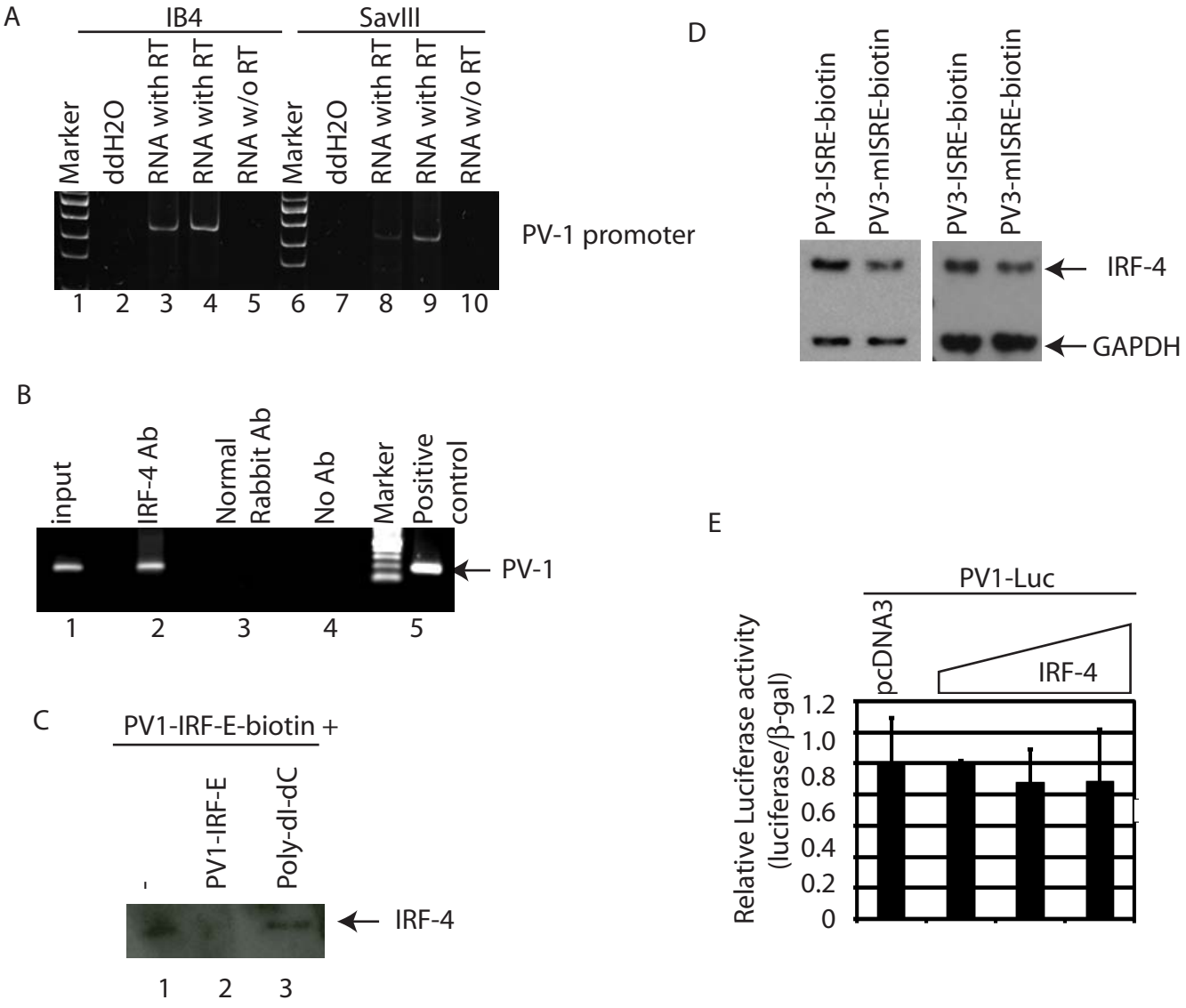
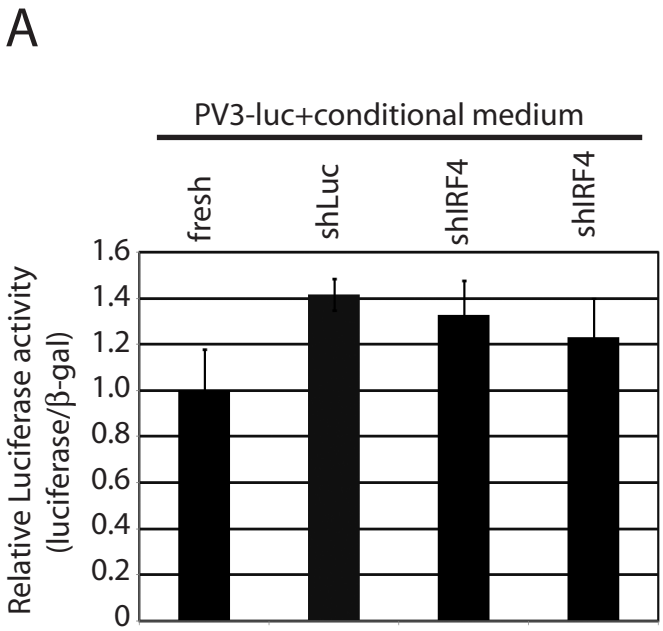
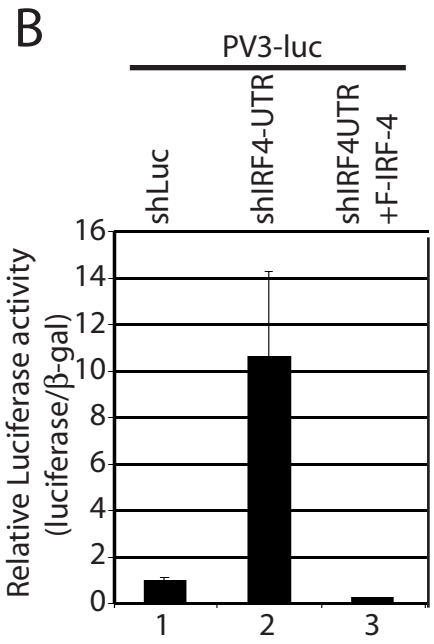
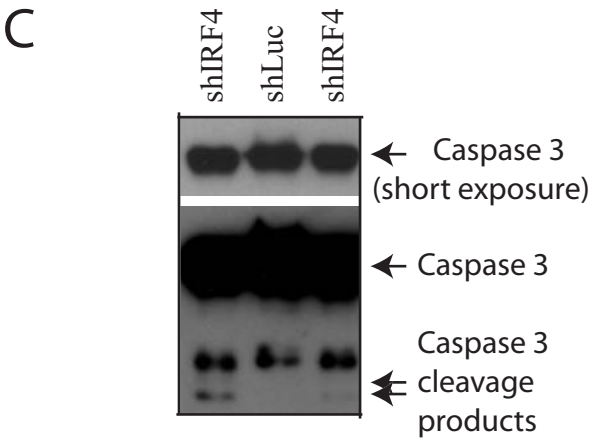


Supplemental Figure 1: IRF-4 binds to IRF-5 PV1 promoter region. A) The PV1 promoter is active in EBV transformed cells. The RNAs were isolated from the EBV transformed cells as shown on the top, and RT-PCR was used to determine the transcriptional activities with the target promoter-specific primers. The specificity of the amplification is indicated. B) IRF-4 binds to IRF-5 PV1 promoter in vivo. IRF-4 antibody was used for ChIP analyses to detect in vivo DNA binding activities to IRF-5 promoter region. Primers for IRF-5 PV-1 promoter regions were used to amplify the DNA from immunoprecipitates. The identity of PV1-promoter amplification is as shown. The plasmid, IRF-5 PV1-luc, was used as positive control. Input DNA represents the PCR amplification from 1/100 the amount of input lysates for immunoprecipitations. PCR amplification of immunoprecipitates from normal rabbit serum (NRS) is also shown as controls. C) IRF-4 binds to PV-1 promoter in vitro. Cell extracts were made and from IB4 cells and incubated with biotinylated oligonucleotides containing the IRF-5 PV-1 IRF-E sequences with or without the un-biotinylated IRF-5 PV1 IRF-E, or poly(dI-dC) as competitors. Western blots were carried out for the detection of IRF-4. D). IRF-4 binds to IRF-5 mutated ISRE weakly in vitro. Cell extracts were made and from IB4 cells and incubated with biotinylated oligonucleotides containing the IRF-5 PV-3 ISRE sequences or its mutated conyterparts. Western blots were carried out for the detection of IRF-4 and GAPDH. E). IRF-4 did not repress IRF-5 PV-1 promoter activity. 293T cells were transfected with various reporter constructs and expression plasmid (0.01, 0.05, and 0.1 g) as shown on the top. Cell lysates were used for the luciferase and -galactosidase assays. Relative promoter reporter activities (luciferase/ -galactosidase) are shown. Standard error bars are shown.







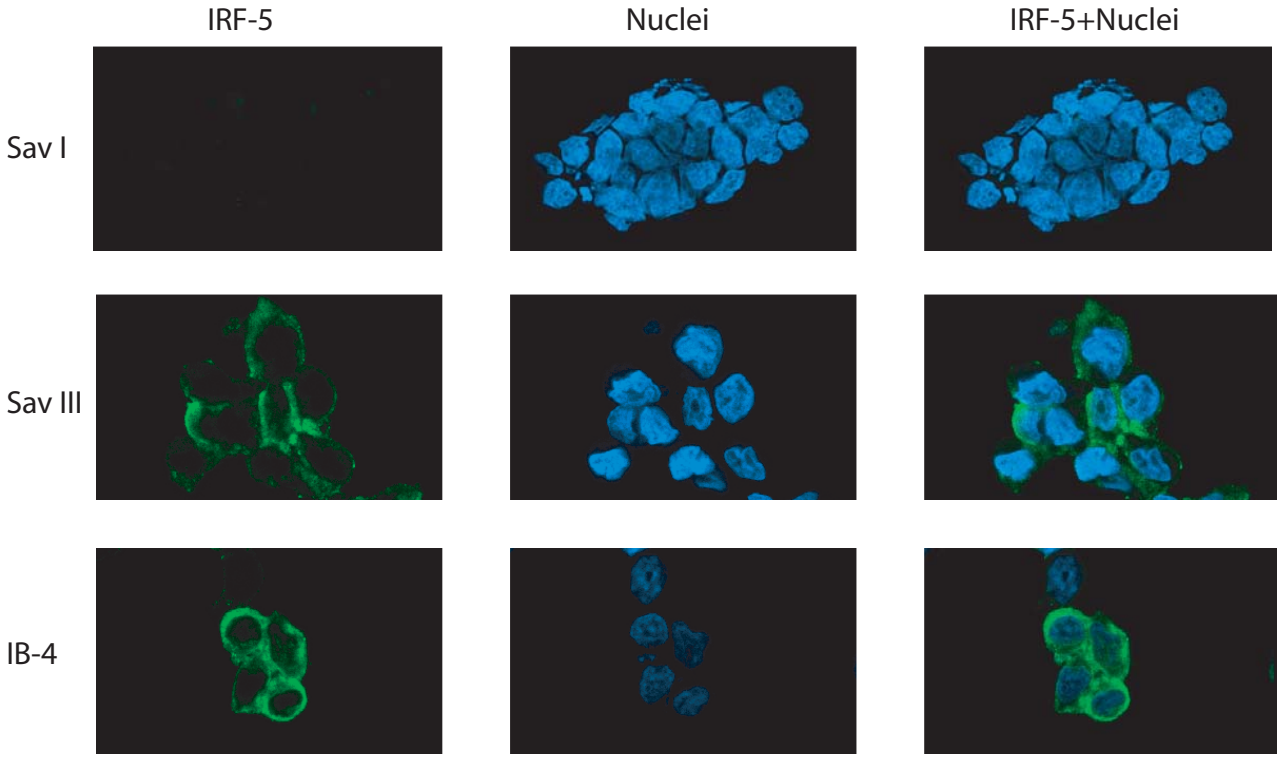
Supplenmenatl Figure 2:

A. Conditional medium from shIRF-4-transfected cells did not activate PV-3 promoter reporter construct. IB4 cells were transfected with shLuc or shIRF4. The medium was collected and passed through a 0.2 mm filter . The conditional medium was used for the treatment. 293T cells were transfected with PV-3 reporter construct for one day. The conditional medium was added (1:1 ratio) as shown on the top. Cell lysates were used for the luciferase and -galactosidase assays one day later. shIRF4 was duplicated.

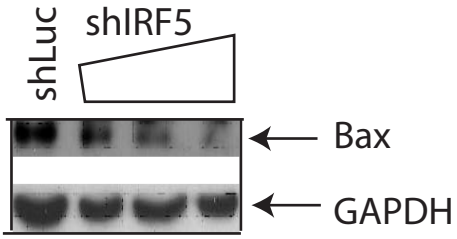
B. Endogenous IRF-4 regulates IRF-5 promoter activity in P2 cells. P2 are STAT-1-null EBV-transformed B lymphocyte line. P2 cells were transfected with indicated plasmid as shown on the top. Two days later, luciferase and -galactosidase activities were measured. Relative promoter reporter activities are shown.

C. Caspase 3 cleavage in IRF-4-knockdown cells. IB4 cells were transfected with shLuc or shIRF4. Two days later, lysates from transfected cells were used for western blot analysis with caspase 3 antibody. shIRF4 was duplicated. The identity of proteins is as shown.

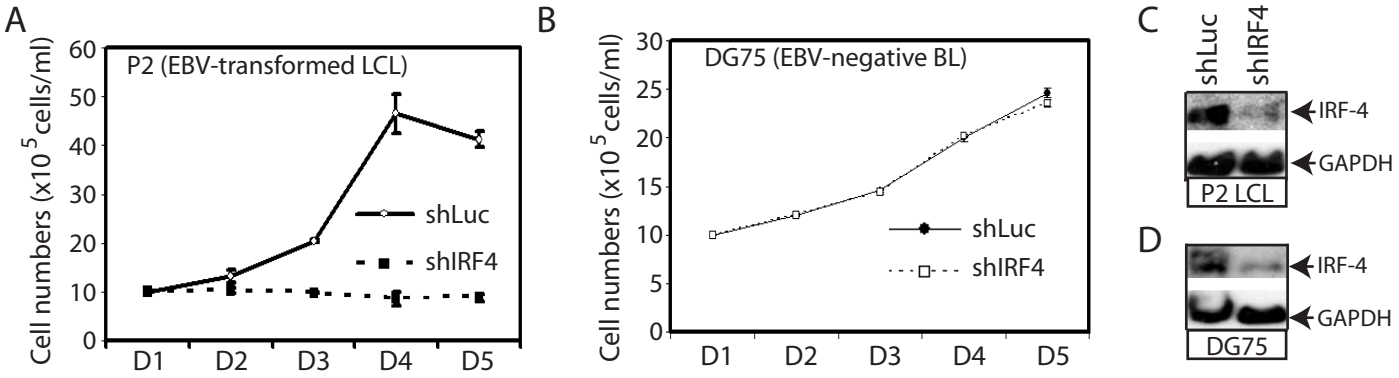
A



B



Supplemental Figure 3. A. Subcellular localization of IRF-5. Cells were stained with anti-IRF-5 and AlexaFlor 488-labeled secondary antibodies were used to determine the subcellular localization of IRF-5 (green). DAPI was used to stain the nuclei (blue). Sav I and Sav III are gentically identical cell lines with different EBV latencies. It is known that IRF-5 is expressed at high levels in type III latency. IB-4 is a type III latency cells.
B. Reduction of IRF-5 reduces the expression of Bax. IB4 cells were transfected with shLuc or shIRF5 . Two days after transfection, cells were collected and western blot was used to examine the expression .



Supplemental Figure 4. Knockdown of IRF-4 inhibits cell growth of EBV-transformed cells.
A) Knockdown of IRF-4 inhibits cell growth of P2 cells. P2 is an EBV-transformed cell line. P2 cells were transfected with shLuc (5 μg), shIRF4 (5 μg) along with CD4 expression plasmid (1μg). One day after the transfection, CD4 expression cells were enriched with a magnetic beads containing CD4 antibody (In vitrogen). At the indicated days after transfection, surviving cells were enumerated. Each point represents the number of live cells (mean ± S.D.). One representative from three independent experiments is shown. B. Knockdown of IRF-4 does not inhibit cell growth of DG75 cells. DG75 is an EBV-negative BL cell line. Similar methods were used as in P2 cells. The expression of IRF-4 in P2 cells (C), or in DG75 cells (D) are shown. The images in the same box are derived from the same membrane.