

APC/C^{CDH1} Synchronizes Ribose-5-Phosphate Levels and DNA Synthesis to Cell Cycle Progression

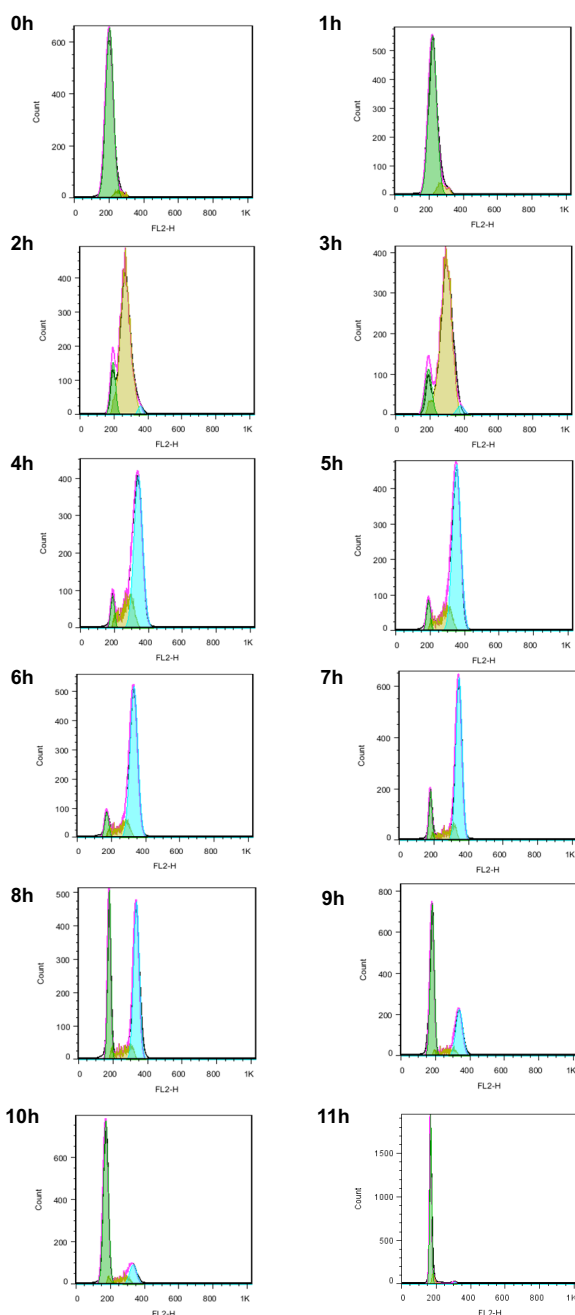
Li et al.

- 1. Supplementary Figures 1-10**
- 2. Supplementary Table 1**

a

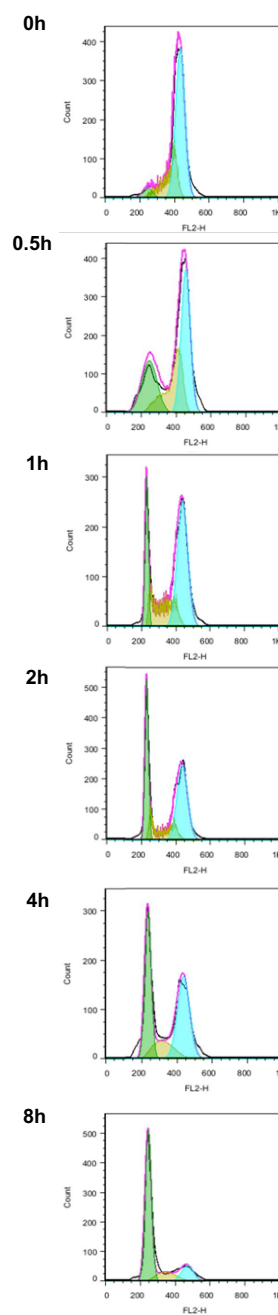
HeLa cells released after Double thymidine synchronization

Hours	0	1	2	3	4	5	6	7	8	9	10	11
G ₁ (%)	96.54	88.86	15.21	15.04	6.5	6.71	6.78	11.62	31.95	59.49	79.07	84.96
S(%)	4.49	8.26	87.53	87	19.96	16.34	13.28	13.19	10.51	9.02	9.69	9.54
G ₂ /M(%)	0.02	0.01	2.66	3.73	71.71	77.83	79.62	76.35	53.98	30.76	13.74	1.36

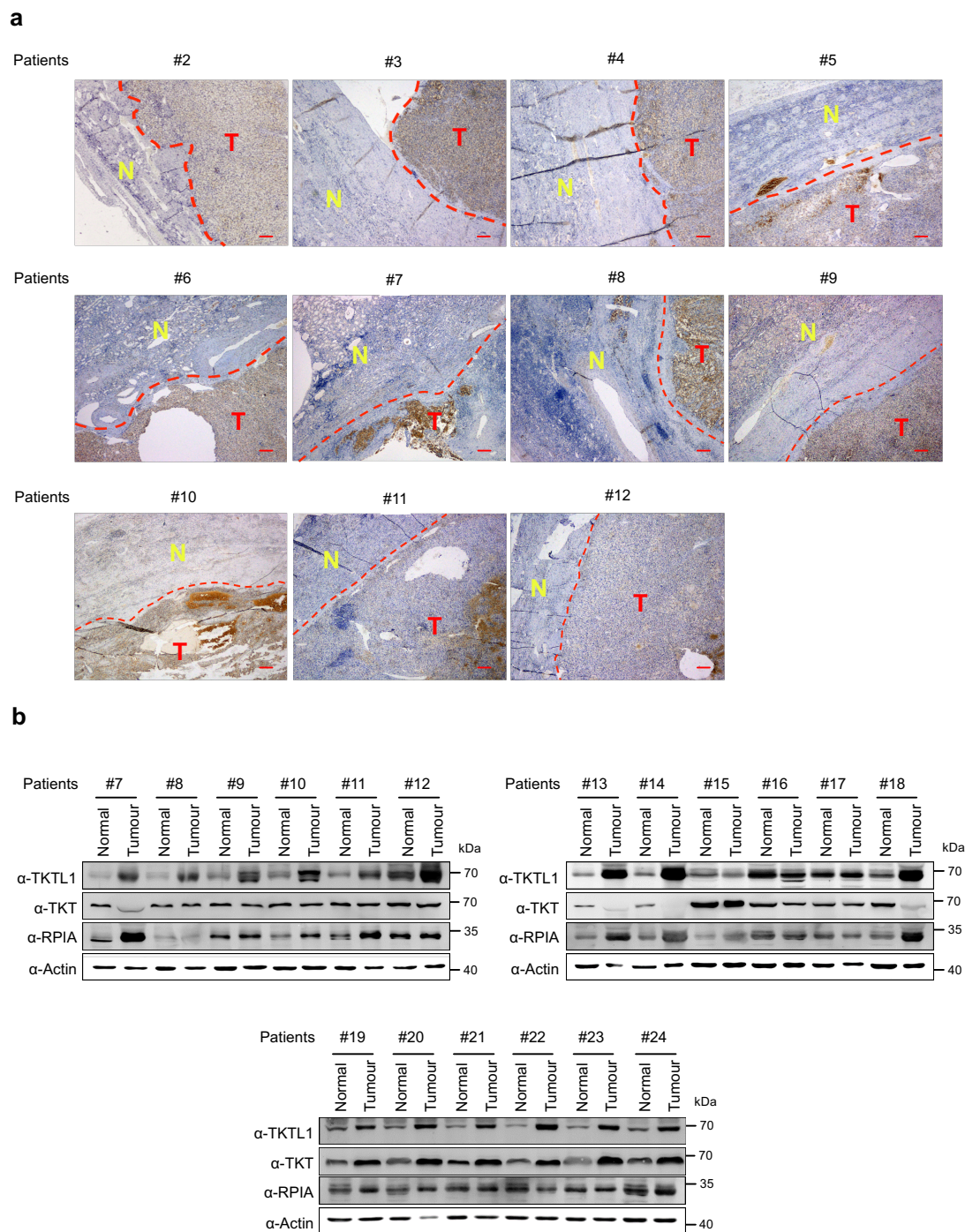
**b**

HeLa cells released after RO3306 synchronization

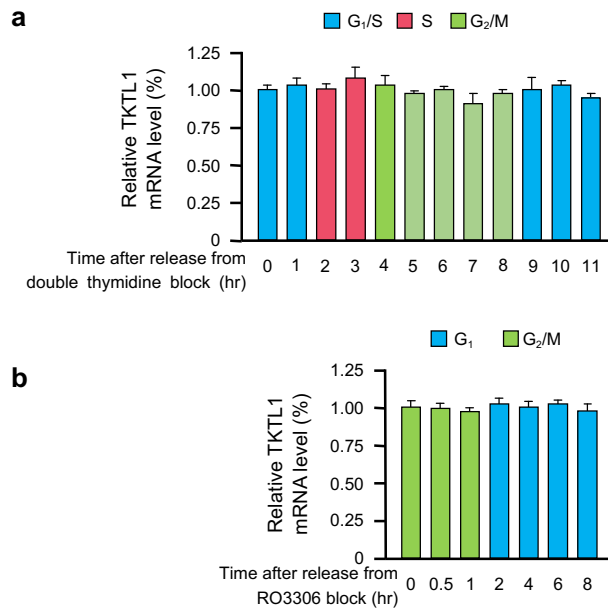
Hours	0	0.5	1	2	4	8
G ₁ (%)	5	18.88	27.64	31.43	37.73	69.26
S(%)	24.28	23.28	30.15	18.1	15.42	13.64
G ₂ /M(%)	70.11	53.05	46.61	46.18	41.41	14.44



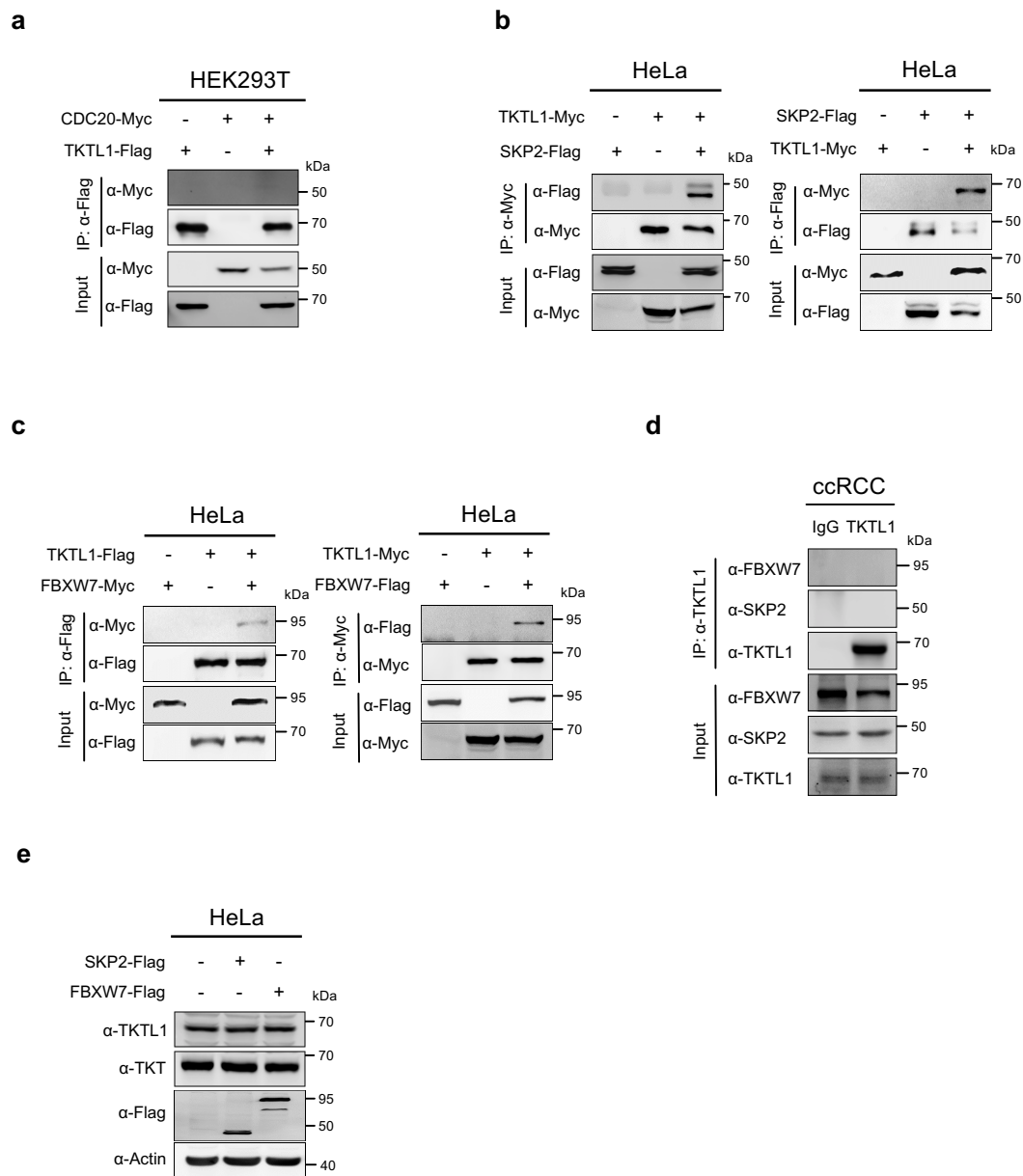
Supplementary Figure 1. Cell phases after release (related to Fig. 1). HeLa cell synchronized by double thymidine block (**a**) or RO3306 block (**b**) were released. Percentage (upper) of cells at different phases was determined by flow cytometric analysis (lower) at times indicated. The gating strategy of flow cytometry was shown in Supplementary Figure 9.



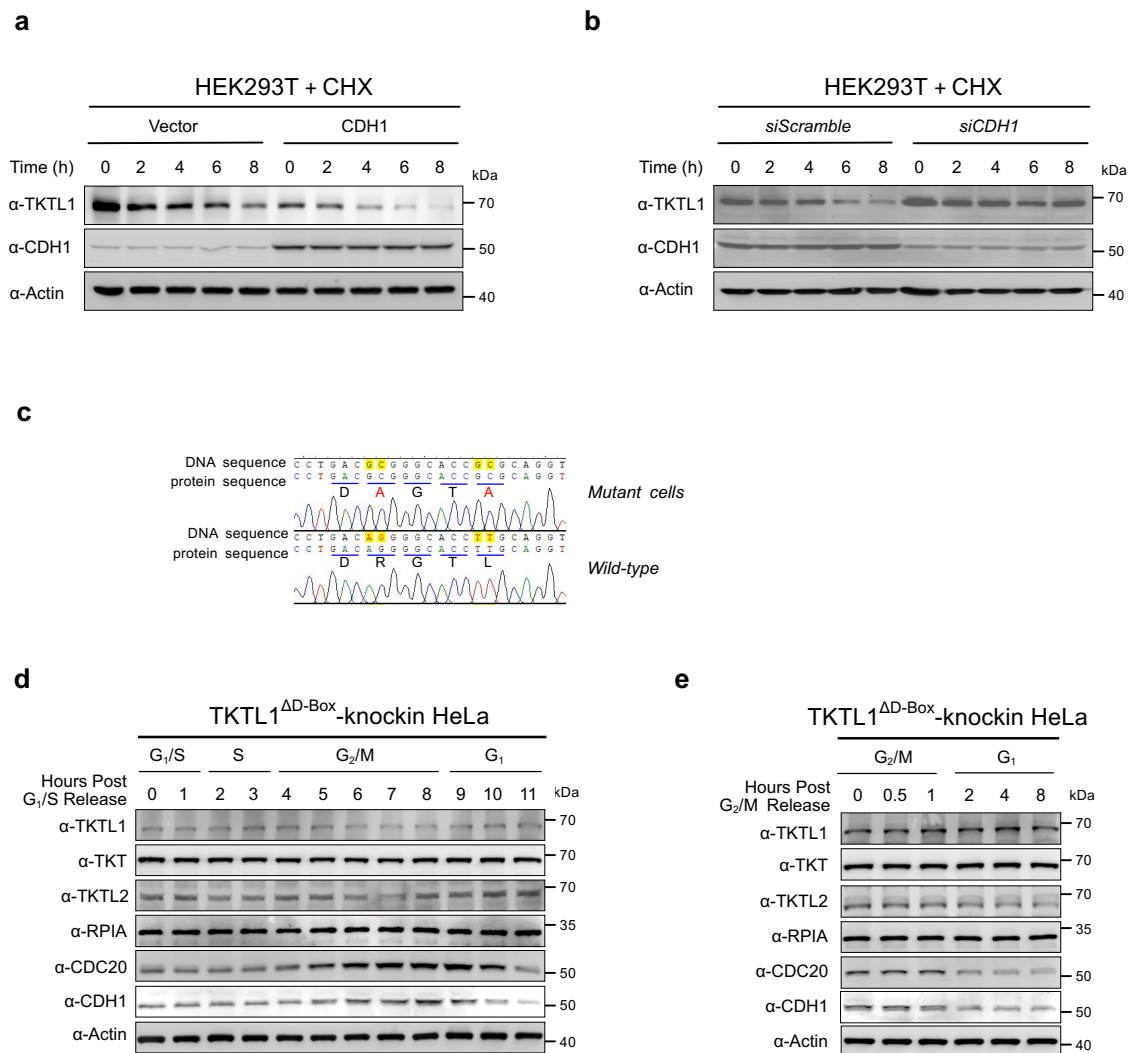
Supplementary Figure 2. Overexpression TKTL1 in ccRCC tissues (related to Fig. 1). **(a)** Immunohistochemically staining analysis of ccRCC and their adjacent non-cancer tissues. T: ccRCC tumor tissue; N: adjacent normal tissue. Scale bars: 200 μ m. Quantitating results are presented in Fig. 1g. **(b)** Western blotting analysis for protein levels in ccRCC and matched adjacent normal tissues. Quantitating results are presented in Fig. 1h.



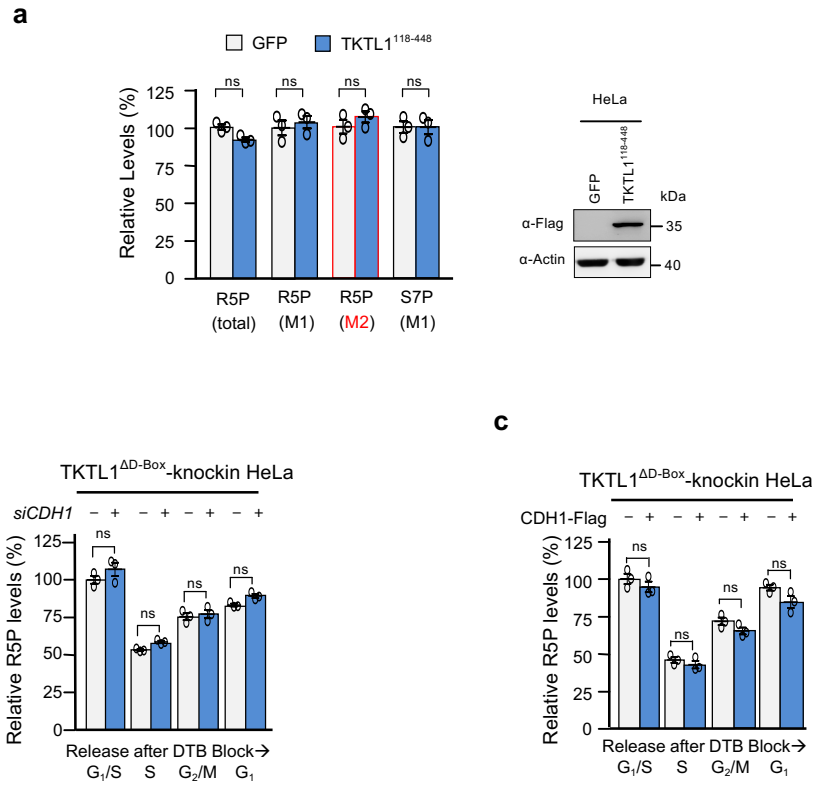
Supplementary Figure 3. TKTL1 is not regulated at transcription level during cell cycle (related to Fig. 2). *TKTL1* mRNA levels of HeLa cells during cell cycle progression released from in double thymidine synchronization (**a**) and RO3306 synchronization (**b**) were determined by qRT-PCR. Each column represents the mean $\pm$ SEM (n=5 biologically independent samples).



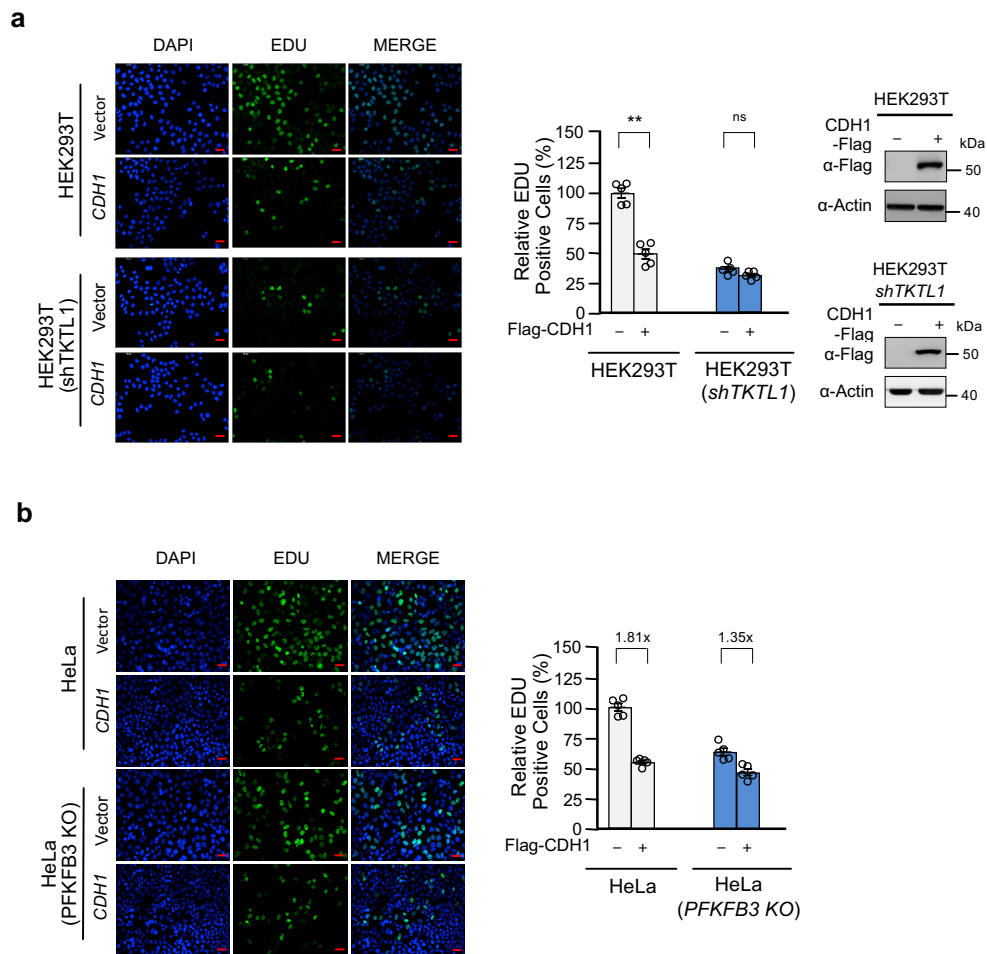
Supplementary Figure 4. Other cell cycle regulating proteins failed to regulate TKTL1 (related to Fig. 2). The interactions between TKTL1 and CDC20 (**a**), SKP2 (**b**), FBWX7 (**c**) were determined by immunoprecipitation. The *in vivo* immunoprecipitation showed TLTL1 did not interact with SKP2 and FBWX7 (**d**). The TKTL1 levels in HeLa and HeLa that overexpressing either SKP2 or FBWX7 were determined by Western blot (**e**).



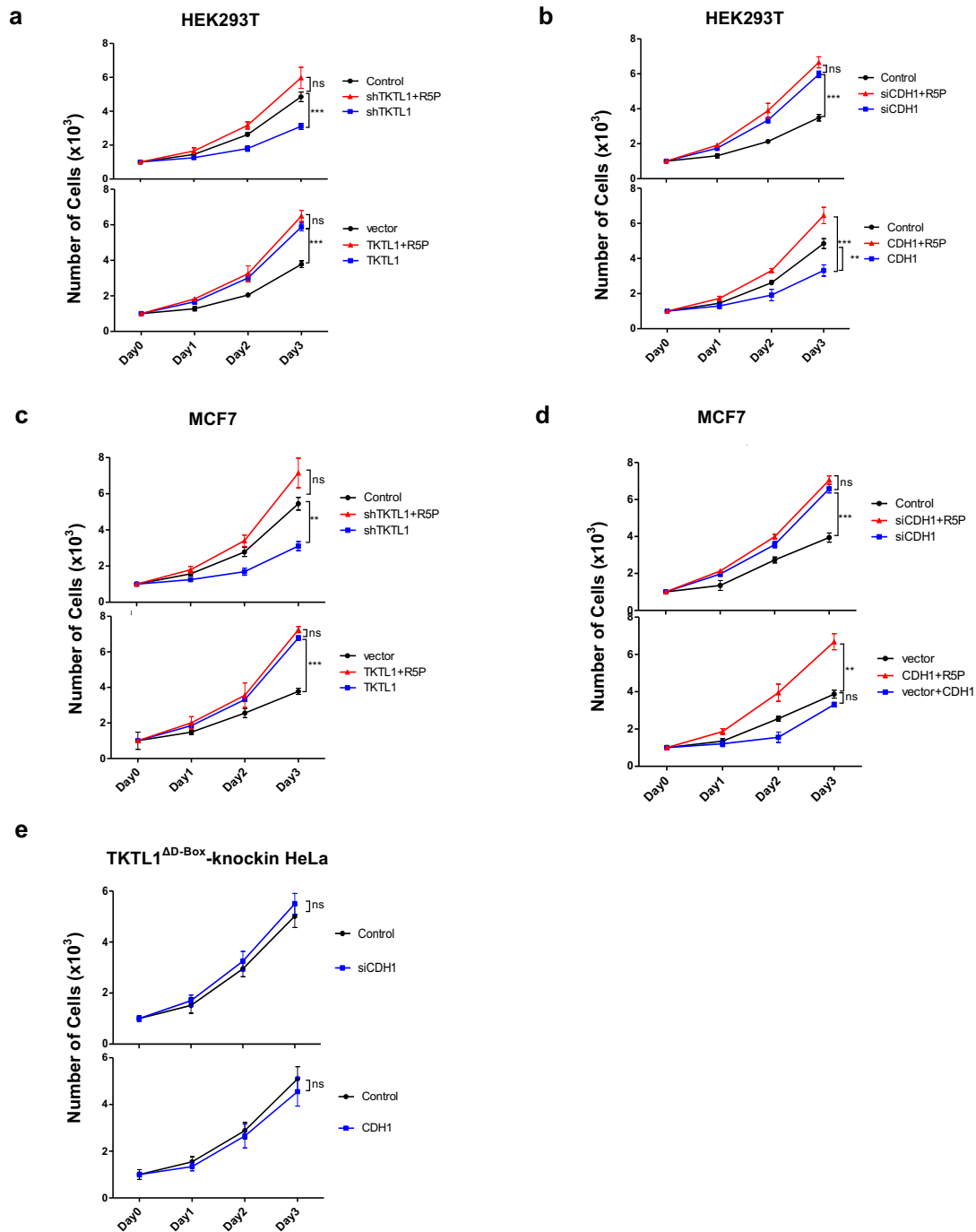
Supplementary Figure 5. CDH1 regulates TKTL1 stability (related to Fig. 2). (**a**, **b**) CDH1 overexpression promotes the degradation of TKTL1 (**a**), and CDH1 knockdown resulted in increased stability of TKTL1 (**b**). (**c**) TKTL1^{ΔD-box}-knockin HeLa sequence identifications are shown. (**d**, **e**) The protein levels of TKTL1, TKT, TKTL2, RPIA, CDC20, and CDH1 were determined at different time points after TKTL1^{ΔD-Box}-knockin-HeLa cells were released from double thymidine (**d**) and RO3306 (**e**) synchronization.



Supplementary Figure 6. TKTL1 mutants didn't reprogram R5P metabolism (related to Fig. 5). **(a)** Relative levels of total, M1 and M2 R5P, and S7P levels were determined in TKTL1¹¹⁸⁻⁴⁴⁸ overexpressing HeLa cells and in HeLa cells. Levels of HeLa cells were set as 100% and data are presented by means $\pm$ SEM of 3 independent experiments, Student's *t* test, ns not significant. **(b, c)** The R5P levels of TKTL1^{ΔD-Box}-knockin HeLa cells were compared with that in CDH1 knocked down HeLa cells **(b)** and CDH1 overexpressing HeLa cells **(c)**. Data are shown by means $\pm$ SEM of 3 independent experiments, Student's *t* test, ns not significant.

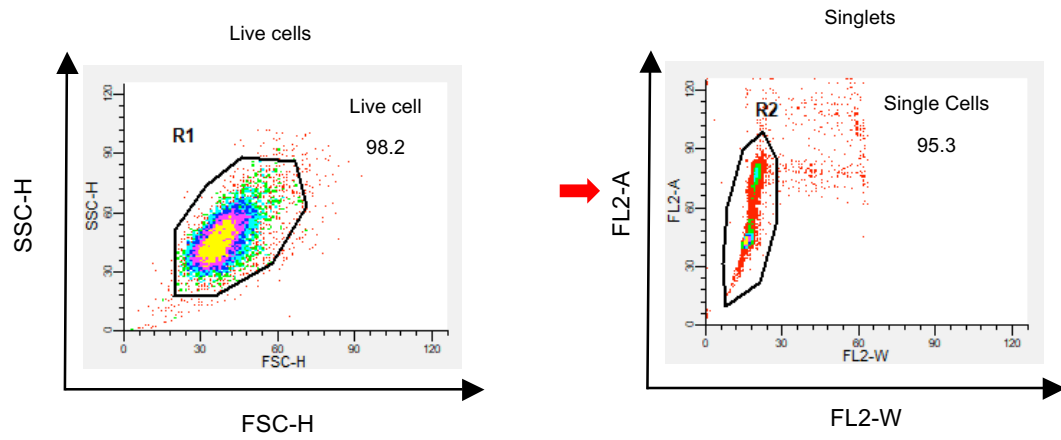


Supplementary Figure 7. APC/C^{CDH1} regulates DNA synthesis through TKTL1 (related to Fig. 7). The CDH1 overexpression effects on DNA synthesis were detected by EDU staining (left) in HEK293T and TKTL1 knockdown HEK293T cells (**a**), and HeLa and PFKFB3 knockout HeLa cells. Data were presented as mean $\pm$ SEM, Student's *t* test, ***p* < 0.01, ns not significant. (**b**). Bar scale: 100 μ m. Five biological independent experiments were used to present the data of the relative EDU staining positive cells (right, to that of control HEK293T cells). Data were presented as mean $\pm$ SEM.

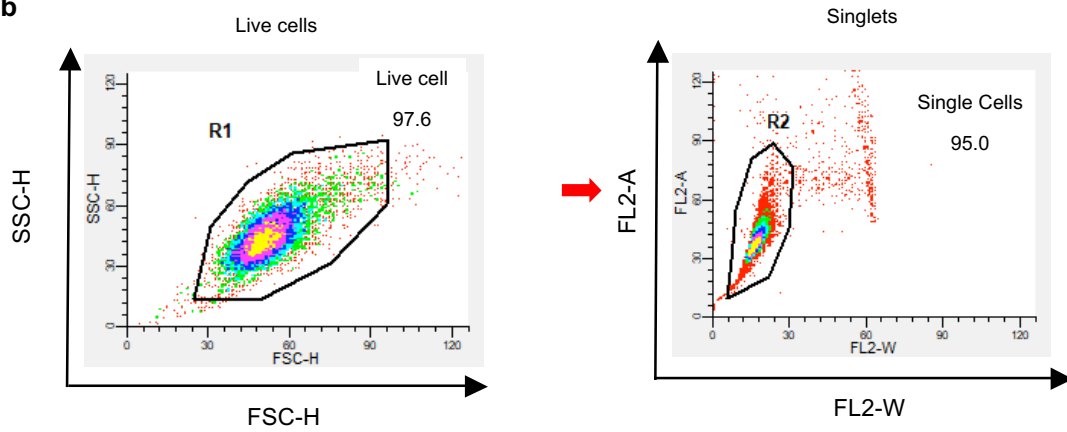


Supplementary Figure 8. APC/C^{CDH1} regulates cell growth through regulating TKTL1 and R5P levels (related to Fig. 7). The growth of HEK293T (**a**, **b**) and MCF cells (**c**, **d**) was determined (n=6 biologically independent samples) under TKTL1 overexpression and knockdown (**a**, **c**), CDH1 overexpression and knockdown (**b**, **d**), and these conditions with 10 mM R5P supplementation in the culture media (**a-d**). (**e**) The growth of TKTL1^{ΔD-Box}-knock-in cells was determined under CDH1 overexpression and knockdown.

a

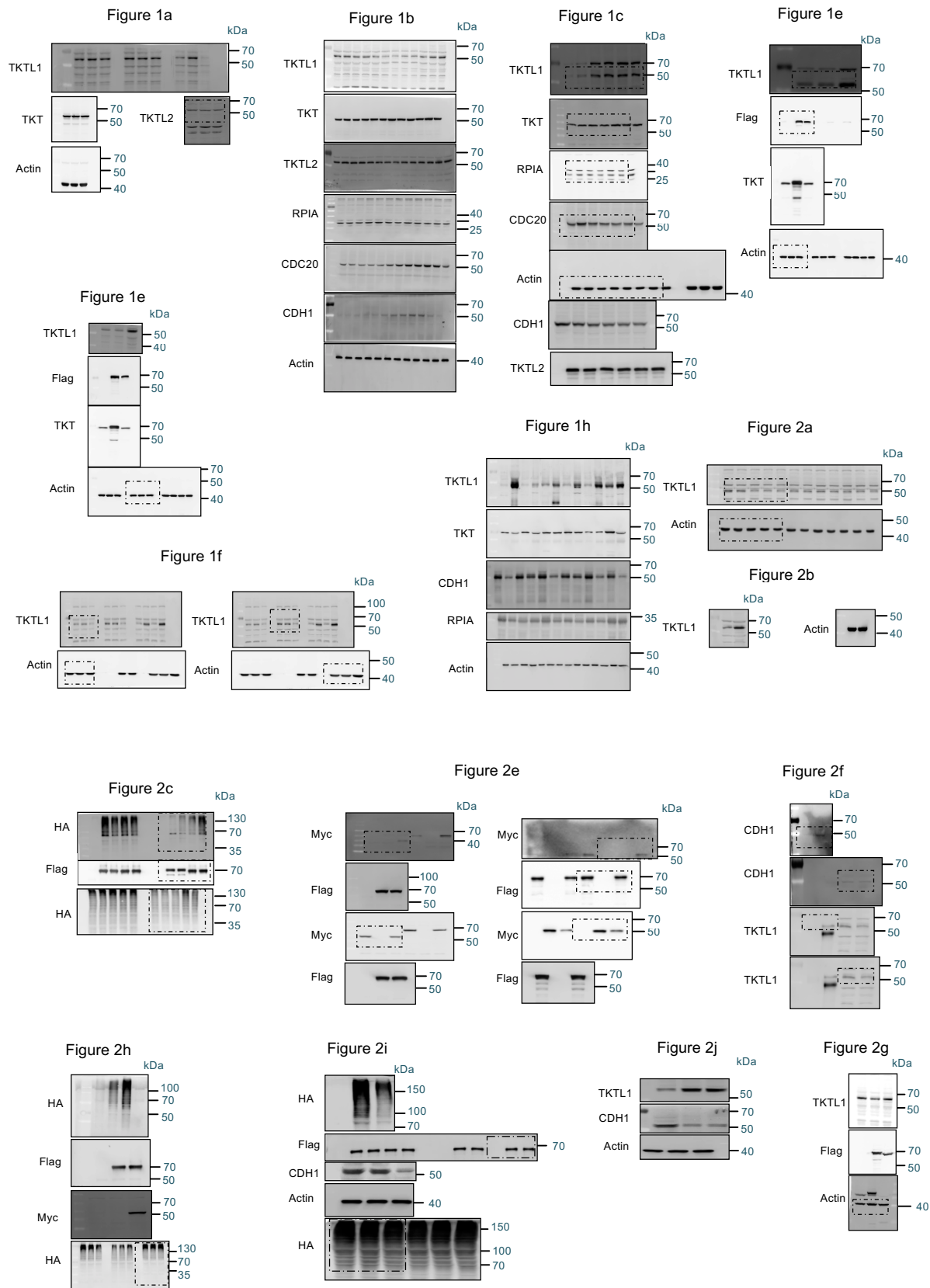


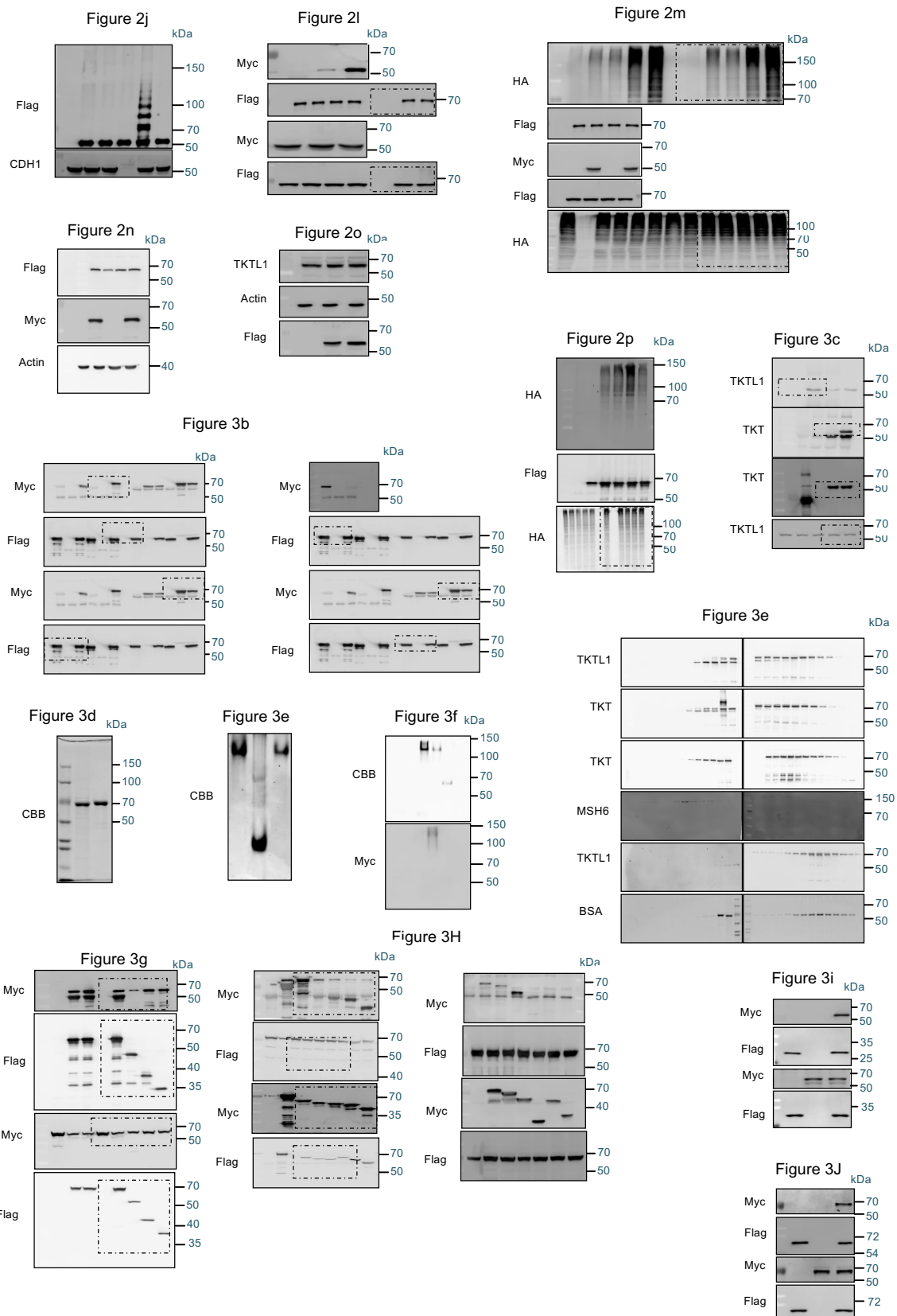
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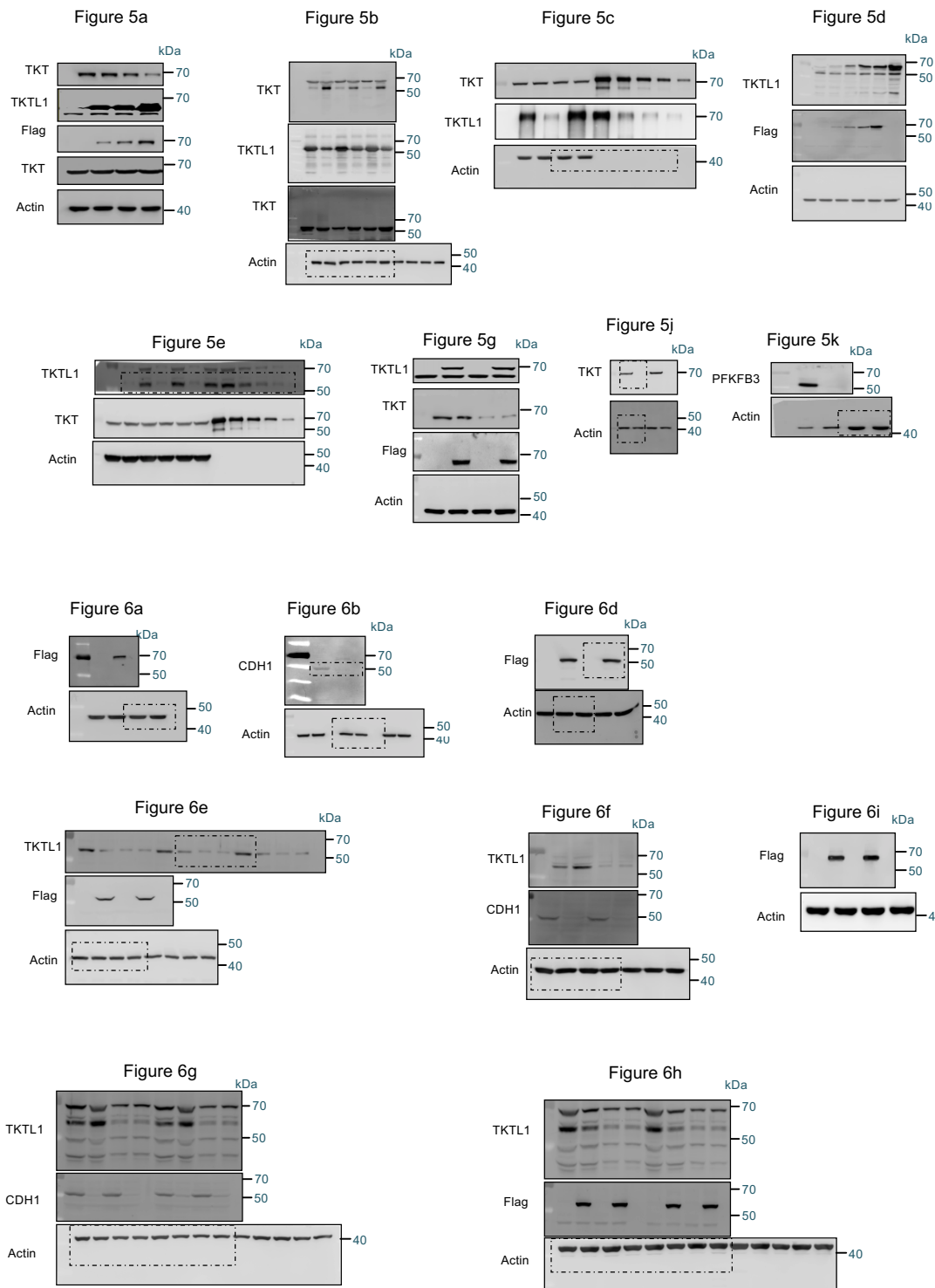


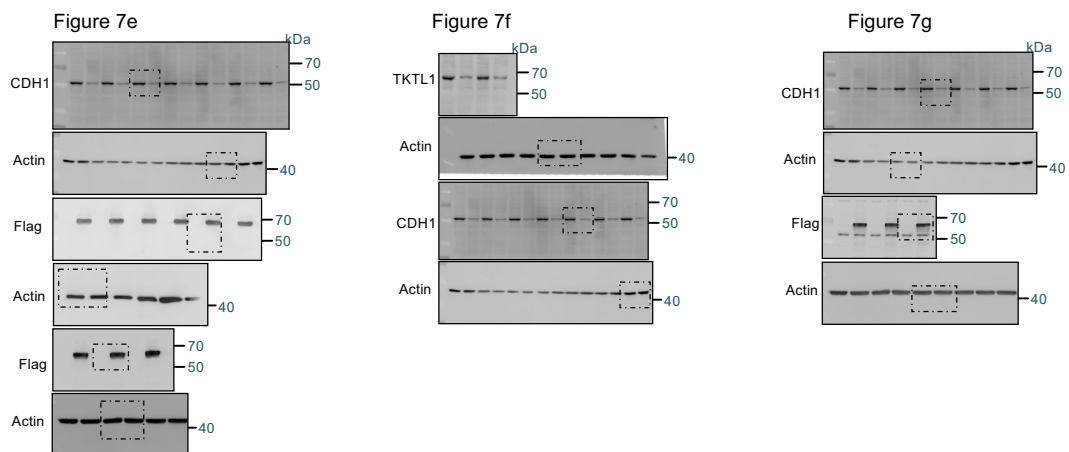
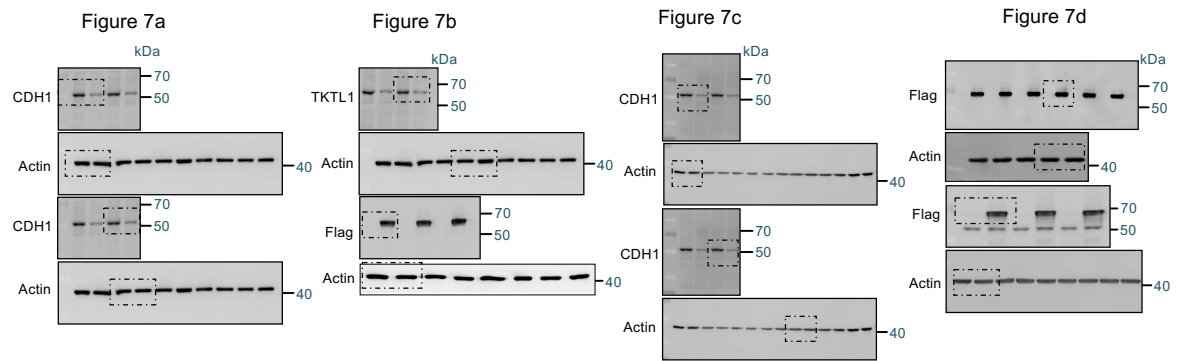
Supplementary Figure 9. Gating strategy of flow cytometry for normal HeLa cells **(a)** and DTB Block Released 0hr HeLa cells **(b)**. The flow cytometry results were shown in Supplementary Figure 1.

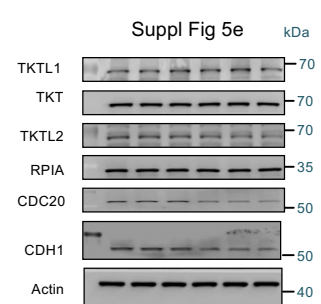
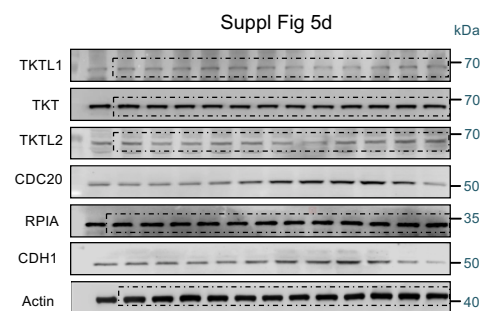
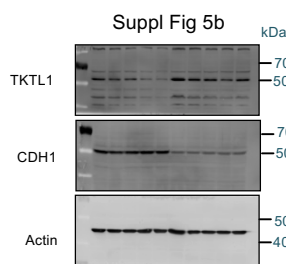
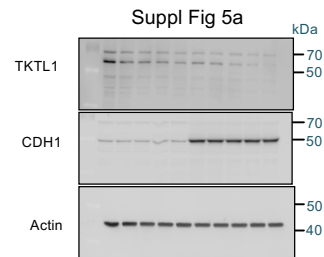
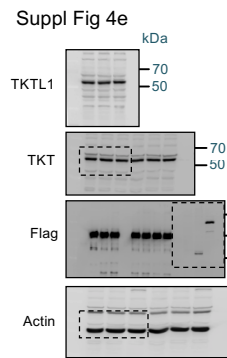
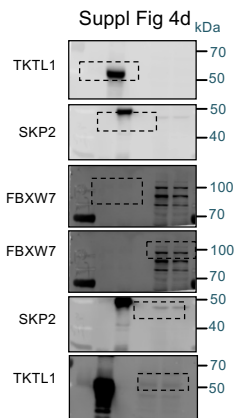
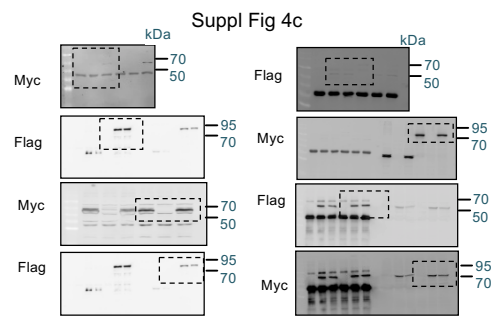
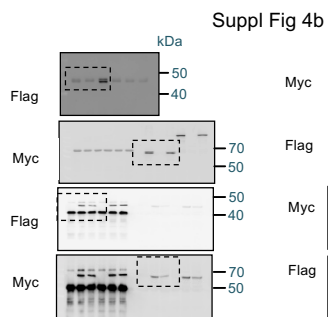
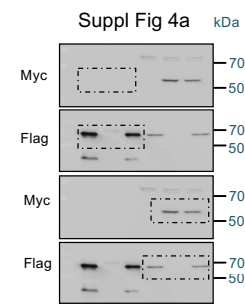
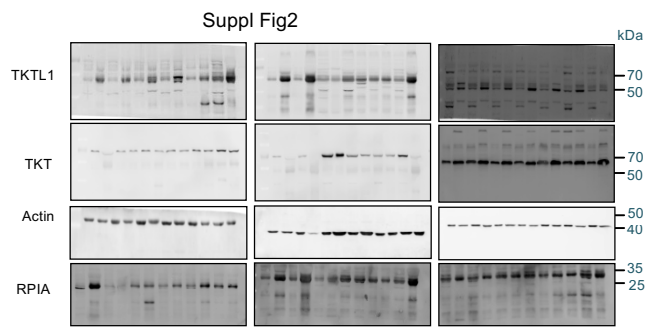
Supplementary Figure 10. Uncropped and unprocessed scans of gels and blots.











Supplementary Table 1

Polymerase chain reaction (PCR) Primers	
TKT-Flag forward:	GCGTCGACATGGAGAGCTACCACAAGCCTGACC
TKT-Flag reverse:	CGGAATTCCTAGGCCTTGGTGATGAGGCCCTC
TKT-Myc forward:	CCCTCGAGATGGAGAGCTACCACAAGCCTGACC
TKT-Myc reverse:	CGGAATTCCTAGGCCTTGGTGATGAGGCCCTC
TKTL1-Flag forward:	CCCAAGCTTATGGCGGATGCTGAGGCGAGG
TKTL1-Flag reverse:	CGGAATTCTTAGTTCAGCAACATGCATTTACGGC
TKTL1-Myc forward:	CGGAATTCATGGCGGATGCTGAGGCGAGG
TKTL1-Myc reverse:	CCCAAGCTTCCTTAGTTCAGCAACATGCATTTACGGC
CDH1-Flag forward:	CCCAAGCTTATGGAGATTTTGATCACGGTAACCG
CDH1-Flag reverse:	CGGGATCCCTAGTCGTCCTCGCCGCCTCCGTAC
CDH1-Myc forward:	CTAGCTAGCATGGACCAGGACTATGAGCGGC
CDH1-Myc reverse:	CGGAATTCCTCGGATCCTGGTGAAGAGGTTGAGC
β TrCP-Flag forward:	CCGCTCGAGATGGACCCGGCCGAGGCGGTGCTGCAAG
β TrCP-Flag reverse:	CGGGATCCGTTCTGGAGATGTAGGTGTATGTTTCGAG
SKP2-Flag forward:	GATGACAAGAAGCTTATGCACAGGAAGCACCTC
SKP2-Flag reverse:	CCATCGATTGAATTCTTATTGTTTTAAACAAG
FBXW7-Flag forward:	GATGACAAGAAGCTTATGAATCAGGAACTGCTC
FBXW7-Flag reverse:	CCATCGATTGAATTCTCACTTCATGTCCACATC
Truncated TKT and TKTL1 Primers	
TKTL1-1-448 reverse	CGGAATTCTTATCGGGTGGTCCGAATGAAGCACATC
TKTL1-1-292 reverse	CGGAATTCTTACCGAGTAGCTATCTTGTCACC
TKTL1-1-218 reverse	CGGAATTCTTACTTGAAGGTCTTGGCCACCACAGCA
TKTL1-84-596 forward	CCCAAGCTTAGACTGTCGTTTGTGGATGTGGCAAC
TKTL1-118-596 forward	CCCAAGCTTTACCGGGTGTCTGCCTCATGAGTG
TKTL1-146-596 forward	CCCAAGCTTCTGGACAATCTTGTGGCAATCTTTG
TKTL1-205-596 forward	CCCAAGCTTGTGAAGCACAAGCCCACTGCTGTGG
TKTL1-235-596 forward	CCCAAGCTTCCAATGCCGAGAGAAAGAGCAGATG
TKTL1-118-448 forward	CCCAAGCTTTACCGGGTGTCTGCCTCATGAGTG
TKTL1-118-448 reverse	CGGAATTCTTATCGGGTGGTCCGAATGAAGCACATC
TKTL1 ^{Δ119-447} forward	AGTTTCTGGGCTGGCCCTGTCGAAGTACTT
TKTL1 ^{Δ119-447} reverse	AGGGCCAGCCCAGAACTATGGTTATTTAC

TKTL1 ^{Δ148-473} forward	ATTTTCTGGGCTGGCCTTGTCGAAGTATTT
TKTL1 ^{Δ148-473} reverse	AAGGCCAGCCCAGAAAAATGCCATCATCTAT
siRNA sequence	
CDH1 siRNA-1:	5'- GCCAGATCGTCATCCAGAA
CDH1 siRNA-2:	5'- CCAACTGGAGCGTGAACCTT
shRNA sequence	
shTKTL1:	CATTCATCCCTAGTTCGGAAATTCA
shTKT:	TCGCCAGCATCTATAAGCTGGACAA
Quantitative RT-PCR Primers	
TKT forward:	5'- GTGGAAAAAGAGGACAGCCA
TKT reverse:	5'- ACAAGCCTGACCAGCAGAAG
TKTL1 forward:	5'- GTCGTTGTCCGGATTCTCTG
TKTL1 reverse:	5'- GAATCCATTCCATCAGGGC
β-actin forward:	5'-GCCGACAGGATGCAGAAGGAGATC A
β-actin reverse:	5'- AAGCATTTGCGGTGGACGATGGA

Supplementary Table 1. Sequences of primers of siRNA, shRNA, PCR and Q-PCR used in this study are listed.