



**S7 Fig: Covalent bond formation assays of 3kptC active site mutants.**

(A)  $3kptC^C + 3kptC^T$ -MBP (wildtype interaction); (B)  $3kptC^C + 3kptC^T(N512A)$ -MBP; (C)  $3kptC^C(K417A) + 3kptC^T$ -MBP; (D)  $3kptC^C(E472Q) + 3kptC^T$ -MBP. The wildtype interaction showed a covalent product increasing over time whereas no covalent product was detected in the active site mutants. Comparative covalent bond formation assays were performed with  $10\mu\text{M}$  final concentration of the reactants. The reactive residues N512 in the  $3kptC^T$  and K417 and E472 in the  $3kptC^C$  were mutated and tested for covalent product formation via SDS-PAGE and subsequent Coomassie Brilliant blue staining. Samples were boiled at  $95^\circ\text{C}$  for 10min prior to gel loading. (A-D): lane 1: catcher input ( $10\mu\text{M}$ ), lane 2: tag input ( $10\mu\text{M}$ ), lane 3: 0h, lane 4: 1h, lane 5: 2h, lane 6: 3h, lane 7: 4h, lane 8: 24h). Same volume of samples were loaded. MW stands for molecular weight (kDa).