CRISPR/Cas9-Mediated Whole Genomic Wide Knockout Screening Identifies Specific Genes Related to Mineral Absorption Involving in PM$_{2.5}$ Liver Toxicity

Jinfu Peng

Central South University Third Xiangya Hospital

Bin Yi

Central South University Third Xiangya Hospital

Mengyao Wang

Central South University Third Xiangya Hospital

Jieqiong Tan

Central South University School of Life Sciences

Zhijun Huang (✉ huangzj@csu.edu.cn)

The Third Xiangya Hospital, Central South University

Abstract

Background: $PM_{2.5}$, also known as fine particles, refers to particulate matter with a dynamic diameter of ≦ 2.5 μm in air pollutants, carrying toxic substances (e.g. heavy metals or minerals), which can pass through the alveolar epithelium and enter the bloodstream and tissues, can have more serious health problems, such as non-alcoholic fatty liver and hepatocellular carcinoma. However, the underlying mechanisms for toxic effect of $PM_{2.5}$ are poorly understood.

Results: Here, we subjected L02 cells to expose of $PM_{2.5}$ and performed a pooled genome-wide CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9) loss of function screen to discover new potential targets for $PM_{2.5}$. Enrichr and KEGG were employed to identify candidate genes which might involve in $PM_{2.5}$ toxicity. We found that ATP1A2(ATPase Na+/K+ transporting subunit alpha 2), MT1M(metallothionein 1M), SLC6A19(solute carrier family 6 member 19) and TRPV6(transient receptor potential cation channel subfamily V member 6) genes contributed to $PM_{2.5}$ toxicity involving in mineral absorption pathway. Moreover, we demonstrated that these candidate genes deficiency increased the cell viability and attenuated apoptosis in cells explored to $PM_{2.5}$.

Conclusions: ATP1A2, MT1M, SLC6A19 and TRPV6 contribute to $PM_{2.5}$ toxicity and may be potential therapeutic targets for $PM_{2.5}$ related diseases.

Background

Particulate Matter (PM) is a mixture of solid and liquid particles that are suspended in the air. $PM_{2.5}$ describes fine inhalable particles, with diameters that are generally 2.5 micrometers and smaller. $PM_{2.5}$ have small diameters, however large surface areas, may therefore be capable of carrying various toxic stuffs (such as heavy metals or minerals: Ca, Cu, K, Mg, Mo, Ps, Pt, Se, and Zn), passing through the filtration of nose hair, reaching the end of the respiratory tract with airflow and accumulate there by diffusion or active transport[1], distributing to other organs through blood, damaging other parts of the body[2]. Many respiratory diseases[1], cardiovascular diseases[3], neurodegenerative diseases[4] and hepatic disease[5] were reported to be related to $PM_{2.5}$.

$PM_{2.5}$ exposure leads to NAFLD which is known as a silent disease and present in around 20–30% of people[6]. What's more, people living in environments with higher $PM_{2.5}$ concentrations have a higher incidence rate ratio of hepatocellular carcinoma (HCC) and patients with HCC has a higher mortality rate[7, 8]. Some studies discovered the mechanism of $PM_{2.5}$ induced liver disease. Oxidative stress and inflammatory in liver cells aggravated by $PM_{2.5}$ contributed to hepatic injury by altering normal lipid metabolism[9], farnesoid X receptor(FXR) [10], ROS/PINK1/Parking signal pathway and NADPH oxidase[11, 12] may mediate the effects of $PM_{2.5}$ exposure on promoting hepatic fibrosis. However, the material basis and detailed mechanism of $PM_{2.5}$ toxicity are still unknown. A comprehensive study is still necessary.

CRISPR (clustered regularly interspaced short palindromic repeats)/ Cas9 (CRISPR-associated protein 9) is an RNA-guided DNA endonuclease and can be easily programmed to target new sites by altering its guide RNA sequence[13, 14]. CRISPR/Cas9 is a powerful gene editing tool that edits DNA in a precise, directed manner. This technology to enable thousands of genes to be modified and their function assessed in a single experiment. This cutting-edge tool can help identify and validate novel drug targets or study the underlying human disease[15]. CRISPR/Cas9 has been used to study the pathogenesis of NAFLD and is also becoming a promising genetic engineering approach for liver cancer[16, 17]. To explore the effect of $PM_{2.5}$ exposure, we firstly applied genome-scale CRISPR/Cas9 knockout (GeCKO) screening technology to discover new regulators involved in $PM_{2.5}$ resistance in human L02 cell line. Moreover, some genes were validated as $PM_{2.5}$ resistance genes, indicating that they were important contributors and potential therapeutic targets for toxic effect of $PM_{2.5}$.

Results

A genome-wide CRISPR/Cas9-mediated screen to identify $PM_{2.5}$ resistance genes

The rapid emergence of CRISPR/Cas9-mediated genome editing technologies has now allowed us to discover additional modulators of $PM_{2.5}$. We introduced a GeCKO library containing 123,411 sgRNAs targeting 19,050 human genes into lentiviral vectors and generated a pool of cells, in which every targeted gene theoretically carried a loss-of-function mutation[18]. After treating the pooled cells with $PM_{2.5}$ exposure insult, viable cells were collected for analysis of the inserted sgRNAs in genome (Fig. 1A). The sgRNAs amplified from the surviving cells' genomes were determined by next-generation sequencing (NGS), and the candidate genes were ranked depending on the number of unique sgRNAs versus NGS reads. As shown in the scatter diagram of sgRNA number and corresponding sequencing reads of genes, the detected genes were well-distributed in every sgRNA (Fig. 1B). Enrichment of multiple sgRNAs was found in our GeCKO screening, suggesting that loss of the cognate genes conferred resistance to $PM_{2.5}$ insult. Of the 19,050 genes tested, 614 top-rank genes with high number (4−6) of unique sgRNAs were involved in $PM_{2.5}$ toxic effect (Additional file 1). We had also discovered that the deficiency of some interesting genes related to mineral absorption, ATP1A2, ATP1B2, MT1M, SLC6A19 and TRPV6, might involve the protection of $PM_{2.5}$ toxic.

KEGG pathway and GO analysis of PM2.5 resistance genes

To understand the roles of these candidate genes in apoptosis and their association with $PM_{2.5}$ resistance, Enrichr (https://maayanlab.cloud/Enrichr/)[19] was used for KEGG pathway and GO enrichment analysis of top-rank genes with high number (4−6) of unique sgRNAs, and the top 10 significant pathways and functions were selected in accordance with p values.

KEGG analysis indicated that some genes (ATP1A2, SLC6A19, MT1M, TRPV6 and ATP1B2) were belong to mineral absorption pathway (p = 0.019602244) (Fig. 2, Additional file 2). ATP1A2, ATP1B2, MT1M, SLC6A19 and TRPV6 are all responsible for absorption of metals and minerals(cadmium, zinc, copper and calcium ions,etc.), which means knocking down ATP1A2, ATP1B2, MT1M, SLC6A19 or TRPV6 may keep L02 cells from transporting metals and minerals in PM2.5. What's more, inflammatory mediator regulation of TRP channels (ADCY9, ASIC5, CALML5, CYP4A22, CALML4, IL1RAP, PIK3R1,PLCG1) was the other pathway affected by PM2.5 in our study which indicated that oxidative stress and inflammatory mediates PM2.5-induced cell damage, and it was well known that $PM_{2.5}$ could cause oxidative stress and inflammatory in liver cells [9].

The GO analysis demonstrated that $PM_{2.5}$ resistance genes were primarily involved in GO biological process: biomineral tissue development(p = 0.0014), skeletal system development(p = 0.0018), inorganic anion transmembrane transport(p = 0.0033), GO molecular function: inorganic anion transmembrane transporter activity(p = 0.0033), inhibitory extracellular ligand-gated ion channel activity(p = 0.0099), chloride channel activity(p = 0.01), transmitter-gated ion channel activity(p = 0.017), and GO cellular component: tertiary granule membrane (p = 0.0069), specific granule membrane(p = 0.06), bicellular tight junction (p = 0.069)etc.(Fig. 2, Additional file 3). It was obvious that minerals played an important role in biomineral tissue development and skeletal system development[20, 21]. We found that other GO functions may also be indirectly related to transcellular transport of ion and minerals (or metals). Thus, it can be seen that $PM_{2.5}$ resistance genes may be responsible for mineral toxicity and mineral absorption. On the other hand, oxidative stress and inflammation are a consequence of the absorption of metal ions in $PM_{2.5}$. In our study, oxidoreductase activity(GO:0016709) and T cell receptor binding (GO:0042608) were found in the GO analysis, which might correspond to $PM_{2.5}$-induced oxidative stress and inflammation[22, 23].

Taken together, our GeCKO screen had identified the $PM_{2.5}$ resistance genes in L02 cells that were involved in mineral absorption pathway and inflammation-related pathway. GO analysis also revealed that $PM_{2.5}$ resistance genes involved the physiological functions of minerals and inflammation, oxidative stress. We were surprised to find that mineral absorption deficiency might be involved in $PM_{2.5}$ exposure protection.

Effect of the mineral absorption related genes from GeCKO screen on PM $_{2.5}$ -induced apoptosis

To validate the top-ranking genes from the GeCKO screen, we next knocked down the expression of the genes by expressing siRNA sequences targeting mineral absorption related genes. The ratios of cell viability and apoptosis of the during $PM_{2.5}$ exposure were compared to that of the control cells that transfected an siRNA control. We observed a significant increase in the ratio of cell viability during $PM_{2.5}$ exposure insult following knockdown of ATP1A2, MT1M, SLC6A19 or TRPV6 (Figure.3). Meanwhile,

knocking down ATP1A2, ATP1B2, MT1M, SLC6A19 or TRPV6 could decrease in the ratio of apoptosis during $PM_{2.5}$ exposure (Figure. 4). The findings for mineral absorption related genes were consistent with the integrative analysis during the genome-wide CRISPR/Cas9-mediated screen, which suggested that these genes might sensitize cells to apoptosis following $PM_{2.5}$ exposure.

Discussion

$PM_{2.5}$ particles are widely believed to be associated with NAFLD[5] and HCC ,etc[7]. As $PM_{2.5}$ exposure induced the activation of apoptotic pathway, there is no doubt that apoptosis is associated with the formation of these diseases and is one of the important pathological features[24, 25]. CRISPR screening facilitates discovery of key genes or genetic sequences that elicit a specific function or phenotype for a cell type, which is conducted to identify gene involving in $PM_{2.5}$ resistance. Of the 19,050 genes tested, our GeCKO screen identified 614 genes with high number (4−6) of unique sgRNAs that may be involved in $PM_{2.5}$ toxic effect. The precent study demonstrates that $PM_{2.5}$ resistance genes were involved in minerals physiological function and mineral absorption, etc., which may be important for liver cell apoptosis and damage.

Although there are some mechanisms of $PM_{2.5}$ induced liver deceases are reported, such as inflammatory, ROS and FXR, they focus on studying the physiological changes and molecular pathways in the body after $PM_{2.5}$ exposure[9, 11, 26]. As Qingzhao Li ,etc. reported main heavy metals of $PM_{2.5}$ were more likely to be distributed in the liver[2]. The cell permeability and transfer of toxic Chemicals(metals and minerals) constituted $PM_{2.5}$ might also play an important role in $PM_{2.5}$ for liver cell apoptosis and damage, but were less explored. In present study, genes related to mineral absorption in GeCKO screening were confirmed by expressing siRNA sequences. KEGG analysis of 614 genes with high number (4−6) of unique sgRNAs showed that some genes (ATP1A2, SLC6A19, MT1M, TRPV6 and ATP1B2) were belong to mineral absorption pathway. What's more, GO analysis confirmed that genes involved in $PM_{2.5}$ toxic effect might produce many biological functions, such as biomineral tissue development, skeletal system development, inorganic anion transmembrane transport(GO Biological Process), inorganic anion transmembrane transporter activity, inhibitory extracellular ligand-gated ion channel activity, transmitter-gated ion channel activity(GO Molecular Function), and tertiary granule membrane (GO Cellular Component), which directly or indirectly related to mineral absorption.

Knocking down ATP1A2, SLC6A19, MT1M and TRPV6 significantly increased cell viability ratio and decreased apoptosis. MT1M is an important member of metallothioneins (MTs) family that contains the short peptide of cysteine and sulfur protein with high affinity to heavy metals, including cadmium, zinc, and copper, and play a vital role in metal ion homeostasis and detoxification[27]. TRPV6 is the most Ca^{2+}-selective members of the TRP ion channel family, play an important role in intestinal Ca^{2+} absorption. Pharmacological interference with TRPV6 in the digestive system is likely to interfere with calcium absorption and bone mineralization as well as with epithelial cell hyperplasia and early stages of malignancies[28]. ATP1A2 encodes the α2 isoform of the Na^+,K^+-ATPase's catalytic subunit, an ion

channel/ion transporter. SLC6A19 encodes the amino acid transporter B(0)AT1, mediating neutral amino acid transport from the luminal compartment to the intracellular space[29]. In generally, MT1M, ATP1A2, SLC6A19 and TRPV6 relates to mineral absorption. When knocking down these genes by transfection of siRNA, the cell activity increased and apoptosis decreased compared to control under the effect of $PM_{2.5}$. As these genes are responsible for mineral absorption, loss of them may stop the absorption of minerals or metals into the cell or imbalance of metal ion homeostasis caused by $PM_{2.5}$, which means these genes/proteins are responsible for transporting metals and minerals involved in $PM_{2.5}$ toxic effect to cells.

On the other hand, $PM_{2.5}$ may induce oxidative stress and inflammation that indirectly mediated $PM_{2.5}$ induced apoptosis and diseases[30]. Studies reported oxidative stress and inflammation in liver cells aggravated by $PM_{2.5}$ contributed to hepatic injury by altering normal lipid metabolism[9]. In our GeCKO screening, loss of some genes related to inflammation and oxidoreductase activity, such as IL1RAP[31], PIK3R1, PLCG1[32], CYP26B1, CYP4F11[26] and MICAL2[33], might relieve $PM_{2.5}$ toxicity[34].

There are some limits in our study that can be improved and explored in the future. First, we focused on the genes of transporters. We did not specify which metals or minerals transported into cells were responsible for the damage of L02 cells and the mechanism of minerals mediated apoptosis. Second, metals or minerals concentration had not been measured in our study. This work will be done in our lab in the future to elucidate the role of metals or minerals transporters in the $PM_{2.5}$ toxicity.

In conclusion, metals are main components of $PM_{2.5}$ [35] and play an important role in $PM_{2.5}$ -induced apoptosis[36], it is reasonable that mineral absorption results apoptosis of cells which induces and deteriorates deceases. In our study, loss of genes involving to mineral absorption protected the L02 cell, which indicated that metals of $PM_{2.5}$ could distribute to liver by transporters and induced apoptosis of several deceases [2]. We can inspect that methods of prevention and treatment may be explored on the genes or proteins of mineral absorption in the future.

Conclusion

$PM_{2.5}$ can damage cells, cause damage to tissues and organs, and cause a very negative impact on human health. This genome-wide CRISPR/Cas9-mediated screen found genes related to mineral absorption (ATP1A2, ATP1B2, MT1M and TRPV6) may be involved in this process, which can provide theoretical support for the prevention and treatment of $PM_{2.5}$ -induced damage.

Methods

Lentiviral production of the sgRNA library.

Lentiviral production was carried out as previously described[37]. Briefly, HEK293T cells were seeded at about 50% confluence 1 day before transfection in DMEM supplemented with 10% fetal bovine serum. 4

µg of GeCKO library (#1000000048, Addgene), 2 µg of pV-SVg (#8454, Addgene,) and 6 ug of psPAX2 (#12260, Addgene) packing plasmids were co-transfected in a 10cm^2-dish using Lipofectamine 2000 (Invitrogen) per manufacturer's protocol. After 48h, the cell culture media was collected and centrifuged at 3,000 rpm at 4 °C for 10 min to pellet cell debris, filtered (0.45-µm pore size), and concentrated by ultracentrifugation (Beckmann) at 24,000 rpm for 2 h at 4°C. The virus preparation was finally resuspended overnight at 4°C in DMEM, divided into aliquots, and stored at −80°C.

Lentiviral transduction of the sgRNA library.

L02 cells were purchased from ATCC, cultured at 37°C, 5% CO$_2$ in the DMEM medium containing 10% fetal bovine serum (Invitrogen). 3x10^8 L02 cells were infected with the GeCKO library at a multiplicity of infection (MOI) of 0.1 aiming for ensure that most cells receive only 1 viral construct with high probability in full DMEM supplemented with 10% fetal bovine serum, 4 mM l-glutamine, and 10 µg/ml penicillin and streptomycin in the presence of 10 µg/ml of Polybrene. 48 hr after infection, the medium was removed and fresh DMEM was added to the cells containing 1 ug/mL puromycin for 7 days culture.

PM$_{2.5}$ resistance gene screen and DNA sequencing

Cells were exposed to PM$_{2.5}$ for 48 hours and then returned to 95% air, 5% CO2, and glucose-containing medium for recovery 6h. The surviving cells were collected. The genomic DNA from surviving cells was isolated using the Blood & Cell Culture DNA Midi Kit (Quiagen, Hilden, Germany) and stored at -20°C. PCR was performed in two steps and resulting amplicons from the second PCR were sequenced using a HiSeq 2500 (Illumina) as described by Dr. Feng Zhang[14].

Forward primer: CTTGTGGAAAGGACGAAACA

Reverse primer: GCCAATTCCCACTCCTTTCA.

The raw sequencing data were processed and analyzed using customized CRISPR- Cas9 library screen pipelines. Briefly, sequencing reads were first de-multiplexed by using the barcode in the reverse primer, and processed by Cutadapt to remove sequences from beginning to sgRNA priming site primers. Trimmed reads were used to map sgRNA sequences to pooled GeCKO v2 libraries A and B. Read counts of sgRNA for each sample were quantified by MAGeCK v5.6.0. Count data were filtered and normalized, and essential sgRNA and genes were ranked by MAGeCK.

Cell culture, RNA interference

Oligo RNA was purchased from GenePharma (Shanghai, China) siRNA and control siRNA transfection was performed with Lipofectamine 2000 (Invitrogen) according to the instructions of the manufacturer, 50 pmol siRNA/well was transfected with 1 µL of Lipofectamine 2000 in the 24 wells for 48h, after RNA transfection, the cells were exposed to PM$_{2.5}$ for 48 hours and then returned to 95% air, 5% CO$_2$, and glucose-containing medium for different recovery times to induce cell apoptosis.

MTT assay

Cell viability was determined by 3, (4,5-diamethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Thus, 30 μL of the MTT solution was added to each well and incubated at 37 °C for 3 h. After 3 h, the wells were aspirated, and the plates were left to dry overnight. The next day, 50 μL of dimethyl sulfoxide (DMSO) was added to each well to solubilize the formazan crystals. The plates were then put on a shaker for 1 h and read spectrophotometrically at 570 nm in a plate reader. The data was then analyzed and is represented as percent cell viability.

Apoptosis assay

Cells were washed with PBS and resuspended in binding buffer before Annexin-V-FITC and propidium iodide (PI) double staining, according to the manufacturers' instructions (BD Biosciences Pharmingen, USA). Briefly, L02 cells were collected and washed twice with PBS, followed by addition of 500 μL binding buffer, 5 μL Annexin V-FITC and 10 μL PI then were added to each group and cultures were incubated at 37°C for 10 min in dark. The apoptosis was analyzed by flow cytometry and Cell QuestPro software (BD Biosciences).

Statistics

The data is presented as mean ± SD. The significance of differences between the groups was determined by paired Student's t-test and/or one-way ANOVA by the GraphPad Prism 6 software, with 0.05 as the level of significance.

Abbreviations

CRISPR: clustered regularly interspaced short palindromic repeats;Cas9:CRISPR-associated protein 9; $PM_{2.5}$: Particulate Matter 2.5; ATP1A2: ATPase Na+/K+ Transporting Subunit Alpha 2 ; ATP1B2:ATPase Na+/K+ transporting subunit beta 2; MT1M: metallothionein 1M ; SLC6A19: Solute Carrier Family 6 Member 19 ; TRPV6: Transient Receptor Potential Cation Channel Subfamily V Member 6 ; GeCKO :genome-scale CRISPR/Cas9 knockout; NGS: next-generation sequencing; sgRNA: single guide RNA; IL1RAP: interleukin 1 receptor accessory protein; PIK3R1: phosphoinositide-3-kinase, regulatory subunit 1 (alpha); PLCG1: phospholipase C gamma 1; MICAL2: Microtubule Associated Monooxygenase, Calponin And LIM Domain Containing 2

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ZH developed the study concept. BY, JP, JT and MW designed and performed the experiments. BY and JP analyzed the data and wrote the manuscript. ZH, JP, JT and BY provided critical revisions. All authors have read and approved the final manuscript.

Funding

References

1. Xing YF, Xu YH, Shi MH, Lian YX. The impact of PM2.5 on the human respiratory system. J. Thorac. Dis. 2016; 8(1): E69-E74. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4740125/

2. Li Q, Liu H, Alattar M, Jiang S, Han J, Ma Y, et al. The preferential accumulation of heavy metals in different tissues following frequent respiratory exposure to PM2.5 in rats. Sci Rep. 2015;5:1–8. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4652264/

3. Rajagopalan S, Al-Kindi SG, Brook RD. Air Pollution and Cardiovascular Disease: JACC State-of-the-Art Review. J. Am. Coll. Cardiol. 2018: 17(72):2054-2070. https://doi.org/10.1016/j.jacc.2018.07.099 https://pubmed.ncbi.nlm.nih.gov/30336830/

4. Zhu X, Ji X, Shou Y, Huang Y, Hu Y, Wang H. Recent advances in understanding the mechanisms of PM2.5-mediated neurodegenerative diseases. Toxicol. Lett. Elsevier Ireland Ltd; 2020: 31–7. https://doi.org/10.1016/j.toxlet.2020.04.017

5. Tarantino G, Capone D, Finelli C. Exposure to ambient air particulate matter and non-alcoholic fatty liver disease. World J Gastroenterol. 2013;19:3951–6. https://pubmed.ncbi.nlm.nih.gov/23840139/

6. Sivell C. Nonalcoholic Fatty Liver Disease: A Silent Epidemic. Gastroenterol Nurs. 2019; 42(5):428-434. https://pubmed.ncbi.nlm.nih.gov/31574071/

7. VoPham T, Bertrand KA, Tamimi RM, Laden F, Hart JE. Ambient PM2.5 air pollution exposure and hepatocellular carcinoma incidence in the United States. Cancer Causes Control. Springer International Publishing; 2018;29:563–72. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5940508/

8. Lee CH, Hsieh SY, Huang WH, Wang IK, Yen TH. Association between ambient particulate matter 2.5 exposure and mortality in patients with hepatocellular carcinoma. Int J Environ Res Public Health. MDPI AG; 2019; 16(14):2490. https://doi.org/10.3390/ijerph16142490

9. Xu MX, Ge CX, Qin YT, Gu TT, Lou DS, Li Q, et al. Prolonged PM2.5 exposure elevates risk of oxidative stress-driven nonalcoholic fatty liver disease by triggering increase of dyslipidemia. Free Radic Biol Med. Elsevier Inc.; 2019;130:542–56. https://doi.org/10.1016/j.freeradbiomed.2018.11.016 https://pubmed.ncbi.nlm.nih.gov/30465824/

10. Wang M, Tan J, Zhou J, Yi B, Huang Z. Farnesoid X receptor mediates hepatic steatosis induced by PM2.5. Environ Sci Pollut Res. Springer; 2020;27:34412–20. https://pubmed.ncbi.nlm.nih.gov/32557026/

11. Qiu YN, Wang GH, Zhou F, Hao JJ, Tian L, Guan LF, et al. PM2.5 induces liver fibrosis via triggering ROS-mediated mitophagy. Ecotoxicol Environ Saf. Academic Press; 2019;167:178–87. https://doi.org/10.1016/j.ecoenv.2018.08.050

12. Zheng Z, Zhang X, Wang J, Dandekar A, Kim H, Qiu Y, et al. Exposure to fine airborne particulate matters induces hepatic fibrosis in murine models. J Hepatol. Elsevier; 2015;63:1397–404. https://pubmed.ncbi.nlm.nih.gov/26220751/

13. Wang H, La Russa M, Qi LS. CRISPR/Cas9 in Genome Editing and Beyond. Annu Rev Biochem. Annual Reviews; 2016; 85:227–64. https://doi.org/10.1146/annurev-biochem-060815-014607 https://pubmed.ncbi.nlm.nih.gov/27145843/

14. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelsen TS, et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. Science; 2014; 343:84–7. https://pubmed.ncbi.nlm.nih.gov/24336571/

15. Huang A, Garraway LA, Ashworth A, Weber B. Synthetic lethality as an engine for cancer drug target discovery. Nat. Rev. Drug Discov. 2020; 19:23–38. https://pubmed.ncbi.nlm.nih.gov/31712683/

16. Gordon DM, Adeosun SO, Ngwudike SI, Anderson CD, Hall JE, Hinds TD, et al. CRISPR Cas9-mediated deletion of biliverdin reductase A (BVRA) in mouse liver cells induces oxidative stress and lipid accumulation. Arch Biochem Biophys. Academic Press Inc.; 2019;672. https://doi.org/10.1016/j.abb.2019.108072

17. Ratan ZA, Son YJ, Haidere MF, Uddin BMM, Yusuf MA, Zaman S Bin, et al. CRISPR-Cas9: A promising genetic engineering approach in cancer research. Ther Adv Med Oncol. SAGE Publications Inc.; 2018;10. https://doi.org/10.1177/1758834018755089 https://pubmed.ncbi.nlm.nih.gov/29434679/

18. Wang T, Wei JJ, Sabatini DM, Lander ES. Genetic screens in human cells using the CRISPR-Cas9 system. Science. 2014;343:80–4. https://pubmed.ncbi.nlm.nih.gov/24336569/

19. Kuleshov M V., Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016;44:W90–7. https://doi.org/10.1093/nar/gkw377 https://pubmed.ncbi.nlm.nih.gov/27141961/

20. Upadhyay RK. Role of Calcium Bio-Minerals in Regenerative Medicine and Tissue Engineering. J Stem Cell Res Ther. 2017; 2(6):166-175.

21. Office of the Surgeon General. 2. The Basics of Bone in Health and Disease. Bone Heal Osteoporos A Rep Surg Gen. 2004;1–16. https://www.ncbi.nlm.nih.gov/books/NBK45504/

22. Deleonardi G, Biondi A, D'Aurelio M, Pich MM, Stankov K, Falasca A, et al. Plasma membrane oxidoreductase activity in cultured cells in relation to mitochondrial function and oxidative stress. BioFactors. IOS Press; 2004; 265–72. https://pubmed.ncbi.nlm.nih.gov/15706061/

23. Cope AP. Exploring the reciprocal relationship between immunity and inflammation in chronic inflammatory arthritis. Rheumatology. 2003;716–31. https://doi.org/10.1093/rheumatology/keg262

24. Peng H, Zhao XH, Bi TT, Yuan XY, Guo J Bin, Peng SQ. PM2.5 obtained from urban areas in Beijing induces apoptosis by activating nuclear factor-kappa B. Mil Med Res. 2017;4:1–10. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5577776/

25. Huang X, Shi X, Zhou J, Li S, Zhang L, Zhao H, et al. The activation of antioxidant and apoptosis pathways involved in damage of human proximal tubule epithelial cells by PM2.5 exposure. Environ Sci Eur. 2020; 32:1–13.

26. Jian T, Ding X, Wu Y, Ren B, Li W, Lv H, et al. Hepatoprotective effect of loquat leaf flavonoids in PM2.5-induced non-alcoholic fatty liver disease via regulation of IRs-1/Akt and CYP2E1/JNK pathways. Int J Mol Sci. MDPI AG; 2018;19. https://doi.org/10.3390/ijms19103005 https://pubmed.ncbi.nlm.nih.gov/30275422/

27. Si M, Lang J. The roles of metallothioneins in carcinogenesis. J. Hematol. Oncol. 2018;11(107):1-20. https://pubmed.ncbi.nlm.nih.gov/30139373/

28. Holzer P. Transient receptor potential (TRP) channels as drug targets for diseases of the digestive system. Pharmacol. Ther. 2011; 131:142-170. https://doi.org/10.1016/j.pharmthera.2011.03.006 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3107431/

29. Cheon CK, Lee BH, Ko JM, Kim HJ, Yoo HW. Novel Mutation in SLC6A19 Causing Late-Onset Seizures in Hartnup Disorder. Pediatr Neurol. 2010;42:369–71. https://pubmed.ncbi.nlm.nih.gov/20399395/

30. Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. Toxicology. 2011;283(2–3):65-87. https://doi.org/10.1016/j.tox.2011.03.001 https://pubmed.ncbi.nlm.nih.gov/21414382/

31. Wood IS, Wang B, Trayhurn P. IL-33, a recently identified interleukin-1 gene family member, is expressed in human adipocytes. Biochem Biophys Res Commun. 2009;384:105–9. https://doi.org/10.1016/j.bbrc.2009.04.081 https://pubmed.ncbi.nlm.nih.gov/19393621/

32. Jiang H, Dong L, Gong F, Gu Y, Zhang H, Fan D, et al. Inflammatory genes are novel prognostic biomarkers for colorectal cancer. Int J Mol Med. Spandidos Publications; 2018;42:368–80. https://doi.org/10.3892/ijmm.2018.3631 https://pubmed.ncbi.nlm.nih.gov/29693170/

33. Mariotti S, Barravecchia I, Vindigni C, Pucci A, Balsamo M, Libro R, et al. MICAL2 is a novel human cancer gene controlling mesenchymal to epithelial transition involved in cancer growth and invasion. Oncotarget. 2016; 7:1808–25. https://doi.org/10.18632/oncotarget.6577 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4811499/

34. Chang H-W, Chen S-Y, Chuang L-Y, Guleria S. Editorial Toxicology and Disease/Cancer Therapy in Reactive Oxygen Species-Mediated Drugs and Treatments. 2015; 2045:1. http://dx.doi.org/10.1155/2015/860563 https://pubmed.ncbi.nlm.nih.gov/25861684/

35. Soleimani M, Amini N, Sadeghian B, Wang D, Fang L. J Environ Sci (China). 2018;72:166–75. https://doi.org/10.1016/j.jes.2018.01.002 https://pubmed.ncbi.nlm.nih.gov/30244743/

36. Dagher Z, Garçon G, Billet S, Gosset P, Ledoux F, Courcot D, et al. Activation of different pathways of apoptosis by air pollution particulate matter (PM2.5) in human epithelial lung cells (L132) in culture. Toxicology. 2006;225:12–24. https://doi.org/10.1016/j.tox.2006.04.038 https://pubmed.ncbi.nlm.nih.gov/16787692/

37. Cai M, Li S, Shuai Y, Li J, Tan J, Zeng Q. Genome-wide CRISPR-Cas9 viability screen reveals genes involved in TNF-α-induced apoptosis of human umbilical vein endothelial cells. J Cell Physiol. Wiley-Liss Inc.; 2019;234:9184–93. https://doi.org/10.1002/jcp.27595 https://pubmed.ncbi.nlm.nih.gov/30317623/
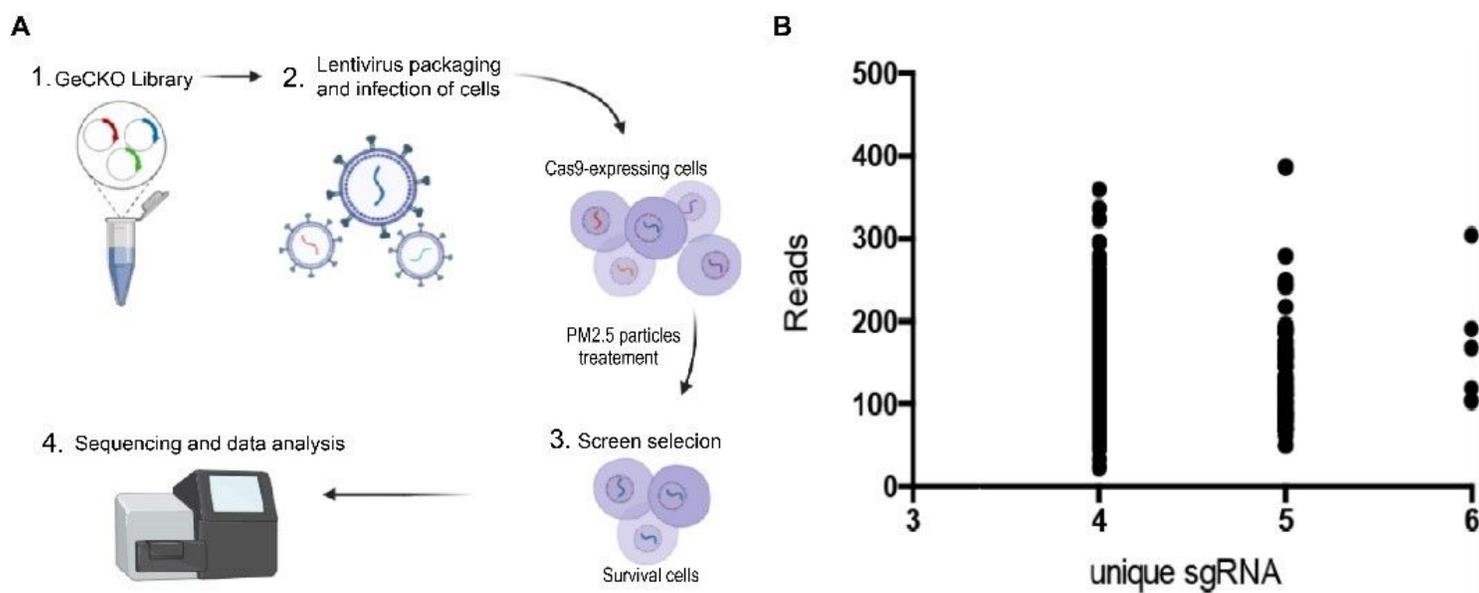
Figures

Figure 1

A genome-wide CRISPR/Cas9-mediated forward genetic screen to identify genes whose loss confers PM2.5 toxic effect. (A) Schematic of forward genetic screens in L02cells using pooled sgRNA libraries. (B) Genes identified in the screen for PM2.5 resistance. The X-axis is the number of unique sgRNAs for each gene, Y-axis is the number of reads change of each sgRNA compared to control.
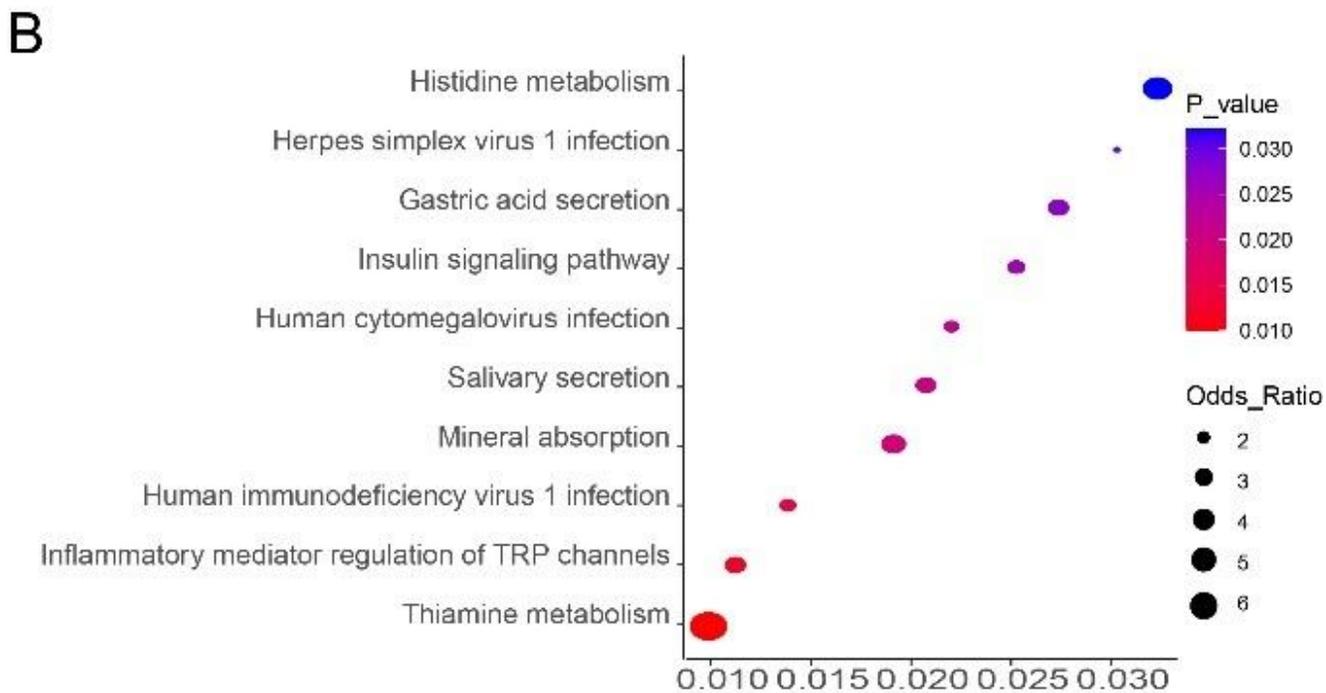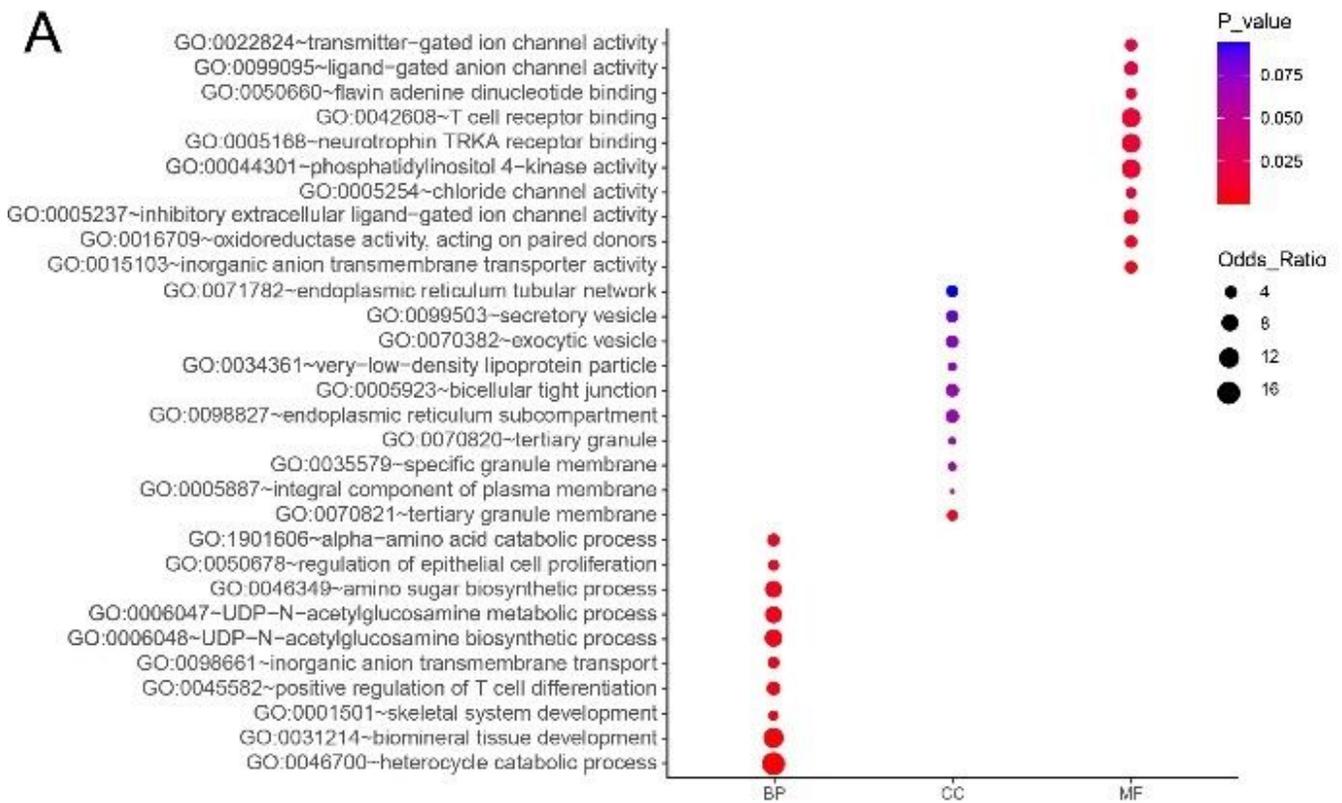
Figure 2

KEGG pathway (A) and GO enrichment (B) analysis of top-rank PM2.5 resistance genes. (Note. The processes and pathways in red are significant. BP: GO Biological Process, MF: GO Molecular Function, CC: GO Cellular Component.)
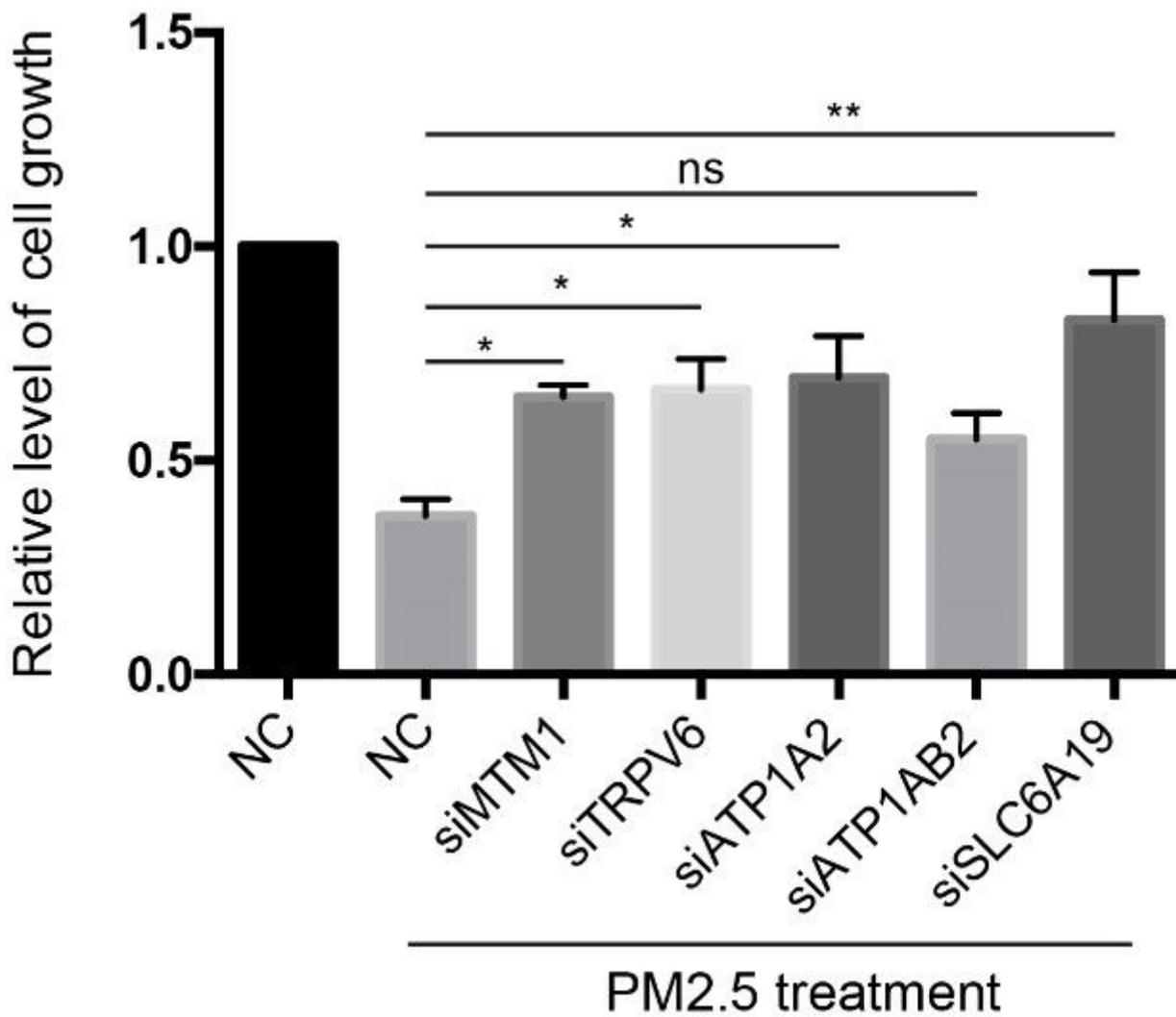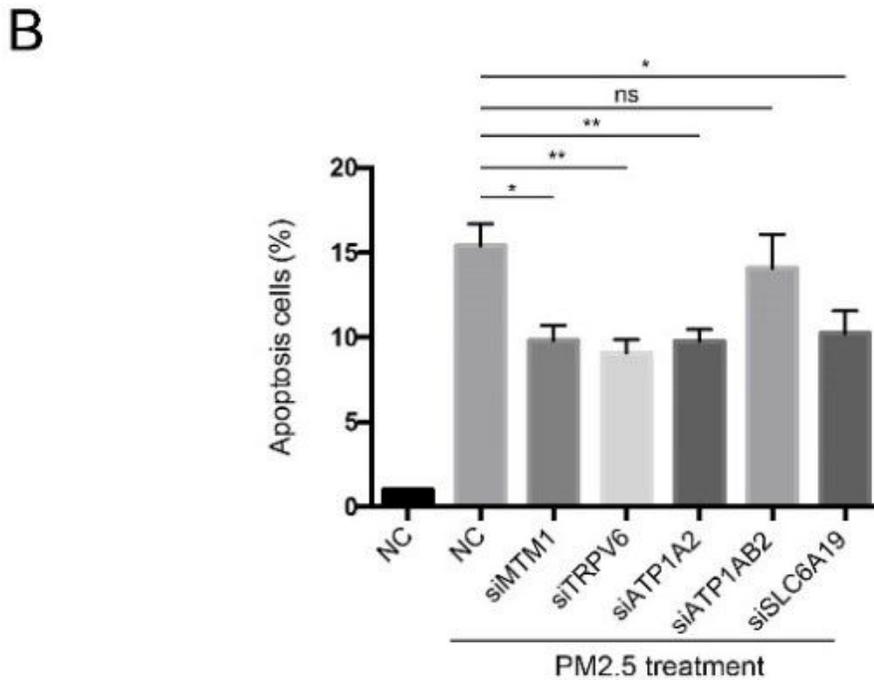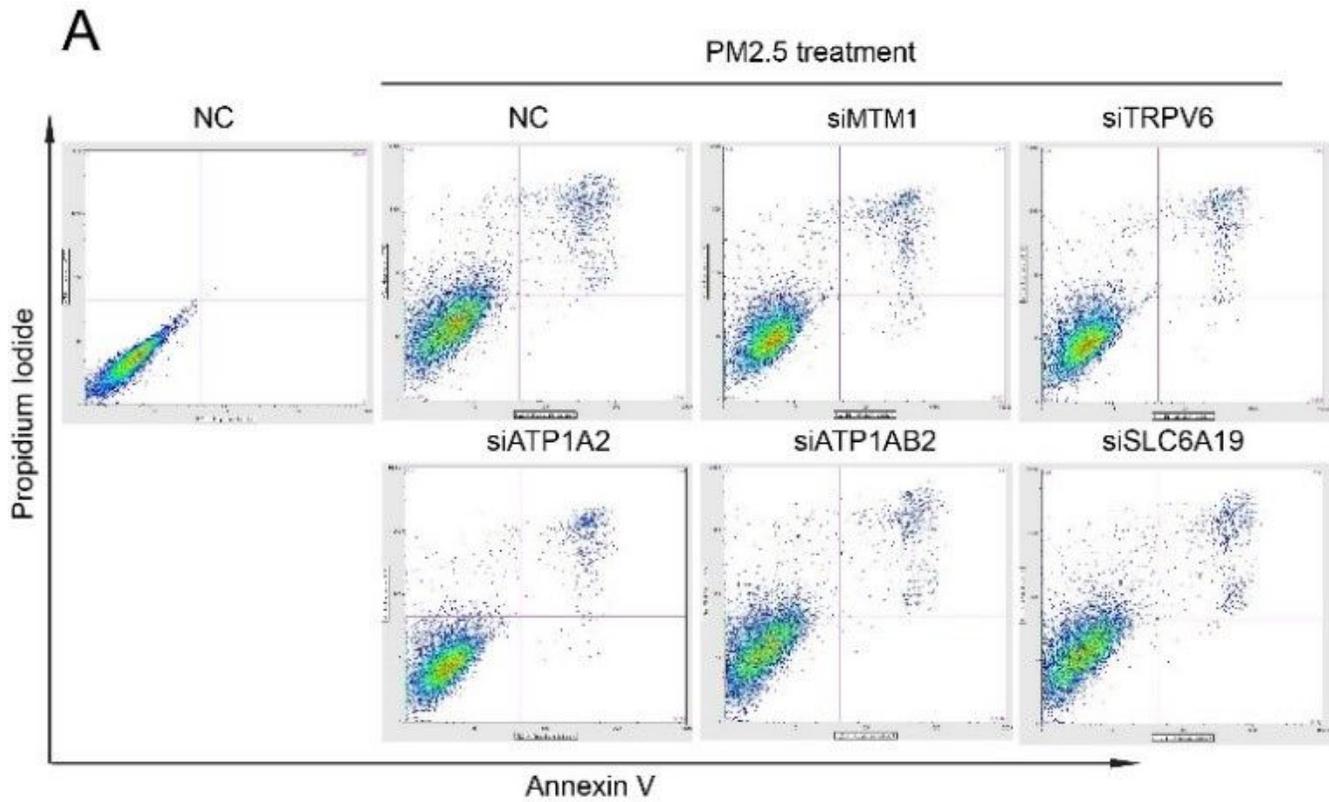
Figure 3

Knockdown of mineral absorption related genes rescued inhibition of cell viability by PM2.5. L02 cells were transfected siRNAs targeting each candidate gene and subjected PM2.5 exposure. The relative levels of cell viability were analyzed. *p<0.05, **p<0.01. ns, no significance.

Figure 4

Knockdown of mineral absorption related genes decreased PM2.5 -induced cell apoptosis. (A) Cells were treated with PM2.5 after knockdown different genes. Apoptosis was detected by Annexin V + PI staining. NC: Negative control. (B) The relative levels of cell apoptosis were analyzed. *p<0.05, **p<0.01. ns, no significance.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.xlsx
- Additionalfile2.xlsx
- Additionalfile3.xlsx