Supplementary Data - Figures S1-S9 & Table S1

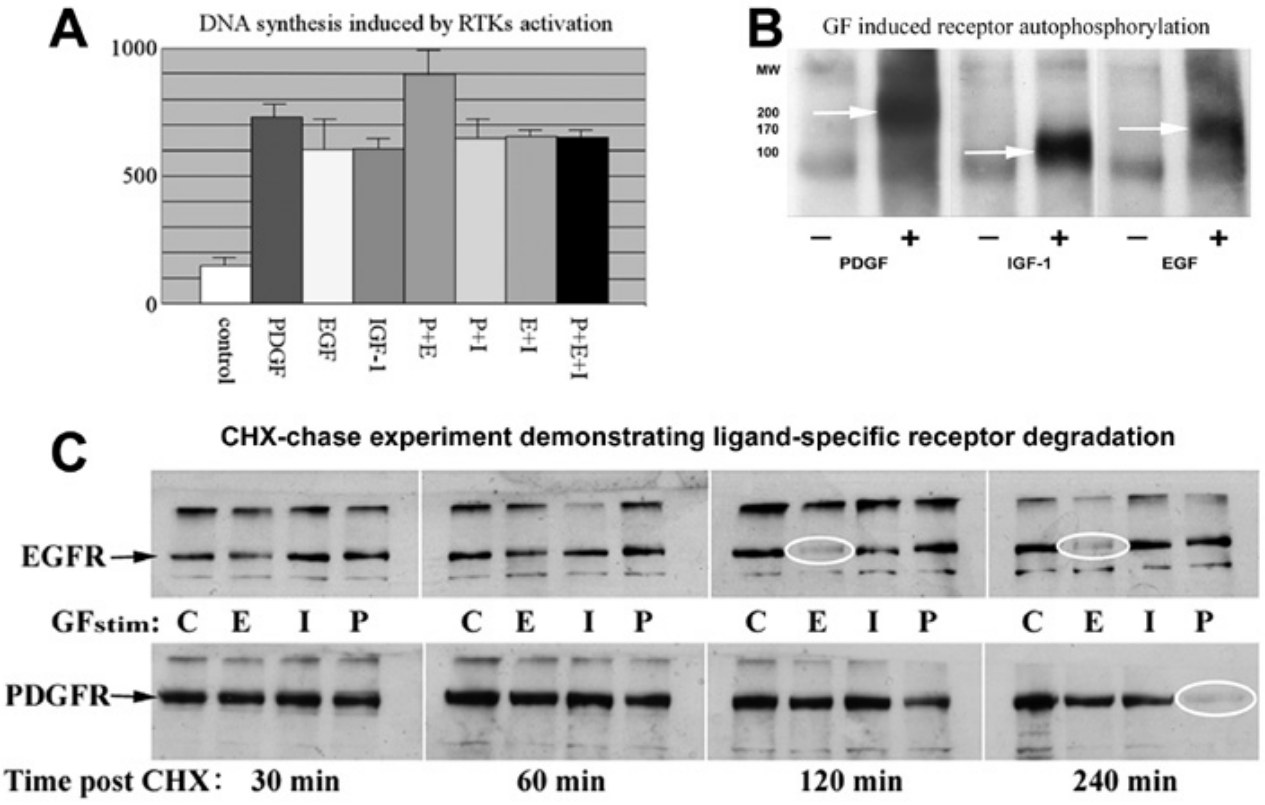

Figure S1 A. Mitogenic effect of PDGF, EGF and IGF-1 monitored by [^{3}H]-thymidine incorporation. **B**. Immunoblot demonstrating tyrosine autophosphorylation of different molecular weight receptors after 15 minutes exposure to the growth factors. The observed molecular weights for phospho-activated PDGFR, IGF-1R, and EGFR were 200 kDa, 170 kDa, and 100 kDa, respectively. **C**. Cycloheximide-chaseimmunoblot analysis of EGFR and PDGFR degradation in growth factor treated cells.

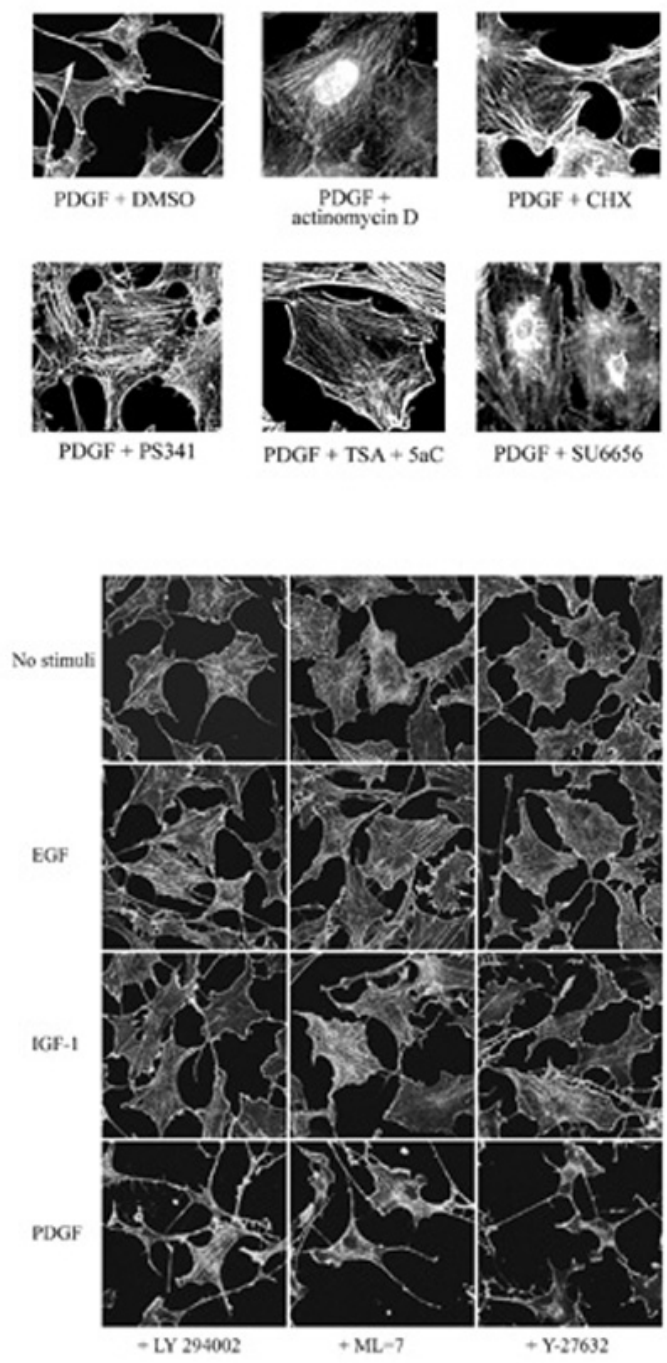

Figure S2 Immunofluorescence staining of the actin cytoskeleton in 3T3 cells stimulated with PDGF for 18 hr in the absence or presence of various inhibitors for the final 3 hr of stimulation. Actin filaments were visualized by rhodamine phalloidin staining.

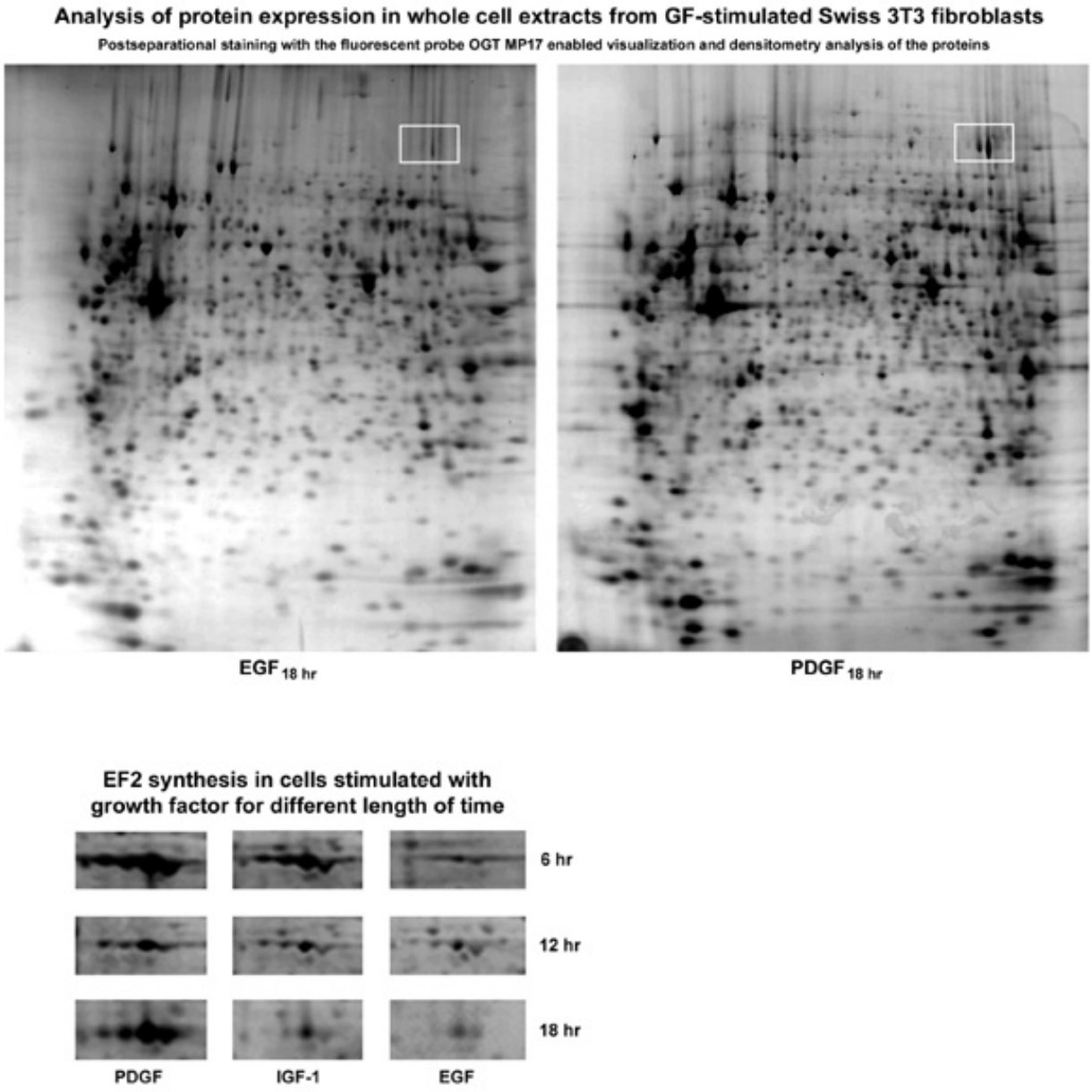

Figure S3 A. 2DE comparison of protein expression in Swiss 3T3 cells treated with EGF or PDGF for 18 hr. Post-separation gel staining with the fluorescent probe OGT MP17 enabled visualization and densitometry analysis of the proteins. EF2 isoforms are boxed by white rectangles. **B**. Enlarged area of ^{35}S-autoradiograms demonstrating variations in EF2 synthesis in response to long-term EGF, IGF1, or PDGF exposure. Both EF2 synthesis and expression levels were up-regulated in PDGF-stimulated cells.

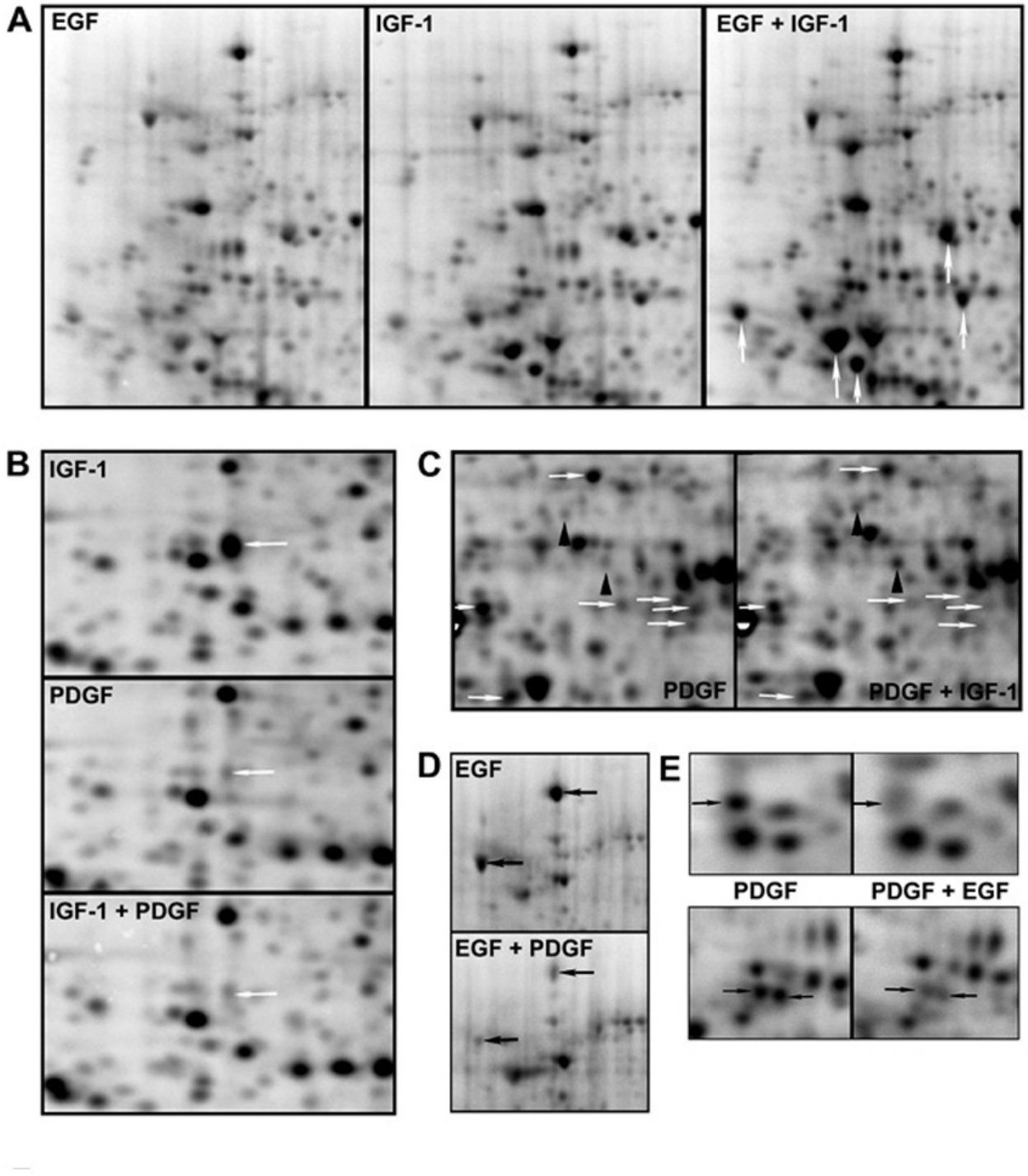

Figure S4 A-E. Examples of the enhancing and suppressive effects receptor co-activation instigate on protein synthesis in Swiss 3T3 fibroblasts. ^{35}S-labelled proteins were extracted by detergent/urea buffer following 18 hr GF-stimulation, separated on 2D gels and visualized by phosphoimaging. Enlarged areas of broad pH-range, non-linear IPG/PAGE gels are shown. Note, the additive, synergistic, and suppressive effects long-term co-stimulations instigate on protein synthesis.

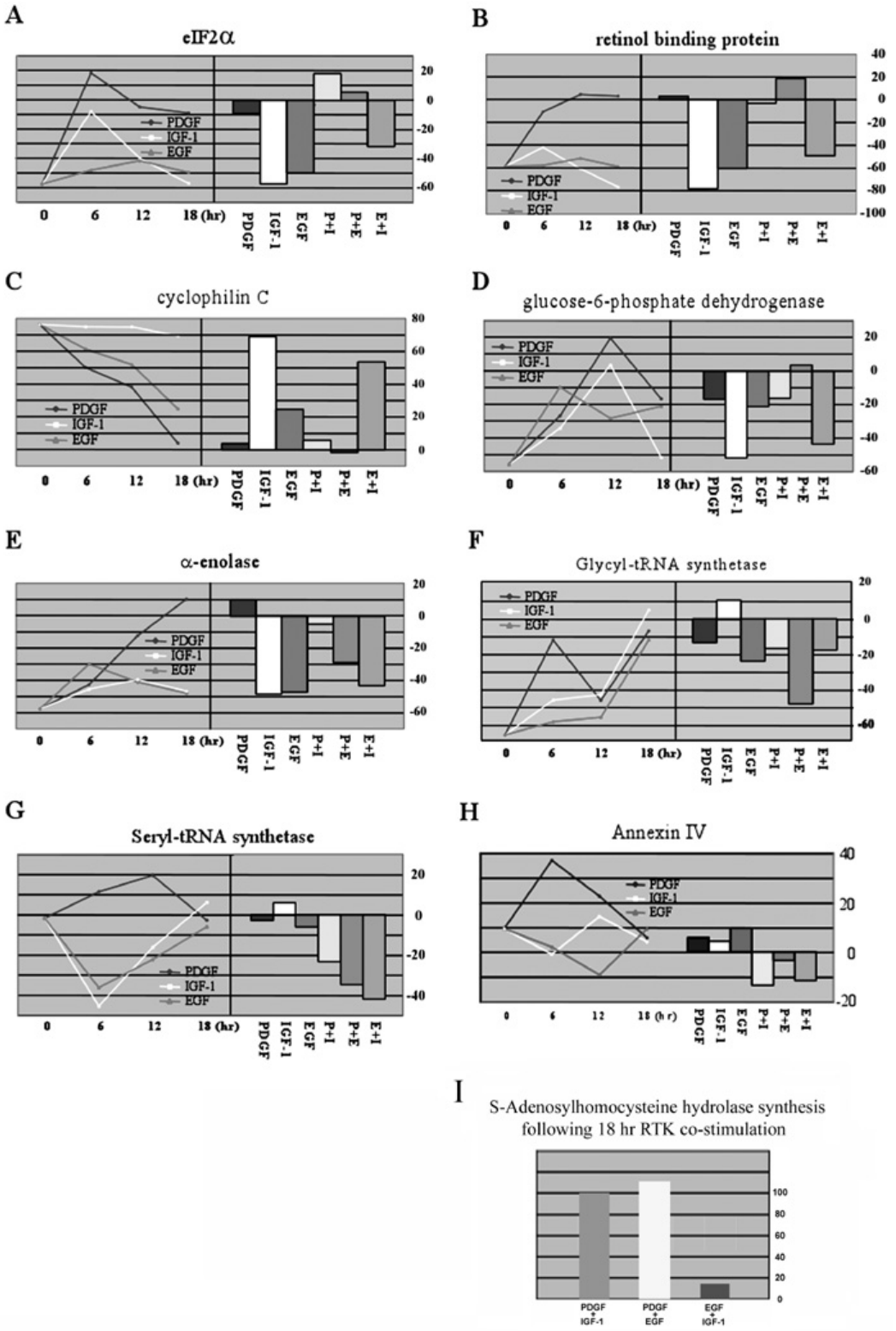

Figure S5 Examples of temporal changes in *de novo* protein synthesis in response to growth factors and their combinations. Effects of individual GF treatments over the time-course are shown to the left, and the effect of co-stimulation after 18 hr are shown to the right. Proteins were selected as examples of the

different responses induced by sustained GF treatment and to illustrate the occasional suppressive effect of co-stimulation. Quantitative values of protein spots were obtained from the average of three independent experiments. The three GFs induced different temporal patterns of synthesis for some proteins. Glucose-6-phosphate dehydrogenase was initially induced by all three GFs, but returned to basal levels following prolonged IGF-1, but not PDGF or EGF stimulation. Similarly, induction of eIF2a and HSP60 are maintained in PDGFtreated cells, but not in IGF-1 or EGF-treated cells, while glycyl-tRNA synthetase, seryl-tRNA synthetase and annexin IV were differentially regulated over the 18 hr time-course. a-enolase and retinol binding proteins were specifically and continuously induced during long-term PDGF treatment, while PDGF triggered transient induction of annexin IV. In contrast, EGF suppression of COP9 subunit 4 synthesis occurred following a 6 hr initial period of slightly increased synthetic rate (Figure S9). In some cases, co-stimulation had little effect on protein synthesis rates. For example, the induction of retinol binding protein by PDGF was unaffected by co-stimulation with IGF-1 or EGF, as was suppression of cyclophilin C synthesis. In both examples the effect of PDGF was dominant over EGF or IGF-1. Interestingly, some ^{35}S-labelled protein spots were suppressed by any kind of co-stimulation, as seen with glycyl-tRNA synthetase, seryl-tRNA synthetase and annexin IV. The PDGF-specific dominant increase in the synthesis of copper binding protein (also known as S-Adenosylhomocysteine hydrolase) is illustrated in Figure S5I. The synthesis rates were detected in cells co-stimulated for 18 hr. The rate of S-Adenosylhomocysteine hydrolase synthesis in cells treated with PDGF for 18 hr is set at 100.

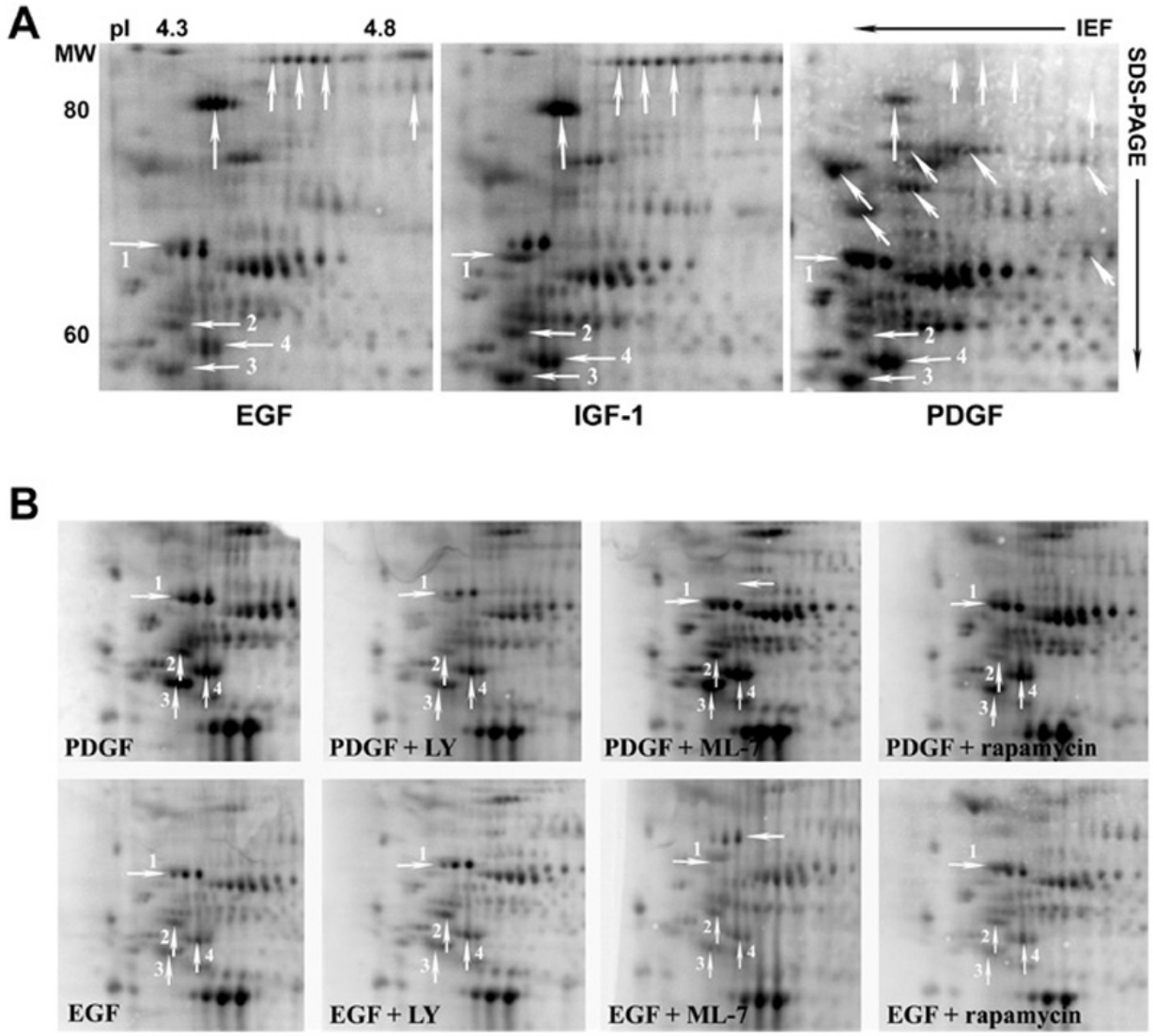

Figure S6 A. ^{33}P-labelled proteins found in Swiss 3T3 cells following 6 hr GF-stimulation. The highest level of ^{33}P-incorporation was detected in PDGF-treated cells. EGF and IGF-1 induced similar patterns of phospho-isotope labelling with some of the induced phospho-modifications barely detectable in the PDGF stimulated cells (white vertical arrows). Conversely, several PDGF-regulated phospho-proteins were barely detectable in cells stimulated with EGF or IGF-1 (oblique upward arrows).
B. ^{33}P-labelling experiment demonstrating the effect of selective kinase inhibition on phospho-signalling PDGF-and EGF-stimulated cells. LY294002 treatment resulted in a reduction in radiolabelling of several PDGF-regulated phosphoproteins (indicated by arrows with numbers). LY294002 treatment did not affect the pattern of ^{33}P labelling in EGF-treated cells. The effects of rapamycin were more subtle (proteins 2 and 3), suggesting that these proteins may be phosphorylated downstream of mTOR kinase. Protein 4 was excised from stained gels and identified as Akt by LC-MS/MS analysis.

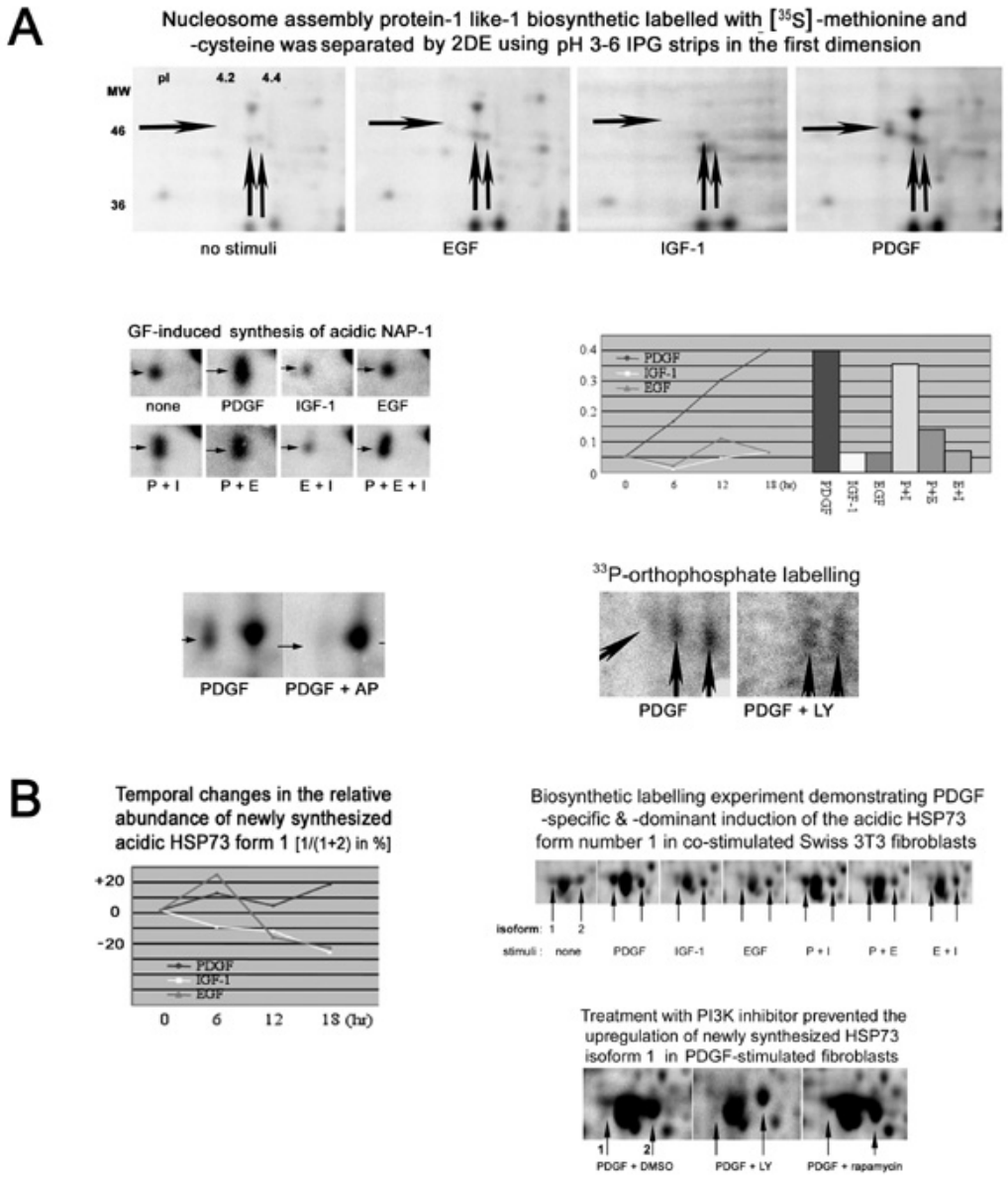

Figure S7 PDGF-specific phosphorylation of NAP1L1 and HSP73 via PI3K/Akt signalling.
A. Autoradiograms showing ^{35}S-biosynthetic labelling of NAP1L1 under different GF treatments (upper). Temporal changes in the synthesis of the most acidic isoform of NAP1L1 monitored as the amount of isotope incorporated during the last 3 hr of 6, 12 and 18 stimulation (middle). ^{35}Slabelled acidic NAP1L1 in extracts from PDGF stimulated cells (18 hr) untreated or treated with 7.5 U of alkaline phosphatase for 30 min prior to 2DE (lower left). ^{33}P-labelled NAP1L1 detected by 2DE autoradiography (lower right). Note that the PDGF-induced acidic forms (arrow) are reduced upon inhibition of PI3K with LY294002.
B. Temporal changes in the biosynthesis of two HSP73 isoforms (represented as the intensity of the acidic isoform (form 1) over the sum of the intensities of both isoforms) following different GF treatments (left). Representative 2D images of the HSP73 isoforms under different GF treatments and decrease in form 1 after LY294002 treatment (right panels).

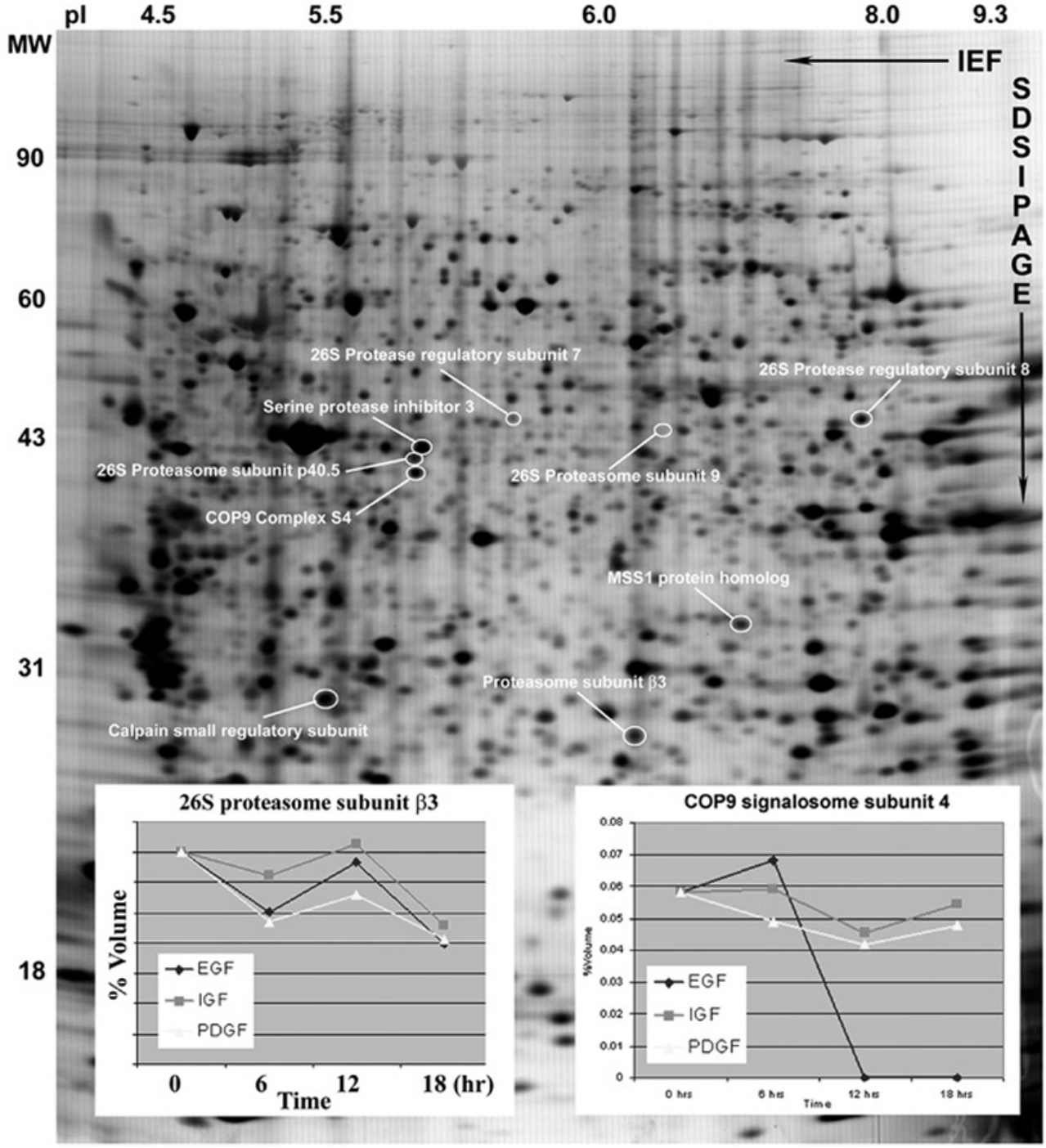

Figure S8 Silver-stained gel image demonstrating the electrophoretic positions of 9 identified proteins involved in the regulation of protein stability. Inserts show fluctuations in the synthesis of 26S proteasome subunit b3 (left) and COP9 signalosome subunit 4 (right) over the 18 hr time course.

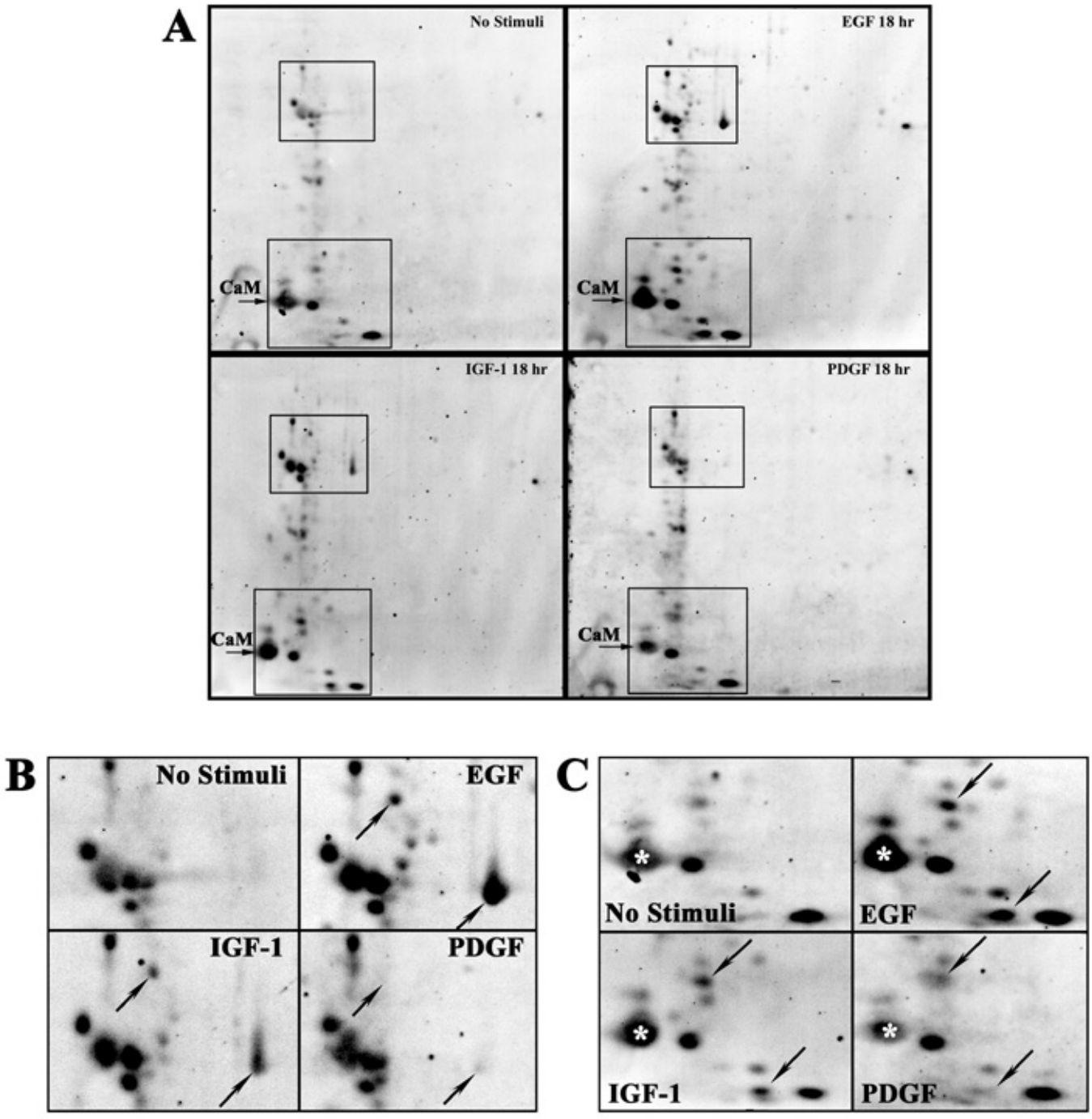

Figure S9 A. 2-DE ^{45}Ca overlay experiment demonstrating up-regulation of calcium binding capacity in cells stimulated with EGF for 18 hr. IGF-1 had an intermediate effect on calcium binding protein (CBP) activity and /or level, while long-term PDGF treatment had least effect on CBP activity, compared to non stimulated controls. Calmodulin (CaM) was identified based on its electrophoretic migration at 17 kDa, pI 3.9 (horizontal arrows). Boxed areas are enlarged in B and C. **B**. Differential regulation of 50 to 80 kDa calcium binding proteins in response to GF treatment. EGF treatment led to a significant up-regulation of the level and/or activity of two CBPs not detected in PDGF treated cells (oblique arrows). **C**. Differentially regulated low molecular weight CBP (oblique arrows). The position of CaM is indicated by white stars.