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# Supplemental Information

# JNK1 Phosphorylation of Cdt1 Inhibits Recruitment of HBO1 Histone Acetylase and Blocks Replication Licensing in Response to Stress

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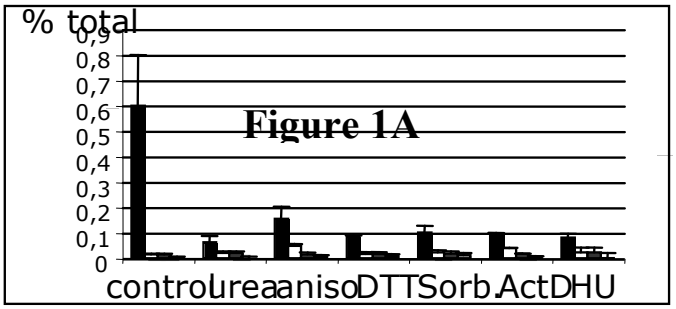
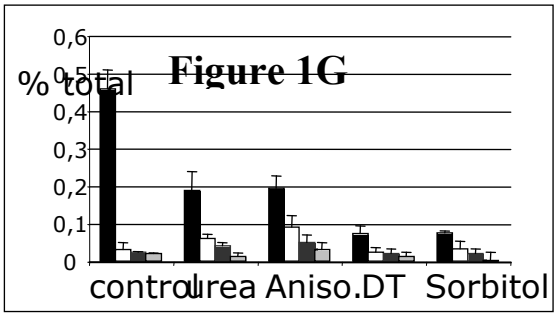
# Figure S1. Related to Figure 1

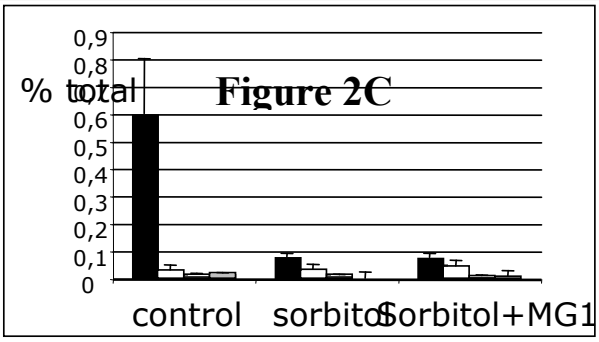
**Representation of HBO1 ChIP-qPCR data in percentage of total DNA**

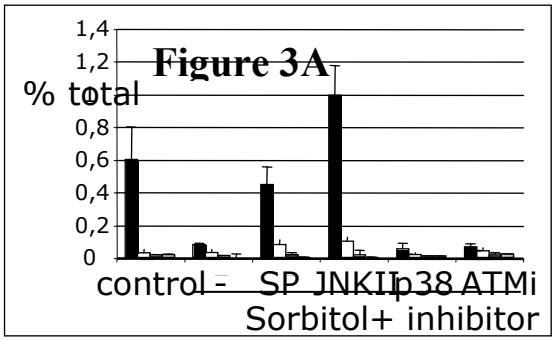
Title of each panel indicate the figure associated with these data. See figure legend for information regarding the samples. In each panel HBO1 ChIP and IgG control data are presented for the MCM4 replication origin and the GAPDH control region.

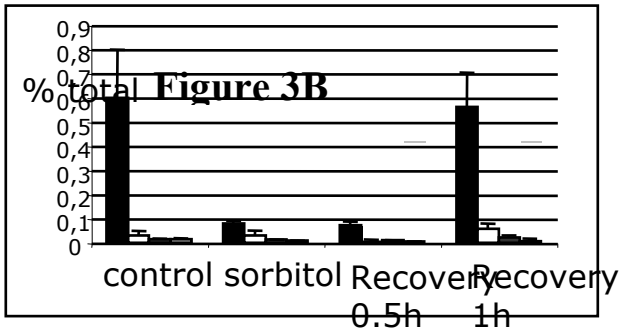
HBO1 ChIP/MCM4 origin
IgG/MCM4 origin
HBO1 ChIP/GAPDH
IgG/GAPDH

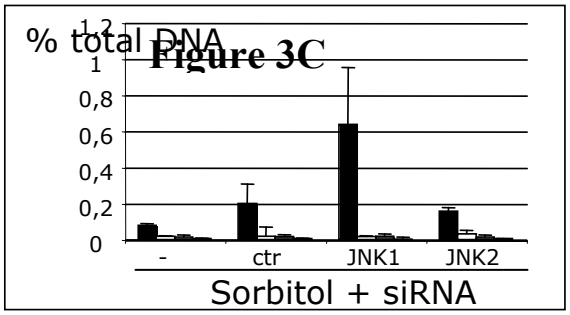


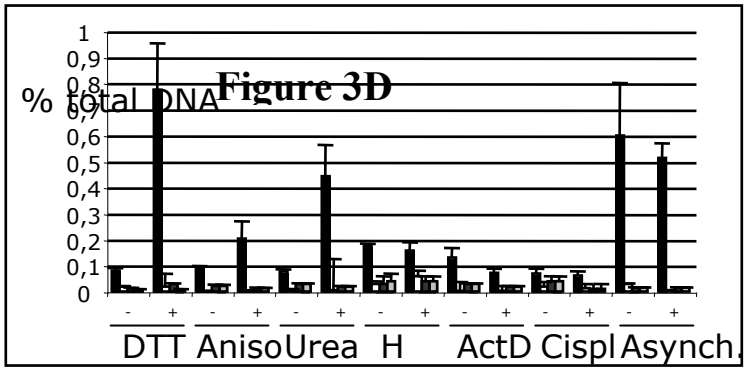



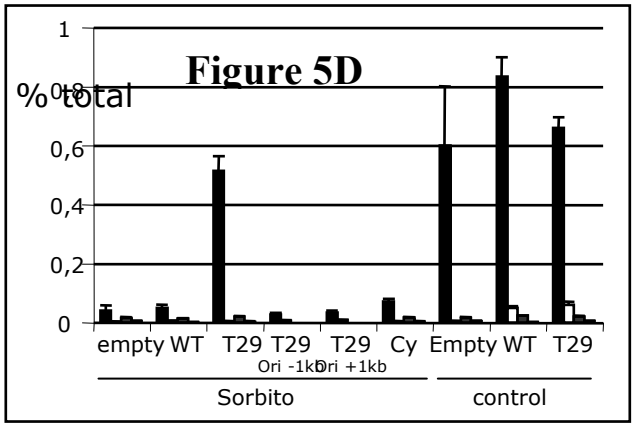



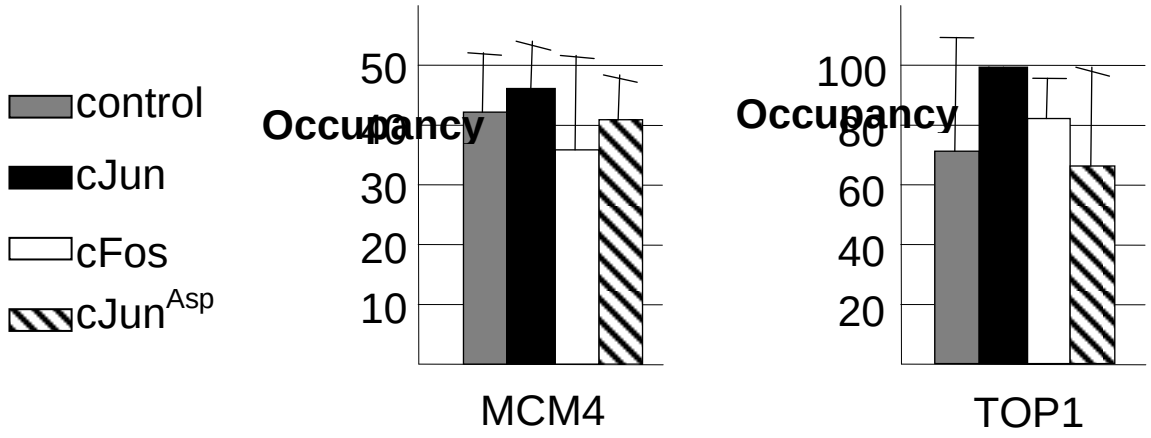



# Figure S2. Related to Figure 1

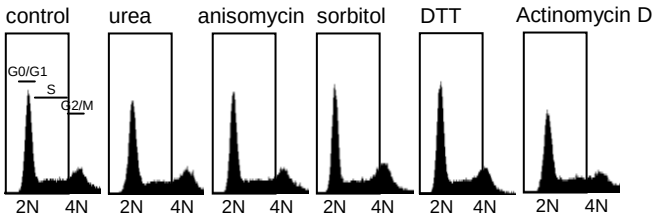
**A**




**Increasing cJun, cFos or a constitutive form of cJun in HeLa cells does not perturb HBO1 binding at origins**

HeLa cells in their exponential phase of growth were transfected with plasmids encoding cJun, cFos or a constitutive JNK-phosphorylated form of cJun. 48 hours after transfection, cells were harvested and cross-linked with formaldehyde. HBO1 binding at MCM4 and TOP1 origins was investigated by ChIP (n=3).

**B**

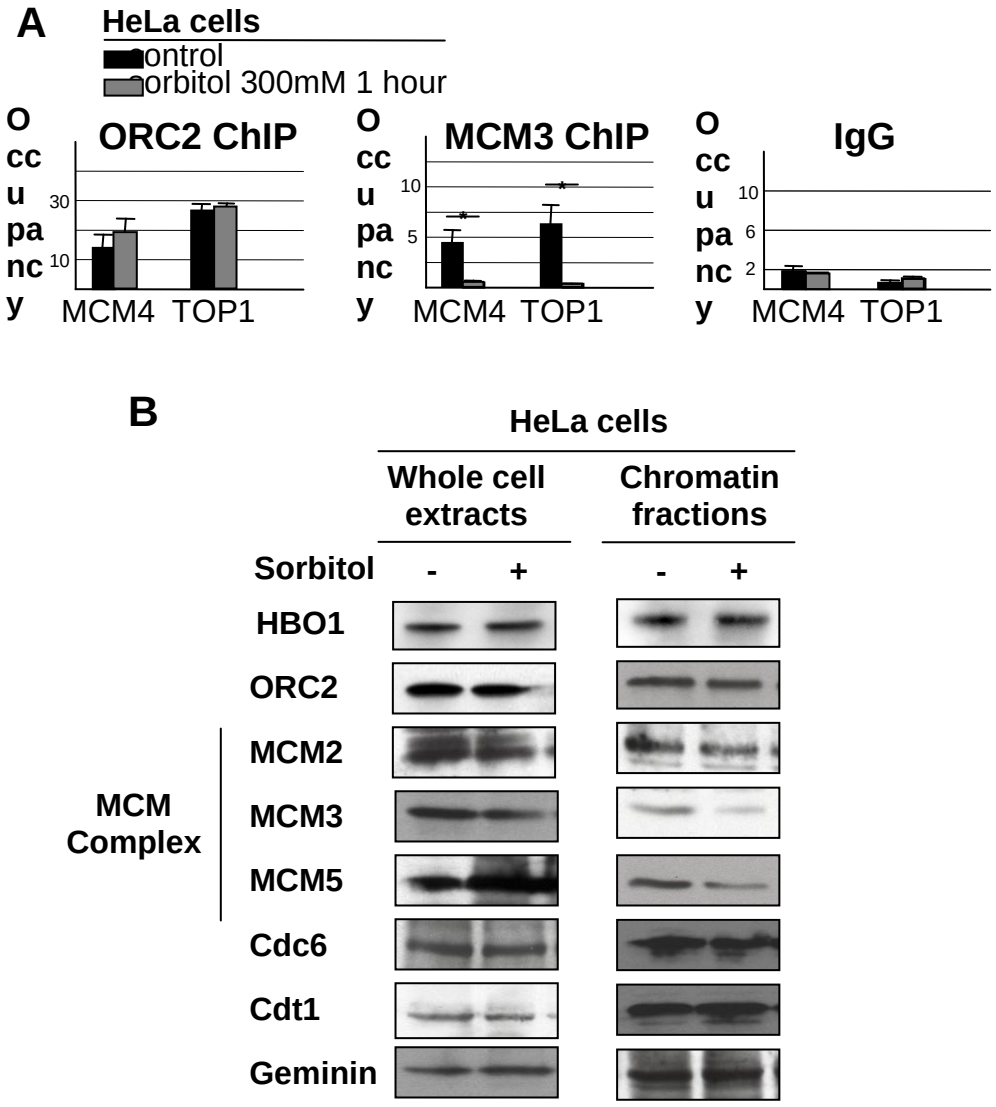



| | %G0/G1 | %S | %G2/M |
|---|---|---|---|
| control | 49.3 | 22.7 | 28 |
| urea | 49 | 24.5 | 26.5 |
| anisomycin | 48.7 | 23.1 | 28.2 |
| sorbitol | 48.2 | 23.1 | 28.7 |
| DTT | 51.3 | 23 | 25.7 |
| Actinomycin D | 51 | 24.7 | 24.3 |

**Cell cycle distribution of HeLa cells exposed to different stress agents**

HeLa cells in their exponential phase of growth were exposed to stress agents (in presence of serum). Cells were stained with 20μg/ml propidium iodide (Sigma) in presence of 50μg/ml of RNase H for 30 minutes at room temperature in the dark and extensively washed in PBS. $1x10^6$ cells were subsequently analyzed for each condition using a FACSCalibur flow cytometer and data were processed using the CellQuest software (Becton Dickinson).

# Figure S3. Related to Figure 1

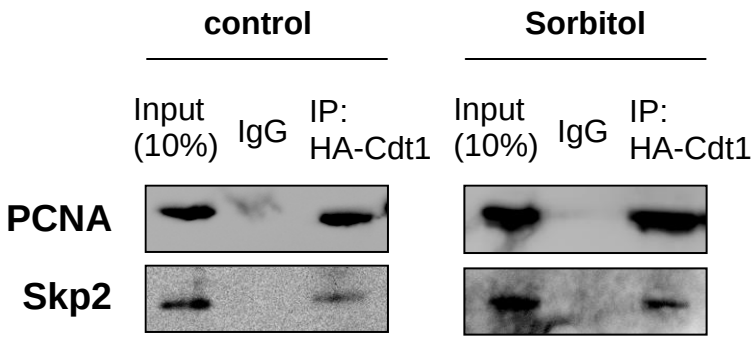



**DNA licensing defect in HeLa cells in hyper-osmotic condition**

(**A**) ChIP analysis of ORC2 and MCM3 binding at MCM4 and TOP1 replication origins in HeLa cells in their exponential phase of growth treated with 300mM sorbitol for 1 hour (n=3).

(**B**) Western blot analysis of DNA licensing factors expression and chromatin loading in HeLa cells treated with sorbitol or left untreated.

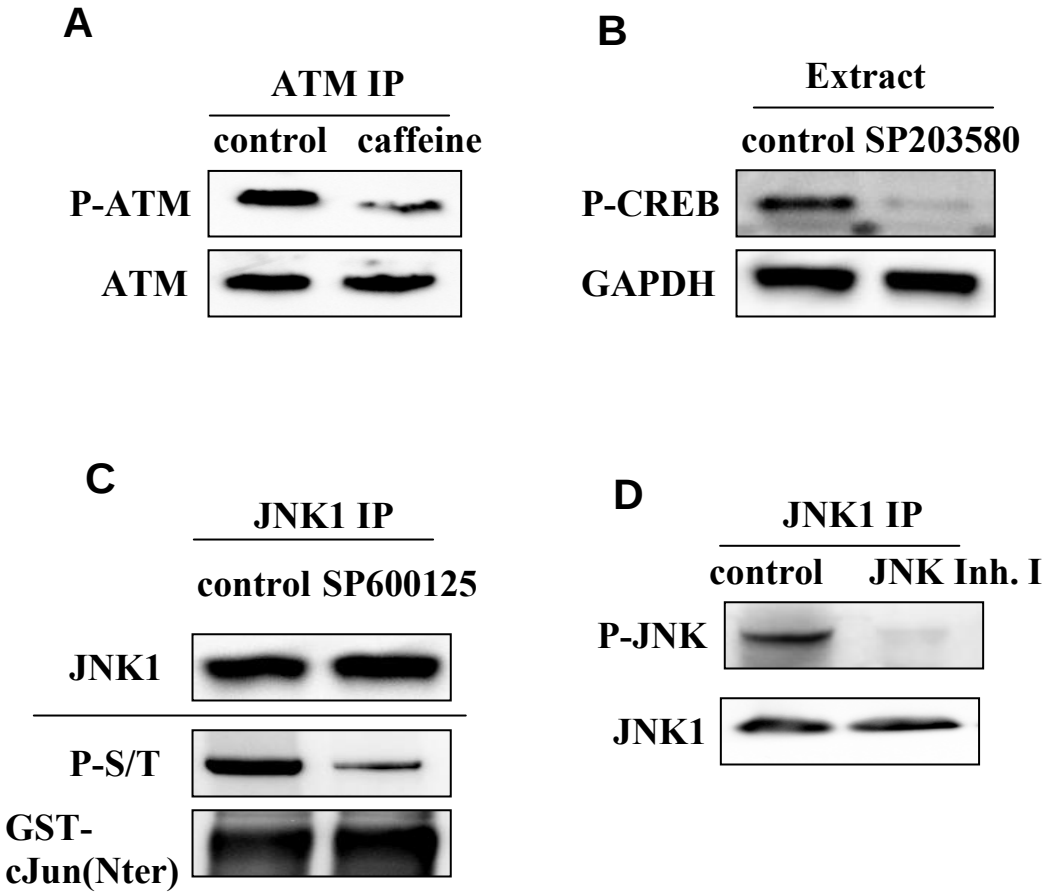
# Figure S4. Related to Figure 2




**Cdt1 interaction with PCNA and Skp2 is not promoted by hyper-osmotic shock**

Western blot analysis of HA-Cdt1 immunoprecipitates in control and sorbitol treated cells. Nuclear extracts from HeLa cells expressing HA-Cdt1 and treated with sorbitol or not were incubated with anti-HA resin. After extensive wash, Skp2 and PCNA presence in the immunoprecipitates was tested by Western blot using specific antibodies.

# Figure S5. Related to Figure 3




**Efficient inhibition of ATM, p38 and JNK activity in cells treated with specific inhibitors prior to sorbitol treatment**

HeLa cells in their exponential phase of growth were treated with ATM, JNK and p38 inhibitors, 30 minutes, prior to exposure to 300mM sorbitol for 1 hour. (**A**) ATM activity was monitored in cells by analysis of its phosphorylation level after immuno-precipitation.

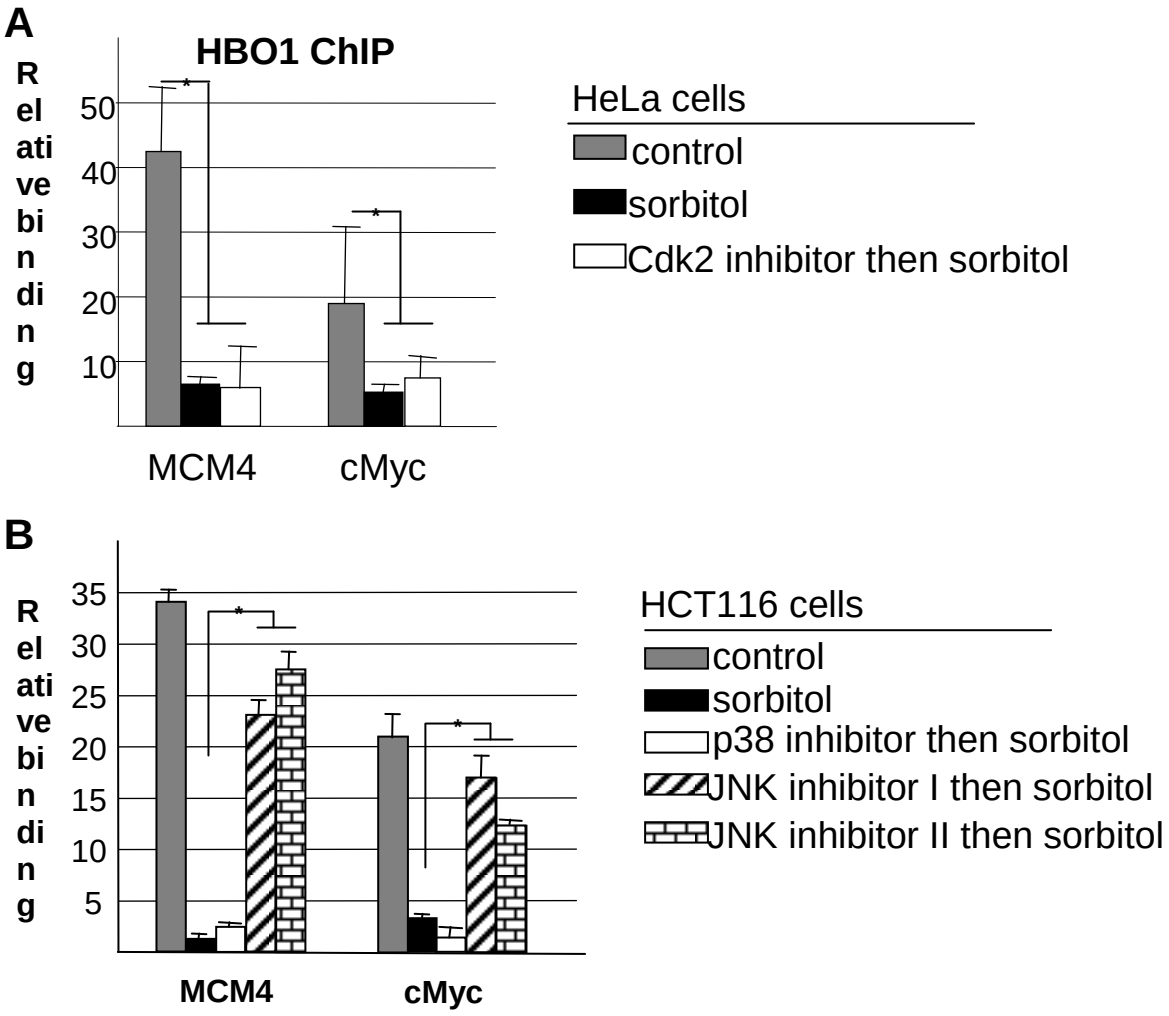
(**B**) p38 activity was assessed by quantifying the phosphorylation of its substrate CREB (Iordanov et al., 1997).

(**C**) JNK activity following SP600125 treatment was assessed by quantifying the phosphorylation of its substrate GST-cJun(Nter) in an IP-Kinase assay.

(**D**) JNK activity following JNK Inhibitor I treatment was monitored in cells by analysis of its phosphorylation level after immuno-precipitation.

The panels are representative of cell samples used in Figure 3A for chromatin immunoprecipitation analysis.

# Figure S6. Related to Figure 3



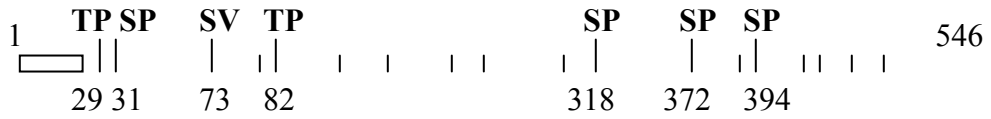

**Effect of Cdk2 kinase and p53 transcription factor on HBO1 binding at MCM4 origin**

(**A**) HeLa cells in their exponential phase of growth were treated with a Cdk2 kinase specific inhibitor (sc-221409; Santa Cruz), prior to exposure to sorbitol. HBO1 binding was analyzed by ChIP-qPCR at the MCM4 origin (n=3).

(**B**) HCT116 cells, p53 positive, in their exponential phase of growth were treated with a JNK or p38 kinase specific inhibitor prior to exposure to sorbitol. HBO1 binding was analyzed by ChIP-qPCR at the MCM4 origin (n=3).

# Figure S7. Related to Figure 5

**A**




**B**

MEQR**RVTDFF ARRRPGPPR**I APPK**LACRTP SPARPALR**AP ASATSGSRKR
**ARPPAAPGRD QARPPARR**RL R**LSVDEVSSP STPEAPDIPA CPSPGQKIKK**
STPAAGQPPH LTSAQDQDTI SELASCLQRA R**ELGARVR**AL KASAQDAGES
CTPEAEGR**PE EPCGEKAPAY QRFHALAQPG LPGLVLPYKY QVLAEMFR**SM
DTIVGMLHNR **SETPTFAKVQ RGVQDMMRRR FEECNVGQIK TVYPASYRFR**
QERSVPTFKD GTRRSDYQLT IEPLLEQEAD GAAPQLTASR **LLQRRQIFSQ**
**KLVEHVKEHH KAFLASLSPA MVVPEDQLTR** WHPRFNVDEV PDIEPAALPQ
PPATEK**LTTA QEVLARARNL ISPRMEKALS QLALRSAAPS SPGSPR**PALP
ATPPATPPAA SPSALKGVSQ DLLER**IRAKE AQKQLAQMTR** CPEQEQRLQR
LERLPELARV LRSVFVSERK PALSMEVACA RMVGSCCTIM SPGEMEKHLL
LLSELLPDWL SLHR**IRTDTY VKLDKAADLA HITARLAHQT** RAEEGL

**C**

MEQR**RVTDFF ARRR**PGPPRI APPK**LACRTP SPARPALR**AP ASATSGSRKR
ARPPAAPGRD QARPPARRRL R**LSVDEVSSP STPEAPDIPA CPSPGQKIKK**
STPAAGQPPH LTSAQDQDTI SELASCLQRA R**ELGARVR**AL KASAQDAGES
CTPEAEGR**PE EPCGEKAPAY QRFHALAQPG LPGLVLPYKY QVLAEMFR**SM
DTIVGMLHNR **SETPTFAKVQ RGVQDMMRRR** FEECNVGQIK TVYPASYRFR
QERSVPTFKD GTRRSDYQLT IEPLLEQEAD GAAPQLTASR LLQRRQIFSQ
KLVEHVKEHH K**AFLASLSPA MVVPEDQLTR** WHPRFNVDEV PDIEPAALPQ
PPATEKLTTA QEVLARARNL ISPRMEK**ALS QLALRSAAPS SPGSPR**PALP
ATPPATPPAA SPSALKGVSQ DLLER**IRAKE AQKQLAQMTR** CPEQEQRLQR
LERLPELARV LRSVFVSERK PALSMEVACA RMVGSCCTIM SPGEMEKHLL
LLSELLPDWL SLHR**IRTDTY VKLDKAADLA HITAR**LAHQT RAEEGL

**Threonine 29 is a JNK- and stress-regulated phosphorylation site**

(**A**) Summary of previously described phosphorylation sites onto Cdt1 (Takeda et al., 2008; Chen et al., 2009; Beausoleil et al., 2004; Dephourne et al., 2008). The following residues of each phosphorylation site is also indicated. Single bars represent additional SP and TP motif, matching the JNK consensus target site.

(**B**) Phosphopeptide analysis of His-Cdt1 incubated with JNK1 in vitro. His-Cdt1 incubated with bulk IP (IgG) was used as a control for non-phospho-sites. Residues recovered during the mass-spectrometry analysis in both samples are indicated in bold and the phospho-sites underlined and in red.

(**C**) Phosphopeptide analysis of Flag-Cdt1 immunoprecipitated from HeLa cells incubated with sorbitol, sorbitol and JNK inhibitor I or left untreated. Residues recovered during the mass-spectrometry analysis in the three samples are indicated in bold. Phospho-sites are underlined and JNK-dependent phospho-site appear in red.