MiRacles for babies with abnormal lung development and Congenital Diaphragmatic Hernia

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The Children's Hospital Research Institute of Manitoba

BIOLOGY OF BREATHING

Shared health Soins communs Manitoba

Health Sciences Centre Winnipeg

University of Manitoba

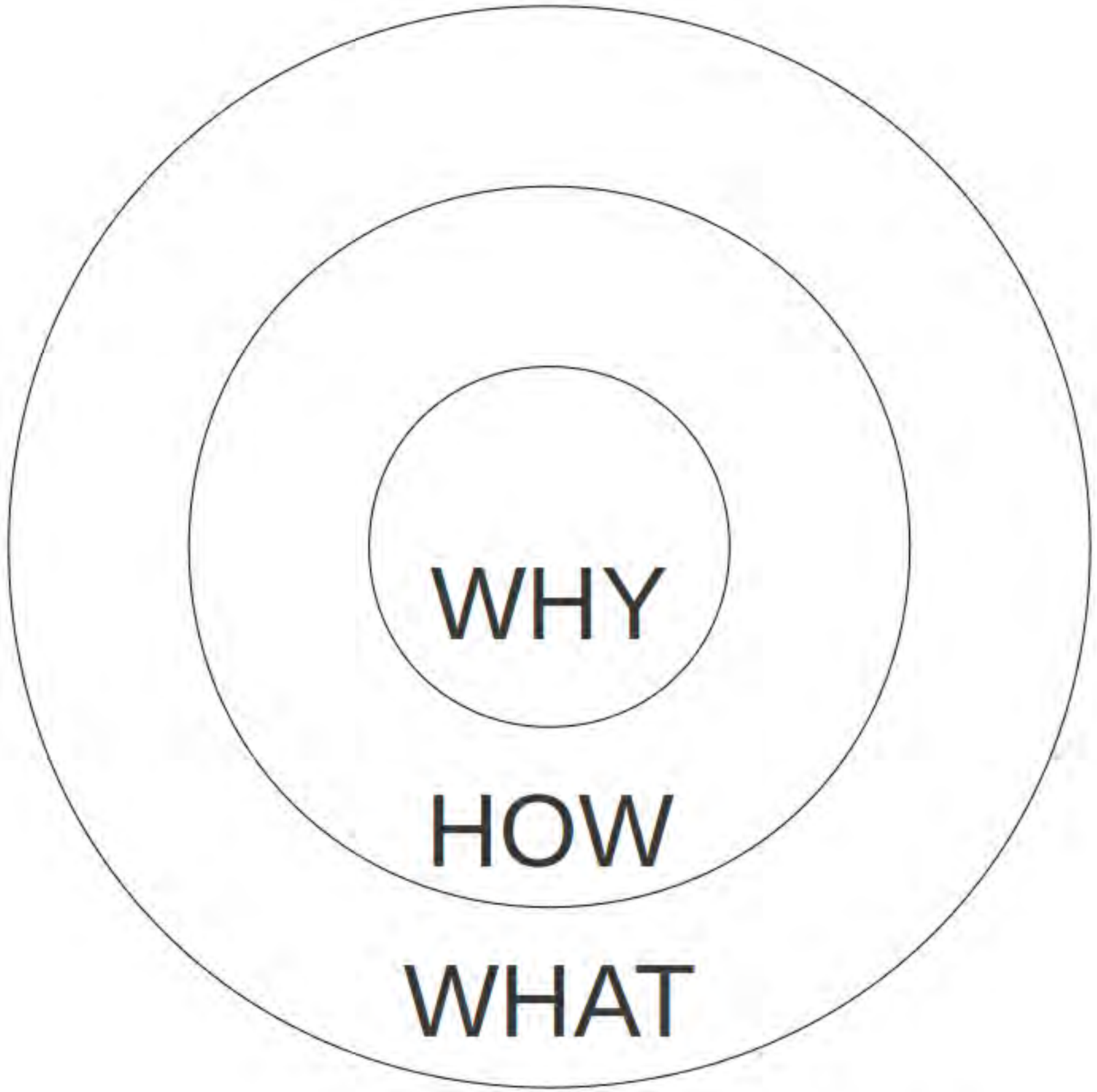
Conflict of Interest Disclosure

I hold a patent application (PCT/CA2015/051028) containing technology described in the presentation

I have filed for a patent to use circular RNAs as biomarkers for abnormal lung development and CDH

How it began

We don’t do chart reviews here, why don’t you go in the lab for the next few months.



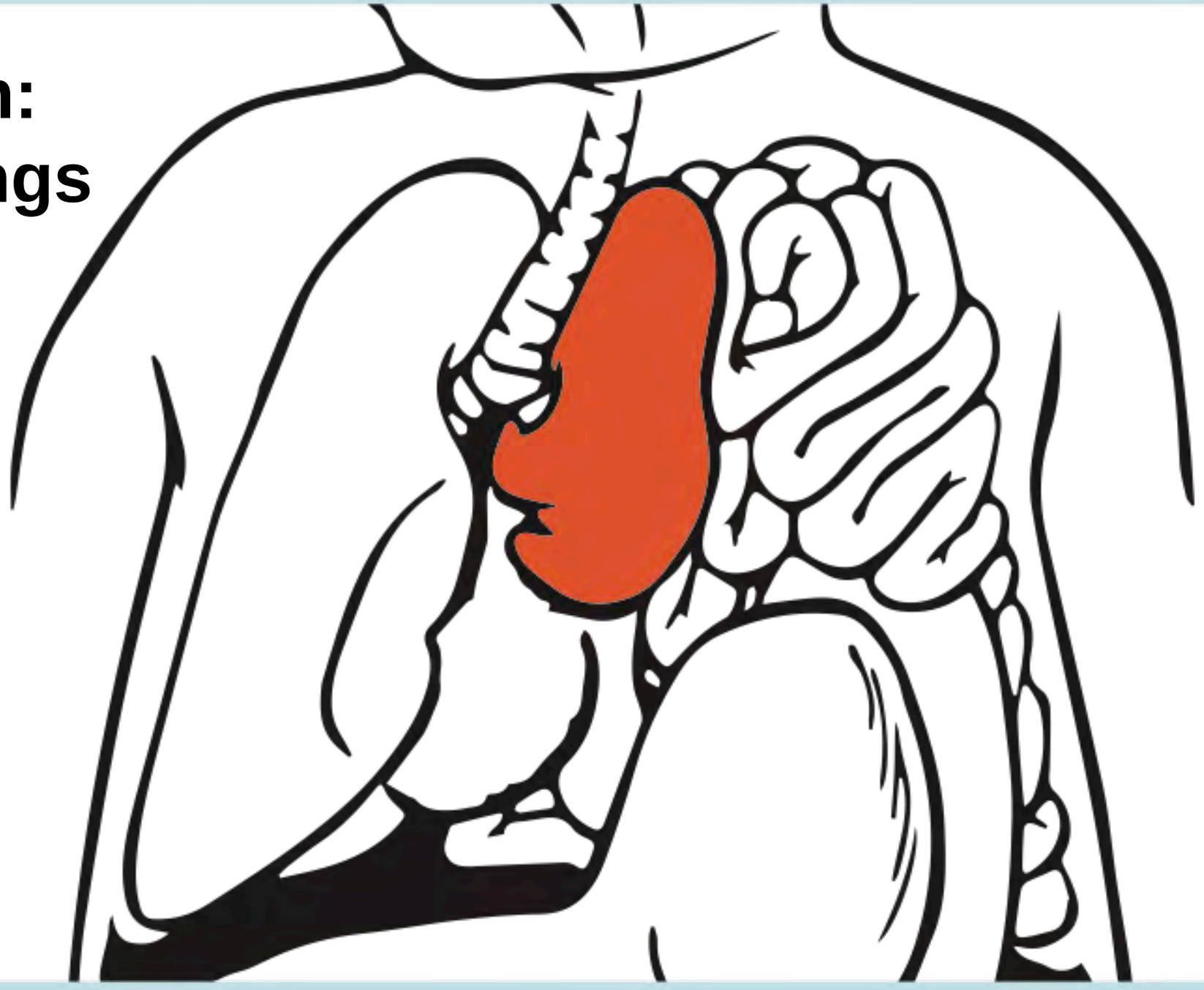
The 'golden circle' from Simon Sinek

http://store.winnipegfreepress.com/photostore/details/139975/

Mortality: >400,000 since 2000

Image: Shutterstock

Main problem:
Abnormal lungs

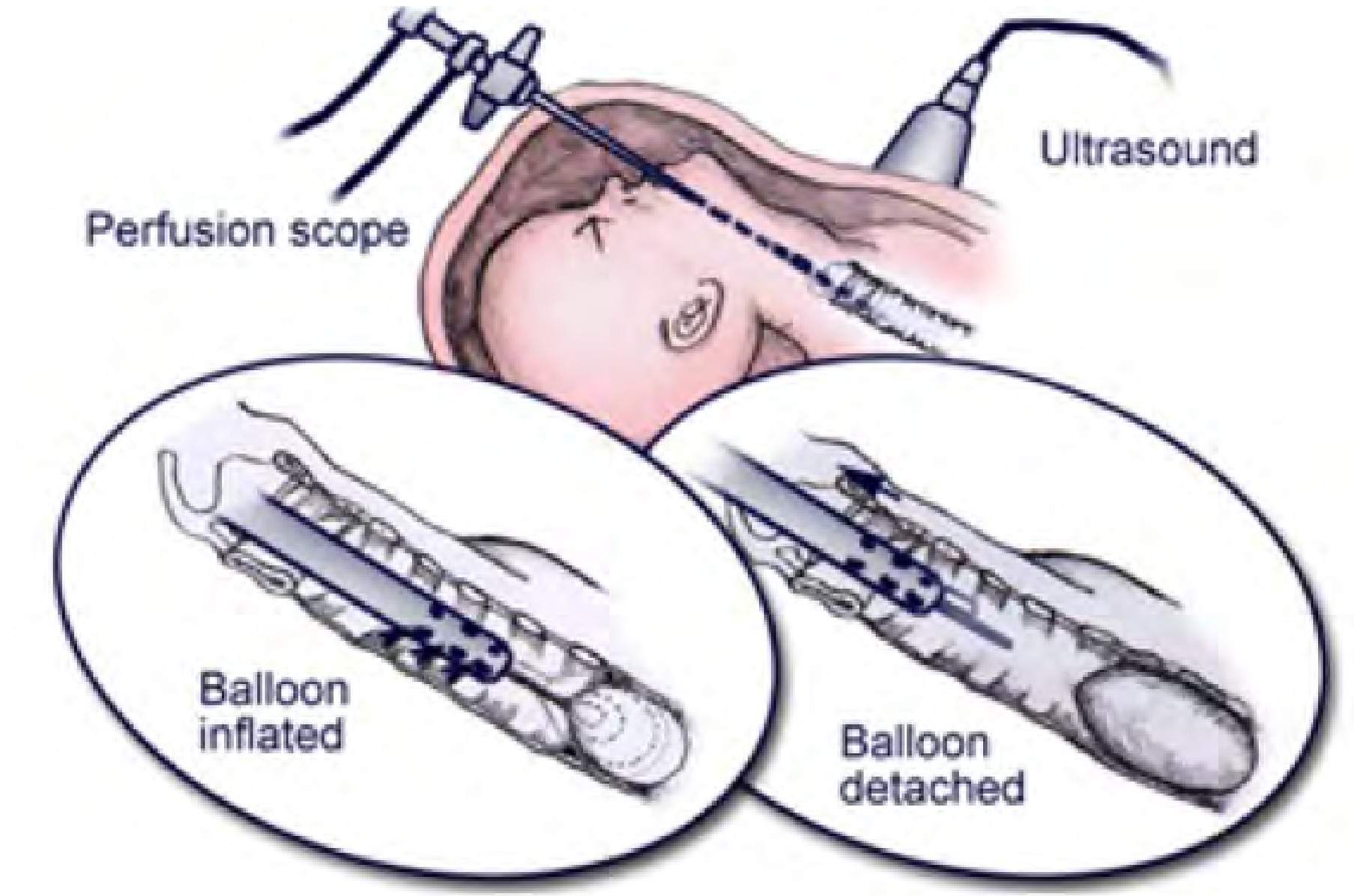


MIRACLA

MicroRNAs • And • Congenital • Lung • Anomalies

What is wrong with CDH lungs?

Can we fix the lungs before birth?



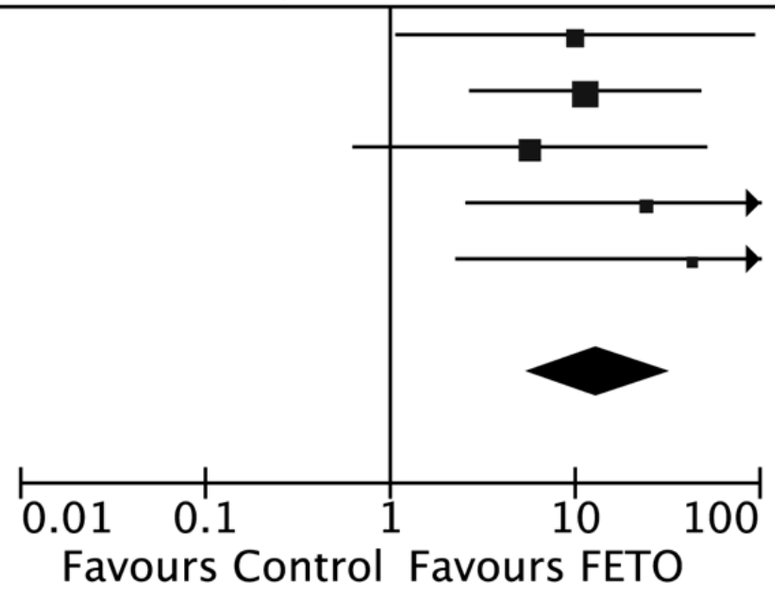
totaltrial.eu

Meta-analysis FETO improves survival in isolated CDH

Study or Subgroup	FETO Events	FETO Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Deprest 2004	10	21	1	12	19.5%	10.00 [1.09, 91.98]
Deprest 2006	12	24	3	37	34.5%	11.33 [2.72, 47.17]
Peralta 2011	9	28	1	13	27.1%	5.68 [0.64, 50.73]
Ruano 2011	10	17	1	18	11.7%	24.29 [2.60, 227.25]
Ruano 2012	10	20	0	21	7.1%	43.00 [2.29, 806.44]
Total (95% CI)		**110**		**101**	**100.0%**	**13.32 [5.40, 32.87]**
Total events	51		6			

Heterogeneity: $Chi^2 = 1.59$, df = 4 (P = 0.81); $I^2 = 0\%$

Test for overall effect: Z = 5.62 (P < 0.00001)



META-ANALYSIS

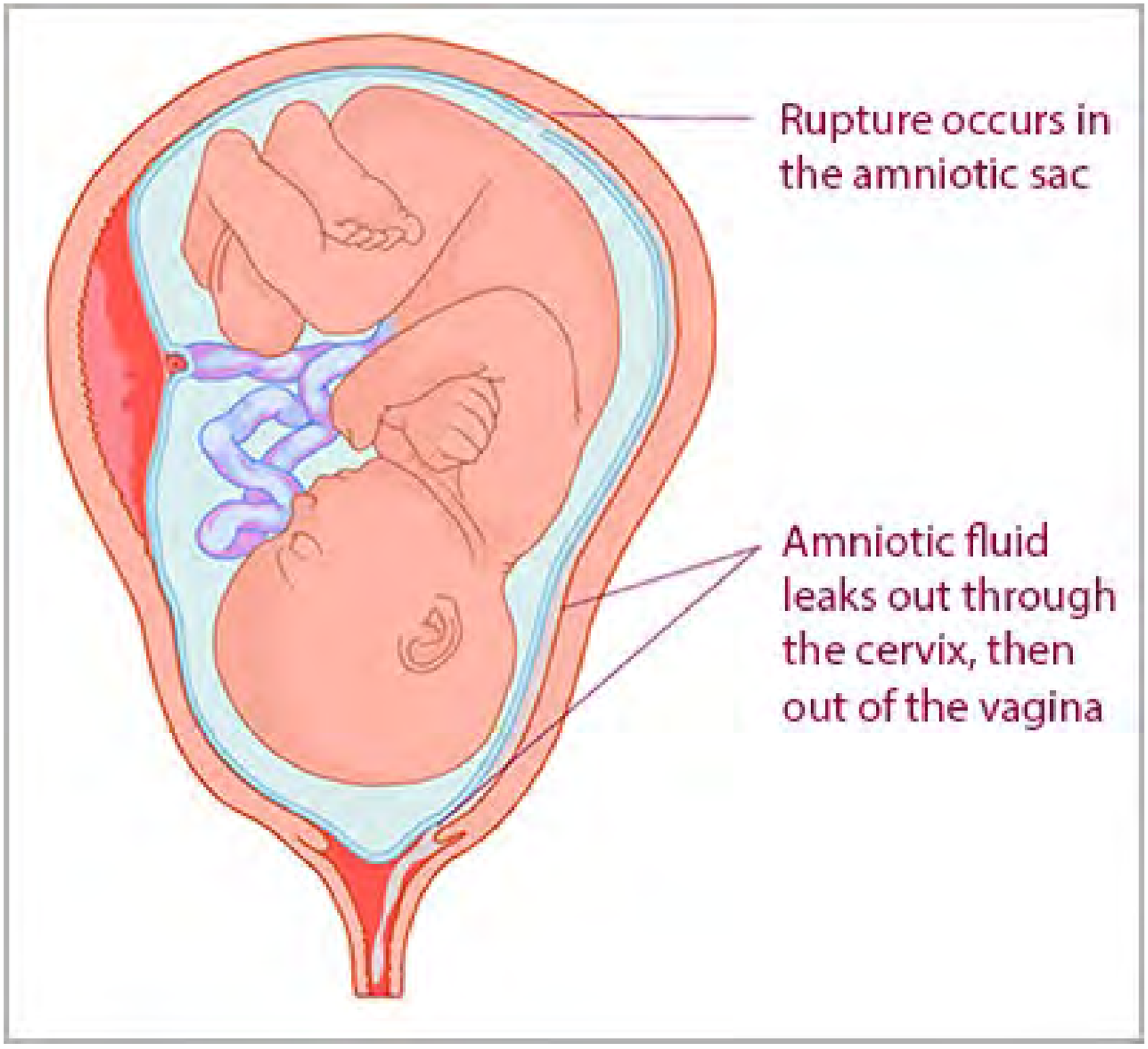
Fetal Tracheal Occlusion for Severe Pulmonary Hypoplasia in Isolated Congenital Diaphragmatic Hernia

A Systematic Review and Meta-analysis of Survival

Jamila Al-Maary, MD, Mary P. Eastwood, MBChB,† Francesca Maria Russo, MD,†*
Jan A. Deprest, PhD,†§ and Richard Keijzer, PhD‡*

Annals of Surgery • Volume 264, Number 6, December 2016

But, negative side effects!



https://www.tommys.org/pregnancy-information/pregnancy-complications/waters-breaking-early-pprom

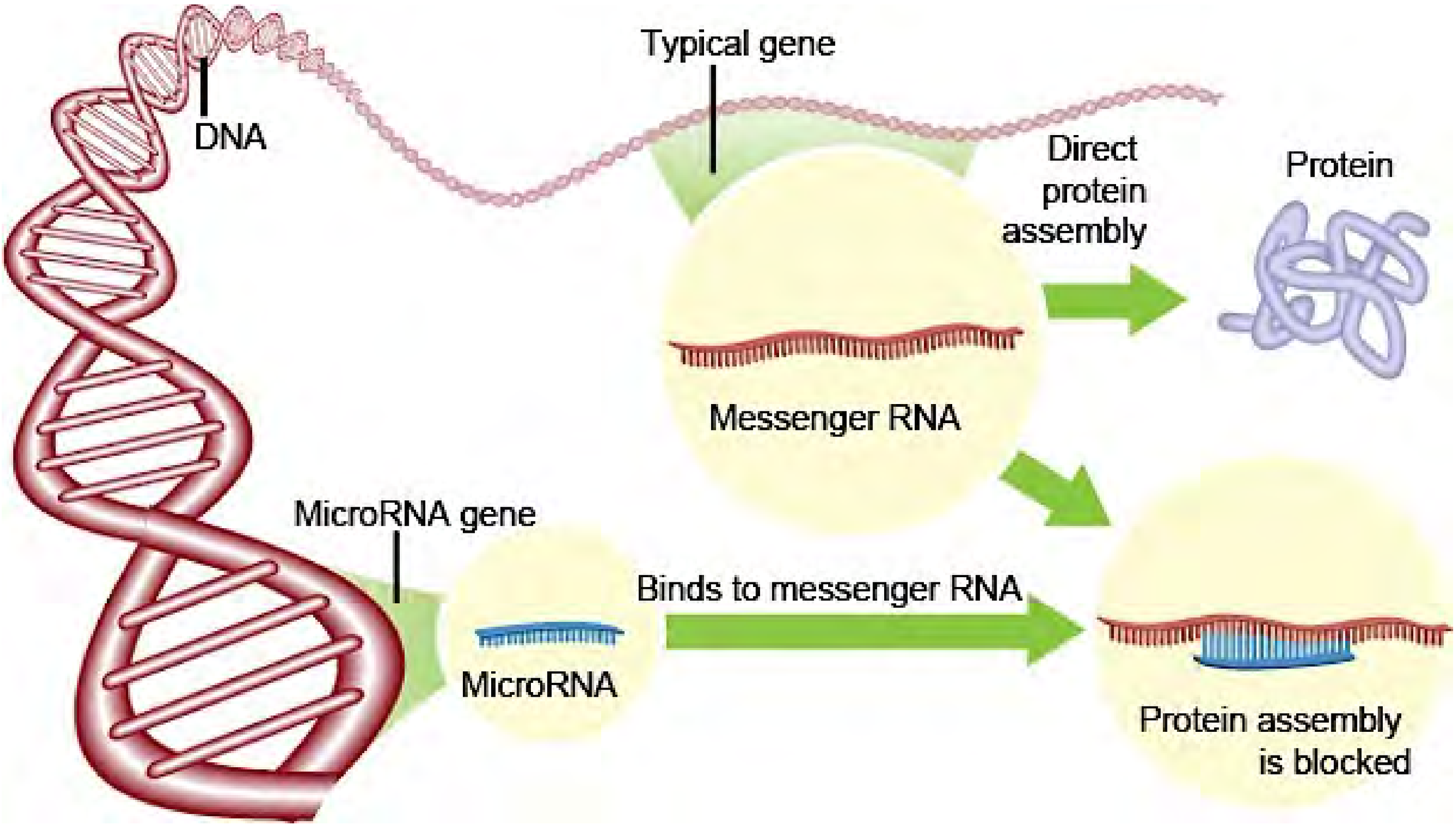
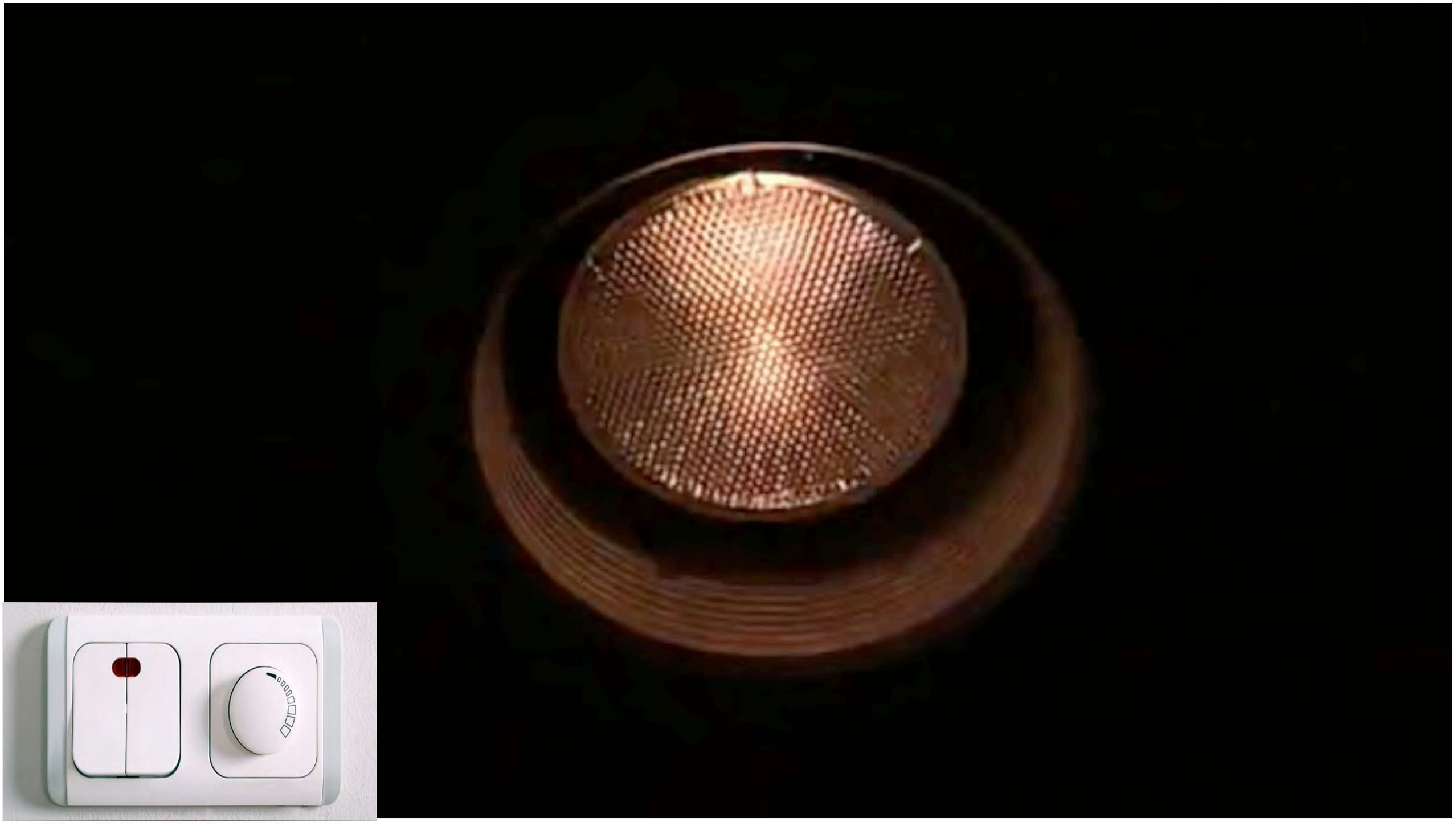
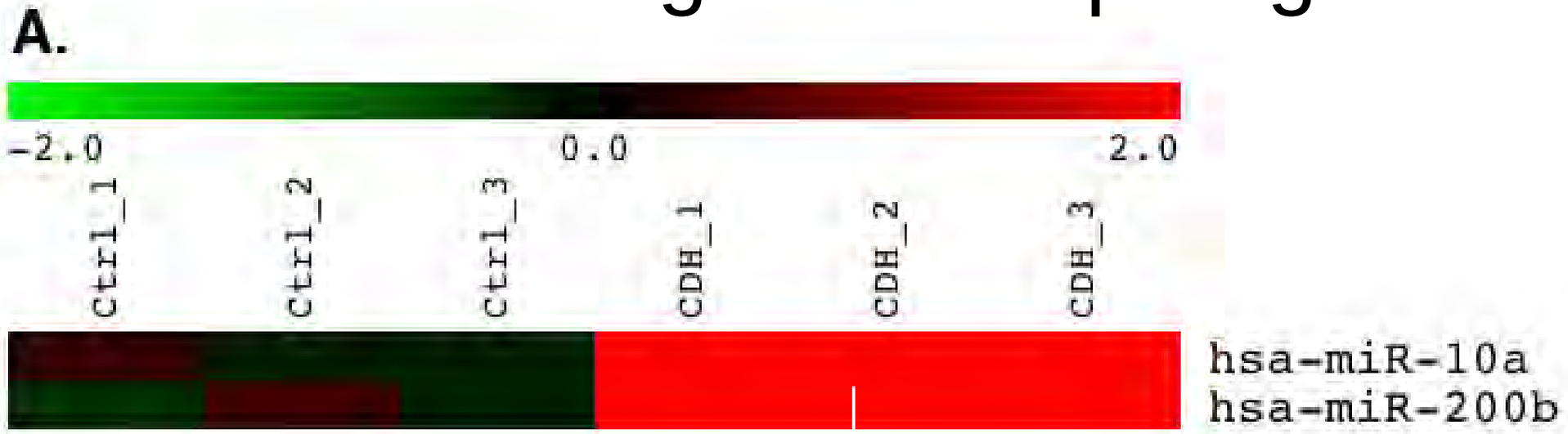


Image: Steve Karp, Discover Magazine



MicroRNAs and Congenital Diaphragmatic Hernia

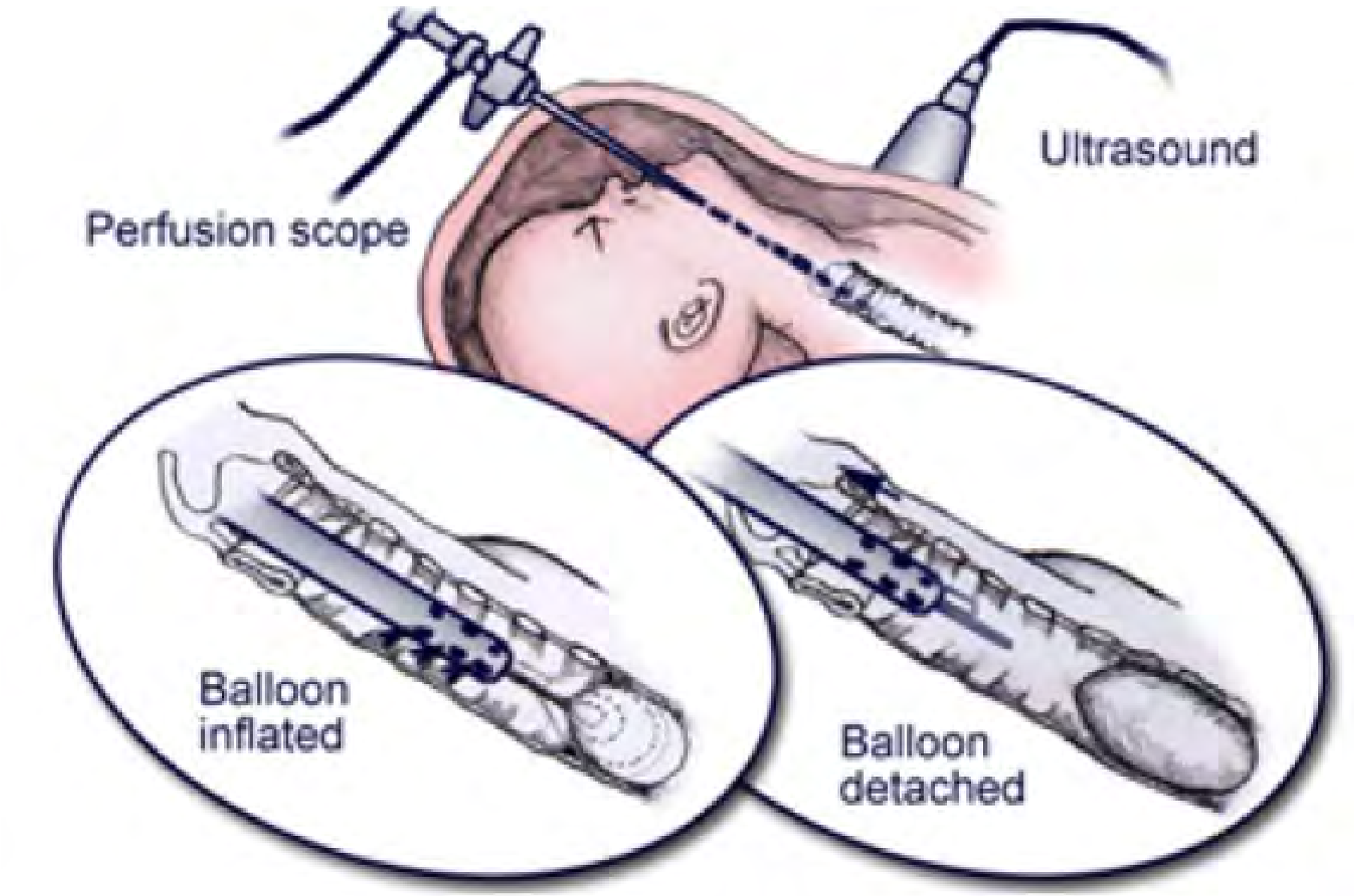


ORIGINAL ARTICLE

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**

Annals of Surgery • Volume 262, Number 6, December 2015



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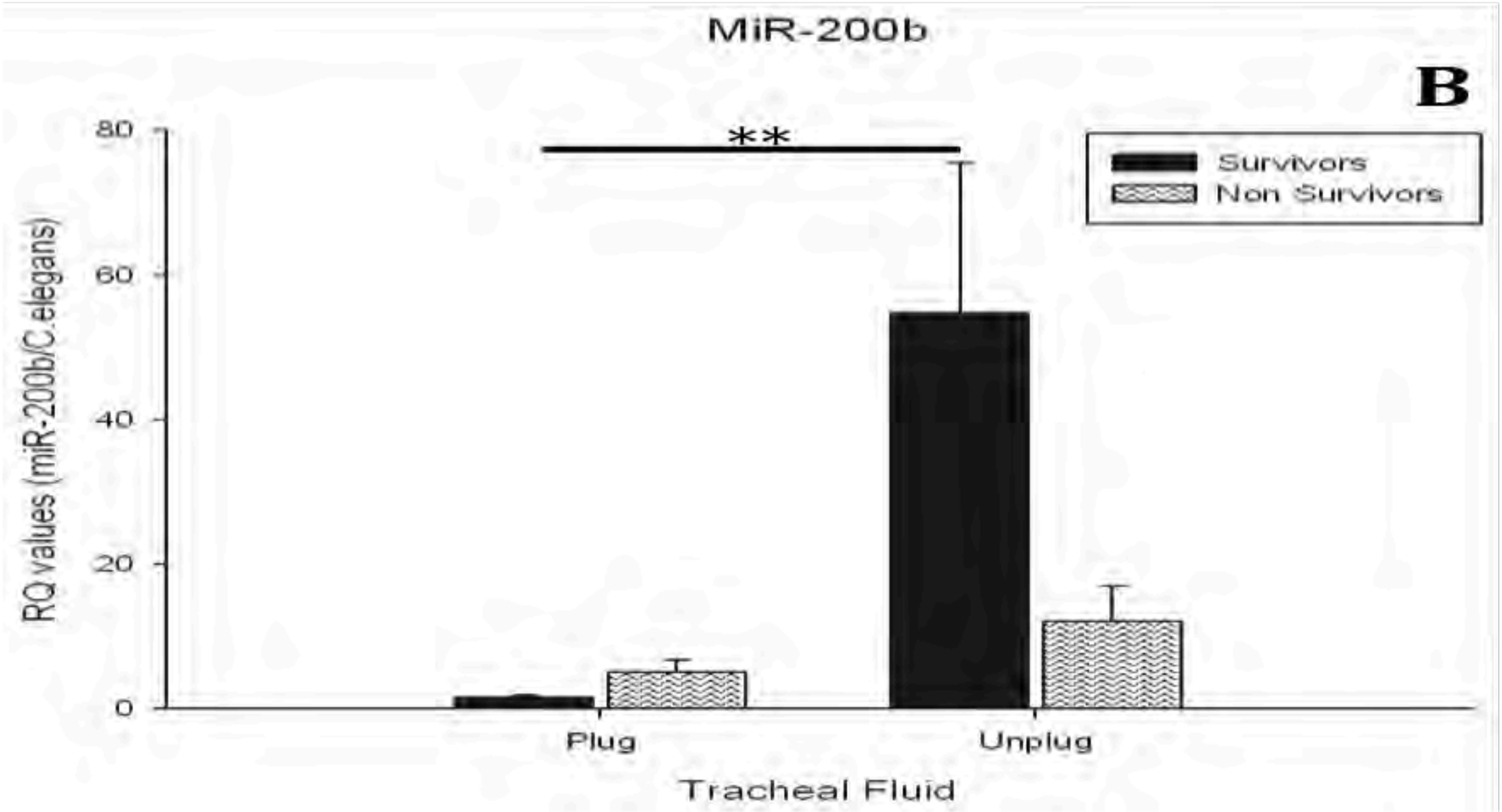
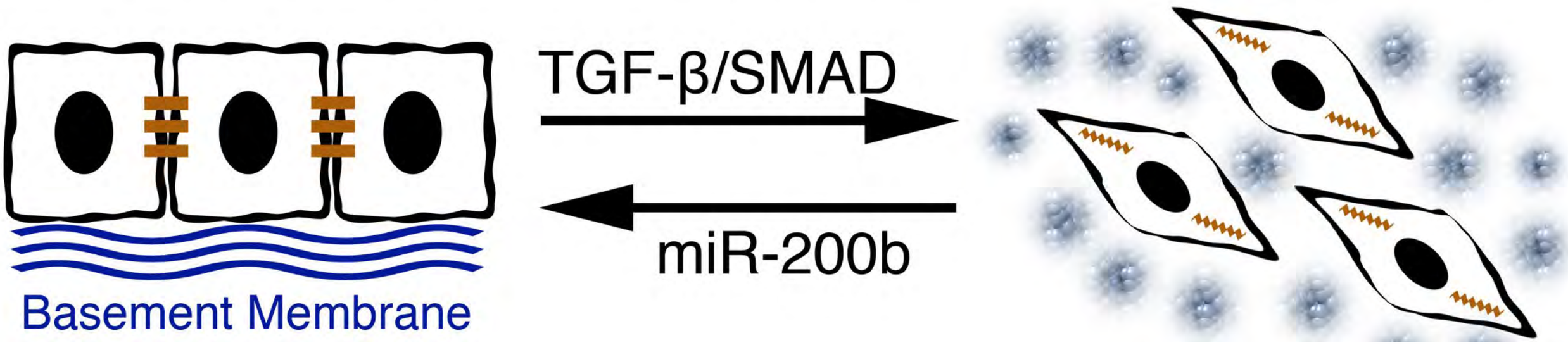
Higher miR-200b has better outcomes



Epithelial-to-Mesenchymal Transition (EMT)



Epithelial cells

Mesenchymal cells

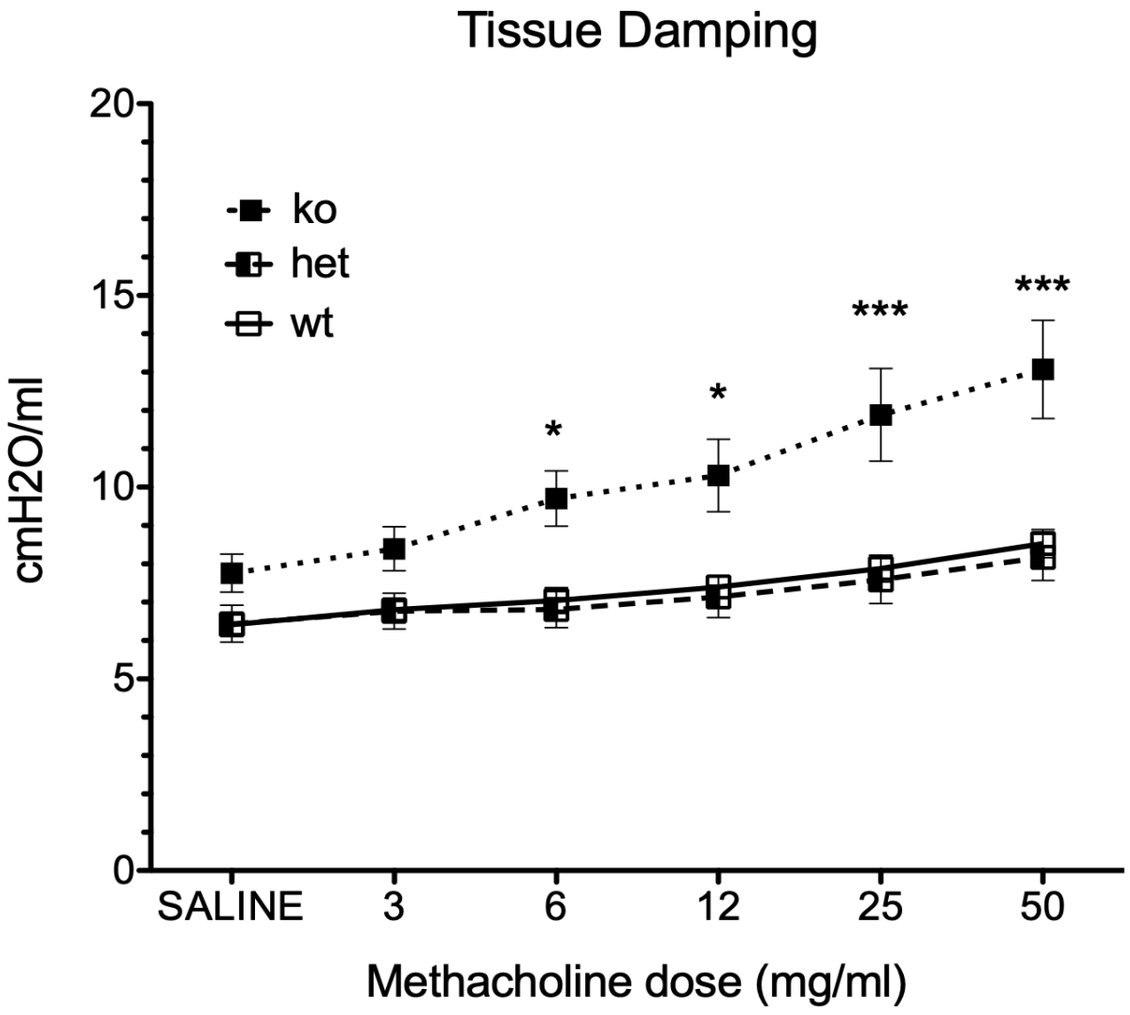
SCIENTIFIC REPORTS

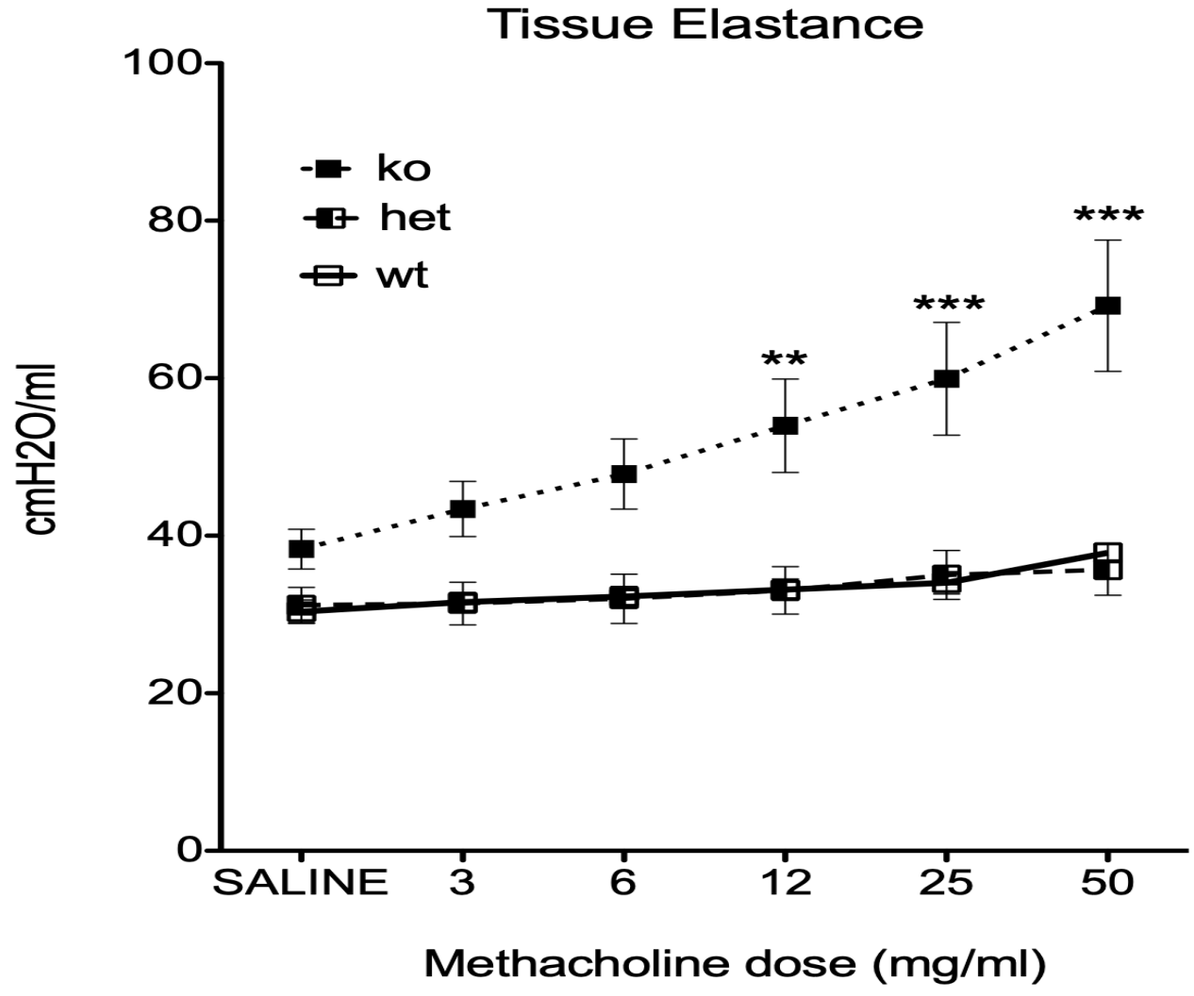
OPEN

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]

miR-200b -/- mice have higher lung tissue damping and elasticity

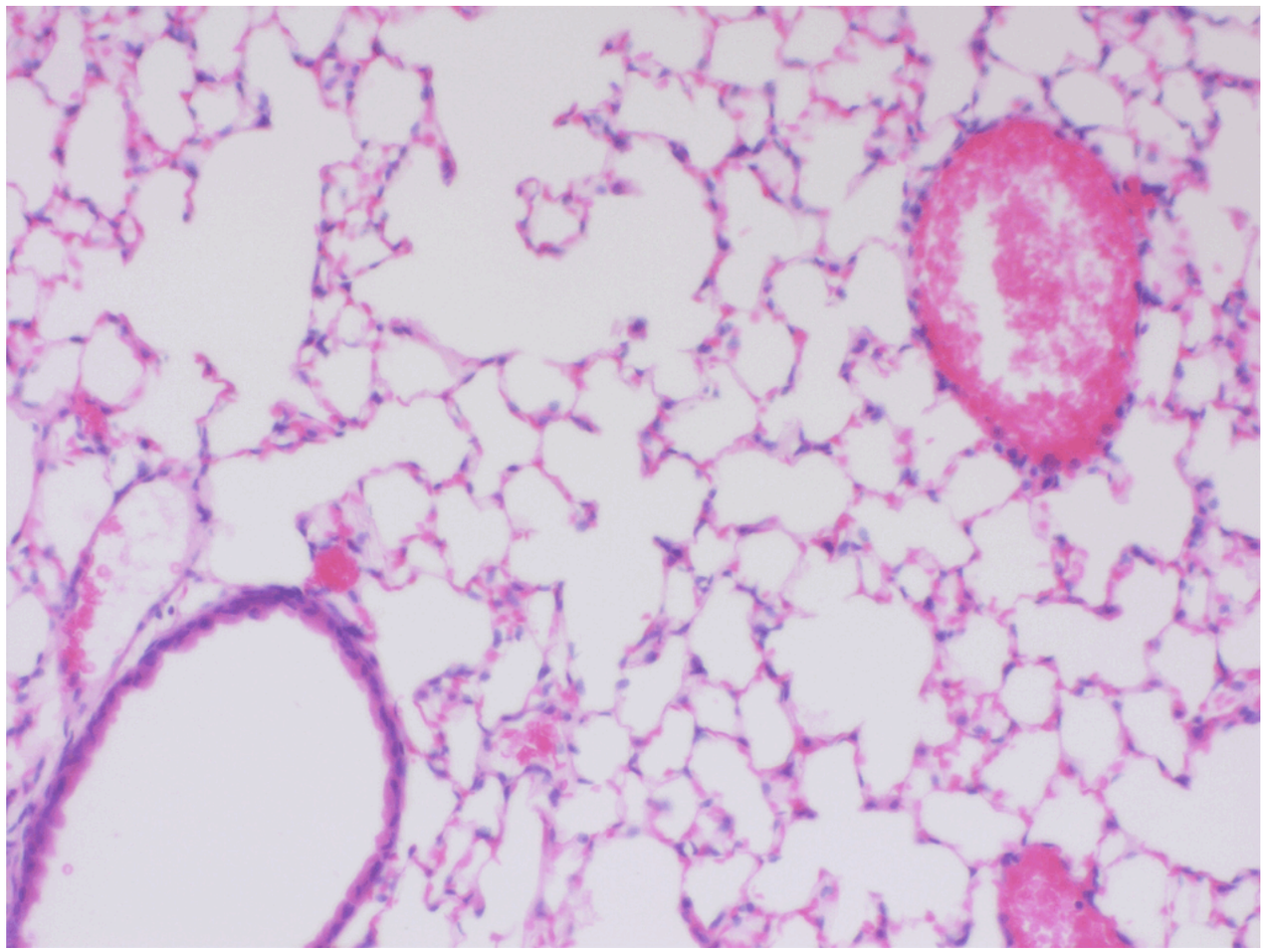




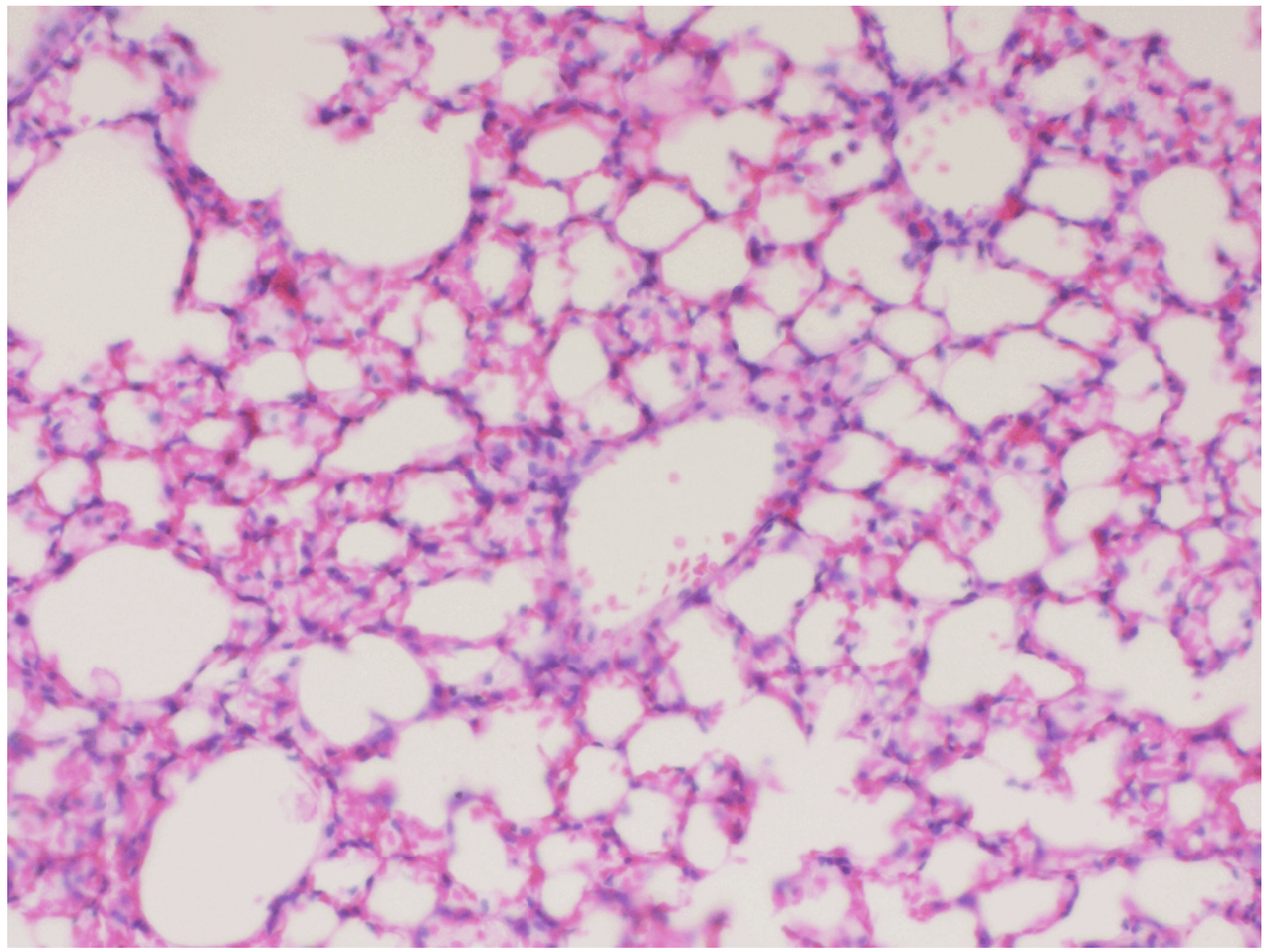
* P< 0.05, ** P< 0.01, *** P< 0.001

miR-200b -/- lungs are hypoplastic

wt

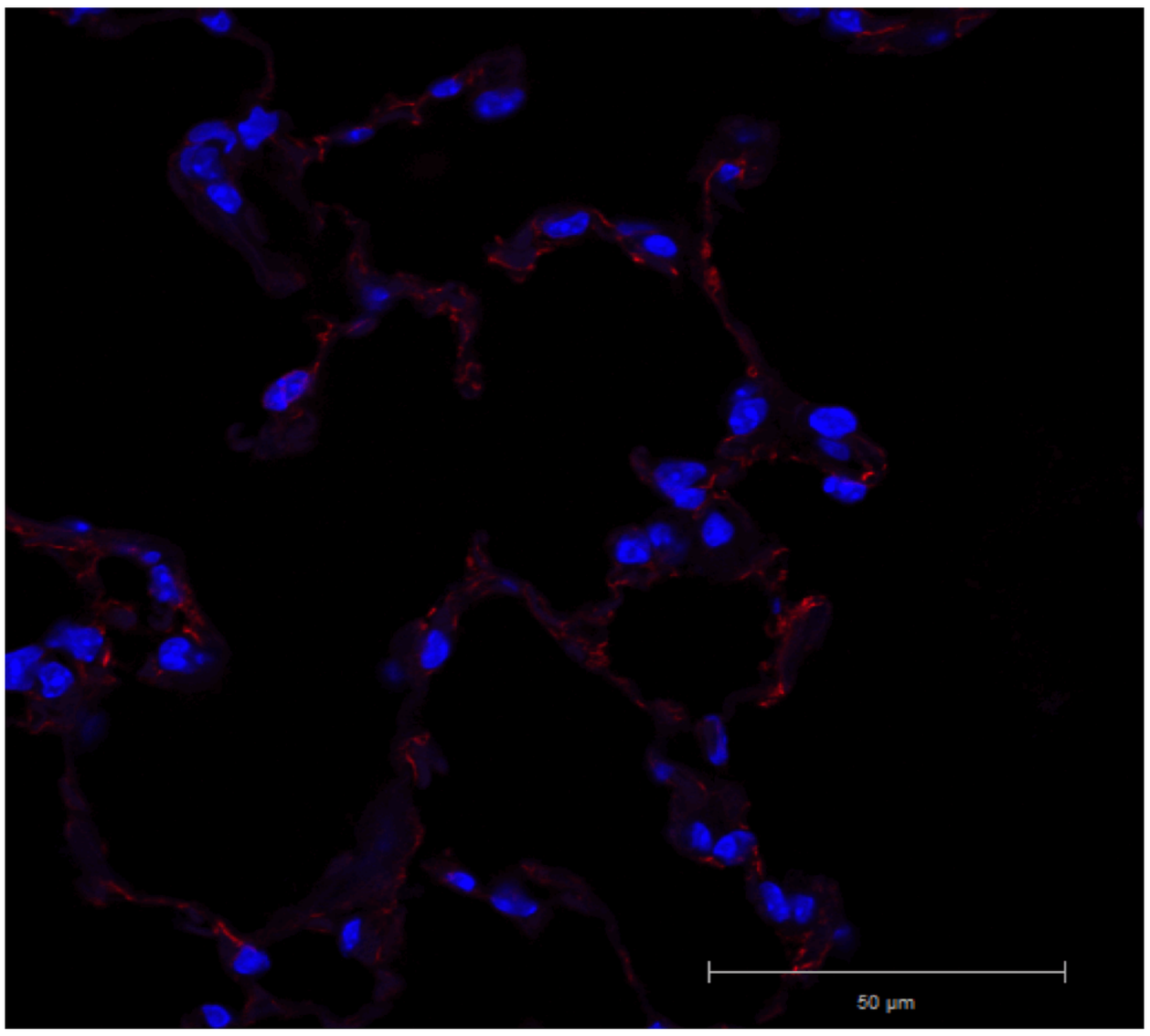


miR-200b -/-

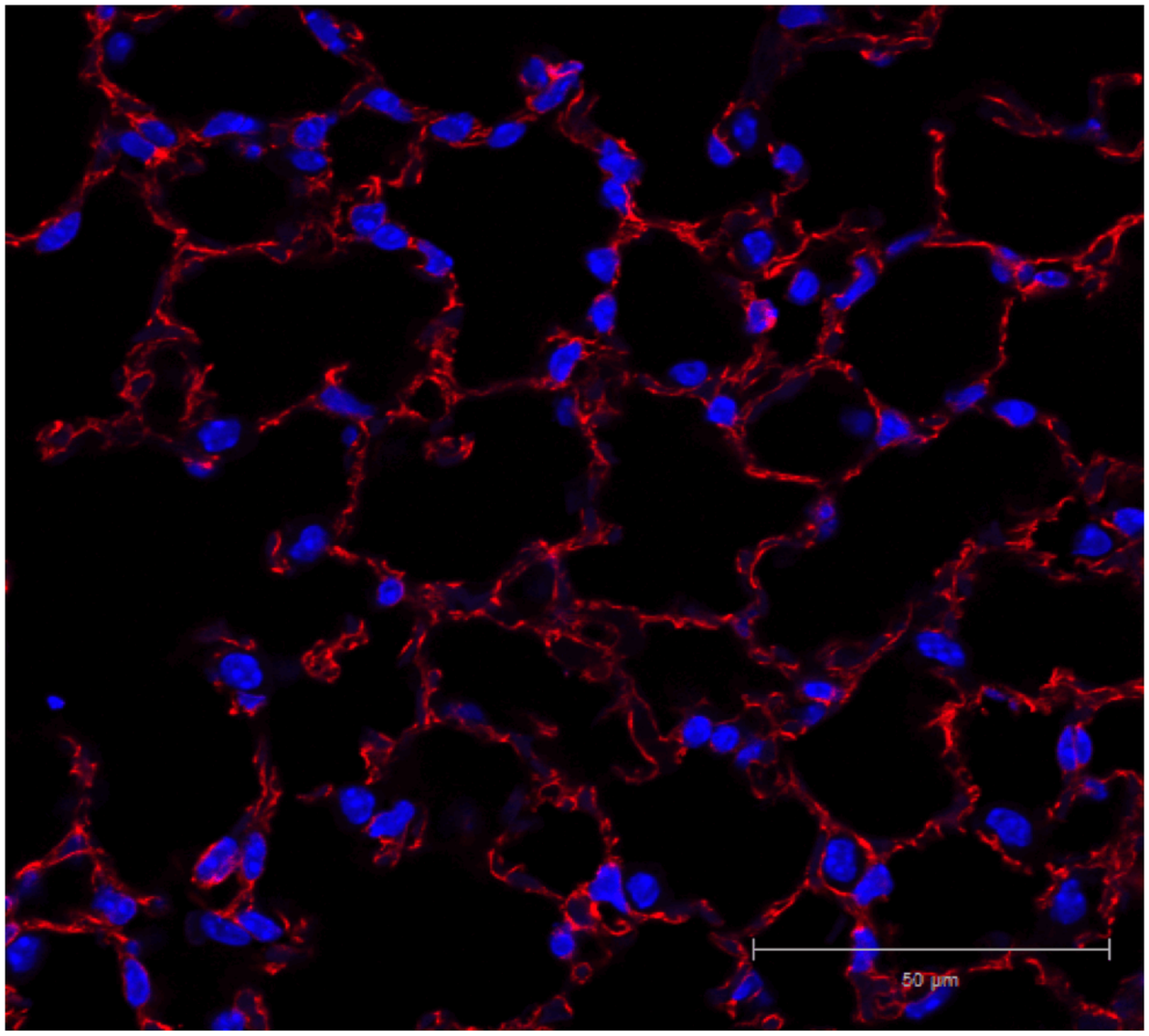


miR-200b -/- lungs have more vimentin

wt



miR-200b -/-

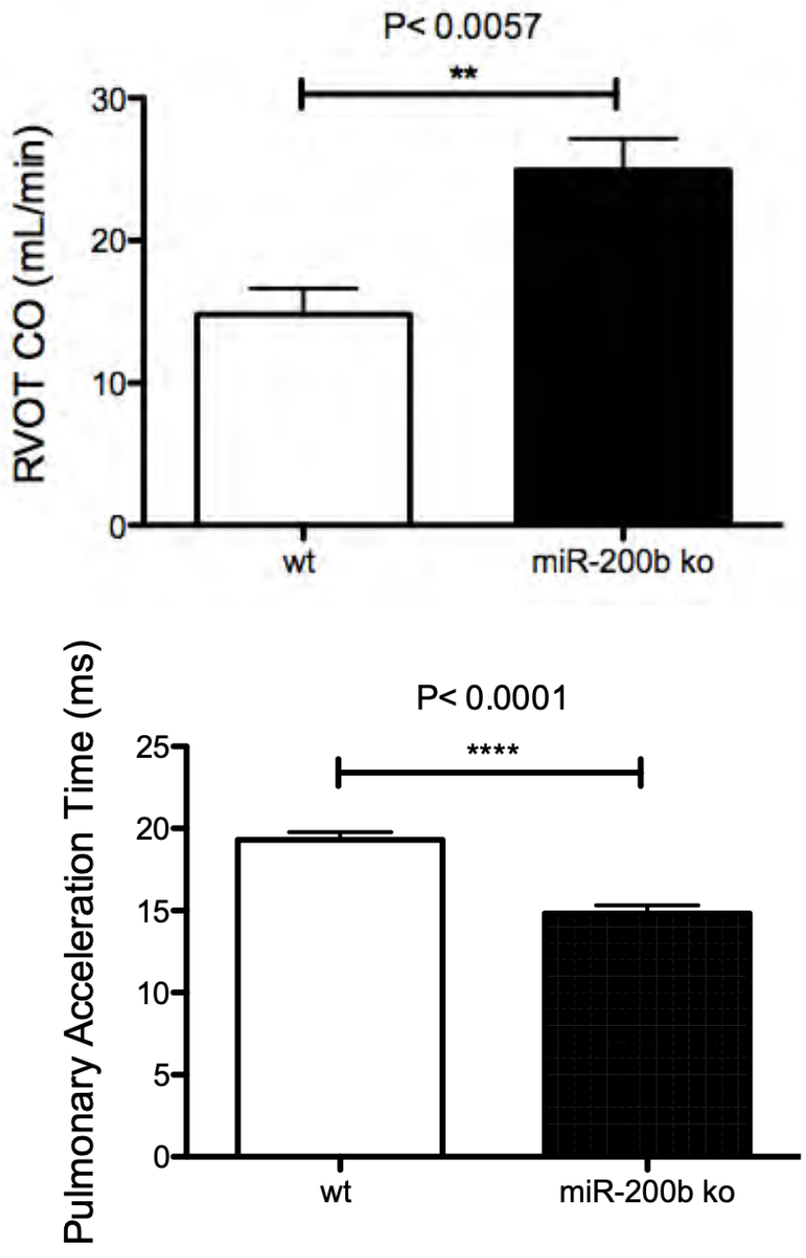


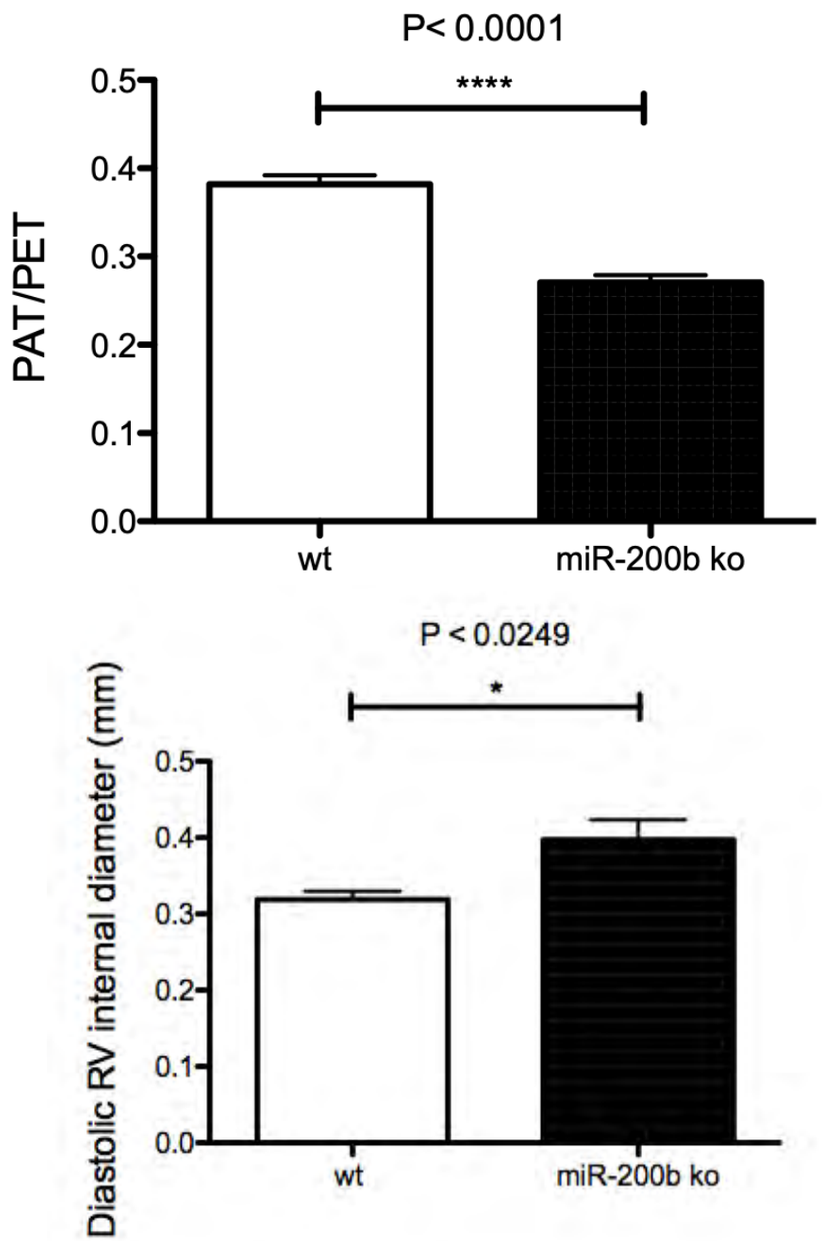
Wildtype

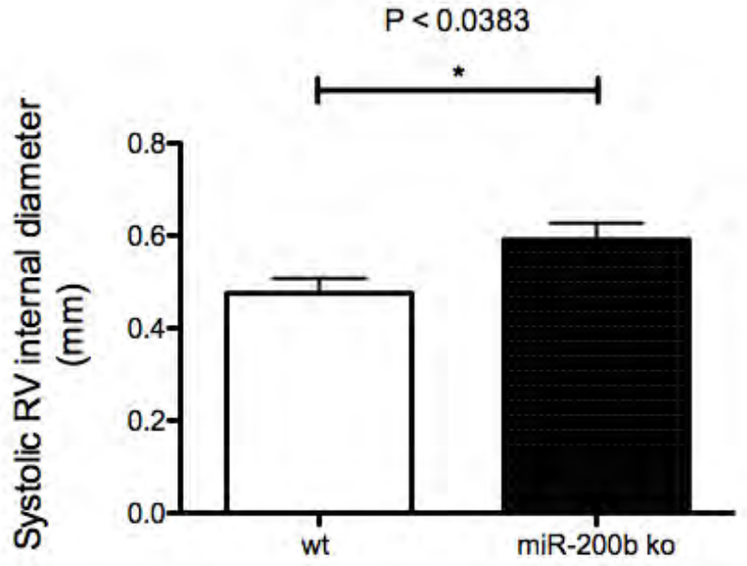
miR-200b -/-

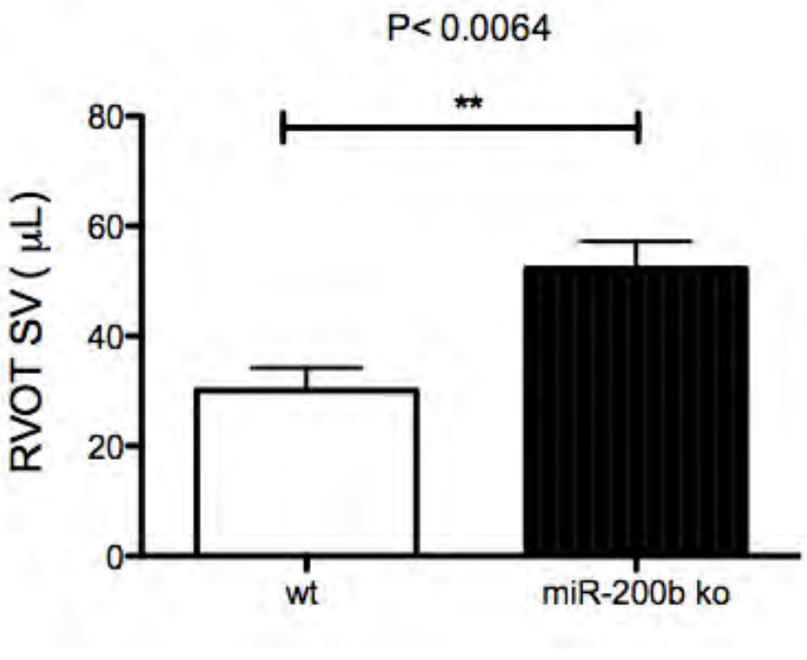
Unpublished results

miR-200b knockout mice have pulmonary hypertension on cardiac echography



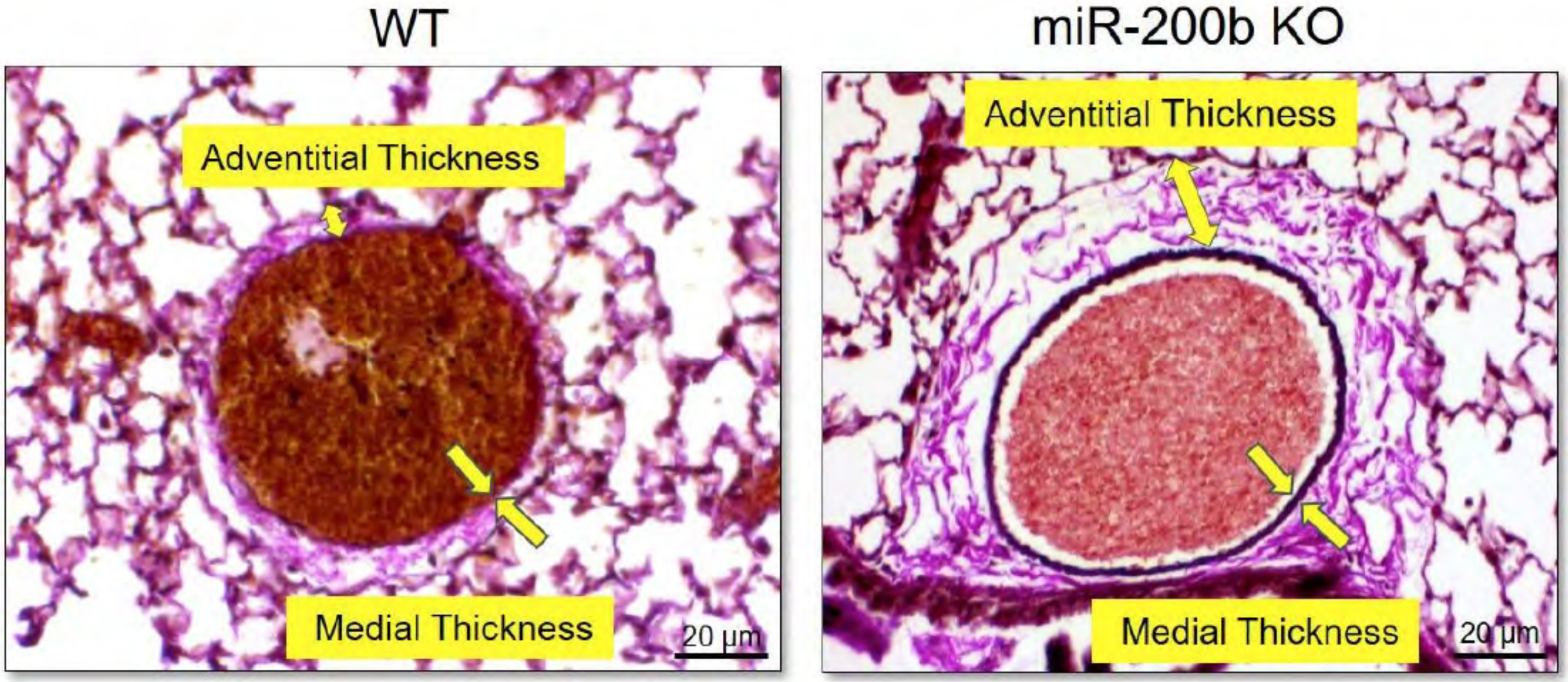






Unpublished results

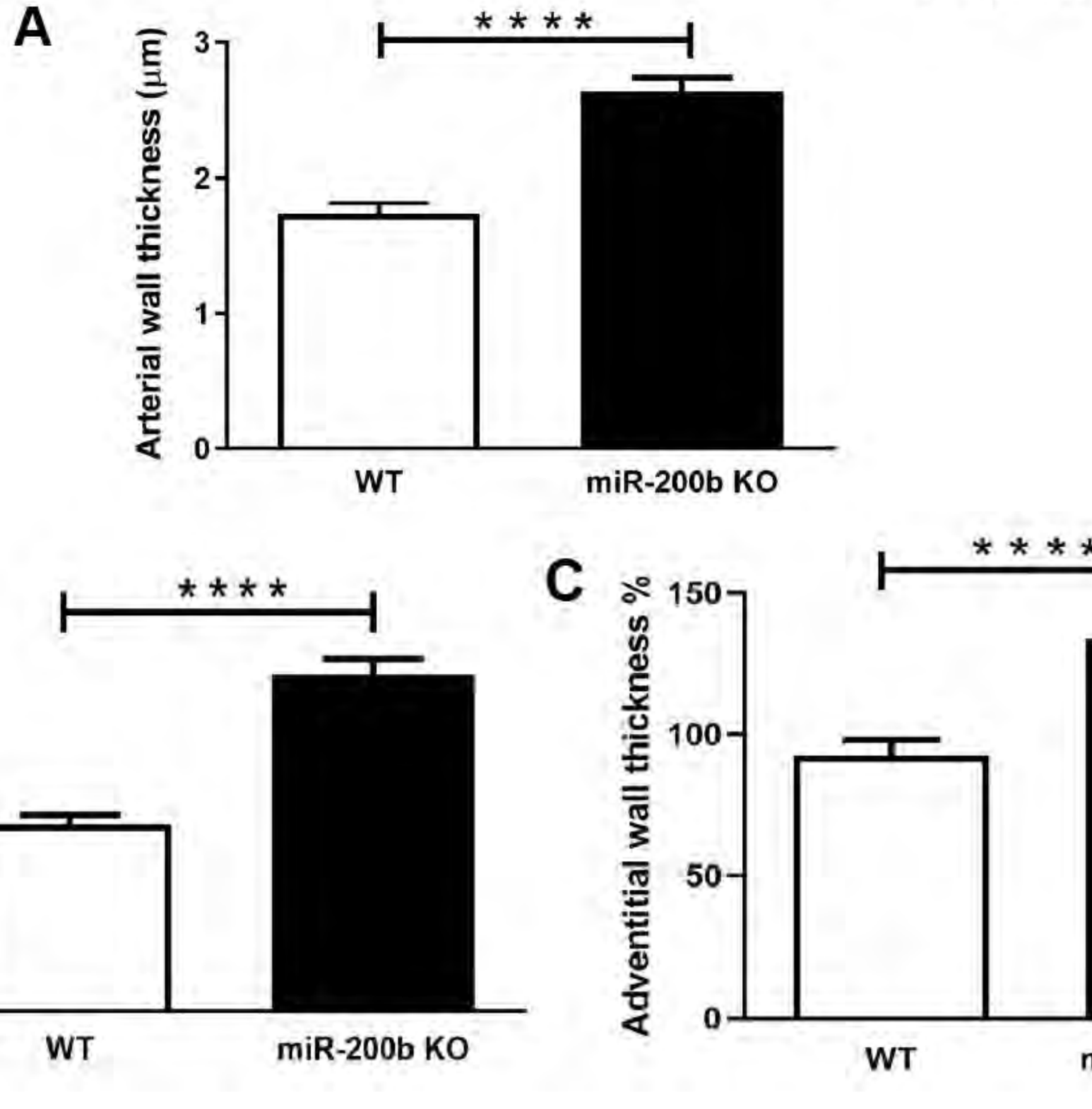
miR-200b-/- lungs have thicker vessel walls



Unpublished results

miR-200b-/- lungs have thicker vessel walls

**** $P < 0.0001$



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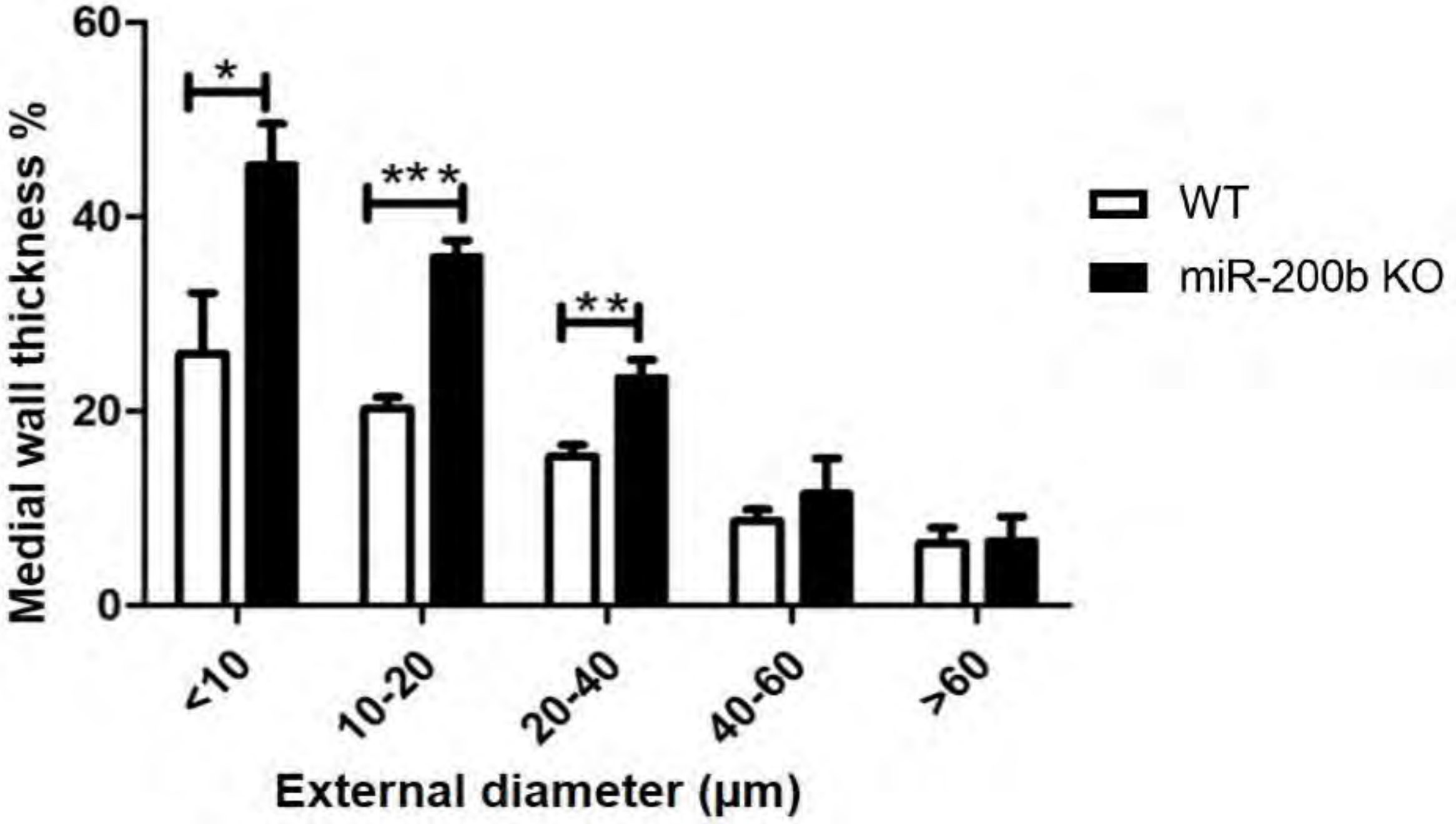

Unpublished results

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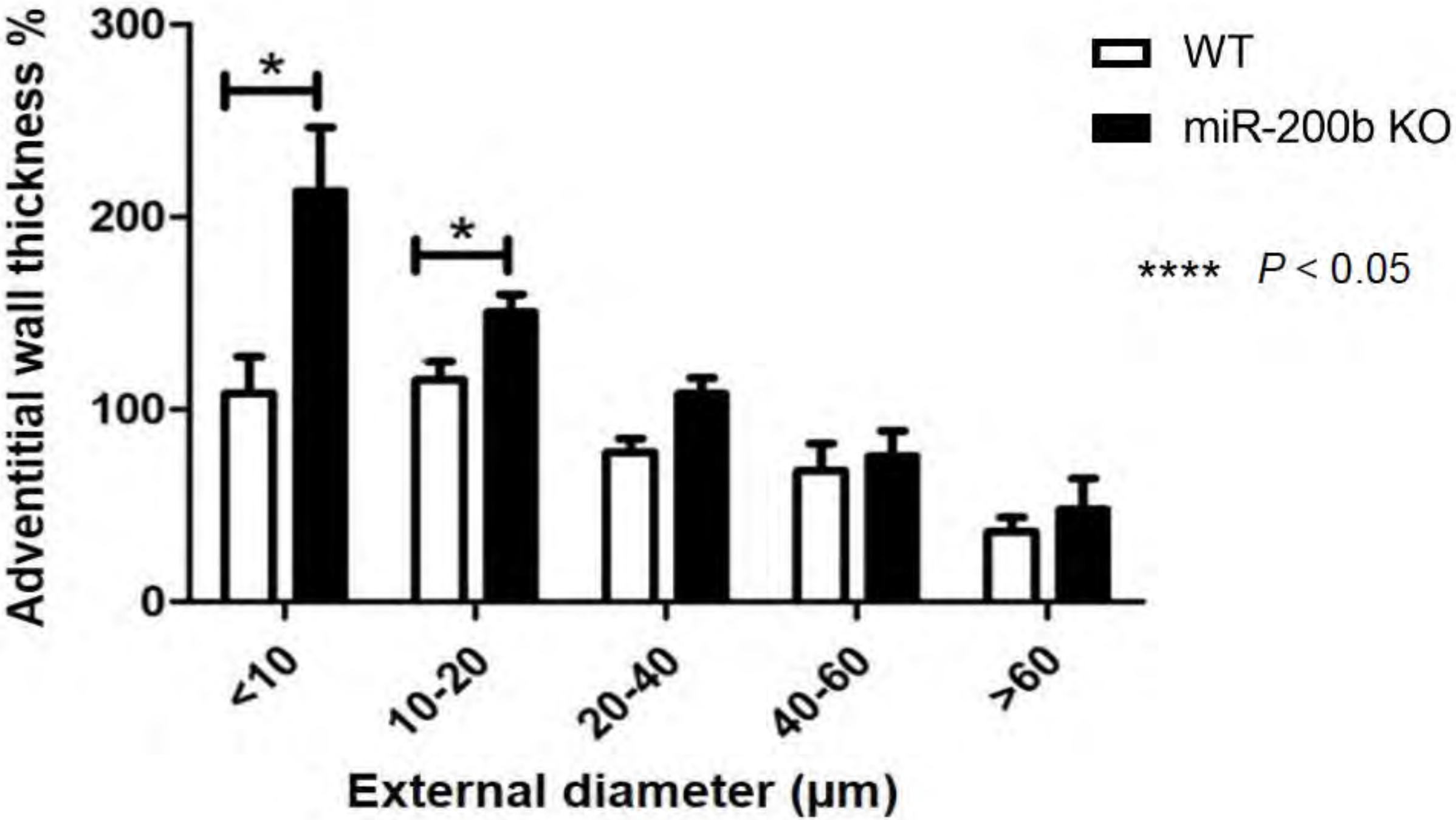


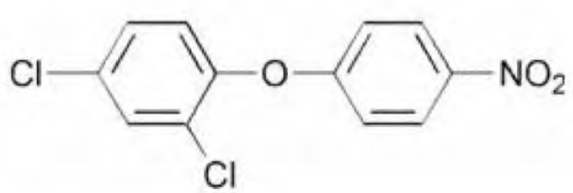
Unpublished results

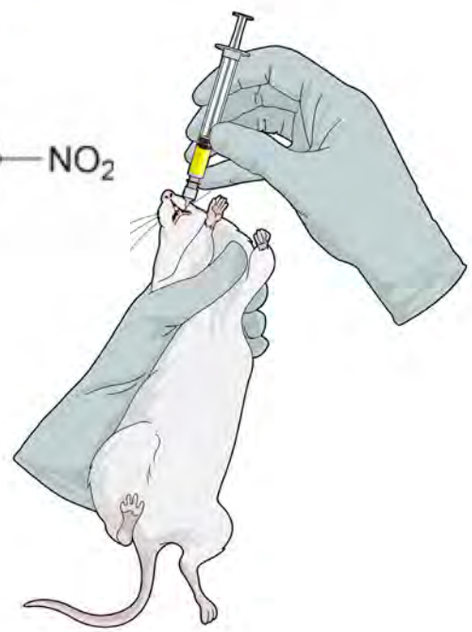
NITROFEN MODEL OF CDH

Lung
Diaphragm
Fetal breathing
Birth

E0 E9 E11 E14 E17 E22

NITROFEN





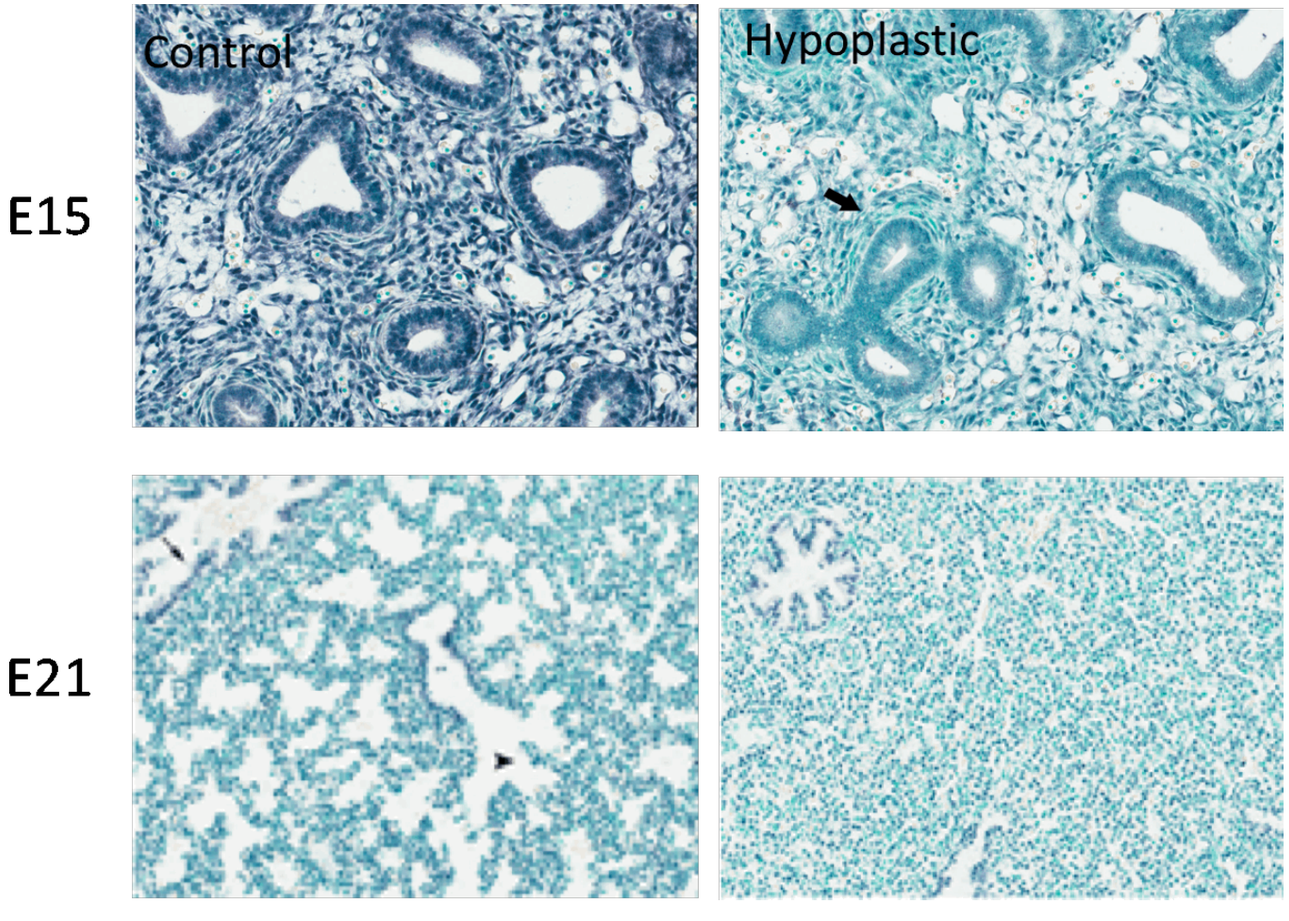
80% CDH
100% PH

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**

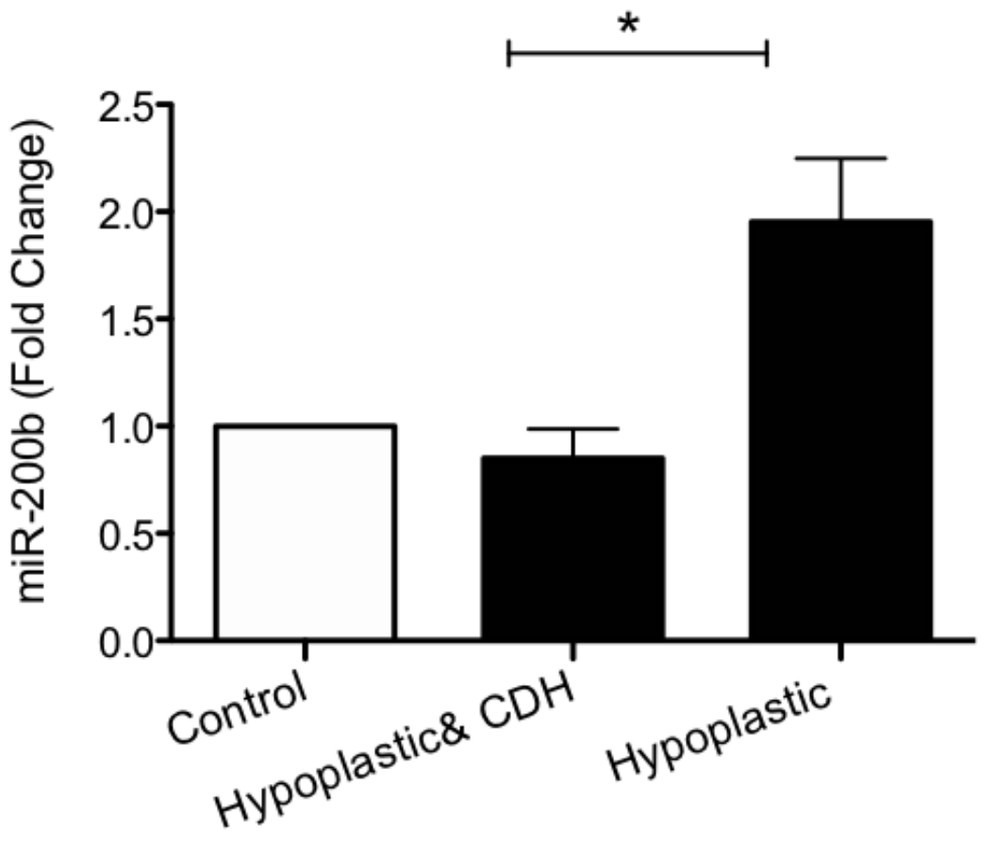
In Situ Hybridization

Control Hypoplastic+ CDH



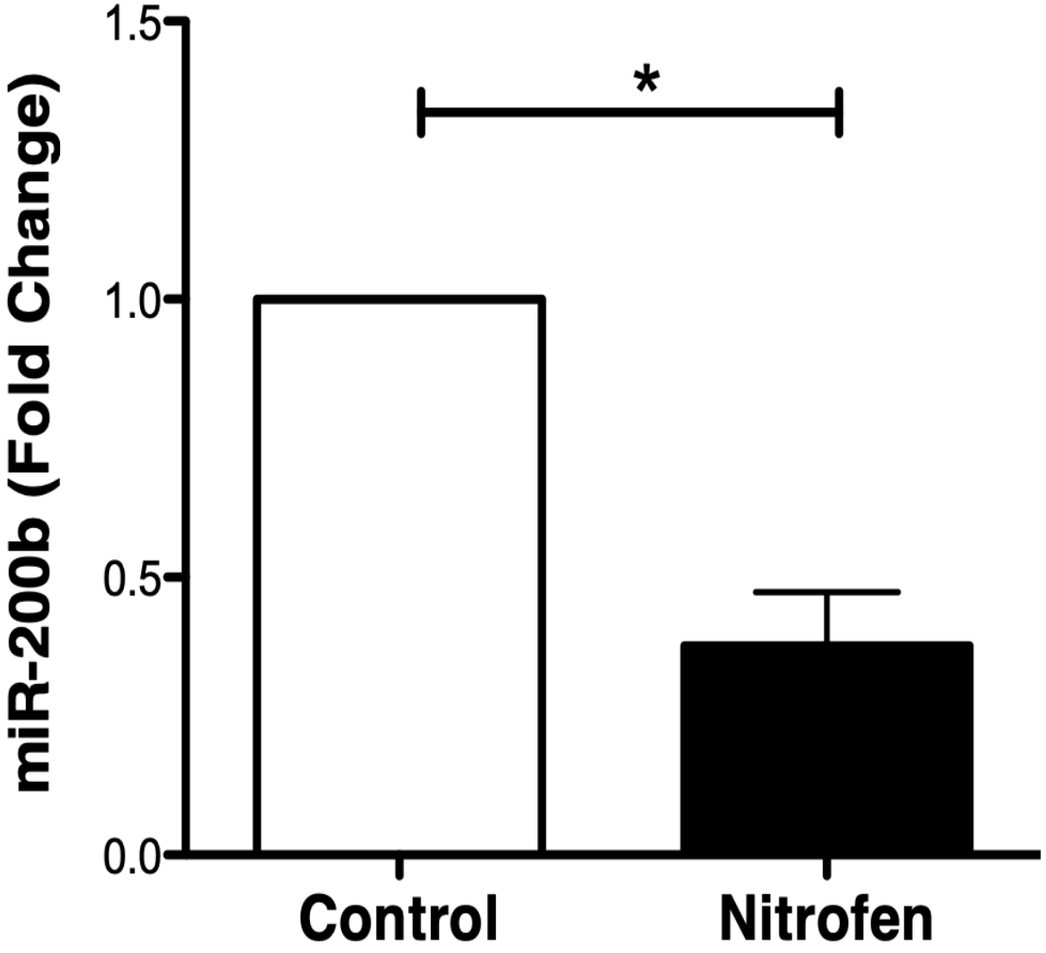
Blue staining: miR-200b

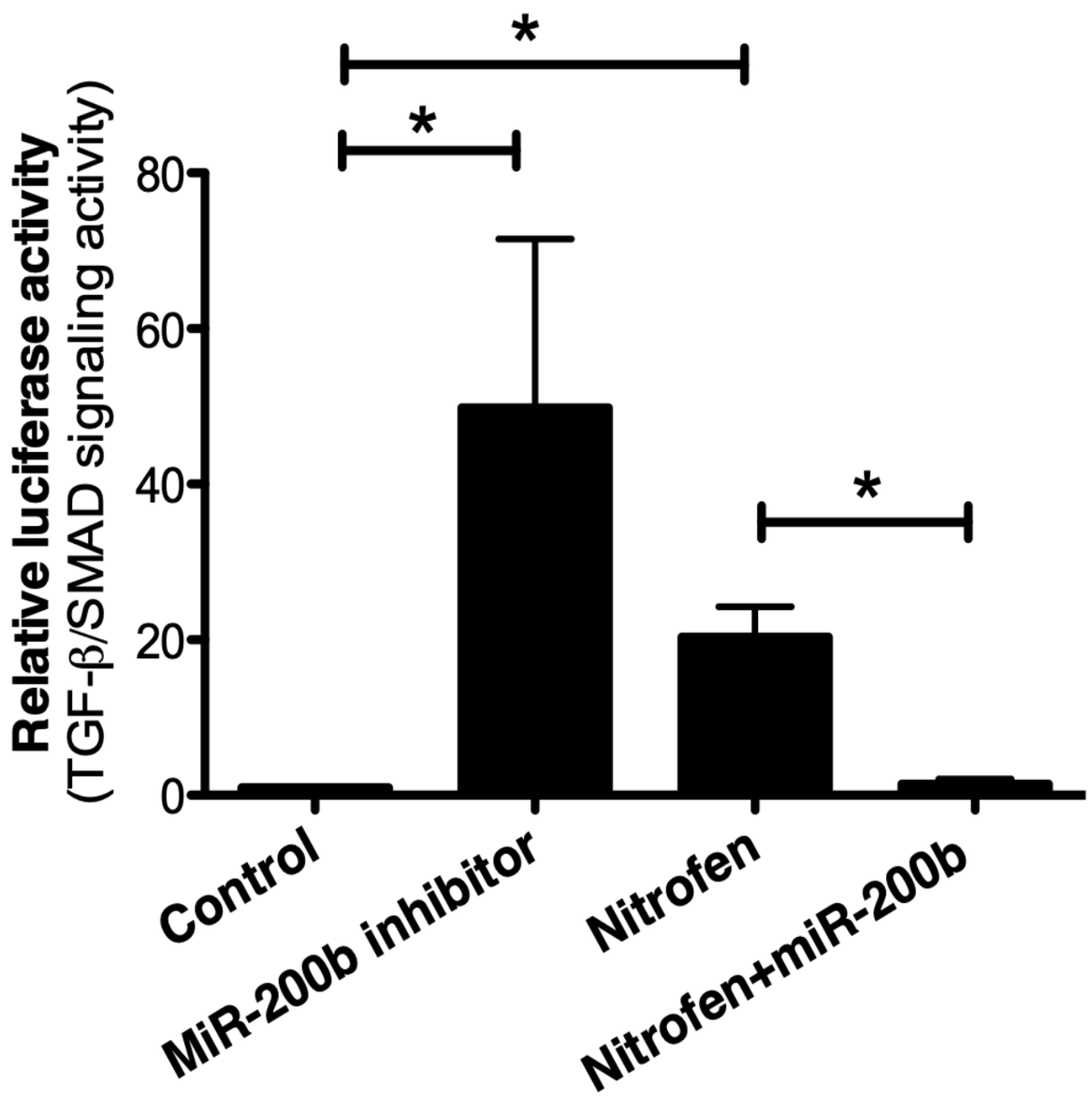
PCR (E21)



* P< 0.05

MiR-200b & Human Bronchial Epithelial Cells





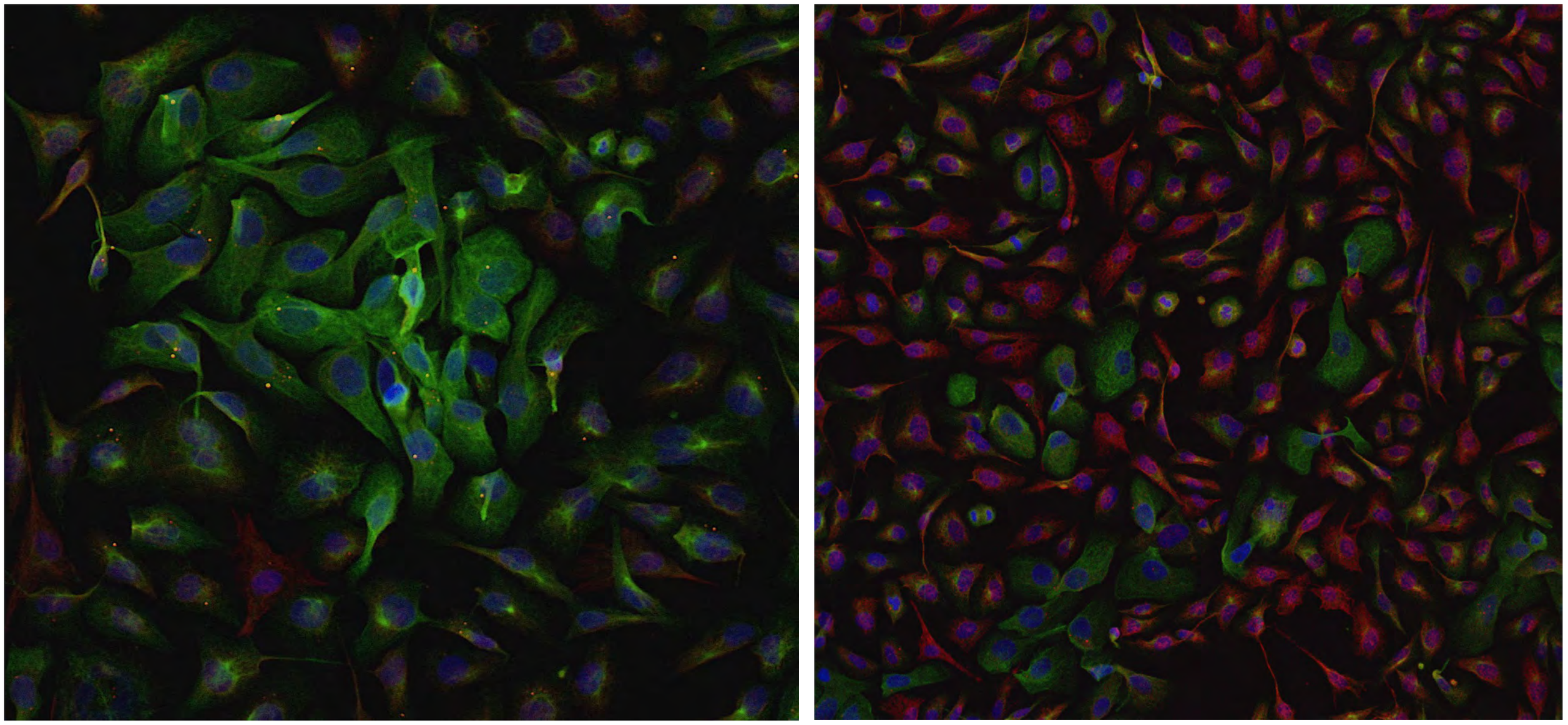
* P< 0.05

Annals of Surgery • Volume 269, Number 5, May 2019

miR-200b maintained epithelial cell phenotype in bronchial epithelial cells

Control

MiR-200b inhibitor



Green : Epithelial Marker (cytokeratin)
Red: Mesenchymal Marker (Vimentin)

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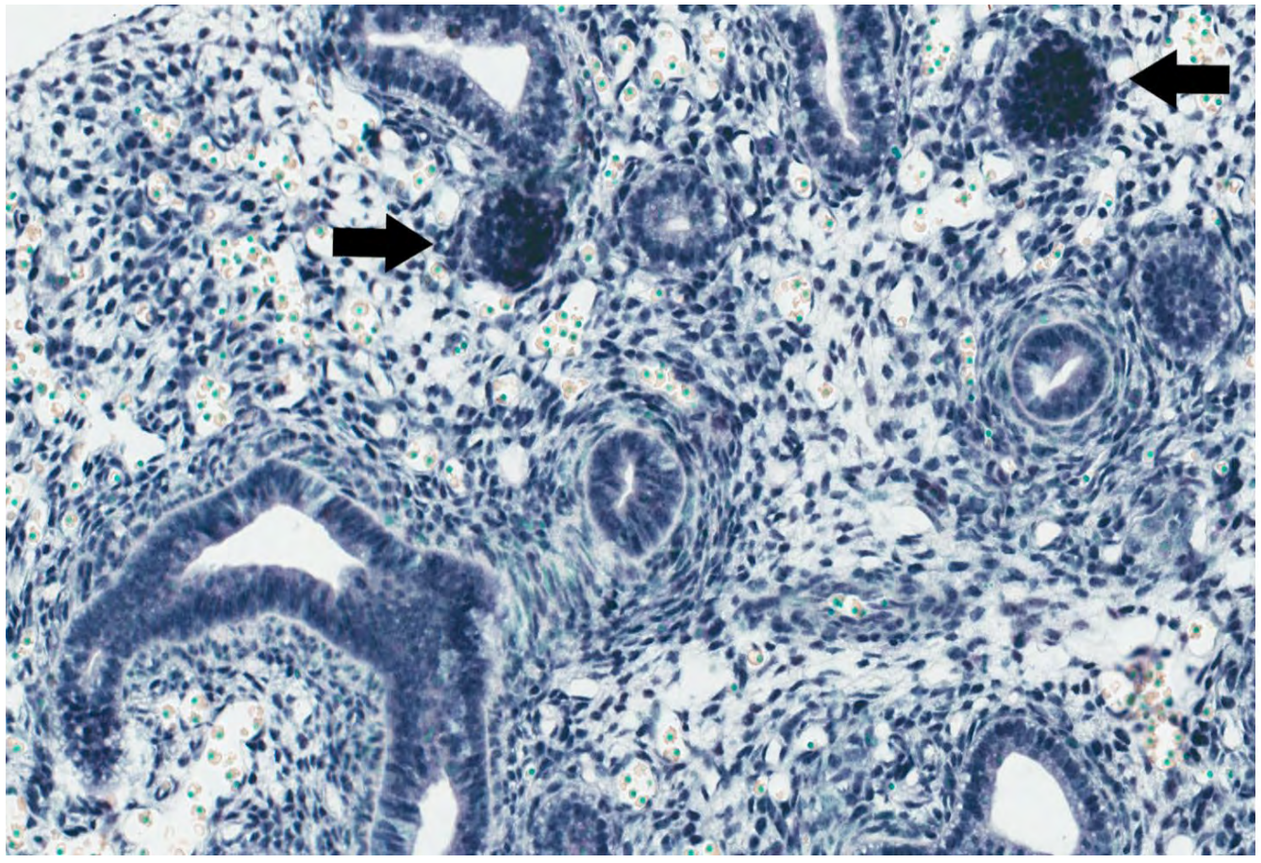
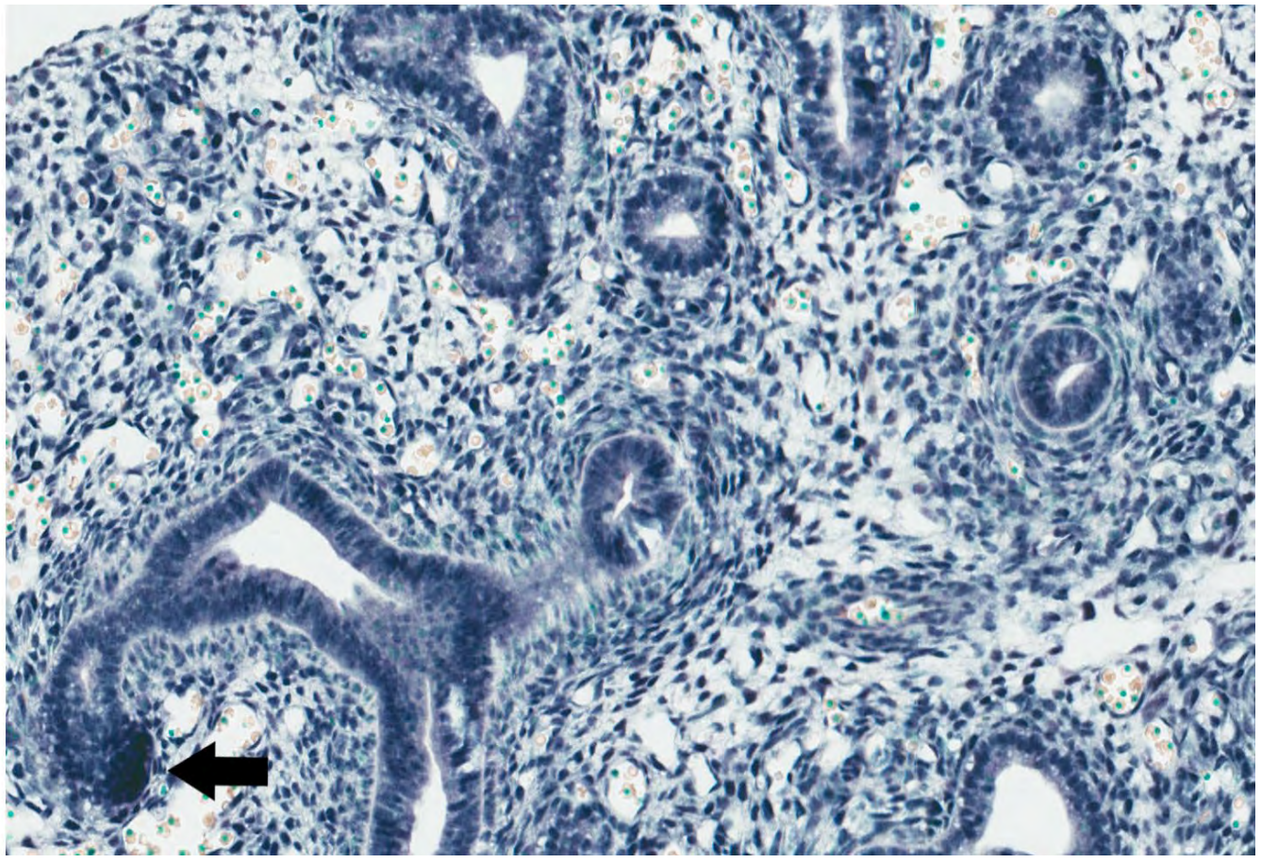
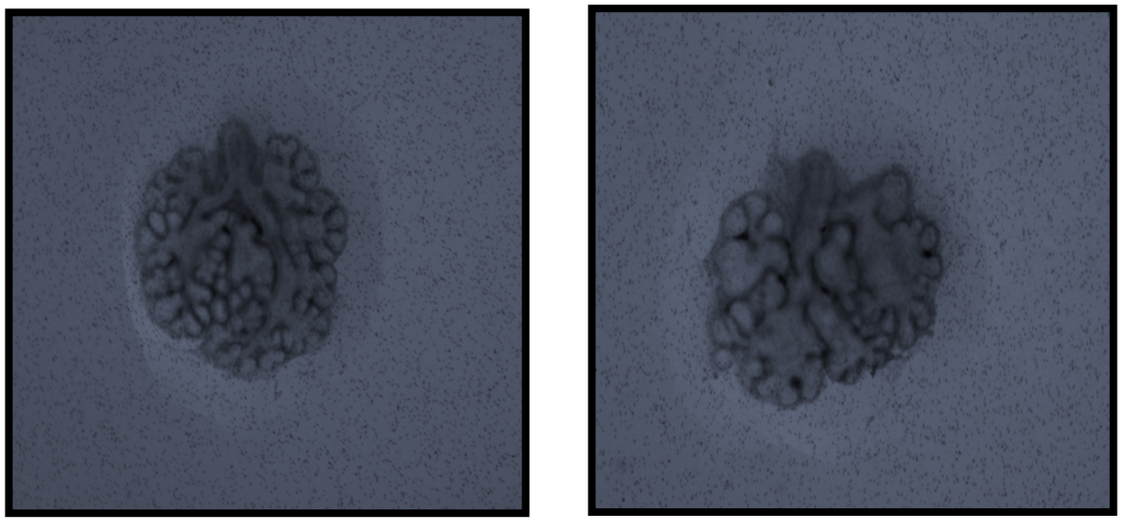
miR-200b expression is high at branching lung tips



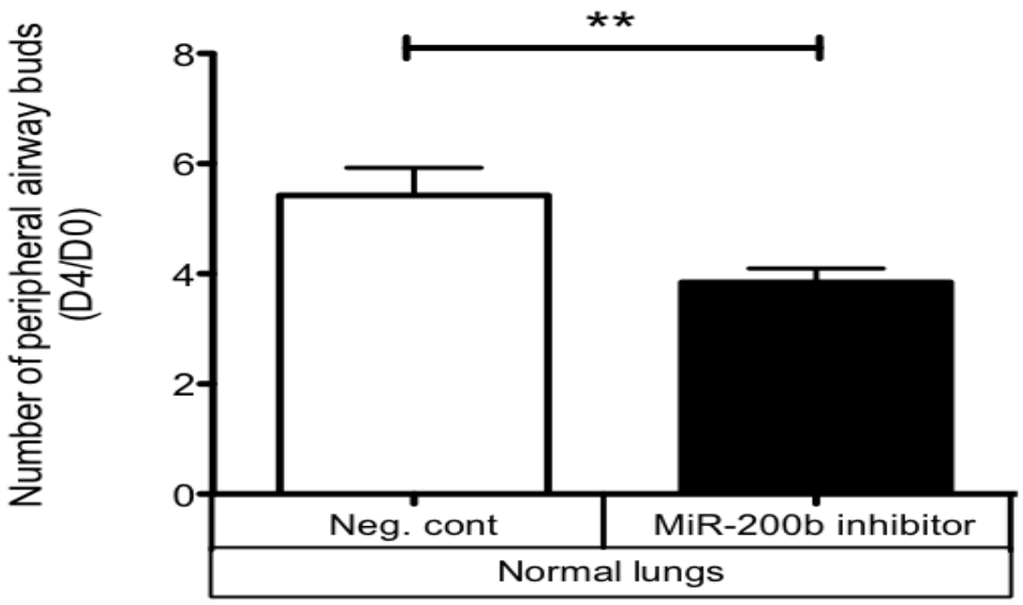


miR-200b improves branching hypoplastic lungs

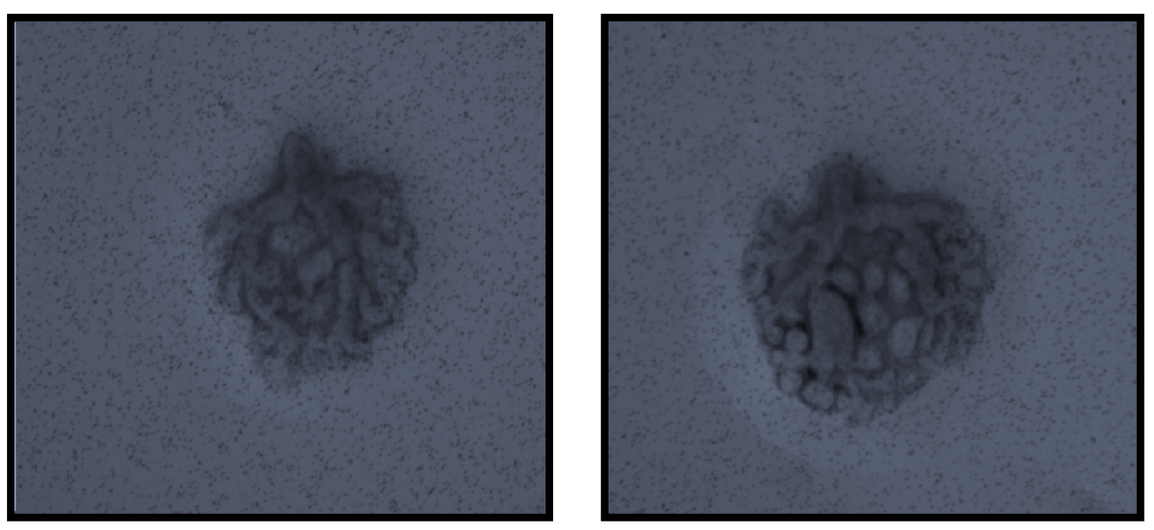
Normal lungs



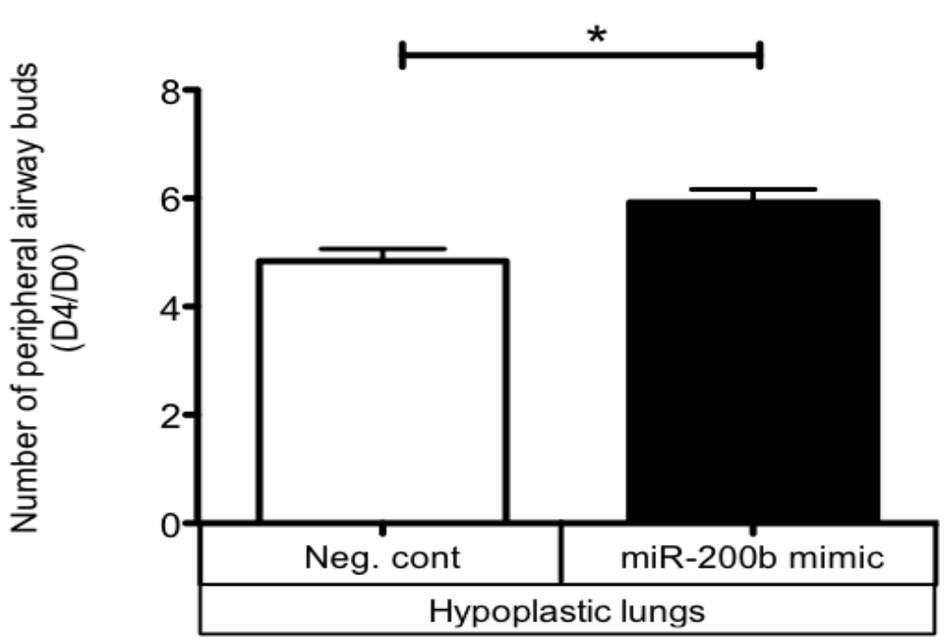
Control 200b inhibitor



Hypoplastic lungs



Hypoplastic Hypoplastic+ miR-200b



* P< 0.05, ** P< 0.01

Annals of Surgery • Volume 269, Number 5, May 2019

NITROFEN MODEL OF CDH

Lung

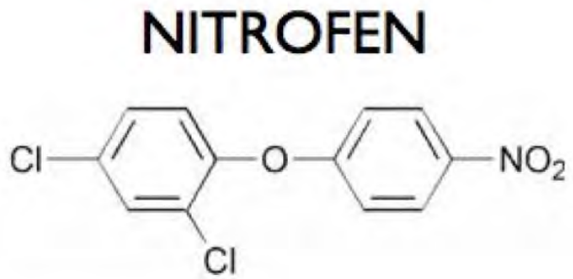
Diaphragm

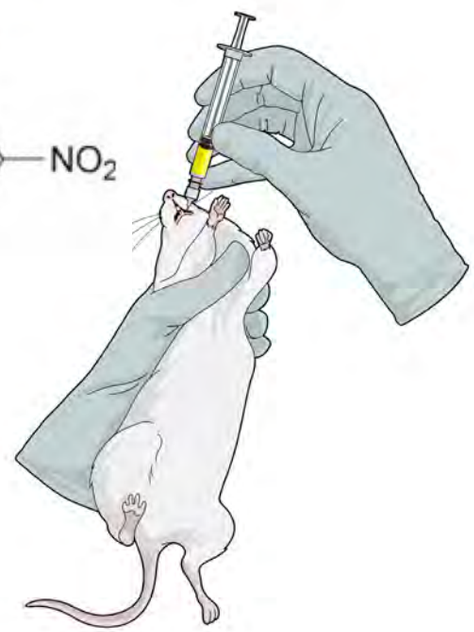
Fetal breathing

Birth

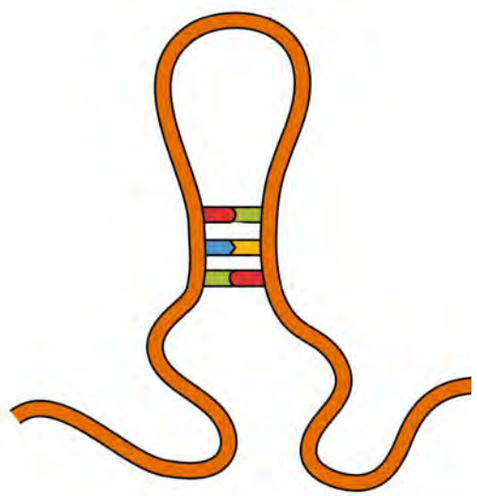
E0 E9 E11 E14 E17 E22

NITROFEN



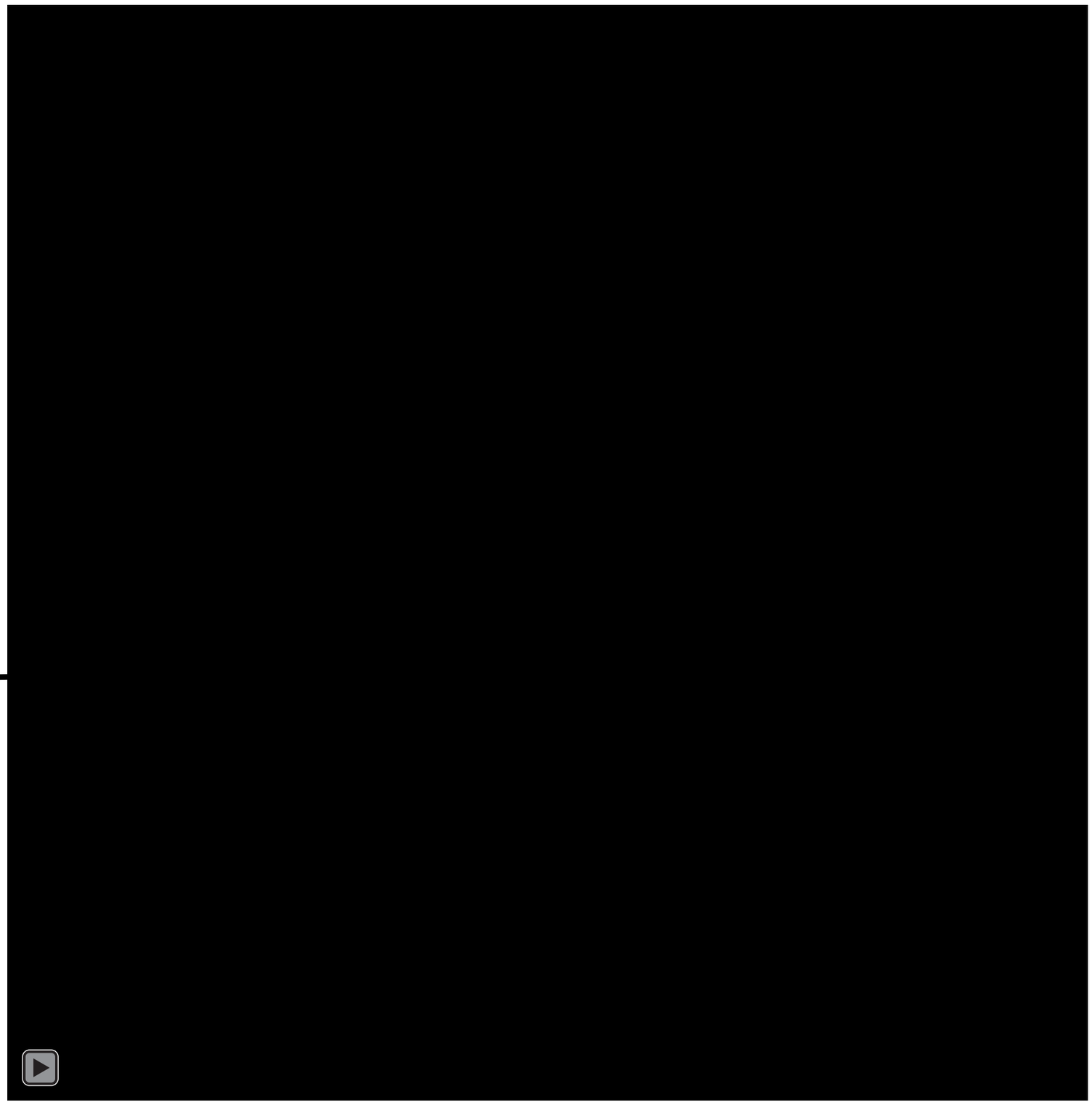


+



?

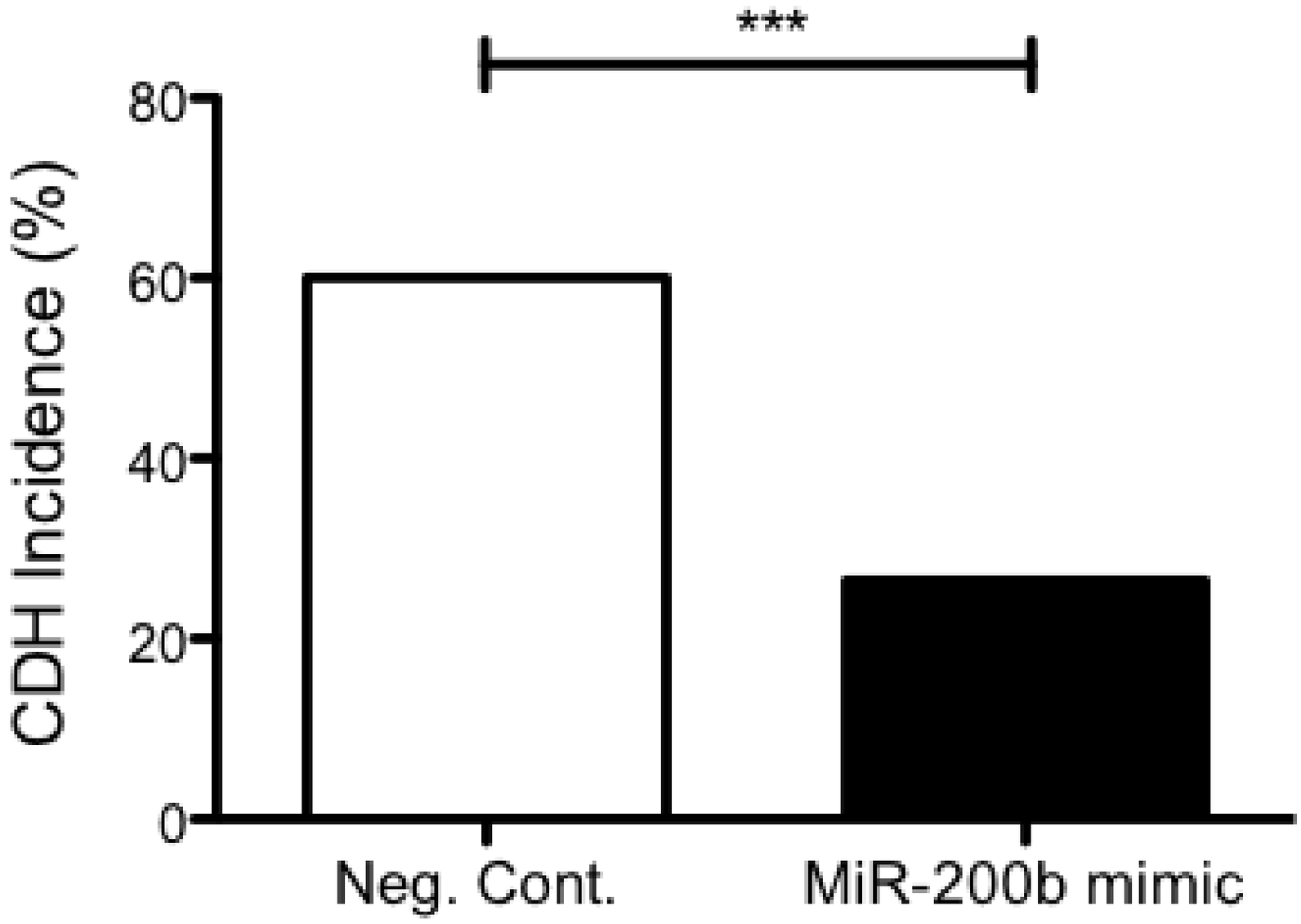
Prenatal miR-200b improves lung hypoplasia



hypoplastic
(80% CDH)

hypoplastic +
miR-200b
(15% CDH)

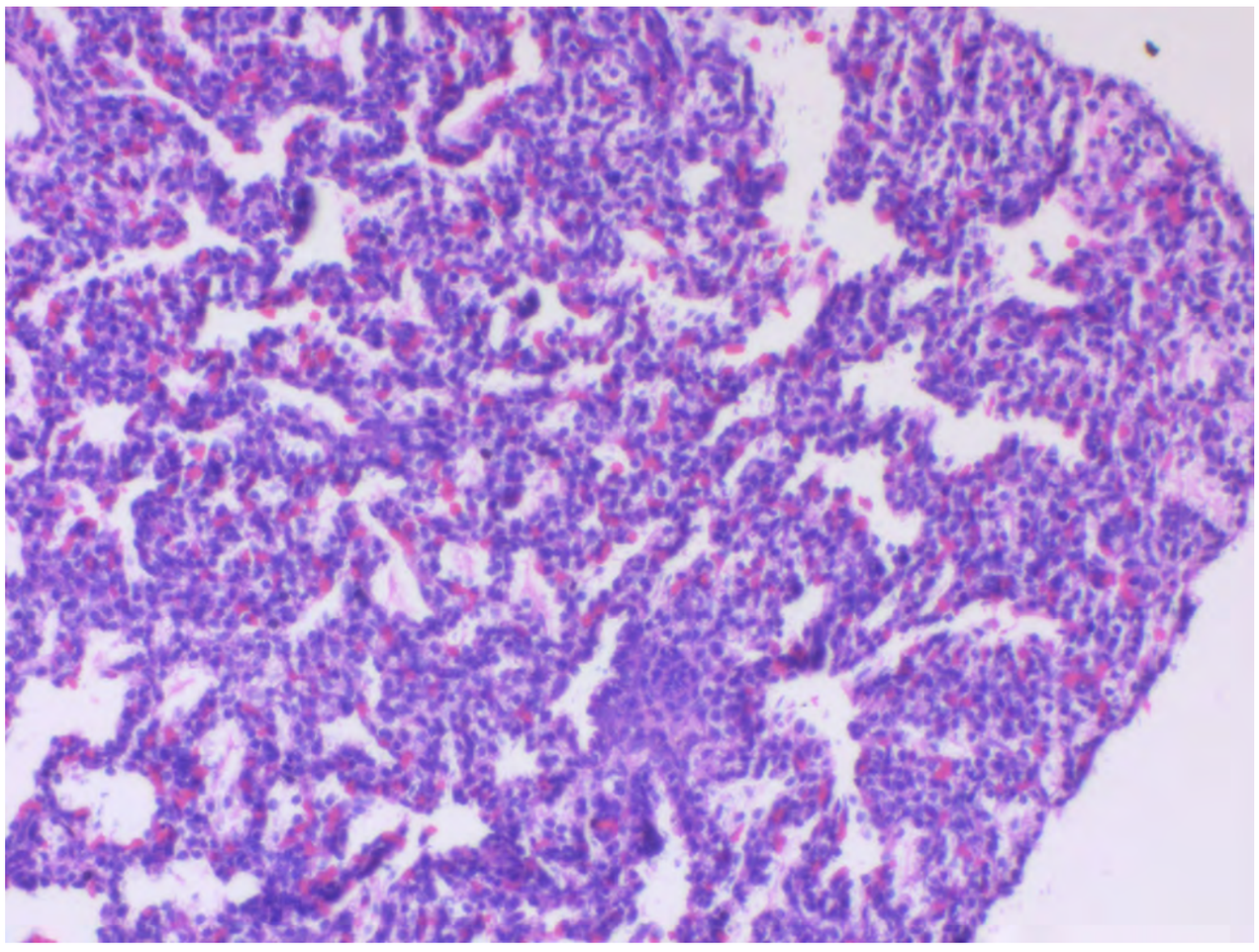
Prenatal miR-200b reduces CDH incidence

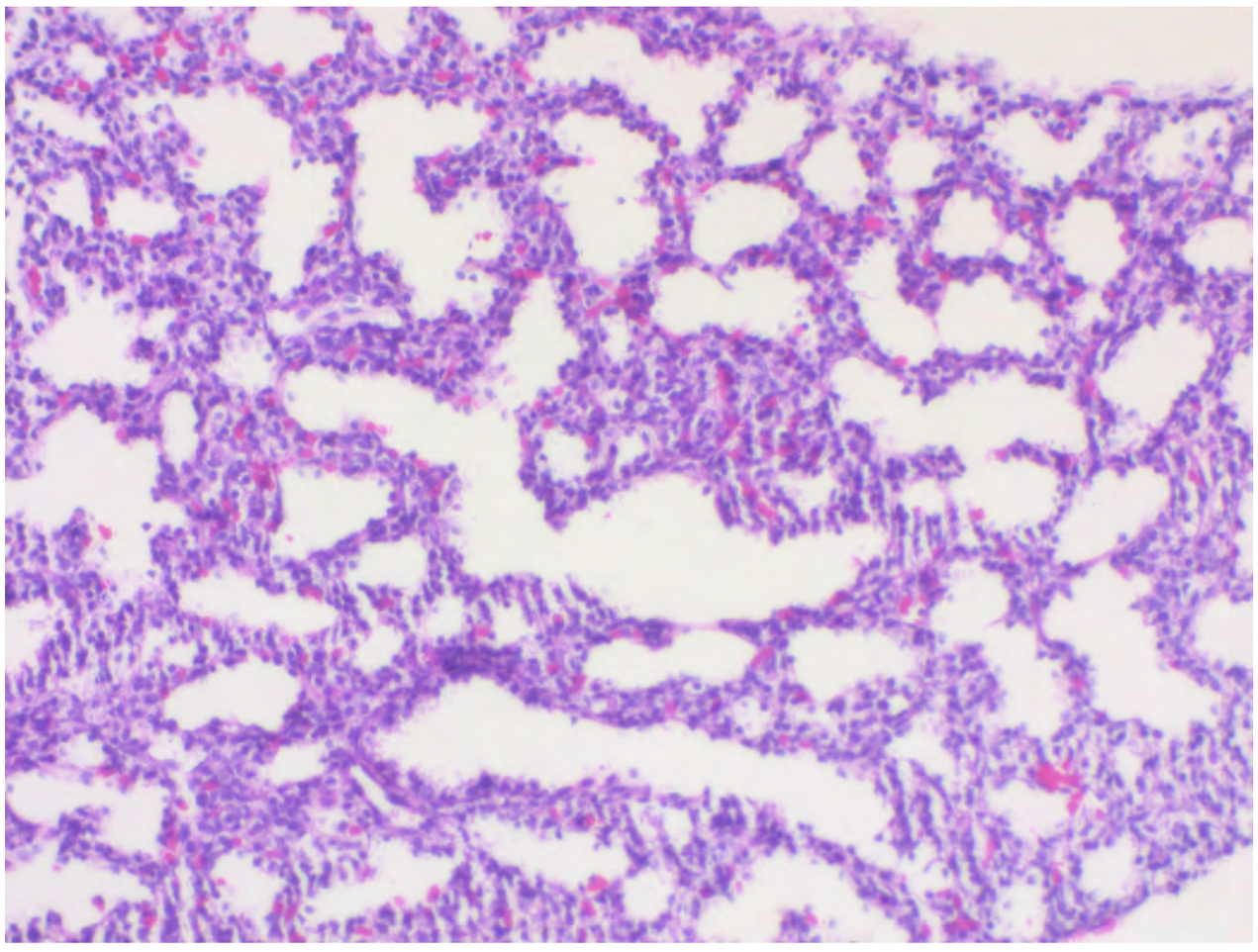


Prenatal miR-200b improves lung hypoplasia

Negative Control

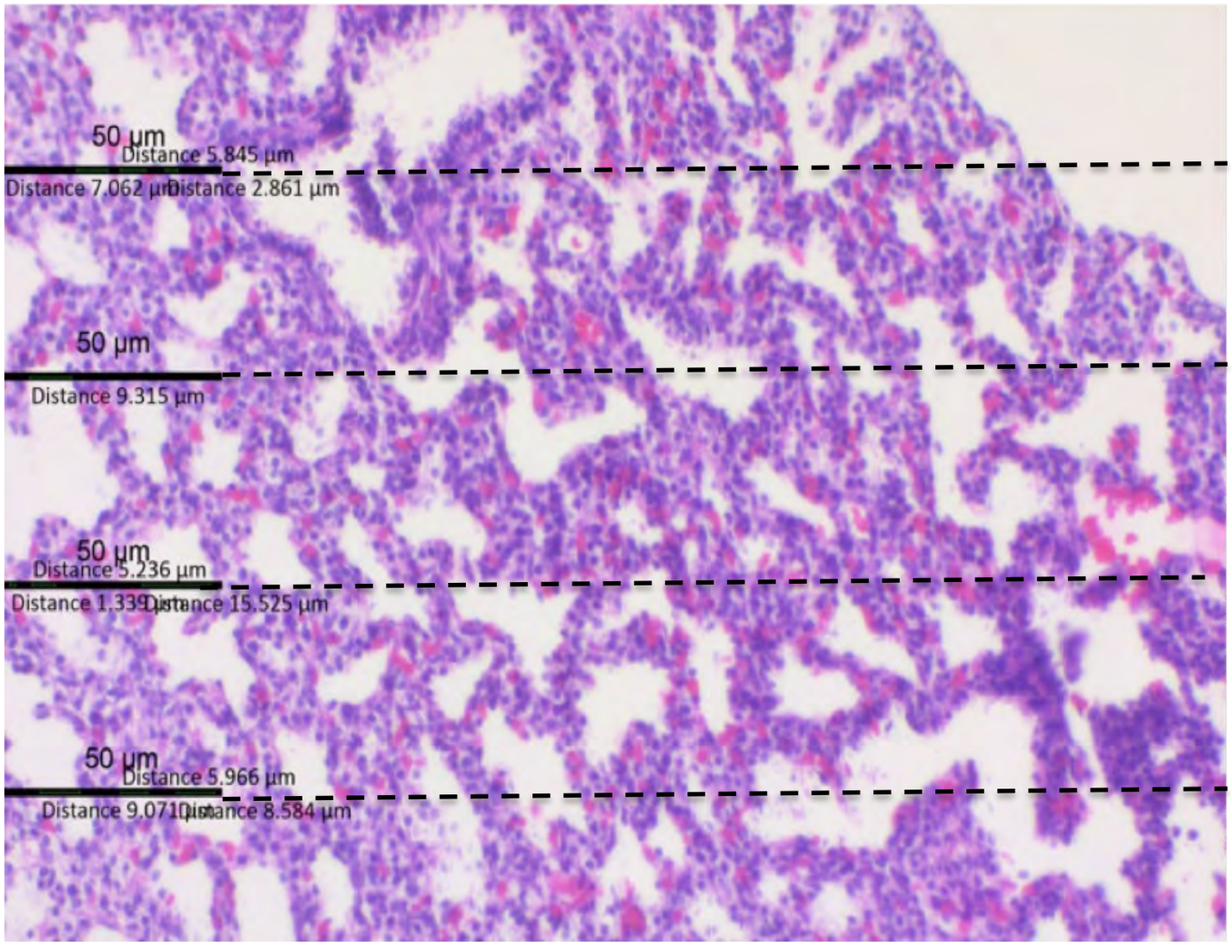
MiR-200b

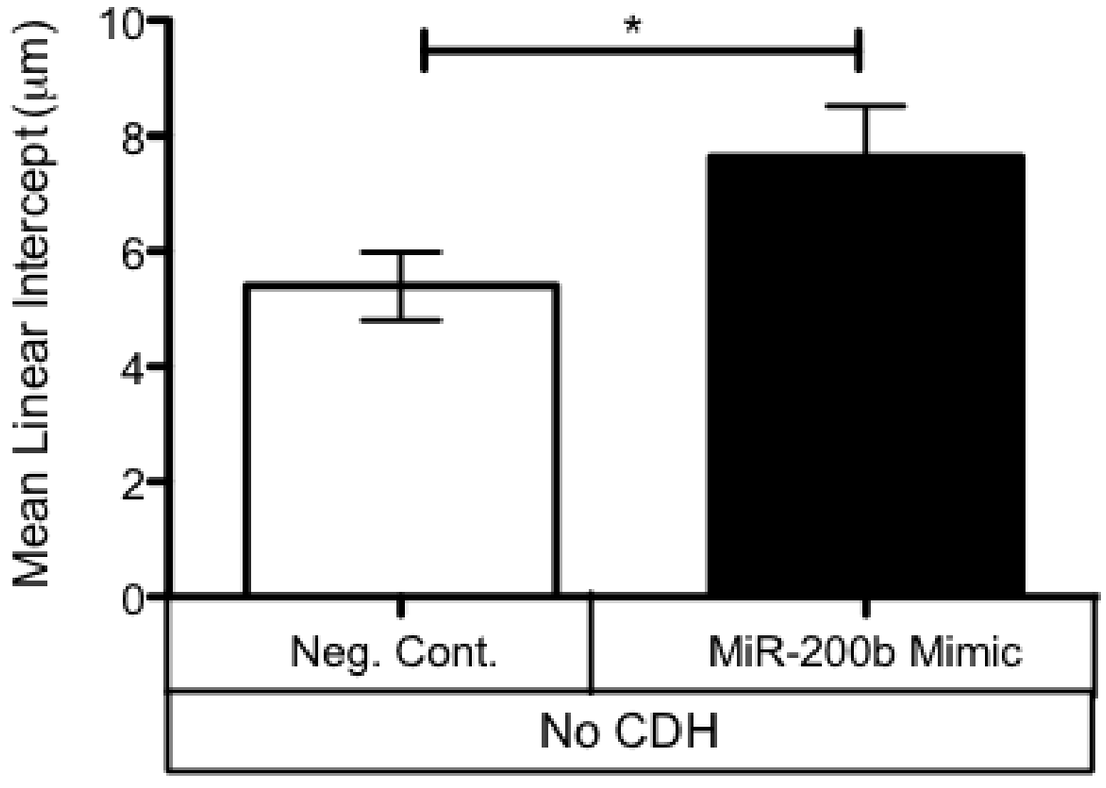




Prenatal miR-200b improves lung hypoplasia

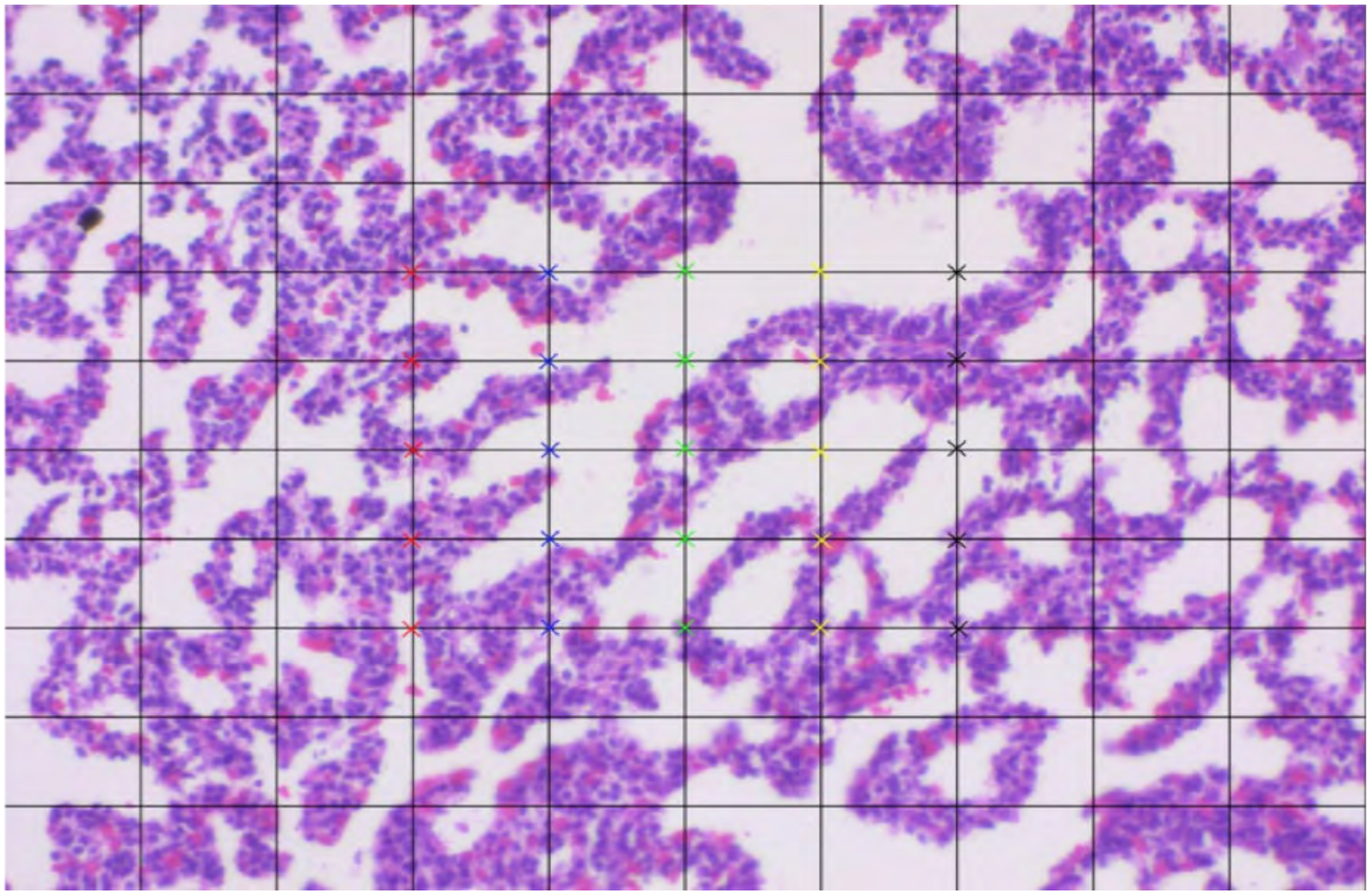
Mean Linear Intercept

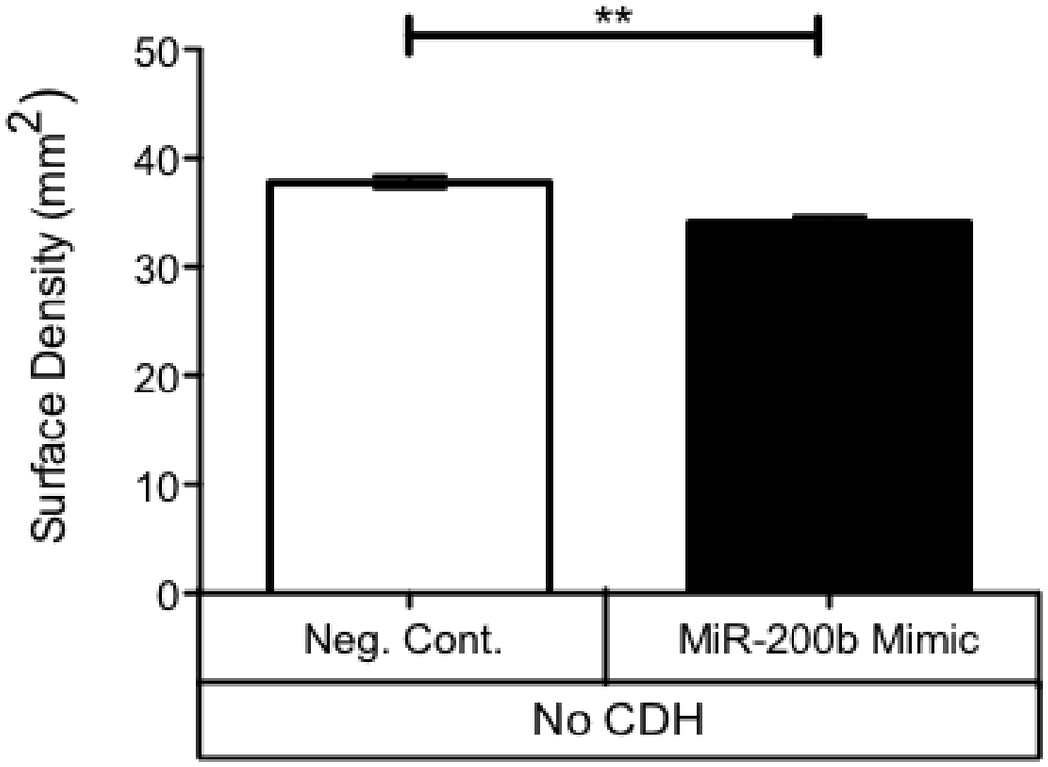




Prenatal miR-200b improves lung hypoplasia

Surface Density





control lung

CDH lung

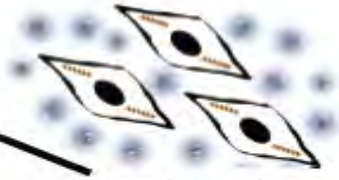
miR-200b ↓

fibroblasts

epithelial cells

epithelial cells

TGF-beta ↑



fibroblasts

E-cadherin ↑

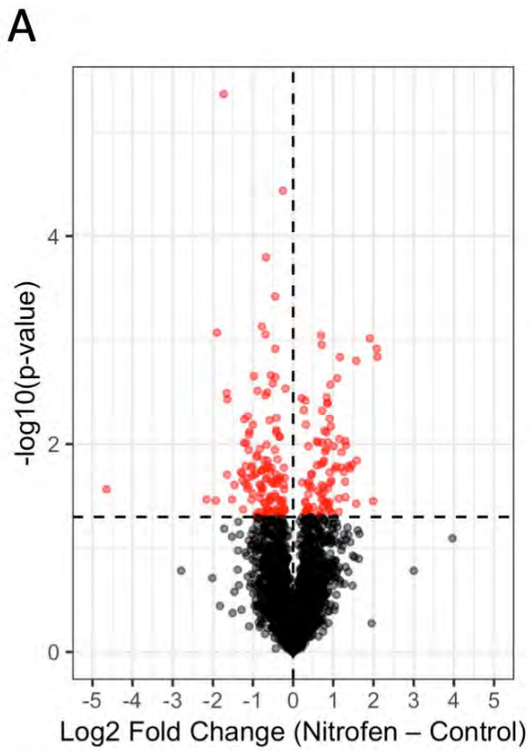
ZEB ↓

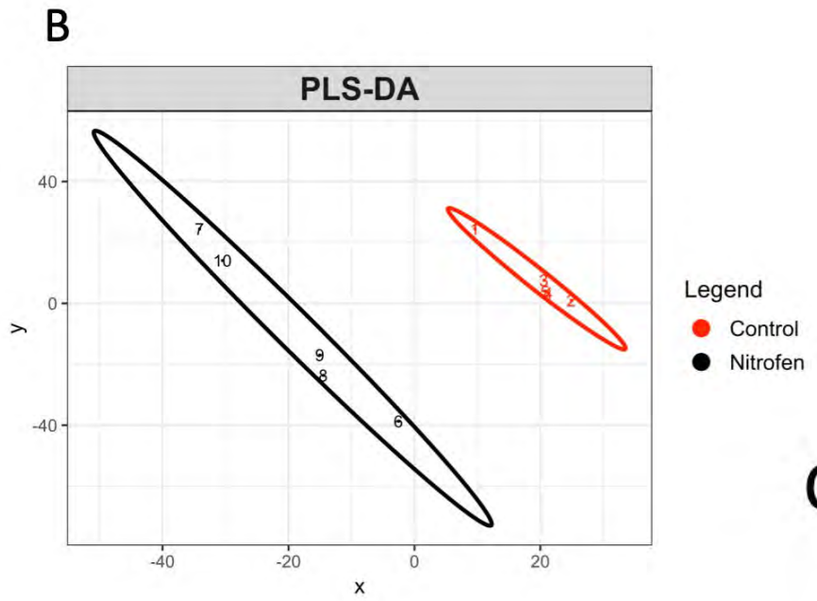
miR-200b ↑

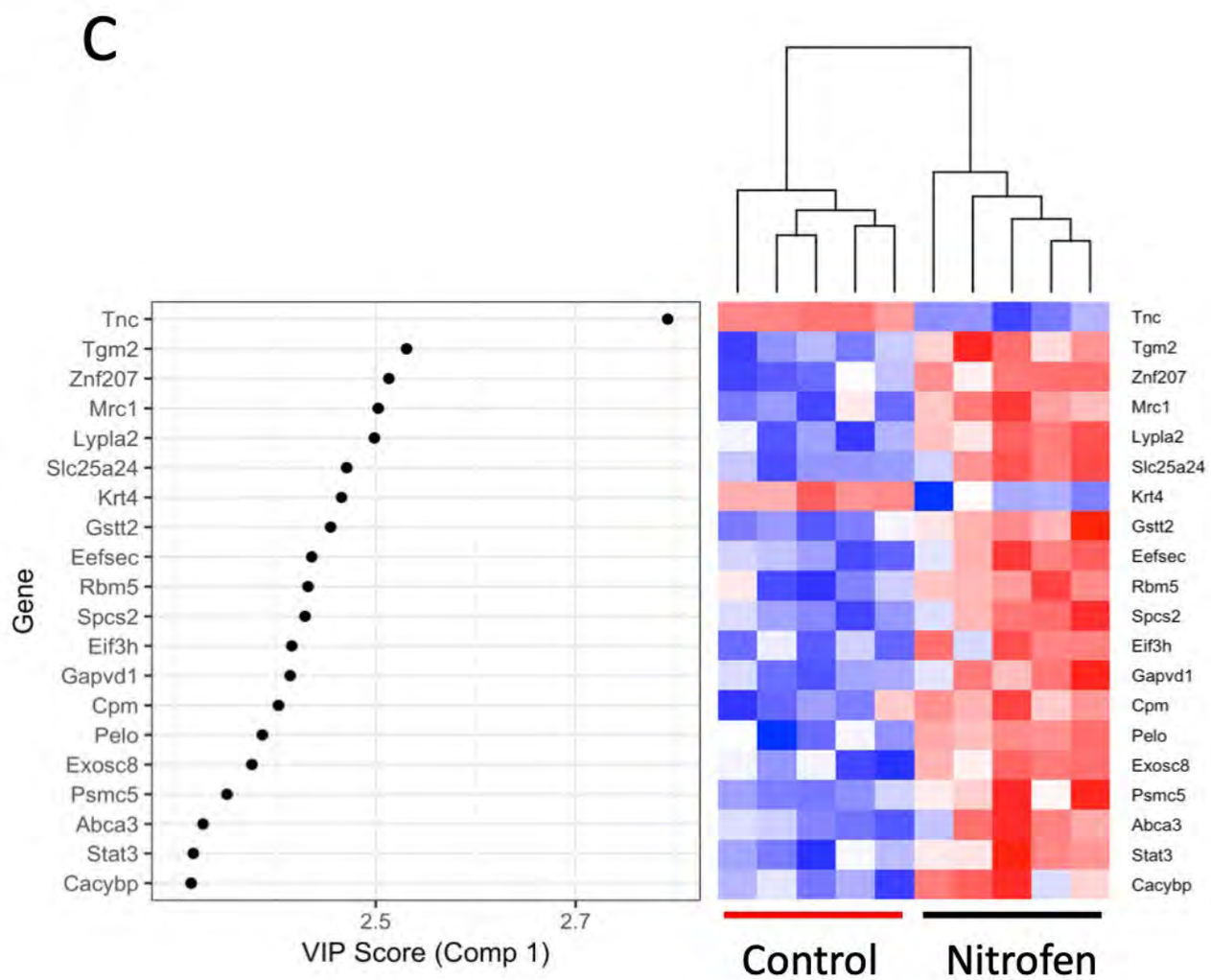
normal lung development

lung hypoplasia

Proteome suggests that nitrofen-induced abnormal lung development is an immune disease?



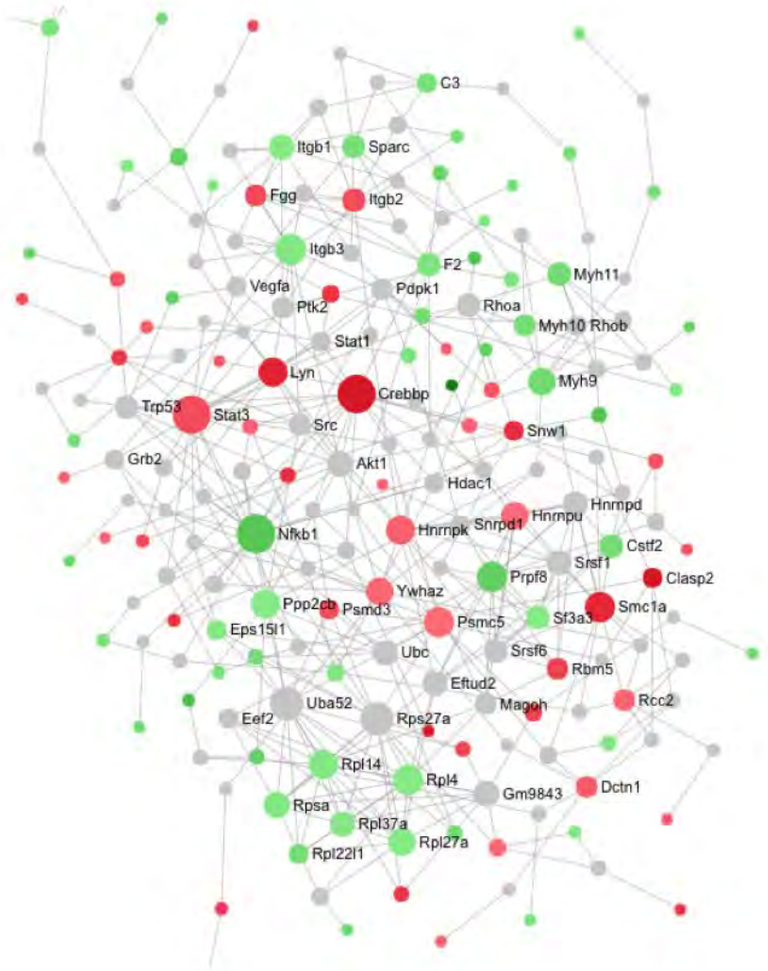




Unpublished results

Proteome suggests that nitrofen-induced abnormal lung development is an immune disease?

A



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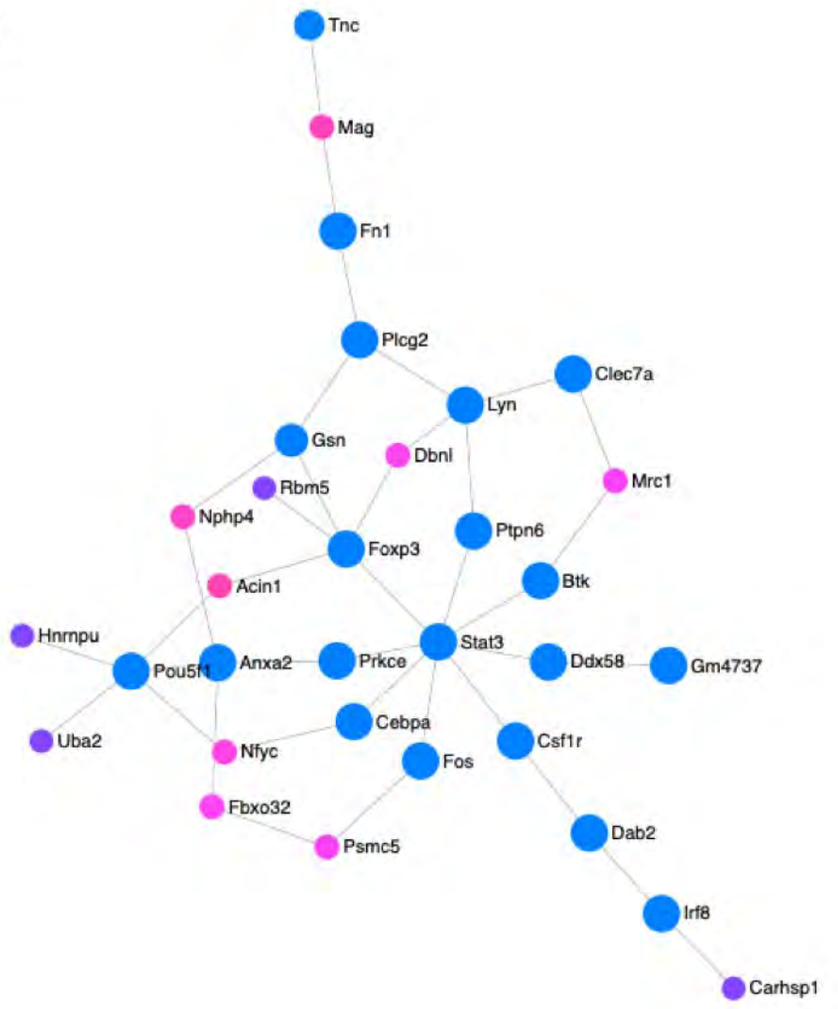
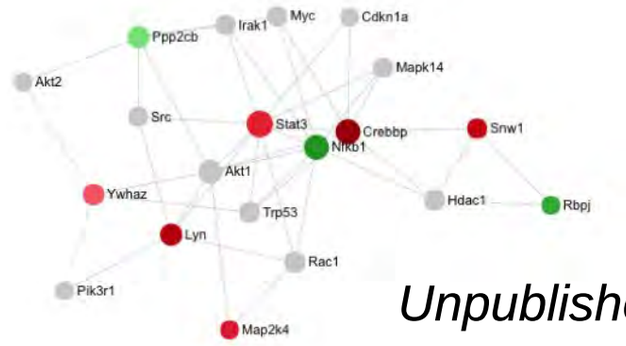


C

Pathway	Total	Expected	Hits	P.Value	FDR
Immune System	1110	27.3	62	4.25E-11	3.33E-08
Signaling by Interleukins	118	2.91	19	5.09E-11	3.33E-08
Cytokine Signaling in Immune system	270	6.66	26	1.62E-09	7.09E-07

D



Unpublished results

Upregulated nodes KEGG

Pathway	Total	Expected	Hits	P.Value	FDR
Epstein-Barr virus infection	114	0.458	4	0.000973	0.207
Cell cycle	127	0.51	3	0.0136	0.75
Phagosome	49	0.197	2	0.0161	0.75
Notch signaling pathway	49	0.197	2	0.0161	0.75
Wnt signaling pathway	147	0.59	3	0.02	0.75
Tuberculosis	170	0.682	3	0.0293	0.75
Salmonella infection	68	0.273	2	0.0299	0.75
Long-term potentiation	69	0.277	2	0.0307	0.75
Phenylalanine, tyrosine and tryptophan biosynthesis	8	0.0321	1	0.0317	0.75
Fc epsilon RI signaling pathway	75	0.301	2	0.0358	0.762
ErbB signaling pathway	87	0.349	2	0.0469	0.829
Malaria	12	0.0482	1	0.0472	0.829

Unpublished results

Does EBV cause CDH?????

JOURNAL OF VIROLOGY, Oct. 2010, p. 10329–10343 Vol. 84, No. 19
0022-538X/10/$12.00 doi:10.1128/JVI.00923-10

Cellular MicroRNAs 200b and 429 Regulate the Epstein-Barr Virus Switch between Latency and Lytic Replication[▽]

Amy L. Ellis-Connell,† Tawin Iempridee, Iris Xu, and Janet E. Mertz*

Tumor and Stem Cell Biology

Cancer Research

Downregulation of MicroRNA-200 in EBV-Associated Gastric Carcinoma

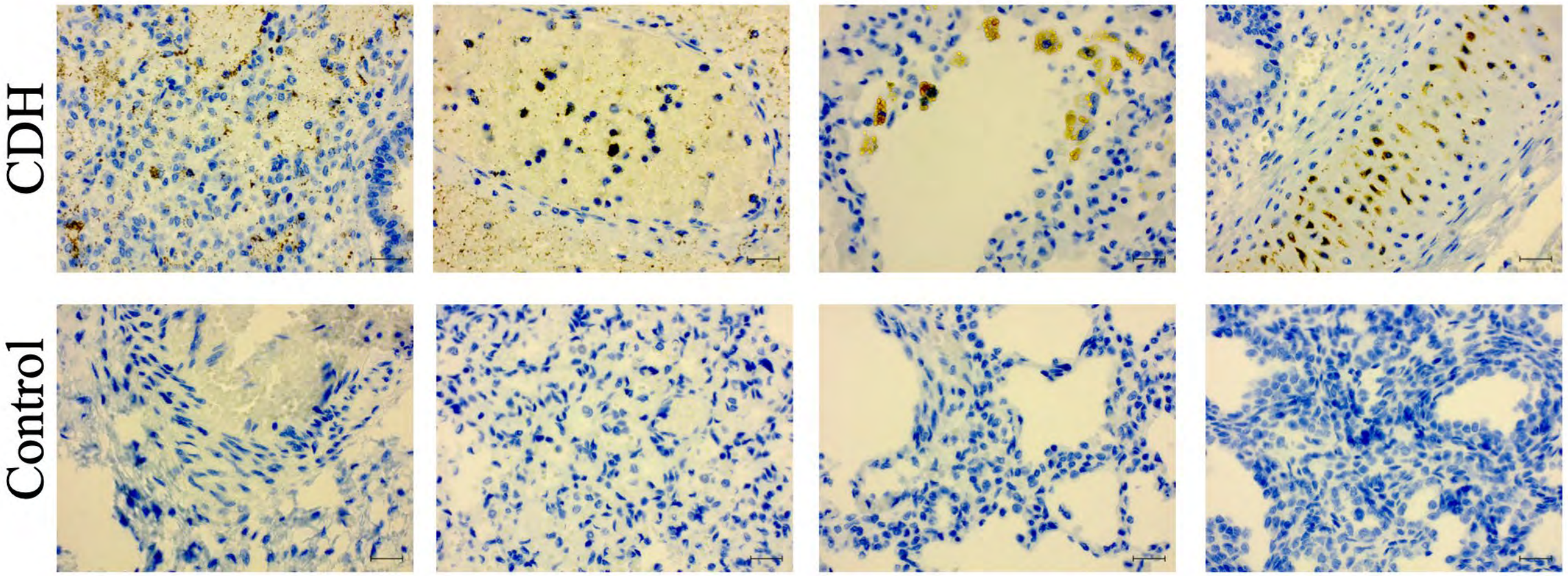
Aya Shinozaki[1], Takashi Sakatani[1], Tetsuo Ushiku[1], Rumi Hino[1], Maya Isogai[1], Shunpei Ishikawa[1], Hiroshi Uozaki[1], Kenzo Takada[2], and Masashi Fukayama[1]

JOURNAL OF VIROLOGY, Aug. 2010, p. 7892–7897 Vol. 84, No. 15
0022-538X/10/$12.00 doi:10.1128/JVI.00379-10

Differential Expression of the miR-200 Family MicroRNAs in Epithelial and B Cells and Regulation of Epstein-Barr Virus Reactivation by the miR-200 Family Member miR-429[▽]

Zhen Lin, Xia Wang, Claire Fewell, Jennifer Cameron, Qinyan Yin, and Erik K. Flemington*

Does EBV cause CDH?????



WiSDOM:

WINNIPEG'S SURGICAL DATABASE & OUTCOMES MANAGEMENT

DEVOTION Legacy Project

Drs. Richard Kaplan, Suyin Lum Min, Melinda Morris, Anna Shawyer, Chelsea Ruth, Manji Brownell, Fiamaccia Rieti, and Robert Bellshaw

ABOUT WiSDOM:

WiSDOM is all about children with congenital anomalies. A congenital anomaly is a birth defect. Some babies are born with a congenital anomaly that requires surgery immediately after birth. Until a few decades ago, most of these babies died. Recent improved surgical and intensive care techniques have resulted in better survival. Currently, we do not know how these babies do later in life when they grow up. To find out, we created a database of almost 800 surgical congenital anomaly patients, recording birth and surgery details. We plan to link our surgical database to population data managed by the Manitoba Centre for Health Policy (MCHP). MCHP databases contain unique information about healthcare, education, and social service utilization for all Manitobans. This linkage will allow us to answer the following questions:

1. **Who** is at greatest risk of giving birth to a baby with a congenital anomaly requiring surgery?
2. **How** do children with surgical congenital anomalies do at school and in life compared to children without a defect?
3. **Why** do some children with surgical congenital anomalies do better than other children with surgical congenital anomalies?

The answers to these questions will help us understand the causes and long-term outcomes of surgical birth defects and improve the care of babies born with surgical congenital anomalies.

OBJECTIVES:

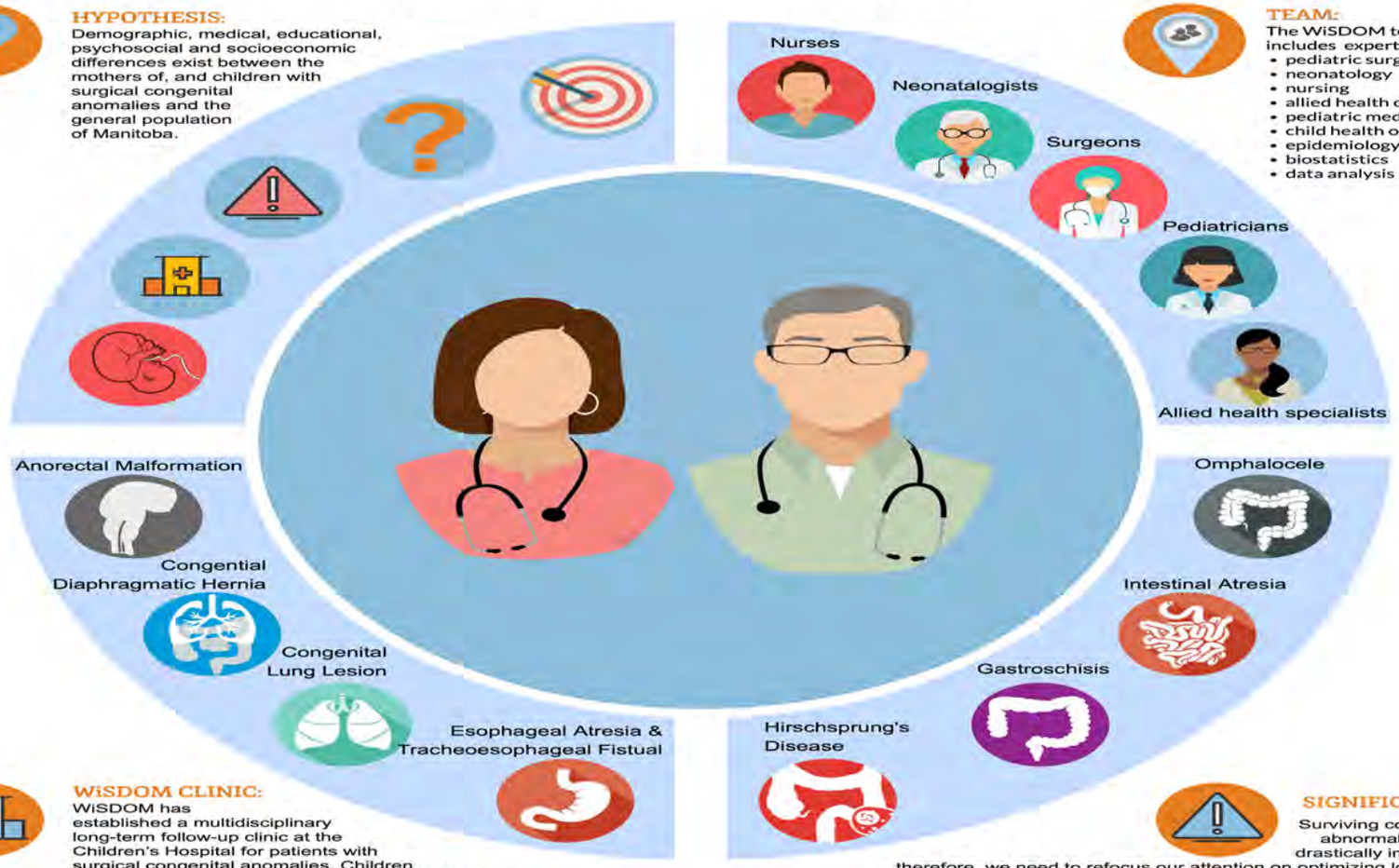
1. WHO is at risk: Identify maternal factors associated with surgical congenital anomalies: Maternal demographic information will be identified using MCHP databases to determine the populations with highest risk of having a baby with surgical congenital anomalies.

2. HOW are children doing: Compare the long-term outcomes of patients with surgical congenital anomalies to those of age-matched controls: Virtual long-term follow-up of each patient in WiSDOM using MCHP linkages to determine their medical, educational, psychosocial, socioeconomic outcomes and comparing them with an age-matched control from MCHP.

3. WHY do some children do better: Determine patient and maternal factors that affect long-term patient outcomes: After defining the maternal risk factors and long-term outcomes for the WiSDOM patient-cohort, subgroups who have the highest risk of poor outcomes will be identified, as well as as the demographic, medical, educational, psychosocial and socioeconomic determinants of favourable long-term outcomes.

HYPOTHESIS:

Demographic, medical, educational, psychosocial and socioeconomic differences exist between the mothers of, and children with surgical congenital anomalies and the general population of Manitoba.



TEAM:

The WiSDOM team includes expertise in:

- pediatric surgery
- neonatology
- nursing
- allied health care
- pediatric medicine
- child health outcomes
- epidemiology
- biostatistics
- data analysis

WiSDOM CLINIC:

WiSDOM has established a multidisciplinary long-term follow-up clinic at the Children's Hospital for patients with surgical congenital anomalies. Children attending the clinic receive evidence-based follow-up for early indentification and intervention for patients and families. Other advantages of the WiSDOM clinic include improved and coordinated care, ongoing education, increased quality of life, and ease of access to health care professionals.

SIGNIFICANCE:

Surviving congenital abnormalities has drastically improved, therefore, we need to refocus our attention on optimizing long-term medical, educational, psychosocial and socioeconomic outcomes. By linking our surgical congenital anomalies patient cohort with the comprehensive information in the MCHP databases we will be able to begin this optimization. Linking databases will provide the opportunity for virtual long-term follow-up for the first time in this patient population. This virtual follow-up will identify the risk factors and outcomes of surgical congenital anomalies. This information will guide the development of preventative strategies to provide better care to babies born with surgical congenital anomalies.

DEVOTION:

1. Provides support for prospective data collection and entry for babies born with surgical congenital anomalies after 2016
2. Facilitates consent and enrollment of participants into the WiSDOM study and collection of research data in the WiSDOM Clinic
3. Helps to distribute information to high-risk populations, healthcare providers and stakeholders using research findings from the WiSDOM database.
4. Aids in developing policies to direct risk mitigation strategies
5. Assists in developing a plan to establish a multidisciplinary, long-term follow-up clinic for WiSDOM children born in Manitoba within the next 5 years
6. DEVOTION provided initial funding for the retrospective data collection for all babies born in Manitoba from 1991 - 2016 in addition to the funding for prospective data collection and linkages to MCHP.

the Children's Hospital Research Institute of Manitoba
DEVOTION Developmental Origins of Chronic Diseases in Children Network
University of Manitoba | Rady Faculty of Health Sciences

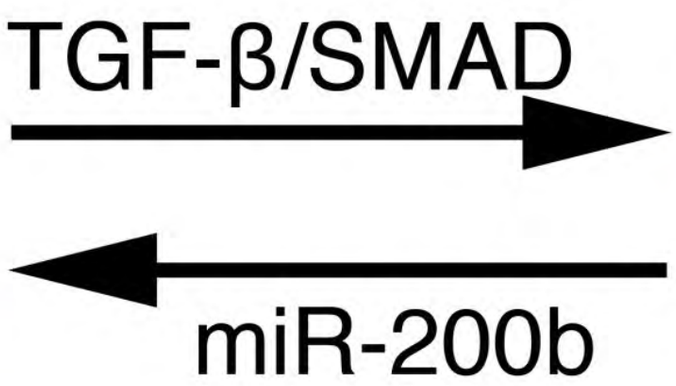
Does EBV cause CDH?????_WiSDOM

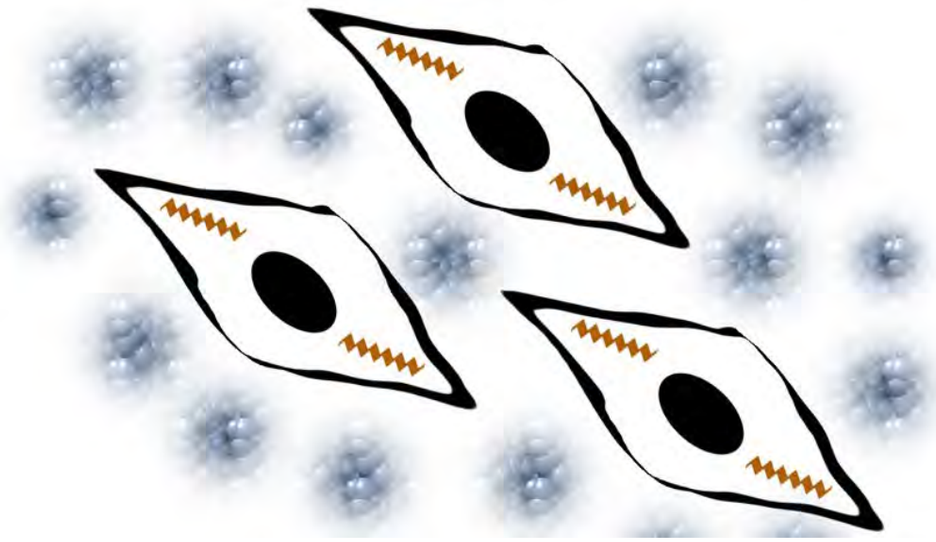
Infectious Mononucleosis (ICD-9:075)

Odds ratio = 0.49 for mothers with CDH baby compared to controls from the general population

95% CI = 0.12-1.36
P-value = 0.2371

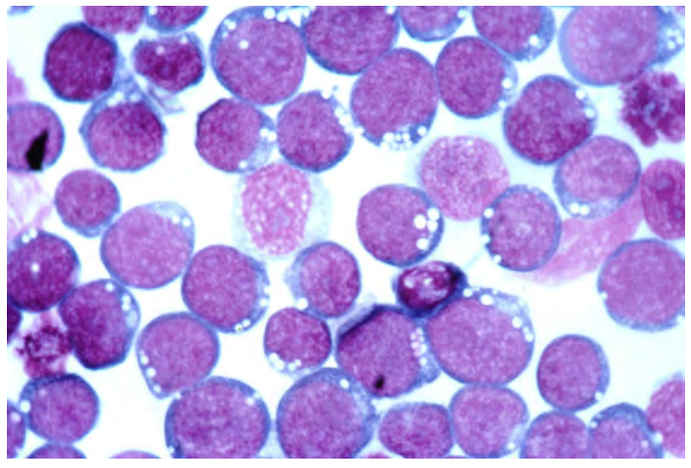
Epithelial-to-Mesenchymal Transition, CDH and EBV





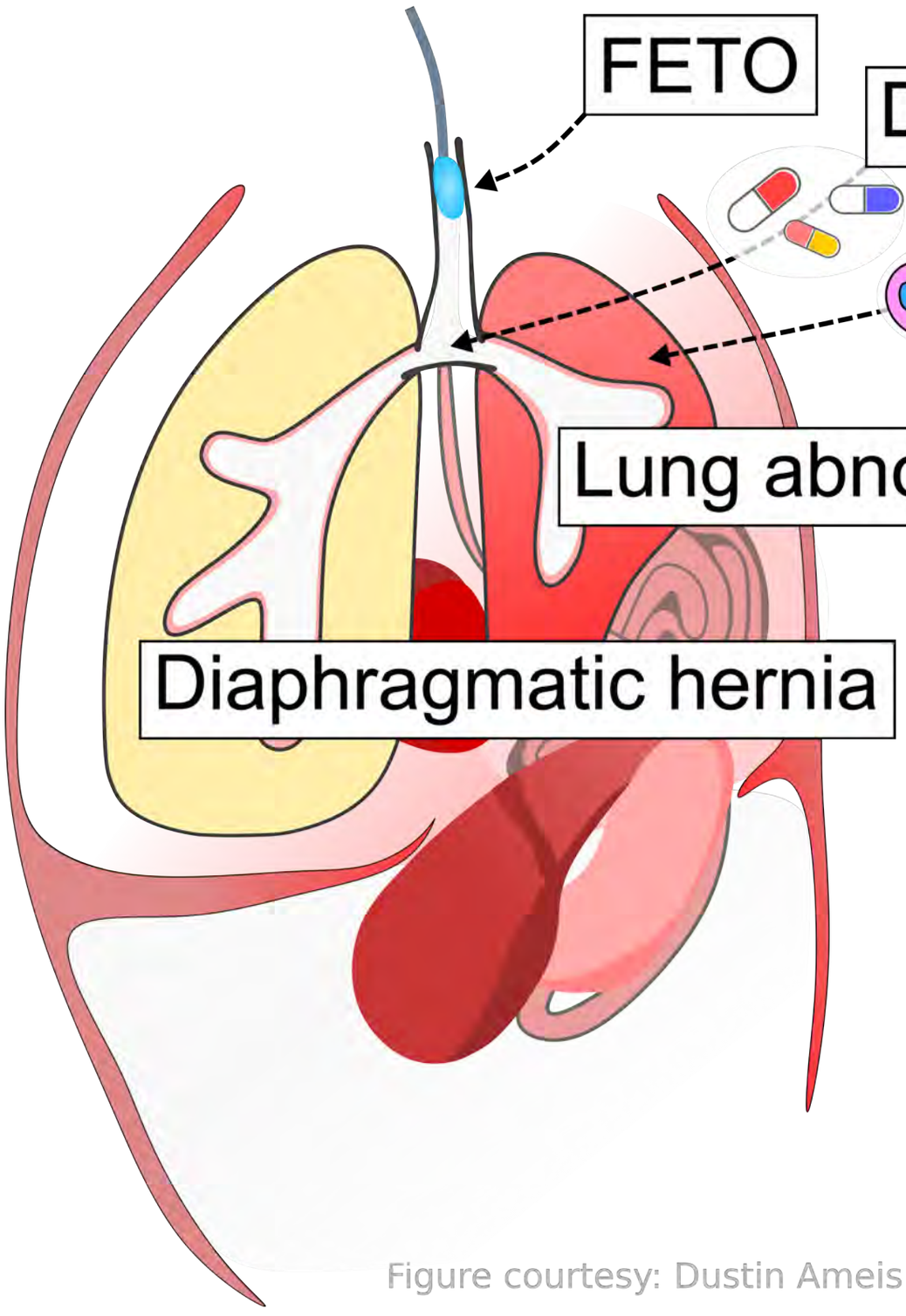
Epithelial cells

Mesenchymal cells



?

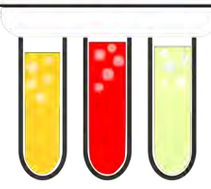
Prenatal treatment



Pathogenesis

Prenatal diagnosis

Biomarkers



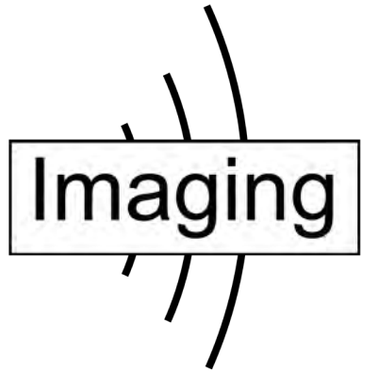
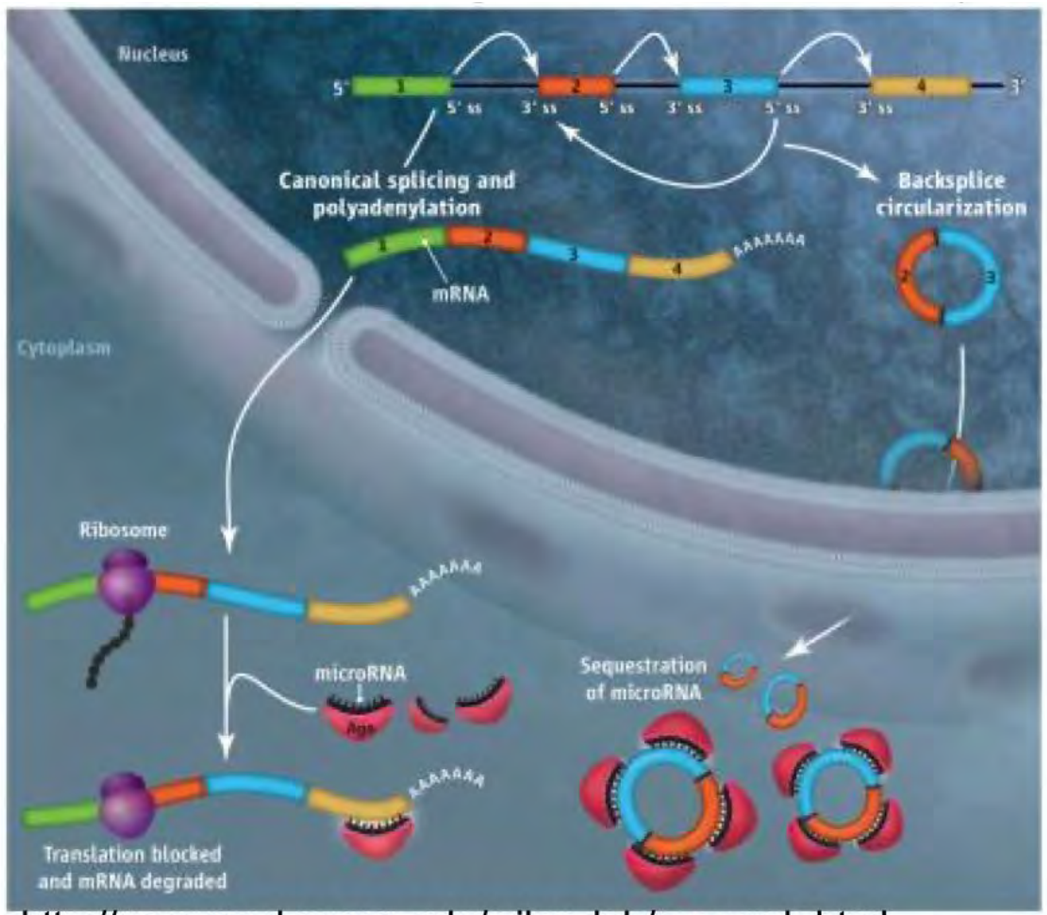


Figure courtesy: Dustin Ameis

CircularRNA

- More stable, more abundant and specific than linear RNAs.
- Regulate gene expression at transcriptional and post-transcriptional level by serving as **microRNA sponges** and interacting with mRNA and proteins.

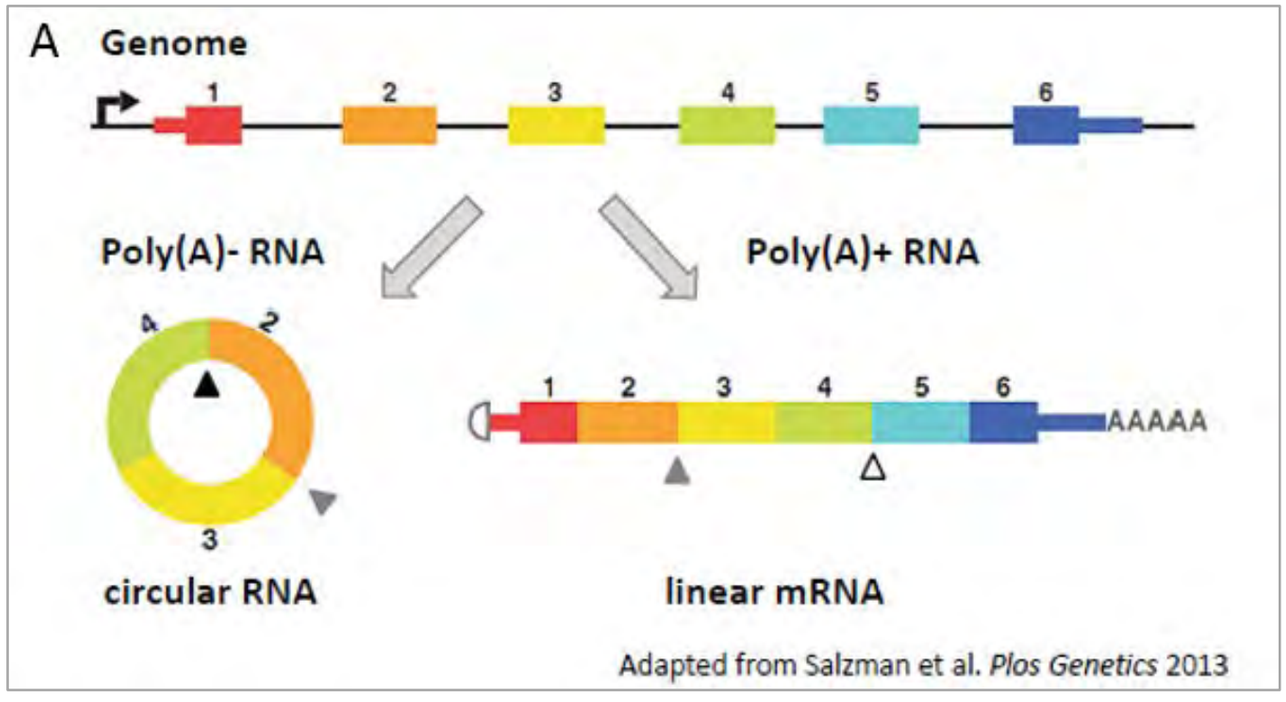


http://www.med.upenn.edu/wiluszlab/research.html

http://www.epibeat.com/developmental-biology-stem-cells/circular-rnas-as-molecular-sponges-for-mirnas/518/

Circular RNAs

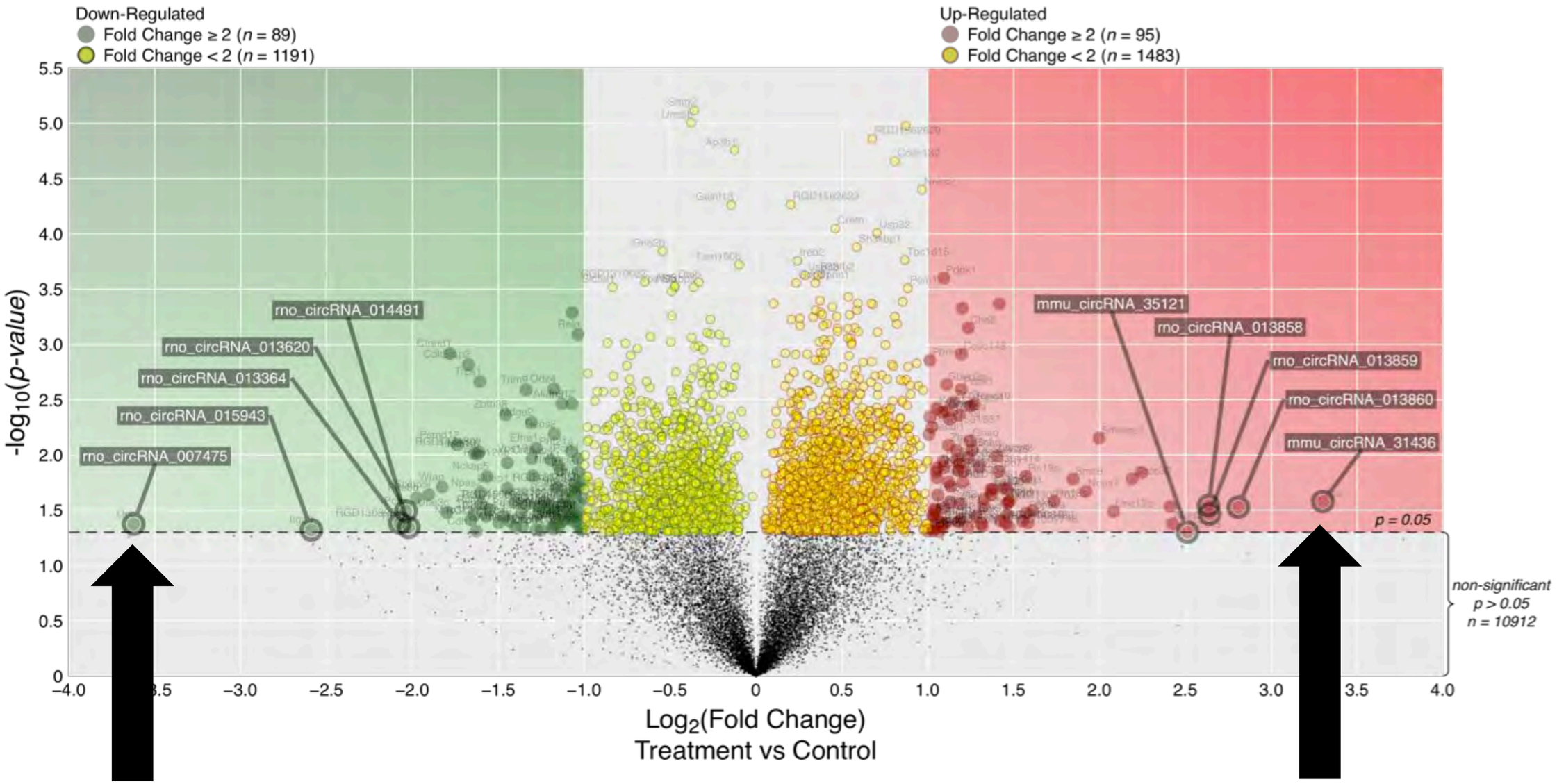
- Upstream regulators of miRNAs (epigenetic regulation)
- “Head-to-tail” splicing



Ideal Biomarkers:

- Stable
- Conserved
- Tissue- and development stage-specific expression
- High abundance in extracellular compartments

Circular RNA profile is dysregulated in E21 nitrofen-induced hypoplastic lungs



BaseScope™ ISH

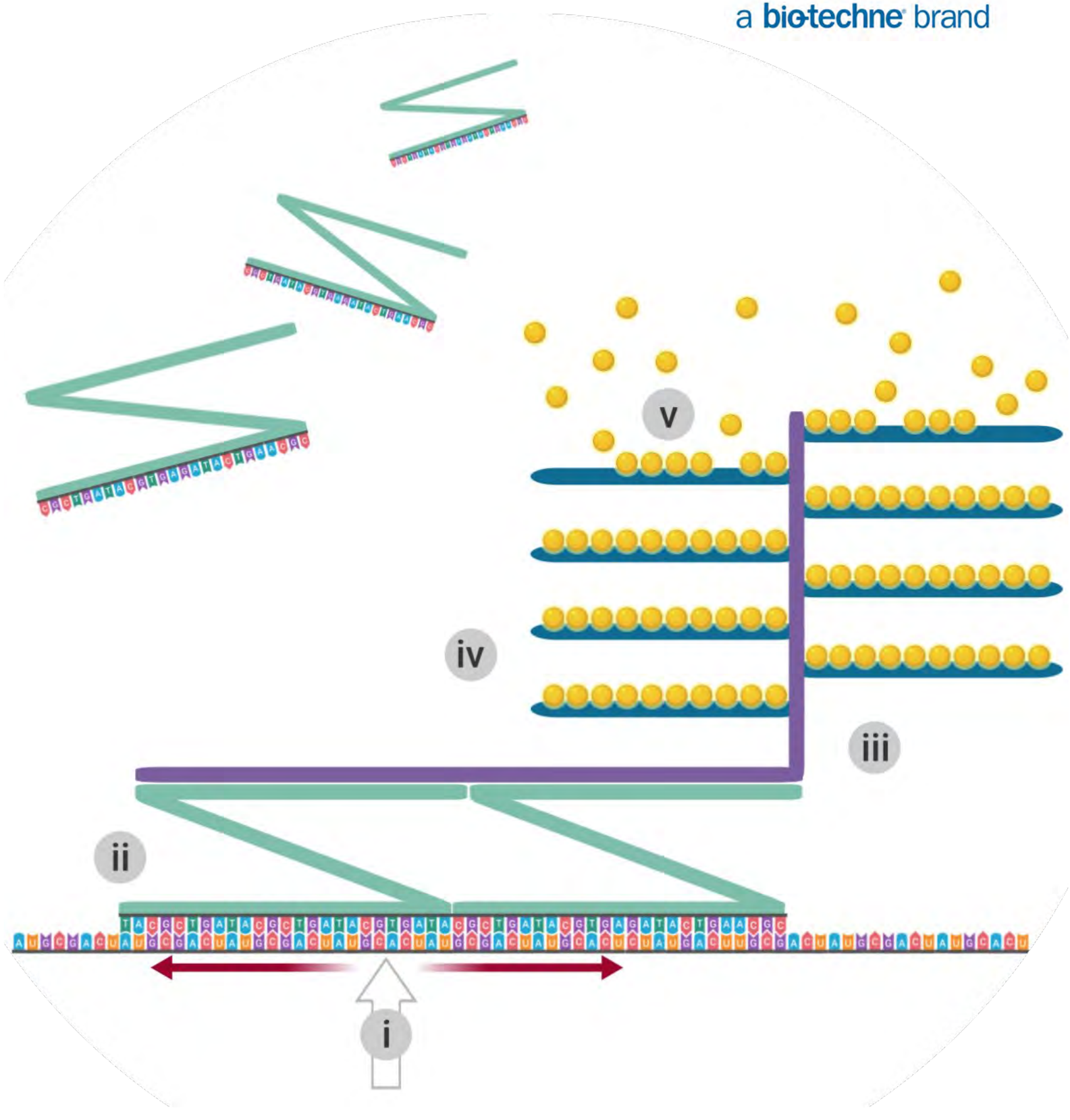
i. Backsplice-specific probe

ii. Adjacent hybridization of probes to circular RNA

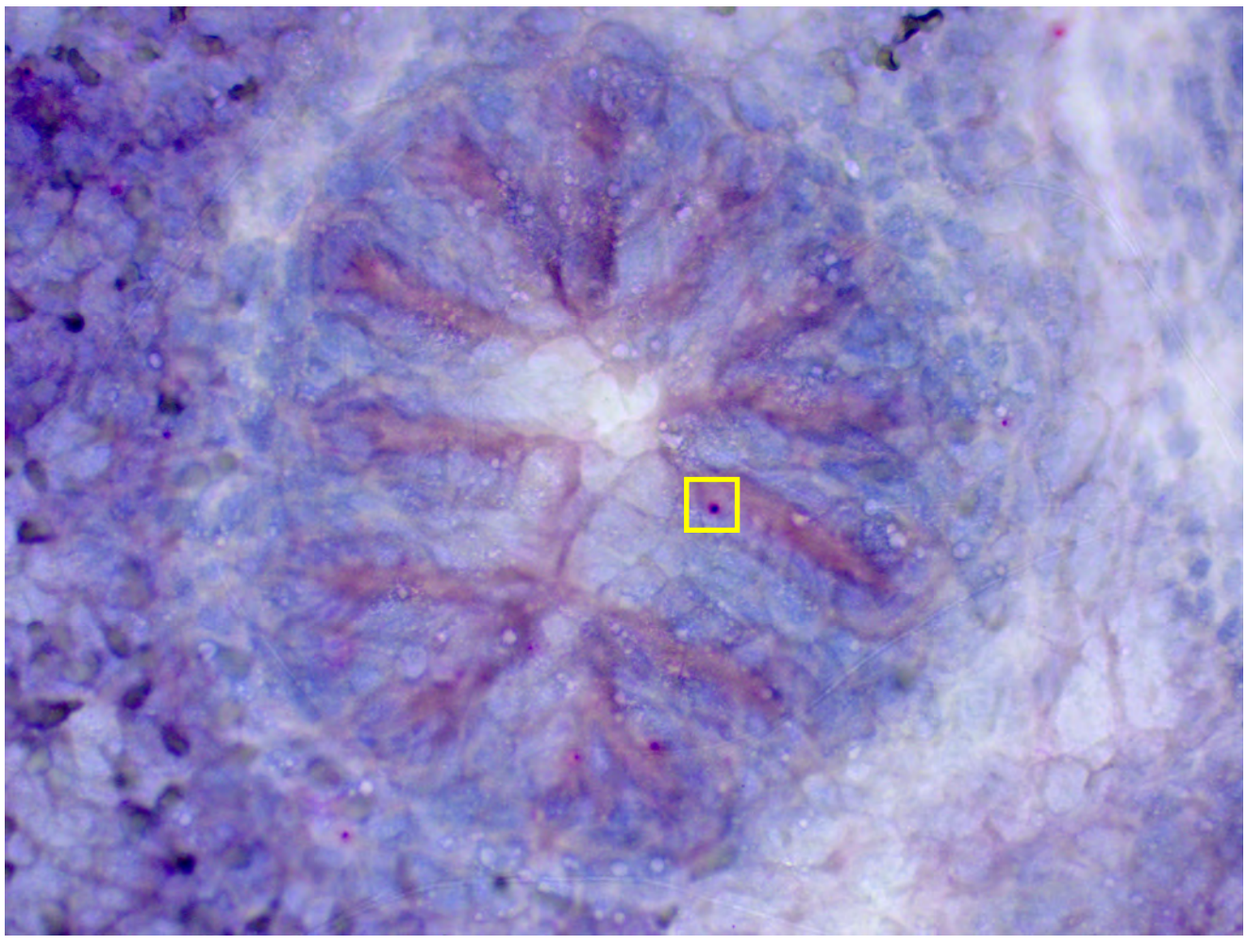
iii. Preamplifier binding

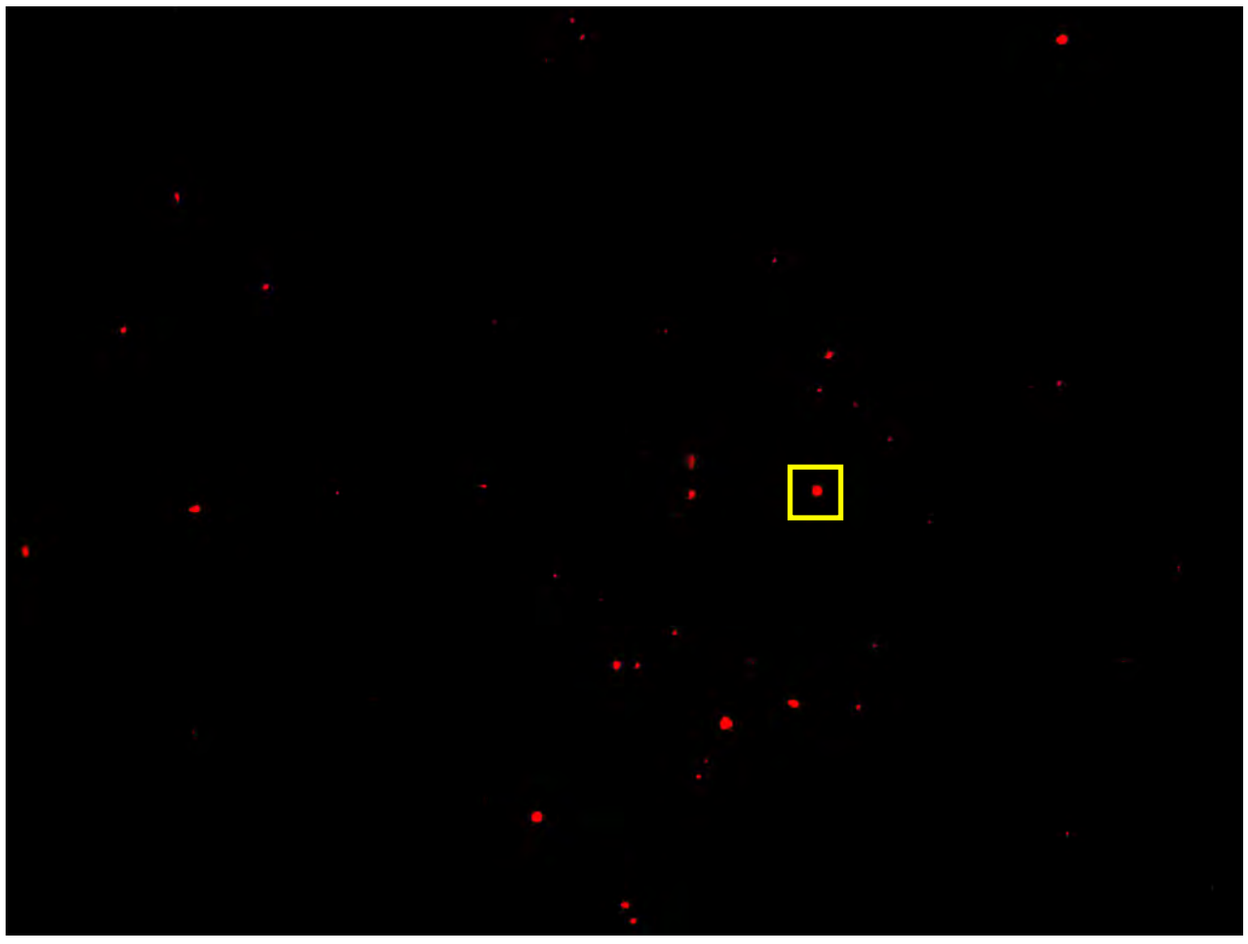
iv. Amplification cascade

v. Label probe binding for fluorescent / chromogenic readout



BaseScope™ E21 Rat Lung





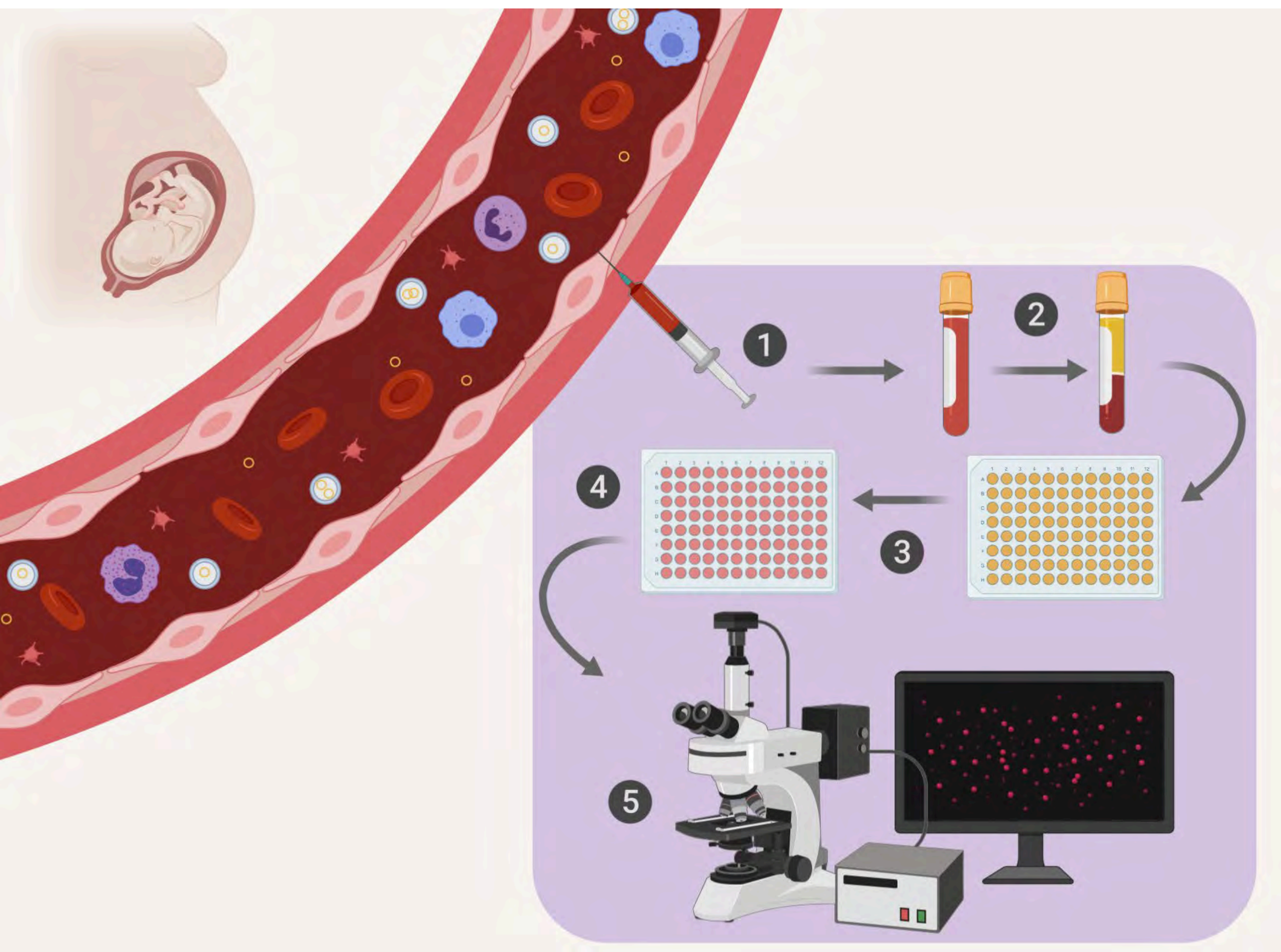
(rno_circRNA_007475)

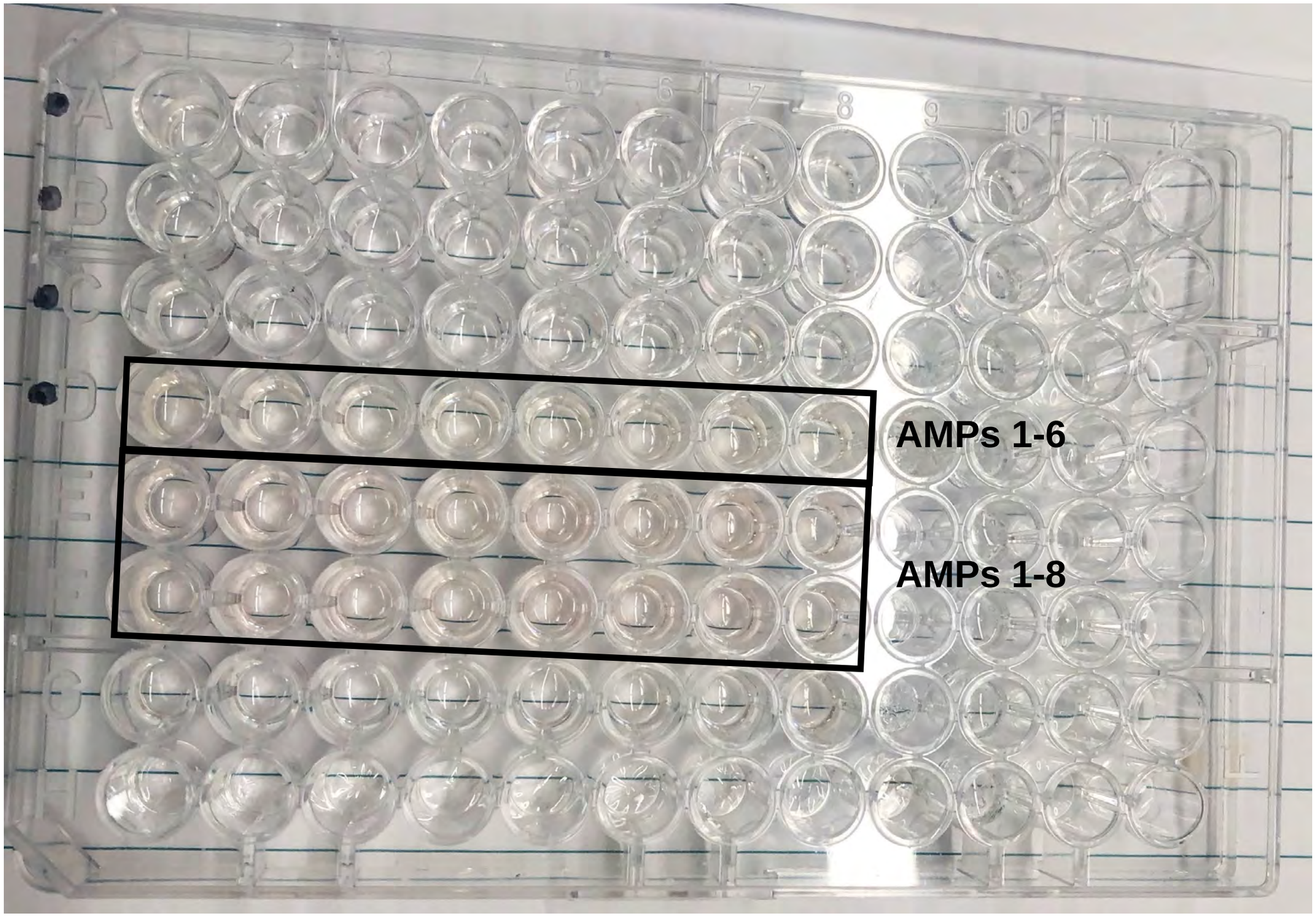
Pediatric Surgery International
https://doi.org/10.1007/s00383-019-04558-2

ORIGINAL ARTICLE

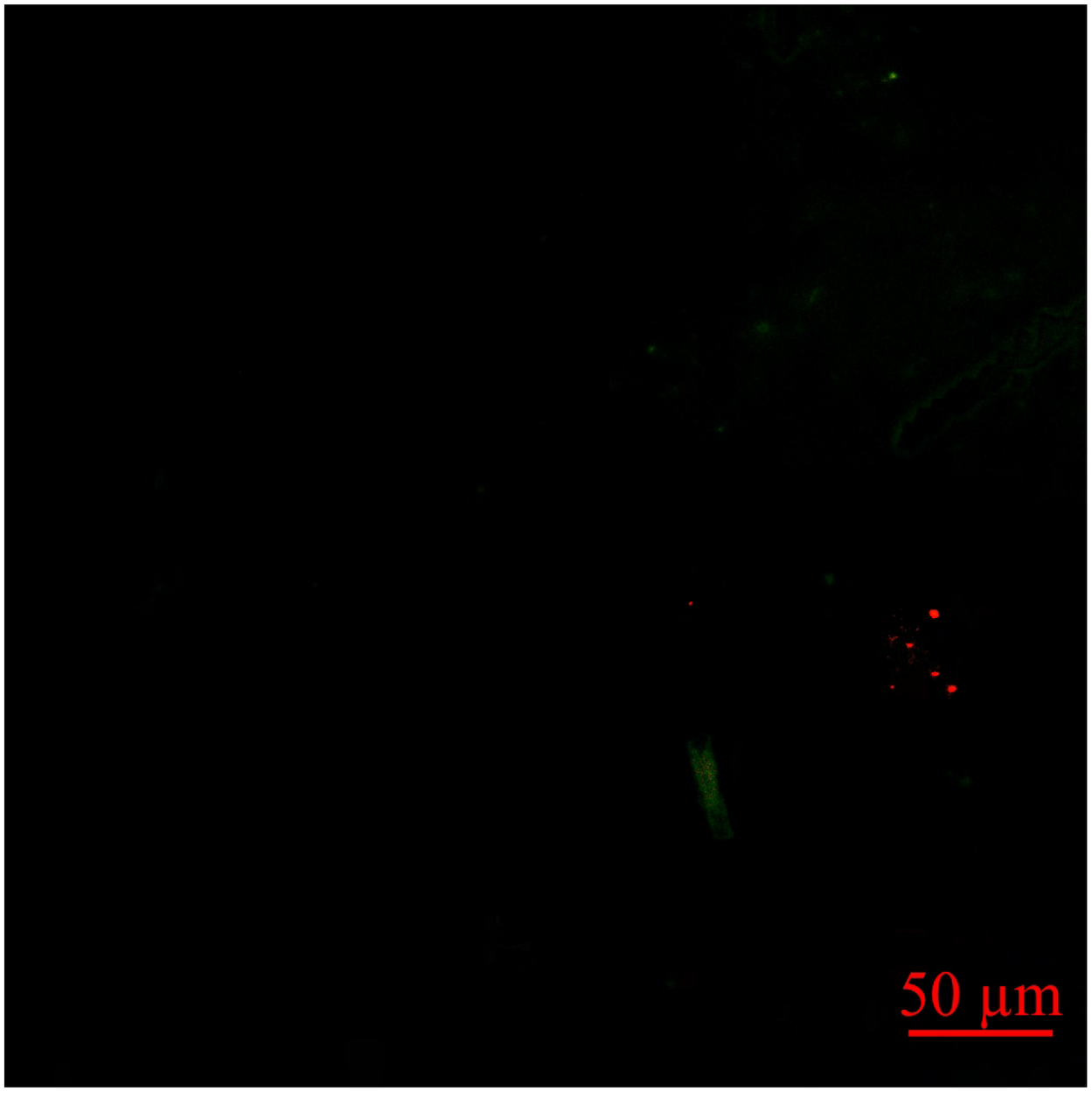
Check for updates

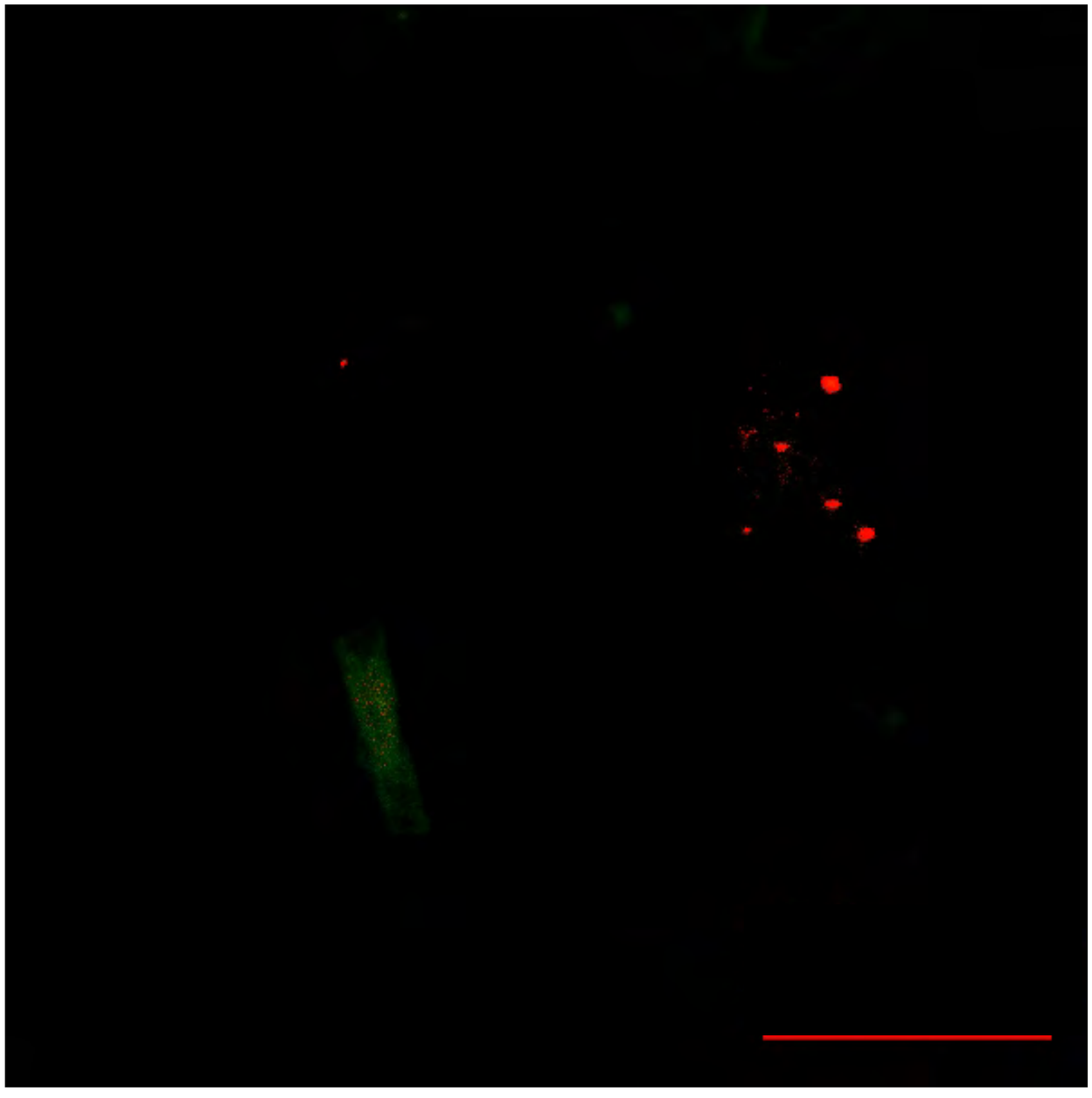
First steps in the development of a liquid biopsy in situ hybridization protocol to determine circular RNA biomarkers in rat biofluids

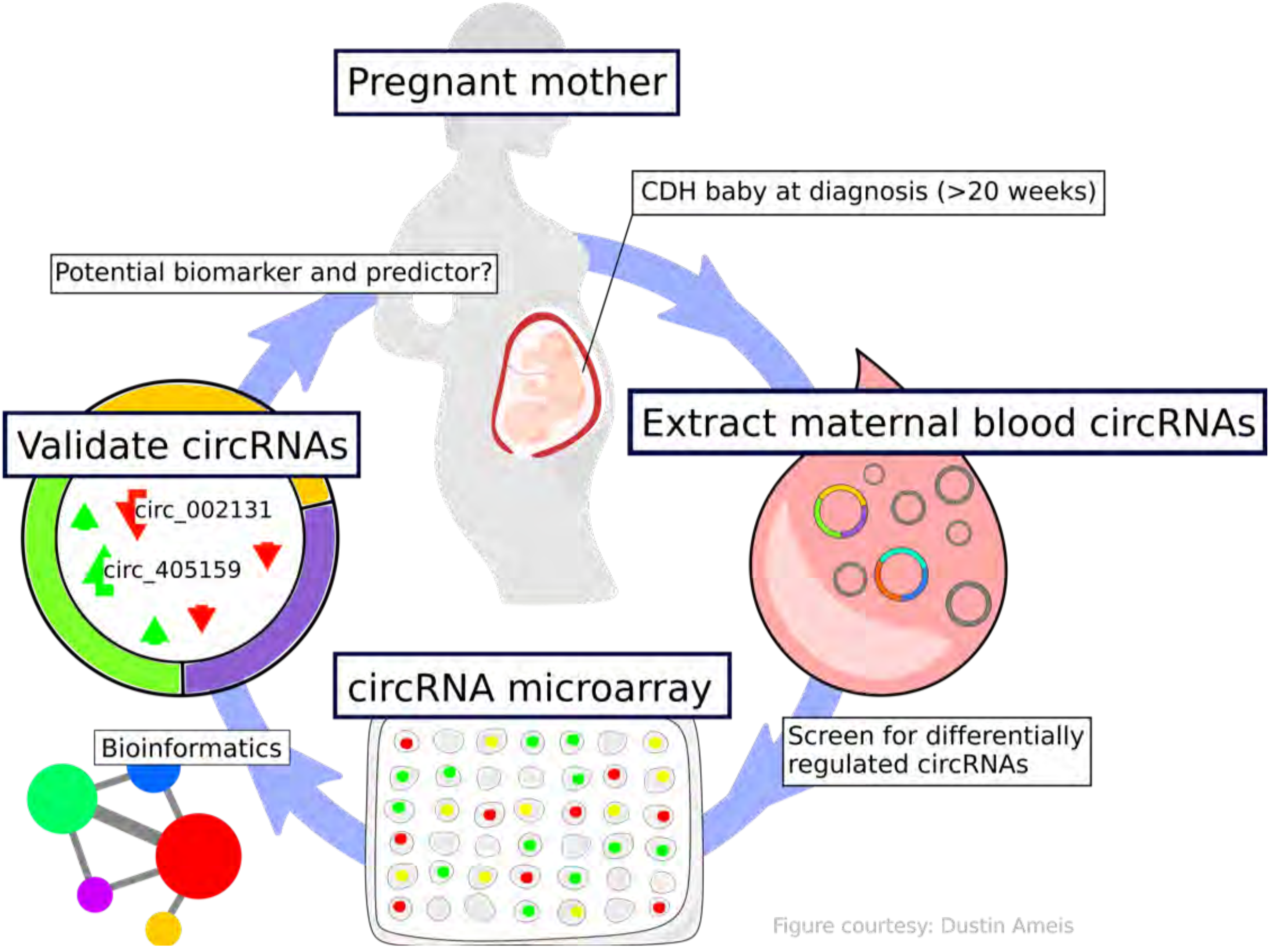

1
2
3
4
5


AMPs 1-6
AMPs 1-8

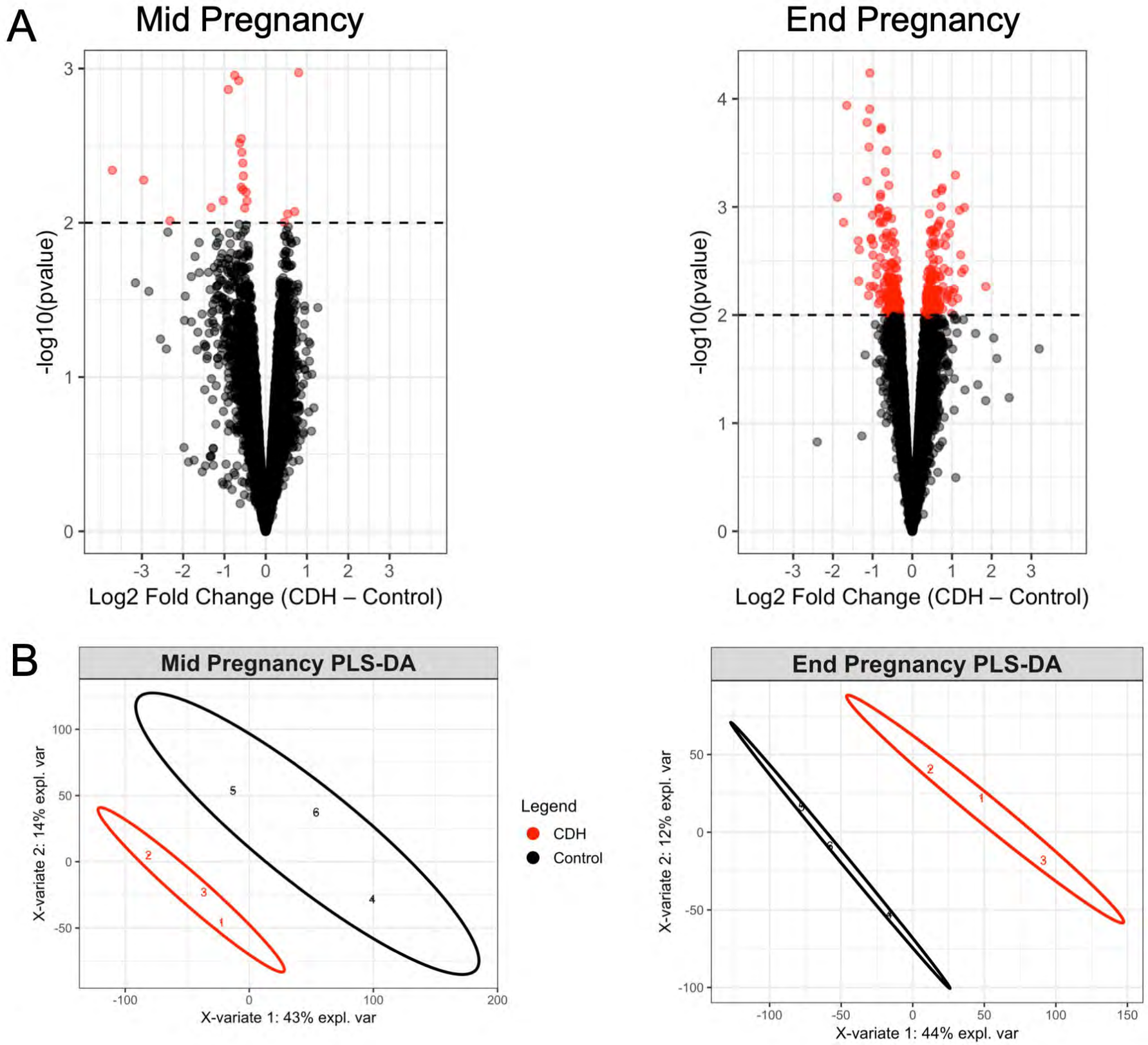
Control Adult Rat Serum: rno_circRNA_007475






Pregnant mother
CDH baby at diagnosis (>20 weeks)
Potential biomarker and predictor?
Validate circRNAs
circ_002131
circ_405159
Extract maternal blood circRNAs
circRNA microarray
Bioinformatics
Screen for differentially regulated circRNAs
Figure courtesy: Dustin Ameis

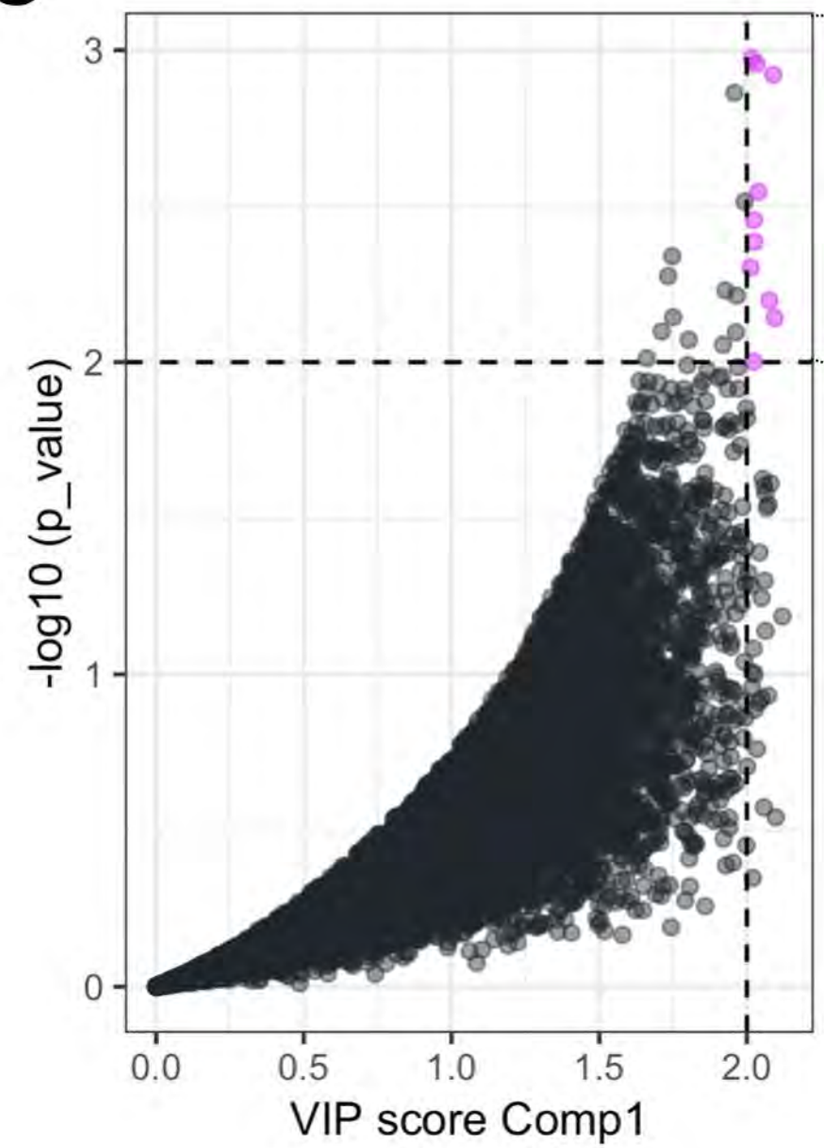
CircRNA profile distinguishes CDH lung from control



Eur Respir J 2020; in press (https://doi.org/10.1183/13993003.00514-2019)

CircRNA profile distinguishes CDH lung from control

C



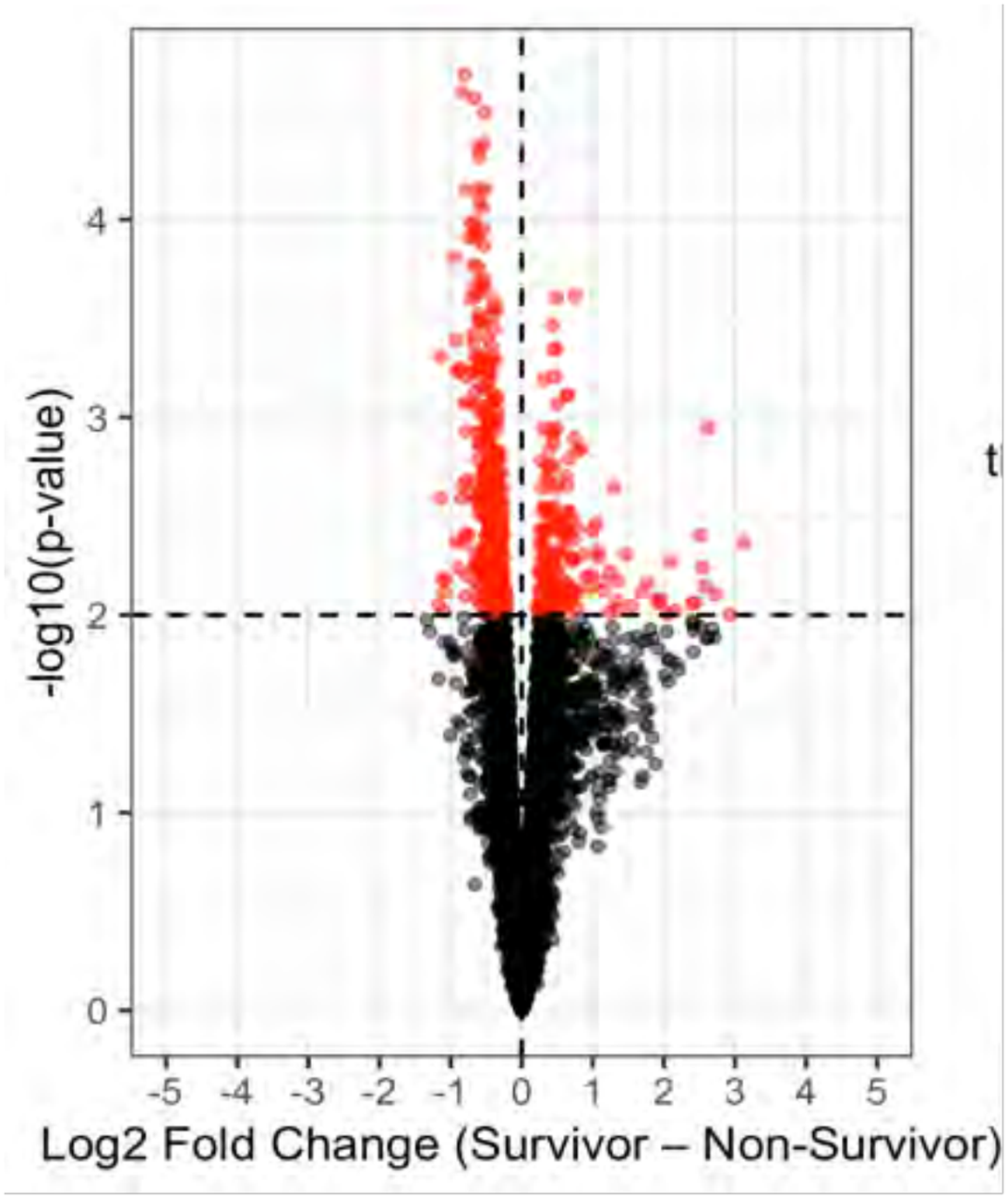
circRNA	VIP Comp1	logFC	P value
hsa_circRNA_001609	2.024	-0.579	0.003
hsa_circRNA_001632	2.089	-0.654	0.001
hsa_circRNA_002074	2.076	-0.475	0.006
hsa_circRNA_002131	2.012	-0.541	0.005
hsa_circRNA_007586	2.023	0.451	0.010
hsa_circRNA_054339	2.094	-0.454	0.007
hsa_circRNA_102068	2.039	-0.593	0.003
hsa_circRNA_103742	2.026	-0.556	0.004
hsa_circRNA_405159	2.016	0.797	0.001
hsa_circRNA_406350	2.033	-0.752	0.001

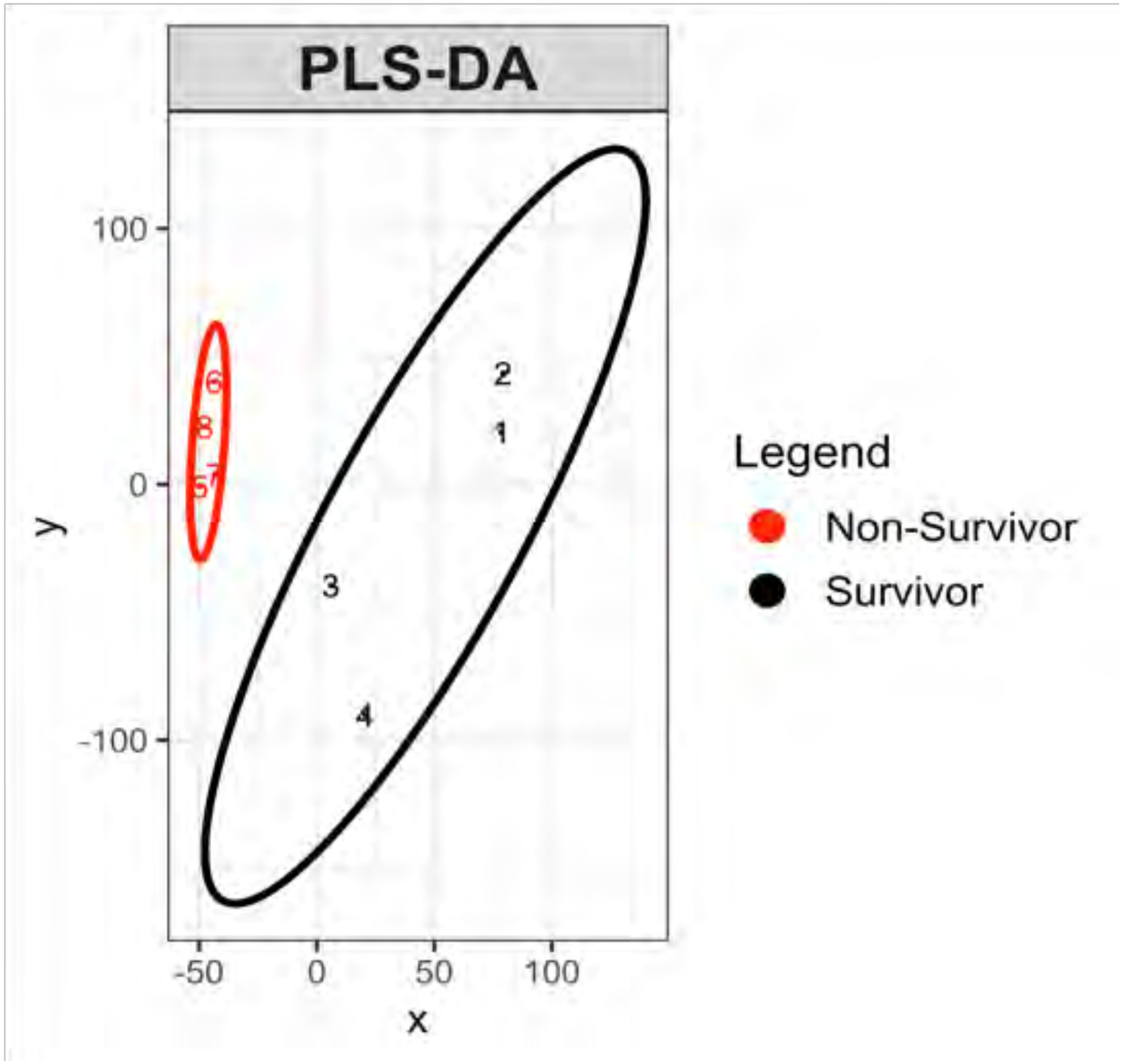
Eur Respir J 2020; in press (https://doi.org/10.1183/13993003.00514-2019)

CircRNA profile can distinguish FETO survivors from non-survivors

	Survivors	**Non-Survivors**	**P-value**
Gestational Age at Plug (weeks)	28.1 (28.7 - 27.1)	27.9 (29.1 - 27.0)	0.84
Observed/ Expected Lung to Head Ratio (%)	22.5 (23.6 - 17.5)	21.6 (24.0 - 15.9)	0.87
Liver herniated	10 (91%)	9 (100%)	1.00
Fetal gender	7 male/ 4 female	4 male/ 5 female	0.65
Birth weight (g)	2780 (3180 - 2160)	3195 (3278 - 2650)	0.3

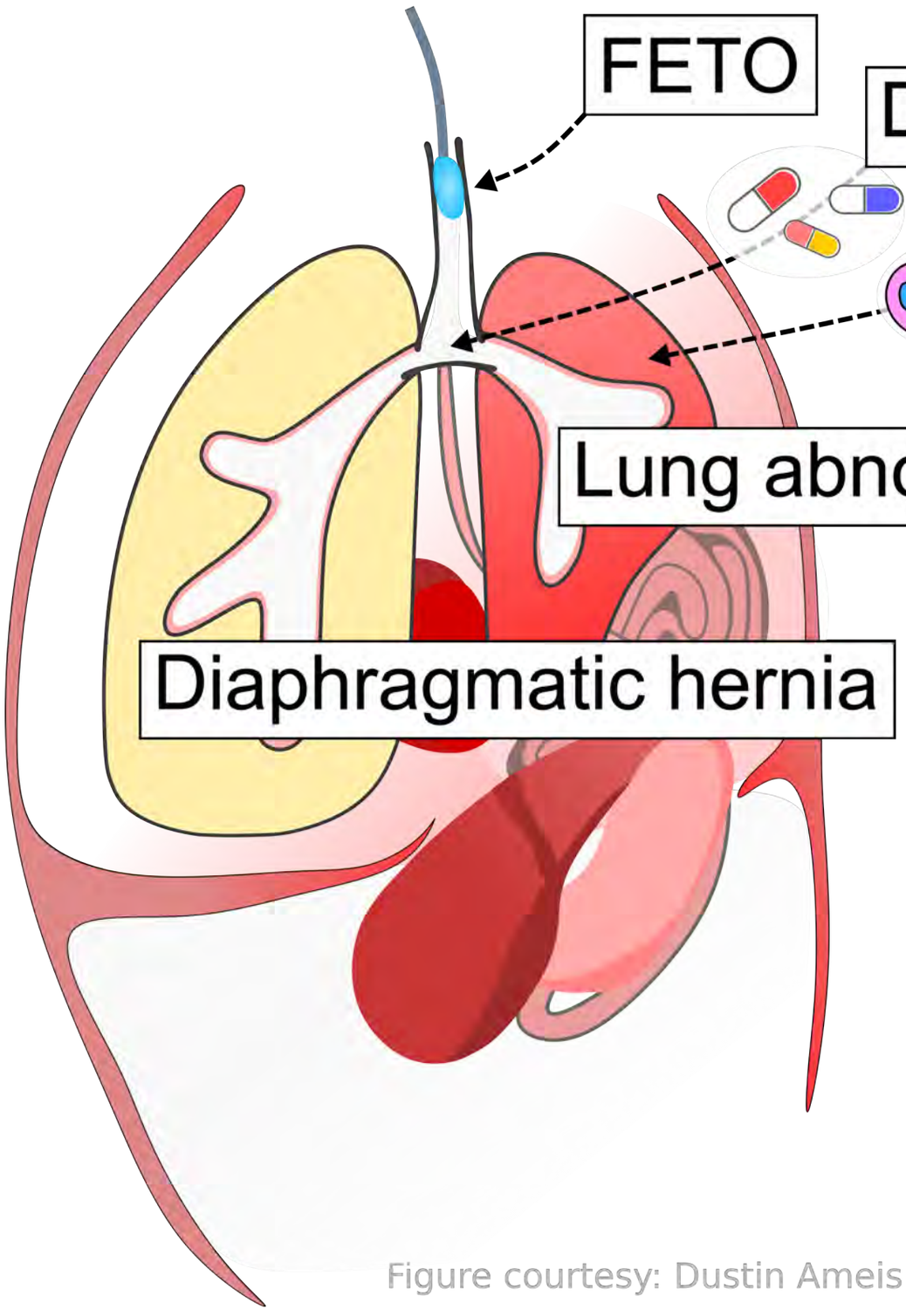
CircRNA profile can distinguish FETO survivors from non-survivors





Unpublished results

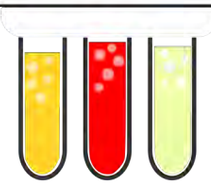
Prenatal treatment



Pathogenesis

Prenatal diagnosis

Biomarkers



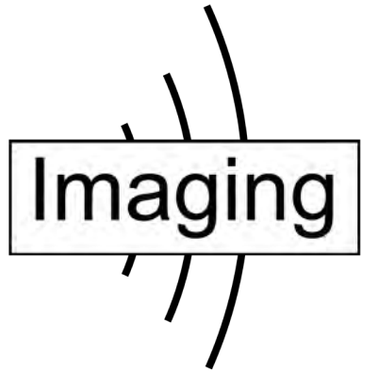


Figure courtesy: Dustin Ameis

http://store.winnipegfreepress.com/photostore/details/139975/

Thank you MIRACLA Lab

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Laboratory members
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BJ Hancock
Nathan Wiseman
Cindy Holland

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Andrew Halayko's group
Malcolm Xing's group
Neeloffer Mookherjee's group

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Dick Tibboel

Martin Post

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Patrice Eastwood
Francesca Russo

Martin Lacher
Richard Wagner

Jorge Correia-Pinto
Patricia Pereira-Terra

Patients and families

GFT Group Academic Surgeons Winnipeg

Molly Towell Perinatal Research Foundation

Canada Foundation for Innovation
Fondation canadienne pour l'innovation

DEVOTION
Developmental Origins of Chronic Diseases in Children Network

EXIQON

CCHCSP PCCCSE

Research Manitoba

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

The Children's Hospital Research Institute of Manitoba

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