Supplementary Fig. 2 (a) Western blot showing specificity of ORL1 staining. The µ, κ and δ opioid receptors were amplified by PCR from human brain cDNA. Each clone was sequenced and full length cDNAs were subcloned into pcDNA3-zeo. ORL1, µ, κ and δ opioid receptors were transfected into tsA-201 cells. Cells were grown for 72 h. and subsequently harvested using a Triton X-100 lysis buffer (150 mM TrisHCl pH 8, 200 mM EDTA pH 8, 3% Triton X-100). Samples were diluted to 5 µg/µl, separated on a 12% SDS-PAGE gel and transferred to a PVDF membrane. Membranes were preblocked overnight (4ºC) with PBS-T (PBS + 0.1% Tween 20) in 5% powdered milk then exposed to the rabbit anti-ORL antibody (PBS-T) at 1:1000 dilution. Membranes were washed, exposed to the HRP tagged anti rabbit secondary antibody (Amersham Biosciences) in PBS-T in 5% powdered milk (1:5000) for 30 minutes, followed by another wash with PBS-T and then exposed using the ECL detection method. Preblocking of the membrane with a ten times higher molar ratio of antigenic peptide (2 µM) resulted in no bands being detected on the membrane. (b) Phase contrast and fluorescent images of two fields of DRG neurons in culture. Top: Positive immunostaining for native ORL1 in the absence of antigenic peptide, arrows indicate DRG neurons without ORL1 immunoreactivity. Right graph, percentage of positive DRG neurons in culture for ORL1 immunostaining (47.5%, n = 80). Bottom: Dramatically reduced ORL1 immunostaining following a pre-incubation with 2 µM of antigenic peptide.