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RESEARCH ARTICLE

Figures and figure supplements

Connectivity map of bipolar cells and photoreceptors in the mouse retina

Christian Behrens *et al*

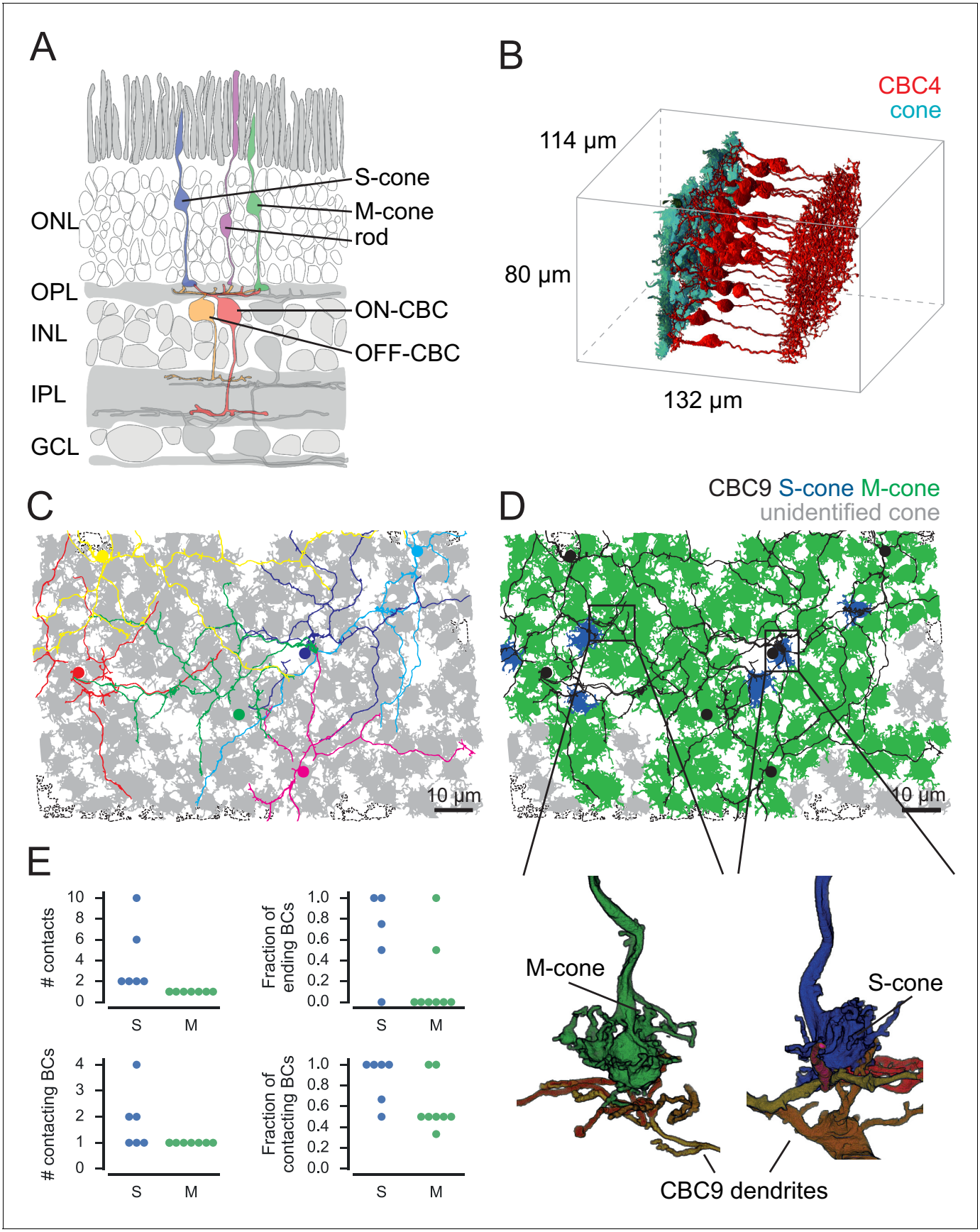
A
S-cone
M-cone
rod
ONL
OPL
INL
IPL
GCL
ON-CBC
OFF-CBC
B
CBC4
cone
114 µm
80 µm
132 µm
C
10 µm
D
CBC9 S-cone M-cone
unidentified cone
10 µm
M-cone
S-cone
CBC9 dendrites
E
contacts
Fraction of ending BCs
contacting BCs
Fraction of contacting BCs
S
M

Figure 1. Identification of S- and M-cones. (**A**) Scheme showing vertical section through the mouse retina. (**B**) Volume-reconstructed cones and all CBC4 cells. (**C**) Cone pedicles (grey) with CBC9s. BC soma localization is indicated by colored dots. Dashed outlines indicate incomplete cones. (**D**) Same as in **C**, but with putative S-cones (blue) and M-cones (green) highlighted. Unidentified cones are shown in grey. Insets indicate the location of the examples shown below of cone pedicles contacted by CBC9 dendrites. (**E**) Contact parameters used for S-cone identification. ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.
DOI: 10.7554/eLife.20041.002

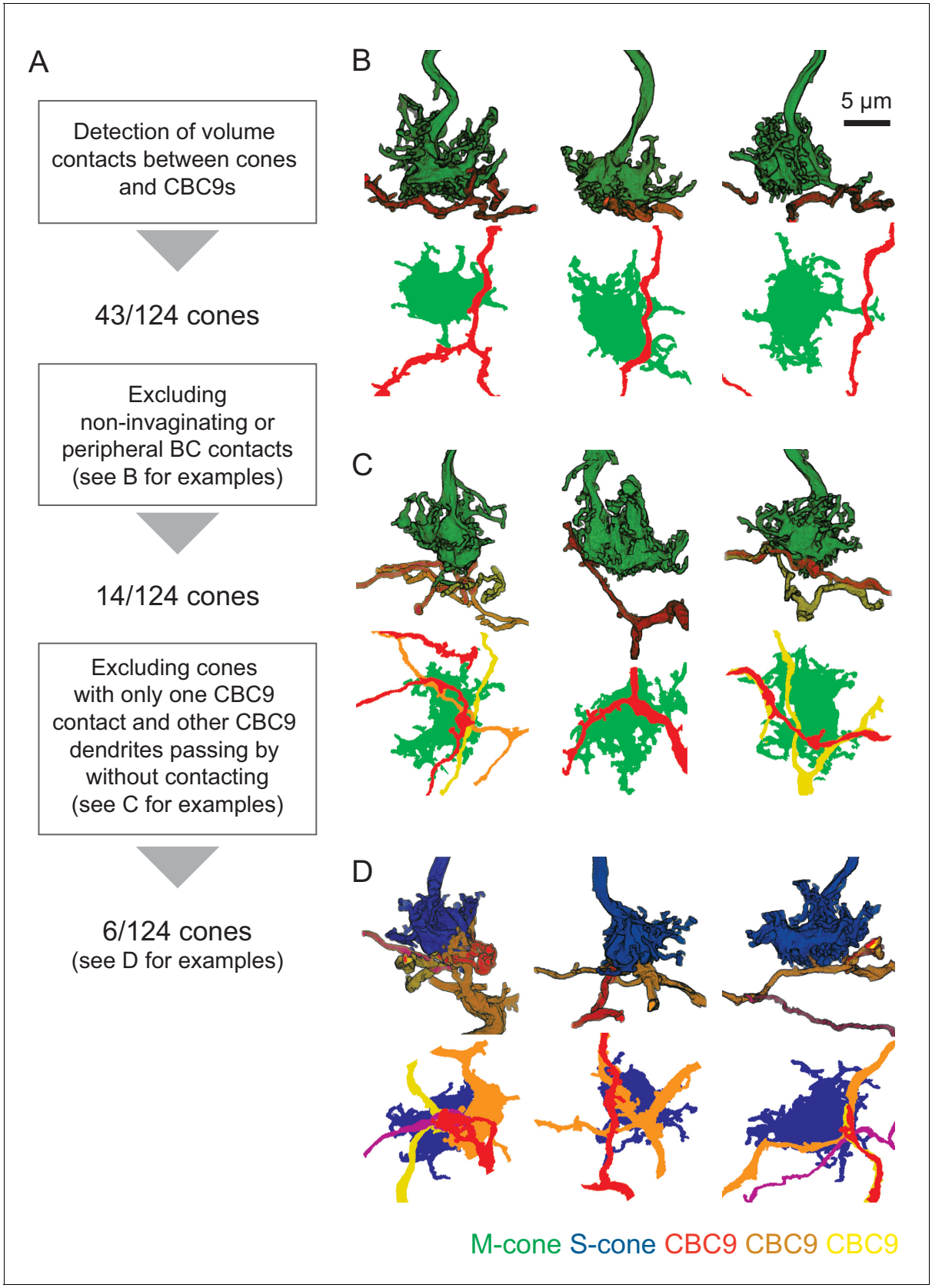

Figure 1—figure supplement 1. Details on the identification of S-cones. (**A**) Diagram showing workflow for identification of S- and M-cones using connectivity with CBC9 cells. (**B–D**) Side view and horizontal projection of representative examples of cone pedicles (green, M-cone; blue, S-cone) with

Figure 1—figure supplement 1 continued on next page

Figure 1—figure supplement 1 continued

CBC9 dendrites (yellow, orange, red) with non-invaginating but peripheral contacts (**B**), with only one CBC9 contact and other CBC9 dendrites passing by (**C**) and 'true' S-cones (**D**).

DOI: 10.7554/eLife.20041.003

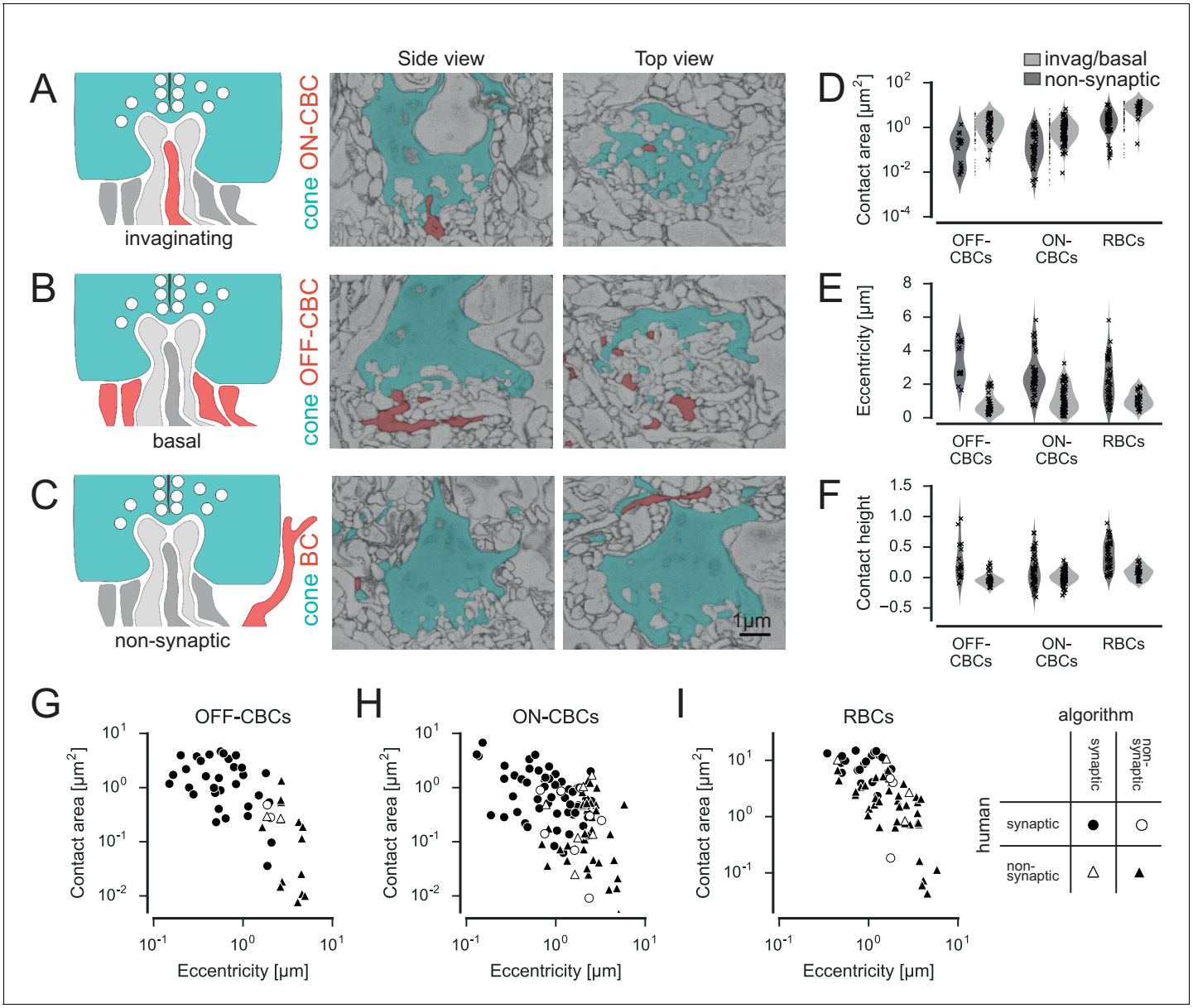

Figure 2. Classification of cone contacts. (**A**) Invaginating ON-CBC contact. Schematic drawing (left), EM side view (center) and top view (right). Red and dark grey, BC dendrites; light grey, horizontal cell dendrites; cyan, cone pedicles. (**B**) Basal/flat OFF-CBC contact as in **A**. (**C**) Peripheral (non-synaptic) BC contact as in **A**. (**D–F**) Contact area (**D**) eccentricity (**E**) contact height (**F**) of invaginating/basal and non-synaptic contacts for OFF-/ON-CBCs and rod bipolar cells (RBCs). (**G–I**) Contact area versus eccentricity for OFF-CBC (**G**), ON-CBC (**H**) and RBC (**I**) contacts indicating correctly and incorrectly classified contacts.

DOI: 10.7554/eLife.20041.004

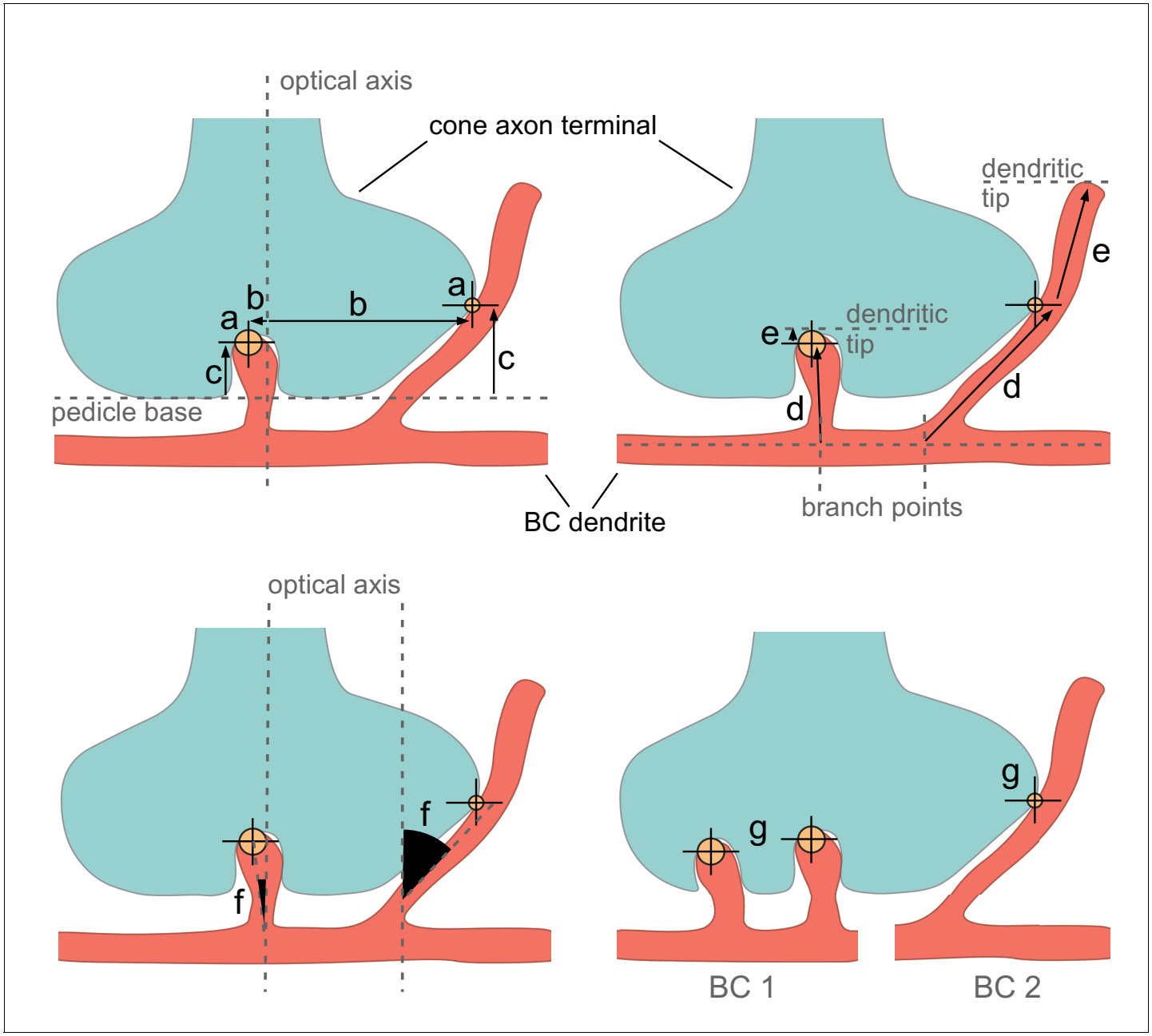

Figure 2—figure supplement 1. Illustration of parameters used for classifying contacts. Cone pedicle schemes showing the parameter used for automated contact classification: Contact area (a), eccentricity (b), contact height (c), distances to branch point (d) and dendritic tip (e), smallest angle between contacting dendrite and optical axis (f) and number of contact points between cone pedicle and BC (g). Example invaginating and peripheral contacts between cone (cyan) and BC dendrite(s) (red) are shown as large and small yellow circles, respectively. The optical axis is defined as a perpendicular through the center of the cone pedicle. BC, bipolar cell.

DOI: 10.7554/eLife.20041.005

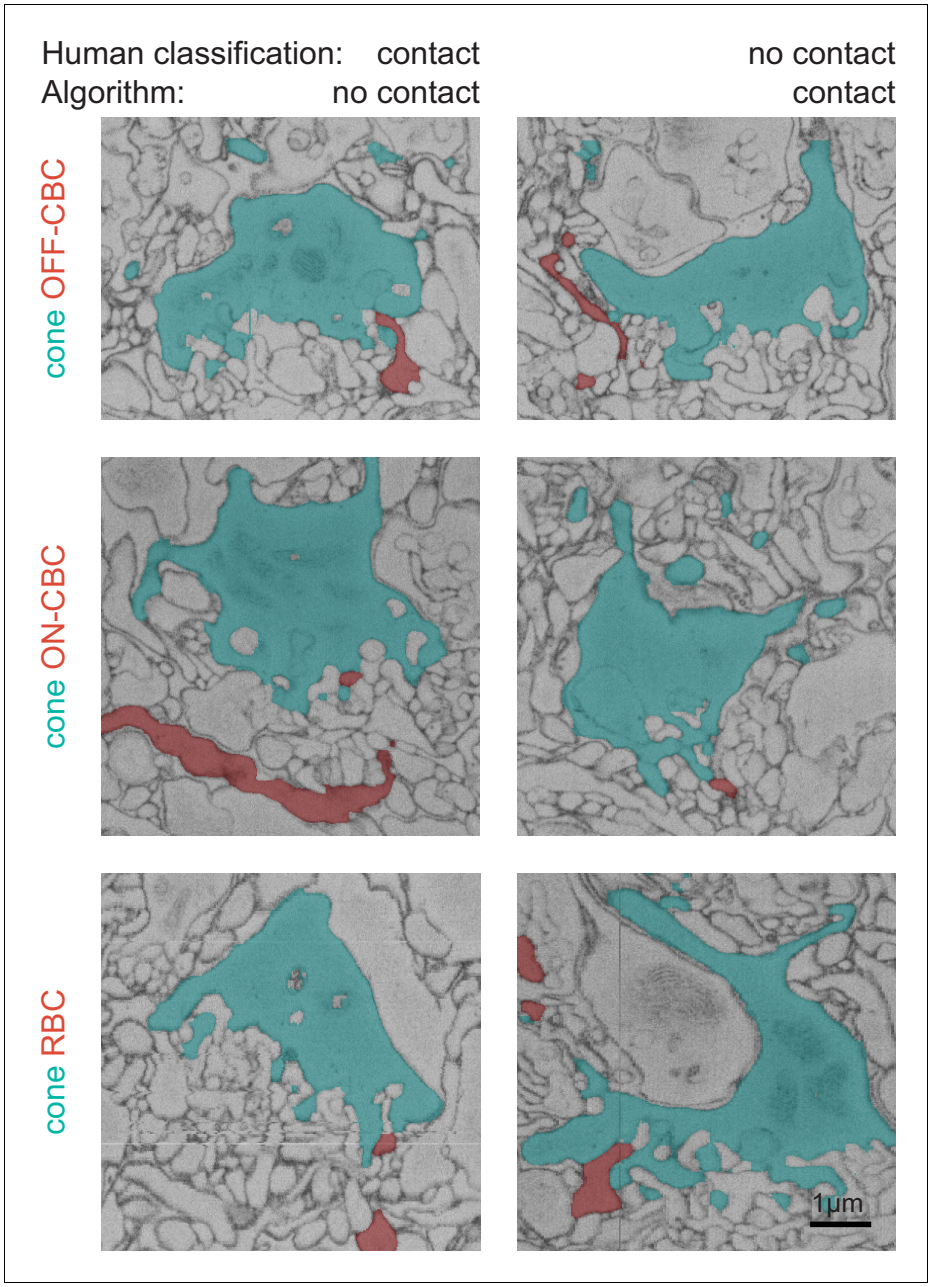

Figure 2—figure supplement 2. Examples for disagreements between human and algorithmic classification. EM slices (side view) showing examples of contacts where the automated contact classification did not match the human label. See also animated versions (***Video 1*** and ***2***).

DOI: 10.7554/eLife.20041.006

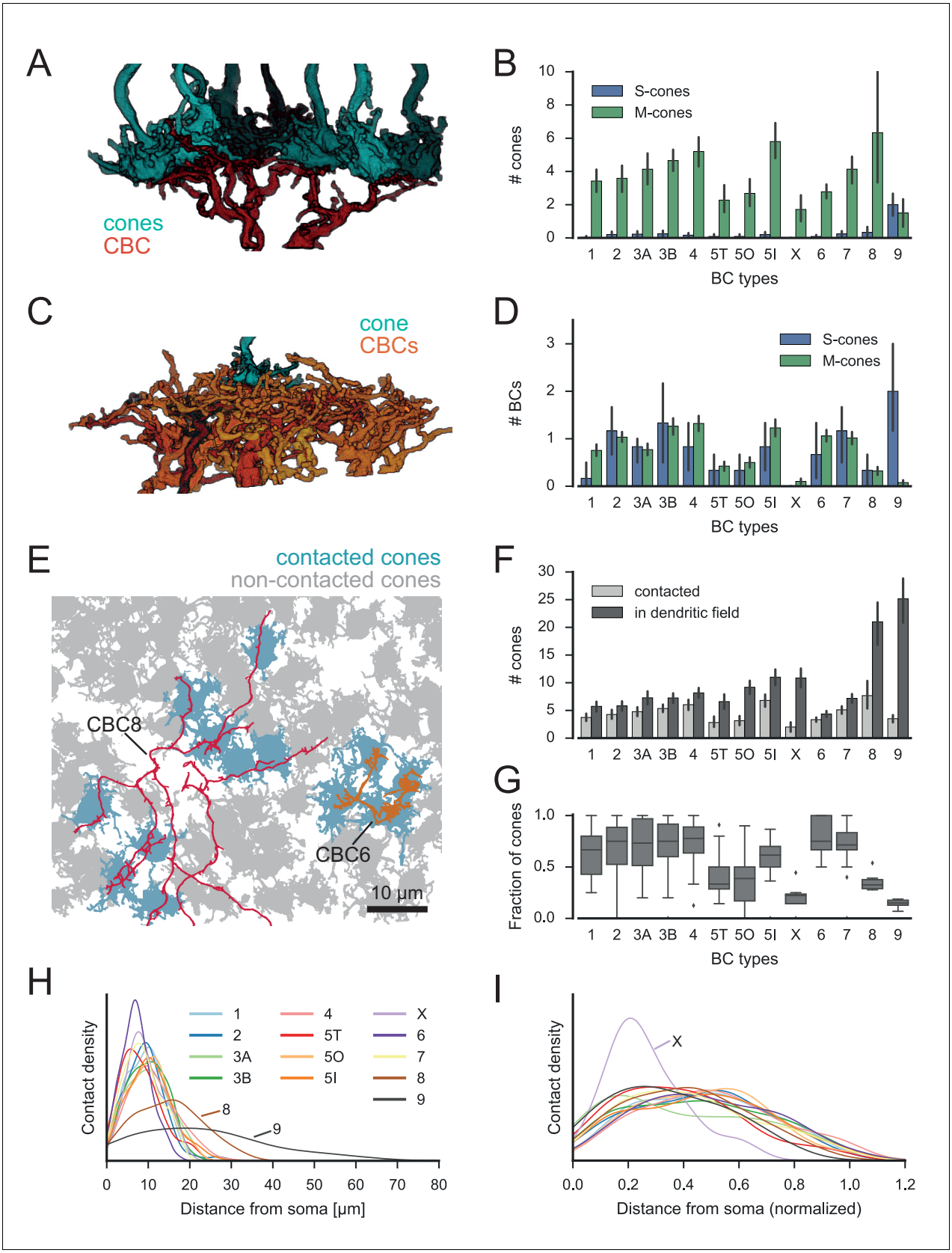

Figure 3. Quantification of cone-to-CBC contacts. (**A**) Volume-reconstructed single BC dendrite (red) contacting numerous cone pedicles (cyan). (**B**) Number of S- and M-cones contacted by different CBC types. (**C**) Volume-reconstructed single cone (cyan) contacted by multiple BCs (orange/red). (**D**)

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Figure 3 continued

Number of CBCs per type contacted by individual S- and M-cones. (**E**) Example cone array with CBC6 and CBC8 contacting cones. Grey, non-contacted cones; blue, contacted cones. (**F**) Number of contacted cones and cones within dendritic field for different CBC types. (**G**) Fraction of contacted cones/cones within the dendritic field. (**H**) Kernel density estimate of the distribution of contacted cones as function of distance from BC somata. (**I**) Same as H. but distance normalized by dendritic field size. Bars in **B**,**D**,**F** indicate 95% CI.

DOI: 10.7554/eLife.20041.010

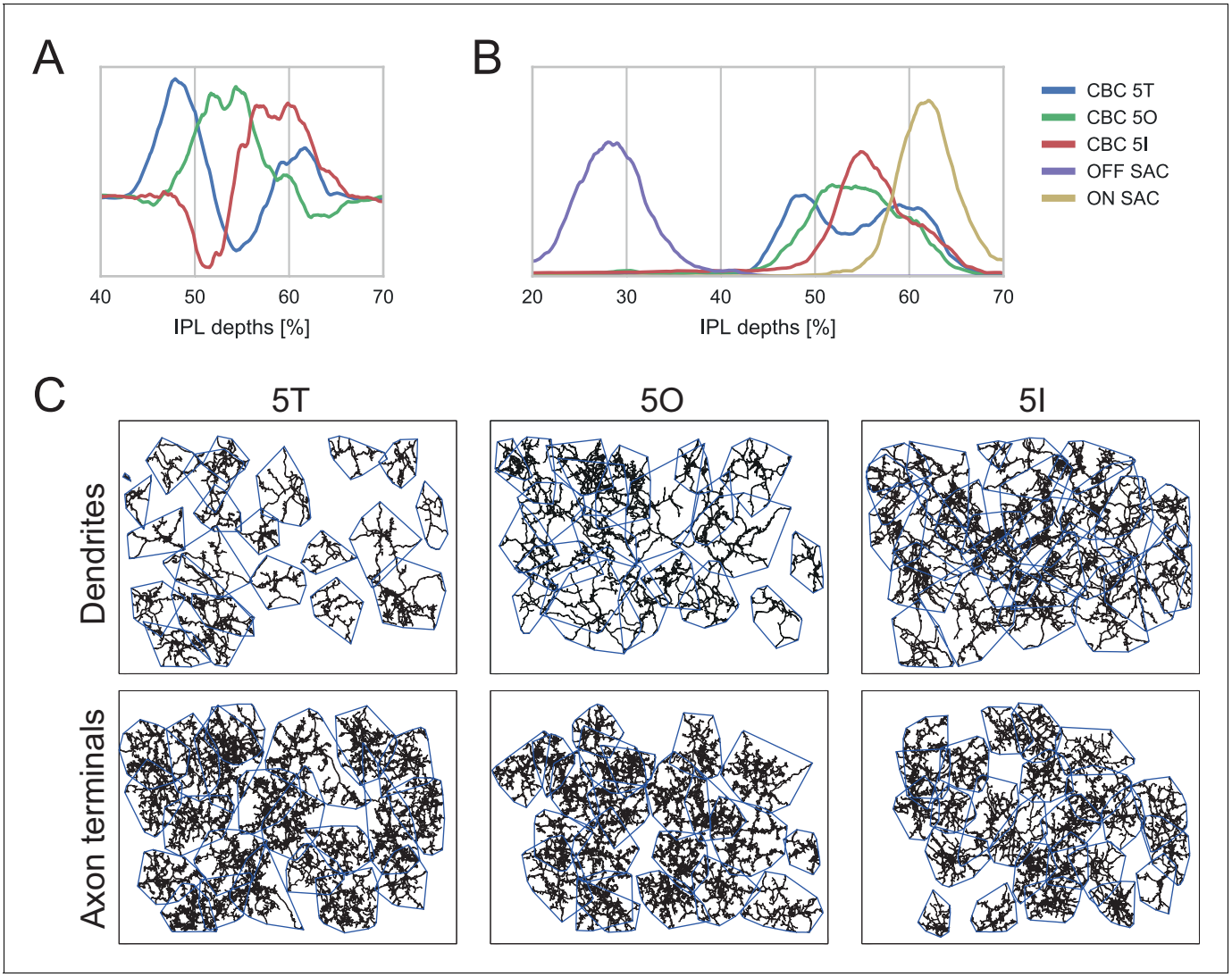

Figure 3—figure supplement 1. Classification of type 5 BCs. (**A**) First three PCA components for CBC5 density profiles in the IPL. (**B**) Stratification depth of CBC5T, 5O and 5I axon terminals in relation to the OFF- and ON-ChAT bands. (**C**) Dendritic (top) and axonal (bottom) mosaics for CBC5T, 5O and 5I cells.

DOI: 10.7554/eLife.20041.011

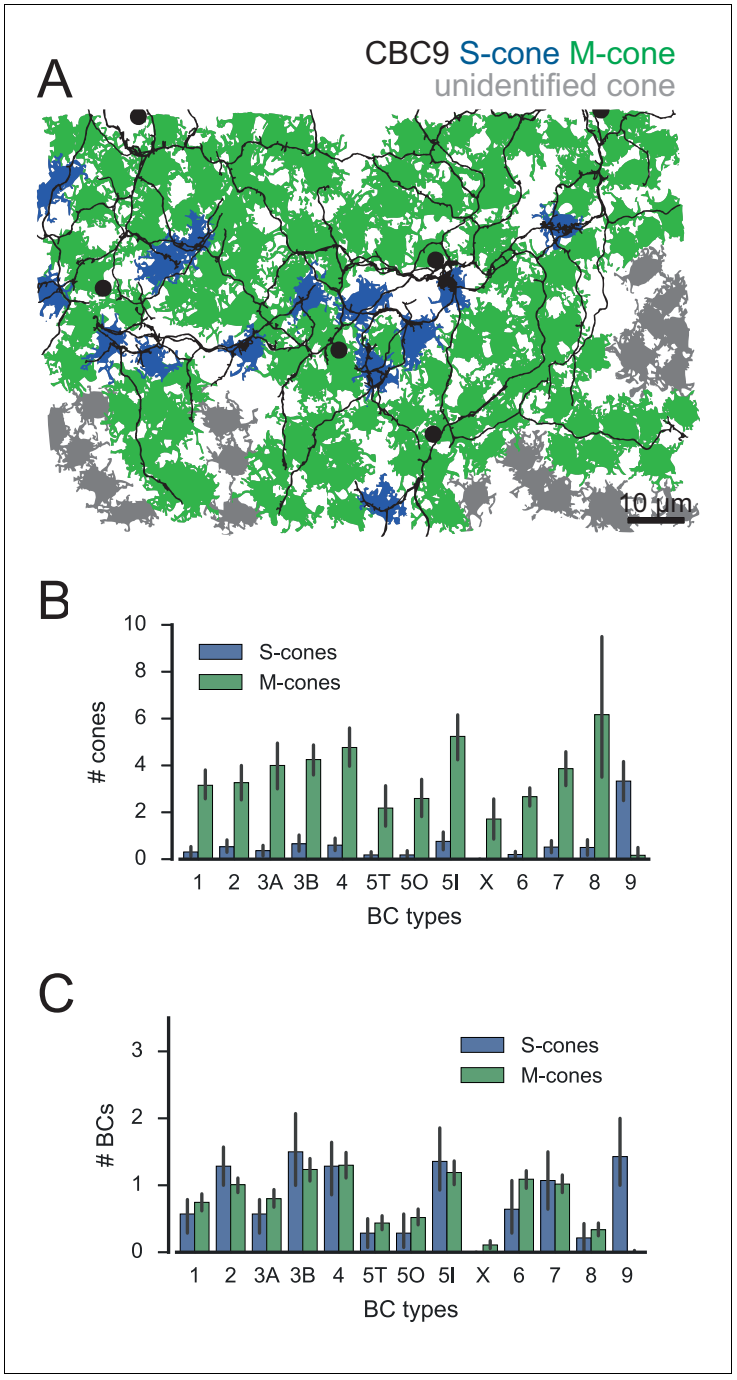

Figure 3—figure supplement 2. Connectivity analysis for alternative s-cone classification. (**A**) Cone pedicle array with CBC9s highlighted showing alternative S-cone classification. CBC9 somata are indicated by black dots, S-cones in blue, M-cones in green and unidentified cones in grey. (**B**) Number of S- and M-cones contacted by different CBC types. (**C**) Number of CBC types contacted by individual S- and M-cones.
DOI: 10.7554/eLife.20041.012

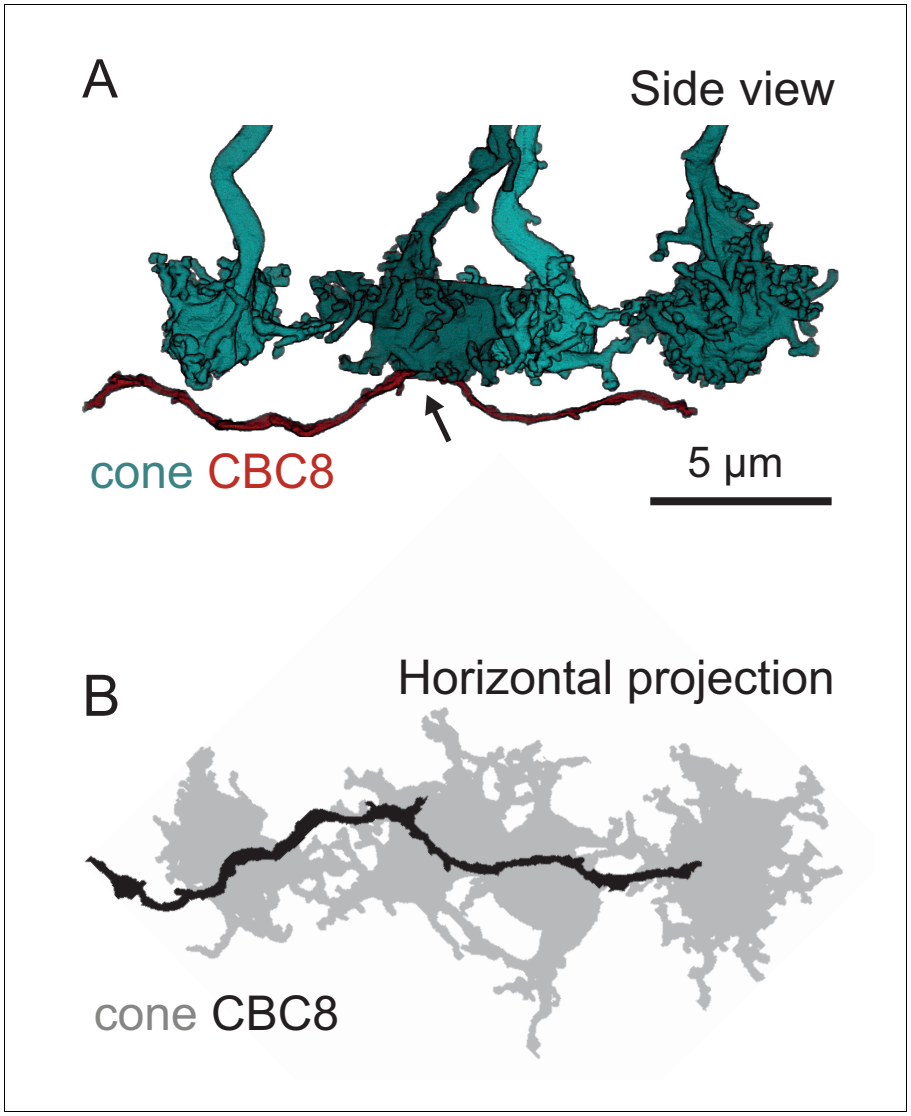

Figure 3—figure supplement 3. Example of a passing dendrite without contacts. (**A**) Side view of four volume-reconstructed cone pedicle (cyan) and CBC8 dendrite (red). (**B**) Horizontal projection of the neurite structures shown in (**A**). Arrow indicates the only invaginating ON-CBC contact along the dendrite.
DOI: 10.7554/eLife.20041.013

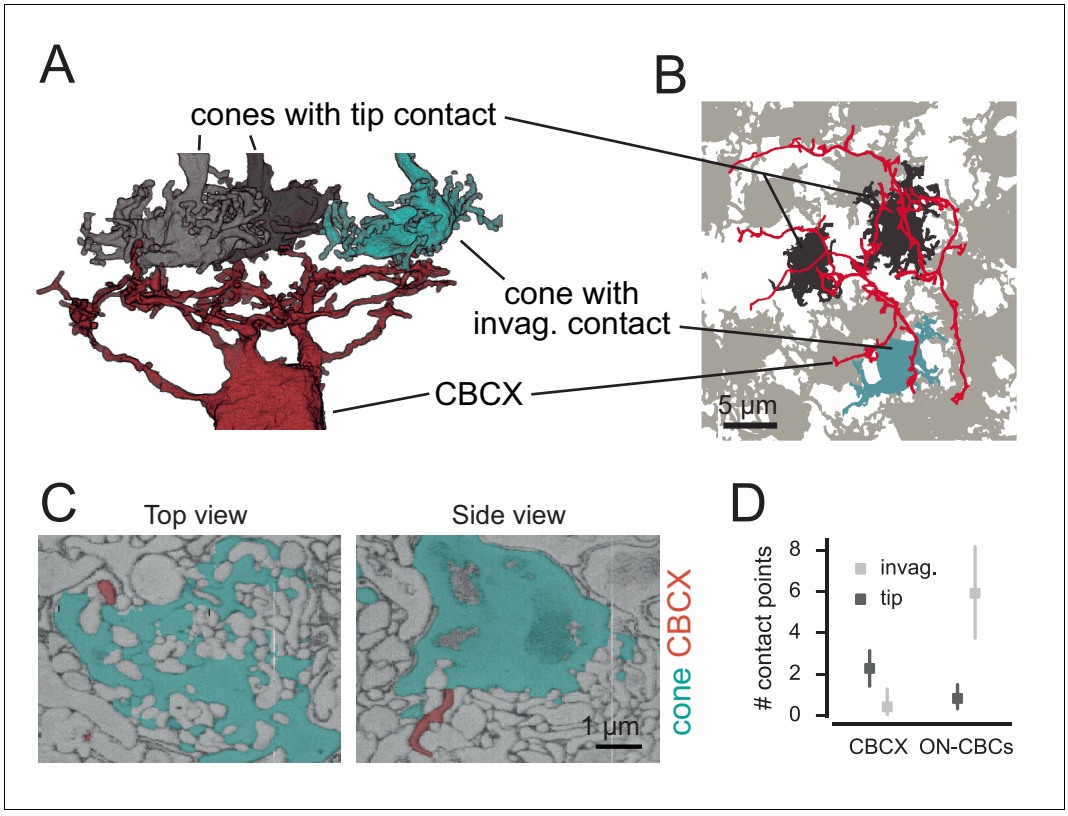

Figure 4. CBCX makes few and atypical cone contacts. (**A**) Volume-reconstructed CBCX dendritic arbor (red) contacting few cone pedicles (cyan, invaginating contact; grey, tip contact). (**B**) Same exemplary cone array as in **A**. with CBCX dendritic arbor contacting cones. Light grey, non-contacted cones; cyan, invaginating contacts; dark grey, tip contacts. (**C**) EM image showing tip contact between CBCX (red) and cone pedicles (cyan), top view (left) and side view (right). (**D**) Invaginating and tip contacts in CBCXs and other ON-CBCs. Bars in **D**. indicate 95% CI.
DOI: 10.7554/eLife.20041.014

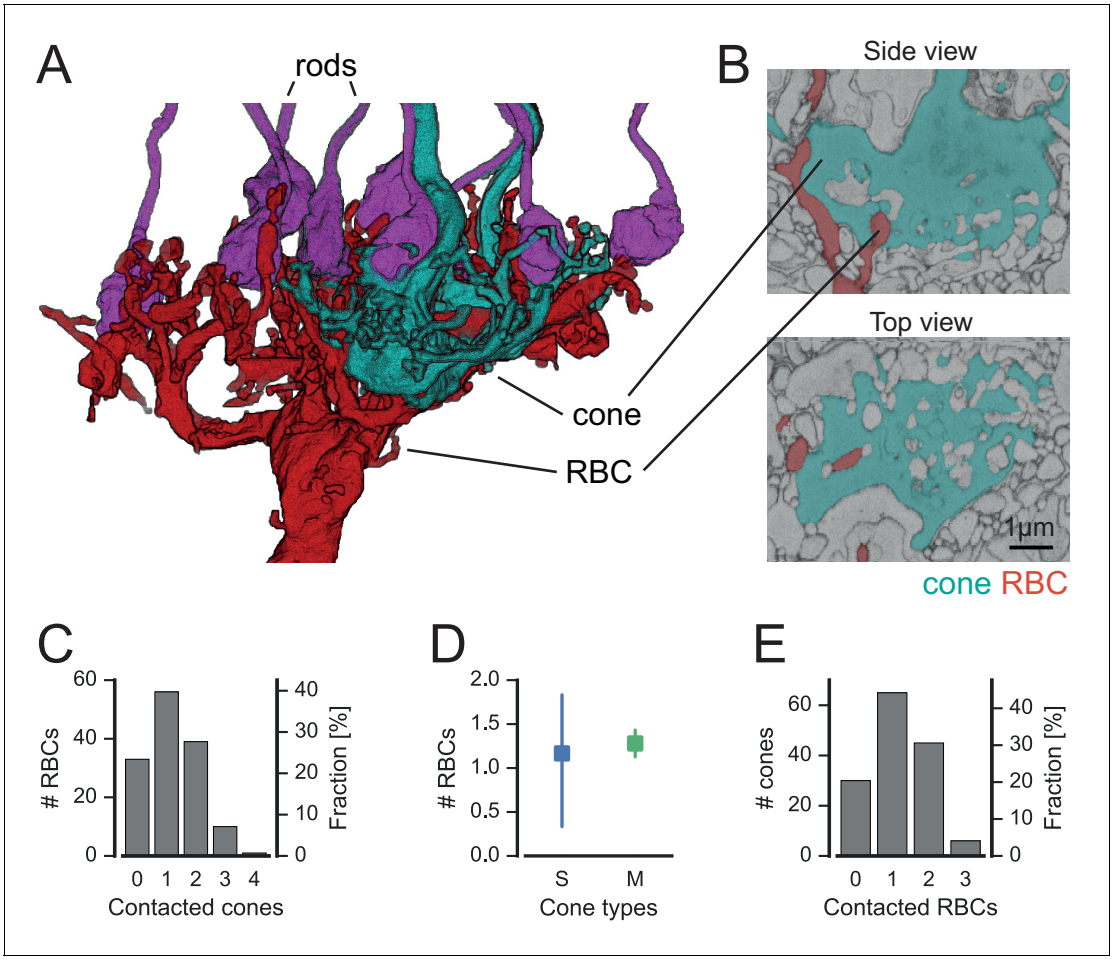

Figure 5. Cones contact rod bipolar cells. (**A**) Volume-reconstructed RBC (red) contacting both rods (magenta) and cone pedicles (cyan). (**B**) EM images showing invaginating contact between cone (cyan) and RBC (red), side view (top) and top view (bottom). (**C**) Number of RBCs contacted by cones. (**D**). Number of RBCs contacted by S- and M cones. (**E**) Number of cones contacted by RBCs. Bars in **D**. indicate 95% CI.

DOI: 10.7554/eLife.20041.015

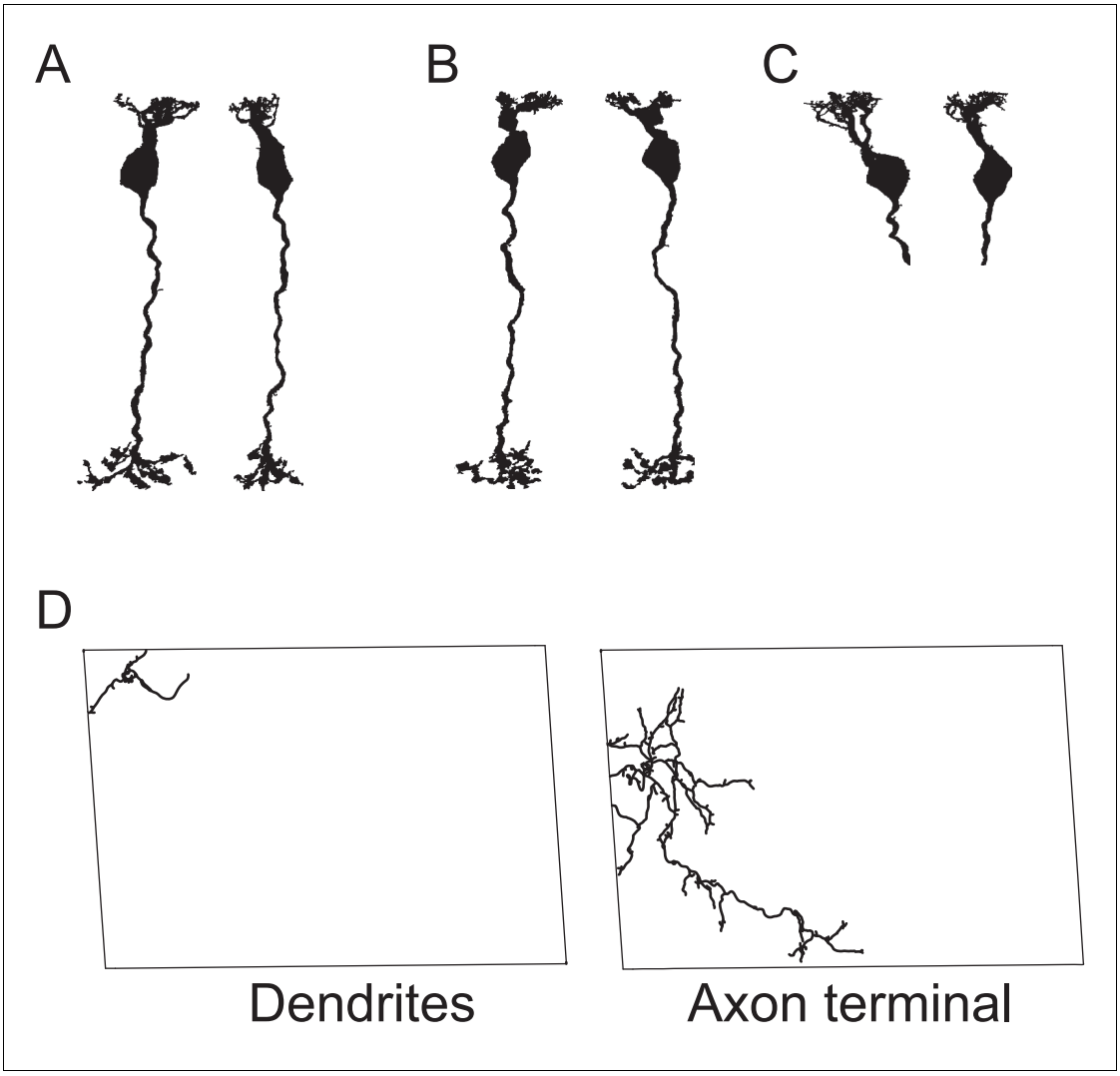

Figure 5—figure supplement 1. Excluded RBCs. (**A–C**) Three BCs classified as RBCs by ***Helmstaedter et al. (2013)*** but not contacting rods in the present study, these cells were therefore excluded from the analysis (for each cell two projections from different angles are shown). (**D**) BC classified as CBC9 but excluded from this study due to lack of complete dendritic tree.
DOI: 10.7554/eLife.20041.016

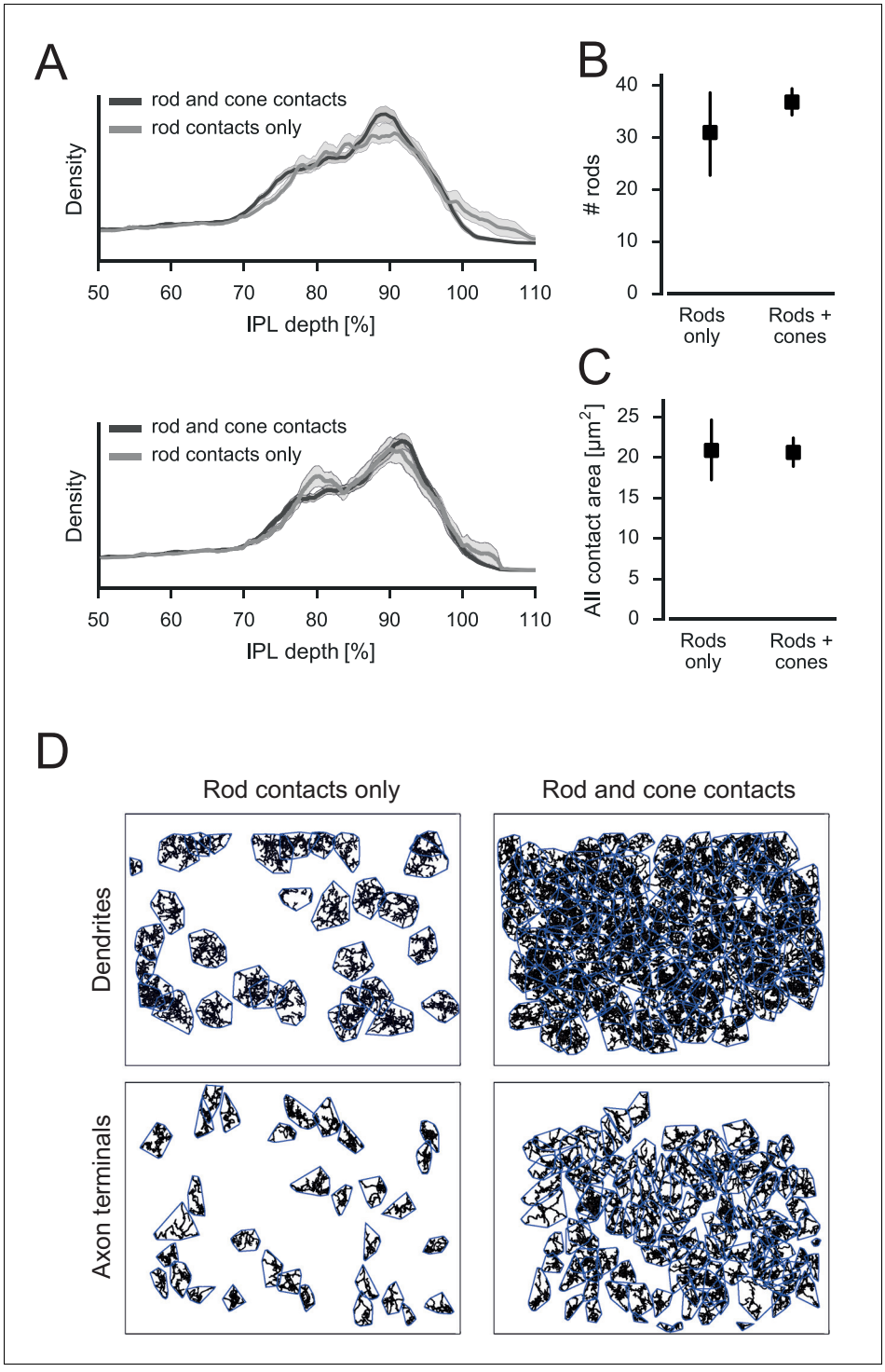

Figure 5—figure supplement 2. No evidence for two RBC subtypes. (**A**) Relative density of RBC spherules in the IPL using both dendritic ON and OFF starburst amacrine cell (SAC) bands (top) and only the dendritic ON SAC band (bottom) for depth correction (shading: SEM). (**B**) Number of rods contacted by RBCs contacting only rods or both rods and cones (95% confidence interval, CI). (**C**) Contact area with AIIs for RBCs contacting only rods or both rods and cones (95% CI). (**D**) Dendritic (top) and axonal (bottom) mosaics for RBCs contacting rods or both rods and cones.

DOI: 10.7554/eLife.20041.017

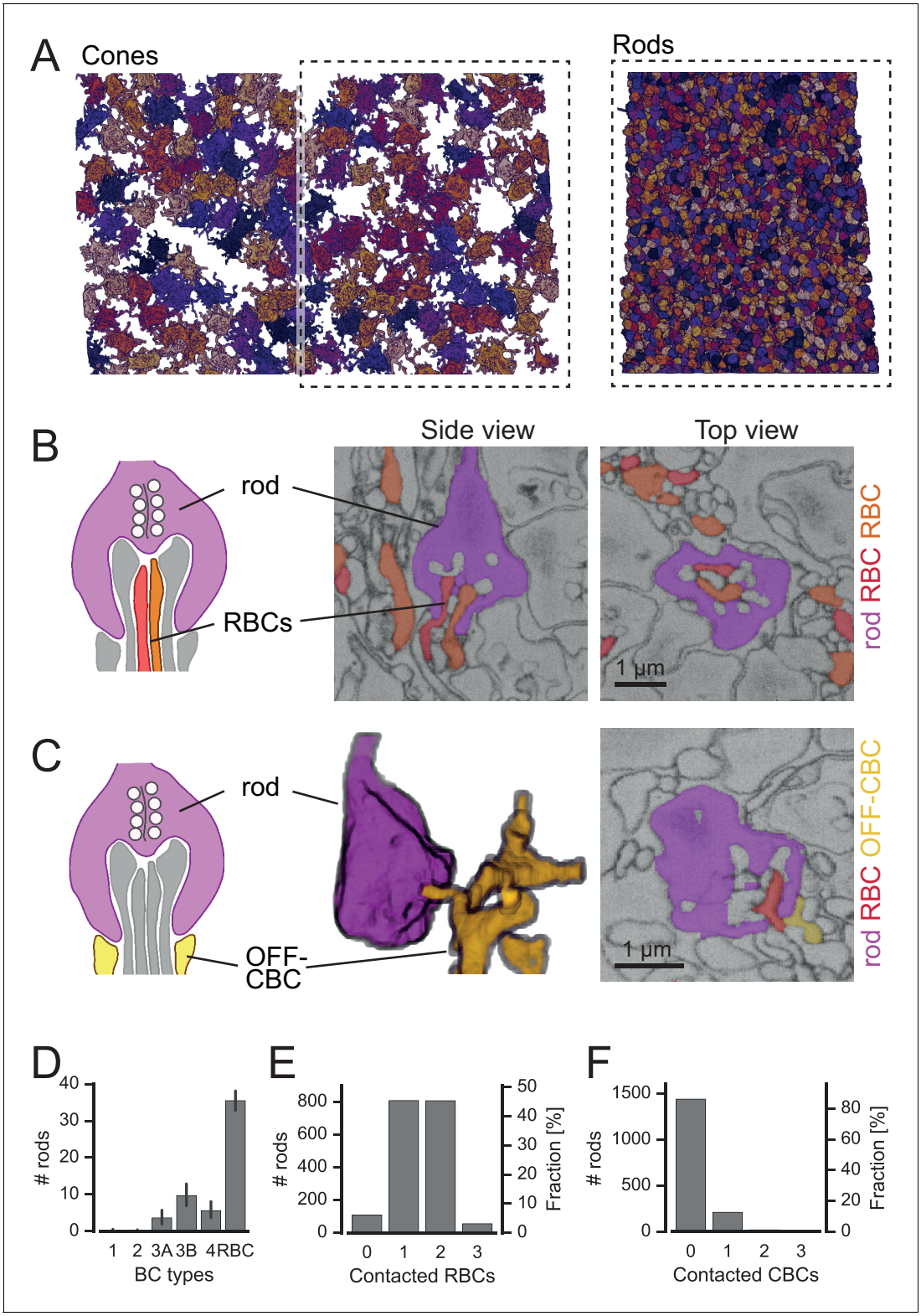

Figure 6. Rods contact RBCs and OFF-CBCs. (**A**) Volume-reconstructed, neighboring rod spherules (right) in one half of the field of the reconstructed cone pedicles (left). (**B**) Rod spherule (magenta) with invaginating dendrites of two RBCs (orange, red). Schematic drawing (left), EM images side view (middle) and top view (right). (**C**) Rod spherule (magenta) with basal contacts by OFF-CBCs (yellow). Schematic (left), volume-reconstructed vertical view (middle), EM image with top view (right). The latter also shows an invaginating RBC dendrite (red). (**D–F**). Number of rods (and fraction) contacted by RBCs (**D**,**E**), and OFF-CBC types (**D**, **F**). Bars in **D**. indicate 95% CI.

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DOI: 10.7554/eLife.20041.018

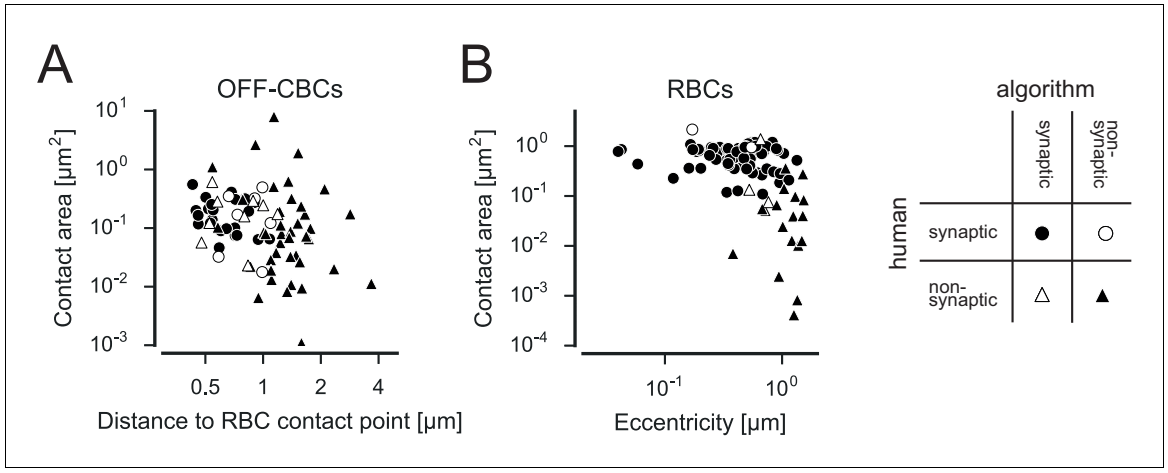

Figure 6—figure supplement 1. Classification of rod contact classification. Contact area versus distance to RBC contact point for OFF-CBC-rod contacts (**A**) and contact area versus eccentricity for RBCs (**B**) contacts indicating correctly and incorrectly classified contacts.
DOI: 10.7554/eLife.20041.019

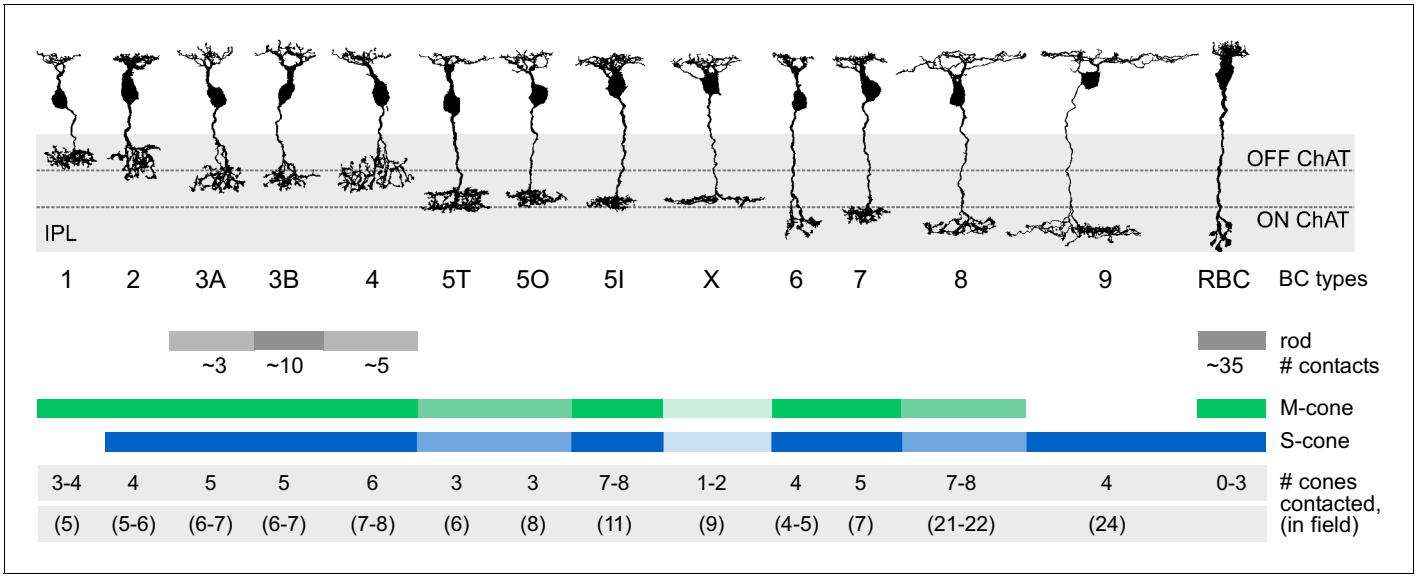

Figure 7. Connectivity between cone and rod photoreceptors and bipolar cells in the mouse retina. Representative examples of bipolar cell types in the mouse retina are shown. The number of cones in the dendritic field number and contacted photoreceptors are given for each type.
DOI: 10.7554/eLife.20041.020