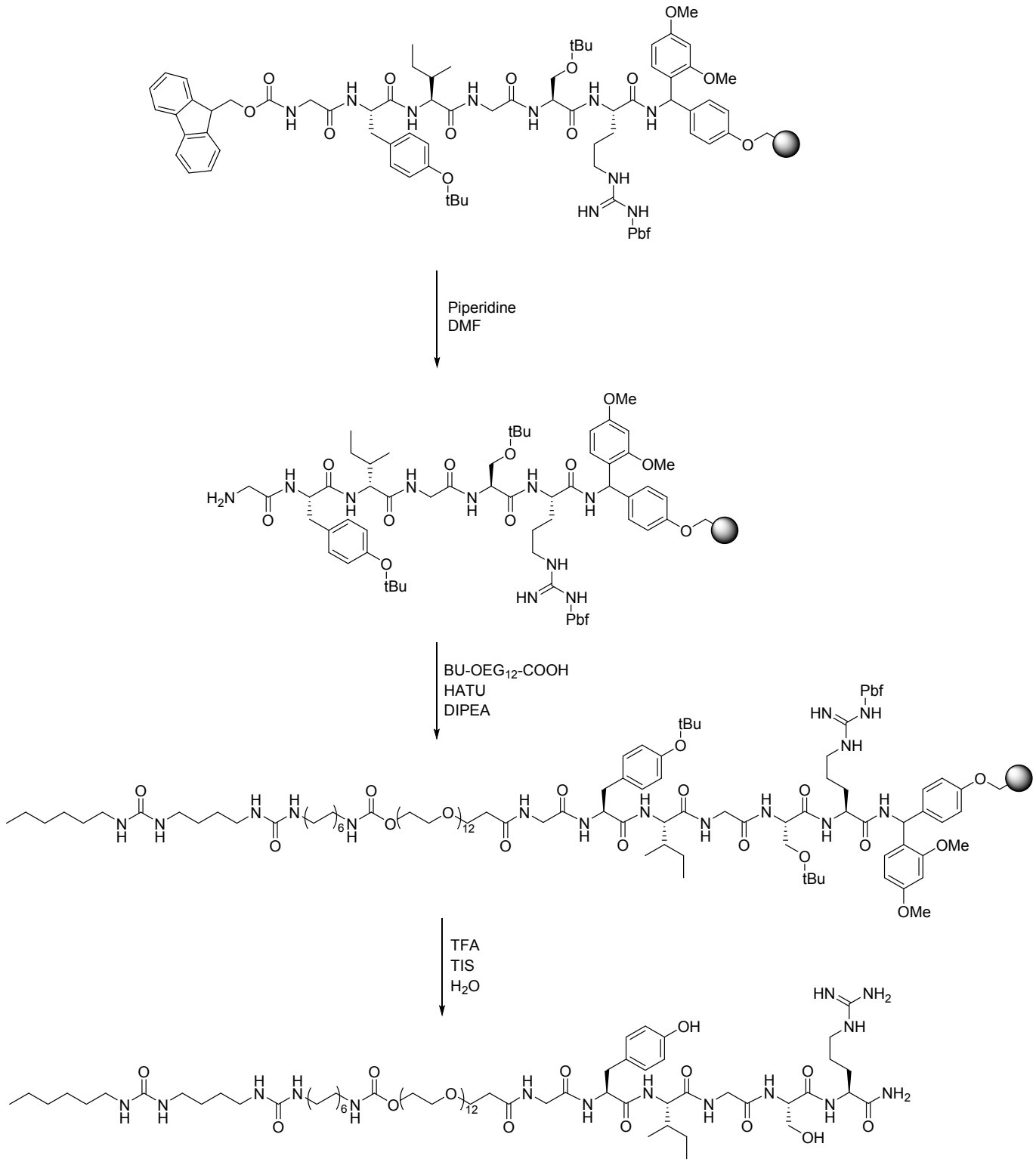
All chemicals were purchesed from Sigma-Aldrich unless otherwise stated. Fmoc-Gly-Asp(OtBu)-Gly-Glu(OtBu)-Ala (40 mg, 0.05 mmol), Fmoc-Gly-Pro-His(Trt)-Ser(tBu)-Arg(Pbf)-Asn(Trt) (96 mg, 0.05 mmol), Fmoc-Gly-Tyr(tBu)-Ile-Gly-Ser(tBu)-Arg(Pbf) (62 mg, 0.05 mmol), or Fmoc-Gly-Gly-Phe-Hyp(tBu)-Gly-Glu(OtBu)-Arg(Pbf) (55 mg, 0.05 mmol) on rink amide resin (0.66 mmol/g) was deprotected 2x15 min with a 20 v/v% piperidine in DMF mixture. BU-OEG_{12}-COOH (108 mg; 0.1 mmol) was dissolved in 4 mL DMF. *N,N*-Diisopropylethylamine (44 µl, 0.25 mmol) and HATU (38 mg, 0.1 mmol) were dissolved in 2 mL DMF, respectively, added to the BU-OEG_{12}-COOH mixture, and stirred for 30 min. After pre-activation, the reaction mixture was added to peptides on resin and reacted overnight under shaking conditions at room temperature. The BU-OEG_{12}-peptides were cleaved from the resin in a 95:2.5:2.5 v/v% TFA:TIS:H_2O mixture. The cleavage mixture was precipitated three times in ice-cold Et_2O. The precipitate was collected, dissolved in a mixture of water/acetonitrile, and freeze-dried yielding 58 mg BU-OEG_{12}-GDGEA (76 %), 49 mg BU-OEG_{12}-GPHSRN (52%), 37 mg BU-OEG_{12}-GYIGSR (43%), and 53 mg BU-OEG_{12}-GGFOGER (58 %) as fluffy white solids. BU-OEG_{12}-GDGEA: LC-MS(ESI) t_r = 6.10 min, m/z calcd ($C_{68}H_{127}N_{11}O_{26}$) 1513.90; found 505.67 $[M+3H]^{3+}$, 758.00 $[M+2H]^{2+}$, 1514.67 $[M+H]^{+}$. BU-OEG_{12}-GPHSRN: LC-MS(ESI) t_r = 4.36 min, m/z calcd ($C_{78}H_{144}N_{18}O_{25}$) 1733.06; found 434.42 $[M+4H]^{4+}$, 578.92 $[M+3H]^{3+}$, 867.58 $[M+2H]^{2+}$, 1733.06 $[M+H]^{+}$. BU-OEG_{12}-GYIGSR: LC-MS(ESI) t_r = 4.65 min, m/z calcd ($C_{80}H_{147}N_{15}O_{25}$) 1718.07; found 573.92 $[M+3H]^{3+}$, 860.42 $[M+2H]^{2+}$, 1718.92 $[M+H]^{+}$. BU-OEG_{12}-GGFOGER: LC-MS(ESI) t_r = 4.65 min, m/z calcd ($C_{83}H_{148}N_{16}O_{27}$) 1801.07; found 473.33 $[M+4Na]^{4+}$, 601.58 $[M+3H]^{3+}$, 901.58 $[M+2H]^{2+}$, 1802.92 $[M+H]^{+}$.



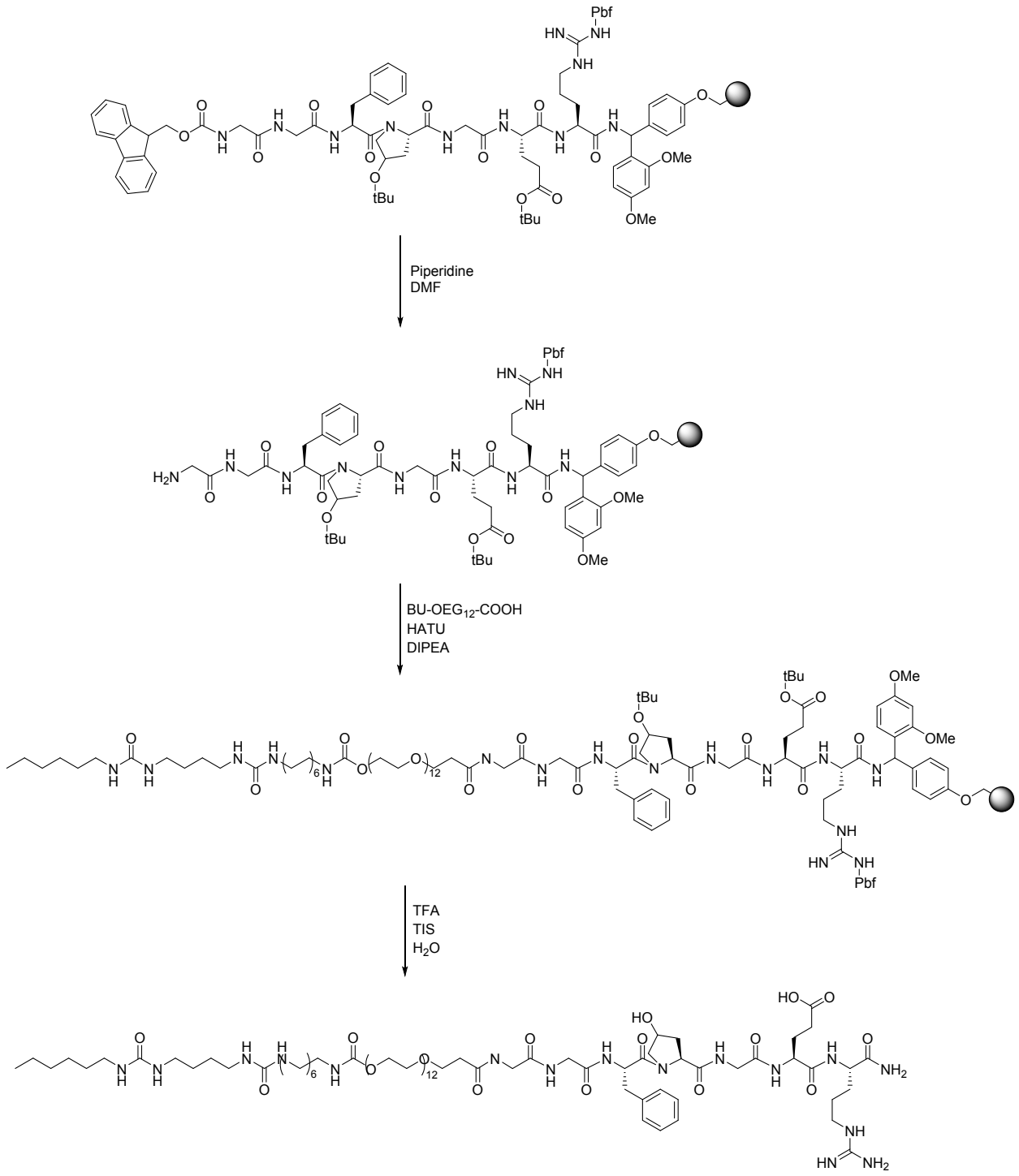
Scheme S2 Synthesis of BU-DGEA.



Scheme S3 Synthesis of BU-PHSRN.



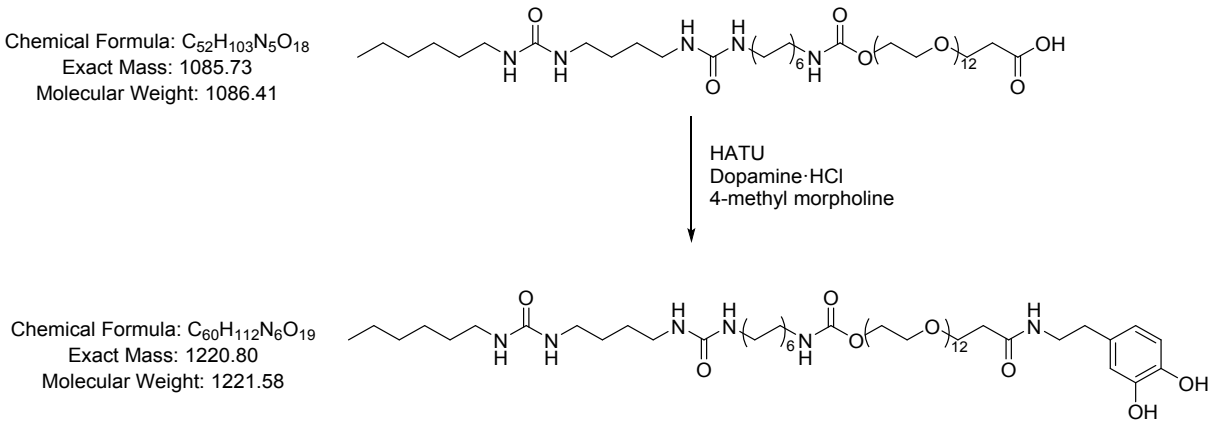
Scheme S4 Synthesis of BU-YIGSR.



Scheme S5 Synthesis of BU-GFOGER.

BU-(OEG)$_{12}$-Catechol

BU-OEG$_{12}$-COOH (100 mg; 0.09 mmol; SyMO-Chem) was dissolved in 4 mL DMF under argon. *N*-methylmorpholine (60 µl, 0.54 mmol) and HATU (50 mg, 0.14 mmol) were added to the reaction mixture and stirred for 30 min. After pre-activation, 3,4-dihydroxyphenethylamine hydrochloride (26 mg, 0.14 mmol) was added and the reaction was stirred overnight under argon. The reaction mixture was precipitated three times in a 1% (v/v) solution of formic acid in water. The precipitate was collected, dissolved in mixture of water/acetonitrile and freeze-dried yielding 92 mg (84%) of the product as a fluffy white solid.



Scheme S6 Synthesis of BU-Catechol.

^{1}H NMR (400 MHz, DMSO) δ = 8.72 (bs, 1H), 8.63 (bs, 1H), 7.84 (NH), 7.16 (NH), 6.61 (d, J = 8.0 Hz, 1H), 6.56 (d, J = 2.0 Hz, 1H), 6.43 (dd, *J* = 8.0, 2.1 Hz, 1H), 5.73 (NH), 4.02 (bs, 2H), 3.60-3.40 (m, 48H), 3.17 (m, 2H), 2.94 (m, 10H), 2.50 (m, 2H), 2.28 (t, J = 6.44 Hz, 2H), 1.40-1.20 (m, 32H), 0.86 (t, J = 6.79 Hz, 3H)

^{13}C NMR (100 MHz, DMSO) δ = 169.84, 158.08, 156.13, 145.03, 143.49, 130.20, 119.17, 115.92, 115.44, 69.78, 69.72, 69.67, 69.52, 68.91, 66.85, 62.98, 39.52, 36.17, 34.68, 31.07, 30.07, 30.03, 29.40, 29.09, 29.03, 28.85, 28.76, 27.61, 26.44, 26.26, 26.09, 22.11, 13.94.

LC-MS(ESI) t_r = 5.29 min, m/z calcd ($C_{60}H_{112}N_6O_{19}$) 1220.80; found 611.42 $[M+2H]^{2+}$, 1221.50 $[M+H]^{+}$.

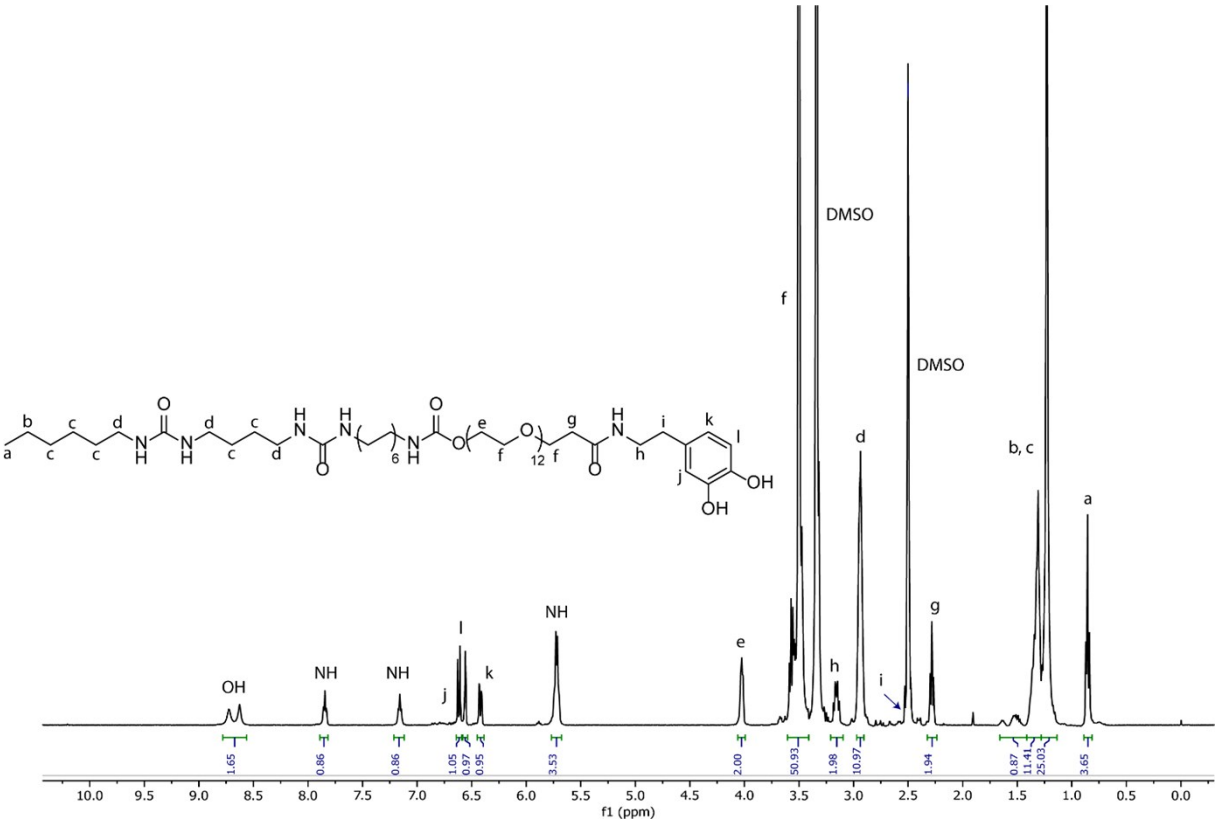


Fig. S5 ^{1}H-NMR spectrum of BU-Catechol.

Table S2 BU-additive library composition. "+" present, "-" absent in combination. Sole presence of PCL-BU is termed Pristine. 1 mol% per BU-peptide and/or 5 mol% per BU-catechol was/were employed.

Run	BU-GFOGER	BU-YIGSR	BU-cRGD	BU-DGEA	BU-Catechol	BU-PHSRN
1	-	-	-	-	-	-
2	+	-	-	-	-	+
3	-	+	-	-	-	+
4	+	+	-	-	-	-
5	-	-	+	-	-	+
6	+	-	+	-	-	-
7	-	+	+	-	-	-
8	+	+	+	-	-	+
9	-	-	-	+	-	+
10	+	-	-	+	-	-
11	-	+	-	+	-	-
12	+	+	-	+	-	+
13	-	-	+	+	-	-
14	+	-	+	+	-	+
15	-	+	+	+	-	+
16	+	+	+	+	-	-
17	-	-	-	-	+	+
18	+	-	-	-	+	-
19	-	+	-	-	+	-
20	+	+	-	-	+	+
21	-	-	+	-	+	-
22	+	-	+	-	+	+
23	-	+	+	-	+	+
24	+	+	+	-	+	-
25	-	-	-	+	+	-
26	+	-	-	+	+	+
27	-	+	-	+	+	+
28	+	+	-	+	+	-
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31	-	+	+	+	+	-
32	+	+	+	+	+	+

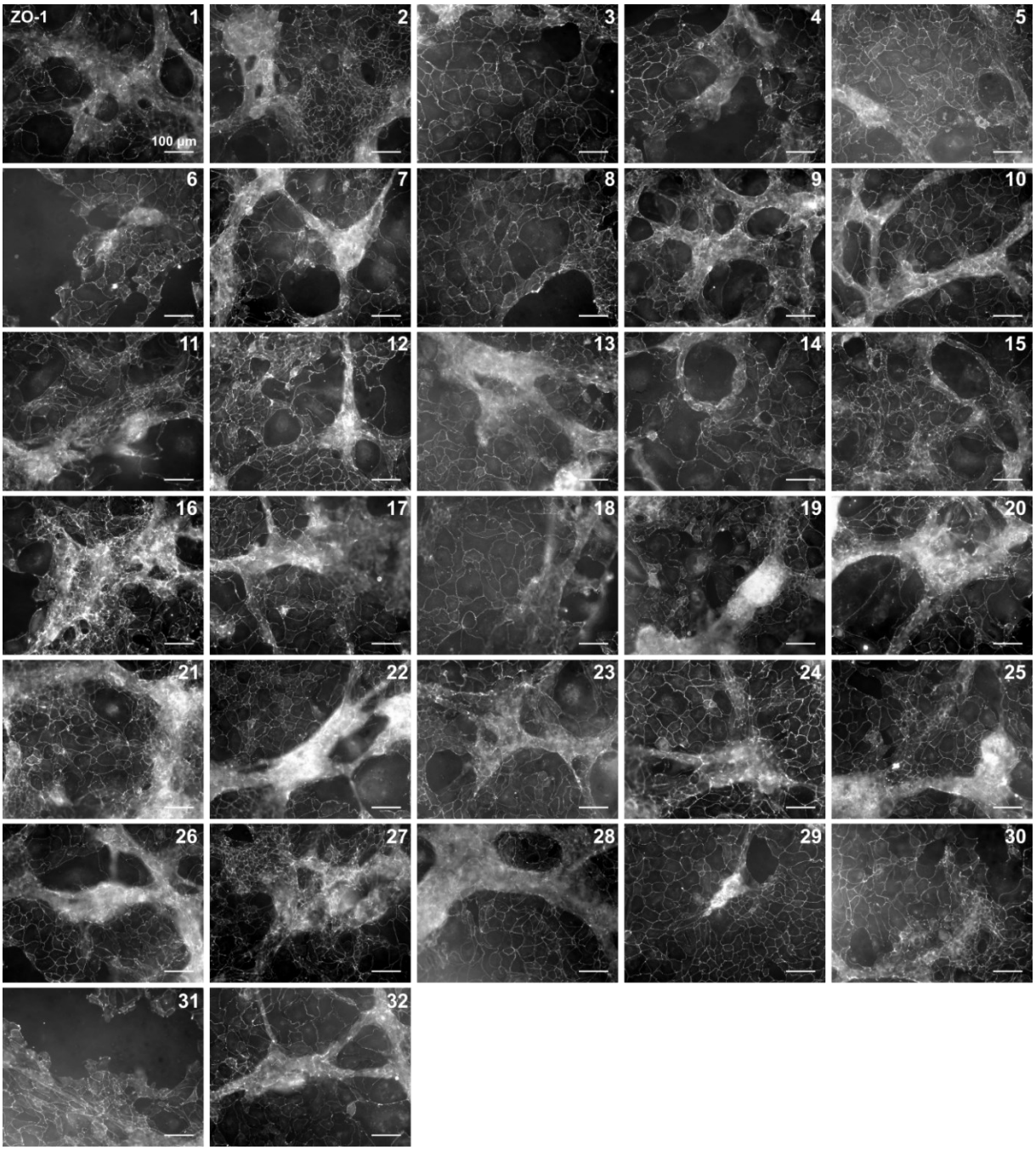

Fig. S6 HK-2 response to all BU-additive library conditions. HK-2 cells cultured for 3 weeks on biomaterial conditions listed in Table S2. Representative images of cells stained for ZO-1, scale bar is 100 µm, n=6.

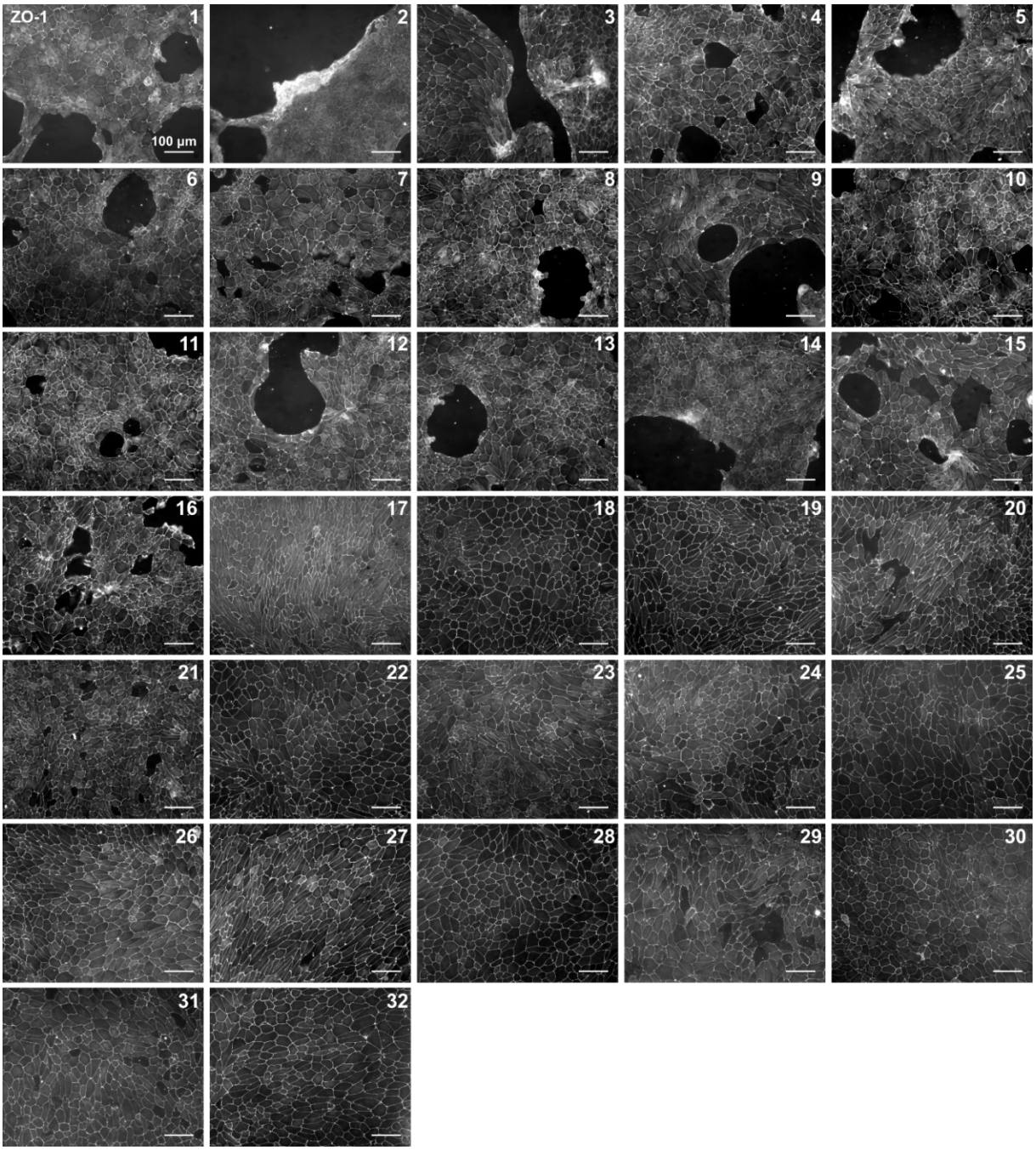

Fig. S7 RPTEC response to all BU-additive library conditions. RPTEC cells cultured for 3 weeks on biomaterial conditions listed in Table S2. Representative images of cells stained for Zona Occludens-1 (ZO-1), scale bar is 100 µm, n=6.

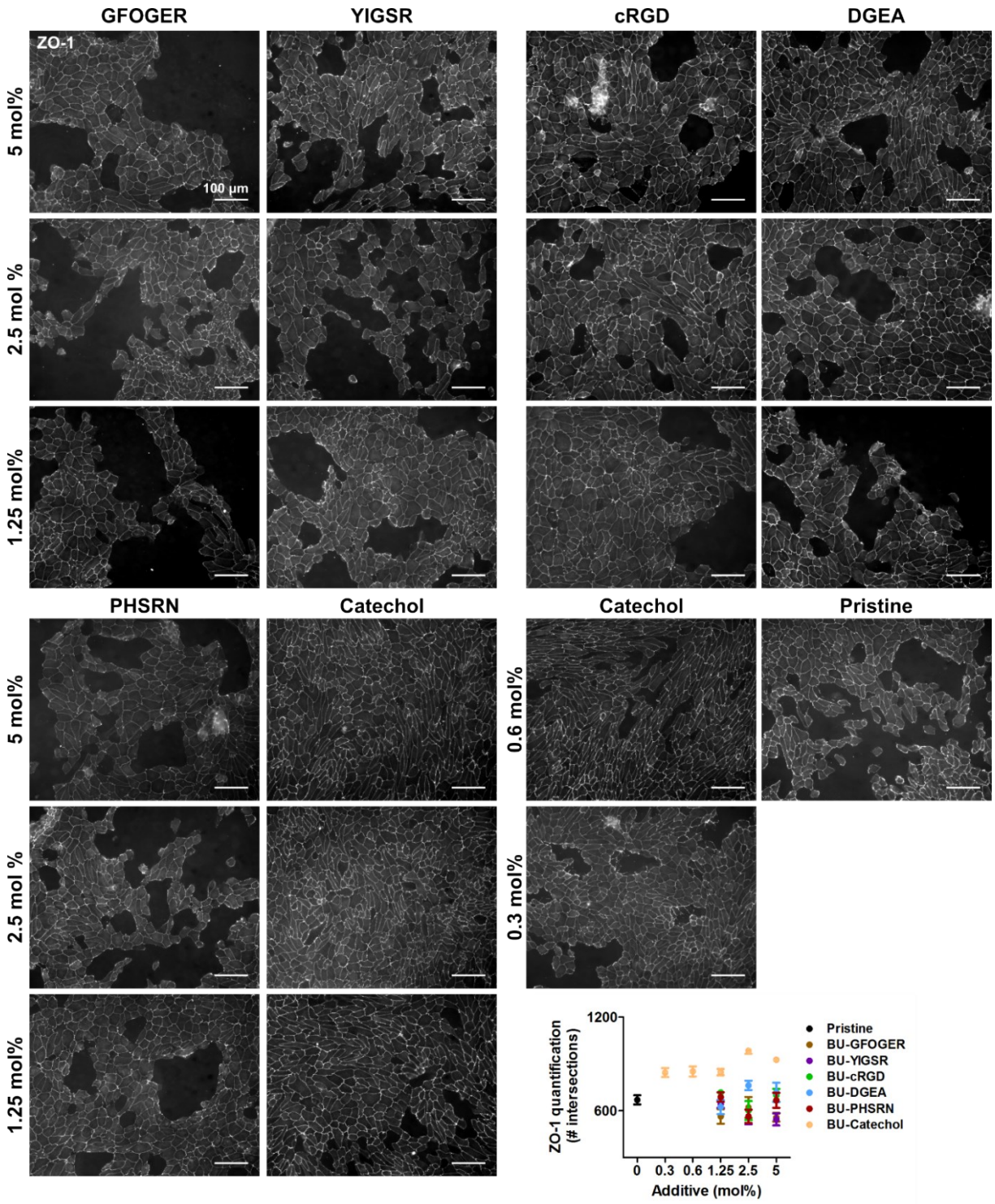

Fig. S8 Concentration range of BU-additives. RPTEC cells cultured for 3 weeks on biomaterials containing different concentrations of BU-additives. Representative images of cells stained for Zona Occludens-1 (ZO-1), and subsequently quantified, n=3.

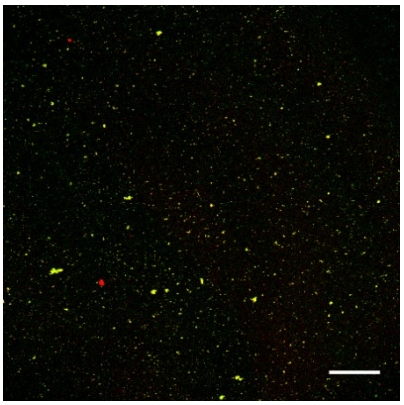

Fig. S9 Staining of L-DOPA with Collagen type IV and Laminin coating before cell culture. Laminin (green), Collagen type IV (red), scale bar is 100 µm.

Table S3. List of employed antibodies.

Antibody against	Supplier, catalogue number	Dilution
ZO-1	BD Biosciences, 610966	1:200
αTubulin	Abcam, ab18251	1:200
Collagen type IV	Abcam ab6586	1:100
Laminin gamma 1	Abcam, ab17792	1:50
Megalin	Abcam, ab76969	1:200
Phalloidin-atto4888	Sigma-Aldrich, 49409	1:500
anti-mouse IgG (H+L) - AF555	Molecular probes, A21424	1:200
anti-mouse IgG (H+L) - AF647	Molecular probes, A21236	1:200
anti-rabbit IgG (H+L) - AF555	Molecular probes, A21428	1:200
anti-rabbit IgG (H+L) - AF647	Molecular probes, A21244	1:200
anti-Rat IgG (H+L) - AF488	Invitrogen, A11006	1:200
anti-Rat IgG (H+L) - AF555	Molecular Probes, A21434	1:200