

## Exosomes Mediate Epithelium-Mesenchyme Crosstalk in Organ Development

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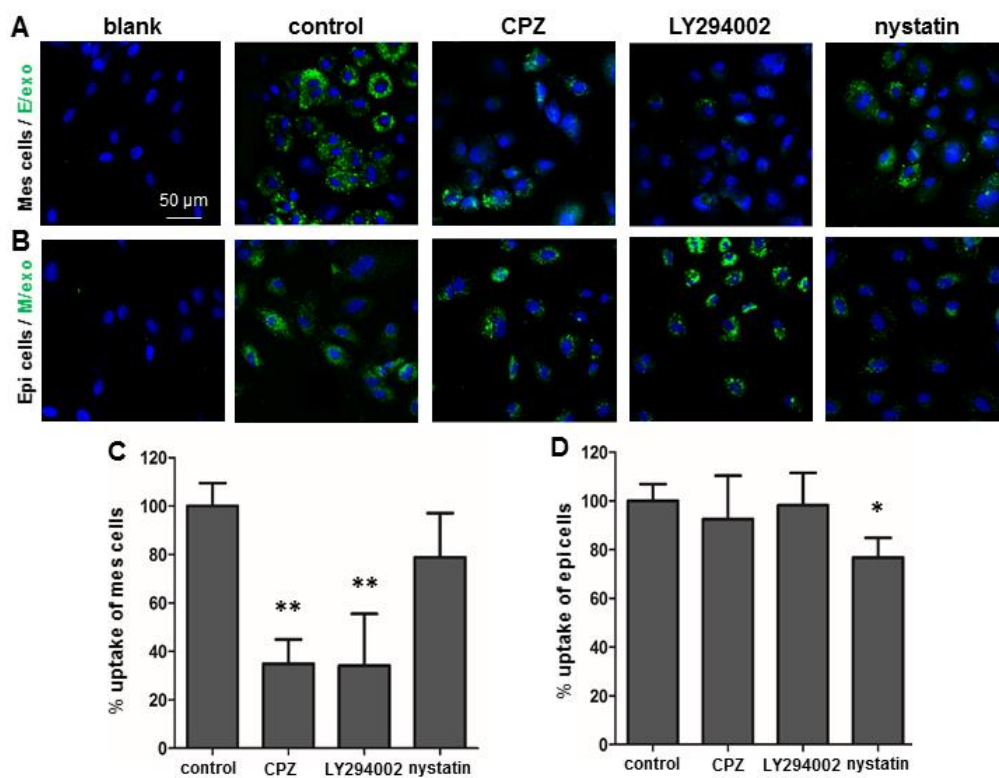
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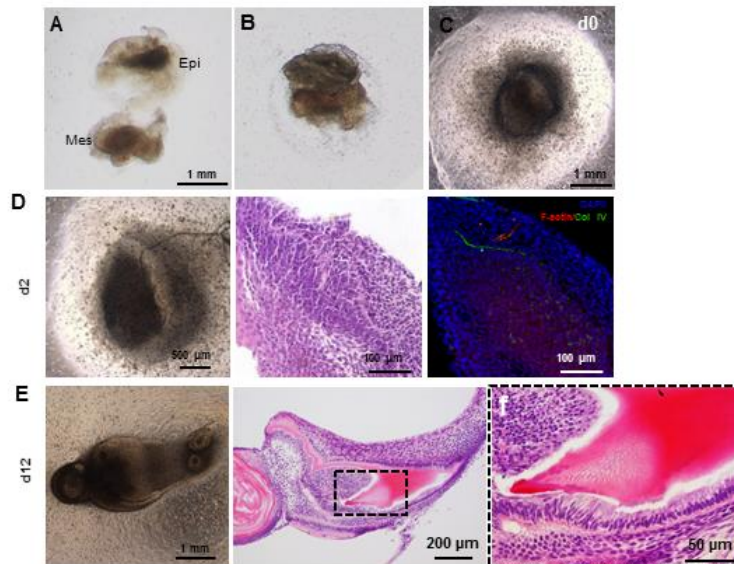
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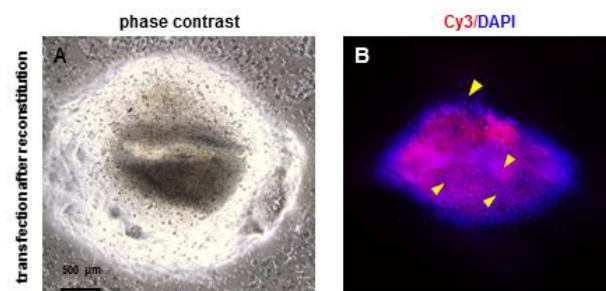
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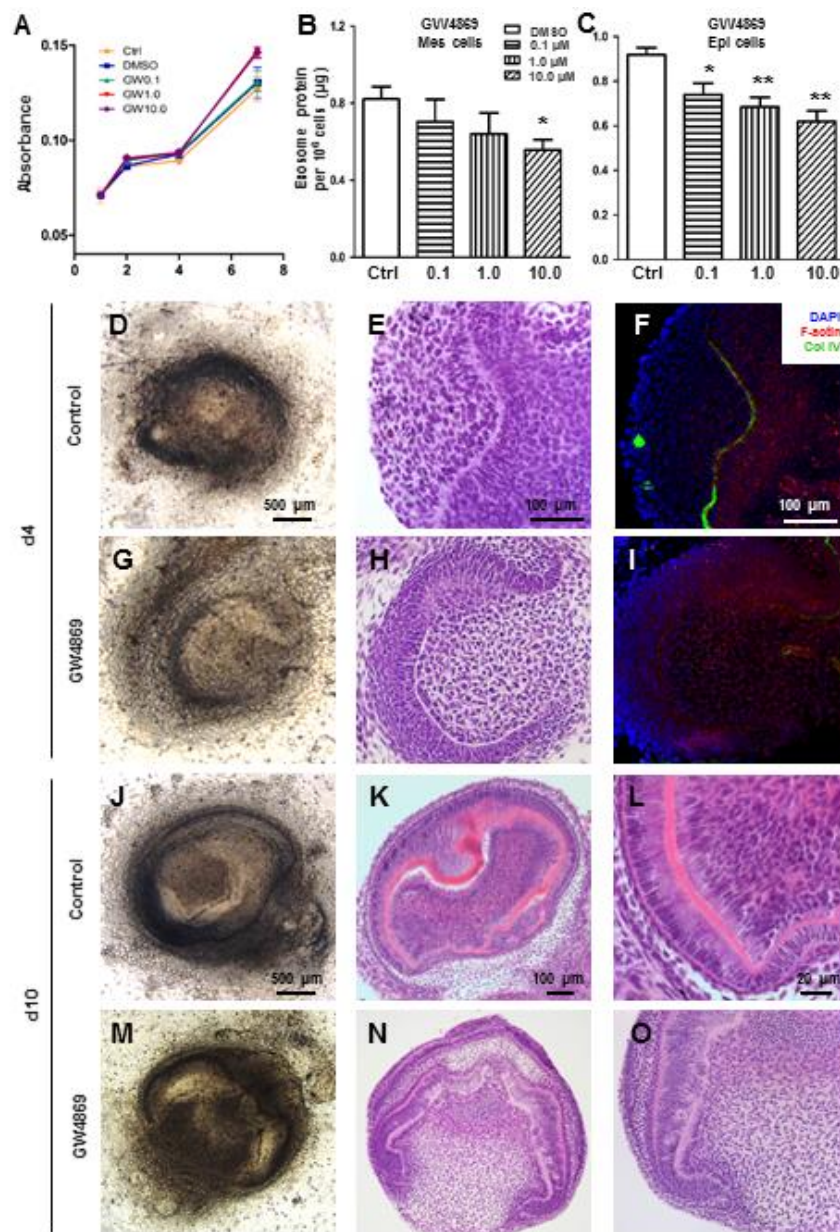
**Supplementary Figure S1.** (A and B) Different endocytosis pathways of epithelium and mesenchyme cells. Immunofluorescence images of dental mesenchymal cells pretreated with PBS (blank), DMSO (control), 10  $\mu$ M CPZ, 50  $\mu$ M LY294002 for 30 min and 5  $\mu$ M nystatin for 120 min, were incubated with labeled epithelium derived exosomes (2  $\mu$ g/ml) except blank group at 37  $^{\circ}$ C for 6 h. (C and D) Exosomes uptake efficiency by recipient cells under various treatments, which is quantified by endocytosis positive cells. The values are normalized to the control. n=6; \*P<0.05, \*\*P<0.01 (one-way ANOVA). All the scale bars are 50  $\mu$ m.



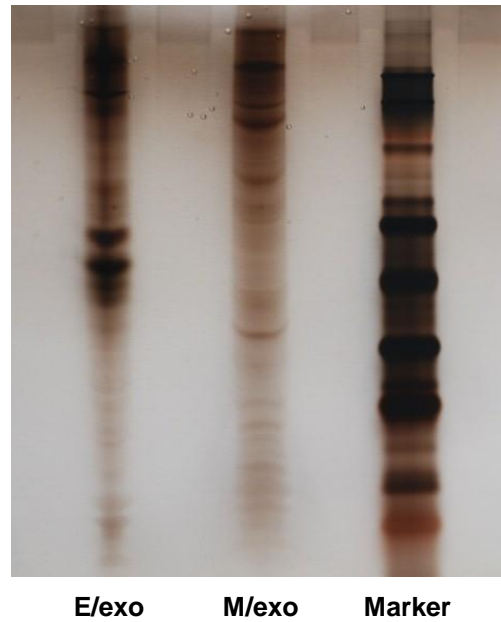
**Supplementary Figure S2.** Reconstituted epithelium and mesenchyme led to a regenerated tooth organ. (A) E16.5 tooth germs digested to remove basement membrane, and separate epithelium (Epi) and mesenchyme (Mes). Epi and Mes reconstituted (B) and cultured in a modified Trowell-type organ culture model (C). (D) Epithelium and mesenchyme attached, with basement membrane components detected by day 2 in immunofluorescence: Col IV (green), F-actin (red) and DAPI (blue). (E) A tooth organ formed in 12 days by reconstituted Epi and Mes tissues, similar to a native late-bell stage tooth germ. H&E staining of 12-day reconstituted tooth organ with dentin formation, cusp structure and polarized ameloblasts and odontoblasts showing in the enlargement area.



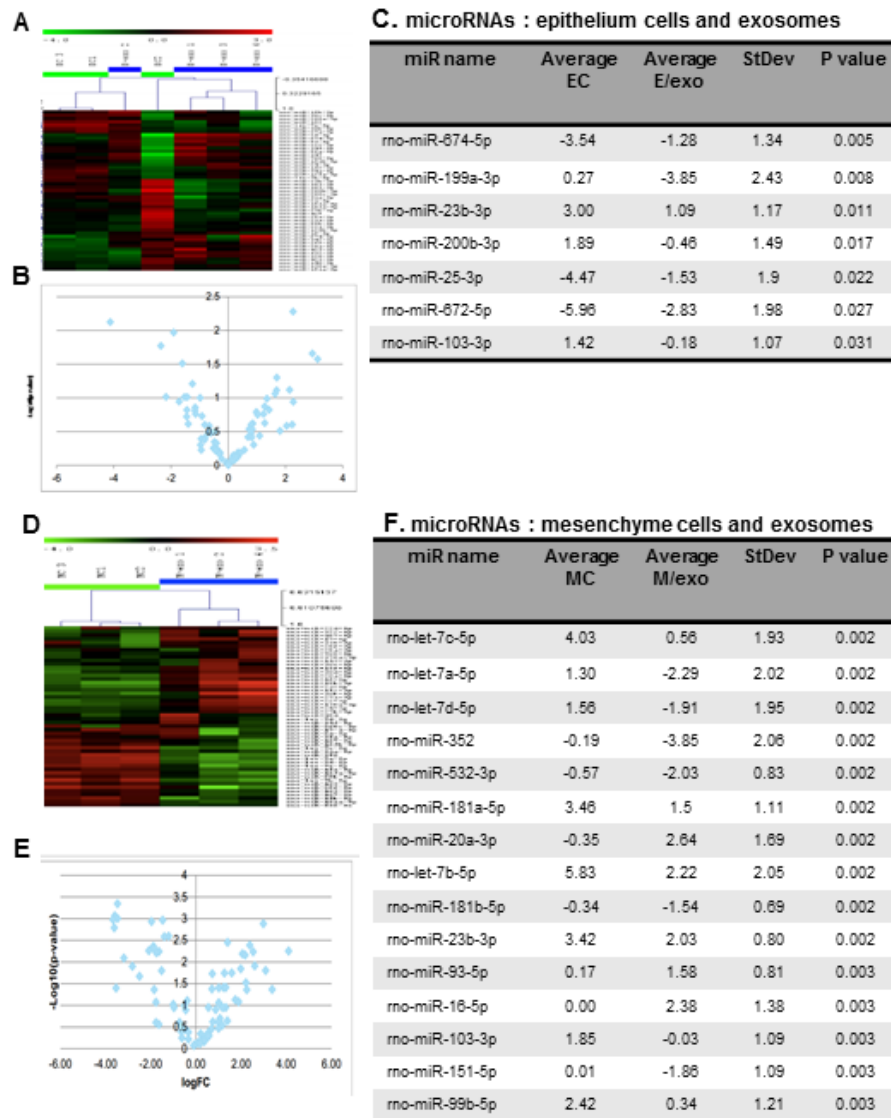
**Supplementary Figure S3.** The siRNA transfection efficiency in reconstituted tooth organ. (A) Phase contrast image of a reconstituted tooth organ at day 1 in the presence of Cy3. (B) Cy3 (red) transfected into reconstituted tooth organ with DAPI staining (blue).



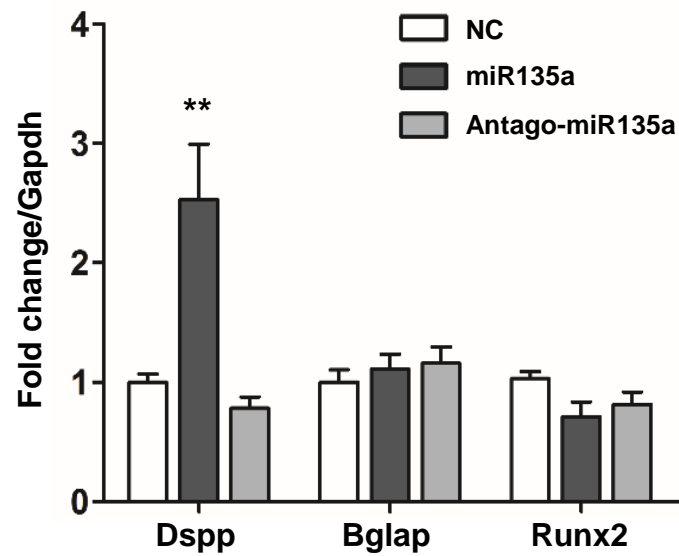
**Supplementary Figure S4.** Attenuation of exosomal secretion by GW4869 causes epithelium-mesenchyme dysmorphogenesis. (A) Proliferation of mesenchyme cells unaffected by GW4869 in the observed 12 days. (B and C) Exosomal protein secretion decreased to ~70% at 10 µg/ml in postnatal 4-5 day mesenchyme (Mes) and epithelium (Epi) cells. (mean±s.d.; four independent experiments). \*P<0.05, \*\*P<0.01 (one-way ANOVA). Epithelium and mesenchyme tissues isolated from E16.5 tooth germs and reconstituted. (D) By day 4, the reconstituted tooth organ formed a basement membrane (E) that was positive for Col IV (F). (G-I) GW4869 at 10 µM attenuated basement membrane formation, with virtual absence of Col IV (I). (J and K) By day 10, the reconstituted tooth organ formed a tooth crown-like structure with dentin formation (L). (M-O) GW4869 virtually abolished dentin mineralization by day 10.



**Supplementary Figure S5.** Exosomal proteins. Proteins extracted from epithelium exosomes (E/exo) and mesenchyme exosomes (M/exo) loaded onto a 4-12% SDS-PAGE gel, followed by silver staining and mass spectrometry.



**Supplementary Figure S6.** miRNAs differentially expressed from those in their parent cells by statistical significance. (A, D) Heat map of microRNA profiles differentially expressed by epithelium/mesenchyme cells and exosomes. (B, E) The volcano plot shows the relation between the p-values and the ddCp of epithelium/mesenchyme cells and exosomes. (C, F) List of microRNAs that significantly differential expressed between parent cells and exosomes.



**Supplementary Figure S7.** mRNA expression of Dsp, Bglap and Runx2 of mesenchymal cells transfected with miRNA mimic, miR135a or Antago-miR135a. (mean $\pm$ s.d.; four independent experiments) \*\*P < 0.01 (one-way ANOVA and LSD test).

**Supplementary Table S1.** Epithelium and mesenchyme exosome proteins (separate Word files)

<b>1) Epi &lt;20KD</b>	
<b>Rat Protein Identified</b>	<b>MW(D)</b>
histone H4 [Homo sapiens]	11360
transgelin-2 [Rattus norvegicus]	22550
albumin, isoform CRA_a [Rattus norvegicus]	53060
cofilin-1 [Rattus norvegicus]	18749
phosphatidylethanolamine-binding protein 1 [Rattus norvegicus]	20902
connective tissue growth factor [Rattus norvegicus]	13386
actin, alpha skeletal muscle [Homo sapiens]	42366
destrin [Rattus norvegicus]	18807
PREDICTED: uncharacterized protein LOC679994 [Rattus norvegicus]	73760
Chain A, Crystal Structure Of A Mammalian 2-Cys Peroxiredoxin, Hbp23.	22250
ribosomal protein P2=iron-binding protein [rats, liver, Peptide Partial, 12 aa, segment 3 of 4]	1242
peptidyl-prolyl cis-trans isomerase A [Rattus norvegicus]	18091
histone H2A type 4 [Rattus norvegicus]	14275
peroxiredoxin-2 [Rattus norvegicus]	21941
rCG45615, isoform CRA_d [Rattus norvegicus]	8201
40S ribosomal protein S18 [Mus musculus]	17708
actin related protein 2/3 complex, subunit 3 (predicted), isoform CRA_a [Rattus norvegicus]	19864
<b>2) Epi 20-40KD</b>	
actin, cytoplasmic 1 [Homo sapiens]	42052
pro1 collagen type III [Rattus norvegicus]	62863
aldose reductase-related protein 2 [Rattus norvegicus]	36510
aldose reductase [Rattus norvegicus]	36230
procollagen, type 1, alpha 1, isoform CRA_a [Rattus norvegicus]	72112
annexin A2 [Rattus norvegicus]	38939
Similar to 14-3-3 protein sigma [Rattus norvegicus]	28698
triosephosphate isomerase [Rattus norvegicus]	27417
nucleolar protein B23.2 [Rattus norvegicus]	28482
histone (H1d) [Rattus norvegicus]	21832
glyceraldehyde 3-phosphate-dehydrogenase [Rattus norvegicus]	36098
unnamed protein product [Rattus rattus]	34350
lipocortin I [Rattus sp.]	39137
14-3-3 protein epsilon [Homo sapiens]	29326
RecName: Full=Heat shock protein beta-1; Short=HspB1; AltName: Full=Heat shock 27 kDa protein; Short=HSP 27	22936
14-3-3 protein eta [Mus musculus]	28365
odontogenic ameloblast-associated protein precursor [Rattus norvegicus]	30424
translation elongation factor 1-delta subunit [Rattus norvegicus]	5176
histone cluster 1, H1b [Rattus norvegicus]	22635
PREDICTED: uncharacterized protein LOC679994 [Rattus norvegicus]	73760
ribosomal protein S4	29892
heat shock protein 90 [Rattus sp.]	83606



14-3-3 protein theta [Mus musculus]	28046
14-3-3 zeta isoform [Rattus norvegicus]	27955
unnamed protein product [Rattus norvegicus]	70670
brain alpha-tropomyosin (TMBr-1) [Rattus norvegicus]	32547
uncharacterized protein LOC364814 [Rattus norvegicus]	28047
<b>3) Epi 40-50KD</b>	
actin, cytoplasmic 2 [Homo sapiens]	42108
unnamed protein product [Rattus norvegicus]	47428
laminin, gamma 2 precursor [Rattus norvegicus]	134651
aldolase A [Rattus norvegicus]	39691
elongation factor-1 alpha [Rattus norvegicus]	50460
rCG31394, isoform CRA_b [Rattus norvegicus]	51370
amelin 1 [Rattus norvegicus]	43455
hemiferrin, transferrin-like protein [Rattus norvegicus]	24874
serine (or cysteine) proteinase inhibitor, clade E, member 2, isoform CRA_b [Rattus norvegicus]	35647
serum albumin precursor [Rattus norvegicus]	70710
elongation factor 1-gamma [Rattus norvegicus]	50371
Chain A, Rat Liver S-Adenosylhomocystein Hydrolase	47889
Chain E, Crystal Structures Of Rat Anionic Trypsin Complexed With The Protein Inhibitors Appi And Bpti	24467
odontogenic ameloblast-associated protein precursor [Rattus norvegicus]	30424
tubulin alpha	50894
ruvB-like 1 [Mus musculus]	50524
Sept11 protein [Mus musculus]	49219
multifunctional protein ADE2 [Rattus norvegicus]	47807
tubulin beta-5 chain [Mus musculus]	50095
CaBP1 [Rattus norvegicus]	47590
follistatin-related protein 1 precursor [Rattus norvegicus]	35740
serine protease HTRA1 precursor [Rattus norvegicus]	52210
histone H4 [Homo sapiens]	11360
<b>4) Epi 50-100KD</b>	
laminin, gamma 2 precursor [Rattus norvegicus]	134651
dnaK-type molecular chaperone hsp72-ps1 - rat	71112
78 kDa glucose-regulated protein precursor [Rattus norvegicus]	72473
heat shock protein HSP 90-beta [Mus musculus]	83571
heat shock protein HSP 90-alpha [Rattus norvegicus]	85161
endoplasmic precursor [Rattus norvegicus]	92998
laminin, beta 3 precursor [Rattus norvegicus]	132908
transitional endoplasmic reticulum ATPase [Rattus norvegicus]	89977
RecName: Full=Nucleolin; AltName: Full=Protein C23	77158
periostin, osteoblast specific factor (predicted), isoform CRA_b [Rattus norvegicus]	77990
alpha actinin [Rattus norvegicus]	102899
alpha-actinin-1 [Rattus norvegicus]	103466

PREDICTED: putative uncharacterized protein C8orf73-like [Rattus norvegicus]	78414
myosin-9 [Rattus norvegicus]	227566
elongation factor 2 [Rattus norvegicus]	96192
actin, alpha skeletal muscle [Homo sapiens]	42366
<b>5) Epi &gt;100KD</b>	
similar to Laminin alpha-3 chain precursor (Nicein alpha subunit), isoform CRA_b [Rattus norvegicus]	192602
laminin-5 alpha 3 chain [Rattus norvegicus]	192555
laminin, beta 3 precursor [Rattus norvegicus]	132908
laminin, gamma 2 precursor [Rattus norvegicus]	134651
tenascin-N precursor [Rattus norvegicus]	174967
fibronectin 1, isoform CRA_d [Rattus norvegicus]	266235
rCG55184, isoform CRA_a [Rattus norvegicus]	171938
filamin, alpha (predicted), isoform CRA_b [Rattus norvegicus]	279491
collagen alpha-1(VII) chain precursor [Rattus norvegicus]	259785
thrombospondin 1 precursor [Rattus norvegicus]	133579
procollagen, type XII, alpha 1, isoform CRA_b [Rattus norvegicus]	310201
<b>6) Mes &lt;20KD</b>	
histone H4 [Homo sapiens]	11360
albumin, isoform CRA_a [Rattus norvegicus]	53060
peptidyl-prolyl cis-trans isomerase B precursor [Rattus norvegicus]	22845
PREDICTED: uncharacterized protein LOC679994 [Rattus norvegicus]	73760
rCG45615, isoform CRA_d [Rattus norvegicus]	8201
histone H2A type 1-C [Homo sapiens]	14097
ribosomal protein P2=iron-binding protein [rats, liver, Peptide Partial, 12 aa, segment 3 of 4]	1242
vimentin, partial [Rattus norvegicus]	8971
dermcidin preproprotein [Homo sapiens]	11391
cofilin-1 [Rattus norvegicus]	18749
<b>7) Mes 20-40KD</b>	
Collagen alpha1 [Rattus norvegicus]	138828
pro1 collagen type III [Rattus norvegicus]	62863
PREDICTED: uncharacterized protein LOC684681 [Rattus norvegicus]	21304
actin, cytoplasmic 1 [Homo sapiens]	42052
annexin II [Rattus sp.]	39236
collagen alpha-2(I) chain precursor [Rattus norvegicus]	129999
tropomyosin beta chain [Mus musculus]	32931
histone cluster 1, H1b [Rattus norvegicus]	22635
<b>8) Mes 40-60KD</b>	
78 kDa glucose-regulated protein precursor [Rattus norvegicus]	72473
heat shock cognate 71 kDa protein [Rattus norvegicus]	71055
lactadherin isoform 2 precursor [Rattus norvegicus]	48522

procollagen C-endopeptidase enhancer 1 precursor [Rattus norvegicus]	50837
M2 pyruvate kinase [Rattus norvegicus]	58314
serpin H1 precursor [Rattus norvegicus]	46602
protein disulfide-isomerase A4 precursor [Rattus norvegicus]	73103
grp75 [Rattus sp.]	73984
PREDICTED: putative uncharacterized protein C8orf73-like [Rattus norvegicus]	78414
elongation factor-1 alpha [Rattus norvegicus]	50460
pigment epithelium-derived factor precursor [Rattus norvegicus]	46493
beta-hexosaminidase subunit beta precursor [Rattus norvegicus]	61888
serum albumin precursor [Rattus norvegicus]	70710
protein disulfide-isomerase A3 precursor [Rattus norvegicus]	57010
<b>9) Mes 60-100KD</b>	
periostin, osteoblast specific factor (predicted), isoform CRA_d [Rattus norvegicus]	90879
tenascin-N precursor [Rattus norvegicus]	174967
heat shock protein HSP 90-beta [Mus musculus]	83571
endoplasmic precursor [Rattus norvegicus]	92998
heat shock protein HSP 90-alpha [Rattus norvegicus]	85161
RecName: Full=Nucleolin; AltName: Full=Protein C23	77158
filamin, alpha (predicted), isoform CRA_b [Rattus norvegicus]	279491
procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2, long variant [Rattus norvegicus]	87527
transitional endoplasmic reticulum ATPase [Rattus norvegicus]	89977
histone (H1d) [Rattus norvegicus]	21832
elongation factor 2 [Rattus norvegicus]	96192
keratin, type II cytoskeletal 5 [Rattus norvegicus]	62060
PREDICTED: keratin, type II cytoskeletal 6A-like isoform 1 [Rattus norvegicus]	59735
actin, alpha skeletal muscle [Homo sapiens]	42366
procollagen, type XII, alpha 1, isoform CRA_b [Rattus norvegicus]	310201
lactadherin isoform 2 precursor [Rattus norvegicus]	48522
<b>10) Mes &gt;100KD</b>	
PREDICTED: collagen alpha-1(XII) chain [Rattus norvegicus]	333977
tenascin-N precursor [Rattus norvegicus]	174967
tenascin N (predicted) [Rattus norvegicus]	88723
AE binding protein 1 (predicted) [Rattus norvegicus]	116181
fibronectin 1, isoform CRA_d [Rattus norvegicus]	266235
filamin, alpha (predicted), isoform CRA_b [Rattus norvegicus]	279491
Tln1 protein [Rattus norvegicus]	163474
periostin, osteoblast specific factor (predicted), isoform CRA_b [Rattus norvegicus]	77990
laminin, gamma 1 [Rattus norvegicus]	175782
PREDICTED: laminin subunit beta-1, partial [Rattus norvegicus]	202701
thrombospondin 2 precursor [Rattus norvegicus]	133522