Supporting Information

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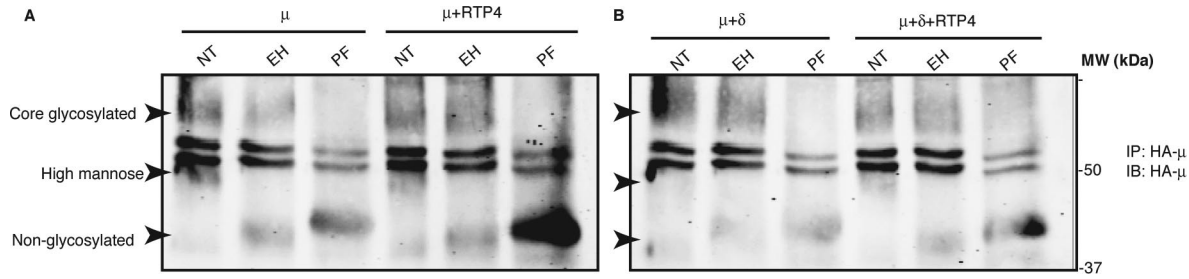

Fig. S1. (*A*) Western blot analysis shows that HA-tagged μ receptors are expressed in Neuro2A cells as a mature form (core glycosylated) and an immature form (high mannose). The latest can be digested by treatment with endoglycosidase H (EH) whereas both bands are digested by treatment by PNGase F (PF) into a nonglycosylated form. (*B*) μ receptors are present as a mature receptor that is resistant to EndoH digestion in all conditions, consistent with our observation that μ receptors are found at the plasma membrane and/or in the Golgi apparatus. Note that in the absence of glycosidase treatment, the high mannose (nonmature) form of the receptor is not detected in μ-δ cells, but is partially recovered in μ-δ+RTP4 cells. NT, not treated.

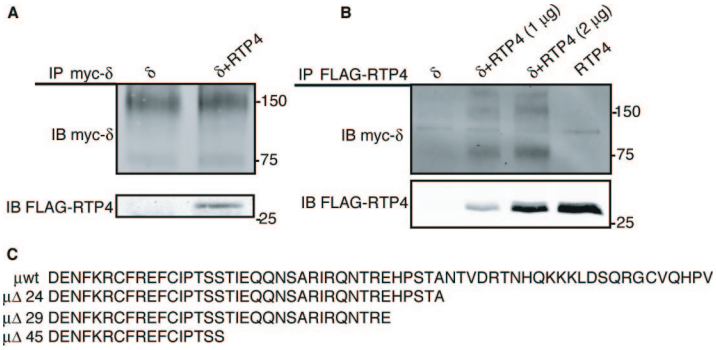

Fig. S2. (*A*) Western blot analysis shows that FLAG-tagged RTP4 coimmunoprecipitates with *myc*-tagged δ receptors. (*B*) Western blot analysis shows that δ coimmunoprecipitates with FLAG-tagged RTP4. (*C*) The amino acid sequence of mouse μ receptor C-tail and truncations used in the study.

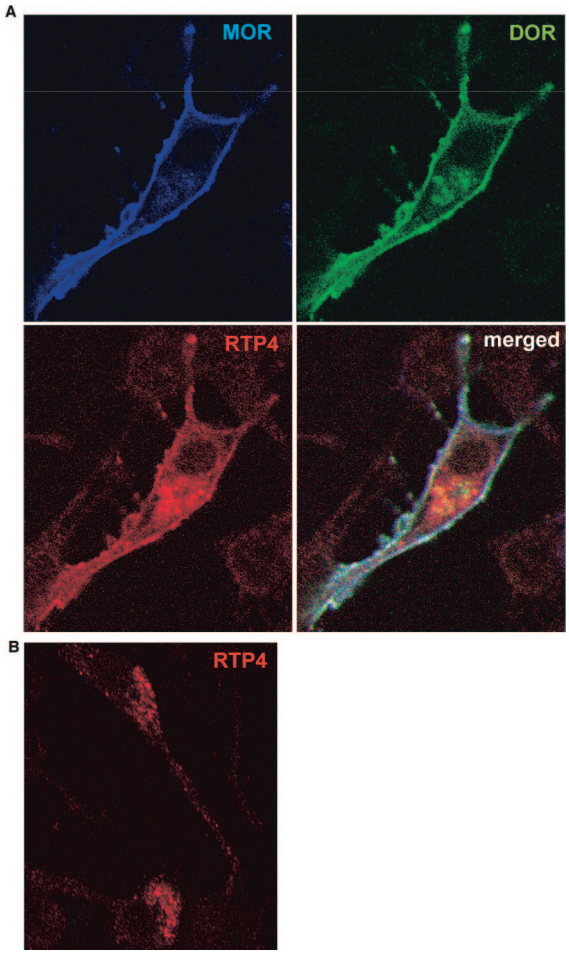

Fig. S3. Colocalization of μ, δ and RTP4 in Neuro2A cells. (*A*) Immunofluorescence images of Neuro2A cells co-expressing HA-tagged μ (*Upper Left*), *myc*-tagged δ (*Upper Right*) and FLAG-tagged RTP4 (*Middle Left*). Immunostaining with anti-HA, anti-*myc* and anti-FLAG antibodies were carried out as described previously. (*B*) Immunofluorescence images of Neuro2A cells expressing only RTP4 where in the absence of receptors δ, RTP4 exhibits a predominantly cytoplasmic localization.

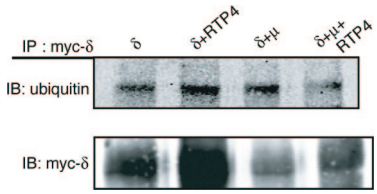

Fig. S4. Western blot analysis shows the level of ubiquitination of δ receptors when co-expressed with μ receptors; co-expression of RTP4 leads to a decrease in the extent of δ receptor ubiquitination.

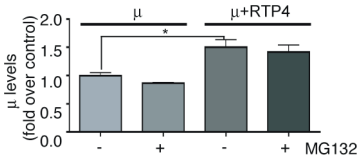

Fig. S5. Co-expression with RTP4 significantly increases μ receptors levels as determined by quantification of HA-tagged μ receptors coexpressed with RTP4 treated without or with 50 μM MG132 at 37°C for 3 h before harvesting.

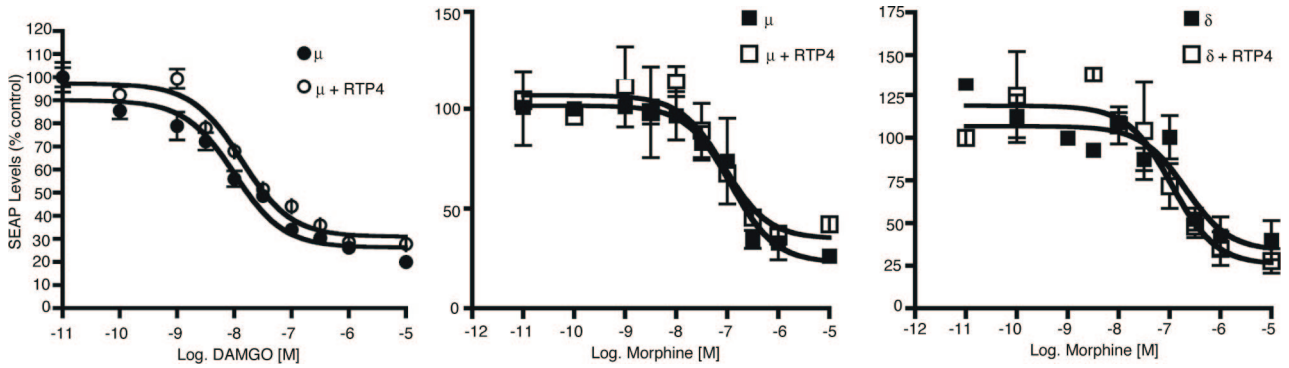

Fig. S6. Co-expression with RTP4 leads to no changes in μ or δ signaling, as seen on the pCRE-SEAP assay. Co-expression of RTP4 leads to no changes in signaling by DAMGO or morphine in cells expressing either μ or δ receptors. Data are given as mean $\pm$ SEM ($n = 3$).

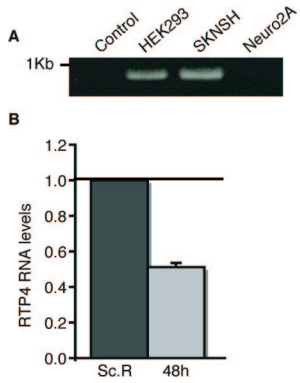

Fig. S7. (*A*) Amplification of human RTP4 in three different cell lines. The two human cell lines (HEK293 and SKNSH) show the presence of hRTP4; mouse Neuro2A cells do not. (*B*) Quantitative RT-PCR showing a decrease in RTP4 transcript 48 h after transfection with siRNA compared with scrambled siRNA (ScR). Data were normalized to the value obtained with scrambled RNA. dData are given as mean ± SEM ($n = 3$).

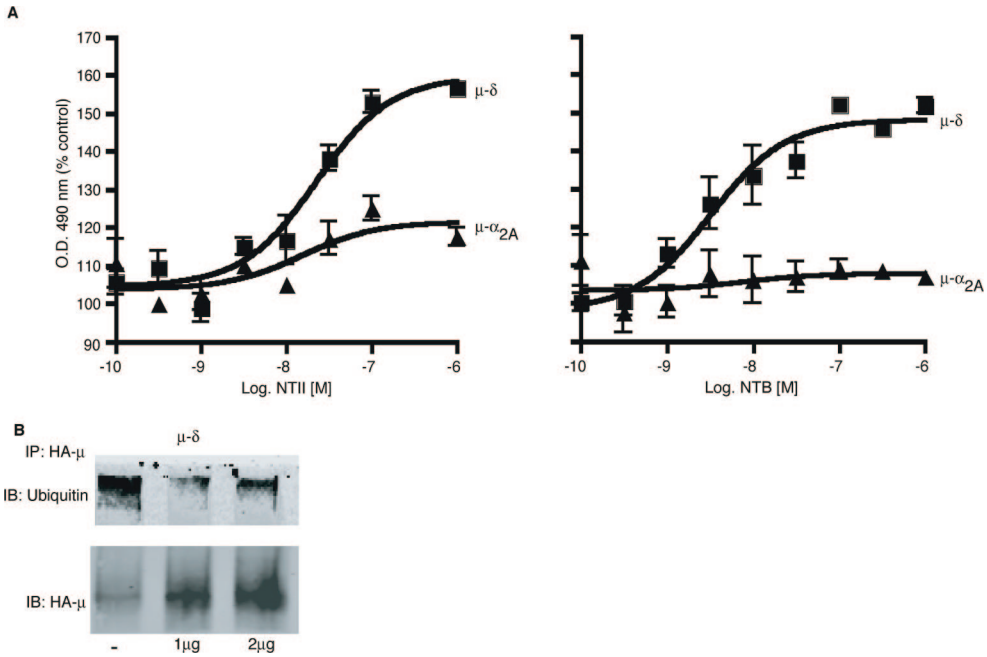

Fig. S8. Treatment with opiates leads to enhanced cell surface expression of μ-δ heterodimers. (*A*) Cells transfected with μ-δ or μ-α_{2A} receptors were treated with indicated concentration of ligands for 24 h, and the level of cell surface receptors was quantitated by ELISA using anti-FLAG antibody to detect μ receptors. (*B*) Treatment with opiates leads to a decreased ubiquitination of the heterodimer. Cells expressing μ-δ receptors were treated with 100 μM naltriben (NTB) for 24h. The level of ubiquitinated receptors was detected by Western blot analysis (with anti-ubiquitin antibody) of the receptor immunoprecipitate (1 or 2 μg) as described in *Materials and Methods*. Ligand treatment leads to enhanced receptor expression at the cell surface and decreased ubiquitination.