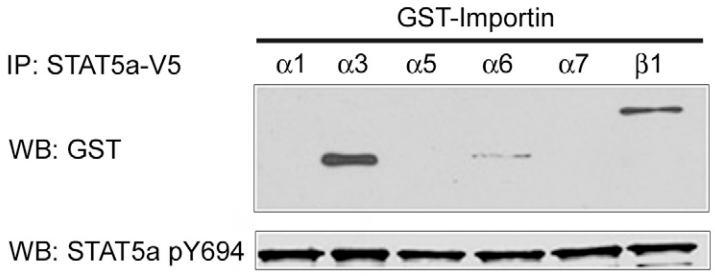
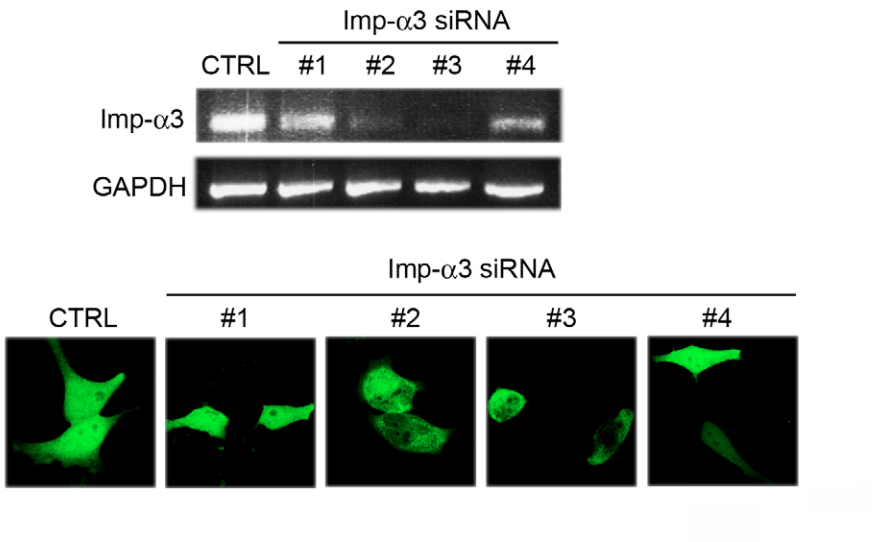
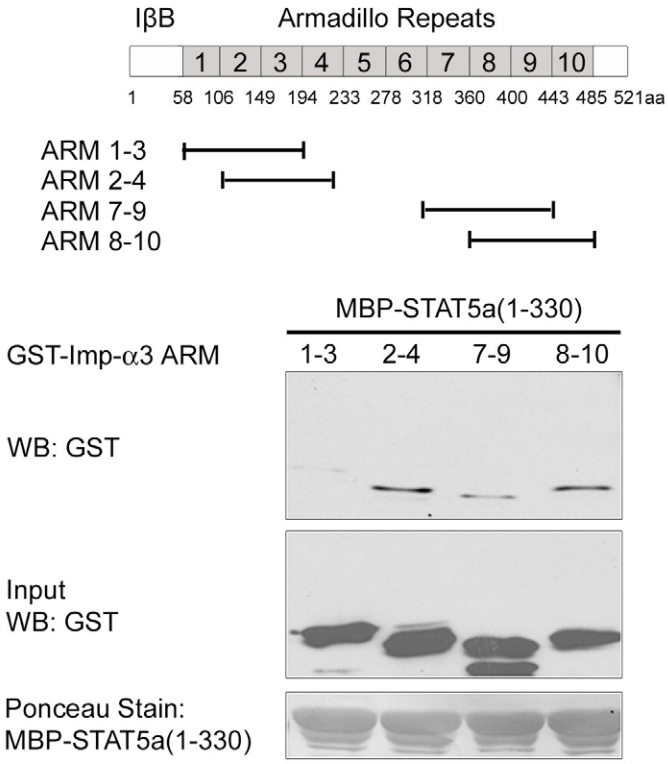


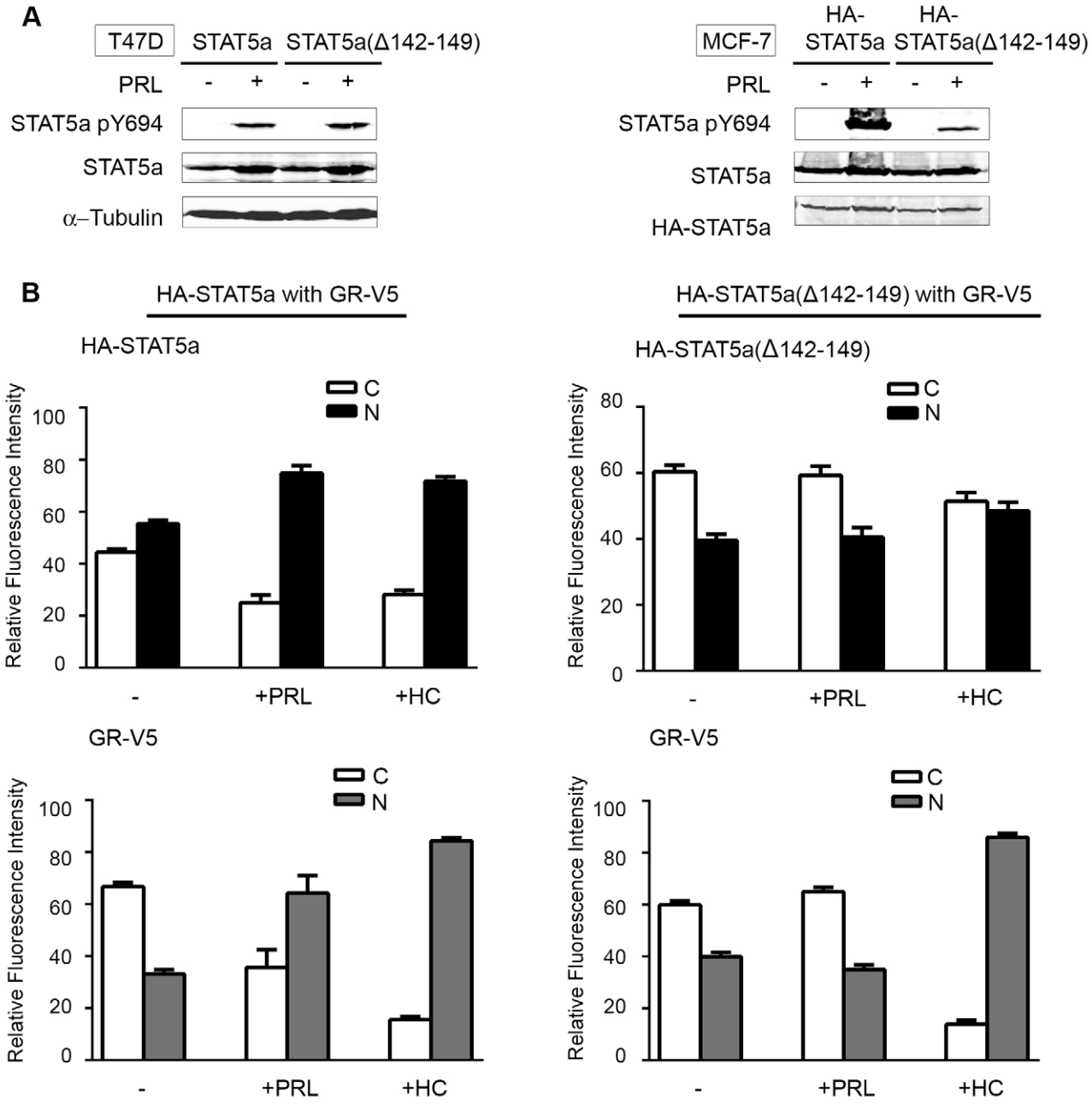
Supplementary Figure 1. *In vitro* binding assay with STAT5a and importins. Cells were transfected with STAT5a-V5 and treated with epidermal growth factor to stimulate STAT5a tyrosine phosphorylation. STAT5a was immunoprecipitated using protein G agarose beads, and incubated with purified GST-importins. Western blot with anti-GST identified importins bound to STAT5a. Input of tyrosine phosphorylated STAT5a bound to the beads shown by Western blot with anti-phosphotyrosine 694 (pY694) antibody. Purified importin input as Figure 3.



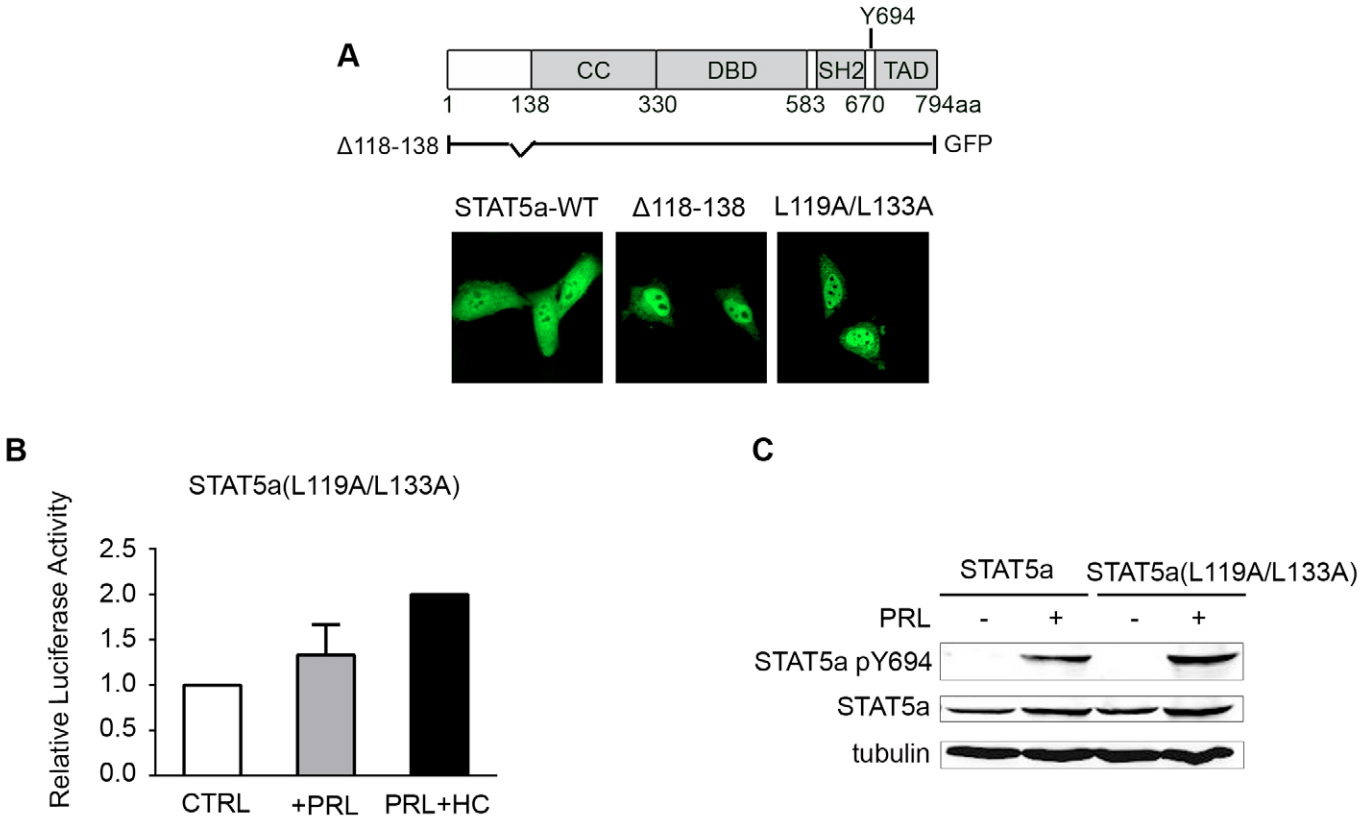
Supplementary Figure 2. Effect of importin-α3 knockdown by individual siRNAs on STAT5a nuclear import. *Top panel*, HeLa cells were transfected with four independent siRNAs targeting importin-α3 (Qiagen) for 48 hrs, and then transfected with STAT5a-GFP for 24 hrs. The effect of siRNAs on endogenous importin-α3 mRNA levels was detected by RT-PCR. Only importin-α3 siRNAs #2 and #3 significantly reduced the endogenous importin-α3 mRNA levels. The level of GAPDH mRNA was evaluated as an internal control. *Lower panel*, Effect of the four independent importin-α3 siRNAs on the cellular localization of STAT5a-GFP. Nuclear accumulation of STAT5a was reduced in the cells treated with siRNA that lowered levels of endogenous importin-α3 mRNA, siRNA #2 and #3. Results support a role of importin-α3 in STAT5a nuclear import.



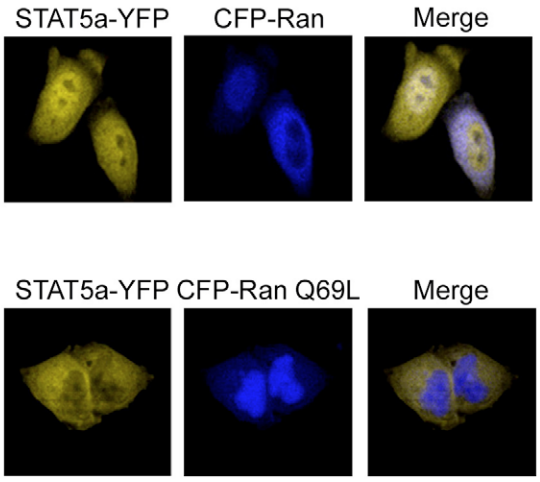
Supplementary Figure 3. ***In vitro*** **binding assay with STAT5a and importin-α3 deletion constructs.** *Top panel*, linear depiction of importin-α3 functional motifs with the constructs of deletion mutations. *Lower panel*, bacterially expressed MBP-STAT5a (1-330) was immobilized on amylase resin and incubated with GST-importin-α3 fragments. Deletions of importin-α3 bound to STAT5a were identified by Western blot using anti-GST antibody. Ten percent of importin inputs are shown in the middle panel, and protein level of STAT5a bound to resin shown in bottom panel.



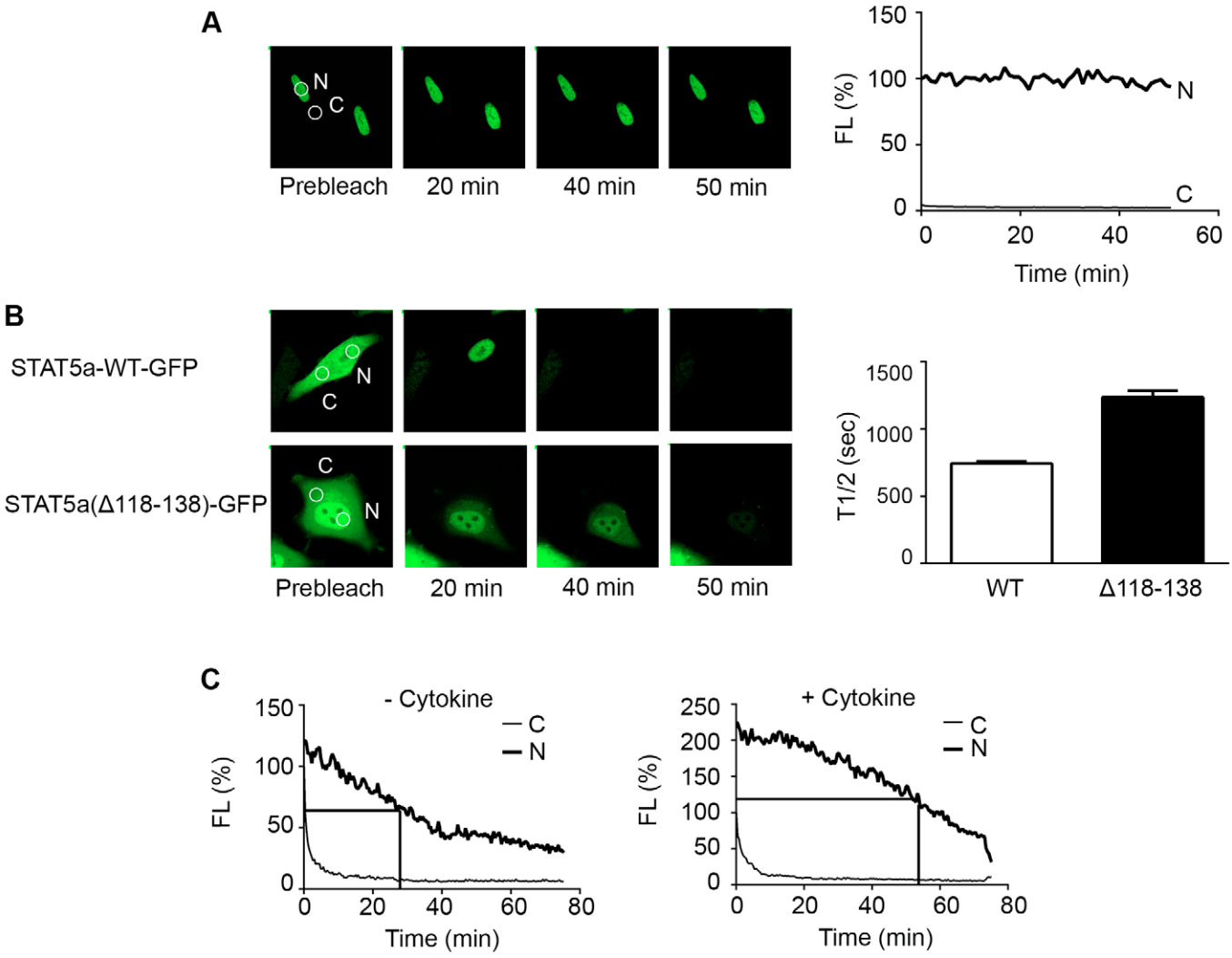
Supplementary Figure 4. Tyrosine phosphorylation of the STAT5a import mutation in response to PRL, and quantitative nuclear/cytoplasmic fluorescence of STAT5a and GR in response to PRL or HC. (A) *Left panel*, T47D cells transfected with pcDNA3-STAT5a or pcDNA3-STAT5a(Δ142-149), and cells untreated or treated with PRL for 16 h after serum starvation. Western blot of cell lysates with anti-STAT5a phosphotyrosine antibody (pY694), anti-STAT5a and anti-Tubulin. *Right panel*, MCF-7 cells transfected with the PRL receptor and HA-STAT5a wild type or HA-STAT5a(Δ142-149). Cells were untreated or treated with PRL. Western blots with anti-STAT5a pY694, anti-STAT5a, or anti-HA. (B) Fluorescence intensity of proteins in nuclear and cytoplasmic regions of Figure 5 images was quantified by LSM Image in multiple cells. Relative nuclear (dark) and cytoplasmic (white) fluorescence intensities are plotted of HA-STAT5a with GR-V5 (left panels), or HA-STAT5a(Δ142-149) with GR-V5 (right panels).



Supplementary Figure 5. Cellular localization of the Crm1 NES deletion or site-directed mutations within full length STAT5a, and transcriptional activity. (A) Linear diagram of STAT5a functional domains and the STAT5a deletion construct. Fluorescence images of the internal deletion of STAT5a (Δ118-138) and the L119A/L133A double mutation in full length STAT5a. (B) T47D cells were transfected with β-casein promoter-luciferase reporter, β-gal, and STAT5a (L119A/L133A). After serum starvation PRL alone or PRL and HC was added to cells for 16 hrs. Luciferase activity was normalized to β-gal activity. (C) Tyrosine phosphorylated STAT5a (L119A/L133A) was detected by Western blot using anti-STAT5a pY694 antibody. STAT5a expression was detected with anti-STAT5a antibody, and anti-tubulin was used as a loading control.



Supplementary Figure 6. STAT5a nucleocytoplasmic transport is dependent on Ran. Cells were co-transfected with STAT5a-YFP and CFP-Ran or CFP-Ran Q69L mutant. Cellular localization of STAT5a and Ran were evaluated by fluorescence microscopy.



Supplementary Figure 7. Cytoplasmic FLIP measurements to evaluate nuclear export. A) Cells were transfected with nuclear construct T-Ag-NLS-GST-2GFP, and a small region in the cytoplasm (C) was continuously bleached with high intensity laser. Time lapse images obtained by fluorescence microscopy are shown in left panel, and fluorescence intensities of nucleus and cytoplasm of the photobleached cell are depicted with time in right panel. B) Continuous high intensity laser was subjected to a small region in the cytoplasm (C) of cells expressing wt STAT5a-GFP or STAT5a(Δ118-138)-GFP. Time lapse fluorescence of each construct is shown in left panel, and the half-time of nuclear fluorescence loss calculated by curve fitting analysis is plotted in the right panel. C) Untreated or cytokine-treated cells expressing STAT5a-GFP were continuously photobleached in a small region of cytoplasm. Relative fluorescence of nucleus and cytoplasm of each photobleached cell is plotted with time.