Epithelial Stress and Apoptosis Underlie Hermansky-Pudlak Syndrome Associated Interstitial Pneumonia

Poornima Mahavadi[1], Martina Korfei[1], Ingrid Henneke[1], Gerhard Liebisch[3], Gerd Schmitz[3], Bernadette R. Gochuico[4], Philipp Markart[1,2], Sevario Bellusci[2], Werner Seeger[1,2], Clemens Ruppert[1,2*] and Andreas Guenther[1,2,5*].

[1]Department of Internal Medicine II and [2]Excellence Cluster "Cardiopulmonary System", University of Giessen Lung Center (UGLC), Giessen, Germany. [2]Institute of Clinical Chemistry and Laboratory Medicine, University of Regensburg, Regensburg, Germany. [3]Medical Genetics Branch, NHGRI, Bethesda, NIH, USA. [5]Lung Clinic Waldhof-Elgershausen, Greifenstain, Germany.

* - both authors equally contributed to the paper

Online Supplement

Supplementary methods:

Genotyping of HPS mice: All HPS mice used in this study were genotyped for the mutated gene with the primers listed below. Genomic DNA for this purpose was isolated with the "DNeasy kit" (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Sentinel mice were tested periodically and were free of known viral and bacterial pathogens. Mice were sacrificed by injecting an overdose of anesthesia, and lungs were isolated according to the standard protocol as described in the following chapter. As described earlier [E1], HPS 1/2, but not any of the other mice, show breeding difficulties, making it difficult to expand the HPS1/2 colony.

List of primers used for genotyping HPS mice.

wt HPS1-forward	5' AGCTAGAACACTGTCCAAAGATAGC 3'
wt HPS1-reverse	5' GTATGAGAGAAGGCACTGGAAGAAG 3'
HPS1-forward	5' AGCTAGAACACTGTCCAAAGATAGC 3'
HPS1-forward	5' ATGTAAGAATAAAGCTTTGTCGCAG 3'
wt HPS6-forward	5' ACATCCTGCTACACCATTGCCCC 3'
wt HPS6-reverse	5' CATGTCCAGTAGTTCCAAGGTGGAG 3'
HPS6-forward	5' CACCCACATCCTGCTACACCTTTTTG 3'
HPS6-reverse	5' CATGTCCAGTAGTTCCAAGGTGGAG 3'
HPS2-forward	5' GAAATGGGGCTGCACATAG 3'
HPS2-reverse	5' GAACCCTCACACAGGACTCG 3'

Sacrificing the mice. Mice were sacrificed by injecting an overdose of anesthesia (Ropum solution - 2% of the injecting solution, 100mg/mL ketavet; a final volume of 100µL per mouse). Mice were dissected, an incision was made in the trachea with a blunt tweezer, into which a tube (braunuele) was inserted and fixed. The left main bronchus was clamped and the right lung was washed (lavaged) three times with 350µL 0.9% NaCl each time. The lavage was done with the same volume and in the same way for all HPS and wt control mice. The obtained lavages/BALF were collected and centrifuged for 10 minutes at 300xg and 4^{o}C. The cell-free supernatants were transferred to new vials and shock frozen in liquid nitrogen. Then, the clamp from the left main bronchus was removed and fixed to the right main bronchus. The bronchus was cut distal from the clamp and the right lung was excised and shock frozen. Before taking the left lung for histology, the lung was flushed to make it free from blood via the right ventricle. Formalin-fixation was achieved by filling the lung with 3.7% formaldehyde solution with a constant hydrostatic pressure of 20 cm H_2O. Following instillation the trachea was ligated, the lung was carefully removed and transferred into a cup with formaldehyde solution. After overnight incubation at 4°C, the lung was transferred into an embedding cassette, buffered in PBS and stored at 4°C. This lung tissue was processed in a vacuum-dryer for dehydration and then embedded in paraffin. Thin sections (3µm) were cut with a microtome for further analysis.

Quantification of lung collagen. Lyophilized mouse lung was weighted and hydrolysed with 4 mL of 6N HCl at 116°C for 16 h. 2mL of the hydrolysate was evaporated and reconstituted in d.H_2O, which was re-evaporated and reconstituted with 2 mL of d.H_2O, followed by adjusting

the pH to 7.0. Standard solutions containing 3, 4-D-L- hydroxyproline (Sigma) were prepared. Samples and standards were oxidized with chloramine-T (Sigma), and reaction was stopped with 3.15 M perchloric acid. *p*-dimethylaminobenzaldehyde (Sigma) solution was added, vortexed and solutions were incubated at 60°C for 20 minutes. Thereafter, the absorbance at 557nm was measured. Finally, the hydroxyproline content was calculated from the standard curve, and is expressed as µg hydroxyproline per mg dry weight.

AECII isolation: Alveolar epithelial type II cells from 3 months old HPS1/2 and wt control mice lungs were isolated according as described elsewhere [E2]. Briefly, mice were anaesthesized and dissected according to the standard dissection methods. A tubus was fixed into the trachea and lung lavage was performed for 3 times. The lung was cleared from blood by flushing with 20mL 0.9% NaCl through the left ventricle. 1.5mL dispase, followed by 0.4mL melted agarose was injected into the lung through the tubus. The lung was then carefully taken out of the thorax and incubated for 45 minutes in 1mL dispase at RT. The heart, trachea, bronchus, etc., were removed, and the tissue was minced (in medium containing 2% glutamine, 1% penicillin/streptomycin, 10mM HEPES, 4% DNase, DMEM) with the help of scissors, and pipetted thoroughly until a cell suspension was reached. Cell suspensions were pooled and filtered through different filter gazes (100µm, 20µm and 10µm), and sufficient medium was used to avoid conglomeration. Filtered suspensions were centrifuged, and the supernatant was carefully removed followed by resuspending the pellet in 1mL medium. Suspensions were pooled (by adding the above mentioned medium, but without DNase) and plated on antibody (CD16/32, CD45, BD Biosciences) coated dishes, which were then incubated for 45 minutes at

$37^{o}C$ / 4% CO_2 followed by transferring the suspensions onto non-coated dishes and incubating them at $37^{o}C$ / 4% CO_2 for 30 minutes. Suspensions were then pooled and centrifuged. The supernatant was then removed and the pellet was resuspended in an appropriate volume of medium. Nile red staining was performed for counting the AECII. Purity of the cells was checked by indirect immunofluorescence for pro-SP-C, and the remaining cells were frozen until used.

Western blot analysis: Immunoblot analysis for pro-SP-B, pro-SP-C, mature SP-B (all from Chemicon/Millipore), mature SP-C (Nycomed), cathepsin D (R&D systems), GADD153 (=CHOP, Santa Cruz Biotechnolgy), ATF4 (Aviva Systems Biology), cleaved caspase-3 (Trevigen) and β-actin (Abcam) were performed according to standard procedures. Blotted membranes were developed with the ECL Plus chemiluminescent detection system (Amersham Biosciences), and the band intensity of exposed film was analyzed by densitometric scanning and quantified using the Alpha EaseFC Imaging System (San Leandro, CA). Integrated density values of targeted proteins were first normalized against those of actin values. The values of controls were then assumed as 100% and the percent of increase or decrease of a target protein was calculated.

Isolation of phospholipids. Phospholipids (PL) were extracted from bronchoalveolar lavage fluids (BALF) or lung homogenates according to Bligh & Dyer [E3]. Upon phase separation, the chloroform phase was transferred to a new tube and dried under nitrogen. A phosphate assay was then performed as reported [E4] and the concentration of phospholipids was calculated by correlation to a standard curve generated with phosphorous. The resultant values were normalized against protein concentration of respective samples.

Isolation of large surfactant aggregates and assessment of surface activity. Characterization of large surfactant aggregate (LA) content and surface activitiy of LA was undertaken as outlined previously [E4]. In brief, BALF aliquots were centrifuged at 48000xg (1h, 4^{o}C) for separation of LA. LA preparations were re-assessed for PL content and related to the originally centrifuged amount of phospholipids (indicating the % of LA in the original BALF). Upon adjustment to a PL concentration of 2 mg/mL, surface tension measurements were performed by means of a pulsating bubble surfactometer (Electronetics, Mew York, USA), as previously described [E5]. In brief, samples were transferred into the sample chamber and adsorption was measured over the initial 12sec period (the γ_{ads} value given refers to the surface tension after 12 sec adsorption). Next, pulsation was started with a cycling rate of 20 times per minute, and minimal and maximum surface tension were recorded over 5 min (the γ_{min} value given here refers to the minimal surface tension-value obtained after 5 min of pulsation).

Cell differentials. Bronchoalveolar lavage cells were pelleted by centrifugation at 400 g for 10 min. Cell differentials were performed on cytospin preparations stained with May-Grünwald-Giemsa, and a total of 2 x 100 cells were counted.

Lipidomics. Lipids were quantified by electrospray ionization tandem mass spectrometry (ESI-MS/MS) in positive ion mode as described previously [E6, E7, E8]. Samples were quantified by direct flow injection analysis using the analytical setup described previously [E7, E8]. A precursor ion scan of *m/z* 184 specific for phosphocholine containing lipids was used for phosphatidylcholine (PC), sphingomyelin (SM) [E7, E8] and lysophosphatidylcholine (LPC) [E8]. Neutral loss scans of *m/z* 141 and *m/z* 185 were used for phosphatidylethanolamine (PE) and

phosphatidylserine (PS), respectively [E6]. PE-based plasmalogens were analyzed according to the principles described by Zemski-Berry [E9]. Phosphatidylglycerol was analyzed using a neutral loss scan of *m/z* 189 of ammonium adduct ions. Ceramide was analyzed similar to a previously described methodology [E10] using N-heptadecanoyl-sphingosine as internal standard. Free cholesterol (FC) and cholesteryl ester (CE) were quantified using a fragment ion of *m/z* 369 after selective derivatization of FC using acetyl chloride [E11]. Correction of isotopic overlap of lipid species as well as data analysis by self programmed Excel Macros was performed for all lipid classes according to the principles described previously [E7].

Immunohistochemistry: For human subjects, tissue was procured from one post-mortem lung specimen and 1 explanted lung sample. Written informed consent was obtained, and subjects were enrolled in a protocol (04-HG-0211) approved by the Institutional Review Board of the National Human Genome Research Institute. All specimens from human and mice were fixed in 4% formaldehyde, embedded in formalin and serial sections (5-6µm for human and 3µm for mice lungs) were performed. Sections were stained routinely by hematoxylin & eosin and by trichrome staining for collagen. AP-fast red kit (Zytochem systems, Berlin, Germany) was used according to the manufacturer's instructions for immunohistochemical localization of the proteins: cathepsin D (R&D systems [mouse] and Abcam [human]), cleaved caspase-3 (Trevigen), ATF4 (Santa Cruz Biotechnology), CHOP (Santa Cruz Biotechnology [mouse], Abcam [human]), and pro-SP-C (Chemicon/Millipore). Antigen retrieval was performed either by detergent method (0.4% Triton X-100 in 1x PBS) or by microwaving the sections in 10mM Sodium citrate buffer, pH 6.0. Hemalaun was used as counter-stain.

Computerized lung morphometry. Following scanning of the whole section with a Mirax Desk scanning device (Carl Zeiss MicroImaging GmbH, Jena, Germany), a digital image of the lung was generated which was subjected to further analysis employing the Axiovision® Software (Carl Zeiss MicroImaging GmbH, Jena, Germany). This software generated a graphic overlay of parallel horizontal and vertical chords over representative areas of the lung section. From each lung slide approximately 20-25 representative captions covering almost the entire subpleural area of the lung were defined, and then subjected to analysis. By this approach practically the entire peripheral lung area of each section was subjected to analysis, and hilar structures like bronchi and large vessels were excluded. The number and length of each chord was calculated, resulting in about 1,000-2,000 single values per caption.

Isolation of RNA. Total RNA was extracted from lungs using guanidine thiocyanate-acid phenol (RNAzol B, WAK-Chemie, Germany). Briefly, snap frozen lung tissue was added to an appropriate volume of RNAzol and homogenized on ice. Then, chloroform (10% of the total volume) was added to the homogenate, immediately mixed and the mixture was incubated on ice for 30min, followed by centrifugation at 5000rpm and 4°C for 45 min. The upper phase containing RNA was collected and precipitated by addition of equal volume of isopropanol and incubated at -20°C for 1 hour, followed by centrifugation at 13,000 rpm and 4°C for 10 min. The supernatant was discarded, and the RNA pellet was washed with 70% ethanol, and centrifuged again for 10 min at 13,000 rpm and 4°C, and dried at room temperature. Finally, the pellet was dissolved in an appropriate volume of RNase free H_2O. After measuring the RNA concentration, the samples were stored at -80°C.

Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). For the preparation of cDNA, 2µg RNA per sample was used. RNA was copied to cDNA using reverse transcriptase (Qiagen, omnitranscript RT kit) with random hexameres according to the kit instructions. cDNA synthesis was performed by incubating the master solution (Random hexameres, RNase inhibitor, buffer, dNTPs, reverse transcriptase) at RT for 15 minutes and then at 37^{o}C for 1 hour. 2µL of cDNA was then subjected to PCR using Taq polymerase and specific primers for the gene of interest. Gene expression of SP-B, SP-C, cathepsin D, glucosylceramide synthase and β-actin was studied using the following primers:

SP-B forward 5' GGAAGATGCTTTCCAGGAAGC 3'

SP-B reverse 5' TGCTCAGAGAAGTCCTGAGTG 3'

SP-C forward 5' TACTGAGATGGTCCTTGAGATG 3'

SP-C reverse 5' GGAAGAATCGGACTCGGAAC 3'

Glucosyl ceramide synthase forward 5' ATGTAGCCG ACAGACAAG 3'

Glucosyl ceramide synthase reverse 5' GTCCACCTGATCATTCTG 3'

Cathepsin D-forward 5' GGTACCTGAGCCAGGACAC 3'

Cathepsin D-reverse 5'CCGTGGTAGTACTTGGAGTC 3'

β – actin-forward 5' CTACAGCTTCACCACCACAG 3'

β – actin-reverse 5' CTCGTTGCCAATAGT GATGAC 3'

The PCR products were analyzed on agarose gels. The size of amplified DNA was determined using a DNA molecular weight standard and ethidium bromide stained agarose gels were visualized by FluoroChem 8900, gel Biodoc-system.

Cloning: The mouse full length cathepsin-D was generated by RT-PCR as described above. *Xba1* and *Not1* restriction sites were introduced with the cathepsin-D (Genebank Accession NP_034113.1) primers: 5'TATATATCTAGAATGAAGACTCCCGGCGTCTTGC3' (forward), 5' ATATA TAGCGGCCGCCTAAGCGAGTGTGACTATGTGTGAG 3' (reverse) and the amplified and restricted product was cloned into pcDNA3.1 mammalian expression vector (Invitrogen). The final clones were purified using endo free plasmid purification kit (Qiagen), restriction digestion was made and checked for insert release and were sequenced for a final confirmation using the primers: 5' CGAAATTAATACGACTCACTATAGGGAG 3' (vector specific forward primer), 5'CAACTAGAAGGCACAGTCGAGG 3' (vector specific reverse primer), 5' GGCTGTGAGGCTATTG TGG 3' (insert specific forward primer).

Cell culture: MLE-12 cells were cultured in DMEM-F12 medium containing 10nM each of hydrocortisone, and hydrobeta estradiole (Sigma), ITS solution (Insulin, Transferin, Sodium Selenite, 5µg/ml, 10µg/ml, 30nM, respectively, PAN Biotech), 10mM HEPES, 2mM L-Glutamine 1% Pencillin/Streptomycin, 2% FCS (Gibco). For transfection, $0.5x10^6$ cells were plated on a 6 well plate and either cathepsin-D plasmid or the empty vector was transfected into MLE-12 cells using Lipofectamine-2000 (Invitrogen) according to manufacturer's instructions. Cells were harvested 24 hours after transfection and the supernatant was spin down for cells where required. NIH-3T3 mouse fibroblast cells were cultured in DMEM-F12 containing 10% FCS, 2%

L-Glutamine and 2% Pencillin/Streptomycin. For proliferation experiments, different cell numbers were plated in triplicates in a 96 well microtiter plate and cultured for 24 hours before applying conditioned medium. 100µl conditioned medium was applied and the cells were further cultured for 48 hours. 10µl of WST-1 reagent per each well was added (Roche) and the absorbance was measure after 1.5 hours at 450nm, (reference wavelength = 690nm). BrdU incorporation assay was performed according to manufacturer's instructions. Briefly, cells were cultured for 48 hours in conditioned medium. BrdU labeling reagent was added to the cells and were reincubated for another 2 hours, followed by fixation and washing. Preoxidase conjugated anti BrdU was added, incubated for 90 minutes at RT, followed by washing. Substrate solution was then added and the reaction was stopped with 1M H_2SO_4. The reaction product was quantified by measuring the absorbance at 450nm (reference wavelength = 690nm).

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Supplementary figure legends:

Supplementary Figure E1. Computerized morphometric analysis: H&E stained lung tissue sections were scanned with a Mirax Desk slide scanning device. Representative lung sections from **a)** HPS1/2 **b)** HPS1/6 and **c)** wt mice are shown. Following scanning of the whole section, the digital image of the lung was subjected to further analysis as described in methods. From each lung slide approximately 20-25 representative captions, each ~ 4,000 x 4,000 pixel in size, were marked in the subpleural area of the lung, exported and subjected to linear intercept analysis. For each lung, a single caption is shown at a higher magnification in **a, b & c.** During

automated measurement, a grid of parallel vertical and horizontal chord (distance of 40μm) was generated and placed over the image. Chords touching solid tissue were indicated in yellow, and chords touching airspace were indicated in blue. Finally, the number and length of all chords were calculated. Approximately 1,000-2,000 chords were analyzed from each of the 20-25 captions per lung. Graphic representation of the length distribution of airspaces is indicated for HPS1/2 mice in **d)**, for HPS1/6 mice in **e)**; length distribution of septal thickness is shown for HPS1/2 mice in **f)** and for HPS1/6 mice in **g)**, in comparison to respective wt controls. Five 9 months old mice per each group were analyzed and the data are represented as frequency distribution of either alveolar diameter (**d, e**) or septal thickness (**f, g**).

Supplementary Figure E2. Hydrophobic surfactant proteins in HPS mice lung tissue: Western blots for pro- and mature forms of SP-B and SP-C were performed using the lung homogenates of **a)** HPS1, **b)** HPS2, **c)** HPS6 and **d)** HPS1/6 mice. Densitometry analysis of these blots, along with that of the HPS1/2 mice was performed and is depicted in Figure 4 of the main manuscript. Per group, n=5 mice were analyzed.

Supplementary Figure E3. Gene expression of hydrophobic surfactant proteins in HPS1/2 mice lungs: a) RNA was isolated from lungs of 9 months old HPS1/2 and wt mice followed by semi-quantitative RT-PCR for SP-B, SP-C and β-actin, in order to study if increase in SP-B/C proteins in HPS1/2 mice could be ascribed to their altered gene regulation. However, no meaningful difference in their gene expression was observed. Per group, n=5 mice were analyzed.

Supplementary Figure E4. Apoptosis of alveolar epithelial type-II cells (AECII) in HPS1/2 mice lungs: Serial sections were obtained from paraffin embedded lungs of all HPS and wt control mice. **a)** Serial sections were either stained for TUNEL assay (upper panel) or immunostained for pro-SP-C (lower panel). Identical regions were identified and processed using Mirax viewer software. Arrows indicate the same cells stained positive for both TUNEL and pro-SP-C in lung tissue sections of HPS1/2 mice. Original magnification of photomicrograph, 200X, bar = 100µm. Representative stainings from each group (n=5 mice) are shown.

Supplementary Figure E5. Lysosomal stress is specifically found in HPS1/2 mice: Western blot analysis for cathepsin D using lung homogenates of 9 months old **a)** HPS1, HPS2 mice and wt controls, **b)** HPS6, HPS1/2 mice and wt controls and **c)** HPS1/6 mice and wt controls. Gels were run, blotted and developed under same conditions for all the samples. **d)** RNA was isolated from 9 months old HPS1/2 and wt mice followed by semi-quantitative RT-PCR for cathepsin D and β-actin. Per group, n=5 mice were analyzed.

Supplementary Figure E6. Control IHC using mouse IgG-isotype control: Representative photomicrographs of paraffin-embedded lung tissue sections from two HPS1/2 mice (**a,b**) and one human HPS patient (**c**) incubated with rabbit IgG isotype control. No staining was observed in such sections. Original magnification of photomicrograph, 400X, bar = 50µm.

	%PC	%SPM	%Dih SPM	%PE	%PE-Pla	%PS	%PG	%LPC	%Cer	%GluCer	%CE	%FC
Bl_6 Controls	40.1 ± 0.98	4.9 ± 0.05	0.2 ± 0.05	3.8 ± 0.05	18.4 ± 0.34	4.9 ± 0.11	0.6 ± 0.05	2.3 ± 0.11	0.28 ± 0.01	0.04 ± 0.001	1.4 ± 0.11	23.5 ± 0.46
HPS1/2	73.0 ± 1.00 ***	2.2 ± 0.17 **	0.2 ± 0.05	2.0 ± 0.05 **	8.2 ± 0.06 ***	2.0 ± 0.05 ***	0.8 ± 0.04	1.3 ± 0.05 ***	0.11 ± 0.01 **	0.03 ± 0.001	0.9 ± 0.05	10.1 ± 0.11 ***
HPS1	47.7 ± 0.75 **	4.1 ± 0.11	0.3 ± 0.05	3.4 ± 0.17	15.4 ± 0.28	4.5 ± 0.28	1.0 ± 0.05	2.0 ± 0.17	0.20 ± 0.02	0.04 ± 0.001	1.6 ± 0.34	20.7 ± 0.41
HPS2	46.5 ± 1.92	4.2 ± 0.17	0.2 ± 0.05	3.3 ± 0.11	16.6 ± 0.63	4.2 ± 0.05	0.9 ± 0.05	2.1 ± 0.05	0.23 ± 0.01	0.03 ± 0.002	1.0 ± 0.11	21.2 ± 0.17
HPS6	41.0 ± 0.28	4.8 ± 0.11	0.2 ± 0.05	3.3 ± 0.05	19.0 ± 0.4	4.8 ± 0.17	0.7 ± 0.04	1.9 ± 0.05	0.24 ± 0.02	0.03 ± 0.001	1.1 ± 0.11	23.6 ± 0.17
HPS1/6 Controls	40.1 ± 1.81	4.3 ± 0.05	0.2 ± 0.06	3.7 ± 0.11	18.4 ± 0.52	4.9 ± 0.28	0.6 ± 0.05	2.6 ± 0.28	0.23 ± 0.01	0.03 ± 0.001	1.9 ± 0.17	23.6 ± 0.63
HPS1/6	57.2 ± 1.51 **	3.1 ± 0.17 **	0.1 ± 0.05	3.0 ± 0.11	12.7 ± 0.52	3.4 ± 0.05 **	1.4 ± 0.05 **	2.4 ± 0.05	0.16 ± 0.01	0.02 ± 0.001	1.5 ± 0.17	16.3 ± 0.70 **

Supplementary Table E1. Phospholipid profile in lung homogenates from 9 months old HPS mice: Depicted here are the relative percentages of different phospholipid classes in the lung tissue of HPS mice. Values are represented as means ± standard error means, p-value summary: *p<0.05, **p<0.01, ***p<0.001. Where significance is not mentioned, values are considered as being not significant. Per group, n=5 mice were analyzed. SPM - Sphingomyelin; DihSPM – Dihydro-Sphingomyelin, PC -Phosphatidylcholine; LPC - Lysophosphatidylcholine; PE - Phosphatidylethanolamine; Pla - Plasmalogens, PE-Pla – PE based plasmalogens; PG - Phosphatidylglycerol, PS – Phosphatidylserine; Cer – Ceramides; GluCer – Glucosylceramides; CE – Cholesteryl esters; FC – Free cholesterol.

	%SPM	%DihSPM	%PE	%PE-Pla	%PS	%PG	%LPC	%Cer	%GluCer	%CE	%FC
Bl_6 Controls	8.1 ± 0.05	0.33 ± 0.05	6.3 ± 0.05	30.5 ± 0.34	8.1 ± 0.11	1.0 ± 0.05	3.8 ± 0.11	0.46 ± 0.01	0.06 ± 0.001	2.32 ± 0.11	39.0 ± 0.46
HPS1/2	7.9 ± 0.17	0.72 ± 0.05	7.2 ± 0.05 **	29.4 ± 0.06	7.2 ± 0.05	2.8 ± 0.04 ***	4.6 ± 0.05 *	0.39 ± 0.01	0.10 ± 0.001 **	3.20 ± 0.05	36.3 ± 0.11
HPS1	7.7 ± 0.11	0.56 ± 0.05	6.3 ± 0.17	29.0 ± 0.28	8.4 ± 0.28	1.8 ± 0.05	3.7 ± 0.17	0.37 ± 0.02	0.07 ± 0.001	3.01 ± 0.34	38.8 ± 0.41
HPS2	7.7 ± 0.17	0.37 ± 0.05	6.1 ± 0.11	30.7 ± 0.63	7.8 ± 0.05	1.6 ± 0.05	3.9 ± 0.05	0.42 ± 0.01	0.05 ± 0.002	1.85 ± 0.11	39.3 ± 0.17
HPS6	8.0 ± 0.11	0.33 ± 0.05	5.5 ± 0.05	31.8 ± 0.40	8.0 ± 0.17	1.2 ± 0.04	3.2 ± 0.05	0.40 ± 0.02	0.05 ± 0.001	1.84 ± 0.11	39.5 ± 0.17
HPS1/6 Controls	7.1 ± 0.05	0.33 ± 0.06	6.1 ± 0.11	30.4 ± 0.52	8.1 ± 0.28	1.0 ± 0.05	4.3 ± 0.28	0.38 ± 0.01	0.05 ± 0.001	3.14 ± 0.17	39.0 ± 0.63
HPS1/6	7.0 ± 0.17	0.22 ± 0.05	6.8 ± 0.11	28.8 ± 0.52	7.7 ± 0.05	3.1 ± 0.05	5.4 ± 0.05	0.36 ± 0.01	0.04 ± 0.001	3.40 ± 0.17	37.0 ± 0.70

Supplementary Table E2: Phospholipid profile in HPS mice without PC: Depicted here are the relative percentages of different phospholipid classes except PC in the lung tissue of HPS mice at the age of 9 months. Values are represented as means ± standard error means, p-value summary: *p<0.05, **p<0.01, ***p<0.001. Where significance is not mentioned, values are considered as being not significant. Per group, n=5 mice were analyzed.