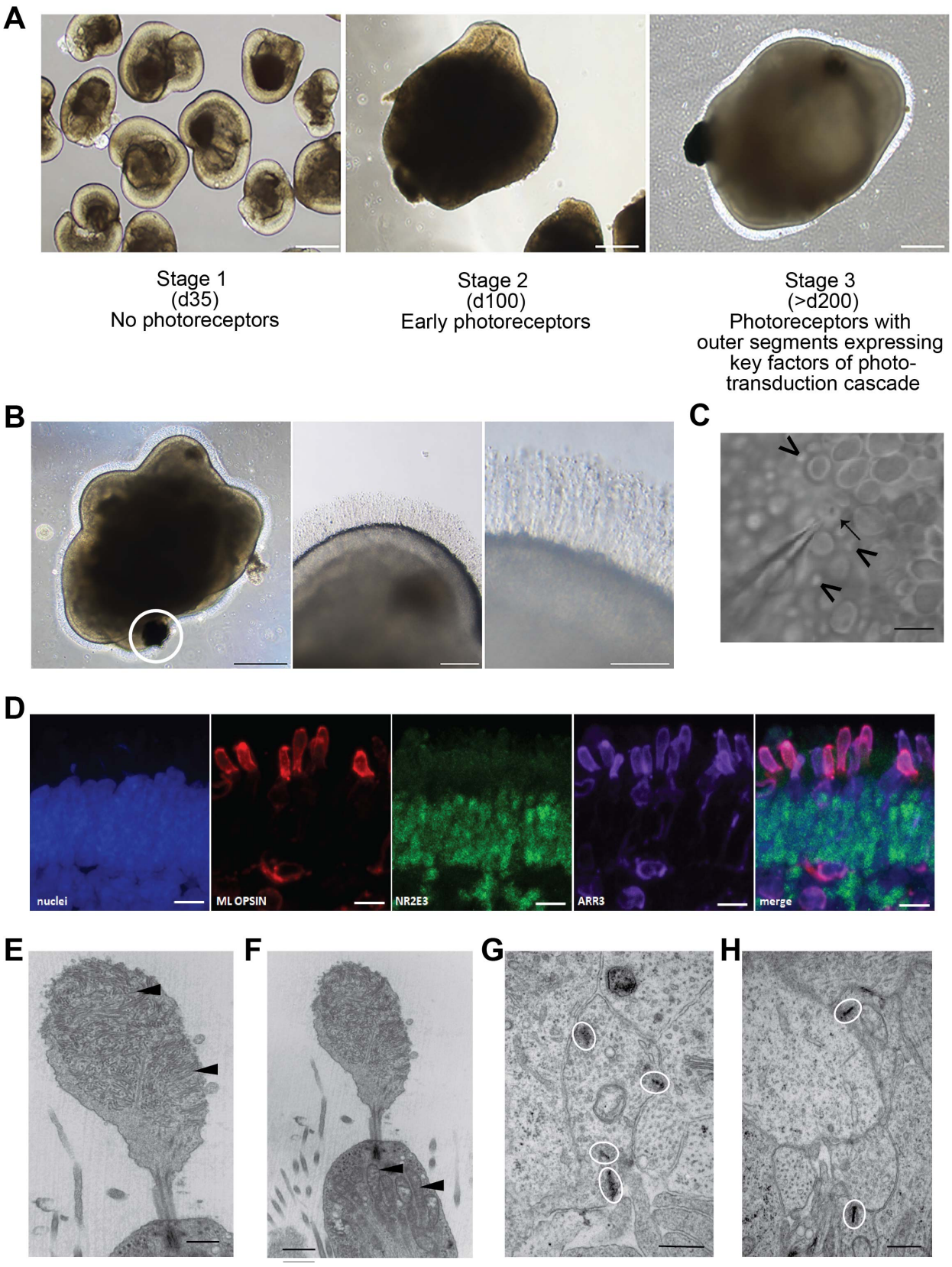

Figure S1. Developmental timeline, photoreceptor density, morphology and ultrastructure of hPSC-retinal organoids, related to Figure 1.

(A) Stage 1, 2 and 3 hPSC-derived ROs relative to timeline of differentiation (scale bar: 250 μm). (B) Stage 3 RO with photoreceptor outer segments and small patch of RPE (circle; left panel). Scale bars: left - 250

μm; center and right – 25 μm. (C) A cone photoreceptor in a stage 3 RO with a patch pipette attached to the inner segment. Arrow pointing to an inner segment of a cone and arrowheads pointing to the smaller inner segments of rods. Scale bar – 6 μm. (D) Confocal image illustrating the 4:1 rod to cone ratio seen in ROs used for this study. blue, nuclei; red, cone marker; ML-Opsin, green, rod marker NR2E3; violet, cone marker ARR3; Merged. Scale bar: 10 μm. (E) Electron micrograph of an outer segment of a stage 3 organoid showing partially stacked disks (black arrowheads) and connecting cilium. Scale bar: 0.5 μm. (F) Lower magnification electron micrograph of the outer segment showing the attached mitochondria rich inner segment (black arrowheads) of the stage 3 organoid cone. Scale bar: 0.8 μm. (G, H) Electron micrographs demonstrating ribbon synapses at photoreceptor terminals in a stage 3 organoid (marked by white ellipses). Scale bar: 0.5 μm.

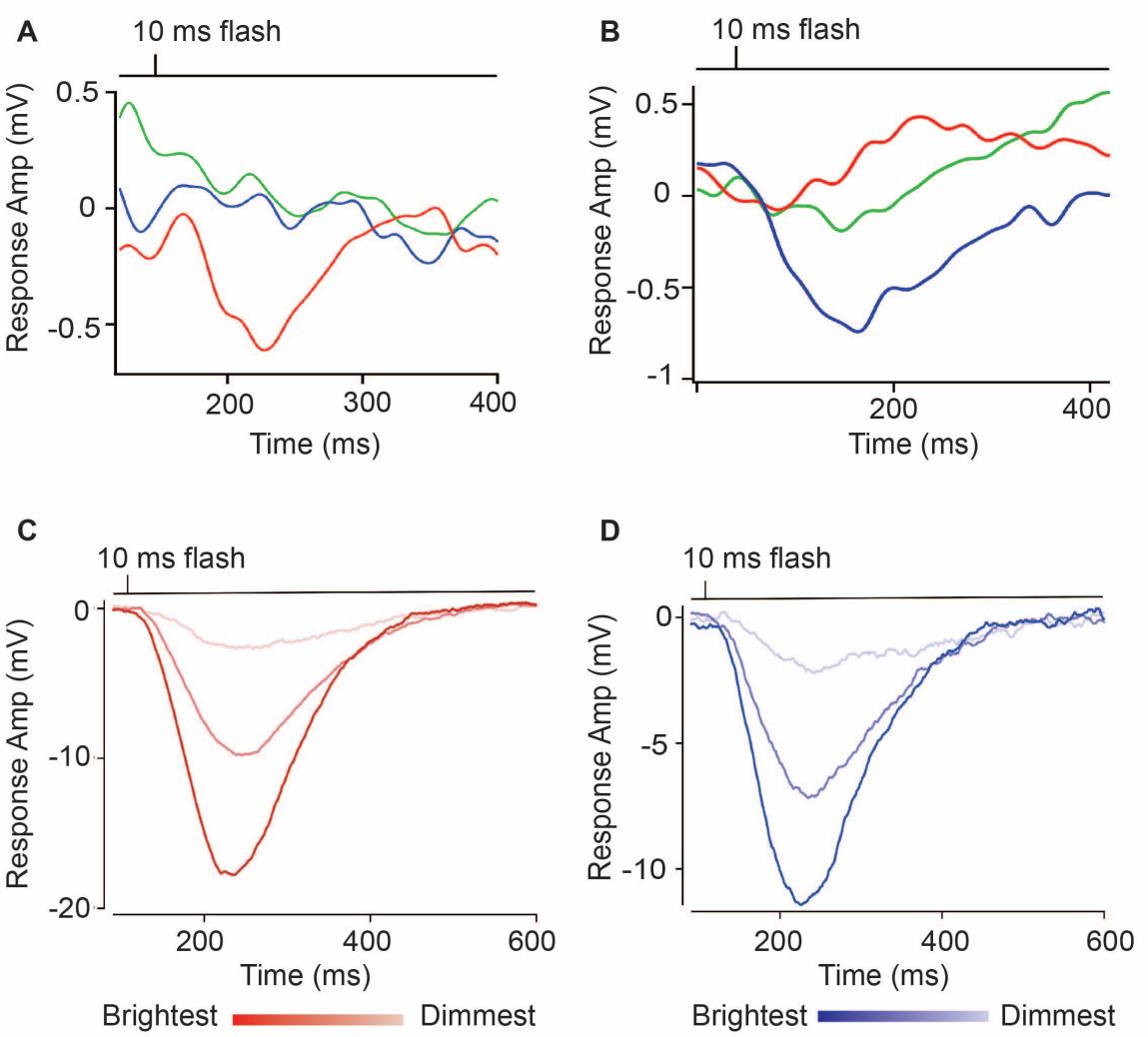

Figure S2. Wavelength-specific light responses of organoid cones, related to Figure 1.

(A, B) Typing flash responses (presented at time = 100ms) of a long wavelength sensitive cone (A) and a short wavelength sensitive cone (B) smoothened by using a binary smoothing function sampling over 10,000 points on Igor Pro 8.0. (C, D) Average voltage response of a long wavelength-sensitive (red; C) and a short wavelength-sensitive (blue; D) cone to increasing intensity (dimmest – 50,000 R*/cone/sec to brightest – 500,000 R*/cone/sec) of a 10 ms light flash (presented at time = 100 ms).

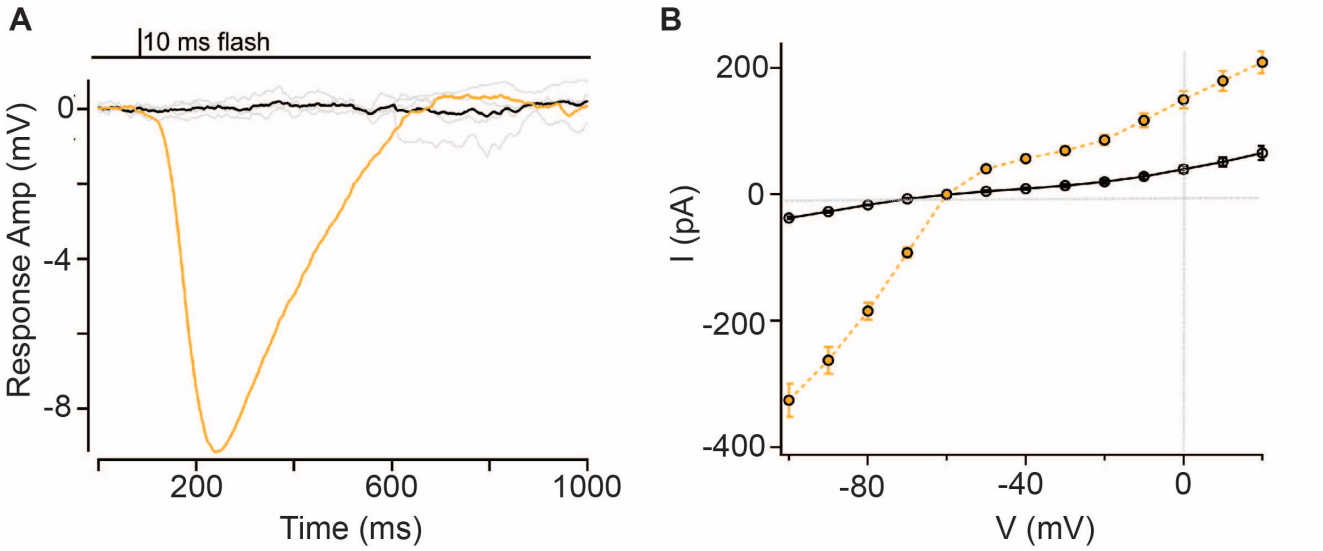

Figure S3. Light-induced responses and current-voltage relationship of organoid rods, related to Fig 1.

(A) Average voltage response of four stage 3 organoid rod photoreceptors (black, average response; grey, individual responses) and an organoid cone (yellow, average response) to a brief (10ms) bright flash of light. (B) Current voltage curve at plateau amplitude for RO rods (black, n=5) and cones (yellow, n=15).

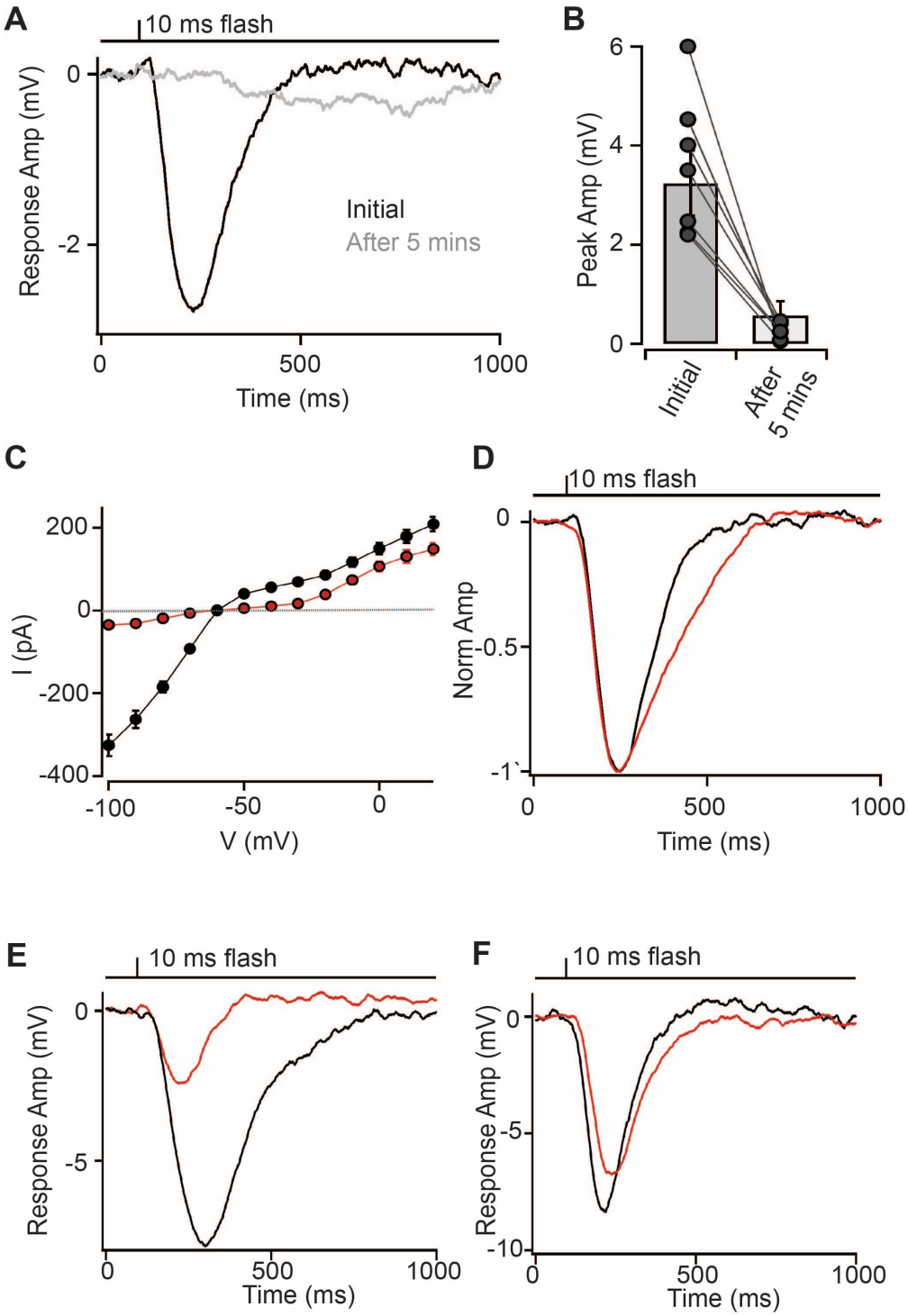

Figure S4. Role of gap-junctional coupling and HCN channels in shaping light-evoked responses of organoid cones, related to Figures 2,3.

(A) Voltage response of an exemplar RO cone, with an intracellular solution lacking ATP and GTP, to a 10 ms brief flash of light (~500,000 R*/cone/s) soon after breaking into the cell (black trace) and 5 mins after (grey trace). (B) Response amplitude was reduced by ~90% (n=6) 5 mins after breaking into the cell. Thus, gap junctional coupling minimally contributes to RO cone responses. (C) Current-voltage curve for plateau current for RO cones before (black) and after (red) application of ZD7288, a specific HCN channel blocker.

(D) Average normalized responses (n=5) to bright light flash before (black) and after (red) application of ZD7288, an HCN channel blocker, to stage 3 RO cones. (E, F) Individual exemplar light responses of stage 3 RO cones before (black) and after (red) blocking HCN channels.

Table S1: Human pluripotent stem cell lines, related to all figures

line	hPSC	gender	rods	cones	% rods	% cones	reference	# of organoids
1013	iPSC	Male	1485	518	74.1	25.8	(Capowski et al., 2019)	3
1581	iPSC	Male	1762	426	80.5	19.4	(Kallman et al., 2020)	2
WA09	hESC	Female	1045	251	80.6	19.4	(Capowski et al., 2019)	14
2429	iPSC	Male	933	246	79.1	20.9		4

Table S2: Quantification of parameters for light responses in Fig 1 and 2.

Parameter	Organoid cone			Primate foveal cone		
	Mean	SEM	n	Mean	SEM	n
Typing flash – peak amplitude	2.512 mV	0.448 mV	27	9.372 mV	0.562 mV	14
Typing flash – time to peak	127.567 ms	9.689 ms	27	62.193 ms	2.773 ms	14
5000 R* background – time to peak	203.753 ms	21.148 ms	15	34.796 ms	1.728 ms	7
5000 R* background – decay time	88.530 ms	8.401 ms	15	20.666 ms	1.035 ms	7
5000 R* background – FWHM	229.505 ms	20.481 ms	15	47.689 ms	1.775 ms	7

Table S3: Quantification of passive membrane properties in Fig 3.

Parameter	Organoid cone			Primate foveal cone		
	Mean	SEM	n	Mean	SEM	n
Resting membrane potential	-34 mV	2.809 mV	19	-51.5 mV	2.703 mV	9
Membrane time constant	1.615 ms	0.097 ms	14	1.051 ms	0.091 ms	8
Input Resistance	35.1 MΩ	2.055 MΩ	10	30.913 MΩ	1.990 MΩ	8
Membrane Capacitance	37.933 pF	4.572 pF	15	38.129 pF	3.765 pF	7