Supporting Information

Particulate matter ($PM_{2.5}$) from biomass combustion induces an anti-oxidative response and cancer drug resistance in human bronchial epithelial BEAS-2B cells

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Supporting Tables

Table S1. Mineralogical composition of $PM_{2.5}$ [†]

Component	percentage portion[b]
Amorphous	30%
Sylvite (KCl)	5%
Syngenite ($K_2Ca(SO_4)_2 \cdot H_2O$	1%
Arcanite (K_2SO_4)	47%
Thenardite (Na_2SO_4)	2%
Merwinite ($Ca_3Mg(SiO_4)_2$)	2%
Larnite (Ca_2SO_4)	5%
Periclase (MgO)	2%
Dolomite ($CaMg(CO_3)_2$)	1%
Portlandite ($Ca(OH)_2$)	2%
Lime (CaO)	1%
Aragonite ($CaCO_3$)	2%

[†] data depicted from Dornhof et al. [1]; values given in wt%

Table S2. Concentrations of PAHs (μg/kg) in $PM_{2.5}$ from biomass combustion [†]

PAH	μg/kg	PAH	μg/kg
Acenaphthene	3.64	Naphtho[1,2-*b*]fluoranthene and Naptho[2,3-*a*]pyrene	0.93
Anthracene	7.38	Fluorene	4.67
Benzo[*a*]anthracene	6.70	1-Methylfluorene	1.24
9-Methylanthracene	0.23	7H-Benzo[*c*]fluorene	1.73
9,10-Dimethylanthracene	<0.50	Naphthalene	22.57
6-Methylbenzo[*a*]anthracene	0.84	2-Methylnaphthalene	11.44
5-Methylbenzo[*a*]anthracene	<0.50	2,6-Dimethylnaphthalene	10.63
7,12-Dimethylbenzo[*a*]anthracene	2.27	1,8-Dimethylnaphthalene	0.17
Dibenzo[*a,j*]anthracene	<0.25	Phenanthrene	36.01
Dibenzo[*a,c*]anthracene	<0.25	9-Methylphenanthrene	11.52
Dibenzo[*a,h*]anthracene	<0.25	1-Methylphenanthrene	7.30
Anthanthrene	<2.50	Benzo[*c*]phenanthrene	1.93
3-Methylcholanthrene	<1.25	Pyrene	41.98
Chrysene	15.27	1-Methylpyrene	14.22
2-Methylchrysene	1.31	Benzo[*e*]pyrene	12.27
6-Methylchrysene	0.12	Benzo[*a*]pyrene	5.29
5-Methylchrysene	0.28	6-Methylbenzo[*a*]pyrene	<1.25
4-Methylchrysene	<0.50	Indeno[1,2,3-*cd*]pyrene	3.86
Coronene	2.10	Naphtho[2,3-*e*]pyrene	<0.38
Fluoranthene	50.67	Dibenzo[*a,l*]pyrene	0.49
Benzo[*b*]fluoranthene	12.23	Dibenzo[*a,e*]pyrene	1.72
Benzo[*k*]fluoranthene	2.74	Dibenzo[*a,i*]pyrene	0.38
Benzo[*ghi*]fluoranthene	10.15	Dibenzo[*a,h*]pyrene	<0.50
Naphtho[1,2-*k*]fluoranthene	<0.38	Perylene	0.73
Naphtho[2,3-*b*]fluoranthene	0.59	Benzo[*ghi*]perylene	2.42
Dibenzo[*a,e*]fluoranthene	<0.38	Retene	33.43
Naphtho[2,3-*k*]fluoranthene	<0.38	Triphenylene	6.41

[†] data depicted from Popadic et al. [2]

Table S3. Expression of cytokine, chemokine, adhesion molecule, and matrix metalloproteinase genes upon long-term exposure to $PM_{2.5}$ [†]

gene symbol	*encoded protein*	*adjusted p-value*	*log2FC*	*FC*
CCL2	chemokine (C-C motif) ligand 2, monocyte chemotactic protein 1 (MCP1)	0.0043	-1.68	0.31
CCL5	chemokine (C-C motif) ligand 5, RANTES	n.s.	0.01	1.01
CCL8	chemokine (C-C motif) ligand 8, monocyte chemotactic protein 2 (MCP2)	n.s.	-0.06	0.96
CXCL1	chemokine (C-X-C motif) ligand 1	n.s.	0.02	1.01
CXCL10	chemokine (C-X-C motif) ligand 10	n.s.	0.00	1.00
ICAM1	intracellular adhesion molecule 1	n.s.	-0.02	0.98
IL12A	interleukin 12 subunit alpha	n.s.	0.10	1.07
IL12B	interleukin 12 subunit beta	n.s.	-0.01	0.99
MMP1	matrix metallopeptidase 1	n.s.	0.20	1.14
MMP9	matrix metallopeptidase 9	n.s.	-0.02	0.98
MMP12	matrix metallopeptidase 12	0.0009	-2.69	0.16
MMP13	matrix metallopeptidase 13	0.0065	-1.15	0.45
TNF	tumor necrosis factor, TNFalpha	n.s.	-0.05	0.97
VCAM1	vascular cellular adhesion molecule 1	n.s.	-0.04	0.97

[†] according to Affymetrix Human Genome U133 Plus 2.0 gene expression arrays; n.s., not significant.

Table S4. Expression of genes, relevant for apoptosis after long-term exposure to $PM_{2.5}$ †

gene symbol	*encoded protein*	*adjusted p-value*	*log2FC*	*FC*
ATG5 ‡	autophagy related 5	n.s.	0.02	1.01
BAD, BCL2L8 §	Bad, BCL2 associated agonist of cell death	n.s.	0.03	1.02
BAK1, BCL2L7 §	Bak, BCL2 antagonist/killer 1	n.s.	0.04	1.03
BAX, BCL2L4 §	Bax, Bcl-2-associated X protein	n.s.	0.04	1.01
BCL2 ¶ £	Bcl-2, B-cell lymphoma 2	n.s.	0.01	0.99
BCL2L1 ¶ £	Bcl-xL, BCL2 like 1	n.s.	0.07	1.05
BECN1 ‡	beclin-1, Zinc finger protein basonuclin-1	n.s.	-0.01	0.99
BID §	Bid, BH3 interacting-domain death agonist	n.s.	0.03	1.01
BIM, BCL2L11 §	Bim, Bcl-2-like protein 11	n.s.	0.01	1.01
BIRC5 ¶	Surviving	n.s.	-0.03	0.98
CFLAR ¶ £	c-Flip, caspase 8 and FADD-like apoptosis regulator	n.s.	-0.01	0.99
HMGB1 ‡	high mobility group protein B1	n.s.	0.01	1.01
MCL1 ¶ £	induced myeloid leukemia cell differentiation protein Mcl1	n.s.	0.03	1.02
XIAP ¶ £	XIAP, X-linked inhibitor of apoptosis protein	n.s.	0.03	1.03

† according to Affymetrix Human Genome U133 Plus 2.0 gene expression arrays; ‡ involved in autophagy, which inhibits apoptosis; § promotes apoptosis; ¶ inhibits apoptosis; £ transcriptional regulation by Akt/NF- B; n.s., not significant.

Table S5. Expression of ABC transporter genes, relevant for multidrug resistance after long-term exposure to $PM_{2.5}$ †

gene symbol	*encoded protein*	*adjusted p-value*	*log2FC*	*FC*
ABCA2	ATP-binding cassette sub-family A member 2	n.s.	-0.01	0.99
ABCB1	P-glycoprotein, P-gp, multi drug resistance protein (MDR)1	0.005	-1.22	0.43
ABCB4	MDR3	n.s.	-0.03	0.98
ABCB11	BSEP (bile salt export pump), sPgp (sister of P-glycoprotein)	n.s.	-0.03	0.98
ABCC1	Multi resistance-associated protein (MRP)1	n.s.	0.03	1.02
ABCC2	Multi resistance-associated protein (MRP)2	0.045	0.50	1.41
ABCC3	Canalicular multispecific organic anion transporter 2, MRP3	n.s.	0.03	1.02
ABCC4	MRP4	n.s.	-0.01	0.99
ABCC5	MRP5	n.s.	-0.03	0.98
ABCC6	MRP6	n.s.	-0.01	1.00
ABCC10	MRP7	n.s.	-0.01	0.99
ABCC11	Multidrug resistance-related protein 8 (MRP8)	n.s.	-0.01	0.99
ABCG2	ATP-binding cassette super-family G member 2	n.s.	0.02	1.01

† according to Affymetrix Human Genome U133 Plus 2.0 gene expression arrays; n.s., not significant.

Table S6. Expression of antioxidant redox-sensitive genes upon long-term exposure to $PM_{2.5}$ †

gene symbol	*encoded protein*	*adjusted p-value*	*log2FC*	*FC*
CAT ‡, §	Catalase	n.s.	0.00	1.00
GPX1 ‡, §	glutathione peroxidase 1	n.s.	0.01	1.00
GPX2 §	glutathione peroxidase 2	n.s.	-0.01	1.00
GPX3 §	glutathione peroxidase 3	n.s.	0.02	1.01
GSR	glutathione reductase	n.s.	0.01	1.00
GSS	glutathione synthetase	n.s.	0.03	1.02
PPARGC1A	peroxisome proliferator-activated receptor gamma coactivator 1-alpha	n.s.	-0.07	0.96
PRDX1 ‡, §	peroxiredoxin 1	n.s.	0.01	1.00
PRDX2 ‡, §	peroxiredoxin 2	n.s.	-0.04	0.99
PRDX3 ‡, §	peroxiredoxin 3	n.s.	0.00	1.00
PRDX4 §	peroxiredoxin 4	n.s.	0.00	1.00
PRDX5 ‡, §	peroxiredoxin 5	n.s.	0.01	1.00
PRDX6 §	peroxiredoxin 6	n.s.	0.00	1.00
TXN ‡	thioredoxin 1	n.s.	0.04	1.03
TXN2	thioredoxin 2	n.s.	-0.01	1.00
TXNRD1	thioredoxin reductase 1	n.s.	0.04	1.03
TXNRD2	thioredoxin reductase 2	n.s.	-0.01	0.99
SOD1 §	superoxide dismutase 1	n.s.	0.02	1.02
SOD2 ‡, §	superoxide dismutase 2	n.s.	0.00	1.00
SOD3 §	superoxide dismutase 3	n.s.	0.00	1.00

† according to Affymetrix Human Genome U133 Plus 2.0 gene expression arrays; ‡ involved in doxorubicin resistance; § involved in H_2O_2 generation/detoxification; n.s., not significant.

Supporting Figures

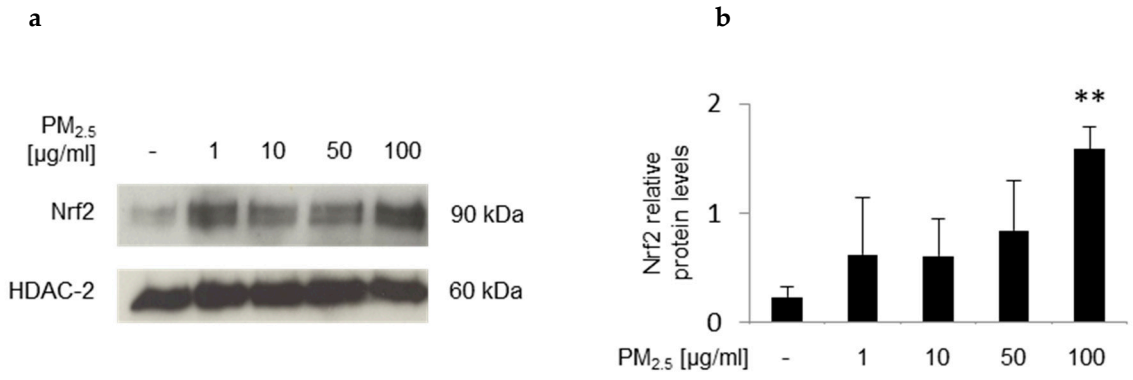

Figure S1. Long-term exposure to $PM_{2.5}$ induces nuclear translocation of Nrf2. BEAS-2B cells were exposed to 1 - 100 g/ml $PM_{2.5}$ for 12 h. (**a**), translocated Nrf2 was detected in nuclear extracts by immunoblotting and normalized to histone deacetylase (HDAC)-2. Cells, cultured in the absence of $PM_{2.5}$ served as controls (first lane). A representative blot of two different experiments is shown. (**b**), quantification of immunoblots relative to control. Statistical analysis was performed by using the R statistical software [3] and determined by analysis of variance (ANOVA), followed by Dunnett's post-hoc test. Values are depicted as means and +standard deviations; **, $p<0.01$.

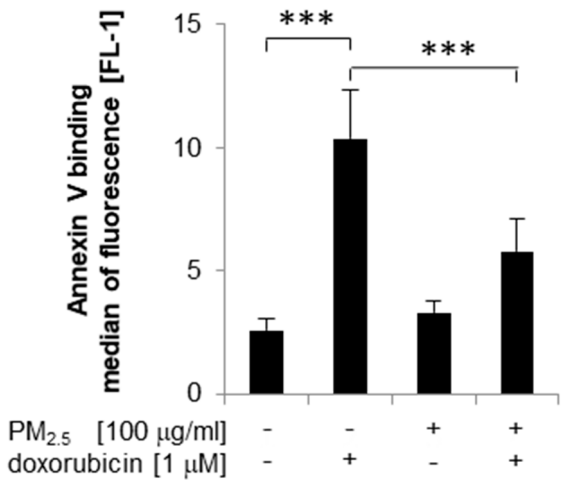

Figure S2. Cell death resistance to doxorubicin is mediated by $PM_{2.5}$. BEAS-2B cells were cultured in the presence of 100 µg/ml $PM_{2.5}$ for 3 to 5 weeks, before final passage and re-exposure of cells to 100 µg/ml $PM_{2.5}$ for 48 h. Doxorubicin (1 µM) was added 24 h after the last addition of $PM_{2.5}$. Equally cultured cells that were never exposed to $PM_{2.5}$ served as controls. Detection of apoptosis by Annexin V staining and flow cytometry (n = 4). Statistical analysis was performed by using the R statistical software [3] and determined by analysis of variance (ANOVA), followed by the Tukey's post-hoc test. Values are depicted as means and +standard deviations; **p<0.01; ***p<0.001.

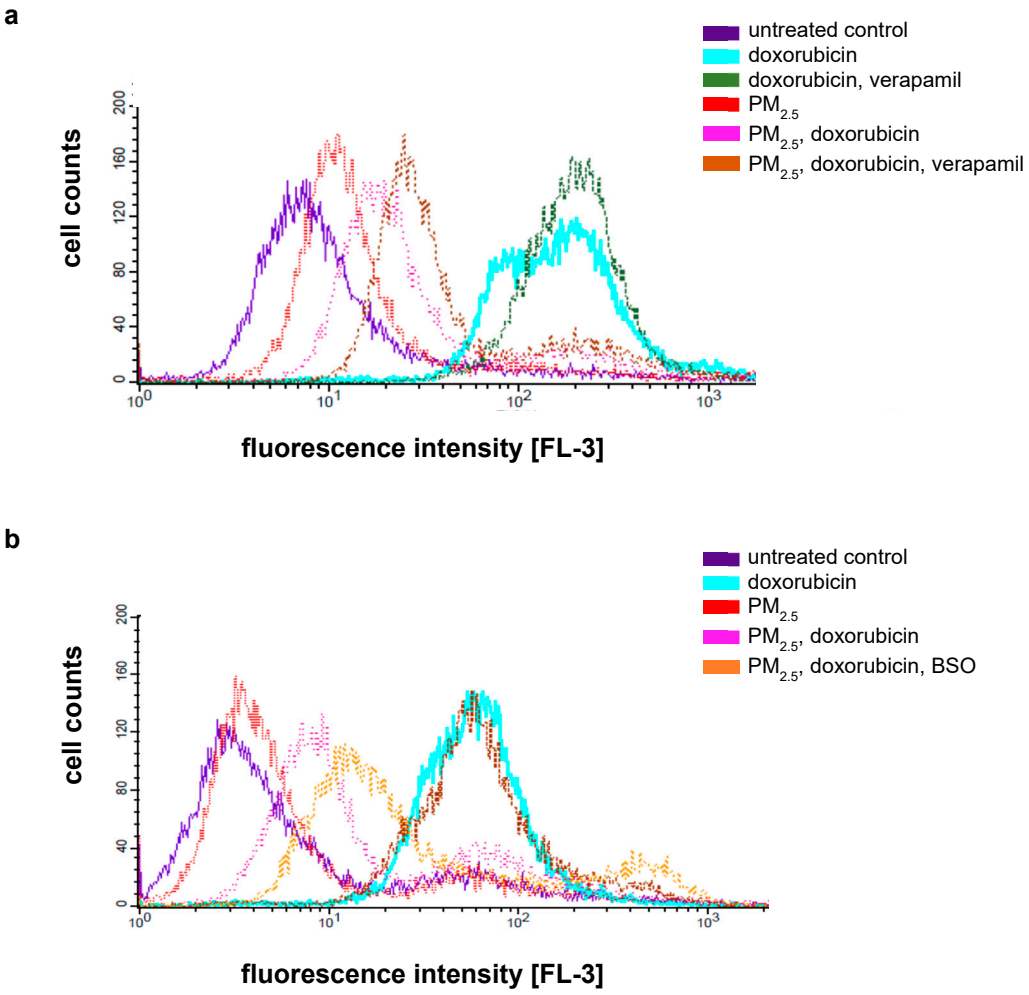

Figure S3. Acquired chemoresistance in $PM_{2.5}$ exposed BEAS-2B cells is mediated by GSH. BEAS-2B cells were cultured in the presence of 100 g/ml $PM_{2.5}$ for 3 to 5 weeks. After final passage, cells were re-exposed to 100 g/ml $PM_{2.5}$ after 24 h. Verapamil (20 M) or BSO (200 M) were added 1 h prior to the last $PM_{2.5}$ exposure. Doxorubicin (1 M) was added 24 h after the last $PM_{2.5}$ exposure for an additional 48 h. Cells that were never exposed to $PM_{2.5}$ served as controls. Intracellular doxorubicin content was determined by flow cytometry and is depicted as histograms. (**a**), inhibition of P-gp/MDR1 by verapamil; (**b**), inhibition of GSH synthesis by BSO.

References

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