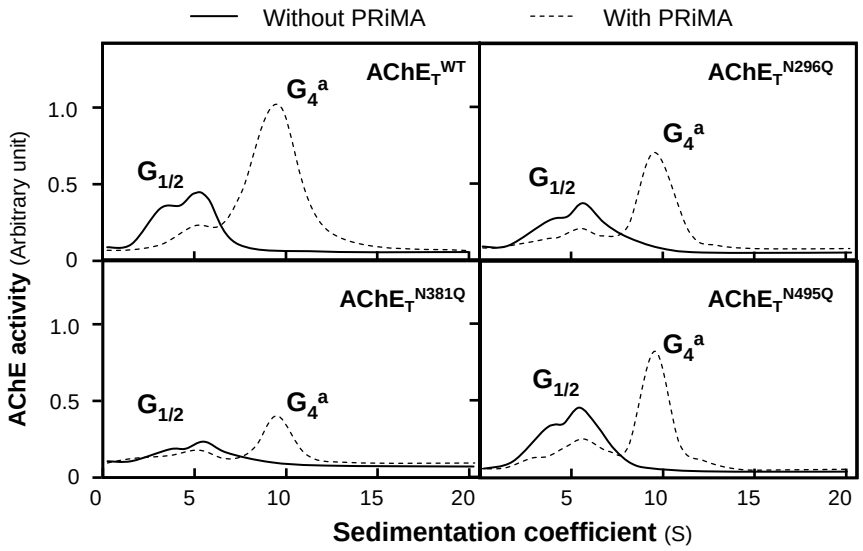
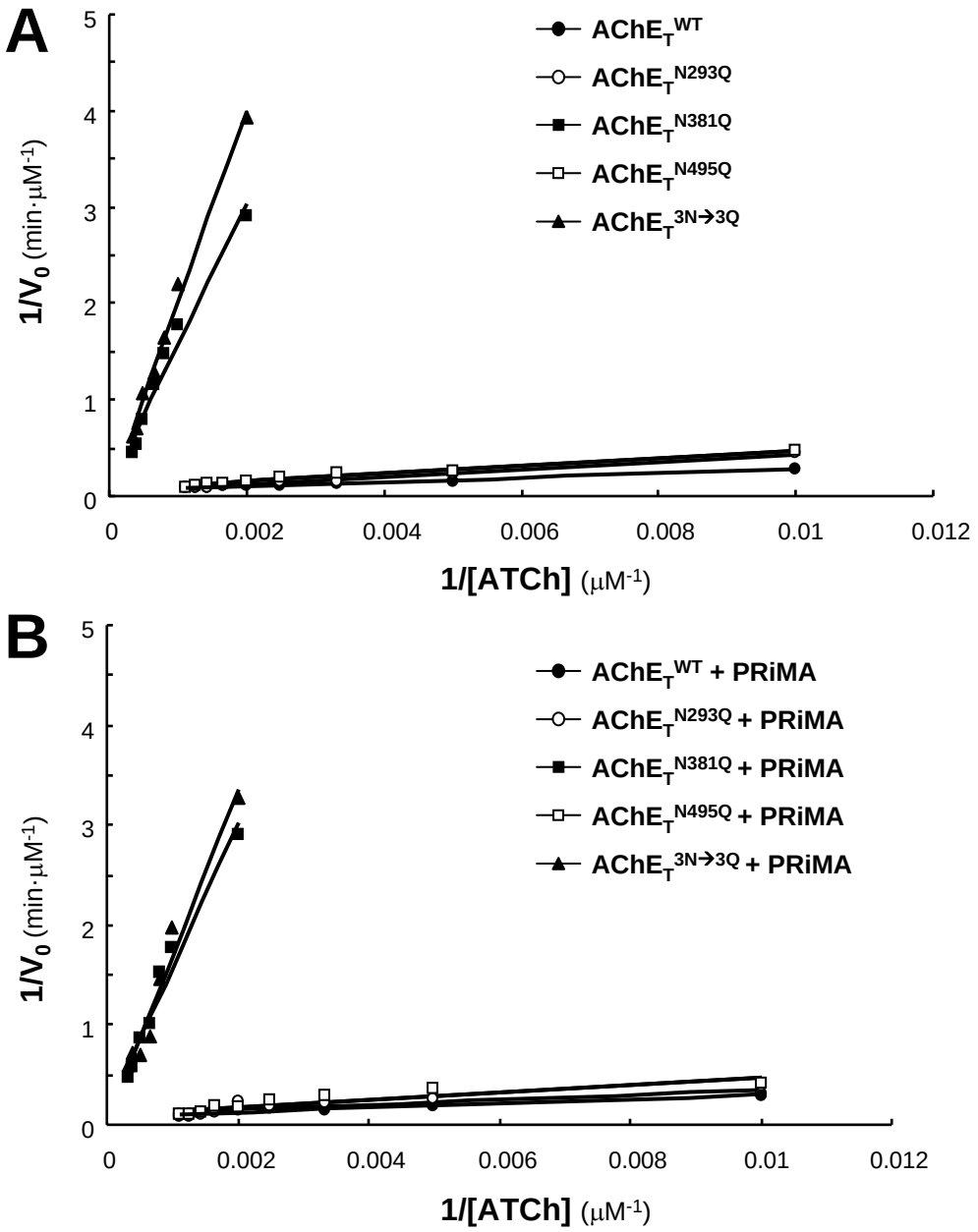


Supplementary Figure 1. N-glycosylation is not required for the assembly with PRiMA.

HEK293T cells were transfected with the cDNA encoding AChE$_T^{WT}$, AChE$_T^{N296Q}$, AChE$_T^{N381Q}$or AChE$_T^{N495Q}$ with or without PRiMA. Cell lysates containing equal amounts of total protein were subjected to sucrose density gradient analysis. The enzymatic activity is plotted as a function of the S value, estimated from the position of the sedimentation markers. The enzymatic activity is expressed in arbitrary units. Representative gradient profiles from 3 independent experiments are shown (n=3).

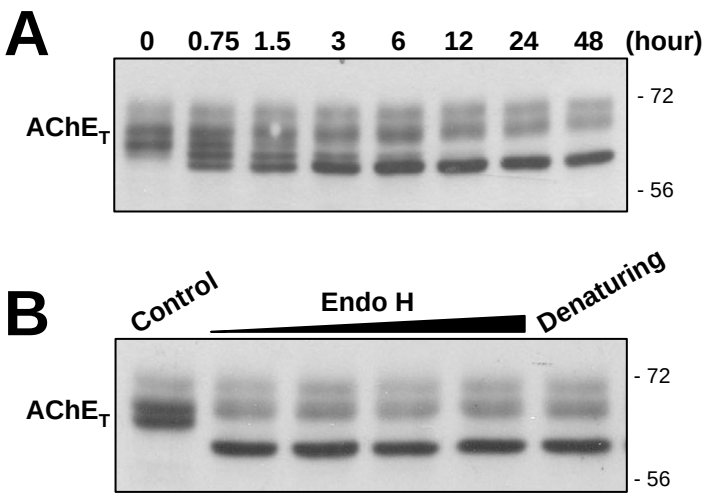


Supplementary Figure 2. Lineweaver-Burk plots of ATCh hydrolysis by $AChE_T$ and its N-glycosylation mutants.

Cell lysates from HEK293T cells expressing $AChE_T^{WT}$, $AChE_T^{N296Q}$, $AChE_T^{N381Q}$, $AChE_T^{N495Q}$ or $AChE_T^{3N→3Q}$ with (mainly G_4) or without (mainly $G_{1/2}$) PRiMA were subjected to Ellman assay using different concentrations of ATCh substrate [S] which would not cause substrate inhibition effect on AChE (900, 800, 700, 600, 500, 400, 300, 200 and 100 μM for $AChE_T^{WT}$, $AChE_T^{N296Q}$ and $AChE_T^{N495Q}$; 2500, 2000, 1500, 1000, 500 and 100 μM for $AChE_T^{N381Q}$ and $AChE_T^{3N→3Q}$). The reaction velocity [V_0] was calculated at each concentration of ATCh. K_m values were determined according to the Lineweaver-Burk plot:

$$\frac{1}{V_0} = \frac{K_M}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

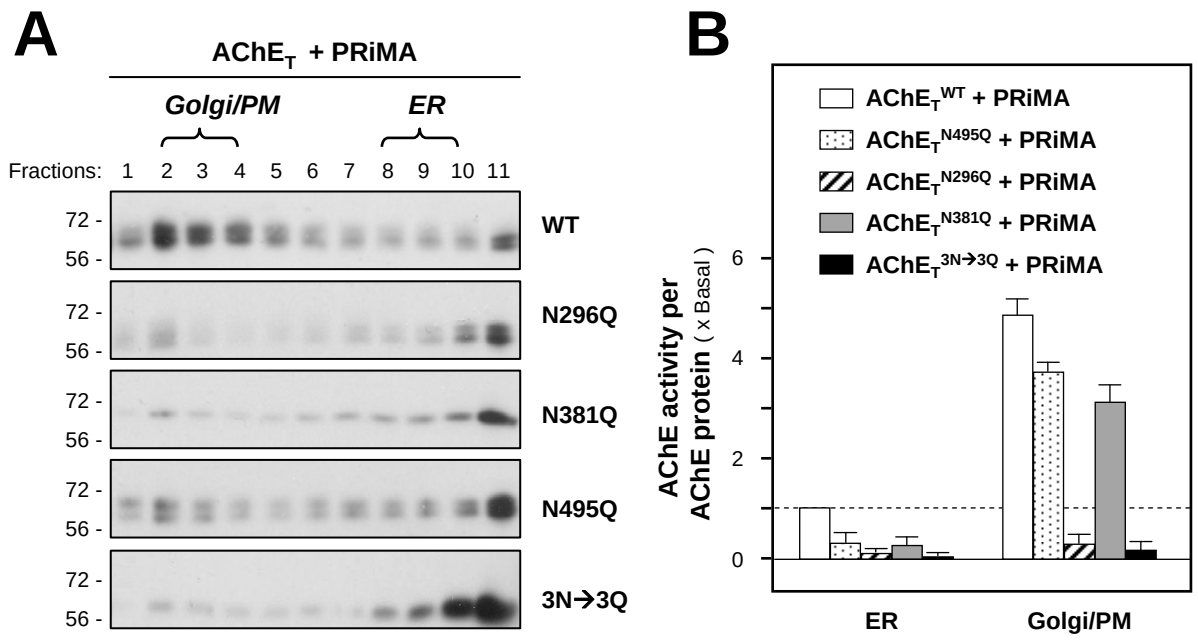
Representative profiles from 9 independent experiments are shown (n=9).



Supplementary Figure 3. N-glycans of AChE$_T$ are fully digested by Endo H in non-denaturing condition.

(A): HEK293T cells were transfected with the cDNA encoding AChE$_T$ and PRiMA. The cell lysates, containing 50 μg of total protein, were treated with 2 μl of Endo H (Roche) under non-denaturing condition (without SDS or β-mercaptoethanol) and incubated for different time. Samples were analyzed by Western blotting with anti-AChE antibody.

(B): The cell lysates from transfected cells (A) were treated with different amounts of Endo H (1, 2, 4 or 8 μl) under non-denaturing condition for 24 hours. The cell lysate treated with 2 μl of Endo H under denaturing condition served as a positive control for the complete digestion by Endo H. Samples were analyzed by Western blotting with anti-AChE antibody.



Supplementary Figure 4. N-glycosylation affects the trafficking of PRiMA-linked AChE.

(A): HEK293T cells were transfected with the cDNA encoding AChE$_T^{WT}$, AChE$_T^{N296Q}$, AChE$_T^{N381Q}$, AChE$_T^{N495Q}$ or AChE$_T^{3N→3Q}$ with PRiMA. After 48 hours, the transfected cells were subjected to subcellular fractionation. The subcellular distribution of AChE$_T$ protein was analyzed by immuno-blotting with anti-AChE antibody.

(B): AChE enzymatic activity in ER- and Golgi/PM-enriched fractions from (A) was determined by Ellman assay. The data are normalized by the amount of AChE$_T$ protein in each fraction, and expressed as the ratio to the value obtained from the ER fractions of cells co-expressing AChE$_T^{WT}$ and PRiMA, which is arbitrary set to 1. Values are means ± SEM obtained from 3 independent experiments (n=3).