Supplemental information

GPCR kinases generate an APH1A phosphorylation barcode to regulate amyloid-β generation

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Title

GPCR kinases generate an APH1A phosphorylation barcode to regulate amyloid-β generation

Authors

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Figure S1. LC-MS/MS analysis identifies sites of APH1 phosphorylation in HEK293 cells. (A) MS2 spectra of identified APH1A phosphorylation at S103 in ICL2. (B) MS2 spectra of identified APH1A phosphorylation at S110 in ICL2. (C) MS2 spectra of identified APH1A phosphorylation at S110 in ICL2. (D) MS2 spectra of identified APH1A phosphorylation at S251 in the C-terminus. (E) MS2 spectra of identified APH1A phosphorylation at S257 in the C-terminus. Related to Figure 1.
Figure S2. GRK and γ-secretase subunit expression in GRK KO cell lines.
(A-D) Western blot analysis of GRK expression in HEK293 CRISPR Control and (A) GRK2 KO, (B) GRK3 KO, (C) GRK5 KO, and (D) GRK6 KO cell lysates from 3 different passages of cells (P1, P2, and P3). (E-H) Western blot analysis of γ-secretase subunit expression in HEK293 CRISPR Control and (E) GRK2 KO, (F) GRK3 KO, (G) GRK5 KO, and (H) GRK6 KO cell lysates from 3 different passages of cells (P1, P2, and P3). Related to Figures 3 and 4 and STAR Methods Generation of HEK293 GRK Knockout Monoclonal Cell lines with CRISPR/Cas9.
Figure S3. SiRNA-mediated knockdown of GRK levels and Aβ generation.
(A) Representative Western blot analysis indicates the specificity of the siRNA-mediated knockdown of endogenous GRK2, GRK3, GRK5, and GRK6 in HEK293 cells. (B) Aβ generation in HEK293 cells following expression of APP-C99 and siRNA-mediated knockdown of endogenous GRK2, GRK3, GRK5, or GRK6. N=4 independent experiments performed in triplicate. Bars are the mean ± SEM. ns – not significant, *P < 0.05 by one-way ANOVA and the Dunnett’s post hoc test. Related to Figure 3.
Figure S4. LC-MS/MS analysis identifies APH1 S105 phosphorylation in GRK2 KO cells and expression of APH1A phosphorylation mutants, corresponding to the PathHunter and Aβ ELISAs in Figure 4.

(A) MS2 spectra of an identified APH1A S105 phosphorylated peptide in GRK2 KO cells. (B) Representative Western blot analysis of C99-FLAG, APH1A WT-PK, and APH1A-PK ICL2 phosphorylation-dead and phosphorylation-mimetic mutants. (C) Representative Western blot analysis of C99-FLAG, APH1A WT-PK, and APH1A-PK C-terminus phosphorylation-dead mutants used for PathHunter and Aβ ELISA experiments in Figure 4.
Figure S5. Interaction between the APH1A ICL2 and C-terminal mutants and βarr2 and enrichment of the γ-secretase complex subunits in DRMs.

(A) Co-immunoprecipitation experiments in HEK293 cells expressing HA-βarr2 and APH1A WT-PK or Aph1A mutants (S103A/S110A, S251A/S257A). (B) Quantification of co-immunoprecipitation experiments shows reduced interaction between the APH1A mutants and HA-βarr2 relative to APH1A WT. N = 3 independent experiments. Data are presented as mean ± SEM. ***P < 0.001 by one-way ANOVA and Dunnett’s post hoc test. (C) Sucrose gradient fractionation of HEK CRISPR control cells and GRK2 KO cells. Equivalent volumes were assessed by Western blot analysis using antibodies that recognize γ-secretase complex subunits or caveolae (Caveolin-1). (D-H) Quantification of the expression of the γ-secretase complex subunits (D) NCT, (E) APH1A, (F) PS1-NTF, (G) PS1-CTF, and (H) PEN2 in fraction 3 from CRISPR control and GRK2 KO cells were normalized to Caveolin-1 (caveolae) expression. N=3 independent experiments. Data are presented as mean ± SEM. ***P < 0.05 by unpaired t-test. Related to Figures 3 and 4.
Figure S6. Modeling of the APH1A-βarr2 complex based on structural alignment against the complex resolved for rhodopsin and the interaction between APH1A and βarr2 with the time evolution of the most stable interfacial contacts for the Alignment and DOCK1 models.

(A) The X-ray structure of rhodopsin bound to arrestin (PDB id 4zwj) was used as a template. The rhodopsin is in green and visual arrestin in yellow. (B) Alignment of APH1A (magenta ribbon, taken from γ-secretase complex; PDB id: 5a63) onto rhodopsin (green ribbon; PDB id: 4zwj) using Cevalign plugin in PyMOL. The two structures exhibit structural similarities between their TM helices, especially TM6 and TM7. (C) Structural model of the complex between APH1A and βarr2 generated after structural alignment of APH1A against rhodopsin and further refinement to optimize interfacial interactions. Additional models generated by docking simulations and the time evolution of the corresponding interfacial contacts in MD simulations are presented in Figures 5 and (D). (D) Alignment and (E) structural model DOCK1 generated by docking simulations followed by MD refinement carried out at full atomic scale in an explicit membrane (shown in gray sticks) and water. Note that the simulations were performed for the intact γ-secretase complex that displayed tight interaction between APH1A (magenta) and βarr2 (salmon), with transient involvement of other γ-secretase complex subunits such as the PS1 (cyan) and NCT (green). (F) Interactions between APH1A and βarr2 for two independent runs (40 ns each) carried out for this model (the first two columns) and corresponding time-evolution of interactions for both runs. The last column displays the cumulative fractional time during which those pairs made contacts. Here, interactions are defined when any pairs of heavy atoms belonging to the two respective proteins are separated by less than 5 Å. Related to Figure 5.
Figure S7. Effect of interfacial double mutations of APH1A on the interactions with βarr2. (A-C) The APH1A mutants were modeled using the complex structure generated by alignment as a starting conformer. (A-C) display representative conformers from MD simulations carried out in duplicates for each system. The mutated residues, S103A, S105D, S110A, and S110D of APH1A, are shown in blue stick representation and labeled. Three important interaction pairs between APH1A and βarr2 are shown in colored stick representation and labeled: D107(red, APH1A)-K285(salmon, βarr2), R109(red, APH1A)-P134(violet, βarr2), and R109(red, APH1A)-Y250(light yellow, βarr2). Regions of βarr2 including the P134 (Q131-A140), Y250 (I242-Y250), and K285 (N281-A289) are also colored. (D) The duration of the contacts of the three pairs is based on duplicate runs (total 80 ns). Here, contacts refer to heavy atom distances closer than 4 Å. Green, cyan, and orange bars are for WT APH1A, and its double mutants S103A/S110A and S105D/S110D, respectively. The mutants S103A/S110A exhibit an overall destabilization at the interface region compared to WT while the mutants S105D/S110D exhibit more stabilization than WT. There are also conformational changes in βarr2 relative to the mutations in APH1A. The system of S105D/S110D shows conformational changes in the three regions around P134 (violet), Y250 (light yellow), and K285 (salmon) compared to WT while S103A/S110A shows slight changes in the loop with P134, which indicates the stronger interface interaction of S105D/S110D induces a larger deformation of βarr2. Related to Figures 5 and 6.
Table S1. DNA sequences for each sgRNA used to generate the CRISPR GRK KO cell lines. Underlined letters indicate additional nucleotides added to the sgRNA sequence corresponding to the Bbs1 restriction site. Related to STAR Methods Generation of HEK293 GRK Knockout Monoclonal Cell lines with CRISPR/Cas9.

| Gene | Target | Direction | sgRNA Sequence |
|------|--------|-----------|----------------|
| Adrbk1 | A | Fwd | 5’-CACCGCGTCGCGCCAGCCACCACGCTCC-3’ |
|       |       | Rev | 5’-AAACCGAGGCAGGGCTGCTGGCCAGAACC-3’ |
|       | B | Fwd | 5’-CACCGGAAGAGACAGCAAGCCACGC-3’ |
|       |       | Rev | 5’-AAACGGCGTGGCCCTGGCTCTCC-3’ |
| Adrbk2 | A | Fwd | 5’-CACCGGTCGCGCTTCTTCTCTCC-3’ |
|       |       | Rev | 5’-AAACTGGAGAAGAGCAAGGCGACC-3’ |
|       | B | Fwd | 5’-CACCGCAGCAAGAGGATCGTCCTG-3’ |
|       |       | Rev | 5’-AAACGCAGAGCGATCTGCTTCG-3’ |
| Grk5 | A | Fwd | 5’-CACCGGAAAAAGCAGCTCCCGAT-3’ |
|       |       | Rev | 5’-AAACCATCGGAGGGCTGCTTTCCC-3’ |
|       | B | Fwd | 5’-CACCGCAGTTGTGTGAAACCAGGCC-3’ |
|       |       | Rev | 5’-AAACAGGCCTTGGTTACAAACTGC-3’ |
| Grk6 | A | Fwd | 5’-CACCGACTGCTCAGAAGCTGCTC-3’ |
|       |       | Rev | 5’-AAACGAGGCTGCTTCTGAGGAGCTGC-3’ |
|       | B | Fwd | 5’-CACCGATTGCTCACCAGGGTGCGG-3’ |
|       |       | Rev | 5’-AAACCCCGACCTGTGGAGCAATC-3’ |
Table S2. Sequencing primers used to validate the CRISPR GRK KO cell lines. Related to STAR Methods Generation of HEK293 GRK Knockout Monoclonal Cell lines with CRISPR/Cas9.

| Gene  | Exon | Direction | Primer Sequence                  |
|-------|------|-----------|----------------------------------|
| Adrbk1| 1    | Fwd       | 5’-CTGGTTCCGGGGTCAGATT-3’        |
|       |      | Rev       | 5’-GTCTGGGGCTTAGGGTCT-3’         |
| Adrbk2| 1    | Fwd       | 5’-AGGAAGAGGAGGAGGT-3’           |
|       |      | Rev       | 5’-ATTTCCAGAGACTGGAAACGAC-3’     |
| Grk5  | 3    | Fwd       | 5’-CAGTGTAATCAGAGAATGTGATG-3’    |
|       |      | Rev       | 5’-CTCCACTTTATAGCAATAGCAAC-3’    |
| Grk6  | 2    | Fwd       | 5’-GTCCCTTCTCCCTTTTCTCTC-3’      |
|       |      | Rev       | 5’-CTCTCTTTTCAAAAGTGAAT-3’      |