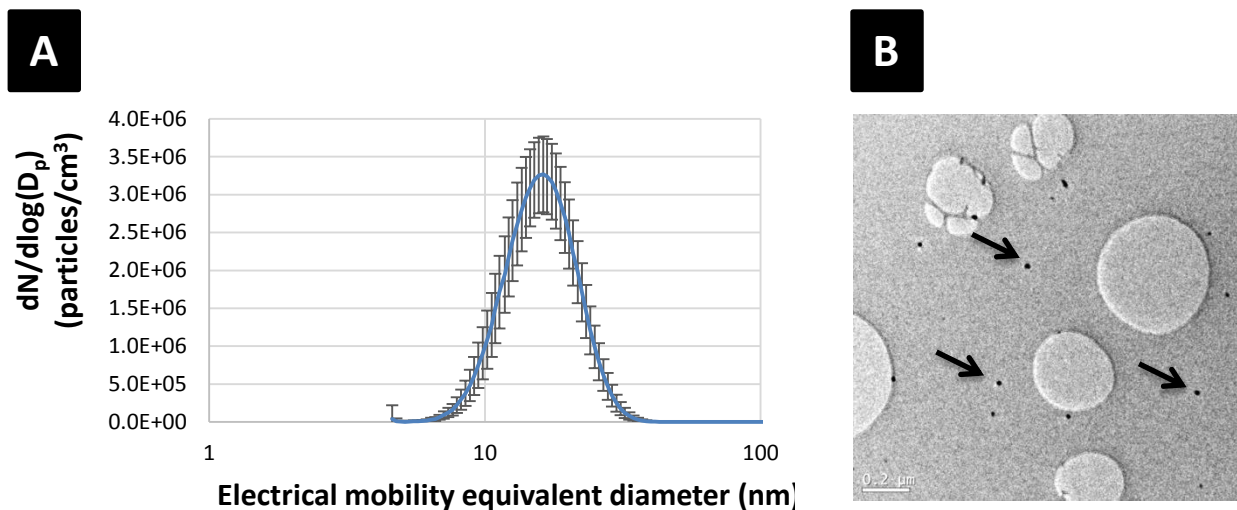
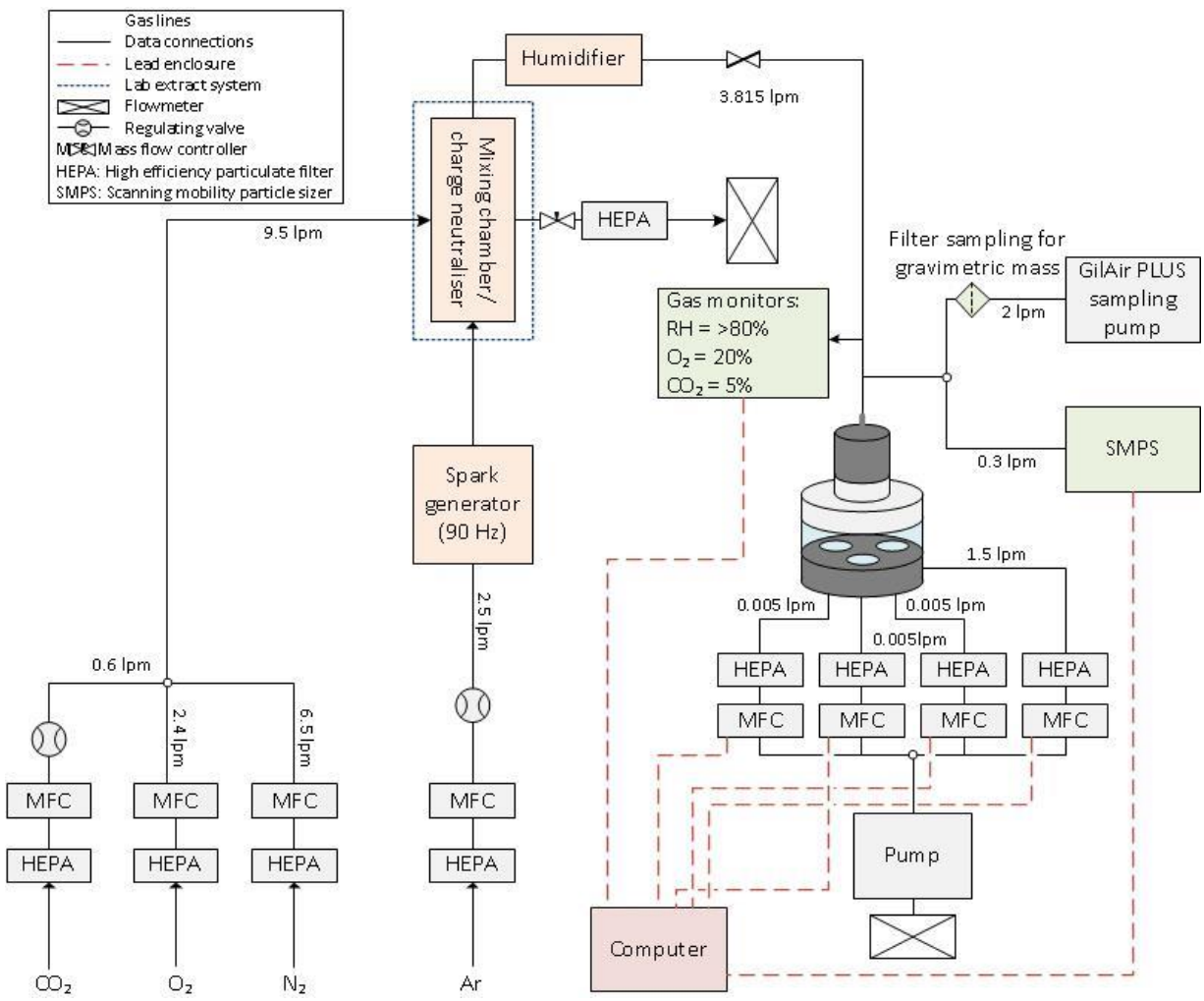


Supplementary Figure 1



Supplementary Figure 1 Characterisation of spark generated AgNP aerosols for in vitro exposures. (A) Average number-weighted aerosol size distribution for all exposures measured with an SMPS. (B) Representative TEM image of particles (indicated by arrows) deposited onto grids placed at the bottom of the wells (with no cells). Larger objects are part of grid structure.

Supplementary Figure 2



AE-ALI System Schematic

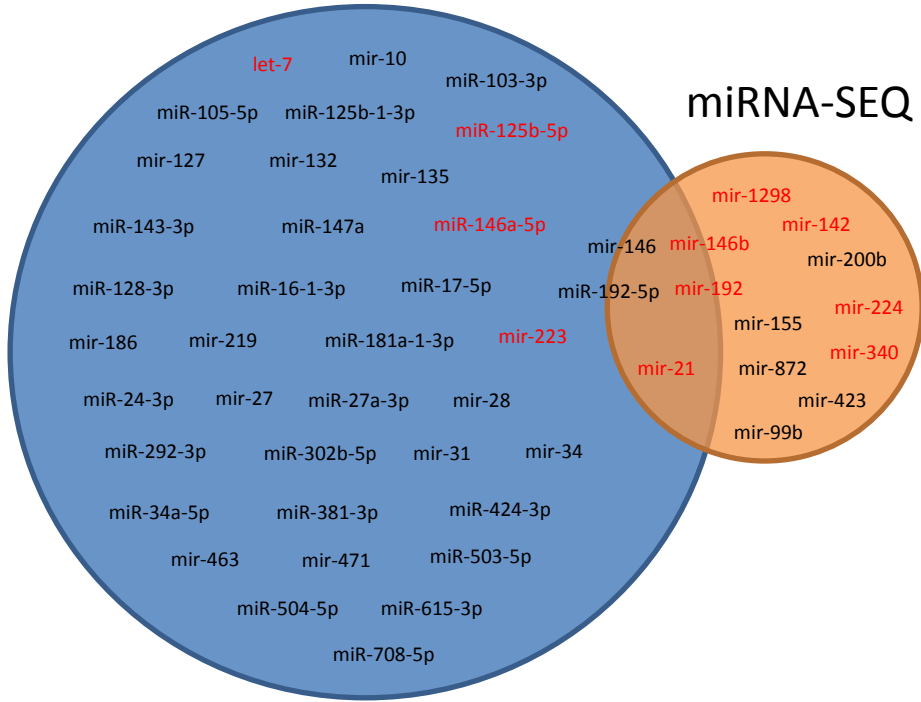
Supplementary Figure 2 Aerosol-exposure air-liquid-interface (AE-ALI) system schematic

Supplementary Figure 3 Full list of mRNA genes as identified as being involved in the in vivo pulmonary response elicited by deposited AgNPs, together with fold change for both Day 1 group comparison (Day 1 Air vs Day 1 NP) and Day 7 group comparison (Day 7 Air vs Day 7 NP).

Feature ID	Day 1 Change	Day 7 Change	Feature ID	Day 1 Change	Day 7 Change	Feature ID	Day 1 Change	Day 7 Change	Feature ID	Day 1 Change	Day 7 Change
Osm1_1	88.8544	12.55123	ENSRNOG00000021166_1	2.737471	3217183	Advrn1_1	1.781103	1.069798	Gtd80_1	1.240788	0.618567
Sic7a6_1	29.9725	4.75062	ENSRNOG00000031947_1	2.73084	0.52569	Icos_1	1.778016	1.405462	Sg6_1	1.235099	2.03831
Lnc2_1	27.30732	4.392821	Ccnb1_1	2.721706	1.319167	Rps24_1_1	1.777993	1.035938	Pfpmr_1	1.221727	2.392864
Ccrl5_1	23.09598	2.822472	Cenp1_1	2.717151	1.57277	ENSRNOG00000029996_1	1.775844	0.855833	Hicr1b_1	1.216333	0.562804
C12orf1_1	21.23532	1.805715	Bubb1_1	2.697492	1.667492	Cdrl1_1	1.77667	1.021929	Hr1b_1	1.21232	2.304016
RGD1565410_1	20.11676	4.56582	Tnf1_1	2.68331	1.519515	Mklnp_1	1.767993	1.285534	RGD1309362_2_1	1.18889	0.553938
Cd177_1	18.04014	3.186922	Rasaf1_1	2.66576	1.23422	C12orf1_1	1.769979	1.158450	Gpr88_2_1	1.186754	1.590465
ENSRNOG0000004783_1	17.68812	1.90965	Ncapg_1	2.646523	1.566253	Plazp7_1	1.766625	0.929237	Rasl1_1	1.159305	1.953198
Mmp12_1	15.19761	1.290658	Tkl_1	2.611008	1.1165	Myh7b_1	1.756313	1.338607	Cmpk2_1	1.145533	0.64245
C11orf1_1	15.04035	4.774685	Sik7a3_1	2.592809	1.058214	Gpr173_1	1.752292	0.979581	Ghsp2_1	1.145159	0.575763
Rnae9_1	11.14308	2.78411	Pap1_1	2.585076	1.233005	Incomp_1	1.742215	1.251885	Ghsp7_1	1.142587	0.52888
Retr1b_1	10.88788	2.026464	Ckbl_1	2.582224	1.348277	Wnt4_1	1.739791	1.006555	Rasl1_1	1.139441	1.982555
Ccrl1_1	10.49879	0.456	Neil3_1	2.564941	1.008258	Ube1c_1	1.73421	0.851225	Shng11_1	1.121297	1.916033
Spp1_1	9.797125	2.709457	Mh2a_1	2.543	2.55241	Patp1p_1	1.733564	1.118613	Stat1_1	1.108462	0.626461
Rnae11_1	9.580593	3.601424	Nr4a1_1	2.52471	1.232871	Cd81_1	1.730322	1.745173	ENSRNOG00000049123_1	1.078041	2.579194
H1r1_1	9.705997	1.920318	Uhrf1_1	2.523856	1.083631	Nr1h3_1	1.727936	1.45178	ENSRNOG000000049123_1	1.073025	1.753536
ENSRNOG00000048564_1	6.953763	0.600894	Kntc1_1	2.510944	1.223838	Sfnf13_1	1.726833	0.917871	Sytl4_1	1.069661	2.85429
Cd171_1	6.791436	1.385694	Htt1_1	2.507374	1.364797	SLC16A11_2	1.725855	1.578216	Lay1_1	1.056757	1.710322
Cd9p_1	6.643137	3.067617	Chc1_1	2.495528	2.325628	Gpr114_1	1.725025	0.856723	Gbps_1	1.054554	0.541775
Erf2_1	6.36432	2.369578	Clec4a_1	2.489191	1.005	Krt11h_1	1.711106	2.0462	Plac8_1	1.054474	0.606693
Gd7_1	6.239028	1.374199	Batf1_1	2.473033	1.057273	Altp1a3_1	1.708759	0.76541	Spre3_1	1.053783	1.790142
Cd2_1	6.162632	1.566707	Bard1_1	2.462054	1.194864	Myo12_1	1.70565	1.569995	Hfr_1	1.046221	1.63841
Tfrc4_1	5.700971	1.414125	Batf1_1	2.458225	0.853022	Frs1_1	1.692408	1.279291	Vaw1_1	1.027256	1.591024
Sik7a11_1	5.517521	1.243218	Ticrr_1	2.443311	1.313764	MYO2_1	1.701172	1.299709	Tap1_1	1.013891	0.644574
Nkox1_1	5.375701	1.555907	Cxcl10_1	2.432851	0.392828	Hfr_1	1.699066	1.068119	Cor1_1	1.013379	2.381957
Rhsm2_1	5.317353	1.624125	Hmox1_1	2.432147	1.142147	Pfz1a1_1	1.698325	0.640355	ENSRNOG00000002619_1	1.008214	1.898134
ENSRNOG00000037076_1	5.154769	2.242089	Trh1_1	2.41915	1.623908	Hfr_1	1.696587	1.074481	Sell_1	1.005638	0.613931
Ccrl1_1	5.132187	1.520132	Nkx211_1	2.41381	1.108802	Tc2n_1	1.69494	1.335497	Rnae6_1	0.993406	0.527607
Fcar_1	5.113399	1.365593	C4bp1_1	2.428947	1.363017	Adrb2b_1	1.693531	1.535683	Gkac_1	0.984578	1.542105
NSD1568	4.978468	1.479648	Fetua_2	2.410515	1.871736	Mitf_1	1.686666	1.757198	ENSRNOG00000000787_1	0.979993	1.537324
Pcp2_1	4.860803	2.640328	Gpr35_1	2.40852	2.23513	Nkx1b_1	1.685305	1.148892	Odn1_1	0.854599	0.613757
C17orf28	4.774268	3.275195	Mcm10_1	2.40577	1.290729	Npm1b_1	1.685046	1.066638	Nrcam_2	0.800058	2.860123
Cd17orf4	4.774083	1.295295	ENSRNOG00000031898_1	2.40577	1.290729	Alm1_1	1.683323	1.067655	ENSRNOG000000007087_1	0.799993	1.537324
C20orf4	4.655239	2.141905	Clec2d2_1	2.384891	0.967289	Mnrl1_1	1.680126	1.05225	ENSRNOG00000007238_1	0.776887	0.465424
Mek1_1	4.686317	1.458866	Rcat1_1	2.381367	1.293919	Cks1b_1	1.676457	1.043034	Kn3j_1	0.764748	0.468486
Sht3p1_1	4.568796	1.317224	Cep72_1	2.369808	1.401721	Mbp_1	1.675771	0.816128	Myh13_1	0.760396	2.600224
Mab21b_1	4.498431	1.587283	ENSRNOG00000002619_1	2.369808	1.401721	Ncf1_1	1.674127	1.114734	Nfya_1	0.742102	0.613931
H1h1_1	4.46093	3.53207	C12orf1_1	2.356604	0.974413	P7256_1	1.672556	1.199249	Nf1c1_1	0.724373	0.385713
Ube2c_1	4.31863	1.357182	Tmem171_1	2.350225	1.838113	Entpd3_1	1.672227	1.366902	Hmbox2_1	0.718209	0.380261
Exo1_1	4.264873	1.42368	Tnfrsf9_1	2.348629	1.164202	ENSRNOG00000038375_1	1.670834	0.980176	Pfz1_1	0.714116	0.438069
1700061919R1_1	4.26285	1.247955	Hsk_1	2.348184	2.536736	Mout1_1	1.670608	1.357389	Wdr1_1	0.712805	2.09768
Mist1b_1	4.20245	1.251207	Slamf8_1	2.335795	2.152323	Nkx1b_1	1.669253	0.883143	Bgntm1_1	0.686851	0.662339
lapp1_1	4.188857	1.805418	Gat1_1	2.33068	0.974301	Nippapab3_1	1.669002	1.161948	Nran1_1	0.666169	0.901352
Rgs1_1	4.152895	1.212626	Rubt1_1	2.317103	1.031023	Rmgp2_1	1.667103	1.711755	Sirt1b_1	0.661718	0.911444
Fgr2b_2	4.111064	1.756308	Abcb4_1	2.316676	1.403753	Fgfr1a_1	1.66485	0.991694	Ier1_1	0.661375	0.993161
Skat1_1	4.094703	1.442105	Ragap1_1	2.315945	1.31288	Npcr_1	1.662883	1.461546	Gn1_1	0.660268	0.866847
Kntm1_1	4.089966	1.39522	Fetua_2	2.311867	1.752118	Chst1_1	1.66175	1.33352	Ecm1_1	0.658463	0.880777
Cenpw_1	4.074147	1.569915	KRXL1_1	2.308488	1.308359	ENSRNOG00000000787_1	1.660348	1.067655	Wdr1_1	0.656731	0.652344
Famil11a_1	4.015862	1.538291	Sod1_1	2.303123	1.118497	Mnrl1_1	1.659569	1.439641	Veg1a_1	0.656454	0.927588
Dl1_2	4.014703	1.347396	Duo1_1	2.301103	2.48377	Chk1_1	1.654621	1.045896	Dmnpb_1	0.656252	1.551283
Cd8a1_1	3.955501	1.42584	Dnrl1_1	2.300503	0.989877	MHO2_1	1.651177	1.053896	NKX2_1	0.654662	0.910857
C2orf1_1	3.877653	1.317787	Irga1_1	2.299914	0.860934	Birc1_1	1.651005	0.870333	RAP1GAP_1	0.653545	1.028256
ENSRNOG00000032086_1	3.86441	0.818377	Fabp5_1	2.291457	1.545776	Polr2h_1	1.643156	0.894383	rXs_1	0.653349	1.203874
Cenph_2	3.858102	1.805418	Gims1_1	2.289277	0.889524	Polr2h_1	1.643156	0.894383	G04897	0.649887	0.966442
Cenph_2	3.858102	1.805418	Dccr1_1	2.271283	1.031023	Pmaip1_1	1.642137	1.738149	ENSRNOG00000000787_1	0.649887	0.966442
Cenp1_1	3.805105	1.305878	Fgl1_1	2.270727	0.874259	Fgfr1a_1	1.637011	1.194587	Dir1_1	0.645727	1.089013
Cd2_1	3.770568	1.517956	Frrb_1	2.262635	1.020941	Klf18a1_1	1.629027	0.845359	Jaag1_1	0.640226	1.066814
ENSRNOG000000028690_1	3.754003	0.979376	Pkar1p_1	2.260909	2.270945	TRPM1_1	1.627236	1.721471	Wdr7_1	0.638402	1.077743
Hfr1_1	3.740531	1.370388	Hax1_1	2.257807	1.020941	Argp1_1	1.626573	1.540765	Duo1_1	0.638293	0.848233
RGD1305807_1	3.730843	2.38053	224c83	2.20839	1.20839	ARGP1_1	1.626577	1.250477	MOXB1_1	0.627153	1.054411
Cenpa_1	3.716658	1.349919	RPS2-PSE_1	2.204729	0.989894	z3h12a1_1	1.625778	1.158549	Pfmr2_1	0.624197	2.138407
S100b_1	3.715908	1.480705	Cenpe_1	2.202597	1.272401	Meif2_1	1.625137	0.948796	ACSM1_1	0.620066	1.007094
Wt1_1	3.689264	1.531223	Argp1_1	2.194134	1.299183	MeiA1_1	1.62331	1.755807	Car1_1	0.614859	1.051068
Tgfr1_1	3.658248	0.853625	Tspan11_1	2.18529	1.98214	Altp13a1_1	1.621753	1.109735	Dlg5_1	0.611259	1.222118
Ank1_1	3.645228	1.793986	Hbb_1	2.175444	1.164282	Fdps_1	1.619352	1.058366	Kn3k2_1	0.610491	0.879552
Sytl2_1	3.630221	1.956565	Fam64b_1	2.173787	1.031023	Lgals1_1	1.617827	1.540765	ENSRNOG00000000787_1	0.607578	1.537324
Myh2_1	3.588242	1.97864	ENSRNOG00000045712_1	2.165851	1.034311	Krk_1	1.61753	1.487202	Balz_1	0.606474	0.914777
E2f1_1	3.586105	1.040644	Trem3_1	2.162831	1.297484	C2_1	1.615423	1.260562	Garem1_1	0.603528	0.822398
Nufr1_1	3.54149	1.257994	Ckap2_1	2.155895	1.216657	Spc24_1	1.610811	1.347881	Hoxd2_1	0.598855	0.958294
Gpr118_1	3.529178	1.030591	Amic1_1	2.156487	1.295147	Amic1_1	1.610114	1.183788	ENSRNOG00000000787_1	0.597346	0.946294
Rna12c_1	3.472356	1.627694	Mcm5_1	2.142609	1.124269	Mcm6_1	1.608811	1.070549	gpn905_1	0.59358	0.936465
Llrb1a_1	3.443962	1.207591	SAA1_1	2.104795	1.00158	Cd121_1	1.607357	1.207479	Zfp385d_1	0.592205	0.716407
C1_1	3.406506	1.484234	LY6L_1	2.10347	1.031023	Tact1_1	1.605713	1.090609	ENSRNOG00000000787_1	0.591623	1.537324
Ccn1_1	3.443496	1.659612	Irgam1_1	2.101818	1.118497	Cd121_1	1.605539	1.212713	CLEC2L_1	0.581206	1.190345
C1_1	3.441645	1.639383	Cdc45_1	2.10085	1.134511	Sic16a1_1	1.605227	1.218584	Tmem200b_1	0.580373	0.933313
Pk1_1	3.426253	1.332084	Cdh17_1	2.097059	1.331028	Cic16a1_1	1.603742	1.218983	Fam107a_1	0.579211	1.076139
Ford1_1	3.410428	2.360254	Gk_1	2.092788	1.356868	RHO1_1	1.603456	1.227066	ENSRNOG00000000787_1	0.579211	1.076139
Exo2_1	3.391539	1.084861	Afhgap11a_1	2.08196	1.090508	Flox1_1	1.59841	0.895617	Adf1_1	0.573388	0.936392
CDK9_1	3.38405	1.169371	Clec10a_1	2.06516	1.321639	Sik13a2_1	1.598114	1.834104	RGD156237b_1	0.572194	1.071113
Sapo2_1	3.377529	1.304637	C1orf1_1	2.053205	1.147906	Hfr1_1	1.597445	1.190645	Pfkfb3_1</		

Supplementary Figure 4

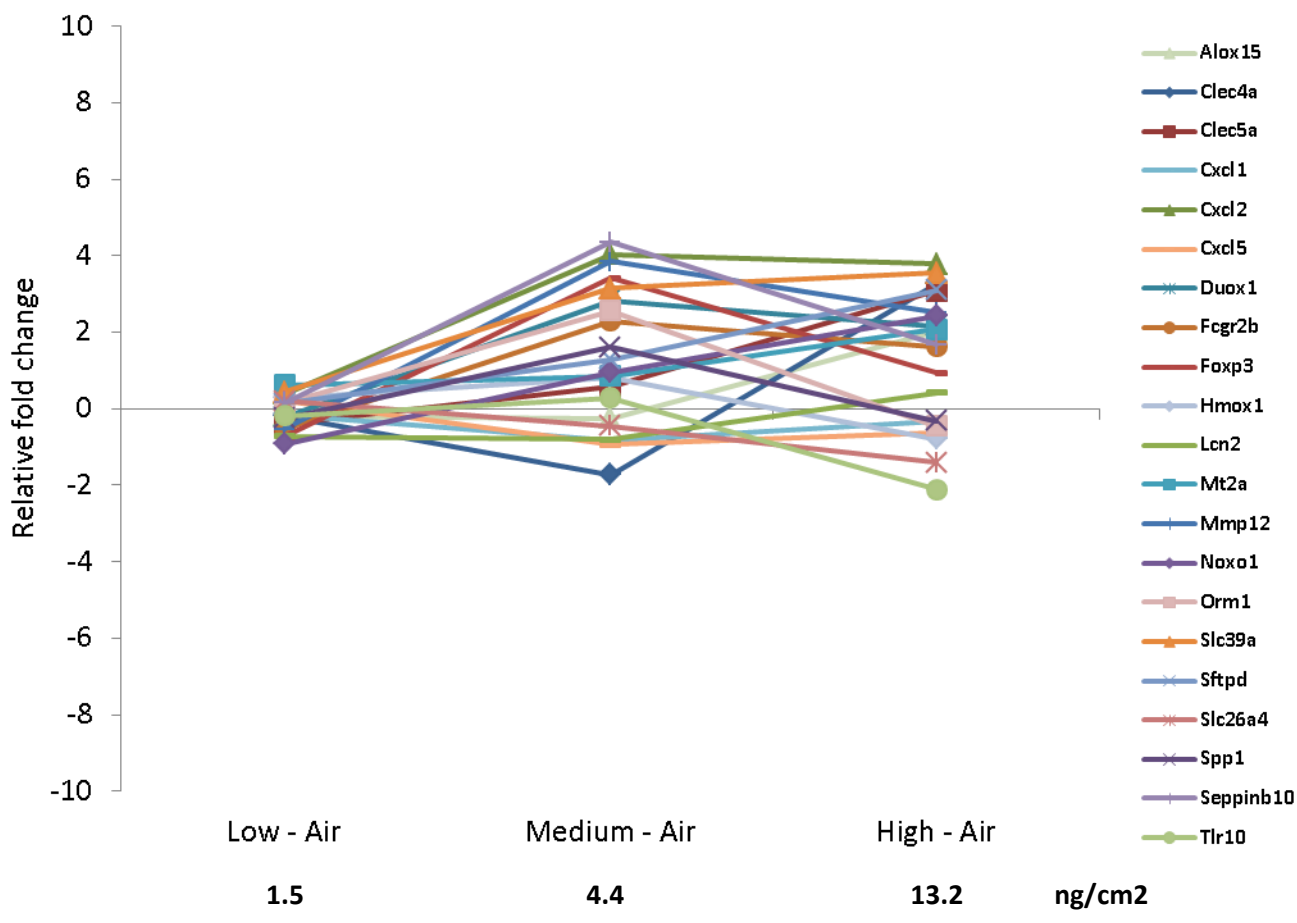
IPA Predicted from mRNA-SEQ



Supplementary Figure 4; Criteria to select miRNA for PCR Analysis:

The gene set (553 mRNA genes, $p < 0.01$ based on groups of Day 1 only) selected from mRNA sequencing analysis was put into IPA to obtain the prediction of relevant miRNA genes. There were 40 miRNA genes output, as shown in the blue part of the Venn diagram. The miRNA genes with activation z-score either above 2 or below -2 are highlighted in red. In addition, the set of 12 miRNA genes extracted from Qlucore analysis ($q < 0.05$) (Figure 2C) were plotted in the orange part of the Venn diagram, (those with fold changes over 1.5 and counts over 50 (based on groups of Day 1 only) are highlighted in red). The Venn diagram was used to depict intersections between the two groups. Three of the miRNA genes (mir146, mir192, and mir21) were highlighted in both analyses and thus were selected for PCR analysis in the in vitro sample analysis.

Supplementary Figure 5



Supplementary Figure 5 Sensitivity of a set of Stress Response genes to air exposure in SmallAIR cultures in AE-ALI system.

Supplementary Figure 6

Transcript ID	Fold Change Day 1	Fold Change Day 1	Consistent change
Mt1a_1	3.689264366	3.79331381	1.028203304
Chia_1	2.495582289	2.621564085	1.050481924
Hk3_1	2.348183669	2.586372925	1.10143553
Klk13_1	2.308358711	3.589327716	1.55492632
Duox1_1	2.301102831	2.453503912	1.066229583
Prkar1b_1	2.260909176	2.571882764	1.1375436
Gpnmb_1	2.246283023	2.287372868	1.018292372
KNG1L1_1	2.132835621	2.038910399	0.955962278
Ly6i_1	2.103740195	2.062465304	0.980380234
Fcrlb_1	1.849340913	2.07372178	1.121330181
Slc7a7_1	1.830903224	1.877904092	1.025670864
Cd68_1	1.730321599	1.821021155	1.052417745
Kng1l1_1	1.711105938	2.283995113	1.334806374
Pmaip1_1	1.642407653	1.848241397	1.125324394
Folr1_1	1.626753054	1.628737609	1.001219948
S100b_1	0.594138534	0.521361793	0.877508802

Supplementary Figure 6. Summary of RNA-SEQ identified transcripts, which were consistently regulated within lung tissue to the same level by AgNPs across Day and Day 7 post exposure.