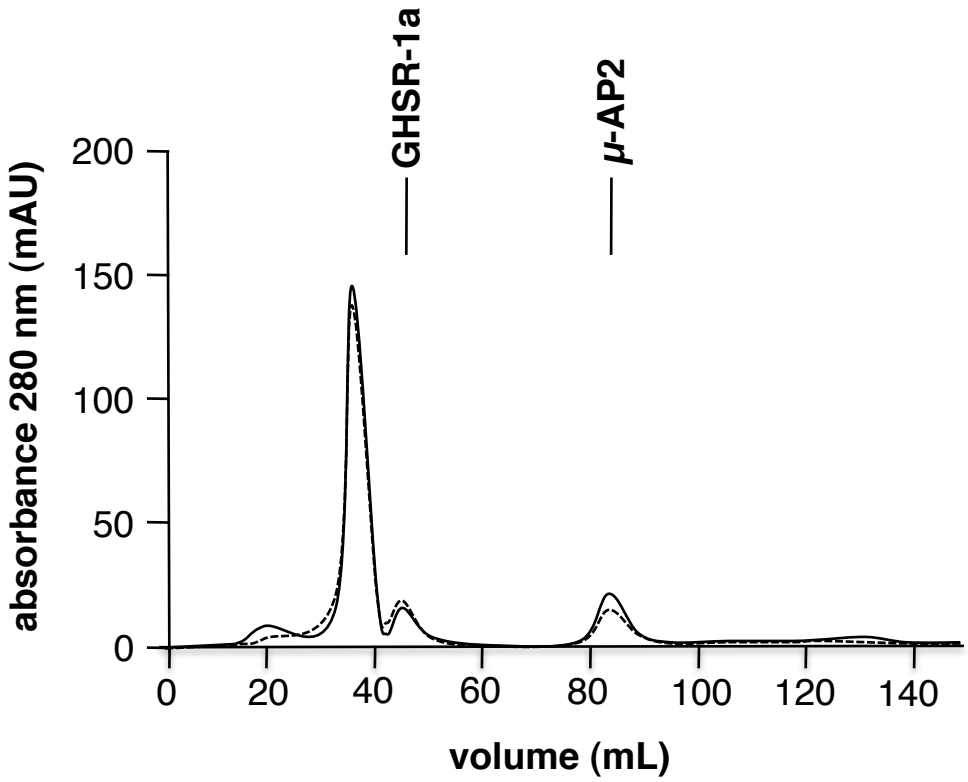
SUPPLEMENTARY DATA 2



Labeling of μ-AP2 with mBBr does not modify its ability to interact with the ghrelin receptor. To assess whether labeling of μ-AP2 with mBBr affected its interaction with the purified ghrelin receptor, we monitored complex formation by size-exclusion chromatography. To this end, the ligand-free GHSR-1a receptor in lipid discs was mixed with unlabeled (plain line) or mBBr-labeled (dotted line) μ-AP2 (protein concentrations in the 10 μM range). After incubation of 1 hour at 4°C, the protein mixture was loaded on a Superose6 column (16x700mm). Elution was carried out at a 0.2 mL/min flow-rate with a 20 mM Tris-HCl, 250 mM NaCl, 0.5 mM EDTA, pH 7.4 buffer. The elution volumes of the individual components of the complex, i.e. the receptor and μ-AP2, were identified from runs carried under the same conditions with the isolated proteins. As evidenced from the elution profile, the complex was similarly formed with either labeled or unlabeled AP2, indicating that labeling of the protein with mBBr does not affect the way it binds to the ghrelin receptor. Identical SEC profiles were also obtained in the presence of ghrelin.