5-HT_4 RECEPTORS CONSTITUTIVELY PROMOTE THE NON-AMYLOIDOGENIC PATHWAY OF APP CLEAVAGE AND INTERACT WITH ADAM10

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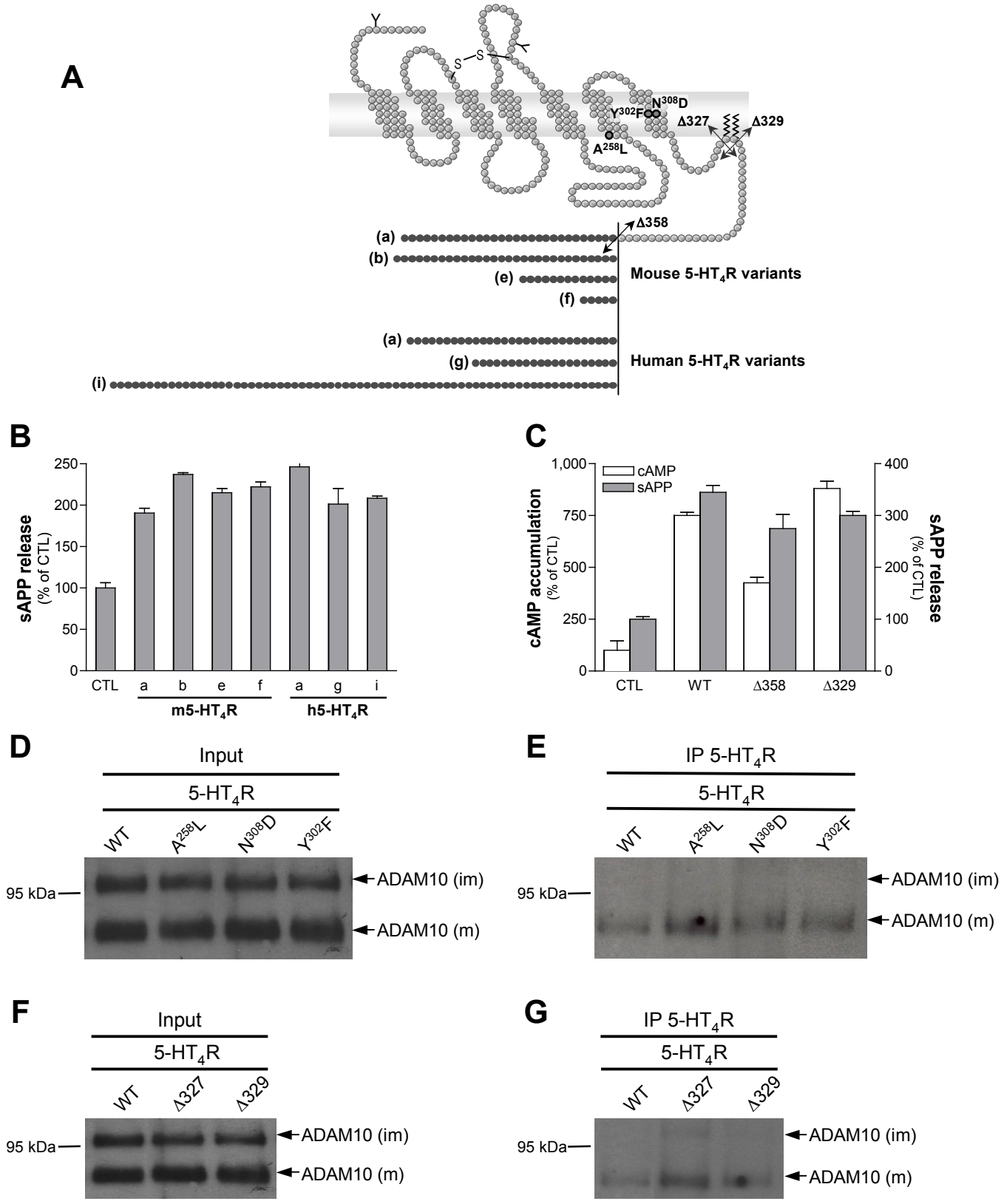
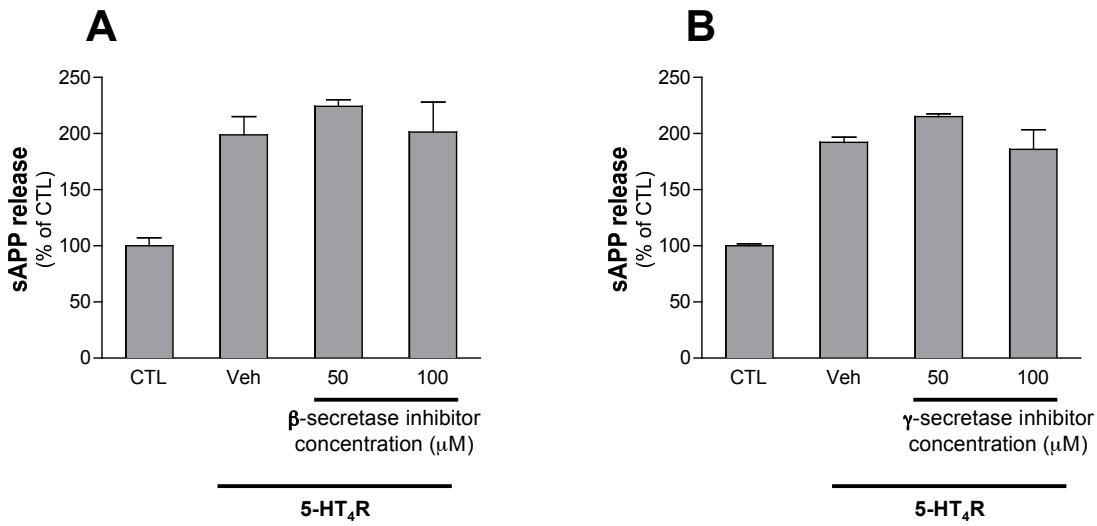


Supplemental Figure 1



Supplemental Figure 2

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: 5-HT_4R variants (mouse a, b, e and f; human a, g and i), truncated forms (Δ358 and Δ329) and mutants ($A^{258}L$, $N^{308}D$ and $Y^{302}F$) promote sAPP release and physically interact with ADAM10.

A, Schematic representation of 5-HT_4R variants and mutants. (*B*-*G*) HEK-293 cells were transiently transfected with the indicated plasmids encoding HA-tagged 5-HT_4R variants, mutants or truncated forms (200 ng/10^7 cells). *B*, *C*, 24 hrs after transfection, cAMP accumulation and sAPP release (alkaline phosphate activity) were measured. Results are the means ± SD of at least three independent experiments. *D*-*G*, Protein lysates were immunoprecipitated with the anti-HA antibody. Whole cell extracts and immunoprecipitated material were analysed by Western blotting using the anti-ADAM10 C-terminal antibody. The results are representative of three independent experiments performed with different sets of cultured cells.

Supplemental Figure 2: sAPP release induced by 5-HT_4R expression is not affected by β- or γ-secretase inhibitors.

HEK-293 cells were transiently transfected with plasmids encoding Myc-tagged 5-HT_4R (250 ng/10^7 cells) and SEAP-tagged APP (500 ng/10^7 cells). 24 hrs after transfection, cells were treated with vehicle alone or the indicated concentrations of a β-secretase (KTEEISEVN-statine-VAEF) (*A*) or a γ-secrease (L-685,458) (*B*) inhibitor for 2 hrs and sAPP release was evaluated by measuring the alkaline phosphatase activity. Data are the means ± SD of at least three independent experiments.