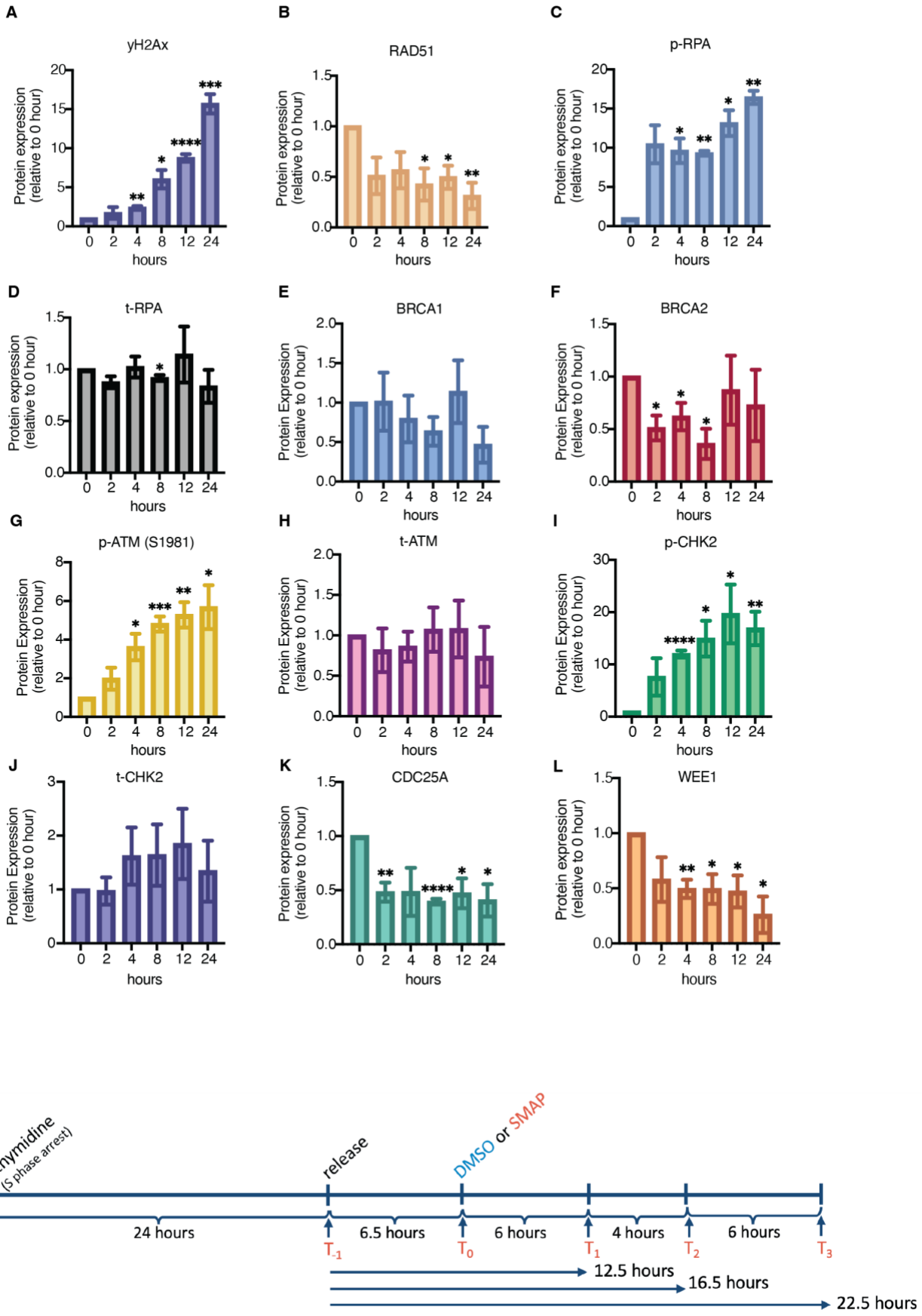
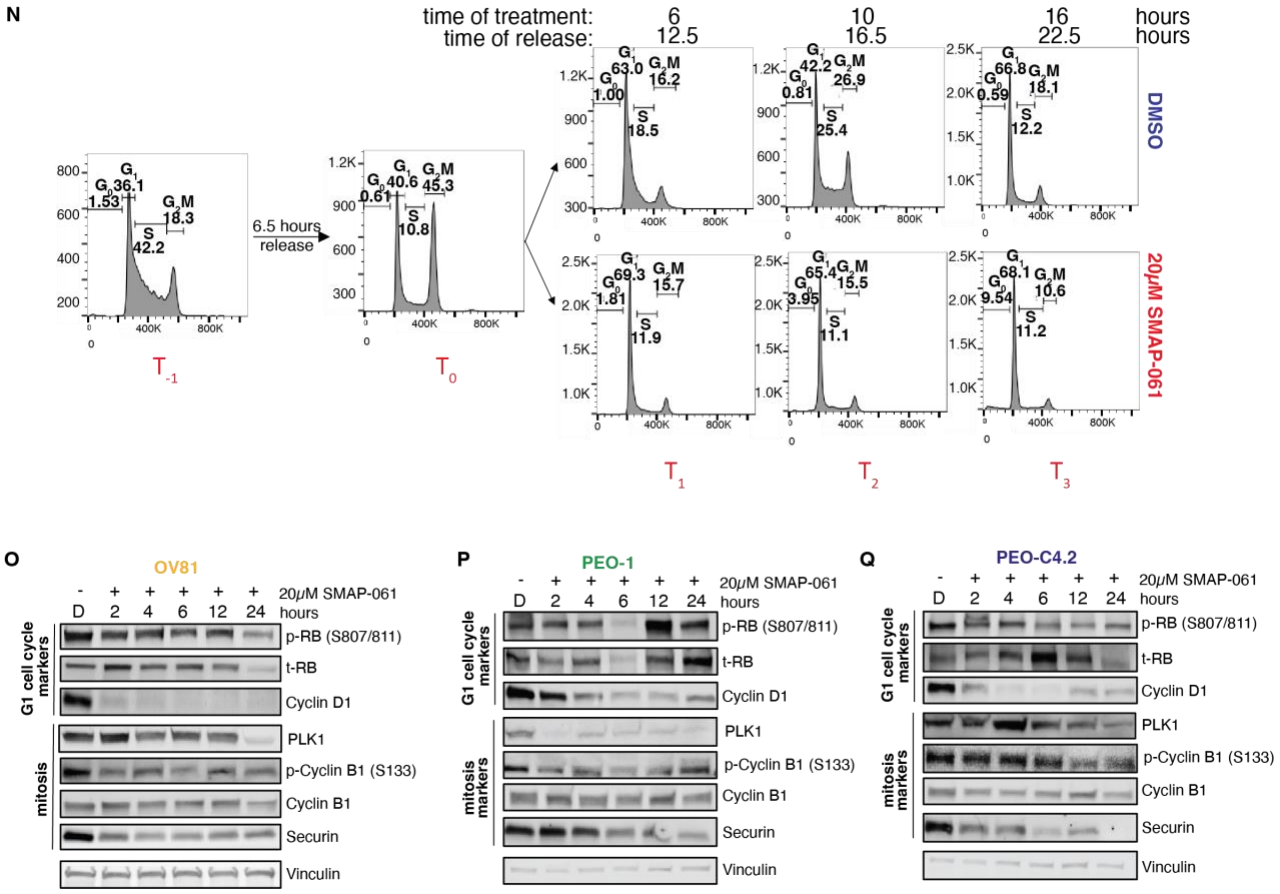
Supplementary Figure 2





Supplementary Figure 2 – SMAP-061 consistently decreases expression of DDR and cell cycle proteins in multiple HGSC models, including OV81, PEO-1 and PEO-C4.2, resulting in unrepaired DNA damage and G1 cell cycle arrest. A) γH2Ax, B) RAD51, C) p-RPA, D) t-RPA, E) BRCA1, F) BRCA2, G) p-ATM, H) t-ATM, I) p-CHK2, J) t-CHK2, K) CDC25A and L) WEE1 protein expression quantification from western blot analysis of OV81 in main Fig. 3B looking at the impact of 20μM SMAP-061 in DDR and HR proteins after 2, 4, 6, 12 and 24 hours of treatment. Data is presented as the mean ± SEM (n=3), (unpaired Student T-tests, comparing each time point relative to zero, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). M) Schematic of the timeline for the treatment and collection of the different time points used for cell cycle flow cytometry analysis. T_{-1} – 24h post 2mM thymidine block; T_0 – 6.5 hours post thymidine release; T_1 – 6 hours post DMSO or SMAP-061 treatment; T_2 – 10 hours post DMSO or SMAP-061 treatment; T_3 – 16 hours post DMSO or SMAP-061 treatment. N) Flow cytometry assay of OV81 evaluating cell cycle profiles. Cells were incubated with 2mM thymidine for 24 hours for synchronization in S phase. Thymidine was then released to allow cells to reengage cell cycle progression, by replacing with drug-free media for 6.5 hours. Consecutively, cells were treated with either DMSO (top) or SMAP-061 (bottom) for 6, 10 and 16 hours and cell cycle profiles were analyzed using FlowJo. (Statistics in Fig. 3G). Western blot analysis of O) OV81, P) PEO-1 and Q) PEO-C4.2 cell lines further validating the impact of 20μM SMAP-061 in cell cycle proteins after 2, 4, 6, 12 and 24 hours of treatment.