Legends to supplementary Figures

Legend to Supplementary Fig. 1. Effect of different protease inhibitors on the expression of EYFP- Ct-Cot in PC12 cells. PC12 cells were untransfected or transiently transfected with EYFP-C-cot and untreated (control) or treated with different protease inhibitors E64-d (50 μM), Pefablock (100 μM), leupeptin (200 μM), MG132 (10 μM) or lactacystin (10 μM) for 12h, as indicated. Graph show the relative fold change on cell fluorescence mean intensity ± S.E.M. from three different experiments. Immunoblots show the expression of EYFP-C-cot in untramsfected, control cells (transfected cells, no treatment) and cells transfected and treated with the different protease inhibitors, as indicated. Note that only treatment with proteasome inhibitors (MG132 and lactacystin) produce an increase in fluorescence and protein expression levels.

Legend to supplementary Fig. 2 Northern blot analysis of the effect of proteasome, transcription and translation inhibitors on the mRNA expression of unstable EGFPd2. PC12 cells untransfected or stably transfected with unstable EGFPd2 where untreated (control) or treated for 12h with proteasome inhibitors (10μM MG132) in the absence or in the presence of 500ng/ml actinomycin D (ActD), or 20 μg/ml cycloheximide (CHX), as indicated. Total RNA was isolated from cells and analyzed by Northern blot. Membranes of transferred RNAs were stained with methylene blue (28S and 18S RNAs) and successively hybridized with ^{32}P-labeled probes for EYFP, GAPDH and PDI. A representative experiment is presented. Graph shows the relative changes in the amount of mRNA for EYFP quantitated using either GAPDH (open bars) or PDI (grey-filled bars) mRNAs as controls, data are mean ± S.E.M from three experiments.

Legend to supplementary Fig. 3. qPCR analysis of the effect of proteasome, transcription and translation inhibitors on the mRNA expression of unstable EGFPd2 and stable EYFP in PC12 cells. PC12 cells stably transfected with unstable EGFPd2 or stable EYFP constructs where untreated (control) or treated for 12h with proteasome inhibitors (10μM MG132) in the absence or in the presence of 500 ng/ml actinomycin D (ActD), or 20 μg/ml cycloheximide (CHX), as indicated. Total RNA was isolated and analyzed by qPCR, as described under "Experimental Procedures". Graphs show the relative fold change and the corresponding ranges (upper and lower bars) as calculated from three different experiments from $-\Delta\Delta$Ct values.

Legend to supplementary Fig. 4. Effect of proteasome, transcription and translation inhibitors on the expression at the mRNA and protein abundance of unstable GFPu. PC12 cells untransfected or stably transfected with either unstable EGFPd2 (a) or stable EGFP (b), where untreated (control) or treated for 12h with proteasome inhibitors (10μM MG132) in the absence or in the presence of 500ng/ml actinomycin D (ActD), or 20 μg/ml cycloheximide (CHX), as indicated. Panel a), graph of cell fluorescence mean intensity analyzed by flow cytometry and expressed as mean ± S.E.M from three different experiments each done by triplicate. Lower pictures show representative experiments of mRNA (EGFP and actin, used as control) analyzed by 1.5% agarose gel and ethidium bromide staining of RT-PCRs products obtained from the respective total cell mRNAs samples; and protein levels (EGFP and tubulin, used as control) by Western and immunoblot with anti-EGFP and anti-tubulin antibodies. Panel b), Total RNA was isolated from cells after the corresponding treatments and analyzed by Northern blot. Membranes of transferred RNAs were stained with methylene blue (28S and 18S RNAs) and successively hybridized with ^{32}P-labeled probes for EYFP, GAPDH and PDI. Graph shows the relative changes in the amount of mRNA for EGFP quantitated using either GAPDH (white bars) or PDI (grey bars) mRNAs as controls.

Legend to supplementary Fig. 5. Fluorescence microscopy analysis of unstable GFPu transfected PC12 cells in response to proteasome, transcriptional and translational inhibitors. Cells stably expressing GFPu were untreated (control) or treated as indicated for 12h with

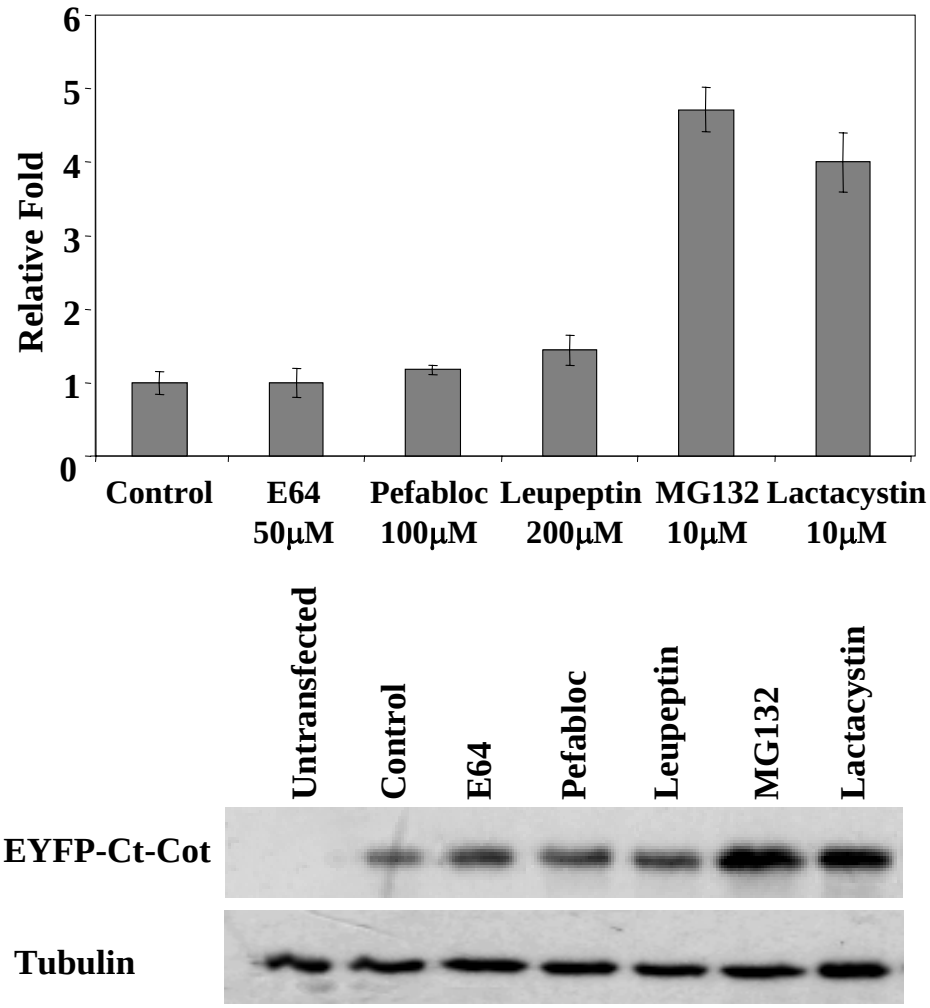
MG132, ActD and CHX or the combinations indicated. Living cells were examined under confocal microscopy with medium magnification (x 400), fluorescence images are superimposed over phase-contrast images of the cells. All fluorescent images were captured with the same settings of the confocal microscope.

Legend to Supplementary Fig. 6. Effect of proteasome, transcription and translation inhibitors on cell fluorescence mRNA and protein levels of PC12 cells stably transfected with EYFP and GFPu. Left panels, EYFP; right panels, GFP. Upper panels (both sides), graphs of cell fluorescence mean intensity analyzed by flow cytometry and expressed as mean ± S.E.M from three different experiments, each run in triplicate. Lower panels (both sides) show representative experiments of the levels of mRNA analyzed by 1.5% agarose gel and ethidium bromide staining of RT-PCRs products obtained from the respective total cell mRNAs samples; and protein (GFP and tubulin, used as control) by immunoblotting with anti-EGFP and anti-tubulin antibodies. PC12 cells stably transfected cell lines (2-7) where untreated (control, 2) or treated for 12h with proteasome inhibitors (10μM MG132, in the absence or in the presence of 5 μg/ml of α-amanitine or 7.5 μg/ml emetine, as indicated (3-7).

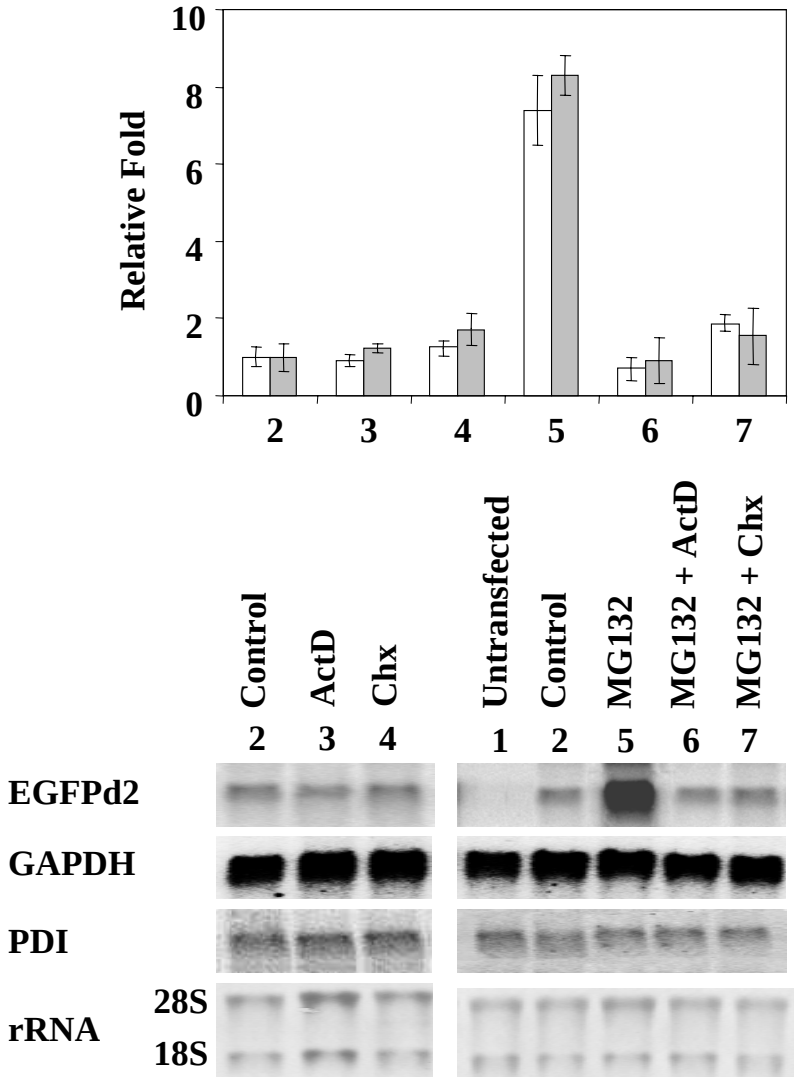
Legend to supplementary Fig. 7. Effect of proteasome, transcription and translation inhibitors on mRNA and protein abundance of Hela cells transiently transfected with EYFP and EGFPd2. Hela cells transiently transfected with EYFP or EGFPd2 constructs were untreated (control) or treated for 14h with proteasome inhibitors (10μM MG132) in the absence, or in the presence of 500 ng/ml actinomycin D (ActD), or 20 μg/ml cycloheximide (CHX), as indicated. Total RNA was isolated and analyzed by qPCR, as described under "Experimental Procedures". Graphs show the relative fold change and the corresponding ranges (upper and lower bars) as calculated from three different experiments from $-\Delta\Delta Ct$ values. Lower panels show protein expression levels for EYFP and EGFPd2, and tubulin, used as control, by Western and immunoblot with anti-EGFP and anti-tubulin antibodies.

Legend to supplementary Fig. 8. DNA dose-dependent expression of flourescent proteins in Hela cells. Hela cells were transiently transfected with increasing amounts of the corresponding pCMV GFPu and pCMV EYFP together with: empty pcDNA3.1 making a final total amount of 500 ng. Transfected cells where untreated (control) or treated for 12h with proteasome inhibitors (10μM MG132). Cell fluorescence was analyzed by flow cytometry and protein levels by immunoblotting with anti-EGFP antibodies. Results presented are average from two different experiments.

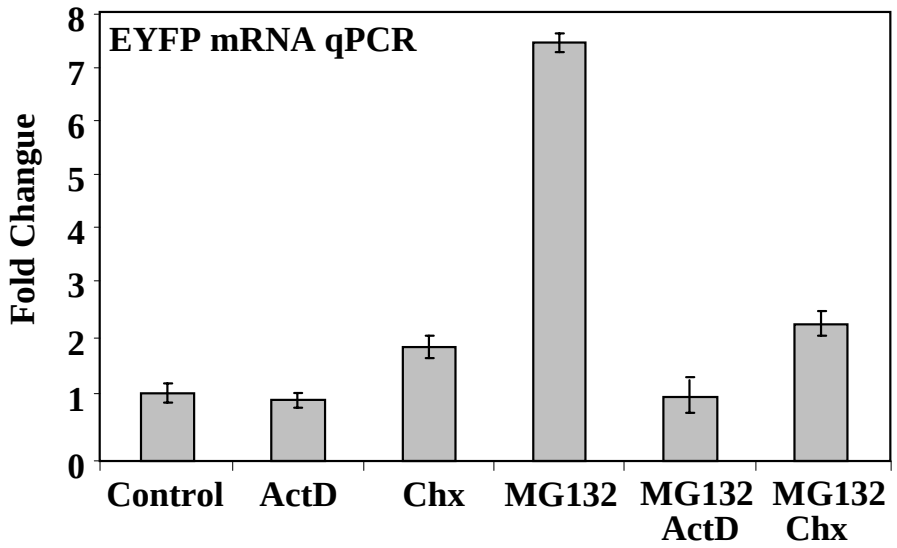
Legend to supplementary Fig. 9. DNA dose-dependent expression of flourescent proteins in Hela cells. Hela cells were transiently transfected with increasing amounts of the corresponding pCMV GFPu (a) and pCMV EYFP (b) together with the indicated amounts of the following plasmids: empty pcDNA3.1, and AP-1, CREB, or NFκB luciferase reporters. Cell fluorescence was analyzed by flow cytometry. Results presented are average from two different experiments.

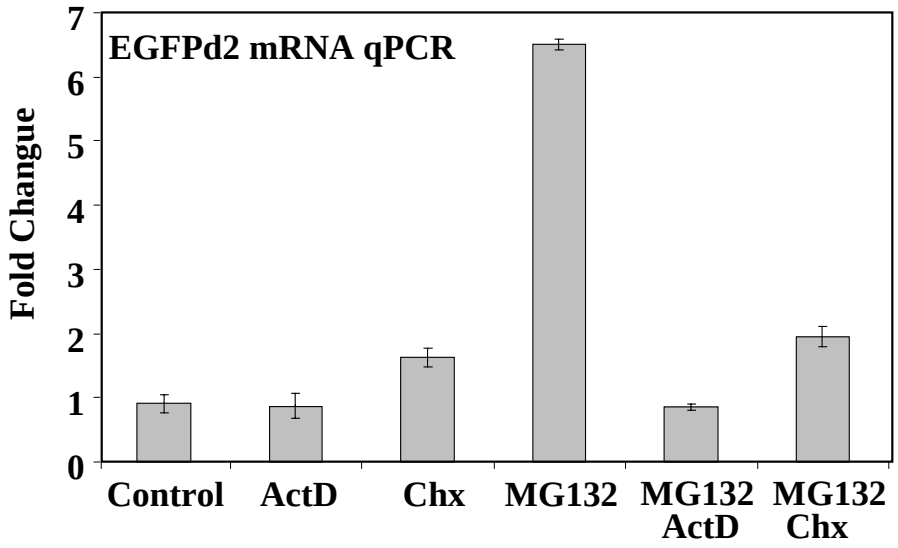


Supplementary Figure 1

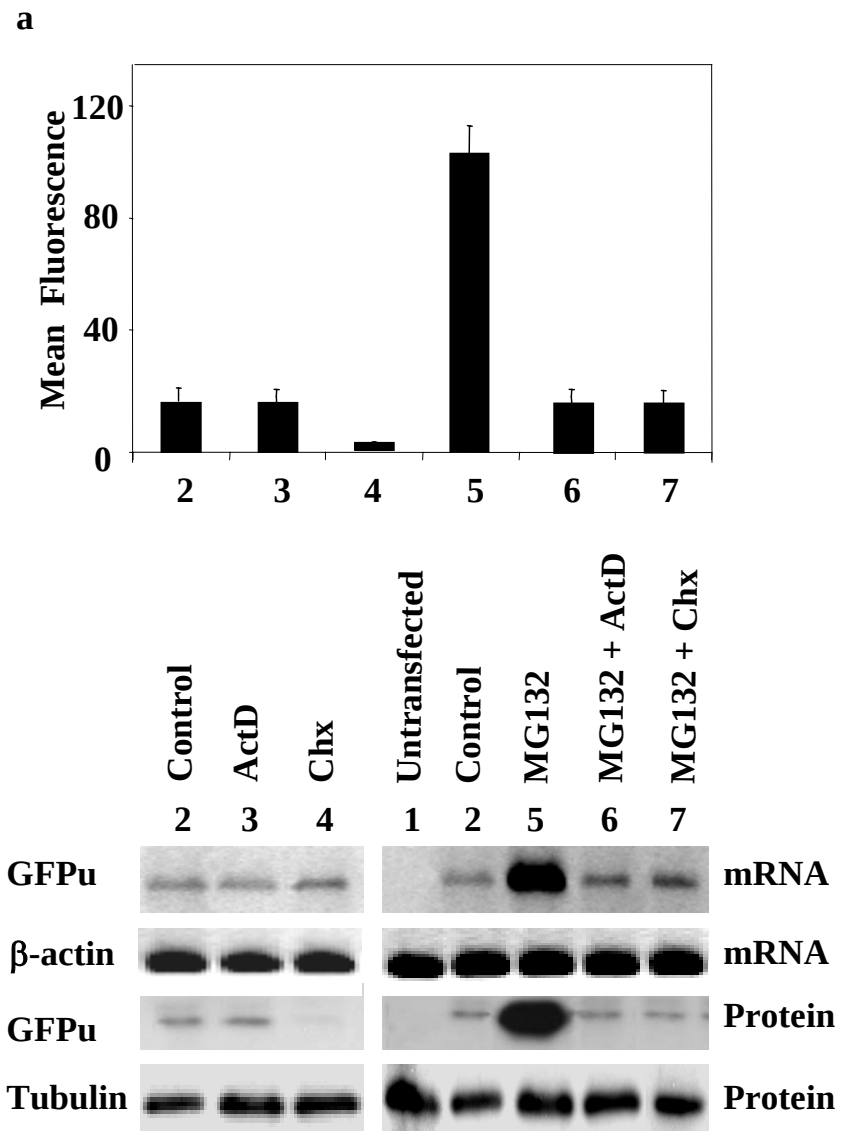


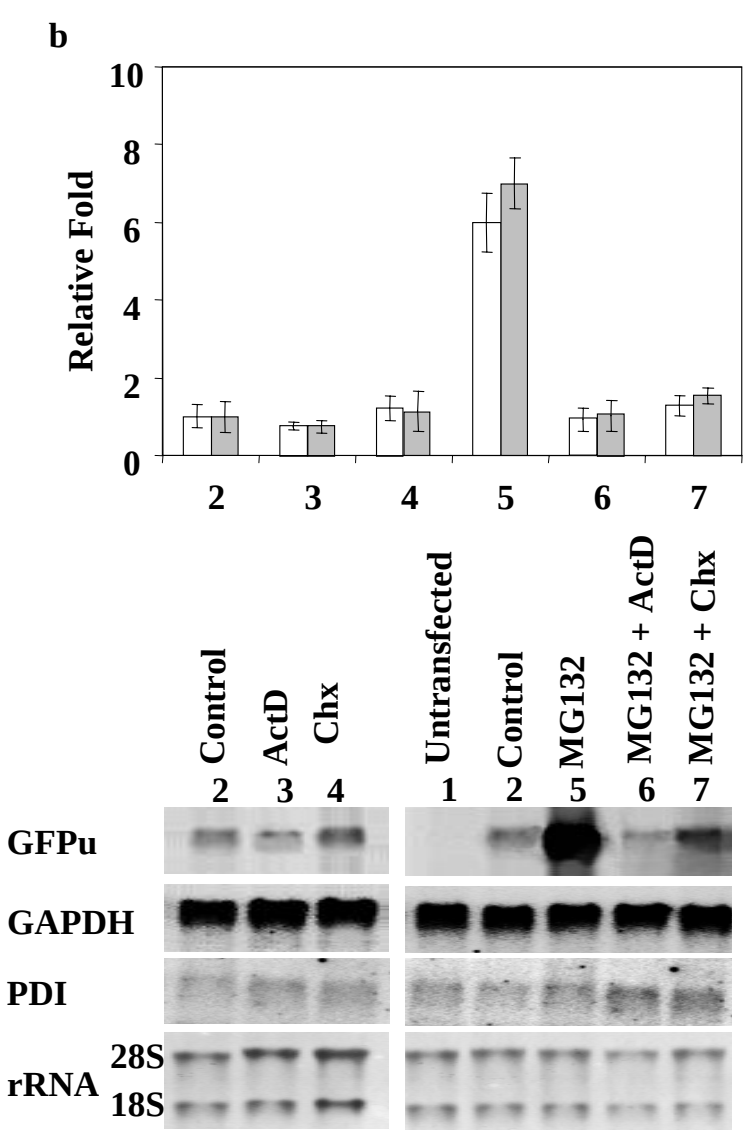
Supplementary Fig. 2





Supplementary Fig. 3





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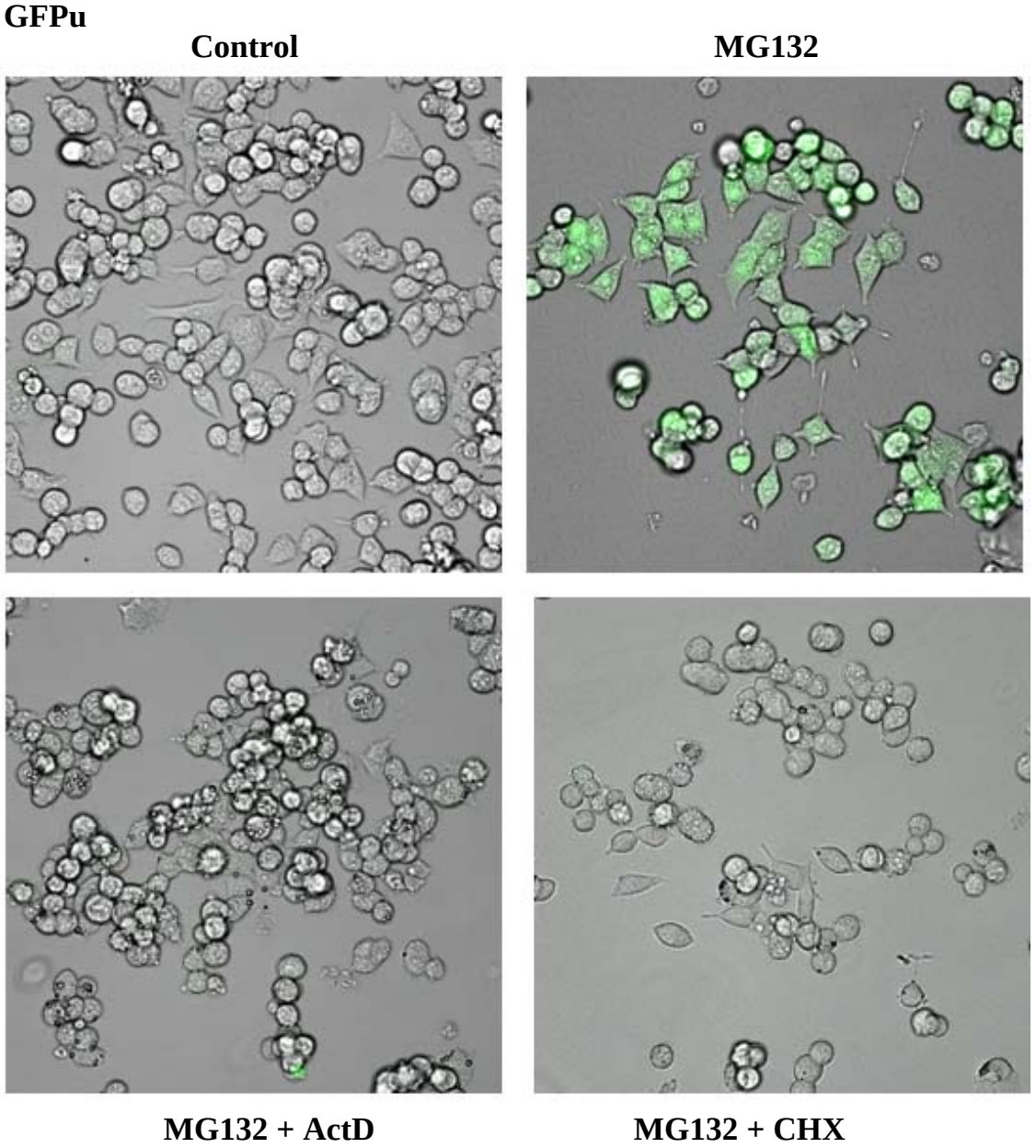

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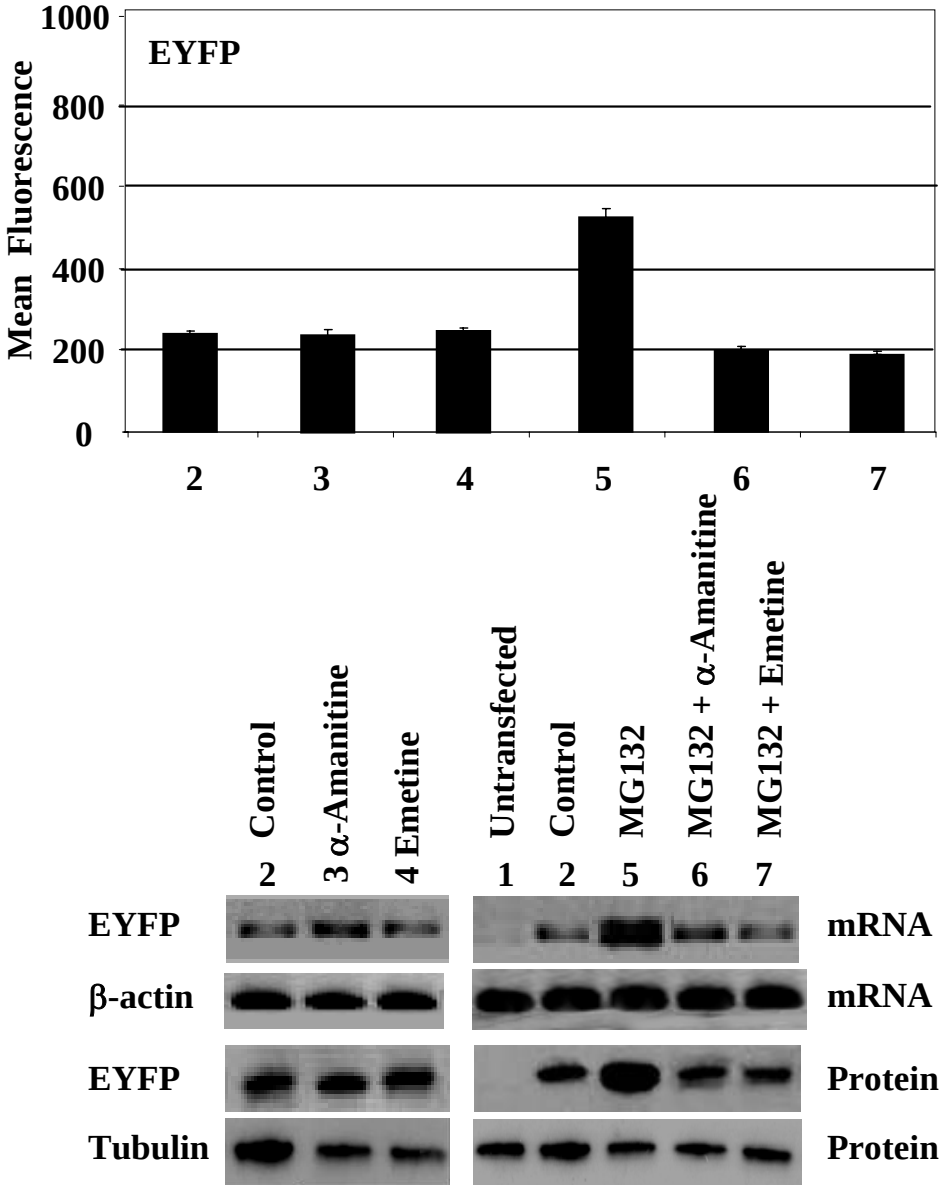
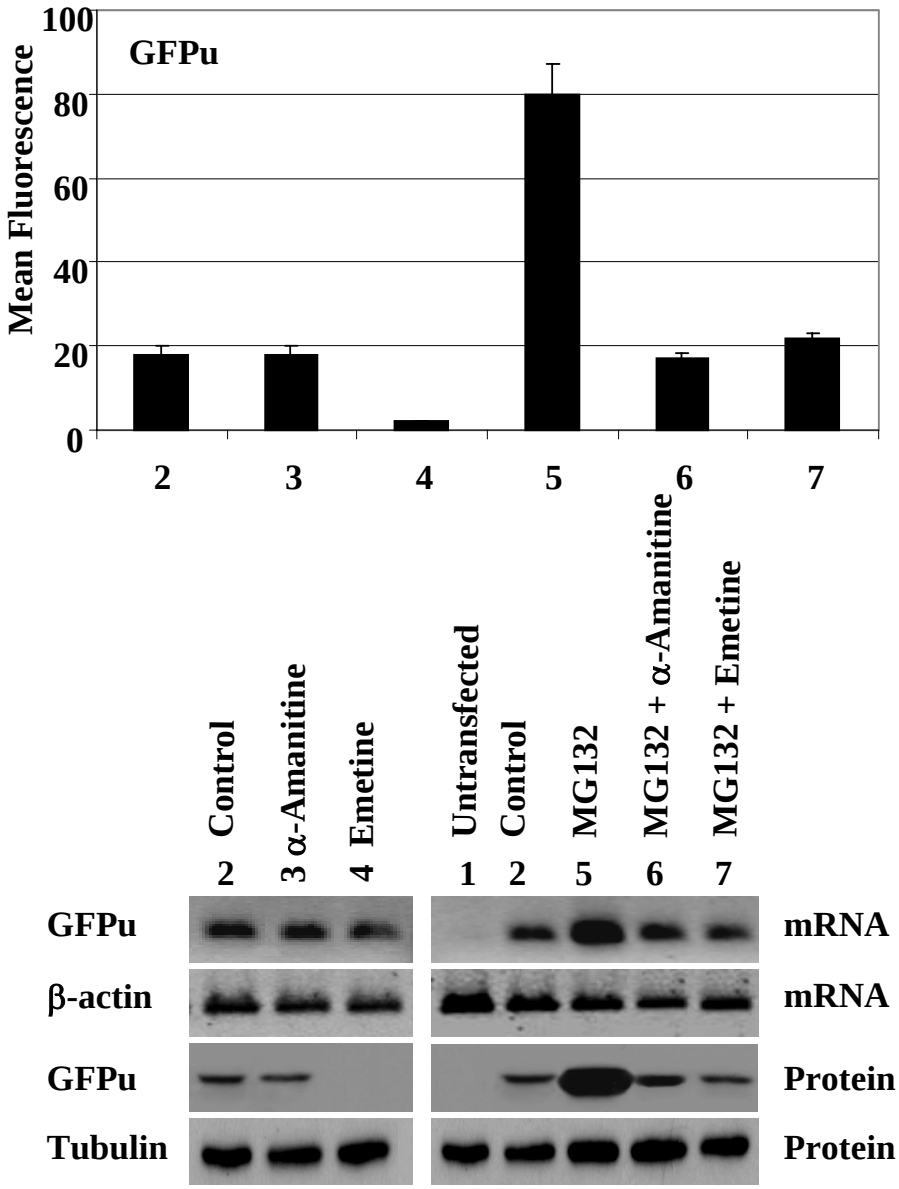
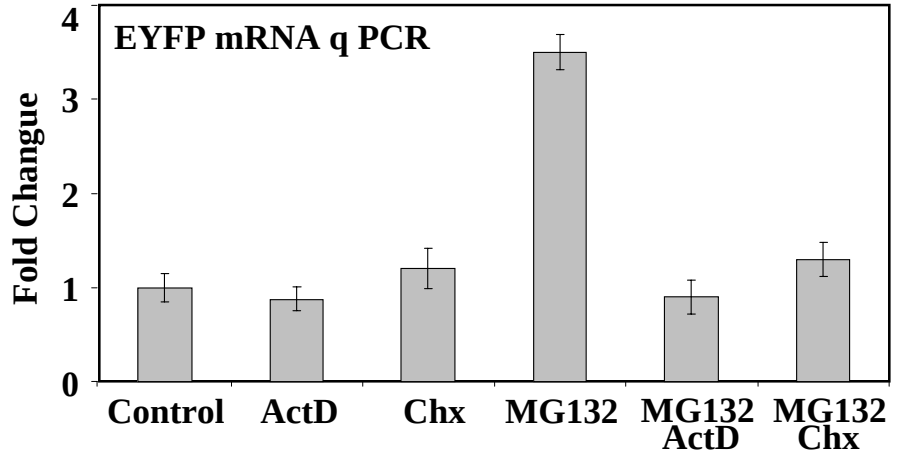
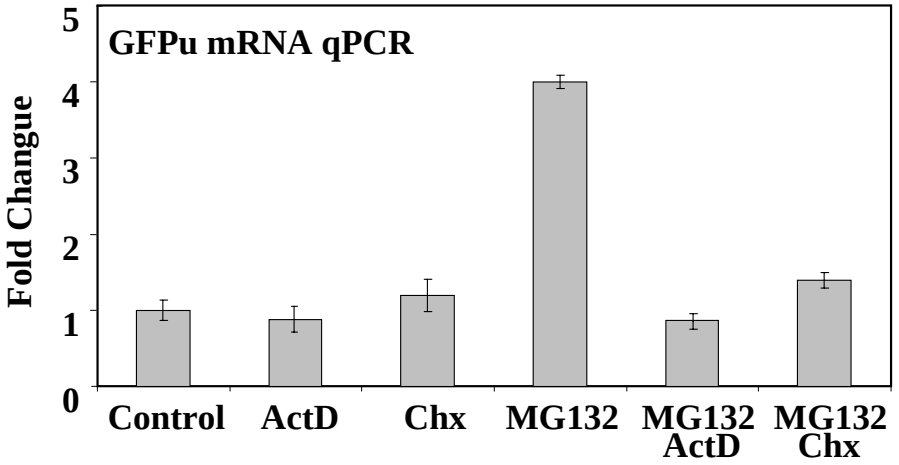
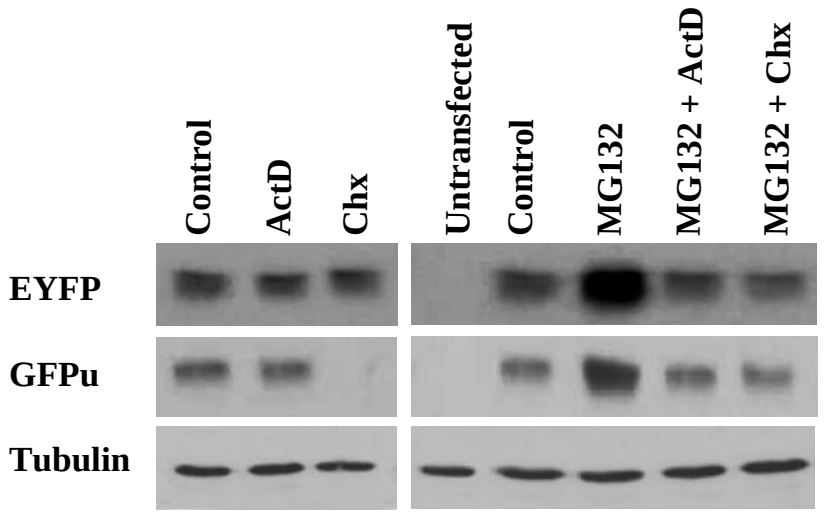




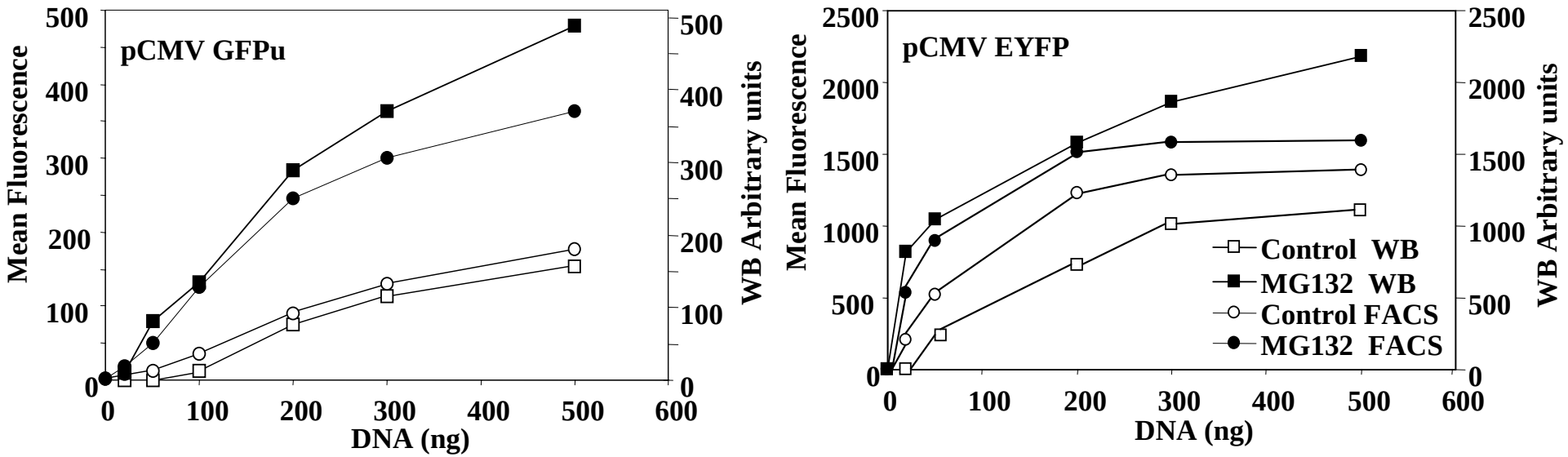
Supplementary Figure 6







Supplementary Fig. 7



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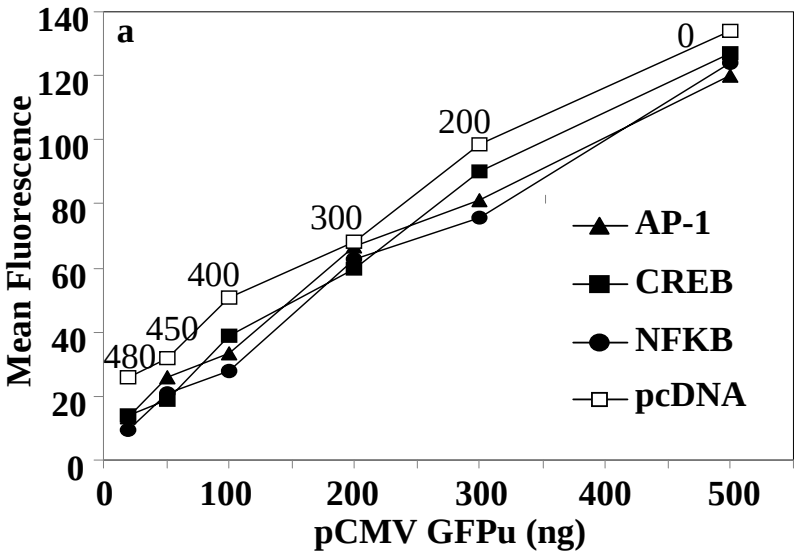

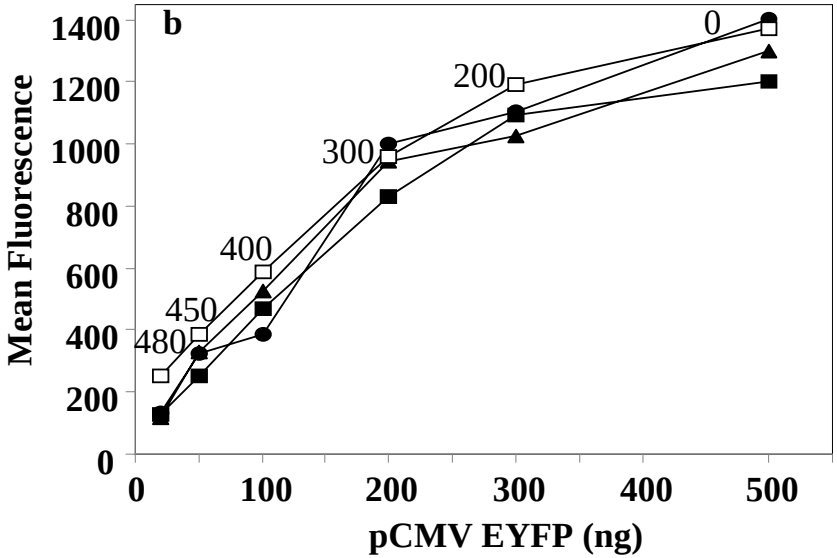

Supplementary Fig. 9