# What basic/ translational breakthroughs are going to change the way we understand CDH and care for these patients in the future?

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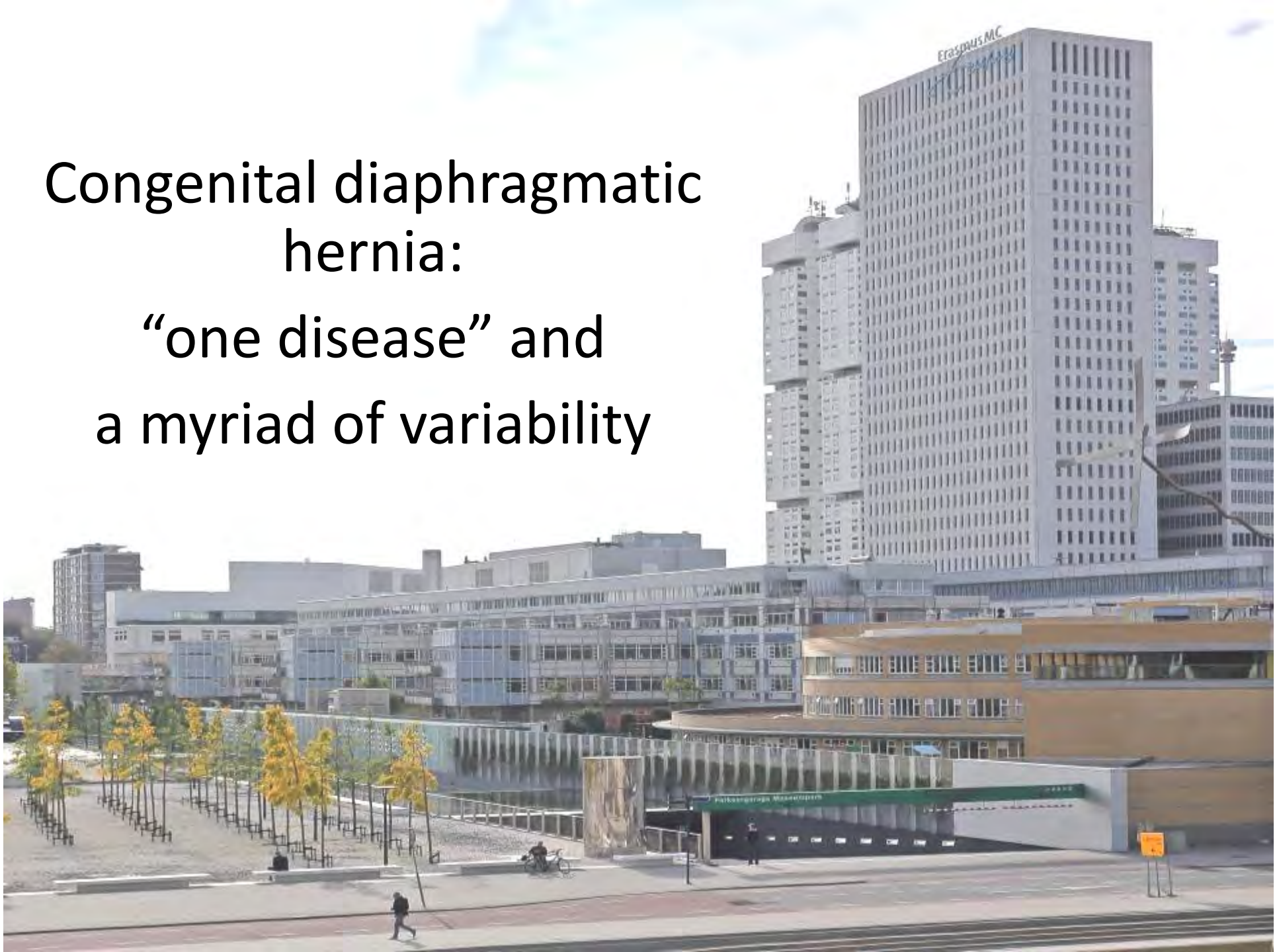
# Congenital diaphragmatic hernia:

## “one disease” and

## a myriad of variability



# Congenital diaphragmatic hernia:“one disease” and a myriad of variability

**Variability in:** (epi)genetics/ etiology

Natural history during fetal development

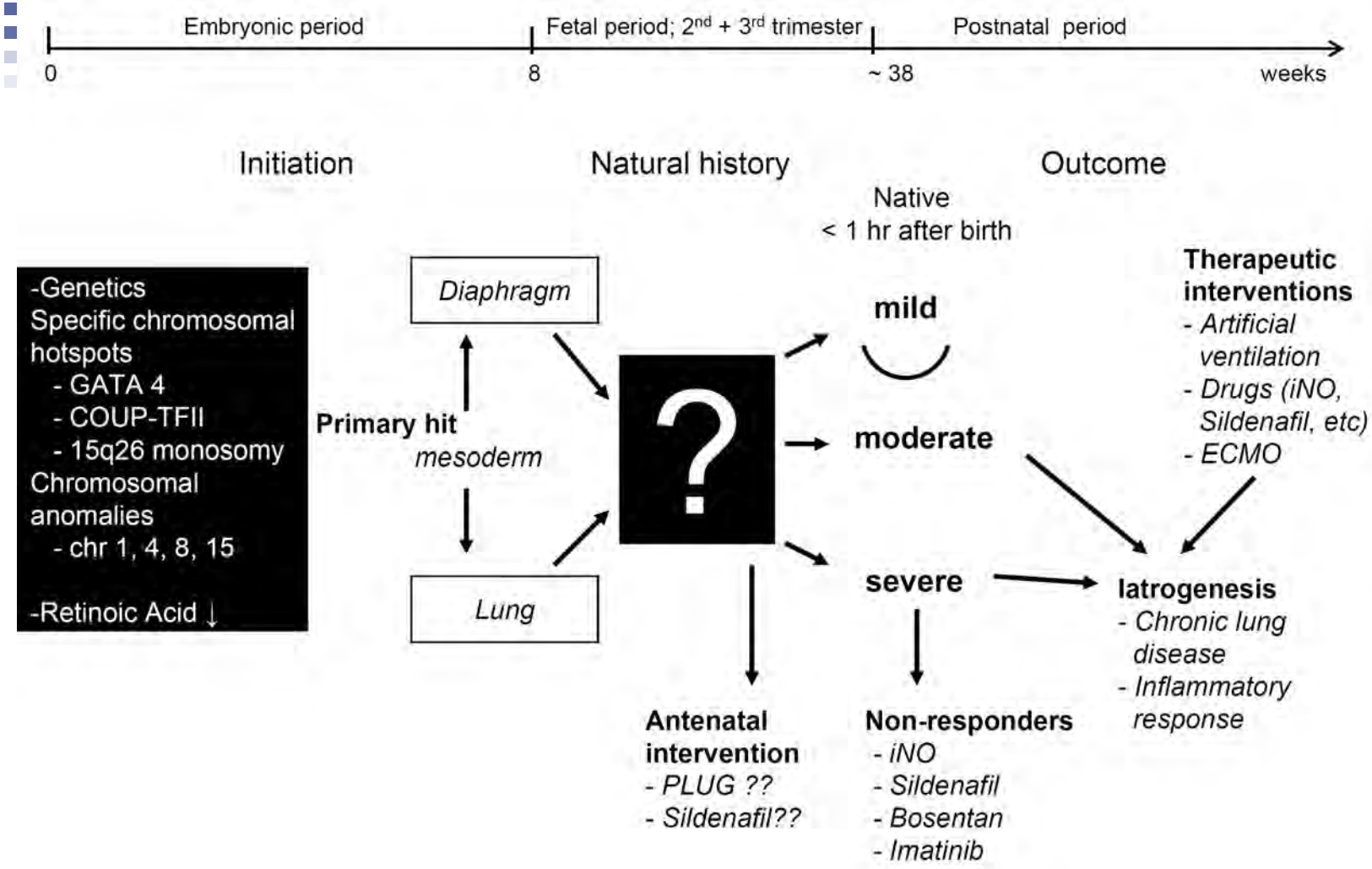
Births and the first hours

Treatment sequences in particular pharmacotherapy related

Iatrogenic insults and specific responses of the lung

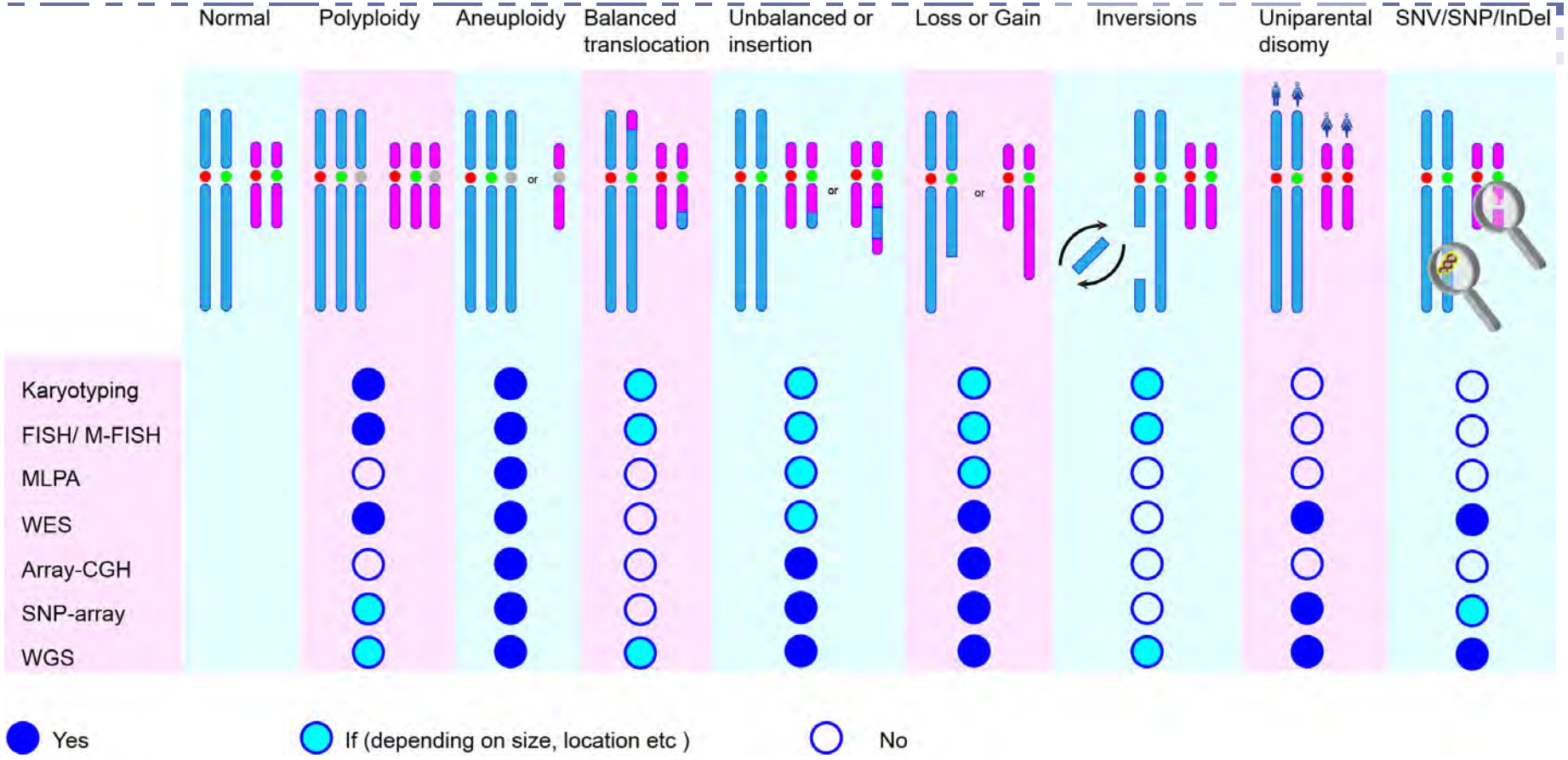
Microbiome effects

# Insight in the black box of CDH




*I. Sluiter, thesis 'CDH: A vascular disease'*

# Genetics: What do I want to detect?




Adapted from Speicher & Carter: **The new cytogenetics: blurring the boundaries with molecular biology**

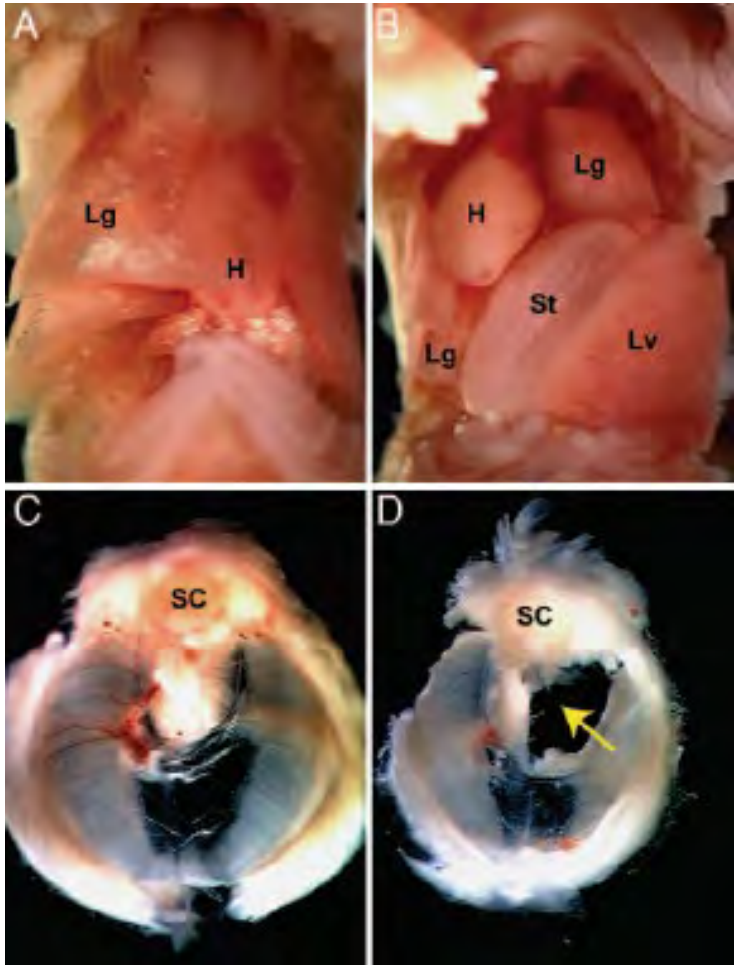
# COUP-TF2 mouse model of CDH

- Tissue specific ablation

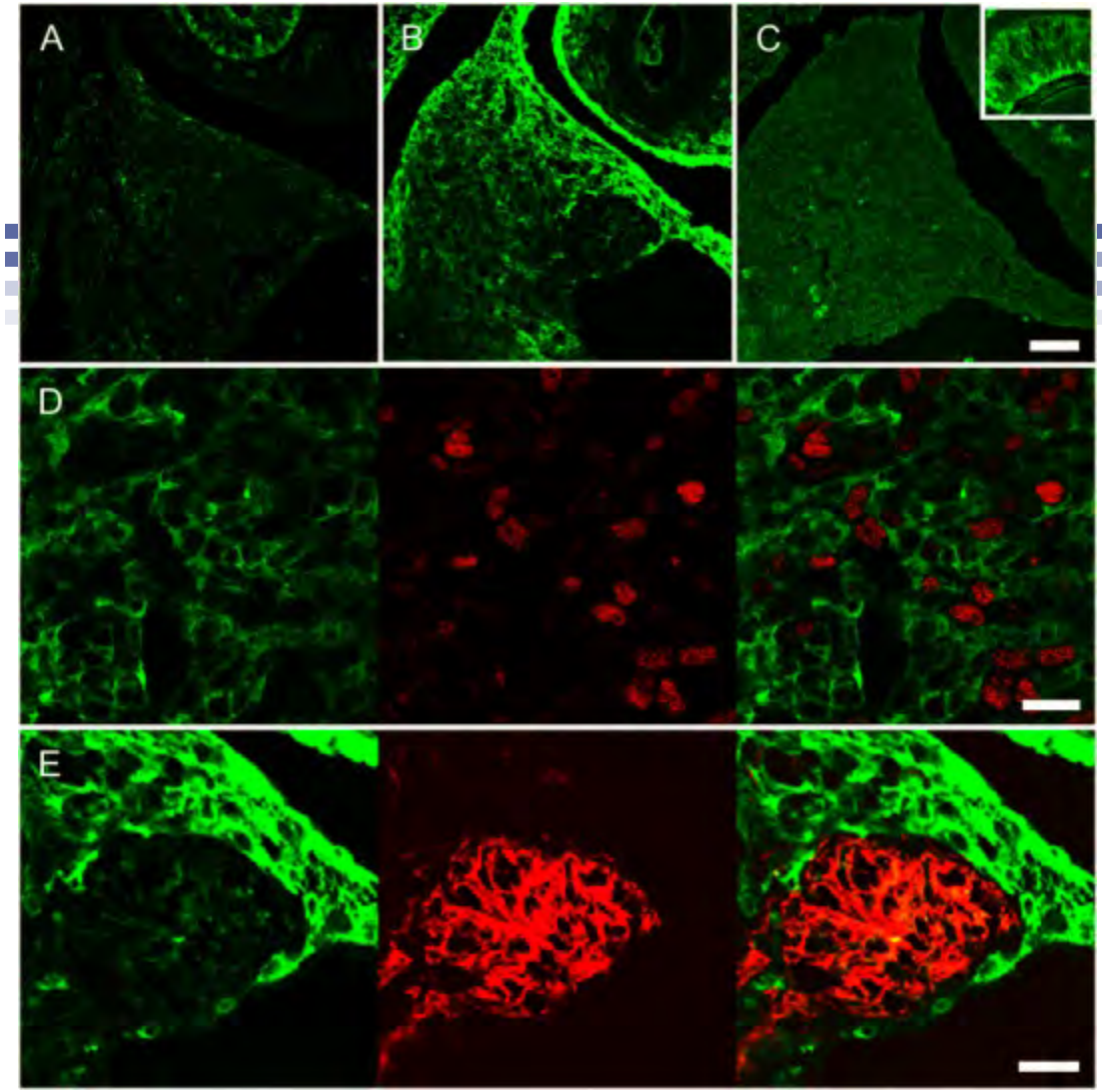
- Ablation in foregut mesoderm

  (incl. posthepatic mesenchymal plate)

  → left-sided CDH



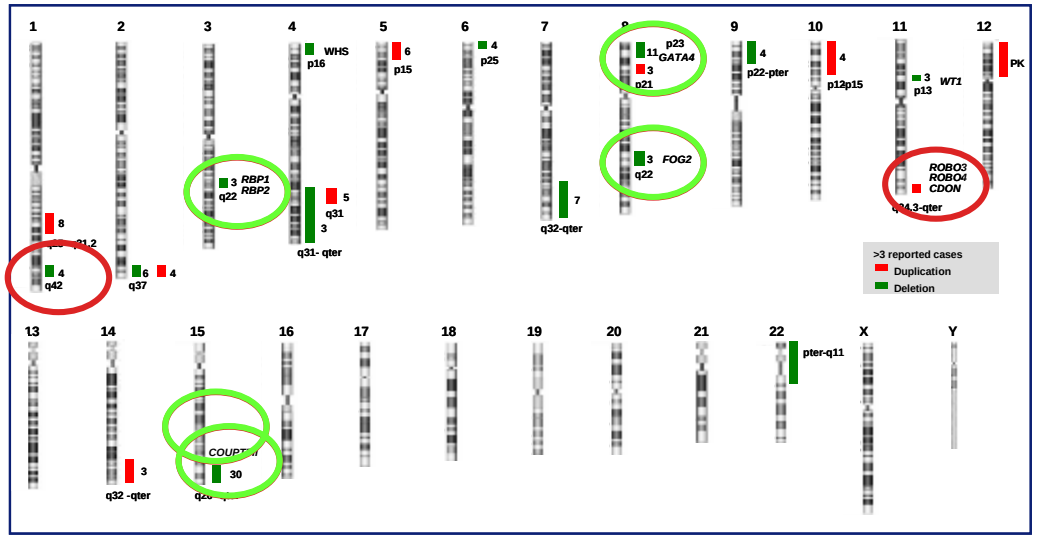

*(You et al., PNAS, 2005)*




# Retinal dehydrogenase (Raldh2) expression in the developing diaphragm at E13.5 (A)

**Robin D. Clugston, Wei Zhang, Susana Alvarez et al.**
**Am J Respir Cell Mol Biol 2010;42:276-285**

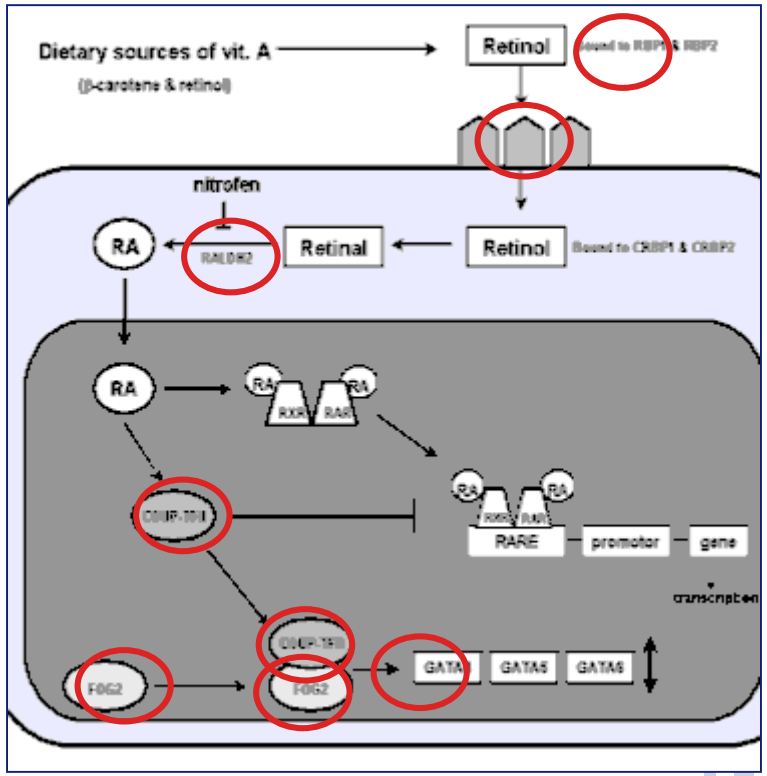
# Insights in molecular mechanisms




>450 chromosomal aberrations

Involved in RA pathway

Candidate genes??




Mutation analysis

*COUPTF* II (Tsai *etal*; KO mouse model)
-150 CDH pt for 15q gene *COUPTF* II
(total all research groups >500 pt for COUPTFII,*GATA4*, *FOG2*, *ROBO3*/4…)
*(STRA6* (Donnai-Barrow) & *LRP2* (PDAC) : recessive mutation)

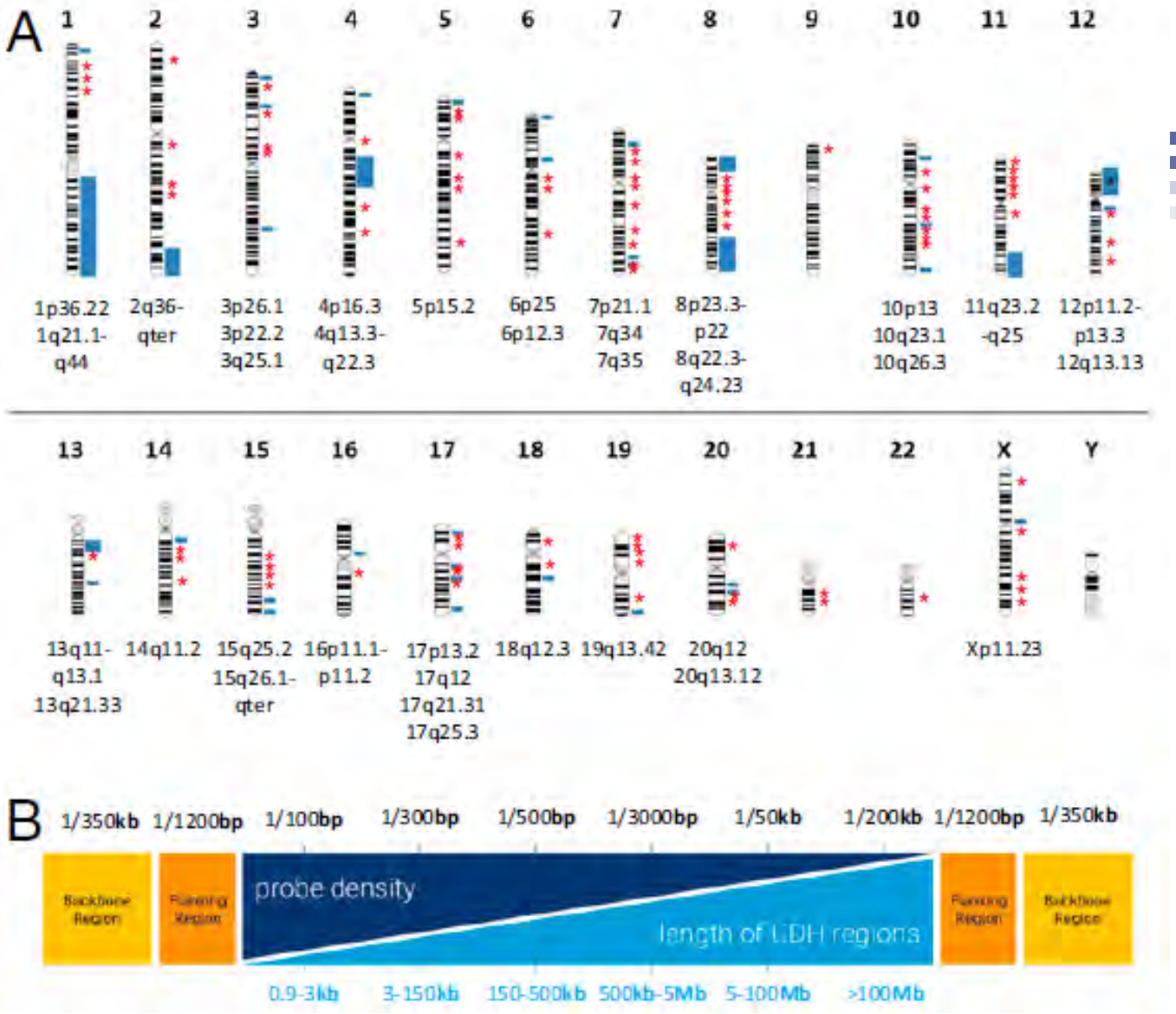
Only sporadic small (bp) changes!

# Systematic analysis of copy number variation associated with congenital diaphragmatic hernia

Qihui Zhu[a,1], Frances A. High[b,c,d,1], Chengsheng Zhang[a,1], Eliza Cerveira[a], Meaghan K. Russell[b], Mauro Longoni[b,d], Maliackal P. Joy[b], Mallory Ryan[a], Adam Mil-homens[a], Lauren Bellfy[a], Caroline M. Coletti[b], Pooja Bhayani[b], Regis Hila[b], Jay M. Wilson[c,d], Patricia K. Donahoe[b,d,2,3], and Charles Lee[a,e,2,3]

**Significance** This study describes the results of a large-scale case control analysis of copy number variants (CNVs) in a cohort of patients with congenital diaphragmatic hernia (CDH) and a large number of healthy population-matched controls. Using a customized array comparative genomic hybridization system, we have identified six CNVs that are associated with CDH with statistical significance ($P < 0.05$). These regions validate several hypothesized CDH candidate genes and identify additional genes and pathways that contribute to the pathogenesis of CDH. The estimated frequency of pathogenic CNVs in this cohort is 13%, which underscores the critical contribution of CNVs in CDH. This study also provides a model approach that is broadly applicable to other structural birth defects and identifies candidates for future functional studies.

*Proc Natl Acad Sci U S A*. 2018;115(20):5247–5252.



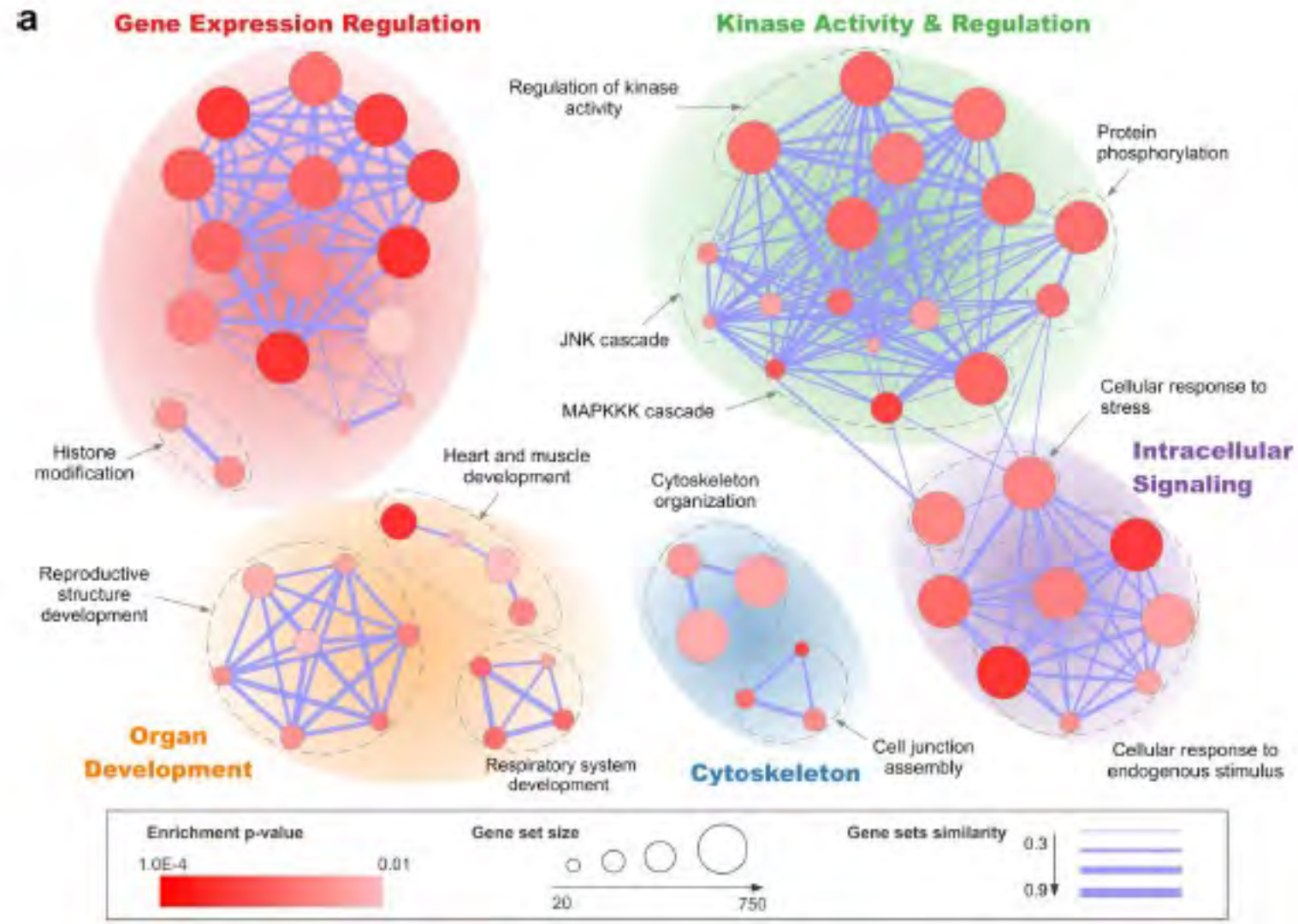

Zhu Q, High FA, Zhang C, et al. 2018

RESEARCH ARTICLE

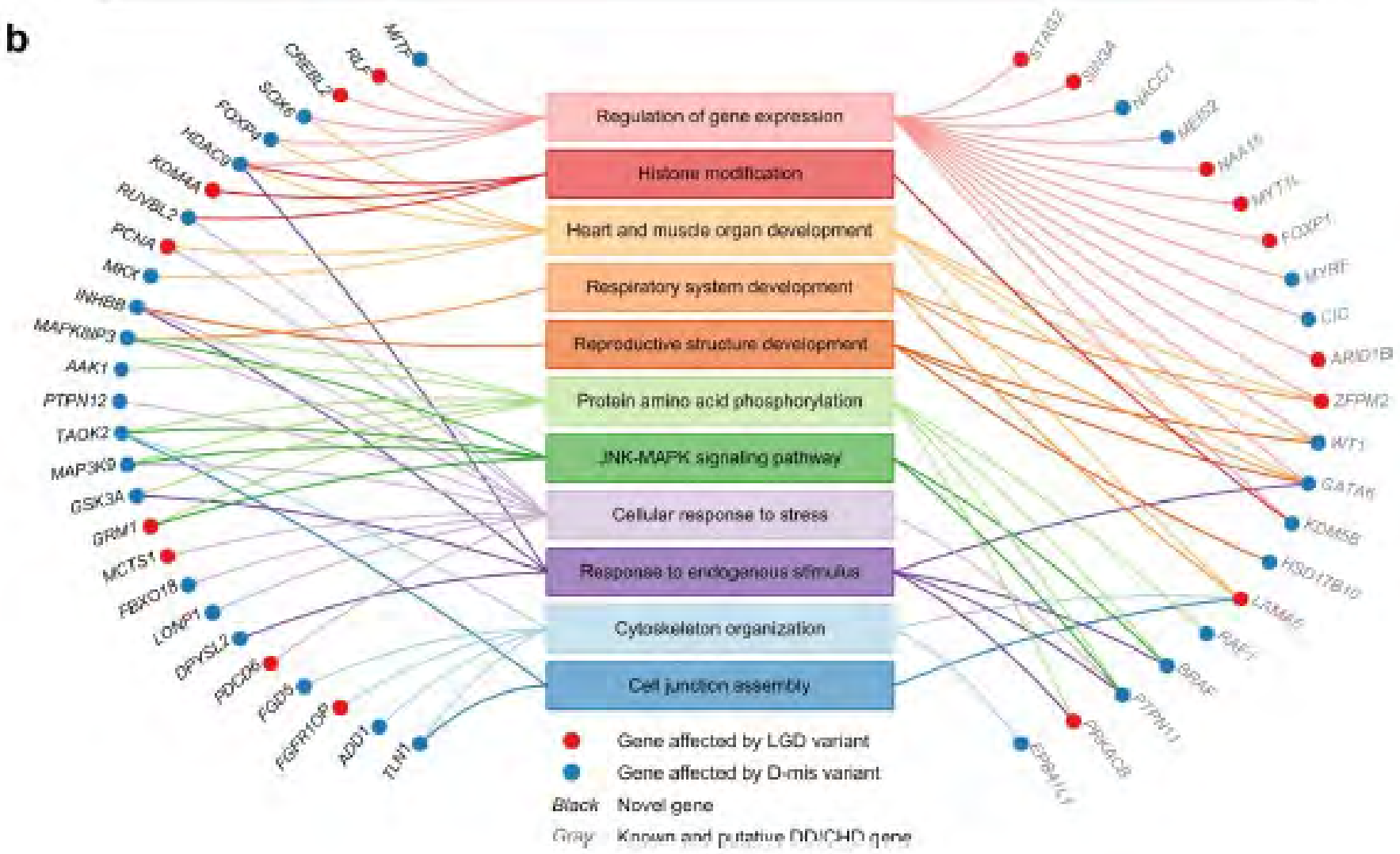
# *De novo* variants in congenital diaphragmatic hernia identify *MYRF* as a new syndrome and reveal genetic overlaps with other developmental disorders

Hongjian Qi[1,2☯], Lan Yu[3☯], Xueya Zhou[1,3☯], Julia Wynn[3], Haoquan Zhao[1,4], Yicheng Guo[1], Na Zhu[1,3], Alexander Kitaygorodsky[1,4], Rebecca Hernan[3], Gudrun Aspelund[5], Foong-Yen Lim[6], Timothy Crombleholme[6], Robert Cusick[7], Kenneth Azarow[8], Melissa E. Danko[9], Dai Chung[9], Brad W. Warner[10], George B. Mychaliska[11], Douglas Potoka[12], Amy J. Wagner[13], Mahmoud ElFiky[14], Jay M. Wilson[15,16], Debbie Nickerson[17], Michael Bamshad[17], Frances A. High[15,16,18], Mauro Longoni[16,18], Patricia K. Donahoe[16,18], Wendy K. Chung[3,19,20]*, Yufeng Shen[1,4,21]*

PLOS genetics, 2018




Qi H, Yu L, Zhou X, Wynn J, Zhao H, Guo Y, et al. (2018)
PLoS Genet 14(12): e1007822




Qi H, Yu L, Zhou X, Wynn J, Zhao H, Guo Y, et al. (2018)
PLoS Genet 14(12): e1007822

Human Molecular Genetics, 2013, Vol. 22, No. 5 1026–1038
doi:10.1093/hmg/dds507
Advance Access published on December 5, 2012

# Deficiency of FRAS1-related extracellular matrix 1 (FREM1) causes congenital diaphragmatic hernia in humans and mice

Tyler F. Beck[1], Danielle Veenma[3,4], Oleg A. Shchelochkov[5], Zhiyin Yu[1], Bum Jun Kim[1], Hitisha P. Zaveri[1], Yolande van Bever[4], Sunju Choi[6], Hannie Douben[4], Terry K. Bertin[1], Pragna I. Patel[6], Brendan Lee[1,7], Dick Tibboel[3], Annelies de Klein[4], David W. Stockton[8,9], Monica J. Justice[1] and Daryl A. Scott[1,2,*]

Human Molecular Genetics, 2012, Vol. 21, No. 18 4115–4125
doi:10.1093/hmg/dds241
Advance Access published on June 20, 2012

# Mouse model reveals the role of SOX7 in the development of congenital diaphragmatic hernia associated with recurrent deletions of 8p23.1

Margaret J. Wat[1], Tyler F. Beck[1], Andrés Hernández-García[1,5], Zhiyin Yu[1], Danielle Veenma[6,7], Monica Garcia[2], Ashley M. Holder[8], Jeanette J. Wat[9], Yuqing Chen[1,3], Carrie A. Mohila[4], Kevin P. Lally[10], Mary Dickinson[2], Dick Tibboel[6], Annelies de Klein[7], Brendan Lee[1,3] and Daryl A. Scott[1,2,*]

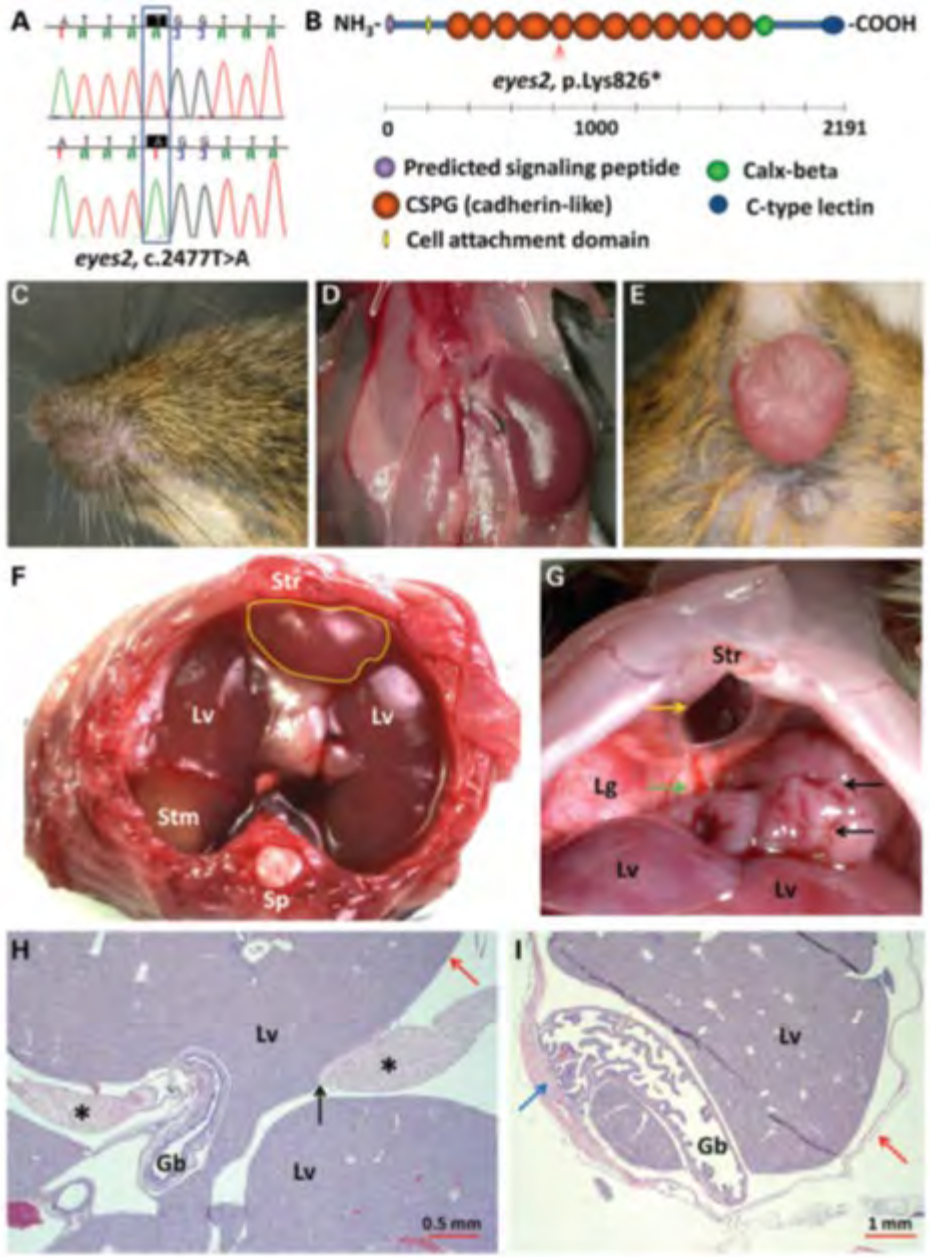



Figure 2. A homozygous truncating mutation in *Frem1* is responsible for the eye, kidney, anal, and diaphragmatic defects seen in *eyes2* mice.



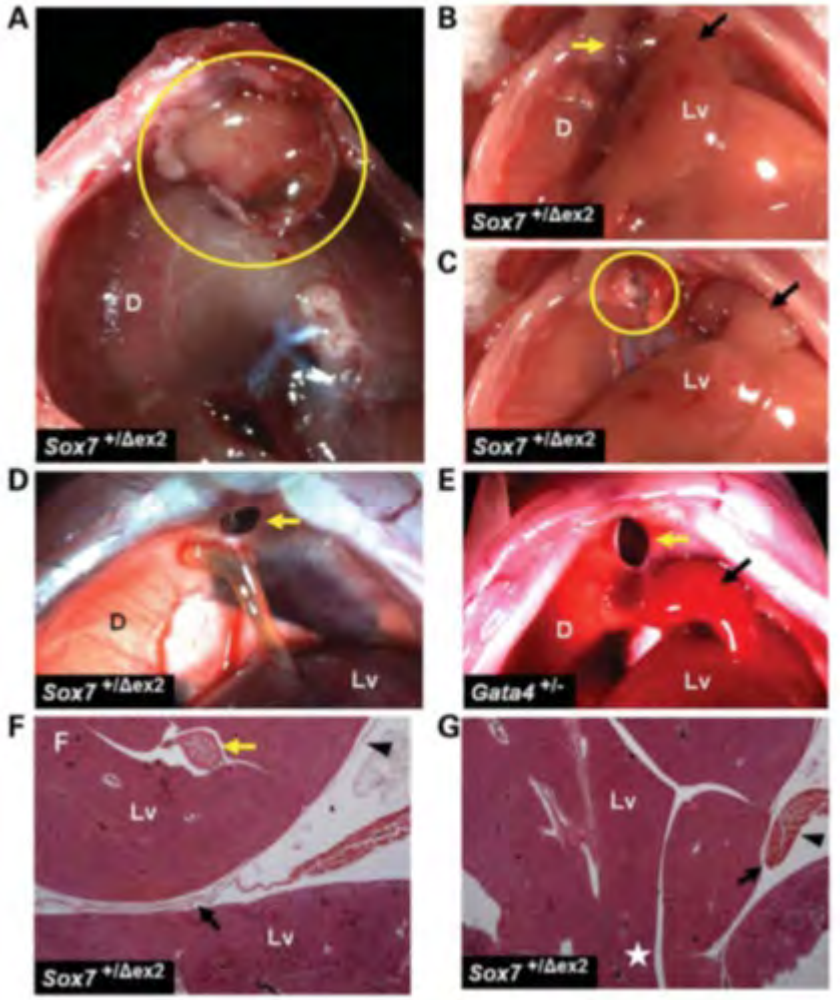
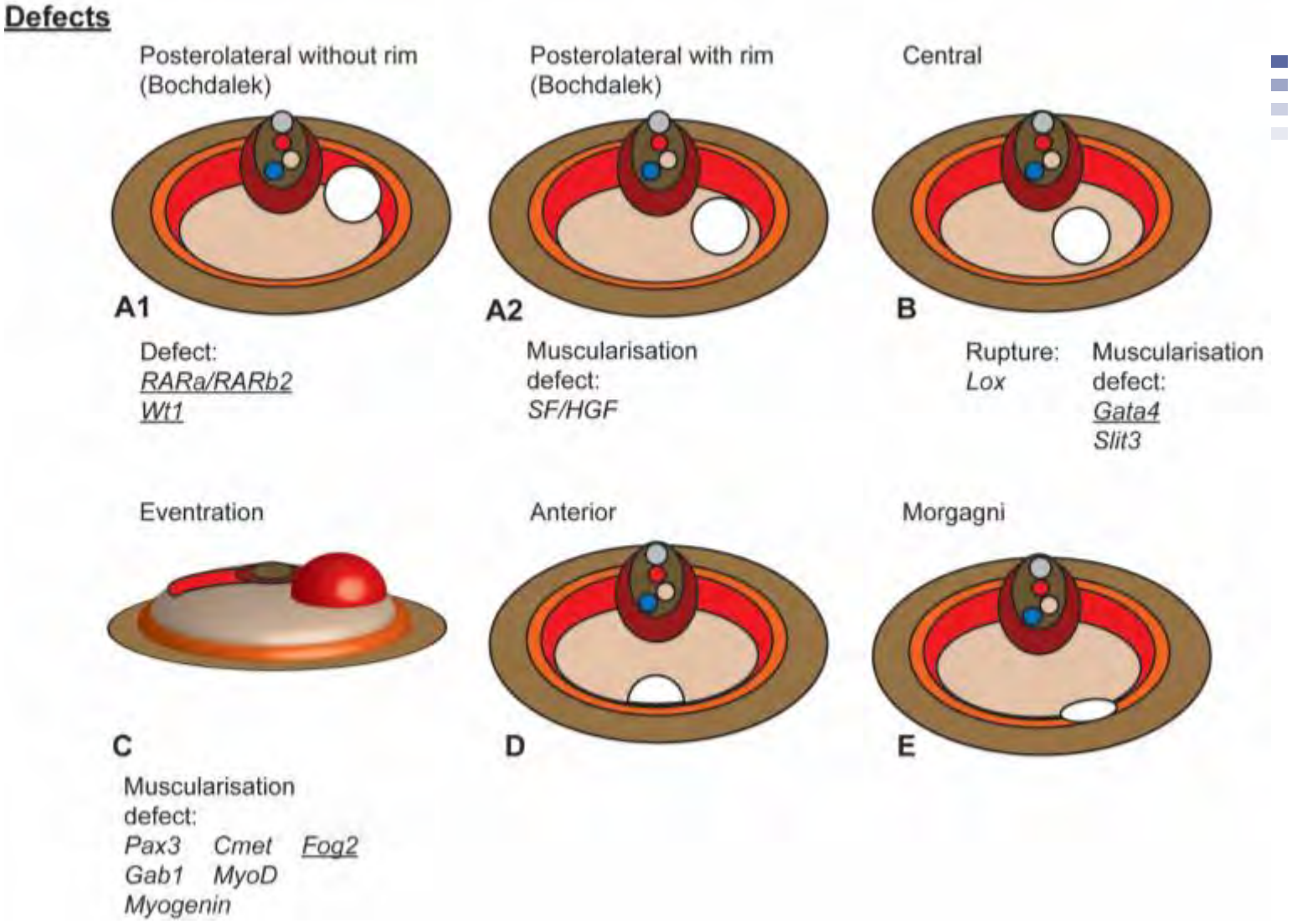



Figure 2. A portion of *Sox7*$^{+/\Delta ex2}$ mice develop retrosternal CDH that is similar to those seen in *Gata4*$^{+/-}$ mice. (

# Congenital Diaphragmatic Hernia




*Adapted from Beurskens et al., 2009 Nutrition Reviews*

REVIEW

# Polygenic Causes of Congenital Diaphragmatic Hernia Produce Common Lung Pathologies

Patricia K. Donahoe,*†‡ Mauro Longoni,*† and Frances A. High*†§¶

*From the Pediatric Surgical Research Laboratories* and the Department of Pediatrics,§ Massachusetts General Hospital, Boston; the Department of Surgery,† Harvard Medical School, Boston; the Broad Institute of the Massachusetts Institute of Technology and Harvard,‡ Cambridge; and the Department of Surgery,¶ Boston Children's Hospital, Boston, Massachusetts*

The American Journal of PATHOLOGY

'Successful treatment of CDH is dependent on the integration of human genomic and genetic data with developmental expression profiling, mouse knockouts, and gene network and pathway modeling, which have generated a large number of candidate genes and pathways for follow-up studies.'

*Am J Pathol* 2016,186: 2532–2543

**Table 1** Mouse Models with Both Diaphragm and Lung Abnormalities

| Symbol | Name | Diaphragmatic phenotype | Lung phenotype |
|---|---|---|---|
| *Atp2a1* | ATPase, $Ca^{++}$ transporting, cardiac muscle, fast twitch 1 | Abnormal diaphragm muscle | Abnormal alveoli (failure to expand, hypercellularity) |
| *Ctnnb1* | Catenin (cadherin associated protein), β1 | Diaphragmatic hernia (Wt1-Cre conditional knockout) | Absent lung buds (Shh-Cre conditional knockout) |
| *Efemp2* | Epidermal growth factor–containing fibulin-like extracellular matrix protein 2 | Diaphragmatic hernia | Abnormal elastic fibers |
| *Eya1* | EYA transcriptional co-activator and phosphatase 1 and 2 | Amuscular diaphragm (double Eya1; Eya2 knockout) | Lung hypoplasia, abnormal epithelium morphologic features |
| *Eya2* | EYA transcriptional co-activator and phosphatase 1 and 2 | Amuscular diaphragm (double Eya1; Eya2 knockout) | Lung hypoplasia, abnormal epithelium morphologic features |
| *Frem1* | Fras1 related extracellular matrix protein 1 | Diaphragmatic hernia | Fused pulmonary lobes |
| *Frem2* | Fras1-related extracellular matrix protein 2 | Diaphragmatic hernia | Fused pulmonary lobes |
| *Fuz* | Fuzzy planar cell polarity protein | Diaphragmatic hernia | Lung hypoplasia |
| *Gata4* | GATA-binding protein 4 | Diaphragmatic hernia | Abnormal saccule morphologic features, abnormal vasculature |
| *Gli2, Gli3* | GLI-Kruppel family member 2 and 3 | Diaphragmatic hernia (double Gli2; Gli3 knockout) | Lung hypoplasia, absent right lung accessory lobe, thick mesenchyme |
| *Hlx* | H2.0-like homeobox | Diaphragmatic hernia | Enlarged lungs with normal structure |
| *Igf2* | Insulin-like growth factor 2 | Thin diaphragm muscle (double Igf2; Myod1 knockout) | Abnormal epithelial proliferation/ differentiation (organ culture) |
| *Kif7* | Kinesin family member 7 | Diaphragmatic hernia, thick diaphragm muscle | Lung hypoplasia |
| *Lmnb1* | Lamin B1 | Thin diaphragm muscle, abnormal phrenic nerve | Abnormal alveoli |
| *Lmnb2* | Lamin B2 | Thin diaphragm muscle, abnormal phrenic nerve (double Lmnb1; Lmnb2 knockout) | Abnormal alveoli |
| *Lox* | Lysyl oxidase | Diaphragmatic hernia, thin diaphragm muscle | Lung hypoplasia, abnormal acini, abnormal elastic fibers |
| *Met* | Met proto-oncogene | Diaphragmatic hernia, thin diaphragm muscle | Abnormal saccule morphologic features (conditional knockout in the respiratory epithelium) |
| *Mmp2, Mmp14* | Matrix metallopeptidase 2, and 14 | Thin diaphragm muscle (double Mmp14; Mmp2 knockout) | Lung hypoplasia, abnormal alveoli, dilated alveolar ducts, abnormal elastic fibers |
| *Myod1* | Myogenic differentiation 1 | Thin diaphragm muscle (MyoD:mdx) | Pulmonary hypoplasia (MyoD:mdx) |
| *Myog* | Myogenin | Thin diaphragm muscle | Lung hypoplasia |
| *Msc* | Musculin | Diaphragmatic hernia (double Msc; Tcf21 knockout) | Lung hypoplasia, abnormal branching, abnormal vasculature |
| *Ndst1* | *N*-deacetylase/*N*-sulfotransferase (heparan glucosaminyl) 1 | Diaphragmatic hernia, thin diaphragm muscle (conditional knockout) | Lung hypoplasia, thick interalveolar septa |
| *Pbx1* | Pre–B-cell leukemia homeobox 1 | Diaphragmatic hernia | Lung hypoplasia |
| *Pdgfra* | Platelet-derived growth factor receptor, α-polypeptide | Diaphragmatic hernia | Lung hypoplasia, abnormal alveoli, increased cell proliferation |
| *Rara, Rarb* | Retinoic acid receptor, α and β | Diaphragmatic hernia (double Rara; Rarb knockout) | Lung hypoplasia, abnormal alveoli (double Rara; Rarb knockout) |
| *Robo1, Robo 2* | Roundabout guidance receptor 1 | Diaphragmatic hernia (double Robo1; Robo2 knockout) | Abnormal alveoli, thick septa |
| *Six1* | Sine oculis homeobox, *Drosophila*, homolog of, 1 | Amuscular diaphragm (double Six1; Six4 knockout) | Lung hypoplasia |
| *Tcf21* | Transcription factor 21 | Diaphragmatic hernia (double Msc; Tcf21 knockout) | Lung hypoplasia, abnormal branching, abnormal vasculature |
| *Wdr35* | WD repeat domain 35 | Diaphragmatic hernia | Pulmonary hypoplasia |
| *Wt1* | Wilms tumor 1 homolog | Diaphragmatic hernia | Lung hypoplasia |
| *Zfpm2* | Zinc finger protein, multitype 2 | Abnormal diaphragm morphologic features | Lung hypoplasia, absent right lung accessory lobe |

The Mouse Genome Database Group 2015.[33]

Donahoe PK, Longoni M, High FA.
*Am J Pathol* 2016,186: 2532–2543

Erasmus MC

# Molecular pathogenesis of congenital diaphragmatic hernia revealed by exome sequencing, developmental data, and bioinformatics

Mauro Longoni[a,b,1], Frances A. High[a,c,1], Meaghan K. Russell[a,b,1], Alireza Kashani[a,d,1], Adam A. Tracy[a], Caroline M. Coletti[a], Regis Hila[a], Ahmed Shamia[a], Julie Wells[e], Kate G. Ackerman[f], Jay M. Wilson[g], Carol J. Bult[e], Charles Lee[h], Kasper Lage[a,b,d], Barbara R. Pober[a,g,i], and Patricia K. Donahoe[a,b,d,2]

**Significance** Congenital diaphragmatic hernia (CDH) is a common birth defect associated with high morbidity and mortality. Focusing on the coding sequence of 51 genes, discovered in human studies and in mouse models, we studied 275 CDH patients and identified multiple variants in CDH-causing genes. Information on gene expression in embryonic mouse diaphragms and protein interactions allowed us to prioritize additional compelling CDHassociated genes. We believe that an improved understanding of the genetics of CDH will be important to design new therapeutic strategies for patients with diaphragmatic defects

*Proc Natl Acad Sci U S A*. 2014;111(34):12450–12455

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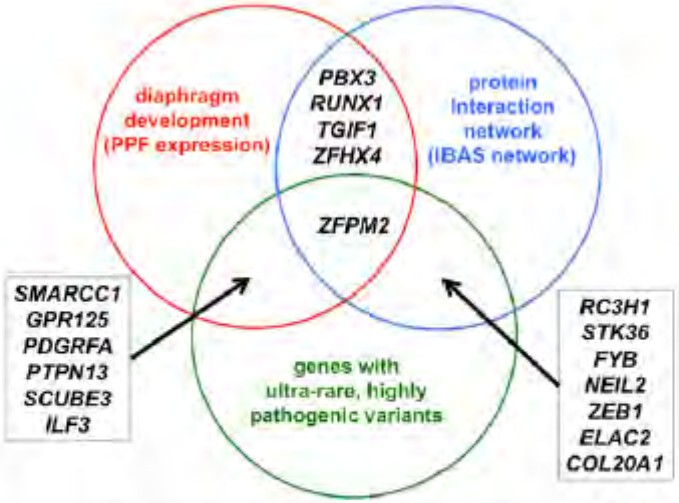
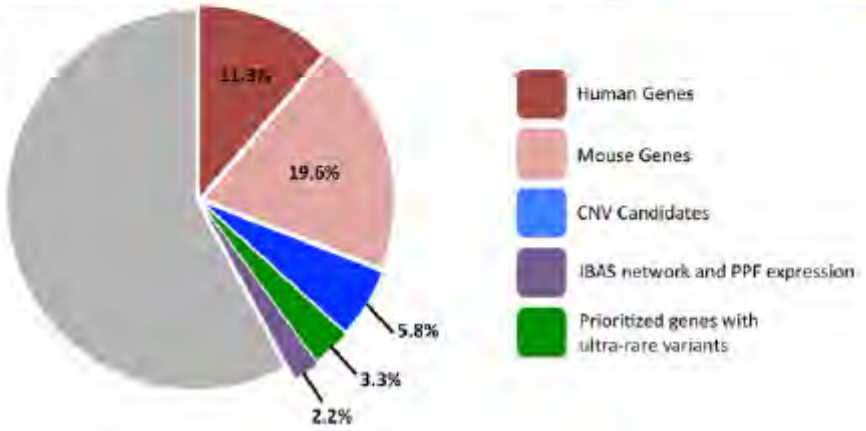


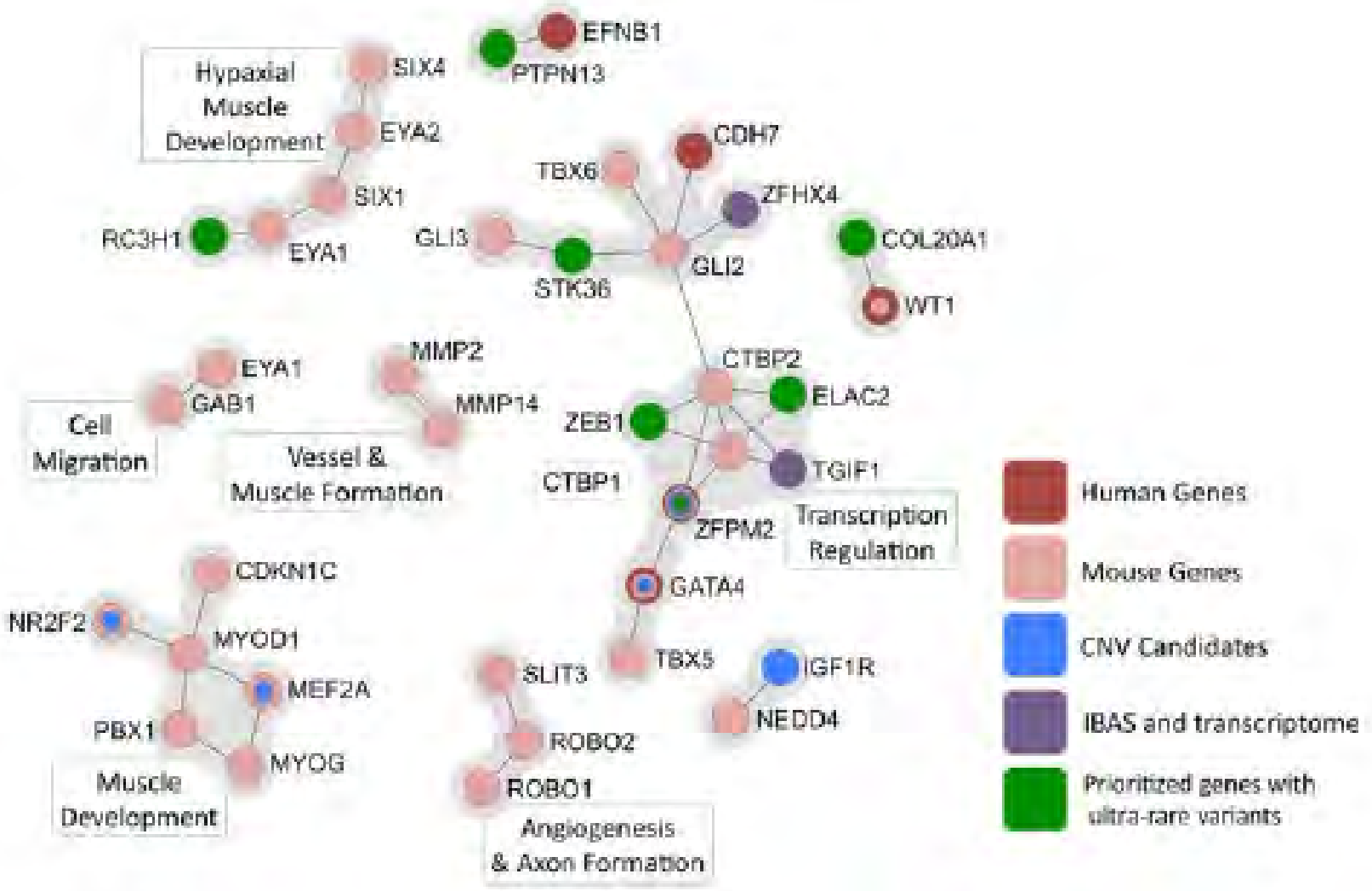

B

| Chr | Position | Ref | Var | Protein Variant | Gene | Cases | Ph. | IBAS network | PPF expr. | ns | fs | sp |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 173910520 | T | - | n/a | *RC3H1* | 1 | C | | | | | |
| 2 | 219563908 | C | G | p.S1193* | *STK36* | 1 | C | | | | | |
| 3 | 47632201 | G | - | p.P1057fs*5 | *SMARCC1* | 1 | I | | | | | |
| 4 | 22444439 | G | A | p.Q252* | *GPR125* | 1 | C | | | | | |
| 4 | 55151624 | C | T | p.R804* | *PDGFRA* | 1 | I | | | | | |
| 4 | 87614764 | G | T | p.E191* | *PTPN13* | 1 | C | | | | | |
| 5 | 39202090 | - | C | p.P335fs*43 | *FYB* | 1 | I | | | | | |
| 6 | 35211445 | GAGA | - | p.R663fs*37 | *SCUBE3* | 1 | I | | | | | |
| 8 | 11628978 | A | - | p.R8fs*51 | *NEIL2* | 1 | I | | | | | |
| 8 | 106431503 | G | T | p.E58* | *ZFPM2* | 1 | I | | | | | |
| 8 | 106815496 | C | - | p.N1062fs*23 | *ZFPM2* | 1 | I | | | | | |
| 10 | 31813042 | G | A | n/a | *ZEB1* | 1 | I | | | | | |
| 17 | 12901805 | C | A | p.E442* | *ELAC2* | 1 | C | | | | | |
| 19 | 10794414 | - | C | p.G656fs*37 | *ILF3* | 1 | C | | | | | |
| 20 | 61947959 | GG | - | p.G861fs*28 | *COL20A1* | 1 | U | | | | | |
| 20 | 61951722 | T | C | n/a | *COL20A1* | 1 | I | | | | | |

C




Longoni M et al. 2014




Longoni M et al. 2014

# MicroRNA-200b regulates distal airway development by maintaining epithelial integrity

Naghmeh Khoshgoo[1,2,3], Robin Visser[1,2], Landon Falk[1,2], Chelsea A. Day[1,2], Dustin Ameis[1,2], Barbara M. Iwasiow[1,2], Fuqin Zhu[1,2], Arzu Öztürk[4,5], Sujata Basu[1,3], Molly Pind[4,5], Agnes Fresnosa[4,5], Mike Jackson[6], Vinaya Kumar Siragam[1,2], Gerald Stelmack[1,3], Geoffrey G. Hicks[4,5], Andrew J. Halayko[1,3] & Richard Keijzer[1,2,3]

MicroRNAs (miRNA) are small, non-coding RNAs that regulate gene expression through mRNA stability and translation[8–10]. They are essential for development and homeostasis of organs[11–14]. More than 1800 microRNAs have been identified in human[15]. Research focusing on the role of microRNAs in lung development and disease is limited. We recently discovered that miR-200b is elevated in abnormal lungs of human CDH babies. In the same study, we found that higher miR-200b expression in the fetal tracheal fluid of CDH fetus is associated with a better response to fetoscopic endoluminal tracheal occlusion (FETO, a prenatal therapy to promote lung growth)

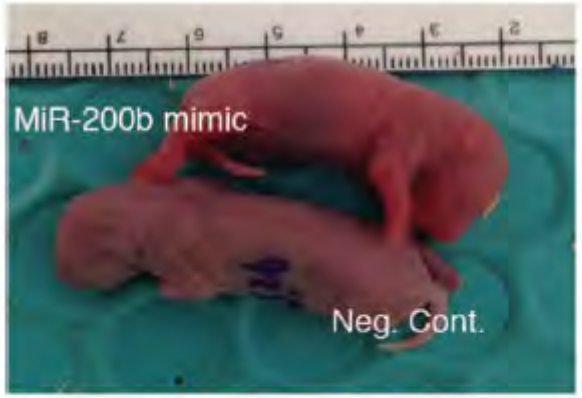
Scientific reports, 2017

# Prenatal microRNA miR-200b Therapy Improves Nitrofen-induced Pulmonary Hypoplasia Associated With Congenital Diaphragmatic Hernia

*Naghmeh Khoshgoo, MSc,* Ramin Kholdebarin, MD, MSc,* Patricia Pereira-Terra, PhD,*†*
*Thomas H. Mahood, MSc,* Landon Falk, BSc,* Chelsea A. Day, BSc,* Barbara M. Iwasiow, MSc,**
*Fuqin Zhu, BSc,* Drew Mulhall, BSc,* Carly Fraser, BSc,* Jorge Correia-Pinto, MD, PhD,†‡*
*and Richard Keijzer, MD, PhD, MSc, FACS**

‘**Conclusions**: Our data indicate that miR-200b improves PH and decreases the incidence of CDH. Future studies will further exploit this newly discovered prenatal therapy for lung hypoplasia and CDH.’

Annals of Surgery. 2019, 269(5):979–987,

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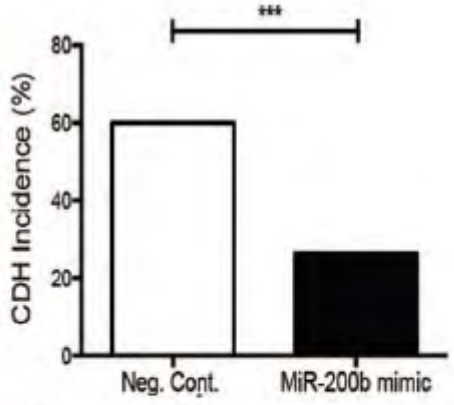


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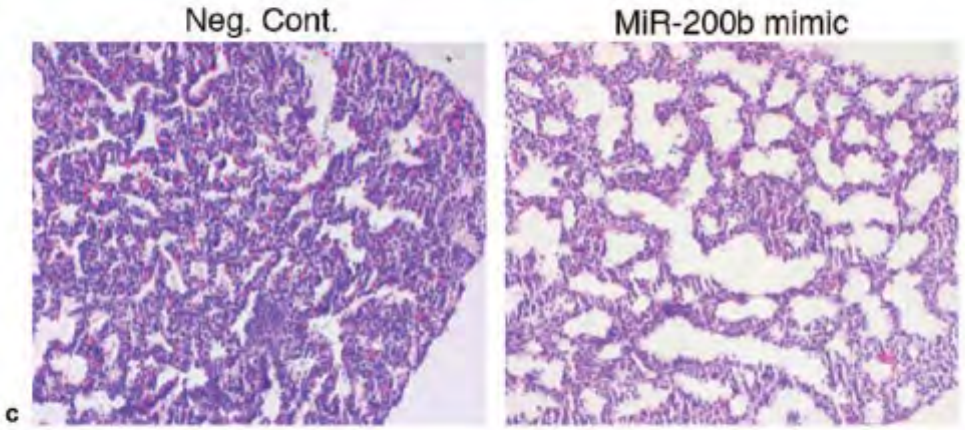


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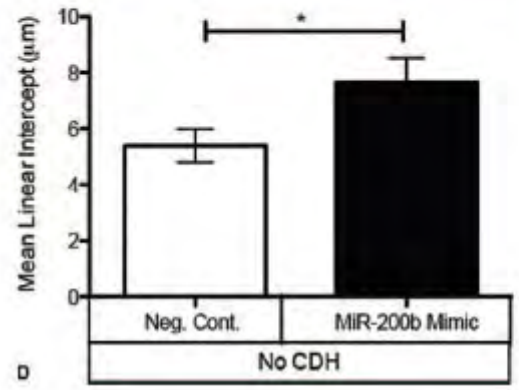


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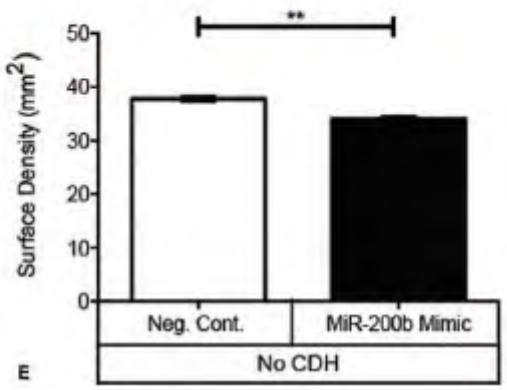



E

Koshgoo N, et al. 2019

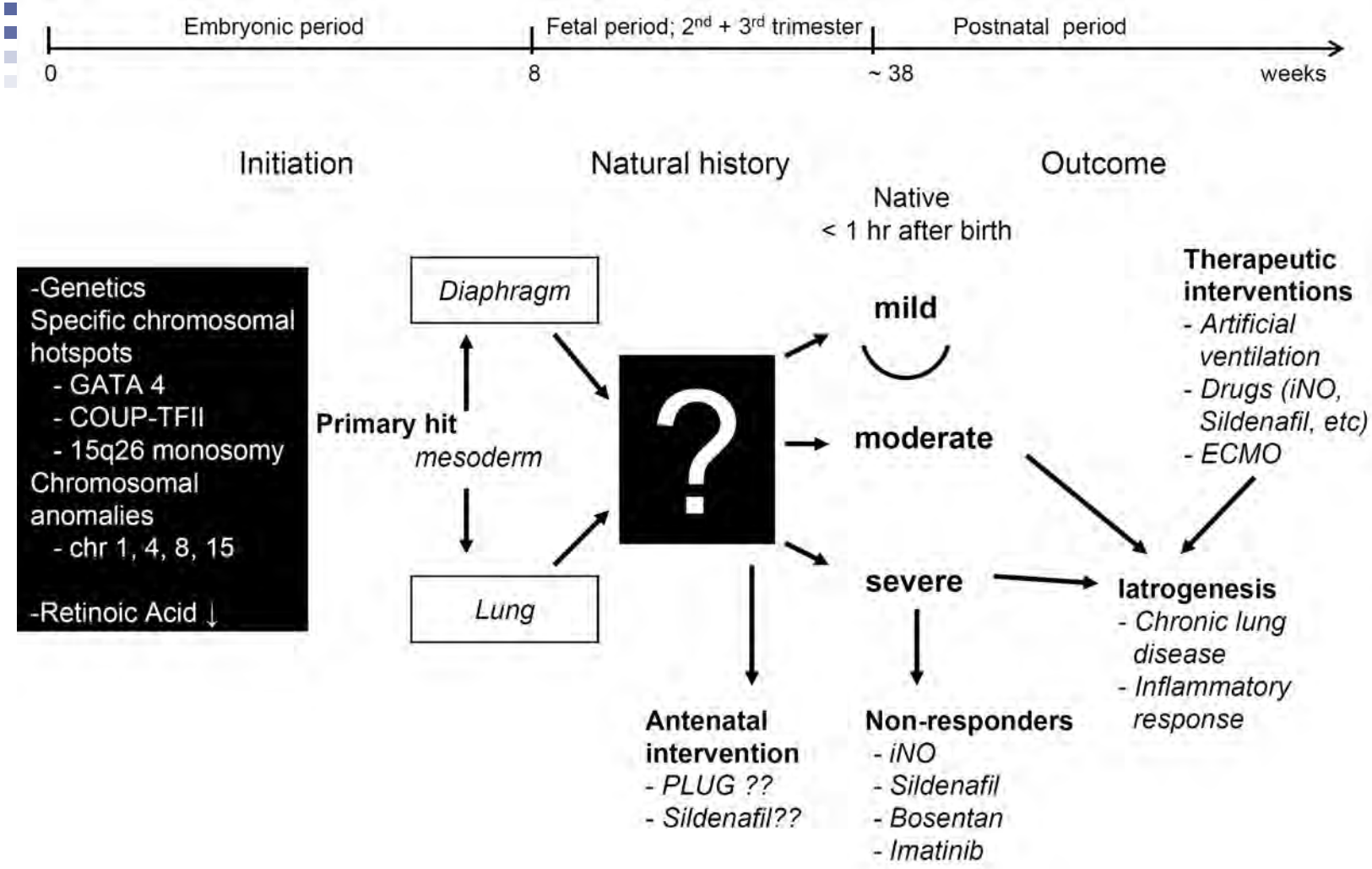
# Unique Tracheal Fluid MicroRNA Signature Predicts Response to FETO in Patients With Congenital Diaphragmatic Hernia

*Patrícia Pereira-Terra, MSc,*† Jan A. Deprest, MD, PhD,‡ Ramin Kholdebarin, MD, MSc,* Naghmeh Khoshgoo, MS,* Philip DeKoninck, MD, PhD,‡ Anne A. Boerema-De Munck,§ Jinxia Wang,¶ Fuqin Zhu,* Robbert J. Rottier, PhD,§ Barbara M. Iwasiow, MSc,* Jorge Correia-Pinto, MD, PhD,† Dick Tibboel, MD, PhD,§ Martin Post, DVM, PhD,¶ and Richard Keijzer, PhD**

**CONCLUSIONS:** Human fetal hypoplastic CDH lungs have a specific miR-200/miR-10a signature. Survival after FETO is associated with increased miR-200 family expression. miR-200b overexpression in CDH lungs results in decreased TGF-β/SMAD signaling.

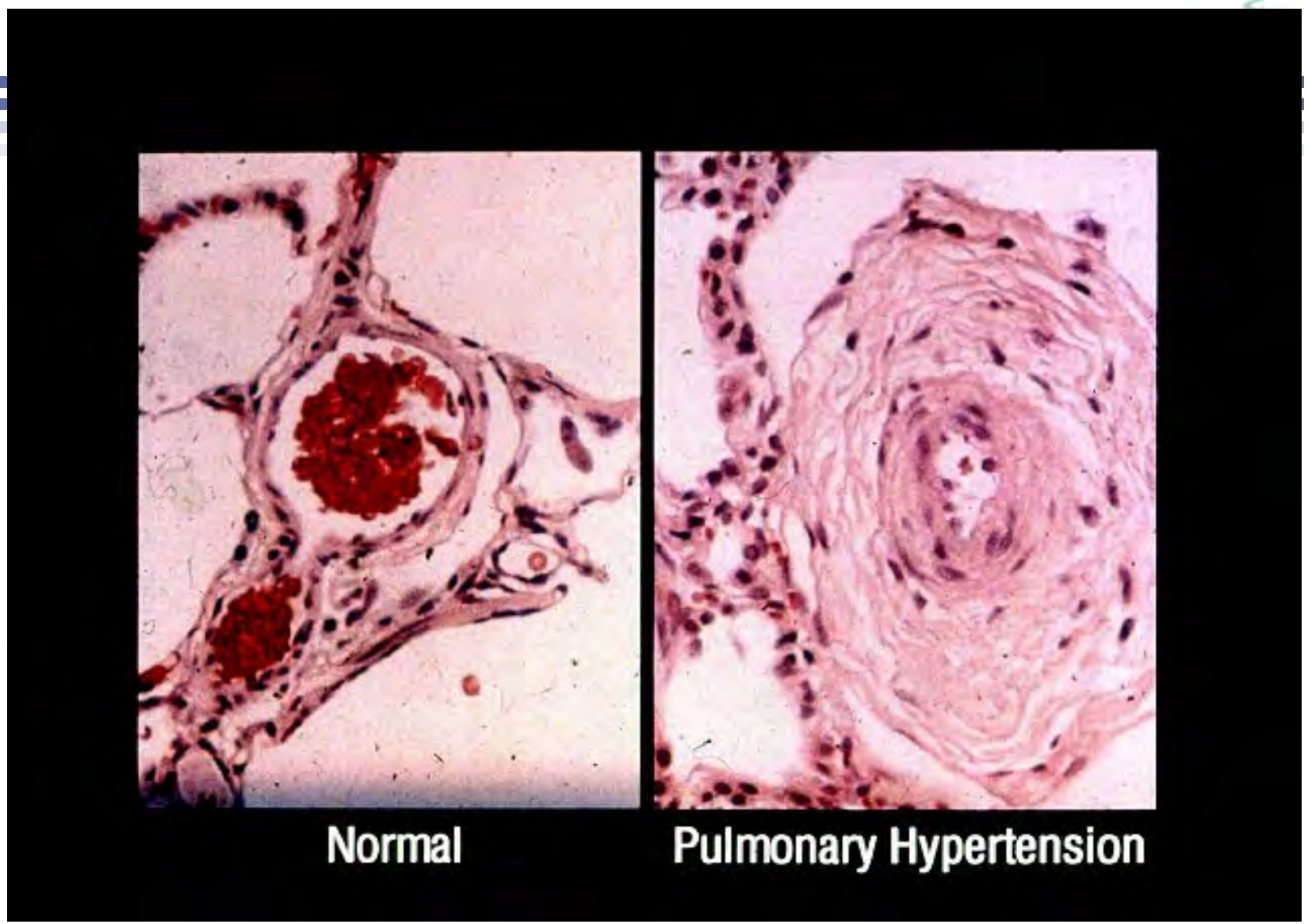
Annals of Surgery. 2015, 262(6):1130–1140,

# Insight in the black box of CDH

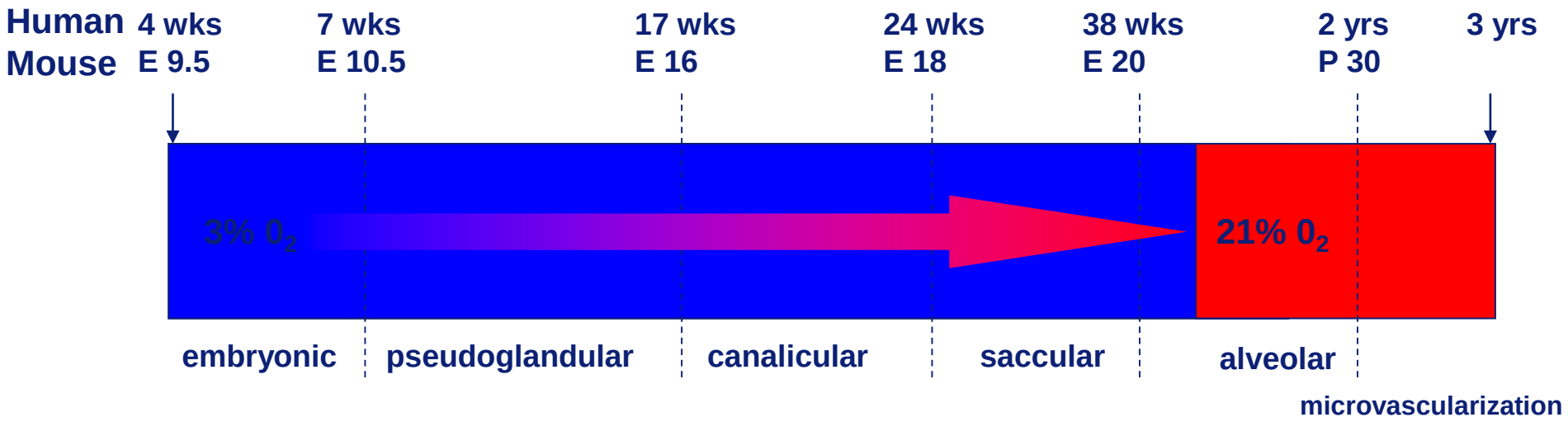



*I. Sluiter, thesis 'CDH: A vascular disease'*

# Characteristic morphological findings

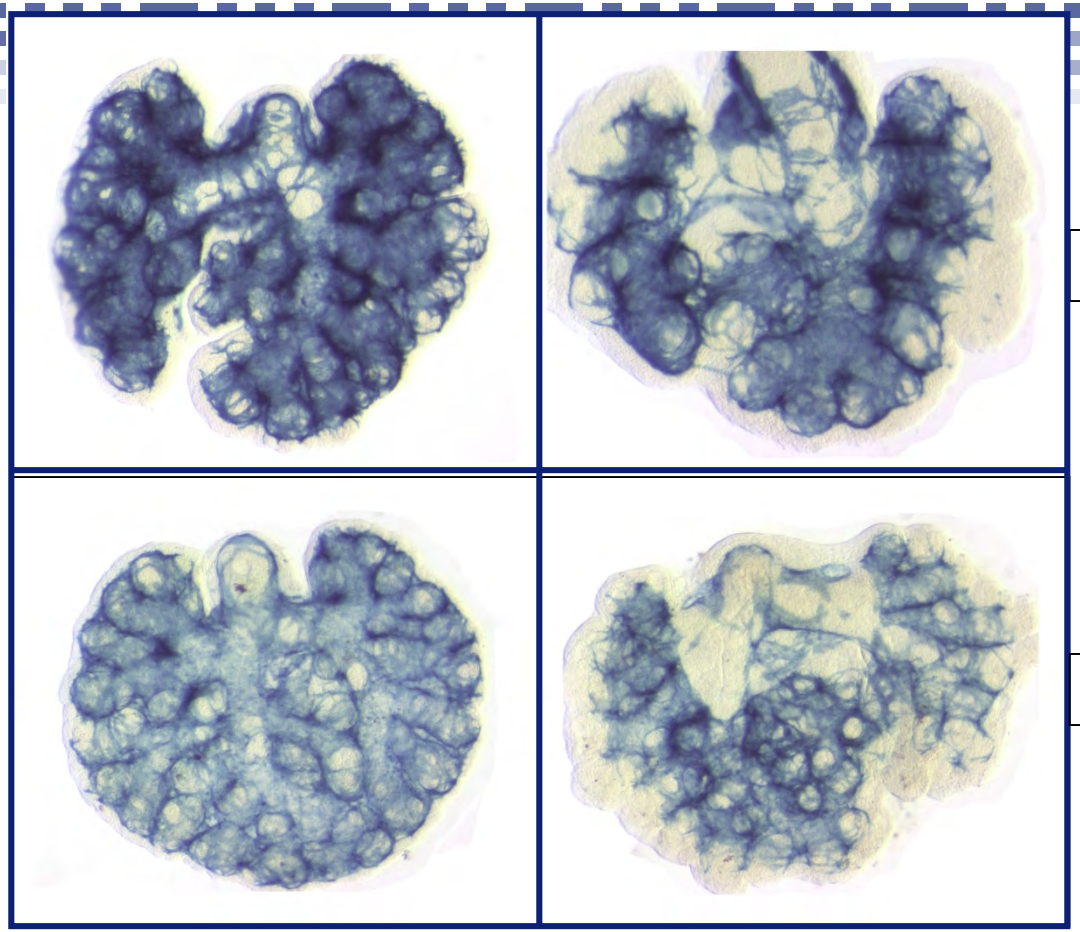


# Oxygen and lung development




## The master switch of life at birth?

# Oxygen and lung development




M van Tuyl et al, Am J Physiol Lung Cell Mol 2005.

Neonatology
formerly Biology of the Neonate

Neonatology 2010;97:346–354
DOI: 10.1159/000261018

Received: March 10, 2009
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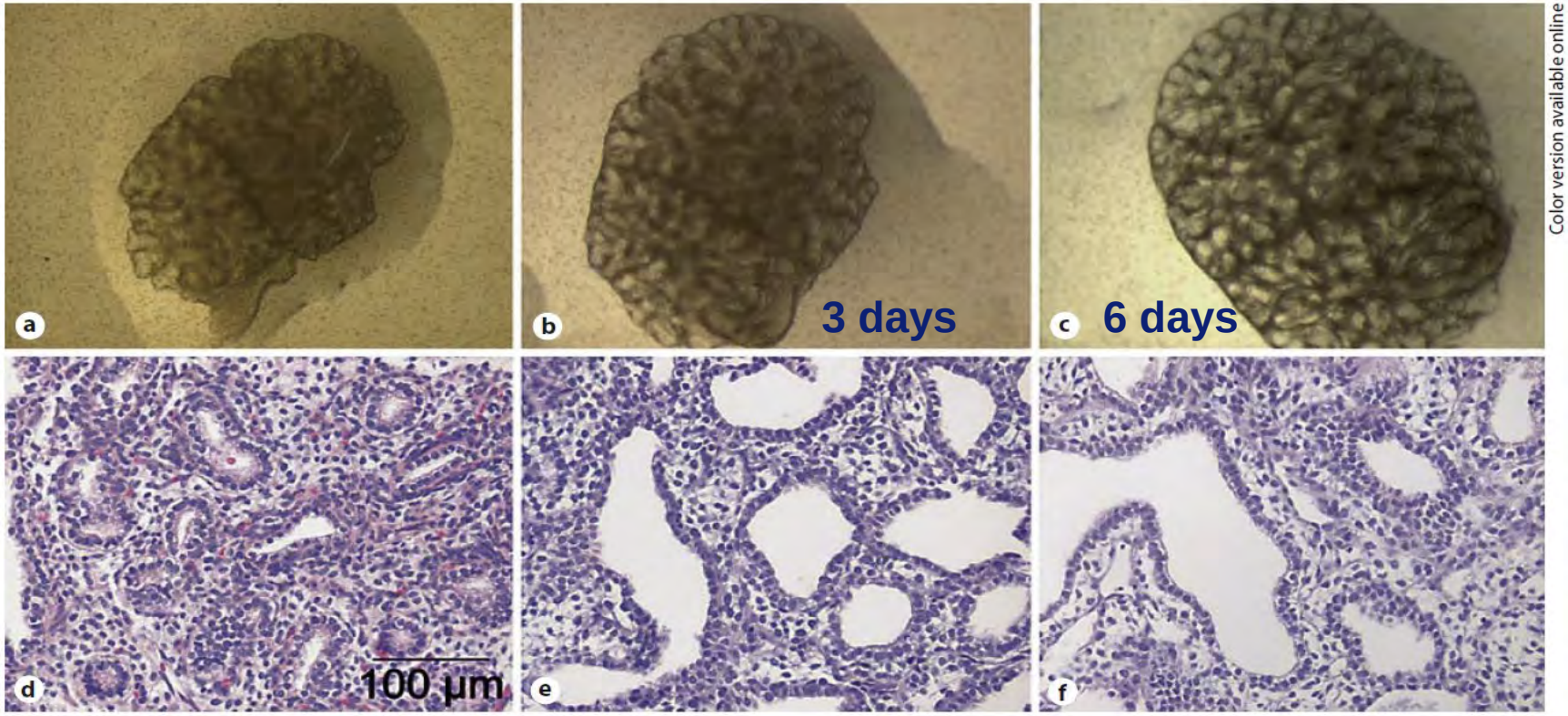
Published online: November 25, 2009

Erasmus MC

# Effect of Oxygen on the Expression of Hypoxia-Inducible Factors in Human Fetal Lung Explants

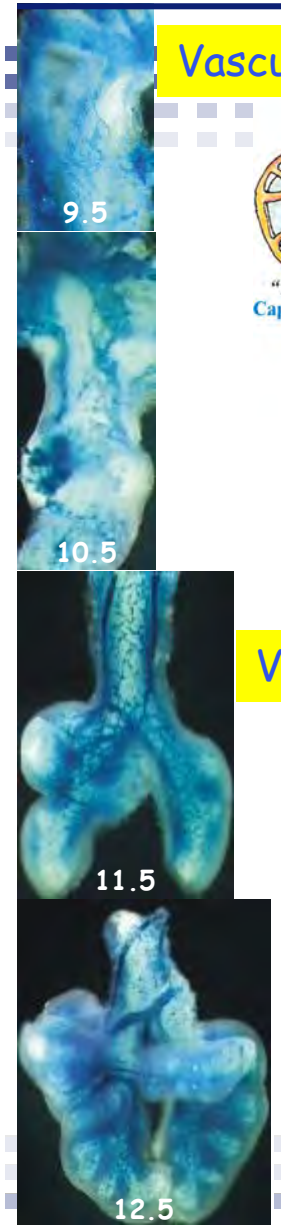
Prapapan Rajatapiti[a, d] Jessica D. de Rooij[a, b] Leonardus W.J.E. Beurskens[a, b]
Richard Keijzer[a] Dick Tibboel[a] Robbert J. Rottier[a, c] Ronald R. de Krijger[b]



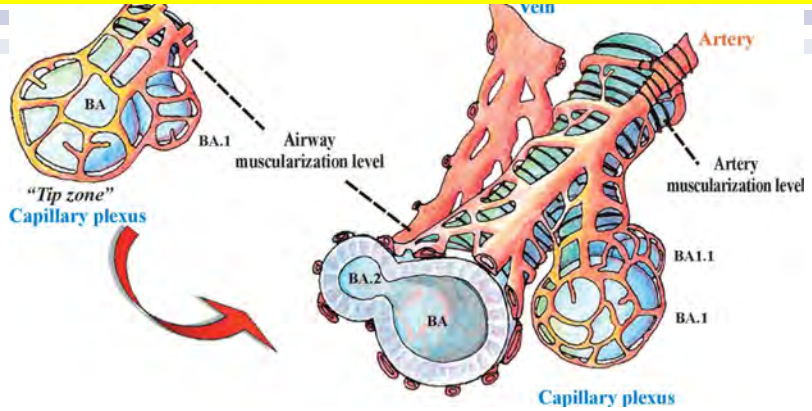

Color version available online

Representative images showing the morphology of human fetal lung explants (gestational age 16 weeks)

# Lung Vascular Development

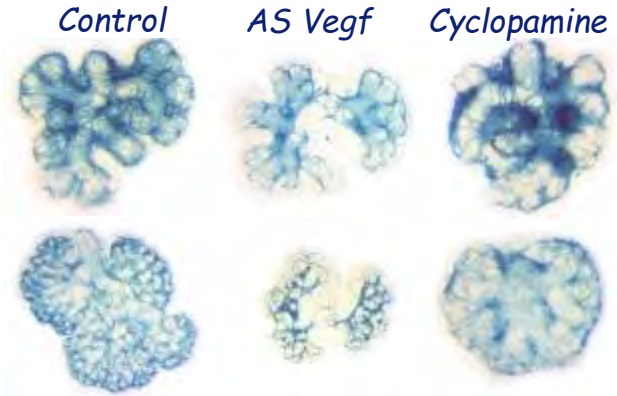



## Vascular expansion through distal angiogenesis



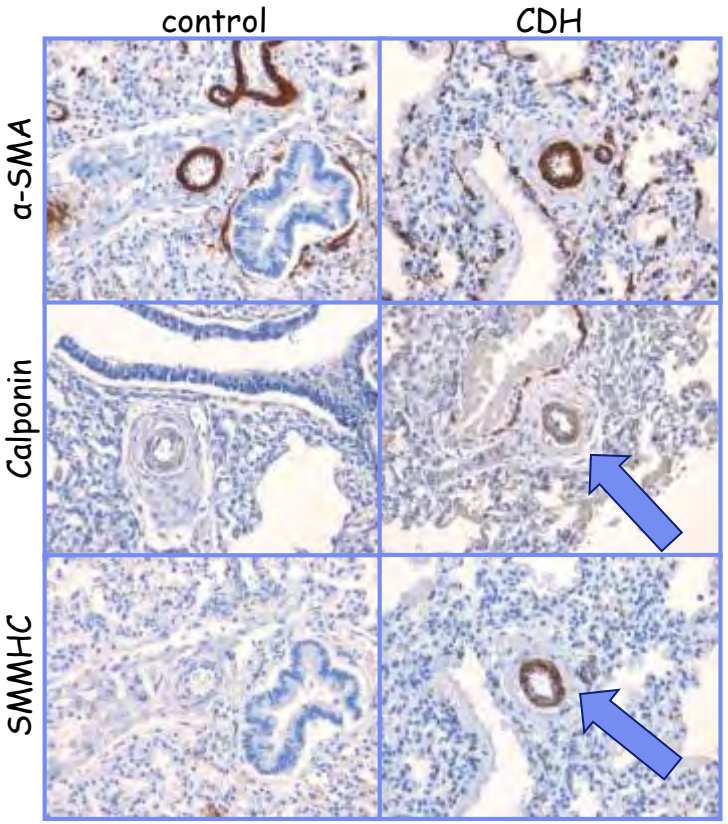

Parera *et al*, Am J Physiol L141-149, 2005

## Vascular growth guides epithelial branching



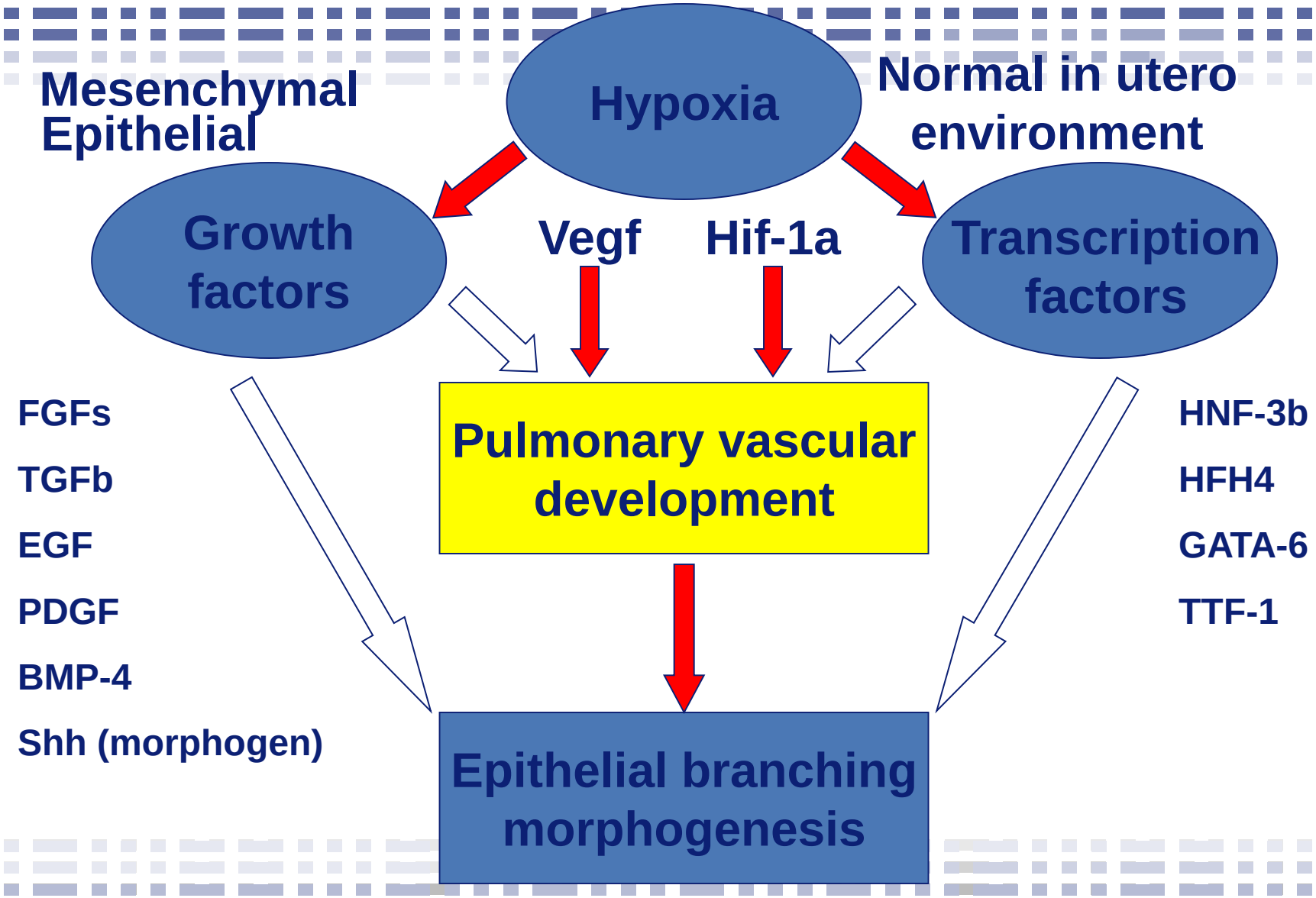

Van Tuyl et al, Am J Physiol 288, L167-L178, 2005

## VSMCs are different in CDH




Sluiter et al., Exp Mol Pathol 94, 195-202, 2012

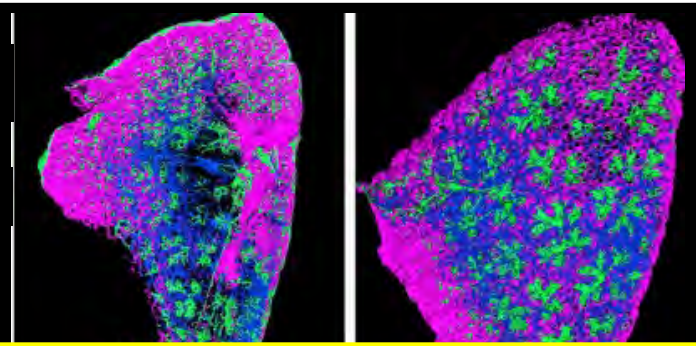
# Working model



# Whole mount analysis of pulmonary vasculature

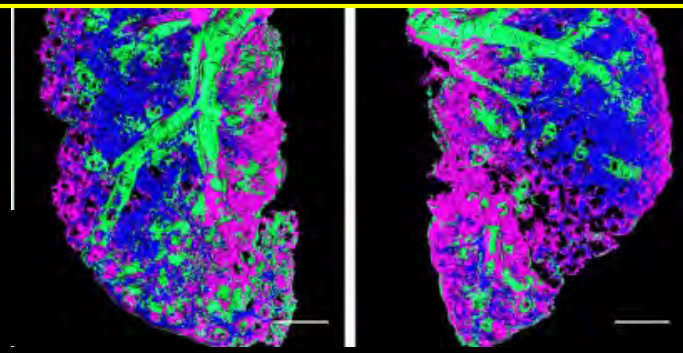
Lungs isolated at E15

Control



**Capillaries in lungs of CDH pups appear less developed**

CDH



NG2: pericytes
ACTA: smooth muscle cells
CD31: endothelial cells

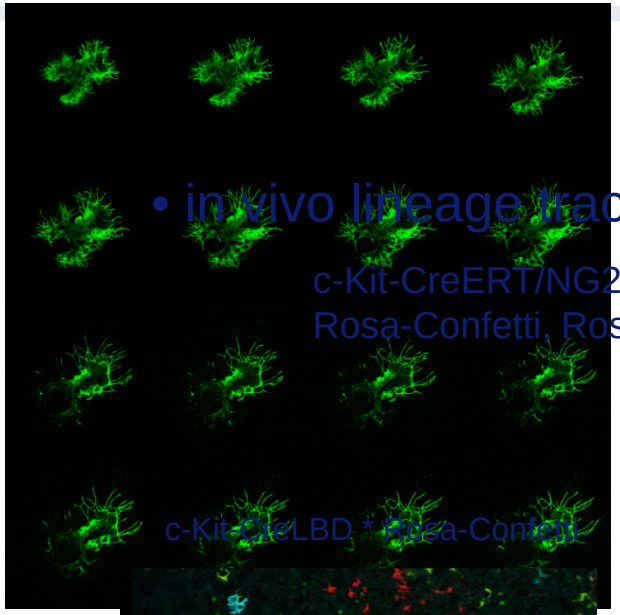
CD31: endothelial cells
NG2: pericytes
ACTA: smooth muscle cells

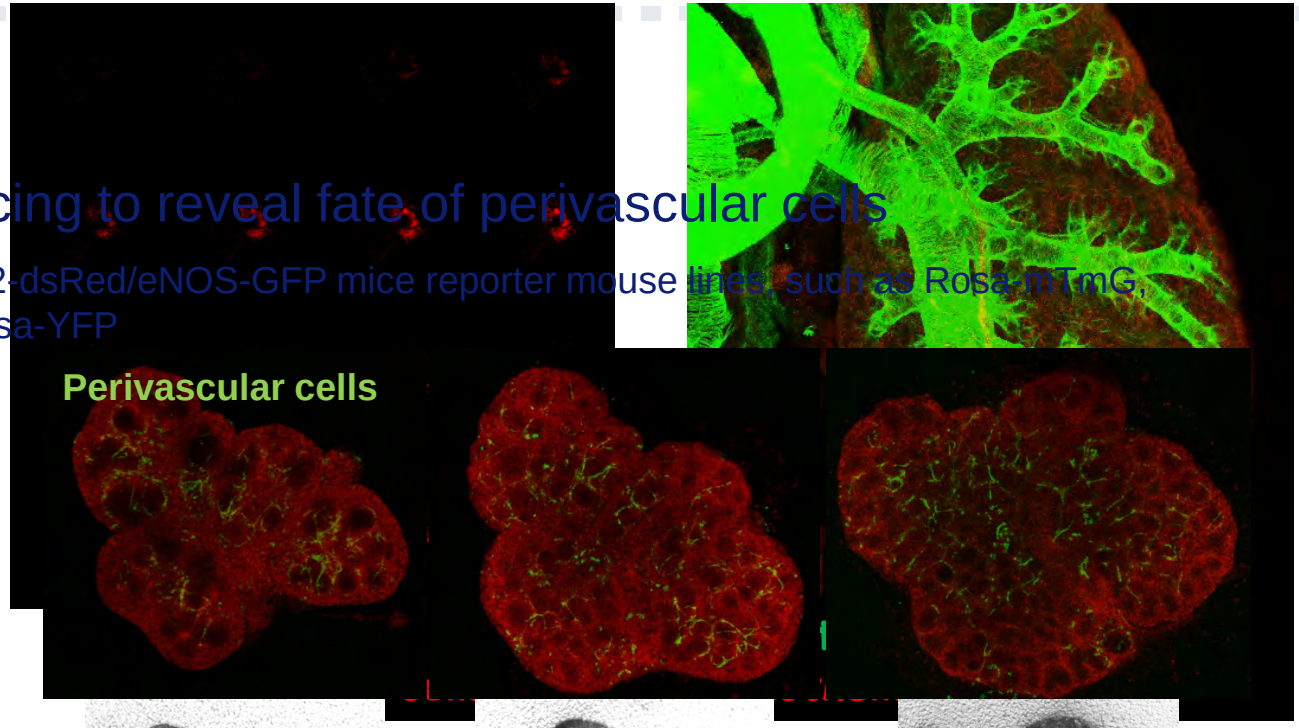
# Origin pulmonary perivascular cells?

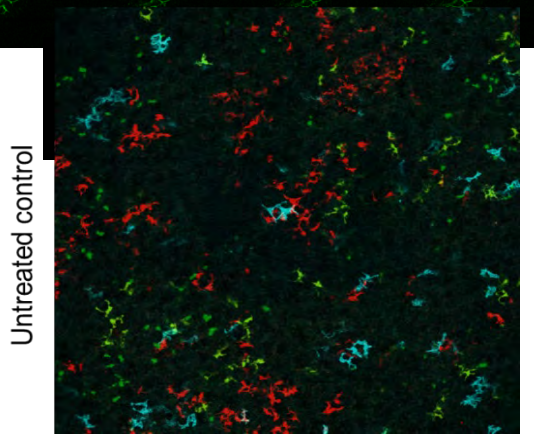
Lineage tracing of pericytes:

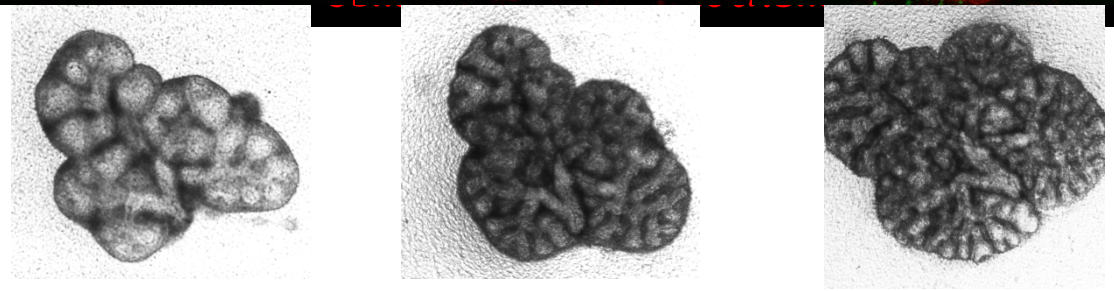
- ex vivo lung explants, whole mount immuno staining, …
- in vivo lineage tracing to reveal fate of perivascular cells

c-Kit-CreERT/NG2-dsRed/eNOS-GFP mice reporter mouse lines, such as Rosa-mTmG, Rosa-Confetti, Rosa-YFP



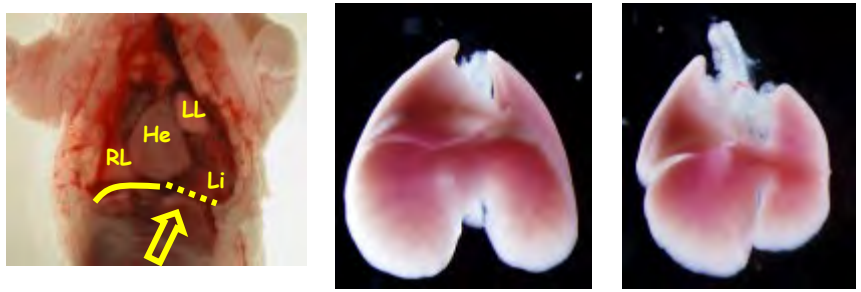



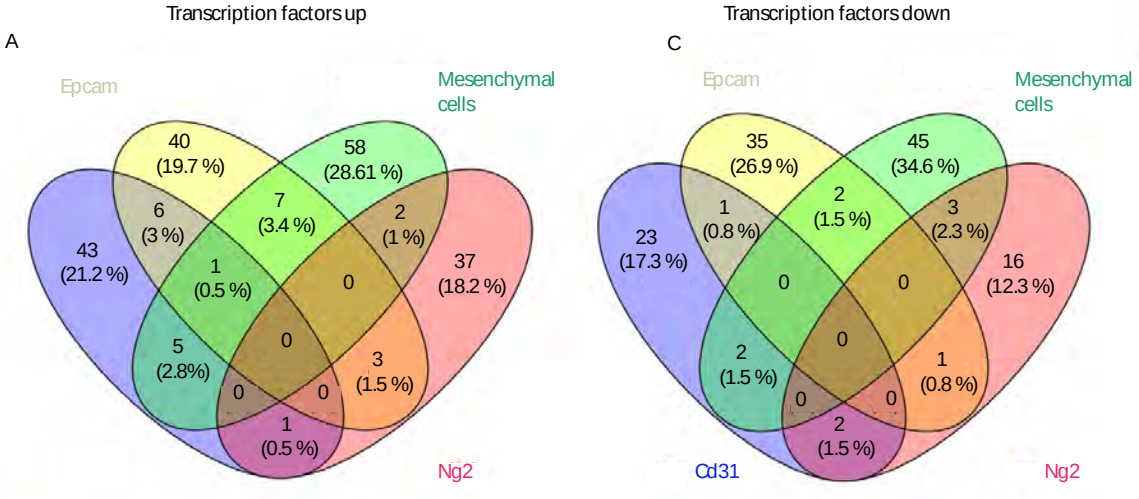
24 Hours 48 Hours 72 Hours

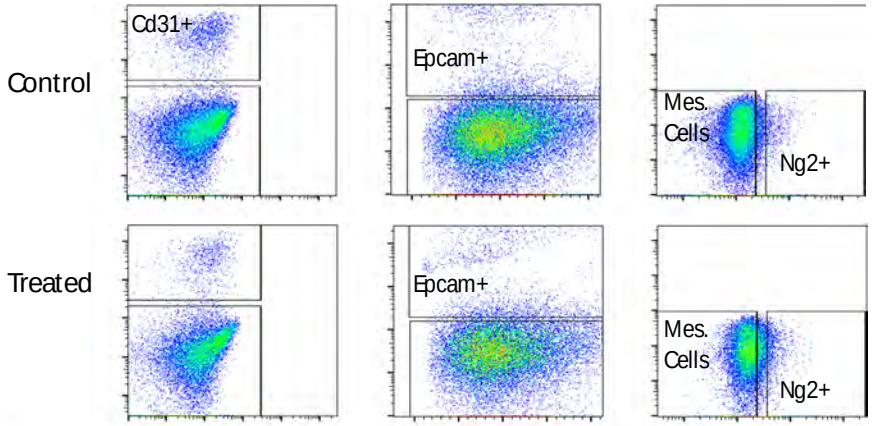
# Origin pulmonary perivascular cells

## Model of pulmonary vascular disease:



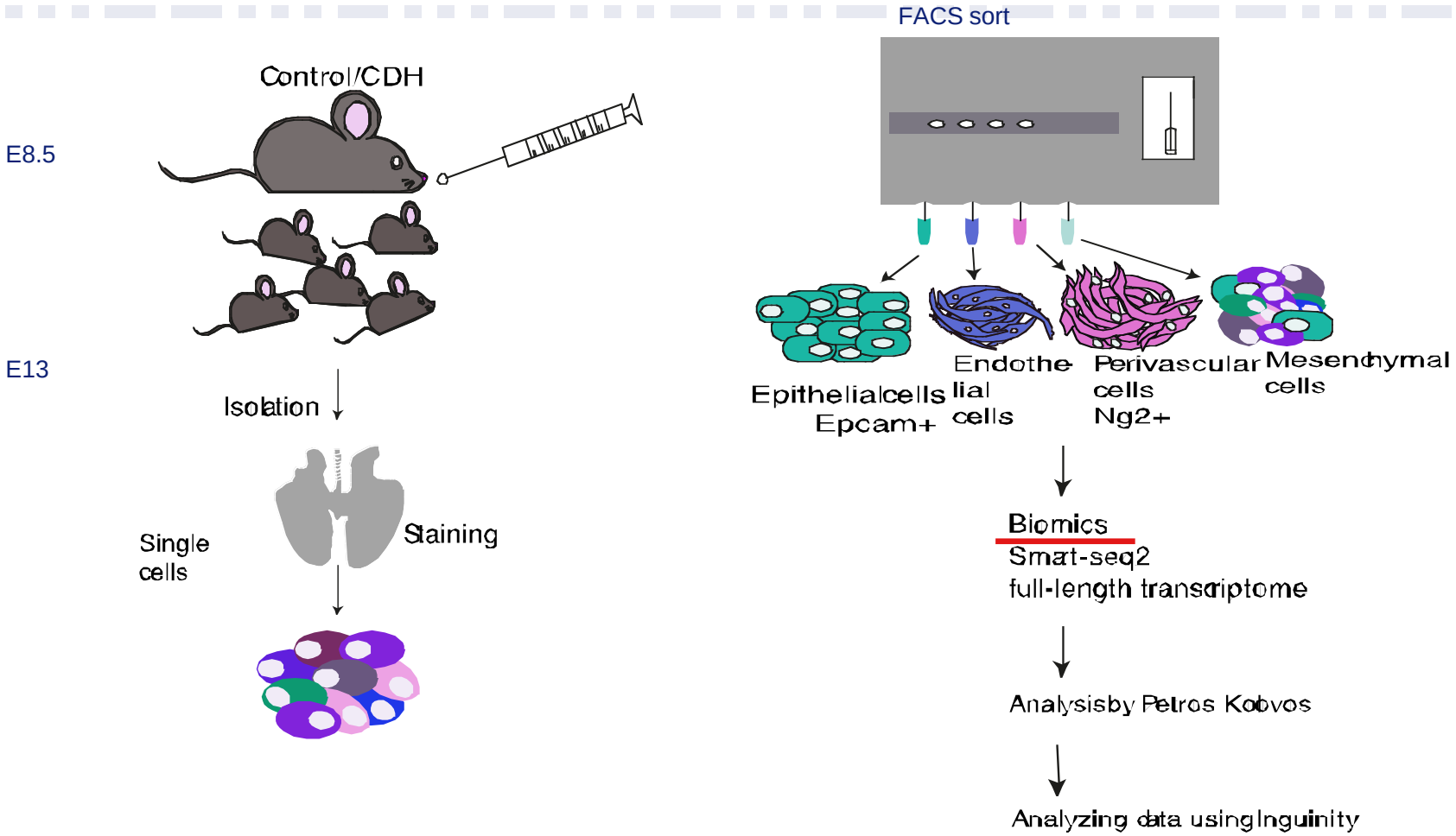

Mouse CDH model (SSWO project 678)



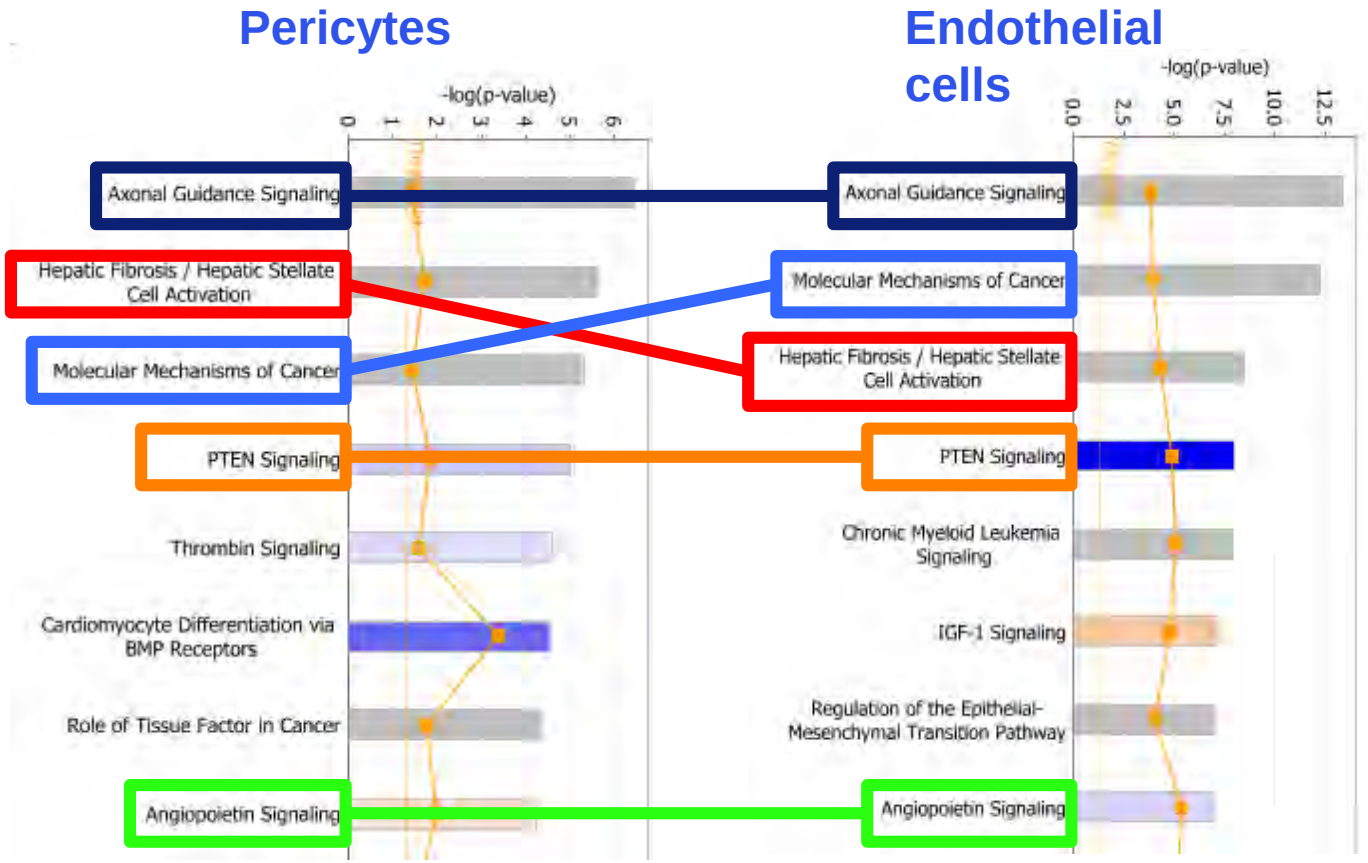




| | Cd31+ | Epcam+ | Ng2+ | Mes. Cells |
|---|---|---|---|---|
| Control | 4.38 | 4.49 | 3.02 | 89.86 |
| Treated | 2.88 | 4.85 | 5.82 | 78.17 |

# RNA sequencing of different cell populations



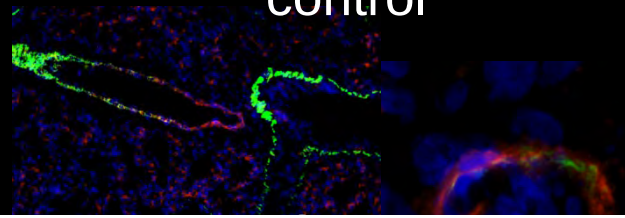
# Top pathways overlap pericytes – endothelial cells



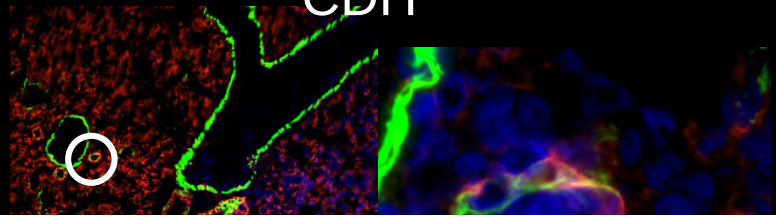
# (Neo)-muscularization and perivascular cells?

Perivascular cells express differentiation markers in CDH

control

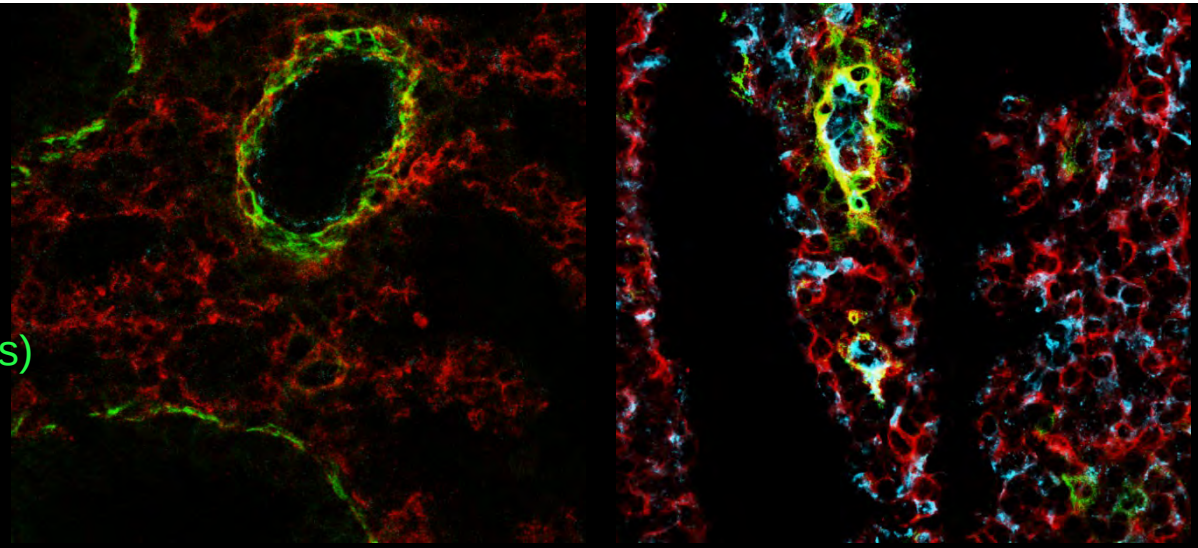


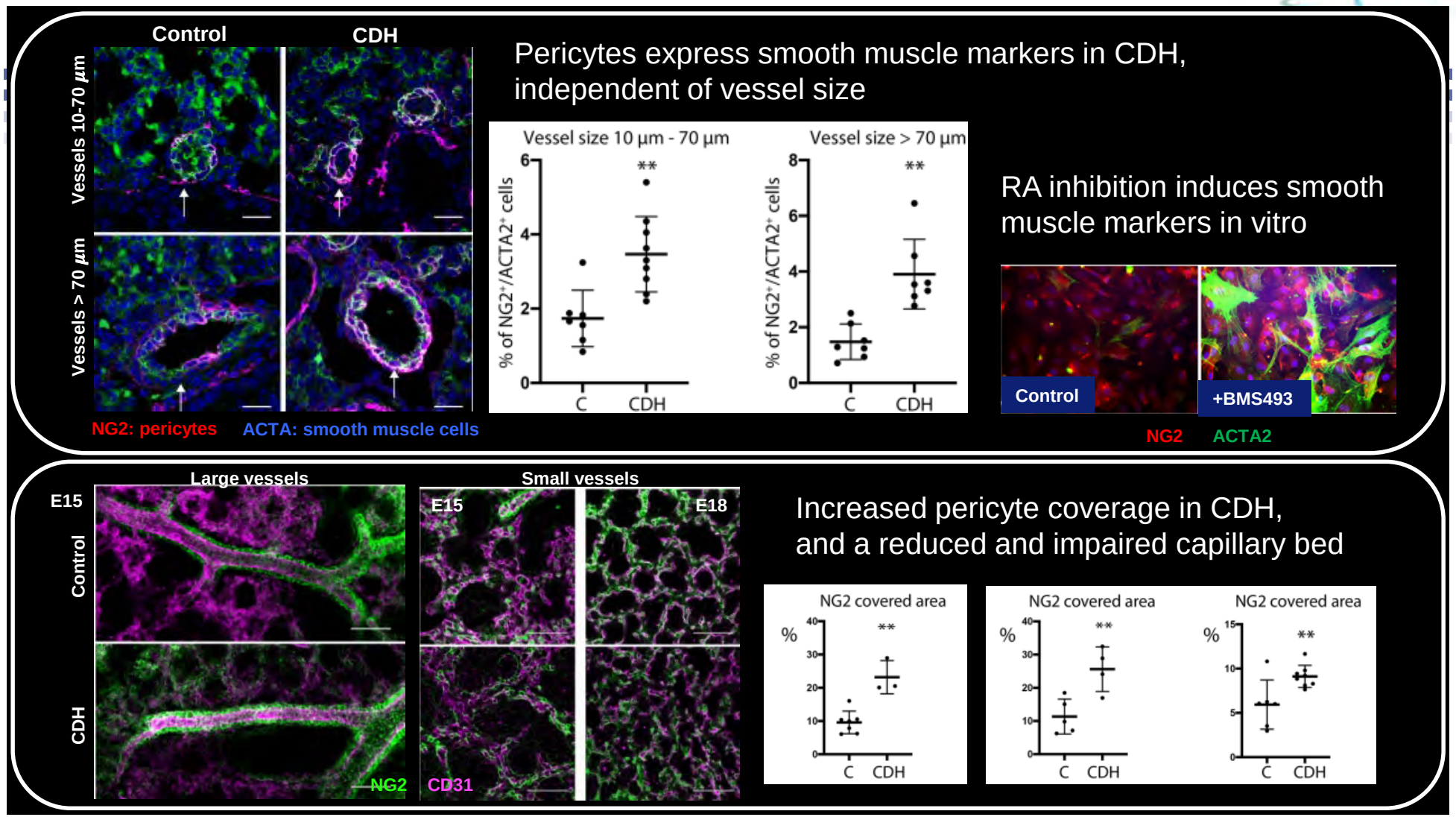
CDH



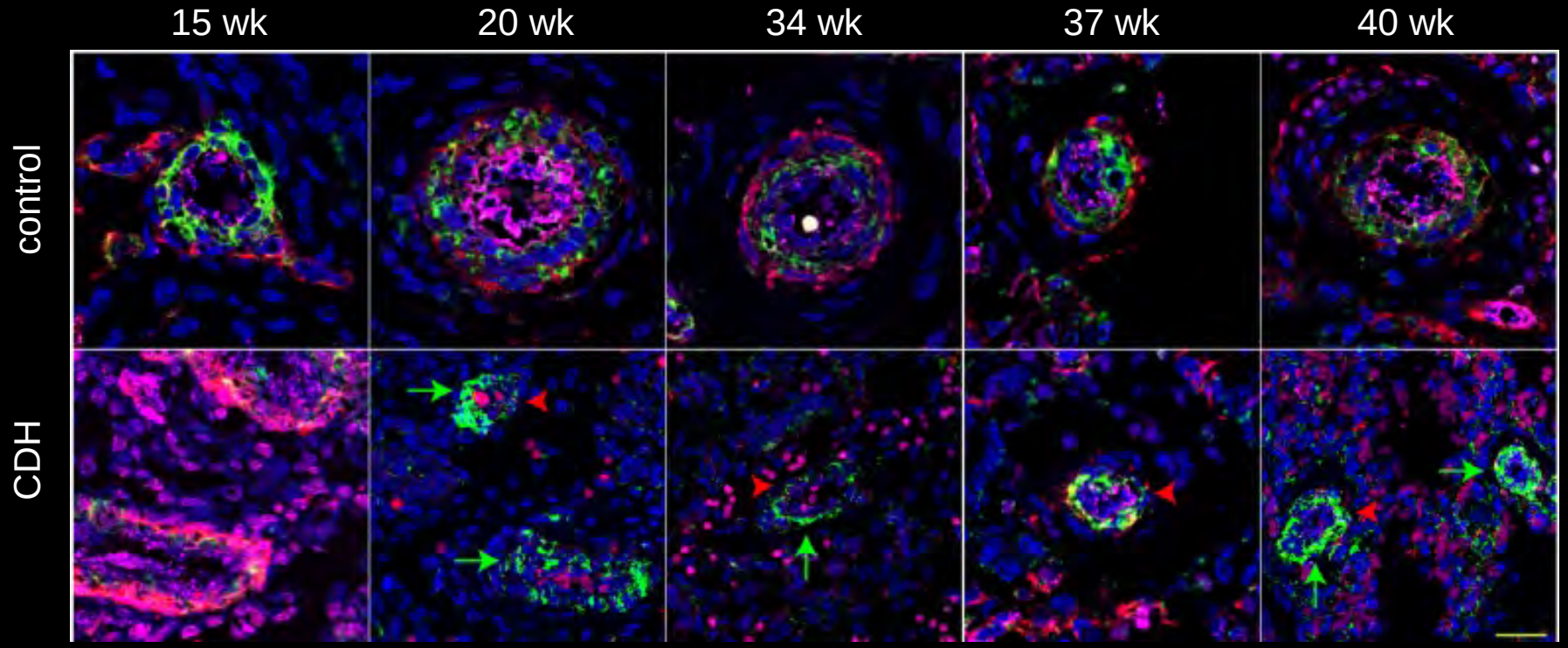
Premature or altered differentiation of pericytes in CDH

NG2
(perivascular cells)
SMA
(Smooth muscle cells)
CD31
(endothelial cells)



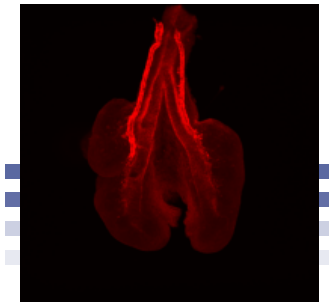

Control
CDH
Vessels 10-70 µm
Vessels > 70 µm
NG2: pericytes
ACTA: smooth muscle cells
Pericytes express smooth muscle markers in CDH, independent of vessel size
Vessel size 10 µm - 70 µm
Vessel size > 70 µm
% of NG2+/ACTA2+ cells
C
CDH
RA inhibition induces smooth muscle markers in vitro
Control
+BMS493
NG2
ACTA2
Large vessels
Small vessels
E15
E18
Control
CDH
NG2
CD31
Increased pericyte coverage in CDH, and a reduced and impaired capillary bed
NG2 covered area
%

# Vascular abnormalities in human CDH



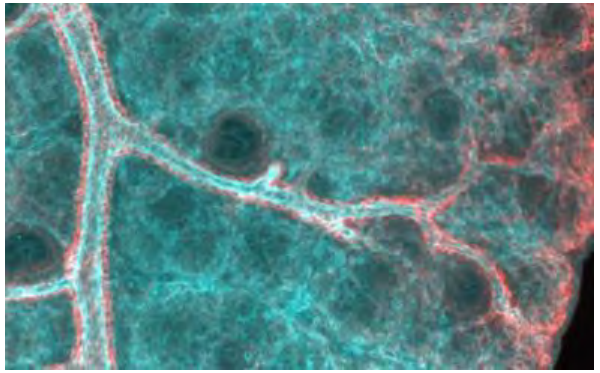

vWF
Collagen IV
NG2

Decreased collagen IV deposition (arrowheads)
Increased pericyte coverage (arrows)

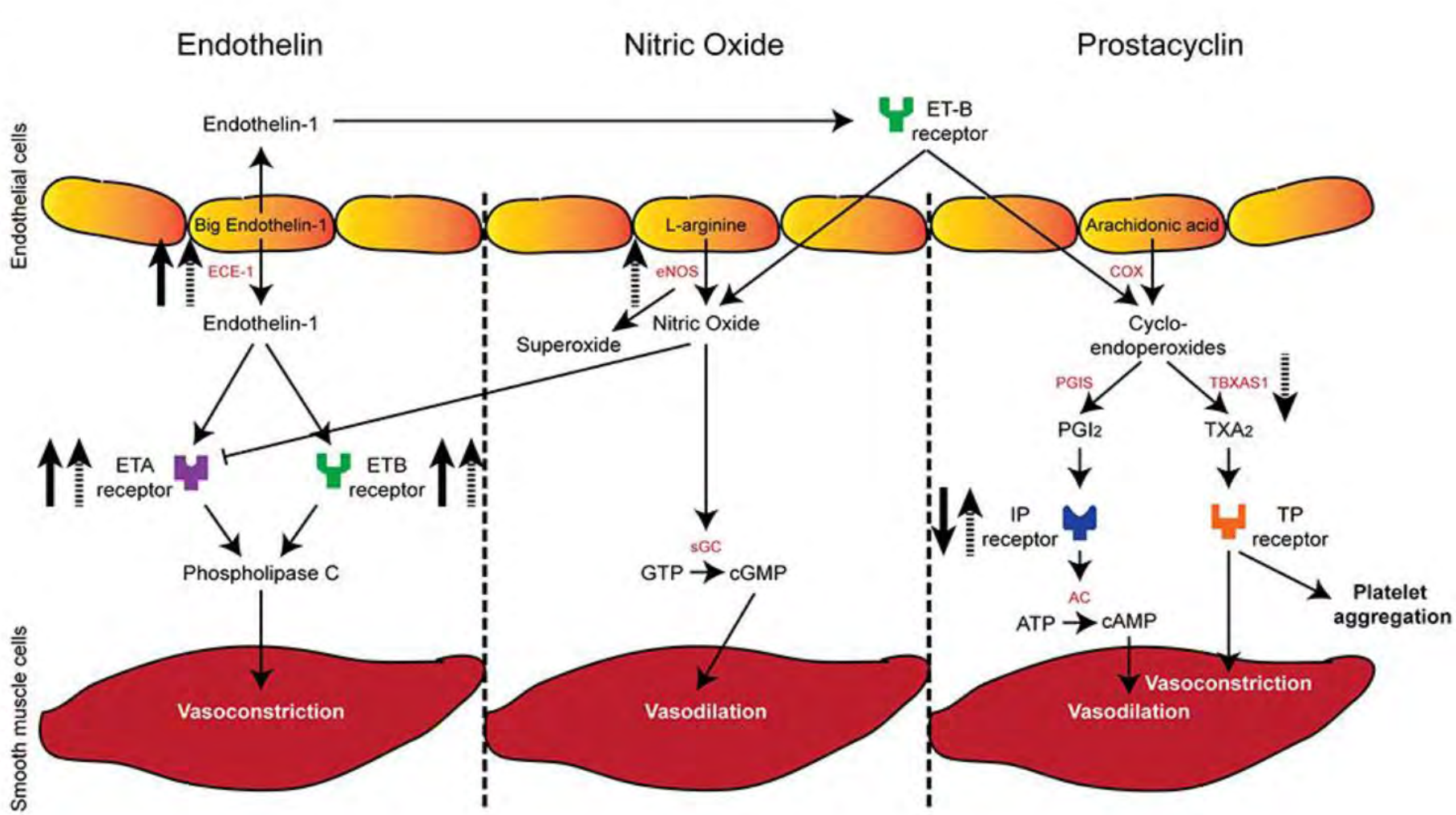


# Thus……

- Pulmonary **vascular development** seems **accelerated** in CDH
- **Pericytes** are different in CDH and **may be the source** of extensive muscularisation
- **Pericytes** may be the **origin** of pulmonary hypertension in CDH
- **Increased** pericyte **coverage** in CDH



# Metabolic pathways of vascular tone




Daphne S Mous; Marjon J Buscop-van Kempen MSc et al European Resp Review 2018

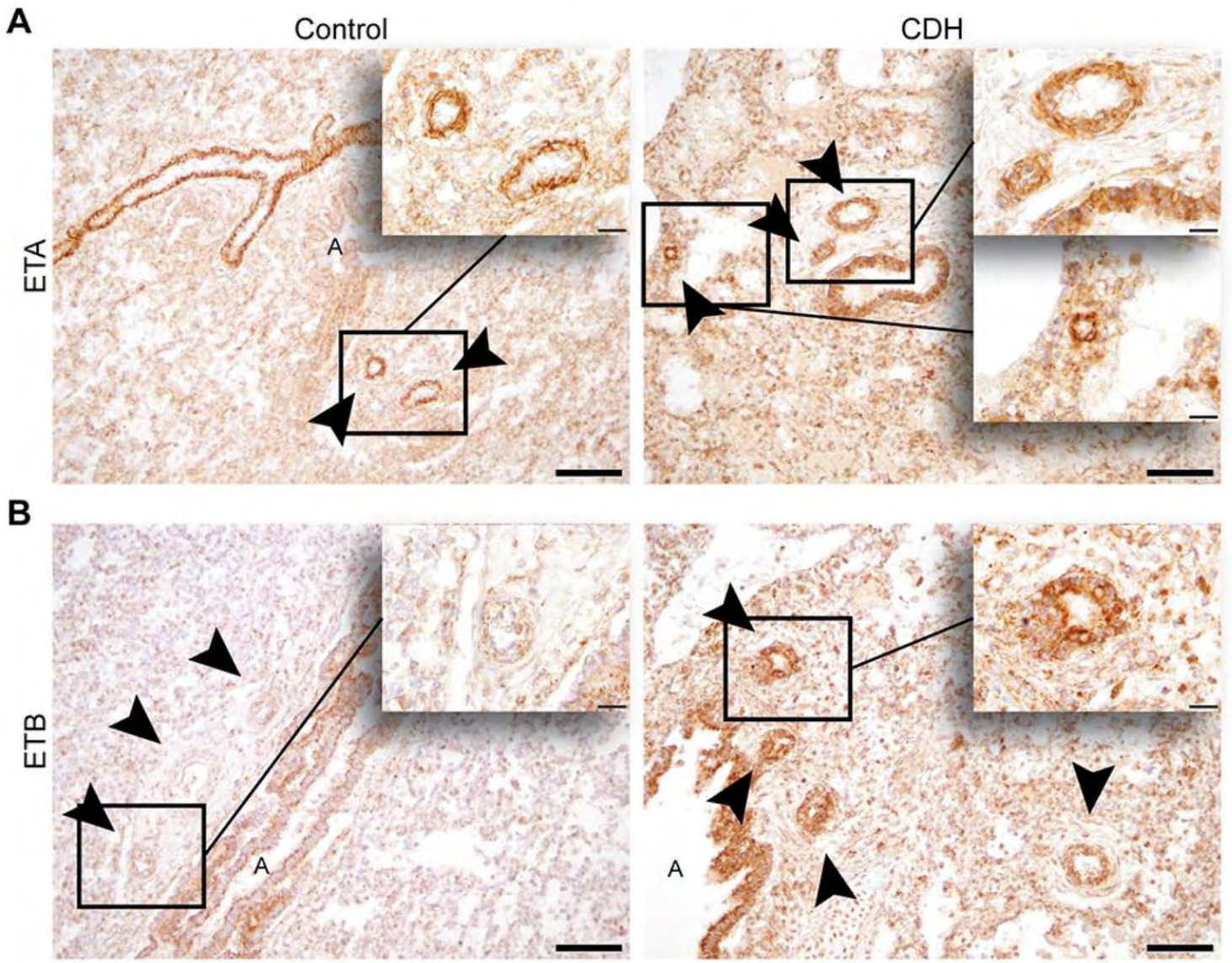



**Figure 3: Increased expression of both the ETA and ETB receptor and endothelin converting enzyme in human CDH.**

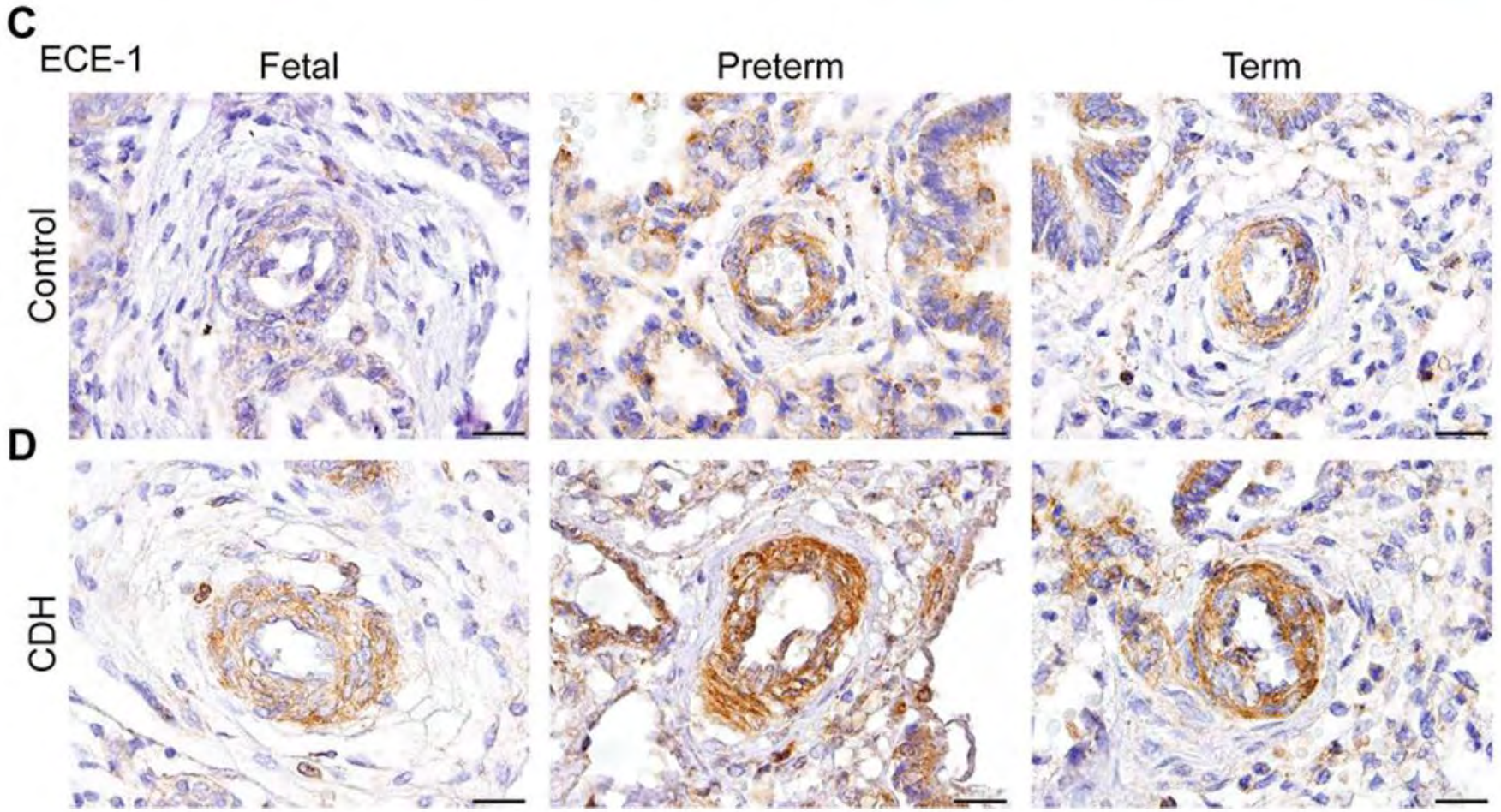



**Figure 3: Increased expression of both the ETA and ETB receptor and endothelin converting enzyme in human CDH.**

Daphne S Mous MD, Dick Tibboel MD PhD, Marjon J Buscop-van Kempen MSc, Rene MH Wijnen MD PhD, Robbert J Rottier PhD

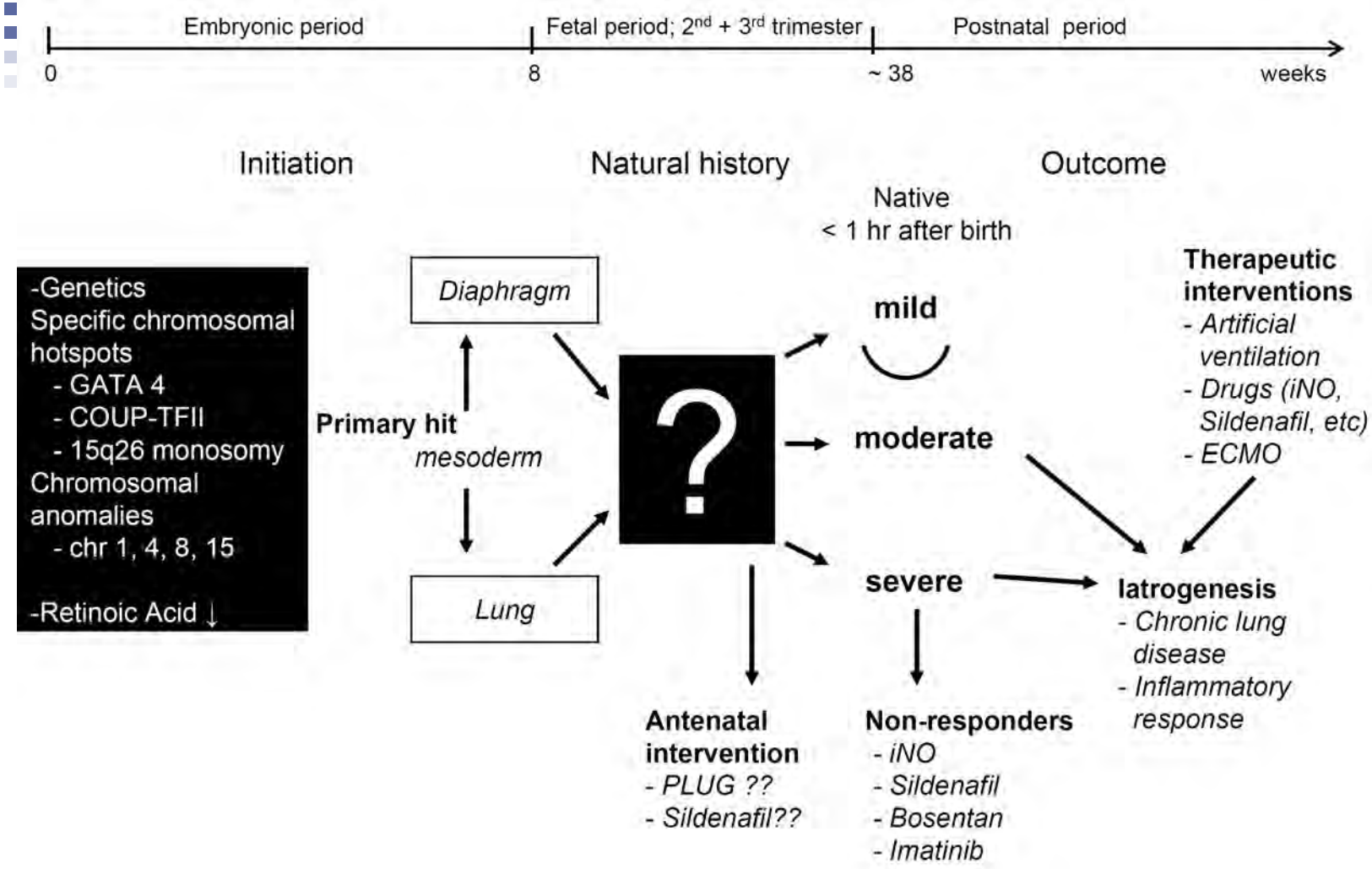
# The next RCT on behalf of the CDH EURO consortium and supported by CDH-UK

Newborns with **Co**ngenital **Di**aphragmatic hernia: inhaled **N**itric **O**xide versus intravenous **S**ildenafil,

an international randomized controlled trial

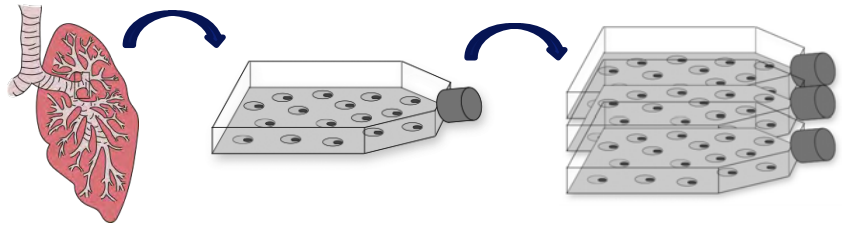
## CoDiNOS Trial

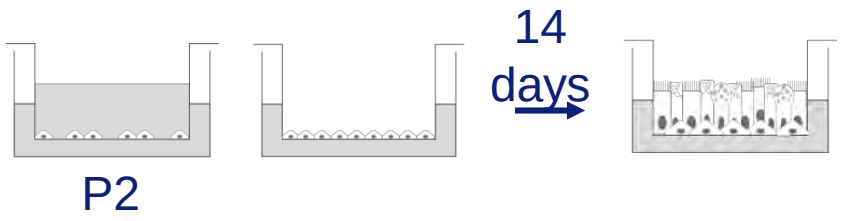
# Insight in the black box of CDH




*I. Sluiter, thesis 'CDH: A vascular disease'*

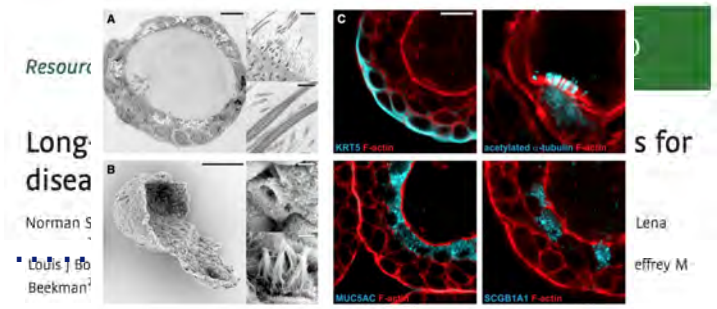
# Study human diseases using *in vitro* cultures

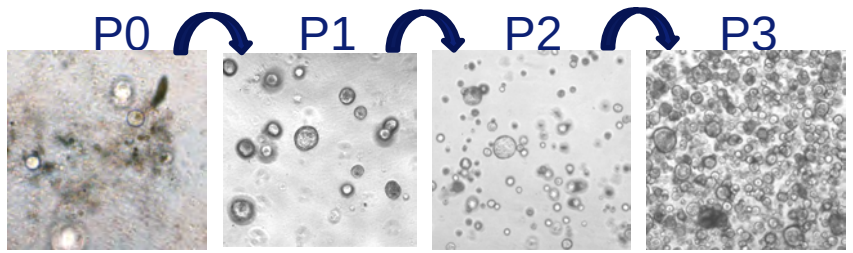





- Sufficient starting material
- Limited expansion (passage 2) +

\+ Air Exposure -



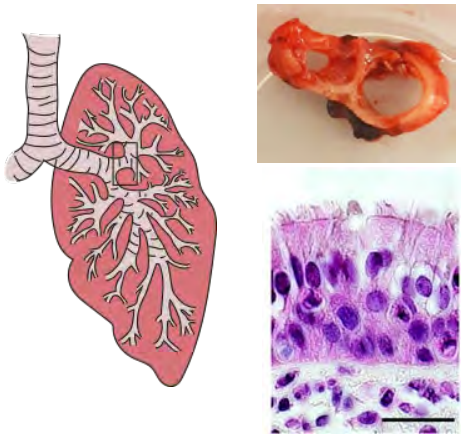



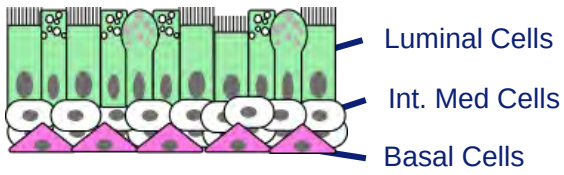

Need only 1 cell to form an organoid
(Un)limited Expansion (Till P19)

No Air Exposure

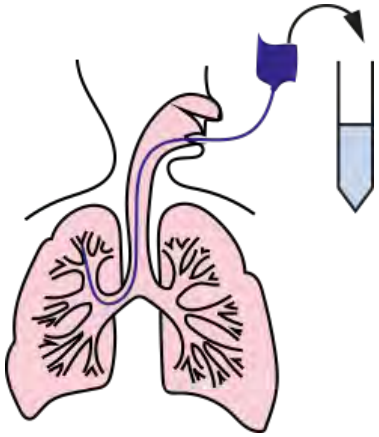
## We obtained material from 3 different sources

### Bronchial Tissue (BT)



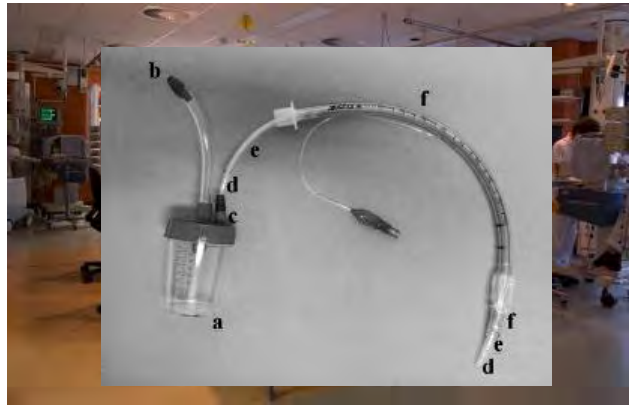



### Broncho Alveolar Lavage (BAL)



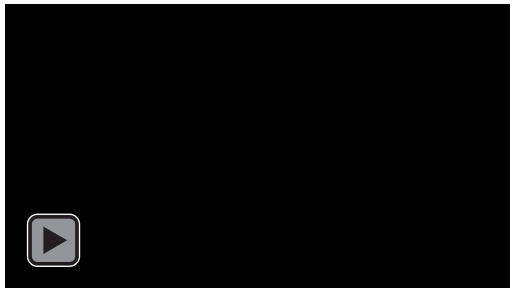
Department of Pulmonology
LUMC
Prof Hiemstra
Sander van Riet

### Tracheal Aspirate (TA)

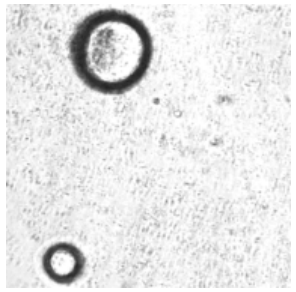



Department of Neonatology
Sophia Children's' hospital
Prof Reiss
Dr Kroon

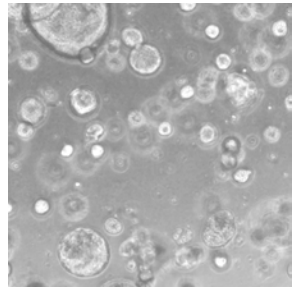
# Tracheal aspirates are a good source for AEC differentiation

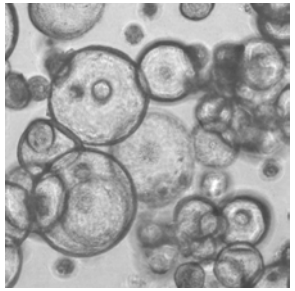


12 days →

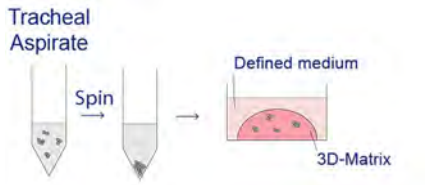


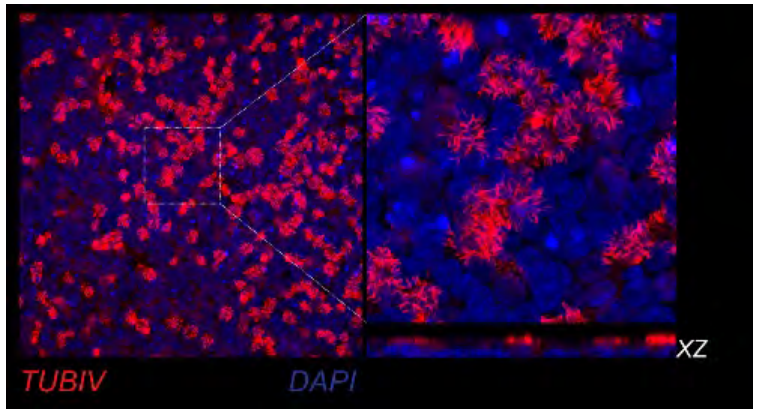
Expansion →





Disrupt ↓

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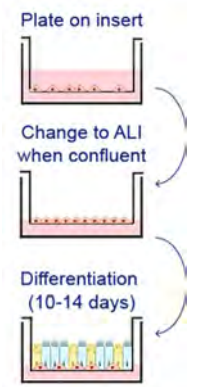
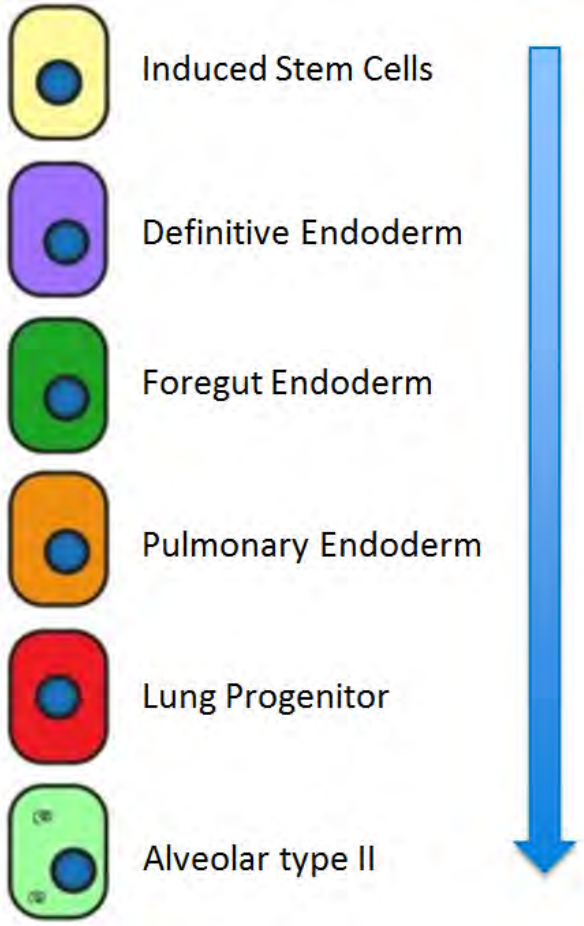
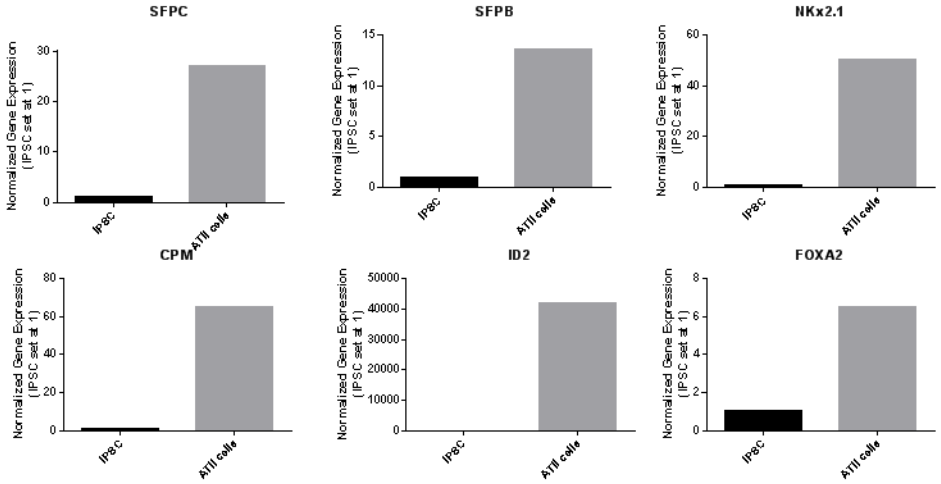


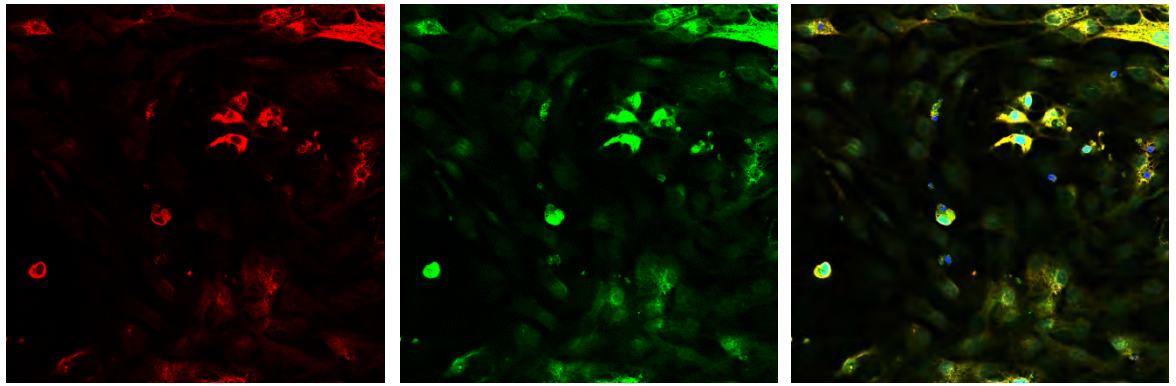
# Generation of alveolar epithelial type 2 (ATII) cells from human induced pluripotent stems cells (hiPSC)




Stages of differentiation




Gene expression of iPSC compared to generated ATII cells

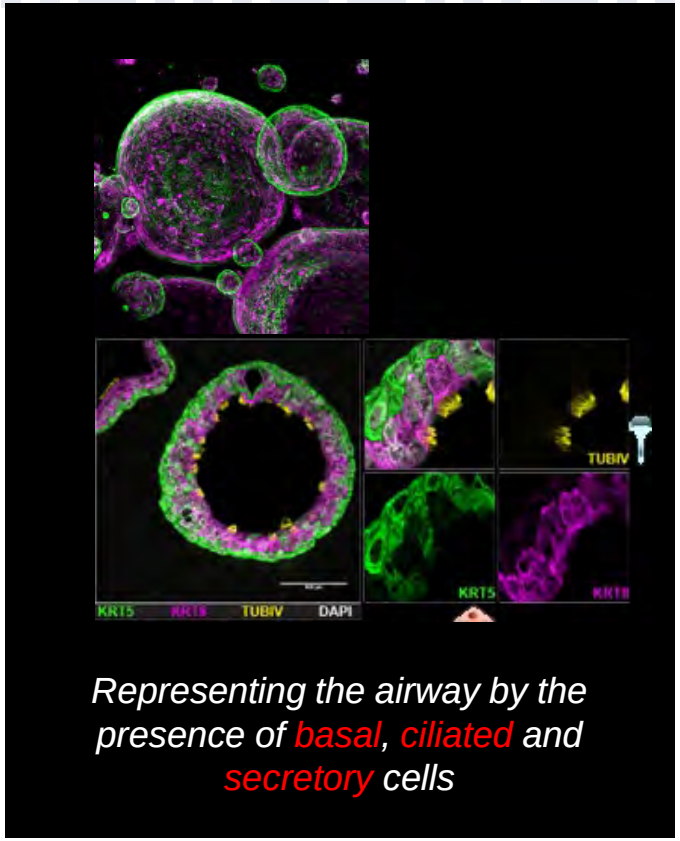


EpCAM SFTPC Merge

Staining of generated ATII cells

Sander van Riet, ms submitted

# Airway organoids, an *in vitro* system to study airway diseases?




*Representing the airway by the presence of basal, ciliated and secretory cells*

- **The amount of primary tissue availability can be limited**
  - An Organoid can be obtained from one single cell

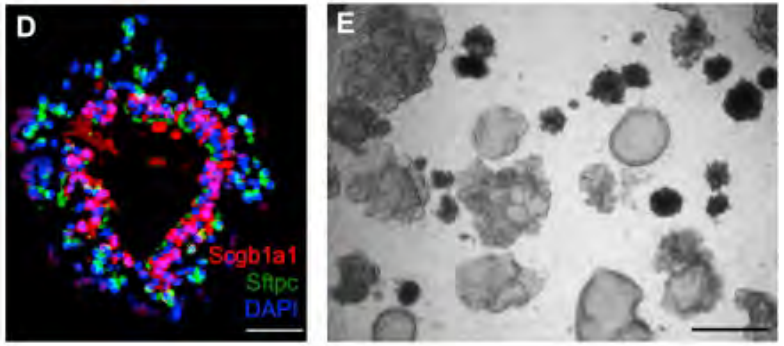
- **Can be obtained from**
  - Conducting airways
  - Tracheal aspirates
  - Nose swaps

**Model and study disease using a small amount of patient material** – Patient specific cultures

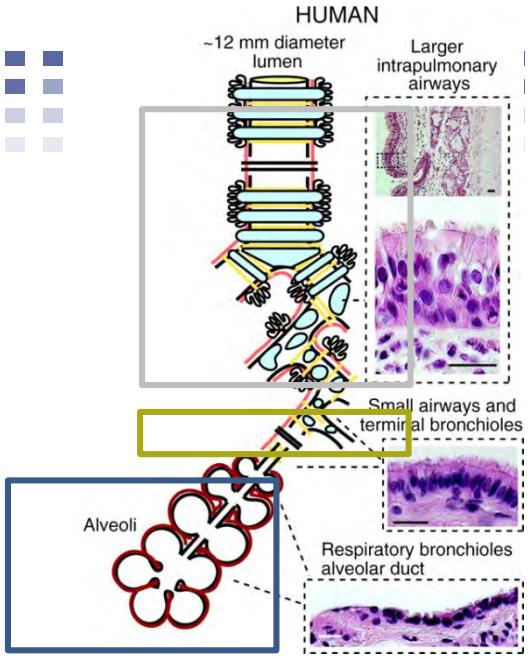
Congenital Pulmonary Airway Malformations

# 3 types of lung organoids: Bronchiolar, Bronchoalveolar, Alveolar

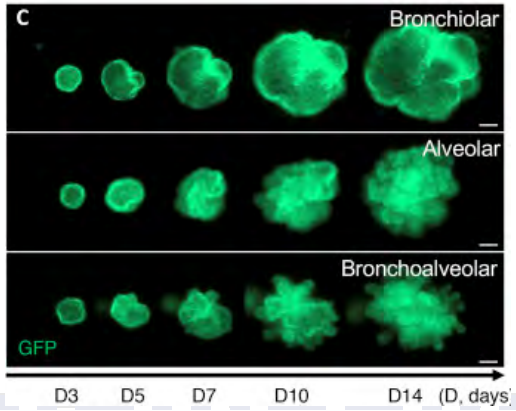
- **Alveolar organoids**
  - Formed from Alveolar type II cells
  - Show presence of both Alveolar type I and type II cells
- **Bronchoalveolar organoids**
  - Formed from Bronchoalveolar stem cells
  - Show presence of Alveolar and Airway cells
- **B**




Scgb1a1: Secretory cells
Sftpc: Alveolar type II cells




Rock et al., 2010 Dis Mod & Mech




Choi et al., 2016 Dev Biol

# Chip requirements:

Mimic the organ of interest

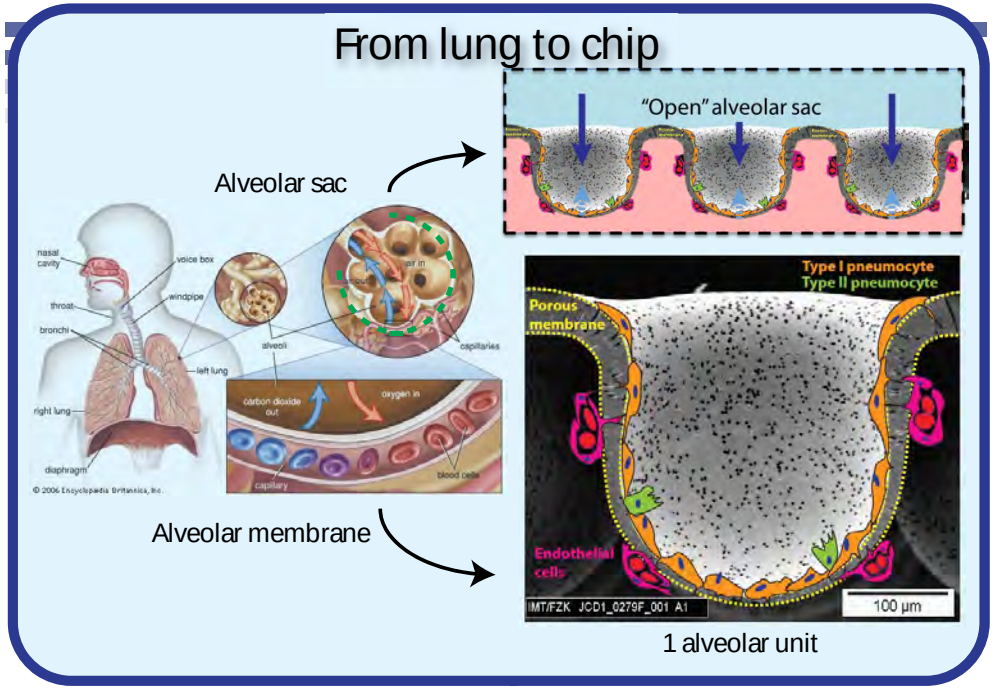
Recapitulate the organ's physiology

Attainable read-out system(s)

For the lung:

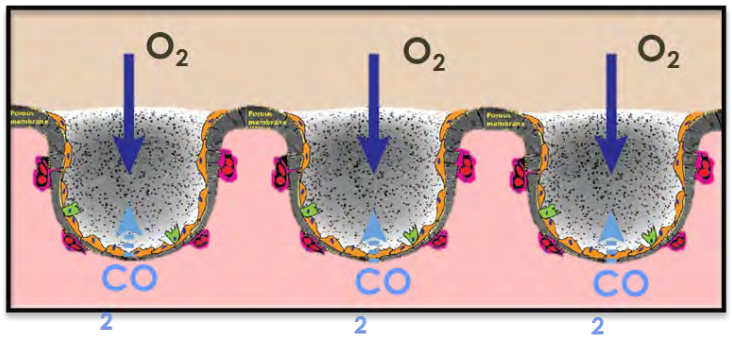
- Stretchable (breathing)
- Air - and blood compartment
- Read out of both compartments (microscopy, $O_2$ sensor, TEER measurement, etc)
- Collect air and "blood" from the chip for analysis
- Recovery of cells post-chip for analysis

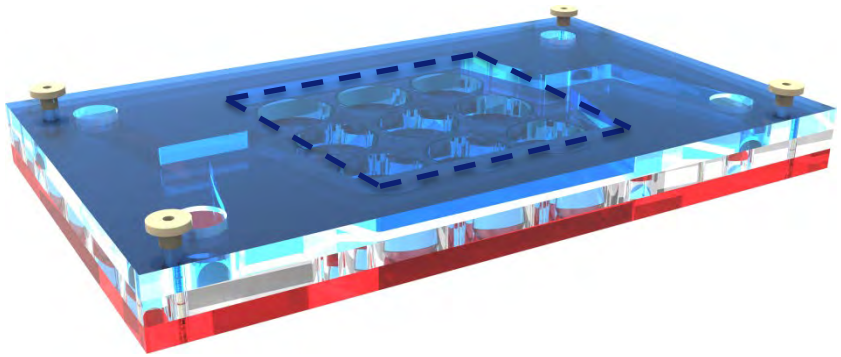
# Epithelial differentiation - application

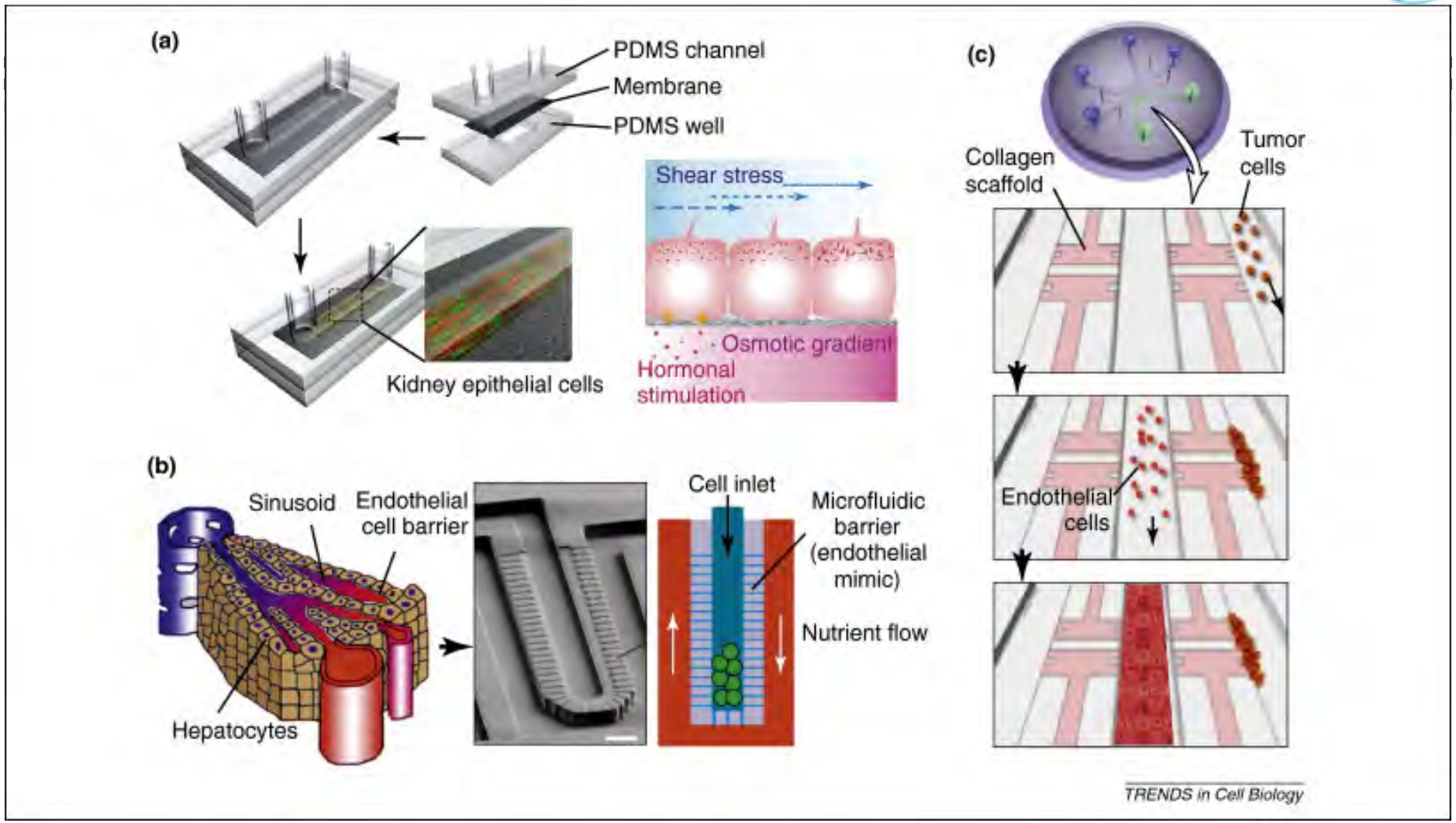



## Dynamic 3D in vitro lung model :

- Mimics micro-anatomy of lung
- Recapitulates alveolar physiology



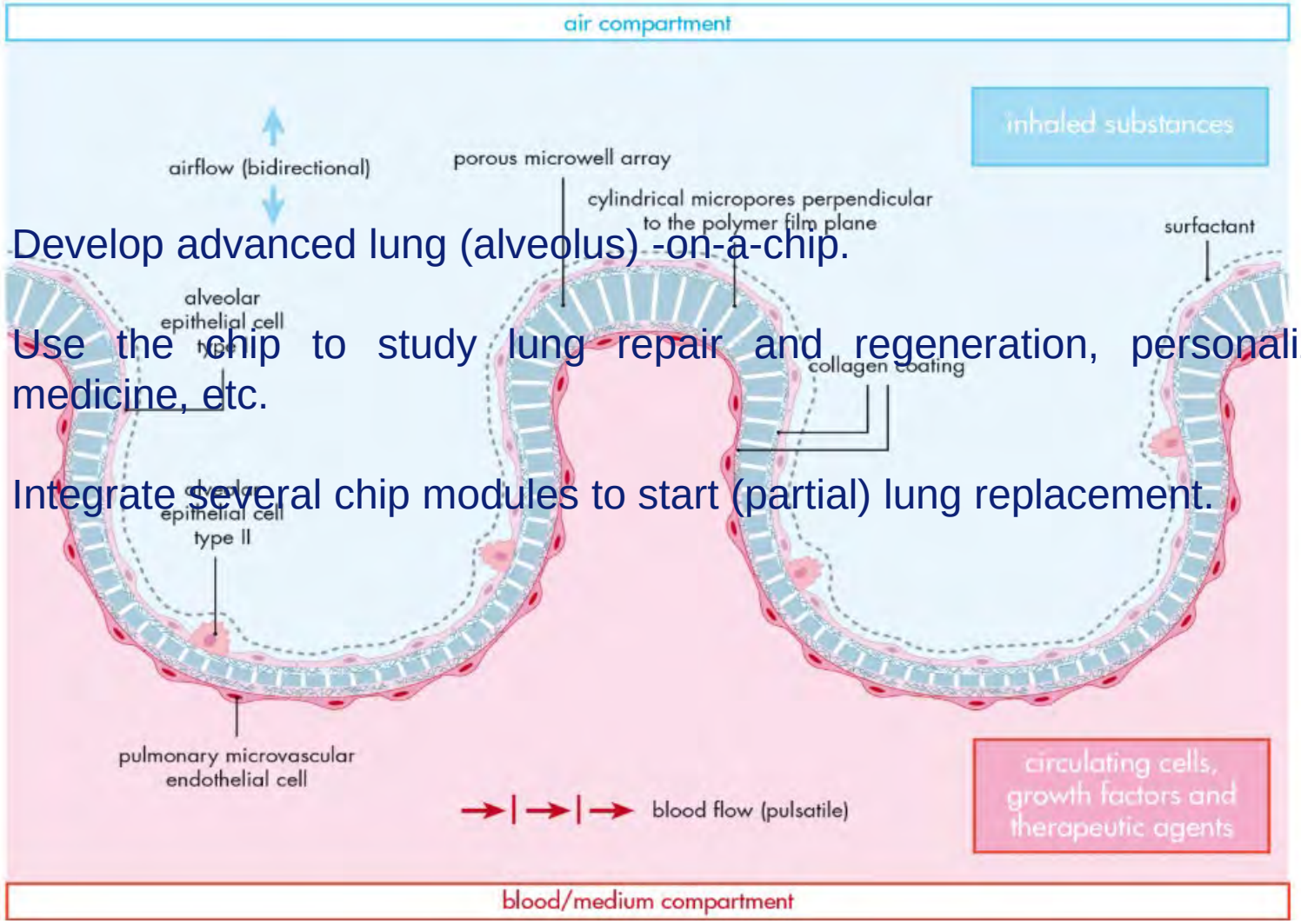






Huh et al, 2011

# Lung-on-a-chip consortium

1. Develop advanced lung (alveolus) -on-a-chip.
2. Use the chip to study lung repair and regeneration, personalized medicine, etc.
3. Integrate several chip modules to start (partial) lung replacement.




**microengineered 3D analogues of alveolar tissue for lung regeneration**

# What basic/ translational breakthroughs are going to change the way we understand CDH and care for these patients in the future?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# CDH in 2025: prenatal

High-risk CDH patient: new risk assessment score integrating both pre- and postnatal characteristics.

Molecular genetic analysis incorporating next generation sequencing and adding this info to the international genetic biobank of CDH.

iPS cells will be generated for further identification of factors known to predict chronic lung disease using organoids and organ on chip techniques.

Single cell sequencing after differentiation will be performed to elucidate developmental abnormalities.

New experimental studies, like the use of microRNAs or stem cell therapy +/- PLUG.

# CDH in 2025: perinatal

At birth, umbilical cord cells will be harvested for endothelial cells responses on vasoactive drugs using vessel-on-a-chip perfusion models.

To investigate potential biomarkers to identify at an early stage patients at risk for developing chronic lung disease as well as adverse comorbidities
.

The best drug therapy for pulmonary hypertension based on in-vitro responses to a variety of drugs

Drug dosing will be tailored based on phenotypic knowledge of drug metabolism as well as known influence of the disease state.

# CDH in 2025: first admission and beyond

All data will be integrated using the infrastructure of the CDH-EURO Consortium; the CDH-registry and ERNICA  taking the FAIR principle into account

Markers of chronic lung disease extracted from tracheal aspirates during artificial ventilation will determine the ventilator settings and will be repeatedly re-evaluated.

Dense monitoring data are collected, notably with respect to brain-related parameters.

An MRI of the brain will be made with special attention for the hippocampus to predict dysfunction in executive functions enabling implementation in an intervention study.

The child and family are invited to join a tailor-made lifelong interdisciplinary follow-up program aiming to decrease long-term morbidity

# CDH EURO CONSORTIUM 2017

Austria
Belgium
Canada
France
Germany
Ireland
Italy
Norway
Poland
Portugal
Scotland
Spain
Sweden
United Kingdom
The Netherlands

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Sophia kinderziekenhuis fonds

Erasmus MC Daf


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Rory Morty (Bad Nauheim)
Jan DePrest (Leuven)
Richard Keijzer (Winnipeg)

# Significant progress and shifts over time

- Prenatal risk stratification and trial design
- Improved survival and definition of standards of care

  Comparative effectiveness trials CDH-EURO Consortium

- Developmental biology of the pulmonary vasculature

# Significant  progress and shifts over time

- Establishment of parent support groups in many countries
- Delayed surgical repair
- Gentle ventilation
- International collaboration such as the CDH registry and the DHREAMS initiative

Erasmus MC

# EUROPEAN CDH DATABASE

## MISSION

Better diagnosis, risk assessment and personalized treatment for CDH

## GOAL

Integration of clinical, molecular and cellular data, to understand the pulmonary vascularization in CDH


**Collect Data/Material**

**European CDH database (WP1)**

## ANALYSIS

**Extract patient data/material for analysis:**

**Molecular:** GWAS/NGS studies, expression studies (-omics based), image analysis (WP1,3,4)

**Cellular:** Isolate stem/progenitor cells, develop iPS cells, differentiation of cells, LCM (WP1,3)

**Clinical:** Prediction model, pathology, link prenatal imaging with postnatal management (WP1,2,4)

Designed by Richard Keijzer 2015

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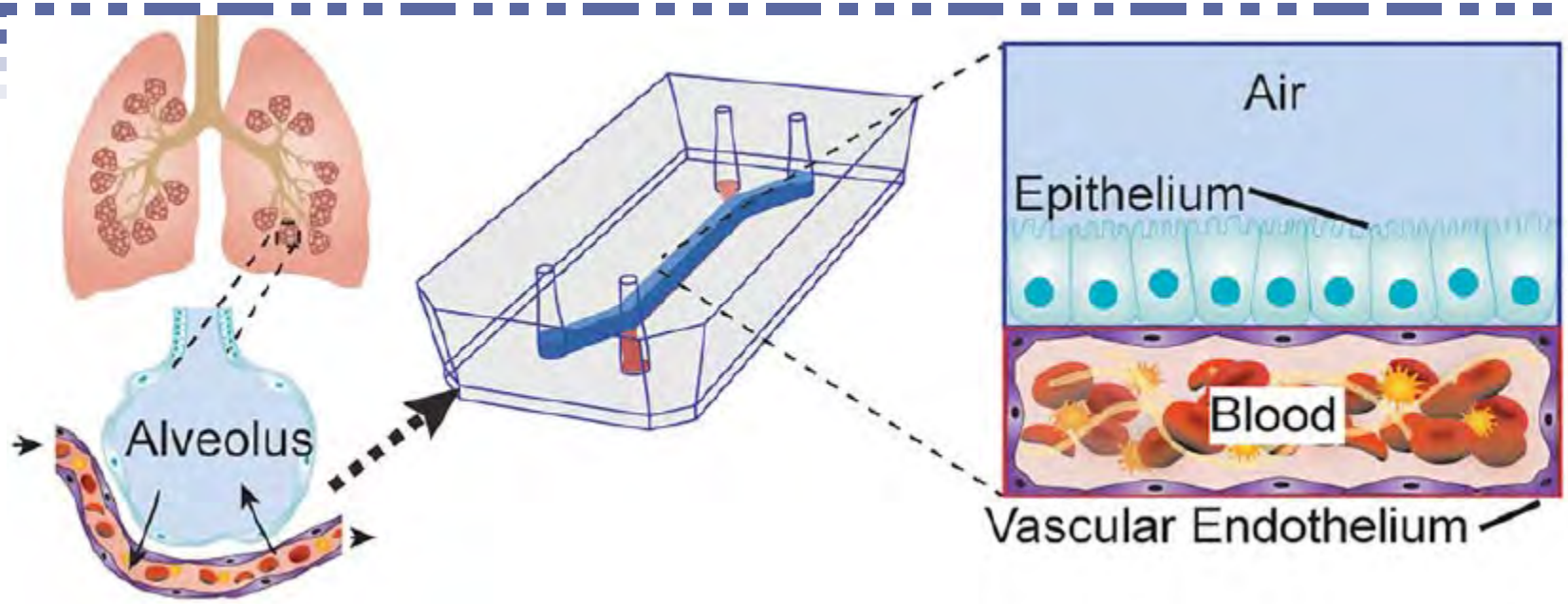
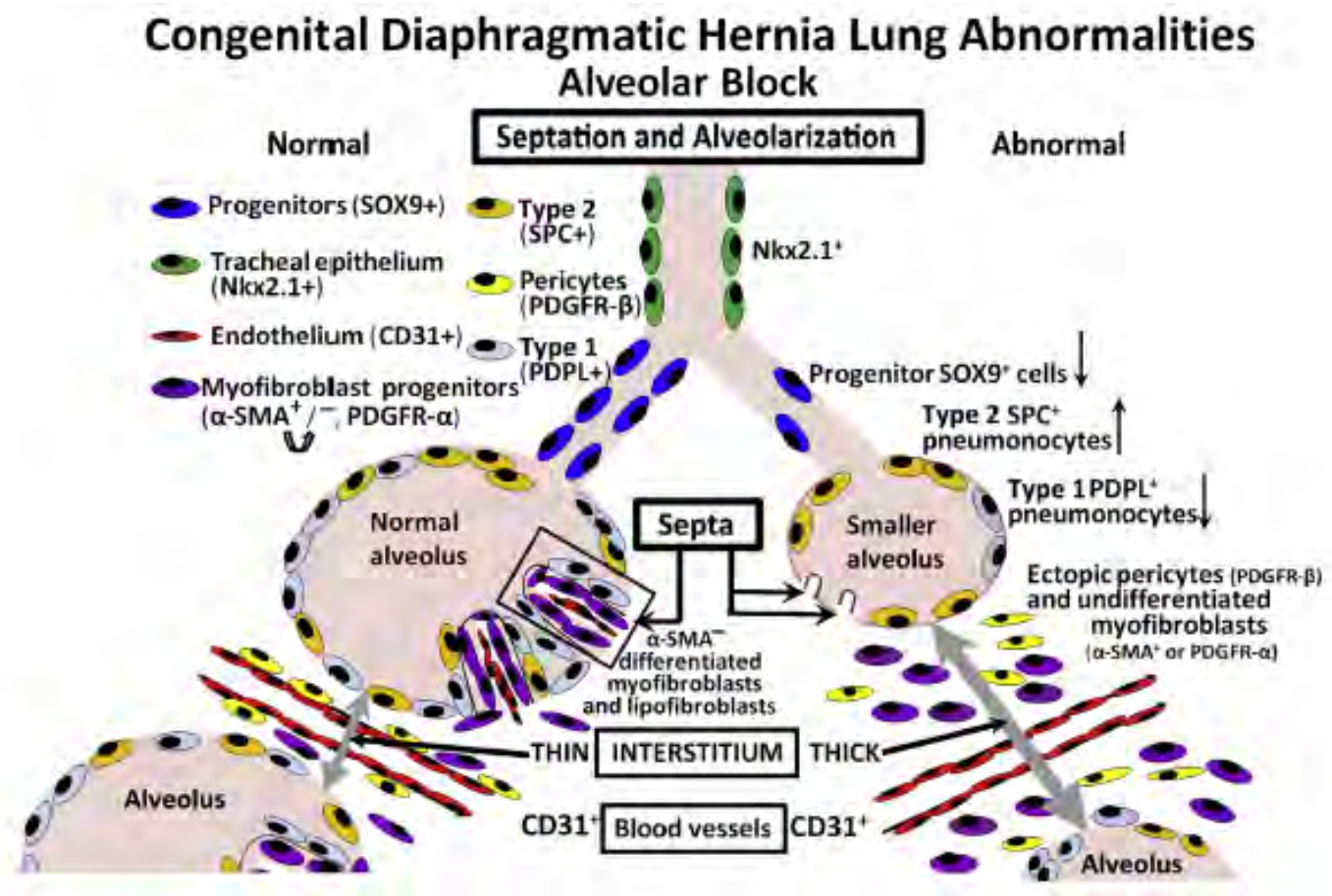

Alveolus
Air
Epithelium
Blood
Vascular Endothelium




Donahoe PK, Longoni M, High FA.
*Am J Pathol* 2016,186: 2532–2543

# Epithelial differentiation - application