Supplementary Note: Exploring the sequence and structural determinants for activating Hsp70

While binding is not sufficient for Hsp70 activation (**Fig. 1b**), we explored if there are structural features unique to JDM37 to gather information about the determinants in activating Hsp70. Using AlphaFold2 structure predictions and crystal structures of DnaJ:DnaK complex, we explored whether structure can discern Hsp70 activators compared to inhibitors. However, there were no clear differences between JDM37 and the other JDMs or native J-domains in terms of Hsp70 interactions and conformation (**Extended Data Fig. 9a-b and Table S1**), although many of these complexes are only predictions. While there is very little sequence similarity between JDM37 and Hsp70 including the absence of the highly conserved HPD motif, JDM37 does have a very similar NPD motif (**Extended Data Fig. 9c-d**). Moreover, the electrostatics involved in Hsp70 binding (helices 2 for native J-domain and JDM37) are also largely conserved (**Extended Data Fig. 9e**). However, JDM37 and the other JDMs have high sequence similarity especially in their charged residue patches (**Extended Data Fig. 2**), thus the pattern of these electrostatic patches is also conserved for the other JDMs. Thus, it is still not obvious what differentiates JDM37 when looking at binding, structure, or sequence so far.

Table S1: Rosetta metrics of JDM:DnaK predicted complexes
Using AF2, each JDM was assessed for their predicted binding to DnaK (*E. coli* Hsp70). Afterwards, each JDM:DnaK complex was scored based on Rosetta and AF2 metrics.