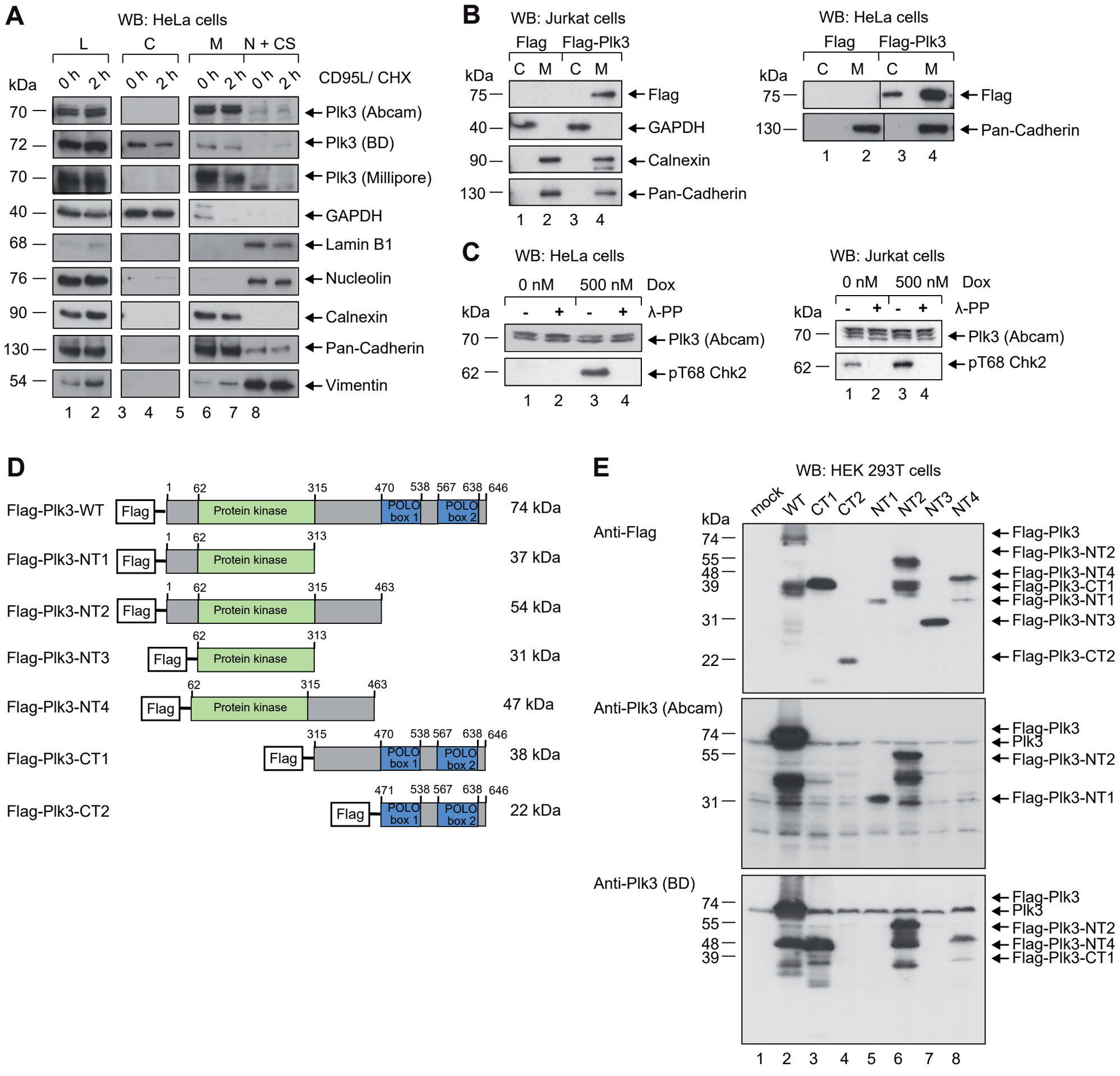
A
WB: HeLa cells
L
C
M
N + CS
kDa
0 h
2 h
CD95L/ CHX
70
72
70
40
68
76
90
130
54
Plk3 (Abcam)
Plk3 (BD)
Plk3 (Millipore)
GAPDH
Lamin B1
Nucleolin
Calnexin
Pan-Cadherin
Vimentin
1 2 3 4 5 6 7 8
B
WB: Jurkat cells
Flag
Flag-Plk3
C M C M
kDa
75
40
90
130
Flag
GAPDH
Calnexin
Pan-Cadherin
1 2 3 4
WB: HeLa cells
Flag
Flag-Plk3
C M C M
kDa
75
130
Flag
Pan-Cadherin
1 2 3 4
C
WB: HeLa cells
0 nM
500 nM
Dox
kDa
- + - +
λ-PP
70
62
Plk3 (Abcam)
pT68 Chk2
1 2 3 4
WB: Jurkat cells
0 nM
500 nM
Dox
kDa
- + - +
λ-PP
70
62
Plk3 (Abcam)
pT68 Chk2
1 2 3 4
D
Flag-Plk3-WT
Flag
1
62
315
470
538
567
638
646
Protein kinase
POLO box 1
POLO box 2
74 kDa
Flag-Plk3-NT1
313
37 kDa
Flag-Plk3-NT2
463
54 kDa
Flag-Plk3-NT3
31 kDa
Flag-Plk3-NT4
47 kDa
Flag-Plk3-CT1
38 kDa
Flag-Plk3-CT2
471
22 kDa
E
WB: HEK 293T cells
mock
WT
CT1
CT2
NT1
NT2
NT3
NT4
kDa
Anti-Flag
74
55
48
39
31
22
Flag-Plk3
Flag-Plk3-NT2
Flag-Plk3-NT4
Flag-Plk3-CT1
Flag-Plk3-NT1
Flag-Plk3-NT3
Flag-Plk3-CT2
Anti-Plk3 (Abcam)
74
55
31
Flag-Plk3
Plk3
Flag-Plk3-NT2
Flag-Plk3-NT1
Anti-Plk3 (BD)
74
55
48
39
Flag-Plk3
Plk3
Flag-Plk3-NT2
Flag-Plk3-NT4
Flag-Plk3-CT1
1 2 3 4 5 6 7 8

Supplementary Information, Figure S2. Analysis of the subcellular localization of Plk3.

(**A**) HeLa cells were treated with CD95L and CHX for the indicated time periods. Cytoplasmic, membrane and nuclear/cytoskeletal proteins were extracted using the Compartmental protein extraction kit. The whole cell lysate (L), cytoplasmic (C), membrane (M) and nuclear/cytoskeletal fractions (N + CS) were immunoblotted against Plk3. Different markers for cellular compartments were used: antibodies for GAPDH (cytoplasm), Lamin B1 (nucleus), Nucleolin (nucleolus), Pan-Cadherin (plasma membrane), Calnexin (ER membrane) and Vimentin (cytoskeleton). (**B**) Jurkat (left panel) or HeLa (right panel) cells were transfected with Flag-tagged full-length Plk3. Cytoplasmic and membrane proteins were extracted, separated and subsequently immunoblotted against Flag, GAPDH, Calnexin and Pan-Cadherin. (**C**) HeLa (left panel) and Jurkat (right panel) cells were treated without or with 500 nM Doxorubicin (Dox) for 0 and 2 h to induce DNA damage. Cell lysates were incubated with or without 1000 U λ-phosphatase for 30 min at 30 °C, and immunoblotted for Plk3 and the DNA damage-specific marker pT68 Chk2. (**D**) Schematic representation of Flag-tagged full-length Plk3 (Flag-Plk3-WT) and truncated forms of Plk3 (NT1, NT2, NT3, NT4, CT1 and CT2) which were used for transfection of HEK 293T cells (E). (**E**) HEK 293T cells were transfected with Flag-Plk3-WT and its truncated forms for 24 h. WB analysis of cell lysates using α-Flag (Sigma-Aldrich), α-Plk3 (BD; aa. 334-607) and α-Plk3 (Abcam; aa. 1-100) antibodies was performed in order to determine the specificity and the binding site of antibody.