

Quantification of tube formation (F) and chemotaxis assay using Boyden chambers (G) of HUVECs incubated with microparticles isolated from MCS-fed mice (control diet) and MCD-fed mice treated with VNN1 siRNA, control siRNA, or PBS (mock) (n = 5 mice per group). VEGF was used as a positive control, and serum-free medium as a negative control. Values in (C), (E), (F), and (G) represent means \pm SD from three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001, Kruskal-Wallis with Bonferroni correction.

Supplemental Figure 1. HepG2 exposed to different saturated and unsaturated free fatty acids. Representative micrographs of Oil red-O staining for lipid droplets in HepG2 exposed to 1% BSA (control) or 0.25 mM of oleic, palmitic and stearic acid for 24 h. 20X magnification was used for acquisition of the pictures.

Supplemental Figure 2. Effect of lipotoxic FFAs is counteracted by non-lipotoxic FFAs. HepG2 were exposed to 0.25 mM of palmitic acid, oleic acid and a mixture of both up to 24 hrs. MPs were isolated from the supernatant and Annexin V+ positive MPs were detected by flow cytometry and quantity is reported in the graph. Values represent mean \pm S.D. * P < 0.05; ** P < 0.01; ***P < 0.001 compared to controls.

Supplemental Figure 3. Cellular localization and molecular function of proteins from hepatocyte-derived MPs. All the proteins obtained by three different proteomics analysis of MPs from HepG2 cells exposed to palmitic acid were organized based on cellular localization (top pie chart) and molecular function (bottom pie chart). Percentages over the total number of proteins were reported in the pie charts.

Supplemental Figure 4. MPs released by fat-laden rat primary hepatocytes are potent inducers of angiogenesis. (A) Representative micrographs of tube formation of HUVECs after exposure up to 6 h to rat primary hepatocytes-derived MPs, MP-free supernatant or controls. (B) HUVECs total tube

length has been measured and reported in the histogram. (C) Representative micrographs of chemotaxis assay (Boyden's chamber assay) of HUVECs were tested with primary hepatocytes-derived MPs, MP-free supernatant or controls, as described in Methods. A 10X magnification was used for microscopic pictures. (D) An average of HUVECs migrated into the filter was measured and reported in the graph. VEGF (100 ng/ml) was used as a positive control. Values represent mean \pm S.D. from three independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to controls.

Supplemental Figure 5. Pro-angiogenic effect of hepatocytes-derived MPs is dose-dependent.

HepG2 were treated with 0.25 mM of palmitic acid up to 24 h and MPs were isolated from the supernatant by ultracentrifugation. MPs samples were quantified by BCA protein assay and the concentration has been determined. Different doses (50, 125, 250 and 500 $\mu\text{g/mL}$) have been used for assessing (A) HUVECs tube formation and (B) chemotaxis assay (Boyden's chamber assay). Quantification graphs are reported in the figure. A dose of 100 ng/mL of VEGF has been used as positive control. Values represent mean \pm S.D. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to controls.

Supplemental Figure 6. Endothelial cells angiogenesis in vitro depends on internalization of MPs into HUVECs. Representative micrographs of tube formation of HUVECs after exposure to HepG2-derived MPs^{Calcein} up to 6 hours. HUVECs tubes were detected by an Olympus FV1000 Spectral Confocal with 20X lens.

Supplemental Figure 7. Genetic suppression of VNN1 expression on MPs significantly reduced the pro-angiogenic effects of MPs on endothelial cell migration and tube formation.

HepG2 were exposed to palmitic acid (PA) for 24 h and then treated with VNN1 siRNA or control RNA (Ctrl RNA). Representative micrographs of (A) tube formation and (B) chemotaxis (Boyden's chamber assay) of HUVECs treated with hepatocyte-derived MPs, MP-free supernatant (MP-free

sup.) or controls for 6 h and 16 h, respectively. A dose of 100 ng/mL of VEGF was used as positive control. A 4X magnification was used for acquisition of pictures.

Supplemental Figure 8. Pro-angiogenic effect of hepatocytes-derived microparticles acts through VNN1-dependent internalization. (A) Fat-laden HepG2-derived MPs were stained with 1 μ M of Calcein AM-FITC (MPs^{Calcein}), washed to removed unspecific bound and incubated with or without a neutralizing antibody anti-VNN1 (4 μ g/mL) (VNN1 nAb) and anti-GAPDH for 30 minutes. MPs were then incubated with HUVECs for 6 hours and FITC-positive events were detected by FACS. (B) Number of FITC-positive HUVECs exposed to MP^{Calcein} with or without VNN1 were reported in graph; (C) tube formation of HUVECs after exposure for 6 hours to HepG2-derived MPs with or without VNN1 or controls; (D) chemotaxis assay of HUVECs was performed with HepG2-derived MPs with or without VNN1 or controls as described in Methods. (E) Protein expression of endothelial cells markers activation, ICAM-1 and VCAM-1, after exposure up to 6 hours with hepatocytes-derived MPs or MPs pre-incubated with a VNN1 neutralizing antibody. GAPDH was used as loading control. (F) Glutathione activity assay analysis in HUVECs treated with HepG2-derived MPs with or without VNN1 or controls. Glutathione activity is reported in RFU. * P < 0.05; ** P < 0.01; ***P < 0.001, compared to controls.

Supplemental Figure 9. Proangiogenic effects of VNN1 positive MPs are not mediated by induction of endothelial cell proliferation or modulation of PPAR- γ expression. Fat-laden HepG2-derived MPs were incubated with or without a rabbit polyclonal antibody anti-VNN1 (4 μ g/mL) for 30 minutes. A BrdU proliferation assay has been performed with HUVECs incubated for 16 h with MPs, MPs and VNN1 neutralizing Ab or controls. (A) Flow cytometry analysis gating (P3) proliferating-FITC-positive HUVECs. A BrdU negative staining has been included as negative control. (B) RNA was isolated from HUVECs that were incubated for 16 h with MPs, MPs and VNN1 nAb or controls in order to determine the expression of PPAR- γ by quantitative PCR. The

housekeeping gene 18S was used as an internal control. Values represent mean \pm S.D. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to controls.

Supplemental Figure 10. Increase of VNN1 in hepatocytes during lipotoxicity is independent

of PPAR α and γ . (A) Quantitative PCR of PPAR γ expression in HepG2 treated with 0.25 mM of palmitic acid (PA) or 1% BSA (vehicle) for 24 h. (B) Quantitative PCR for PPAR α and (C) VNN1 in HepG2 treated with 1% BSA, 0.25 mM of palmitic acid (PA), oleic acid (OA) or palmitic acid/oleic acid mix for 24 h. (D) Quantitative PCR for PPAR α and (E) VNN1 in HepG2 exposed to 1% BSA, 0.25 mM of palmitic acid (PA), oleic acid (OA) or palmitic acid/oleic acid mix for 24 h and treated with PPAR α siRNA or control RNA (Ctrl RNA).

Supplementary Figure 11. Release of circulating microparticles depends on the stage of

NASH. C57/B6 mice (n=5) were put on a MCD, high fat/high carbohydrates (HF/HCarb) or chow diet for 6 weeks. (A) Circulating MPs were isolated as described in Methods and Annexin V+ MPs were detected by flow cytometry. (B) H-E staining. (C) NAFLD activity score. Values represent mean \pm S.D. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to controls.

Supplementary Figure 12. Circulating MPs from mice with NASH stimulate angiogenesis ex

vivo. C57/B6 mice were put on a MCD, high fat/high carbohydrates (HF/HCarb) and chow diet for 6 weeks, platelet-free plasma was harvested and MPs were isolated as described in Methods. MPs and MPs-free supernatant were used to induce HUVECs tube formation and oriented migration (Boyden's chamber assay) ex vivo. Quantification of (A) HUVECs tube formation (total tube length) and (B) chemotaxis assay (Boyden's chamber assay) is reported in the graph. A dose of 100 ng/mL of VEGF has been used as positive control. Values represent mean \pm S.D. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to controls.

Supplemental Figure 13. Liver specific in vivo silencing of VNN1 does not affect VNN1

expression in other tissues. Expression of VNN1 was assessed in (A) kidney, (B) intestine and (C)

spleen harvested from MCD-fed mice treated with VNN1 siRNA, control RNA (CTRL RNA) or PBS (Mock) to confirm the liver-specific knockdown of VNN1. The housekeeping gene 18S was used as an internal control. Values represent mean \pm S.D. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to controls.

Supplemental Figure 14. Casp3^{-/-} mice are protected from MCD-induced pathological angiogenesis and liver fibrosis. (A) Representative micrographs for H-E staining, immunostaining for CD-31, vonWillebrand factor (vWF) and Sirius red staining of liver specimens harvested from WT and Caspase-3 KO mice fed the MCD or control diet (MCS) diet for 6 weeks. A 10X magnification was used for micrographs. (B) Total area in pixel (px) is reported in the graph to show the quantification of CD31 immunostaining. (C) Percentage of fibrosis was measured by using Image J. (C) RNA was isolated from liver samples of C57BL/6 Casp3^{-/-} and WT mice, fed a MCD or MCS diet. Analysis of the transcripts for the pro-angiogenic genes (VEGF-B and FGF- β) and pro-fibrogenic genes (Collagen type I and α -SMA) was assessed by quantitative PCR. The housekeeping gene 18S was used as an internal control. Values represent mean \pm S.D. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to controls.

Supplemental Table 1. Identification of the whole hepatocytes-derived microparticles proteins based on the LC-MS/MS-derived sequences. Pure hepatocytes-derived microparticles were isolated and processed for a complete proteomics analysis as described in the 'Methods' paragraph. The proteins detected are listed in the table with the corresponding uniprot accession code, number of peptides, biological function and cellular localization.

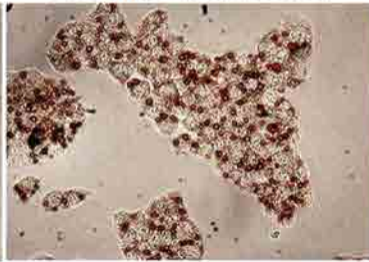
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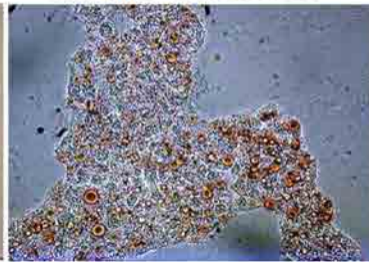
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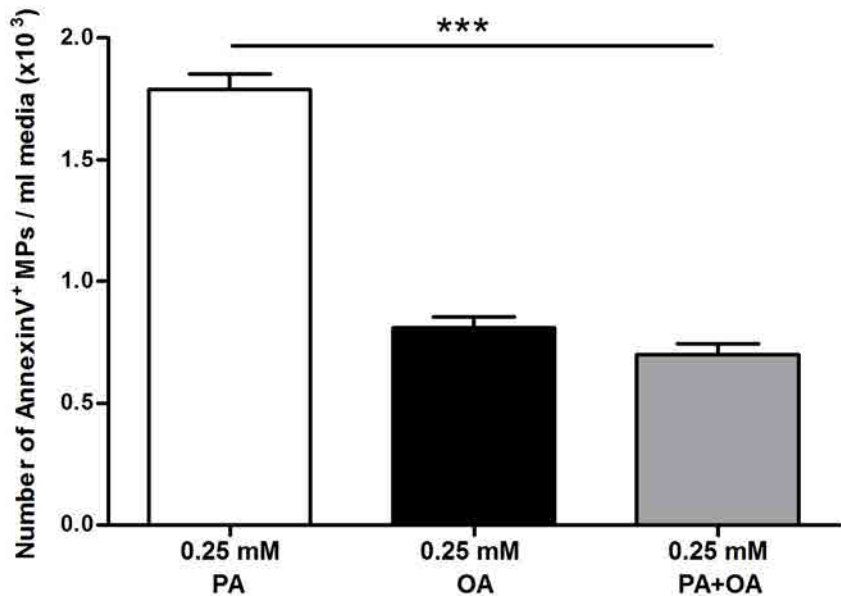


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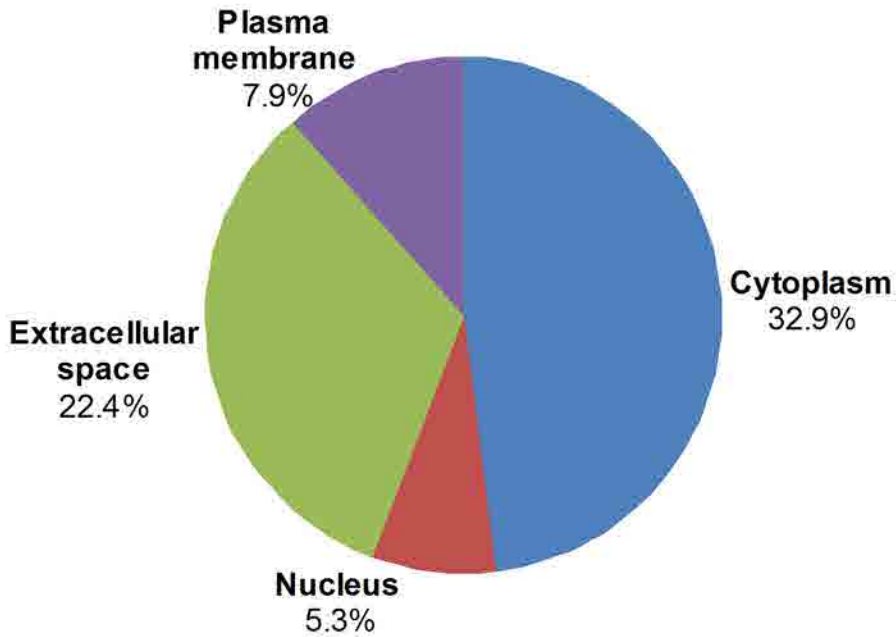


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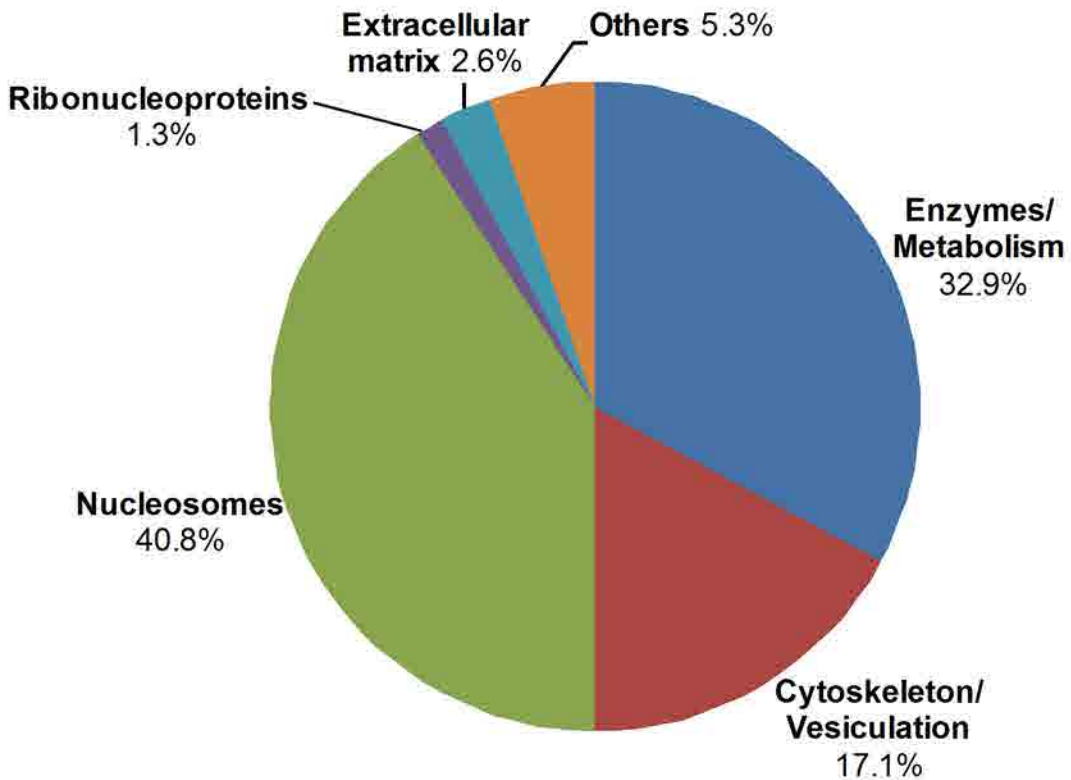


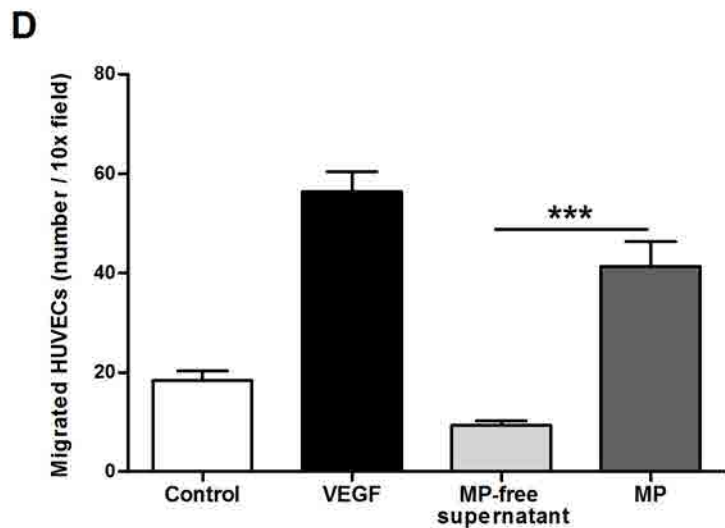
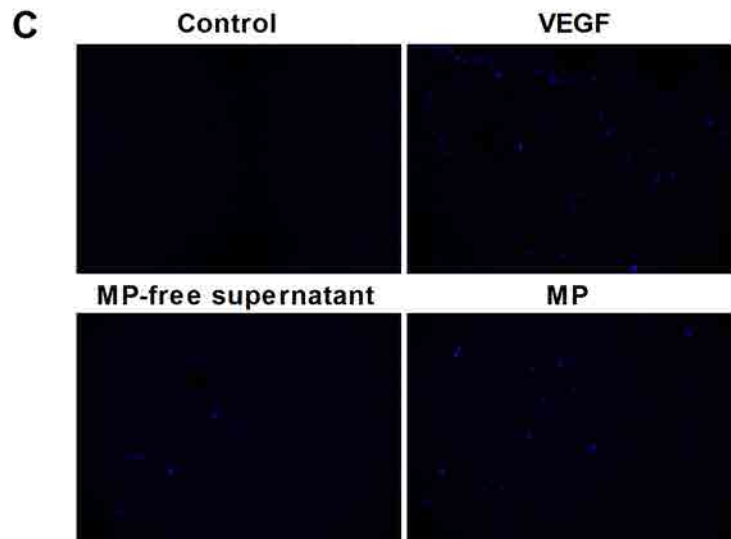
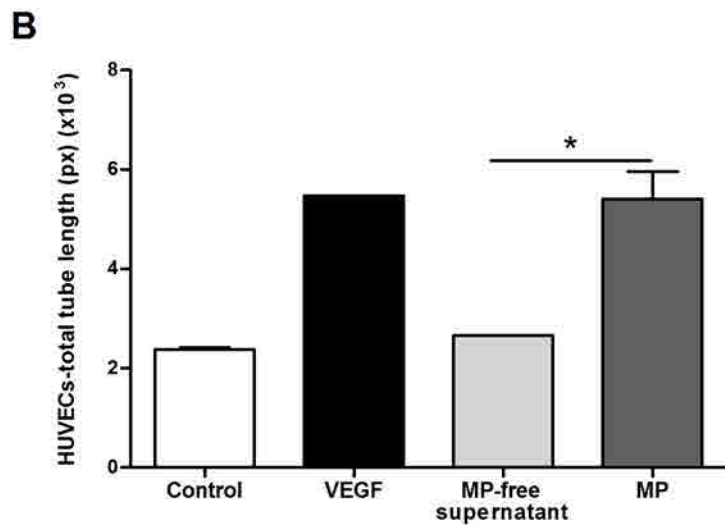
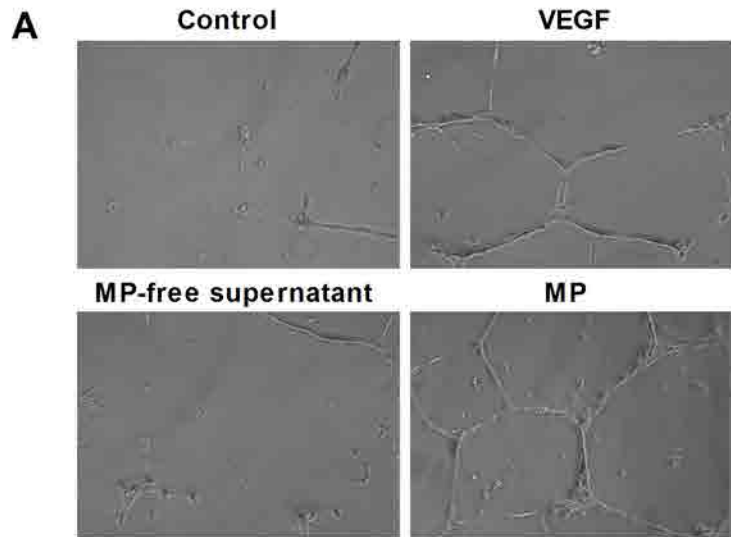


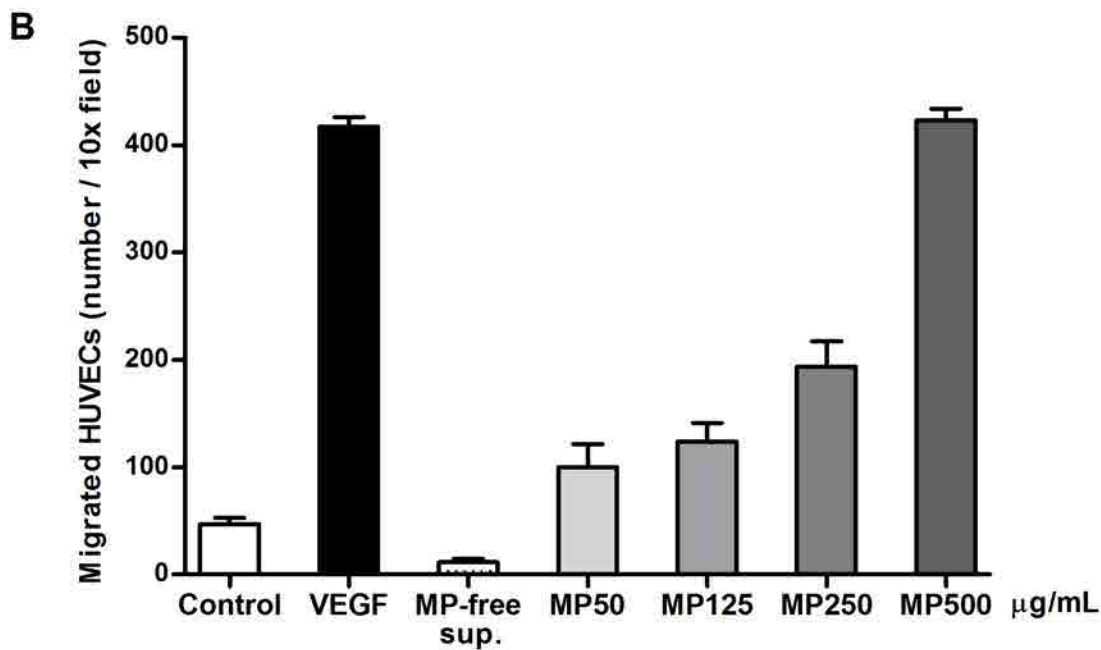
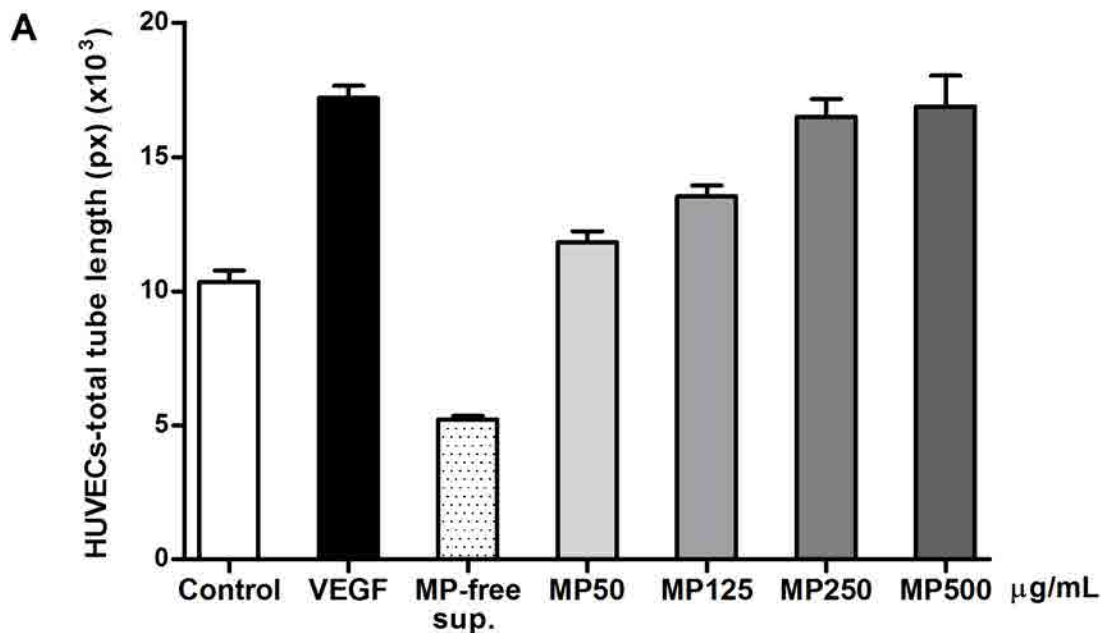
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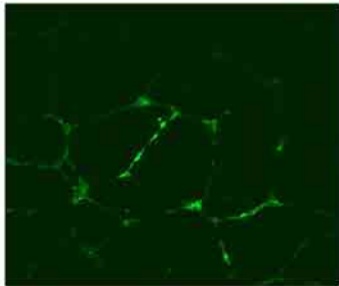
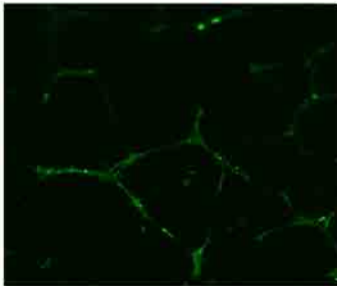
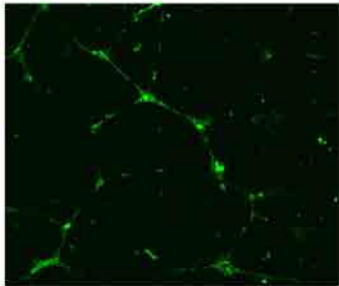
Molecular function







Calcein⁺ HUVECs tubes after treatment with MPs ^{Calcein}



A

Control

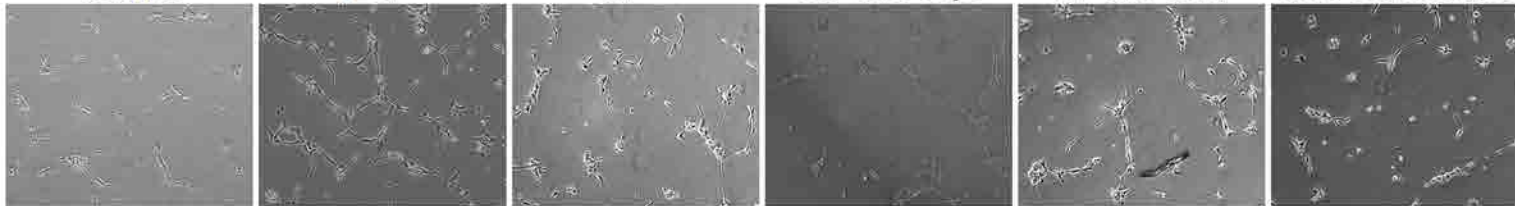
VEGF

MP

MP-free sup.

MP Ctrl RNA

MPs VNN1 siRNA

**B**

Control

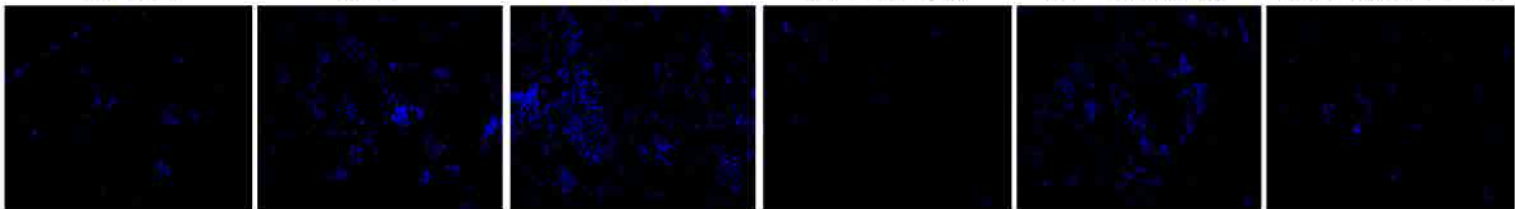
VEGF

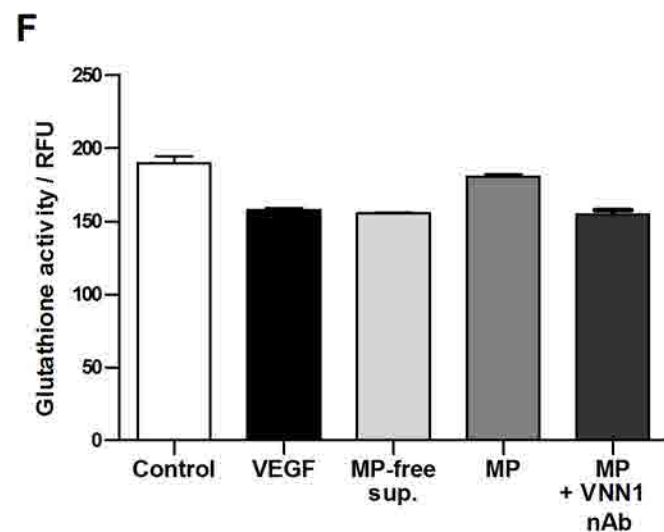
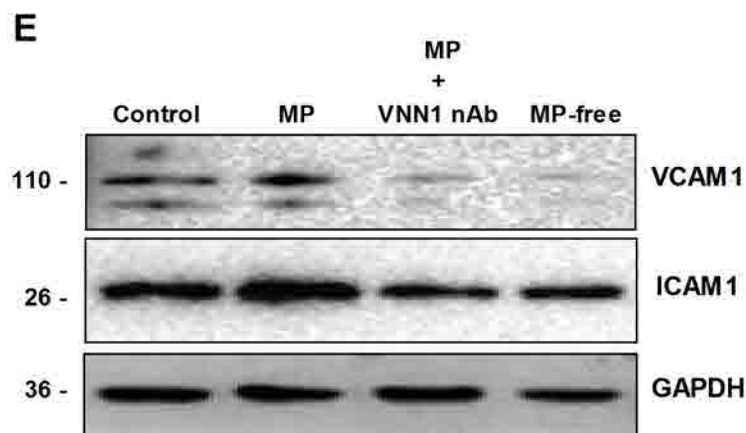
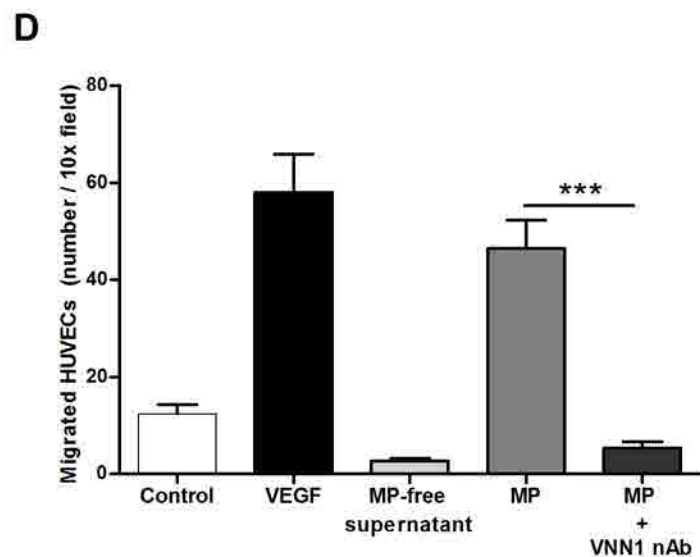
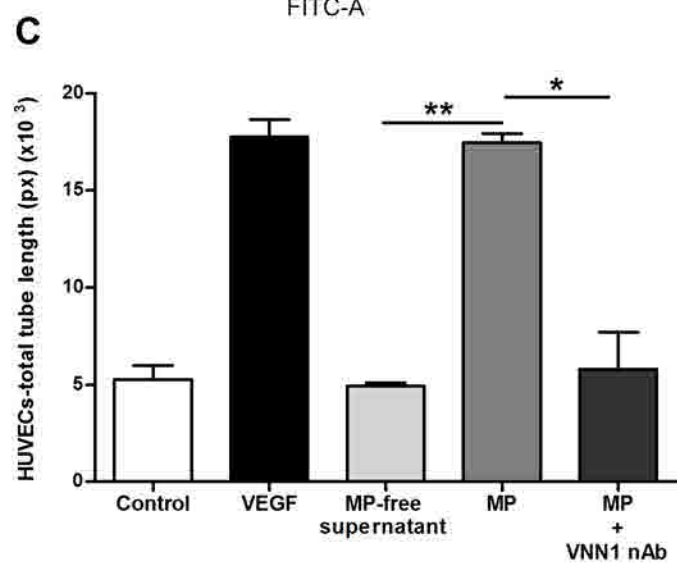
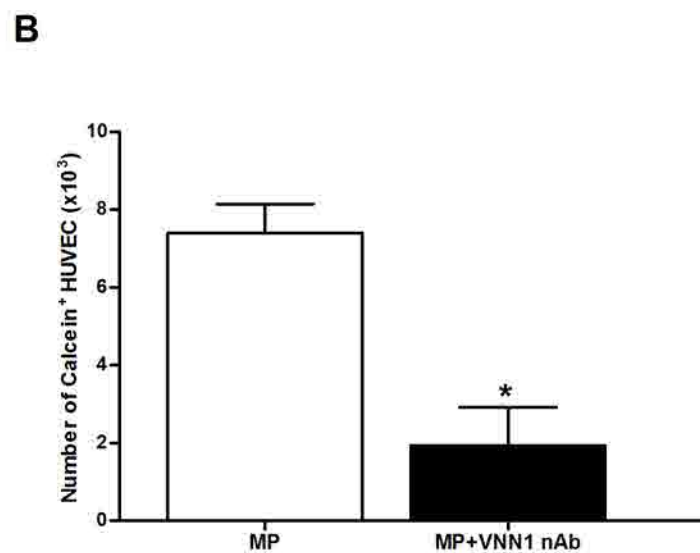
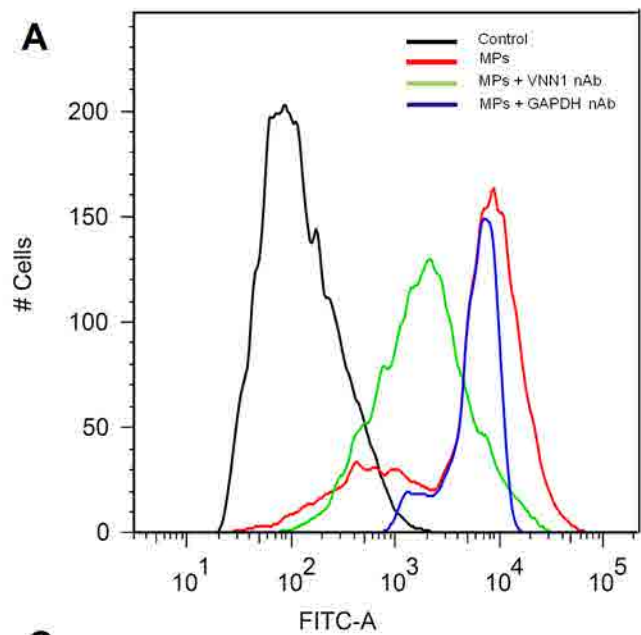
MP

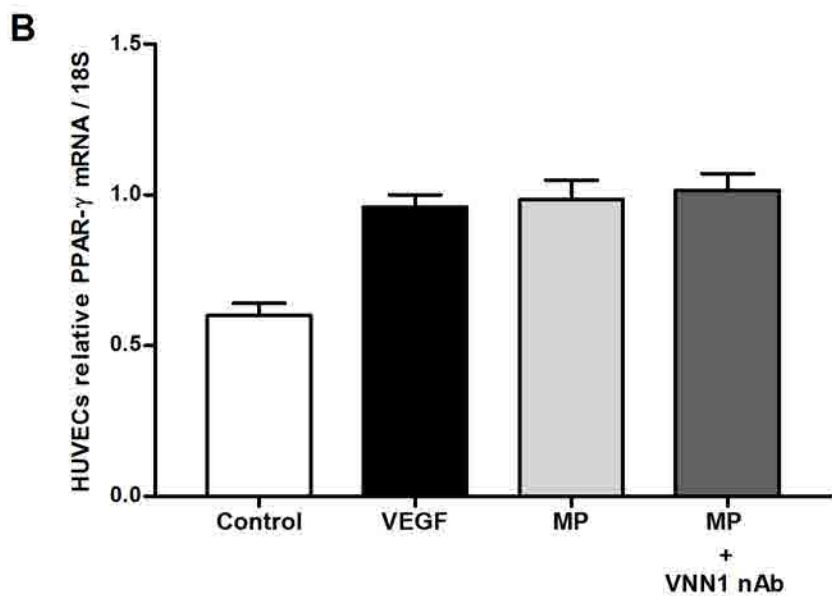
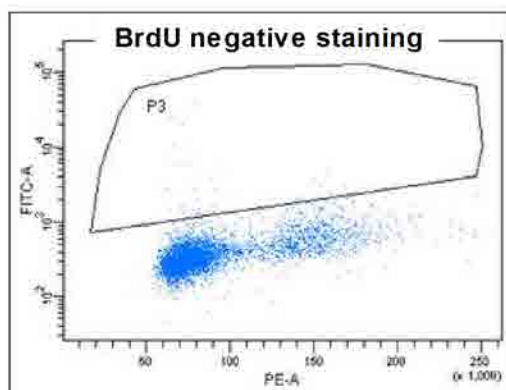
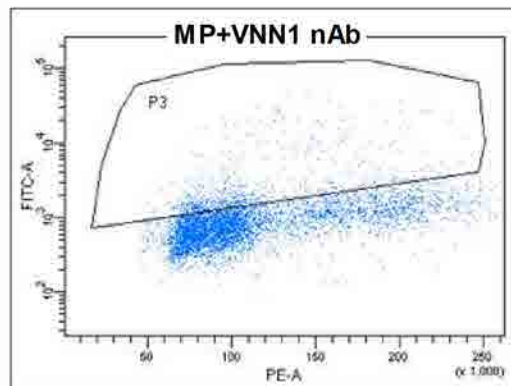
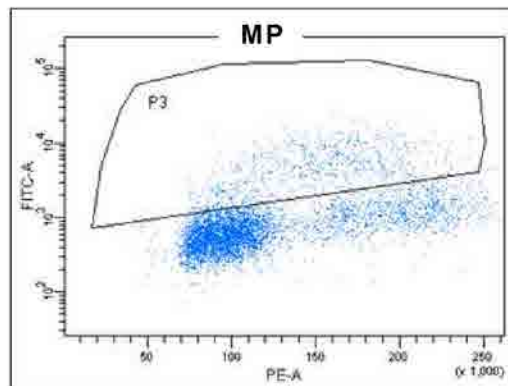
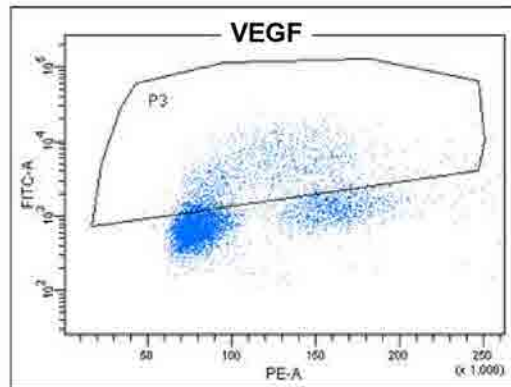
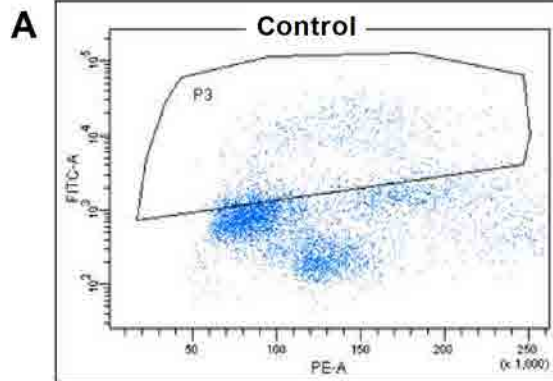
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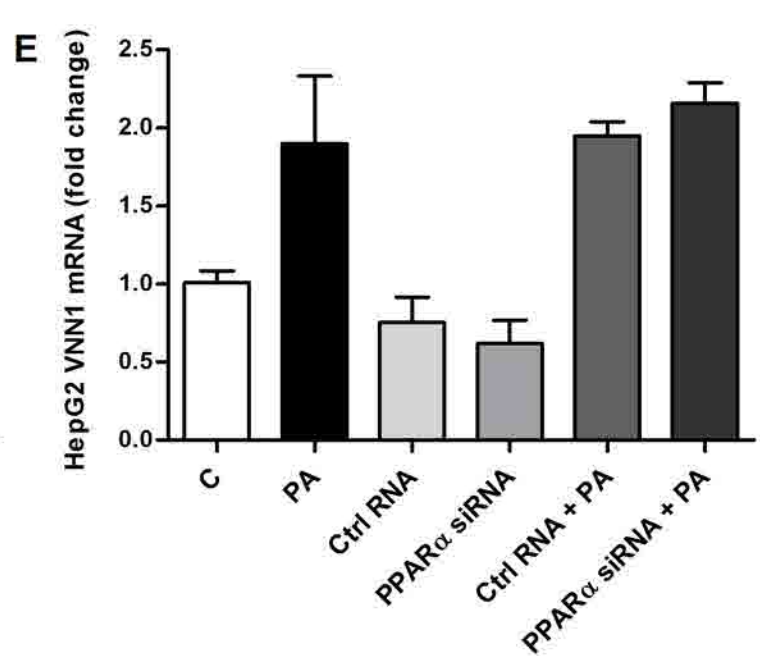
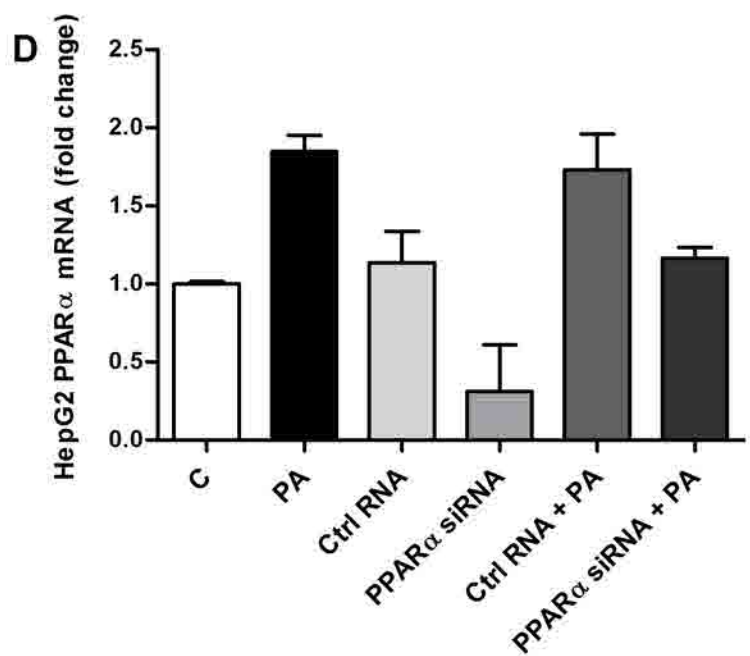
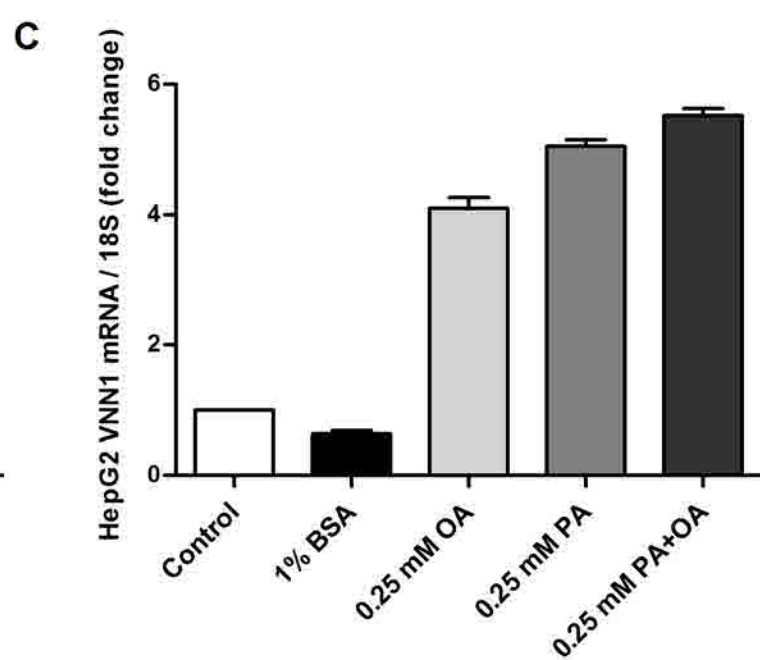
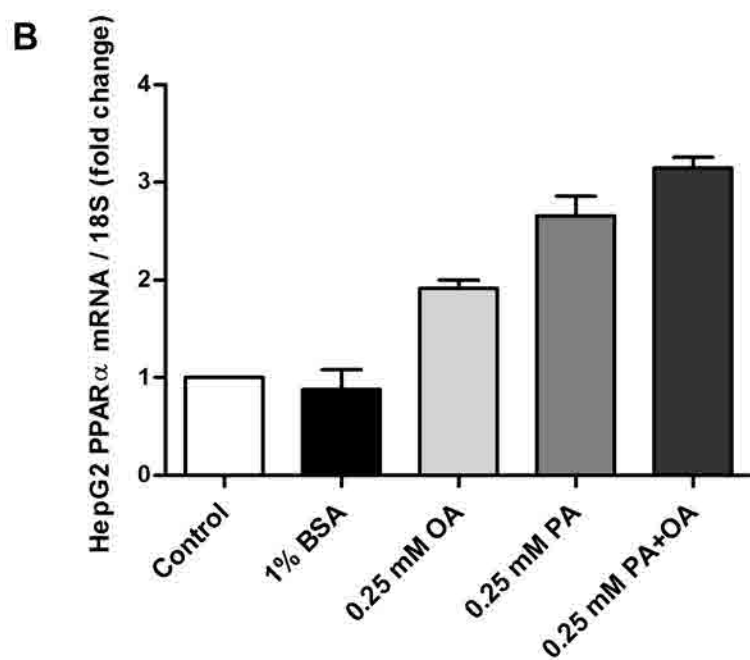
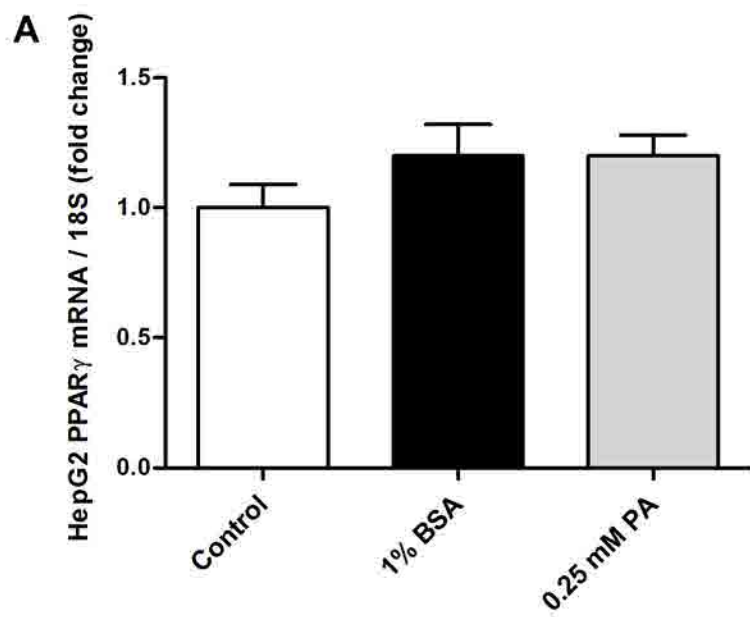
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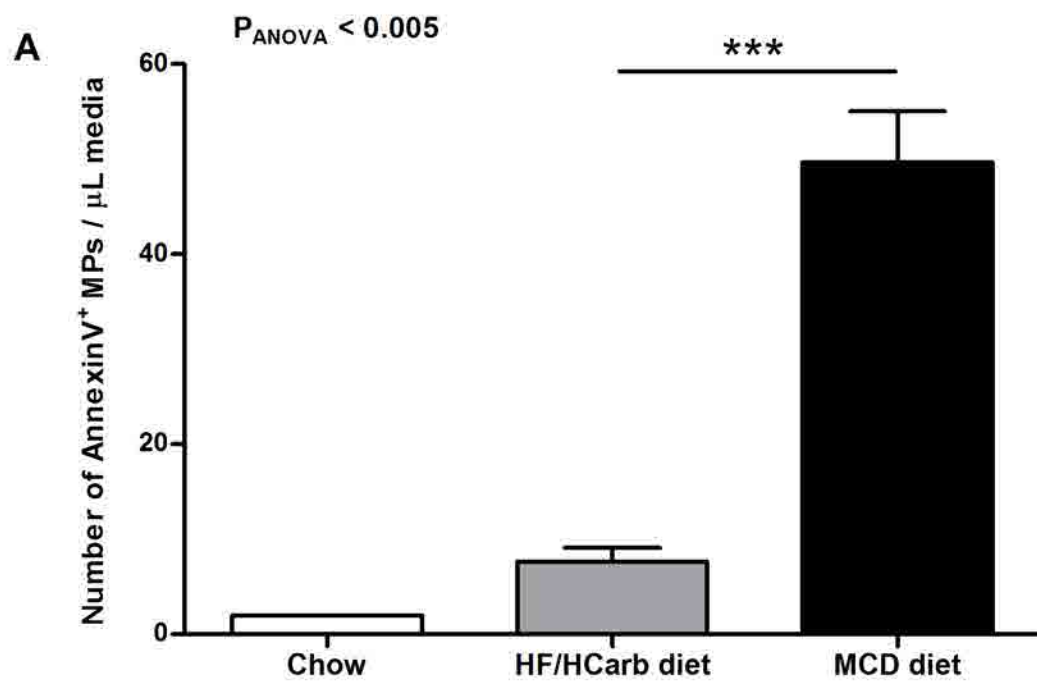
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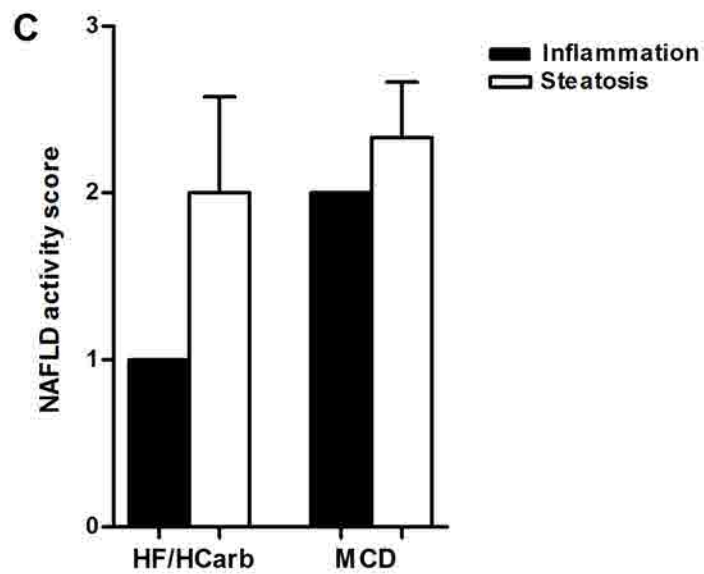
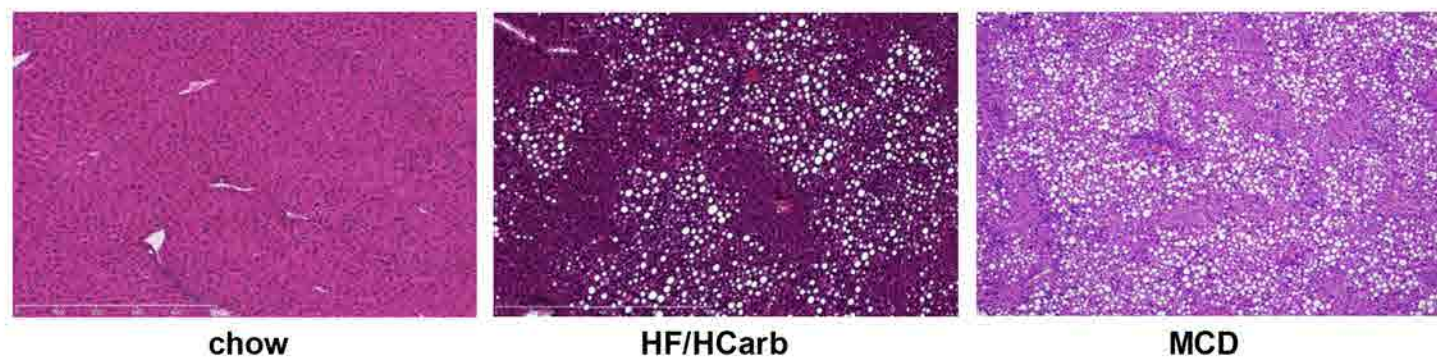


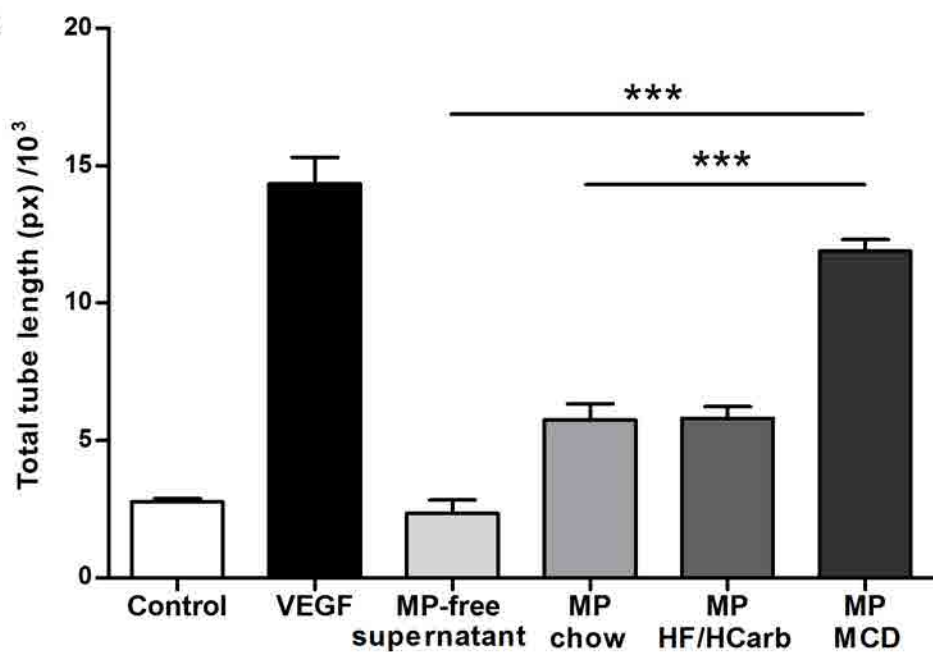
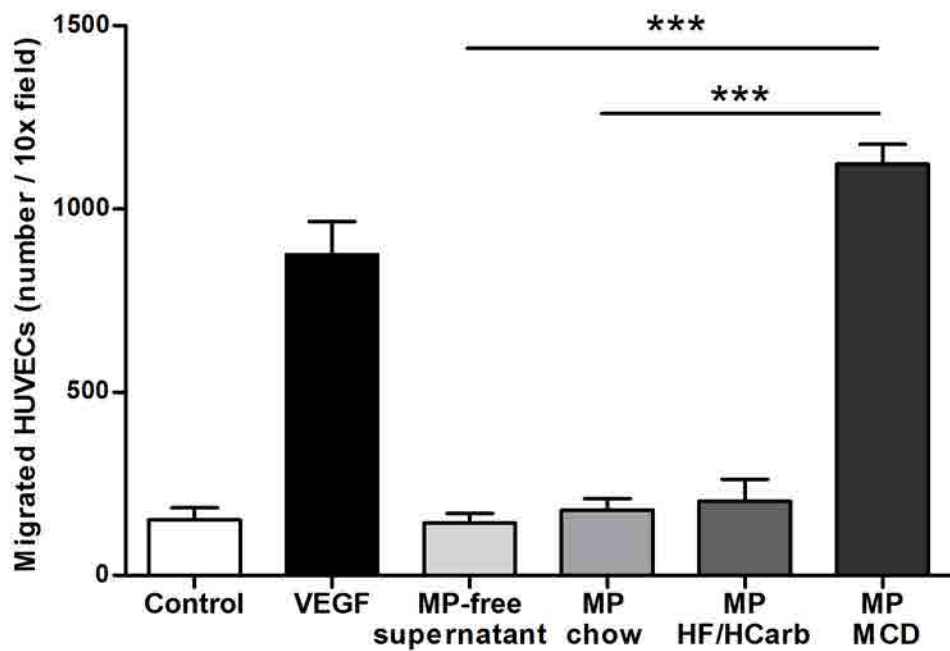


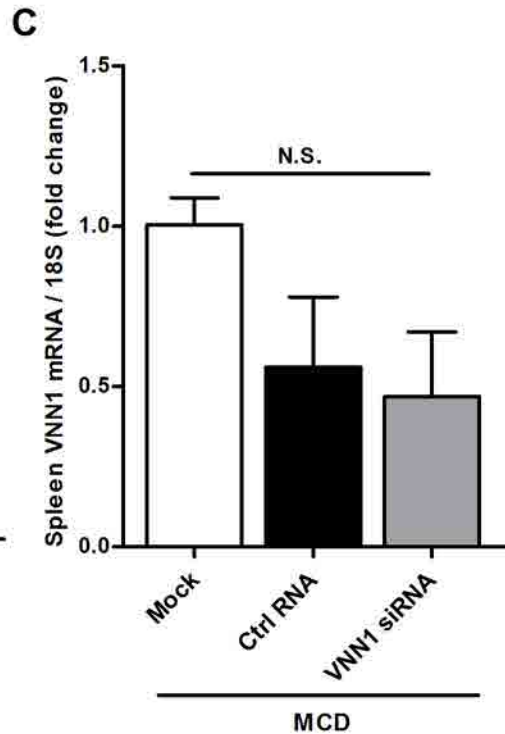
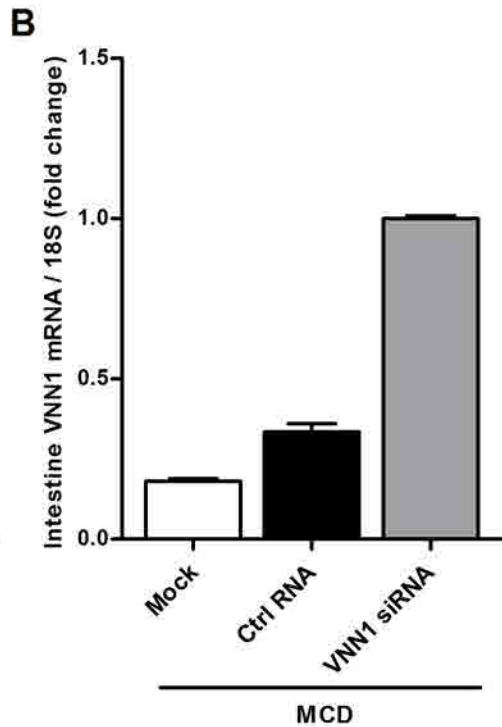
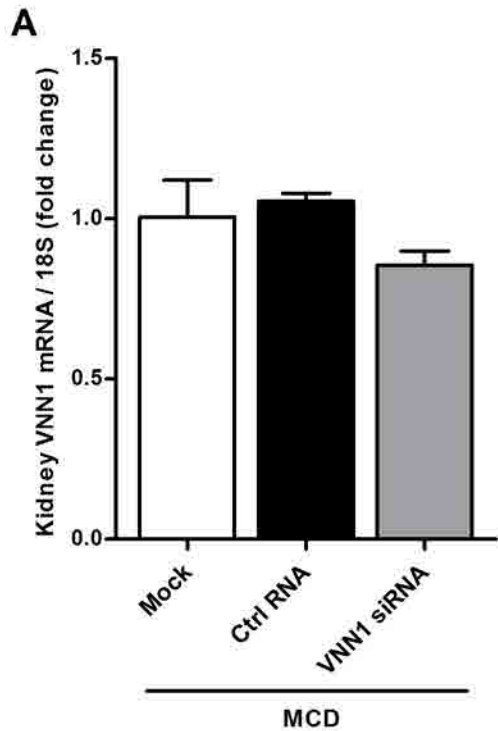


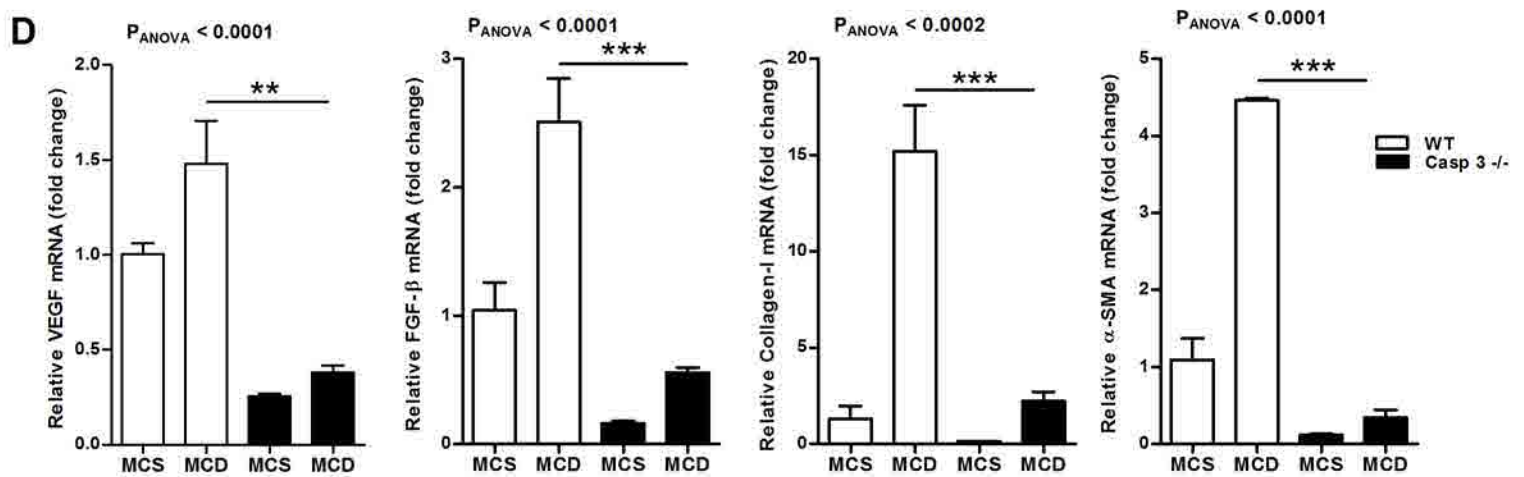
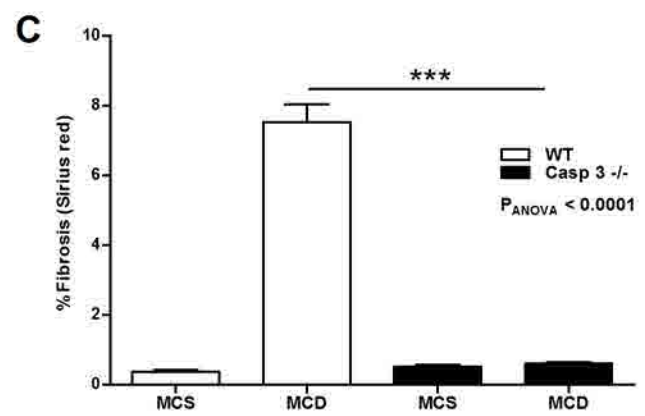
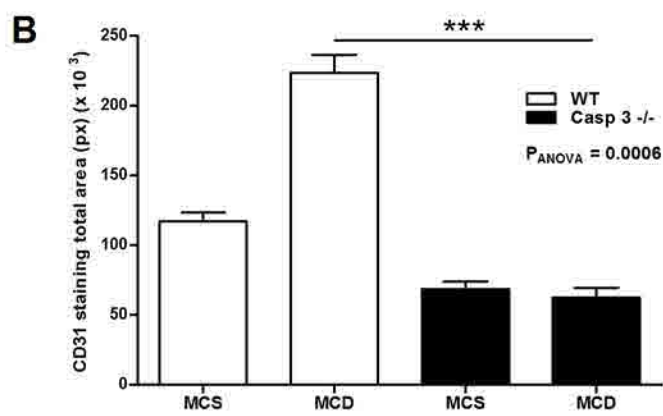
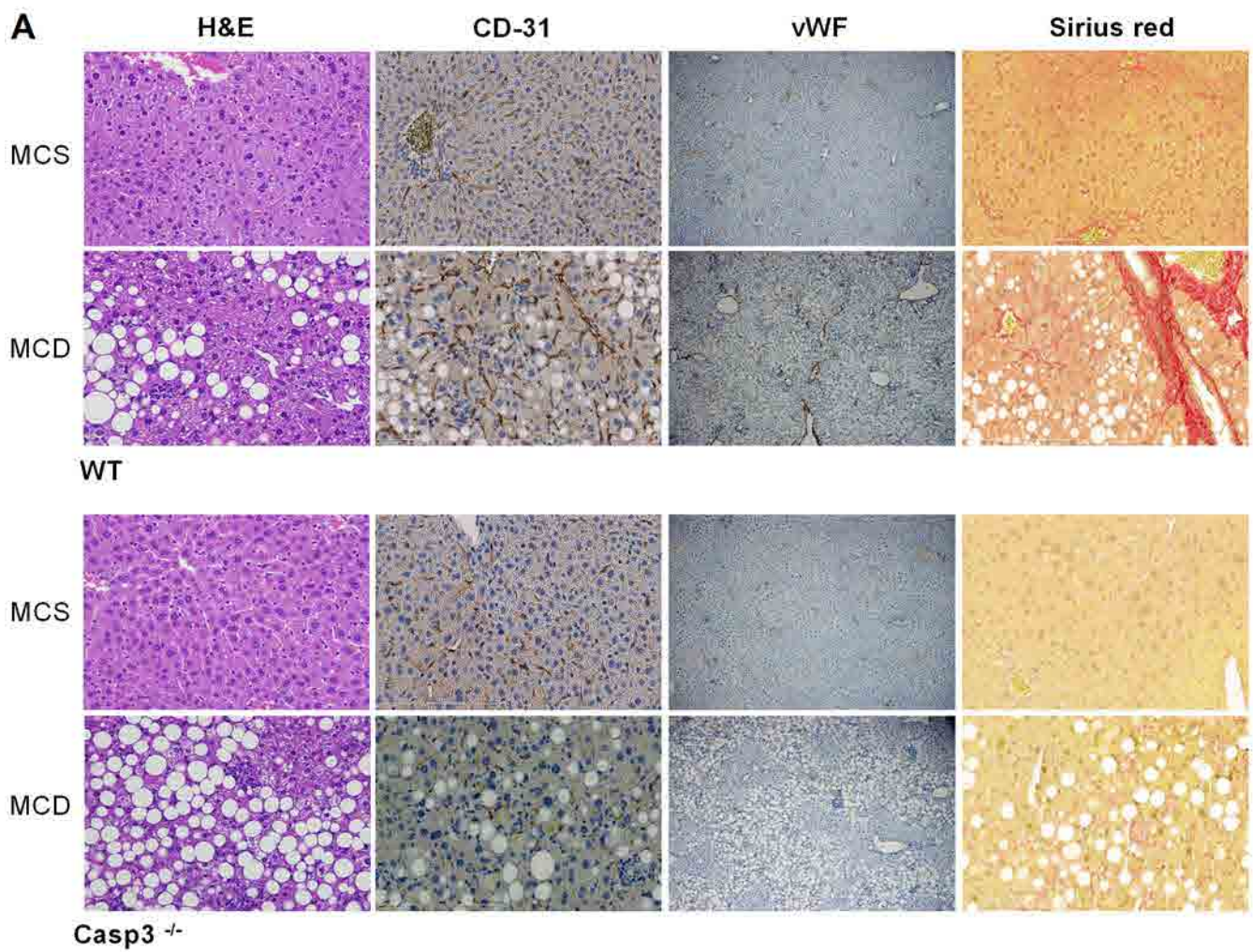


B



A**B**





Supplementary Table 1. Identification of the whole hepatocytes-derived microparticles proteins based on the LC-MS/MS-derived sequences

Protein name	Accession code	Number of peptides	Molecular function	Biological process	Cellular localization
CYTOSKELETON / VESICULATION / ENDOCYTOSIS					
Tubulin, alpha, ubiquitous	gi 57013276	2	Nucleotide binding	Cytoskeleton organisation Mitosis / transport / protein folding / cytoskeleton organisation	Cytoplasm
Tubulin, alpha 1a	gi 17986283	2	Nucleotide binding	Cytoskeleton organisation	Cytoplasm
Tubulin alpha 6	gi 14389309	2	Nucleotide binding	Cytoskeleton organisation	Cytoplasm
Tubulin, alpha 3e	gi 46409270	1	Nucleotide binding	Cytoskeleton organisation	Cytoplasm
Tubulin, alpha 3c	gi 17921993	1	Nucleotide binding	Cytoskeleton organisation	Cytoplasm
Tubulin, alpha 3d	gi 156564363	1	Nucleotide binding	Cytoskeleton organisation	Cytoplasm
Tubulin, beta, 2	gi 5174735	1	Nucleotide binding	Cytoskeleton organisation	Cytoplasm
Tubulin, beta	gi 29788785	1	Nucleotide binding	Cytoskeleton organisation	Cytoplasm
Tubulin, beta 8	gi 42558279	1	Nucleotide binding	Cytoskeleton organisation	Cytoplasm
Tubulin, beta 2B	gi 29788768	1	Nucleotide binding	Cytoskeleton organisation	Cytoplasm
Tubulin, beta 4	gi 21361322	1	Nucleotide binding	Cytoskeleton organisation	Cytoplasm
Tubulin, beta polypeptide 4, member Q	gi 55770868	1	Nucleotide binding	Cytoskeleton organisation	Cytoplasm
Tubulin, beta 6	gi 14210536	1	Nucleotide binding	Cytoskeleton organisation	Cytoplasm
ENZYMES / METABOLIC PROCESSES					
Albumin precursor	gi 4502027	42	Protein binding	Transport / response to stress / lipid metabolism	Extracellular space/ cytoplasm
Maltase-glucoamylase	gi 4758712	25	Glucosidase	Carbohydrate metabolism	Plasma membrane
Ceruloplasmin precursor	gi 4557485	55	Ferroxidase	Ion transport	Extracellular space

Apolipoprotein E precursor	gi 4557325	6	Lipid/protein binding	Lipid metabolism	Extracellular space / cytoplasm
Amine oxidase, copper containing 3 precursor	gi 4502119	12	Oxidase / binding	Catecholamine metabolism / adhesion / inflammation	Cytoplasm / plasma membrane
Vitamin D-binding protein precursor	gi 32483410	7	Protein binding	Vitamin D transport	Extracellular space
Isocitrate dehydrogenase 1 (NADP+), soluble	gi 28178825	4	Dehydrogenase	Isocitrate oxidative decarboxylation	Cytoplasm / mitochondrion / peroxisome
Fumarylacetoacetate hydrolase	gi 4557587	5	Hydrolase	Tyrosine catabolism	Cytoplasm
Apolipoprotein A-I preproprotein	gi 4557321	3	Lipid/protein binding	Lipid metabolism / platelet activation / endothelial cell proliferation	Extracellular space / cytoplasm / plasma membrane
Vanin-1 precursor	gi 4759312	3	Hydrolase / GPI anchor binding	Inflammatory response / anti-apoptosis / adhesion / cell migration	Plasma membrane
Eukaryotic translation elongation factor 1 alpha 1	gi 4503471	2	Nucleotide binding	Transcription / translation regulation	Cytoplasm / Nucleus
Clustein isoform 2	gi 42740907	2	ATPase / misfolded protein binding	Apoptosis regulation	Extracellular space / cytoplasm / mitochondrion
Clusterin isoform 1	gi 42716297	2	ATPase / misfolded protein binding	Apoptosis regulation	Extracellular space / cytoplasm / mitochondrion
Haptoglobin	gi 4826762	2	Catalytic activity	Hemoglobin binding / catabolic process	Extracellular space
Haptoglobin-related protein	gi 45580723	1	Catalytic activity	Hemoglobin binding	Extracellular space
Glyceraldehyde-3-phosphate dehydrogenase	gi 7669492	3	Dehydrogenase	Glycolysis	Cytoplasm
Dipeptidylpeptidase IV	gi 18765694	1	Aminopeptidase	Endothelial cell migration / adhesion / proteolysis	Plasma membrane / Golgi apparatus
Alpha 1 globin	gi 4504347	1	Transporter	Oxygen transport	Extracellular space
Alpha 2 globin	gi 4504345	1	Transporter	Oxygen transport	Extracellular space

Plasma glutamate carboxypeptidase	gi 7706387	3	Peptidase	Proteolysis	Cytoplasm / plasma membrane / nucleus
Eukaryotic translation elongation factor 1 alpha 2	gi 4503475	1	Nucleotide binding	Translation / anti-apoptosis	Cytoplasm / nucleus
Hornerin	gi 57864582	3	Isomerase	Tryptophan metabolic process	Extracellular space
Aspartate aminotransferase 1	gi 4504067	1	Transaminase	Amino acid degradation and biosynthesis	Cytoplasm
Lysozyme precursor Serine (or cysteine) proteinase inhibitor, clade A, member 7	gi 4557894	1	Hydrolase / Lysozyme activity	Inflammatory response / cytolysis	Extracellular space
	gi 4507377	1	Protein binding	Regulation of proteolysis	Extracellular space

RIBONUCLEOPROTEINS

Ribosomal protein S10	gi 4506679	1	Protein binding	Translation	Nucleus
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EXTRACELLULAR MATRIX

Transforming growth factor, beta-induced, 68kDa	gi 4507467	3	Extracellular matrix binding	Angiogenesis / cell adhesion	Extracellular space
Vitronectin precursor	gi 88853069	2	Extracellular matrix binding	Cell-matrix adhesion / migration / integrin binding	Extracellular space

NUCLEOSOMES

Histone cluster 2, H4b	gi 77539758	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 2, H4a	gi 4504323	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H4i	gi 4504321	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H4l	gi 4504317	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H4e	gi 4504315	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H4b	gi 4504313	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H4h	gi 4504311	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H4c	gi 4504309	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H4k	gi 4504307	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H4f	gi 4504305	2	DNA binding	Nucleosome assembly	Nucleus

Histone cluster 1, H4d	gi 4504303	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H4a	gi 4504301	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 4, H4	gi 28173560	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H4j	gi 11415030	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 2, H2be	gi 4504277	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 3, H2bb	gi 28173554	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H2bj	gi 20336754	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H2bo	gi 16306566	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H2bb	gi 10800140	2	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 2, H2bf	gi 66912162	1	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H2bi	gi 4504271	1	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H2bh	gi 4504269	1	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H2bf	gi 4504265	1	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H2bm	gi 4504263	1	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H2bn	gi 4504261	1	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H2bl	gi 4504259	1	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H2bg	gi 4504257	1	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H2be	gi 21396484	1	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H2bc	gi 21166389	1	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H2bd	gi 20336752	1	DNA binding	Nucleosome assembly	Nucleus
Histone cluster 1, H2bk	gi 18105048	1	DNA binding	Nucleosome assembly	Nucleus

OTHER PROTEINS

Fibrinogen, alpha polypeptide isoform alpha-E preproprotein	gi 4503689	1	Protein binding	Coagulation	Extracellular space
Fibrinogen, alpha polypeptide isoform alpha preproprotein	gi 11761629	1	Protein binding	Coagulation Chemotaxis / inflammation / signal transduction	Extracellular space
Chemokine (C-C motif) ligand 20	gi 4759076	1	Chemokine	Inflammation response / apoptosis	Extracellular space
Growth differentiation factor 15	gi 153792495	1	Growth factor (TGF- β family)		Cytoplasm