

Kindler Syndrome

Skin blistering and
cancer development



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Kindler syndrome is a skin condition with some severe phenotypes. Some of the following images may be graphic.

What is Kindler Syndrome?

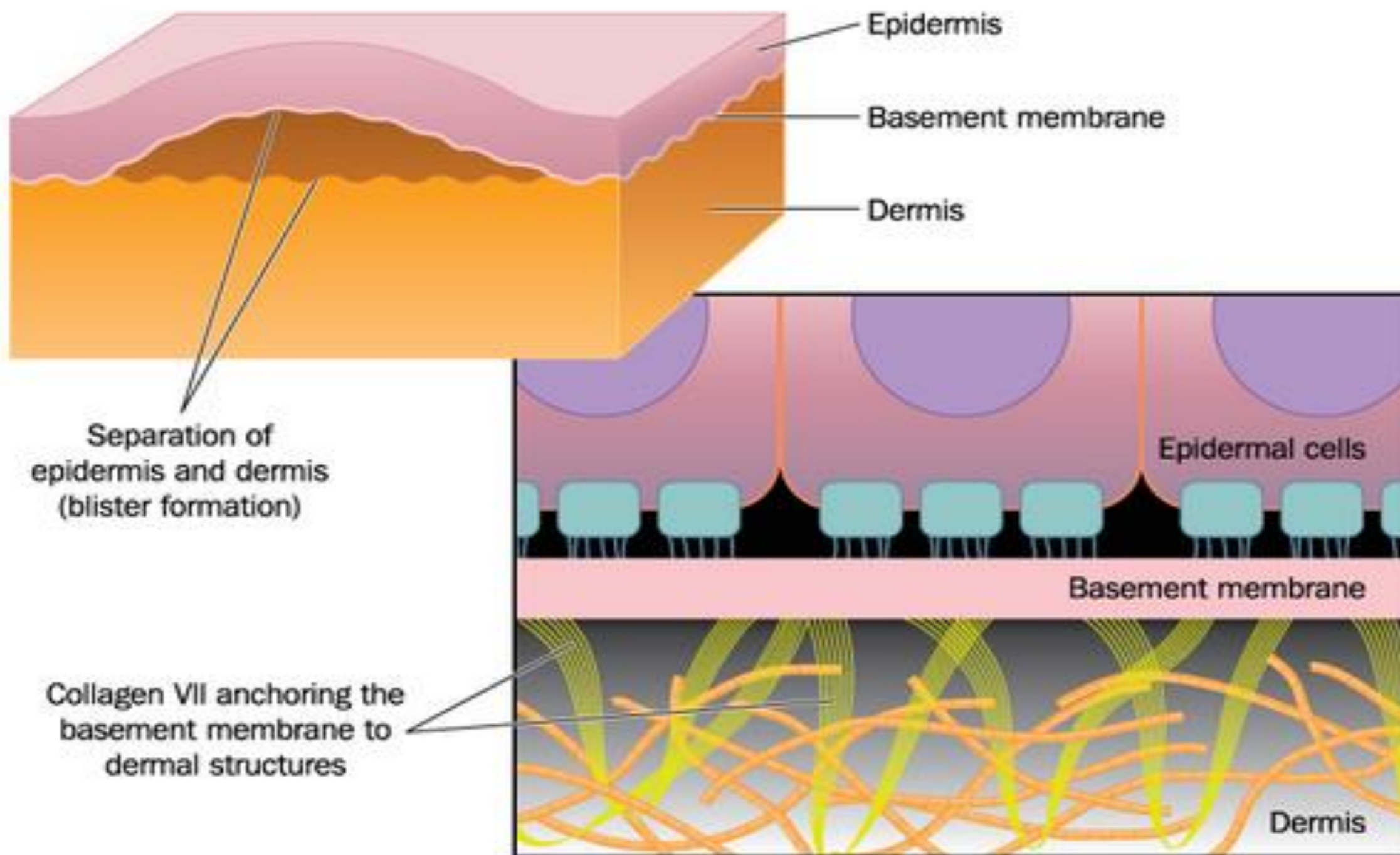


Skin blistering



**Squamous-cell
carcinoma**

How do these blisters form in Kindler Syndrome?



Older Kindler syndrome patients develop skin cancer

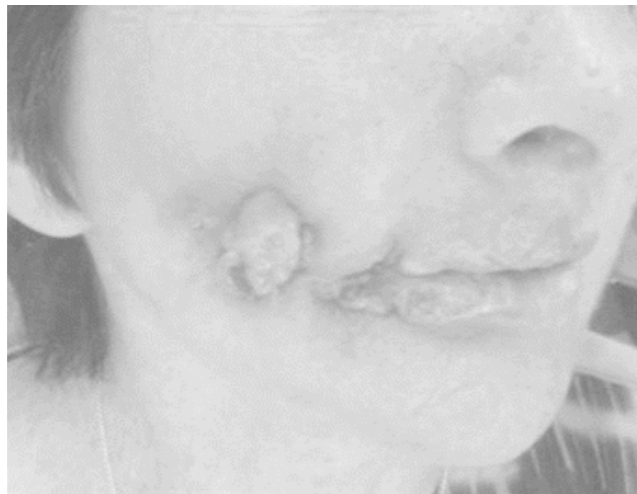
Youth



Skin blistering



Adult



Squamous cell carcinoma



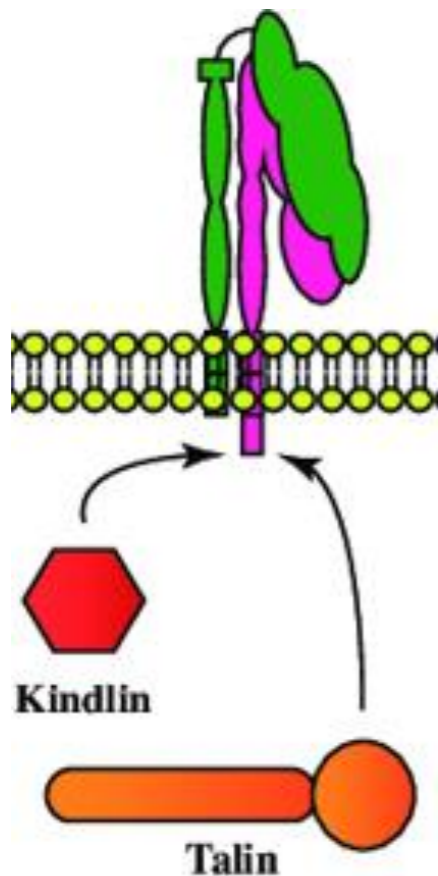
What gene causes Kindler Syndrome?

FERMT1

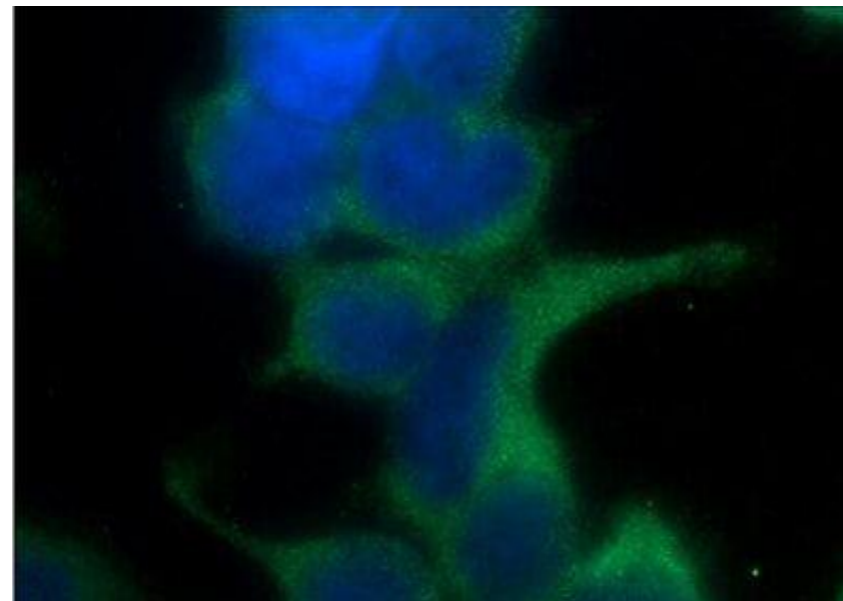
Kindlin-2-N

FERM

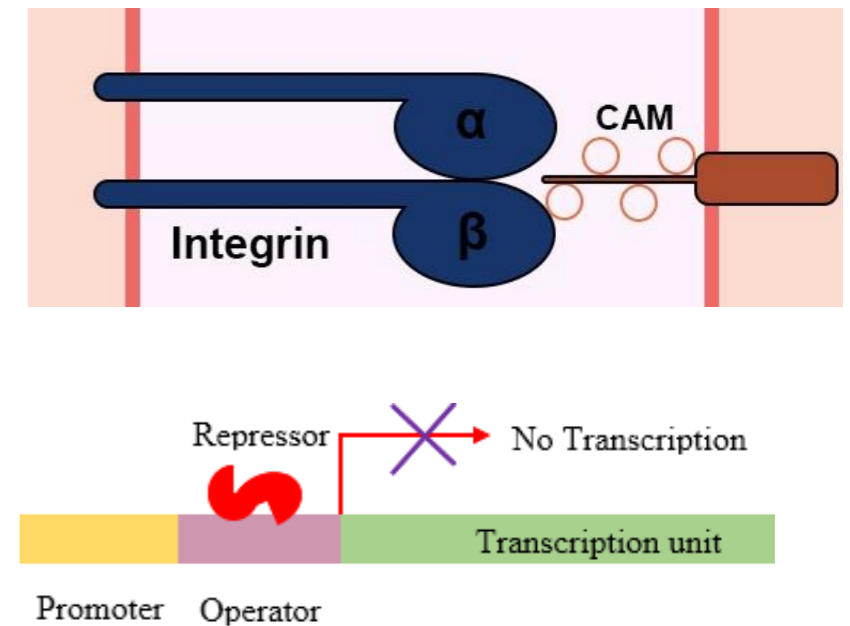
678 AA



Molecular Function

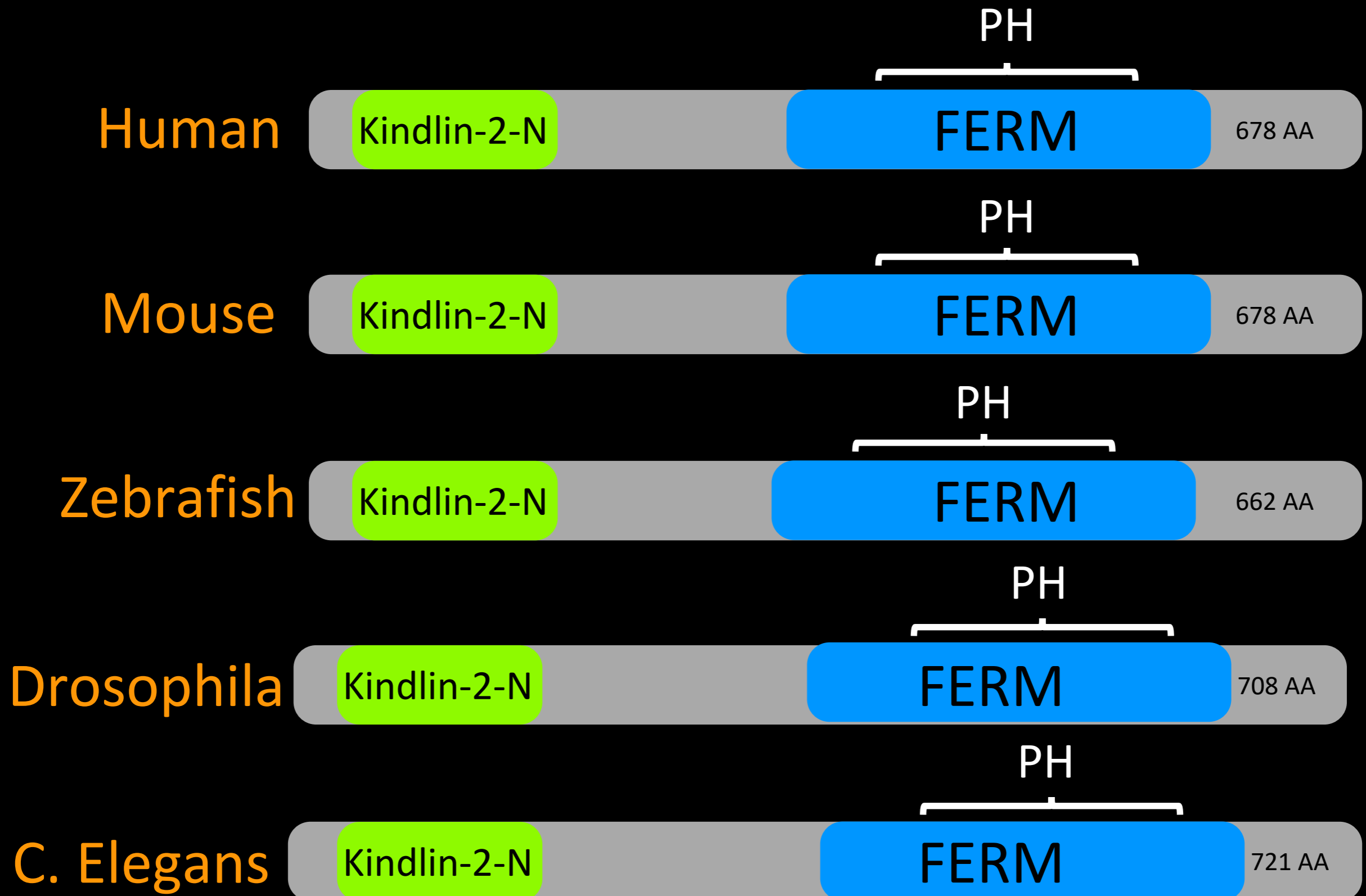


Cellular Component

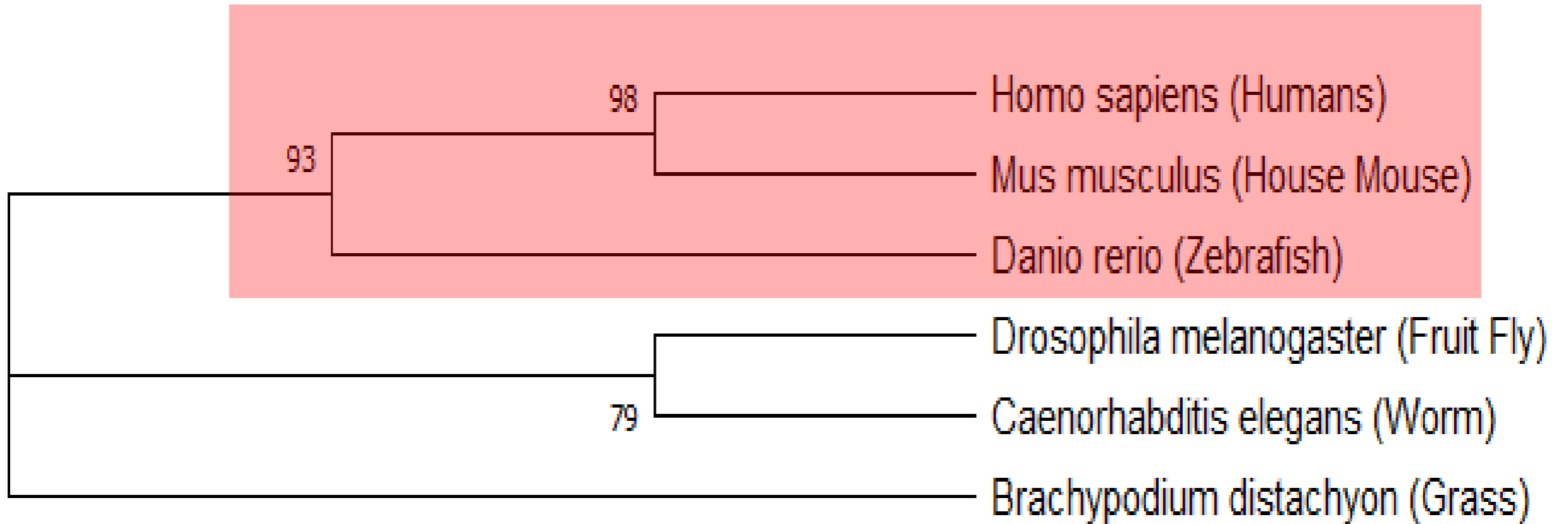


Biological Function

FERMT1 is highly conserved across model organisms

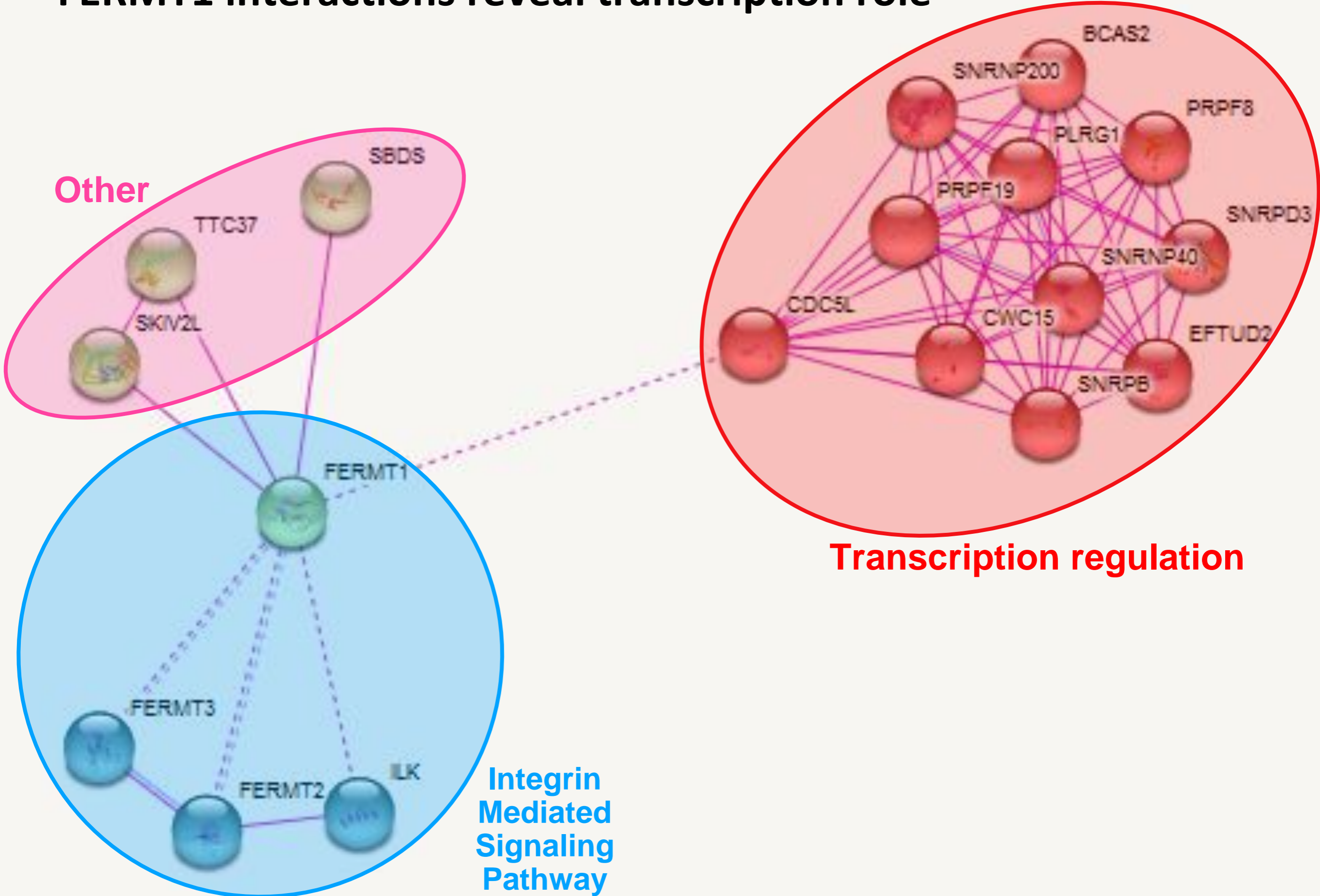


FERMT1 in mice and zebrafish is most closely related to humans



FERMT1 phylogeny

FERMT1 interactions reveal transcription role



Zebrafish will be used to address my goals



Danio rerio



Inexpensive

Inexpensive

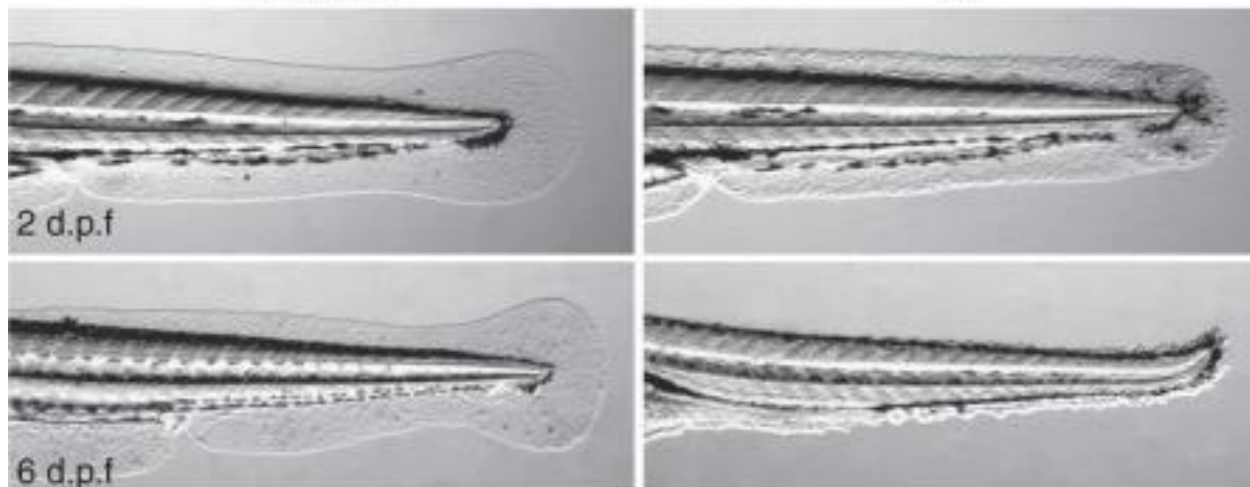
Shows **BLISTERING** phenotype

Visualize cell division easily

Tumor development

Wild Type

kindlin mutant



GAP: Why does loss of Kindlin-1 lead to development of squamous-cell carcinomas in older patients?

Human

Kindlin-2-N

FERM

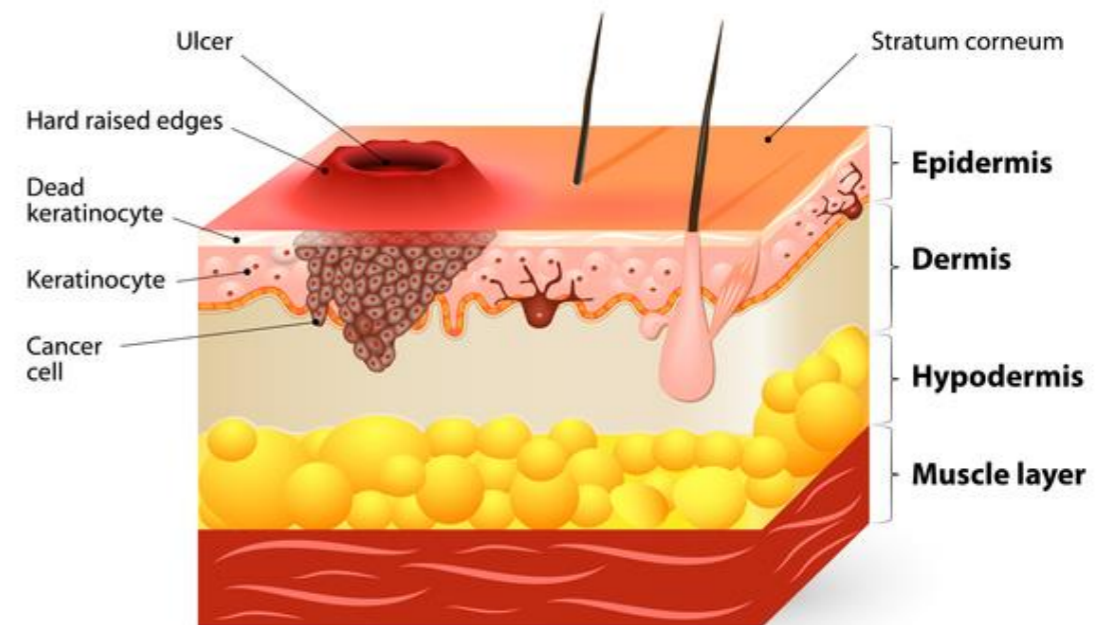
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VS.



Squamous-cell carcinoma



GOAL: Determine the role of FERMT1 in mediating cell proliferation

Human

Kindlin-2-N

FERM

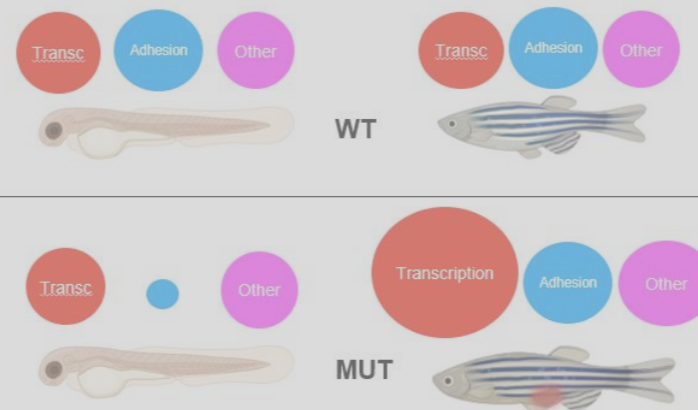
678 AA

AIM 1

Species/Abbrev	Protein Sequences
1. Homo_sapiens_(Humans)	KQWNVHWEIRGVVIEFDQNVFIAFTCLSDCKIVVHEYIGGYIFLSTRSKDQNETLDEDFHKLGGDD
2. Mus_musculus_(House_Mouse)	KQWNVHWEIRGVVIEFDQNVVIAFTCLSDCKIVVHEYIGGYIFLSTRSKDQNETLDEDFHKLGGDD
3. Danio_zebra_(Zebrafish)	KQWNVHWEIRGVVIEFDQNVVIAFTCLSDCKIVVHEYIGGYIFLSTRSKDQNETLDEDFHKLGGDD
4. Drosophila_melanogaster_(Fruit_Fly)	KAWNVHWEIRKMMVQLQD-EHIVFVQSDCKVVVHEFVGGYIFLSTRSKENQTLDEEFHKLGGDD
5. Caenorhabditis_elegans_(Worm)	KKNHVVWEIRHLKIRFED-EDIEFKPLSDCKVVVHEFVGGYIFLSTRSKENQTLDEEFHKLGGDD
6. Brachypodium_distachyon_(Grass)	CDTGLGCTPVVGSGLG

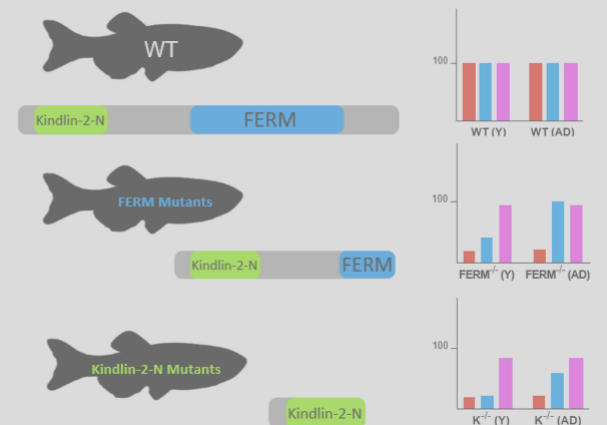
Identify highly conserved residues of FERMT1 that maintain cell proliferation in skin in **older patients**

AIM 2



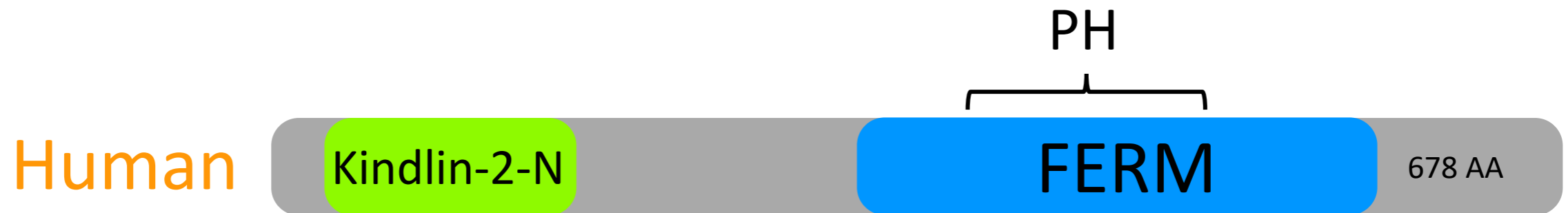
Identify differentially expressed genes in **adult zebrafish mutants** versus adolescent

AIM 3



Identify protein interactions of FERMT1 in **adult zebrafish mutants** versus adolescent

AIM1: Determine which amino acids/domain are important for cell proliferation in the skin in older fish

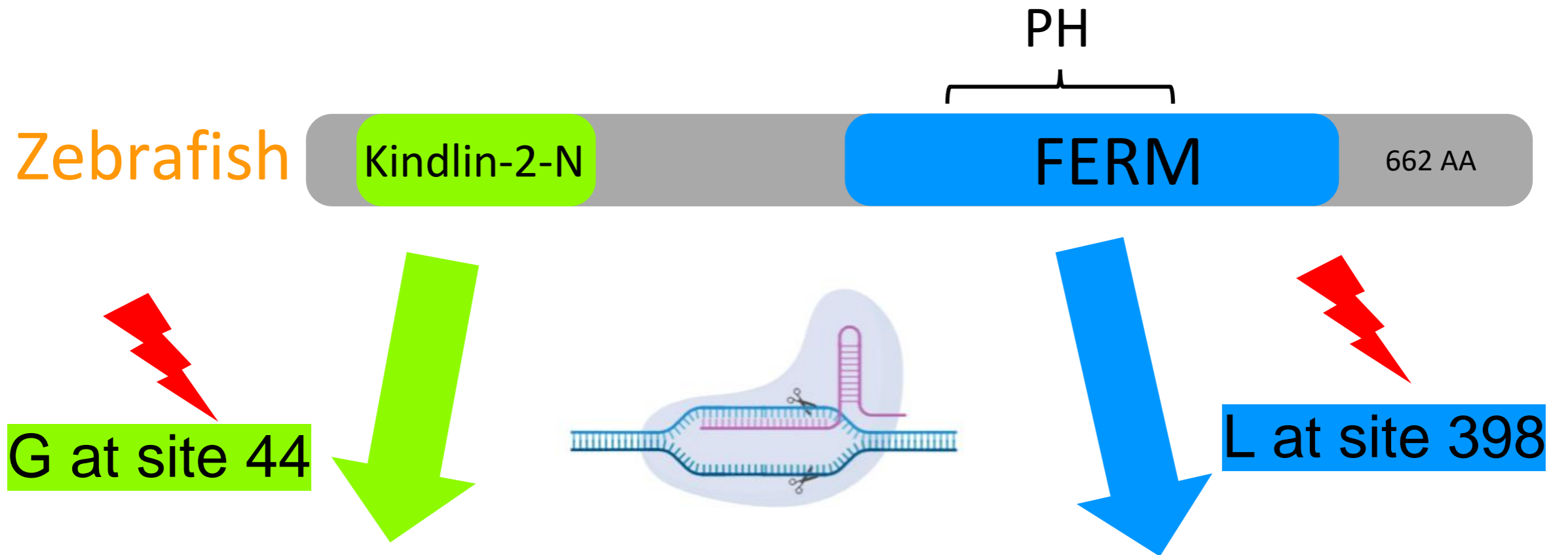


Protein Sequences

Species/Abbrv												*		*																					
1. Homo sapiens (Humans)	D	F	A	-	-	-	-	-	-	G	E	S	E	V	D	E	I	E	A	A	L	S	N	L	E	V	T	L	E	G	G	K	A	D	
2. Mus musculus (House Mouse)	D	F	A	-	-	-	-	-	-	T	K	S	E	V	D	E	V	E	A	A	L	S	S	L	E	V	T	L	E	G	G	K	A	D	
3. Danio rerio (Zebrafish)	D	N	S	-	-	-	-	-	-	D	E	K	E	V	D	E	V	E	A	A	L	S	N	L	E	T	H	L	E	G	R	N	A	D	
4. Drosophila melanogaster (Fruit Fly)	I	D	S	G	I	D	T	S	S	Q	E	T	G	G	E	D	D	I	D	S	A	L	N	E	L	Q	I	T	L	E	G	P	G	G	G
5. Caenorhabditis elegans (Worm)	-	-	-	-	-	-	-	-	-	-	E	E	N	N	K	D	D	V	D	I	L	L	D	E	L	E	Q	N	L	D	A	A	A	L	N
6. Brachypodium distachyon (Grass)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	E	D	M	E	S	H	L	D	N	L	K	A	M	L	R	V	G	K	G	S

The amino acids highly conserved in all species will be important for cell proliferation in older fish

AIM1: Truncation of kindlin-1 via CRISPR-Cas9 mutation



Young



Old



AIM1: Expected phenotypes in young and old FERMT1 mutants

Young



Old



Both **young** mutants will show **blistering** phenotype

Both **old** mutants will grow **tumors**

GOAL: Determine the role of FERMT1 in mediating cell proliferation

Human

Kindlin-2-N

FERM

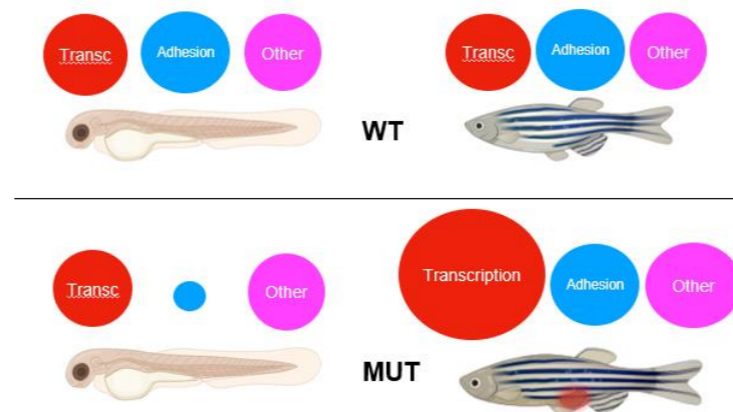
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AIM 1

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3. Danio_zebra_(Zebrafish)	KQWVHWEIRGVVIEFDQVFAFTCLADCKIVHEYIGGYIFLSTRKQNETLDEDFHKLGGQD
4. Drosophila_melanogaster_(Fruit_Fly)	KAWVHWEIKCMVQLDQ-ENIVFVQSDCKVVEYIGGYIFLSTRKQNETLDEDFHKLGGQD
5. Caenorhabditis_elegans_(Worm)	KKWVHWEIRLKIIFED-EDIEFKLADCKVVEYIGGYIFLSTRKQNETLDEDFHKLGGQA
6. Brachypodium_distachyon_(Grass)	-----CCTDGLG-----PVGKSSGQU-----

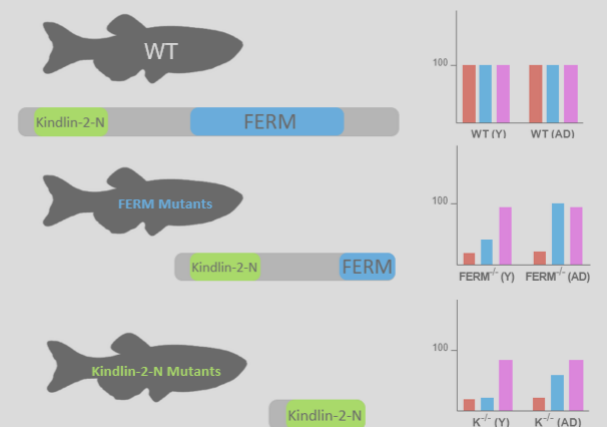
Identify highly conserved residues of FERMT1 that maintain cell proliferation in skin in **older patients**

AIM 2



Identify differentially expressed genes in **adult zebrafish mutants** versus adolescent

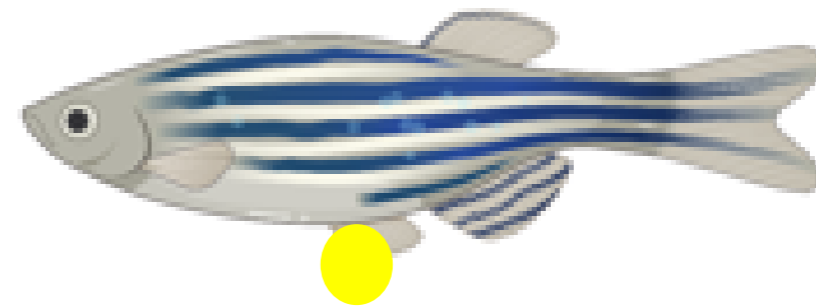
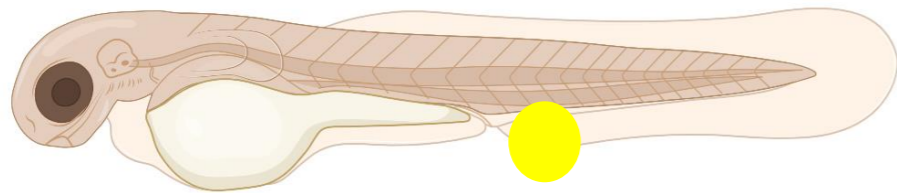
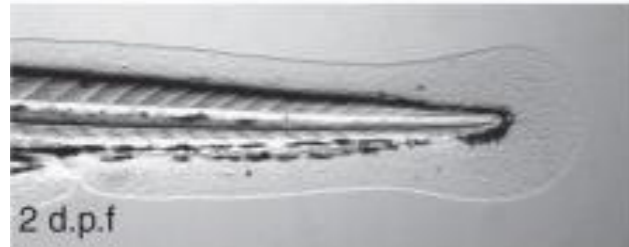
AIM 3



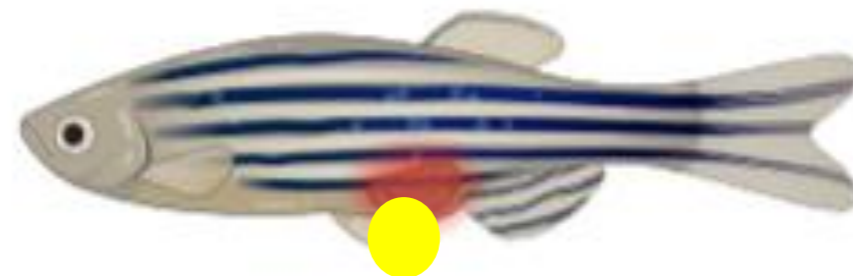
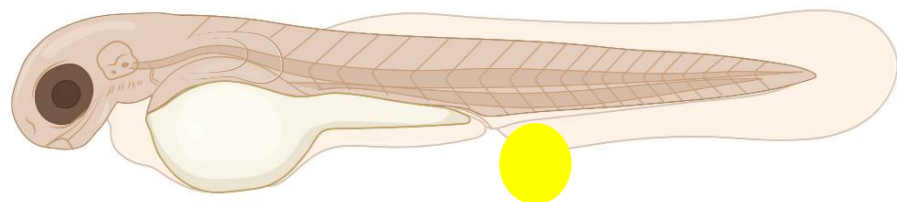
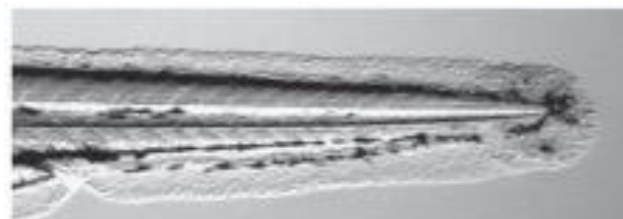
Identify protein interactions of FERMT1 in **adult zebrafish mutants** versus adolescent

AIM2: Single-cell RNA Seq on young and old fish

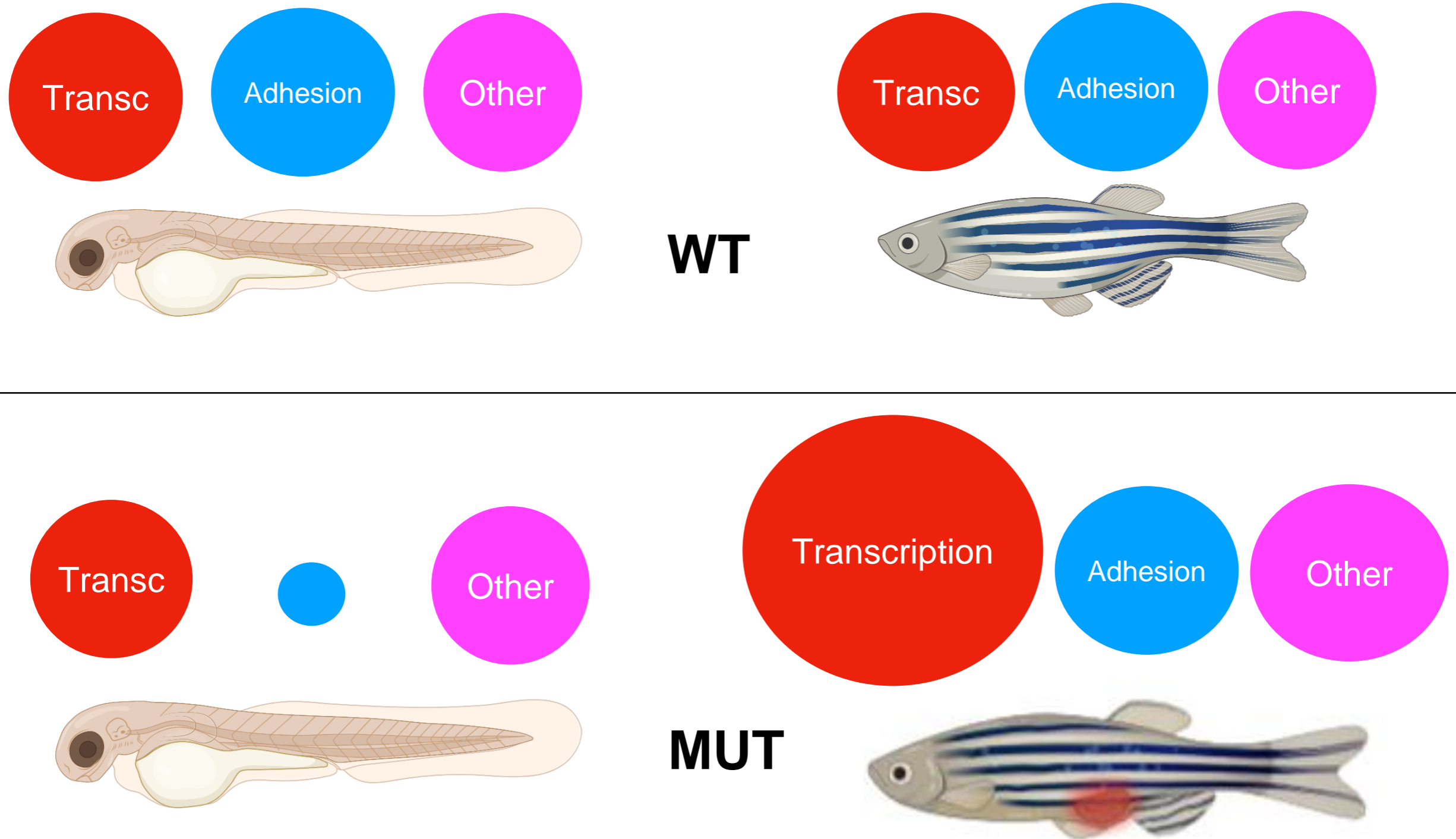
Wild Type



kindlin mutant



AIM2: Identify differentially expressed genes in adult mutants



Transcription related and **adhesion related** gene expression will **increase** significantly in **adult mutants**

GOAL: Determine the role of FERMT1 in mediating cell proliferation

Human

Kindlin-2-N

FERM

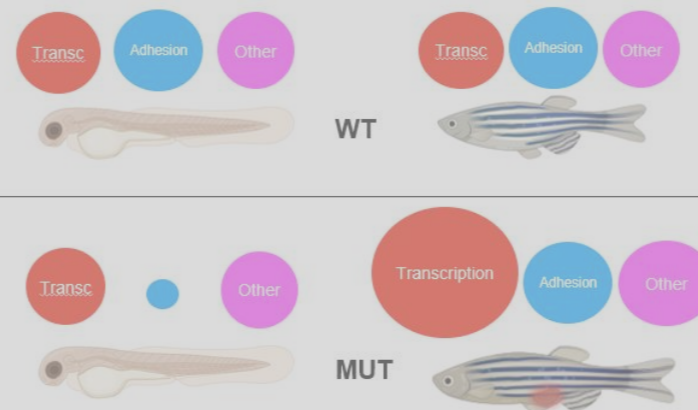
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AIM 1

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4. Drosophila_melanogaster_(Fruit_Fly)	KAWVHVEIRKMMIQDQENIVFVQSDCKVVEHFGGYIFMWRKKNQTLDEDFHKLGGQA
5. Caenorhabditis_elegans_(Worm)	KKWHVVEIRLKIIFEDQIEFKPLADCKVVEHFGGYIFLWRKKEHSQLDEDFHKLGGQA
6. Brachypodium_distachyon_(Grass)CCTDGLG.....PVGKSSGQL.....

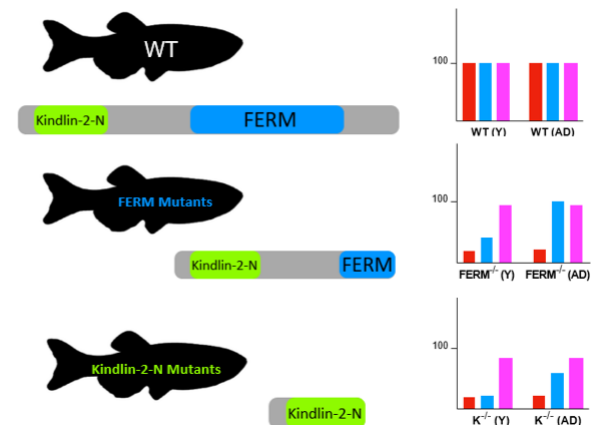
Identify highly conserved residues of FERMT1 that maintain cell proliferation in skin in **older patients**

AIM 2



Identify differentially expressed genes in **adult zebrafish mutants** versus adolescent

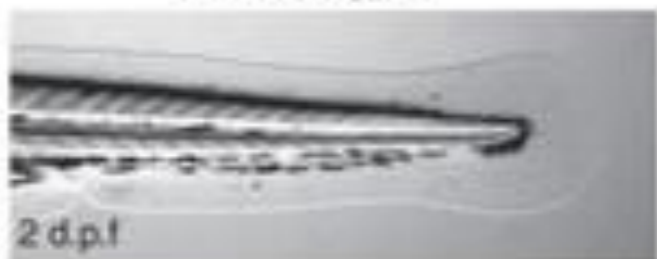
AIM 3



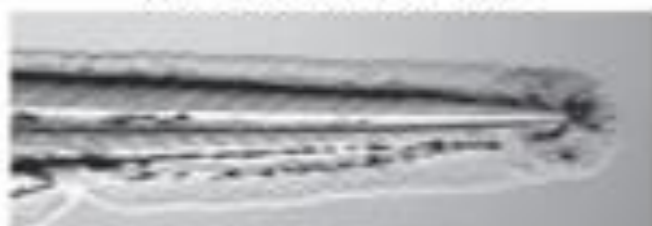
Identify protein interactions of FERMT1 in **adult zebrafish mutants** versus adolescent

AIM3: Sample protein interaction in fins of FERMT1 mutants *in vivo*

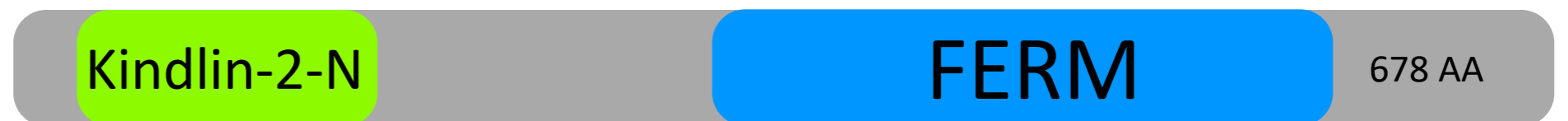
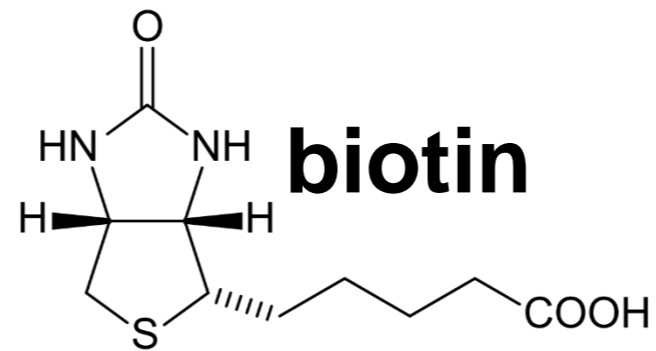
Wild Type



kindlin mutant



AIM3: Adding biotin to FERMT1

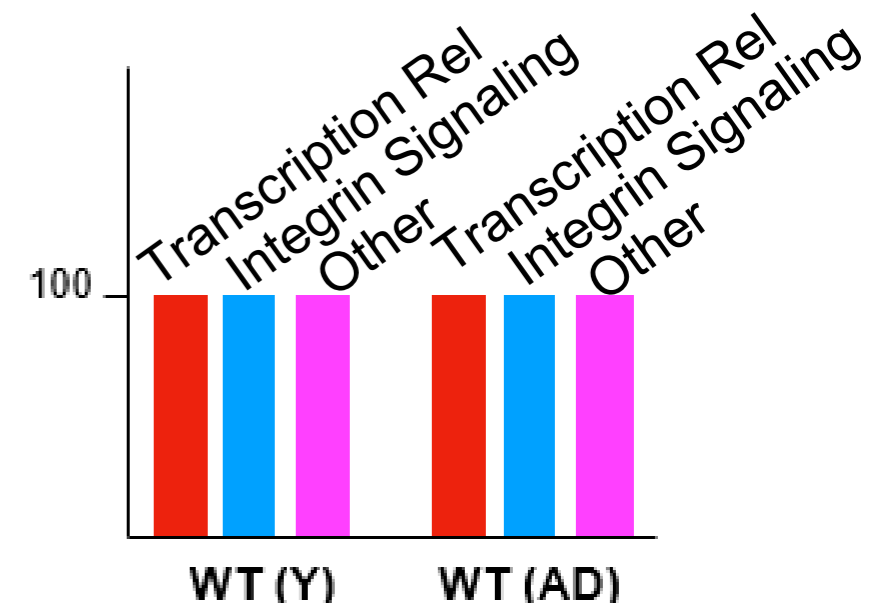
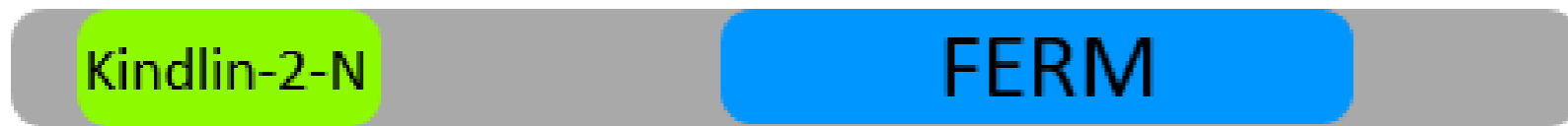


Add biotin near the N-terminus so it is in the same place for all mutants

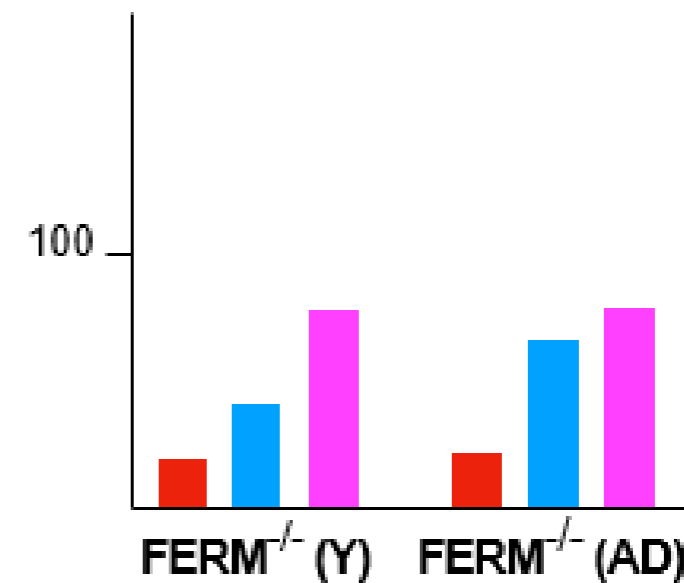
AIM3: Use BioID to track networks from young to old fish in mutants



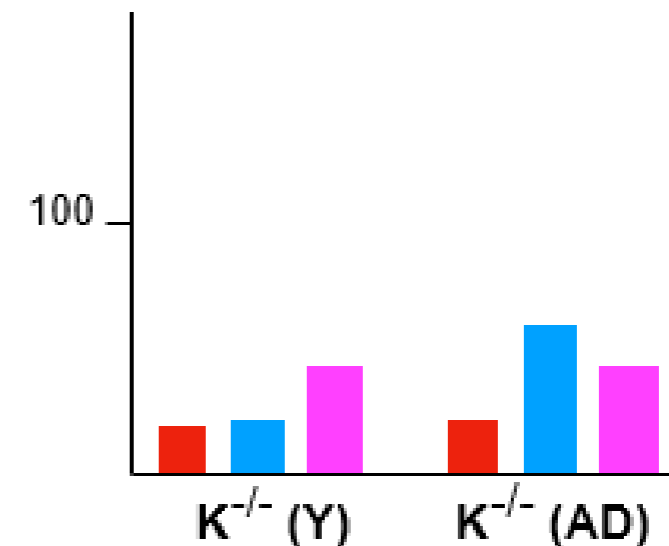
WT



FERM Mutants



Kindlin-2-N Mutants



Summary

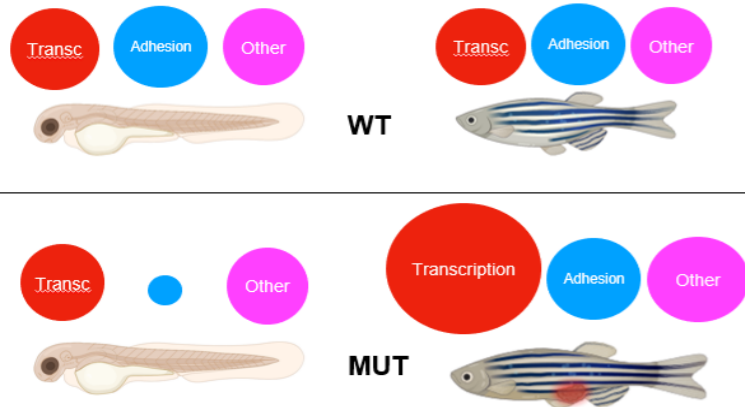
AIM 1: Analyze protein sequence for conserved sights

Protein Sequences

Species/Abbrv	Sequence
1. Homo_sapiens_(Humans)	KDQNVVWEIRRVYVIEFDQVVFAPCLSAQCKIVHVEYIIGYIFLSRSKQDNELDDELFRKLGQGG
2. Mus_musculus_(House_Mouse)	KDQNVVWEIRRVYVIEFDQVVFAPCLSAQCKIVHVEYIIGYIFLSRSKQDNELDDELFRKLGQGG
3. Danio_rerio_(Zebrafish)	KDQNVVWEIRRVYVIEFDQVVFAPCLSAQCKIVHVEYIIGYIFLSRSKQDNELDDELFRKLGQGG
4. Drosophila_melanogaster_(Fruit_Fly)	KANRVVWEIRKVMIGLQD-ELIVFVDSADCKVVHVEYIIGYIFLSRSKQDNELDDELFRKLGQGG
5. Caenorhabditis_elegans_(Worm)	KKNRVVWEIRHKLIDFED-ESIEFPLSADCKVVHVEYIIGYIFLSRSKQDNELDDELFRKLGQGG
6. Brachyopodium_distachyon_(Grass)	-----CDCTDGLCG-----PVSQKSSQGL

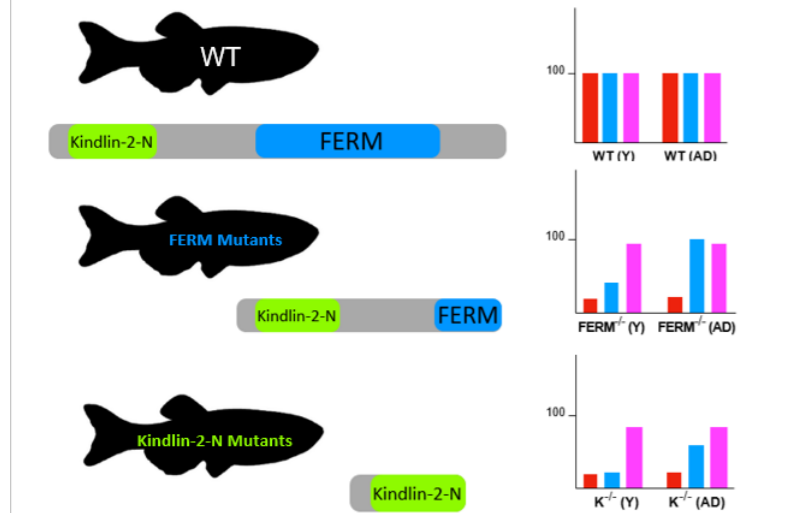
Identify highly conserved regions of the kindlin-1 to be mutated

AIM2: Identify differentially expressed genes in adult mutants



Transcription related and adhesion related gene expression will increase significantly in adult mutants

AIM3: Use BioID to track networks from young to old fish in mutants

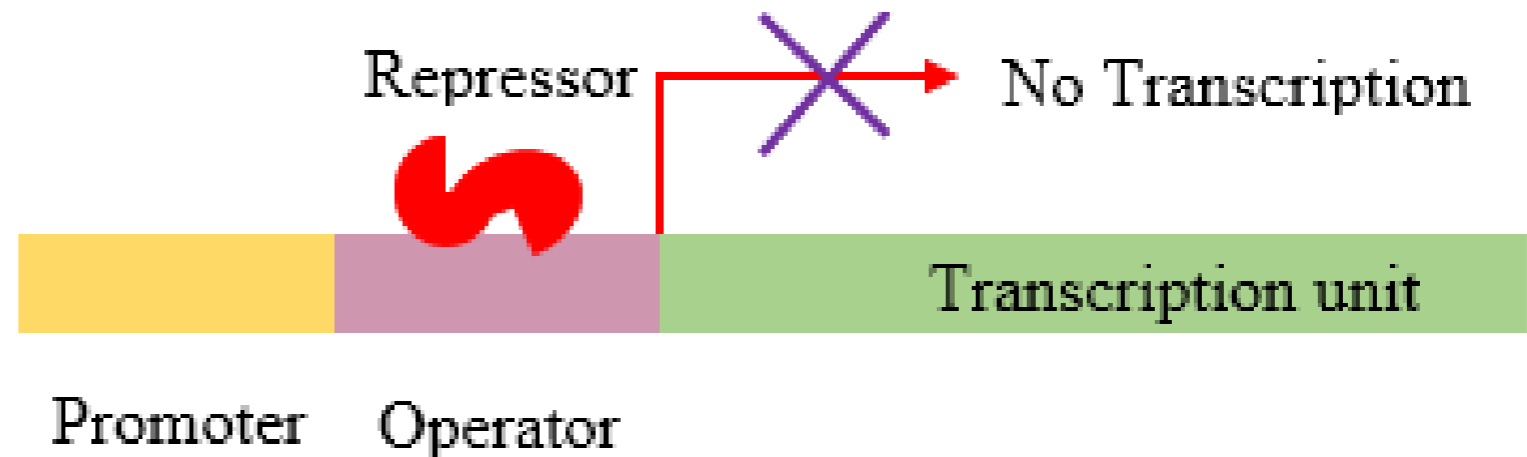


AIM1: Identify highly conserved residues of FERMT1 that maintain cell proliferation in skin in **older patients**

AIM2: Identify differentially expressed genes in **adult zebrafish mutants** versus adolescent

AIM1: Identify protein interactions of FERMT1 in **adult zebrafish mutants** versus adolescent

Future Directions



Does FERMT2 have a role in regulating cell proliferation?

FERMT2 rescues FERMT1 mutations

References

Epidermis Picture: <https://ghr.nlm.nih.gov/condition/kindler-syndrome>

Kindler Syndrome picture: <https://www.debra.org.uk/uk-funded-projects/sonnenberg-kindler-syndrome>

Kindler Knee: https://www.researchgate.net/figure/Clinical-features-of-Kindler-syndrome-a-b-Poikiloderma-with-hyperpigmentation-and_fig1_229161951

How to treat: <https://www.lybrate.com/topic/how-to-treat-kindler-s-syndrome/4dd0715e83fa2628d34f85008f42581e>

Worm: <http://haasegen564s17.weebly.com/homology.html>

Hands: <https://www.vectorstock.com/royalty-free-vector/silhouette-hand-helping-hand-vector-631062>

Kindler hands: https://link.springer.com/chapter/10.1007/978-3-662-45698-9_43

Zebrafish: https://www.google.com/url?sa=i&url=https%3A%2F%2Fgtgc2016.sciencesconf.org%2Fconference%2Fgtgc2016%2FYvesClement_2016.07.01_GTGC.pdf&psig=AOvVaw3OSzzPEah2nDhbn3ibyZrP&ust=1582740105733000&source=images&cd=vfe&ved=0CAMQjB1qFwoTCliz08Ck7ecCFQAAAAAdAAAAABBP

Kindlin-1 function: https://www.researchgate.net/figure/Molecular-mechanisms-for-integrin-activation-Integrins-exist-in-two-activation-states-on_fig4_323191947

Zebrafish embryo: <http://sitn.hms.harvard.edu/art/2014/zebrafish-embryo-development/>

Zebrafish adult: <http://www.sleepreviewmag.com/2019/10/zebrafish-study-sheds-light-sleep-regulated-brain/>

Squamous Cell Carcinoma: <https://www.sciencedirect.com/science/article/pii/S1507136716300517>