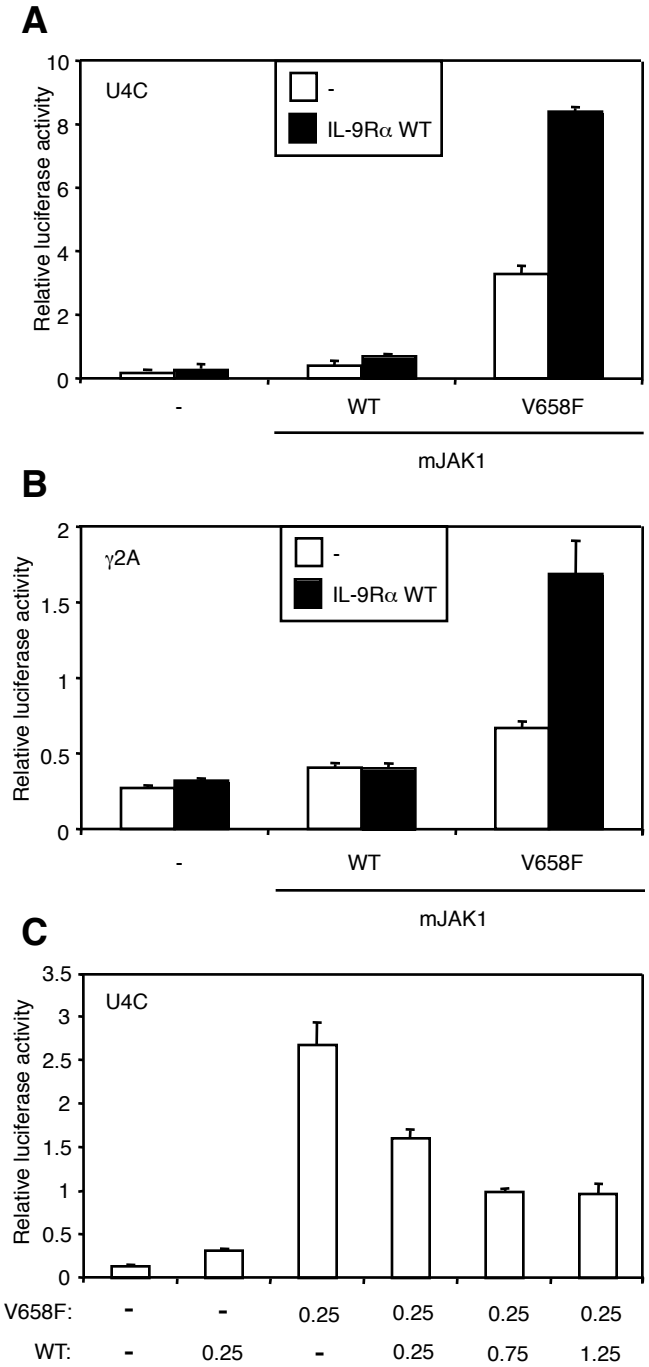
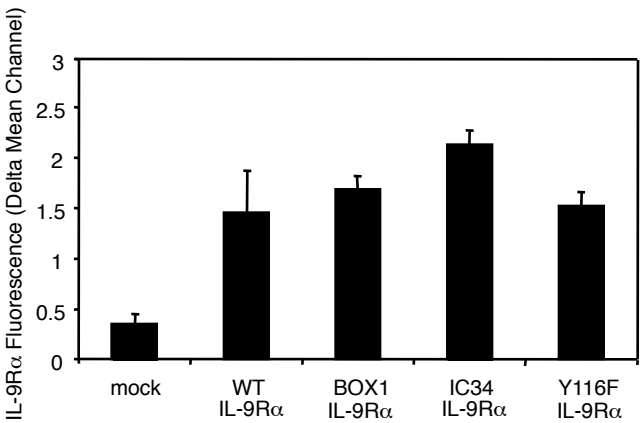


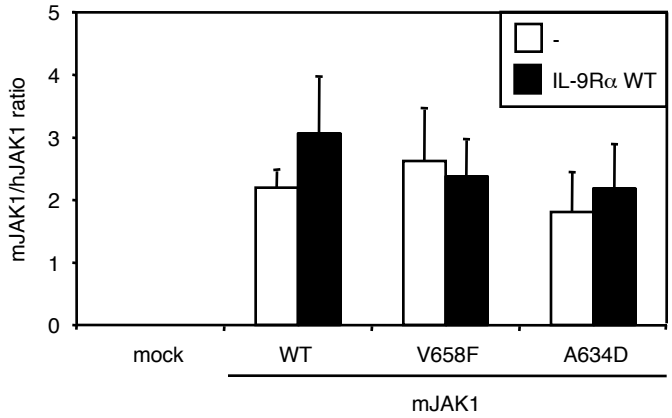
Supplementary Figure 1

Co-expression of wild-type JAK1 inhibits ability of JAK1 V658F to constitutively activate STAT3. A) JAK1-deficient U4C human fibrosarcoma cells were transiently transfected with empty vector, JAK1 wild-type or V658F with or without IL-9Rα chain (in the absence of JAK3 and γc), in addition to the STAT3-responsive luciferase reporter pGL3-pap1. 24 hours post-transfection, cells were subjected to a luciferase assay. Results are mean ± S.D. of triplicate samples. Similar results were obtained in 2 independent experiments. B) The same experiment was performed with JAK2-deficient γ2A human fibrosarcoma cell line. Results are mean ± S.D. of triplicate samples. Similar results were obtained in 2 independent experiments. C) JAK1-deficient U4C human fibrosarcoma cells were transiently transfected with 0.25 μg of empty vector, JAK1 wild-type and V658F alone or co-transfected with increasing concentration of wild-type JAK1 (0.25 μg, 0.75 μg or 1.25 μg), in addition to the STAT3-responsive luciferase reporter pGL3-pap1. 24 hours post-transfection, cells were subjected to a luciferase assay. Results are mean ± S.D. of triplicate samples. Similar results were obtained in 2 independent experiments.



Supplementary Figure 2

Cell surface expression of wild-type and mutated IL-9Rα constructs in HEK293 cells.
HEK293 cells were transiently transfected with empty vector, wild-type or mutated IL-9Rα constructs. One day after transient transfection, an aliquot of the cells was used to assess cell surface expression of IL-9Rα by FACS analysis using biotinylated anti-human IL-9Rα antibody followed with phycoerythrin-conjugated Streptavidin. The rest of the transfected cells was ~~sta~~ined only with phycoerythrin-conjugated Streptavidin as a control. After FACS analysis was performed, the geometric mean channel of the fluorescence of anti-IL-9R-SAPE stained and SAPE-only stained cells was compared and used to calculate the delta mean channel. Results are mean ± variation of duplicate transfections.



Supplementary Figure 3

Expression of wild-type and mutated murine JAK1 in human HEK293 cells.
Human HEK293 cells were transiently transfected with empty vector, wild-type, V658F or A634D murine JAK1 with or without IL-9Rα chain, in addition to the STAT3-responsive luciferase reporter pGL3-pap1. 24 hours post-transfection, an aliquot of cells was used for the RNA extraction and cDNA synthesis. Quantitative PCR was performed using mouse or human JAK1-specific primers and a series of dilutions of the respective cloned cDNA as a standard. Results are mean of duplicate transfections ± variation. Similar results were obtained in 2 independent experiments.