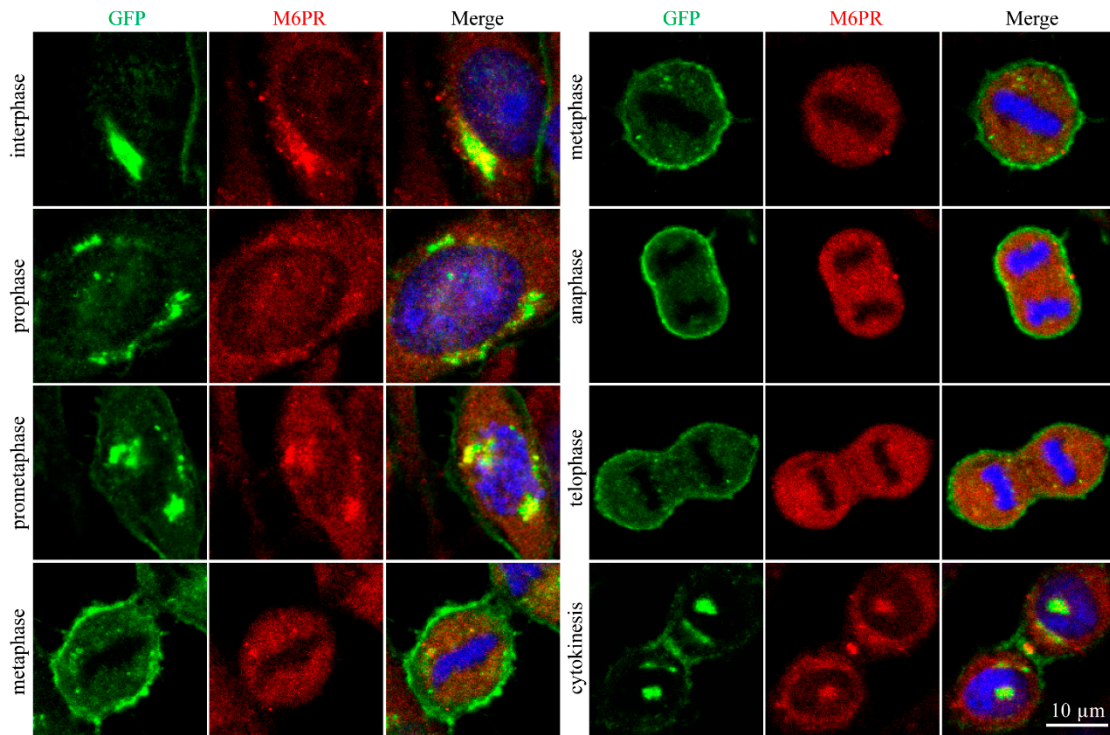
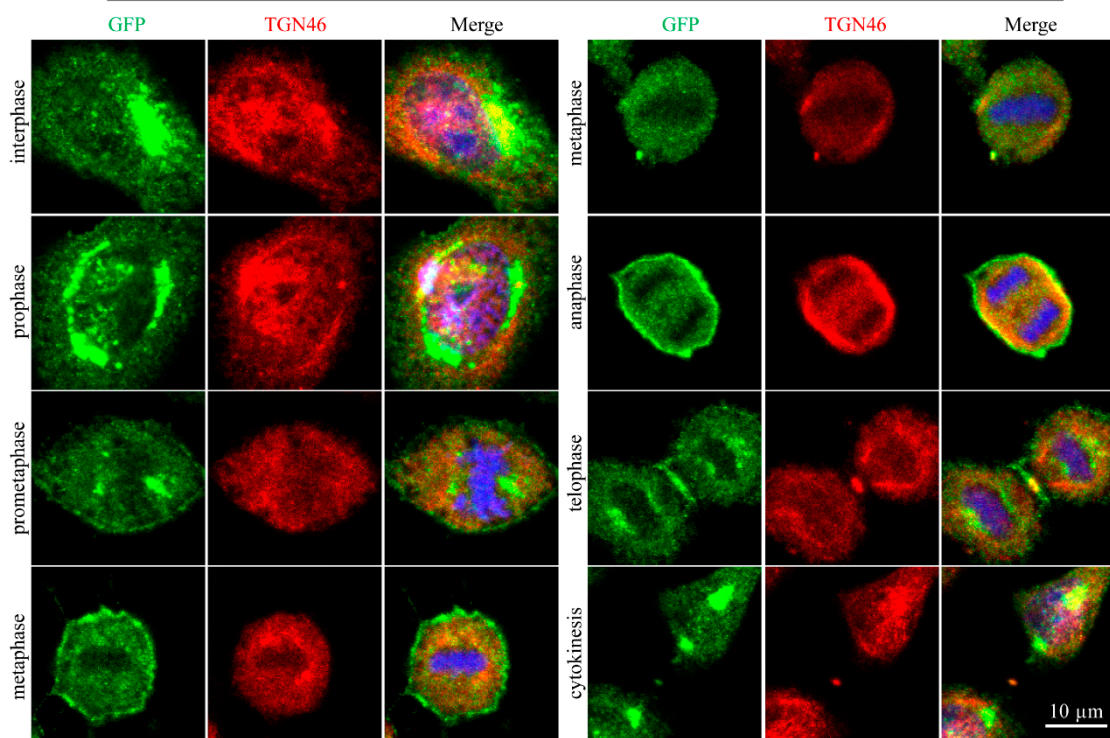


### HeLa-STK16-GFP-FLAG



**Figure S1.** The subcellular localization of STK16 throughout the cell cycle. HeLa cells stably expressing STK16-GFP-FLAG were fixed and then subjected to anti-GFP antibody and anti-M6PR antibody staining. GFP staining of STK16 is shown in green, M6PR staining is shown in red, and DAPI staining of the nucleus is shown in blue. Experiments were repeated at least three times and representative results are shown. Scale bar, 10 μm.

### HeLa-STK16-GFP-FLAG

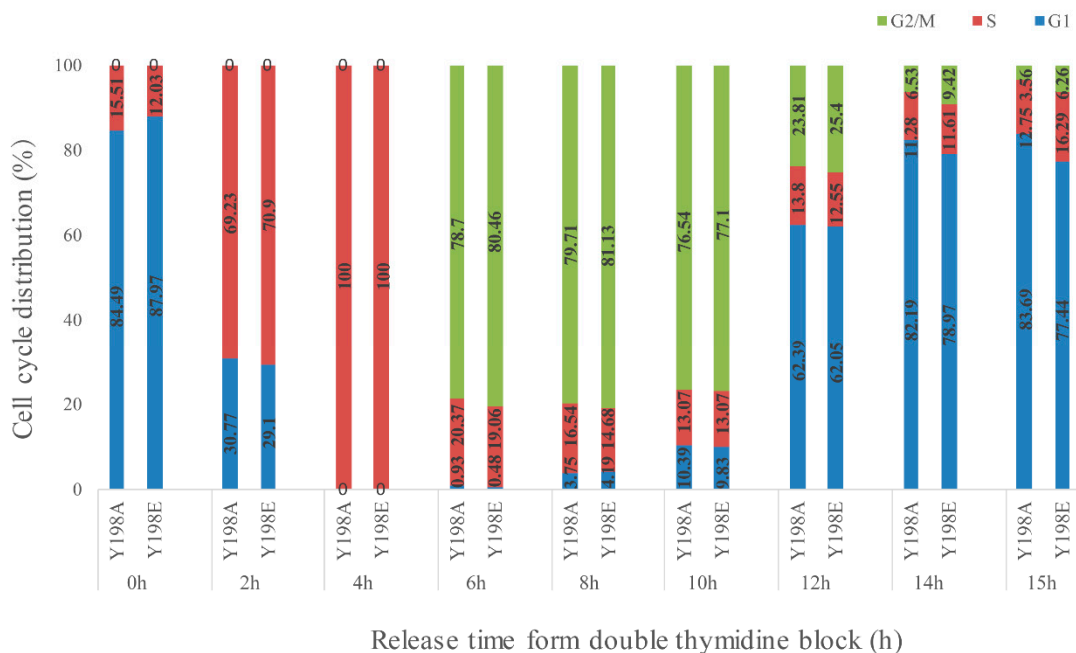


**Figure S2.** The subcellular localization of STK16 throughout the cell cycle. HeLa cells stably expressing STK16-GFP-FLAG were fixed and then subjected to anti-GFP antibody and anti-TGN46 antibody staining. GFP staining of STK16 is shown in green, TGN46 staining is shown in red, and DAPI staining of the nucleus is shown in blue. Experiments were repeated at least three times and representative results are shown. Scale bar, 10  $\mu$ m.

**Table S1.** Oligonucleotides used to construct plasmids in this study.

Oligonucleotide	Sequence (5'-3')
STK16-WT-GFP-FLAG FP	ACTGACCTCGAGGCCACCATGGGCCACGCGCTGTGTGC
STK16-WT-GFP-FLAG RP	ACTGACGAATTCGATTGGGTAGTATGTTGCCAGGAGC
STK16-T185E-FMP	CAGGCTC <b>TGG</b> AACTGCAGGACTGGGCAGCCCAGC
STK16-T185E-RMP	GTCCTGCAG <b>TTC</b> CAGAGCCTGGCGGGAGCCCTC
STK16-T185A-FMP	CAGGCTCTG <b>GCG</b> CTGCAGGACTGGGCAGCCCAGC
STK16-T185A-RMP	GTCCTGCAG <b>CGC</b> CAGAGCCTGGCGGGAGCCCTC
STK16-S197E-FMP	GGCTCGGTA <b>TTC</b> GATGGTGCACCGCTGGGCTGC
STK16-S197E-RMP	TGCACCATC <b>GAA</b> TACCGAGCCCCAGAGCTCTTCT
STK16-S197A-FMP	GGCTCGGTA <b>GGC</b> GATGGTGCACCGCTGGGCTGC
STK16-S197A-RMP	TGCACCATC <b>GCC</b> TACCGAGCCCCAGAGCTCTTCT
STK16-Y198E-FMP	CTGGGGCTCG <b>TTC</b> GGAGATGGTGCACCGCTGGGCT
STK16-Y198E-RMP	CACCATCTCC <b>GAA</b> CGAGCCCCAGAGCTCTTCTCTGTGC
STK16-Y198A-FMP	CTGGGGCTCG <b>GCC</b> GGAGATGGTGCACCGCTGGGCT
STK16-Y198A-RMP	CACCATCTCC <b>GCCC</b> CGAGCCCCAGAGCTCTTCTCTGTGC
STK16-S197E-Y198E-FMP	TGGGGCTCG <b>TTCTTC</b> GATGGTGCACCGCTGGGCTGCCCA
STK16-S197E-Y198E-RMP	TGCACCATC <b>GAAGAA</b> CGAGCCCCAGAGCTCTTCTCTGTGC
STK16-S197A-Y198A-FMP	TGGGGCTCG <b>GCCAGC</b> GATGGTGCACCGCTGGGCTGCCCA
STK16-S197A-Y198A-RMP	TGCACCATC <b>GCTGCC</b> CGAGCCCCAGAGCTCTTCTCTGTGC

Point mutations are indicated in bold type, and affected codon(s) are underlined and marked in red. FP, forward primer; RP, reverse primer; FMP, forward mutant primer; RMP, reverse mutant primer.



**Figure S3.** HeLa-STK16 Y198E-GFP-FLAG and HeLa-STK16 Y198A-GFP-FLAG cell lines were synchronized to the G1/S phase by double thymidine, and the cell cycle was detected by FACS at the times indicated.