Ligands of SidJ in the cryo-EM density:

Black et al., have noted that SidJ has two active centers, one canonical pocket that is sandwiched between the N- and C-lobes of the kinase like domain and another is a migrated pocket in the C-lobe of the kinase domain (Extended data Fig. 9A)[1]. According to the model proposed by Black et al., these two active sites in SidJ perform AMPylation of SdeA E860, followed by glutamylation respectively. Interestingly, Black et al., observed AMP bound in the migrated pocket while the canonical pocket is occupied with only PPi (Extended data Fig. 9A). In agreement with this, in our Cryo-EM map, we also observe density in the migrated pocket that most likely represent AMPPNP (Added to the sample prior to plunge freezing) (Extended data Fig. 9B). Moreover, we also found additional density in the canonical pocket that although less detailed to unambiguously place a nucleotide, can accommodate an AMP (Extended data Fig. 9C). Possible binding of nucleotide at the canonical kinase active site provides structural evidence for the proposed two active sites model for SidJ catalysis [1].

1. Black, M. H. *et al.* Bacterial pseudokinase catalyzes protein polyglutamylation to inhibit the SidE-family ubiquitin ligases. *Science* **364,** 787–792 (2019).