**Supplementary Materials**

# Olfactory ensheathing cell-derived extracellular vesicles protect the degenerated retinal pigment epithelium by delivering phagocytosis-related proteins

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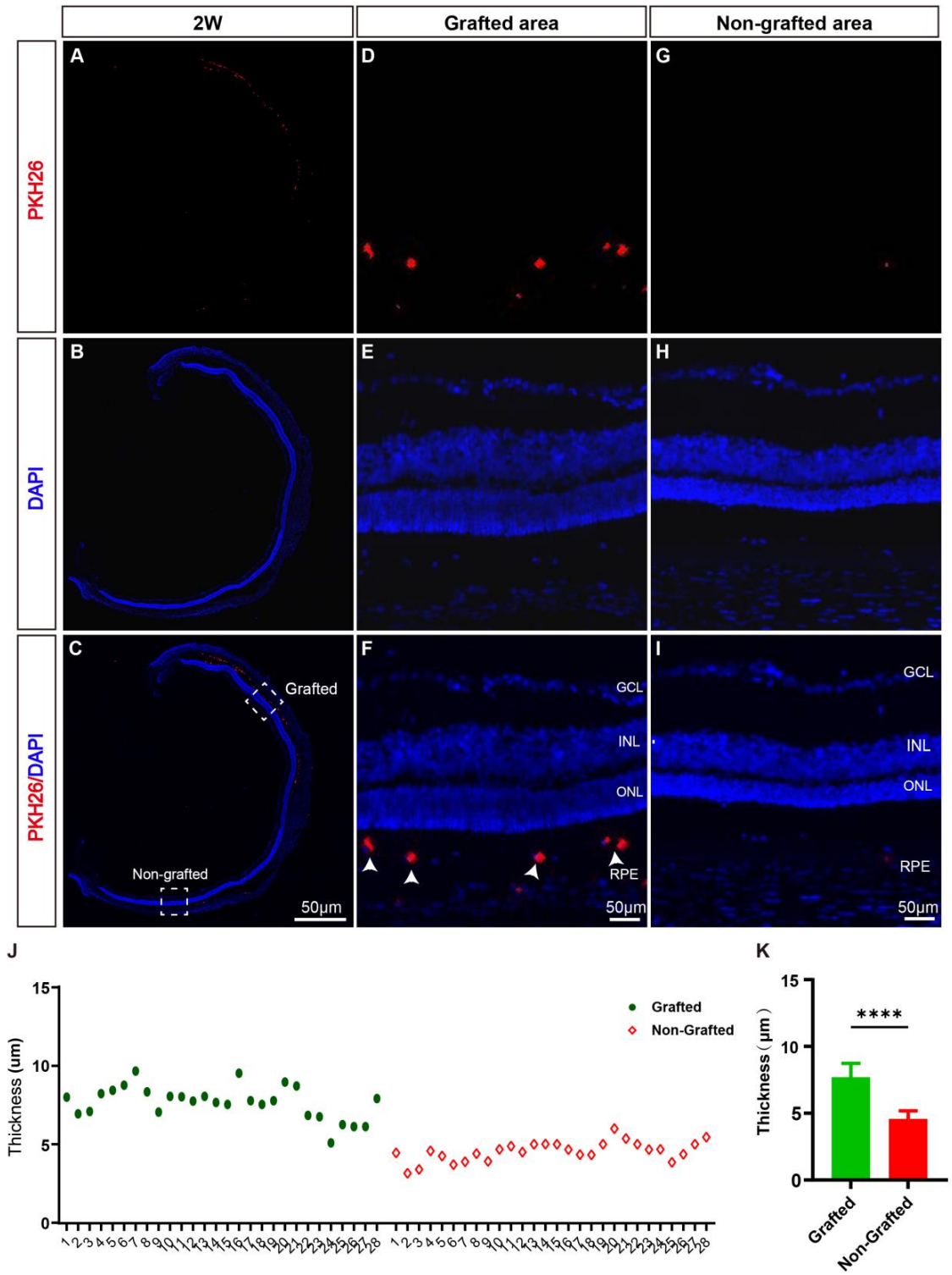
**Supplementary Table 1. Antibody information**

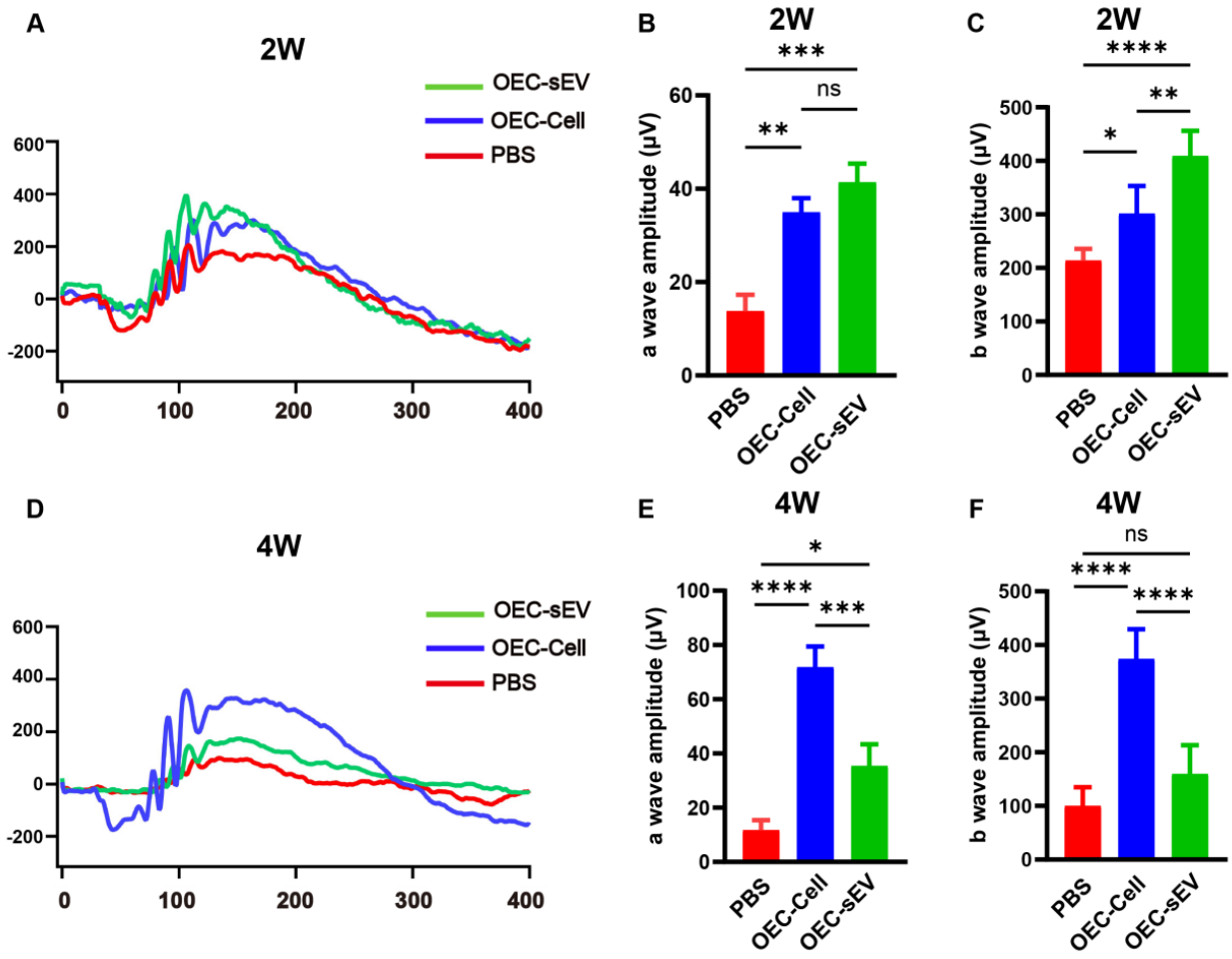
| Antibody | Company | Product Number | Species | Dilution |
|---|---|---|---|---|
| S100 | Beyotime | AF1945 | Rabbit | 1:150 |
| P75 | Beyotime | AF1033 | Rabbit | 1:150 |
| PKCa | Abcam | ab32376 | Mouse | 1:500 |
| RPE65 | Santa Cruz | sc-390787 | Mouse | 1:100 |
| F-actin | Invitrogen | MA1-80729 | Mouse | 1:1000 |
| Arresttin | Sigma-Aldrich | ab15282 | Rabbit | 1:500 |
| CD63 | Invitrogen | MA5-35208 | Rabbit | 1:200 |
| CD9 | Invitrogen | MA5-31980 | Rabbit | 1:200 |
| TSG101 | bioworld | BS6042 | Rabbit | 1:1000 |
| GM130 | Boster | M05865-2 | Mouse | 1:1000 |
| Opsin | Thermo Fisher | PA1-9517 | Chicken | 1:200 |
| Tunel | Roche | 12156792910 | / | 1:300 |
| 647 Phalloidin | Abcam | AB176759 | / | 1:2000 |
| goat-rabbit 488 | Invitrogen | A11008 | / | 1:500 |
| goat-mouse 488 | Invitrogen | A11011 | / | 1:500 |
| goat-chicken 488 | Invitrogen | A11039 | / | 1:500 |
| goat-mouse 647 | Invitrogen | A21235 | / | 1:500 |
| goat-rabbit 568 | Invitrogen | A11036 | / | 1:500 |
| goat-mouse 568 | Invitrogen | A11031 | / | 1:500 |

**Supplementary Table 2. Primer sequences**

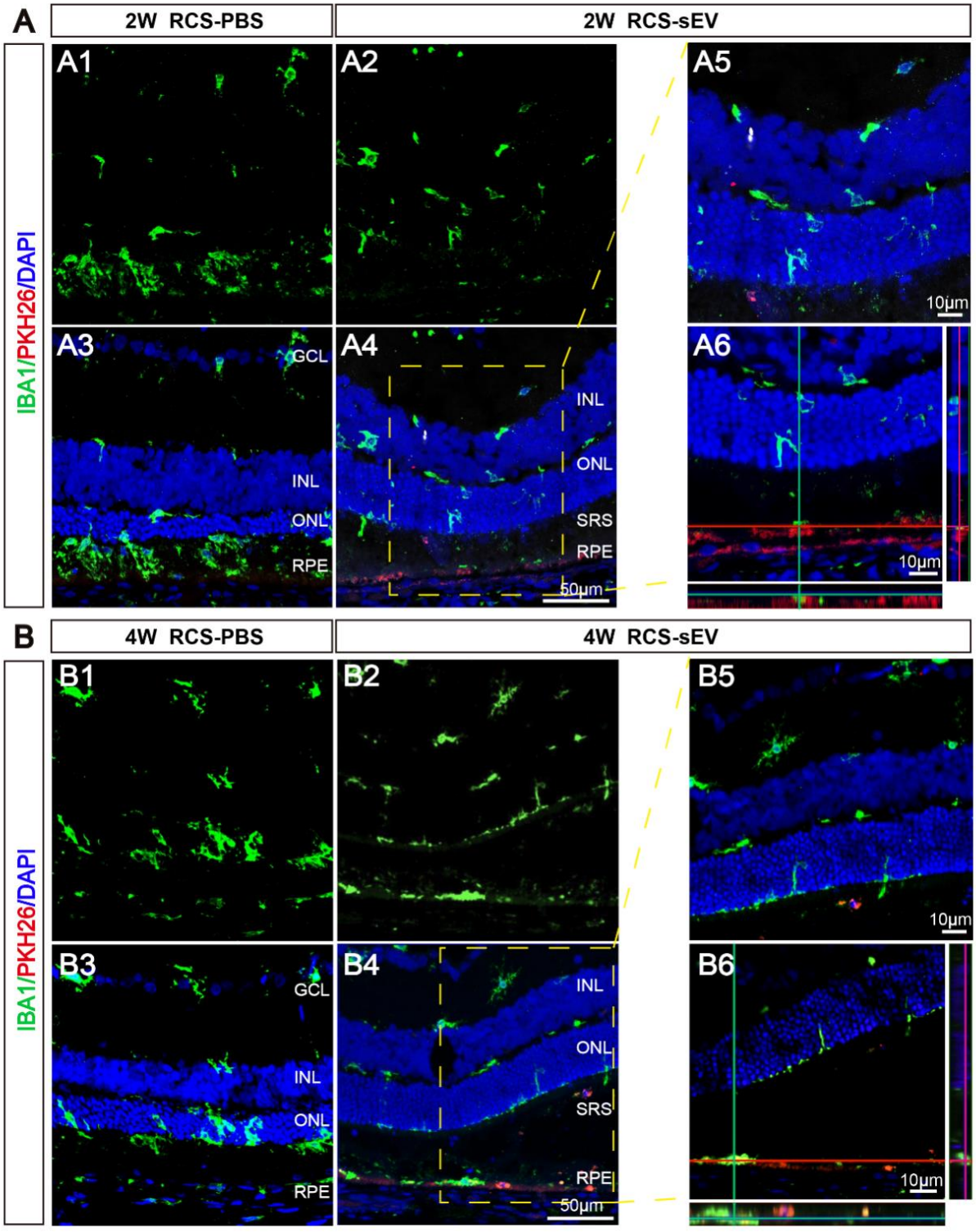
| primers | species | Forward 5'-3' | Reverse 5'-3' |
|---|---|---|---|
| Rab5a | Rat | CTTCAAAGGCAAGCAAGTCC | GCCTGGATTTTGTGGTTCGT |
| Rab7a | Rat | GGAGGTGATGGTGGATGACA | TGGGGGCAGTCACATCAAAC |
| FAK | Rat | CCAAGTGCAGACCATCCAGT | AGGTCAGCCATGTTCTCTGC |
| Mertk | Rat | CGCTCAGACAATGGGTCGTA | CCAGAAGATGTTGACGGGCT |
| Gas6 | Rat | GCCAGGTCTGCCATAACAAA | AGTGTACCCCTTGTCGCAGA |
| PROS1 | Rat | AGCTAAGGAGGCCGTGATGA | TGCACTTTCCACCTTCCGAG |
| β-Actin | Rat | ACAACCTTCTTGCAGCTCCTC | CTGACCCATACCCACCATCAC |



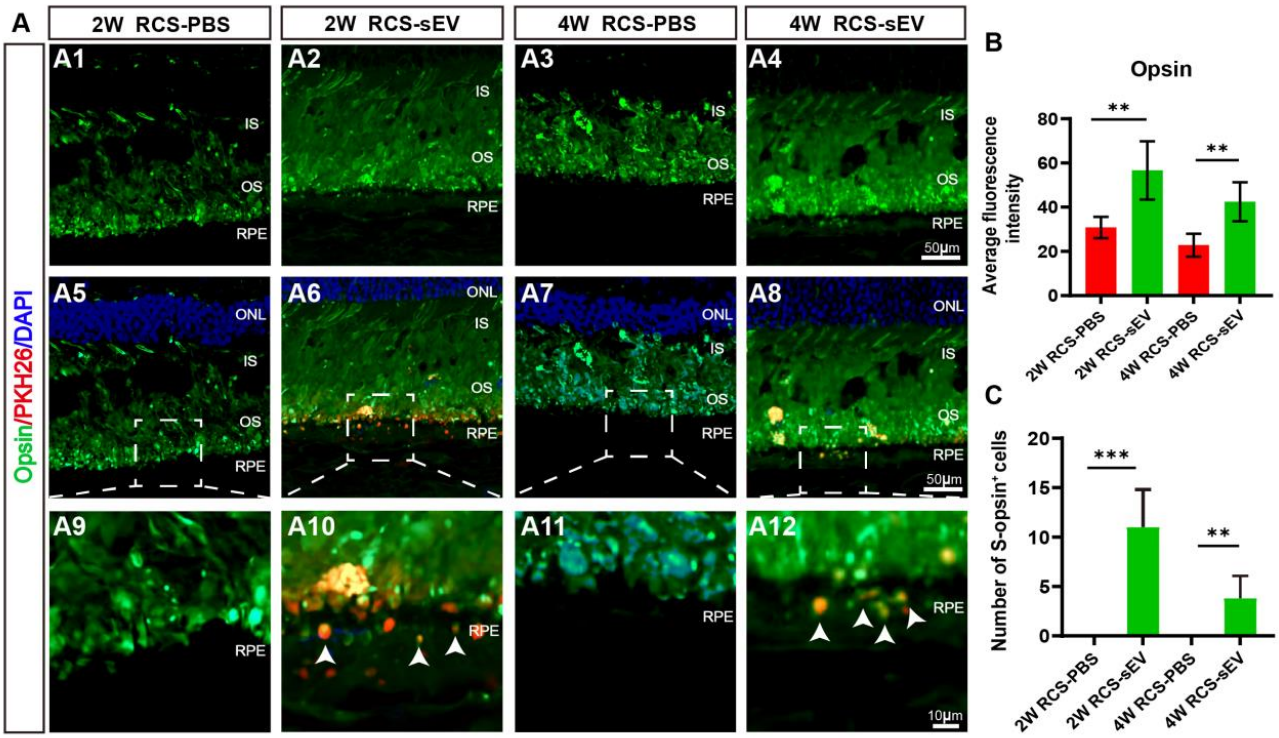

**Supplementary Figure 1.** Distribution areas and protective effects of OEC-sEVs on outer nuclear layer (ONL) post injection. (A-I) Comparison of PHK26-labeled OEC-sEVs grafted area and non-grafted area. Scale bar = 50 μm; (J-K) Comparison of thickness of ONL in Grafted group and Non-Grafted groups. Arrow shows the presence of PKH26-labeled OEC-sEVs within RPE layer. *n = 4* biologically independent rats per group. Data are represented as mean ± SD. ****$P < 0.0001$. Student's *t*-test was applied.



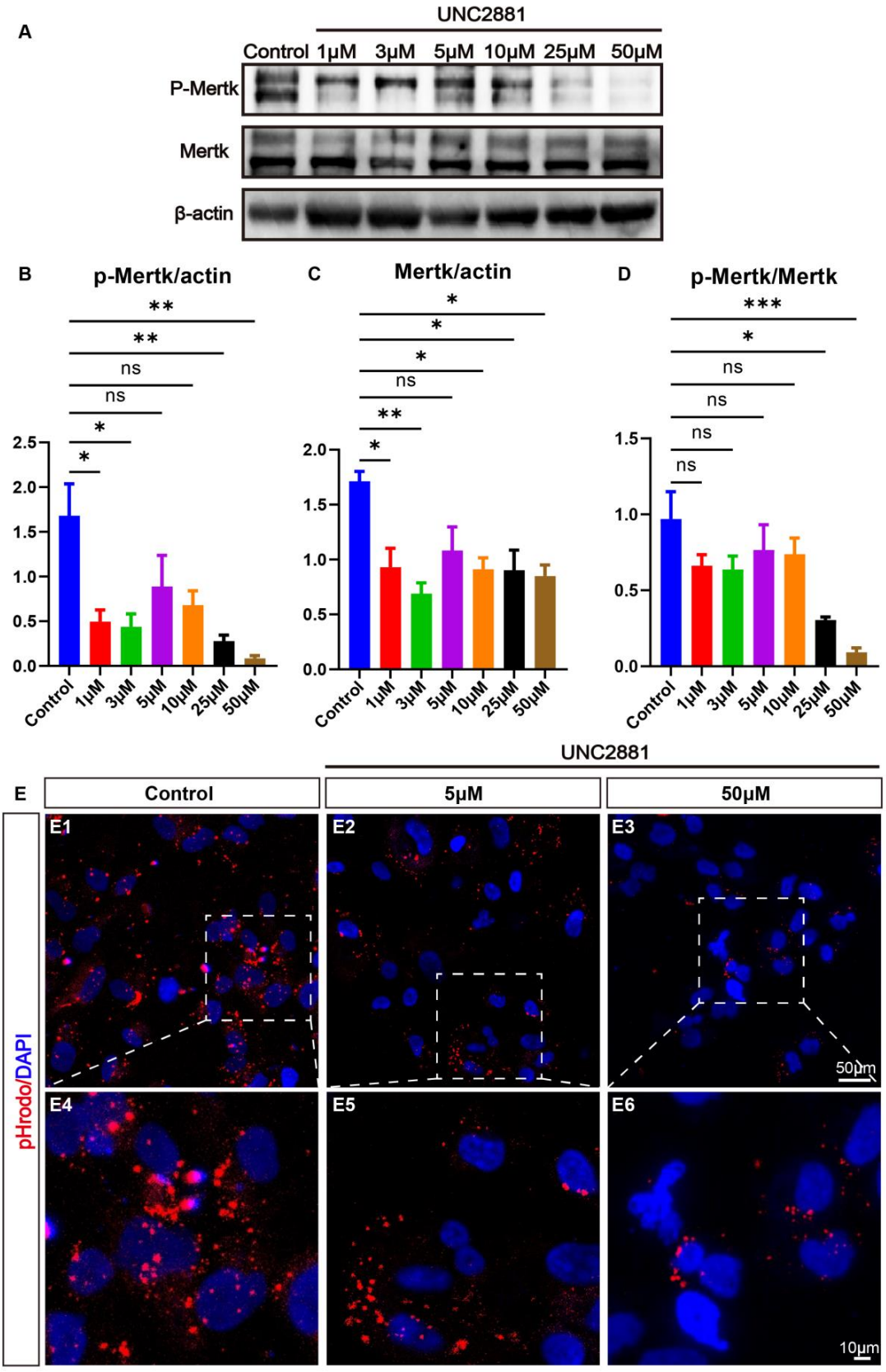

**Supplementary Figure 2.** Comparative neuroprotective effects of OEC-sEVs and OECs in RCS rats. (A and D) Representative ERG graphs at 2 and 4 weeks post-injection. (B, C, E, and F) Quantitative analysis of a-wave and b-wave amplitudes. $n$ = 4 biologically independent rats per group. Data are represented as mean ± SD. *$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001, ****$P$ < 0.0001, ns, no statistical difference. One-way ANOVA, followed by Tukey's multiple comparisons test, was conducted for multiple comparisons.




**Supplementary Figure 3.** The phagocytic uptake of OEC-sEVs by microglial cells. (A) Distribution of PKH26-labeled OEC-sEVs (red) within the retina and their co-localization with Iba1-positive microglia (green) at week 2. (A1-A4) Scale bar = 50 μm. (A5 and A6) Scale bar = 10 μm; (B) Distribution of PKH26-labeled OEC-sEVs (red) within the retina and their co-localization with Iba1-positive microglia (green) at week 4. (B1-B4) Scale bar = 50 μm. (B5 and B6) Scale bar = 10 μm.

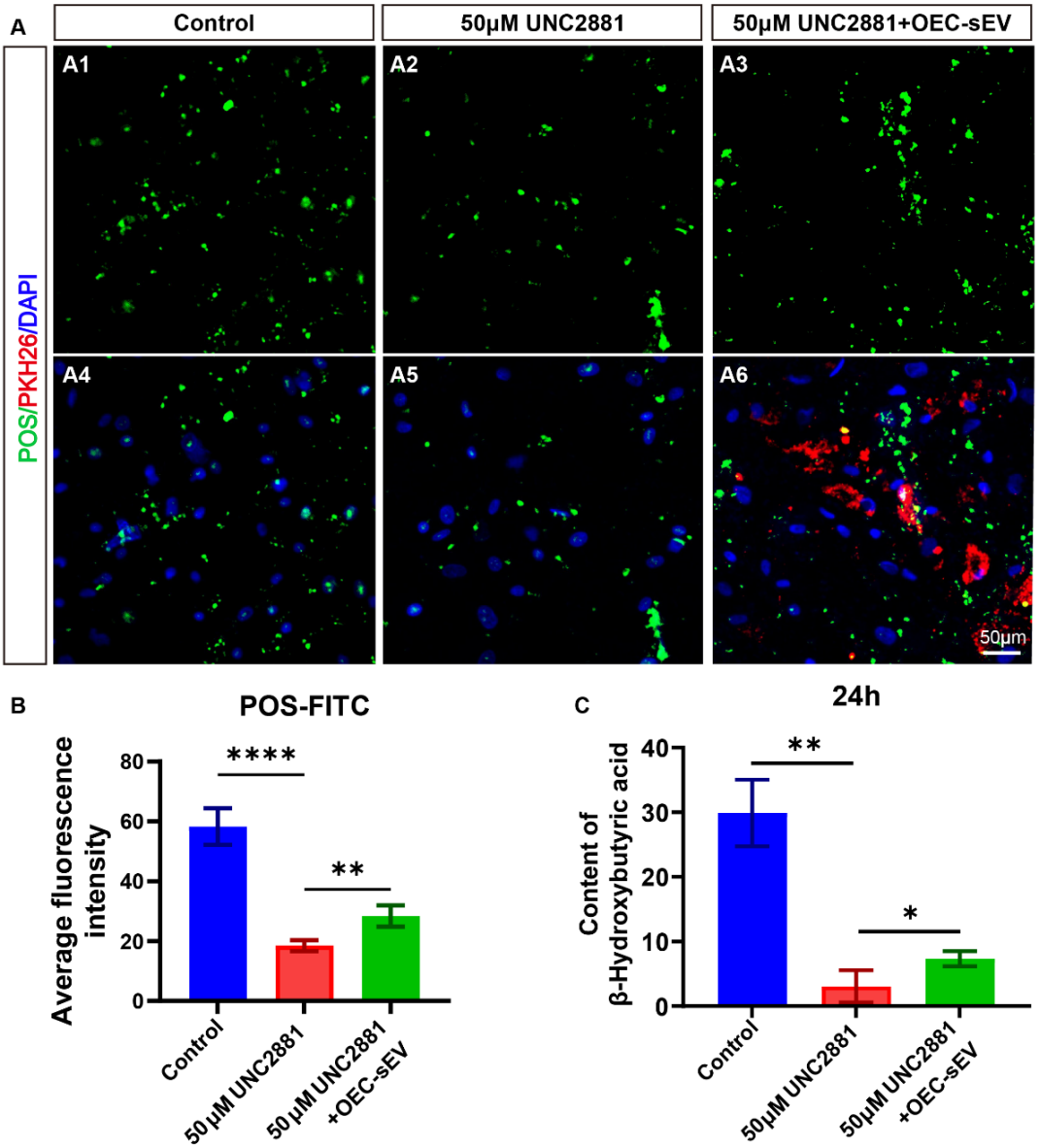



**Supplementary Figure 4.** Co-localization of OEC-sEVs with photoreceptors in the retinal pigment epithelial (RPE) layer of RCS rats. (A) PKH26-labeled OEC-sEVs (red) and Immunofluorescence staining of S-opsin (green) and DAPI (blue) at week 2 and week 4. (A1-A8) Scale bar = 50 μm. (A9-A12) Scale bar = 10 μm; (B) Average fluorescence intensity analysis of S-opsin in RCS-PBS and RCS-sEV groups at week 2 and 4; (C) Quantitative analysis of number of S-opsin positive cells within RPE layer in different groups. Arrow shows the co-localization of PKH26-labeled OEC-sEVs and S-opsin within RPE layer. $n = 4$ biologically independent rats per group. Data are represented as mean ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$. Student's *t*-test was applied.

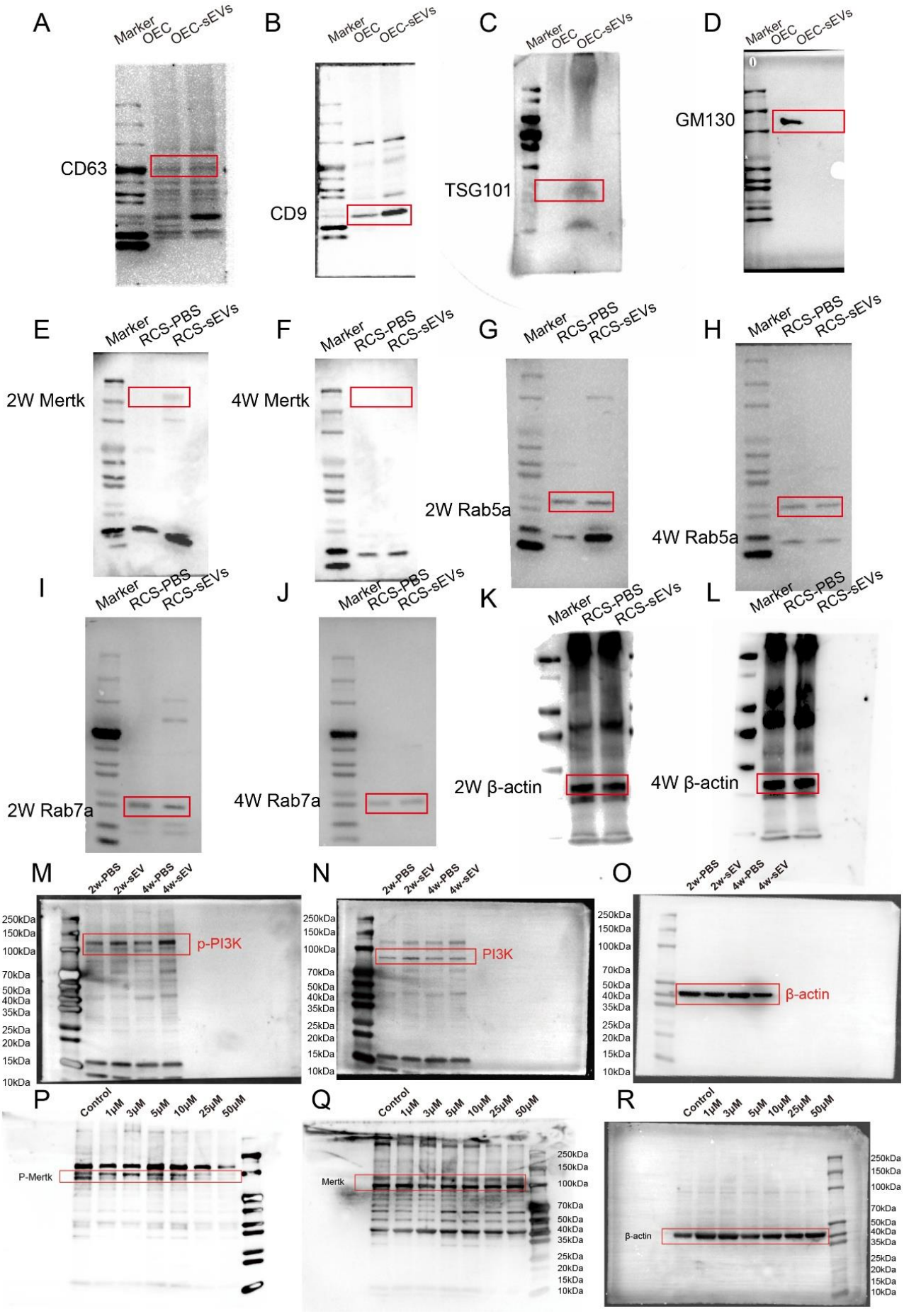



**Supplementary Figure 5.** Concentration gradient exploration of specific Mer tyrosine kinase inhibitor UNC2881. (A-D) Western blot analysis of p-Mertk and Mertk protein levels in ARPE-19 cells treated with UNC2881 (0, 1, 3, 5, 10, 25, and 50 μM); (E) Suppression effect of UNC2881 on phagocytic

activity of ARPE-19 cells. (E1 and E4) Control group without UNC2881 treatment. (E2 and E5) ARPE-19 cells treated with 5 μM UNC2881. (E3 and E6) ARPE-19 cells treated with 50 μM UNC2881. (E1-E3) Scale bar = 50 μm. (E4-E6) Scale bar = 10 μm. $n$ = 3 biologically independent cell cultures per group. For each biological replicate, three technical replicates were performed. Data are represented as mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns, no statistical difference. Student's $t$-test was applied.




**Supplementary Figure 6.** OEC-sEVs enhance phagocytosis of POS in ARPE-19 cells under UNC2881 suppression. (A) Internalization of PKH26-labeled OEC-sEVs in rat retina and FITC-labeled POS in different groups. Scale bar = 50 μm; (B) Quantification of FITC-labeled POS fluorescence intensity across groups; (C) β-hydroxybutyric acid levels in all three groups after 24 h of treatment. $n$ = 3 biologically independent cell cultures per group. Data are represented as mean ± SD. *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$. Student's $t$-test was applied.


A
Marker
OEC
OEC-sEVs
CD63
B
CD9
C
TSG101
D
GM130
E
RCS-PBS
RCS-sEVs
2W Mertk
F
4W Mertk
G
2W Rab5a
H
4W Rab5a
I
2W Rab7a
J
4W Rab7a
K
2W β-actin
L
4W β-actin
M
2w-PBS
2w-sEV
4w-PBS
4w-sEV
p-PI3K
250kDa
150kDa
100kDa
70kDa
50kDa
40kDa
35kDa
25kDa
20kDa
15kDa
10kDa
N
PI3K
O
β-actin
P
Control
1μM
3μM
5μM
10μM
25μM
50μM
P-Mertk
Q
Mertk
R
β-actin

**Supplementary Figure 7.** Full Western blot membrane. The uncropped Western blot membrane corresponding to CD63 (A, related to Figure 1G), CD9 (B, related to Figure 1G), TSG101 (C, related to Figure 1G), GM130 (D, related to Figure 1G), 2w Mertk (E, related to Figure 6D), 4w Mertk (F, related to Figure 6F), 2w Rab5a (G, related to Figure 6D), 4w Rab5a (H, related to Figure 6F), 2w Rab7a (I, related to Figure 6D), 4w Rab7a (J, related to Figure 6F), 2w β-actin (K, related to Figure 6D) and 4w β-actin (L, related to Figure 6F), p-PI3K (M, related to Figure 6H), PI3K (N, related to Figure 6H), β-actin (O, related to Figure 6H), p-Mertk (P, related to Supplementary Figure 5A), Mertk (Q, related to Supplementary Figure 5A), β-actin (R, related to Supplementary Figure 5A).