SUPPLEMENTARY METHODS

AE-ALI system exposure duration estimation

The previous *in vivo* AgNP exposure study (Seiffert *et al.*, 2016) estimated an alveolar dose of 19 µg AgNPs (equivalent to approximately 1.3 x 10^{12} particles) per rat. Values in the literature for rat lung surface areas show some variation (e.g. 0.29 m^2 to 0.55 m^2 Frochlich *et al.,* 2016), however, assuming an indicative alveolar surface area of 0.4 m^2, (Ji and Yu, 2012) gives a deposition of approximately 5 ng/cm^2 or 3 particles/µm^2 which, assuming a cell diameter of around 20 µm, equates to approximately one thousand particles per cell. The objective for the AE-ALI exposures was to expose cell cultures to three different doses: a level approximating the *in vivo* study and a lower (x 0.3) and higher (x 3) dose. The intention was to use the same aerosol for all exposures so the deposited dose was controlled by varying the exposure duration. The required exposure duration in each case, T (mins), was estimated using the following formula: T = (D x A)/(MC*FL*DEff), where D (ng/cm^2) is the desired dose level, A (cm^2) is the surface area of the exposed area of cells (diameter 6.5 mm), MC is the aerosol mass concentration (µg/m^3), FL (L/min) is the aerosol flow rate to the cells (0.005 L/min), and DEff is the deposition efficiency, 0.39, which was derived from (Aufderheide *et al.*, 2011). Mass concentration was calculated as described (Seiffert *et al.*, 2016) gravimetrically using Pallflex® emfab™ filters (Pall Life Sciences, Ann Arbor, MI, USA) with the aerosol drawn at 2 L min −1 with continuous monitoring using a TEOM™ ambient particulate monitor (Model 1400a, Thermo Scientific, Franklin, MA, USA).

Laser ablation ICP-MS measurements

Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) analyses of samples of lung tissue sections mounted on microscope slides were undertaken using a New Wave Research NWR213 laser ablation system (Electro Scientific Industries, Portland, Oregon, USA) linked to a iCAP Q ICPMS (Thermo Fisher Scientific, Hemel Hempstead, UK). Laser ablation conditions comprised 5 µm diameter spot size, fluence 10 J/cm^2, 25 µms^{-1} scan speed and a repetition rate of 20Hz. The cell gas was helium which was run at a flow of 0.9 mLmin^{-1}. Helium was also used as the collision gas in KED mode. Sample was introduced into the ICP-MS via a micro flow nebuliser. The ICP-MS was run in KED mode and the isotopes monitored were ^{13}C and ^{107}Ag. Dwell time for each isotope was 0.05 s. Scan log files were generated to allow reconstruction of the data into an image. Image generation was achieved using Iolite v3 (Paton *et al.*, 2011) within Igor Pro 6.36 (Wavemetrics Inc. Oregon, USA). Basically, scan log file was aligned with the ICP-MS data exported from Qtegra as an excel .csv file, to identify data of interest. Baseline values were then subtracted using the data reduction scheme using

^{13}C as the index channel. For quantification of Ag relative to control ^{107}Ag and ^{13}C content were analysed across 4 different transverse lines through 3 different airway walls for both bronchiolar and alveolar structures (n=3 animals). ^{13}C was used as a spatial quantitative reference for the amount of tissue present within the ablated samples. Ag was used as a representative isotope of Ag present as either AgNPs or Ag+ ions.

Ag content determined by ICP-MS

Transwell inserts (diameter in 6.5 mm) without cells were exposed to AgNPs in the AE-ALI system. The membranes were digested by acidification of each sample with 1 mL of 20% nitric acid and using an UltraWAVE ™ microwave digester with 22 position rack, 660 terminal and easyCONTROL software. Then the samples were heated for 25 min at 200°C, a pressure of 160 bar and a power of 1500 W. Once cooled, samples were filtered with a 200 nm syringe filter, diluted with 10 mL deionized water and stored at room temperature. Total Ag content was measured by an iCAP Q ICP-MS (Thermo Fisher Scientific, Hemel Hempstead, UK). The ICP-MS was run in KED mode and the isotopes monitored were ^{107}Ag and Ir-193, which was used as an internal standard. Calibration standards (0 – 10 µg/L) were prepared from a Spex CertPrep 10 mg/L stock solution.

Lactate Assay

The lactate assay is based on the conversion of lactate to pyruvate by lactate dehydrogenase (LDH) activity reducing NAD to NADH. NADH reduces N-Methylphenazonium methyl sulphate (PMS) to PMSH which reduces p-iodonitrotetrazolium violet (INT) to INTH. The lactate reaction mixture was TRAM buffer (82 mM Triethanolamine HCl, 8.2 mM $EDTA.Na_2$, 32 mM $MgCl_2.6H_20$) and colour reagent (0.3 mM PMS and 0.75 mM INT, 9.5% ethanol, 0.4% Triton-X-100, 4.5 mM β-NAD and 2 U/ml LDH). Lactate was determined by adding 20 µl supernatant to 150 µl reaction mixture for 30 min. The absorption of the colour product (INTH) was measured at 490 nm. A lactate standard curve with a spline fit curve was used to determine lactate concentrations from the unknown values. Assays were performed in 96-well black-side and clear-bottom plates.

PCR Primer details

Table Primers applied in real-time PCR analysis for mRNA gene expression

HPRT1 F	TCAGGCAGTATAATCCAAAGATGGT	HPRT1 R	AGTCTGGCTTATATCCAACACTTCG

Alox15 F	CCGACCTCGCTATCAAAGACT	Alox15 R	CACCAGAAAATCCGGTTGAAGT
Batf F	ATCTGATGATGTGAGAAGAGTTC	Batf R	TTCTGTGTCTGCCTCTGT
C3 F	GAGCCAGGAGTGGACTATGTGTA	C3 R	CAATGGCCATGATGTACTCG
Ccl17 F	GAAGAAGAGCCACAGTGA	Ccl17 R	AGGATAAAGACTTTAATCTGGG
Ccl2 F	TTTAGATACAGAGACTTGGG	Ccl2 R	CAAAGACCCTCAAAACATC
Ccl22 F	TCCTTGTGCTCCCAAAGTGC	Ccl22 R	AGGAAATGTAAGCAGACACG
Ccl24 F	CCTAAGGCAGGAGTGGTCTT	Ccl24 R	AGGGCTTTGGTGCTCATTG
Ccl7 F	CCAATGCATCCACATGCTGC	Ccl7 R	GCTTCCCAGGGACACCGAC
Cd177 F	CCTTGCTACCCTGTGTCCCAGC	Cd177 R	GGCAACCCTCGCTAACCTCGC
Cep55 F	AAATAACACAGTTGGAATCCTTGAA	Cep55 R	TCAGTCTCTCCTTGGAAAGTGAC
Chi3l1 F	TGCCCTTGACCGCTCCTCTGTACC	Chi3l1 R	GAGCGTCACATCATTCCACTC
Chia F	GTCAACTCGGCCATCAGGTT	Chia R	CAAGGTCAAGGCCGTCAAA
Clec10a F	AGCTGAGGTGGAGGGTTTC	Clec10a R	GTGTGCCTTCTGCGTAGTG
Clec4a F	GACCCTCACACTCAGATCATC	Clec4a R	ACGCTGGCTCAGCCACTC
Clec5a F	GGACATAACTGATGCTGAG	Clec5a R	ATTCTGATTCTGATTGGTAACA
Cxcl1 F	AACCGAAGTCATAGCCACAC	Cxcl1 R	GTTGGATTTGTCACTGTTCAGC
Cxcl2 F	CGCTGTCAATGCCTGAAG	Cxcl2 R	GGCGTCACACTCAAGCTCT
Cxcl5 F	TGGACGGTGGAAACAAGG	Cxcl5 R	CTTCCCTGGGTTCAGAGAC
Defb4 F	GAAGCTCCCAGCCATCAGCCR	Defb4 R	GTCGCACGTCTCTGATGAGGGA
Duox1 F	TAAAGTCCATGAAGGGTCTC	Duox1 R	CCAACCAGAAGTGTCCAT
Fabp5 F	AATTAGTGGTGGAGTGTG	Fabp5 R	AGAAAGAAACAGTATGGAGAT
Fcgr2b F	CCCTAGCTCCCAGCTCTTCA	Fcgr2b R	TGCAGTAGATCAAGGCCACTACA
Foxp3 F	TGCAGGGCAGCTAGGTACTTG	Foxp3 R	TCGGAGATCCCCTTTGTCTTATC
Hmox1 F	TCACTGTGTCCCTCTCTC	Hmox1 R	ATTGCCTGGATGTGCTTT
Il1b F	CCACATTCTGATGAGCAACC	Il1b R	GAGAGCACACCAGTCCAA
Il1r2 F	TTTCTGCCTTCACCCTTCAG	Il1r2 R	GGCACCTCAGGGCTACAG
Itgam F	AAACCACAGTCCCGCAGAGA	Itgam R	CGTGTTCACCAGCTGGCTTA
Lcn2 F	AGACAAAGACCCGCAAAAG	Lcn2 R	TGGCAACCTGGAACAAAAG
Lilrb4 F	CTGTGTCAGTCACGGAGCC	Lilrb4 R	GCAGGTAGTGGGAGAAGCC
Mmp12 F	GCTAGAAGCAACTGGGCAAC	Mmp12 R	ACCGCTTCATCCATCTTGAC
Mreg F	GAGCCCCTCGTCAGTGATAA	Mreg R	TGAGCTTCTGCCACTCCTCT
Mt1a F	GCAAATGCAAAGAGTGCAAA	Mt1a R	CAGCTGCACTTCTCTGATGC
Mt2A F	TGCAGCGATCTCTCGTTGAT	Mt2A R	AGCAGGATCCATCTGTGGCA
Muc1 F	AGAGAAGTTCAGTGCCCAGC	Muc1 R	TGACATCCTGTCCCTGAGTG
Noxo1 F	AGATCAAGAGGCTCCAAACG	Noxo1 R	AGGTCTCCTTGAGGGTCTTC
Orm1 F	ATGTCAACTGGTTCCGGAAGGACA	Orm1 R	TTTCAGGTTCAGGGATCCTCCTT
Rnase9 F	CTGGTGCAGTTTCAAGAGGTG	Rnase9 R	TCCCCCGGGCTAGGGCGATATGA
S100a9 F	GCTCCTCGGCTTTGACAG	S100a9 R	TGATGGTCTCTATGTTGCGTTC
Serpinb10 F	GTGACCCTGAAAGTGAAA	Serpinb10 R	TATATCGCATTGGCTGTT
Sftpd F	GCTGCTTTCTCTCAGTATAAG	Sftpd R	TGCCTCCGTAAATGGTTT
Slc26a4 F	CATCAAGACATATCTCAGTTGGACCT	Slc26a4 R	ACAGTTCCATTGCTGCTGGAT
Slc39a2 F	GTTTGCCCTGTTGGCTCTCA	Slc39a2 R	ATCAATCTGGAACCATTTGAAGC
Spp1 F	CCCACAGACCCTTCCAAGTA	Spp1 R	GTCAATGGAGTCCTGGCTGT
Tac1 F	ACTGTCCGTCGCAAAATC	Tac1 R	GGGCCACTTGTTTTTCAA
Tac4 F	GTAGGGCAGGAGGAAGAAGATGT	Tac4 R	CAAGGTGGTCAAAATGATGATTGT
Tlr10 F	GCCCAAGGATAGGCGTAAATG	Tlr10 R	ATAGCAGCTCGAAGGTTTGCC
Tnfrsf8 F	GCTGGGACTGATTCATTCATTC	Tnfrsf8 R	CGGACGACCAACCTCCCATG
Trem1 F	GGCCACACCAACCTTCTG	Trem1 R	AGTGCCTGCCTCAATGTCTCCA
Trem2 F	GCATCTTTCTCATCAAGATTCTAGC	Trem2 R	CTGGCAGAGTTTGGAGCTGATAC

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