ORIGINAL ARTICLE

Inhalable Textile Microplastic Fibers Impair Airway Epithelial Differentiation

Shanshan Song[1,3*], Fransien van Dijk[1,3*], Gwenda F. Vasse[1,3], Qiongliang Liu[6], Irene F. Gosselink[7], Ellen Weltjens[7], Alex H. V. Remels[7], Marina H. de Jager[1], Sophie Bos[1], Chenxi Li[6], Tobias Stoeger[6], Markus Rehberg[6], David Kutschke[6], Gail W. A. van Eck[1], Xinhui Wu[1,3], Suzanne H. Willems[1], Devin H. A. Boom[8], Ingeborg M. Kooter[7,8], Diana Spierings[4*], René Wardenaar[4], Matthew Cole[9], Martijn C. Nawijn[3,5], Anna Salvati[2], Reinoud Gosens[1,3], and Barbro N. Melgert[1,3]

[1]Department of Molecular Pharmacology, [2]Department of Nanomedicine & Drug Targeting, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, the Netherlands; [3]Groningen Research Institute for Asthma and COPD, [4]European Research Institute for the Biology of Ageing, and [5]Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands; [6]Comprehensive Pneumology Center, Institute of Lung Health and Immunity, Helmholtz Center Munich, German Research Center for Environmental Health, Neuherberg, German Center for Lung Research (DZL), Munich, Germany; [7]Department of Pharmacology and Toxicology, School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center+, Maastricht, the Netherlands; [8]The Netherlands Organization for Applied Scientific Research (TNO), Utrecht, the Netherlands; and [9]Plymouth Marine Laboratory, Plymouth, United Kingdom

ORCID IDs: 0000-0002-0851-8387 (S.S.); 0000-0003-3623-7045 (G.F.V.); 0000-0002-2790-0389 (T.S.); 0000-0003-1195-5470 (X.W.); 0000-0001-9891-1897 (R.W.); 0000-0001-5910-1189 (M.C.); 0000-0003-3372-6521 (M.C.N.); 0000-0002-9339-0161 (A.S.); 0000-0002-5595-152X (R.G.); 0000-0001-7091-907X (B.N.M.).

Abstract

Rationale: Microplastics are a pressing global concern, and inhalation of microplastic fibers has been associated with interstitial and bronchial inflammation in flock workers. However, how microplastic fibers affect the lungs is unknown.

Objectives: Our aim was to assess the effects of 12 × 31 μm nylon 6,6 (nylon) and 15 × 52 μm polyethylene terephthalate (polyester) textile microplastic fibers on lung epithelial growth and differentiation.

Methods: We used human and murine alveolar and airway-type organoids as well as air–liquid interface cultures derived from primary lung epithelial progenitor cells and incubated these with either nylon or polyester fibers or nylon leachate. In addition, mice received one dose of nylon fibers or nylon leachate, and, 7 days later, organoid-forming capacity of isolated epithelial cells was investigated.

Measurements and Main Results: We observed that nylon microfibers, more than polyester, inhibited developing airway organoids and not established ones. This effect was mediated by components leaching from nylon. Epithelial cells isolated from mice exposed to nylon fibers or leachate also formed fewer airway organoids, suggesting long-lasting effects of nylon components on epithelial cells. Part of these effects was recapitulated in human air–liquid interface cultures. Transcriptomic analysis revealed upregulation of *Hoxa5* after exposure to nylon fibers. Inhibiting Hoxa5 during nylon exposure restored airway organoid formation, confirming Hoxa5's pivotal role in the effects of nylon.

Conclusions: These results suggest that components leaching from nylon 6,6 may especially harm developing airways and/or airways undergoing repair, and we strongly encourage characterization in more detail of both the hazard of and the exposure to microplastic fibers.

Keywords: lung epithelial repair; airway organoids; polyester; polyethylene terephthalate; nylon

(Received in original form November 14, 2022; accepted in final form November 16, 2023)

*Co–first authors.

Supported by ZonMw Microplastics and Health grant 458001013 [B.N.M./R.G./A.S./M.C./F.v.D.] and ZonMw/Health Holland consortium grant MOMENTUM 458001101 [B.N.M./S.S./G.F.V./I.M.K./A.H.V.R./I.F.G.]).

Author Contributions: S.S., F.v.D., G.F.V., and B.N.M.: study design, manuscript writing, collection of data, and data analysis. Q.L., I.F.G., E.W., M.H.d.J., S.B., C.L., D.K., G.W.A.v.E., S.H.W., D.H.A.B., D.S., and R.W.: collection of data and data analysis. A.H.V.R., T.S., M.R., X.W., I.M.K., M.C., M.C.N., A.S., and R.G.: experimental support, critical reading, and revision. All authors contributed to the interpretation of data and manuscript preparation.

Correspondence and requests for reprints should be addressed to Barbro N. Melgert, Ph.D., Department of Molecular Pharmacology, Groningen Research Institute of Pharmacy, University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, the Netherlands. E-mail: b.n.melgert@rug.nl.

This article has a related editorial.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

Am J Respir Crit Care Med Vol 209, Iss 4, pp 427–443, Feb 15, 2024

Originally Published in Press as DOI: 10.1164/rccm.202211-2099OC on November 16, 2023
Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the Subject: Microplastics are present in the air we breathe and have been found in lung tissue. Experimental studies on animals have demonstrated microplastic-induced inflammatory responses and histological changes in lungs, whereas epidemiological studies have suggested links between airborne microplastics and respiratory health issues in humans. In particular, studies on occupational exposures have shown that 15–40% of workers exposed to synthetic fibers develop work-related airway and interstitial lung disease. However, a comprehensive understanding of the biological mechanisms underlying these associations remains elusive.

What This Study Adds to the Field: The goal of the present study was to investigate if and how inhalable microplastics could affect epithelial repair mechanisms. Using a combination of murine and human lung organoids, experimental animals and air–liquid interface cultures, we demonstrate that nylon microplastic fibers inhibit airway epithelial differentiation, thereby interfering with lung repair processes, through transcription factor Hoxa5. A still unknown chemical leaching from nylon was found to be responsible for these effects. Our results are a first step toward generating data for risk assessment and establishing much-needed safe exposure levels in humans.

Plastic pollution is a pressing global concern, and microplastics are a significant part of this problem (1, 2). High amounts of microplastics have been found in marine environments, air, soils, plants, and animals, which illustrates how omnipresent this pollutant is (3, 4). Microplastics can enter the human body via ingestion, dermal contact, and/or inhalation. Confirmation of human exposure was provided in a recent study by Leslie and colleagues that showed an average of 1.6 μg/ml of plastic in human blood (5).

Synthetic textile fibers are the most prevalent type of microplastic observed in indoor air (6–10), and concentrations indoors generally are two to five times higher than outdoors (11, 12). These fibers are typically composed of polyester or nylon and are released into the environment by wear and tear and during washing and drying of garments (13). The ubiquitous nature of microplastics indoors is unavoidably leading to human exposure via inhalation. Indeed, the presence of microplastic fibers in human lung tissue was shown in three separate studies (14–16), with polyester being the most commonly identified polymer. Furthermore, combinations of degraded and fragmented textile fibers, with an average size of almost 2 mm, were found in BAL fluid samples, suggesting that large textile fibers are also able to reach the lower airways (17). Studies of workers in synthetic textile, flock, and polyvinyl chloride industries indicate that inhalation of concentrations of approximately 7 mg/m^3 or 1 million fibers/m^3 of such microfibers can be harmful (18). Up to 40% of factory workers were found to develop work-related airway and interstitial lung disease (19, 20).

Unfortunately, health effects of long-term exposure to inhaled microplastics are greatly understudied, and explanations for the effects in plastic industry workers are lacking. In this study, we therefore investigated the effects of long-term exposure to nylon and polyester microfibers on growth and differentiation of lung epithelial cells using a murine and human lung organoid model, human air–liquid interface (ALI) cultures, and a mouse model of fiber exposure. To extend the applicability of our findings, we investigated both standardized and environmentally relevant fibers. These ranged from uniform, well-defined nylon 6,6 (nylon) and polyethylene terephthalate (polyester) fibers to fibers generated from locally sourced undefined nylon and polyester fabrics and irregularly shaped nylon particles generated within the Momentum (Microplastics and Human Health) consortium (https://momentummicroplastics.nl) by grinding and sieving of larger pieces of nylon. Some of the results of these studies were previously reported in the form of abstracts (21, 22) and a preprint (23).

Methods

Full experimental details are available in the online supplement.

Production of Fibers and Leachate

Microfibers of standardized dimensions and Nile red–labeled nylon were produced as described before (24) from polyethylene terephthalate (ES305710; Goodfellow, Huntingdon, UK) or nylon 6,6 (AM325705; Goodfellow). Environmental polyester and nylon microfibers were prepared from commercially available fabrics. Microfibers were characterized using scanning electron microscopy, and dimensions were determined as described in the online supplement. Nylon leachate was produced by incubation of nylon in phosphate-buffered saline for 7 days at 37°C. Leachate was characterized by mass spectrometry as described in the online supplement. Nylon microplastic particles in sizes 1–5 μm and 5–10 μm were supplied by the Momentum consortium (https://momentummicroplastics.nl).

Estimation of Exposure Concentrations

Exposure concentrations were extrapolated from occupational exposures of approximately 7 mg/m^3 (18), a deposition efficiency of approximately 20% for cylindrical particles (25), and the assumption that a factory worker would inhale approximately 8,000 L of air per 8-hour shift (26). Assuming a perfect distribution over a pair of lungs of approximately 1 kg would result in a concentration of approximately 11 μg/g of lung tissue each day, corresponding to roughly 11 μg/ml of culture medium in organoid cultures. We therefore investigated 2,000–5,000 fibers per well of organoids corresponding to 16–39 μg/ml of nylon and 49–122 μg/ml of polyester. For the Momentum nylon particles (https://momentummicroplastics.nl), we tested 1, 10, and 100 μg/ml.

Animal Experiments

All experiments were performed according to strict governmental and international guidelines on animal experimentation under licenses IVD 15303-01-004 (Groningen) and ROB-55.2Vet-2532.Vet_02-19-150 (Munich). C57BL/6 mice (males and females aged 8–14 wk) for organoid experiments were bred at the central animal facility of the University Medical Center Groningen. Female C57BL/6 mice for *in vivo* exposure experiments were purchased from Charles River (Sulzfeld, Germany). These mice received an intratracheal instillation of Nile red–labeled nylon or unlabeled nylon fibers or leachate suspended in 50 μl of phosphate-buffered

Table 1. Demographics and Clinical Characteristics of Patients Whose Lung Tissue Was Used for Isolation of Epithelial Cells for Organoids or Air–Liquid Interface Cultures

Type	Sex (F/M)	Age (*yr*)	Smoking Status	Pack-Years (*yr*)	FEV_1/FVC ratio	COPD GOLD Stage
Lung tissue used for lung organoid cultures						
Non-COPD	0/1	68	ES	15	73	NA
COPD	2/4	62 (52–65)	CS/ES (1), CS (1), ES (4)	25 (15–58)	46 (21–58)	I (1), II (2), IV (3)
Lung tissue used for airway organoid cultures						
COPD	4/3	58 (54–61)	ES (7)	25 (14–32)	28 (21–30)	IV (7)
Lung tissue used for air–liquid interface cultures						
Non-COPD	0/2	73 (72–73)	ES	Unknown	75 (72–78)	NA
Healthy	3 unknown	Unknown	Unknown	Unknown	Unknown	NA

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; CS = current smoking; ES = ex-smoking; GOLD = Global Initiative for Chronic Obstructive Lung Disease; NA = not applicable.
Values are median (interquartile range).

saline containing 0.05% bovine serum albumin. After 7 days, mice were killed, and their lung tissues were collected for lung organoid cultures.

Human Lung Tissue

Epithelial cells for organoids were isolated from nontumorous lung tissue anonymously donated by individuals with or without chronic obstructive pulmonary disease (COPD) undergoing surgery for lung cancer or lung transplant for COPD and not objecting to the use of their tissue. Primary bronchial epithelial cells for ALI cultures were isolated from resected lung tissue from patients without COPD or healthy tracheal-bronchial tissue donors. Known clinical characteristics of all patients are listed in Table 1. The study protocols were consistent with the Research Code of the University Medical Center Groningen or the scientific board of the Maastricht Pathology Tissue Collection and the local medical ethics committees, as well as with Dutch national ethical and professional guidelines (www.federa.org).

Lung Organoid Cultures

Lung organoids were grown from primary human or mouse lung $CD31^-CD45^-Epcam^+$ cells cocultured with human MRC5 or mouse CCL206 fibroblasts as previously described with minor modifications (27, 28). For most experiments, 5,000 polyester or nylon reference or environmental microfibers were used per condition. Fibers were directly mixed with Matrigel/cell suspensions, added on top of Matrigel, or equivalent amounts of fiber leachate or 5 μM LE135 (2021; Tocris Bioscience) were added to the medium during organoid culture. The number and size of organoids was manually quantified using a light microscope (Eclipse Ti; Nikon Instrument), only including organoids larger than 50 μm in diameter.

Human Airway Organoids

A total of 20,000 human $CD31^-CD45^-Epcam^+$ cells were resuspended with 5,000 nylon fibers in droplets of 50 μl of growth factor reduced BME2 (3533-010-02; R&D Systems) and added on prewarmed 24-well suspension culture plates (M9312; Greiner Bio-One) with 500 μl of airway organoid medium with or without 2.5 μM LE135 per well (29). After 21 days, the total number and the size of organoids were quantified, only including organoids larger than 100 μm in diameter (28, 29).

ALI Cultures

Primary bronchial epithelial cells were seeded in coated Transwell inserts, grown submerged until reaching 95% confluency, and subsequently airlifted and cultured for 26–32 days. ALI cultures were continuously exposed to 40–50 μg/ml nylon microfibers starting either 1 day after seeding or upon airlifting. Transepithelial electrical resistance was monitored at an approximately 7-day interval.

PCR

Total RNA was extracted from ALI cultures that were exposed to 40 μg/ml nylon microfibers starting 1 day after seeding and cultured at ALI for 26 days. RNA was reverse transcribed into cDNA for real-time quantitative PCR. Gene expression levels of keratin 5, P63, FoxJ1, and secretoglobin family 1A member 1 (Scgb1a1) were normalized to the average expression of housekeeping genes cyclophilin A and RPL13A by using the $2^{-\Delta\Delta Ct}$ method.

Histology

Organoid cultures in Matrigel were stained for acetylated α-tubulin (ACT) and pro–surfactant protein C (pro-SPC) with primary antibodies as described previously (27). Isolated organoids and ALI cultures were stained using immunohistochemistry for P63, ACT, and Scgb1a1 (organoids and ALI cultures); ProSPC and Ki67 (organoids); and FoxJ1 (ALI cultures) according to sample- and antibody-specific protocols.

Isolation of Epithelial Cells and Fibroblasts from Organoid Cultures and RNA Sequencing

$Epcam^+$ cells (epithelial cells) were isolated from fresh lung tissue and 7-day-old organoid cultures using anti-Epcam microbeads. These were used together with the $Epcam^-$ cells (fibroblasts) from 7-day-old organoid cultures for RNA isolation and subsequent RNA sequencing (RNAseq) as previously described (30). The RNAseq data have been deposited to the Gene Expression Omnibus with dataset identifier GSE238065.

Data Analyses

For RNAseq data, principal component analyses were performed in R using the R package DESeq2 (version 1.26.0) to visualize the overall effect of experimental covariates as well as batch effects (function plotPCA). Differential gene expression analyses (treated vs. nontreated) were performed with the same R package (default settings; Negative

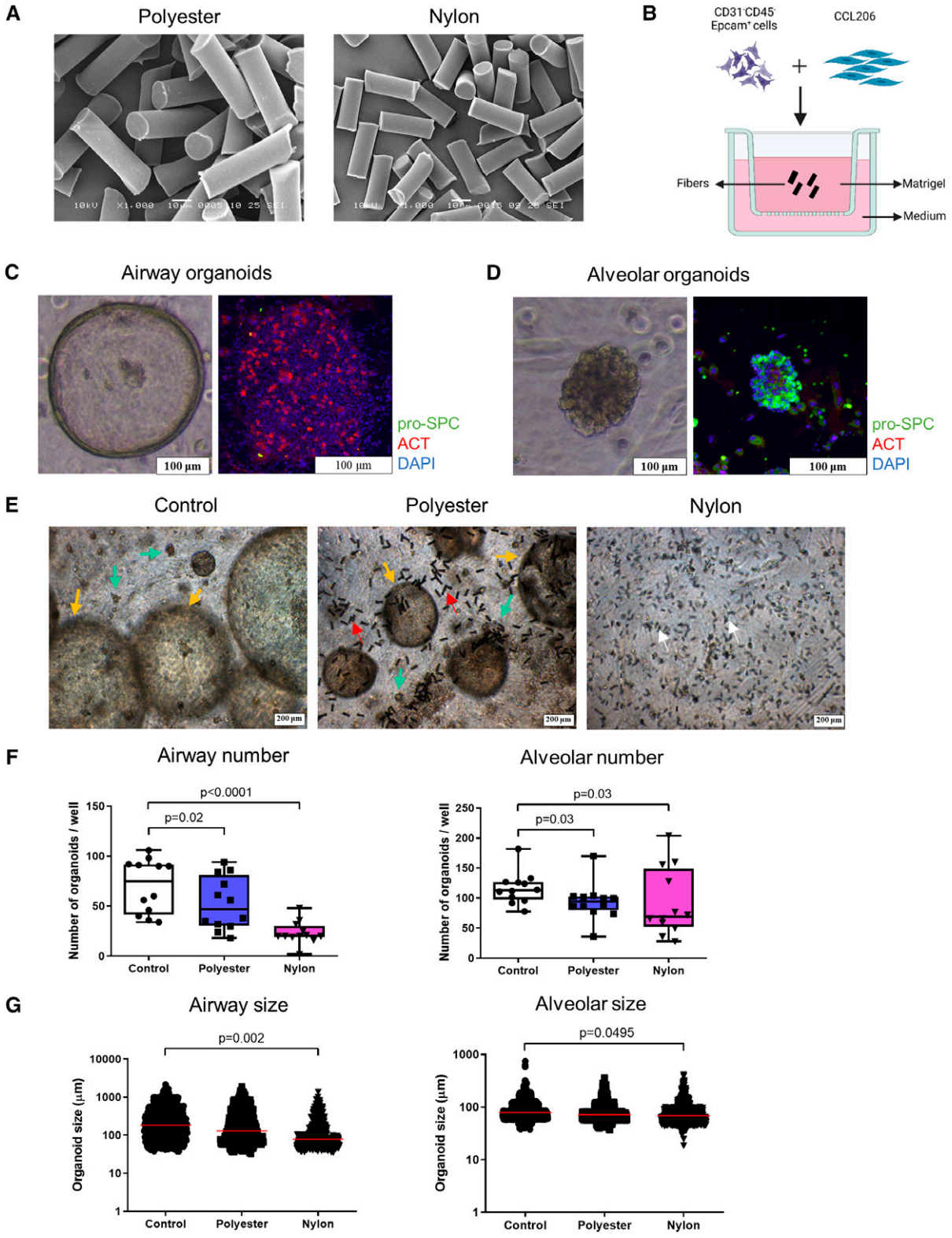

Figure 1. Effects of microplastic fibers on outgrowth of murine lung organoids. (*A*) Morphology of microplastic fibers of standardized dimensions. Representative scanning electron microscopy micrographs of polyester microfibers (15 × 52 μm) and nylon microfibers (12 × 31 μm). (*B*) Murine lung organoid coculture model: 5,000 polyester or 5,000 nylon fibers (equivalent to 122 μg/ml polyester or 39 μg/ml nylon) were mixed together with Epcam$^+$ cells, CCL206 fibroblasts, and Matrigel. (*C* and *D*) Light microscopy images and fluorescence images of ACT$^+$ airway organoids (red) and pro-SPC$^+$ alveolar organoids (green). Nuclei were counterstained with DAPI (blue). (*E*) Representative light

Binomial GLM fitting and Wald statistics; design = ~mouse + condition) following standard normalization procedures. Genes with differential expression >2 (nylon-treated vs. nontreated epithelial cells or fibroblasts) and a false discovery rate smaller than 0.05 were considered differentially expressed for that specific cell type. Volcano plots were made in R studio using ggplot2, and clustering heat maps were made using BioJupies. Pathway analysis was done using Metascape. Cellular deconvolution of bulk RNAseq data from Epcam$^+$ cells was done using MuSIC (31) with a single-cell sequencing dataset from Angelidis and colleagues (32) for freshly isolated epithelial cells and a dataset from Choi and colleagues (33) for epithelial cells isolated from 7-day organoid cultures.

Statistics

Statistical analyses were performed in either GraphPad Prism 9.0 or RStudio (RStudio 2022.02.2 + 485 Prairie Trillium Release). For data $n < 8$, nonparametric testing was used to compare groups, whereas for $n > 8$, parametric testing was used if data were normally distributed as assessed from QQ plots. For comparison of multiple groups, a Kruskal-Wallis or Friedman test was used for nonpaired or paired nonparametric data, respectively, with Dunn's correction for multiple testing, or a paired/unpaired one-way ANOVA for parametric data with Sidak's correction for multiple testing. Differences in organoid size between groups were tested by using the average size of the organoids per independent replicate. Data are presented as median ± range, and P values <0.05 were considered significant.

Results

Nylon Microfibers Inhibit Growth of Murine Lung Organoids

Fibers (aspect ratio 3:1) with a median size of 15 × 52 μm for polyester and 12 × 31 μm for nylon (Figure 1A) were extensively characterized (*see* Figure E1 and Table E1 in the online supplement) before use in organoid cultures (Figure 1B). On the basis of pilot results (Figure E2), we exposed organoids to 5,000 polyester or nylon fibers per well (equivalent to 122 μg/ml polyester or 39 μg/ml nylon). This concentration was conceivable in occupational exposure situations, had clear effects, and was on the lower end of the spectrum of concentrations used in other studies (34). Murine lung organoids commonly develop into ACT$^+$ airway organoids or pro-SPC$^+$ alveolar organoids, and 14-day exposure to either polyester or nylon fibers resulted in significantly fewer and smaller organoids than the control condition (Figures 1C–1G). The inhibitory effect of nylon was most profound on airway organoids (Figures 1E–1G). In addition, we tested nylon 6,6 particles from the Dutch Momentum research consortium (https://momentummicroplastics.nl/) and found these irregular sphere-shaped particles to be inhibitory in concentrations as low as 1 μg/ml (Figure E3). This demonstrated that the harmful effects of nylon fibers are also present at much lower doses and with differently shaped nylon.

To exclude cytotoxicity as a cause, we first assessed the viability of primary epithelial cells in the presence of 39 μg/ml nylon fibers or leachate and found no effects on the metabolic activity or proliferation of these cells (Figure E4A). Furthermore, the number and size of developing organoids during the survival and proliferation phase of organoids at Day 7 was not affected by nylon (Figure E4B). These results all suggest that nylon is not directly cytotoxic.

Nylon Microfibers Inhibit Growth of Human Lung Organoids

Similar results were observed in human lung organoids that mainly develop into alveolar organoids or mixed alveolar or airway organoids (Figures 2A and 2B). Exposure to nylon fibers resulted in significantly fewer human lung organoids, whereas the effects of polyester were less profound (Figures 2C and 2D). Organoid size was not affected by the presence of fibers. To further confirm effects on airway development, we specifically tested nylon on dedicated human airway organoids (Figures 2E and 2F). Again, we found that nylon significantly inhibited the number of organoids but had no effects on size (Figure 2G). Because lung tissue was mostly obtained from patients with COPD, we assessed whether having COPD influenced the effects of microplastics exposure. We compared outcomes of cultures from COPD Global Initiative for Chronic Obstructive Lung Disease stage IV with cultures from no COPD or mild COPD (Global Initiative for Chronic Obstructive Lung Disease stages I and II) and found no differences in outcomes when treated with or without microplastics (data not shown).

Environmental Microplastic Fibers Also Impair Lung Organoid Growth

To test if environmentally relevant polyester and nylon fibers had effects similar to those of pristine fibers, we also tested fibers made from textile fabrics on murine lung organoids. We first characterized the morphology (Figure 3A) and chemical composition of these environmental fibers (Figure E5). For polyester, we observed a median size of 17 × 63 μm (Table E2), and for nylon, we observed a median size of 57 × 20 μm (Table E2). Similar to reference fibers, 14-day exposure to 5,000 environmental nylon fibers resulted in significantly fewer and smaller airway and alveolar organoids, whereas the effect of polyester was again less profound (Figures 3B–3D).

Nylon Leachate Causes a Reduction in Lung Organoid Growth

We then investigated whether the inhibition by nylon was caused by interactions of cells with fibers or by leaching components from nylon. Pristine nylon microfibers were added to organoid cultures either in or on top of the Matrigel, or equivalent amounts of nylon fiber leachate were added to organoid medium (Figure 4A). Both the presence of nylon microfibers on top of the Matrigel and the presence of leachate resulted in significantly fewer and smaller airway organoids than the control condition. This was not the case for alveolar organoids, which increased significantly in number with nylon leachate (Figures 4B–4D). These data suggest that airway epithelial growth is specifically inhibited by components leaching from nylon but that alveolar epithelial growth may not be.

Figure 1. (*Continued*). microscopy images of the different treatment conditions. Yellow arrows indicate airway organoids, cyan arrows indicate alveolar organoids, and red and white arrows indicate polyester and nylon fibers, respectively. (*F* and *G*) Quantification of numbers and sizes of airway and alveolar lung organoids after 14-day exposure ($n = 12$). Groups were compared using a Friedman test with Dunn's correction for multiple testing. $P < 0.05$ was considered significant. ACT$^+$ = acetylated α-tubulin; pro-SPC$^+$ = pro-surfactant protein C.

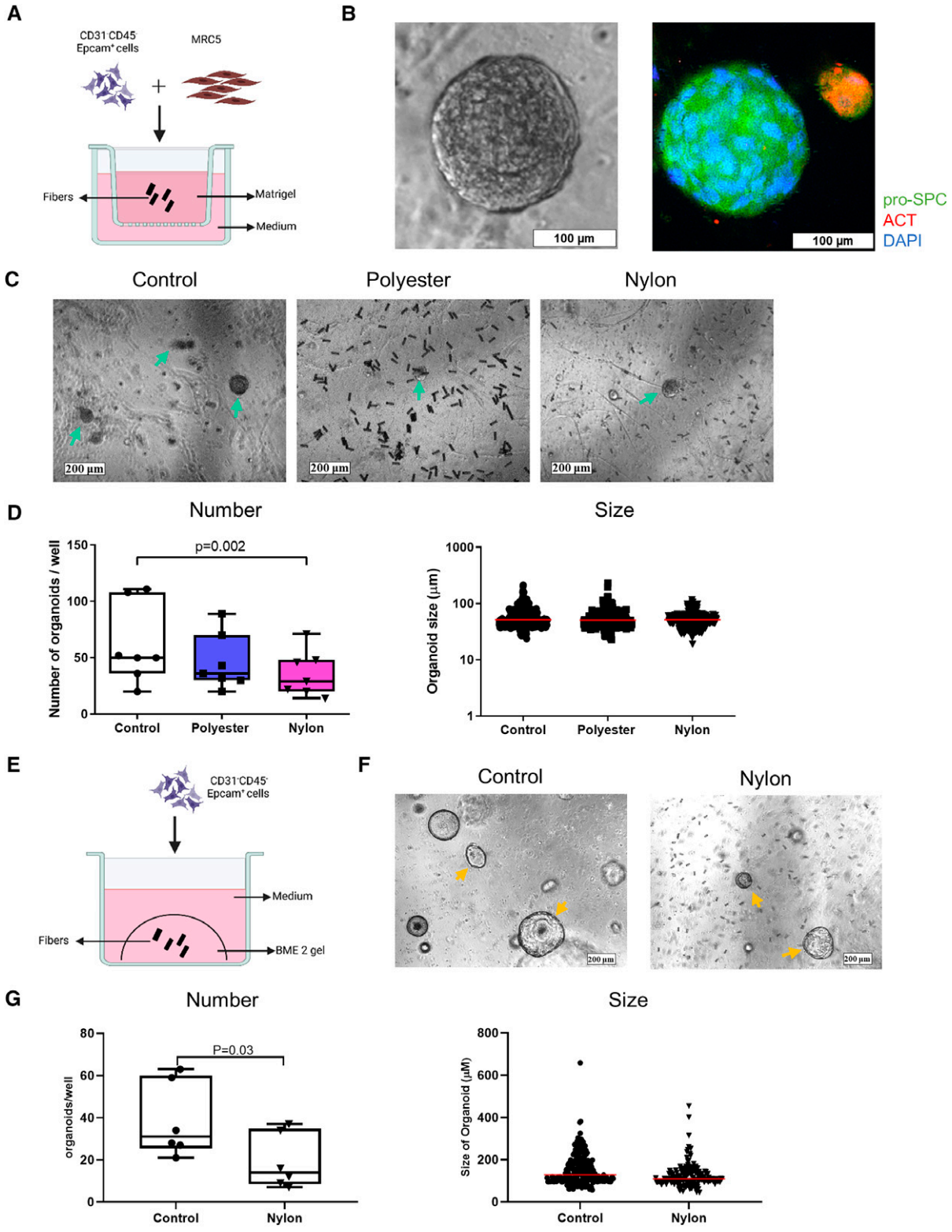

Figure 2. Influence of microplastic fibers on outgrowth of human lung and airway organoids. (*A*) Human lung organoid coculture model. A total of 5,000 polyester fibers measuring 15 × 52 μm or 5,000 nylon fibers measuring 12 × 31 μm (equivalent to 122 μg/ml polyester or 39 μg/ml nylon) were mixed with $CD31^{-}CD45^{-}Epcam^{+}$ cells, MRC5 fibroblasts and Matrigel. (*B*) The morphology of alveolar pro-SPC1 = pro–surfactant protein C. ($pro\text{-}SPC^{+}$) organoids (green) and mixed acetylated a-tubulin $(ACT)^{+}/pro\text{-}SPC1^{+}$ organoids (orange) as shown by light and fluorescence microscopy. Nuclei were counterstained with DAPI (blue). (*C*) Representative light microscopy images of all treatment conditions. Cyan arrows indicate lung organoids. (*D*) Quantification of numbers and sizes of human lung organoids after 14-day exposure to either no

The strong effects observed with nylon leachate suggested that some components and/or degradation products may leak and/or form during culture. Mass spectrometry analysis revealed high concentrations of cyclic nylon oligomers in the leachate (Figure E6A). However, 53.6 μg/ml of these oligomers separately or in combination had no effects on either the number or the size of organoids (Figure E6B). In addition, PEG600 was detected in leachate, but 1 μM up to 10 mM also did not affect outgrowth of organoids (Figure E6C). Recent studies showed the most abundant chemicals leaching from nylon in seawater were bisphenol A and benzophenone-3 (10, 34). However, we could not detect these in our leachate, and, importantly, preliminary experiments with different concentrations of bisphenol A (2 ng/ml–2,000 ng/ml) or benzophenone-3 (1 nM–10 mM) showed no effects on lung organoid growth (Figures E6D and E6E).

Nylon Leachate Mainly Affects Airway Epithelial Differentiation

We then investigated if nylon fibers affect developing or established organoids differently. Lung organoid cultures have a clearly defined survival and proliferation phase (Days 0 to 7), a differentiation phase (Days 7 to 14), and an established phase (>14 d) (35) (Figure 5A). In contrast to the strong effects observed for developing organoids, we found that fibers on top of the Matrigel or leachate had no effects on established organoids (*see* Figures 5B and E7A for representative pictures of the cultures). However, organoid cultures exposed to nylon leachate or fibers on top of the Matrigel during the differentiation phase from Days 7 to 14 also contained fewer airway organoids. No effects on size were found (Figures E7B and E7C). This suggests that nylon leachate specifically inhibits differentiation of airway epithelial cells and is not directly toxic to cells.

To elucidate how differentiation was affected by nylon, we investigated several markers of differentiation and proliferation in 14-day control and nylon-exposed organoids (Figure 5C). Visually, the cultures were different, with many more big and hollow organoids (airway-like structures) with multilayer walls in control cultures than in nylon-treated cultures. The latter contained more smaller clumplike structures (alveolar-like structures), and the airway-like organoids tended to be smaller, more branched or disorganized, and with thinner walls. We stained sections of these organoid cultures for several markers that indicate differentiation to more terminally differentiated epithelial cell types: P63 for basal cells, ACT for ciliated cells, Scgb1a1 (also known as CC10) for club cells, and pro-SPC for type II cells. In addition, we also stained for proliferation marker Ki67 to again exclude effects of nylon on proliferation of cells. The hollow airway-like organoids in control cultures clearly expressed airway markers P63, ACT, and Scgb1a1 and not alveolar marker pro-SPC. The branched and thinner-walled airway-like organoids in nylon-treated cultures contained fewer cells expressing ACT and Scgb1a1 and no expression of pro-SPC. P63 expression was abundantly present in nylon-treated organoids (in both airway- and alveolar-like organoids) but was more present in the cytoplasm of cells and less localized to the nucleus than control organoids. In addition, the more clumplike alveolar-like structures did not express either ACT or Scgb1a1 but did express pro-SPC in some structures. Last, in both control and nylon-treated cultures, we found Ki67-positive proliferating cells, with no obvious differences between the two conditions. This again confirmed that nylon was not inhibiting proliferation on Day 14 of culture.

We then also investigated the effects of nylon leachate on human airway epithelial differentiation in ALI cultures of primary bronchial epithelial cells. Basolateral exposure of these cultures to 40–50 μg/ml nylon fibers, either 1 day after seeding or upon airlifting, resulted in thinner epithelial layers, but only in donors that developed well in untreated control conditions, highlighting interdonor variability (Figure 5D). Both treatment strategies did not affect transepithelial resistance in a negative way (Figure E7D). We then stained for airway epithelial markers a well-developed and a less-developed donor that were exposed to nylon upon airlifting. After nylon exposure, only the well-developed donor had less staining for ciliated cells (ACT and FOXJ1), basal cells ($P63^+$), and club cells ($Scgb1a1^+$), again indicating that to visualize effects of nylon, cells in control conditions need to be differentiating well (Figures 5E and E8). In addition, we found more mRNA expression of basal cell markers keratin 5 (*Krt5*) and P63 (*Trp63*), less expression of ciliated cell marker *Foxj1*, and no changes in club cell marker *Scgb1a1* in cultures exposed from the start (Figure E7E). In summary, treatment with nylon does not impair epithelial proliferation but specifically inhibits differentiation to more terminally differentiated airway epithelial cells.

Exposure to Nylon Inhibits Airway Epithelial Developmental Pathways and Stimulates Expression of Hox Family Genes

To better understand the mechanisms behind the observed effects on epithelial differentiation, we performed bulk RNAseq analysis on freshly isolated epithelial cells as well as on epithelial cells and fibroblasts isolated from 7-day organoid cultures exposed to 2,000 or 5,000 nylon fibers. This time point was chosen to capture transcriptomic changes before cellular composition changes had set in.

Cellular deconvolution of bulk RNAseq data from freshly isolated epithelial cells showed that approximately 70% of epithelial cells going into the organoid assay were type II epithelial cells, ±15% were ciliated cells, and ±10% were type I epithelial cells (Figure E9A), which was consistent with data using flow cytometry to identify cell types (36). After 7 days of organoid culture, these identities had changed dramatically to approximately 70% proliferating epithelial cells and 30% alveolar intermediates positive for keratin 8 (Figure 6A). The latter can differentiate into alveolar epithelial cells after damage *in vivo* (37). Treatment with 2,000

Figure 2. (*Continued*). microfibers, 5,000 polyester fibers, or 5,000 nylon fibers ($n = 7$). Groups were compared using a Friedman test with Dunn's correction for multiple testing. $P < 0.05$ was considered significant. (*E*) Human airway organoid model: 5,000 nylon fibers were mixed with $CD31^-CD45^-Epcam^+$ cells and BME2 gel. (*F*) Representative light microscopy images of control or nylon-treated airway organoids. Yellow arrows indicate airway organoids. (*G*) Quantification of numbers and sizes of human airway organoids after 21-day exposure ($n = 6$). Groups were compared using a Wilcoxon test. $P < 0.05$ was considered significant.

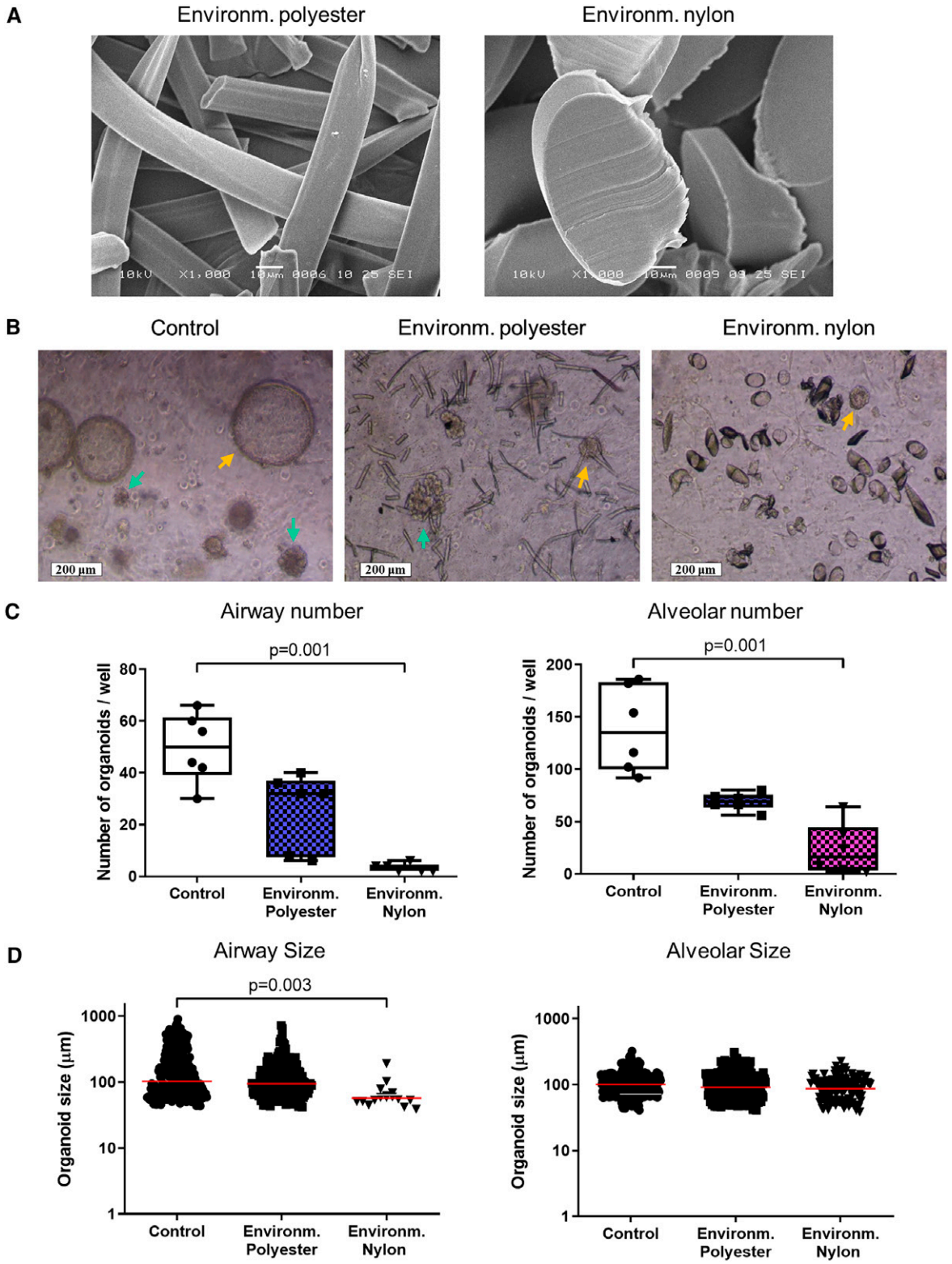

Figure 3. Influence of environmentally relevant textile fibers on growth of murine lung organoids. (*A*) Morphology of environmental (environm.) microplastic fibers. Representative scanning electron microscopy micrographs of polyester microfibers ($17 \times 63\,\mu m$) and nylon microfibers ($57 \times 20\,\mu m$). (*B*) Representative light microscopy images of all treatment conditions. Yellow arrows indicate airway organoids, whereas cyan arrows indicate alveolar organoids. (*C* and *D*) Quantification of numbers and sizes of airway and alveolar organoids ($n = 6$) after 14-day exposure to either no microfibers, 5,000 polyester microfibers, or 5,000 nylon microfibers (equivalent to 189 µg/ml polyester or 531 µg/ml nylon). Groups were compared using a Friedman test with Dunn's correction for multiple testing. $P < 0.05$ was considered significant.

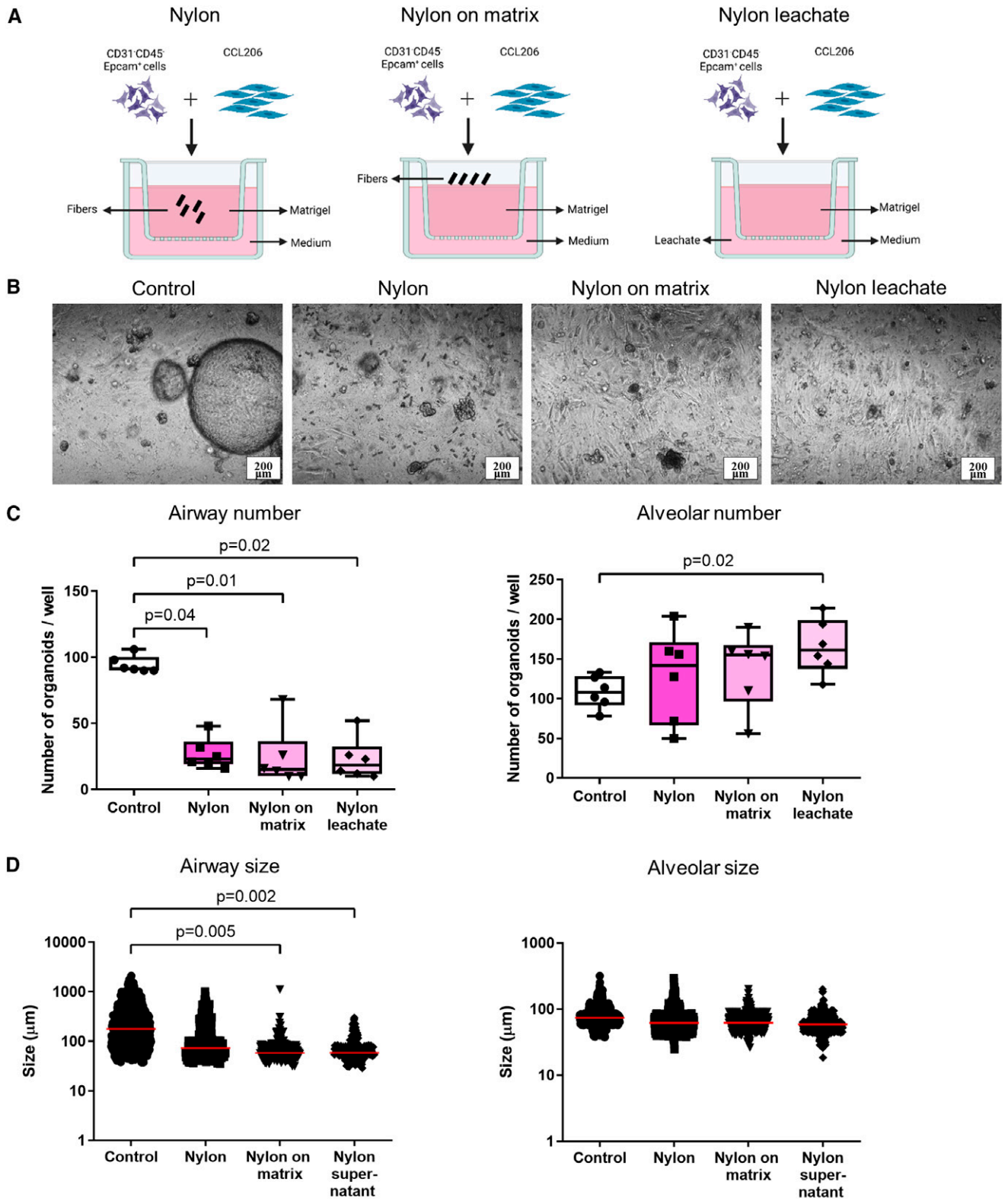

Figure 4. Impact of nylon microfibers or leachate on growth of murine lung organoids. (*A*) Experimental scheme. Nylon microfibers measuring 12×31 μm were added in or on top of Matrigel, or leachate of these fibers was added to the basolateral medium. (*B*) Representative light microscopy images of all treatment conditions. (*C* and *D*) Quantification of numbers and sizes of airway and alveolar organoids after either direct exposure to 5,000 nylon microfibers (equivalent to 39 μg/ml) in Matrigel or indirect exposure ($n = 6$). Groups were compared using a Friedman test with Dunn's correction for multiple testing. $P < 0.05$ was considered significant.

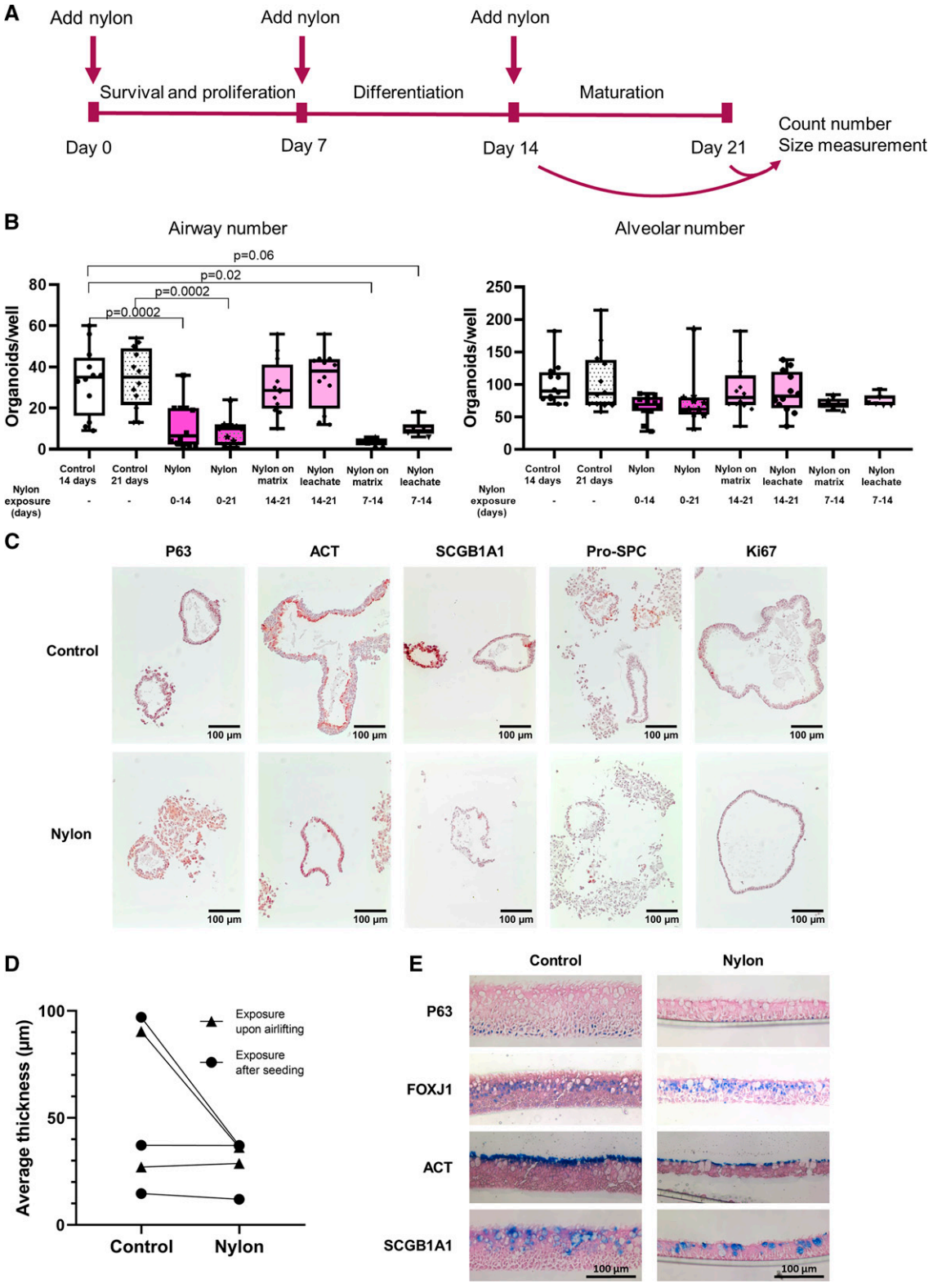

Figure 5. Nylon leachate has no effect on established lung organoids but inhibits airway epithelial differentiation. (*A*) Experimental scheme. Organoids were exposed at Day 0, 7, or 14 to 12 × 31 μm nylon fibers/leachate for 7–21 days. (*B*) Quantification of numbers of airway and alveolar organoids after exposure to no or 5,000 nylon microfibers (equivalent to 39 μg/ml) or leachate for 7, 14, or 21 days ($n = 6$–12). Groups were compared using one-way ANOVA with Holm-Sidak correction for multiple testing. $P < 0.05$ was considered significant. (*C*) Representative images of immunohistochemical staining for differentiation and proliferation markers expressed by control lung organoids and lung organoids

nylon fibers did not affect those proportions, but treatment with 5,000 nylon fibers resulted in fewer alveolar intermediates and a concomitant increase in stromal cells. This may be an indication of epithelial-to-mesenchymal transition (EMT) of these epithelial cells because they were positively selected for Epcam and were therefore unlikely to be fibroblasts. We investigated expression of EMT markers *Cdh2* (N-cadherin), *Vim* (vimentin), *Tbx3* (T-box transcription factor 3), *Snail2* (Snail family transcriptional repressor 2), *Twist1* (Twist family BHLH transcription factor 1), and *Acta2* (α-smooth muscle actin) and found all of them to be expressed at higher levels in organoids treated with 5,000 nylon fibers, whereas expression of epithelial adhesion marker *Cdh1* (E-cadherin) was lower (Figure E9B), all of which points to the development of EMT (38).

Both concentrations of nylon had an enormous impact on epithelial gene expression as depicted in volcano plots (Figures 6B and 6C) and unsupervised clustering heat maps (Figure E9C). Pathways for downregulated genes were highly enriched for epithelial development and function, whereas those identified for upregulated genes were highly enriched for mRNA translation and protein synthesis (Figures 6D and 6E and *see* Tables E4 and E5 for full lists of pathways).

We then investigated the expression of individual genes in both top-five enriched pathways in more detail. Many of the downregulated genes represent important epithelial populations in the lung. The genes associated with specific epithelial populations are listed in Table E3. The expression of these genes correlated well with our histological findings that airway epithelial cells were most affected by exposure to nylon fibers, whereas alveolar epithelial cell growth was less affected (Figures 6F and 6G). Proliferation markers in epithelial cells were not greatly affected, confirming that nylon mainly affects epithelial differentiation and not proliferation (Figure 6H). Expression of genes for signaling molecules essential for epithelial differentiation and growth were also dose dependently downregulated by nylon (Figure E9D). The genes prominently upregulated after nylon exposure were mostly encoding for ribosomal proteins, including *Rpl38* (Figure E9E), which regulates expression of *Hox* (homeobox) genes that are important for cell and tissue identities (39). Interestingly, *Hoxa4*, *Hoxa5*, *Hoxc9*, and *Hoxb3* were all significantly higher after nylon exposure (Figure 6I), with *Hoxa5* being most profoundly induced.

To exclude the possibility that these effects on epithelial cells were mediated by fibroblasts, we separately analyzed the resorted fibroblast fraction for expression of proliferation genes and important growth factors. None of these genes were inhibited by nylon in fibroblasts (Figures E10A and E10B).

Hoxa5 Inhibitor LE135 Restores Airway Organoid Formation in the Presence of Nylon

To confirm RNA data, we investigated protein expression of Hoxa5 in organoids. Hoxa5 protein was highly expressed in many epithelial organoids (Figure 7A) and most nuclei of nylon-exposed organoids, but it was hardly expressed in control organoids (Figure 7B). To further assess the role of *Hoxa5* in defective airway epithelial development, we used a Hoxa5 inhibitor (LE135), a retinoic acid receptor β–specific antagonist, which inhibits retinoic acid–induced Hoxa5 expression (40). Our data showed that exposure to LE135 in the presence of nylon rescued the development of murine airway organoids (Figures 7C and 7D). In addition, we found similar effects in human airway organoids (Figures 7E and 7F). These results indicate that nylon inhibited differentiation of airway organoids through Hoxa5.

In Vivo Exposure of Mice to Nylon Fibers Results in Long-Lasting Inhibition of Airway Epithelial Differentiation as Assessed by Organoid Formation

To investigate the effects of nylon on lung epithelial repair, we exposed mice once to either 75,000 or 150,000 fibers or leachate, and we isolated lung epithelial cells after 7 days for organoid culture (Figure 8A). *Ex vivo* fluorescence imaging on excised lungs of mice exposed to labeled nylon fibers revealed that 3 days after exposure, many fibers were still present in all areas of the lung. Seven days after exposure, some fibers were obviously cleared, and the remaining ones had accumulated centrally (Figure 8B). Nylon treatment did not result in body weight loss (Figure E11). The number of airway organoids growing from these *in vivo* exposed mice was significantly lower than that for the control exposure, indicating that being exposed once to nylon fibers or its leachate *in vivo* is sufficient for long-lasting inhibition of cell differentiation (Figures 8C and 8D). Nylon exposure did not affect the number of alveolar organoids (Figures 8C and 8E). To investigate whether Hoxa5 inhibition could alleviate the effects of *in vivo* nylon exposure, we treated organoid cultures from either vehicle- or nylon-exposed mice with LE135 for 14 days (Figures 8C–8E). This resulted in significantly more but smaller airway organoids than untreated organoid cultures derived from nylon- or nylon leachate–exposed mice (Figures 8D and E12A and E12C). The number of alveolar organoids was lower and their size was smaller after LE135 treatment (Figures 8E and E12B and E12D).

Discussion

Recent reports have shown that manmade fibers are ubiquitously present in indoor air (6, 11, 41, 42). We are therefore continuously exposed to this airborne microplastic pollution (41), but the consequences of common household exposure on our lungs are unclear. Evidence from textile factory workers indicates detrimental effects on lung tissue during high-exposure conditions at concentrations of approximately 7 mg/m^3 or 1 million fibers/m^3 (18). Our data now show that both polyester and nylon fibers can impair differentiation of human and murine lung epithelial cells, with nylon being the

Figure 5. (*Continued*). exposed to nylon for 14 days. Positive staining is red, and nuclei are blue. (*D*) Average thickness of air–liquid interface cultures treated with 40 or 50 μg/ml nylon microfibers on the basolateral side for 4 weeks 1 day after seeding or upon airlifting compared with vehicle-treated control cultures. (*E*) Representative images of immunohistochemical staining for proliferation and differentiation of well-developed air–liquid interface cultures treated with vehicle or 50 μg/ml nylon microfibers on the basolateral side upon airlifting for 4 weeks. Positive staining is blue. ACT = acetylated α-tubulin: ciliated cell marker; Ki67 = proliferation marker; P63 = basal cell marker; Pro-SPC = pro-surfactant protein C: type II cell marker; Scgb1a1 = secretoglobin family 1A family member 1: club cell marker.

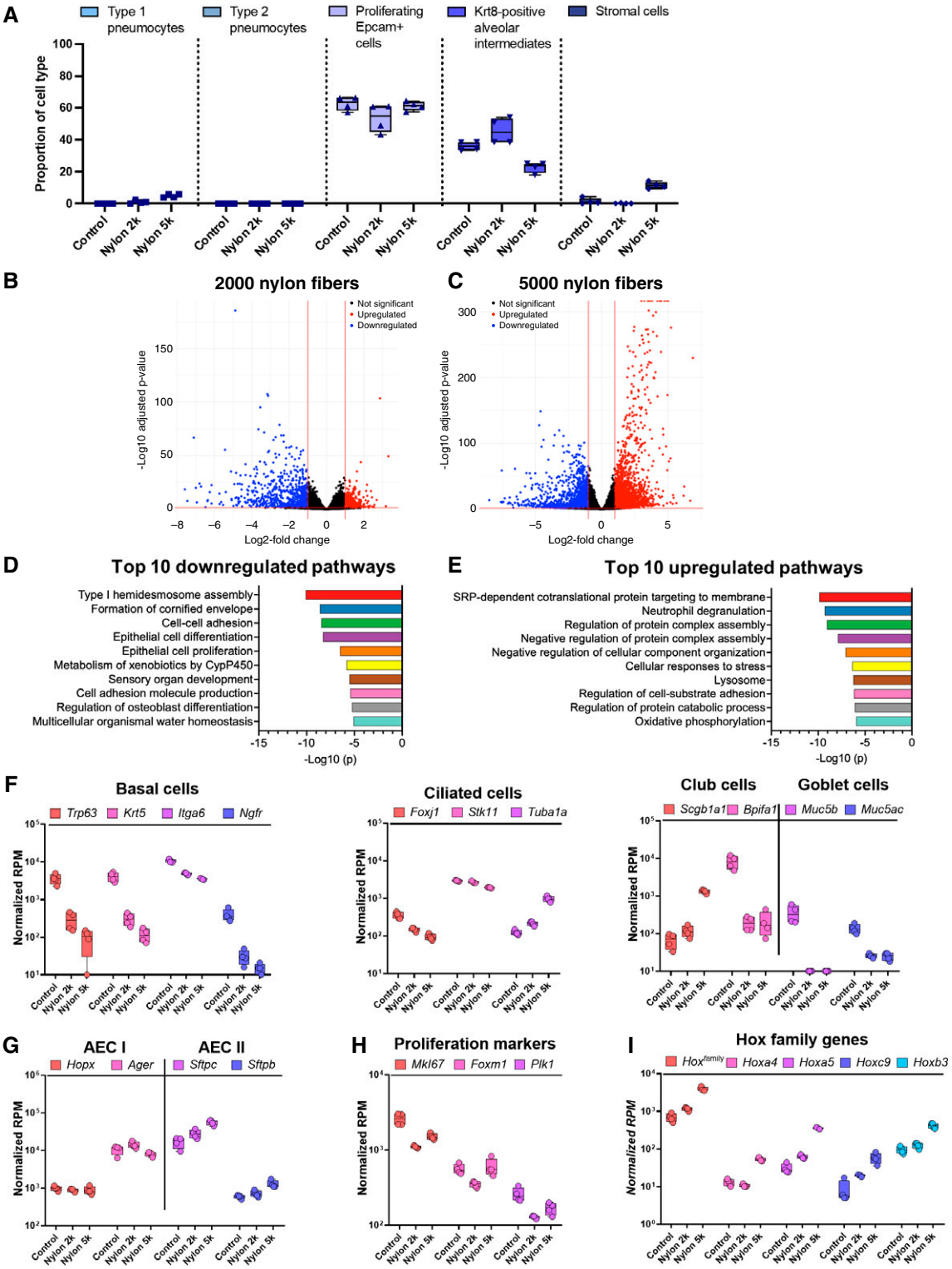

Figure 6. RNA-sequencing analysis of epithelial cells exposed to nylon. (*A*) Estimated proportions of cell types present in Epcam^{+} cells isolated from organoids on Day 7 of culture with or without 2,000 (2k; equivalent to 16 μg/ml nylon) or 5,000 (5k; equivalent to 39 μg/ml nylon) 12 × 31 μm nylon fibers. (*B* and *C*) Volcano plots of differentially expressed genes by epithelial cells exposed to 2,000 or 5,000 nylon fibers measuring 12 × 31 μm or not. Upregulated genes are marked in red, downregulated genes in blue. Genes were selected with thresholds of fold change greater than two and false discovery rate <0.05 ($n = 4$). (*D*) Pathway analysis of genes downregulated in epithelial cells by nylon. (*E*) Pathway analysis of genes upregulated in epithelial cells by nylon. (*F*) Genes associated with airway epithelial cells. (*G*) Genes associated with alveolar epithelial cells. (*H*) Genes associated with cell proliferation. (*I*) Genes encoding Hox (homeobox) family proteins. AECs = alveolar epithelial cells.

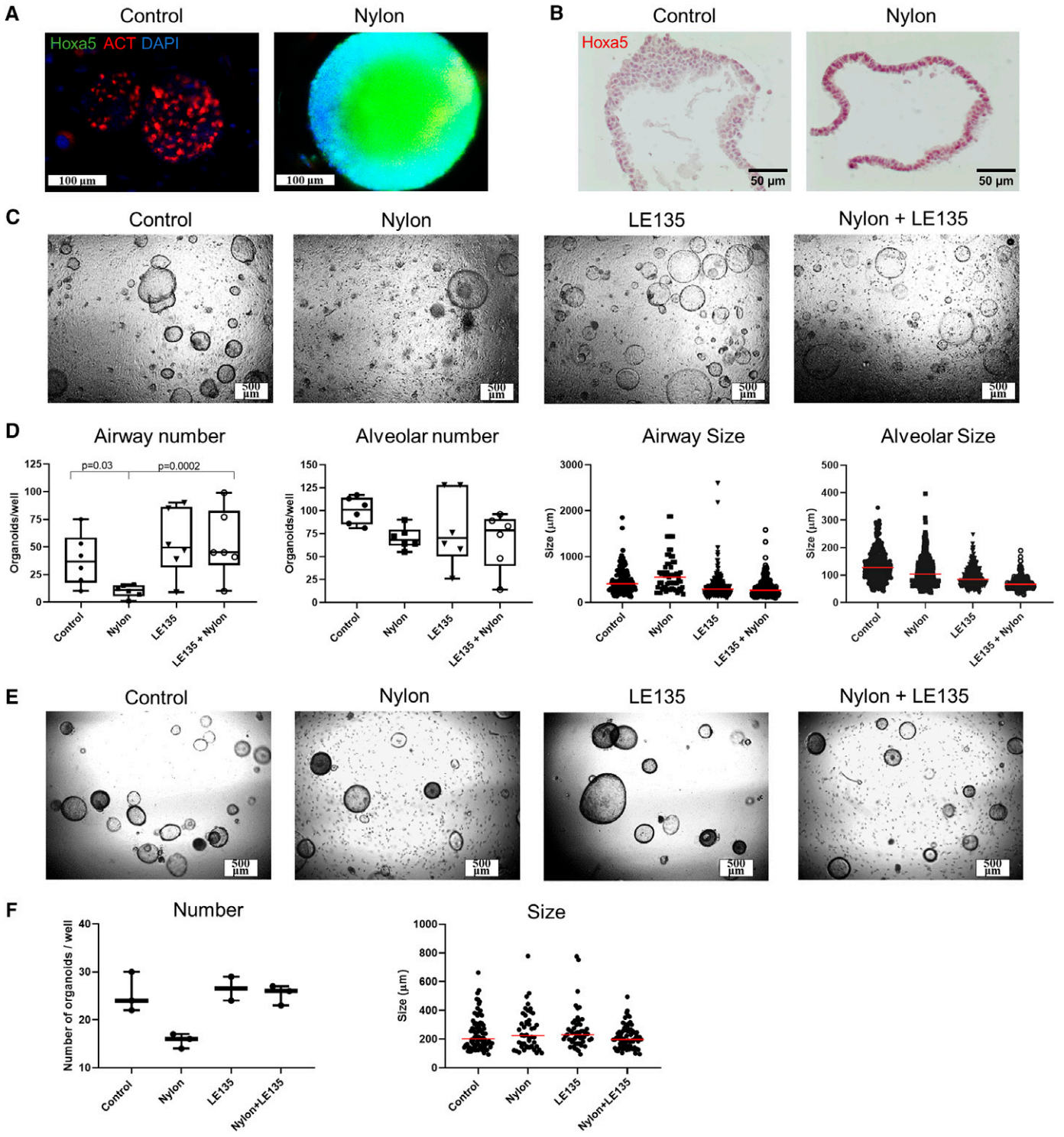

Figure 7. Hoxa5 is essential for airway differentiation in organoids after nylon exposure. (*A*) Hoxa5 expression in murine organoids treated with or without nylon. Fluorescence images of ACT staining (red) and Hoxa5 expression (green). Nuclei were counterstained with DAPI (blue). (*B*) Representative images of immunohistochemical staining for Hoxa5 expressed by control lung organoids and lung organoids exposed to nylon for 14 days. Positive (nuclear) staining is red, and nuclei are blue. (*C*) Representative images of murine lung organoids treated with nylon and/or Hoxa5 inhibitor LE135. (*D*) Quantification of the number and size of murine airway or alveolar organoids treated with no or 5,000 nylon microfibers measuring 12×31 μm (equivalent to 39 μg/ml) and/or LE135 ($n = 6$). Groups were compared using a Friedman test with Dunn's correction for multiple testing. $P < 0.05$ was considered significant. (*E*) Representative images of human airway organoids treated with nylon and/or Hoxa5 inhibitor LE135. (*F*) Quantification of the number and size of human airway organoids treated with nylon and/or LE135 ($n = 3$, not statistically compared because of small sample size). ACT = acetylated α-tubulin.

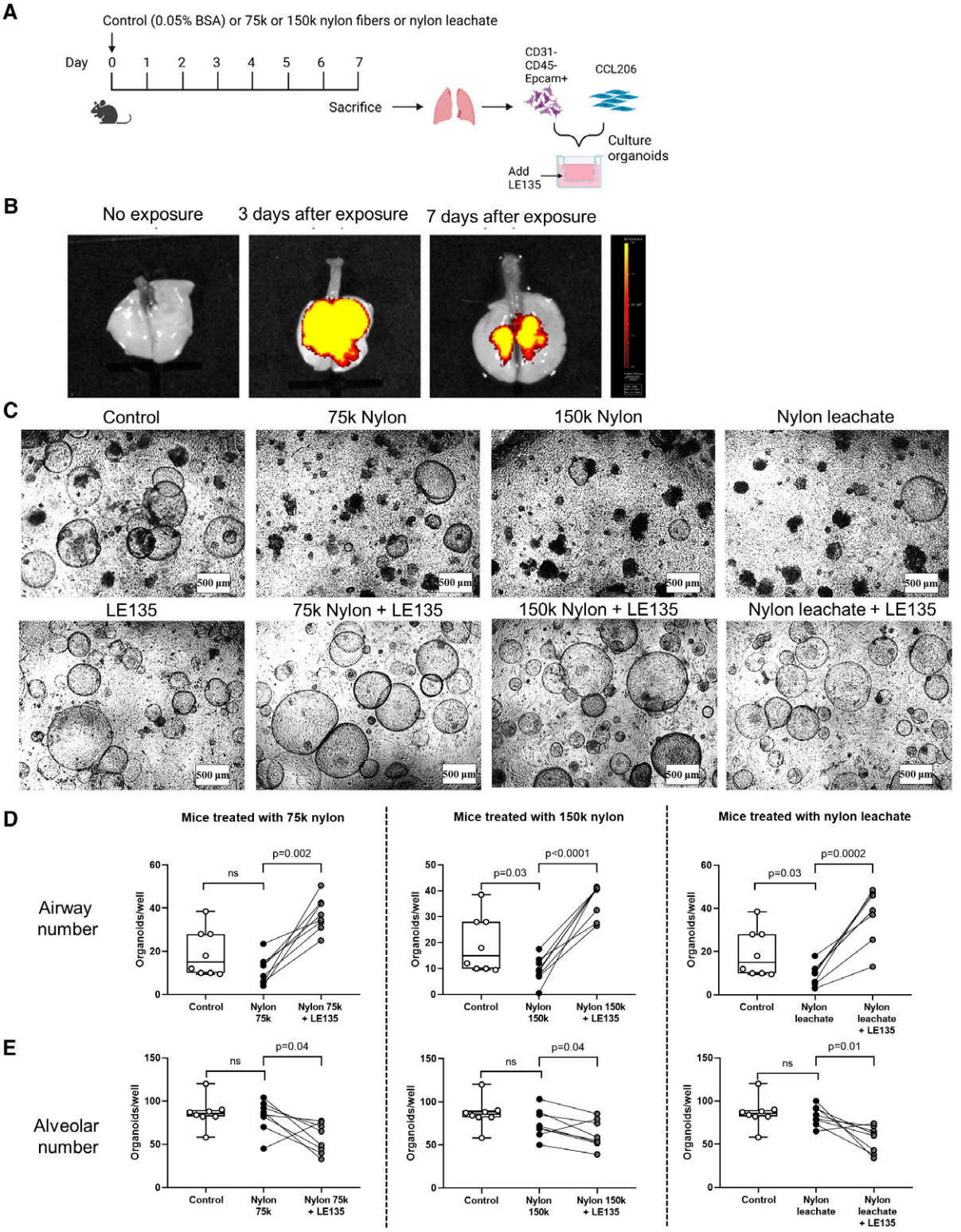

Figure 8. *In vivo* exposure of mice to nylon fibers results in long-lasting inhibition of airway epithelial differentiation as assessed by organoid formation. (*A*) Experimental scheme of *in vivo* nylon exposure. (*B*) *Ex vivo* fluorescence imaging of Nile red–labeled nylon. (*C*) Representative images of airway and alveolar organoids derived from epithelial cells of mice exposed to 75,000 (75k; equivalent to 298 μg of nylon) or 150,000 nylon fibers measuring 12 × 31 μm (150k; equivalent to 597 μg of nylon) or the equivalent amount of 150,000 leachate with or without *in vitro* treatment with 5 μM Hoxa5 inhibitor LE135 ($n = 8$). (*D* and *E*) Quantification of numbers of airway and alveolar organoids from all treatments. Groups of nylon-treated mice were compared using one-way ANOVA with Dunnett's correction for multiple testing. Organoid cultures derived from these mice and treated with LE135 were compared with a paired Student's *t* test. $P < 0.05$ was considered significant.

most harmful for airway epithelial cell differentiation. The effects of polyester were much milder and confirm previous findings by Winkler and colleagues demonstrating that lower concentrations of polyester microplastics (50 μg/ml) had no effects on human airway organoid growth (28). The effects of nylon were mediated by compounds leaking from nylon that upregulate *Hoxa5*, which in turn specifically inhibits differentiation of airway epithelial cells.

Components leaching from nylon were found to be consistently harmful for growth of airway organoids rather than alveolar organoids, which may explain the bronchiolitis found in nylon flock workers and rats exposed to nylon (43–45). Because we were unable to identify the specific culprit(s) responsible for this effect of nylon, we concentrated on elucidating the pathway responsible for the observed inhibition of airway development and found a role for Hoxa5. Our data suggest that upregulation of Hoxa5 in epithelial cells inhibits terminal differentiation of airway epithelial cells and instead keeps them in an aberrant basal phenotype with EMT characteristics. These findings are supported by previous studies showing that increased expression of Hoxa5 drives EMT in alveolar epithelial cells (46), studies showing that nylon enhances EMT (47–49), and studies showing that loss of Hoxa5 drives epithelial differentiation toward goblet cells (50). Therefore, upregulation of Hoxa5 in epithelial cells by nylon appears to be a determining factor in the impairment of airway organoid differentiation, and this finding was confirmed when we inhibited Hoxa5 and found restoration of airway organoid development. Because stromal cells are also known to express Hoxa5, the Hoxa5 upregulation with downstream effects we found could theoretically also be caused by the fibroblasts present in the mixed lung organoid cultures. However, we think this is an unlikely explanation for the following reasons. First, the growth-inhibited fibroblasts present in murine organoid cultures expressed *Hoxa5* mRNA, but this expression was actually downregulated by nylon, whereas nylon upregulated *Hoxa5* mRNA expression in epithelial cells. Second, even human airway organoid cultures that were grown without fibroblasts present were inhibited by nylon and rescued by Hoxa5 inhibitor LE135. Incidentally, the inhibition of Hoxa5 also resulted in fewer and smaller alveolar-like organoids. Our hypothesis for this finding is that inhibition of Hoxa5 allows more progenitors to develop toward airways and that this may reduce the number available for alveolar development.

Whether nylon components directly or indirectly upregulate Hoxa5 remains an open question. The upregulation of Hoxa5 may also be the result of inhibition of Wnt (Wingless/Integrated) signaling by nylon components. Ordóñez-Morán and colleagues previously showed a feedback loop between Hoxa5 and Wnt in intestinal stem cells, with Wnt signaling suppressing Hoxa5 expression to maintain stemness (51). Therefore, inhibition of Wnt family members by nylon components could indirectly result in upregulation of Hoxa5. Support for this hypothesis is found in our RNAseq data, which showed downregulation of both *Wnt4* and *Wnt7* concomitant with upregulation of *Hoxa5* mRNA by nylon.

Our data uniquely show that nylon fibers especially inhibit differentiating (airway) epithelial cells. However, nylon did not affect already developed airway organoids or proliferation of epithelial cells. This suggests that nylon is not directly toxic, but that it only interferes with developmental and/or repair pathways. It is therefore likely that the potential adverse health effects of nylon are especially relevant for young children with developing airways and patients with chronic or acute lung disease, who rely on epithelial repair. Recent studies have already shown potential health impacts of microplastics in early life and in patients with asthma (52, 53). Of note, a recent study by Soltani and colleagues estimated possible household exposure in different types of individuals and found that young children (<0.5 yr of age) could inhale twice as much microplastics as adults, averaging 45 ng/kg of body weight per day (12). In addition, microplastics were shown to be present in human placenta, and, in mice, this resulted in reproductive toxicity (54, 55). Therefore, the presence of inhalable microplastic or nylon fibers in indoor environments is a matter of great concern for children and fetuses whose lungs are still growing.

Nevertheless, this concern is only relevant when concentrations of these fibers are high enough to cause adverse effects, and this is where data are severely lacking. Studies have shown microplastic fibers to be ubiquitously present in houses with an average of five fibers per cubic meter of air (8, 56), but actual lung deposition of these microplastic fibers has not been quantified yet. Vianello and colleagues used a breathing thermal manikin to estimate adult human lung deposition by inhalation in daily life (41). They showed that, on average, nine particles per cubic meter of air were inhaled by this device. Because humans process approximately 11 m^3 of air per day, exposure could be approximately 100 particles per day. This is similar to estimations in Australian homes of approximately 13,000 fibers per year (12). Another comprehensive review estimated that annual inhalation of microplastic particles could be between 40,000 and 62,000 particles/yr (57). However, this may be an underestimation because high-exposure activities such as unloading a tumble drier, working with textile materials, or crawling around on carpets were not considered. Nor were ventilation habits considered, and these factors could all greatly determine exposure, although vacuum cleaning was estimated to reduce exposure (12). Therefore, microplastic concentrations in indoor environments need to be assessed more widely during a variety of activities and conditions to make better estimations of human exposure possible. These can then be compared with occupational exposures known to cause adverse effects and animal studies such as ours and previous ones (43, 45). For instance, concentrations of 7 mg/m^3 or 10^6 fibers per cubic meter of air were found in the nylon flocking industry, with many employees reporting respiratory symptoms (18, 58). Furthermore, rats exposed once to 10 mg/kg body weight developed bronchiolitis (43), but 4-week exposure to 57 fibers/cm^3 did not lead to adverse effects in rats (45).

A strength of using lung organoids is the opportunity to directly translate *ex vivo* findings to humans (28, 59). Because we found comparable negative effects of nylon fibers on epithelial differentiation and growth in murine and human organoids, this demonstrates that our results are of high relevance for the human population. Despite this advantage, lung organoids are a relatively simple model of lung tissue and lack the immune and endothelial compartment present *in vivo*. Especially having the immune cells present could alter how lung tissue responds to these microplastic fibers. For example, innate immune cells such as macrophages are also among the first cells to come into contact with microplastic fibers after inhalation and can respond strongly to inhaled particles and fibers (60). Therefore, future studies should also focus on more

complex models involving an immune component as well.

A limitation in our work is not having access to a specific Hoxa5 inhibitor. Even though inhibiting retinoic acid receptor-β with LE135 has a fairly specific effect on Hoxa5 expression (40), we cannot exclude the possibility that other proteins were affected by this treatment and contributed to the effect.

In conclusion, as plastic use continues to expand, there may be a corresponding rise in health risks for humans. This study underscores the pressing need to delve deeper into the dangers and prevalence of microplastic fibers. Those outcomes will be invaluable to advisory organizations such as the World Health Organization and Science Advice for Policy by European Academies, which recently called for more data on effects of microplastics on human health (2). Future research endeavors should prioritize analyzing the concentration and prevalence of these fibers in indoor settings and within human lung tissues to provide a more accurate assessment of their potential threat to human health. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank Habibie, Jesse Mulder, Imco Sibum, Paul Hagedoorn, and Anko Eissens (University of Groningen); Andreas W. Ehlers (University of Amsterdam); and Elena Höppener (TNO) for their technical assistance and ZonMw for their financial support with the Microplastics and Health grant 458001013 (awarded to B.N.M., R.G., A.S., and M.C.) and the ZonMw/Health Holland consortium grant MOMENTUM (458001101, momentummicroplastics.nl) led by Prof. Dr. J. Legler and Prof. Dr. D. Vethaak and awarded to B.N.M. and I.M.K., among others. The authors also acknowledge Prof. Jeroen den Hertog (Hubrecht Institute) for providing Noggin-producing cells and Dr. Calvin Kuo (Stanford University) for providing R-spondin1–producing cells.

References

1. Vethaak AD, Legler J. Microplastics and human health. *Science* 2021;371: 672–674.
2. World Health Organization (WHO). Dietary and inhalation exposure to nano- and microplastic particles and potential implications for human health. Geneva: WHO; 2022.
3. Gasperi J, Wright SL, Dris R, Collard F, Mandin C, Guerrouache M, *et al.* Microplastics in air: are we breathing it in? *Curr Opin Environ Sci Health* 2018;1:1–5.
4. Rochman CM. Microplastics research—from sink to source in freshwater systems. *Science* 2018;360:28–29.
5. Leslie HA, van Velzen MJM, Brandsma SH, Vethaak AD, Garcia-Vallejo JJ, Lamoree MH. Discovery and quantification of plastic particle pollution in human blood. *Environ Int* 2022;163:107199.
6. Zhang J, Wang L, Kannan K. Microplastics in house dust from 12 countries and associated human exposure. *Environ Int* 2020;134:105314.
7. Dris R, Gasperi J, Saad M, Mirande C, Tassin B. Synthetic fibers in atmospheric fallout: a source of microplastics in the environment? *Mar Pollut Bull* 2016;104:290–293.
8. Yao Y, Glamoclija M, Murphy A, Gao Y. Characterization of microplastics in indoor and ambient air in northern New Jersey. *Environ Res* 2022; 207:112142.
9. Liao Z, Ji X, Ma Y, Lv B, Huang W, Zhu X, *et al.* Airborne microplastics in indoor and outdoor environments of a coastal city in Eastern China. *J Hazard Mater* 2021;417:126007.
10. Salthammer T. Microplastics and their additives in the indoor environment. *Angew Chem Int Ed Engl* 2022;61:e202205713.
11. Gaston E, Woo M, Steele C, Sukumaran S, Anderson S. Microplastics differ between indoor and outdoor air masses: insights from multiple microscopy methodologies. *Appl Spectrosc* 2020;74:1079–1098.
12. Soltani NS, Taylor MP, Wilson SP. Quantification and exposure assessment of microplastics in Australian indoor house dust. *Environ Pollut* 2021;283:117064.
13. Browne MA, Crump P, Niven SJ, Teuten E, Tonkin A, Galloway T, *et al.* Accumulation of microplastic on shorelines woldwide: sources and sinks. *Environ Sci Technol* 2011;45:9175–9179.
14. Pauly JL, Stegmeier SJ, Allaart HA, Cheney RT, Zhang PJ, Mayer AG, *et al.* Inhaled cellulosic and plastic fibers found in human lung tissue. *Cancer Epidemiol Biomarkers Prev* 1998;7:419–428.
15. Amato-Lourenço LF, Carvalho-Oliveira R, Júnior GR, Dos Santos Galvão L, Ando RA, Mauad T. Presence of airborne microplastics in human lung tissue. *J Hazard Mater* 2021;416:126124.
16. Jenner LC, Rotchell JM, Bennett RT, Cowen M, Tentzeris V, Sadofsky LR. Detection of microplastics in human lung tissue using μFTIR spectroscopy. *Sci Total Environ* 2022;831:154907.
17. Baeza-Martínez C, Olmos S, González-Pleiter M, López-Castellanos J, García-Pachón E, Masiá-Canuto M, *et al.* First evidence of microplastics isolated in European citizens' lower airway. *J Hazard Mater* 2022;438:129439.
18. Wright SL, Kelly FJ. Plastic and human health: a micro issue? *Environ Sci Technol* 2017;51:6634–6647.
19. Atis S, Tutluoglu B, Levent E, Ozturk C, Tunaci A, Sahin K, *et al.* The respiratory effects of occupational polypropylene flock exposure. *Eur Respir J* 2005;25:110–117.
20. Eschenbacher WL, Kreiss K, Lougheed MD, Pransky GS, Day B, Castellan RM. Nylon flock–associated interstitial lung disease. *Am J Respir Crit Care Med* 1999;159:2003–2008.
21. Song S, Van Dijk F, Eck G, Wu X, Bos S, Boom D, *et al.* Inhalable textile microplastic fibers impair lung repair [abstract]. *ERJ Open Res* 2022;8(Suppl 8):69.
22. Van Dijk F, Van Eck G, Cole M, Salvati A, Bos S, Gosens R, *et al.* Exposure to textile microplastic fibers impairs epithelial growth [abstract]. *Eur Respir J* 2020;56(Suppl 64):1972.
23. Van Dijk F, Song S, van Eck GWA, Wu X, Bos IST, Boom DHA, *et al.* Inhalable textile microplastic fibers impair lung repair [preprint]. bioRxiv; 2021 [accessed 2023 Nov 13]. Available from: https://doi.org/10.1101/2021.01.25.428144.
24. Cole M. A novel method for preparing microplastic fibers. *Sci Rep* 2016; 6:34519.
25. Islam MS, Rahman MM, Larpruenrudee P, Arsalanloo A, Beni HM, Islam MA, *et al.* How microplastics are transported and deposited in realistic upper airways? *Phys Fluids (1994)* 2023;35:063319.
26. Pleil JD, Ariel Geer Wallace M, Davis MD, Matty CM. The physics of human breathing: flow, timing, volume, and pressure parameters for normal, on-demand, and ventilator respiration. *J Breath Res* 2021;15: 042002.
27. Wu X, Bos IST, Conlon TM, Ansari M, Verschut V, van der Koog L, *et al.* A transcriptomics-guided drug target discovery strategy identifies receptor ligands for lung regeneration. *Sci Adv* 2022;8:eabj9949.
28. Winkler AS, Cherubini A, Rusconi F, Santo N, Madaschi L, Pistoni C, *et al.* Human airway organoids and microplastic fibers: a new exposure model for emerging contaminants. *Environ Int* 2022;163:107200.
29. Sachs N, Papaspyropoulos A, Zomer-van Ommen DD, Heo I, Böttinger L, Klay D, *et al.* Long-term expanding human airway organoids for disease modeling. *EMBO J* 2019;38:1–20.
30. Ng-Blichfeldt J-P, de Jong T, Kortekaas RK, Wu X, Lindner M, Guryev V, *et al.* TGF-β activation impairs fibroblast ability to support adult lung epithelial progenitor cell organoid formation. *Am J Physiol Lung Cell Mol Physiol* 2019;317:L14–L28.
31. Wang X, Park J, Susztak K, Zhang NR, Li M. Bulk tissue cell type deconvolution with multi-subject single-cell expression reference. *Nat Commun* 2019;10:380.
32. Angelidis I, Simon LM, Fernandez IE, Strunz M, Mayr CH, Greiffo FR, *et al.* An atlas of the aging lung mapped by single cell transcriptomics and deep tissue proteomics. *Nat Commun* 2019;10:963.
33. Choi J, Park J-E, Tsagkogeorga G, Yanagita M, Koo B-K, Han N, *et al.* Inflammatory signals induce AT2 cell-derived damage-associated transient progenitors that mediate alveolar regeneration. *Cell Stem Cell* 2020;27:366–382.e7.

34. Sørensen L, Groven AS, Hovsbakken IA, Del Puerto O, Krause DF, Sarno A, *et al.* UV degradation of natural and synthetic microfibers causes fragmentation and release of polymer degradation products and chemical additives. *Sci Total Environ* 2021;755:143170.
35. Ng-Blichfeldt JP, Schrik A, Kortekaas RK, Noordhoek JA, Heijink IH, Hiemstra PS, *et al.* Retinoic acid signaling balances adult distal lung epithelial progenitor cell growth and differentiation. *EBioMedicine* 2018; 36:461–474.
36. Hu Y, Ng-Blichfeldt JP, Ota C, Ciminieri C, Ren W, Hiemstra PS, *et al.* Wnt/β-catenin signaling is critical for regenerative potential of distal lung epithelial progenitor cells in homeostasis and emphysema. *Stem Cells* 2020;38:1467–1478.
37. Strunz M, Simon LM, Ansari M, Kathiriya JJ, Angelidis I, Mayr CH, *et al.* Alveolar regeneration through a $Krt8^{+}$ transitional stem cell state that persists in human lung fibrosis. *Nat Commun* 2020;11:3559.
38. Rout-Pitt N, Farrow N, Parsons D, Donnelley M. Epithelial mesenchymal transition (EMT): a universal process in lung diseases with implications for cystic fibrosis pathophysiology. *Respir Res* 2018;19:136.
39. Taniguchi Y. Hox transcription factors: modulators of cell-cell and cell-extracellular matrix adhesion. *BioMed Res Int* 2014;2014:591374.
40. Chen H, Zhang H, Lee J, Liang X, Wu X, Zhu T, *et al.* HOXA5 acts directly downstream of retinoic acid receptor β and contributes to retinoic acid-induced apoptosis and growth inhibition. *Cancer Res* 2007;67:8007–8013.
41. Vianello A, Jensen RL, Liu L, Vollertsen J. Simulating human exposure to indoor airborne microplastics using a breathing thermal manikin. *Sci Rep* 2019;9:8670.
42. Zhang Q, Zhao Y, Du F, Cai H, Wang G, Shi H. Microplastic fallout in different indoor environments. *Environ Sci Technol* 2020;54: 6530–6539.
43. Porter DW, Castranova V, Robinson VA, Hubbs AF, Mercer RR, Scabilloni J, *et al.* Acute inflammatory reaction in rats after intratracheal instillation of material collected from a nylon flocking plant. *J Toxicol Environ Health A* 1999;57:25–45.
44. Sauler M, Gulati M. Newly recognized occupational and environmental causes of chronic terminal airways and parenchymal lung disease. *Clin Chest Med* 2012;33:667–680.
45. Warheit DB, Webb TR, Reed KL, Hansen JF, Kennedy GL Jr. Four-week inhalation toxicity study in rats with nylon respirable fibers: rapid lung clearance. *Toxicology* 2003;192:189–210.
46. Fujino N, Kubo H, Maciewicz RA. Phenotypic screening identifies Axl kinase as a negative regulator of an alveolar epithelial cell phenotype. *Lab Invest* 2017;97:1047–1062.
47. Yang B, Cao G, Cai K, Wang G, Li P, Zheng L, *et al.* VEGF-modified PVA/silicone nanofibers enhance islet function transplanted in subcutaneous site followed by device-less procedure. *Int J Nanomedicine* 2020;15:587–599.
48. Li X, Yan S, Dai J, Lu Y, Wang Y, Sun M, *et al.* Human lung epithelial cells A549 epithelial-mesenchymal transition induced by PVA/collagen nanofiber. *Colloids Surf B Biointerfaces* 2018;162:390–397.
49. Yan S, Li X, Dai J, Wang Y, Wang B, Lu Y, *et al.* Electrospinning of PVA/sericin nanofiber and the effect on epithelial-mesenchymal transition of A549 cells. *Mater Sci Eng C* 2017;79:436–444.
50. Boucherat O, Chakir J, Jeannotte L. The loss of Hoxa5 function promotes Notch-dependent goblet cell metaplasia in lung airways. *Biol Open* 2012;1:677–691.
51. Ordóñez-Morán P, Dafflon C, Imajo M, Nishida E, Huelsken J. HOXA5 counteracts stem cell traits by inhibiting Wnt signaling in colorectal cancer. *Cancer Cell* 2015;28:815–829.
52. Lu K, Lai KP, Stoeger T, Ji S, Lin Z, Lin X, *et al.* Detrimental effects of microplastic exposure on normal and asthmatic pulmonary physiology. *J Hazard Mater* 2021;416:126069.
53. Sripada K, Wierzbicka A, Abass K, Grimalt JO, Erbe A, Röllin HBA, *et al.* A children's health perspective on nano- and microplastics. *Environ Health Perspect* 2022;130:15001.
54. Ragusa A, Svelato A, Santacroce C, Catalano P, Notarstefano V, Carnevali O, *et al.* Plasticenta: first evidence of microplastics in human placenta. *Environ Int* 2021;146:106274.
55. Hu J, Qin X, Zhang J, Zhu Y, Zeng W, Lin Y, *et al.* Polystyrene microplastics disturb maternal-fetal immune balance and cause reproductive toxicity in pregnant mice. *Reprod Toxicol* 2021;106:42–50.
56. Xumiao L, Prata JC, Alves JR, Duarte AC, Rocha-Santos T, Cerqueira M. Airborne microplastics and fibers in indoor residential environments in Aveiro, Portugal. *Environ Adv* 2021;6:100134.
57. Lee S, Kang KK, Sung SE, Choi JH, Sung M, Seong KY, *et al.* In vivo toxicity and pharmacokinetics of polytetrafluoroethylene microplastics in ICR mice. *Polymers (Basel)* 2022;14:2220.
58. Burkhart J, Piacitelli C, Schwegler-Berry D, Jones W. Environmental study of nylon flocking process. *J Toxicol Environ Health A* 1999;57: 1–23.
59. Fatehullah A, Tan SH, Barker N. Organoids as an in vitro model of human development and disease. *Nat Cell Biol* 2016;18:246–254.
60. Kuroda E, Ozasa K, Temizoz B, Ohata K, Koo CX, Kanuma T, *et al.* Inhaled fine particles induce alveolar macrophage death and interleukin-1α release to promote inducible bronchus-associated lymphoid tissue formation. *Immunity* 2016;45:1299–1310.