

Supplementary information

Glycome analysis of extracellular vesicles derived from human induced pluripotent stem cells using lectin microarray

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Table S1. Cells used for the preparation of EVs

Name	Cell type	Supply	Cat# (Lot#)	Passage
hFibs(P11)	Human fibroblasts	ATCC	PCS-201-012 (58605481)	11
ADSC#2117(P6)	Human adipose-derived mesenchymal stem cells	ThermoFisher SCIENTIFIC	R7788110 (2117)	6
ADSC#2117(P24)	Human adipose-derived mesenchymal stem cells	ThermoFisher SCIENTIFIC	R7788110 (2117)	24
ADSC#2118(P5)	Human adipose-derived mesenchymal stem cells	ThermoFisher SCIENTIFIC	R7788110 (2118)	5
ADSC#2118(P27)	Human adipose-derived mesenchymal stem cells	ThermoFisher SCIENTIFIC	R7788110 (2118)	27
Yub621c(P7)	Human chondrocytes from polydactylous fingers	RIKEN BRC	RBRC-HMS0013	7
Yub621c(P27)	Human chondrocytes from polydactylous fingers	RIKEN BRC	RBRC-HMS0013	27
Yub625(P5)	Human chondrocytes from polydactylous fingers	RIKEN BRC	RBRC-HMS0038	5
Yub625(P18)	Human chondrocytes from polydactylous fingers	RIKEN BRC	RBRC-HMS0038	18
201B7(P46)	Human induced pluripotent stem cells	RIKEN BRC	HPS0063	46
201B7(P48)	Human induced pluripotent stem cells	RIKEN BRC	HPS0063	48
201B7(P55)	Human induced pluripotent stem cells	RIKEN BRC	HPS0063	55
201B7(P57)	Human induced pluripotent stem cells	RIKEN BRC	HPS0063	57

SupplementaryTable 2. Lectins used for lectin microarray¹

Name	Species	Origin	Source ³	Rough specificity ²
1 LFA	<i>Limax flavus</i>	Natural	EY Lab.	Sia
2 WGA	<i>Triticum vulgaris</i>	Natural	EY Lab.	(GlcNAc) _n , polySia
3 PVL	<i>Psathyrella velutina</i>	Natural	Wako	Sia, GlcNAc
4 MAL	<i>Maackia amurensis</i>	Natural	Seikagaku	α2-3Sia
5 MAH	<i>Maackia amurensis</i>	Natural	Vector	α2-3Sia
6 ACG	<i>Agrocybe cylindracea</i>	Natural	JOM	α2-3Sia
7 rACG	<i>Agrocybe cylindracea</i>	<i>E. coli</i>	AIST	α2-3Sia
8 rGal8N	<i>Homo sapiens</i>	<i>E. coli</i>	AIST	α2-3Sia
9 SNA	<i>Sambucus nigra</i>	Natural	Seikagaku	α2-6Sia
10 SSA	<i>Sambucus sieboldiana</i>	Natural	Vector	α2-6Sia
11 TJA1	<i>Trichosanthes japonica</i>	Natural	Vector	α2-6Sia
12 rPSL1a	<i>Polyporus squamosus</i>	<i>E. coli</i>	AIST	α2-6Sia
13 PHAL	<i>Phaseolus vulgaris</i>	Natural	Seikagaku	GlcNAcβ1-6Man (Tetraantenna)
14 DSA	<i>Datura stramonium</i>	Natural	Seikagaku	GlcNAcβ1-6Man (Tetraantenna)
15 TxLcI	<i>Tulipa gesneriana</i>	Natural	JOM	Galactosylated N-glycans up to triantenna
16 ECA	<i>Erythrina cristagalli</i>	Natural	Seikagaku	βGal
17 RCA120	<i>Ricinus communis</i>	Natural	Vector	βGal
18 rGal7	<i>Homo sapiens</i>	<i>E. coli</i>	AIST	Type1 LacNAc, chondroitin polymer
19 rGal9N	<i>Homo sapiens</i>	<i>E. coli</i>	AIST	GalNAcα1-4Gal (A), PolyLacNAc
20 rGal9C	<i>Homo sapiens</i>	<i>E. coli</i>	AIST	PolyLacNAc, Branched LacNAc
21 rC14	<i>Gallus gallus domesticus</i>	<i>E. coli</i>	AIST	Branched LacNAc
22 rDiscoidin II	<i>Dictyostelium discoideum</i>	<i>E. coli</i>	AIST	LacNAc, Galβ1-3GalNAc (T), GalNAc (Tn)
23 BPL	<i>Bauhinia purpurea alba</i>	Natural	Vector	Galβ1-3GlcNAc (GalNAc), α/βGalNAc
24 rCGL2	<i>Homo sapiens</i>	<i>E. coli</i>	AIST	GalNAcα1-3Gal (A), PolyLacNAc
25 PHAE	<i>Phaseolus vulgaris</i>	Natural	Vector	bisecting GlcNAc
26 GSLII	<i>Griffonia simplicifolia</i>	Natural	Vector	GlcNAcβ1-4Man
27 rSRL	<i>Sclerotium rolfsii</i>	<i>E. coli</i>	AIST	Core1,3, agalacto N-glycan
28 UDA	<i>Urtica dioica</i>	Natural	Vector	(GlcNAc) _n
29 PWM	<i>Phytolacca americana</i>	Natural	Vector	(GlcNAc) _n
30 rF17AG	<i>Escherichia coli</i>	<i>E. coli</i>	AIST	GlcNAc
31 rGRFT	<i>Griffithia sp.</i>	<i>E. coli</i>	AIST	Man
32 NPA	<i>Narcissus pseudonarcis</i>	Natural	Seikagaku	Manα1-3Man
33 ConA	<i>Canavalia ensiformis</i>	Natural	Vector	M3, Manα1-2Manα1-3 (Manα1-6)Man, GlcNAcβ1-2Manα1-3 (Manα1-6)Man
34 GNA	<i>Galanthus nivalis</i>	Natural	Vector	Manα1-3Man, Manα1-6Man
35 HHL	<i>Hippeastrum hybrid</i>	Natural	Vector	Manα1-3Man, Manα1-6Man
36 ASA	<i>Allium sativum</i>	Natural	JOM	Galβ1-4GlcNAcβ1-2Man
37 DBA1	<i>Dioscorea batatas</i>	Natural	JOM	High-man
38 CCA	<i>Castanea crenata</i>	Natural	JOM	Galactosylated N-glycans up to triantenna
39 Heltuba	<i>Helianthus tuberosus</i>	Natural	JOM	Manα1-3Man
40 rHeltuba	<i>Helianthus tuberosus</i>	<i>E. coli</i>	AIST	Manα1-3Man
41 ADA	<i>Allomyrina dichotoma</i>	Natural	JOM	α2-6Sia, Forssman, A, B
42 VVAII	<i>Vicia villosa</i>	Natural	JOM	Man, Agalacto
43 rOrysata	<i>Oryza sativa</i>	<i>E. coli</i>	AIST	Manα1-3Man, Highman, biantenna
44 rPALa	<i>Phlebodium aureum</i>	<i>E. coli</i>	AIST	Man5, biantenna
45 rBanana	<i>Musa acuminata</i>	<i>E. coli</i>	AIST	Manα1-2Manα1-3 (6)Man
46 rCalsepa	<i>Calystegia sepium</i>	<i>E. coli</i>	AIST	Biantenna with bisecting GlcNAc
47 rRSL	<i>Ralstonia solanacearum</i>	<i>E. coli</i>	AIST	αMan, α1-2Fuc (H), α1-3Fuc (Lex), α1-4Fuc (Lea)
48 rBC2LA	<i>Burkholderia cenocepac</i>	<i>E. coli</i>	AIST	aMan, High-man
49 AOL	<i>Aspergillus oryzae</i>	Natural	Vector	α1-2Fuc (H), α1-3Fuc (Lex), α1-3Fuc (Lea)
50 AAL	<i>Aleuria aurantia</i>	Natural	Vector	α1-2Fuc (H), α1-3Fuc (Lex), α1-4Fuc (Lea)
51 rAAL	<i>Aleuria aurantia</i>	<i>E. coli</i>	AIST	α1-2Fuc (H), α1-3Fuc (Lex), α1-3Fuc (Lea)
52 rPAIIL	<i>Pseudomonas aeruginosa</i>	<i>E. coli</i>	AIST	aMan, a1-2Fuc (H), a1-3Fuc (Lex), a1-4Fuc (Lea)
53 rRSIIL	<i>Ralstonia solanacearum</i>	<i>E. coli</i>	AIST	α1-2Fuc (H), α1-3Fuc (Lex), α1-3Fuc (Lea)
54 rPTL	<i>Pholiota terrestris</i>	<i>E. coli</i>	AIST	α1-6Fuc
55 PSA	<i>Pisum sativum</i>	Natural	Seikagaku	α1-6Fuc up to biantenna
56 LCA	<i>Lens culinaris</i>	Natural	Vector	α1-6Fuc up to biantenna
57 rAOL	<i>Aspergillus oryzae</i>	<i>E. coli</i>	AIST	α1-2Fuc (H), α1-3Fuc (Lex), α1-3Fuc (Lea)
58 rBC2LCN	<i>Burkholderia cenocepac</i>	<i>E. coli</i>	AIST	Fuc α1-2Galβ1-3GlcNAc (GalNAc)
59 LTL	<i>Lotus tetragonolobus</i>	Natural	Seikagaku	Lex, Ley
60 UEA1	<i>Ulex europaeus</i>	Natural	Vector	α1-2Fuc
61 TJAII	<i>Trichosanthes japonica</i>	Natural	Vector	α1-2Fuc
62 MCA	<i>Momordica charantia</i>	Natural	JOM	α1-2Fuc
63 GSLI	<i>Griffonia simplicifolia</i>	Natural	Seikagaku	αGalNAc (A, Tn), aGal (B)
64 PTLI	<i>Psophocarpus tetragono</i>	Natural	Tokyo Kasei	αGalNAc (A, Tn)
65 GSLIA4	<i>Griffonia simplicifolia</i>	Natural	EY Lab.	αGalNAc (A, Tn)
66 rGC2	<i>Geodia cydonium</i>	<i>E. coli</i>	AIST	α1-2Fuc (H), αGalNAc (A), αGal (B)
67 GSLIB4	<i>Griffonia simplicifolia</i>	Natural	Vector	αGal (B)
68 rMOA	<i>Marasmius oreades</i>	<i>E. coli</i>	AIST	αGal (B)
69 EEL	<i>Euonymus europaeus</i>	Natural	Vector	αGal (B)
70 rPAIL	<i>Pseudomonas aeruginosa</i>	<i>E. coli</i>	AIST	α,βGal, αGalNAc (Tn)
71 LEL	<i>Lycopersicon esculentum</i>	Natural	Vector	Polylactosamine, (GlcNAc) _n
72 STL	<i>Solanum tuberosum</i>	Natural	Seikagaku	Polylactosamine, (GlcNAc) _n

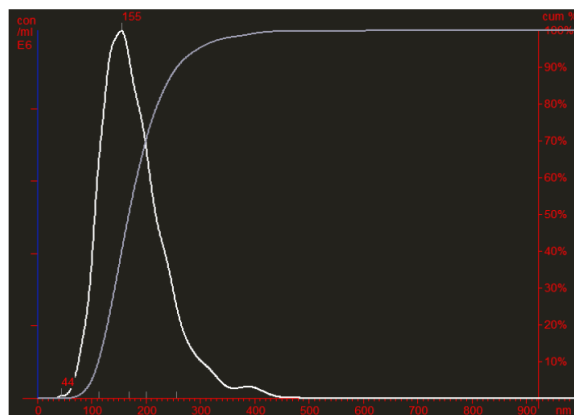
73 rGal3C	<i>Homo sapiens</i>	<i>E. coli</i>	AIST	LacNAc, polylactosamine
74 rLSLN	<i>Laetiporus sulphureus</i>	<i>E. coli</i>	AIST	LacNAc, polylactosamine
75 rCGL3	<i>Coprinopsis cinerea</i>	<i>E. coli</i>	AIST	LacDiNAc
76 PNA	<i>Arachis hypogaea</i>	Natural	Vector	Galβ1-3GalNAc (T)
77 ACA	<i>Amaranthus caudatus</i>	Natural	Vector	Galβ1-3GalNAc (T)
78 HEA	<i>Hericium erinaceum</i>	Natural	JOM	Galβ1-3GalNAc (T)
79 ABA	<i>Agarics bisporus</i>	Natural	Vector	Galβ1-3GalNAc (T), GlcNAc
80 Jacalin	<i>Artocarpus integrifoli.</i>	Natural	Seikagaku	Galβ1-3GalNAc (T), GalNAca (Tn)
81 MPA	<i>Maclura pomifera</i>	Natural	Seikagaku	Galβ1-3GalNAc (T), GalNAca (Tn)
82 HPA	<i>Helix pomatia</i>	Natural	Seikagaku	αGalNAc (A, Tn)
83 VVA	<i>Vicia villosa</i>	Natural	Vector	α,βGalNAc (A, Tn, LacDiNAc)
84 DBA	<i>Dolichos biflorus</i>	Natural	Vector	α,βGalNAc (A, Tn, LacDiNAc)
85 SBA	<i>Glycine max</i>	Natural	EY Lab.	α,βGalNAc (A, Tn, LacDiNAc)
86 rPPL	<i>Pleurocybella porrigen.</i>	<i>E. coli</i>	AIST	α,βGalNAc (A, Tn, LacDiNAc)
87 rCNL	<i>Clitocybe nebularis</i>	<i>E. coli</i>	AIST	α,βGalNAc (A, Tn, LacDiNAc)
88 rXCL	<i>Xerocomus chrysenteron</i>	<i>E. coli</i>	AIST	Core1,3, agalacto N-glycan
89 VVA I	<i>Vicia villosa</i>	Natural	JOM	GalNAcβ1-3(4)Gal
90 WFA	<i>Wisteria floribunda</i>	Natural	Vector	Terminal GalNAc, LacDiNAc
91 rABA	<i>Agarics bisporus</i>	<i>E. coli</i>	AIST	Galβ1-3GalNAc (T), GlcNAc
92 rDiscoidin I	<i>Dictyostelium Discodeu</i>	<i>E. coli</i>	AIST	Gal
93 DBAIII	<i>Dioscorea batatas</i>	Natural	JOM	Maltose
94 rMalectin	<i>Homo sapiens</i>	<i>E. coli</i>	AIST	Glcα1-2Glc
95 CSA	<i>Oncorhynchus keta</i>	Natural	JOM	Rhamnose, Galα1-4Gal
96 FLAG-EW29Ch-E20K	<i>Lumbricus terrestris</i>	<i>E. coli</i>	AIST	6-sulfo-Gal

¹Abbreviations: Gal (D-galactose), GalNAc (N-acetyl-galactosamine), GlcNAc (N-acetyl-glucosamine), Fuc (L-fucose), Glc (D-glucose), Sia (Sialic acid), LacNAc (N-acetyl-lactosamine).

²Specificity data was obtained by frontal affinity chromatography and glycoconjugate microarray.

³Abbreviations: JOM (J-OIL MILLS, INC), Vector (VECTOR LABORATORIES, INC), Seikagaku (SEIKAGAKU CORPORATION), EY (EY LABORATORIES, INC), AIST (National Institute of Advanced Industrial Science and Technology)

201B7(P48)

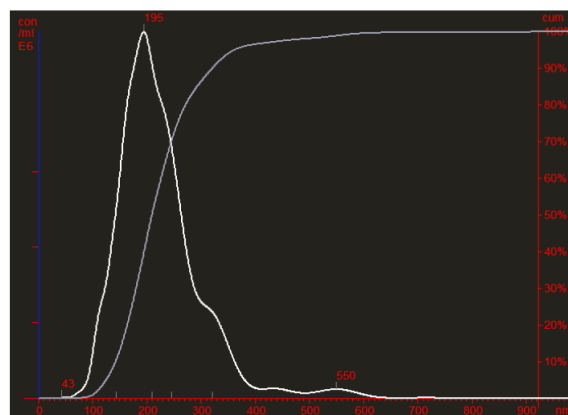


Particle Size / Concentration

Results

Mean: 180 nm
 Mode: 155 nm
 SD: 60 nm
 D10: 113 nm
 D50: 169 nm
 D90: 257 nm
 User Lines: 0 nm, 0 nm
 Concentration: 6.46 E8 particles/ml
 Completed Tracks: 1128

ADSC#2118(P8)

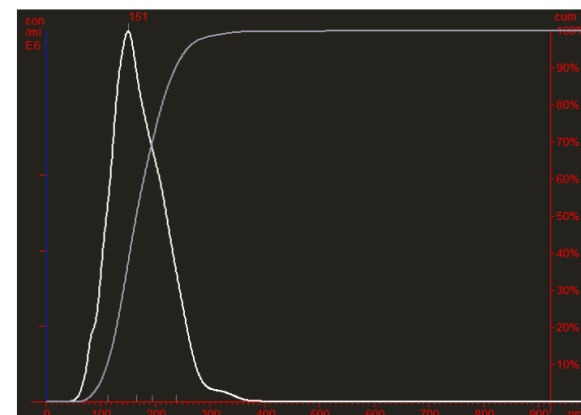


Particle Size / Concentration

Results

Mean: 225 nm
 Mode: 195 nm
 SD: 81 nm
 D10: 143 nm
 D50: 210 nm
 D90: 321 nm
 User Lines: 0 nm, 0 nm
 Concentration: 6.98 E8 particles/ml
 Completed Tracks: 961

hFibs(P15)



Particle Size / Concentration

Results

Mean: 172 nm
 Mode: 151 nm
 SD: 48 nm
 D10: 113 nm
 D50: 166 nm
 D90: 238 nm
 User Lines: 0 nm, 0 nm
 Concentration: 5.60 E8 particles/ml
 Completed Tracks: 926

Fig. S1. Analysis of the particle sizes of EVs analyzed by Nanosight LM10 system.

73 rGal3C	<i>Homo sapiens</i>	<i>E. coli</i>	AIST	LacNAc, polylactosamine
74 rLSLN	<i>Laetiporus sulphureus</i>	<i>E. coli</i>	AIST	LacNAc, polylactosamine
75 rCGL3	<i>Coprinopsis cinerea</i>	<i>E. coli</i>	AIST	LacDiNAc
76 PNA	<i>Arachis hypogaea</i>	Natural	Vector	Galβ1-3GalNAc (T)
77 ACA	<i>Amaranthus caudatus</i>	Natural	Vector	Galβ1-3GalNAc (T)
78 HEA	<i>Hericium erinaceum</i>	Natural	JOM	Galβ1-3GalNAc (T)
79 ABA	<i>Agarics bisporus</i>	Natural	Vector	Galβ1-3GalNAc (T), GlcNAc
80 Jacalin	<i>Artocarpus integrifoli.</i>	Natural	Seikagaku	Galβ1-3GalNAc (T), GalNAca (Tn)
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92 rDiscoidin I	<i>Dictyostelium Discodeu</i>	<i>E. coli</i>	AIST	Gal
93 DBAIII	<i>Dioscorea batatas</i>	Natural	JOM	Maltose
94 rMalectin	<i>Homo sapiens</i>	<i>E. coli</i>	AIST	Glcα1-2Glc
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¹Abbreviations: Gal (D-galactose), GalNAc (N-acetyl-galactosamine), GlcNAc (N-acetyl-glucosamine), Fuc (L-fucose), Glc (D-glucose), Sia (Sialic acid), LacNAc (N-acetyl-lactosamine).

²Specificity data was obtained by frontal affinity chromatography and glycoconjugate microarray.

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Fig. S2

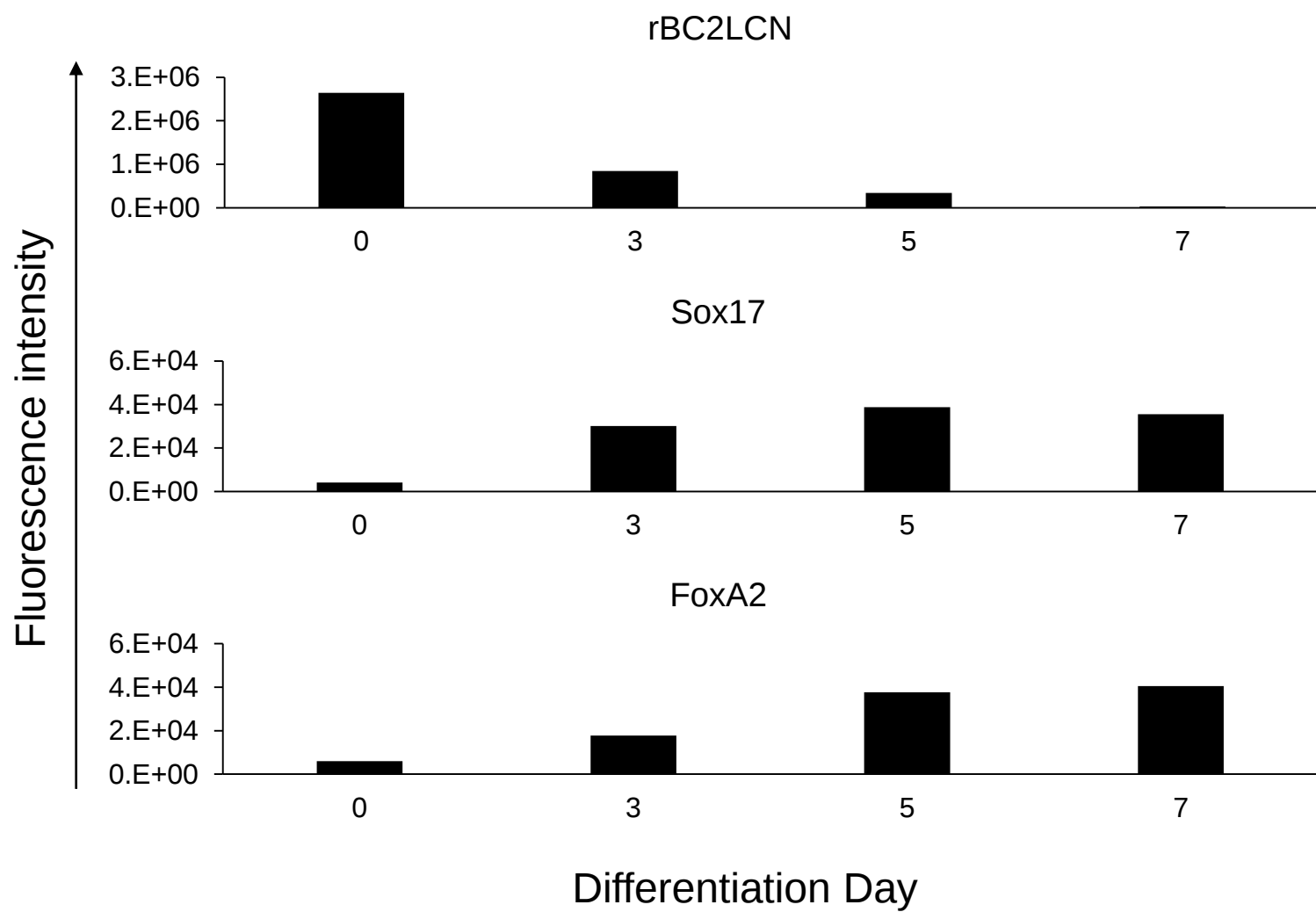


Fig. S2. Expression of rBC2LCN ligands and endoderm markers such as Sox17 and FoxA2 by flow cytometry. Data are shown as the mean fluorescence intensity.