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Electronic Supplementary Information

Budded baculoviruses as a receptor display system to quantify ligand binding at single-molecule resolution with TIRF microscopy

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SUPPORTING EXPERIMENTAL

Image analysis and the selection of threshold and sigma

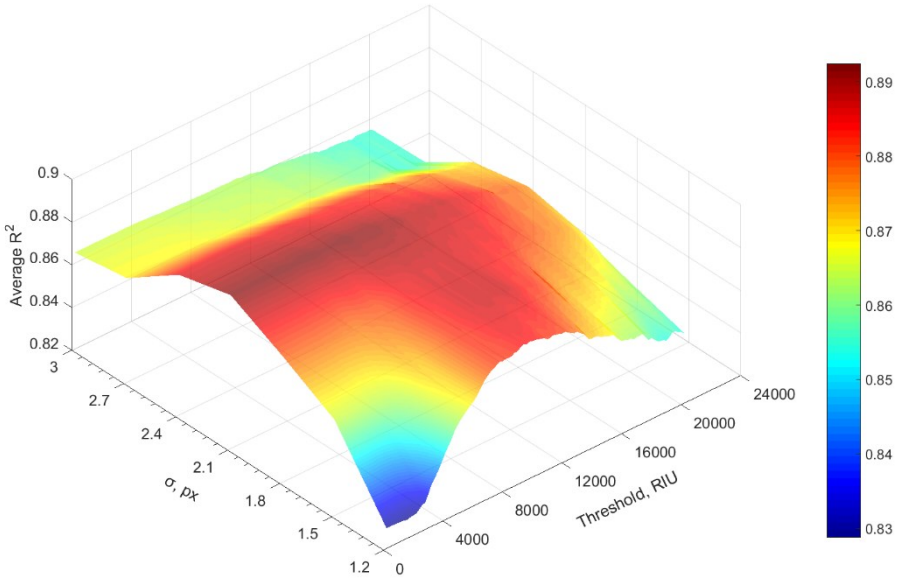

Fig. S1 Coefficient of determination (R^2) dependence on the image analysis parameters. The spot detection image analysis pipeline parameters sigma and the intensity cutoff threshold were varied and used for the analysis of the entire dataset (twelve competition binding experiments, three Y_1R, and three UR-MC026 saturation binding experiments). The corresponding models (three parameter logistic function for competition binding and one site total and nonspecific binding model for saturation binding) were used for calculating the R^2 value for data from each experiment. The average R^2 obtained by averaging the R^2 values from all 20 experiments.

Saturation binding assay with FA

Global fitting of the data from both binding isotherms was analysed as previously in[1], resulted in the apparent K_d ± SEM value of 120 ± 30 pM and estimated receptor stock concentration after extrusion R_{stock} ± SEM of 70 ± 4 nM.

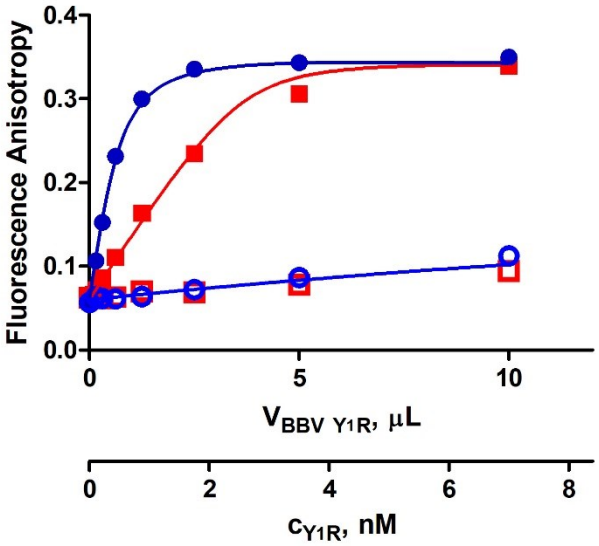

Fig. S2 The equilibrium binding curves of UR-MC026 to the Y_1R. Fixed concentration of 0.5 nM (circles), 3 nM (squares) fluorescent UR-MC026 were incubated with the increasing amount of the Y_1R receptor budded baculovirus preparation (0 - 10 µL/well, as indicated on the upper x-axis) in the absence (filled symbol, total binding) or presence (open symbols, nonspecific binding) of 0.5 µM or 3 µM non-labeled UR-MK299. Fluorescence intensities were measured, and the background was corrected, and anisotropy values were calculated as previously described by [1]. The lines correspond to the global fit of the binding data as described by [1]. This is a representative graph from two separate experiments performed in duplicates, and the results are presented as mean ± SEM.

Competition binding assay with FA

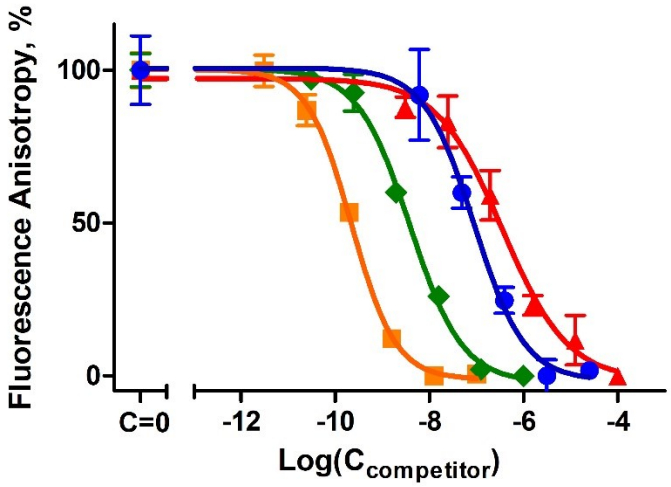

Fig. S3 Inhibition of UR-MC026 binding to Y_1R by different ligands measured in FA assay. Different concentrations of Y_1R ligands UR-MK299 (□), BIBO3304 (◆), pNPY (●), PYY (▲) together with 0.5 nM UR-MC026, 50 nM neutravidin and 50 nM biotin-PEG-cholesterol were incubated with BBV preparation (0.32 nM Y_1R). The lines represent the three parameter logistic function fit and the data presented are from representative competition binding experiments from three independent experiments that were performed in duplicates. Data are normalised so that 100 % is FA without any competitor, and 0 % is the lowest FA value for each competitor separately. Error bars represent SEM of measurements performed in duplicates.

References

1 S. Veiksina, S. Kopanchuk and A. Rinken, *Biochim. Biophys. Acta - Biomembr.*, 2014, **1838**, 372–381.