Warner and Longmore, http://www.jcb.org/cgi/content/full/jcb.200901029/DC1

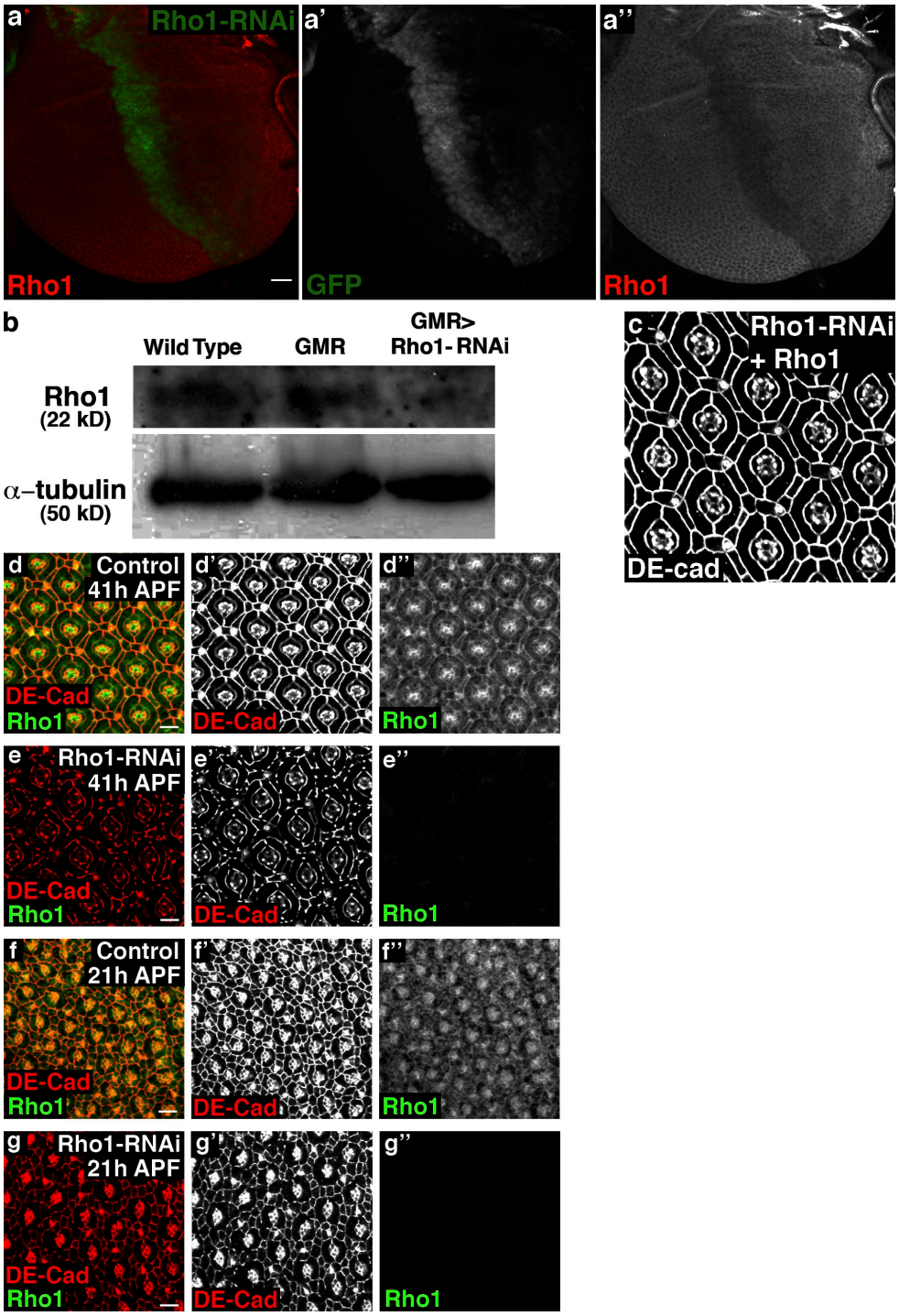


Figure S1. **Rho1-RNAi specifically affects Rho1.** (a–a″) Confocal immunofluorescent localization of Rho1 (a and a″) in larval wing disc coexpressing GFP and Rho1-RNAi using *patched-gal4*. (b) Western blot analysis of 41 h–APF pupal eyes. (c) Confocal immunofluorescent localization of DE-cadherin (DE-cad) in pupal eye coexpressing Rho1-RNAi and Rho1 with *GMR-gal4*. (d and e) Confocal immunofluorescent localization of DE-cadherin (d, d′, e, and e′) and Rho1 (d, d″, e, and e″) in control or Rho1-RNAi–expressing pupal eye 41 h APF. (f and g) Confocal immunofluorescent localization of DE-cadherin (f, f′, g, and g′) and Rho1 (f, f″, g, and g″) in control or Rho1-RNAi–expressing pupal eye 21 h APF. Bars, 10 µm.

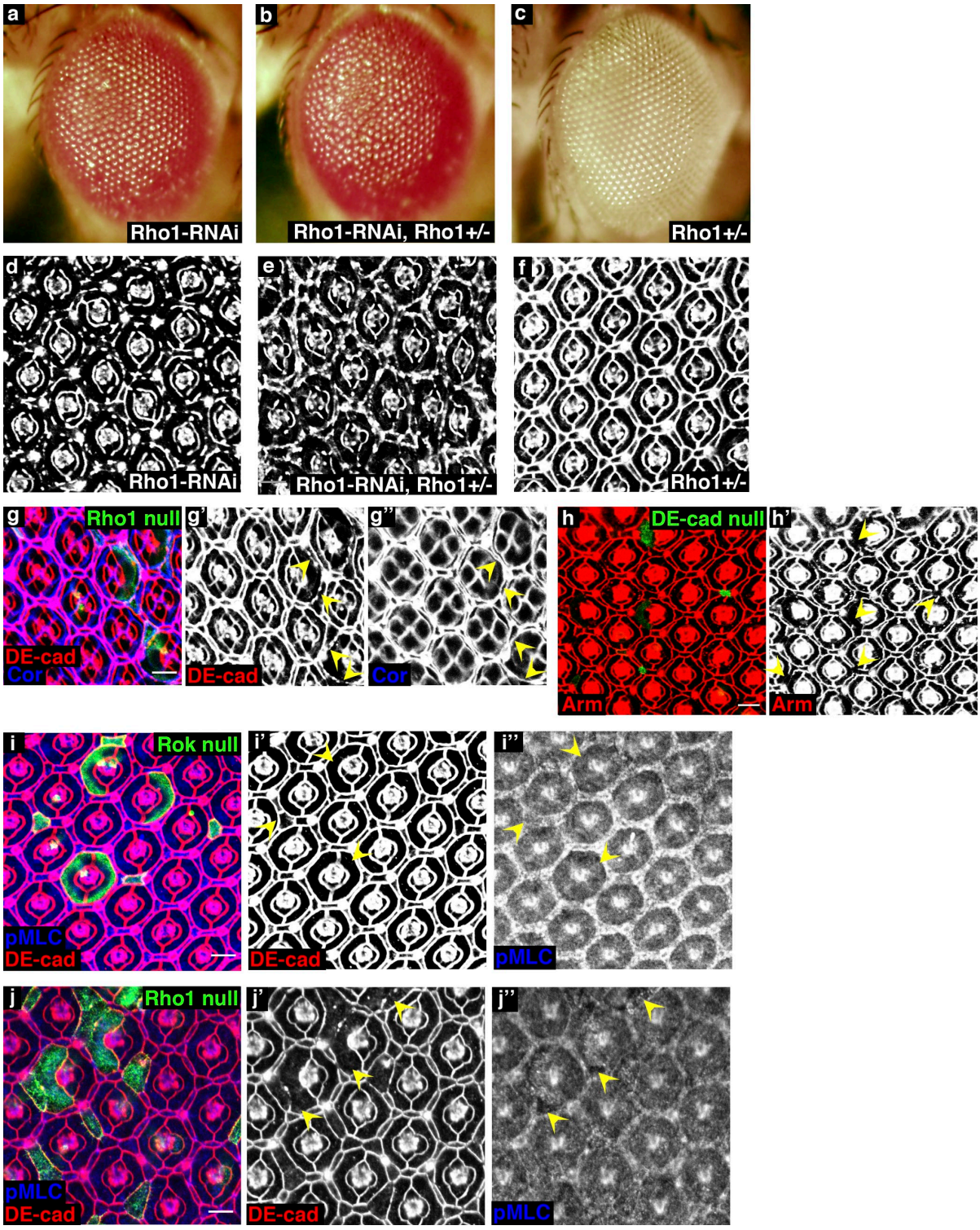


Figure S2. **GMR>Rho1-RNAi phenotypes are enhanced in a Rho1 heterozygous background, Rho1 depletion does not affect localization of the SJ protein Cor, Armadillo localization is lost between DE-cadherin–null and wild-type cells, and Rho1 and Rok depletion decreases phospho-MLC levels.** (a and b) Adult eyes expressing Rho1-RNAi in a wild-type (a) or *Rho1*^{72F} heterozygous background (b). (c) Adult eye heterozygous for *Rho1*^{72F}. (d and e) Confocal immunofluorescent localization of DE-cadherin in pupal eye expressing Rho1-RNAi in a wild-type (d) or *Rho1*^{72F} heterozygous background (e). (f) Confocal immunofluorescent localization of DE-cadherin in pupal eye heterozygous for *Rho1*^{72F}. (g–g″) Confocal immunofluorescent localization of DE-cadherin (DE-cad; g and g′) and Cor (g and g″) in *Rho1*^{72} MARCM clones. Arrowheads identify AJs and SJs between two Rho1-null cells. (h) Confocal immunofluorescent localization of Armadillo (Arm) in *shg*^{R69} MARCM clones. Arrowheads identify single-cell DE-cadherin–null clones. (i and j) Confocal immunofluorescent localization of DE-cadherin (i, i′, j, and j′) and phospho-MLC (pMLC; i, i″, j, and j″) in *Rho1*^{72} and *rok*^{2} MARCM clones. Arrowheads identify clonal cells. Bars, 10 µm.

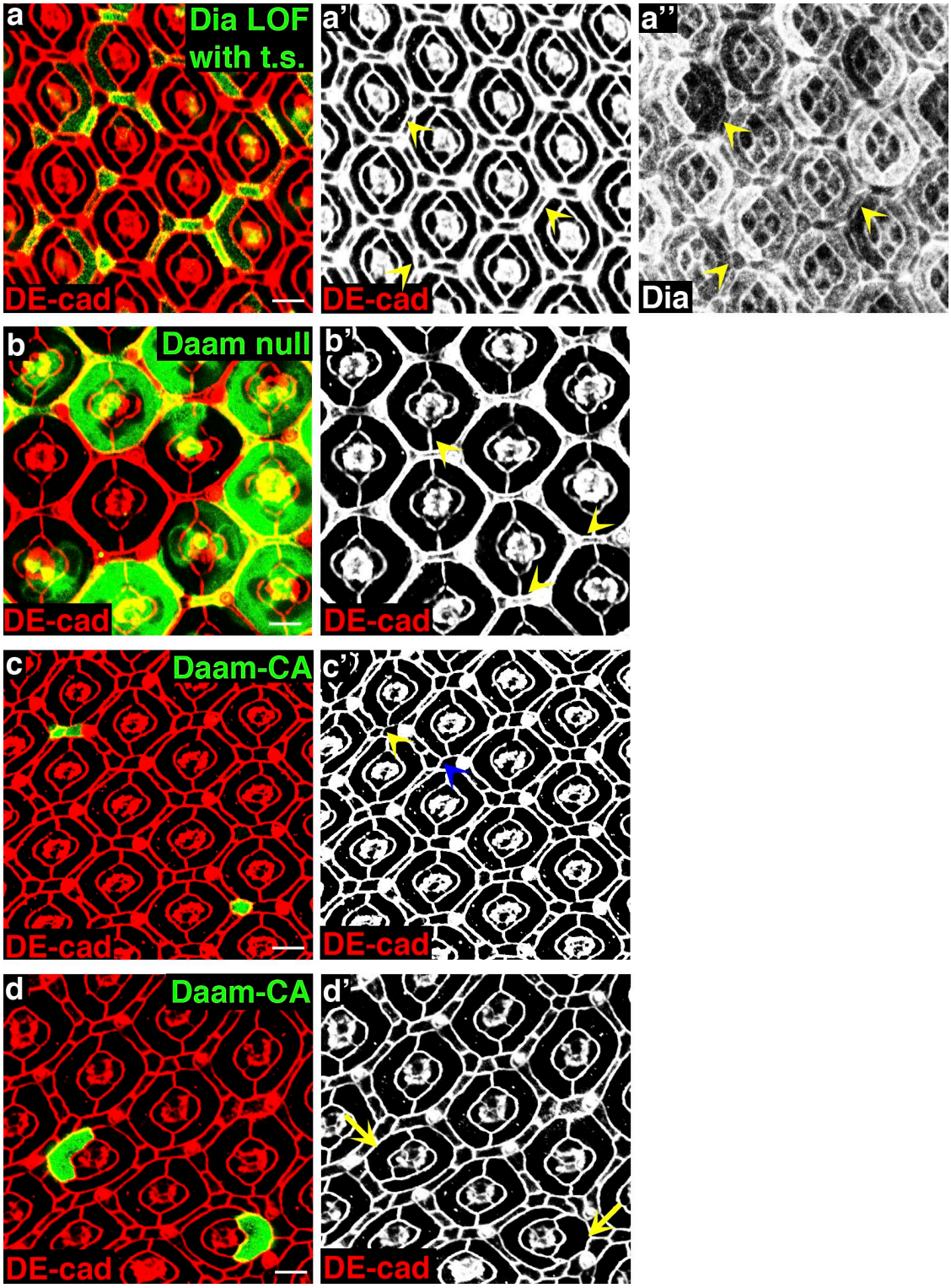


Figure S3. **Dia and Daam do not maintain formed AJs.** (a–a″) Confocal immunofluorescent localization of DE-cadherin (DE-cad; a and a′) and Dia (a″) in *dia*^{5} MARCM clones after a 30-h temperature shift (t.s.) at 29°C. (b) Confocal immunofluorescent localization of DE-cadherin in Daam^{Ex68} (Daam null) MARCM clones. (a and b) Arrowheads identify AJs between clonal cells. (c and d) Confocal immunofluorescent localization of DE-cadherin in clones expressing CA Daam (Daam-CA). (c) The yellow arrowhead identifies an AJ between two clonal cells, whereas the blue arrowhead identifies an AJ between two analogous wild-type cells. (d) Yellow arrows identify clonal cells. Bars, 10 µm.

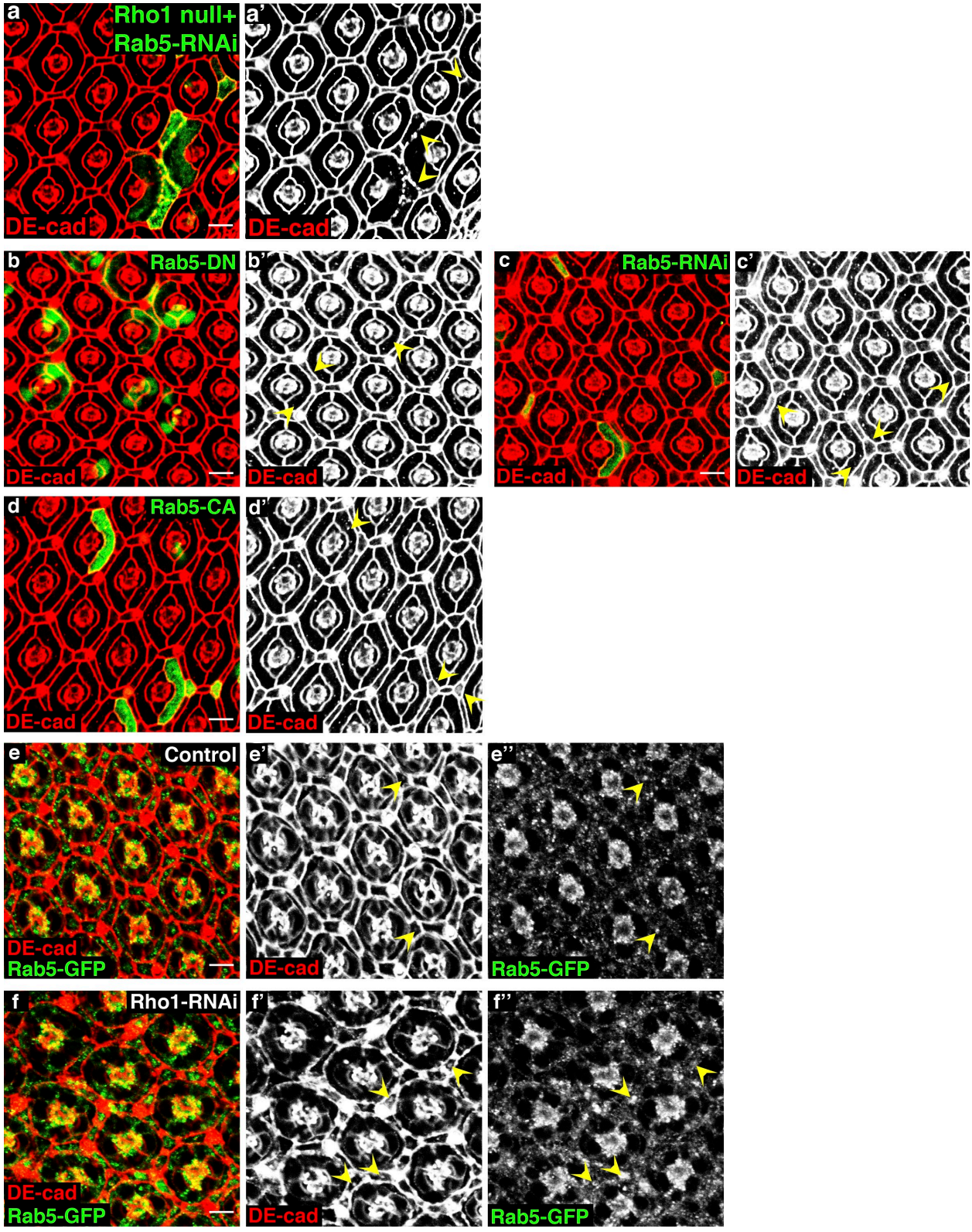


Figure S4. **Rab5-RNAi expression partially rescues AJ disruptions between Rho1-null cells; Rab5-DN, Rab5-RNAi, or Rab5-CA expression alone does not affect AJs or apical area; and Rho1 depletion increases intracellular DE-cadherin in the endocytic compartment.** (a) Confocal immunofluorescent localization of DE-cadherin (DE-cad) in *Rho1*^{72} MARCM clones expressing Rab5-RNAi. Arrowheads identify AJs between clonal cells. (b–d) Confocal immunofluorescent localization of DE-cadherin in flippase-out clones expressing Rab5-DN (b), Rab5-RNAi (c), or Rab5-CA (d). Arrowheads identify clonal cells. (e and f) Confocal immunofluorescent localization of DE-cadherin (e, e', f, and f') in pupal eyes expressing either Rab5-GFP alone (e) or Rab5-GFP and Rho1-RNAi (f) with *GMR-gal4*. Arrowheads identify intracellular DE-cadherin that colocalizes with Rab5-GFP. This image is 0.75 µm basal compared with other pupal eye images. Bars, 10 µm.

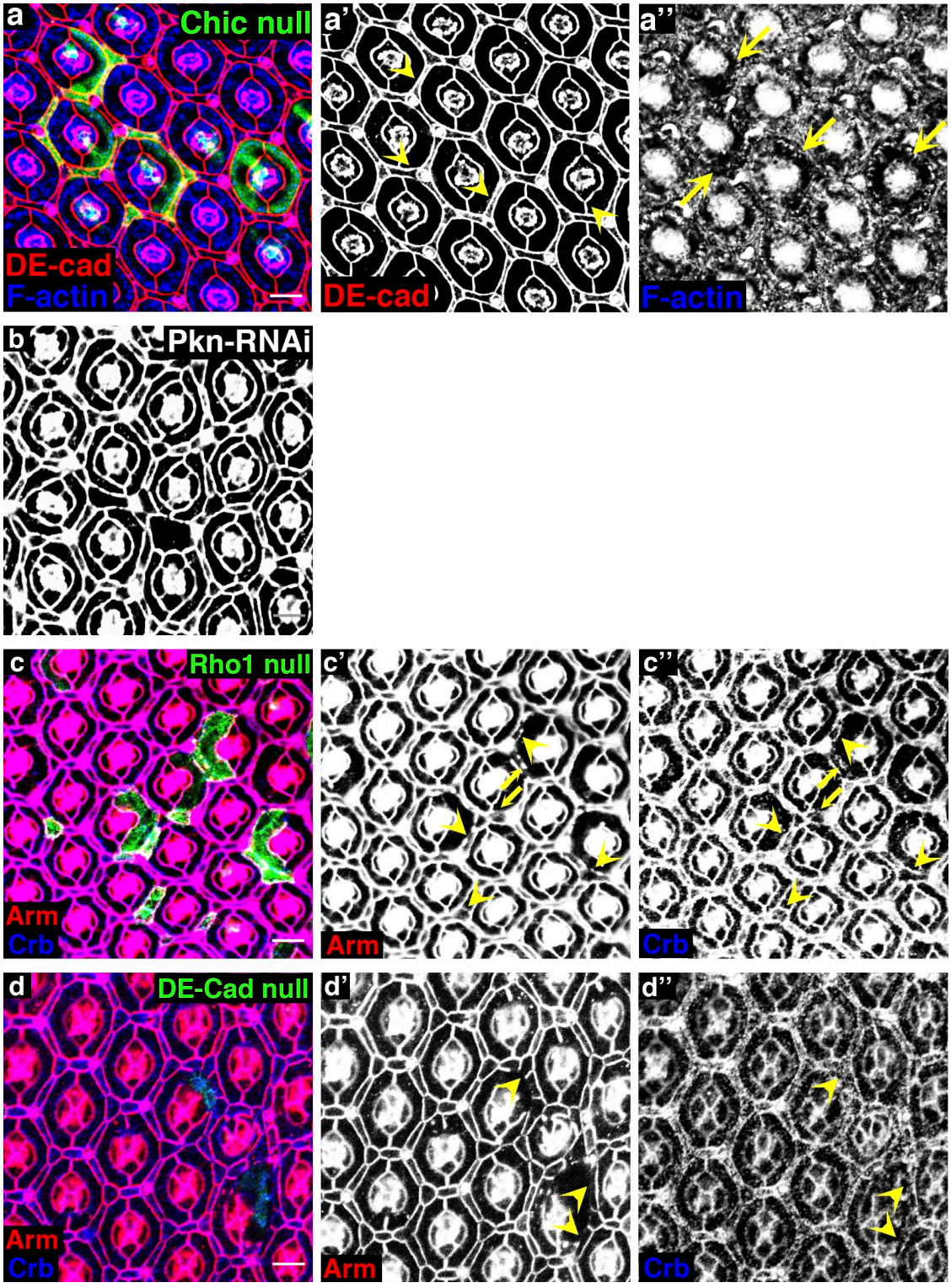


Figure S5. **Depletion of Chickadee disrupts F-actin but not AJs, expression of PKN-RNAi disrupts pupal eye patterning but not AJs, and Crumbs localization is disrupted by Rho1 depletion or DE-cadherin depletion.** (a–a″) Confocal immunofluorescent localization of DE-cadherin (DE-cad; a and a′) and phalloidin staining (a and a″) in *chic*^{221} MARCM clones. Arrowheads identify AJs between clonal cells, and arrows identify clonal cells. Chic, Chickadee. (b) Confocal immunofluorescent localization of DE-cadherin in pupal eye expressing PKN-RNAi (GMR>PKN-RNAi). (c and d) Confocal immunofluorescent localization of Armadillo (Arm; c, c′, d, and d′) and Crumbs (Crb; c, c″, d, and d″) in *Rho1*^{72} MARCM clones (c) or *shg*^{R69} MARCM clones (d). Arrowheads identify disrupted AJs between Rho1-null cells (c) or around DE-cadherin–null cells (d). Arrows indicate where Crumbs colocalizes with fragmented DE-cadherin. Bars, 10 µm.

Table S1. **Apical area index quantification of *Rho1*^{72}, Rho1-RNAi, *rok*^{2}, and *dia*^{5} clones**

Genotype	Apical area index mean	SD	*n*	P-value
Wild type	0.9975	0.0286	25	NA
Rho1^{72O}	1.9820	0.1517	40	0.006320
Rho1^{72F}	1.8932	0.1711	23	0.010338
Rho1-RNAi	1.5477	0.0262	67	0.000017
rok^{2}	1.5236	0.0691	35	0.002064
dia^{5}	1.0279	0.0272	76	0.253950
dia^{5} + Dia-RNAi	1.0356	0.0528	43	0.350822
rok^{2} + Dia-RNAi	1.8258	0.1928	38	0.022038[a]
Rho1^{72} + Rho1	0.8448	0.0792	34	0.065477

NA, not applicable. The apical area index is the ratio of a clonal cell apical area divided by an analogous, neighboring nonclonal cell apical area. Quantifications were performed using ImageJ version 1.38. P-values were calculated using an unpaired, two-sided Student's *t* test against wild-type clones.
[a]The p-value for *rok2* + Dia-RNAi was calculated against *rok*^{2}.

Table S2. **F-actin index quantification**

Genotype	F-actin index mean	SD	*n*	P-value
Wild type	1.0125	0.1233	26	NA
Rho1^{72}	0.7794	0.1829	38	0.018798
rok^{2}	0.9686	0.1084	36	0.620749
dia^{5}	1.0742	0.2761	21	0.131398
dia^{5} + Dia-RNAi	1.0046	0.1396	15	0.857209
chic^{221}	0.6400	0.0279	43	0.000370

NA, not applicable. The F-actin index is the ratio of phalloidin-staining pixel intensity in a clonal cell divided by an analogous, neighboring nonclonal cell. Quantifications were performed using ImageJ version 1.38. P-values were calculated using an unpaired, two-sided Student's *t* test against wild-type clones.

Table S3. **Phospho-MLC index quantification**

Genotype	Phospho-MLC index mean	SD	*n*	P-value (wild type)	P-value (*Rho1*^{72})
Wild type	1.0694	0.0396	22	NA	NA
Rho1^{72}	0.7954	0.0863	40	0.006184	NA
rok^{2}	0.8234	0.0732	42	0.007296	0.637941

NA, not applicable. The phospho-MLC index is the ratio of phospho-MLC immunofluorescence pixel intensity in a clonal cell divided by an analogous, neighboring nonclonal cell. Quantifications were performed using ImageJ version 1.38. P-values were calculated using an unpaired, two-sided Student's *t* test against either wild-type clones or *Rho1*^{72} clones.

Table S4. **AJ index quantification**

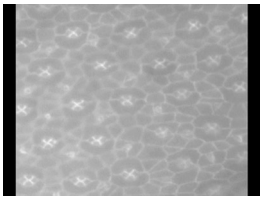
Genotype	AJ index mean	SD	*n*	P-value
Rho1^{72}	0.2668	0.0756	41	NA
Rho1^{72} + Rab5-DN	0.7661	0.1382	54	0.000066
Rho1^{72} + Rab5-RNAi	0.7071	0.1263	36	0.000351
Rho1^{72} + Rab11-DN	0.3487	0.0492	15	0.114392
Rho1^{72} + Rab7-DN	0.3264	0.0646	12	0.394211
Rho1^{72} + Rab8-DN	0.3413	0.0147	12	0.093595
Rho1^{72} + Cdc42-RNAi	0.8946	0.0045	11	0.004461
Rho1^{72} + Dia-CA	0.3646	0.0412	37	0.153122
Rho1^{72} + Rho1	1.0000	0.0000	15	0.000027

NA, not applicable. The AJ index is the ratio of the border length positive for DE-cadherin immunofluorescence divided by the total border length between two clonal cells. Quantifications were performed using ImageJ version 1.38. P-values were calculated using an unpaired, two-sided Student's *t* test against *Rho1*^{72} clones.

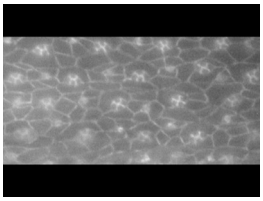
Table S5. **Apical area index quantification in *Rho1*^{72} clones expressing DN Rab transgenes, Rab5-RNAi, or Cdc42-RNAi**

Genotype	Apical area index mean	SD	*n*	P-value
Wild type	0.9975	0.0286	25	NA
Rho1^{72}	1.9376	0.1526	63	NA
Rho1^{72} + Rho1	0.8448	0.0792	34	0.000003
Rho1^{72} + Rab5DN	1.9681	0.2906	60	0.839399
Rho1^{72} + Rab5-RNAi	1.8237	0.2366	29	0.435798
Rho1^{72} + Rab11DN	1.9218	0.2022	22	0.912045
Rho1^{72} + Rab7DN	1.9426	0.1964	35	0.971204
Rho1^{72} + Rab8DN	2.0238	0.1738	20	0.610625
Rho1^{72} + Cdc42-RNAi	1.7791	0.3344	35	0.622521

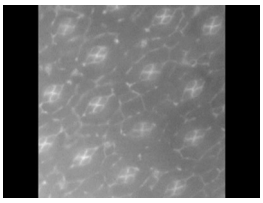
NA, not applicable. The apical area index is the ratio of a clonal cell apical area divided by an analogous, neighboring nonclonal cell apical area. Quantifications were performed using ImageJ version 1.38. P-values were calculated using an unpaired, two-sided Student's *t* test against *Rho1*^{72} clones.



Video 1. **Live imaging of AJs in wild-type pupal eye 20–28 h APF.** Time-lapse imaging of α-catenin–GFP expressed with *GMR-gal4* in pupal eye 20–28 h APF. Imaging was performed using a 63× NA 1.4 objective. Every 20 min, a series of images was captured at different focal planes and subsequently compiled into a composite image for each time point using Photoshop. The video is shown at 1 frame/0.8 s.



Video 2. **Live imaging of AJs in pupal eyes expressing Rho1-RNAi 20–23 h APF.** Time-lapse imaging of α-catenin–GFP and Rho1-RNAi expressed with *GMR-gal4* in pupal eye 20–23 h APF. Imaging was performed using a 63× NA 1.4 objective. Every 15 min, a series of images was captured at different focal planes and subsequently compiled into a composite image for each time point using Photoshop. The video is shown at 1 frame/0.8 s.



Video 3. **Live imaging of AJs in pupal eyes expressing Rho1-RNAi 23–28 h APF.** Time-lapse imaging of α-catenin–GFP and Rho1-RNAi expressed with *GMR-gal4* in pupal eye 23–28 h APF. Imaging was performed using a 63× NA 1.4 objective. Every 15 min, a series of images was captured at different focal planes and subsequently compiled into a composite image for each time point using Photoshop. The video is shown at 1 frame/0.8 s.