Supplemental Material

Supplementary Table 1: Primers employed in the generation of the truncated A_{2A} and H_3 receptors

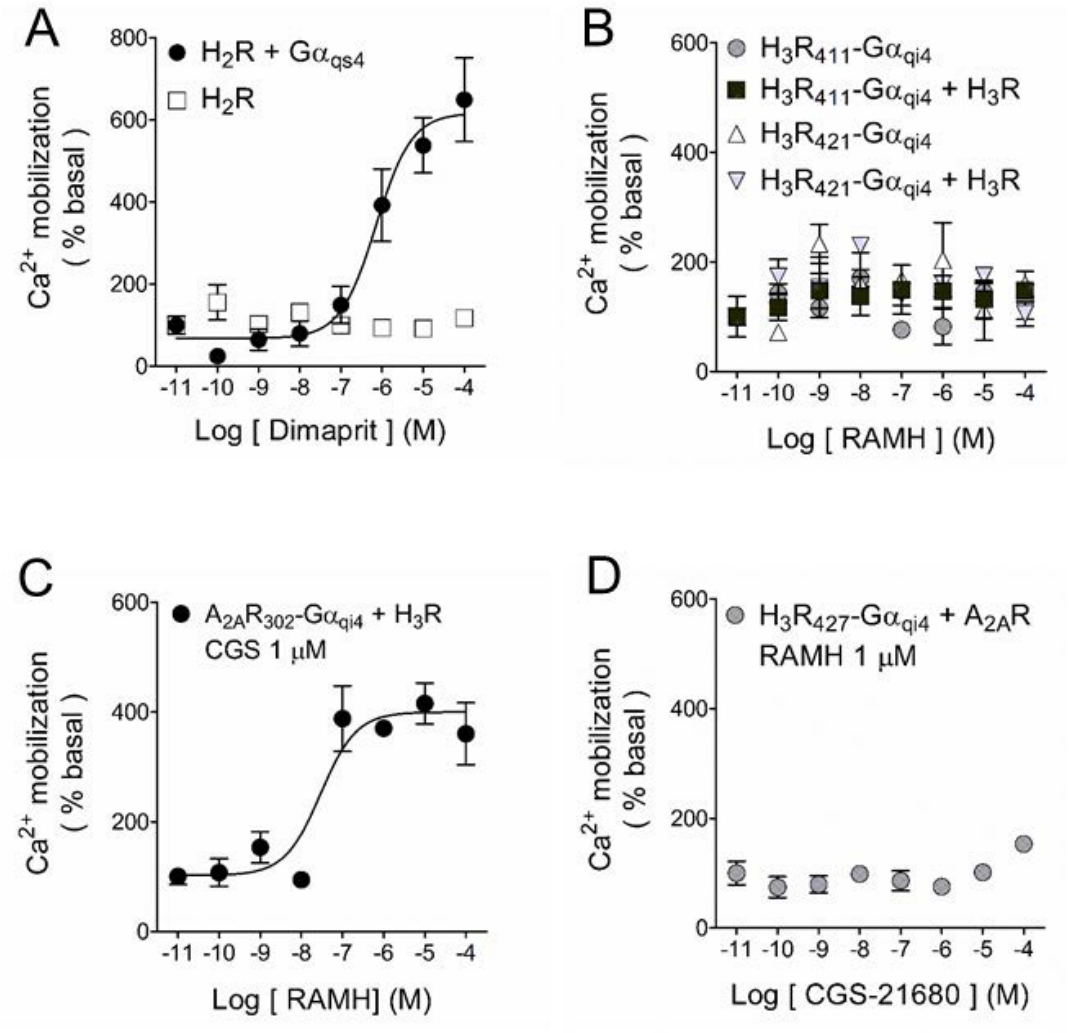
Fwd_3xHA + HindIII	5'-AAAAAGCTTATGTACCCATACGATGTTCC-3
Rev_3xHA-A_{2A}R-302 + BamHI	5'-AAAGGATCCGATCTTGCGGAAGG-3'
Rev_3xHA-H_3R-427 + BamHI	5'-AAAGGATCCCAGCAGCTTGGTG-3'
Rev_3xHA-H_3R-411 + BamHI	5'-AAGGATCCAGGGTAGAGGACAGGGTTGAC-3'
Rev_3xHA-H_3R-421 + BamHI	5'-AAAGGATCCCCGGCGGAAGCTGTGGTG-3'

Supplementary Table 2. Pharmacological data of the functional complementation assays performed.

Transfection	Agonist	Emax (%)	pEC_{50}	EC_{50} (nM)
H_3R	RAMH	-	-	-
	Histamine	-	-	-
$H_3R + G\alpha_{qi4}$	RAMH	419 ± 25	7.73 ± 0.31	19.95
	Histamine	504 ± 22	6.19 ± 0.17	645.65
$A_{2A}R$	CGS	-	-	-
$A_{2A}R + G\alpha_{qs4}$	CGS	-	-	-
$A_{2A}R_{302}$-$G\alpha_{qi4}$	CGS	-	-	-
$A_{2A}R_{302}$-$G\alpha_{qs4}$	CGS	-	-	-
$A_{2A}R_{302}$-$G\alpha_{qi4} + A_{2A}R$	CGS	-	-	-
$A_{2A}R_{302}$-$G\alpha_{qs4} + A_{2A}R$	CGS	-	-	-
H_3R_{427}-$G\alpha_{qi4}$	RAMH	753 ± 41	7.14 ± 0.21	72.44
H_3R_{427}-$G\alpha_{qs4}$	RAMH	-	-	-
H_3R_{427}-$G\alpha_{qi4} + H_3R$	RAMH	523 ± 41	7.38 ± 0.34	41.68
H_3R_{427}-$G\alpha_{qs4} + H_3R$	RAMH	-	-	-
H_3R_{411}-$G\alpha_{qi4}$	RAMH	-	-	-
H_3R_{421}-$G\alpha_{qi4}$	RAMH	-	-	-
H_3R_{421}-$G\alpha_{qi4} + H_3R$	RAMH	-	-	-
H_{411}-$G\alpha_{qi4} + H_3R$	RAMH	-	-	-

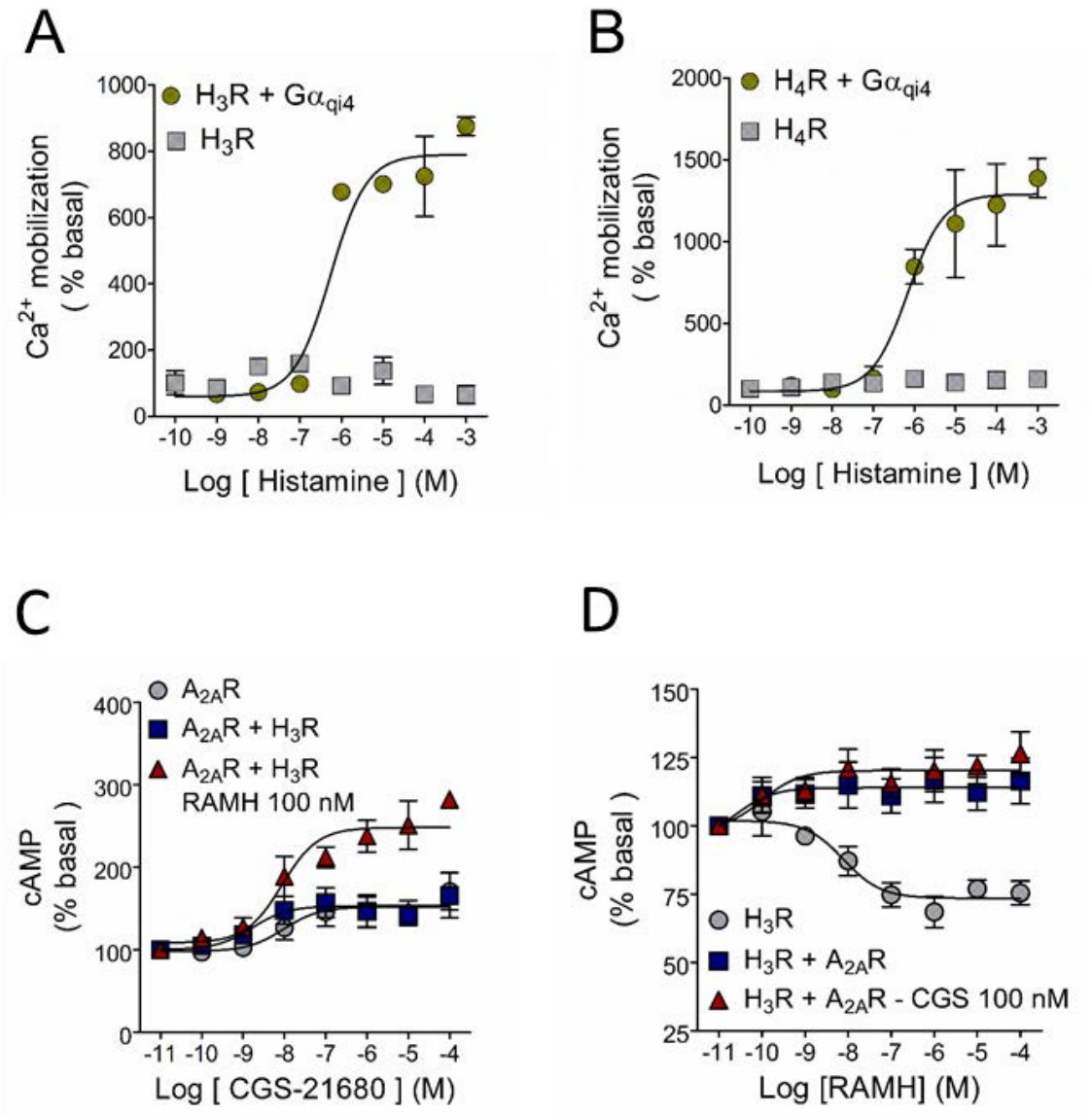
$A_{2A}R_{302}$-Gα_{qi4} + H_3R	RAMH	449 ± 25	7.31 ± 0.23	48.97
	CGS	429 ± 14	7.63 ± 0.15	23.44
	+ RAMH			
$A_{2A}R$-Gα_{qs4} + H_3R	RAMH	-	-	-
H_3R_{427}-Gα_{qs4} + $A_{2A}R$	CGS	-	-	-
H_3R_{427}-Gα_{qi4} + $A_{2A}R$	CGS	-	-	-
	CGS	-	-	-
	+ RAMH			
H_4R	Histamine	-	-	-
	RAMH	-	-	-
H_4R + Gα_{qi4}	Histamine	725 ± 51	6.35 ± 0.25	446.68
	RAMH	551 ± 27	6.15 ± 0.16	794.32
$A_{2A}R_{302}$-Gα_{qi4} + H_3R	Histamine	-	-	-
$A_{2A}R_{302}$-Gα_{qi4} + H_4R	Histamine	-	-	-
$A_{2A}R_{302}$-Gα_{qi4} + D_2R	Quinpirole	612 ± 34	6.47 ± 0.22	338.84
D_2R	Quinpirole	-	-	-
H_2R + Gα_{qs4}	Dimaprit	860 ± 37	6.15 ± 0.12	707.94
H_2R	Dimaprit	-	-	-

Data are means ± SEM from 3 experiments. RAMH and CGS-21680 were tested at 100 nM. RAMH: R-α-methylhistamine; CGS: CGS-21680.



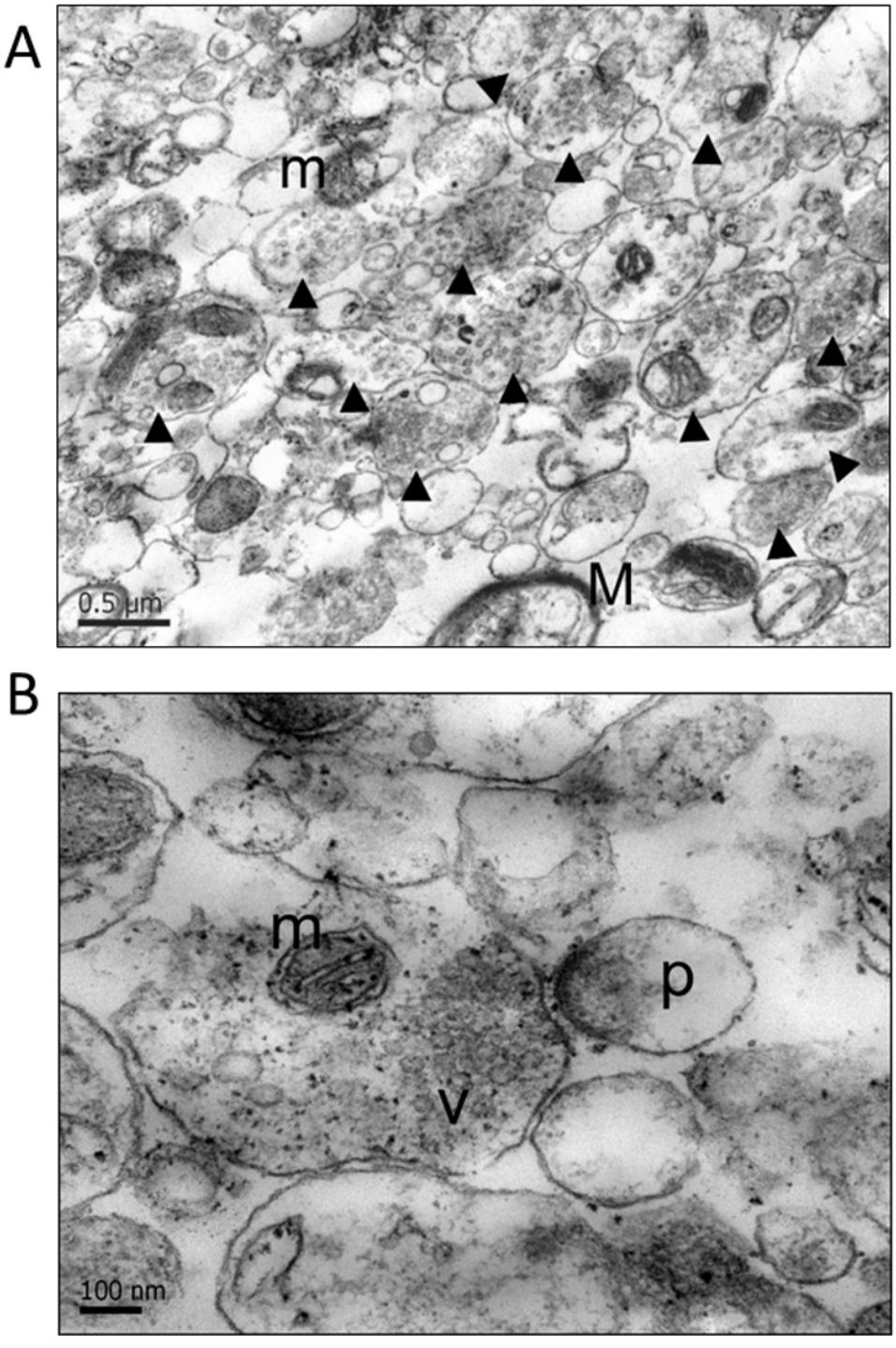
Supplementary Figure 1. A. The activation of the $G\alpha_s$-coupled histamine H_2 receptor (H_2R) did not induce Ca^{2+} mobilization when transfected alone, but did so upon co-expression with $G\alpha_{qs}$, proving the functionality of the chimeric protein. B. The truncated H_3Rs of 421 and 411 residues were unable to induce Ca^{2+} mobilization and functional complementation was not observed by homodimerization with either receptor. C. Functional complementation assay showing that the co-activation of the WT-H_3R with increasing concentrations of RAMH and the $A_{2A}R_{302}$-$G\alpha_{qs4}$ with a fixed concentration of CGS-21680 did not modified the response. D. The co-activation of the H_3R_{427}-$G\alpha_{qi4}$ and

the WT-$A_{2A}R$ did not recover the Ca^{2+} response observed when the second construct was transfected alone. For all graphs data are means ± SEM from 3 replicates from representative experiments. Where SEM bars are not visible, they are smaller than the symbol size. The quantitative analysis is shown in Supplementary Table 2.

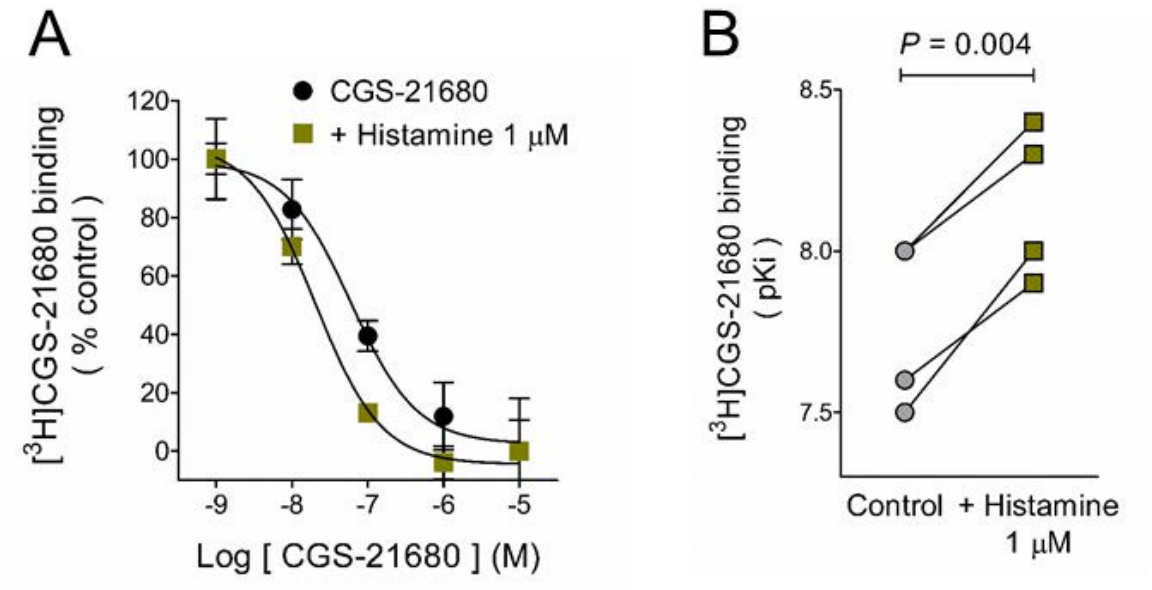


Supplementary Figure 2. Effect of histamine and RAMH on H_3R- and H_4R-mediated Ca^{2+} mobilization and cAMP formation induced by the $A_{2A}R/H_3R$ heterodimer A. Histamine induced Ca^{2+} mobilization by activation of the H_3R only when the $G\alpha_{qi4}$ was expressed. B. Activation of the H_4R with histamine caused a Ca^{2+} response promoted by the $G\alpha_{qi4}$. No response was observed by the solely transfection of the H_4R. C. $A_{2A}R$-mediated cAMP formation is increased by co-activation of the H_3R. No change in the CGS-21680 efficacy or potency was observed when the H_3R was co-expressed with the $A_{2A}R$. D. $A_{2A}R$ co-

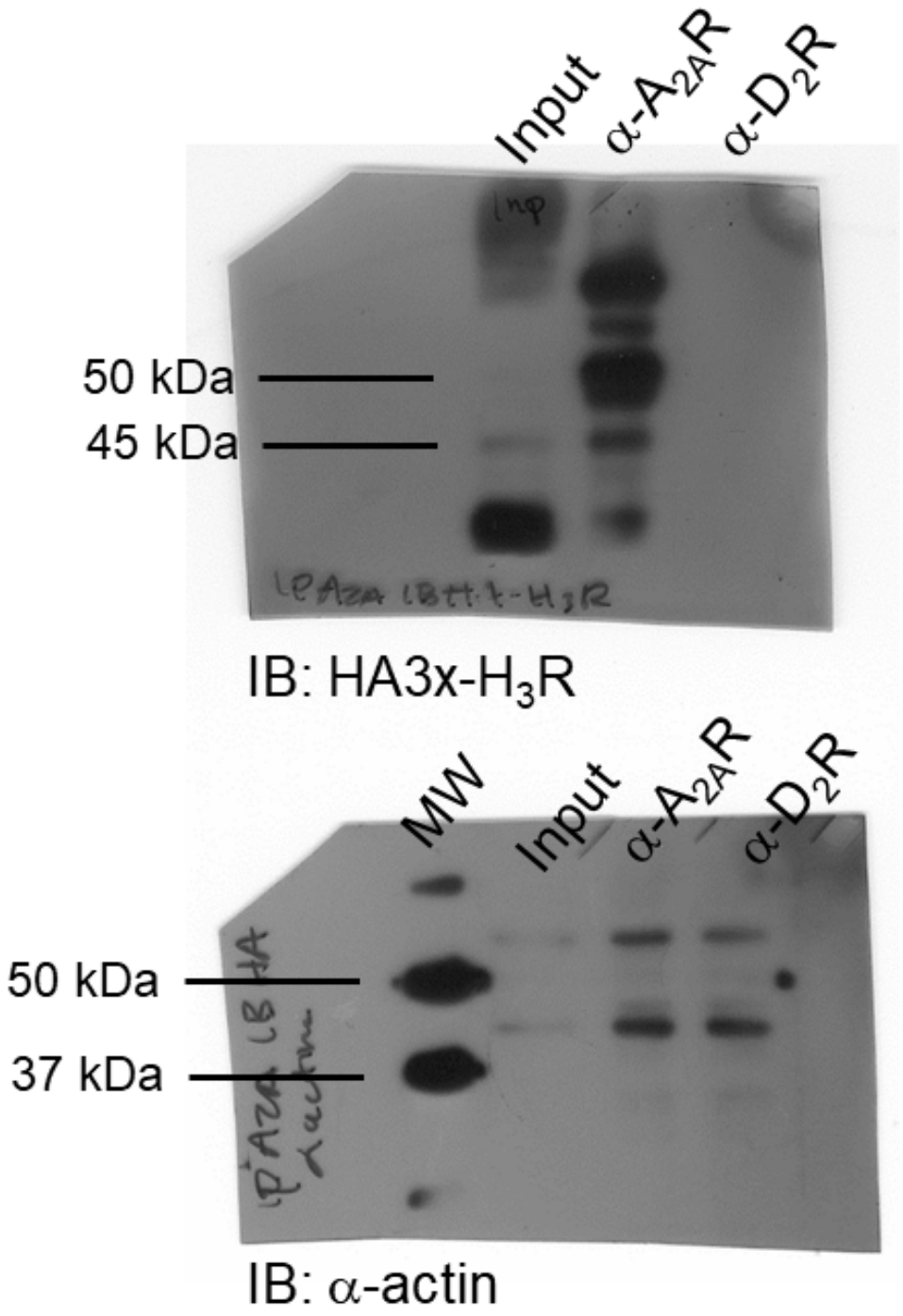
expression and co-activation prevented H_3R-mediated cAMP inhibition, suggesting the prevalence of $A_{2A}R$ signaling in the heterodimer. For A and B panels, data are means ± SEM from 3 replicates from representative experiments. The quantitative analysis is shown in Supplementary Table 2. For C and D panels, cAMP formation is expressed as percentage of the basal. Means ± SEM are shown in Table 2. Where SEM bars are not visible, they are smaller than the symbol size.


A
m
M
0.5 µm
B
m
p
v
100 nm

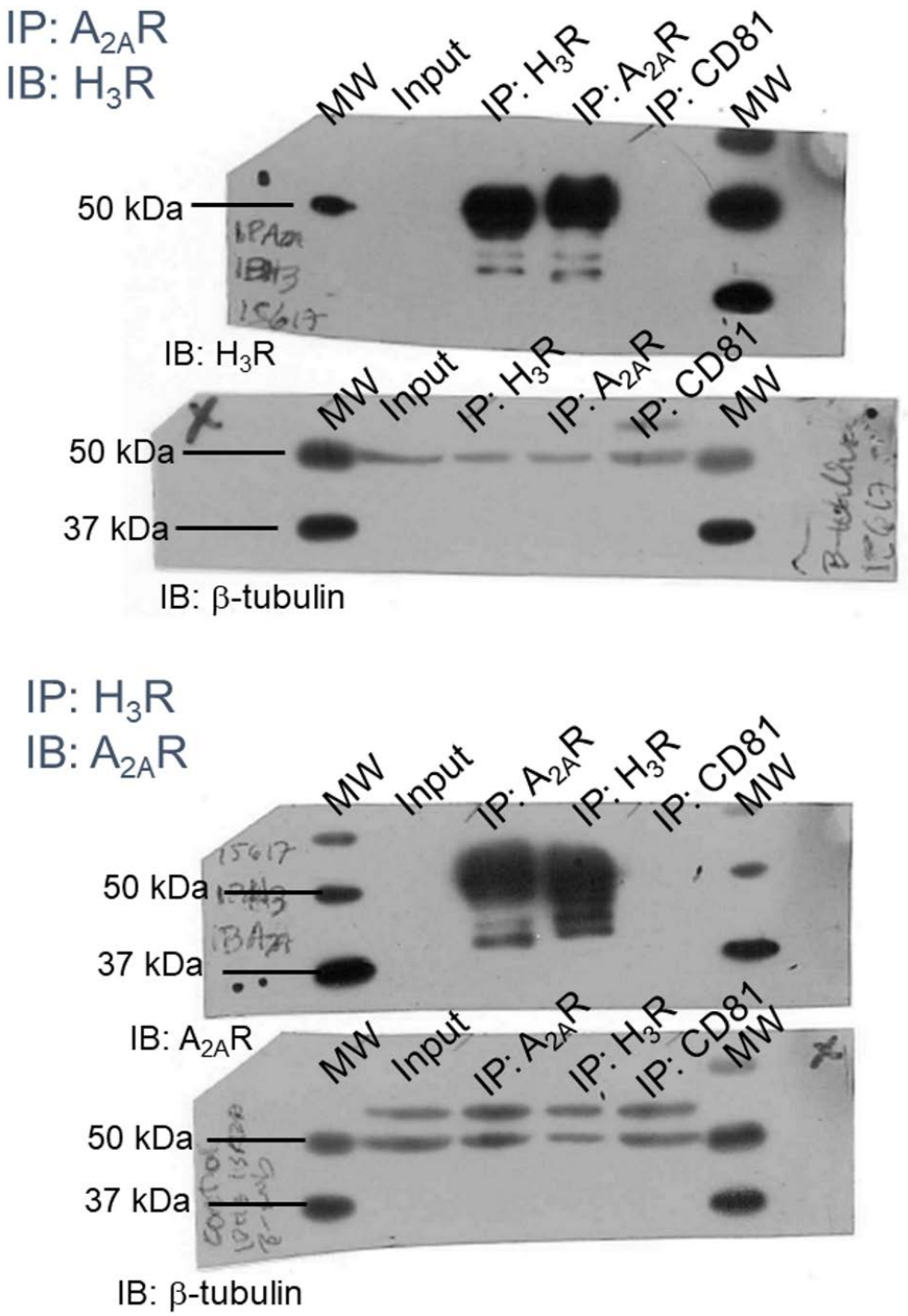
Supplementary Figure 3. Transmission electron microscopy of rat striatal synaptosomes. A. The presence of isolated terminals is indicated by the arrow heads. Other common components such as mitochondrion (m) and myelin fragments (M) can also be observed. B. Close view of a pre-synaptic terminal, distinguishable by the synaptic vesicles (v) and mitochondria, making contact with a post-synaptic element (p). The pre-synaptic active zone and the post-synaptic density can be distinguished.



Supplementary Figure 4. Effect of the endogenous H_3R agonist histamine on the $A_{2A}R$ affinity for its agonist [^{3}H]CGS-21680. A. Histamine (1 μM) increased the $A_{2A}R$ affinity for CGS-21680. Values are means ± SEM from 3 replicates from a representative experiment. B. Analysis of 4 independent experiments. The statistical analysis was performed with Student's paired *t* test.



Supplementary Figure 5. Blots of the co-immunoprecipitation experiments in HEK-293 cells. Upper blot: Co-immunoprecipitation of the $A_{2A}R$ and immuno-detection of the HA3x-H_3R. A D_2R antibody was used as a negative control. Lower blot: loading control (α-actin).



Supplementary Figure 6. Blots of the $A_{2A}R$ and H_3R co-immunoprecipitation in striatal synaptosomal samples and loading control (β-tubulin).