Zebrafish modeling of variants of unknown significance: preliminary results

Andrew Prendergast

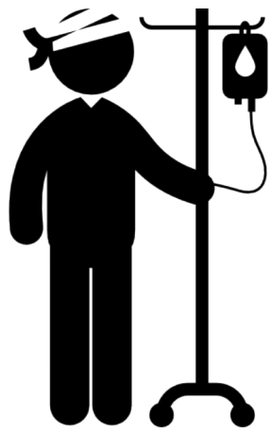
no disclosures

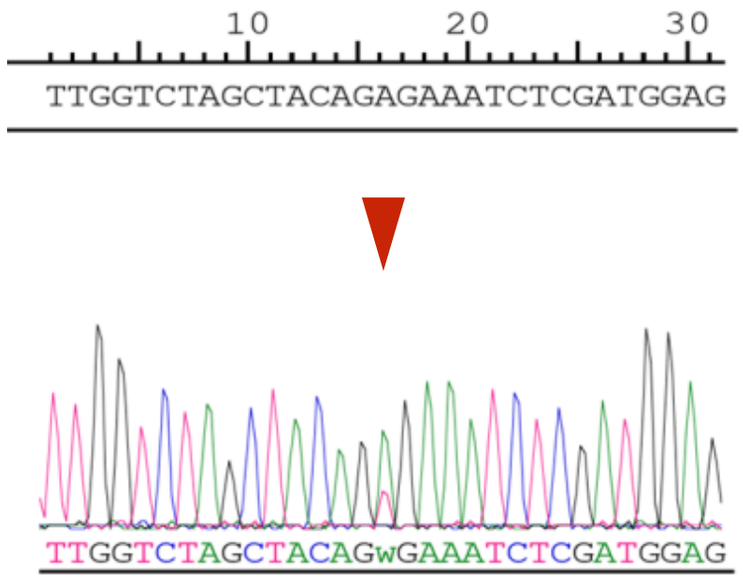
22 Jun 2022

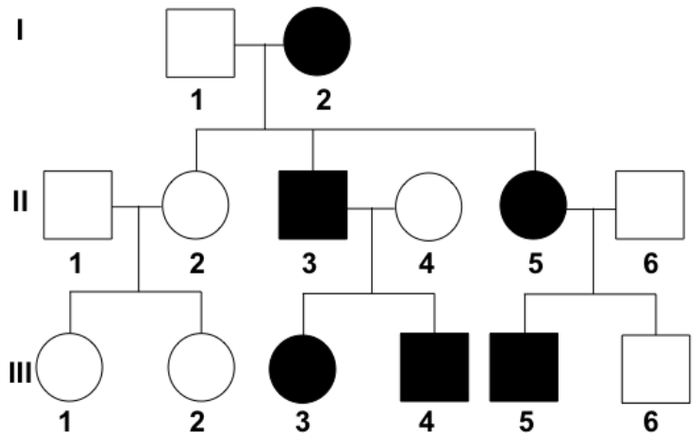
IMAD Meeting

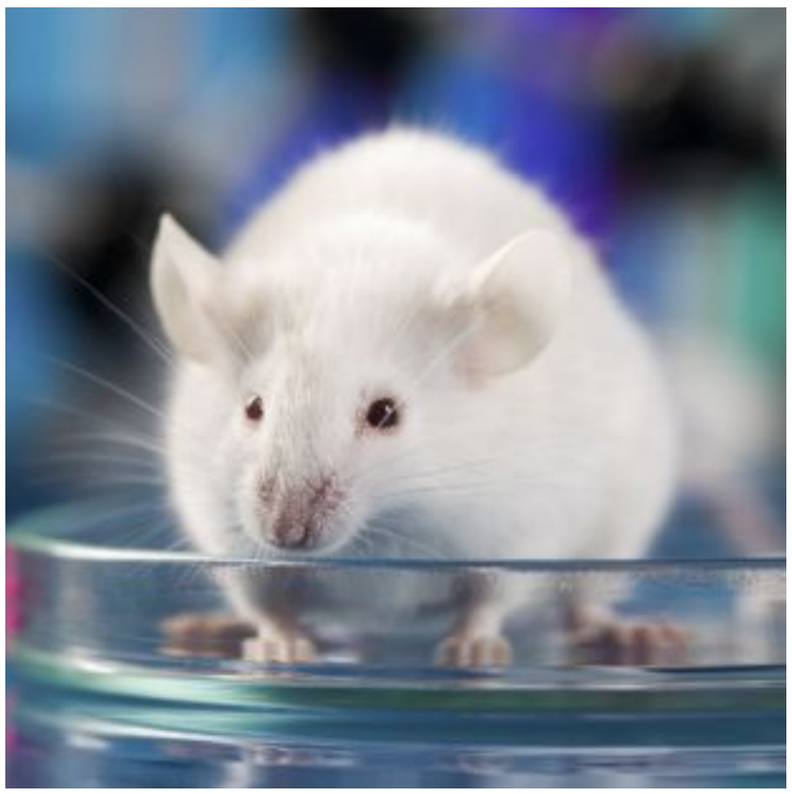
Yale Zebrafish Research Core

The lag between candidacy and causation



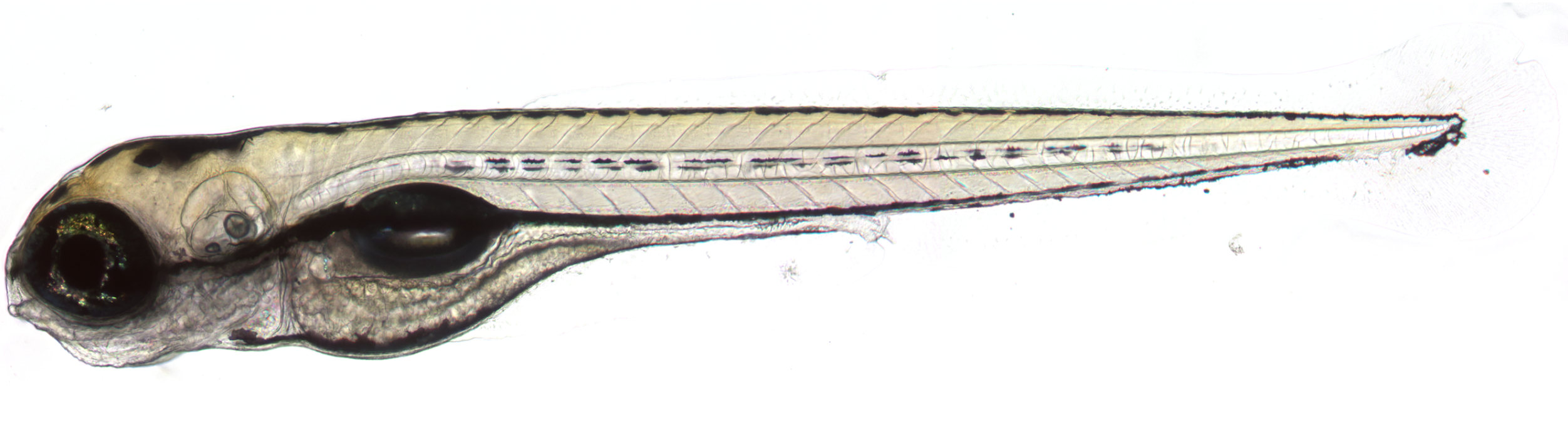






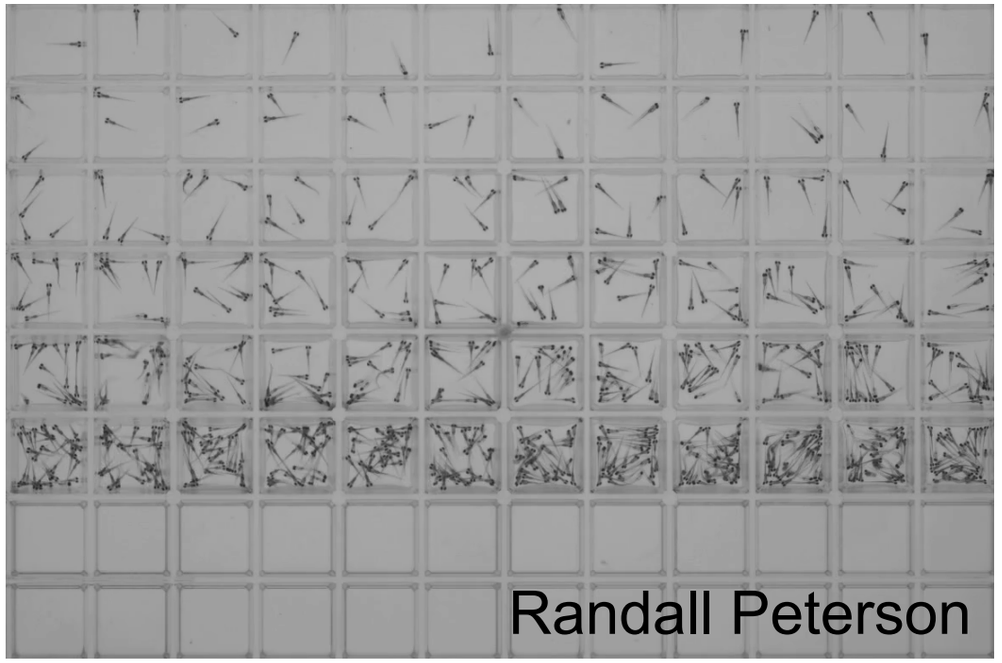
- Limited by number of testable genes
- Limited by time/money
- Limited by sample size
- You can’t see through them (usually)

Zebrafish for rapid vascular analysis



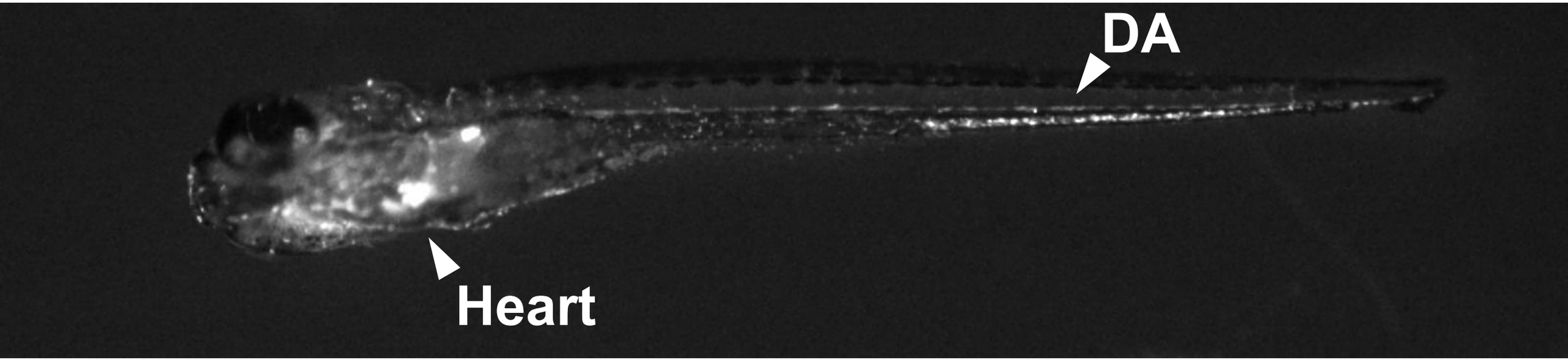
Transparency, scale, and transgenics

David Schoppik

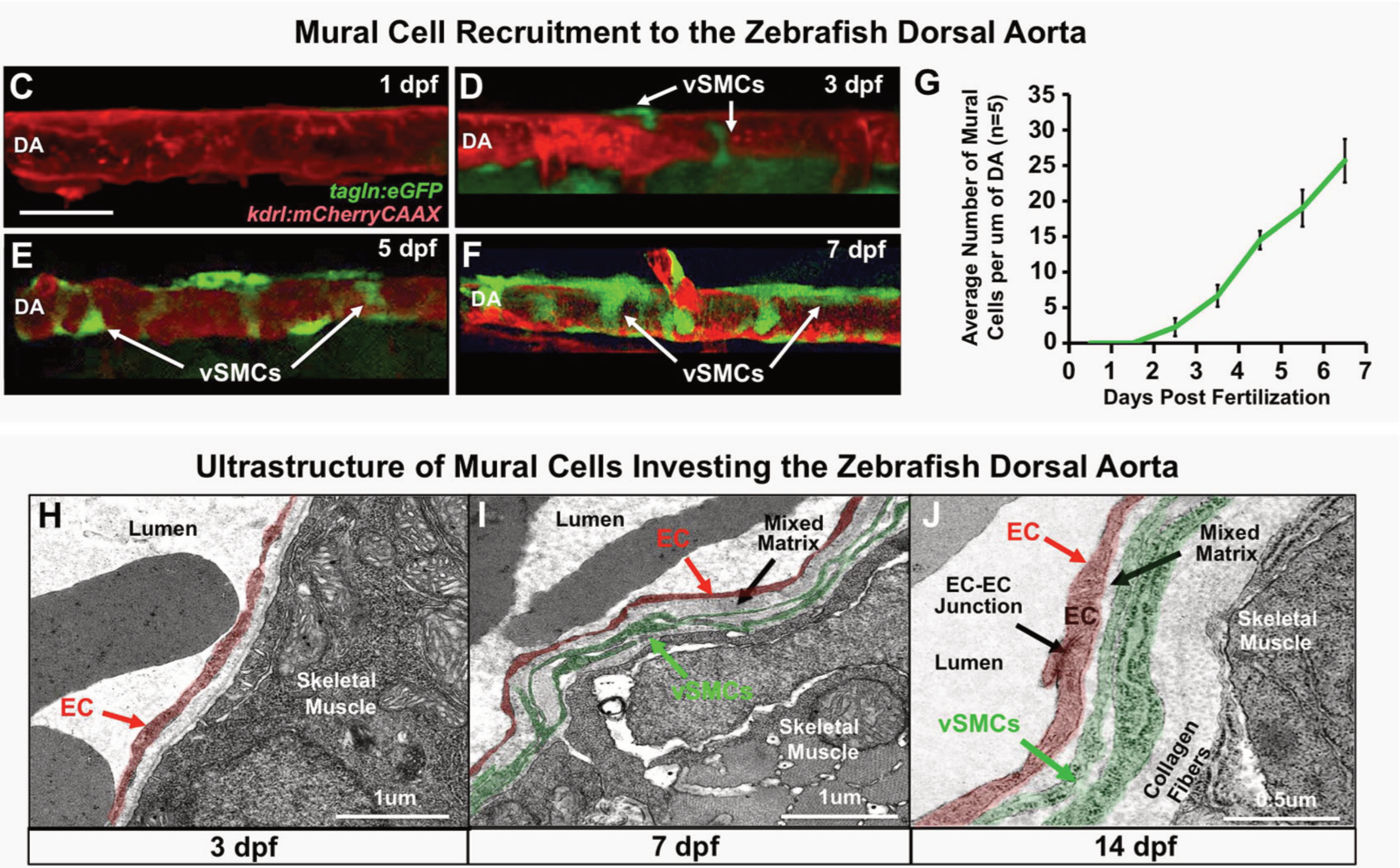


Randall Peterson

Tg(gata1:dsRed)

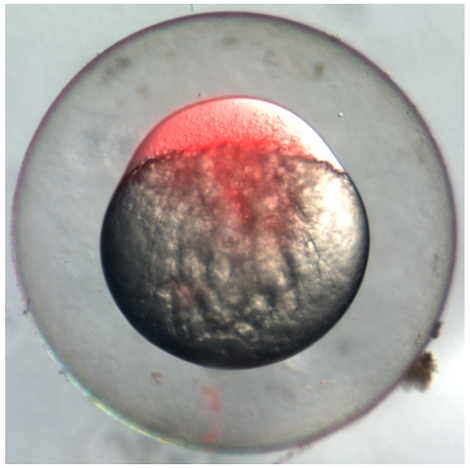


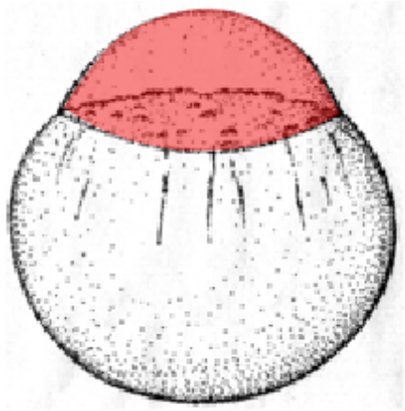
How is fish aorta different?

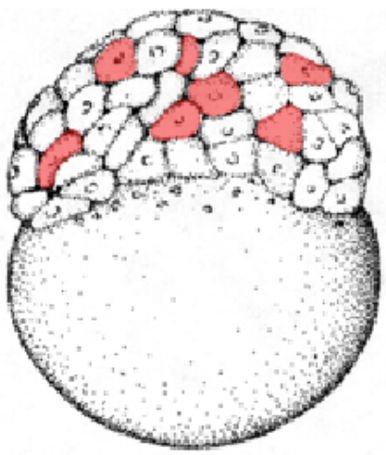


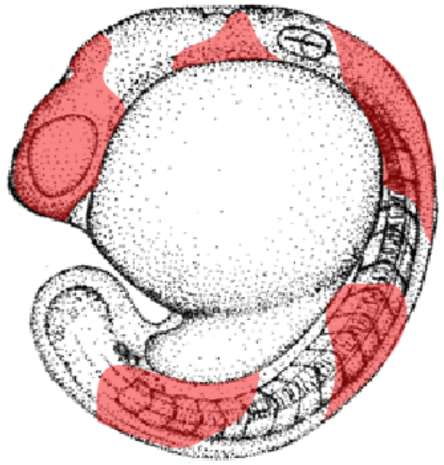
Stratman *et al.* (2017)

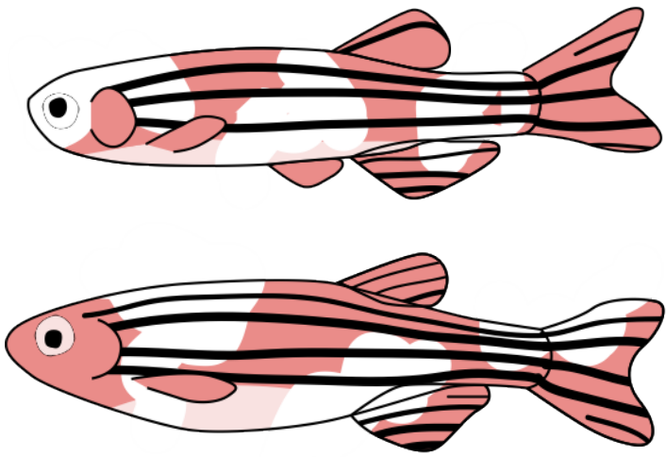
Approaches to loss of function









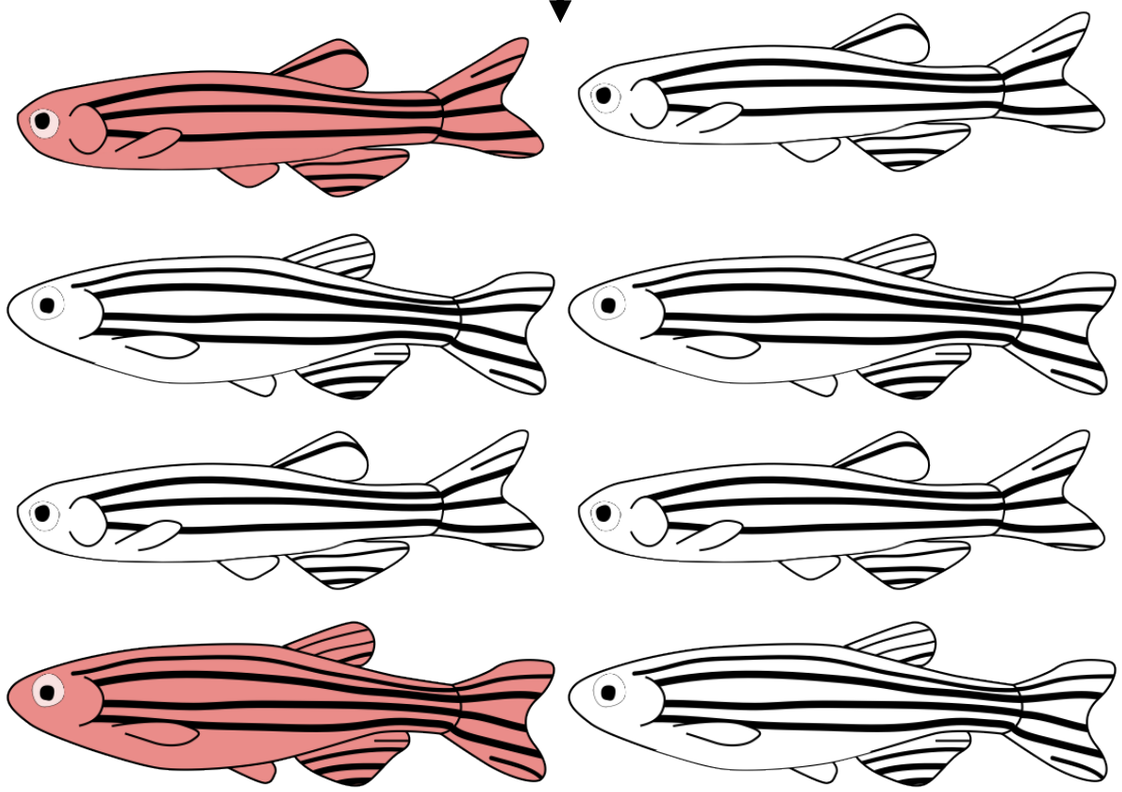


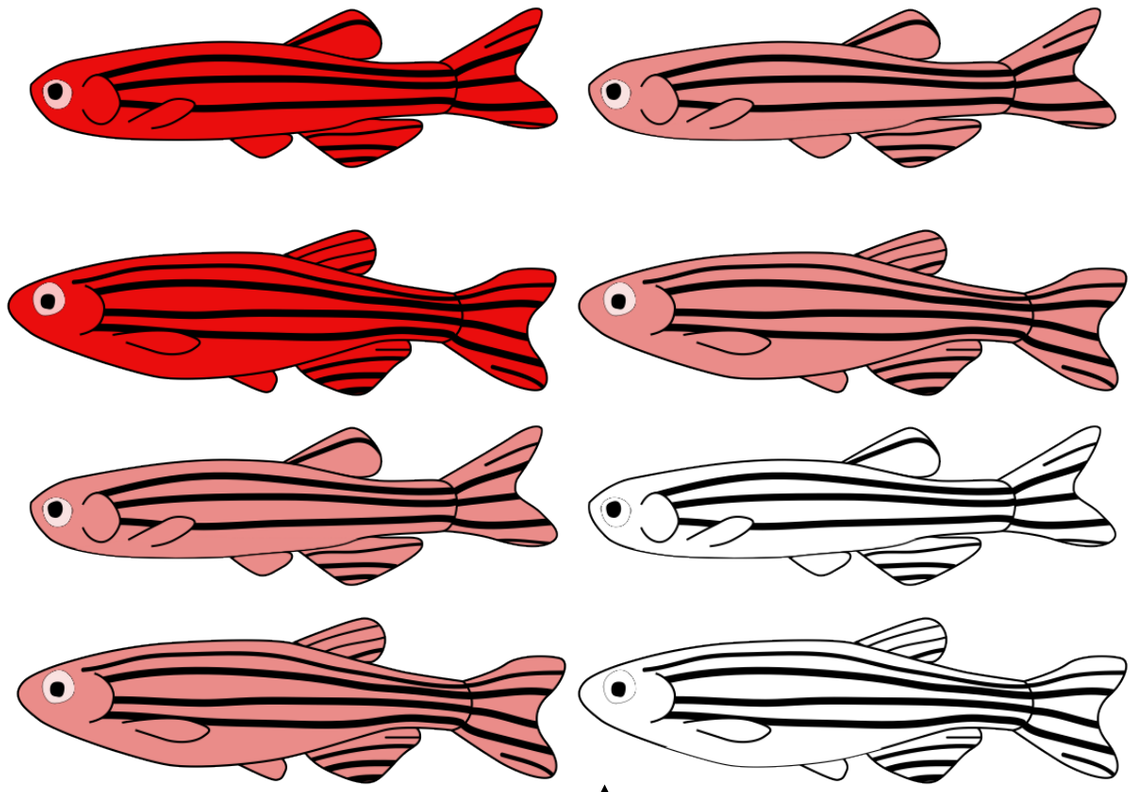
3 months

F_0

F_1

3 months

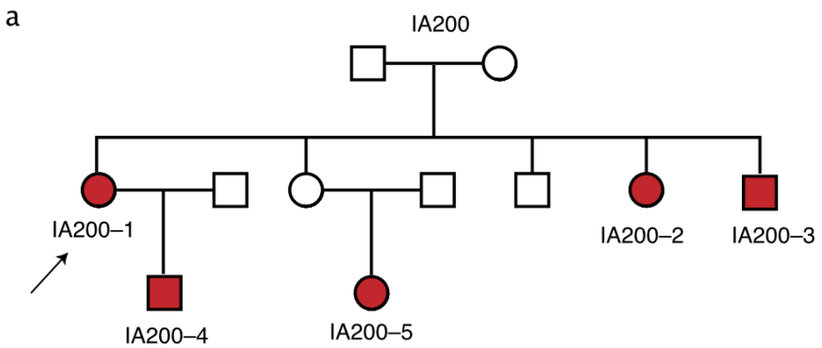


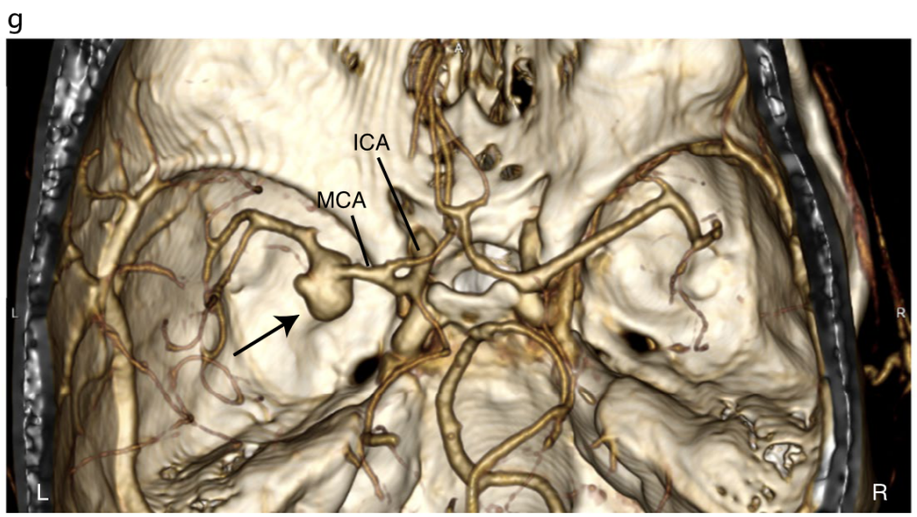


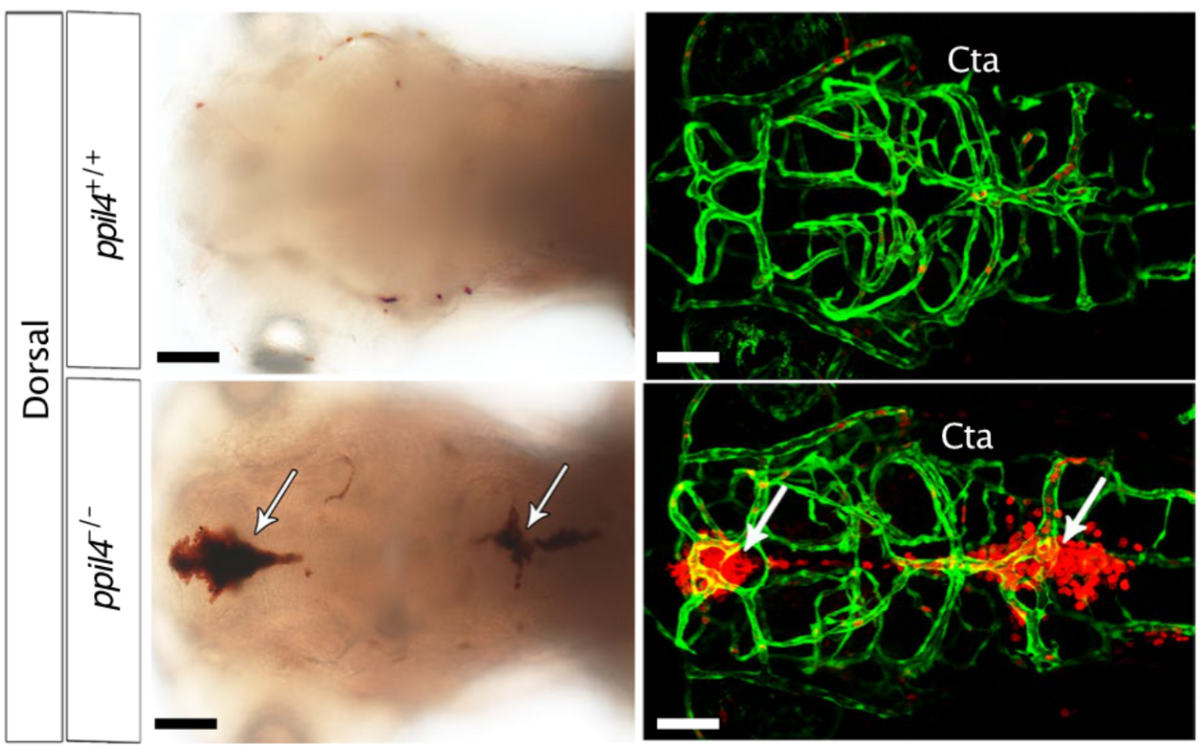
3 months

F_2

Disease model: IAD (in F_2)



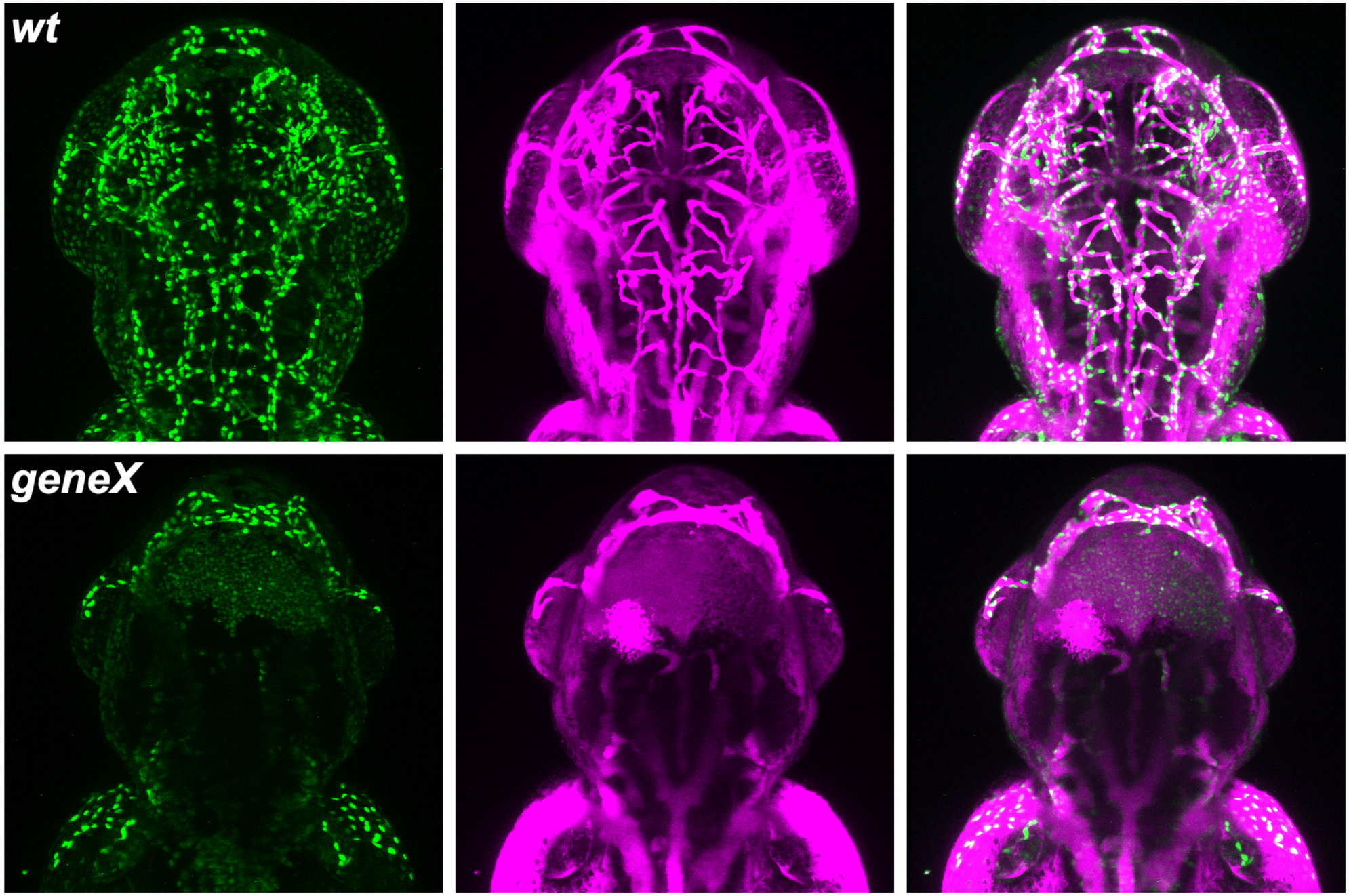




- Only allele to meet selection criteria is *ppil4* G132S
- Knockout of zebrafish *ppil4* in exon 5 causes arborization defects, aneurysms, and hemorrhages

Barak *et al.* (2021)

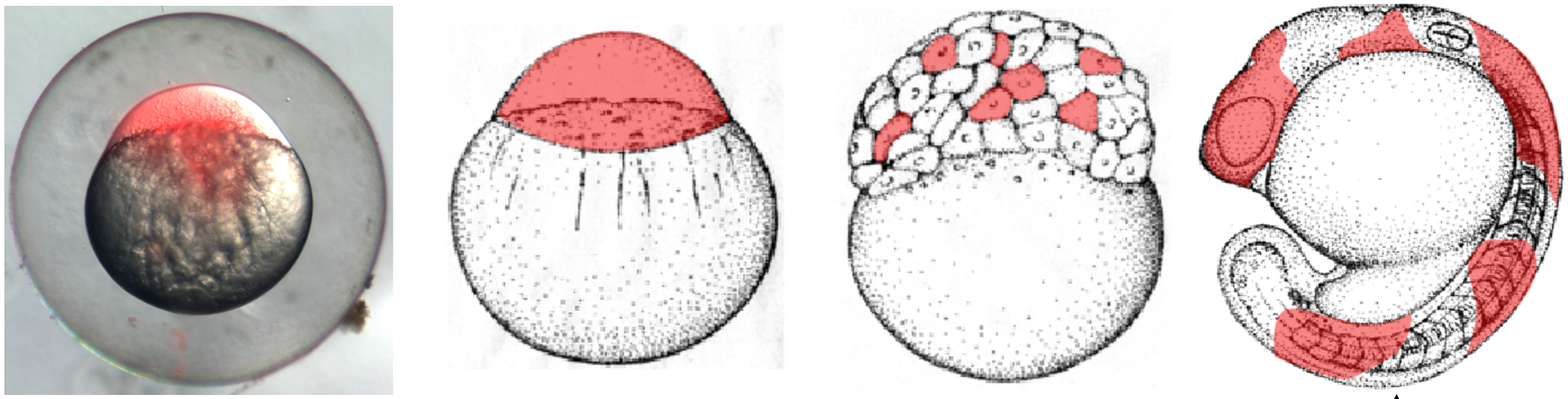
Disease model: IAD (in F_2)



unpublished

Elapsed time start to F_2: <u>17 months</u>

Approaches to loss of function



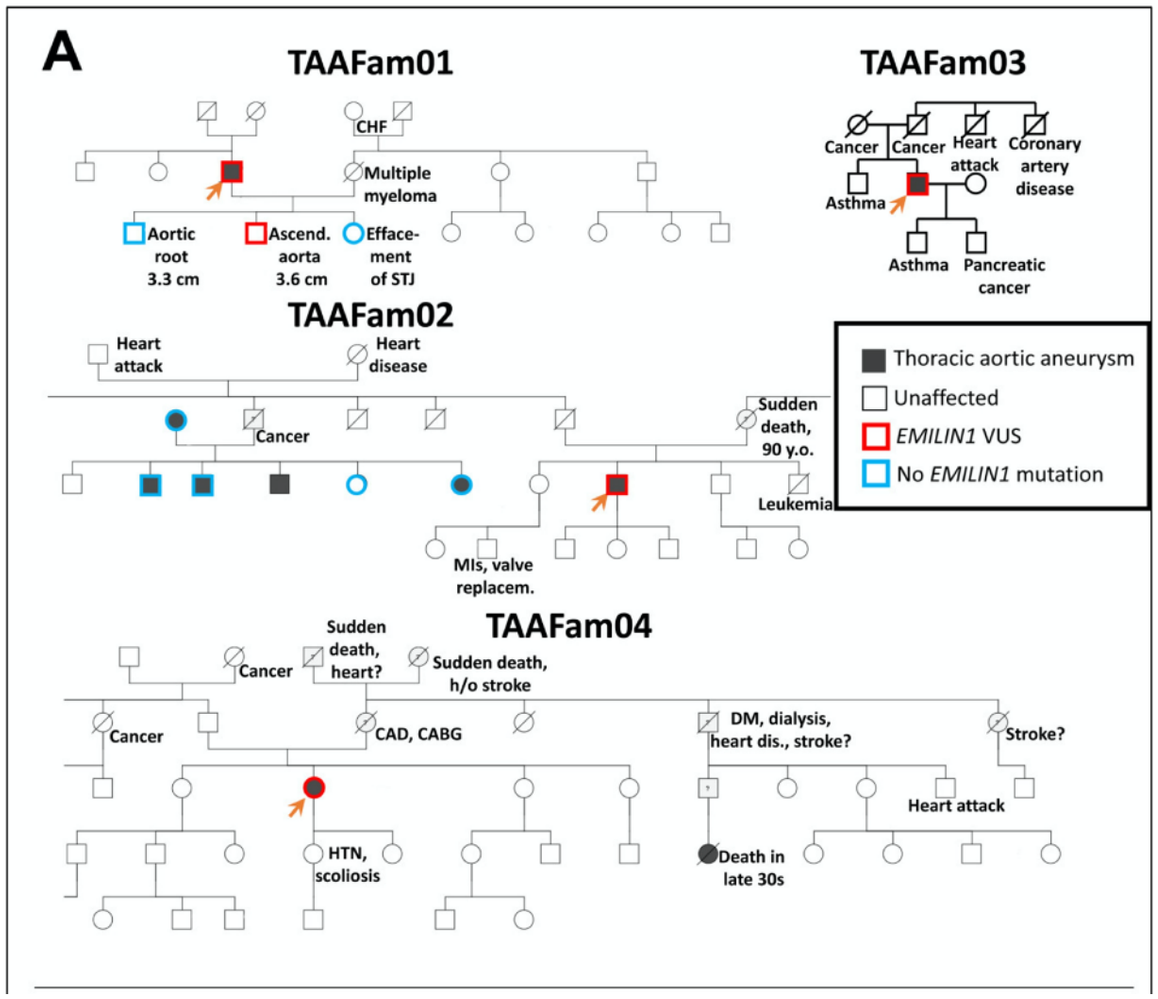
fbn1 — Marfan syndrome

col1a2
col5a1
col5a2a/b — Ehlers-Danlos syndrome

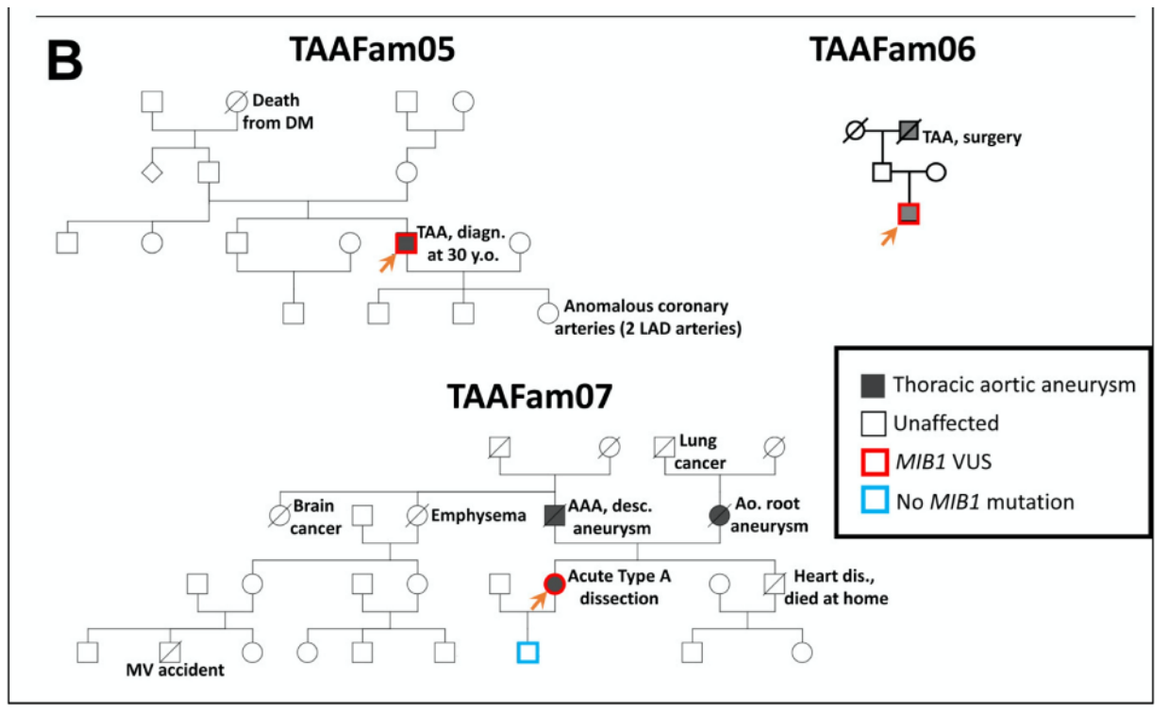
emilin1a/b
mib1 — Thoracic aortic aneurysm

Disease model: TAA (in F_0)

emilin1

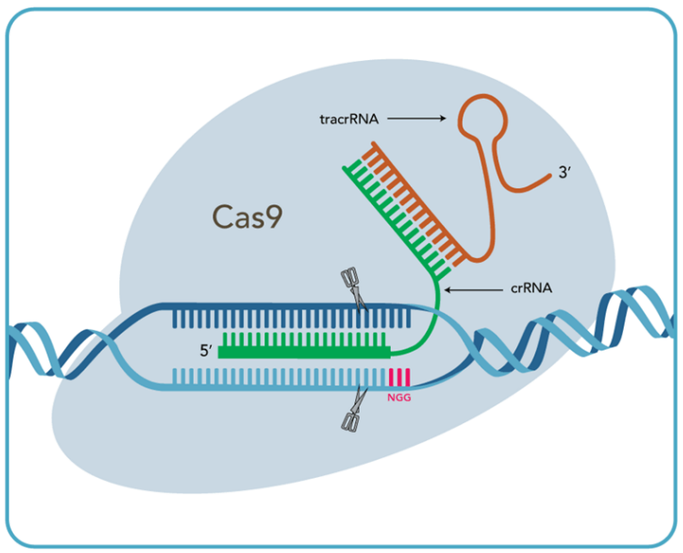


mib1

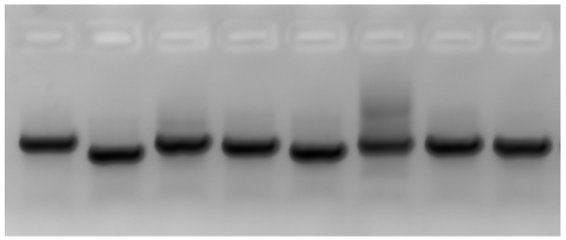


Bulat Ziganshin

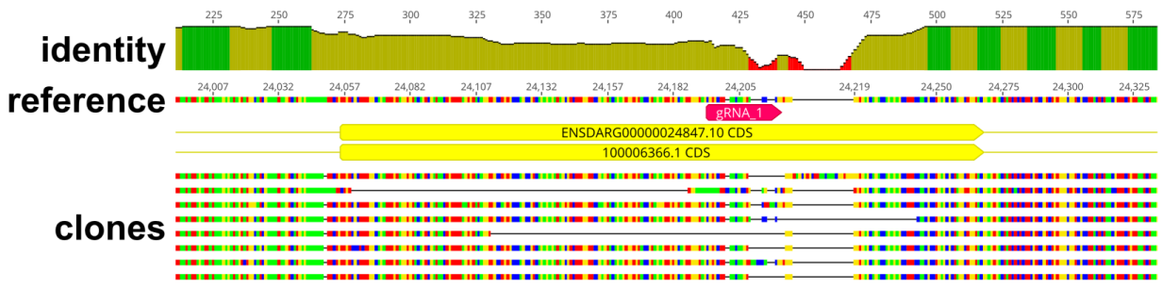
CRISPR/Cas9 to generate LOF models



B *col5a2b* 040 clones (gRNA1)



C *col5a2b* 040 (gRNA1, 100% efficacy)

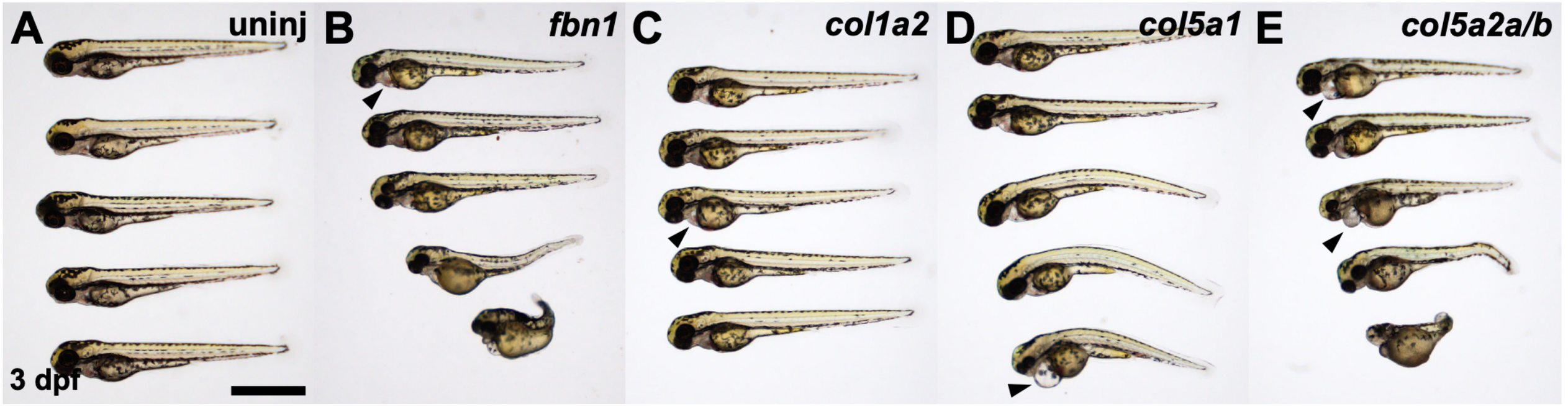


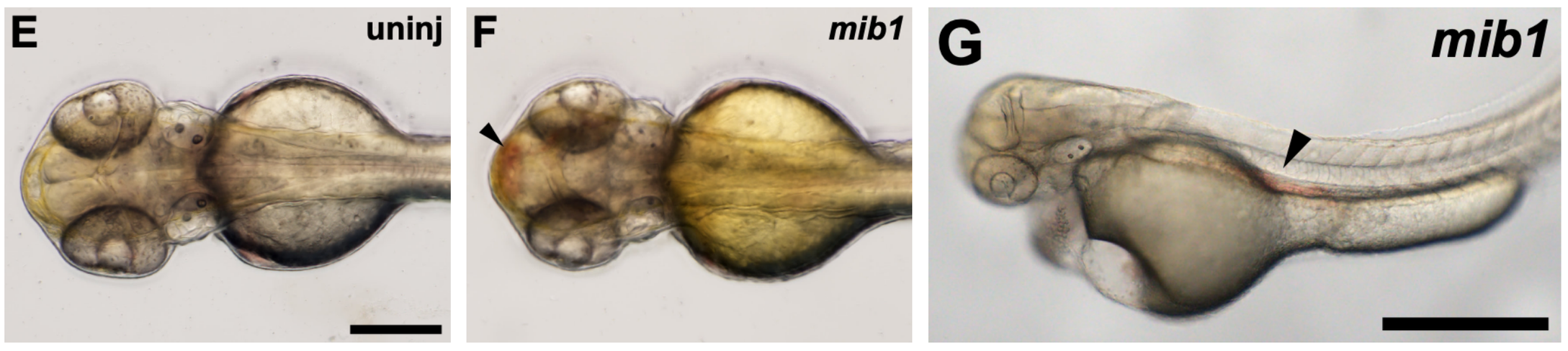
D

Gene	Target	Sequences	nº *wt*	nº mutant	Efficacy
emilin1a	0015	6	0	6	100%
emilin1a	0016	5	0	5	100%
emilin1b	0017	5	0	5	100%
emilin1b	0018	8	8	0	0%
mib1	0019	6	0	6	100%
mib1	0020	3	0	3	100%
col1a2	0036	1	0	1	100%
col1a2	0037	5	0	5	100%
col5a1	0038	4	2	2	50%
col5a1	0039	6	0	6	100%
col5a2b	0040	8	0	8	100%
col5a2b	0041	2	0	2	100%
col5a2a	0042	6	0	6	100%
col5a2a	0043	5	0	5	100%
fbn1	0044	0	0	0	N/A
fbn1	0045	1	0	1	100%
TOTAL		71	10	61	86%

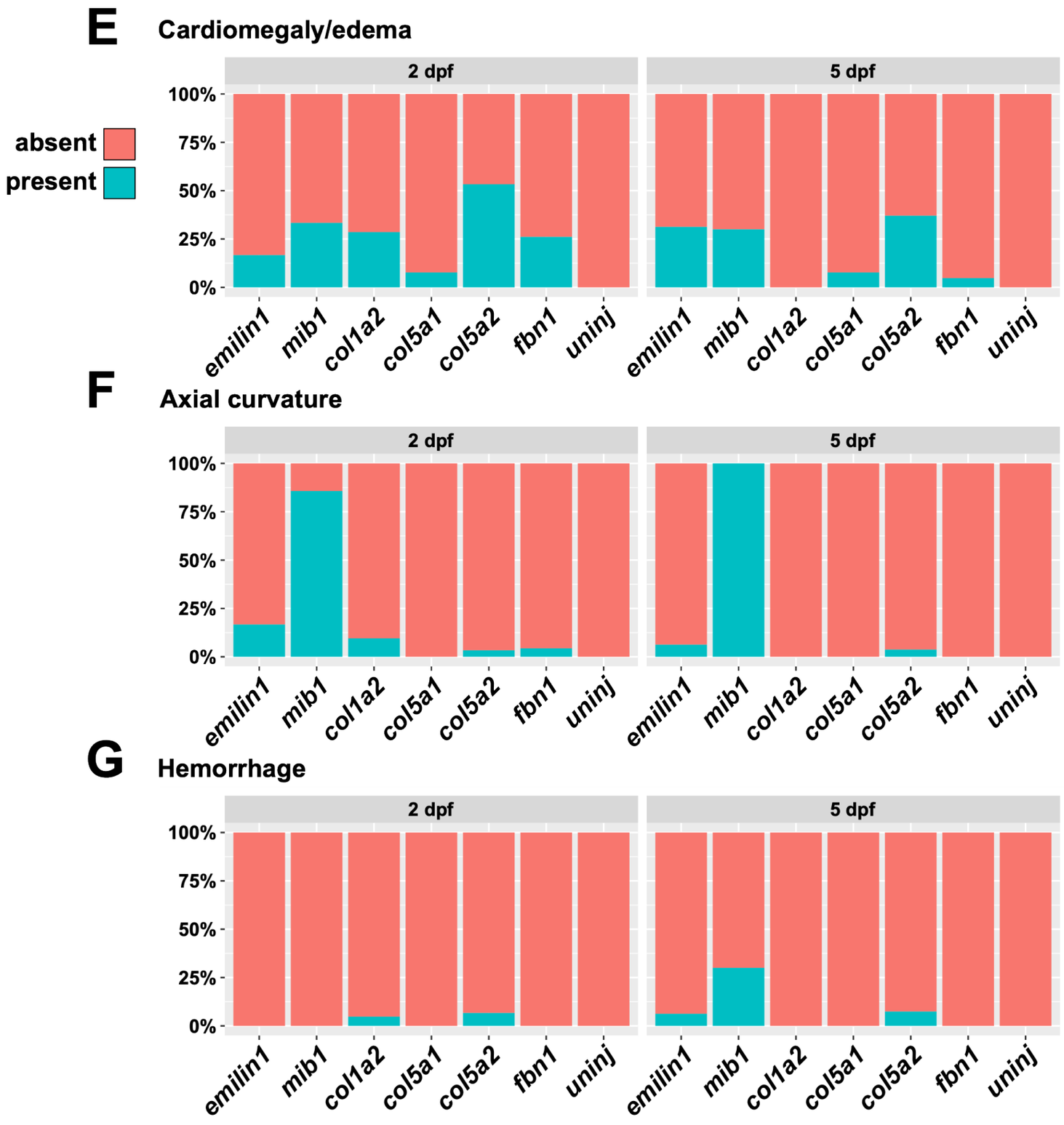
Prendergast *et al.* 2022

Observed phenotypes





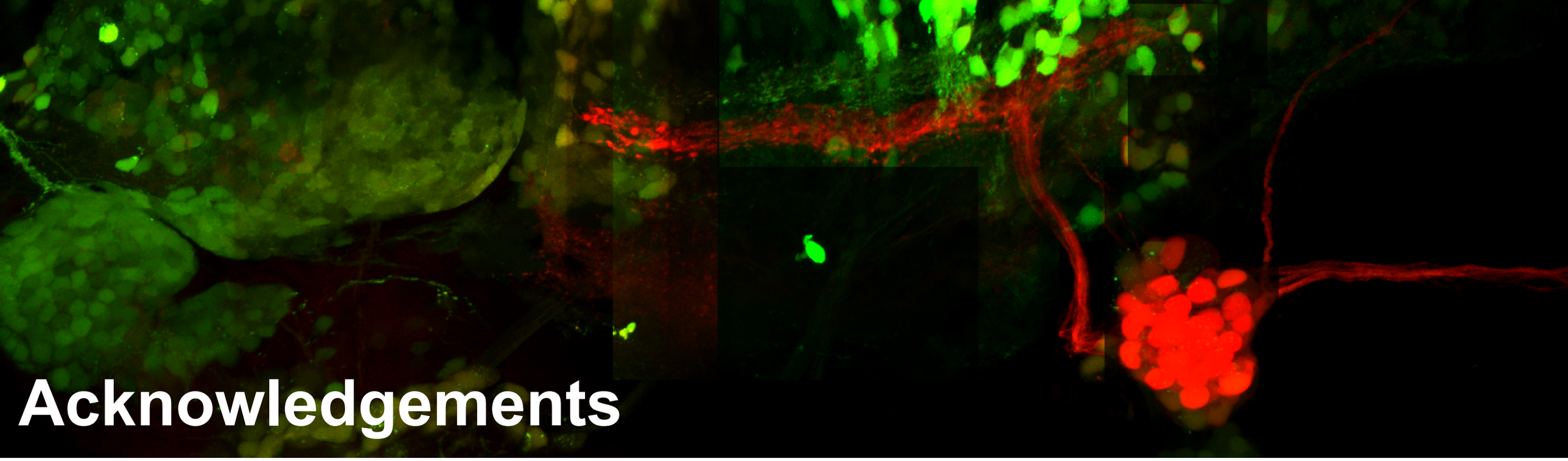
Incidence of phenotypes



Elapsed time: 39 days

Conclusions and questions

- F_0 phenotypic is possible, fast, can screen dozens of candidates rapidly
- What does TAA mean in an animal that does not have substantial aortic lamination? Focus on hemorrhagic incidence?
- Can we do things to push appearance of phenotypes farther forward? (Epinephrine, etc.)
- Rule-in, not rule-out



Acknowledgements

YZRC Staff

- Yue Yang
- Ramil Noche

YZRC Administration

- Stefania Nicoli
- Pat Preisig

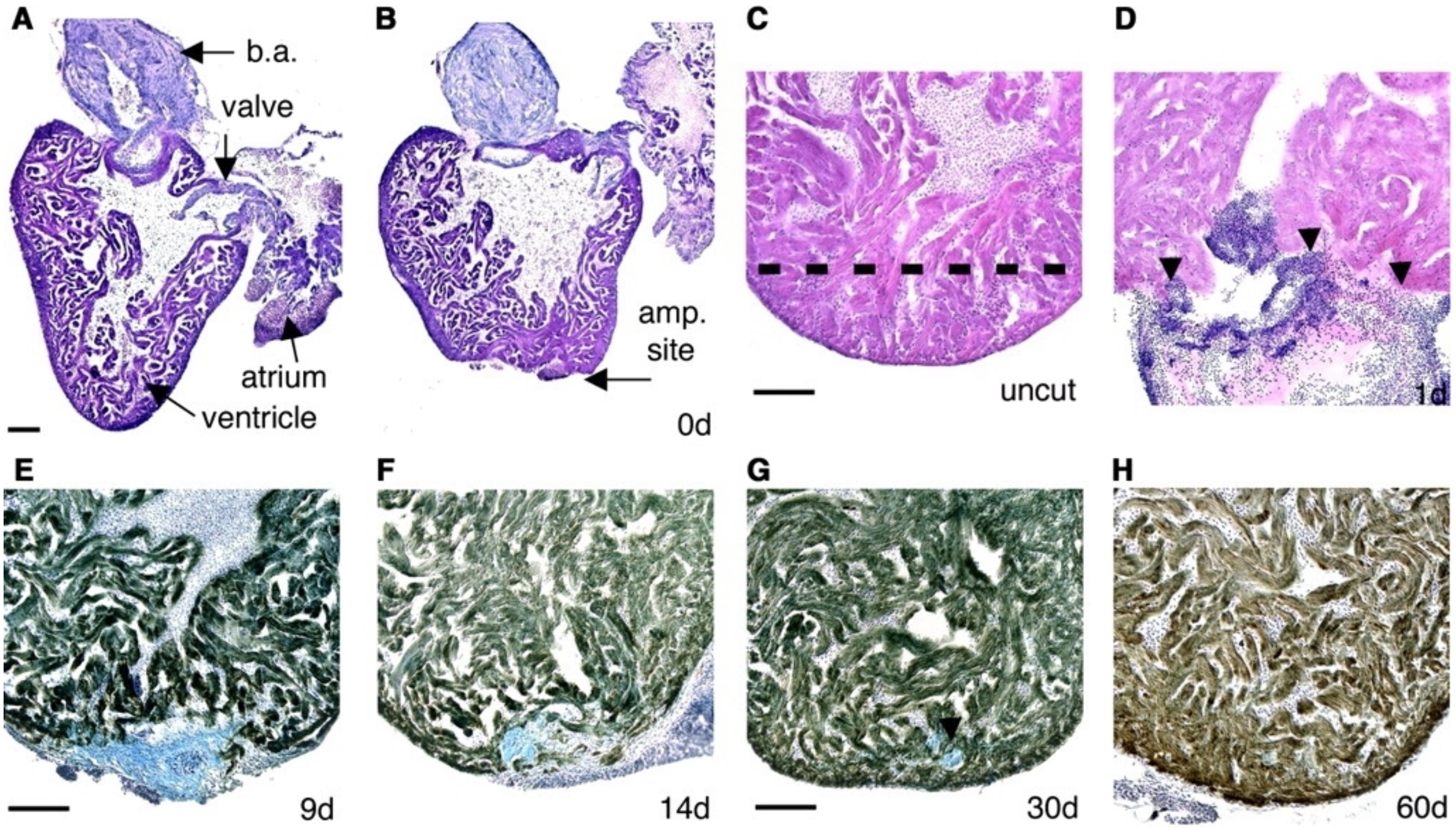
Yale Aortic Institute

- John Elefteriades
- Bulat Ziganshin
- Mohammad Zafar
- Dimitria Papanikolaou
- Sandip Mukherjee

Talk goals

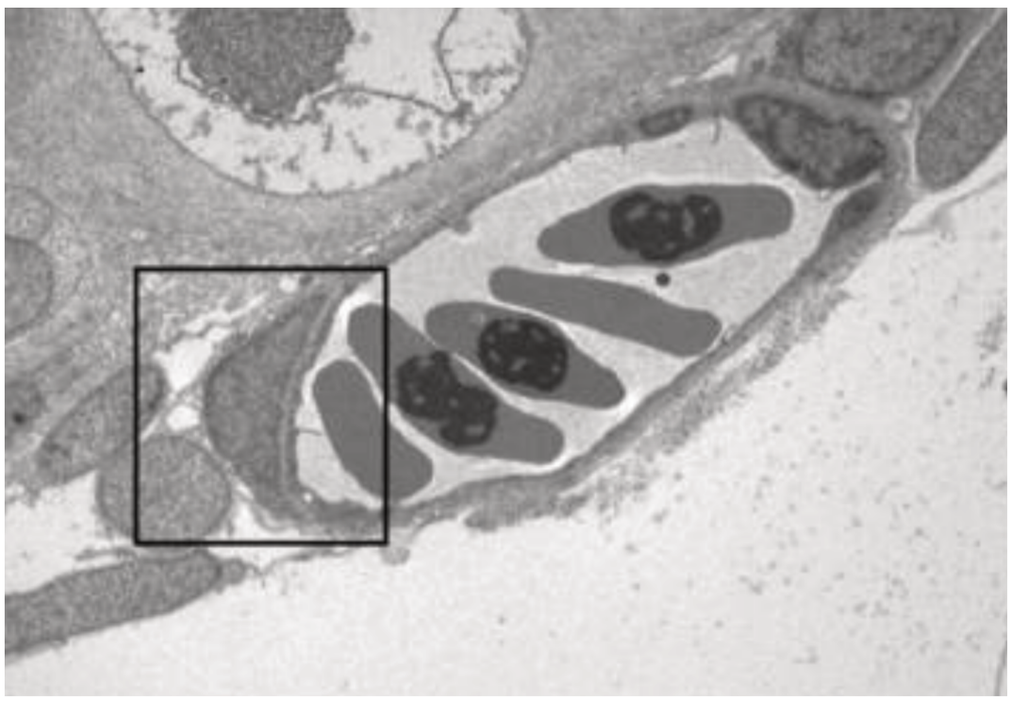
- Sell zebrafish as a vascular disease model
- Show a few "traditional" (F_2) mutants we have generated for IAD and what we learned
- Show an approach for rapid (F_0) loss-of-function fish we have generated for TAA

Fish vascular tissue regenerates well



Poss *et al.* 2002

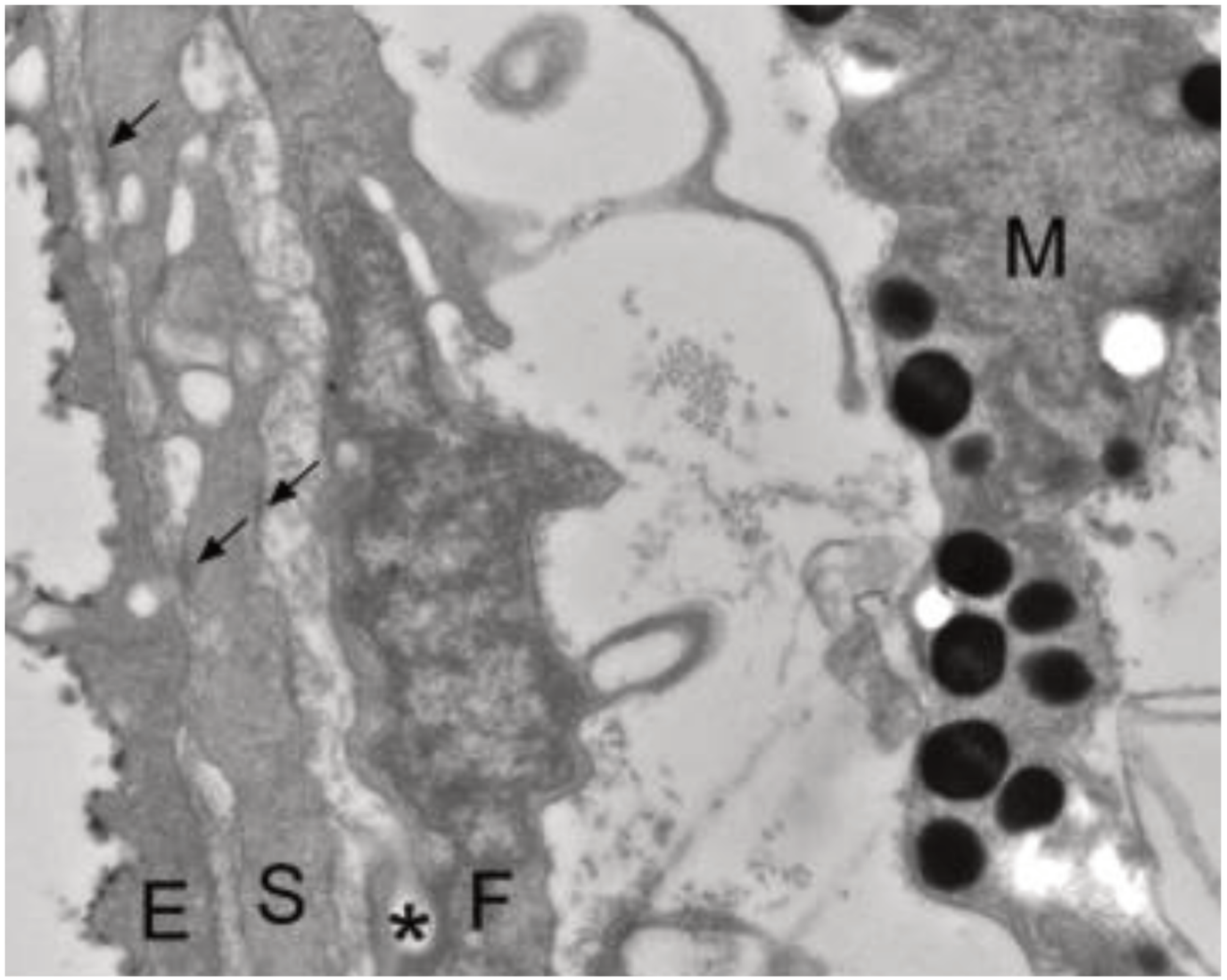
How is zebrafish different?



- 7 dpf aorta is characterized by endothelial cell layer with closely juxtaposed mural cells
- No internal elastic lamina at this stage
- Note nucleated RBCs!

Miano *et al.* (2013)

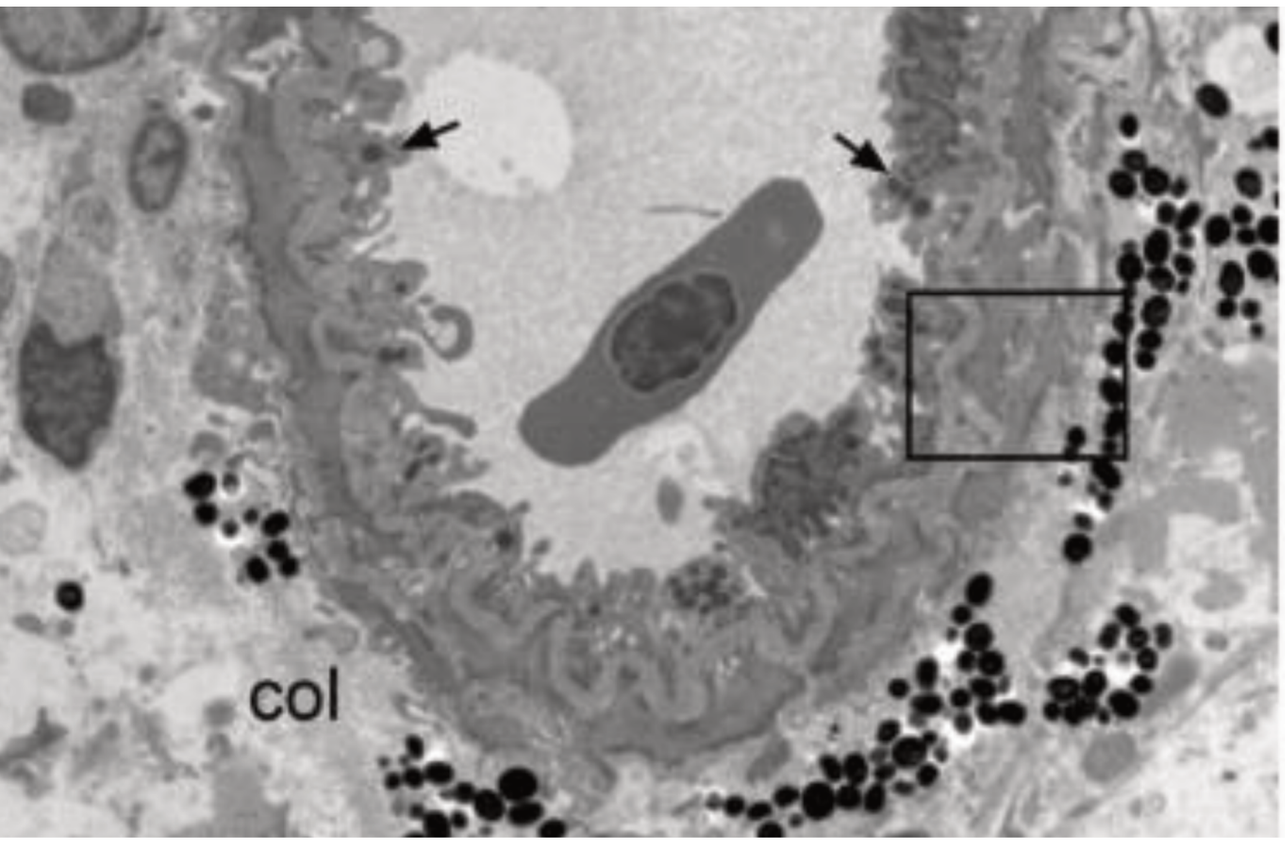
How is zebrafish different?

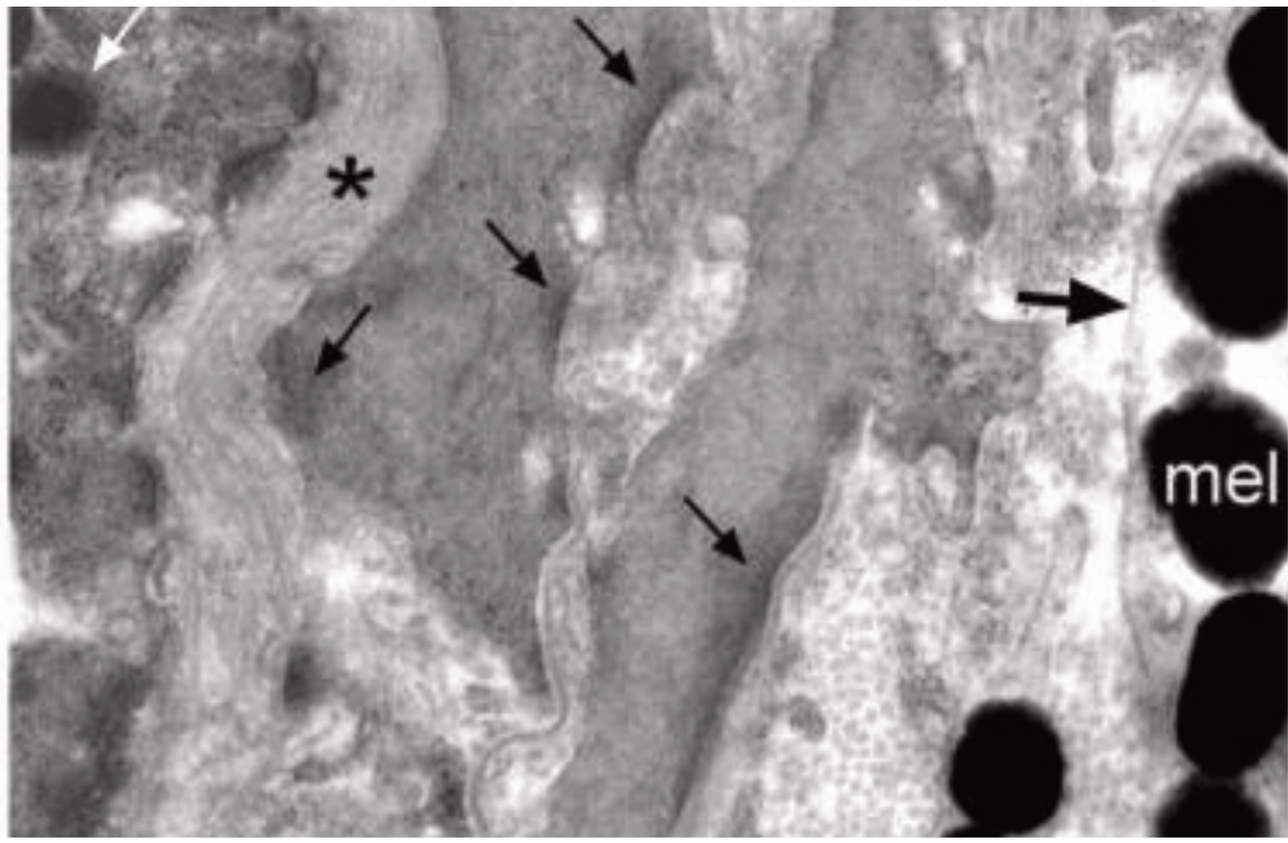


- 1 mpf aorta exhibits 4 cell types: endothelial (**E**), VSMCs (**S**), adventitial fibroblasts (**F**), and melanocytes (**M**)
- Layered structure is evident

Miano *et al.* (2013)

How is zebrafish different?





- 3 mpf (adult) aorta has a clear internal elastic lamina (*) and differentiated VSMCs (arrows indicate plaques)

Miano *et al.* (2013)