Effect of microculture on cell metabolism and biochemistry: Do cells get stressed in microchannels?

Supporting Information

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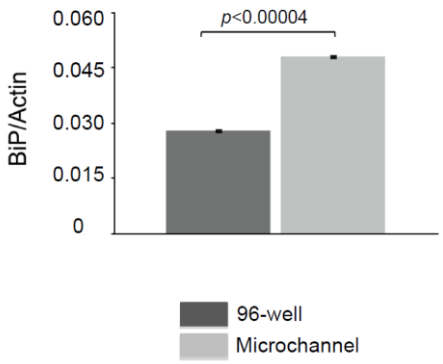

Figure S-1. BiP mRNA expression is upregulated in NMuMG cells in microchannel cell culture compared to 96-well cell culture under standard culture conditions. NMuMG cells obtained from ATCC were cultured for 24 h in DMEM containing 10% FBS, 4.5 g/L glucose, 10 µg/mL insulin, 100 units/mL penicillin, and 100 µg/mL streptomycin. RT-qPCR analysis showed BiP mRNA expression was significantly higher in microchannels than 96-wells as evaluated by a two-sample t-test. Results are the average ± standard error (SE); n=3.

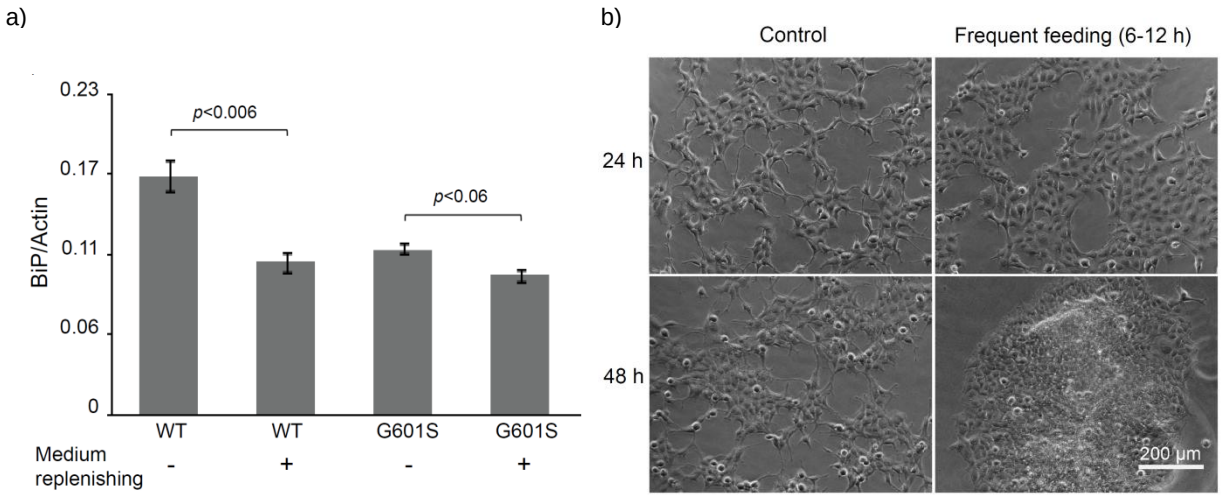

Figure S-2. Effects of medium replenishment on cell stress and morphology in microchannels. Cells were seeded at a density of 630 cells/mm^2 and cultured in media containing 10% FBS and 1 g/L glucose. a) Medium replenishment decreases BiP mRNA expression. Where indicated (+), cell culture media was changed 21 h post seeding, and BiP mRNA levels were measured using RT-qPCR 3 h after the media change. Medium replenishment resulted in a decrease in BiP levels in comparison to cells cultured without medium replenishment (-); the reduction was significant in WT-hERG-HEK cells. Statistical significance was evaluated using two-sample t-tests. Data are the mean ± SE; n=5. b) Overfeeding (media changes in 6-12 h intervals) leads to a change in cell morphology. Phase contrast images show G601S-hERG-HEK cells cultured in microchannels after 24 and 48 h with media changes every 24 h (control) or 6-12 h (frequent feeding). Images are representative of 4 samples.

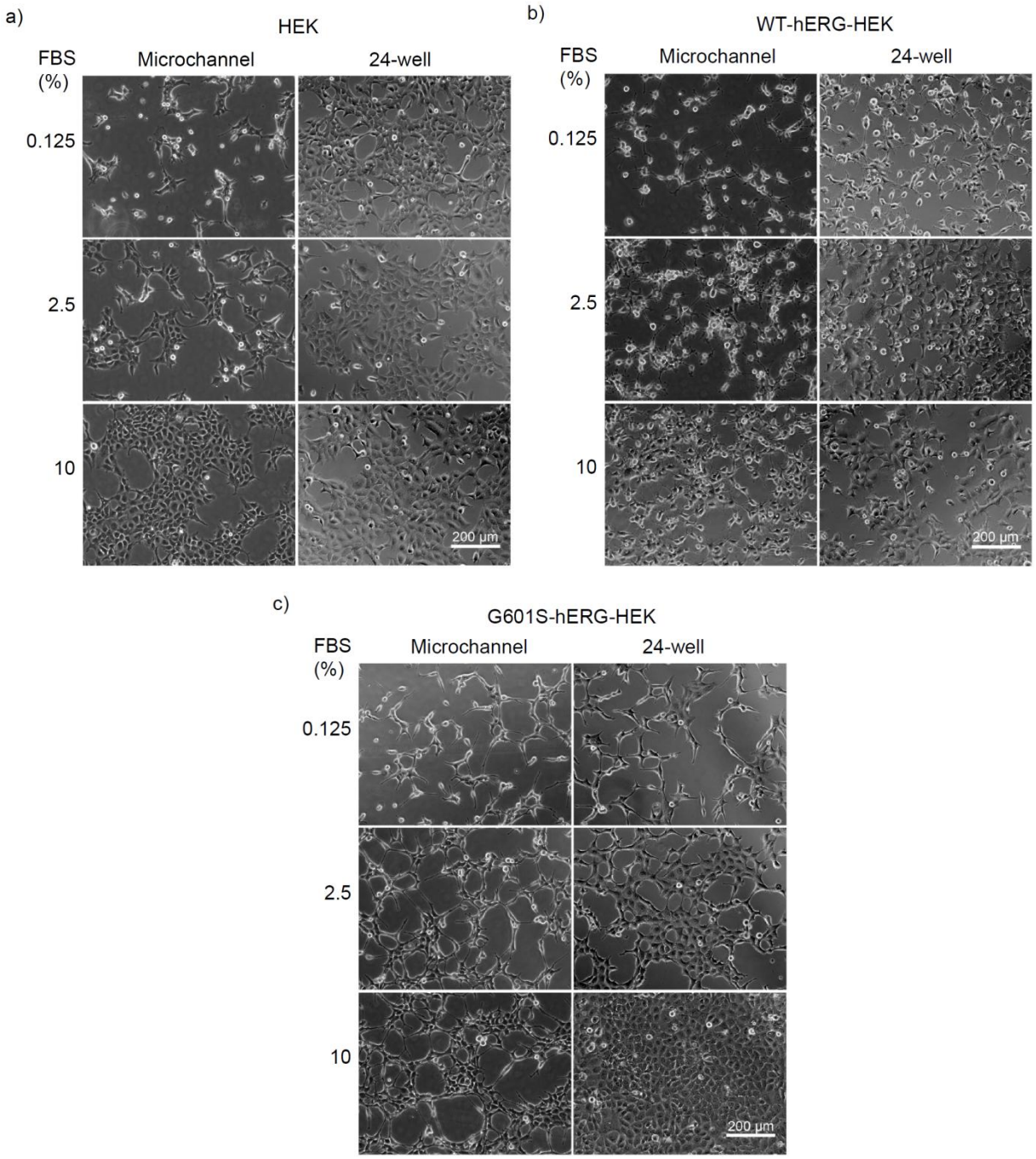

Figure S-3. Phase contrast images of parental HEK cells (a), WT-hERG-HEK cells (b), and G601S-hERG-HEK cells (c). Cells were cultured for 24 h in a 24-well plate or in microchannels. Cell spreading and attachment was reduced in channels compared to wells for all three cell lines, particularly at low serum concentrations. Cell spreading was reduced in 24-wells at the lowest serum concentration (0.125% serum). Cells were seeded at a density of 630 cells/mm^2 and cultured in media containing 1 g/L glucose. Images are representative of 1 well and 2 channels from 3 independent experiments.

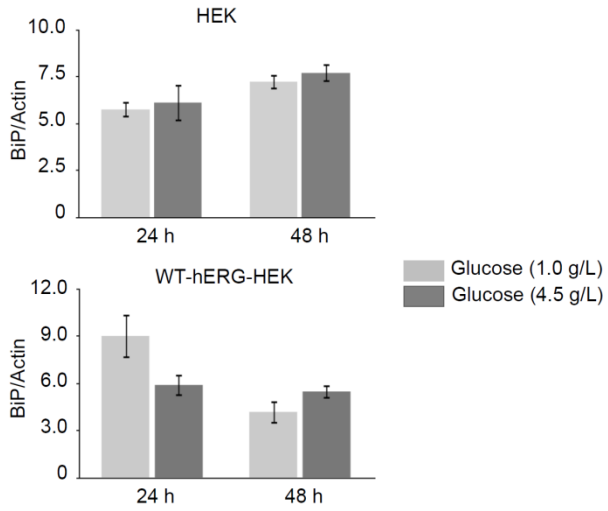

Figure S-4. The effects of glucose concentration on BiP mRNA expression in microchannel culture. Increased glucose concentration (from 1 g/L to 4.5 g/L) did not reduce BiP mRNA transcription in microchannels in parental HEK cells or WT-hERG-HEK cells. *P*-values from two-sample t-tests for all comparisons (comparing the two glucose concentrations within a given time point and comparing across time points within a glucose concentration) are >0.05. Cells were seeded at a density of 630 cells/mm^2 and cultured in media containing 10% FBS. Data are the mean ± SE; n=4.