SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Plaque formation assay reveals partial rescue of the *Ddnhe1*$^{-}$ phenotype by expression of *Dd*Aip1-FL. Cells were plated together with heat-killed bacteria on PB buffer agar plates and incubated for 2 days. Representative images of plaques are shown. Bar, 1 mm. Ax2 cells formed large plaques whereas *Ddnhe1*$^{-}$ cells formed smaller plaques without aggregates. Expression of *Dd*Aip1-Δ382 made plaques size of *Ddnhe1*$^{-}$ cells smaller, but expression of *Dd*Aip1-FL rescue partially plaque size and aggregation defect of *Ddnhe1*$^{-}$ cells.

Supplemental Figure 2. Immunoblot analysis reveals comparable expression of *Dd*Aip1 mutants in Ax2 and *Ddnhe1*$^{-}$ cells. (A) Expression of *Dd*Aip1-Δ382 tagged with a FLAG epitope in Ax2 and *Ddnhe1*$^{-}$ cells, determined with antibodies to FLAG. (B) Expression of full-length *Dd*Aip1 (FL) and *Dd*Aip1-4X (4X) tagged with GFP in Ax2 and *Ddnhe1*$^{-}$ cells, determined with antibodies to GFP. Immunoblotting for actin was used as a loading control.

Supplemental Figure 3. (A) Localization of F-actin, determined by rhodamine-phalloidin staining, was restricted to the leading edge of migrating Ax2 cells but around the cell periphery in *Ddnhe1*$^{-}$ cells. In *Ddnhe1*$^{-}$/*Dd*Aip1-FL cells, F-actin was localized at the front and at the lateral edges of migrating cells. (B) Quantitative analysis of F-actin indicates a predominant localization at the leading edge of Ax2 cells but not *Ddnhe1*$^{-}$ or *Ddnhe1*$^{-}$/*Dd*Aip1-FL cells. Fluorescence intensity of rhodamine-phalloidin was measured in 20 sectors along the cell perimeter starting and ending at the rear of the cell (-180 and +180, respectively), with 0 degree indicating the cell front oriented toward the cAMP source. Data are for cells shown in A and are representative of more than 80% of chemotaxing cells for each clone.

Supplemental Figure 4. Quadruple mutant of Aip1 (Aip1-4X) binds cofilin and F-actin. (A) GST-pull down assay indicates the comparable binding of Aip1-FL (FL) and Aip1-4X (4X) to cofilin in the absence of actin. 0.5 μM GST-fusion of Aip1-FL and Aip1-4X was incubated with various concentrations of cofilin. (B) Actin co-sedimentation assay indicates binding of Aip1-4X to F-actin. 10 μM of polymerized actin was incubated with 0.5 μM of Aip1-4X for 1 h. F-actin was pelleted by ultracentrifugation and proteins in supernatant (S) and the pellet (P) fractions were resolved by SDS-PAGE.

Supplemental Video Legends:

Supplemental Video 1. Chemotaxis of wild-type Ax2 cells.

Supplemental Video 2. Chemotaxis of *Ddnhe1*$^{-}$ cells.

Supplemental Video 3. Chemotaxis of Ax2/*Dd*Aip1-Δ382 cells.

Supplemental Video 4. Chemotaxis of *Ddnhe1*$^{-}$/*Dd*Aip1-Δ382 cells.

Supplemental Video 5. Chemotaxis of Ax2/*Dd*Aip1-FL cells.

Supplemental Video 6. Chemotaxis of *Ddnhe1*$^{-}$/*Dd*Aip1-FL cells.

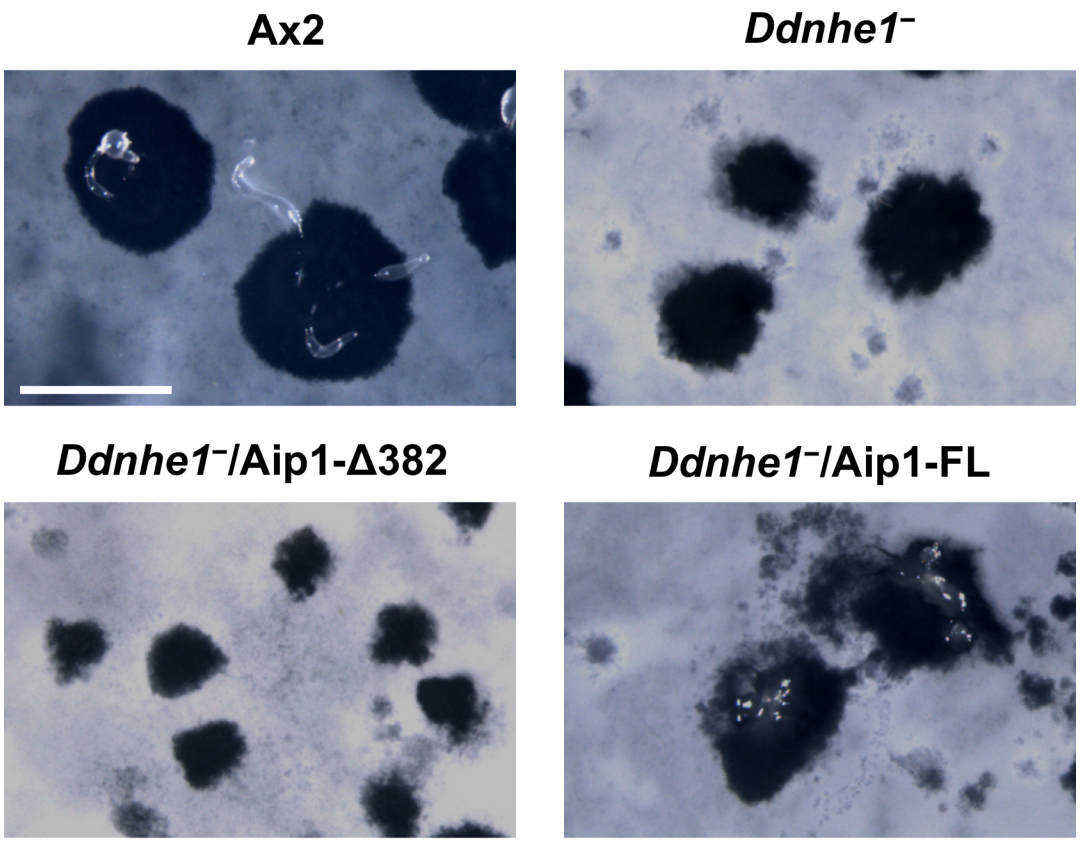
Supplemental Video 7. Submerged development of Ax2 cells.

Supplemental Video 8. Submerged development of *Ddnhe1*$^{-}$ cells.

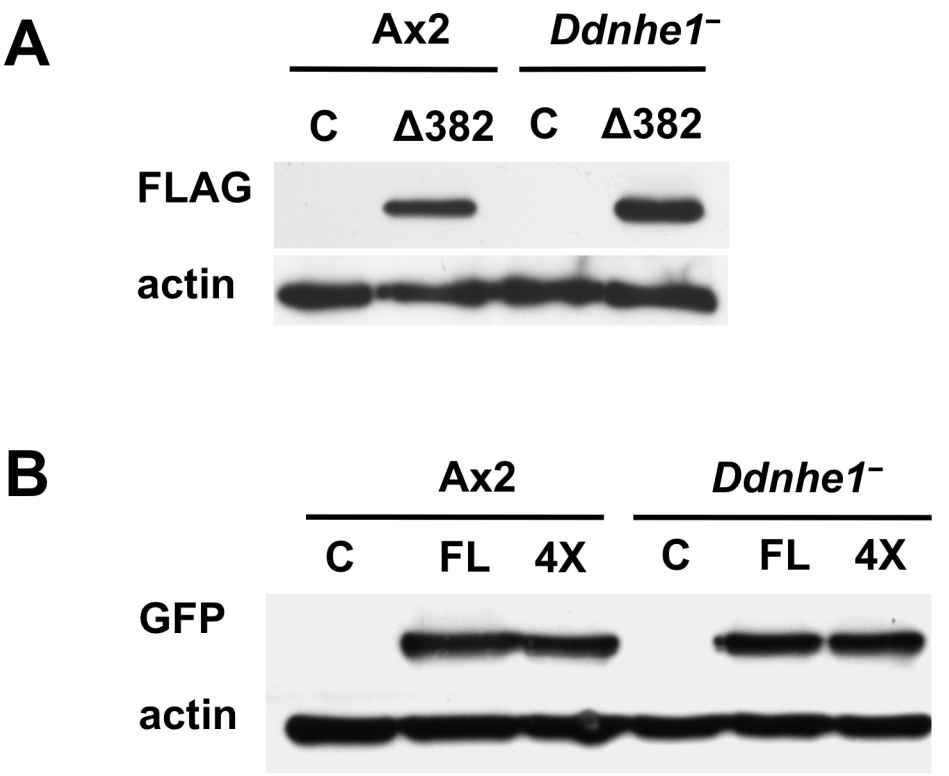
Supplemental Video 9. Submerged development of *Ddnhe1*$^{-}$/*Dd*Aip1-FL cells.

Supplemental Video 10. Submerged development of *Ddnhe1*$^{-}$/*Dd*Aip1-4X cells.

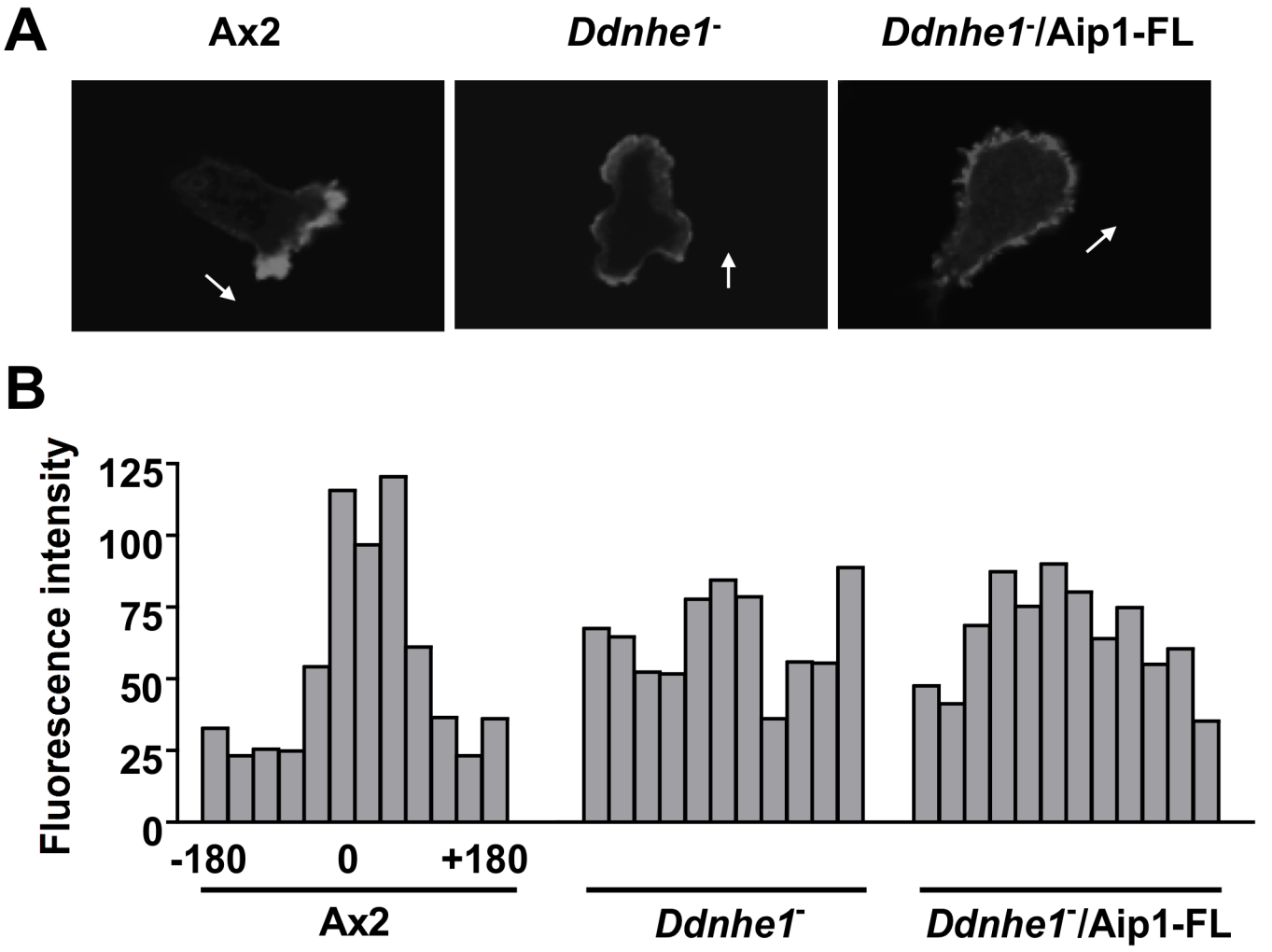
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4