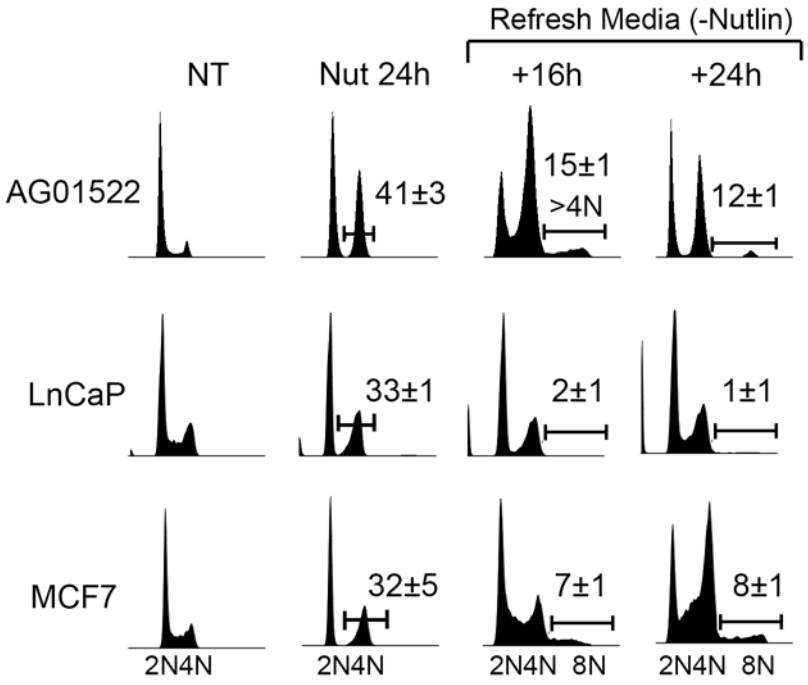
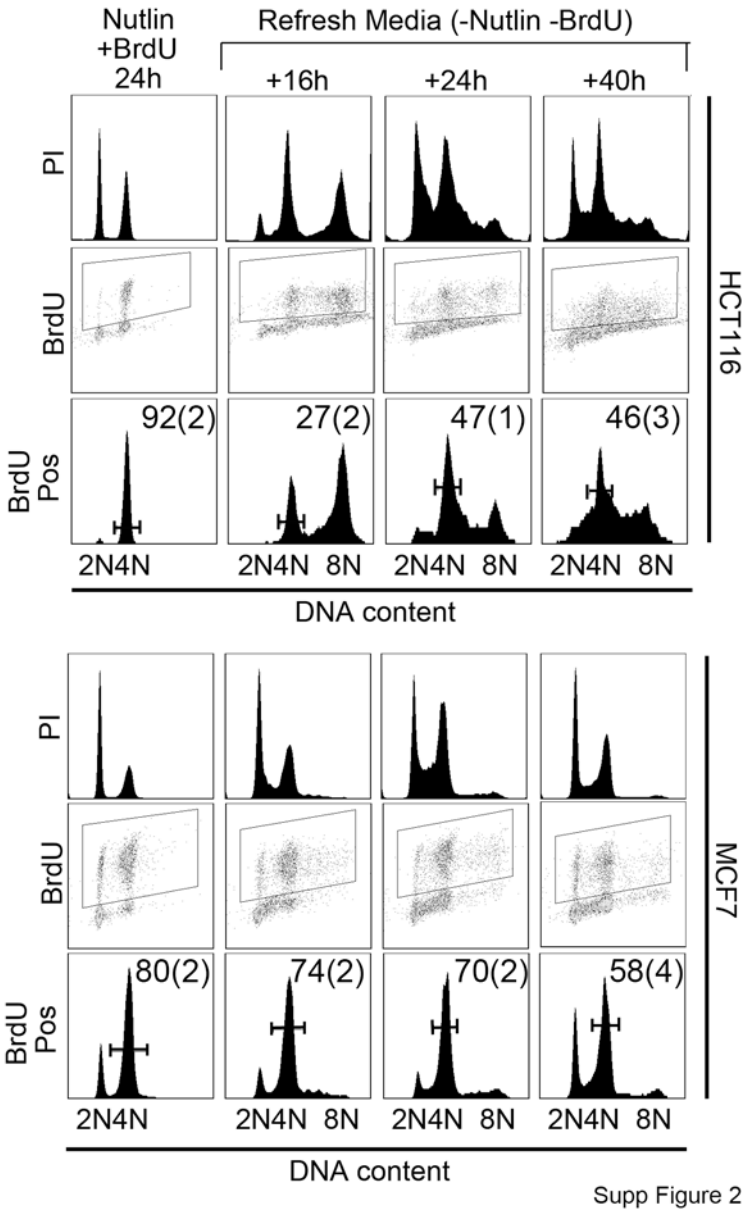
SUPPLEMENTAL FIGURE 1**. Transient Nutlin treatment induces the appearance of cells with >4N DNA content in AG01522, MCF7 and LnCaP cells.** AG01522, MCF7 and LnCaP cells were either untreated *(NT)* or treated with Nutlin (*Nut*; 10 μmol/L) for 24 hrs followed by Nutlin removal. The cells were harvested at the indicated time points after Nutlin removal. Fixed cells were stained with propidium iodide (25 μg/mL) and subjected to flow cytometry analysis. Shown is the representative DNA profile histograms analyzed using FlowJo (cell count versus propidium iodide/DNA content). The position of 2N, 4N, and 8N cells is indicated. Percentage of cells in 4N state was determined at Nut 24h time point. Percentage of cells in >4N state was determined at +16h and +24h time point after Nutlin removal. The numbers represent averages from three independent experiments for the gated population. Mean+/- SE.

SUPPLEMENTAL FIGURE 2. **Transient Nutlin treatment promotes a prolonged 4N arrest in MCF7 cells.** HCT116 (*A*) and MCF7 (*B*) cells were treated with 10 μM Nutlin for 24 hrs. BrdU (20 μM) was added 6 hrs after Nutlin addition. At the 24 hrs time point, cells were rinsed with PBS and refed with normal medium (minus Nutlin and BrdU). The analysis was done as described in FIGURE 4.


Refresh Media (-Nutlin)
NT
Nut 24h
+16h
+24h
AG01522
41±3
15±1
>4N
12±1
LnCaP
33±1
2±1
1±1
MCF7
32±5
7±1
8±1
2N4N
2N4N
2N4N 8N
2N4N 8N

Supp Fig 1




Supp Figure 2