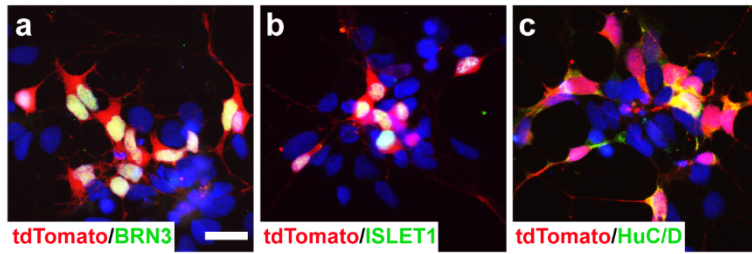


Three-Dimensional Retinal Organoids Facilitate the Investigation of Retinal Ganglion Cell Development, Organization and Neurite Outgrowth from Human Pluripotent Stem Cells

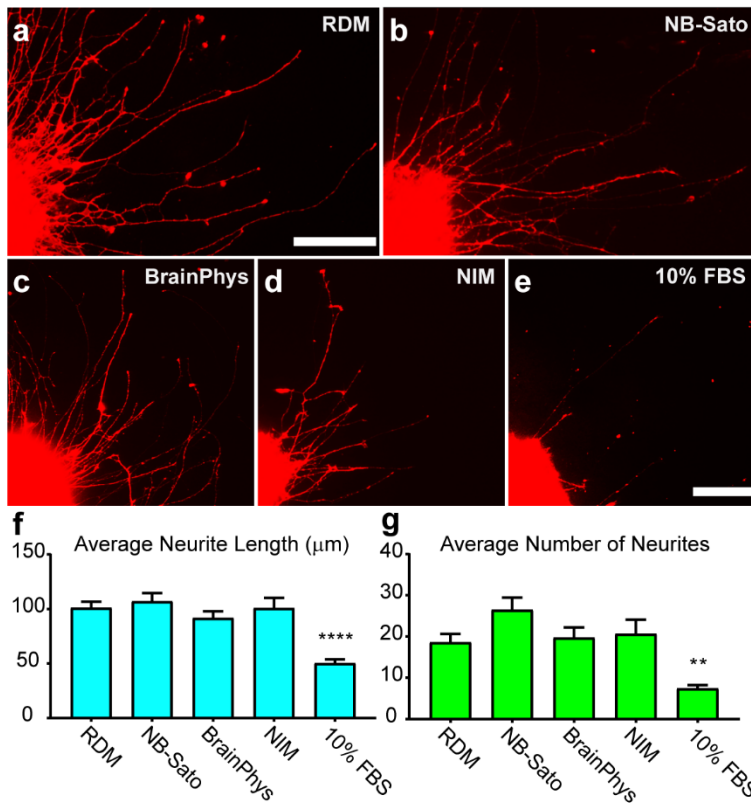
Clarisse M. Fligor¹, Kirstin B. Langer¹, Akshayalakshmi Sridhar^{1*}, Yuan Ren^{2,3}, Priya K. Shields¹, Michael C. Edler¹, Sarah K. Ohlemacher¹, Valentin M. Sluch⁴, Donald J. Zack⁴⁻⁷, Chi Zhang⁸, Daniel M. Suter^{2,3}, and Jason S. Meyer^{*1, 8, 9}

Supplementary Table S1. Primary antibodies used for immunocytochemistry.

Antibody	Species	Source	Catalog Number	Dilution
BRN3	Goat	Santa Cruz Biotechnology	SC-6026	1:200
CHX10	Goat	Santa Cruz Biotechnology	SC-21690	1:200
HuC/D	Mouse	Molecular Probes	A-21271	1:200
ISLET1	Mouse	DSHB	40.2D6	1:200
Ki-67	Mouse	BD Biosciences	550609	1:500
MAP2	Rabbit	Santa Cruz Biotechnology	SC-20172	1:200
mCHERRY	Rabbit	Invitrogen	PA5-34974	1:500
NeuN	Mouse	Millipore	MAP377	1:100
OTX2	Goat	R&D Systems	AF1979	1:2000
RECOVERIN	Rabbit	Millipore	AB5585	1:2000
SMI32	Mouse	Calbiochem	CBL171	1:100
TUJ1	Mouse	Sigma	T8660	1:800



Supplementary Figure S1. Identification of RGCs using a BRN3B:tdTomato reporter. tdTomato-expressing presumptive RGCs co-expressed characteristic markers including BRN3, ISLET1, and HuC/D. Scale bar: 20 μm .



Supplementary Figure S2. Optimizing culture media formulations for enhanced neurite outgrowth. (a-e) mCherry-positive RGCs were analyzed for neurite outgrowth in various culture media including RDM (n=51), NB-Sato (n=56), BrainPhys (n=60), NIM (n=38), and 10% FBS (n=47). (f-g) Results indicated that RDM, NB-Sato, BrainPhys, and NIM were all comparable for average neurite length and average neurite number, with only 10% FBS significantly lower than all others in both length and number. One-way ANOVA was used to compare media, **p<0.01, ****p<0.001. Error bars represent s.e.m. Scale bar: 100 μm .