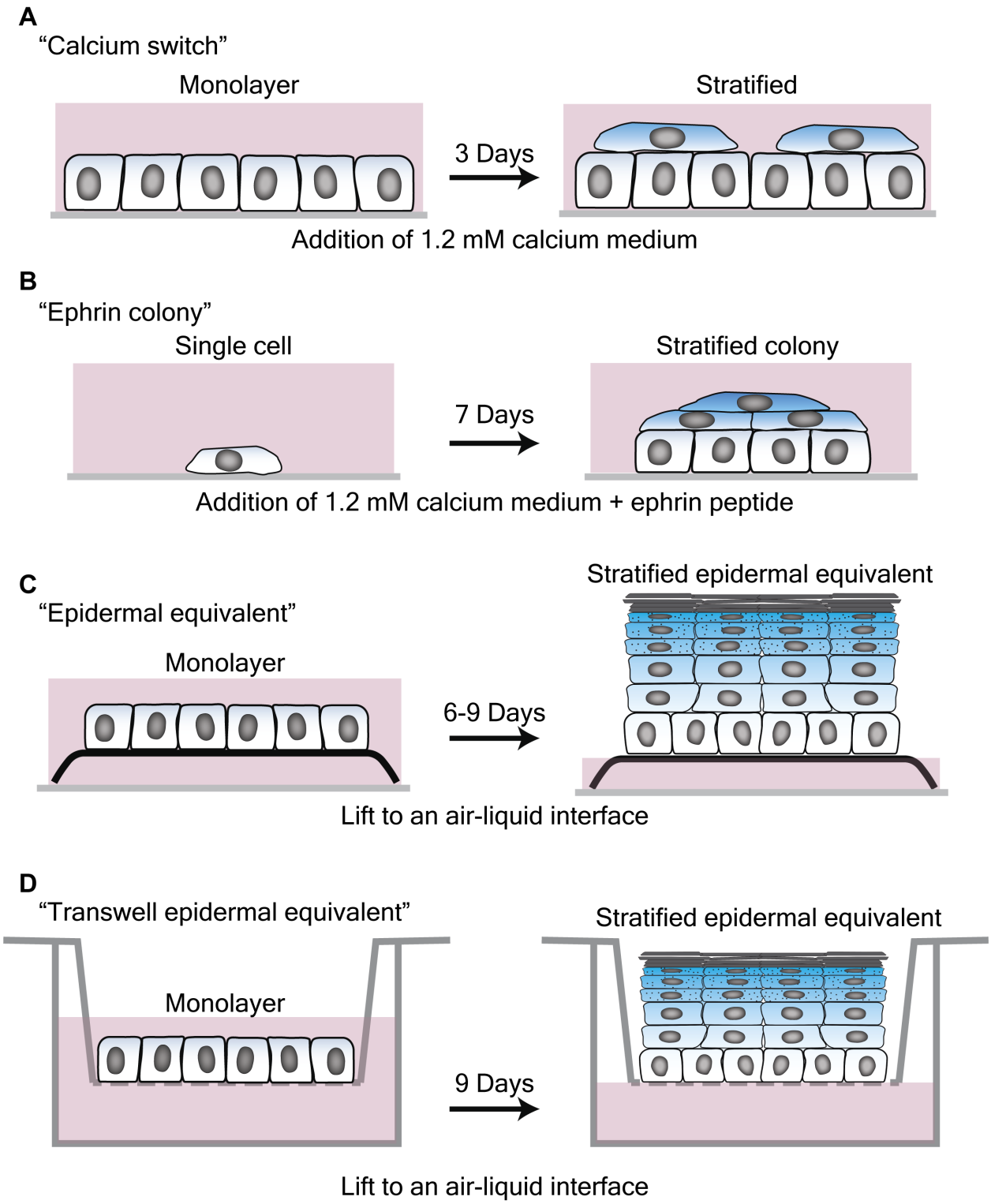

Figure S1. Cell culture models used in this study; Related to Figures 1-7
A) Calcium switch experiment- NHEKs are seeded at confluence in 0.07 mM calcium containing medium. The following day, the medium is changed to 1.2 mM calcium

containing medium to induce stratification and differentiation. After 3 days, unless otherwise noted, cells were harvested for analysis.
B) Ephrin colony experiment- NHEKs are seeded at low confluence such that they are initially sparse fields of single cells that subsequently grow into individual colonies. Culture medium contains 1.2 mM calcium and 1 μg/ml ephrin-A1 peptide to induce stratification and differentiation. After 7 days, unless otherwise noted, cells were harvested for analysis.
C) Epidermal equivalent experiment- monolayers of NHEKs were seeded on fibroblast containing collagen I gels at confluence. Subsequently, the gels were lifted to an air-liquid interface by placing them onto a metal grid with culture medium only filling the space up to the bottom of the metal grid. Cultures were allowed to stratify and differentiate for 6-9 days prior to harvesting, unless otherwise noted.
D) Transwell epidermal equivalent experiment- monolayers of NHEKs were seeded onto transwell inserts at confluence. The cultures were exposed to an air liquid interface by removing the medium from the top chamber of the transwell. Cultures were allowed to stratify and differentiate for 9 days prior to harvesting, unless otherwise noted. Transepidermal electrical resistance measurements were performed by adding medium into the top chamber, taking measurements, then promptly removing medium to restore the air-liquid interface.

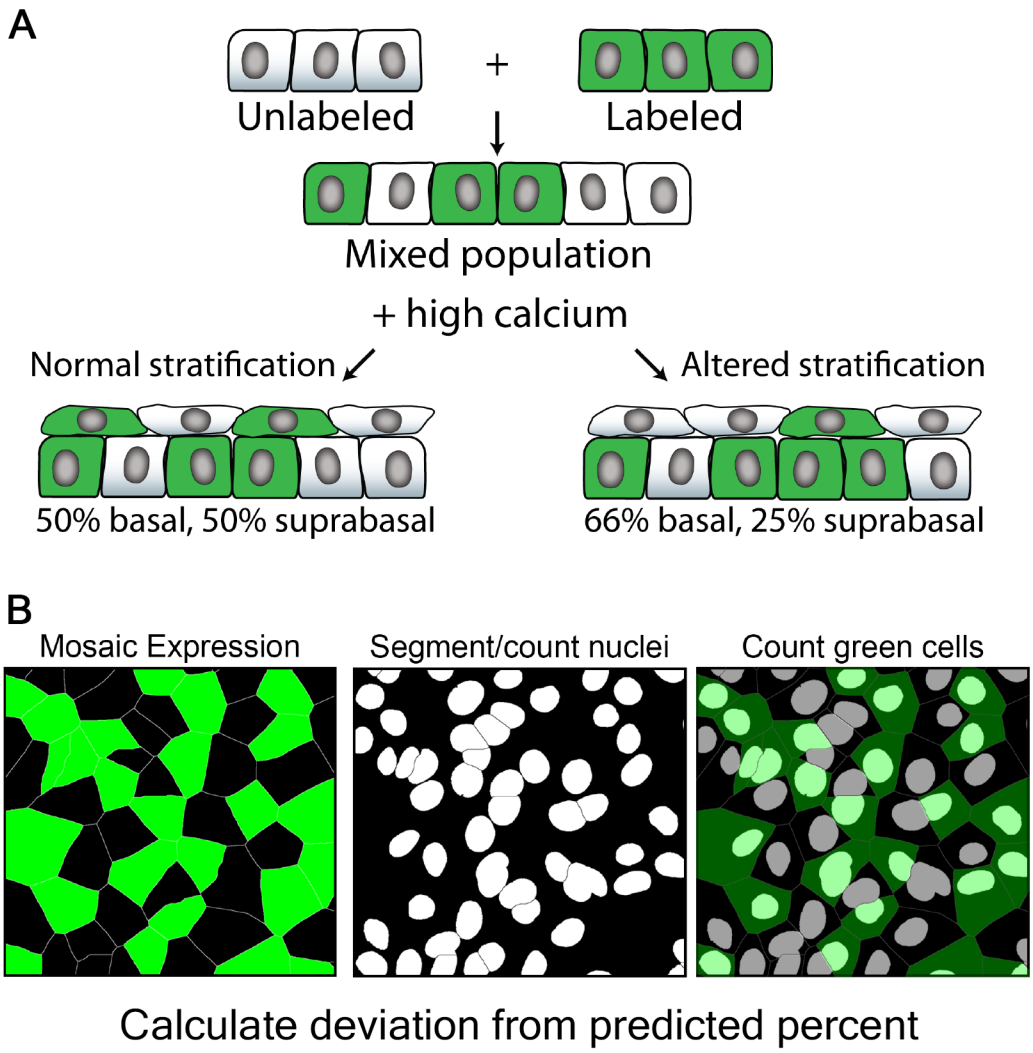

Figure S2. Schematic of stratification assay; Related to Figure 2
A) Wild type NHEKs were mixed at known ratios with genetically-modified NHEKs that expressed GFP. These combined populations were switched to 1.2 mM calcium (high calcium) containing medium and allowed to stratify for 3 days. An example in which 50% of cells are expressing GFP and 50% are wild type is presented. If stratification occurs normally, 50% of both basal and suprabasal cells will be expressing GFP. If stratification is inhibited, there will be an underrepresentation of GFP-positive cells in the suprabasal layer.
B) To quantify the percentage of cells expressing GFP, total cells were segmented (using a nuclear dye for example) and the percentage of GFP positive cells was calculated and then compared to the predicted percentage based on the initial ratio of cells plated.

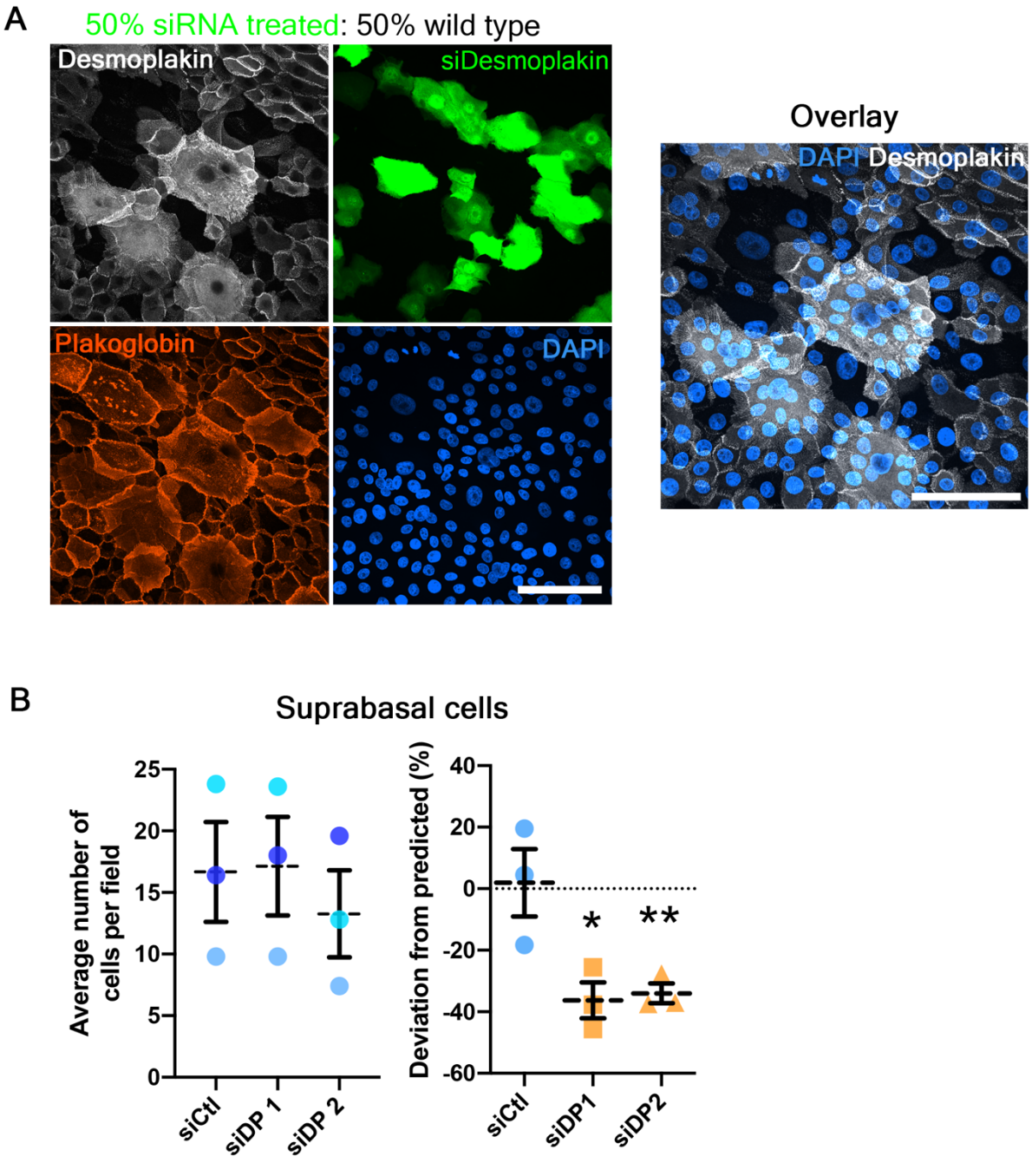

Figure S3. RNAi-mediated depletion of desmoplakin impairs stratification; Related to Figure 2

A) A representative sorting assay for desmoplakin (DP) depleted cells is shown. GFP-positive cells treated with either non-targeting (siCtl) or two DP-targeting siRNA pools (siDP) were mixed with wild type cells and induced to stratify. Immunostaining for DP indicates level of knockdown and the percentage of suprabasal cells (total cells

identified with plakoglobin in red and DAPI in blue) that were GFP positive was calculated. Bar is 100 μm.
B) Quantification for the average number of suprabasal cells as well as the deviation from the predicted percentage of GFP positive cells in the suprabasal layer for control (siCtl) and DP-depleted (siDP1 and siDP2) conditions is shown. Dashed lines indicate the mean of 3 independent experiments and error bars are SEM. *p=0.025, **p=0.0087, one sample t test with theoretical mean of 0.

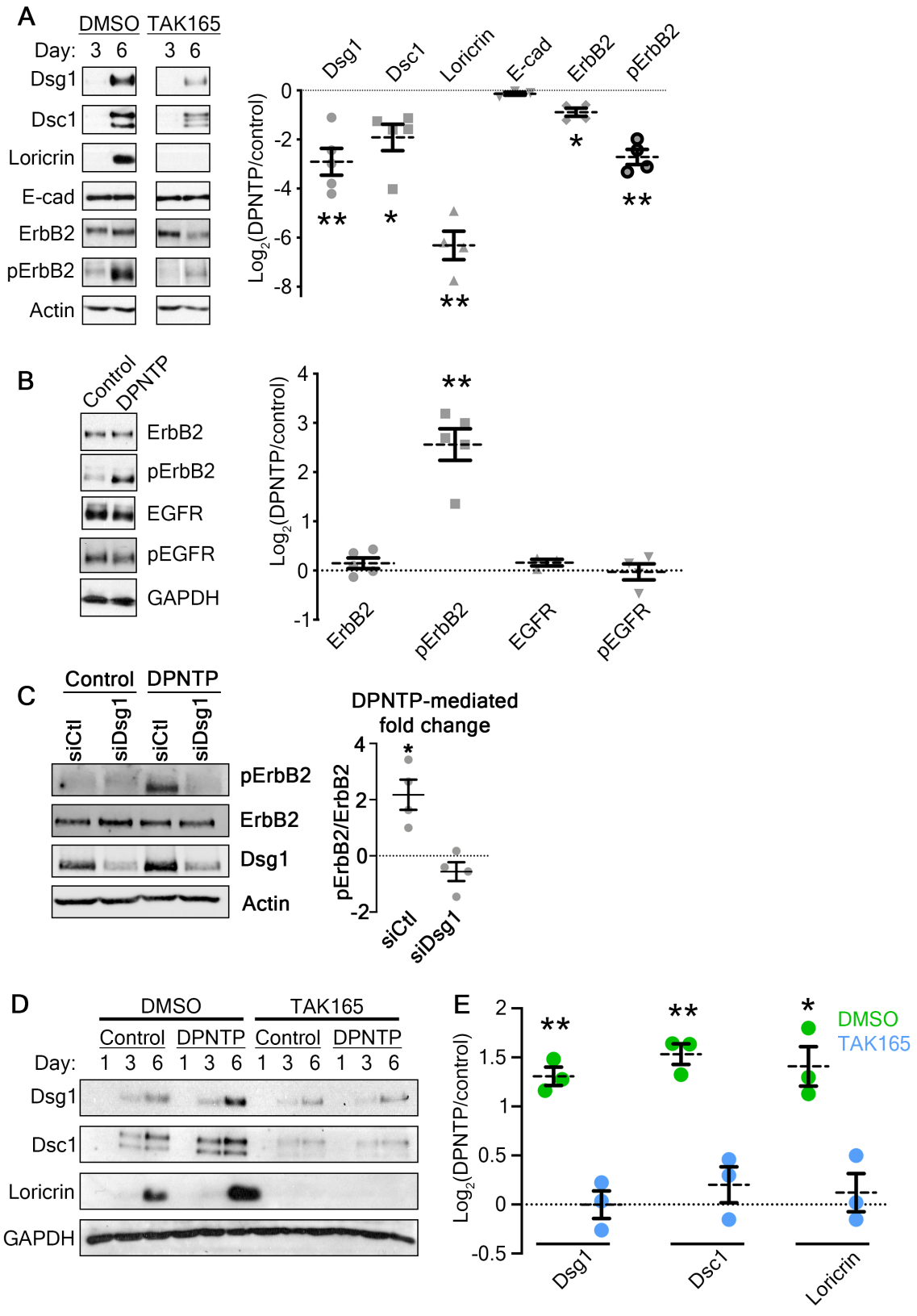

Figure S4. The DPNTP-mediated effects on differentiation are dependent on ErbB2; Related to Figure 3

A) Left, western blots show the expression of the indicated proteins in control (DMSO) or ErbB2 inhibitor (TAK165) treated keratinocytes at the indicated times after addition of 1.2 mM calcium medium. Right, quantification of the fold change (Log_2-transformed) of TAK165 treated over control are shown for the indicated protein expression. Dashed lines indicate the mean of 3-5 independent experiments and error bars are SEM. *$p \leq 0.02$, **$p \leq 0.006$, one sample t test with theoretical mean of 0.
B) Left, western blots show the expression of the indicated proteins in GFP (Control) or DPNTP-GFP expressing keratinocytes differentiated in 1.2 mM calcium medium for 6 days. Right, quantification of the fold change (Log_2-transformed) of DPNTP-expressing over control are shown for the indicated protein expression. pErbB2 is Y877-phosphorlated ErbB2 and pEGFR is Y1068-phosphoylated EGFR. Dashed lines indicate the mean of 3-5 independent experiments and error bars are SEM. **p=0.0013, one sample t test with theoretical mean of 0.
C) Left, western blots show the expression of the indicated proteins in GFP (Control) or DPNTP-GFP expressing keratinocytes treated with the indicated siRNA and differentiated in 1.2 mM calcium medium for 6 days. Right, quantification of the fold change (Log_2-transformed) of DPNTP-expressing over control are shown for the indicated protein expression. pErbB2 is Y877-phosphorlated ErbB2. Means are from 3 independent experiments and error bars are SEM. **p=0.0385, one sample t test with theoretical mean of 0.
D) Western blots show a time course of the expression of the indicated proteins in differentiated GFP (Control) or DPNTP-GFP expressing keratinocytes either treated with DMSO as a control or the ErbB2 inhibitor TAK165.
E) Quantification of the fold change (Log_2-transformed) of DPNTP-expressing over GFP control keratinocytes are shown for the indicated protein expression for both DMSO and TAK165 treatment. Dashed lines indicate the mean of 3 independent experiments and error bars are SEM. *p=0.02, **$p \leq 0.05$, one sample t test with theoretical mean of 0.

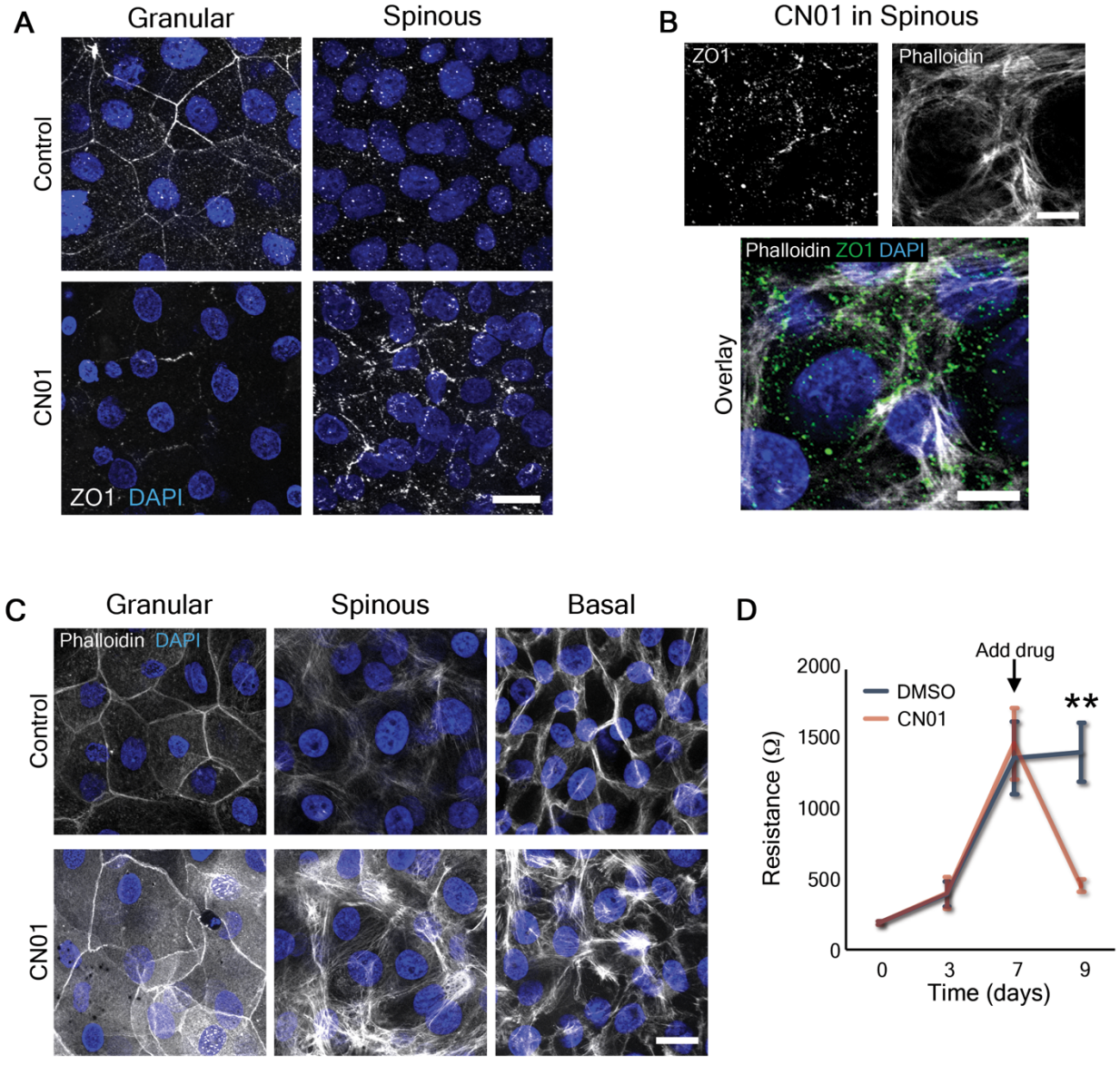

Figure S5. Excessive actomyosin contraction impairs epidermal barrier function; Related to Figure 5

A) Maximum projection micrographs of day 9 transwell epidermal equivalent cultures that were treated with either DMSO (Control) or a Rho activator (CN01, 1 unit/mL) at day 7-9 show immunostaining for the tight junction protein ZO1 and staining for nuclei in blue (DAPI) within the indicated cell layers. Bar is 20 μm.

B) Maximum projection micrographs of day 9 transwell epidermal equivalent cultures that were treated with a Rho activator (CN01, 1 unit/mL) at day 7-9 show immunostaining for the tight junction protein ZO1, staining for nuclei in blue (DAPI), and F-actin (phalloidin) within the spinous cell layer. Bar is 10 μm.

C) Maximum projection micrographs of day 9 transwell epidermal equivalent cultures that were treated with either DMSO (Control) or a Rho activator (CN01, 1 unit/mL) at

day 7-9 show staining for nuclei in blue (DAPI) and F-actin (phalloidin) within the indicated cell layers. Bar is 20 μm.
D) Quantification of resistance measurements from TEER experiments performed on a time course of transwell epidermal equivalent cultures that were treated with either DMSO or a Rho activator (CN01, 1 unit/mL) at day 7. Data are presented as mean ± SEM. **p=0.0029, paired t test from 6 independent experiments.

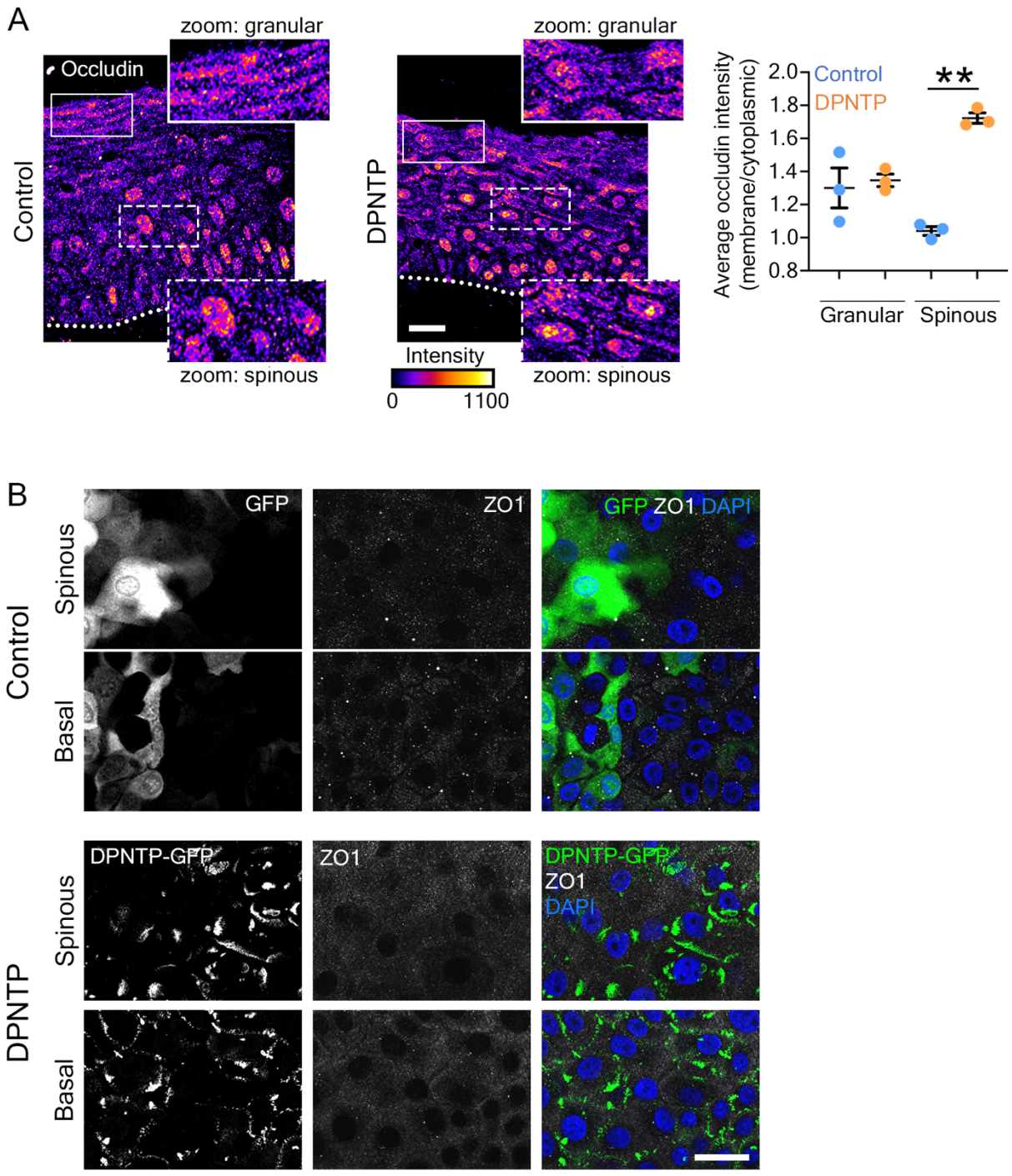

Figure S6. Expression of DPNTP and layer-specific tight junction component staining; Related to Figure 5

A) Left, micrographs of transverse sections of day 6 human epidermal equivalent cultures expressing either GFP (Control) or DPNTP-GFP immunostained for the tight junction component occludin are shown. Dotted line marks the bottom of the basal layer. Bar is 20 μm. Intensity is displayed with the indicated lookup table. Right, quantification of the average membrane to cytoplasmic ratio of occludin staining in the

indicated cell layers is shown. Means are from 3 independent experiments and error bars are SEM. **p=0.0014, paired t test.
B) Fluorescence micrographs show the expression of GFP (Control) and DPNTP-GFP in the spinous and basal layer of day 9 transwell epidermal equivalent cultures (same as Figure 4D) that were immunostained for the tight junction component ZO1. Nuclei are stained with DAPI in blue. Bar is 25 μm.

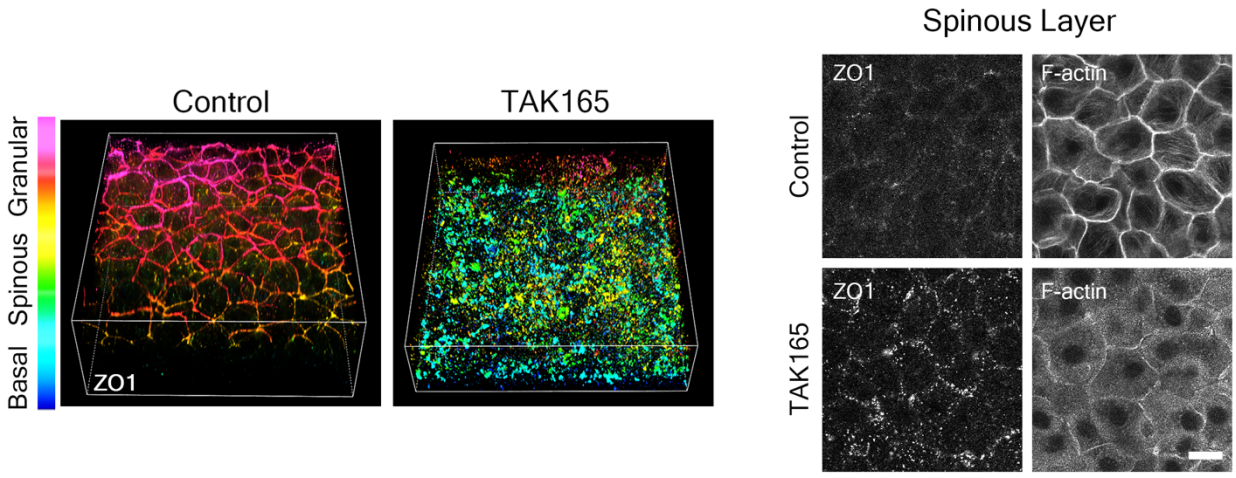

Figure S7. Treating epidermal equivalent cultures with an ErbB2 inhibitor disrupts polarized ZO1 immunostaining; Related to Figure 6

3D renderings of whole mount immunostaining of tight junction component ZO1 in control (DMSO) and TAK165 treated day 9 epidermal equivalent cultures are presented with a look-up table that depicts z-depth in the indicated colors. Right panels show representative ZO1 immunostaining and F-actin (phalloidin staining) in the spinous layer. Cultures were allowed to grow normally until day 7 and then treated with DMSO or TAK165 for 2 subsequent days. Bar is 20 μm.