Toxicity Evaluations of Superparamagnetic Iron Oxide Nanoparticles: Cell "Vision" Versus Physicochemical Properties of Nanoparticles

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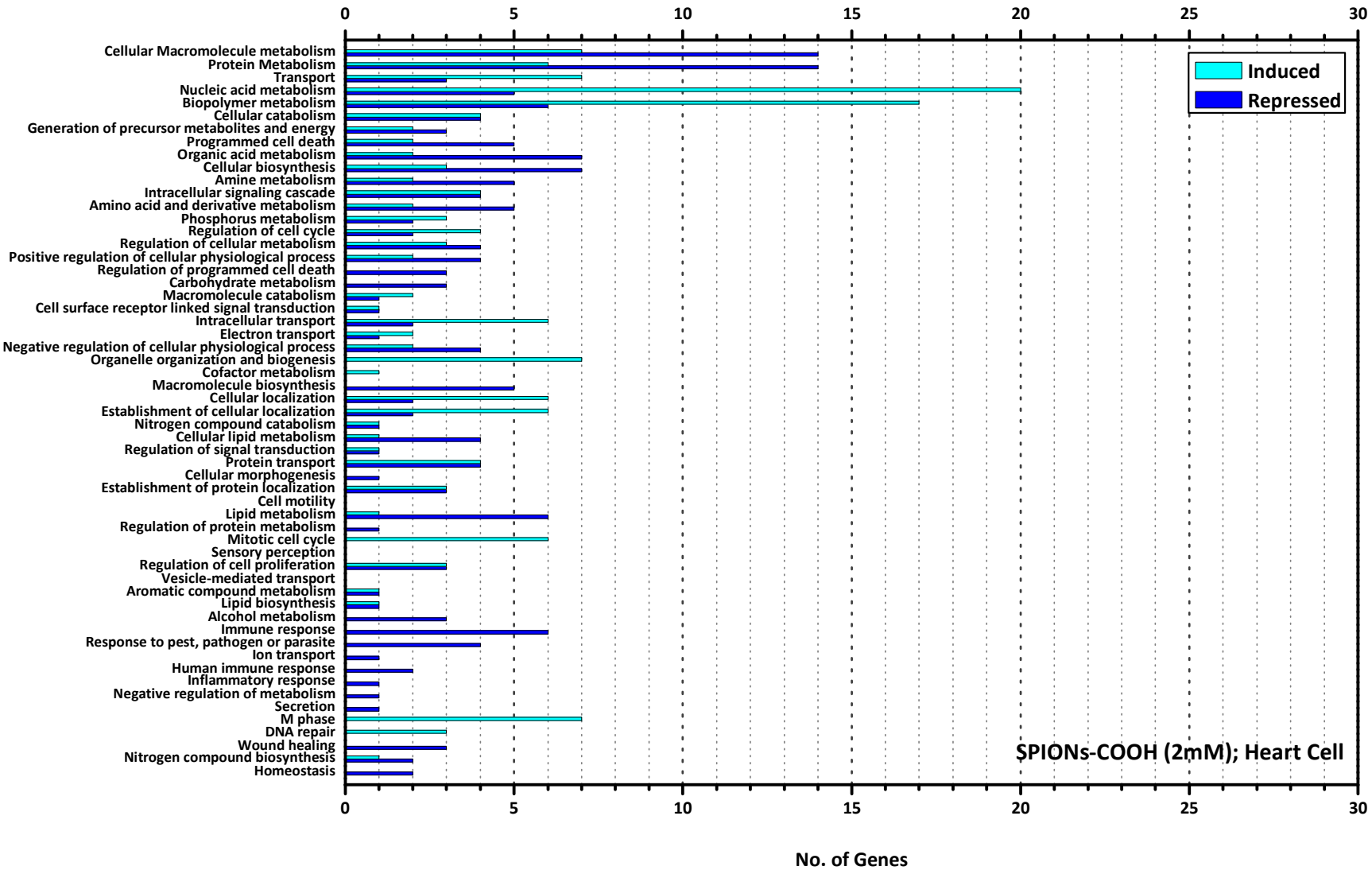
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Cellular Macromolecule metabolism
Protein Metabolism
Transport
Nucleic acid metabolism
Biopolymer metabolism
Cellular catabolism
Generation of precursor metabolites and energy
Programmed cell death
Organic acid metabolism
Cellular biosynthesis
Amine metabolism
Intracellular signaling cascade
Amino acid and derivative metabolism
Phosphorus metabolism
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Regulation of cellular metabolism
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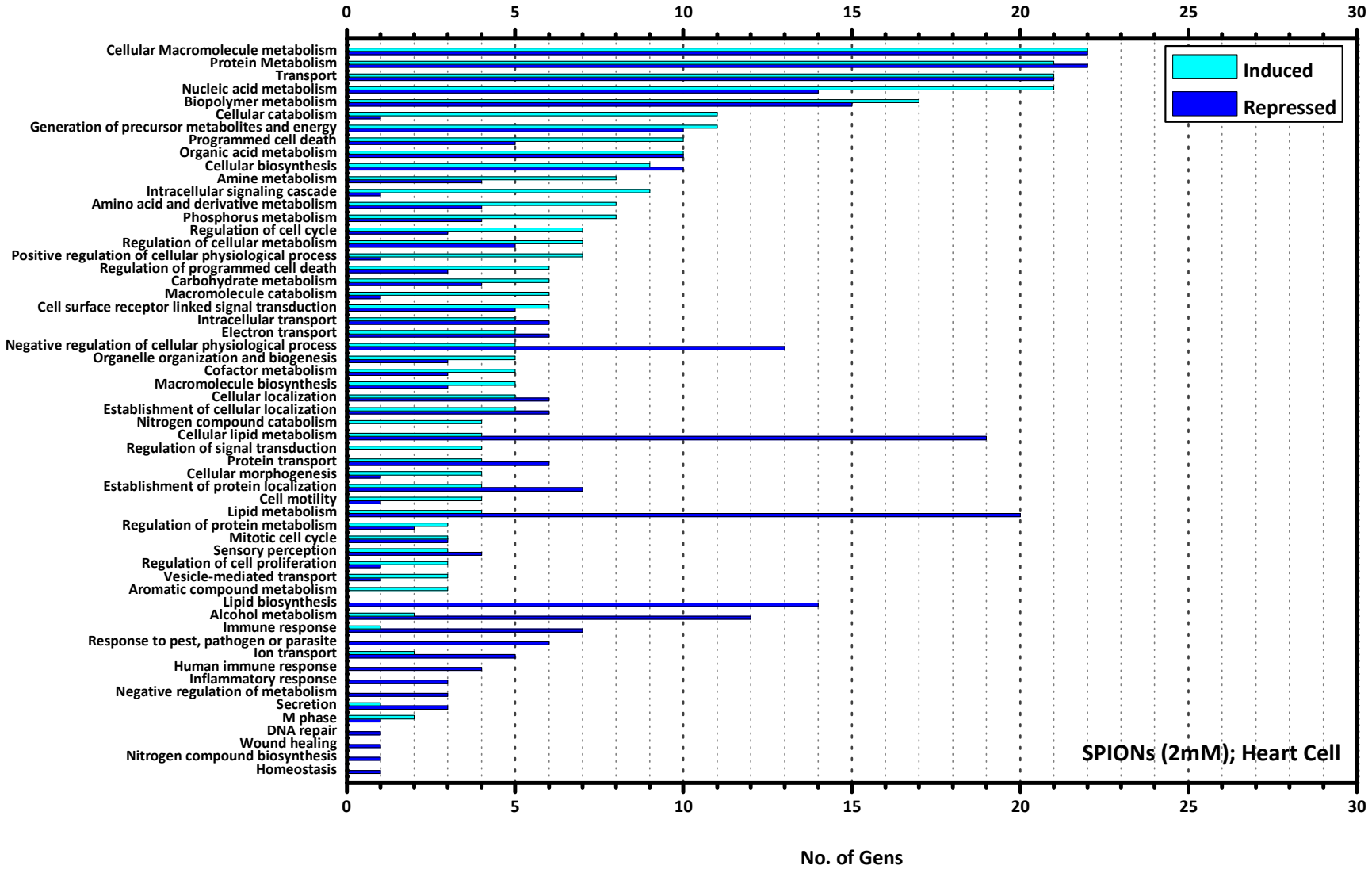
DNA repair
Wound healing
Nitrogen compound biosynthesis
Homeostasis
Induced
Repressed
SPIONs-COOH (2mM); Heart Cell
No. of Genes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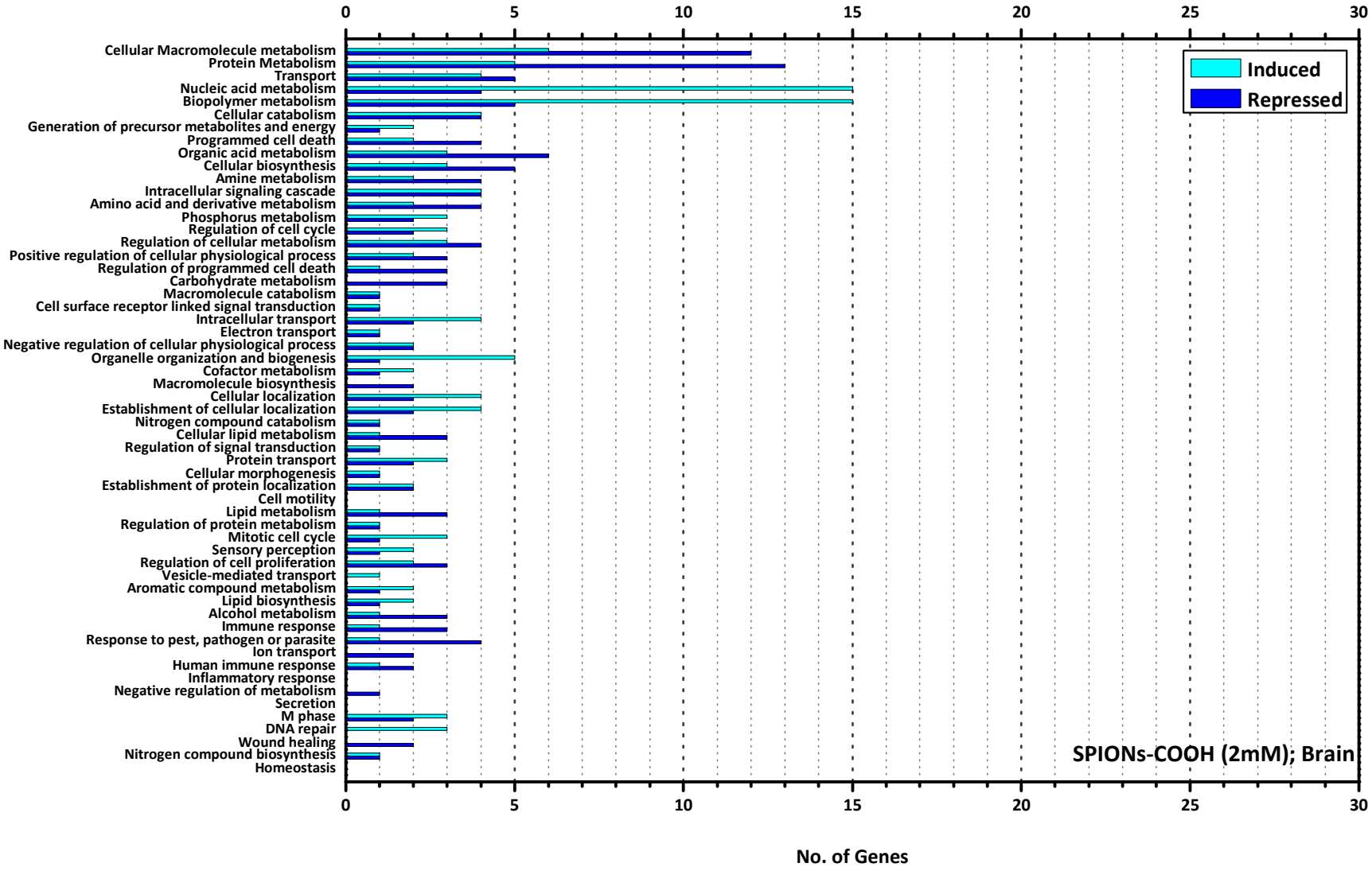
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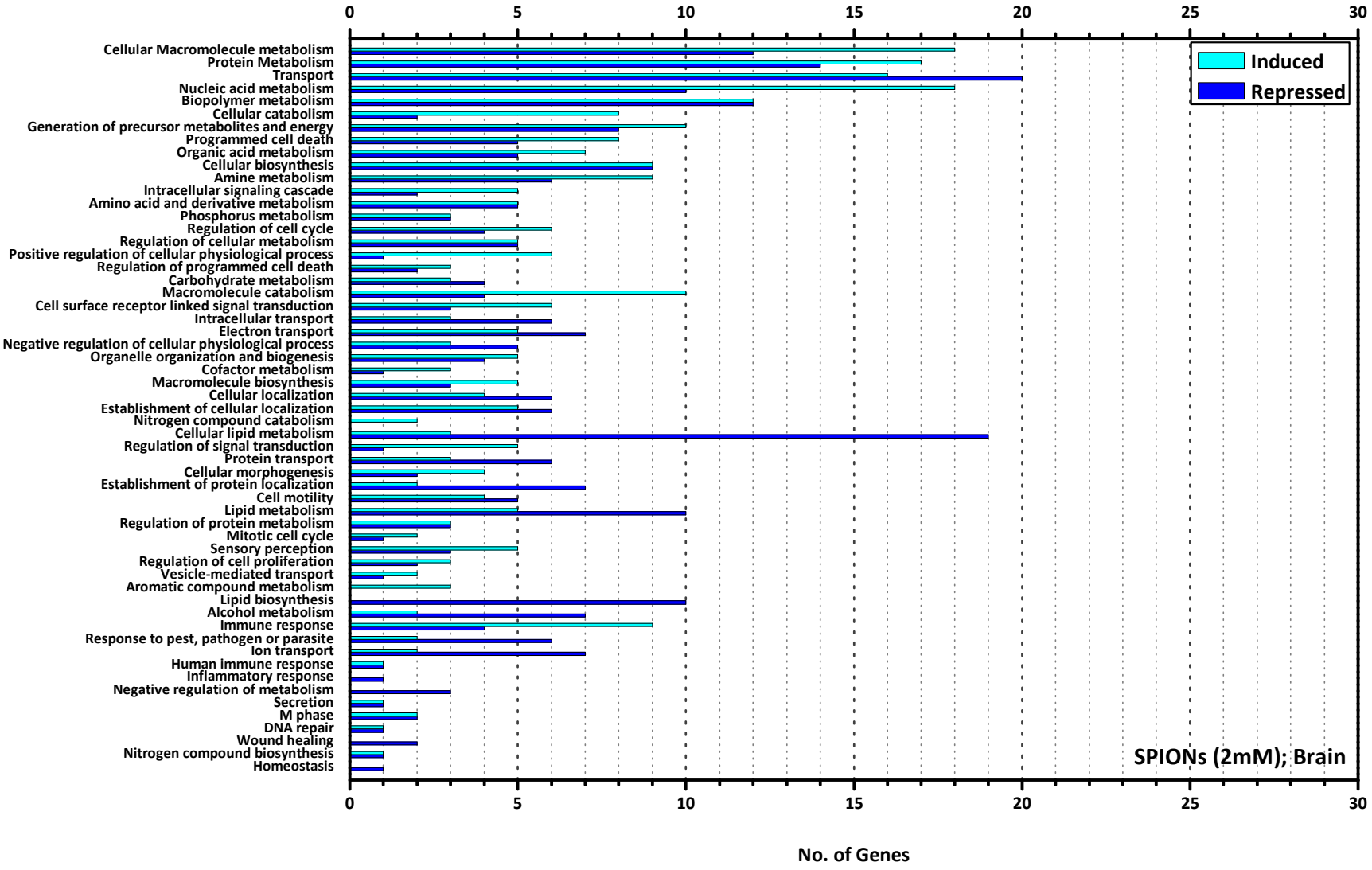
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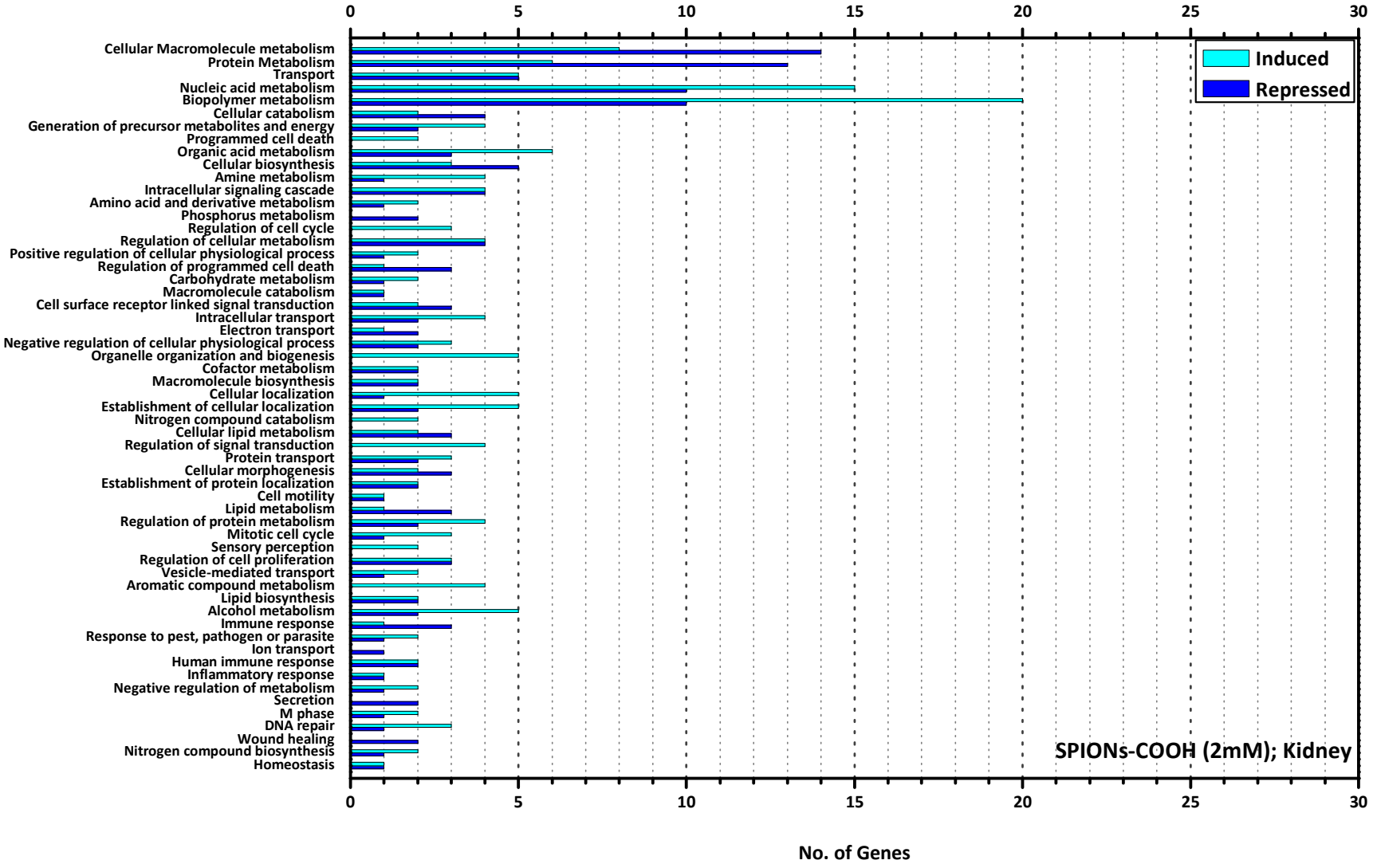
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DNA repair
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Nitrogen compound biosynthesis
Homeostasis
SPIONs-COOH (2mM); Kidney
No. of Genes

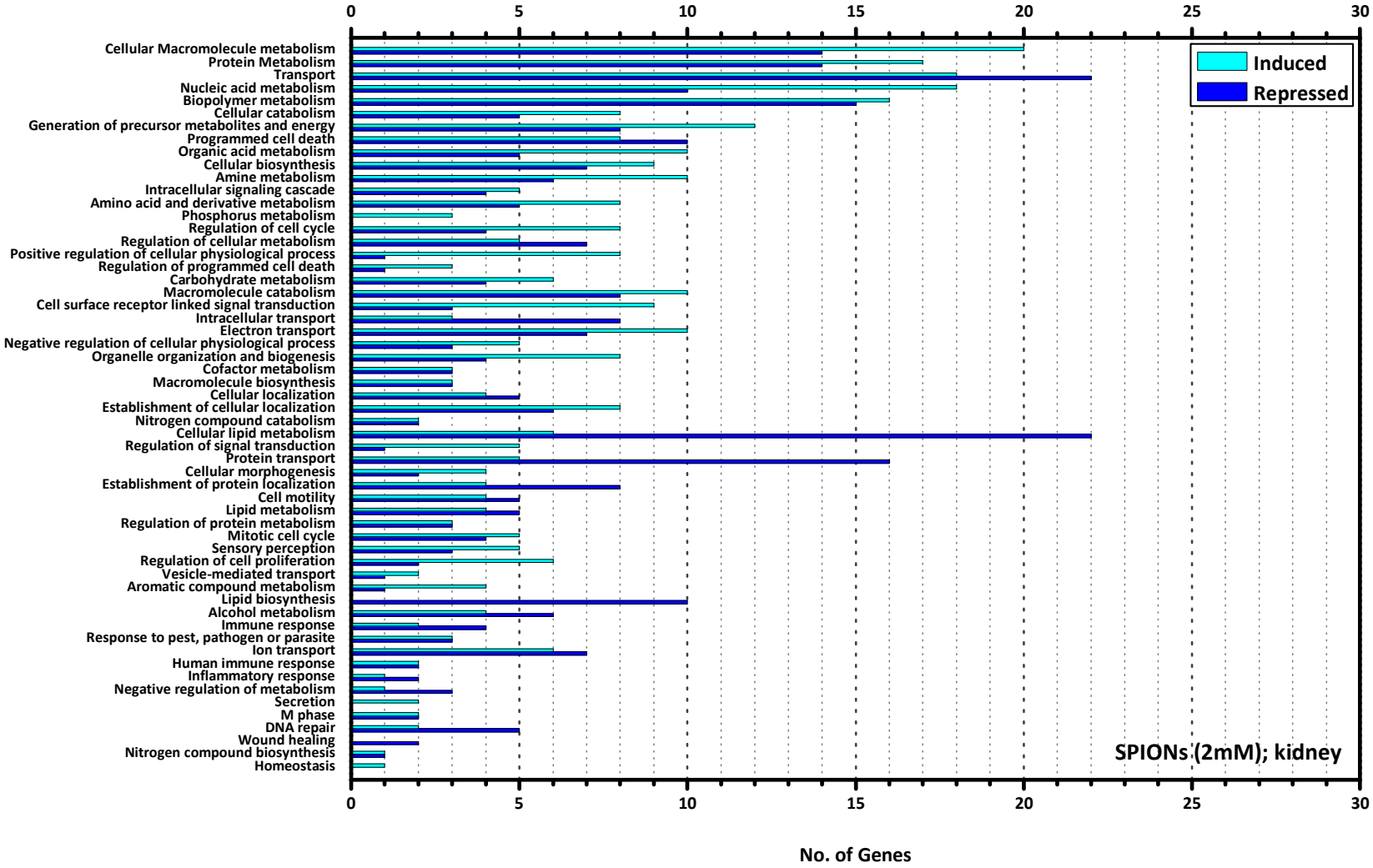

Figure S1: DNA microarray results for incubated HCM, BE-2-C, and 293T cells with 2mM of negative-SPIONs (i.e. SPIONs-COOH) and Bare-SPIONs.

Table S1: The 50 SPIONs-COOH specific genes most highly induced and repressed

Genes (Induction)	Heart	Kidney	Brain	Genes (Repression)	Heart	Kidney	Brain
SERPINE2	3.21 (<0.0001)	0.40 (0.0733)	1.67 (0.0009)	UBD	-3.21 (0.0121)	1.56 (0.646)	0.56 (0.4092)
ABCC3	2.57 (0.0016)	0.84 (0.0982)	0.97 (0.0590)	FABP1	-3.16 (0.0006)	0.04 (0.9016)	-0.30 (0.3493)
LGALS3	2.30 (0.0012)	0.01 (0.9644)	0.28 (0.3796)	CRYAA	-2.95 (0.0047)	0.12 (0.8284)	-0.14 (0.7883)
GCLC	2.24 (0.0081)	0.16 (0.5948)	0.92 (0.0619)	EBP	-2.67 (0.0038)	-0.03 (0.9513)	-1.38 (0.0369)
CTSH	2.16 (<0.0001)	0.67 (0.0023)	0.92 (0.0007)	LSS	-2.67 (0.0104)	0.01 (0.9932)	-0.51 (0.4401)
AQP3	2.14 (0.0075)	-2.22 (0.0075)	-0.18 (0.6964)	RELN	-2.59 (0.0012)	-0.93 (0.0376)	-0.34 (0.2937)
BLVRB	2.14 (0.0026)	0.08 (0.8163)	-0.03 (0.9302)	PDIA4	-2.54 (0.0015)	-0.44 (0.2356)	-0.55 (0.1940)
HPD	2.14 (0.0005)	0.27 (0.2974)	0.64 (0.0396)	HSD17B2	-2.51 (0.0001)	-1.12 (<0.0001)	-0.89 (0.0001)
CAPN2	2.12 (0.0001)	-0.99 (0.0012)	-0.54 (0.0068)	NFKBIA	-2.64 (0.0002)	-0.57 (0.0330)	-0.15 (0.4604)
ENO2	2.07 (0.0001)	0.70 (0.0093)	0.90 (0.0072)	FDPS	-2.43 (0.0058)	0.52 (0.3093)	-0.72 (0.1836)
GLRX	2.02 (0.0051)	-0.71 (0.1824)	-0.57 (0.1876)	HMGCR	-2.40 (0.0109)	-0.07 (0.8983)	-0.92 (0.1579)
DFNA5	1.95 (0.0005)	0.48 (0.0601)	0.42 (0.1014)	PROM1	-2.36 (0.0021)	-0.03 (0.8575)	0.21 (0.3002)
PHLDA2	1.95 (0.0007)	-0.86 (0.0407)	0.37 (0.3232)	SQLE	-2.35 (0.0030)	-0.36 (0.3670)	-0.68 (0.1377)
HPCAL1	1.91 (<0.0001)	-0.12 (0.4707)	-0.24 (0.2654)	FDFT1	-2.34 (0.0157)	0.21 (0.7413)	-0.17 (0.7886)
GCNT3	1.89 (0.0015)	0.83 (0.0278)	0.15 (0.5678)	LIPA	-2.26 (0.0014)	-0.40 (0.2406)	-0.80 (0.0533)
CIDEC	1.83 (0.0163)	-0.25 (0.5891)	-0.13 (0.7914)	DHCR7	-2.23 (0.0127)	-0.45 (0.4471)	-0.66 (0.2764)
CES1	1.83 (0.0145)	0.72 (0.1962)	0.39 (0.4437)	A2M	-2.13 (<0.0001)	-0.76 (0.0047)	-0.37 (0.0425)
CDKN1A1	1.8 (0.0073)	0.11 (0.7963)	0.86 (0.0998)	ARMET	-2.12 (0.0218)	-0.65 (0.3279)	-0.37 (0.5577)
ABCG2	1.69 (0.0027)	1.01 (0.0173)	1.31 (0.0105)	TRA2A	-2.07 (0.0042)	0.31 (0.3641)	-0.15 (0.7480)
STX3A	1.68 (0.0090)	0.26 (0.4930)	0.45 (0.2600)	HSD17B7	-2.02 (0.0016)	-0.07 (0.8012)	-0.64 (0.0586)
FAH	1.67 (0.0004)	0.80 (0.0066)	0.68 (0.0124)	C14orf1	-2.01 (0.0323)	0.17 (0.7990)	-1.32 (0.0998)
ENPP1	1.66 (0.0143)	0.40 (0.3827)	0.99 (0.0784)	ALDH18A1	-1.97 (0.0001)	-0.85 (0.0012)	-0.24 (0.1316)

ASNS	1.65 (0.0074)	-1.51 (0.0101)	0.06 (0.8670)	HMGCS1	-1.93 (0.0407)	0.25 (0.7155)	0.00 (0.9980)
IFTIM2	1.60 (0.0020)	0.17 (0.4937)	1.24 (0.0064)	NDRG2	-1.92 (0.0006)	-0.93 (0.0051)	-0.07 (0.7081)
FEZ2	1.60 (0.0001)	0.43 (0.0129)	0.45 (0.0133)	PBP	-1.89 (0.0001)	-0.68 (0.0089)	-0.80 (0.0153)

Table S2: The 50 bare-SPIONs specific genes most highly induced and repressed

Genes (Induction)	Heart	Kidney	Brain	Genes (Repression)	Heart	Kidney	Brain
CCNB2	2.81 (0.0124)	2.44 (0.0202)	0.89 (0.2563)	CLGN	-2.22 (0.0023)	-1.64 (0.0059)	-1.62 (0.0022)
MCM2	2.67 (0.0037)	2.56 (0.0042)	2.02 (0.0102)	OS-9	-2.09 (0.0003)	-1.57 (0.0003)	-1.01 (0.0017)
UBE2C	2.66 (0.0030)	2.58 (0.0033)	0.95 (0.0835)	SERPINC1	-2.04 (0.0001)	-0.95 (0.0005)	-1.48 (0.0007)
ZWINT	2.54 (0.0055)	2.56 (0.0053)	0.80 (0.1626)	TNFSF10	-2.04 (0.0025)	-1.00 (0.0124)	-1.84 (0.0069)
HIST1H4C	2.14 (<0.0001)	2.48 (<0.0001)	0.86 (0.0101)	IFRD1	-1.86 (0.0031)	-1.09 (0.0230)	-0.91 (0.0375)
RNASEH2A	2.13 (0.0010)	1.91 (0.0012)	0.83 (0.0366)	AZGP1	-1.85 (<0.0001)	-1.09 (<0.0001)	0.28 (0.0965)
TRIP13	2.04 (0.0004)	2.16 (0.0004)	0.93 (0.0106)	SLC1A2	-1.75 (0.0014)	-1.34 (0.0089)	-0.72 (0.0428)
PPIH	1.85 (0.0005)	1.08 (0.0015)	0.67 (0.0163)	ASNS	-1.75 (0.0065)	1.51 (0.0101)	0.06 (0.8670)
ADM	1.82 (0.0006)	0.62 (0.0050)	0.47 (0.0127)	16. Sep	-1.56 (0.0066)	-1.28 (0.0174)	-1.09 (0.0082)
HMGN2	1.82 (0.0027)	1.33 (0.0087)	0.76 (0.0711)	CALR	-1.50 (0.0027)	-0.20 (0.0408)	0.58 (0.0106)
RACGAP1	1.67 (0.0021)	1.78 (0.0006)	-0.12 (0.6853)	GHITM	-1.49 (0.0108)	-1.49 (0.1273)	-0.77 (0.2546)
TUBA3	1.67 (0.0168)	0.54 (0.2131)	0.22 (0.5897)	CDH2	-1.49 (0.0007)	0.29 (0.5353)	0.13 (0.6481)

ITGB3BP	1.62 (0.0002)	1.81 (0.0001)	1.06 (0.0001)	PSPH	-1.42 (0.0086)	-0.97 (0.0230)	-1.59 (0.0089)
SNRPA	1.60 (0.0050)	0.85 (0.0299)	0.65 (0.0628)	ALDH6A1	-1.40 (0.0025)	-1.34 (0.0005)	-1.16 (0.0003)
SMC4L1	1.59 (0.0019)	1.87 (0.0008)	0.52 (0.1063)	BCAT1	-1.39 (0.0120)	-.094 (0.0196)	-0.18 (0.7083)
PC4	1.55 (0.0024)	0.18 (0.6953)	0.52 (0.2988)	TST	-1.37 (0.0006)	-0.67 (0.0164)	-0.33 (0.1924)
NUP107	1.55 (0.0015)	1.45 (0.0017)	0.86 (0.0199)	CLU	-1.37 (0.0003)	-0.42 (0.0050)	-0.24 (0.0319)
TYMS	1.53 (0.0008)	1.73 (<0.0001)	1.16 (0.0001)	EPHX1	-1.37 (0.0206)	-1.15 (0.0237)	-0.37 (0.2255)
SFRS7	1.52 (0.0065)	0.72 (0.0706)	0.83 (0.0624)	BF	-1.35 (0.0088)	-1.24 (0.0114)	-0.61 (0.1143)
CKS2	1.52 (0.0009)	0.90 (0.0003)	0.38 (0.0856)	C6orf48	-1.34 (0.0007)	-1.16 (0.0011)	-0.46 (0.0803)
DKK1	1.47 (0.0071)	2.08 (0.0040)	0.72 (0.0561)	ST6GALNAC4	-1.31 (0.0148)	-1.34 (0.0165)	0.38 (0.1820)
SFRS2	1.45 (0.0044)	0.91 (0.0138)	1.11 (0.0063)	ARSE	-1.26 (0.0001)	-0.65 (0.0002)	-0.22 (0.0577)
GTPBP6	1.44 (0.0015)	0.88 (0.0055)	1.08 (0.0071)	ITIH3	-1.25 (0.0111)	-0.47 (0.1769)	-0.32 (0.3277)
RFC2	1.38 (0.0034)	1.01 (0.0177)	0.70 (0.1067)	TSPAN7	-1.25 (0.0012)	-0.23 (0.4827)	-0.80 (0.0079)
CDKN3	1.37 (0.0018)	1.41 (0.0018)	0.55 (0.0459)	IGSF1	-1.24 (0.0054)	-0.19 (0.4596)	-0.45 (0.1426)

Cellular organelle's staining

CellPainterTM Organelle Markers are GFP- or RFP-tagged TrueORF cDNA clones that encode organelle-specific or structure-specific proteins (Origine, USA). The proteins are fused in-frame with different fluorescent proteins and allow clear visualization of the organelles or structures. All CellPainterTM Organelle marker plasmids are constructed using OriGene's TrueORF destination vectors. The fluorescent protein tags (turboGFP or turboRFP) are fused at the N- or C-terminus of the marker protein. All of the vectors can be selected with ampicillin (100ug/ml) in *E.coli* and with the neomycin analog, G418, in mammalian cells.

Protocol for transient transfection (adherent cells)

On the day before transfection, the cells were plated at a density of 1-3 x 10^5 cells in complete growth medium per well of a 6-well plate to obtain 50-70% confluence on the following day. After overnight incubation of the cells, they were transformed to a sterile plastic tube, and100 uL of serum free medium was added. An appropriate amount of TurboFectin 8.0 (2-6 uL per 1 ug DNA) was added into tube and mixed completely by gentle pipetting followed by incubation at room temperature for 5 minutes. Plasmid DNA (1-3 ug per well) were poured to the TurboFectin-containing media prepared above and mixed by gentle pipetting. The mixture was incubate at room temperature for 30 min. The transfection was done in complete culture medium and the mixture was added dropwise to the cells followed by incubation for 48 hours.

Protocol for Confocal Microspcopy:

At 30 hrs post-transfection, the cells were washed with cold PBS and then fixed with 4% formaldehyde in PBS for 20 min at room temperature. The cover slip was carefully transferred from well with cells face down to a glass slide with one drop of commercial mounting medium (VECTASHIELD®).

Protocol for Stable Transfection:

At 24 hrs post-transfection, the cells were transferred into fresh growth medium containing the selective agent, G418. After 1-2 weeks, a large number of the cells will be killed; the cells that remain growing in the selective medium have retained the expression plasmid, which stably integrates into the genome of the targeted cells. The mock control is also monitored to ensure the cells are dying.