Supplemental material

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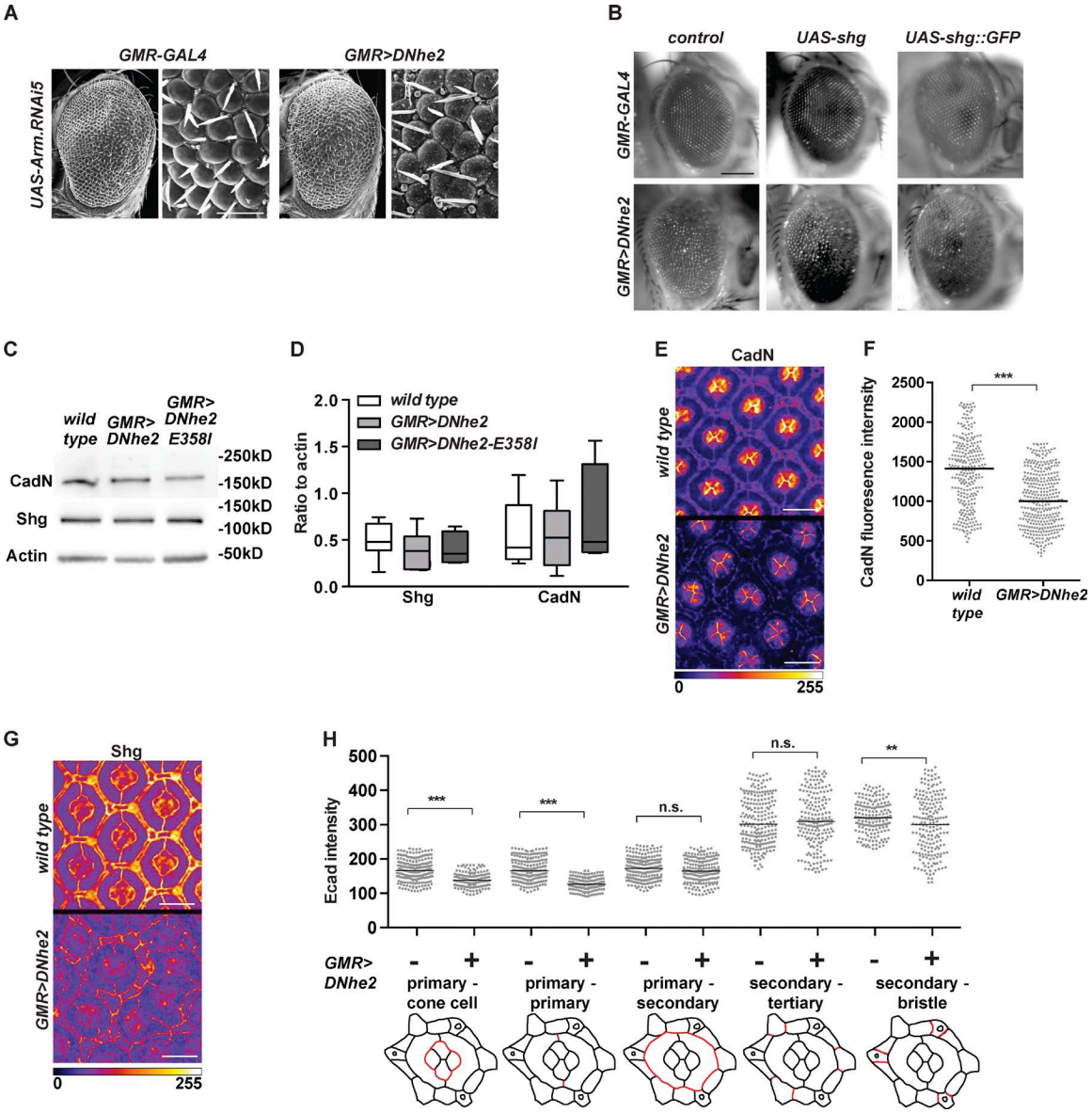

Figure S1. **Overexpression of *DNhe2* has no effect on total cadherins and mixed effects on cadherin localization at cell–cell contacts. (A)** Scanning electron micrographs of adult *Drosophila* eyes depicting genetic interactions between *control* (*GMR-GAL4*) and overexpression of *DNhe2* (*GMR>DNhe2*) with RNAi-mediated knockdown of Arm (*UAS-Arm.RNAi5*) using a distinct hairpin sequence. **(B)** Representative images show no suppression of the *GMR>DNhe2* overexpression phenotype with coexpression of two different *UAS-shg* transgenic lines (*UAS-shg* and *UAS-shg::GFP*). **(C)** Representative immunoblots showing total protein levels for Shg (*Drosophila* E-cadherin), CadN, and actin from adult *Drosophila* head lysates made from three *Drosophila* lines: *WT*, overexpression of *DNhe2* (*GMR>DNhe2*), and overexpression of an inactive *DNhe2* mutant (*GMR>DNhe2^{E358I}*). **(D)** Quantitative analysis of replicate Western blots performed as described in C. *WT* and *GMR>DNhe2*, N = 8; *GMR>DNhe2^{E358I}*, N = 4. **(E)** Confocal micrographs of pupal retinae from *WT* and *GMR>DNhe2*-expressing flies labeled for CadN and pseudocolored to show pixel intensities. **(F)** Quantitative measurements of CadN fluorescence intensity (in AU) measured at cone cell–cone cell contacts. *n* = 267–360 junctions, N = 4 individual flies per condition. **(G)** Confocal micrographs of pupal retinae from *WT* and *GMR>DNhe2*-expressing flies labeled for Shg. Bars: 25 µm (A and B); 10 µm (E and G). **(H)** Quantitative measurements of Shg fluorescence intensity (in AU) measured at distinct cell–cell junctions. Labeled schematics show which cell junctions (labeled in red) were measured. *n* = 267–360 junctions, N = 4 individual flies per condition. In D, Tukey boxplots are shown, and significance was determined using an unpaired, two-tailed Student's *t* test with Holm-Sidak's multiple comparisons correction. In F and H, medians are shown, and significance was determined using the Mann–Whitney test. **, P < 0.01; ***, P < 0.001.

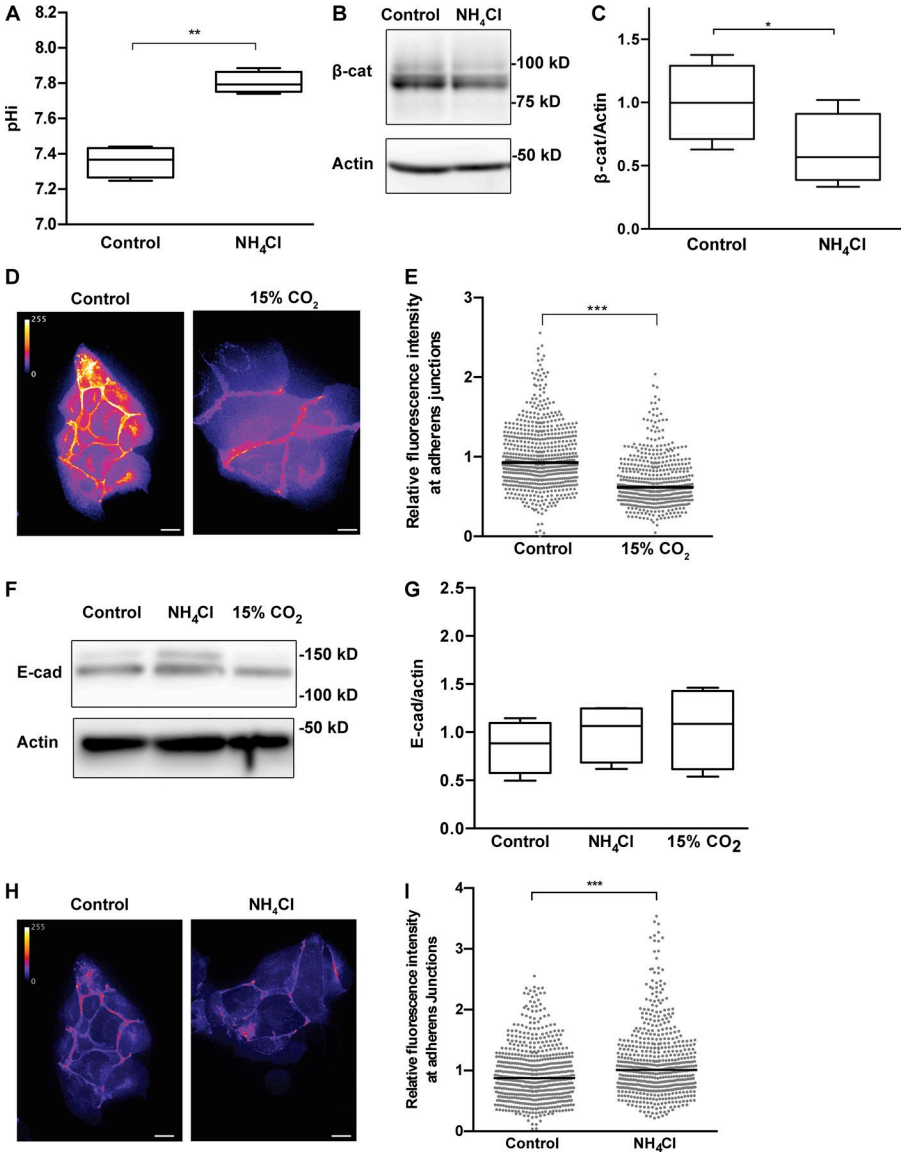

Figure S2. **Higher pHi decreases total and junctional β-catenin abundance in clonal NMuMG epithelial cells and MDCK cells; while E-cadherin total levels are unchanged with higher pHi, junctional localization is altered. (A)** NMuMG cells maintained for 24 h with 5 mM NH_4Cl have a higher pHi than control untreated cells. *n* = 4. **(B)** Representative immunoblots showing total protein levels for β-catenin from NMuMG cells grown under conditions as described in A. **(C)** Quantitative analysis of replicate Western blots performed as described in B with β-catenin intensity normalized to actin for each replicate. *n* = 4. **(D)** Confocal images of MDCK cells grown under normal conditions (control) or acclimated to 15% CO_2 for two passages, fixed and immunolabeled for β-catenin, and pseudocolored to show fluorescence intensities. **(E)** Quantitative measurements of β-catenin fluorescence intensity at adherens junctions under conditions described in D. *n* = 3, *n* = 508–631 junctions. **(F)** Representative immunoblots showing total protein levels for E-cadherin from MDCK cells grown under normal conditions (control), acute 24-h treatment with 5 mM NH_4Cl, or acclimated to 15% CO_2 for two passages. **(G)** Quantitative analysis of immunoblots with E-cadherin intensity normalized to actin for each replicate. Control and NH_4Cl, *n* = 6; 15% CO_2, *n* = 4. **(H)** Confocal images of MDCK cells grown under conditions described in F, fixed and immunolabeled for E-cadherin, and pseudocolored to show fluorescence intensities. Bars, 20 µm. **(I)** Quantitative measurements of images collected as described in H. Data were normalized to the mean fluorescence intensity in control cells for each replicate. Control and NH_4Cl, *n* = 3, *n* = 581–617 junctions. In A, C, and G, Tukey boxplots are shown, and significance was determined by unpaired, two-tailed Student's *t* tests. In E and I, medians are shown, and significance was determined using the Mann–Whitney test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

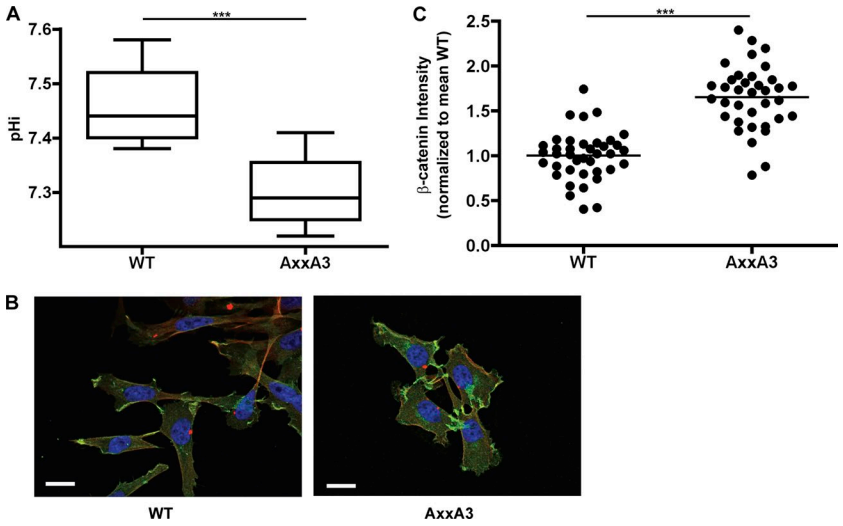

Figure S3. **Lower pHi increases β-catenin abundance at membrane protrusions. (A)** pHi measurements in PS120 cells stably expressing WT Nhe1 (WT) or mutant Nhe1 (AxxA3). *n* = 4. **(B)** Representative immunofluorescence images of cells treated as described in A showing β-catenin– (green), phalloidin- (red), and DAPI-stained nuclei (blue). Bars, 20 µm. **(C)** Quantitative analysis of β-catenin intensity at membrane protrusions. *n* = 3. In A, Tukey boxplots are shown; in C, means are shown; in A and C, significance was determined by unpaired, two-tailed Student's *t* tests. ***, P < 0.001.

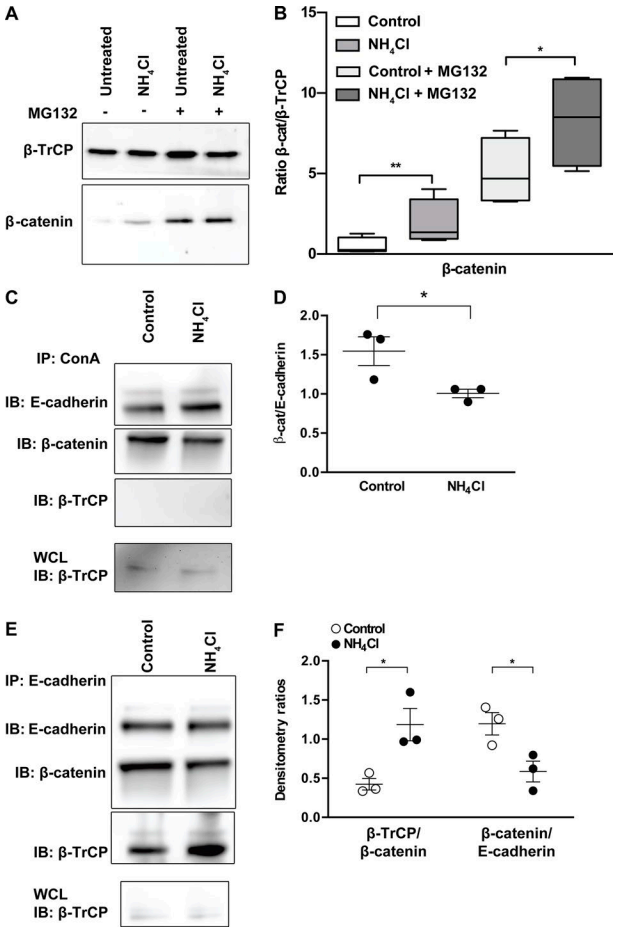

Figure S4. **Higher pHi increases association of β-catenin with the E3 ligase β-TrCP and increases the amount of β-TrCP associating with cadherin-associated β-catenin. (A)** Representative immunoblots (IBs) of β-catenin and β-TrCP abundance in endogenous β-TrCP immune complexes (IP) from MDCK cells untreated (control) or treated with 5 mM NH_4Cl for 24 h in the absence and presence of proteasome inhibitor MG132 (see Materials and methods for details). **(B)** Quantitative analysis of immunoblot replicates from A. *n* = 4. **(C)** Representative immunoblots of E-cadherin, β-catenin, and β-TrCP abundance in ConA precipitations from MDCK cells untreated (control) or treated with 5 mM NH_4Cl for 24 h. Immunoblot of β-TrCP in whole-cell lysates (WCLs) shows overall abundance. **(D)** Quantitative analysis of immunoblot replicates from C. *n* = 3. **(E)** Representative immunoblots of E-cadherin, β-catenin, and β-TrCP abundance in endogenous E-cadherin immune complexes (IP) from MDCK cells untreated (control) or treated with 5 mM NH_4Cl. Immunoblot of β-TrCP in whole-cell lysates shows overall abundance. **(F)** Quantitative analysis of immunoblot replicates from E. *n* = 3. In B, Tukey boxplots are shown. In D and F, scatterplots and means are shown. In B, D, and F, significance was determined using an unpaired, two-tailed Student's *t* test. *, P < 0.05; **, P < 0.01.

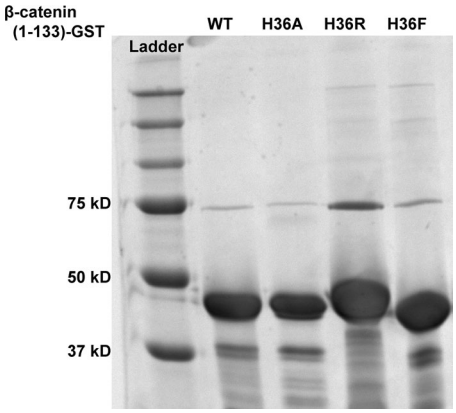

Figure S5. **Coomassie of purified β-catenin–(1–133)-GST proteins.**