Supplementary Information:

Supplementary Figure 1. D2R mutants and sensors activation. D2R dimerization is specific in the cell surface. a and **b**. HEK 293T-rex Aequorin cells expressing the different D2R constructs used in this study were used to study agonist mediated receptor activation. The system will be described in detail elsewhere (Hang et al, manuscript in preparation) - aequorin produces luminescence in a calcium dependent manner in the presence of the substrate coelenterazine, and it has been used to create a luminescence readout for receptor-mediated PLC activation through a chimeric Gq_{i5}. **c**. Selected representative regions of interest of confocal micrographs of cells expressing different Venus split combinations. Green channel illustrates the level and the localization of Venus complementation and far-red detection (represented in magenta) shows the anti-flag-AF647 staining for Flag-D2R, highlighting only the D2R present at the cell surface. **d**. BiFC and FACS (inset) of cells expressing a constant amount of TSHr-V1 and increasing amounts of D2-V2. The BiFC and the FACS were performed as described in the main text. BA8 monoclonal antibody was used to label TSHr. **e**. BRET titration experiment performed as described in the main text in cells expressing the endogenous C4.58 in the presence and in the absence of CuP. Increasing amounts of the Venus tagged construct were used to titrate the constant amount of the RLuc8 construct transiently expressed in HEK 293T cells.

Supplementary Figure 2. D2R oligomerization is specific. **Split RLuc8**: Cells coexpressing different combinations of the split RLuc8 constructs and the full length Venus fused to either the C-terminus of the D2R or the TSHr. **a** and **b**. Coexpressing constant amounts of the split donor and the full length Venus species. The data in the insets are the extension of the relative expression ratios (x axis) highlighting the right-shifted $BRET_{50}$ when the D2-Venus was used as an acceptor with either D2-L1 and TSHr-L2 or TSHr-L1 and TSHr-L2 as complemented donors. **Split mVenus**: **c**. Coexpression of different combinations of split Venus with constant amounts of either D2R-RLuc8 or TSHr-RLuc8. BRET was performed and analyzed as described above. In all the cases BRET titration curves show the results of pooled data sets from 3 independent experiments each with duplicate samples.

Supplementary Figure 3. D2R oligomerization in D2R native environment. CAD cells were transiently transfected with TM1 $Y37^{1.35}C$, the endogenous TM4 $C168^{4.58}$, the background construct without the $C168^{4.58}$ or the double Cys construct $Y37^{1.35}C$ / $C168^{4.58}$. 24 h post-transfection CuP crosslinking was performed as described in the manuscript (CuP at 1:2 mM at 25 C for 10 min, reaction stopped with addition of 20 mM NEM for 20 min at RT). The blot is representative of an experiment performed twice.

Supplementary Figure 4. Rates of higher order crosslinking are independent of the level of expression.

Cells stably expressing the double Cys construct Y37$^{1.35}$C / C168$^{4.58}$ were crosslinked as explained in the main text but for 1 to 10 min with CuP. Tetracycline induction was for 4 hr or 21hr as indicated. Representative blots are shown, and the fraction of monomer, dimer, trimer, and tetramer + higher order from each lane is shown as mean ± SEM from two independent experiments.

Supplementary Figure 5. Scheme illustrating possible combinations of coexpressed split receptor constructs. Color indicates complementation and a bold line around the acceptor (full length or split) indicates efficient resonance energy transfer.

Supplementary Figure 6. **Comparison of TM1-TM1 dimeric configurations of GPCRs** based on: **a** results of our D2R crosslinking experiments, **b** inferences from atomic force microscopy data from native murine disc membranes (PDBID: 1N3M), **c** a model based on cryoelectron microscopy of metarhodopsin I, **d** crystal packing of a photoactivated rhodopsin (PDBID: 2I36) and **e** crystal packing of B2AR receptor (PDBID: 2RH1). Most rhodopsin 3D crystal structures (PDBIDs: 1F88, 1L9H, 1GZM, 1U19, 2J4Y) are non-physiological dimers of which two rhodopsin molecules are associated at TM1 or TM5 in an antiparallel configuration (not shown). In the recent photoactivated rhodopsin crystal structure [39] (PDBID: 2I36), the dimer is oriented in a parallel configuration with

TM1, TM2 and H8 at the interface. The interface of this dimer, however, differs significantly from ours due to a pronounced tilting of one protomer with respect to the other, and to the presence of a symmetric interaction at the top of TM1 centered at position 1.34. Finally, the TM1-TM1 configuration of the recent high-resolution crystal structure of B2AR (PDBID: 2RH1) differs from ours due to a different orientation of TM1 and H8 helices in the two molecules of the dimer.