

## Supplemental Figure 1. Replacement of $\alpha 1$ subunits with $\alpha 3$ subunits did not caused apparent changes on the endo H digestion patterns of $\beta 2$ subunits without or with $\gamma 2S$ subunit coexpression.

Surface proteins of HEK cells coexpressing  $\alpha 3\beta 2$  (left panel) or  $\alpha 3\beta 2\gamma 2S$  (right panel) subunits were biotinylated and pulled-down with streptavidin beads. The purified surface proteins were undigested (U) or digested with endo H (H) or PNGaseF (F) endoglycosidase. Surface proteins were then resolved by SDS-PAGE and probed with polyclonal anti- $\beta 2$  subunit antibodies.



Supplemental Figure 2. Endo H digestion did not significantly decrease the total IDV.

A, Membrane proteins of HEK cells coexpressing  $\alpha 1\beta 2$  (left panel) or  $\alpha 1\beta 2\gamma 2S$  (right panel) subunits were undigested (U) or digested with endo H (H) or PNGaseF (F) endoglycosidase. Proteins were subjected to western blotting using anti- $\beta 2$  subunit cytoplasmic loop antibodies. **B-D**, The same as in A, but the  $\beta 2$  subunits contained an Asn to Gln mutation of N32 (B), N104 (C) or N173 (D) glycosylation site. The combinations of subunit coexpression are indicated along the top of the panels. **E**, The total IDVs of endo H digested subunits were expressed as % of total IDVs of their corresponding undigested subunits. The white bars and black bars represented binary and ternary subunit coexpression, respectively. The  $\beta 2$  subunit mutations are indicated along the bottom of the graph.