



**S7 Fig. c-di-GMP specifically binds to RavS to enhance the RavS-RavR phosphotransfer.** (a) Expression of truncated RavS and RavR proteins. Numbers indicate the sites of amino acid residues of RavS or RavR. (b) RavS<sup>ΔN</sup> specifically interacts with fl-c-di-GMP. The interactions of RavS<sup>ΔN</sup>, RavA, VgrS and RavR<sup>ΔEAL</sup> with fl-c-di-GMP were measured by microscale thermophoresis (MST). Twenty nanomolar fluorescein-labelled nucleotide and increasing concentrations of proteins were co-incubated and potential interactions were measured by MST. The dissociation constant ( $K_d$ ) was determined to estimate the binding affinity. Averages and standard deviations are shown ( $n = 3$ ). (c) Time course analyses of the impact of c-di-GMP on the velocity of RavS-RavR phosphotransfer. Representative pictures in Fig 5b are shown. Each experiment was repeated three times.