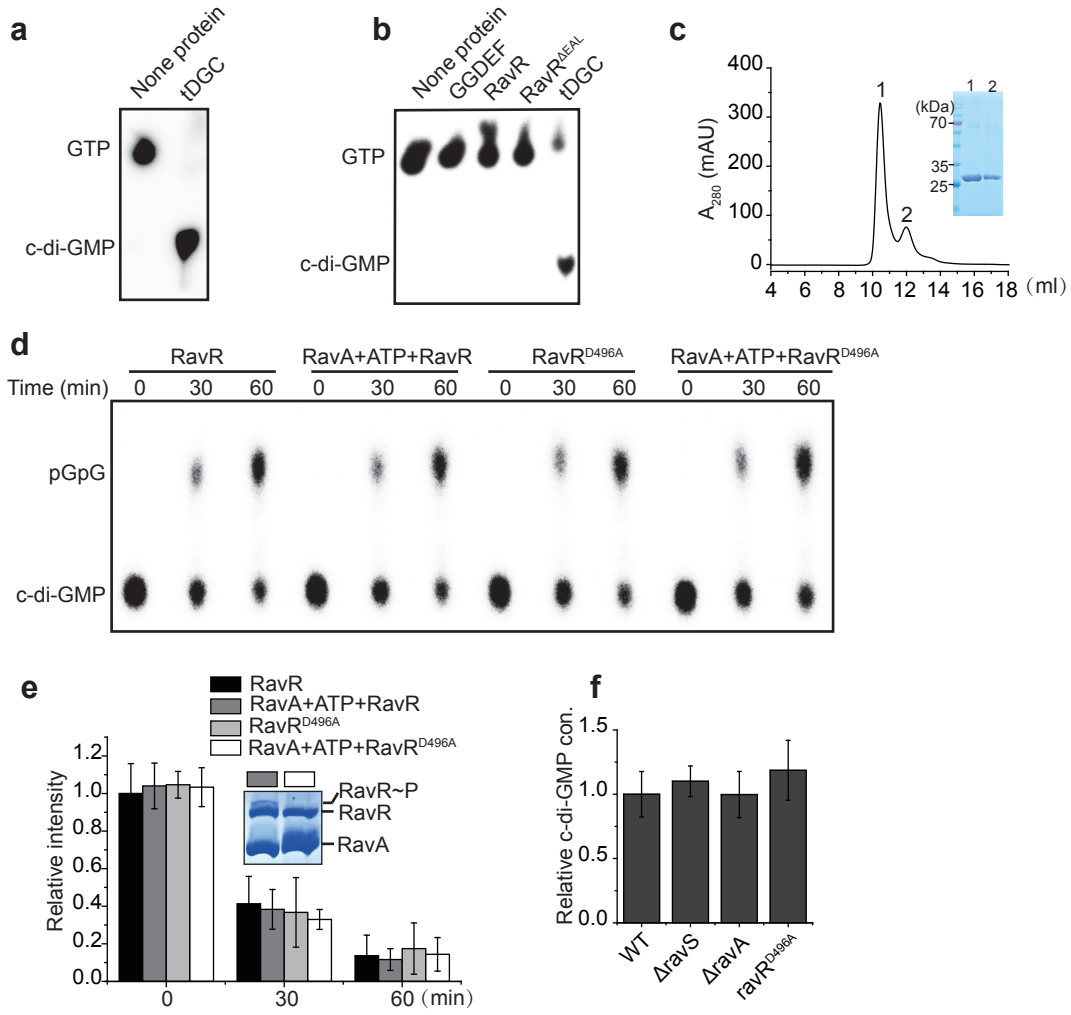

S5 Fig. EAL domain of RavR has phosphodiesterase activity. (a) Synthesis of 32P-labelled c-di-GMP by tDGC cyclase in vitro. c-di-GMP was synthesized and labelled by using tDGC cyclase from [^{32}P]GTP. Samples were analysed on thin-layer chromatography (TLC) plates. (b) RavR did not possess diguanylate cyclase (DGC) activity. None or 5 μM affinity purified proteins including GGDEF, RavR, RavR$^{\Delta EAL}$ or tDGC were added to the DGC reaction mixture with α-^{32}P-labeled GTP. After incubation at 28 °C for 60 min, samples were analysed by TLC assays. (c) Recombinant EAL-domain containing protein naturally forms homodimer. The purified EAL protein was separated by a molecular sieve and the molecular weight of each fraction was determined by analytic ultra-centrifugation. (d) Phosphorylation of RavR did not affect its PDE activity. The enzymatic reactions contained 25 μM ^{32}P-labelled c-di-GMP were analysed by TLC. RavR or RavR^{D496A} (5 μM), RavA (10 μM) and ATP (2 mM) were added as indicated. (e) Intensity of the signals in (d), measured by ImageJ. (f) Intracellular c-di-GMP concentrations of various strains detected by LC-MS/MS analysis. All the experiments were repeated independently three times.