

Electronic supplementary information

Evaluation of DMSA-coated iron nanoparticles immunotargeted to amyloid-beta as MRI contrast agents for the diagnosis of Alzheimer's disease

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Supplementary method

Surface Plasmon Resonance Analysis for Anti-Amyloid Antibody and A β (1-42) Peptide Binding

BiaCore T200 (Cytiva) was used to measure the binding kinetic of A β (1-42) peptide (Sigma A9810) to anti-amyloid antibody. A β (1-42) peptide was immobilised on a CM5 sensor using amine coupling chemistry. Prior to the ligand immobilisation step, BiaCore T200 was primed with the immobilisation running buffer, HBS-EP (0.15 M NaCl, 10 mM HEPES, 3 mM EDTA, 0.005 % (v/v) P20 surfactant, pH 7.4). The surfaces of two flow cells were activated for 7 min with a 1:1 mixture of 0.1 M NHS (N-hydroxysuccinimide) and 0.1 M EDC (3-(N, N-dimethylamino) propyl-N-ethylcarbodiimide) at a flow rate of 10 μ L/min. The A β (1-42) peptide at a concentration of 4 μ M in 10 mM sodium acetate, pH 4 was injected on flow cell 2 of the activated sensor surface for 7 min. 95.4 RU of ligand density was achieved on flow cell 2, whereas flow cell 1 was left blank to be used as a reference channel. The unbound sensor surfaces were blocked with 7 min injection of 1 M ethanolamine, pH 8.

For kinetic measurement, anti-amyloid antibody in HBS-EP+ (0.15 M NaCl, 10 mM HEPES, 3 mM EDTA, 0.05 % (v/v) P20 surfactant, pH 7.4) was injected at concentrations of 1.2, 3.6, 11.11, 33.33, 100 nM to the ligand immobilised sensor at a flowrate of 30 μ L/min. The association and dissociation of anti-amyloid antibody to A β (1-42) peptide were measured for 120 s and 480 s, respectively. The sensor surface was regenerated using 10 mM glycine-HCl, pH 2. The collected data were fit to 1:1 interaction model using the global data analysis in Biacore T200 Evaluation Software.

Biolayer Interferometry Analysis for NP-Ab and A β (1-42) Peptide Binding

Kinetic binding of A β (1-42) peptide (Sigma A9810) to NP-Ab was assessed using a BLItz system, which is a label-free biosensor technology that measures protein-protein interactions in real-time. BLItz Protein G (ProG) biosensors were incubated in running buffer (PBS containing 0.005 % Tween-20) for a minimum of 10 min to dissolve the sucrose coating on the biosensors. The baseline of the activated BLItz ProG biosensor was established with the running buffer for 30 seconds followed by dipping into the NP-Ab sample to capture nanoparticles for 180 seconds. A β (1-42) was diluted in the running buffer at 1, 4 and 10 μ M, and the NP-Ab captured ProG biosensor tip was then dipped into A β (1-42) for 180 seconds, followed by a dissociation step in the running buffer for 120 seconds. The association and dissociation of each interaction were recorded in real-time. The association and dissociation rate constants (k_a and k_d), equilibrium dissociation constant (KD), and maximum binding response (Δ nm) were calculated for each interaction.

| | Step Type | Duration (s) | Position |
|---|------------------------|--------------|----------|
| 1 | Initial Baseline | 30 | Tube |
| 2 | Loading (NP-Ab) | 180 | Drop |
| 3 | Baseline | 30 | Tube |
| 4 | Association (Peptides) | 180 | Drop |
| 5 | Dissociation | 120 | Tube |

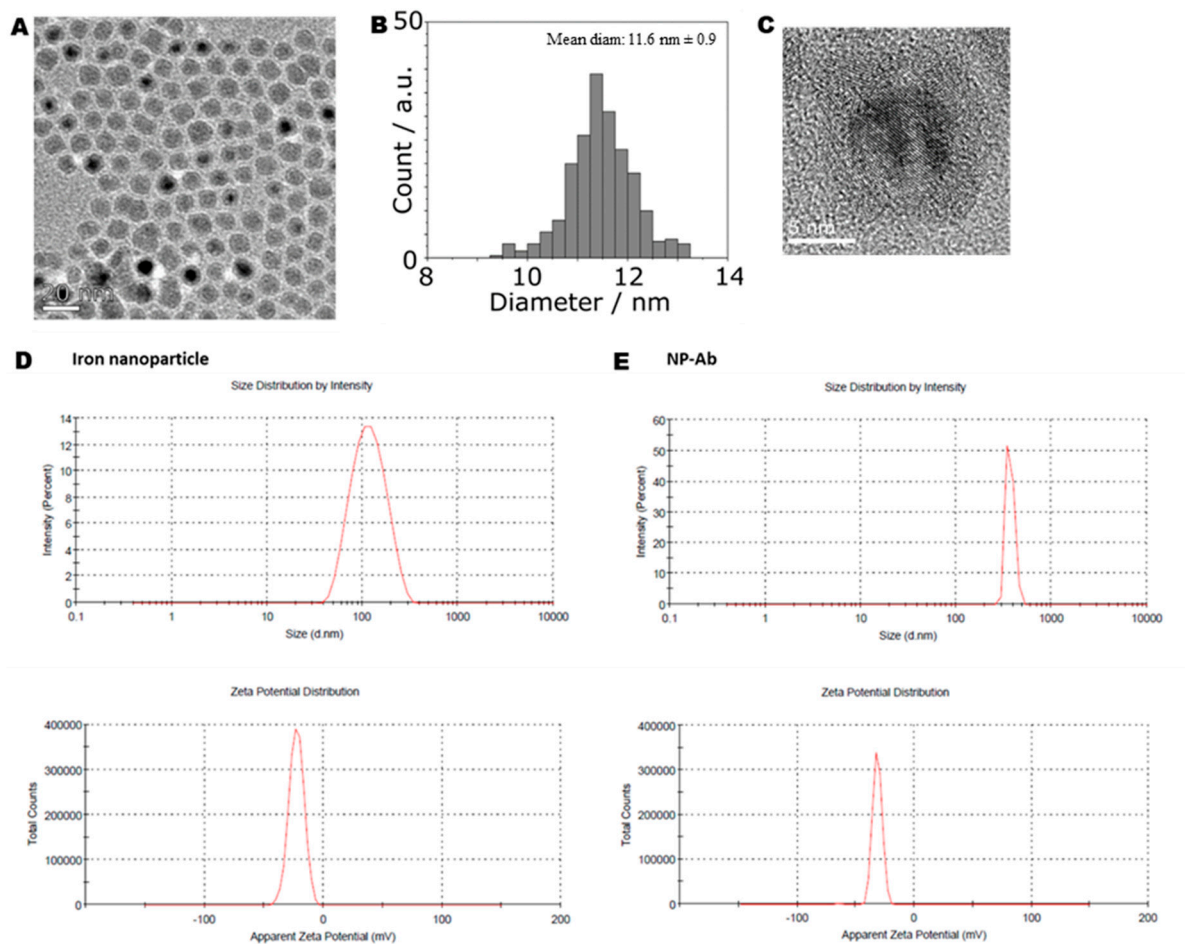


Figure S1. (A) Transmission electron micrograph (TEM) of iron nanoparticles. (B) Size distribution of dry diameter. (C) High resolution TEM image showing the darker crystalline core, and lighter oxide shell of one iron nanoparticles. (D, E) DLS and zeta potential of iron nanoparticles (D) and NP-Ab (E)

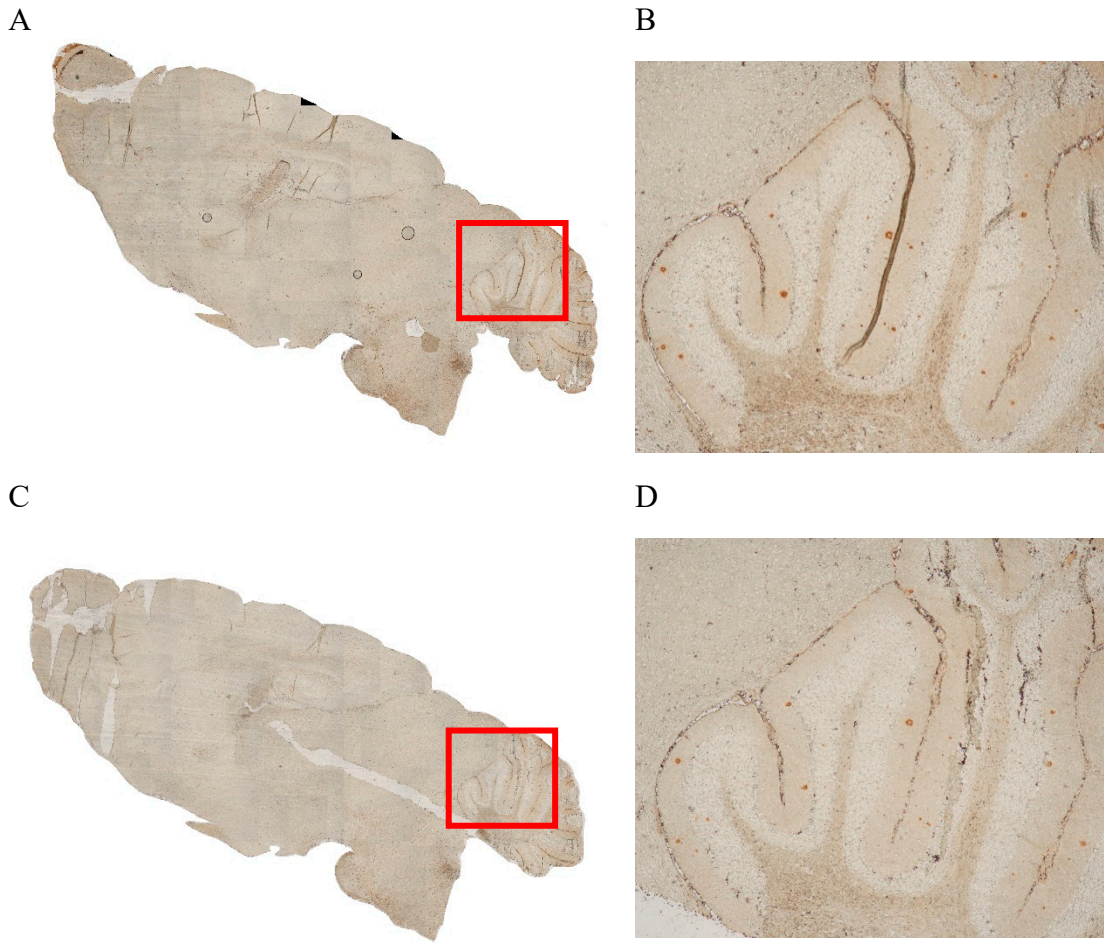


Figure S2. Micrographs from a light microscope of adjacent brain sections of APP/PS1 mice immunostained for amyloid-beta plaques with NP-Ab (A, B) and an anti-amyloid-beta antibody (C, D) as control. DAB was used as a chromogen and plaques appear as dark brown circles on image. Plaques present in corresponding regions on control section confirmed presence of antibodies on nanoparticles.

| Conc (nM) | KD (M) | ka (1/Ms) | kd (1/s) |
|----------------|----------|-----------|----------|
| Global fitting | | | |
| 1.2 | 2.14E-09 | 1.44E+05 | 3.08E-04 |
| 3.6 | | | |
| 11.11 | | | |
| 33.33 | | | |
| 100 | | | |

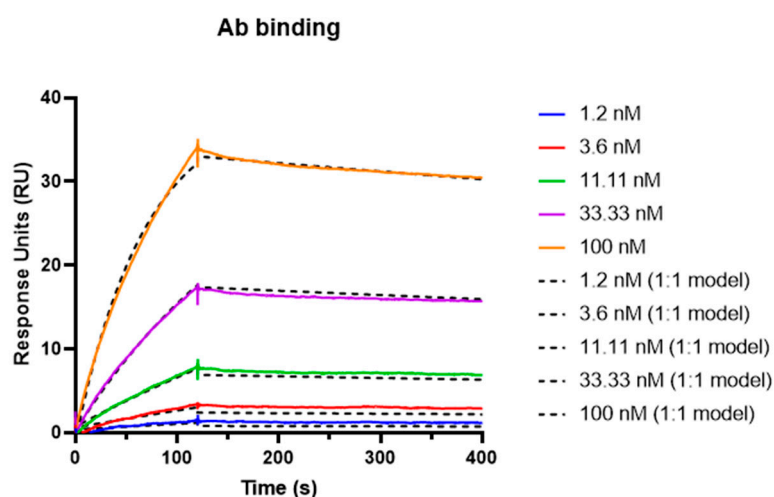
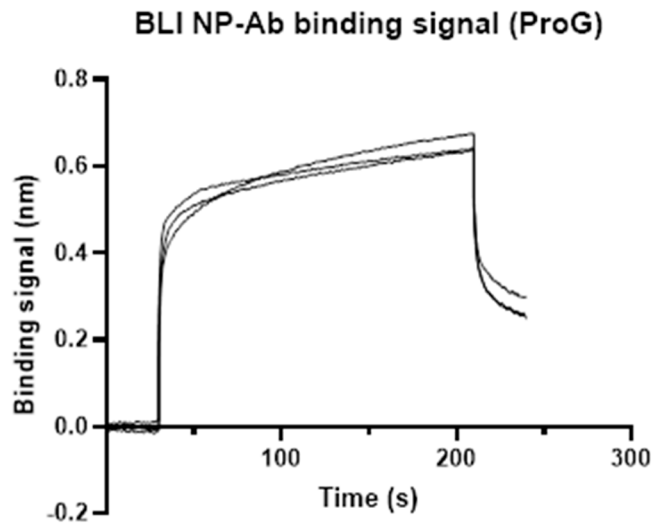


Figure S3. Binding affinity of anti- $A\beta$ antibody to $A\beta_{(1-42)}$ peptide immobilised CM5 sensor different concentrations. Binding kinetics of anti- $A\beta$ antibody to $A\beta_{(1-42)}$ peptide were monitored by BiaCore T200. The data were fitted to a 1:1 binding model to calculate the equilibrium dissociation constant (KD).

A



B

| Conc (uM) | KD (M) | ka (1/Ms) | kd (1/s) |
|----------------|----------|-----------|----------|
| Global fitting | | | |
| 1 | 2.395E-6 | 4.288E3 | 1.027E-2 |
| 4 | | | |
| 10 | | | |

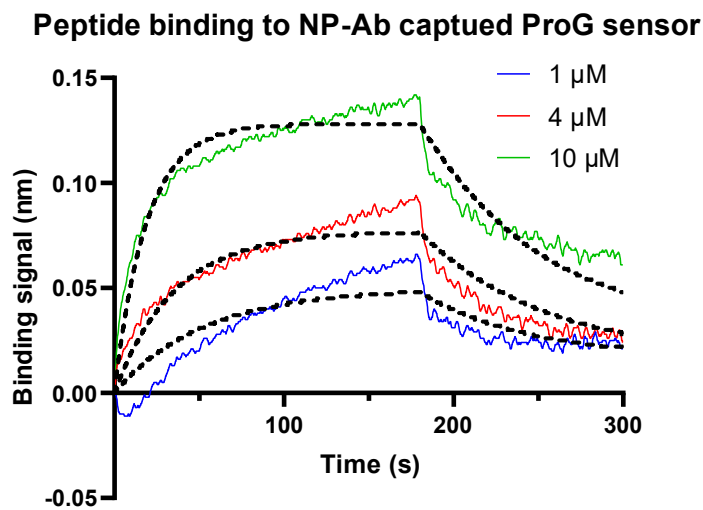


Figure S4. Binding affinity of NP-Ab to ProG sensors at different concentrations. **(A)** NP-Ab binding to ProG sensors. **(B)** Kinetics of $A\beta_{(1-42)}$ peptide binding to NP-Ab captured ProG sensors (colored lines). The data were fitted to a 1:1 model to calculate the equilibrium dissociation constant (KD).

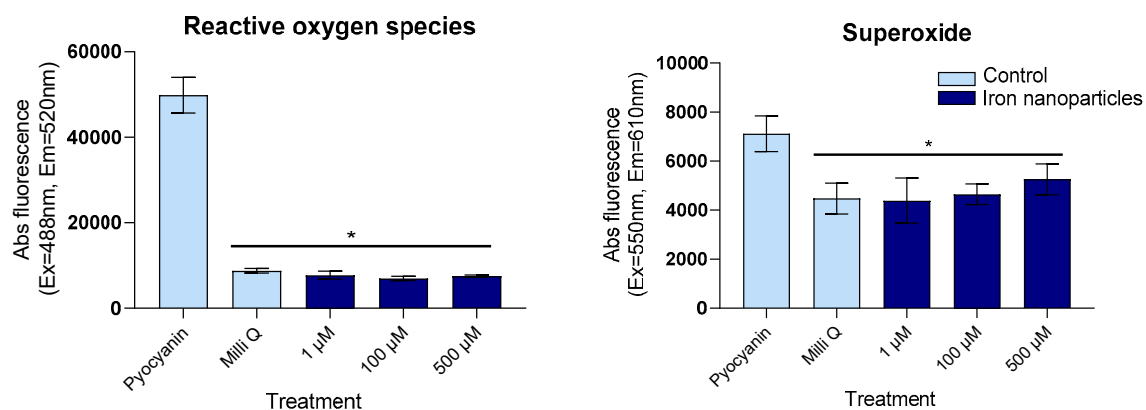


Figure S5. Level of reactive oxygen species (ROS) and superoxide production in U-251 cells following 24 h treatment with iron nanoparticles at three different concentrations. Pyocyanin was used as a positive control ROS inducer, Milli Q water was used as a vehicle control. Data represent mean \pm SD ($n = 5$), *: significant difference from positive control ($p < 0.05$).

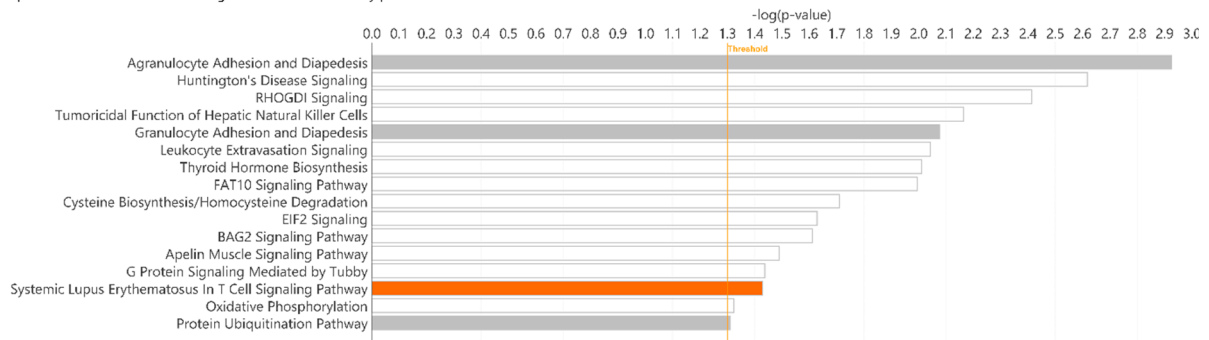
Table S1 Mutagenicity of iron nanoparticles and NP-Ab in *Salmonella typhirium*. *: $p < 0.05$ based on reference table https://www.biotoxicity.com/images/Toxicity%20PDF/muta-ChromoPlate_Ames_Test.pdf.

| Treatment | Number of positive wells | | Total testable wells (48 - excluded wells) | | Proportion of positive wells (%) | | Average (%) | SD |
|-------------------------------|--------------------------|----------|---|----------|----------------------------------|----------|---------------|------|
| | Sample 1 | Sample 2 | Sample 1 | Sample 2 | Sample 1 | Sample 2 | | |
| Negative control | 2 | 3 | 48 | 48 | 4.17 | 6.25 | 5.21 | 1.47 |
| Positive control | 48 | 40 | 48 | 40 | 100.00 | 100.00 | 100.00 | 0.00 |
| 50 μ M Iron nanoparticle | 5 | 6 | 45 | 40 | 5.33 | 15.00 | 10.17 | 6.84 |
| 100 μ M Iron nanoparticle | 11 | 10 | 37 | 37 | 14.27 | 27.03 | 20.65* | 9.02 |
| 50 μ M NP-Ab | 7 | 4 | 33 | 29 | 10.18 | 13.79 | 11.99 | 2.55 |

A

Analysis: CvsNS

■ positive z-score ■ z-score = 0 ■ negative z-score ■ no activity pattern available

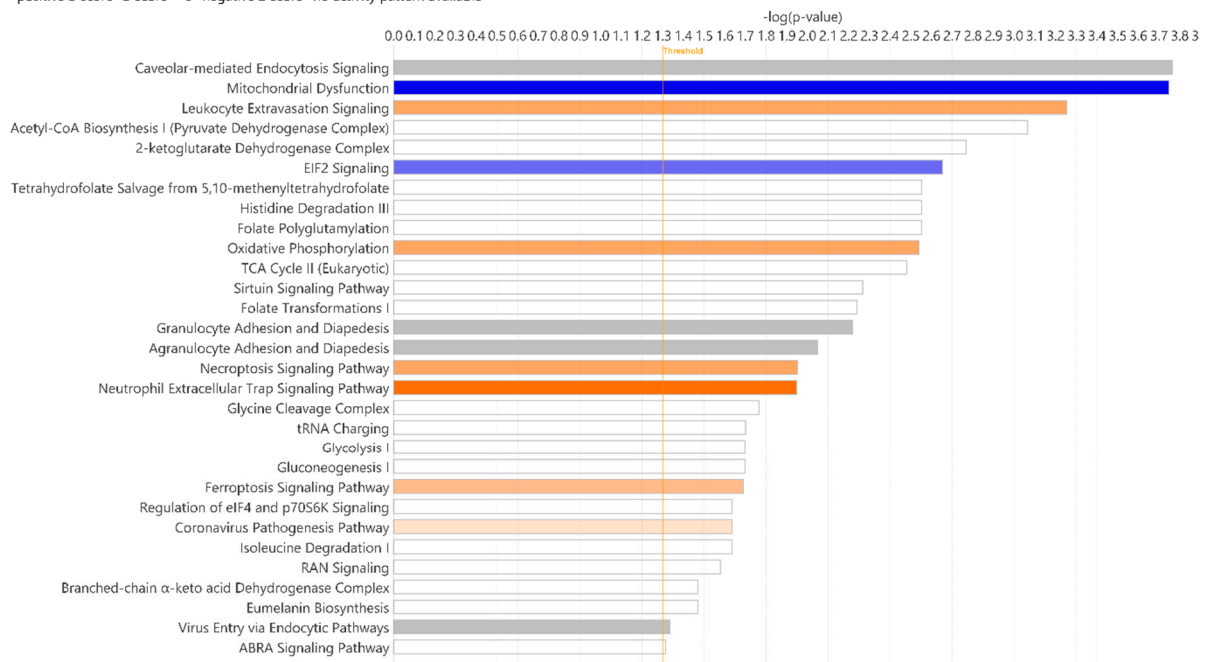


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B

Analysis: CvsNAb

■ positive z-score ■ z-score = 0 ■ negative z-score ■ no activity pattern available



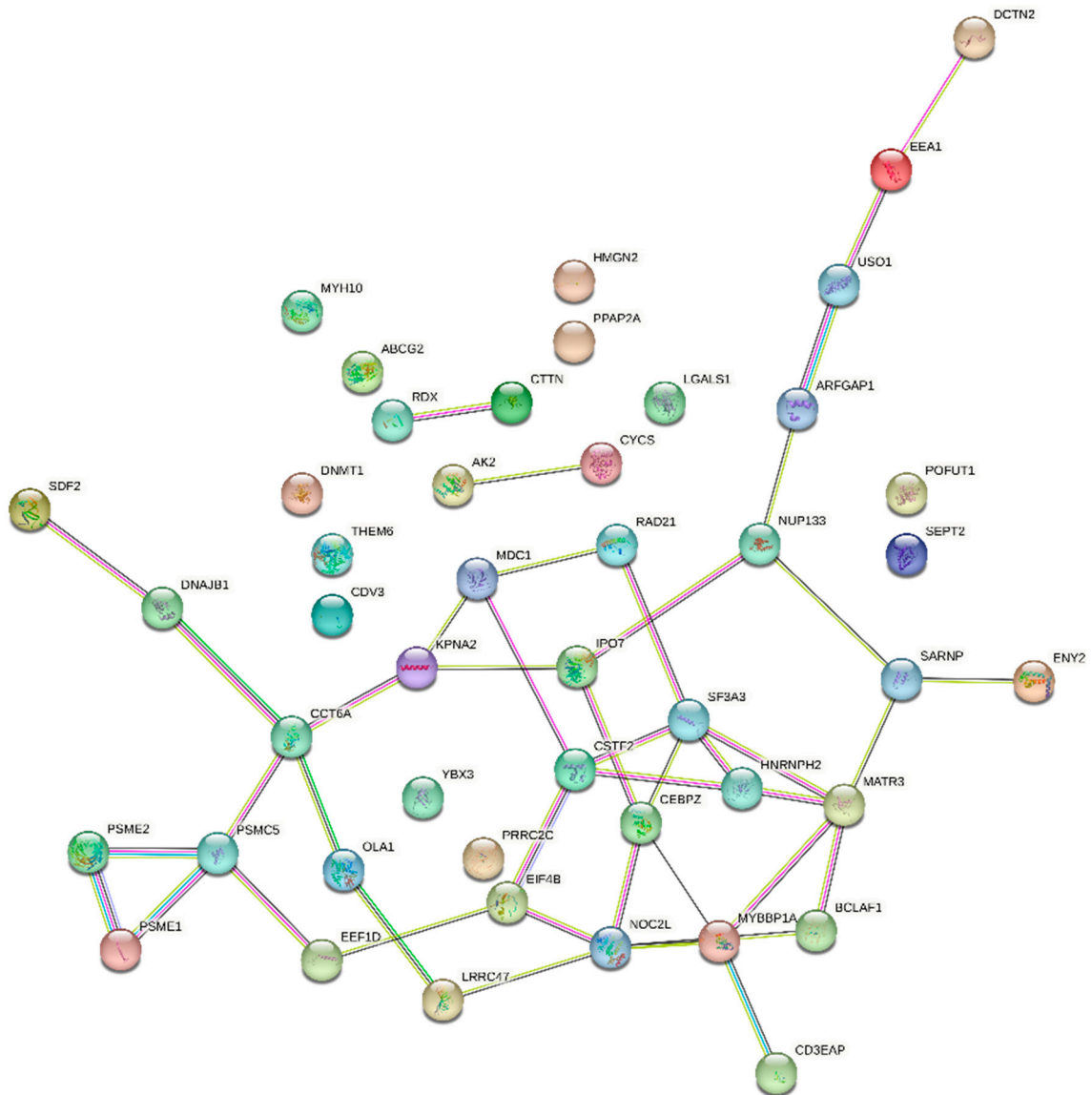
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C

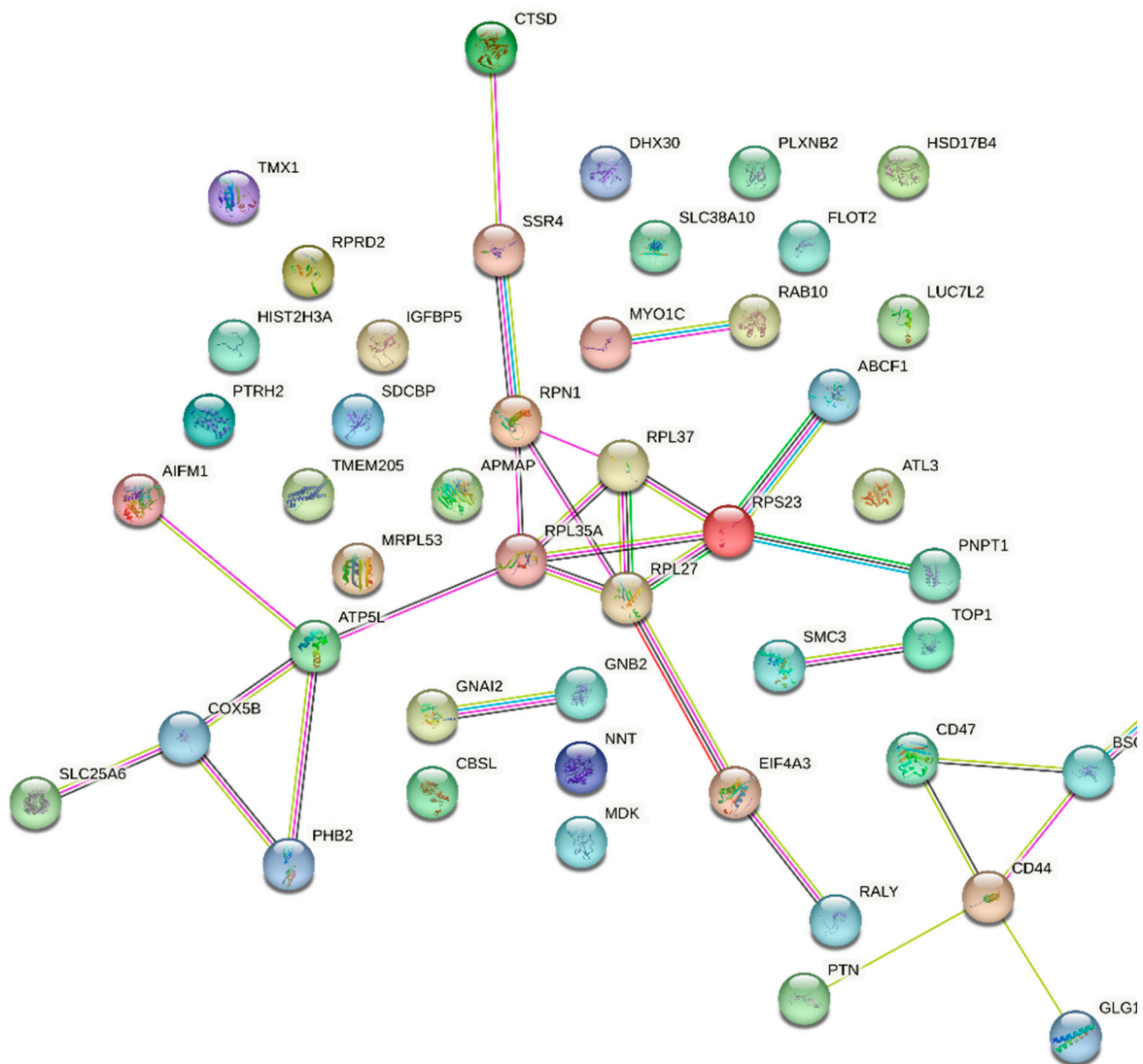


Figure S6: Canonical pathways of iron nanoparticles vs. no treatment (A), NP-Ab vs. no treatment (B), and comparison between groups (C).

A



B



C



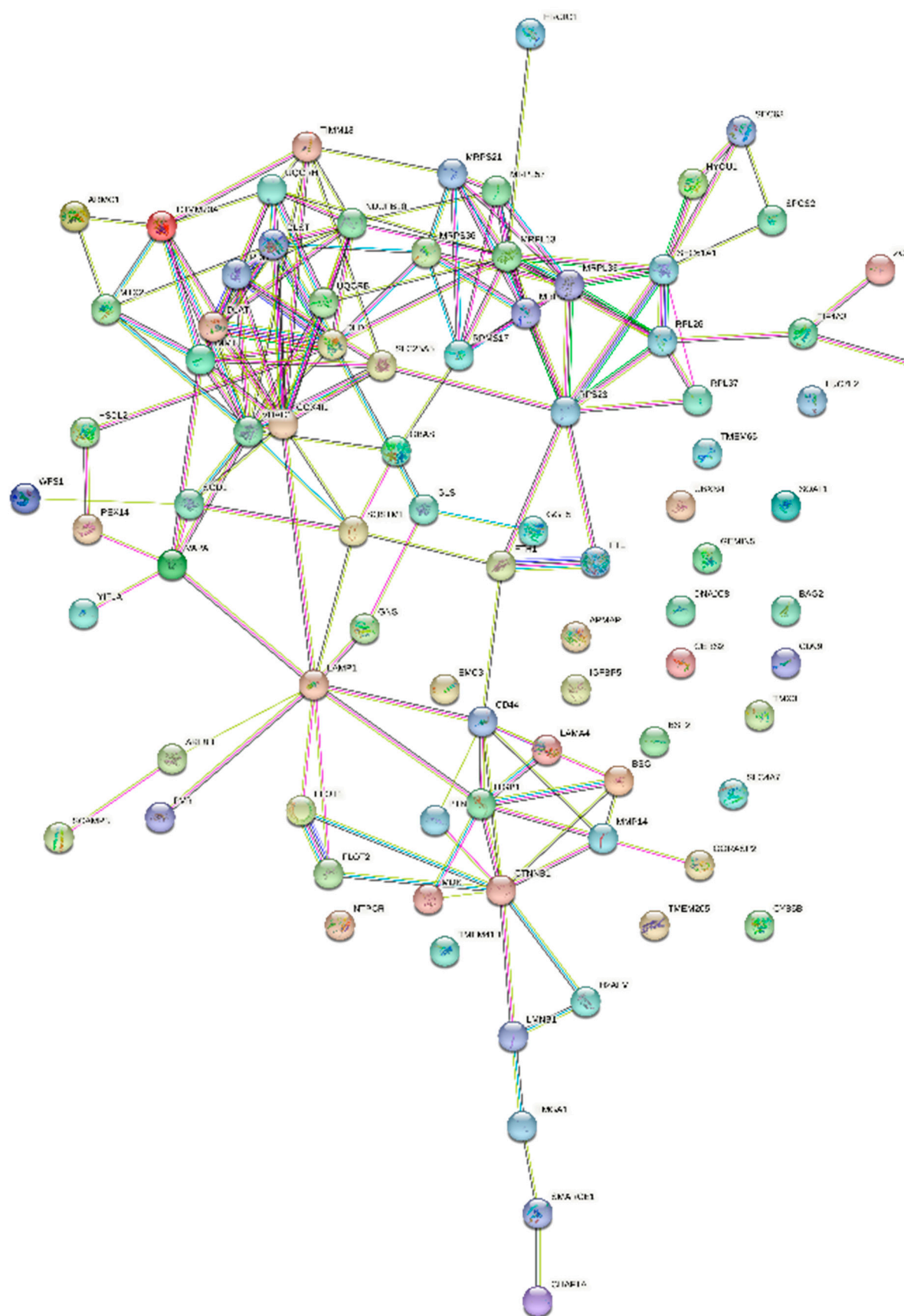


Figure S7: STRING diagrams of iron nanoparticles vs. no treatment (**A, B**), NP-Ab vs. no treatment (**C, D**), down (**A, C**) and up (**B, D**) regulated proteins.

Table S2: List of significantly differentially expressed proteins in iron nanoparticles vs. no treatment.

| Protein names | Gene | T-test (p-value) | log₂(Fold change) |
|---|-------------|-------------------------|-------------------------------------|
| GDP-fucose protein O-fucosyltransferase 1 | POFUT1 | 0.0006 | -1.9038 |
| ATP-binding cassette sub-family G member 2 | ABCG2 | 0.0007 | -3.9147 |
| Leukocyte surface antigen CD47 | CD47 | 0.0010 | 2.5729 |
| Protein CDV3 homolog | CDV3 | 0.0010 | -1.2559 |
| Plexin-B2 | PLXNB2 | 0.0013 | 1.5739 |
| RNA-binding protein Raly | RALY | 0.0027 | 0.3115 |
| Putative sodium-coupled neutral amino acid transporter 10 | SLC38A10 | 0.0043 | 1.3994 |
| Cytochrome c oxidase subunit 5B, mitochondrial | COX5B | 0.0045 | 4.8150 |
| ATP-binding cassette sub-family F member 1 | ABCF1 | 0.0050 | 0.3956 |
| Eukaryotic initiation factor 4A-III | EIF4A3 | 0.0050 | 2.3533 |
| Atlastin-3 | ATL3 | 0.0052 | 0.7937 |
| Lipid phosphate phosphohydrolase 1 | PPAP2A | 0.0053 | -0.5620 |
| Large neutral amino acids transporter small subunit 1 | SLC7A5 | 0.0057 | 0.7714 |
| Adipocyte plasma membrane-associated protein | APMAP | 0.0059 | 0.3165 |
| Midkine | MDK | 0.0066 | 2.9811 |
| Double-strand-break repair protein rad21 homolog | RAD21 | 0.0072 | -0.4798 |
| Myosin-10 | MYH10 | 0.0077 | -0.7161 |
| Prohibitin-2 | PHB2 | 0.0079 | 0.6104 |
| 39S ribosomal protein L53, mitochondrial | MRPL53 | 0.0095 | 2.0910 |
| Proteasome activator complex subunit 2 | PSME2 | 0.0096 | -2.1093 |
| Transmembrane protein 205 | TMEM205 | 0.0105 | 1.8023 |
| SAP domain-containing ribonucleoprotein | CIP29 | 0.0113 | -1.9063 |
| Peptidyl-tRNA hydrolase 2, mitochondrial | PTRH2 | 0.0113 | 0.7309 |
| CCAAT/enhancer-binding protein zeta | CEBPZ | 0.0120 | -1.0376 |
| Heterogeneous nuclear ribonucleoprotein H2 | HNRNPH2 | 0.0124 | -0.4662 |
| Non-histone chromosomal protein HMG-17 | HMG2 | 0.0139 | -0.8954 |

| | | | |
|--|---------|--------|---------|
| Regulation of nuclear pre-mRNA domain-containing protein 2 | RPRD2 | 0.0143 | 0.7157 |
| Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2 | GNB2 | 0.0143 | 0.4417 |
| Src substrate cortactin | CTTN | 0.0147 | -0.4255 |
| 60S ribosomal protein L37 | RPL37 | 0.0153 | 1.9032 |
| Transcription and mRNA export factor ENY2 | ENY2 | 0.0154 | -0.5103 |
| Guanine nucleotide-binding protein G(i) subunit alpha-2 | GNAI2 | 0.0159 | 0.6008 |
| Proteasome activator complex subunit 1 | PSME1 | 0.0162 | -1.4754 |
| Protein THEM6 | THEM6 | 0.0164 | -1.3676 |
| Insulin-like growth factor-binding protein 5 | IGFBP5 | 0.0172 | 1.6481 |
| Stromal cell-derived factor 2 | SDF2 | 0.0176 | -1.1884 |
| Splicing factor 3A subunit 3 | SF3A3 | 0.0181 | -1.1735 |
| NAD(P) transhydrogenase, mitochondrial | NNT | 0.0190 | 0.6090 |
| Myb-binding protein 1A | MYBBP1A | 0.0195 | -0.9194 |
| Adenylate kinase 2, mitochondrial | AK2 | 0.0195 | -0.7063 |
| Nucleolar complex protein 2 homolog | NOC2L | 0.0201 | -0.5056 |
| Mediator of DNA damage checkpoint protein 1 | MDC1 | 0.0209 | -0.5937 |
| ADP/ATP translocase 3 | SLC25A6 | 0.0218 | 0.5081 |
| Putative ATP-dependent RNA helicase DHX30 | DHX30 | 0.0228 | 1.1354 |
| Septin-2 | SEPT2 | 0.0247 | -0.6835 |
| DNA (cytosine-5)-methyltransferase 1 | DNMT1 | 0.0251 | -0.6731 |
| Apoptosis-inducing factor 1, mitochondrial | AIFM1 | 0.0252 | 0.2921 |
| Nuclear pore complex protein Nup133 | NUP133 | 0.0252 | -1.1591 |
| galectin 1 | LGALS1 | 0.0254 | -2.3125 |
| Peroxisomal multifunctional enzyme type 2 | HSD17B4 | 0.0261 | 0.5641 |
| Leucine-rich repeat-containing protein 47 | LRRC47 | 0.0267 | -1.2543 |
| Translocon-associated protein subunit delta | SSR4 | 0.0268 | 0.3816 |
| ATP synthase subunit g, mitochondrial | ATP5L | 0.0273 | 0.5592 |
| Elongation factor 1-delta | EEF1D | 0.0279 | -0.3744 |
| ADP-ribosylation factor GTPase-activating protein 1 | ARFGAP1 | 0.0286 | -1.3690 |

| | | | |
|--|----------|--------|---------|
| Early endosome antigen 1 | EEA1 | 0.0300 | -1.3751 |
| Protein PRRC2C | PRRC2C | 0.0303 | -0.8528 |
| Putative RNA-binding protein Luc7-like 2 | LUC7L2 | 0.0307 | 0.8823 |
| Pleiotrophin | PTN | 0.0308 | 1.7302 |
| Importin-7 | IPO7 | 0.0309 | -1.3591 |
| Thioredoxin-related transmembrane protein 1 | TMX1 | 0.0313 | 0.7077 |
| Flotillin-2 | FLOT2 | 0.0329 | 0.6724 |
| DNA-directed RNA polymerase I subunit RPA34 | CD3EAP | 0.0339 | -0.5885 |
| Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 | RPN1 | 0.0348 | 0.4404 |
| Ras-related protein Rab-10 | RAB10 | 0.0348 | 0.5369 |
| Syntenin-1 | SDCBP | 0.0348 | 1.6728 |
| Cytochrome c | CYCS | 0.0364 | -1.5270 |
| Importin subunit alpha | KPNA2 | 0.0368 | -0.4933 |
| 60S ribosomal protein L35a | RPL35A | 0.0373 | 0.5335 |
| Bcl-2-associated transcription factor 1 | BCLAF1 | 0.0374 | -0.4765 |
| Eukaryotic translation initiation factor 4B | EIF4B | 0.0378 | -2.0060 |
| CD44 antigen | CD44 | 0.0392 | 0.3942 |
| Unconventional myosin-Ic | MYO1C | 0.0401 | 0.3105 |
| 26S protease regulatory subunit 8 | PSMC5 | 0.0402 | -1.1658 |
| Polyribonucleotide nucleotidyltransferase 1, mitochondrial | PNPT1 | 0.0407 | 0.4901 |
| Obg-like ATPase 1 | OLA1 | 0.0407 | -1.1111 |
| General vesicular transport factor p115 | USO1 | 0.0412 | -0.7585 |
| Dynactin subunit 2 | DCTN2 | 0.0428 | -0.8899 |
| Cleavage stimulation factor subunit 2 | CSTF2 | 0.0440 | -0.8107 |
| Golgi apparatus protein 1 | GLG1 | 0.0443 | 0.4408 |
| 40S ribosomal protein S23 | RPS23 | 0.0450 | 0.6056 |
| Histone H3.2 | HIST2H3A | 0.0450 | 0.3266 |
| 60S ribosomal protein L27 | RPL27 | 0.0450 | 0.3076 |
| T-complex protein 1 subunit zeta | CCT6A | 0.0458 | -1.0169 |

| | | | |
|---|---------|--------|---------|
| DNA topoisomerase 1 | TOP1 | 0.0462 | 0.3740 |
| Matrin-3 | MATR3 | 0.0463 | -0.3351 |
| Cystathionine beta-synthase | CBS | 0.0470 | 1.4284 |
| DnaJ homolog subfamily B member 1 | DNAJB1 | 0.0471 | -0.7371 |
| Y-box-binding protein 3 | CSDA | 0.0475 | -0.3591 |
| Basigin | EMMPRIN | 0.0476 | 0.2804 |
| Cathepsin D | CTSD | 0.0488 | 0.2758 |
| Structural maintenance of chromosomes protein | SMC3 | 0.0495 | 0.3513 |
| Radixin | RDX | 0.0498 | -0.8543 |

Table S3: List of significantly differentially expressed proteins in NP-Ab vs. no treatment.

| Protein names | Gene | T-test (p-value) | log ₂ (Fold change) |
|---|---------|------------------|--------------------------------|
| 28S ribosomal protein S17, mitochondrial | MRPS17 | 0.0001 | 3.4772 |
| ATP-binding cassette sub-family G member 2 | ABCG2 | 0.0001 | -4.0280 |
| Eukaryotic initiation factor 4A-III | EIF4A3 | 0.0002 | 2.5383 |
| Poliovirus receptor | PVR | 0.0002 | 1.0296 |
| Midkine | MDK | 0.0003 | 3.4510 |
| SAP domain-containing ribonucleoprotein | CIP29 | 0.0003 | -1.5195 |
| Superoxide dismutase [Cu-Zn] | SOD1 | 0.0004 | 2.3078 |
| Protein THEM6 | THEM6 | 0.0004 | -1.9948 |
| galectin 1 | LGALS1 | 0.0005 | -2.5652 |
| Peroxisomal membrane protein PEX14 | PEX14 | 0.0005 | 2.2424 |
| Lysosome-associated membrane glycoprotein 1 | LAMP1 | 0.0005 | 3.6663 |
| Pleiotrophin | PTN | 0.0006 | 1.8087 |
| Adipocyte plasma membrane-associated protein | APMAP | 0.0006 | 0.3352 |
| Transmembrane protein 41B | TMEM41B | 0.0008 | 2.4630 |
| Transmembrane protein 205 | TMEM205 | 0.0008 | 1.8339 |
| BAG family molecular chaperone regulator 2 | BAG2 | 0.0011 | 0.4071 |
| Mitochondrial import inner membrane translocase subunit Tim13 | TIMM13 | 0.0011 | 3.2074 |
| Filamin-B | FLNB | 0.0012 | -0.5015 |
| Ferritin | FTH1 | 0.0012 | 1.2108 |
| Stromal cell-derived factor 2-like protein 1 | SDF2L1 | 0.0013 | -1.8811 |
| High mobility group protein HMG-I/HMG-Y | HMGA1 | 0.0016 | 0.8241 |
| 28S ribosomal protein S18b, mitochondrial | MRPS18B | 0.0017 | -2.9979 |

| | | | |
|---|---------|--------|---------|
| mRNA export factor | RAE1 | 0.0019 | -1.9188 |
| Cytochrome c-type heme lyase | HCCS | 0.0021 | -1.7306 |
| Poly(rC)-binding protein 2 | PCBP2 | 0.0028 | -0.6162 |
| Flap endonuclease 1 | FEN1 | 0.0029 | -1.5829 |
| SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5 | SMARCA5 | 0.0030 | -0.7712 |
| 28S ribosomal protein S21, mitochondrial | MRPS21 | 0.0032 | 1.5755 |
| ADP-ribosylation factor-like protein 8B | ARL8B | 0.0033 | 0.8229 |
| Gamma-glutamyltransferase 5 | GGT5 | 0.0034 | 2.4295 |
| Insulin-like growth factor-binding protein 5 | IGFBP5 | 0.0036 | 1.4291 |
| Translocation protein SEC63 homolog | SEC63 | 0.0039 | 0.7977 |
| 28S ribosomal protein S5, mitochondrial | MRPS5 | 0.0041 | -1.9747 |
| 39S ribosomal protein L33, mitochondrial | MRPL33 | 0.0045 | 1.5243 |
| Valine--tRNA ligase | VAR5 | 0.0052 | -1.8495 |
| Protein disulfide-isomerase TMX3 | TMX3 | 0.0053 | 2.6733 |
| Nuclear fragile X mental retardation-interacting protein 2 | NUFIP2 | 0.0054 | -0.3719 |
| Eukaryotic translation initiation factor 3 subunit H | EIF3H | 0.0054 | -1.2525 |
| SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1 | SMARCE1 | 0.0057 | 1.3343 |
| Endoplasmic reticulum-Golgi intermediate compartment protein 1 | ERGIC1 | 0.0058 | 1.8188 |
| C-1-tetrahydrofolate synthase, cytoplasmic | MTHFD1 | 0.0058 | -2.2323 |
| ATP-binding cassette sub-family F member 1 | ABCF1 | 0.0059 | -1.9886 |
| Anion exchange protein | SLC4A7 | 0.0061 | 0.7393 |
| Lipid phosphate phosphohydrolase 1 | PPAP2A | 0.0066 | -1.4439 |
| Armadillo repeat-containing protein 1 | ARMC1 | 0.0068 | 1.9002 |

| | | | |
|---|---------|--------|---------|
| Coatomer subunit beta | COPB2 | 0.0070 | -2.3003 |
| T-complex protein 1 subunit beta | CCT2 | 0.0073 | -0.8644 |
| 60S ribosomal protein L13 | RPL13 | 0.0075 | -0.3719 |
| Ceramide synthase 2 | CERS2 | 0.0084 | 2.2992 |
| Splicing factor 3B subunit 3 | SF3B3 | 0.0090 | -1.5410 |
| Isocitrate dehydrogenase [NAD] subunit, mitochondrial | IDH3B | 0.0090 | -1.6173 |
| Cancer-related nucleoside-triphosphatase | NTPCR | 0.0093 | 1.3784 |
| Methionine--tRNA ligase, cytoplasmic | MARS | 0.0098 | -0.6055 |
| Secretory carrier-associated membrane protein 3 | SCAMP3 | 0.0102 | 0.6350 |
| Early endosome antigen 1 | EEA1 | 0.0102 | -1.6817 |
| Flotillin-2 | FLOT2 | 0.0107 | 0.6411 |
| Flotillin-1 | FLOT1 | 0.0107 | 1.3345 |
| 40S ribosomal protein S27 | RPS27 | 0.0111 | -0.7153 |
| Rac GTPase-activating protein 1 | RACGAP1 | 0.0114 | -1.0788 |
| Y-box-binding protein 3 | CSDA | 0.0118 | -0.5607 |
| 40S ribosomal protein S23 | RPS23 | 0.0122 | 0.8086 |
| SPATS2-like protein | DNAPTP6 | 0.0123 | -0.4884 |
| Thyroid hormone receptor-associated protein 3 | THRAP3 | 0.0126 | -0.4759 |
| NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 11, mitochondrial | NDUFB11 | 0.0127 | -3.3131 |
| Eukaryotic translation initiation factor 4H | EIF4H | 0.0127 | -2.3031 |
| Heterogeneous nuclear ribonucleoprotein F | HNRPF | 0.0134 | -0.5895 |
| Protein transport protein Sec23A | SEC23A | 0.0136 | -1.6195 |
| D-3-phosphoglycerate dehydrogenase | PHGDH | 0.0140 | -0.6366 |
| Protein NipSnap homolog 2 | GBAS | 0.0147 | 1.0788 |

| | | | |
|---|---------|--------|---------|
| Putative RNA-binding protein Luc7-like 2 | LUC7L2 | 0.0152 | 1.1515 |
| Laminin subunit alpha-4 | LAMA4 | 0.0154 | 0.7832 |
| Lamin-B1 | LMNB1 | 0.0161 | 0.2827 |
| Golgi reassembly-stacking protein 2 | GORASP2 | 0.0164 | 1.7081 |
| Prolyl 3-hydroxylase 1 | LEPRE1 | 0.0167 | -1.2940 |
| Dihydrolipoyl dehydrogenase | DLD | 0.0167 | 0.3026 |
| Calpain-2 catalytic subunit | CAPN2 | 0.0171 | -1.3587 |
| Collagen alpha-2(V) chain | COL5A2 | 0.0171 | -1.4330 |
| 60S ribosomal protein L26 | RPL26 | 0.0178 | 0.6681 |
| Actin-related protein 3 | ACTR3 | 0.0179 | -0.4475 |
| Polymerase delta-interacting protein 3 | POLDIP3 | 0.0179 | -0.9405 |
| Nucleolar complex protein 2 homolog | NOC2L | 0.0185 | -0.9860 |
| Septin-2 | SEPT2 | 0.0185 | -0.3581 |
| 28S ribosomal protein S18a, mitochondrial | MRPS18A | 0.0188 | 0.6309 |
| UBX domain-containing protein 4 | UBXN4 | 0.0189 | 0.7451 |
| Transmembrane protein 65 | TMEM65 | 0.0198 | 1.2945 |
| Copine-3 | CPNE3 | 0.0199 | -1.5767 |
| Cytochrome c oxidase subunit 4 isoform 1, mitochondrial | COX4I1 | 0.0200 | 0.7576 |
| glutaminase | GLS | 0.0202 | 0.9063 |
| N-acetylglucosamine-6-sulfatase | GNS | 0.0204 | 2.5720 |
| Histone H2A.V | H2AFV | 0.0207 | 0.6423 |
| Cell cycle control protein 50A | TMEM30A | 0.0209 | -1.0582 |
| 28S ribosomal protein S36, mitochondrial | MRPS36 | 0.0209 | 0.4672 |
| Transgelin-2 | TAGLN2 | 0.0211 | -1.2663 |

| | | | |
|--|---------------|--------|---------|
| Putative helicase MOV-10 | MOV10 | 0.0213 | -0.9536 |
| Src substrate cortactin | CTTN | 0.0214 | -0.3318 |
| Importin subunit alpha | KPNA2 | 0.0215 | -0.3920 |
| Cytochrome b-c1 complex subunit 7 | UQCRB | 0.0216 | 0.5363 |
| Sequestosome-1 | SQSTM1 | 0.0216 | 0.4922 |
| Matrix metalloproteinase-14 | MMP14 | 0.0220 | 1.0464 |
| Acetyl-CoA acetyltransferase, cytosolic | ACAT2 | 0.0220 | -1.4965 |
| SUN domain-containing protein 2 | SUN2 | 0.0223 | -1.4274 |
| Major vault protein | MVP | 0.0224 | -0.3699 |
| MICOS complex subunit MIC60 | IMMT | 0.0224 | 0.3500 |
| Non-histone chromosomal protein HMG-17 | HMGN2 | 0.0232 | -0.6104 |
| Obg-like ATPase 1 | OLA1 | 0.0239 | -1.5918 |
| Glucose-6-phosphate isomerase | GPI | 0.0243 | -2.1884 |
| 40S ribosomal protein S24 | RPS24 | 0.0266 | -0.5799 |
| Gem-associated protein 5 | GEMIN5 | 0.0268 | 0.8453 |
| Pyruvate dehydrogenase E1 component subunit alpha | PDHA1 | 0.0268 | 0.3149 |
| Serine/threonine-protein phosphatase PP1-alpha catalytic subunit | PPP1CA | 0.0271 | -0.4546 |
| Wolframin | WFS1 | 0.0278 | 1.4713 |
| Nucleoside diphosphate kinase | NME1- NME2 | 0.0279 | -0.6432 |
| D-dopachrome decarboxylase | DDT | 0.0281 | -1.1471 |
| Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial | DLST | 0.0294 | 0.4810 |
| Protein YIF1A | YIF1A | 0.0296 | 1.6141 |
| 39S ribosomal protein L13, mitochondrial | MRPL13 | 0.0303 | 0.9888 |

| | | | |
|--|---------|--------|---------|
| Hypoxia up-regulated protein 1 | HYOU1 | 0.0305 | 0.3840 |
| Zinc finger CCCH domain-containing protein 14 | ZC3H14 | 0.0305 | 1.0385 |
| NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10 | NDUFB10 | 0.0307 | 0.5275 |
| Voltage-dependent anion-selective channel protein 1 | VDAC1 | 0.0308 | 0.4137 |
| Mitochondrial import receptor subunit TOM70 | TOMM70A | 0.0312 | 0.4007 |
| Insulin-like growth factor 2 mRNA-binding protein 3 | IGF2BP3 | 0.0315 | -0.3846 |
| Metaxin-2 | MTX2 | 0.0324 | 1.2495 |
| Basigin | EMMPRIN | 0.0331 | 0.4975 |
| Arginine--tRNA ligase, cytoplasmic | RARS | 0.0331 | -0.8051 |
| Heat shock protein beta-1 | HSPB1 | 0.0337 | -0.6896 |
| 60S ribosomal protein L37 | RPL37 | 0.0339 | 1.8160 |
| Heterogeneous nuclear ribonucleoprotein H2 | HNRNPH2 | 0.0342 | 0.4639 |
| Vesicle-associated membrane protein-associated protein A | VAPA | 0.0342 | 0.3434 |
| Eukaryotic translation initiation factor 5B | EIF5B | 0.0343 | -0.4981 |
| T-complex protein 1 subunit zeta | CCT6A | 0.0360 | -0.6830 |
| major histocompatibility complex, class I, C | HLA-C | 0.0363 | -0.4223 |
| Importin subunit beta-1 | KPNB1 | 0.0371 | -0.4539 |
| ADP/ATP translocase 2 | SLC25A5 | 0.0383 | 0.4003 |
| Ferritin light chain | FTL | 0.0391 | 0.7215 |
| Bone marrow stromal antigen 2 | BST2 | 0.0391 | 1.6869 |
| Protein transport protein Sec61 subunit alpha isoform 1 | SEC61A1 | 0.0397 | 0.9844 |
| DnaJ homolog subfamily C member 8 | DNAJC8 | 0.0400 | 1.4623 |
| CD44 antigen | CD44 | 0.0402 | 0.2670 |
| AP-2 complex subunit alpha-1 | AP2A1 | 0.0408 | -1.0962 |

| | | | |
|--|---------|--------|---------|
| Cytochrome b5 type B | CYB5B | 0.0410 | 0.6904 |
| Cytochrome b-c1 complex subunit 6, mitochondrial | UQCRH | 0.0414 | 0.2658 |
| Integrin beta | ITGB1 | 0.0418 | 0.3131 |
| tRNA-splicing ligase RtcB homolog | RTCB | 0.0418 | -1.3176 |
| ER membrane protein complex subunit 3 | EMC3 | 0.0421 | 0.4650 |
| Endoplasmic reticulum aminopeptidase 2 | ERAP2 | 0.0424 | -0.5146 |
| Ribosomal protein 63, mitochondrial | MRP63 | 0.0428 | 1.1748 |
| Signal peptidase complex subunit 2 | SPCS2 | 0.0430 | 0.4859 |
| Catenin beta-1 | CTNNB1 | 0.0432 | 0.3634 |
| Radixin | RDX | 0.0432 | -0.3131 |
| Bifunctional purine biosynthesis protein PURH | ATIC | 0.0434 | -1.1070 |
| Chromatin assembly factor 1 subunit A | CHAF1A | 0.0435 | 1.0982 |
| CD99 antigen | CD99 | 0.0445 | 2.5502 |
| Sterol O-acyltransferase 1 | SOAT1 | 0.0445 | 0.6573 |
| Mitochondrial import inner membrane translocase subunit TIM44 | hTIM44 | 0.0447 | 0.4991 |
| Ribonuclease inhibitor | RNH1 | 0.0466 | -1.5893 |
| 40S ribosomal protein SA | RPSA | 0.0467 | -0.5067 |
| Serine/arginine-rich splicing factor 6 | SRSF6 | 0.0469 | -1.2163 |
| Hydroxysteroid dehydrogenase-like protein 2 | HSDL2 | 0.0472 | 0.3804 |
| Monofunctional C1-tetrahydrofolate synthase, mitochondrial | MTHFD1L | 0.0478 | -0.5552 |
| CD166 antigen | ALCAM | 0.0483 | -0.6400 |
| Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial | DLAT | 0.0485 | 0.6243 |
| 5-3 exoribonuclease 2 | XRN2 | 0.0496 | -0.4001 |

| | | | |
|-------------------------|-------|--------|---------|
| Dynactin subunit 2 | DCTN2 | 0.0497 | -0.8707 |
| Phosphoglycerate kinase | PGK1 | 0.0498 | -0.6840 |

Table S4: List of overlapped DEPs across different nanoparticle treatment (iron nanoparticles and NP-Ab).

| Protein names | Gene | Iron nanoparticle vs. control | | NP-Ab vs. control | |
|--|--------|-------------------------------|--------------------------------|-------------------|--------------------------------|
| | | T-test (p-value) | log ₂ (Fold change) | T-test (p-value) | log ₂ (Fold change) |
| ATP-binding cassette sub-family G member 2 | ABCG2 | 0.0007 | -3.9147 | 0.0001 | -4.0280 |
| T-complex protein 1 subunit zeta | CCT6A | 0.0458 | -1.0169 | 0.0360 | -0.6830 |
| SAP domain-containing ribonucleoprotein | CIP29 | 0.0113 | -1.9063 | 0.0003 | -1.5195 |
| Y-box-binding protein 3 | CSDA | 0.0475 | -0.3591 | 0.0118 | -0.5607 |
| Src substrate cortactin | CTTN | 0.0147 | -0.4255 | 0.0214 | -0.3318 |
| Dynactin subunit 2 | DCTN2 | 0.0428 | -0.8899 | 0.0497 | -0.8707 |
| Early endosome antigen 1 | EEA1 | 0.0300 | -1.3751 | 0.0102 | -1.6817 |
| Non-histone chromosomal protein HMG-17 | HMGN2 | 0.0139 | -0.8954 | 0.0232 | -0.6104 |
| Importin subunit alpha | KPNA2 | 0.0368 | -0.4933 | 0.0215 | -0.3920 |
| galectin 1 | LGALS1 | 0.0254 | -2.3125 | 0.0005 | -2.5652 |
| Nucleolar complex protein 2 homolog | NOC2L | 0.0201 | -0.5056 | 0.0185 | -0.9860 |
| Obg-like ATPase 1 | OLA1 | 0.0407 | -1.1111 | 0.0239 | -1.5918 |
| Lipid phosphate phosphohydrolase 1 | PPAP2A | 0.0053 | -0.5620 | 0.0066 | -1.4439 |
| Radixin | RDX | 0.0498 | -0.8543 | 0.0432 | -0.3131 |
| Septin-2 | SEPT2 | 0.0247 | -0.6835 | 0.0185 | -0.3581 |
| Protein THEM6 | THEM6 | 0.0164 | -1.3676 | 0.0004 | -1.9948 |
| Adipocyte plasma membrane-associated protein | APMAP | 0.0059 | 0.3165 | 0.0006 | 0.3352 |
| CD44 antigen | CD44 | 0.0392 | 0.3942 | 0.0402 | 0.2670 |
| Eukaryotic initiation factor 4A-III | EIF4A3 | 0.0050 | 2.3533 | 0.0002 | 2.5383 |
| Flotillin-2 | FLOT2 | 0.0329 | 0.6724 | 0.0107 | 0.6411 |

| | | | | | |
|--|---------|--------|--------|--------|--------|
| Insulin-like growth factor-binding protein 5 | IGFBP5 | 0.0172 | 1.6481 | 0.0036 | 1.4291 |
| Putative RNA-binding protein Luc7-like 2 | LUC7L2 | 0.0307 | 0.8823 | 0.0152 | 1.1515 |
| Midkine | MDK | 0.0066 | 2.9811 | 0.0003 | 3.4510 |
| Pleiotrophin | PTN | 0.0308 | 1.7302 | 0.0006 | 1.8087 |
| 60S ribosomal protein L37 | RPL37 | 0.0153 | 1.9032 | 0.0339 | 1.8160 |
| 40S ribosomal protein S23 | RPS23 | 0.0450 | 0.6056 | 0.0122 | 0.8086 |
| Transmembrane protein 205 | TMEM205 | 0.0105 | 1.8023 | 0.0008 | 1.8339 |
| Basigin | EMMPRIN | 0.0476 | 0.2804 | 0.0331 | 0.4975 |

Table S5: Gene ontology enrichment analysis of DEPs (iron nanoparticles vs. control).

| Term | | Count | % | Genes | Fold Enrichment | p-value |
|---------------------------|---|-------|--------|---|-----------------|---------|
| Biological Process | | | | | | |
| GO:0007062 | sister chromatid cohesion | 3 | 3.5294 | RAD21, SMC3, PHB2 | 27.5399 | 0.0052 |
| GO:0002181 | cytoplasmic translation | 4 | 4.7059 | RPL35A, RPL27, RPL37, RPS23 | 10.6080 | 0.0062 |
| GO:0006606 | protein import into nucleus | 4 | 4.7059 | NUP133, KPNA2, PHB2, IPO7 | 9.1800 | 0.0092 |
| GO:0006915 | apoptotic process | 8 | 9.4118 | BCLAF1, LGALS1, AIFM1, PTRH2, RAD21, CYCS, NOC2L, SLC25A6 | 3.1457 | 0.0128 |
| GO:0006412 | translation | 5 | 5.8824 | RPL35A, RPL27, RPL37, RPS23, ABCF1 | 5.3515 | 0.0138 |
| GO:0034089 | establishment of meiotic sister chromatid cohesion | 2 | 2.3529 | RAD21, SMC3 | 119.3395 | 0.0165 |
| GO:1904707 | positive regulation of vascular smooth muscle cell proliferation | 3 | 3.5294 | DNMT1, IGFBP5, GNAI2 | 12.7864 | 0.0225 |
| GO:0030335 | positive regulation of cell migration | 5 | 5.8824 | SDCBP, MYO1C, MDK, RDX, GNAI2 | 4.5204 | 0.0240 |
| GO:0006406 | mRNA export from nucleus | 3 | 3.5294 | ENY2, NUP133, EIF4A3 | 12.3455 | 0.0241 |
| GO:0008360 | regulation of cell shape | 4 | 4.7059 | RDX, PLXNB2, PTN, MYH10 | 6.1995 | 0.0261 |
| GO:0034087 | establishment of mitotic sister chromatid cohesion | 2 | 2.3529 | RAD21, SMC3 | 68.1940 | 0.0286 |
| GO:2000210 | positive regulation of anoikis | 2 | 2.3529 | MYBBP1A, PTRH2 | 68.1940 | 0.0286 |
| GO:1905460 | negative regulation of vascular associated smooth muscle cell apoptotic process | 2 | 2.3529 | SLC7A5, DNMT1 | 68.1940 | 0.0286 |
| GO:0002232 | leukocyte chemotaxis involved in inflammatory response | 2 | 2.3529 | MDK, PTN | 59.6698 | 0.0326 |

| | | | | | | |
|---------------------------|--|----|---------|--|---------|---------|
| GO:0006930 | substrate-dependent cell migration, cell extension | 2 | 2.3529 | SDCBP, CTTN | 53.0398 | 0.0366 |
| GO:2000347 | positive regulation of hepatocyte proliferation | 2 | 2.3529 | MDK, PTN | 43.3962 | 0.0446 |
| GO:0045727 | positive regulation of translation | 3 | 3.5294 | EIF4A3, PLXNB2, ABCF1 | 8.5243 | 0.0474 |
| GO:0032330 | regulation of chondrocyte differentiation | 2 | 2.3529 | MDK, GLG1 | 39.7798 | 0.0486 |
| GO:0043065 | positive regulation of apoptotic process | 5 | 5.8824 | BCLAF1, LGALS1, AIFM1, PTN, CTSD | 3.5946 | 0.0489 |
| Molecular Function | | | | | | |
| GO:0003723 | RNA binding | 30 | 35.2941 | DNMT1, CSTF2, USO1, RPN1, EIF4A3, NOC2L, DHX30, BCLAF1, LGALS1, RALY, RPL37, HMGN2, KPNA2, EIF4B, ABCF1, SF3A3, LRRC47, PNPT1, RDX, RPL35A, PRRC2C, CCT6A, MYBBP1A, RPL27, CEBPZ, HNRNPH2, MATR3, TOP1, LUC7L2, RPS23 | 4.7026 | <0.0001 |
| GO:0005515 | protein binding | 71 | 83.5294 | MDC1, EIF4A3, OLA1, SMC3, NOC2L, IPO7, PHB2, EEA1, LGALS1, AIFM1, APMAP, MDK, FLOT2, HMGN2, KPNA2, CTSD, LRRC47, IGFBP5, NUP133, ARFGAP1, MRPL53, SLC7A5, CTTN, TMX1, EEF1D, PTRH2, PSME1, PSME2, RPL27, MATR3, HNRNPH2, CD47, TOP1, LUC7L2, CD44, SLC25A6, ABCG2, ENY2, DNMT1, DCTN2, ATL3, USO1, RPN1, CSTF2, AK2, PTN, COX5B, GNAI2, DNAJB1, SDCBP, DHX30, BCLAF1, CBS, TMEM205, RAD21, RALY, MYH10, EIF4B, ABCF1, SF3A3, PNPT1, RDX, RPL35A, RAB10, CCT6A, PSMC5, MYO1C, GNB2, CYCS, PLXNB2, RPS23 | 1.2974 | <0.0001 |
| GO:0003682 | chromatin binding | 8 | 9.4118 | DHX30, DNMT1, ENY2, RAD21, TOP1, SMC3, NOC2L, HMGN2 | 3.9332 | 0.0040 |
| GO:0016887 | ATPase activity | 7 | 8.2353 | CCT6A, DHX30, PSMC5, EIF4A3, OLA1, SMC3, MYH10 | 4.5596 | 0.0041 |
| GO:0045296 | cadherin binding | 6 | 7.0588 | DNAJB1, CTTN, EEF1D, USO1, RDX, OLA1 | 4.3782 | 0.0115 |
| GO:0008022 | protein C-terminus binding | 5 | 5.8824 | SDCBP, MDC1, MYO1C, PRRC2C, PHB2 | 5.5164 | 0.0124 |
| GO:0045545 | syndecan binding | 2 | 2.3529 | SDCBP, PTN | 76.8618 | 0.0254 |
| GO:1904399 | heparan sulfate binding | 2 | 2.3529 | MDK, PTN | 65.8815 | 0.0296 |
| GO:0035374 | chondroitin sulfate binding | 2 | 2.3529 | MDK, PTN | 46.1171 | 0.0420 |
| GO:0061133 | endopeptidase activator activity | 2 | 2.3529 | PSME1, PSME2 | 41.9246 | 0.0461 |

| | | | | | | |
|---------------------------|---|----|---------|---|----------|--------|
| GO:0003735 | structural constituent of ribosome | 4 | 4.7059 | RPL35A, RPL27, RPL37, RPS23 | 4.8801 | 0.0476 |
| Cellular Component | | | | | | |
| GO:0042788 | polysomal ribosome | 3 | 3.5294 | PNPT1, RPS23, ABCF1 | 22.2727 | 0.0078 |
| GO:0008537 | proteasome activator complex | 2 | 2.3529 | PSME1, PSME2 | 163.3333 | 0.0121 |
| GO:0005743 | mitochondrial inner membrane | 7 | 8.2353 | MRPL53, AIFM1, NNT, CYCS, COX5B, PHB2, SLC25A6 | 3.5804 | 0.0129 |
| GO:0005739 | mitochondrion | 13 | 15.2941 | ENY2, PNPT1, AK2, COX5B, PHB2, SDCBP, DHX30, MRPL53, AIFM1, NNT, PTRH2, CYCS, SLC25A6 | 2.1950 | 0.0133 |
| GO:0016363 | nuclear matrix | 4 | 4.7059 | RAD21, MATR3, SMC3, PHB2 | 7.5385 | 0.0157 |
| GO:0045121 | membrane raft | 5 | 5.8824 | SDCBP, MYO1C, CTSD, GNAI2, ABCG2 | 4.8419 | 0.0192 |
| GO:0045202 | synapse | 7 | 8.2353 | SDCBP, RPL35A, RPL27, RPL37, ARFGAP1, GNAI2, RPS23 | 3.2543 | 0.0197 |
| GO:0005694 | chromosome | 5 | 5.8824 | MDC1, RAD21, TOP1, SMC3, NOC2L | 4.7852 | 0.0200 |
| GO:0000502 | proteasome complex | 3 | 3.5294 | PSMC5, PSME1, PSME2 | 12.8947 | 0.0222 |
| GO:0098591 | external side of apical plasma membrane | 2 | 2.3529 | SLC7A5, ABCG2 | 81.6667 | 0.0240 |
| GO:0034991 | nuclear meiotic cohesin complex | 2 | 2.3529 | RAD21, SMC3 | 81.6667 | 0.0240 |
| GO:0022625 | cytosolic large ribosomal subunit | 3 | 3.5294 | RPL35A, RPL27, RPL37 | 12.2500 | 0.0245 |
| GO:0030864 | cortical actin cytoskeleton | 3 | 3.5294 | CTTN, RDX, FLOT2 | 12.2500 | 0.0245 |
| GO:0005791 | rough endoplasmic reticulum | 3 | 3.5294 | RPN1, RPL27, RPS23 | 11.8548 | 0.0260 |
| GO:0031982 | vesicle | 4 | 4.7059 | MYO1C, DCTN2, GNB2, FLOT2 | 5.6322 | 0.0334 |
| GO:0005912 | adherens junction | 4 | 4.7059 | RAB10, SDCBP, RDX, FLOT2 | 5.5682 | 0.0343 |
| GO:0005902 | microvillus | 3 | 3.5294 | MYO1C, RDX, CD44 | 9.9324 | 0.0360 |
| GO:0098978 | glutamatergic synapse | 6 | 7.0588 | EEA1, DNAJB1, EIF4A3, FLOT2, KPNA2, PHB2 | 3.2379 | 0.0366 |
| GO:0030496 | midbody | 4 | 4.7059 | RAD21, RDX, MYH10, GNAI2 | 5.2973 | 0.0389 |

| | | | | | | |
|------------|--|----|---------|---|---------|--------|
| GO:0042564 | NLS-dependent protein nuclear import complex | 2 | 2.3529 | MYBBP1A, KPNA2 | 49.0000 | 0.0396 |
| GO:0005783 | endoplasmic reticulum | 10 | 11.7647 | CTTN, ATL3, SSR4, EEF1D, POFUT1, USO1, RPN1, PTN, SDF2, RPS23 | 2.1416 | 0.0406 |
| GO:0030027 | lamellipodium | 4 | 4.7059 | CTTN, RDX, FLOT2, MYH10 | 5.1042 | 0.0427 |
| GO:0009986 | cell surface | 7 | 8.2353 | LGALS1, APMAP, PLXNB2, CD47, PTN, PHB2, CD44 | 2.6713 | 0.0451 |
| GO:0035145 | exon-exon junction complex | 2 | 2.3529 | BCLAF1, EIF4A3 | 40.8333 | 0.0473 |

Table S6: Gene ontology enrichment analysis of DEPs (NP-Ab vs. control).

| Term | | Count | % | Genes | Fold Enrichment | p-value |
|--------------------|--|-------|---------|---|-----------------|---------|
| Biological Process | | | | | | |
| GO:0006412 | translation | 17 | 11.4865 | MRPS17, MRPS18B, MRPS18A, MRPS21, RPSA, MRPL13, MRPS5, MRPL33, RPS27, GEMIN5, RPL13, IGF2BP3, RPL26, RPL37, RPS24, RPS23, ABCF1 | 10.3790 | <0.0001 |
| GO:0002181 | cytoplasmic translation | 7 | 4.7297 | RPS27, RPL13, RPSA, RPL37, RPL26, RPS24, RPS23 | 10.5893 | 0.0001 |
| GO:0032543 | mitochondrial translation | 7 | 4.7297 | MRPS17, MRPS18B, MRPS18A, MRPS21, MRPL13, MRPS5, MRPL33 | 9.9275 | 0.0001 |
| GO:0006099 | tricarboxylic acid cycle | 5 | 3.3784 | PDHA1, MRPS36, IDH3B, DLST, DLAT | 20.6285 | 0.0001 |
| GO:0006103 | 2-oxoglutarate metabolic process | 4 | 2.7027 | MRPS36, IDH3B, DLST, DLD | 30.2551 | 0.0003 |
| GO:0006086 | acetyl-CoA biosynthetic process from pyruvate | 3 | 2.0270 | PDHA1, DLAT, DLD | 68.0739 | 0.0008 |
| GO:0006446 | regulation of translational initiation | 4 | 2.7027 | EIF5B, EIF4H, HSPB1, EIF3H | 17.5675 | 0.0015 |
| GO:0006888 | ER to Golgi vesicle-mediated transport | 6 | 4.0541 | COPB2, SEC23A, VAPA, HYOU1, YIF1A, ERGIC1 | 6.2358 | 0.0027 |
| GO:0050821 | protein stabilization | 7 | 4.7297 | CCT6A, CCT2, BAG2, LAMP1, WFS1, FLOT1, FLOT2 | 4.3320 | 0.0056 |
| GO:0006164 | purine nucleotide biosynthetic process | 3 | 2.0270 | SLC4A7, ATIC, MTHFD1 | 25.5277 | 0.0059 |
| GO:0009257 | 10-formyltetrahydrofolate biosynthetic process | 2 | 1.3514 | MTHFD1, MTHFD1L | 136.1479 | 0.0145 |
| GO:0009060 | aerobic respiration | 4 | 2.7027 | UQCRB, NDUFB10, NDUFB11, UQCRH | 7.7799 | 0.0146 |
| GO:0006364 | rRNA processing | 5 | 3.3784 | RPS27, XRN2, EIF4A3, RPL26, RPS24 | 5.1965 | 0.0156 |
| GO:0017148 | negative regulation of translation | 4 | 2.7027 | IGFBP5, EIF4A3, IGF2BP3, MRPL13 | 6.5613 | 0.0228 |
| GO:0045727 | positive regulation of translation | 4 | 2.7027 | POLDIP3, EIF4A3, RPL26, ABCF1 | 6.4832 | 0.0236 |

| | | | | | | |
|---------------------------|---|---|--------|--|---------|--------|
| GO:0034976 | response to endoplasmic reticulum stress | 4 | 2.7027 | GORASP2, WFS1, FLOT1, HYOU1 | 6.4070 | 0.0243 |
| GO:0032212 | positive regulation of telomere maintenance via telomerase | 3 | 2.0270 | CCT6A, CCT2, CTNNB1 | 12.0130 | 0.0254 |
| GO:0007405 | neuroblast proliferation | 3 | 2.0270 | ITGB1, RACGAP1, CTNNB1 | 11.3457 | 0.0283 |
| GO:0090114 | COPII-coated vesicle budding | 2 | 1.3514 | SEC23A, VAPA | 54.4592 | 0.0359 |
| GO:0061732 | mitochondrial acetyl-CoA biosynthetic process from pyruvate | 2 | 1.3514 | PDHA1, DLD | 54.4592 | 0.0359 |
| GO:0061077 | chaperone-mediated protein folding | 3 | 2.0270 | CCT6A, CCT2, HSPB1 | 9.9620 | 0.0360 |
| GO:0045333 | cellular respiration | 3 | 2.0270 | UQCRB, COX4I1, UQCRH | 9.7248 | 0.0376 |
| GO:0046718 | viral entry into host cell | 4 | 2.7027 | ITGB1, LAMP1, RPSA, PVR | 5.3391 | 0.0387 |
| GO:0015031 | protein transport | 8 | 5.4054 | SEC61A1, SEC23A, MTX2, MVP, TIMM13, YIF1A, SCAMP3, ARL8B | 2.4811 | 0.0422 |
| GO:1901526 | positive regulation of macromitophagy | 2 | 1.3514 | VDAC1, SLC25A5 | 45.3826 | 0.0430 |
| GO:0006880 | intracellular sequestering of iron ion | 2 | 1.3514 | FTH1, FTL | 45.3826 | 0.0430 |
| GO:0001765 | membrane raft assembly | 2 | 1.3514 | FLOT1, FLOT2 | 45.3826 | 0.0430 |
| GO:2000675 | negative regulation of type B pancreatic cell apoptotic process | 2 | 1.3514 | WFS1, SRSF6 | 45.3826 | 0.0430 |
| GO:0030335 | positive regulation of cell migration | 6 | 4.0541 | ITGB1, SUN2, MMP14, MDK, RDX, CPNE3 | 3.0943 | 0.0444 |
| GO:0032355 | response to estradiol | 4 | 2.7027 | GGT5, GPI, CTNNB1, PTN | 4.8624 | 0.0487 |
| GO:2000391 | positive regulation of neutrophil extravasation | 2 | 1.3514 | MDK, CD99 | 38.8994 | 0.0500 |
| Molecular Function | | | | | | |

| | | | | | | |
|------------|--|-----|---------|---|----------|---------|
| GO:0003723 | RNA binding | 46 | 31.0811 | NUFIP2, POLDIP3, RTCB, EIF4A3, HSPB1, NTPCR, NOC2L, MRPL13, LGALS1, PCBP2, EIF4H, RPL13, FLNB, IGF2BP3, CPNE3, RPL37, HMG2, KPNA2, ZC3H14, RAE1, ABCF1, SEC63, SMARCE1, EIF5B, RDX, HMGA1, IMMT, MRPS21, RPSA, MRPS5, CCT6A, BST2, MOV10, RPS27, THRAP3, XRN2, GEMIN5, EIF3H, HNRNPH2, RPL26, SRSF6, SLC25A5, RPS24, LUC7L2, KPNB1, RPS23 | 4.0498 | <0.0001 |
| GO:0003735 | structural constituent of ribosome | 14 | 9.4595 | MRPS17, MRPS18B, MRPS18A, MRPS21, RPSA, MRPL13, MRPS5, MRPL33, RPS27, RPL13, RPL26, RPL37, RPS24, RPS23 | 9.5931 | <0.0001 |
| GO:0005515 | protein binding | 127 | 85.8108 | RNH1, TMEM41B, RTCB, EIF4A3, NOC2L, PVR, LGALS1, ALCAM, APMAP, LAMP1, FTH1, CAPN2, CPNE3, HMG2, KPNA2, SEC63, IGFBP5, MRPS18B, HLA-C, RPSA, ARMC1, TMX3, MTHFD1, SOAT1, XRN2, GEMIN5, TAGLN2, VDAC1, RPL26, SRSF6, SQSTM1, KPNB1, ARL8B, FTL, ABCG2, COPB2, NDUFB10, UQCRB, NDUFB11, MRPL13, GNS, ACAT2, UQCRH, BAG2, RACGAP1, PCBP2, PGK1, ZC3H14, CD99, PDHA1, SDF2L1, WFS1, RDX, SMARCA5, HMGA1, CCT6A, BST2, MOV10, RPS27, HYOU1, RPS23, ITGB1, MRPS17, GPI, FEN1, COX4I1, OLA1, HSPB1, AP2A1, HCCS, LMNB1, EEA1, SEC61A1, CHAF1A, MDK, FLOT1, FLOT2, DLAT, UBXLN4, ACTR3, CCT2, SUN2, EIF5B, MMP14, CTTN, GORASP2, THRAP3, HNRNPH2, DNAJC8, SLC25A5, DLD, ERGIC1, LUC7L2, CD44, CERS2, SEC23A, SF3B3, DCTN2, NUFIP2, POLDIP3, MVP, TIMM13, DLST, PTN, TMEM65, TMEM205, EMC3, EIF4H, RPL13, FLNB, IGF2BP3, RAE1, ABCF1, CYB5B, SMARCE1, TMEM30A, MTX2, IMMT, PEX14, YIF1A, PPP1CA, SOD1, SLC4A7, SPCS2, VAPA, CTNNB1, EIF3H | 1.3034 | <0.0001 |
| GO:0045296 | cadherin binding | 10 | 6.7568 | ITGB1, ATIC, CTTN, VAPA, RDX, EIF4H, OLA1, CTNNB1, TAGLN2, FLNB | 4.0983 | 0.0008 |
| GO:0034604 | pyruvate dehydrogenase (NAD+) activity | 3 | 2.0270 | PDHA1, DLAT, DLD | 64.7534 | 0.0009 |
| GO:0043022 | ribosome binding | 5 | 3.3784 | SEC61A1, OLA1, GEMIN5, RPSA, ABCF1 | 8.9935 | 0.0023 |
| GO:0031625 | ubiquitin protein ligase binding | 8 | 5.4054 | CCT2, GPI, BAG2, WFS1, PCBP2, SLC25A5, SQSTM1, SCAMP3 | 3.3748 | 0.0096 |
| GO:0003729 | mRNA binding | 7 | 4.7297 | POLDIP3, PCBP2, EIF4A3, IGF2BP3, SRSF6, MRPL13, LUC7L2 | 3.7002 | 0.0116 |
| GO:0008320 | protein transmembrane transporter activity | 3 | 2.0270 | SEC61A1, PEX14, SEC63 | 16.1884 | 0.0144 |
| GO:0004329 | formate-tetrahydrofolate ligase activity | 2 | 1.3514 | MTHFD1, MTHFD1L | 129.5068 | 0.0153 |
| GO:0043236 | laminin binding | 3 | 2.0270 | ITGB1, LGALS1, RPSA | 14.9431 | 0.0168 |
| GO:0051082 | unfolded protein binding | 5 | 3.3784 | CCT6A, CCT2, CHAF1A, HSPB1, HYOU1 | 4.9430 | 0.0184 |

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|---------------------------|--|----|---------|--|---------|---------|
| GO:0035255 | ionotropic glutamate receptor binding | 3 | 2.0270 | FLOT1, FLOT2, SQSTM1 | 13.8757 | 0.0194 |
| GO:0001618 | virus receptor activity | 4 | 2.7027 | ITGB1, LAMP1, RPSA, PVR | 6.7276 | 0.0214 |
| GO:0004488 | methylenetetrahydrofolate dehydrogenase (NADP+) activity | 2 | 1.3514 | MTHFD1, MTHFD1L | 64.7534 | 0.0303 |
| GO:0019899 | enzyme binding | 8 | 5.4054 | LAMP1, PCBP2, HMGA1, CAPN2, CTNNB1, SQSTM1, KPNB1, LUC7L2 | 2.6566 | 0.0309 |
| GO:0051287 | NAD binding | 3 | 2.0270 | IDH3B, PHGDH, DLD | 9.7130 | 0.0377 |
| GO:0048487 | beta-tubulin binding | 3 | 2.0270 | RACGAP1, PEX14, ARL8B | 9.2505 | 0.0412 |
| GO:0019901 | protein kinase binding | 9 | 6.0811 | RACGAP1, DCTN2, MVP, HSPB1, CTNNB1, AP2A1, VDAC1, PTN, SQSTM1 | 2.2809 | 0.0434 |
| Cellular Component | | | | | | |
| GO:0016020 | membrane | 66 | 44.5946 | ITGB1, GPI, FEN1, COX4I1, EIF4A3, OLA1, AP2A1, HCCS, PVR, LMNB1, EEA1, SEC61A1, ATIC, APMAP, LAMP1, FLOT1, FLOT2, KPNA2, SEC63, ACTR3, HSDL2, HLA-C, RPSA, MTHFD1, SOAT1, GORASP2, XRN2, GEMIN5, HNRNPH2, VDAC1, RPL26, SLC25A5, ERGIC1, KPNB1, CERS2, ARL8B, FTL, ABCG2, DCTN2, NUFIP2, LAMA4, MVP, DLST, PTN, NTPCR, MTHFD1L, TMEM205, PCBP2, EMC3, EIF4H, PGK1, RPL13, FLNB, ABCF1, CYB5B, SDF2L1, TMEM30A, IMMT, PEX14, SLC4A7, BST2, CTNNB1, EIF3H, HYOU1, RPS24, RPS23 | 2.4222 | <0.0001 |
| GO:0070062 | extracellular exosome | 49 | 33.1081 | ITGB1, GPI, RNH1, OLA1, HSPB1, EEA1, ALCAM, ATIC, LGALS1, LAMP1, FTH1, CAPN2, FLOT1, FLOT2, CPNE3, PHGDH, ACTR3, CCT2, HLA-C, RPSA, SCAMP3, MTHFD1, THRAP3, TAGLN2, VDAC1, RPL26, SQSTM1, CD44, KPNB1, ARL8B, FTL, DCTN2, LAMA4, MVP, GNS, ACAT2, RACGAP1, DDT, PCBP2, PGK1, FLNB, RDX, SOD1, PPP1CA, CCT6A, BST2, CTNNB1, EIF3H, HYOU1 | 3.0706 | <0.0001 |
| GO:0005925 | focal adhesion | 19 | 12.8378 | ITGB1, ACTR3, RDX, HMGA1, HSPB1, PVR, MMP14, ALCAM, CTTN, PCBP2, FLOT1, CAPN2, FLOT2, CTNNB1, FLNB, HYOU1, CPNE3, CD44, CD99 | 6.2165 | <0.0001 |
| GO:0005829 | cytosol | 73 | 49.3243 | GPI, RNH1, COX4I1, RTCB, EIF4A3, OLA1, HSPB1, AP2A1, HCCS, NOC2L, LMNB1, EEA1, ATIC, LGALS1, LAMP1, FTH1, CAPN2, CPNE3, PHGDH, RPL37, UBXLN4, KPNA2, ACTR3, EIF5B, CCT2, RPSA, ARMC1, MMP14, MTHFD1, CTTN, GEMIN5, TAGLN2, HNRNPH2, RPL26, DNAJC8, SQSTM1, CD44, KPNB1, ARL8B, FTL, COPB2, SEC23A, DCTN2, NUFIP2, POLDIP3, MVP, DLST, ACAT2, NME1-NME2, MTHFD1L, BAG2, RACGAP1, PCBP2, EIF4H, PGK1, RPL13, FLNB, IGF2BP3, ABCF1, SMARCA5, HMGA1, PEX14, | 1.8389 | <0.0001 |

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| | | | | SOD1, PPP1CA, CCT6A, BST2, MOV10, RPS27, VAPA, CTNNB1, EIF3H, RPS24, RPS23 | | |
| GO:0005743 | mitochondrial inner membrane | 18 | 12.1622 | MRPS17, NDUFB10, UQCRB, MRPS36, NDUFB11, COX4I1, MRPS18B, TIMM13, MRPS18A, IMMT, MRPS21, HCCS, MRPL13, MRPS5, UQCRH, MRPL33, TMEM65, SLC25A5 | 5.2254 | <0.0001 |
| GO:0005739 | mitochondrion | 31 | 20.9459 | FEN1, NDUFB11, MRPS36, COX4I1, TIMM13, DLST, HCCS, MRPL13, UQCRH, ACAT2, MRPL33, TMEM65, MTHFD1L, IDH3B, CPNE3, DLAT, EIF5B, HSDL2, PDHA1, MTX2, MRPS18B, MRPS18A, IMMT, MRPS5, SOD1, ARMC1, MTHFD1, VDAC1, SLC25A5, DLD, SQSTM1 | 2.9708 | <0.0001 |
| GO:0005840 | ribosome | 10 | 6.7568 | MRPS17, RPS27, MRPS18B, MRPS18A, RPL13, RPL37, MRPL13, RPS24, RPS23, ABCF1 | 7.9008 | <0.0001 |
| GO:0022626 | cytosolic ribosome | 7 | 4.7297 | RPS27, RPL13, RPSA, RPL37, RPL26, RPS24, RPS23 | 12.6413 | <0.0001 |
| GO:0044754 | autolysosome | 4 | 2.7027 | LAMP1, FTH1, SQSTM1, FTL | 50.5651 | 0.0001 |
| GO:0005763 | mitochondrial small ribosomal subunit | 5 | 3.3784 | MRPS17, MRPS18B, MRPS18A, MRPS21, MRPS5 | 21.0688 | 0.0001 |
| GO:0005783 | endoplasmic reticulum | 21 | 14.1892 | SDF2L1, TMEM30A, ERAP2, WFS1, HLA-C, PTN, SEC61A1, TMX3, CTTN, VAPA, SOAT1, CAPN2, RPL13, HYOU1, UBXL4, SQSTM1, ERGIC1, RPS24, CERS2, RPS23, SEC63 | 2.5526 | 0.0002 |
| GO:0005789 | endoplasmic reticulum membrane | 20 | 13.5135 | COPB2, SEC23A, SDF2L1, ERAP2, TMEM41B, WFS1, RTCB, HLA-C, YIF1A, SEC61A1, SPCS2, TMX3, VAPA, SOAT1, GORASP2, EMC3, UBXL4, KPNA2, ERGIC1, CERS2 | 2.5145 | 0.0003 |
| GO:0045254 | pyruvate dehydrogenase complex | 3 | 2.0270 | PDHA1, DLAT, DLD | 69.5270 | 0.0007 |
| GO:0035577 | azurophil granule membrane | 5 | 3.3784 | BST2, TMEM30A, VAPA, LAMP1, CPNE3 | 11.9874 | 0.0008 |
| GO:0030864 | cortical actin cytoskeleton | 5 | 3.3784 | CTTN, RDX, FLOT1, CAPN2, FLOT2 | 11.5878 | 0.0009 |
| GO:0005967 | mitochondrial pyruvate dehydrogenase complex | 3 | 2.0270 | PDHA1, DLAT, DLD | 52.1453 | 0.0014 |
| GO:0042788 | polysomal ribosome | 4 | 2.7027 | NUFIP2, EIF3H, RPS23, ABCF1 | 16.8550 | 0.0017 |
| GO:0016600 | flotillin complex | 3 | 2.0270 | FLOT1, CTNNB1, FLOT2 | 46.3514 | 0.0018 |
| GO:0045121 | membrane raft | 8 | 5.4054 | ITGB1, BST2, FLOT1, CAPN2, PGK1, VDAC1, SLC25A5, ABCG2 | 4.3970 | 0.0023 |
| GO:0030027 | lamellipodium | 7 | 4.7297 | ACTR3, ITGB1, CTTN, RDX, FLOT1, CTNNB1, FLOT2 | 5.0697 | 0.0026 |
| GO:0005635 | nuclear envelope | 7 | 4.7297 | SUN2, RTCB, UBXL4, RAE1, KPNA1, LMNB1, ABCF1 | 5.0174 | 0.0027 |
| GO:0022627 | cytosolic small ribosomal subunit | 4 | 2.7027 | RPS27, RPSA, RPS24, RPS23 | 12.0917 | 0.0043 |
| GO:0005912 | adherens junction | 6 | 4.0541 | RDX, FLOT1, CTNNB1, FLOT2, PVR, PPP1CA | 4.7405 | 0.0088 |

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|------------|------------------------------------|----|---------|---|----------|--------|
| GO:1990904 | ribonucleoprotein complex | 6 | 4.0541 | MVP, PCBP2, EIF4A3, HNRNPH2, RPL26, ZC3H14 | 4.5591 | 0.0102 |
| GO:1904115 | axon cytoplasm | 4 | 2.7027 | HSPB1, ZC3H14, ARL8B, SOD1 | 8.5572 | 0.0113 |
| GO:1904813 | ficolin-1-rich granule lumen | 5 | 3.3784 | GPI, MVP, FTH1, GNS, KPNB1 | 5.6070 | 0.0121 |
| GO:0045202 | synapse | 10 | 6.7568 | SLC4A7, EIF5B, RPL13, CTNNB1, VDAC1, RPL37, RPL26, RPS24, RPS23, ARL8B | 2.6386 | 0.0136 |
| GO:0008043 | intracellular ferritin complex | 2 | 1.3514 | FTH1, FTL | 139.0541 | 0.0142 |
| GO:0015935 | small ribosomal subunit | 3 | 2.0270 | RPSA, RPS24, RPS23 | 14.3849 | 0.0181 |
| GO:0005654 | nucleoplasm | 40 | 27.0270 | GPI, FEN1, SF3B3, RNH1, NUFIP2, POLDIP3, COX4I1, RTCB, EIF4A3, DLST, NOC2L, LMNB1, RACGAP1, PCBP2, CPNE3, KPNA2, RAE1, ABCF1, SMARCE1, SMARCA5, HMGA1, MRPS18B, RPSA, SOD1, PPP1CA, RPS27, THRAP3, XRN2, GEMIN5, CTNNB1, HNRNPH2, RPL26, DNAJC8, SRSF6, SQSTM1, ERGIC1, RPS24, KPNB1, RPS23, ABCG2 | 1.4046 | 0.0187 |
| GO:0101004 | cytolytic granule membrane | 2 | 1.3514 | LAMP1, ARL8B | 92.7027 | 0.0213 |
| GO:0005788 | endoplasmic reticulum lumen | 7 | 4.7297 | LGALS1, SDF2L1, IGFBP5, ERAP2, WFS1, COL5A2, HYOU1 | 3.1810 | 0.0228 |
| GO:0005758 | mitochondrial intermembrane space | 4 | 2.7027 | TIMM13, CAPN2, IMMT, SOD1 | 6.1802 | 0.0267 |
| GO:0010494 | cytoplasmic stress granule | 4 | 2.7027 | MOV10, NUFIP2, IGF2BP3, KPNB1 | 6.1802 | 0.0267 |
| GO:0005737 | cytoplasm | 52 | 35.1351 | ITGB1, RNH1, RTCB, EIF4A3, OLA1, HSPB1, PVR, EEA1, LGALS1, LAMP1, MDK, FTH1, CAPN2, CPNE3, RPL37, HMGN2, KPNA2, ACTR3, EIF5B, RPSA, MMP14, CTTN, GEMIN5, RPL26, SQSTM1, KPNB1, ARL8B, FTL, DCTN2, NUFIP2, POLDIP3, MVP, ACAT2, DDT, PCBP2, RPL13, FLNB, IGF2BP3, ZC3H14, RAE1, CD99, ABCF1, ERAP2, MTX2, RDX, SOD1, PPP1CA, CCT6A, BST2, CTNNB1, RPS24, RPS23 | 1.2928 | 0.0269 |
| GO:0032839 | dendrite cytoplasm | 3 | 2.0270 | FLOT2, ZC3H14, SOD1 | 11.5878 | 0.0272 |
| GO:0045252 | oxoglutarate dehydrogenase complex | 2 | 1.3514 | DLST, DLD | 69.5270 | 0.0283 |
| GO:0016323 | basolateral plasma membrane | 6 | 4.0541 | SLC4A7, FLOT1, CTNNB1, FLOT2, AP2A1, CD44 | 3.4194 | 0.0310 |
| GO:0042470 | melanosome | 4 | 2.7027 | ITGB1, MMP14, LAMP1, FLOT1 | 5.3482 | 0.0386 |
| GO:0098685 | Schaffer collateral - CA1 synapse | 4 | 2.7027 | ITGB1, EEA1, CTNNB1, PTN | 5.1502 | 0.0424 |
| GO:0098978 | glutamatergic synapse | 8 | 5.4054 | ITGB1, EEA1, EIF4A3, FLOT1, CTNNB1, FLOT2, KPNA2, PPP1CA | 2.4503 | 0.0447 |

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|------------|--|---|--------|----------------|---------|--------|
| GO:0005757 | mitochondrial permeability transition pore complex | 2 | 1.3514 | VDAC1, SLC25A5 | 39.7297 | 0.0489 |
|------------|--|---|--------|----------------|---------|--------|