

Electronic Supplementary Material – Online Resource 1

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Article title: Caffeine inhibits PI3K and mTORC2 in *Dictyostelium* and differentially affects multiple other cAMP chemoattractant signaling effectors.

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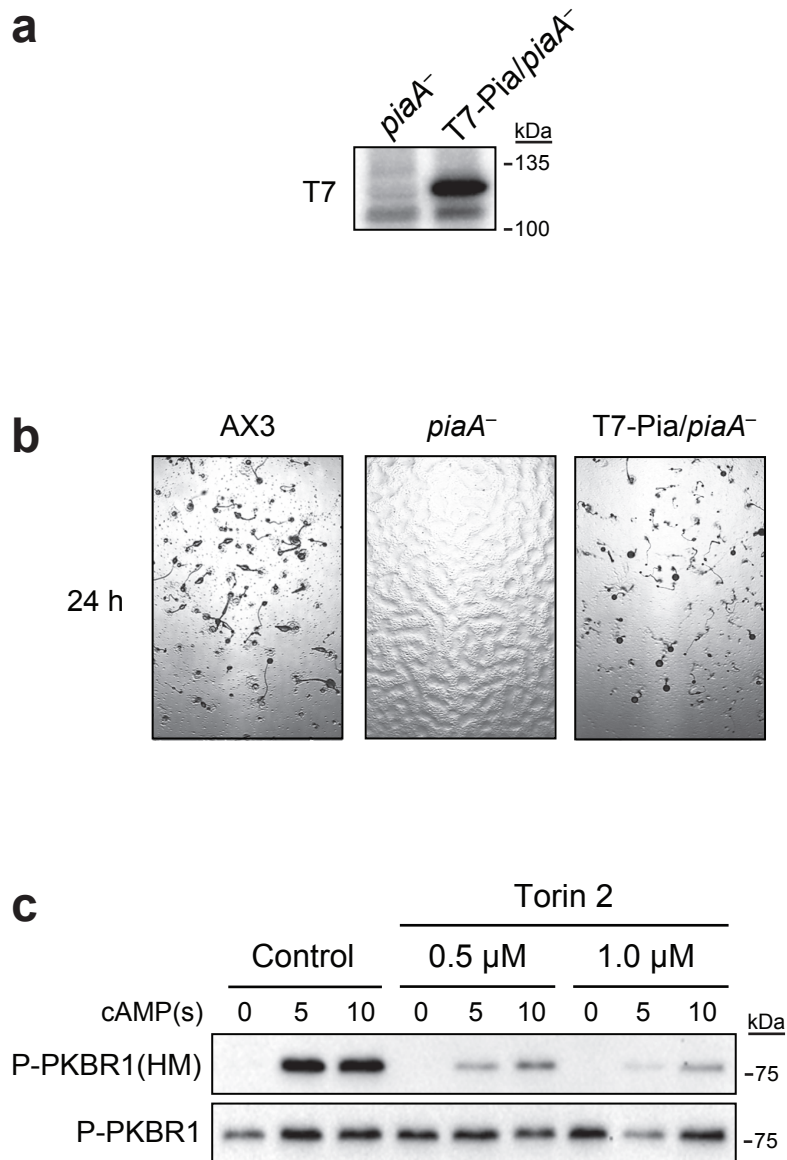
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Online Resource 1. a, T7-Pia was expressed in *piaA* null (*piaA*⁻) cells and detected by T7 immunoblot. **b**, T7-Pia rescues the developmental defect of *piaA* null cells, demonstrating that the construct is functional. Pictures shown were taken at 24 h after the onset of development triggered by starving the cells on non-nutrient agar. Fruiting bodies are seen on plates of wild-type AX3 and T7-Pia/*piaA*⁻ cells and absent on plates of *piaA*⁻ cells. **c**, Torin 2-mediated inhibition of mTORC2 in cells. Torin 2 was incubated with developed AX3 cells at 0.5 and 1.0 μ M for 30 min before cAMP stimulation for the time indicated. mTORC2 activity was assessed by evaluating its phosphorylation of PKBR1 at its hydrophobic motif (HM).