Electronic Supplementary Material (ESI) for Toxicology Research.

Supplementary data

1 Particle preparation and characterization

1.1 SWCNT preparation

The purchased SWCNTs (P2-SWNTs, Carbon Solutions, Inc. CA, USA) have a poor dispersibility and colloidal stability in aqueous medium. To make well-dispersed SWCNTs in aqueous medium with a proper colloidal stability for the duration of the experiments, the purchased SWCNTs were accurately weighted and suspended in dimethyl sulfoxide (DMSO) to 0.125 mg/mL, followed by ultrasonication for 30 minutes in a water bath sonicator (B3510, Branson Ultrasonics, 40KHz). In the last minute of ultrasonication, the SWCNTs-DMSO suspension was rapidly diluted 5 times by injecting a stabilization buffer (5 mg/mL BSA and 10 mM NaCl in MilliQ water). The mixture, referred to as as-dispersed SWCNTs (AD-SWCNTs), resulted in a clear dark-brown suspension. No sedimentation of AD-SWCNTs was observed for weeks at room temperature indicating a good colloidal stability. To remove the majority of DMSO and free BSA, AD-SWCNTs were pelleted by centrifugation at 16,000 g, 4 °C for 30 min followed by three times wash with 10 mM NaCl in MilliQ water under the same conditions. The colloidal stability and surface charge of SWCNTs at each step were monitored by dynamic light scattering analysis (DLS) (see below). The depletion of DMSO and BSA was monitored by UV-Visible absorption analysis (see below). The SWCNTs aggregates and metallic impurities were characterized by TEM and TEM-EDX (see below). After the washing steps, SWCNTs were re-dispersed in MilliQ water with 10 mM NaCl to about 0.4 mg/mL, which was used in the experiments and here referred to as prepared SWCNTs. The accurate concentration of prepared SWCNTs was determined by near-infrared (NIR) spectrometry, where the standard curve (see below) was made from a set of AD-SWCNTs with known concentrations.

1.2 SWCNT characterization – methods, results and discussion

1.2.1 TEM and TEM-EDX

As-received SWCNTs were dispersed in MilliQ water to about 0.1 mg/mL by ultrasonication in a water bath sonicator (B3510, Branson Ultrasonics, 40KHz). After sonication 20 µL suspension were dropped onto a grid (200 mesh copper grid with formvar/carbon support film, TED PELLA, Inc.) and left to dry at room temperature. The sample was observed by TEM (CM20 PHILIPS, LaB6 filament) at 200 keV. The images were captured by a CCD camera. EDX spectrum was collected at the area with visible metallic impurities by TEM at 200 keV and analyzed with EDAX GENESIS software (EDAX Inc. USA). For prepared SWCNTs as used in the experiments, a proper amount of the stock suspension was diluted in MilliQ water to 0.1 mg/mL and 20 µL of the suspension was dropped onto a grid. The grid was left to dry and observed by TEM as describe above.

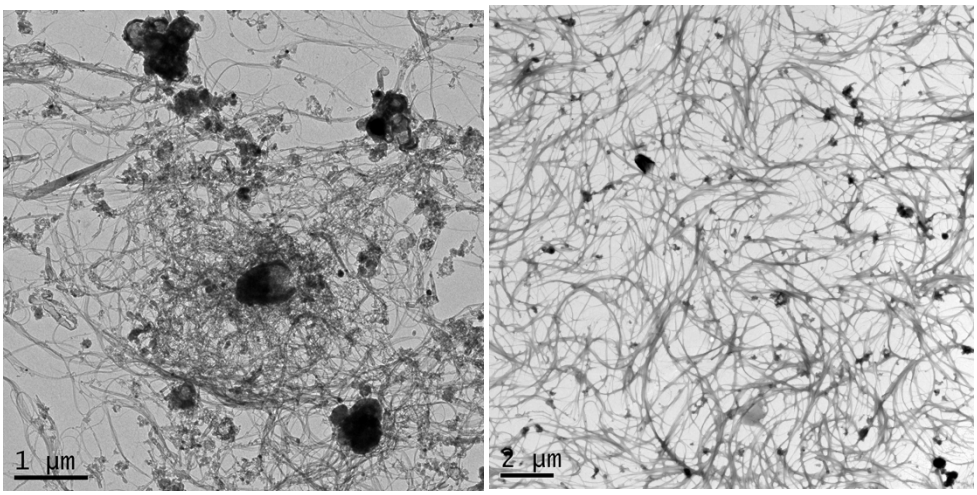

Figure S1: *TEM images of as-received SWCNTs (left) and prepared SWCNTs (right). The comparison of TEM images of as-received and prepared SWCNTs shows a significant improvement on the dispersibility of prepared SWCNTs in aqueous medium.*

1.2.2 SEM

Prepared SWCNTs were diluted to 10 µg/mL in MilliQ water and dropped on a 5 x 5 mm^2 silicon wafer to form a thin layer of water. After drying at room temperature, the sample was observed with FEI Magellan XHR SEM in high vacuum mode at 20 keV. Images were captured by a CCD camera. The length and diameter of 49 representative SWCNTs were measured by ImageJ 1.47.

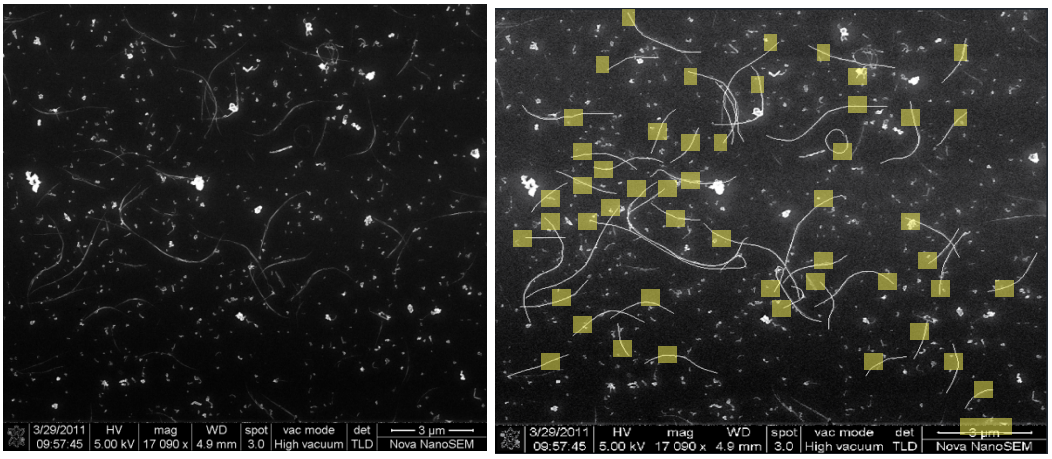

Figure S2: *SEM image of prepared SWNTs. The length and diameter distributions were measured for 49 representative bundles and are 2.97 ± 1.30 µm and 31.79 ± 8.66 nm in average, respectively.*

1.2.3 DLS

SWCNTs samples were diluted in MilliQ water or cell culture medium to 100 µg/mL and incubated for different time periods (up to 25 hours). The SWCNTs were measured by DLS (Zetasizer Nano, Malvern) with 173° backscatter angle, utilizing the high-resolution model, while zeta potentials were measured with the smoluchwski model (F (Ka) = 1.5).

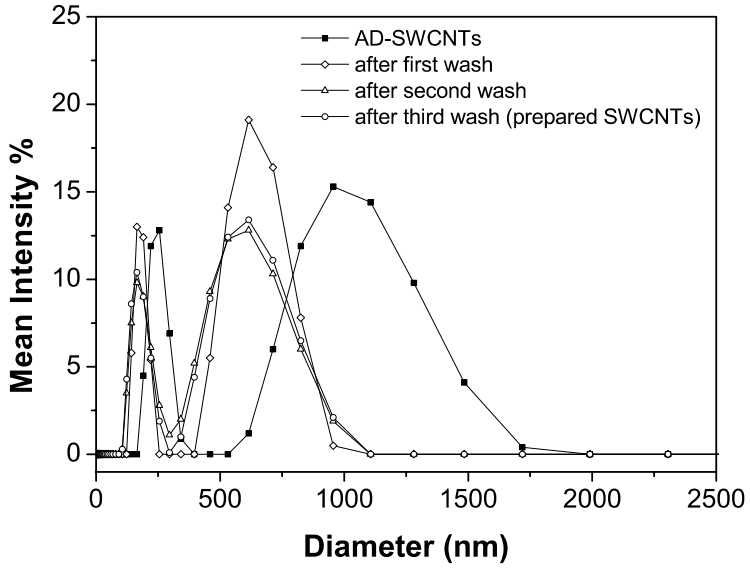

Figure S3: *Hydrodynamic size distribution of SWCNTs at different preparation steps, measured by DLS. A significant shift after first wash and a minor shift after second wash of "diameter" towards smaller size indicate a possible removal of surrounding BSA on SWCNTs. The lack of significant size change observed between second wash and prepared SWCNTs indicates a good re-dispersibility of prepared SWCNTs. AD-SWCNTs - as-dispersed SWCNTs (see supplementary data chapter 1.1)*

Table S1: *Zeta potential of SWCNTs at different preparation steps and in cell culture medium, measured by DLS. Zeta deviations were generated from the fitting of the model.*

	AD-SWCNTs	First wash	Second wash	Prepared SWCNTs	In medium
Zeta potential	-19.6	-46.6	-54.3	-50.3	-14.4
± Deviation (mV)	± 3.7	± 5.3	± 5.1	± 5.2	± 0.0

The zeta potential changes of SWCNTs at different steps are matching the changes of size distribution indicating removal of extra BSA from the SWCNTs surface. In cell culture medium the surface charge of SWCNTs dropped to -14.4 mV, indicating adsorption of protein onto the SWCNT surface.

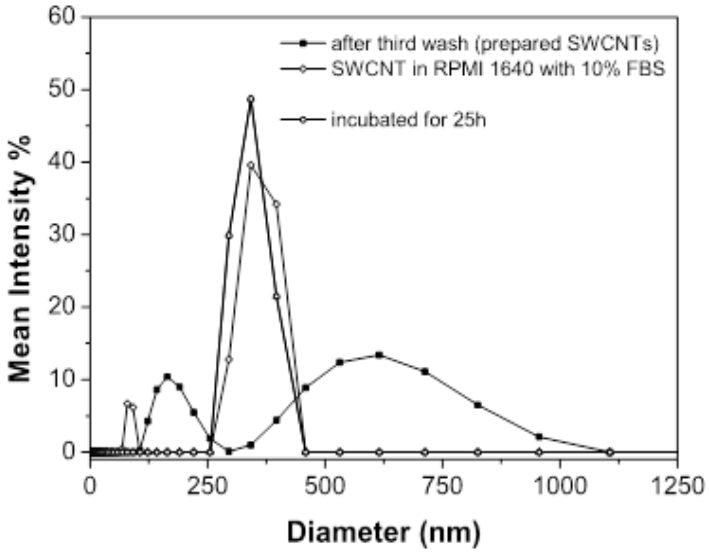

Figure S4: *Hydrodynamic size distribution of SWCNTs after incubation in cell culture medium. Only a slight increase in size was observed after 25h incubation.*

The size distribution readout from DLS does not necessarily reflect the hydrodynamic size of SWCNTs in solution due to the string-like shape of SWCNTs. However, the alteration in size profile of SWCNTs at different conditions indicates the stability of SWCNTs in aqueous mediums. As shown in Figure S4, only a slight increase in size was observed after 25h incubation, even though the surface charge of SWCNTs is low (-14.4 mV), indicating a good colloidal stability of prepared SWCNTs in cell culture medium.

1.2.4 UV-Vis-NIR spectrum analysis

The absorption spectrum of SWCNTs from 200 nm to 1200 nm wavelength was measured with a UV-Vis-NIR spectrophotometer (UV-3600, Shimadzu), and analyzed by UVprobe 2.21 software (Shimadzu). To generate a standard curve for the quantification, AD-SWCNTs was diluted in stabilization buffer to different concentrations ranging from 2.5 μg/mL to 25 μg/mL The absorption spectra from 200 nm to 1200 nm wavelength were

measured using stabilization buffer as reference. The intensity of absorption peak (E_{11}(7,5)) at 1048 nm wavelength was chosen to calculate the extinction coefficient of SWCNTs according to Beer-Lambert law using linear regression.

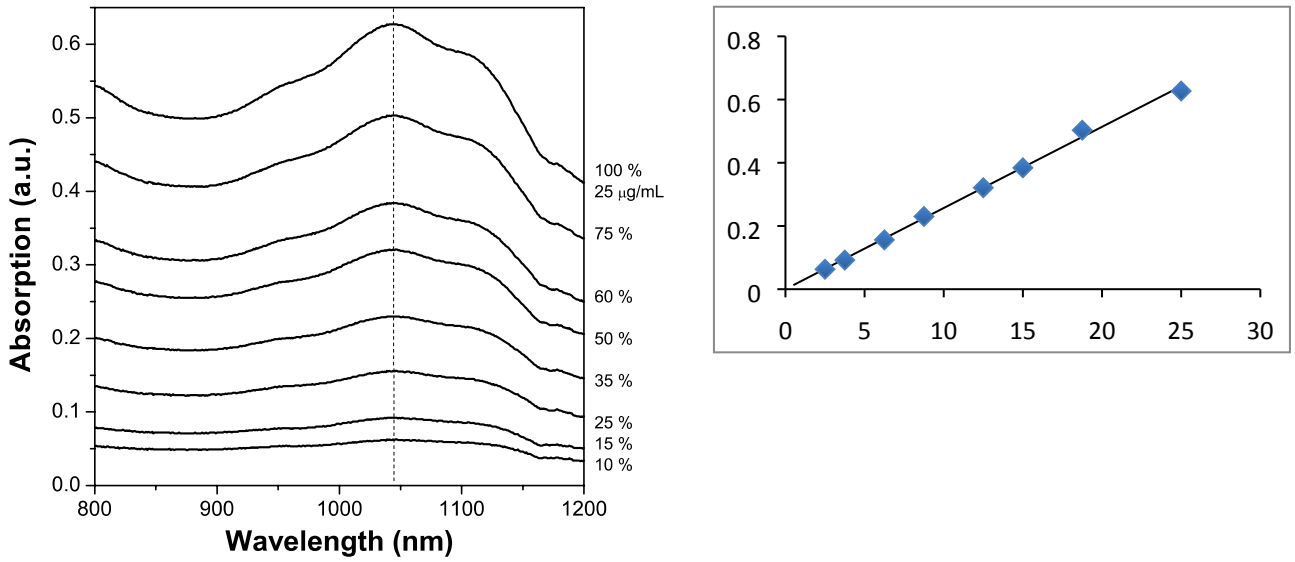

Figure S5: *Standard curve of AD-SWCNTs and linear regression of absorption peak intensity at 1048 nm. The extinction coefficient of SWCNTs was calculated as 25.7 $mg^{-1}cm^2$ applying Beer-Lambert law.*

2 Tables S2 – S4: Microarray results

Table S2: *Complete list of all differentially regulated genes in A549 cells after 24 hours exposure to 10µg/ml SWCNTs*

Gene symbol	Fold change SWCNT vs Control	Transcripts Cluster Id	Genbank #	Gene description
ADM2	3,29	8074063	AK090635	adrenomedullin 2
ALDH1L2	2,47	7965979	BC103934	aldehyde dehydrogenase 1 family, member L2
ANK2	3,99	8096959	BX537758	ankyrin 2, neuronal
ANO1	0,49	7942135	AY728143	anoctamin 1, calcium activated chloride channel
APOH	0,37	8017766	BC026283	apolipoprotein H (beta-2-glycoprotein I)
AQP3	0,40	8160670	AK095363	aquaporin 3 (Gill blood group)
ARRDC4	3,69	7986350	BC028704	arrestin domain containing 4
BAAT	0,43	8162870	BC107424	bile acid CoA: amino acid N-acyltransferase (glycine N-choloyltransferase)
C12orf36	0,23	7961413	BC101220	chromosome 12 open reading frame 36
C12orf39	2,93	7954398	AK075342	chromosome 12 open reading frame 39
C6orf48	2,74	8178090	BC000706|BT007334|BC017660	chromosome 6 open reading frame 48
C6orf48	2,74	8179326	BC000706|BT007334|BC017660	chromosome 6 open reading frame 48
CAPN5	2,21	7942697	AK128124	calpain 5
CCL2	0,37	8006433	BC009716	chemokine (C-C motif) ligand 2
CD38	0,37	8094240	D84276	
CDH6	0,36	8104663	D31784	cadherin 6, type 2, K-cadherin (fetal kidney)
CHAC1	2,76	7982868	BC019625	ChaC, cation transport regulator homolog 1 (E. coli)
CLDN2|RIPPLY1	0,41	8169210	BC071747|BC105692	claudin 2 | ripply1 homolog (zebrafish)
CRISPLD2	0,37	7997642	AY358790	cysteine-rich secretory protein LCCL domain containing 2
CYP1B1	0,35	8051583	U03688	cytochrome P450, family 1, subfamily B, polypeptide 1
DDIT3	2,88	7964460	BC003637	DNA-damage-inducible transcript 3
DLEU2	0,41	7971653	AF264787	deleted in lymphocytic leukemia 2 (non-protein coding)

ECM2	2,84	8162404	BX537976	extracellular matrix protein 2, female organ and adipocyte specific
FAM111B	0,43	7940147	AY457926	family with sequence similarity 111, member B
FAM129A	8,91	7922846	AF288391	family with sequence similarity 129, member A
FGFBP1	0,33	8099467	BC008910	fibroblast growth factor binding protein 1
GDA	0,23	8155802	AF019638	guanine deaminase
GTPBP2	2,22	8126629	BC064968|AK290267|AB024574|AJ420518|BC032315	GTP binding protein 2
HAS3|CHTF8	0,45	7996883	BC021853|AF232772|AF234839|AK291400	hyaluronan synthase 3 | CTF8, chromosome transmission fidelity factor 8 homolog (S. cerevisiae)
HIST1H2BJ	0,39	8124484		histone cluster 1, H2bj
HMGCS1	0,34	8111941	BC083514	3-hydroxy-3-methylglutaryl-CoA synthase 1 (soluble)
IL11	2,35	8039484	BC012506	interleukin 11
INSIG1	0,34	8137526	BC001880	insulin induced gene 1
IQGAP2	0,40	8106354	AK291066	IQ motif containing GTPase activating protein 2
KCNG1	2,35	8067029	BC046629|AK290689|AF033383|BC006367|AK128721	potassium voltage-gated channel, subfamily G, member 1
KCNG1	2,61	8067033	BC006367|BC046629|AK290689|AF033383	potassium voltage-gated channel, subfamily G, member 1
KRT4	0,47	7963534	AK056254	keratin 4
LOH3CR2A	0,40	8077499	AF086709|AK054898	loss of heterozygosity, 3, chromosomal region 2, gene A
LY6K	0,42	8148553	AJ001348	lymphocyte antigen 6 complex, locus K
MAFK|TMEM184A	2,33	8131091	AK092414	v-maf musculoaponeurotic fibrosarcoma oncogene homolog K (avian) transmembrane protein 184A
MGAM	0,13	8136662	AF016833	maltase-glucoamylase (alpha-glucosidase)
MMP7	0,36	7951217	BC003635	matrix metallopeptidase 7 (matrilysin, uterine)
MRAP2	0,43	8120961	BC010003	melanocortin 2 receptor accessory protein 2
OLFML3	0,31	7904158	AY358954	olfactomedin-like 3
PCK2	2,30	7973530	BC001454	phosphoenolpyruvate carboxykinase 2 (mitochondrial)
PRDM1	2,80	8121257	AY198414	PR domain containing 1, with ZNF domain
S100P	2,36	8093950	AF539739	S100 calcium binding protein P
SESN2	3,44	7899436	AY123223	sestrin 2
SFRP4	0,35	8139087	AF026692	secreted frizzled-related protein 4
SLFN5	3,45	8006531	AK303299	schlafen family member 5
SMOC1	3,18	7975390	AK289988	SPARC related modular calcium binding 1
SMOX	2,35	8060745	EF032141	spermine oxidase
SNTB1	3,18	8152606	AK292655	syntrophin, beta 1 (dystrophin-associated protein A1, 59kDa, basic component 1)
SPANXB1|SPANXB2|SPANXF1	0,46	8170241	AF098307|BC054023	SPANX family, member B1 | SPANX family, member B2 |

				SPANX family, member F1
SPANXB1|SPANXB2|SPANXF1	0,46	8170244	AF098307|BC054023	SPANX family, member B1 | SPANX family, member B2 | SPANX family, member F1
TAGLN|PCSK7	2,22	7944082	BC004927	transgelin | proprotein convertase subtilisin/kexin type 7
TIPARP	0,47	8083569	BC050350	TCDD-inducible poly(ADP-ribose) polymerase
TPPP3	0,33	8002020	BC000691	tubulin polymerization-promoting protein family member 3
TRIB3	3,56	8060344	AY247738	tribbles homolog 3 (Drosophila)
TXNIP	6,57	7904726	S73591	thioredoxin interacting protein
ULBP1	2,46	8122724	BC035416	UL16 binding protein 1
VSIG1	0,36	8169263	BC043216	V-set and immunoglobulin domain containing 1
ZHX2	2,18	8148149	BC042145	zinc fingers and homeoboxes 2

Table S3: *Complete list of all differentially regulated genes in monocyte derived THP-1 macrophages after 24 hours exposure to 10µg/ml SWCNTs*

Gene symbol	Fold change SWCNT vs Control	**Transcripts Cluster Id**	**Genbank #**	**Gene description**
AMPD3	2,56	7938396	BC126118	adenosine monophosphate deaminase 3
AQP9	2,27	7983910	AF016495	aquaporin 9
BCL3	2,37	8029465	BC064993	B-cell CLL/lymphoma 3
BIRC3	5,17	7943413	AF070674	baculoviral IAP repeat-containing 3
CAV1	2,16	8135594	AK290871	caveolin 1, caveolae protein, 22kDa
CCL20	3,79	8048864	BC020698	chemokine (C-C motif) ligand 20
CCL3	2,82	8014369	M23452	chemokine (C-C motif) ligand 3
CCL3L1|CCL3L3	2,82	8014391	BC007783|D00044|X52149	chemokine (C-C motif) ligand 3-like 1 | chemokine (C-C motif) ligand 3-like 3 | chemokine (C-C motif) ligand 3
CCL3L1|CCL3L3	2,82	8014414	BC007783|D00044|X52149	chemokine (C-C motif) ligand 3-like 1 | chemokine (C-C motif) ligand 3-like 3 | chemokine (C-C motif) ligand 3
CCL3L1|CCL3L3	2,82	8019731	BC007783|D00044|X52149	chemokine (C-C motif) ligand 3-like 1 | chemokine (C-C motif) ligand 3-like 3 | chemokine (C-C motif) ligand 3
CCL4	3,95	8006602	AY766446	chemokine (C-C motif) ligand 4
CCL4L1|CCL4L2	9,59	8006608	AY766452|AY766446	chemokine (C-C motif) ligand 4-like 1 | chemokine (C-C motif) ligand 4-like 2 | chemokine (C-C motif) ligand 4
CCL4L1|CCL4L2	9,60	8006621	AY766447|AY766446	chemokine (C-C motif) ligand 4-like 1 | chemokine (C-C motif) ligand 4-like 2 | chemokine (C-C motif) ligand 4

CCL4L1|CCL4L2	9,60	8019651	AY766447|AY766446	chemokine (C-C motif) ligand 4-like 1 | chemokine (C-C motif) ligand 4-like 2 | chemokine (C-C motif) ligand 4
CD274	2,85	8154233	AF233516	
CD48	2,77	7921667	BC016182	
CD80	3,67	8089771	BC042665	
CHAC1	3,01	7982868	BC019625	ChaC, cation transport regulator homolog 1 (E. coli)
CHST2	3,56	8083214	AB021124	carbohydrate (N-acetylglucosamine-6-O) sulfotransferase 2
CXCL1	4,50	8095697	BC011976|M57731	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
CXCL10	6,21	8101126	BC010954	chemokine (C-X-C motif) ligand 10
CXCL11	2,90	8101131	BC012532	chemokine (C-X-C motif) ligand 11
CXCL12	3,08	7933194	L36033	chemokine (C-X-C motif) ligand 12
DLL4	2,83	7982854	AB036931	delta-like 4 (Drosophila)
EBI3	3,91	8024792	L08187	Epstein-Barr virus induced 3
FABP6	2,46	8109629	BC022489	fatty acid binding protein 6, ileal
FEZ1	2,48	7952490	BC009545	fasciculation and elongation protein zeta 1 (zygin I)
GCH1	2,42	7979269	U66097	GTP cyclohydrolase 1
GJB2	3,82	7970441	M86849	gap junction protein, beta 2, 26kDa
HLA-DQA1	2,18	8118556	AB209628	major histocompatibility complex, class II, DQ alpha 1
HLA-DQA2|HLA-DQA1	2,16	8118564		major histocompatibility complex, class II, DQ alpha 2 | major histocompatibility complex, class II, DQ alpha 1
HLA-DQA2|HLA-DQA1	2,16	8178205		major histocompatibility complex, class II, DQ alpha 2 | major histocompatibility complex, class II, DQ alpha 1
HP	0,41	7997188	BC121125|K00422|L29394|BC107587|BC121124|BC017862|BC058031|BC070299|AK055872|CR616676	haptoglobin
HPR	0,37	7997192		haptoglobin-related protein
IL1B	2,46	8054722	BC008678	interleukin 1, beta
IL21R	2,19	7994292	BC004348	interleukin 21 receptor
IL32	5,38	7992828	BC009401	interleukin 32
IL4I1|NUP62	2,05	8038487	DQ079587|AK125857	interleukin 4 induced 1 | nucleoporin 62kDa
IL8	3,82	8095680	M17017	interleukin 8
JAM2	2,29	8068024	AY016009	junctional adhesion molecule 2
NFKBIA	2,36	7978644	BC002601	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
PDCD1LG2	2,15	8154245	AF344424	programmed cell death 1 ligand 2
RAB44	0,46	8119096	AK125083	
RELB	2,57	8029580	BC028013	v-rel reticuloendotheliosis viral oncogene homolog B

RNF144B	3,06	8117106	AB076367	ring finger protein 144B
RTN1	2,24	7979455	L10333	reticulon 1
SEMA3A	2,53	8140668	BC111416	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A
SERPINB10	0,41	8021645	BC096217	serpin peptidase inhibitor, clade B (ovalbumin), member 10
SLC2A6	3,00	8164918	BC013740|AK294974|AK096630|AB209108	solute carrier family 2 (facilitated glucose transporter), member 6
SOD2	3,65	8130556	BC012423	superoxide dismutase 2, mitochondrial
SULF2	0,44	8066822	AB033073	sulfatase 2
TNFAIP3	2,62	8122265	M59465	tumor necrosis factor, alpha-induced protein 3
TNFAIP6	18,39	8045688	BC030205	tumor necrosis factor, alpha-induced protein 6
TNFRSF9	3,03	7912145	L12964	tumor necrosis factor receptor superfamily, member 9
VCAM1	2,97	7903358	BC017276	vascular cell adhesion molecule 1
VIT	0,42	8041467	AY358338	vitrin
ZC3H12A	2,16	7900146	BC005001	zinc finger CCCH-type containing 12A

Table S4: *Complete list of all differentially regulated genes in J774 macrophages after 24 hours exposure to 10µg/ml SWCNTs*

Gene symbol	Fold change SWCNT vs Control	Transcripts Cluster Id	Genbank #	gene description
Abi3|Phospho1	0,36	10390186	BC058260	ABI gene family, member 3 | phosphatase, orphan 1
Acap1	0,22	10387699	BC031462	ArfGAP with coiled-coil, ankyrin repeat and PH domains 1
Acy1	0,35	10596465	BC005631	aminoacylase 1
Acyp2	2,29	10384936	BC027642	acylphosphatase 2, muscle type
Adamts7	2,78	10587748	BC141173|BC145491|AY551090|AK052239	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 7
Adap1	0,40	10535065	BC144874	ArfGAP with dual PH domains 1
Akna	0,42	10513666	BC092227	AT-hook transcription factor
Aldh3b1	0,47	10464560	BC046597	aldehyde dehydrogenase 3 family, member B1
Alox5	0,14	10547153	BC139102	arachidonate 5-lipoxygenase
Ambp	0,38	10513630	BC021660	alpha 1 microglobulin/bikunin
Arhgap30	0,48	10351603	BC082573	Rho GTPase activating protein 30

Arhgap4	0,31	10605143	AF443826	Rho GTPase activating protein 4
Arhgef1	0,46	10551030	BC012488	Rho guanine nucleotide exchange factor (GEF) 1
Asb2	0,23	10402325	BC031161	ankyrin repeat and SOCS box-containing 2
Atp10d	0,44	10522335	BC141328	ATPase, class V, type 10D
Atp2a3	0,33	10378216	BC017639	ATPase, Ca++ transporting, ubiquitous
Bank1	0,29	10502335	AY178734	B-cell scaffold protein with ankyrin repeats 1
BC005685	0,45	10401937	AF263910|BC052501|AF043688|M11024	
Bex6	0,24	10435009	AY833559	brain expressed gene 6
Bin2	0,25	10432640	BC127021	bridging integrator 2
C130050O18Rik	0,30	10526947	BC100320	RIKEN cDNA C130050O18 gene
C1qa	0,36	10517517	BC002086	complement component 1, q subcomponent, alpha polypeptide
C1s|Gm5077	2,49	10547740	AF459019	complement component 1, s subcomponent | predicted gene 5077
Calcrl	0,29	10484371	AB015595	calcitonin receptor-like
Card9	0,26	10480956	BC151023	caspase recruitment domain family, member 9
Ccl5	2,88	10389207	BC033508	chemokine (C-C motif) ligand 5
Ccl8	3,89	10379535	BC117101	chemokine (C-C motif) ligand 8
Cd300ld	0,38	10392808	BC019814	CD300 molecule-like family member d
Cd300lg	0,24	10381514	AB243063	CD300 antigen like family member G
Cd74	0,42	10456005	BC096435	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)
Cfp	0,33	10603860	BC132369	complement factor properdin
Clec12a	0,41	10542164	BC094904	C-type lectin domain family 12, member a
Clec5a	0,42	10544273	BC104364	C-type lectin domain family 5, member a
Coro1a	0,38	10568024	AF143955	coronin, actin binding protein 1A
Coro7	0,44	10437399	BC061006	coronin 7
Cp	2,06	10490989	BC062957	ceruloplasmin
Crct1	2,50	10499952	BC060280	cysteine-rich C-terminal 1
Crisp1	3,62	10450930	BC011150	cysteine-rich secretory protein 1
Cryl1	0,41	10420372	AF351609	crystallin, lambda 1
Cxcl1	3,25	10523151	BC037997|AF352782	chemokine (C-X-C motif) ligand 1
Cxcl11	2,49	10531420	AF179872	chemokine (C-X-C motif) ligand 11
Cysltr1	0,46	10606355	BC027102	cysteinyl leukotriene receptor 1
Dgkg	0,43	10438639	BC015278	diacylglycerol kinase, gamma

Dok2	0,20	10416334	AF030627	docking protein 2
Dok3	0,31	10409502	BC116922	docking protein 3
Dpep2	0,36	10581434	AF488552	dipeptidase 2
Endod1	0,44	10590909	BC096490	endonuclease domain containing 1
Enpp2	3,51	10428619	BC058759	ectonucleotide pyrophosphatase/phosphodiesterase 2
F11r	0,43	10351623	BC021876	F11 receptor
F9	0,40	10599826		coagulation factor IX
Fam46c	2,38	10500610	BC046309	family with sequence similarity 46, member C
Fcgr2b	0,43	10360028	M16367	Fc receptor, IgG, low affinity IIb
Fermt3	0,34	10465587	BC066803	fermitin family homolog 3 (Drosophila)
Fmnl1	0,36	10381708	AF006466	formin-like 1
G530011O06Rik	0,37	10607950		RIKEN cDNA G530011O06 gene
Gas7	0,40	10377215	U19860	growth arrest specific 7
Gbp1|Gbp5	2,79	10496555	EF494422|AF422243	guanylate binding protein 1 | guanylate binding protein 5
Gbp2	3,32	10496592	AF077007	guanylate binding protein 2
Gdpd1	3,49	10389590	BC016541	glycerophosphodiester phosphodiesterase domain containing 1
Gm10554	0,45	10453759		predicted gene 10554
Gm10639|Gsta2|Gsta1|Gm3776	4,77	10587339	BC061134|J03958	predicted gene 10639 | glutathione S-transferase, alpha 2 (Yc2) | glutathione S-transferase, alpha 1 (Ya) | predicted gene 3776
Gm885	0,41	10382106	AB542953	predicted gene 885
Gm8995	0,43	10566583	AK172683	predicted gene 8995
Gngt2|Abi3	0,42	10380571	BC061005	guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 2 | ABI gene family, member 3
Gpd1l	0,45	10597477	BC138976	glycerol-3-phosphate dehydrogenase 1-like
Gpnmb	2,69	10538187	BC026375	glycoprotein (transmembrane) nmb
Gpr84	0,32	10433101	BC023249	G protein-coupled receptor 84
Gsta1|Gm10639|Gsta2|Gm3776	4,77	10587331	BC061134|J03958	glutathione S-transferase, alpha 1 (Ya) | predicted gene 10639 | glutathione S-transferase, alpha 2 (Yc2) | predicted gene 3776
Gsta1|Gsta2|Gm10639|Gm3776	4,78	10587323	BC061134|J03958	glutathione S-transferase, alpha 1 (Ya) | glutathione S-transferase, alpha 2 (Yc2) | predicted gene 10639 | predicted gene 3776
Gsta2|Gm10639|Gsta1|Gm3776	4,73	10595148	BC132572|J03958	glutathione S-transferase, alpha 2 (Yc2) | predicted gene 10639 | glutathione S-transferase, alpha 1 (Ya) | predicted gene 3776
Gsta4	2,69	10587315	BC012639	glutathione S-transferase, alpha 4
Gstk1	0,29	10537712	AY279096	glutathione S-transferase kappa 1
H2-DMb2|H2-DMb1	0,38	10444236	U35330|BC052864	histocompatibility 2, class II, locus Mb2 | histocompatibility 2, class II, locus Mb1
H2-M2	2,75	10450880	AY302217	histocompatibility 2, M region locus 2

Hal	2,22	10365769	BC157999	histidine ammonia lyase
Hck	0,36	10477250	BC010478	hemopoietic cell kinase
Hs3st1	0,27	10529732	AF019385	heparan sulfate (glucosamine) 3-O-sulfotransferase 1
Hs3st3b1	0,47	10386951	AF168992	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1
Icosl	0,48	10364361	BC029227	icos ligand
Ifi27l2a	0,47	10402347	BC100588	interferon, alpha-inducible protein 27 like 2A
Ifit1	2,56	10462623	BC003768	interferon-induced protein with tetratricopeptide repeats 1
Ifit3	2,30	10462618	BC089563	interferon-induced protein with tetratricopeptide repeats 3
Ikzf1	0,40	10374333	BC138789|L03547|AY536269|AK220235|AK077815|AK154175|AK040268	IKAROS family zinc finger 1
Inpp5d	0,41	10348244	BC108328	inositol polyphosphate-5-phosphatase D
Irg1	2,34	10416837	L38281	immunoresponsive gene 1
Itga2	2,26	10412267	BC065139	integrin alpha 2
Itga4	0,43	10473125	AF109136	integrin alpha 4
Itga9	0,45	10590031	BC150824	integrin alpha 9
Itgal	0,36	10557591	BC145096	integrin alpha L
Itgam	0,33	10557862		integrin alpha M
Itgb7	2,42	10432957	M68903	integrin beta 7
Lass4	0,39	10569972	BC003946	LAG1 homolog, ceramide synthase 4
Lfng	0,31	10527012	BC115680	LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase
Lifr	0,36	10422822	D26177	leukemia inhibitory factor receptor
Lix1	0,28	10442069	BC052700	limb expression 1 homolog (chicken)
Lmo2	0,38	10474201	BC057880	LIM domain only 2
Lpar5	0,37	10541799	BC117528	lysophosphatidic acid receptor 5
Lpcat2	0,31	10573939	AB244716	lysophosphatidylcholine acyltransferase 2
Lrmp	0,40	10542691	BC052909	lymphoid-restricted membrane protein
Lrrc33	0,27	10439058	BC027411	leucine rich repeat containing 33
Lsp1	0,44	10559207	D49691	lymphocyte specific 1
Ltb	0,40	10444752	U16985	lymphotoxin B
Lyl1	0,38	10573419	BC005736	lymphoblastomic leukemia 1
Map4k1	0,40	10551666	BC005433	mitogen-activated protein kinase kinase kinase kinase 1
Mfng	0,39	10430389	BC010983	MFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase
Mgp	0,37	10548879	BC036991	matrix Gla protein

Mical1	0,34	10362767	BC034682	microtubule associated monoxygenase, calponin and LIM domain containing 1
Mmp10	2,62	10583090	Y13185	matrix metallopeptidase 10
Mmp12	3,50	10583056	BC019135	matrix metallopeptidase 12
Mmp13	2,87	10583044	BC125320	matrix metallopeptidase 13
Mmp14	2,55	10415052	BC076638	matrix metallopeptidase 14 (membrane-inserted)
Mocs1	0,45	10445826	BC027444	molybdenum cofactor synthesis 1
Ms4a6b	0,46	10461622	AF237909	membrane-spanning 4-domains, subfamily A, member 6B
Msn	0,45	10600836	BC047366	moesin
Mycl1	2,61	10507798	BC053059	v-myc myelocytomatosis viral oncogene homolog 1, lung carcinoma derived (avian)
Myo1g	0,40	10384154	BC113755	myosin IG
Nagpa	0,46	10437541	BC039790	N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase
Napsa	0,44	10552697	D88899	napsin A aspartic peptidase
Nbeal2	0,45	10597182	BC157956	neurobeachin-like 2
Ncf2	0,40	10412123	BC076609	neutrophil cytosolic factor 2
Ncf4	0,41	10425053	AB002665	neutrophil cytosolic factor 4
Ndst4	2,16	10495878	AB036838	N-deacetylase/N-sulfotransferase (heparin glucosaminyl) 4
Nefm	0,46	10421100	DQ201636	neurofilament, medium polypeptide
Nfam1	0,27	10430931	BC150488	Nfat activating molecule with ITAM motif 1
Nfatc2	0,39	10489961	EU887584	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2
Nfe2l3	2,32	10538275	AB013852	nuclear factor, erythroid derived 2, like 3
Nipsnap1	0,45	10373950	BC010837	4-nitrophenylphosphatase domain and non-neuronal SNAP25-like protein homolog 1 (C. elegans)
Nlrp3	0,45	10376513	AY495376	NLR family, pyrin domain containing 3
Nov	2,55	10424119	BC003774	nephroblastoma overexpressed gene
Nr0b2	0,27	10508887	BC019540	nuclear receptor subfamily 0, group B, member 2
Nuak2	0,38	10349782	BC033302	NUAK family, SNF1-like kinase, 2
Ogfrl1	0,39	10353524	BC019747	opioid growth factor receptor-like 1
Pag1	0,48	10497237	BC145761	phosphoprotein associated with glycosphingolipid microdomains 1
Parvg	0,34	10425866	BC011200|AF312712	parvin, gamma
Penk	2,85	10511363	M55181	preproenkephalin
Pik3cd	0,40	10518686	BC035203|U86587|CT010349	phosphatidylinositol 3-kinase catalytic delta polypeptide
Pik3cg	0,43	10399924	AJ249280	phosphoinositide-3-kinase, catalytic, gamma polypeptide

Pilra	0,30	10534927	BC115565	paired immunoglobin-like type 2 receptor alpha
Pilrb1	0,40	10534935	BC145880	paired immunoglobin-like type 2 receptor beta 1
Pilrb2|Pilrb1	0,32	10534940	BC147406|BC145880	paired immunoglobin-like type 2 receptor beta 2 | paired immunoglobin-like type 2 receptor beta 1
Pin4	2,28	10552284	BC119355	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting, 4 (parvulin)
Pin4	2,28	10601272	BC119355	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting, 4 (parvulin)
Pkd1l1	2,16	10384266	AB061684	polycystic kidney disease 1 like 1
Pld5	2,59	10360463	BC100428	phospholipase D family, member 5
Ppp1r12c	0,41	10559524	BC114396	protein phosphatase 1, regulatory (inhibitor) subunit 12C
Ppp1r13b	0,45	10402730	BC053092	protein phosphatase 1, regulatory (inhibitor) subunit 13B
Prdm1	3,06	10368970	U08185	PR domain containing 1, with ZNF domain
Prkch	0,42	10396402	BC031121	protein kinase C, eta
Prl2c3|Prl2c4|Prl2c2	4,57	10407797	BC100299|K03235|BC100300	prolactin family 2, subfamily c, member 3 | prolactin family 2, subfamily c, member 4 | prolactin family 2, subfamily c, member 2
Prl2c5|Prl2c2	2,96	10403579	AF128884	prolactin family 2, subfamily c, member 5 | prolactin family 2, subfamily c, member 2
Prss16	0,20	10408097	BC125481	protease, serine, 16 (thymus)
Prune2	0,41	10461878	BC094224	prune homolog 2 (Drosophila)
Ptgir	0,30	10550394	D26157	prostaglandin I receptor (IP)
Ptpn18	0,44	10345370	U35124	protein tyrosine phosphatase, non-receptor type 18
Ptpn6	0,44	10547769	BC012660	protein tyrosine phosphatase, non-receptor type 6
Pycard	0,42	10568355	AB032249	PYD and CARD domain containing
Qpct	3,11	10447056	BC151027	glutaminyl-peptide cyclotransferase (glutaminyl cyclase)
Qprt	0,35	10568169	BC011191	quinolinate phosphoribosyltransferase
Rab19	0,38	10537428	BC032936	RAB19, member RAS oncogene family
Rab27a	0,33	10587023	AF304376	RAB27A, member RAS oncogene family
Rab3il1	0,39	10461408	BC020147	RAB3A interacting protein (rabin3)-like 1
Raet1d|Raet1e|Raet1b	0,33	10362091	AF257520|AY056835|BC132022	retinoic acid early transcript delta | retinoic acid early transcript 1E | retinoic acid early transcript beta
Rasa4	0,36	10526459	BC049381	RAS p21 protein activator 4
Rasal3|A530088E08Rik	0,38	10449893	BC132341	RAS protein activator like 3 | RIKEN cDNA A530088E08 gene
Rasgef1a	2,31	10541114	BC138284	RasGEF domain family, member 1A
Rasgrp3	0,41	10446965	BC066127	RAS, guanyl releasing protein 3
Rassf4	0,35	10547177	BC060709	Ras association (RalGDS/AF-6) domain family member 4
Rassf5	0,36	10357594	BC089605	Ras association (RalGDS/AF-6) domain family member 5

Rcsd1	0,31	10359762	BC025872	RCSD domain containing 1
Rgs14	0,39	10405432	BC030321	regulator of G-protein signaling 14
Rgs3	0,41	10607113	BC033449	regulator of G-protein signaling 3
Rhof|Tmem120b	0,40	10533603	BC132355	ras homolog gene family, member f | transmembrane protein 120B
Rinl	0,42	10551587	BC046530	Ras and Rab interactor-like
Rnase4|Ang	0,36	10414537	AY762362|BC055355	ribonuclease, RNase A family 4 | angiogenin, ribonuclease, RNase A family, 5
Rock2	0,38	10394731	BC056964	Rho-associated coiled-coil containing protein kinase 2
Rsad2	2,89	10399710	BC057868	radical S-adenosyl methionine domain containing 2
Samsn1	0,39	10440393	BC052906	SAM domain, SH3 domain and nuclear localization signals, 1
Selplg	0,40	10532744	BC003874	selectin, platelet (p-selectin) ligand
Serpinb10-ps|Serpinb2	0,13	10349166	BC029736	serine (or cysteine) peptidase inhibitor, clade B (ovalbumin), member 10, pseudogene | serine (or cysteine) peptidase inhibitor, clade B, member 2
Sgk3	0,33	10344750	AF312007	serum/glucocorticoid regulated kinase 3
Sh3d20|Arhgap27	0,40	10391914	BC138292	SH3 domain containing 20 | Rho GTPase activating protein 27
Siglece	0,42	10562720	BC023280	sialic acid binding Ig-like lectin E
Slamf9	0,45	10351792	BC019477	SLAM family member 9
Slc16a6	0,38	10392440	BC017129	solute carrier family 16 (monocarboxylic acid transporters), member 6
Slc25a38	0,37	10590245	BC010801	solute carrier family 25, member 38
Slc7a11	2,73	10498024	AY766236	solute carrier family 7 (cationic amino acid transporter, y+ system), member 11
Slc9a9	0,35	10587854	BC099942	solute carrier family 9 (sodium/hydrogen exchanger), member 9
Slco2b1	0,26	10565819	BC019209	solute carrier organic anion transporter family, member 2b1
Smox	0,39	10476301	BC004831	spermine oxidase
Snora20	0,41	10441813		small nucleolar RNA, H/ACA box 20
Snx20	0,45	10580504	BC039809	sorting nexin 20
Spn	0,44	10568174	BC100741	sialophorin
Spsb3	0,45	10442629	BC072556|AF403038|AK167474|AK156579|BC026523|AK212011|AK199452|AK208775|AK199344|AK186791	splA/ryanodine receptor domain and SOCS box containing 3
Ssh2	0,34	10378855		slingshot homolog 2 (Drosophila)
Stard8	0,44	10600921	BC062944	START domain containing 8
Saa3	5,55	10563597	BC055885	serum amyloid A 3
Tagap	0,33	10441601	BC030886	T-cell activation Rho GTPase-activating protein
Tcf19	0,44	10450519	BC004617	transcription factor 19
Thsd4	2,91	10594199	BC139329|AK148408	thrombospondin, type I, domain containing 4

Tifab	0,37	10409567	BC031130	TRAF-interacting protein with forkhead-associated domain, family member B
Tm6sf1|Hdgfrp3	0,34	10554574	BC023123	transmembrane 6 superfamily member 1 | hepatoma-derived growth factor, related protein 3
Tmc8	0,43	10382956	AY263160|BC138254|BC145210|AY236501|AK220211	transmembrane channel-like gene family 8
Tnfaip8l2	0,42	10500100	BC094616	tumor necrosis factor, alpha-induced protein 8-like 2
Tnfrsf9	2,26	10510580	DQ832278|DQ832279|BC028507|J04492	tumor necrosis factor receptor superfamily, member 9
Tnfsf14	0,41	10452307	AB029155	tumor necrosis factor (ligand) superfamily, member 14
Topors	2,37	10512088	AB072395	topoisomerase I binding, arginine/serine-rich
Trem1	0,26	10445746	BC111875	triggering receptor expressed on myeloid cells 1
Trem3	0,43	10445753	AF241220	triggering receptor expressed on myeloid cells 3
Trf	0,33	10596148	BC092046	transferrin
Trim65	0,43	10393113	BC057094	tripartite motif-containing 65
Unc119	0,32	10379176	BC001990	unc-119 homolog (C. elegans)
Was	0,43	10603440	U29673	Wiskott-Aldrich syndrome homolog (human)
Wdfy4	0,39	10418868		WD repeat and FYVE domain containing 4

3 Information on the investigated cytokines

Table S5: *Overview over the by multiplex assay investigated cytokines and their function*

			Multiplex assay	
Name	**Synonym**	**Function**	**Human**	**Mice**
CCL2	MCP-1	Recruits dendritic cells, monocytes and T cells to the sites of inflammation [1, 2]	x	x
CCL3	MIP-α	Recruits and activates leukocytes, part of the acute inflammatory state, crucial for immune response to inflammation, induce release of other pro-inflammatory cytokines [3, 4]	x	x
CCL4	MIP-β	Activates granulocytes, can lead to acute neutrophilic inflammation, induce release of other pro-inflammatory cytokines, chemoattractant for a variety of immune cells [5]	x	x
CCL5	RANTES	Recruits monocytes and T cells to the sites of inflammation [6]		x
CCL7	MCP-3	One of the most broadly active chemoattractants, attracts monocytes, eosinophils, basophils, NK cells, T lymphocytes, and neutrophils, promotes monocyte mobilization to inflammatory sites [7, 8, 9]		x
CXCL8	IL-8	Attracts leukocytes to sites of acute inflammation, activates monocytes [6]	x	
CXCL9	MIG	Is is induced by IFN-γ and functions as a T-cell chemoattractant [10]	x	
CSF2	GM-CSF	Promotes the differentiation, growth and effector functions of neutrophils and macrophages [11, 12]		x
CSF3	G-CSF	Promotes the expansion and maturation of neutrophils, mobilizes hemopoietic stem cells to peripheral blood, has immunregulatory effects [13, 14, 15]	x	

Abbreviations: CCL - chemokine (C-C motif) ligand; CSF - colony stimulating factor; CXCL - CXC-Motive-Chemokine; G-CSF - granulocyte colony-stimulating factor; GM-CSF - Granulocyte-macrophage colony-stimulating factor; IL-8 - interleukin 8; MCP- monocyte chemotactic protein; MIG - Monokine induced by gamma interferon; MIP - macrophage inflammatory protein; RANTES - Regulated on Activation, Normal T cell Expressed and Secreted

4 Investigation of the effect of SWCNT exposure on the cell morphology of A549, THP-1 and J774 cells.

To show the influence of the SWCNT exposure to the cell morphology, A549, THP-1 (in differentiation medium) and J774 cells were seeded two days before the experiment, aiming for 70 - 80% confluence at the start of exposure. The cells were exposed to 3 mL 10 µg/mL SWCNT in CO_2 independent DMEM (GIBCO) supplemented with 10% FBS during the time lapse experiment. Live-cell imaging was started directly after the initial exposure, using a Leica DMIL LED microscope equipped with a 37°C heated stage with mounted Leica DFC 295 camera and Leica Application Suite Multitime-Movie-Timelapse software (both from Leica Microsystems, Switzerland), taking one picture every 30 seconds for a total duration of 2 hours at 200x magnification. After 2 hours, the CO_2 independent medium was replaced with normal growth medium with or without 10 µg/mL SWCNTs and incubated at 37°C, 5% CO_2 for 22 hours. After 24 hours of total exposure the medium was replaced with 3 mL CO_2 independent DMEM (GIBCO) supplemented with 10%FBS with and without 10 µg/mL SWCNT followed by 2 hours live-cell imaging as described before. ImageJ software [16] was used for editing the time lapse images and data are presented as still images (Fig. S6).

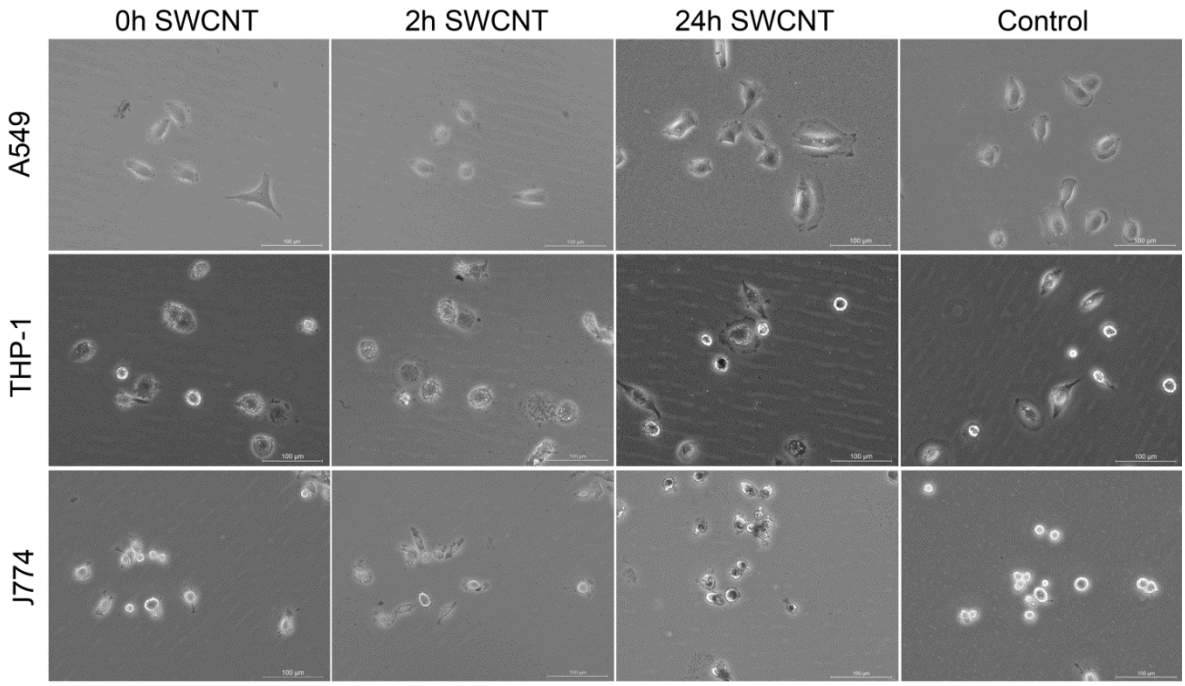

Figure S6: *Investigation of the effect of SWCNT exposure to the cell morphology of A549, THP-1 and J774 cells. The cells were untreated (control) or exposed to 10µg/ml SWCNTs for 0, 2 and 24 hours. Original magnification 200x.*

5 References Supplementary Data

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