

Supplementary Fig. 2.

GISP increases levels of GABA<sub>B2</sub> receptors. (A) Images of a field of HEK cells transfected with GISP (0.1 µg) + GABA<sub>B2</sub> (0.5 µg) GISP expressing cells (green) display increased levels of GABA<sub>B2</sub> (red), indicated by arrowhead. Scale bar 20 µm. The data are representative of 4 separate experiments. Note: at 0.5 µg DNA expression of GABA<sub>B2</sub> is at the minimal limits of detection with the anti-GABA<sub>B2</sub> antibody in the absence of GISP. Methods: Immunocytochemistry was performed as previously described [11]. For double staining the concentration of the antibodies used were as follows; rabbit anti-GISP (10 µg/ml), mouse anti-HA (Sigma; 10 µg/mL) and secondary antibodies were used at 2 µg/ml. Briefly, HEK cells were washed in PBS and were fixed for 10 min in a 4% paraformaldehyde solution before permeabilisation with 5% horse serum, 0.1% Triton X-100 in PBS. Cells were incubated for an hour with primary antibodies in PBS containing 5% horse serum and 0.1% Triton X-100. Subsequently cells were incubated for 45 min with the appropriate secondary antibodies coupled to Cy2 or Cy3 described in the figure legends. Samples were analysed by confocal laser-scanning microscopy using a Zeiss LSM510 META confocal system (Obelkochen, Germany). There was no bleed through between the channels under the conditions used and the same physical parameters were used for all images. Images were processed using Photoshop 7.0 (Adobe Systems, San Jose, CA, USA).

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