Figures and figure supplements

Increasing β-catenin/Wnt3A activity levels drive mechanical strain-induced cell cycle progression through mitosis

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Figure 1. Mechanical strain is sufficient to drive cell cycle re-entry, but not entry into mitosis. (A) Distribution of mAG-Geminin in Fucci MDCK monolayers 0 hr or 24 hr after the application of No Strain or High Strain (~8.5%) using the biaxial live cell stretcher. Scale bar: 150 μm. (B) Distribution of mitotic events (white arrow heads) in Fucci MDCK monolayers 12 hr or 24 hr after the application of No Strain or High Strain (~8.5% Strain) using the biaxial live cell stretcher. Scale bar: 100 μm. (C) Quantification of percent Geminin positive cells in Fucci-MDCK monolayers; high strain is statistically significant (p<0.05) relative to no strain from 1–24 hr. (D) Number of mitotic events per hour in Fucci-MDCK monolayers; there is no statistically significant difference at any time point. (E) Single cell tracking quantification of the number of cell objects accumulated in S/G2 (red to green fluorescence, left) or passed through S/G2 and divided (red to green to red fluorescence, right) during 24 hr. All quantifications were from at least 3 independent experiments and included analysis of at least 9500 cells. Quantifications were mean +/- SEM; unpaired t-test p values<0.001 (**). DOI: 10.7554/eLife.19799.003

The following source data is available for figure 1:

Source data 1. Data used to construct graphs in Figure 1 and Figure 1—figure supplement 1. DOI: 10.7554/eLife.19799.004
Figure 1—figure supplement 1. Design and calibration of bi-axial live cell stretcher. Schematic representation of the side view (A) and top view (B) of the biaxial live cell stretcher. Representative image (C) and quantification (D) of displacement of immobilized fluorescent microspheres on the live cell.
stretcher imaging membrane with applied pressure of 0–65 kPa. Representative image of distance to nearest neighbor calculation (E) and quantification of average distance to nearest neighbor in Fucci MDCK monolayer at 0 (Vacuum Off) and 65 kPa (Vacuum On) of applied pressure (F). Quantifications were from at least 3 independent experiments and distance to nearest neighbor calculations included analysis of 6943 cells (65 kPa) or 8142 cells (0 kPa). Quantifications are mean ± SEM; Kolmogorov-Smirnov test p values<0.001 (**). DOI: 10.7554/eLife.19799.005
Figure 1—figure supplement 2. Mitotic events and distance to nearest neighbor in fixed MDCK monolayers after mechanical strain. (A) Nuclear stain (Hoescht) of fixed MDCK monolayers 2–24 hr after application of mechanical strain using the ISA. (B) Quantification of average distance to nearest neighbor (B) or number of mitotic figures observed (C) 2–24 hr after application of mechanical strain. Quantifications were from at least 3 independent experiments and distance to nearest neighbor calculations included analysis of 3350–5215 cells. Quantifications are mean ± SEM; Kolmogorov-Smirnoff test p values 0.05 (*). DOI: 10.7554/eLife.19799.006
**Figure 2.** Mechanical strain does not induce significant DNA damage or activation of DNA repair. Distribution of phospho-γH2A.X (A), p53 (C), and p53BP1 (E) in MDCK monolayers 24 hr after No Strain or High Strain (15%) using the ISA, or in actively cycling cells treated with DMSO or MMS.

*Figure 2 continued on next page.*
Figure 2 continued

Quantification of percent cells phospho-γH2A.X- (B), p53- (D), or p53BP1-positive (F) in each condition. Quantifications were from 3 independent experiments and included analysis of 397–3135 cells per experiment. Quantifications were mean +/- SEM; unpaired t-test p values<0.05 (*), <0.01 (**), and <0.001 (***)

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The following source data is available for figure 2:

Source data 1. Data used to construct graphs in Figure 2.
DOI: 10.7554/eLife.19799.010
Figure 3. Mechanical strain induces a Src-dependent increase in Y654 phosphorylated β-catenin and β-catenin transcriptional activity. Distribution of TOPdGFP at 8 hr (A), β-catenin at 8 hr (A, insets), EdU at 24 hr (C), and pY654 β-catenin at 8 hr (E) in MDCK monolayers after No Strain or High Strain Figure 3 continued on next page
Figure 3 continued

(15%) applied by the ISA, treated with either DMSO or the Src inhibitor SU6656 (10 μM). Scale bars: 25 μm. Quantification of percent cells TOPdGFP-
(\textit{B}) or EdU- (\textit{D}) positive and quantification of average pY654 β-catenin intensity per pixel (\textit{F}); note that the small surface area (0.81 cm²) of the ISA does
not provide sufficient cell numbers for biochemical characterization. Quantifications were from at least 3 independent experiments and for the
TOPdGFP and EdU quantifications included analysis of 677–1168 cells per experiment. Quantifications were mean +/- SEM, unpaired t-test (\textit{B,D}) or
Kolmogorov-Smirnoff (\textit{F}) test p values<0.05 (*), <0.01 (**), and <0.001 (**). DOI: 10.7554/eLife.19799.011

The following source data is available for figure 3:

\textbf{Source data 1.} Data used to construct graphs in Figure 3 and Figure 3—figure supplement 1.
DOI: 10.7554/eLife.19799.012
Figure 3—figure supplement 1. Distribution of β-catenin in monolayers treated with either DMSO or the Src Inhibitor SU6656 8 hr after the application of No Strain or High Strain (15%) using the ISA. Scale bars: 25 μm.
DOI: 10.7554/eLife.19799.013
Figure 3—figure supplement 2. The Src Inhibitor PP2 blocks strain-induced increases in Y654 phosphorylated β-catenin and β-catenin transcriptional activity. Distribution of TOPdGFP at 8 hr (A), β-catenin at 8 hr (A, insets), EdU at 24 hr (C), and pY654 β-catenin at 8 hr (E) in MDCK Monolayers after No

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Strain or High Strain (15%) applied by the ISA, treated with either DMSO or the Src inhibitor PP2. Quantification of percent cells TOPdGFP- (B) or EdU- (D) positive and quantification of average pY654 β-catenin intensity per pixel (F). Quantifications were from at least 3 independent experiments and for the TOPdGFP and EdU quantifications included analysis of 707–1478 cells per experiment. Quantifications were mean +/- SEM; unpaired t-test (B, D) or Kolmogorov-Smirnoff (F) test p values<0.05 (*), <0.01 (**), and <0.001 (**).
Figure 3—figure supplement 3. Distribution of β-catenin in monolayers treated with either DMSO or the Src Inhibitor PP2 8 hr after the application of No Strain or High Strain (15%) using the ISA. Scale bars: 25 μm.
DOI: 10.7554/eLife.19799.015
Figure 3—figure supplement 4. EGFR inhibition reduces an increase in pY654 β-catenin following mechanical strain, but does not affect β-catenin transcriptional activity or cell cycle re-entry. Distribution of TOPdGFP at 8 hr (A), β-catenin at 8 hr (A, insets), EdU at 24 hr (C), and pY654 β-catenin at 8
hr (E) in MDCK monolayers after No Strain or High Strain (15%) applied by the ISA and treated with either DMSO or the EGFR inhibitor PD153035. Scale bars: 25 μm. Quantification of percent cells TOPdGFP- (B) or EdU- (D) positive and quantification of average pY654 β-catenin intensity per pixel (F). Quantifications were from at least 3 independent experiments and for the TOPdGFP and EdU quantifications included analysis of 677–1226 cells per experiment. Quantifications were mean +/- SEM; unpaired t-test (B, D) or Kolmogorov-Smirnov (F) test p values<0.05 (*), <0.01 (**), and <0.001 (**). DOI: 10.7554/eLife.19799.016
Figure 3—figure supplement 5. Distribution of β-catenin in monolayers treated with either DMSO or the EGFR Inhibitor PD153035 8 hr after the application of No Strain or High Strain (15%) using the ISA. Scale bars: 25 μm.
DOI: 10.7554/eLife.19799.017
Figure 4. Inhibition of Casein Kinase I (CKI) increases β-catenin transcriptional activity in MDCK quiescent monolayers, independent of mechanical strain. Distribution of TOPdGFP at 8 hr (A), β-catenin at 8 hr (A, insets), EdU at 24 hr (C), and pY654 β-catenin at 8 hr (E) in MDCK Monolayers after No Strain High Strain.

Figure 4 continued on next page
Strain or High Strain (15%) applied by the ISA and treated with either DMSO or the CKI inhibitor D4476. Quantification of percent cells TOPdGFP- (B) or EdU- (D) positive and average pY654 β-catenin intensity per pixel (F). Quantifications were from at least 3 independent experiments and, for the TOPdGFP and EdU quantifications, included analysis of 832–1364 cells per experiment. Quantifications were mean +/- SEM; unpaired t-test (B, D) or Kolmogorov-Smirnoff (F) test p values <0.05 (*), <0.01 (**), and <0.001 (**).
Figure 4—figure supplement 1. Distribution of β-catenin in monolayers treated with either DMSO or the CKI Inhibitor D4476 8 hr after the application of No Strain or High Strain (15%) using the ISA. Scale bars: 25 μm.
DOI: 10.7554/eLife.19799.020
Figure 5. Inhibition of β-catenin degradation promotes progression from G1 into S/G2 following mechanical strain. (A) Distribution of mAG-Geminin in Fucci MDCK monolayers treated with DMSO, D4476, or D4476 and iCRT3 0 hr or 24 hr after the application of No Strain or High Strain (~8.5%) using the biaxial live cell stretcher. Scale bar: 150 μm. Quantification of percent cells Geminin positive in Fucci-MDCK monolayers after No Strain (B) or High Strain (C); percent cells Geminin positive in D4476 treated monolayers are statistically significant (p<0.05) relative to DMSO monolayers following mechanical strain at 16–24 hr. (D) Single cell tracking quantification of number of cell objects accumulated in S/G2 (red to green fluorescence, left). All quantifications were from 2–4 independent experiments and included analysis of at least 15,000 cells. Quantifications were mean +/- SEM; unpaired t-test (B, C) or Kolmogorov-Smirnoff (D) test p values<0.01 (**), and <0.001 (***).

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Figure 5 continued

DOI: 10.7554/eLife.19799.021

The following source data is available for figure 5:

**Source data 1.** Data used to construct graphs in *Figure 5.*

DOI: 10.7554/eLife.19799.022
Figure 6. Inhibition of β-catenin degradation promotes mitotic entry following mechanical strain. (A) Distribution of mitotic events (white arrow heads) in Fucci MDCK monolayers 12 hr or 24 hr after the application of No Strain or High Strain (~8.5%) using the biaxial live cell stretcher and treatment with DMSO, D4476, or D4476 and iCRT3. Scale bar: 100 μm. (B) Number of mitotic events per hour in treated Fucci-MDCK monolayers following No Strain; mitotic events in D4476 treated monolayers were statistically significant (p<0.05) compared to DMSO treated monolayers from 6–16 hr, and at 22 and 24 hr (C) Number of mitotic events per hour in treated Fucci-MDCK monolayers following High Strain; mitotic events in D4476 treated monolayers were statistically significant (p<0.05) when compared to DMSO treated monolayers from 5–24 hr. (D) Single cell tracking quantification of number of cell objects that passed through S/G2 and divided (red to green to red fluorescence, right) during 24 hr. All quantifications were from 2–4 independent
Figure 6 continued

experiments and included analysis of at least 15,000 cells. Quantifications were mean +/- SEM; Kolmogorov-Smirnoff test p values<0.05 (*), <0.01 (**), and <0.001 (***)

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The following source data is available for figure 6:

Source data 1. Data used to construct graphs in Figure 6.
DOI: 10.7554/eLife.19799.026
**Figure 7.** Mechanical strain induced increase in the progression from G1 into S/G2 in the presence of Wnt3A. (A) Distribution of mAG-Geminin in Fucci MDCK monolayers treated with control or Wnt3A-conditioned media 0 hr or 24 hr after the application of No Strain or High Strain (~8.5%) using the biaxial live cell stretcher. Scale bar: 150 μm. Quantification of percent cells Geminin positive in Fucci-MDCK monolayers after No Strain (B) or High Strain (15%) (C); percent cells Geminin positive in Wnt3A treated monolayers were statistically significant (p<0.05) relative to control monolayers following mechanical strain at 14–24 hr. (D) Single cell tracking quantification of number of cell objects accumulated in S/G2 (red to green fluorescence, left) during 24 hr. All quantifications were from 2–3 independent experiments and included analysis of at least 15,000 cells. Quantifications were mean +/- SEM; unpaired t-test (B, C) or Kolmogorov-Smirnoff (D) test p values<0.05 (*), <0.01 (**), and <0.001 (**). DOI: 10.7554/eLife.19799.029

The following source data is available for figure 7:

**Source data 1.** Data used to construct graphs in Figure 7 and Figure 7—figure supplement 1.
DOI: 10.7554/eLife.19799.030
Figure 7—figure supplement 1. Wnt3A induces increased β-catenin transcriptional activity, independent of mechanical strain. Distribution of TOPdGFP at 8 hr (A) and pY654 β-catenin at 8 hr (C) in MDCK monolayers after No Strain or High Strain (15%) using the ISA, treated with either control or Wnt3A-conditioned media. Quantification of percent cells TOPdGFP-positive (B) and quantification of average pY654 β-catenin intensity per pixel (D). Quantifications were from at least 3 independent experiments and, for the TOPdGFP quantifications, included analysis of 1004–1479 cells per experiment. Quantifications were mean +/- SEM; unpaired t-test (B) or Kolmogorov-Smirnoff (D) test p values<0.05 (*), <0.01 (**), and <0.001 (**). DOI: 10.7554/eLife.19799.031
Figure 8. Mechanical strain induced increased mitotic entry in the presence of Wnt3A. (A) Distribution of mitotic events (white arrow heads) in Fucci MDCK monolayers 12 hr or 24 hr after the application of No Strain or High Strain (~8.5%) using the biaxial live cell stretcher in the presence of control or Wnt3A-conditioned media. Scale bar: 100 μm. (B) Number of mitotic events per hour in treated Fucci-MDCK monolayers following No Strain; mitotic events in Wnt3A treated monolayers were statistically significant (p<0.05) compared to control monolayers from 4–14 hr (C) Number of mitotic events per hour in treated Fucci-MDCK monolayers following High Strain; mitotic events in Wnt3A treated monolayers were statistically significant (p<0.05) compared to control monolayers from 4–24 hr. (D) Single cell tracking quantification of number of cell objects that passed through S/G2 and divided (red to green to red fluorescence, right) during 24 hr. All quantifications were from 2–3 independent experiments and included analysis of at least 15,000 cells. Quantifications were mean +/- SEM; Kolmogorov-Smirnov test p values<0.001 (***).

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The following source data is available for figure 8:

Source data 1. Data used to construct graphs in Figure 8.

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