Figure. S4

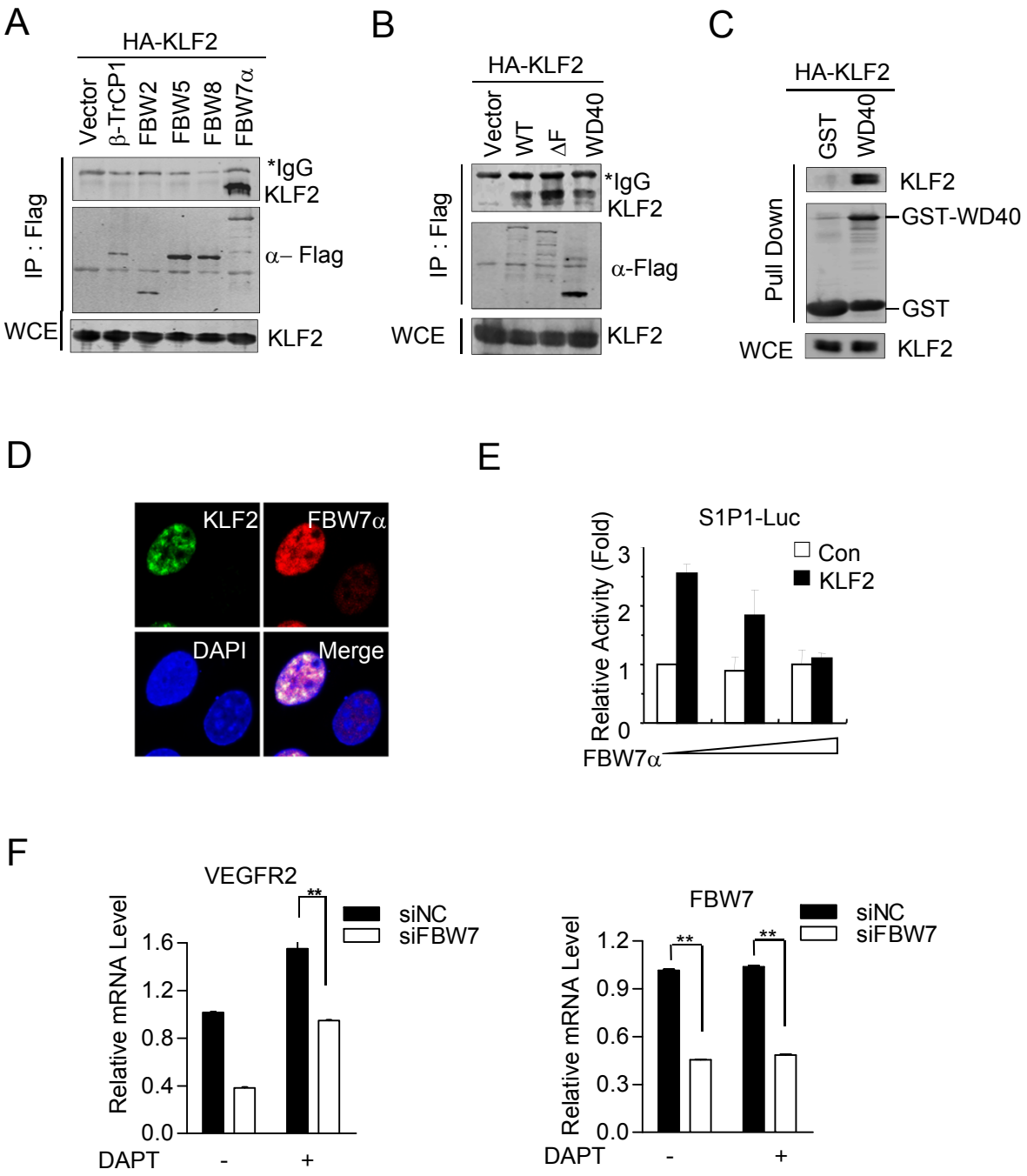
A
HA-KLF2
Vector
β-TrCP1
FBW2
FBW5
FBW8
FBW7α
IP : Flag
*IgG
KLF2
α– Flag
WCE
KLF2
B
HA-KLF2
Vector
WT
ΔF
WD40
IP : Flag
*IgG
KLF2
α-Flag
WCE
KLF2
C
HA-KLF2
GST
WD40
Pull Down
KLF2
GST-WD40
GST
WCE
KLF2
D
KLF2
FBW7α
DAPI
Merge
E
S1P1-Luc
Relative Activity (Fold)
Con
KLF2
FBW7α
F
VEGFR2
Relative mRNA Level
siNC
siFBW7
DAPT
FBW7
Relative mRNA Level
siNC
siFBW7
DAPT

Fig. S4. Interaction of KLF2 with FBW7. (A). HA-KLF2 were transfected into 293T cells with empty vector, β-TrCP1, FBW2, FBW5, FBW8, or FBW7. Transfected cells were lysed after treatment with 10 μM MG132 for 6 hours, immunoprecipitated with an anti-Flag antibody, and the immunoprecipitates (IP) and the original whole-cell extracts (WCE) were analyzed by Western blotting using an anti-HA or anti-Flag antibody. *, IgG band. (B). Interaction of KLF2 with FBW7 deletion mutants. *, IgG band. (C). Purified GST or GST-WD40 proteins were incubated with the cell lysates containing HA-KLF2. The products of GST pull-down were analyzed by Western blotting using anti-HA or anti-GST antibodies. (D). Colocalization of KLF2 and FBW7 in the nucleus. GFP-KLF2 and Flag-FBW7α were co-expressed in 293T cells. Transfected cells were treated with MG132, fixed, and FBW7 was stained with an anti-Flag antibody. (E). FBW7 inhibited the KLF2 transcriptional activity. KLF2 was cotransfected with S1P1 promoter reporter gene in the presence of increasing amount FBW7. The activity was measured using a luciferase assay.(F). HUVECs transfected with control or FBW7 siRNA were treated with or without DAPT(10 μM) for 24 hours, the expression levels of VEGFR2 and FBW7 mRNA were analyzed using qRT-PCR. **, $P < 0.01$.