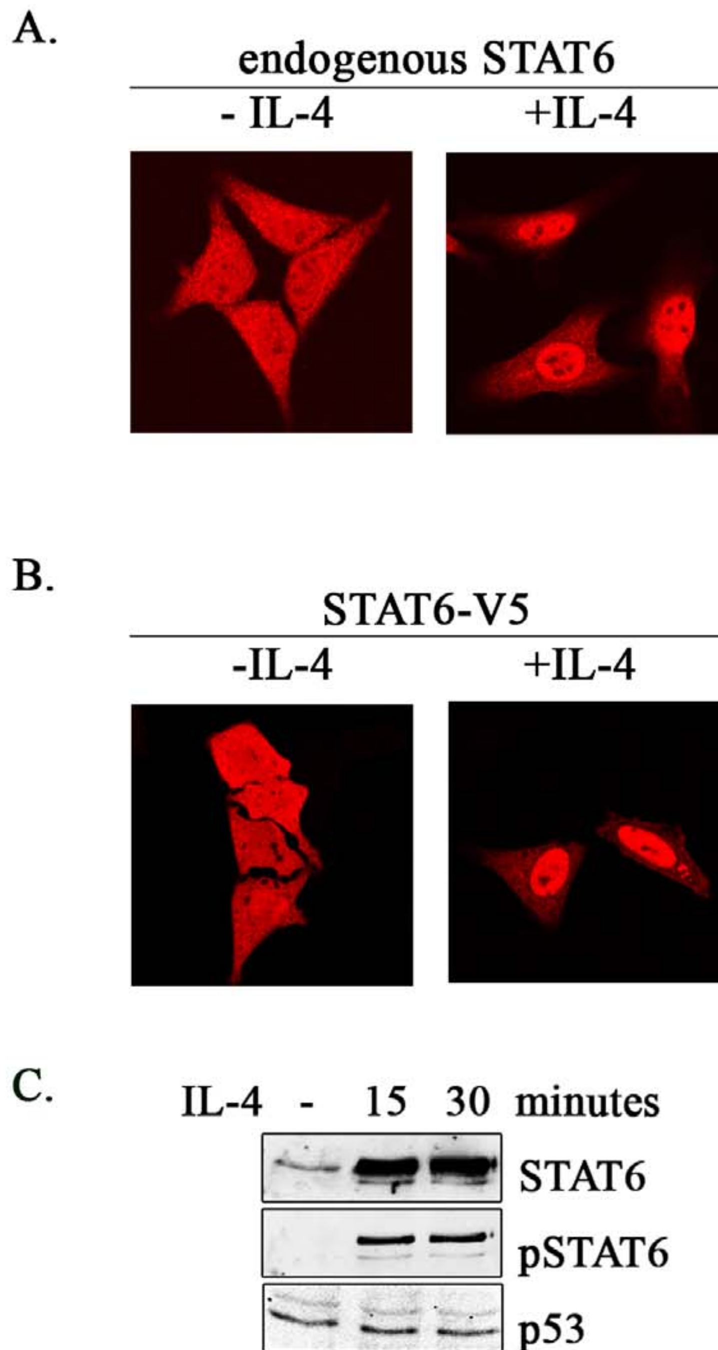

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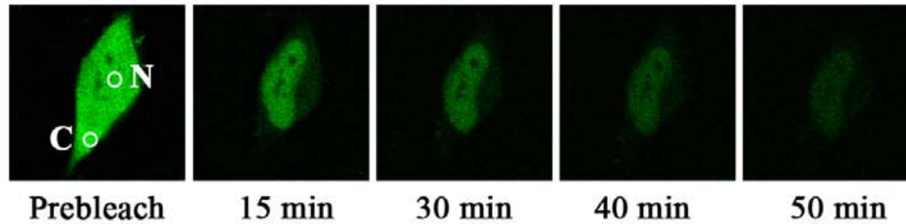
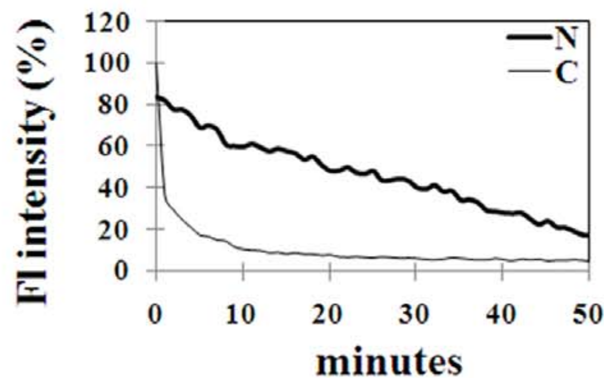
Supplemental Figure 1: Localization of STAT6. A) Endogenous STAT6 was detected in HeLa cells by immunofluorescence. Cells were serum starved and untreated (-IL-4) or treated with IL-4 (+IL-4) for 30 min, fixed with paraformaldehyde, permeabilized with Triton X-100, and stained with anti-STAT6 antibody and TRITC-conjugated secondary antibody. Imaging with Zeiss LSM 5 laser scanning microscope using a 40x oil objective [Plan-Neofluar, numerical aperture 1.3, DIC objective]. B) HeLa cells were transfected with a plasmid encoding V5-tagged STAT6 and stained with anti-V5 antibody and secondary as in (A). C) Primary human lymphocytes were purified from peripheral venous blood from healthy donors with Accu-Prep™ Lymphocytes, Human, Cell Separation Media. Cells were untreated or treated with IL-4 for 15 or 30 min, and resuspended in 10mM HEPES pH 7.9, 0.1mM EDTA, 10mM KCl and 0.5% Nonidet P40. Nuclei were collected by centrifugation and proteins were extracted from nuclei in 20mM HEPES pH7.9, 1mM EDTA, and 400mM NaCl. 10 μg protein was analyzed by Western blot with anti-STAT6, anti-phosphotyrosine STAT6, or p53 as a nuclear marker.





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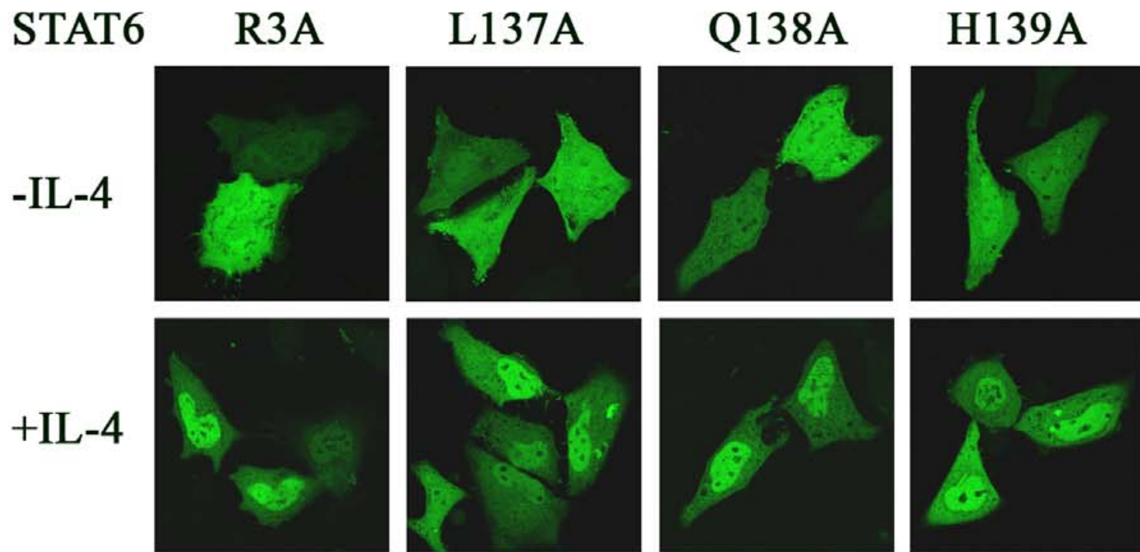
Supplemental Figure 2: Tyrosine phosphorylated STAT6 DNA binding mutant is exported from the nucleus with kinetics similar to unphosphorylated wtSTAT6 (Figure 3). Cytoplasmic FLIP was performed with cells expressing STAT6(KR)-GFP in the presence of IL-4. A small region in the cytoplasm (C) was subjected to continuous high intensity laser. Fluorescence loss was monitored with time in the bleached area in the cytoplasm and in a region of the nucleus (N). The quantitative data of relative fluorescent intensity (FI) with time of both regions is shown. Experiments are representative of more than three independent studies. Imaging with Zeiss LSM 5 laser scanning microscope.



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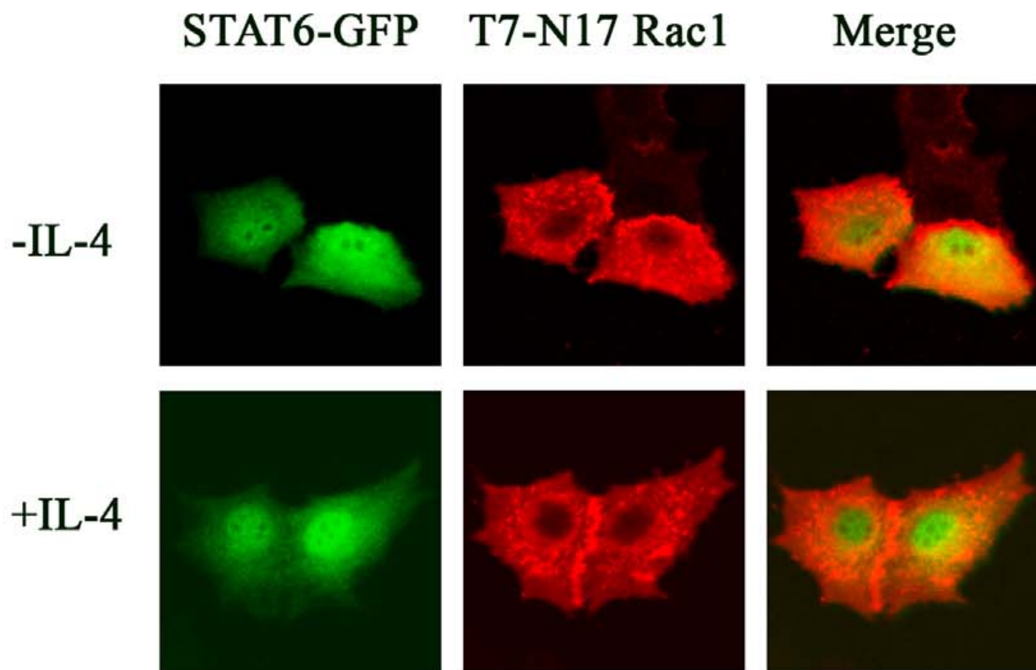
Supplemental Figure 3: Effect of point mutants within amino acids 135-140 on STAT6 nuclear import. Each amino acid within 135-140 (RRLQHR) was mutated individually to alanine in full length STAT6 to generate STAT6R3A-GFP, STAT6L137A-GFP, STAT6Q138A-GFP and STAT6H139A-GFP. Cellular localization of the mutants was evaluated in HeLa cells that were serum starved (-IL-4) or stimulated with IL-4 (+IL-4) for 30 minutes. GFP-tagged protein was observed with a Zeiss LSM 5 laser scanning microscope using a 40x oil objective [Plan-Neofluar, numerical aperture 1.3, DIC objective].



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Supplemental Figure 4: N17 Rac does not block nuclear import of STAT6. HeLa cells were co-transfected with STAT6-GFP and T7 tagged N17 Rac1 and serum starved overnight. Cells were left untreated (-IL-4) or treated with IL-4 for 30 min (+IL-4), fixed and stained by immunofluorescence with anti-T7 primary antibody and TRITC-conjugated secondary antibody. Cellular localization of STAT6-GFP (green) and T7-N17 Rac1 (red) was visualized by Zeiss LSM 5 laser scanning microscope using a 40X oil objective [Plan-Neofluar, numerical aperture 1.3, DIC objective].