Figure. S3

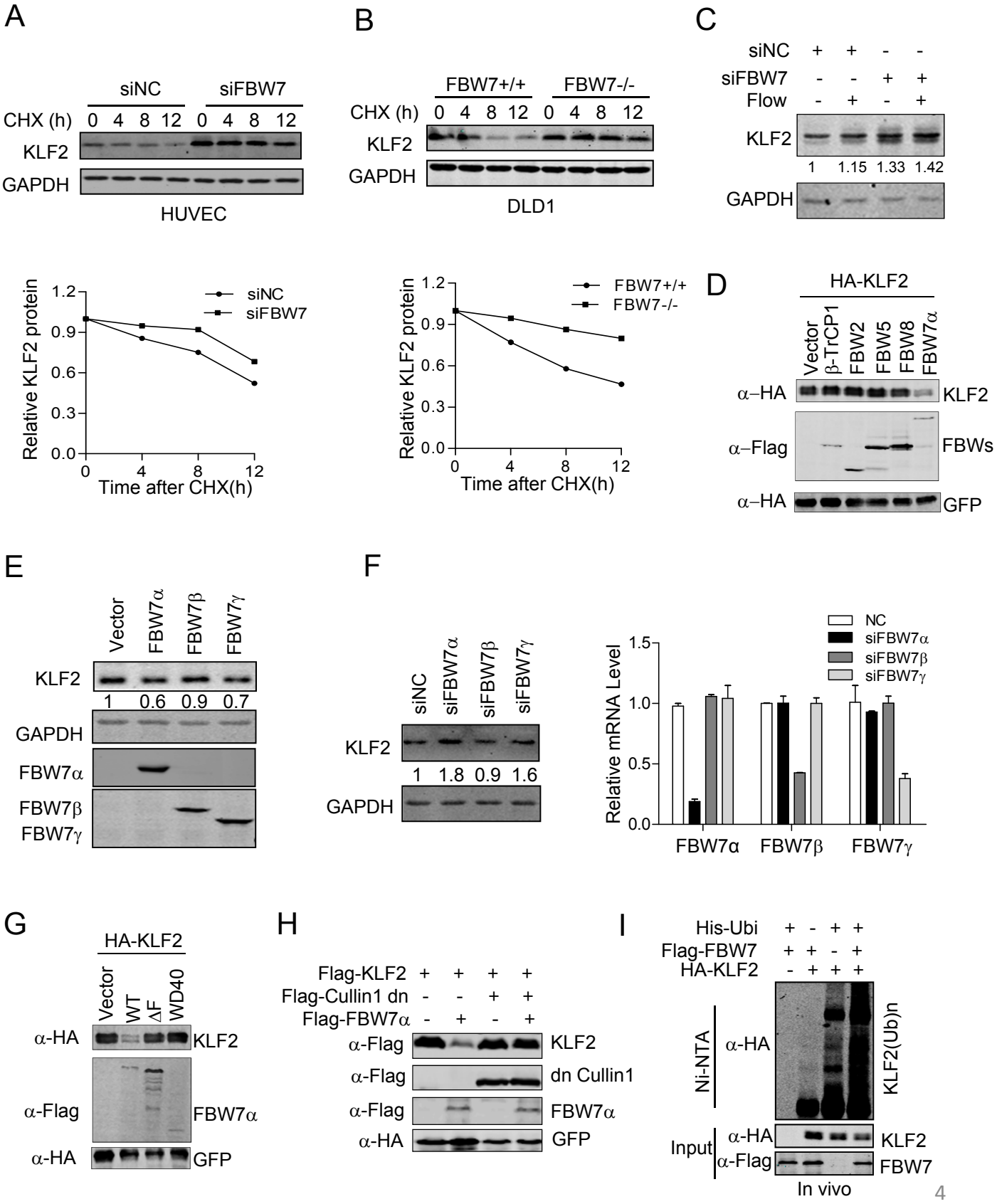
A
siNC
siFBW7
CHX (h) 0 4 8 12 0 4 8 12
KLF2
GAPDH
HUVEC
Relative KLF2 protein
siNC
siFBW7
Time after CHX(h)
B
FBW7+/+
FBW7-/-
CHX (h) 0 4 8 12 0 4 8 12
KLF2
GAPDH
DLD1
Relative KLF2 protein
FBW7+/+
FBW7-/-
Time after CHX(h)
C
siNC + + - -
siFBW7 - - + +
Flow - + - +
KLF2
1 1.15 1.33 1.42
GAPDH
D
HA-KLF2
Vector
β-TrCP1
FBW2
FBW5
FBW8
FBW7α
α–HA
KLF2
α–Flag
FBWs
α–HA
GFP
E
Vector
FBW7α
FBW7β
FBW7γ
KLF2
1 0.6 0.9 0.7
GAPDH
FBW7α
FBW7β
FBW7γ
F
siNC
siFBW7α
siFBW7β
siFBW7γ
KLF2
1 1.8 0.9 1.6
GAPDH
NC
siFBW7α
siFBW7β
siFBW7γ
Relative mRNA Level
FBW7α
FBW7β
FBW7γ
G
HA-KLF2
Vector
WT
ΔF
WD40
α-HA
KLF2
α-Flag
FBW7α
α-HA
GFP
H
Flag-KLF2 + + + +
Flag-Cullin1 dn - - + +
Flag-FBW7α - + - +
α-Flag
KLF2
α-Flag
dn Cullin1
α-Flag
FBW7α
α-HA
GFP
I
His-Ubi + - + +
Flag-FBW7 + + - +
HA-KLF2 - + + +
Ni-NTA
α-HA
KLF2(Ub)n
Input
α-HA
KLF2
α-Flag
FBW7
In vivo

Fig. S3. FBW7-mediated degradation of KLF2. (A). Knockdown of FBW7 in HUVECs reduced the degradation of KLF2. (B) Deficiency of FBW7 increases the KLF2 protein stability. Wild-type or FBW7 deficient DLD1 cells were treated with cycloheximide for the indicated times. The protein levels were analyzed using Western blotting. (C). Effect of FBW7 knockdown on the flow stress-induced KLF2 protein level. HUVECs were seeded on a parallel-plate flow chamber embedded with collagen and subjected to a shear stress of 15 dyn/cm^2 up to 16 hours. HUVECs transfected with either control siRNA or FBW7-silencing siRNA, Western blotting analysis of KLF2 in HUVECs, GAPDH was used as a loading control. Quantification of KLF2 was normalized against the GAPDH. The relative densitometric data are presented below the corresponding blots. (D). HA-KLF2 was cotransfected with empty vector, β-TrCP1, FBW2, FBW5, FBW8, or FBW7α in 293T cells. All transfections contained equivalent amounts of HA-GFP to monitor transfection efficiency. Transfected cells were lysed and the levels of KLF2 were detected using a Western blotting with an anti-HA antibody. Expression of FBW proteins and GFP were detected using anti-Flag and anti-HA antibodies respectively. (E). Degradation of endogenous KLF2 by FBW7 isoforms. HUVECs were infected virus expressing FBW7α, β, or γ. The protein levels were examined using Western blotting. Quantification of KLF2 was normalized against the GAPDH. The relative densitometric data are presented below the corresponding blots. (F). Regulation of endogenous KLF2 protein by endogenous FBW7. HUVECs were transfected with siRNA against FBW7α, β, or γ. The protein levels were examined using Western blotting. Quantification of KLF2 was normalized against the GAPDH. The relative densitometric data are shown below the corresponding blots. The mRNA levels of FBW7 were detected using qRT-PCR. (G). Domains of FBW7 required for KLF2 degradation. HA-KLF2 was cotransfected with FBW7 mutants and the degradation of KLF2 was monitored by Western blotting. (H). Coexpression of dominant negative form of Cullin (dn Cullin1) blocked the FBW7-mediated KLF2 destruction. Flag-KLF2 was cotransfected with empty vector, dn Cullin1, FBW7α into 293T cells. All transfections contained equivalent amounts of GFP to monitor transfection efficiency. Transfected cells were lysed and the levels of KLF2 were detected by Western blotting using an anti-Flag antibody. (I). Ubiquitination of KLF2 by FBW7. 293T cells were transfected as indicated and treated with MG132. The ubiquitination of KLF2 was measured by an in vivo ubiquitination assay.