SUPPORTING INFORMATION

Sensitivity study for the key parameters in Heterospheroid Preparation with Insulin-Secreting β-Cells and Mesenchymal Stem Cells

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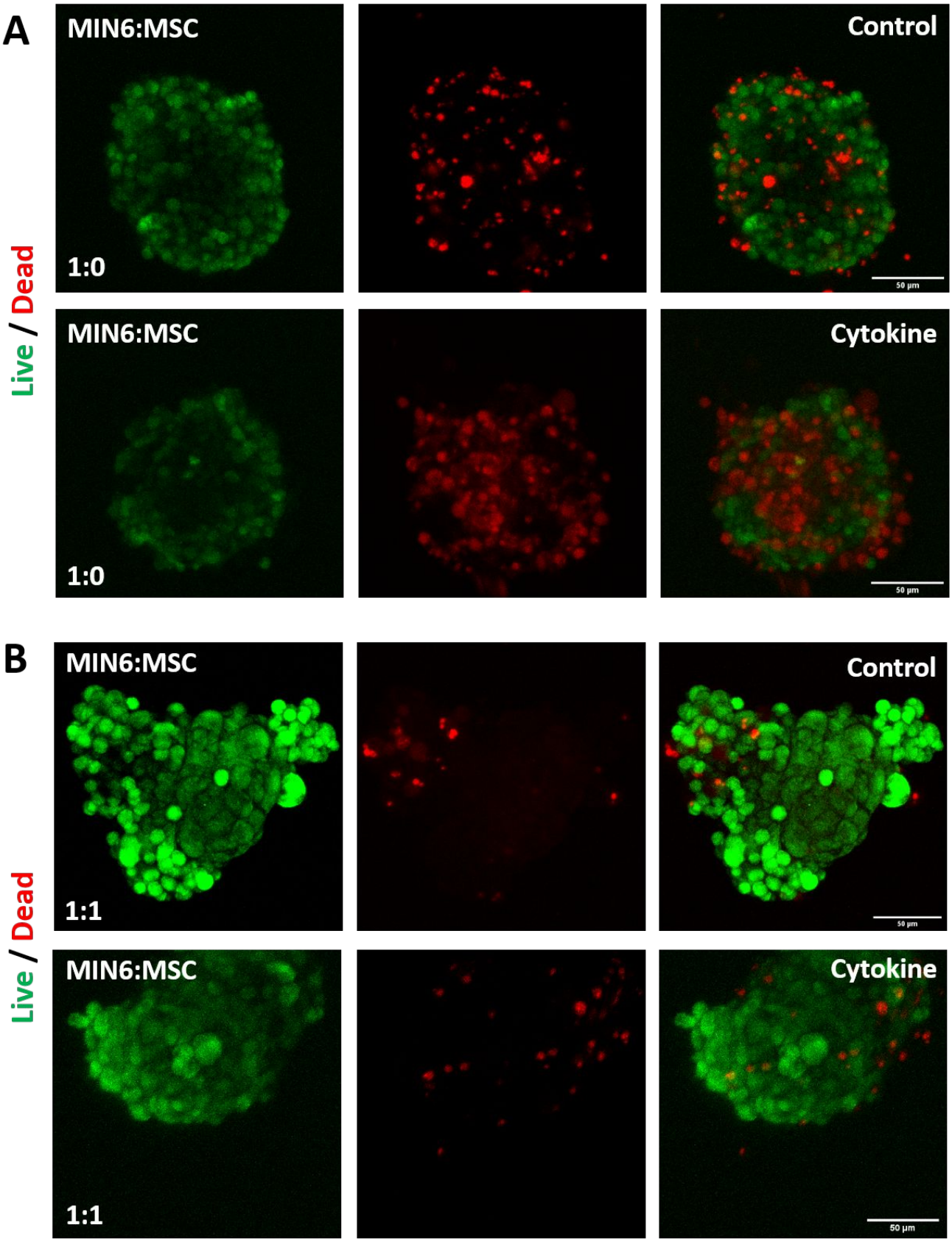

Figure S1. FDA/PI staining of (hetero)spheroids for 500 initial cell number after cytokine cocktail treatment. Confocal microscopy images of control and cytokine treated A) MIN6 homospheroids (1:0) and B) MIN6:MSC heterospheroids (1:1). Scale bars: 50µm.

Table S1. Percent viability of each group (control and cytokine treated) for 1:0 and 1:1 MIN6:MSC ratios was calculated as average of percent viabilities of multiple z-stack images analyzed by ImageJ software.

Experimental Groups	Percent Viability
1:0 cytokine treated	36%
1:0 control	90%
1:1 cytokine treated	89%
1:1 control	99%

Table S2. Tukey's multiple comparison test for viability (Fig. 4A) and intracellular ATP amount (Fig. 4B) of (hetero)spheroids of 200 or 500 initial cell numbers. ****p<0.0001.***p<0.001. **p<0.01. *p<0.05.

Experimental Groups	Viability	Intracellular ATP
200:1:0 vs. 200:0:1	****	ns
200:1:0 vs. 200:1:1	****	ns
200:1:0 vs. 200:2:1	***	ns
200:1:0 vs. 200:5:1	***	ns
200:1:0 vs. 200:10:1	ns	ns
200:1:0 vs. 500:1:0	***	ns
200:1:0 vs. 500:0:1	****	ns
200:1:0 vs. 500:1:1	***	**
200:1:0 vs. 500:2:1	****	*
200:1:0 vs. 500:5:1	ns	**
200:1:0 vs. 500:10:1	ns	ns
200:0:1 vs. 200:1:1	ns	ns
200:0:1 vs. 200:2:1	ns	ns
200:0:1 vs. 200:5:1	ns	ns
200:0:1 vs. 200:10:1	***	ns
200:0:1 vs. 500:1:0	****	ns
200:0:1 vs. 500:0:1	ns	ns
200:0:1 vs. 500:1:1	ns	*
200:0:1 vs. 500:2:1	ns	ns
200:0:1 vs. 500:5:1	**	*
200:0:1 vs. 500:10:1	*	ns
200:1:1 vs. 200:2:1	ns	ns
200:1:1 vs. 200:5:1	ns	ns
200:1:1 vs. 200:10:1	*	ns
200:1:1 vs. 500:1:0	****	ns
200:1:1 vs. 500:0:1	ns	ns
200:1:1 vs. 500:1:1	ns	*
200:1:1 vs. 500:2:1	ns	ns
200:1:1 vs. 500:5:1	ns	*
200:1:1 vs. 500:10:1	ns	ns
200:2:1 vs. 200:5:1	ns	ns

200:2:1 vs. 200:10:1	*	ns
200:2:1 vs. 500:1:0	****	ns
200:2:1 vs. 500:0:1	ns	ns
200:2:1 vs. 500:1:1	ns	*
200:2:1 vs. 500:2:1	ns	ns
200:2:1 vs. 500:5:1	ns	*
200:2:1 vs. 500:10:1	ns	ns
200:5:1 vs. 200:10:1	ns	ns
200:5:1 vs. 500:1:0	****	ns
200:5:1 vs. 500:0:1	ns	ns
200:5:1 vs. 500:1:1	ns	ns
200:5:1 vs. 500:2:1	ns	ns
200:5:1 vs. 500:5:1	ns	ns
200:5:1 vs. 500:10:1	ns	ns
200:10:1 vs. 500:1:0	****	ns
200:10:1 vs. 500:0:1	**	ns
200:10:1 vs. 500:1:1	ns	*
200:10:1 vs. 500:2:1	**	*
200:10:1 vs. 500:5:1	ns	*
200:10:1 vs. 500:10:1	ns	ns
500:1:0 vs. 500:0:1	****	ns
500:1:0 vs. 500:1:1	****	ns
500:1:0 vs. 500:2:1	****	ns
500:1:0 vs. 500:5:1	****	ns
500:1:0 vs. 500:10:1	****	ns
500:0:1 vs. 500:1:1	ns	ns
500:0:1 vs. 500:2:1	ns	ns
500:0:1 vs. 500:5:1	**	ns
500:0:1 vs. 500:10:1	*	ns
500:1:1 vs. 500:2:1	ns	ns
500:1:1 vs. 500:5:1	ns	ns
500:1:1 vs. 500:10:1	ns	ns
500:2:1 vs. 500:5:1	*	ns
500:2:1 vs. 500:10:1	ns	ns
500:5:1 vs. 500:10:1	ns	ns

Table S3. Tukey's multiple comparison test for stimulation index of (hetero)spheroids of 200 and 500 initial cell numbers (Fig. 4C).

Experimental Groups	Stimulation index
200:1:0 vs. 200:1:1	ns
200:1:0 vs. 200:2:1	ns
200:1:0 vs. 500:1:0	ns
200:1:0 vs. 500:1:1	ns
200:1:0 vs. 500:2:1	ns
200:1:1 vs. 200:2:1	ns
200:1:1 vs. 500:1:0	ns
200:1:1 vs. 500:1:1	ns
200:1:1 vs. 500:2:1	ns
200:2:1 vs. 500:1:0	ns
200:2:1 vs. 500:1:1	ns
200:2:1 vs. 500:2:1	ns
500:1:0 vs. 500:1:1	ns
500:1:0 vs. 500:2:1	ns
500:1:1 vs. 500:2:1	ns

Table S4. Tukey's multiple comparison test for viability of heterospheroids after cytokine treatment for initial cell numbers 200 (Fig. 5B) and 500 (Fig. 5E). ****p<0.0001. **p<0.01. *p<0.05.

Experimental Groups	Viability (200 cells)	Viability (500 cells)
control:1:0 vs. control:0:1	****	****
control:1:0 vs. control:1:1	**	****
control:1:0 vs. control:2:1	**	****
control:1:0 vs. cytokine:1:0	****	****
control:1:0 vs. cytokine:0:1	*	****
control:1:0 vs. cytokine:1:1	ns	ns
control:1:0 vs. cytokine:2:1	*	****
control:0:1 vs. control:1:1	ns	*
control:0:1 vs. control:2:1	ns	ns
control:0:1 vs. cytokine:1:0	****	****
control:0:1 vs. cytokine:0:1	ns	ns
control:0:1 vs. cytokine:1:1	*	****
control:0:1 vs. cytokine:2:1	****	****
control:1:1 vs. control:2:1	ns	ns
control:1:1 vs. cytokine:1:0	****	****
control:1:1 vs. cytokine:0:1	ns	ns
control:1:1 vs. cytokine:1:1	ns	****
control:1:1 vs. cytokine:2:1	****	****
control:2:1 vs. cytokine:1:0	****	****
control:2:1 vs. cytokine:0:1	ns	ns
control:2:1 vs. cytokine:1:1	ns	****
control:2:1 vs. cytokine:2:1	****	****
cytokine:1:0 vs. cytokine:0:1	****	****
cytokine:1:0 vs. cytokine:1:1	****	****
cytokine:1:0 vs. cytokine:2:1	****	****
cytokine:0:1 vs. cytokine:1:1	ns	****
cytokine:0:1 vs. cytokine:2:1	****	****
cytokine:1:1 vs. cytokine:2:1	****	****

Table S5. Tukey's multiple comparison test for viability (Fig. 7B) and stimulation index (Fig. 7C) of encapsulated (heterospheroids). ****p<0.0001.

Experimental Groups	Viability	Stimulation Index
control:1:0 vs. control:1:1	****	ns
control:1:0 vs. glp:1:0	ns	ns
control:1:0 vs. glp:1:1	****	ns
control:1:1 vs. glp:1:0	****	ns
control:1:1 vs. glp:1:1	ns	ns
glp:1:0 vs. glp:1:1	****	ns

Table S6. Tukey's multiple comparison test for apoptotic activity of encapsulated (hetero)spheroids after cytokine treatment (Fig. 8). *p<0.05.

Experimental Groups	Apoptotic Index
1:0 control:without glp vs. 1:0 control:with glp	ns
1:0 control:without glp vs. 1:0 cytokine:without glp	ns
1:0 control:without glp vs. 1:0 cytokine:with glp	*
1:0 control:with glp vs. 1:0 cytokine:without glp	ns
1:0 control:with glp vs. 1:0 cytokine:with glp	ns
1:0 cytokine:without glp vs. 1:0 cytokine:with glp	ns
1:1 control:without glp vs. 1:1 control:with glp	ns
1:1 control:without glp vs. 1:1 cytokine:without glp	ns
1:1 control:without glp vs. 1:1 cytokine:with glp	ns
1:1 control:with glp vs. 1:1 cytokine:without glp	ns
1:1 control:with glp vs. 1:1 cytokine:with glp	ns
1:1 cytokine:without glp vs. 1:1 cytokine:with glp	ns

Supporting Video Legends

Video S1:
Visualization of z-stack images of MIN6 homospheroid (1:0) after FDA/PI staining without cytokine treatment (control)

Video S2:
Visualization of z-stack images of MIN6 homospheroid (1:0) after FDA/PI staining with cytokine treatment

Video S3:
Visualization of z-stack images of MIN6:MSC heterospheroid (1:1) after FDA/PI staining without cytokine treatment (control)

Video S4:
Visualization of z-stack images of MIN6:MSC heterospheroid (1:1) after FDA/PI staining with cytokine treatment