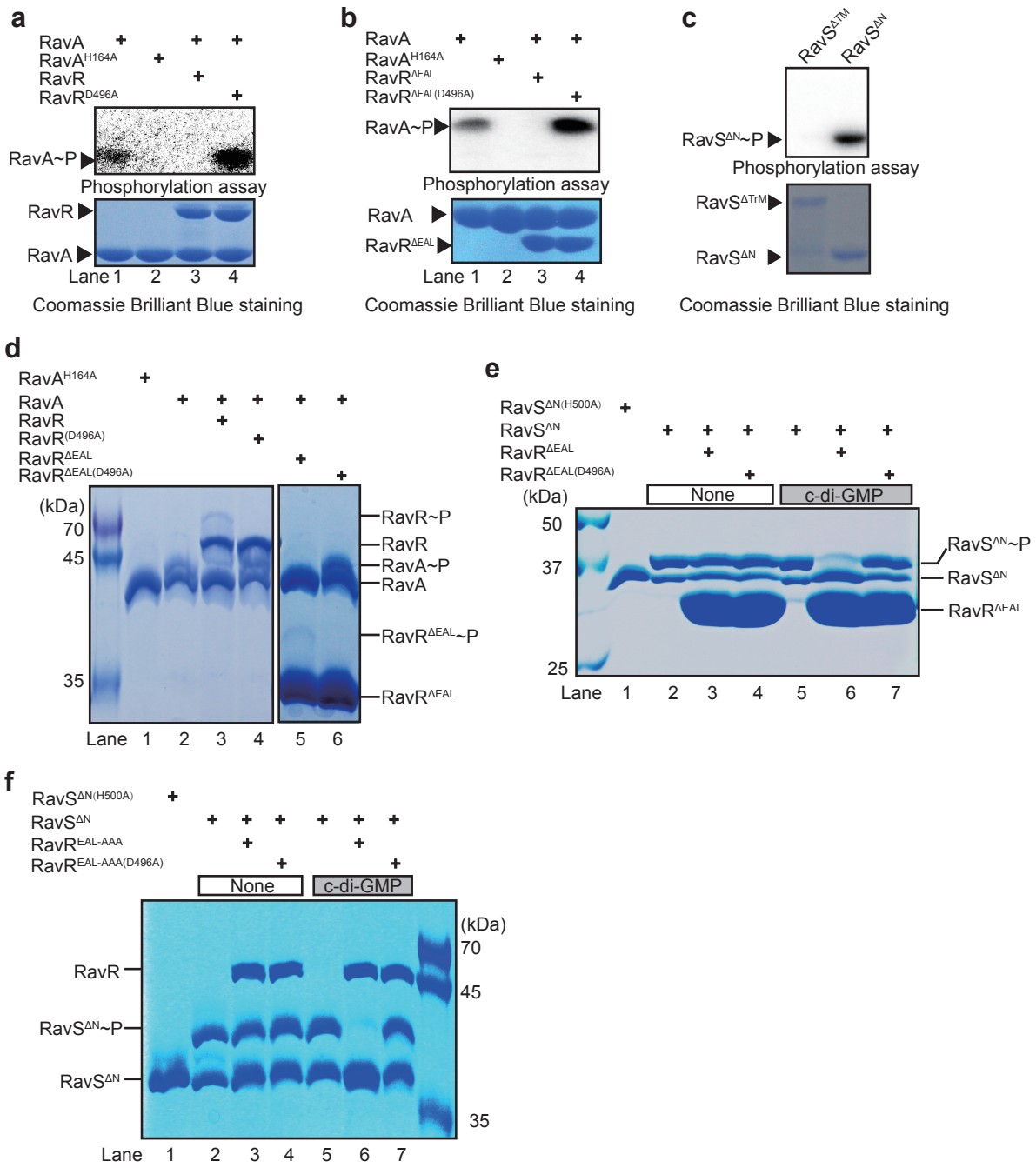

S6 Fig. Both RavA and RavS have autokinase activity and transfer the phosphoryl group to RavR in a RavR^{D496} dependent manner. (a) RavA phosphorylates RavR in vitro. Autophosphorylation of the histidine kinase RavA and its recombinant derivative RavA^{H164A} in the presence of [γ-^{32}P]ATP at room temperature for 10 min. RavA phosphotransfer to RavR was then carried out for 30 s. Assays contained 10 μM of soluble protein RavA or RavA^{H164A}. Ten micromolar RavR or RavR^{D496A} was added into the mixtures as indicated. The experiment was repeated three times. (b) RavA phosphorylated the recombinant protein RavR$^{\Delta EAL}$ in vitro. Assays contained 15 μM of soluble protein RavA or RavA^{H164A}. Five micromolar RavR$^{\Delta EAL}$ or RavR$^{\Delta EAL(D496A)}$ was added as indicated. (c) RavS$^{\Delta N}$ but not RavS$^{\Delta TrM}$ possesses robust autophosphorylation activity. Five micromolar of RavS$^{\Delta TrM}$ or RavS$^{\Delta N}$ was added into the in vitro autophosphorylation mixture in the presence of [γ-^{32}P]ATP at room temperature for 10 min. (d–f) Detection phosphorylated HK and RR using Phos-tag acrylamide gel. (d) RavA phosphorylates RavR or RavR$^{\Delta EAL}$ in vivo. RavA or RavA^{H164A} was incubated with 2 mM ATP at 28 °C for 15 min, respectively. RavR (or RavR^{D496A}) or RavR$^{\Delta EAL}$ (or RavR$^{\Delta EAL(D496A)}$) was then added into mixtures for 2 min. (e – f) RavS$^{\Delta N}$ phosphorylates RavR$^{\Delta EAL}$ or RavR$^{EAL-AAA}$ in vitro. RavS$^{\Delta N}$ was autophosphorylated with 2 mM ATP at 28 °C for 15 min. A total of 20 μM various recombinant RavR proteins, including RavR$^{\Delta EAL}$, RavR$^{\Delta EAL(D496A)}$, RavR$^{EAL-AAA}$ or RavR$^{EAL-AAA(D496A)}$ were added and incubated for 30 min at 28°C. A total of 100 μM c-di-GMP were added as indicated. The reactions were stopped with 3 × SDS loading buffer and the products were separated by 8% or 12% acrylamide gels at 4 °C, the gels were stained with Coomassie brilliant blue (d–f). Each experiment was repeated three times.