Chen et al., http://www.jcb.org/cgi/content/full/jcb.201202105/DC1

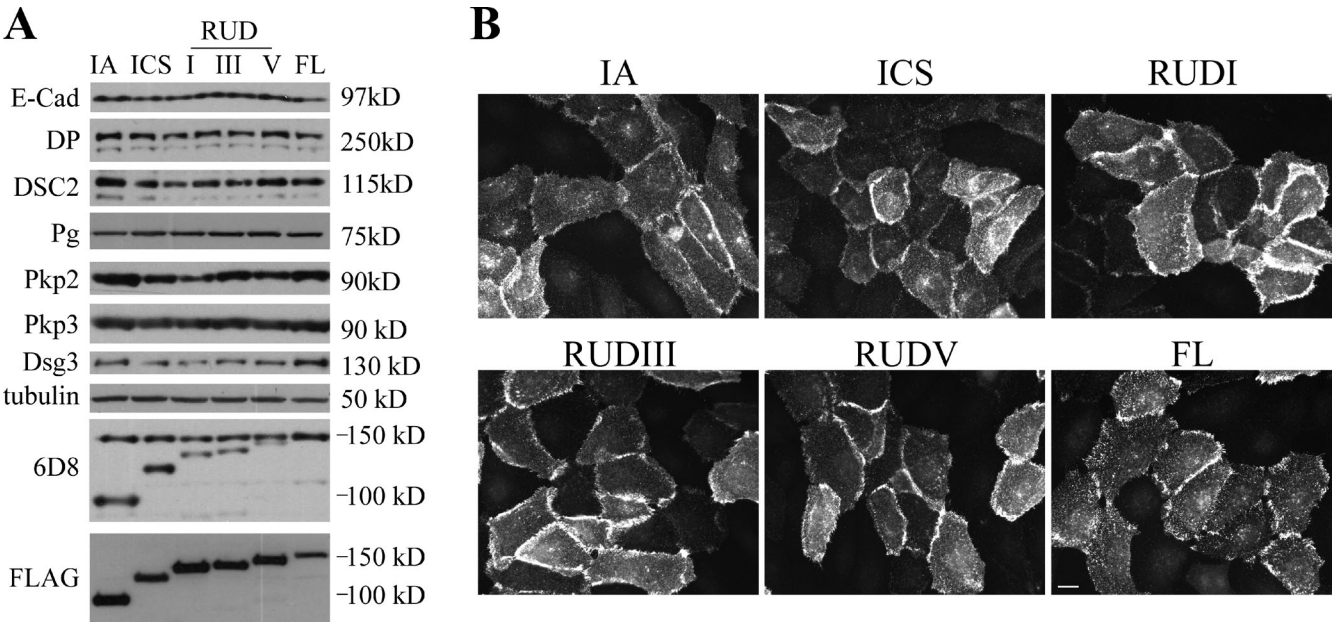

Figure S1. **Dsg2 mutants can be delivered to cell–cell borders, and the expression level of most of the junctional proteins remains unchanged.** (A) Lysates from SCC68 cells expressing Dsg2 mutants were immunoblotted with the indicated antibodies. E-cad, E-cadherin; DP, desmoplakin. (B) Immunofluorescence of SCC68 cells expressing Dsg2 mutant at 1.0 mM Ca^{2+} stained with anti-FLAG antibody. Bar, 20 µm.

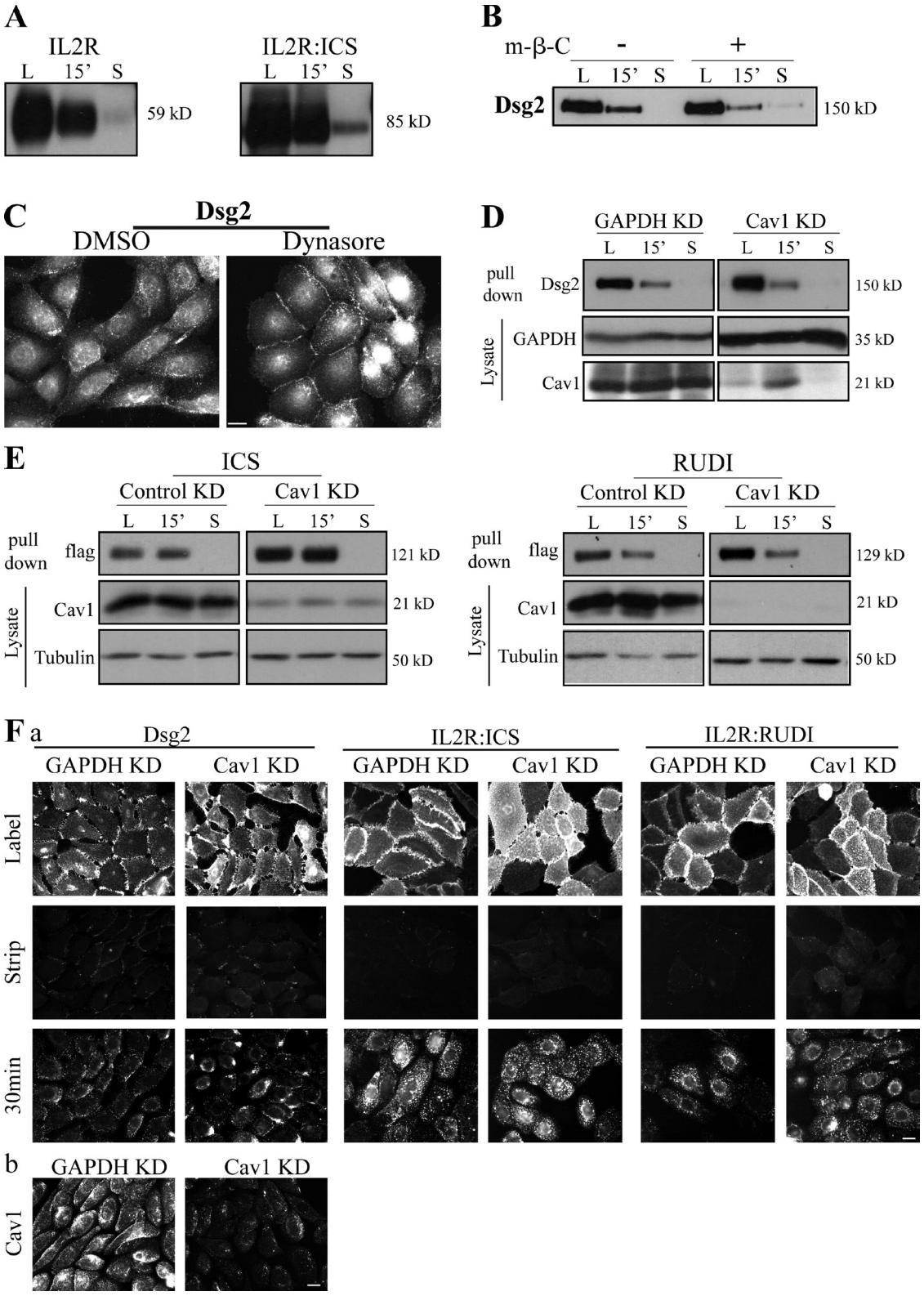

Figure S2. **Cholesterol and dynamin promote the internalization of endogenous Dsg2, whereas Cav1 has no effect on the internalization of either endogenous Dsg2 or Dsg2 mutants.** (A) Biotinylation assay of IL2R-FLAG and IL2R:ICS-FLAG at 0.25 mM Ca^{2+}. (B) Parental SCC68 cells were pretreated with 5 mM methyl-β-cyclodextrin (m-β-C) for 30 min before being subjected to the biotinylation assay. The immunoblot was probed with the 4B2 antibody. (C) Parental SCC68 cells grown in medium with 0.25 mM Ca^{2+} were treated with 80 µM dynasore for 30 min and then stained for FLAG. (D and E) Parental SCC68 (D) or SCC68 cells expressing Dsg2.ICS or Dsg.RUDI (E) were transfected with 20 nM siRNA oligos targeting GAPDH, caveolin-1 (Cav1), or a nonspecific sequence (control). (D–F, a) Biotinylation assay (D and E) and antibody internalization assay (F, a) were conducted 72 h after transfection. (F, b) Immunofluorescence analysis of SCC68 cells was performed 72 h after GAPDH or Cav1 siRNA transfection and stained for Cav1. KD, knockdown; L, surface pool of the Dsg2 mutants; S, residual Dsg2 mutant protein left on the cell surface after stripping. Bars, 20 µm.

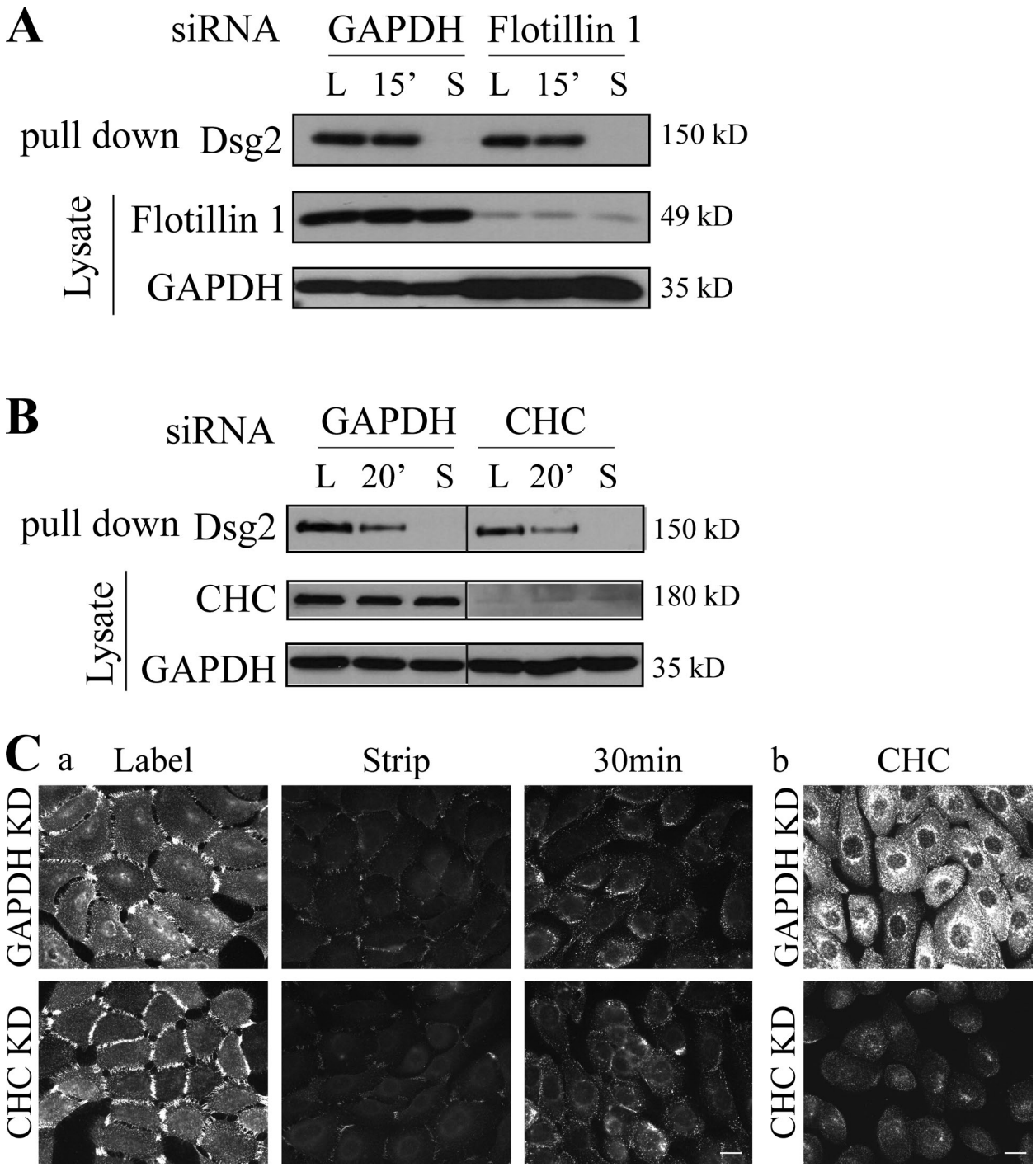

Figure S3. **The internalization of endogenous Dsg2 remains unchanged when the function of flotillin-1 and clathrin heavy chain is disrupted.** (A and B) Parental SCC68 cells were transfected with 20 nM siRNA targeting GAPDH, flotillin-1 (A), or clathrin heavy chain (CHC; B). Biotinylation assay was conducted 72 h after transfection. Immunoblots were probed with the indicated antibodies. Black line indicates that intervening lanes have been spliced out. (C, a) Antibody internalization assay was conducted 72 h after siRNA transfection. 6D8 was used to label Dsg2. (b) Immunofluorescence analysis of SCC68 was performed 72 h after GAPDH or clathrin heavy chain siRNA transfection and stained for clathrin heavy chain. KD, knockdown; L, surface pool of the Dsg2 mutants; S, residual Dsg2 mutant protein left on the cell surface after stripping. Bar, 20 μm.

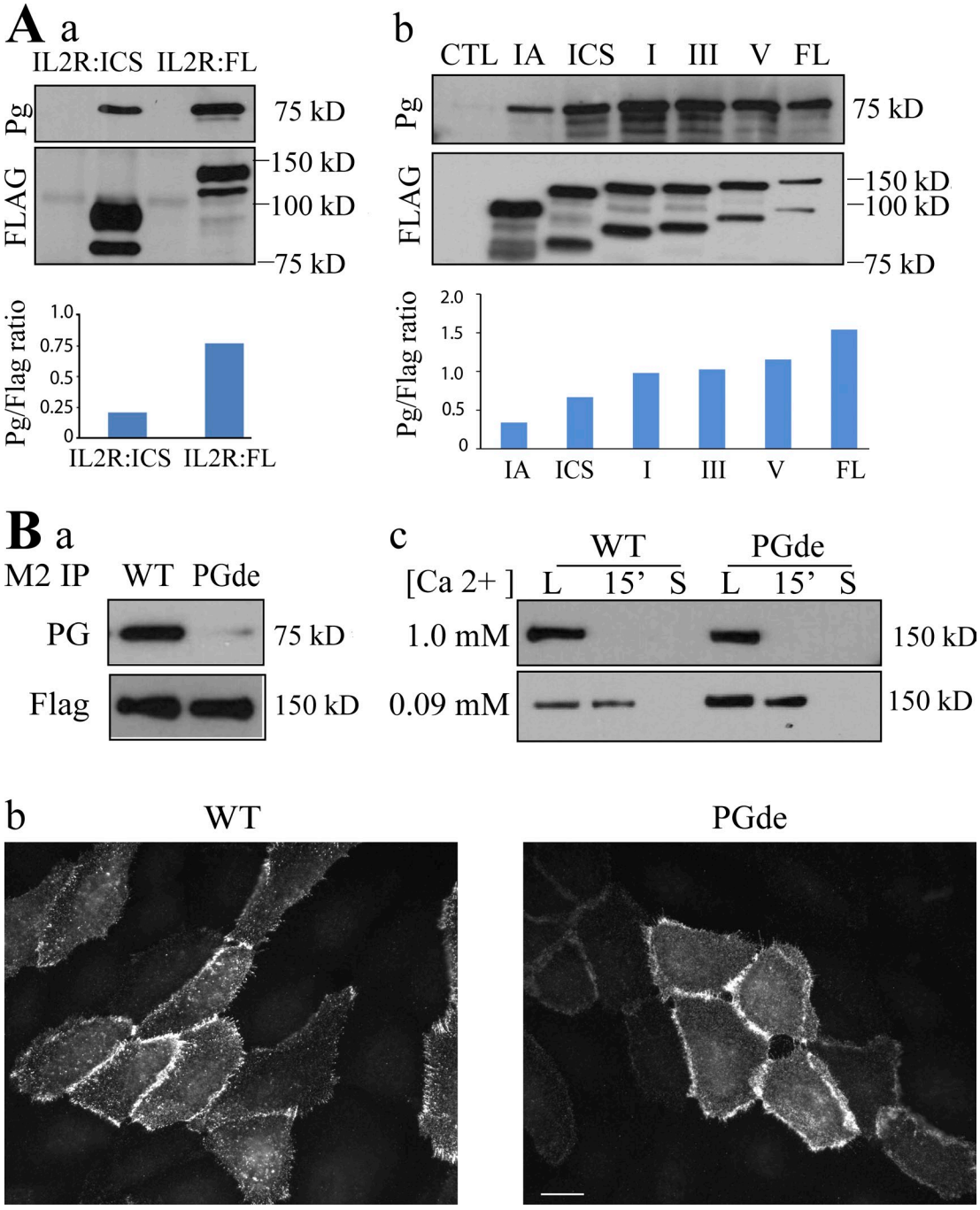

Figure S4. **DUR promotes Pg association with Dsg2, but disruption of the Pg–Dsg2 interaction has no effect on Dsg2 internalization.** (A) Parental (control [CTL]) SCC68 cells or SCC68 cells expressing Dsg2 chimeras (a) or mutants (b) were lysed in RIPA buffer. Protein complexes were immunoprecipitated by M2 affinity gel and probed for Pg or FLAG. Densitometric analysis was performed using ImageJ to determine the ratio of Pg/FLAG intensity. The experiment in A was performed once, and B is one representative experiment out of three independent repeats. (B, a) SCC68 cells expressing Dsg2-WT or Dsg2-PGde were lysed in RIPA buffer. Protein complexes were immunoprecipitated by M2 affinity gel and probed for Pg or FLAG. (b) SCC68 cells expressing Dsg2-WT or Dsg2-PGde were grown in medium with 1.0 mM Ca^{2+} and stained with anti-FLAG antibody. Bar, 20 µm. (c) Biotinylation assays were performed in mutant-expressing cells grown in medium with 0.09 mM Ca^{2+} or 1.0 mM Ca^{2+}. IP, immunoprecipitation; L, surface pool of the Dsg2 mutants; S, residual Dsg2 mutant protein left on the cell surface after stripping.