Supplementary Figure 1. KLF6 binds to and activates the ATF3 promoter in PC-3 cells. (A) PC-3 cells were cotransfected with reporter plasmids and pCMV-Tag2-KLF6. Cells were incubated in media containing 0.1% serum after transfection. Cell extracts were assayed for luciferase activity. The experiment has been repeated three times. (B) ChIP assay was performed with KLF-6-transfected PC-3 cells using anti-KLF6 antibody. Promoter regions RND (-1418 to -1219) and KB (-370 to -120) were amplified by PCR.

Supplementary Figure 2. KLF6 splicing variants also up-regulate ATF3 and induce apoptosis. (A) Overexpression of KLF6-SV2 up-regulated ATF3 protein in PC-3 cells. Cells were transfected with empty pCMV-Tag2 or pCMV-Tag2-KLF6-SV2 vectors. Western blotting was done with anti-Flag and anti-ATF3 antibodies. (B) KLF6-SV2 induced apoptosis in PC-3 cells. Cells were transfected with empty pCMV-Tag2 or pCMV-Tag2-KLF6-SV2 vectors. Apoptosis was analyzed using the Cell Death Detection Elisa^{PLUS} kit. The average results from three independent experiments were shown. (C) Knocking-down of KLF6-SV1 by siRNA. KLF6-SV1 targeting siRNA and negative control siRNA were transfected into PC-3 cells. Protein samples were collected at 48h after siRNA transfection. Western blot was performed with anti-KLF6 antibody. (D) KLF6-SV1 siRNA blocked STS-induced ATF3 expression and apoptosis. PC-3 cells were transfected with KLF6-SV1 targeting siRNA or negative control siRNA. At 24h after siRNA transfection, cells were treated with 1 μM STS for additional 8h. ATF3 protein level was determined by western blotting. Apoptosis was analyzed using the Cell Death Detection Elisa^{PLUS} kit. The average result from three independent experiments was shown.

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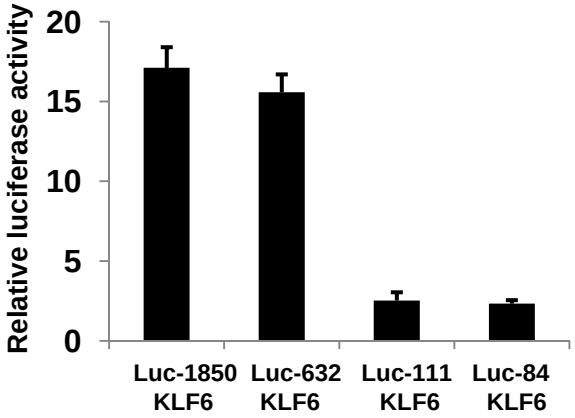
A.



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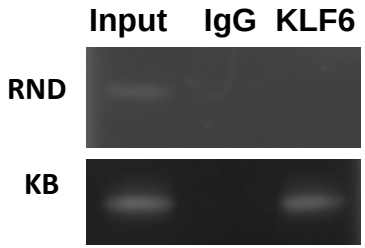

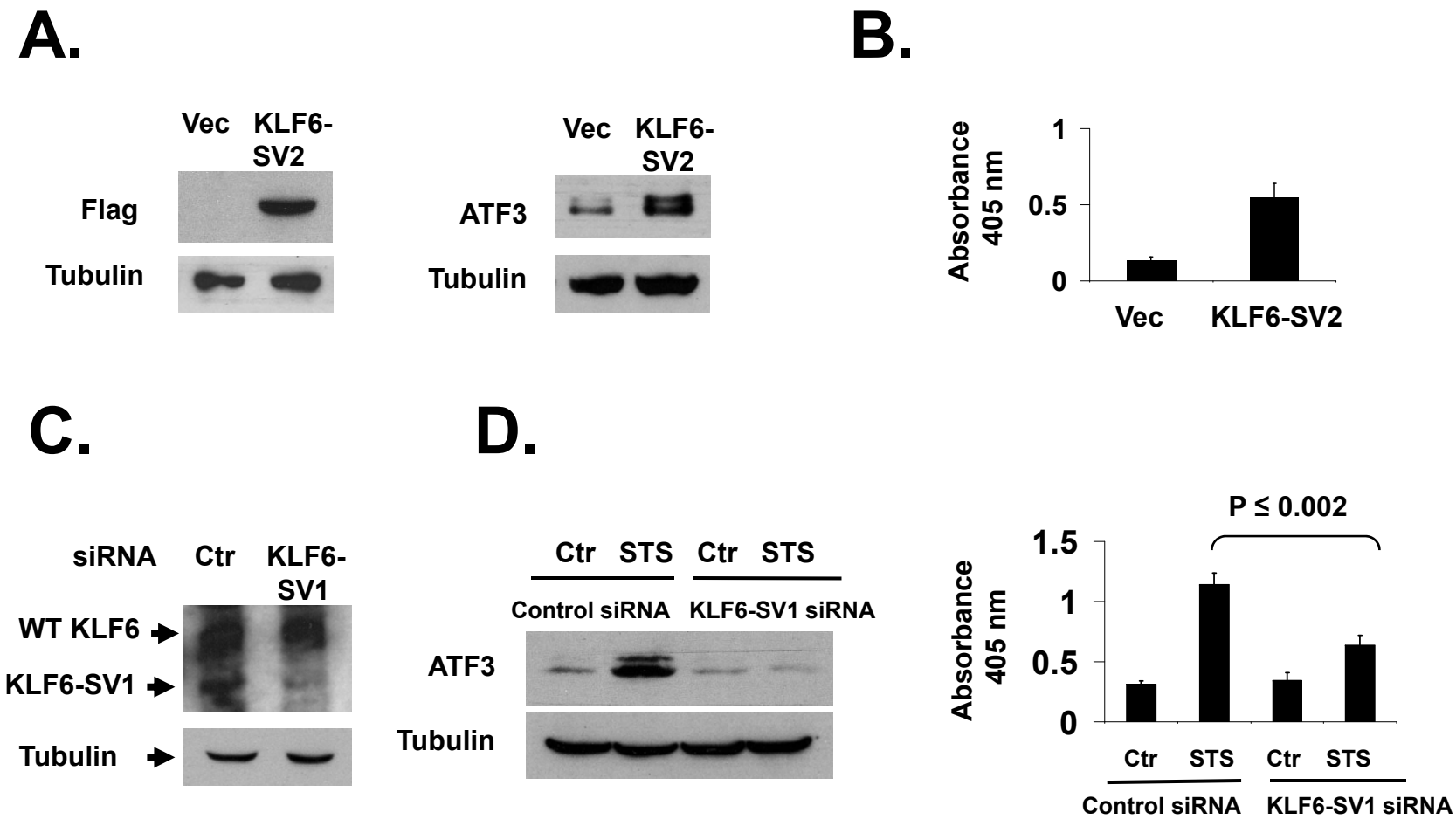

Huang et al Supplementary Figure 2