

Supplemental Figure 1. Time-dependent changes in p53 and related proteins after APAP exposure. C6 glioma cells were treated with 5 mM APAP for different time points as indicated. Equal amounts of the whole cell homogenates (30 µg protein/well of mini-gels) were separated on 12% SDS-polyacrylamide gels followed by immunoblot analysis using the specific antibody against p53 (top), phospho-p53 (second), p21 (third), mdm2 (fourth), or actin (bottom). This result represents a typical result of two independent experiments except for mdm2.



Supplemental Figure 2. Little changes in the levels of p53 and p21 by CMZ. C6 glioma cells were untreated (lane 1) or treated with 20  $\mu$ M CMZ (lane 2) or DMSO (lane 3) for 16 h before cell harvest. Equal amounts of the whole cell homogenates (30  $\mu$ g protein/well of minigels) were separated on 12% SDS-polyacrylamide gels followed by immunoblot analysis using the specific antibody against p53 (top), p21 (middle), or actin (bottom). This result represents a typical result of two independent experiments.



Supplemental Figure 3. Increased binding of APAP moiety to p53 protein after exposure to APAP. C6 glioma cells were treated with DMSO (lane 1) or 5 mM APAP (lane 2) for 24 h as indicated. Equal amounts of the soluble fraction (1 mg protein) were used to immunoprecipitate (IP) p53 protein using the specific antibody to p53 protein as described under Materials and Methods. The immunoprecipitated p53 protein was washed twice with 1 x PBS with 1% CHAPS and subjected to 12% SDS-PAGE followed by immunoblot analysis (IB) with the specific antibody against APAP. An arrow indicates the specific protein band recognized with the antibody to APAP (lane 2). This result represents a typical result of two independent experiments.