Supplementary Information

Active Site Gate Dynamics Modulate the Catalytic Activity of the Ubiquitination Enzyme E2-25K

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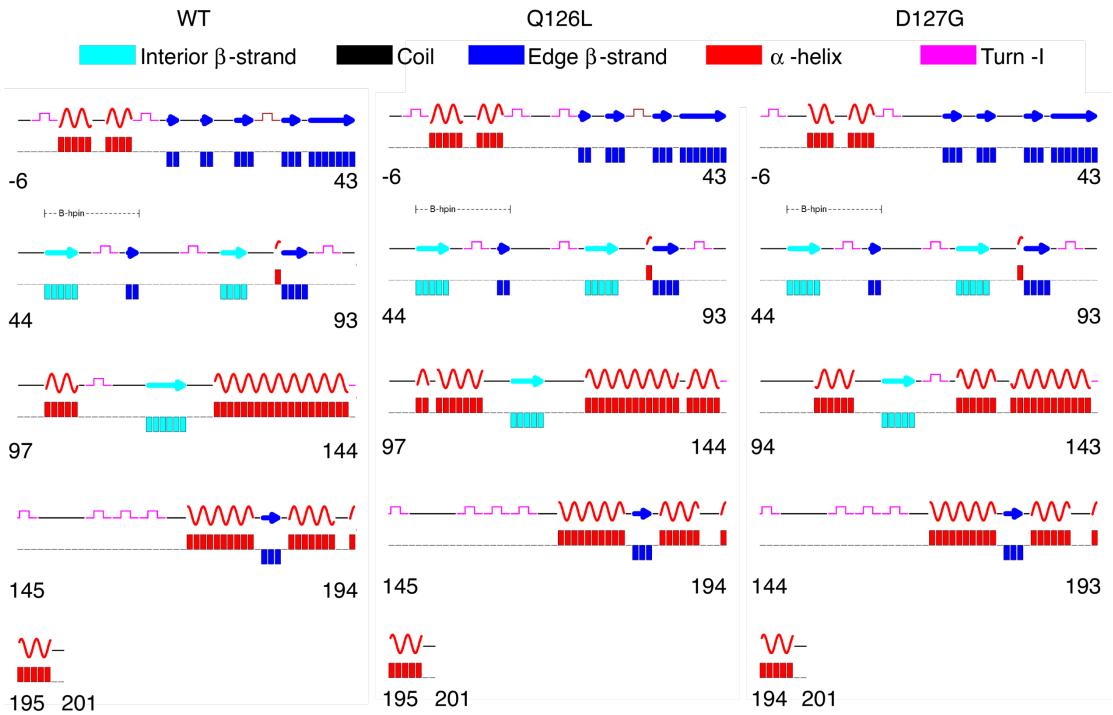
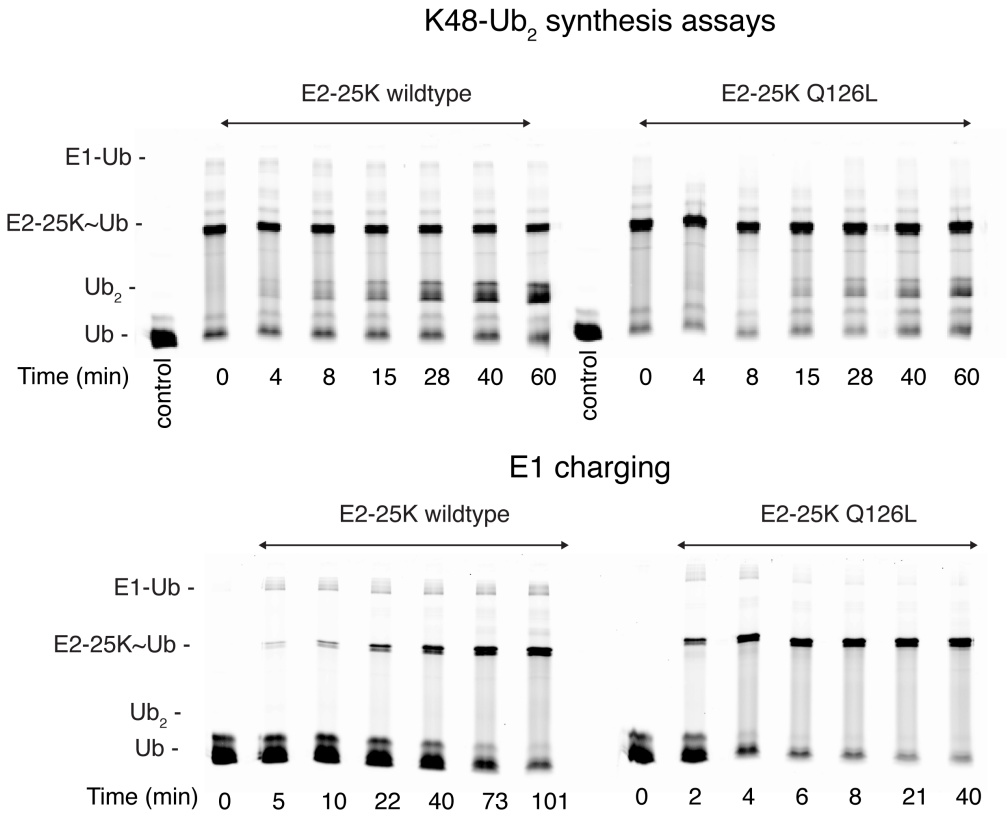
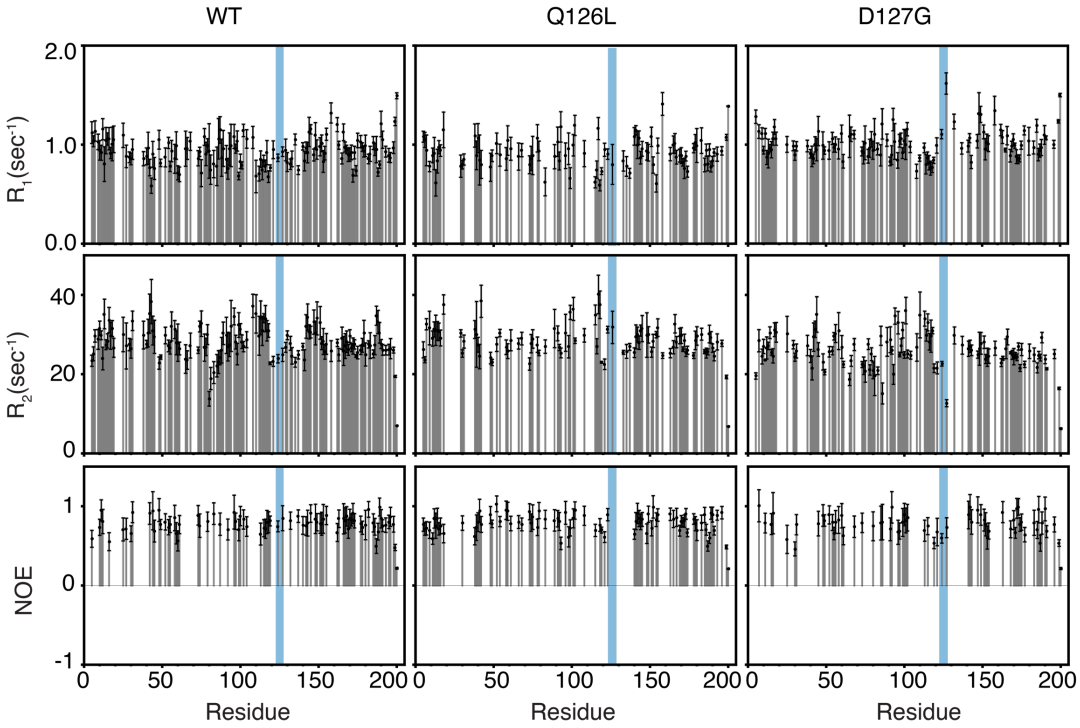


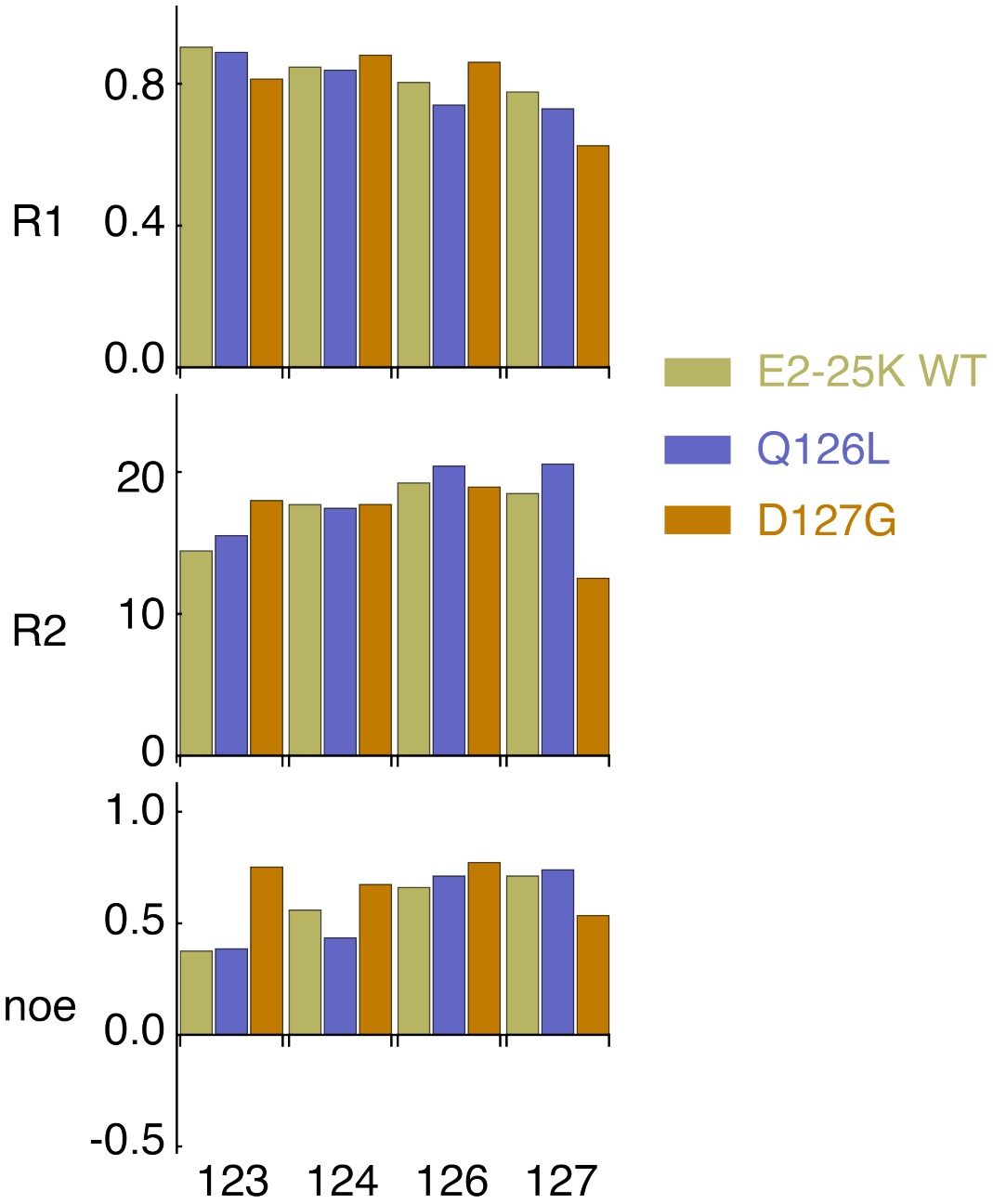
Supplementary Figure S1: Predicted secondary structures calculated using ^{15}N, $^{1}H^{N}$, CO and CA chemical shifts for wildtype, Q126L, and D127G E2-25K. The overall secondary structures of both the proteins remain similar to wildtype.



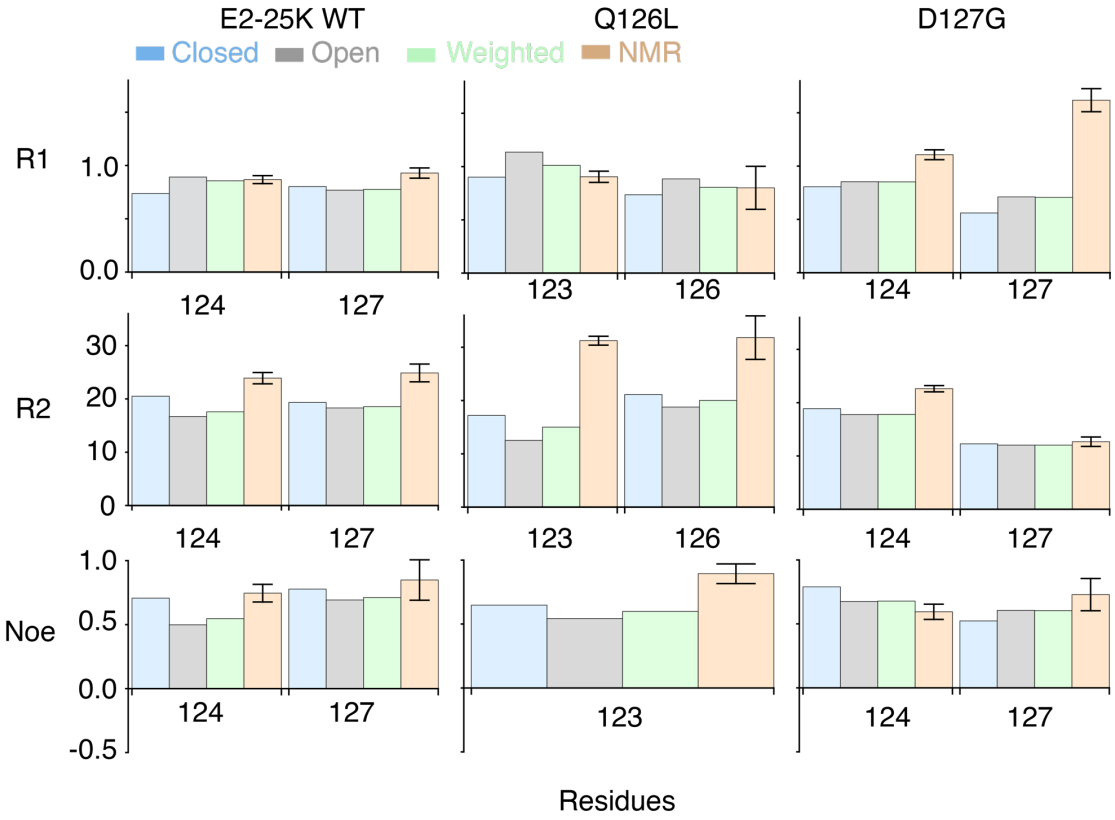
Supplementary Figure S2: Representative SDS-PAGE results for E2-25K enzyme assays for catalysis of the synthesis K48-Ub_2 chains (top) and E1-catalyzed charging of E2-25K to form a thioester with the C-terminus of Ub (bottom). Data for wildtype enzyme and the Q126L mutant are shown.



Supplementary Figure S3. 600 MHz backbone ^{15}N relaxation data for E2-25K wildtype, Q126L and D127G. Residues from 123-127 from the active site loop are highlighted with a blue bar.



Supplementary Figure S4. MD-derived 600 MHz backbone ^{15}N relaxation data for E2-25K wildtype, Q126L and D127G.



Supplementary Figure S5. Comparison of experimental and calculated 600 MHz backbone ^{15}N relaxation data for E2-25K wildtype, Q126L and D127G. The active site loop encompasses residues 123-127.