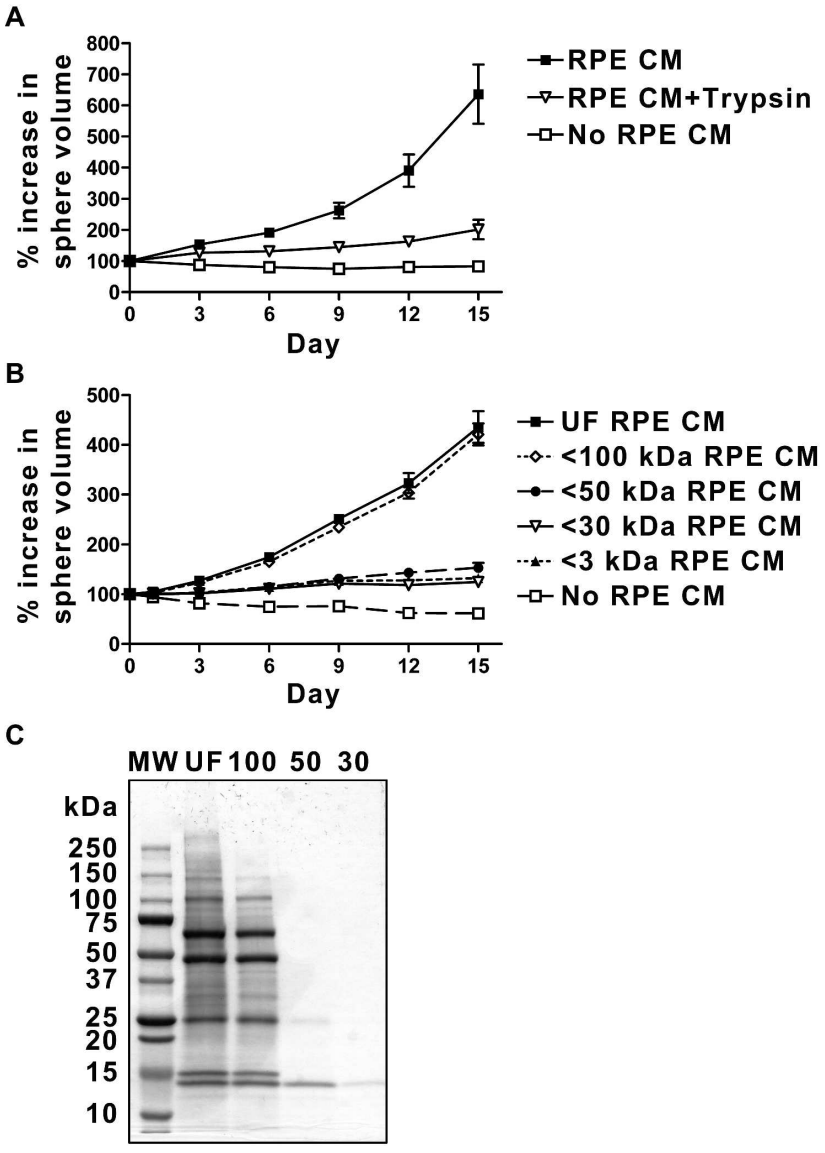
Gamm Supplemental Figure S1 Top



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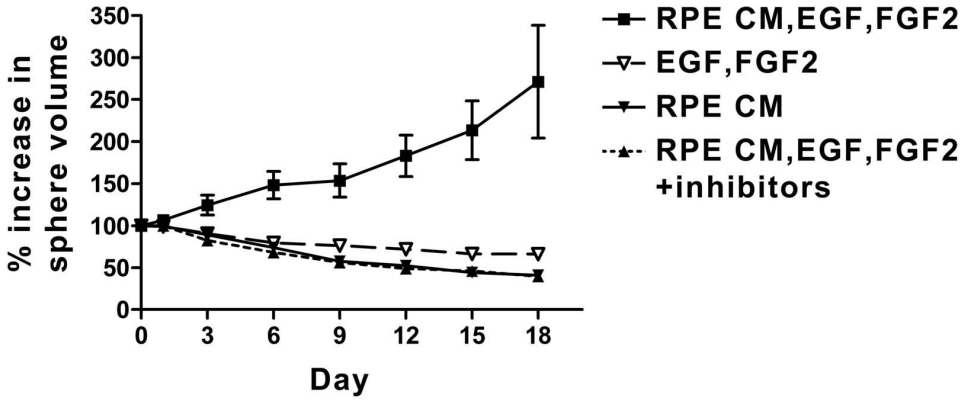
Gamm Supplemental Figure S2 Top



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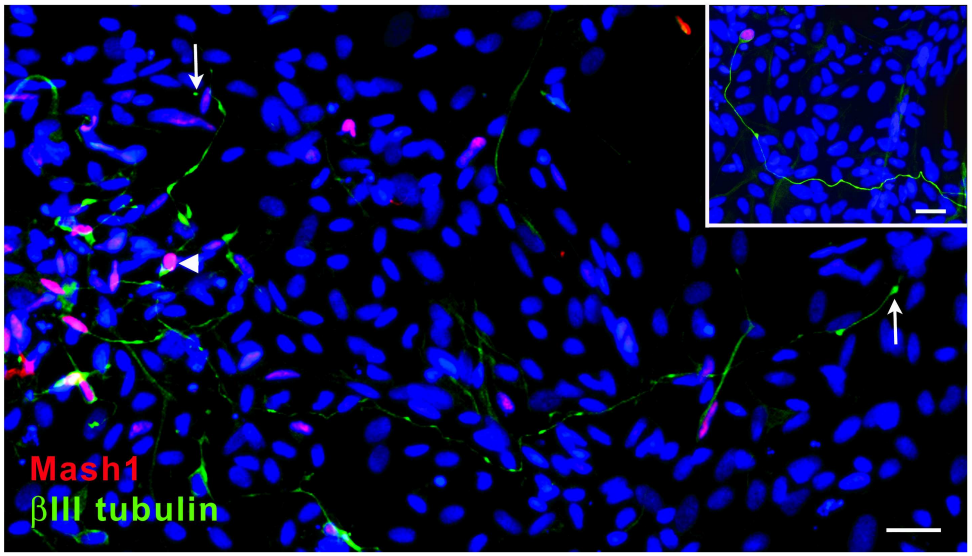
Gamm Supplemental Figure S3 Top



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Supplemental Figure Legends

Supplemental Figure S1. RPE CM contains proteinaceous factors necessary for retinal neurosphere growth. **(A)** Neurosphere growth assay comparing the effect of RPE CM to that of standard medium or trypsin-digested RPE CM. **(B)** Neurosphere growth assay comparing the effect of RPE CM fractions separated by molecular weight using size exclusion filtration. **(C)** Coomassie-stained SDS-PAGE comparing the protein bands present in unfractionated (UF) RPE CM versus fractionated RPE CM. MW: molecular weight standards.

Supplemental Figure S2. Inhibition of EGF receptor (EGFR) and FGF2 receptor (FGFR1) activity abolishes the mitogen-dependent growth effect of RPE CM. Neurosphere growth assay comparing the effects of RPE CM with mitogens (20 ng/ml EGF + 20 ng/ml FGF2) (*filled squares*), standard medium with mitogens (*open triangles*), RPE CM without mitogens (*downward filled triangles*), and RPE CM with mitogens and 10 μM EGFR and FGFR1 inhibitors (AG1517 and SU5402, respectively) (*upward filled triangles*).

Supplemental Figure S3. Occasional long projection neurons are generated after Mash1 infection. Photomicrograph of lenti-Mash1 infected cells maintained for 12 days in RPE CM and immunostained with anti-Mash1 (red) and anti-βIII tubulin (green). Nuclei were visualized with Hoechst dye. *Arrows* designate both end feet of one long projection neuron whose Mash1-positive nucleus is demarcated by an *arrowhead*. The relative

paucity of Mash1-positive nuclei in this culture is due to continued cell division of non-infected cells in RPE CM. Inset: photomicrograph of a second lenti-Mash1 infected long projection neuron. Scale bars = 20 μm.

Supplemental Table 1. *Primary antibodies used for immunocytochemistry.*

Antibody	Type	Source	Dilution
Calretinin	Goat polyclonal	Chemicon	1:2000
Doublecortin	Guinea pig polyclonal	Chemicon	1:300
GFAP	Rabbit polyclonal	Dako	1:1000
HuC/D	Mouse monoclonal	Molecular Probes	5 µg/ml
Ki67	Mouse monoclonal	BD Pharmingen	1:500
MAP2ab	Mouse monoclonal	Sigma-Aldrich	1:250
Mash1	Mouse monoclonal	BD Pharmingen	1:100
Nestin	Rabbit polyclonal	Chemicon	1:200
Parvalbumin	Mouse monoclonal	Chemicon	1:300
PhosphoCREB	Mouse monoclonal	Upstate	1:1500
PKCα	Rabbit polyclonal	Santa Cruz	1:50
Rx	Rabbit polyclonal	Abcam	1:1000
Recoverin	Rabbit polyclonal	Chemicon	1:2000
Sox2	Goat polyclonal	R and D Systems	1:500
βIII tubulin	Mouse monoclonal	Sigma-Aldrich	1:5000
βIII tubulin	Rabbit polyclonal	Covance	1:5000
vimentin	Mouse monoclonal	DSHB*	1:100

*Developmental Studies Hybridoma Bank

Supplemental Table 2. *Primers used for RT-PCR.*

Gene amplified	Forward	Reverse	Size (bp)
Sox1	CTC ACT TTC CTC CGC GTT GCT TCC	TGC CGT GGT CTT TGT CCT TCA TCC	848
Sox2	CCC CCG GCG GCA ATA GCA	TCG GCG CCG GGG AGA TAC AT	448
Dlx1	GGC AAG GCG GTG TTT ATG	ATG TAG GGG CTG GAT GCG TG	207
Dlx2	AGG ACC TTG AGC CTG AAA TTC GGA	ACA TCT TCT TGA ACT TGG ACC GGC	225
Pax6	CCA GGC AGA GCC AGC ATG CAG AAC A	GGT TGG TAG ACA CTG GTG CTG AAA CT	949
Six6	ATT TGG GAC GGC GAA CAG AAG ACA	ATC CTG GAT GGG CAA CTC AGA TGT	385
Hes1	TCT GAA GAA AGA TAG CTC GCG GCA	ATT GAT CTG GGT CAT GCA GTT GGC	262
Rx	TCA GAG GAG GAA CAG CCC AAG AAA	TCA TGG AGG ACA CTT CCA GCT TCT	229

Supplemental Table 3. *Primers used for quantitative RT-PCR.*

Gene amplified	Forward	Reverse	Size (bp)
EGFR	AAC TGT GAG GTG GTC CTT GGG AAT	ACT GTG TTG AGG GCA ATG AGG ACA	119
FGFR1	AGG ATC GAG CTC ACT GTG GAG TAT	CTC CAC ATC CCA GTT CTG CAG TTA	80
β-Actin	GCG AGA AGA TGA CCC AGA TC	CCA GTG GTA CGG CCA GAG G	102

EGFR, epithelial growth factor receptor (recognizes all isoforms); FGFR1, fibroblast growth factor receptor 1.

Supplemental Methods

Trypsin treatment of RPE CM. RPE CM was treated with 0.5% Trypsin-EDTA solution (Sigma-Aldrich) for 2 hours at 37ºC on a rotating platform. Thereafter, an excess of soybean trypsin inhibitor (Sigma-Aldrich) was added and the solution was incubated for an additional hour at 37ºC. Control samples of RPE CM and standard medium were treated in an identical manner with the exception that no trypsin was added. Following treatment, all samples were supplemented with 2% B27, 20 ng/ml EGF, 20 ng/ml FGF2 and 5 μg/ml heparin prior to being used in neurosphere growth assays

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Size exclusion filtration of RPE CM. Standard medium conditioned for 24 hr by RPE was collected and 1:200 protease inhibitor cocktail (Sigma-Aldrich) was added prior to fractionation. RPE CM was then fractionated using Amicon Centriplus Centrifugal Filter Devices (Millipore, Billerica, MA) with 100-, 50-, 30-, or 3-kDa cutoffs according to the manufacturer's instructions. Filtrates were supplemented with 2% B27, 20 ng/ml EGF, 20 ng/ml FGF2 and 5 μg/ml heparin before being used in neurosphere growth assays. For SDS-PAGE, the filtrate was concentrated (80-fold) using a 3-kDa cutoff centrifugal filter and total protein in the retentate was quantified using the DC Protein Assay (Bio-Rad).

SDS-PAGE. Twenty micrograms of protein from concentrated RPE CM were separated on 4-20% gradient Tris-Cl gels (Bio-Rad). The gels were either stained with Coomassie

Blue or silver stained using the Plus One Silver Staining® kit (Amersham Biosciences, Piscataway, NJ) according to the manufacturer's instructions.

Cloning of human Mash1. RNA was isolated from human fetal tissue by Trizol extraction and reverse transcribed using the Superscript III First Strand® synthesis kit (Invitrogen). The full length cDNA for human Mash1 was amplified using a specific primer set (forward primer: 5'-CGTCCCGGATCGCTCTGA-3'; reverse primer: 5'-GGCATGCCTCGCTTAGTTG-3') and the Expand High Fidelity PCR® kit and buffer system 1 (Roche). The amplified fragment corresponding to human Mash1 was cloned into the SIN-W-PGK lentiviral vector [44] and sequenced prior to lentivirus production.

Titration of lentivirus suspensions. The p24 content of the viral suspension was measured using an enzyme-linked immunosorbent assay (PerkinElmer Life Sciences, MA, USA). To determine viral infectivity, 6 wells containing $5x10^4$ HeLa cells were transduced with a suspension volume containing 3.3 and 10 ng of p24. On day 8 post-infection, genomic DNA was extracted from the expanded cell population using a DNeasy Blood & Tissue kit (Qiagen, CA, USA) and the number of integrated copies was measured by TaqMan quantitative PCR on a Rotor-Gene™ 2000 instrument (Corbett Life Science, Australia). The following primers specific for the WPRE sequence and the human albumin gene were used to determine the number of integrated lentiviral genomes per human genome, respectively: WPRE forward: 5'-CCGTTGTCAGGCAACGTG-3'; WPRE reverse: 5'-AGCTGACAGGTGGTGGCAAT-3'; WPRE probe: 5'-FAM-TGCTGACGCAACCCCCACTGGT-TAMRA-3'; Albumin forward: 5'-

TGAAACATACGTTCCCAAAGAGTTT-3'; Albumin reverse: 5'-CTCTCCTTCTCAGAAAGTGTGCATAT-3'; Albumin probe: 5'-FAM-TGCTGAAACATTCACCTTCCATGCAGA-TAMRA-3'. Titers obtained for the Mash1 lentivirus suspensions were 2,000 to 7,500 p24 ng per ml and measured infectivity was in the range of 10 to 15 transducing units per p24 pg.