Astuti et al., Supplementary Figures

Figure S1: Over expression of cdc25B overcomes the TPA induced G2 delay .

HeLa cells arrested at G2 by etoposide were transfected with vector control and myc-cdc25B3. TPA was added 3h post transfection and the cells were harvested at the indicated times post transfection and probed with pMEKT286 as a marker of mitotic entry.

Figure S2: Gö6976 overcomes the TPA induced G2 delay

Thymidine synchronised HeLa cells were treated either without (diamonds) or with TPA (squares) and the PKC inhibitor Gö6976 (triangles) at 7 h after synchrony release. Cells were followed by time lapse microscopy and cumulative mitotic index determined.

Figure S3: TPA does not induced DNA damage

HeLa cells, either asynchronously growing or synchronised in G2 phase were treated with TPA for 2 h, or etoposide (Etop) overnight as a positive control for DNA damage. Whole cell lysates were immunoblotted for γH2Ax, a marker of DNA damage. MEK1 is a loading control.

Figure S4: Chk2 is not required for the TPA induced G2 phase delay.

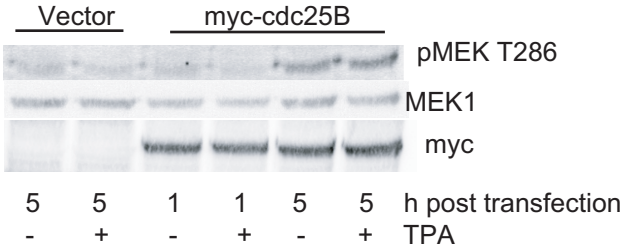
Asynchronous HeLa cells were transfected with Chk2 directed (triangles), or scrambled control (circles) siRNA for 24 h, treated with (open symbols) or without (closed symbols)

TPA then followed by time lapse microscopy and the cumulative mitotic indexed assessed. The degree of knockdown of each Chk2 is shown. MEK1 is a loading control.

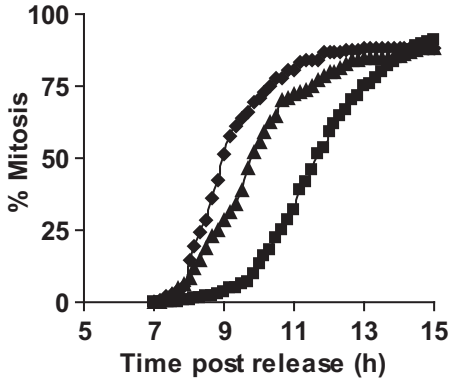
Figure S5: TPA induced G2 delay is independent of p21 expression.

HeLa were transfected with p21 (open symbols) or scrambled (closed symbols) siRNA, synchronised then treated without (diamonds) or with TPA (triangles) at 7 h or the histone deacetylase inhibitor suberic bishydroxamic acid (SBHA, 100 μg/ml; circles) at 1 h after synchrony release. The cells were followed by time lapse microscopy and the cumulative mitotic index assessed. The level of p21 knockdown is shown. α-Tubulin is shown as a loading control.

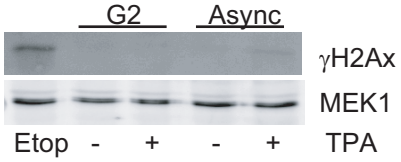
S1


Vector
myc-cdc25B
pMEK T286
MEK1
myc
5 5 1 1 5 5 h post transfection
- + - + - + TPA

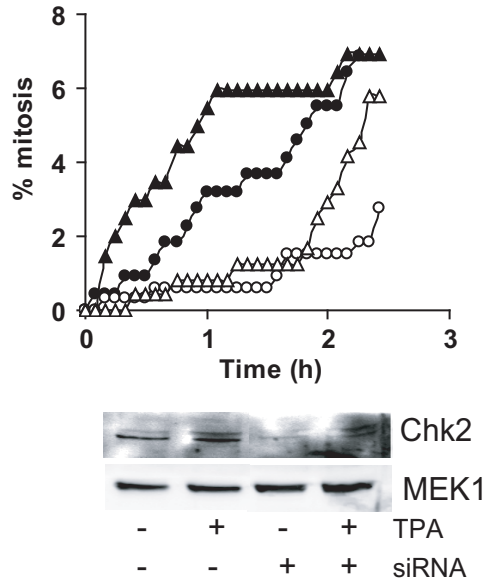
S2


100
75
50
25
0
% Mitosis
5 7 9 11 13 15
Time post release (h)

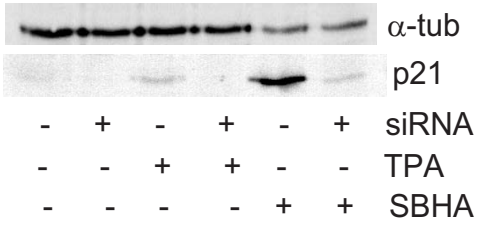
S3

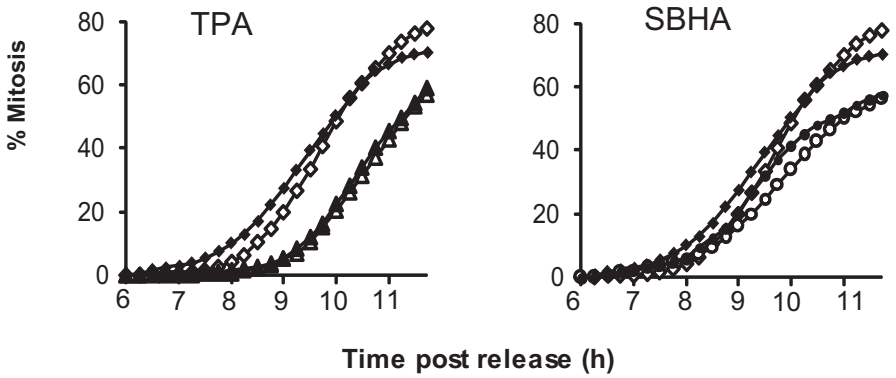

G2
Async
γH2Ax
MEK1
Etop - + - + TPA

S4


% mitosis
Time (h)
Chk2
MEK1
- + - + TPA
- - + + siRNA

S5


α-tub
p21
- + - + - + siRNA
- - + + - - TPA
- - - - + + SBHA


% Mitosis
TPA
SBHA
Time post release (h)