Association Rule Mining of Cellular Responses induced by Metal and Metal Oxide Nanoparticles

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Supporting Information

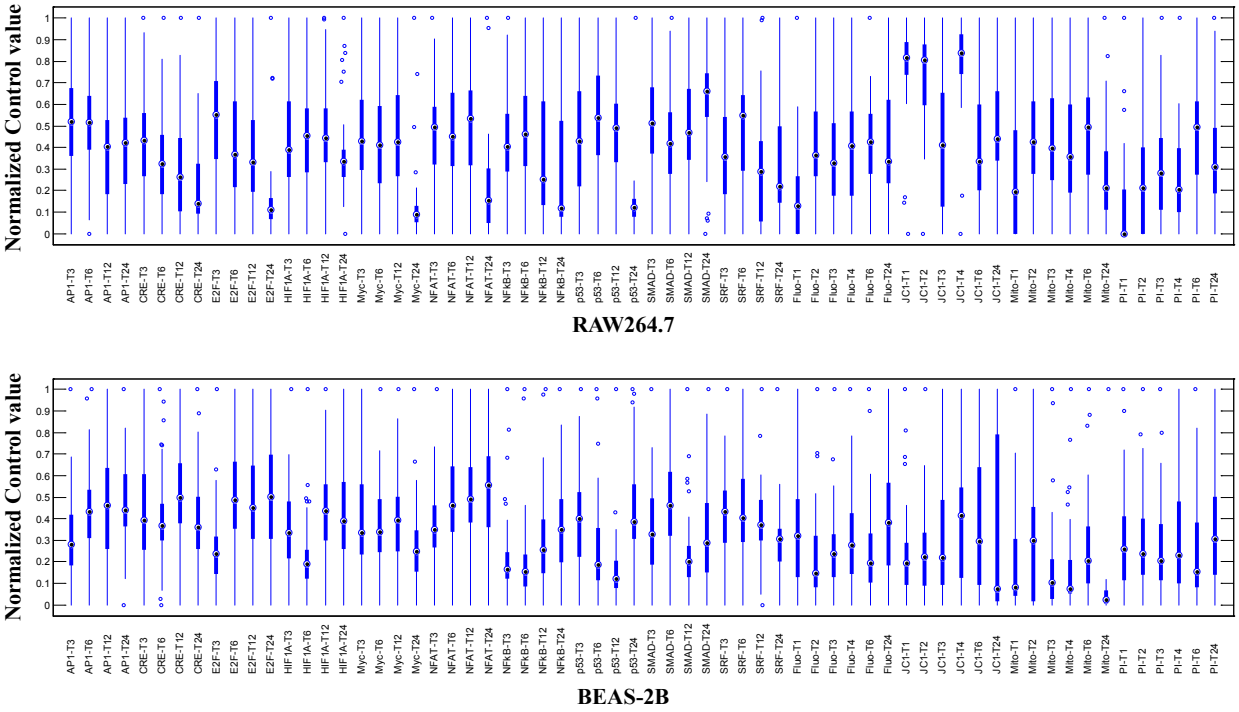

Figure S1. Distribution of the control values of each HTS plate (the control values of each HTS plate were normalized over [0, 1] so that they can be displayed in the same plot). The exposure time is identified by the "-Tx" appended to a cellular response. The upper and lower boundaries of a solid bar identify the first quartile (Q1) and the third quartile (Q3) of the data, the symbol "ϵ" inside the solid bar represents the median of the data; ends of the whiskers are the smallest and largest observations within the range defined by [Q1-1.5(Q3-Q1), Q3+1.5(Q3-Q1)]. Control values outside the range are considered as outliers (marked by "○") since they are abnormally deviated from the majorities of control populations. It is noted that only a small percent of outliers were identified in control data (1.5% and 2.8% for RAW264.7 and BEAS-2B cell lines, respectively) via the box-plots.

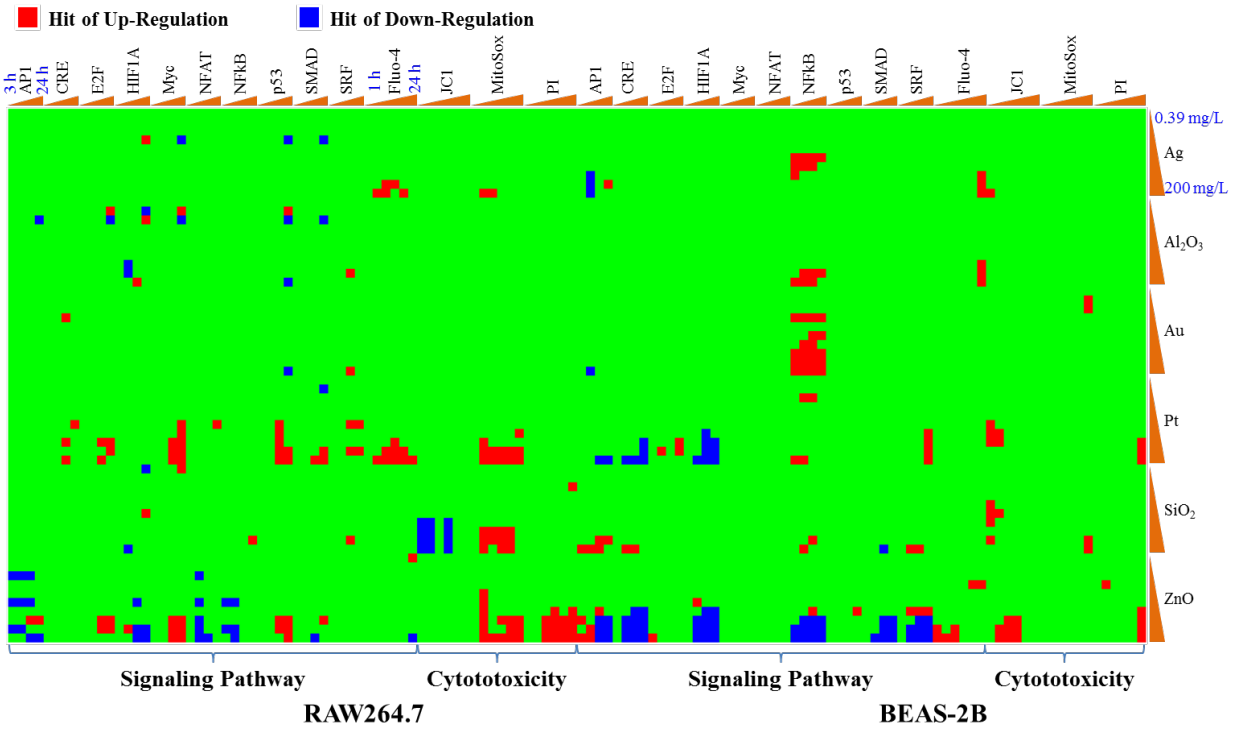

Figure S2. Identified significant cellular responses (nanoparticle concentration: 0.39-200 mg/L, exposure period: 3-24 h or 1-24 h (denoted by the wedges)).

Table S1. Summary of the ten luciferase-reporter based and the four fluorescence-based cell response assays

TF	Pathway	Significance
CRE	cAMP/PKA	cAMP response element is a point of convergence for many extracellular and intracellular signals, including cAMP, calcium, G-protein coupled receptors (GPCR) and neurotrophins
E2F/ DP1	Cell Cycle	Regulator of cell-cycle checkpoints in mammalian cells-major target of the retinoblastoma gene product (Rb) and the activity of E2F/pRb is intimately connected with the G1-S transition of the cell cycle
Myc/ Max	c-myc	Transcription factor that heterodimerizes with an obligatory partner, Max, and regulates the transcription of genes important for cell proliferation, differentiation, and apoptosis.
NFkB	NFkB	Nuclear factor-kappaB plays a key role in inflammation, immune response, cell proliferation and protection against apoptosis
SMAD2/ SMAD3/ SMAD4	TGF-beta	Transforming growth factor β (TGFβ) signaling pathway is involved in many cellular processes, including cell cycle arrest, differentiation, homeostasis, and immunosuppression. TGFβ signaling induces phosphorylation and activation of the SMAD2 and SMAD3 proteins, which then form complexes with the mediator SMAD4. These SMAD complexes then translocate to the nucleus, where they activate the expression of TGFβ-responsive genes
p53	p53/DNA Damage	Role in DNA repair, cell cycle arrest, and apoptosis.
Elk-1/ SRF	MAPK/ERK	TCR and Elk-1, form a complex with the SRF over the serum response element (SRE), and activate gene expression. The Elk-1 protein is phosphorylated by mitogen-activated protein kinase (MAPK), causing increased DNA binding, ternary complex formation, and transcriptional activation of target genes
HIF1A	Hypoxia	Hypoxia-inducible factor-1 protein is a key regulator of oxygen homeostasis and plays significant roles in cancer progression as well as in cardiovascular diseases
AP1	MAPK/JNK	Activator protein-1 (AP1) transcription factor is a hetero- or homo-dimeric complex that comprises members of the proto-oncogene Jun protein family (c-Jun, JunB and JunD) and Fos protein family (c-Fos, Fos B, Fra-1 and Fra-2). The stress-activated protein kinase/Jun N-terminal kinase (SAPK/JNK) signal transduction pathway is responsible for the phosphorylation and activation of Jun, which in turn activates AP1
NFAT	PKC/Ca++	NFAT family of transcription factors plays a role in the transcriptional regulation of cytokine genes and other genes critical for the immune response. Several pathways are associated with activation of the NFAT enhancer element, including calcineurin and protein kinase C
Probe	**pathway**	**Significance**
Fluo-4	Intracellular calcium	Detect intracellular calcium influx. Calcium ions in cellular cytoplasm are detected by increased fluorescence of Fluo-4.
JC1	Mitochondrial membrane potential	Detect the level of mitochondria membrane depolarization.
MitoSox	Mitochondrial superoxide generation	Detect the superoxide radicals in mitochondria.
PI	Cell membrane	Propidium Iodide (PI) Detects the cell membrane integrity.