Supplemental Research Design and Methods

Protein analysis by MS/MS

ELV proteins were run in PAGE on 10% SDS gels. Coomassie-stained gels were cut into 10 strips and trypsinized. The digested peptides were loaded on a 100 nm ×10 cm capillary column packed in-house with C18 Monitor 100 A-spherical silica beads and eluted by a one h gradient of 10–100% acetonitrile, 0.1% TFA. Mass spectrometric analysis was performed and analyzed in the UAB Proteomic Core Facility using an LTQ XL spectrometer (Thermo Finnigan).

UniProt protein IDs were enriched for GO terms using the Protein Information and Property Explorer (http://pipe.systemsbiology.net/pipe/#summary). Search and calculation of the number of proteins falling into different categories according to their cellular location and biological processes was done.

ELV fatty acid profile

Fatty acids from adipose ELVs are isolated and methylated according to the method of Moser and Moser (1). Briefly, 0.5 mg of adipose ELVs isolated from the medium of 30 min cultures were resuspended in PBS and mixed with 1 ml methanol:dichloromethane (3:1 v/v). After addition of an internal standard (50 nmol of heptadecanoic acid), 200 µl acetyl chloride was added while vortexing, and the sample was incubated at 75^{o}C for 1 h. After cooling, the reaction solution was neutralized with 4 ml of 7% K_2CO_3 and the lipids were extracted into hexane. The hexane fraction was washed with acetonitrile and concentrated under nitrogen. The fatty acid methyl ester (FAME) mixture was then

resuspended in hexane and analyzed by gas chromatography-mass spectroscopy (GC-MS). GC-MS analysis was performed on a Hewlett-Packard Series II 5890 gas chromatograph coupled to an HP-5971 mass spectrometer equipped with a Supelcowax SP-10 capillary column. The oven temperature was maintained at 150^{o}C for 2 min, ramped at 10^{o}C/min to 200^{o}C and held for 4 min, ramped again at 5^{o}C/min to 240^{o}C and held for 3 min, and then finally ramped to 270^{o}C at 10^{o}C/min and maintained for 5 min. The injector and detector were maintained at 260^{o}C and 280^{o}C, respectively. Carrier gas flow rate was maintained at a constant 0.8ml/min. Total ion monitoring was performed, encompassing mass ranges from 50-550 amus. Peak identification was based upon comparison of both retention time and mass spectra of the unknown peak to that of known standards within the GC-MS database library. FAME mass was determined by comparing areas of unknown FAMEs to that of a fixed concentration of the 17:0 internal standard. Response factors were determined for each individual FAME to correct for GC-MS total ion chromatograph discrepancies in quantification. These factors were determined through the use of a GLC reference standard which contained known masses of FAMEs ranging from 14-24 carbons. The response ratio of each FAME was corrected to a fixed amount ratio for each FAME relative to 17:0. The concentration of individual fatty acid was expressed as the percentage of total fatty acids isolated from ELVs.

Antibodies

Mouse monoclonal antibodies anti-CD9, anti-CD63, anti-HSP90, and rat monoclonal antibody anti-Lamp-1, and polyclonal rabbit anti-calnexin antibodies were obtained from Santa Cruz Biotechnology (CA). The mouse monoclonal antibody anti-Akt and the rabbit

polyclonal anti-phosphorylated Akt was purchased from Cell Signaling Technology (Danvers, MA).

Western Blotting

ELVs and cell lysates were separated on SDS-polyacrylamide gels by electrophoresis (Bio-Rad Laboratories, Hercules, CA). Proteins were transferred to polyvinylidene difluoride membranes (Amersham) and blocked with 5% BSA in PBS. Membranes were incubated with a primary antibody overnight at 4°C, followed by washing with TBS-Tween-20. The membrane was subsequently incubated with 1:10,000 fluorescent secondary antibody in blocking solution containing 0.1% Tween-20 and IRDye 800 anti-mouse (Molecular Probes, Rockland Immunochemicals, PA) and Alexa Fluor 700 anti-rabbit (Molecular Probes, OR) probes. Blotted proteins were detected and quantified using an Odyssey infrared imaging system (LI-COR, Lincoln, Nebraska).

Isolation of leukocytes from liver and adipose tissues

For isolation of liver leukocytes, the liver was perfused with RPMI 1640 medium, and leukocytes were obtained by homogenizing the organ with a Potter-Elvehjem homogenizer. The homogenate was layered on a 33% (v/v) Percoll solution (Pharmacia Piscataway, NJ) and centrifuged for 20 min at 1200x*g*. Erythrocytes in the isolated leukocyte suspension were lysed in ACK lysis buffer (0.15 M NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2EDTA). Cells were adjusted to 2×10^7/ml in FACS staining buffer. For isolation of adipose tissue leukocytes, adipose tissue from wild-type (wt) and ob/ob mice was subjected to collagenase digestion (1 mg collagenase/g adipose tissue) in RPMI 1640

media at 37°C for 1 h. The cell digest was strained through a 200-μm mesh filter and was centrifuged at 500x*g* for 5 minute. Erythrocytes were lysed by treatment with ACK buffer. Cells were collected and centrifuged at 1,200xg for 5 min. The cell pellets were resuspended in 8 ml of 40% Percoll, pipetted onto 3 ml of a 70% Percoll solution and centrifuged at 2,000xg for 20 min at 22°C. The cells were washed in PBS containing 2% (w/v) BSA and 0.2% (w/v) NaN_3 before staining with fluorescent-conjugated antibodies. The methods used for preparation of peripheral blood samples and bone marrow cells for FACS analysis have been described previously (2).

Labeling of ELVs and analysis of their target cells in vivo

ELVs were labeled with the PKH67 green fluorescent dye using a commercially available kit (Sigma-Aldrich) and according to a previously described protocol (3). The efficiency of labeling of the ELVs (>92%) with PKH67 was determined by FACS analysis as described previously (3). B6 male mice fed a high fat diet or standard diet for 3 months starting at 2 months of age were injected i.v. with PKH67-labeled ELVs (30 μg/mouse) obtained from adipose tissues of ob/ob mice or with unlabeled ELVs obtained from the same tissues.

Twenty-four h after injection the mice were sacrificed and the peripheral blood and liver, lymph nodes, and spleen tissues were collected. Single-cell suspensions of each tissue were prepared in RPMI 1640 medium and subjected to FACS analysis. The percentages of cells containing ELVs were determined by counting green fluorescent-positive cells.

Labeling macrophages with fluorescent dyes and macrophage trafficking in vivo

PKH67 and PKH26 kits were used for labeling bone marrow derived macrophages (BMDM) according to the manufacturer's instructions (Sigma). BMDMs were generated from primary cultures of femoral bone marrow from 6- to 8-wk-old female wild-type (wt) mice as described previously (2). In brief, BM cells flushed from the femurs of 6- to 8-wk-old mice were cultured at 37°C in a 5% CO_2 atmosphere in the presence of 20 ng/ml recombinant mouse MCSF (PeproTech). Since ELVs are released from tissues continuously, wtELVs or obELVs (10μg/ml) were repeatedly added to the BM cell culture medium on days 0, 3, 5 and 7. At day 8, FACS sorted F4/80 macrophages pretreated with wtELVs or obELVs were labeled with PKH67 and PKH26, respectively. For tracking labeled macrophage trafficking in vivo, macrophages pretreated with obELVs labeled with PKH26 (red dye) or wt-ELVs labeled with PKH67 (green dye) were mixed in equal numbers ($2x10^6$, each) and injected i.v. into B6 mice (5 mice/group) fed a high fat diet over 3 months. The trafficking of injected macrophages was monitored by FACS analysis of labeled macrophages in adipose tissue, liver, spleen and bone marrow.

Bone marrow-derived cell culture and differentiation

Bone marrow cells were isolated from the femurs and tibias of 7-10 week-old mice by flushing the medullary cavity with RPMI medium. After washing, the cells were seeded in tissue culture plates and differentiated into either bone marrow-derived macrophages (BMDMs) or bone marrow-derived dendritic cells (BMDCs). Differentiation into BMDMs or BMDCs was accomplished by suspending the BM cells in RPMI medium

supplemented with 10% low endotoxin FBS and streptomycin/penicillin and containing 20 ng/ml of murine recombinant M-CSF or 20 ng/ml of recombinant GM-CSF, respectively. BMDM and BMDC differentiation was complete 7 days after cell plating; this was confirmed using FACS to detect the expression of CD11b and F4/80, a marker preferentially expressed by mature macrophages, and detection of CD11b and CD11c, a cell surface marker for dendritic cells (data not shown).

Flow cytometric analysis

Prior to flow cytometric analysis, all cells were preincubated with 10 μg/ml anti-CD16/32 antibody (4.2G2, PharMingen, San Diego, CA, USA) at 4°C for 30 min before staining with the following specific antibodies. All antibodies used for FACS analyses, including mouse monoclonal anti-CD11b, anti-ICAM-1, anti-MHCII, and anti-BrdU (E-bioscience); anti-CD204 and anti-F4/80 (AbD Serotec) were purchased. 50 μl of the cell suspension were used for staining with fluorescent antibodies. FITC- or PE-conjugated hamster IgG (PharMingen), rat IgG1 (PharMingen), rat IgG2a (PharMingen and Serotec Ltd., Oxford, UK), and rat IgG2b (PharMingen) served as control antibodies. All antibodies were used at a final concentration of 10 μg/ml. Cells were incubated with the antibodies for 30 min at 4°C and washed with PBS. The samples were fixed with 1% paraformaldehyde/PBS and data acquired with a FACSCalibur (Becton Dickinson) and analyzed with FlowJo or WinMDI software.

ELISA for IL-6, TNF-α, MCSF, IL-10, and anti-ELV exosomes antibody

Cell culture supernatants from a 96-well tissue culture plate and sera harvested from mice were used in an ELISA to detect expression of IL-6, TNF-α, MCSF, and IL-10. Reagents for the

ELISA were purchased from E-Bioscience; the ELISA was done according to the manufacturer's instructions.

Ninety-six well-plates were coated with obELVs (10 μg/ml) in a volume of 100 μl/well of carbonate buffer (pH 9.6) and incubated overnight at 4°C. After 3 washes with PBS, 100 μl/well of blocking solution (PBS containing 0.5% BSA) were added at room temperature for 1 hour. Following 3 washes in PBS, a 2-fold diluted sera collected from mice were added in a final volume of 50 μl and incubated for 1 hour at 37°C. After 3 washes with PBS, the plate was incubated with 100 μl of HRP-conjugated anti-mouse antibody (Pierce) diluted 1:50,000 in blocking solution for 1 hour at room temperature. After the final 3 washes with PBS, the reaction was developed for 15 min, blocked with H_2SO_4 and optical densities were recorded at 450 nm.

Glucose tolerance and insulin tolerance test

Mice were injected intravenously with ELVs (30 μg/mouse) every three days for 21 days. After the last injection, following a 16-h fast, baseline blood glucose levels (mg/dl) were determined using 10 μl of tail vein blood and an Accu-Chek Advantage glucose meter and Accu-Chek test strips (Roche). Glucose (2 mg dextrose/g body weight) in sterile PBS was injected intraperitoneally and blood glucose measured 30, 60, 90, 120, 150 and 180 min after injection. Insulin tolerance was conducted using the same glucometer. Mice were fasted for 4 h prior to starting the procedure. After the baseline glucose values were established, mice were given recombinant human insulin (1 U/kg i.p., Eli Lilly). Clearance of plasma glucose was subsequently monitored at the indicated times post-injection.

Glucose uptake assay

C2C12 myocytes were purchased from ATCC and cultured in α-minimal essential medium supplemented with 10% FBS. Once 80% confluency was reached, the C2C12 myocyte culture medium was replaced with conditioned media (CM) from bone marrow precursors cells that had been pretreated at day 0 of culture with wtELVs, obELVs or thymus exosomes (10 μg/ml) in the presence of GM-CSF (20 ng/ml) and conditioned media was harvested at day 14 of the cultures. C2C12 myocytes were incubated for an additional 24 h with BMDM-CM. The cells were then starved for 3 h before being stimulated with insulin (100 nM) for 20 min at 37°C. The cell lysates (50 μg/lane) were used for western blot analysis of Akt activation using a previously described method (4). To neutralize TNF-α and IL-6 in the conditioned media (CM) before adding the CM to the C2C12 myocyte cultures, the harvested CM was preincubated at 37 C for 1 h with a rat anti-TNF-α (R&D system), a rat anti-IL-6 (R&D System) or with a mixture of both antibodies. The neutralizing $dose_{50}$ for the anti-TNF-α antibody was 0.2 μg/ml in the presence of 0.25 ng/ml of recombinant mouse TNF-α, therefore, 3 μg/ml of anti-TNF-α was used based on the concentration of TNF-α detected in the CM shown in figure 2b., , For rat anti-IL-6 antibody 1.0 μg/ml was used based on the neutralizing $dose_{50}$ provided by R&D system., A normal rat IgG at the same concentration as the anti-TNF-α and anti-IL-6 antibodies was used as a control. To assay for glucose uptake, 24 h after treated with CM with/without preincubation with the antibodies as described above, the C2C12 myocyte were serum starved for 3 h in α-minimal essential medium containing 0.5% BSA and then glucose-starved for 30 min in KRP buffer (130 mM NaCl, 5 mM KCl,

1.3 mM $MgSO_4$, 1.3 mM $CaCl_2$, and 10 mM Na_2HPO_4, pH 7.4). The cells were stimulated with insulin (100 nM) for 20 min at 37°C; tracer glucose (1,2-^{3}H-2-deoxy-D-glucose, 0.5 μCi) was added for 10 min. Four independent experiments were run. After the 10 min incubation, the C2C12 cells were washed and solubilized in 1 N NaOH and 0.1% SDS. Using a TopCount liquid scintillation counter (Packard-Perkin Elmer), [^{3}H]glucose uptake was detected in 0.1 ml cell lysate mixed with scintillation fluid. Non-specific deoxyglucose uptake was measured in the presence of 60 μM cytochalasin B and was subtracted to yield insulin-specific glucose uptake for each data point.

Macrophages treated with a recombinant mouse RBP4

Recombinant mouse RBP4 (R&D, catalog number 3476-LC) was purchased and possible entotoxin contamination was determined using a LAL assay kit (Cambrex Bio Science, Walkersville, MD). All reagents used in this experiment were endotoxin-free based on detection limits of the assay (data not shown). To make control buffer used for diluting RBP4 more comparable with the RBP4, RBP4 protein was dialyzed in a buffer containing 10 mM HEPES buffer, 100 mM NaCl, and the dialysate solution containing no RBP4 was used as a vehicle control for the experiments. The mouse dialysed RBP4 (5 μg/ml) or the dialysate solution at an equal volume as the recombinant mouse RBP4 was used for stimulating BMDMs ($1x10^5$/100 μl in RPMI160 medium). The macrophages were cultured in RPMI 1640 medium supplemented with 10% FBS for 24 h. The cell culture supernatants were harvested and assayed for TNF-α and IL-6 using an ELISA. To determine the in vivo effects of a mouse RBP4 on the induction of IL-6 and TNF-α, wild-type B6 mice or TLR4 KO B6 mice were injected I.V. with a mouse RBP4 (250 μg/mouse in 200 ul of PBS). 6 h after the injection, serum levels of IL-6 and TNF-

α were measured using a standard ELISA. To determine whether the cells pretreated with recombinant mouse RBP4 respond to subsequent ELVs RBP4 stimulation, the BMDMs treated with a mouse RBP4 were washed with PBS 3x, and then cultured in the presence of wtELVs or obELVs (10 μg/ml) for an additional 24 h. TNF-α and IL-6 in the supernatants was quantified using an ELISA.

References:

1. Moser HW, and A.B. Moser: Measurement of saturated very long chain fatty acids in plasma. *A Laboratory Manual* Techniques in Diagnostic Human Biochemical Genetics: , 1991
2. Yu S, Liu C, Su K, Wang J, Liu Y, Zhang L, Li C, Cong Y, Kimberly R, Grizzle WE, Falkson C, Zhang HG: Tumor exosomes inhibit differentiation of bone marrow dendritic cells. *J Immunol* 178:6867-6875, 2007
3. Liu C, Yu S, Zinn K, Wang J, Zhang L, Jia Y, Kappes JC, Barnes S, Kimberly RP, Grizzle WE, Zhang HG: Murine mammary carcinoma exosomes promote tumor growth by suppression of NK cell function. *J Immunol* 176:1375-1385, 2006
4. Zhang HG, Wang Y, Xie JF, Liang X, Liu D, Yang P, Hsu HC, Ray RB, Mountz JD: Regulation of tumor necrosis factor alpha-mediated apoptosis of rheumatoid arthritis synovial fibroblasts by the protein kinase Akt. *Arthritis Rheum* 44:1555-1567, 2001

Supplementary figure legends

Supplementary Figure S1. Characterization of adipose ELVs. (A) Visceral adipose tissues isolated from ob/ob mice and B6 mice fed a high fat diet or regular diet over three months were cultured for 6 h. ELVs were isolated from the supernatants of ex-vivo cultured tissues at 30 min, 3h, and 6 h of culture. The amounts of ELVs released from visceral adipose tissue were quantified using a BCA protein assay kit (Bio-Rad, Hercules, CA). The data are presented as means of three independent experiments with 10 replicas.

**$P < 0.01$. (B) Representative electron micrographs of sucrose gradient purified ELVs (magnification, x 80,000; scale bar = 100 nm). (C) Western blot analysis to determine which proteins are enriched in obELVs. 50 μg of obELVs or whole adipose tissue lysates were run on 10% SDS PAGE and immunoblotted with antibodies as indicated on Figure 1B. Results are representative of 3 independent experiments.

Supplementary Figure S2. (A) 30 μg of PKH67 labeled or unlabeled obELVs were injected via the tail vein into B6 mice that had been fed a high fat diet for 3 months. 24 h after injection, the mice were sacrificed and peripheral blood and total leukocytes isolated from livers were collected for FACS analysis of PKH67 positive cells (A), or (B) within the gated R1 region, the percentages of $PKH67^+CD11b^+F4/80^+$ cells were determined. Data are representative of 5 independent experiments performed with 5 mice for each treatment and the means were calculated for each experiment (**, $p < 0.01$).

Supplementary Figure S3. (A) ObELVs or wtELVs (10 μg/ml) were added to the erythrocyte-depleted BM cells at day 0 after the addition of the GM-CSF (20 ng/ml) to the cultures. After 6 days of culture, the cells were analyzed by FACS for the expression of CD11bF4/80. (B) Sorted $CD11b^+F4/80^+$ cells from the 6-day culture were continuously cultured for an additional 7 days without the addition of GM-CSF, and the proliferation of sorted cells was determined by FACS analysis of $BrdU^+CD11b^+F4/80^+$ cells. (C) The concentration of MCSF, TNF-α, IL-6, and IL-10 in culture supernatants was determined using an ELISA. Data are the mean ± SEM of 3 experiments with 3 replicates in each experiment (A-C). To determine whether thymus ELVs have similar

effects as obELVs on the differentiation and activation of bone marrow precursor cells, identical protocols as described in S3A-C were used for quantifying percent of $CD11b^{+}F4/80^{+}$ cells (D) and cytokine induction (E). Data are the mean ± SEM of 3 experiments with 3 replicates in each experiment (A-E). $^{**}P < 0.01$.

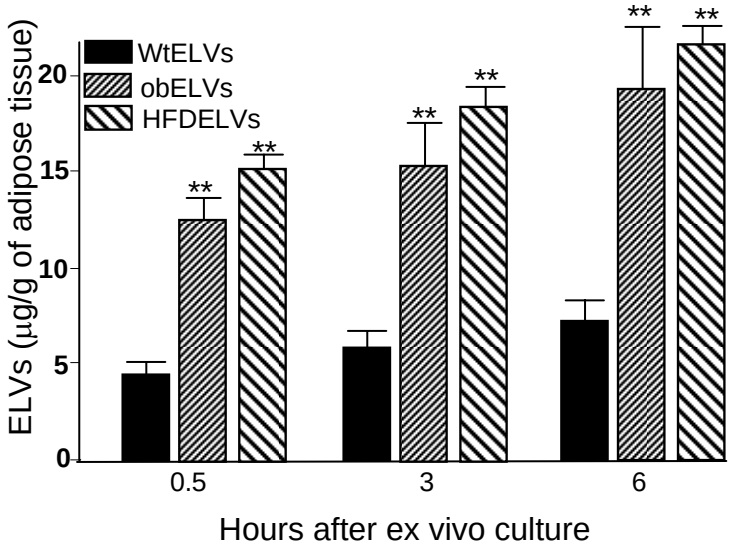
Supplementary Figure S4. To determine the concentration effects of obELVs, bone marrow precursor cells were treated using identical protocols as described in Figure S3 A-C with elevated doses of obELVs from 0 to 100 μg/ml in culture. The production of $CD11b^{+}F4/80^{+}$ cells (A), proliferation (B), and induction of IL-6 (C) were quantified. Data are the mean ± SEM of 3 experiments with 3 replicates of each. $^{**}P < 0.01$.

Supplementary Figure S5. To determine the effects of IL-6 and TNF-α released in the CM on glucose up take and insulin response, TNF-α and IL-6 neutralization assays were carried out by adding anti-TNF-α antibody (3 μg/ml), or anti-IL6 (1 μg/ml) antibody or a mixture of anti-TNF-α and anti-IL6 antibodies or rat IgG served as a control to the CM harvested from bone marrow precursors cells that had been pretreated on day 0 with wtELVs or obELVs (10 μg/ml) in the presence of GM-CSF (20 ng/ml). CM was harvested on day 14 of the cultures.. The treated CM was incubated for 1 h at 37 C before adding to C2C12 cell cultures. The glucose up take and insulin response was determined using identical protocols as described in figure 2B. Data are the mean ± SEM of 3 experiments with 2 replicates of each. $^{*}P < 0.05$.

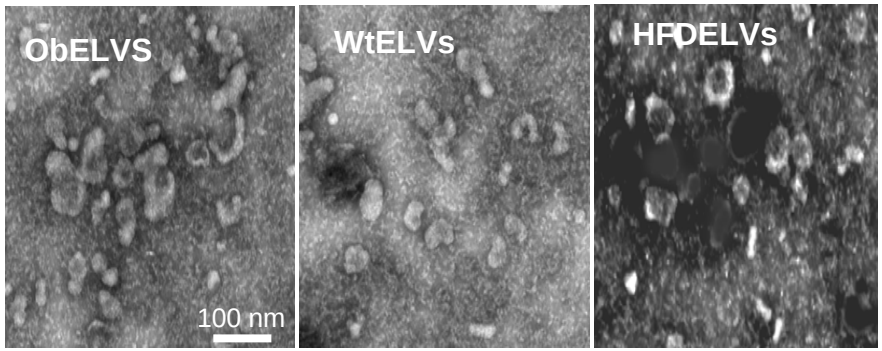
Supplementary Figure S6. ObELV-mediated and TLR4-dependent effect on the expression of CD204 of macrophages. BMDMs ($5x10^5$) were treated with wtELVs or obELVs (10 μg/ml) for 24 h. The expression of CD204 was quantified by FACS analysis. The results represent the mean ± SEM of triplicate cultures, $^{**}P < 0.01$.

Supplementary Figure S7. BMDMs from wild-type B6 mice were treated with increasing concentrations of a mouse RBP4. The quantity of IL-6 and TNF-α produced was determined from the supernatants of 24-h cultures. The results represent the mean ± SEM of triplicate cultures (A), $*p < 0.05$, $**p < 0.01$. 7-week old wild-type B6 mice or TLR4 KO of B6 mice were injected intravenously with a recombinant RBP4 (250 μg/mouse in 200 ul of dialyzed buffer) or dialyzed buffer as a control. 6 h after the injection, peripheral blood was collected from treated mice, and the concentration of IL-6 and TNF-α in the sera was determined using a standard ELISA. Data are given as the means ± SEM obtained for five samples in two independent experiments (B), $**$, $p < 0.001$. BMDMs from wild-type B6 mice or NF-κB p50 KO B6 mice were treated with a mouse RBP4 (5 μg/ml) or PBS as a control for 6 h. The supernatants were harvested and IL-6 and TNF-α quantified by ELISA. Data represent the mean ± SEM of five replicate wells (C), $**p < 0.01$.

A



B



C

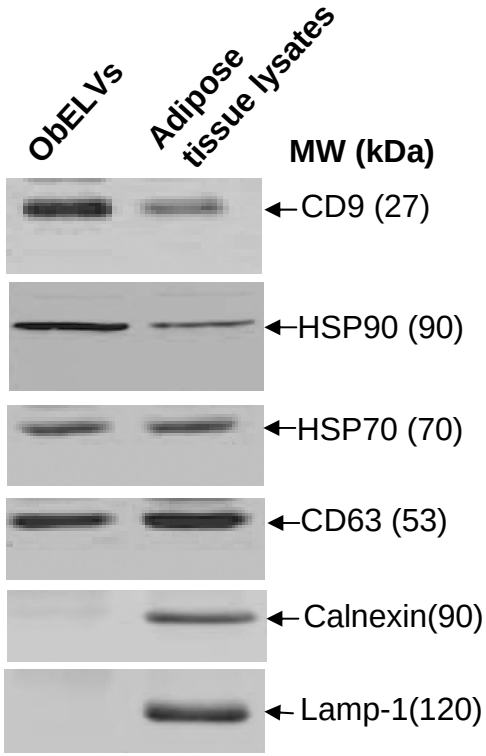


Figure S1

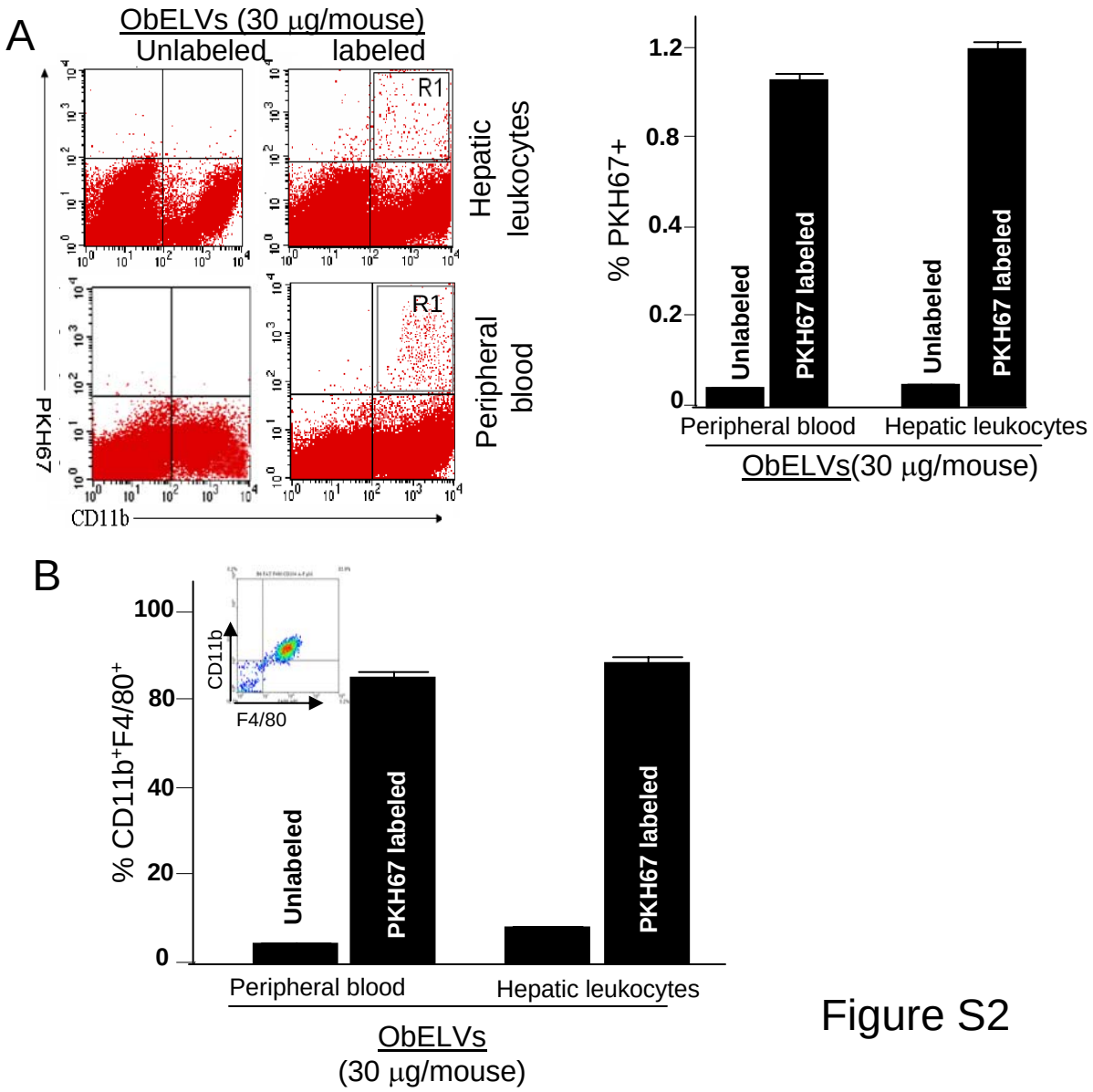

A
ObELVs (30 μg/mouse)
Unlabeled
labeled
R1
Hepatic leukocytes
Peripheral blood
PKH67
CD11b
% PKH67+
1.2
0.8
0.4
0.2
0
Unlabeled
PKH67 labeled
Peripheral blood
Hepatic leukocytes
ObELVs(30 μg/mouse)
B
% CD11b+F4/80+
100
80
40
20
0
CD11b
F4/80
Unlabeled
PKH67 labeled
Peripheral blood
Hepatic leukocytes
ObELVs
(30 μg/mouse)

Figure S2

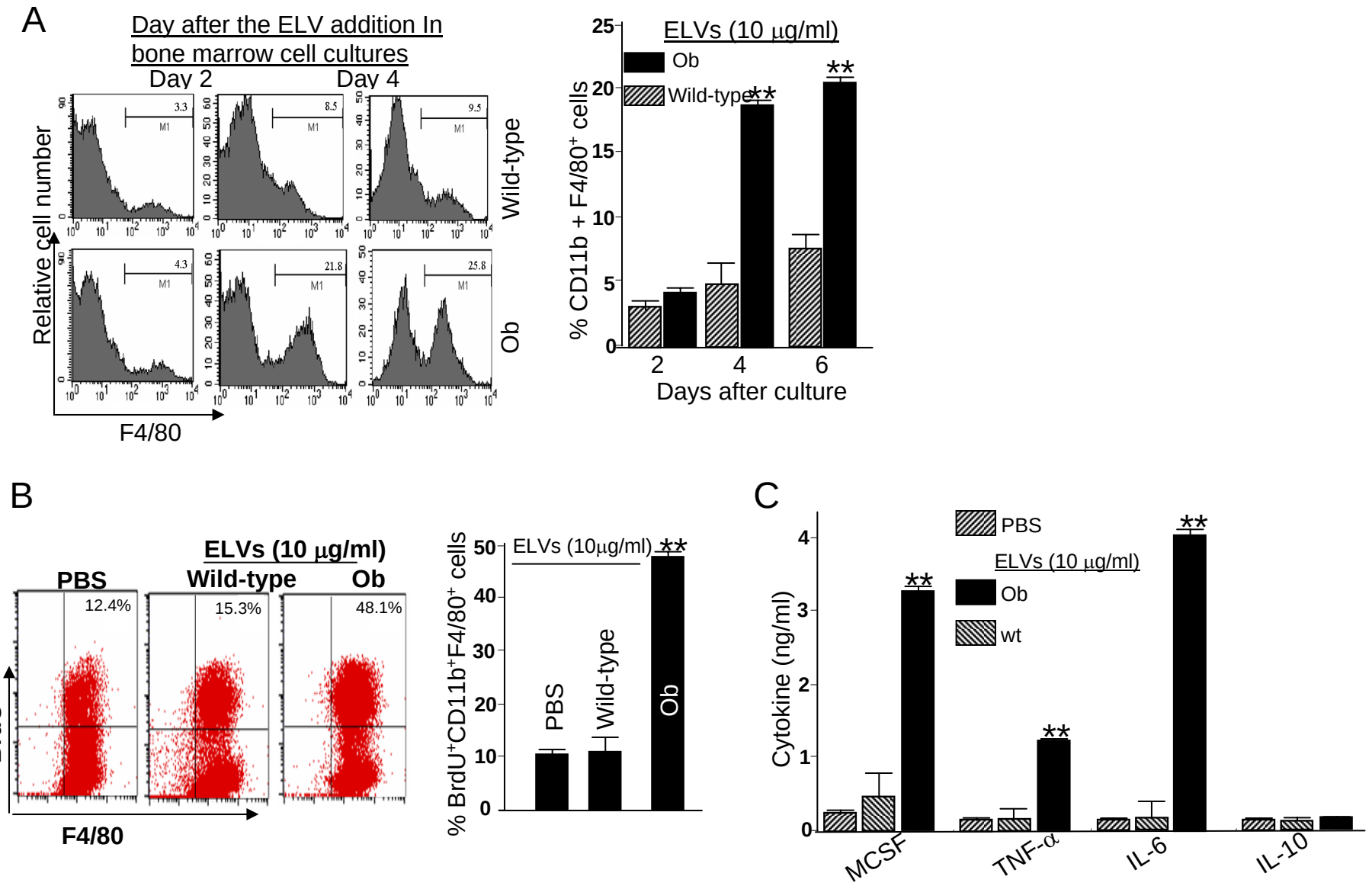

A
Day after the ELV addition In bone marrow cell cultures
Day 2
Day 4
Wild-type
Ob
Relative cell number
F4/80
3.3
8.5
9.5
4.3
21.8
25.8
M1
ELVs (10 μg/ml)
Ob
Wild-type
% CD11b + F4/80+ cells
Days after culture
**
B
ELVs (10 μg/ml)
PBS
Wild-type
Ob
12.4%
15.3%
48.1%
BrdU
F4/80
% BrdU+CD11b+F4/80+ cells
ELVs (10μg/ml)
C
PBS
ELVs (10 μg/ml)
Ob
wt
Cytokine (ng/ml)
MCSF
TNF-α
IL-6
IL-10

Figure S3

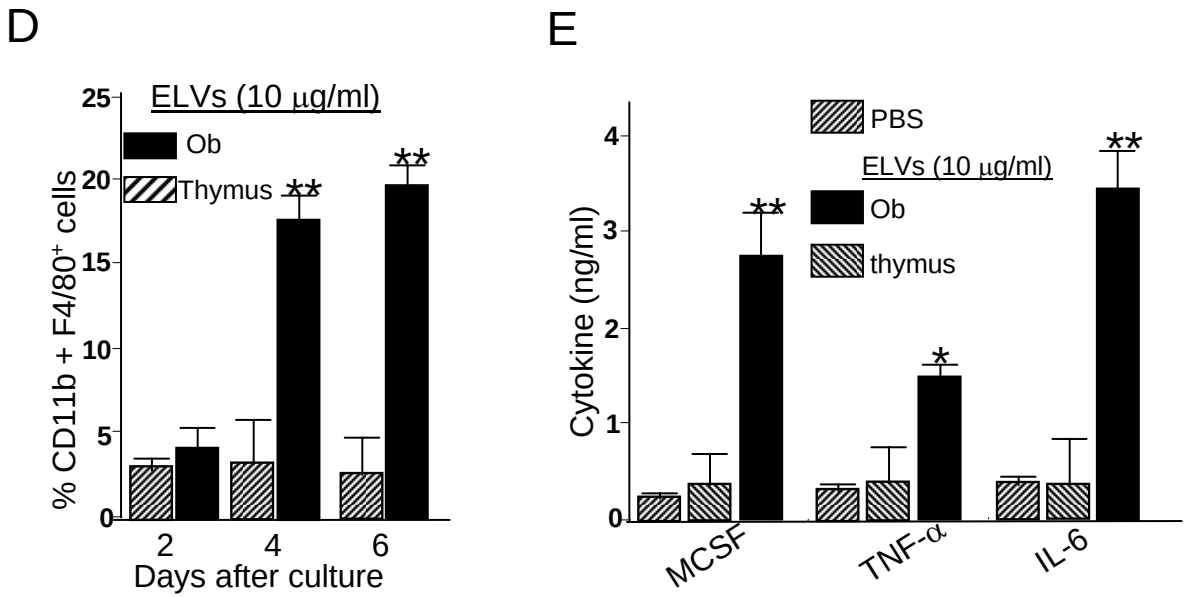

D
ELVs (10 μg/ml)
Ob
Thymus
% CD11b + F4/80+ cells
25
20
15
10
5
0
2
4
6
Days after culture
**
**
E
PBS
ELVs (10 μg/ml)
Ob
thymus
Cytokine (ng/ml)
4
3
2
1
0
**
*
**
MCSF
TNF-α
IL-6

Figure S3

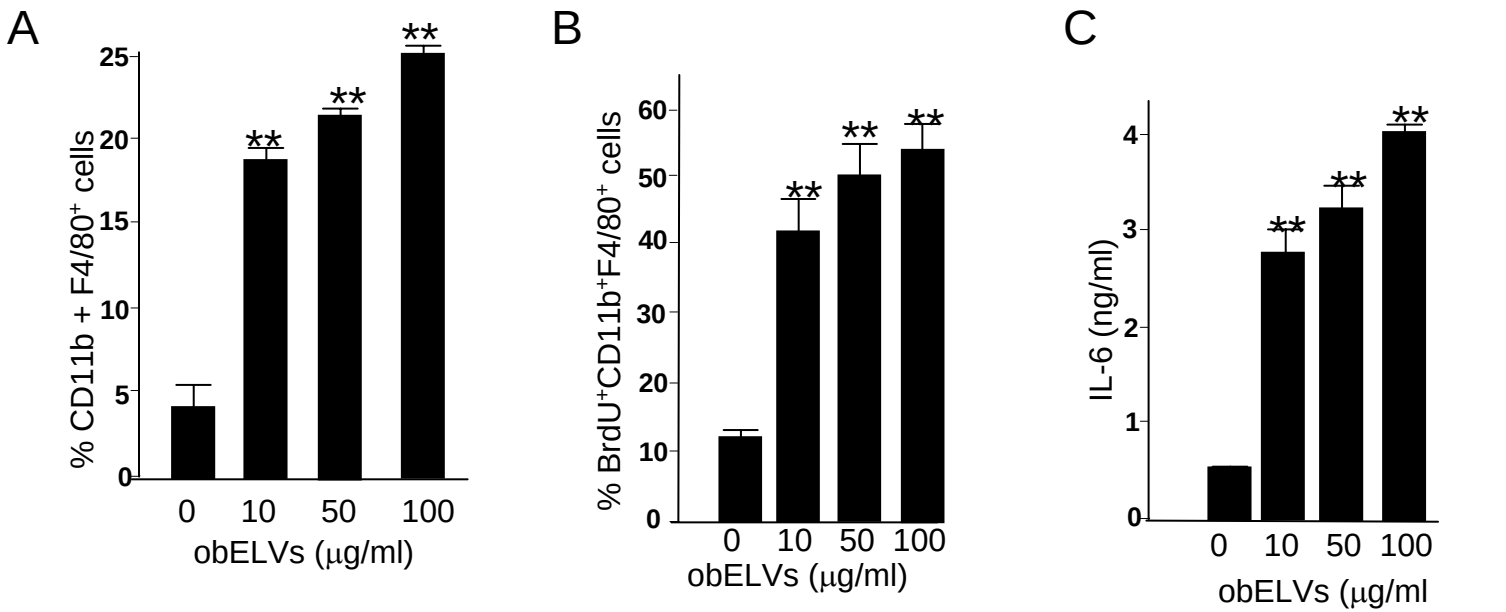

A
% CD11b + F4/80+ cells
25
20
15
10
5
0
**
**
**
0
10
50
100
obELVs (μg/ml)
B
% BrdU+CD11b+F4/80+ cells
60
50
40
30
20
10
0
**
**
**
0
10
50
100
obELVs (μg/ml)
C
IL-6 (ng/ml)
4
3
2
1
0
**
**
**
0
10
50
100
obELVs (μg/ml

Figure S4

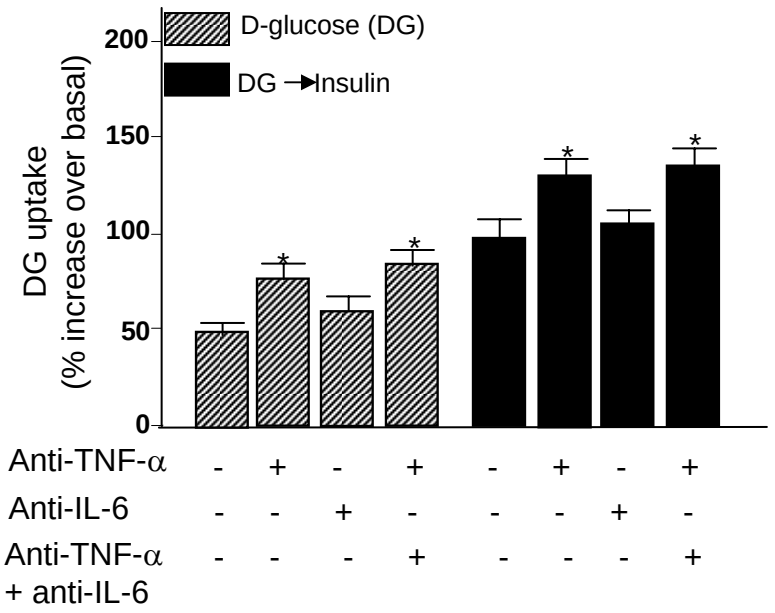
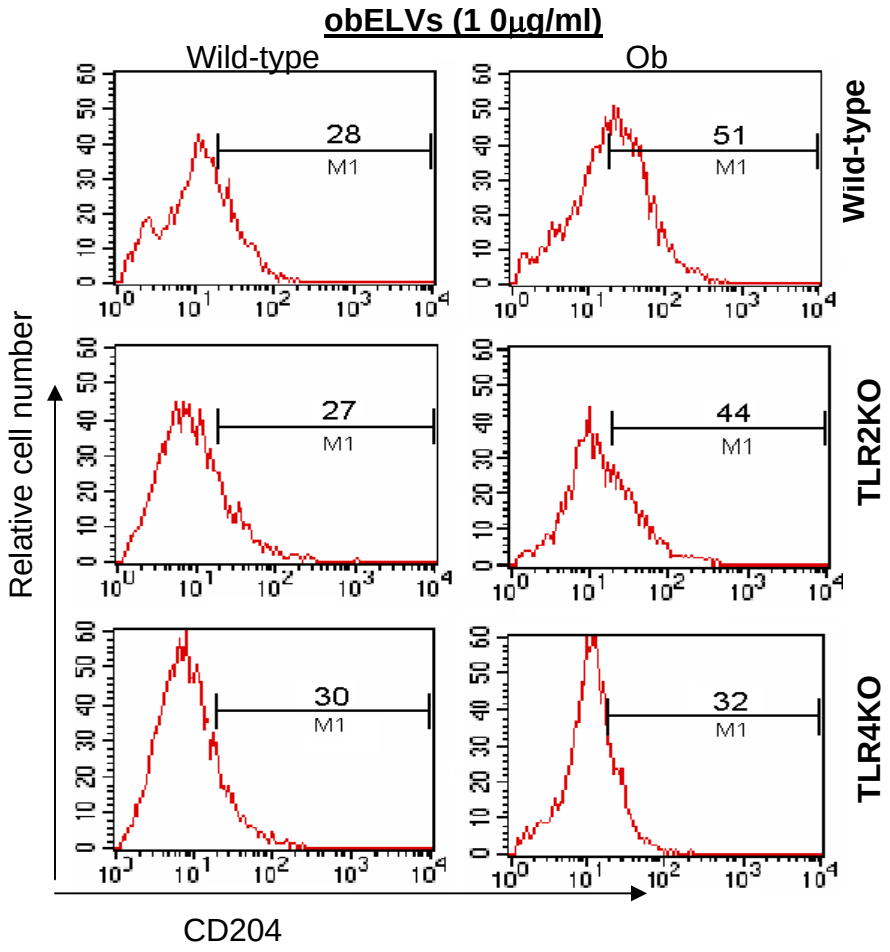

D-glucose (DG)
DG →Insulin
DG uptake
(% increase over basal)
200
150
100
50
0
*
*
*
*
Anti-TNF-α - + - + - + - +
Anti-IL-6 - - + - - - + -
Anti-TNF-α + anti-IL-6 - - - + - - - +

Figure S5


obELVs (1 0μg/ml)
Wild-type
Ob
Wild-type
TLR2KO
TLR4KO
28
M1
51
M1
27
M1
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M1
30
M1
32
M1
Relative cell number
CD204

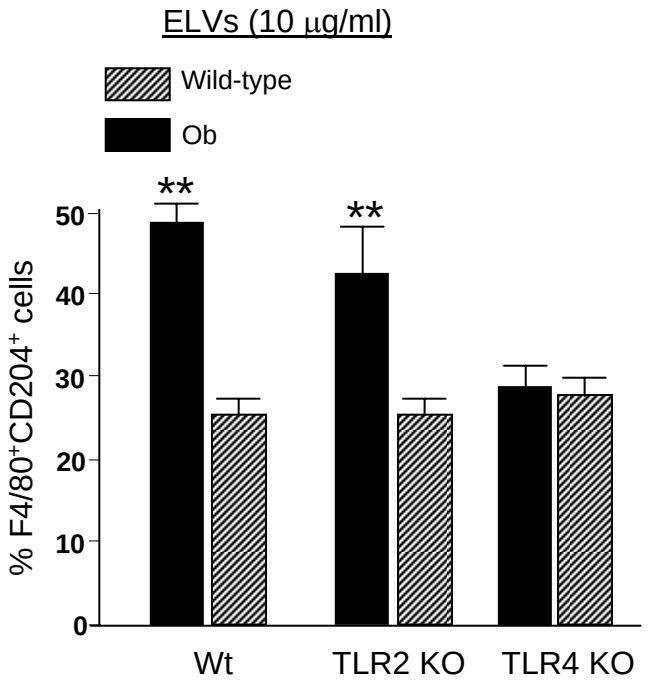
ELVs (10 μg/ml)
Wild-type
Ob
% F4/80+CD204+ cells
**
**
Wt
TLR2 KO
TLR4 KO

Figure S6

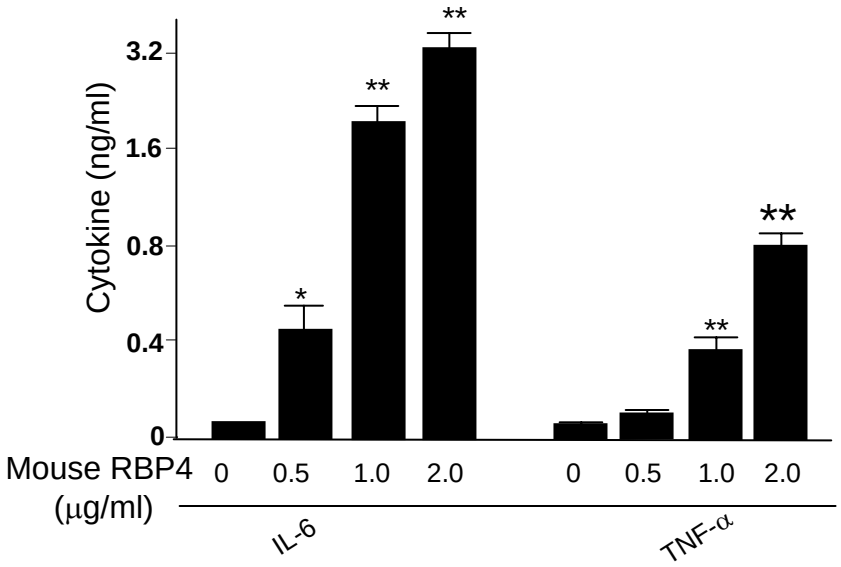
Cytokine (ng/ml)
3.2
1.6
0.8
0.4
0
**
**
*
**
**
Mouse RBP4 (μg/ml)
0
0.5
1.0
2.0
0
0.5
1.0
2.0
IL-6
TNF-α

Figure S7A

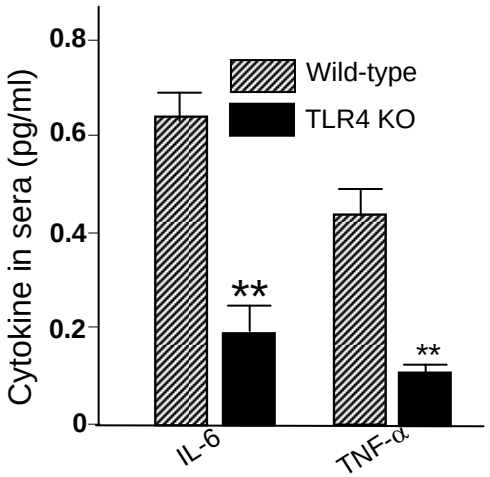

Figure S7B

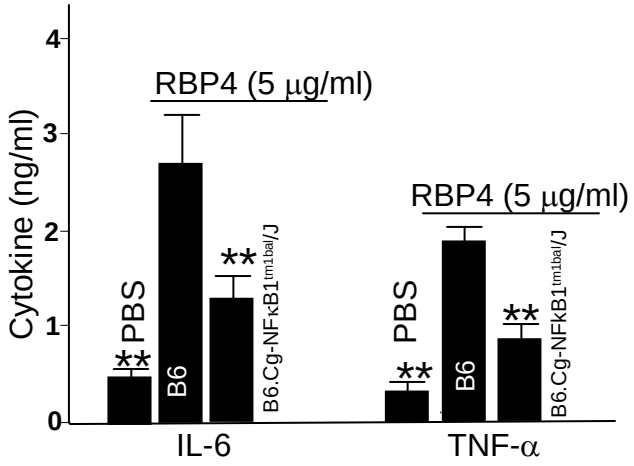
Cytokine (ng/ml)
4
3
2
1
0
RBP4 (5 μg/ml)
RBP4 (5 μg/ml)
PBS
B6
B6.Cg-NFκB1tm1ba/J
PBS
B6
B6.Cg-NFκB1tm1ba/J
**
**
**
**
IL-6
TNF-α

Figure S7C

Table 1. Possible biological roles of proteins identified in adipose exosomes-like vesicles

1. Metabolic process

Accession No	Identified protein	ob	HFD	wt
UniRef100_O35083	1-acylglycerol-3-phosphate O-acyltransferase 1	+	+	+
UniRef100_Q8K3K7	1-acylglycerol-3-phosphate O-acyltransferase 2	+	+	+
UniRef100_Q8C0N2	1-acylglycerol-3-phosphate O-acyltransferase 9	+	+	+
UniRef100_Q9CQ62	2,4-dienoyl CoA reductase 1, mitochondrial	+	+	-
UniRef100_Q3THN2	2-deoxyribose-5-phosphate aldolase homolog (C. elegans)	+	+	+
UniRef100_Q3TDR0	3-ketodihydrosphingosine reductase	+	+	+
UniRef100_Q61753	3-phosphoglycerate dehydrogenase	+	+	-
UniRef100_Q922Z5	abhydrolase domain containing 5	+	+	+
UniRef100_Q3TQP7	acetyl-Coenzyme A acetyltransferase 1	+	+	+
UniRef100_Q3UPU8	acetyl-Coenzyme A acyltransferase 1A; acetyl-Coenzyme A acyltransferase 1B	+	+	-
UniRef100_Q5SWU9	acetyl-Coenzyme A carboxylase alpha	+	+	-
UniRef100_Q99KI0	aconitase 2, mitochondrial	-	+	-
UniRef100_P41216	acyl-CoA synthetase long-chain family member 1	+	+	+
UniRef100_Q3UKS0	acyl-CoA synthetase long-chain family member 5	+	+	+
UniRef100_P51174	acyl-Coenzyme A dehydrogenase, long-chain	+	+	-
UniRef100_Q07417	acyl-Coenzyme A dehydrogenase, short chain	+	+	+
UniRef100_Q3THT3	adenylate kinase 2	-	+	-
UniRef100_Q60994	adiponectin, C1Q and collagen domain containing	+	+	+
UniRef100_P00329	alcohol dehydrogenase 1 (class I)	+	+	+
UniRef100_Q8CIF2	aldehyde dehydrogenase 1 family, member L1	+	+	+
UniRef100_Q571I9	aldehyde dehydrogenase 16 family, member A1	+	+	+
UniRef100_P47738	aldehyde dehydrogenase 2, mitochondrial	+	+	+

UniRef100_Q80VQ0	aldehyde dehydrogenase 3 family, member B1	+	+	+
UniRef100_P47740	aldehyde dehydrogenase family 3, subfamily A2	+	+	+
UniRef100_P05064	aldolase 1, A isoform	+	+	+
UniRef100_A1A4T2	alpha glucosidase 2 alpha neutral subunit	+	+	+
UniRef100_O70423	amine oxidase, copper containing 3	+	+	+
UniRef100_O08739	AMP deaminase 3	+	-	+
UniRef100_P09470	angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	+	+	+
UniRef100_O08855	apolipoprotein A-I	+	+	+
UniRef100_A0AUP0	apolipoprotein A-IV	+	+	+
UniRef100_P08226	apolipoprotein E	+	-	+
UniRef100_Q8BUE4	apoptosis-inducing factor, mitochondrion-associated 2	+	+	+
UniRef100_Q3TED3	ATP citrate lyase	+	+	+
UniRef100_P56480	ATP synthase, H+ transporting mitochondrial F1 complex, beta subunit	+	+	+
UniRef100_Q03265	ATP synthase, H+ transporting, mitochondrial F1 complex,	+	+	+
UniRef100_Q69Z96	ATPase type 13A1	+	+	+
UniRef100_Q8R429	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1	+	+	-
UniRef100_O55143	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	+	+	+
UniRef100_O70228	ATPase, class II, type 9A	+	+	+
UniRef100_P50516	ATPase, H+ transporting, lysosomal V1 subunit A	+	+	+
UniRef100_P62814	ATPase, H+ transporting, lysosomal V1 subunit B2; ATPase, H transporting, lysosomal V1 subunit B2	+	+	+
UniRef100_Q3TXF9	ATPase, Na+/K+ transporting, alpha 1 polypeptide	+	+	+
UniRef100_Q6PIE5	ATPase, Na+/K+ transporting, alpha 2 polypeptide	+	+	+
UniRef100_Q8CIF4	biotinidase	+	+	+
UniRef100_P16015	carbonic anhydrase 3	-	-	+
UniRef100_Q3U3I1	carbonic anhydrase 4	+	+	-

UniRef100_Q8VCT4	carboxylesterase 3	+	+	+
UniRef100_P24270	catalase	+	+	+
UniRef100_P49817	caveolin, caveolae protein 1	+	+	+
UniRef100_P80314	chaperonin subunit 2 (beta)	+	+	+
UniRef100_P80318	chaperonin subunit 3 (gamma)	+	+	+
UniRef100_P80315	chaperonin subunit 4 (delta)	+	+	+
UniRef100_P80316	chaperonin subunit 5 (epsilon)	+	+	+
UniRef100_P80317	chaperonin subunit 6a (zeta)	+	+	+
UniRef100_Q3THH8	chaperonin subunit 7 (eta)	+	+	+
UniRef100_P42932	chaperonin subunit 8 (theta)	+	+	+
UniRef100_Q922Q9	chitinase domain containing 1	+	+	+
UniRef100_Q3TLJ6	coenzyme Q3 homolog, methyltransferase (yeast)	+	+	+
UniRef100_P07310	creatine kinase, muscle	+	+	+
UniRef100_Q9CXR1	dehydrogenase/reductase (SDR family) member 7	+	+	+
UniRef100_P53395	dihydrolipoamide branched chain transacylase E2	+	+	+
UniRef100_Q8BMF4	dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex)	+	+	+
UniRef100_Q9D2G2	dihydrolipoamide S-succinyltransferase (E2 component of 2-oxo-glutarate complex)	+	+	+
UniRef100_Q148B3	ectonucleotide pyrophosphatase/phosphodiesterase 3	+	+	+
UniRef100_Q9CZX0	elongation protein 3 homolog (S. cerevisiae)	+	+	+
UniRef100_Q3TYW7	eukaryotic translation initiation factor 2B, subunit 4 delta	+	+	+
UniRef100_Q6IQY1	fatty acid desaturase 3	+	+	-
UniRef100_P19096	fatty acid synthase	+	+	+
UniRef100_Q8K2I3	flavin containing monooxygenase 2	+	+	+
UniRef100_Q8BZW3	galactosidase, beta 1-like 2	+	+	+
UniRef100_P06745	glucose phosphate isomerase 1	+	+	+

UniRef100_P15105	glutamate-ammonia ligase (glutamine synthetase)	+	+	+
UniRef100_P47856	glutamine fructose-6-phosphate transaminase 1	+	+	+
UniRef100_O09131	glutathione S-transferase omega 1	+	+	+
UniRef100_P13707	glycerol-3-phosphate dehydrogenase 1 (soluble)	+	+	+
UniRef100_Q3TAV1	glycoprotein (transmembrane) nmb	+	+	+
UniRef100_Q9CY27	glycoprotein, synaptic 2	+	+	+
UniRef100_A2A607	GNAS (guanine nucleotide binding protein, alpha stimulating) complex locus	+	+	+
UniRef100_P29416	hexosaminidase A	+	+	+
UniRef100_A2A7A7	hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase)	+	+	-
UniRef100_Q61425	hydroxyacyl-Coenzyme A dehydrogenase	+	+	+
UniRef100_Q5U5Y5	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit	+	+	-
UniRef100_Q99JY0	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit	+	+	-
UniRef100_O70503	hydroxysteroid (17-beta) dehydrogenase 12	+	+	-
UniRef100_P51660	hydroxysteroid (17-beta) dehydrogenase 4	+	+	+
UniRef100_O88736	hydroxysteroid (17-beta) dehydrogenase 7	+	+	-
UniRef100_P50172	hydroxysteroid 11-beta dehydrogenase 1	+	+	-
UniRef100_Q91VA7	isocitrate dehydrogenase 3 (NAD+) beta	+	+	-
UniRef100_P06151	lactate dehydrogenase A	+	+	+
UniRef100_Q8BLN5	lanosterol synthase	+	+	+
UniRef100_Q3TTA8	leucyl/cystinyl aminopeptidase	+	+	+
UniRef100_P54310	lipase, hormone sensitive	+	+	+
UniRef100_P11152	lipoprotein lipase; similar to Lipoprotein lipase precursor (LPL)	+	+	+
UniRef100_Q3UZK0	lysophosphatidylglycerol acyltransferase 1	+	+	+
UniRef100_P08249	malate dehydrogenase 2, NAD (mitochondrial)	+	+	+

UniRef100_P45700	mannosidase 1, alpha	+	+	+
UniRef100_P27046	mannosidase 2, alpha 1	+	+	+
UniRef100_Q3TBQ3	mannosidase, alpha, class 2C, member 1	+	+	+
UniRef100_Q80W94	monoacylglycerol O-acyltransferase 2	+	+	+
UniRef100_Q9CTZ0	muscle glycogen phosphorylase	+	+	+
UniRef100_Q7TPY3	NAD(P) dependent steroid dehydrogenase-like	+	+	+
UniRef100_Q60597	oxoglutarate dehydrogenase (lipoamide)	+	+	+
UniRef100_Q8BJ56	patatin-like phospholipase domain containing 2	+	+	+
UniRef100_Q4V9U2	perilipin	-	-	+
UniRef100_Q3UAG2	phosphogluconate dehydrogenase	+	+	+
UniRef100_O70250	phosphoglycerate mutase 2	+	+	+
UniRef100_Q3UWT7	phospholipase D1	+	+	-
UniRef100_Q3TI27	phosphoribosyl pyrophosphate synthetase 1	+	+	+
UniRef100_Q8C5R8	phosphoribosyl pyrophosphate synthetase 1-like 1	+	+	+
UniRef100_Q8BK29	phosphoribosyl pyrophosphate synthetase-associated protein 1	+	+	+
UniRef100_Q8BK37	phosphoribosyl pyrophosphate synthetase-associated protein 2	+	+	+
UniRef100_A2AI87	phosphorylase kinase alpha 1	+	+	+
UniRef100_Q3UV76	phosphorylase kinase beta	+	+	+
UniRef100_Q3TF18	poly (ADP-ribose) polymerase family, member 1	+	+	+
UniRef100_Q9R0E1	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3	+	+	+
UniRef100_P31324	protein kinase, cAMP dependent regulatory, type II beta	+	+	+
UniRef100_Q05920	pyruvate carboxylase	+	+	+
UniRef100_Q9D051	pyruvate dehydrogenase (lipoamide) beta	+	+	+
UniRef100_Q8BKZ9	pyruvate dehydrogenase complex, component X	+	+	+
UniRef100_P49194	Retinoid binding protein 4	+	+	-

UniRef100_Q9QYF1	retinol dehydrogenase 11	+	+	+
UniRef100_Q149J8	retinol saturase (all trans retinol 13,14 reductase)	-	-	+
UniRef100_Q8BVA5	RIKEN cDNA 1110057K04 gene	+	+	+
UniRef100_Q3TN26	solute carrier family 27 (fatty acid transporter), member 1	+	+	+
UniRef100_P10852	solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	+	+	+
UniRef100_Q3UDJ2	sphingosine phosphate lyase 1	+	+	-
UniRef100_P13011	stearoyl-Coenzyme A desaturase 2	+	+	+
UniRef100_P50427	steroid sulfatase	+	+	+
UniRef100_O09164	superoxide dismutase 3, extracellular	+	+	+
UniRef100_P11983	t-complex protein 1	+	+	+
UniRef100_Q64511	topoisomerase (DNA) II beta	+	+	+
UniRef100_Q02053	ubiquitin-like modifier activating enzyme 1	+	+	+
UniRef100_Q6ZQM8	UDP glucuronosyltransferase 1 family, polypeptide A7C	+	+	+
UniRef100_Q3TXD3	vesicle amine transport protein 1 homolog (T californica)	+	+	+

2. Protein transport

UniRef100_P17426	adaptor protein complex AP-2, alpha 1 subunit	+	+	+
UniRef100_P17427	adaptor protein complex AP-2, alpha 2 subunit	+	+	+
UniRef100_Q6A0C9	adaptor protein complex AP-2, mu1	+	+	+
UniRef100_Q5SWR1	adaptor-related protein complex 2, beta 1 subunit	+	+	+
UniRef100_O54774	adaptor-related protein complex 3, delta 1 subunit	+	+	+
UniRef100_Q99K28	ADP-ribosylation factor GTPase activating protein 2	+	+	+
UniRef100_Q5XJY5	archain 1	+	+	-
UniRef100_A2ALM8	B-cell receptor-associated protein 31	+	+	+
UniRef100_Q68FD5	clathrin, heavy polypeptide (Hc)	+	+	+
UniRef100_O08585	clathrin, light polypeptide (Lca)	+	+	+

UniRef100_Q63ZW9	coatomer protein complex subunit alpha	+	+	+
UniRef100_Q3UE02	coatomer protein complex, subunit beta 1	+	+	+
UniRef100_O55029	coatomer protein complex, subunit beta 2 (beta prime)	+	+	+
UniRef100_O89079	coatomer protein complex, subunit epsilon	+	+	+
UniRef100_Q8BP96	coatomer protein complex, subunit gamma	+	+	+
UniRef100_Q3UGB8	Der1-like domain family, member 1	+	+	-
UniRef100_Q9D4H1	exocyst complex component 2	+	+	+
UniRef100_Q9CR60	golgi transport 1 homolog B (S. cerevisiae)	+	+	+
UniRef100_Q3TIY6	guanosine diphosphate (GDP) dissociation inhibitor 2	+	+	+
UniRef100_P70168	karyopherin (importin) beta 1	+	+	+
UniRef100_Q8BJL4	lectin, mannose-binding 2	+	+	+
UniRef100_Q3U944	lectin, mannose-binding, 1	+	+	+
UniRef100_P24668	mannose-6-phosphate receptor, cation dependent	+	+	+
UniRef100_Q64331	myosin VI	+	+	+
UniRef100_Q9DB05	N-ethylmaleimide sensitive fusion protein attachment protein alpha	+	+	+
UniRef100_Q8C1T5	N-ethylmaleimide sensitive fusion protein attachment protein gamma	+	+	+
UniRef100_Q3UB66	RAB1, member RAS oncogene family	+	+	+
UniRef100_Q3U621	RAB10, member RAS oncogene family	+	+	+
UniRef100_P46638	RAB11B, member RAS oncogene family	+	+	+
UniRef100_Q50HW9	RAB14, member RAS oncogene family	+	+	-
UniRef100_P35293	RAB18, member RAS oncogene family	+	+	-
UniRef100_Q9D1G1	RAB1B, member RAS oncogene family	-	-	+
UniRef100_P35278	RAB5C, member RAS oncogene family	+	+	+
UniRef100_P55258	RAB8A, member RAS oncogene family	+	+	+
UniRef100_P61028	RAB8B, member RAS oncogene family	+	+	+

UniRef100_A2AVJ7	ribosome binding protein 1	+	+	+
UniRef100_Q6ZQ26	sec1 family domain containing 1	+	+	+
UniRef100_Q3UZ06	SEC22 vesicle trafficking protein homolog B (S. cerevisiae)	+	+	+
UniRef100_Q01405	SEC23A (S. cerevisiae)	+	+	+
UniRef100_A2AA71	SEC24 related gene family, member A (S. cerevisiae)	+	+	+
UniRef100_Q3U882	SEC24 related gene family, member B (S. cerevisiae)	+	+	+
UniRef100_Q3UY11	secretory carrier membrane protein 1	+	+	+
UniRef100_O35609	secretory carrier membrane protein 3	+	+	+
UniRef100_O09044	synaptosomal-associated protein 23	+	+	+
UniRef100_Q3TDG9	syntaxin 12	+	+	+
UniRef100_Q0VGN4	syntaxin 16	+	+	-
UniRef100_P70452	syntaxin 4A (placental)	+	+	-
UniRef100_O70439	syntaxin 7	+	+	+
UniRef100_O88983	syntaxin 8	+	+	+
UniRef100_Q5SVW9	transmembrane emp24 protein transport domain containing 4	+	+	+
UniRef100_Q9D1D4	transmembrane emp24-like trafficking protein 10 (yeast)	+	+	-
UniRef100_Q3TJ43	vacuolar protein sorting 35	+	+	+
UniRef100_P97390	vacuolar protein sorting 45 (yeast)	+	+	+

3. Electron carrier

UniRef100_P51174	acyl-Coenzyme A dehydrogenase, long-chain	+	+	-
UniRef100_Q07417	acyl-Coenzyme A dehydrogenase, short chain	+	+	+
UniRef100_Q8BUE4	apoptosis-inducing factor, mitochondrion-associated 2	+	+	+
UniRef100_Q9CY59	cytochrome b5 reductase 3	+	+	+
UniRef100_O08749	dihydrolipoamide dehydrogenase	+	+	+
UniRef100_Q99LC5	electron transferring flavoprotein, alpha polypeptide	+	+	+

UniRef100_P50285	flavin containing monooxygenase 1	+	+	+
UniRef100_Q14CG9	monoamine oxidase B	+	+	+
UniRef100_Q3TIU7	NADH dehydrogenase (ubiquinone) Fe-S protein 1	+	+	+
UniRef100_Q9DCT2	NADH dehydrogenase (ubiquinone) Fe-S protein 3	+	+	+
UniRef100_P37040	P450 (cytochrome) oxidoreductase	+	+	-
UniRef100_Q3TVT6	prenylcysteine oxidase 1	+	+	-
UniRef100_A2ASQ2	prostaglandin E synthase 2	+	+	-
UniRef100_Q8BKZ9	pyruvate dehydrogenase complex, component X	+	+	+
UniRef100_Q149J8	retinol saturase (all trans retinol 13,14 reductase)	-	-	+
UniRef100_Q9D710	similar to thioredoxin-related transmembrane protein 2; predicted gene, EG433144; thioredoxin domain containing 14	+	+	-
UniRef100_Q8K2B3	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	+	+	+
UniRef100_Q3TE45	succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	+	+	+

4. Signal transduction

UniRef100_Q9WTQ5	A kinase (PRKA) anchor protein (gravin) 12	+	+	+
UniRef100_Q60994	adiponectin, C1Q and collagen domain containing	+	+	+
UniRef100_P10107	annexin A1	+	+	+
UniRef100_P49817	caveolin, caveolae protein 1	+	+	+
UniRef100_Q5DY47	dedicator of cyto-kinesis 2	+	+	+
UniRef100_Q8R1Q8	dynein cytoplasmic 1 light intermediate chain 1	+	+	+
UniRef100_Q571D6	engulfment and cell motility 1, ced-12 homolog (C. elegans)	+	+	+
UniRef100_Q8K0E8	fibrinogen, B beta polypeptide	+	+	+
UniRef100_Q3UER8	fibrinogen, gamma polypeptide	+	+	+
UniRef100_A2A607	GNAS (guanine nucleotide binding protein, alpha stimulating) complex locus	+	+	+
UniRef100_Q3TXK7	guanine nucleotide binding protein (G protein), alpha inhibiting 2	+	+	-

UniRef100_Q3U1B1	guanine nucleotide binding protein (G protein), beta 1	+	+	-
UniRef100_P27601	guanine nucleotide binding protein, alpha 13	+	+	+
UniRef100_P18872	guanine nucleotide binding protein, alpha O	+	+	+
UniRef100_P21279	guanine nucleotide binding protein, alpha q polypeptide	+	+	+
UniRef100_Q3TIY6	guanosine diphosphate (GDP) dissociation inhibitor 2	+	+	-
UniRef100_P10833	Harvey rat sarcoma oncogene, subgroup R	+	+	+
UniRef100_Q05CQ4	IQ motif containing GTPase activating protein 1	+	+	+
UniRef100_P08556	neuroblastoma ras oncogene	+	+	+
UniRef100_Q3V2V2	prohibitin 2	+	+	+
UniRef100_P31324	protein kinase, cAMP dependent regulatory, type II beta	+	+	+
UniRef100_Q3UB66	RAB1, member RAS oncogene family	+	+	+
UniRef100_Q3U621	RAB10, member RAS oncogene family	+	+	+
UniRef100_P46638	RAB11B, member RAS oncogene family	+	+	+
UniRef100_Q50HW9	RAB14, member RAS oncogene family	+	+	-
UniRef100_P35293	RAB18, member RAS oncogene family	+	+	-
UniRef100_Q9D1G1	RAB1B, member RAS oncogene family	+	+	+
UniRef100_P35278	RAB5C, member RAS oncogene family	+	+	+
UniRef100_P55258	RAB8A, member RAS oncogene family	+	+	-
UniRef100_P61028	RAB8B, member RAS oncogene family	+	+	-
UniRef100_Q3TN61	ras homolog gene family, member A	+	+	+
UniRef100_Q3TLP8	RAS-related C3 botulinum substrate 1	+	+	+
UniRef100_Q05144	RAS-related C3 botulinum substrate 2	+	+	+
UniRef100_Q3U7U8	RAS-related protein-1a	+	+	-
UniRef100_P63321	v-ral simian leukemia viral oncogene homolog A (ras related)	+	+	+
		+	+	+

5. Ion transport

UniRef100_P14824	annexin A6	+	+	+
UniRef100_O54984	arsA (bacterial) arsenite transporter, ATP-binding, homolog 1	+	+	+
UniRef100_P56480	ATP synthase, H+ transporting mitochondrial F1 complex, beta subunit	+	+	+
UniRef100_Q3TJD4	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b, isoform 1	+	+	+
UniRef100_Q03265	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1	+	+	+
UniRef100_Q91VR2	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1	+	+	+
UniRef100_Q69Z96	ATPase type 13A1	+	+	+
UniRef100_Q8R429	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1	+	+	-
UniRef100_O55143	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	+	+	+
UniRef100_Q05CJ5	ATPase, Ca++ transporting, plasma membrane 1	+	+	+
UniRef100_A2A5A0	ATPase, H+ transporting, lysosomal V0 subunit A1	+	+	-
UniRef100_P51863	ATPase, H+ transporting, lysosomal V0 subunit D1	+	+	-
UniRef100_Q80SY3	ATPase, H+ transporting, lysosomal V0 subunit D2	+	+	+
UniRef100_P50516	ATPase, H+ transporting, lysosomal V1 subunit A	+	+	+
UniRef100_P62814	ATPase, H+ transporting, lysosomal V1 subunit B2; ATPase, H transporting, lysosomal V1 subunit B2	+	+	+
UniRef100_Q3TG21	ATPase, H+ transporting, lysosomal V1 subunit C1	+	+	-
UniRef100_Q8BVE3	ATPase, H+ transporting, lysosomal V1 subunit H	+	+	+
UniRef100_Q3TXF9	ATPase, Na+/K+ transporting, alpha 1 polypeptide	+	+	+
UniRef100_Q6PIE5	ATPase, Na+/K+ transporting, alpha 2 polypeptide	+	+	+
UniRef100_P97370	ATPase, Na+/K+ transporting, beta 3 polypeptide	+	+	+
UniRef100_A3KPC9	calcium channel, voltage-dependent, L type, alpha 1S subunit	+	+	+
UniRef100_P49817	caveolin, caveolae protein 1	+	+	+
UniRef100_Q61147	ceruloplasmin	+	+	-
UniRef100_P09528	ferritin heavy chain 1	+	+	+
UniRef100_P29391	ferritin light chain 1	+	+	+

UniRef100_Q6WVG3	potassium channel tetramerisation domain containing 12	+	+	+
UniRef100_Q99JR1	sideroflexin 1	+	+	+
UniRef100_P04919	solute carrier family 4 (anion exchanger), member 1	+	+	+
UniRef100_P70302	stromal interaction molecule 1	+	+	-
UniRef100_Q921I1	transferrin	+	+	-
UniRef100_P50518	VATPase, H+ transporting, lysosomal V1 subunit E1	+	+	+
UniRef100_Q3THL7	voltage-dependent anion channel 1	+	+	+
UniRef100_Q3TTN3	voltage-dependent anion channel 3	+	+	+

6. Cell adhesion

UniRef100_O70423	amine oxidase, copper containing 3	+	+	+
UniRef100_P56480	ATP synthase, H+ transporting mitochondrial F1 complex, beta subunit	+	+	+
UniRef100_Q08857	CD36 antigen	+	+	-
UniRef100_Q61735	CD47 antigen (Rh-related antigen, integrin-associated signal transducer)	+	+	+
UniRef100_P40240	CD9 antigen	+	+	+
UniRef100_Q04857	collagen, type VI, alpha 1	+	+	+
UniRef100_Q02788	collagen, type VI, alpha 2	+	+	+
UniRef100_Q80X19	collagen, type XIV, alpha 1	+	+	+
UniRef100_P70412	CUB and zona pellucida-like domains 1	+	+	+
UniRef100_Q3UMA4	fermitin family homolog 2 (Drosophila)	+	+	+
UniRef100_P11276	fibronectin 1	+	+	-
UniRef100_Q3TAV1	glycoprotein (transmembrane) nmb	+	+	+
UniRef100_P09055	integrin beta 1 (fibronectin receptor beta)	+	+	+
UniRef100_P02469	laminin B1 subunit 1	+	+	+
UniRef100_Q60675	laminin, alpha 2	+	+	+
UniRef100_P97927	laminin, alpha 4	+	+	+

UniRef100_Q61292	laminin, beta 2	+	+	+
UniRef100_P02468	laminin, gamma 1	+	+	+
UniRef100_Q3UNG0	laminin, gamma 1	+	+	+
UniRef100_Q8R2Y2	melanoma cell adhesion molecule	+	+	+
UniRef100_Q3U8S9	milk fat globule-EGF factor 8 protein	+	+	+
UniRef100_Q5XKE0	myosin binding protein C, fast-type	+	+	+
UniRef100_Q3UHT9	myosin, heavy polypeptide 9, non-muscle	+	+	+
UniRef100_P10493	nidogen 1	+	+	+
UniRef100_Q3U4U1	periostin, osteoblast specific factor	+	+	-
UniRef100_Q05793	perlecan (heparan sulfate proteoglycan 2)	+	+	+
UniRef100_A6H585	predicted gene, EG665033	+	+	+
UniRef100_A6H584	predicted gene, EG665033	+	+	+
UniRef100_Q3TN61	ras homolog gene family, member A	+	+	+
UniRef100_Q3TLP8	RAS-related C3 botulinum substrate 1	+	+	+
UniRef100_A6H588	RIKEN cDNA E330026B02 gene	+	+	+
UniRef100_O35114	scavenger receptor class B, member 2	+	+	+
UniRef100_Q3TLV2	transglutaminase 2, C polypeptide	+	+	+

7. Apoptosis

UniRef100_Q8BUE4	apoptosis-inducing factor, mitochondrion-associated 2	+	+	+
UniRef100_O88738	baculoviral IAP repeat-containing 6	+	+	+
UniRef100_A2ALM8	B-cell receptor-associated protein 31	+	+	+
UniRef100_P59017	BCL2-like 13 (apoptosis facilitator)	+	+	+
UniRef100_O35864	COP9 signalosome complex subunit 5	+	+	+
UniRef100_Q571D6	engulfment and cell motility 1, ced-12 homolog (C. elegans)	+	+	+
UniRef100_P10126	eukaryotic translation elongation factor 1 alpha 1	+	+	+

UniRef100_Q3TIN2	glutaminyl-tRNA synthetase	+	+	+
UniRef100_Q8C3K0	lectin, galactose binding, soluble 12	+	+	+
UniRef100_P12813	nuclear receptor subfamily 4, group A, member 1	+	+	+
UniRef100_P63028	predicted gene, OTTMUSG00000016691; similar to tumor protein, translationally-controlled 1; tumor protein, translationally-controlled 1	+	+	+
UniRef100_P12815	programmed cell death 6	+	+	-
UniRef100_P67778	prohibitin	+	+	-
UniRef100_P27773	protein disulfide isomerase associated 3	+	+	+
UniRef100_Q9WTX2	protein kinase, interferon inducible double stranded RNA dependent activator	+	+	+
UniRef100_O35295	purine rich element binding protein B	+	+	+
UniRef100_Q3TN61	ras homolog gene family, member A	+	+	-
UniRef100_Q9ES97	reticulon 3	+	+	-
UniRef100_Q8BH78	reticulon 4	+	+	-
UniRef100_P42669	similar to hCG45299; purine rich element binding protein A	+	+	+
UniRef100_Q3UDJ2	sphingosine phosphate lyase 1	+	+	-
UniRef100_Q3THL7	voltage-dependent anion channel 1	+	+	+

8. Vesicle-mediated transport

UniRef100_P17426	adaptor protein complex AP-2, alpha 1 subunit	+	+	+
UniRef100_P17427	adaptor protein complex AP-2, alpha 2 subunit	+	+	+
UniRef100_Q6A0C9	adaptor protein complex AP-2, mu1	+	+	+
UniRef100_Q5SWR1	adaptor-related protein complex 2, beta 1 subunit	+	+	+
UniRef100_O54774	adaptor-related protein complex 3, delta 1 subunit	+	+	+
UniRef100_Q99K28	ADP-ribosylation factor GTPase activating protein 2	+	+	+
UniRef100_Q5XJY5	archain 1	+	+	+
UniRef100_A2ALM8	B-cell receptor-associated protein 31	+	+	+

UniRef100_Q68FD5	clathrin, heavy polypeptide (Hc)	+	+	+
UniRef100_O08585	clathrin, light polypeptide (Lca)	+	+	+
UniRef100_Q63ZW9	coatomer protein complex subunit alpha	+	+	+
UniRef100_Q3UE02	coatomer protein complex, subunit beta 1	+	+	+
UniRef100_O55029	coatomer protein complex, subunit beta 2 (beta prime)	+	+	+
UniRef100_O89079	coatomer protein complex, subunit epsilon	+	+	+
UniRef100_Q8BP96	coatomer protein complex, subunit gamma	+	+	+
UniRef100_Q9DC16	endoplasmic reticulum-golgi intermediate compartment (ERGIC) 1	+	+	+
UniRef100_P13020	gelsolin	+	+	+
UniRef100_Q9CR60	golgi transport 1 homolog B (S. cerevisiae)	+	+	+
UniRef100_Q3U944	lectin, mannose-binding, 1	+	+	+
UniRef100_Q9DB05	N-ethylmaleimide sensitive fusion protein attachment protein alpha	+	+	+
UniRef100_Q8C1T5	N-ethylmaleimide sensitive fusion protein attachment protein gamma	+	+	+
UniRef100_Q3UB66	RAB1, member RAS oncogene family	+	+	+
UniRef100_Q9ES97	reticulon 3	+	+	-
UniRef100_Q6ZQ26	sec1 family domain containing 1	+	+	+
UniRef100_Q3UZ06	SEC22 vesicle trafficking protein homolog B (S. cerevisiae)	+	+	+
UniRef100_Q01405	SEC23A (S. cerevisiae)	+	+	+
UniRef100_A2AA71	SEC24 related gene family, member A (S. cerevisiae)	+	+	+
UniRef100_Q3U882	SEC24 related gene family, member B (S. cerevisiae)	+	+	+
UniRef100_O09044	synaptosomal-associated protein 23	+	+	+
UniRef100_Q0VGN4	syntaxin 16	+	+	+
UniRef100_O70439	syntaxin 7	+	+	+
UniRef100_Q9ES56	trafficking protein particle complex 4	+	+	+
UniRef100_Q9D1D4	transmembrane emp24-like trafficking protein 10 (yeast)	+	+	+

UniRef100_P97390	vacuolar protein sorting 45 (yeast)	+	+	+

9. Differentiation

UniRef100_O54774	adaptor-related protein complex 3, delta 1 subunit	+	+	+
UniRef100_P97449	alanyl (membrane) aminopeptidase	+	+	+
UniRef100_P49817	caveolin, caveolae protein 1	+	+	+
UniRef100_P11609	CD1d1 antigen	+	+	+
UniRef100_Q7TMB8	cytoplasmic FMR1 interacting protein 1	+	+	+
UniRef100_Q3U878	DAZ associated protein 1	+	+	+
UniRef100_A2A607	GNAS (guanine nucleotide binding protein, alpha stimulating)	+	+	+
UniRef100_P27601	guanine nucleotide binding protein, alpha 13	+	+	+
UniRef100_P21279	guanine nucleotide binding protein, alpha q polypeptide	+	+	+
UniRef100_A0PJ91	heat shock protein 90, alpha (cytosolic), class A member 1	+	+	+
UniRef100_P09055	integrin beta 1 (fibronectin receptor beta)	+	+	+
UniRef100_Q64331	myosin VI	+	+	+
UniRef100_Q3UHT9	myosin, heavy polypeptide 9, non-muscle	+	+	+
UniRef100_Q9DB05	N-ethylmaleimide sensitive fusion protein attachment protein alpha	+	+	+
UniRef100_Q5SSW0	proteasome (prosome, macropain) activator subunit 4	+	+	+
UniRef100_Q4ADG5	PRP19/PSO4 pre-mRNA processing factor 19 homolog (S. cerevisiae)	+	-	+
UniRef100_O35295	purine rich element binding protein B	+	+	+
UniRef100_Q3TN61	ras homolog gene family, member A	+	+	+
UniRef100_O70551	serine/arginine-rich protein specific kinase 1	+	+	+
UniRef100_Q99JR1	sideroflexin 1	+	+	+
UniRef100_P42669	similar to hCG45299; purine rich element binding protein A	+	+	+
UniRef100_Q3UHN1	slit homolog 3 (Drosophila)	-	+	+
UniRef100_Q6PHU5	sortilin 1	+	+	+

UniRef100_Q9ERD7	tubulin, beta 3	+	+	+
UniRef100_P25911	Yamaguchi sarcoma viral (v-yes-1) oncogene homolog	+	+	+

10. Lipid biosynthetic

UniRef100_O35083	1-acylglycerol-3-phosphate O-acyltransferase 1 (lysophosphatidic acid acyltransferase, alpha)	+	+	+
UniRef100_Q8K3K7	1-acylglycerol-3-phosphate O-acyltransferase 2 (lysophosphatidic acid acyltransferase, beta)	+	+	+
UniRef100_Q8C0N2	1-acylglycerol-3-phosphate O-acyltransferase 9	+	+	+
UniRef100_Q5SWU9	acetyl-Coenzyme A carboxylase alpha	+	+	+
UniRef100_Q3TED3	ATP citrate lyase	+	+	+
UniRef100_Q8VDP6	CDP-diacylglycerol--inositol 3-phosphatidyltransferase (phosphatidylinositol synthase)	+	+	+
UniRef100_Q9CY59	cytochrome b5 reductase 3	+	+	+
UniRef100_Q6IQY1	fatty acid desaturase 3	+	+	-
UniRef100_P19096	fatty acid synthase	+	+	+
UniRef100_Q9CY27	glycoprotein, synaptic 2	+	+	+
UniRef100_O70503	hydroxysteroid (17-beta) dehydrogenase 12	+	+	+
UniRef100_O88736	hydroxysteroid (17-beta) dehydrogenase 7	+	+	+
UniRef100_Q8BLN5	lanosterol synthase	+	+	+
UniRef100_Q3UZK0	lysophosphatidylglycerol acyltransferase 1	+	+	+
UniRef100_Q91V01	membrane bound O-acyltransferase domain containing 5	+	+	+
UniRef100_Q80W94	monoacylglycerol O-acyltransferase 2	+	+	+
UniRef100_Q7TPY3	NAD(P) dependent steroid dehydrogenase-like	+	+	+
UniRef100_A2ASQ2	prostaglandin E synthase 2	+	+	-
UniRef100_Q4ADG5	PRP19/PSO4 pre-mRNA processing factor 19 homolog (S. cerevisiae)	+	-	+
UniRef100_Q05920	pyruvate carboxylase	+	+	+
UniRef100_P13011	stearoyl-Coenzyme A desaturase 2	+	+	+

12. Immune response

UniRef100_A0AUP0	apolipoprotein A-IV	+	+	+
UniRef100_A2ALM8	B-cell receptor-associated protein 31	+	+	+
UniRef100_P11609	CD1d1 antigen	+	+	+
UniRef100_Q62479	complement component (3b/4b) receptor 1-like	+	+	+
UniRef100_P98086	complement component 1, q subcomponent, alpha polypeptide	+	+	+
UniRef100_P14106	complement component 1, q subcomponent, beta polypeptide	+	+	+
UniRef100_Q6DI63	complement component 1, q subcomponent, C chain	+	+	+
UniRef100_Q6GTP5	endoplasmic reticulum aminopeptidase 1	+	+	+
UniRef100_P14483	histocompatibility 2, class II antigen A, beta 1	+	+	+
UniRef100_P01872	immunoglobulin heavy chain 6 (heavy chain of IgM)	+	+	+
UniRef100_P01592	immunoglobulin joining chain	+	+	+
UniRef100_A7VMS6	predicted gene, EG667977	+	+	+
UniRef100_P39429	TNF receptor-associated factor 2	+	+	+
UniRef100_Q8BQL7	toll interacting protein	+	+	+

13. Endocytosis

UniRef100_P17426	adaptor protein complex AP-2, alpha 1 subunit	+	+	+
UniRef100_P17427	adaptor protein complex AP-2, alpha 2 subunit	+	+	+
UniRef100_Q9WVC3	caveolin 2	+	+	+
UniRef100_P49817	caveolin, caveolae protein 1	+	+	+
UniRef100_Q8BH64	EH-domain containing 2	+	+	+
UniRef100_P01872	immunoglobulin heavy chain 6 (heavy chain of IgM)	+	+	+
UniRef100_Q91ZX7	low density lipoprotein receptor-related protein 1	+	+	+
UniRef100_Q2HZ94	mannose receptor, C type 1	+	+	+
UniRef100_Q64331	myosin VI	+	+	+

UniRef100_O88531	palmitoyl-protein thioesterase 1	+	+	+
UniRef100_Q3TD51	phosphatidylinositol binding clathrin assembly protein	+	+	+
UniRef100_P35278	RAB5C, member RAS oncogene family	+	+	+
UniRef100_Q3TLP8	RAS-related C3 botulinum substrate 1	+	+	+
UniRef100_Q6PHU5	sortilin 1	+	+	+

14. Insulin

UniRef100_Q07113	insulin-like growth factor 2 receptor	+	+	+
UniRef100_Q3ULI3	proteasome (prosome, macropain) 26S subunit, non-ATPase, 9	+	+	+
UniRef100_P14142	solute carrier family 2 (facilitated glucose transporter), member 4	+	+	+
UniRef100_Q6PHU5	sortilin 1	+	+	+

Notes: “+” Detected in the ELVs, “-” not detected in the ELVs.

Table 2. Identities of exosome proteins found in exosomes-like microvesicles isolated from adipocyte tissue

Accession no.	Identified protein
UniRef 100_Q3TCE7	actin related protein 2/3 complex, subunit 1B
UniRef 100_Q9CVB6	actin related protein 2/3 complex, subunit 2
UniRef 100_Q9JM76	actin related protein 2/3 complex, subunit 3
UniRef 100_Q7TPD9	actin related protein 2/3 complex, subunit 4
UniRef 100_Q61264	actin, alpha 1, skeletal muscle
UniRef 100_Q3UA89	actin, beta, cytoplasmic
UniRef 100_Q9QZ83	actin, gamma, cytoplasmic 1
UniRef 100_O88990	actinin alpha 3
UniRef 100_P14602	heat shock protein 1
UniRef 100_Q8K0U4	heat shock protein 12A
UniRef 100_P20029	heat shock protein 5; heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)
UniRef 100_P38647	heat shock protein 9
UniRef 100_P17879	heat shock protein 70
UniRef 100_A0PJ91	heat shock protein 90, alpha (cytosolic), class A member 1
UniRef 100_P08113	heat shock protein 90, beta (Grp94), member 1
UniRef 100_P11499	heat shock protein 90kDa alpha (cytosolic),
UniRef 100_P09055	integrin beta 1 (fibronectin receptor beta)
UniRef 100_Q99KA2	tubulin, alpha 1B
UniRef 100_P68373	tubulin, alpha 1C
UniRef 100_Q9D9Y2	tubulin, alpha 3B
UniRef 100_Q62363	tubulin, beta 2a; tubulin, beta 2b
UniRef 100_Q9CVR0	tubulin, beta 2c
UniRef 100_Q9ERD7	tubulin, beta 3
UniRef 100_Q3U9U3	tubulin, beta 6
UniRef 100_Q02053	ubiquitin-like modifier activating enzyme 1
UniRef 100_Q921I1	transferrin
UniRef 100_Q3UB66	RAB1, member RAS oncogene family
UniRef 100_Q3U621	RAB10, member RAS oncogene family
UniRef 100_P46638	RAB11B, member RAS oncogene family
UniRef 100_Q50HW9	RAB14, member RAS oncogene family
UniRef 100_P35293	RAB18, member RAS oncogene family
UniRef 100_Q9D1G1	RAB1B, member RAS oncogene family
UniRef 100_P35278	RAB5C, member RAS oncogene family
UniRef 100_P55258	RAB8A, member RAS oncogene family
UniRef 100_P61028	RAB8B, member RAS oncogene family
UniRef 100_Q6S385	plectin 1

UniRef 100_Q3TD51	phosphatidylinositol binding clathrin assembly protein
UniRef 100_P08122	collagen, type IV, alpha 2
UniRef 100_Q04857	collagen, type VI, alpha 1
UniRef 100_Q02788	collagen, type VI, alpha 2
UniRef 100_Q80X19	collagen, type XIV, alpha 1
UniRef 100_Q68FD5	clathrin, heavy polypeptide (Hc)
UniRef 100_O08585	clathrin, light polypeptide (Lca)
UniRef 100_P35762	CD 81 antigen
UniRef 100_P11609	CD1d1 antigen
UniRef 100_Q08857	CD36 antigen
UniRef 100_Q61735	CD47 antigen
UniRef 100_P41731	CD63 antigen
UniRef 100_P40240	CD9 antigen
UniRef 100_Q02013	aquaporin 1
UniRef 100_P10107	annexin A1
UniRef 100_P97384	annexin A11
UniRef 100_P07356	annexin A2
UniRef 100_O35639	annexin A3
UniRef 100_P97429	annexin A4
UniRef 100_P48036	annexin A5
UniRef 100_P14824	annexin A6
UniRef 100_O08855	apolipoprotein A-I
UniRef 100_A0AUP0	apolipoprotein A-IV
UniRef 100_P08226	apolipoprotein E
UniRef 100_P19096	fatty acid synthase

Table 3. Fatty acid composition of exosome like vesicles isolated from adipose tissue

Fatty acid	14:0	15:0	**16:0**	16:1	**18:0**	18:1w9	18:1w7	18:2w6	19:0	18:3w6	18:3w3	20:0	20:1w9	20:1w7	20:2w6	20:3w9	20:3w6	20:3w3	20:4w6	20:5w3	22:0	22:1w9	23:0	22:4w6	22:5w6	22:5w3	24:0	24:1	22:6w3
%	0.15	0.06	**42.81**	0.00	**55.00**	0.00	0.00	0.00	0.28	0.00	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.00	0.27	0.00	0.00	0.00	0.60	0.00	0.00