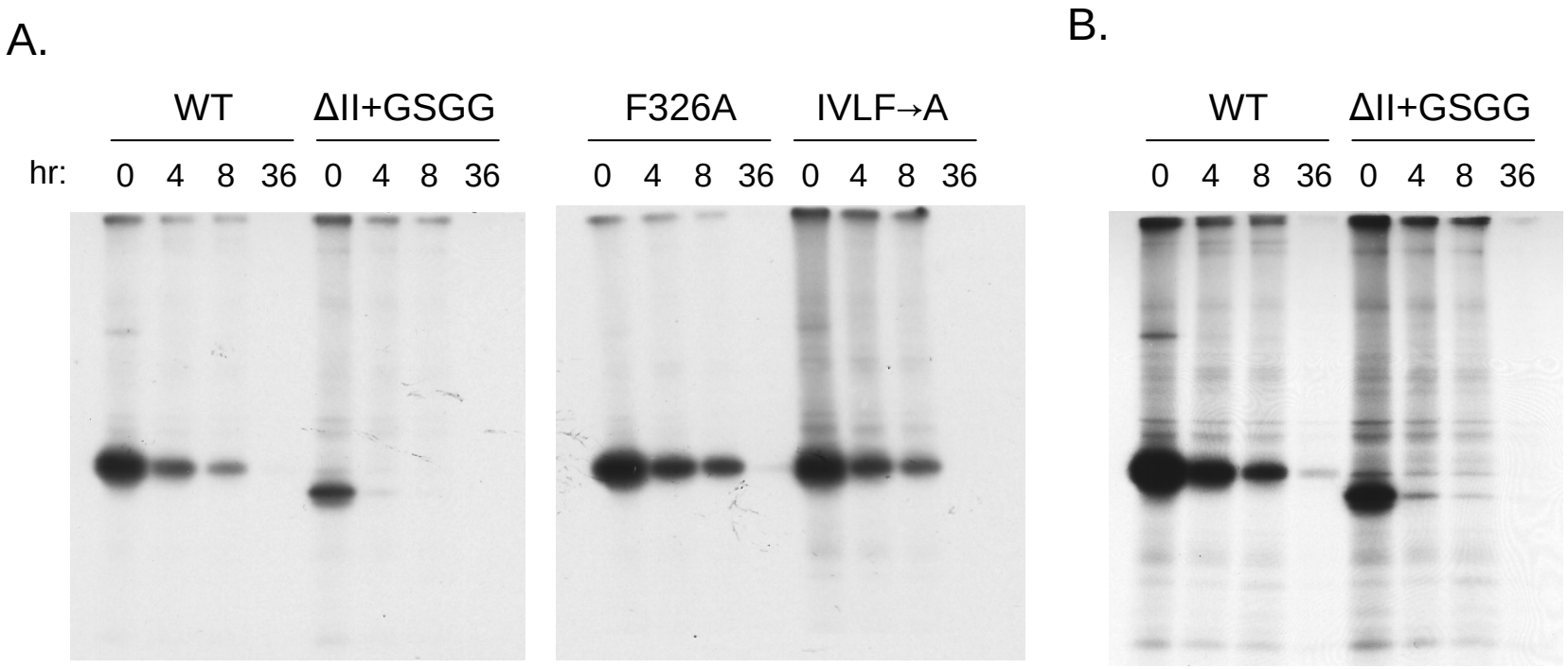

Figure 1S. Point mutations that affect substrate binding do not alter the half-life of the corresponding mutant protein, whereas deletion of domain II has modest effects on protein stability. COS-1 cells were transfected with the indicated ERdj3 constructs and pulse labeled with 35S methionine and cysteine. After chasing for the indicated times, cell lysates were immunoprecipitated with an anti-HA antibody and analyzed by SDS-PAGE and signals were detected by autoradiography to determine the turnover rate. Panel B represents a longer exposure of the gel in panel A.

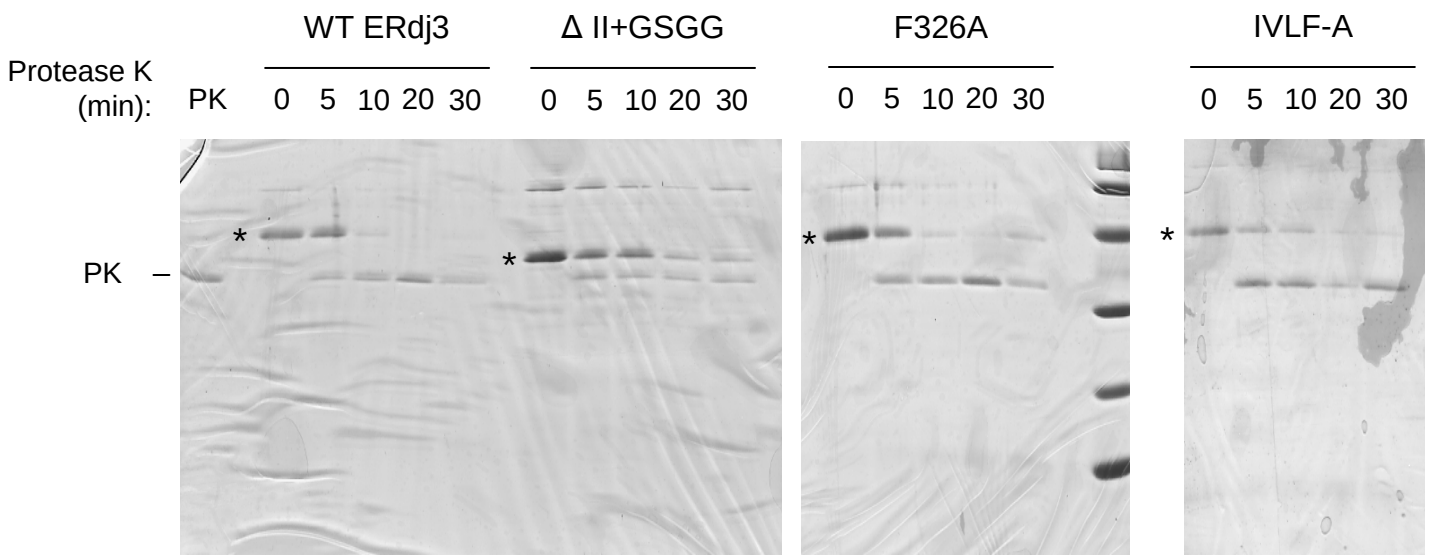

Figure 2S. Mutations that affect substrate binding do not alter overall structure of ERdj3 point mutants, but deletion of domain II decreases stability. The indicated recombinant ERdj3 proteins were incubated in the presence or absence of Proteinase K for the indicated times. Samples were analyzed by reducing SDS-PAGE. The band representing Proteinase K is shown and the migration of each full length mutant protein is indicated with an asterisk.

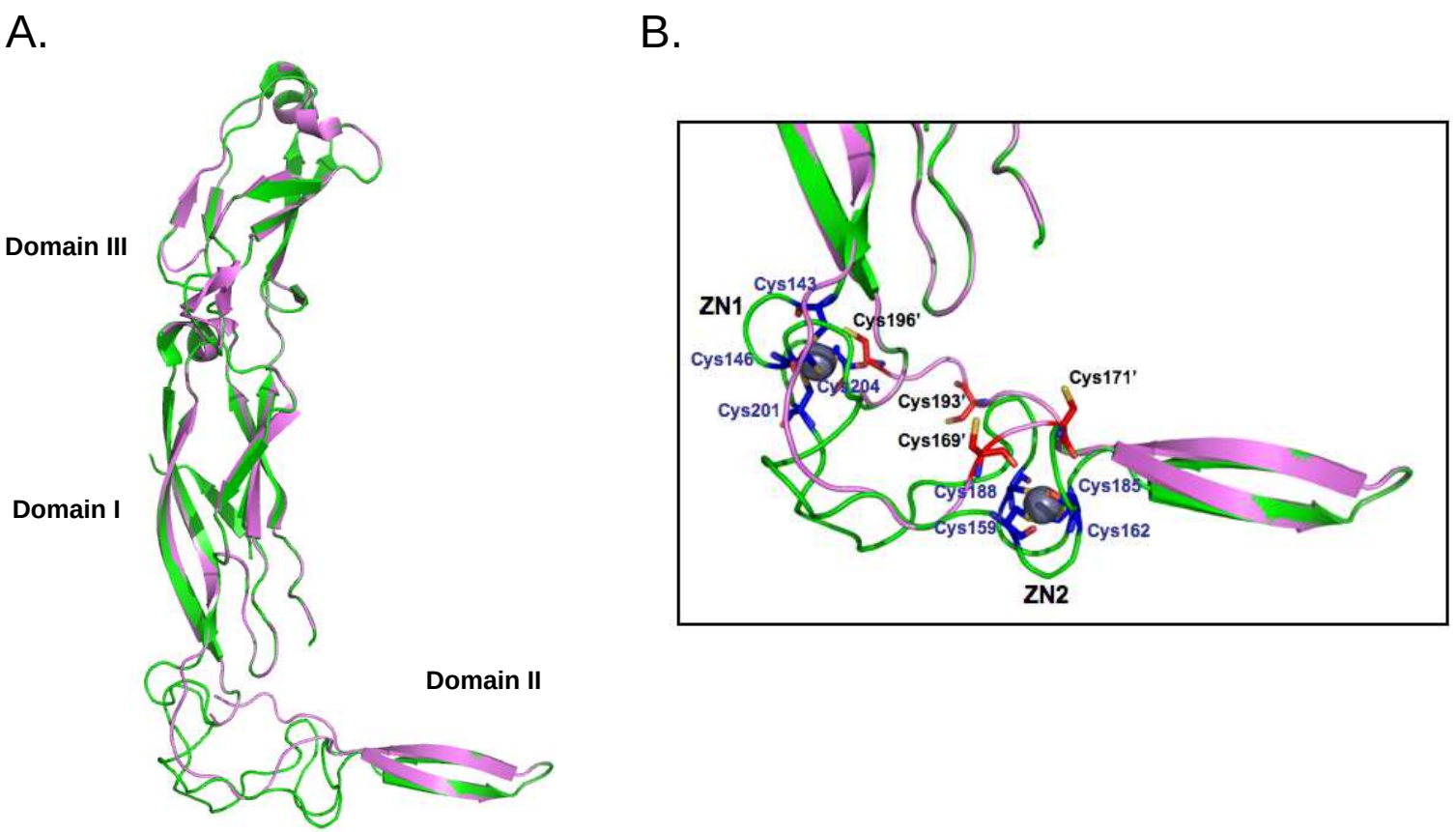

Figure 3S. Predicted structure of domains I-III of ERdj3. (A) The substrate binding domains of ERdj3 and Ydj1 may have a similar overall structure. Crystal structure of Ydj1 (PDB id 1NTL) is shown in green and the structure model of ERdj3 is in pink. The ERdj3 structure model is generated with SWISS_MODEL, using Ydj1 crystal structure (1NTL) as the template. The structures are aligned and displayed with PyMOL. **(B)** The region corresponding to domain II of Ydj1 and ERdj1 of the model are shown in close up. In both cases, two β sheets are predicted to form, although the flexible linkers are different. Cysteines that form two zinc binding sites on Ydj1 are displayed with side chains and highlighted in blue. Zinc atoms are shown in grey. The four cysteines on ERdj3 that form intradomain disulfide bonds *in vivo* are colored in red.