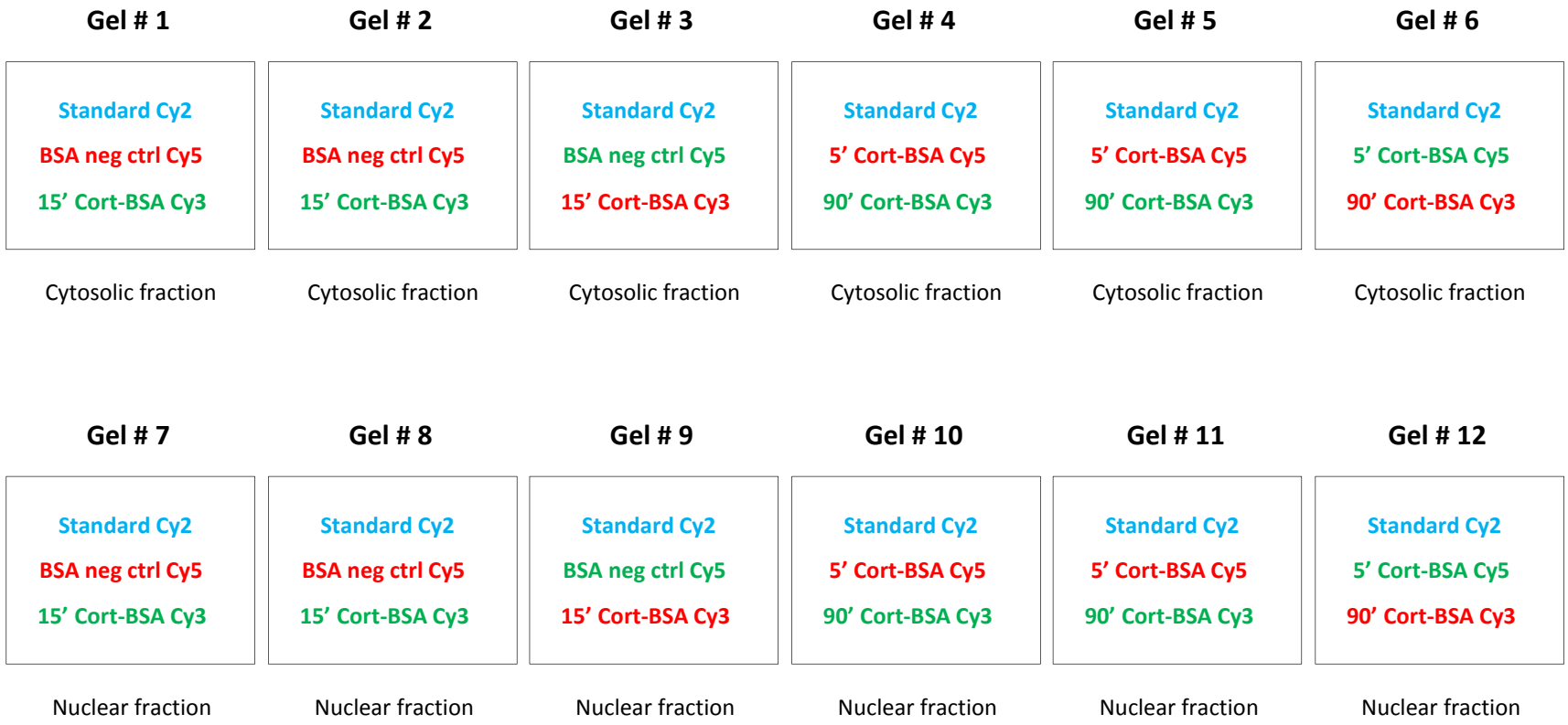
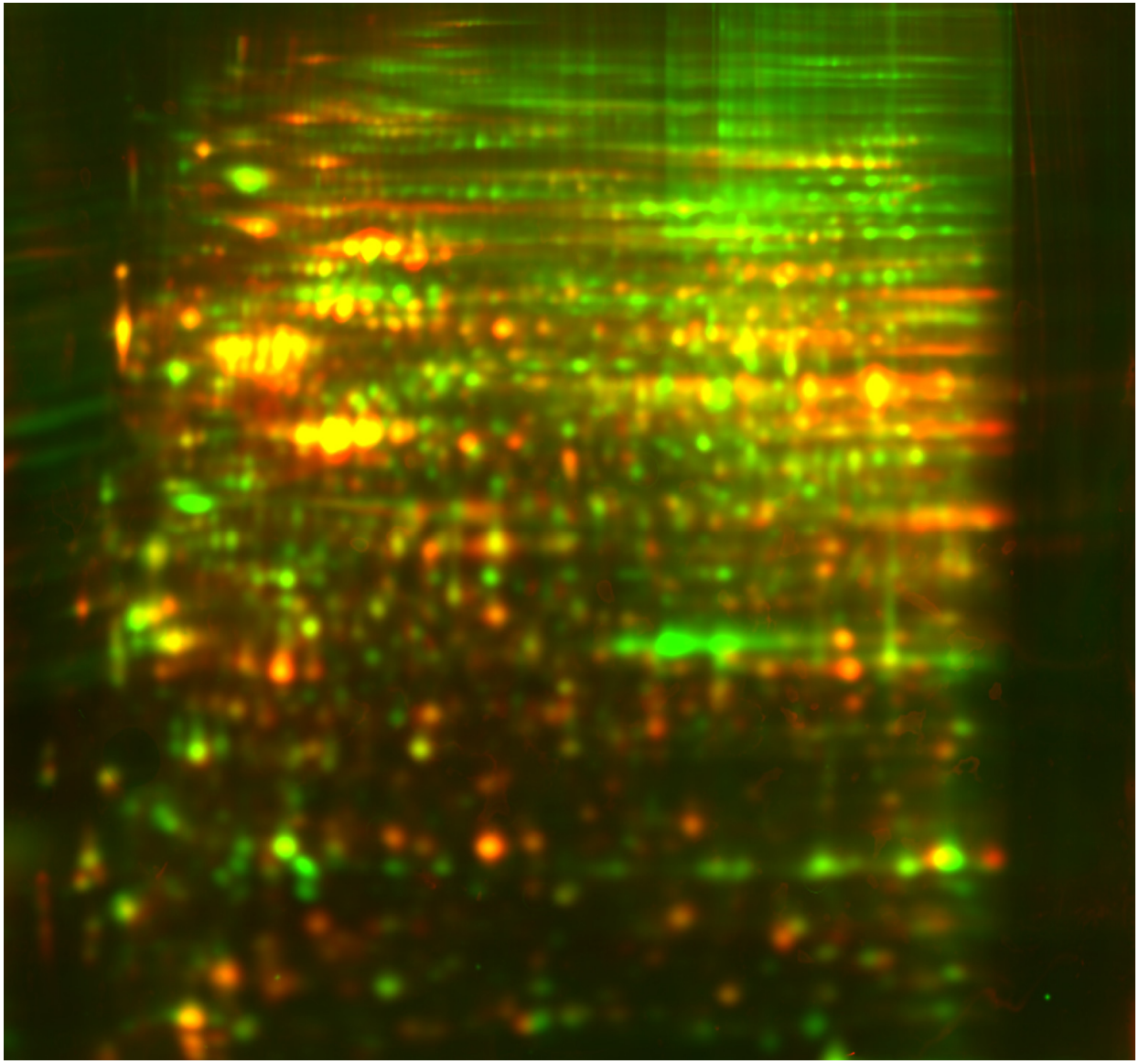
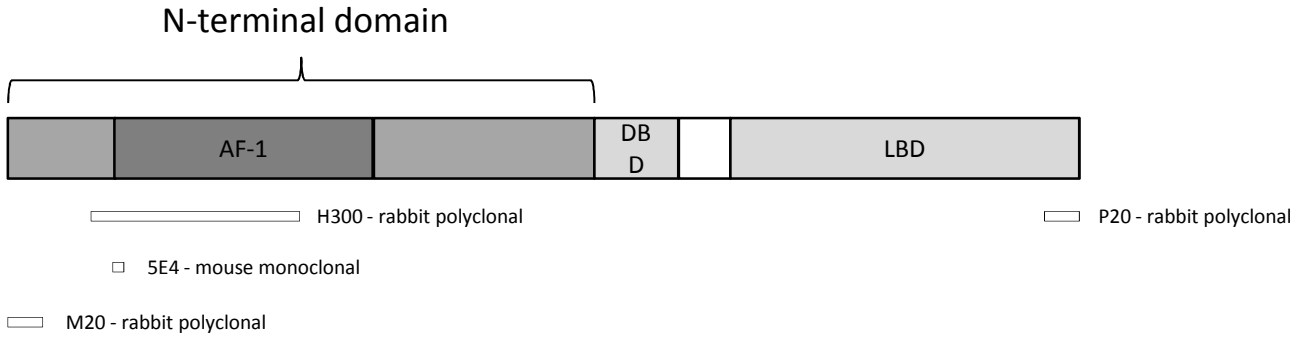


Supplementary Fig. 1

Cytosolic and nuclear fractions were fluorescence-labeled as follows: BSA negative control and 5min Cort-BSA by Cy5 dye, 15min and 90 min Cort-BSA by Cy3. A total of 3 analytical gels were performed for each of the following comparisons: CYTO neg vs. CYTO 15min; CYTO 5 min vs. CYTO 90min; NUCL neg vs. NUCL 15min; NUCL 5min vs. 90min. In each analytical gel a common Cy2-labelled control, obtained by pooling all the samples in equal proportion, was loaded and used as internal standard. To account for variations between the labeling, the dyes were swapped in one of the three replicatesCytosolic and nuclear fractions were fluorescence-labeled as follows: BSA negative control and 5min Cort-BSA by Cy5 dye, 15min and 90 min Cort-BSA by Cy3. A total of 3 analytical gels were performed for each of the following comparisons: CYTO neg vs. CYTO 15min; CYTO 5 min vs. CYTO 90min; NUCL neg vs. NUCL 15min; NUCL 5min vs. 90min. In each analytical gel a common Cy2-labelled control, obtained by pooling all the samples in equal proportion, was loaded and used as internal standard. To account for variations between the labeling, the dyes were swapped in one of the three replicates



Supplementary Fig. 2 Bidimensional gel showing the ovelay of cytosolic and nuclear fractions.

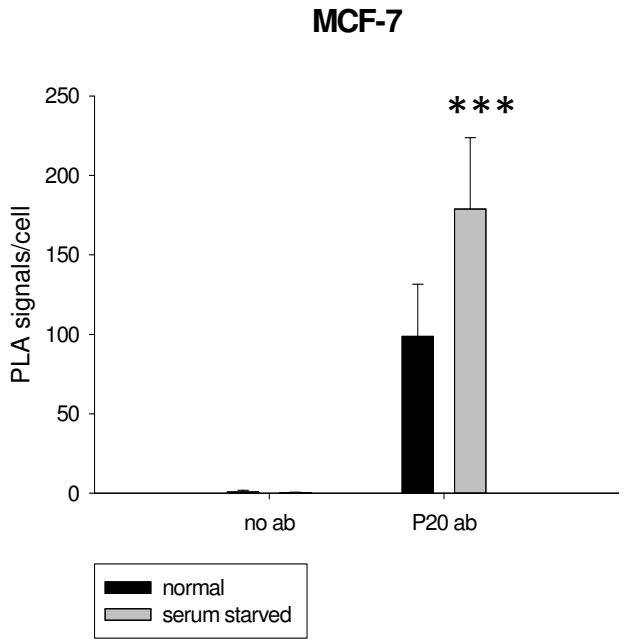


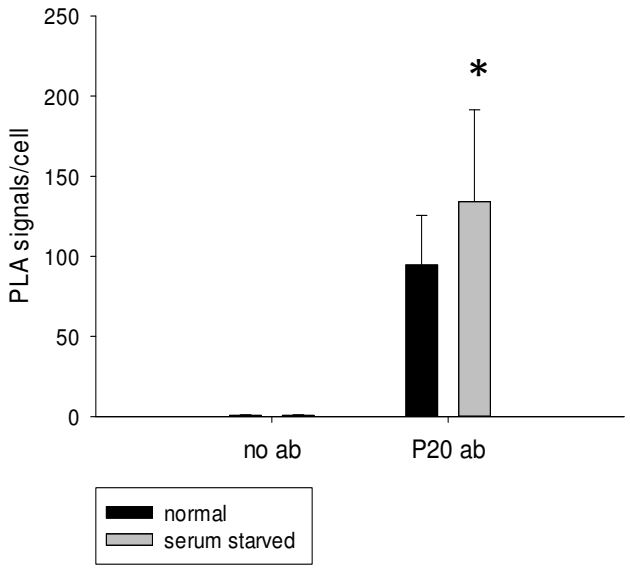
Supplementary Fig. 3

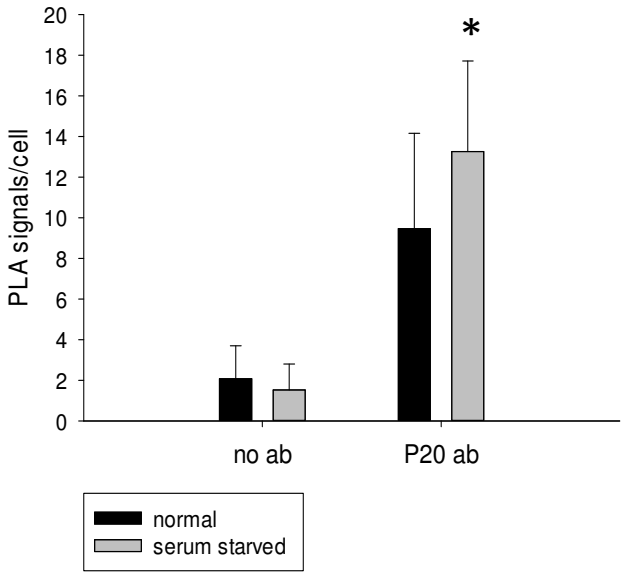
Schematic representation of the epitopes targeted by the four anti-GR antibodies used in the in situ PLA experiments.

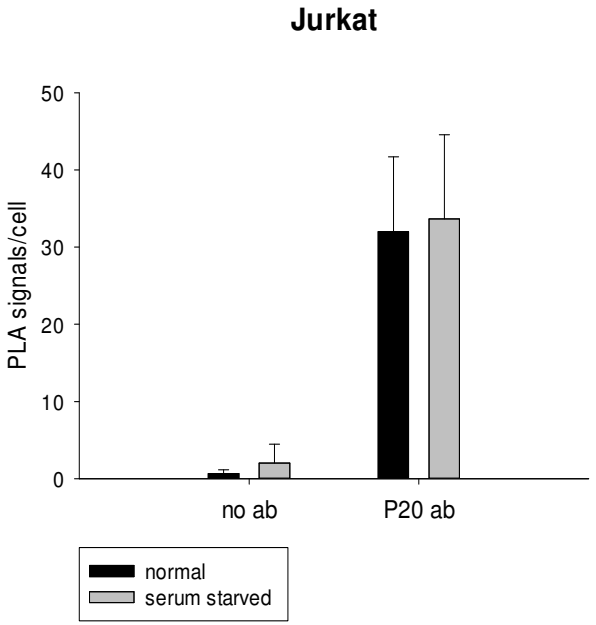
Supplementary Fig. 4

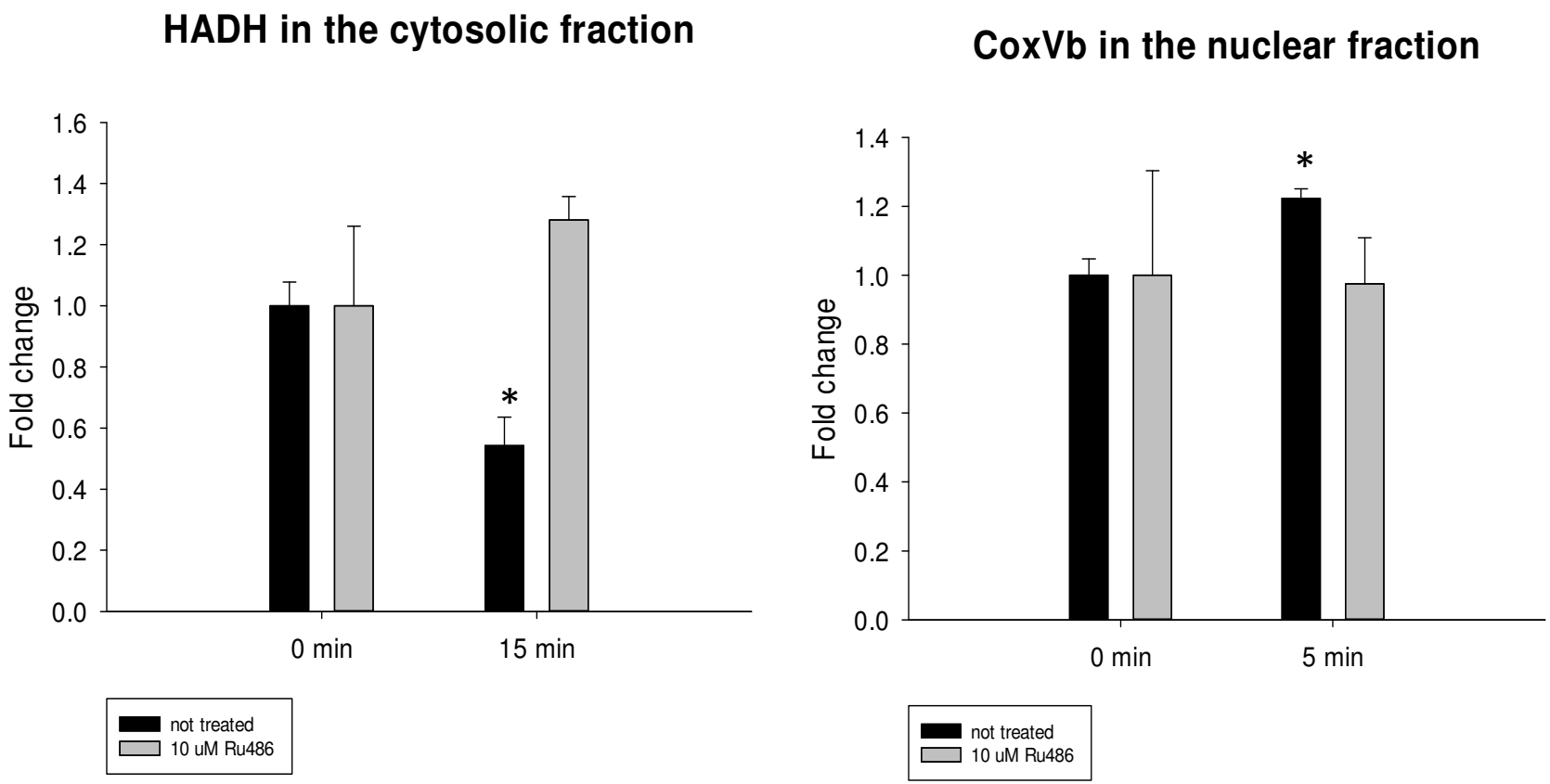
Cells were washed three times in Dulbecco's phosphate-buffered saline and incubated for 24h in their growth medium containing 10% charcoal-stripped FBS. Membrane GR expression was subsequently tested by in situ PLA using the P20 antibody. Glucocorticoid withdrawal significantly increased mGR expression in all cell lines, except Jurkat cells.











Supplementary Fig. 5 GR signalling was pharmacologically blocked in CCRF-CEM cells. Prior to Cort-BSA stimulation cells were incubated with 10 μM Ru486 for 15 minutes. The modulatory effects of Cort-BSA on both CoxVb and HADH were lost in treated cells, indicating that a functional GR is necessary for Cort-BSA to exert its function.