Supplemental Figure 3  Northern blots showing the effect of γn on Ca2.2 mRNA

![Northern blots diagram](image)

Left: Northern blots were prepared using mRNA isolated from COS-7 cells expressing γn alone (left lane), γn plus Ca2.2:0.3UTR (middle lane) or Ca2.2:0.3UTR alone (right lane) and hybridized with a biotinylated probe against the I-II loop of Ca2.2 (top). The blot was stripped and re-probed for γn (bottom). Right: bar chart showing data analysed using ImageQuant. Amount of Ca2.2 (open bar) or γn (grey bar) is shown as a percentage of control, either Ca2.2 or γn alone. Numbers of separate experiments are given in parentheses. ** P < 0.01 (Student's t-test).

Measurement of mRNA levels by Northern blot. COS-7 cells were transfected with cDNA encoding γn alone, γn with Ca2.2:0.3UTR, or Ca2.2:0.3UTR alone using GenePorter (Gene Therapy Systems, San Diego, CA). Cells were harvested after 48 hours. Total RNA was purified using the RNeasy mini kit (Qiagen, Crawley, UK). mRNA was then purified from 30 μg total RNA using Dynabeads (Dynal, Fremont, CA).

Samples were denatured, run on a 1% formaldehyde gel and transferred onto positively-charged membranes by downward capillary suction. RNA was cross-linked onto the membrane using UV. DNA fragments were made using the following primers: Ca2.2, 5'-ACT CGT TGC TAG TCC TCA and 5'-GTC GCT TCT CTT CCT GGC; γn, 5'-TTC TGA AGA CAG TGC GCA CG and 5'-TCA TTT GGA TGG ACA CGT CG. The probes were labelled with polyclonal-biotin using the BrightStar labelling kit (Ambion, Inc, Texas, USA) stored at -80°C and denatured at 80°C for 10 min immediately before use. Membranes were prehybridized in Ultrahyb buffer (Ambion) at 42°C for 6h, and then hybridized with the biotinylated probe at 50°C overnight. After washing membranes in 0.5 x saline sodium citrate (SSC), 100 mM NaCl, 15 mM Na acetate, pH 7 with 0.1% sodium dodecyl sulfate (SDS) at 65°C, the BrightStar assay was carried out following kit instructions. Bands were detected using autoradiography. Data were analysed using ImageQuant (Molecular Dynamics) after scanning autoradiographs.