

Supplemental Tables/Figures

Table S.I. Spheroid elasticities and heights. Values shown represent geometric means \pm standard deviations of each condition.

<i>Elastic (kPa)</i>		Cells only	~0.25 kPa	~1 kPa	~2 kPa	~10 kPa
Control	Day 1	1.8 \pm 0.6	2.0 \pm 0.7	2.0 \pm 0.9	1.9 \pm 0.9	1.9 \pm 0.9
	Day 7	1.8 \pm 0.7	2.2 \pm 0.5	2.2 \pm 0.7	2.4 \pm 1.0	2.3 \pm 1.1
	Day 14	2.3 \pm 1.1	1.4 \pm 0.4	2.2 \pm 0.6	0.6 \pm 0.4	0.7 \pm 0.3
	Day 21	2.0 \pm 1.0	1.2 \pm 0.9	0.8 \pm 1.0	0.3 \pm 0.1	1.0 \pm 1.3
Adipo	Day 1	0.8 \pm 0.9	0.8 \pm 1.0	1.0 \pm 0.7	1.0 \pm 1.0	0.8 \pm 1.0
	Day 7	1.6 \pm 0.7	1.8 \pm 0.8	2.0 \pm 0.7	2.0 \pm 0.7	1.8 \pm 1.0
	Day 14	1.0 \pm 0.5	1.6 \pm 0.9	2.2 \pm 1.3	2.1 \pm 1.4	1.3 \pm 0.9
	Day 21	1.0 \pm 0.4	1.6 \pm 0.7	1.7 \pm 0.8	2.0 \pm 1.0	2.0 \pm 1.3

<i>Height (μm)</i>		Cells only	~0.25 kPa	~1 kPa	~2 kPa	~10 kPa
Control	Day 1	140 \pm 30	140 \pm 40	140 \pm 30	130 \pm 40	110 \pm 30
	Day 7	100 \pm 20	140 \pm 30	130 \pm 30	130 \pm 30	120 \pm 30
	Day 14	80 \pm 30	130 \pm 40	130 \pm 40	100 \pm 40	90 \pm 20
	Day 21	60 \pm 20	140 \pm 20	110 \pm 30	130 \pm 20	90 \pm 20
Adipo	Day 1	130 \pm 30	150 \pm 40	150 \pm 40	140 \pm 30	110 \pm 30
	Day 7	170 \pm 40	170 \pm 40	170 \pm 50	140 \pm 40	130 \pm 30
	Day 14	140 \pm 30	140 \pm 40	150 \pm 50	120 \pm 30	110 \pm 40
	Day 21	170 \pm 40	170 \pm 40	140 \pm 30	150 \pm 40	110 \pm 30

Table S.II. Additional mechanical property measurements. This table summarizes the viscoelastic properties of spheroids characterized by AFM.

E_R (kPa)		Just Cells	~0.25 kPa	~1 kPa	~2 kPa	~10 kPa
Control	Day 1	0.7±0.3	0.9±0.5	0.9±0.5	0.7±0.4	0.7±0.5
	Day 7	0.8±0.4	1.0±0.3	1.1±0.3	1.1±0.6	1.0±0.6
	Day 14	1.0±0.6	0.8±0.2	1.1±0.4	0.4±0.2	0.4±0.2
	Day 21	0.9±0.5	0.7±0.4	0.5±0.4	0.3±0.1	0.6±0.8
Adipo	Day 1	0.3±0.5	0.3±0.5	0.5±0.4	0.4±0.6	0.3±0.5
	Day 7	0.8±0.5	0.9±0.5	0.9±0.4	0.9±0.5	0.8±0.5
	Day 14	0.5±0.3	0.8±0.6	1.1±0.8	0.9±0.9	1.4±1.0
	Day 21	0.5±0.3	0.8±0.4	0.8±0.5	1.0±0.5	1.0±0.7
E_0 (kPa)		Just Cells	~0.25 kPa	~1 kPa	~2 kPa	~10 kPa
Control	Day 1	1.3±0.5	1.6±0.6	1.6±0.7	1.4±0.7	1.4±0.7
	Day 7	1.4±0.6	1.7±0.4	1.7±0.6	1.9±0.8	1.7±0.9
	Day 14	1.7±0.9	1.2±0.3	1.7±0.5	0.5±0.3	0.6±0.3
	Day 21	1.5±0.8	1.1±0.7	0.7±0.8	0.3±0.1	0.8±1.1
Adipo	Day 1	0.6±0.7	0.6±0.8	0.8±0.6	0.8±0.9	0.6±0.8
	Day 7	1.3±0.6	1.4±0.7	1.6±0.5	1.5±0.6	1.4±0.8
	Day 14	0.8±0.4	1.3±0.8	1.8±1.1	1.6±1.1	1.1±0.6
	Day 21	0.8±0.3	1.3±0.6	1.4±0.6	1.6±0.8	1.6±1.1
μ (kPa*s)		Just Cells	~0.25 kPa	~1 kPa	~2 kPa	~10 kPa
Control	Day 1	2.9±1.2	2.7±1.2	2.9±1.4	2.8±1.7	2.9±1.5
	Day 7	2.9±1.0	4.4±3.2	3.5±1.5	3.7±1.8	3.5±2.4
	Day 14	3.3±1.9	2.4±1.0	3.5±0.9	0.7±0.7	1.5±0.5
	Day 21	2.9±2.0	2.3±3.2	0.8±2.9	0.4±0.3	1.4±1.1
Adipo	Day 1	1.2±1.6	1.3±1.6	1.3±1.1	1.4±1.9	1.3±1.9
	Day 7	2.4±1.5	2.5±1.7	2.7±1.1	2.7±1.3	2.7±1.9
	Day 14	1.4±0.8	2.4±1.5	3.2±1.8	2.8±1.9	0.1±1.6
	Day 21	1.5±0.7	2.1±1.8	2.5±1.4	3.4±1.7	2.7±1.9

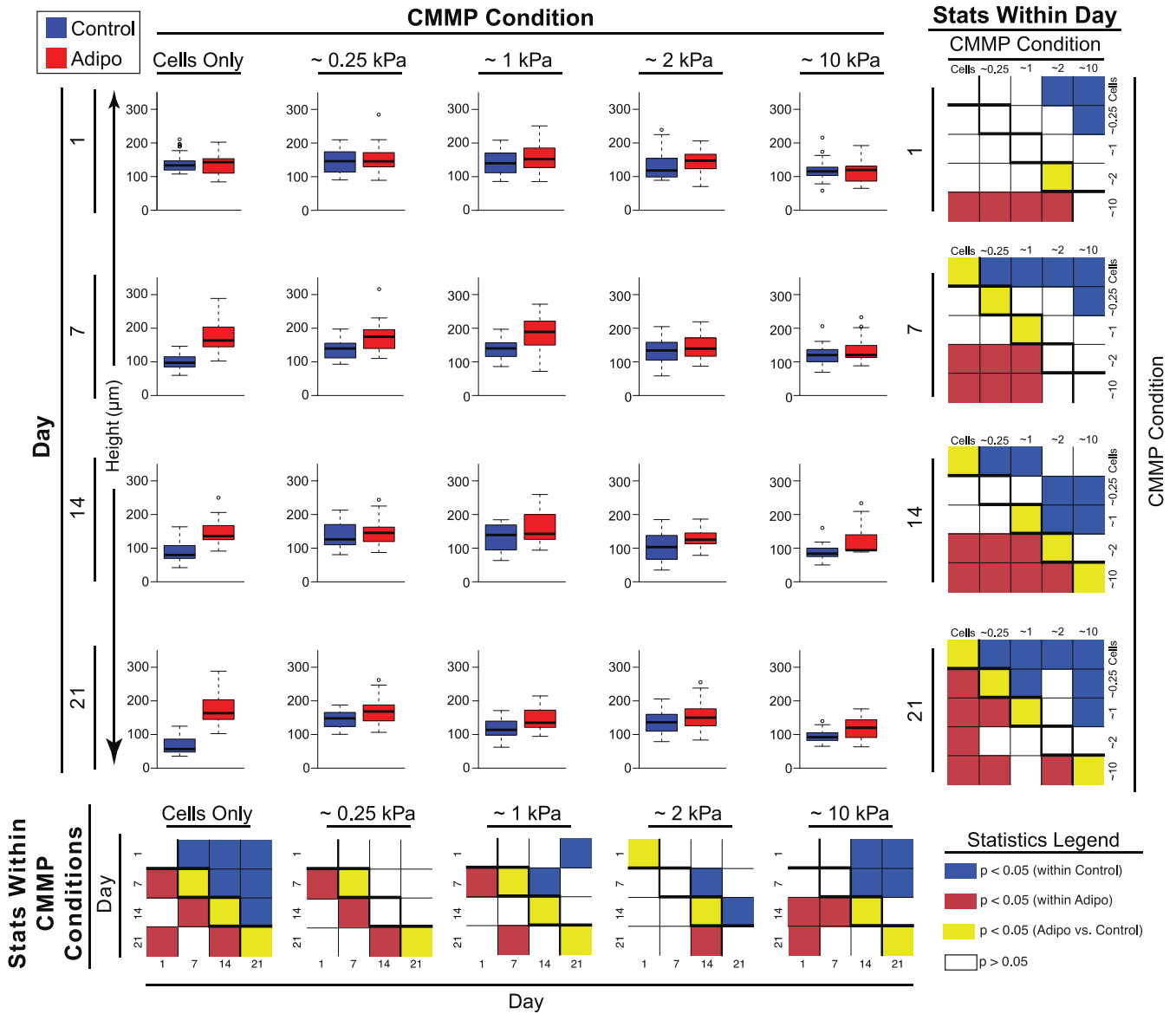


Figure S1. Temporal changes in the height of composite spheroids. These box and whisker plots display the spheroid heights in a matrix where descending rows illustrate later time points while columns represent the various CMMP conditions. The blue (control) and red (adipogenic) colored regions represent the 25-75% quartile ranges, the central black line represents the median of the data, and the whiskers represent the data with outliers (circles) removed. The smaller matrices at the end of each row and column depict the statistical comparisons using raw p-values across CMMP conditions and day, respectively. Significant differences within either control or adipogenic groups are represented by blue and red boxes, respectively. Differences across media environments are denoted by yellow boxes, while comparisons that were not significantly different are represented by white boxes.

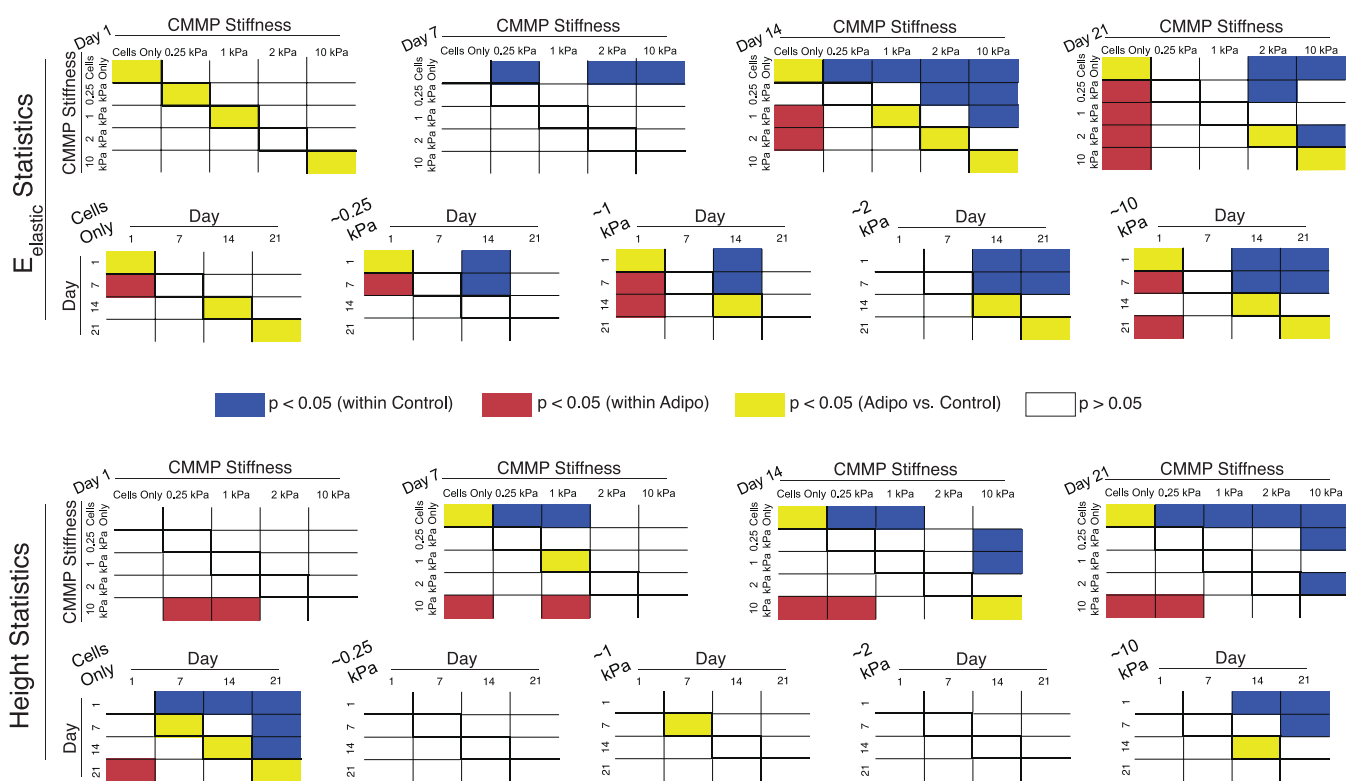


Figure S2. Conservative statistical comparisons of spheroid elasticities and heights. Illustrated here are significance charts representing more stringent, Holm-Bonferroni corrected p-values, which account for potential type-1 error for multiple comparisons, returned from statistical tests between various CMMP stiffnesses at each time point (top) and across time points within single CMMP conditions (bottom) for both spheroid elasticity and height. Significant differences within either control or adipogenic groups are represented by blue and red boxes, respectively. Differences across media environments are denoted by yellow boxes, while comparisons that were not significantly different are represented by white boxes.

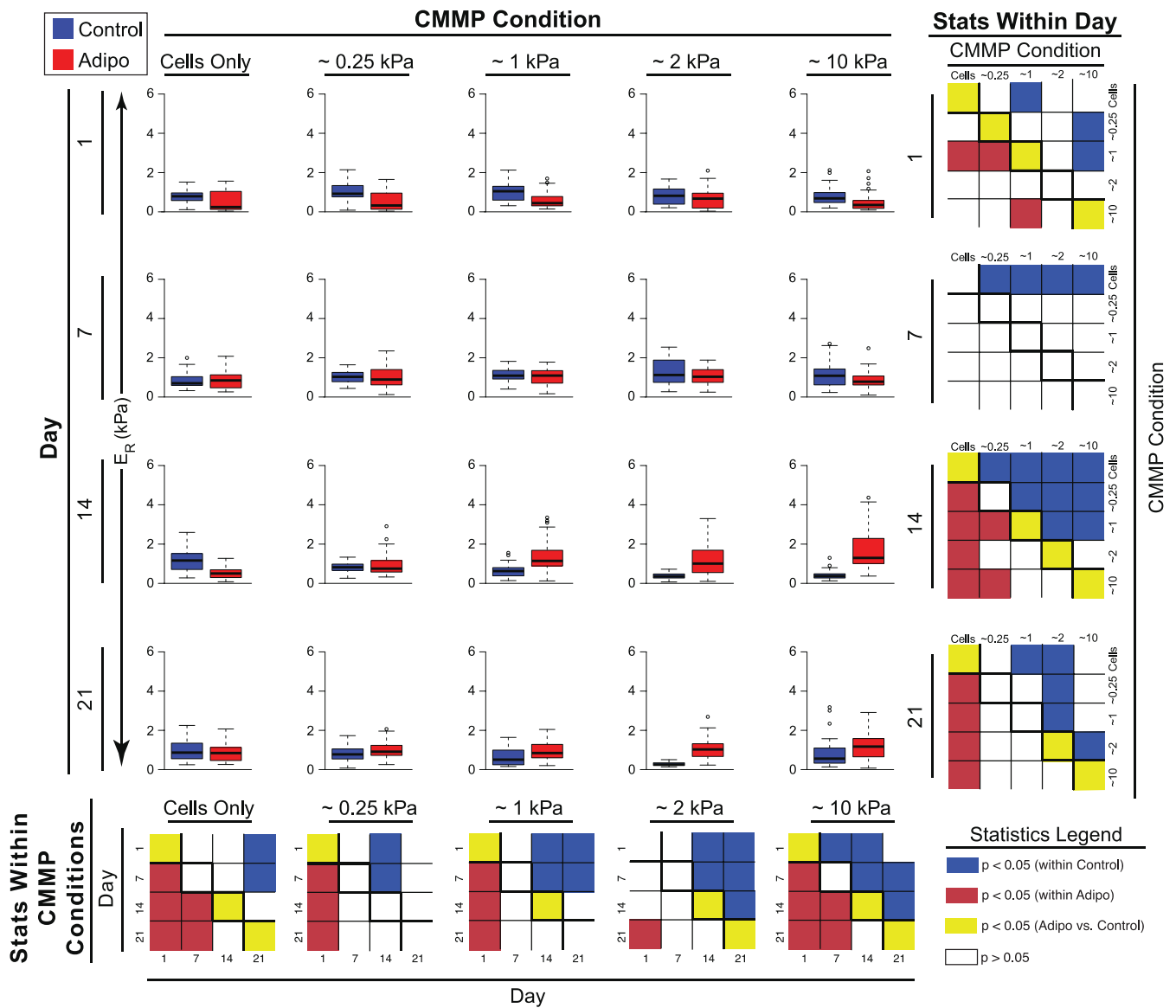


Figure S3. Temporal changes in the relaxed modulus of composite spheroids. These box and whisker plots display the relaxed modulus (E_R) of spheroids in a matrix where descending rows illustrate later time points while columns represent the various CMMP conditions. The blue (control) and red (adipogenic) colored regions represent the 25-75% quartile ranges, the central black line represents the median of the data, and the whiskers represent the data with outliers (circles) removed. **The smaller matrices at the end of each row and column depict the statistical comparisons using raw p-values across CMMP conditions and day, respectively. Significant differences within either control or adipogenic groups are represented by blue and red boxes, respectively. Differences across media environments are denoted by yellow boxes, while comparisons that were not significantly different are represented by white boxes.**

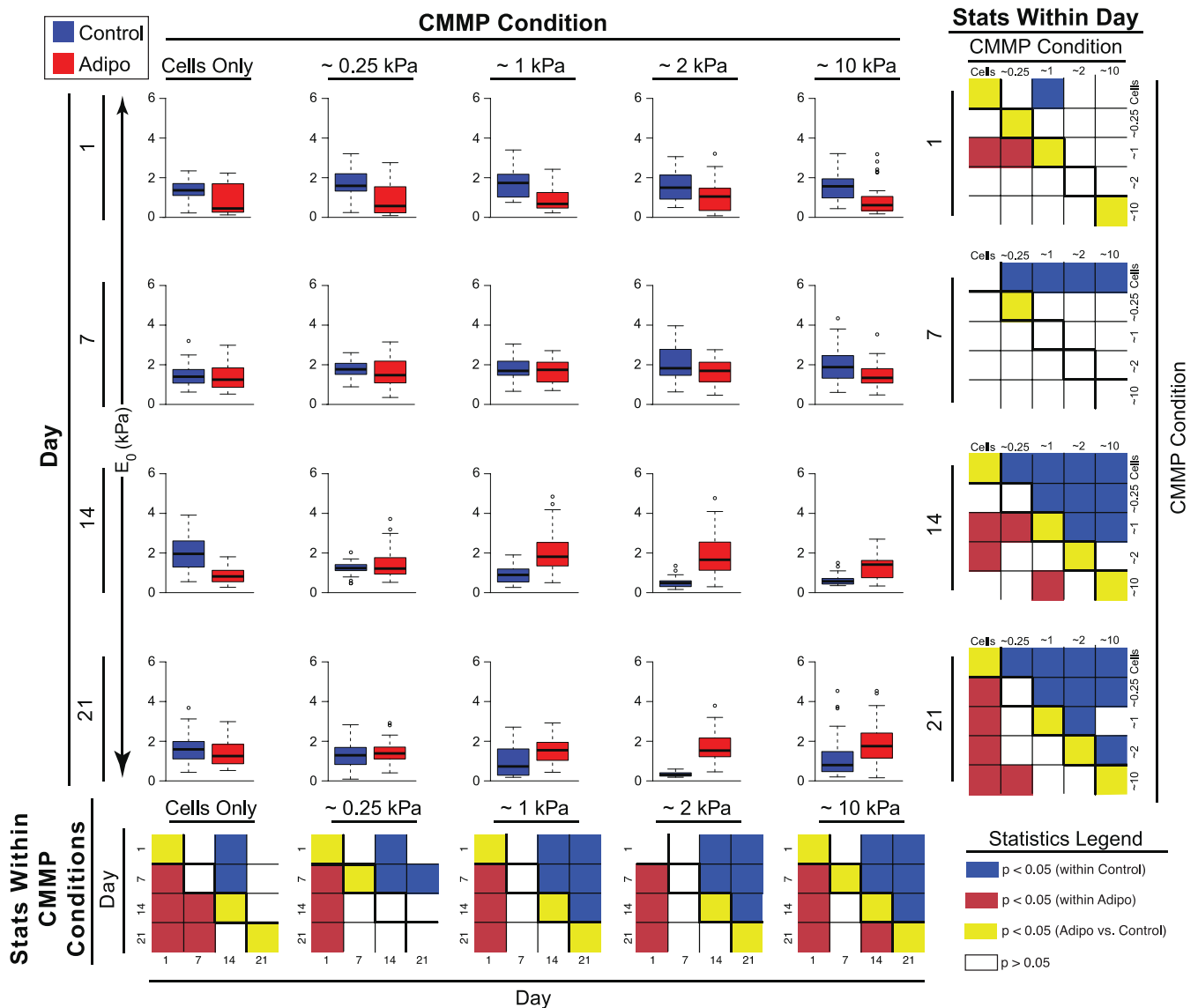


Figure S4. Temporal changes in the instantaneous modulus of composite spheroids. These box and whisker plots display the instantaneous modulus (E_0) of spheroids in a matrix where descending rows illustrate later time points while columns represent the various CMMP conditions. The blue (control) and red (adipogenic) colored regions represent the 25-75% quartile ranges, the central black line represents the median of the data, and the whiskers represent the data with outliers (circles) removed. The smaller matrices at the end of each row and column depict the statistical comparisons using raw p-values across CMMP conditions and day, respectively. Significant differences within either control or adipogenic groups are represented by blue and red boxes, respectively. Differences across media environments are denoted by yellow boxes, while comparisons that were not significantly different are represented by white boxes.

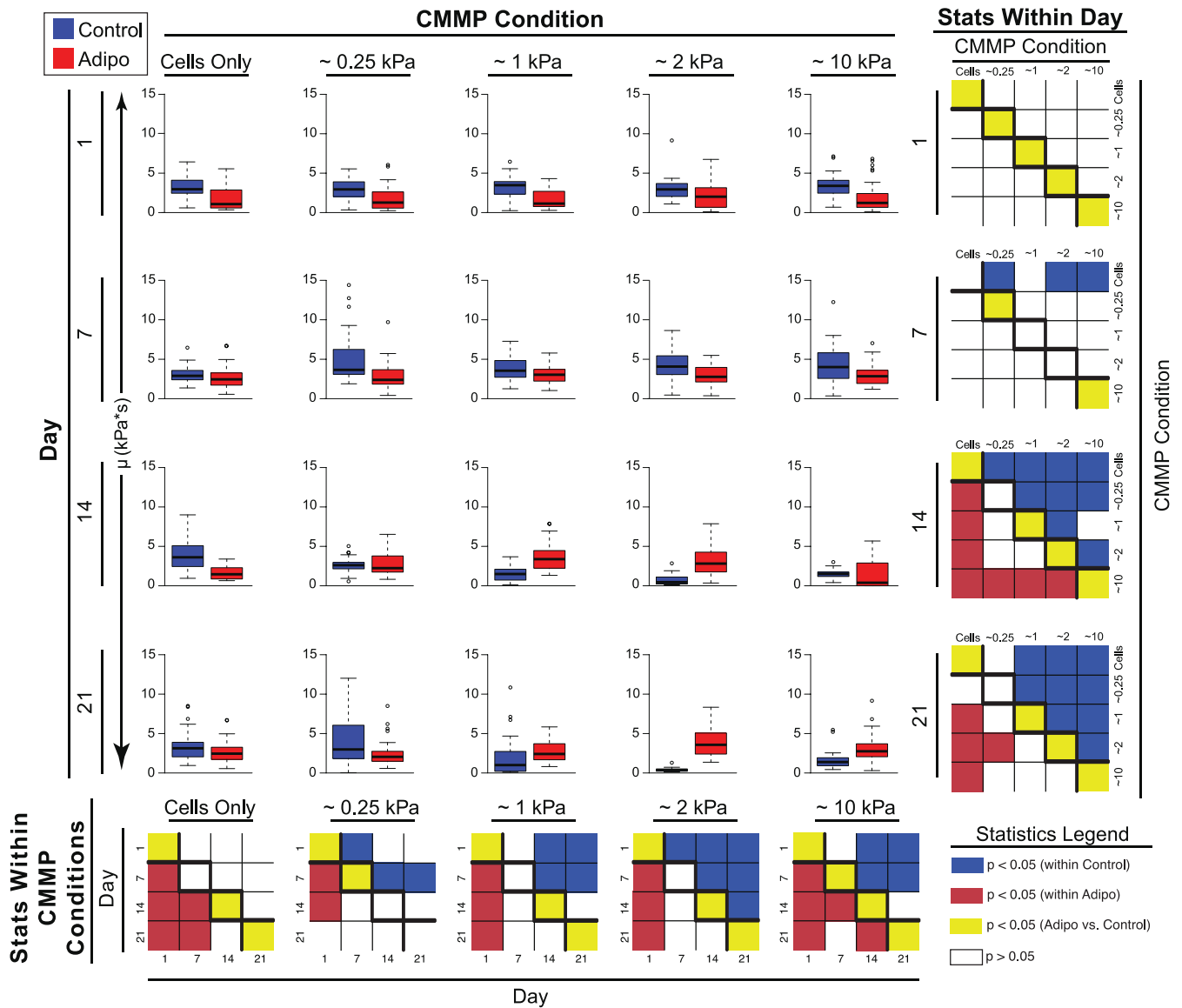


Figure S5. Temporal changes in the apparent viscosity of composite spheroids. These box and whisker plots display the apparent viscosity (μ) of spheroids in a matrix where descending rows illustrate later time points while columns represent the various CMMP conditions. The blue (control) and red (adipogenic) colored regions represent the 25-75% quartile ranges, the central black line represents the median of the data, and the whiskers represent the data with outliers (circles) removed. The smaller matrices at the end of each row and column depict the statistical comparisons using raw p-values across CMMP conditions and day, respectively. Significant differences within either control or adipogenic groups are represented by blue and red boxes, respectively. Differences across media environments are denoted by yellow boxes, while comparisons that were not significantly different are represented by white boxes.

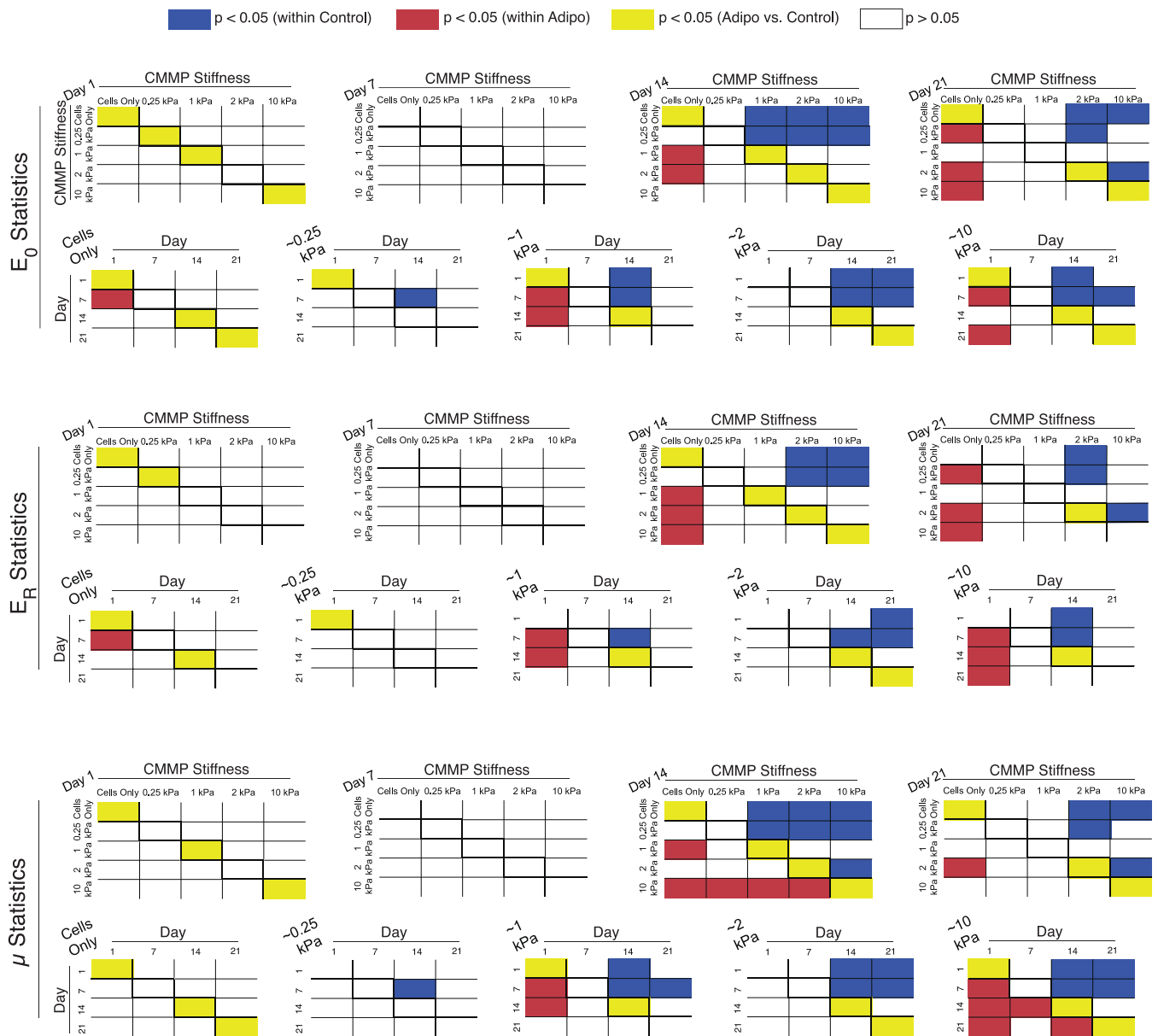


Figure S6. Type-1 error-adjusted statistical comparisons of spheroid viscoelastic properties. Illustrated here are significance charts representing more stringent, Holm-Bonferroni corrected p-values, which account for potential type-1 error for multiple comparisons, returned from statistical tests between various CMMP stiffnesses at each time point (top) and across time points within single CMMP conditions (bottom) for instantaneous modulus (E_0), relaxed modulus (E_R), and apparent viscosity (μ). Significant differences within either control or adipogenic groups are represented by blue and red boxes, respectively. Differences across media environments are denoted by yellow boxes, while comparisons that were not significantly different are represented by white boxes.

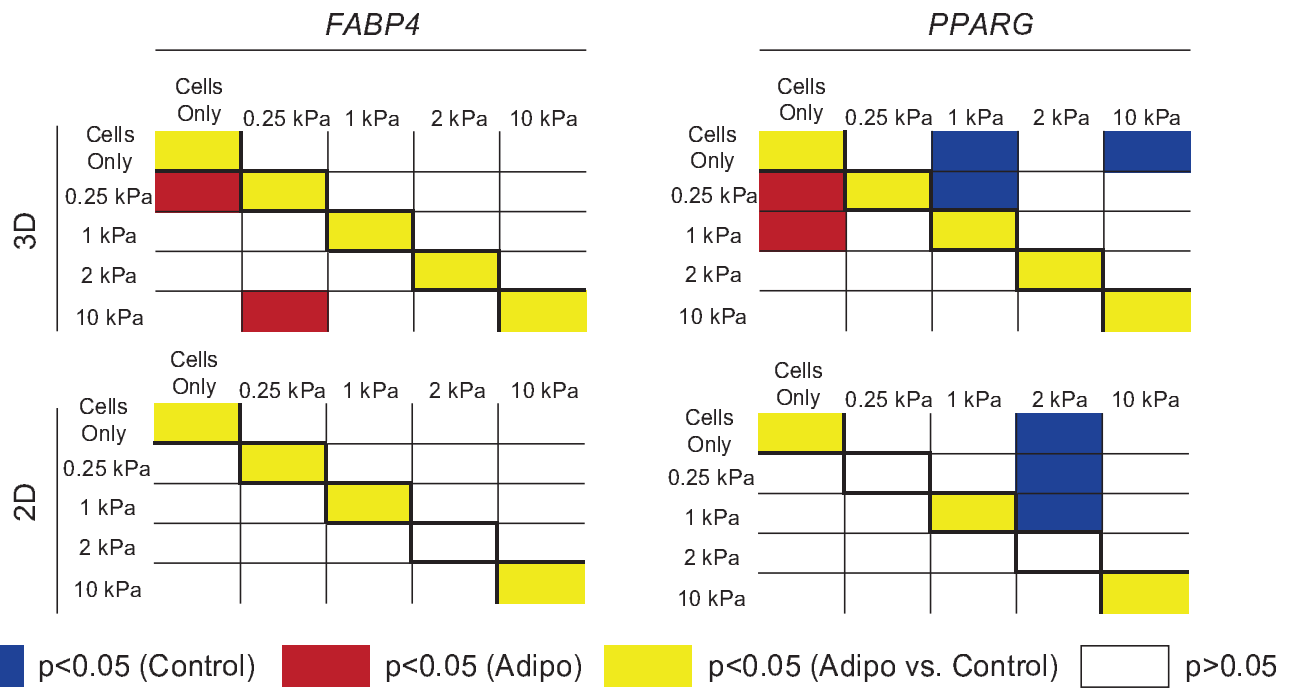


Figure S7. Statistical comparisons of lineage-specific mRNA expression. This matrix illustrates the significant, raw p-values for statistical comparisons across CMMP stiffnesses of 3D (top) and 2D (bottom) cultures for the genes *FABP4* (left) and *PPARG* (right). The coloring of the boxes represents a significant difference ($p < 0.05$) within either control (blue) or adipogenic (red) medium, as well as across these media conditions (yellow). White boxes signify that the comparison showed no statistically significant differences.

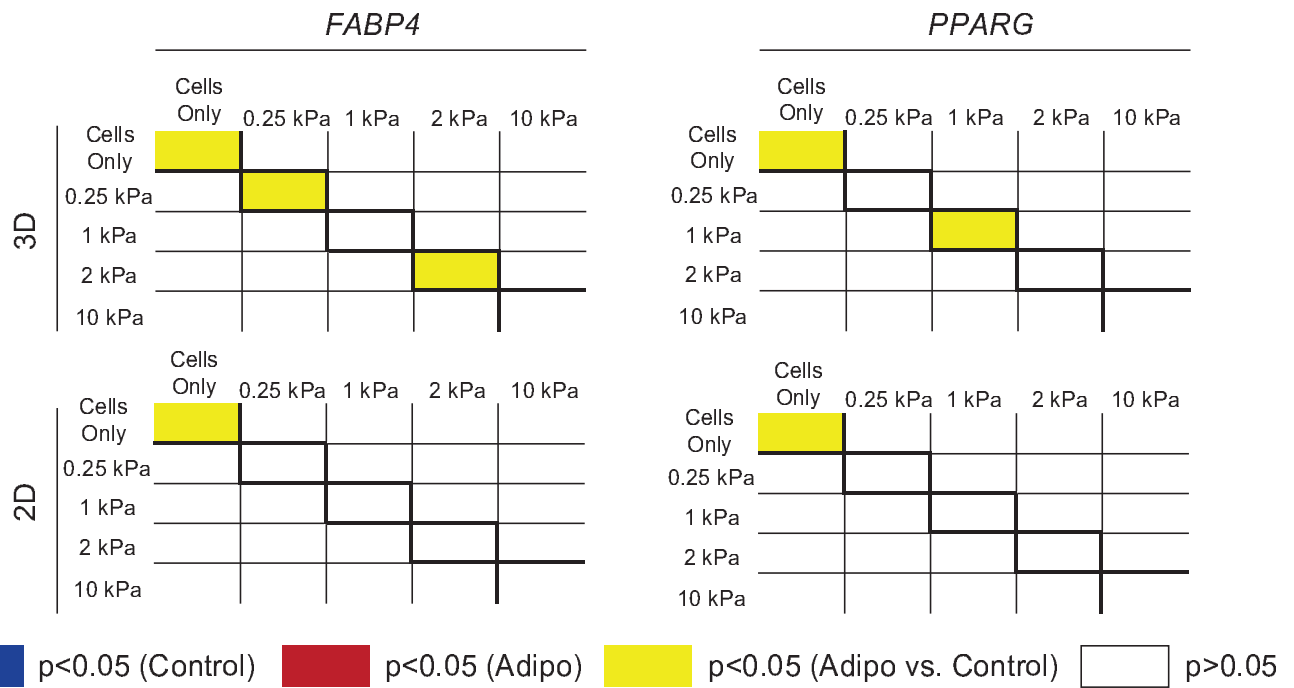


Figure S8. Conservative statistical comparisons of lineage-specific mRNA expression. This matrix illustrates the significant, Holm-Bonferroni corrected p-values, providing a more stringent adjustment for type-1 error, for statistical comparisons across CMMP stiffnesses of 3D (top) and 2D (bottom) cultures for the genes *FABP4* (left) and *PPARG* (right). The coloring of the boxes represents a significant difference ($p < 0.05$) within either control (blue) or adipogenic (red) medium, as well as across these media conditions (yellow). White boxes signify that the comparison showed no statistically significant differences.

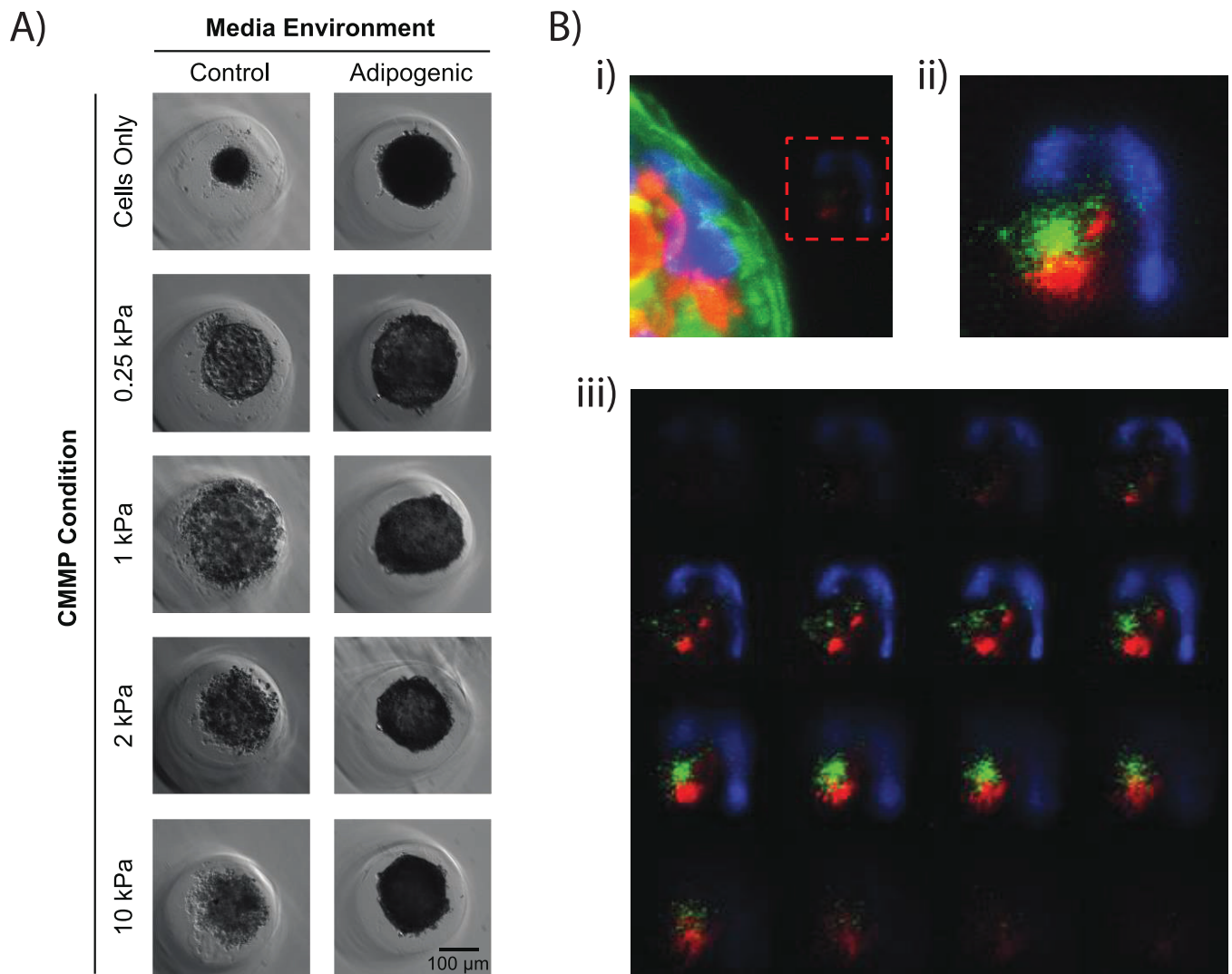


Figure S9. Evidence of spheroid dissociation. (A) These images illustrate control (left) and adipogenic (right) spheroids within agarose microwells after 18 days of culture for each CMMP condition. (B) (i) Image of a single CMMP interacting with cells separate from the larger, intact Day 21 adipogenic spheroid. (ii) Magnification of the red, boxed region depicting a 3D projection of the dissociated CMMP/cell unit. (iii) A montage of confocal image slices (21 μ m thickness, 1.33 μ m steps) shows 1-2 cells interacting with a single CMMP.