Figure 1: The decrease in Tip60 levels upon Mdm2 expression is not dependent on Tip60 phosphorylation.

U2OS cells were transfected with 100 ng of pCMV luciferase reporter vector, 1 µg of pCDNA3 Tip60 or pCDNA3 Tip60 AA (which expressed a non phosphorylatable Tip60 (our unpublished results)), as indicated, 1 µg of pCMV 2N3T Ku80, in the presence (lanes 1 and 3) or absence (lane 2 and 4) of 2 µg of pXJ Mdm2. The amount of promoters in the transfection was kept constant by the addition of empty vectors. 24 hours after transfection, luciferase activity was measured. After standardisation for luciferase activity, total cell extracts were directly loaded and subjected to an anti-HA western blot to detect exogenous Tip60 and Ku80, as indicated.
Figure 2: Transfected Tip60 is an unstable protein with a half life around 30 min in pulse chase experiments.
A) U2OS cells were transfected with 10 µg of pCMV2N3T Ku80 and 10 µg of pCMV 2N3T Tip60 (lanes 1, 3) or none (lane 2). 24 hours after transfection, transfected cells were labelled with $^{35}$S methionine, and subjected to a chase period of 75 min (lane 3) or not (lanes 1, 2). Cells were lysed in RIPA buffer, and extracts were immunoprecipitated using the anti-HA antibody. Immunoprecipitates were analysed by SDS-PAGE and detected by fluorography. Note that Tip60 is a highly unstable protein, since there is no Tip60 left after the chase period. The star (*) indicates a non specific band.
B) Same experiment as in A, except that the chase was conducted for 15, 30 or 60 min. In this experiment, the half life of Tip60 was about 30 min.
Figure 3: Tip60 259-396 domain is sufficient to interact with Mdm2.
U2OS cells were transfected with 10 µg of pCMV NeoBam Mdm2 and either 10 µg of pCDNA3 Tip60 fl, pcDNA3 Tip60 1-258, or pCDNA3 Tip60 259-396. 24 hours after transfection, whole cell extracts were prepared, and subjected to immunoprecipitation with either the anti-HA antibody (lanes 2, 5, 8), or an irrelevant antibody (anti myc 9E10, lanes 3, 6, 9), as indicated. Immunoprecipitates were tested for the presence of Mdm2 by western blotting. In lanes labelled inp (input), 10 µl of whole cell extracts were directly loaded. In lanes 10-12, 10 µl of whole cell extracts were subjected to an anti-HA western blot, to monitor the quantity of the various mutants in the extracts. Note that Tip60 1-258 is present at far higher amounts than Tip60 fl or 259-396, because these latter proteins are poorly extractible from cells. The stars indicate two non specific bands. Note that HA-Tip60 259-396 corresponds to a faint band migrating just above a non specific band.

Figure 4: Tip60 259-396 domain is sufficient for Mdm2-induced degradation

U2OS cells were transfected as in Fig 5B with expression vectors for the indicated Tip60 deletion mutant (in pCDNA3, 1 µg), pCMV luciferase reporter vector (100 ng), in the presence or absence of pCMV NeoBam Mdm2 (2 µg), as indicated. The amount of promoters in the transfection was kept constant using empty vectors. 24 hours after transfection, luciferase activity was measured. After standardisation according to luciferase activity, the steady state levels of HA-tagged proteins were assessed by western blot.
Figure 5: UV irradiation of Jurkat cells induced Mdm2 degradation.
Jurkat cells were irradiated as in Figure 7 for the indicated period of time, and Mdm2 levels were assayed by western blot. Note the decrease in Mdm2 levels that precedes the appearance of the Tip60 band (figure 7).