Supporting Information

Legends to supplementary figures

Figure S1. Neutralization of HCV infectivity with SR-BI-specific antibodies. Huh7.5MAVS-gfpNLS cells (1) were seeded onto 24-well dishes one day prior to infection. Cells were preincubated either with an SR-BI-specific antibody or the corresponding isotype-matched control at a 1:20 dilution for 1 h at 37° C. Subsequently cells were infected with constant amounts of each virus fraction and 24 h later cells were fixed with 2 % PFA and analyzed by fluorescence microscopy. Infection was scored by nuclear translocation of the GFP-MAVS-NLS reporter as a result of proteolytic cleavage by the NS3/4A protease (2). For each datapoint 250 - 500 cells from at least 2 different micrographs were counted. (A) Percentage of HCV-positive cells reflecting infectivity contained in each fraction. (B) SR-BI specific neutralization as determined by normalization to the isotype-matched control. Note that Jc1E2^{Flag} is more sensitive to neutralization with this antibody.

Figure S2. **Molecular lipid species distributions of PC (A), PE (B), SM (C) and CE (D) in HCV particles.** Lipids from highly purified Jc1E2^{Flag} particles were extracted and subjected to quantitative lipid analysis by nano-ESI-MS/MS. aPC, acyl-linked phosphatidylcholines; ePC, ether-linked phosphatidylcholines; PE, phosphatidylethanolamines; SM, sphingomyelins; CE, cholesteryl esters.

Figure S3. Comparison of lipid composition of Huh7.5 cells and purified Jc1E2^{Flag} particles. Lipids from Huh7.5 cells and highly purified Jc1E2^{Flag} particles were extracted and subjected to quantitative lipid analysis by nano-ESI-MS/MS. (A) Detection of acyl-linked phosphatidylcholine (aPC) and sphingomyelin (SM) species in Huh7.5 cells (upper panel) and viral particles (lower panel) (precursor ion scans of m/z 184). (B) Detection of cholesteryl ester (CE) species in Huh7.5 cells (upper panel) and viral particles (lower panel) (precursor ion scans of m/z 369). Major lipid species are annotated giving number of C atoms : number of double bonds in fatty acids. IS, internal standard; Mass spectra of virus preparations (identical to the ones in Fig. 5) are presented for ease of comparison.

Figure S4. Morphological analysis of gradient-purified Jc1 particles by negative staining and transmission electron microscopy. (A) Schematic representation of Jc1 virus production and purification. Huh7.5 cells were transfected with the Jc1 genome or plain PBS (mock). Culture supernatants were concentrated by ultrafiltration and virus particles were sedimented through a 10% Optiprep phase onto an 80 % cushion (for details see materials and methods). Concentrated samples collected at the interphase were layered onto a pre-formed Optiprep step gradient. After ultracentrifugation 500 µl fractions were collected from the top of the gradient and used for analyses. (B) Jc1 containing fractions were used for immunolabeling with a rabbit polyclonal E2-specific antibody (detected with 10 nm gold; panel a-f), with a rabbit apoE-specific monoclonal antibody (detected with 10 nm gold; panel g-i) or with both antibodies (E2 10 nm, apoE 15 nm, panel j-l). Samples were negatively stained with uranyl acetate and examined with a Zeiss EM-10 microscope. (C) Samples derived from mock-transfected cells were processed and analyzed in parallel using exactly the same procedure. Note that structures contained in these preparations did not react with the E2-specific antibody, but with anti-apoE, arguing that many of these structures correspond to lipoprotein particles. (D-F) Statistics were performed by counting structures and immunogold particles bound to each structure on at least 2 different EM grids of ≥ 2 preparations each. (D) Mean diameters of all counted structures as well as of structures that were labeled either with the E2- or apoE-specific antibody or with both antibodies (Double (+)). (E) Relative proportion of structures that were labeled with the J6E2- or the apoE-specific antibody or with both antibodies ((% Double (+)). (F) Labeling background of the E2- or the apoE-specific antibody. Values refer to the number of gold particles per µm^2. Error bars in panels (D-F) indicate the standard deviation (n = 121).

Figure S5. Comparison of negative staining procedures used for structure preservation, contrast and antigenicity of HCV particles. Jc1E2^{Flag} particles were prepared and purified as

specified in Fig. 3 and processed for negative staining. Several combinations of uranyl acetate (UA), methylcellulose (MC) and osmium tetroxide (OsO4) fixation and sample preparation methods were assessed for their ability to preserve structure and antigenicity of $Jc1E2^{Flag}$ particles. Of note, enhanced structure preservation as achieved by OsO4 reduced immunolabeling efficiency. MC increased preservation but reduced the contrast. Scale bars represent 50 nm.

Supplementary Table S1. Antibodies used in this study. For details, see Materials and Methods.

Antibody specificity (name)	Species	Specificity	Neutralization[a]	Capture[b]	Immuno-EM[c]	Source
mouse Fc	rabbit	pc	n.d.	n.d.	1:150	Dako Cytomation, Hamburg, Germany
apoAI (AB740)	goat	pc	1:40	1:50	n.d.	Millipore, Schwalbach, Germany
apoB (178467)	goat	pc	n.d.	1:50	n.d.	Merck (Calbiochem), Darmstadt, Germany
apoB (AB20737)	rabbit	pc	1:40	n.d.	1:5	AbCam, Cambridge, UK
apoB (AB742)	goat	pc	n.d.	1:50	n.d.	Millipore (Chemicon), Schwalbach, Germany
apoCI (AB79867)	rabbit	pc	n.d.	1:50	n.d.	AbCam, Cambridge, UK
apoE	mouse	mc	1:40	n.d.	n.d.	BD Transduction, Heidelberg, Germany
apoE (178479)	goat	pc	1:40 or 2 µg/ml	n.d.	n.d.	Merck (Calbiochem), Darmstadt, Germany
apoE (AB52607)	rabbit	mc	n.d.	n.d.	1:10	AbCam, Cambridge, UK
apoE (AB947)	goat	pc	n.d.	1:50	n.d.	Millipore (Chemicon), Schwalbach, Germany
apoE3 (61086)	mouse	mc	1:40	1:50	n.d.	Progen, Heidelberg, Germany
SR-BI	goat	pc	1:20	n.d.	n.d.	kindly provided by T. Baumert, University of Strasbourg, France, (3)
Core (C830)	rabbit	pc	n.d.	n.d.	1:10	(4)
Dengue-E (3H5-1)	mouse	mc	1:40	1:50	n.d.	ATCC, HB-46, LGC Standards, Wesel, Germany
E2 (1:7)	human	mc	n.d.	1:50	1:10	kindly provided by Mats Persson, Karolinska Institute Stockholm, Sweden, (5)
E2 (AP33)	mouse	mc	1:40	1:50	1:5	kindly provided by A. Patel, Glasgow university, U.K., (6)
E2 (J6E2)	rabbit	pc	1:40 or 10 µg/ml	1:50	1:10	Molecular Virology, University of Heidelberg
Flag (F7425)	rabbit	pc	1:40	n.d.	1:50	Sigma-Aldrich, Munich, Germany
Flag (F3165) M2	mouse	mc	1:40	n.d.	n.d.	Sigma-Aldrich, Munich, Germany
NS3 (2E3)	mouse	mc	n.d.	n.d.	n.d.	generated in co-operation with H. Tang, Florida State University, USA, (7)
NS5A (9E10)	mouse	mc	n.d.	n.d.	1:10	kindly provided by C.M. Rice, Rockefeller university, New York, (8)

[a] antibody dilution used for neutralization assays (Fig. 4)

[b] antibody dilution used to coat protein G beads for virus capture assay (Fig. 1D)

[c] antibody dilution used for immuno electron microscopy (Fig. 6, Fig. S3 and S4)

pc, polyclonal antiserum; mc, monoclonal antibody nd, not determined

Supplementary Table S2. Molecule numbers of lipid species per viral RNA-containing Jc1E2^{Flag} particle.

Lipid molecules per viral RNA (Average ± SEM, n = 2)	
aPC	42,042 ± 559
ePC	6,886 ± 1,452
SM	26,872 ± 3,600
PE	6,399 ± 118
PS	732 ± 311
PI	2,472 ± 829
PG	1,022 ± 400
Chol	51,267 ± 15,665
CE	109,896 ± 14,919
TAG	not determined
Total	247,589 ± 37,231

Lipids were extracted and analyzed for containing lipid species as desribed in Materials and Methods. Values are expressed as mol percentage of a given lipid to total lipids quantified.

Mean values and standard error of the means (SEM) are given.

Supplementary references

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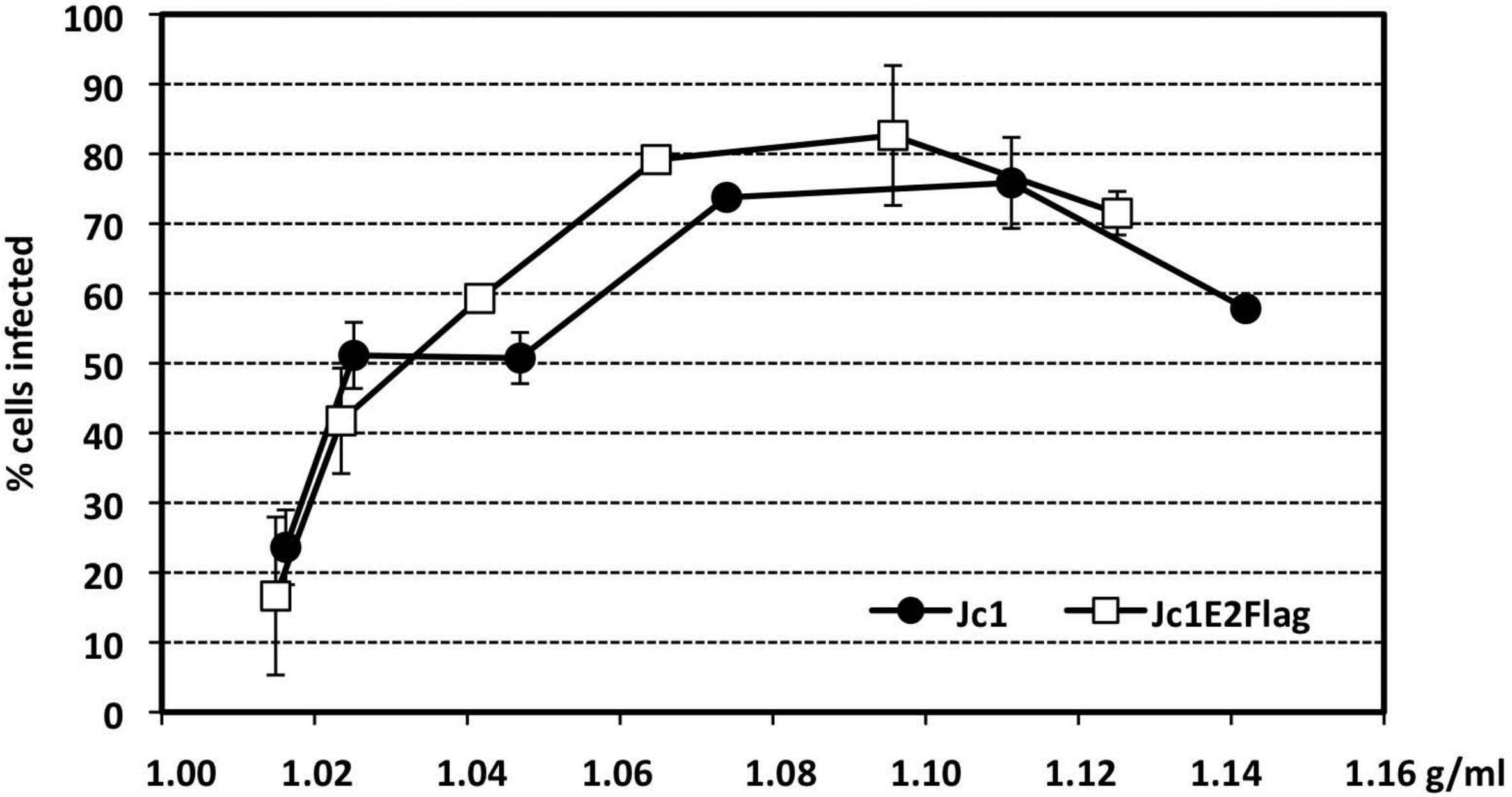
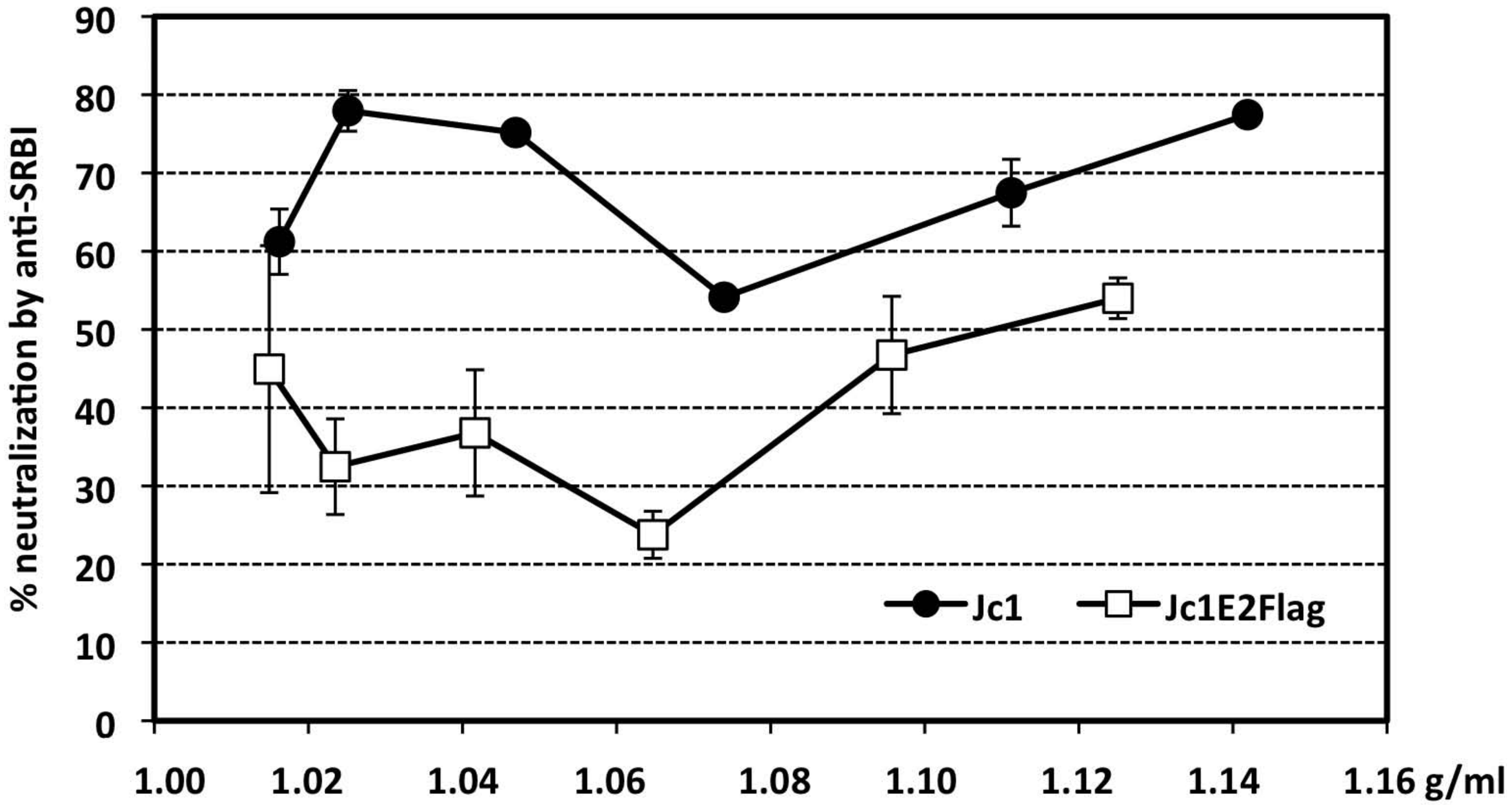
A


100
90
80
70
60
50
40
30
20
10
0
% cells infected
Jc1
Jc1E2Flag
1.00
1.02
1.04
1.06
1.08
1.10
1.12
1.14
1.16 g/ml

B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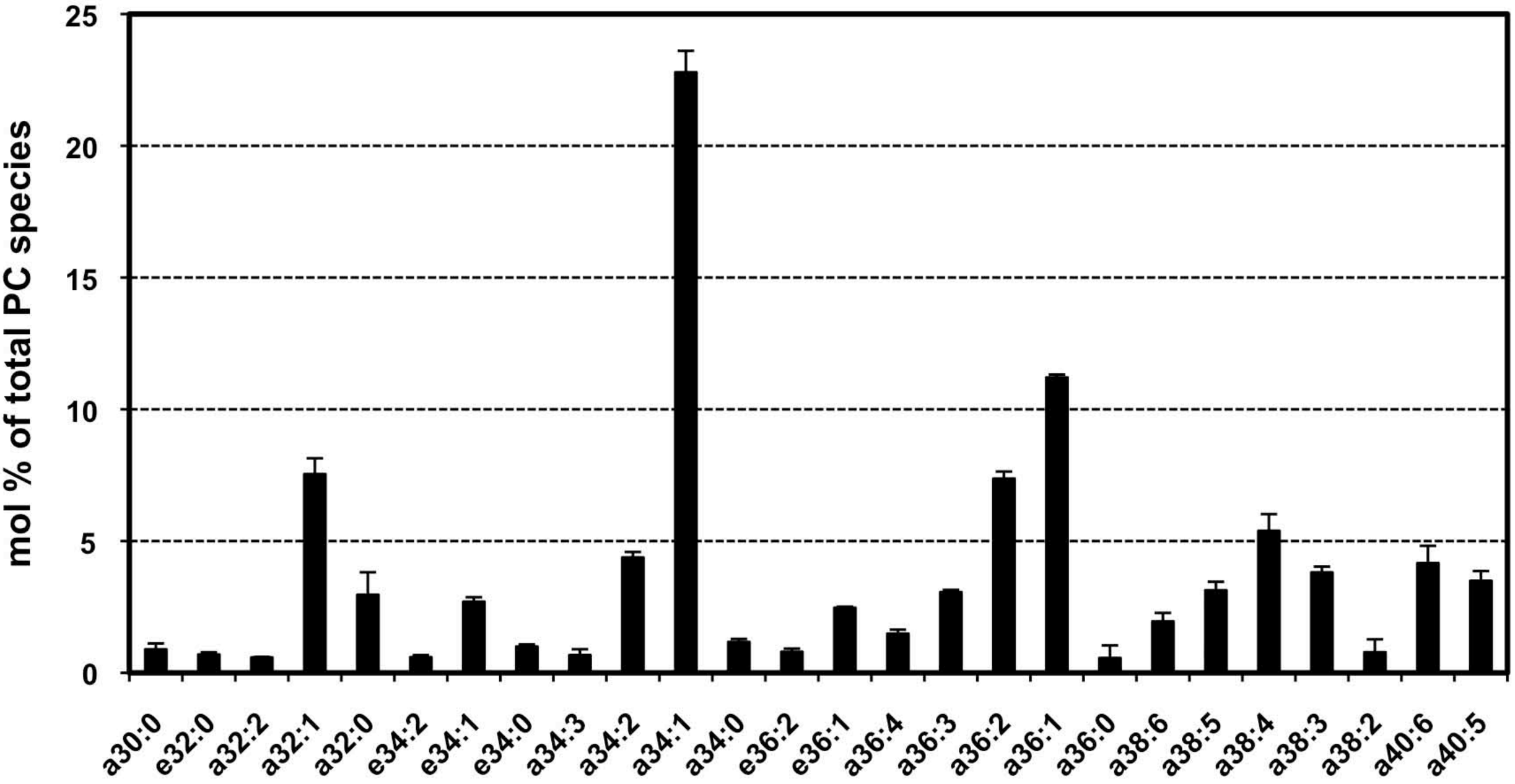
1.08
1.10
1.12
1.14
1.16 g/ml

A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15
10
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e34:1
e34:0
a34:3
a34:2
a34:1
a34:0
e36:2
e36:1
a36:4
a36:3
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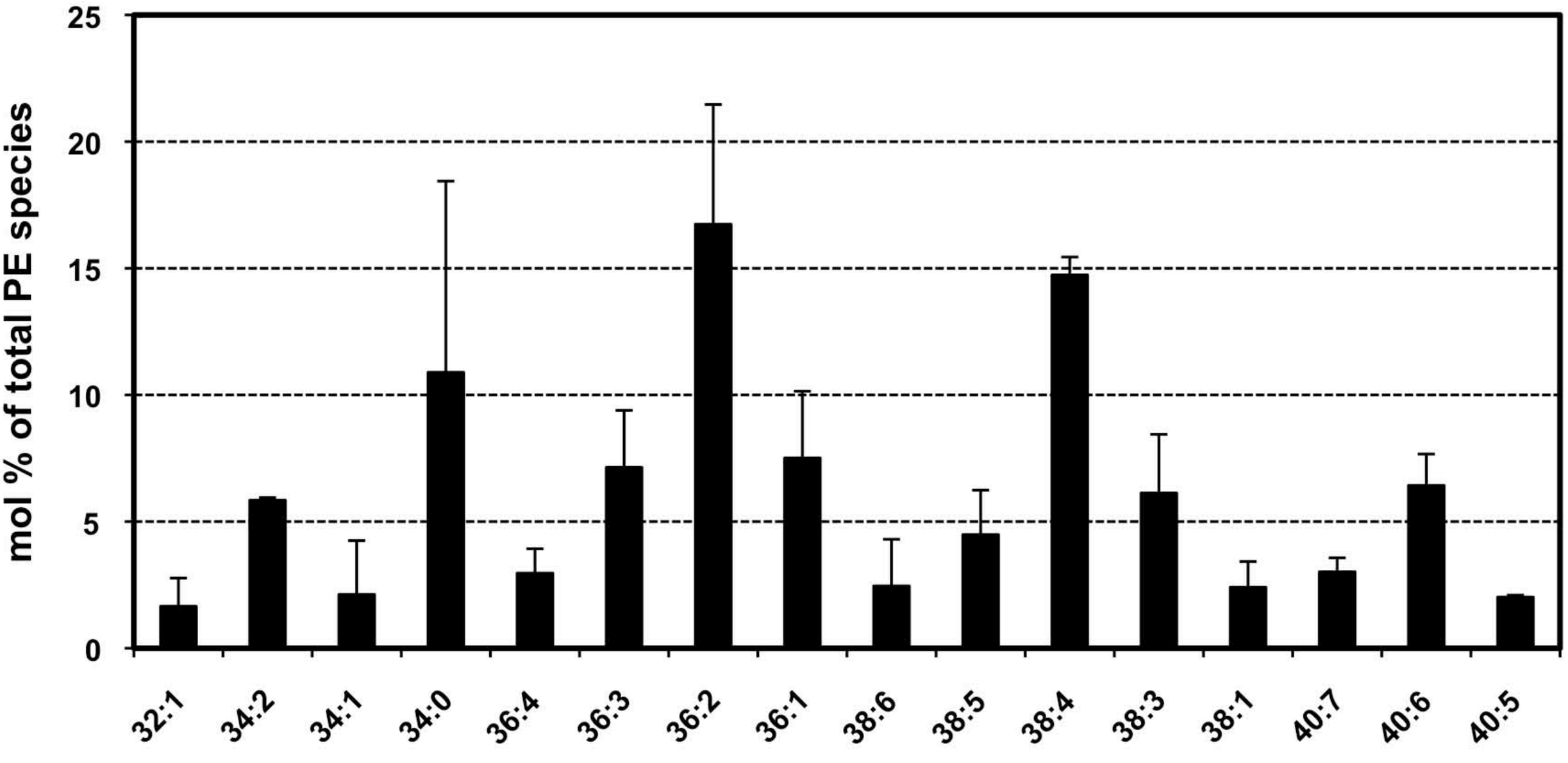
a40:5

B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15
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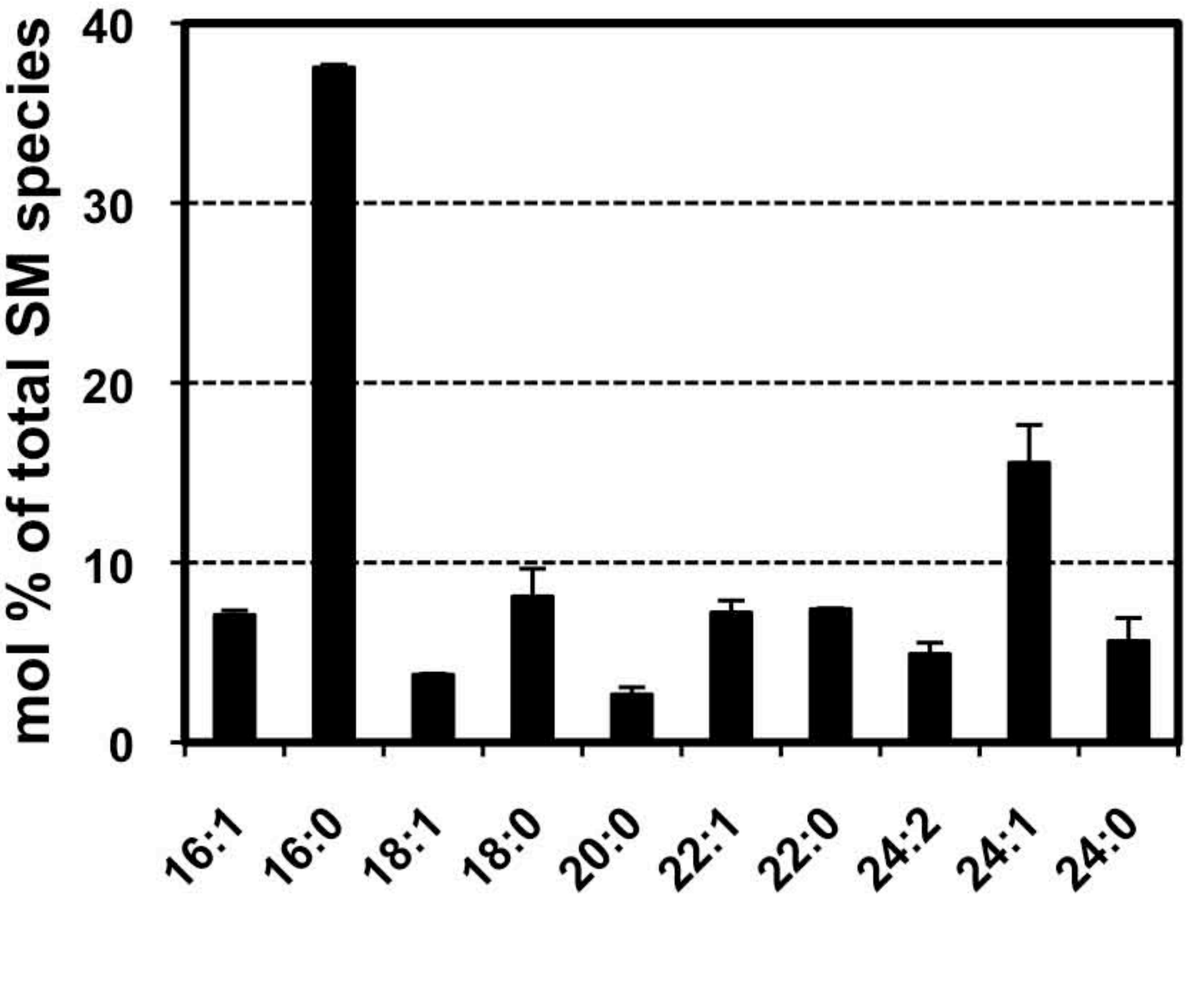
40:7
40:6
40:5

C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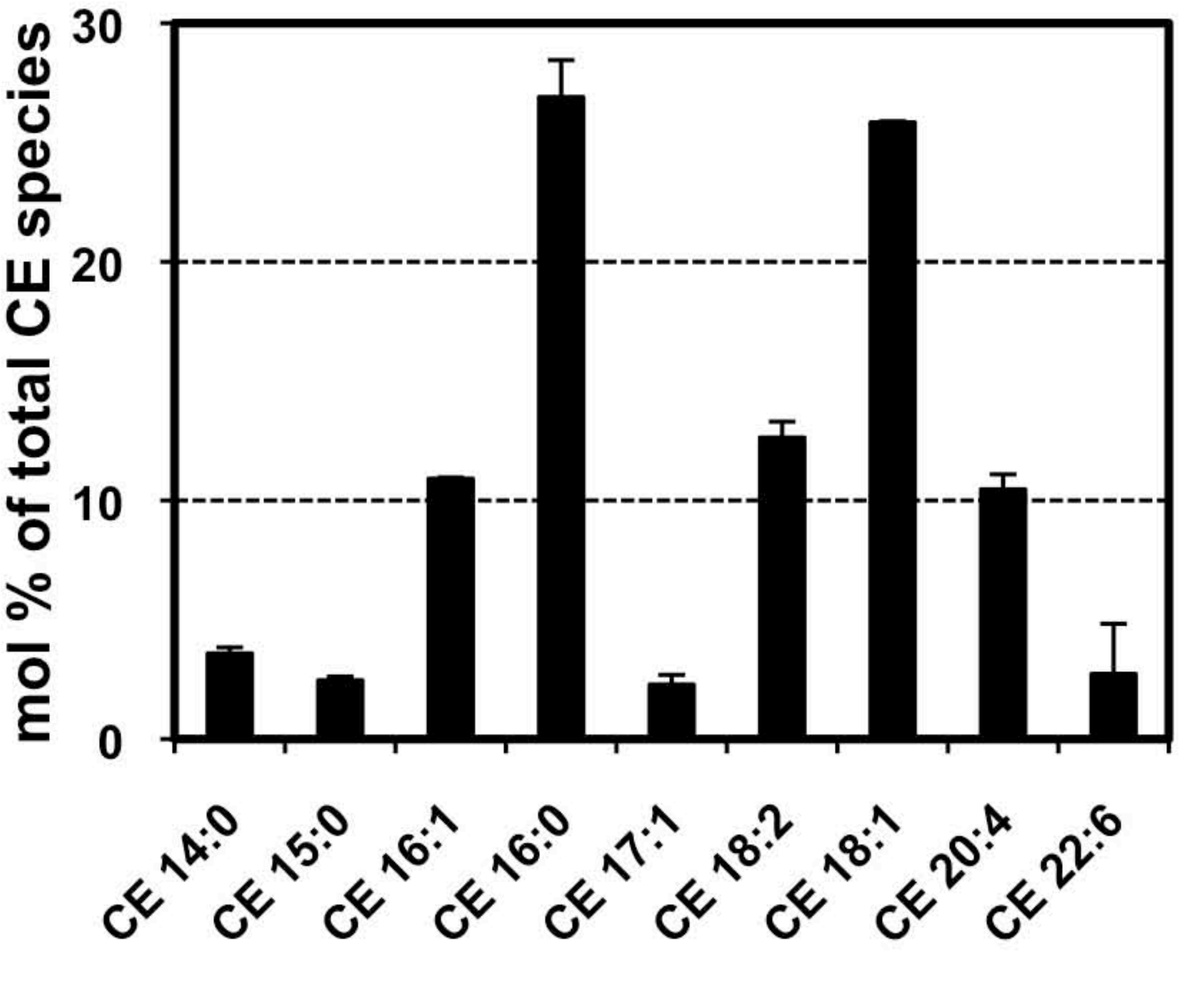
24:1
24:0

D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20
10
0
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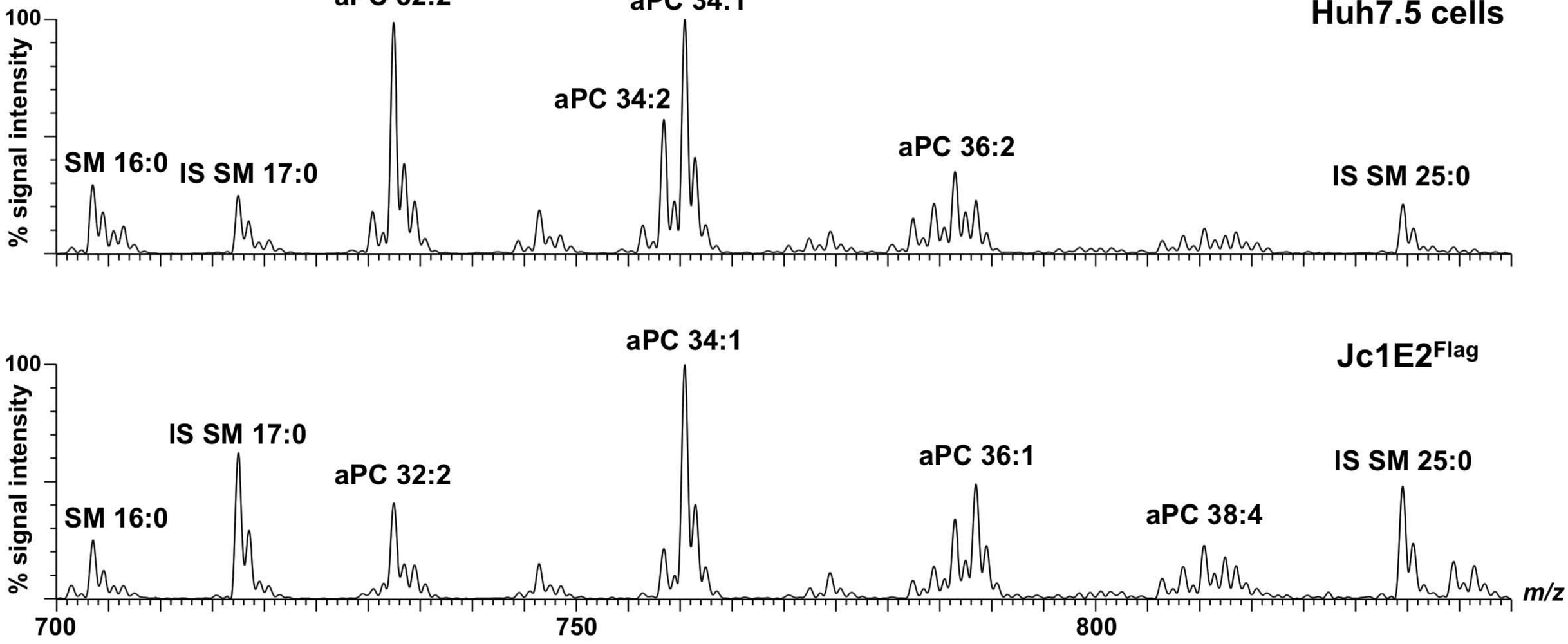
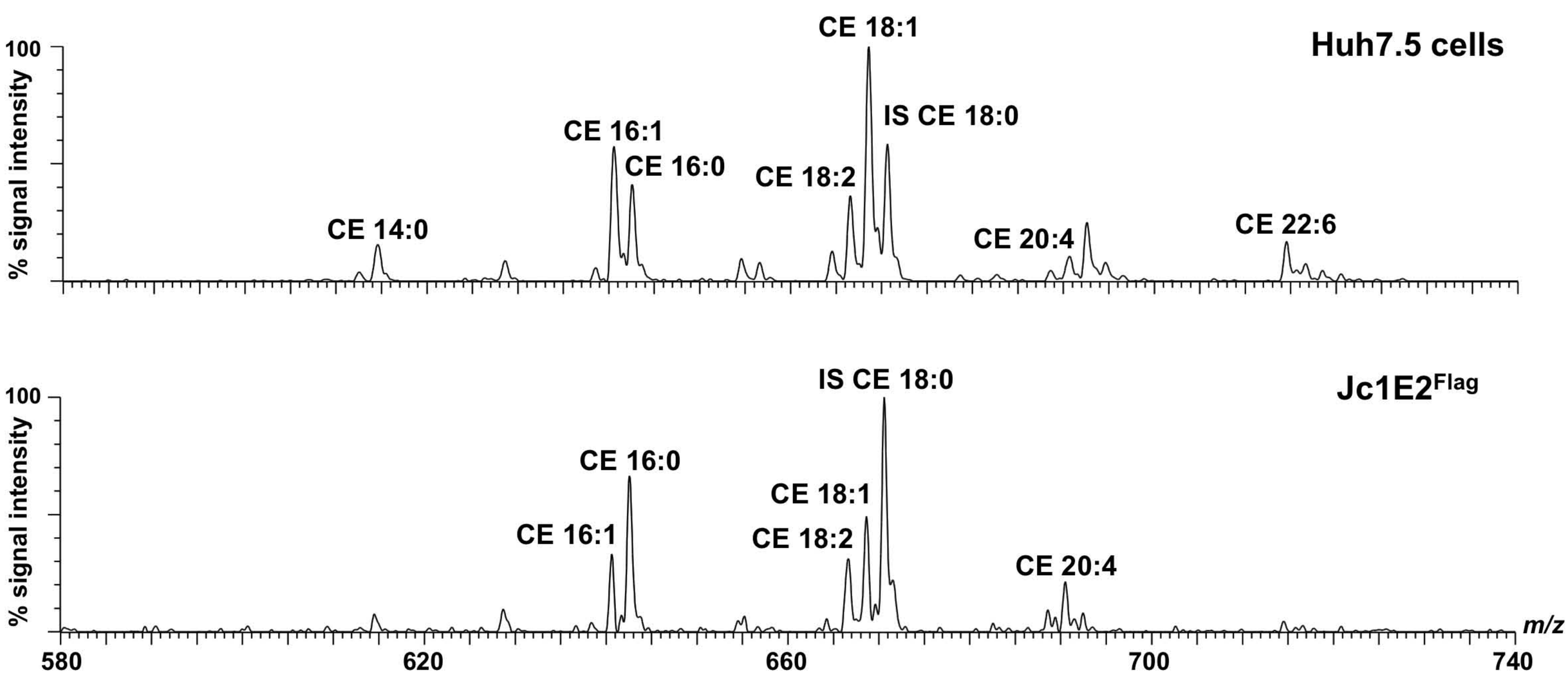
CE 18:2
CE 18:1
CE 20:4
CE 22:6

A


Huh7.5 cells
% signal intensity
100
SM 16:0
IS SM 17:0
aPC 32:2
aPC 34:2
aPC 34:1
aPC 36:2
IS SM 25:0
Jc1E2Flag
% signal intensity
100
SM 16:0
IS SM 17:0
aPC 32:2
aPC 34:1
aPC 36:1
aPC 38:4
IS SM 25:0
m/z
700
750
800

B


Huh7.5 cells
% signal intensity
100
CE 14:0
CE 16:1
CE 16:0
CE 18:2
CE 18:1
IS CE 18:0
CE 20:4
CE 22:6
Jc1E2Flag
% signal intensity
100
CE 16:1
CE 16:0
CE 18:2
CE 18:1
IS CE 18:0
CE 20:4
m/z
580
620
660
700
740

A

Jc1/mock

Huh7.5

ultra-filtration

1st uc

80%

2nd uc

0%

80%

collect fractions

B

Jc1 (1.1 g/ml)

C

Mock (1.1 g/ml)

E2 (▲)

apoE (−)

E2 (10 nm) apoE (15 nm)

(a) (b) (c) (d) (e) (f) (g) (h) (i) (j) (k) (l)

50 nm

200 nm

(a) (b) (c) (d)

50 nm

200 nm

100 nm

D

Mean Diameter (nm)

120 100 80 60 40 20 0

Jc1

Total

E2 (+)

apoE (+)

Double (+)

E

Percentage

20 15 10 5 0

Jc1

E2 (+)

apoE (+)

Double (+)

F

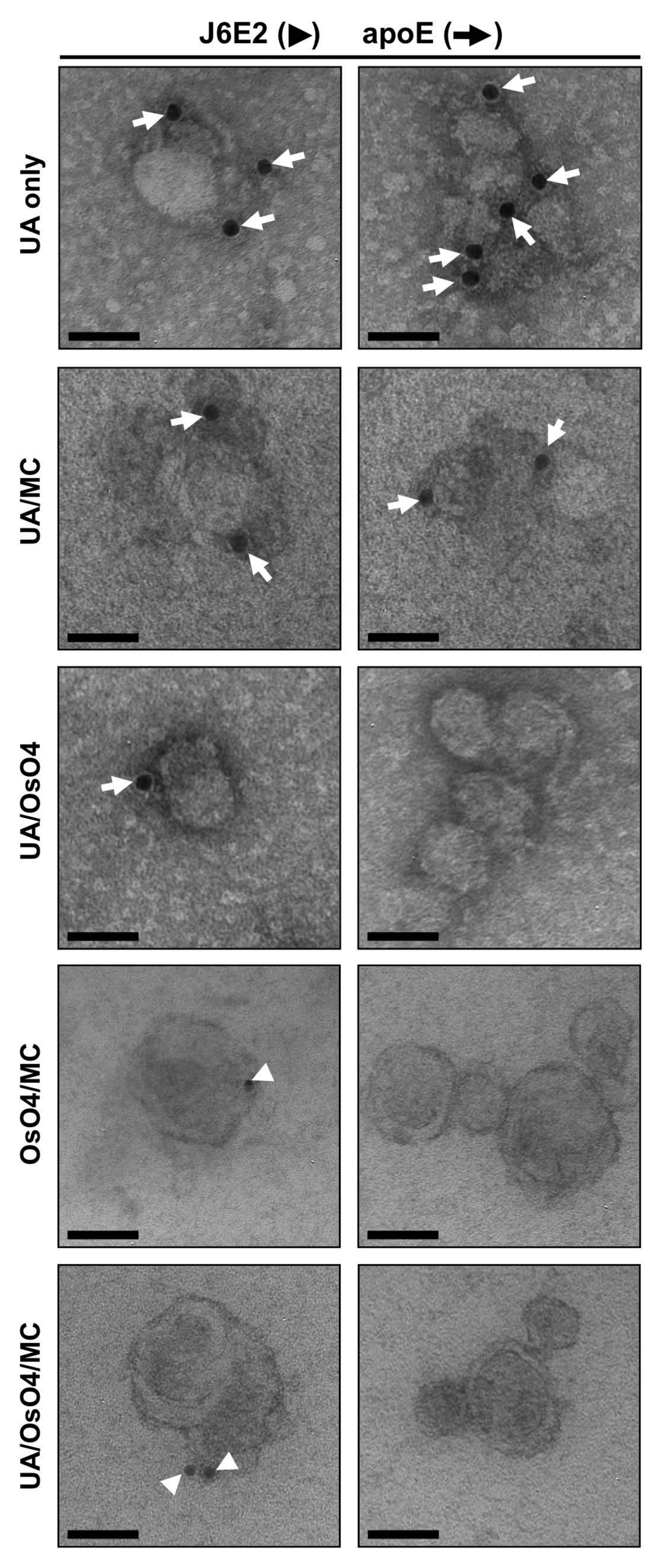
labelling background (gold/μm²)

5 4 3 2 1 0

Jc1

E2

apoE


J6E2 (▶)
apoE (➡)
UA only
UA/MC
UA/OsO4
OsO4/MC
UA/OsO4/MC