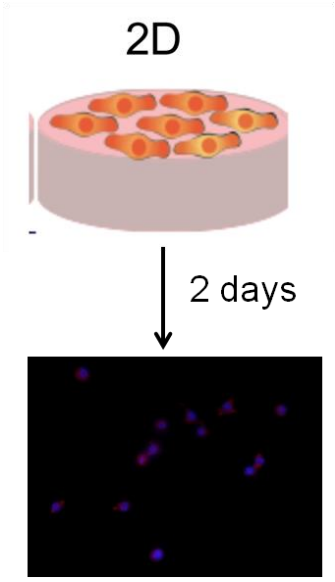
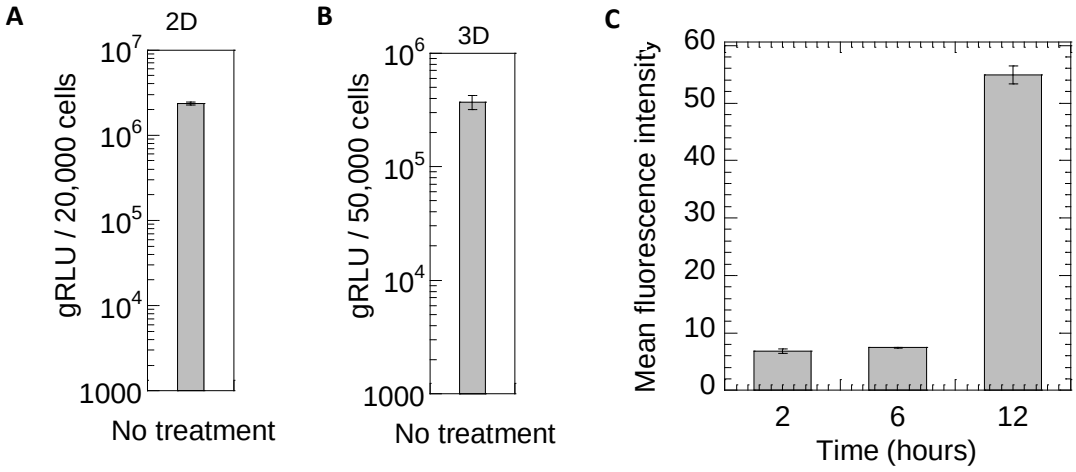
Electronic Supplementary Material (ESI) for Integrative Biology

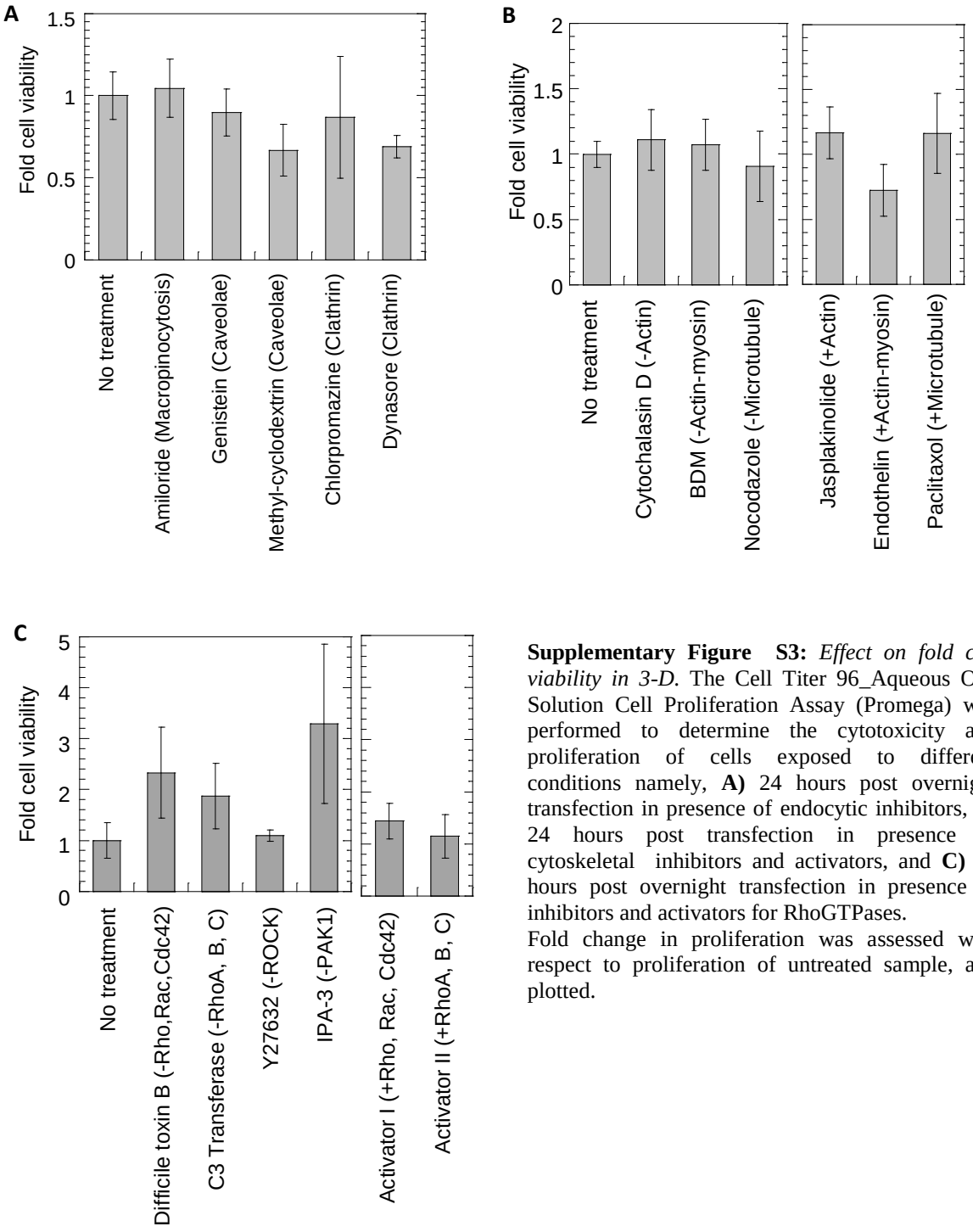
Supplementary material



Supplementary Figure S1: *Schematic representing cells plated on top HA-RGD hydrogels*. 10,000 cells were plated on top of 10µl gels placed per well in a 96-well plate. Cell spreading was analyzed after 2-days through actin and DNA staining using Alexa488 conjugated phalloidin, and Hoechst dye, respectively. Cells were observed to not spread well when seeded on top of HA-RGD hydrogelsand resulted in clumping and detachment.



Supplementary Figure S2: *Transfection in 2-D and 3-D.* **A)** In 2-D, 20,000 cells were cultured per well in a 48 well plate for 14-15 hours before transfection. Transgene expression was analyzed 48 post addition of polyplexes. **B)** In 3-D, 50,000 cells were cultured per 10 µl hydrogel per well in a 96 well plate for 2 days before transfection. Cells were transfected overnight, and transgene expression was analyzed 24 hours after overnight transfection. **C)** 50,000 cells were cultured per 10 µl hydrogel per well in a 96 well plate in 3-D for 2 days. Cells were transfected with YOYO-1 labeled polyplexes, and internalization was analyzed after 2 hours, 6 hours, and overnight (12-13 hours).



Supplementary Figure S3: *Effect on fold cell viability in 3-D.* The Cell Titer 96_Aqueous One Solution Cell Proliferation Assay (Promega) was performed to determine the cytotoxicity and proliferation of cells exposed to different conditions namely, **A)** 24 hours post overnight transfection in presence of endocytic inhibitors, **B)** 24 hours post transfection in presence of cytoskeletal inhibitors and activators, and **C)** 24 hours post overnight transfection in presence of inhibitors and activators for RhoGTPases.
Fold change in proliferation was assessed with respect to proliferation of untreated sample, and plotted.

Supplementary Table 1: Effect of endocytic inhibitors on gene transfer in 2-D vs 3-D

	% Transgene expression			% Internalization	
	2-D	3-D		2-D	3-D
-Caveolae I	49%↓, ***	99.1%↓, ***		72.9%↓, ***	48.6%↓,**
-Caveolae II	91%,↓, ***	98%↓, ***		9.5%↓	107%↑
-Clathrin I	69%↓, ***	95.4%↓, *		28.3%↓, ***	89.4%↓, **
-Clathrin II	79%↓, ***	94%↓, ***		17.53%↓, **	102%↑
-Macropinocytosis	124%↑, *	54.6%↓, *		2.2%↓	104%↑

Supplementary Table 2: Effect of cytoskeletal inhibitors and activators on gene transfer in 2-D vs 3-D

	% Transgene expression			% Internalization	
	2-D	3-D		2-D	3-D
-Actin	32.4%↓	94.4%↓, ***		172.2%↑, ***	47.9%↓,*
-Microtubule	1012%↑, ***	92%↓, ***		101.3%↑	16%↓
-Actin-myosin	21.5%↓	24.5%↓, *		111.4%↑	27.8%↓
+Actin	67%↓, *	70%↓		61.4%↓, ***	52%↓,**
+Microtubule	750%↑, ***	3552%↑, ***		126%↑, **	136%,↑
+Actin-myosin	195↑%, **	139%↑		25.8%↓, **	116.7%,↑

Supplementary Table 3: Effect of RhoGTPase mediated signaling on gene transfer in 2-D vs 3-D

	% Transgene expression			% Internalization	
	2-D	3-D		2-D	3-D
-Rho, Rac, Cdc42	23.7%↓	64%↓		10.3%↓	30.8%↓
-RhoA,B,C	99.5%↓, ***	95.3%↓		112.4%↑, *	134.6%↑
-ROCK	119.7%↑,	81%↓		128.1%↑, ***	91%↓,***
-PAK1	198%↑, ***	387.7%↑, ***		11.1%, ↓ *	129%↑
+Rho, Rac, Cdc42	1516%↑,*	536%↑		177.3%↑,**	15.5%↓
+RhoA,B,C	112.3%↑	33%↓		21.3%↓	30.5%↓

Supplementary Table 1,2,3: *Non-viral gene transfer in 2-D vs 3-D. The % change in transgene expression or internalization on treatment with respect to untreated sample, has been plotted for each condition.* ↓ or ↑ indicates a percent decrease or increase for each treated sample with respect to untreated. Statistical analysis was done using tukey Multiple Comparison test, which compares all columns with each other for 2-D and 3-D, respectively. The symbols *, ** and *** represents a significant % change to the level of $p<0.05$, $p<0.01$ and $p<0.001$, respectively, as compared to untreated sample for 2-D and 3-D respectively.