Supplementary Material

Supplemental Tables

Table S1. Forward and reverse primer sequences used in rtPCR validation of microarray expression data in transformed D-SWCNT, D-MWCNT and ASB human small airway epithelial cells.

Gene	GenBank	Forward (5' → 3')	Reverse (3' → 5')
ANTXR1	NM_053034	GCTCTCCTCTGGTGGTTCTG	TGGAGGGACCTCCTTGATAA
BCL2L2	NM_004050.3	GATGGTGGCCTACCTGGAG	CGTCCCCGTATAGAGCTGTG
CASP8	NM_033358	ACTATGAAGAATTCAGCAAAGAGAGA	GTATCCCCGAGGTTTGCTTT
COL1A1	NM_000088.3	GGGATTCCCTGGACCTAAAG	GGAACACCTCGCTCTCCA
FEZ1	NM_005103.3	GGCAACTGCTCTGACACTGA	AGGCTCCTCGTTGATACCG
FOS	NM_005252.3	CTACCACTCACCCGCAGACT	AGGTCCGTGCAGAAGTCCT
IL8	NM_000584	AGACAGCAGAGCACACAAGC	ATGGTTCCTTCCGGTGGT
LAMA4	NM_002290	TGCACAGGACCTTCAACAAG	TGTACCAGCCCGTTCATATCT
MYC	NM_002467.	CACCAGCAGCGACTCTGA	GATCCAGACTCTGACCTTTT

	4		GC
NFKB2	NM_002502.3	CACATGGGTGGAGGCTCT	ACTGGTAGGGGCTGTAGGC
PLAU	NM_002658	TTGCTCACCACAACGACATT	GGCAGGCAGATGGTCTGTAT
PPARG	NM_005037.5	GACAGGAAAGACAACAGACAAATC	GGGGTGATGTGTTTGAACTTG

Table S2. Top-ranked disease functions for saline (SAL), D-UFCB, D-SWCNT, D-MWCNT, and ASB-exposed small airway epithelial cells compared to dispersant-only exposed cells.

Disease function	- Log p-value	Number of genes
SAL		
Cancer	20.36	308
Genetic Disorder	17.76	36
Neurological Disease	12.18	102
Gastrointestinal Disease	12.00	109
Immunological Disease	10.92	189
Reproductive System Disease	10.78	103
D-UFCB		
Cancer†	10.01	310
Genetic Disorder	8.71	548
Neurological Disease	6.51	334

Reproductive System Disease	5.67	46
Gastrointestinal Disease	4.80	86
Immunological Disease	4.25	229
D-SWCNT		
Cancer	27.24	401
Genetic Disorder	24.29	722
Neurological Disease	18.54	455
Gastrointestinal Disease	14.00	131
Hematological Disease	11.69	251
Reproductive System Disease	9.71	127
D-MWCNT		
Cancer	21.96	277
Neurological Disease	17.03	318
Genetic Disorder	14.60	471
Gastrointestinal Disease	12.13	94
Hematological Disease	11.29	179

Skeletal and Muscular Disorders	11.16	237
ASB		
Cancer	19.68	228
Inflammatory Response*	16.04	116
Connective Tissue Disorders	14.40	128
Inflammatory Disease*	14.40	128
Skeletal and Muscular Disorders	14.40	128
Immunological Disease	11.32	147

* and † indicate diseases predicted to be activated/inhibited, respectively ($Z \geq \pm 2$).

Supplementary Figures:-

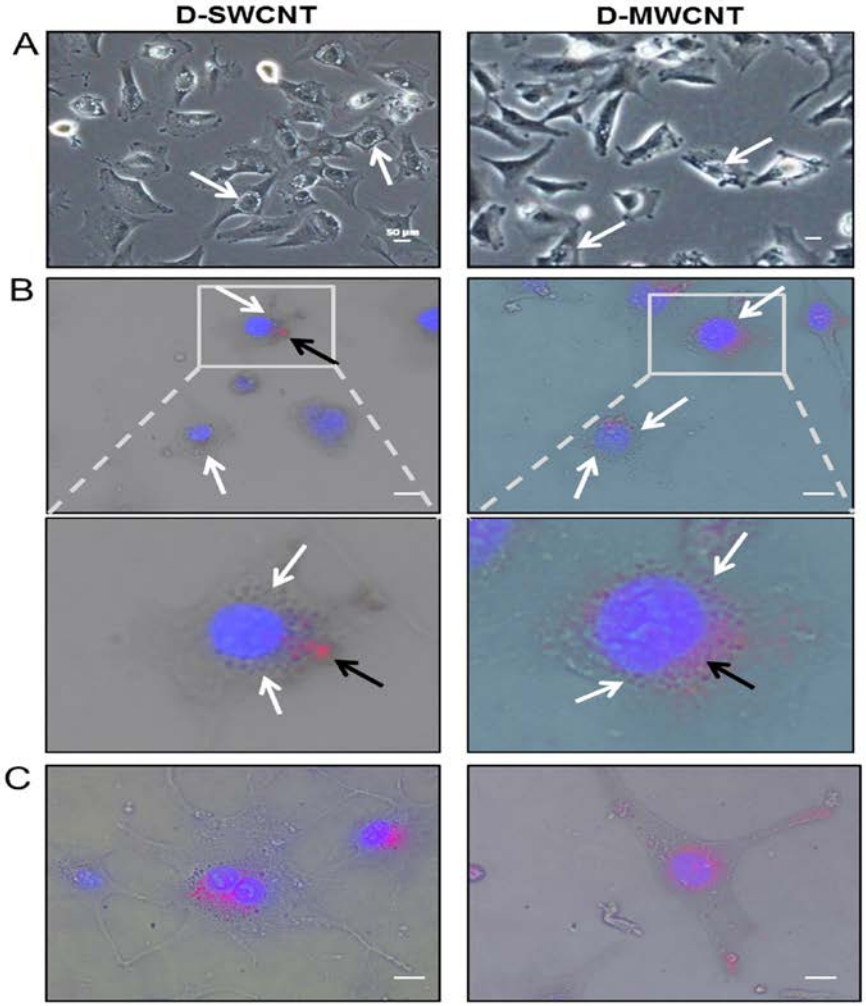


Figure S1. Morphological characteristics of D-SWCNT and D-MWCNT exposed (6-month) human small airway epithelial cells. A) Both D-CNT cells displayed numerous intracellular vesicles surrounding nucleus (white arrows). B) Fluorescent images of agglutinin-stained Golgi (Invitrogen; black arrows) overlaid with blue Hoescht 33342-stained nucleus did not co-localize with vesicles. Both Golgi apparatus (B; lower panels) and lysosome staining (LysoTracker; Invitrogen; not shown) showed close proximity to vesicles. C) Giant cell morphology in D-CNT cells. Reverse-phase digital micrographs were captured on an Olympus X70 scope with a mounted Retiga camera. Reverse-phase and fluorescent images were overlaid in Photoshop CS5. Bar = 50 µm.

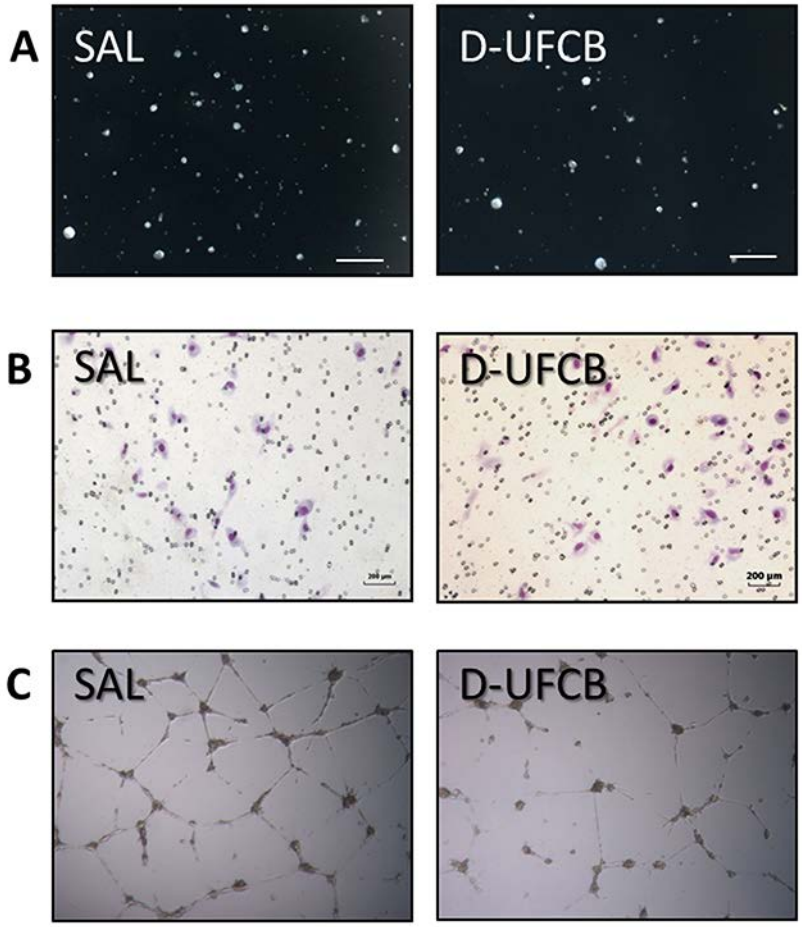


Figure S2. Representative transformed phenotype microphotographs for SAL and D-UFCB SAECs. A) Anchorage-independent growth; bar = 500 µm B) Transwell chemotaxis invasion assay C) Angiogenesis assay.

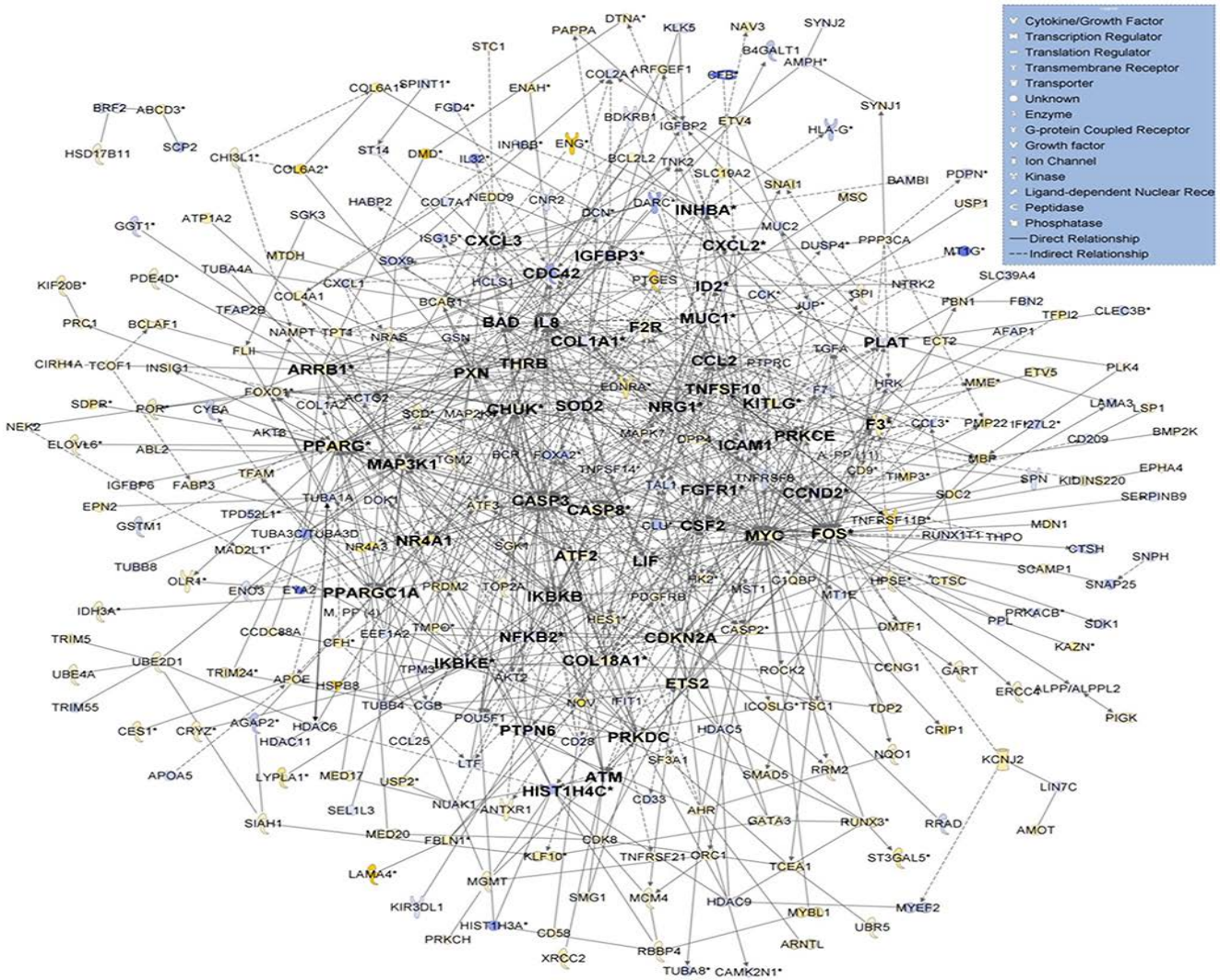


Figure S3. Cancer promotion related gene signaling network (GSN) in human small airway epithelial cells subchronically exposed to dispersed SWCNT. Genes were included in the network if they affect cancer, were over-expressed and promote cancer, or down-regulated and inhibit cancer. Genes passing the network filter, but with no signaling relationships are not shown. Large font genes indicate hubs in the GSN. Yellow and blue indicate over- and under-expressed genes compared to dispersant-only control cells.

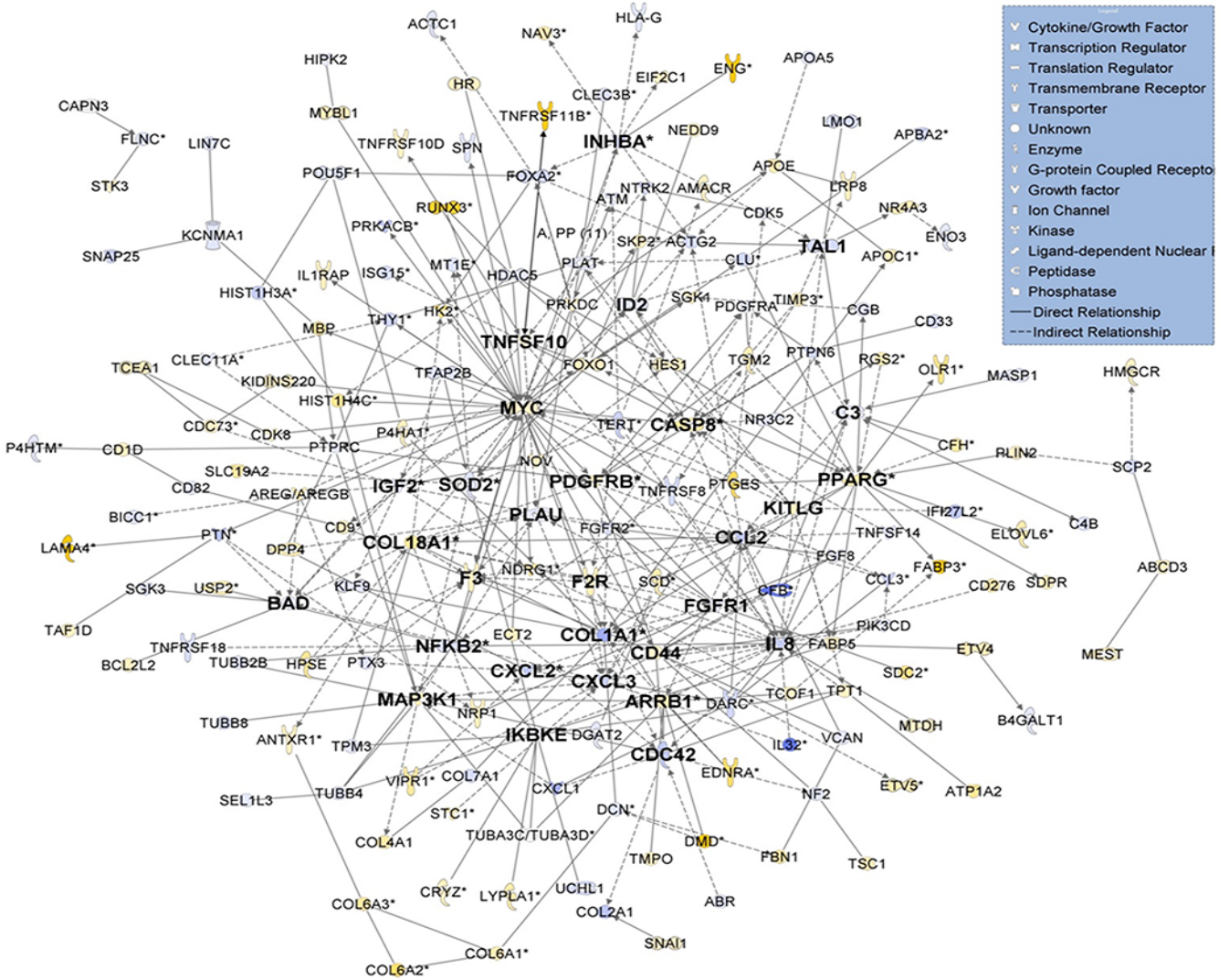


Figure S4. Cancer promotion related gene signaling network (GSN) in human small airway epithelial cells subchronically exposed to dispersed MWCNT. Genes were included in the network if they affect cancer, were over-expressed and promote cancer, or down-regulated and inhibit cancer. Genes passing the network filter, but with no signaling relationships are not shown. Large font genes indicate hubs in the GSN. Yellow and blue indicate over- and under-expressed genes compared to dispersant-only control cells.

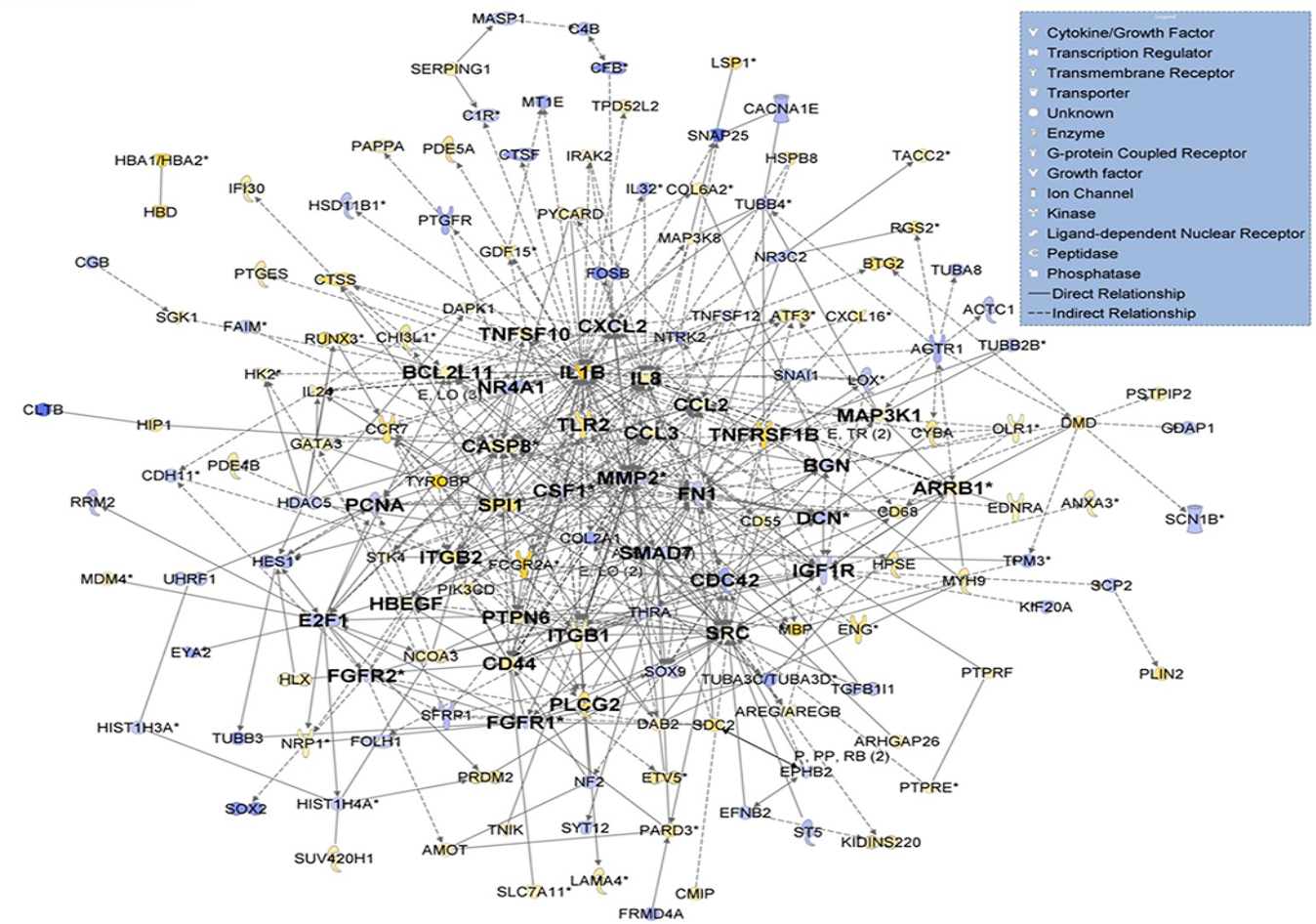
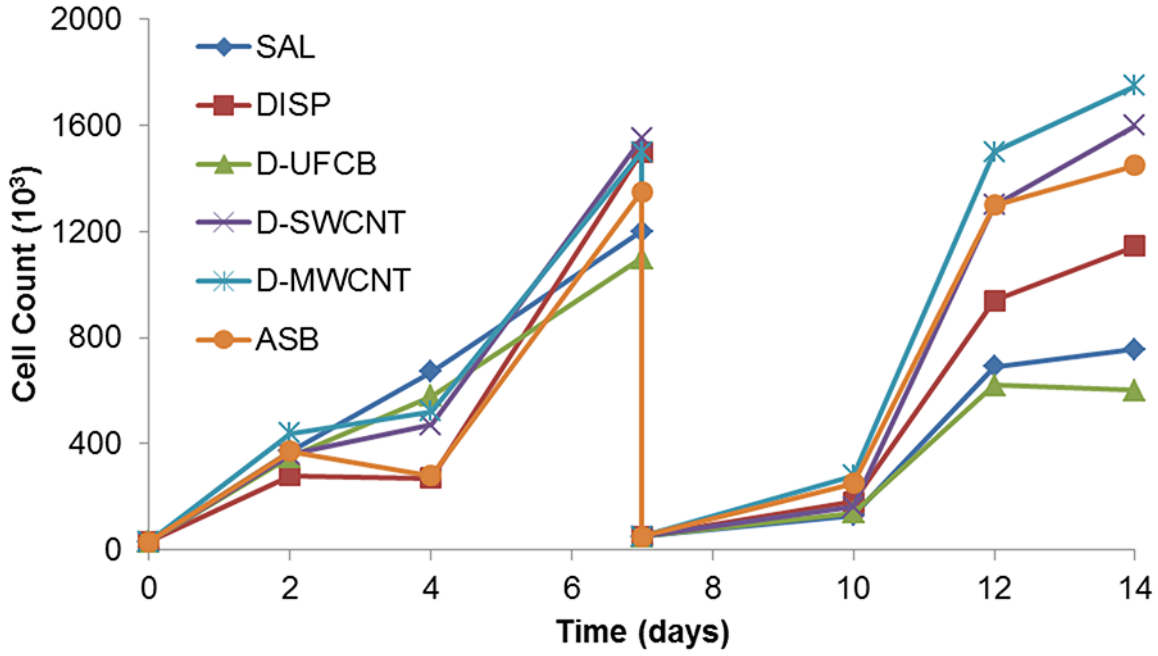


Figure S5. Cancer promotion related gene signaling network (GSN) in human small airway epithelial cells subchronically exposed to dispersed crocidolite asbestos. Genes were included in the network if they affect cancer, were over-expressed and promote cancer, or down-regulated and inhibit cancer. Genes passing the network filter, but with no signaling relationships are not shown. Large font genes indicate hubs in the GSN. Yellow and blue indicate over- and under-expressed genes compared to dispersant-only control cells.



FOR REVIEW ONLY

Figure S6. Representative growth curves over a two week period of human small airway epithelial cells during subchronic exposure (0.02 μg/cm^2) to dispersed ultrafine carbon black (D-UFCB), single- (D-SWCNT), multi-walled carbon nanotubes (D-MWCNT) and crocidolite asbestos (ASB). Passaged saline (SAL) and dispersant-only(DISP) cells served as controls. 5x10^4 cells were plated into 6-well plates in triplicate for each particle exposure. Every two days, one replicate from each treatment was suspended via trypsinization, spun down and resuspended in 1 mL of culture medium. Suspended cells were assayed for live cell number by trypan blue exclusion staining. On Day 7, all triplicates were repeated at initial seeding density.