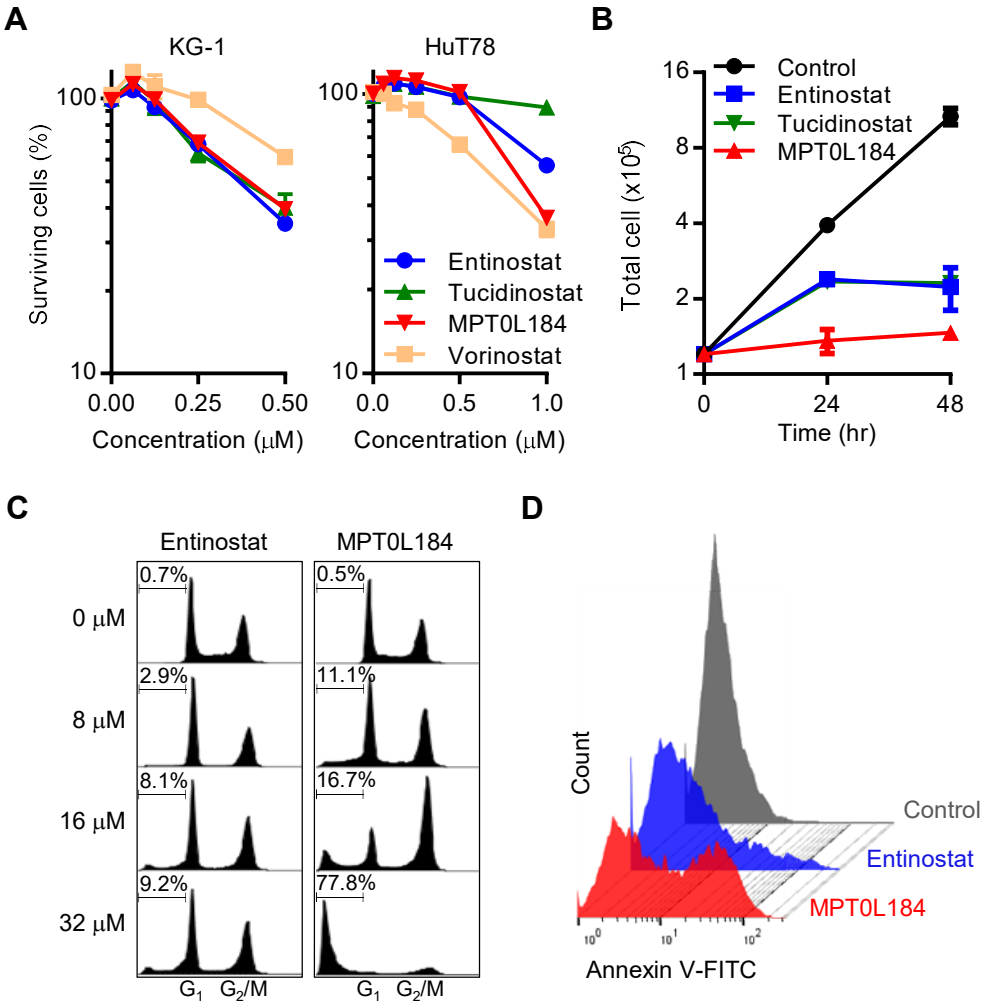
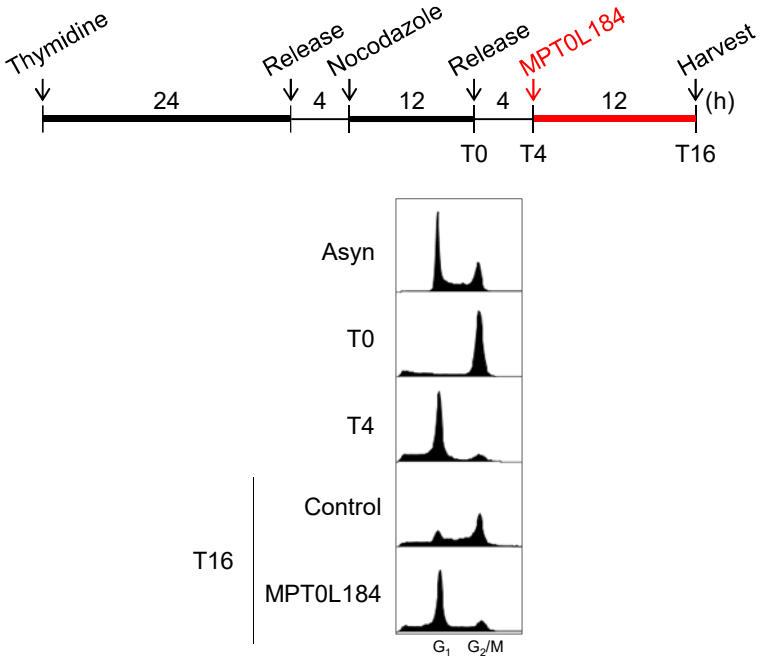
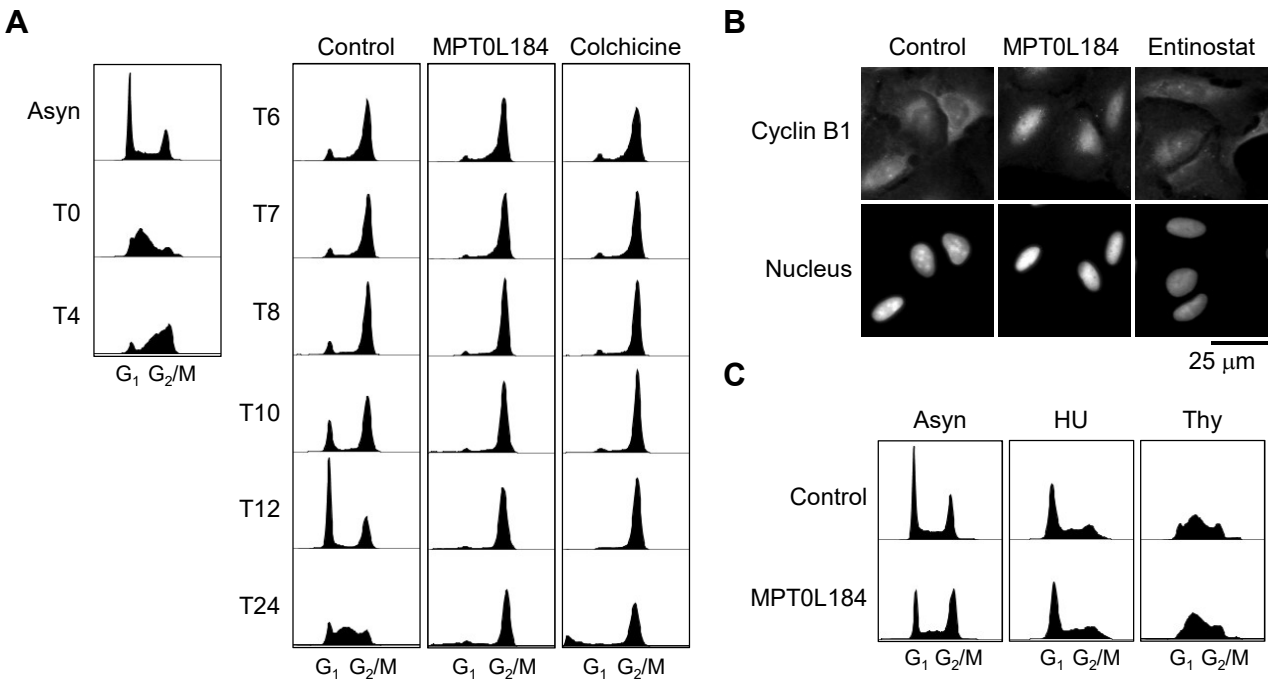


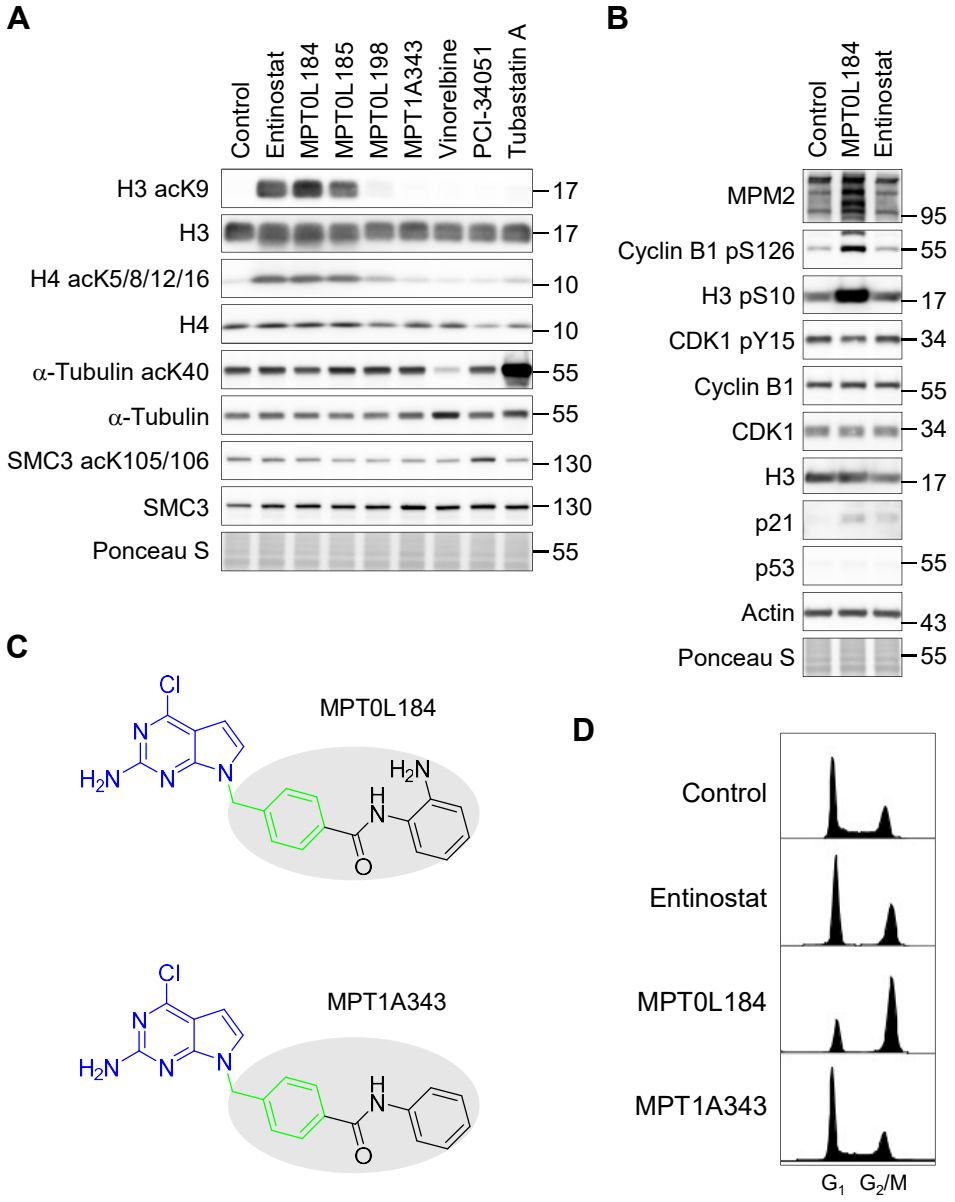
Supplementary Fig S1. MPT0L184 exhibits potent inhibitory activity on cell proliferation. (A) MTS analyses of KG-1 (left) and HuT78 (right) cells treated with MPT0L184, entinostat, tucidinostat, or vorinostat for 72 hours. Percentages of surviving cells were calculated and results from one of two biological replicates are shown with means and SD (n = 3). (B) Trypan-blue exclusion assays of MDA-MB-231 cells treated as Fig. 2C. Numbers of total cells were calculated and results from one of two biological replicates are shown with means and SD (n = 3). (C) Cell-cycle profiles of MDA-MB-231 cells treated with MPT0L184 or entinostat for 48 hours. Representative results from one of two biological replicates are shown. Percentages of the sub-G_1 population were determined using Flowjo. (D) Flow cytometry analysis of MPT0L184- or entinostat-treated MDA-MB-231 cells stained with annexin V-FITC. Representative results from one of two biological replicates are shown.



Supplementary Fig 2. MPT0L184 treatment induces cell-cycle arrest in the G_1 phase.
Cell-cycle profiles of G_1-synchronized MDA-MB-231 cells treated with 16 μM of MPT0L184. The experimental design (upper) and representative results from one of two biological replicates (lower) are shown.

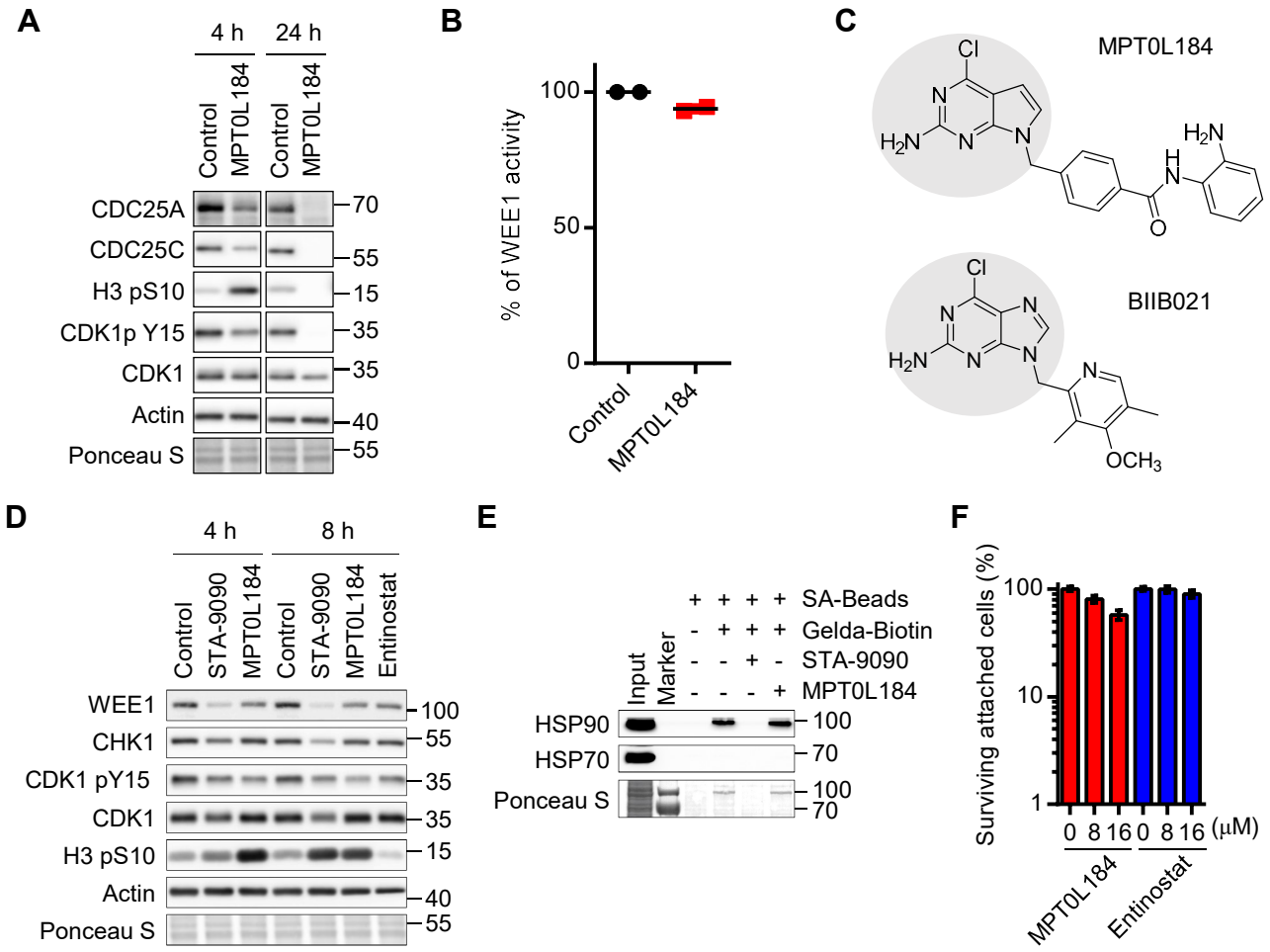


Supplementary Fig 3. MPT0L184 treatment induces cell-cycle arrest in the G_2 phase.
(A) Cell-cycle profiles of S-synchronized MDA-MB-231 cells treated as in Fig. 4A. Representative results from one of two biological replicates are shown. (B) Immunofluorescence staining of cyclin B1 in synchronized U-2 OS cells treated with 16 μM of MPT0L184 or entinostat for 4 hours. Representative results from one of two biological replicates are shown. (C) Cell-cycle profiles of S-phase-arrested MDA-MB-231 cells treated as in Fig. 4C. Representative results from one of two biological replicates are shown. Asyn: asynchronized; HU: hydroxyurea; Thy: thymidine.



Supplementary Fig 4. MPT0L184 dysregulates mitotic control depending on its HDAC-inhibitory activity but independent of p53 expression.

(A) Western blot analysis of acetylation levels of HDAC substrates in MDA-MB-231 cells treated with 16 μM of entinostat derivatives, 40 μM of PCI-34051, or 4 μM tubastatin A for 6 hours. Representative results from one of two biological replicates are shown. (B) Western blot analysis of mitotic markers in NCI-H1299 cells treated with 16 μM of MPT0L184 or entinostat for 6 hours. Representative results from one of two biological replicates are shown. (C) Structures of MPT0L184 and MPT1A343. (D) Cell-cycle profiles of MDA-MB-231 cells treated with 16 μM of MPT0L184, entinostat, or MPT1A343 for 24 hours. Representative results from one of two biological replicates are shown.



Supplementary Fig 5. MPT0L184 down regulates expression of CDC25 and WEE1.
(A) Western blot analysis of CDC25A/C in MDA-MB-231 cells treated with 20 μM of MPT0L184. Representative results from one of two biological replicates are shown. (B) *In vitro* WEE1 inhibition analysis of MPT0L184 at a concentration of 10 μM (n = 2). (C) Structures of MPT0L184 and BIIB021. Consensus groups are highlighted in gray. (D) Western blot analysis of WEE1 and CHK1 in MDA-MB-231 cells treated with 16 μM of MPT0L184, entinostat or STA-9090. Representative results from one of two biological replicates are shown. (E) *In vitro* HSP90-binding analysis of geldanamycin in the absence or presence of 20 μM of MPT0L184 or STA-9090. Streptavidin (SA) beads were used to isolate proteins associated with geldanamycin (Gelda)-biotin. (F) MTT analysis of MDA-MB-231 cells treated with indicated concentrations of MPT0L184/entinostat for 2 hours and then released for 72 hours. Percentages of surviving attached cells were calculated and results from two biological replicates are shown with means and SD ($n \geq 6$).