Supplemental Figure Legends

Fig. 1. Western blot testing of the rabbit polyclonal DOR antisera from Chemicon (AB1560). No detectable labeling for the DOR was observed in COS-7 cell lysates (lanes 1-5) as these cells do not endogenously express DORs, whereas the expected 36 kDa band was observed in NG108-15 cell lysates (lanes 7-11) which have been shown to endogenously express DORs (Barg et al., 1984; Kieffer et al., 1992; Persson et al., 2005).

Supplemental Methods

For western blot analysis of DOR labeling specificity, COS-7 (American Type Cell Culture) and NG108-15 cells were seeded in 6-well plates at a density of 3 x 10^5 cells per well and allowed to grow at 37°C for 3 d as previously described (Akama & McEwen, 2003). Samples were washed in warm PBS and harvested in 400 μl 1 x sample buffer. Lysates then were scrape collected into tubes, briefly sonicated and denatured at 60°C for 20 min before separation by SDS-PAGE. An equal volume of prepared lysate was run per condition and transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore, Bedford, MA). After blocking in 5% non-fat dry milk with 0.1% Tween 20 (blocking buffer), the membranes were incubated in rabbit polyclonal DOR antisera (1:18000) in blocking buffer overnight at 4°C followed by HRP-conjugated goat anti-rabbit IgG secondary antisera (1:18000) (Jackson Immunoresearch) for 1 hr at room temperature, and visualized by enhanced chemiluminescence (SuperSignal West Dura Extended Duration Substrate; Thermo Scientific, Rockford, IL).