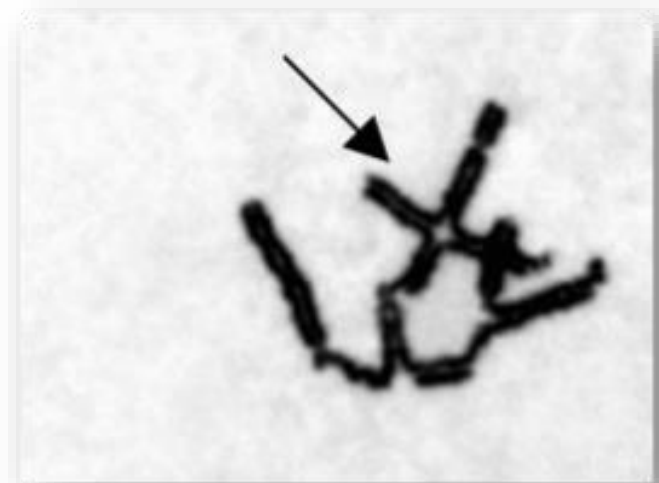




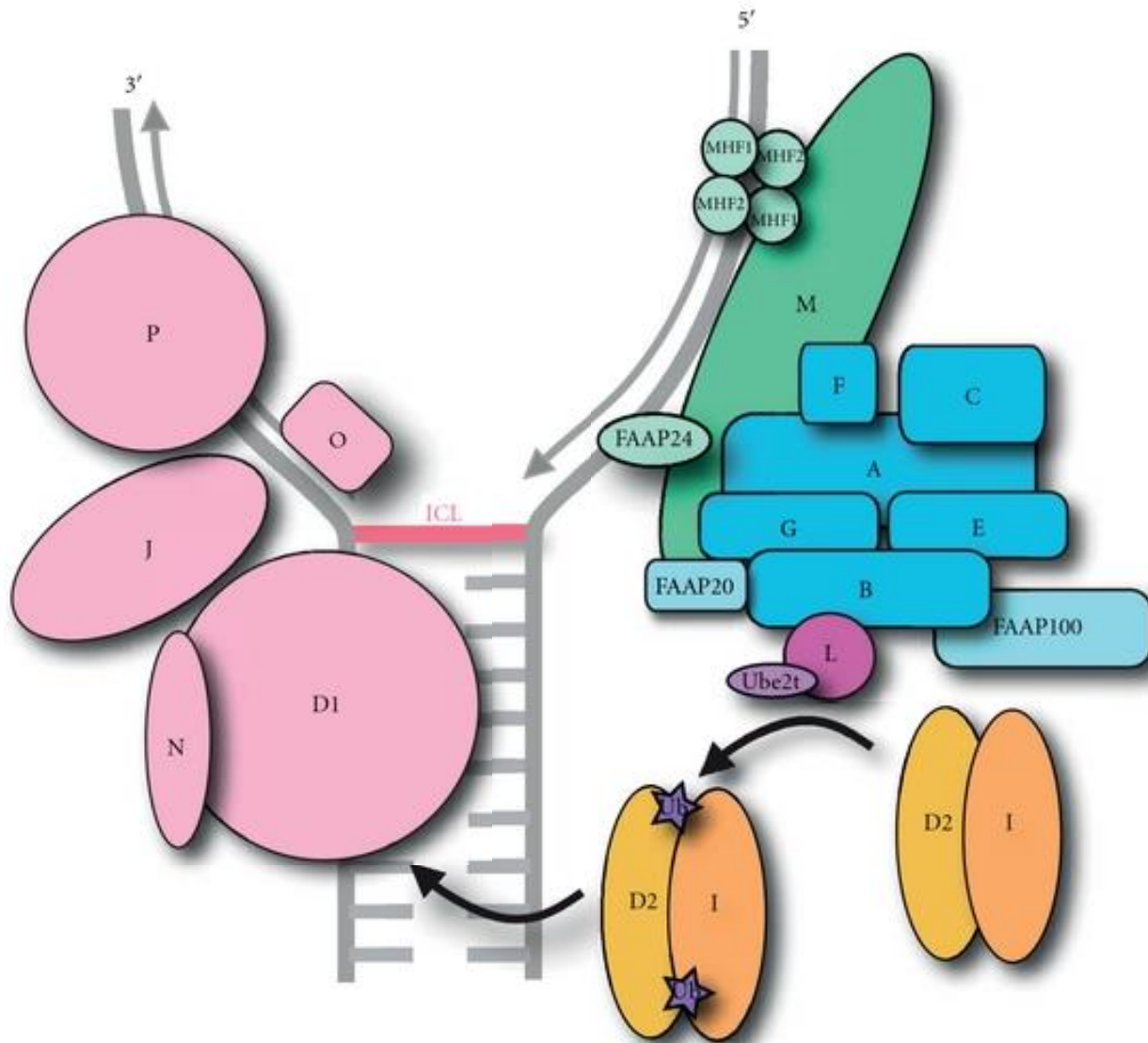
Fanconi Anemia

Anthony Winchell

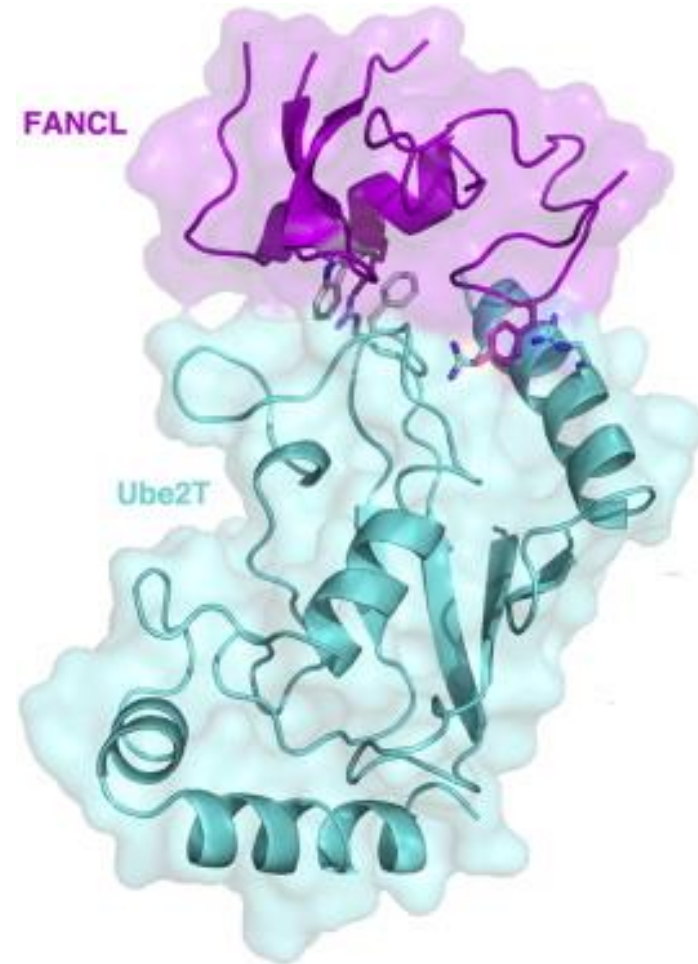
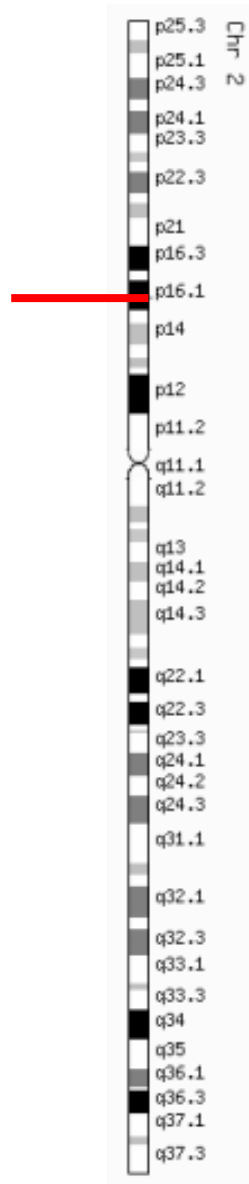
What is Fanconi Anemia?



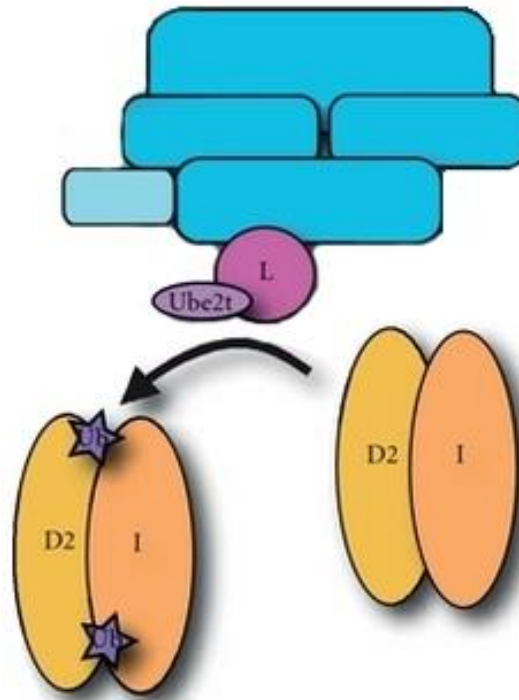
What is the Fanconi Anemia pathway?



What is Fanconi Anemia Complementation Group L?



What role does FANCL play?



Cellular Components

Fanconi Anemia nuclear complex

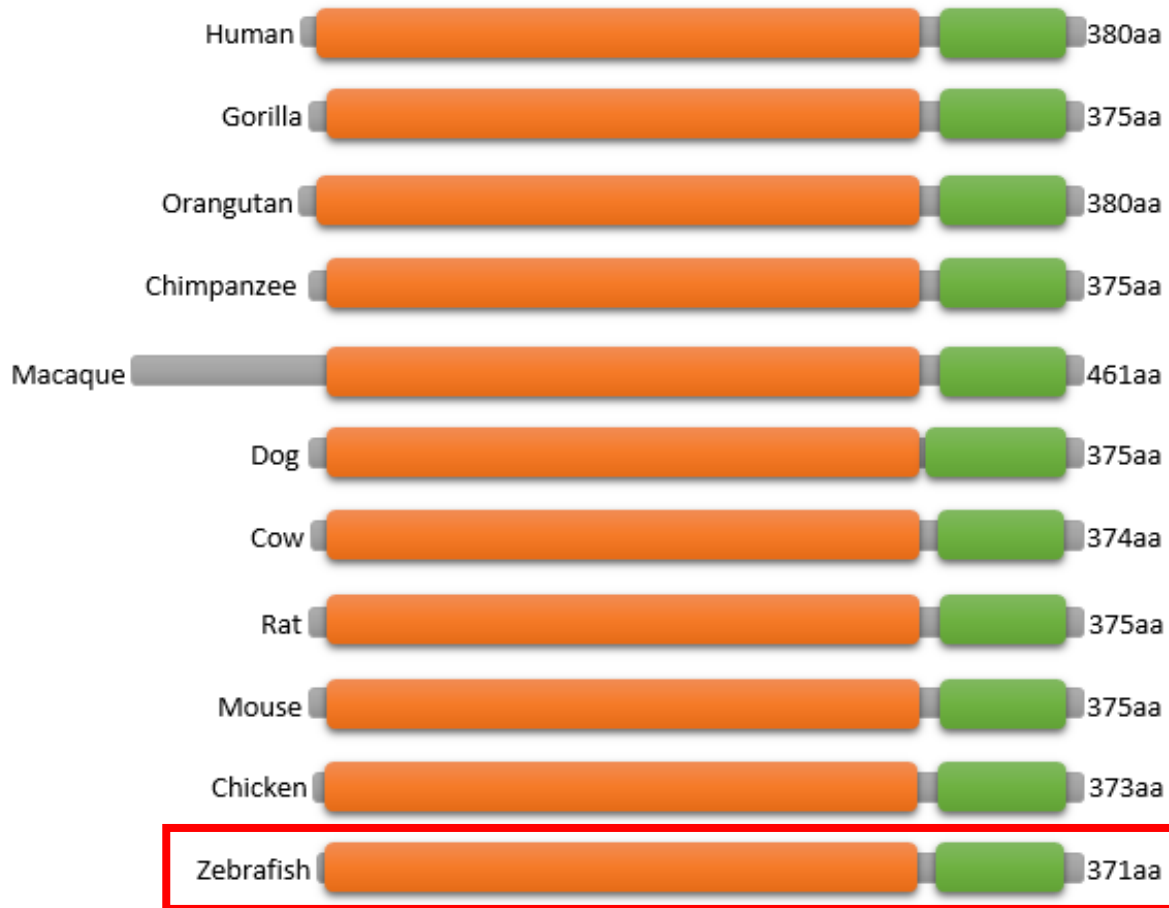
Molecular Function

Ubiquitin-protein transferase activity

Biological Processes

Protein ubiquitination
DNA Repair

How well conserved is FANCL?



WD Repeat Domain



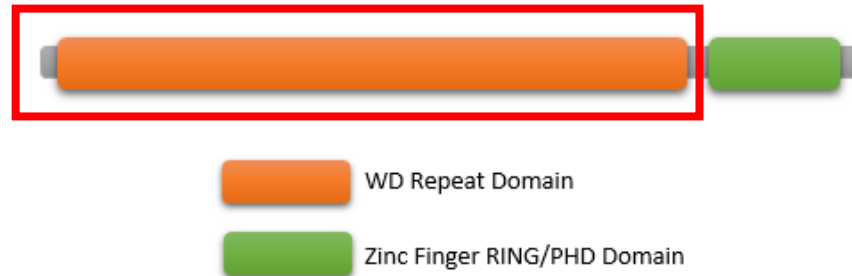
Zinc Finger RING/PHD Domain

Knowledge Gap : Why do FANCL-caused FA patients have such varying phenotypes?



Understand how different mutations affect phenotype and ID complex ubiquitination.

Hypothesis



Mutations occurring in the WD40 domain will have a greater effect on phenotype than mutations to the RING finger domain.

Aim I

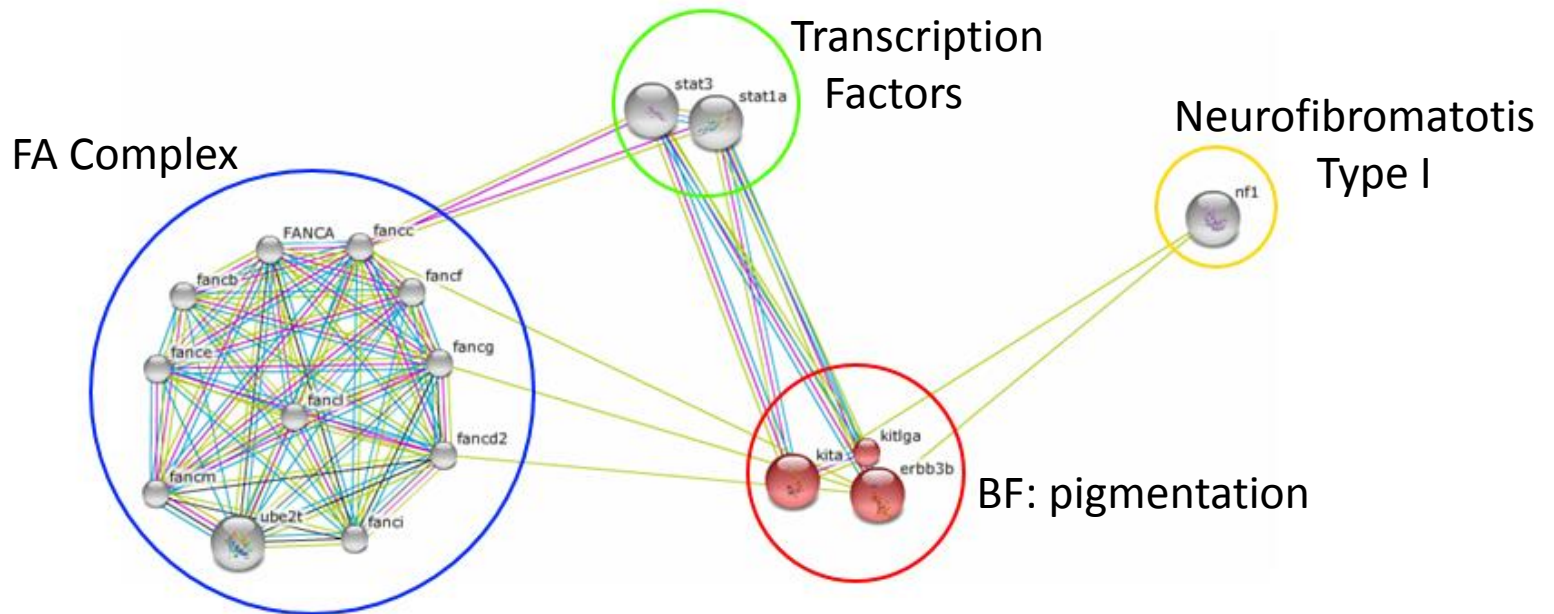
Aim II

Aim III

**Identify binding partners of FANCL
that have a role in skin pigmentation**

Aim I: Results

kita identified as key pigmentation gene



Aim I

Aim II

Aim III

Determine which domain plays a more prominent role in pigmentation

Aim II: Approach

Identify conserved regions in the WD40 and RING domains.

WD-Repeat Domain

	200	210	220	230	240	250	260
<i>Zebrafish/1-371</i>	YYSQLITEMEILG	WDKLLFIDAEFRTLQLKAEDSAGRQHAITVKLKS	KYPAEAEF	SADLP	IPLVIT	WT	
<i>Chicken/1-373</i>	YYSCLIRDLEILG	WNVKVAIVDTGLTTVKLKAEDSRGRQHLITLKLNAKYPTEPPDCVVDFFVQFAISW					
<i>Brown_Rat/1-375</i>	FCKDLLTEIGAIG	WDLKACVDTSFTTIKLLKADDASGRKHLITVKL	KAKYPVEPPDCLVDFPIPF	SVSWT			
<i>House_Mouse/1-375</i>	FCKDLLTEIGAIG	WDLKACVVESSFSTIKLLKADDASGRKHLITVKL	KAKYPVEPPDCVVDFFVPPF	SVSWT			
<i>Cattle/1-374</i>	FYSSLIGEIRTLG	WDLKLVFVDPCFSTIKLKA-DVSGREHLITVKL	KAKYPAESPDCVVDFFVPPF	SVSWT			
<i>Dog/1-375</i>	FYSSLMEEIGTLG	WDLKLVYVDTCLSTIKLKAEDASGRKHLITLKL	KTKYPTESPDCVVDFFVPSF	SVSWT			
<i>Macaque/1-461</i>	FYSSLIEEIGTLG	WDLRVYVDTCFSTIKLKAEDASGREHLITLKL	KAKYPAESPDCFVDFVPPF	SASWT			
<i>Orangutan/1-380</i>	FYSSLIEEIGTLG	WDLKLVYADTCFSTIKLKAEDASGREHLITLKL	KAKYPAESPDYFVDFVPPF	CASWT			
<i>Chimpanzee/1-375</i>	FYSSLIEEIGTLG	WDLKLVYADTCFSTIKLKAEDASGREHLITLKL	KAKYPAESPDYFVDFVPPF	CASRT			
<i>Human/1-380</i>	FYSSLIEEIGTLG	WDLKLVYADTCFSTIKLKAEDASGREHLITLKL	KAKYPAESPDYFVDFVPPF	CASWT			
<i>Gorilla/1-375</i>	FYSSLIEEIGTLG	WDLKLVYADTCFSTIKLKAEDASGREHLITLKL	KAKYPAESPDYFVDFVPPF	CASWT			

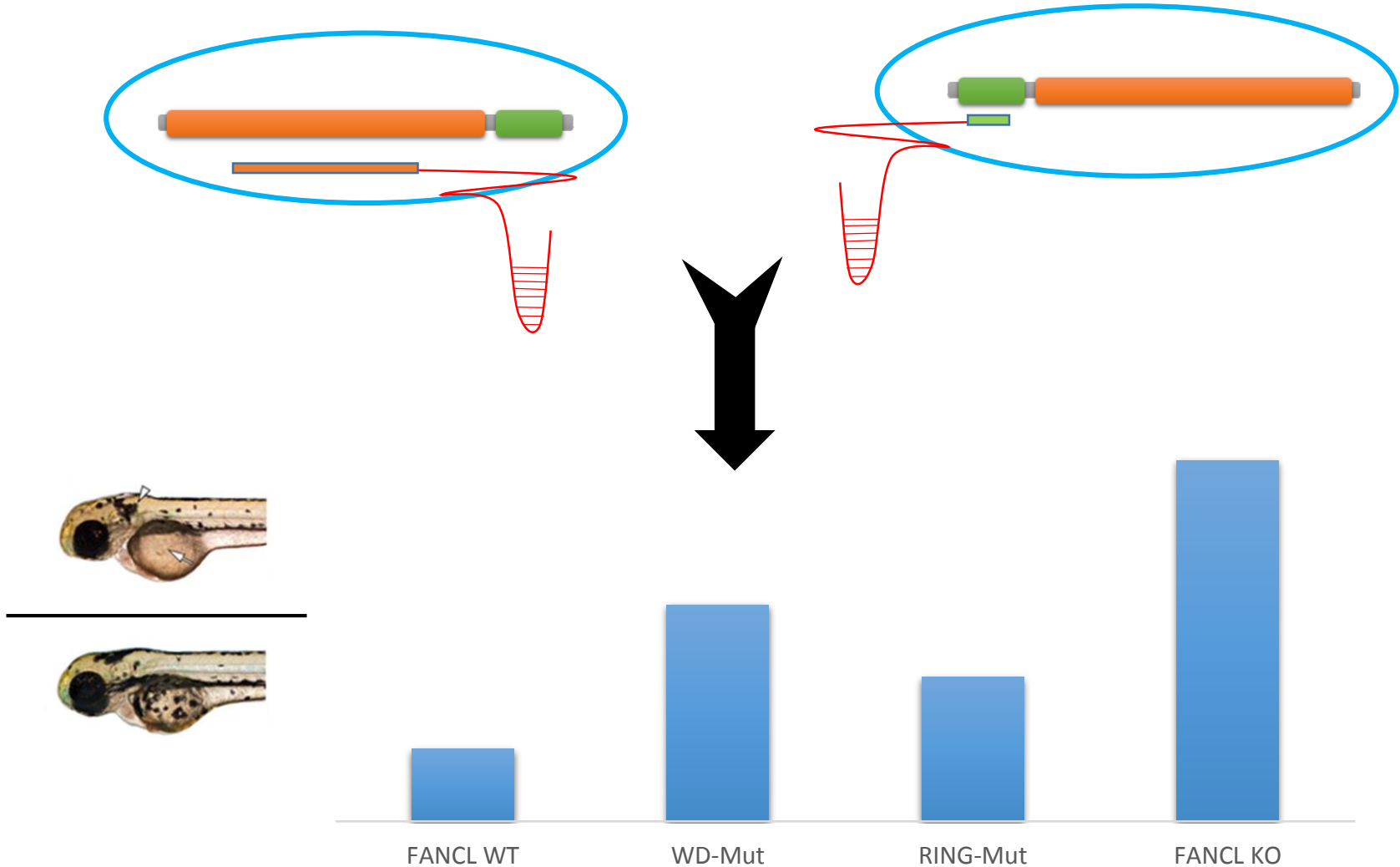
RING Domain

	400	410	420	430	440	450	460
<i>Zebrafish/1-371</i>	FSVECGICYSYRLESAIPDQVCNDPRCGQPFHQVCLYE	WLRGLP	TSRQSFNIVFG	ECPYCSKPI	TVK	MV	
<i>Chicken/1-373</i>	FTKDCGICAYARLNGTTPDQVCNEPRCGQPFHQACLYE	WLRGLP	SSRQSFNVIFG	ECPYCNKPL	TVK	SS	
<i>Brown_Rat/1-375</i>	FSMDCGICYARHLNGAIPDQVCNPNPQCGQLFHQMCLYE	WLRGLS	SSRQSFNVFFG	ECPYCSKPI	TLK	MS	
<i>House_Mouse/1-375</i>	FSMDCGICYARHLNGAIPDQVCNPNPQCGQPFHEICLYE	WLRGLS	TSRQSFNVFFG	DCPYCSKPI	TLK	MS	
<i>Cattle/1-374</i>	FSMDCGICYAYQLDGAIPDQVCDNSQCGQSFHYICLYE	WLRGLL	TSRQSFNIFG	ECPYCSKPI	TLK	MS	
<i>Dog/1-375</i>	FSMDCGICYAYQLDGAIPDQVCDNLQCGQPFHQICLYE	WVRGLL	TSRQSFNIFG	ECPYCSKPI	TLK	MS	
<i>Macaque/1-461</i>	FTMDCGICYAYQLDGAIPDQVCDNSQCEQPFHQICLYE	WLRGLL	TSRQSFNIFG	ECPYCSKPI	TLK	MS	
<i>Orangutan/1-380</i>	FTMDCGICYAYQLDGTIPDQVCDNSQCGQPFHQICLYE	WLRGLL	TSRQSFNIFG	ECPYCSKPI	TLK	MS	
<i>Chimpanzee/1-375</i>	FTMDCGICYAYQLDGTIPDQVCDNSQCGQPFHQICLYE	WLRGLL	TSRQSFNIFG	ECPYCSKPI	TLK	MS	
<i>Human/1-380</i>	FTMDCGICYAYQLDGTIPDQVCDNSQCGQPFHQICLYE	WLRGLL	TSRQSFNIFG	ECPYCSKPI	TLK	MS	
<i>Gorilla/1-375</i>	FTMDCGICYAYQLDGTIPDQVCDNSQCGQPFHQICLYE	WLRGLL	TSRQSFNIFG	ECPYCSKPI	TLK	MS	

Utilize the CRISPER/Cas9 system to induce site specific mutations

Aim II: Expected Results

WD40-mutant transgenic lines will have more mutant Zebrafish



Aim I

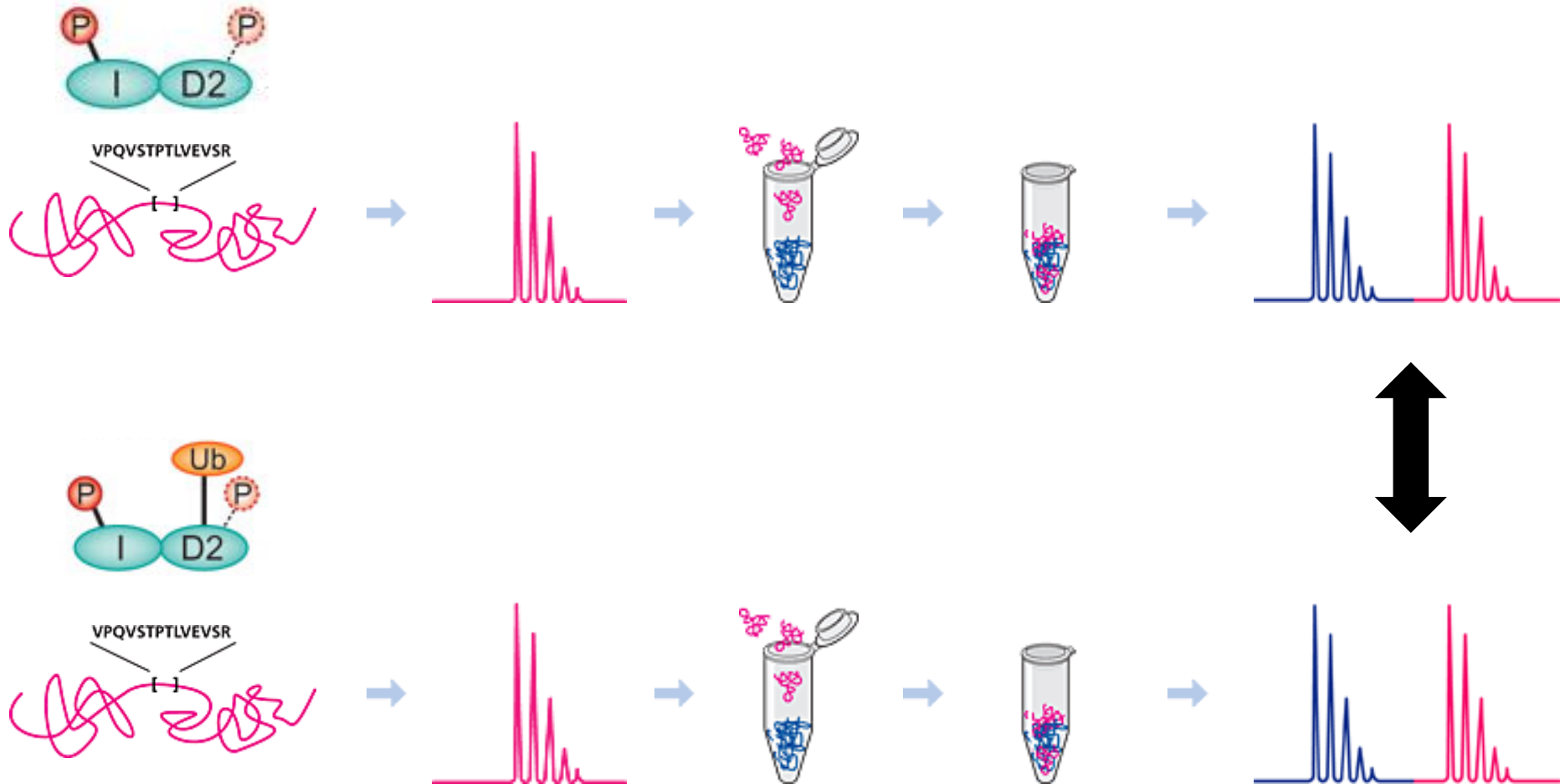
Aim II

Aim III

**Quantify the level of ubiquitination
in each transgenic line**

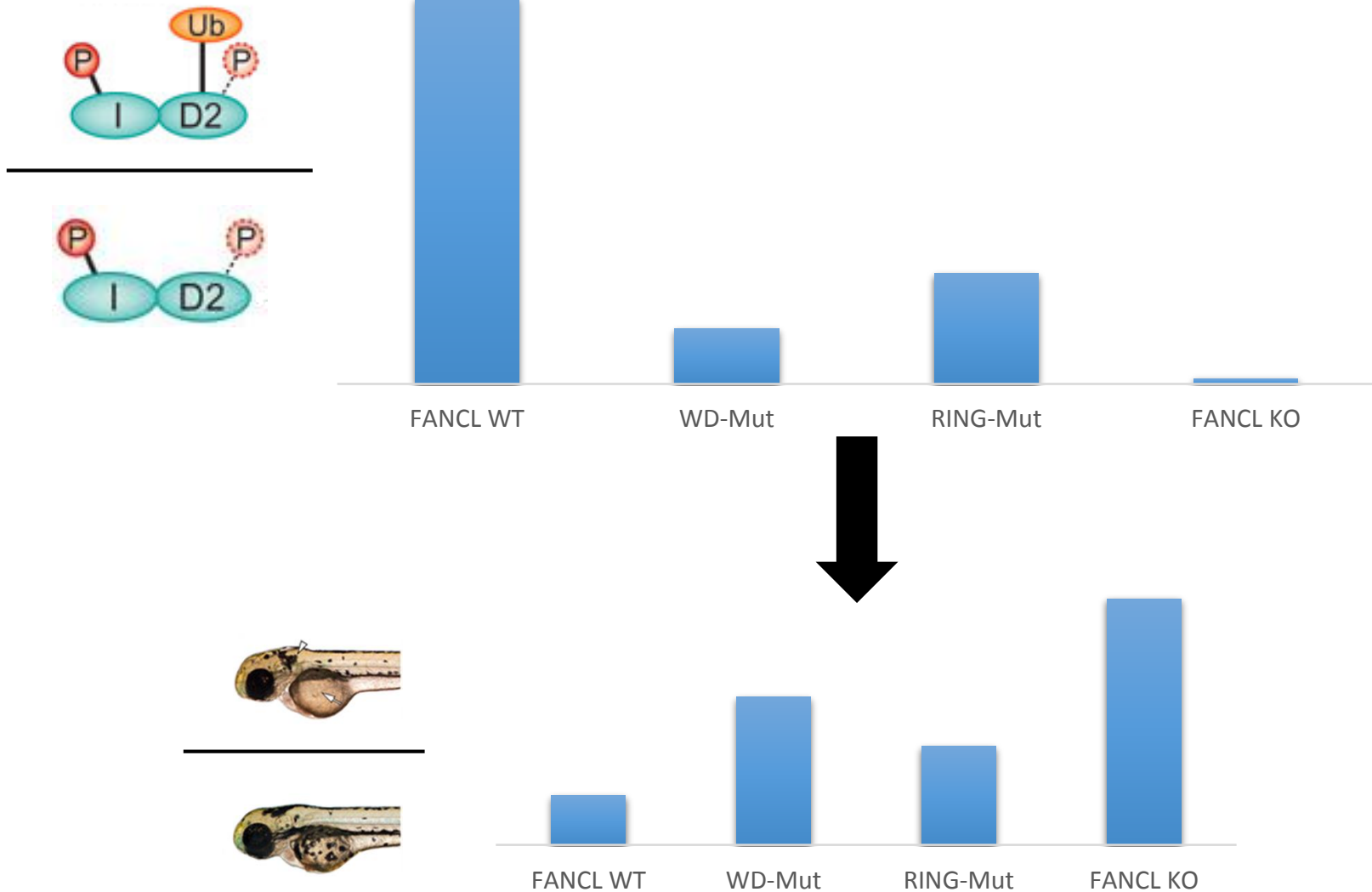
Aim III: Approach

Utilize quantitative mass spec to quantify levels of ubiquitination



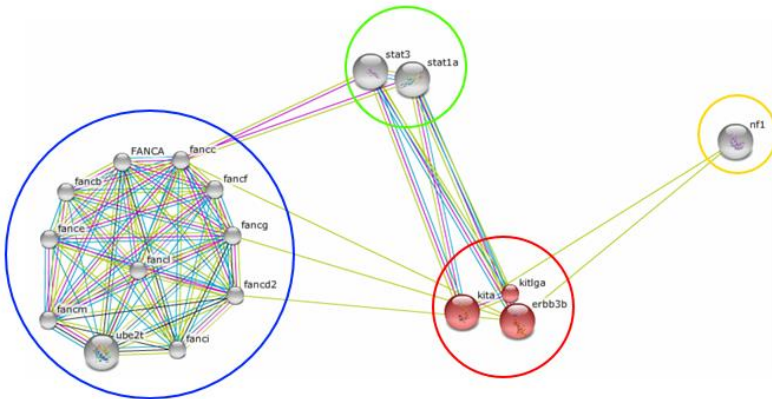
Aim III: Expected Results

WD40-mutant transgenic lines will have reduced ubiquitination

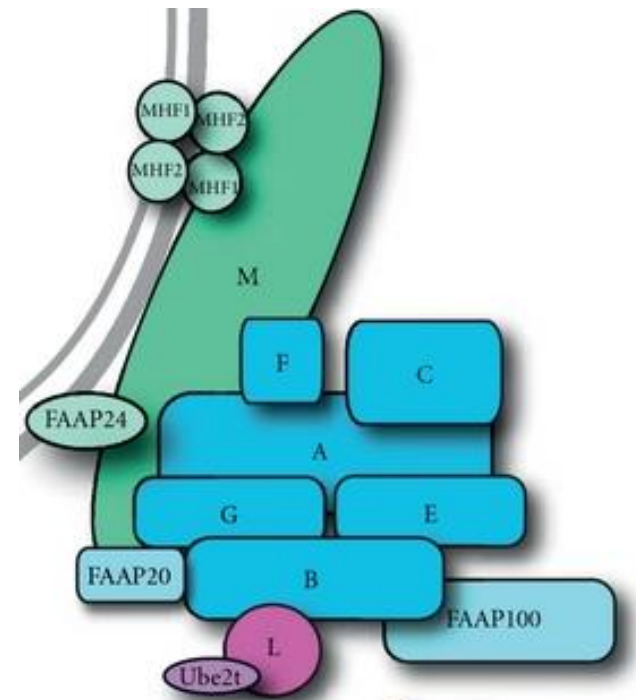


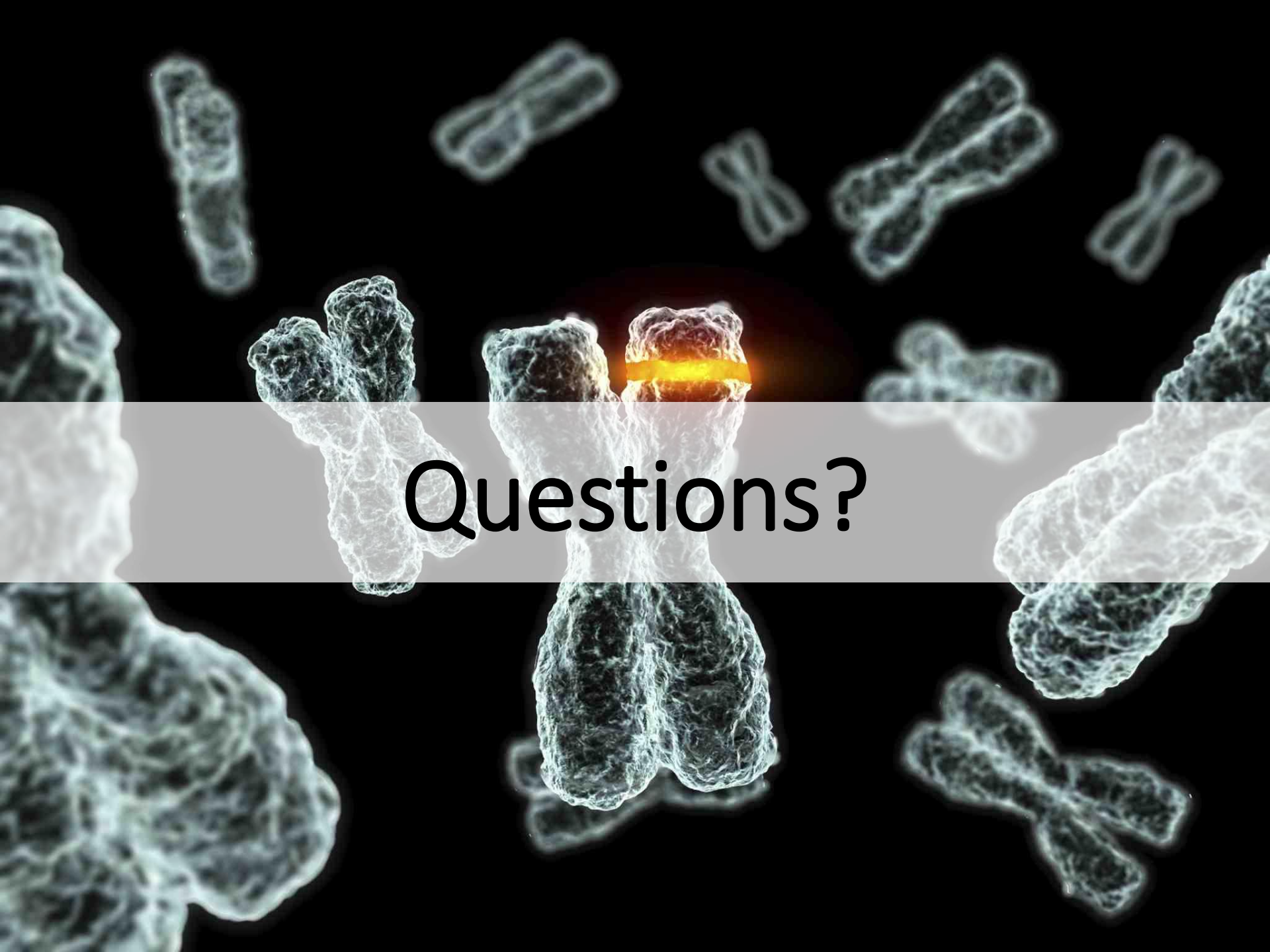
Future Directions

Relationship between FA,
KIT, and pigmentation



Binding between core
complex proteins





Questions?