Supporting Information

Mechanistic insight into the transforming potential of SHP2/T507K mutation

Ruo-Yu Zhang[†], Zhi-Hong Yu[†], Lan Chen, Chad D. Walls, Sheng Zhang, Li Wu, and Zhong-Yin Zhang*

Departments of Medicinal Chemistry and Molecular Pharmacology and of Chemistry, Center for Cancer Research, and Institute for Drug Discovery, Purdue University, 720 Clinic Drive, West Lafayette, IN 47907.

[†]These authors contributed equally to this work.

*To whom correspondence should be addressed. Phone: (765) 494-1403; fax: (765) 494-1414; E-mail: zhang-zy@purdue.edu.

Figure S1

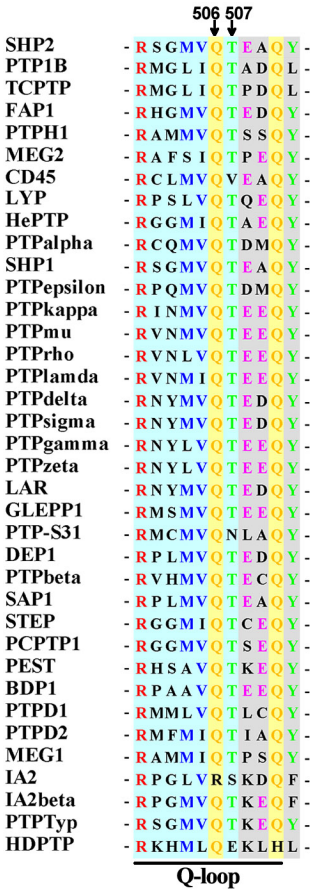

Figure S1. Sequence alignment of the Q-loop among classical PTPs. SHP2 Thr507 is highly conserved with a 89.2% (33 out of 37) identity among the 37 classical PTPs.

Figure S2

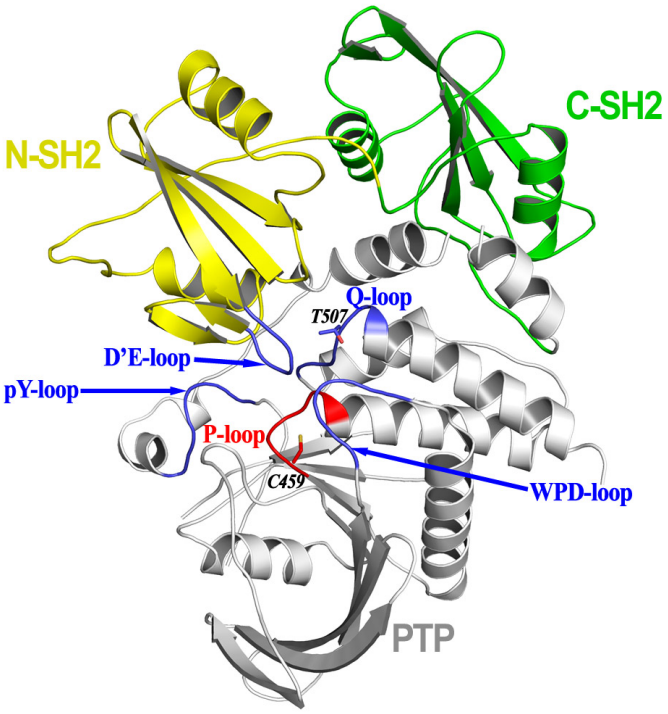

Figure S2. The SHP2 crystal structure in ribbon representation with key loops and residue Thr507 highlighted. Thr507 is located within the catalytic Q-loop and close to the D'E autoinhibition loop in the N-SH2 domain.

Figure S3

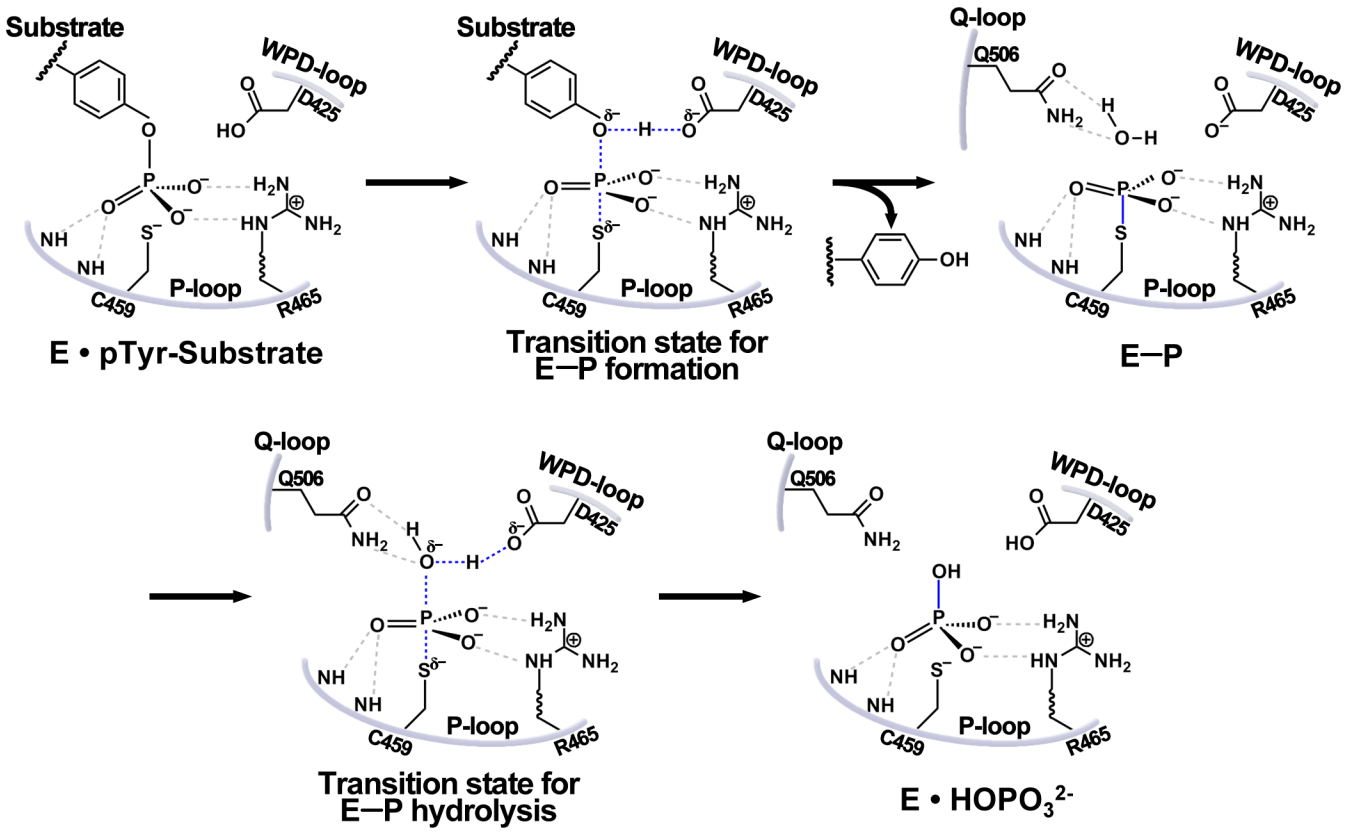

Figure S3. The chemical mechanism of SHP2 catalyzed reaction. Q506 located within the Q-loop is important for efficient hydrolysis of the phosphoenzyme intermediate, given its vital role of precisely holding both the general base Asp425 and the nucleophilic water in place.

Figure S4

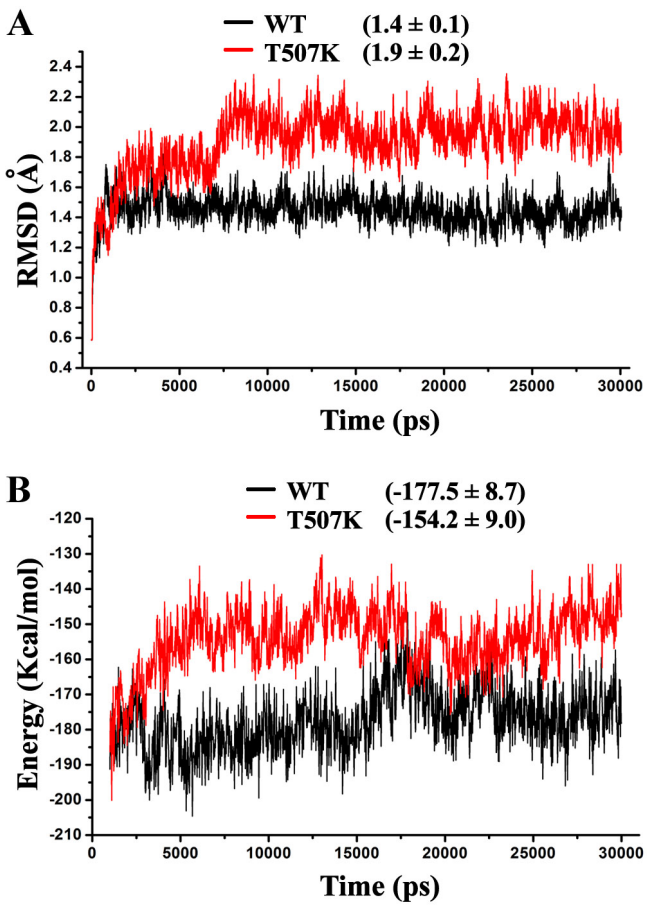

Figure S4. Molecular dynamics (MD) simulation-based N-SH2/PTP interdomain interaction energy calculation. (**A**) The plot of RMSD value of all backbone atoms during a 30-ns MD simulation. The relative larger mean and standard deviation value for SHP2/T507K suggest that it's more dynamic relative to wild-type SHP2. (**B**) The plot of N-SH2/PTP interdomain interaction energy. The N-SH2/PTP interaction energy is ~24 kcal/mol higher for the SHP2/T507K mutant, supporting the prediction from structural analyses that the T507K mutation would weaken the N-SH2/PTP interaction due to SH2 domain shifting away from the PTP domain.

Figure S5

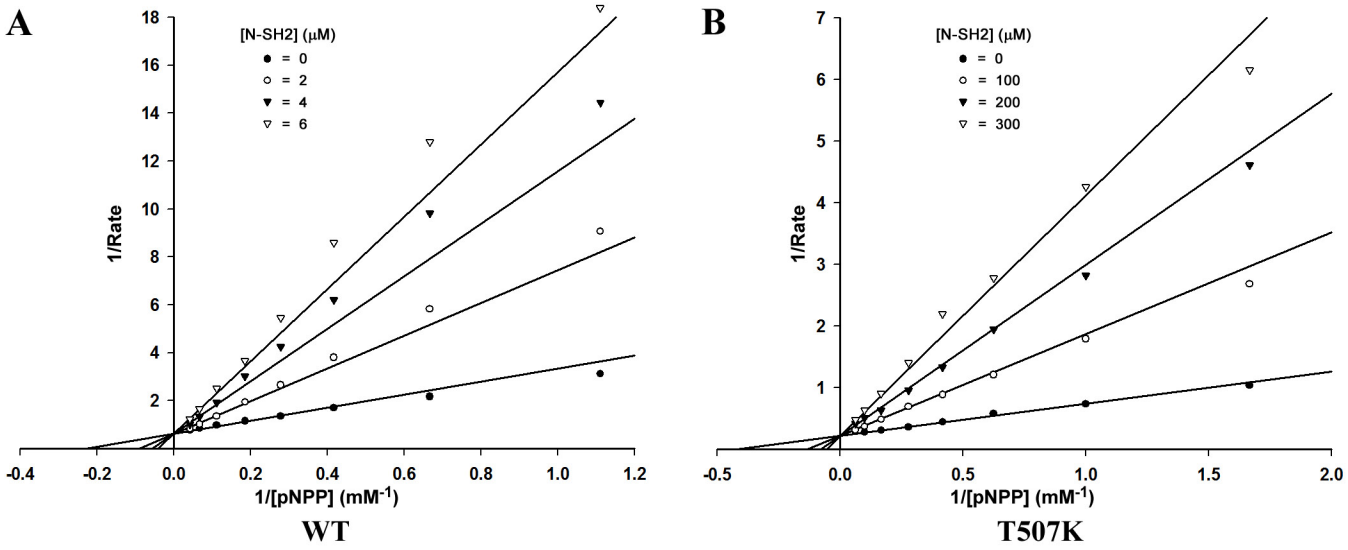

Figure S5. The Lineweaver-Burk plot of N-SH2 domain inhibiting (**A**) WT or (**B**) T507K mutant catalytic domain catalyzed *p*NPP hydrolysis. The isolated N-SH2 domain acts as a competitive inhibitor against the wild-type SHP2 or T507K mutant catalytic domain catalyzed *p*NPP hydrolysis with a K_i value of 1.3 ± 0.1 μM and 46.3 ± 1.9 μM, respectively.

Figure S6

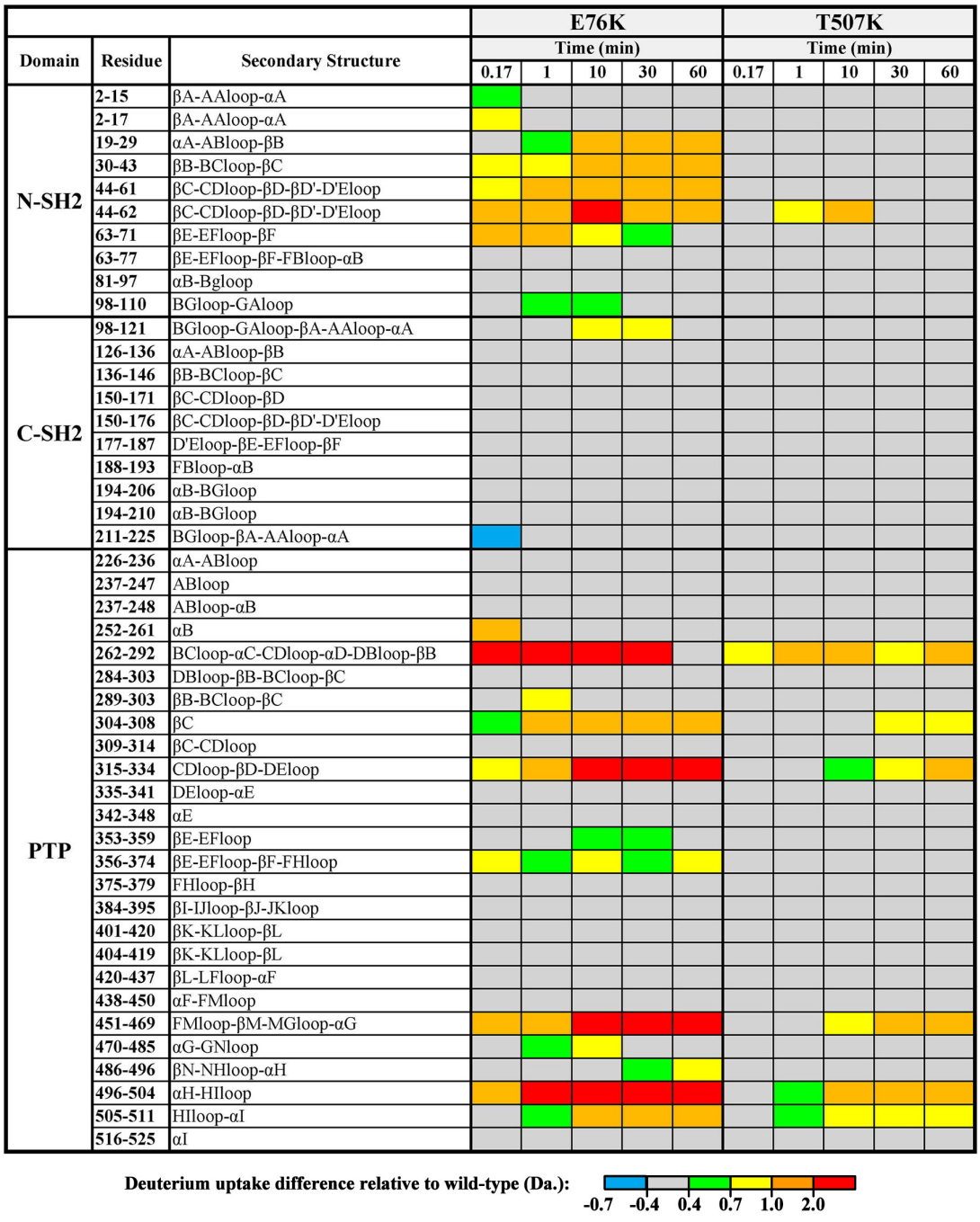

Figure S6. Two dimensional "heat map" for the E76K and T507K mutants displaying differential H/D exchange relative to the wild-type.

Figure S7

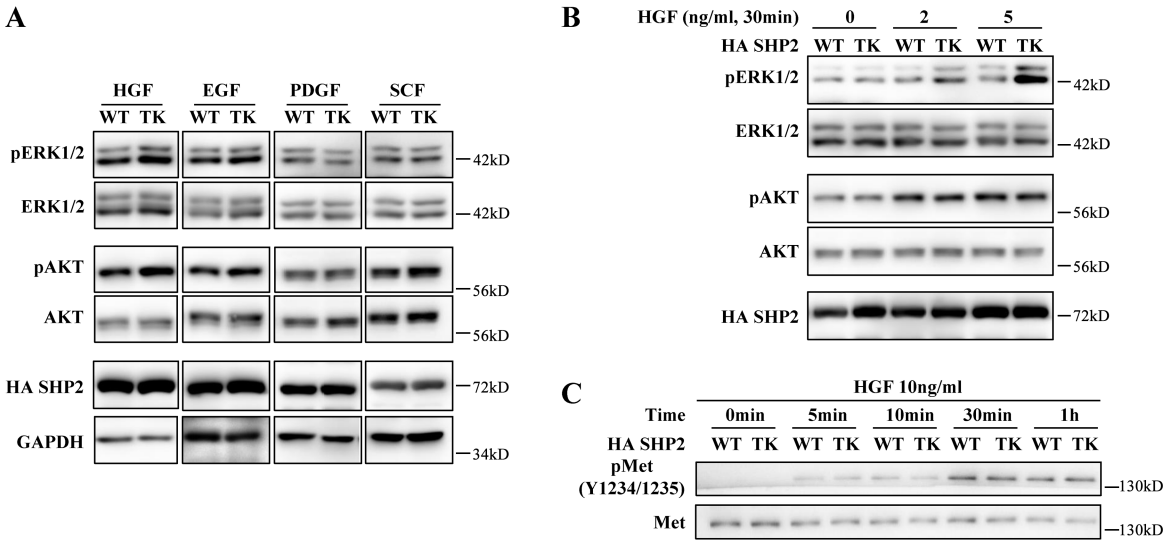

Figure S7. (**A**). SHP2/T507K mutant shows the most overactivation (vs. wild-type SHP2) of ERK1/2 and AKT upon HGF stimulation. Wild-type SHP2 or SHP2/T507K transiently transfected HEK293 cells were stimulated with 10n g/ml HGF, 5 ng/ml EGF, 100 ng/ml PDGF or 100 ng/ml SCF. (**B**). SHP2/T507K mutant is more sensitive to HGF stimulation. (**C**). SHP2/T507K mutant does not alter Met expression or activation as measured by Met phosphorylation at Y1234/1235.

Figure S8

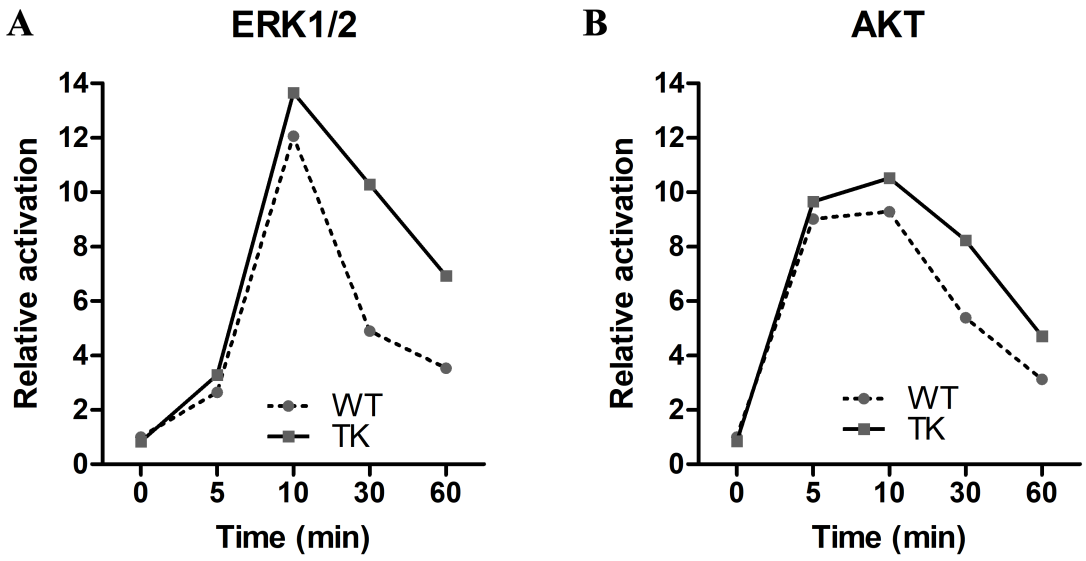

Figure S8. The kinetics for the HGF induced activation of ERK1/2 (**A**) and AKT (**B**) in wild-type SHP2 and SHP2/T507K cells. The ratio of pERK/ERK (or pAKT/AKT) for WT at time 0 (i.e. the first lane) in Figure 5A was set as a reference, then the corresponding ratio for all other lanes were normalized to the reference to obtain the relative activation (i.e. the fold change), which was plotted along time for WT and T507K for comparison.

Figure S9

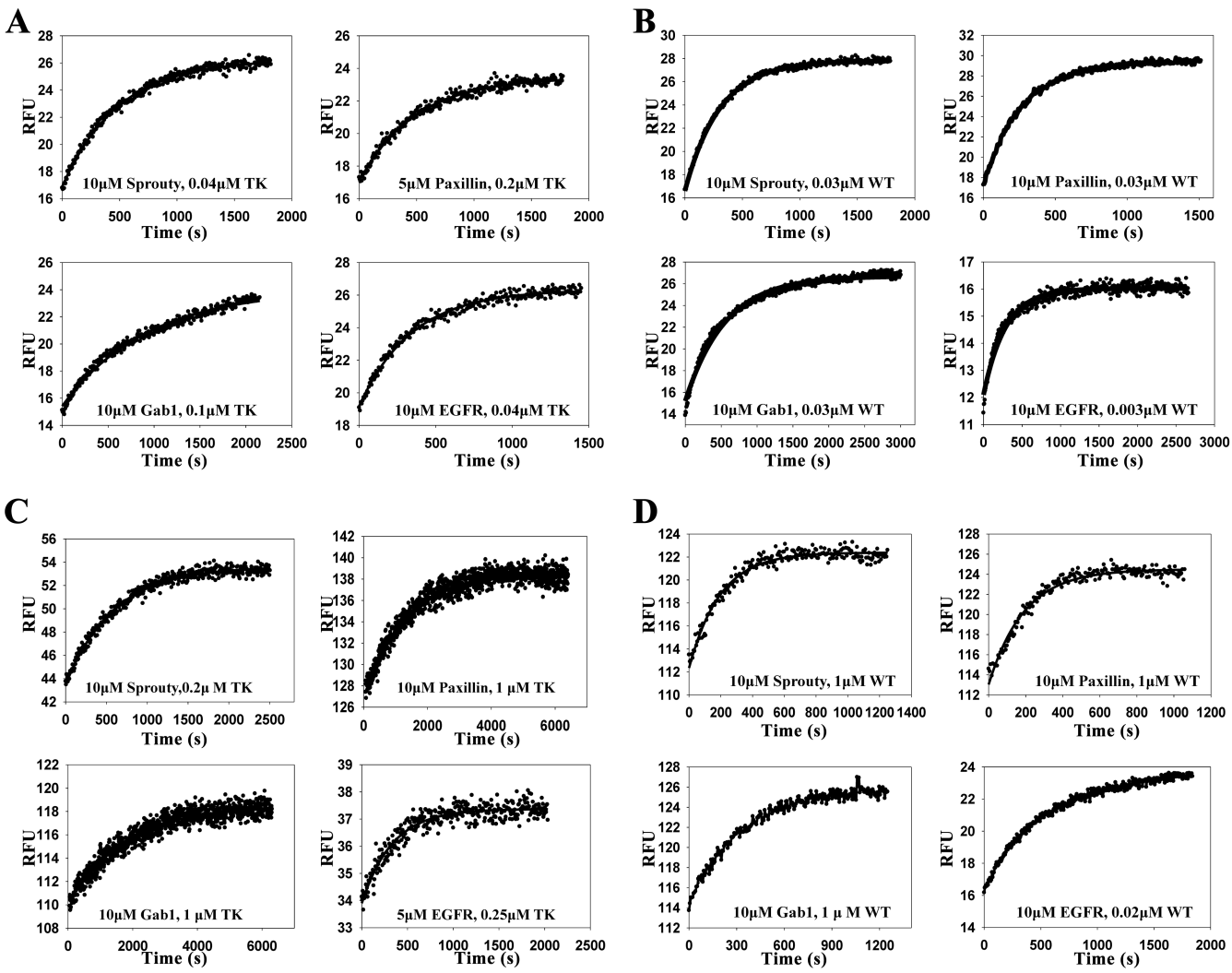

Figure S9. The reaction progress curves for SHP2/T507K mutant CD (**A**), wild-type SHP2 CD (**B**), SHP2/T507K mutant FL (**C**), or wild-type SHP2 FL (**D**) catalyzed dephosphorylation of pY-peptides from potential SHP2 substrates. The fluorescence of the dephosphorylated peptide product at 305 nm with excitation at 280 nm was monitored on a Perkin Elmer Luminescence Spectrometer LS50B.