Supplemental methods

The hESCs line AND-2 was obtained from the "Biobanco de células madre de Granada" (ISCIII, Spain); passages 26-40. Mouse embryonic fibroblasts (MEFs) were obtained at 13.5 days *post-coitum* from C57BL/6 mice as described previously (1). MEFs were mitotically inactivated by an overnight treatment with 2 µg/mL of mitomycin C (cat.#M4287; Sigma-Aldrich) and plated at a density of approximately 16,000 cells/cm^2. hESCs were cultured along with MEFs under standard conditions (http://www.stembook.org). The maintenance medium was composed of KO-DMEM (cat.#10829-018 Gibco; Life Technologies), 20% KO serum replacement (cat.#10828010 Gibco; Life Technologies), 0.1 mM β-mercaptoethanol (cat.#21985-023 Gibco; Life Technologies), 2 mM Glutamax (cat.#35050-061,Gibco; Life Technologies), nonessential aminoacids (cat.#11140-050 Gibco; Life Technologies and primocin (cat.#12I05MM; InvivoGen). The medium was filtered by using 0.22-µ pore filter systems (cat.#431097; Corning); 10 ng/mL recombinant human basic Fibroblast Growth Factor (hbFGF) (cat.#PHG6015; Invitrogen) and 10 µM Y-27632 (cat.#1254; Tocris R&D Systems) were added before use. The medium was changed in a daily basis and cells were passaged either by enzymatic (collagenase IV method) (collagenase IV: cat.#11140050; Gibco; Life Technologies) or mechanical procedures (http://stembook.org). Cells were maintained in an undifferentiated state in a 5% CO_2/air environment. The differentiation process was carried-out under hypoxic conditions in a 5% CO_2/5% O_2/95% N_2 environment [Galaxy 48R incubator (New Brunswick)] or in normoxia as indicated in the corresponding differentiation step.

Primitive streak formation and induction of definitive endoderm (DE)

Induction of endoderm was performed as previously described. Primitive streak formation (day 0; 24h) and endoderm induction (days 1-4) were performed in serum-free differentiation (SFD) medium. SFD medium was composed of a mix of IMDM:F12 (3:1) media (cats.#B12-722F and 10-080 CVR; Corning), supplemented with N2 (cat.#17502-048, Gibco; Life Technologies), B27

(cat.#17504-044, Gibco; Life Technologies), 2 mM Glutamax (cat.#35050-061 Gibco; Life Technologies), 1% penicillin-streptomycin (DE17-602E; Lonza), and 0.05% bovine serum albumin (BSA) (cat.#A7906; Sigma-Aldrich). The medium was filtered using a 0.22 µ-pore filter system (cat.#431097; Corning); 50 µg/mL ascorbic acid (cat.#A4554; SigmaAldrich) and 0.04 µL/mL monothioglycerol (stock >97%) (cat.#M6145; Sigma-Aldrich) were added before use. MEFs were depleted by passaging hESCs lines onto Matrigel^{TM}-coated (cat.#354230; Life Technologies) plates for at least 48h. Cells were briefly trypsinized into small 3-10 cell clumps and the reaction was halted with stop medium [IMDM medium (BE12722F) supplemented with 50% foetal bovine serum (F7524, Sigma-Aldrich), 2 mM Glutamax, 1% penicillin-streptomycin and 30 ng/mL DNase I (cat.#260913-10MU; Calbiochem)]. Cells were then centrifuged 5 min at 850 rpm and washed carefully two times with an excess of SFD medium. To form embryoid bodies (EBs), the clumps were plated onto low-attachment 6-well plates (cat.#3471; Corning) and maintained in SFD medium in a 5% CO_2/5% O2/95% N_2 environment (Galaxy 48R incubator; New Brunswick).

For primitive streak formation, 10 µM Y-27632, 10 ng/mL Wnt3a (cat.#5036-WN; R&D Systems) and 3 ng/mL human BMP4 (cat.#314-BP; R&D Systems) were used. EBs were collected, resuspended carefully in endoderm induction medium containing 10 µM Y-27632, 0.5 ng/mL human BMP4, 2.5 ng/mL hbFGF, and 100 ng/mL human Activin (cat.# 338-AC; R&D Systems). Cells were fed after 36–48 h, depending on cell density, by removing half the old medium and adding half fresh medium.

Induction of anterior foregut endoderm (AFE)

AFE (days 4, 5 or 5) was induced as previously described. EBs were dissociated into single cells with trypsin. Dissociated cells were transferred to a conical tube containing stop medium to neutralize trypsin. Cells were centrifuged for 5 min at 850 rpm, washed carefully twice with SFD medium and counted. For AFE induction, 25,000-30,000 cells/cm^2 were plated on fibronectin-coated (F0895; Sigma-Aldrich) 12-well tissue culture plates in AFE induction medium 1 [SFD

medium supplemented with 10 mM SB-431542 (cat.#1614; Tocris) and 100 ng/mL of NOGGIN (cat.#6057;, R&D Systems). After 24h of incubation, the medium was aspirated and AFE induction medium 2 [SFD medium supplemented with 1 µM IWP2 (cat.#3533; Tocris) and 10 µM of SB-431542] was added to the cultures. This process was carried out under hypoxic conditions only for the bidimensional cultures.

Lung progenitors induction and expansion

Lung progenitor induction and expansion was carried out as previously described. On day 6,5-7, AFE cultures treated for 20 days with the ventralization medium consisting of SFD medium supplemented with 3 µM CHIR99021 (cat.#04; Tocris), 10 ng/mL human FGF10 (cat.#345-FG; R&D Systems), 10 ng/mL human KGF (cat.#251KG-010; R&D Systems), 10 ng/mL human BMP4 (cat.#314-BP; R&D Systems), 10 ng/mL murine EGF (cat.#2028-EG-200; R&D Systems) and 50 nM all-trans retinoic acid (cat.#R2625; Sigma-Aldrich). The culture medium was changed every two days. At a time point between days 8 to 12 cultures were incubated under normoxic conditions. At day 16, cultures were briefly digested with trypsin in order to remove potential nonectodermal contaminating cells. Supernatant of this brief digestion containing single cells and small clumps were removed. The remaining cell clumps were replated onto fibronectin-coated MW12 plates at 1:3 dilutions in fresh medium after trypsin neutralization and careful washing. Plates were returned to the hypoxic conditions (5% CO_2/5%O_2/95%N_2 environment).

Lung and airway epithelial cells maturation

At day 26 cultures were incubated with SFD medium supplemented with 3 µM CHIR99021, 10 ng/mL human FGF10, 10 ng/mL human FGF10, 0,1 mM 8-bromocAMP (cat.# B5386; Sigma-Aldrich), 0.1 mM IBMX (3,7-dihydro-1-methyl-3-(2methylpropyl)-1H-purine-2,6-dione; cat.# I5879; Sigma-Aldrich)) and 60 nM dexamethasone (cat.#D5902; Sigma-Aldrich). The medium was changed every two days and plates were maintained under hypoxic conditions (5%CO_2/5%O_2/95%N_2 environment). Cultures were carried further under these conditions until

their experimental use at >60 days. Treatments were performed in minilungs maintained in day 26 medium as indicated in the corresponding experiments. The specific conditions for the different hydrogels are described in the main text.