Fig. S1. Treatment with genotoxic stress reagents Dox (A) or hydroxyurea (B) led to S6K1 activation and Mdm2 S163 phosphorylation. MEFs were treated with Dox for different periods of time or with different doses of HU for 8 hrs, and the phosphorylation and protein levels of S6K1, mTOR, Mdm2, and MAPKs were analyzed by Western blot.
Fig. S2. Transient down-regulation of Mdm2 in response to DNA damage occurred at the mRNA level. (A) MEFs were treated with Dox for different periods of time, mRNA levels of Mdm2 were determined by real-time PCR. (B) MEFs were pretreated with or without MG132 for 1hr before treatment of Dox for different periods of time. The protein levels of Mdm2 was analyzed by Western blot.
Fig. S3. (A) Serum-induced Mdm2 S163 phosphorylation required mTOR. MEFs were pretreated with or without rapamycin for 1 hr and serum-starved for 2 hrs, before serum was added to 10% and incubated for 2 or 4 hrs. The phosphorylation and protein levels of Mdm2 and S6K1 were analyzed by Western blot. The value of p-Mdm2 S163 at time 0 in the presence of RAP was set at 1.0. (B) Akt1-induced Mdm2 S163 phosphorylation could be blocked by rapamycin. Mdm2 and different amounts of Akt1 constructs were co-transfected into 293T cells. One set of experiments were treated with rapamycin for 24 hours. Mdm2 phosphorylation was determined by Western blot analysis.
Fig. S4. The effects of MAPK inhibitors on Mdm2 phosphorylation. MEFs were pretreated with 10 μM SB203580 (p38 inhibitor) (A) or 20 μM U0126 (MEK inhibitor) (B) for 1 hr, and followed by Dox for different periods of time. Mdm2 S163 phosphorylation and its protein levels were analyzed by Western blot.
Fig. S5. Atm and Atr are not required for genotoxic stress-induced Mdm2 phosphorylation, p38 MAPK activation, or S6K1 activation. Atr was knocked down in Atm+/+ or Atm-- MEFs, followed by Dox treatment. Mdm2, p-Mdm2, p38, p-p38, Atm, Atr protein levels were analyzed by Western blot.
Fig. S6. TSC tumor samples did not show much nuclear translocation of Hdm2. Three normal tissues (A-C) and four TSC tumors (A-D) were separated into cytoplasmic and nuclear fractions using a fractionation kit. Mdm2 phosphorylation and protein levels were analyzed by Western blot.
Fig. S7. S6K knockdown did not affect the transcription and translation rates of p53. (A) S6K1/2 knockdown showed no effect on p53 mRNA levels. S6K1 and S6K2 were knocked down in MEFs for 48 hrs and followed by Dox treatment for 4 hrs. Total RNA was extracted, cDNA synthesis was carried out and p53 mRNA levels were determined by real-time PCR. (B) S6K1/2 deficiency showed no effect on p53 translation rates. After knockdown of S6K1 in MEFs, cells were treated with Dox for different periods of time and then labeled with 35S methionine for 30 min before being harvested. p53 was immunoprecipitated and 35S-labeled p53 was detected by radioautography.