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Review

The Role of Small Molecules and Their Effect on the Molecular Mechanisms of Early Retinal Organoid Development

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Supplementary Data

Gene	F/R	Sequence	Product BP
EEF1a	F	CAAAGCGACCCAAAGGTGGAT	219
	R	AAATAAGCGCCGGCTATGCC	
NANOG	F	TTTGGAAGCTGCTGGGGAAG	194
	R	GATGGGAGGAGGGGAGAGGA	
PAX6	F	AACAGACACAGCCCTCACAAACA	275
	R	CGGGAACTTGAACTGGAACTGAC	
RAX	F	GCGAAACTGTCAGAGGAGGAA	236
	R	ATGGAGGACACTTCCAGCTTC	
VSX2	F	CCATGCCTAAAGCCCATTGC	240
	R	TCTGGACTCCACTGATGGGT	
MITF	F	TTGCAACGAGAACAGCAACG	225
	R	ATGCTGAAGGAGGTCTTGGC	
ATOH7	F	CAGCCTGGTCATCCAGTAGAACA	100
	R	GAGCAAATAAGTCATAAACAAAGCAAC	

Supplementary Table S1. Primers used for RT-PCR analysis

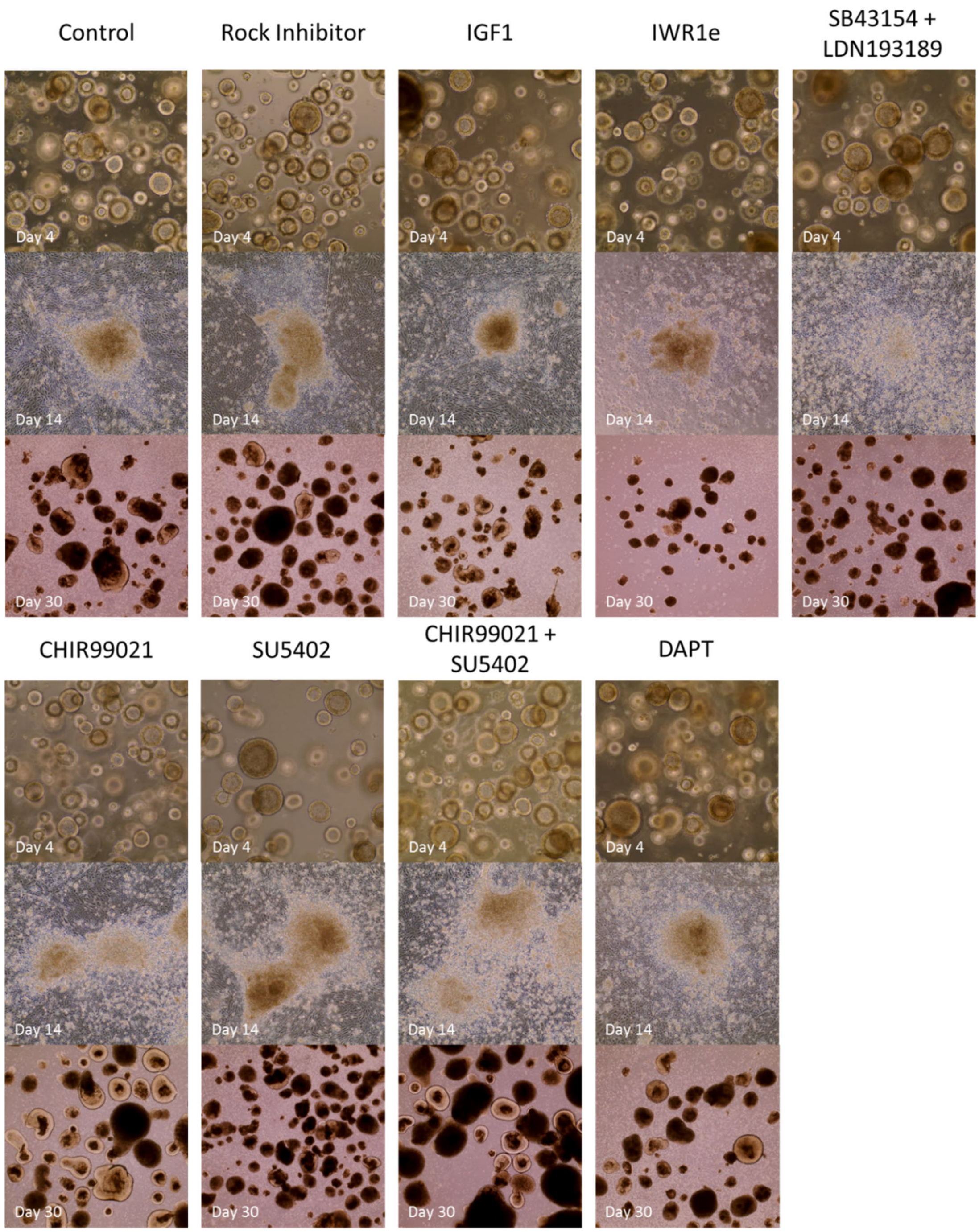
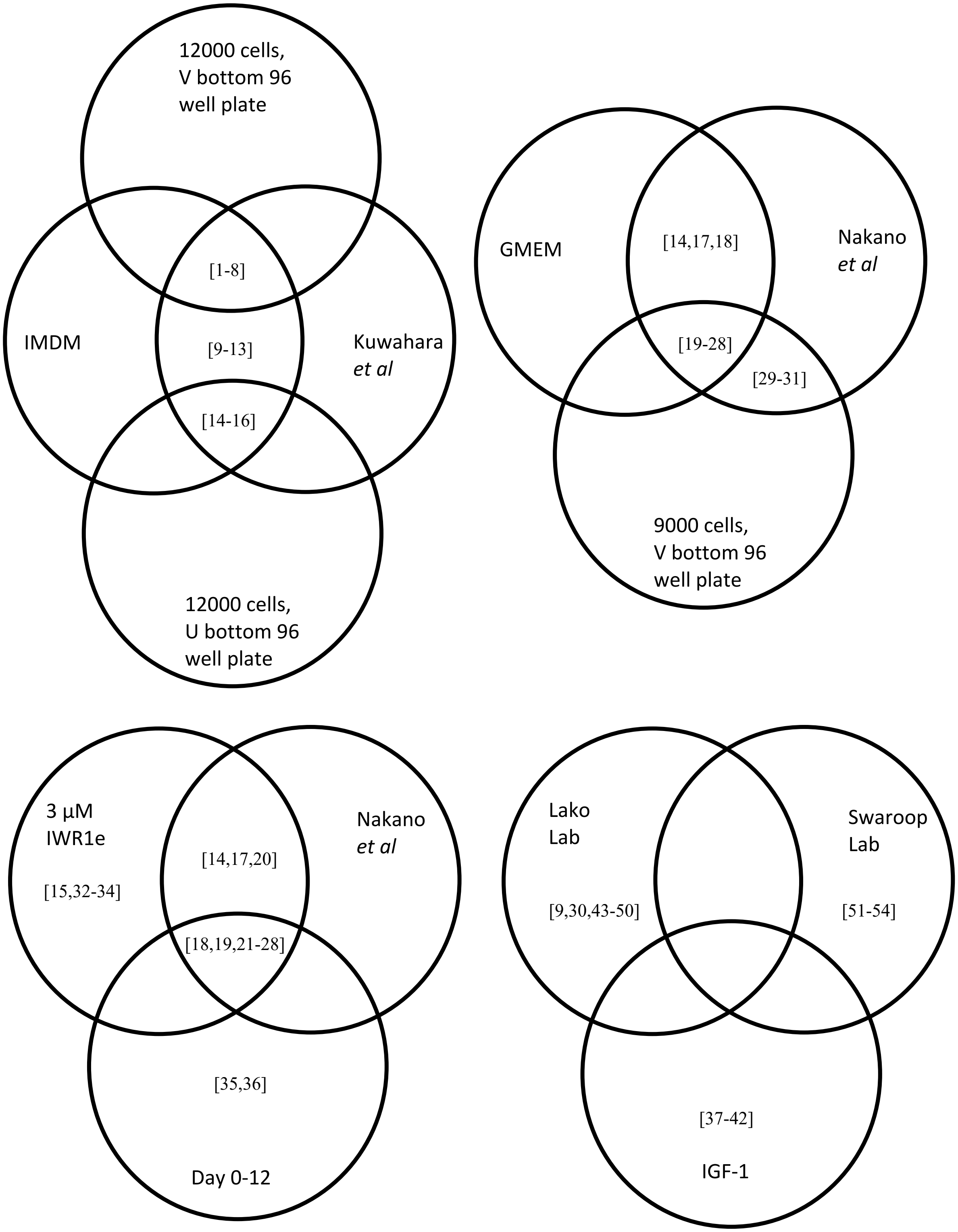


Figure S1. Retinal organoid development over time was analyzed for all conditions through brightfield microscopy. From left to right we present the 9 different conditions (Top: Control, Rock Inhibitor, IGF1, IWR1e, SB431542+LDN193189. Bottom: CHIR99021, SU5402, CHIR99021+SU5402, DAPT). These were analyzed by microscopy throughout development. All conditions were comparable by day 4, with some changes in Rock Inhibitor, IWR1e and SB431542+LDN193189 treated cells by day 14. At day 30, the differences in retinal organoid yield were apparent, with some conditions generating more retinal organoids than others.


12000 cells,
V bottom 96
well plate
IMDM
Kuwahara
et al
[1-8]
[9-13]
[14-16]
12000 cells,
U bottom 96
well plate
GMEM
Nakano
et al
[14,17,18]
[19-28]
[29-31]
9000 cells,
V bottom 96
well plate
3 µM
IWR1e
[15,32-34]
Nakano
et al
[14,17,20]
[18,19,21-28]
[35,36]
Day 0-12
Lako
Lab
[9,30,43-50]
Swaroop
Lab
[51-54]
[37-42]
IGF-1

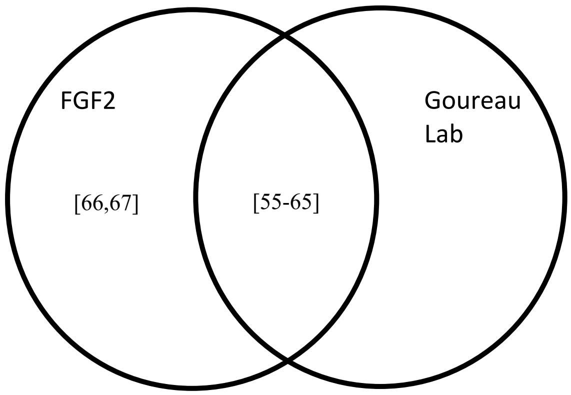


Figure S2. The specificity of certain methods and additions. A large variety of types and combinations of culture plates and medium were used in different studies, as indicated by the Venn diagrams above. Some plate-type and additions have been unique to a few studies or laboratories (FGF2 and IGF-1). For example, FGF2 and IGF-1 have been largely used only by the lab of Goureau (FGF2), and the labs of Lako and Swaroop (IGF-1). The use of other specific methods, such as the use of IMDM and GMEM, were introduced by Kuwahara *et al* and by Nakano *et al*. Subsequently, many groups followed-up using the same methods, making them become more wide-spread in the scientific community.

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