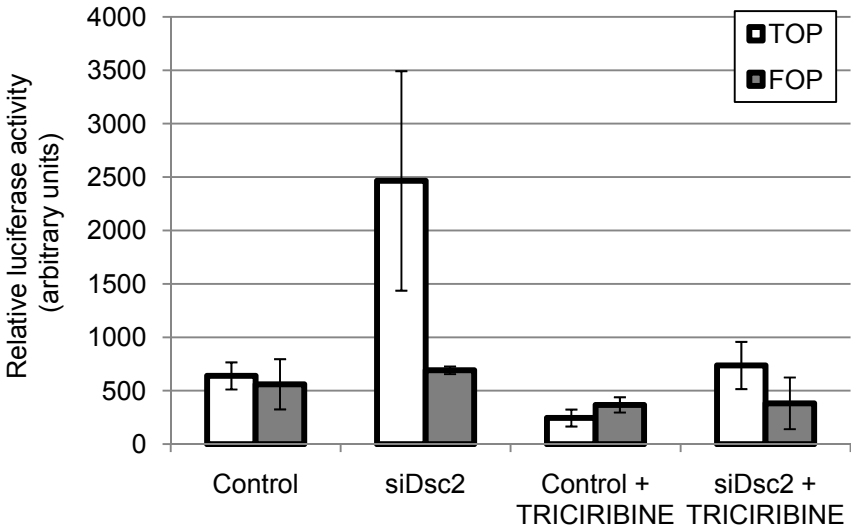
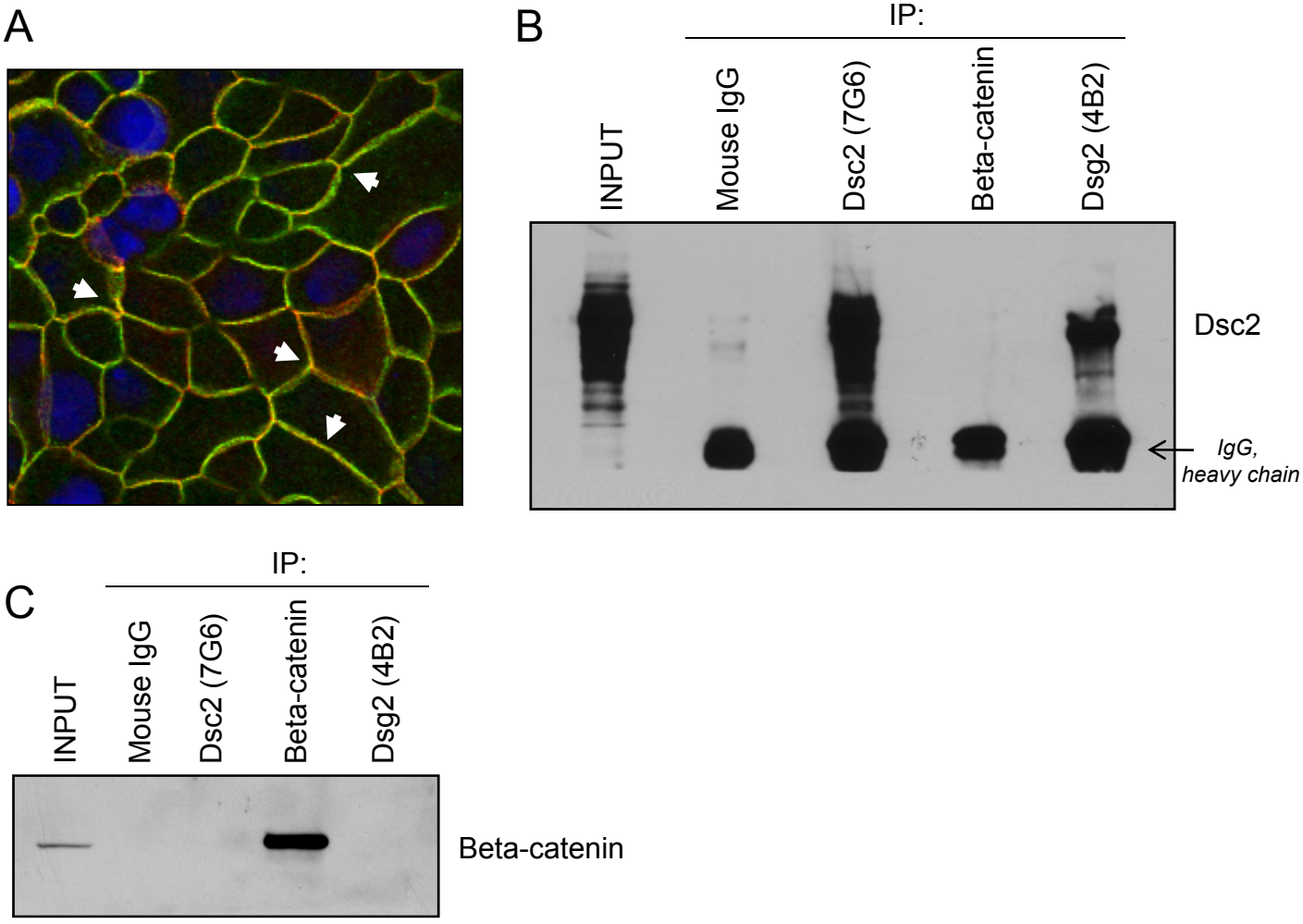
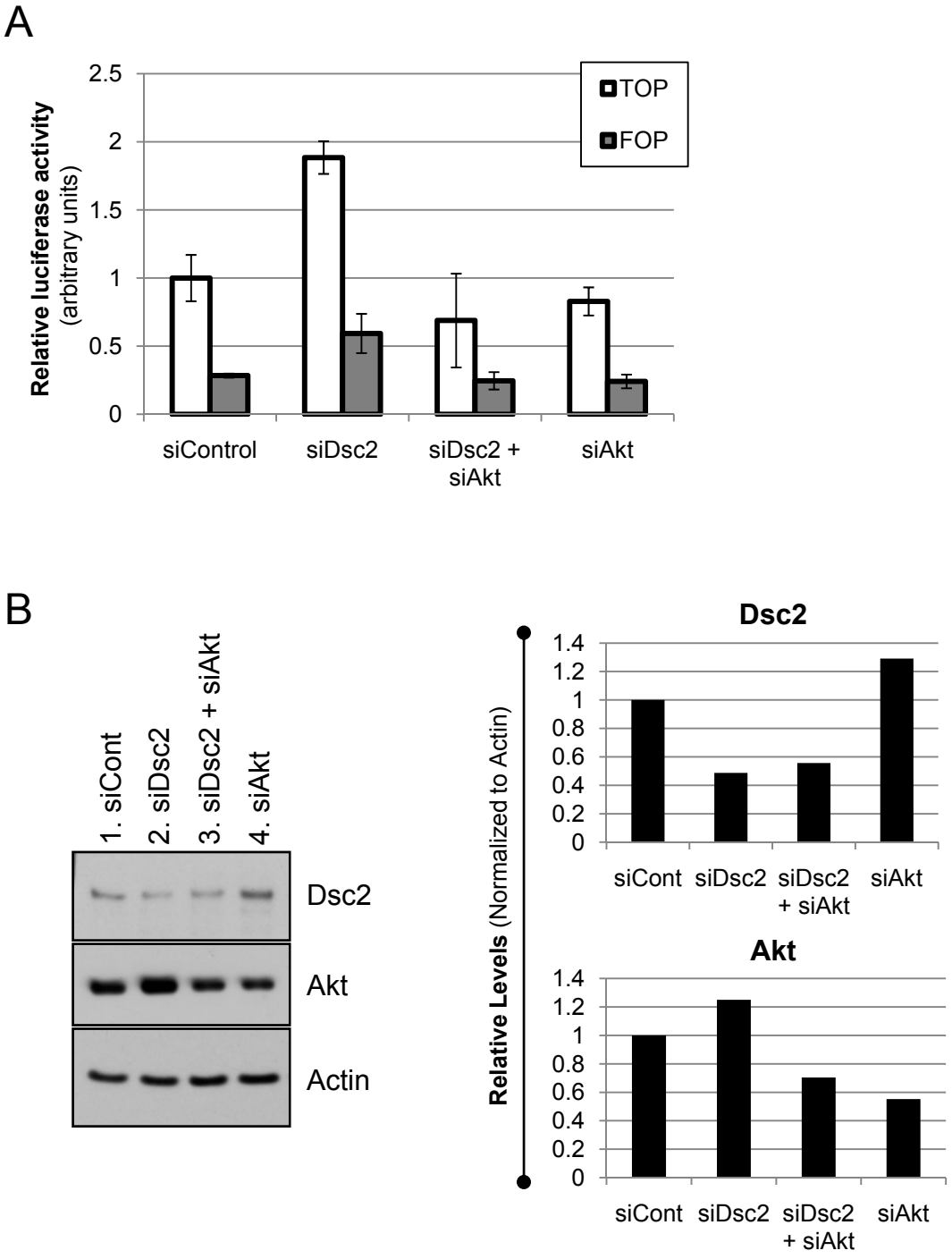


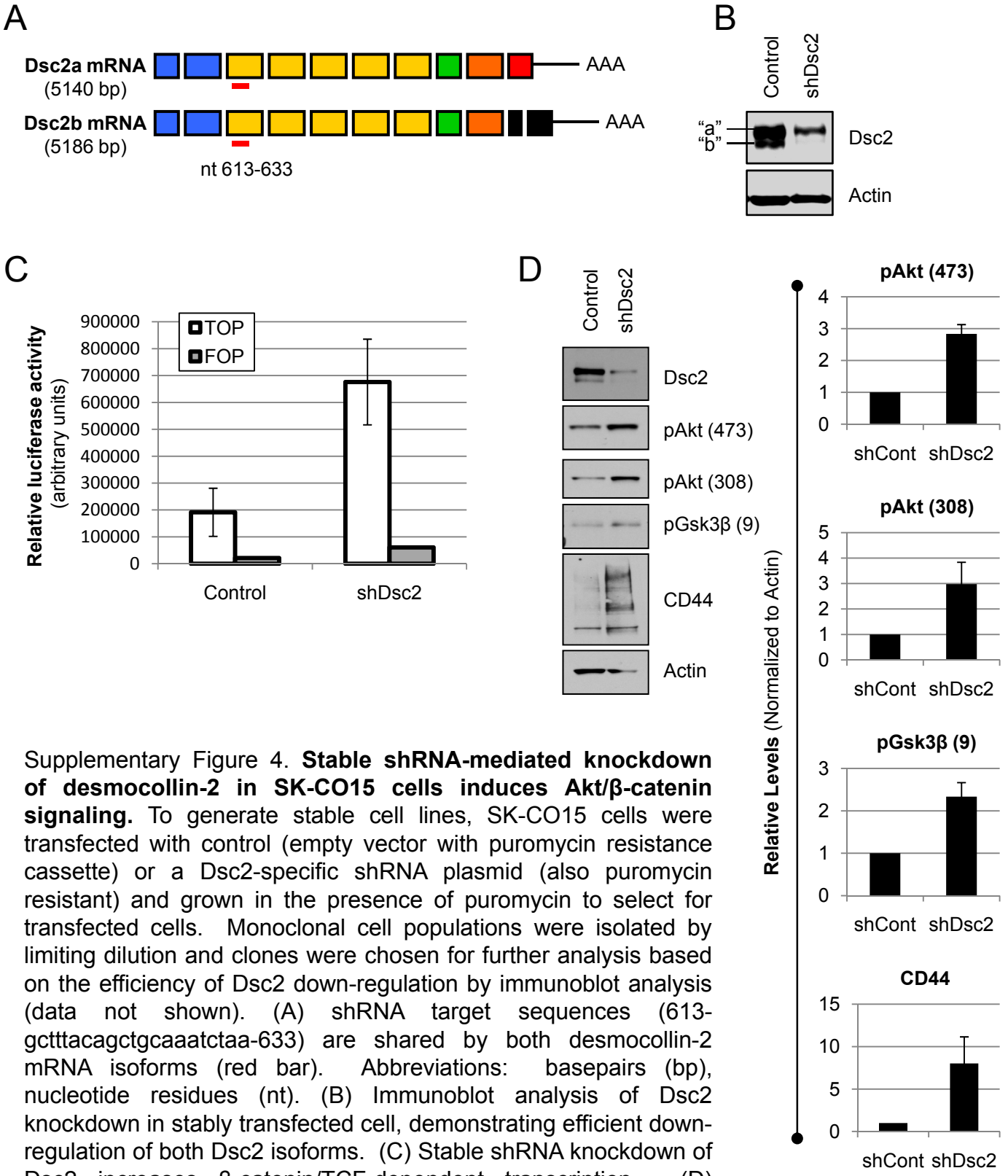
Supplementary Figure 1. **Loss of desmocollin-2 in Caco-2 cells leads to activation of β-catenin/TCF-dependent transcription.** Luciferase-based reporter assay for β-catenin/TCF-dependent transcription (TOP Flash). Caco-2 cells were transfected with the indicated siRNAs along with the TOP or FOP luciferase reporter plasmids. Twelve hours after transfection, the cells were treated with the Akt inhibitor triciribine or vehicle alone (DMSO). At 72 hours post-transfection, luciferase activity was assessed.



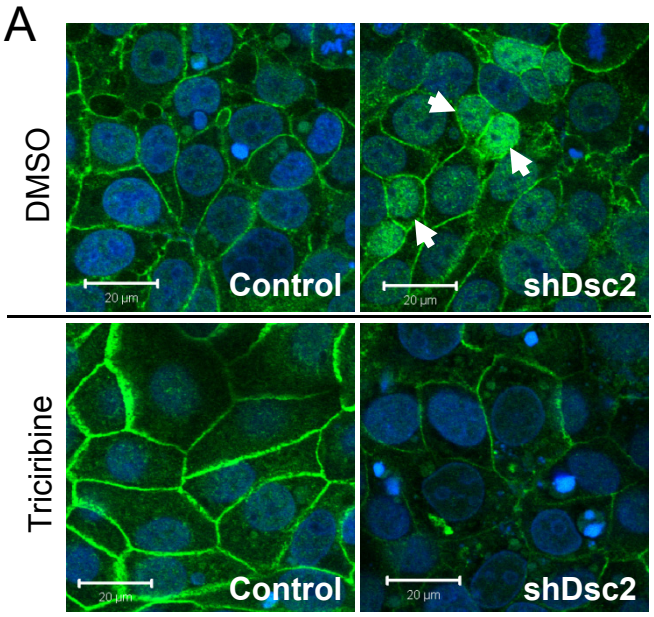
Supplementary Figure 2. **Desmocollin-2 and β-catenin do not form a complex in SK-CO15 cells.** (A) SK-CO15 cells stained for Dsc2 (green) and β-catenin (red). These proteins co-localize at some areas along the lateral membrane of SK-CO15 cells (arrows). (B) and (C) Co-immunoprecipitation (IP) experiments using SK-CO15 cell lysates. Antibody used for IP is indicated across the top of the blot; membranes were probed with Dsc2 (B) or B-catenin (C). There is no evidence of complex formation between Dsc2 and β-catenin. As expected, an association between Dsg2 and Dsc2 was detected (positive control).

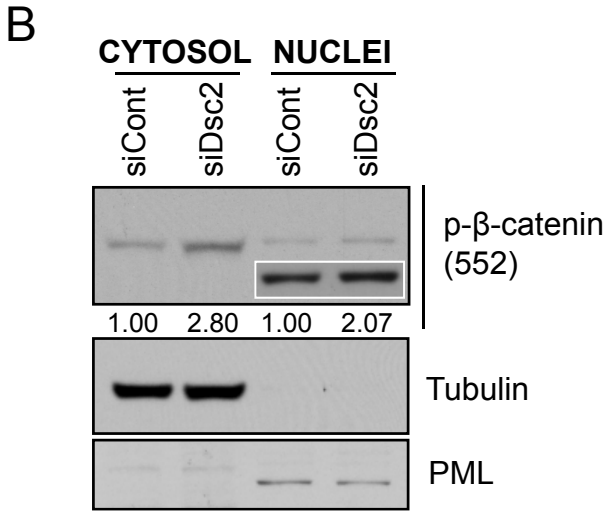


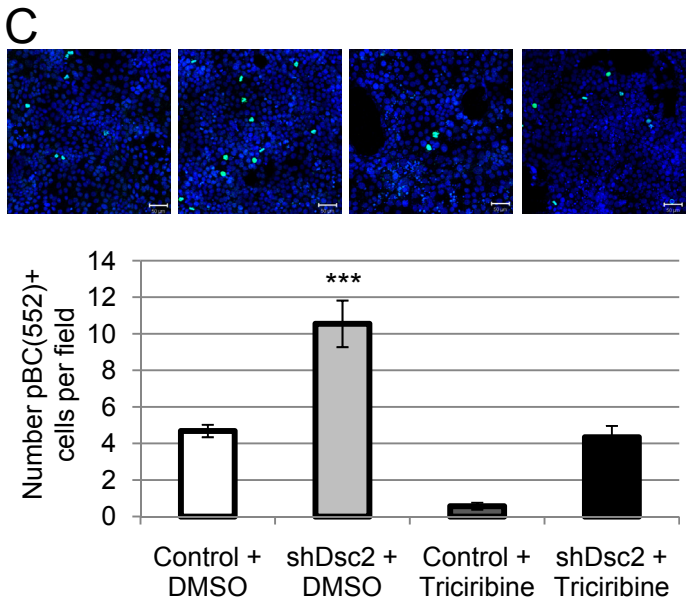
Supplementary Figure 3. **Targeted depletion of Akt using specific siRNA recapitulates the effect of triciribine on β-catenin-dependent transcription following Dsc2 knockdown.** (A) SK-CO15 cells were transfected with the indicated siRNAs along with the TOP or FOP luciferase reporter plasmids. At 48 hours post-transfection, luciferase activity was assessed. The graph is representative of at least three independent experiments. (B) Immunoblot analysis of Dsc2 and Akt protein levels following treatment with siRNA specific for Dsc2, Akt, or both (left panel). Densitometric quantification of the western blot data, normalized to total actin levels.

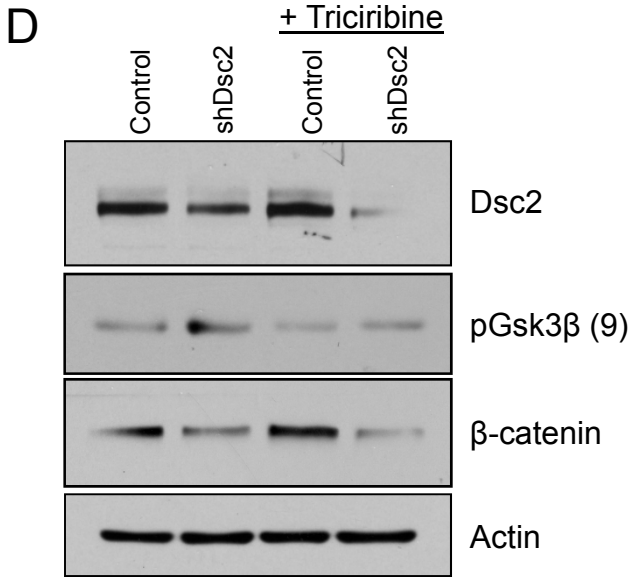


Supplementary Figure 4. **Stable shRNA-mediated knockdown of desmocollin-2 in SK-CO15 cells induces Akt/β-catenin signaling.** To generate stable cell lines, SK-CO15 cells were transfected with control (empty vector with puromycin resistance cassette) or a Dsc2-specific shRNA plasmid (also puromycin resistant) and grown in the presence of puromycin to select for transfected cells. Monoclonal cell populations were isolated by limiting dilution and clones were chosen for further analysis based on the efficiency of Dsc2 down-regulation by immunoblot analysis (data not shown). (A) shRNA target sequences (613-gctttacagctgcaaatctaa-633) are shared by both desmocollin-2 mRNA isoforms (red bar). Abbreviations: basepairs (bp), nucleotide residues (nt). (B) Immunoblot analysis of Dsc2 knockdown in stably transfected cell, demonstrating efficient down-regulation of both Dsc2 isoforms. (C) Stable shRNA knockdown of Dsc2 increases β-catenin/TCF-dependent transcription. (D) Western blot analysis of phosphorylated Akt (Thr308 and Ser473, active), phospho-Gsk3β (Ser9, inactive), and CD44 in control and shDsc2 knockdown cells. Actin is included as a loading control. Densitometric analysis of at least three experiments. Graph represents the mean ± SEM for each protein analyzed.



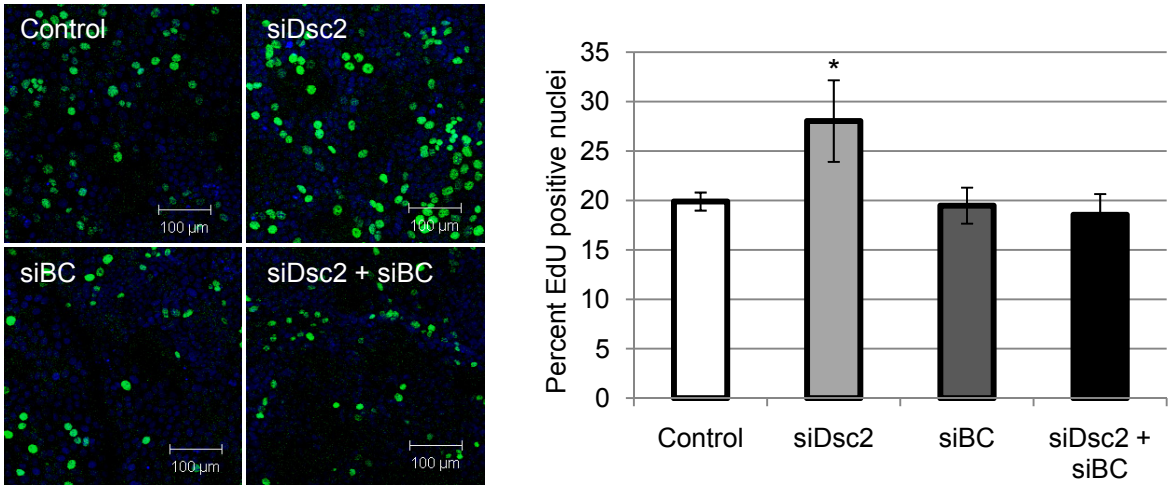




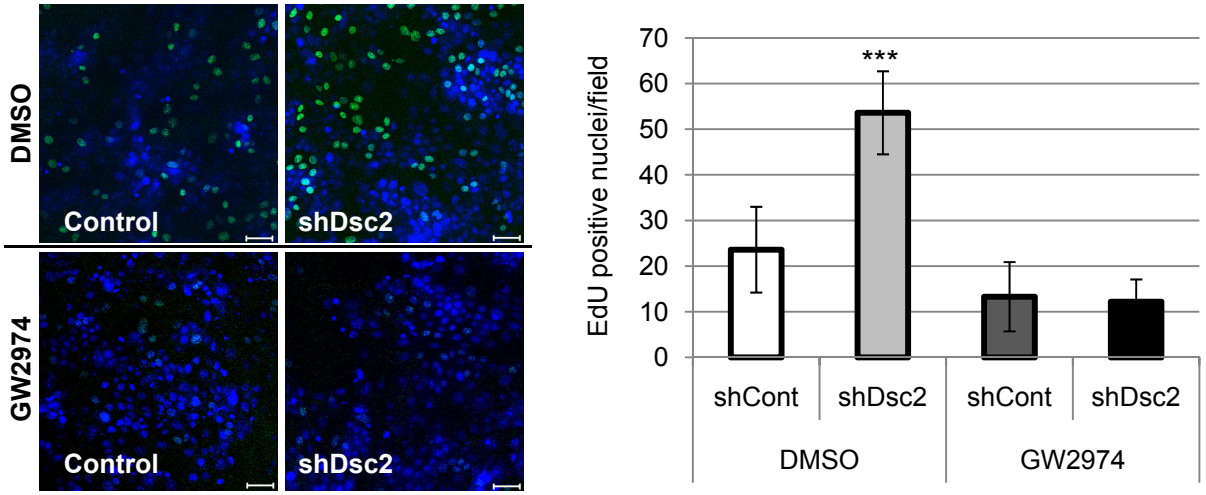


Supplementary Figure 5. **Down-regulation of desmocollin-2 induces nuclear accumulation of p-(Ser552)-β-catenin through an Akt-dependent mechanism.** (A) Immunofluorescence labeling/confocal microscopy of β-catenin in control and shDsc2 cells after Akt inhibition, nuclear accumulation is noted by the arrows (green, β-catenin; blue, nuclei; scale bar is 20 µm). (B) Nuclear and cytosolic fractionation of siControl and siDsc2 transfected cells, at 24 hours post-transfection. Tubulin and PML mark the cytosolic and nuclear compartments, respectively. Signal intensities were determined using densitometry and the relative difference for each fraction in indicated. Inset is a darker exposure of nuclear immunoblot. Results are representative of three independent experiments. (C) Immunofluorescence labeling/confocal microscopy of phospho-β-catenin (Serine 552, active; pBC(552)) in control and shDsc2 cells after Akt inhibition (green, pBC(552); blue, nuclei; scale bar is 10 µm). The histogram shows the mean ± SEM of pBC(552) positive nuclei per field from at least 10 different fields. (***p< 0.0001, ANOVA). The results are representative of at least three independent experiments. (D) Cells stably expressing Dsc2-specific shRNA (shDsc2) or vector alone control plasmid (control) were grown in the presence of triciribine or vehicle only (DMSO). Protein extracts from each condition were assayed for expression of phosphorylated Gsk3β (Serine 9, inactive) and total β-catenin by immunoblot. Actin is included as a loading control.

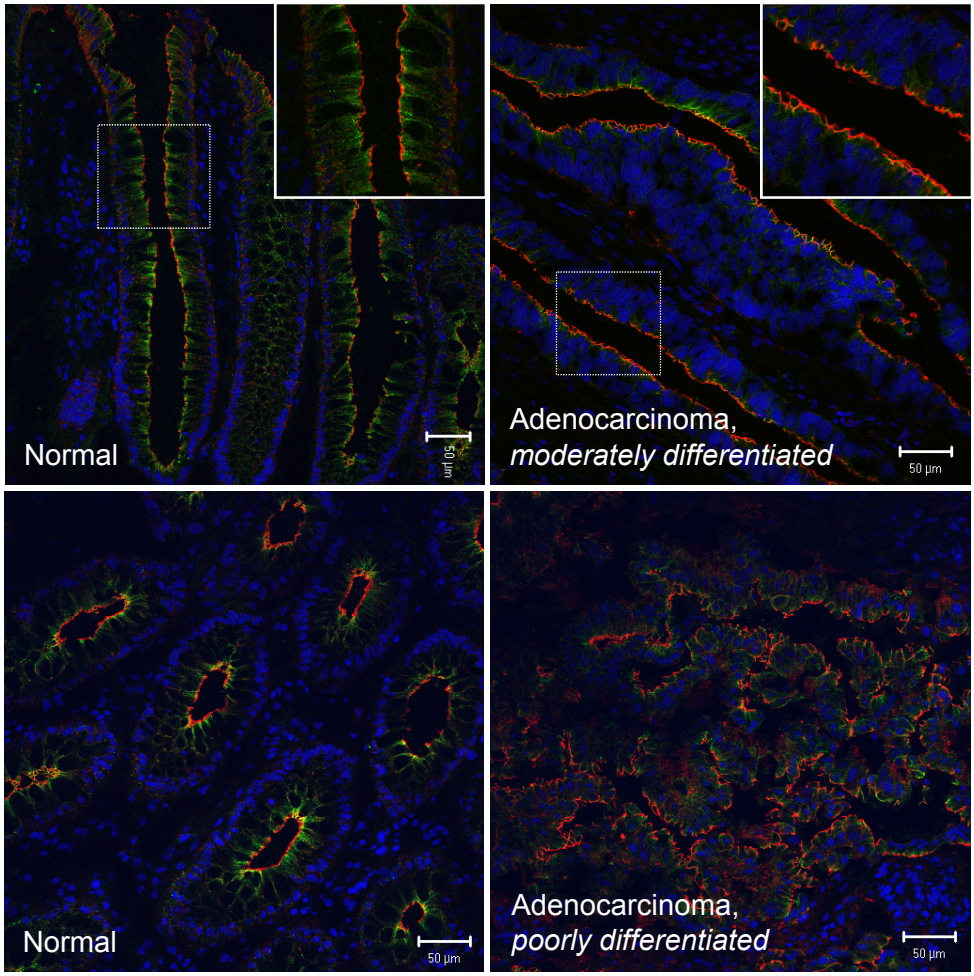
A



B



Supplementary Figure 6. **Desmocollin-2 loss enhances cell proliferation through an EGFR and β-catenin-dependent mechanism.** (A) SK-CO15 cells transfected with control, Dsc2, β-catenin (BC), or Dsc2 and BC-specific siRNAs. At 72 hours post-transfection, cells were pulse-labeled with EdU for 1 hour and processed to detect incorporated EdU. Images were obtained using confocal microscopy and the percent of EdU positive nuclei was scored. The histogram shows the mean ± SEM of the percent of EdU positive nuclei from at least 10 different fields. The results are representative of three independent experiments. (EdU, green; nuclei, blue). (*p< 0.05, ANOVA) (B) EdU incorporation assay for cells stably expressing Dsc2-specific shRNA (shDsc2) or control shRNA plasmid (control) and grown in the presence of the EGFR inhibitor GW2974 or vehicle only (DMSO). The histogram shows the mean ± SD of EdU positive nuclei from at least 10 different fields. (***p< 0.0001, ANOVA).



Supplementary Figure 7. **Desmocollin-2 is down-regulated in moderately and poorly differentiated colonic carcinomas.** Dsc2 distribution in human colonic epithelium (Normal, left panels) and adenocarcinoma of the colon (right panels) using confocal microscopy. Co-staining with anti-JAM-A delineates the apical/lumenal domain of the epithelium. (Dsc2, green; JAM-A, red; nuclei, blue).