Heart Valve Malformations:

The role of NOTCH1 in Aortic Valve Disease (AoVD1)

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Gen 564

Genetics
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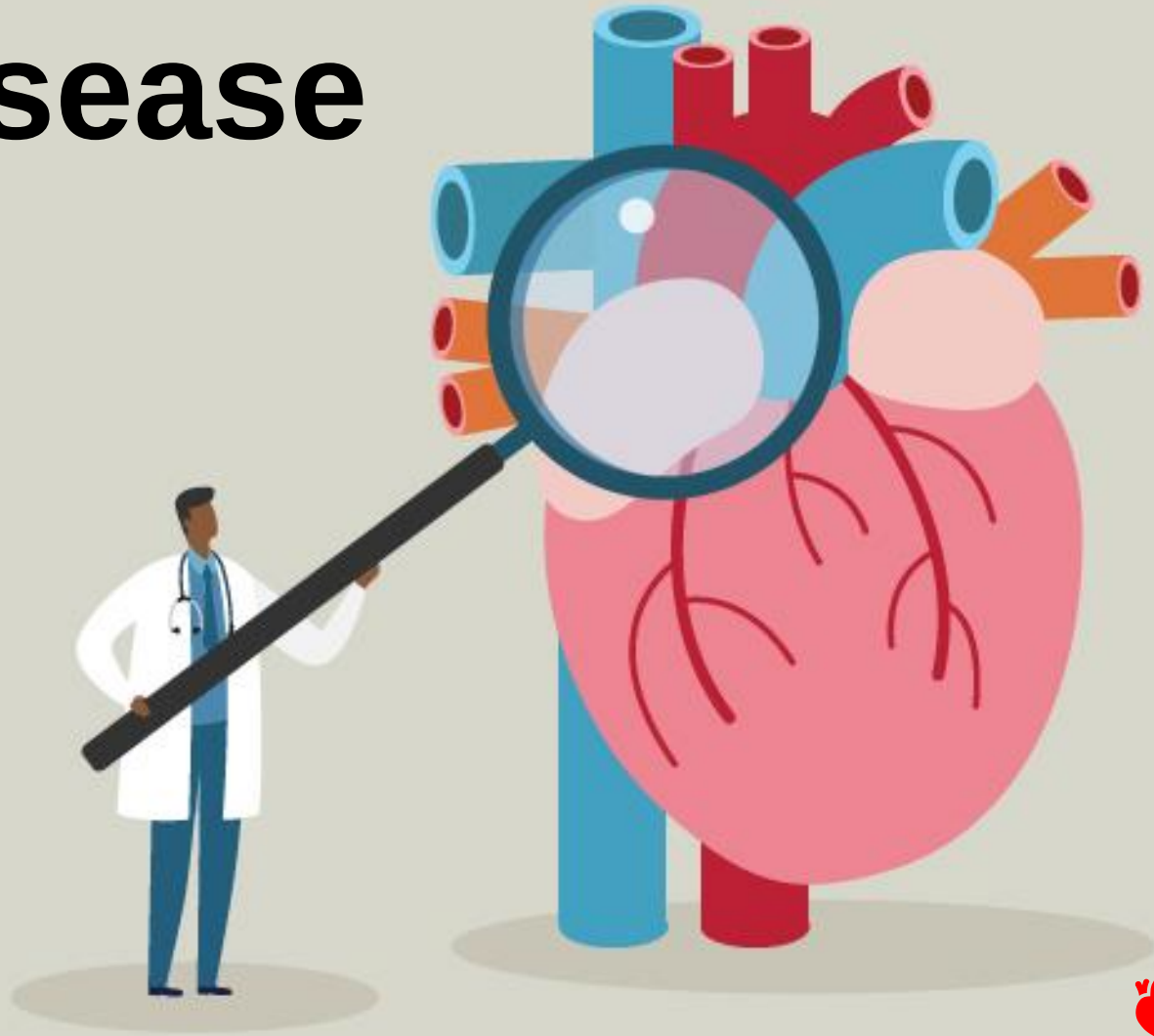
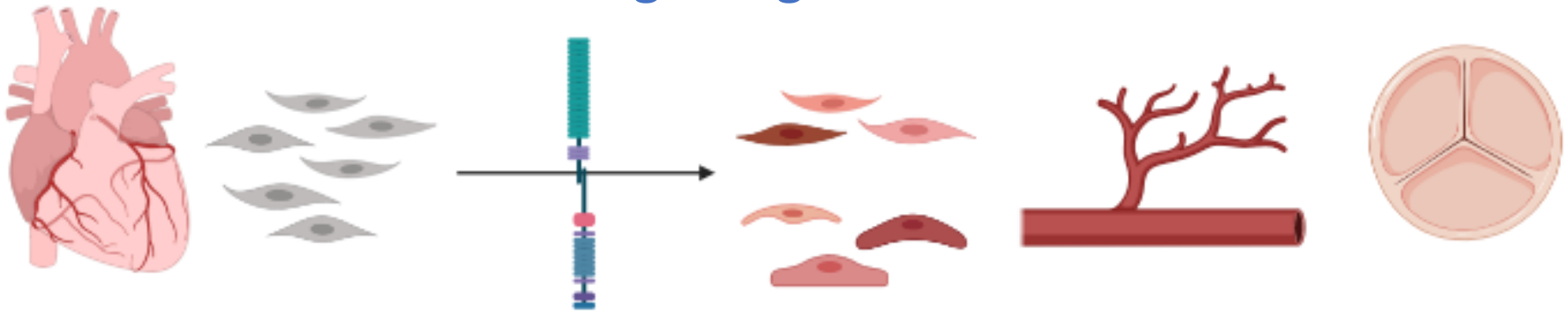


What is Aortic Valve Disease 1 (AoVD1)?

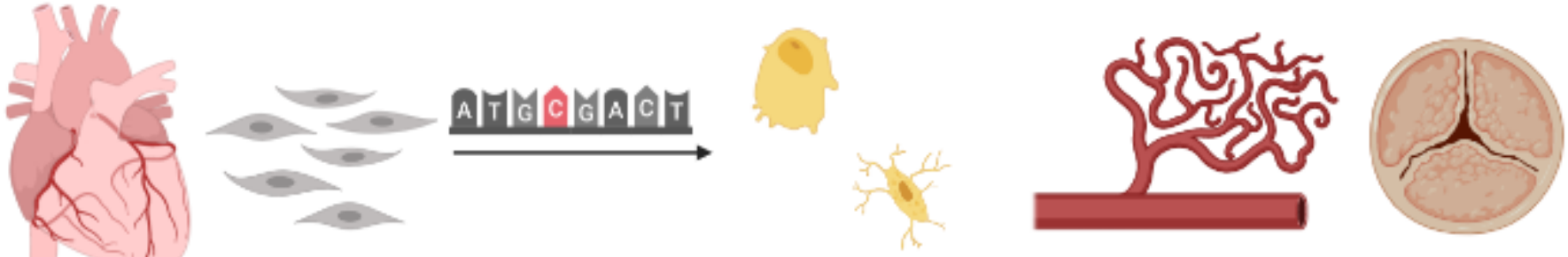
NOTCH1 signaling



Developing heart

Proper aortic valve formation and vascularization

NOTCH1 mutation



Developing heart

Osteoblast formation and aortic valve calcification

How does AoVD1 manifest?

Heart Murmur

Chest pain

Arrhythmia

Short of breath

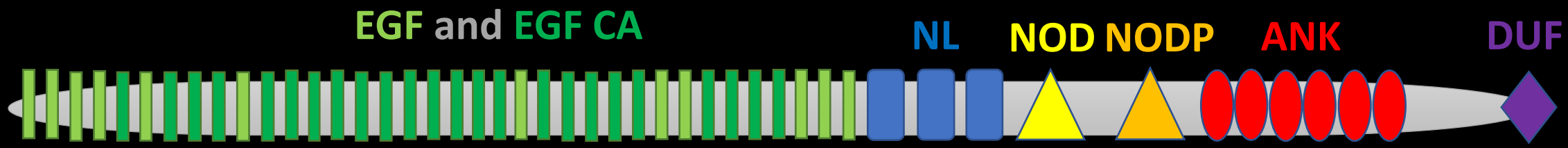
Why did I pick AoVD1?

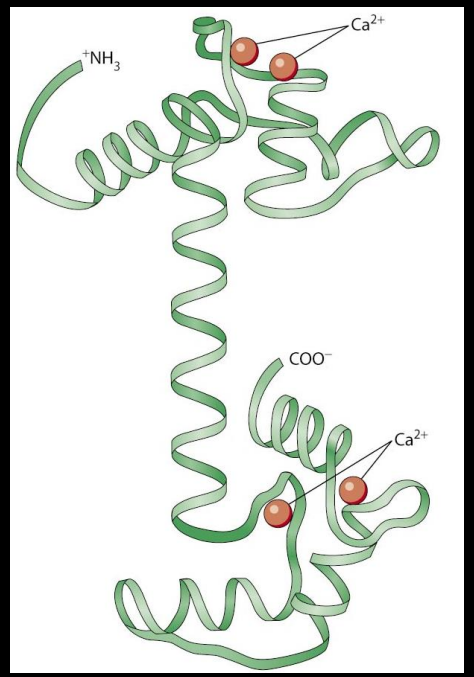
Transapical

Transaortic

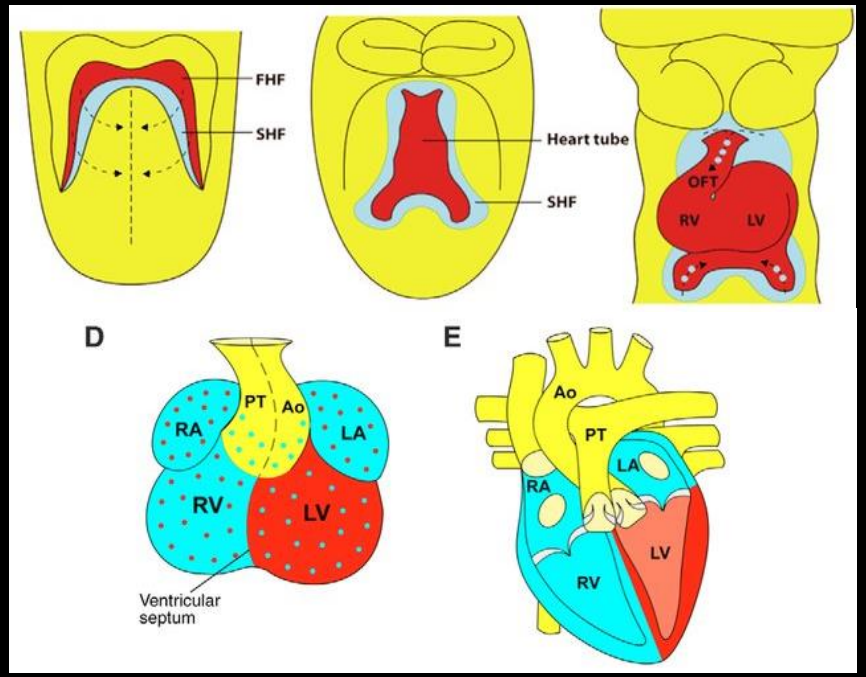
Transfemoral

NOTCH1 is associated with AoVD1

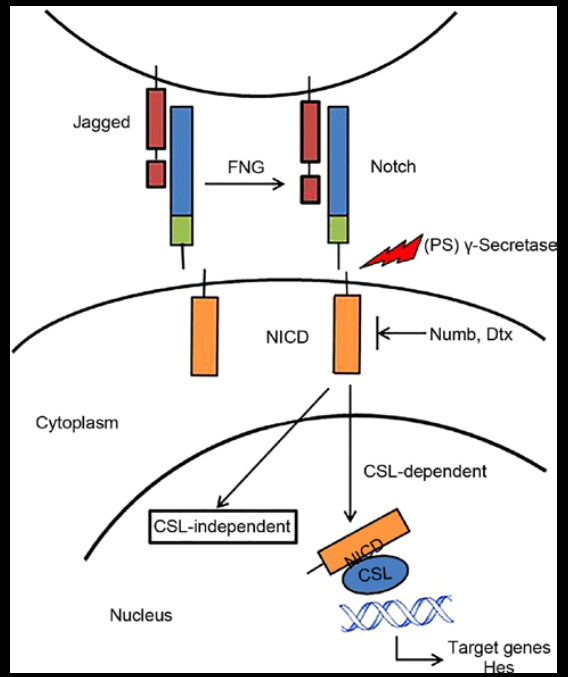




Molecular function

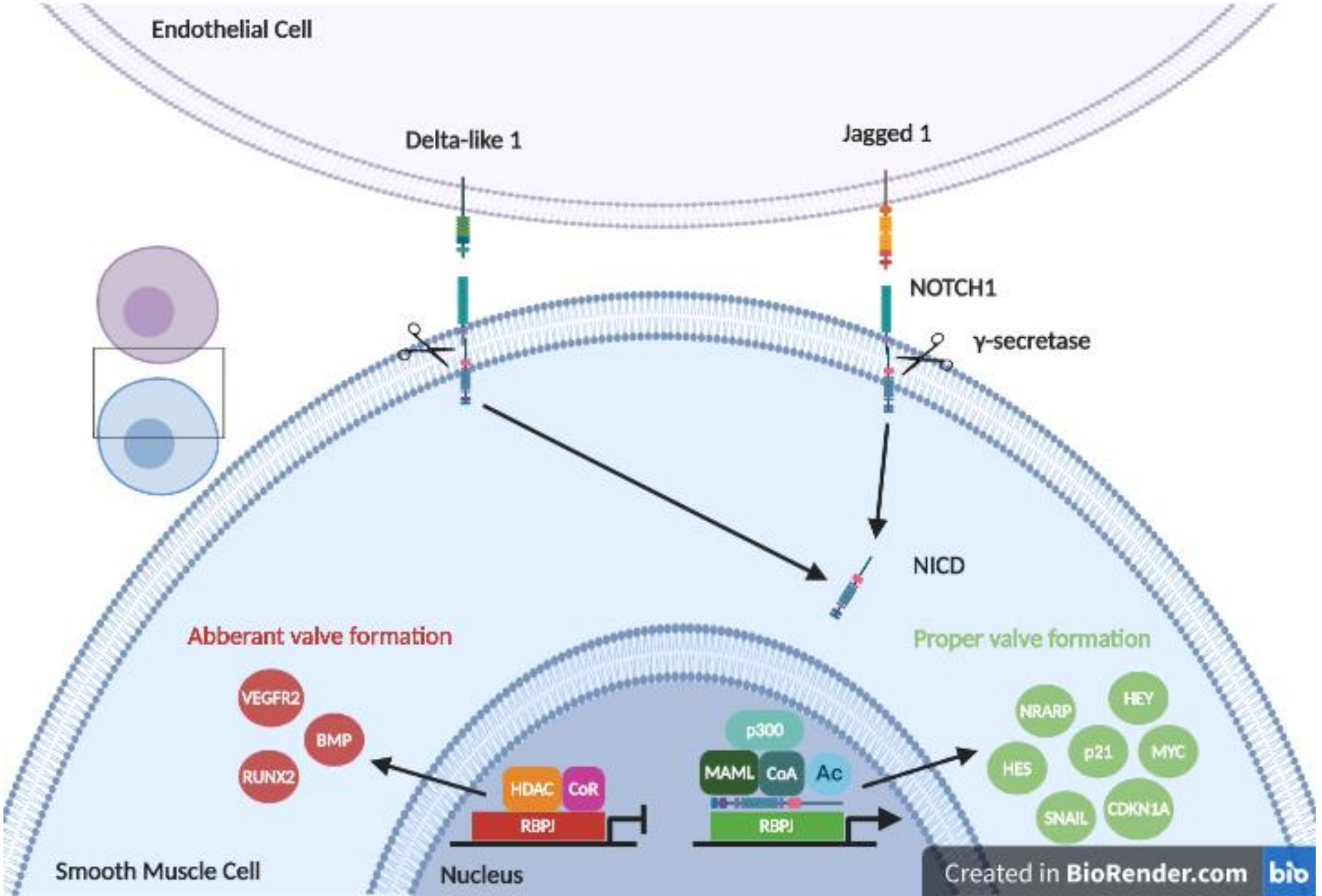


Biological process

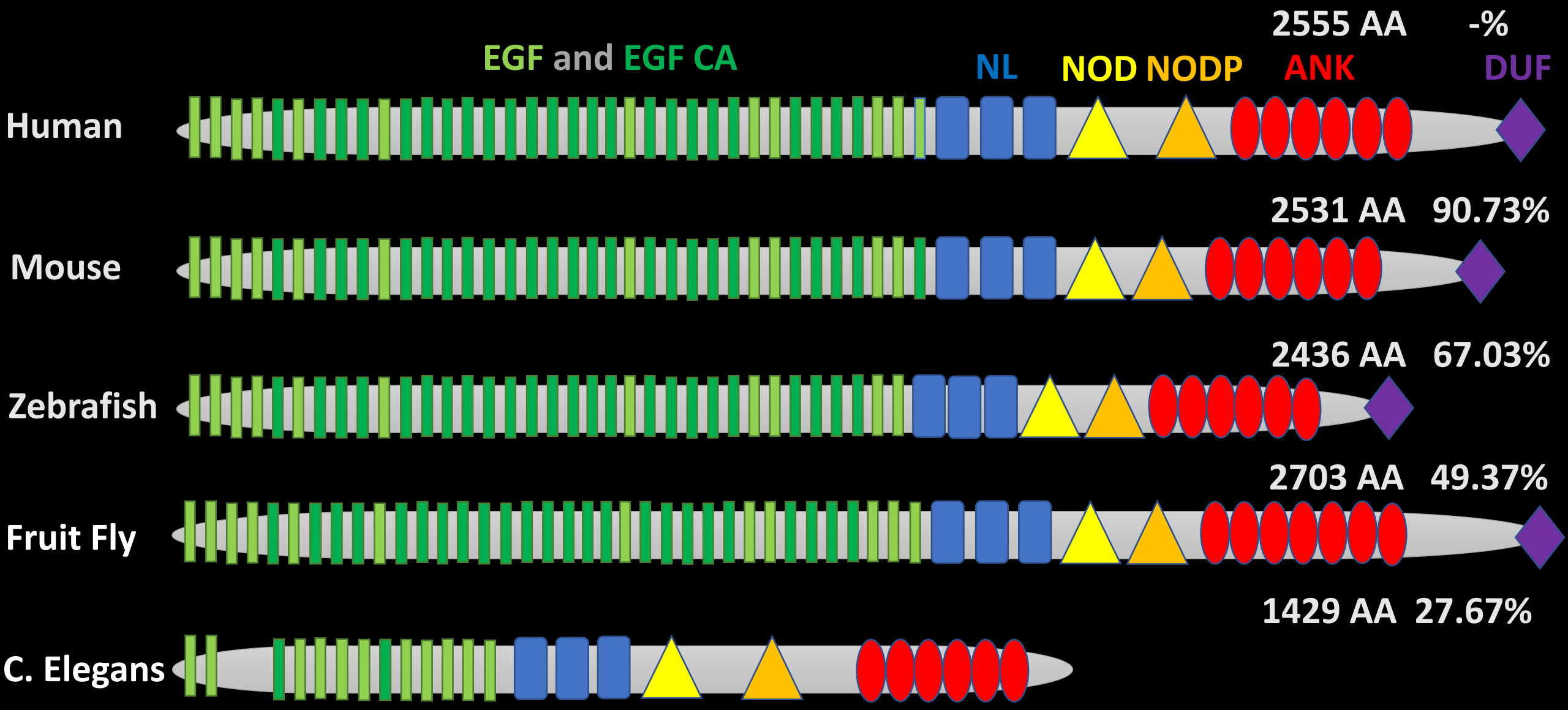


Cellular component

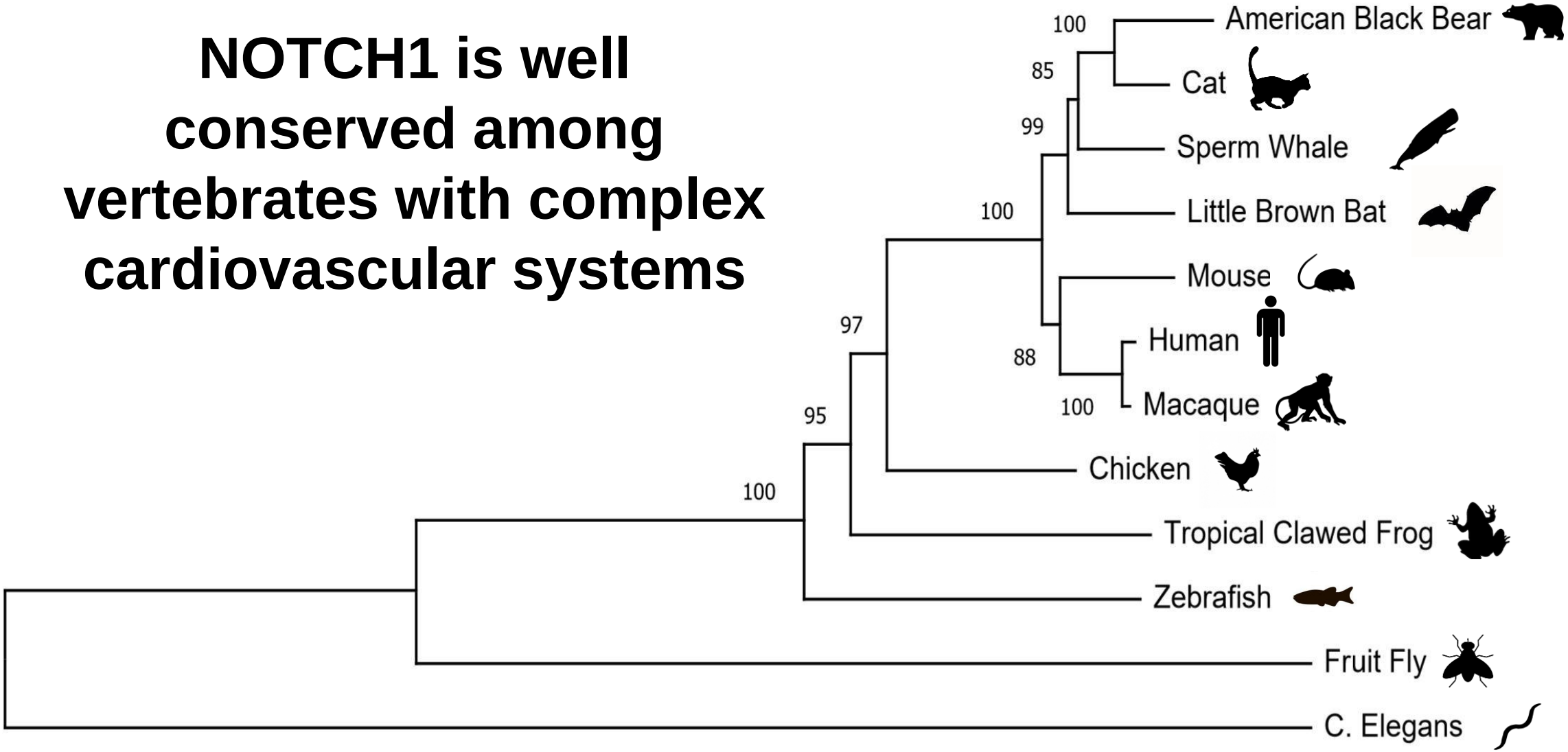
The NOTCH Signaling Pathway



NOTCH1 is a conserved transmembrane receptor

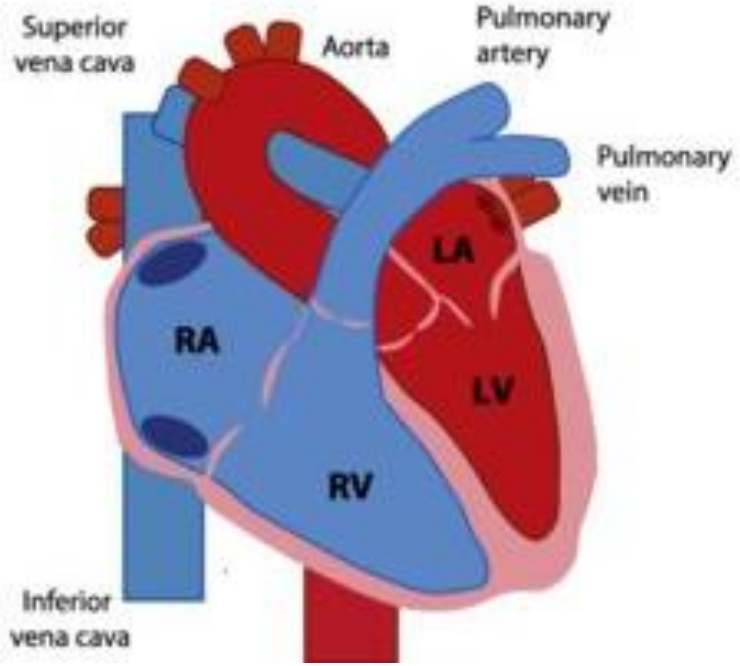


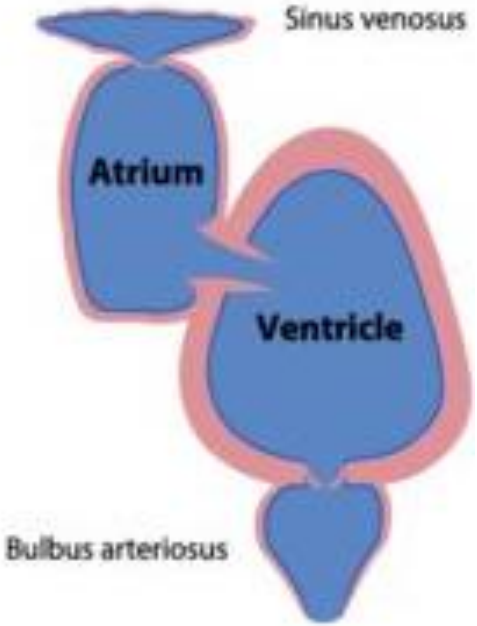
NOTCH1 is well conserved among vertebrates with complex cardiovascular systems



Muscle aligned neighbor-joining method

Zebrafish can be used to study heart development

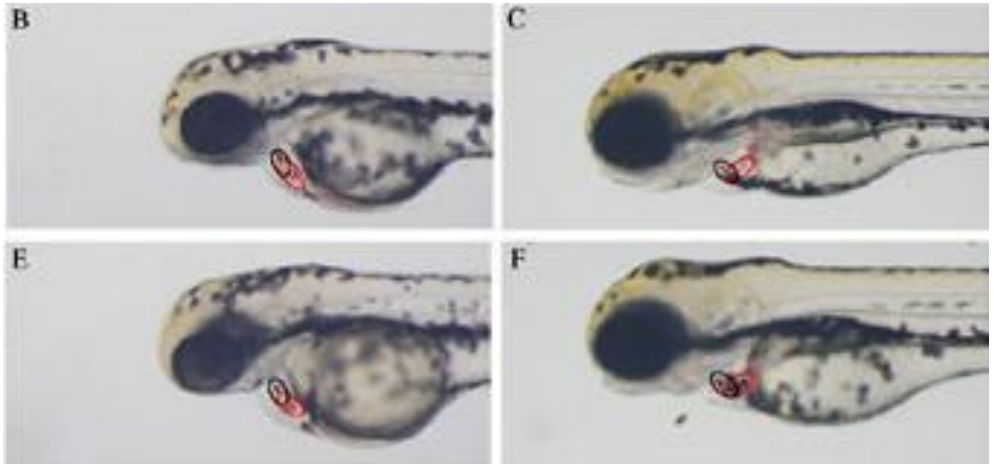


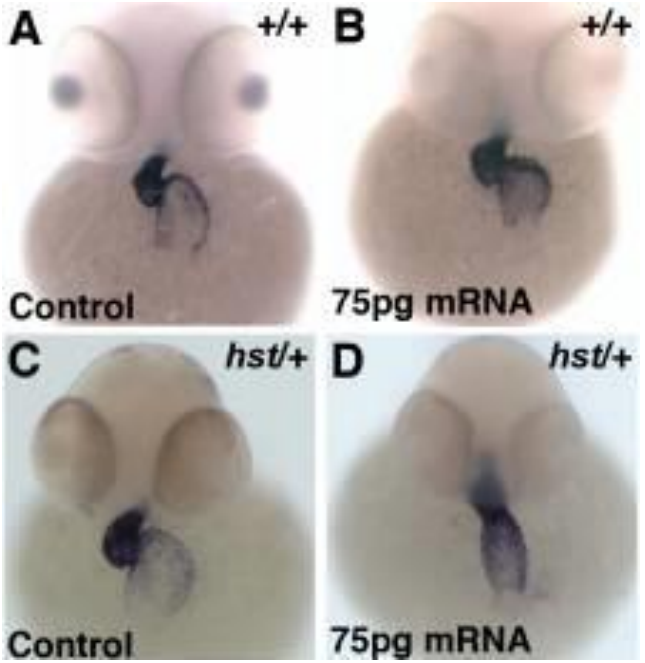


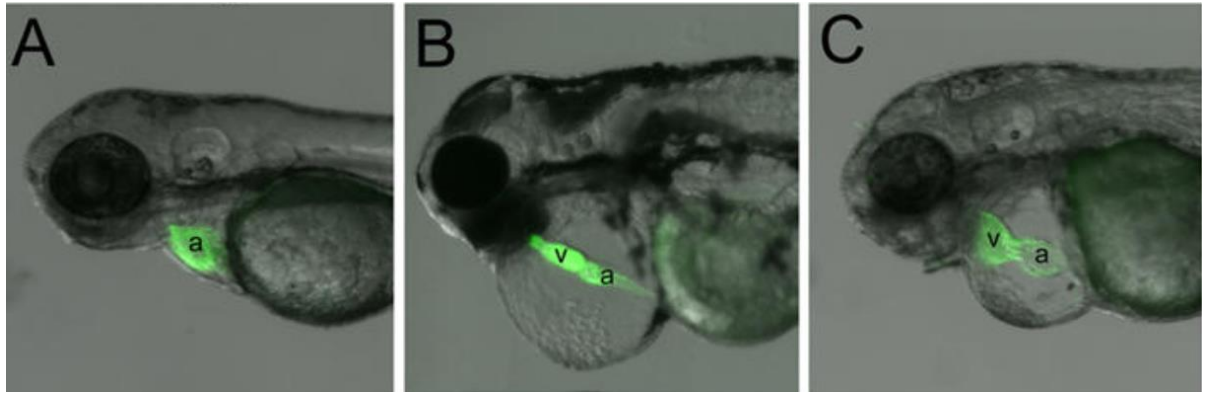
Human

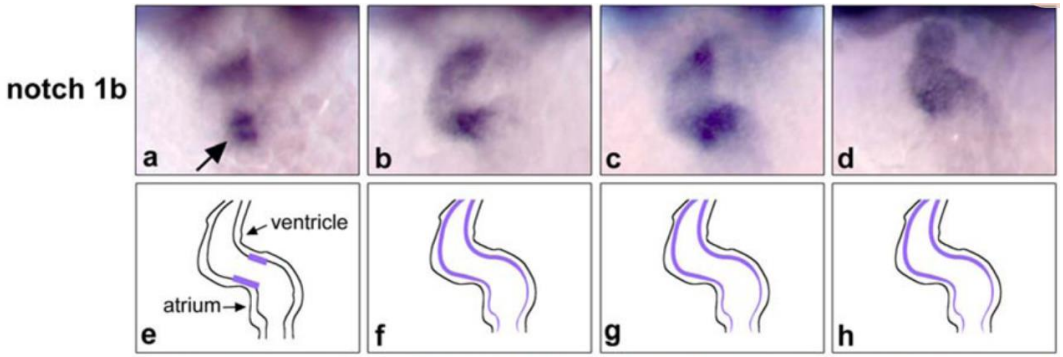
Zebrafish

Zebrafish can be used to study heart development

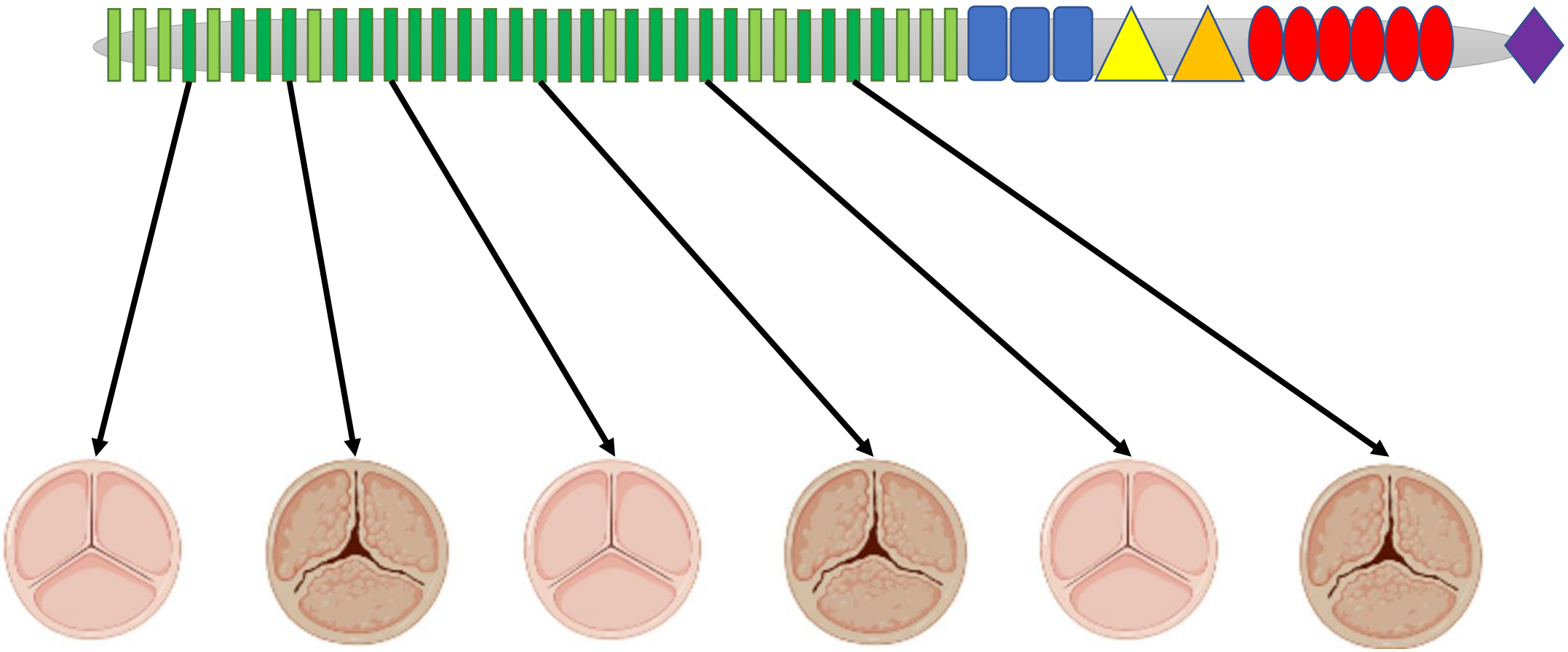








What is the gap in knowledge?



Which EGF-like calcium binding domains are key to aortic valve formation and why?

What is my primary goal?

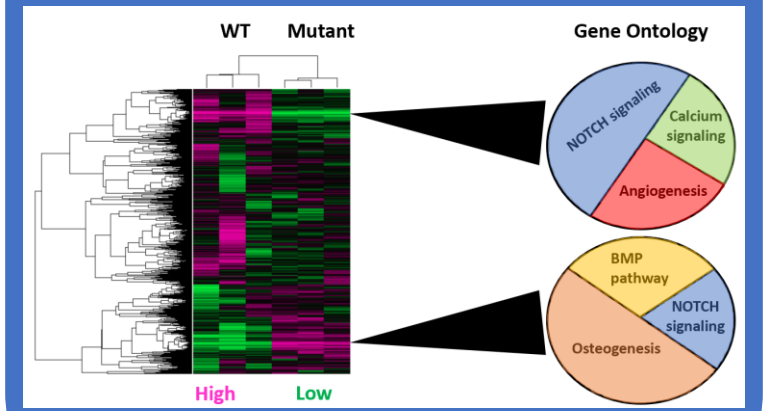
Determine how mutations in the EGF-like calcium binding domains lead to aortic valve calcification

AIM 1

Identify and mutate conserved amino acids in EGF-like domains

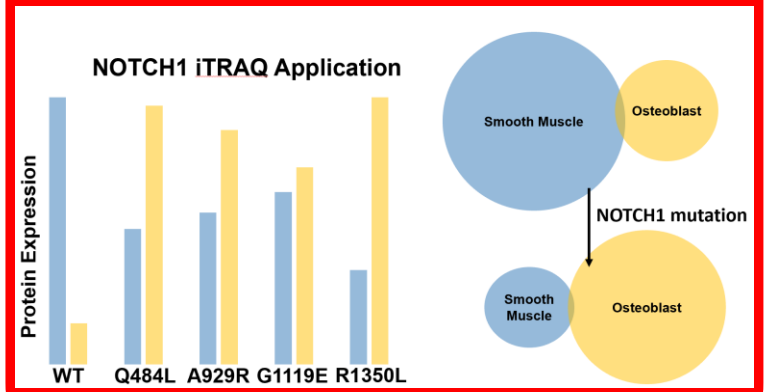
AIM 2

Identify expression changes in response to EGF-like mutations



AIM 3

Identify protein changes in response to EGF-like mutations



Hypothesis 1: NOTCH1 EGF-like calcium binding domains conserved in organisms with complex cardiovascular systems (hearts) are critical for aortic valve development

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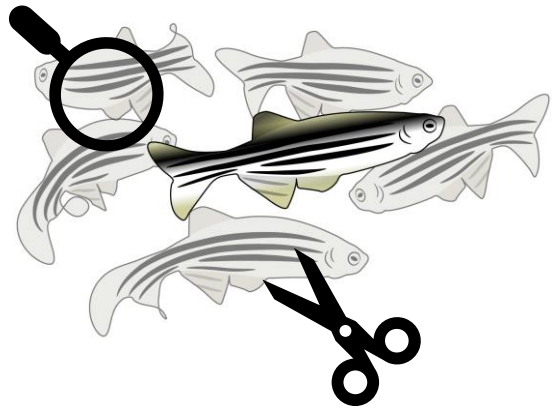
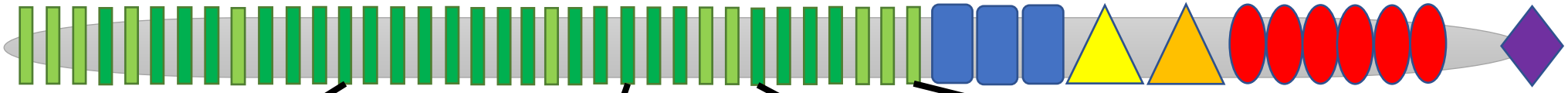
1. Human	S	F	E	C	Q	C	L	Q	G	Y	T	G	P
2. Macaque	S	F	E	C	Q	C	L	Q	G	Y	T	G	P
3. Mouse	S	F	E	C	Q	C	L	Q	G	Y	T	G	P
4. Tropical Clawed Frog	S	F	Q	C	N	C	P	Q	G	Y	A	G	P
5. Zebrafish	S	F	Q	C	K	C	L	Q	G	Y	V	G	A
6. Fruit Fly	S	Y	R	C	N	C	S	Q	G	F	T	G	P
7. C. Elegans	S	Y	W	C	R	C	D	E	G	F	G	G	E



FASTA → MEGA → CRISPR → SCREEN

AIM 1: Determine which EGF-like calcium binding domains are necessary for aortic valve calcification



1. Human	S	F	E	C	Q	C	L	Q	G	Y	T	G	P
2. Macaque	S	F	E	C	Q	C	L	Q	G	Y	T	G	P
3. Mouse	S	F	E	C	Q	C	L	Q	G	Y	T	G	P
4. Tropical Clawed Frog	S	F	Q	C	N	C	P	Q	G	Y	A	G	P
5. Zebrafish	S	F	Q	C	K	C	L	Q	G	Y	V	G	A
6. Fruit Fly	S	Y	R	C	N	C	S	Q	G	F	T	G	P
7. C. Elegans	S	Y	W	C	R	C	D	E	G	F	G	G	E

Q484

1. Human	H	G	A	S	C	Q	N	T	H	G	G
2. Macaque	H	G	A	S	C	Q	N	T	H	G	G
3. Mouse	H	G	A	S	C	Q	N	T	N	G	S
4. Tropical Clawed Frog	N	G	A	M	C	Q	N	T	N	G	S
5. Zebrafish	N	D	A	I	C	Q	N	S	I	G	S
6. Fruit Fly	N	G	A	S	C	L	N	V	P	G	S
7. C. Elegans	N	N	G	T	C	V	N	L	P	G	S

A929

1. Human	C	D	S	S	P	C	K	N	G	G	K	C	W
2. Macaque	C	D	S	S	P	C	K	N	G	G	K	C	W
3. Mouse	C	D	S	A	P	C	K	N	G	G	R	C	W
4. Tropical Clawed Frog	C	D	S	S	P	C	K	N	G	G	K	C	W
5. Zebrafish	C	K	P	S	P	C	K	N	G	G	I	C	R
6. Fruit Fly	C	G	Q	S	P	C	E	N	G	A	T	C	S
7. C. Elegans	C	L	S	D	P	C	M	N	N	A	T	C	I

G1119

1. Human	E	C	R	A	G	H	T	G	R	R	C	E
2. Macaque	E	C	R	A	G	H	T	G	R	R	C	E
3. Mouse	E	C	R	A	G	H	T	G	R	R	C	E
4. Tropical Clawed Frog	E	C	R	Q	G	F	T	G	R	R	C	D
5. Zebrafish	E	C	R	T	G	Y	T	G	Q	H	C	D
6. Fruit Fly	N	C	R	P	G	H	M	G	R	H	C	E
7. C. Elegans	-	-	-	-	-	-	-	-	-	-	-	-

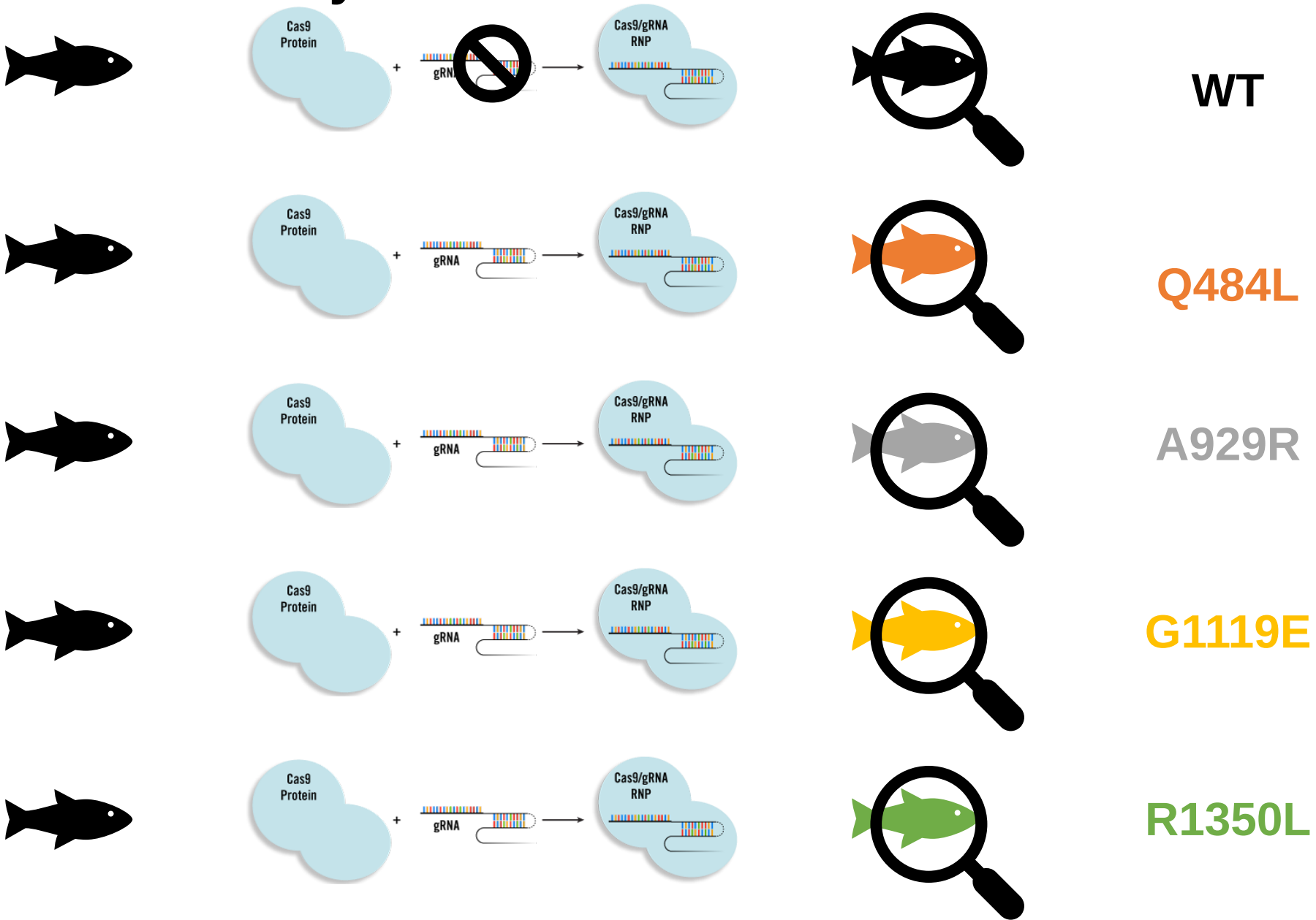
R1350

Heart

No heart

1. Human	S	F	E	C	Q	C	L	Q	G	Y	T	G	P
2. Macaque	S	F	E	C	Q	C	L	Q	G	Y	T	G	P
3. Mouse	S	F	E	C	Q	C	L	Q	G	Y	T	G	P
4. Tropical Clawed Frog	S	F	Q	C	N	C	P	Q	G	Y	A	G	P
5. Zebrafish	S	F	Q	C	K	C	L	Q	G	Y	V	G	A
6. Fruit Fly	S	Y	R	C	N	C	S	Q	G	F	T	G	P
7. C. Elegans	S	Y	W	C	R	C	D	E	G	F	G	G	E

AIM 1: Determine which EGF-like calcium binding domains are necessary for aortic valve calcification



Hypothesis 2: Mutation of conserved NOTCH1 EGF-like calcium binding domains leads to a shift from smooth muscle expression to osteoblast expression

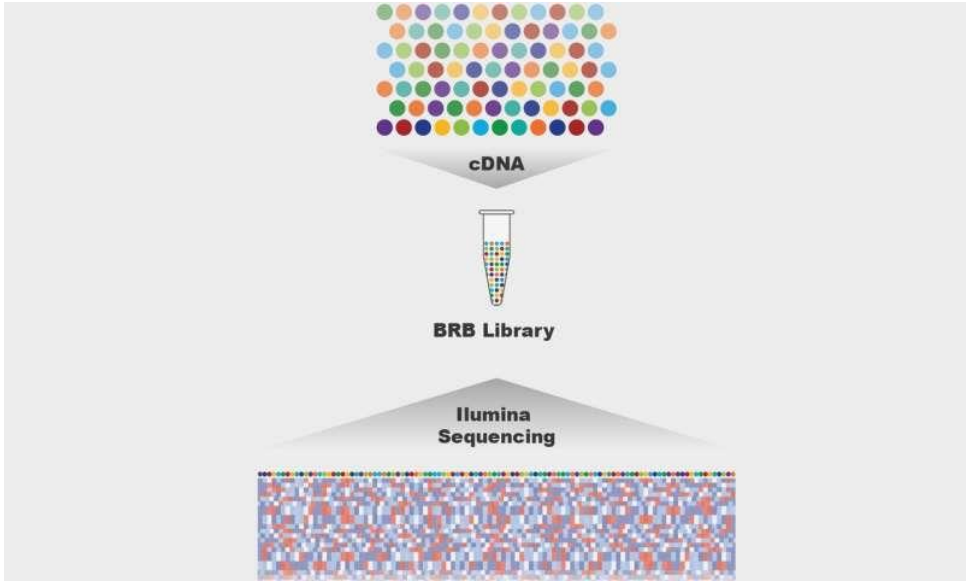
WT

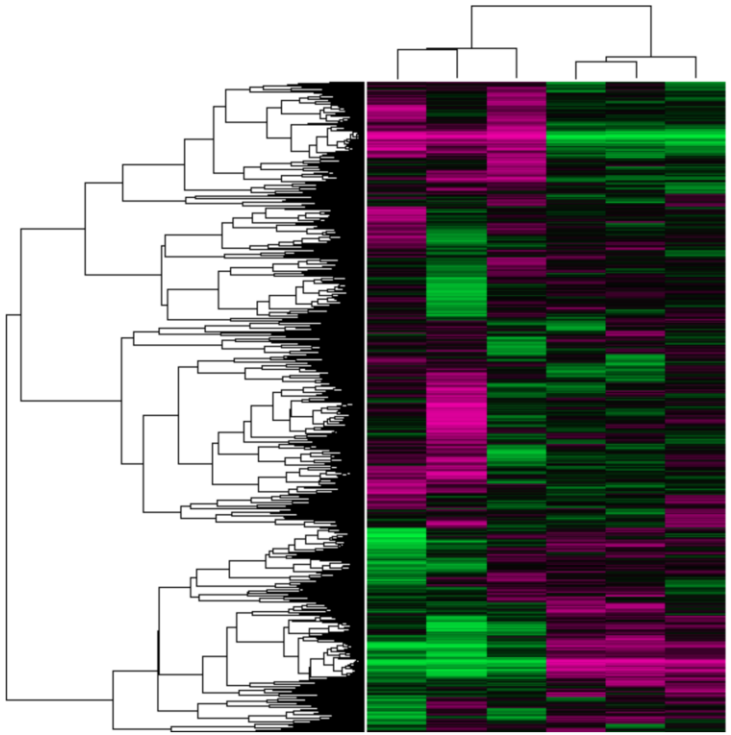
Q484L

A929R

G1119E

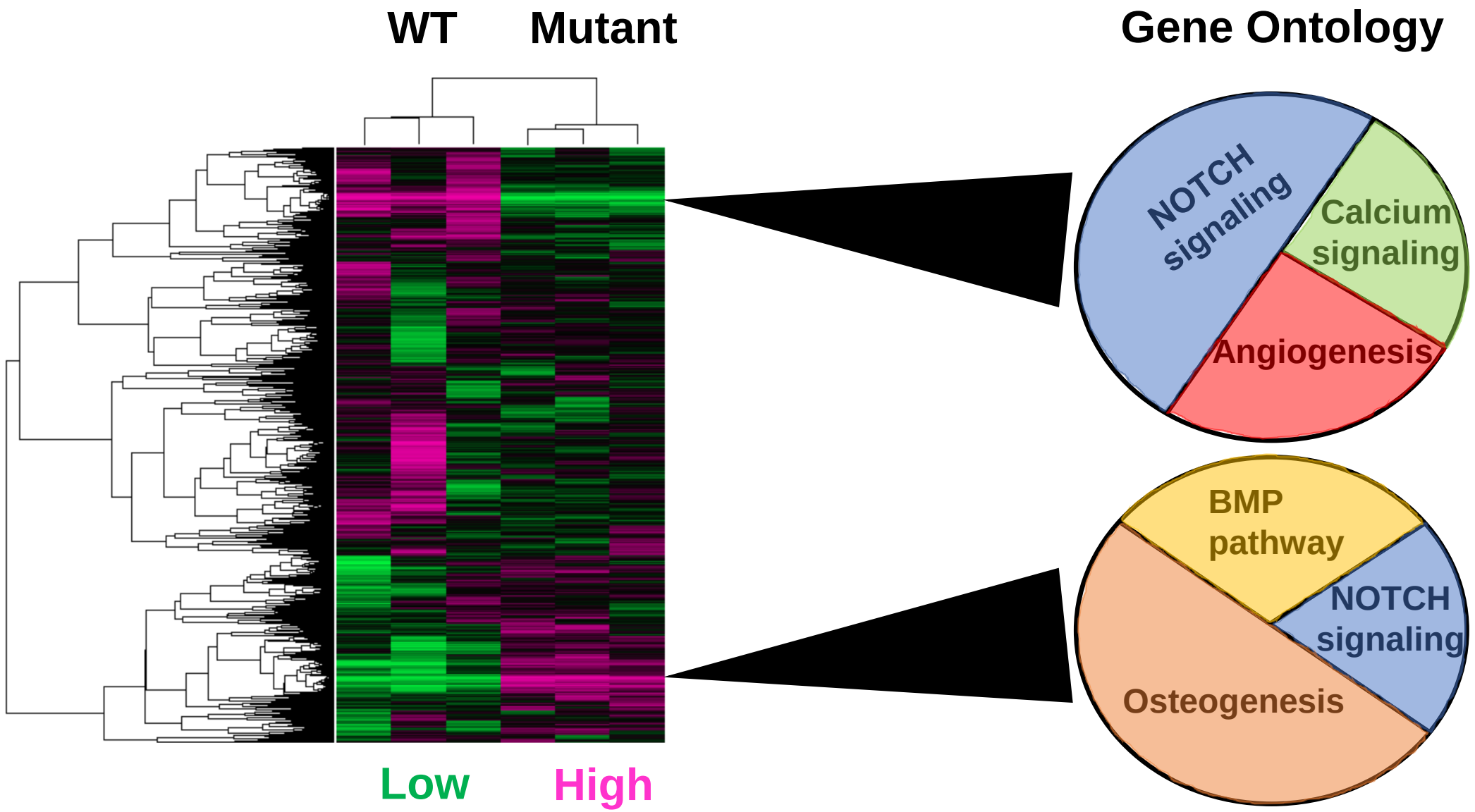
R1350L





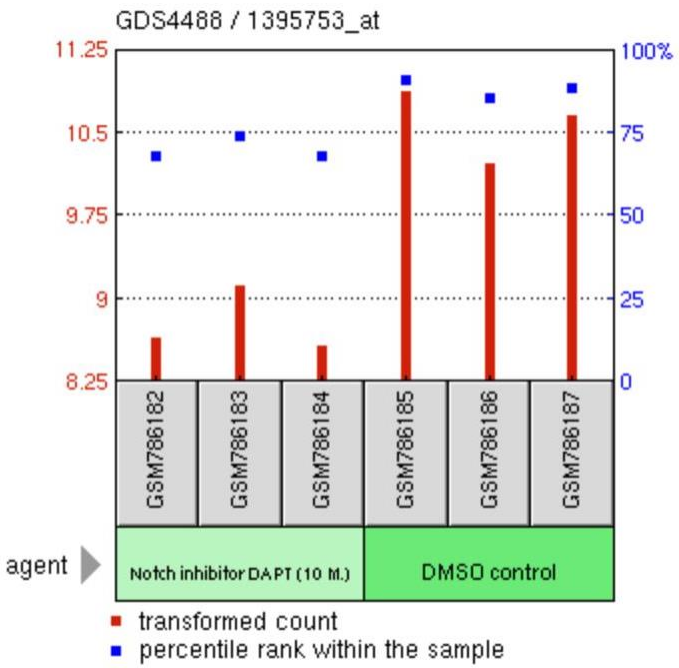
CRISPR → LIBRARY → RNA-SEQ → GEO

AIM 2: Identify which EGF-like calcium binding domain is necessary for proper smooth muscle expression

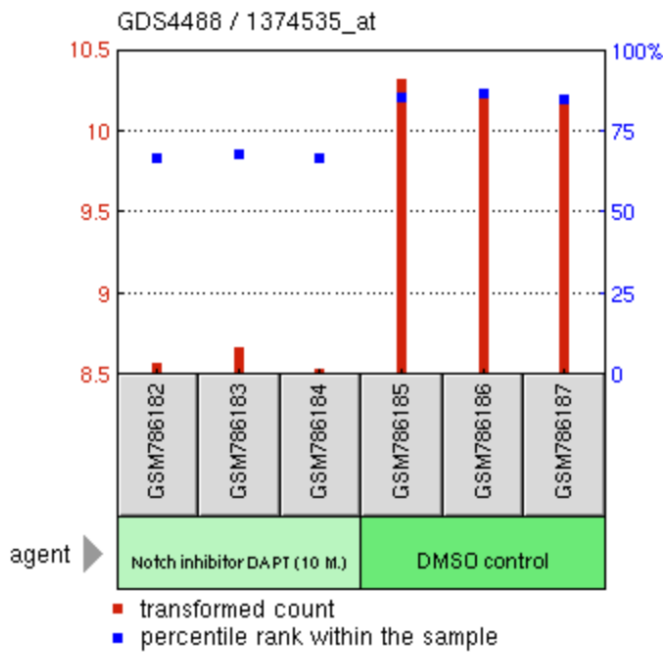


AIM 2: Identify which EGF-like calcium binding domain is necessary for proper smooth muscle expression

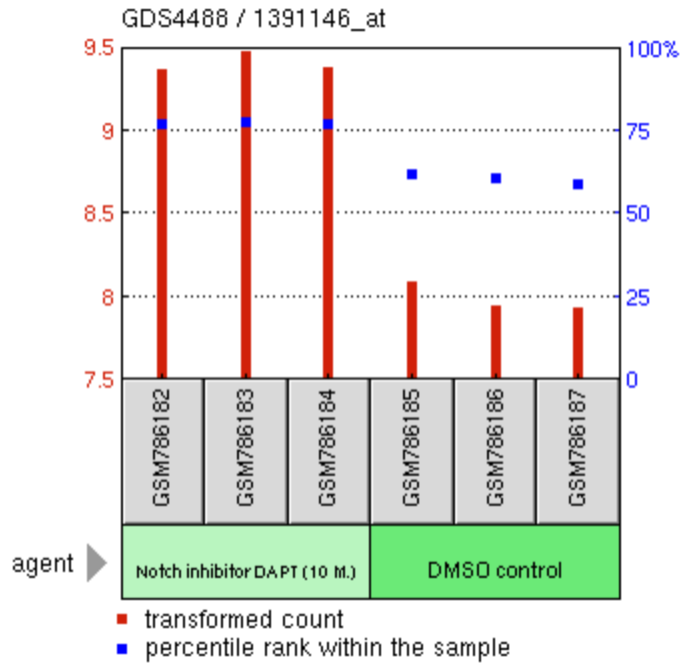
ELN



AFAP1L2



CDH11



From “Notch1 signaling inhibition effect on aortic valve interstitial cells” (GDS4488)

Hypothesis 3: Mutation of conserved NOTCH1 EGF-like calcium binding domains leads to a shift from smooth muscle proteins to osteoblast proteins

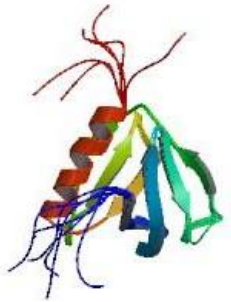
WT

Q484L

A929R

G1119E

R1350L



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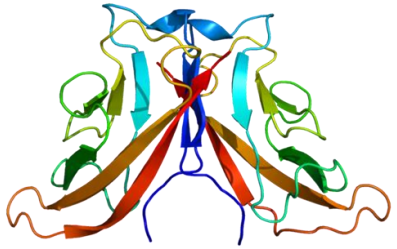
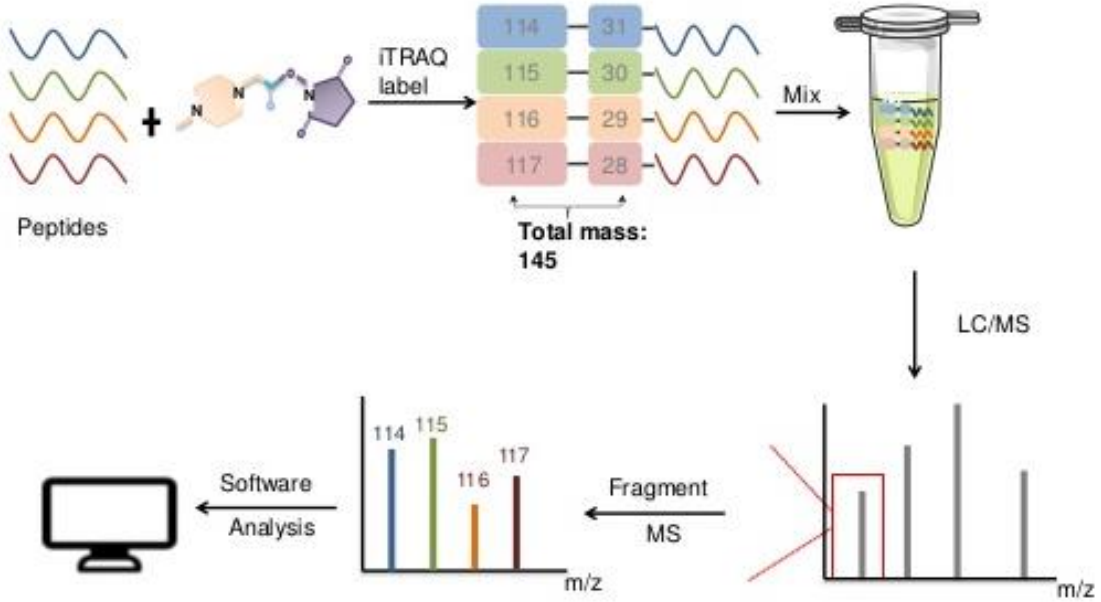
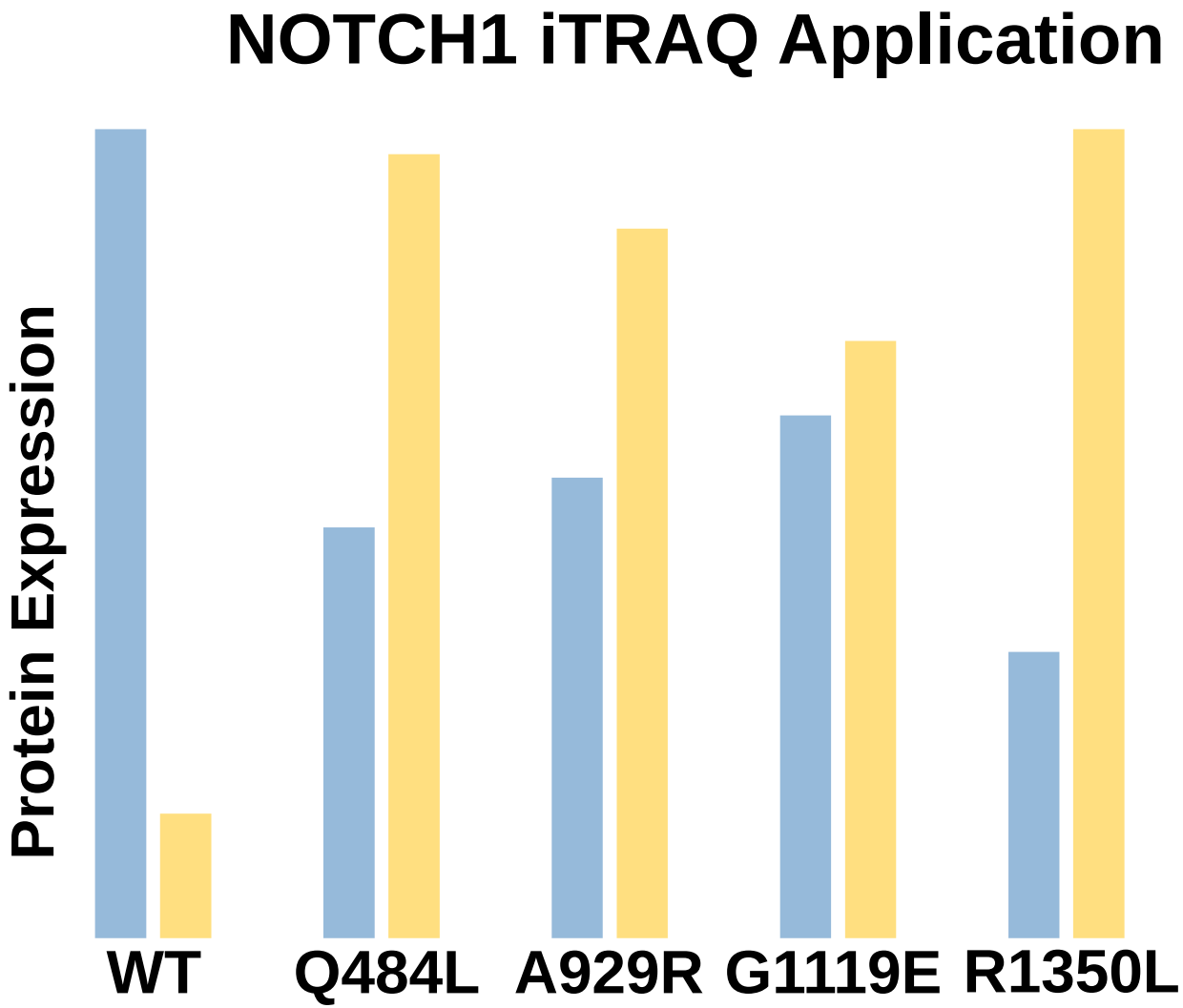


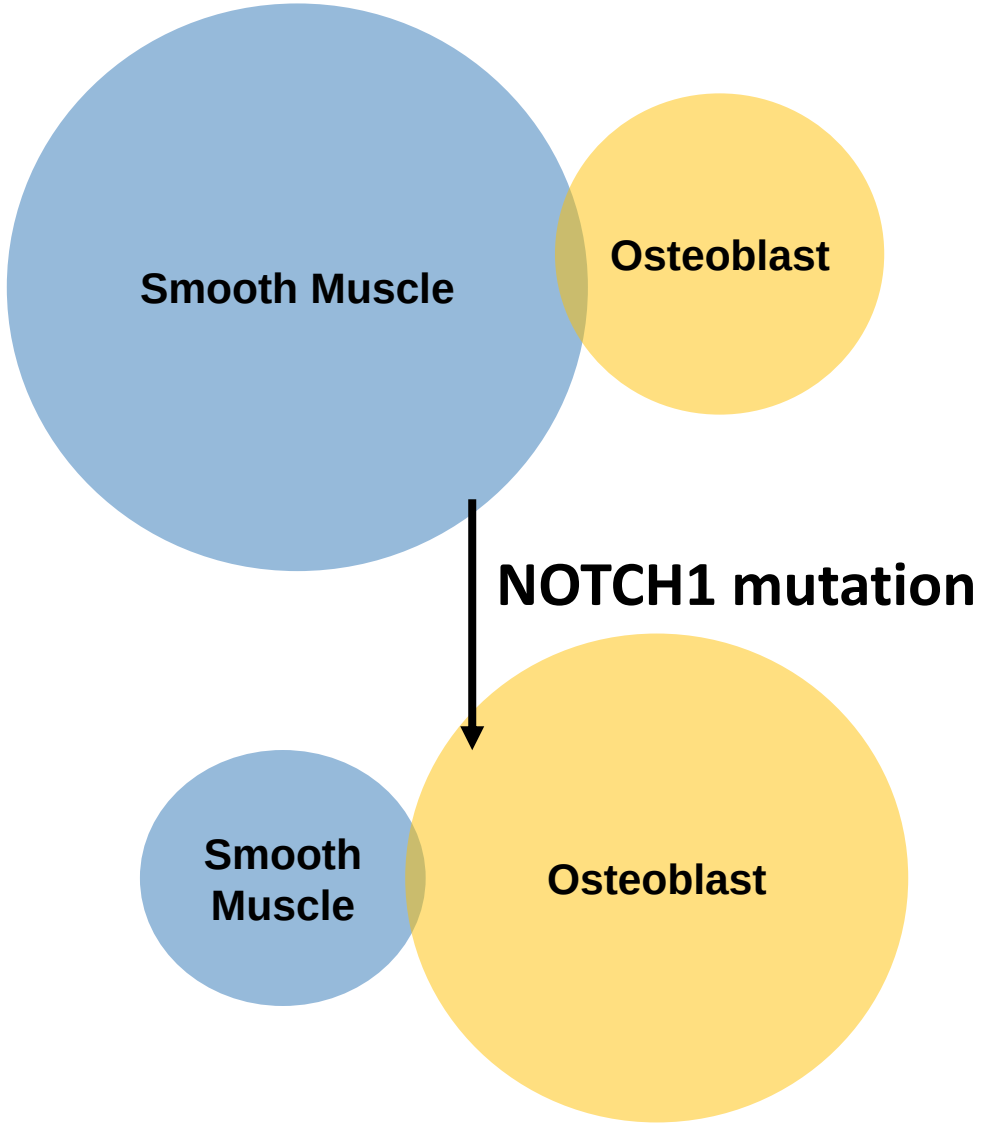
CDH11



CRISPR → iTRAQ → LC/MS

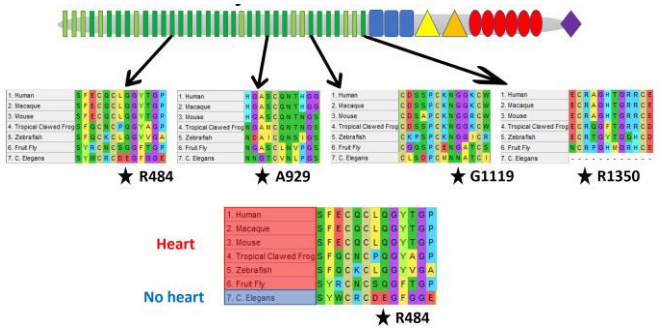
AIM 3: Determine which EGF-like calcium binding domain crucial for smooth muscle protein expression



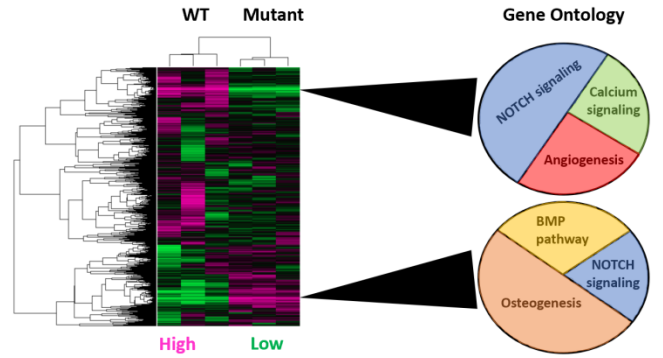


Conclusions

Mutations in NOTCH1 cause Aortic Valve Disease (AoVD1)

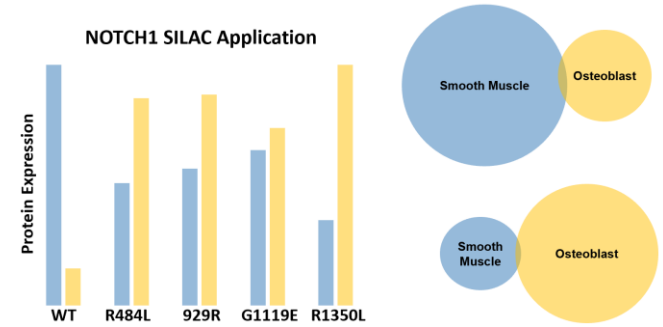


Loss in NOTCH1 signaling shifts transcription programming from smooth muscle expression to osteoblast expression



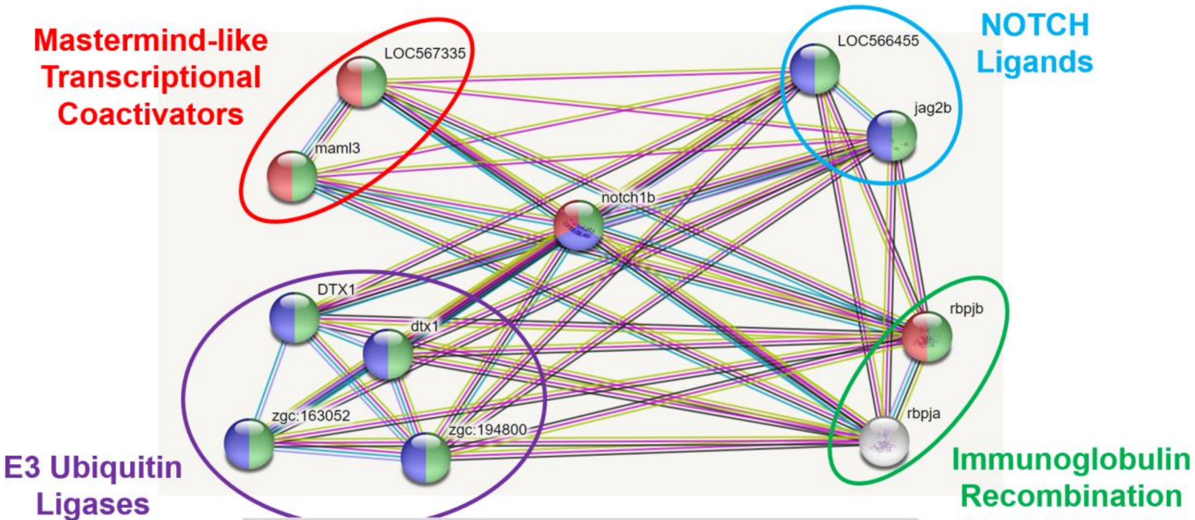
EGF-like like calcium binding domains are highly conserved in animals with complex cardiovascular systems

CRISPR, RNA-seq, and iTRAQ can be utilized to isolate which domains are necessary for proper aortic valve development



Future Goals

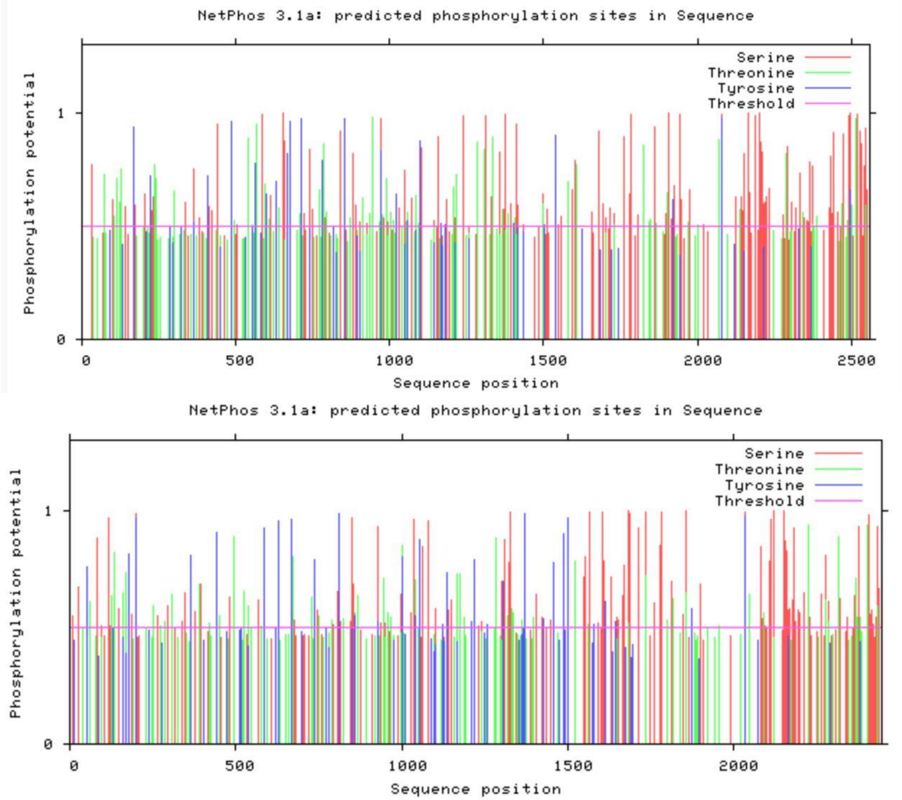
Interactions



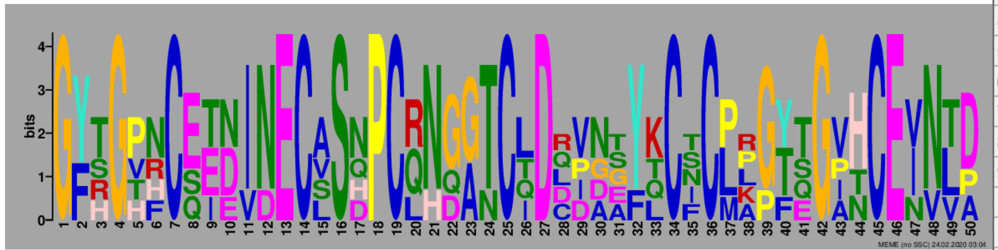
Reactome Pathways

pathway	description	count in gene set	false discovery rate
DRE-1980143	Signaling by NOTCH1	10 of 21	2.45e-28
DRE-2122948	Activated NOTCH1 Transmits Signal to the Nucleus	7 of 12	7.12e-20
DRE-8941856	RUNX3 regulates NOTCH signaling	4 of 12	1.27e-10

PTMs



Motifs



1. Human	G H C D S Q C N S A G C L F D G F D C Q R A E G Q C
2. American Black Bear	G R C D S Q C N S A G C L F D G F D C Q R A E G Q C
3. Cat	G R C D S Q C N S A G C L F D G F D C Q R A D G Q C
4. Chicken	G K C D S Q C N N A G C L Y D G F D C Q K Y E G Q C
5. Macaque	G H C D S Q C N S A G C L F D G F D C Q R A E G Q C
6. Little Brown Bat	G H C D S Q C N S A G C L F D G F D C Q R A E G Q C
7. Mouse	G H C D S Q C N S A G C L F D G F D C Q L T E G Q C
8. Sperm Whale	G R C D S Q C N S A G C L F D G F D C Q R A E G Q C
9. Tropical Clawed Frog	G K C D S Q C N N S G C L Y D G F D C Q K V E V Q C
10. Zebrafish	G K C D E Q C H N T G C L Y D G F D C Q R V E A Q C
11. Fruit Fly	G K C N E E C N N A A C H Y D G H D C E R K L K S C

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