Supplementary Figures

Fig. S1. Cycling properties of H358 cells after HU release. H358 cells were synchronized with HU as described in "Materials and Methods". HU was removed by extensive washing, and cell cycle profiles were determined by flow cytometry at the indicated times. *A.* Cell cycle distribution after HU release. Data are expressed as mean ± S.D. (n=3). Three additional experiments gave similar results. *B.* Schematic representation of the protocol used in most studies. PMA was added in most cases 2 h after HU release (t=2 h), which corresponds to late G1-early S phase.

Figure S2. $p21_{Cip1}$ or PKCα RNAi does not affect H358 cell synchronization with HU. H358 cells were transfected with $p21_{Cip1}$, PKCα or control RNAi duplexes, and 24 h later subject to synchronization with HU. Cell cycle distribution was determined by flow cytometry at the end of the synchronization period (t=0 h). Data are expressed as mean ± S.D. Similar results were observed in two additional experiments.

Figure S3. Expression and translocation of PKCs in H358 cells during S phase. H358 cells were synchronized with HU, released by extensive washing, and then treated for 30 min with 100 nM PMA, 2 h after HU release. Cell lysates were prepared at different times after PMA treatment. *A.* Expression of PMA-responsive PKC isozymes at different times after HU release. *B.* Translocation of PKC isozymes by PMA from soluble (cytosolic) to particulate fractions.

Figure S4. PKCα over-expression has no effect on cell cycle. H358 cells were infected with either PKCα or LacZ (control) AdVs (MOI = 100 pfu/cell), and 24 h later synchronized with HU. Cell cycle distribution was determined by flow cytometry at the end of the synchronization period (t=0 h). Data are expressed as mean ± S.D. Similar results were observed in two additional experiments.

Figure S1

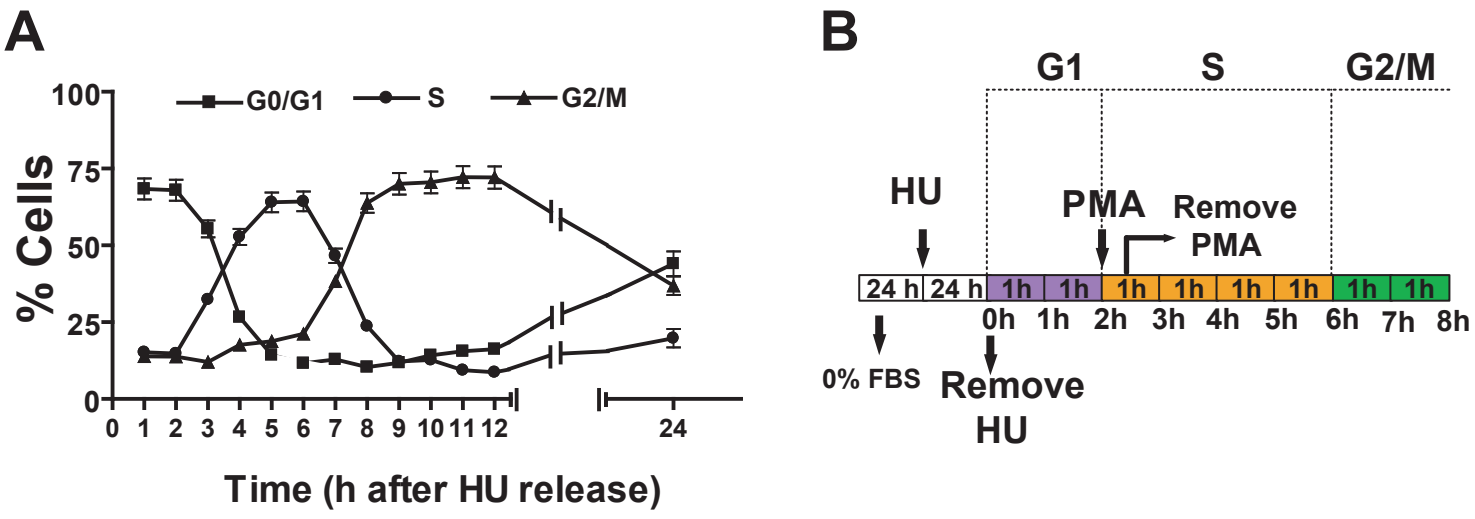
A
100
75
50
25
0
% Cells
G0/G1
S
G2/M
0 1 2 3 4 5 6 7 8 9 10 11 12
24
Time (h after HU release)
B
G1
S
G2/M
HU
PMA
Remove
PMA
24 h
24 h
1h
1h
1h
1h
1h
1h
1h
1h
0h 1h 2h 3h 4h 5h 6h 7h 8h
0% FBS
Remove
HU

Figure S2

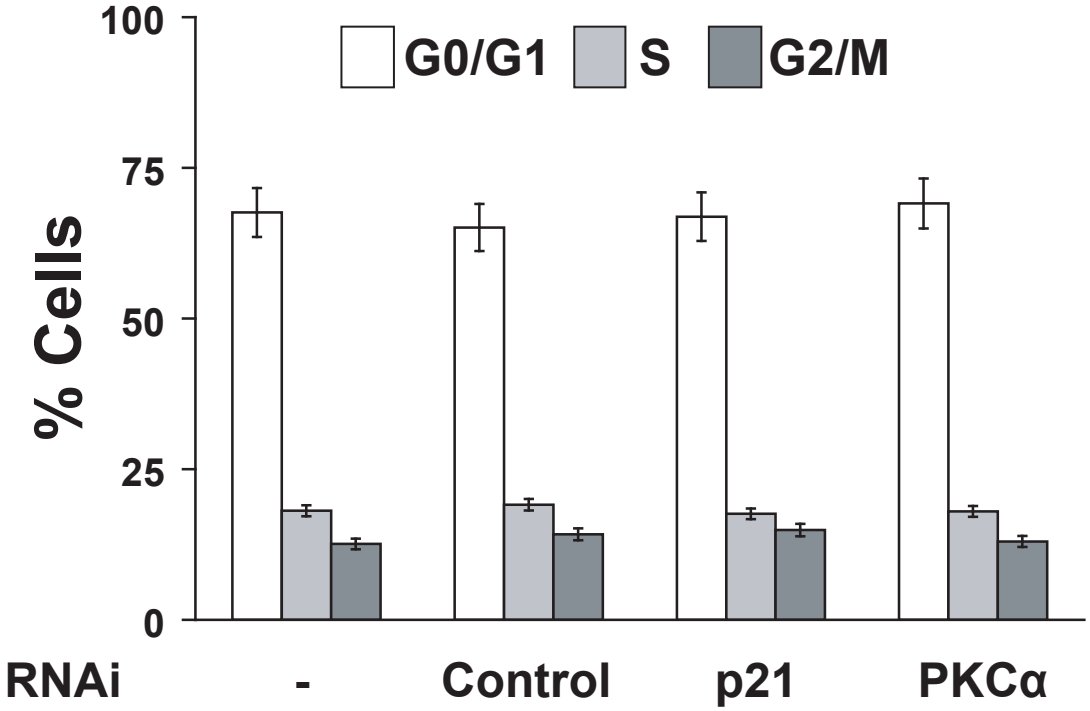

Figure S3

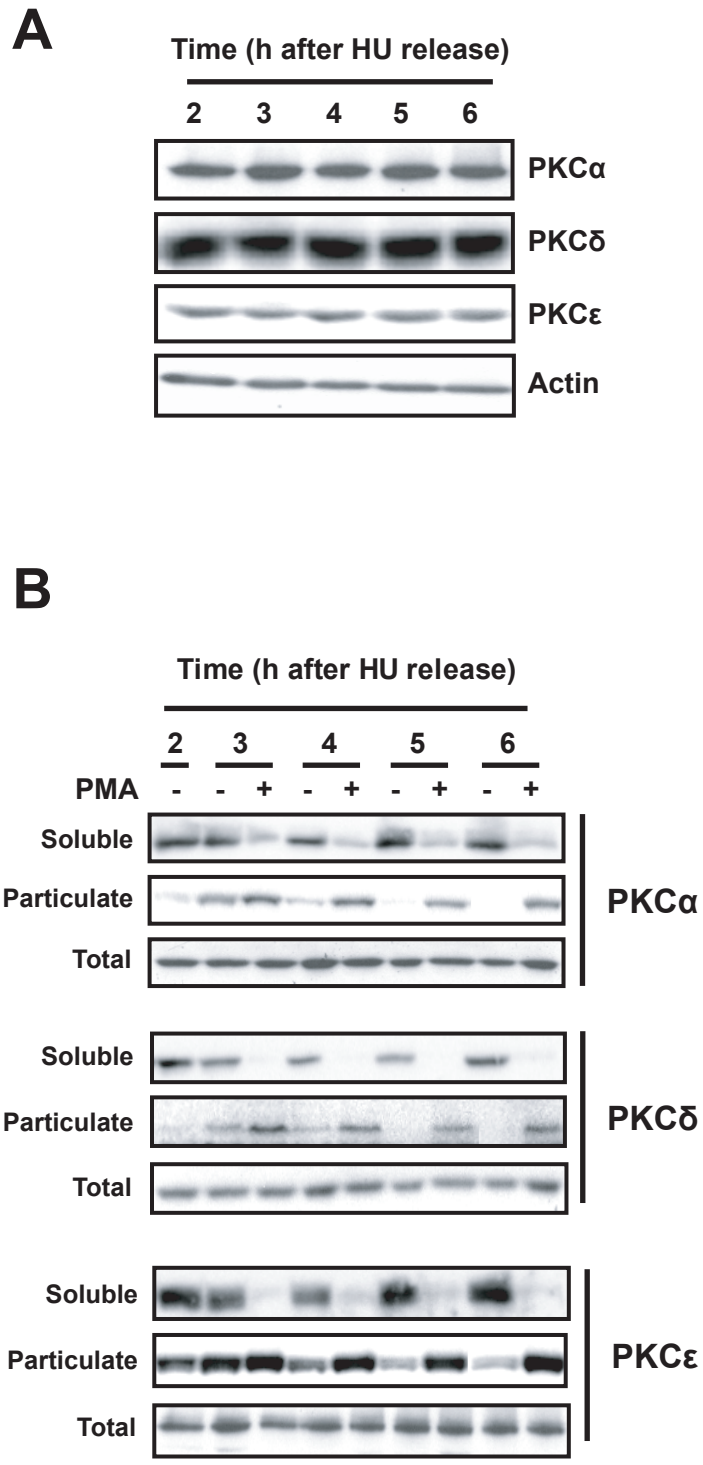
A
Time (h after HU release)
2 3 4 5 6
PKCα
PKCδ
PKCε
Actin
B
Time (h after HU release)
2 3 4 5 6
PMA - - + - + - + - +
Soluble
Particulate
Total
PKCα
Soluble
Particulate
Total
PKCδ
Soluble
Particulate
Total
PKCε

Figure S4