SUPPORTING INFORMATION

Differential Role of Serines and Threonines in Intracellular Loop 3 and C-terminal Tail of the Histamine H_4 Receptor in β-arrestin and G Protein-Coupled Receptor Kinase Interaction, Internalization, and Signaling.

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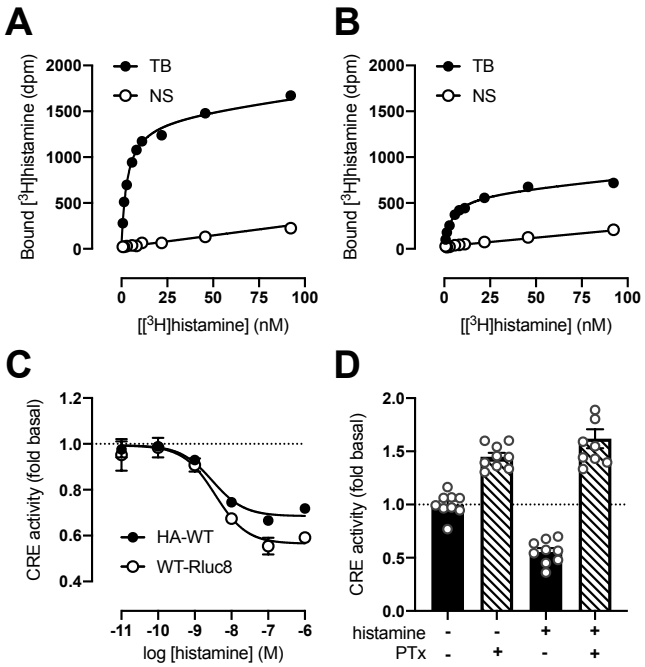

Figure S1. Pharmacological characterization of WT H_4R-Rluc8 fusion protein. Total and non-specific binding of increasing concentrations [^{3}H]histamine to HEK293T cell homogenates expressing HA-H_4R (A) or H_4R-RLuc8 (B). Graphs are representative of 3 independent experiments that were performed in triplicate. Data are shown as mean ± SEM. Inhibition of forskolin-induced CRE-driven reporter gene activity in HEK293T cells expressing H_4R-RLuc8 or HA-H_4R in response to increasing concentrations histamine (B) or 100 nM histamine following pretreatment with 100 ng/mL PTx for 16 h (C). Data are shown as mean ± SEM from at least 3 independent experiments performed in triplicate and presented as fold over vehicle-stimulated cells (i.e. basal).

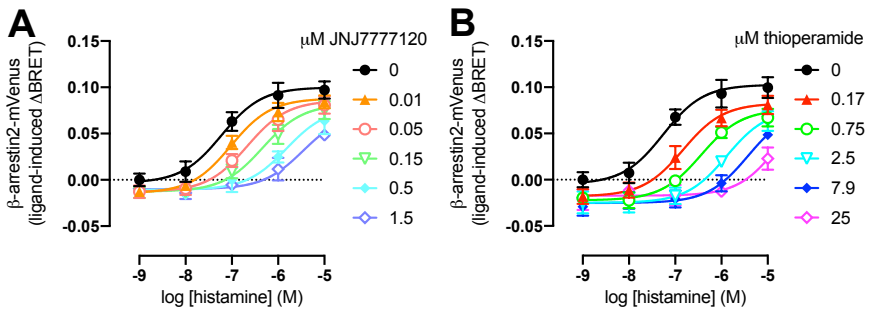

Figure S2. β-arrestin2 recruitment to H_4R. (A-C) JNJ7777120 (A) and thioperamide (B) inhibited histamine-induced β-arrestin2 recruitment in HEK293T cells coexpressing H_4R-Rluc8 and β-arrestin2-mVenus in a concentration-dependent manner. Data are shown as mean ± SEM from 3 independent experiments performed in duplicate. Ligand-induced BRET changes (ΔBRET) were calculated by subtracting BRET ratio of vehicle-treated cells.

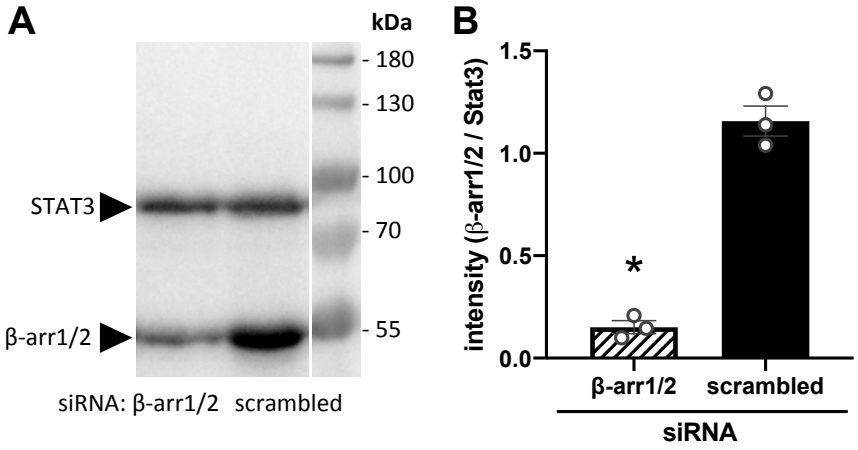

Figure S3. β-arrestin1/2 protein levels after knockdown by siRNA. (A) Representative immunoblot to evaluate β-arrestin1/2 expression in HEK293T cells cotransfected with H_4R-Rluc8 and Venus-Rab5a encoding plasmids, and scrambled or β-arrestin1/2 siRNA. STAT3 expression was used as loading control. (B) Grouped densitometric measurements from 3 independent experiments using ImageJ software (National Institutes of Health, MD, USA). Bars show means ± SEM from 3 independent experiments. Scatter plots show individual data. Statistical difference ($p < 0.05$) compared to scrambled siRNA treated cells was determined using unpaired t-test and is indicated by an asterisk (*).

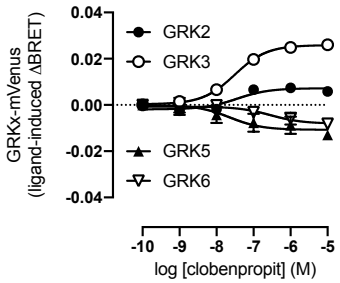

Figure S4. Effect of clobenpropit on the interaction between H_4R and GRKs. BRET measurements in HEK293T cells expressing H_4R-Rluc8 in combination with GRK2-mVenus, GRK3-mVenus, GRK5-mVenus, or GRK6-mVenus, after 30 min incubation with increasing concentrations clobenpropit. Data are shown as mean ± SEM from 3 independent experiments performed in triplicate. Ligand-induced BRET changes (ΔBRET) were calculated by subtracting BRET ratio of vehicle-treated cells.

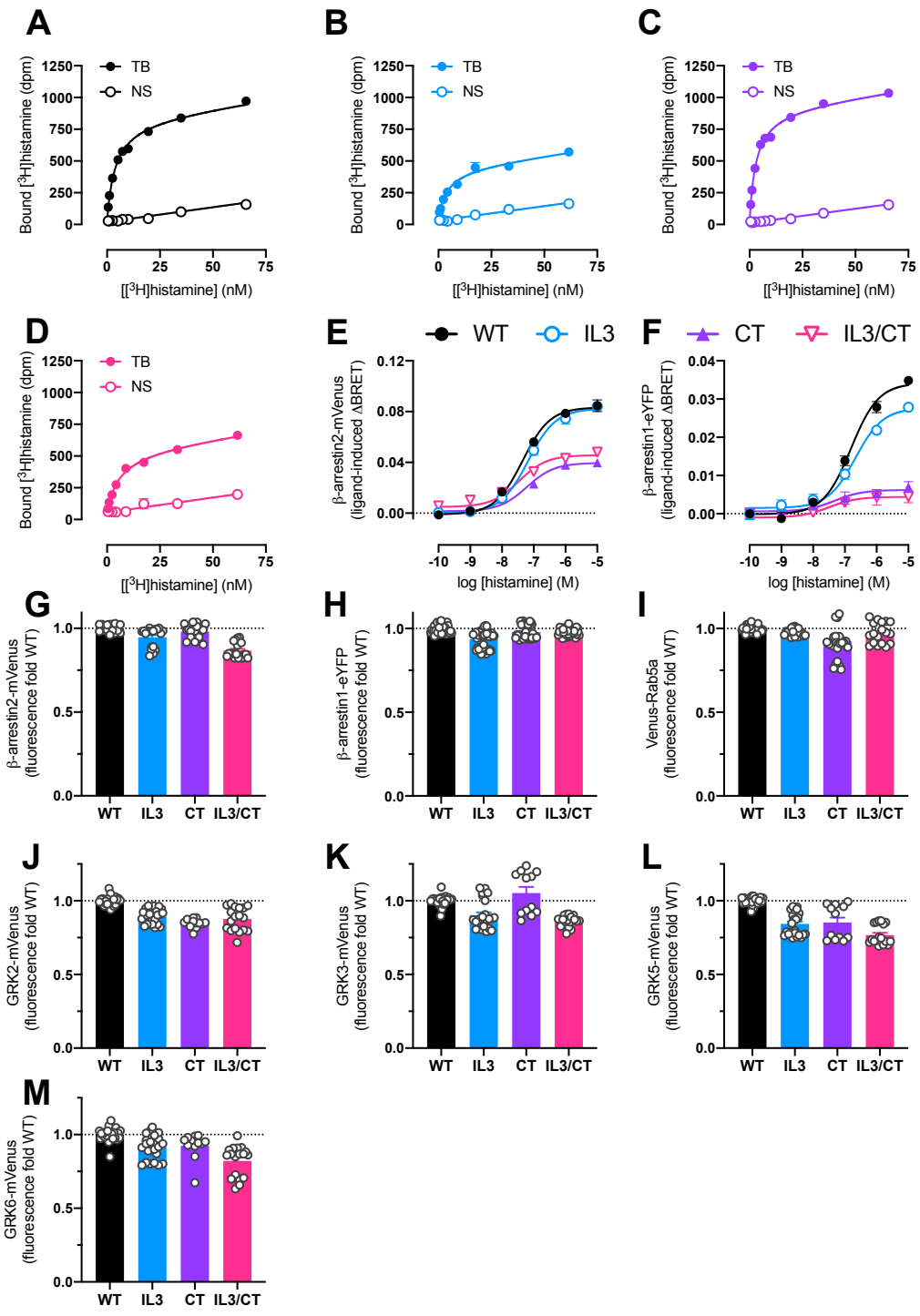

Figure S5. Pharmacological characterization of IL3 and/or CT H_4R mutants and BRET acceptor expression. Total and non-specific binding of increasing concentrations [^{3}H]histamine to HEK293T cell homogenates expressing H_4R-Rluc8 (A), H_4R-IL3-Rluc8 (B), H_4R-CT-Rluc8 (C), or H_4R-IL3/CT-Rluc8 (D). Graphs are representative of 3 independent experiments that were performed in triplicate. Data are shown as mean ± SEM. BRET measurements in HEK293T cells expressing H_4R-Rluc8 WT or mutants in combination with β-arrestin2-mVenus (E) or β-arrestin1-eYFP (F), after 30 min incubation

with increasing concentrations histamine. Data are shown as mean ± SEM from 3 independent experiments performed in triplicate. Ligand-induced BRET changes (ΔBRET) were calculated by subtracting BRET ratio of vehicle-treated cells. (G-M) Fluorescence measurements (excitation at 485 nm and emission at 535 nm) to detect expression levels of β-arrestin2-mVenus (G), β-arrestin1-eYFP (H), Venus-Rab5a (I), GRK2-mVenus (J), GRK3-mVenus (K), GRK5-mVenus (L), GRK6-mVenus (M) in HEK293T cells that co-express H_4R-Rluc8, H_4R-IL3-Rluc8, H_4R-CT-Rluc8, or H_4R-IL3/CT-Rluc8. Data are shown as mean ± SEM from 3 independent experiments and expressed as fold over WT H_4R-Rluc8.

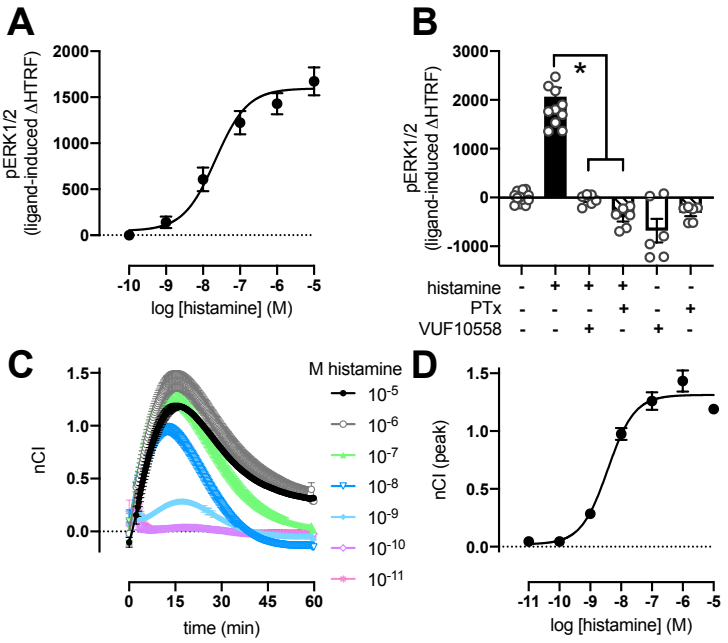

Figure S6. H_4R-induced ERK1/2 activation and cellular impedance. HA-tagged H_4R was stably expressed in HEK293 cells. Histamine-induced ERK1/2 phosphorylation was measured using an HTRF-based detection kit after 5 min incubation (A) and following pretreatment with 100 ng/mL PTx for 16 h or 100 µM H_4R antagonist VUF10558 (B). Data are shown as mean ± SEM from 3 independent experiments in duplicate, and presented as histamine-induced HTRF changes (ΔHTRF) by subtracting the HTRF ratio of vehicle-treated cells. Statistical differences between histamine-induced ERK1/2 in the absence or presence of PTx or VUF10558 were analyzed using one-way ANOVA with Dunnett's multiple comparisons test (* p<0.05). Cellular impedance changes in response to stimulation with increasing concentrations histamine in time (C) and peak response (D). Data are shown as mean ± SEM from 4 independent experiments in duplicate.