Supplementary Figures

The growth hormone receptor interacts with transcriptional regulator HMGN1 upon GH-induced nuclear translocation in RL95-2 cells

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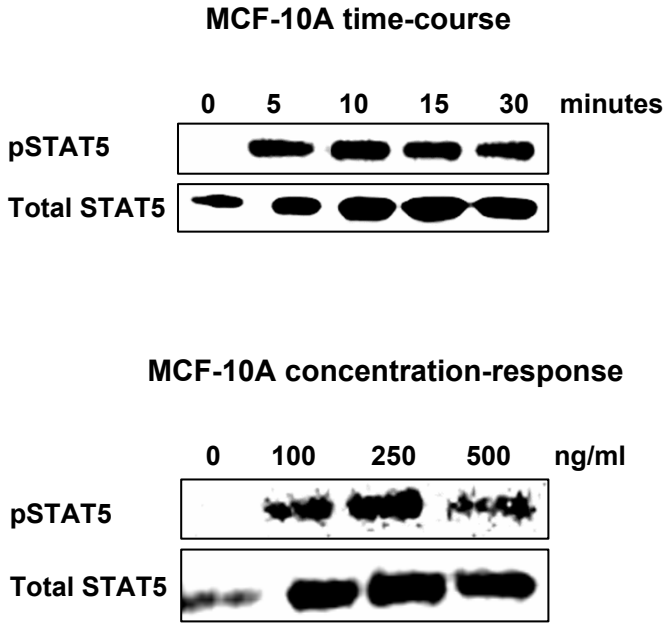
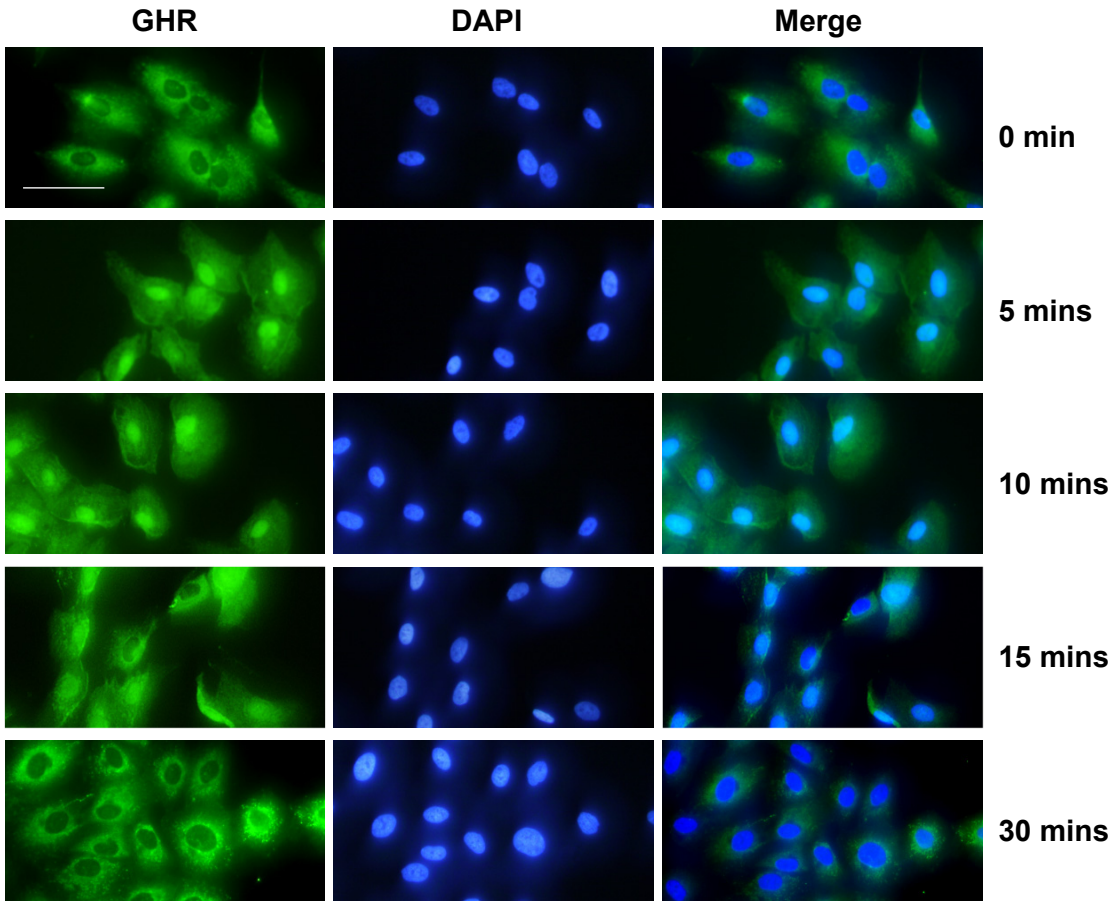
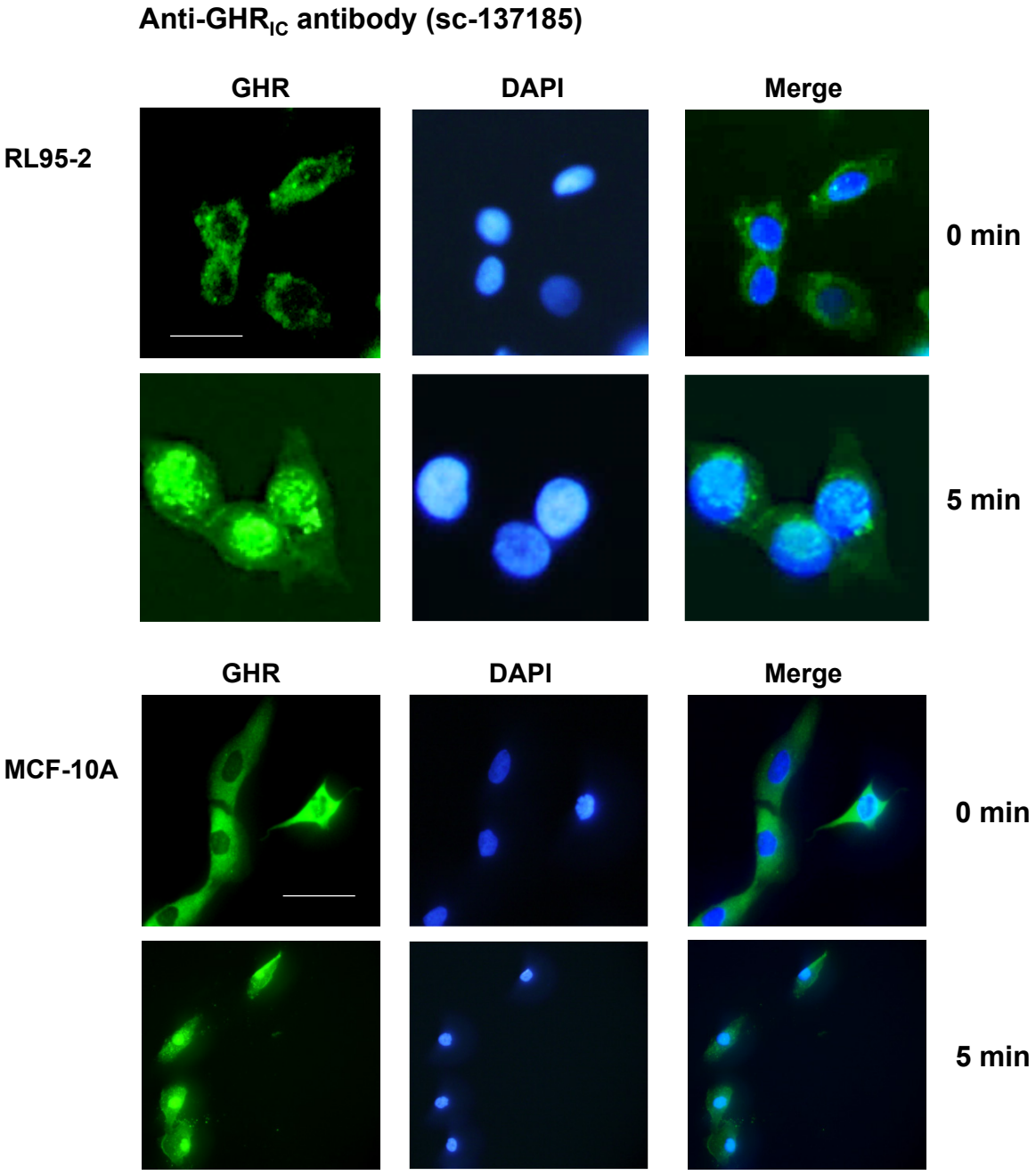


Jain *et al.* Supplementary Figure 1: GH increases STAT5 phosphorylation in MCF-10A cells. (A) GH treatment time-course in MCF-10A cells. Serum-starved MCF-10A cells were treated with 500 ng/ml recombinant human GH for 0, 5, 10, 15 and 30 min. (B) GH treatment concentration response in MCF-10A cells. Serum-starved MCF-10A cells were treated with varying doses of recombinant human GH, 0, 100, 250 and 500 ng/ml for 15 min. Cell lysates were immunoblotted for phosphorylated STAT5 (pSTAT5) and total STAT.

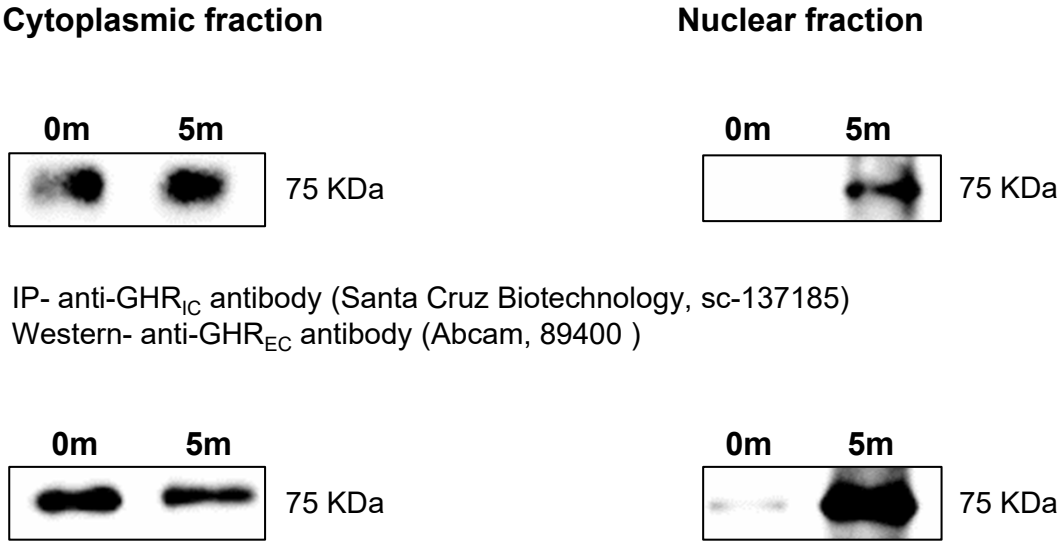
MCF-10A: Anti-GHR_{EC} antibody (Abcam, 89400)



Jain *et al.* Supplementary Figure 2: Nuclear localisation of the GHR in MCF-10A cells. MCF-10A cells were grown on coverslips, serum-starved overnight, and were treated with 250 ng/ml recombinant human GH for 0, 5, 10, 15 and 30 min. Cells were then fixed, permeabilised, blocked and immuno-stained with the anti-GHR_{EC} antibody (Abcam, 89400) and fluorescent secondary antibody. The slides were visualised using fluorescence microscopy. Green (alexa-fluor 488) represents GHR staining and blue (DAPI) is a nuclear stain. The scale bar represents 100 μm.



Jain *et al.* Supplementary Figure 3: GHR nuclear localisation in RL95-2 and MCF-10A cells using the anti-GHR$_{IC}$ antibody. Cells were grown on coverslips then serum starved and were treated with 250 ng/ml (MCF-10A) or 500 ng/ml (RL95-2) recombinant human GH for 0 and 5 min. Cells were then fixed, permeabilised, blocked and immuno-stained with the anti-GHR$_{IC}$ antibody (sc-137185) and fluorescent secondary antibody. The slides were visualised using fluorescence microscopy. Green (alexa-fluor 488) represents GHR staining and blue (DAPI) is a nuclear stain. The scale bar represents 100 μm.



Jain *et al.* Supplementary Figure 4: Serum-starved RL95-2 cells were treated with 500 ng/ml recombinant human GH for 0 and 5 min. Cell lysates were fractionated into cytoplasmic and nuclear fractions, immunoprecipitated and immunoblotted for GHR using the anti-GHR$_{EC}$ or anti-GHR$_{IC}$ antibodies (ab89400 and sc-137185, respectively), as indicated.