Supplementary Information

Par3 and aPKC regulate BACE1 endosome-to-TGN trafficking through PACS1

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Supplementary Figures and Legends

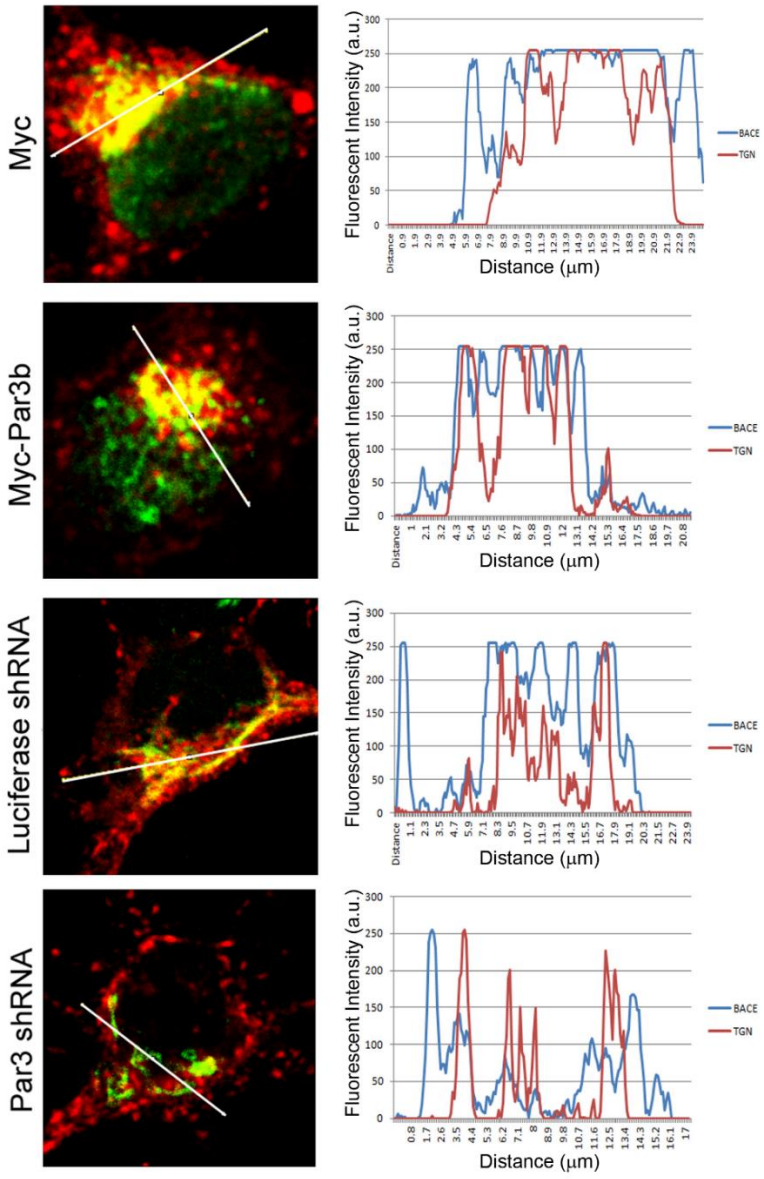

Figure S1. Line scan graphs of neurons expressing different Par3 constructs. Hippocampal neurons expressing BACE1-FLAG (red) were immuostained with an antibody to TGN (green). Line scan fluorescent intensity profiles were plotted along the white lines and the graphs are shown on the right.

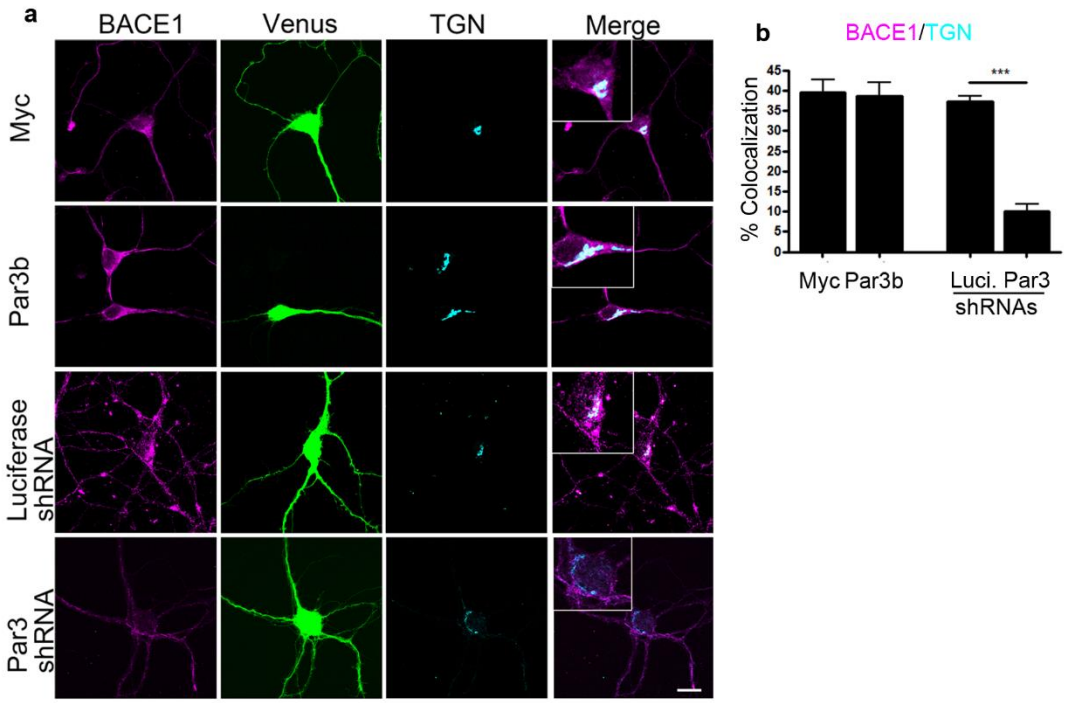

Figure S2. Effects of Par3 on endogenous BACE1 localization to the TGN in hippocampal neurons. (a) Hippocampal neurons were transfected with indicated constructs together with Venus (green). At DIV11 neurons were immunostained for endogenous BACE1 (magenta) and TGN (cyan). Merge shows the overlap between BACE1 and TGN. Scale bar: 10μm. (b) Quantification of colocalization of BACE1 and TGN. Data were expressed as Mean ± SEM with Student's t test: *** $p<0.001$.

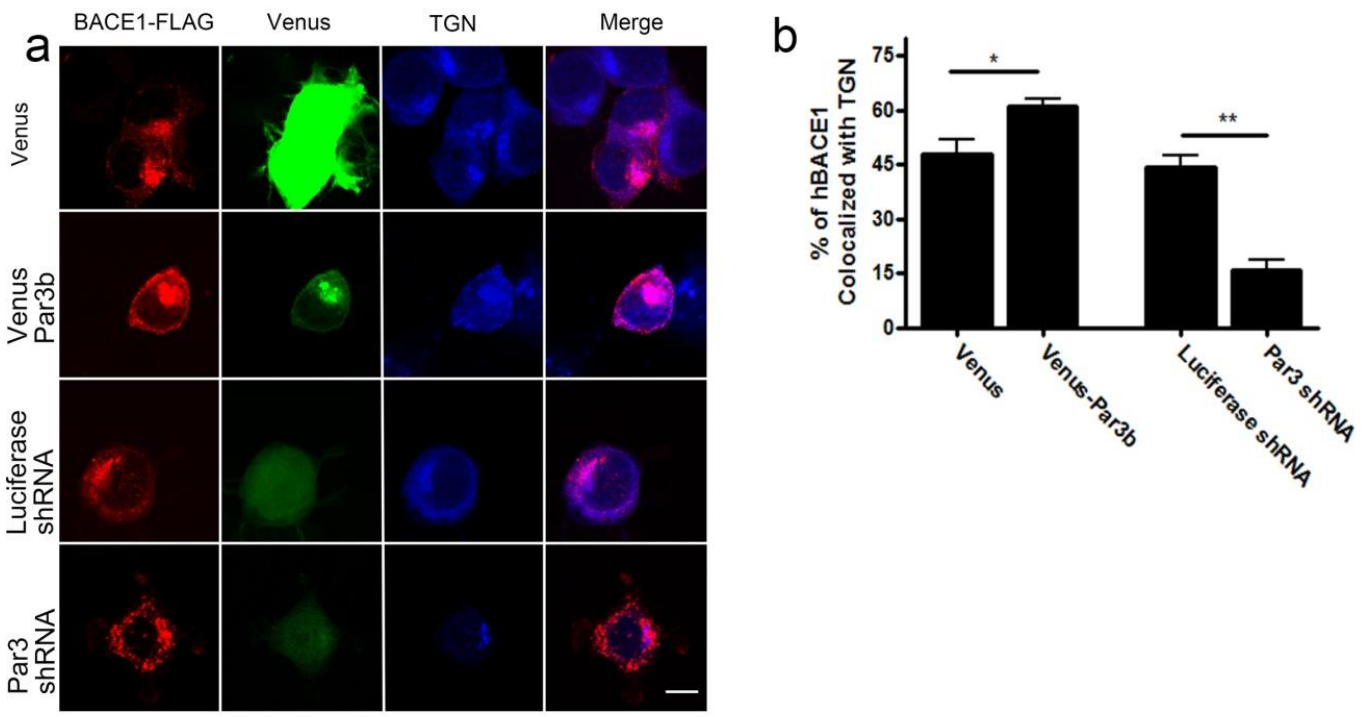

Figure S3. Effects of Par3 on BACE1 localization to the TGN in N2a cells. (a) N2a cells were transfected with indicated constructs together with BACE1-FLAG. Three days after transfection, N2a cells were immunostained for BACE1-FLAG (red) and TGN (blue). Merge shows the overlap between BACE1 and TGN. Scale bar: 20 μm. (b) Quantification of colocalization of BACE1 and TGN. Data were expressed as Mean ± SEM with Student's t test: *$p<0.05$; ** $p<0.01$.

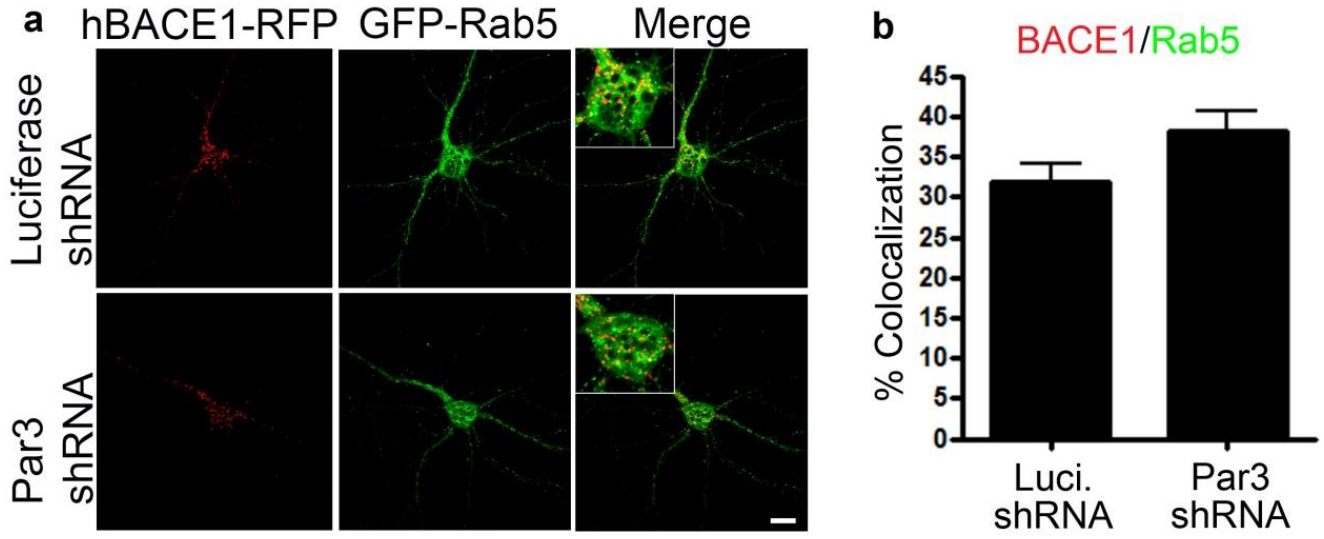

Figure S4. **Depletion of Par3 does not significantly affect BACE1 localization to Rab5 positive early endosomes in hippocampal neurons.** (a) Hippocampal neurons were transfected with indicated constructs together with hBACE1-RFP and GFP-Rab5 and imaged live on DIV11. Scale bar: 20 μm. (b) Quantification of colocalization of BACE1-RFP and GFP-Rab5.

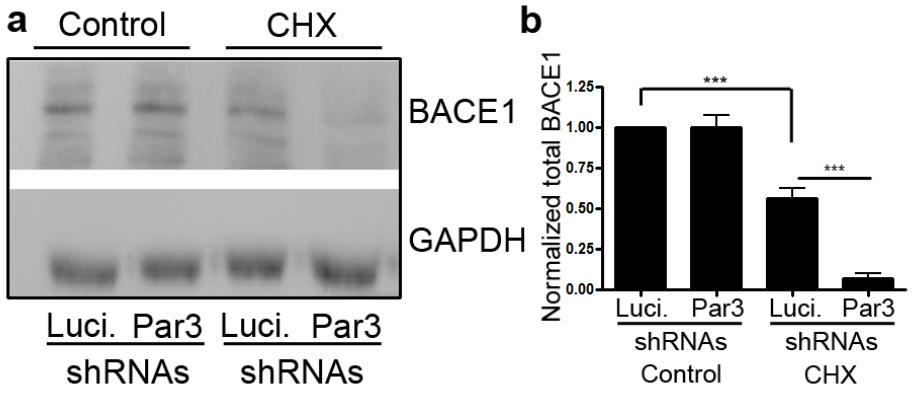

Figure S5. Par3 depletion facilitates BACE1 turnover. (a) N2a cells expressing luciferase or Par3 shRNA were treated with vehicle (Control) or 50 μM cycloheximide (CHX) for 20 hours. Cells were lysed and analyzed by Western blotting with the indicated antibodies. (b) Quantification of the blots shown in (a). ***p<0.001 by two way ANOVA, n=3.

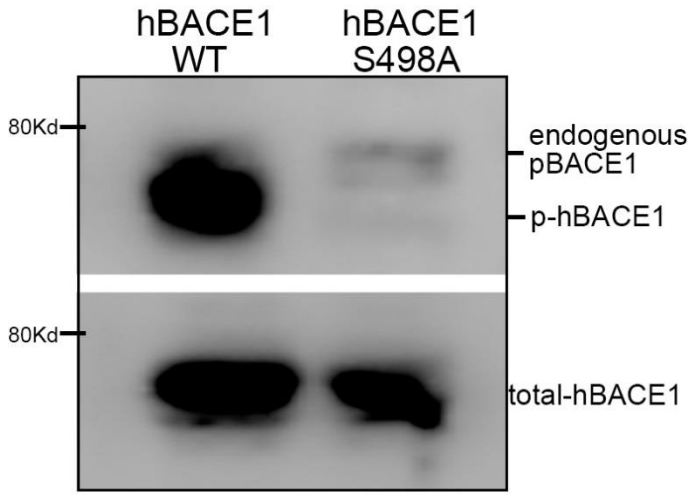

Figure S6. **Specificity of the pSer498 BACE1 antibody**. N2a cells were transfected with BACE1 wt and BACE1 S498A. Forty-eight hours later cells were lysed and subjected to Western blotting to detect phospho-Ser498 BACE1 levels.

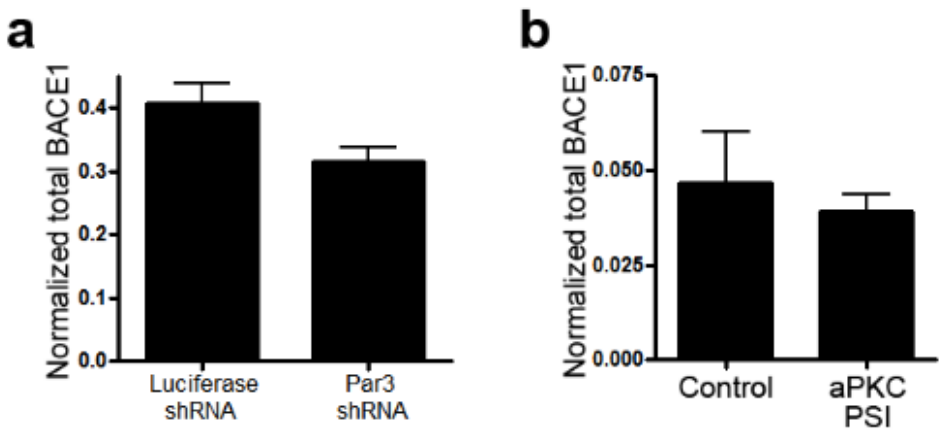

Figure S7. Total BACE1 levels were not significantly altered by Par3 shRNA or aPKC pseudosubstrate inhibitor (PSI) treatment. (a) Primary hippocampal neurons were infected with Luciferase shRNA or Par3 shRNA expressing lentivirus at DIV 0. On DIV 7 neurons were lysed and analyzed by Western blotting. Blots were quantified for total BACE1 levels normalized to GAPDH, n=3. (b) Primary hippocampal neurons were treated with aPKC pseudosubstrate inhibitor (PSI) (2µg/ml) at DIV 10 and lysed on DIV 13. Lysates were analyzed by Western blotting. Blots were quantified for total BACE1 levels normalized to GAPDH, n=3.

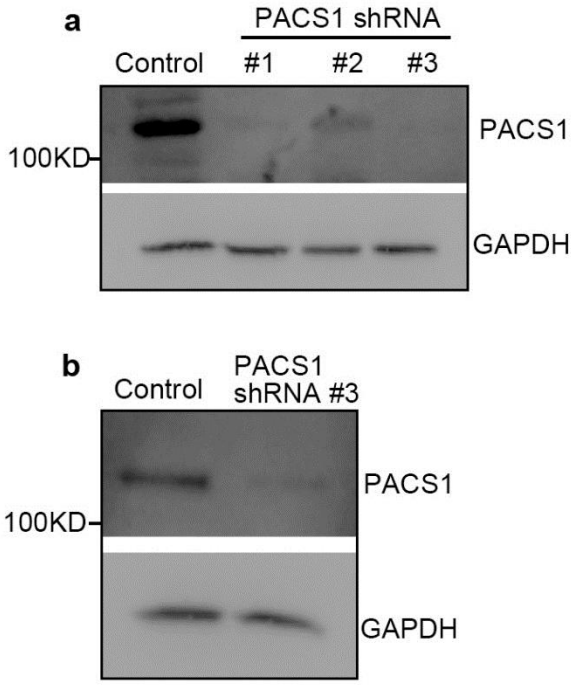

Figure S8. **Efficiency of the PACS1 shRNAs.** (a) N2a cells were transfected with indicated PACS1 shRNA and lysed 72 hours later. Total PACS1 protein levels were detected by Western blotting. (b) Rat2 cells were transfected with PACS1 shRNA #3 and lysed 72 hours later. Total PACS1 protein levels were detected by Western blotting.

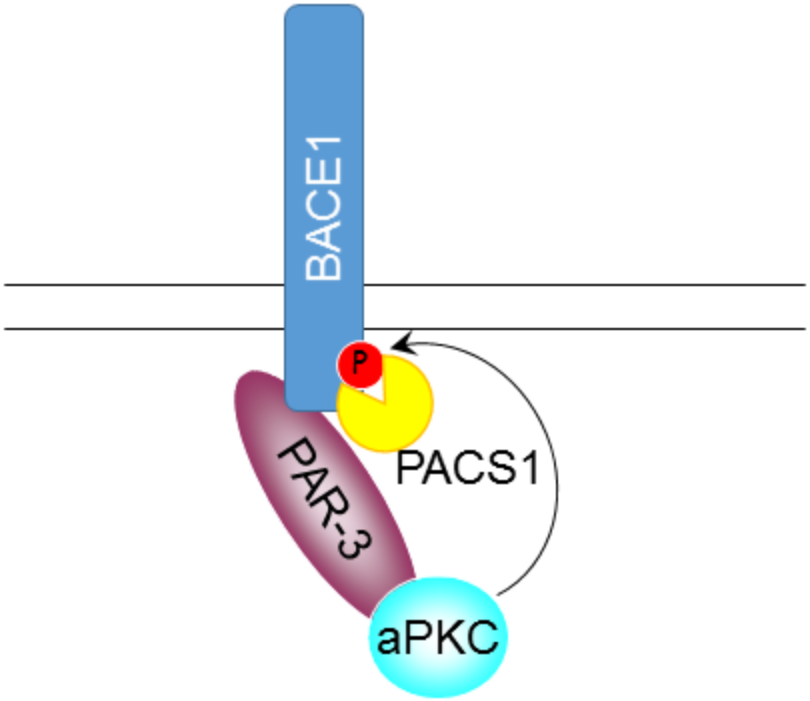

Figure S9. Working model. Par3 forms a complex with BACE1 and recruits aPKC to promote phosphorylation of BACE1 on Ser498. Phosphorylated BACE1 interacts with PACS1 and is retrogradely trafficked from the endosomes to the TGN. In the absence of Ser498 phosphroylation, BACE1 will be targeted to late endosomes/lysosomes.