Supplemental data

Time of Exposure to Curcumin

We determined how long of an exposure to curcumin was required for inhibition of mammosphere formation (Figure 5). We found that as little as 4 hr exposure to curcumin 5 μM caused a 50% inhibition in mammosphere number and 10μM curcumin completely inhibited mammosphere formation. Greater than 25 μM curcumin concentration caused complete inhibition of sphere formation and generalized cell death with 4 hours of exposure (Data not shown). The addition of 10 μM piperine to 5 μM curcumin concentration with 4 hr of exposure inhibited sphere number to less than 20% and completely inhibited sphere formation at exposure times of 24 hrs or more.

Effect of Curcumin and Piperine on β Catenin by Immunohistochemistry Staining

We also qualitatively demonstrated effect of piperine and curcumin on Wnt signaling using β catenin immunohistochemistry staining (supplemental data Figure 6). We found that curcumin 10 μM and piperine 10 μM completely inhibited intracellular β catenin expression compared to DMSO vehicle.

Figure 5 Time of Exposure to curcumin necessary to downregulate mammosphere formation (C5=curcumin 5 μM;, C10=curcumin 10 μM; C10+P10=curcumin 5 μM and piperine 10 μM). Note that no spheres were formed at the C10+P10 concentrations.

Figure 6. β catenin staining in MCF 7 cells treated with curcumin and piperine.

Figure 5

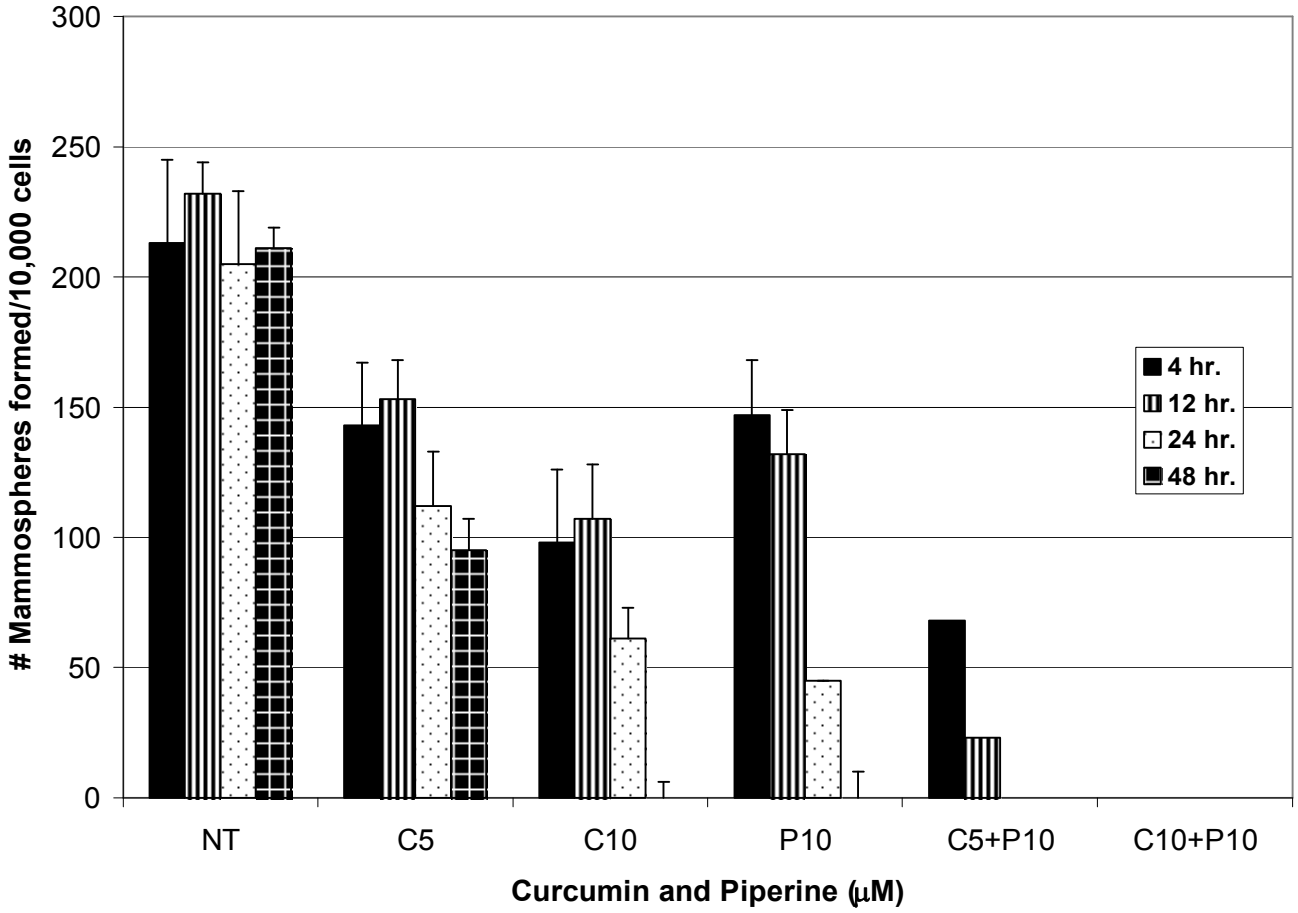
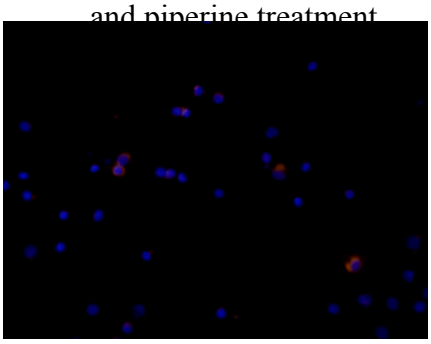
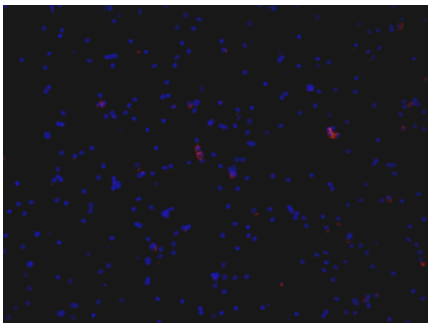

300
250
200
150
100
50
0
Mammospheres formed/10,000 cells
4 hr.
12 hr.
24 hr.
48 hr.
NT
C5
C10
P10
C5+P10
C10+P10
Curcumin and Piperine (μM)

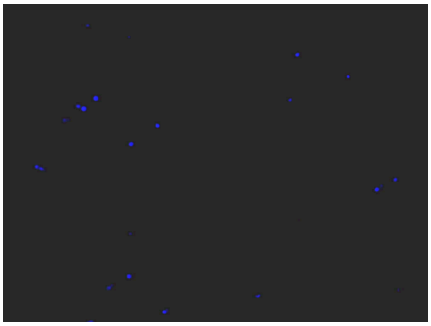
Figure 6 β Catenin staining in MCF7 cells with DMSO control compared to curcumin and piperine treatment



β Catenin/DAPI staining with control



Curcumin 10 μM-β Catenin/DAPI staining



Piperine 10μM β Catenin/DAPI staining