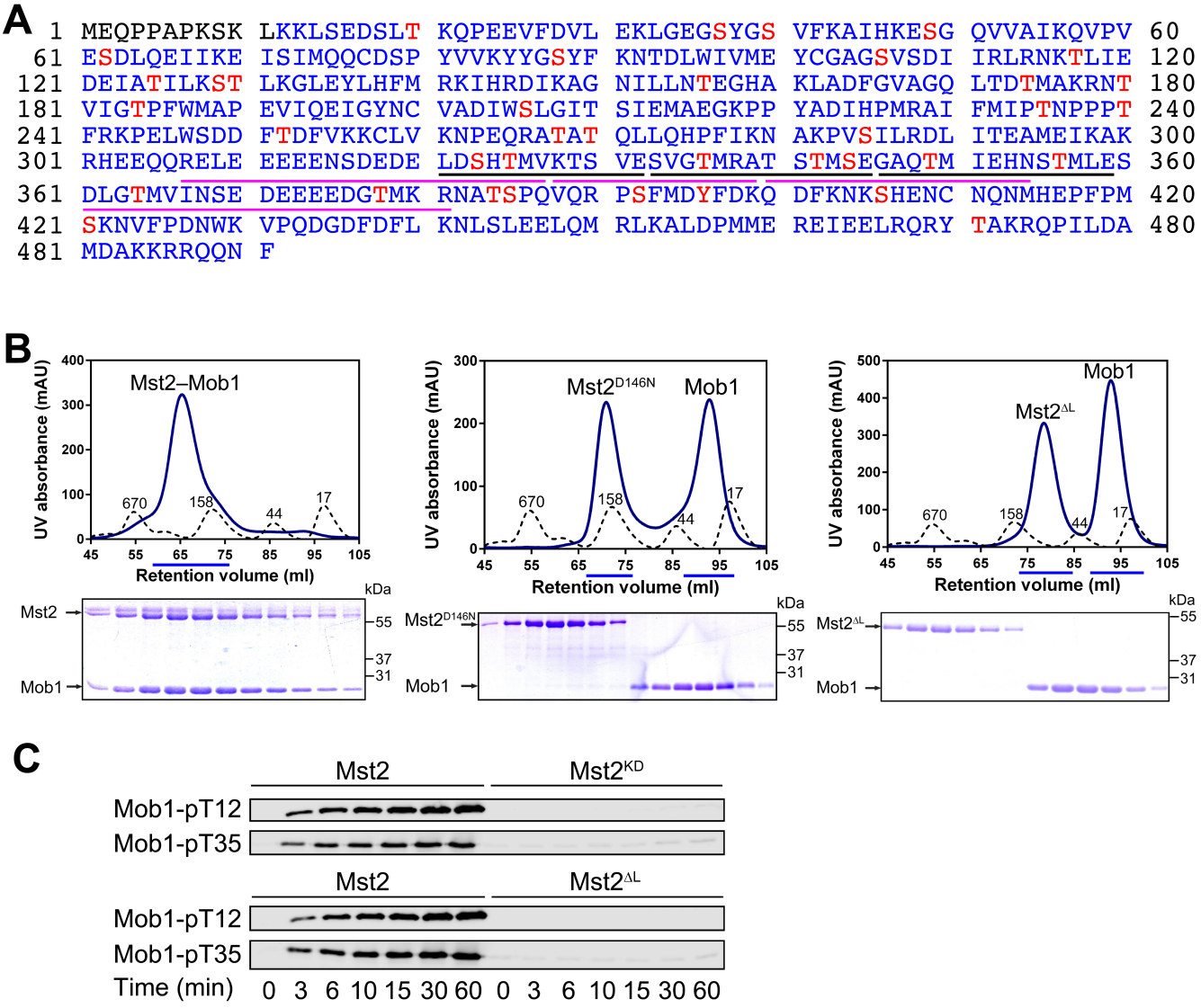
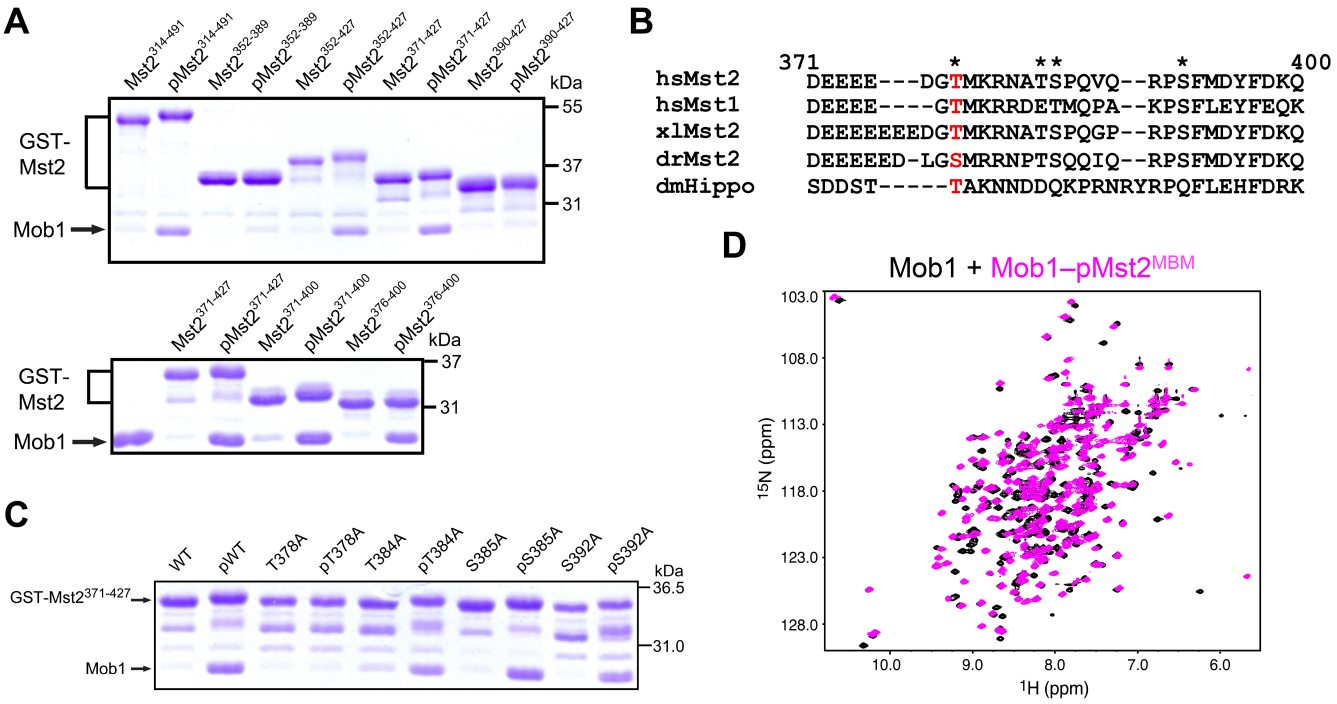
Supplemental Figures and Tables

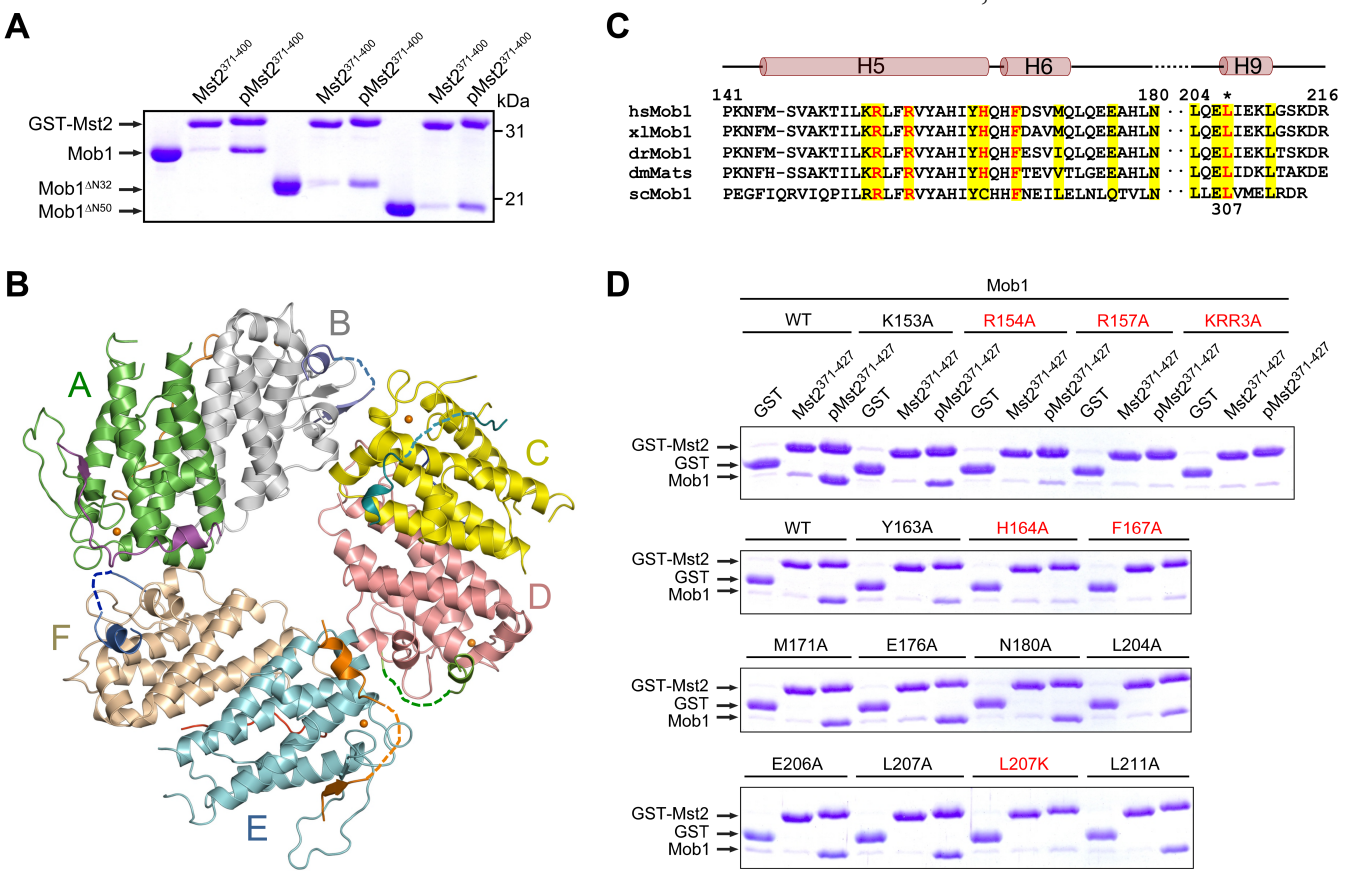


Supplemental Figure 1. Binding of Mob1 to the autophosphorylated Mst2 linker promotes Mob1 phosphorylation by Mst2. (*A*) Summary of the mass spectrometry analysis of recombinant full-length human Mst2. The Mst2 sequence covered by the identified peptides is colored blue. Identified phosphorylation sites (all sites with ModLS >29 and ID probability >95%) are colored red. To obtain more complete protein coverage, we used two proteases (elastase and Glu-C) to digest Mst2. For the linker region, the peptides identified from the elastase digest and the Glu-C digest are indicated by magenta and black lines, respectively. The total protein coverage is 97.7%. (*B*) UV traces of molecular weight standards (dashed line) and the indicated Mst2 proteins mixed with Mob1 at 1:2 molar ratio

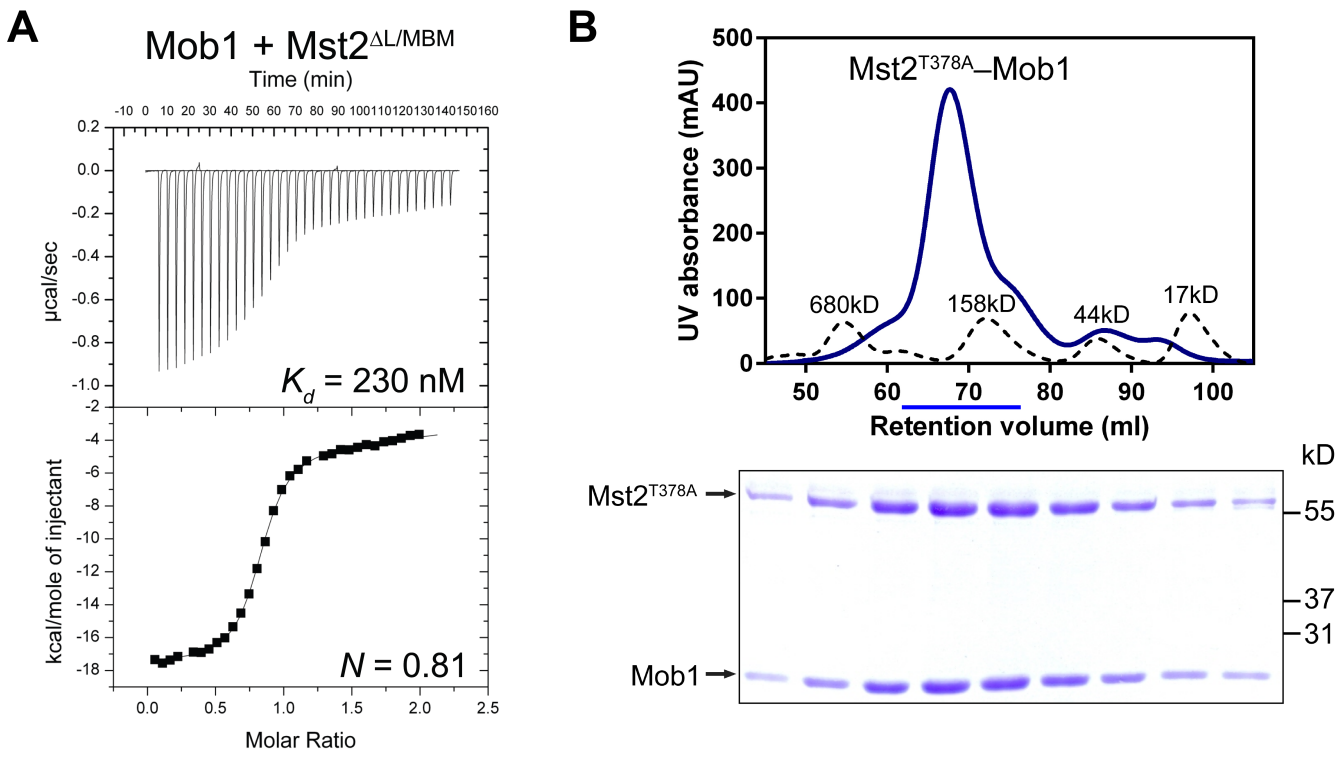
(solid line) fractionated on a Superdex 200 column. The underlined fractions were separated on SDS-PAGE and stained with Coomassie. (*C*) Quantitative immunoblots of kinase reactions containing Mob1 and the indicated Mst2 proteins. KD, kinase domain.



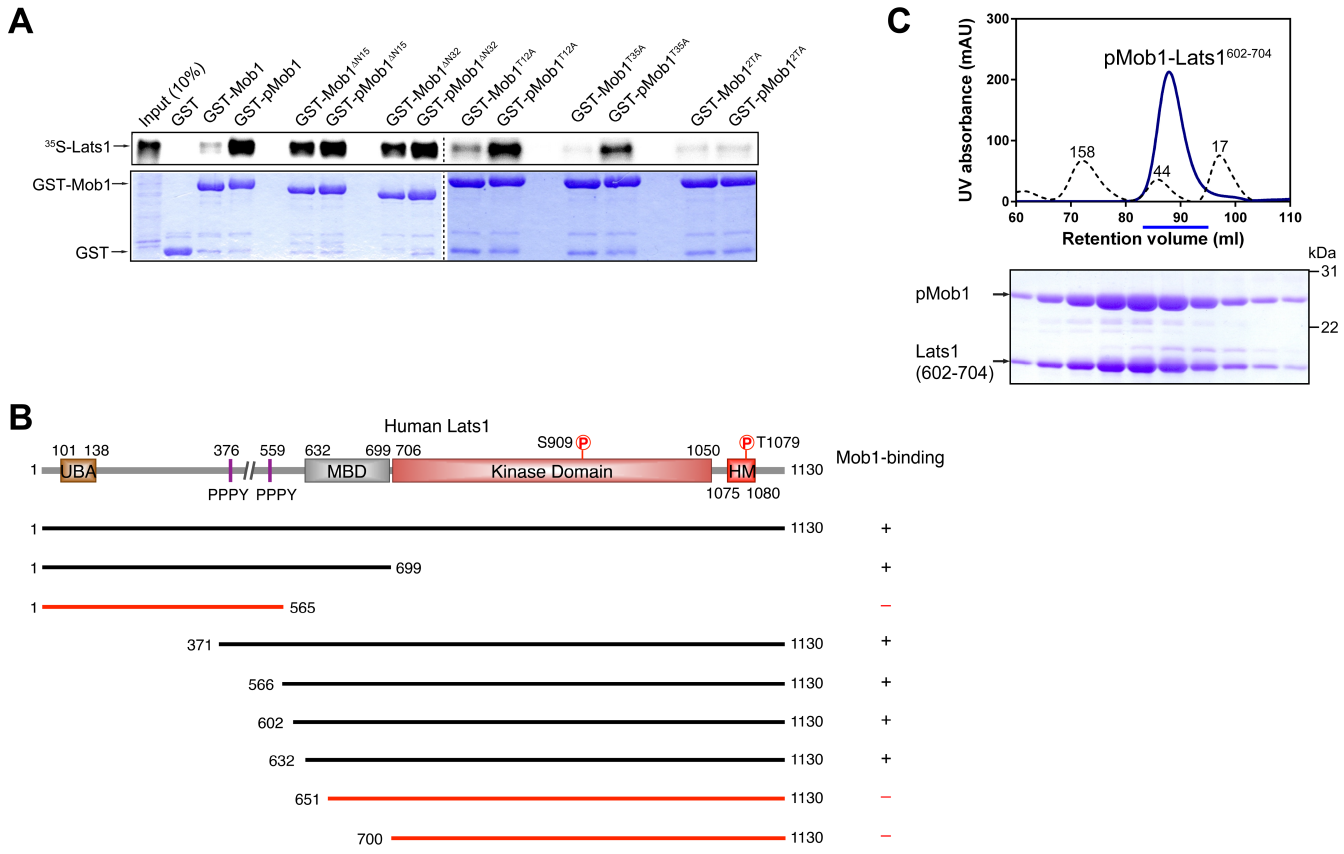
Supplemental Figure 2. Phosphorylated Mob1-binding motif (MBM) of Mst2 is sufficient for Mob1 binding. (*A*) Coomassie stained gel of Mob1 bound to beads containing the indicated GST-Mst2 and phospho-Mst2 (pMst2) proteins. The positions of GST-Mst2 proteins and Mob1 are indicated. (*B*) Sequence alignment of the Mob1-binding motif (MBM) of metazoan Mst2 proteins. Human Mst2 residue numbers are labeled. Four S/T residues are indicated with asterisks. The conserved residue T378 is colored red. (*C*) Coomassie stained gel of Mob1 bound to beads containing the indicated GST-Mst2 and phospho-Mst2 (pMst2) proteins. The positions of GST-Mst2 proteins and Mob1 are indicated. (*D*) Overlay of $^{1}H/^{15}N$ HSQC spectra of ^{15}N-labeled Mob1 (black) and ^{15}N-labeled Mob1 bound to unlabeled $pMst2^{MBM}$ (magenta).



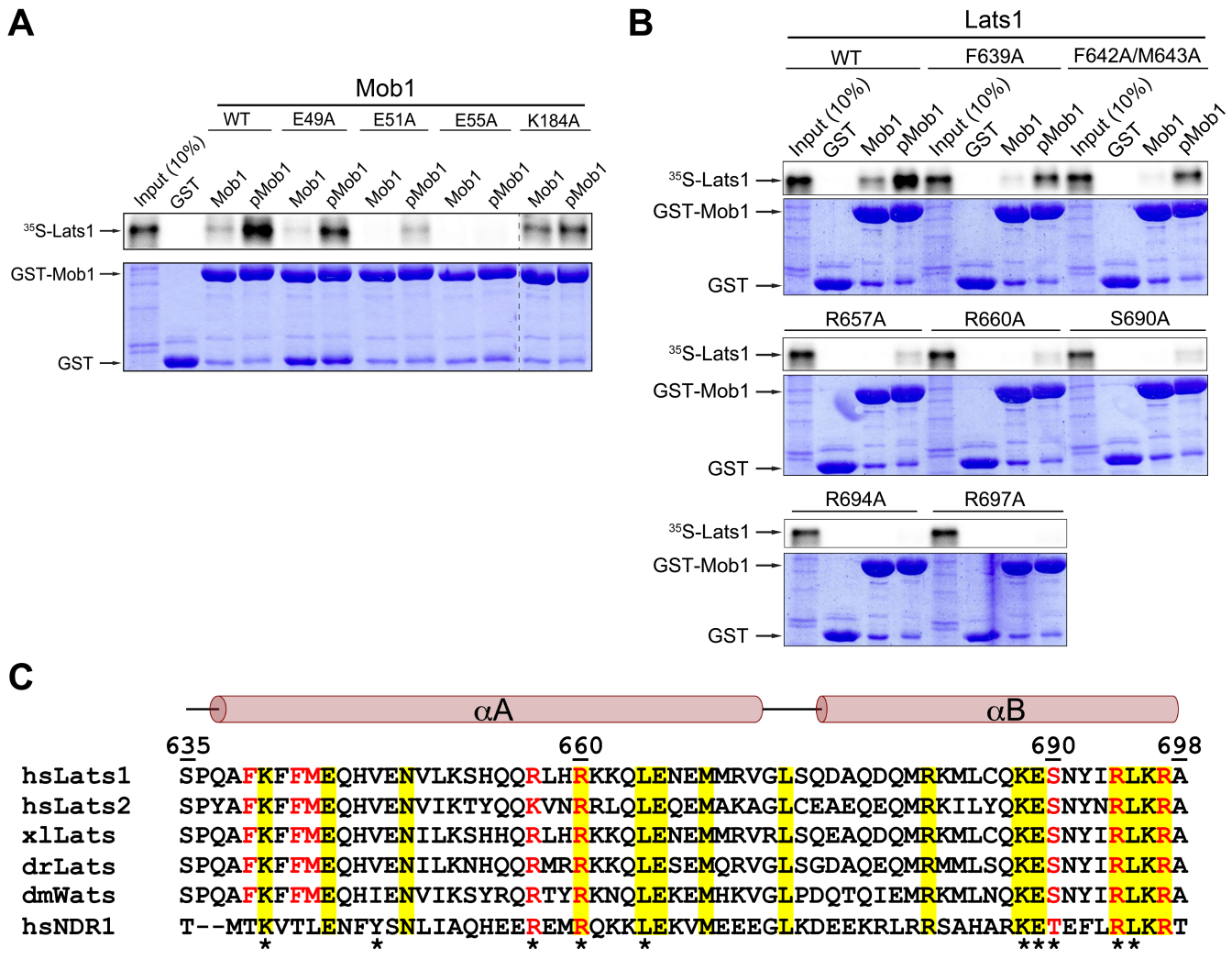
Supplemental Figure 3. The pT and HS binding sites of Mob1 are critical for pMst2 binding. (*A*) Coomassie stained gel of the indicated Mob1 proteins bound to beads containing the indicated GST-Mst2 and phospho-Mst2 (pMst2) proteins. The positions of GST-Mst2 and Mob1 proteins are indicated. (*B*) Cartoon drawing of six protomers in the asymmetric unit of Mob1–pMst2. Only the pMst2 peptide in protomer A had clear electron density for the connecting loop between the pTM and HS motifs. The connecting loops for the other five pMst2 peptides in protomers B to F were not visible and shown as dashed lines. (*C*) Sequence alignment of the pT (H5) and HS (H6 and H9) binding sites of Mob1. The secondary structure elements of Mob1 are drawn above the sequences and colored in salmon. Mutated Mob1 residues are colored yellow. Mutations that abolished pMst2 binding are colored red. Residue 307 in yeast Mob1 is labeled with an asterisk. (*D*) Coomassie stained gel of the indicated Mob1 proteins bound to beads containing the indicated GST, GST-Mst2, and phospho-Mst2 (pMst2) proteins. The positions of GST, GST-Mst2, and Mob1 proteins are indicated.



Supplemental Figure 4. The Mst2 linker contains multiple functional Mob1-binding elements. (*A*) ITC curves of the binding between Mob1 and Mst2$^{\Delta L/MBM}$, with K_d and binding stoichiometry (*N*) indicated. (*B*) UV traces of molecular weight standards (dashed line) and the Mst2^{T378A} protein mixed with Mob1 at 1:2 molar ratio (solid line) fractionated on a Superdex 200 column. The underlined fractions were separated on SDS-PAGE and stained with Coomassie.



Supplemental Figure 5. Interactions between pMob1 and Lats1. (*A*) Autoradiograph of ^{35}S-labeled Lats1 bound to beads coupled with the indicated Mob1 proteins (top panel). The positions of GST and GST-Mob1 proteins on the Coomassie-stained SDS-PAGE gel are indicated (bottom panel). (*B*) Schematic drawing of Lats1 fragments used in this study. The Mob1-binding activities of these Lats1 fragments were summarized on the right. Lats1 fragments deficient in Mob1-binding are colored red. (*C*) UV traces of molecular weight standards (dashed line) and the indicated pMob1–Lats1 complex (solid line) fractionated on a Superdex 200 column. The underlined fractions were separated on SDS-PAGE and stained with Coomassie.



Supplemental Figure 6. Binding interface of pMob1 and Lats1. (*A*) Autoradiograph of ^{35}S-labeled Lats1 bound to beads coupled with the indicated Mob1 proteins (top panel). The positions of GST and GST-Mob1 proteins on the Coomassie-stained SDS-PAGE gel are indicated (bottom panel). (*B*) Autoradiograph of the indicated ^{35}S-labeled Lats1 proteins bound to beads coupled with the indicated Mob1 proteins (top panel). The positions of GST and GST-Mob1 proteins on the Coomassie-stained SDS-PAGE gel are indicated (bottom panel). (*C*) Sequence alignment of the Mob1-binding domain (MBD) of Lats1. Secondary structures of Lats1 MBD are drawn above the sequences and colored in salmon. Identical residues in the alignment are colored yellow. Lats1 mutants used in this study are colored red. The human NDR1 mutants that were shown to affect its kinase activity are labeled with asterisks.

Table S1. Data collection and refinement statistics for pMst2–Mob1 structure

Data collection	
Space group	$P3_121$
Wavelength (Å)	0.97918
Unit cell	
a, b, c (Å)	142.19, 142.19, 135.98
β (°)	120.0
Resolution range (Å)	40.00 – 2.65 (2.70 – 2.65)
Unique reflections	46,880 (2,285)
Multiplicity	11.0 (5.9)
Data completeness (%)	99.5 (97.1)
R_{merge} (%)[a]	9.0 (100.0)
I/σ (I)	25.5 (1.8)
Wilson B-value (Å^2)	38.3
Refinement statistics	
Resolution range (Å)	39.29 – 2.64 (2.71 – 2.64)
No. of reflections R_{work}/R_{free}	42,216/2,440 (1,581/79)
Data completeness (%)	89.7 (50.0)
Atoms (non-H protein/solvent/metal)	8,906/85/6
R_{work} (%)	23.7 (31.7)
R_{free} (%)	27.4 (35.2)
R.m.s.d. bond length (Å)	0.009
R.m.s.d. bond angle (°)	1.11
Mean B-value (Å^2) (protein/solvent/metal)	54.8/48.8/56.9
Ramachandran plot (%) (favored/additional/disallowed)[b]	96.9/3.0/0.1

Statistics for the highest-resolution shell are shown in parentheses.

[a]$R_{merge} = 100\ \Sigma_h\Sigma_i|I_{h,i} - \langle I_h\rangle|/\Sigma_h\Sigma_i I_{h,i}$, where the outer sum (h) is over the unique reflections and the inner sum (i) is over the set of independent observations of each unique reflection.

[b]As defined by the validation suite MolProbity.

Table S2. Data collection, structure determination and refinement statistics for pMob1–Lats1 structure

Data collection	
Crystal	SeMet (Peak)[a]
Space group	C2
Wavelength (Å)	0.97915
Resolution range (Å)	50.00 – 2.30 (2.34 – 2.30)
Unique reflections	15,456 (625)
Multiplicity	8.0 (5.9)
Data completeness (%)	95.9 (80.1)
R_{merge} (%)[b]	12.6 (42.8)
I/σ (I)	16.5 (1.8)
Wilson B-value (Å^2)	27.7
Phase determination	
Anomalous scatterer	selenium (5 of 5 possible sites)
Figure of merit (50-2.30 Å)	0.39
Refinement statistics	
Resolution range (Å)	41.06 – 2.29 (2.34 – 2.29)
No. of reflections R_{work}/R_{free}	13,818/1381 (589/71)
Data completeness (%)	80.9 (37.0)
Atoms (non-H protein/solvent/metal)	2,133/64/1
R_{work} (%)	18.6 (21.6)
R_{free} (%)	22.1 (25.9)
R.m.s.d. bond length (Å)	0.004
R.m.s.d. bond angle (°)	0.80
Mean B-value (Å^2) (protein/solvent/metal)	43.7/41.5/30.9
Ramachandran plot (%) (favored/additional/disallowed)[c]	97.2/2.8/0.0

Statistics for the highest-resolution shell are shown in parentheses.

[a]Bijvoet pairs were kept separate for data processing.

[b]$R_{merge} = 100\ \Sigma_h\Sigma_i|I_{h,i} - \langle I_h\rangle|/\Sigma_h\Sigma_i I_{h,i}$, where the outer sum (h) is over the unique reflections and the inner sum (i) is over the set of independent observations of each unique reflection.

[c]As defined by the validation suite MolProbity.