Supplementary Figure Legends

Supplementary Figure 1. FBI-1 represses transcription of the Rb gene. (A) RT-PCR and western blot analysis of Rb gene repression by FBI-1 at mRNA and protein levels in stable HeLa cells overexpressing FBI-1 or β -galactosidase. GAPDH, control for RT-PCR and western blot analysis. (B) Western blot analysis of RNA interference of endogenous FBI-1 mRNA and protein expression. Knock-down of FBI-1 mRNA by siRNA increased Rb gene expression both at the mRNA and protein levels in HEK293A cells.

Supplementary Figure 2. ChIP assays. FBI-1 and Sp1 compete with each other for binding to FRE3 and GC-box 2 *in vivo*. (A) ChIP assays on the endogenous Rb gene promoter. Human HEK293A cells were transfected with increasing amount of Flag-FBI-1 expression vector $(1, 2, 4 \mu g)$ and analyzed for Sp1 and FBI-1 binding using the antibodies indicated. Sp1 and FBI-1 compete with each other both on the distal and proximal promoter regions. (B) ChIP assays of binding competition between Sp1 and FBI-1 for the distal (bp -370 to -147) and proximal promoter (bp, -131 to +93) elements (probably FRE3 and GC-box 2) in Drosophila SL2 cells. SL2 cells were co-transfected with pGL2-Rb-Luc reporter plasmid, pPac-Sp1 $(1 \mu g)$, and increasing amounts of pPac-FBI-1 $(1, 2, 4 \mu g)$ and analyzed for promoter binding by ChIP. (C) ChIP assays on the endogenous Rb gene promoter after knock-down of endogenous FBI-1 expression in HEK293A cells. Knock-down of FBI-1 resulted in an increase in Sp1 binding to the endogenous Rb gene promoter, both on the proximal and distal promoters.

Supplementary Figure 3. Mouse C2C12 myoblast differentiation, and Rb and FBI-1 expression. (A) Differentiation of control mouse C2C12 myoblasts by 2% horse serum treatment. Pictures of the cells were taken at day 0-4 days after treatment with 2% horse serum differentiation medium. Differentiation into myotubes was observed at day 3, and 4. **(B)** Western blot analysis of the C2C12 cell lysates using the antibodies indicated. Rb expression was gradually increased in control cells with concomitant decrease in FBI-1 (LRF). LRF is a mouse homologue of human FBI-1. Cell extracts (40 μg) were analyzed by western blot analysis. GAPDH control; Rb, retinoblastoma protein.







