Turn-over and steady-state levels of Dsg3 and associated PG in subcellular fractions. Dsg3 and PG were assessed in subcellular compartments of metabolic pulse-chase labeled wild-type mouse keratinocytes treated with PV IgG or nhlgG. Within the first hour, PV IgG binding markedly and transiently increased the turn-over of non-keratin anchored (Triton X-100 soluble) Dsg3 and associated PG both at the cell surface (Triton / IP-Protein G, 35S) as well as in the cytosol (Triton / IP-PV IgG, 35S) reminiscent of a receptor-like signaling event. Despite enhanced turn-over, the steady state Dsg3 at the cell surface did not decrease over 24 h while PG started to decline at 24 h. However, the cytosolic vesicular pool was rapidly depleted suggesting that the latter initially served to replenish the Dsg3 and PG at the plasma membrane. Up to 24 h, the turn-over of PG and the steady-state of Dsg3 in the desmosomal fraction were not significantly changed (SDS). Together these results indicate that PV IgG initially targets cell surface-exposed soluble Dsg3 and associated PG, triggers their enhanced but transient turn-over that results in rapid depletion of these molecules from the cytosolic reservoir. Despite the continuous presence of PV IgG, these molecules then remain at the steady-state levels of cells around the calcium switch.

Experimental procedure
At confluency, keratinocytes were incubated in high calcium medium for 3 h, starved with methionine/cysteine-free EMEM medium for 1 hr and pulse labeled with 200 μCi/ml [35S-methionine (ICN Pharmaceuticals, Inc.) in EMEM for 2 h. Surplus radioactivity was then chased with high calcium medium containing a 10’000 fold excesses of cold methionine and cysteine in the presence of nhlgG or PV IgG (1mg/ml) for up to 24 hr. To evaluate the half-life of PV IgG-targeted, cell surface-exposed non-keratin anchored Dsg3 and associated PG, all cultures were incubated with additional PV IgG (1mg/ml) at 4°C for 1 h prior to lysis with 0.5% Triton X-100 containing buffer. The in vivo PV IgG-bound, cell surface-exposed soluble Dsg3/PG in the supernatant was precipitated three times by direct addition of Protein G-Sepharose 4 fast flow without additional antibody (Amersham/Pharmacia, Biotech AB). In that way all in vivo bound PV IgG were collected (confirmed by western blotting of the remaining supernatant; data not shown). Note that chemical cross-linking of PV antibodies prior to lysis gave the same result (data not shown). The remaining Dsg3 and associated PG in the supernatant (referred to as “cytosolic-vesicular pool”) was subsequently immunoprecipitated by addition of new PV IgG (15 mg/ml). The desmosomal fraction obtained by solubilization of the pellet with SDS from the same cell cultures was diluted 10 times with 0.5% Triton X-100 containing buffer and subjected to immunoprecipitation with PV IgG (15 mg/ml) or PG antibodies. Note that PV IgG did not efficiently precipitate Dsg3 from the SDS fraction. Immunoprecipitation of untreated cells (0h) with PG antibodies (IP-PG) or beads alone served as specific and negative control, respectively. The location of Dsg3 and PG was confirmed by incubating the 35S-labeled protein blots (35S) with Dsg3 and PG antibodies (WB). Note that PV IgG specifically precipitates two major proteins from radiolabeled Triton-X-100 soluble lysates, one co-migrating with Dsg3 and the second with PG. One representative experiment of two is shown.