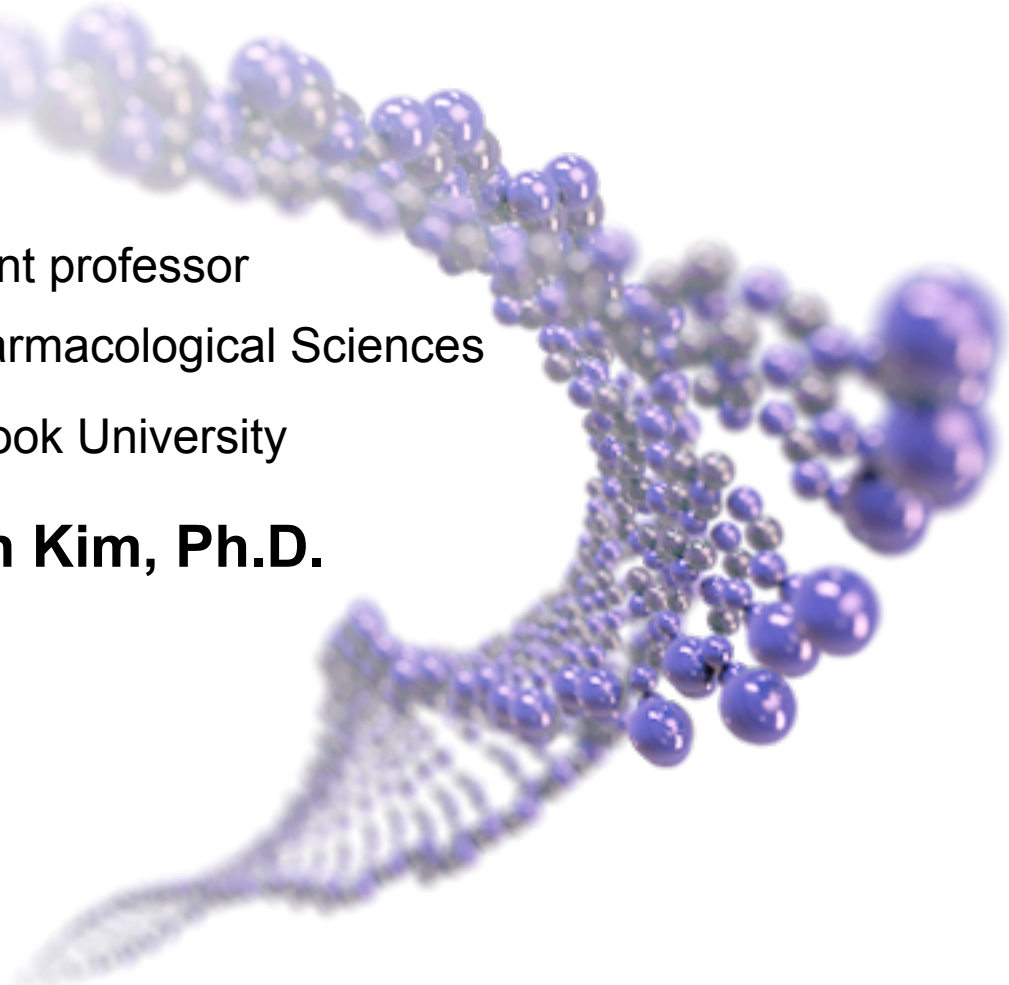
Stony Brook Medicine

Posttranslational Regulation of the Fanconi Anemia Cancer Susceptibility Pathway



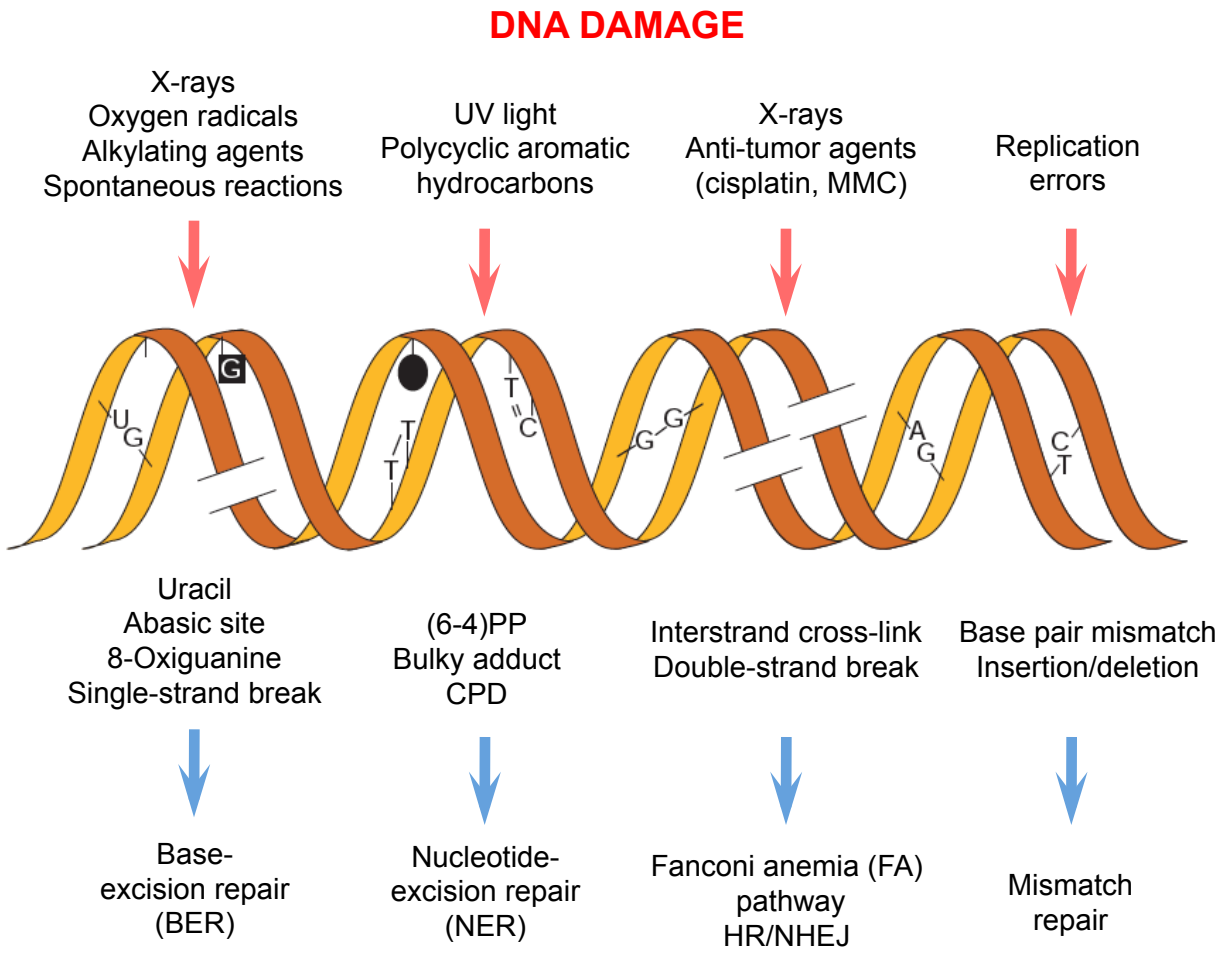
Assistant professor

Department of Pharmacological Sciences

Stony Brook University

Hyungjin Kim, Ph.D.

DNA Repair System Preserves Genome Stability



DNA DAMAGE RESPONSE

Cell-cycle arrest

Cell death

DNA REPAIR DEFECT

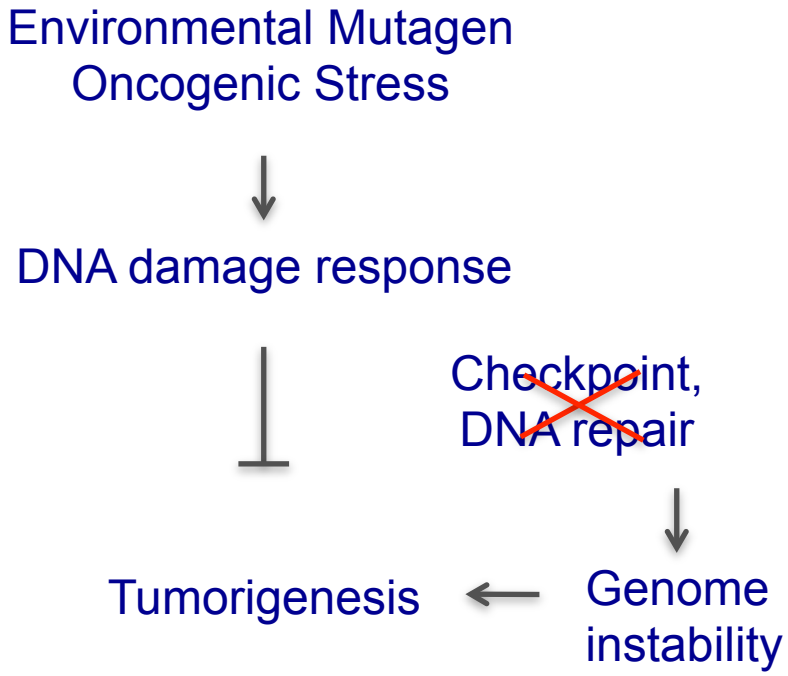
Cancer

- Mutations
- Chromosome aberrations

Adapted from Hoeijmakers (2001) Nature

DNA Repair Mechanism as a Tumor Suppressor Network

Cancer is a disease of DNA repair



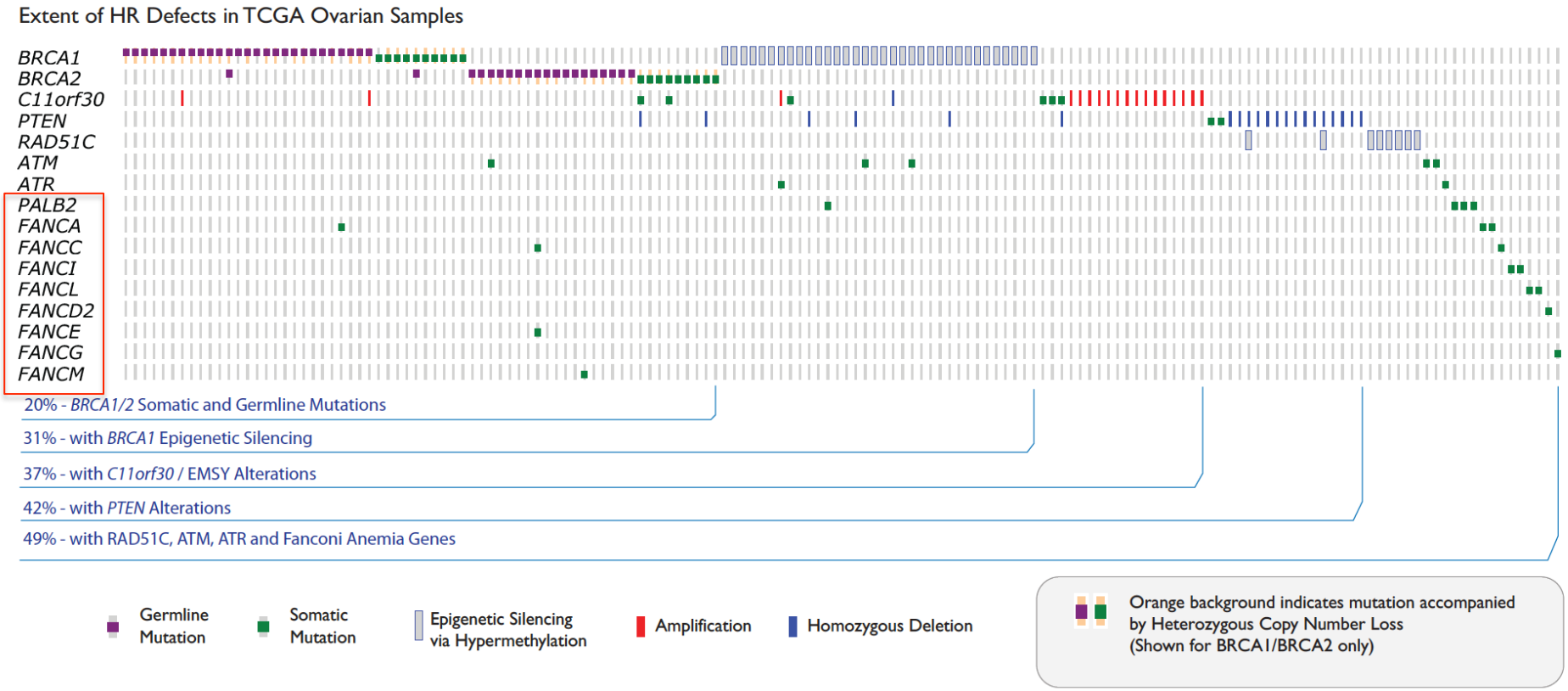
- *Hereditary* cancers: mutations in DNA repair genes (*BRCA1*, *BRCA2*, *MSH2*, etc)

- *Sporadic* cancers:
  - Oncogene-induced replication stress
  - Inaccurate DNA repair caused by selection of somatic mutations in DNA repair genes

Germ-line and Somatic Disruption of DNA Repair Is Prevalent in Cancer

The Cancer Genome Atlas (TCGA):

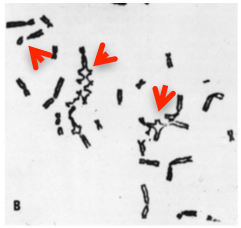
Genomic characterization and sequence analysis of various tumors from patient samples



TCGA network, (2011) Nature

Clinical and Cellular Features of Fanconi Anemia (FA)

Fanconi Anemia



Chromosome instability syndrome

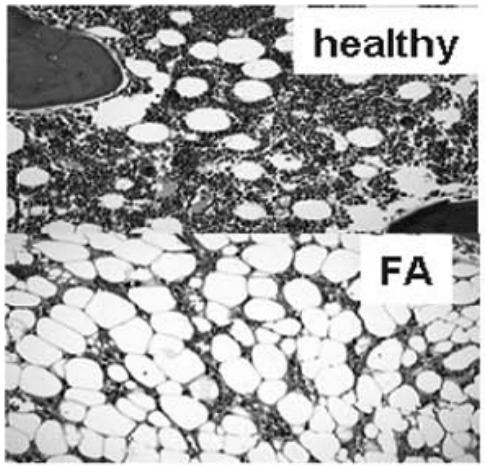
Mutation in 17 genes that cooperate in DNA interstrand cross-link (ICL) repair
i.e. the FA pathway

Disease of defective DNA repair

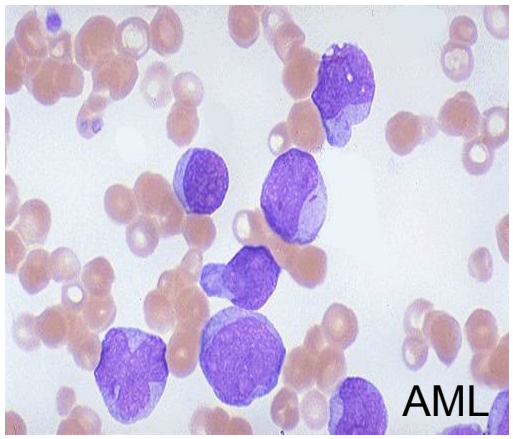
Model for cancer pathogenesis

Developmental defect

Bone marrow failure



Increased cancer risks

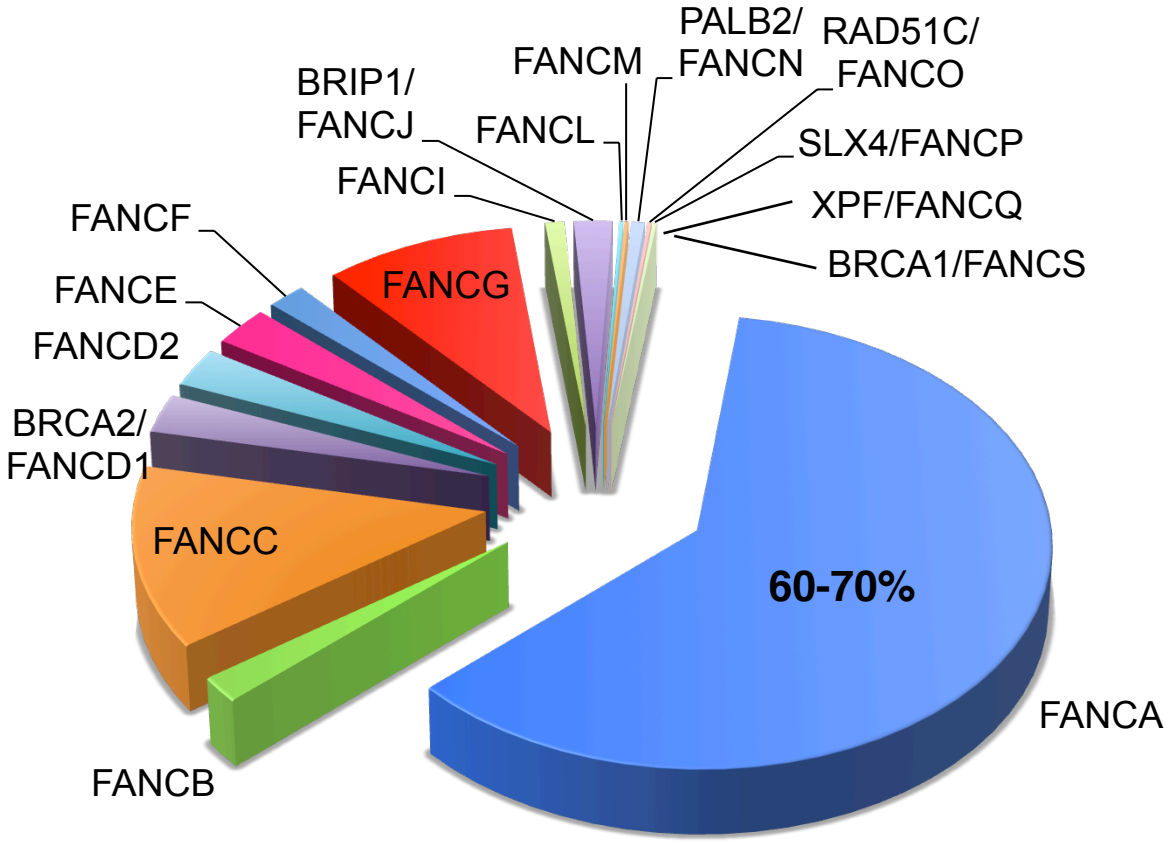


The Seventeen Fanconi Anemia Genes in the FA/BRCA Pathway

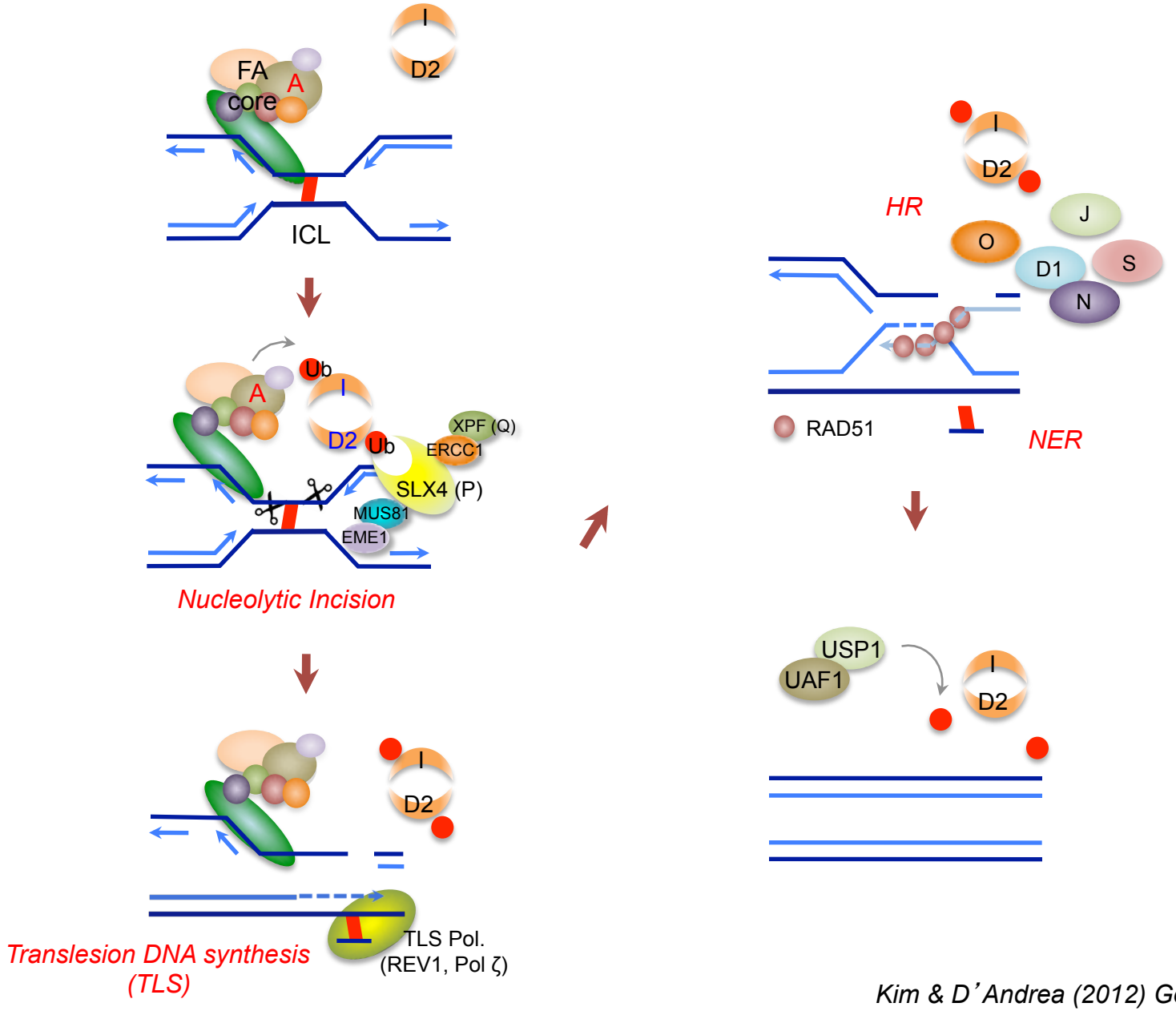
17 FANC genes
(Complementation group)

FANCA
FANCB
FANCC
FANCD1
FANCD2
FANCE
FANCF
FANCG
FANCI
FANCJ
FANCL
FANCM
FANCN
FANCO
FANCP
FANCQ
FANCS

Frequencies of FA complementation group mutations

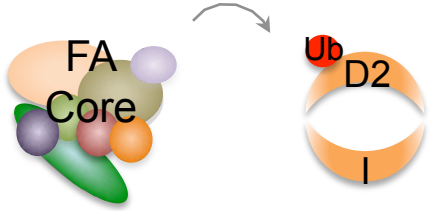


The FA Pathway Regulates Interstrand Cross-link (ICL) Repair

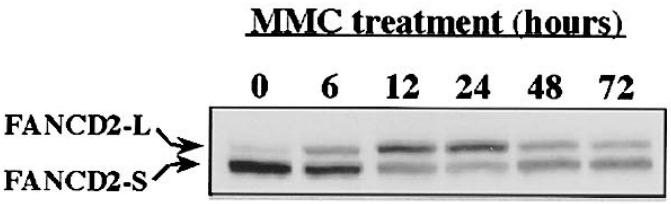


Kim & D'Andrea (2012) Genes & Dev

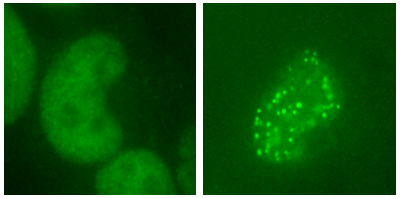
Regulation of FANCD2 Mono- and De-Ubiquitination



FANCD2-L: FANCD2-Ub



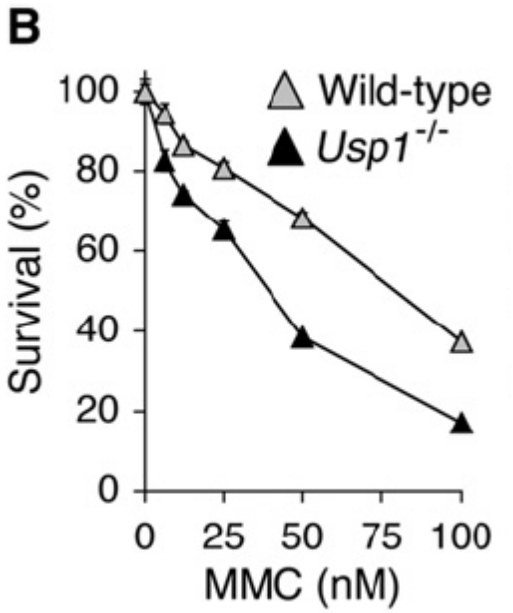
+ damage



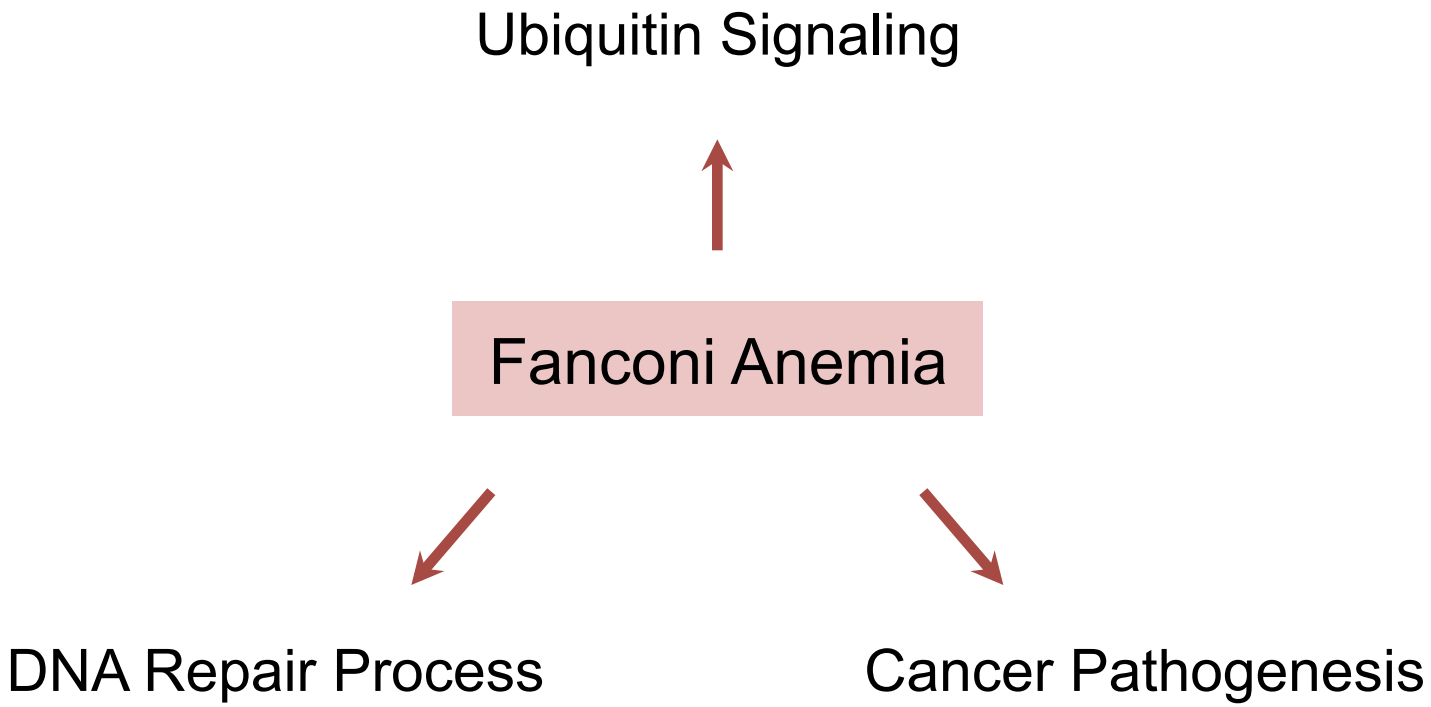
α FANCD2 IF

Garcia-Higuera et al., (2001) Mol. Cell

USP1: Deubiquitinating enzyme

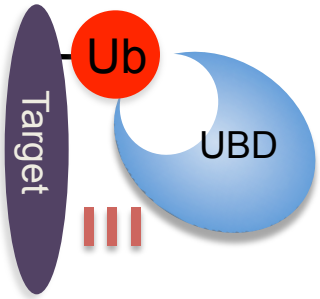


Kim et al., (2009) Dev. Cell

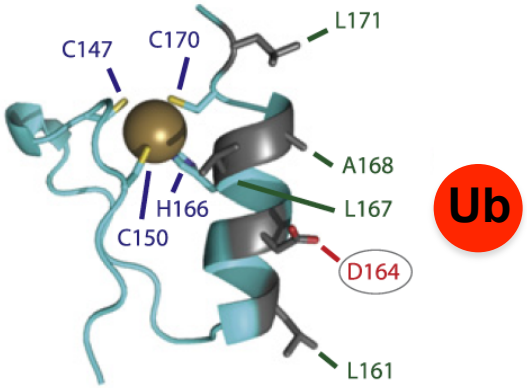

Ubiquitin Signaling
Fanconi Anemia
DNA Repair Process
Cancer Pathogenesis

Bioinformatic Search of UBZ4-Containing Proteins

UBD: Ubiquitin Binding Domain



UBZ4 (Ub-Binding Zn-finger 4)



UBZ4 domain from TLS polymerase κ

QILTCPVCFRAQGCISLEALNKHVDECLDGPS

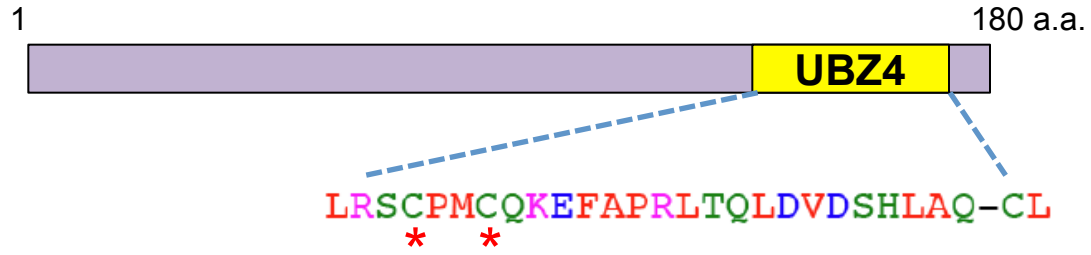
Hidden Markov Model (HMM)

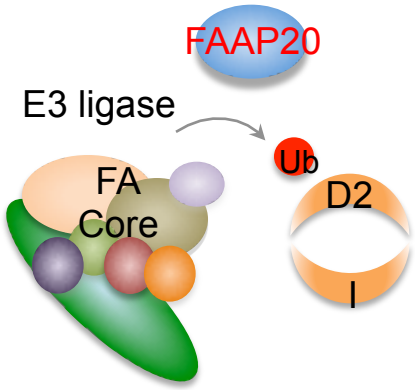
```
Polκ-1    ILTCPVCFRAQG-CISLEALNKHVDE-CL--
Polκ-2    ALVCPVCNVEQK-TSDLTLFNVHVDV-CL--
RAD18     KVDCPVCGVN----IPESHINKHLDS-CL--
SLX4-1    LFFCQICQKNLS-AMNVTRREQHVNR-CL--
SLX4-2    IPECPICGKPF---LTLKSRTSHLKQ-CA--
FAN1      KLACPVCSKM----VPRYDLNRHLDEMCANN
RAP80     QVSCPLCD-Q---CFPPTKIERHAMY-CN--
SNM1A     DGYCPNCQMPFS-SLIGQTPRWHVFE-CL--
UBZ4 #1   PFRCPNCGQRF---ETENLVVEHMSS-CL--
UBZ4 #2   -LHCPWCTLN---CRKLYSLLKHLKL-CH--
UBZ4 #3   LRSCPMCQKEFAPRLTQLDVDSHLAQ-CL-- FAAP20
              *   *           *     *
```

Predominantly found in DNA repair factors

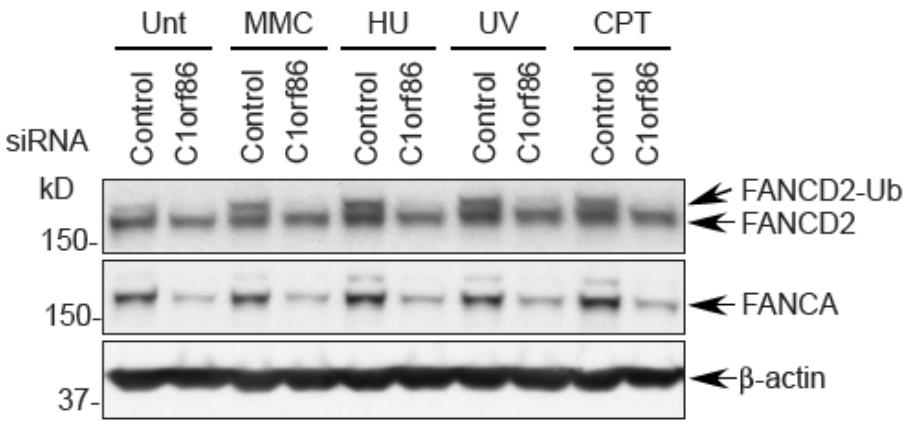
FAAP20 Regulates FANCD2 Activation in the FA Pathway

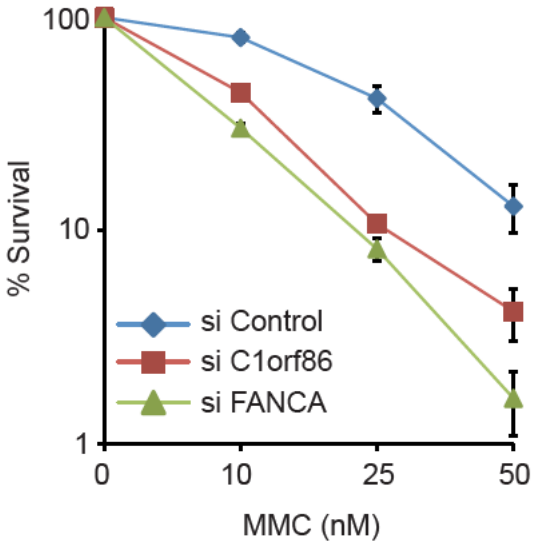
FAAP20 (C1orf86): Fanconi anemia-associated protein 20 kD



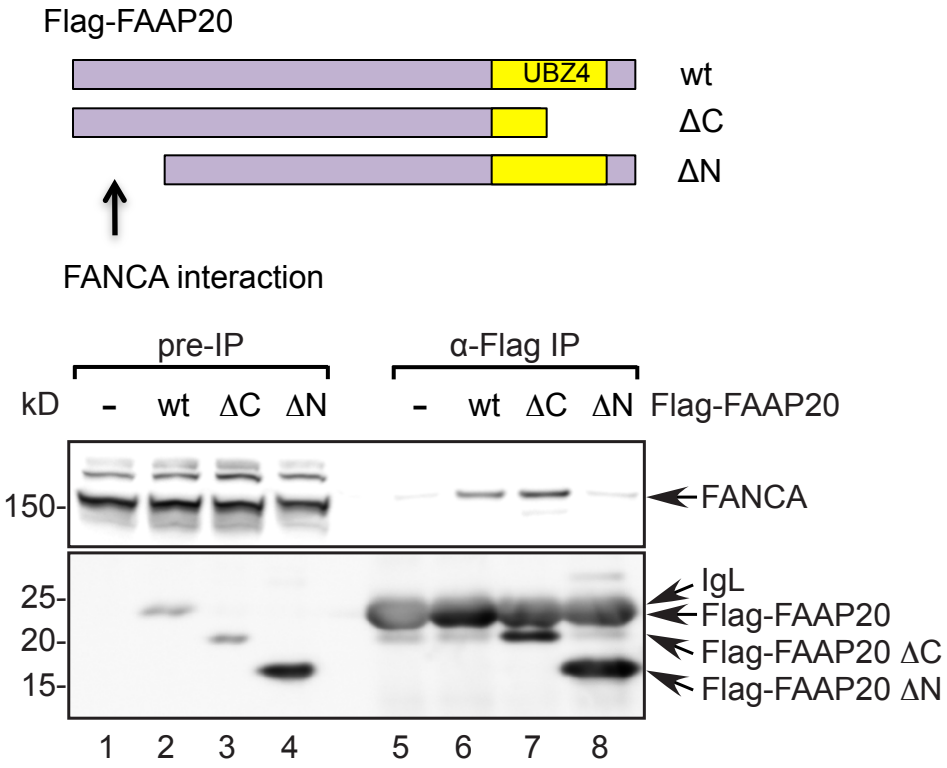


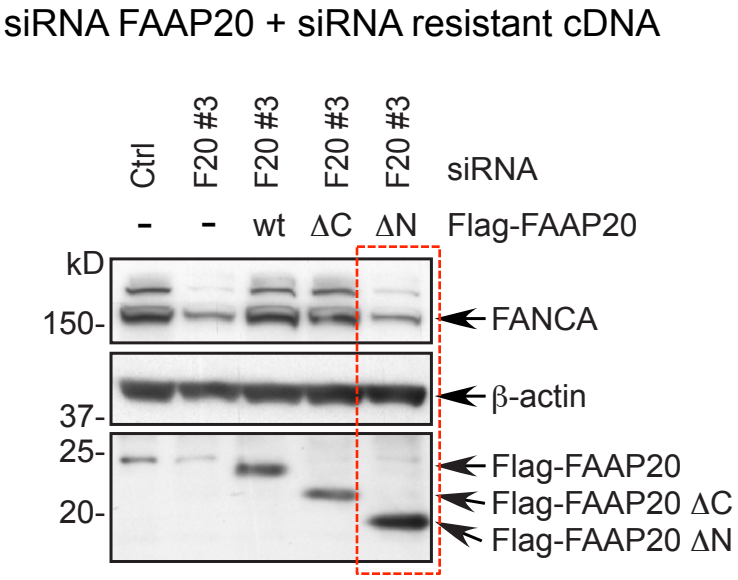
FANCA: FAAP20



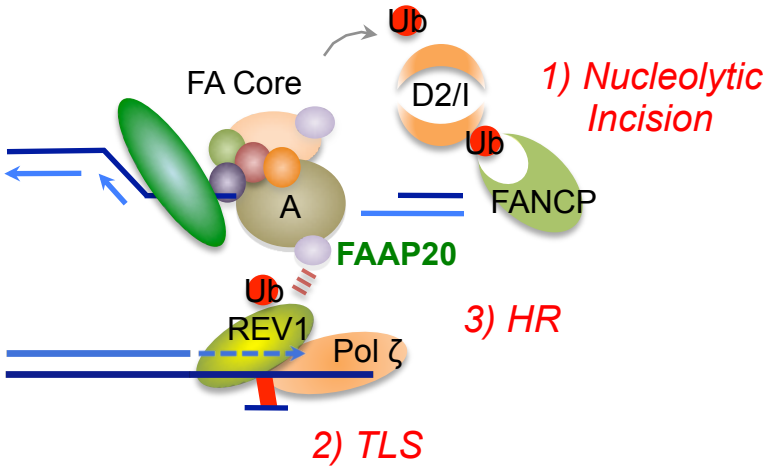


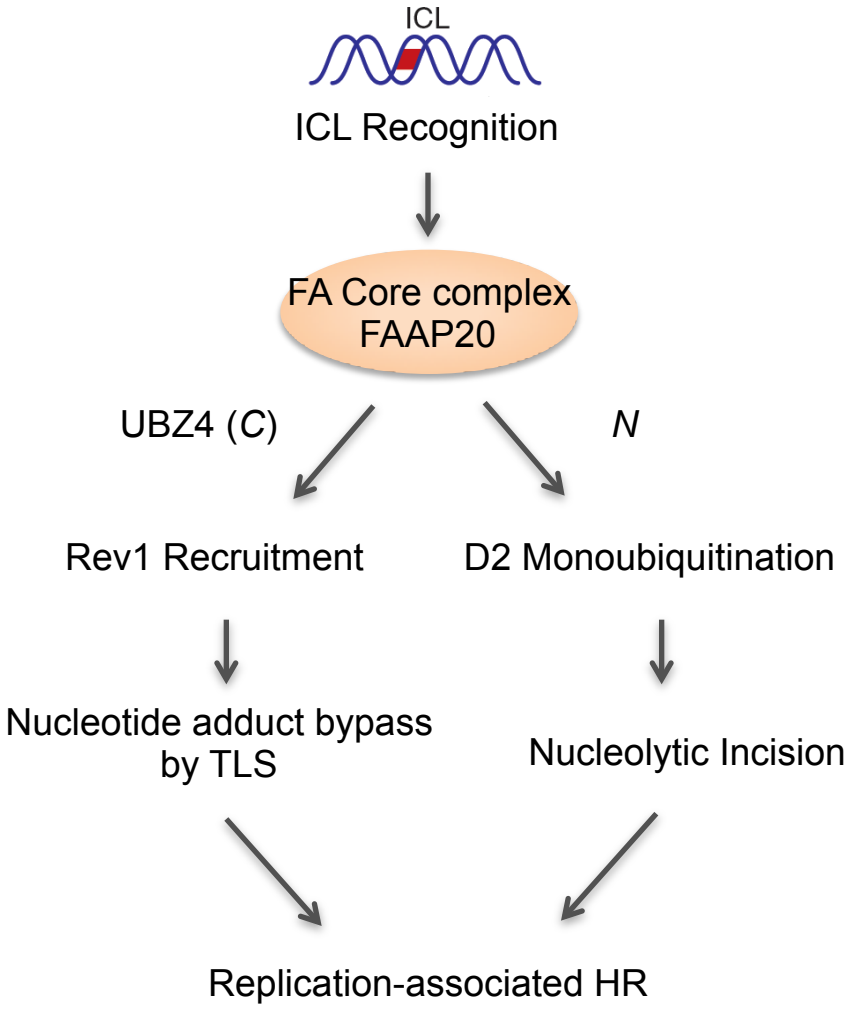
The FAAP20-FANCA Interaction Is Required for Maintaining FANCA Level





FAAP20 Connects the FA Pathway with Translesion DNA Synthesis

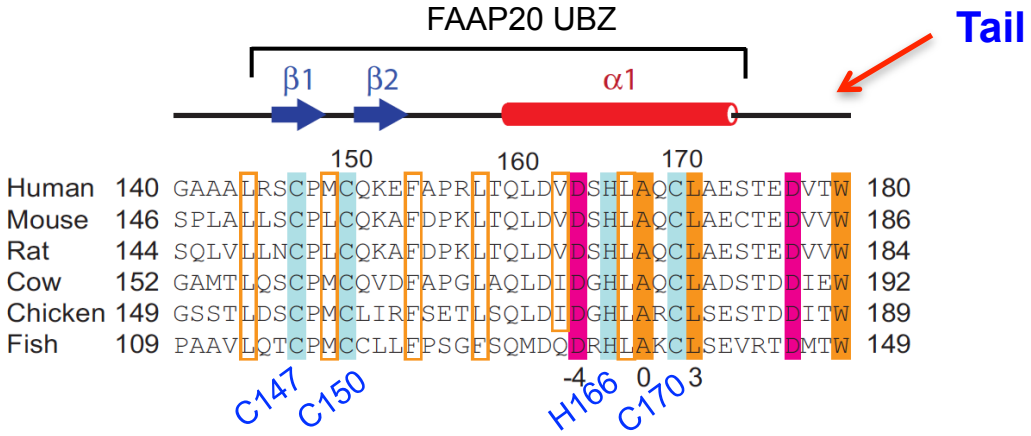


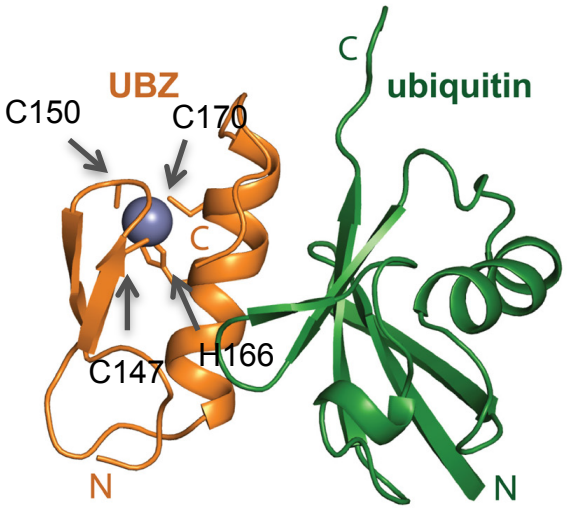


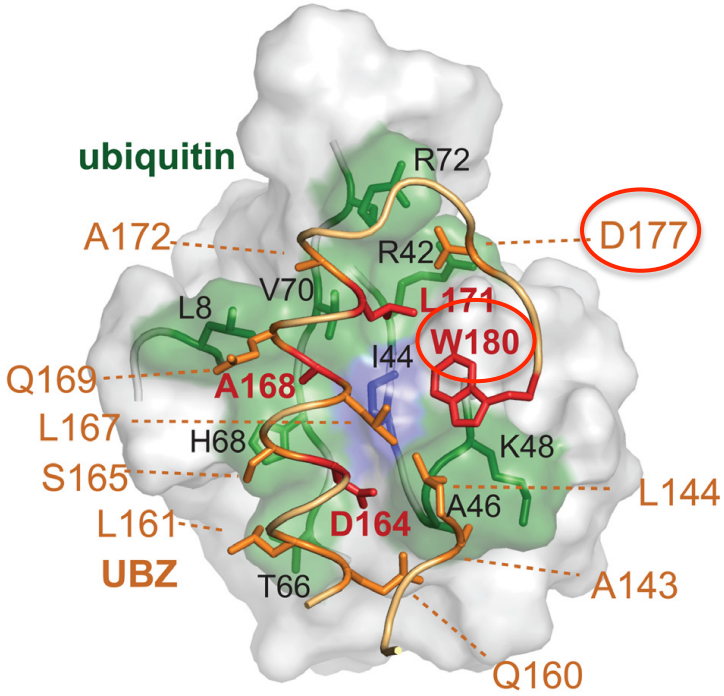
Kim et al., (2012) Nat. Struc. Mol. Biol.

Solution Structure of the FAAP20-Ubiquitin Complex

The disordered C-terminal tail of FAAP20 is involved in ubiquitin binding



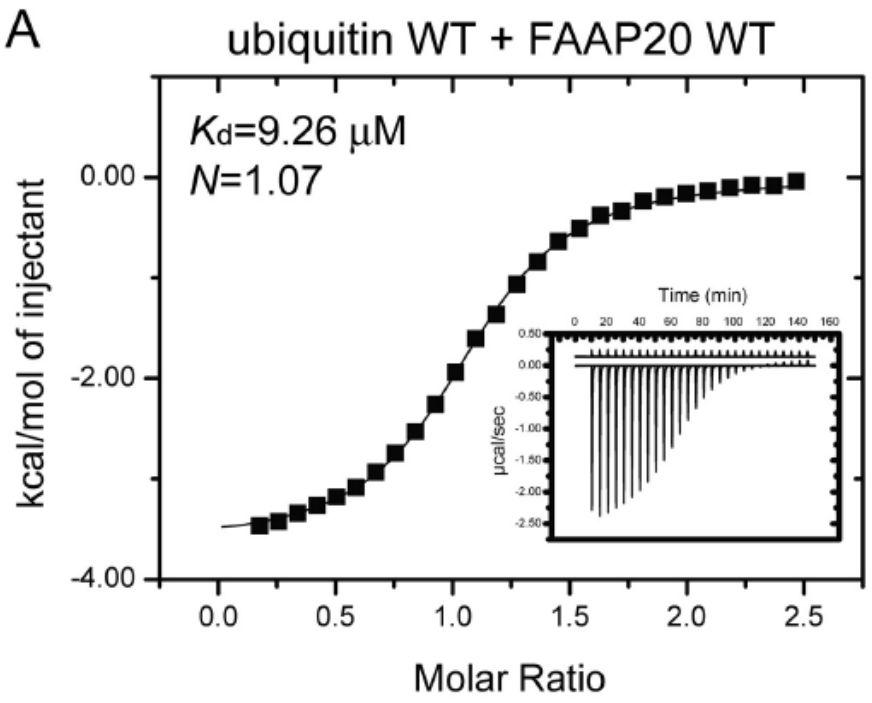


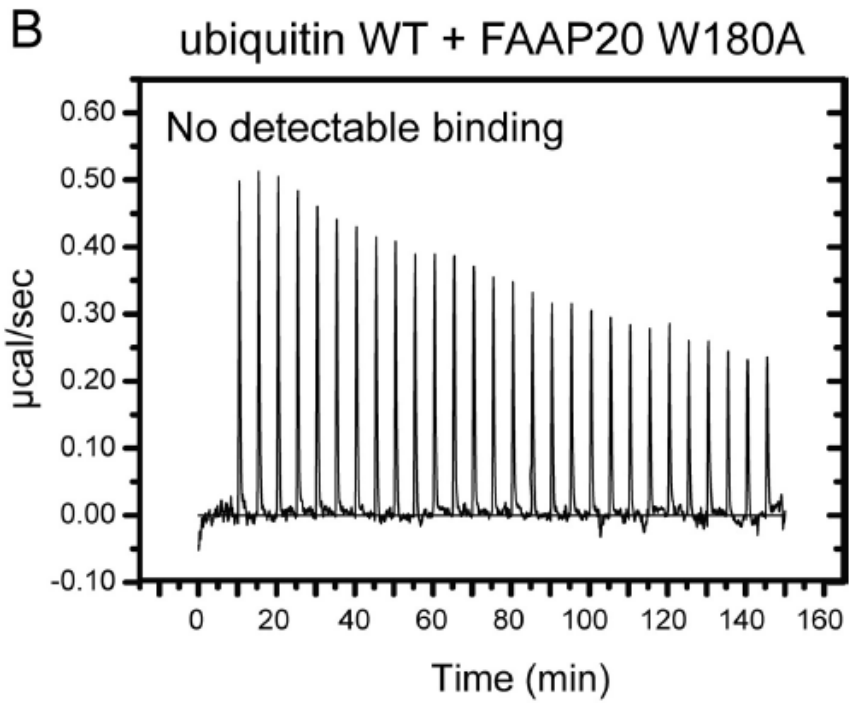


Wojtaszek et al., (2014) Nucleic Acids Res.

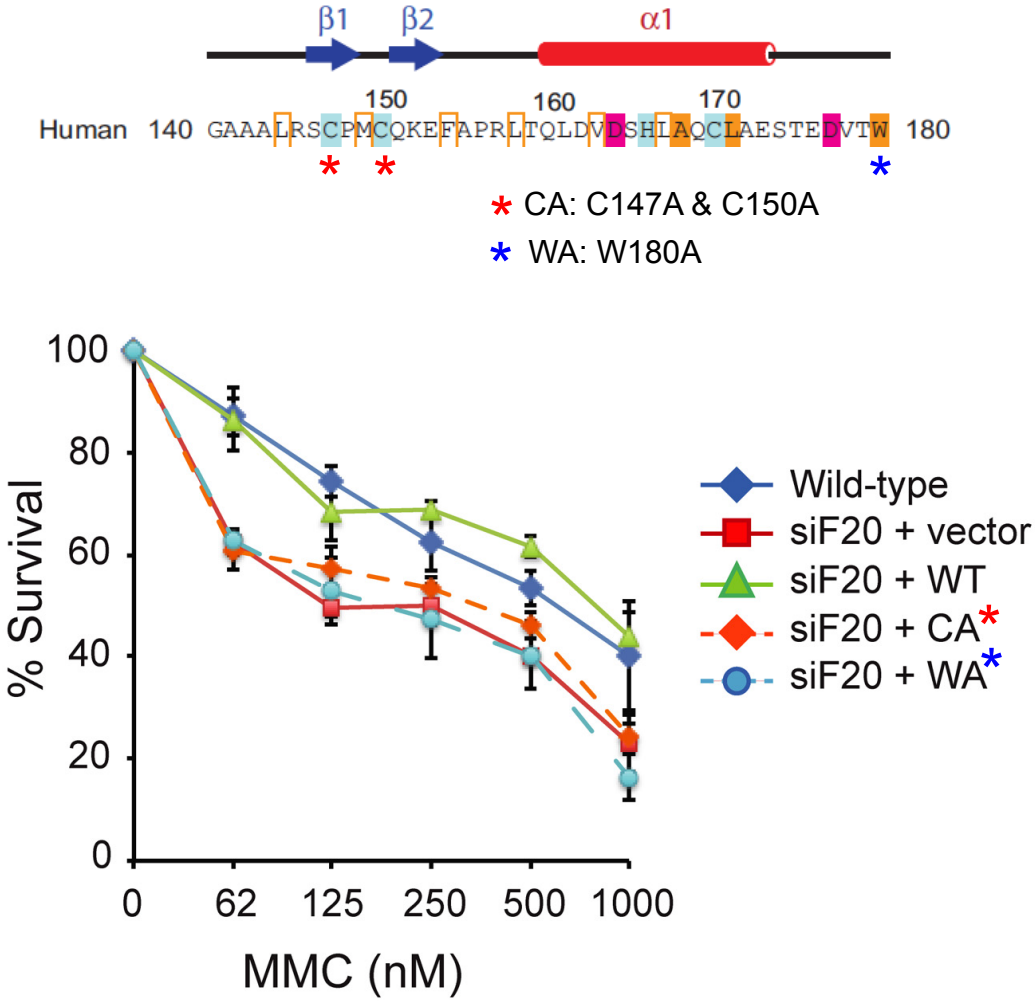
The Very C-terminal Tryptophan Is Required for Ubiquitin Binding

Isothermal Titration Calorimetry (ITC)

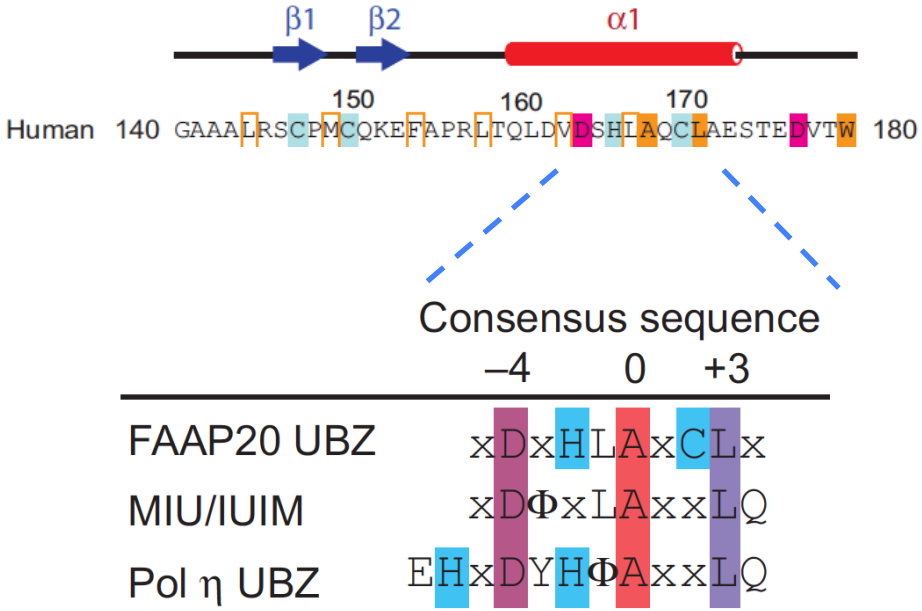




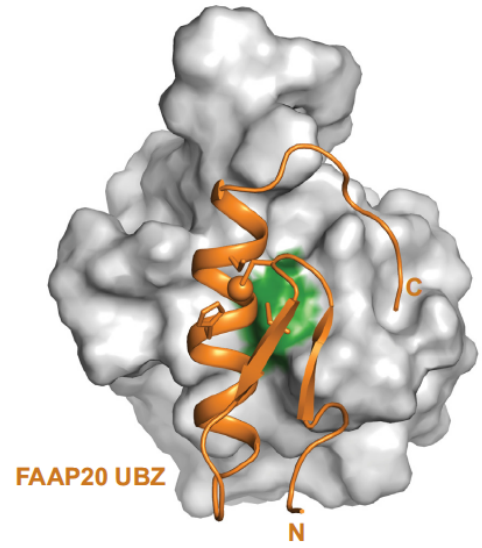
The Very C-terminal Tryptophan Is Required for DNA ICL Repair

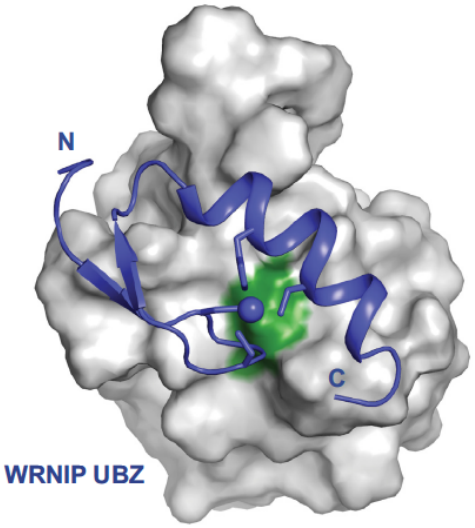


Ubiquitin Recognition of FAAP20 Is Distinct from Canonical UBZ Module

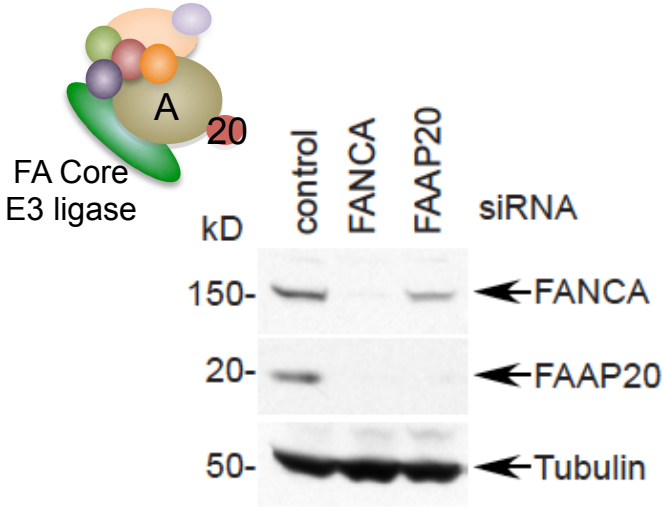


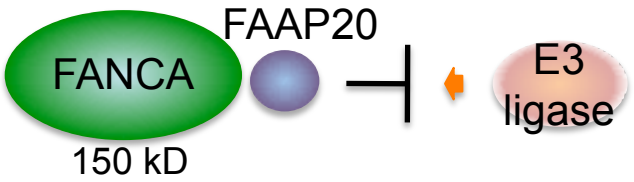
Unconventional FAAP20 UBZ motif





Regulation of the FA Pathway by the FANCA-FAAP20 Axis

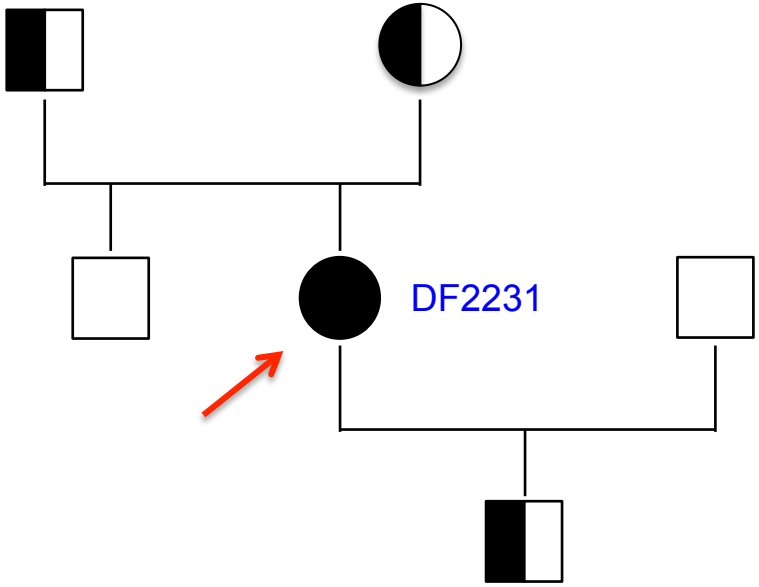




- Mechanism of maintaining FANCA stability by FAAP20?
- Mechanism of FANCA degradation and its implication in tumorigenesis?

Characterization of a FA-like Breast Cancer Patient

- 33 year-old triple negative breast cancer (TNBC) patient (not a FA patient *per se*)
- Features of FA (i.e. short stature, café au la spot, mild macrocytic anemia)



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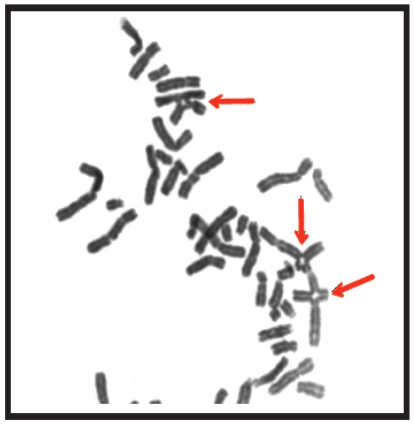
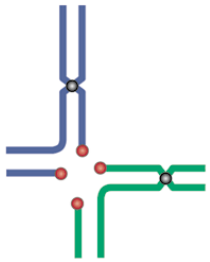
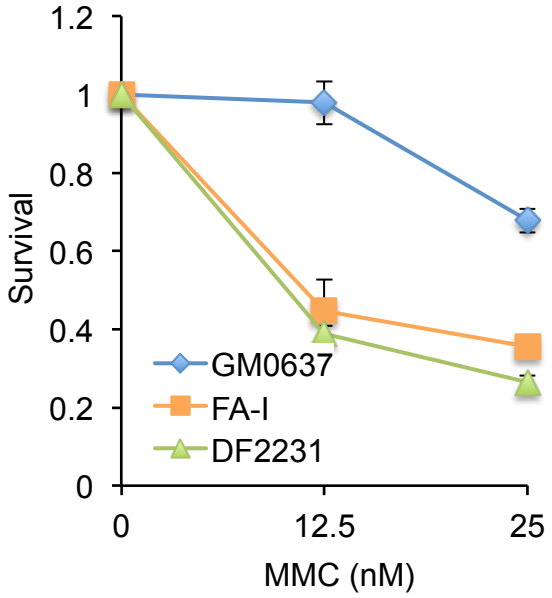


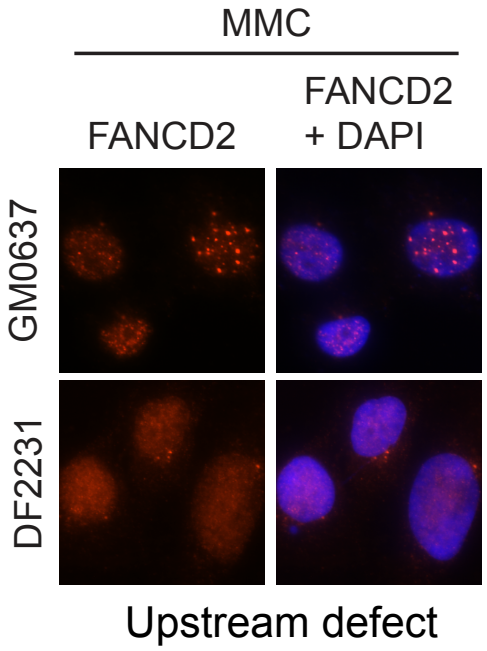
20 ng/mL MMC

Quadriradial chromosome



Characterization of a FA-like Breast Cancer Patient

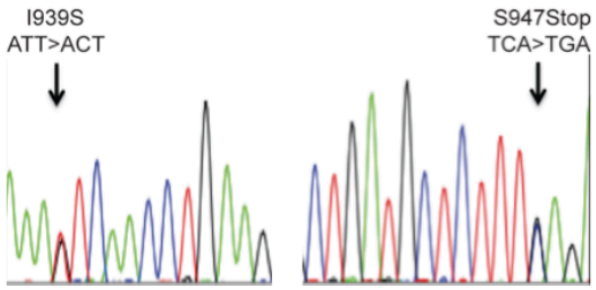


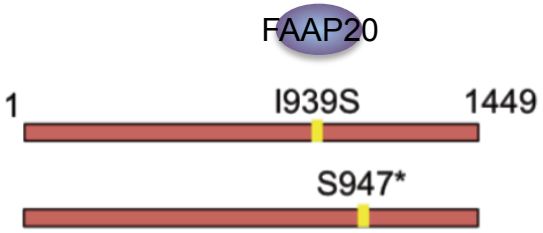


Upstream defect

The I939S Point Mutation in Patient FANCA Destabilizes FANCA

FANCA gene

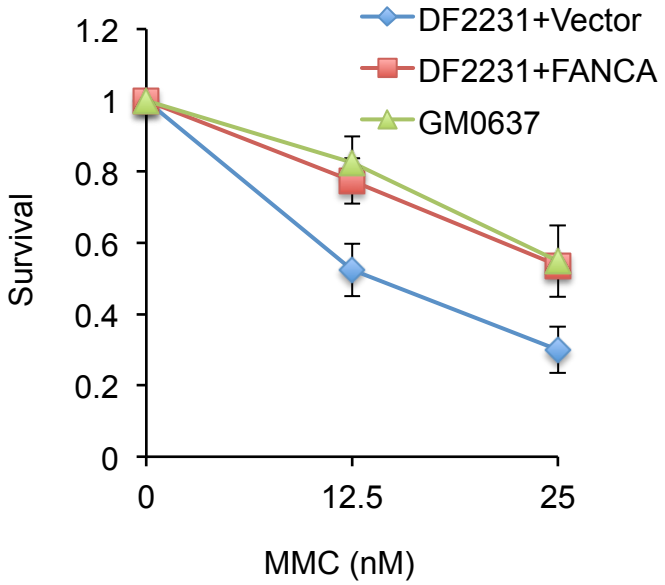


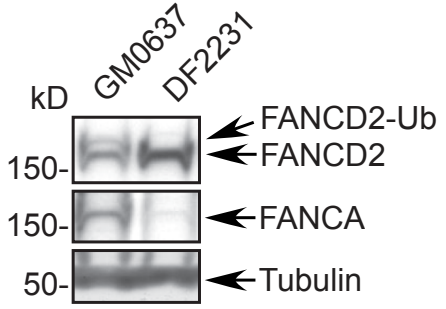


2816T>G; Ile939Ser

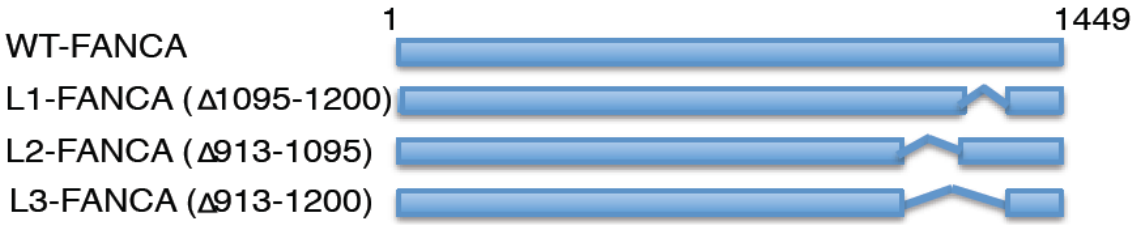
2840C>G; Ser947stop

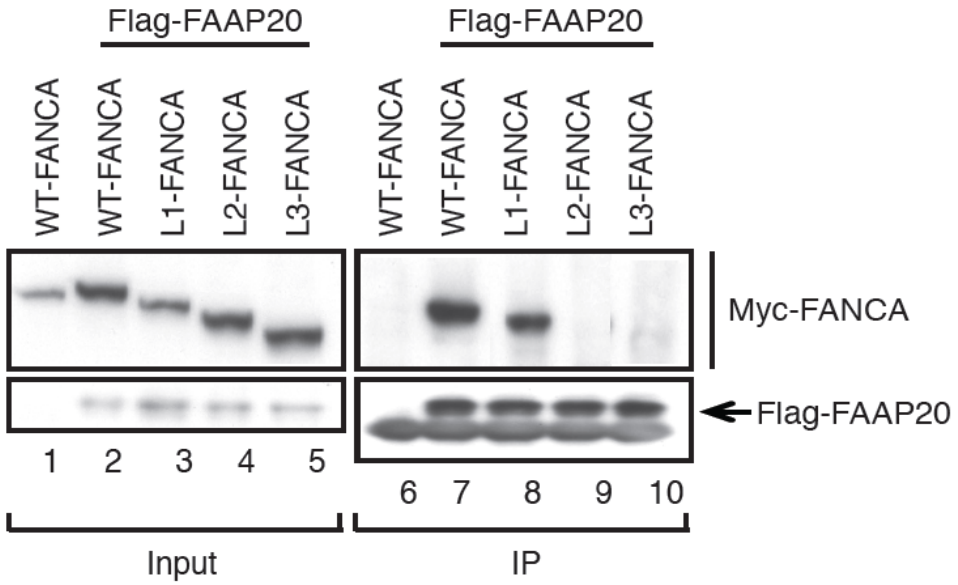
(*Savoia et al. 1996, Levran et al. 1997, Savino et al. 2003*)



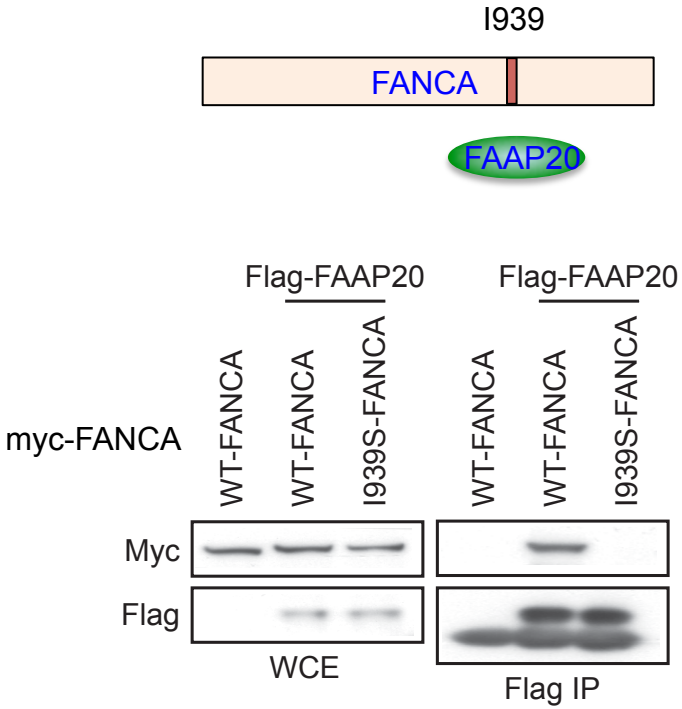


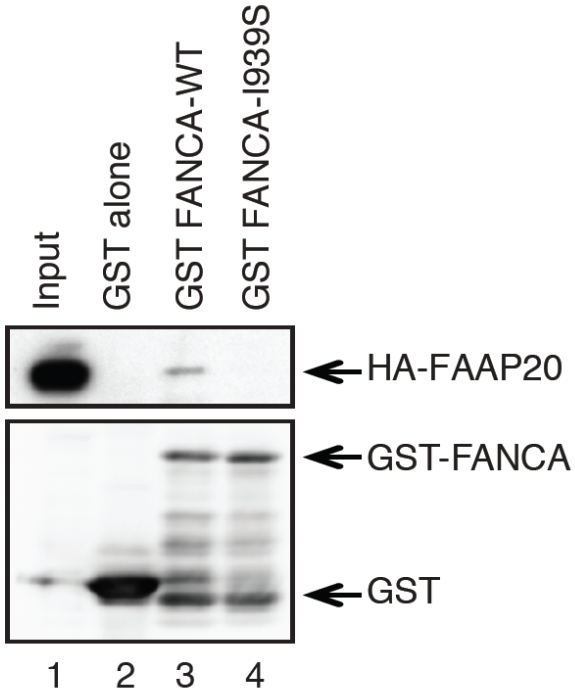
The Amino Acid 913-1095 Region of FANCA Is Required for FAAP20 Interaction



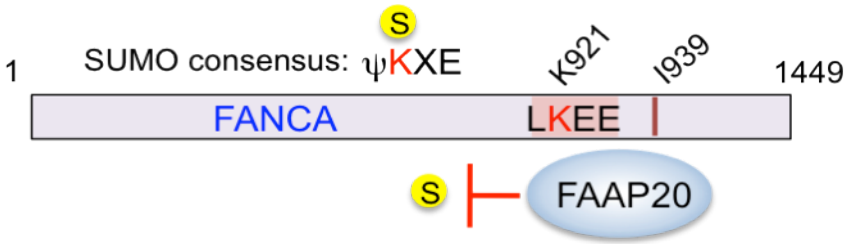


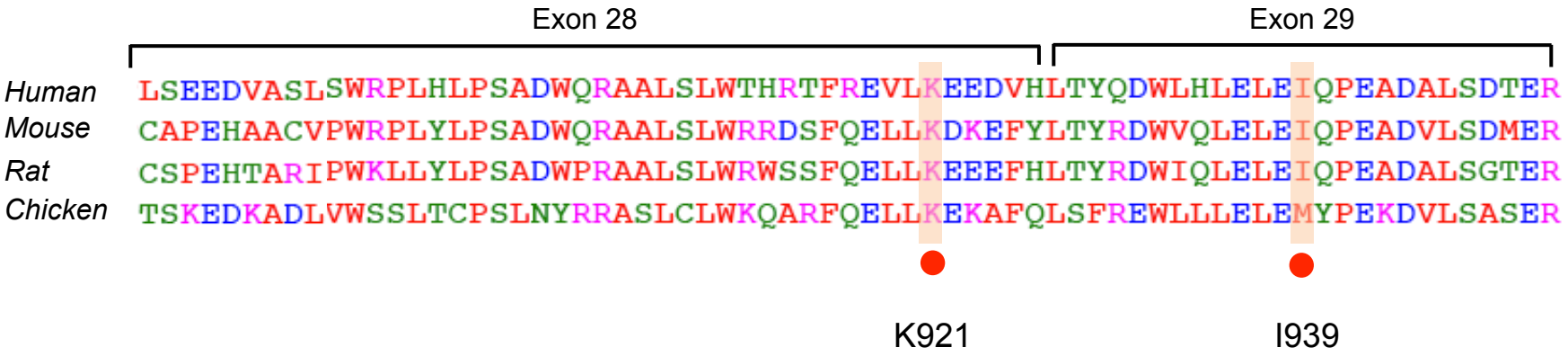
FANCA-I939S Mutation Disrupts FAAP20 Binding





Identification of SUMOylation Site of FANCA Near the FAAP20 Binding Region



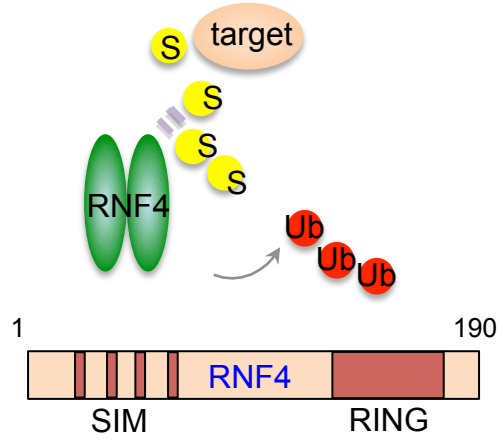


Regulation of Protein Stability by Coupled SUMO-Ubiquitin Signaling

1) RNF4 is a Ubiquitin E3 ligase which regulates the DNA damage response (Galanty, Y et al, *Genes Dev* 26, 2012; Yin, Y, et al, *Genes Dev* 26, 2012)

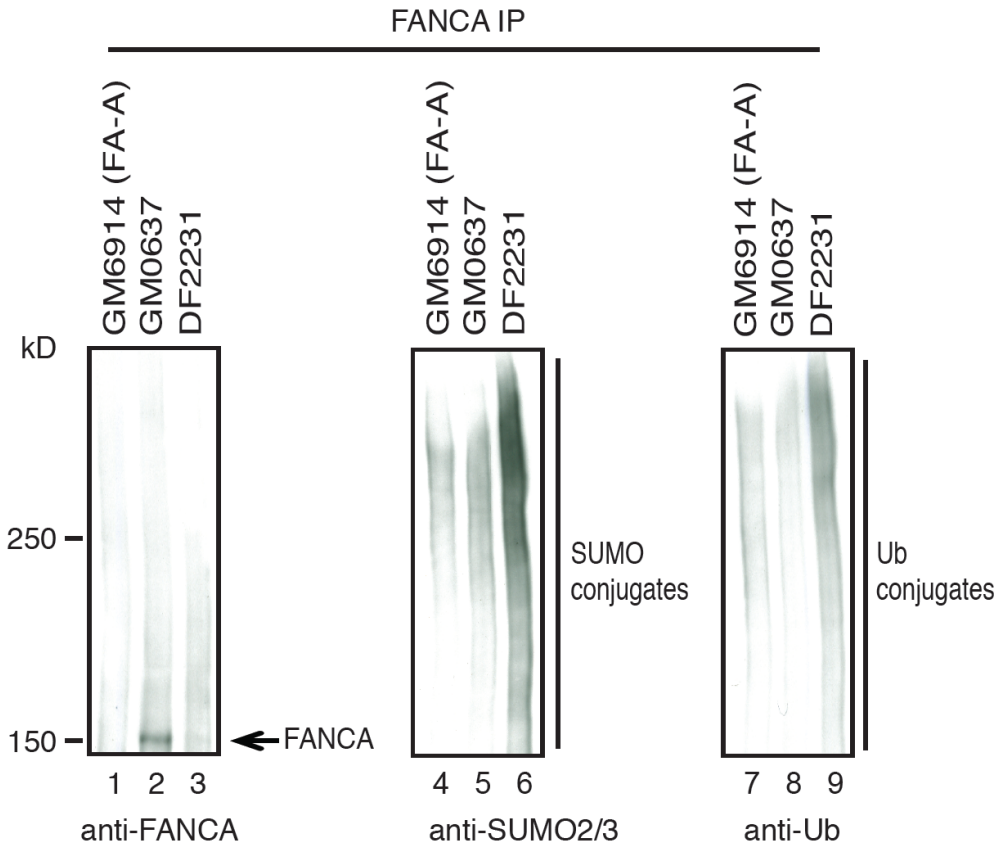
2) RNF4 is the human ortholog of the yeast proteins, Slx5 and Slx8 (discovered in the same screen which identified Slx4; SLX4/FANCP)

STUbL (SUMO-Targeted Ubiquitin Ligase)

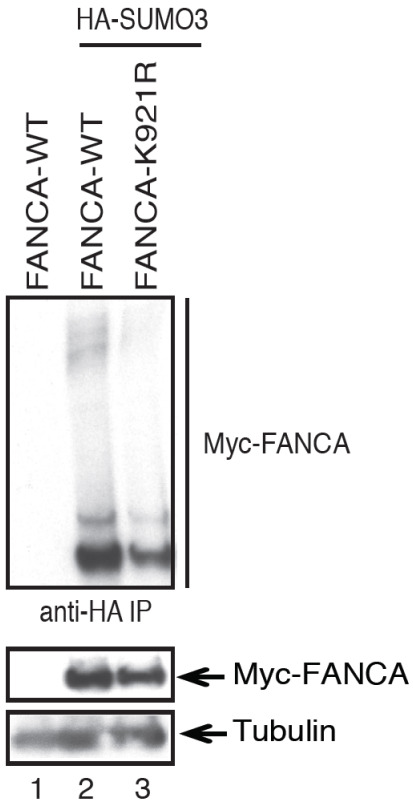


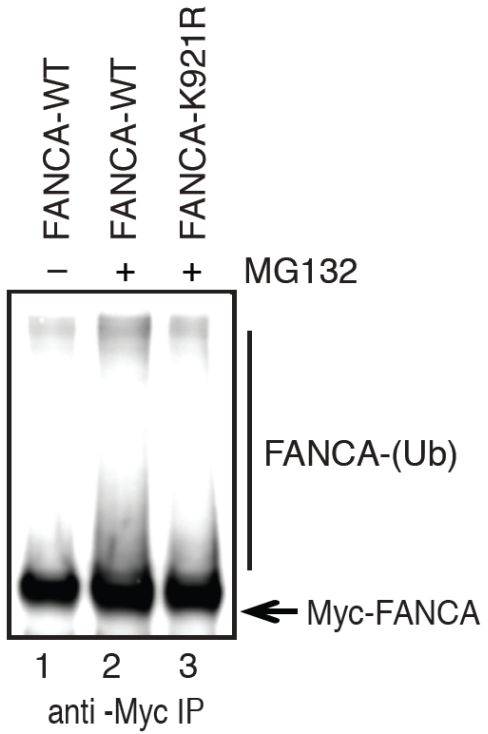
SIM: SUMO-interacting motif

FANCA from the Patient Shows Increased SUMOylation and Polyubiquitination

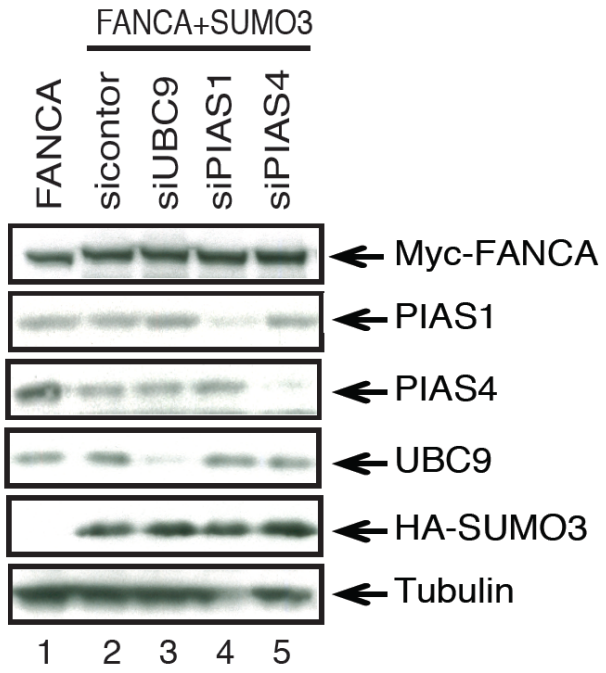


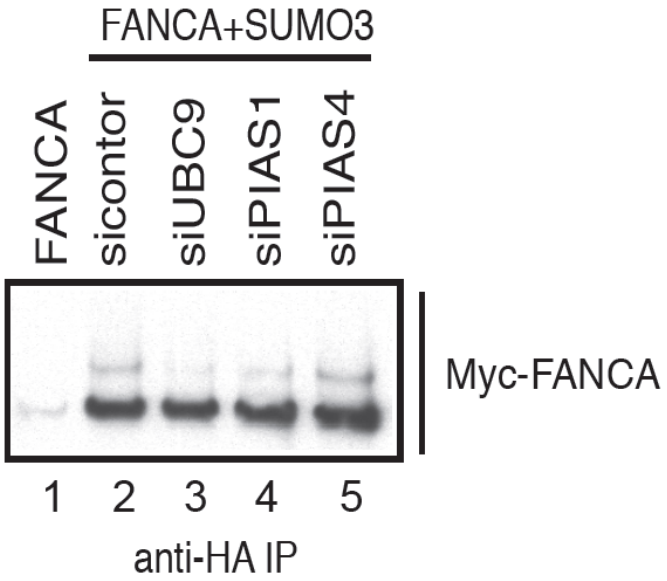
FANCA SUMOylation at K921 Is Required for Polyubiquitination of FANCA





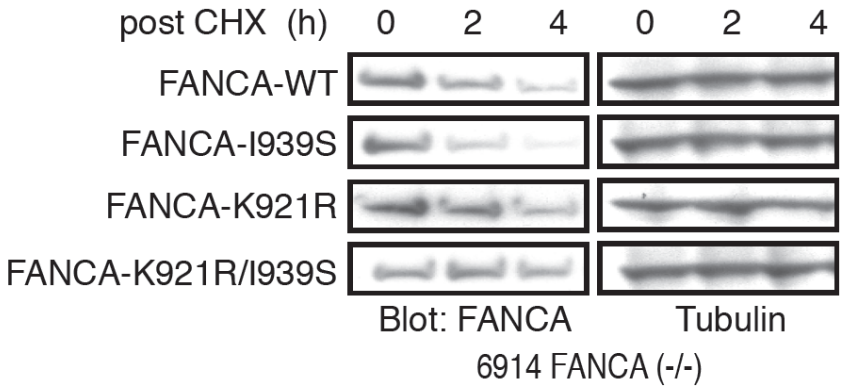
UBC9 and PIAS1 Are Required for FANCA Sumoylation



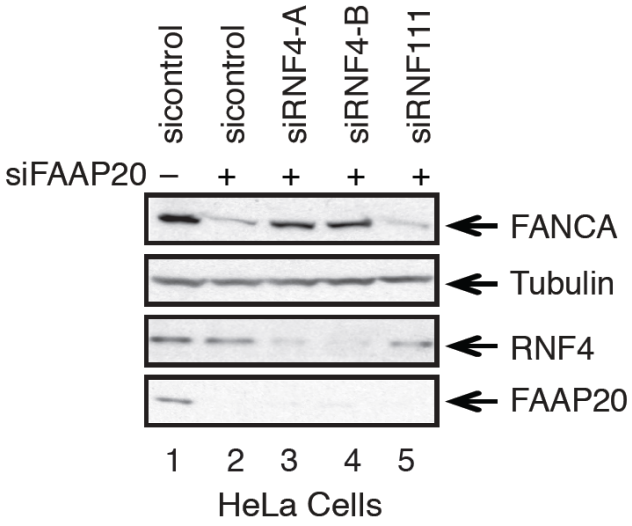


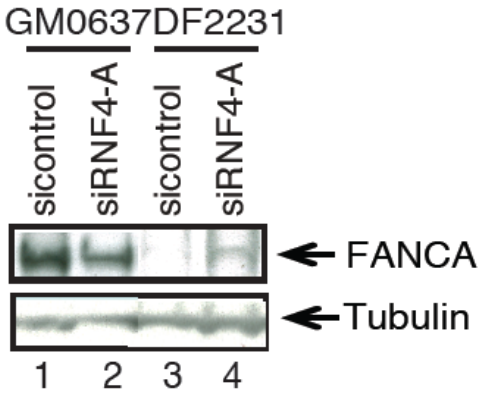
FANCA-K921R SUMO-Defective Mutant Exhibits Increased Half-life

Cycloheximide blocking



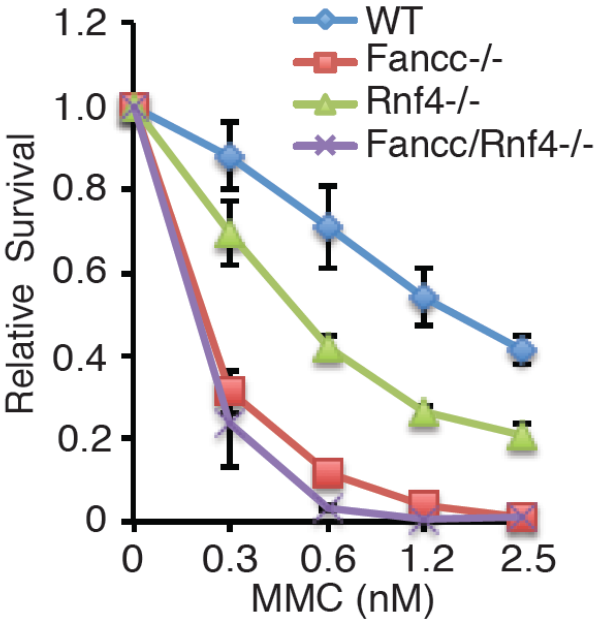
RNF4 Regulates FANCA Stability

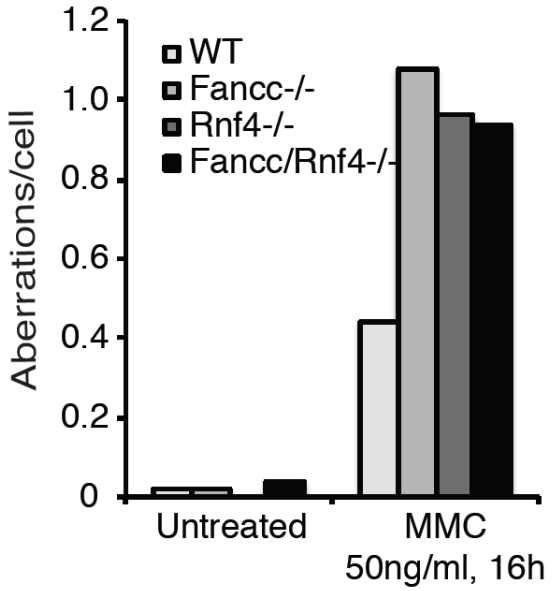




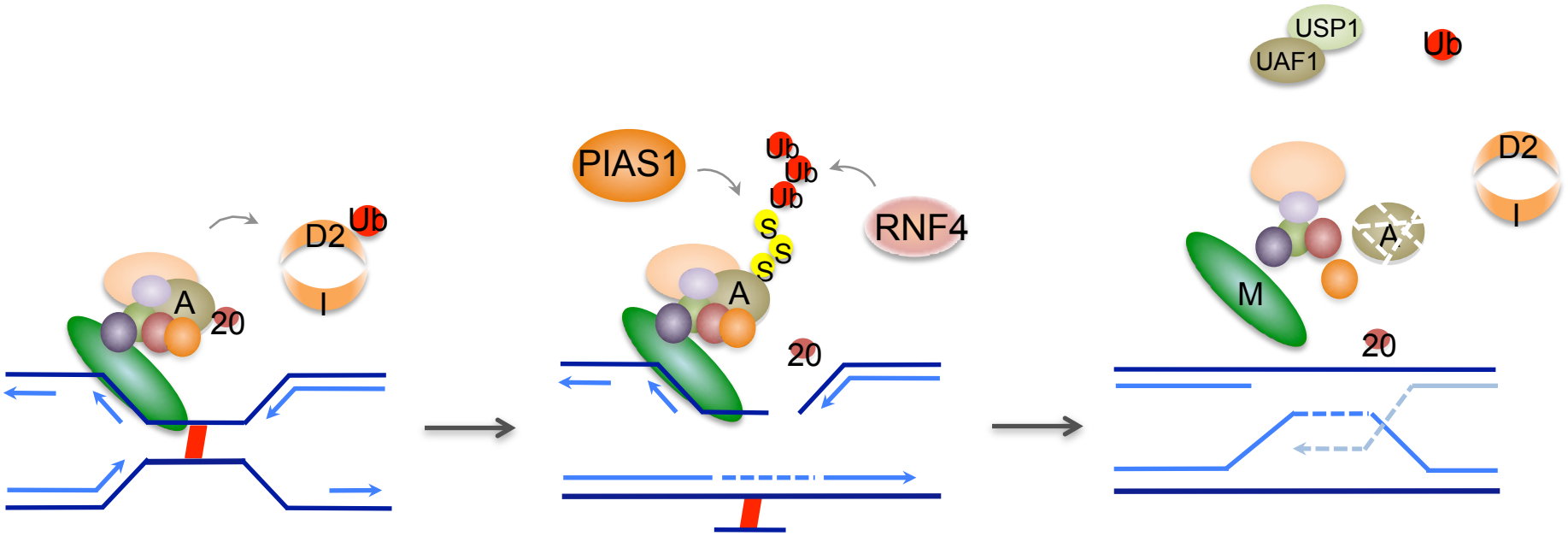
FANCA I939S + siRNF4

RNF4 Is a New Player in the FA Pathway



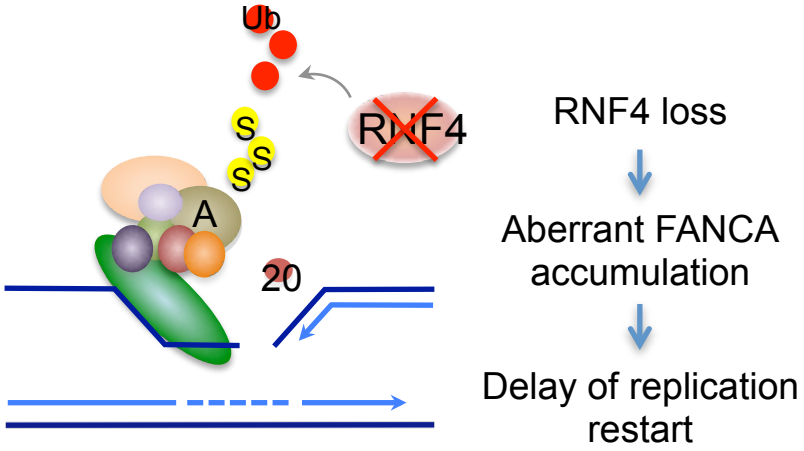


Regulation of DNA ICL Repair by Integrated Ubiquitin-SUMO Signaling



- ✓ FANCD2 deubiquitination (USP1)
- ✓ FANCM release
- ✓ FANCA degradation

Regulation of DNA ICL Repair by Integrated Ubiquitin-SUMO Signaling

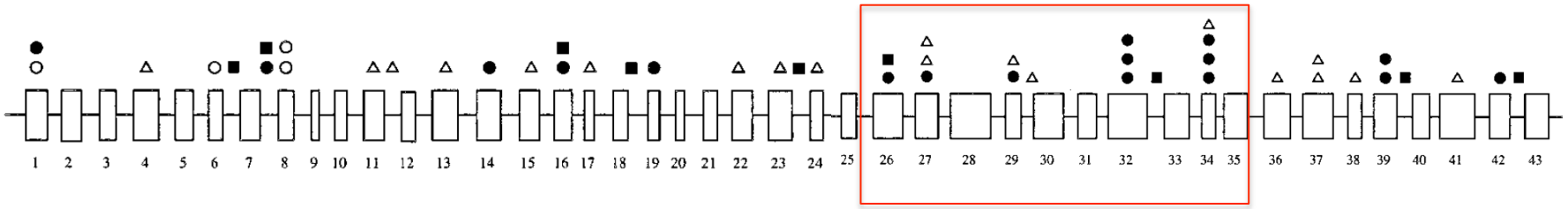


The FANC Proteins Are Predicted to be Extensively SUMOylated

Fanconi Anemia Gene	Consense SUMO Conjugation sites
FANCM	K867, K1316
FANCA	K921
FANCB	K68, K321, K681, K779
FANCE	K425, K447
FANCI	K646, K715
FANCJ	K1037, K1186
FANCP	K359, K1575
FANCN	K25, K203, K623

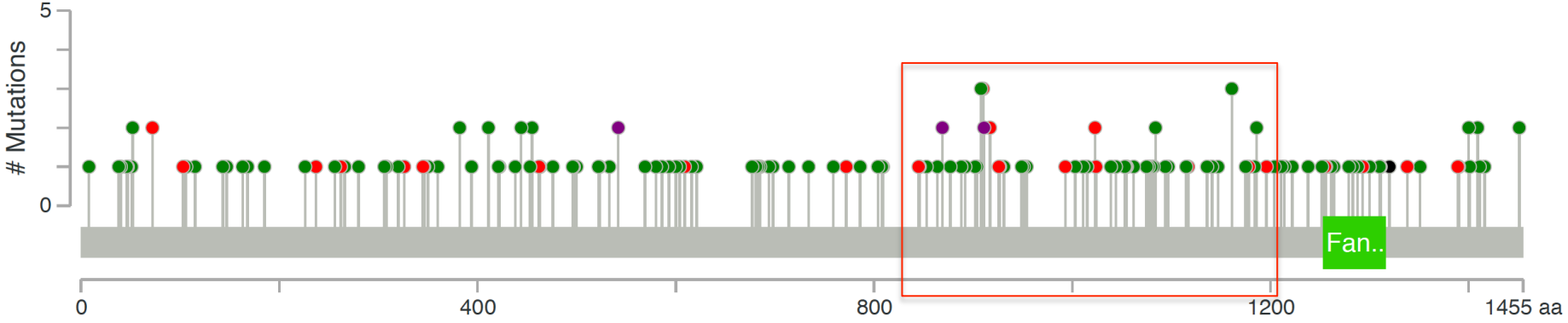
Mutational Hotspot of *FANCA* Corresponds to the FAAP20 Interacting Region

FANCA gene mutations from 97 FA patients



Levran et al., (1997) PNAS

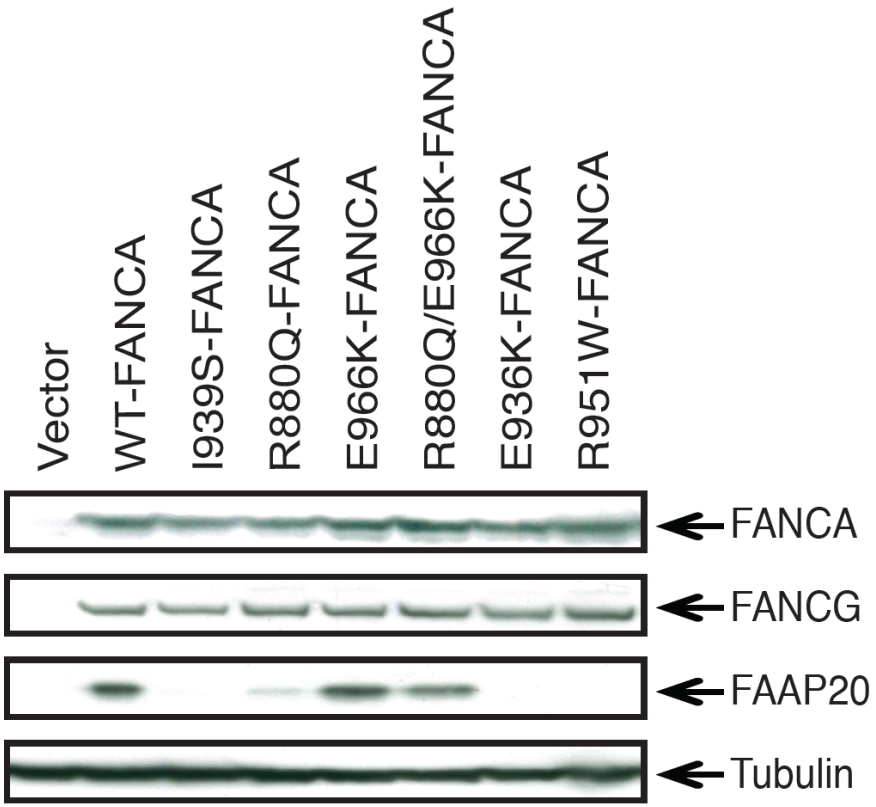
221 FANCA mutations from 89 studies including TCGA



cBioPortal for Cancer Genomics

FANCA Mutations From FA and/or Cancer Patients Disrupt FAAP20 Interaction

FANCA null FA patient cells + FANCA variants



Conclusions

1. DNA repair mechanisms preserve genome integrity and their deregulation contributes to tumorigenesis

2. The FA pathway removes DNA ICL encountered during S phase, and its defect leads to cancer-prone disease, Fanconi anemia

3. Complex interplay of post-translational modification network including ubiquitination/ SUMOylation controls DNA repair pathways

- FAAP20 ubiquitin binding in regulating ICL repair

- FANCA degradation by Ubiquitin plus SUMO

4. Compound heterozygosity of *FANCA* leads to (or contribute to) TNBC

- Disruption of the DNA repair activity (germ-line or somatic) could be a major driving force for tumorigenesis

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