

min phosphatase treatment

0,0,0,0000

0,0,0,000

0,0,0,000

0,0,0,0,0

0,0,0,000

0,0,0,000

Supplementary Figure 7. Mutations at the KD–AID interface abolish AMP-dependent allosteric kinase activation, but not protection against activation loop dephosphorylation. Repeat sets of the representative data shown in Fig. 4f and g. (a) Mutations that disrupt the KD–AID interaction make AMPK constitutively active. [ $\gamma$ -32P]-ATP kinase assays with His<sub>6</sub>GST-SAMS peptide as substrate (n=3, error bars, s.d.). (b) Mutations that disrupt the KD–AID interaction still show AMP-dependent stabilization of activation loop phosphorylation. WT and mutant AMPK were incubated with human PP2C $\alpha$  for the indicated amount of time in the absence or presence of 0.2 mM AMP. Activation loop T174-phosphorylation was determined by immunoblotting (n=2, error bars, s.d.).

а

0,0,0,000

0,0,0,0,000