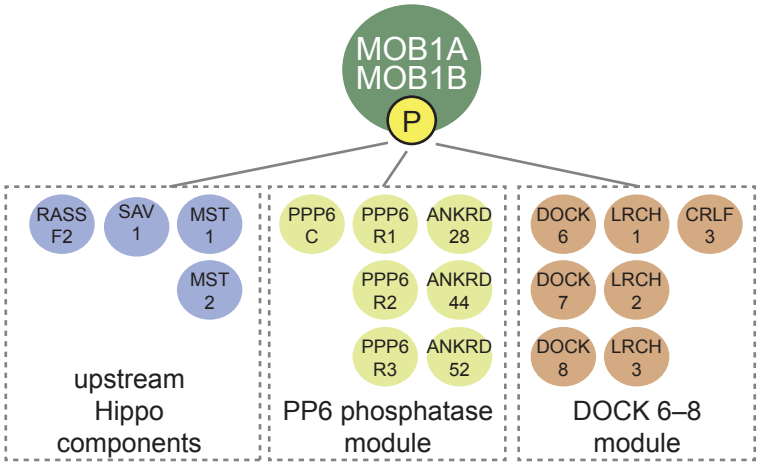
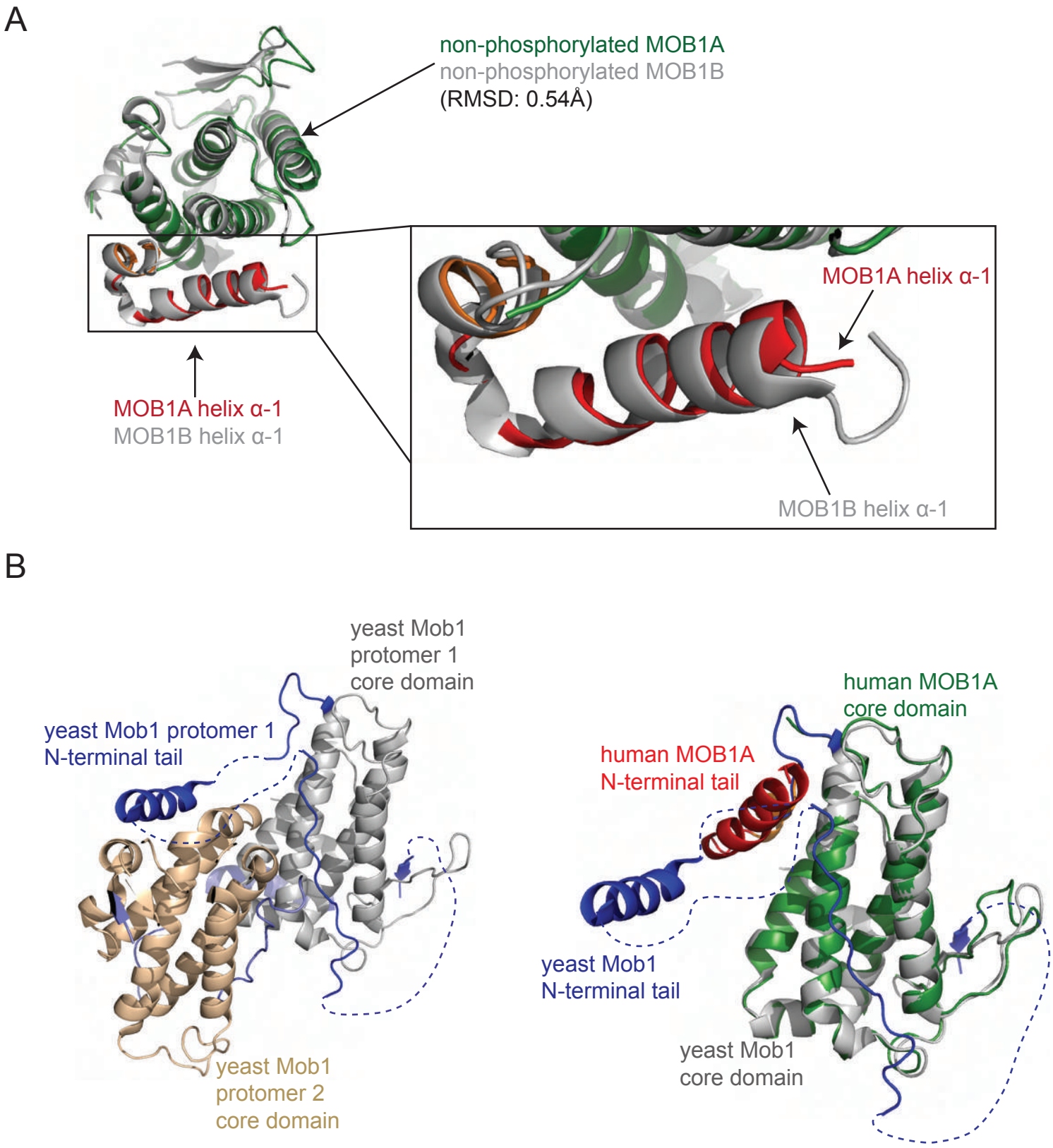
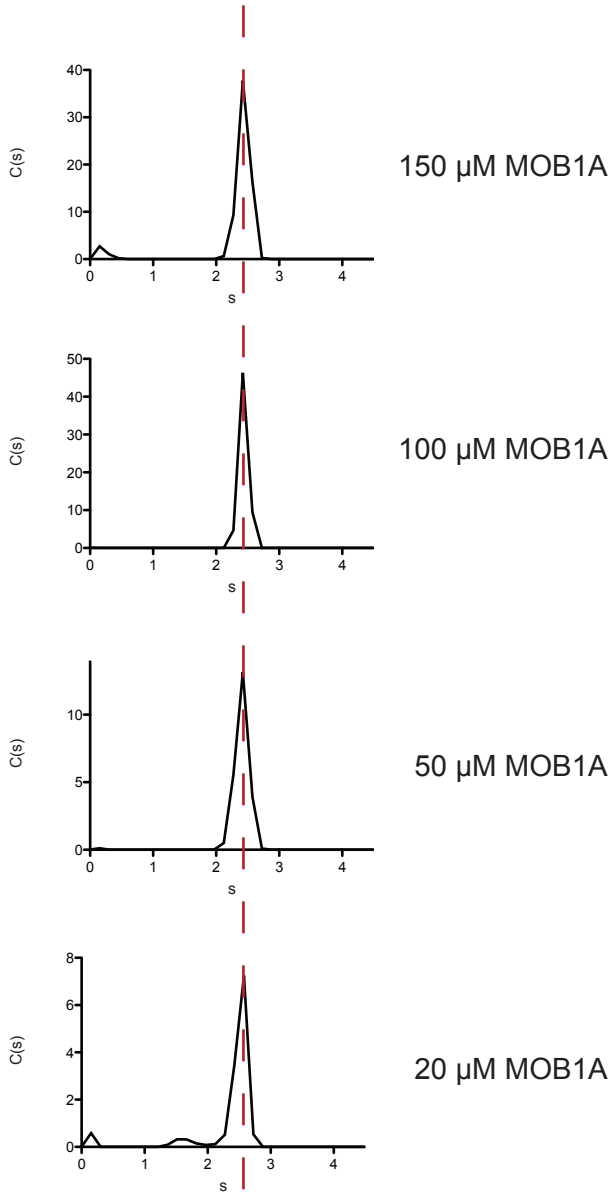


Alias	Gene	Module	Function
MST1	STK4	upstream Hippo components	Kinase
MST2	STK3	upstream Hippo components	Kinase
WW45	SAV1	upstream Hippo components	Scaffold
RASSF2	RASSF2	upstream Hippo components	Scaffold
PP6	PPP6C	PP6 phosphatase module	Phosphatase catalytic subunit
SAPS1	PPP6R1	PP6 phosphatase module	Phosphatase regulatory subunit
SAPS2	PPP6R2	PP6 phosphatase module	Phosphatase regulatory subunit
SAPS3	PPP6R3	PP6 phosphatase module	Phosphatase regulatory subunit
PP6-ARS-A	ANKRD28	PP6 phosphatase module	Phosphatase regulatory subunit
PP6-ARS-B	ANKRD44	PP6 phosphatase module	Phosphatase regulatory subunit
PP6-ARS-C	ANKRD52	PP6 phosphatase module	Phosphatase regulatory subunit
DOCK6	DOCK6	DOCK6-8 module	Guanine nucleotide exchange factor
DOCK7	DOCK7	DOCK6-8 module	Guanine nucleotide exchange factor
DOCK8	DOCK8	DOCK6-8 module	Guanine nucleotide exchange factor
LRCH1	LRCH1	DOCK6-8 module	Putative scaffold
LRCH2	LRCH2	DOCK6-8 module	Putative scaffold
LRCH3	LRCH3	DOCK6-8 module	Putative scaffold
CRLF3	CRLF3	DOCK6-8 module	Unknown

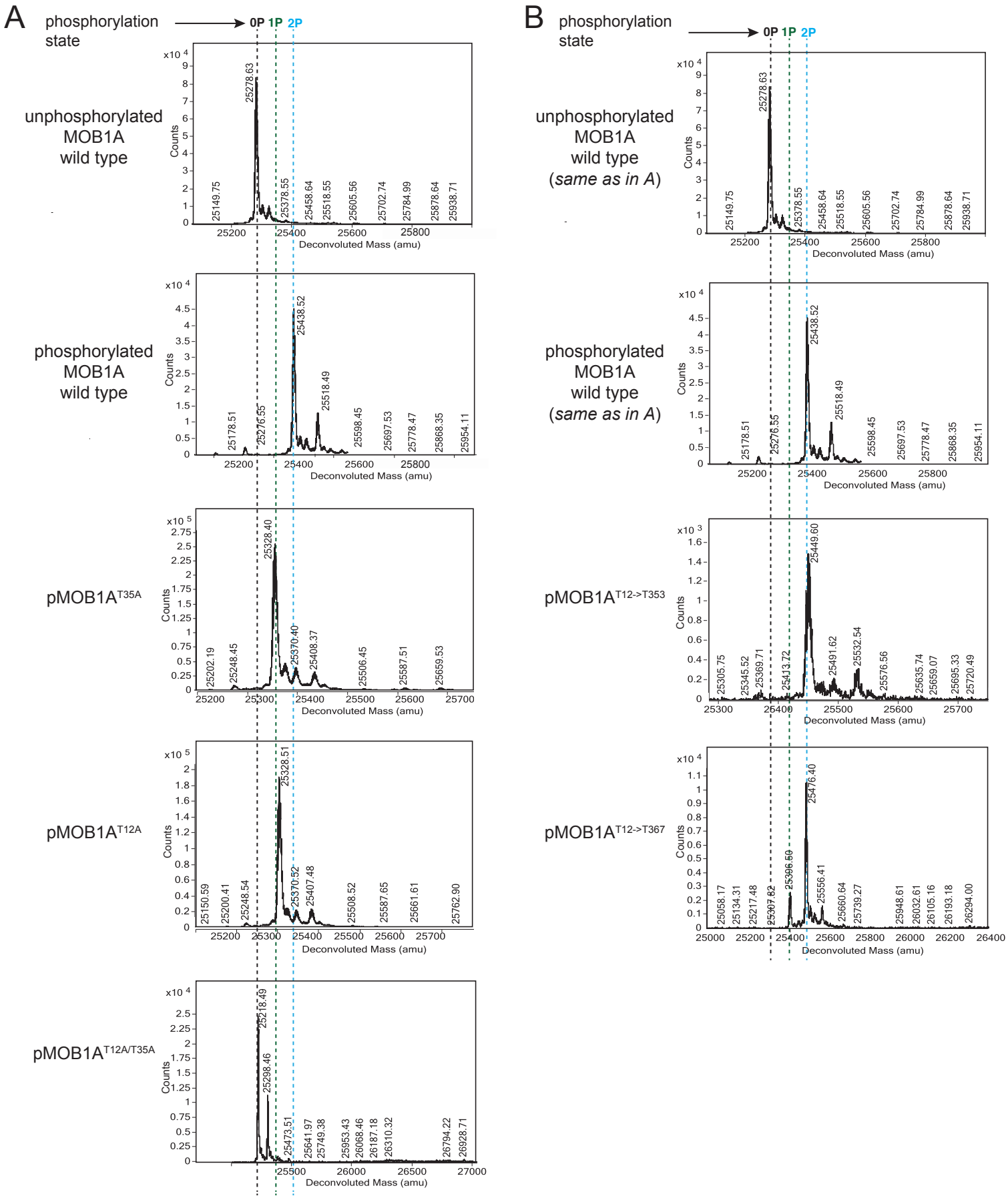
Supplemental Fig. 1. Phospho-dependent recruitment to MOB1A and MOB1B of Hippo, DOCK6–8 and PP6 modules previously discovered in Couzens *et al*. (2013, *Sci Signal*, **6:**rs15). A, Okadaic acid treatment stimulates the association of at least three mutually exclusive modules to MOB1A or MOB1B. B, List of the genes associated with the three phosphorylation-dependent MOB1 binding modules, alongside protein aliases and molecular function.



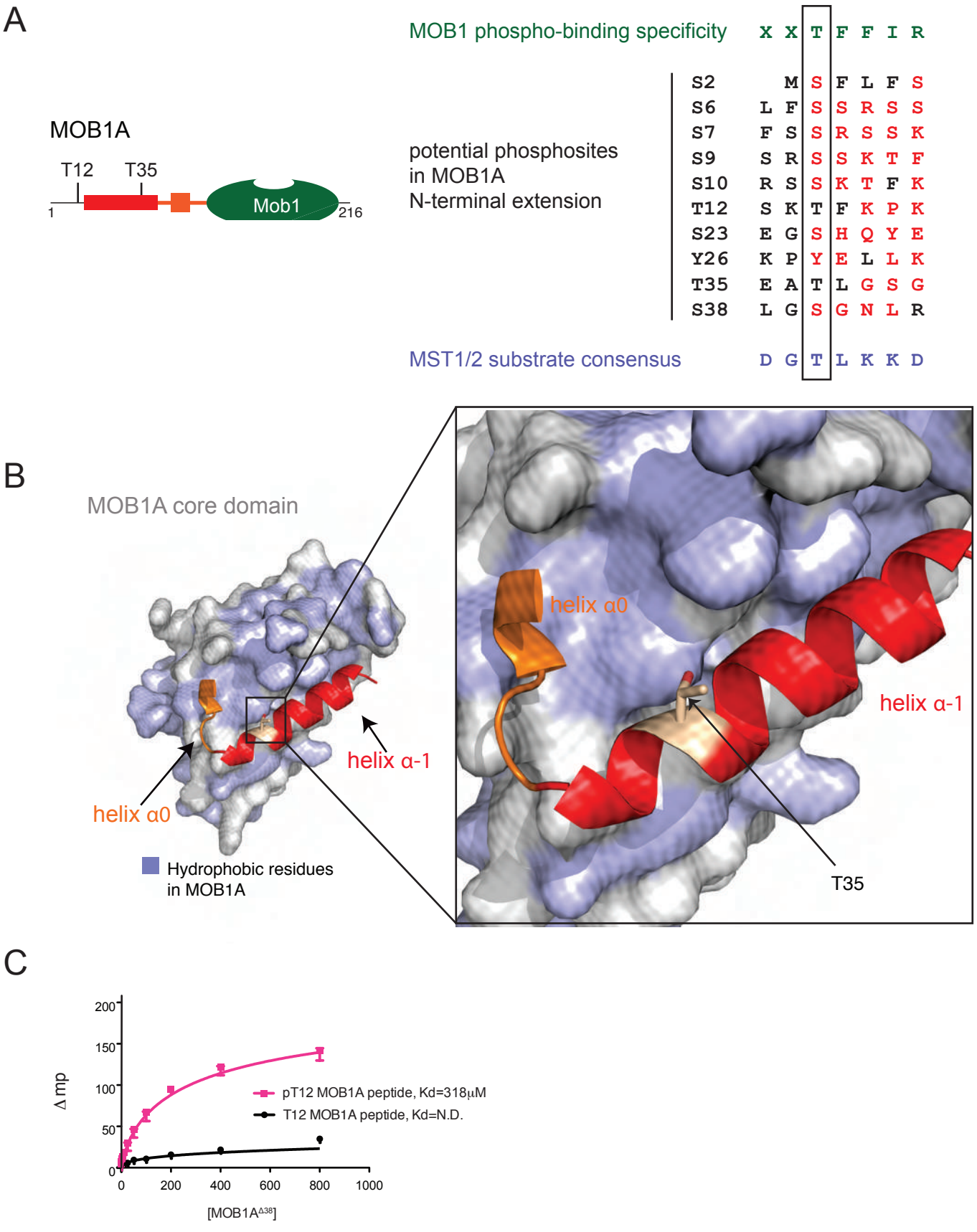
Supplemental Fig. 2. Comparison of full length MOB1A structures highlighting similarities and difference in the N-terminal extension. A, Superposition of non-phosphorylated full-length human MOB1A (solved here) and non-phosphorylated full-length mouse MOB1B (PDB: 5B5V; Kim *et al*., 2016, *Sci Rep*, **6:**28488). B, Left: Crystal structure of yeast Mob1 dimer; Right: Superimposition of non-phosphorylated full length MOB1A (solved here) and non-phosphorylated yeast Mob1 (PDB: 2HJN; Mrkobrada *et al*., 2016, *J. Mol. Biol.*, **362:**430-40).



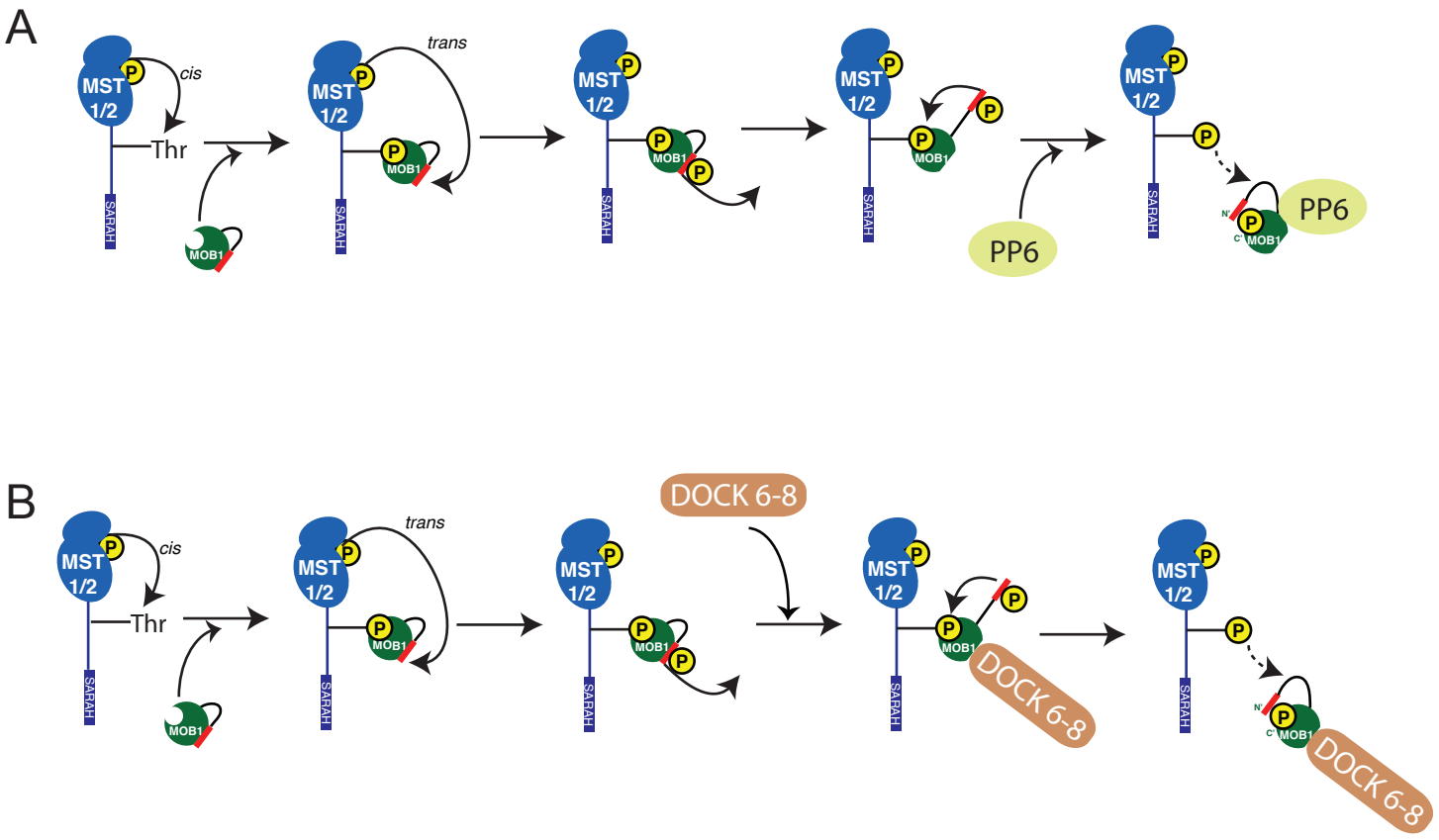
Supplemental Fig. 3. Oligomer state analysis of MOB1A by analytical ultracentrifugation. The dashed red line indicates the monomeric MOB1A form for each of the range of concentrations (20-150 μM) tested.



Supplemental Fig. 4. Phosphorylation status of MOB1A assessed by intact mass analysis. A, Intact mass spectra for non-phosphorylated MOB1 and MST1-phosphorylated MOB1 wild-type or proteins harboring alanine substitutions at T12, T35 or both. B, Intact mass spectra for non-phosphorylated MOB1 and MST1-phosphorylated MOB1 wild-type or proteins harboring substitutions of the T12 sequence to match the high affinity MST1 T353 or T367 sequences. Note that the wild-type non-phosphorylated and MST1-phosphorylated MOB1 (top two panels) are identical for A and B and only duplicated to facilitate comparison with the mutants.



Supplemental Fig. 5. Binding analysis of non-phosphorylated and phosphorylated variants of T12 and T35 MOB1A peptides to the MOB1A core domain in trans. A, Sequence alignment of putative phospho-regulatory sites in the N-terminus of MOB1A highlighting good (black) and poor (red) residue matches to the MOB1A phosphopeptide binding consensus and the MST1 and MST2 phosphorylation consensus (see accompanying paper by Couzens *et al.*, 2017). B, Structural representation of full-length non-phosphorylated MOB1A with the position of T35 indicated. T35 contributes to the interaction of helix α-1 with the MOB1A core. C, Fluorescence polarization binding analysis of phosphorylated and non-phosphorylated MOB1A T12 (*left*) and T35 (*right*) peptides to the MOB1A core domain. Data is plotted as the mean ± SEM (n=3).



Supplemental Fig. 6. Models for the phosphoregulated interactions of MOB1 with the PP6 and DOCK6–8 modules. A, Possible model of recruitment of the PP6 phosphatase module to MOB1A: PP6 components preferentially associate with MOB1A under conditions where the N-terminal tail of MOB1A occupies the MOB1A phosphopeptide binding pocket. B, Possible model of recruitment of the components of the DOCK6–8 module. Our interaction proteomics data (Fig. 5) are best explained by a model whereby DOCK6–8 and their interaction partners are recruited to MOB1A following phosphorylation-dependent release of auto-inhibitory interactions between the N-terminal tail of MOB1 and the NDR and LATS binding site on the MOB1 core domain.