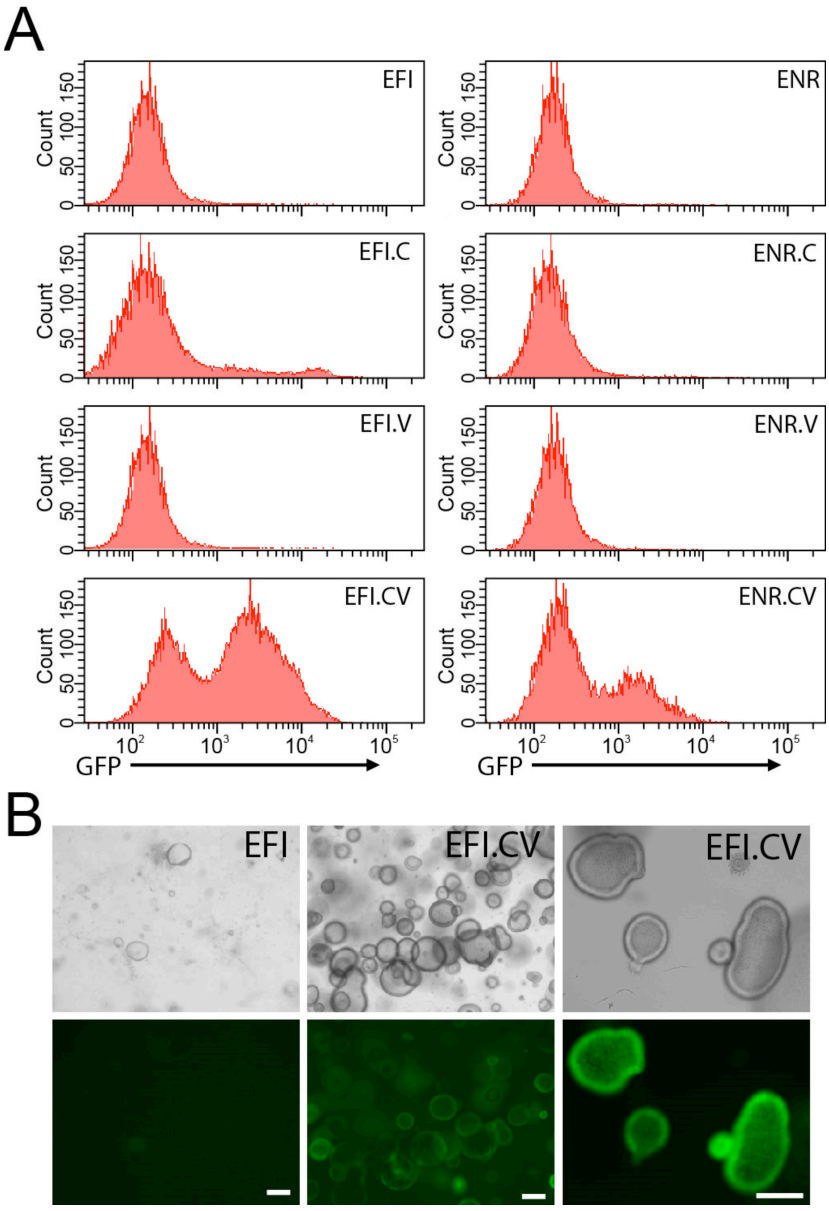
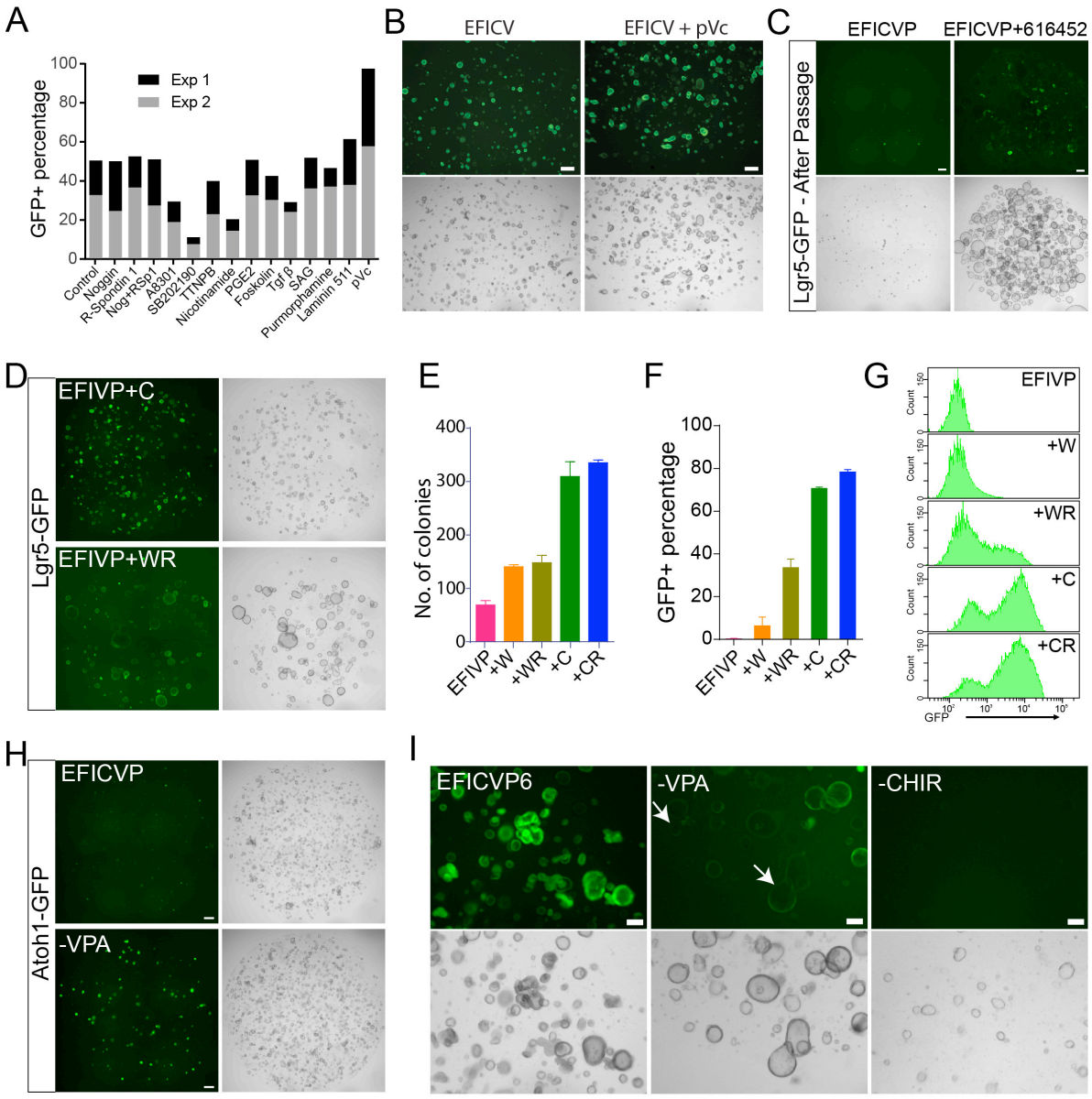
SUPPLEMENTAL INFORMATION



Supplemental Figure 1. Related to Figure 2. Wnt Activation Combined with HDAC Inhibition Increased the Number of Inner Ear Lgr5+ Cells in Culture

A) FACS histograms showing GFP expression of isolated single Lgr5-GFP inner ear progenitor cells cultured for 7 days under multiple conditions. E: EGF. F: bFGF. I: IGF1. W: Wnt3a. R: R-Spondin 1. C: CHIR99021. V: VPA. N: Noggin. n>3 experiments.

B) GFP fluorescence and bright-field images of Lgr5-GFP inner ear progenitor cells cultured with or without CHIR and VPA. Scale bars: 100 μm. n>3 experiments.



Supplemental Figure 2. Related to Figure 2. Small Molecules Increased the Number and Purity of Lgr5+ Cells and Enabled Passaging

A) Screen for supportive factors for inner ear progenitor cells. The percentage of Lgr5-GFP inner ear progenitor cells cultured in multiple conditions is shown. Small molecules were added based on the control (EFICV) condition. The percentage of Lgr5-GFP cells is shown. Laminin was added to Matrigel. Two batches of cells were used for screening as shown as Exp 1 and Exp 2.

B) GFP fluorescence and bright-field images of Lgr5-GFP inner ear progenitor cells cultured for 7 days in EFICV, with or without the addition of 2-phospho-L-ascorbic acid (pVc, P). Scale bars: 400 μm.

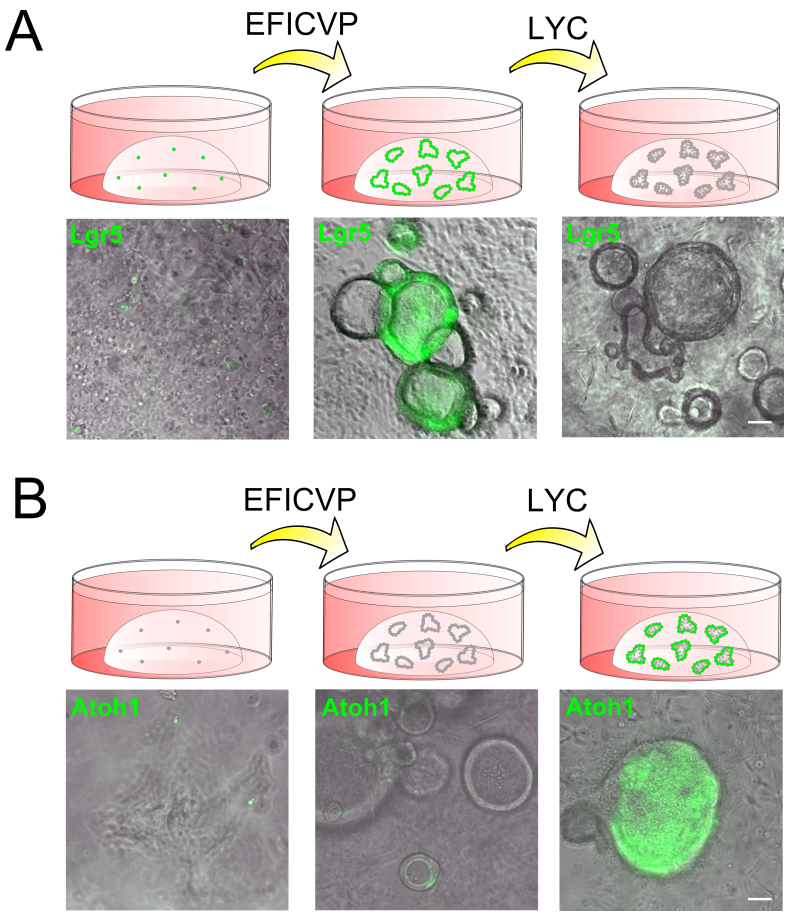
C) Fluorescence and bright-field images of Lgr5-GFP cells at day 10 of passage 2 in EFICVP, with or without the addition of 616452. The addition of 616452 permitted the passage of cultured Lgr5-GFP cells. Scale bars: 400 μm.

D) Lgr5-GFP expression of inner ear progenitor cells cultured for 7 days with Wnt pathway activators. EFIVP was added in all conditions. W: Wnt3a. R: R-Spondin 1. C: CHIR99021.

E, F, G) Number of colonies (E), percentage of Lgr5-GFP cells (F), and FACS histogram (G) of cultures obtained with equal numbers of Lgr5-GFP cells after 7 days. EFIVP was added in all conditions. W, Wnt3a, R, R-Spondin 1. C: CHIR99021. n=2 duplicated wells.

H) GFP fluorescence and bright-field images of progenitor cells isolated from Atoh1-nGFP+ mice and cultured for 7 days in EFICVP with or without VPA. Scale bars: 400 μm.

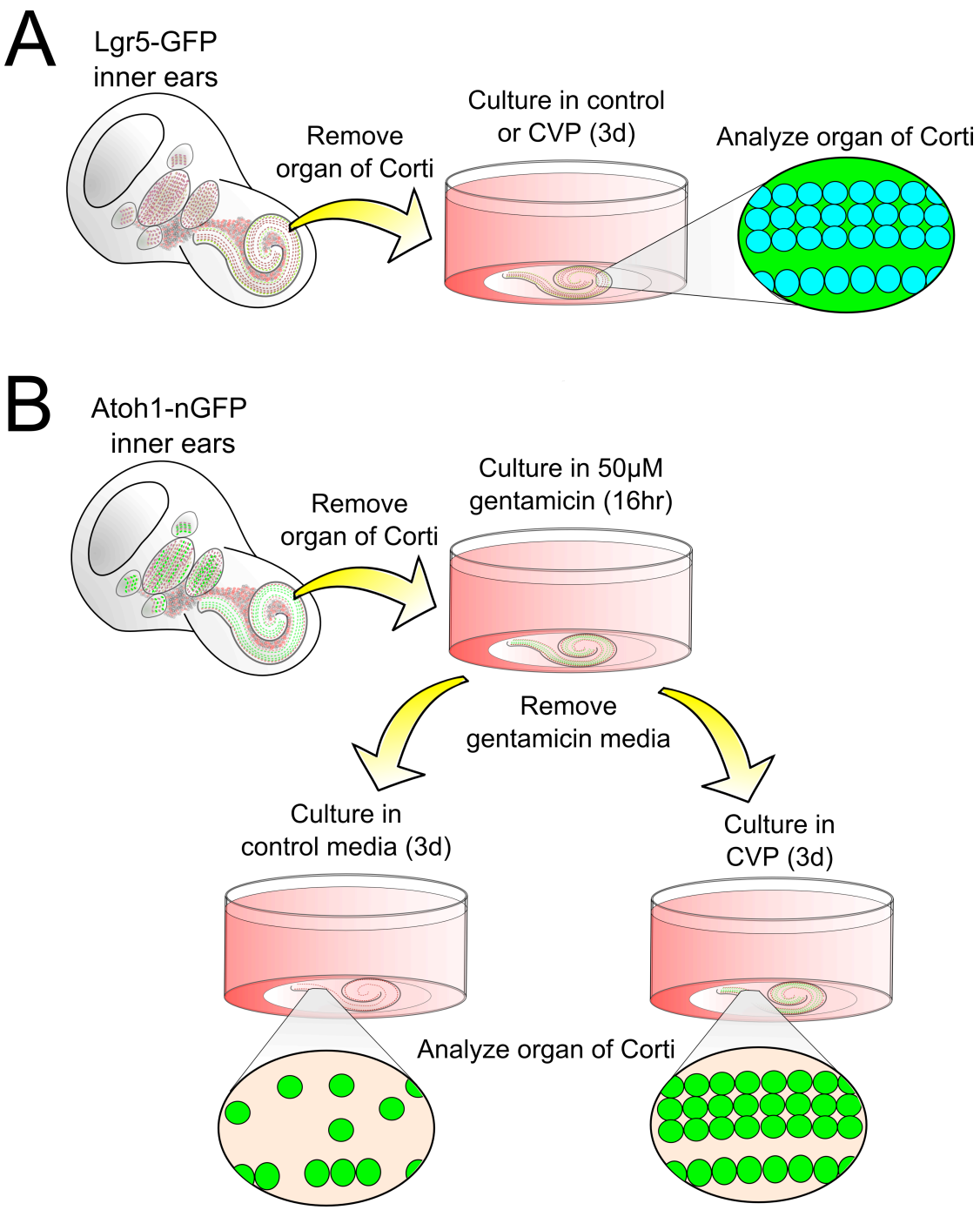
I) GFP fluorescence and bright-field images of Lgr5-GFP inner ear progenitor cells cultured for 10 days in the indicated conditions. Arrows indicate colonies with diminished Lgr5-GFP expression. Scale bars: 200 μm.



Supplemental Figure 3. Related to Figure 4. Wnt and Notch Pathway Manipulation Promoted the Proliferation of Supporting Cells and their Conversion into Hair Cells

A) Cultures were subjected to expansion for ~10 days (EFICVP), followed by differentiation for ~10 days (LYC). The expansion protocol resulted in large increases in Lgr5+ cells. Lgr5 expression was halted by the differentiation protocol. n = 24. Scale bar: 50 μm.

B) Atoh1-nGFP+ cells did not proliferate during expansion but were obtained after the treatment used for differentiation. n = 24. Scale bar: 50 μm.



Supplemental Figure 4. Related to Figure 7. Methods for Cochlear Explant Experiments

A) Methods used for the treatment of intact cochlear explants.

B) Methods used for the treatment of hair cell-damaged cochlear explants.

Supplemental Video 1. Related to Figure 5. Progenitor cells treated with CHIR and LY411575

Confocal scanning after drug treatment shows nearly complete conversion of Lgr5+ cells in the organoid to myosin VIIa+ cells (cyan) with actin-rich protrusions (red).

Supplemental Table 1. Related to Figures 3-7. Growth Factors and Small Molecules

Reagent Name	Abbreviation	Final concentration	Vendor
EGF	E	50 ng/ml	Life Technologies
bFGF	F	50 ng/ml	Life Technologies
IGF1	I	50 ng/ml	Life Technologies
CHIR99021	CHIR/C	3 μM	LC Labs
Valproic Acid Sodium Salt	VPA/V	1 mM	Sigma
2-phospho-L-ascorbic acid	pVc/P	100 μg/ml	Sigma
616452	6	2 μM	Calbiochem
LY411575	L	5 μM	Sigma

Supplemental Table 2. Related to Figures 3-7. Antibodies

Primary antibody	Dilution	Source	Vendor
Myosin VIIa	1:500	Rabbit Polyclonal	Proteus Biosciences
Sox2	1:300	Goat polyclonal	Santa Cruz
vGlut3	1:1000	Guinea Pig polyclonal	Chemicon
Prestin	1:400	Goat Polyclonal	Santa Cruz
Phalloidin	1:100	Fluorescent labeled toxin	Invitrogen
CtBP2	1:100	Mouse monoclonal	BD Biosciences
FM1-43FX	5μM	Fluorescent dye	Life Technologies

Supplemental Table 3. Related to Figure 5. Primers

Gene	Forward primer	Reverse primer
Myo7a	GGAGGCCATCCAACATAAGA	CGGAAGACTTGAGCAGCAG
Lgr5	TCTCCTACATCGCCTCTGCT	GCACTTTGAGGCTGTGAAGG
Cdh23	ATCATCACGGACATGCAAGA	TCCCTTATCCTGGTCCACAG
Pcdh15	CCCAGGACTGCAGAACTCAC	TCGTCTAGTATTTCGATGTGC
Myo1c	TGGAGAAGTTGGAGGACACTG	TGTAGGTCACCTCTCCAGCA
Tmc1	CTGTCCCACCCTGTTTGACT	TCACGAAACATGCTCTGAGG
Cav1.3	AAGGGCTACCTGGACTGGAT	CCACACACCACAAAGCAATC
Ribeye	GCACCTCTTGGAGACAGCA	CTACCCCAGCTTGTGAAGGA
Prestin	ACAGTGTGGATGTCGTTGGA	CAGGTTGACGATCACAATGG
Oncomodulin	TTCTGAGCGCTGATGACATT	TGGCAGACATCTTGGAGAGG
vGlut3	TGGACCTTCTATTTGCTCCTG	GCACCACGATTGTCATCACC
Chrna9	GGAACCAGGTGGACATATTCAAT	GCAGCCGTAGGAGATGACG