E3 ubiquitin ligase APC/C^{Cdh1} negatively regulates FAH protein stability by promoting its polyubiquitination

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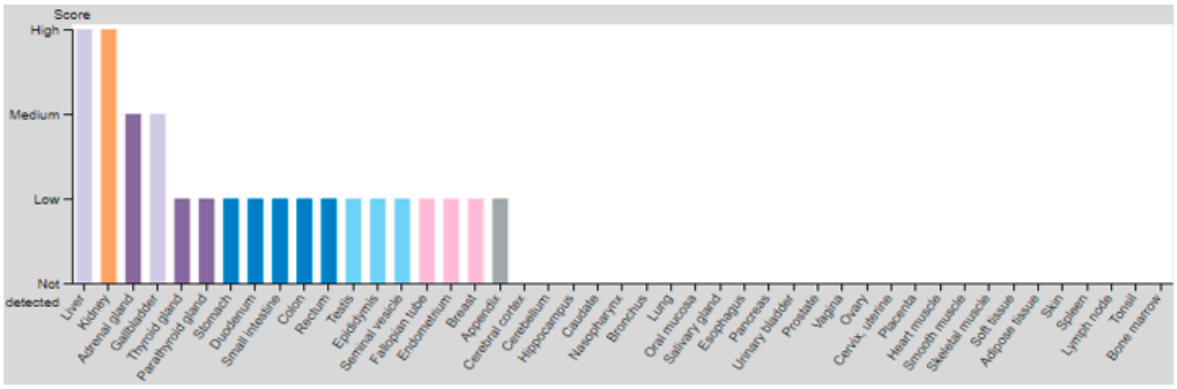
Figure S4. The original uncropped images for immunoblots in the main figures.

Figure S5. Triplicate images of western blots for significance value.

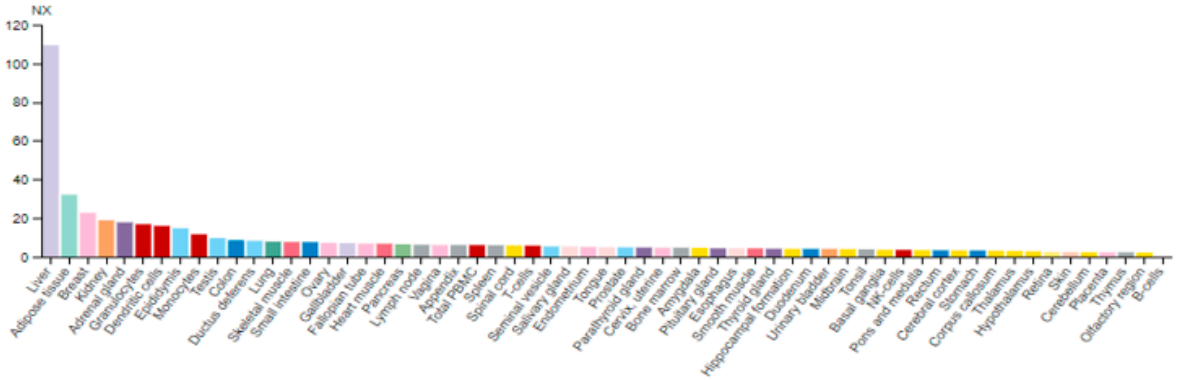
Figure S1

(A)



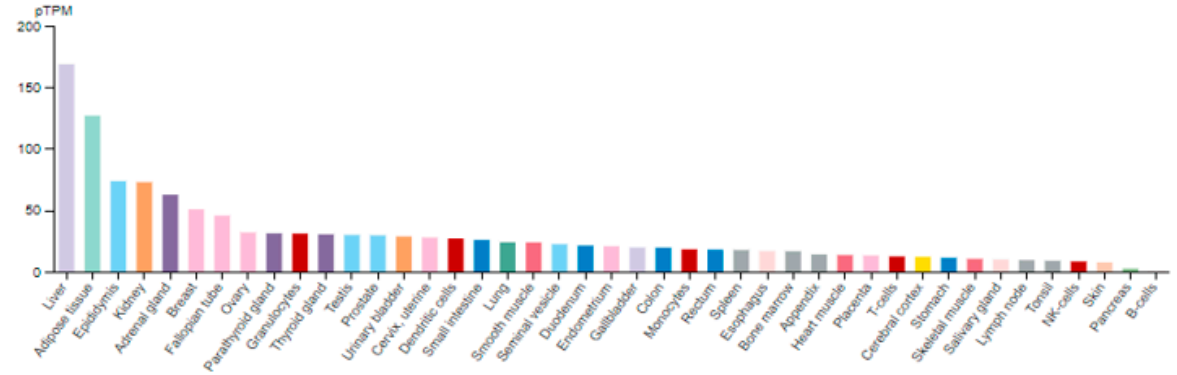
Protein Expression Overview

(B)



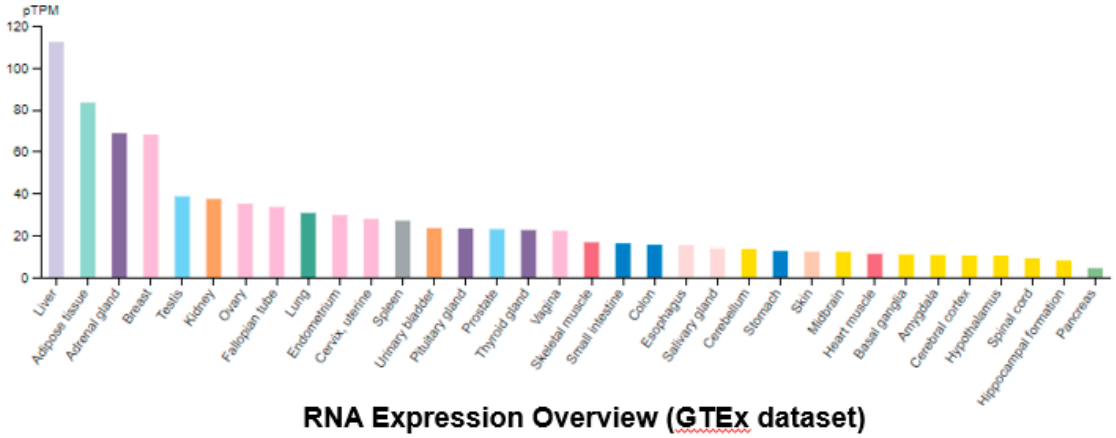
RNA Expression Overview (Consensus dataset)

(C)



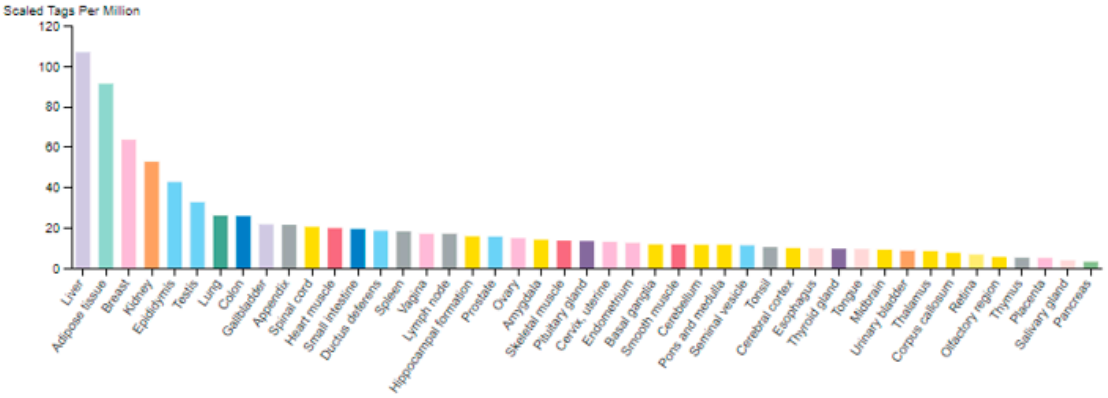
RNA Expression Overview (HPA dataset)

(D)



RNA Expression Overview (GTEx dataset)

(E)



RNA Expression Overview (FANTOM5 dataset)

Figure S1. (A) Comparison of FAH protein expression in different tissue types obtained from The Human Protein Atlas. Comparison of mRNA expression of FAH in different tissue types obtained from (B) Consensus dataset (C) HPA dataset (D) GTEx dataset (E) FANTOM5 dataset.

Figure S2

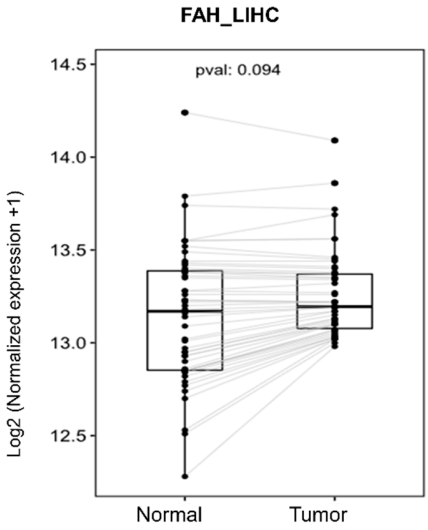


Figure S2. Expression box plot showing significant differential expression of FAH in normal vs. tumor tissues using normal-tumor matched liver cancer patient RNAseq data from TCGA. P value < 0.05 was considered to be statistically significant

Figure S3

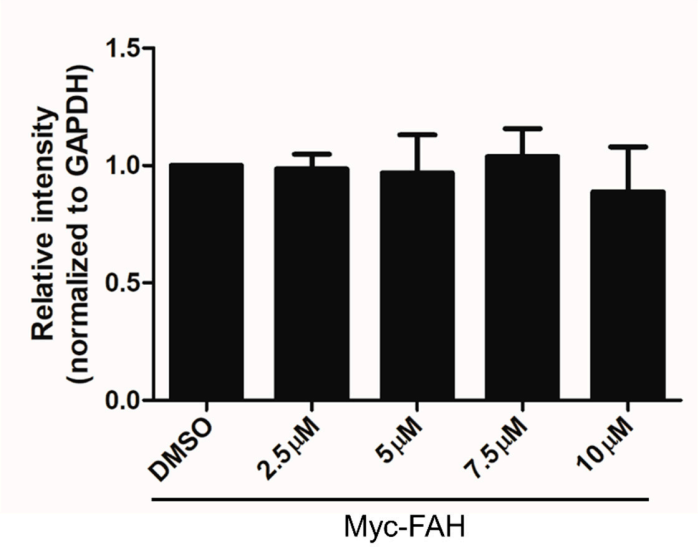


Figure S3: mRNA expression levels of Myc-FAH. Reverse transcription-quantitative PCR was used to assess the mRNA expression levels of Myc-FAH when HEK293 cells are treated with MG132 in a dose-dependent manner.

Table S1: Oligonucleotides used for sgRNA plasmid construction**.**

Gene	sgRNA	Direction	Sequence (5' to 3')
Cdh1	sgRNA1	FP	GCAGTACACGGAGCACCTGG
		RP	CCAGGTGCTCCGTGTACTGC
	sgRNA2	FP	CGCTTCTGGAACACGCTGAC
		RP	GTCAGCGTGTTCCAGAAGCG

Table S2: Oligonucleotide sequences used to get PCR amplicon for T7E1 assay.

Gene	sgRNA	Direction	Sequence (5' to 3')
Cdh1	sgRNA1 and sgRNA2	FP	AACGACAACAAGGTACCCCC
		FP1	GCTGCTGGTCTGGAATCACT
		RP	CTATGCCTGCTGCCTCACAT
		RP1	CCATCTCCCTTCAGAGCGAC

Table S3: PCR amplicon and cleavage sizes after T7E1 assay.

Gene	sgRNA	PCR size	Cleavage size	Orientation
Cdh1	sgRNA1	355	299+56	Sense
	sgRNA2	355	151+196	Sense

Figure S4: **The original uncropped images for immunoblots in the main figures.**

Fig 2A

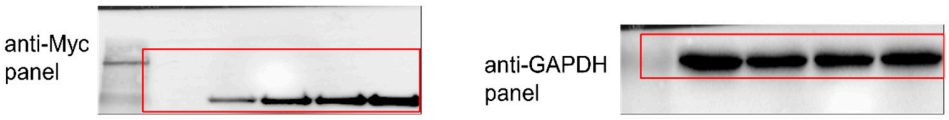


Fig 2B

Fig 2C

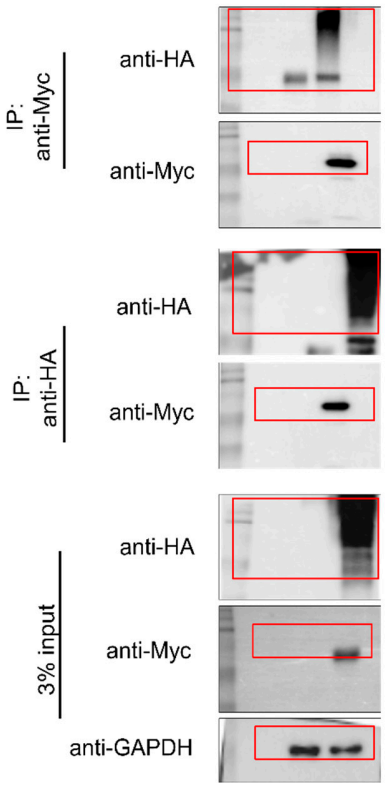


Fig 2D

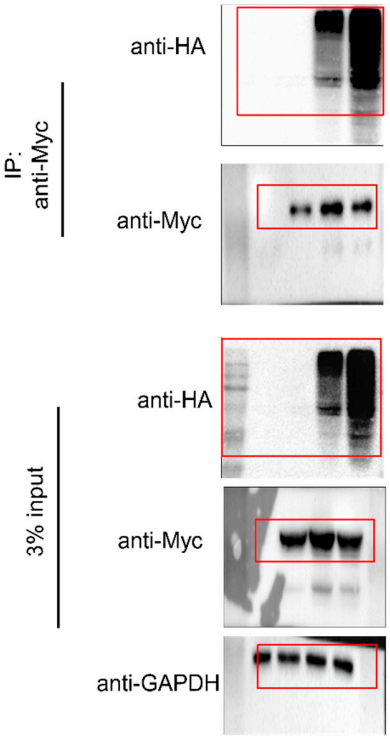


Fig 2E

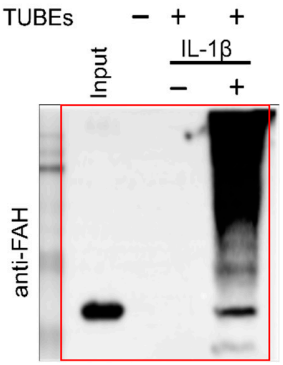


Fig 3A

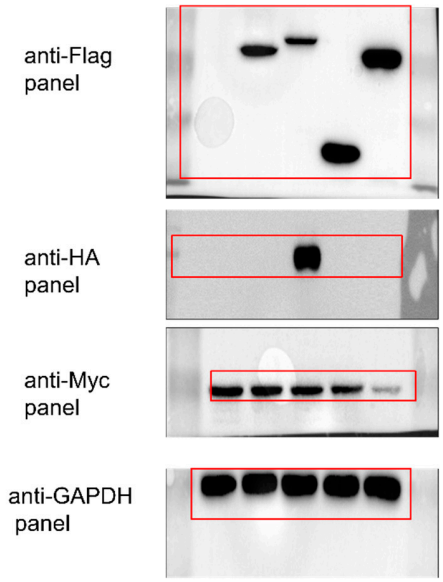


Fig 3D

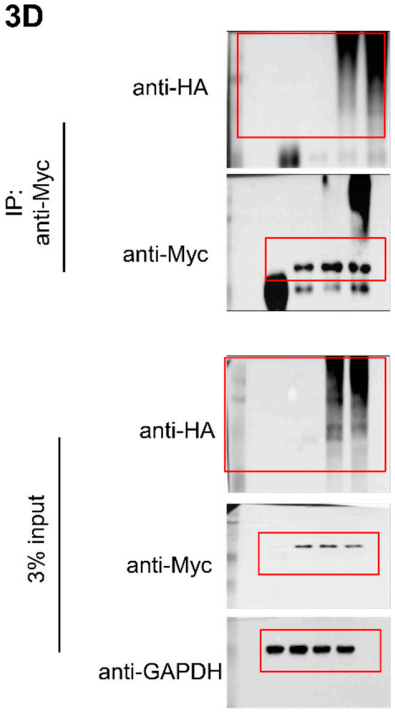


Fig 3B

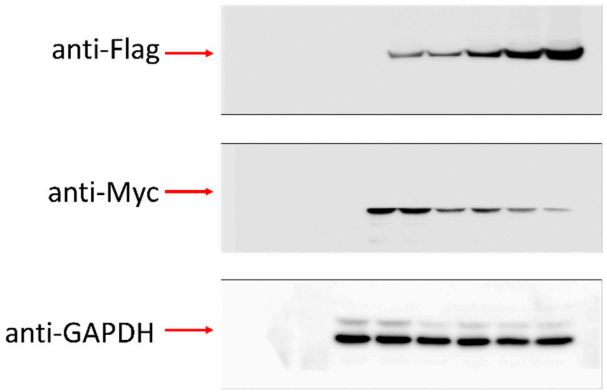


Fig 3C

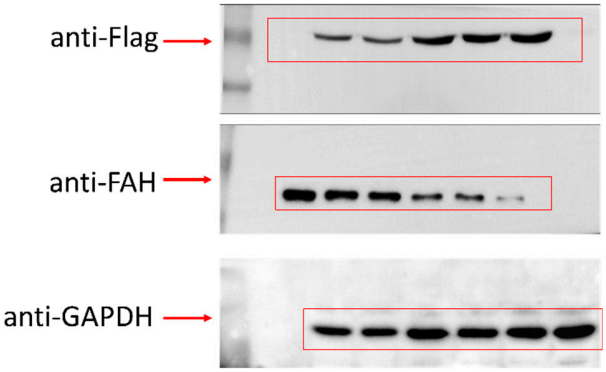


Fig 4C

Fig 4D

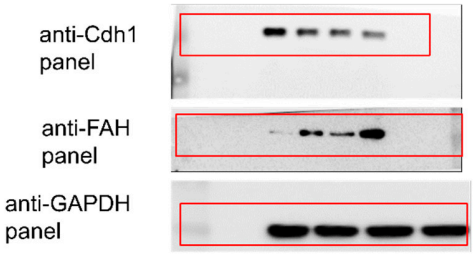


Fig 4E

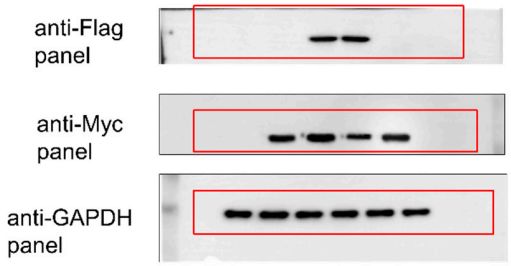


Fig 4F

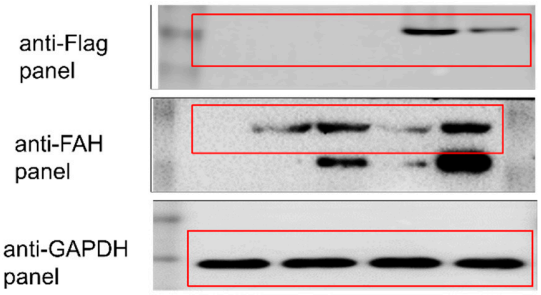


Figure 5

A

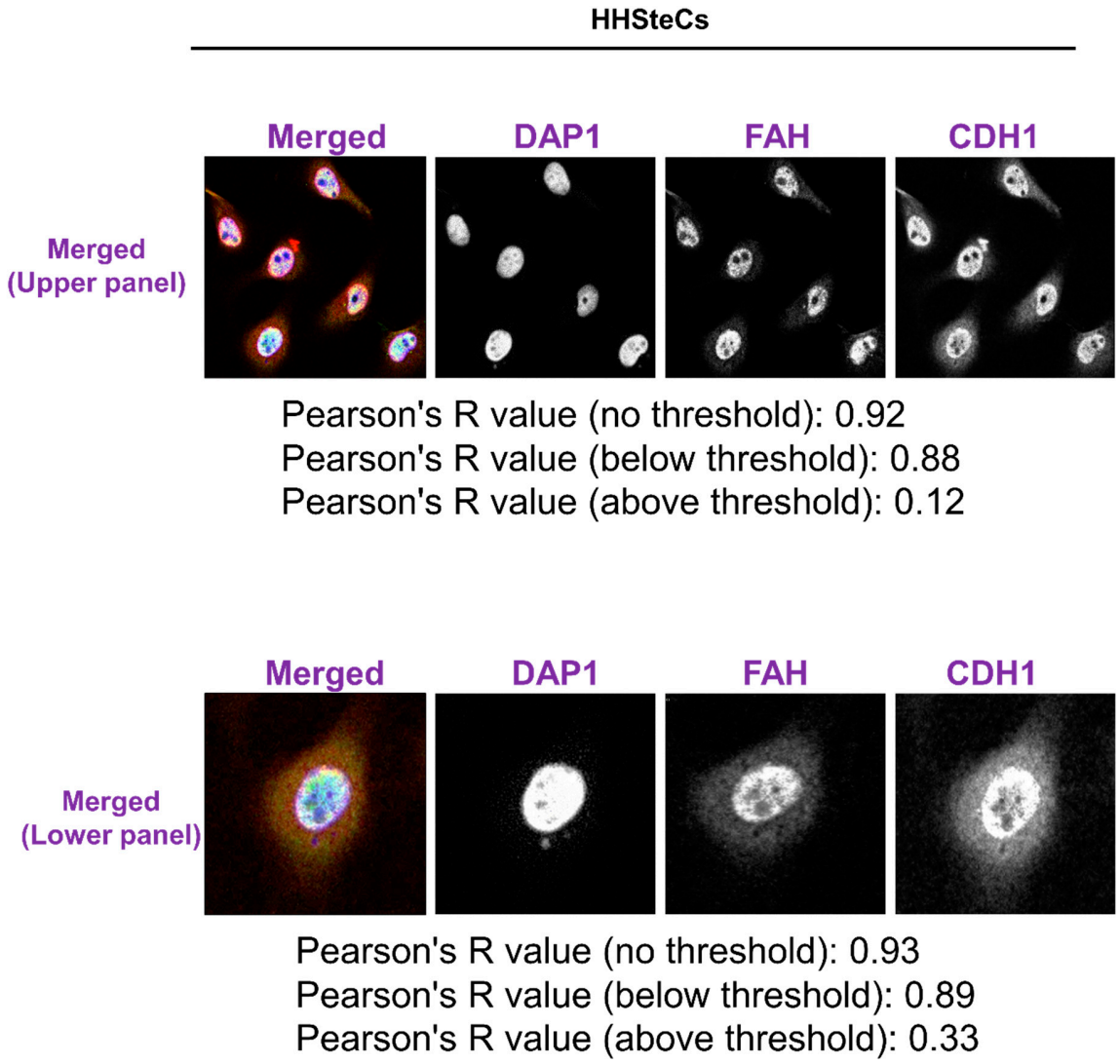

HHSteCs
Merged
DAP1
FAH
CDH1
Merged
(Upper panel)
Pearson's R value (no threshold): 0.92
Pearson's R value (below threshold): 0.88
Pearson's R value (above threshold): 0.12
Merged
DAP1
FAH
CDH1
Merged
(Lower panel)
Pearson's R value (no threshold): 0.93
Pearson's R value (below threshold): 0.89
Pearson's R value (above threshold): 0.33

Fig 5B

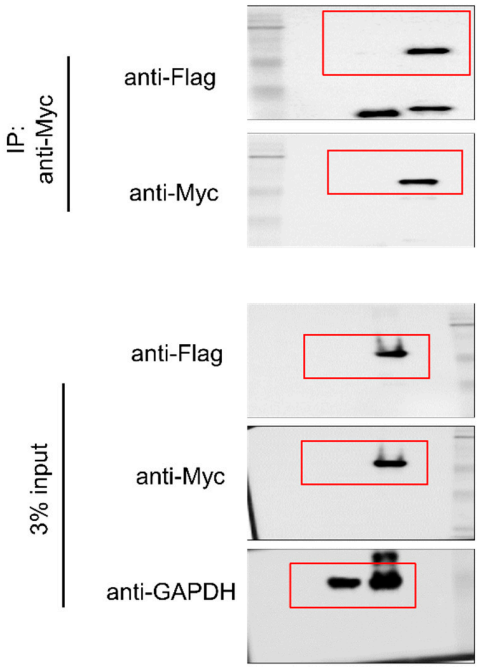


Fig 5C

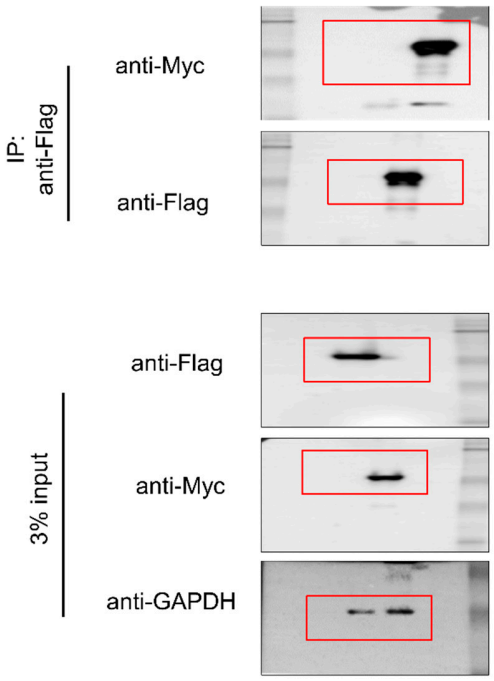


Fig 5D (upper panel)

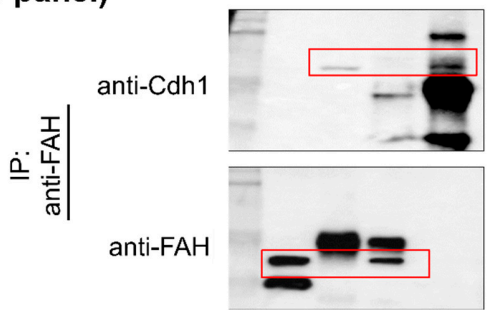


Fig 5D (lower panel)

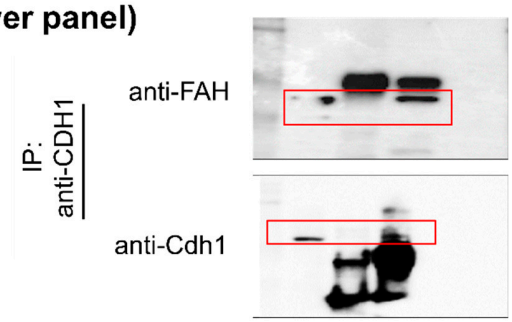


Fig 6A

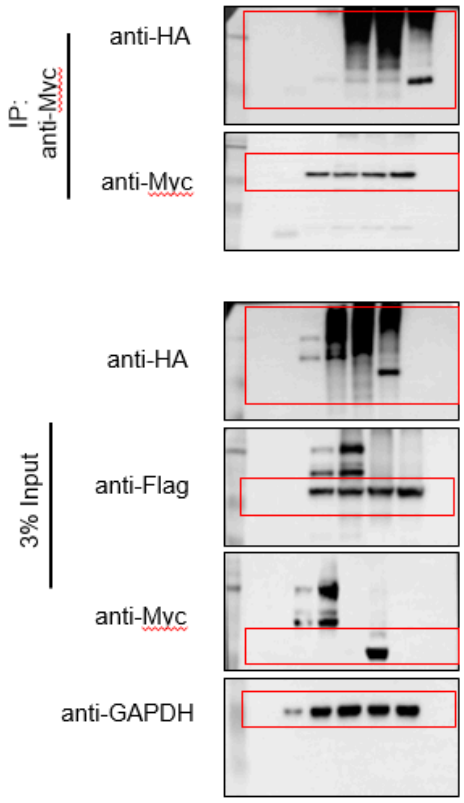


Fig 6B

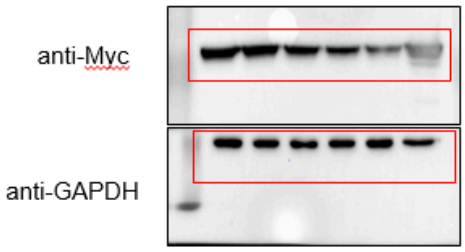


Fig 6C

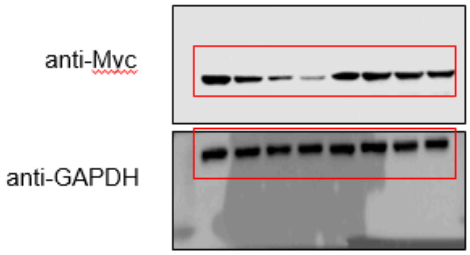


Fig 6D

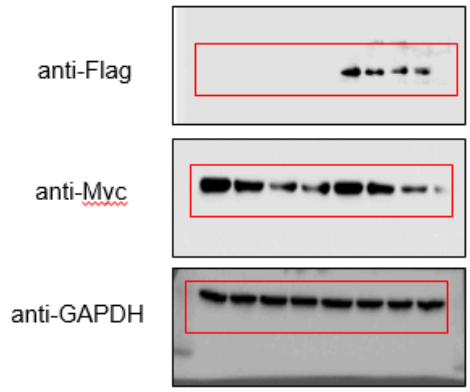


Figure S5. Triplicate images of western blots for significance value.

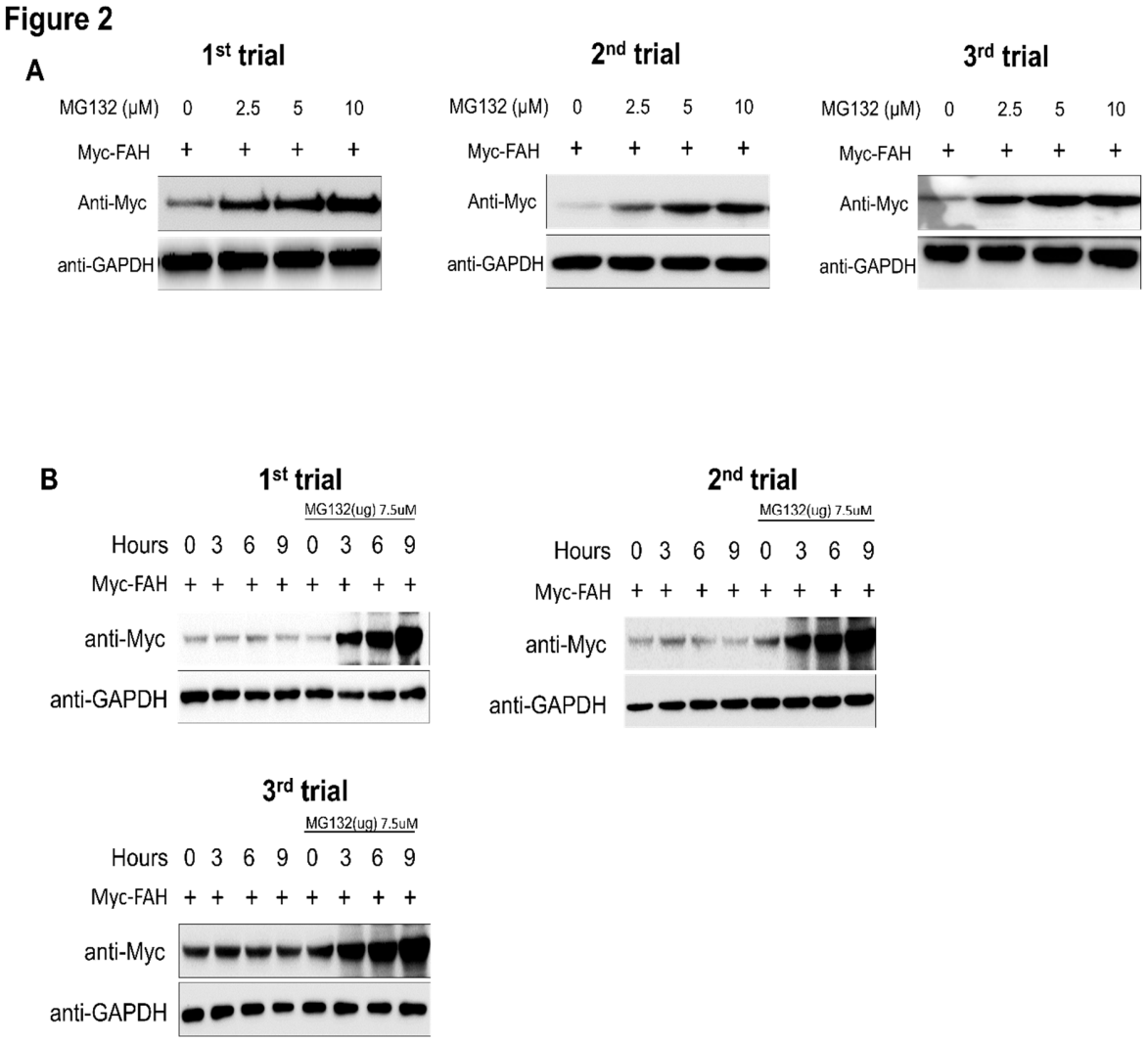


Figure 3

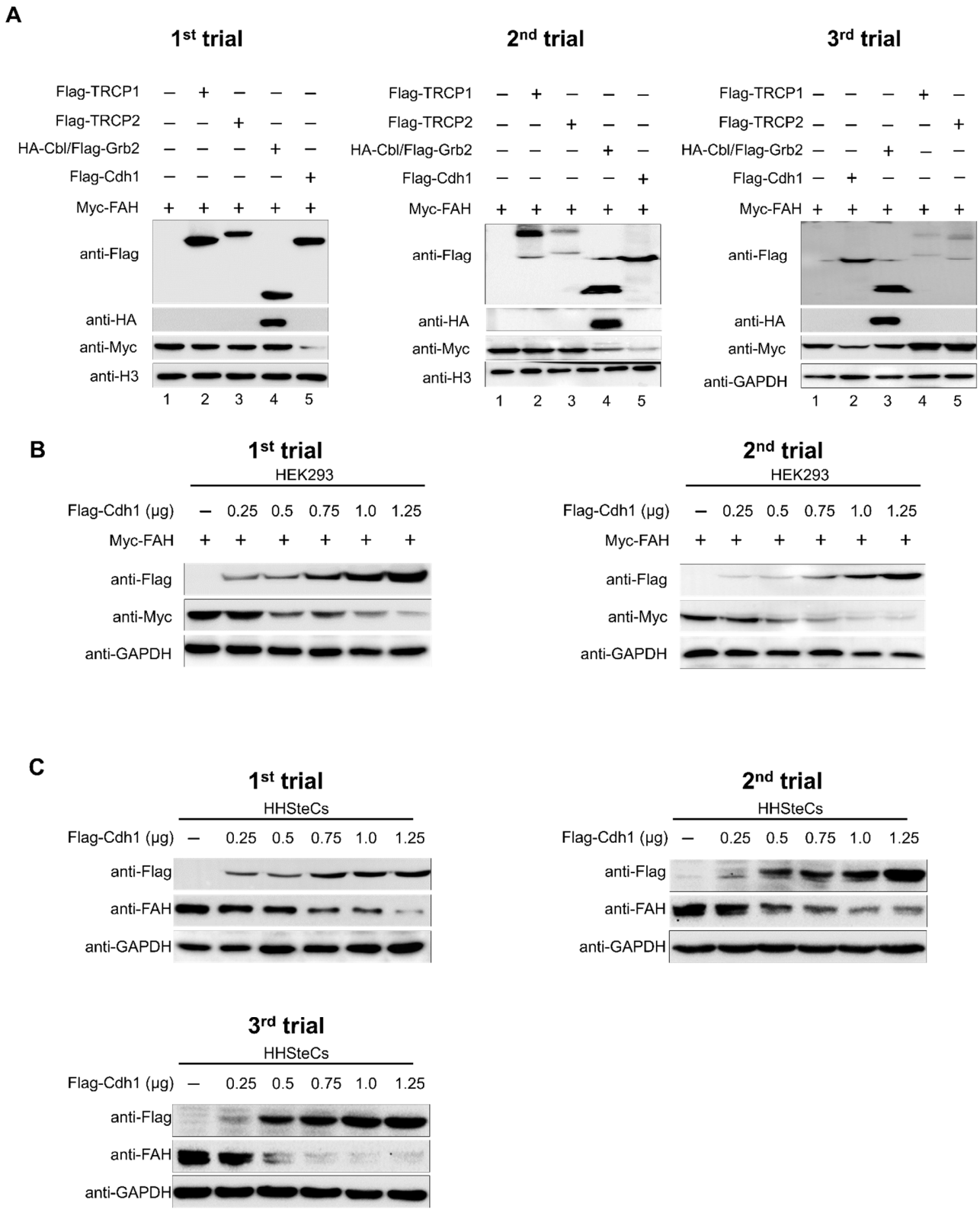

A
1st trial
2nd trial
3rd trial
Flag-TRCP1
Flag-TRCP2
HA-Cbl/Flag-Grb2
Flag-Cdh1
Myc-FAH
anti-Flag
anti-HA
anti-Myc
anti-H3
anti-GAPDH
1 2 3 4 5
B
1st trial
2nd trial
HEK293
Flag-Cdh1 (μg) – 0.25 0.5 0.75 1.0 1.25
Myc-FAH + + + + + +
anti-Flag
anti-Myc
anti-GAPDH
C
1st trial
2nd trial
3rd trial
HHSteCs
Flag-Cdh1 (μg) – 0.25 0.5 0.75 1.0 1.25
anti-Flag
anti-FAH
anti-GAPDH

Figure 4

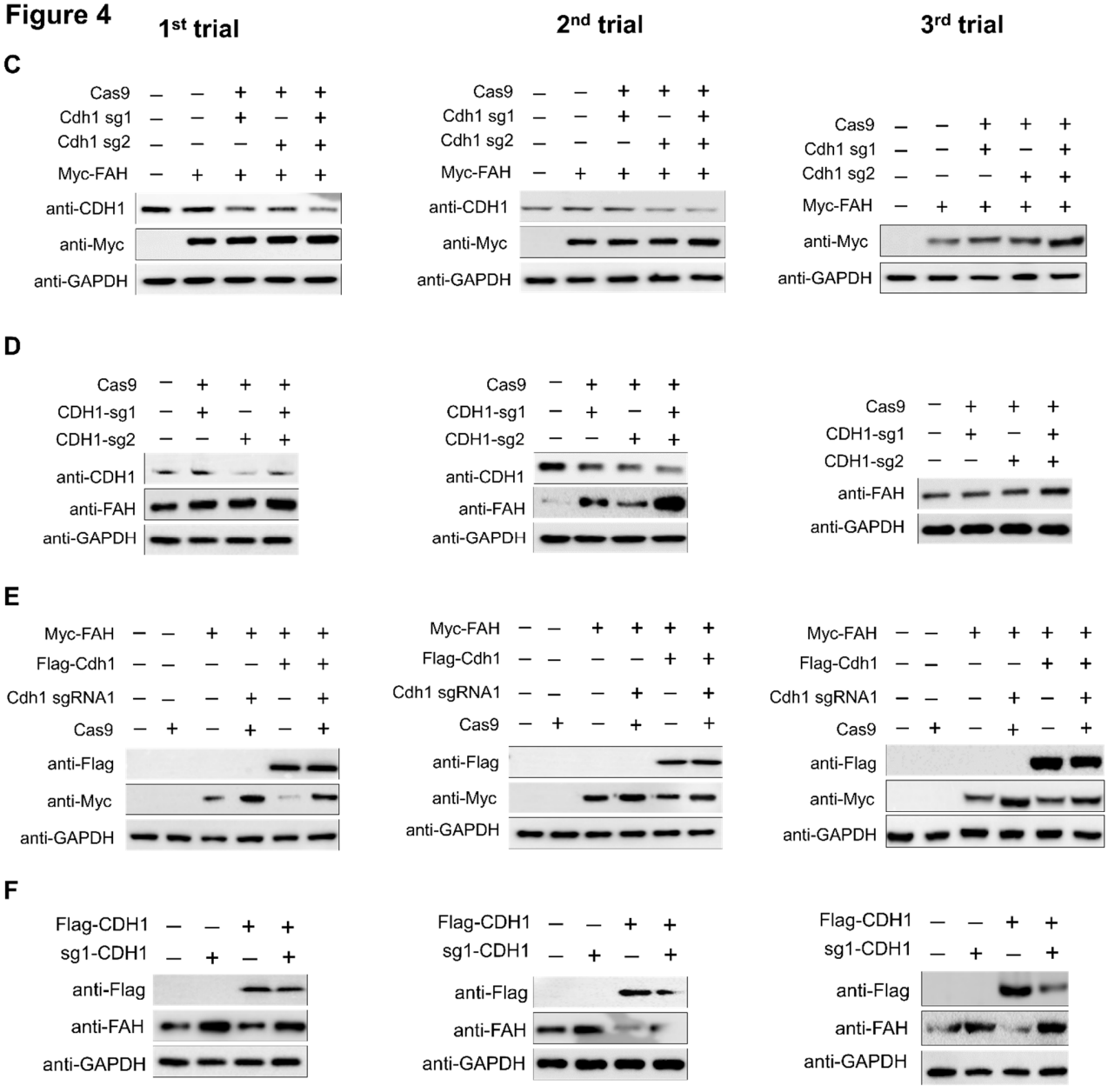
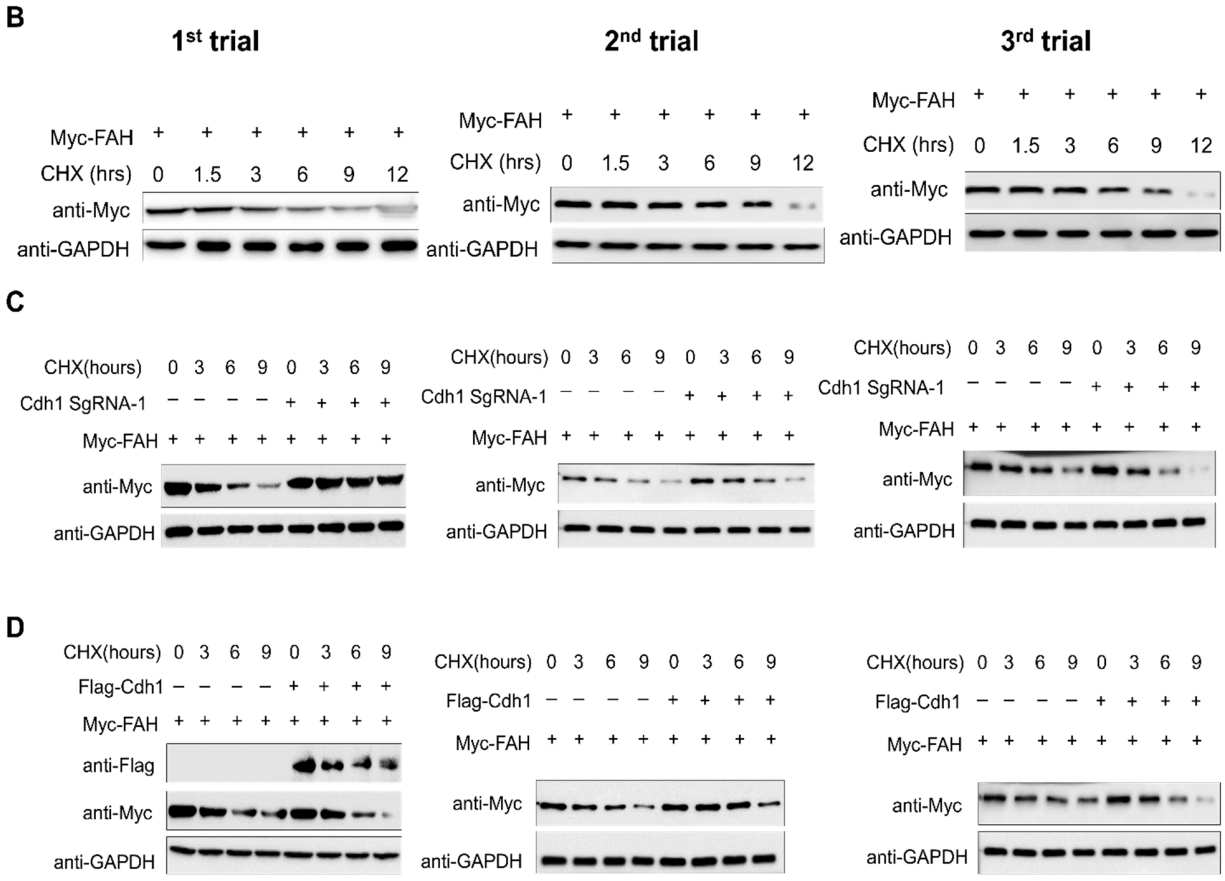

1st trial
2nd trial
3rd trial
C
Cas9
Cdh1 sg1
Cdh1 sg2
Myc-FAH
anti-CDH1
anti-Myc
anti-GAPDH
D
Cas9
CDH1-sg1
CDH1-sg2
anti-CDH1
anti-FAH
anti-GAPDH
E
Myc-FAH
Flag-Cdh1
Cdh1 sgRNA1
Cas9
anti-Flag
anti-Myc
anti-GAPDH
F
Flag-CDH1
sg1-CDH1
anti-Flag
anti-FAH
anti-GAPDH

Figure 6


B
1st trial
2nd trial
3rd trial
Myc-FAH + + + + + +
CHX (hrs) 0 1.5 3 6 9 12
anti-Myc
anti-GAPDH
C
CHX(hours) 0 3 6 9 0 3 6 9
Cdh1 SgRNA-1 − − − − + + + +
Myc-FAH + + + + + + + +
anti-Myc
anti-GAPDH
D
CHX(hours) 0 3 6 9 0 3 6 9
Flag-Cdh1 − − − − + + + +
Myc-FAH + + + + + + + +
anti-Flag
anti-Myc
anti-GAPDH