

Supplemental Materials

Molecular Biology of the Cell

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Supplemental Figure 1: The Dsg1(G578R) mutant is inserted into the membrane in the correct orientation and is present on the cell surface at levels similar to WT Dsg1. A) Immunofluorescence localization of WT Dsg1.GFP and Dsg1(G578R).GFP in cells fixed with or without Triton-X 100 permeabilization using antibodies directed against the cytoplasmic GFP tag. The c-terminal GFP tag for both the Dsg1 WT and the Dsg1(G578R) SAM mutant is accessible only in cells permeabilized with Triton X-100, indicating that the Dsg carboxyl terminus is intracellular for both WT and mutant Dsg1. Scale bar = 20 μ m. B) A431 cell surface proteins were biotinylated and captured using streptavidin beads followed by western blot analysis for Dsg1 WT or Dsg1(G578R) SAM mutant. Whole cell lysates (WCL) represent total Dsg1 protein levels in unbiotinylated cells. Dsg1 WT and mutant were detected using the cytoplasmic GFP tag. C) Dsg1 WT and mutant levels at the cell surface (biotinylated) were normalized to E-cadherin cell surface levels and the ratio of surface protein to whole cell lysate was calculated. Quantification represents three independent experiments.

