

Supporting Information

Magnetite (Fe₃O₄) Nanocrystals Affect The Expression of Genes Involved in TGF-beta Signaling Pathway

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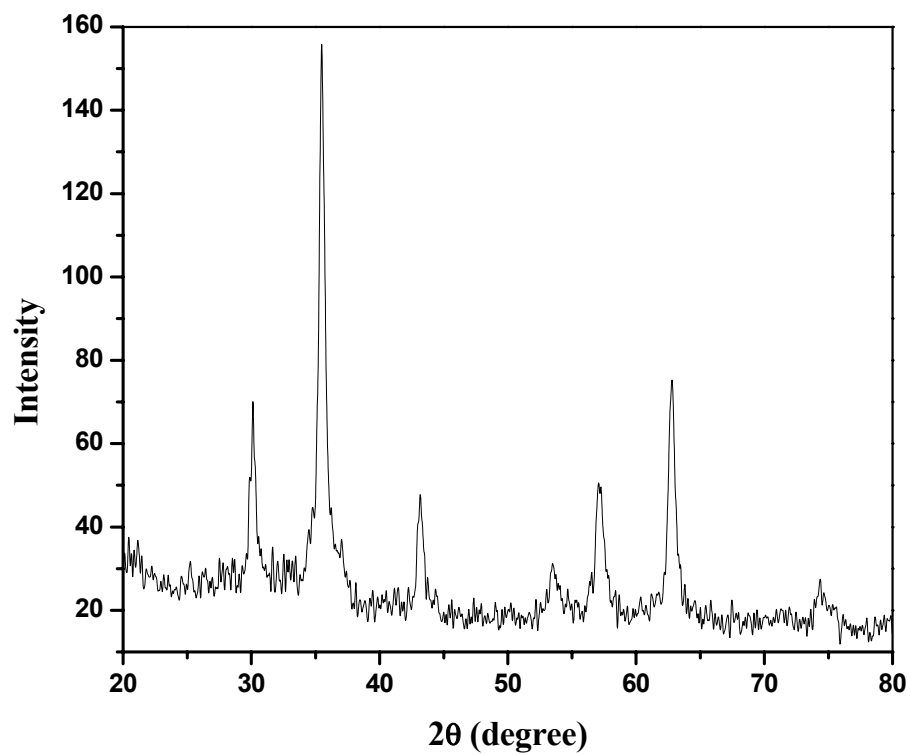


Figure SI.1: X-ray diffraction pattern of naked magnetite nanocrystals

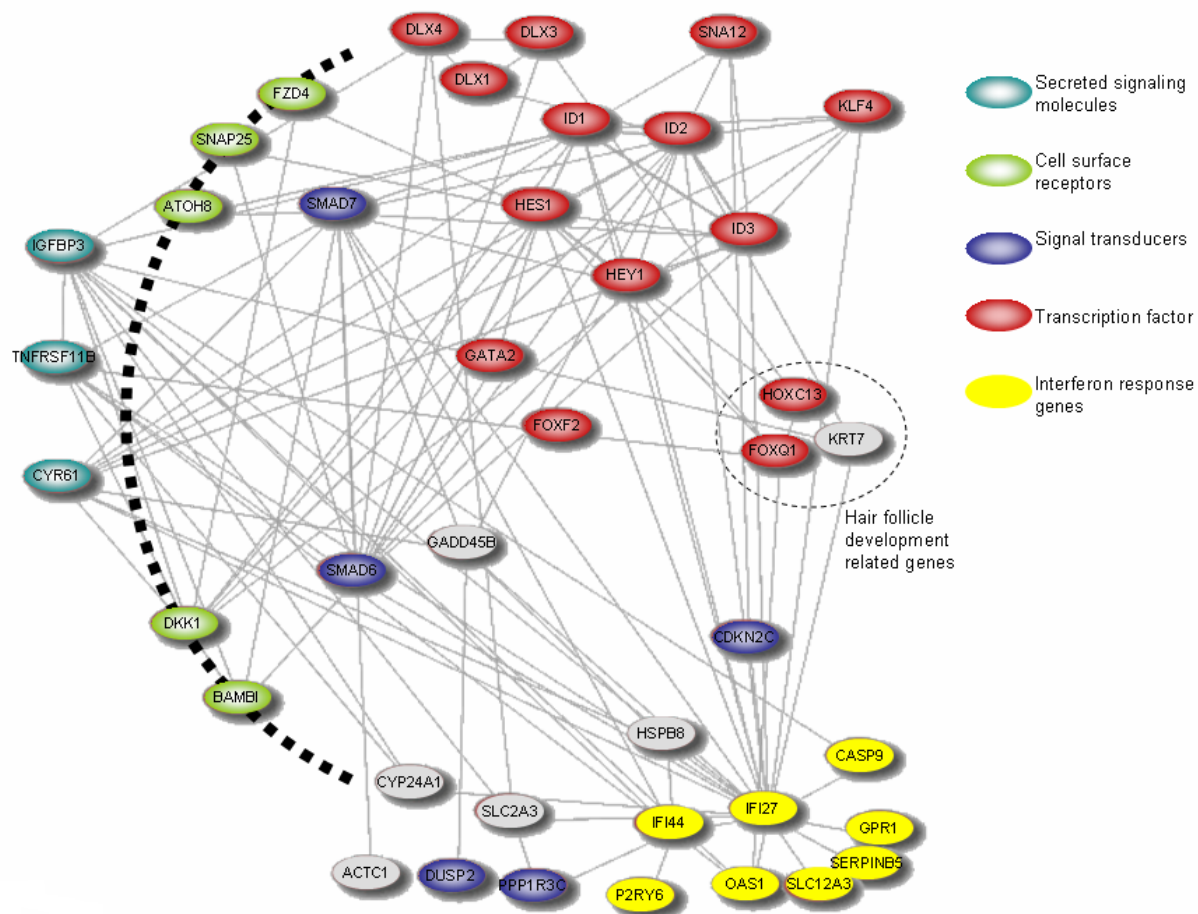


Figure SI.2: Gene Interaction network based on co-occurrence of genes in published literature: The list of 69 differentially expressed genes (supplementary Table SI.2) was cut down to 67 genes since two of the genes did not have any traceable gene name/annotation associated with them. The list of genes was used to search literature, using Pubgene software, and color coded according to molecular function. Genes that did not fall into major classes were marked grey. Three genes involved in hair follicle development are encircled by dashed line. The thick dotted line connecting cell surface receptors represents cell membrane. Thin grey lines connect genes with at least one instance of co-occurrence in literature.

Table SI.1: Sequences of the primers for Real Time PCR Experiments

Gene	Primer	Sequence
ID1	FP	CTACGACATGAACGGCTGTTACTC
	RP	CTTGCTCACCTTGCGGTTCT
ID2	FP	TCAGCCTGCATCACCAGAGA
	RP	CTGCAAGGACAGGATGCTGATA
ID3	FP	TCAGCTTAGCCAGGTGGAAATC
	RP	TGGCTCGGCCAGGACTAC
SMAD6	FP	TCTCCTCGCGACGAGTACAAG
	RP	GGAGCAGTGATGAGGGAGTTG
SMAD7	FP	AGAGGCTGTGTTGCTGTGAATC
	RP	GCAGAGTCGGCTAAGGTGATG
B2M	FP	TGCTGTCTCCATGTTTGATGTATCT
	RP	TCTCTGCTCCCCACCTCTAAGT

Table SI.2: Differentially expressed Genes in MNC treated cells with p-value < 0.05.

Accession No.	Symbol	Definition	Diff Pval	Fold Change
NM_004418.2	DUSP2	Homo sapiens dual specificity phosphatase 2 (DUSP2), mRNA.	4.90E-20	0.113844
NM_002166.4	ID2	Homo sapiens inhibitor of DNA binding 2, dominant negative helix-loop-helix protein (ID2), mRNA.	3.26E-19	0.160793
NM_194286.2	KIAA1853	Homo sapiens KIAA1853 (KIAA1853), mRNA.	5.11E-19	0.142492
NM_181353.1	ID1	Homo sapiens inhibitor of DNA binding 1, dominant negative helix-loop-helix protein (ID1), transcript variant 2, mRNA.	2.09E-17	0.224446
NM_005159.3	ACTC	Homo sapiens actin, alpha, cardiac muscle (ACTC), mRNA.	3.79E-17	0.200464
NM_005220.2	DLX3	Homo sapiens distal-less homeobox 3 (DLX3), mRNA.	1.56E-16	0.108622
NM_152486.2	SAMD11	Homo sapiens sterile alpha motif domain containing 11 (SAMD11), mRNA.	3.64E-15	0.253359
NM_005585.2	SMAD6	Homo sapiens SMAD, mothers against DPP homolog 6 (Drosophila) (SMAD6), mRNA.	6.07E-15	0.252655
NM_032827.3	ATOH8	Homo sapiens atonal homolog 8 (Drosophila) (ATOH8), mRNA.	2.25E-12	0.26244
NM_033260.2	FOXQ1	Homo sapiens forkhead box Q1 (FOXQ1), mRNA.	3.73E-12	0.314029
NM_015419.1	MXRA5	Homo sapiens matrix-remodelling associated 5 (MXRA5), mRNA.	2.54E-10	0.305755
NM_032638.3	GATA2	Homo sapiens GATA binding protein 2 (GATA2), mRNA.	9.36E-10	0.362878
NM_022165.2	LIN7B	Homo sapiens lin-7 homolog B (C. elegans) (LIN7B), mRNA.	6.07E-08	0.37653
NM_002167.2	ID3	Homo sapiens inhibitor of DNA binding 3, dominant negative helix-loop-helix protein (ID3), mRNA.	1.42E-07	0.439205
NM_000782.3	CYP24A1	Homo sapiens cytochrome P450, family 24, subfamily A, polypeptide 1 (CYP24A1), nuclear gene encoding mitochondrial protein, mRNA.	2.25E-07	0.224381
NM_002546.2	TNFRSF11B	Homo sapiens tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin) (TNFRSF11B), mRNA.	4.91E-07	0.32991
NM_014365.2	HSPB8	Homo sapiens heat shock 22kDa protein 8 (HSPB8), mRNA.	5.71E-07	0.391759
NM_032867.1	MICALCL	Homo sapiens MICAL C-terminal like (MICALCL), mRNA.	9.69E-07	0.2252
NM_014899.2	RHOBTB3	Homo sapiens Rho-related BTB domain containing 3 (RHOBTB3), mRNA.	1.15E-06	0.453833
NM_000142.2	FGFR3	Homo sapiens fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism) (FGFR3), transcript variant 1, mRNA.	1.84E-06	0.299151

Accession No.	Symbol	Definition	Diff Pval	Fold Change
NM_005904.2	SMAD7	Homo sapiens SMAD, mothers against DPP homolog 7 (Drosophila) (SMAD7), mRNA.	1.87E-06	0.432974
NM_001452.1	FOXF2	Homo sapiens forkhead box F2 (FOXF2), mRNA.	1.87E-06	0.45895
NM_000339.1	SLC12A3	Homo sapiens solute carrier family 12 (sodium/chloride transporters), member 3 (SLC12A3), mRNA.	1.87E-06	0.459448
NM_003068.3	SNAI2	Homo sapiens snail homolog 2 (Drosophila) (SNAI2), mRNA.	5.12E-06	0.356594
NM_006869.1	CENTA1	Homo sapiens centaurin, alpha 1 (CENTA1), mRNA.	1.31E-05	0.226087
NM_181726.1	ANKRD37	Homo sapiens ankyrin repeat domain 37 (ANKRD37), mRNA.	1.75E-05	0.485657
NM_004753.4	DHRS3	Homo sapiens dehydrogenase/reductase (SDR family) member 3 (DHRS3), mRNA.	2.33E-05	0.437411
NM_005532.3	IFI27	Homo sapiens interferon, alpha-inducible protein 27 (IFI27), mRNA.	5.51E-05	0.501769
NM_178120.3	DLX1	Homo sapiens distal-less homeo box 1 (DLX1), mRNA.	7.54E-05	0.481572
NM_012258.2	HEY1	Homo sapiens hairy/enhancer-of-split related with YRPW motif 1 (HEY1), mRNA.	8.05E-05	0.331584
NM_176797.1	P2RY6	Homo sapiens pyrimidinergic receptor P2Y, G-protein coupled, 6 (P2RY6), transcript variant 1, mRNA.	0.00013	1.69846
NM_181712.2	ANKRD38	Homo sapiens ankyrin repeat domain 38 (ANKRD38), mRNA.	0.00013	0.455382
NM_173362.2	LOC317671	Homo sapiens LOC317671 (LOC317671), mRNA.	0.000239	0.506275
NM_024554.2	PGBD5	Homo sapiens piggyBac transposable element derived 5 (PGBD5), mRNA.	0.000386	0.522677
NM_003081.2	SNAP25	Homo sapiens synaptosomal-associated protein, 25kDa (SNAP25), transcript variant 1, mRNA.	0.000421	0.529917
NM_016307.3	PRRX2	Homo sapiens paired related homeobox 2 (PRRX2), mRNA.	0.000569	0.436785
NM_012342.2	BAMBI	Homo sapiens BMP and activin membrane-bound inhibitor homolog (Xenopus laevis) (BAMBI), mRNA.	0.000873	0.557372
NM_002304.1	LFNG	Homo sapiens lunatic fringe homolog (Drosophila) (LFNG), mRNA.	0.00161	0.558764
NM_000710.2	BDKRB1	Homo sapiens bradykinin receptor B1 (BDKRB1), mRNA.	0.002094	0.510206
XM_376018.3	KIAA1644	PREDICTED: Homo sapiens KIAA1644 protein (KIAA1644), mRNA.	0.002094	0.556893
NM_015675.1	GADD45B	Homo sapiens growth arrest and DNA-damage-inducible, beta (GADD45B), mRNA.	0.003203	0.527743
NM_002639.2	SERPINB5	Homo sapiens serpin peptidase inhibitor, clade B (ovalbumin), member 5 (SERPINB5), mRNA.	0.003394	0.586929

Accession No.	Symbol	Definition	Diff Pval	Fold Change
NM_001032409.1	OAS1	Homo sapiens 2',5'-oligoadenylate synthetase 1, 40/46kDa (OAS1), transcript variant 3, mRNA.	0.003508	0.557544
NM_078626.1	CDKN2C	Homo sapiens cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4) (CDKN2C), transcript variant 2, mRNA.	0.003697	0.578428
NM_017410.2	HOXC13	Homo sapiens homeobox C13 (HOXC13), mRNA.	0.004871	0.474712
NM_006417.2	IFI44	Homo sapiens interferon-induced protein 44 (IFI44), mRNA.	0.005905	0.579062
NM_001554.3	CYR61	Homo sapiens cysteine-rich, angiogenic inducer, 61 (CYR61), mRNA.	0.00616	0.583433
NM_025214.1	CCDC68	Homo sapiens coiled-coil domain containing 68 (CCDC68), mRNA.	0.008176	0.465831
NM_005279.2	GPR1	Homo sapiens G protein-coupled receptor 1 (GPR1), mRNA.	0.009273	0.386195
NM_005556.3	KRT7	Homo sapiens keratin 7 (KRT7), mRNA.	0.010387	0.607844
NM_004669.2	CLIC3	Homo sapiens chloride intracellular channel 3 (CLIC3), mRNA.	0.011595	0.589831
NM_004235.3	KLF4	Homo sapiens Kruppel-like factor 4 (gut) (KLF4), mRNA.	0.011645	0.588791
NM_020734.1	FAM80B	Homo sapiens family with sequence similarity 80, member B (FAM80B), mRNA.	0.011794	0.530095
NM_019000.2	FLJ20152	Homo sapiens hypothetical protein FLJ20152 (FLJ20152), mRNA.	0.014697	0.54545
NM_001934.2	DLX4	Homo sapiens distal-less homeobox 4 (DLX4), transcript variant 2, mRNA.	0.015465	0.41464
NM_005738.2	ARL4	Homo sapiens ADP-ribosylation factor-like 4 (ARL4), transcript variant 1, mRNA.	0.015601	0.523949
BU659183		cl44f11.z1 Hembase; Erythroid Precursor Cells (LCB:cl library) Homo sapiens cDNA clone cl44f11 5, mRNA sequence	0.016179	0.461992
NM_016619.1	PLAC8	Homo sapiens placenta-specific 8 (PLAC8), mRNA.	0.016179	0.620699
NM_152553.2	IBRDC1	Homo sapiens IBR domain containing 1 (IBRDC1), mRNA.	0.016179	0.56492
NM_012242.2	DKK1	Homo sapiens dickkopf homolog 1 (Xenopus laevis) (DKK1), mRNA.	0.019385	0.470827
NM_015556.1	SIPA1L1	Homo sapiens signal-induced proliferation-associated 1 like 1 (SIPA1L1), mRNA.	0.024069	0.584835
NM_001013398.1	IGFBP3	Homo sapiens insulin-like growth factor binding protein 3 (IGFBP3), transcript variant 1, mRNA.	0.02729	0.62321
NM_001229.2	CASP9	Homo sapiens caspase 9, apoptosis-related cysteine peptidase (CASP9), transcript variant alpha, mRNA.	0.02764	0.56053
BM724647		UI-E-EJ0-ais-e-15-0-UI.r1 UI-E-EJ0 Homo sapiens cDNA clone UI-E-EJ0-ais-e-15-0-UI 5, mRNA sequence	0.033027	0.537131

Accession No.	Symbol	Definition	Diff Pval	Fold Change
NM_006931.1	SLC2A3	Homo sapiens solute carrier family 2 (facilitated glucose transporter), member 3 (SLC2A3), mRNA.	0.033027	0.635309
NM_005524.2	HES1	Homo sapiens hairy and enhancer of split 1, (Drosophila) (HES1), mRNA.	0.041602	0.512536
NM_012193.2	FZD4	Homo sapiens frizzled homolog 4 (Drosophila) (FZD4), mRNA.	0.041602	0.566376
NM_005398.3	PPP1R3C	Homo sapiens protein phosphatase 1, regulatory (inhibitor) subunit 3C (PPP1R3C), mRNA.	0.041602	0.640479
NM_052943.2	FAM46B	Homo sapiens family with sequence similarity 46, member B (FAM46B), mRNA.	0.042424	0.613257

Table SI.3: Molecular Function GO Categories with statistically significant (p -value < 0.05) enrichment in differentially expressed genes identified by *OntoExpress*.

GO ID	Function Name	Genes Names	No of genes	Total No. of Ref Genes	Corrected P-Value
GO:0003700	Transcription factor activity	HOXC13, HEY1, FOXQ1, GATA2, PRRX2, DLX4, DLX3, DLX1, FOXF2, SMAD7, SMAD6, KLF4	12	884	0.000556
GO:0030617	Transforming growth factor beta receptor, inhibitory cytoplasmic mediator activity	SMAD7, SMAD6	2	2	0.000633
GO:0030528	transcription regulator activity	HEY1, ATOH8, ID3, ID2, ID1	5	114	0.000796
GO:0043565	Sequence-specific DNA binding	HOXC13, FOXQ1, GATA2, PRRX2, DLX4, DLX3, DLX1, FOXF2	8	476	0.001643
null	UnknownF	PGBD5, KIAA1853, BAMBI, FAM46B, CCDC68, FAM134B, MICALCL, ANKRD37, PLAC8, IFI44, SAMD11, KANK4	13	9808	0.019029
GO:0005520	Insulin-like growth factor binding	CYR61, IGFBP3	2	21	0.042908

Table SI.4: Summary of Pathway Analysis of differentially expressed Genes

Rank	Pathway Name	Impact Factor	Total No of Genes in Pathway	No of Diff. Expressed Genes in Pathway	Corrected p-value
1	TGF-beta signaling pathway	13.557	89	5	4.1667E-06
2	p53 signaling pathway	12.187	68	4	3.3893E-05
3	Basal cell carcinoma	8.147	55	1	0.13757436
4	Maturity onset diabetes of the young	7.056	25	1	0.06250493
5	Regulation of actin cytoskeleton	6.754	211	2	0.10588244
6	Apoptosis	6.173	84	1	0.2024254
7	Calcium signaling pathway	5.429	175	1	0.37291402
8	Wnt signaling pathway	5.117	148	2	0.06012291
9	Cell cycle	5.088	112	2	0.03651655
10	Colorectal cancer	5.067	84	2	0.02148037
11	Bladder cancer	4.759	42	1	0.10684315
12	Melanogenesis	4.638	101	1	0.23818297
13	MAPK signaling pathway	4.551	265	3	0.03348438
14	Neuroactive ligand-receptor interaction	4.328	254	2	0.14601322
15	Insulin signaling pathway	3.554	138	1	0.30877404
16	SNARE interactions in vesicular transport	3.536	38	1	0.09229818
17	Notch signaling pathway	3.266	46	1	0.11641201
18	Endometrial cancer	3.256	52	1	0.13057631
19	Non-small cell lung cancer	3.221	54	1	0.13524786
20	VEGF signaling pathway	2.982	71	1	0.17174101
21	Pancreatic cancer	2.944	73	1	0.17841261
22	Complement and coagulation cascades	2.899	69	1	0.16726367
23	Small cell lung cancer	2.786	87	1	0.20885349
24	Prostate cancer	2.776	89	1	0.21098483
25	Cell Communication	2.504	138	1	0.3069031
26	Adherens junction	2.475	75	1	0.18283091
27	Vibrio cholerae infection	2.387	59	1	0.14220888
28	Cytokine-cytokine receptor interaction	1.413	259	1	0.49929734