

## **Supplementary material**

### **Oxidative stress response in pulmonary cells exposed to different fractions of PM<sub>2.5-0.3</sub> from urban, traffic and industrial sites**

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Table S1: Chemical composition ( $\mu\text{g/g}$  of  $\text{PM}_{2.5-0.3}$ ) of the water-soluble fraction (WSE) of  $\text{PM}_{2.5-0.3}$  particles collected under urban influence –seasonal and yearly average (U-Sp, U-Su, U-A, U-W, U-Y), industrial influence (I), and traffic influence (T). Water-soluble versus Total concentration ratio (%) in  $\text{PM}_{2.5-0.3}$  is also indicated for detected elements in both fractions.

	<b>U-Sp</b>	<b>U-Su</b>	<b>U-A</b>	<b>U-W</b>	<b>U-Y</b>	<b>T</b>	<b>I</b>
Al	22.6 <i>0.1%</i>	16.3 <i>0.1%</i>	10.4 <i>0.0%</i>	6.9 <i>0.0%</i>	11.2 <i>0.0%</i>	27.2 <i>0.2%</i>	54.6 <i>0.2%</i>
As	< DL	< DL	< DL	< DL	< DL	< DL	< DL
Ba	46.8 <i>17.2%</i>	34.5 <i>10.1%</i>	36.8 <i>9.2%</i>	31 <i>9.1%</i>	41.5 <i>12.5%</i>	126.1 <i>13.5%</i>	38.1 <i>13.4%</i>
Cd	< DL	< DL	< DL	< DL	< DL	< DL	< DL
Co	< DL	< DL	< DL	< DL	< DL	< DL	< DL
Cr	< DL	< DL	< DL	< DL	< DL	< DL	< DL
Cu	22.1 <i>3.8%</i>	13.5 <i>2.5%</i>	20.2 <i>2.5%</i>	12.9 <i>1.9%</i>	14.2 <i>2.2%</i>	53.7 <i>5.8%</i>	17.7 <i>6.4%</i>
Fe	< DL	< DL	< DL	< DL	< DL	56.6 <i>0.1%</i>	10.1 <i>0.0%</i>
Mn	343 <i>18.2%</i>	691 <i>34.8%</i>	524 <i>42.4%</i>	676 <i>35.2%</i>	627 <i>35.5%</i>	169 <i>30.5%</i>	1347 <i>20.1%</i>
Mo	9 <i>37.5%</i>	11.4 <i>37.4%</i>	6.1 <i>15.2%</i>	4.5 <i>14.7%</i>	9.9 <i>32.7%</i>	3.9 <i>10.9%</i>	7.5 <i>33.6%</i>
Ni	2.5 <i>1.8%</i>	4.4 <i>4.0%</i>	13.4 <i>12.8%</i>	10.6 <i>11.1%</i>	4.7 <i>4.1%</i>	4.5 <i>7.6%</i>	6.9 <i>1.5%</i>
P	23.4 <i>1.8%</i>	9.8 <i>0.5%</i>	5.4 <i>0.4%</i>	< DL <i>0.0%</i>	9.9 <i>0.7%</i>	26.5 <i>2.7%</i>	27.2 <i>2.6%</i>
Pb	< DL	< DL	< DL	< DL	< DL	< DL	< DL
Sn	< DL	< DL	< DL	< DL	< DL	2.3 <i>0.9%</i>	< DL
Sr	116 <i>77.9%</i>	80.6 <i>69.5%</i>	72.3 <i>73.6%</i>	99.7 <i>83.1%</i>	97.2 <i>77.1%</i>	115 <i>74.9%</i>	80.4 <i>57.1%</i>
Ti	< DL	< DL	< DL	< DL	< DL	< DL	< DL
V	< DL	< DL	< DL	< DL	< DL	< DL	< DL
Zn	9.7 <i>0.8%</i>	13 <i>1.2%</i>	157 <i>11.8%</i>	143 <i>9.9%</i>	24.2 <i>1.9%</i>	31.3 <i>2.5%</i>	12.3 <i>0.7%</i>

Table S2: IL-6 concentration reported to total protein and control, in BEAS-2B cells exposed to different fractions of PM<sub>2.5-0.3</sub> samples collected under urban influence –seasonal and yearly average (U-Sp, U-Su, U-A, U-W, U-Y), industrial influence (I), and traffic influence (T), at the dose 24 µg/cm<sup>2</sup>, represented by means and standard deviations over 3 biological replicates; Mann-Whitney U-test vs. controls; \*=  $p < 0.05$ .

	<b>Fraction</b>			
	<b>PM</b>	<b>WSE</b>	<b>OE</b>	<b>dPM</b>
U-Sp	1.8* ± 0.2	0.9* ± 0.1	1.2* ± 0.1	3.1* ± 0.5
U-Su	1.9* ± 0.2	0.9 ± 0.2	1.4* ± 0.1	1.4* ± 0.1
U-A	2.0* ± 0.8	0.8* ± 0.1	1.1* ± 0.1	1.9* ± 0.4
U-W	2.5* ± 0.5	0.9 ± 0.1	1.2* ± 0.2	1.5* ± 0.2
U-Y	1.8* ± 0.4	0.5* ± 0.1	0.9* ± 0.1	3.6* ± 0.4
T	1.6* ± 0.3	0.5* ± 0.1	1.3* ± 0.1	4.9* ± 0.2
I	1.8* ± 0.1	0.5* ± 0.1	0.9* ± 0.1	3.2* ± 0.3

Table S3: Correlation coefficient (Spearman) oxidative stress parameters and PAHs (n=48), and metal (n=24) concentrations. Only correlations with  $p < 0.05$  are reported (\*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

	<b>IL-6</b>	<b>ROS</b>	<b>Carbonylated proteins</b>	<b>8-OHdG</b>	<b>8-isoprostane</b>
<b>PAHs</b>			Acy: 0.33	Nap: 0.37	Ace: 0.56***
			Ace: 0.38**	Ace: 0.46**	Phe: 0.42***
					Ant: 0.37**
					Fla: 0.36
					Pyr: 0.35
<b>Elements</b>	Ba: 0.43	Ca: 0.44	Al: 0.42		Al: 0.54**
	Mn: 0.48	Cd: 0.43	As: 0.50		As: 0.48
	Mo: 0.57**	Sr: 0.55**	Cu: 0.45		
	Na: 0.43		K: 0.42		
	P: 0.45		Pb: 0.43		
	Sn: 0.45				
	Cl <sup>-</sup> : 0.57**				
	SO <sub>4</sub> <sup>2-</sup> : 0.86***				
	NH <sub>4</sub> <sup>+</sup> : 0.76***				

Figure S1: PAH contents ( $\mu\text{g/g}$ ) for  $\text{PM}_{2.5-0.3}$  collected under urban influence (U-Sp, U-Su, U-A, U-W, U-Y), industrial influence (I), and traffic influence (T).

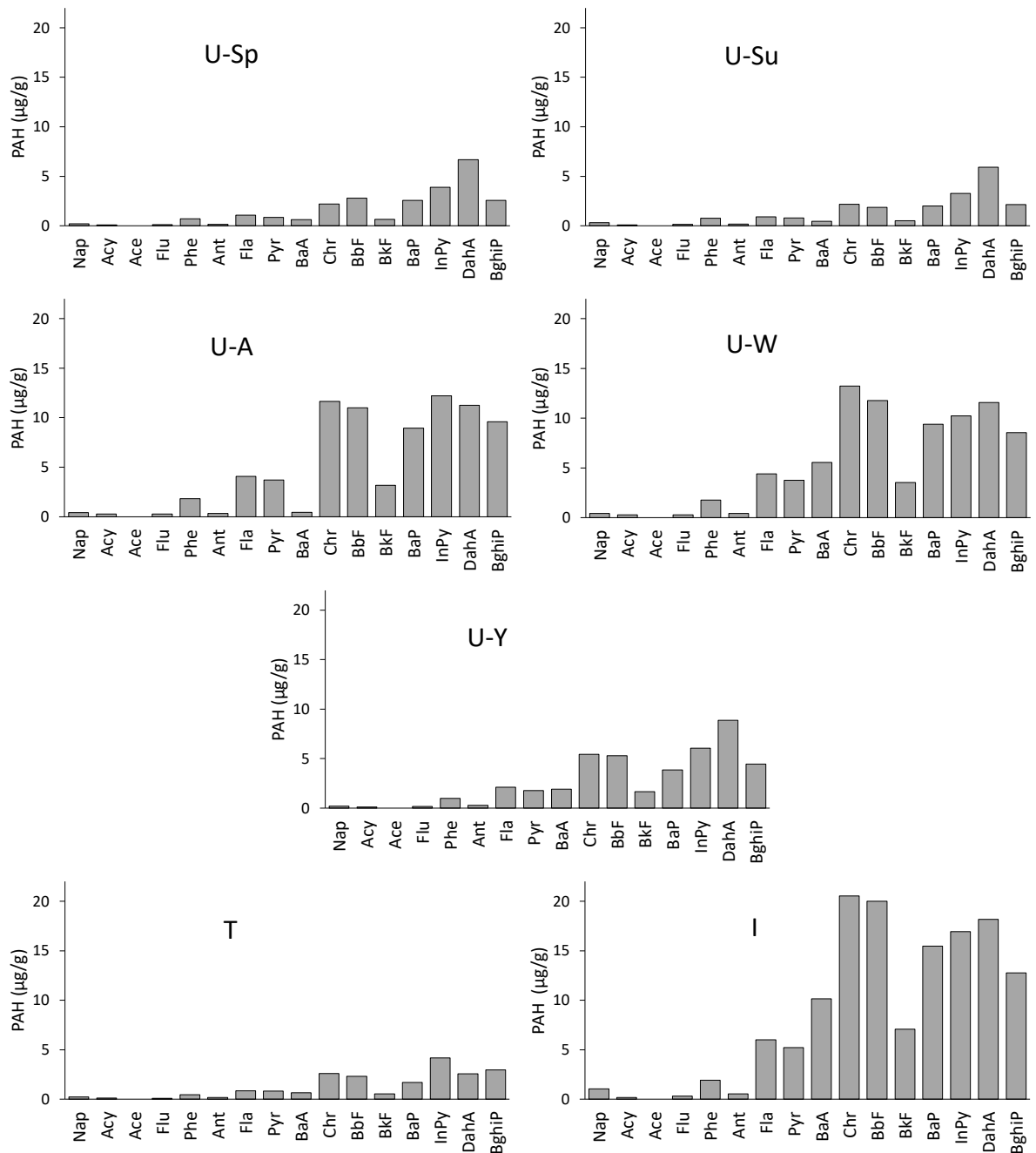


Figure S2: IL-6 concentration relative to total protein and control in BEAS-2B cells exposed to the different fractions of PM (dPM, OE, WSE, PM) samples at 24  $\mu\text{g}/\text{cm}^2$ . Results are represented by median and interquartile ranges (n=21 for  $\text{PM}_{2.5-0.3}$ ); Mann-Whitney test: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

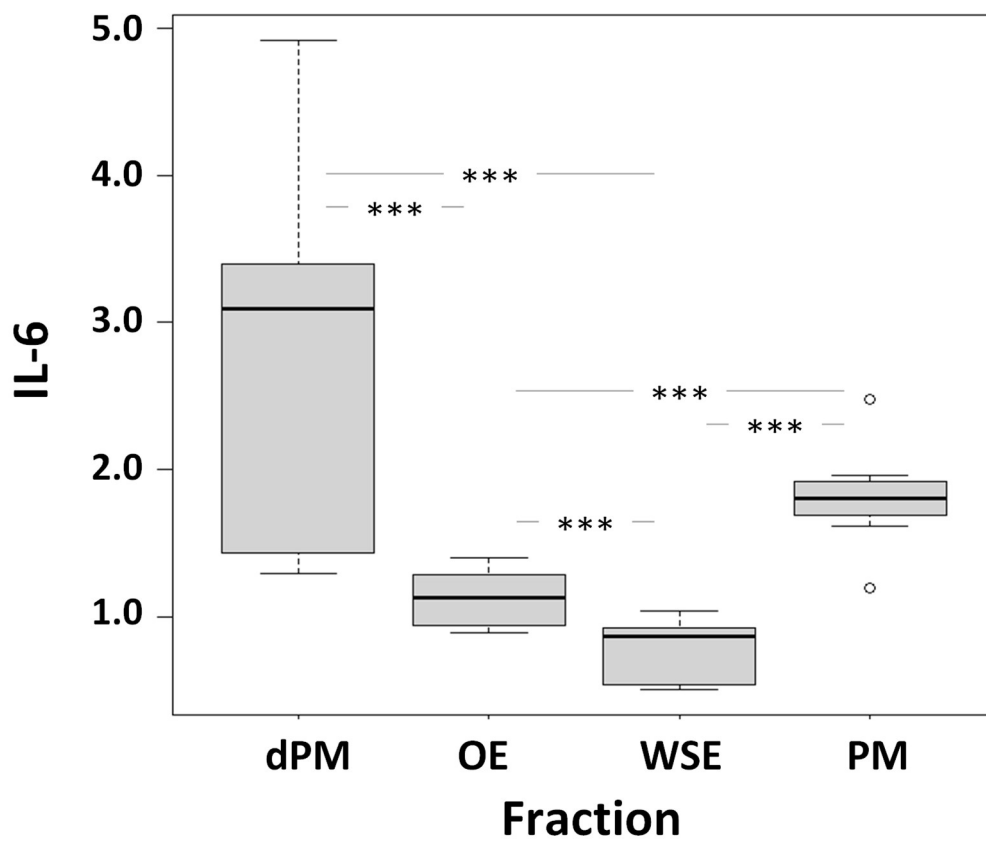


Figure S3: PCA performed considering the 4 parameters ROS, 8-OHdG, carbonylated proteins, and 8-isoprostane, on n=84 samples - Representation of the samples in the (1,2) plane after classification.

