Figure S4: Dimerization of MC4R/CB1R chimeras (chim1-3) lacking cell surface expression and CB1R homo- and heterodimerization.
To investigate dimerization by sandwich ELISA COS-7 cells were transiently co-transfected with equal amounts N-terminally HA-tagged constructs and C-terminally FLAG-tagged constructs. The FLAG-tagged receptors were captured in the FLAG-antibody-coated 96-well plate. The dimerization capacities were measured via the N-terminally HA-tagged receptor as an increase in optical density [mean absorption (492/620)]. The N-terminally HA-tagged MC4R transfected alone served as negative control (MC4R-WT-NHA, white bar). N-terminally HA and C-terminally FLAG-tagged MC4R (MC4R-WT-HAF) and the MC4R-WT homodimer (MC4R-WT-NHA + MC4R-WT-FLAG) served as positive control (black bars). For the detection of the MC4R-WT/CB1R-WT heterodimer averaged values for both tagged variance are shown. The CB1R-WT is not able to form homodimers (23 %) (Rediger, et al. 2009), chim1 show slightly decreased (72 %) and chim2 and chim3 comparable dimerization capacity compared to WT. The continuous line marked 100 % homodimer formation of the MC4R-WT and the dotted line indicate the negative control. The values are calculated per mg/ml of protein and shown as percentage of the MC4R-WT homodimer [absorption (492/620)/ 1 mg/ml of protein: 0.7 ± 0.15]. Data are means ± SEM of five or more independent experiments performed in triplicates.