A homogeneous single-label quenching resonance energy transfer assay for δ-opioid receptor ligand using intact cells

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SUPPORTING INFORMATION

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Quencher structures. Homogeneous QRET was performed using two quenchers in the last optimization state. Both (A) Leucoberbelin Blue I (LLB I) and (B) Malachite Green (MG) share the same basic structure which was critical in current assay (Figure S-1). MG was selected because it gives more stable signal than LBB. Also with MG, variation between assays was better, because of better solubility.

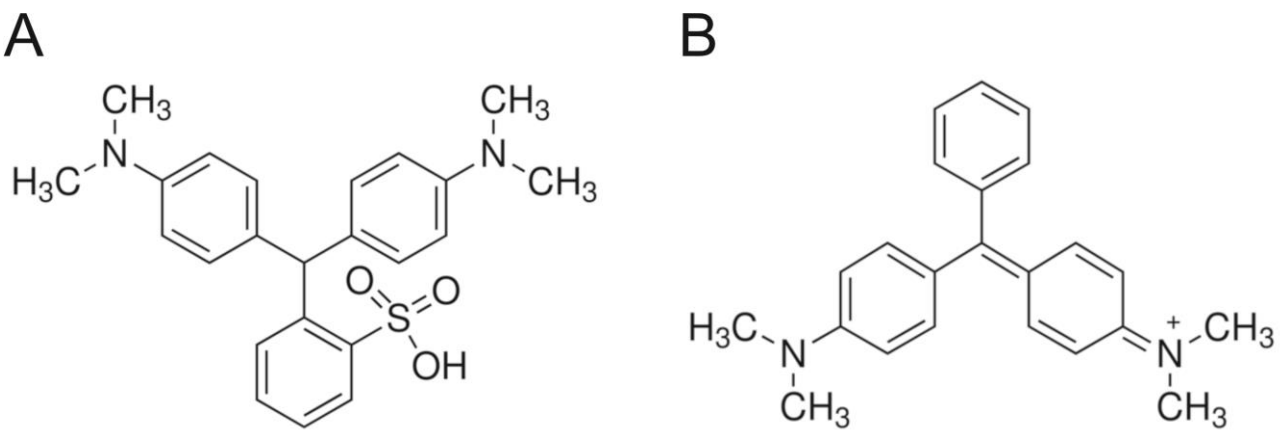

Figure S-1. Structures of the soluble quenchers. Structures of the used quenchers, Leucoberbelin Blue I (A) and Malachite Green (B). Malachite Green was selected in final assays.

Heterogeneous Eu-AX0 titration. Heterogeneous Eu-AX0 titration was done using six different Eu-AX0 concentrations from 3 nM to 2500 nM (Figure S-2). $HEK293_i$ cell number $4x10^5$ was used to detect maximal Eu-AX0 binding to δOR. K_d value for Eu-AX0 was determined using heterogeneous TRL assay because of problematic K_d value determination in homogeneous assay format. Based on heterogeneous saturation titration we could calculate the K_d value for Eu-AX0 which we can use in K_i value calculations. The K_d value calculated for Eu-AX0 was 198 ± 12 nM.

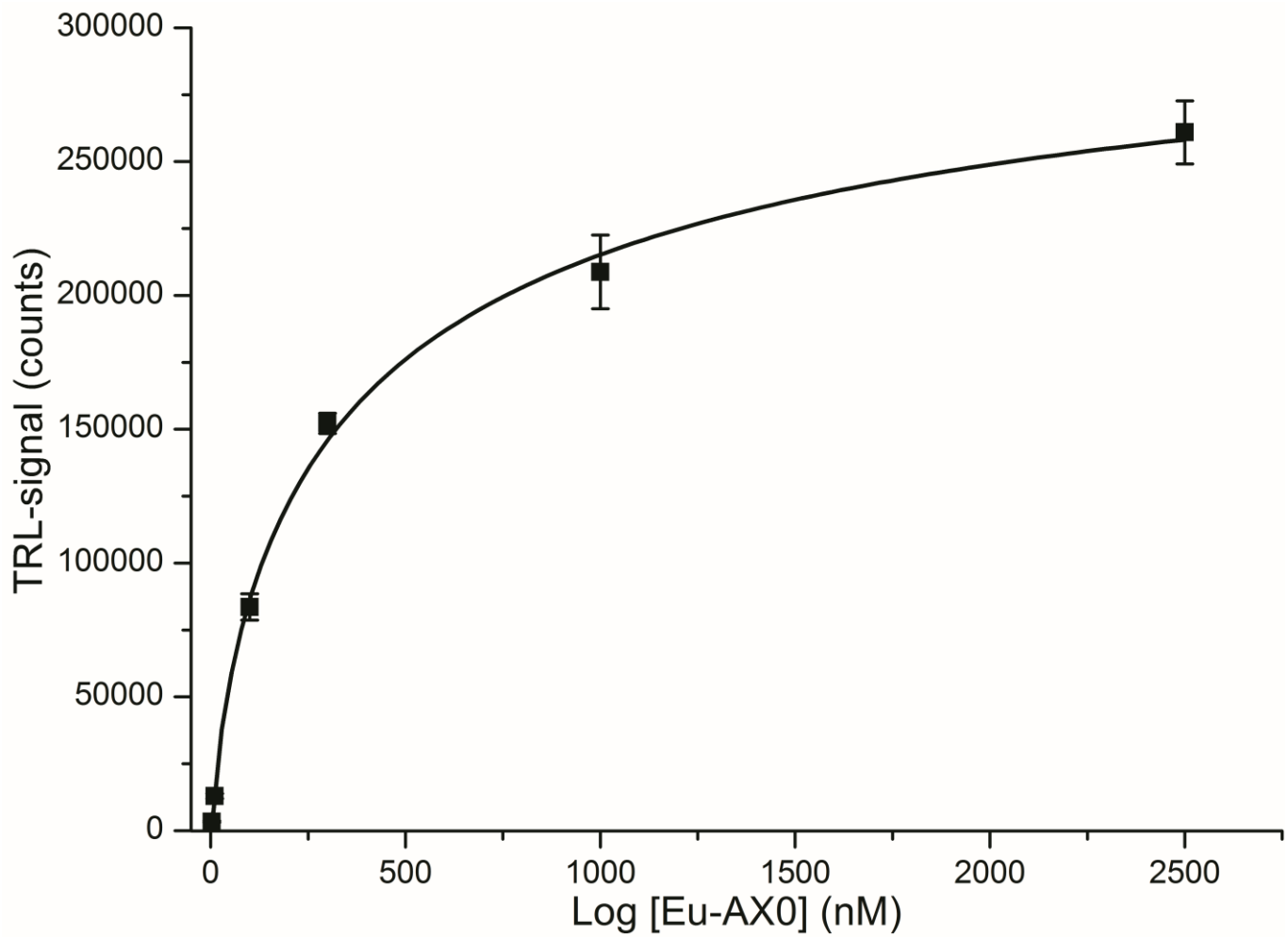

Figure S-2. Heterogeneous Eu-AX0 peptide titration. K_d value of Eu-AX0 peptides were calculated using heterogeneous assay. Based on maximal Eu-AX0 binding to δOR, the Kd value calculated for Eu-AX0 was 198 ± 12 nM. The data are shown as means ± SD of three replicates.

Cell number optimization. Different HEK293$_i$ cell concentrations were tested in QRET assay. Cell number from 0 cells to 8x10^5 cells in well were used in optimization (Figure S-3). Cell concentration of 4x10^5 was selected because it resulted the highest signal-to-background ration.

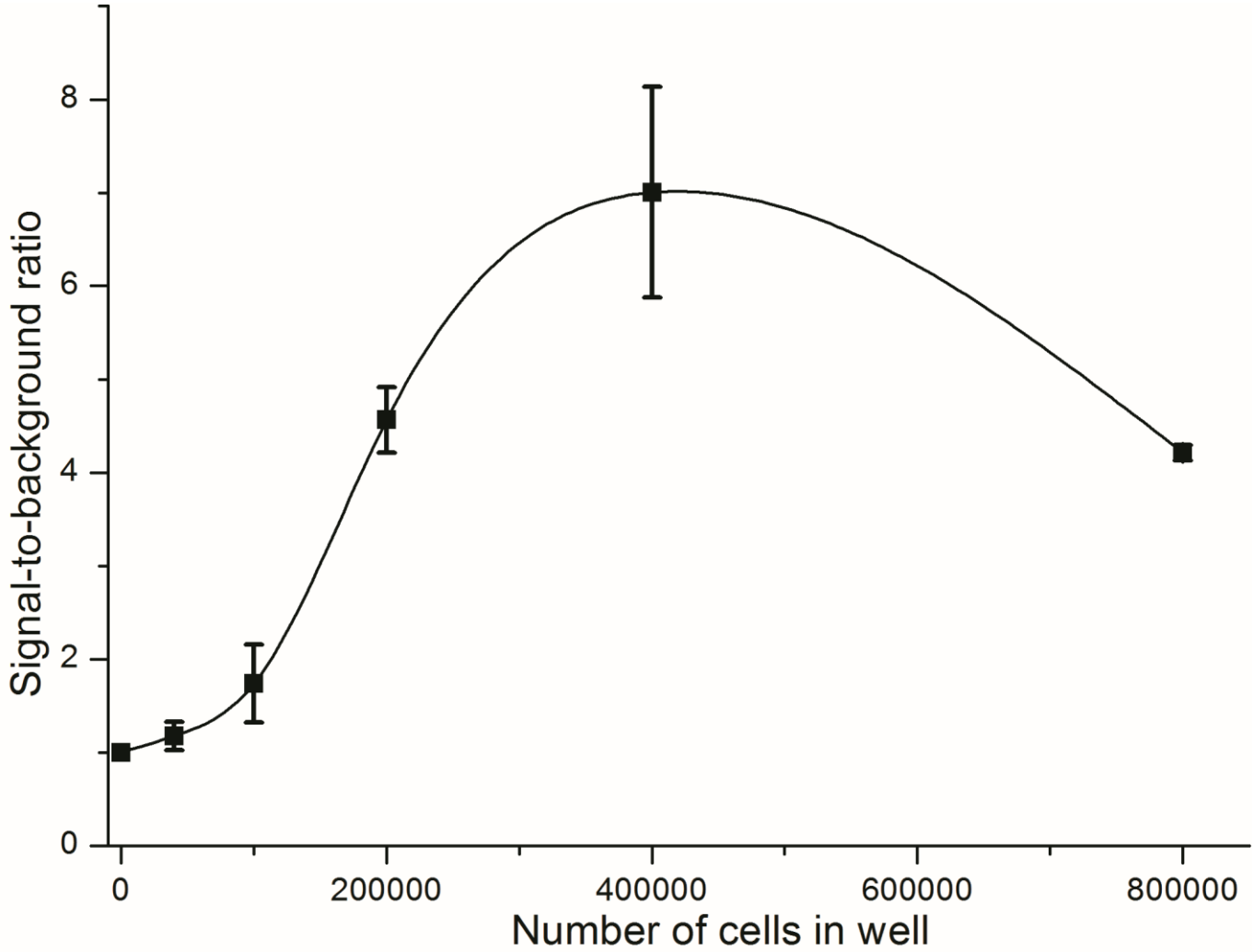

Figure S-3. The influence of cell number on Eu-AX0 peptide binding to δOR. Cell number optimization for the quenching resonance energy transfer (QRET) assay was carried out in six different cell concentrations from zero to 8x105 cells per well. The data are shown as means ± SD of three replicates.

DMSO tolerance titration. The influence of DMSO concentration was tested. DMSO is widely used solvent, and most of the drug compounds libraries are stored in DMSO. Screening method should tolerate comparable high DMSO concentration. The influence of DMSO in QRET assay was low even in high concentration (Figure S-4). Despite only minor effect, the DMSO concentration was kept as low as possible in all assays.

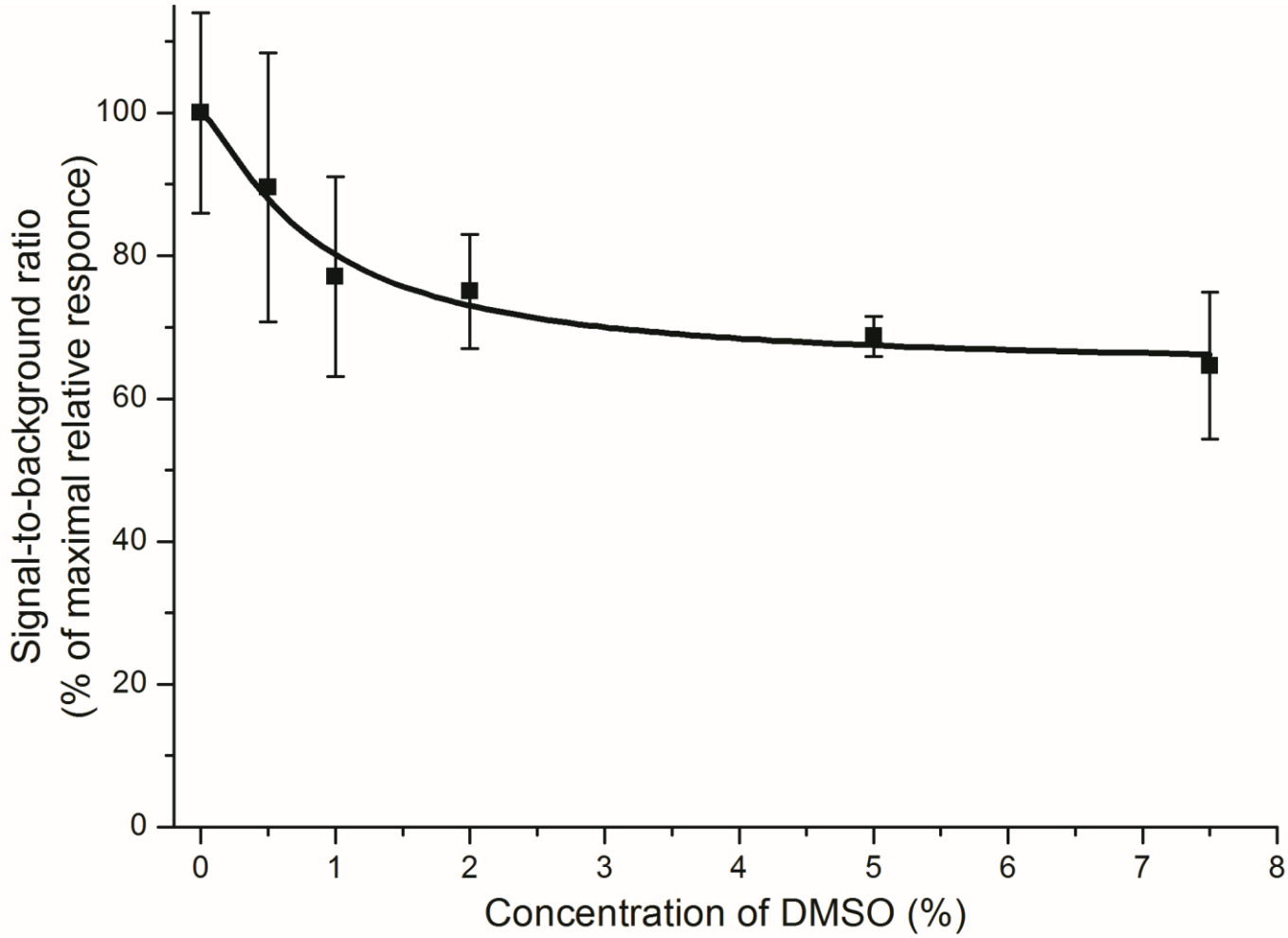

Figure S-4. The influence of DMSO concentration on Eu-AX0 peptide binding to δOR. The relative effect of DMSO to the maximal signal-to-background ratio was less than 35% in the QRET assay, even in high DMSO concentration. The data are shown as means ± SD of three replicates.