Supplementary Figure 1. MerTK expression in ARPE19 and D407 cells. (A) RT-PCR analysis of MerTK. MerTK expression in different cells were analyzed by RT-PCR using the primers of 5’-CAGCATCCGTAACAGCAGCCTGC-3’ and 5’-GCACGTAGCATTGTGGACTTG-3’. (B) Western blot analysis of MerTK. MerTK was immunoprecipitated using anti-MerTK antibodies and analyzed by Western blot using the same antibodies. HeLa and HEK293 cells were included as negative controls.
**A** Plasmid      | Catalog #      | Sense sequence targeted                          
shRNA-1       | V3LMM_439055  | 5’-ACGGCAGAAGTTCACGAGA-3’                         
shRNA-2       | V2LMM_31546   | 5’-CTGTTATATTCCCGATTAA-3’                         
shRNA-3       | V3LMM_439057  | 5’-TCGAGGAGTTCTCAGCGA-3’                          
shRNA-4       | V3LMM_439060  | 5’-GGGCTCAGATTCTGTCCA-3’                          
shRNA-5       | V3LMM_439059  | 5’-CGGAACATAAAAATGTCAA-3’                          

**Supplementary Figure 2.** MerTK is necessary for tubby- or Tulp1-mediated macrophage phagocytosis. **(A)** Sequence information of shRNA plasmids from Open Biosystems. **(B)** MerTK knockdown by shRNA. J774 macrophage cells were transfected with shRNA plasmid(s), selected with puromycin, and analyzed by RT-PCR. Additionally, MerTK in the cell lysates was directly analyzed by Western blot. **(C)** Tubby- or Tulp1-mediated macrophage phagocytosis is blocked by MerTK silencing. J774 cells were transfected with the mixture of all five shRNA plasmids, selected with puromycin, and used for phagocytosis analysis, as described (Caberoy et al., 2010a). J774 cells transfected with mock plasmid were included as a control. Confocal images of phagocytosed green fluorescence signals, DAPI signals and bright fields are superimposed. Bar = 10 µm for all. **(D)** Percentage of macrophages with phagocytosed Jurkat cells in (C) were quantified (±SEM, n≥9, *p<0.001, **p<0.005, control vs. shRNA).
Supplementary Figure 3. MerTK phosphorylation by purified tubby and Tulp1. D407 cells (5 x 10^5 cells) were treated with purified tubby and Tulp1 (~50 nM) as described in Figure 1D. MerTK was immunoprecipitated with anti-MerTK antibodies and analyzed by Western blot using anti-phospho-MerTK or anti-MerTK antibodies. Gas6 (50 nM) was included as positive control.

Supplementary Figure 4. Tubby and Tulp1 bind to phagocytosis preys. (A) Conditioned medium was collected from Neuro-2a cells expressing FLAG-tagged tubby or Tulp1 and incubated with membrane vesicles prepared from control Neuro-2a cells. After extensive washing, the vesicles were analyzed for RPE phagocytosis as in Figure 1A. Bar = 10 µm. (B) Relative fluorescence intensity per cells in (A) was quantified in more than 100 cells per group (± SEM, n>100, *p<0.001, vs. control).
**Supplementary Figure 5.** Analysis of early and later apoptosis. Apoptosis of Jurkat cells was induced with different reagents, including staurosporine for 3 h or etoposide for 16 h. Apoptotic cells were analyzed by staining with FITC-labeled annexin V and propidium iodide, and analyzed by flow cytometry.

**Supplementary Figure 6.** PPBD of Tulp1 binds to apoptotic HEK293 cells. HEK293 cells were treated with staurosporine (1 µg/ml) for 3 h to induce apoptosis. The apoptotic and healthy HEK293 cells were incubated with purified FLAG-tagged Tulp1 and Tulp1-ΔC44 mutant, and analyzed by flow cytometry as in Figure 5B and 5C.
Supplementary Figure 7. Endogenous tubby associates with purified POS vesicles. Purified POS vesicles were analyzed by Western blot using anti-Tubby-N, anti-Tubby-C and anti-β-actin antibodies. Cell lysate prepared from HEK293 cells expressing FLAG-tubby and total retinal homogenate were used as positive controls (~20 µg protein/lane).
Supplementary Figure 8. RPE phagocytosis of POS vesicles mediated by endogenous tubby is blocked by anti-Tubby-N and anti-Tubby-C antibodies. RPE phagocytosis of POS vesicles was performed as in Figure 6A in the presence or absence of anti-Tubby-N or anti-Tubby-C antibody (40 µg/ml). Control rabbit IgG was included as a negative control. Bar = 10 µm for all.
Supplementary Figure 9. Purification of FLAG-tagged tubby, Tulp1 and their mutants. FLAG-tagged proteins were expressed in HEK293 cells, purified using anti-FLAG mAb affinity columns, eluted with FLAG peptide, dialyzed and analyzed by SDS-PAGE. Estimated ~2 – 3 µg protein/lane. (A) Tulp1 and its mutants; (B) Tubby and its mutants.