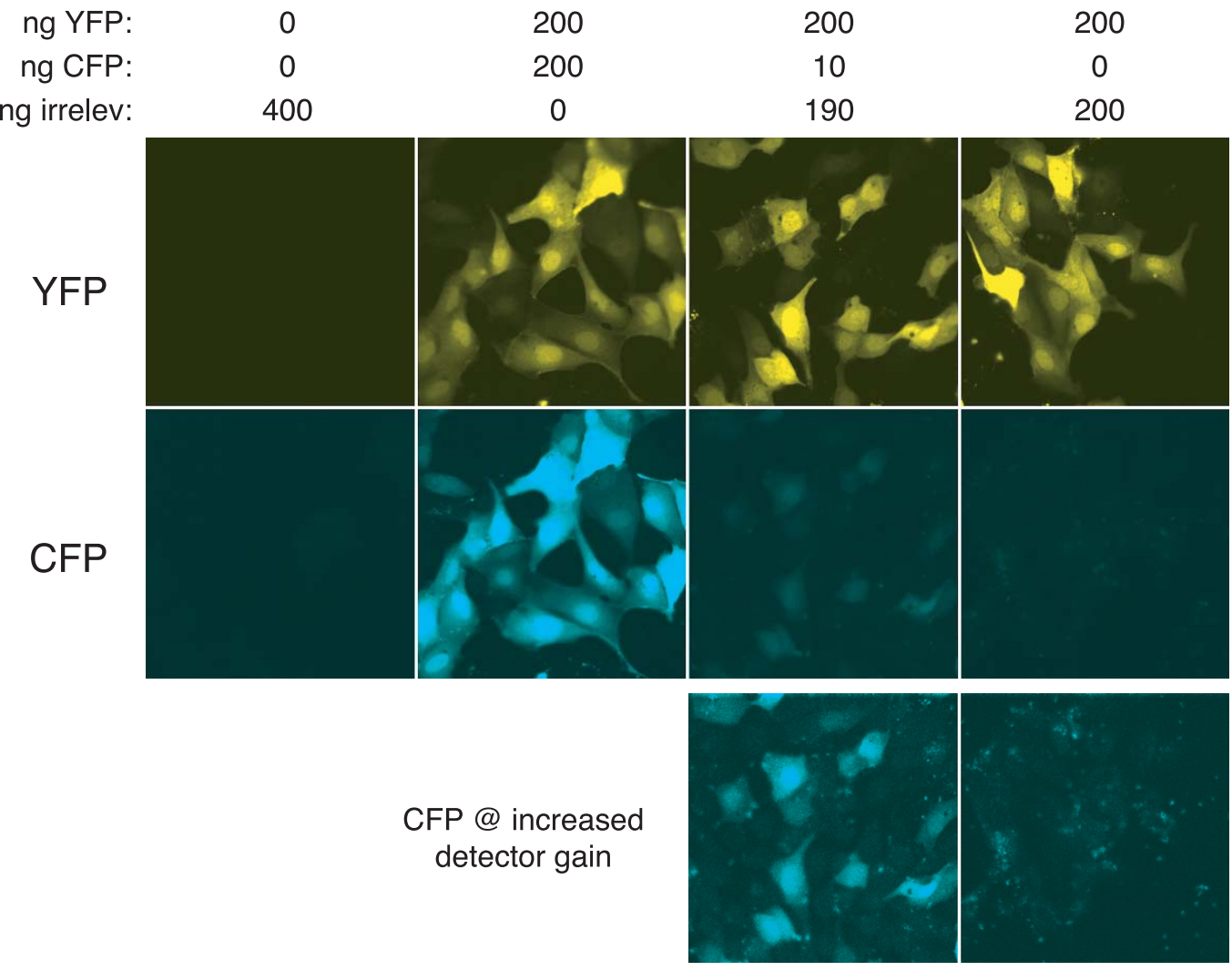

Figure S1. Analysis of expression levels on a per cell basis.

MDCK cells growing on 8-well chambered coverglasses were transfected with 400 ng plasmid DNA using lipofectamine as recommended by the manufacturer (Invitrogen). The composition of the 400 ng is indicated above each set of images and includes CMV promoter-containing expression plasmids for ECFP and EYFP (the cyan and yellow color variants of enhanced green fluorescent protein; Clonetech). An irrelevant plasmid (encoding luciferase) was used to make up the difference. The CFP and YFP proteins (represented by blue and yellow images, respectively) were visualized 24 hours after transfection. Shown in the top panels are the YFP images, captured at identical settings for the four sets of transfected cells. The middle panels show the corresponding CFP images of the same fields of cells, again captured at identical settings for the four sets of cells. Note that little or no fluorescence was seen in the YFP or CFP channel if the respective plasmid was omitted. Importantly, the CFP fluorescence on a per cell basis decreased markedly when the amount of transfected plasmid was reduced from 200 ng to 10 ng. In these same cells, the YFP fluorescence remained the same. We confirmed using more sensitive imaging conditions (lower panels) that indeed, at the lower expression of CFP, all cells were nonetheless transfected with both plasmids.

A

... ACC ATG GAC AGC AAA GGT TCG TCG CAG AAA GGG TCC CGC CTG CTC CTG CTG CTG GTG GTG
TCA AAT CTA CTC TTG TGC CAG GGT GTG GTC TCC AAG TTC ACC ATA GTT TTT CCA CAC AAC CAA
AAA GGA AAC TGG AAA AAT GTT CCT TCC AAT TAC CAT TAT TGC CCG TCA AGC TCA GAT TTA AAT
TGG CAT AAG GAC TTA ATA GGC ACA GCC TTA CAA GTC AAA ATG CCC AAG ...

B

... ACC ATG GCG AAC CTT AGC TAC TGG CTG CTG GCA CTC TTT GTG GCT ATG TGG ACT GAT GTT
GGC CTC TGC AAG TTC ACC ATA GTT TTT CCA CAC AAC CAA AAA GGA AAC TGG AAA AAT GTT CCT
TCC AAT TAC CAT TAT TGC CCG TCA AGC TCA GAT TTA AAT TGG CAT AAG GAC TTA ATA GGC ACA
GCC TTA CAA GTC AAA ATG CCC AAG ...

Figure S2. Sequences of Prl-VSV-GFP (panel A) and PrP-VSV-GFP (panel B).

The 5' untranslated region is in black lettering, the signal sequence in green, and the mature region in red. The first ATG sequence in the transcript is indicated with a double box. Note that this ATG has an ideal Kozak's consensus sequence for translational initiation and is identical for the two constructs. Other in-frame ATG codons are indicated with single boxes. Out of frame ATG sequences are underlined. Note that for Prl-VSV-GFP, which shows a cytosolic distribution in neurons, the second in-frame start codon does not appear until ~225 nucleotides after the first. Between these two, there is an out of frame ATG. Thus, in order to synthesize a version of Prl-VSV-GFP lacking a signal sequence, two ATGs, one with an ideal and another with an acceptable Kozak's consensus, would need to be bypassed by the ribosome, making this an unlikely explanation for the observed cytosolic distribution in neurons. Furthermore, this bypassing would have to occur only for Prl-VSV-GFP and not for PrP-VSV-GFP despite the fact they have identical contexts for their first ATGs, further making a translational mechanism for the observed results unlikely.

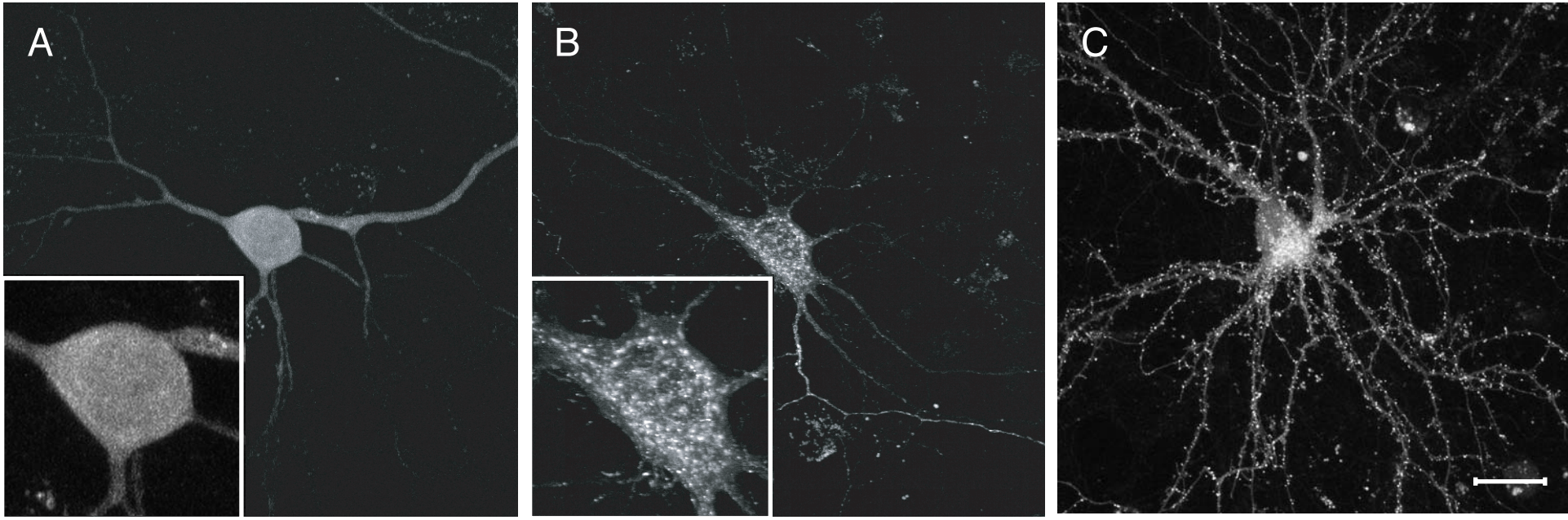

Figure S3. Effect of the signal sequence on VSV-GFP compartmentalization in neurons.

Primary hippocampal neurons growing on a feeder layer of glial cells were transfected with Prl-VSV-GFP (panel A) or PrP-VSV-GFP (panels B and C) and visualized by confocal microscopy. In the experiment in panels A and B, cells were maintained for 4 hours at 40° to prevent ER to Golgi trafficking of VSV-GFP. They were then removed to a microscope stage at 38° and imaged immediately. PrP-VSV-GFP was evidently in the ER since very shortly after reducing the temperature, it is located in transport carriers seen as widely distributed punctate structures (panel B). By contrast, Prl-VSV-GFP analyzed in parallel under the identical conditions remains diffuse throughout the nucleocytoplasmic compartment (panel A). Higher magnification images are shown in the insets. At later times, the transport carriers seen for PrP-VSV-GFP in panel B are observed to traffic to the Golgi (e.g., Fig. 7E), after which they leave the Golgi for the cell periphery. Shown in panel C is the distribution for PrP-VSV-GFP after two days at 33°C, where it has been given sufficient time to become trafficked throughout the axon and dendrites. By contrast, Prl-VSV-GFP always remains primarily diffuse as seen in panel A and Fig. 7E, with very few transport carriers or other membrane structures.