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## Defining the Sites of Interaction of the FANCD2, FANCE, and FANCL proteins

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# Defining the Sites of Interaction of the FANCD2, FANCE, and FANCL proteins

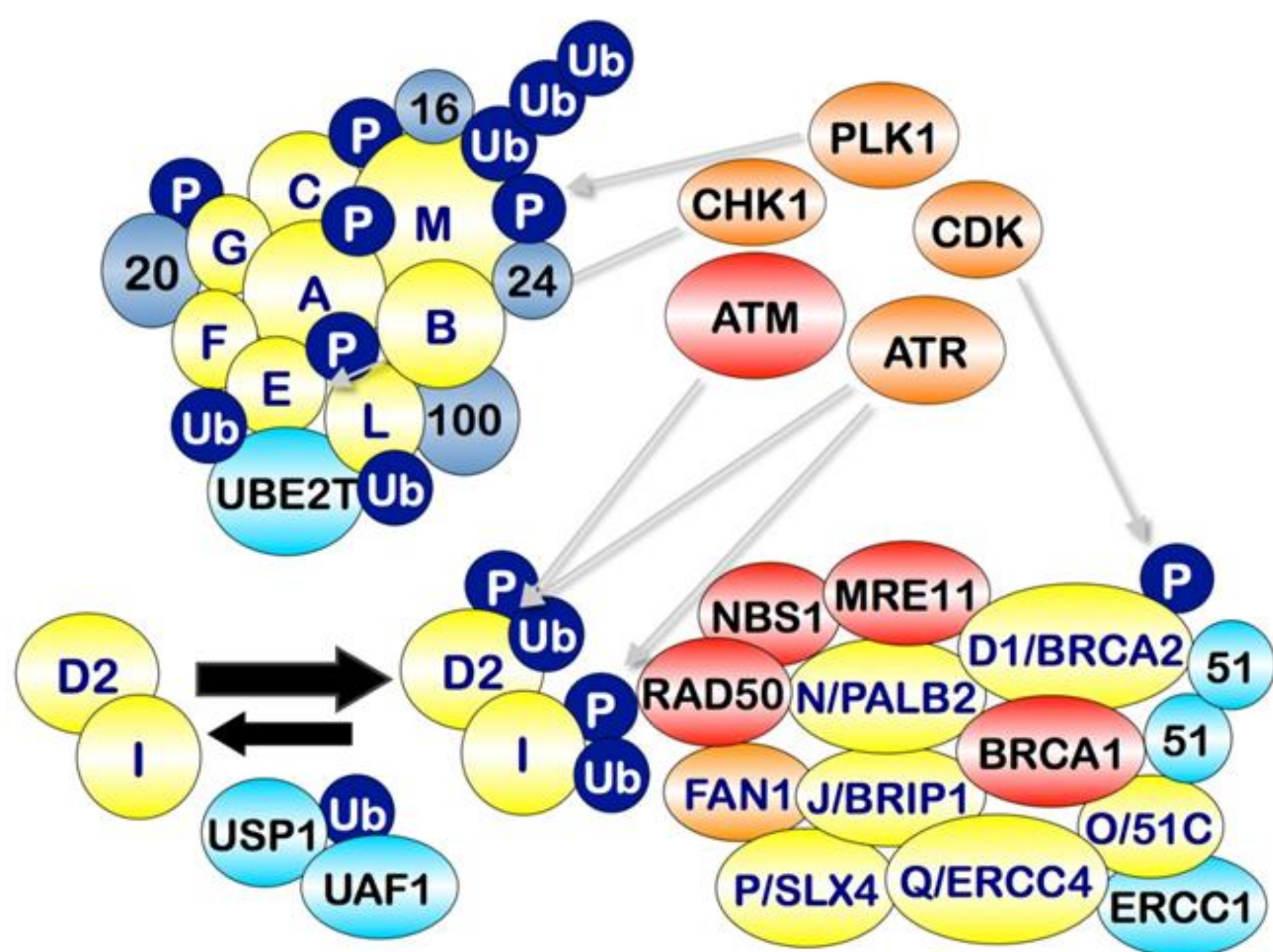
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Karissa L. Paquin, Paul A. Azzinaro; Faculty Sponsor Dr. Niall G. Howlett

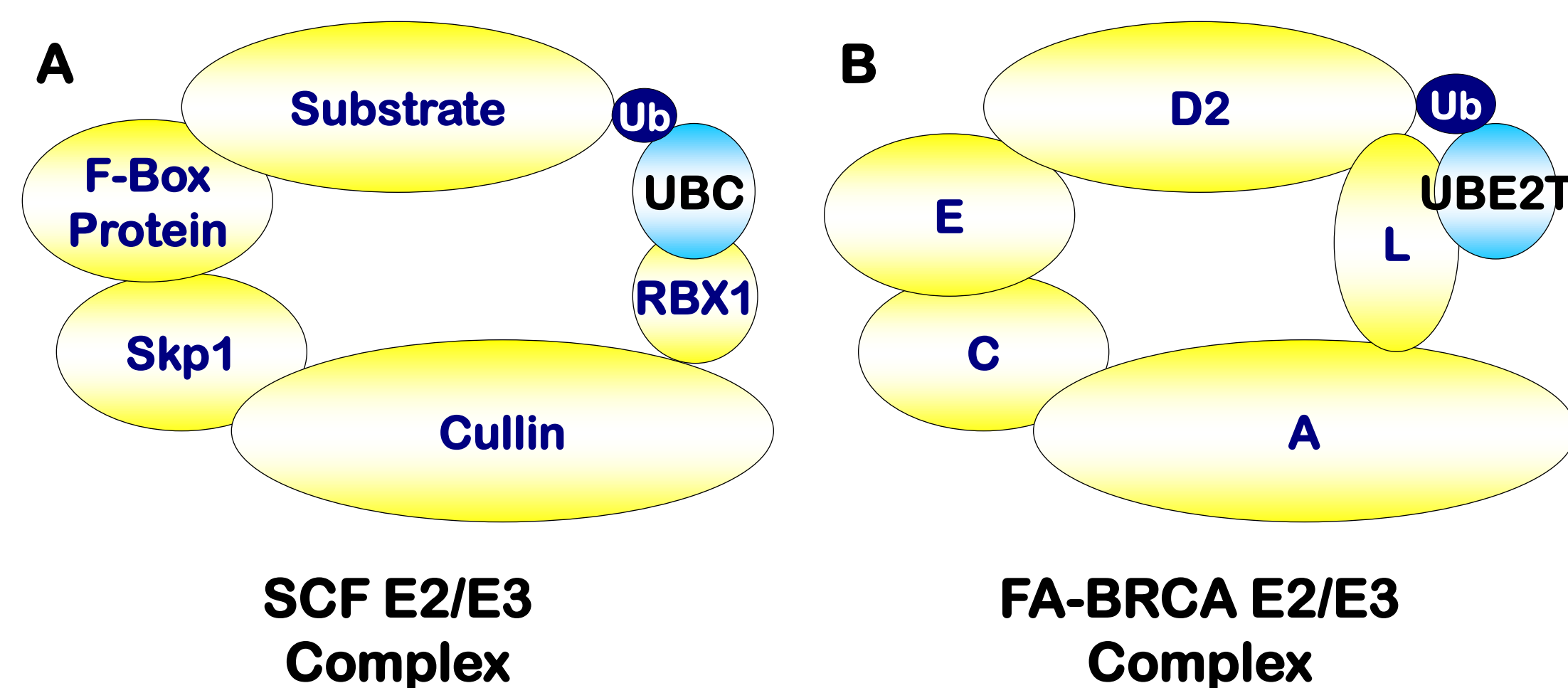
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## Background

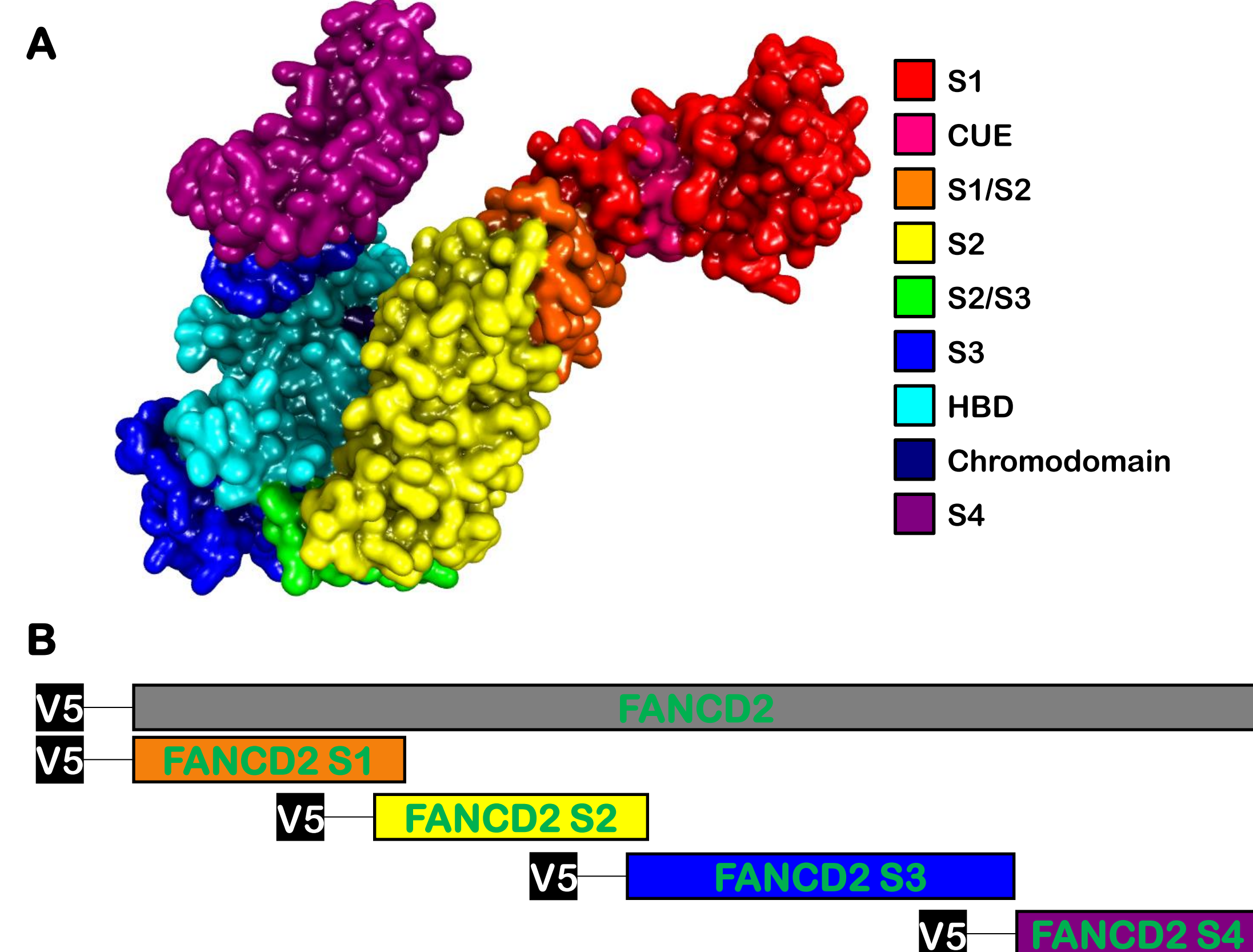
Fanconi anemia (FA) is a rare X-linked and autosomal disease characterized by bone marrow failure, congenital defects, and increased cancer susceptibility. FA is caused by mutations in any 1 of 16 genes. The protein products of these genes work together in the FA-BRCA DNA damage response pathway to repair DNA interstrand crosslinks (ICLs). The FA-BRCA pathway is a critical tumor suppressor pathway.



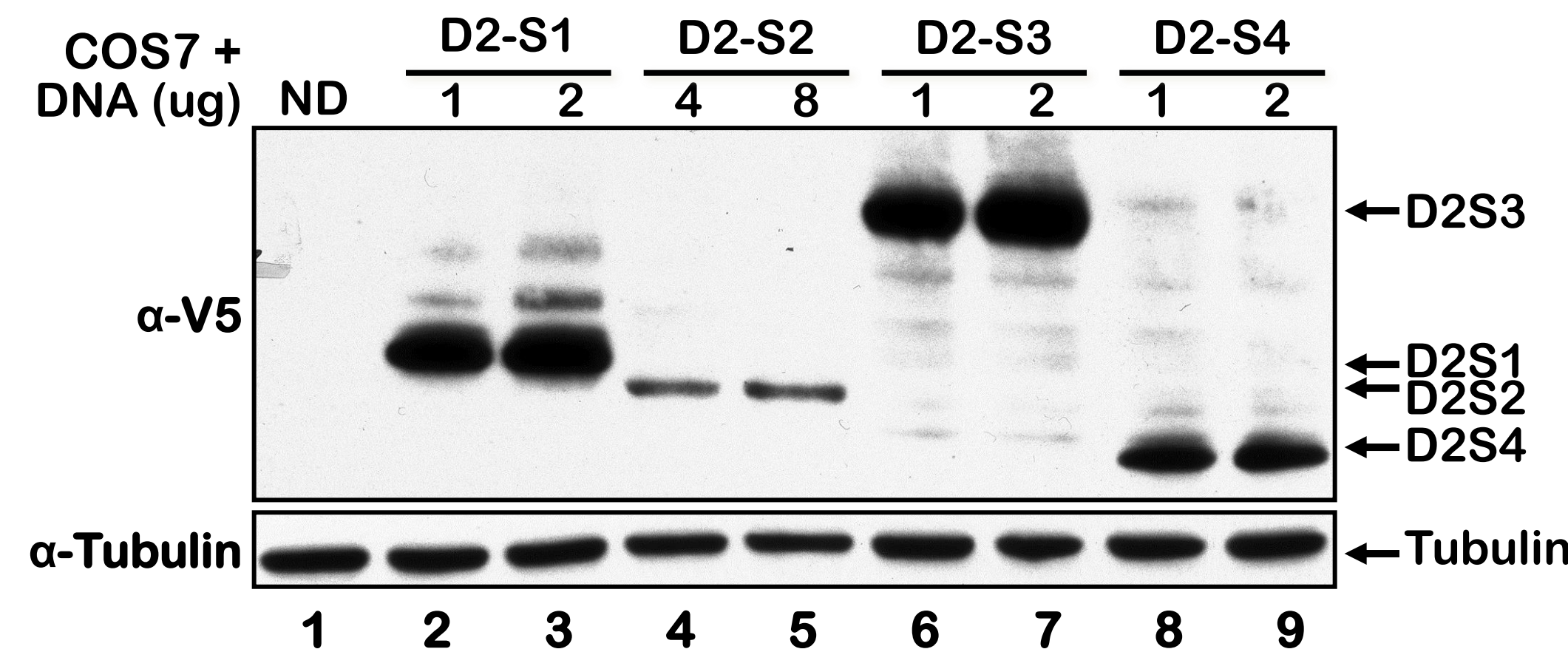
**Figure 1. Schematic model of the FA-BRCA pathway.** Upon ICL formation, the FA core complex monoubiquitinates FANCD2 and FANCI. Upon monoubiquitination FANCD2 and FANCI localize to sites of DNA damage where they interact with known DNA repair proteins.



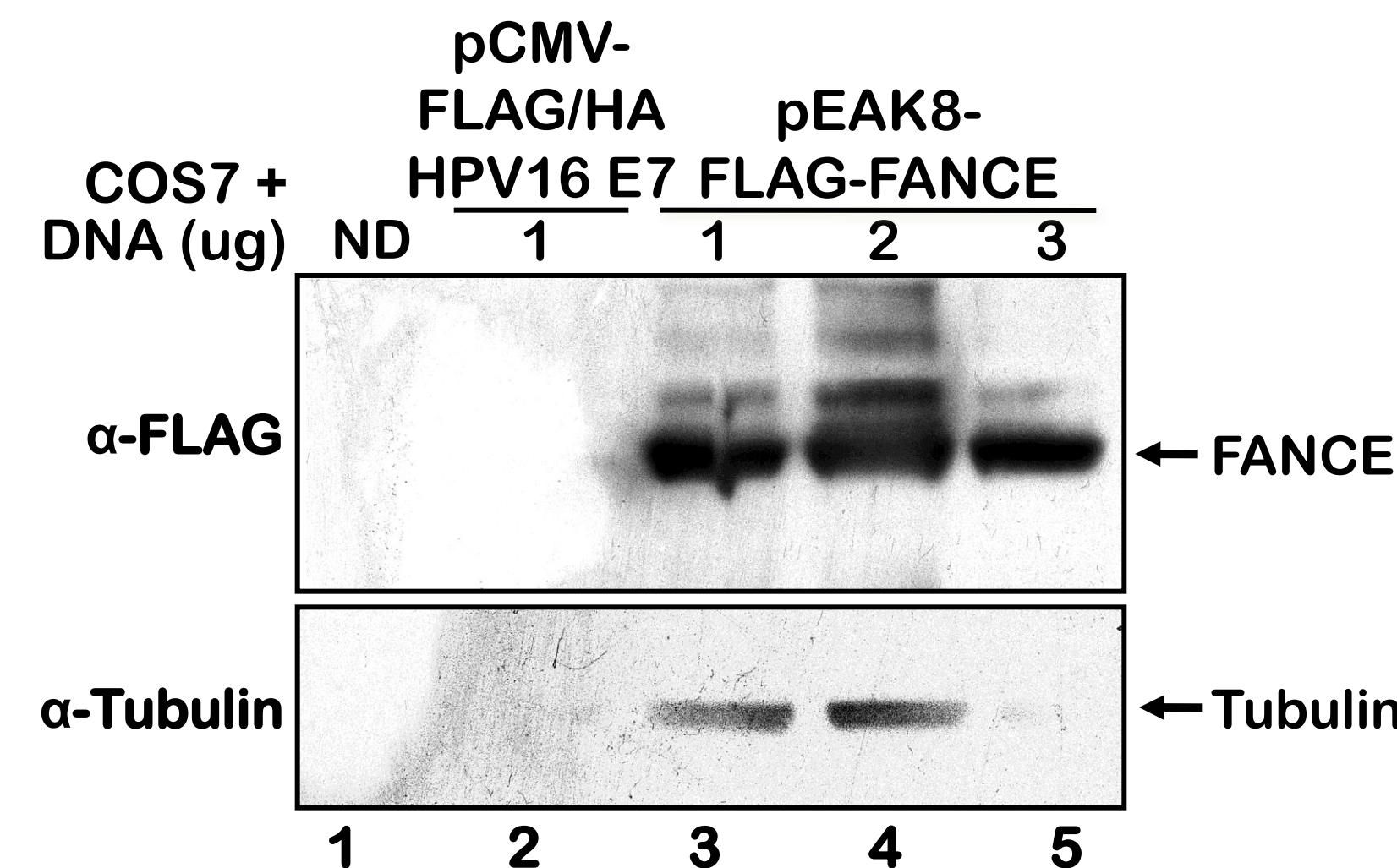
**Figure 2. Comparison of SCF E2/E3 and the FA-BRCA E2/E3 ubiquitin ligase complexes.** The SCF (for Skp1-Cul1-F-box protein) E3 ubiquitin ligase (A) is a large complex made up of the RBX1 E3 ubiquitin ligase, UBC E2 ubiquitin-conjugating enzyme, and the F-box protein which recognizes the substrate. Here, we propose a similar model for the ubiquitination of FANCD2 by the FA core complex (B), where FANCL is the E3 ligase, UBE2T is the E2 ubiquitin-conjugating enzyme, and FANCE is the substrate (FANCD2) recognition protein.



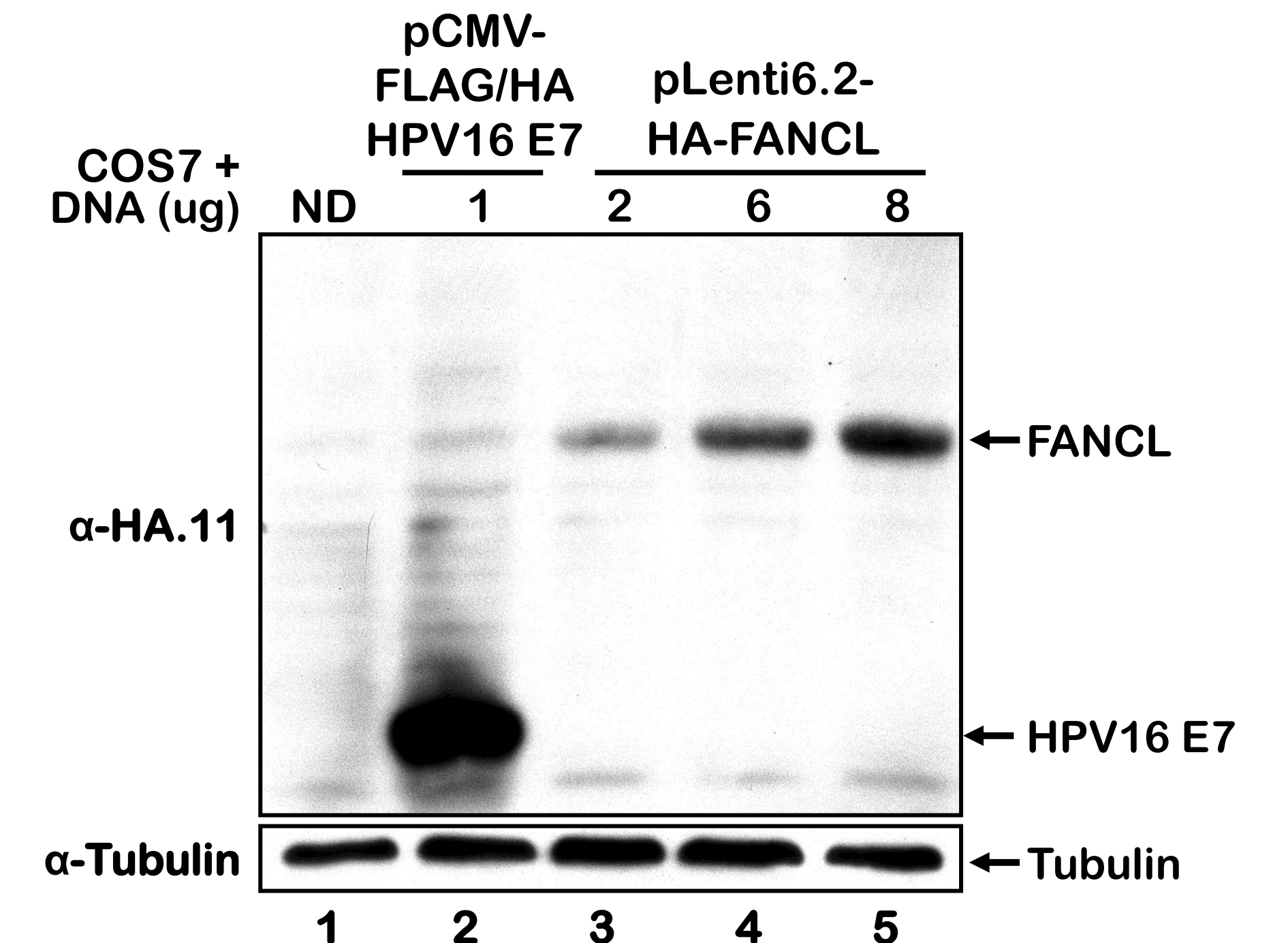
**Figure 3. Generation of overlapping fragments of FANCD2.** Four FANCD2 fragments were generated, each with a N-terminal V5 epitope tag. The four fragments are based on the four solenoids of FANCD2, designated S1-S4.



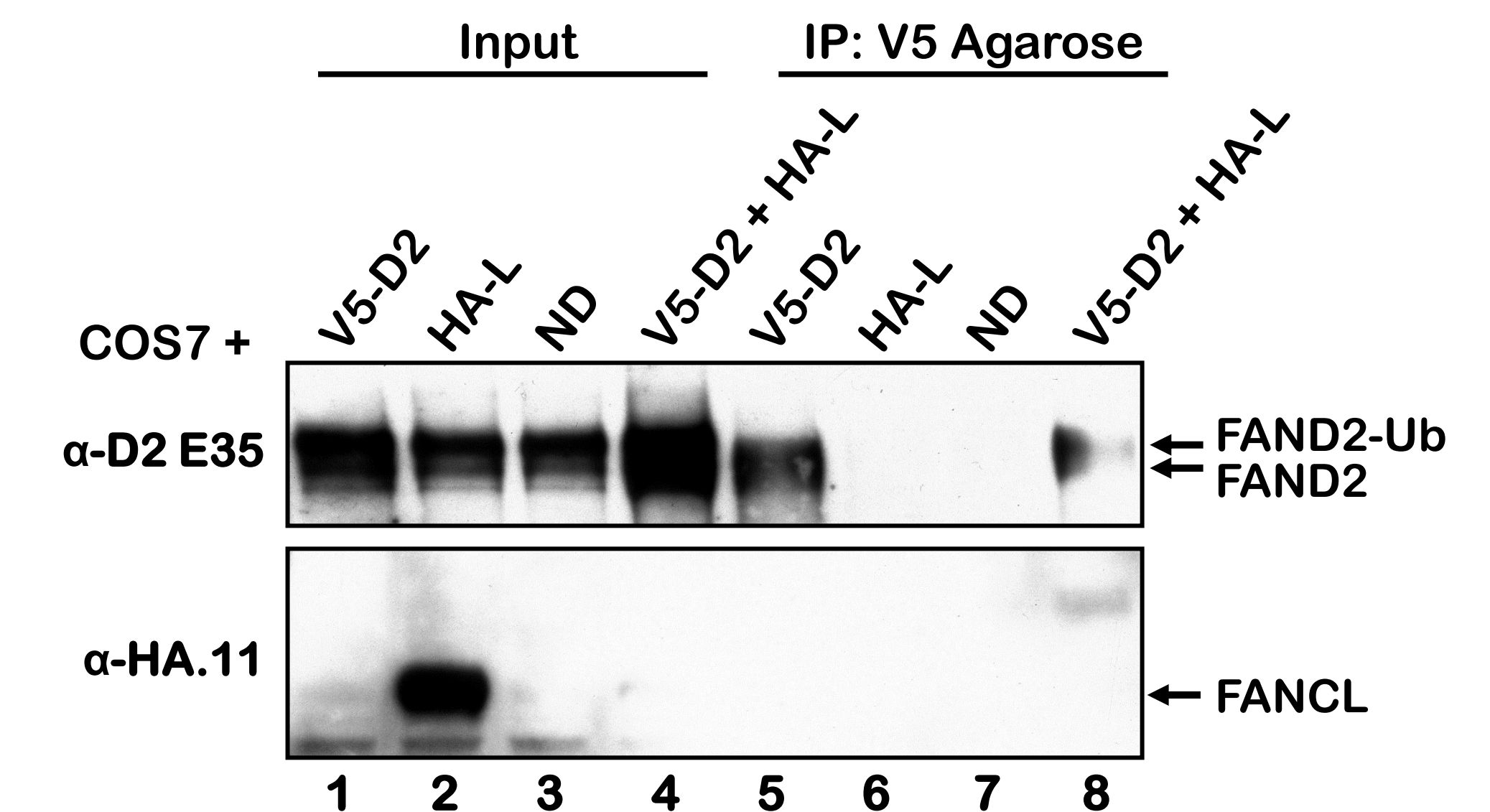
**Figure 4. Expression of pcDNA3.1-V5-FANCD2 Fragments.** COS7 cells were transfected with pcDNA3.1-V5-FANCD2 fragments or no DNA (ND). Cells were harvested and lysed in 2% SDS lysis buffer, and analyzed via Western blotting using anti-V5 and anti-tubulin antibodies.



**Figure 5. Expression of FLAG-FANCE.** A FLAG-tagged FANCE construct was also obtained. COS7 cells were transfected with pEAK8-FLAG-FANCE or no DNA (ND). Cells were harvested and lysed in 2% SDS lysis buffer, and analyzed via Western blotting using anti-FLAG and anti-tubulin antibodies.



**Figure 6. Expression of pLenti6.2 HA-FANCL.** COS7 cells were transfected with pLenti6.2-HA-FANCL, a FLAG/HA HPV16 control, or no DNA (ND). Cells were harvested and lysed in 2% SDS lysis buffer and analyzed via Western blotting using anti-HA and anti-tubulin antibodies.



**Figure 7. Co-Immunoprecipitation of V5-FANCD2 and HA-FANCL.** A co-IP was performed to determine if FANCD2 and FANCL interact. COS7 cells were transfected with no DNA (ND; lanes 3 and 7), pcDNA3.1-V5-FANCD2 (V5-D2; lanes 1 and 5), pLenti6.2-HA-FANCL (HA-L; lanes 2 and 6), or both (V5-D2 + HA-L; lanes 4 and 8). Samples were lysed in low stringency buffer and immune complexes isolated with anti-V5 agarose. Samples were eluted and analyzed using Western blotting with anti-HA and anti-FANCD2 antibodies.

## Conclusions

We have successfully generated mammalian expression constructs for four overlapping V5-tagged FANCD2 fragments and HA-tagged FANCL. We have also successfully optimized conditions for their ectopic expression in COS7 cells and also optimized the FLAG-tagged FANCE construct. In addition, we have begun to perform co-IP experiments to determine the specific regions of FANCD2 to which FANCL and FANCE bind. Mapping these sites of interaction will generate important mechanistic insight into the regulation of this critical tumor suppressor pathway.