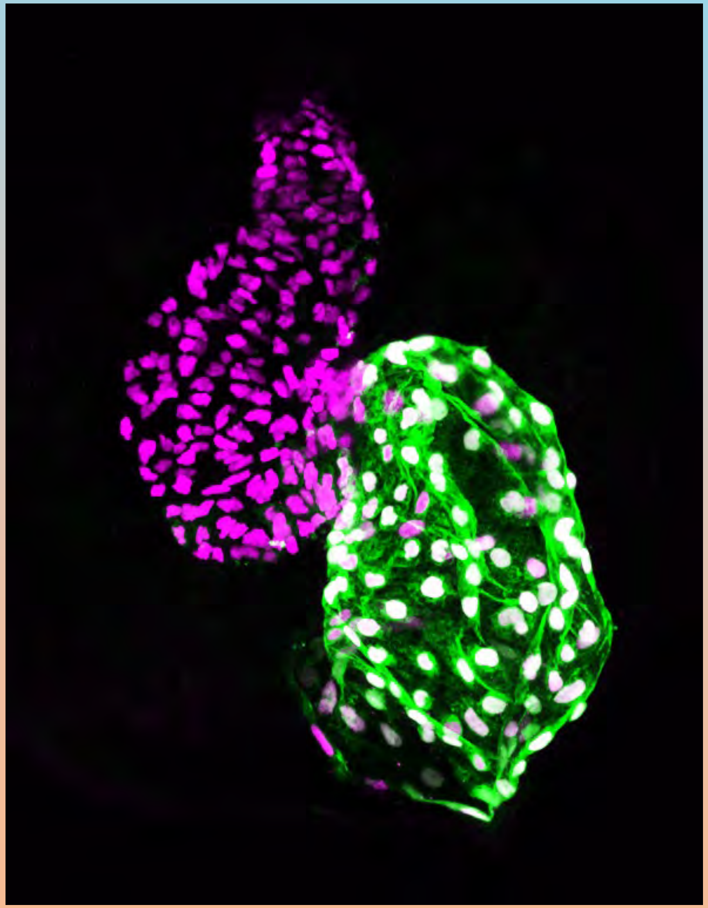
Innovative zebrafish research for investigating CHDs
or
Using zebrafish to understand how to build a heart



Hank Farr, Kimia Imani,
Darren Pouv, Lisa Maves

Center for Developmental Biology
and Regenerative Medicine
Seattle Children's Research Institute

Division of Cardiology
UW Department of Pediatrics

Seattle Children's
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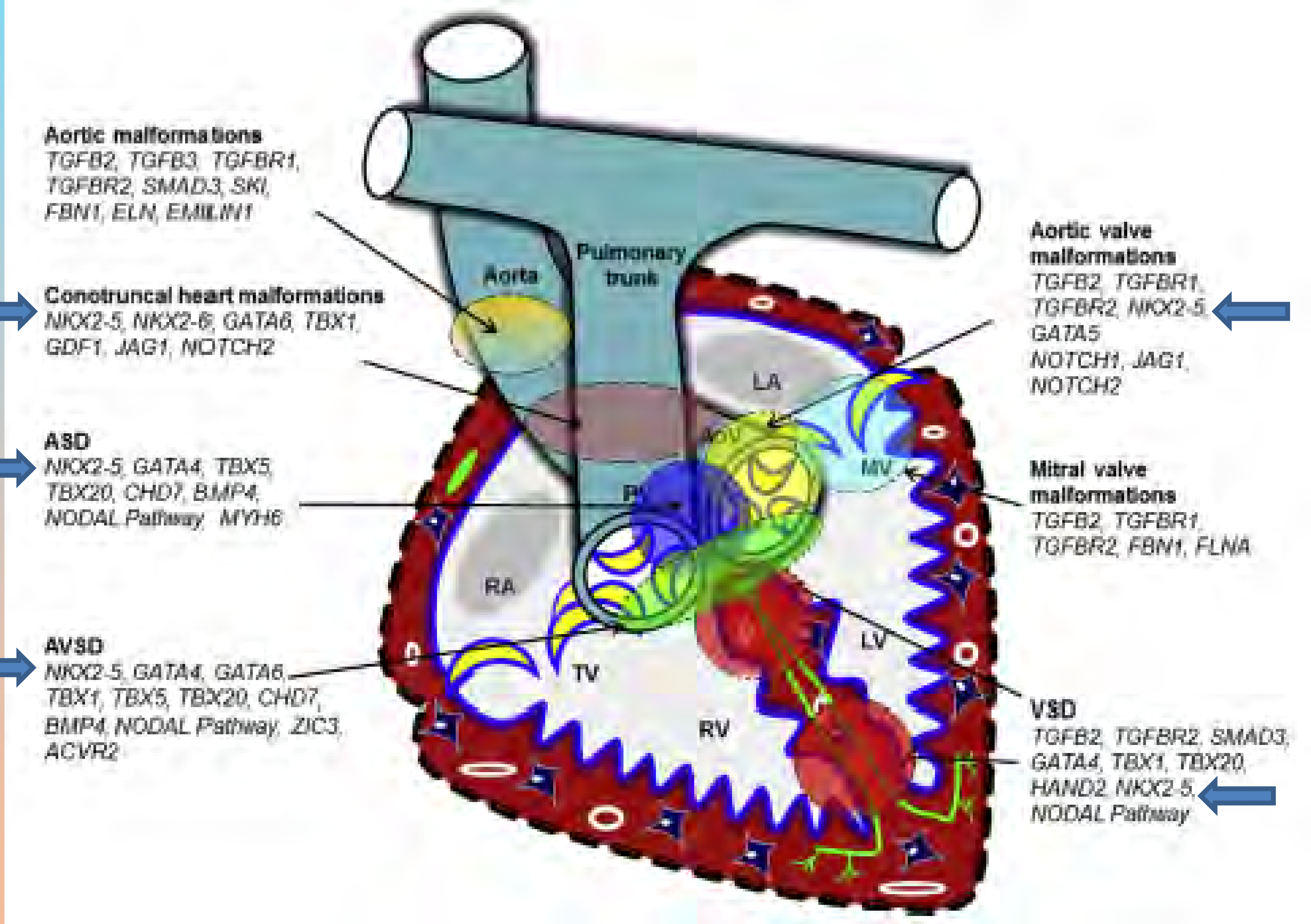
Genetics of congenital heart defects



Azhar and Ware, 2015

Estimate of 400+ CHD risk genes (Jin et al., 2017, *Nature Genetics*)

Roles for modifier genes?

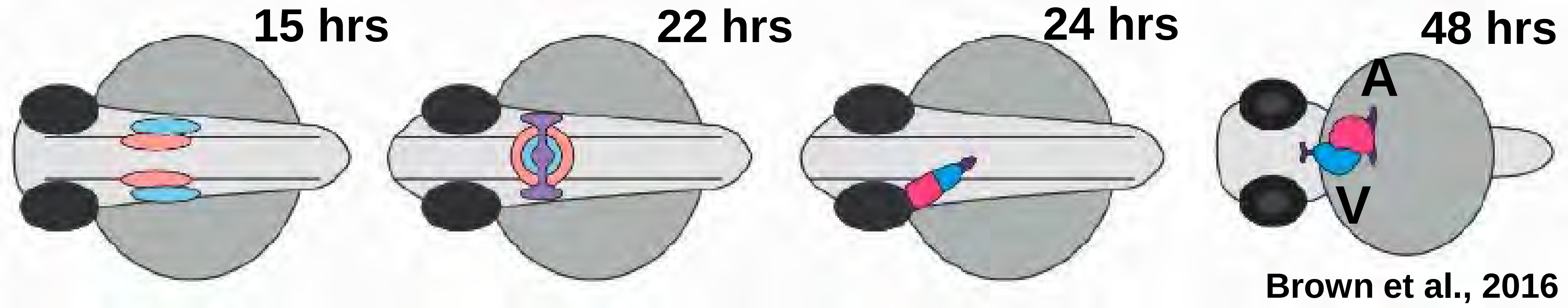
Goals

1. Advantages and challenges of zebrafish models

2. Genome editing in zebrafish to characterize a genetic variant in congenital heart defects

3. Roles for Pbx and Meis factors in heart development and cardiomyocyte differentiation

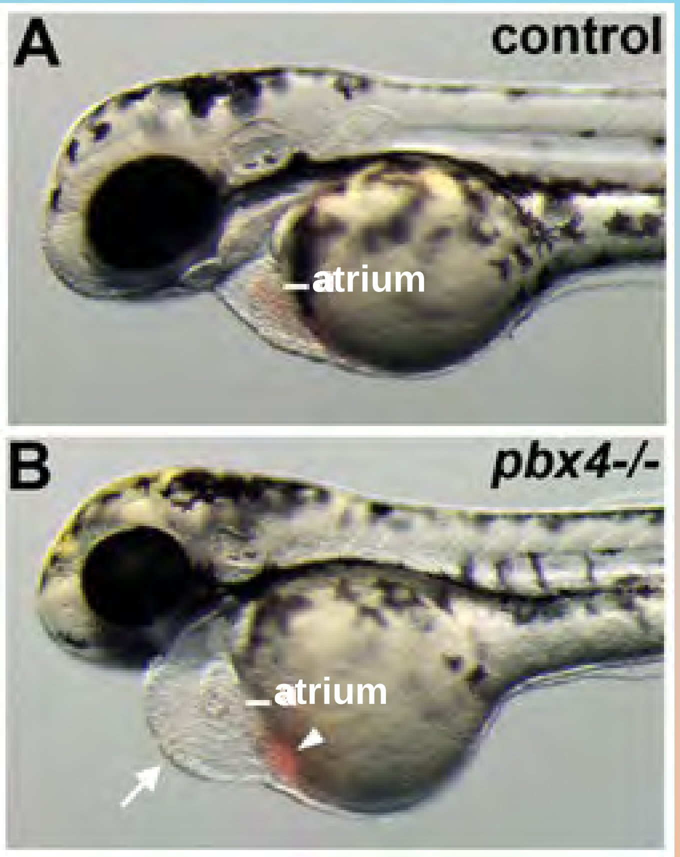
Zebrafish heart development



- Rapid, external development
- Hundreds of embryos/day
- Genome editing

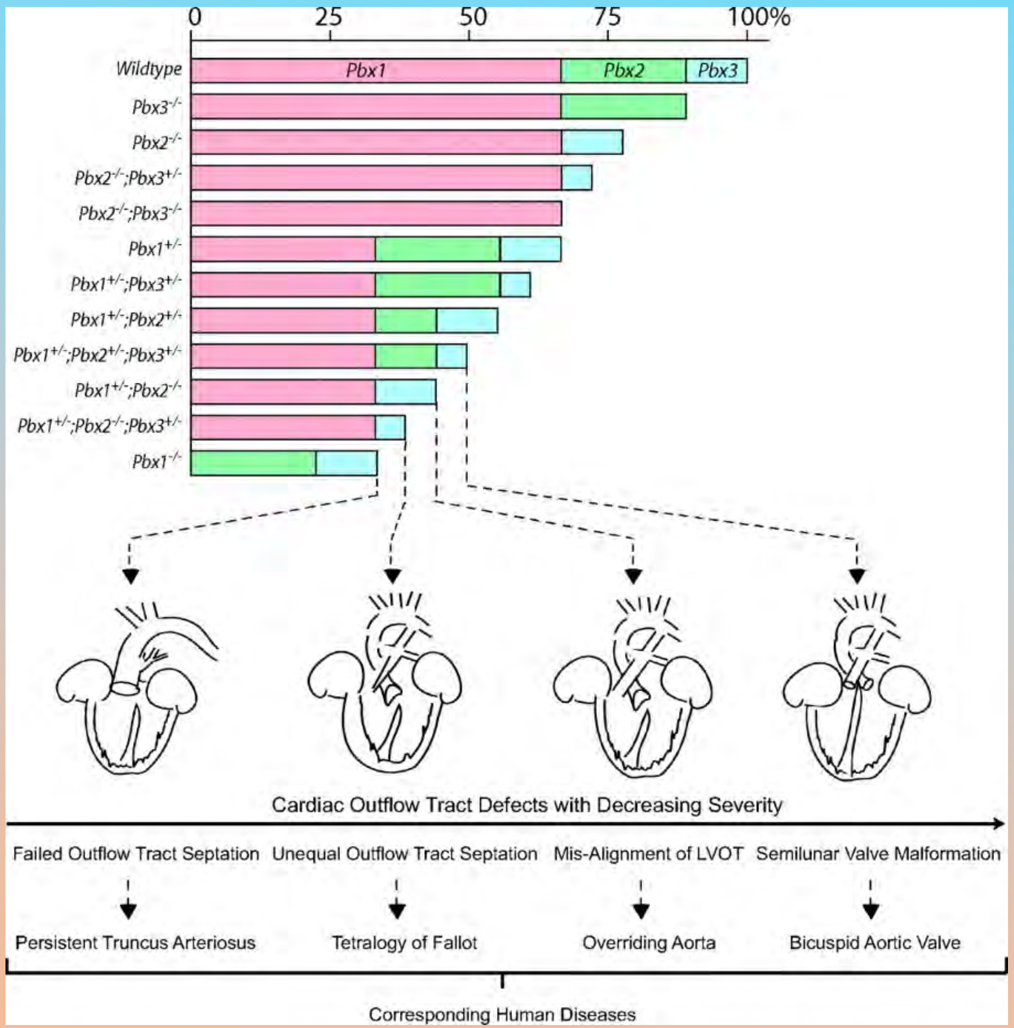
Pbx mutant animal models have heart defects

Zebrafish mutants:



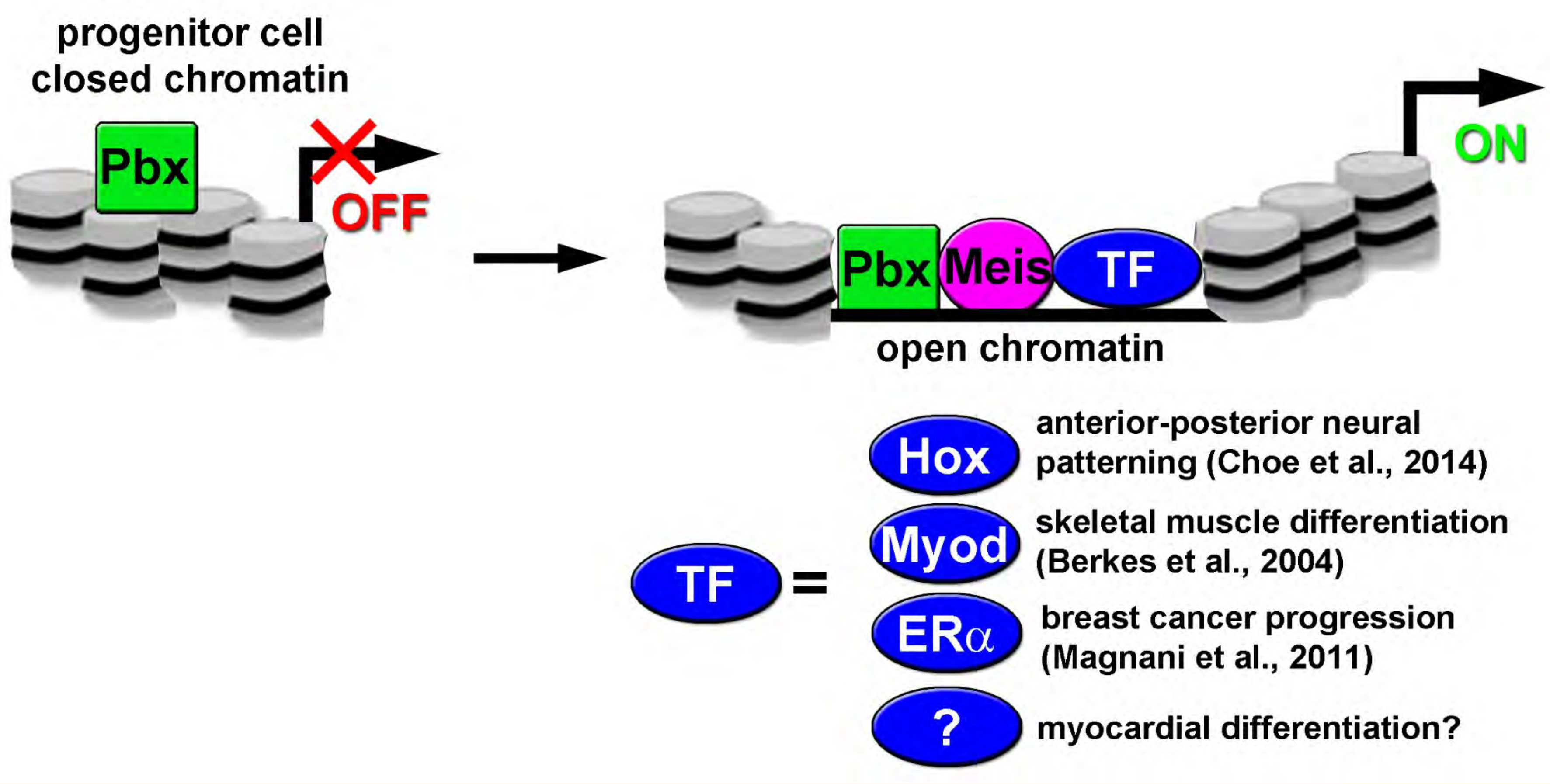
Maves et al., 2009 *Dev Bio*
Kao et al., 2015 *J Dev Bio*

Mouse mutants:

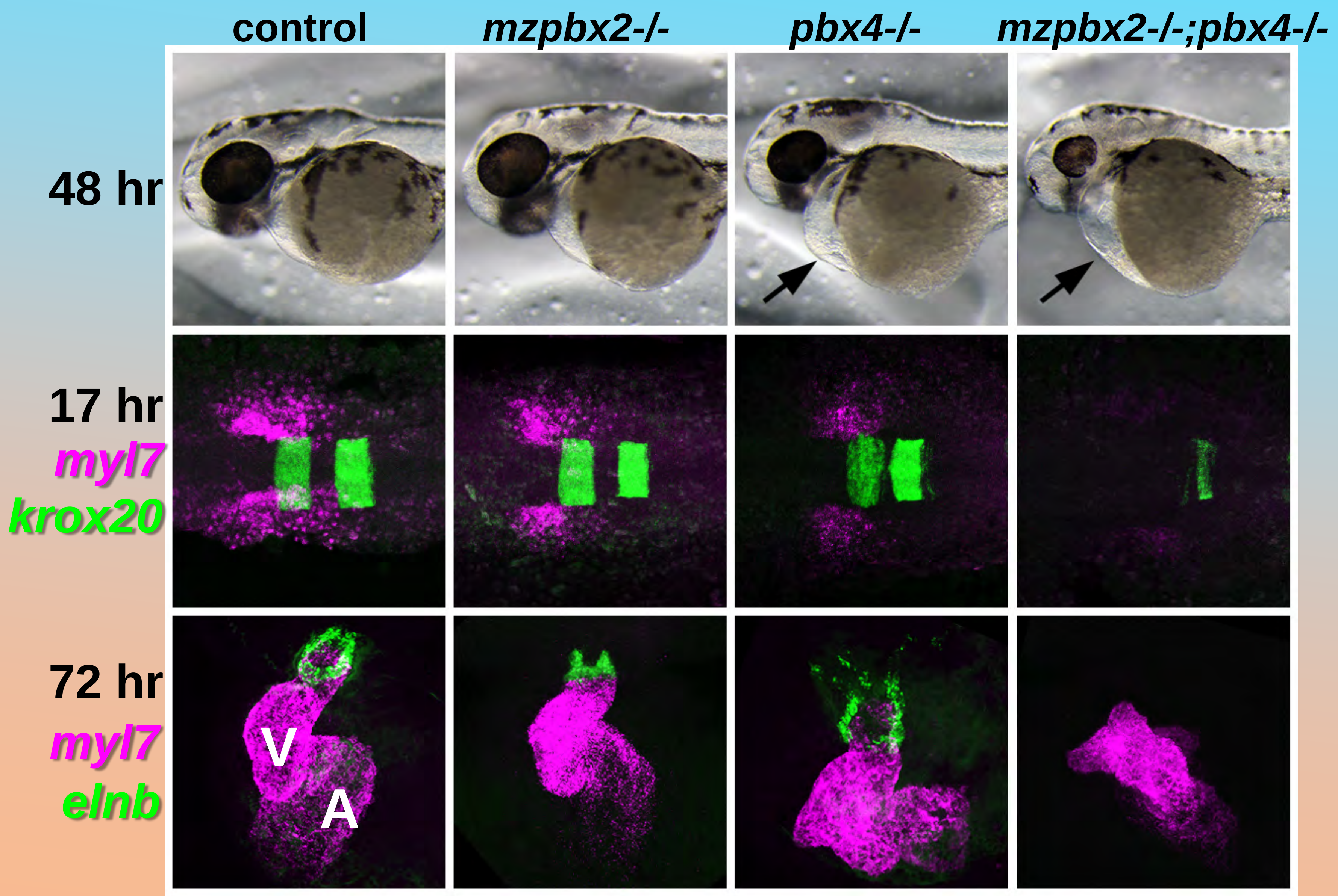


Stankunas et al., 2008 *Circ Res*

Pbx proteins are pioneer factors that promote regulatory transcription factor binding



Pbx proteins are required for myocardial differentiation and outflow tract formation



Human *PBX* gene variants linked to CHDs

De novo, deleterious sequence variants that alter the transcriptional activity of the homeoprotein PBX1 are associated with intellectual disability and pleiotropic developmental defects

Anne Slavotinek[1,2,*,†], Maurizio Risolino[2,3,†], Marta Losa[2,3], Megan T. Cho[4], Kristin G. Monaghan[4], Dina Schneidman-Duhovny[5,6], Sarah Parisotto[7], Johanna C. Herkert[8], Alexander P.A. Stegmann[9,10], Kathryn Miller[11], Natasha Shur[11], Jacqueline Chui[12], Eric Muller[12], Suzanne DeBrosse[13], Justin O. Szot[14,15], Gavin Chapman[14,15], Nicholas S. Pachter[16,17], David S. Winlaw[18,19], Bryce A. Mendelsohn[1,2], Joline Dalton[20], Kyriakie Sarafoglou[21], Peter I. Karachunski[22], Jane M. Lewis[23], Helio Pedro[7], Sally L. Dunwoodie[14,15], Licia Selleri[2,3,‡] and Joseph Shieh[1,2,‡]

Human Molecular Genetics, 2017, Vol. 26, No. 24

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Contents lists available at SciVerse ScienceDirect

European Journal of Medical Genetics

journal homepage: http://www.elsevier.com/locate/ejmg

Short report

Non-synonymous variants in pre-B cell leukemia homeobox (PBX) genes are associated with congenital heart defects

Cammon B. Arrington[a], Benjamin R. Dowse[a], Steven B. Bleyl[b], Neil E. Bowles[a,*]

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Short report

Non-synonymous variants in pre-B cell leukemia homeobox (PBX) genes are associated with congenital heart defects

Cammon B. Arrington [a], Benjamin R. Dowse [a], Steven B. Bleyl [b], Neil E. Bowles [a,*]

Table 1
Non-synonymous variants identified in the patients with OFT malformations and in controls.

Gene	Exon	Number of patients (Allelic frequency)	Phenotypes	Variant	SIFT prediction (Score)	Panther prediction (Score)	Polyphen2 prediction (Score)	Allelic f requency in controls	ESP database allelic frequency[a]
PBX3	8	5 (2.6%)	TOF AVSD PTA BAV/COA HLHS	c.407C > T (p.Ala136Val)	Tolerated (0.10)	Deleterious (-4.68688)	Probably Damaging (0.980)	5/760 (0.66%)	18/2484 (0.72%)
PBX4	1	1 (0.5%)	AVSD	c.19C > T (p.Pro7Ser)	Tolerated (0.43)	No prediction	Unknown	0/760 (0%)	0/2700 (0%)
	3	1 (0.5%)	COA	c.308G > A (p.Gly103Glu)	Tolerated (0.34)	Benign (−1.18809)	Benign (0.034)	0/760 (0%)	0/2700 (0%)
	4	2 (1.1%)	COA/TGA HLHS	c.461C > T (p.Thr154Met)	Tolerated (0.08)	Benign (−2.56188)	Benign (0.104)	3/190 (1.58%)	8/2696 (0.30%)
MEIS1	8	1 (0.5%)	DORV	rs61752693 (p.Arg272His)	Tolerated (0.08)	Benign (−2.49403)	Benign (0.006)	0/760 (0%)	0/2100 (0%)
MEIS3	4	1 (0.5%)	AS	c.330G> T (p.Arg117Leu)	Damaging (0.00)	Benign (−2.9254)	Possibly Damaging (0.802)	1/760 (0.13%)	0/2650 (0%)
PKNOX1	11	1 (0.5%)	DORV	c.1238A > G (p.Glu413Gly)	Tolerated (0.22)	Benign (−1.83073)	Benign (0.000)	0/760 (0%)	0/2650 (0%)

TOF: Tetralogy of Fallot; AVSD: Atrioventricular Septal Defect; PTA: Persistent Truncus Arteriosus; BAV: Bicuspid Aortic Valve; COA: Coarctation of the Aorta; HLHS: Hypoplastic Left Heart Syndrome; TGA: Transposition of the Great Arteries; AS: Aortic Stenosis; DORV: Double Outlet Right Ventricle.

Pbx sequence associated with CHD variant is highly conserved

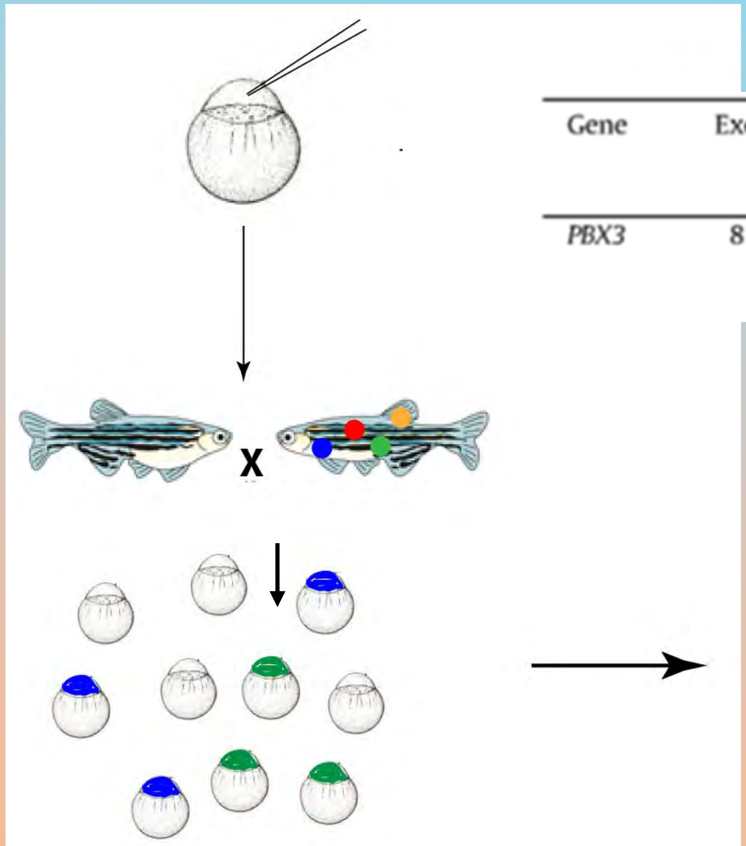
Pbx3

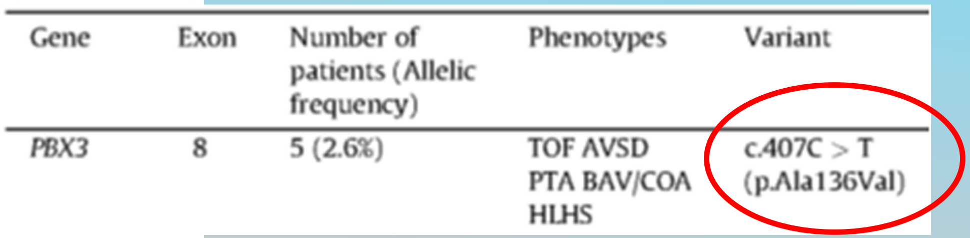
		V136
Human	SGPEKGGGSAAAAAAAAASGGSSDNS	
Rhesus monkey	SGPEKGGGSAAAAAAAAASGGSSDNS	
Mouse	SGPEKGGGSAAAAAAAAASGGSSDNS	
Chicken	SGPEKGGGSAAAAAAAAASGGSSDNS	
Zebrafish	AGPEKGGGSAAAAAAAAAAGSPTDNS	
Platypus	SGPEKGGGSAAAAAAAAASGGSSDNS	
Cow	SGPEKGGGSAAAAAAAAASGGSSDNS	
Horse	AGPEKGGGSAAAAAAAAASGGAGSDNS	
Xenopus	AGPEKGGGSAAAAAAAAASGGVSPDNS	
Human Pbx1	AGPEKGGGSAAAAAAAAASGGAGSDNS	
Human Pbx2	AGPEKGGGSAAAAAAAAASGGVSPDNS	
Zebrafish Pbx4	**SGPEKGGGSAAAAAAAAAAGGSPNDGS**	

Arrington et al., 2011

Using CRISPR/Cas9 to engineer zebrafish model of human *PBX* gene variant

Inject Cas9 + *pbx4* guide RNA + oligo with *pbx4* C>T variant





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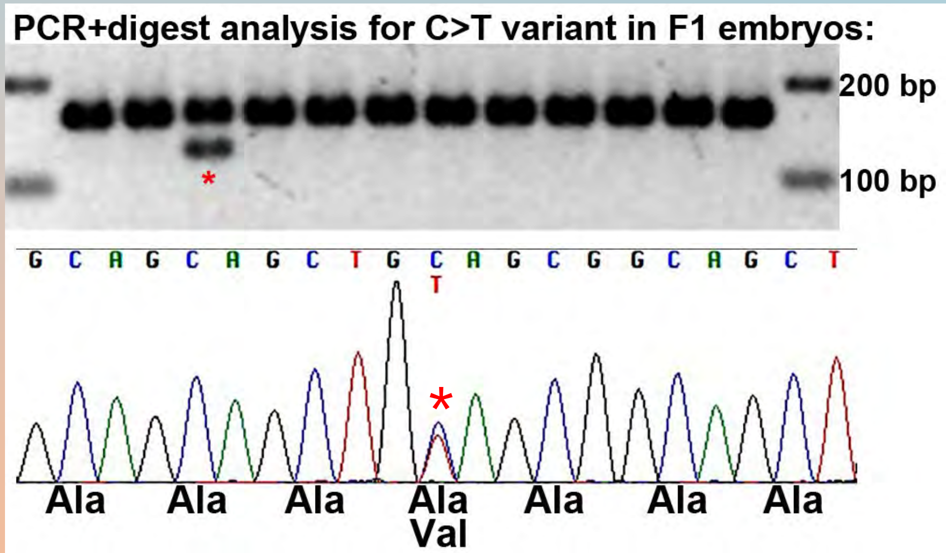

Fish homozygous for C>T variant (A131V allele) show no heart defects

PBX3(A136V) - a modifier allele in congenital heart defects?

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Non-synonymous variants identified in the patients with OFT malformations and in controls.

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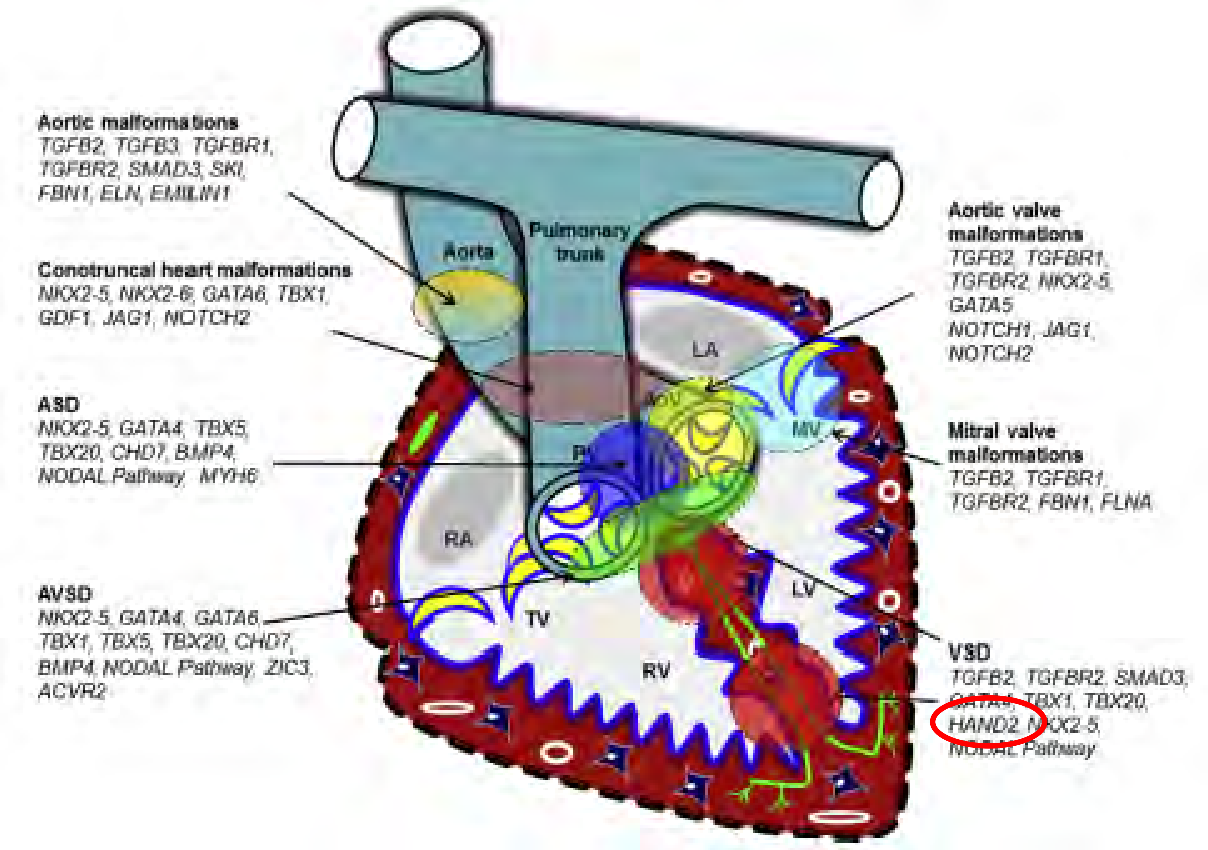
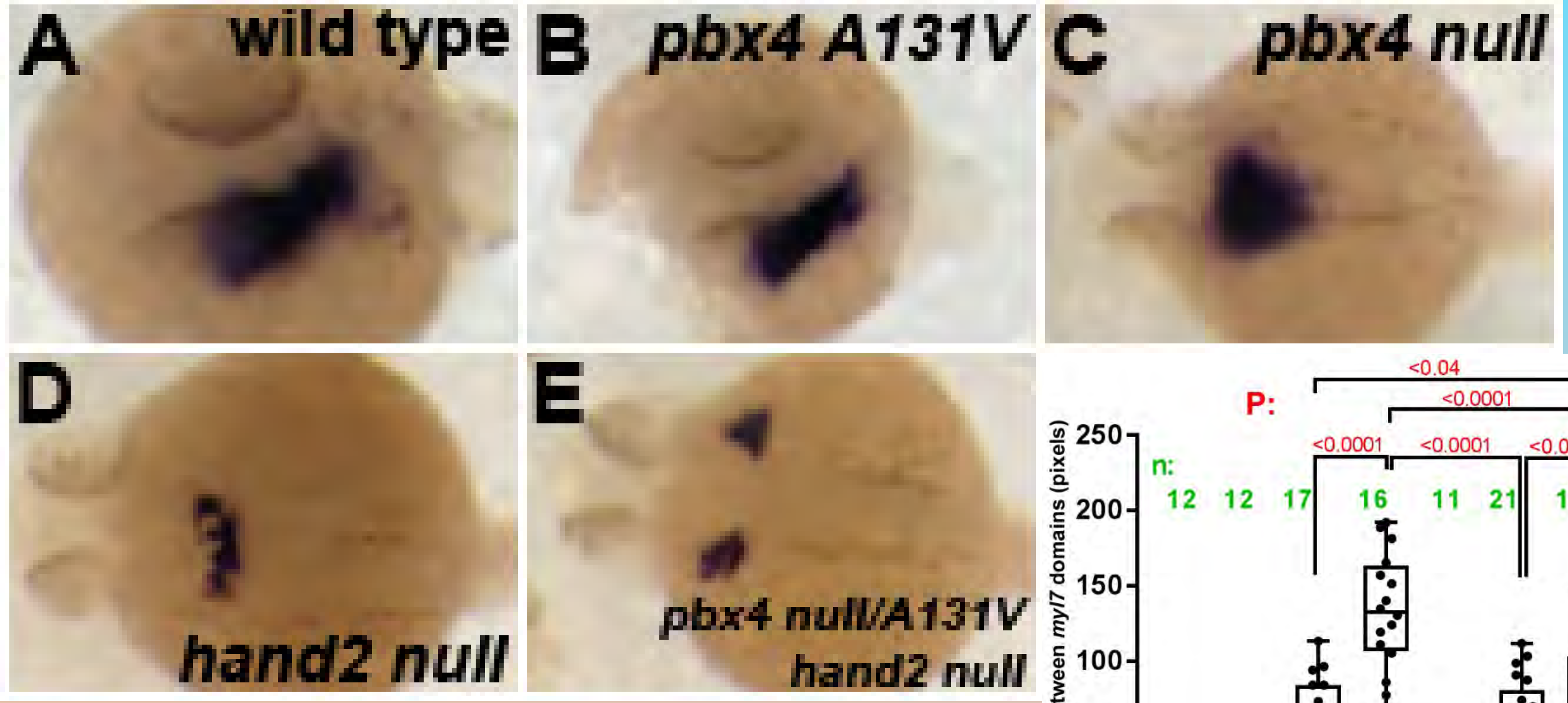
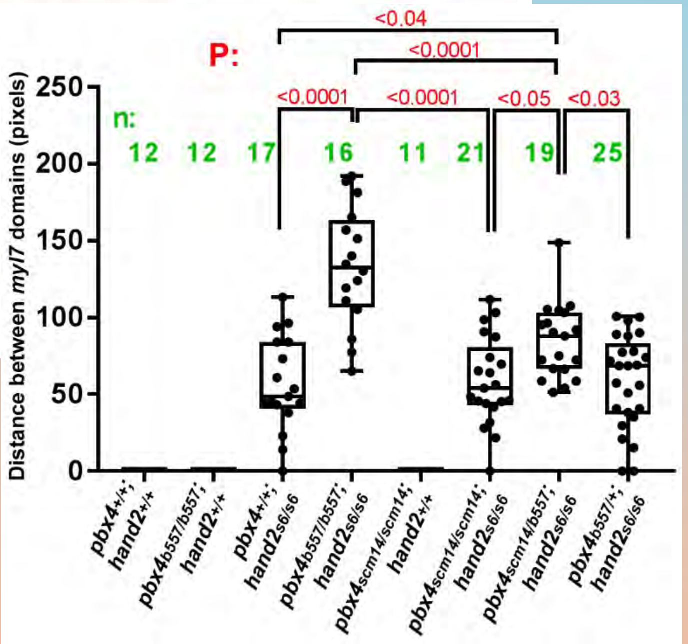


Do *pbx* alleles enhance the effects of mutations in congenital heart defect genes?

pbx4A131V+null alleles enhance heart morphogenesis defects caused by loss of *hand2*





- PBX3A136V is a potential modifier of CHD in humans
- We can make a patient's fish model and test candidate genetic variants

Farr et al., 2018 *Dis Mod Mech*

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EDITORIAL

Zebrafish knock-ins swim into the mainstream

Sergey V. Prykhozhij[1] and Jason N. Berman[1,2,3,*]

© 2018. Published by The Company of Biologists Ltd | Disease Models & Mechanisms (2018) 11, dmm035972. doi:10.1242/dmm.035972

RESEARCH ARTICLE

Functional testing of a human *PBX3* variant in zebrafish reveals a potential modifier role in congenital heart defects

Gist H. Farr, III[1], Kimia Imani[1,2], Darren Pouv[1,2] and Lisa Maves[1,3,*]

© 2018. Published by The Company of Biologists Ltd | Disease Models & Mechanisms (2018) 11, dmm035352. doi:10.1242/dmm.035352

RESEARCH ARTICLE

CRISPR/Cas9-mediated homology-directed repair by ssODNs in zebrafish induces complex mutational patterns resulting from genomic integration of repair-template fragments

Annekatrien Boel, Hanna De Saffel, Wouter Steyaert, Bert Callewaert, Anne De Paepe, Paul J. Coucke and Andy Willaert*

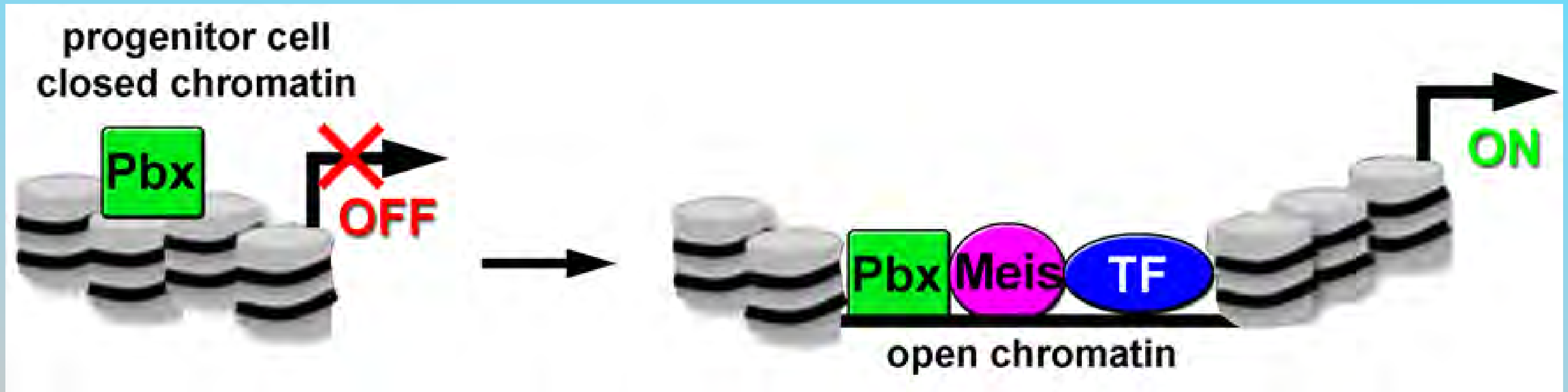
© 2018. Published by The Company of Biologists Ltd | Disease Models & Mechanisms (2018) 11, dmm035469. doi:10.1242/dmm.035469

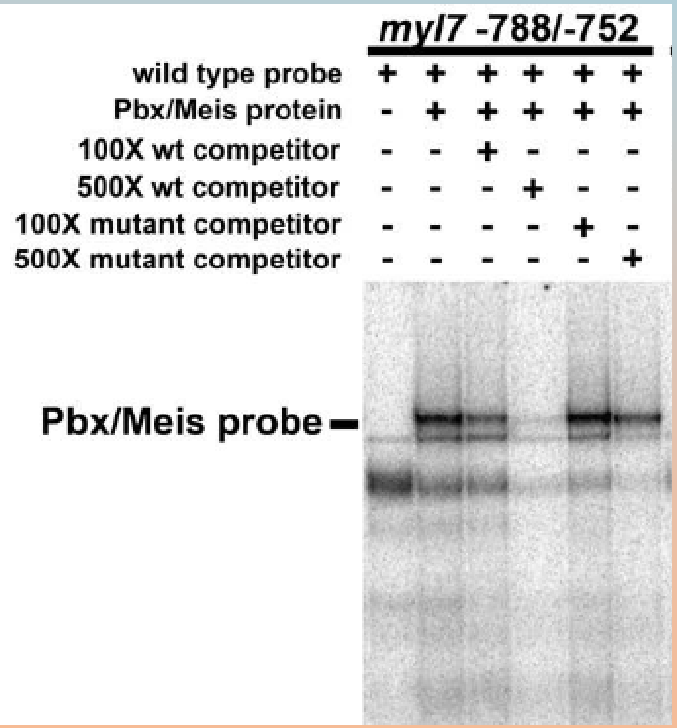
RESEARCH ARTICLE

Effective CRISPR/Cas9-based nucleotide editing in zebrafish to model human genetic cardiovascular disorders

Federico Tessadori[1,*], Helen I. Roessler[2,*], Sanne M. C. Savelberg[2,*], Sonja Chocron[1], Sarah M. Kamel[1], Karen J. Duran[2], Mieke M. van Haelst[2,3,4], Gijs van Haaften[2,‡] and Jeroen Bakkers[1,5,‡]

Are Meis proteins required for heart development?





Pbx and Meis bind the promoter of the myocardial gene *myl7*.

Maves et al., 2009 *Dev Bio*

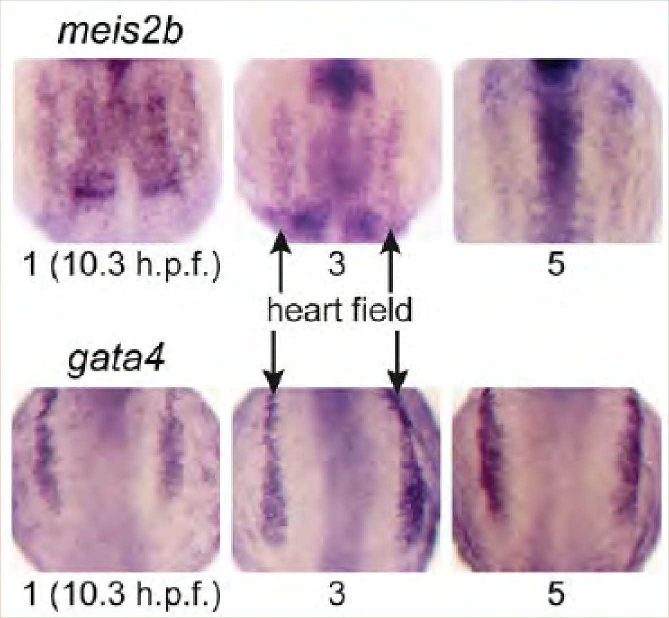
Meis2b is required for cardiac morphogenesis

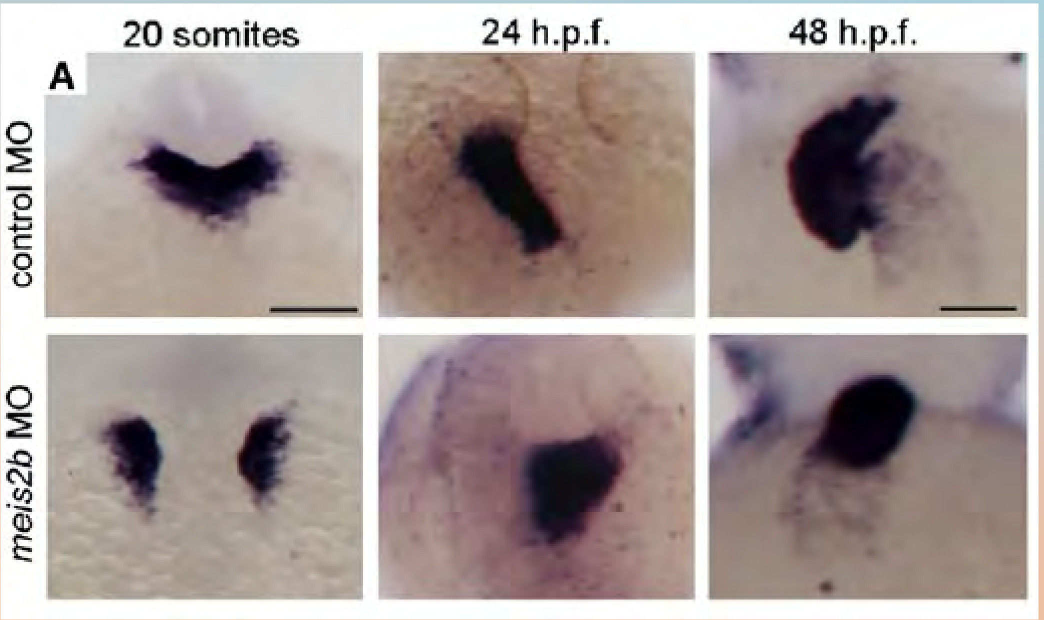
Resource

Cell

A Temporal Chromatin Signature in Human Embryonic Stem Cells Identifies Regulators of Cardiac Development

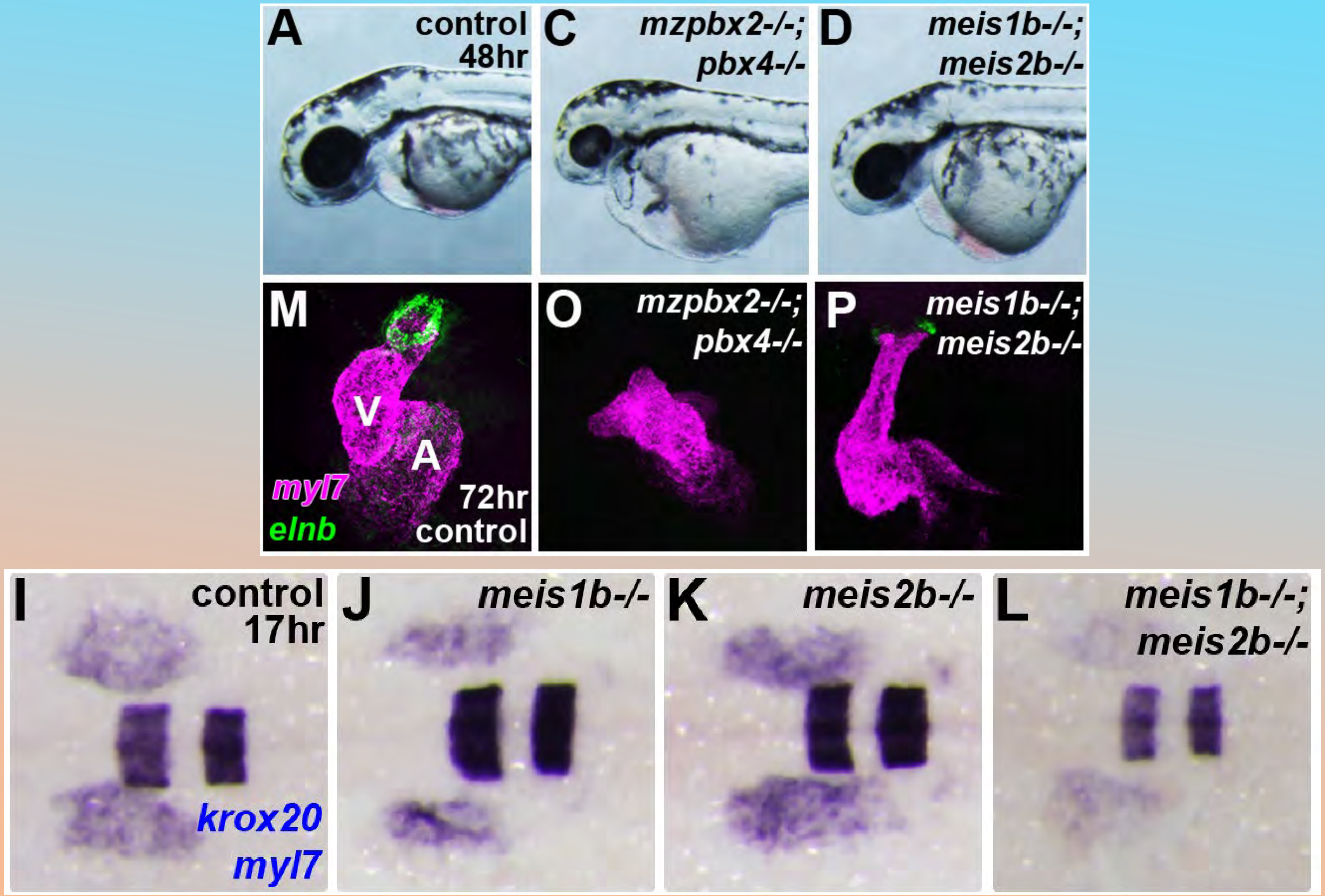
Sharon L. Paige,[1,2,3,12] Sean Thomas,[9,12] Cristi L. Stoick-Cooper,[2,4,12] Hao Wang,[5,12] Lisa Maves,[10] Richard Sandstrom,[5] Lil Pabon,[1,2,3] Hans Reinecke,[1,2,3] Gabriel Pratt,[1,2,3] Gordon Keller,[11] Randall T. Moon,[2,5] John Stamatoyannopoulos,[5,6,*] and Charles E. Murry[1,2,3,7,8,*]



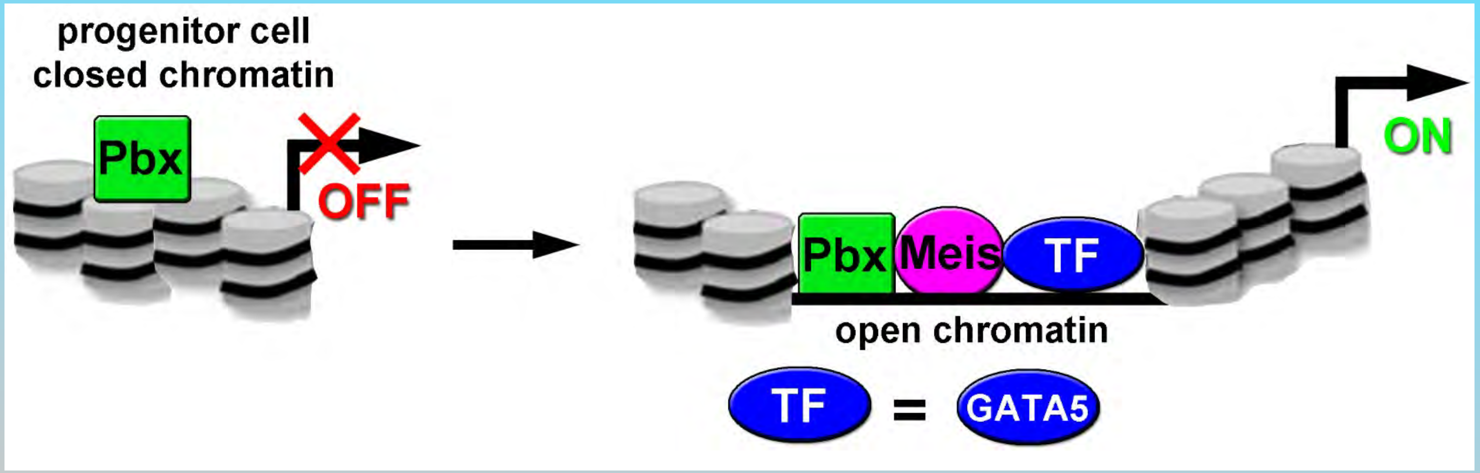


Paige et al., 2012 *Cell*

Meis1b and Meis2b are required for cardiac morphogenesis and myocardial differentiation



Can Pbx and Meis promote myocardial differentiation?



control

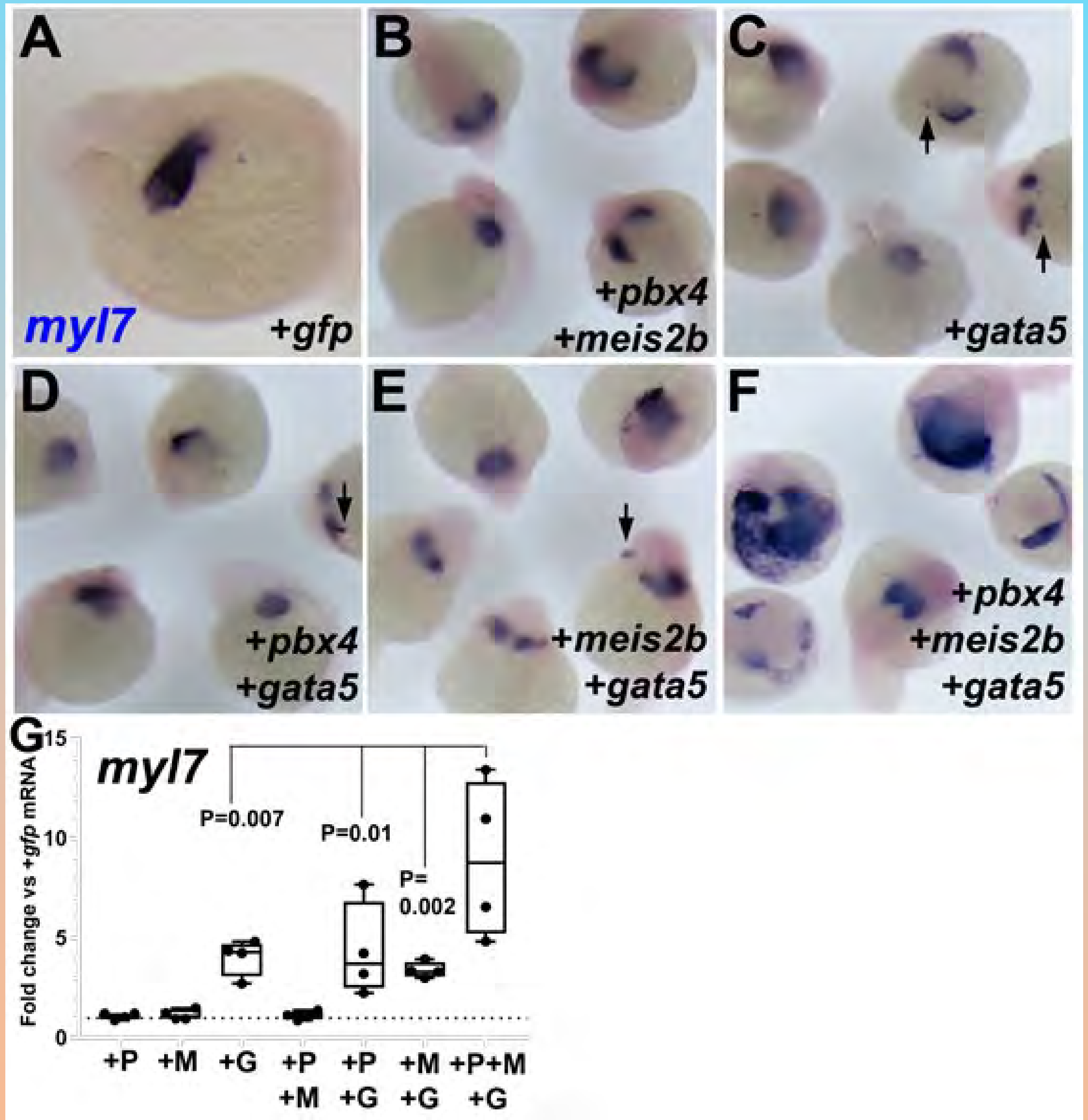
+*gata5* mRNA overexpression

myocardial *myl7* expression

Reiter et al., 1999 *Genes Dev*

gata5 **over-expression induces extra myocardial differentiation in zebrafish embryos**

Pbx and Meis enhance Gata5 induction of myocardial differentiation



Approaches in zebrafish to understand heart development and CHDs: Conclusions

1. Genome editing in zebrafish to characterize a genetic variant in congenital heart defects

    -early heart morphogenesis defects
    -large numbers of embryos
    -example of editing a human variant in zebrafish

2. Roles for Pbx and Meis factors in heart development and myocardial differentiation

    -insight into the control of cardiomyocyte differentiation
    -framework for new transcription and chromatin factors

Thanks!

Maves lab:
- *Hank Farr
- UW undergraduates:
  - Kimia Imani
  - Darren Pouv

Collaborators:
- Cecilia Moens, FHCRC
- Chuck Murry, UW
- Cole Trapnell, UW
- Debbie Yelon, UCSD

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NIAMS
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American Heart Association
Learn and Live

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