Figure 1

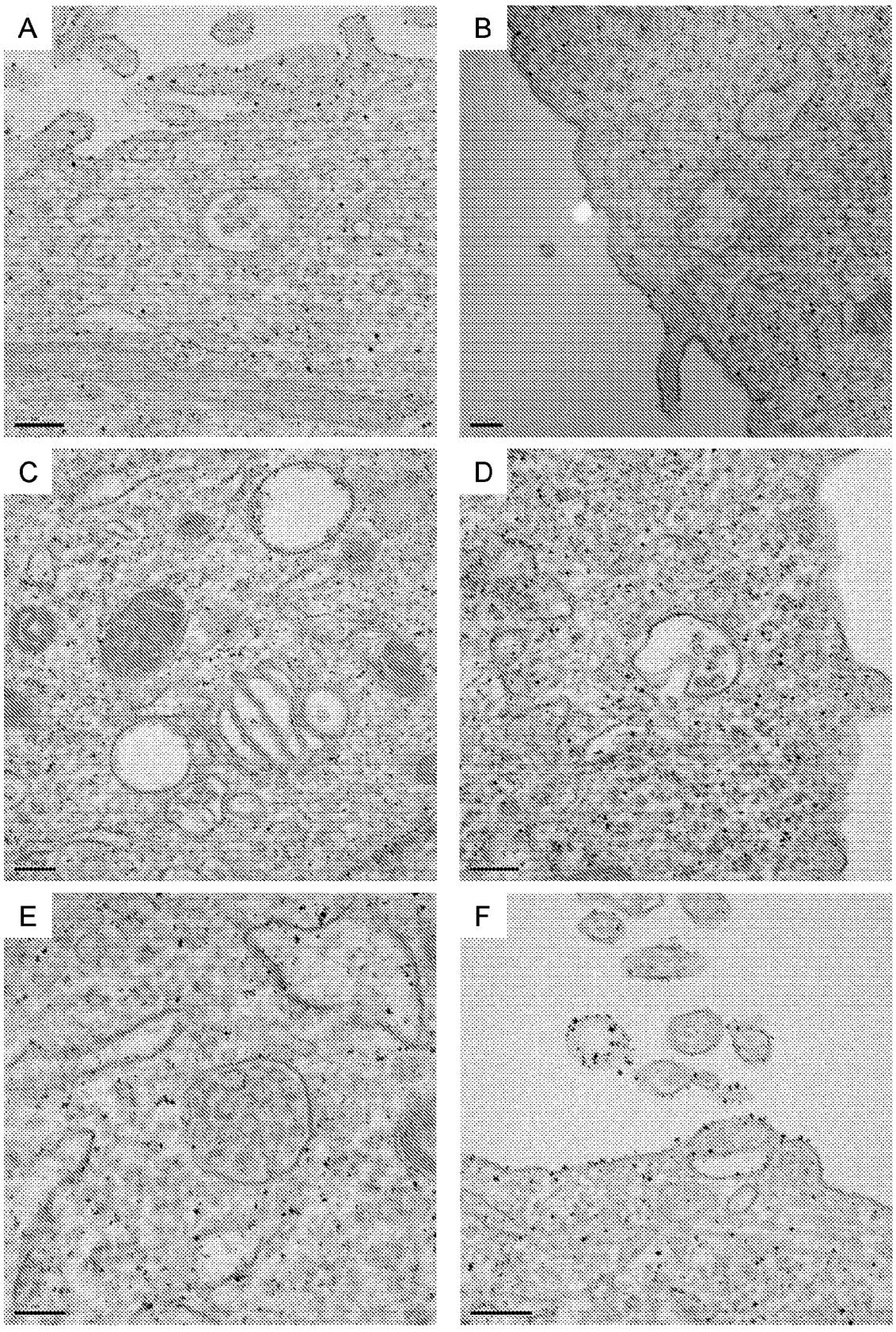


Figure 2

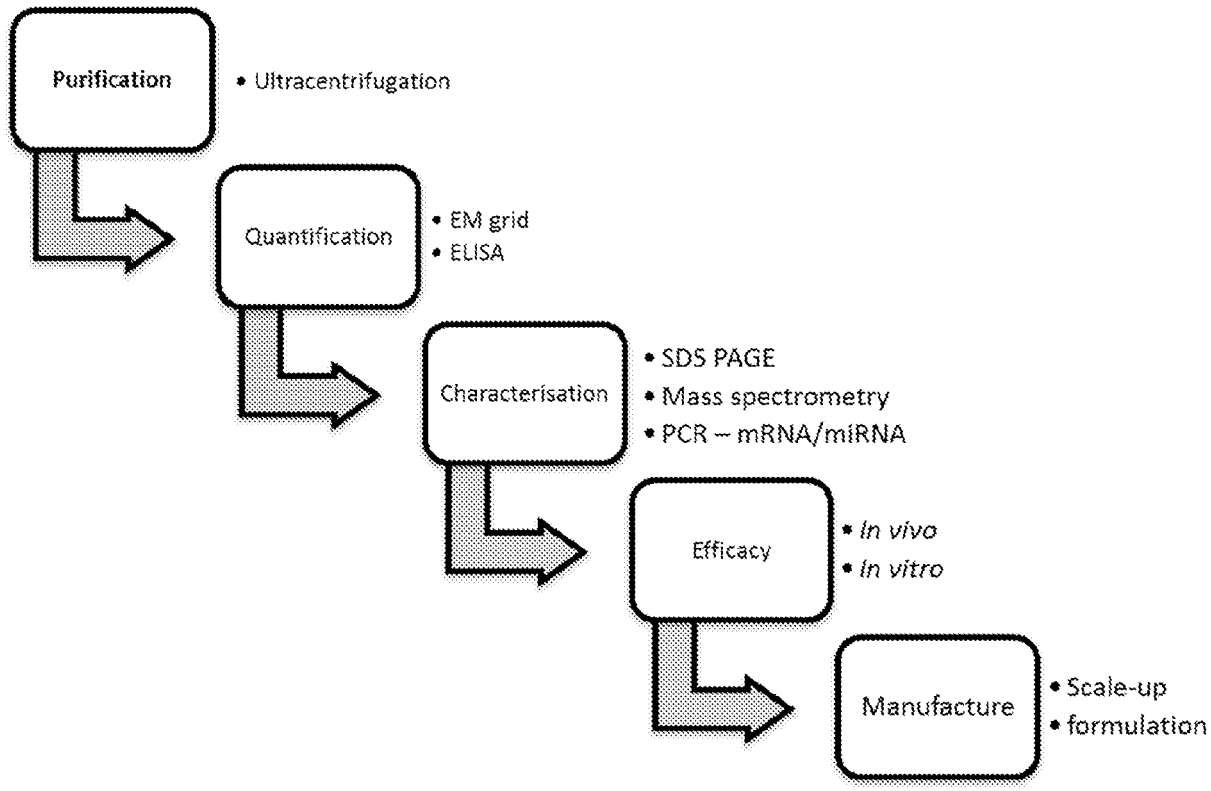

Purification
• Ultracentrifugation
Quantification
• EM grid
• ELISA
Characterisation
• SDS PAGE
• Mass spectrometry
• PCR – mRNA/miRNA
Efficacy
• In vivo
• In vitro
Manufacture
• Scale-up
• formulation

Figure 3

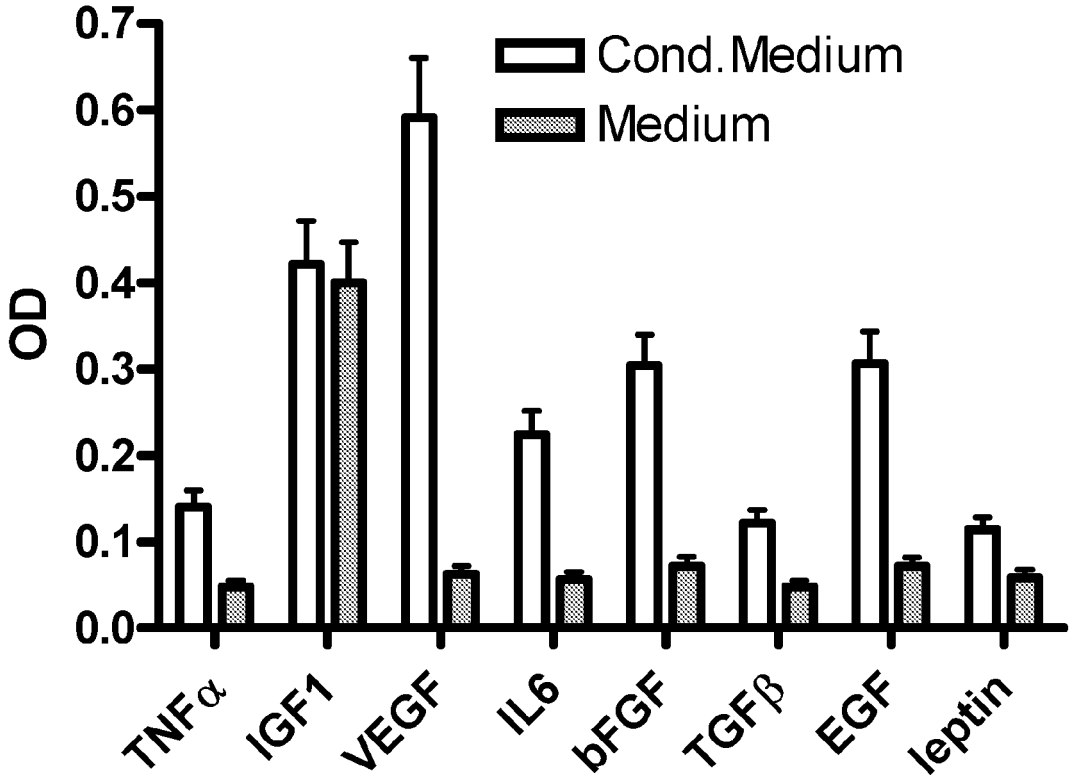

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
OD
Cond.Medium
Medium
TNFα
IGF1
VEGF
IL6
bFGF
TGFβ
EGF
leptin

Figure 4A

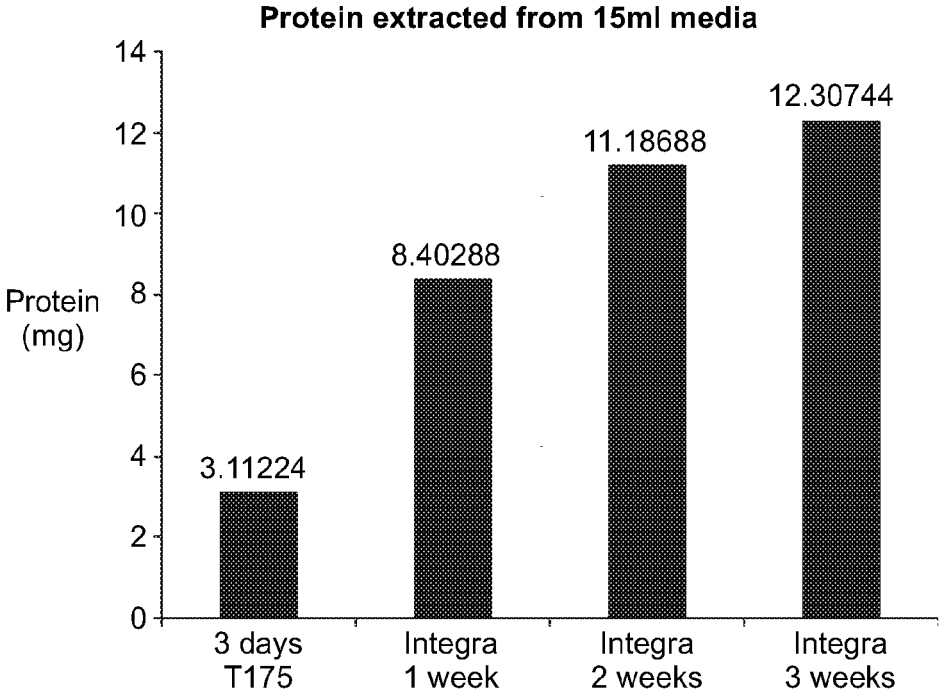


Figure 4B

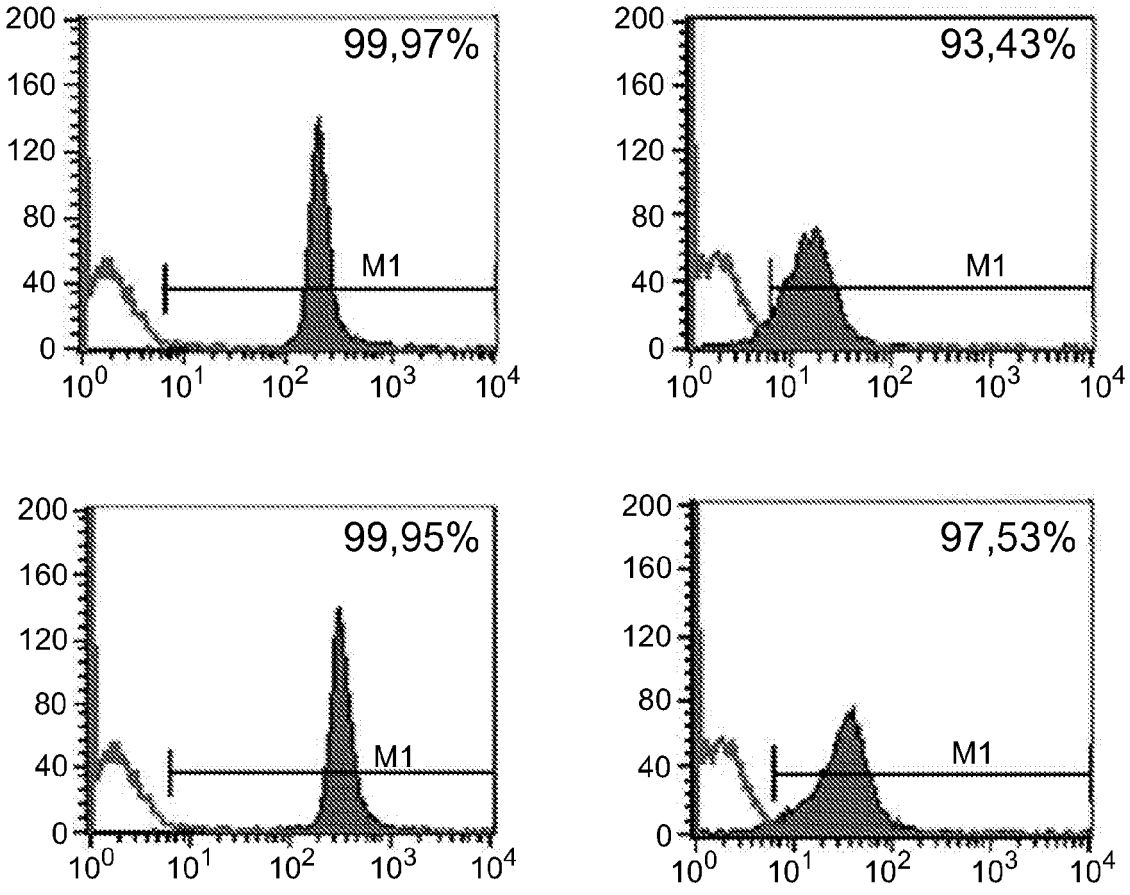


Figure 5

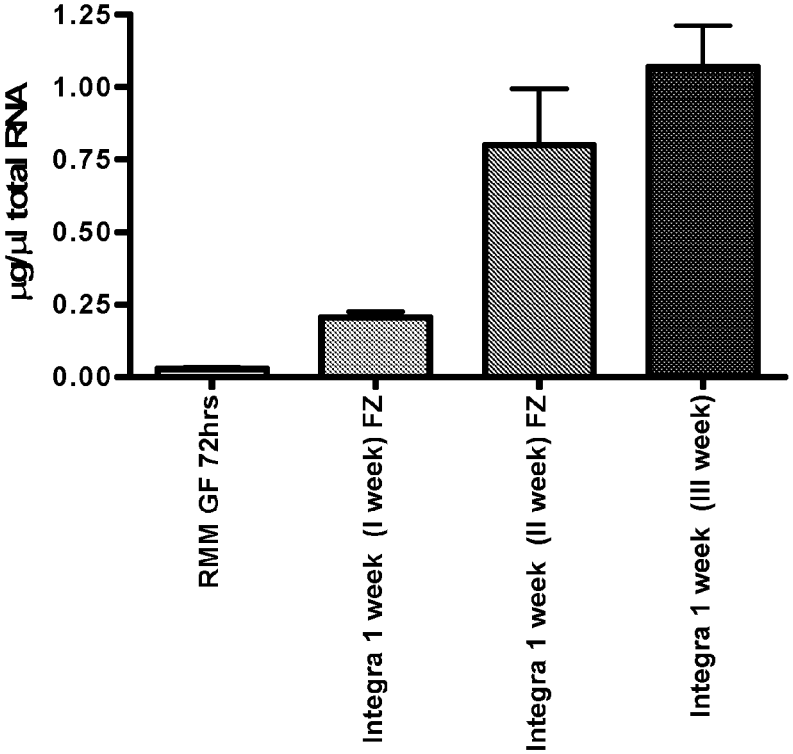

1.25
1.00
0.75
0.50
0.25
0.00
µg/µl total RNA
RMM GF 72hrs
Integra 1 week (I week) FZ
Integra 1 week (II week) FZ
Integra 1 week (III week)

Figure 6A

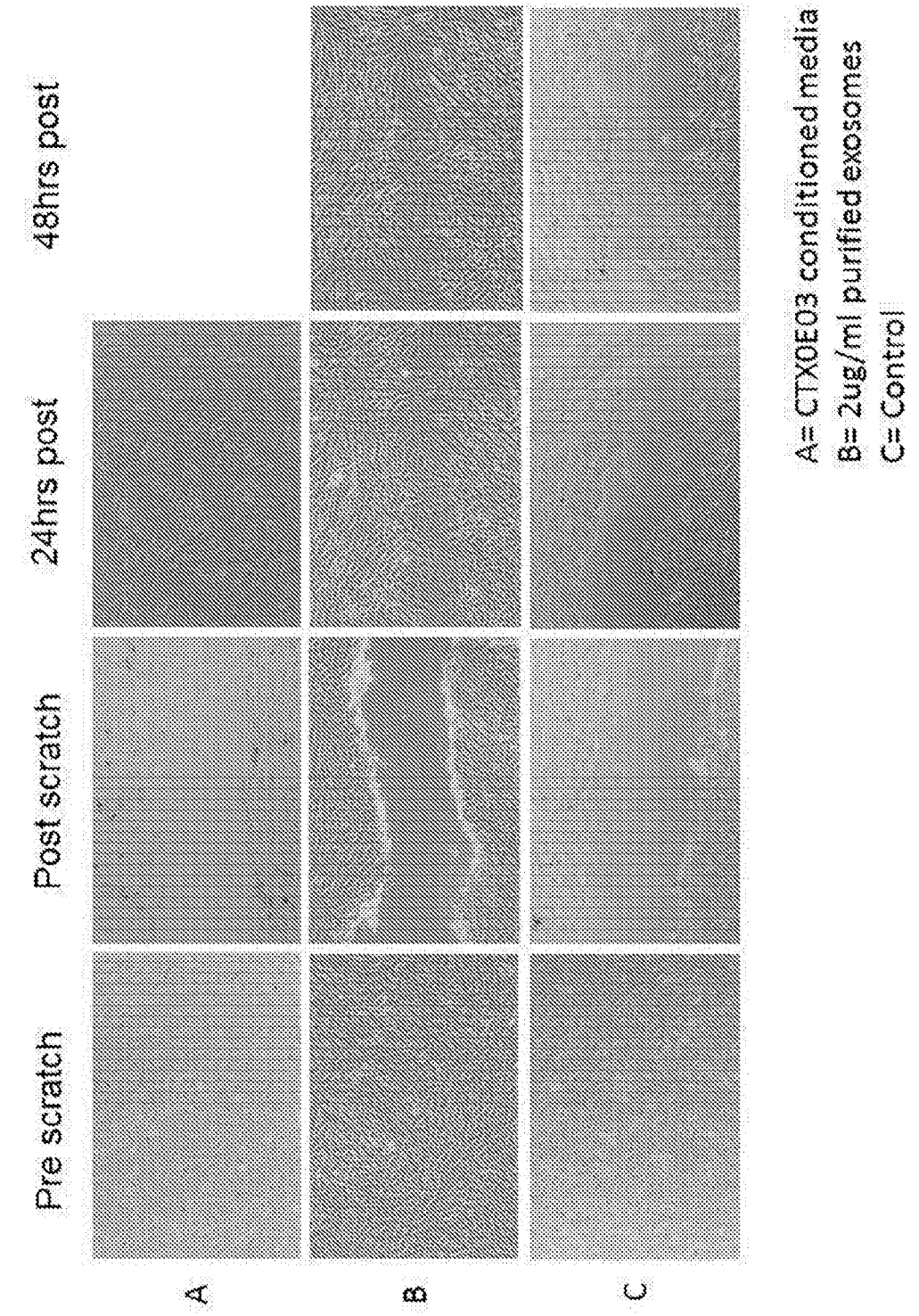

Pre scratch
Post scratch
24hrs post
48hrs post
A
B
C
A= CTX0E03 conditioned media
B= 2ug/ml purified exosomes
C= Control

Figure 6B



Figure 6C

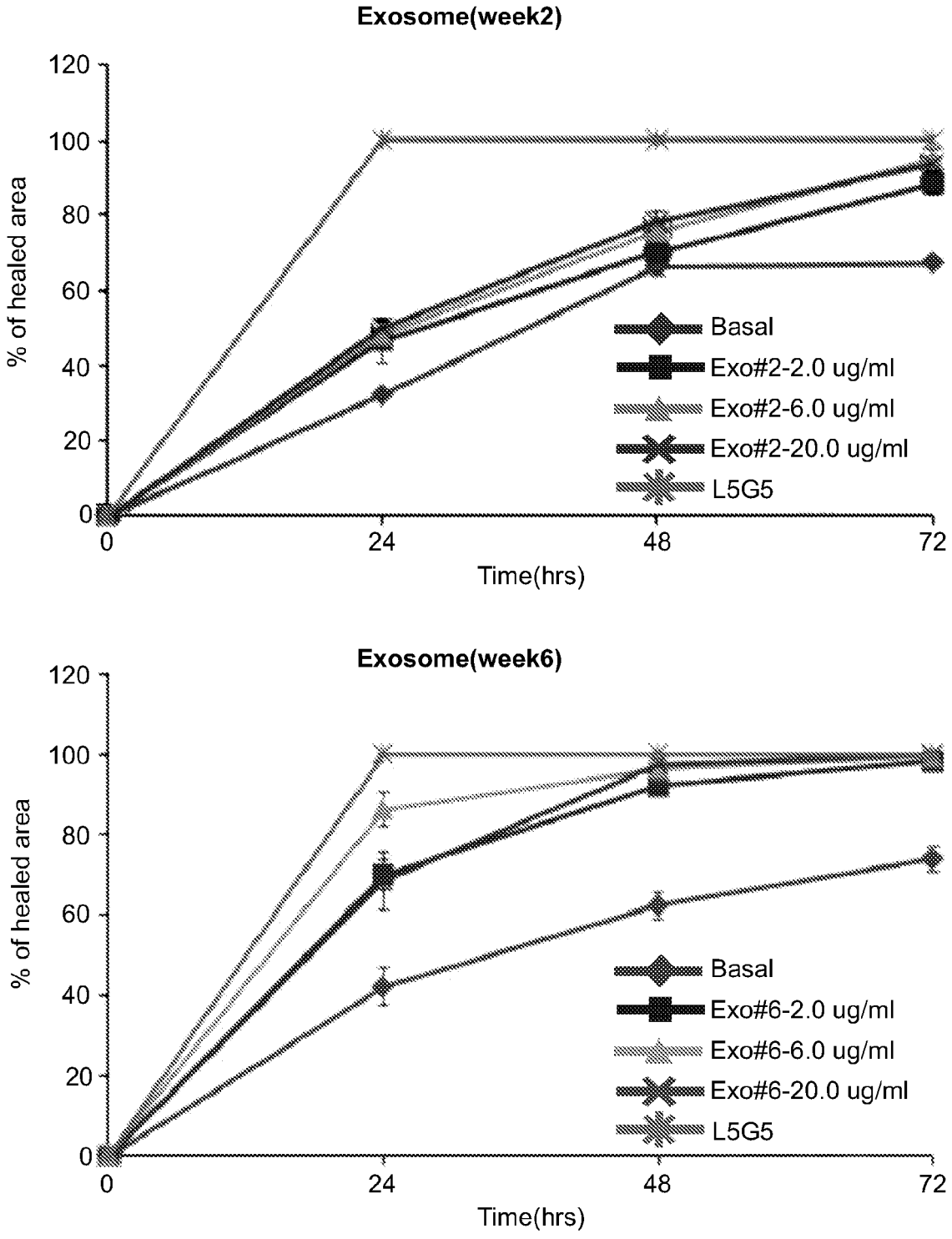

Exosome(week2)
120
100
80
60
40
20
0
% of healed area
0
24
48
72
Time(hrs)
Basal
Exo#2-2.0 ug/ml
Exo#2-6.0 ug/ml
Exo#2-20.0 ug/ml
L5G5
Exosome(week6)
120
100
80
60
40
20
0
% of healed area
0
24
48
72
Time(hrs)
Basal
Exo#6-2.0 ug/ml
Exo#6-6.0 ug/ml
Exo#6-20.0 ug/ml
L5G5

Figure 6D

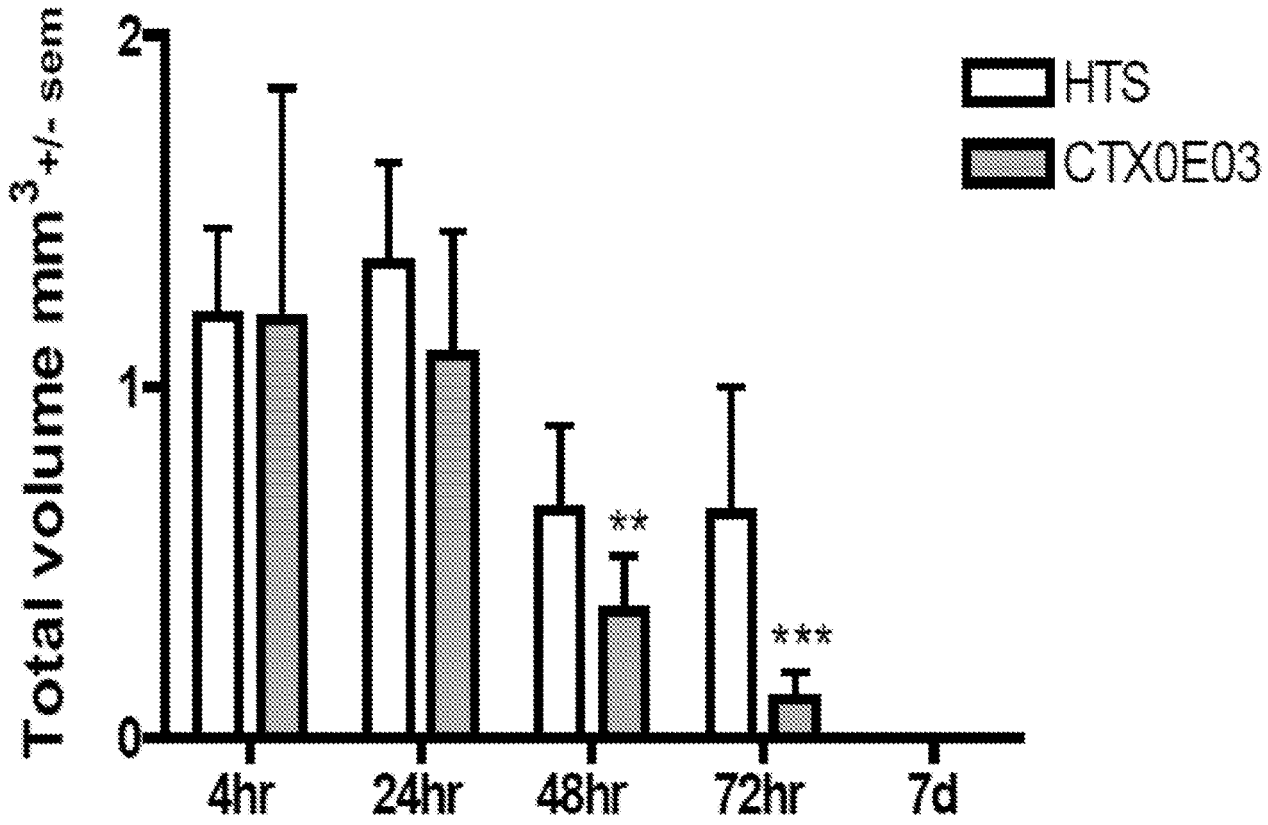


Figure 7

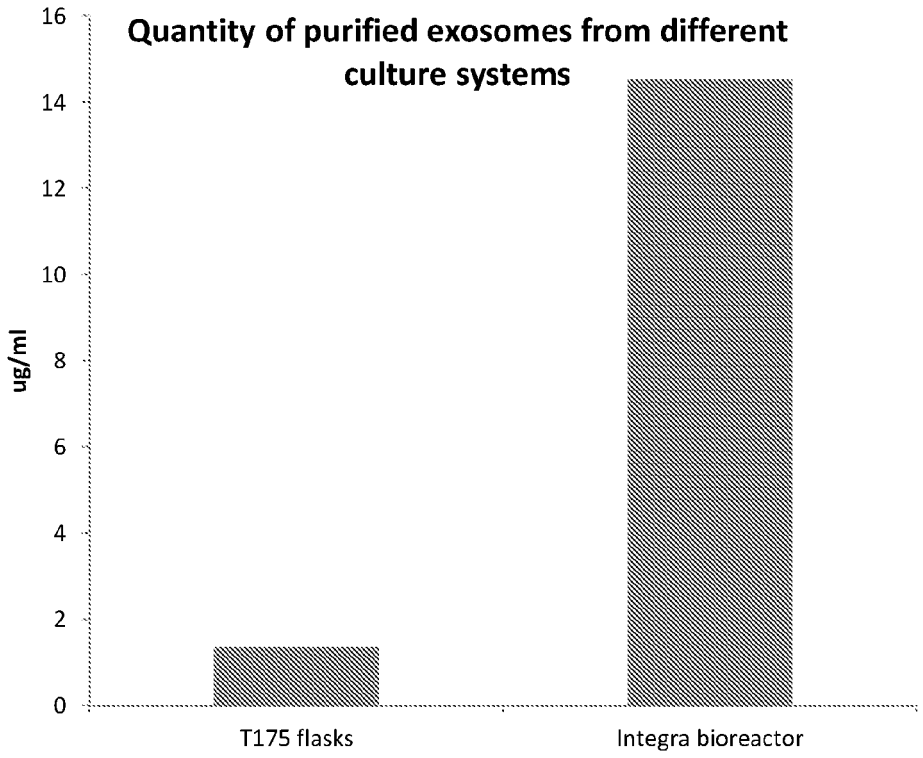

Quantity of purified exosomes from different culture systems
ug/ml
16
14
12
10
8
6
4
2
0
T175 flasks
Integra bioreactor

Figure 8A

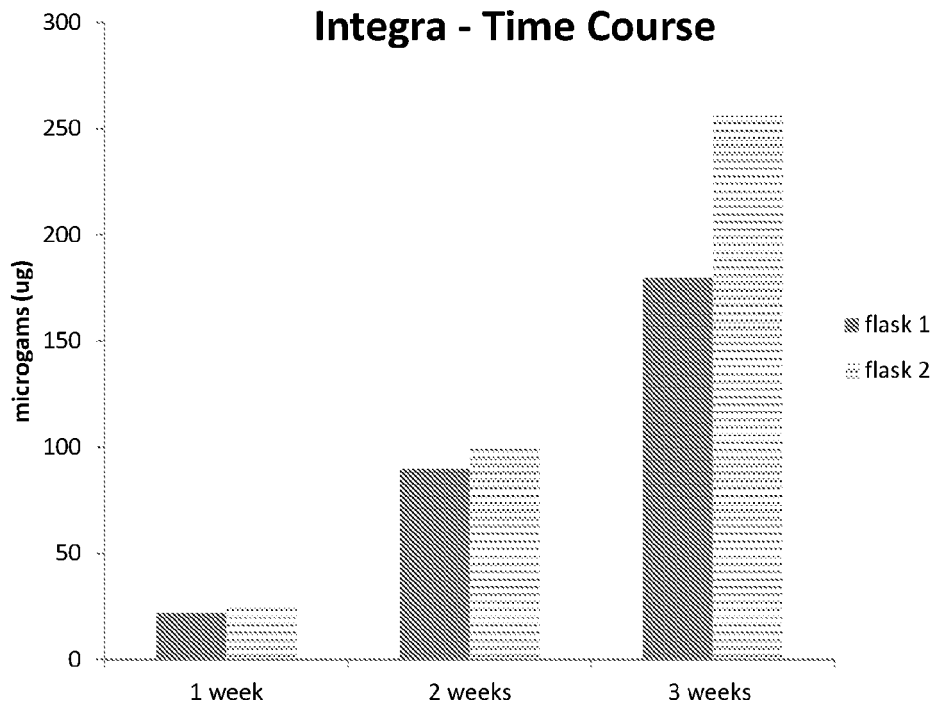


Figure 8B

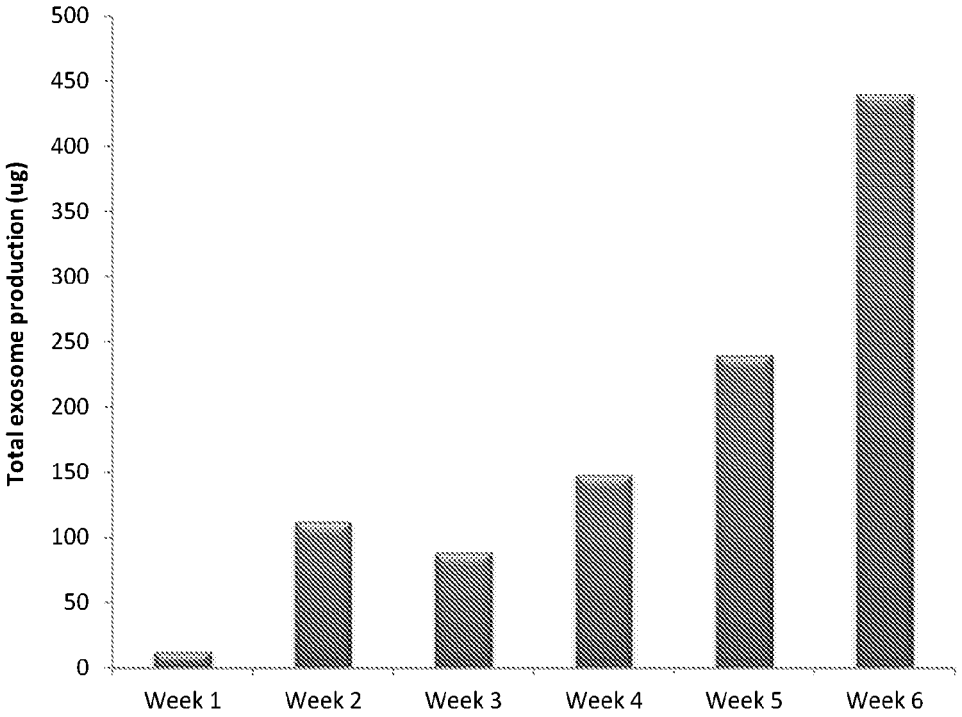


Figure 9

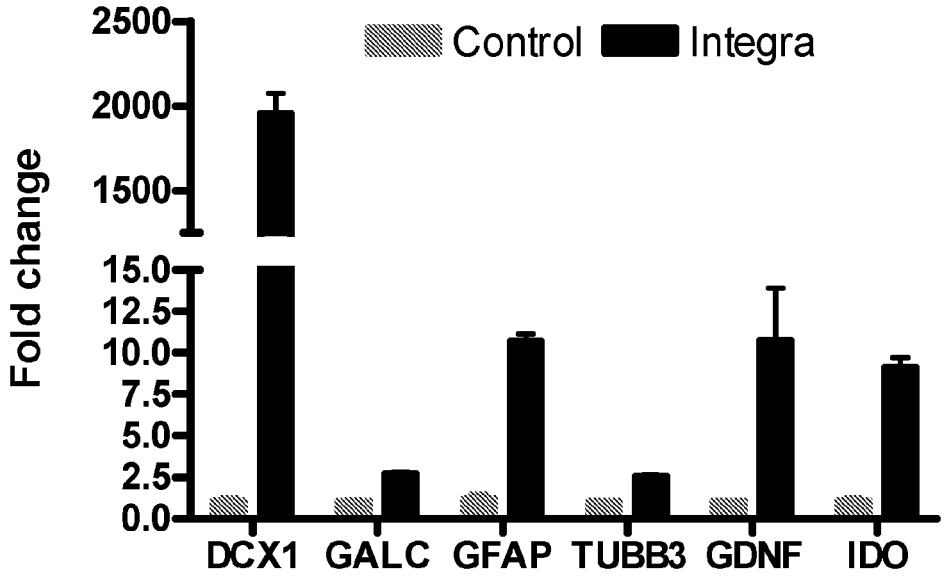

Control
Integra
Fold change
2500
2000
1500
15.0
12.5
10.0
7.5
5.0
2.5
0.0
DCX1
GALC
GFAP
TUBB3
GDNF
IDO

Figure 10

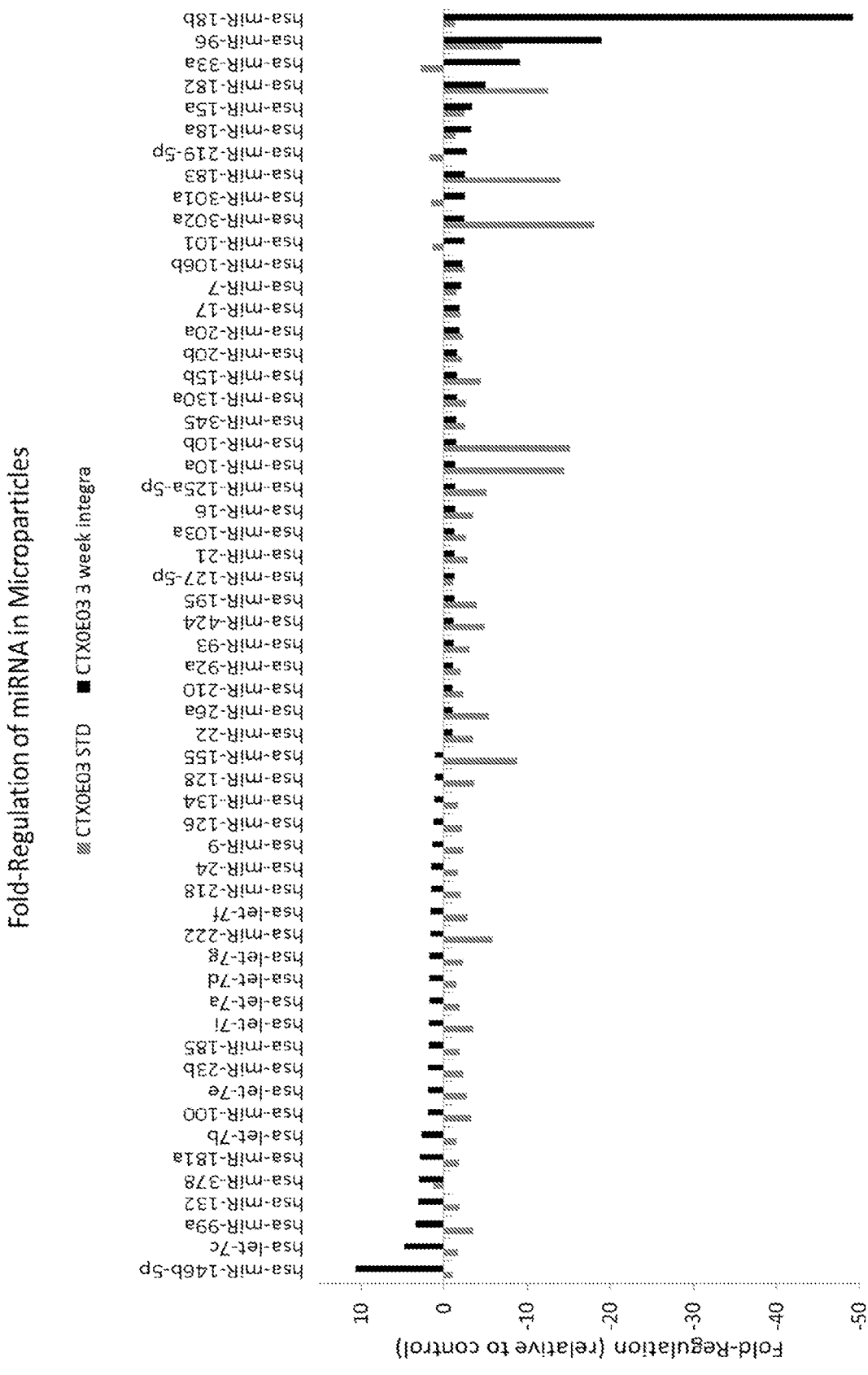

Fold-Regulation of miRNA in Microparticles
CTX0E03 STD
CTX0E03 3 week integra
Fold-Regulation (relative to control)
10
0
-10
-20
-30
-40
-50
hsa-miR-146b-5p
hsa-let-7c
hsa-miR-99a
hsa-miR-132
hsa-miR-378
hsa-miR-181a
hsa-let-7b
hsa-miR-100
hsa-let-7e
hsa-miR-23b
hsa-miR-185
hsa-let-7i
hsa-let-7a
hsa-let-7d
hsa-let-7g
hsa-miR-222
hsa-let-7f
hsa-miR-218
hsa-miR-24
hsa-miR-9
hsa-miR-126
hsa-miR-134
hsa-miR-128
hsa-miR-155
hsa-miR-22
hsa-miR-26a
hsa-miR-210
hsa-miR-92a
hsa-miR-93
hsa-miR-424
hsa-miR-195
hsa-miR-127-5p
hsa-miR-21
hsa-miR-103a
hsa-miR-16
hsa-miR-125a-5p
hsa-miR-10a
hsa-miR-10b
hsa-miR-345
hsa-miR-130a
hsa-miR-15b
hsa-miR-20b
hsa-miR-20a
hsa-miR-17
hsa-miR-7
hsa-miR-106b
hsa-miR-101
hsa-miR-302a
hsa-miR-301a
hsa-miR-183
hsa-miR-219-5p
hsa-miR-18a
hsa-miR-15a
hsa-miR-182
hsa-miR-33a
hsa-miR-96
hsa-miR-18b

Figure 11a

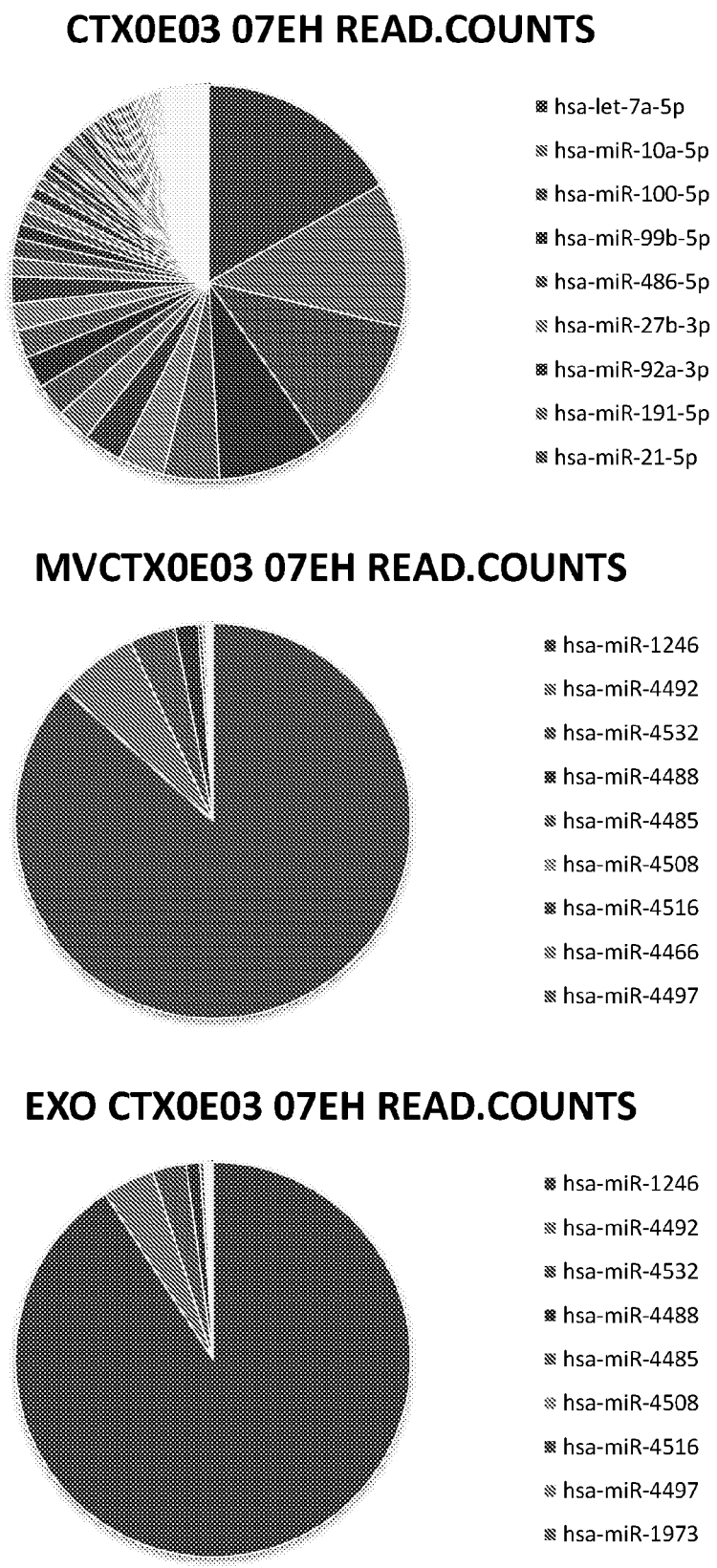

CTX0E03 07EH READ.COUNTS
hsa-let-7a-5p
hsa-miR-10a-5p
hsa-miR-100-5p
hsa-miR-99b-5p
hsa-miR-486-5p
hsa-miR-27b-3p
hsa-miR-92a-3p
hsa-miR-191-5p
hsa-miR-21-5p
MVCTX0E03 07EH READ.COUNTS
hsa-miR-1246
hsa-miR-4492
hsa-miR-4532
hsa-miR-4488
hsa-miR-4485
hsa-miR-4508
hsa-miR-4516
hsa-miR-4466
hsa-miR-4497
EXO CTX0E03 07EH READ.COUNTS
hsa-miR-1246
hsa-miR-4492
hsa-miR-4532
hsa-miR-4488
hsa-miR-4485
hsa-miR-4508
hsa-miR-4516
hsa-miR-4497
hsa-miR-1973

Figure 11b

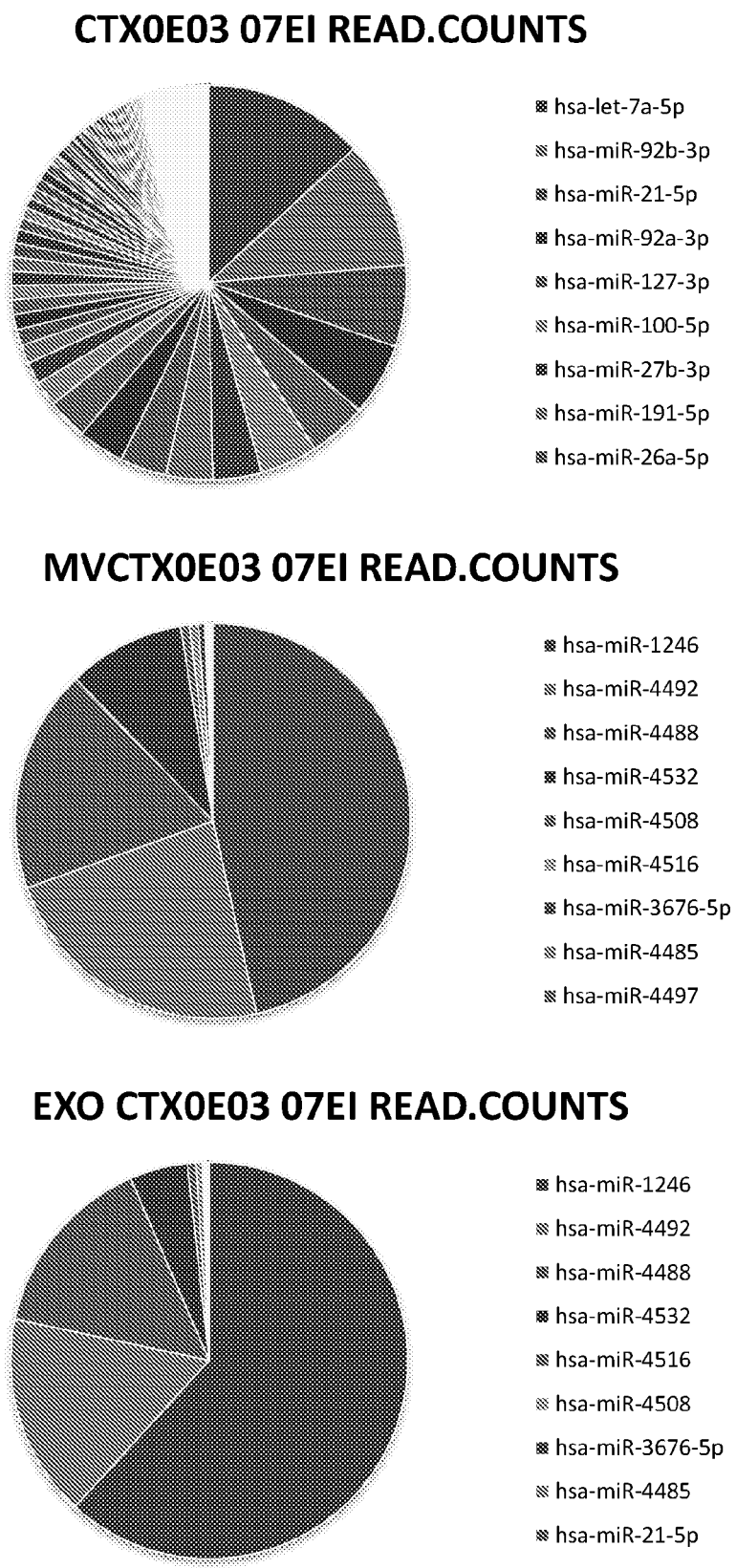

CTX0E03 07EI READ.COUNTS
hsa-let-7a-5p
hsa-miR-92b-3p
hsa-miR-21-5p
hsa-miR-92a-3p
hsa-miR-127-3p
hsa-miR-100-5p
hsa-miR-27b-3p
hsa-miR-191-5p
hsa-miR-26a-5p
MVCTX0E03 07EI READ.COUNTS
hsa-miR-1246
hsa-miR-4492
hsa-miR-4488
hsa-miR-4532
hsa-miR-4508
hsa-miR-4516
hsa-miR-3676-5p
hsa-miR-4485
hsa-miR-4497
EXO CTX0E03 07EI READ.COUNTS
hsa-miR-1246
hsa-miR-4492
hsa-miR-4488
hsa-miR-4532
hsa-miR-4516
hsa-miR-4508
hsa-miR-3676-5p
hsa-miR-4485
hsa-miR-21-5p

Figure 12A

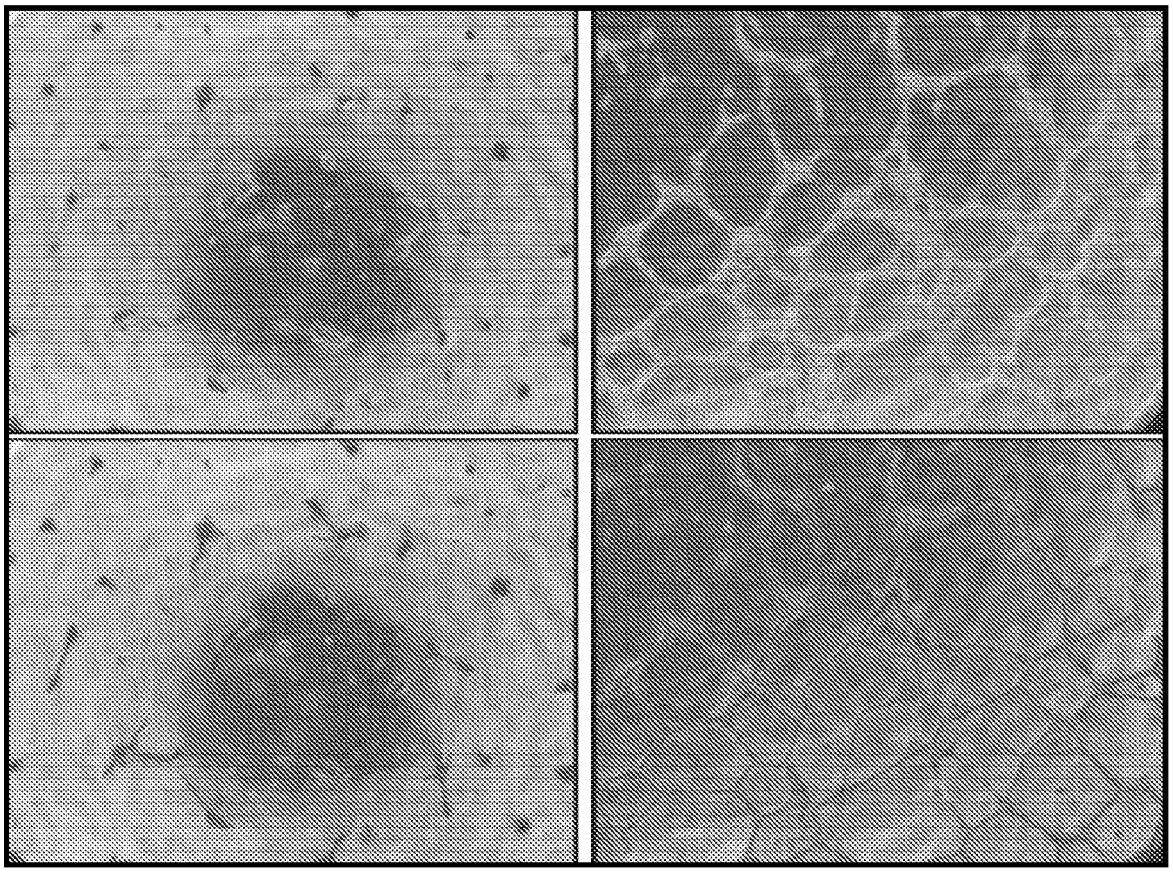


Figure 12B

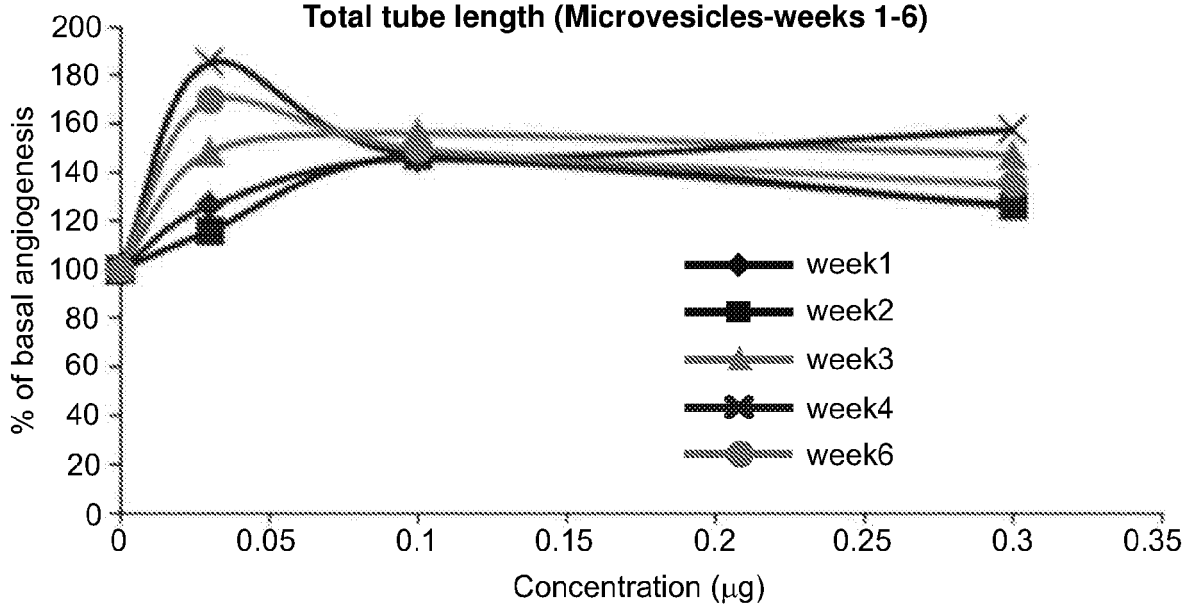


Figure 12C

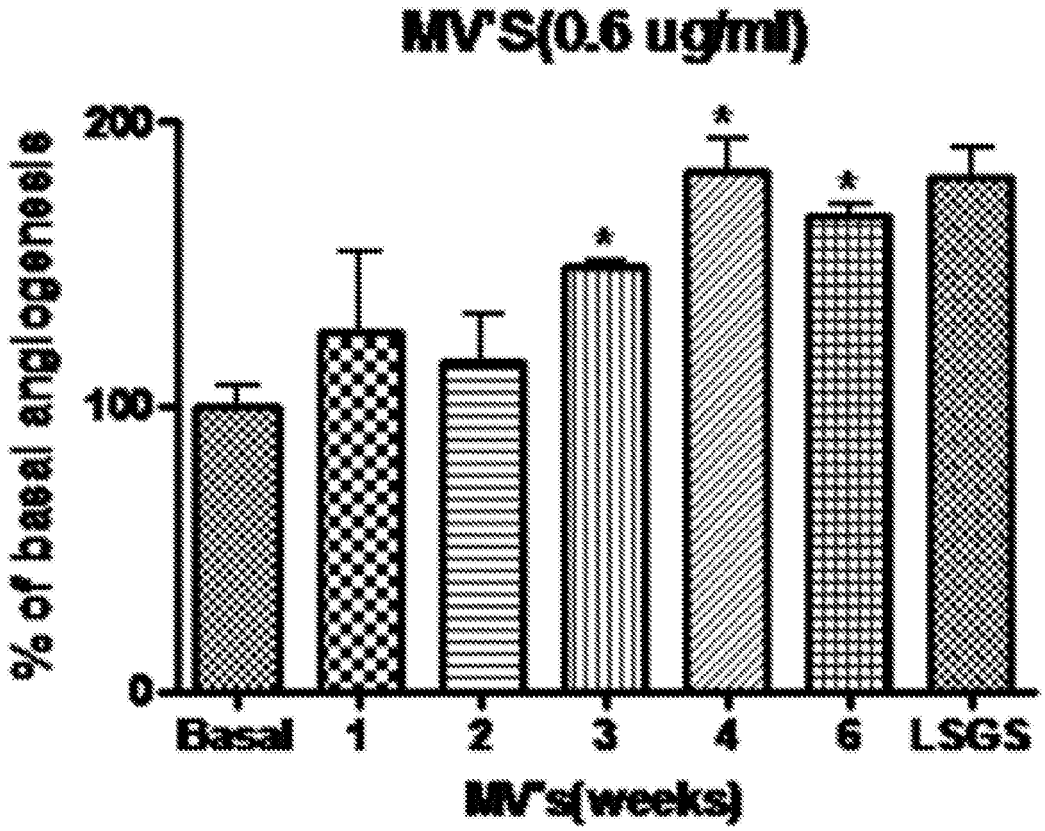
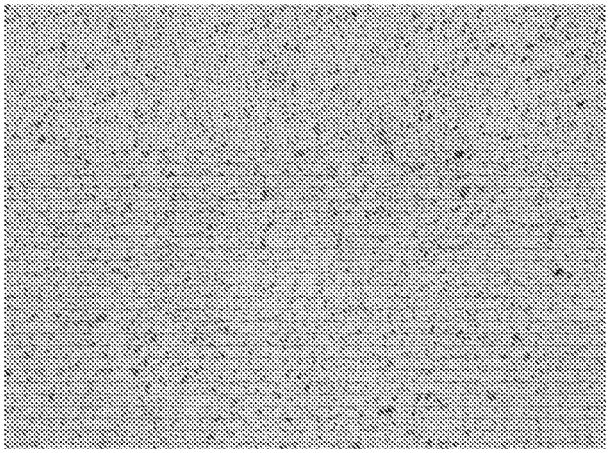
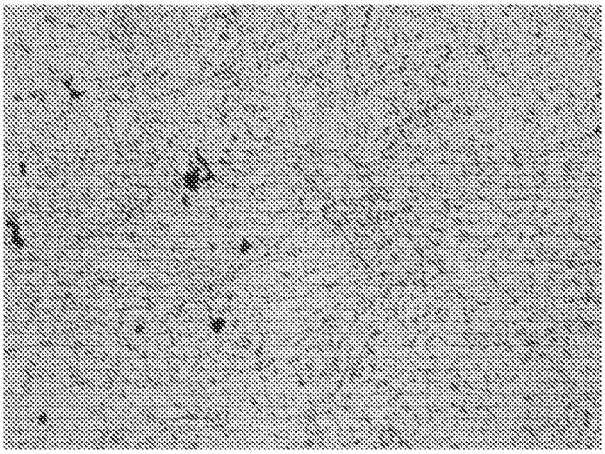

MV'S(0.6 ug/ml)
% of basal angiogeneele
200
100
0
*
*
*
Basal
1
2
3
4
6
LSGS
MV's(weeks)

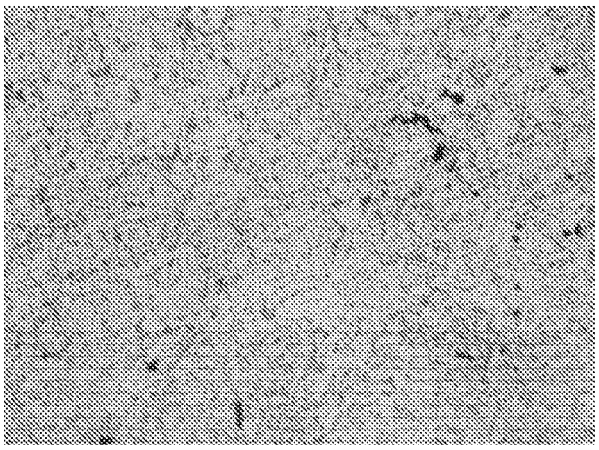
Figure 13



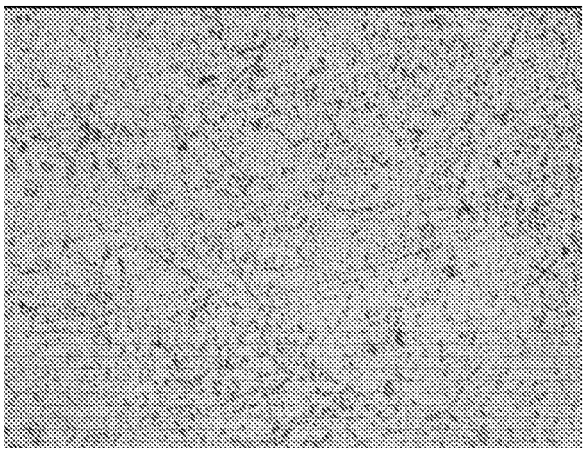
Basal



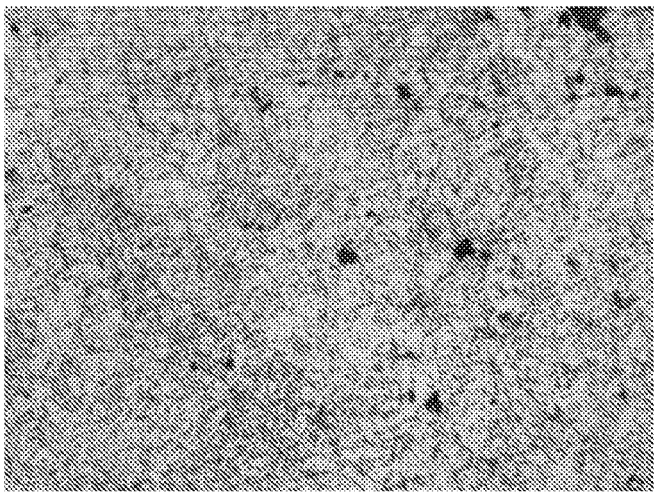
100ng/ml NGF



0.03µg week 2 Integra microvesicles + 100ng/ml NGF



0.3µg week 2 Integra microvesicles + 100ng/ml NGF



3µg week 2 Integra microvesicles + 100ng/ml NGF

Figure 14

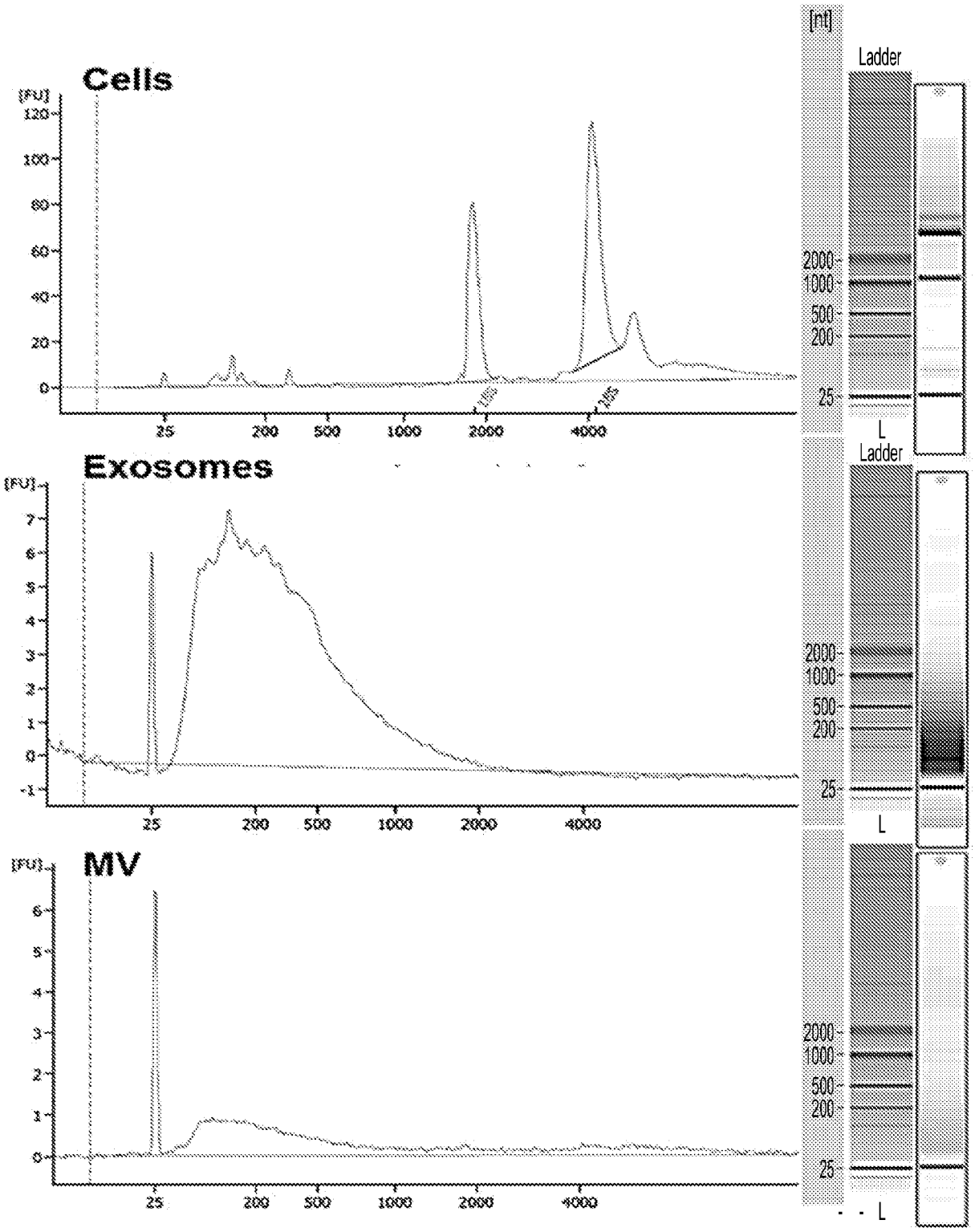

Cells
[FU]
120
100
80
60
40
20
0
25
200
500
1000
2000
4000
Exosomes
[FU]
7
6
5
4
3
2
1
0
-1
MV
[FU]
[nt]
Ladder
2000
1000
500
200
25
L

Figure 15

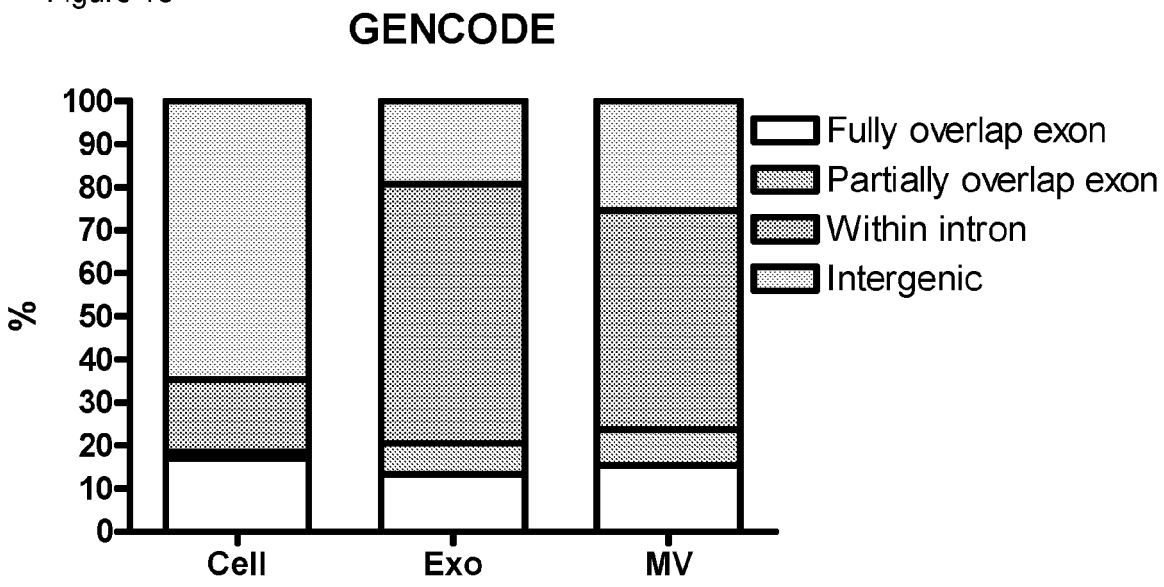


Figure 16

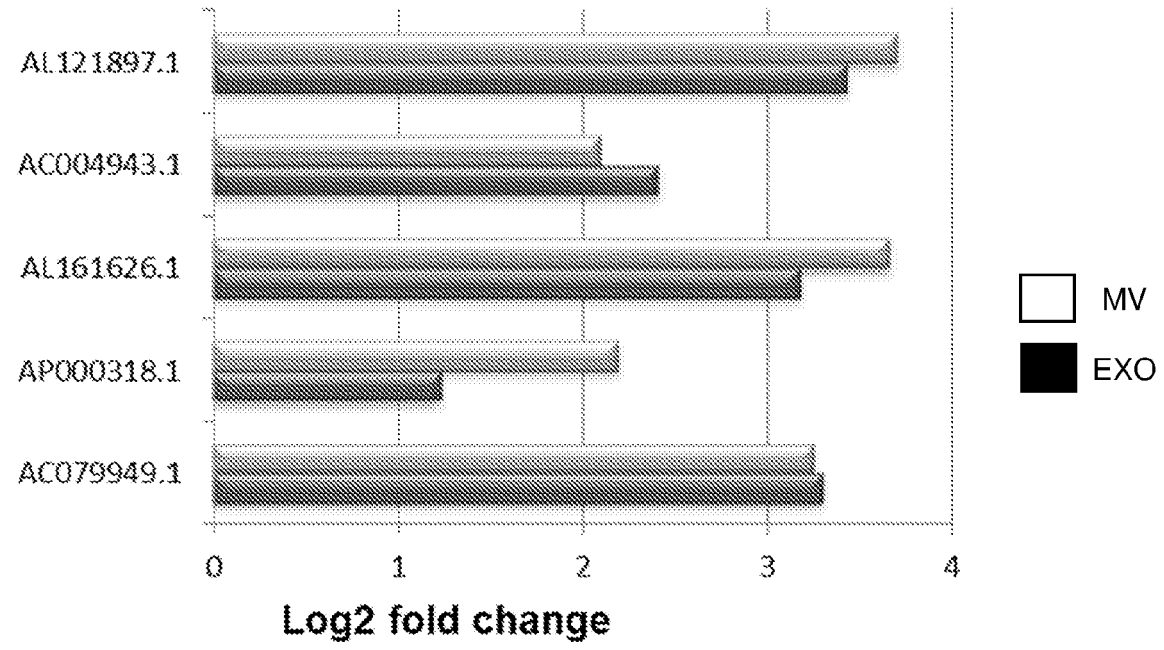


Figure 17A

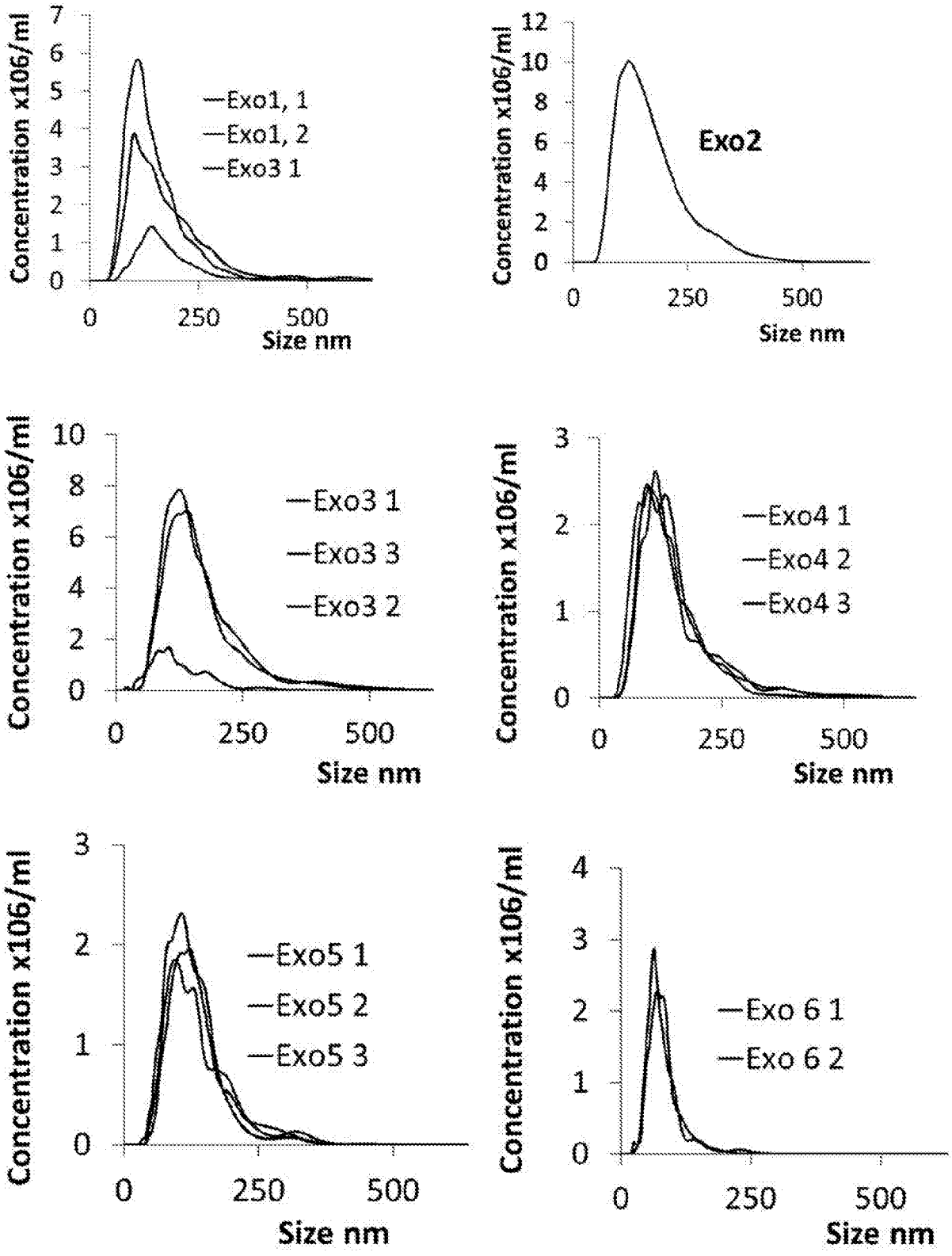

Concentration x106/ml
Size nm
—Exo1, 1
—Exo1, 2
—Exo3 1
Exo2
—Exo3 1
—Exo3 3
—Exo3 2
—Exo4 1
—Exo4 2
—Exo4 3
—Exo5 1
—Exo5 2
—Exo5 3
—Exo 6 1
—Exo 6 2

Figure 17B

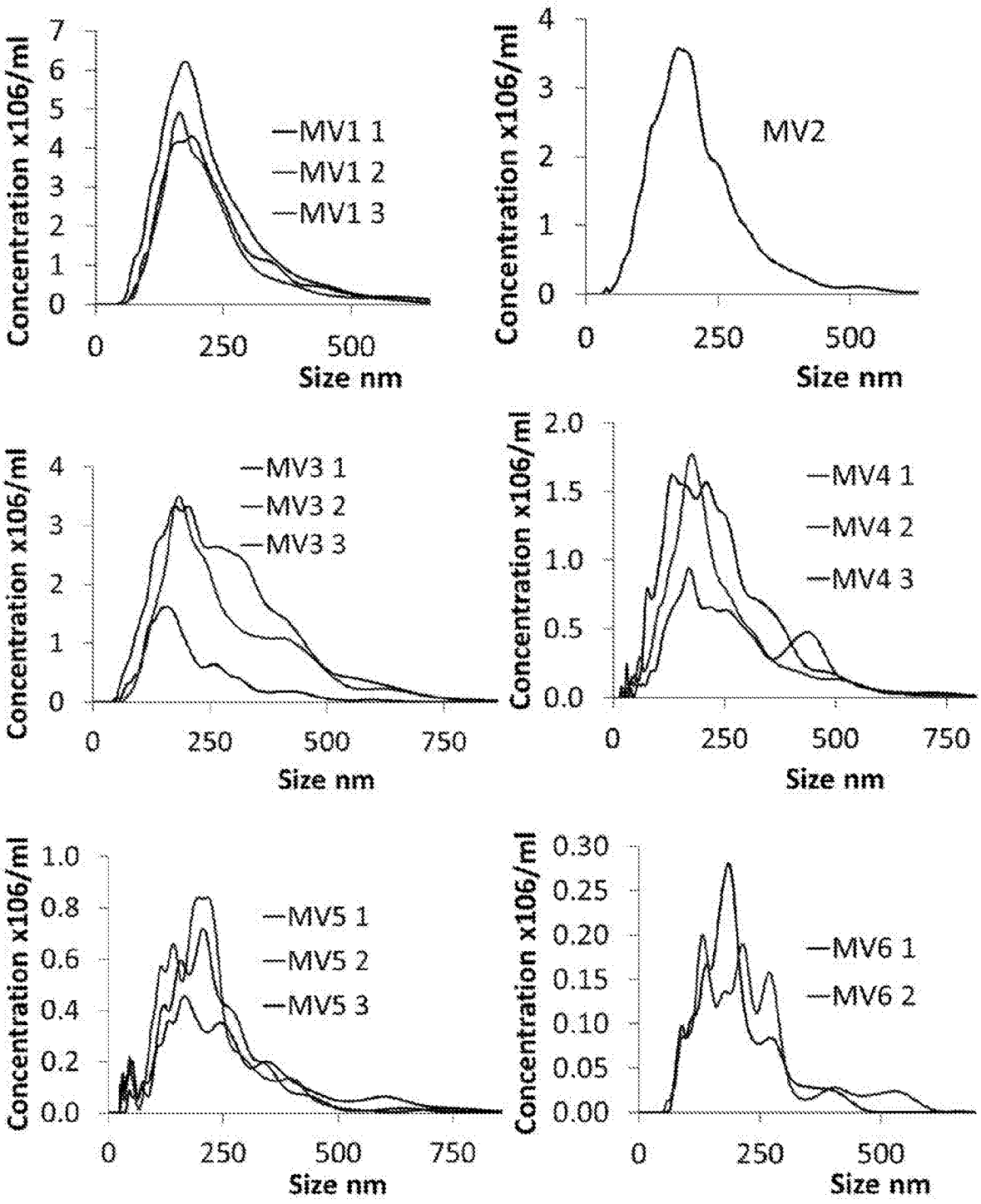

Concentration x106/ml
Size nm
—MV1 1
—MV1 2
—MV1 3
MV2
—MV3 1
—MV3 2
—MV3 3
—MV4 1
—MV4 2
—MV4 3
—MV5 1
—MV5 2
—MV5 3
—MV6 1
—MV6 2

Figure 18A

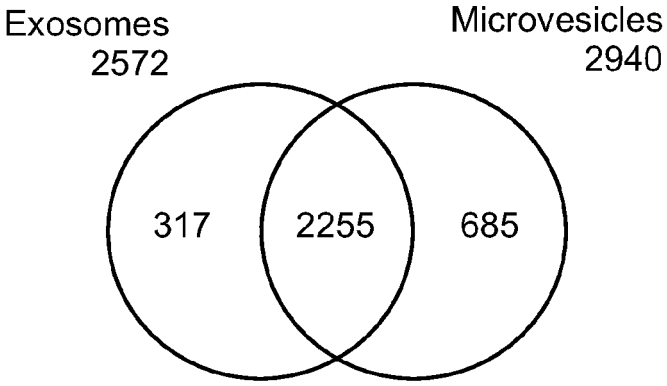


Figure 18B

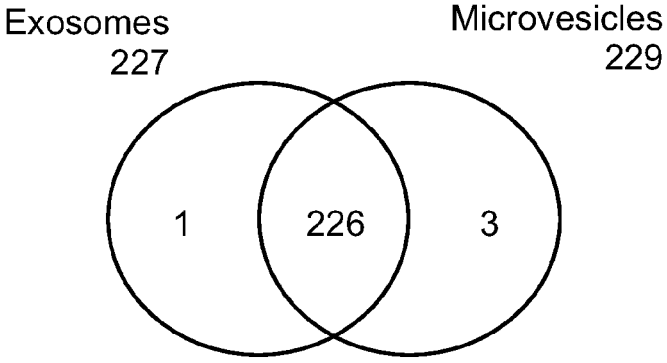


Figure 18C

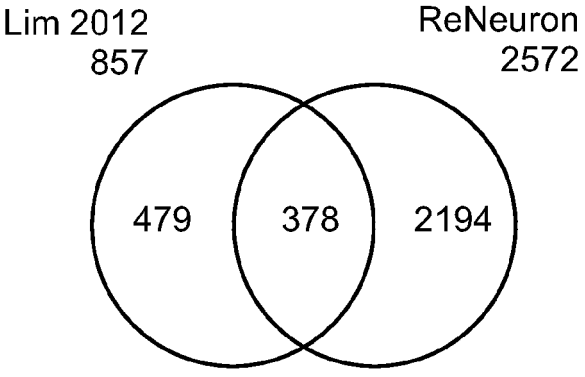
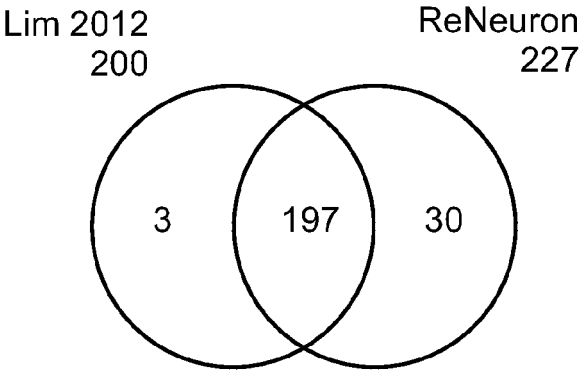


Figure 18D



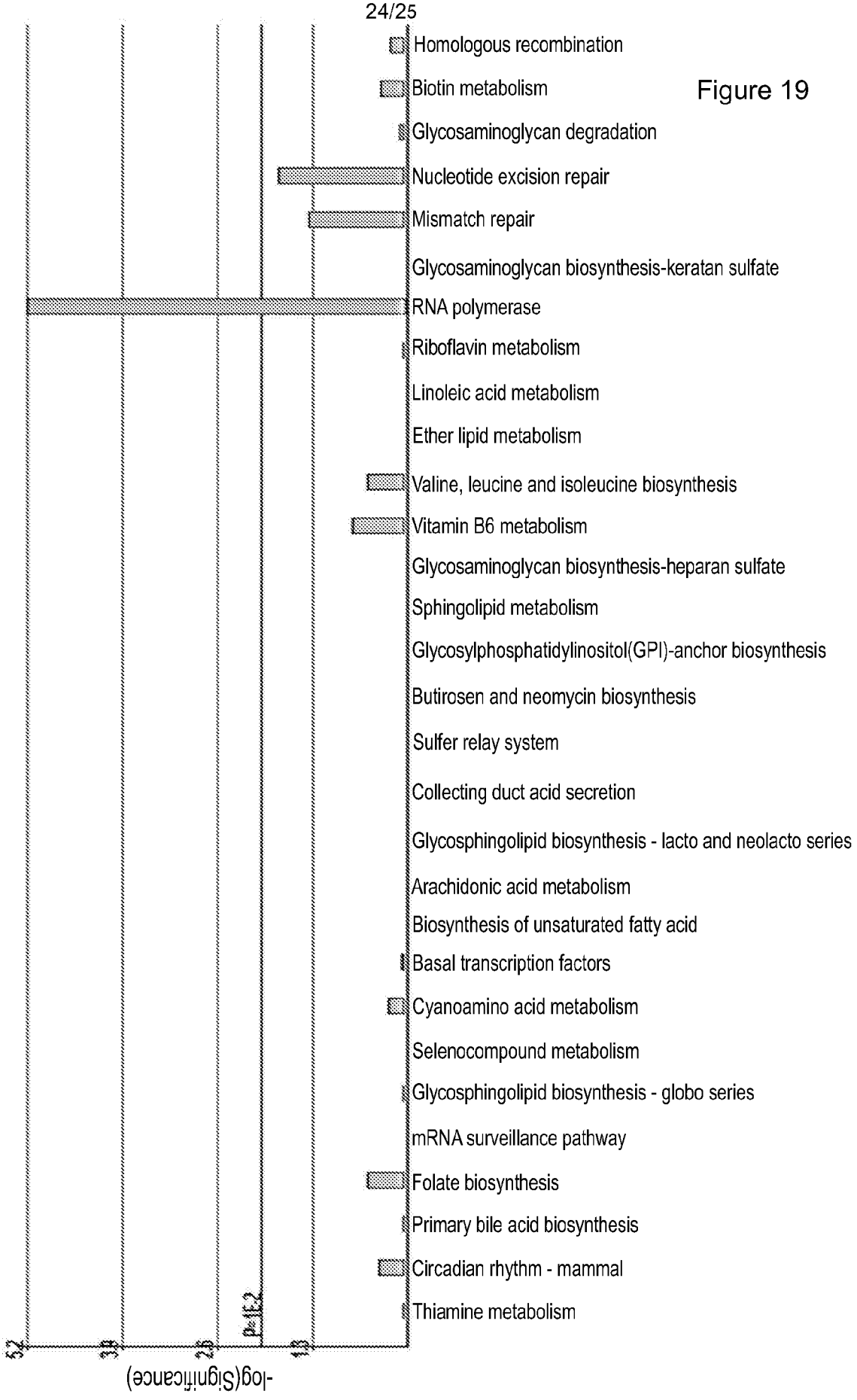
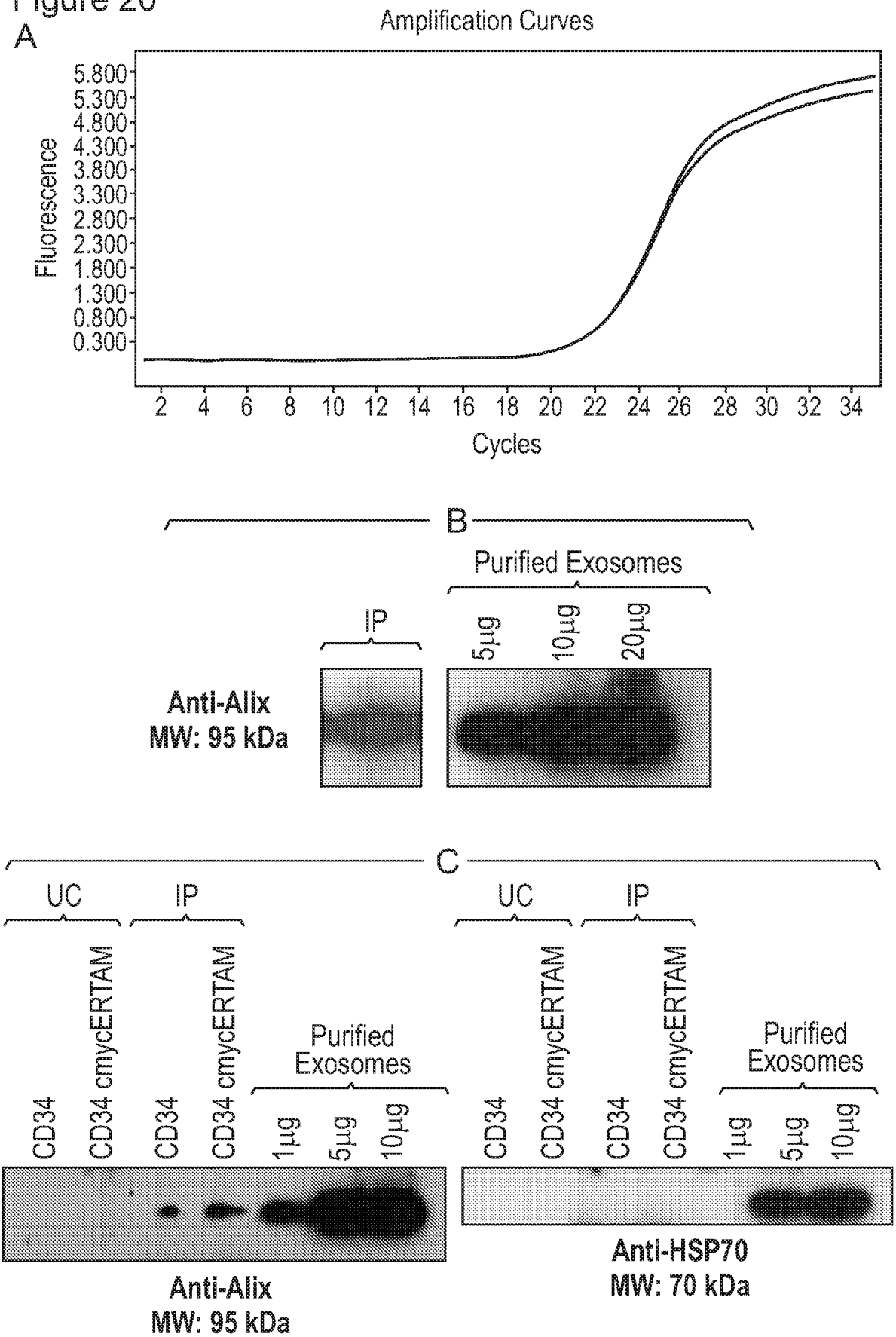

Homologous recombination
Biotin metabolism
Glycosaminoglycan degradation
Nucleotide excision repair
Mismatch repair
Glycosaminoglycan biosynthesis-keratan sulfate
RNA polymerase
Riboflavin metabolism
Linoleic acid metabolism
Ether lipid metabolism
Valine, leucine and isoleucine biosynthesis
Vitamin B6 metabolism
Glycosaminoglycan biosynthesis-heparan sulfate
Sphingolipid metabolism
Glycosylphosphatidylinositol(GPI)-anchor biosynthesis
Butirosen and neomycin biosynthesis
Sulfer relay system
Collecting duct acid secretion
Glycosphingolipid biosynthesis - lacto and neolacto series
Arachidonic acid metabolism
Biosynthesis of unsaturated fatty acid
Basal transcription factors
Cyanoamino acid metabolism
Selenocompound metabolism
Glycosphingolipid biosynthesis - globo series
mRNA surveillance pathway
Folate biosynthesis
Primary bile acid biosynthesis
Circadian rhythm - mammal
Thiamine metabolism
p=1E-2
5.2
3.9
2.6
1.3
-log(Significance)

Figure 19

Figure 20


A
Amplification Curves
Fluorescence
5.800
5.300
4.800
4.300
3.800
3.300
2.800
2.300
1.800
1.300
0.800
0.300
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
Cycles
B
Purified Exosomes
IP
5µg
10µg
20µg
Anti-Alix
MW: 95 kDa
C
UC
IP
UC
IP
CD34
CD34 cmycERTAM
CD34
CD34 cmycERTAM
Purified Exosomes
1µg
5µg
10µg
CD34
CD34 cmycERTAM
CD34
CD34 cmycERTAM
Purified Exosomes
1µg
5µg
10µg
Anti-Alix
MW: 95 kDa
Anti-HSP70
MW: 70 kDa