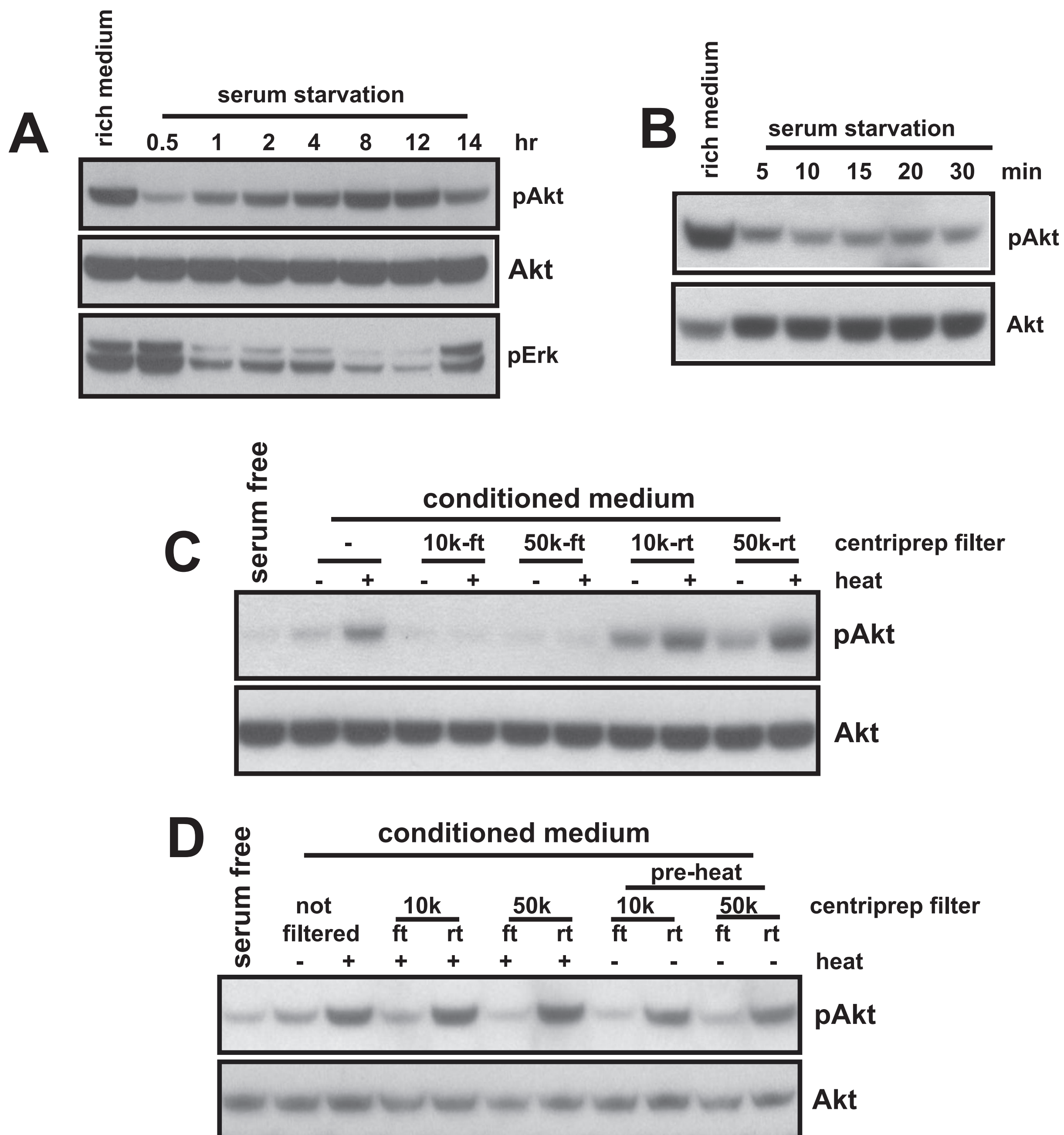
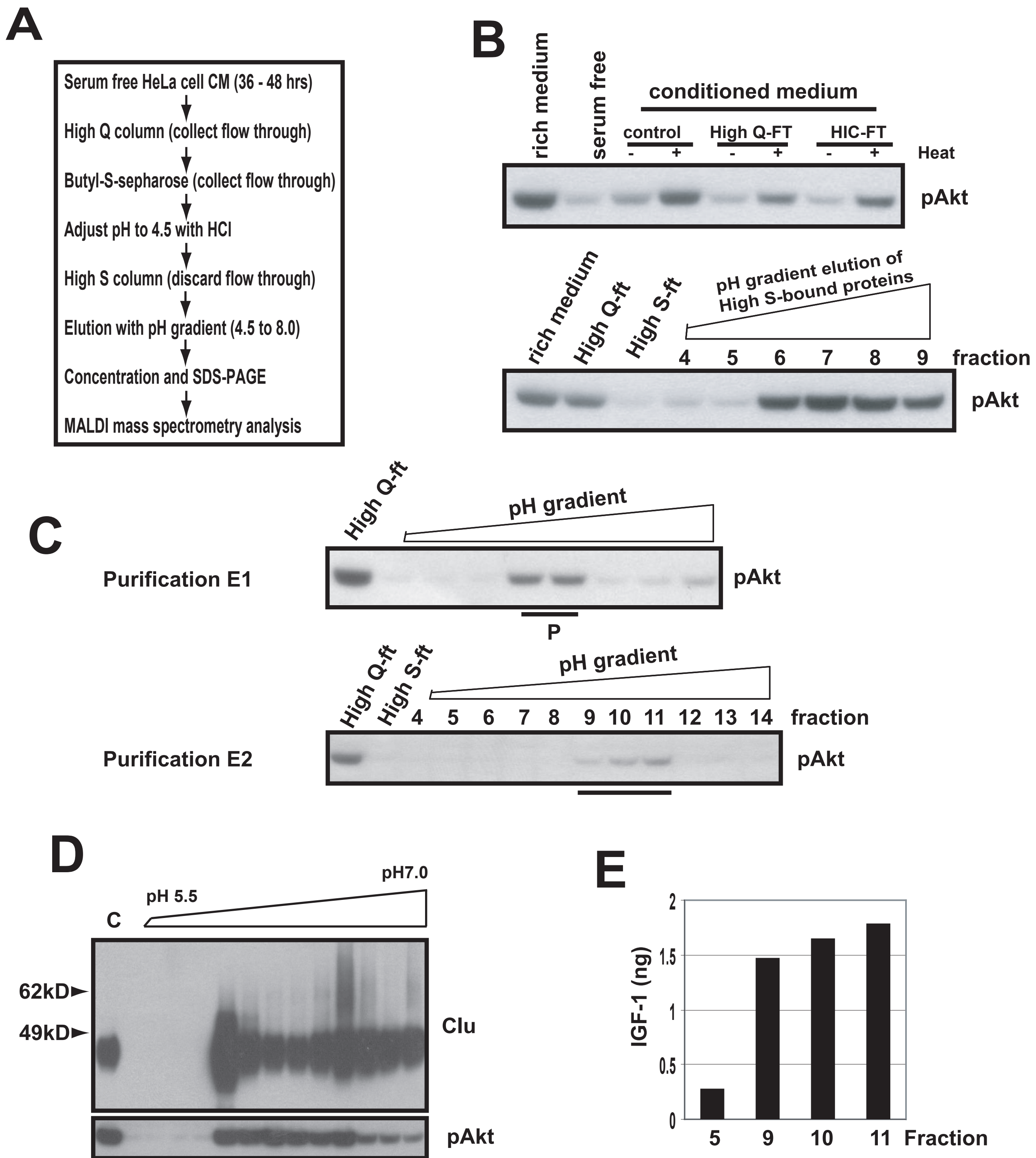
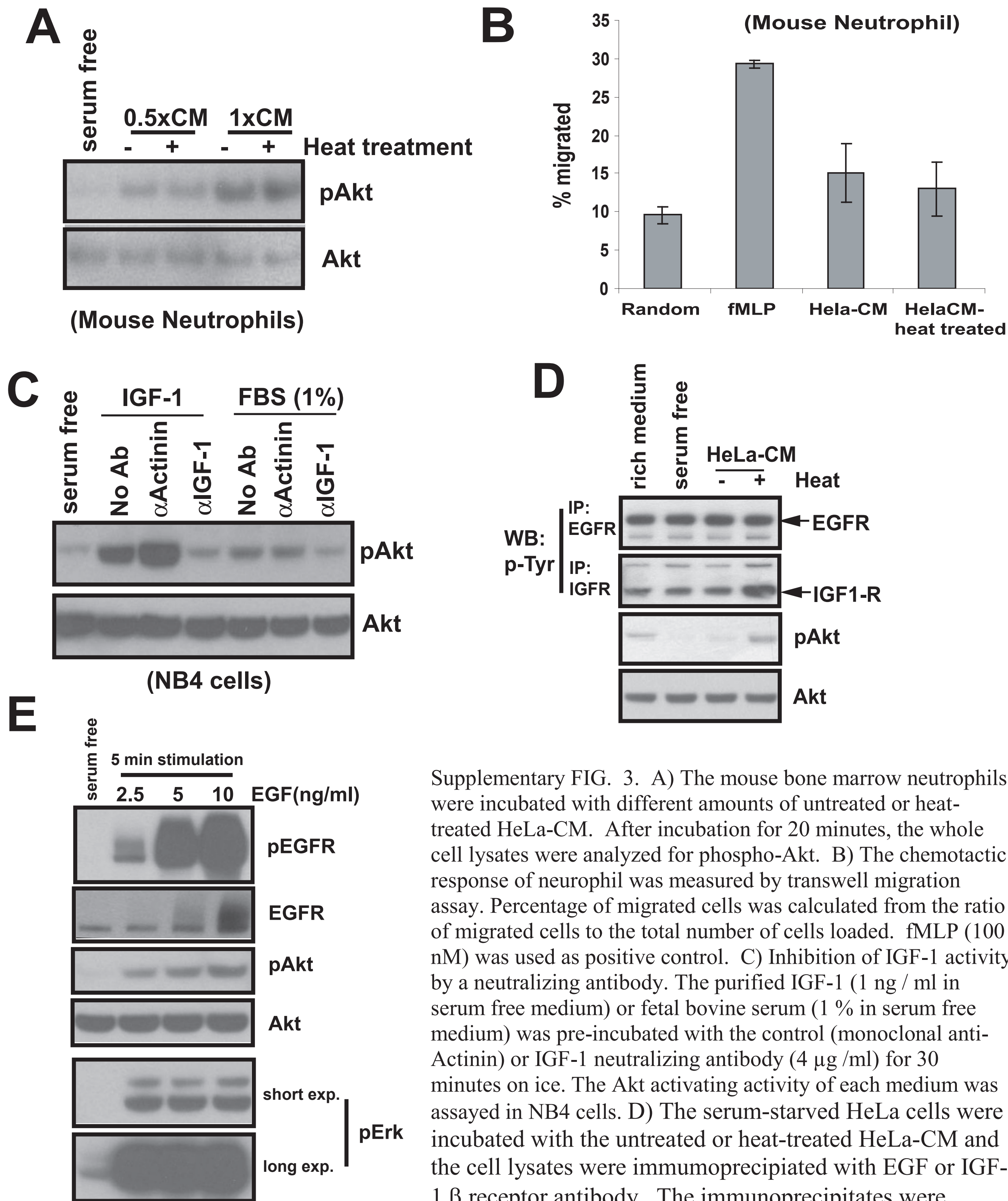


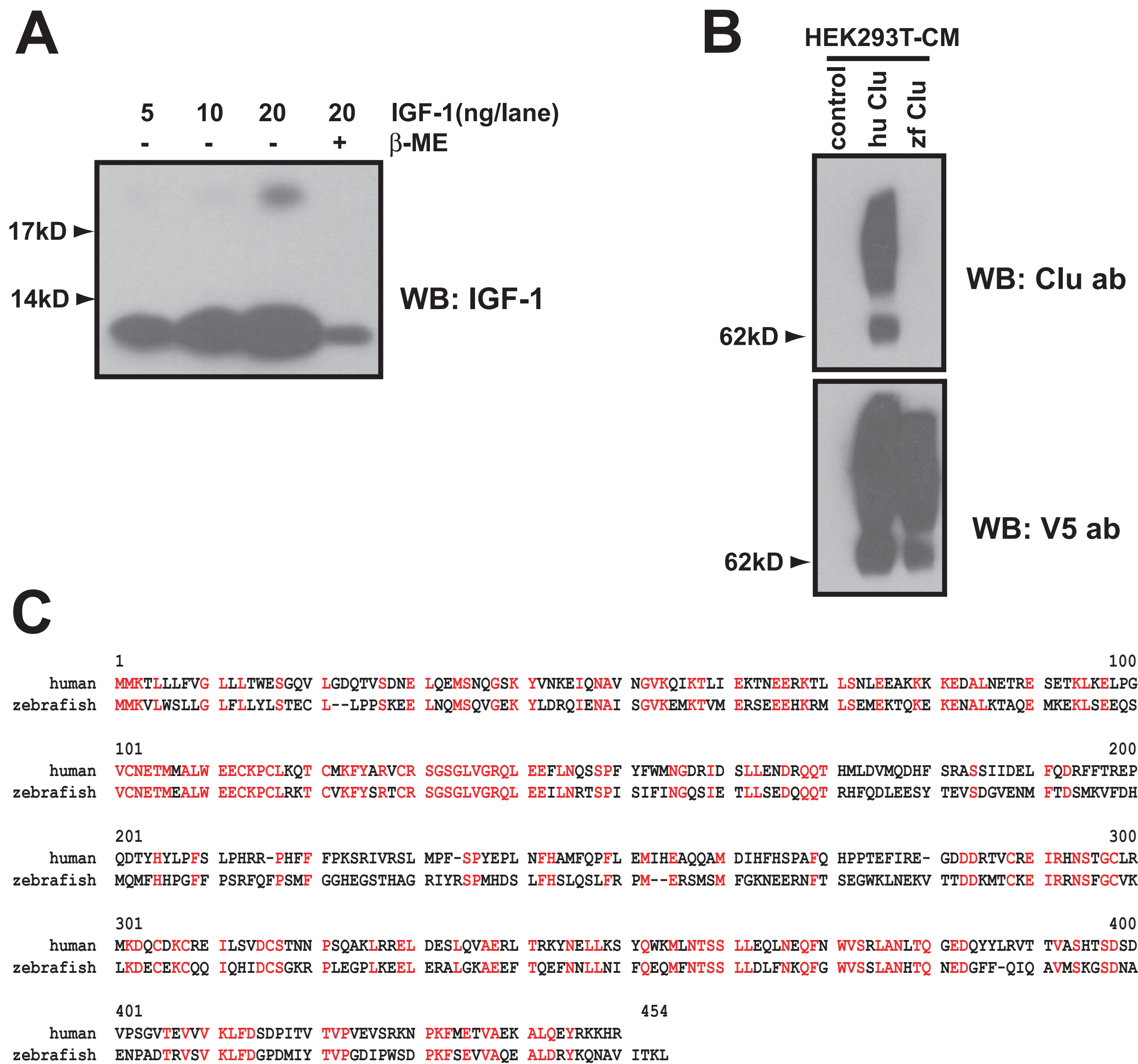
Supplementary FIG. 1. A) HeLa cells grown in serum rich medium for overnight were replenished with serum free medium, and the phospho-Akt and phospho-Erk from the whole cell lysates were examined at the indicated time point. B) A rapid decrease of phospho-Akt level in the initial phase of serum deprivation of HeLa cells. HeLa cells grown overnight in serum rich medium (10% FBS) were briefly washed in PBS and left in serum free medium. The whole cell lysates were analyzed for phospho-Akt level. C) A rapid decrease of phospho-Akt level in the initial phase of serum deprivation of HeLa cells. HeLa cells grown overnight in serum rich medium (10% FBS) were briefly washed in PBS and left in serum free medium. The whole cell lysates were analyzed for phospho-Akt level. D) HeLa-CM was concentrated using the Centriprep-10K or 50K filteration column (from 10 ml to 1 ml) and diluted to initial volume with serum free medium. The flowthrough (ft) or retentate (rt) of each column was either untreated or heat-treated, and assayed for their ability to activate Akt in HeLa cells. E) The untreated or heat-treated HeLa-CM was concentrated by Centriprep-10K or 50K cut-off filter. The flow-through and the retentate (diluted to initial volume with serum free medium) of each column were assayed for the Akt activating activity in HeLa cells.



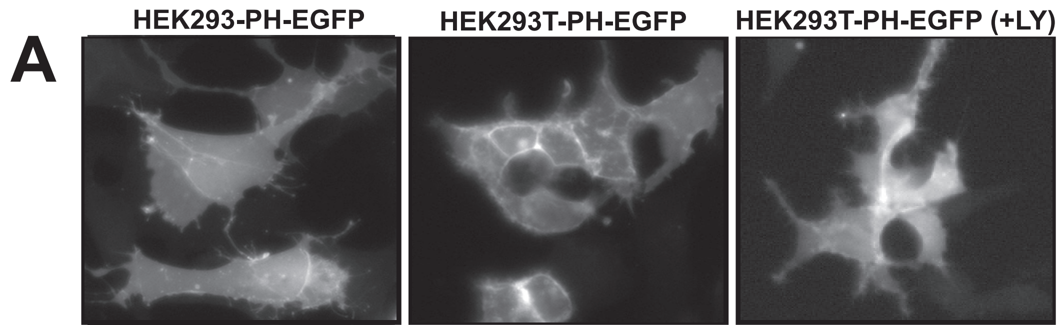
Supplementary FIG. 2. A) A flow chart of biochemical purification scheme. B) The control HeLa-CM and the flow-through fractions of High Q column (High Q-ft) or Butyl-S-sepharose hydrophobic interaction column (HIC-ft) were left untreated or heat-treated. The Akt phosphorylating activity was assayed in NB4 cells (top). The High Q flow-through fraction was collected and pH-adjusted, and then loaded onto High S column. The flow-through (High S-ft) and the bound proteins eluted with a pH gradient were assayed for their Akt phosphorylating activity in NB4 cells (bottom). C) The activity of High S bound proteins from two independent purifications. The underlined fractions correspond to the fractions used in Fig 4A. D) The High S bound-proteins were eluted by the pH gradient and each fraction was mixed with serum free medium (1:1) and analyzed. The corresponding fractions were tested for their activity to activate Akt in NB4 cells (bottom). E) IGF-1 ELISA from the fractions in purification experiment 2. The fraction number corresponds to that of in C).

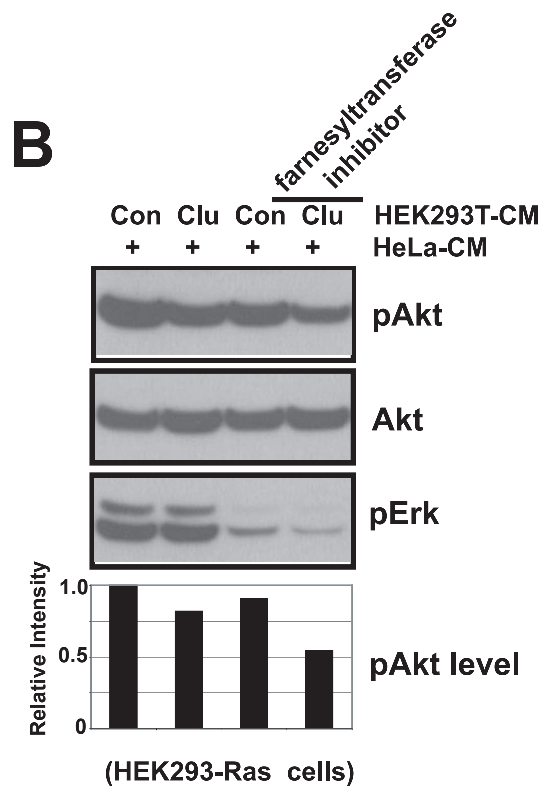


Supplementary FIG. 3. A) The mouse bone marrow neutrophils were incubated with different amounts of untreated or heat-treated HeLa-CM. After incubation for 20 minutes, the whole cell lysates were analyzed for phospho-Akt. B) The chemotactic response of neurophil was measured by transwell migration assay. Percentage of migrated cells was calculated from the ratio of migrated cells to the total number of cells loaded. fMLP (100 nM) was used as positive control. C) Inhibition of IGF-1 activity by a neutralizing antibody. The purified IGF-1 (1 ng / ml in serum free medium) or fetal bovine serum (1 % in serum free medium) was pre-incubated with the control (monoclonal anti-Actinin) or IGF-1 neutralizing antibody (4 μg /ml) for 30 minutes on ice. The Akt activating activity of each medium was assayed in NB4 cells. D) The serum-starved HeLa cells were incubated with the untreated or heat-treated HeLa-CM and the cell lysates were immumoprecipiated with EGF or IGF-1 β receptor antibody. The immunoprecipitates were analyzed by phosphotyrosine antibody. An aliquot of the lysate was also analyzed for phospho-Akt. The representative result of at least three independent experiments was shown. E) The serum-starved HeLa cells were stimulated with different amounts of purified EGF, analyzed for activation of EGF receptor, Akt, and Erk.

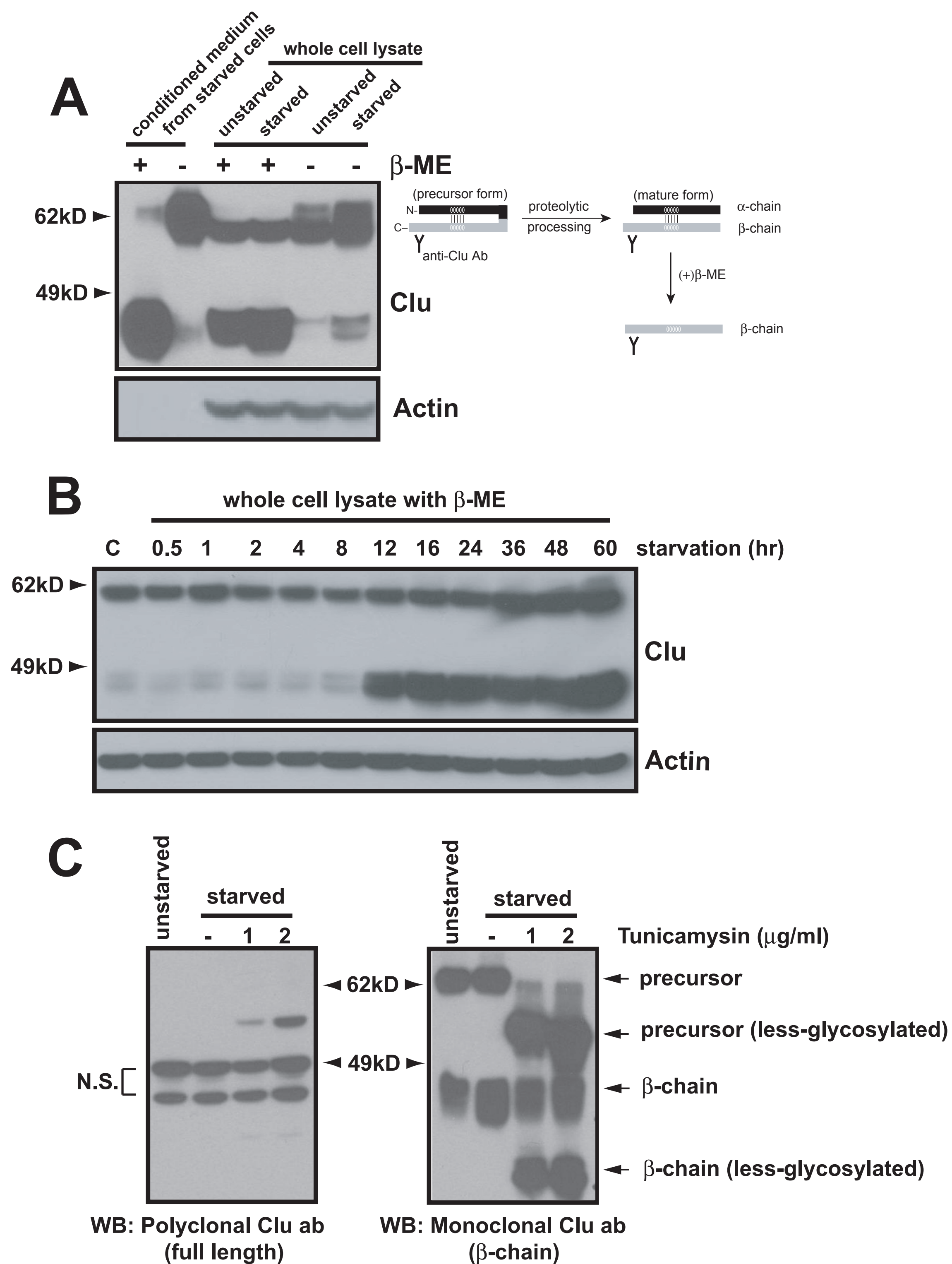


Supplementary FIG. 4. A) The indicated amount of purified IGF-1 was analyzed by Western blot with a monoclonal IGF-1 antibody (CBL52, Chemicon International). B) HEK293T-CM transfected with the control, the V5-6 x His tagged human or zebrafish Clusterin vector was analyzed by Western blot with a monoclonal human Clustern antibody or monoclonal V5 antibody (Invitrogen). C) The amino acid sequence alignment of human and zebrafish Clusterin. The letters in red indicate the identical amino acid.

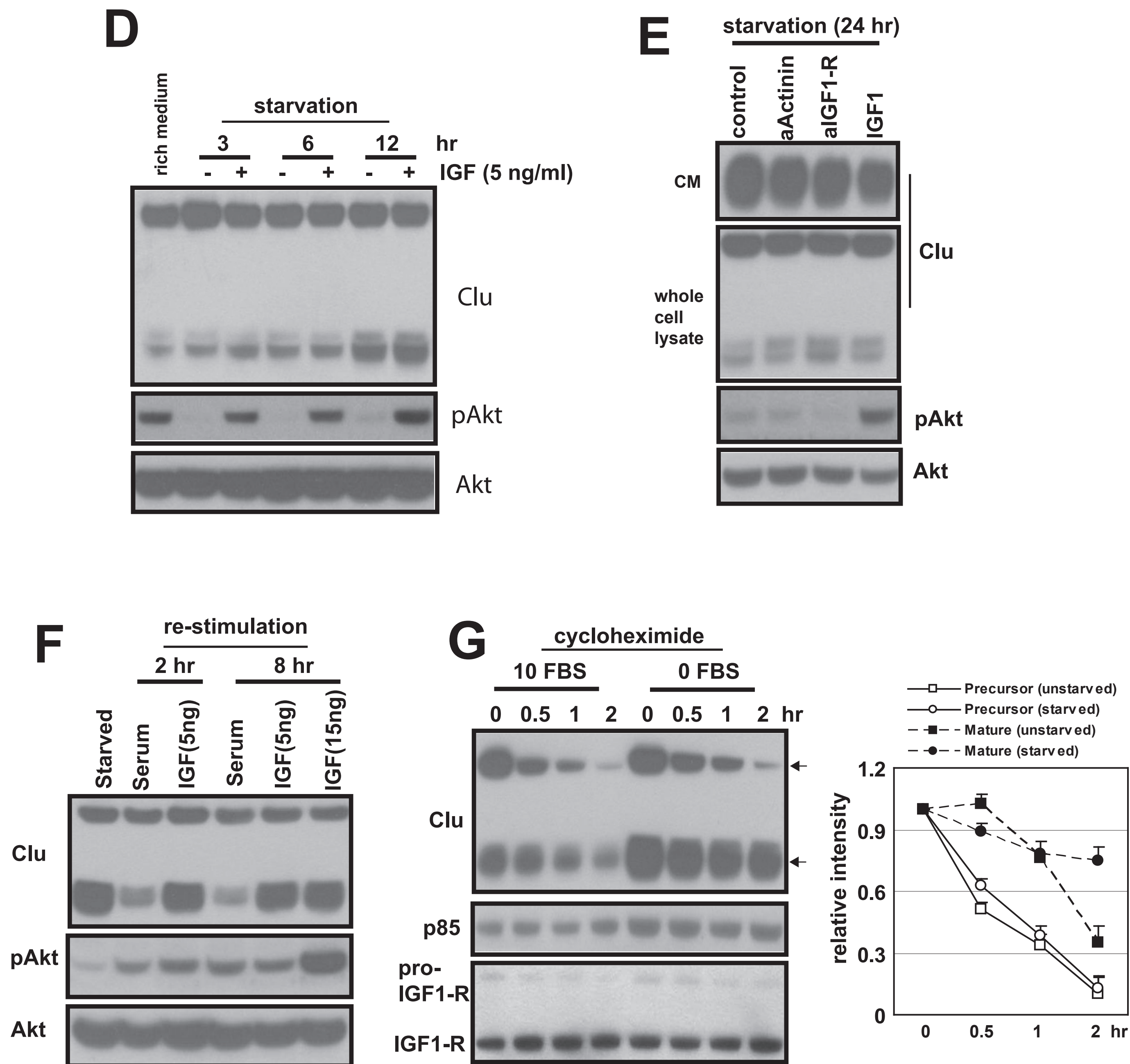




Supplementary FIG. 5. A) The PH-EGFP construct was transfected into HEK293 or HEK293T cells grown in serum rich medium. Cells were treated with LY294002 (20 μM) for 90 minutes before fixation and imaging. B) HEK293-Ras cells grown in serum rich medium with or without farnesyltransferase inhibitor (125 ng / ml for 36 hrs) were serum-starved for 30 min, then treated with HeLa-CM mixed (1:1) with the control (Con) or Clusterin (Clu) containing HEK293T-CM. The level of phospho-Akt was analyzed. The phospho-Erk level was measured to confirm the inhibition of Ras function by farnesyltransferase inhibitor.



Supplementary FIG. 6. A) The conditioned medium and the whole cell lysates of HeLa cells grown in serum rich medium or serum-starved for overnight were treated with or without β-mercaptoethanol (β-ME) prior to SDS-PAGE. The resolved proteins were analyzed for Clusterin. Schematic representation of precursor and mature forms of Clusterin is shown on the right. B) The whole cell lysates of HeLa cells grown in serum free medium for the indicated time were analyzed for Clusterin. C) The whole cell lysate of HeLa cells grown in serum rich or serum free condition with the indicated amount of tunicamysin (an inhibitor of N-glycosylation) was analysed with the polyclonal or monoclonal Clusterin antibody.



Supplementary FIG. 6. D) HeLa cells were serum starved for the indicated time in the presence or absence with IGF-1, the whole cell lysate was analyzed for Clusterin. E) The conditioned medium and whole cell lysates of HeLa cells serum-starved in the presence of indicated reagents were analyzed for Clusterin. F) The serum-starved HeLa cells were re-stimulated with serum or IGF-1 for the indicated time, and the whole cell lysates were analyzed for Clusterin. G) HeLa cells grown in serum rich or serum free condition was treated with cycloheximide (25 μg/ml) for the indicate time, and the whole cell lysates were analyzed for Clusterin, p85 subunit of PI3K, and IGF-1 receptor.

Supplementary FIG. 7. HeLa-PH cells were transfected with the indicated amount of Clusterin shRNA vector, the whole cell lysate of serum starved (12 hr) cells were analyzed for Clusterin. The same blot was analyzed for PARP and Caspase-3 to check for apoptosis.