

Supplemental Figures and Legends:

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Reaction 1 Formula for 150ml (100ml Bioreactor and 50ml for media change)	
150 ml Essential 8™ Flex with Supplement	ThermoFisher Scientific A2858501
1.5ml Penicillin-Streptomycin (10000 U/mL)	ThermoFisher Scientific 15140122
Reaction 8 Formula for 150ml (100ml Bioreactor and 50ml for media change)	
150 ml Essential 8™ Flex with Supplement	ThermoFisher Scientific A2858501
1.5ml Penicillin-Streptomycin (10000 U/mL)	ThermoFisher Scientific 15140122
15ul Heparin (Stock 1mg ml ⁻¹)	Sigma-Aldrich H3149-50KU
3ml PVA (Stock 50mg ml ⁻¹)	Sigma-Aldrich P8136-250G
150 ul Dextran Sulfate (Stock 100 mg ml ⁻¹)	Sigma-Aldrich 42867
1.5ml Pluronic F68 (Stock 100mg ml ⁻¹)	ThermoFisher Scientific 24040032
Reaction 19 Formula for 150ml (100ml Bioreactor and 50ml for media change)	
150 ml Essential 8™ Flex with Supplement	ThermoFisher Scientific A2858501
1.5ml Penicillin-Streptomycin (10000 U/mL)	ThermoFisher Scientific 15140122
7.5ul Heparin (Stock 1mg ml ⁻¹)	Sigma-Aldrich H3149-50KU
1.5ml PVA (Stock 50mg ml ⁻¹)	Sigma-Aldrich P8136-250G
75 ul Dextran Sulfate (Stock 100 mg ml ⁻¹)	Sigma-Aldrich 42867
0.75ml Pluronic F68 (Stock 100mg ml ⁻¹)	ThermoFisher Scientific 24040032
7.5ml PEG (Stock 100mg ml ⁻¹)	Sigma-Aldrich 89510-250G-F

B

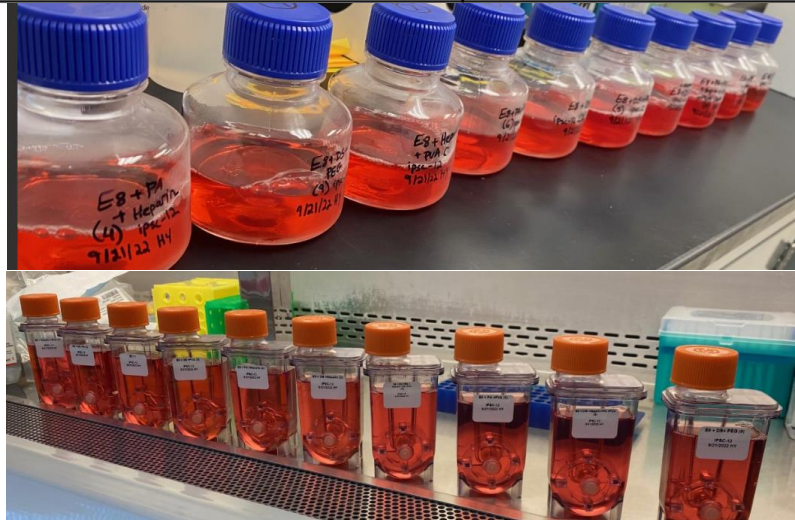


Figure S1: Reaction Additives and Experimental Set Up

- (A) Examples of reaction additives for a control bioreactor (Rxn1), a 4 additive reaction Rxn8) and a mid-point replicate reaction (Rxn19)
- (B) Media and bioreactor set up for the first batch of experiments.

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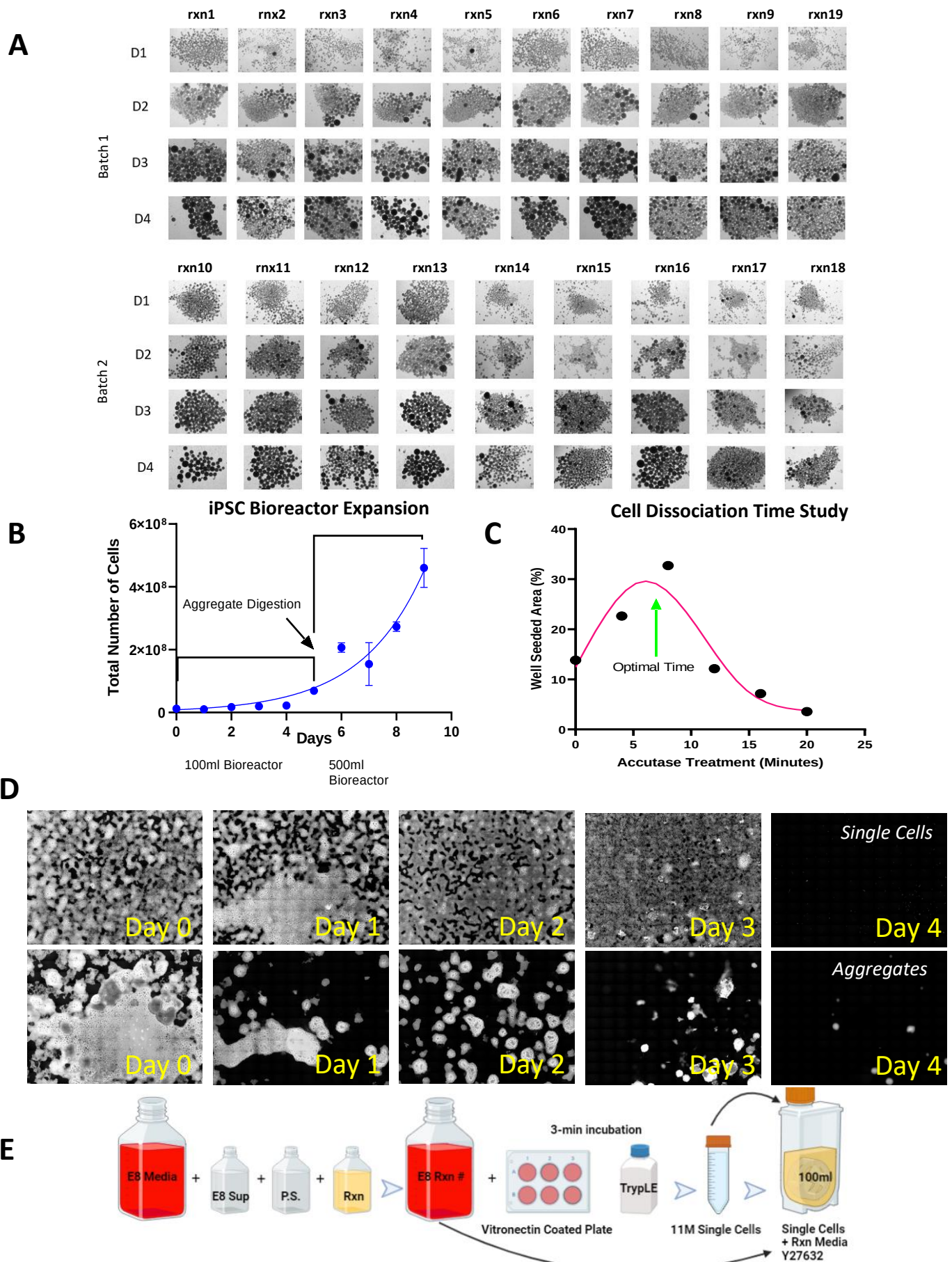
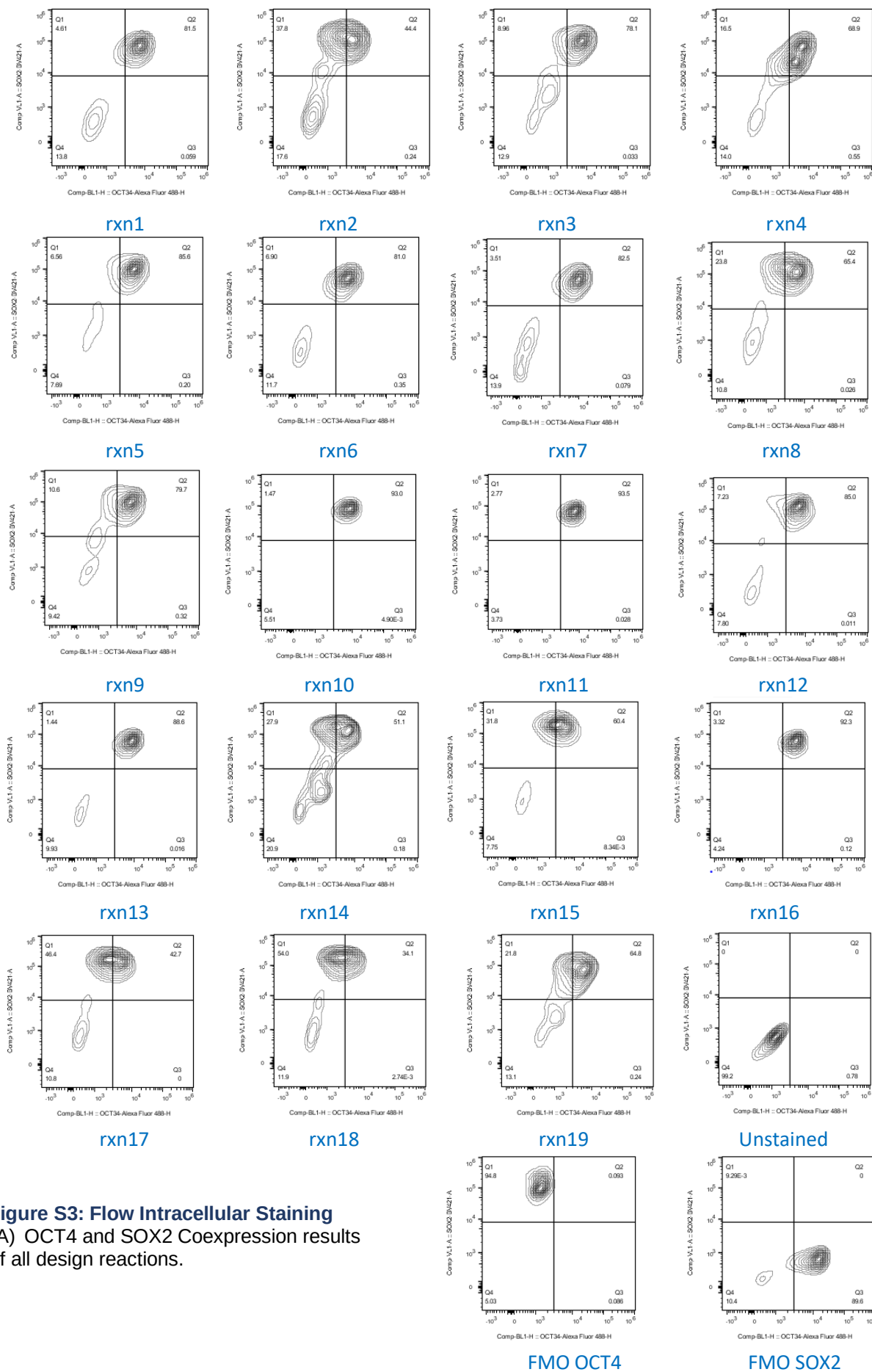


Figure S2: Aggregate Outputs and Optimization of Bioreactor Process Parameters

- (A) Daily aggregate images of all samples
- (B) Continuous growth rate curve after day 4 passaging between bioreactors
- (C) Digestion time optimization for optimal recovery rate between passages
- (D) Growth time optimization shows that passaging needs to be done on day 3-4 of culture.
- (E) A schematic of the method used to seed the bioreactors.

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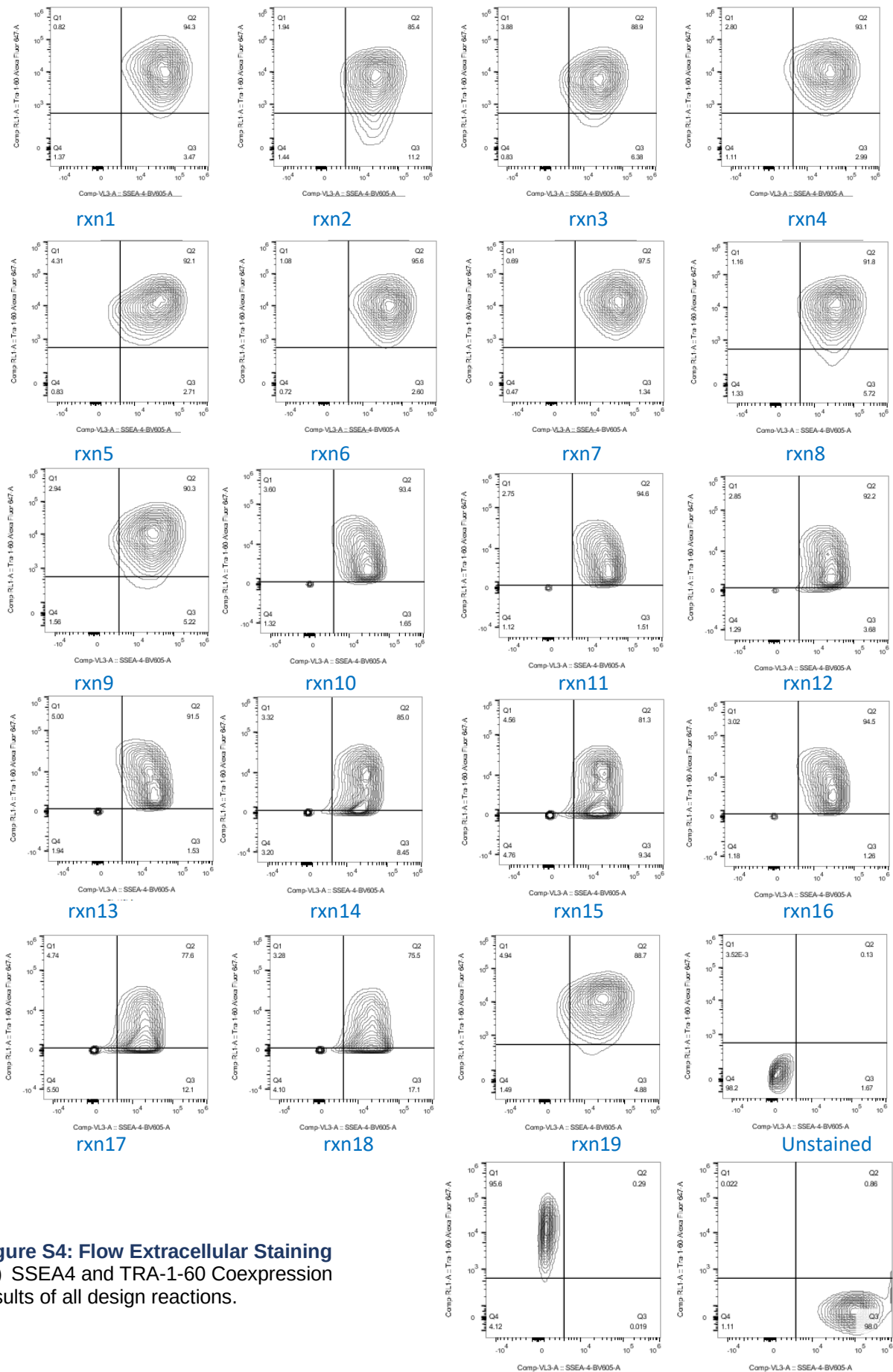
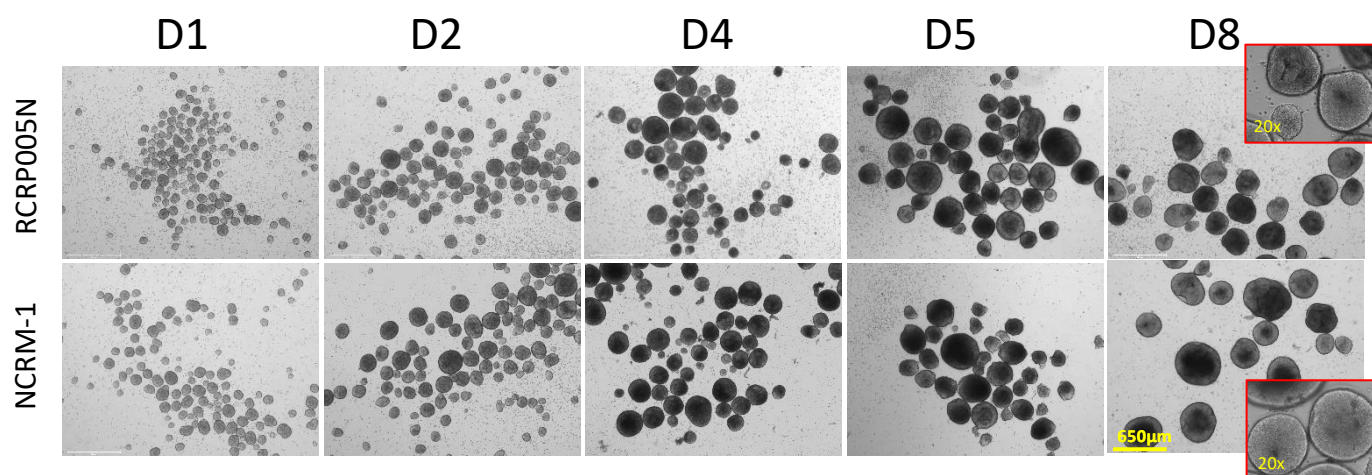


Figure S4: Flow Extracellular Staining
(A) SSEA4 and TRA-1-60 Coexpression results of all design reactions.

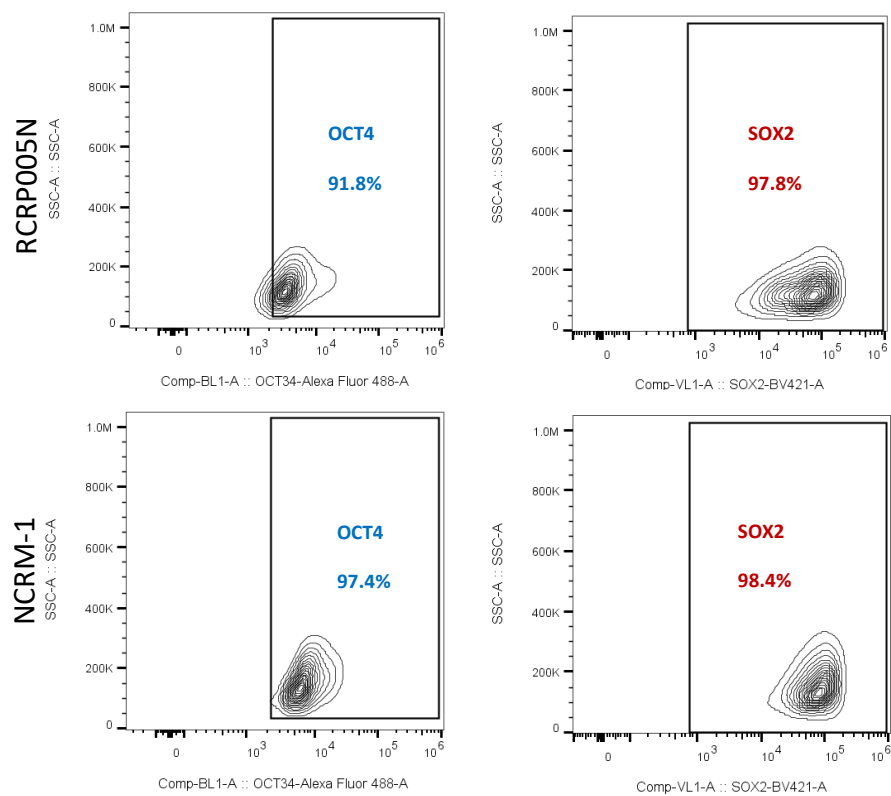
FMO SSEA4

FMO TRA-1-60

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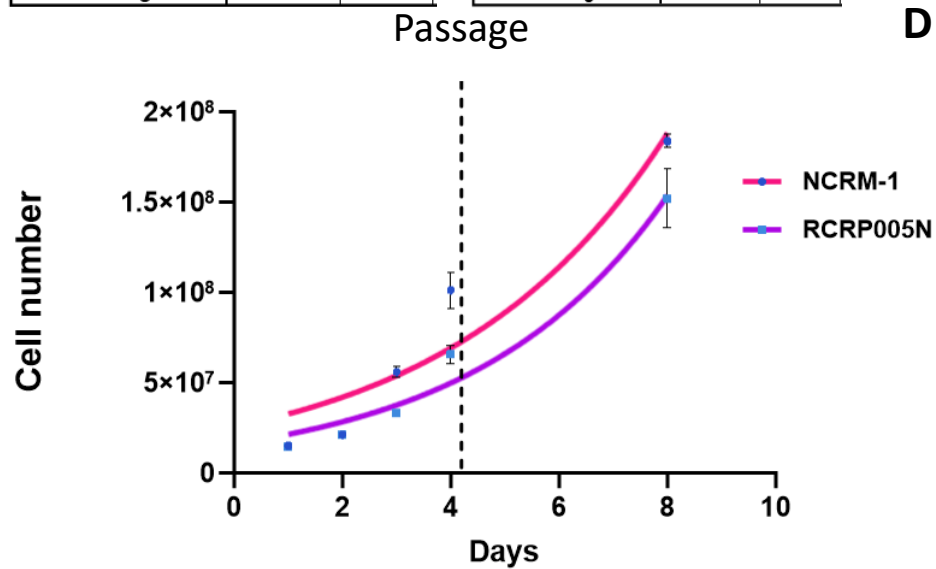
B



	CR0001	Repro
Doubling Time	1.025	1.222

	CR0001	Repro
Doubling Time	1.293	1.434

C



D

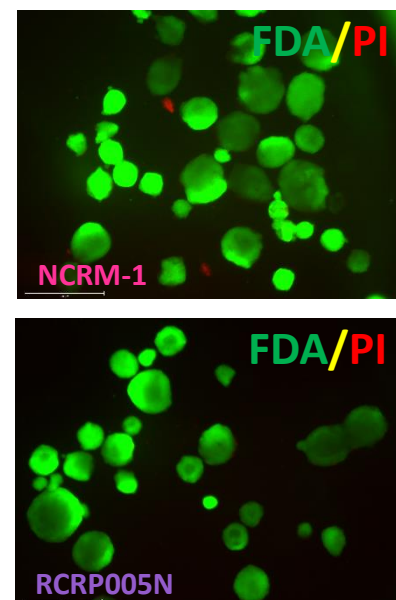


Figure S5: Validation with different cell line and passaging

- (A) Aggregate images throughout the culture assay on two cell lines
- (B) Flow Cytometry data on OCT4 and SOX2 intracellular markers on day 8 of culture
- (C) Measured growth curve before and after passaging
- (D) Live/Dead staining on day 8 of culture using Fluorescein diacetate (FDA) for live cells and Propidium iodide (PI) for dead cells.

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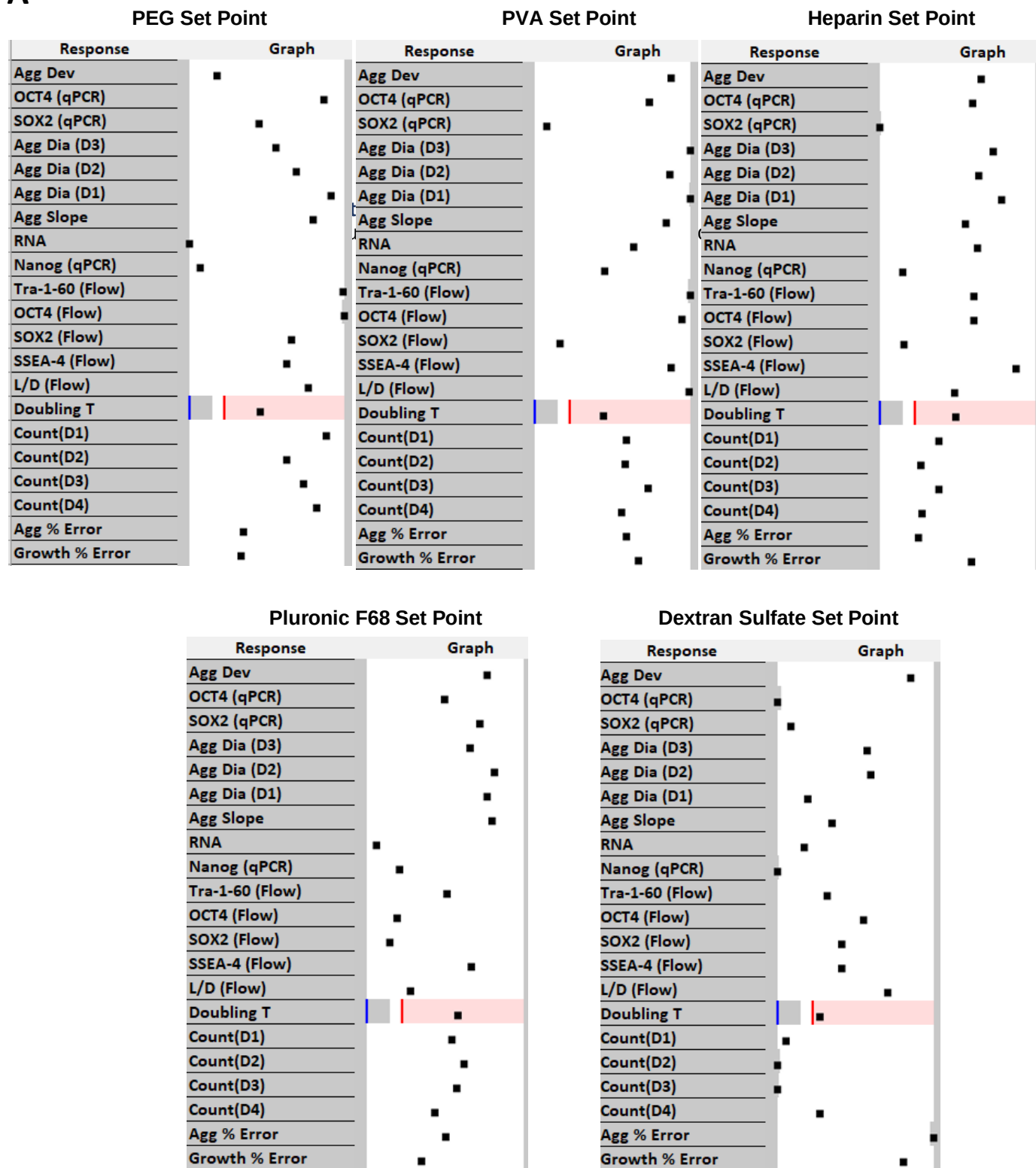


Figure S6: Individual Component Set Point Analysis

(B) Component set point analysis using MODDE showing individual effector impact on the responses measured.

Supplemental Tables:**Table S1: List of Primers used for qPCR.**

Gene	Forward Primer	Reverse Primer
Nanog	GGATGGTCTCGATCTCCTGA	CCTCCCAATCCCAAACAATA
SOX2	GCTACAGCATGATGCAGGACCA	TCTGCGAGCTGGTCATGGAGTT
POU5F1	GAACCAGTATCGAGAACCG	TCAGTTTGAATGCATGGGAG
GAPDH	TATCGTGGAAGGACTCATGACC	TAGAGGCAGGGATGATGTTCTG

Table S2: List of antibodies and reagents used.

ANTIBODIES	SOURCE	IDENTIFIER
POU5F1	Santa Cruz Biotechnology	Cat# sc-5279: RRID:AB_628051
SSEA4	DSHB	Cat#MC-813-70: RRID:AB_528477
SOX2	Abcam	Cat# ab5603: RRID:AB_304980
NANOG	Thermo Fisher Scientific	Cat# PA1-097: RRID:AB_2539867
488-Donkey-a-Mouse	Jackson Immuno Research	Cat# 715-546-151, RRID:AB_2340850
594-Donkey-a-Rabbit	Jackson Immuno Research	Cat# 711-586-152, RRID:AB_2340622
Alexa Fluor® 488 Mouse anti-Oct3/4	BD Biosciences	Cat# 560253, RRID:AB_1645304
BV605 Mouse Anti-SSEA-4	BD Biosciences	Cat# 563119, RRID:AB_2738015
Brilliant Violet 421™ anti-SOX2 Antibody	Biolegend	Cat# 656114, RRID:AB_2566262
Alexa Fluor® 647 Mouse anti-Human TRA-1-60 Antigen	BD Biosciences	Cat# 560850, RRID:AB_10565983
REAGENTS		
Essential 8™ Flex	ThermoFisher Scientific	A2858501
Vitronectin	Thermo Fisher Scientific	A31804
Y-27632 dihydrochloride	MedChem Express	HY-10583
DPBS (without calcium and magnesium)	Thermo Fisher Scientific	14190144
TrypLE	Thermo Fisher Scientific	12563-029
Accutase	Thermo Fisher Scientific	A1110501
CELL LINES		
RCRP5005N	REPROCELL	StemRNA™ Human iPSC 771-3G
NCRM-1	iXCells	CR0000001