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Supplemental Data

The Amoebal MAP Kinase Response to *Legionella pneumophila* Is Regulated by DupA

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Sup. Fig. 1

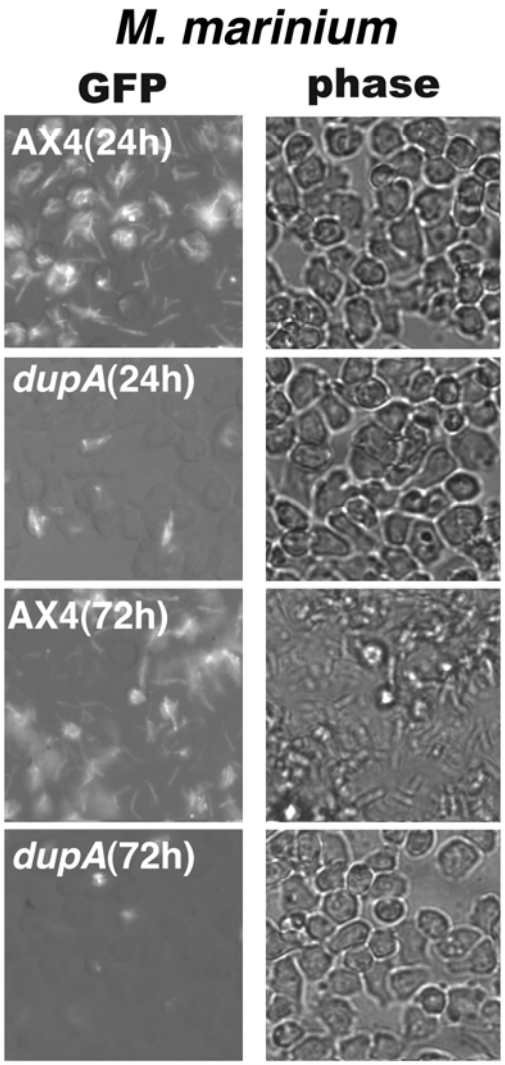

Figure S1. Defective *M. marinum* growth within the *dupA(F6)* mutant. *Dictyostelium* AX4 and *dupA(F6)* cells were challenged with *M. marinum msp12:gfp* at MOI = 1.0. Images of live cells were obtained using phase contrast or fluorescence microscopy for GFP**.** Shown are typical images at noted timepoints. After 24 h. of incubation with *M. marinum*, a much lower percentage of infected amoebae was observed in *dupA(F6)* compaired to AX4, indicating either a strong defect in uptake of bacteria, degradation of bacteria, or selective death of infected amoebae in the mutant. After 72 h. infection, the wild type AX4 supported large amounts of *M. marinum* growth and suffered widespread host cell lysis. In contrast, the monolayer of the *dupA*$^{-}$ mutant remained intact, and there was little obvious growth of bacteria. *M. marinum* strain was a kind gift from L. Ramakrishnan (University of Washington Medical School). The *msp12*::*gfp* strain constitutively expresses GFP and grows efficiently in macrophages (Chan et al., 2002). *M. marinum* were grown in media and maintained as described (Chan et al., 2002; Solomon et al., 2003).

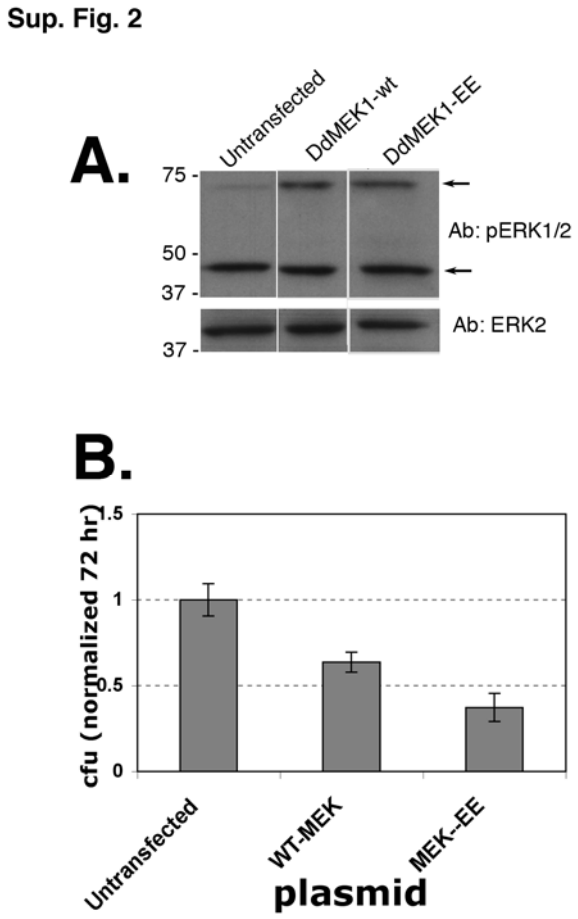

Figure S2. Enhanced phosphorylation of *D. discoideum* ERK1 is associated with lowered *L. pneumophila* intracellular growth. **(A)**. *D. discoideum* harboring plasmids encoding either DdMEK1 (DdMEK1-wt) or the constitutively active ddMEK1:S444E,T448E derivative (DdMEK1-EE; [Sobko, 2002 #268]) were gel fractionated blotted and probed with anti phosphoERK (top panel). Top arrow denotes band migrating with apparent molecular weight of ERK1 and bottom arrow denotes band migrating with apparent molecular weight of ERK2. The DdMEK1-wt strain expresses shows about 4.5X more phospho-ERK1, while DdMEK1-EE showed approximately 3X more phospho-ERK1. In bottom panel, the blot in the top panel was stripped and probed with anti-ERK2 antibody (Experimental Procedures). Region of gel that overlays the 40 kDal phosphoprotein band is displayed. **(B)**. Lowered intracellular growth in

the presence of increased phosphoERK1. AX4 derivatives were challenged with *L. pneumophila* at MOI = 0.05. Growth of *L. pneumophila* was monitored by determining CFU 72 hrs post inoculation. Data shown are mean of three identical experiments performed on different days, normalizing to the yield of bacteria from the AX4(untransfected) control, which is set at 1 for each experiment.

SUPPLEMENTAL REFERENCES

Chan, K., Knaak, T., Satkamp, L., Humbert, O., Falkow, S., and Ramakrishnan, L. (2002). Complex pattern of *Mycobacterium marinum* gene expression during long-term granulomatous infection. Proc. Natl. Acad. Sci. USA *99*, 3920-3925.

Sobko, A., Ma, H., and Firtel, R. A. (2002). Regulated SUMOylation and ubiquitination of DdMEK1 is required for proper chemotaxis. Dev. Cell. *2*, 745-756.

Solomon, J. M., Leung, G. S., and Isberg, R. R. (2003). Intracellular replication of *Mycobacterium marinum* within *Dictyostelium discoideum*: efficient replication in the absence of host coronin. Infect. Immun. *71*, 3578-3586.