Supplementary Figure S3- E2-induced DNA damage is dependent upon cell cycle progression. (A/B) HCC-1428 and MDA-415/Luc cells were treated with HD x 3 d prior to seeding. All cell lines were then treated ± E2 for 21 h, and labeled with BrdU in culture medium for another 3 h prior to harvest. Floating and attached cells were harvested and stained for γH2AX, BrdU, and cleaved PARP, and with propidium iodide. Cells were analyzed by flow cytometry. Cleaved PARP-positive cells were considered apoptotic and excluded from analysis. Representative flow cytometry results are shown in (A). Proportions of cells with DNA breaks (γH2AX-positive) that were or were not in S-phase (i.e., did or did not incorporate BrdU) were plotted in (B). Data are shown as mean of triplicates + SD. Proportions of γH2AX+/BrdU+ cells were statistically compared between treatment groups as indicated with brackets. (C) T47D/pInd20-*ESR1* cells were pretreated with HD x 7 d, and then treated with HD + dox x 14 d prior to seeding. All cell lines were then treated ±E2 ± abemaciclib as indicated x 24 h, fixed, and stained for γH2AX and with DAPI. γH2AX foci were counted in ≥100 nuclei per group. *p<0.05, **p<0.005, ****p<0.0001.