

SUPPLEMENTAL MATERIAL

MicroRNA-338-5p modulates pulmonary hypertension-like injuries caused by SO₂, NO₂ and PM_{2.5} co-exposure through targeting the HIF-1 α /Fhl-1 pathway

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Table S1 Primers sequences used for real-time RT-PCR

miRNA	Sequences (5'-3')
mmu-miR-142-5p	CGCATAAAGTAGAAAGCACT
mmu-miR-338-5p	CAATATCCTGGTGCTGAGTG
mmu-miR-450b-3p	ATTGGGAACATTTTGCAT
U6	TCGCTTCGGCAGCACATA

Table S2 Pearson correlation coefficient (R) of signal densities between two replicates for each treat group

	C1	C2	C3	L1	L2	L3	H1	H2	H3
C1	1	0.9892	0.9777	0.9769	0.9773	0.9650	0.9751	0.9743	0.9723
C2	0.9892	1	0.9768	0.9765	0.9803	0.9708	0.9768	0.9703	0.9713
C3	0.9777	0.9768	1	0.9673	0.9670	0.9755	0.9634	0.9670	0.9722
L1	0.9769	0.9765	0.9673	1	0.9747	0.9447	0.9598	0.9747	0.9608
L2	0.9773	0.9803	0.9670	0.9747	1	0.9583	0.9715	0.9781	0.9687
L3	0.9650	0.9708	0.9755	0.9447	0.9583	1	0.9714	0.9529	0.9718
H1	0.9751	0.9768	0.9634	0.9598	0.9715	0.9714	1	0.9810	0.9760
H2	0.9743	0.9703	0.9670	0.9747	0.9781	0.9529	0.9810	1	0.9758
H3	0.9723	0.9713	0.9722	0.9608	0.9687	0.9718	0.9760	0.9758	1

^aNote. "C1", "C2" and "C3"represents replicate 1, 2 and 3 of control group, respectively. "H1", "H2" and "H3"represents replicate 1, 2 and 3 of high concentration group, respectively.

Table S3 miRNAs with significant expression levels in the lungs after co-exposure to air pollutants

miRNAs	SO ₂ +NO ₂ +PM _{2.5} (low)	miRNAs	SO ₂ +NO ₂ +PM _{2.5} (high)
	Fold Change(p-value)		Fold Change(p-value)
Down-regulated (n=3)		Down-regulated (n=12)	
mmu-miR-3095-3p	0.72(0.0023)	mmu-miR-1196-3p	0.41(0.0437)
mmu-miR-375-3p	0.86(0.0238)	mmu-miR-1894-3p	0.51(0.0369)
mmu-let-7e-5p	0.90(0.0234)	mmu-miR-3070b-3p	0.56(0.0458)
		mmu-miR-1927	0.64(0.0133)
		mmu-miR-504-3p	0.68(0.0078)
		mmu-miR-674-5p	0.72(0.0057)
		mmu-miR-500-3p	0.74(0.0279)
		mmu-miR-338-5p	0.84(0.0108)
		mmu-miR-450b-3p	0.84(0.0182)
		mmu-miR-185-5p	0.86(0.0175)
		mmu-miR-374c-5p	0.87(0.0039)
		mmu-miR-425-5p	0.90(0.0063)
up-regulated (n=3)		up-regulated (n=6)	
mmu-miR-34b-5p	1.13(0.0055)	mmu-miR-3096b-3p	1.16(0.0291)
mmu-miR-30e-5p	1.13(0.0173)	mmu-miR-34b-5p	1.19(0.0296)
mmu-miR-3096b-3p	1.19(0.0069)	mmu-miR-142-5p	1.20(0.0239)
		mmu-miR-1249-3p	1.21(0.0129)
		mmu-miR-434-3p	1.45(0.0482)
		mmu-miR-205-5p	5.95(0.0395)

Table S4 Predicted potential targets for miR-338-5p using five bioinformatics software namely TargetScan, miRanda, PicTar, MirTarget2 and PITA

Accession No.	Gene ID	description
NM_031166	15904	Mus musculus inhibitor of DNA binding 4 (Id4), mRNA
NM_001083806	14800	Mus musculus glutamate receptor, ionotropic, AMPA2 (alpha 2) (Gria2), transcript variant 1, mRNA
NM_027900	71750	Mus musculus R3H domain containing 2 (R3hdm2), transcript variant 2, mRNA
NM_001098171	18526	Mus musculus protocadherin 10 (Pcdh10), transcript variant 3, mRNA
NM_001079883	58208	Mus musculus B cell leukemia/lymphoma 11B (Bcl11b), transcript variant 1, mRNA
NM_001037863	320940	Mus musculus ATPase, class VI, type 11C (Atp11c), transcript variant 1, mRNA
NM_008512	16971	Mus musculus low density lipoprotein receptor-related protein 1 (Lrp1), mRNA
NM_026420	67869	Mus musculus polyadenylate-binding protein-interacting protein 2 (Paip2), mRNA
NM_001081300	110796	Mus musculus teashirt zinc finger family member 1 (Tshz1), mRNA
NM_177747	245595	Mus musculus zinc finger protein 711 (Zfp711), mRNA
NM_010879	17974	Mus musculus non-catalytic region of tyrosine kinase adaptor protein 2 (Nck2), mRNA
NM_010698	16826	Mus musculus LIM domain binding 2 (Ldb2), transcript variant 1, mRNA
NM_198862	216856	Mus musculus neuroligin 2 (Nlgn2), mRNA
NM_001081196	68693	Mus musculus heterogeneous nuclear ribonucleoprotein U-like 2 (Hnrnpul2), mRNA
NM_018745	54375	Mus musculus antizyme inhibitor 1 (Azin1), transcript variant 2, mRNA
NM_145575	109624	Mus musculus caldesmon 1 (Cald1), mRNA
NM_021605	59125	Mus musculus NIMA (never in mitosis gene a)-related expressed kinase 7 (Nek7), mRNA
NM_009427	22057	Mus musculus transducer of ErbB-2.1 (Tob1), mRNA
NM_019634	21912	Mus musculus tetraspanin 7 (Tspan7), mRNA
NM_011364	20400	Mus musculus SH2 domain protein 1A (Sh2d1a), mRNA
NM_008891	18949	Mus musculus pinin (Pnn), mRNA
NM_011898	24066	Mus musculus sprouty homolog 4 (Drosophila) (Spry4), mRNA
NM_178357	194655	Mus musculus Kruppel-like factor 11 (Klf11), mRNA
NM_172684	229675	Mus musculus rosbins, round spermatid basic protein 1 (Rsb1), mRNA
NM_010825	17536	Mus musculus Meis homeobox 2 (Meis2), transcript variant 2,

		mRNA
NM_018824	54338	Mus musculus solute carrier family 23 (nucleobase transporters), member 2 (Slc23a2), mRNA
NM_198300	208922	Mus musculus cytoplasmic polyadenylation element binding protein 3 (Cpeb3), mRNA
NM_028810	74194	Mus musculus Rho family GTPase 3 (Rnd3), mRNA
NM_020007	56758	Mus musculus muscleblind-like 1 (Drosophila) (Mbnl1), transcript variant 1, mRNA
NM_172436	78830	Mus musculus solute carrier family 25 (mitochondrial carrier, Aralar), member 12 (Slc25a12), nuclear gene encoding mitochondrial protein, mRNA
NM_008679	17979	Mus musculus nuclear receptor coactivator 3 (Ncoa3), mRNA
NM_025951	67073	Mus musculus phosphatidylinositol 4-kinase type 2 beta (Pi4k2b), transcript variant 1, mRNA
NM_010431	15251	Mus musculus hypoxia inducible factor 1, alpha subunit (Hif1a), mRNA
NM_177387	338362	Mus musculus uronyl-2-sulfotransferase (Ust), mRNA
NM_212450	329506	Mus musculus CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase like 2 (Ctdspl2), mRNA
NM_019796	56403	Mus musculus synaptotagmin binding, cytoplasmic RNA interacting protein (Syncrip), transcript variant 2, mRNA
NM_009289	20874	Mus musculus STE20-like kinase (Slk), transcript variant 1, mRNA
NM_010914	18045	Mus musculus nuclear transcription factor-Y beta (Nfyb), mRNA
NM_016851	54139	Mus musculus interferon regulatory factor 6 (Irf6), mRNA
NM_027295	100972	Mus musculus RAB28, member RAS oncogene family (Rab28), mRNA
NM_011376	20464	Mus musculus single-minded homolog 1 (Drosophila) (Sim1), mRNA
NM_013556	15452	Mus musculus hypoxanthine guanine phosphoribosyl transferase (Hprt), mRNA
NM_001081034	225055	Mus musculus F-box protein 11 (Fbxo11), mRNA
NM_001081477	382236	Mus musculus bromodomain and WD repeat domain containing 3 (Brwd3), mRNA
NM_008090	14461	Mus musculus GATA binding protein 2 (Gata2), mRNA
NM_008057	14369	Mus musculus frizzled homolog 7 (Drosophila) (Fzd7), mRNA
NM_178890	99382	Mus musculus ankyrin repeat and BTB (POZ) domain containing 2 (Abtb2), mRNA
NM_007705	12696	Mus musculus cold inducible RNA binding protein (Cirbp), mRNA
NM_194055	207920	Mus musculus epithelial splicing regulatory protein 1 (Esrp1), mRNA
NM_009817	12380	Mus musculus calpastatin (Cast), mRNA

NM_010897	18015	Mus musculus neurofibromatosis 1 (Nf1), mRNA
NM_053075	19744	Mus musculus Ras homolog enriched in brain (Rheb), mRNA
NM_009627	11535	Mus musculus adrenomedullin (Adm), mRNA
NM_010217	14219	Mus musculus connective tissue growth factor (Ctgf), mRNA
NM_177821	328572	Mus musculus E1A binding protein p300 (Ep300), mRNA
NM_144802	72692	Mus musculus heterogeneous nuclear ribonucleoprotein L-like (Hnrpll), mRNA
NM_031179	81898	Mus musculus splicing factor 3b, subunit 1 (Sf3b1), mRNA
NM_001037099	12298	Mus musculus calcium channel, voltage-dependent, beta 4 subunit (Cacnb4), transcript variant 1, mRNA
NM_001081395	75723	Mus musculus angiomin-like 1 (Amotl1), mRNA
NM_011809	23872	Mus musculus E26 avian leukemia oncogene 2, 3' domain (Ets2), mRNA
NM_013635	19027	Mus musculus synaptophysin-like protein (Sypl), transcript variant 1, mRNA
NM_010933	18159	Mus musculus natriuretic peptide type C (Nppc), mRNA
NM_145515	226778	Mus musculus MAP/microtubule affinity-regulating kinase 1 (Mark1), mRNA
NM_009859	235072	Mus musculus septin 7 (Sept7), transcript variant 1, mRNA
NM_011957	26427	Mus musculus cAMP responsive element binding protein 3-like 1 (Creb3l1), mRNA
NM_019840	18578	Mus musculus phosphodiesterase 4B, cAMP specific (Pde4b), transcript variant 1, mRNA
NM_145148	232288	Mus musculus FERM domain containing 4B (Frmd4b), mRNA
NM_019812	93759	Mus musculus sirtuin 1 (silent mating type information regulation 2, homolog) 1 (S. cerevisiae) (Sirt1), transcript variant 1, mRNA
NM_011445	20679	Mus musculus SRY-box containing gene 6 (Sox6), transcript variant 1, mRNA
NM_009843	12477	Mus musculus cytotoxic T-lymphocyte-associated protein 4 (Ctla4), mRNA
NM_029924	109241	Mus musculus methyl-CpG binding domain protein 5 (Mbd5), mRNA
NM_001080931	327987	Mus musculus mediator complex subunit 13 (Med13), mRNA
NM_029456	52036	Mus musculus protein phosphatase 6, regulatory subunit 3 (Ppp6r3), transcript variant 2, mRNA
NM_013885	29876	Mus musculus chloride intracellular channel 4 (mitochondrial) (Clic4), nuclear gene encoding mitochondrial protein, mRNA
NM_011768	22764	Mus musculus zinc finger protein X-linked (Zfx), transcript variant 2, mRNA
NM_010834	17700	Mus musculus myostatin (Mstn), mRNA
NM_001025572	106585	Mus musculus ankyrin repeat domain 12 (Ankrd12), mRNA
NM_144825	216965	Mus musculus TAO kinase 1 (Taok1), mRNA
NM_007604	12343	Mus musculus capping protein (actin filament) muscle Z-line,

		alpha 2 (Capza2), mRNA
NM_175836	20742	Mus musculus spectrin beta, non-erythrocytic 1 (Sptbn1), transcript variant 1, mRNA
NM_001045536	195018	Mus musculus zinc finger, ZZ-type with EF hand domain 1 (Zzef1), mRNA
NM_177371	326623	Mus musculus tumor necrosis factor (ligand) superfamily, member 15 (Tnfsf15), mRNA
NM_029441	75796	Mus musculus chromodomain protein, Y chromosome-like 2 (Cdy12), mRNA
NM_133188	70248	Mus musculus DAZ associated protein 1 (Dazap1), transcript variant 1, mRNA
NM_029791	76895	Mus musculus bicaudal D homolog 2 (Drosophila) (Bicd2), transcript variant 2, mRNA
NM_001081348	94253	Mus musculus HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1 (Hecw1), mRNA
NM_008740	18195	Mus musculus N-ethylmaleimide sensitive fusion protein (Nsf), mRNA
NM_007865	13388	Mus musculus delta-like 1 (Drosophila) (Dl1), mRNA
NM_027470	70584	Mus musculus p21 protein (Cdc42/Rac)-activated kinase 4 (Pak4), mRNA
NM_001001798	320940	Mus musculus ATPase, class VI, type 11C (Atp11c), transcript variant 2, mRNA
NM_172664	228012	Mus musculus tousled-like kinase 1 (Tlk1), mRNA
NM_020005	18519	Mus musculus K(lysine) acetyltransferase 2B (Kat2b), transcript variant 1, mRNA
NM_009640	11600	Mus musculus angiopoietin 1 (Angpt1), mRNA
NM_019737	56386	Mus musculus UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 6 (B4galt6), mRNA
NM_015791	50753	Mus musculus F-box protein 8 (Fbxo8), mRNA
NM_008541	17129	Mus musculus SMAD family member 5 (Smad5), transcript variant 1, mRNA
NM_133219	14538	Mus musculus glucosaminyl (N-acetyl) transferase 2, I-branching enzyme (Gcnt2), transcript variant 3, mRNA
NM_026476	67958	Mus musculus U2 snRNP-associated SURP domain containing (U2surp), transcript variant 2, mRNA
NM_001040106	269774	Mus musculus AP2 associated kinase 1 (Aak1), transcript variant 1, mRNA
NM_025483	66315	Mus musculus SUMO1/sentrin specific peptidase 7 (Senp7), transcript variant 1, mRNA
NM_007726	12801	Mus musculus cannabinoid receptor 1 (brain) (Cnr1), mRNA
NM_028459	73178	Mus musculus Wiskott-Aldrich syndrome-like (human) (Wasl), transcript variant 1, mRNA
NM_001081356	67048	Mus musculus VMA21 vacuolar H ⁺ -ATPase homolog (S. cerevisiae) (Vma21), mRNA

NM_013464	11622	Mus musculus aryl-hydrocarbon receptor (Ahr), mRNA
NM_172882	72145	Mus musculus WD repeat and FYVE domain containing 3 (Wdfy3), mRNA
NM_139146	212712	Mus musculus special AT-rich sequence binding protein 2 (Satb2), mRNA
NM_011866	23984	Mus musculus phosphodiesterase 10A (Pde10a), mRNA
NM_019831	56364	Mus musculus zinc finger, MYM-type 3 (Zmym3), transcript variant 1, mRNA
NM_011808	23871	Mus musculus E26 avian leukemia oncogene 1, 5' domain (Ets1), transcript variant 1, mRNA
NM_145556	230908	Mus musculus TAR DNA binding protein (Tardbp), transcript variant 1, mRNA
NM_011935	26381	Mus musculus estrogen-related receptor gamma (Esrrg), transcript variant 1, mRNA
NM_008253	15354	Mus musculus high mobility group box 3 (Hmgb3), mRNA
NM_001001182	407823	Mus musculus bromodomain adjacent to zinc finger domain, 2B (Baz2b), mRNA
NM_172958	268783	Mus musculus myotubularin related protein 12 (Mtmr12), mRNA

Table S5 Predicted potential targets for miR-450b-3p using five bioinformatics software namely TargetScan, miRanda, PicTar, MirTarget2 and PITA

Accession No.	Gene ID	description
NM_007664	12558	Mus musculus cadherin 2 (Cdh2), mRNA
NM_015753	24136	Mus musculus zinc finger E-box binding homeobox 2 (Zeb2), transcript variant 2, mRNA
NM_013927	30952	Mus musculus cyclic nucleotide gated channel beta 3 (Cngb3), mRNA
NM_009223	20621	Mus musculus stannin (Snn), mRNA
NM_177224	109151	Mus musculus chromodomain helicase DNA binding protein 9 (Chd9), mRNA
NM_008166	14803	Mus musculus glutamate receptor, ionotropic, delta 1 (Grid1), mRNA
NM_001077265	11991	Mus musculus heterogeneous nuclear ribonucleoprotein D (Hnrnpd), transcript variant 1, mRNA
NM_028320	72674	Mus musculus adiponectin receptor 1 (Adipor1), mRNA
NM_011749	22661	Mus musculus zinc finger protein 148 (Zfp148), mRNA
NM_026697	68365	Mus musculus RAB14, member RAS oncogene family (Rab14), mRNA
NM_011327	20280	Mus musculus sterol carrier protein 2, liver (Scp2), mRNA
NM_175009	223527	Mus musculus enhancer of yellow 2 homolog (Drosophila) (Eny2), mRNA
NM_144812	213988	Mus musculus trinucleotide repeat containing 6b (Tnrc6b), transcript variant 1, mRNA
NM_026211	67511	Mus musculus transmembrane emp24 protein transport domain containing 9 (Tmed9), mRNA
NM_145711	252838	Mus musculus thymocyte selection-associated high mobility group box (Tox), mRNA
NM_008638	17768	Mus musculus methylenetetrahydrofolate dehydrogenase (NAD+ dependent), methenyltetrahydrofolate cyclohydrolase (Mthfd2), nuclear gene encoding mitochondrial protein, mRNA

