

Supplemental Figures

Figure S1A. Representative phosphopeptides identified by mass spectrometry analysis.

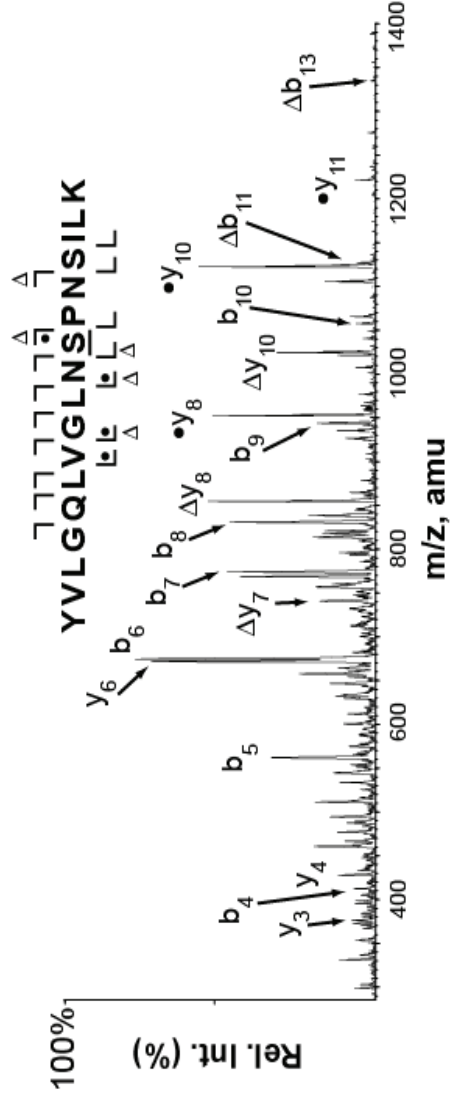
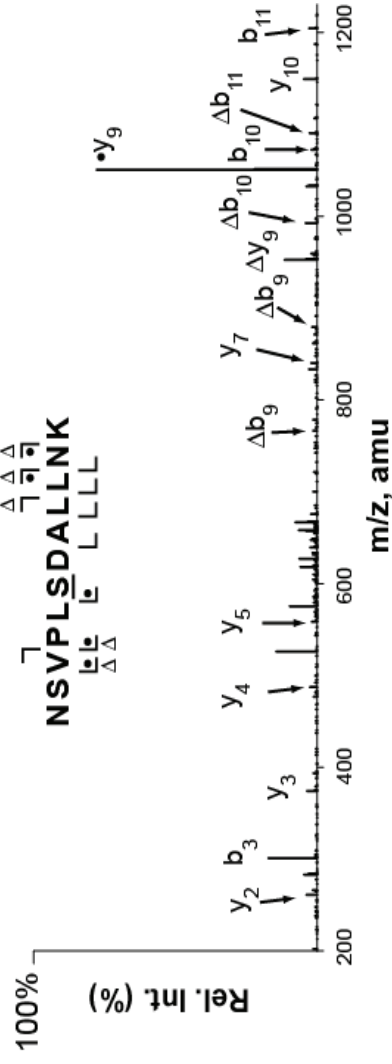
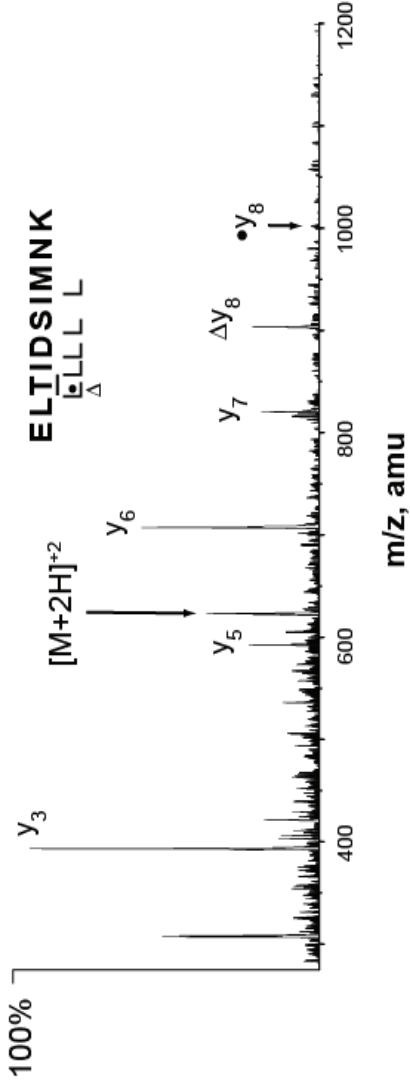
Figure S1B. A list of phosphopeptides identified in kinase-deficient Mps1 mutant in the presence and absence of ATP. The number of phosphopeptides identified is identical with or without ATP incubation.

Figure S2. Mutation of the N-terminal autophosphorylation sites does not affect Mps1 kinase activity. Flag-tagged Mps1, Mps1KD and Mps1^{T12S15→AA} were cotransfected with Flag-Smad2 into 293T cells. Forty-eight hours after transfection cells were lysed and blotted with phospho-Smad2 and Flag antibodies.

Figure S3. Phosphorylation of wild-type or T12 & S15 mutant Mps1 in nocodazole arrested mitotic cells. SW480 cells expressing wild-type or mutant T12S15 Mps1 were synchronized by double thymidine treatment and released into phosphate-free media containing nocodazole for 4h before labeling with 1 mCi/ml ³²P-orthophosphate for 4h. Phosphopeptide mapping analysis was performed with wild-type and mutant Mps1. (A) Wild-type and mutant YFP-Mps1 were immunoprecipitated from cell lysates and analyzed by SDS-PAGE. (B) Illustration of two dimensional peptide mapping analysis. The labeled spot that is present in YFP-Mps1 but missing in YFP-Mps1^{T12S15→AA} is by arrows. (a) 2D phosphopeptide analysis of YFP-Mps1. (b) 2D phosphopeptide analysis of YFP-Mps1^{T12S15→AA}.

Figure S4. A proposed model for regulation of kinetochore targeting of Mps1 by phosphorylation. Upon mitotic entry, Mps1 forms a dimer or oligomers which result in autophosphorylation of Mps1 at both N-terminus and C-terminus of protein. Kinetochore targeting signaling located at the N-terminal region of Mps1 is exposed upon phosphorylation and recognized by unidentified factor(s) that mediate

kinetochore relocation of Mps1 by interacting with Hec1/Ndc80 complex localized on the outer kinetochores.



Summary of Mps1KD Phosphopeptides

Peptide	Sites identified
²⁰² NLSASTVL T AQESFSGSLGHLQNR ²²⁵	S210
²⁷⁴ VPVNL LNS PDVDK ²⁸⁷	S281
⁴⁵³ T PSSNTLDDYMSCFR ⁴⁶⁷	T453
⁸¹¹ YVLGQLVGLN S PNSILK ⁸²⁷	S821

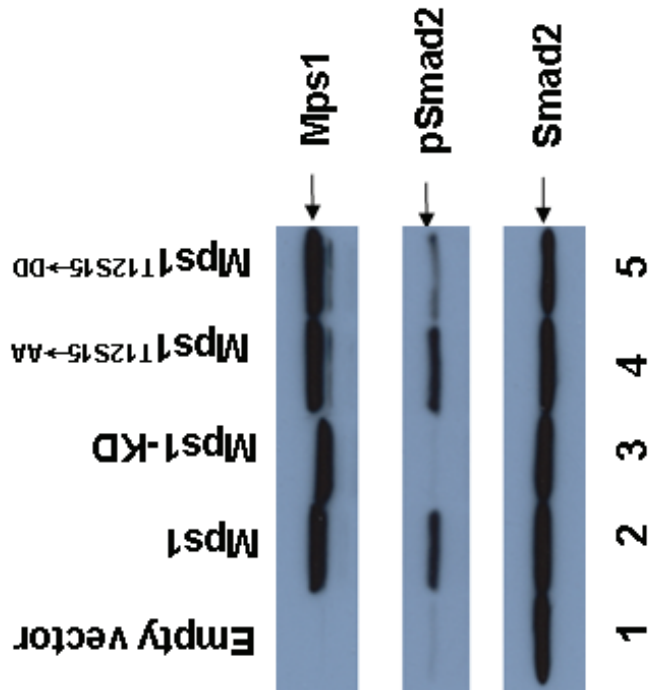


Figure S2

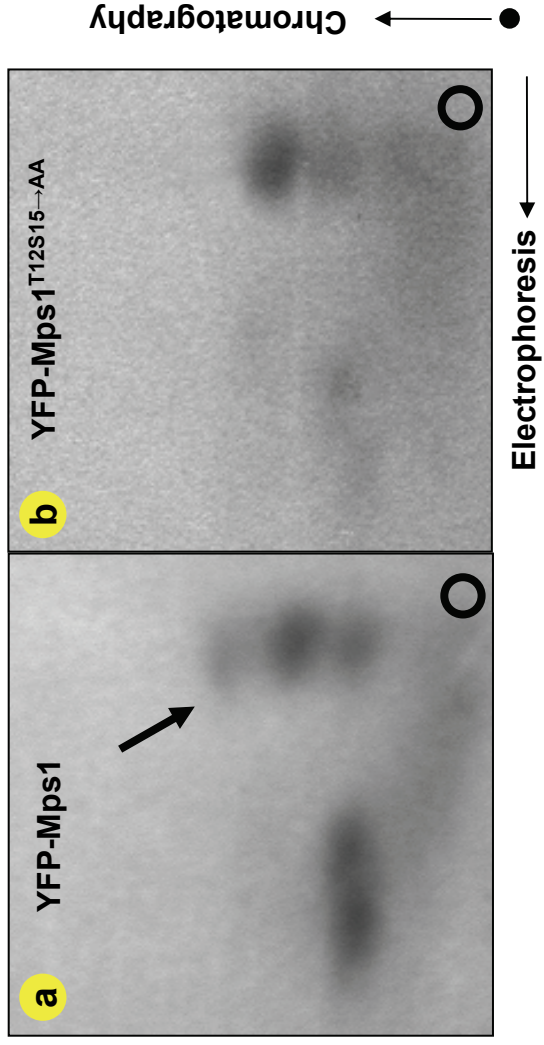
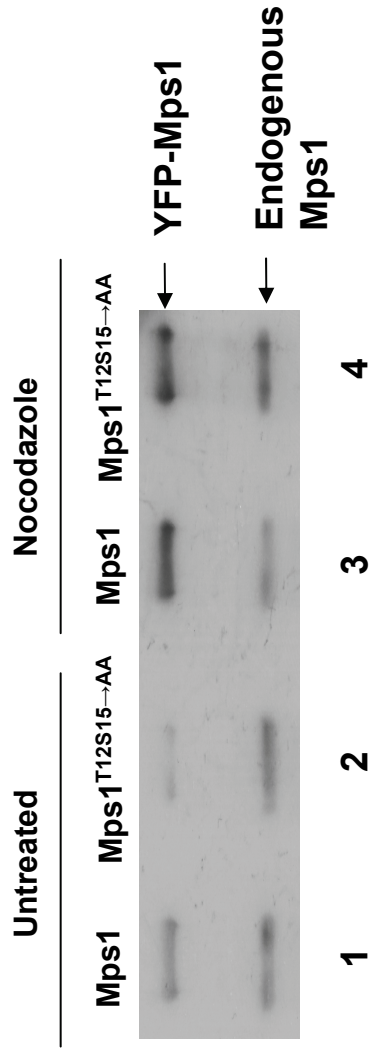


Figure S3

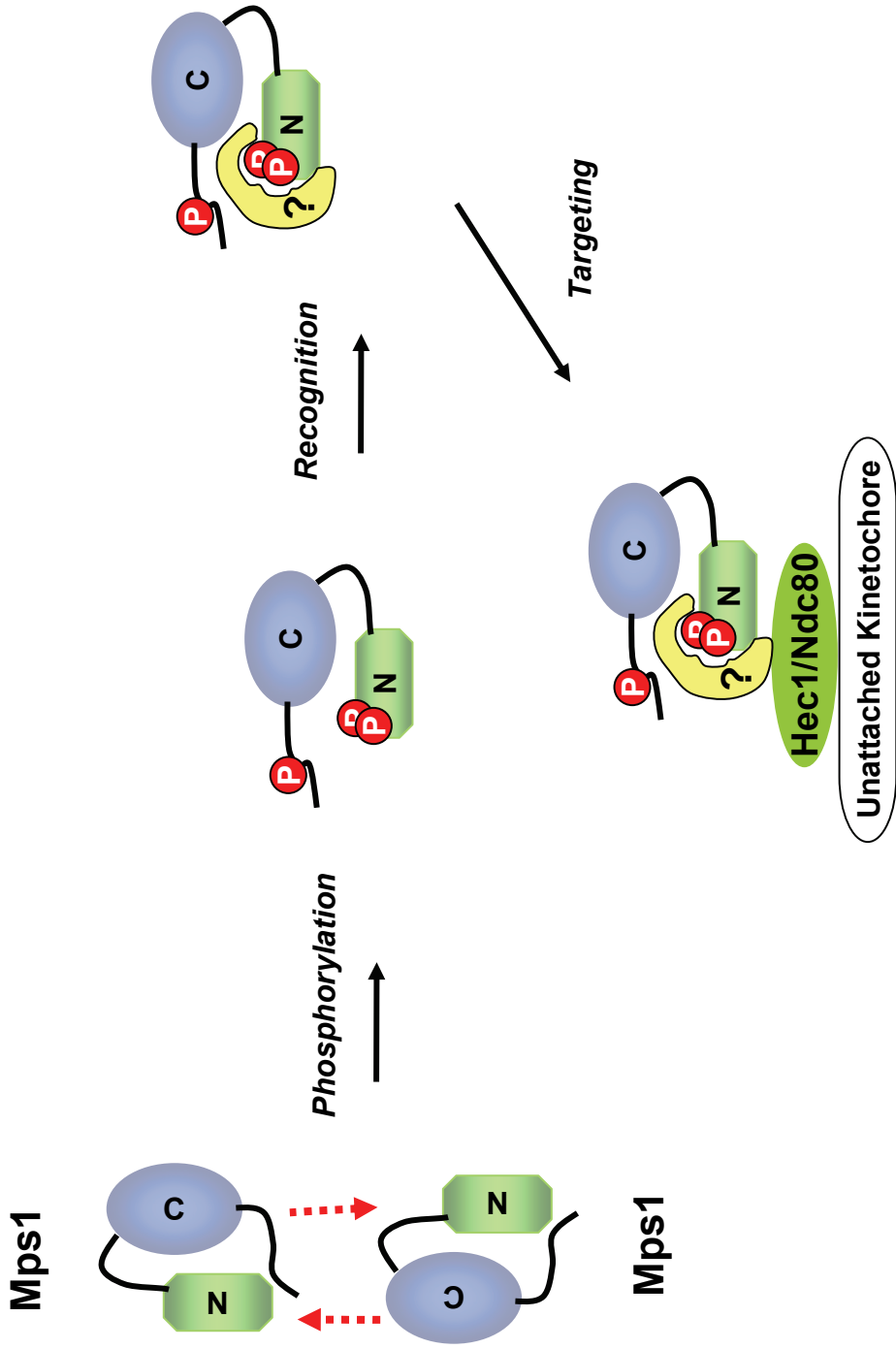


Figure S4