E08-04-0356 Green

Supplemental Figure 1: MMP and EGFR inhibition block the accumulation of an internalized pool of Dsg2. (A) SCC68 cells were incubated overnight in starvation media containing 1.0 mM calcium. Dsg2 and E-cadherin (E-cad) were labeled with antibody and internalization was assessed after 60 min in the presence of EGF. (B) Internalization of Dsg2 and E-cad was assessed in SCC68 cells grown overnight in 1.0 mM media containing growth supplements. *Bar,* 20 μm. Internalized Dsg2 and E-cad were not observed in the PKI-treated cells. Internalized E-cadherin was only marginally reduced when compared to Dsg2 in the GM and TAPI-treated cells.

Supplemental Figure 2: Extracellular cleavage of Dsg2 generates a 100 kDa fragment. (A) Schematic illustrating the Dsg2 antibodies used to map the Dsg2 cleavage fragment. 6D8 and G129 recognize extracellular domain epitopes, while Dg3.1 and 4B2 recognize epitopes within the cytoplasmic tail. (B) RIPA lysates generated from SCC68 cells cultured overnight in 0.25 mM calcium were incubated with beads, non-specific IgG, 6D8, G129, Dg3.1 or 4B2. Immunoprecipitates were analyzed by Western blotting for Dsg2 with 4B2. Full-length 160 kDa Dsg2, but not the 100 kDa fragment, was immunoprecipitated using G129.

Supplemental Figure 3: Dsg2 processing is regulated by multiple ADAMs. SCC68 cells were transfected with GAPDH, ADAM9, 15 or 17 siRNA in 0.09 mM calcium. After 48 hrs, cultures were switched into 0.25 mM calcium containing media with PMA and samples were harvested the next day. Western blot analysis using antibodies against

Dsg2, GAPDH, the respective ADAMs, and tubulin was performed. Note that while ADAM17 and 15 KD did not have major off target effects on the other ADAMs, they both reduced Dsg2 cleavage, as did ADAM9 to a slightly lesser extent.

Supplemental Figure 4: EGFR inhibition promotes an increase in ADAM17 expression and segregation of Dsg2 and ADAM17 localization. SCC68 cells were treated overnight with DMSO or PKI in 0.25 mM calcium. (A) Cell lysates were analyzed by Western blot. Premature and mature ADAM17 expression was increased in the PKI-treated cells. (B) Dsg2 and ADAM7 localization was visualized using indirect immunofluorescence. In PKI-treated cells, Dsg2 staining was observed at sites of cell-cell contact, whereas ADAM17 staining was localized at the dorsal surface and/or in intracellular vesicles.

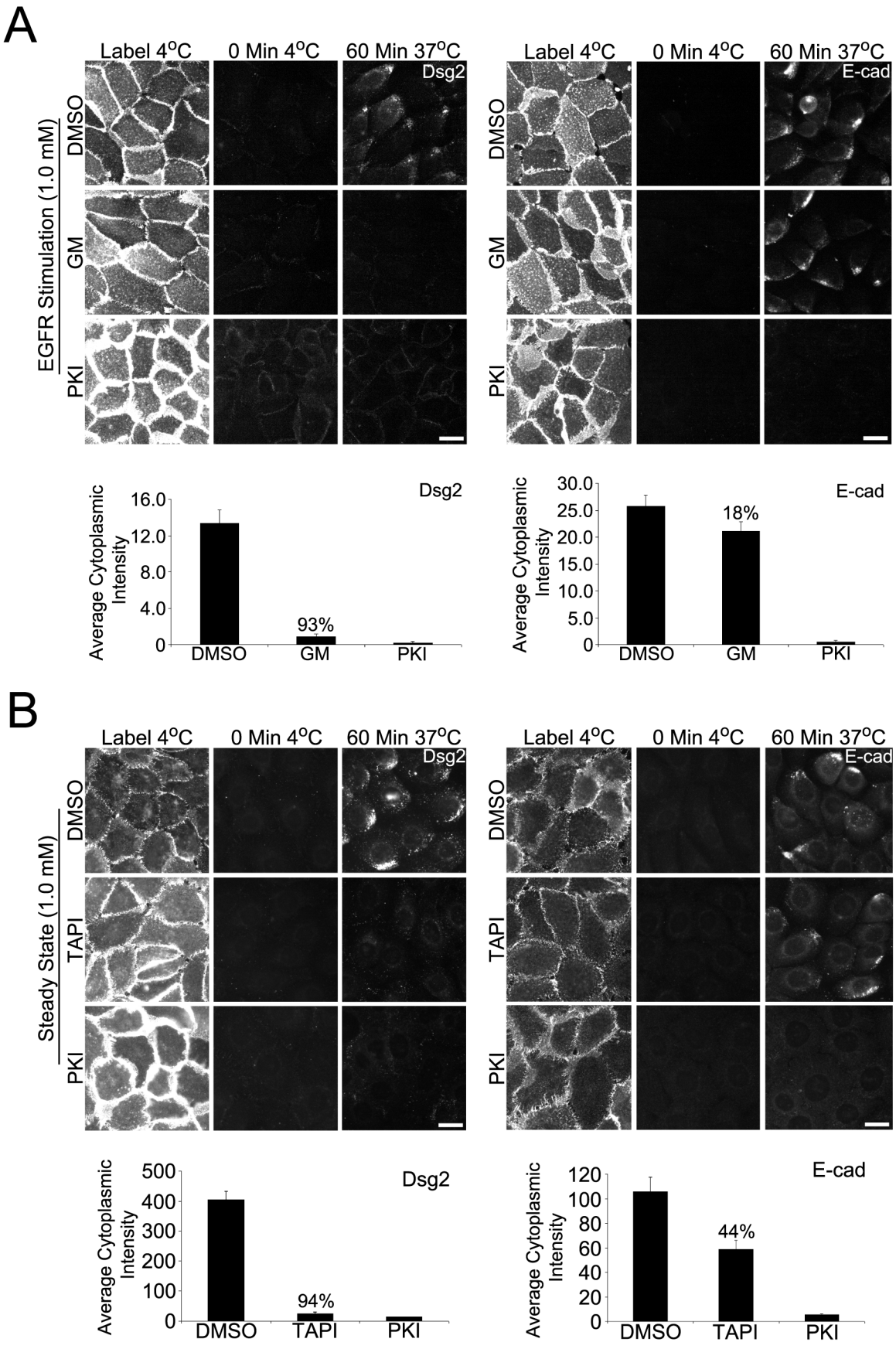
Online Video captions

Figure 4 -Video 1. Movie of SCC68 cells expressing Dsg2-GFP. After growing overnight in KSFM containing 0.09 mM calcium, cells were switched to 0.25 mM calcium and treated with a vehicle control, DMSO. Single-plane time lapse images were taken at 3 sec intervals. Green circles represent particles containing Dsg2-GFP that move in a retrograde manner originating from the cell-cell interface. Figure 4 DMSO represent stills from this movie.

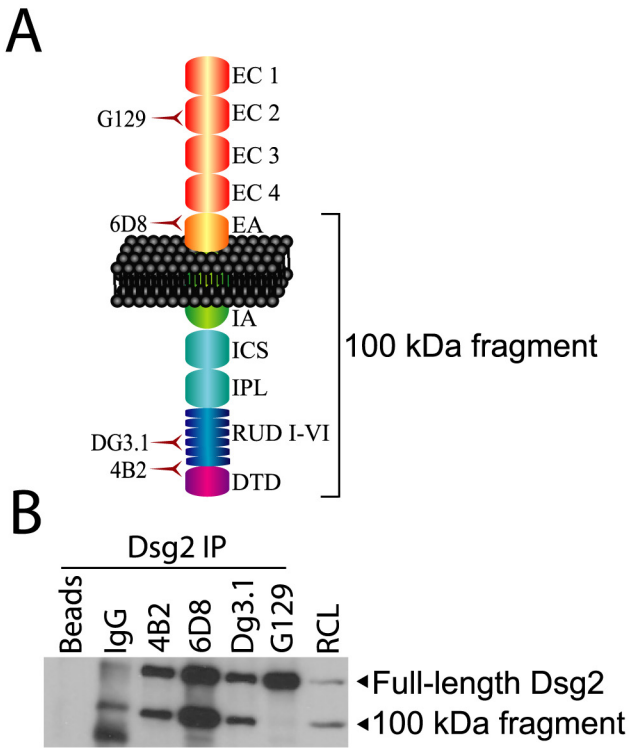
Figure 4 -Video 2. Movie of SCC68 cells expressing Dsg2-GFP. After growing overnight in KSFM containing 0.09 mM calcium, cells were switched to 0.25 mM calcium and treated with TAPI. Single-plane time lapse images were taken at 3 sec

intervals. The retrograde movement of Dsg2-GFP particles seen in control movie was not observed in TAPI treated cells. Figure 4 TAPI represent stills from this movie.

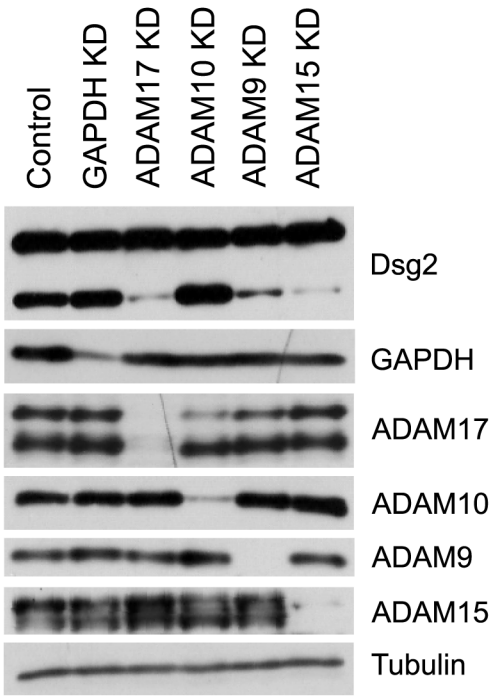
Figure 4 -Video 3. Movie of SCC68 cells expressing Dsg2-GFP. After growing overnight in KSFM containing 0.09 mM calcium, cells were switched to 0.25 mM calcium and treated with PKI. Single-plane time lapse images were taken at 3 sec intervals. The retrograde movement of Dsg2-GFP particles seen in control movie was not observed in PKI treated cells. Figure 4 PKI represent stills from this movie.



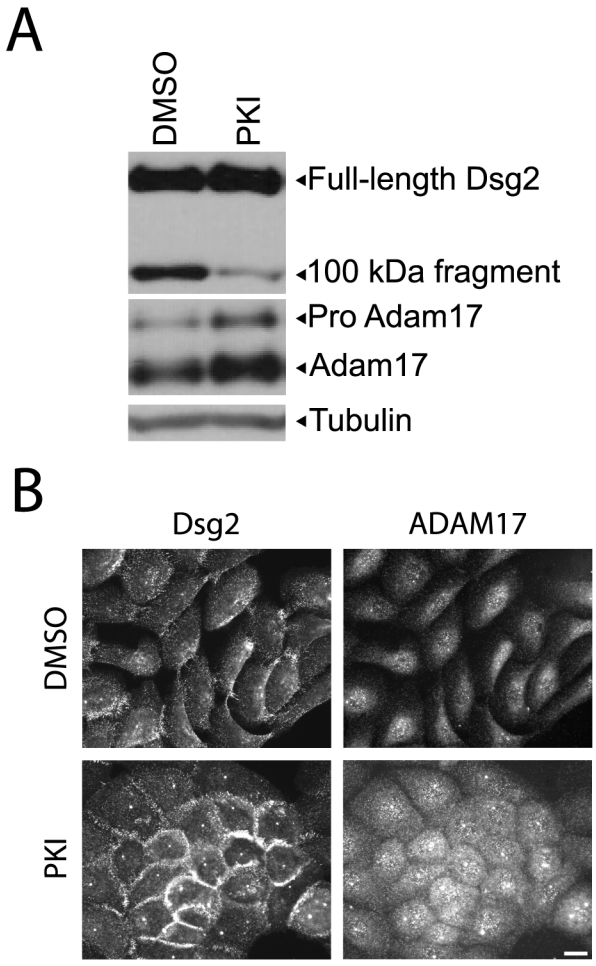
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4