Detection of GPR50 homo- and heterodimers by SDS-PAGE. Lysates from HEK 293 cells expressing either transiently Flag-GPR50-Rluc or Flag-MT\textsubscript{1} alone or together were separated by SDS-PAGE and analysis performed by western blot using an anti-Flag M2 antibody. Immunoreactive forms with apparent molecular weights of 45 kDa and 90 kDa could be attributed to the monomeric and the dimeric forms of MT\textsubscript{1} (lanes 1 and 3) as reported previously. Additional species at ~100, 200, and >200 kDa most likely corresponded to monomeric, dimeric and higher order oligomeric forms of Flar-GPR50-Rluc, respectively (lanes 2 and 3). An additional immunoreactive form with a molecular weight of 150 kDa was only detected in cells coexpressing both receptors (lane 3) suggesting that this species corresponds to the GPR50/MT\textsubscript{1} heterodimer. The three immunoreactive bands between 100 and 200 kDa observed in all lanes correspond to non-specific bands typically detected with this antibody in whole cell lysates.
Detection of MT₁/MT₂-C113A heterodimers by co-immunoprecipitation and BRET. (A) Crude membranes were prepared from HEK 293 cells expressing MT₂-C113A-YFP alone or with Flag-MT₁. MT₂-C113A-YFP was immunoprecipitated with a monoclonal anti-GFP antibody. Membranes and immunoprecipitates were then separated by SDS-PAGE and analysis performed by Western blot using polyclonal anti-Flag antibodies. Similar results were obtained in two additional experiments. mb=membrane; IP=immunoprecipitation, M=monomer; D=dimer. (B) MT₁-Rluc or MT₂C113A-Rluc were coexpressed transiently in HEK 293 cells with the indicated YFP fusion proteins expressed at comparable amounts as determined by direct fluorescence measurements. BRET measurements were performed in living cells by adding 5µM coelenterazine. Data are means ± S.E.M. of at least three independent experiments each performed in duplicate.
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G protein immunoprecipitation. Membranes were incubated with 10 µM melatonin for 1h at 25°C. Membranes were solubilized in 1% digitonin and the soluble fractions immunoprecipitated with the monoclonal anti-GFP antibody (4µg/ml). Immunoblot analysis were carried out with the anti-G$_{i3}$ C-10 (0.4 µg/ml), anti-G$_{a12}$ E-17 (0.4µg/ml), anti-G$_{a12}$ S-20 (0.4µg/ml), and anti-G$_{b}$ T-20 (0.4µg/ml) (all from Santa-Cruz Biotech) and anti-G$_{s}$ ((0.5µg/ml). antibodies Immunoreactivity was revealed using appropriate secondary antibodies coupled to horseradish peroxidase and the ECL chemi-luminescent reagent (Amersham, Aylesbury, UK).