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Supplemental information

Dual pancreatic adrenergic and dopaminergic signaling as a therapeutic target of bromocriptine

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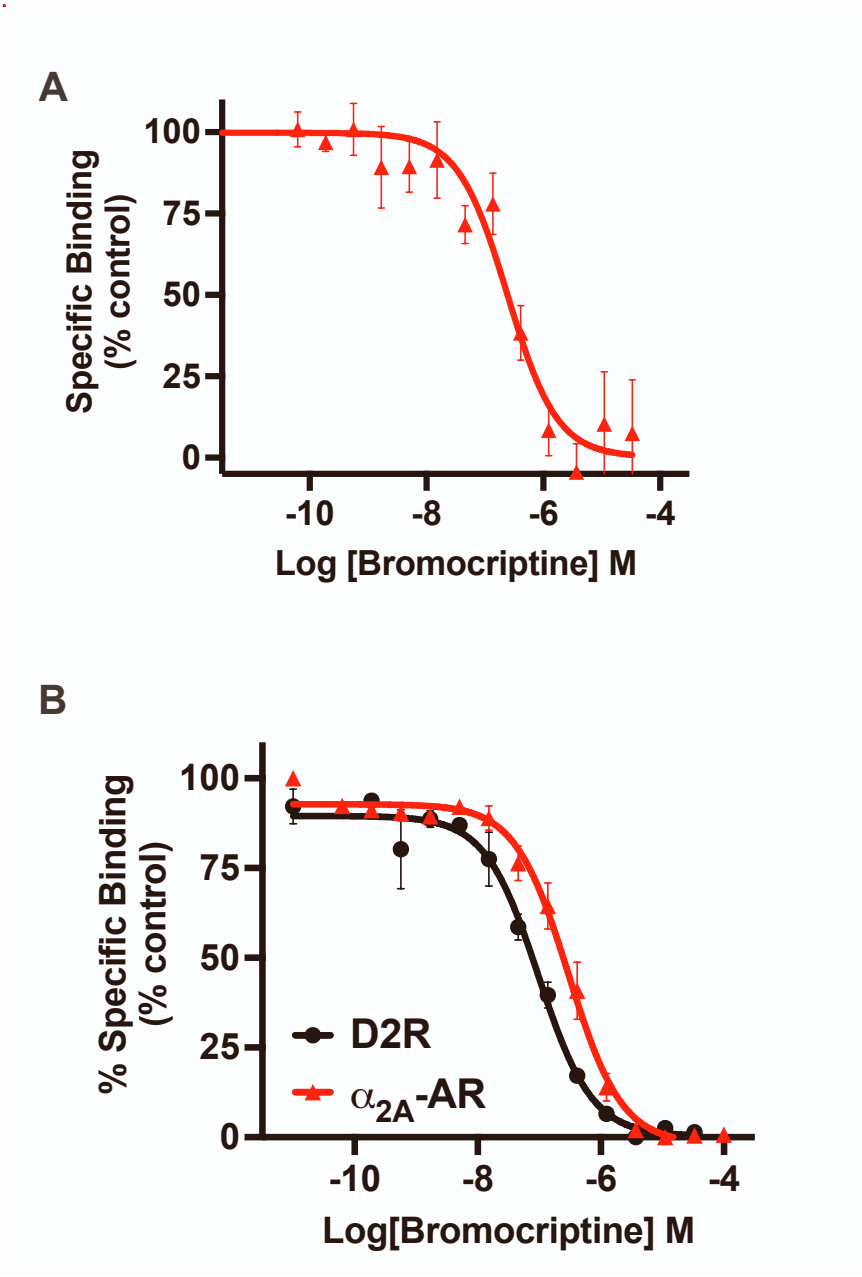

Figure S1. Bromocriptine binds directly to D2R and α_{2A}-AR, related to Figure 1. (A) Radioligand competition with bromocriptine at endogenously expressed α_{2A}-AR in INS-1E cells. Competition binding curve of the α_{2A}-AR antagonist [^{3}H]-RX821002 versus increasing concentrations of bromocriptine showed binding of bromocriptine to endogenous α_{2A}-AR expressed in INS-1E cells (K_i=161.8 nM). Radioligand experiments were normalized to % maximal binding. **(B)** Competition binding curves featuring increasing concentrations of bromocriptine using membranes prepared from HEK-293 cells transiently overexpressing α_{2A}-AR (in red) or D2R (in black) versus [^{3}H]-RX821002 or [^{3}H]-N-methylspipirone, respectively. Bromocriptine demonstrated 4.6-fold higher affinity for D2R (K_i= 43.2 nM) compared to α_{2A}-AR (K_i= 198.7 nM). Data represent mean ± SEM performed in triplicate from n=4 independent experiments.

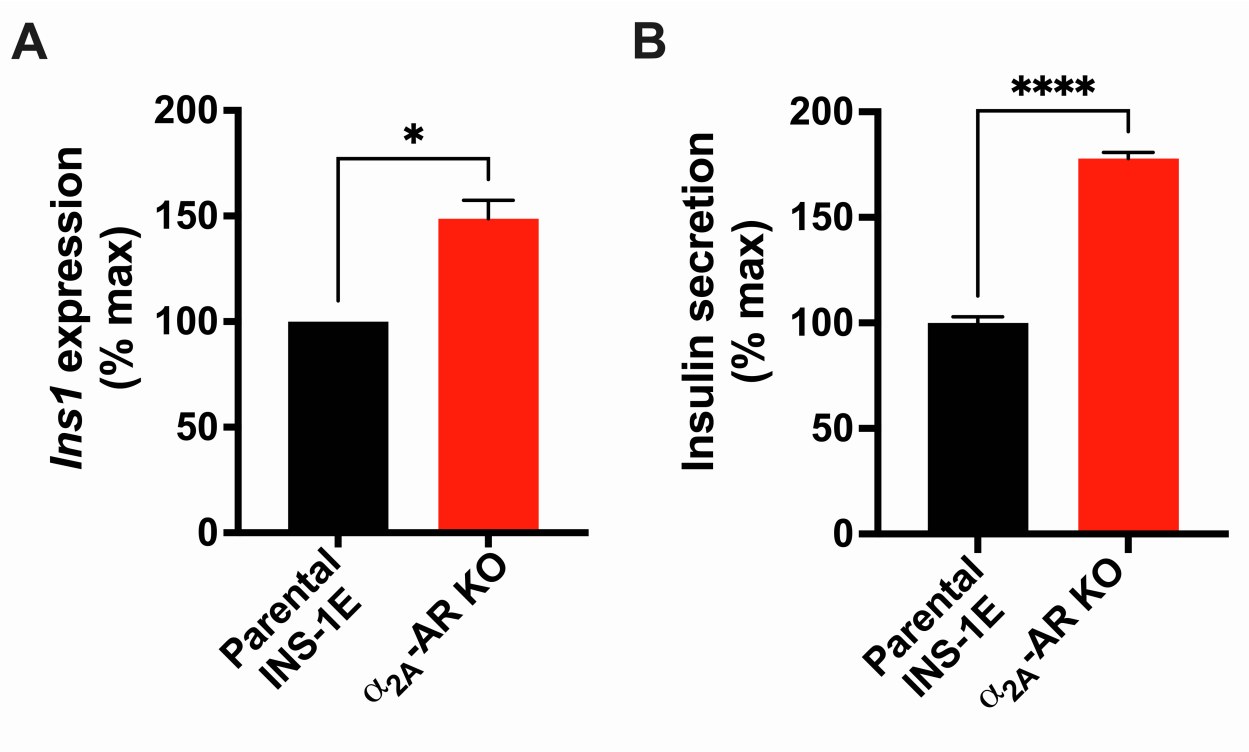

Figure S2. α_{2A}-AR KO upregulates *Ins1* expression and increases basal insulin secretion, related to Figure 1. (A) qPCR analysis comparing *Ins1* expression in α_{2A}-AR KO cells versus control parental INS-1E cells. *Ins1* mRNA expression was significantly upregulated in α_{2A}-AR KO cells (in red) compared to the parental INS-1E cells (in black; 48.8% increase, $p = 0.030$). Results were normalized to % *Ins1* expression in the parental INS-1E cells. Assays were independently performed on two separate experimental days. Data are represented as mean ± SEM; two-tailed Student's t-test, *$p < 0.05$. **(B)** α_{2A}-AR KO cells (in red) demonstrated significantly enhanced basal insulin secretion in the absence of high glucose stimulation compared to the parental INS-1E cells (in black; 77.9% increase, $p < 0.0001$). Assays were performed in triplicate from n≥3 independent experiments. Insulin data were normalized to % maximal secreted insulin. Data are represented as mean ± SEM; two-tailed Student's t-test, ****$p < 0.0001$.

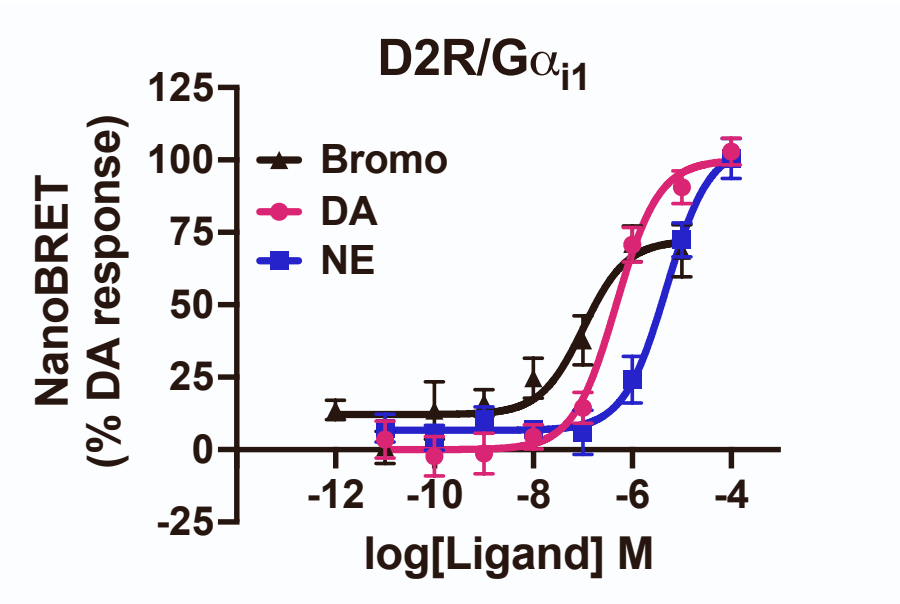

Figure S3. Bromocriptine stimulation of D2R results in $G\alpha_{i1}$ recruitment, related to Figure 4, Table 5, Table 6. Concentration-response nanoBRET assays examining drug-stimulated recruitment of Nano-Luc-labeled $G\alpha_{i1}$ in HEK-293T cells expressing HaloTag-labeled D2R. Cells were treated with either bromocriptine (in black), DA (in purple), or NE (in blue). Bromocriptine was more potent in recruiting $G\alpha_{i1}$ to D2R (EC_{50}=103.8 nM) compared to DA (EC_{50}=471.1 nM) or NE (EC_{50}=4897.5 nM), though with reduced efficacy versus these endogenous catecholamines (see **Tables 5**, **6**). NanoBRET data were baseline-corrected and normalized to % maximal DA response. Assays were performed in triplicate from n=3 independent experiments. Data are represented as mean ± SEM. NanoBRET data from DA and NE dose responses showing coupling of D2R with $G\alpha_{i1}$ were previously published in Aslanoglou et al., 2021.

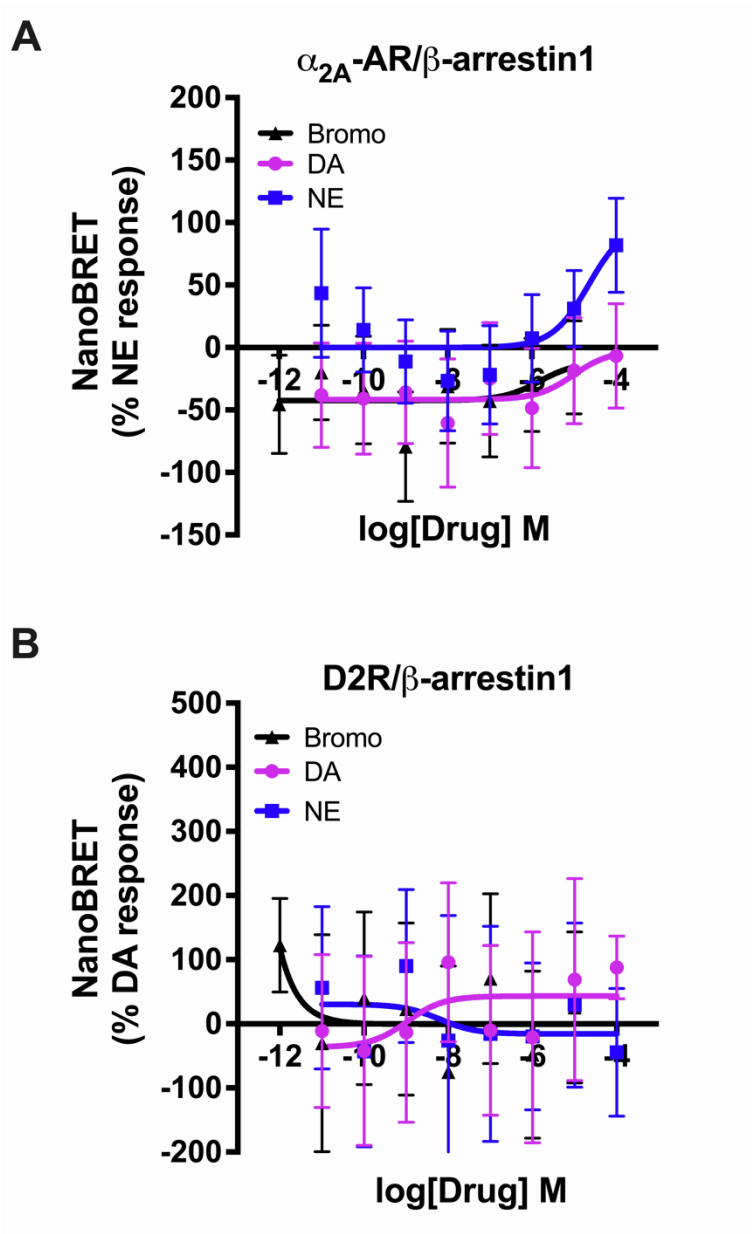

Figure S4. Bromocriptine stimulation results in negligible β-arrestin1 recruitment to D2R and α_{2A}-AR, related to Figure 5. Concentration response curves by nanoBRET examining ligand-stimulated recruitment of β-arrestin1 to D2R versus α_{2A}-AR. **(A)** Stimulation of α_{2A}-AR with either bromocriptine, DA, or NE caused only negligible β-arrestin1 receptor recruitment. **(B)** There was no significant dose-dependent β-arrestin2 recruitment to D2R in response to treatment with either bromocriptine, DA, or NE. NanoBRET data were baseline-corrected and normalized to either % maximal DA response for D2R or to % maximal NE response for α_{2A}-AR. Assays were performed in triplicate from n≥3 independent experiments. Data are represented as mean ± SEM.

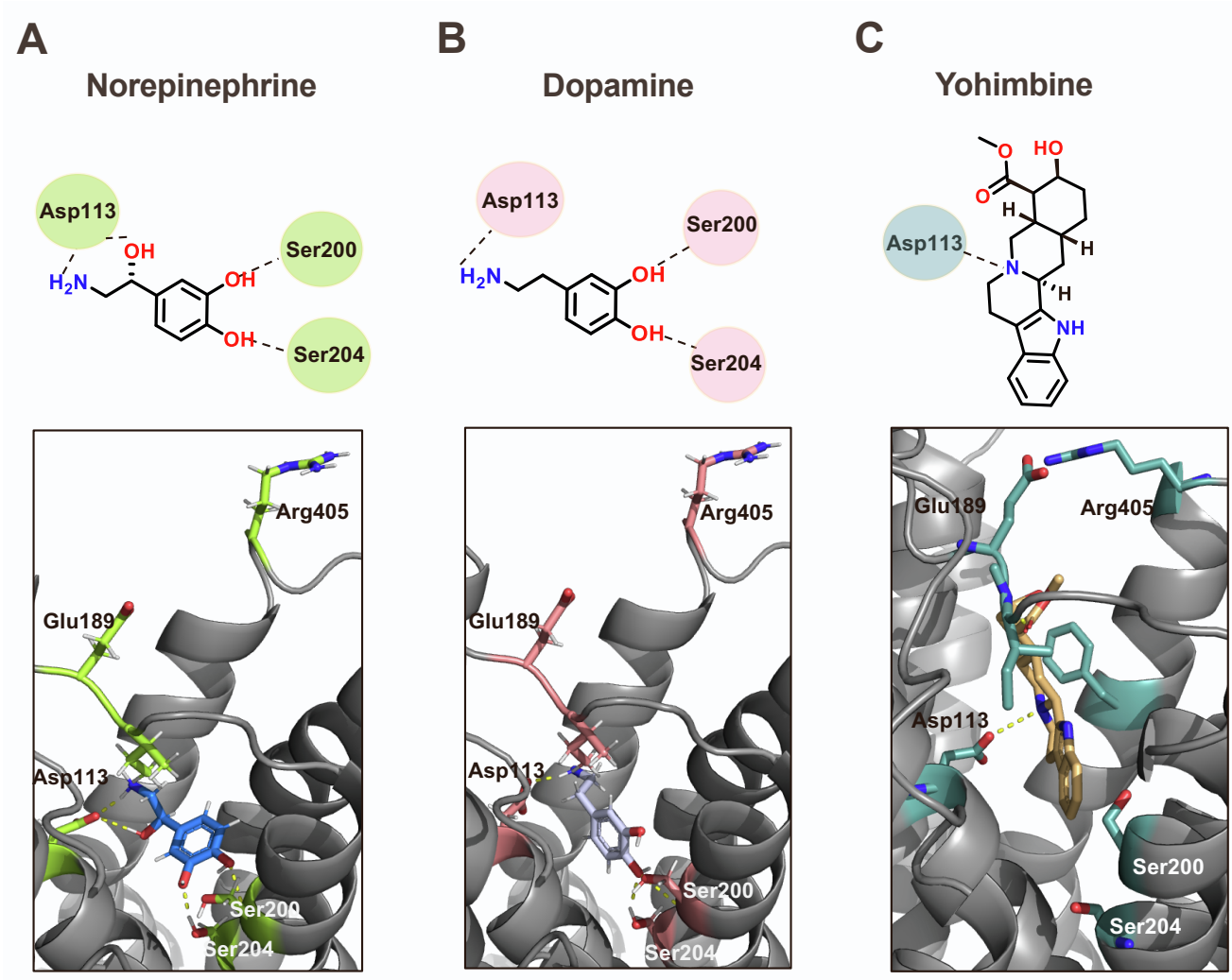

Figure S5. Predicted binding poses of ligands at α_{2A}-AR in two- and three-dimensions, related to Figure 6. Predicted binding poses of three α_{2A}-AR agonists: **(A)** NE, **(B)** DA and **(C)** α_{2A}-AR antagonist yohimbine using ROSETTALIGAND docking. Upper panels feature the respective two-dimensional views of ligand binding to α_{2A}-AR via docking and highlight key binding pocket residues. Lower panels show the predicted binding interactions in three-dimensional models with key amino acid residues highlighted within the α_{2A}-AR binding pocket.