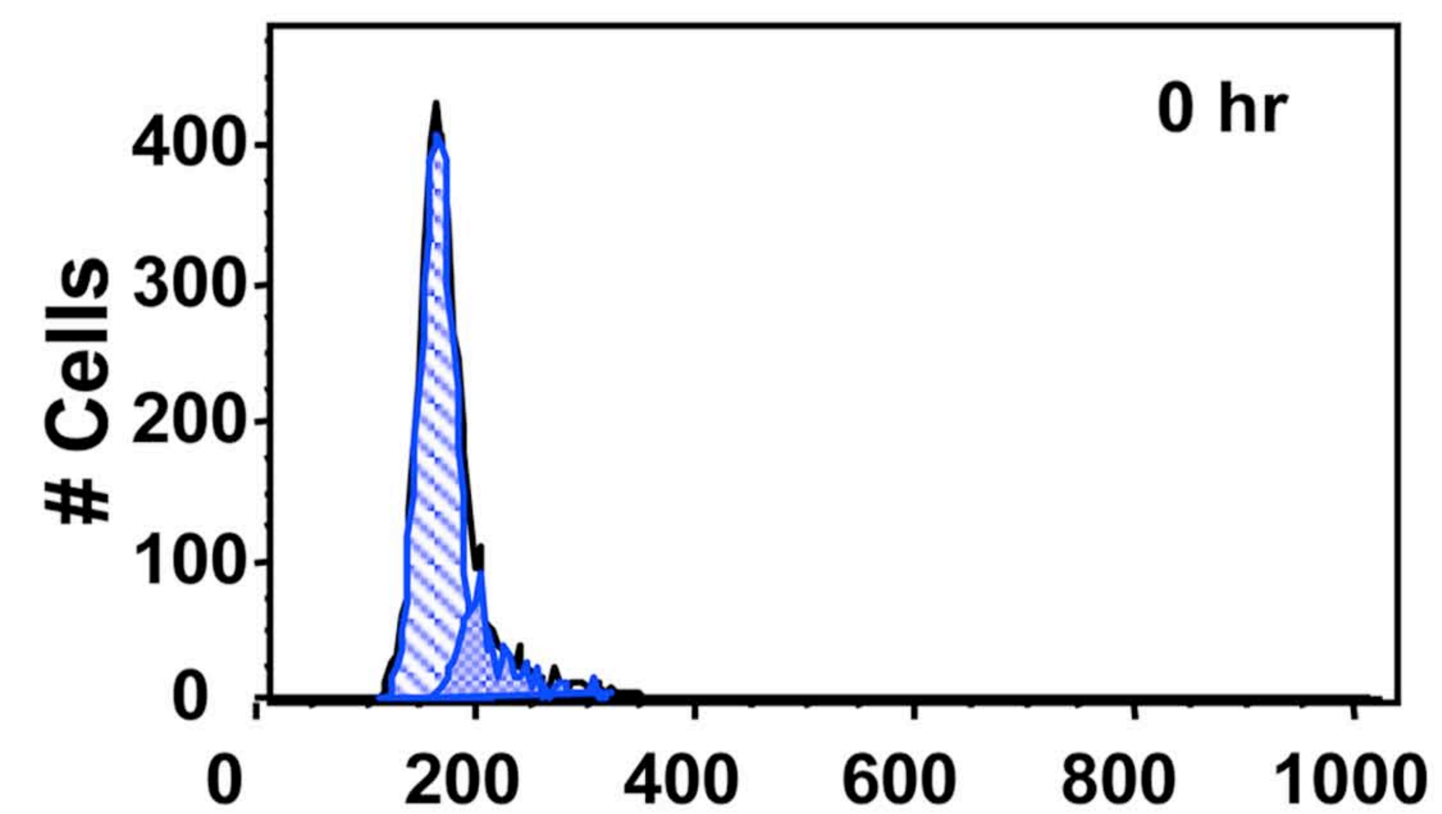

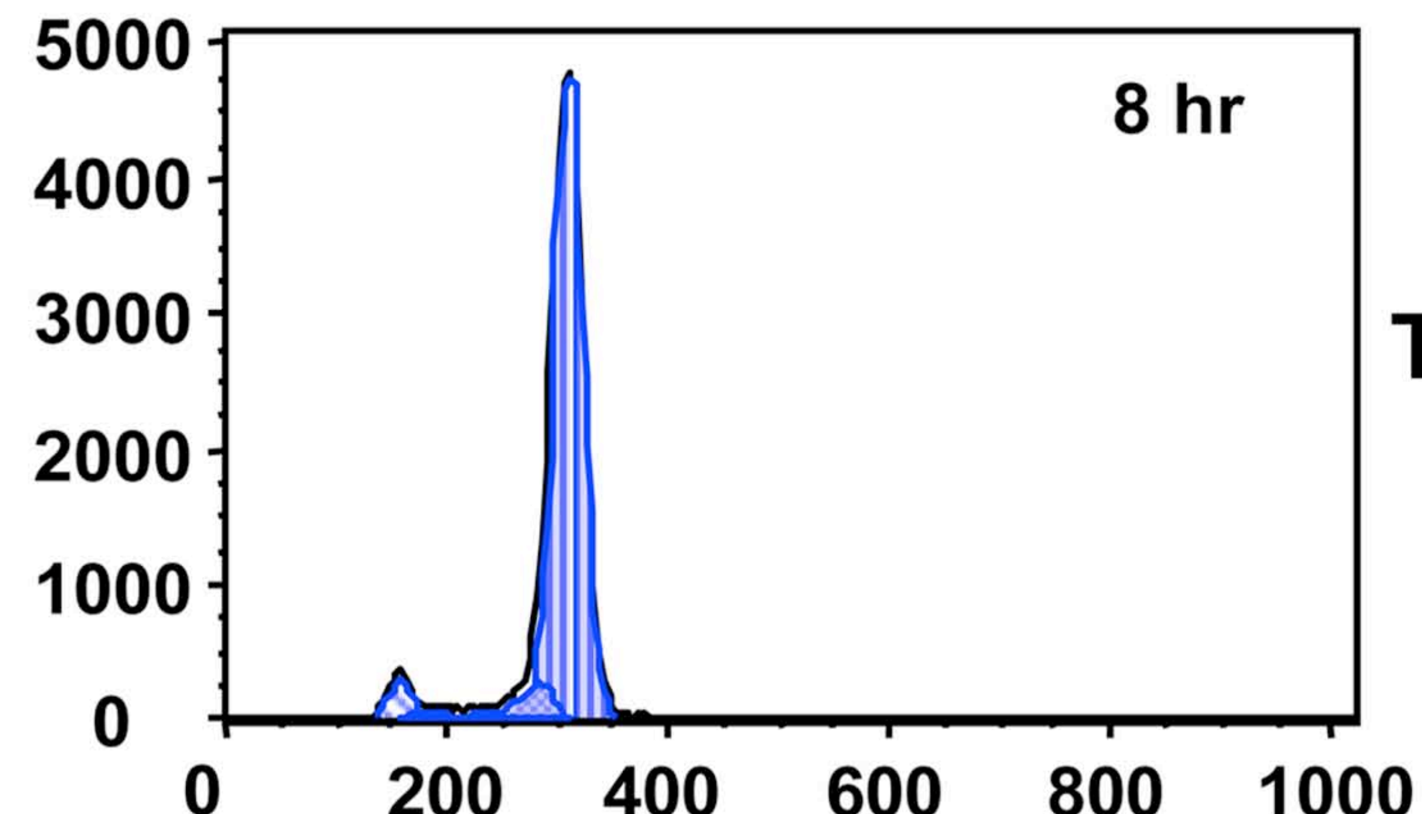

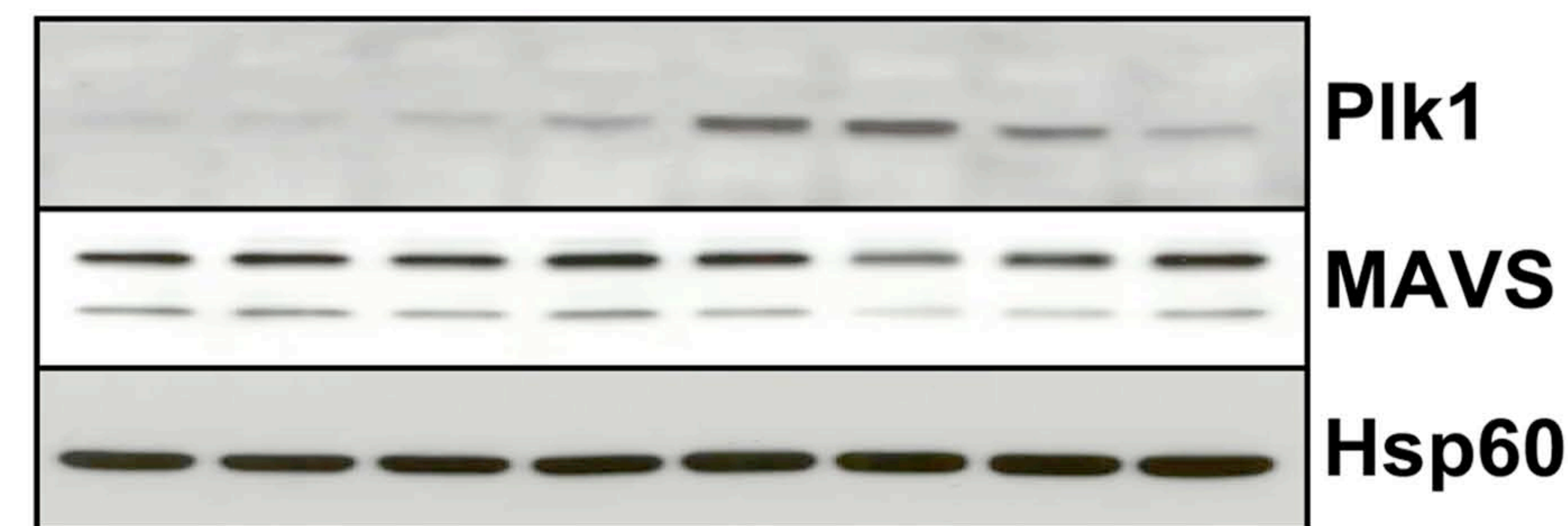

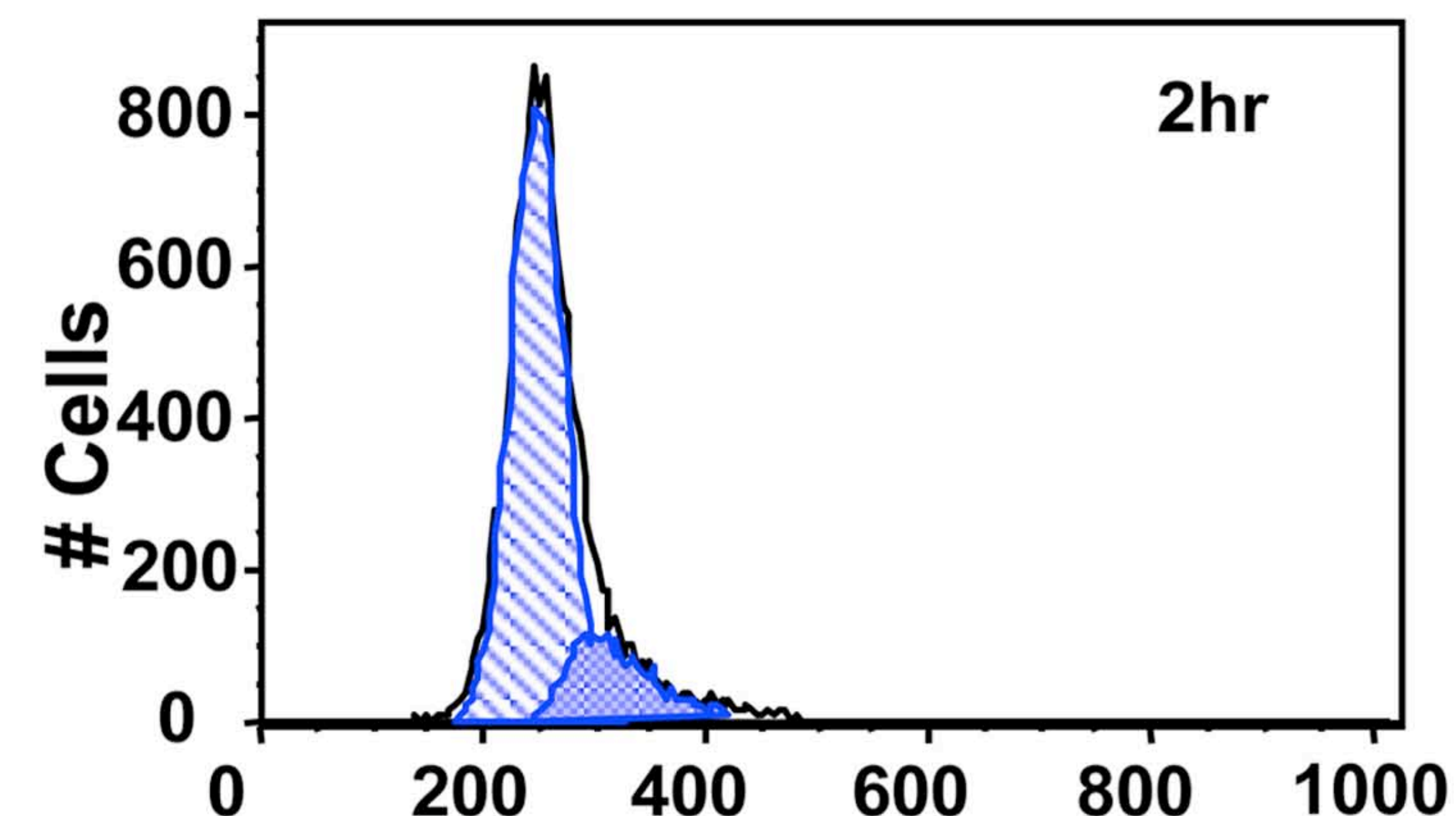

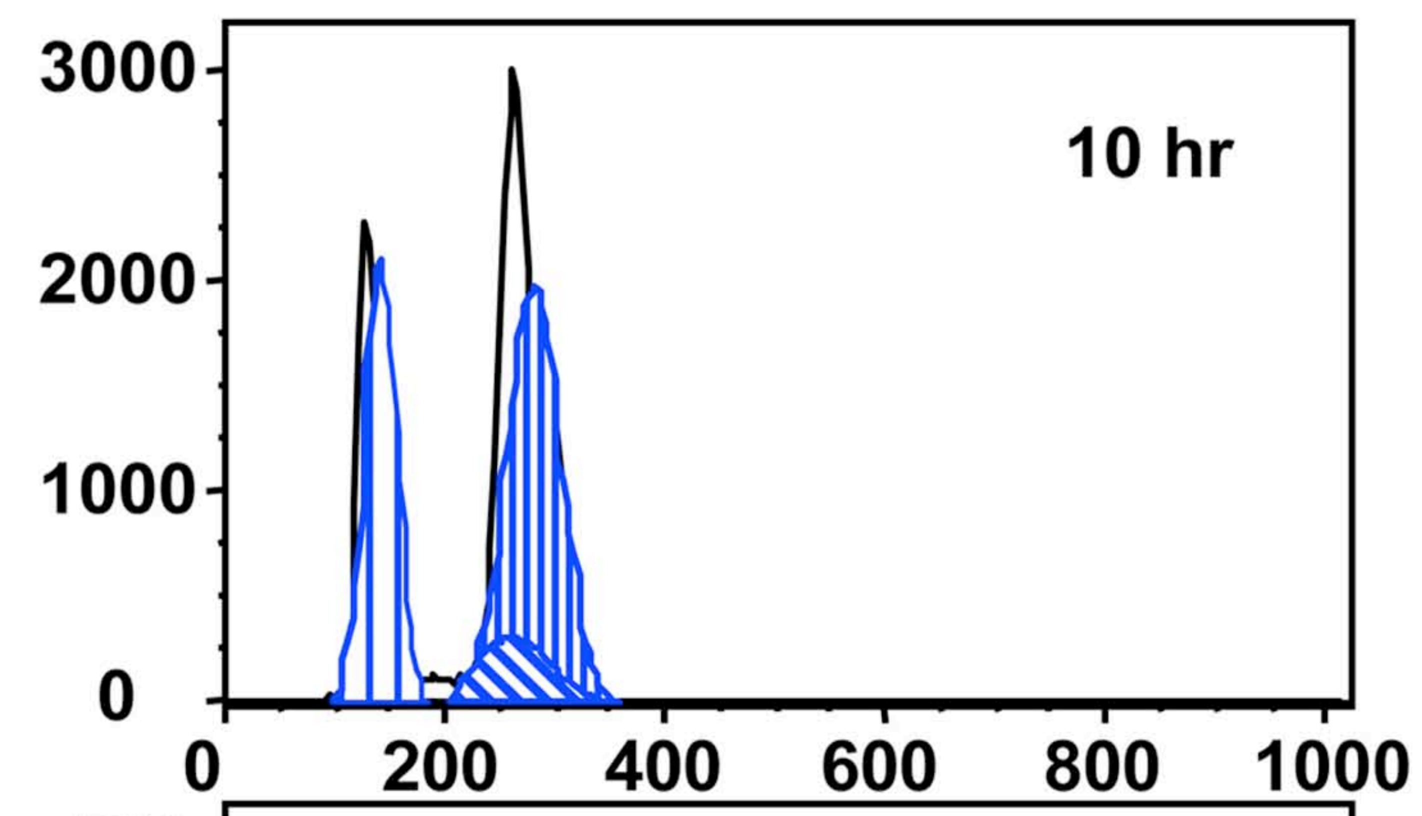

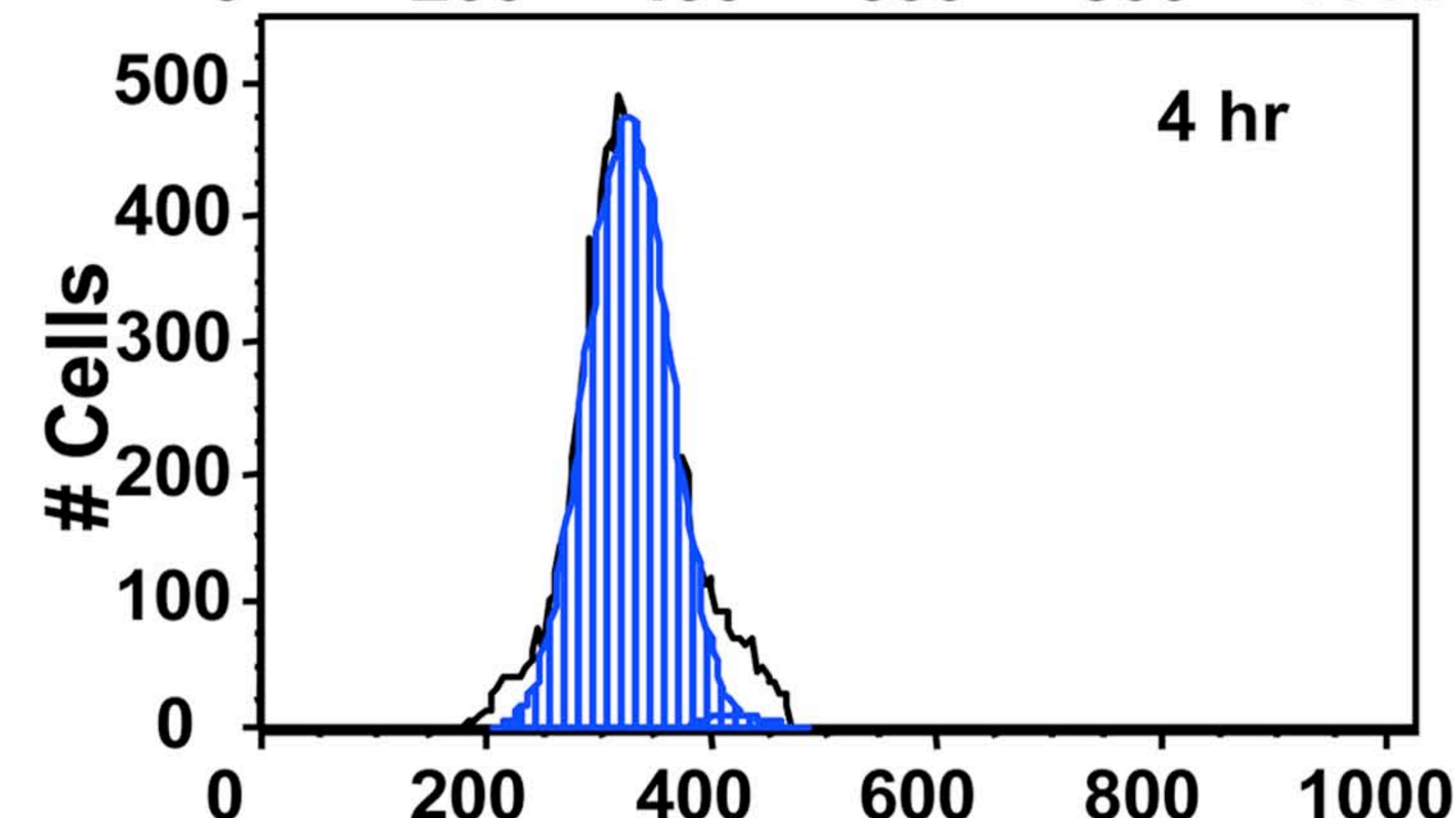

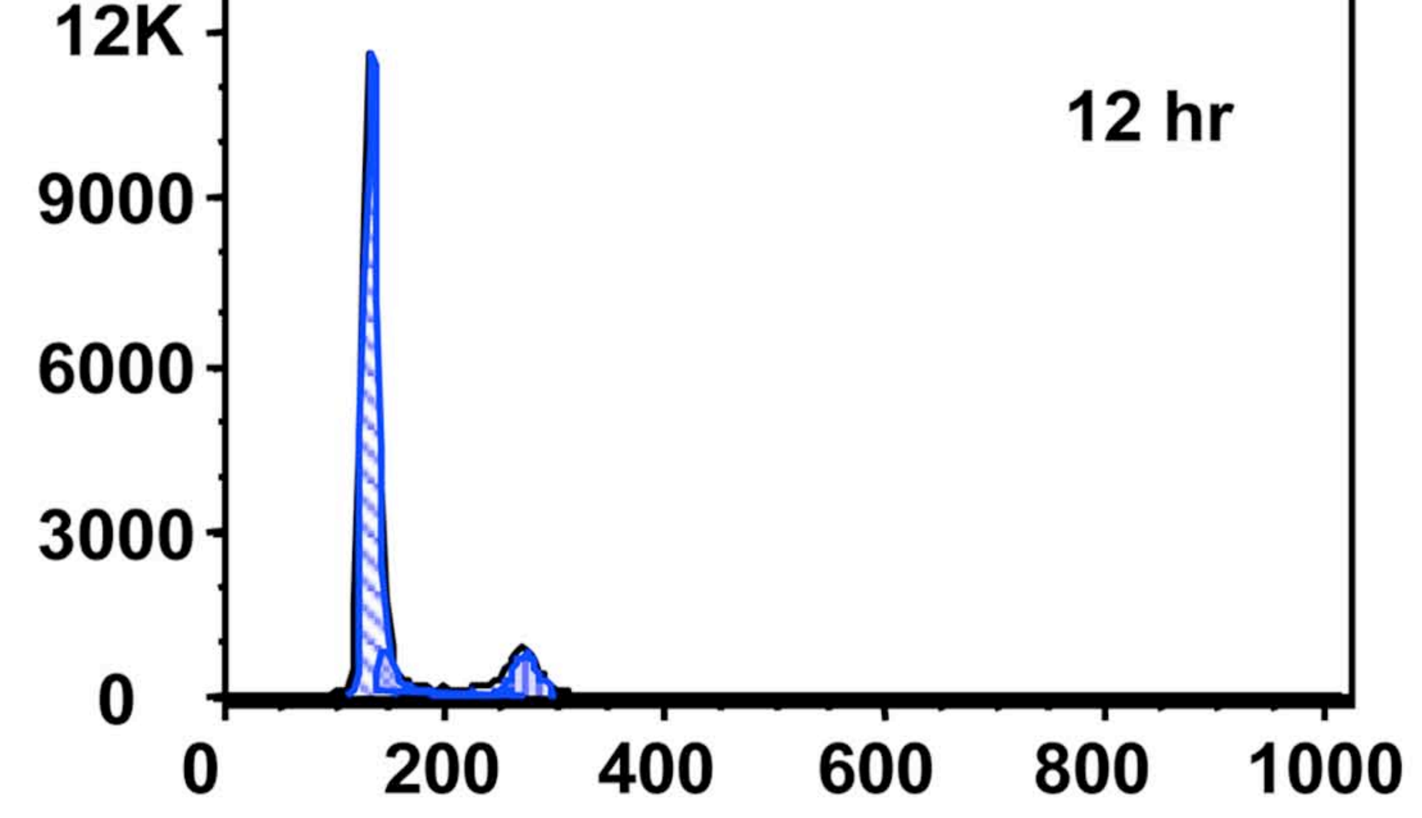

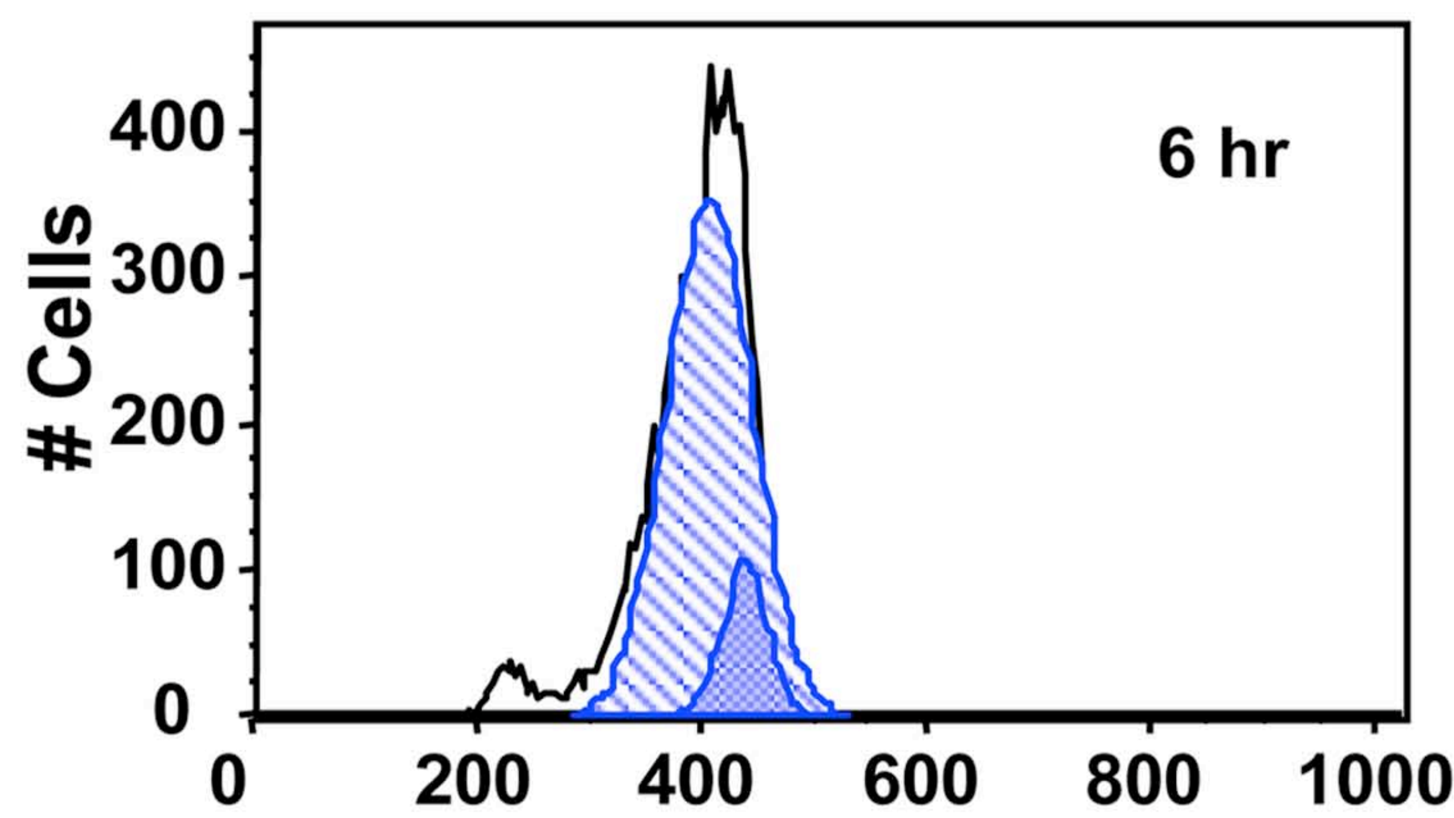

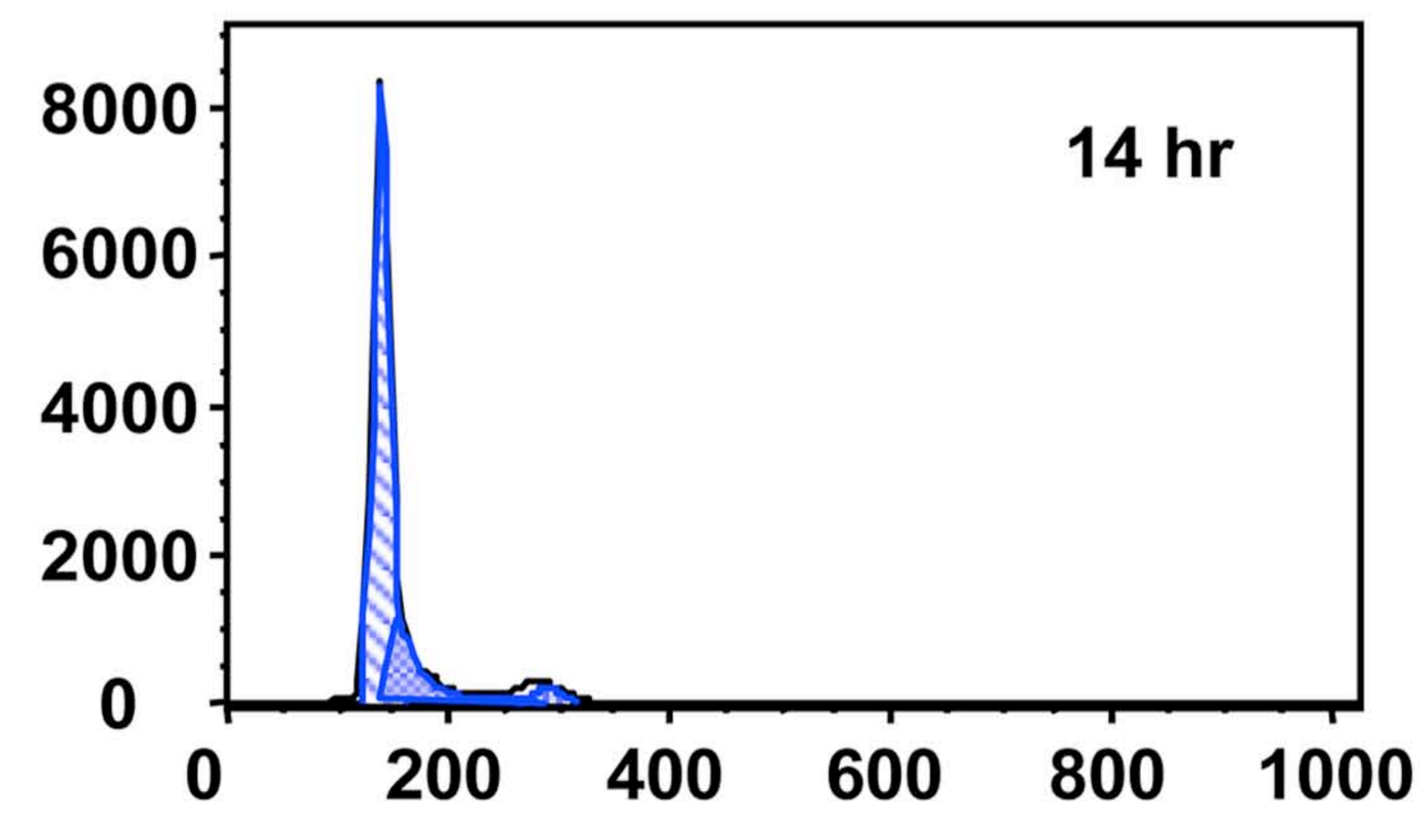

FL2-A: DNA-Area

% cells in	G1	S	G2
0 hr	78.2	19.3	0.0
2 hr	77.7	17.7	0.0
4 hr	92.5	0.91	0.0
6 hr	88.0	13.9	0.0
8 hr	4.2	9.4	85.3
10 hr	36.3	9.1	58.5
12 hr	74.3	12.7	10.6
14 hr	70.1	22.4	4.71

```
1   MPFAEDKTYKYICRNFSNFCNVDVVEILPYLPCLTARDQDRPRATCTLSG 50
51  NRDTLWHLFNTLQRRPGWVEYFIAALRGCELVDLADEVASVYQSYQPRTS 100
101 DRPPDPLEPPSLPAERPGPPTPAAAHSIPYNSCREKEPSYPMPVQETQAP 150
151 ESPGENSEQALQTLSPRAIPRNPDGGPLESSSDLAALSPLTSSGHQEQDT 200
201 ELGSTHTAGATSSLTPSRGPVSPSVSFQPLARSTPRASRLPGPTGSVVST 250
251 GTSFSSSSPGLASAGAAEGKQGAESDQAEPIICSSGAEAPANSLPSKVPT 300
301 TLMPVNTVALKVPANPASVSTVPSKLPTSSKPPGAVPSNALTNPAPSKLP 350
351 INSTRVGMVPSKVPTSMVLTKVPASTVPTDGSSRNEETPAAPTPAGATGG 400
401 SSAWLDSSSENRGLGSELSKPGVLASQVDSPFSGCFEDLAISASTSLGMG 450
451 PCHGPEENEYKSEGTFGIH VAENPSIQLLEGNPGPPADPDGGPRPQADRK 500
501 FQEREVPCHRPSPGALWLQVAVTGVLVVTLLVVLYRRRLH 540
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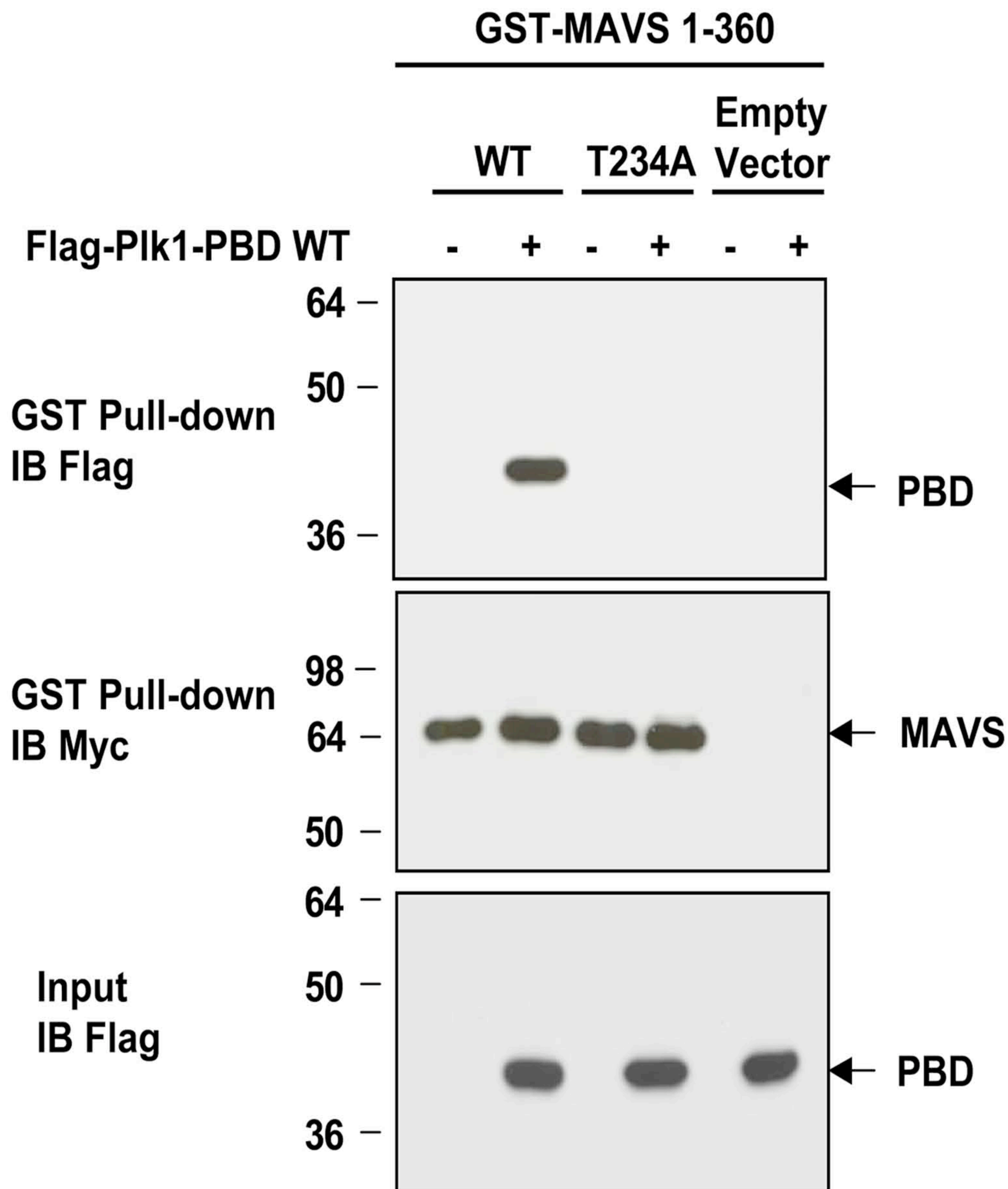
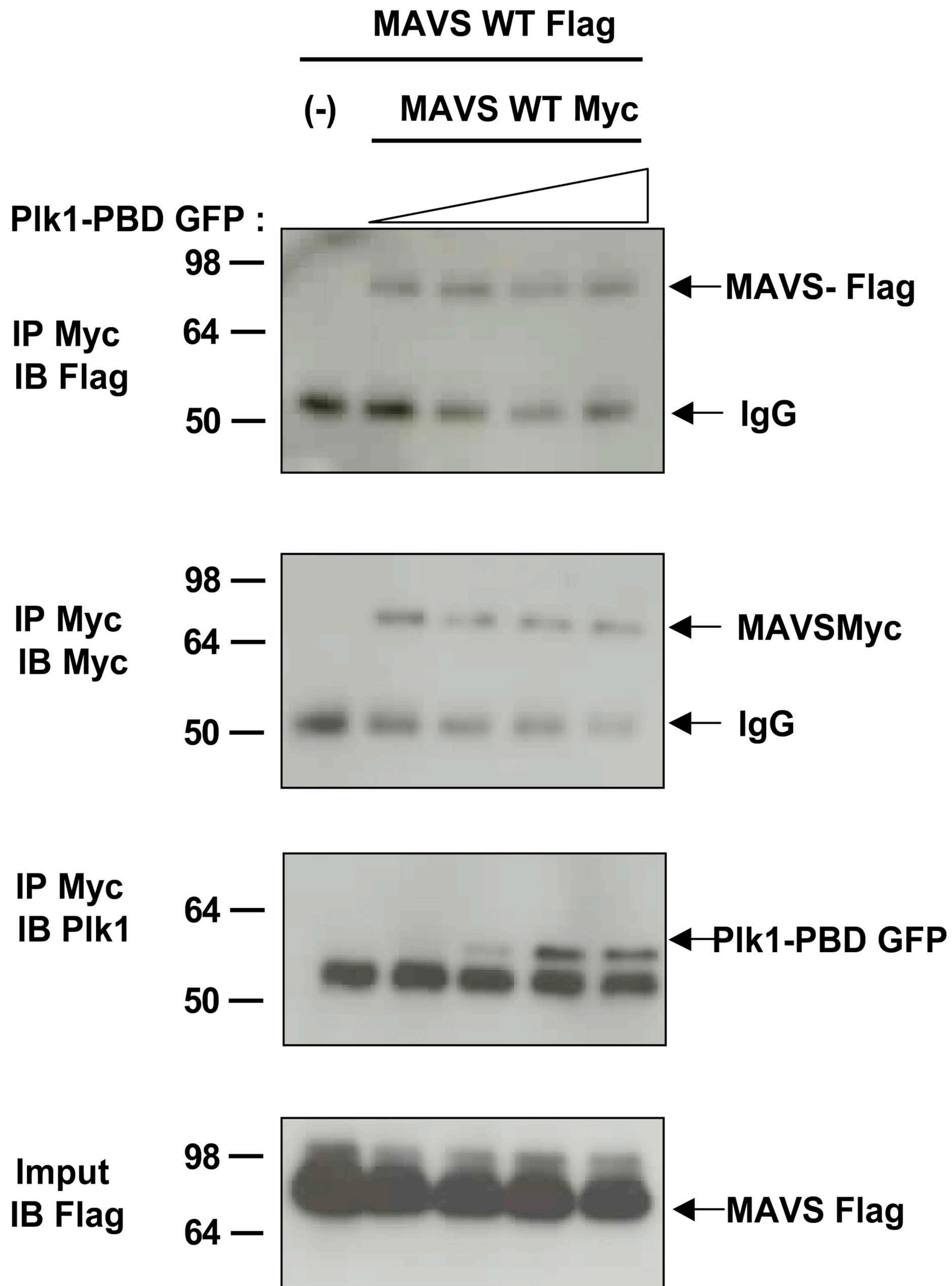


GST-MAVS 1-360
WT
T234A
Empty Vector
Flag-Plk1-PBD WT
- + - + - +
64
50
36
GST Pull-down
IB Flag
PBD
98
64
50
GST Pull-down
IB Myc
MAVS
64
50
36
Input
IB Flag
PBD


MAVS WT Flag
(-)
MAVS WT Myc
Plk1-PBD GFP :
98
64
50
IP Myc
IB Flag
MAVS- Flag
IgG
98
64
50
IP Myc
IB Myc
MAVSMyc
IgG
IP Myc
IB Plk1
64
50
Plk1-PBD GFP
Imput
IB Flag
98
64
MAVS Flag

SUPPLEMENTARY INFORMATION

Supplementary Fig 1. Respective expression of PLK1 and MAVS as function of the cell cycle.
Hela cells were synchronized by double thymidine block as described (58).The medium was removed and cells were washed three times in growth medium. At different times after thymidine release, cells were trypsinized and fixed in ethanol for FACS analysis to study DNA content, and protein extracts were prepared in RIPA buffer (10mM Tris-HCl pH 7.4, 150mM NaCl, 5mM EDTA, 0.1% SDS, 0.5% sodium deoxycholate, 1% NP-40) for immunoblot analysis of PLK1, MAVS and Hsp60 (as control of mitochondria expression). The percentage of cells in the different phases of cell cycle are listed in a table.

Supplementary Fig 2. MAVS amino acid sequence.
The domains 1-157 and 470-540 are represented in grey and correspond to regions that do not bind PLK1. In the 157-470 domain, the 180-280 sequence that specifically recruits the phosphopeptide binding site of PLK1 is framed, as well as the STP motif containing the T234 residue.

Supplementary Fig 3. Requirement of MAVS T234 for specific binding to PLK1-PBD.
HEK 293T cells were transfected with pFLAG-PLK1-PBD WT in the presence of pcDNA3.1(+) (Empty vector) or pDEST-GST-MAVS1-360, in which the sequence was either unmodified (WT) or carrying the mutation T234A. 24 hrs after transfection, the cells were lysed in buffer B minus glycerol and submitted to GST pull-down of the GST-MAVS constructs. The presence of PLK1-PBD and MAVS retained in the GST pull-down and expression of PLK1 in the total cell extracts was revealed by immunoblot using anti-FLAG and anti-MAVS antibodies.

Supplementary Fig 4. PLK1 does not affect MAVS dimerization
HEK 293T cells were transfected with pMyc-MAVS, pFLAG-MAVS and increasing amounts of pEGFP-PLK1-PBD. After 24 hr, Myc-MAVS was immunoprecipitaed as in Fig5A. The presence of FLAG-MAVS (upper panel), Myc-MAVS and PLK1-PBD (middle panels) in the immunocomplexes was revealed by immunoblot using anti-FLAG, anti-c-Myc or anti-PLK1 antibodies. Expression of FLAG-MAVS was controlled in the total cell extracts using anti-FLAG antibody (lower panel).