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Supplemental Material

Air Pollution Particulate Matter Exposure and Chronic Cerebral Hypoperfusion and Measures of White Matter Injury in a Murine Model

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Table S12. nPM and BCAS networks.

Figure S1. The average particle size distribution of the exposure aerosol. Data represented as mean ± standard error. A total of 107 measured size distributions are shown. Dp(nm): particle diameter.

Figure S2. Size distribution of the aerosol inside the suspension. (*A*-*B*) Size distribution of the aerosol inside the suspension was measured by means of Dynamic Light Scattering (Dynamic Light Scattering BI-200SM (Brookhaven Instruments Inc). To analyze the possibility of agglomeration, 2 stages of filtration were performed and compared, 1000 nm filtration (upper plot) and 450 nm filtration (lower plot). There is little difference between these two plots, indicating that the number concentration is dominated by ultrafine size ranges. The green bar is manually set at the first size channel after 200 nm for each scan and the “cumulative” fraction of particles corresponding to the green bar is shown in the upper left of the figures. For 1000 nm filtration, 95% of particles were smaller than 224 nm and for 450 nm filtration ~100% of particles were below 243 nm. This indicates that only about 5% of the total number concentration is in the size ranges above 243 nm. Mode diameter is also below 100 nm. Diam: diameter; Rel Int: relative light scattering intensity; Cum. Int: cumulative intensity.

Figure S3. qPCR validation of a cohort of genes from RNA seq. Data represented as mean ± standard error. n=7 per group (FA, nPM, FA+BCAS, nPM+BCAS). qPCR: quantitative real-time polymerase chain reaction; RNAseq: ribonucleic acid sequencing. Summary qPCR data shown in Table S11. Summary RNA seq data shown in Tables S8-10.

References

Table S1. **Exposure number and corresponding data analysis.**

Exposure Number	Results	PM Mass concentration (ug/m3) [a]	Total PNC (#/cm3) [b]	PN mode diameter (nm)	TOM% (µg TOM/µg PM mass) [c]	Mortality Rate (%)
1[d] *(batch 2015a)*[e]	Behavior, Iba1, GFAP, KB, C5, C5α	330± 18	357,969	53.3	63.3±7.8[f]	0.0 (0/36)
2[d] *(batch 2016a)*[e]	Behavior, Iba1, GFAP, KB, C5, C5α	295± 22	348,341	57.3	50.1±6.2	30.6 (11/36)
3 *(batch 3)*[e]	RNA seq, qPCR	303± 26	359,660	49.5	28.1±4.3	13.9 (5/36)
4 *(batch 4)*[e]	KB, dMBP, CD88, 8-OHdG	301± 28	357,286	45.5	48.4±5.6	13.9 (5/36)
5 *(batch 5)*[e]	Western/Nitrate/Nitrite	310± 22	370,414	50.6	32.4±4.4	19.4 (7/36)
6 *(batch 6)*[e]	MRI	300± 28	368,715	50.4	26.9±2.4	44.4 (16/36)
7 *(batch 7)*[e]	MRI	300± 27	369,535	51.2	31.7±4.8	41.7 (15/36)

PNC: particle number concentration; TOM: total organic matter; PM: particulate matter; nPM: nanoscale particulate matter; KB: Klüver-Barrera; dMBP: degraded myelin basic protein; Iba-1: ionized calcium-binding adaptor protein-1; GFAP: glial fibrillary acidic protein; C5: complement component 5; C5α: complement component 5α; CD88: complement component C5α receptor; 8-OHdG: 8-oxo-2'-deoxyguanosine; qPCR: quantitative real-time polymerase chain reaction; RNAseq: ribonucleic acid sequencing.

[a] The average values reported for PM are the average of our DustTrack observations in the mid-way of each exposure day. The variation (SD) through different days are less than 10% of the reported average values.

[b] Total PNC values are recorded by SMPS for each nPM batch used for the inhalation exposure in the beginning of each exposure campaign.

[c] To account for the contributions of non-carbon atoms (e.g. oxygen, hydrogen) and effect of PM oxygenation in the ambient through photochemistry, total mass of Organic matter (OM) is reported by multiplying TOC% by a factor of 1.6 ± 0.2 recommended for urban aerosols(Turpin and Lim 2001).

[d] There were not exposure differences between Exposure 1 and 2.

[e] nPM exposure batch numbers correspond to those listed in Zhang et al(Zhang et al. 2021).

[f] Variation of the data is shown as standard deviation (SD) in the table.

Table S2. **Primary and secondary antibodies used in western blot assays**.

Primary Antibody	Type	Dilution	Supplier	Catalog Number	Secondary Antibody Type	Dilution	Supplier
C5	Mouse	1:100	Hycult Biotech	HM1073	Goat Anti-Mouse HRP	1:4000	Bio-Rad
C5α	Goat	1:200	Santa Cruz Biotech	sc21941	Mouse Anti-Goat HRP	1:2000	Santa Cruz Biotech sc2354
CD88	Mouse	1:1000	Biolegend	-	Goat Anti-Mouse HRP	1:4000	Bio-Rad
TLR4	Mouse	1:1000	Santa Cruz Biotech	sc293072	Goat Anti-Mouse HRP	1:4000	Bio-Rad
GAPDH	Rabbit	1:20000	Sigma	9545	Goat Anti-Rabbit HRP	1:4000	Bio-Rad

C5: complement component 5; C5α: complement component 5α; CD88: complement component C5α receptor; TLR4: Toll-like receptor 4; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

Table S3. **Antibody dilutions and protocols used in immunohistochemical and immunofluorescence assays.**

Primary Antibody	Type	Supplier	Dilution	Antigen Retrieval Method	Secondary Antibody
IHC					
Iba-1	Rabbit	Wako, Japan	1:200	Dako target retrieval solution	Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) and DAB
GFAP	Rabbit	Dako, Denmark	1:10,000	Dako target retrieval solution	Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) and DAB
IF					
dMBP	Rabbit	EMD Millipore	1:500	10mM sodium citrate	Alexa Fluor 568, Invitrogen, Carlsbad, CA
C5	Mouse	Hycult Biotech	1:200	Dako target retrieval solution	Alexa Fluor 568, Invitrogen, Carlsbad, CA
C5α	Goat	Santa Cruz Biotech	1:50	Dako target retrieval solution	Alexa Fluor 568, Invitrogen, Carlsbad, CA
CD88	Rat	Hycult Biotech	1:200	Dako target retrieval solution	Alexa Fluor 568, Invitrogen, Carlsbad, CA
8-OHdG	Goat	Abcam	1:500	Dako target retrieval solution	Alexa Fluor 647, Invitrogen, Carlsbad, CA

Iba-1: ionized calcium-binding adaptor molecule; GFAP: glial fibrillary acidic protein; DAB: diaminobenzidine; dMBP: degraded myelin basic protein; C5: complement component 5; C5α: complement component 5α; CD88: complement component C5α receptor; 8-OHdG: 8-oxo-2'-deoxyguanosine; IHC: Immunohistochemistry; IF: Immunofluorescence

Table S4. **Quantitative real-time Polymerase Chain Reaction (qPCR) primer pairs.**

Name	**Sequence**	**Scale**	**Purification**
GAPDH Forward	CCAATGTGTCCGTCGTGGATCT		
GAPDH Reverse	GTTGAAGTCGCAGGAGACAACC		
Pon2 Forward	GAGGCTCTTCGTGTACCACC	**25 nmol**	**desalted**
Pon2 Reverse	ATGGTCGCCACAGAACTTCC	**25 nmol**	**desalted**
Rnd3 Forward	AGCGGTGTCTCCCTTAAACC	**25 nmol**	**desalted**
Rnd3 Reverse	AGAAAGCACTTGCCAGCGA	**25 nmol**	**desalted**
Trpc4 Forward	ATGAGGAACCTGGTGAAGCG	**25 nmol**	**desalted**
Trpc4 Reverse	CGCGTTGGCTGACTGTATTG	**25 nmol**	**desalted**
Vgf Forward	CAGACGGGAAAGGCTGTTCT	**25 nmol**	**desalted**
Vgf Reverse	CTATTTCCTCCGTGAGGGGT	**25 nmol**	**desalted**
Cd200 Forward	GCTTTGTTCTGAAGACATCGTGA	**25 nmol**	**desalted**
Cd200 Reverse	TCCAGTTCAACAATGGAAACAACT	**25 nmol**	**desalted**
Cdk19 Forward	AAAGCCAGGACTGAGATGTCC	**25 nmol**	**desalted**
Cdk19 Reverse	GGTGCGTGCAGAAAATGACC	**25 nmol**	**desalted**
Tmod1 Forward	GGATCAGACCACCAAGGCAC	**25 nmol**	**desalted**
Tmod1 Reverse	GGACCCAGACCTTTCCTCGT	**25 nmol**	**desalted**
Fbxo32 Forward	TGAGCGACCTCAGCAGTTAC	**25 nmol**	**desalted**
Fbxo32 Reverse	GCGCTCCTTCGTACTTCCTT	**25 nmol**	**desalted**

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

Table S5. **Mass fractions of organic carbon, trace elements, and metals (in units of ng/µg of PM mass) during exposures**.

Species	Exposure 1	Exposure 2	Exposure 3	Exposure 4	Exposure 5	Exposure 6	Exposure 7
Total Organic Carbon (TOC)	395.6	312.9	175.4	302.4	202.6	168.1	198.0
S	37.68848	37.39218	21.93128	32.92845	8.74261	26.24012	21.23598
Na	36.88259	36.25006	31.22	30.21433	6.71169	11.40668	45.62672
Ca	33.28122	30.25656	27.11785	28.02345	11.27689	10.81711	43.26844
Mg	10.33886	8.29025	8.72142	6.26883	1.97507	3.21959	12.87839
Al	8.84705	0.34266	4.94123	0.39204	4.70309	9.84518	23.14781
Fe	8.64647	0.07821	4.27492	0.06523	3.56151	3.47217	39.38073
K	6.67181	5.51256	2.68593	4.11652	1.78827	6.70077	26.80310
Zn	2.98281	2.60414	0.55804	2.14104	1.50917	0.59947	5.99470
P	0.98802	0.40049	0.19328	0.34419	1.15911	0.44515	0.38551
Ba	0.74808	0.46081	0.00027	0.41134	0.00026	0.00062	1.63285
Cu	0.58003	0.28747	0.24550	0.22417	0.33939	0.26993	1.34965
Ti	0.34601	0.00653	0.19129	0.00442	0.22813	0.17596	0.59326
Mn	0.33301	0.24310	0.07903	0.20321	0.06432	0.05932	0.67426
B	0.30197	0.28301	0.15966	0.25026	0.08734	0.13835	0.61619
Ni	0.25772	0.20169	0.05041	0.19954	0.06871	0.04303	0.60265
Cr	0.16459	0.01917	0.06012	0.01727	0.05946	0.03371	0.55343
Sb	0.12628	0.09871	0.019645	0.07831	0.21027	0.15066	0.16709
Pb	0.11019	0.00492	0.06773	0.00398	1.02298	0.00014	1.17308
Sn	0.06625	0.00847	0.00049	0.00789	0.00188	0.00168	0.43030
Mo	0.05397	0.04402	0.24451	0.03925	0.07783	0.00169	0.09037
Se	0.04485	0.03947	0.00789	0.03573	0.00551	0.00504	0.00456
V	0.03795	0.02041	0.00755	0.02319	0.0114	0.00359	0.14389
Li	0.01611	0.01131	0.01573	0.01017	0.00466	0.01377	0.05508
Rb	0.0143	0.0056	0.00510	0.00487	0.00521	0.01540	0.02658
As	0.01406	0.01044	0.00631	0.01267	0.00262	0.00485	0.04032
Co	0.01186	0.00766	0.00237	0.00686	0.00347	0.00154	0.06167
Ce	0.00678	0.00029	0.00148	0.00025	0.00196	0.00347	0.02428
W	0.00499	0.00312	0.00115	0.00262	0.00111	0.00041	0.00047
La	0.00394	0.00009	0.19212	0.00007	0.21489	0.40821	0.00926
Cd	0.00369	0.00262	0.00081	0.00198	0.00117	0.00065	0.01062
Nd	0.00257	0.00009	0.00028	0.00006	0.00033	0.00060	0.00678

Y	0.00209	0.00016	0.00077	0.00013	0.00095	0.01690	0.00674
Sc	0.001	0.00003	0.00045	0.00002	0.00049	0.00055	0.00267
Nb	0.00082	0.00003	0.00044	0.00003	0.00057	0.00077	0.00259
Cs	0.00078	0.00023	0.02733	0.00018	0.02518	0.04177	0.00369
Ag	0.00076	0.00004	0.00113	0.00004	0.00049	0.00022	0.00241
Pr	0.00067	0.00002	0.00314	0.00002	0.00387	0.00664	0.00250
U	0.00059	0.00012	0.00099	0.00017	0.00024	0.00066	0.00074
Th	0.00057	0.00002	0.00033	0.00002	0.00034	0.27095	0.30347
Tl	0.00047	0.00041	0.00006	0.00028	0.00008	0.00002	0.00002
Sm	0.00046	0.00002	0.00107	0.00002	0.00125	0.00231	0.00139
Pd	0.00035	0.00013	0.00008	0.00009	0.00006	0.00002	0.00116
Dy	0.00034	0.00002	0.00025	0.00002	0.00025	0.00016	0.00018
Hf	0.00028	0.00003	0.00001	0.00003	0.00002	0.00002	0.00002
Yb	0.00017	0.00002	0.00003	0.00002	0.00003	0.00005	0.00006
Eu	0.00016	0.00003	0.00018	0.00003	0.00021	0.00040	0.00058
Ho	0.00007	0.00000	0.00014	0.00000	0.00015	0.00029	0.00033
Pt	0.00004	0.00003	0.00001	0.00002	0.00005	0.00278	0.00318
Rh	0.00003	0.00002	0.01142	0.00000	0.00754	0.02259	0.00021
Lu	0.00003	0.00000	0.00010	0.00000	0.00011	0.00014	0.00016

The following elements are important in terms of redox-active responses: transition metals Fe, Cu, Ti, Mn; Post transition: Al, Zn, Pb, Sn; Alkali and earth metals: Na, Ca, Mg, K; Non-metals: S, P, Se(Haghani et al. 2020).

Table S6. **Effect of behavioral testing on assessed outcomes**.

Outcome	Cohort with Behavior	Cohort without Behavior	p-value
KB	0.41±0.12, n=24	0.33±0.10, n=12	0.70
Iba-1	103.8±5.56, n=24	114.7±6.93, n=12	0.25
GFAP	40.11±5.56, n=24	43.57±8.82, n=12	0.73
C5	9.35±0.59, n=24	9.74±1.06, n=12	0.73
C5α	10.79±1.34, n=24	12.84±1.20, n=12	0.34

Data represented as mean ± standard error. N represents sample size. Two-tailed unpaired student's t-tests were used to calculate p-values. Units: Klüver-Barrera (white matter injury score); Iba-1, GFAP (positive cells/0.1 mm^2); C5, C5α in the corpus callosum (integrated density/μm^2). KB: Klüver-Barrera; Iba-1: ionized calcium-binding adaptor molecule; GFAP: glial fibrillary acidic protein; C5: complement component 5; C5α: complement component 5α.

Table S7. **Outcomes for mice that underwent behavioral testing.**

Outcome	FA	nPM	FA+BCAS	nPM+BCAS	FA vs. nPM	FA vs. FA+BCAS	FA vs. nPM+BCAS	nPM vs. nPM+BCAS	FA+BCAS vs. nPM+BCAS
Klüver-Barrera	0.33 ± 0.14	0.48 ± 0.2	0.63 ± 0.17	1.33 ± 0.12	0.91	0.57	0.0003	0.0025	0.016
Iba-1	90 ± 8.3	117.5 ± 5.2	137.6 ± 8.4	161 ± 4.7	0.03	<0.0001	<0.0001	0.0003	0.09
GFAP	65.6 ± 2.8	66.9 ± 3.8	107.9 ± 4.5	110.9 ± 7.5	1.0	<0.0001	<0.0001	<0.0001	0.97
C5	7.4 ± 0.64	11.3 ± 0.59	9.5 ± 0.8	19.7 ± 1.2	0.009	0.28	<0.0001	<0.0001	<0.0001
C5α	6.9 ± 0.98	14.7 ± 2.0	12.0 ± 0.8	18.4 ± 0.4	0.0003	0.02	<0.0001	0.15	0.003

Table 1 displays outcomes for all analyzed mice. Data represented as mean ± standard error. Twelve mice per group (FA, nPM, FA+BCAS, nPM+BCAS) underwent behavioral testing. Pairwise comparisons used Tukey Kramer adjustment. Iba-1, GFAP cell count were assessed using immunohistochemistry. C5, C5α in the corpus callosum were assessed using immunofluorescent staining. Units: Klüver-Barrera (white matter injury score); Iba-1, GFAP (positive cells/0.1 mm^2); C5, C5α in the corpus callosum (integrated density/μm^2). FA: filtered air; nPM: nanoscale particulate matter; BCAS: bilateral carotid artery stenosis; C5: complement component 5; C5α: complement component 5α; Iba-1: Ionized calcium-binding adaptor protein-1; GFAP: Glial fibrillary acidic protein.

Table S8. **Differential gene expression in nPM vs. filter**.

Gene Name	Base Mean	Log2Fold Change	lfcSE	stat	p-value	padj (q)
Rnd3	348.409118	-0.3534428	0.0781115	-4.5248493	6.04E-06	0.04774648
Pon2	904.865003	0.19270917	0.04272744	4.51019739	6.48E-06	0.04774648
Ccl28	45.1257184	0.4100058	0.0949317	4.31895546	1.57E-05	0.07704694
Gm14827	86.5722715	-0.3991778	0.09474504	-4.2131785	2.52E-05	0.09281418

Transcriptomic response (RNA seq) analysis, demonstrating differential expression of genes in the nPM exposure group when compared to the FA control group. Table S8 provides summary data for differential gene expression between nPM and FA cohorts (Figure 6B). baseMean: the average of the normalized counts taken over all samples; log2FoldChange: log2 fold change between the groups; lfcSE: standard error of the log2FoldChange estimate; stat: Wald statistic; p-value: Wald test p-value; padj: Benjamini-Hochberg adjusted p-value. nPM: nanoscale particulate matter; FA: filtered air.

Table S9. **Differential gene expression in BCAS vs. filter.**

Gene Name	Base Mean	Log2Fold Change	lfcSE	stat	p-value	padj
Vps13c	1432.64437	-0.2828455	0.05745	-4.9233337	8.51E-07	0.01255048
Trpc4	424.944751	-0.3557448	0.08333318	-4.2689453	1.96E-05	0.09088037
St6galnac5	748.597873	-0.2863538	0.06636342	-4.3149348	1.60E-05	0.09088037
Dnajc8	1273.93729	0.16225942	0.03846799	4.21803686	2.46E-05	0.09088037

Transcriptomic response (RNA seq) analysis, demonstrating differential expression of genes in the FA+BCAS exposure group when compared to the FA control group. Table S9 provides summary data for differential gene expression between BCAS and FA cohorts. baseMean: the average of the normalized counts taken over all samples; log2FoldChange: log2 fold change between the groups; lfcSE: standard error of the log2FoldChange estimate; stat: Wald statistic; p-value: Wald test p-value; padj: Benjamini-Hochberg adjusted p-value. BCAS: bilateral carotid artery stenosis; FA: filtered air.

Table S10. **Differential gene expression in nPM+BCAS vs. filter**.

Gene Name	Base Mean	Log2Fold Change	lfcSE	stat	p-value	padj
Fbxo32	1184.46382	0.46740235	0.08223013	5.68407667	1.32E-08	0.00019378
Vgf	8125.84076	-0.3282587	0.0628768	-5.2206649	1.78E-07	0.0013134
A330023F24Rik	785.63376	-0.4054382	0.08359649	-4.8499429	1.23E-06	0.00259944
Cdk19	2453.30949	0.30885454	0.06356493	4.85888263	1.18E-06	0.00259944
Ccl28	46.9604946	0.5682689	0.11487136	4.94700233	7.54E-07	0.00259944
Rbm12b2	138.768504	-0.3647122	0.07382161	-4.9404532	7.79E-07	0.00259944
Sgsm1	3175.4787	-0.3810977	0.07772099	-4.9034083	9.42E-07	0.00259944
2310061I04Rik	869.016538	-0.1970278	0.04218744	-4.6702948	3.01E-06	0.00553939
Tmc4	68.3677457	-0.5025894	0.10894672	-4.6131671	3.97E-06	0.00649244
Slc7a3	131.677671	-0.4552735	0.10073939	-4.5193196	6.20E-06	0.00914078
Susd2	204.341723	-0.457135	0.10216422	-4.4745117	7.66E-06	0.00944067
Coprs	229.81648	-0.347135	0.07759515	-4.4736686	7.69E-06	0.00944067
Ankrd24	412.80572	-0.3332673	0.07490132	-4.4494182	8.61E-06	0.00975881
Podxl2	2536.36826	-0.3464072	0.07819779	-4.4298845	9.43E-06	0.00992267
Gm14827	84.3451452	-0.497554	0.11334819	-4.3896072	1.14E-05	0.01115418
Stmn4	3026.29545	0.20813635	0.0480264	4.3337905	1.47E-05	0.01349668
Camk1g	668.671188	-0.3989569	0.09386685	-4.2502426	2.14E-05	0.01565033
Zfp57	71.0777322	-0.4863573	0.11387876	-4.2708336	1.95E-05	0.01565033
Zbtb46	95.7816651	-0.4031103	0.09506282	-4.2404619	2.23E-05	0.01565033
Atg16l2	178.649395	-0.3568453	0.08410711	-4.2427488	2.21E-05	0.01565033
Zfp612	990.865609	-0.2103073	0.04934817	-4.2617033	2.03E-05	0.01565033
Paxbp1	729.386014	-0.2253161	0.05381072	-4.187198	2.82E-05	0.01809204
Lin7c	1530.02988	0.19535555	0.046623	4.19011098	2.79E-05	0.01809204
Slc1a6	125.874024	-0.4527808	0.10890449	-4.1575953	3.22E-05	0.01974449
Meg3	40999.8621	-0.4074876	0.09965722	-4.0888921	4.33E-05	0.02365289
Mdn1	1674.18271	-0.3163305	0.07735273	-4.0894547	4.32E-05	0.02365289
Cda	22.6261313	-0.4713083	0.11518534	-4.0917384	4.28E-05	0.02365289
Ppp1cb	5347.12303	0.12988201	0.03188028	4.07405547	4.62E-05	0.02431187
Map6d1	1382.22621	0.29688067	0.07321315	4.05501821	5.01E-05	0.0254697
Taf1a	162.836647	-0.3092259	0.07684211	-4.0241728	5.72E-05	0.028081
Tubb2a	3021.64487	-0.2419101	0.06027188	-4.0136485	5.98E-05	0.02841635
Hcn3	338.482138	-0.4168202	0.10440992	-3.992151	6.55E-05	0.0301479
Cct8	2643.13379	-0.1393957	0.03512449	-3.9686192	7.23E-05	0.03227652
Tmod1	1344.20599	0.31950775	0.08068778	3.95980344	7.50E-05	0.03250644
Atic	534.938196	-0.1820216	0.04617263	-3.9421979	8.07E-05	0.03398852
Tuba1b	1509.90022	-0.2473299	0.06313492	-3.917482	8.95E-05	0.03574176

Rfc1	737.334141	0.15795978	0.04032941	3.91673947	8.98E-05	0.03574176
Midn	1610.61689	-0.3201814	0.08204373	-3.9025699	9.52E-05	0.03690353
Comtd1	141.775843	-0.3821779	0.09809638	-3.8959429	9.78E-05	0.03695493
Per2	846.05357	-0.3664675	0.09521191	-3.8489672	0.00011862	0.04064422
Jag2	764.899341	-0.2169864	0.05629396	-3.8545227	0.00011596	0.04064422
Mettl17	228.467616	-0.3068368	0.07959111	-3.8551646	0.00011565	0.04064422
Bmi1	830.222433	0.16328376	0.04242153	3.8490776	0.00011856	0.04064422
BC017158	373.635313	-0.2564761	0.06684339	-3.8369698	0.00012456	0.04171122
Lrrc16b	786.067645	-0.3566706	0.09321786	-3.8262047	0.00013013	0.04260879
Tbc1d14	1668.65895	0.1272151	0.03342211	3.80631536	0.00014105	0.04449926
Dus2	171.022012	-0.2551638	0.06706457	-3.8047487	0.00014195	0.04449926
Mbd2	1014.44721	0.18937868	0.04997847	3.78920513	0.00015113	0.04639064
Dos	7891.71231	-0.1885165	0.05021171	-3.7544324	0.00017373	0.04875026
Cep83os	505.597164	0.27389043	0.07274224	3.76521848	0.0001664	0.04875026
Nhp2	405.212375	0.22348858	0.05947277	3.75783038	0.00017139	0.04875026
Prr3	397.322468	-0.2109673	0.05604653	-3.7641462	0.00016712	0.04875026
Disp2	7487.31806	-0.2711497	0.07226616	-3.7520984	0.00017536	0.04875026
Hrh1	206.551319	-0.3871012	0.10342083	-3.7429719	0.00018186	0.0496199
Dennd5a	7710.12447	0.147706	0.03961583	3.72845938	0.00019265	0.05161024
Tmem106b	4131.86019	0.15103537	0.04059009	3.72099125	0.00019844	0.05221159
Nmbr	26.7075083	-0.4290125	0.11639392	-3.6858671	0.00022793	0.05247267
Cdh23	42.6519053	-0.4181589	0.11295536	-3.7019842	0.00021392	0.05247267
Cd200	1395.03826	-0.2750901	0.074585	-3.6882767	0.00022578	0.05247267
Brd3	1354.87583	-0.1846807	0.05001691	-3.6923659	0.00022218	0.05247267
Eif2a	709.585087	0.15505863	0.04194829	3.69642327	0.00021866	0.05247267
Pigk	1363.93238	-0.1357616	0.03682923	-3.6862459	0.00022759	0.05247267
Gpr3	157.575568	-0.4026696	0.1085116	-3.7108443	0.00020657	0.05247267
Amt	219.117478	-0.2822799	0.07636524	-3.6964447	0.00021864	0.05247267
Trim39	382.272991	-0.2001336	0.05444901	-3.6756152	0.00023728	0.0537852
Resp18	980.376019	-0.4239745	0.11591582	-3.657607	0.00025458	0.05436227
Opa1	3792.25584	0.1465587	0.03995439	3.66815024	0.00024431	0.05436227
Gnas	24912.1774	-0.216675	0.05922296	-3.6586322	0.00025356	0.05436227
P2ry12	537.317844	0.25294434	0.06901417	3.66510734	0.00024723	0.05436227
Inpp5d	316.866473	0.27327309	0.07501017	3.64314718	0.00026932	0.05589057
Nsfl1c	1267.67547	-0.1304686	0.0357893	-3.6454629	0.00026691	0.05589057
Zcchc18	4677.27198	-0.2317248	0.06373884	-3.6355347	0.0002774	0.05676778
Ankrd28	1299.77178	0.24510274	0.06753864	3.62907437	0.00028444	0.05740998
Tubb3	6098.29544	-0.1821642	0.0503638	-3.6169668	0.00029808	0.05934926
Spry4	359.831136	-0.3767515	0.10436856	-3.6098179	0.00030641	0.06019568

Snapc1	486.793186	-0.2075015	0.05769953	-3.5962431	0.00032285	0.06102117
Dpysl2	4335.38453	-0.1934925	0.05380638	-3.5960879	0.00032304	0.06102117
Mfap3l	1204.12057	0.22147658	0.06154242	3.59876311	0.00031973	0.06102117
Fam172a	827.7401	0.16697369	0.04649402	3.59129419	0.00032904	0.06136803
BC031361	149.618017	0.31267985	0.08736609	3.57896122	0.00034496	0.0619839
Cdc7	167.385927	-0.2924018	0.08155859	-3.5851754	0.00033685	0.0619839
Ankrd10	582.314246	-0.2659406	0.07428516	-3.5799962	0.0003436	0.0619839
Cacna1g	1683.07877	-0.3598062	0.10069609	-3.57319	0.00035266	0.06260328
Snn	3884.97064	0.265297	0.0743949	3.5660643	0.00036238	0.06356362
Hgsnat	917.231903	0.23058835	0.06481783	3.557483	0.00037443	0.06414864
Cstf2	1694.68751	-0.1430295	0.04019584	-3.5583163	0.00037324	0.06414864
Msrb1	301.453191	0.22796437	0.06429392	3.54566015	0.00039163	0.06565245
Col16a1	314.652	-0.3495139	0.09858418	-3.5453349	0.00039211	0.06565245
Metap1	599.123331	-0.1456432	0.04113645	-3.5404912	0.00039938	0.06611808
Cenpb	3909.85836	0.19940423	0.05653904	3.52684149	0.00042055	0.06809188
H13	1331.97594	-0.2072172	0.05875323	-3.5269071	0.00042044	0.06809188
Rbm5	1445.76109	-0.2101374	0.05978971	-3.5146081	0.0004404	0.07053155
Tmem86b	78.1397209	-0.3457148	0.09857131	-3.5072561	0.00045275	0.07172975
P2rx5	21.1553087	-0.4067964	0.11610213	-3.5037803	0.0004587	0.07173611
Hdac5	4079.10478	-0.1289561	0.0368967	-3.4950584	0.00047396	0.07173611
Mirg	175.367035	-0.3678668	0.10537196	-3.4911266	0.00048099	0.07173611
Ankrd54	238.227308	-0.2651375	0.0759584	-3.4905619	0.00048201	0.07173611
Yeats2	576.575885	-0.2337743	0.06681649	-3.4987519	0.00046744	0.07173611
Dynlt1a	99.534516	-0.3275333	0.09366392	-3.4968994	0.0004707	0.07173611
Rab30	243.473055	0.24134801	0.06923318	3.48601641	0.00049027	0.07210158
Prss53	41.7546592	-0.3951166	0.11341363	-3.4838542	0.00049425	0.07210158
Vwf	426.865475	0.37529418	0.10780853	3.48111776	0.00049933	0.07212812
Prmt2	947.331816	-0.3465891	0.0997937	-3.4730564	0.00051457	0.07220603
Hacd2	663.074873	0.14043815	0.04040236	3.47598882	0.00050897	0.07220603
Lyrm2	247.167557	0.23292754	0.06705281	3.47379247	0.00051316	0.07220603
Pcdh15	361.985805	-0.3469776	0.1000827	-3.4669092	0.00052648	0.07318067
Ppp6r3	1598.99993	0.11180129	0.03227385	3.4641443	0.00053192	0.07324603
Ankrd23	44.5138094	-0.3732915	0.10807058	-3.4541453	0.00055204	0.07436172
Cited4	192.043049	0.32123103	0.0930397	3.45262305	0.00055516	0.07436172
Tm6sf2	22.5834932	-0.4010913	0.11612451	-3.4539765	0.00055239	0.07436172
Lrp12	1246.04597	0.16213535	0.04704116	3.44667033	0.00056754	0.0745443
N4bp2l1	361.357951	-0.2288426	0.06642386	-3.4451861	0.00057067	0.0745443
Grin2d	503.537493	-0.3872168	0.11240962	-3.4446944	0.00057171	0.0745443
Kdm6b	1126.62741	-0.2818314	0.08188588	-3.4417588	0.00057795	0.0746969

Chga	2993.15547	-0.1922746	0.05631208	-3.4144474	0.00063912	0.07616908
Slc5a3	958.307763	-0.3444744	0.10071994	-3.4201209	0.00062593	0.07616908
Dnase1l2	43.7726462	-0.386503	0.11322342	-3.4136315	0.00064103	0.07616908
Pprc1	542.712539	-0.2079923	0.06088328	-3.4162463	0.00063491	0.07616908
Wwp1	1282.97324	0.16712466	0.04885789	3.42062816	0.00062477	0.07616908
Rasgef1b	1473.21091	0.36112315	0.10561462	3.41925333	0.00062793	0.07616908
Hspb1	219.964453	-0.3941568	0.1149064	-3.4302421	0.00060304	0.07616908
Cfp	110.724923	-0.3457312	0.10108262	-3.4202837	0.00062556	0.07616908
Morf4l2	3153.66412	-0.1403439	0.04099336	-3.4235755	0.00061803	0.07616908
Shroom2	1850.65469	0.1456589	0.04256174	3.42229685	0.00062094	0.07616908
Dusp11	1138.93728	-0.2271184	0.06660668	-3.4098437	0.00065	0.07635206
Kcna1	6774.74457	0.32140672	0.09429247	3.40861482	0.00065294	0.07635206
Safb2	1054.38583	-0.2232581	0.06562186	-3.4021911	0.00066848	0.07755406
Cul4a	1316.2054	0.12890728	0.0379411	3.39756302	0.00067989	0.07826163
Smcr8	1194.89378	0.1468434	0.04333147	3.38883958	0.00070189	0.07894394
Ddx39b	2039.033	-0.099449	0.0293337	-3.3902637	0.00069825	0.07894394
Secisbp2l	4301.46703	0.17382702	0.05124868	3.39183369	0.00069427	0.07894394
Cacybp	1197.03038	-0.1988256	0.05874534	-3.3845341	0.00071299	0.0793296
Rmst	34.794523	-0.393161	0.11631091	-3.3802589	0.00072418	0.0793296
Ccnl1	456.240734	-0.2147906	0.06354779	-3.3799857	0.0007249	0.0793296
Usp27x	382.101581	-0.3217001	0.09519886	-3.3792435	0.00072686	0.0793296
Lmnb2	407.111707	-0.2365031	0.07012174	-3.3727494	0.00074422	0.07945859
Capza2	3019.62639	0.19083636	0.05656078	3.37400528	0.00074083	0.07945859
Ptov1	2639.8363	-0.1436433	0.04254993	-3.3758762	0.00073581	0.07945859
Adam1a	100.151239	-0.3742545	0.11115841	-3.366857	0.0007603	0.08059191
Nop2	412.47762	-0.1761736	0.05236031	-3.3646411	0.00076643	0.08066157
Ablim3	581.614367	-0.2845778	0.08466686	-3.3611476	0.00077619	0.08088839
Acot7	3982.26572	-0.1680653	0.05004906	-3.35801	0.00078506	0.08088839
Dgkq	710.781175	-0.2564169	0.07633345	-3.3591681	0.00078178	0.08088839
Glt8d1	387.492243	-0.2524111	0.075221	-3.3555934	0.00079195	0.08103179
Mob1b	296.575011	0.22211384	0.06632793	3.3487225	0.00081185	0.08249522
Plxnc1	1178.66792	-0.3378988	0.10104624	-3.3440019	0.00082579	0.08333708
Adamts10	280.06903	-0.3310135	0.09917227	-3.3377631	0.00084456	0.08465107
Ulk2	2479.25727	0.15795982	0.04742612	3.3306505	0.00086643	0.08625693
Mpv17l	1113.46624	0.20006904	0.06031366	3.31714332	0.00090943	0.08992976
BC037034	1028.7297	-0.1374826	0.04148681	-3.3138872	0.00092009	0.09037698
Disp1	171.122448	-0.2505472	0.07574923	-3.3075877	0.00094103	0.0916744
Nktr	1813.91735	-0.2327583	0.07040076	-3.3061903	0.00094574	0.0916744
Tspan9	852.413626	0.18928534	0.05730483	3.3031308	0.00095612	0.09207477

Slc2a4rg-ps	134.334393	-0.3524918	0.10678705	-3.300885	0.0009638	0.09221224
Prkar1a	13188.3768	-0.0814712	0.024738	-3.2933627	0.00098997	0.09291855
Trib2	1008.83029	-0.2582975	0.07843066	-3.2933234	0.00099011	0.09291855
Nxf1	1109.93088	-0.196119	0.05951453	-3.2953127	0.00098312	0.09291855
Larp1	3705.19805	-0.108045	0.03283601	-3.2904416	0.0010003	0.09323301
Ccnk	776.016857	-0.1707938	0.05193174	-3.2888121	0.00100611	0.09323301
Map2k3	164.816949	-0.2912101	0.0887563	-3.2810082	0.00103437	0.0934992
Cpne7	643.859146	-0.3822335	0.11635379	-3.2850971	0.00101947	0.0934992
Slc37a2	80.7963055	0.38134216	0.11622705	3.28101039	0.00103436	0.0934992
Pja1	3554.30351	-0.1571748	0.0478919	-3.2818666	0.00103122	0.0934992
Osbp	1762.901	0.12920898	0.03941456	3.27820412	0.0010447	0.09351447
Efna1	72.9319654	0.33627486	0.10265381	3.27581462	0.00105358	0.09351447
Leprot	445.890229	0.1731869	0.05286251	3.27617639	0.00105223	0.09351447
Pafah1b3	139.20183	-0.3270047	0.09991402	-3.2728607	0.00106465	0.0939314
Per1	2610.67968	-0.301146	0.09206439	-3.2710364	0.00107154	0.09393275
Abhd2	1469.13164	0.29537272	0.09034214	3.26949009	0.00107742	0.09393275
P4ha2	182.584278	-0.3302337	0.10111326	-3.265978	0.00109087	0.09445828
Fbxl3	1617.75798	0.1357196	0.04157337	3.26458005	0.00109626	0.09445828
Cdc40	685.108952	0.17022678	0.05226645	3.25690358	0.00112635	0.09504545
Rtn4	11975.5832	0.13040049	0.04004603	3.25626522	0.00112888	0.09504545
Fezf1	15.3150758	-0.211057	0.06480875	-3.2566123	0.0011275	0.09504545
Hyou1	2610.59466	-0.2365181	0.07257999	-3.2587229	0.00111915	0.09504545
Fam193b	1227.42001	-0.2726286	0.08392895	-3.2483259	0.00116086	0.09627453
Impact	5120.04899	-0.2990022	0.09205551	-3.248064	0.00116193	0.09627453
Rprd1b	614.646983	-0.1526681	0.04702996	-3.2461876	0.00116962	0.09627453
Tshz2	794.495082	-0.3619013	0.11144933	-3.2472274	0.00116535	0.09627453
Mblac2	470.706738	-0.2338459	0.07214327	-3.241409	0.0011894	0.09682143
Hsf1	682.493396	-0.1921951	0.05929223	-3.2414881	0.00118907	0.09682143
Gabrd	1443.48025	0.2847223	0.08791588	3.23857656	0.00120128	0.09725069
Slc16a6	418.888571	0.29724912	0.09191316	3.23402142	0.0012206	0.09774111
Acsl1	1181.82934	0.17860089	0.05520539	3.23520728	0.00121554	0.09774111
Frs2	1351.19159	0.13088517	0.04051103	3.23085229	0.00123422	0.09829705

Transcriptomic response (RNA seq) analysis, demonstrating differential expression of genes in the nPM+BCAS exposure group when compared to the FA control group. Table S10 provides summary data for differential gene expression between FA and nPM+BCAS cohorts (Figure 6C). baseMean: the average of the normalized counts taken over all samples; log2FoldChange: log2 fold change between the groups; lfcSE: standard error of the log2FoldChange estimate; stat: Wald statistic; p-value: Wald test p-value; padj: Benjamini-Hochberg adjusted p-value. FA: filtered air.

Table S11. **qPCR summary data**.

Gene	FA	nPM	FA+BCAS	nPM+BCAS
RND3	1.0 ± 0.09	0.80 ± 0.03	1.0 ± 0.09	0.84 ± 0.13
Pon2	1.0 ± 0.03	1.1 ± 0.02	1.1 ± 0.04	1.08 ± 0.05
Tcpr4	1.0 ± 0.06	0.67 ± 0.08	0.65 ± 0.08	0.78 ± 0.17
Vgf	1.0 ± 0.03	0.80 ± 0.04	0.96 ± 0.08	0.71 ± 0.02
CD200	1.0 ± 0.04	1.0 ± 0.1	1.1 ± 0.2	0.84 ± 0.05
FBXO32	1.0 ± 0.1	1.2 ± 0.13	0.90 ± 0.08	1.5 ± 0.04
CDK19	1.0 ± 0.04	1.1 ± 0.08	1.1 ± 0.09	1.3 ± 0.03
Tmod1	1.0 ± 0.06	1.2 ± 0.07	1.1 ± 0.08	1.3 ± 0.05

Data represents gene induction fold relative to the FA cohort. Data shown as mean ± standard error. Data displayed in Figure S3. n=7 per group (FA, nPM, FA+BCAS, nPM+BCAS). qPCR: quantitative real-time polymerase chain reaction; RNAseq: ribonucleic acid sequencing.

Table S12. **nPM and BCAS networks.**

ID	Analysis	Molecules in Network	Score	Focus Molecules	Top Diseases and Functions
1	nPM	B3GNT2,B4GALT1,Ca2+,CACNA2D2,CCL11,CCL28,CCR3,CCR10,chemokine,chenodeoxycholic acid,CTSF,DGAT1,ENPP1,F7,FANCC,FCER1A,GDF15,IFIH1,Iga,lipid peroxide,LRIG1,M6PR,Madcam1,MADCAM1,MR1,NET1,PGF,PON2,RGS14,RND3,SELPLG,ST3GAL6,TNF,UBR2,VLDL	9	3	Cellular Movement, Immune Cell Trafficking, Hematological System Development and Function
1	BCAS	AMPK,APP,Ca2+,Calmodulin,CAV1,CCND1,CDH5,CREB1,CTNNB1,DICER1,dihydrotestosterone,DNAJC8,EGF,EGR1,FN1,FYN,GH1,HGF,HSPA8,HUWE1,IFNG,KIFC1,SELENOW,SF3A1,SLC9A3R1,SRC,SRPK1,ST6GALNAC5,TARBP2,TAZ,tretinoin,TRPC4,UBC,USP7,Vegf	8	3	Cellular Development, Embryonic Development, Hair and Skin Development and Function
2	BCAS	CD24,D-glucose,DNAJC7,EED,FAF2,LGALS12,NPHP4,UBXN4,VPS13C	3	1	Carbohydrate Metabolism, Molecular Transport, Small Molecule Biochemistry
1	nPM+BCAS	ACSL1, Actin, Akt, Alpha tubulin, Beta Tubulin CCT8, CDK19, CDK4/6, DPYSL2, EFNA1 FBXL3, FSH, GNAS, HYOU1, IMPACT, KCNA1 Lh, LMNB2, NOP2,OSBP,P4HA2,PAFAH1B3 PER1,PER2,PPP1CB,PRKAR1A,RTN4,STMN4 TUBA1B,TUBB3,TUBB2A,tubulin,tubulin (family VGF,WWP1	51	26	Developmental Disorder, Neurological Disease, Organismal Injury and Abnormalities
2	nPM+BCAS	CACNA1G,CCNK,CD3,CENPB,CITED4,Ck2 CSTF2,CUL4A,DNASE1L2,FBXO32,Hdac HDAC5,HISTONE,Histone h3,HSF1,Hsp90 KDM6B,LARP1,MBD2,NFkB (complex),NKTR OPA1,P-TEFb,PAXBP1,PEPCK,PLXNC1 PRMT2,PTOV1,RBM5,RNA polymerase II SLC5A3,TAF1A,TMOD1,TSPAN9,YEATS2	48	25	Cell Cycle, Cardiovascular System Development and Function, Cell Morphology
3	nPM+BCAS	ADCY,ADRB,Calcineurin protein(s),Camk,CAMK1G CaMKII,caspase,CD200,CDC7,CHGA,COL16A1 COMMD3-BMI1,Creb,Cyclin A,ERK1/2,FEZF1,Fgf FRS2,GABRD,GRIN2D,Hsp27,IFN Beta,Igm,INPP5D,MAP2K1/2,Mek,Pka catalytic	29	17	Neurological Disease, Cardiovascular Disease, Heart Failure

		subunit,PP2A,RFC1,SLC16A6,SNAPC1,SNN,Sos,SPRY4,VWF			
4	nPM+BCAS	AMOTL1,AMOTL2,ANKRD10,ARG2,BRD3,C7orf43,CAPZA2,CARMIL1,CFP,CNOT11,DENND5A,DUSP11,ELAVL1,FAM172A,GAS2L3,GBE1,GCLC,HACD2,HCN3,IL4,LAMP2,LDLRAD3,LIN7C,LRRFIP2,METAP1,MGST3,MHCII,MRPS10,PLEKHG5,PPFIA2,PRR3,PXDC1,SPATA2,ZBTB46,ZCCHC18	24	15	Amino Acid Metabolism, Small Molecule Biochemistry, Hereditary Disorder
5	nPM+BCAS	ACOT7,ALDOC,ANKRD24,BRWD1,CCND1,CDC40,CDC45,CDCA2,CPNE7,DDX5,DLG4,EPOP,ERBB2,EZH2,FGF9,JARID2,KCNA1,KCNJ12,LIN28A,LIN7B,MBLAC2,MED12L,Meg3,METTL17,Mirg,MYO7A,PCDH15,PER2,SAFB2,SECISBP2L,SLC1A6,SMCR8,SNRPF,WSB2,ZNF622	24	15	Cellular Development, Reproductive System Development and Function, Auditory Disease
6	nPM+BCAS	AMPK,CACYBP,Collagen(s),DYNLT1,EIF2A,ERK Gpcr,GPR3,HRH1,HSPB1,IgG,Immunoglobulin,Insulin,Interferonalpha,JAG2,Jnk,LDL,MAP2K3,Mapk,MIDN,Nfat (family),NHP2,NMBR,NXF1,P2RY12,P38 MAPK,p85(pik3r),PI3K(complex),Pka,Pkc(s),Proinsulin,TCR,Tnf (family),TRIB2,Vegf	22	14	Cardiovascular Disease, Neurological Disease, Organismal Injury and Abnormalities
7	nPM+BCAS	ANKRD54,ATXN3L,CDH23,CNTN2,COMTD1,GLT8D1,GP1BB,LAPTM4A,LRP12,LYN,MDN1,OTUD6A,PDLIM5,PJA1,PTOV1,RACK1,ROBO4,SCARB1,SERPINB12,STK11IP,TMC4,TMCO4,TMEM245,TMEM106B,TP73,TRIM39,TSHZ2,TTYH3,UBC,UBTD2,ULK2,USP28,USP51,USP27X,USP9Y	22	14	Post-Translational Modification, Developmental Disorder, Endocrine System Disorders
8	nPM+BCAS	ADAM2,Adam3,ADAM28,Adam1a,ADAMTS10,AMT,C1QBP,CCL28,COL16A1,Cxcl3,DGKQ,DUS2,ESM1,F2,F3,F9,F13A1,FGFR1,FGFRL1,HNF4A,ITGA9,ITGB,LEPROT,LYRM2,Madcam1,MADCAM1,MSRB1,P2RX5,P2RX6,PODXL2,PPP2R3C,RAB30,RPRD1B,SMN1/SMN2,TNF	22	14	Cardiovascular System Development and Function, Cell-To-Cell Signaling and Interaction, Cellular Assembly and Organization
9	nPM+BCAS	ABHD5,ALOX5AP,ANKRD23,APOD,beta-estradiol CRABP2,DISP1,ENPP2, epoprostenol,FAAH,FABP	18	12	Carbohydrate Metabolism, Molecular Transport, Lipid Metabolism

		GCLC,GK,KBKG,KITLG,LAPTM5,MAP6D1,MC1R MED7,MPZ,N4BP2L1,NHP2,NR4A1,NSFL1C,NUCB2,oleicacid,PPBP,PPRC1,RESP18,RPL7,Rps3a1,SLC37A2,SUSD2,TBC1D14,WWP1			
10	nPM+BCAS	ABLIM3,ACTB,ADCYAP1R1,APP,ARGLU1,ATIC C16orf58,CAND2,CCNL1,CCR10,CDC42SE1,CREB3,DISP2,EED,FAM50A,GABRA4,GPR3,GSTO1,HCAR2,HM13,IMPA1,Lamin,LPAR6,MORF4L2,MPV17L,P2RX6,RBM19,RBM12B,SERP1,SLC7A3,TAS1R3,TGFB1,THOP1,TRAM1,ZNF23	18	12	Cell Cycle, Cardiovascular System Development and Function, Cell Signaling
11	nPM+BCAS	ABCA4,ABHD2,ANKRD28,ANKRD44,ANKRD52 ATG16L2,C6orf136,CA3,CLCN3,CRLF3,DDX39B DUSP11,ESR1,FBXO6,H19,LRCH1,LRCH2,MOB1B MTMR3,PCDH18,PIGK,PLK1,PLOD2,PPP6R2 PPP6R3,PUM2,RASGRF1,SHROOM2,SNRPN SRC,TECPR1,TERF2,TNS3,UGT8,ZFP57	16	11	Gene Expression, Cellular Compromise, Ophthalmic Disease
12	nPM+BCAS	ADCY,ADCYAP1,ADCYAP1R1,adenosine,ADRB,BMP6,BTG2,Calcineurinprotein(s),CD80,CDA,CETN3,CHGA,CHGB,COPRS,CREB1,CXCL2,GNRH,histamine,K+,mir122,NEUROD1,NOS1,NPY,NR4A2,NR4A3,NTRK2,OSBPL3,Pias,POU1F1,POU4F1,RASGEF1B,SGSM1,SLC6A3,TH,voltage-gated calcium channel	6	5	Molecular Transport, Small Molecule Biochemistry, Behavior
13	nPM+BCAS	alkenylglycerophosphocholine hydrolase alkenylglycerophosphoethanolamine hydrolase BCL2L13,PTF1A,TMEM86B	2	1	Cellular Development, Cellular Function and Maintenance, Cellular Growth and Proliferation

Pathways of highly interconnected genes were identified by statistical likelihood according to the methods described in Calvano et al (Calvano et al. 2005). Gene networks were built using Ingenuity Pathway Analysis to connect key genes and enriched categories of diseases and functions based on correlations among differentially expressed genes. Networks were then sorted by score values. Focus genes were determined as the genes having direct interactions with other genes reported in the dataset. Each focus gene's specificity of connections was determined by calculating the percentage of its connections with other significant genes. The initiation and growth of pathways started with, and proceeded from, genes with the highest specificity of connections (Calvano et al. 2005). Table S12 provides summary data for Figure 6G, H, and I.

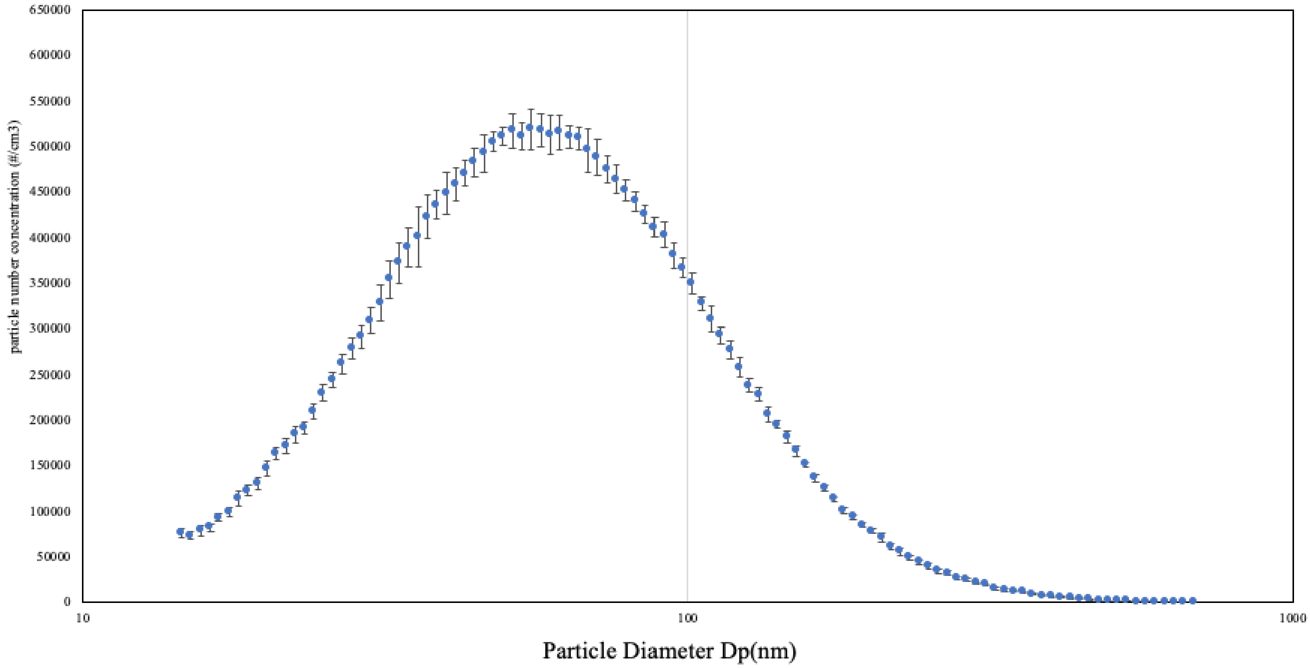
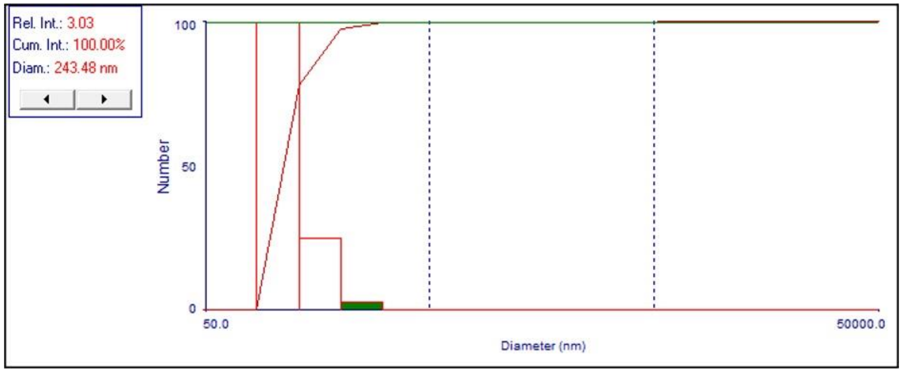


Figure S1. **The average particle size distribution of the exposure aerosol.** Data represented as mean ± standard error. A total of 107 measured size distributions are shown. Dp(nm): particle diameter.

A


Rel. Int.: 3.03
Cum. Int.: 100.00%
Diam.: 243.48 nm
◀ ▶
100
50
0
Number
50.0
50000.0
Diameter (nm)

B

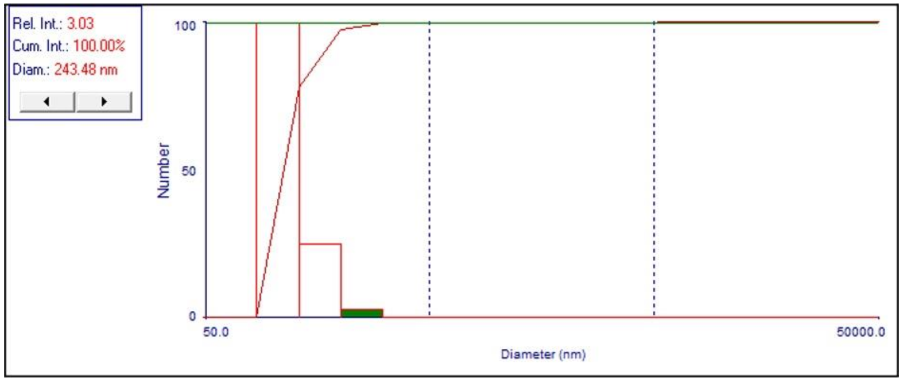

Rel. Int.: 3.03
Cum. Int.: 100.00%
Diam.: 243.48 nm
◀ ▶
100
50
0
Number
50.0
50000.0
Diameter (nm)

Figure S2. **Size distribution of the aerosol inside the suspension.** (*A*-*B*) Size distribution of the aerosol inside the suspension was measured by means of Dynamic Light Scattering (Dynamic Light Scattering BI-200SM (Brookhaven Instruments Inc). To analyze the possibility of agglomeration, 2 stages of filtration were performed and compared, 1000 nm filtration (upper plot) and 450 nm filtration (lower plot). There is little difference between these two plots, indicating that the number concentration is dominated by ultrafine size ranges. The green bar is manually set at the first size channel after 200 nm for each scan and the “cumulative” fraction of particles corresponding to the green bar is shown in the upper left of the figures. For 1000 nm filtration, 95% of particles were smaller than 224 nm and for 450 nm filtration ~100% of particles were below 243 nm. This indicates that only about 5% of the total number concentration is in the size ranges above 243 nm. Mode diameter is also below 100 nm. Diam: diameter; Rel Int: relative light scattering intensity; Cum. Int: cumulative intensity.

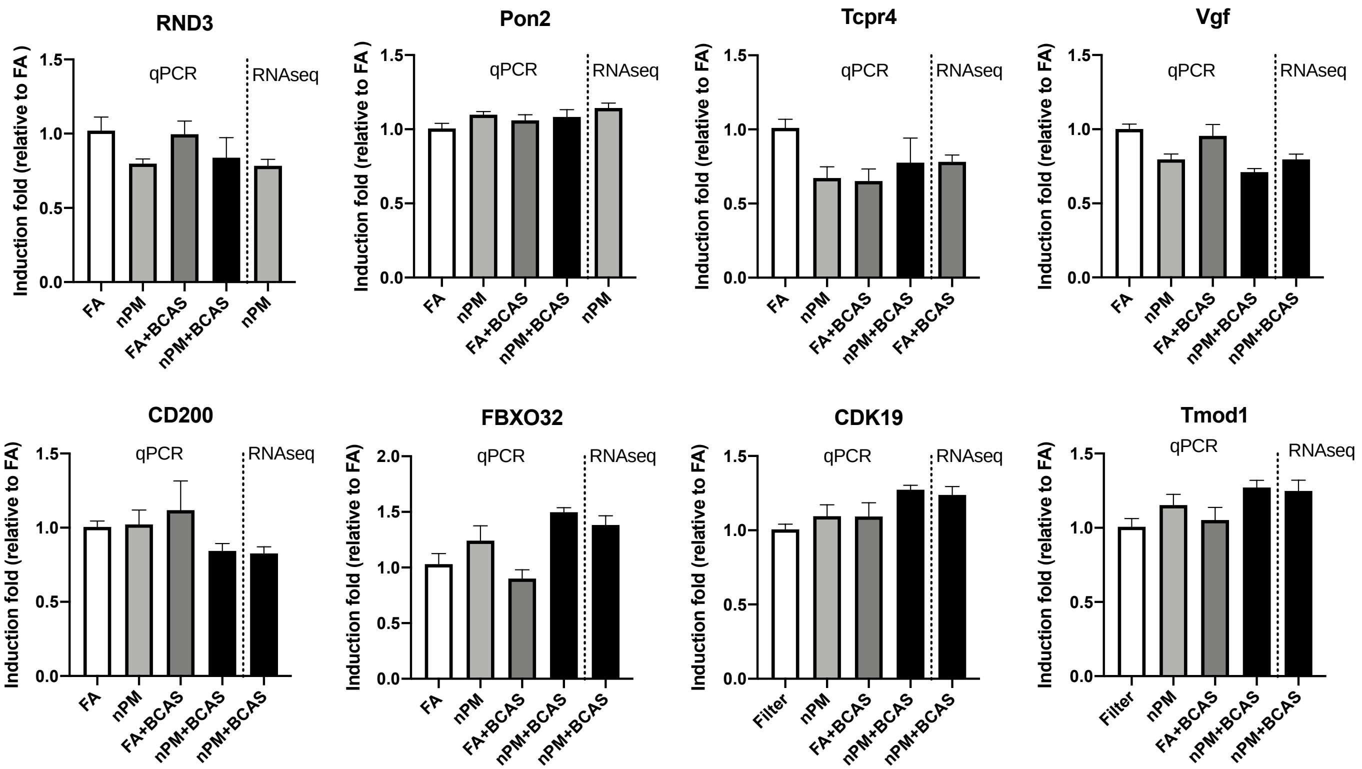


Figure S3. **qPCR validation of a cohort of genes from RNA seq.** Data represented as mean ± standard error. n=7 per group (FA, nPM, FA+BCAS, nPM+BCAS). qPCR: quantitative real-time polymerase chain reaction; RNAseq: ribonucleic acid sequencing. Summary qPCR data shown in Table S11. Summary RNA seq data shown in Tables S8-10.

References

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