

### Supplemental Figure 1. Additional stimuli for probing feature selectivity of RGCs.

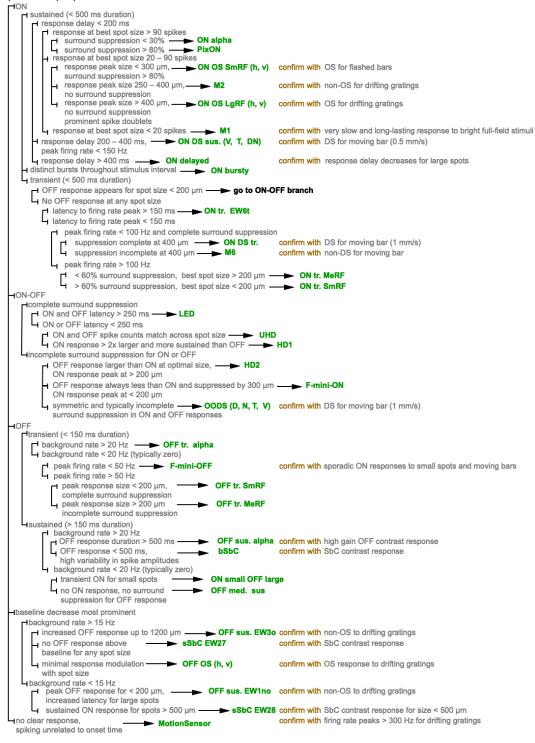
(A) Polar plot of spike responses to moving bar stimuli for an ON-OFF DS Temporal RGC (top) and an ON DS sustained Ventral RGC (bottom). Arrows in the lower left corner show dorsal (D), ventral (V), nasal (N), and temporal (T) directions on the retina. See **Methods** for parameters pertaining to each of the visual stimuli.

(**B**) Polar plot of spike responses to drifting grating stimuli for Horizontal (top) and Vertical (bottom) OFF OS RGCs. Note, angles on polar plots correspond to grating movement direction which is orthogonal to bar orientation.

(C) Spike responses for bars flashed at different orientations for a Horizontal (top) and Vertical (bottom) ON OS SmRF RGC.

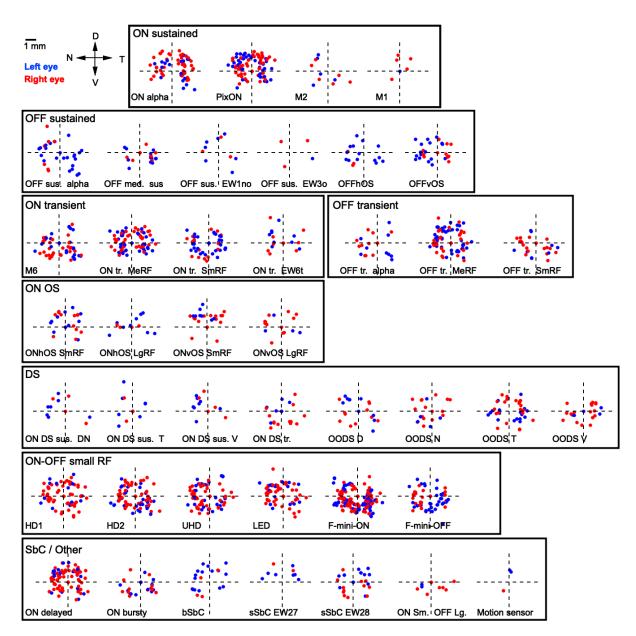
(**D**) Baseline subtracted spike responses for contrast series in two different subtypes of suppress-by-contrast RGCs, the bSbC (top), and the sSbC EW28 (bottom).

#### Response to 200 µm spot



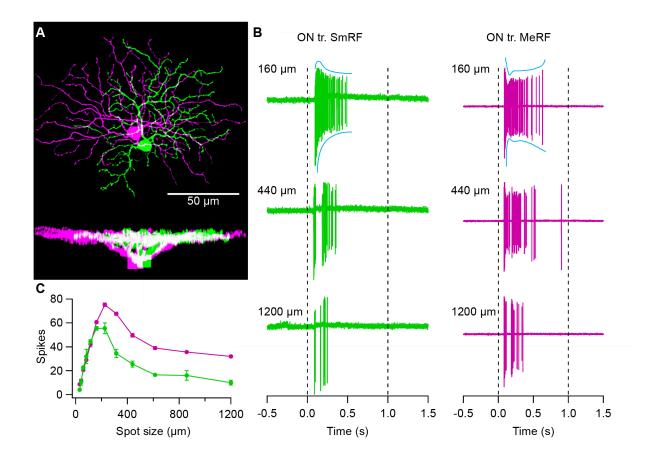
#### Supplemental Figure 2. Decision tree for functional classification.

Branches from each decision point are indicated by connected lines. Arrows indicate terminal decision points to RGC types, marked in green text. Decisions are based solely on data from flashed spot stimuli (as in **Figure 2**), but confirmatory tests using additional stimuli are listed following "confirm with" in brown text.



## Supplemental Figure 3. Position map of physiologically-typed RGCs.

The location of physiologically-typed RGCs are plotted in absolute retinal space, with (0,0) representing the optic nerve. Typology was consistent across retinal space and from both left (blue) and right (red) eyes.

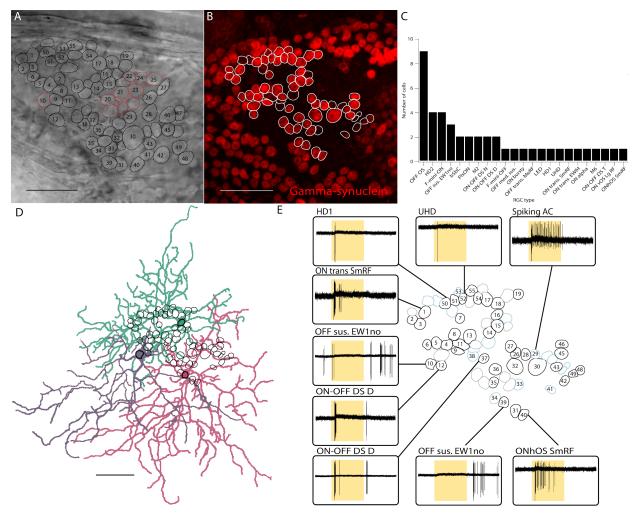


# Supplemental Figure 4. Morphological and physiological discrimination between two similar ON transient RGC types.

(A) Images of neighboring RGCs filled with AlexaFluor dyes, *en face* and side views. The green cell is classified as ON tr. SmRF, Eyewire type 6sn, and the magenta cell is classified as ON tr. MeRF, Eyewire type 6sw.

(B) Spike responses from the cells in A for spots of 3 different sizes. A consistent distinction between these cell types is seen in the shape of the spike waveform envelopes (blue).

(C) Response amplitude versus spot size for the cells in (A), (B). Error bars are s.e.m. across trials (n=3).



## Supplemental Figure 5. Classifying most RGCs in a retinal region

(A) Image with recorded cells outlined. Numbered cells had spike responses to our standard stimuli. Red outlines indicate cells for which the soma was not visible in the fixed tissue.

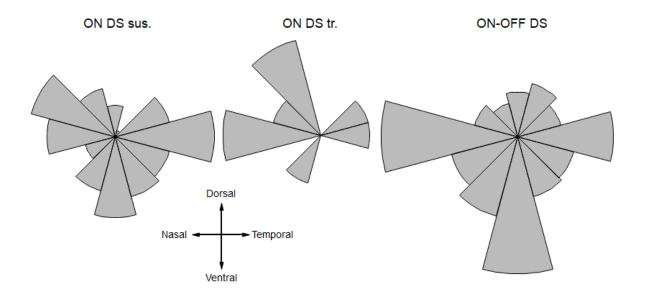
(B) Confocal image of the same region stained with an antibody against the pan-RGC marker gamma-synuclein.

(C) Histogram of RGC types identified in the region in (A,B).

(D) Reconstructions of 3 dye-filled RGC's of type 1no from this region. Soma outlines from (B) are included.

(E) Examples of light responses from some of the recorded cells in this region. Cell outlines are colored according to the outcome of classification and gamma-synuclein staining. Black outlines indicate cells identified as RGCs that were synuclein-gamma positive (n = 42). Cyan outlines indicate gamma-synuclein negative cells that were either identified as spiking amacrine cells (n = 6, numbered) or non-spiking (n = 10, not numbered). Gray outlines indicate gamma-synuclein positive cells for which we were unable to elicit spikes (n = 12).

Scale bars in (A), (B), and (D) indicate 50 µm.



# Supplemental Figure 6. DS rose plots

Histograms showing the direction preferences of each type of DS using the optic nerve and a ventral cut to orient retinas (n = 110 ON DS sus., 8 ON DS tr., 252 OODS). We observe three major categories of ON DS sus. (Dorsonasal, Temporal, and Ventronasal). ON DS tr. were rarely observed, with a tendency to prefer dorsonasal movement. There are known to be four major categories of OODS cells preferring each cardinal direction; while we observe fewer OODS-D than expected, all four types are seen in our dataset.