Figure S5: Heterodimerization of the MC4R-chim6 and chim7 with the rM3R.
To confirm that the rM3R is not able to form heterodimers with the MC4R-WT, with the MC4R-chim6 or with the chim7 and offer an adequate control for the BiFC method, we investigate dimerization capacities by sandwich ELISA like described before. 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, indicated by the broken line). 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). The MC4R-WT, chim6 and chim7 are not able to dimerize with rM3R (32 %, 12 % and 23 % dimerization capacity compared to the MC4R-WT homodimer). For the detection of the heterodimers averaged values for both tagged variance are shown. The values are calculated per 1 mg/ml of protein and shown as percentage of the MC4R-WT homodimer [absorption (492/620)/ mg/ml of protein: 0.5 ± 0.05]. Data are means ± SEM of three independent experiments performed in triplicates.