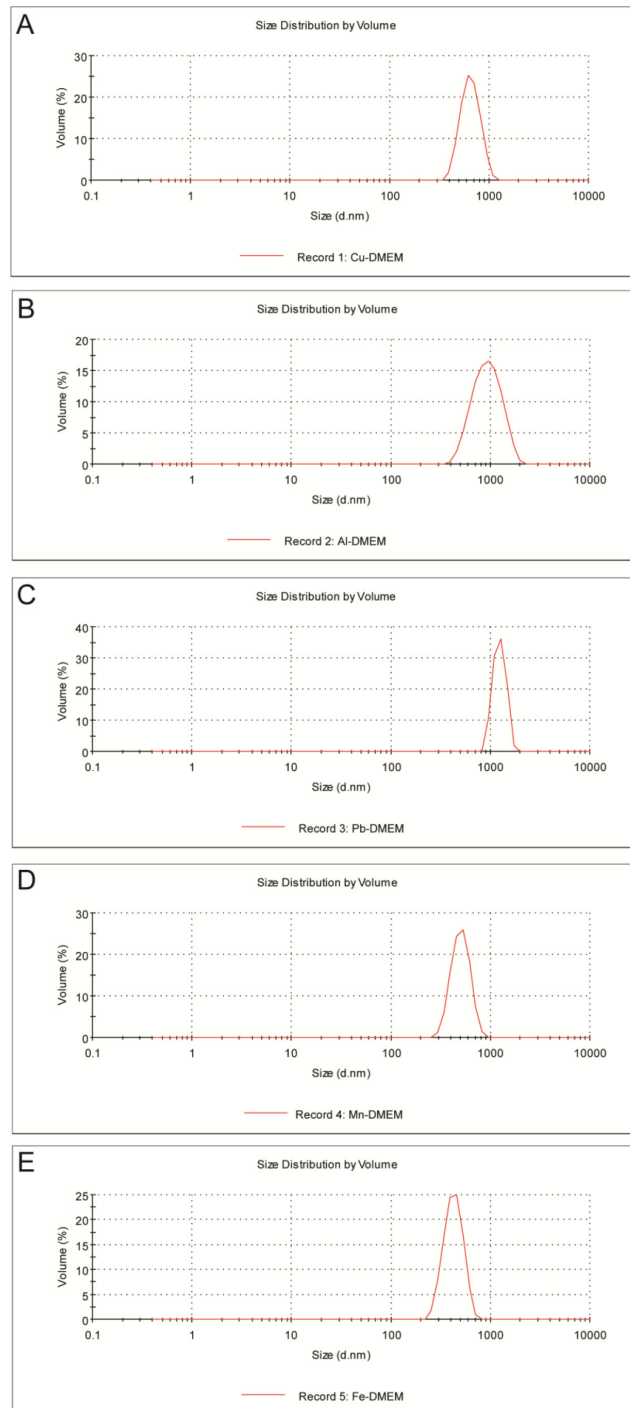


# Role of microRNA-4516 involved autophagy associated with exposure to fine particulate matter

## Supplementary Materials



**Supplementary Figure S1: Size distribution of metal particles.** The metal particles were suspended in DMED culture medium with a final concentration of 1 mg/ml, and dispersed by ultrasonication for 15 min. Then the size distribution of metal particles were immediately evaluated by a zetasizer (nano-zs90, Malvern Instruments, UK). (A) Mean size of Cu particles is 632.7 nm. (B) Mean size of Al particles is 964 nm. (C) Mean size of Pb particles is 1242 nm. (D) Mean size of Mn particles is 511.7 nm. (E) Mean size of Fe particles is 683.5 nm.

**Supplementary Table S1: miRNA microarray predicted modulation of miRNA in PM<sub>2.5</sub> treated A549 cells**

microRNA	Fold change	FDR
hsa-miR-4516	9.687	0.031
hsa-miR-4732-5p	5.867	0.034
hsa-miR-4778-5p	5.563	0.037
hsa-miR-4449	5.352	0.014
hsa-miR-3201	5.278	0.014
hsa-miR-675-5p	4.360	0.037
hsa-miR-638	3.944	0.014
hsa-miR-1469	3.907	0.014
hsa-miR-204-3p	3.212	0.037
hsa-miR-4750-5p	2.967	0.037
hsa-miR-3940-5p	2.848	0.014
hsa-miR-652-5p	2.710	0.041
hsa-miR-3611	2.660	0.022
hsa-miR-802	0.172	0.049
hsa-miR-4319	0.246	0.037
hsa-miR-1322	0.253	0.049
hsa-miR-3176	0.260	0.014
hsa-miR-933	0.260	0.037
hsa-miR-4536-3p	0.317	0.034
hsa-miR-4445-3p	0.345	0.049

**Supplementary Table S2: miR-4516 expression by individuals' characteristics**

Variable	good air quality city			Moderate air pollution city		
	<i>n</i>	miR-4516 (mean, 95% CI)	<i>P</i> -value	<i>n</i>	miR-4516 (mean, 95% CI)	<i>P</i> -value
Age, years						
< 40	38	13.87 (13.29, 14.45)		42	9.776 (8.941, 10.61)	
40–50	44	13.38 (12.87, 13.89)		40	9.771 (8.978, 10.56)	
> 50	38	13.32 (12.75, 13.90)	0.3144	38	10.86 (10.03, 11.70)	0.1022
Sex						
Male	60	13.48 (13.01, 13.96)		60	9.713 (9.075, 10.35)	
Female	60	13.50 (13.07, 13.93)	0.9590	60	10.52 (9.830, 11.21)	0.0881

**Supplementary Table S3: Down-regulations of proteins encoded by miR-4516 target genes**

Gene symbol	mRNA encoded protein function	Fold Change	
		Low dose PM <sub>2.5</sub>	High dose PM <sub>2.5</sub>
RPL37	60S ribosomal protein L37	0.865	0.39
FSTL1	follistatin-related protein 1 precursor	0.895	0.666
PER1	PREDICTED: period circadian protein homolog 1 isoform X2	0.75	0.593
U2AF2	splicing factor U2AF 65 kDa subunit isoform b	0.521	0.33
AKAP17A	A-kinase anchor protein 17A isoform 1	0.82	0.633
PRKCB	protein kinase C beta type isoform 1	0.958	0.614
SUB1	activated RNA polymerase II transcriptional coactivator p15	0.734	0.449
BET1	BET1 homolog	0.729	0.595
LIX1L	LIX1-like protein	0.925	0.645
CSF1	macrophage colony-stimulating factor 1 isoform c precursor	0.769	0.576
PPP2R3A	serine/threonine-protein phosphatase 2A regulatory subunit B'' subunit alpha isoform 3	0.854	0.335
PRPF38B	pre-mRNA-splicing factor 38B	0.652	0.598
LUC7L	putative RNA-binding protein Luc7-like 1 isoform b	0.464	0.213
POLR1D	DNA-directed RNA polymerases I and III subunit RPAC2 isoform 3	0.954	0.557
PLOD2	procollagen-lysine,2-oxoglutarate 5-dioxygenase 2 isoform 1 precursor	0.75	0.659
MTUS1	PREDICTED: microtubule-associated tumor suppressor 1 isoform X3	0.734	0.655
UBA52	ubiquitin-60S ribosomal protein L40 precursor	0.858	0.31
RSRC2	PREDICTED: arginine/serine-rich coiled-coil protein 2 isoform X4	0.72	0.589
FAM105A	inactive ubiquitin thioesterase FAM105A	0.902	0.623
HIF1A	hypoxia-inducible factor 1-alpha isoform 1	0.79	0.649
C1orf35	multiple myeloma tumor-associated protein 2	0.81	0.622
PPIL4	peptidyl-prolyl cis-trans isomerase-like 4	0.966	0.612
MELK	maternal embryonic leucine zipper kinase isoform 2	0.756	0.568
C19orf53	leydig cell tumor 10 kDa protein homolog	0.795	0.464
LLPH	protein LLP homolog	0.86	0.637

### Supplementary Table S4: Sub-ontology molecular function enrichment of miR-4516 target genes

Enrichment Term	Involved Genes
GO:0046872 metal ion binding	PPP2R3A, PLOD2, RPL37, FSTL1, LUC7L, UBA52, PRKCB
GO:0043169 cation binding	PPP2R3A, PLOD2, RPL37, FSTL1, LUC7L, UBA52, PRKCB
GO:0043167 ion binding	PPP2R3A, PLOD2, RPL37, FSTL1, LUC7L, UBA52, PRKCB
GO:0046914 transition metal ion binding	PLOD2, RPL37, LUC7L, UBA52, PRKCB
GO:0000166 nucleotide binding	U2AF2, PPIL4, MELK, PRKCB
GO:0008270 zinc ion binding	RPL37, LUC7L, UBA52, PRKCB
GO:0046983 protein dimerization activity	HIF1A, POLR1D, CSF1
GO:0003723 RNA binding	U2AF2, PPIL4, RPL37
GO:0005509 calcium ion binding	PPP2R3A, FSTL1, PRKCB
GO:0030528 transcription regulator activity	HIF1A, SUB1, UBA52
GO:0003677 DNA binding	HIF1A, POLR1D, SUB1

### Supplementary Table S5: Sub-ontology enrichment terms of biological process (BP)

Term	Genes
GO:0051240 positive regulation of multicellular organismal process	HIF1A, CSF1, UBA52
GO:0045639 positive regulation of myeloid cell differentiation	HIF1A, CSF1
GO:0010647 positive regulation of cell communication	HIF1A, CSF1, UBA52
GO:0016202 regulation of striated muscle tissue development	LUC7L, UBA52
GO:0048634 regulation of muscle development	LUC7L, UBA52
GO:0045637 regulation of myeloid cell differentiation	HIF1A, CSF1

### Supplementary Table S6: Sub-ontology enrichment terms of cellular component (CC)

Term	Genes
GO:0022626 cytosolic ribosome	RPL37, UBA52
GO:0033279 ribosomal subunit	RPL37, UBA52
GO:0044445 cytosolic part	RPL37, UBA52

### Supplementary Table S7: Increment of miR-4516 expression between baseline and postexposure levels by individuals' characteristics

Variable	good air quality city		
	<i>n</i>	miR-4516 (mean, 95% CI)	<i>P</i> -value
Age, years			
< 40	10	1.43 (-0.96, 3.81)	
40–50	9	1.90 (0.31, 3.48)	
> 50	11	0.48 (-1.44, 2.40)	0.5259
Sex			
Male	15	0.77 (-0.51, 2.04)	
Female	15	1.67 (-0.11, 3.46)	0.3820