Electronic Supplementary Material (ESI) for Metallomics. This journal is © The Royal Society of Chemistry 2019

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Supplementary material

2 Supplementary file 1: Extraction of BAD from filters

3 The ventilation duct filter contained a yearly collection of abrasion dusts from commercial vehicle (CV) drum brakes that were manufactured by large companies (such as Volvo and Scania) and used in 4 5 European buses and trucks. The filter contained particles produced during the year 2008 but the 6 specific formula and raw materials that were used in the components cannot be disclosed as this is 7 intellectual property of the manufacturers. Braking was performed in an environmental chamber, fed 8 with filtered air, across a range of speeds and temperatures using a standard CV dynamometer with 9 flywheels, an electric engine and a CV brake installation. Consequently, the sample represents the 10 brake abrasion products of heavy traffic under urban driving and high-speed braking conditions. Air 11 within the chamber was maintained at approximately 20°C and 50% humidity. BAD was extracted 12 from 1 cm³ sections of filter through submersion in 10 mL of high performance liquid 13 chromatography grade methanol with 10 minutes of vortexing. The methanol-solutions were 14 sonicated for 10 minutes at an amplitude of 15 microns, decanted into a pre-weighed 50 ml Falcon 15 tube and dried under a stream of nitrogen gas at 37°C. The dried extract was allowed to equilibrate to room temperature for 24hour prior to re-weighing to establish the extracted mass. Stock solutions 16 were prepared in sterile PBS (1 mg/ml) and stored at -80°C. 17 18 19 20 21

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Supplementary Figure 1: Depletion of (**A**) ascorbate and (**B**) glutathione from a synthetic RTLF model following 4h incubations with BAD, SRM-2975 (diesel) or SRM-1648a (an urban PM_{2.5} positive control) all at a final concentration of 50 µg/ml. Values are displayed as percentages of a particle-free control and represent the mean \pm SE of 3 replicate incubations. Significance differences between values and particle-free control were detected by 1-way ANOVA tests with Bonferroni correction for multiple testing, * p ≤ 0.05, *** p ≤ 0.001

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Supplementary Figure 2: Representative bright field images of U937 cells after treatment with
particle-free media (A) BAD (B) and SRM-2975 (C) (4 µg/ml) for 24 h. These images demonstrate
that particles were ingested by the macrophages (scale bar 5 µm). The images were acquired using an
IN Cell Analyser 6000 (GE Healthcare) with a 40x objective. Image analysis was performed using IN
Cell Developer V1.9.3 (GE Healthcare).



Supplementary Figure 3: Concentrations of IL-8 (**A**, **B**), TNF- α (**C**, **D**) and IL-10 (**E**, **F**) measured within the supernatants of U937s after 24 h exposure to BAD (**A**, **C**, **E**) and SRM-2975 (**B**, **D**, **F**) in the presence of metal chelator desferroxamine (5 µg/ml). Values are expressed as percentages of particle-free controls and normalised to total cellular protein concentrations with error bars depicting the SEM generated during 5-7 replicates. Significant differences in cytokine concentration were identified between control and particle-treated cells using 1-way ANOVA tests with Bonferroni correction. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001.





Supplementary Figure 4: Concentrations of IL-8 (**A**, **B**), TNF- α (**C**, **D**) and IL-10 (**E**, **F**) measured within the supernatants of U937s after 24 h exposure to BAD (**A**, **C**, **E**) and SRM-2975 (**B**, **D**, **F**) and subsequent incubation in particle-free media for 24 h. Values are expressed as percentages of particlefree controls and normalised to total cellular protein concentrations with error bars depicting the SEM generated during 5-7 replicates. Significant differences in cytokine concentration were identified between control and particle-treated cells using 1-way ANOVA tests with Bonferroni correction. * p ≤ 0.05 , ** p ≤ 0.01 , *** p ≤ 0.001 .



Supplementary Figure 5: The impact of BAD and SRM-2975 exposure on *S. aureus* growth. Data
points represent the number of *S.aureus* colonies that grew in MH broth (grey) or MH broth
supplemented with 25 µg/ml BAD (A), 25 µg/ml SRM-2975 (B) or 50 µg/ml gentamicin (A and B)
after 1,2 or 3 hours of incubation at 37°C. Data are shown as a percentage of the time 0 colony counts
for their respective treatment group and error bars represent the SE (n=4). 2-way ANOVA tests with
Bonferroni corrections indicated that there were no significant differences (p values > 0.05) in colony
counts between treatment groups at any time point.



77Supplementary Figure 6: Quantities of S. aureus ingested by U937s over a 2h period subsequent to7824 h incubation with 1 µg/ml LPS or 1 µg/ml benzopyrene. Values were normalised to concentration79of total cellular proteins and presented as percentages of a particle-free control and represent mean \pm 80SEM of n=6. Significant differences in CFU were identified between control and LPS treated cells81using 1-way ANOVA tests with Bonferroni correction. * p \leq 0.05.



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