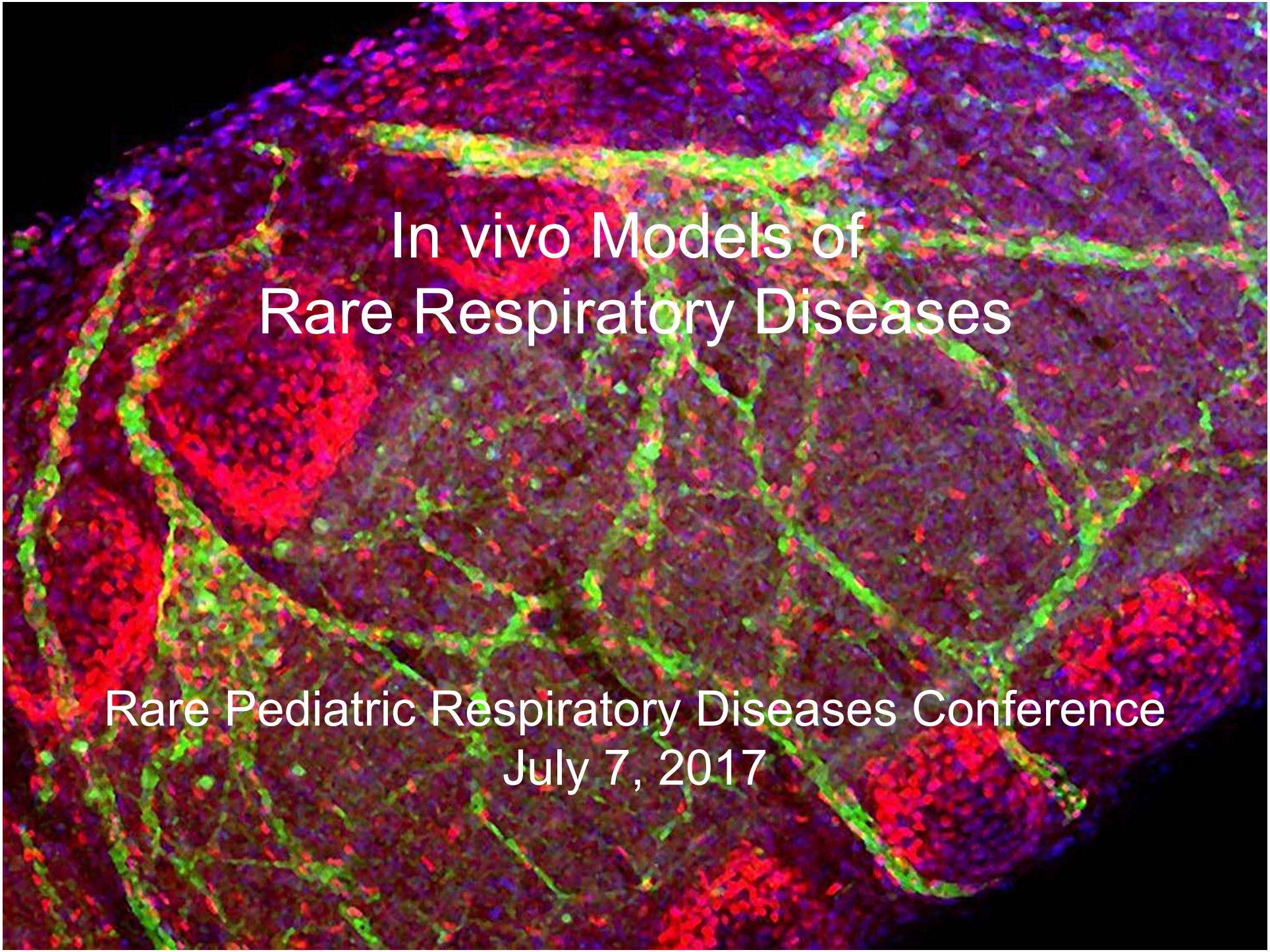
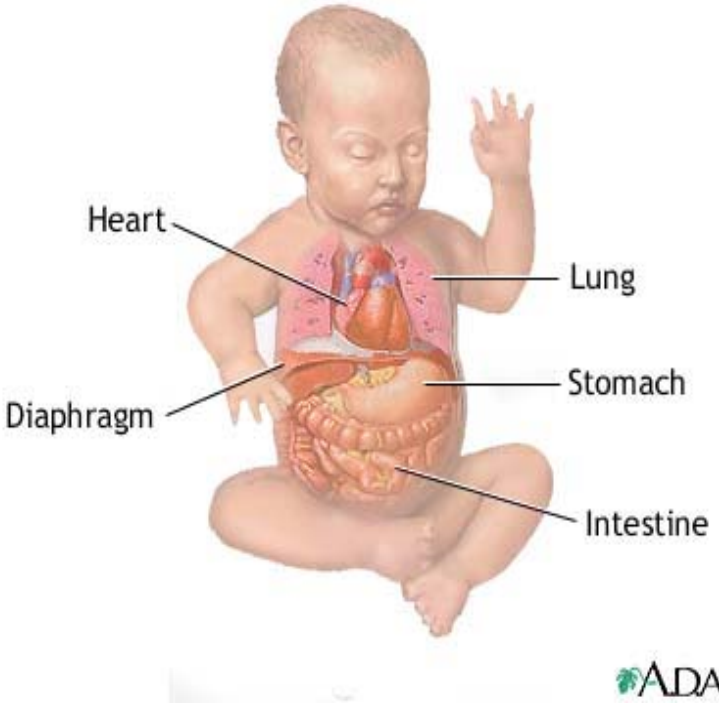
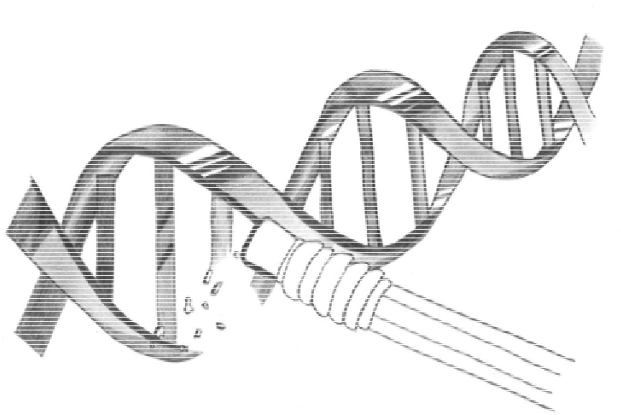


In vivo Models of Rare Respiratory Diseases

Rare Pediatric Respiratory Diseases Conference
July 7, 2017

Genetic basis of pediatric lung diseases





Congenital diaphragmatic hernia
Trachea-esophageal fistrula
Tracheomalacia
Bronchopulmonary dysplasia
Pulmonary hypertension
Asthma

Gene $\xrightarrow{?}$ Disease

Disease Mechanism

New Modes of Treatment

Novel Biology

CDH is a common birth defect with high mortality rate

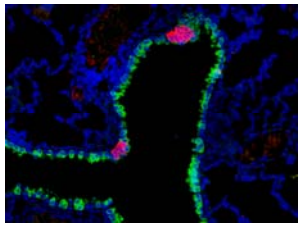
CDH occurs in 1 in 2,500 babies.

Often associated with pulmonary hypertension, and respiratory infection.

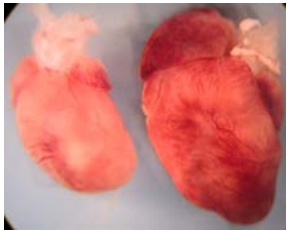
Commonly believed that malformation of the diaphragm is the cause of the disease. And the associated organ herniation/compression leads to lung dysfunction.

Is there a lung-intrinsic cause that is independent of the mechanical compression?

Outline:

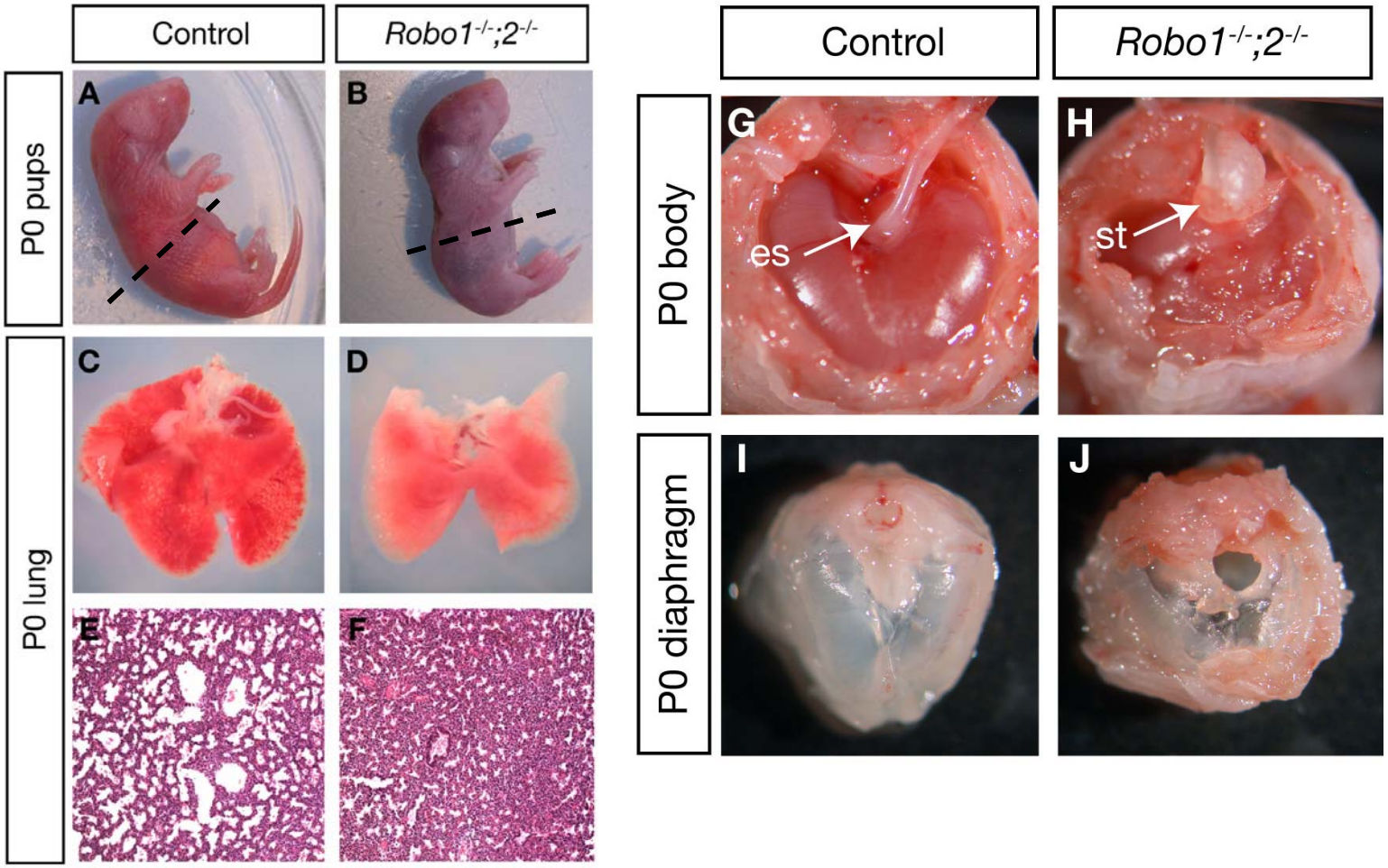


Loss of *Robo* leads to heightened immune response in congenital diaphragmatic hernia.



Loss of *Pbx* leads to pulmonary hypertension in congenital diaphragmatic hernia.

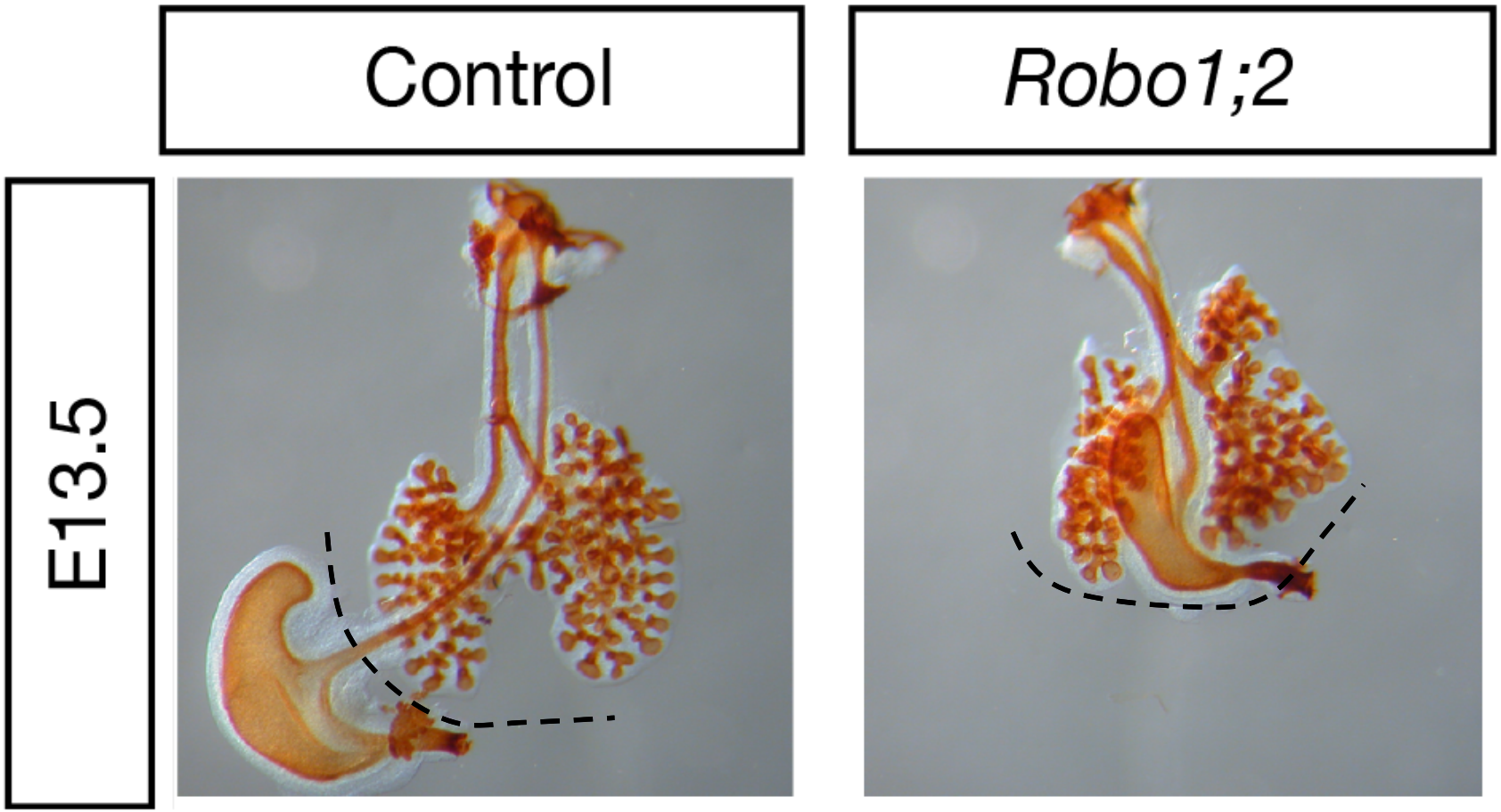
Robo1;2 mutants exhibit CDH



Deletion of chromosome region spanning *ROBO1;2*, as well as point mutation in *ROBO1* have been reported in human CDH.

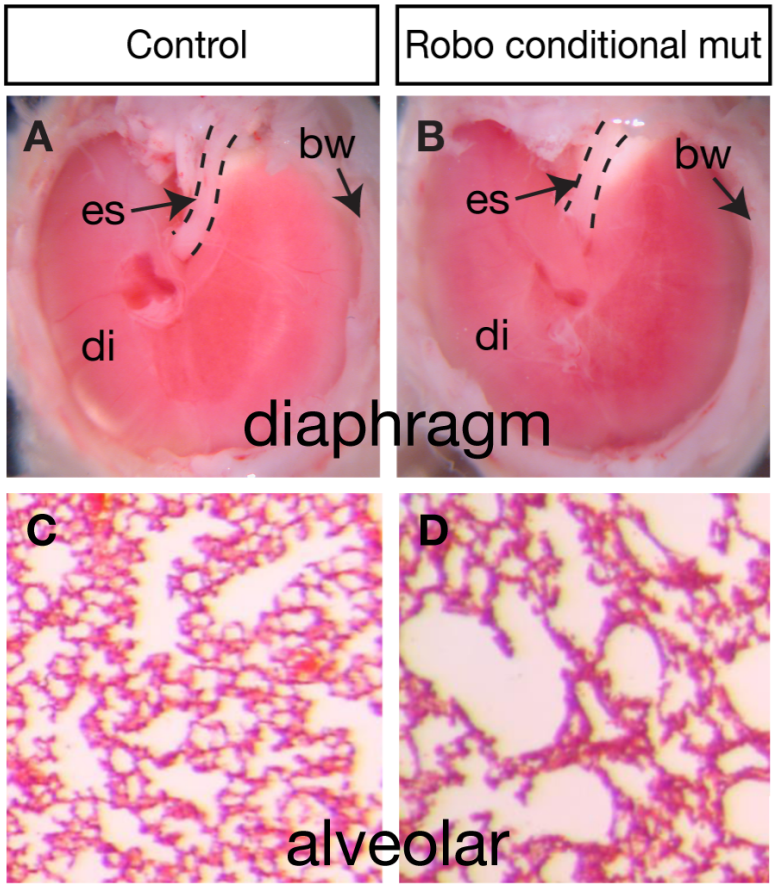
Holder et al., 2007, Pfeiffer et al, 1998; Longoni et al., 2014.

Organ positioning defect precedes diaphragm formation in *Robo* mutants



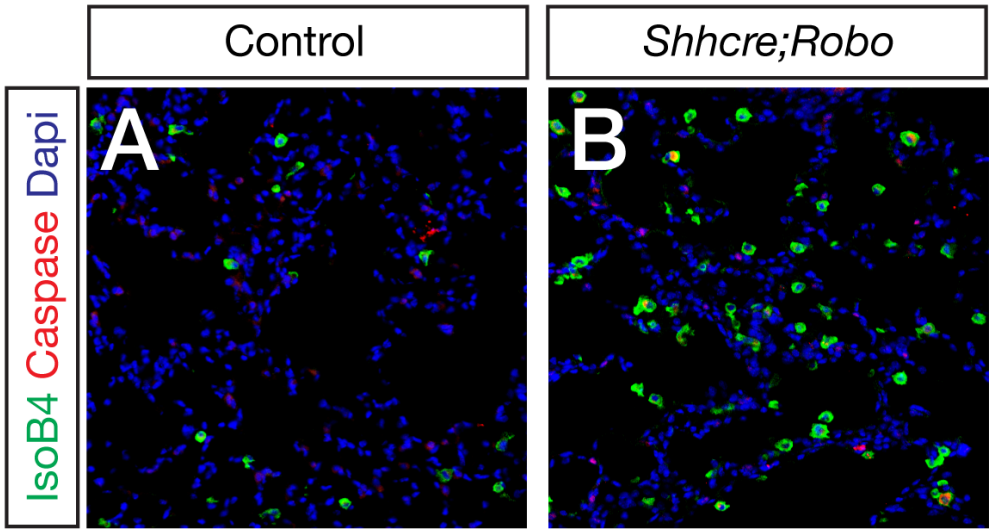
Domyan, Branchfield, et al., DevCell, 2013.

Bypassing organ herniation: epithelium-specific *Robo* mutants do survive, but exhibit alveolar simplification



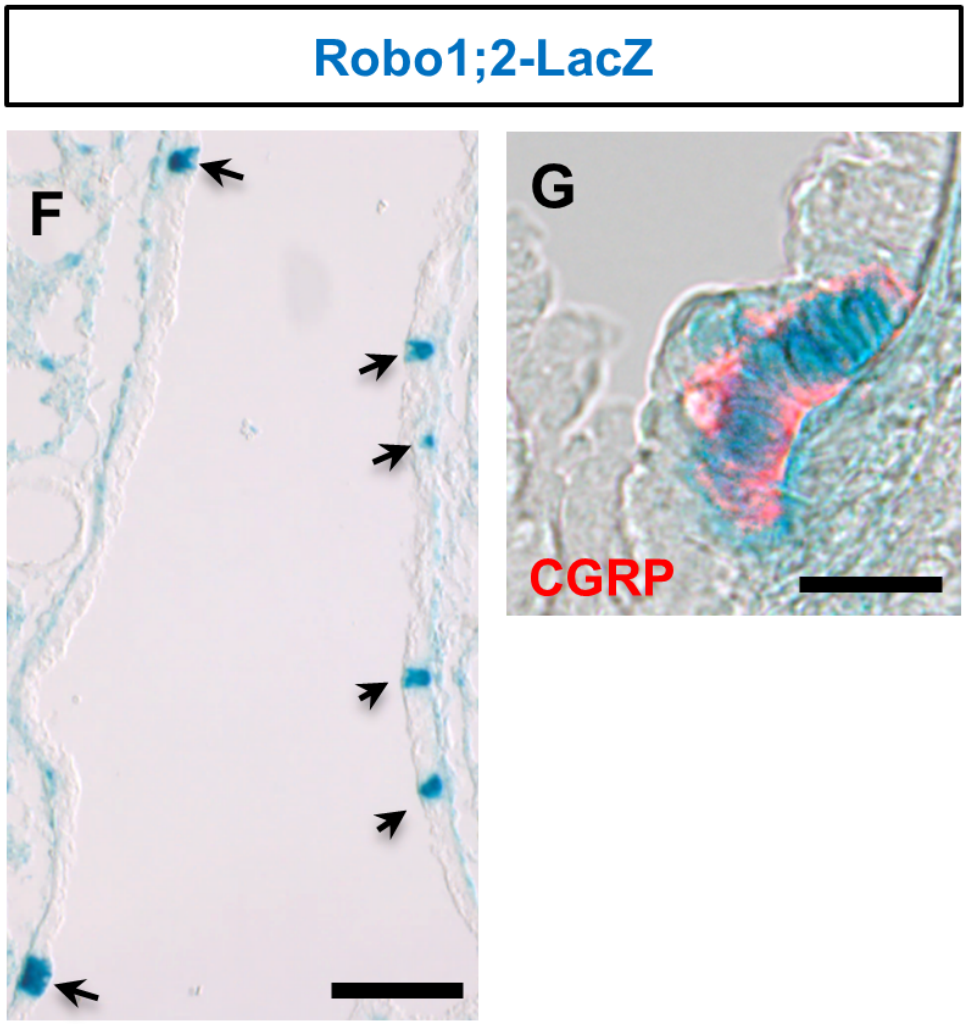
Epithelial *Robo* mutants exhibit heightened immune signature

Immune/inflammatory response	
Gene ID	**Fold Change**
Saa3	+ 27.93
Ccl3	+ 21.79
Cxcl2	+ 15.92
Irg1	+ 12.57
Serpina3k	+ 11.70
Orm1	+ 11.66
Amwap	+ 10.74
Ear1	+ 10.41
Clec4d	+ 7.11
Il1b	+ 7.02
Ly6i	+ 6.85
Chi3l3	+ 6.25
Lcn2	+ 5.67
Clec4e	+ 5.42
Il1r2	+ 5.01

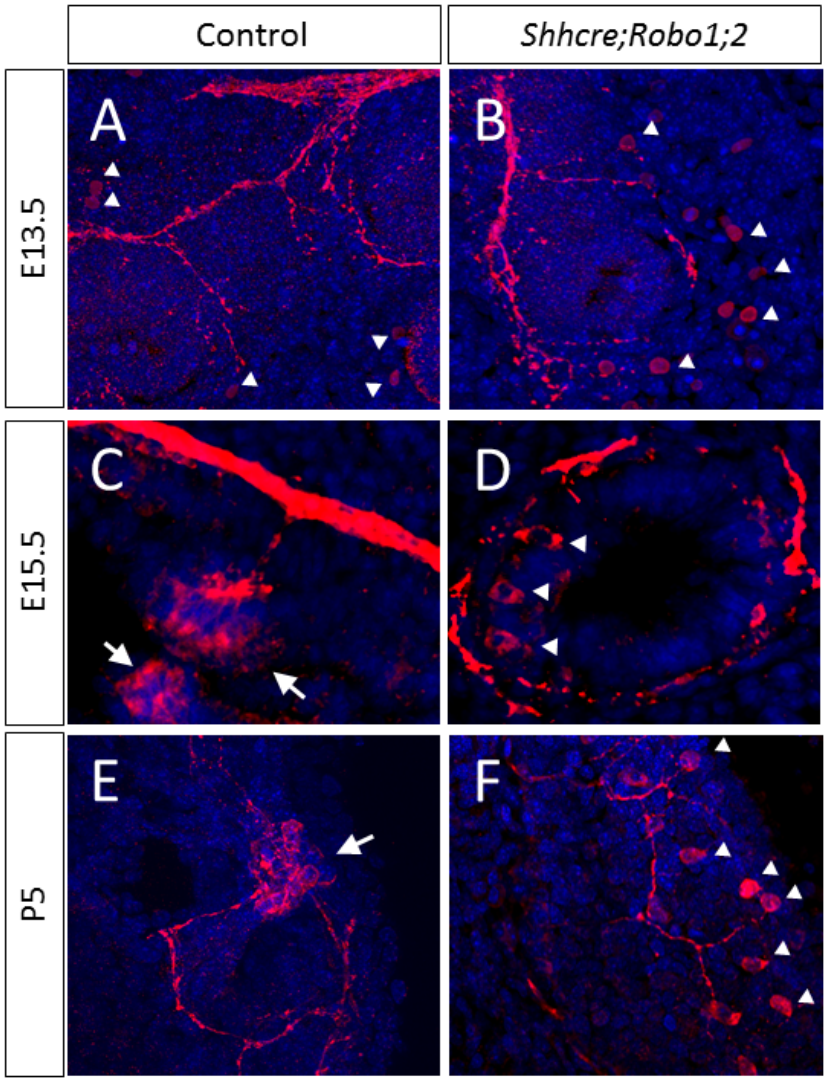


Hypothesis of *Robo* function in lung

Robo genes are expressed in pulmonary neuroendocrine cells (PNECs)

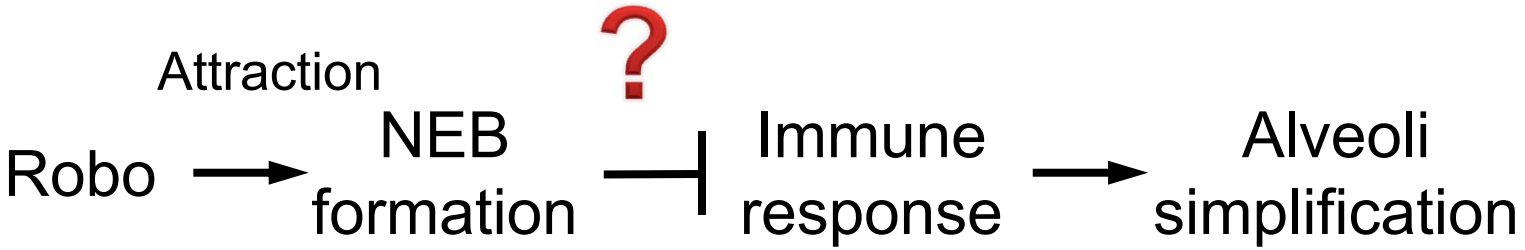


Inactivation of *Robo* led to failed PNEC clustering into NEBs

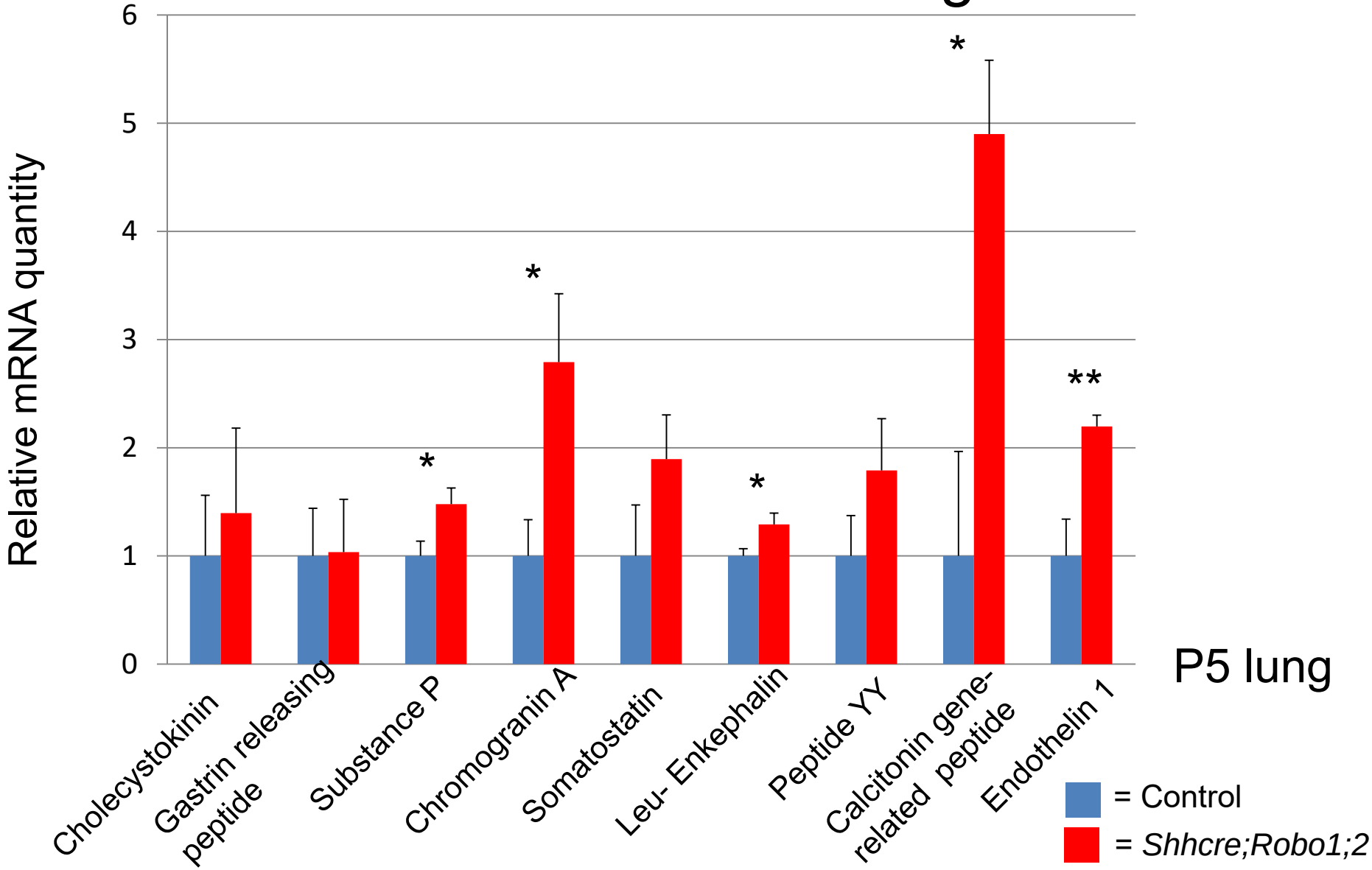


Synaptophysin

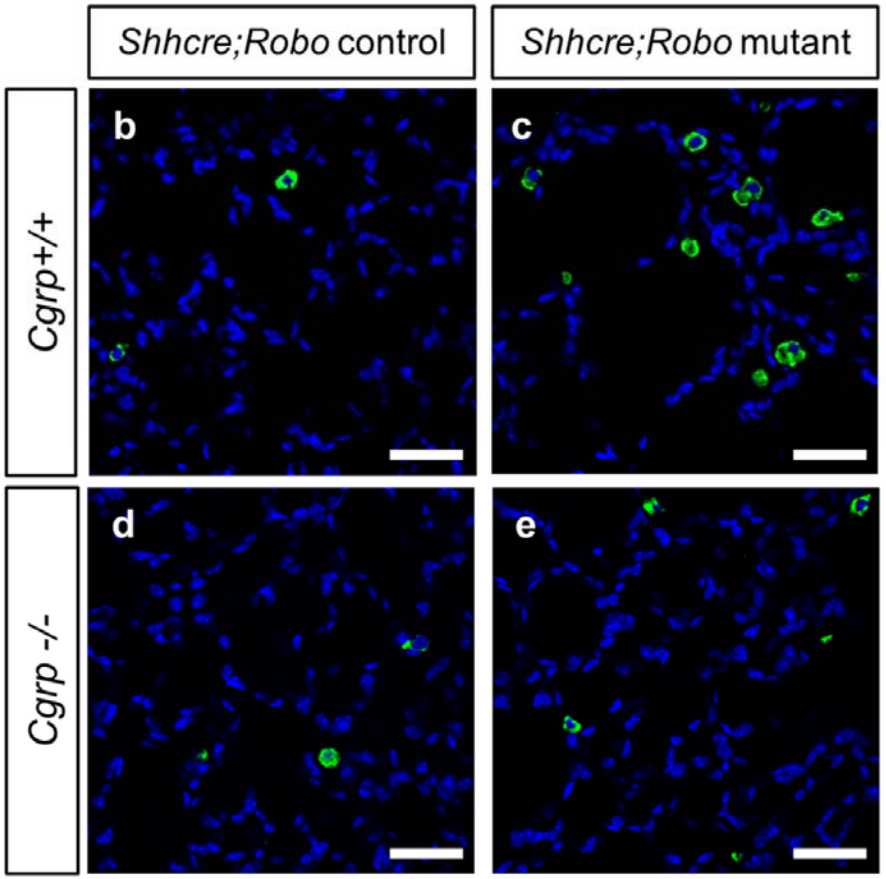
Hypothesis of Robo function in lung

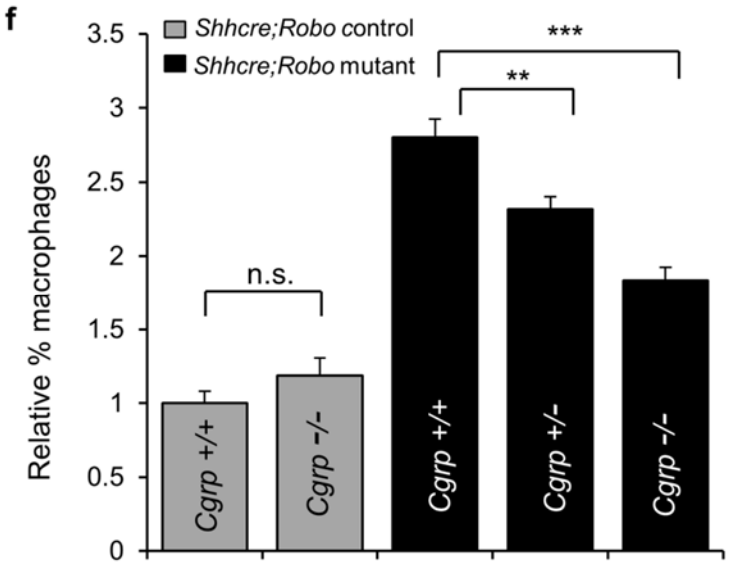


PNEC peptides are upregulated in P5 *Robo* mutant lungs

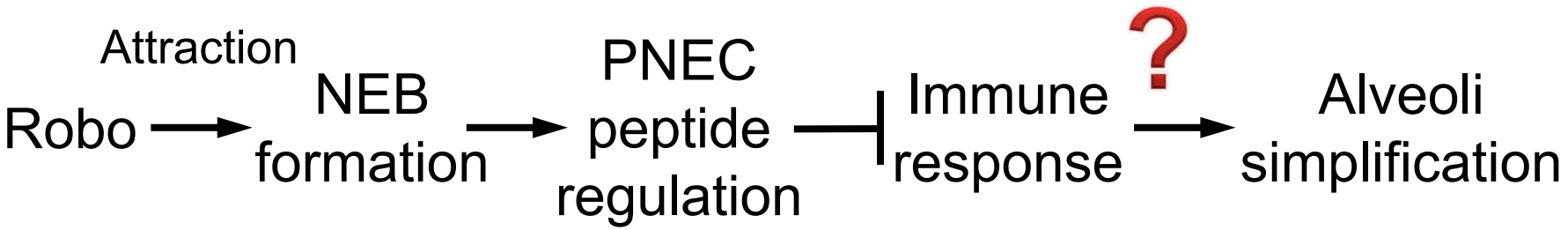


Reducing *Cgrp* dosage attenuates immune cell increase

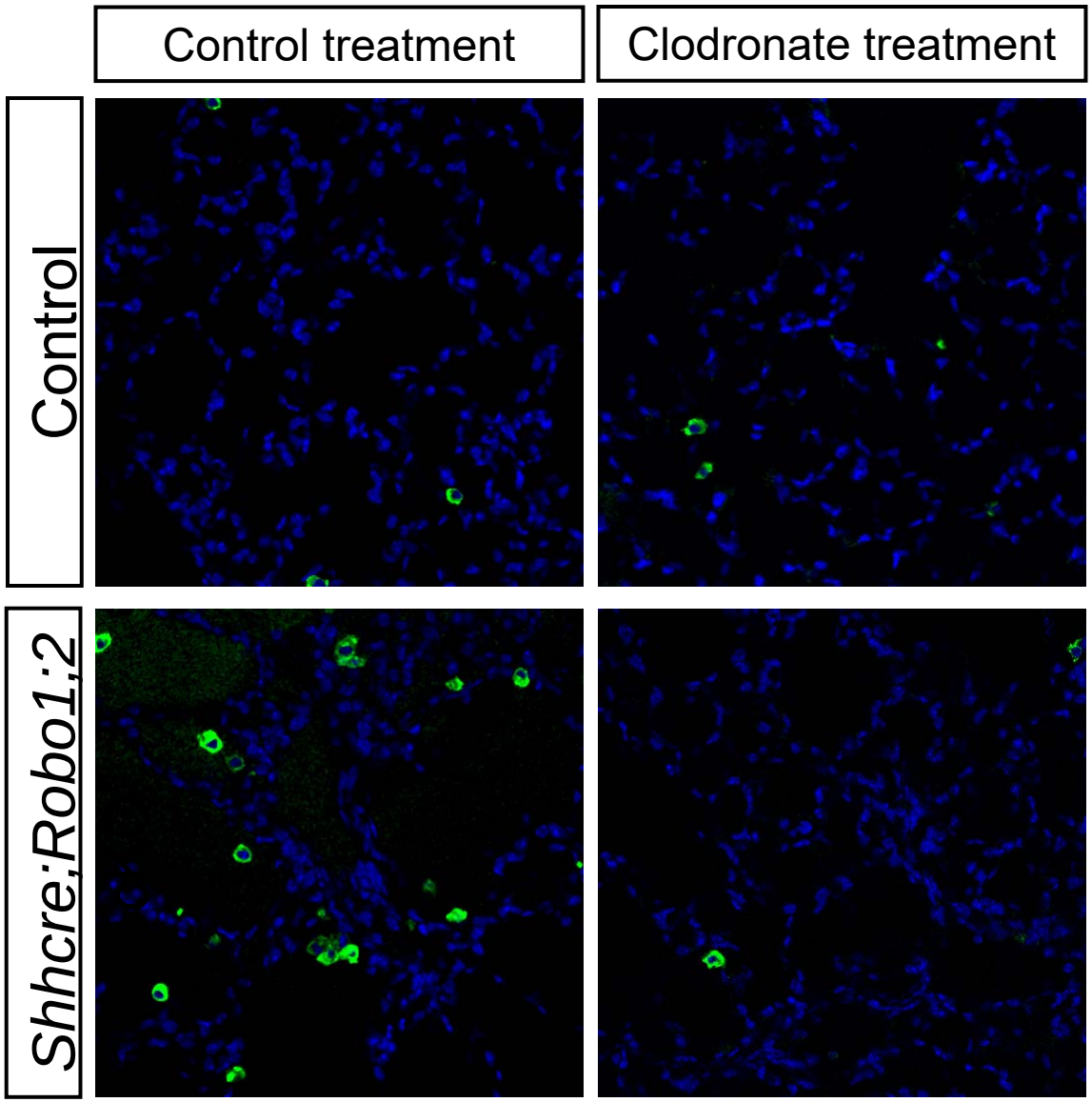


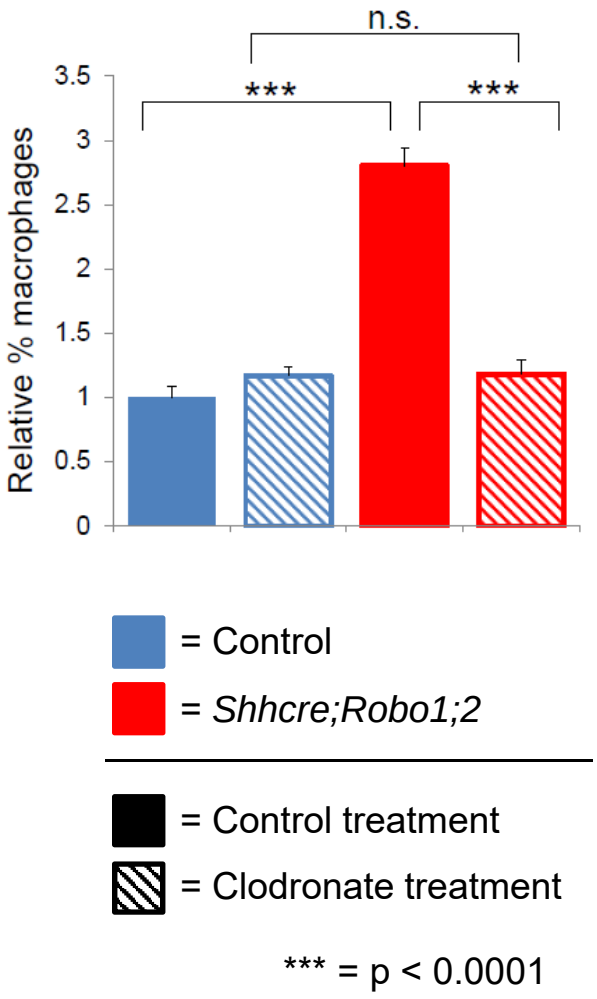


Hypothesis of *Robo* function in lung

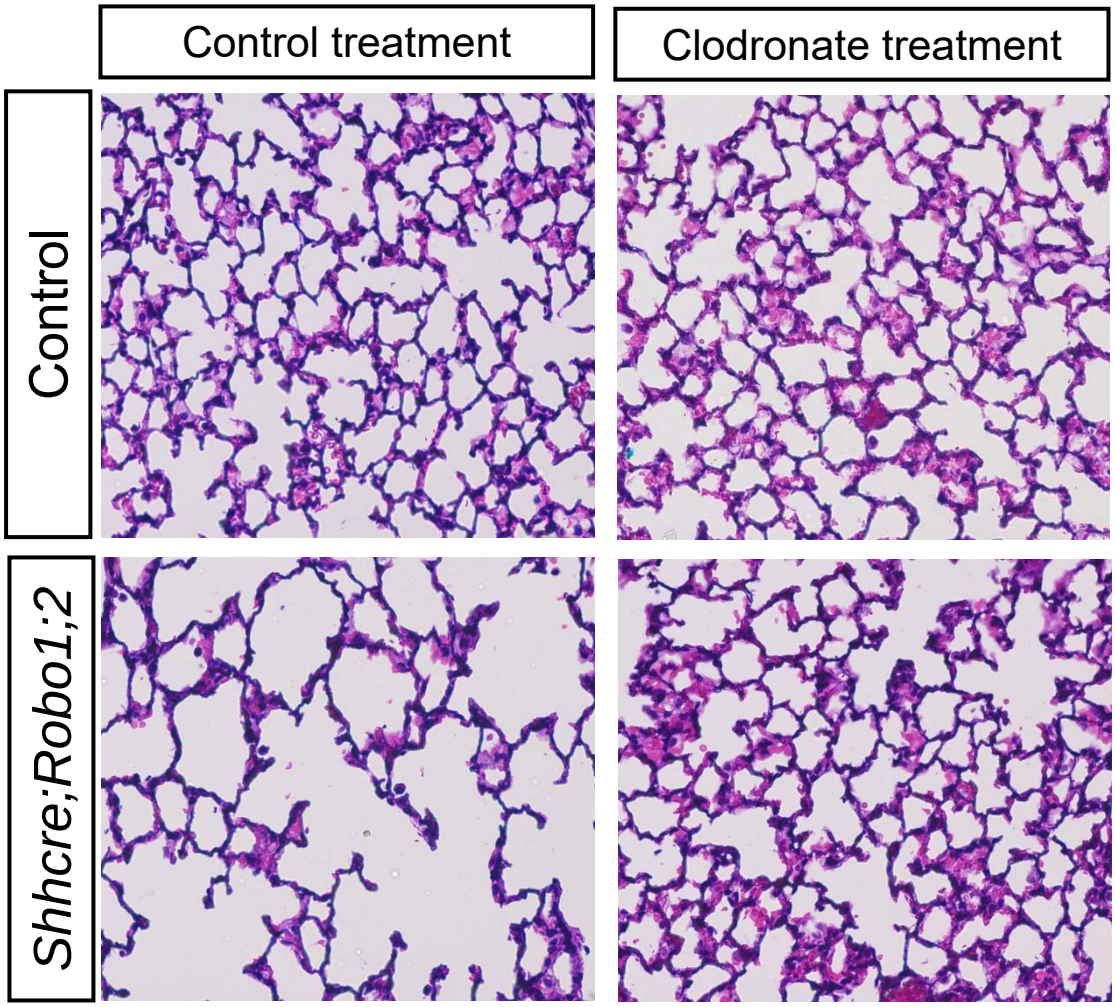


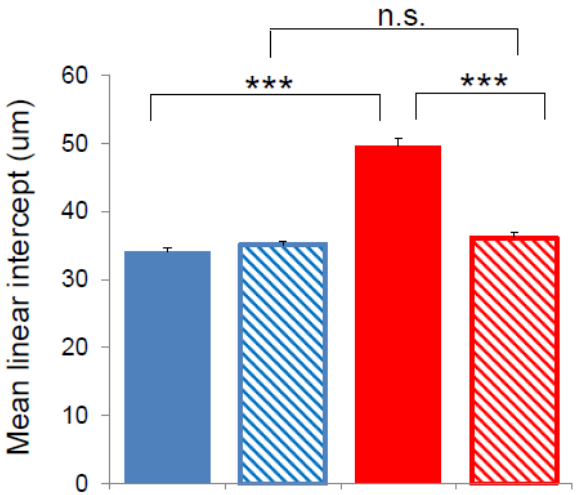
Clodronate treatment attenuates macrophage infiltration in *Robo* mutant lungs





Clodronate treatment attenuates alveolar simplification in *Robo* mutant lungs





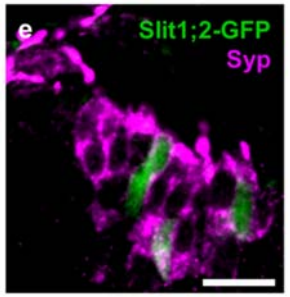
= Control

= *Shhcre;Robo1;2*

= Control treatment

= Clodronate treatment

*** = p < 0.0001

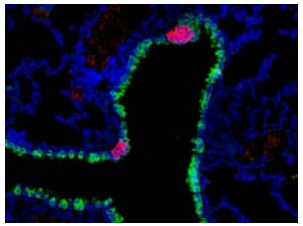


Summary I

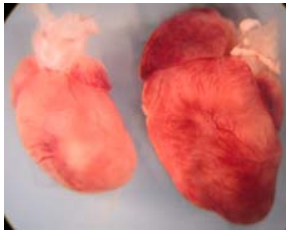
Robo —Attraction→ NEB formation → PNEC peptide regulation ⊣ Increased macrophages → Alveoli simplification

- *Diaphragm independent cause of lung defects in CDH*: PNEC malformation, neuropeptide dysregulation, lung inflammation and alveolar simplification.
- *Clinical implication*: early prevention of neuropeptide hyperactivity or immune inflammation may prevent life-long lung structural damage.
- *Novel biology*: PNECs act as sensors on the airway wall that senses inputs and in turn control pulmonary responses.

Outline:

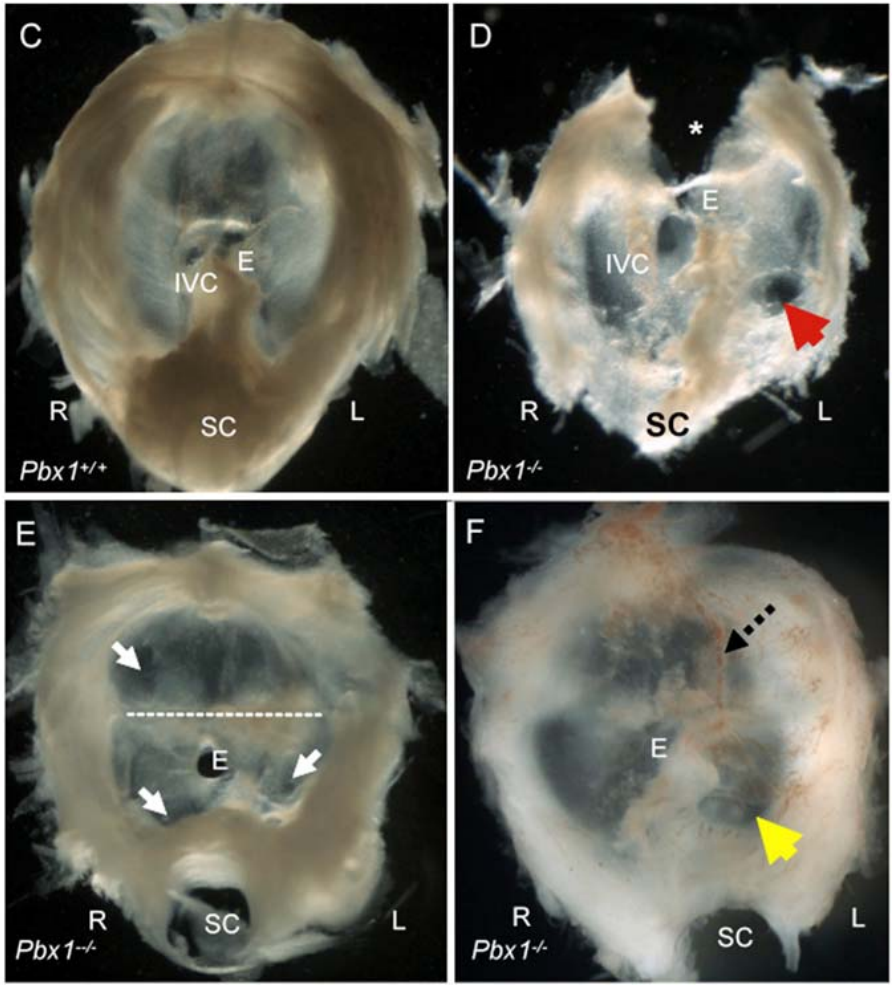


Loss of *Robo* leads to heightened immune response in congenital diaphragmatic hernia.



Loss of *Pbx* leads to pulmonary hypertension in congenital diaphragmatic hernia.

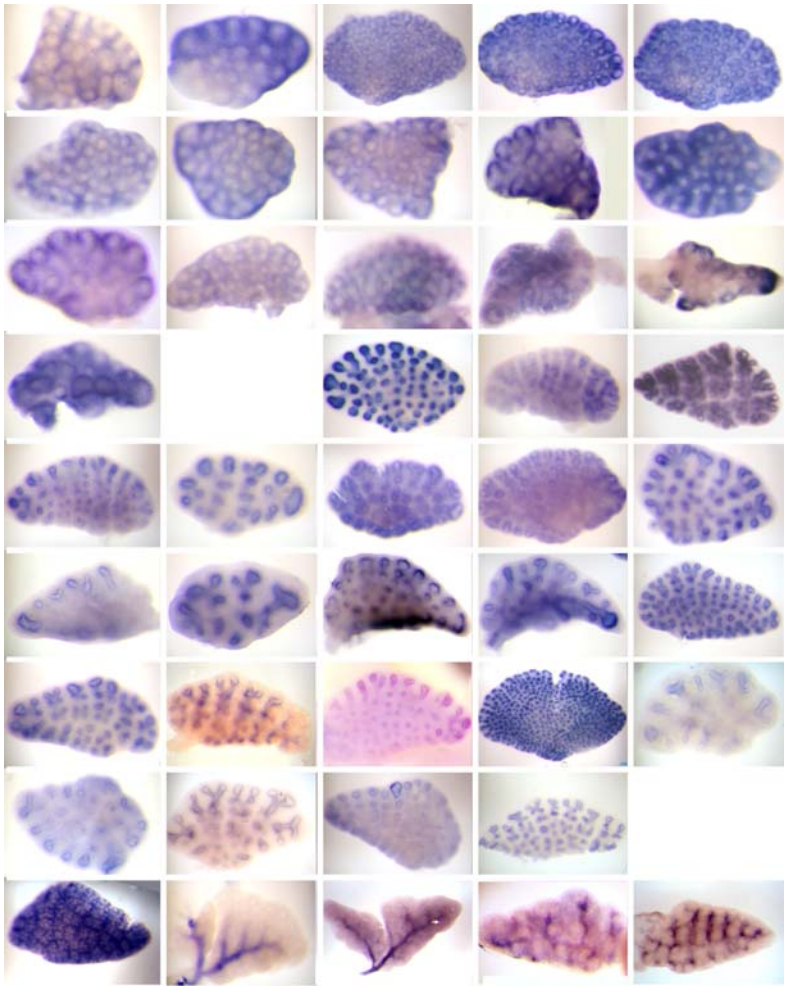
Global inactivation of *Pbx1* led to CDH and die at birth



Russel and Donahoe et al. 2012

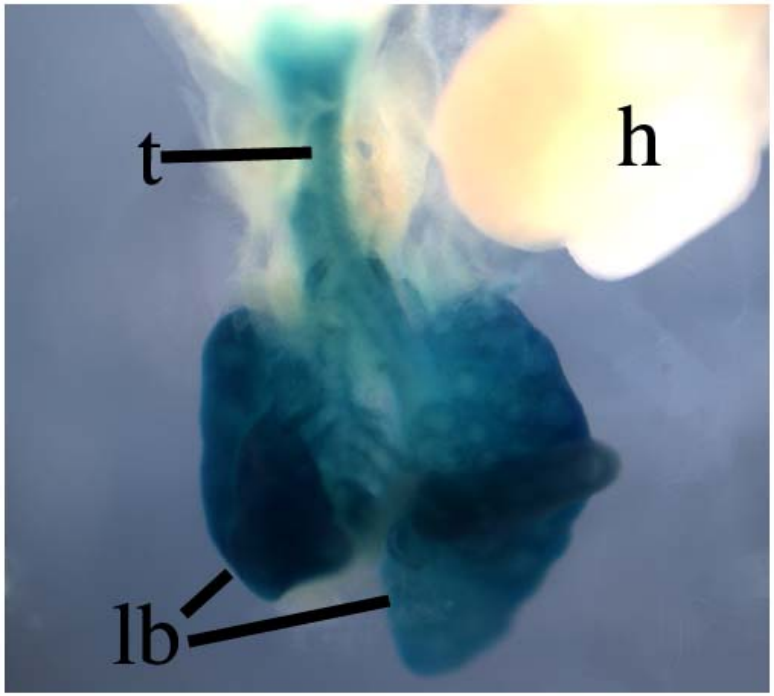
Genome-scale RNA in situ screen identified *Pbx1* as a gene expressed in lung mesenchyme

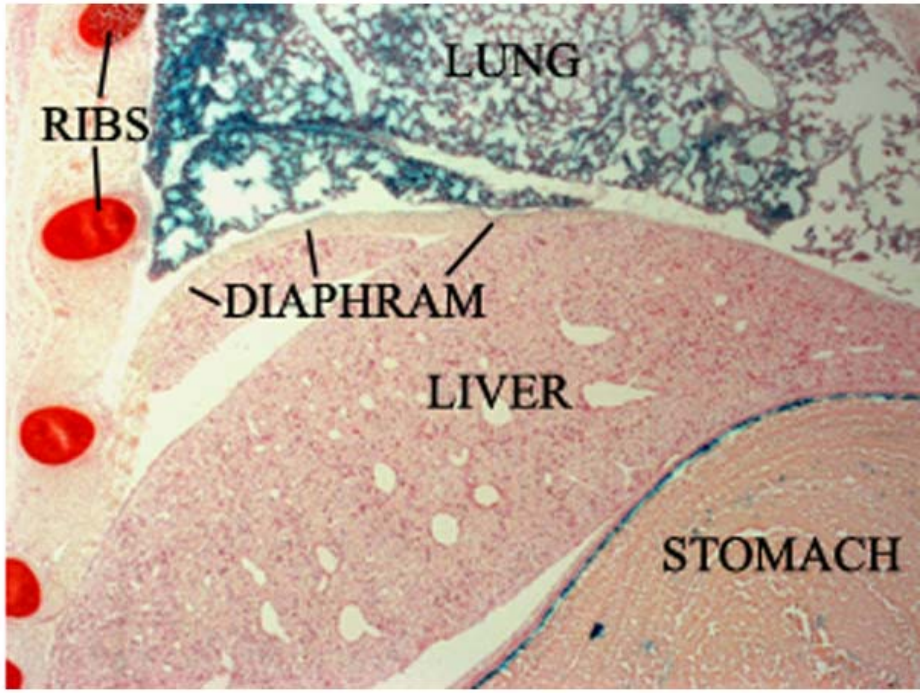
Screened 1,100 transcription factor genes (90%)



Herriges, Yi et al, 2012.

Tbx4cre is active in the lung mesenchyme but not in the diaphragm or heart

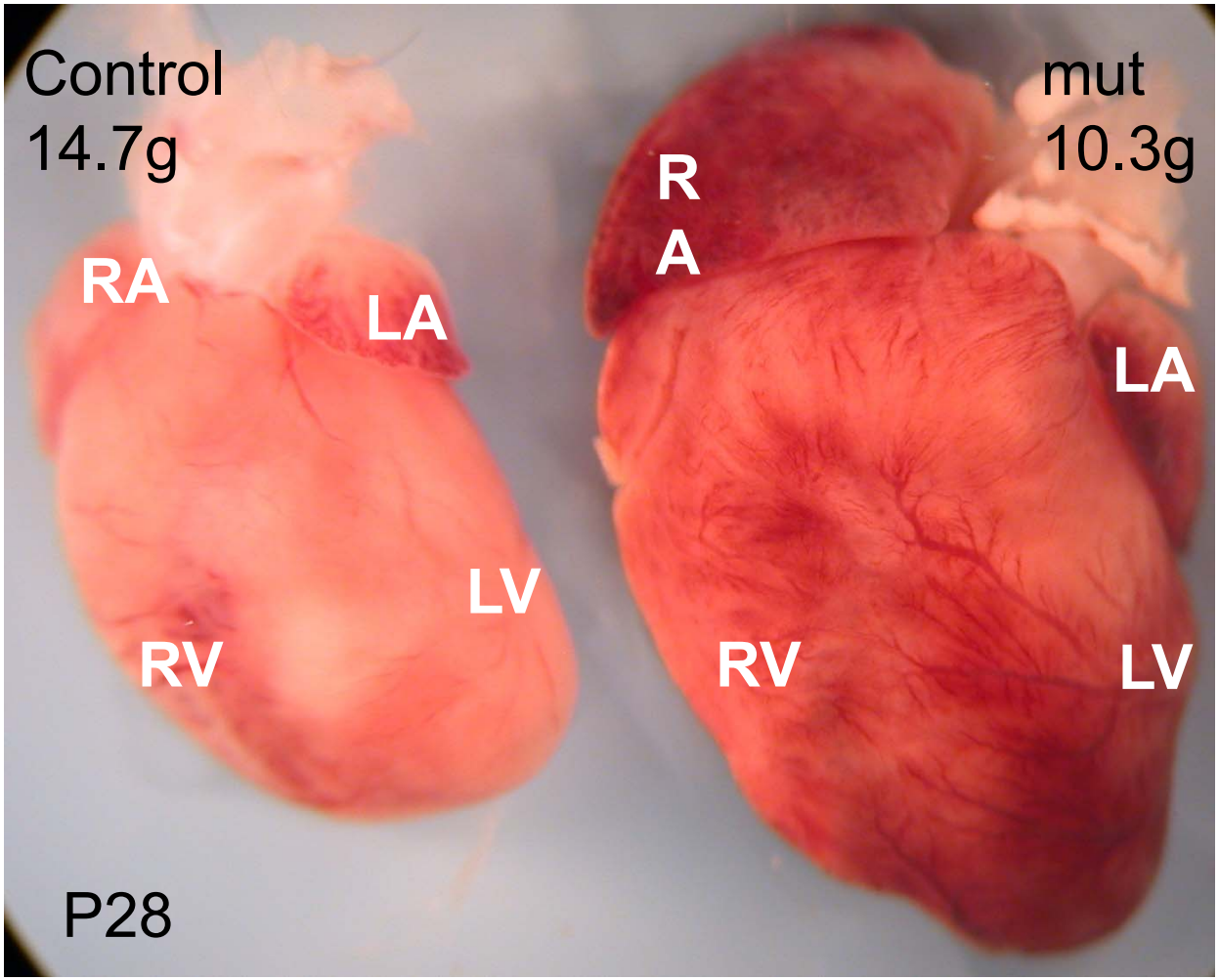




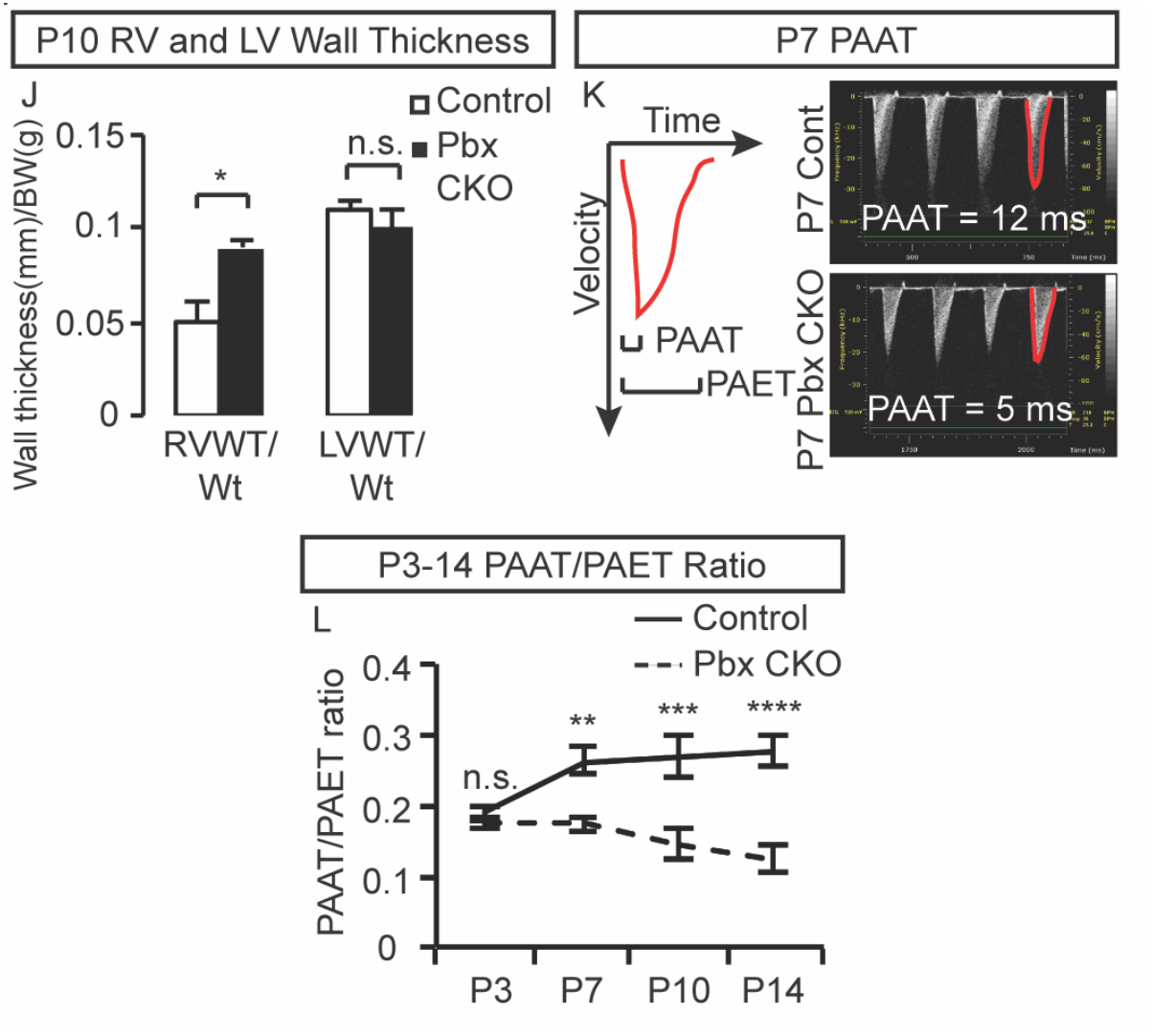
Naiche et al., 2011.

Tbx4cre;Pbx1;2 mutants survive birth, but exhibit tachypnea and die around weaning

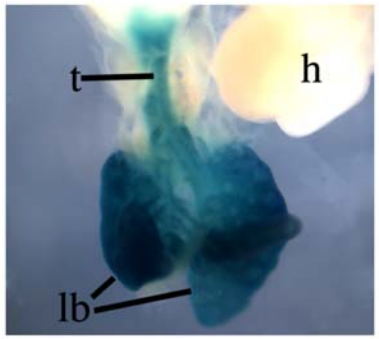
Tbx4cre;Pbx mutants exhibit enlarged heart



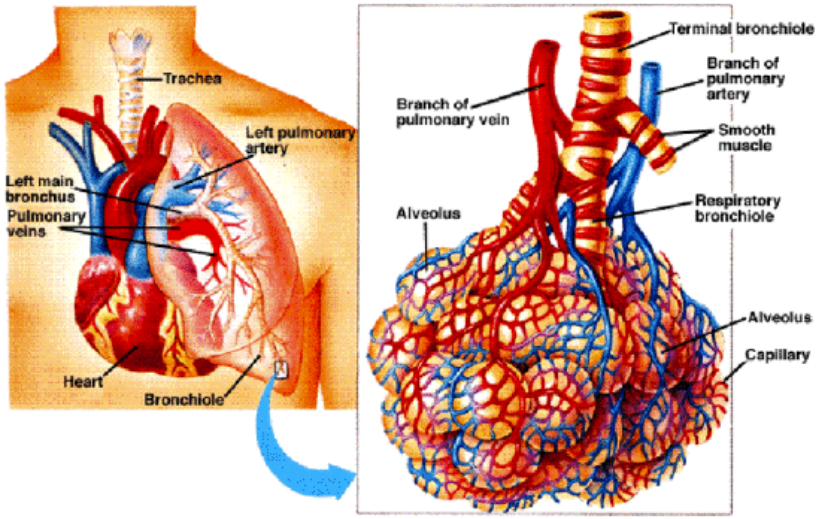
Echocardiography indicates pulmonary hypertension



Cardiac phenotype is secondary to lung defects



Lung-specific Inactivation → Lethal form of Pulmonary Hypertension



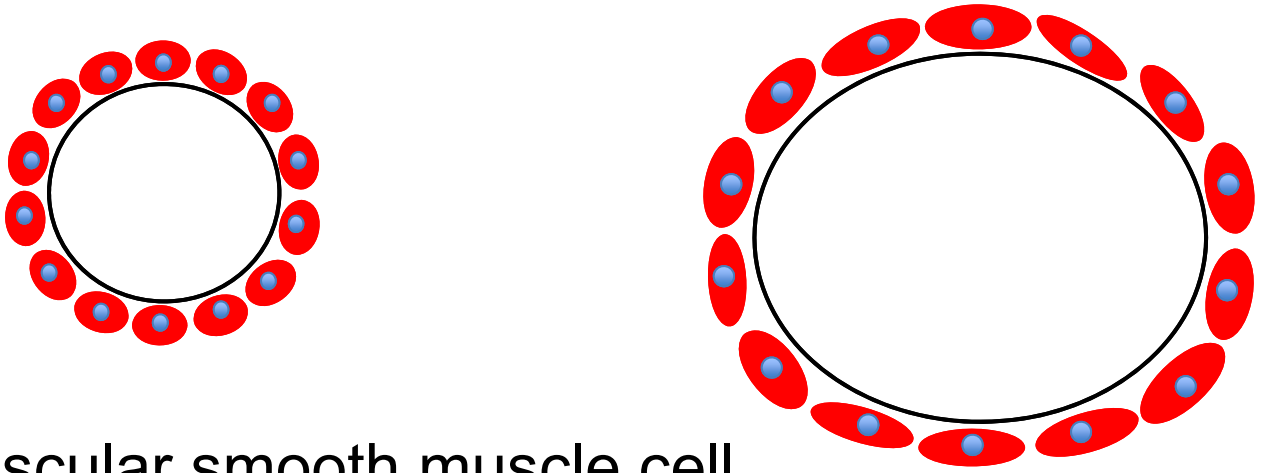
-Decrease in pulmonary artery/vein number;

-Increase in vascular smooth muscle number;

-Decrease in pulmonary capillaries density;

-Increase in vascular smooth muscle constriction.

Pulmonary vascular smooth muscle relax at birth, allows increase in blood flow

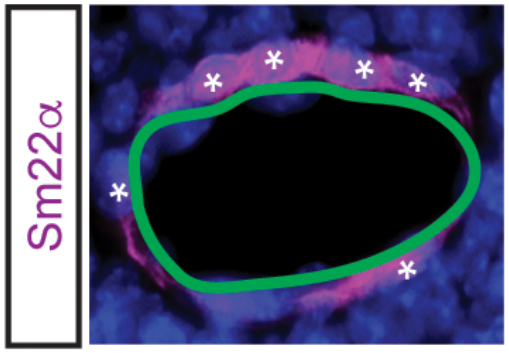


Vascular smooth muscle cell

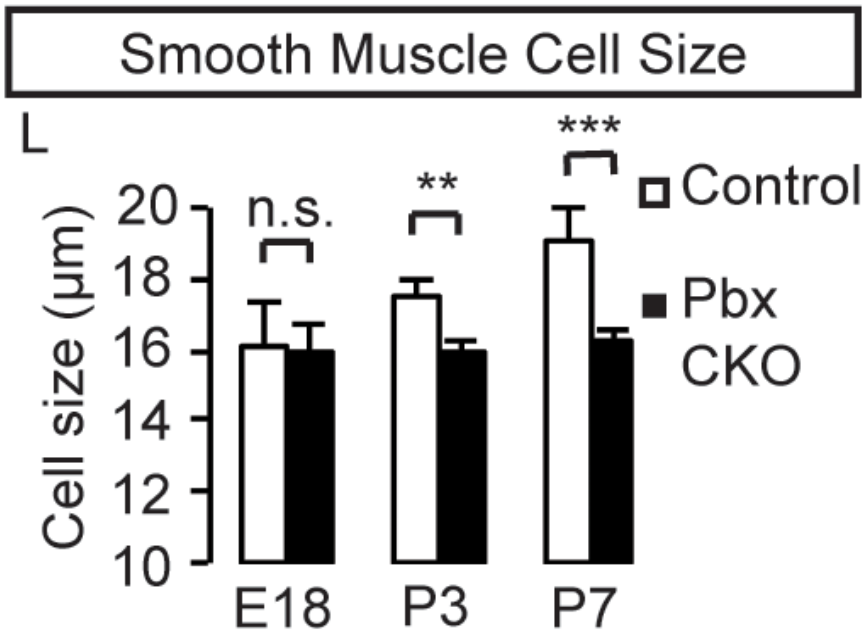
Prior to birth pulmonary vascular smooth muscle is constricted.

After birth pulmonary vascular smooth muscle relaxes.

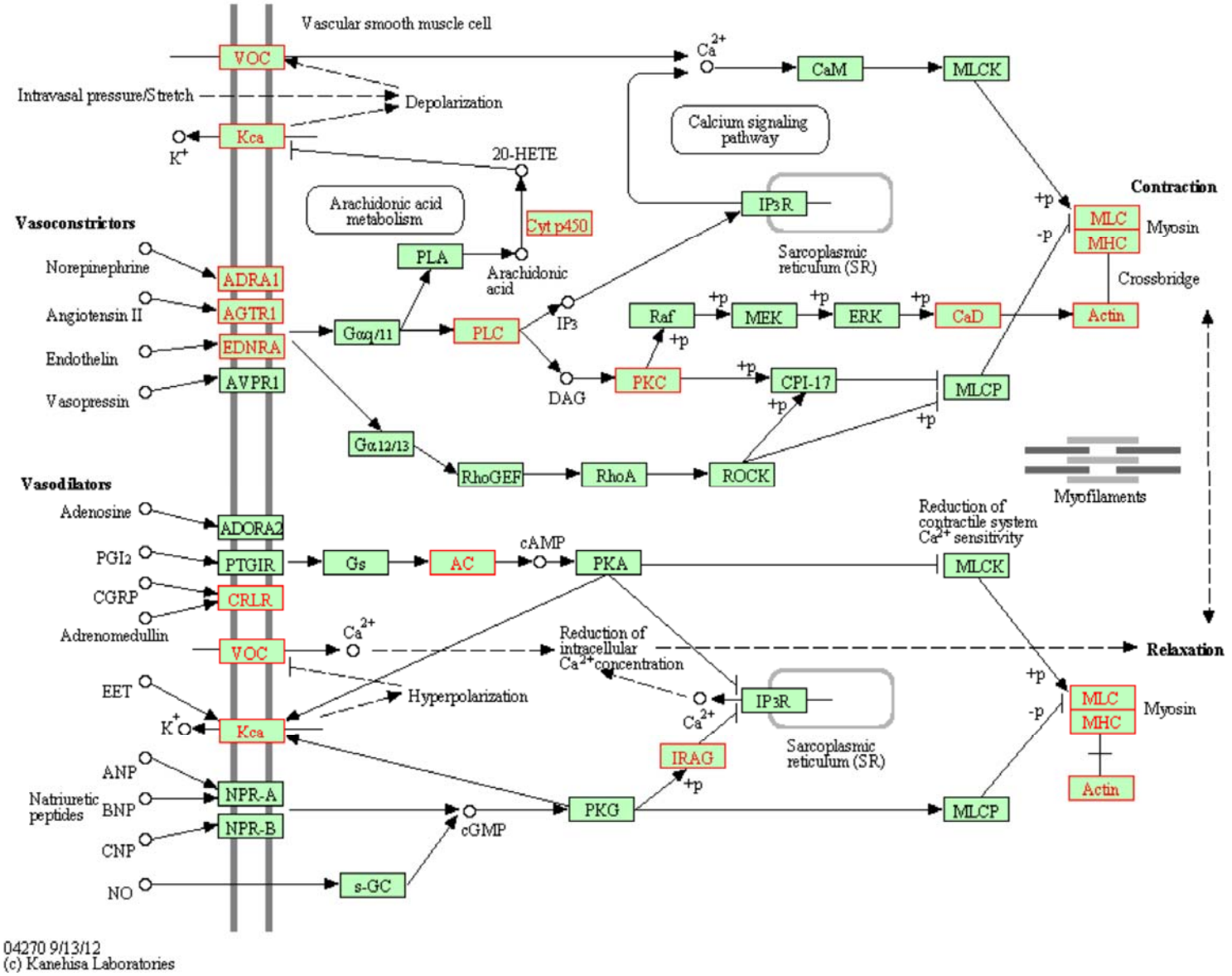
Pulmonary vascular smooth muscle cells do not relax after birth in *Pbx* mutants



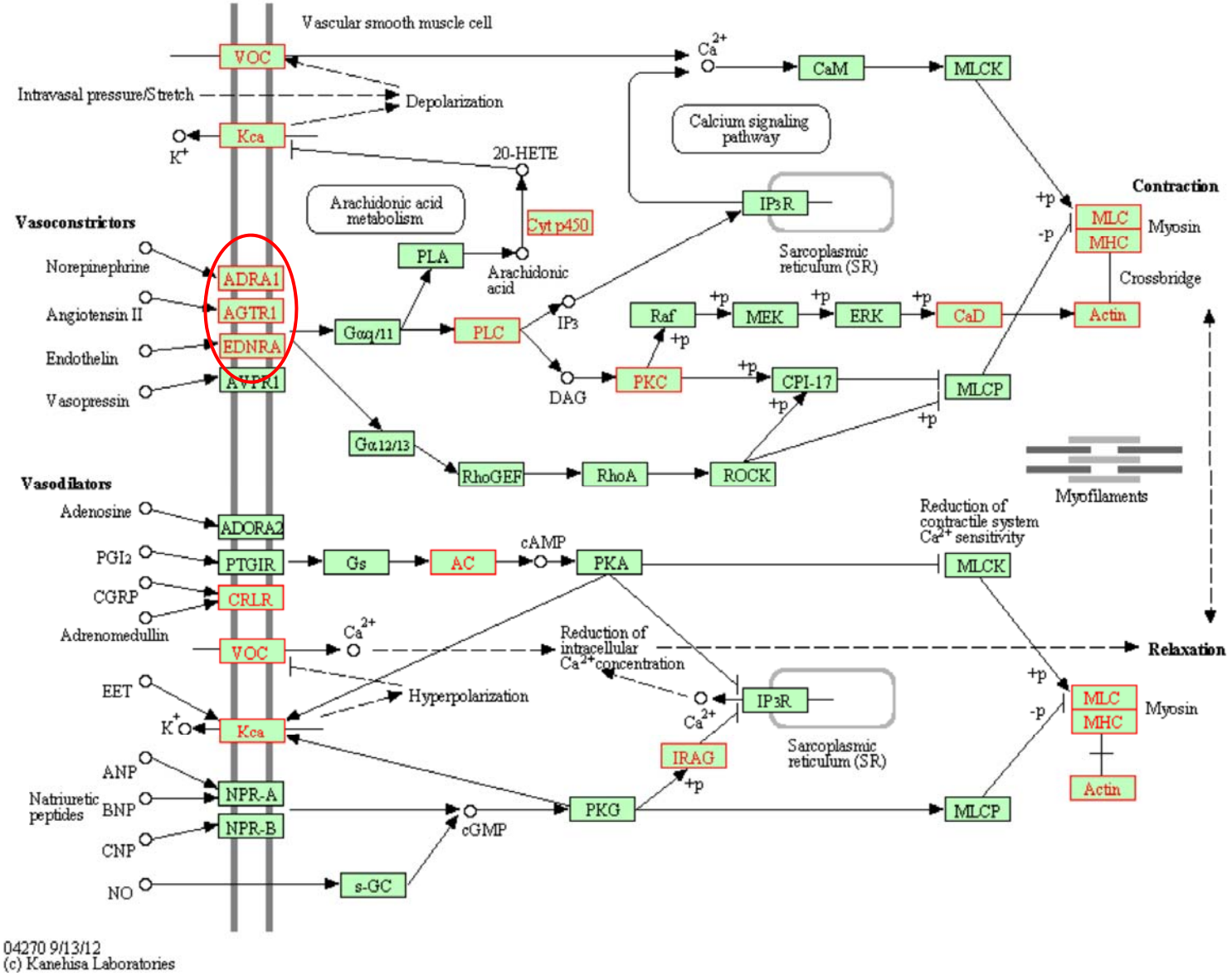
Smooth muscle cell size =
Vessel circumference ÷
Sm22α nuclei number



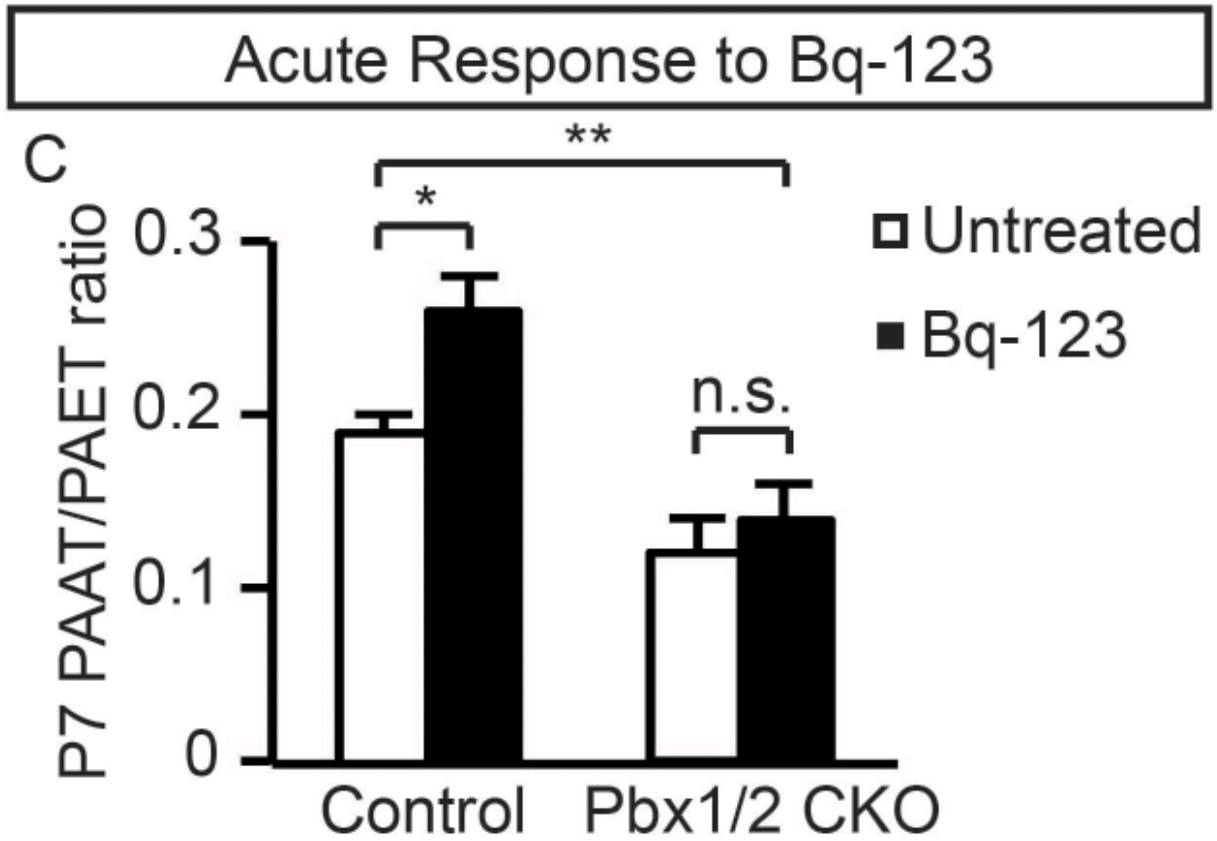
Expression of multiple genes in the vascular smooth muscle contraction network is altered



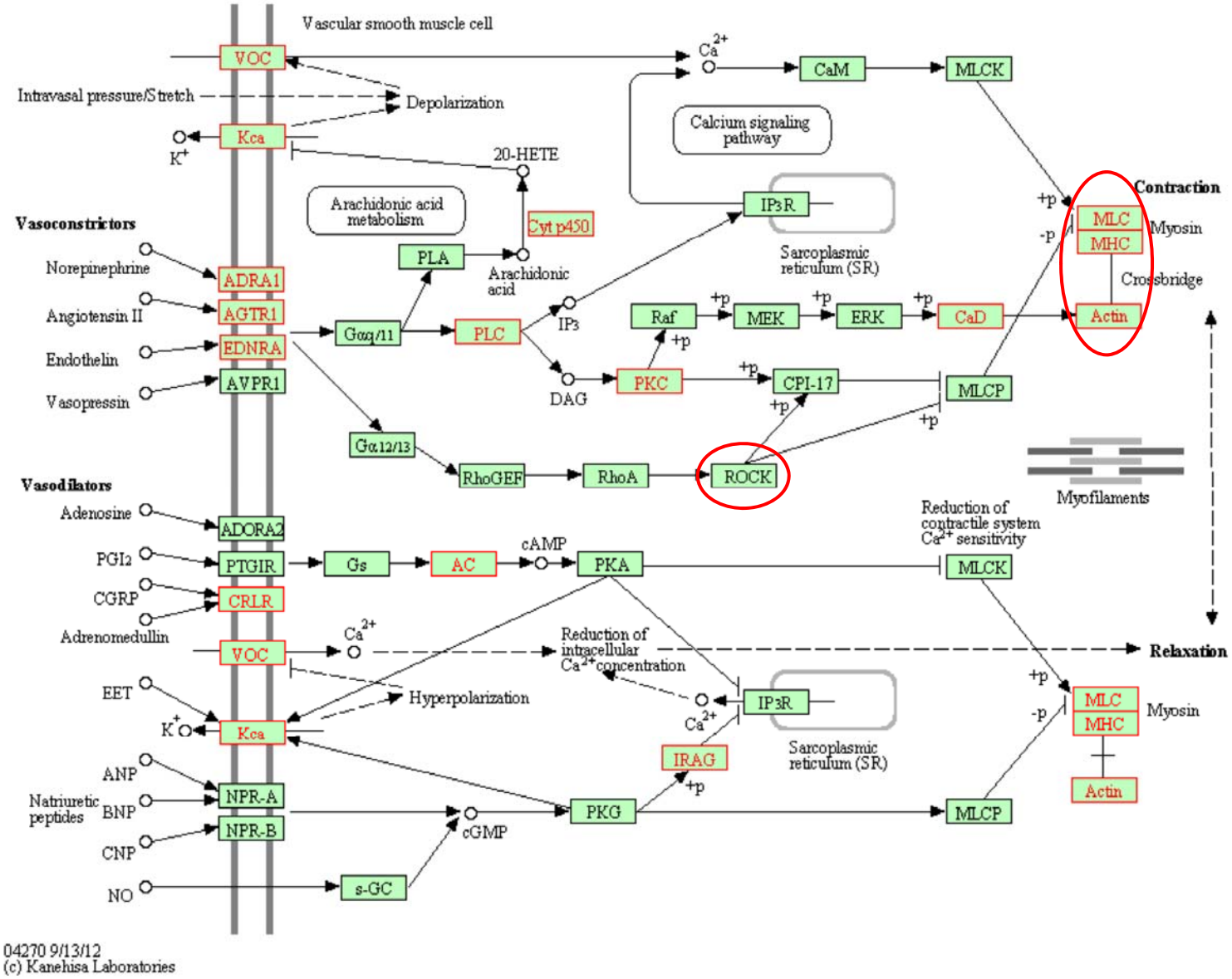
Upstream signals are common targets of treatment



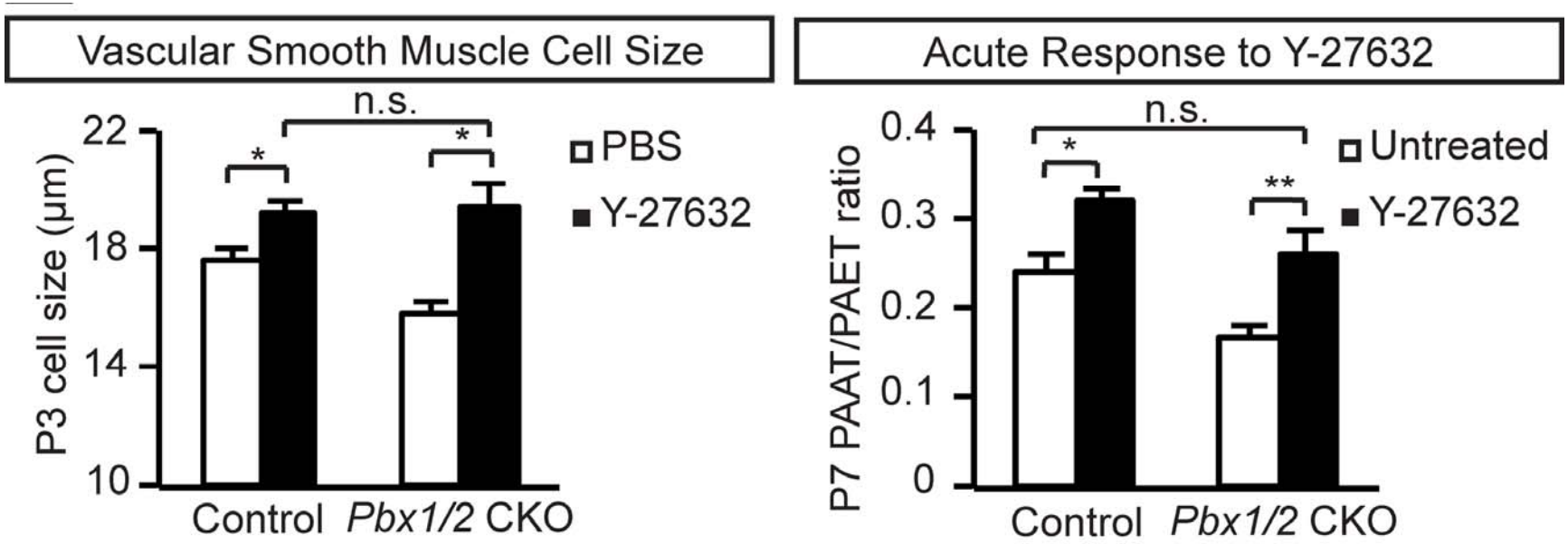
Treatment with Endothelin Receptor A inhibitor did not reverse hypertension in mutants

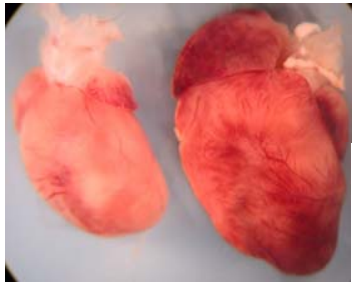


Downstream effectors are also affected



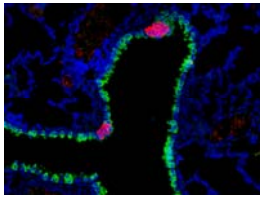
Acute application of ROCK inhibitor attenuates vSMC and pulmonary hypertension phenotypes





Summary II

- *Diaphragm-independent causes of lung defects in CDH:* lethal pulmonary hypertension can occur due to genetic defect that is intrinsic to the lung.
- *Clinical implication:* For CDH cases that are associated with lung-intrinsic loss of gene function, diaphragm repair surgery at birth is not sufficient to prevent pulmonary hypertension.
- *Novel biology:* PBX controls both vasoconstrictors and dilators and coordinates the rapid adaptation of the pulmonary vasculature to the extrauterine environment.



Whole genome/exome sequencing of CDH: a large number of variants

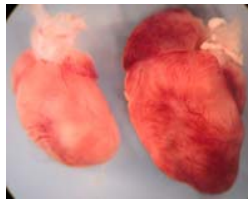


Table 1. Genes with confirmed *de novo* nonsense variants, indels or missense variants predicted deleterious (D) by MetaSVM in CDH probands

Proband ID	Gene	Variant	Amino acid change	MetaSVM prediction (score)	MDD expressed genes
01–0460	*ARFGEF2*	c.G3326A	p.R1109H	D (0.332)	Expressed
01–0761	*CDO1*	c.259delG	p.D87fs	N/A	Highly expressed
01–0568	*CLCN4*	c.G43A	p.D15N	D (0.644)	
01–0109	*DLST*	c.297_298del	p.99_100del	N/A	Highly expressed
01–0562	*GATA6*	c.C1366T	p.R456C	D (0.881)	Highly expressed
05–0011	*INHBB*	c.C1055G	p.T352R	D (0.507)	
01–0057	*LONP1*	c.C1325T	p.T442M	D (1.087)	Expressed
03–0001	*PPAPDC2*	c.T824A	p.V275E	D (0.427)	Expressed
01–0634	*PRKACB*	c.C277T	p.R93X	N/A	Highly expressed
01–0215	*PTPN12*	c.C77T	p.T26M	D (0.465)	Highly expressed
01–0450	*SIN3A*	c.1570_1577del	p.Y524Vfs*26	N/A	Highly expressed
01–0562	*SLC5A9*	c.C172T	p.R58C	D (0.856)	Expressed
01–0147	*STAG2*	c.C1840T	p.R614X	N/A	Highly expressed
01–0083	*TLN1*	c.G98A	p.R33H	D (0.351)	Highly expressed

Yu, Chung, et al. 2015.
Longini and Donahoe, et al. 2014.

CDH is of many distinct causes. Genetic diagnosis combined with detailed phenotyping will direct patient-specific treatment.

Acknowledgements

Sun lab:

Kelsey Branchfield
David McCulley

Sarah Ardell
Leanne Go
Rongbo Li
Arshia Uttma
Jamie Verheyden
Albert Xu
Randee Yang
Tina Zhang

Collaborators:

Dr. Le Ma
Dr. Marc Tessier-Lavigne
Dr. Licia Selleri
Dr. Naomi Chesler
Dr. Tim Hacker

Funding sources:

NIHOD 02385701
NHLBI HL113870
NHLBI HL119946
NHLBI HL122406