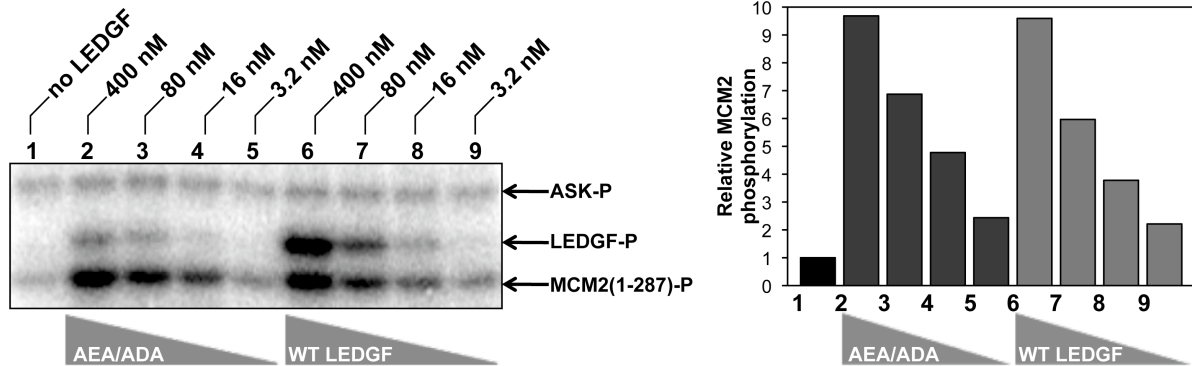


Supplemental Fig. S1. LEDGF IBD competes with full-length LEDGF for binding to Cdc7:ASK. Cdc7:ASK (100 nM) was incubated with 200 nM LEDGF, 4 mM ATP, and S protein agarose in the absence (*lane 5*) or presence of 120-960 nM GST-LEDGF(347-471) (GST-IBD, *lanes 6-9*) or GST (*lanes 10-13*). Proteins bound to the beads were separated by SDS PAGE and visualized by staining with Coomassie Blue (top) or western blotting with anti-LEDGF antibody (bottom). *Lanes 1-4* contained input proteins. Migration of protein molecular weight standards is indicated to the left of the Coomassie-stained gel; migration of LEDGF and GST-IBD are indicated to the right.



Supplemental Fig. S2. Stimulation of Cdc7:ASK kinase activity is independent of the phosphorylation state of LEDGF. Phosphorylation of GST-MCM2(1-287) *in vitro* by Cdc7:ASK in the absence (*lane 1*) or presence of 400-3.2 nM WT (*lanes 6-9*) or AEA/ADA LEDGF (*lanes 2-5*). Reaction products, resolved by SDS PAGE, were visualized by phosphorescence imaging (left). Migration positions of phosphorylated ASK (ASK-P), LEDGF (LEDGF-P) and GST-MCM2(1-287) (MCM2(1-287)-P) are indicated. Quantification of GST-MCM2(1-287) phosphorylation, relative to the condition without LEDGF (*lane 1*), is shown on the right.

SUPPLEMENTAL TABLE S1.**DNA constructs used in this work**

Name	Purpose	Host¹	Reference²
pGM-Mel18-cTAP	cTAP fusion vector	M	(1)
pGM-hLEDGF(326-530)-cTAP	Expression of LEDGF(326-530)-cTAP	M	This work
pCPHA-NLS	HA-fusion vector	M	(2)
pCPHA-38	Expression of HA-LEDGF(326-530)	M	This work
pBHA75	Expression of human HA-LEDGF	M	(2)
pHcRed1-p75	Expression of human LEDGF with N-terminal HcRed1 tag	M	(3)
pHcRed1-p52	Expression of human p52 with N-terminal HcRed1 tag	M	(3)
PCPHA-HRP2	Expression of human HA-HRP2	M	(2)
pLB(N)CX-mp75-HA	Expression of mouse LEDGF-HA	M	This work
pLB(N)CX-mp52-HA	Expression of mouse p52-HA	M	This work
pTRE2-75-hyg	Tetracycline-responsive expression of non-tagged human LEDGF	M	This work
pTRE2-HA75-hyg	Tetracycline-responsive expression of HA-tagged human LEDGF	M	This work
pIRES2-LEDGF-eGFP	Expression of non-tagged WT human LEDGF	M	(4)
pIRES2-LEDGFAIBD-eGFP	Expression of non-tagged human LEDGF lacking residues 347-430	M	This work
pIRES2-LEDGF(EEE)-eGFP	Expression of full-length human LEDGF K401E/K402E/R405E mutant	M	(4)
pcDNA3.1-Flag-Cdc7	Expression of Flag-Cdc7	M	This work
pEGFP-Cdc7	Expression of EGFP-Cdc7	M	This work
pCPHA-Dbf4	Expression of human HA-ASK	M	This work
pCPHA-Dbf4(1-624)	Expression of human HA-ASK, lacking 50 C-terminal residues	M	This work
pQFlag-MCM2-8xHis	Retroviral vector for expression of human Flag-MCM2-His ₈	M	This work
pCG-GAG-POL	Gammaretroviral packaging construct	M	(5)
pCG-VSV-G	Envelope expression for retroviral vector production	M	(5)
pRSF-CDC7-S-tag	Expression of human Cdc7 with C-terminal S tag	E	This work
pCDF-His-Dbf4	Expression of full-length human ASK with an N-terminal His ₆ tag	E	This work
pCDF-His-Dbf4(1-350)	Same for ASK(1-350)	E	This work
pCDF-His-Dbf4(174-350)	Same for ASK(174-250)	E	This work
pCDF-His-Dbf4(174-674)	Same for ASK(174-674)	E	This work
pCDF-His-Dbf4(1-624)	Same for ASK(1-624)	E	This work
pCDF-His-Dbf4(1-541)	Same for ASK(1-541)	E	This work
pFT1-LEDGF	Expression of full-length human LEDGF with a cleavable His ₆ tag	E	(6)
pCPH6P-LEDGF(146-530)	Same for LEDGF(146-530)	E	This work
pCPH6P-LEDGF(226-530)	Same for LEDGF(226-530)	E	This work
pCPH6P-LEDGF(249-530)	Same for LEDGF(249-530)	E	This work
pCPH6P-LEDGF(291-530)	Same for LEDGF(291-530)	E	This work
pCPH6P-LEDGF(347-530)	Same for LEDGF(347-530)	E	This work
pFT1-LEDGFAIBD	Same for LEDGF lacking residues 347-430	E	This work
pFT1-LEDGF(SDA)	Expression of full-length human LEDGF S275A mutant	E	This work
pFT1-LEDGF(ADA)	Same for LEDGF S273A/275A	E	This work
pFT1-LEDGF(AEA)	Same for LEDGF S206A/S208A	E	This work
pFT1-LEDGF(AEA/ADA)	Same for LEDGF S206A/S208A/S273A/S275A	E	This work
pFT1-LEDGF(S206A)	Same for LEDGF S206A	E	This work
pFT1-LEDGF(S208A)	Same for LEDGF S208A	E	This work
pCP-GST-81	Expression of GST-LEDGF(347-471)	E	(2)
pCP-GST-HRP2-IBD	Expression of GST-HRP2(470-593)	E	(2)
pCP-GST-MCM2(1-287)	Expression of GST-MCM2(1-287)	E	This work

¹Host for expression: M, mammalian; E., *Escherichia coli*.²See supplemental References

SUPPLEMENTAL TABLE S2.**Cdc7-specific peptides identified in the material co-purified with LEDGF(326-530)-cTAP**

Cdc7-specific peptides were detected and confirmed by LC-MS/MS; their locations within the sequence of human Cdc7 and experimental masses are shown.

Location	Sequence
91-100	HLIPTSHPIR
177-187	DVKPSNFLYNR
228-244	SHITGNKIPLSGPVPK
258-268	RPYTNAQIIK
313-319	TVDVLSR
417-431	ASDDLTAQAQIMTIR
475-492	LTSDIQGHASHQPAISEK
549-557	LLDLNPASR

SUPPLEMENTAL TABLE S3.**LEDGF phosphopeptides and their locations within the amino acid sequence**

Tryptic peptides were derived from recombinant LEDGF phosphorylated *in vitro* with Cdc7:ASK, or HA-LEDGF isolated from asynchronously growing or S-phase H3 cells. Phosphorylation sites, compatible with results of tandem mass spectrometry analyses, are underlined, and those assigned with confidence are printed in bold type.

Location	<i>In vitro</i> phosphorylation with Cdc7:ASK	Asynchronous cells	S-phase cells
57-67 101-113	DIFPY <u>SEN</u> KEK ¹	QSNASS <u>D</u> VEVEEK ¹	QSNASS <u>D</u> VEVEEK ¹ QSNASS <u>D</u> VEVEEK ²
101-113/127		QSNASSDVEVEEKETS <u>VSKED</u> <u>TD</u> HEEK ¹ QSNASSDVEVEEKETS <u>VSKED</u> <u>TD</u> HEEK ¹ QSNASS <u>D</u> VEVEEK ¹	
114/128-135		ETS <u>VSKED</u> <u>TD</u> HEEK <u>AS</u> NEDVTK ¹ ETS <u>VSKED</u> <u>TD</u> HEEK <u>AS</u> NEDVTK ²	ETS <u>VSKED</u> <u>TD</u> HEEK ¹ ETS <u>VSKED</u> <u>TD</u> HEEK ¹ <u>AS</u> NEDVTK ¹ AVDIT <u>TPK</u> ¹
136-143 156-179		AVDIT <u>TPK</u> ¹ QVETEEAGVVTTATASVNLKV <u>SPK</u> ¹	AVDIT <u>TPK</u> ¹ QVETEEAGVVTTATASVNLKV <u>SPK</u> ¹
202-218 267-286	QPCP <u>SE</u> DIITEEDKSK ¹ TGVSTSD <u>SE</u> EEGDDQEGEK ¹	TGVSTSD <u>SE</u> EEGDDQEGEK ¹ TGVSTSD <u>SE</u> EEGDDQEGEK ²	QPCP <u>SE</u> DIITEEDK ¹ TGVSTSD <u>SE</u> EEGDDQEGEK ¹ TGVSTSD <u>SE</u> EEGDDQEGEK ²
344-351 510/511-524 518-530		KKP <u>SE</u> EERETEISLK ¹	RET <u>SM</u> DSR ¹ KPS <u>SE</u> EERETEISLK ¹

¹ singly-phosphorylated

² doubly-phosphorylated

SUPPLEMENTAL TABLE S4.**LEDGF phosphopeptides reported in global phosphoproteome studies (7,8) and their locations within the amino acid sequence**

Assigned phosphorylation sites are printed in bold type and underlined.

Location	Olsen <i>et al.</i> 2006 (7)¹ (HeLa)	Dephoure <i>et al.</i>, 2008 (8)¹ (Hela, G1)	Dephoure <i>et al.</i>, 2008 (8)¹ (HeLa, G2)
101-113	QSNASSDVEVEEK QSNASSDVEVEEK	QSNASSDVEVEEK	QSNASSDVEVEEK
101-127			QSNASSDVEVEEKETS ¹ VSKED ¹ IDHEEK
114-127	ETSVSKED ¹ IDHEEK		
136-143	AVDIT ¹ TPK	AVDIT ¹ TPK	AVDIT ¹ TPK
267-286/288	<u>TGVT</u> <u>STSD</u> SEEEGDDQEGEK TGVTST ¹ <u>SD</u> SEEEGDDQEGEK	<u>TGVT</u> <u>STSD</u> SEEEGDDQEGEK TGVT ¹ <u>STSD</u> SEEEGDDQEGEKKR TGVT ¹ <u>STSD</u> SEEEGDDQEGEK TGVTST ¹ <u>SD</u> SEEEGDDQEGEKK	<u>TGVT</u> <u>STSD</u> SEEEGDDQEGEK TGVT ¹ <u>STSD</u> SEEEGDDQEGEKKR TGVT ¹ <u>STSD</u> SEEEGDDQEGEK TGVTST ¹ <u>SD</u> SEEEGDDQEGEKK
425-448			NMFLVGE ¹ GDSVITQVLN ¹ KSLAEQR NMFLVGE ¹ GDSVITQVLN ¹ KSLAEQR
510-524	KKPSSEERE ¹ TEISLK		
518-530			ETEIS ¹ LKD ¹ STLDN ETEIS ¹ LKD ¹ STLDN ETEIS ¹ LKD ¹ STLDN

¹See supplemental References**SUPPLEMENTAL REFERENCES**

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