Uncovering caffeine's adenosine A_{2A} receptor inverse agonism in experimental parkinsonism

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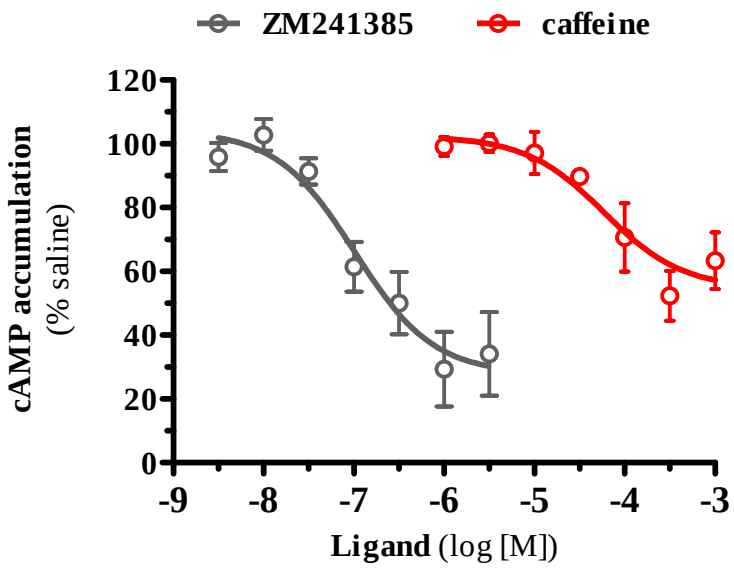

Figure S1. Dose-dependent effects of caffeine and ZM241385 in reducing cAMP levels. HEK293 cells expressing $A_{2A}R$ were incubated with saline or increasing concentrations of caffeine or ZM241385. Saline-stimulated cAMP was set as 100%, and the maximum inhibitory response obtained with the prototypic inverse agonist ZM241385 was set to 0%. Data represent the mean ± s.e.m. of three independent experiments.

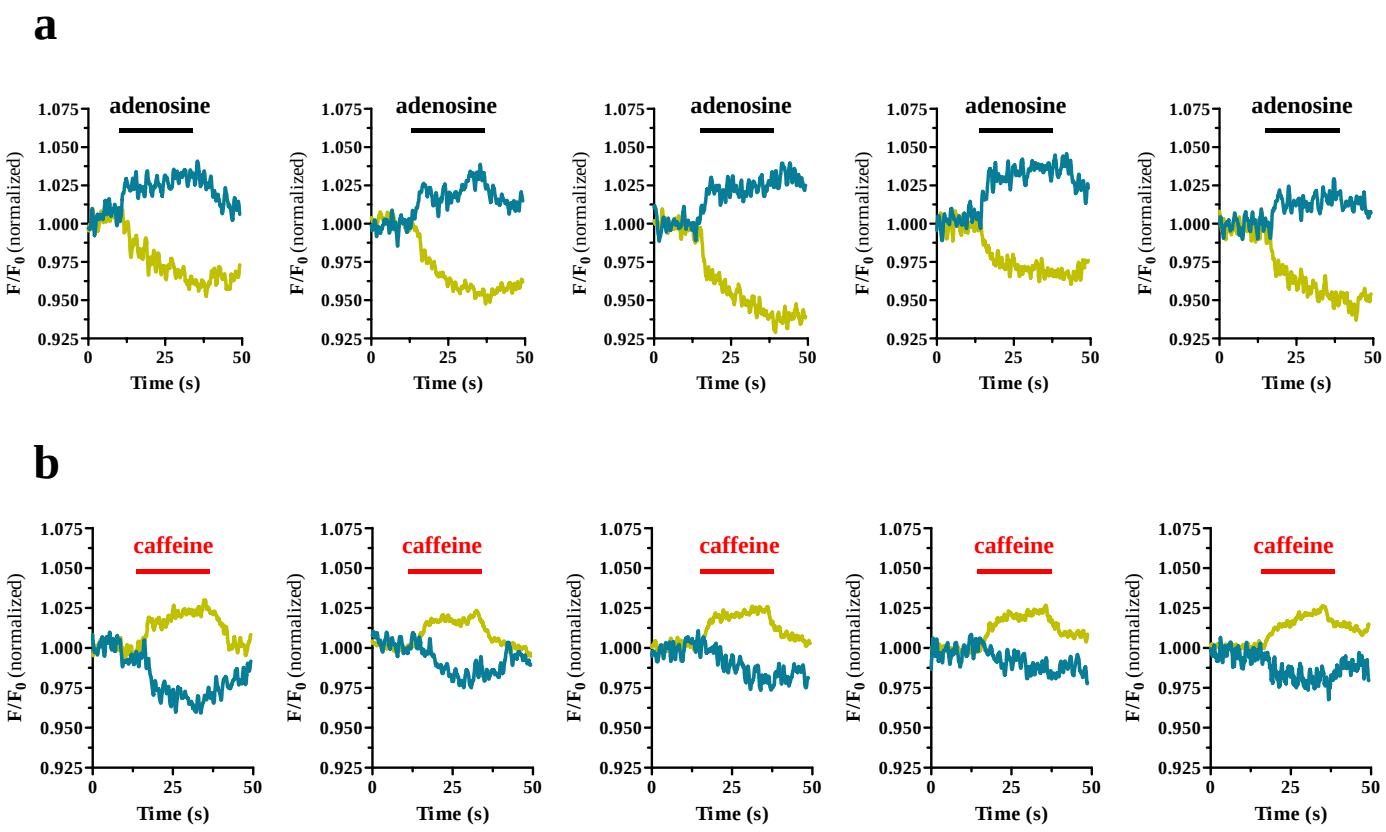

Figure S2. FRET changes of the $A_{2A}R^{FlAsH/CFP}$ in response to a full or an inverse agonist. Shown are the changes on the FlAsH (FlAsH, yellow trace) and CFP (FCFP, blue trace) signals induced by rapid superfusion of (a) adenosine (100 µM, black) or (b) caffeine (300 µM, red).

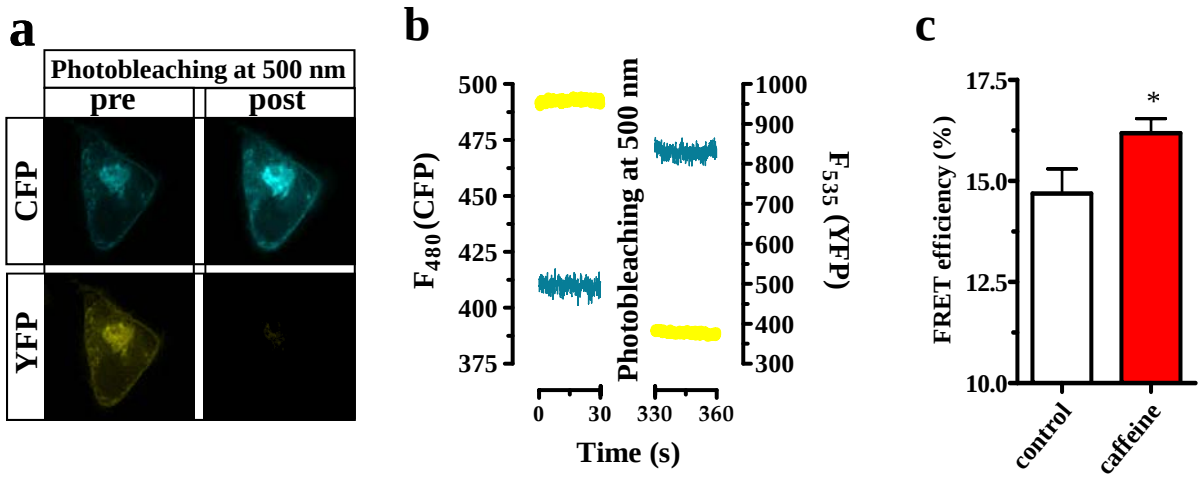

Figure S3. Evaluation of energy transfer efficiency of the $A_{2A}R^{FlAsH/CFP}$ biosensor. (a) Fluorescence images and (b) Emission intensities of CFP (480 nm, blue) and FlAsH (535 nm, yellow) recorded before (pre) and after (post) FlAsH was photobleached by 5 min of exposure to light at 500 nm. Images and traces are representative of the experiments performed upon saline or caffeine continuous superfusion. Scale bar: 10 µm. (c) Quantification of the FRET efficiency of the $A_{2A}R^{FlAsH/CFP}$, determined photodestroying FlAsH in the absence (saline) or the continuous superfusion of caffeine (300 µM). Data indicate the mean ± s.e.m. (saline, n=10; caffeine, n=11). An asterisk designates a significant difference between groups ($P < 0.05$).