

## Supplementary materials

### Materials Methods

#### Cytotoxicity and cell viability

Cytotoxicity of aSiNP-116 was determined by EZ-Cytox cell viability assay kit (Daeil Lab Service, Korea) based on the cleavage of the tetrazolium salt to water-soluble formazan by succinate-tetrazolium reductase. Approximately,  $5 \times 10^3$  cells/well were seeded in 96-well plates 24 h prior to treatment and exposed to a range of concentrations (from 5 mg/L to 200 mg/L) of aSiNP-116 for next 24 h in total MEM. In addition, the cells (in 96-well plates) were exposed to aSiNP-116 (100 mg/L) for different time point (from 4 h to 72 h). After completion of exposure 10  $\mu$ L of EZ-Cytox reagent was added to each well including treated and control (without aSiNP). Absorbance ( $OD_{450}$ ) was detected at 450 nm after 3 h of incubation at 37°C.

Appropriate blanks were used for each concentration to validate the absorbance.

The cell viability was measured using standard trypan blue (Invitrogen) staining method and the total numbers of stained and unstained cells counted using a hemocytometer. All the viability measured in three biological replicates each with duplicates.

#### Total RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA from aSiNP-116 treated cell samples (100 mg/L for 24 h) were extracted by using RNA extraction kit (NucleoSpin, Macherey-Nagel) and the quantity and quality of RNAs were detected in nanodrop spectrophotometer (ND-1000, NanoDrop Technologies, Inc., DE, USA) as well as with agarose gel separation.

Synthesis of cDNAs was performed by a reverse transcriptase (RT) reaction and PCR amplification was carried out with a thermal cycler (Bio-Rad). Real time RT-PCR analysis was

accomplished with CFX manager (Bio-Rad) using the IQ<sup>TM</sup> SYBR Green SuperMix (Bio-Rad). The primers were constructed (by Primer3plus) based on sequences available in NCBI and the qRT-PCR conditions were optimized (efficiency and sensitivity tests) for each primer prior to the experiment (Supplementary Table S1). Three biological replicates each with triplicate were used for each qRT-PCR analysis. Analysis of negative control reactions (minus RT and all reagents minus template) confirmed no DNA contamination. The gene expressions were normalized by using GAPDH and  $\beta$ -Actin as housekeeping gene.

### **Microarray and Global gene expression Analysis**

Cells were exposed to aSiNP-116 (100 mg/L) for 24 h and then total RNA were extracted from treated and control cells by using RNeasy Mini Kits (Qiagen, Hilden, Germany). RNA quality was assessed by Agilent 2100 bioanalyser using the RNA 6000 Nano Chip (Agilent Technologies, Amstelveen, The Netherlands), and quantity was determined by ND-1000 Spectrophotometer (NanoDrop Technologies, Inc., DE, USA). Next, RNA samples were hybridized to the Affymetrix Gene Chip according to the manufacturer's instructions (<http://www.affymetrix.com>). Briefly, 300 ng of total RNA from each sample was converted to cDNA, after amplification, the double strands of cDNA was labeled with biotin-labeling (Ambion® WT Expression kit). The amplified RNA was fragmented using 4.8  $\mu$ L of 5X fragmentation buffer (GeneChip® WT Terminal labeling kit; Affymetrix;P/N 900671). Fragmentation was checked on Agilent 2100 bioanalyser and hybridized to the gene containing over 33,290 transcripts, as described in the Gene Chip Expression Analysis Technical Manual (Affymetrix). After hybridization, the chips were stained and washed in a Genechip Fluidics

Station 450 (Affymetrix) and scanned by using a Genechip Array scanner 3000 7G (Affymetrix).

Data Analysis - Affymetrix GeneChip® Human Gene 1.0 ST Array™ array was scanned using Affymetrix Model 3000 G7 scanner and the image data was extracted through Affymetrix Command Console software 1.1. The data were subjected to normalization, background correction and data summarization using Affymetrix Expression Console software with the standard Robust Multi-array Average (RMA) algorithm. Data were deposited in Gene Expression Omnibus (GSE51276). Only the probes with a detection P value < 0.01 in more than 50% of arrays were included for further analyses. The web-based tool, DAVID (the Database for Annotation, Visualization, and Integrated Discovery) was used to perform the biological interpretation of differentially expressed genes. Functional annotation of genes were performed based on Gene Ontology (GO), classified into three ontologies - Biological Process, Molecular Function and Cellular Component, as extracted from DAVID database.

### **Network analysis using Pathway studio**

Pathway analysis was performed by two approaches – one with Gene set enrichment analysis (GSEA) software using the complete set of gene expression data from the microarray experiments, and the KEGG pathway exploiting the DAVID database using the list of more than 1.5 fold DGE. The network analysis was carried out by pathway studio (pathway Studio v 9.0 program, Ariadne Transcriptomics, Inc.).

### **GC-MS based lipid analysis**

Sample preparation – The aSiNPs (aSiNP-189, aSiNP-116, aSiNP-26, aSiNP-8) treated and untreated control samples were collected with standard trypsinization and after by PBS washing, 600  $\mu$ l of MeOH/CHCl<sub>3</sub> mix (2:1, v/v) were added to the samples, followed by 15 min in an ice-bath and centrifuged at 13,000 g for 15 min. Next, 400  $\mu$ L of methanol and distilled water (1:1, v/v) mix was added and centrifuged 3000 rpm for 10 min. After that, organic layers were extracted and dried under nitrogen gas. The lipid metabolites in extract were dissolved with 30  $\mu$ l of ethyl acetate and then vortexed for 30 sec. The sample was trimethylsilylated with 30  $\mu$ l of MSTFA at 80 °C for 30 min.

GC–MS/MS conditions - The sample analysis was carried out with an Agilent-6890N gas chromatography (Palo Alto, CA, USA) with DB-5MS capillary column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m film, J&W Scientific Folsom, CA, USA) and coupled with an Agilent-5973 mass selective detector. Ultra-high purity helium was used as a carrier gas at a flow rate of 1.0 mL/min. One  $\mu$ L of sample was introduced by split injection mode (split ratio 10:1). The temperature conditions of the GC injection port and the MS interface were set at 280° C and 270° C, respectively. The oven temperature was held at 80° C for 3 min, elevated to 200° C at 15° C/min, and then ramped to 280° C at 10° C/min held for 10 min. The mass spectrometer was run in the electron ionization (EI) mode with electron energy of 70 eV. The mass scan ranged from 70 to 650 amu. The manifold temperature was maintained at 230° C.

Metabolite Identification and Statistical Analyses - Most of peaks in total ion chromatograms were identified by comparing the retention times and mass spectra of authentic standards (malic acid, phosphoric acid, proline, o-hydroxybenzaldehyde, dibutylphthalate, palmitic acid, stearic acid, and cholesterol). Some of minor components were characterized and tentatively identified by correlating data with similarities higher than 90% in accordance with NIST/Wiley library.

The amounts of the individual components observed in total ion chromatogram were expressed as the peak area. Here, we assumed that the response factors of the individual components were identical. The mean amount and relative standard deviation of the individual components were obtained from triplicate analyses.

### **Metabolomics pathway analysis and integrated Transcriptomics-metabolomics analysis**

Metabolomics pathway analysis as well as enrichment based pathway analysis were performed by using MetaboAnalyst 3.0 software (a web service for metabolomics data analysis) (Xia and Wishart, 2011) with the metabolites that displayed >1.5 fold changes in aSiNP s treatment. Integrated Transcriptomics and metabolomics pathway analysis were carried out with freely available IMPaLA (<http://impala.molgen.mpg.de/>) software (Brink-Jensen *et al.*, 2013; Kamburov *et al.*, 2011) with the microarray DGE (>1.5 fold) as well as significantly altered metabolites (>1.5 fold) due to aSiNP treatment.

### **Total cholesterol measurement**

The total cholesterol measurements were performed with colorimetric total cholesterol assay kit (Cell Biolabs Inc., Catalogue No: STA-384) by following the manufacturer's instructions. Briefly, cell extracts were prepared with chloroform: isopropanol: NP40 (7:11:0.1) from control and aSiNP (aSiNP-189, aSiNP-116, aSiNP-26 and aSiNP-8) treated (with 10, 25, 50, 100 and 200 mg/L of doses for 24 h) HepG2 cells. Next, the extracted organic phases were air dried at 50° C and the dried lipids were dissolved in 1X assay diluents. In each well of a 96 well plates, 50 µl of samples were mixed with 50 µl cholesterol reaction reagents (cholesterol oxidase – 1:50, HRP – 1:50, Colorimetric probe – 1:50 and cholesterol esterase 1:250 in 1X assay diluent). After

well mixing, the plates were incubated at 37°C for 45 min and the absorbance was measured at 570 nm in ELISA plate reader. The calculations of the total cholesterol deduced from standard curve. The experiments were carried out in three biological replicates each with duplicate.

**Table S1:** Primers used for the amplification of the human cDNA

<b>Gene Names</b>	<b>Primer sequences</b>
CYP51A1	5' GCGTCCTTTGACTGATGAT 3' 5' AGAAGCCCATCCAAGCACTA 3'
CDK1	5' TTTTCAGAGCTTTGGGCACT 3' 5' AGGCTTCCTGGTTCCATTT 3'
KCNJ10	5' GCGGAGAGTCCTGACAAAAG 3' 5' CCACTGCATGTCAATGAAGG 3'
ACSS2	5' TTCTACACAGCACCCACAGC 3' 5' TTCACCCACTGTGCCTAACA 3'
MVD	5' GCGATGAAGAGCTGGTTCTG 3' 5' TGAAGTCCTTGCTGATGACG 3'
FDPS	5' TTTAGGAGTACCCGCCAACA 3' 5' GAAGACCCCCACAGATCTCA 3'
HMGCS1	5' AAGGTGCAGGGGAGTTAGGT 3' 5' GAACTGTCTGCCAGGTGAT 3'
HMGCR	5' ACCACAGAGGCTATGATTGAGG 3' 5' ATGGCGGTGACAATGTTTGC 3'
SREBF1	5' CTGCTGTCCACAAAAGCAAA 3'

	5' GGTCAGTGTGTCCTCCACCT 3'
SREBF2	5' TGGATGATGCAAAGGTCAAA 3' 5' GAGAGGCACAGGAAGGTGAG 3'
ACTB	5' GATGAGATTGGCATGGCTTT 3' 5' CACCTTCACCGTTCCAGTTT 3'
GAPDH	5'CATCAAGAAGGTGGTGAAGCAGG3' 5'TGTCGCTGTTGAAGTCAGAGGAGA3'

## Results

**Table S2:** Functional Annotation Clustering (Enrichment Score > 3.00) performed with microarray DGE due to aSiNP-116 exposure (>1.5 fold) by using DAVID

<b>Enrichment Score: 4.49</b>	<b>Count</b>	<b>p-Value</b>
Steroid biosynthesis	7	1.4E-7
steroid metabolic process	11	3.3E-7
cholesterol biosynthetic process	6	4.0E-7
sterol biosynthetic process	6	1.9E-6
lipid synthesis	8	2.3E-6
cholesterol metabolic process	7	1.8E-5
lipid biosynthetic process	11	2.2E-5
<b>Enrichment Score: 3.26</b>	<b>Count</b>	<b>p-Value</b>
Glutathione S-transferase, alpha class	4	2.2E-6
Metabolism of xenobiotics by cytochrome P450	6	8.2E-5
Drug metabolism	6	9.6E-5
glutathione transferase activity	4	3.2E-4
transferase activity, transferring alkyl or aryl (other than methyl) groups	5	3.3E-4
Glutathione metabolism	4	6.4E-3
Thioredoxin fold	4	2.4E-2



**Table S3:** Selected genes with the strong differential expression (>1.5 fold) due to aSiNP-116 exposure at 100 mg/L dose for 24 h

Gene Accession	Gene Symbol	Gene Description	absolute fold change	GO Biological Process Term	GO Cellular Component Term	GO Molecular Function Term
<b>Selected Genes up regulated (&gt;1.5 fold) due to aSiNP exposure</b>						
NM_005165	ALDOC	aldolase C, fructose-bisphosphate	2.0150589	response to hypoxia, fructose metabolic process, glycolysis, apoptosis, aging, response to organic nitrogen, response to organic cyclic substance, fructose 1,6-bisphosphate metabolic process, organ regeneration, protein homotetramerization, protein heterotetramerization	Cytoplasm, mitochondrion, cytoskeleton, axon	fructose-bisphosphate aldolase activity, protein binding, cytoskeletal protein binding, lyase activity
NM_018677	ACSS2	acyl-CoA synthetase short-chain family member 2	1.9162529	lipid biosynthetic process	Nucleus, nucleolus, cytoplasm, cytosol, intracellular membrane-bounded organelle	nucleotide binding, acetate-CoA ligase activity, protein binding, ATP binding, AMP binding, ligase activity
NM_004265	FADS2	fatty acid desaturase 2	1.8102513	unsaturated fatty acid biosynthetic process, transport, electron transport chain	membrane fraction, endoplasmic reticulum, endoplasmic reticulum membrane, integral to plasma	stearoyl-CoA 9-desaturase activity oxidoreductase activity, acting on paired donors, with oxidation of a pair of donors resulting in the reduction of molecular oxygen to

					membrane, membrane	two molecules of water, heme binding
NM_0010 05179	OR56A4	olfactory receptor, family 56, subfamily A, member 4	1.8039 12	sensory perception of smell, response to stimulus	plasma membrane, integral to membrane	receptor activity, olfactory receptor activity
NR_0293 72	NT5C3	5'- nucleotidase, cytosolic III	1.7966 221	pyrimidine nucleoside metabolic process, nucleotide metabolic process	cytoplasm mitochondrio n, endoplasmic reticulum	nucleotide binding, magnesium ion binding, 5'- nucleotidase activity, 2'- phosphotransferase activity, hydrolase activity
NM_0013 60	DHCR7	7- dehydrochole sterol reductase	1.7800 157	blood vessel development, cholesterol biosynthetic process, post- embryonic development, cell differentiation lung development, multicellular organism growth, regulation of cell proliferation, regulation of cholesterol biosynthetic process, oxidation reduction	nuclear outer membrane, endoplasmic reticulum, endoplasmic reticulum membrane, membrane, integral to membrane	protein binding, oxidoreductase activity, 7- dehydrocholesterol reductase activity
NM_0036 70	BHLHE 40	basic helix- loop-helix family, member e40	1.7746 727	regulation of transcription, DNA- dependent, circadian rhythm, response to light stimulus, entrainment of circadian clock, regulation of	nucleus	DNA binding, sequence-specific DNA binding transcription factor activity, protein binding, transcription repressor activity

				neuronal synaptic plasticity		
NM_007128	VPREB1	pre-B lymphocyte 1	1.7719636	immune response	extracellular region	antigen binding, protein binding
NM_002461	MVD	mevalonate (diphospho) decarboxylase	1.7683134	cholesterol biosynthetic process, positive regulation of cell proliferation, isoprenoid biosynthetic process, response to drug	soluble fraction, peroxisome peroxisomal matrix, cytosol,	nucleotide binding diphosphomevalonate decarboxylase activity, ATP binding, kinase activity, lyase activity, Hsp70 protein binding, protein homodimerization activity
NM_001005522	OR2T8	olfactory receptor, family 2, subfamily T, member 8	1.7589287	sensory perception of smell, response to stimulus	plasma membrane, integral to membrane	receptor activity, olfactory receptor activity
NM_002340	LSS	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	1.7175106	steroid biosynthetic process, cholesterol biosynthetic process, steroid metabolic process, sterol metabolic process	endoplasmic reticulum, endoplasmic reticulum membrane, microsome, membrane	lanosterol synthase activity, intramolecular transferase activity
NM_000602	SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	1.7116052	response to reactive oxygen species, chronological cell aging, regulation of receptor activity, negative regulation of plasminogen activation, negative regulation of endopeptidase activity,	extracellular region, extracellular space, soluble fraction, plasma membrane, extracellular matrix	protease binding, serine-type endopeptidase inhibitor activity, receptor binding, protein binding, peptidase inhibitor activity

				negative regulation of smooth muscle cell migration, positive regulation of blood coagulation, negative regulation of blood coagulation, negative regulation of cell migration, positive regulation of interleukin-8 production, negative regulation of cell adhesion mediated by integrin, response to cytokine stimulus, positive regulation of leukotriene production involved in inflammatory response, regulation of cell proliferation, tissue regeneration, glucose homeostasis, negative regulation of apoptosis, positive regulation of angiogenesis, positive regulation of receptor-		
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				mediated endocytosis, positive regulation of inflammatory response, defense response to Gram-negative bacterium, response to glucocorticoid stimulus, negative regulation of fibrinolysis, negative regulation of vascular wound healing, cellular response to chemical stimulus, cellular response to lipopolysaccharide, positive regulation of monocyte chemotaxis		
NM_133459	CCBE1	collagen and calcium binding EGF domains 1	1.7070922	Lymphangiogenesis, sprouting angiogenesis, multicellular organismal development, venous blood vessel morphogenesis	extracellular region	calcium ion binding
NM_002297	LCN1	lipocalin 1 (tear prealbumin)	1.7053856	Proteolysis, response to stimulus, sensory perception of taste	extracellular region	cysteine-type endopeptidase inhibitor activity, transporter activity, protein binding
NM_0005	LDLR	low density	1.6650	protein O-	Lysosome,	transmembrane

27		lipoprotein receptor	667	linked glycosylation, lipid metabolic process, lipid transport, endocytosis, steroid metabolic process, cholesterol metabolic process, intestinal cholesterol absorption, cholesterol transport, low-density lipoprotein particle clearance, lipoprotein catabolic process, cholesterol homeostasis, response to estrogen stimulus, interspecies interaction between organisms	early endosome, plasma membrane, integral to plasma membrane, caveola, coated pit, external side of plasma membrane, endosome membrane, clathrin-coated endocytic vesicle membrane, low-density lipoprotein particle	receptor activity, low-density lipoprotein receptor activity, calcium ion binding, protein binding, very-low-density lipoprotein receptor activity
NM_001004481	OR13C2	olfactory receptor, family 13, subfamily C, member 2	1.6521263	sensory perception of smell, response to stimulus	plasma membrane, integral to membrane	receptor activity, olfactory receptor activity
NM_015234	GPR116	G protein-coupled receptor 116	1.6499371	G-protein coupled receptor protein signaling pathway, neuropeptide signaling pathway	plasma membrane, integral to membrane	G-protein coupled receptor activity
NM_005031	FXYD1	FXYD domain containing	1.6494328	ion transport, chloride transport,	plasma membrane, integral to	ion channel activity, chloride channel activity, protein

		ion transport regulator 1		cellular calcium ion homeostasis, muscle contraction	plasma membrane, chloride channel complex	binding
NM_005584	MAB21L1	mab-21-like 1 (C. elegans)	1.6484579	multicellular organismal development, positive regulation of cell proliferation, anatomical structure morphogenesis, camera-type eye development	nucleus	---
NM_000044	AR	androgen receptor	1.6482888	in utero embryonic development, transcription, DNA-dependent, transport, signal transduction, cell-cell signaling, sex differentiation, cell death, cell proliferation, male gonad development, positive regulation of gene-specific transcription from RNA polymerase II promoter, cell growth, male somatic sex determination, prostate gland development, response to insulin stimulus, positive	Nucleus, nucleus, cytoplasm, axon, dendrite	DNA binding, sequence-specific DNA binding transcription factor activity, receptor activity, androgen receptor activity, receptor binding, androgen binding, protein binding, beta-catenin binding, transcription factor binding, zinc ion binding, promoter binding, transcription activator activity, metal ion binding, protein dimerization activity, POU domain binding

				regulation of estrogen receptor signaling pathway, positive regulation of MAPKKK cascade, positive regulation of insulin-like growth factor receptor signaling pathway, male sex differentiation, regulation of developmental growth, organ formation, negative regulation of epithelial cell proliferation, regulation of catalytic activity, activation of prostate induction by androgen receptor signaling pathway, morphogenesis of an epithelial fold, lateral sprouting involved in mammary gland duct morphogenesis, regulation of prostatic bud formation, prostate gland growth, prostate gland		
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				epithelium morphogenesis, epithelial cell differentiation involved in prostate gland development, mammary gland alveolus development		
NM_001002841	MYL4	myosin, light chain 4, alkali; atrial, embryonic	1.6338075	regulation of the force of heart contraction, muscle organ development, positive regulation of ATPase activity, cardiac muscle contraction	muscle myosin complex, myosin complex, A band	motor activity, actin monomer binding, calcium ion binding, structural constituent of muscle, myosin II heavy chain binding, actin filament binding
NM_002241	KCNJ10	potassium inwardly-rectifying channel, subfamily J, member 10	1.6095116	ion transport, potassium ion transport	integral to plasma membrane, membrane	nucleotide binding, voltage-gated ion channel activity, potassium channel activity, protein binding, ATP binding, ATP-activated inward rectifier potassium channel activity
NM_052995	CLRN1	clarin 1	1.6069366	sensory perception of sound, photoreceptor cell maintenance, response to stimulus, sensory perception of light stimulus, equilibrium	Membrane, integral to membrane	---
NM_002900	RBP3	retinol binding protein 3,	1.5969698	Proteolysis, lipid metabolic	extracellular region, interphotorec	retinoid binding, serine-type peptidase activity,

		interstitial		process, transport, visual perception	ceptor matrix	retinal binding
NM_1775 51	GPR109 A	G protein- coupled receptor 109A	1.5857 485	neutrophil apoptosis, apoptosis, positive regulation of neutrophil apoptosis, negative regulation of lipid catabolic process, positive regulation of adiponectin secretion	plasma membrane, integral to membrane	receptor activity, GTP binding, purinergic nucleotide receptor activity, G-protein coupled, nicotinic acid receptor activity
NM_0049 44	DNASE 1L3	deoxyribonuc lease I-like 3	1.5808 582	DNA metabolic process, DNA catabolic process, DNA fragmentation involved in apoptotic nuclear change, apoptosis	nucleus	DNA binding, endodeoxyribonucle ase activity, deoxyribonuclease activity, calcium ion binding, hydrolase activity
NM_0027 73	PRSS8	protease, serine, 8	1.5806 073	Proteolysis, positive regulation of sodium ion transport, response to drug, response to peptide hormone stimulus, response to mineralocortic oid stimulus	extracellular region, extracellular space, plasma membrane, integral to membrane	serine-type endopeptidase activity, peptidase activity, sodium channel regulator activity
NM_0528 90	PGLYR P2	peptidoglycan recognition protein 2	1.5727 365	peptide amidation, peptidoglycan catabolic process, detection of bacterium,	extracellular region, intracellular, membrane	protein binding, N- acetylmuramoyl-L- alanine amidase activity, peptidoglycan receptor activity, hydrolase activity,

				innate immune response, defense response to Gram-positive bacterium		peptidoglycan binding, metal ion binding
NM_018935	PCDHB15	protocadherin beta 15	1.5709268	cell adhesion, homophilic cell adhesion, nervous system development	plasma membrane, integral to plasma membrane	calcium ion binding
NM_002668	PLP2	proteolipid protein 2 (colonic epithelium-enriched)	1.5691725	ion transport, chemotaxis, cytokine-mediated signaling pathway	membrane fraction, endoplasmic reticulum, endoplasmic reticulum membrane, plasma membrane, integral to membrane	protein binding, ion transmembrane transporter activity, chemokine binding
NM_002004	FDPS	farnesyl diphosphate synthase	1.5608198	cholesterol biosynthetic process, isoprenoid biosynthetic process, interspecies interaction between organisms	cytoplasm mitochondrion	dimethylallyltransferase activity, geranyltransferase activity, transferase activity, metal ion binding
NM_002191	INHA	inhibin, alpha	1.5452369	skeletal system development, ovarian follicle development, induction of apoptosis, cell cycle arrest, signal transduction, cell surface receptor linked signaling pathway, cell-	photoreceptor outer segment, photoreceptor inner segment, extracellular region, extracellular space, inhibin-beta glycan-ActRII complex, neuronal cell body, inhibin	signal transducer activity, receptor binding, cytokine activity, hormone activity, protein binding, growth factor activity, protein heterodimerization activity, inhibin beta-A binding

				cell signaling, nervous system development, male gonad development, response to external stimulus, cell differentiation , erythrocyte differentiation , regulation of cell proliferation, negative regulation of phosphorylati on, hemoglobin biosynthetic process, negative regulation of interferon- gamma biosynthetic process, negative regulation of B cell differentiation , negative regulation of macrophage differentiation , negative regulation of cell cycle, positive regulation of follicle- stimulating hormone secretion, negative regulation of follicle- stimulating hormone secretion,	A complex	
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				regulation of cell cycle		
NM_016371	HSD17B7	hydroxysteroid (17-beta) dehydrogenase 7	1.5425422	steroid biosynthetic process, oxidation reduction	endoplasmic reticulum membrane, plasma membrane, integral to membrane	3-keto sterol reductase activity, estradiol 17-beta-dehydrogenase activity, binding, oxidoreductase activity
NM_206809	MOG	myelin oligodendrocyte glycoprotein	1.5410301	cell adhesion, central nervous system development, positive regulation of MyD88-dependent toll-like receptor signaling pathway	plasma membrane, integral to membrane	---
NM_020061	OPN1LW	opsin 1 (cone pigments), long-wave-sensitive	1.5395812	signal transduction, visual perception, phototransduction, protein-chromophore linkage, response to stimulus	integral to plasma membrane, membrane	G-protein coupled receptor activity, photoreceptor activity
NM_139164	STARD4	StAR-related lipid transfer (START) domain containing 4	1.5333393	lipid transport	---	lipid binding
NM_000786	CYP51A1	cytochrome P450, family 51, subfamily A, polypeptide 1	1.5313138	cholesterol biosynthetic process, oxidation reduction	endoplasmic reticulum, endoplasmic reticulum membrane, microsome, membrane, integral to membrane	monooxygenase activity, sterol 14-demethylase activity, electron carrier activity, heme binding, metal ion binding
NM_000567	CRP	C-reactive protein, pentraxin-related	1.5289466	acute-phase response, inflammatory response,	extracellular region, extracellular space	Binding, protein binding, low-density lipoprotein binding, choline binding,

				complement activation, classical pathway, opsonization, negative regulation of macrophage derived foam cell differentiation, negative regulation of lipid storage		metal ion binding, Gram-positive bacterial cell surface binding
NM_001098272	HMGCS1	3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)	1.5200618	lipid metabolic process, cholesterol biosynthetic process, isoprenoid biosynthetic process, response to organic nitrogen, response to drug	soluble fraction, cytoplasm, cytosol	hydroxymethylglutaryl-CoA synthase activity, transferase activity
NM_002976	SCN7A	sodium channel, voltage-gated, type VII, alpha	1.509404	ion transport, sodium ion transport, muscle contraction, transmembrane transport	voltage-gated sodium channel complex, membrane, integral to membrane	voltage-gated ion channel activity, voltage-gated sodium channel activity
NM_005448	BMP15	bone morphogenetic protein 15	1.508356	ovarian follicle development, female gamete generation, positive regulation of transcription, granulosa cell development	extracellular region, extracellular space	cytokine activity, growth factor activity
NM_003802	MYH13	myosin, heavy chain 13, skeletal muscle	1.5062654	muscle contraction	Cytoplasm, muscle myosin complex, myofibril,	microfilament motor activity, nucleotide binding, actin binding, protein binding, calmodulin

					myosin filament	binding, ATP binding
NM_173078	SLITRK4	SLIT and NTRK-like family, member 4	1.5058551	axonogenesis	Membrane, integral to membrane	protein binding
NM_006931	SLC2A3	solute carrier family 2 (facilitated glucose transporter), member 3	1.504112	carbohydrate metabolic process, carbohydrate transport, glucose transport, transmembrane transport	Membrane, integral to membrane	transporter activity, glucose transmembrane transporter activity, substrate-specific transmembrane transporter activity
NM_014513	KIR2DS5	killer cell immunoglobulin-like receptor, two domains, short cytoplasmic tail, 5	1.5028844	immune response	plasma membrane, integral to plasma membrane	receptor activity, HLA-C specific inhibitory MHC class I receptor activity

**Selected Genes down regulated (>1.5 fold) SiO2 NP**

ENST00000361453	ND2	MTND2	2.4947081	mitochondrial electron transport, NADH to ubiquinone, oxygen and reactive oxygen species metabolic process, transport, electron transport chain, ATP synthesis coupled electron transport	Mitochondrion, mitochondrial inner membrane, mitochondrial respiratory chain complex I, postsynaptic density, membrane, integral to membrane, respiratory chain	NADH dehydrogenase (ubiquinone) activity, oxidoreductase activity
BC073772	IGKC	immunoglobulin kappa constant, immunoglobulin kappa constant	2.4923765	immune response, immune response	extracellular region	antigen binding, protein binding
AK172782	GPAM	glycerol-3-	2.39623	fatty acid	mitochondri	glycerol-3-

		phosphate acyltransferase, mitochondrial	77	<p>metabolic process, acyl-CoA</p> <p>metabolic process, glycerophospholipid</p> <p>metabolic process, metabolic process, phospholipid biosynthetic process, response to glucose stimulus, response to fructose stimulus, positive regulation of triglyceride biosynthetic process, response to activity, triglyceride biosynthetic process, response to nutrient levels, cellular response to insulin stimulus, positive regulation of multicellular organism growth, positive regulation of activated T cell proliferation, response to cadmium ion, regulation of cytokine</p>	<p>on mitochondrial outer membrane, mitochondrial inner membrane, membrane, integral to membrane</p>	<p>phosphate O-acyltransferase activity, acyltransferase activity, transferase activity, glycerol-3-phosphate O-acyltransferase activity</p>
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				secretion, defense response to virus, fatty acid homeostasis, phospholipid homeostasis, negative regulation of activation- induced cell death of T cells, interleukin-2 secretion		
NM_003561	PLA2G1 0	phospholipase A2, group X	2.13631 29	phospholipid metabolic process, axon guidance, positive regulation of macrophage derived foam cell differentiation , positive regulation of lipid storage, lipid catabolic process, arachidonic acid metabolic process, positive regulation of cellular protein metabolic process, positive regulation of prostaglandin secretion, regulation of macrophage activation, lysophospholi pid transport	extracellular region	phospholipase A2 activity, calcium ion binding, hydrolase activity

AF284753	UIMC1	ubiquitin interaction motif containing 1	1.9853302	double-strand break repair, response to ionizing radiation, negative regulation of transcription, chromatin modification, G2/M transition DNA damage checkpoint, positive regulation of DNA repair, histone H2A K63-linked deubiquitination	Nucleus, BRCA1-A complex	protein binding, histone binding, K63-linked polyubiquitin binding
NM_000846	<b>GSTA2</b>	glutathione S-transferase alpha 2	1.8979619	metabolic process	cytoplasm	glutathione transferase activity, transferase activity
NM_000670	ADH4	alcohol dehydrogenase 4 (class II), pi polypeptide	1.8768352	retinoid metabolic process, alcohol metabolic process, ethanol oxidation, cellular aldehyde metabolic process, quinone cofactor metabolic process, retinol metabolic process, alcohol catabolic process, oxidation reduction	cytoplasm	NADPH:quinone reductase activity, alcohol dehydrogenase (NAD) activity, alcohol dehydrogenase activity, zinc-dependent, retinol dehydrogenase activity, binding, all-trans retinal binding, zinc ion binding, oxidoreductase activity, oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor, benzaldehyde dehydrogenase

						activity, retinol binding, metal ion binding, NAD or NADH binding
NM_024674	LIN28A	lin-28 homolog A (C. elegans)	1.81133 33	regulation of transcription, DNA-dependent, miRNA catabolic process, stem cell maintenance, gene silencing by RNA, pre-microRNA processing, RNA 3'-end processing, positive regulation of translation, stem cell differentiation	cytoplasmic mRNA processing body, nucleus, nucleolus, cytoplasm, stress granule	DNA binding, RNA binding, mRNA binding, protein binding, zinc ion binding, translation initiation factor binding, metal ion binding
NM_003096	SNRPG	small nuclear ribonucleoprotein polypeptide G	1.78090 55	spliceosome assembly, spliceosomal snRNP assembly, mRNA processing, RNA splicing	Nucleus, nucleoplasm, spliceosomal complex, U7 snRNP, cytosol, small nuclear ribonucleoprotein complex	RNA binding, protein binding
NM_145740	GSTA1	glutathione S-transferase alpha 1	1.73613 6	glutathione metabolic process, metabolic process	cytoplasm	glutathione transferase activity, transferase activity
NM_138374	ZNF845	zinc finger protein 845	1.71546 89	regulation of transcription, DNA-dependent	Intracellular, nucleus	DNA binding, zinc ion binding, metal ion binding
NM_001166292	PTCH2	patched 2	1.71452 98	protein complex assembly, spermatogenesis, epidermis	integral to plasma membrane, membrane	transmembrane receptor activity, hedgehog receptor activity

				development, hair cycle, skin development		
NM_005276	GPD1	glycerol-3-phosphate dehydrogenase 1 (soluble)	1.6811809	carbohydrate metabolic process, gluconeogenesis, NADH oxidation, triglyceride biosynthetic process, glycerol-3-phosphate catabolic process, glycerolipid metabolic process, oxidation reduction	soluble fraction, cytoplasm, mitochondrion, cytosol, glycerol-3-phosphate dehydrogenase complex	glycerol-3-phosphate dehydrogenase [NAD+] activity, glycerol-3-phosphate dehydrogenase activity, binding, protein homodimerization activity, NAD or NADH binding
NM_018260	ZNF701	zinc finger protein 701	1.6715703	regulation of transcription, DNA-dependent	Intracellular, nucleus, nucleolus, centrosome	DNA binding, zinc ion binding, metal ion binding
NM_013391	DMGDH	dimethylglycine dehydrogenase	1.6701851	glycine metabolic process, glycine catabolic process, choline metabolic process, oxidation reduction	Cytoplasm, mitochondrion, mitochondrial matrix	aminomethyltransferase activity, folic acid binding, electron carrier activity, oxidoreductase activity, dimethylglycine dehydrogenase activity, FAD or FADH2 binding
NR_027279	USP17L6P	ubiquitin specific peptidase 17-like 6 (pseudogene)	1.6537292	ubiquitin-dependent protein catabolic process	Nucleus, cytoplasm	ubiquitin thiolesterase activity, peptidase activity, cysteine-type peptidase activity
AK301230	ZNF724P	zinc finger protein 724, pseudogene	1.6330806	regulation of transcription, DNA-dependent	Intracellular, nucleus	DNA binding, zinc ion binding, metal ion binding
NM_001144989	ZNF814	zinc finger protein 814	1.6276867	regulation of transcription, DNA-	intracellular	nucleic acid binding, zinc ion binding, metal ion

				dependent		binding
NM_032117	MND1	meiotic nuclear divisions 1 homolog (S. cerevisiae)	1.6238732	DNA recombination, meiosis	nucleus	DNA binding, protein binding
NM_002491	NDUFB3	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 3, 12kDa	1.6154936	mitochondrial electron transport, NADH to ubiquinone, transport, electron transport chain	Mitochondrion, mitochondrial inner membrane, mitochondrial respiratory chain complex I, membrane, integral to membrane, respiratory chain	NADH dehydrogenase (ubiquinone) activity
ENST00000357491	ZNF729	zinc finger protein 729	1.5924025	regulation of transcription, DNA-dependent	Intracellular, nucleus	DNA binding, zinc ion binding, metal ion binding
NM_001114120	DEPDC1	DEP domain containing 1	1.5782831	signal transduction	Intracellular, nucleus	GTPase activator activity
NM_001005216	OR2J3	olfactory receptor, family 2, subfamily J, member 3	1.5708201	sensory perception of smell, response to stimulus	plasma membrane, integral to membrane	receptor activity, olfactory receptor activity
NM_152355	ZNF441	zinc finger protein 441	1.5660714	regulation of transcription, DNA-dependent	Intracellular, nucleus	DNA binding, zinc ion binding, metal ion binding
NM_018365	MNS1	meiosis-specific nuclear structural 1	1.5621987	meiosis	Nucleus, nuclear envelope, intermediate filament	---
NM_001130862	RAD51AP1	RAD51 associated protein 1	1.5577438	double-strand break repair via homologous recombination, DNA repair, DNA recombination	nucleus	DNA binding, double-stranded DNA binding, single-stranded DNA binding, RNA binding, protein binding
NM_153699	GSTA5	glutathione S-transferase	1.5575311	metabolic process	cytoplasm	glutathione transferase

		alpha 5				activity, transferase activity
NM_203307	ZNF321	zinc finger protein 321	1.557367	regulation of transcription, DNA-dependent	intracellular	nucleic acid binding
NM_001786	CDK1	cyclin-dependent kinase 1	1.5500718	protein complex assembly, protein phosphorylation, anti apoptosis, mitosis, mitotic cell cycle G2/M transition DNA damage checkpoint, small GTPase mediated signal transduction, Ras protein signal transduction, cell aging, response to toxin, positive regulation of gene expression, response to organic cyclic substance, response to amine stimulus, response to activity, intracellular signaling pathway, chromosome condensation, organ regeneration, anaphase-promoting complex-	Nucleus, nucleoplasm, cytoplasm, cytosol, spindle microtubule, midbody	nucleotide binding, protein kinase activity, cyclin-dependent protein kinase activity, protein binding, ATP binding, RNA polymerase II carboxy-terminal domain kinase activity, transferase activity, cyclin binding, Hsp70 protein binding, histone kinase activity

			dependent proteasomal ubiquitin- dependent protein catabolic process, positive regulation of protein import into nucleus, translocation, protein localization to kinetochore, response to drug, response to ethanol, positive regulation of DNA replication, positive regulation of mitotic cell cycle, response to cadmium ion, response to copper ion, response to axon injury, cell division, positive regulation of ubiquitin- protein ligase activity involved in mitotic cell cycle, ventricular cardiac muscle cell development, positive regulation of cardiac muscle cell proliferation,	
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				cellular response to hydrogen peroxide, cellular response to protein stimulus		
NM_031292	PUS7L	pseudouridylate synthase 7 homolog ( <i>S. cerevisiae</i> )-like	1.5481175	pseudouridine synthesis, tRNA processing	---	RNA binding, pseudouridine synthase activity, isomerase activity
NM_001077	UGT2B17	UDP glucuronosyltransferase 2 family, polypeptide B17	1.538956	metabolic process, steroid metabolic process	membrane fraction, endoplasmic reticulum, endoplasmic reticulum membrane, microsome, membrane, integral to membrane	retinoic acid binding, glucuronosyltransferase activity
NM_033625	RPL34	ribosomal protein L34	1.5367559	Translation, translational elongation	Intracellular, cytosol, ribosome, cytosolic large ribosomal subunit	RNA binding, structural constituent of ribosome, protein binding
NM_005420	SULT1E1	sulfotransferase family 1E, estrogen-preferring, member 1	1.5356219	female pregnancy, steroid metabolic process, estrogen metabolic process, sulfation	Cytoplasm, cytosol	estrone sulfotransferase activity, steroid binding, transferase activity, flavonol 3-sulfotransferase activity, steroid sulfotransferase activity
L23320	RFC1	replication factor C (activator 1) 1, 145kDa	1.525963	DNA-dependent DNA replication, nucleotide-excision repair, DNA gap filling, telomere maintenance	Intracellular, nucleus, nucleoplasm, DNA replication factor C complex	nucleotide binding, DNA clamp loader activity, protein binding, ATP binding, enzyme activator activity, nucleoside-triphosphatase activity



				via telomerase, regulation of transcription		
NM_000782	CYP24A1	cytochrome P450, family 24, subfamily A, polypeptide 1	1.5255178	osteoblast differentiation, steroid metabolic process, response to vitamin D, vitamin D metabolic process, oxidation reduction, vitamin D receptor signaling pathway, vitamin D receptor signaling pathway	Mitochondrial inner membrane	25-hydroxycholecalciferol-24-hydroxylase activity, electron carrier activity, oxidoreductase activity, oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NADH or NADPH as one donor, and incorporation of one atom of oxygen, heme binding, 1-alpha,25-dihydroxyvitamin D3 24-hydroxylase activity, metal ion binding
NM_001005216	OR2J3	olfactory receptor, family 2, subfamily J, member 3	1.5223784	sensory perception of smell, response to stimulus	plasma membrane, integral to membrane	receptor activity, olfactory receptor activity
NM_001005216	OR2J3	olfactory receptor, family 2, subfamily J, member 3	1.5223784	sensory perception of smell, response to stimulus	plasma membrane, integral to membrane	receptor activity, olfactory receptor activity
NM_000847	GSTA3	glutathione S-transferase alpha 3	1.5218192	metabolic process	cytoplasm	glutathione transferase activity, transferase activity
NM_006865	LILRA3	leukocyte immunoglobulin-like receptor, subfamily A	1.5192223	defense response, signal transduction	extracellular region, plasma membrane	antigen binding, receptor activity

		(without TM domain), member 3				
NM_003080	SMPD2	sphingomyelin phosphodiesterase 2, neutral membrane (neutral sphingomyelinase)	1.5082734	sphingomyelin metabolic process, intracellular protein kinase cascade, induction of apoptosis by extracellular signals, response to mechanical stimulus, ceramide biosynthetic process	membrane fraction, integral to plasma membrane, caveola, membrane	sphingomyelin phosphodiesterase activity, hydrolase activity, metal ion binding
NM_006451	PAIP1	poly(A) binding protein interacting protein 1	1.5062508	translational initiation, regulation of translation, RNA metabolic process, mRNA stabilization	cytoplasm	RNA binding, protein binding, translation activator activity

**Table S4:** Gene Ontology analysis of DGE >1.5 fold due to aSiNP-116 exposure at 100 mg/L dose for 24 h

<b>Biological Processes</b>			
<b><u>Term</u></b>	<b><u>Count</u></b>	<b><u>%</u></b>	<b><u>p-Value</u></b>
steroid metabolic process	11	7.1	3.3E-7
cholesterol biosynthetic process	6	3.8	4.0E-7
sterol biosynthetic process	6	3.8	1.9E-6
steroid biosynthetic process	7	4.5	1.1E-5
cholesterol metabolic process	7	4.5	1.8E-5
lipid biosynthetic process	11	7.1	2.2E-5
sterol metabolic process	7	4.5	3.1E-5
isoprenoid metabolic process	4	2.6	2.3E-3
isoprenoid biosynthetic process	3	1.9	6.3E-3
oxidation reduction	9	5.8	3.7E-2
muscle contraction	4	2.6	6.4E-2
fibrinolysis	2	1.3	7.0E-2
G2/M transition DNA damage checkpoint	2	1.3	7.5E-2
muscle system process	4	2.6	8.0E-2
alcohol catabolic process	3	1.9	8.5E-2
G2/M transition checkpoint	2	1.3	9.2E-2
<b>Cellular component</b>			
<b><u>Term</u></b>	<b><u>Count</u></b>	<b><u>%</u></b>	<b><u>p-Value</u></b>
endoplasmic reticulum membrane	5	3.2	8.2E-2

nuclear envelope-endoplasmic reticulum network	5	3.2	9.5E-2
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<b>Molecular Function</b>			
<b>Term</b>	<b>Count</b>	<b>%</b>	<b>p-Value</b>
glutathione transferase activity	4	2.6	3.2E-4
transferase activity, transferring alkyl or aryl (other than methyl) groups	5	3.2	3.3E-4
Hsp70 protein binding	2	1.3	2.7E-2
heme binding	4	2.6	4.9E-2
antigen binding	3	1.9	5.5E-2
tetrapyrrole binding	4	2.6	5.7E-2
retinal binding	2	1.3	7.8E-2

**Table S5:** Real-time RT-PCR validation of some selected genes from microarray results of aSiNP-116 exposure<sup>a</sup>

	<b>Gene Name</b>	<b>Microarray DGE</b>	<b>qRT-PCR</b>
Cholesterol	CYP51A1	+ 1.53	+ 1.73
Biosynthesis	ACSS2	+ 1.91	+ 1.92
Pathway	MVD	+ 1.77	+ 1.97
	FDPS	+ 1.56	+ 1.78
	HMGCS1	+ 1.52	+ 1.76
	HMGCR	+1.4	+ 2.87
	SREBF1	+ 1.22	+ 1.59
	SREBF2	+1.41	+ 2.38
	Others	CDK1	-1.55
	KCNJ10	+ 1.61	+ 2.17

<sup>a</sup>: The (+) sign indicated the up-regulation and (-) indicated down-regulation of mRNA levels

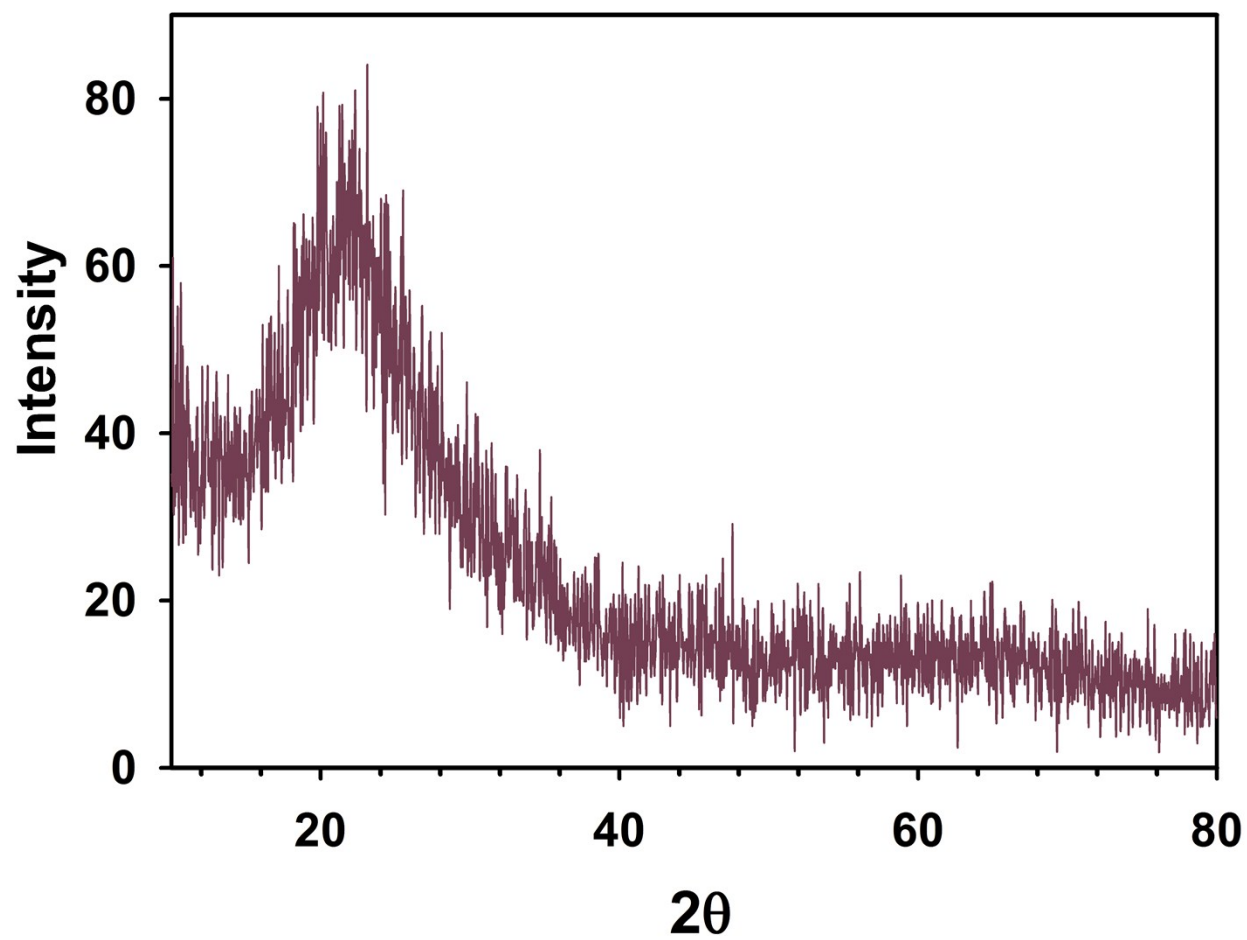


Figure S1

Figure S1: X-ray diffraction (XRD) pattern of aSiNP-116.

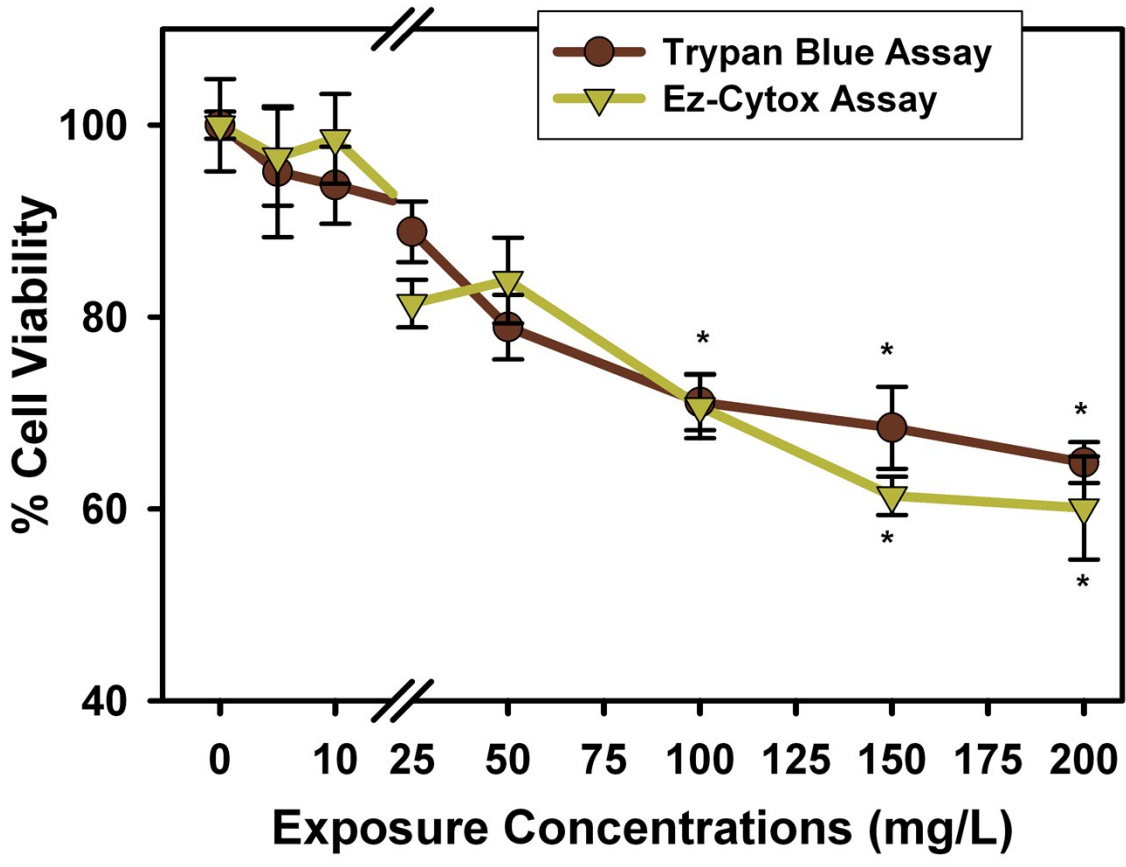
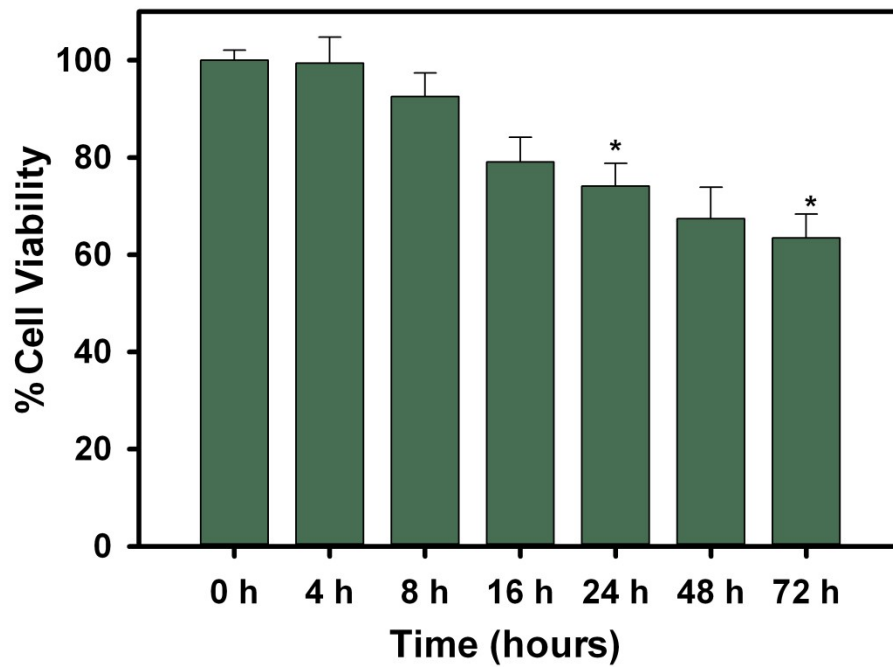


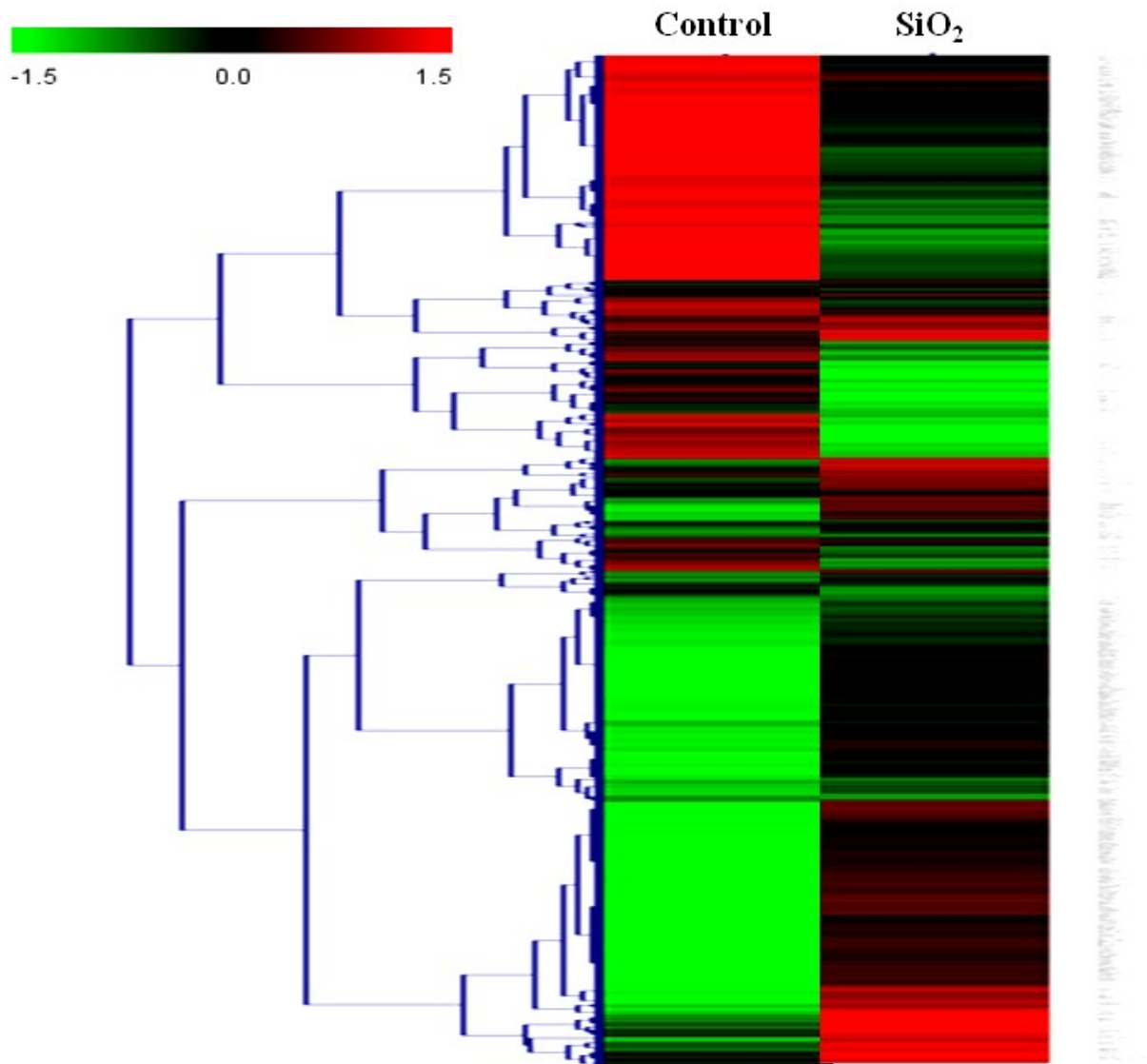
Figure S2A



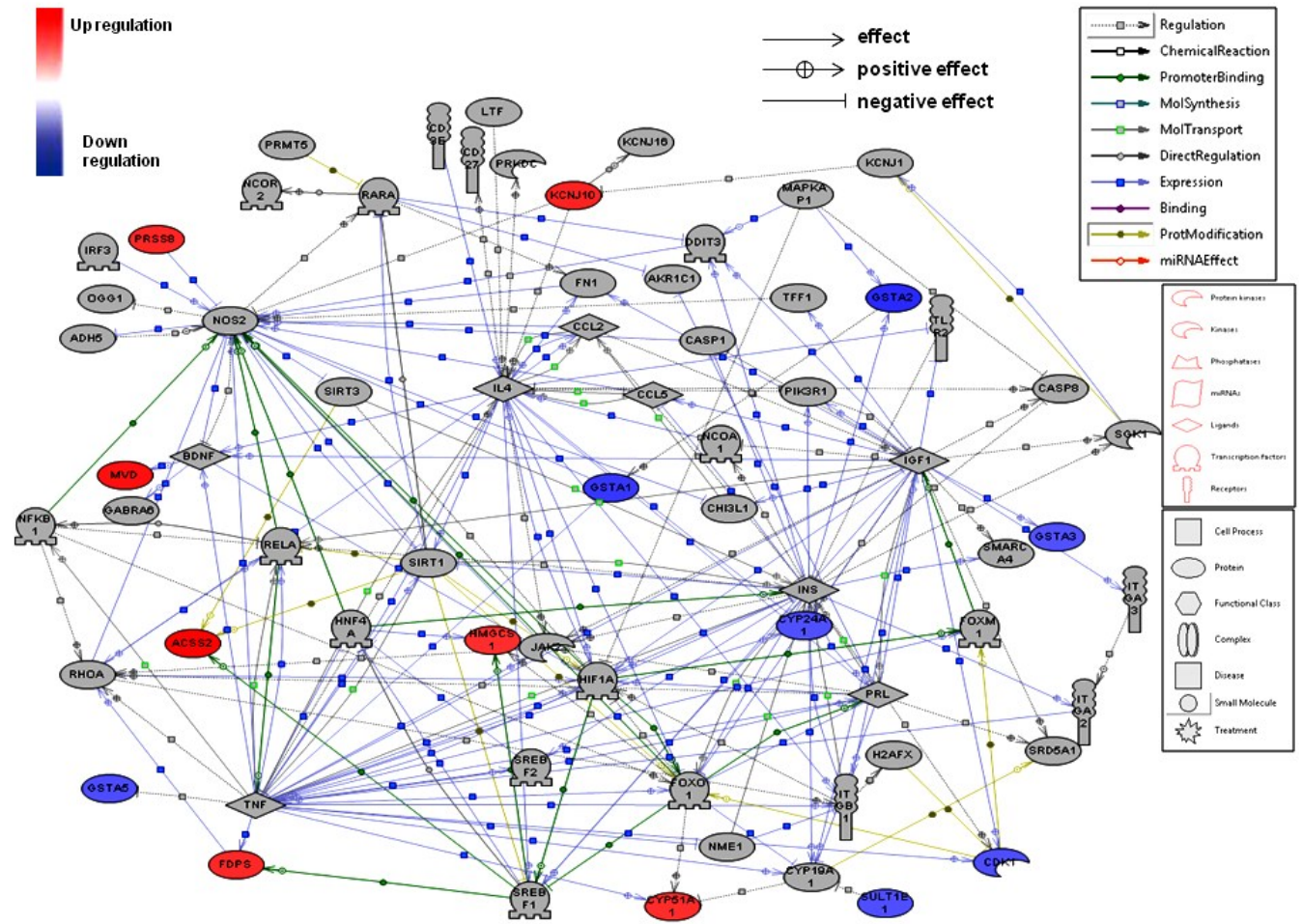
**Figure S2B**

**Figure S2:** Cytotoxicity of aSiNP-116 in HepG2 cells. **A.** Cytotoxicity determined by Ez-cytox assay and trypan blue exclusion method. **B.** Cells exposed at 100 mg/L at different time points and cytotoxicity determined by Ez-cytox assay. Data presented as mean  $\pm$  SEM., \* $p < 0.05$ .

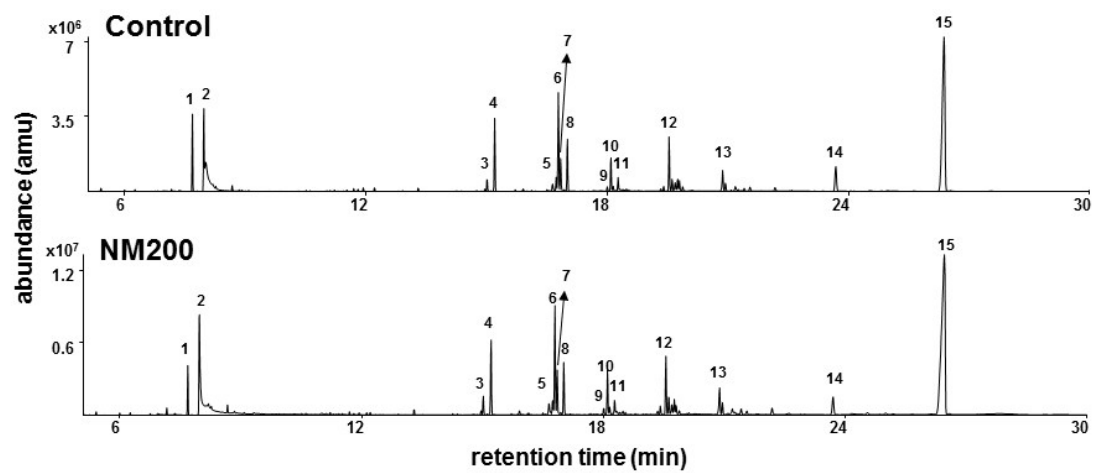




**Figure S3:** Hierarchical cluster analysis of genes with more than 1.5-fold change in expression in aSiNP-116 exposed cells compared to untreated control cells. The bar relates the color code to the expression value.

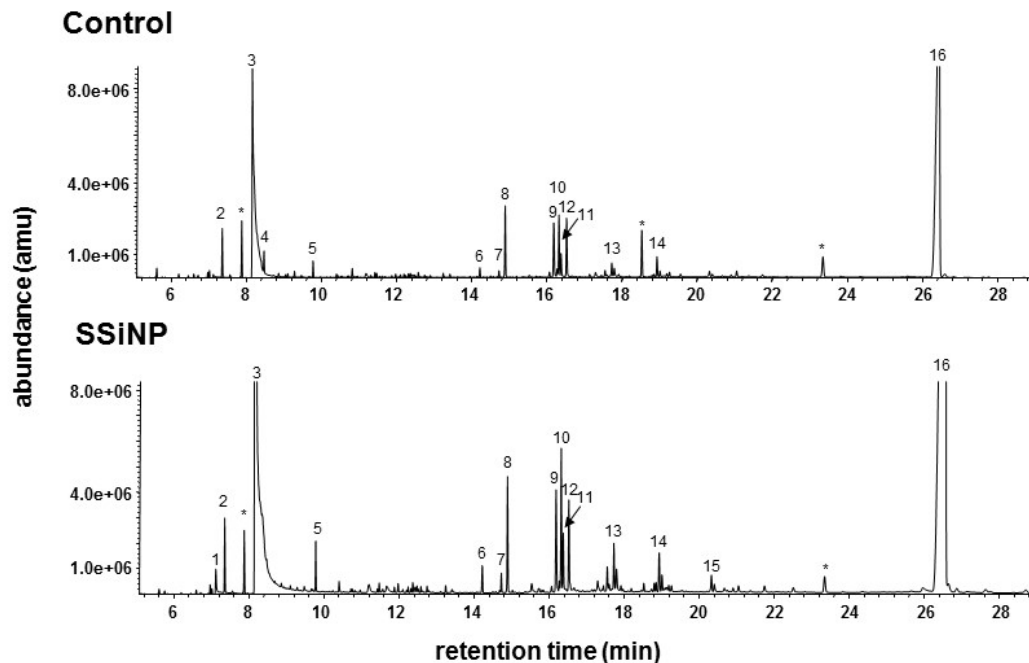


**Figure S4:** Network analysis by pathway studio of aSiNP-116 mediated biological response in HepG2 cells.



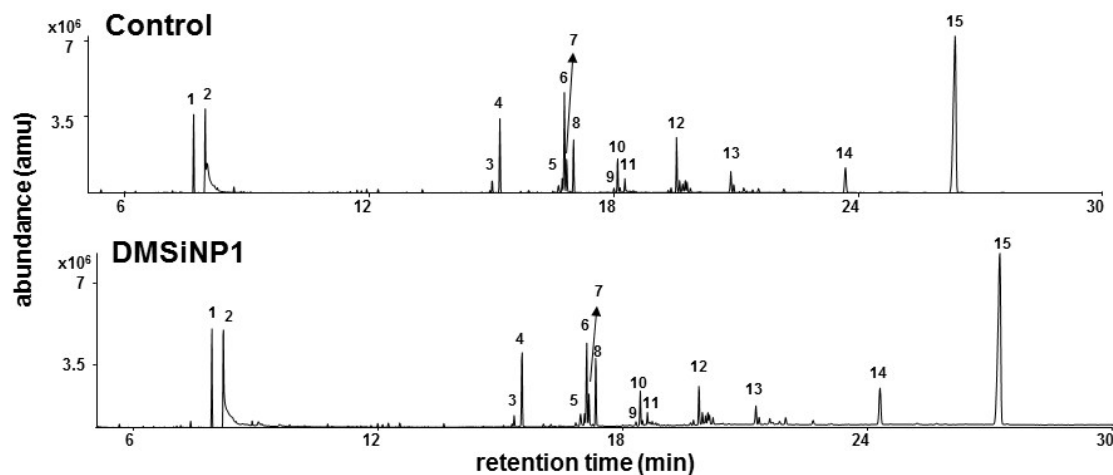
1. Diethylene glycol; 2. Silanol; 3. palmitic acid; 4. palmitoleic acid; 5. oleic acid (isomer); 6. oleic acid; 7. cis-octadecanoic acid; 8. stearic acid; 9. myristic acid; 10. arachidonic acid; 11. eicosatrienoic acid; 12. monopalmitin; 13. 1-monooleoylglycerol; 14. cholesta-3,5-diene; 15. cholesterol

**Figure S5A**



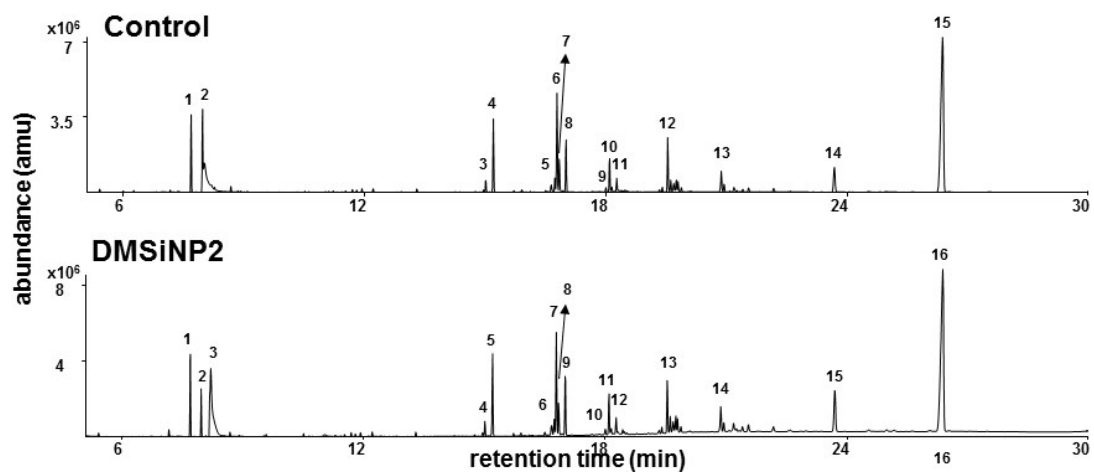
1. monomethylphosphate; 2. malic acid; 3. phosphoric acid; 4. proline; 5. *O*-hydroxybenzaldehyde; 6. dibutylphthalate; 7. palmitoleic acid; 8. palmitic acid; 9. unknown; 10. oleic acid; 11. oleic acid (isomer); 12. stearic acid; 13. di(2-ethylhexyl) adipate; 14. monopalmitin; 15. monostearin; 16. cholesterol; \* byproducts of TMS

Figure S5B



1. Diethylene glycol; 2. Silanol; 3. palmitic acid; 4. palmitoleic acid; 5. oleic acid (isomer); 6. oleic acid; 7. *cis*-octadecanoic acid; 8. stearic acid; 9. myristic acid; 10. arachidonic acid; 11. eicosatrienoic acid; 12. monopalmitin; 13. 1-monooleoylglycerol; 14. cholesta-3,5-diene; 15. cholesterol

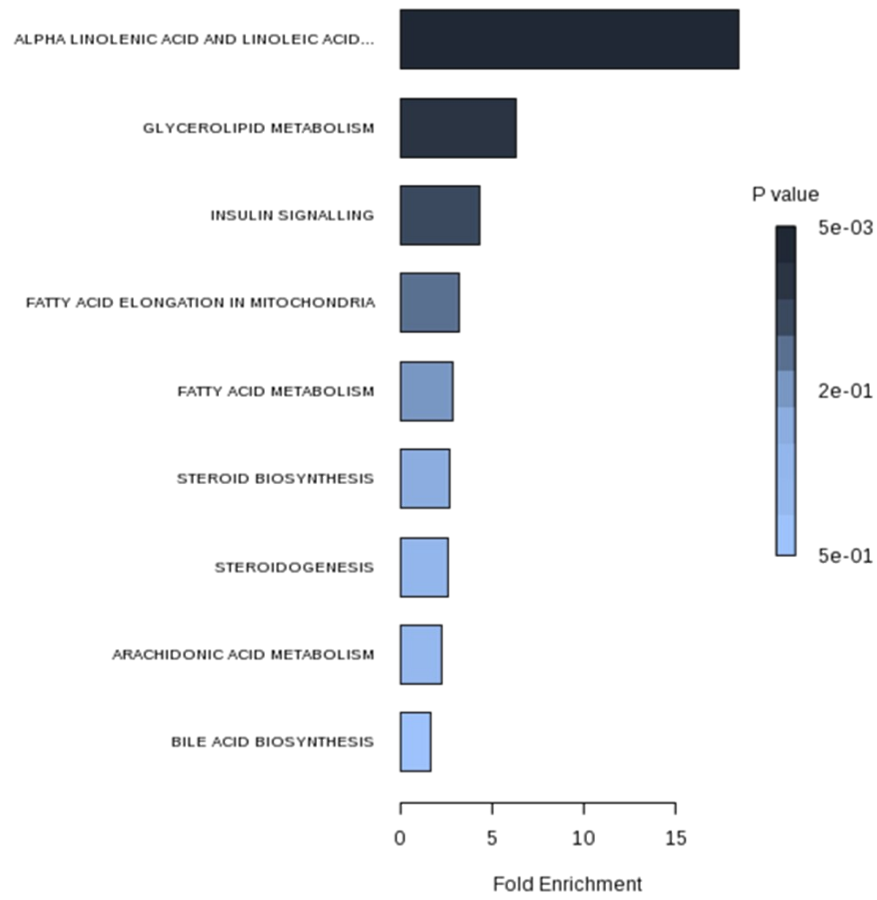
Figure S5C



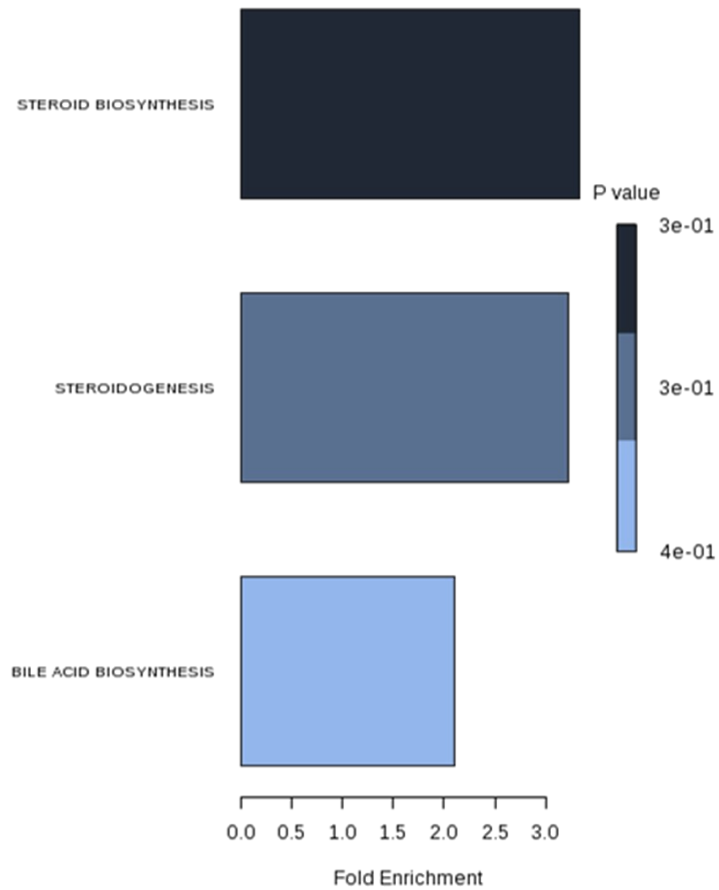
1. Diethylene glycol; 2. glycerol; 3. Silanol; 4. palmitic acid; 5. palmitoleic acid; 6. oleic acid (isomer); 7. oleic acid; 8. cis-octadecanoic acid; 9. stearic acid; 10. myristic acid; 11. arachidonic acid; 12. eicosatrienoic acid; 13. monopalmitin; 14. 1-monooleoylglycerol; 15. cholesta-3,5-diene; 16. cholesterol

## Figure S5D

**Figure S5:** GC-MS spectra of fatty acid and lipid metabolites from HepG2 cells treated (100 mg/L for 24 h) and untreated (control) with aSiNPs. **A.** GC-MS spectra of aSiNP-189. **B.** GC-MS spectra of aSiNP-116. **C.** GC-MS spectra of aSiNP-26. **D.** GC-MS spectra of aSiNP-8.



**Figure S6A**



**Figure S6B**

**Figure S6: Pathway enrichment analysis** performed by Metaboanalyst 3.0 **based on** GC-MS (>1.5 folds). **A.** altered metabolites due to aSiNP-189 exposure (100 mg/L) for 24 h. **B.** Altered metabolites due to aSiNP-116 exposure (100 mg/L). No pathways were found for aSiNP-26 and aSiNP-8.

