

Supplementary Information

The cellular response of the bronchial epithelium shapes the protein corona of inhaled nanoparticles

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Table of content

- Isolation of the protein corona of AgNPs in bronchial mucus
- Epithelial barrier integrity and metabolic activity of Calu-3 cells exposed to AgNPs
- Proteomic analysis of the cellular protein corona
- Reactome pathway analysis of the cellular protein corona
- Composition of the acellular protein corona
- Composition of the cellular protein corona
- Evolution of the apical secretome of Calu-3 cells exposed to AgNPs

Associated files

The full lists of proteins identified in the protein corona of AgNPs in acellular and cellular conditions are provided in the Excel file.

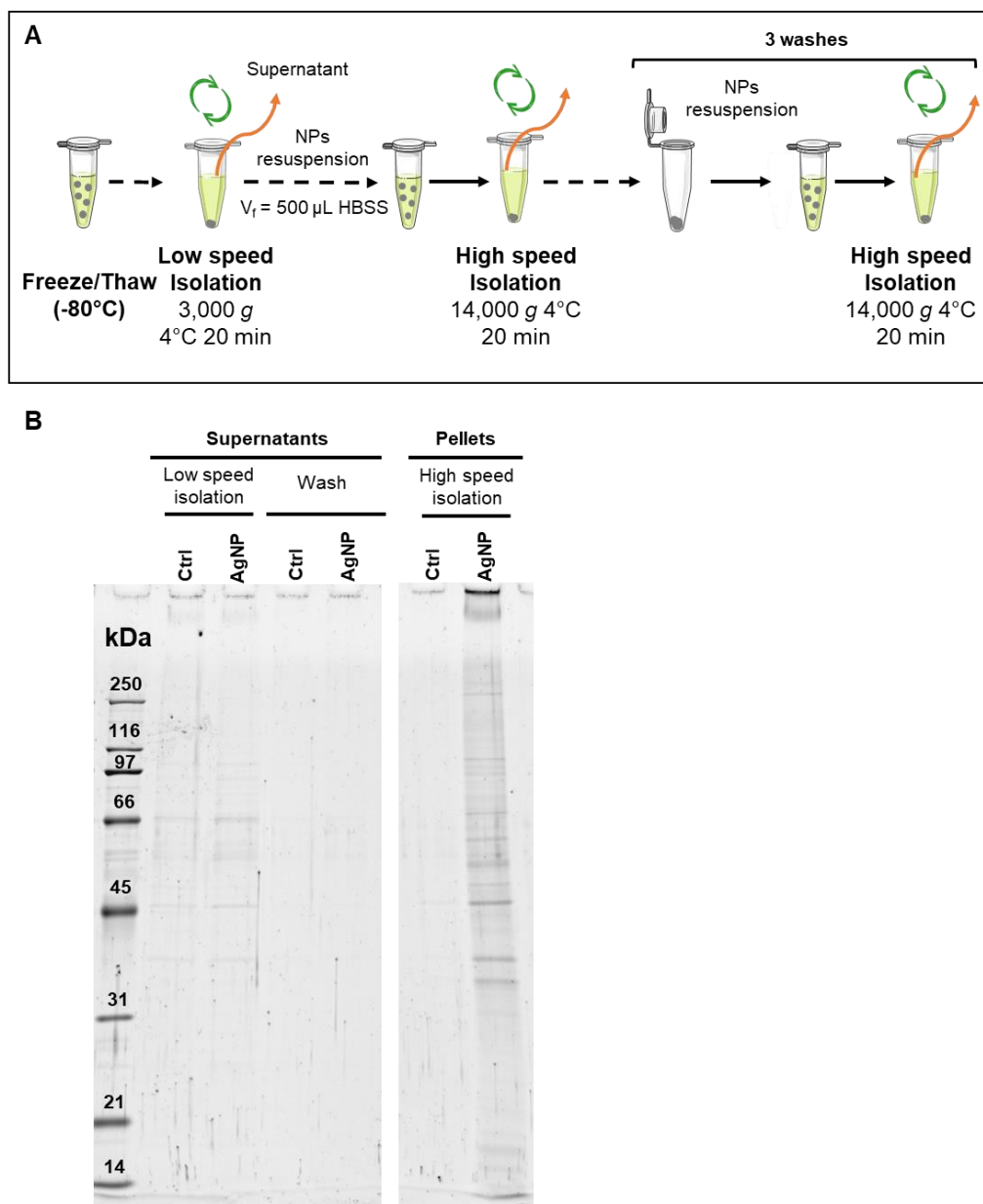


Figure S1. Isolation of the protein corona of AgNPs in bronchial mucus. (A) Protocol for the separation of AgNPs from free proteins in the apical secretome of Calu-3 cells. (B) SDS-PAGE of apical secretome supernatants and pellets with and without AgNPs after isolation protocol. The final pellets were analyzed by LC-MS/MS in both conditions to identify proteins forming the protein corona. Proteins detected in the pellet of control samples were excluded from the analysis.

Materials and methods

SDS-PAGE. AgNPs were separated from free proteins in solution by centrifugation at 3,000 g x 2 for 20 min at 4°C, followed by 3 washes in HBSS^{Ca2+/Mg2+} and centrifugation at 14,000 g for 20 min at 4°C. The pellet was resuspended in lithium dodecyl sulphate (LDS) loading buffer (NP0007, Invitrogen) supplemented with 50 mM DTT (Thermo Fisher, France) and heated to 100°C for 5 min. SDS-PAGE was performed at 120 V for 1h. Proteins were fixed with 50% methanol and 7% acetic acid in milliQ water for 30 min and stained with SYPRO® Ruby (S12000, Molecular Probes).

Table S1. Sequence of primers used for RT-qPCR

Gene name	Forward (5'-3')	Reverse (5'-3')
<i>rpl19</i>	GGCTCGCCTCTAGTGCCTC	GGCTCGCCTCTAGTGCCTC
<i>muc5ac</i>	AACTACTCCCAGCCCTGTTC	GTAGAGGCAGGGGTTGTTCT
<i>alb</i>	TAGAGAAGTGCTGTGCCGCT	AGTTGGAGTTGACACTTGGGGT
<i>hmx-1</i>	CGTTCCTGGTCAACATCC	CTGTCGCCACCAGAAAG
<i>mt1x</i>	ACTCCTGCTTCTCCTTGCCTC	TGCACTTGTCTGACGTCCCTT
<i>mt2a</i>	TCCTGCAAATGCAAAGAGTGC	AAAATCCAGGTTTGTGGAAGTCG
<i>il-6</i>	TGGTCTTTGGAGTTTGAGG	CGCAGAATGAGATGAGTTGTC
<i>il-8</i>	AGACAGCAGAGCACACAAGC	ATGGTTCCTCCGGTGGT

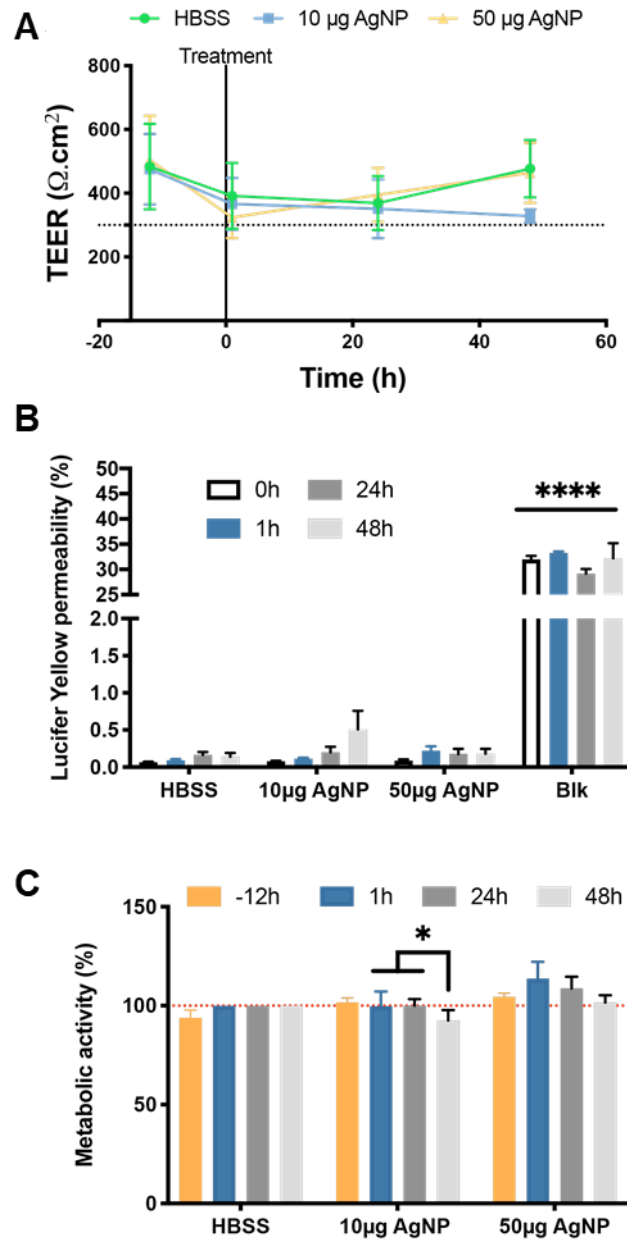


Figure S2. Epithelial barrier integrity and metabolic activity of Calu-3 cells exposed to 10 and 50 $\mu\text{g}/\text{cm}^2$ AgNPs at ALI for 1, 24, and 48 h. Control cells were treated with HBSS buffer. (A) TEER (Trans Epithelial Electric Resistance) measured 12h before treatment and after 1, 24, 48h exposure to AgNPs. The minimum value of 300 $\Omega \cdot \text{cm}^2$ for a tight epithelium is indicated by a dashed line. (B) Lucifer yellow permeability assay (Blk: empty well). (C) Alamar blue cell viability assay: metabolic activity normalized to HBSS control measured 12h before treatment and after 1, 24, 48 h exposure to AgNPs. N = 3 (* p < 0.05).

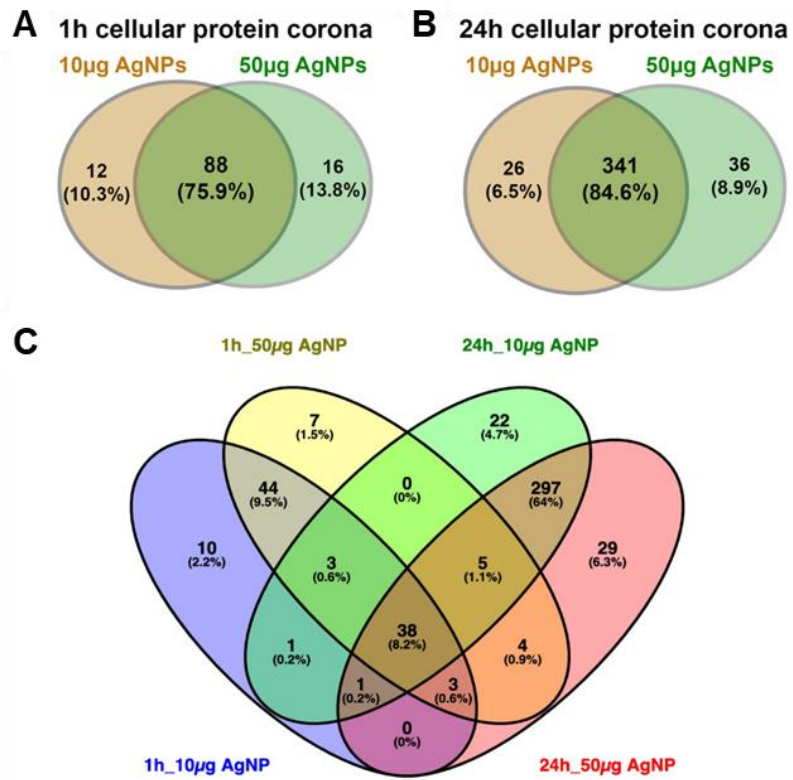


Figure S3. Quantitative proteomic analysis of the cellular protein corona after exposure of the bronchial epithelium model to 10 and 50 µg/cm². Comparison of the corona (A) after 1 h exposure as a function of AgNP concentration, (B) after 24 h exposure as a function of AgNP concentration, (C) as a function of both exposure time and AgNP concentration.

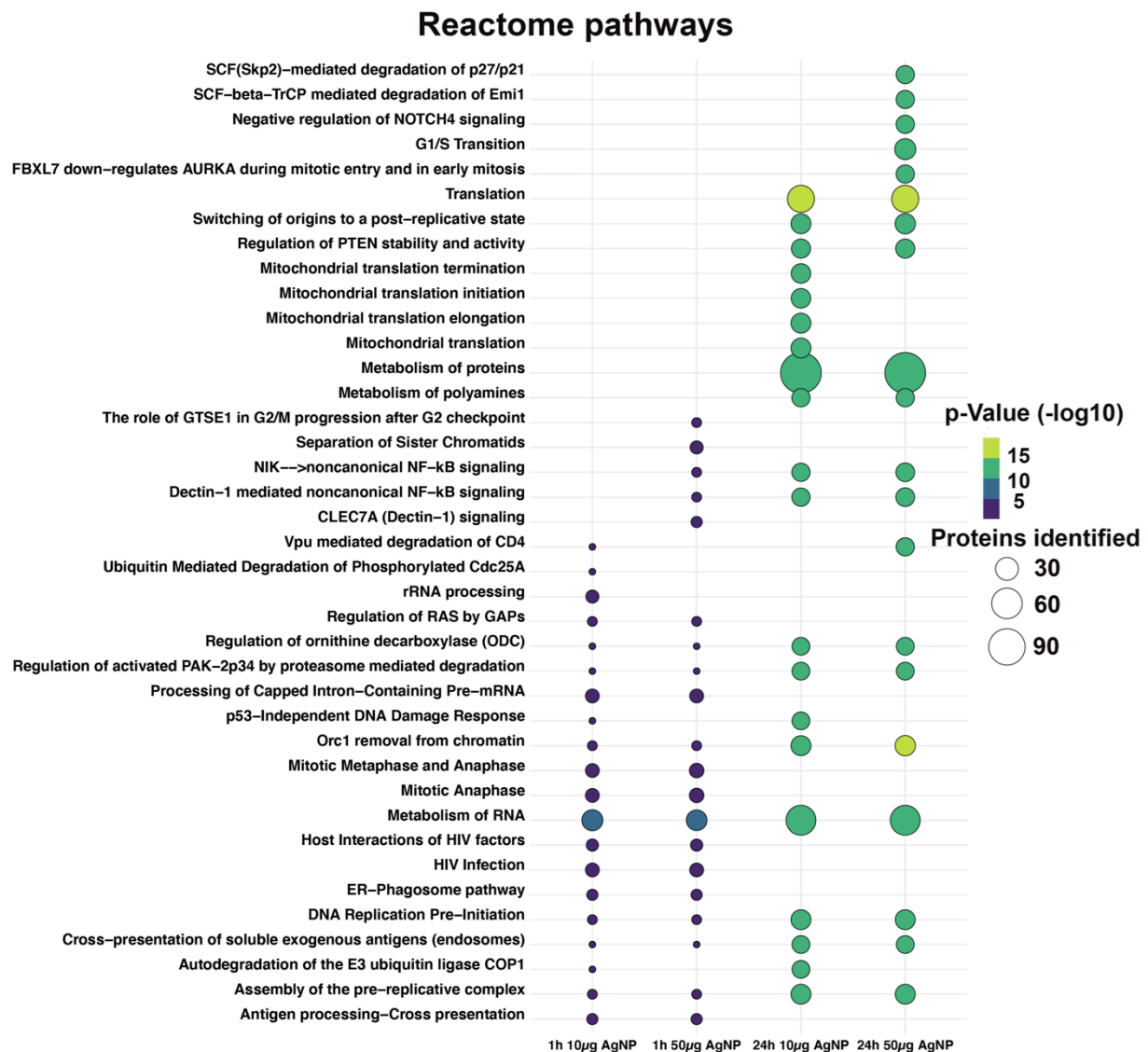


Figure S4. Reactome pathway functional enrichment analysis for the cellular protein corona after exposure of Calu-3 cells to 10 and 50 µg/cm² AgNPs for 1 and 24 h. Circle size reflects the number of proteins associated with each pathway. A minimum of 10 different proteins must be identified to validate a functional pathway. Only pathways significantly enriched in the corona are shown (p < 0.05).

Table S2. List of the 10 most abundant proteins in the acellular corona after 1 h incubation of 10 and 50 μg AgNPs in the apical secretome of Calu-3 cells (E: extracellular, I: intracellular).

Condition	Gene name	Accession	Extra-cellular	Intra-cellular	Description
10 μg AgNP	PSMB3	P49720	E	I	Proteasome subunit beta type-3
	PCBD1	P61457	E	I	Pterin-4-alpha-carbinolamine dehydratase
	PSMB4	P28070	E	I	Proteasome subunit beta type-4
	ANP32B	Q92688	E	I	Acidic leucine-rich nuclear phosphoprotein 32 family member B
	MDK	P21741	E		Midkine
	EWSR1	Q01844		I	RNA-binding protein EWS
	PCMT1	P22061	E	I	Protein-L-isoaspartate(D-aspartate) O-methyltransferase
	PSAT1	Q9Y617	E	I	Phosphoserine aminotransferase
	API5	Q9BZZ5		I	Apoptosis inhibitor 5
	FAH	P16930	E	I	Fumarylacetoacetase
50 μg AgNP	EWSR1	Q01844		I	RNA-binding protein EWS
	PSMB3	P49720	E	I	Proteasome subunit beta type-3
	FKBP1A	P62942		I	Peptidyl-prolyl cis-trans isomerase FKBP1A
	ANP32B	Q92688	E	I	Acidic leucine-rich nuclear phosphoprotein 32 family member B
	YBX1	P67809	E	I	Y-box-binding protein 1
	PCBD1	P61457	E	I	Pterin-4-alpha-carbinolamine dehydratase
	TFPI	P10646	E		Tissue factor pathway inhibitor
	PSMB4	P28070	E	I	Proteasome subunit beta type-4
	HMGB3	O15347		I	High mobility group protein B3
	KPNA4	O00629		I	Importin subunit alpha-3

Table S3. List of the 10 most abundant proteins in the cellular corona after 1 h exposure of Calu-3 cells to 10 and 50 $\mu\text{g}/\text{cm}^2$ AgNPs (E: extracellular, I: intracellular).

Condition	Gene name	Accession	Extra-cellular	Intra-cellular	Description
10 $\mu\text{g}/\text{cm}^2$ AgNP	RHOC	P08134	E	I	Rho-related GTP-binding protein RhoC
	DEK	P35659		I	Protein DEK
	TAF15	Q92804		I	TATA-binding protein-associated factor 2N
	NUP205	Q92621		I	Nuclear pore complex protein Nup205
	STX7	O15400	E	I	Syntaxin-7
	NDUFB10	O96000		I	NADH dehydrogenase 1 beta subcomplex subunit 10
	CLDN4	O14493		I	Claudin-4
	RCC2	Q9P258		I	Protein RCC2
	NDUFB6	O95139		I	NADH dehydrogenase 1 beta subcomplex subunit 6
	RPL36	Q9Y3U8		I	60S ribosomal protein L36
50 $\mu\text{g}/\text{cm}^2$ AgNP	YBX1	P67809	E	I	Nuclease-sensitive element-binding protein 1
	SLTM	Q9NWH9		I	SAFB-like transcription modulator
	MTX1	Q13505		I	Metaxin-1
	SUB1	P53999	E	I	Activated RNA polymerase II transcriptional coactivator p15
	SEC61G	P60059		I	Protein transport Sec61 subunit gamma
	RPL36	Q9Y3U8		I	60S ribosomal protein L36
	EIF3E	P60228	E	I	Eukaryotic translation initiation factor 3 subunit E
	SNRPD2	P62316	E	I	Small nuclear ribonucleoprotein Sm D2
	PPL	O60437	E	I	PPL
	NUP205	NUP205		I	Nuclear pore complex protein

Table S4. List of the 10 most abundant proteins in the cellular corona after 24 h exposure of Calu-3 cells to 10 and 50 $\mu\text{g}/\text{cm}^2$ AgNP (E: extracellular, EV: exosomes, I: intracellular).

Condition	Gene name	Accession	Extra-cellular	Intra-cellular	Description
10 $\mu\text{g}/\text{cm}^2$ AgNP	TUBB8	Q3ZCM7	E	I	Tubulin beta-8 chain
	CYB5B	H3BUX2		I	Cytochrome b5 type B
	ALDOC	P09972	E	I	Fructose-bisphosphate aldolase C
	SF3A3	Q12874		I	Splicing factor 3A subunit 3
	C1QBP	Q07021		I	Complement component 1 Q subcomponent-binding protein
	HSP90AB2P	Q58FF8	EV	I	Putative heat shock protein HSP 90-beta 2
	CCL20	P78556	E		C-C motif chemokine 20
	SAR1A	Q9NR31	EV	I	GTP-binding protein SAR1a
	HCFC1	A6NEM2		I	Host cell factor 1
	SNRPD3	P62318		I	Small nuclear ribonucleoprotein Sm D3
50 $\mu\text{g}/\text{cm}^2$ AgNP	TUBB8	Q3ZCM7	E	I	Tubulin beta-8 chain
	HSP90AB2P	Q58FF8	EV	I	Putative heat shock protein HSP 90-beta 2
	SF3A3	Q12874		I	Splicing factor 3A subunit 3
	CYB5B	H3BUX2		I	Cytochrome b5 type B
	ALDOC	P09972	E	I	Fructose-bisphosphate aldolase C
	DDAH1	O94760	E	I	N(G),N(G)-dimethylarginine dimethylaminohydrolase1
	UBE2I	P63279		I	SUMO-conjugating enzyme UBC9
	TXN	P10599		I	Thioredoxin
	SSB	P05455		I	Lupus La protein
	ANP32A	P39687		I	Acidic leucine-rich nuclear phosphoprotein 32 family member A

Table S5. Proteins significantly overexpressed in the apical secretome of Calu-3 cells exposed to 50 $\mu\text{g}/\text{cm}^2$ AgNPs for 48 h, based on the datasets from [1] (E: extracellular, EV: exosomes, I: intracellular).

Condition	Gene name	Accession	Extra-cellular	Intra-cellular	Description
Secretome 50 $\mu\text{g}/\text{cm}^2$ AgNP	SMPDL3B	Q92485	E/EV		Acid sphingomyelinase-like phosphodiesterase 3b
	VCL	P18206	E/EV	I	Vinculin
	CAP1	Q01518	E/EV	I	Adenylyl cyclase-associated protein 1
	HNRNPL	P14866	EV	I	Heterogeneous nuclear ribonucleoprotein L
	YWHAZ	P63104	E/EV	I	14-3-3 protein zeta/delta
	YWHAE	P62258	EV	I	14-3-3 protein epsilon
	GAPDH	P04406	EV	I	Glyceraldehyde-3-phosphate dehydrogenase
	EPRS	P07814		I	Glutamate/proline-tRNA ligase

Table S6. Proteins common to the protein corona formed *in situ* during Calu-3 exposure to AgNPs (50 µg/cm², 24 h) and to the apical secretome of Calu-3 cells exposed to AgNPs (50 µg/cm², 48 h), based on the dataset from [1]. Only proteins unique to cells exposed to AgNPs were considered.

Condition	Gene name	Description
50 µg/cm ² AgNP	SEC23A	Protein transport Sec23A
	KIF5B	Kinesin-1 heavy chain
	EIF5	Translation initiation factor 5
	PCBD1	Pterin-4-alpha-carbinolamine dehydratase
	HUWE1	E3 ubiquitin-protein ligase
	DIAPH1	Protein diaphanous homolog 1
	SMS	Spermine synthase
	UBE2L3	Ubiquitin-conjugating enzyme
	PSMC1	26S protease regulatory subunit 4
	SERPINB6	Serpin B6
	GMDS	GDP-mannose 4,6 dehydratase
	PSMB1	Proteasome subunit beta type-1
	PSMD7	26S proteasome non-ATPase regulatory subunit 7
	ANP32A	Acidic leucine-rich nuclear phosphoprotein 32 family member A
	C1QBP	Complement component 1 Q subcomponent-binding protein
	EIF3B	Translation initiation factor 3 subunit B
	AHSA1	Activator of 90 kDa heat shock protein ATPase homolog 1
	TSTA3	GDP-L-fucose synthase
	NUTF2	Nuclear transport factor 2
	HSP90AB2P	Heat shock protein HSP 90-beta 2

References

1. Chivé C, Mc Cord C, Sanchez-Guzman D, Brookes O, Joseph P, Lai Kuen R, et al. 3D model of the bronchial epithelial barrier to study repeated exposure to xenobiotics: Application to silver nanoparticles. *Environ Toxicol Pharmacol.* 2023;103:104281; doi: 10.1016/j.etap.2023.104281.