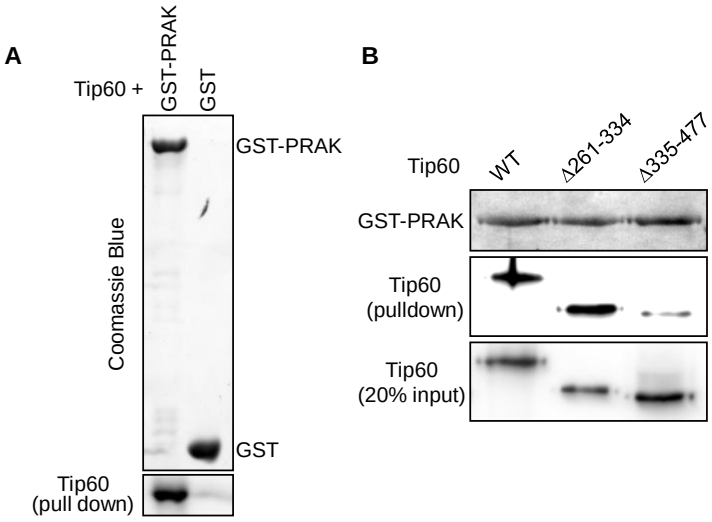

Figure S1, related to Figure 1

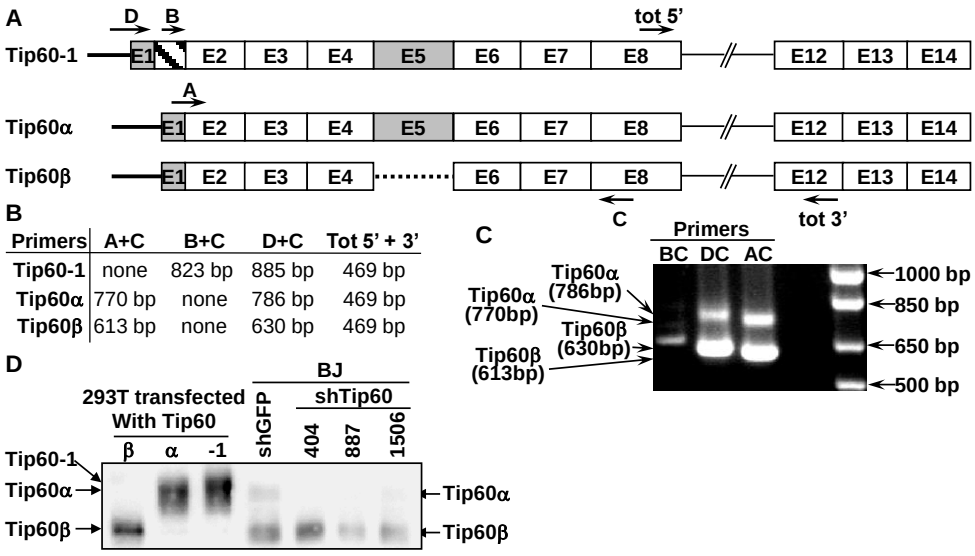

Figure S2, related to Figure 2

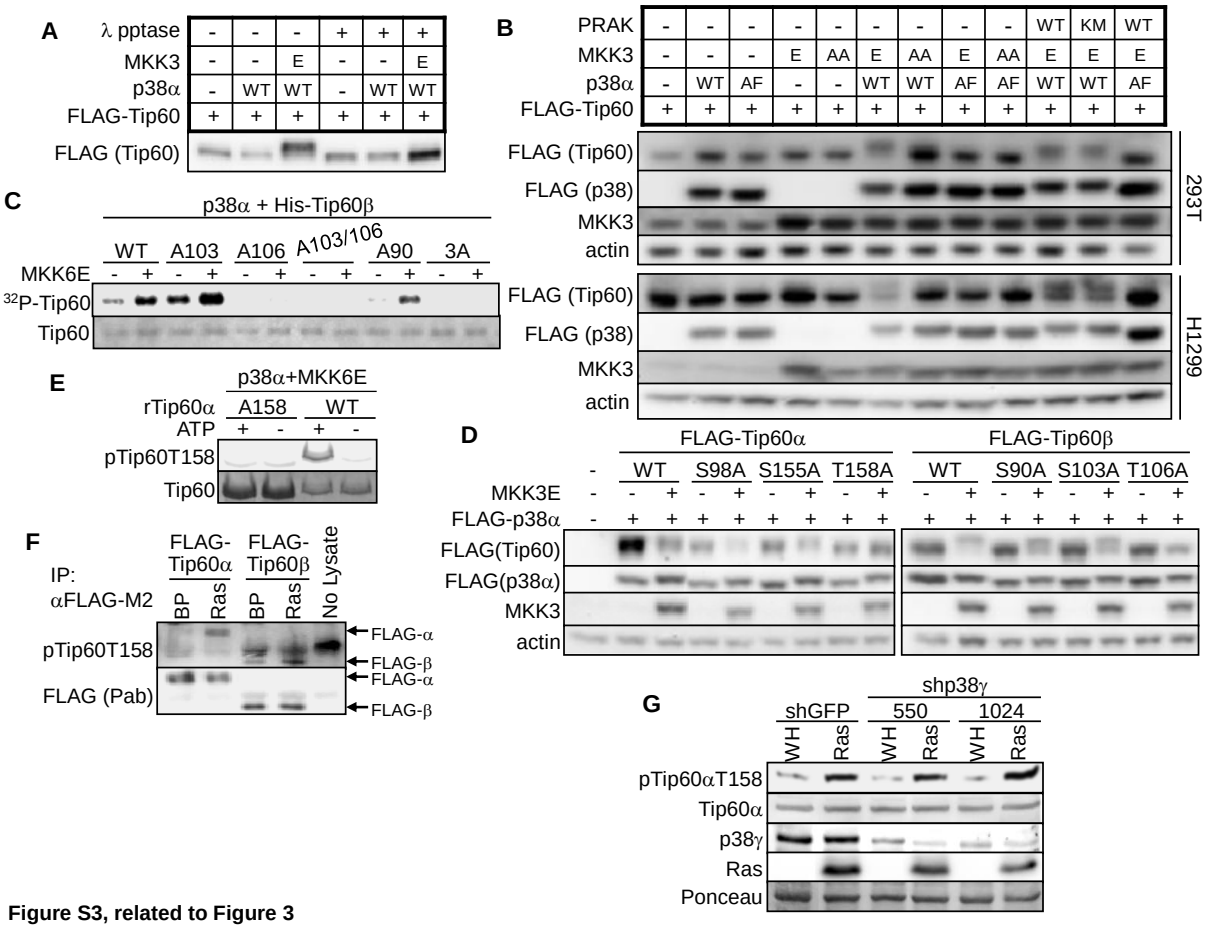

Figure S3, related to Figure 3

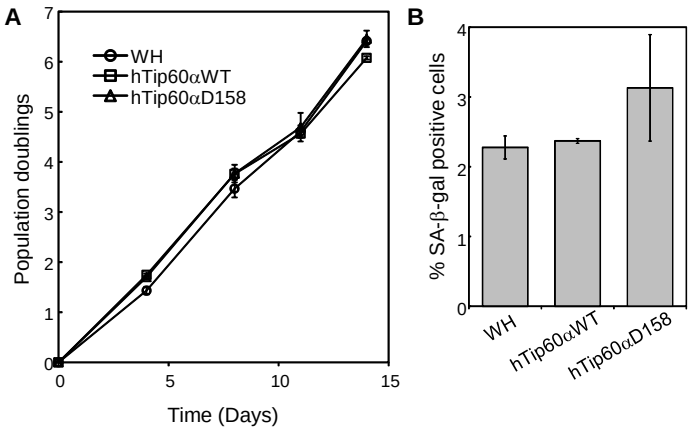

Figure S4, related to Figure 4

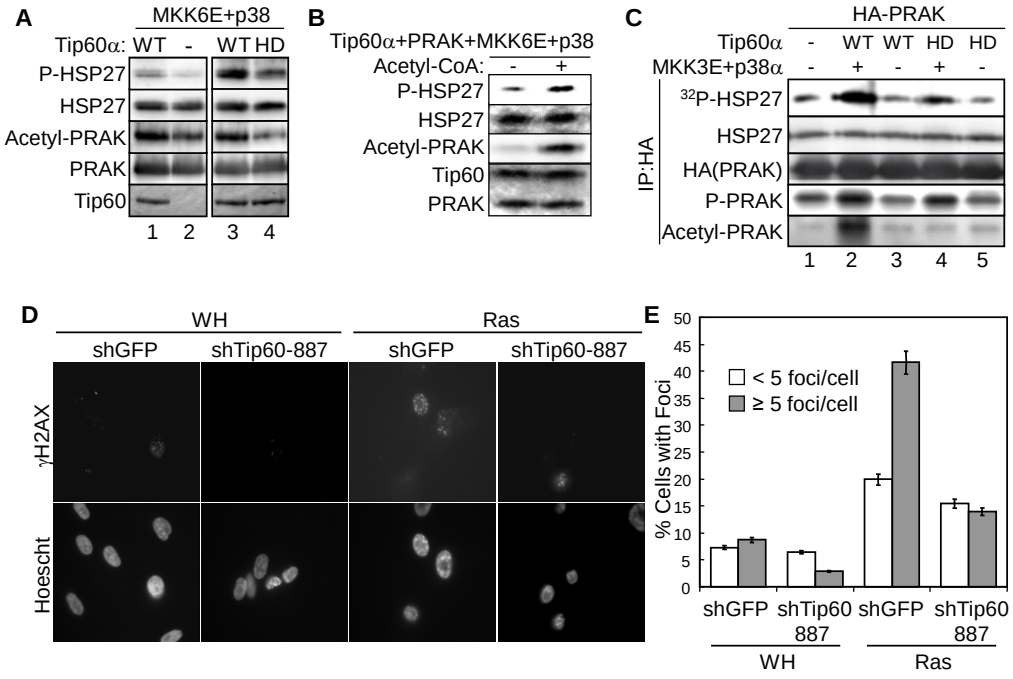

Figure S5, related to Figures 5, 6 and 7

Supplemental Figure legends

Fig. S1, related to Fig. 1 PRAK directly binds to the HAT domain of Tip60.

(A) GST-PRAK or GST was incubated with His-Tip60α, and then with Glutathione-agarose beads.

(B) GST-PRAK was incubated with wild type His-Tip60α (WT) or His-Tip60α carrying indicated deletion mutations, and then with Glutathione-agarose beads.

(A, B) Proteins bound to beads after washing were eluted with 2X Laemmli buffer and resolved by SDS-PAGE. GST-PRAK and GST proteins bound to the beads were visualized by Coomassie Brilliant Blue R staining. Tip60 proteins bound to the beads and Tip60 input were detected by Western blotting.

Fig. S2, related to Fig. 2. Tip60α and Tip60β are the major Tip60 isoforms expressed in primary BJ human fibroblasts.

(A) Schematic diagrams of three Tip60 isoforms generated by alternative splicing, showing the exon arrangement and locations of the PCR primers used to detect these Tip60 isoforms in the study.

(B) Expected sizes of the amplified fragments for each Tip60 isoform in reverse transcription (RT)-PCR analysis using indicated combinations of primers.

(C) RT-PCR analysis of the mRNA levels of Tip60 isoforms using indicated combination of primers. The sizes of DNA ladder are shown on the right. The sizes of the PCR products corresponding to each detectable Tip60 isoform are shown on the left.

(D) Western blot analysis of BJ cells transduced with GFP shRNA or indicated Tip60 shRNA. 293T cells transfected with Tip60-1, Tip60α or Tip60β were analyzed in parallel as positive controls for the positions of the bands corresponding to different Tip60 isoforms.

Fig. S3, related to Fig. 3. Tip60 is phosphorylated at Thr158/106 by activated p38 and during oncogenic *ras*-induced senescence.

(A) FLAG-Tip60 was immunoprecipitated by an agarose-conjugated anti-FLAG antibody M2 from 293T cells transfected with FLAG-Tip60α, p38α (WT) or vector (-), and MKK3E (E) or vector (-), treated with λ protein phosphatase (+) or left untreated (-), and subjected to Western blot analysis for FLAG-Tip60.

(B) 293T (upper panels) or H1299 (lower panels) cells were transfected with FLAG-Tip60 in combination with wild type (WT) or inactive mutant (AF) of p38α or vector (-); active (E) or inactive (AA) mutant of MKK3 or vector (-); and wild type (WT) or kinase dead mutant (KM) of PRAK or vector (-). Protein lysates were prepared 2 days after transfection and subjected to Western blot analysis.

(C) Wild type His-Tip60β (WT) or His-Tip60β carrying S103A (A103), T106A (A106), S90A (A90), S103A/T106A (A103/106) or S90A/S103A/T106A (3A) mutations was incubated with p38α with (+) or without (-) MKK6E in the presence of [γ-^{32}P]ATP. The reactions were resolved by SDS-PAGE. Phosphorylated Tip60 was detected by autoradiography. Tip60 input was detected by staining with Coomassie Brilliant Blue R.

(D) 293T cells were cotransfected with FLAG-Tip60α (left panels) or FLAG-Tip60β (right panels) that either was wild type (WT) or carried indicated mutations or vector (-), MKK3E (+)

or vector (-), and p38α (+) or vector (-). Protein lysates were prepared 2 days after transfection and subjected to Western blot analysis.

(E) Wild type Tip60α (WT) or Tip60α carrying the T158A mutation (A158) was incubated with p38α and MKK6E with (+) or without (-) ATP. The reactions were resolved by SDS-PAGE and subjected to Western blot analysis using an antibody that specifically recognized Tip60 phosphorylated at Thr158 (pTip60T158) or an antibody recognizing total Tip60.

(F) FLAG-Tip60 was immunoprecipitated from BJ cells transduced with FLAG-Tip60α or FLAG-Tip60β and HaRasV12 (Ras) or vector (BP), and subjected to Western blot analysis using the pTip60T158 antibody and an antibody against FLAG tag.

(G) Western blot analysis of BJ cells transduced with shRNA for GFP (shGFP) or p38γ (550 and 1024) and HaRasV12 or vector (WH).

Fig. S4, related to Fig. 4 The Tip60α-D158 mutation fails to induce senescence.

(A) Population doublings of BJ cells transduced with wild type human Tip60α (hTip60αWT), human Tip60α-D158 (hTip60α-D158) or vector (WH) over 14 days, starting on day-5 post Tip60 transduction. Values are mean ± SD for triplicates.

(B) % of SA-β-gal positive cells in cell populations described in (A). Values are mean ± SD for triplicates.

Fig. S5, related to Fig. 5, 6, 7 Acetylation of PRAK by Tip60 stimulates the protein kinase activity of PRAK in vitro and in cells, and Tip60 deficiency reduces oncogenic *ras*-induced DNA damage foci.

(A) PRAK was incubated first with wild type (WT) or HAT-defective (HD) Tip60α or buffer (-) in the presence of p38α, MKK6E, acetyl-CoA and ATP, and then with HSP27. Phosphorylation of HSP27 and acetylation of PRAK were detected by Western blot analysis using an anti-pHSP27-S82 and anti-acetyl-Lys antibody, respectively. Total PRAK, Tip60 and HSP27 proteins were detected by Ponceau staining.

(B) PRAK was incubated first with wild type Tip60α, p38α, MKK6E, and ATP with (+) or without (-) acetyl-CoA, and then with HSP27. Phosphorylation of HSP27 and acetylation of PRAK were detected by Western blot analysis using an anti-pHSP27-S82 and anti-acetyl-Lys antibody, respectively. Total PRAK, Tip60 and HSP27 proteins were detected by Ponceau staining.

(C) HA-PRAK was immunoprecipitated from293T cells transfected with HA-PRAK; wild type (WT) or HAT-defective (HD) Tip60α or vector (-); and p38α and MKK3E (+) or vector (-), and incubated with HSP27 in the presence of [γ-^{32}P]ATP. The proteins were resolved by SDS-PAGE and transferred to nitrocellulose membrane. Phosphorylation of HSP27 was detected by autoradiography. The HSP27 input was detected by Ponceau staining. Total, phosphorylated and acetylated PRAK was detected by Western blot analysis.

(D) BJ cells transduced with shRNA for GFP (shGFP) or Tip60 (shTip60-887) and HaRasV12 or vector (WH) were stained for DNA damage foci using an antibody against γH2AX and a FITC-coupled secondary antibody. Nuclei were stained with Hoescht 33342.

(E) Quantification of % of cells containing < 5 or ≥ 5 γH2AX foci in the experiment in (D). Values are mean ± SD for triplicates.

Supplemental Experimental Procedures

Yeast 2-hybrid screen Yeast 2-hybrid screen was performing with the Matchmaker system (Clontech) using PRAK as bait, following manufacturer's protocols. pGBKT7-PRAK was transformed into the PJ69-ZA strain. The PRAK-expression clones were selected and transformed with a HeLa cell cDNA library in pACT2. The positive clones were selected on the Dex-His-Leu-Trp plates and confirmed on Dex-Ade-His-Leu-Trp plates. The plasmid DNA was isolated from the positive yeast clones, and the pACT2 plasmids were recovered in bacteria. These pACT2 plasmids were retransformed into PJ69-ZA-PRAK cells to retest for their positivity. The identity of the positive plasmids was determined by sequencing analysis. The interaction between PRAK and the proteins encoded by the identified plasmids was further confirmed by co-transforming pGBKT7-PRAK and these plasmids into PJ69-ZA.

Plasmids cDNAs encoding Tip60 isoforms were obtained from Dr. Didier Trouche (Université de Toulouse, Toulouse, France), and were cloned into pcDNA3 or retroviral vectors (Babe-puro or WZL-hygro) after adding an N-terminal FLAG-tag by PCR. The deletion mutants of Tip60 (Δ261-334, Δ335-477, Δ478-492 and Δ261-492) were generated by PCR. Expression vectors for Ha-RasV12, MKK3E, MKK6E, and PRAK (human and mouse) (Sun et al., 2007), and those for wild type and constitutive active mutants of p38 isoforms (Kwong et al., 2009) have been published previously. Point mutants of Tip60α (S90A, S98A, S155A, T158A, S90A/S98A, S155A/T158A, Q377E/G380E), Tip60β (S90A, S103A, T106A, S103A/T106A), and PRAK (K51R, K364R and K51R/K364R) were generated by site-directed mutagenesis using

QuikChange Kit (Stratagene). Oligonucleotides for shRNA targeting Tip60 isoforms were designed and cloned into pSUPER.retro according to the published protocol (Brummelkamp et al., 2002). The target sequences for these Tip60 shRNAs are the following, 275: AGAGAGAGGTGAAACGGAA; 404: GCGGGAAGACCTTGCCAAT; 887: GCGAGTTCTGCCTCAAGTA; 1506: GGGCCAGTACATCCTCACA. shRNAs for PRAK (shPK-1: GCTGGAATTAGTGGTCCAG and shPK-2: GTGTCTATATCCACGACCA) and GFP have been described previously (Sun et al., 2007).

Western blot analysis Western blotting was performed with lysates prepared 6-8 days after transduction of Ras or MKK3/6E from sub-confluent cells as described (Wang et al., 2002). Primary antibodies were from Covance (HA-11), Sigma (actin, HA-H6908, FLAG-M5, FLAG-F7425), Santa Cruz (Ras-C-20, MKK3-C-19, MKK6-V-20, Tip60-N-17), Cell Signaling (phospho-p38-Thr180/Tyr182, lamin A/C, pHSP27-Ser82, HSP27, acetyl-Lys-Ac-K-103 mouse mAb, acetyl-Lys-Ac-K2-100 rabbit mAb), Millipore-Upstate (Tip60-07-038, acetyl-Lys-06-933 rabbit pAb). Antibodies specific for each p38 isoform (Kwong et al., 2009) and those for phospho-PRAK-T182 (Yoshizuka et al., 2012) and PRAK (Sun et al., 2007) were described previously. The rabbit polyclonal antibody for phospho-Tip60-T158 was raised in Epitomics, Inc. (Burlingame, CA). Signals were detected using enhanced chemiluminescence and captured by the FluorChem HD2 Imaging System (Cell Biosciences).

Reverse transcription-coupled PCR RNA was isolated using TRIzol reagent (Life Technologies). RT-PCR was performed using One-Step RT-PCR kit (Qiagen), with 0.5 μg of RNA and 25 pmoles of each primer in 50 μl of volume. The reactions were incubated at 50°C for

30 min and then at 94° C for 15 min, followed by 32 (for Tip60) or 28 (for talin1) cycles of 94° C for 0.5 min, 53° C for 0.5 min and 72° C for 0.5 min. Primers used for PCR amplification of Tip60 isoforms are the following (Fig. S2), Tot5': TGTGCGAGTTCTGCCTCAAGTAC; Tot 3': CAGTAGCTTCGATAGGATAGGAG; A: GGCGGAGGTGGGGGAGATAATCG; B: AGGGGAGGTGGGTAGAGCC; C: CAATGTGGTGAGTTCCTGTGGGTACG; D: GTGGAGGGAGGGAAGATGGCGGAG. Primers used to amplify talin1 are CTCGAGATGGCAAGCTTAAACCCT (forward) and CCAGAAGAAGTTTGCTTGAAGACATG (reverse).

Recombinant proteins N-terminal His-tagged wild type and mutant Tip60α and Tip60β proteins and GST-PRAK were expressed from pET28 and pGEX, respectively, in BL-21. Transformed bacteria was cultured at 30° C until OD 600 reaches 0.8, and induced with 1 mM IPTG at 28 °C for 6 hours. Bacteria were pelleted, frozen at -20° C, and lysed with lysis buffer (300 mM NaCl, 20 mM Tris-HCl, pH7.4, 0.5% TritonX-100, 2 mM β-mercaptoethanol, 2 mM PMSF, 2 mM imidazole, 10% glycerol) on ice for 1 hour and then sonicated. The lysates were cleared by centrifugation at 18,000g, 4° C for 1 hour, filtered through 0.45 uM PES membrane, and then incubated with cobalt agarose (Clontech, CA) (for His-Tip60) or Glutathione-agarose beads (Sigma, MO) (for GST-PRAK) at 4°C for 1 hour with shaking. The beads were washed 3 times with lysis buffer and then twice with wash buffer (150 mM NaCl, 20 mM Tris-Cl, pH7.4, 2 mM β-mercaptoethanol, 1 mM PMSF, 10 mM imidazole, 10% glycerol). Tip60 was eluted with elution buffer [150 mM NaCl, 20 mM Tris-Cl, pH7.4, 2 mM β-mercaptoethanol, 1 mM PMSF, 200 mM imidazole (for His-Tip60) or 15 mM reduced glutathione (for GST-PRAK),

10% glycerol], dialyzed against dialysis buffer (150 mM NaCl, 20 mM Tris-Cl, pH7.4, 2 mM β-mercaptoethanol, 50% glycerol), and stored at -20 or -80^{o} C.

Co-Transfection in 293T and H1299 cells 293T and H1299 cells were in general transfected by Lipofectamine 2000 (Life Technologies) according to manufacturer's protocol. In some cases, 297T cells were transfected with calcium phosphate. One day before transfection, 293T cells were seeded into 10cm culture dishes at ~70-80% confluence. 1-4 hours before transfection each dish were replaced with 10 ml fresh medium. For every 10cm dish co-transfection, pCDNA3-HA-hPRAK (15μg), pEF3-FLAG-Tip60α (10μg) and pBabe-p38 (5μg) plasmids were mixed with 100 μl of $CaCl_2$ solutions (2.5 M $CaCl_2$, 10 mM HEPES pH5.5) and diluted with ddH2O to 1ml. The DNA-$CaCl_2$ mixture was mixed with 1 ml of 2X BBS solution (280 mM $NaCl_2$, 1.5 mM Na_2HPO_4, 50 mM BES, pH 6.875) and incubated at room temperature for 20 minutes. After incubation, the complex solution was added to cells drop-wisely with gently swirling. Cells were lysed for analysis 24-30 hours later.

Immunoprecipitation Immunoprecipitation was performed in 293T cells transfected with Tip60, PRAK and other appropriate genes, or from BJ cells stably transduced relevant genes. Cells were washed twice with ice cold PBS and lysed with lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 0.5 mM EDTA, 0.5 mM EGTA, 50 mM NaF, 40 mM β-glycerophosphate, 5 mM tetrasodium pyrophosphate, 0.1 mM sodium vanadate, 2 mM phenylmethylsulfonyl fluoride, 1X protease inhibitor cocktail, 1μM TSA). Cell lysates were sonicated and then cleared by centrifugation at 18,000g, 4^{o} C for 10 minutes. Protein concentrations were determined by BCA protein assay (Pierce). Typically 15-30 μg of protein

lysates was subjected to direct Western blot analysis. For immunoprecipitation, 200-500 μg of cell lysates were mixed with 1 μg of antibody in 500 μl of total volume, incubated at 4° C for overnight with rotating, and then incubated with 10 μl bead volume of pre-washed protein A- or G-agarose beads (Sigma) for additional 2 hours. The beads were spun down at 2,000g, 4° C for 1 minute and washed twice with lysis buffer. The beads were subjected to Western blot analysis after resuspended in 2X Laemmli buffer and heated at 95° C, or to IP-coupled enzymatic assays. For IP-Western analysis of endogenous PRAK acetylation, 4-6 mg total cell lysates in the above lysis buffer were incubated with 40-80 μl (8-16 μg) of a PRAK mouse monoclonal antibody (A-7, Santa Cruz) at 4°C for overnight, after which 20-40 μl of protein G agarose (Pierce) was added and incubated for additional 2 hours. The beads were washed 2X with lysis buffer and boiled with SDS loading buffer. The eluate was resolved by SDS-PAGE for western blot. Acetylated PRAKs were detected by a rabbit monoclonal acetylated-Lys antibody (1:250 dilution, Ac-K2-100, Cell Signaling) and total PRAKs were probed by a rabbit polyclonal PRAK antibody (1:1000 dilution, A302-612A, Bethyl Laboratories).

PRAK kinase assay For acetylation-coupled PRAK kinase assay, 0.5 μg of recombinant PRAK was incubated in a 30 μl volume with 0.4 μg of recombinant Tip60α, 30 ng of MKK6E and 100 ng of p38α in a universal reaction buffer (10 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.4, 0.2 mM DTT, 1 mM ATP, 0.5 mM Acetyl-CoA) at 30° C for 1 hour. 2 μg of HSP27 was then added and the reactions were incubated for additional 15 minutes. For PRAK IP kinase assay, beads with freshly immunoprecipitated PRAK (see above) were washed twice with kinase reaction buffer (10 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.4, 0.2 mM DTT, 1 mM ATP) and incubated in 30 μl volume with 2 μg of HSP27 in kinase reaction buffer for 15 minutes at 30° C. At the end of

incubation with HSP27, all kinase reaction s were mixed with 10 μl of 4X Laemmli buffer, heated at 95° C, and resolved on SDS-PAGE. Phosphorylated HSP27 was detected by Western blotting using a phospho-specific HSP27 antibody (pHSP27-Ser82, Cell Signaling). Total HSP27 protein input was determined by Ponceau staining or by Western blotting using an anti-HSP27 antibody (Cell Signaling). In some cases, the reactions were performed in the presence of [γ-^{32}P]ATP; phosphorylated HSP27 was detected by autoradiography.

Tip60 acetyltransferase assay For phosphorylation-coupled Tip60 acetyltransferase assay, 0.3 μg of recombinant Tip60 was incubated in 30 μl volume with 30 ng of MKK6E and 100 ng of p38α proteins in the universal reaction buffer (10 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.4, 0.2 mM DTT, 1 mM ATP, 0.5 mM Acetyl-CoA) at 30^o C for 20 minutes. 4 μg of histones (Sigma or Millipore) or 0.8-1 μg of His-PRAK was then added and the reactions were incubated for another 1 hour. For IP-coupled Tip60 acetyltransferase assay, beads containing freshly immunoprecipitated Tip60 proteins (see above) were washed twice with universal reaction buffer and incubated in 30 μl volume with 4 μg of histones or 0.8-1 μg of His-PRAK in the reaction buffer at 30^o C for 1 hour. If necessary, ATP was omitted from the reactions and sometimes 133 μM of SB203580 was added before addition of Tip60 or PRAK. After incubation with substrates all acetylation reaction samples were analyzed by Western blotting. While acetylation of histones and PRAK from the recombinant Tip60 assay was detected using anti-acetyl-Lys-Ac-K-103 mouse mAb (Cell Signaling), anti- acetyl-Lys-Ac-K2-100 rabbit mAb (Cell Signaling), or anti-acetyl-Lys-06-933 rabbit pAb (Millipore), that from assays for Tip60 immunoprecipitated from cells (using a mouse monoclonal antibody) was detected using the anti-acetyl-Lys-06-933 rabbit pAb (Millipore).

Mass spectrometry His-Tip60α phosphorylated by p38α and MKK6E in vitro and His-PRAK acetylated by Tip60α in vitro in the presence of p38α and MKK6E were first denatured in 8 M urea and then reduced and alkylated with 10 mM Tris (2-carboxyethyl) phosphine hydrochloride and 55 mM iodoacetamide , respectively.

For phospho-peptide mapping of Tip60, samples were split and digested overnight with either trypsin, Glu-C or elastase , and pressure-loaded onto a 250 micron i.d. fused silica capillary column packed with 3 cm of 5 micron Partisphere strong cation exchange resin and 3 cm of 5 micron C18 resin. After desalting, this bi-phasic column was connected to a 100 micron i.d. fused silica capillary analytical column packed with 10 cm of 5 micron C18 resin. The MudPIT column was placed inline with an Eksigent pump and the eluted peptides were electrosprayed directly into an LTQ Orbitrap mass spectrometer. The buffer solutions used were 5% acetonitrile/0.1% formic acid (buffer A), 80% acetonitrile/0.1% formic acid (buffer B) and 500 mM ammonium acetate/5% acetonitrile/0.1% formic acid (buffer C). A five-step MudPIT was run with salt pulses of 0%, 20%, 40%, 100% and 100% buffer C and a 120 minute elution gradient. A cycle consisted of one full scan mass spectrum (300-1600 m/z) followed by five data-dependent collision induced dissociation (CID) MS/MS spectra. MS/MS spectra were extracted using RawXtract (version 1.9.9) (McDonald et al., 2004). MS/MS spectra were searched with the ProLuCID algorithm (Eng, 1994) against a human IPI database concatenated to a decoy database in which the sequence for each entry in the original database was reversed (Peng et al., 2003). The ProLuCID search was performed using full enzyme specificity, static modification of cysteine due to carboxyamidomethylation (57.02146) and differential modification of serine and threonine due to phosphorylation (79.9663).

For acetyl-peptide mapping of PRAK, samples were digested overnight with trypsin, and pressure-loaded onto a 250 micron i.d. fused silica capillary column packed with 3 cm of 5 micron C18 resin. After desalting, this column was connected to a 100 micron i.d. fused silica capillary analytical column packed with 10 cm of 5 micron C18 resin. The column was placed inline with a 1200 quaternary HPLC pump and the eluted peptides were electrosprayed directly into an LTQ Orbitrap Velos mass spectrometer. The buffer solutions used were 5% acetonitrile/0.1% formic acid (buffer A) and 80% acetonitrile/0.1% formic acid (buffer B). The 180 minute elution gradient had the following profile: 10% buffer B beginning at 15 minutes to 40% buffer B at 105 minutes to 70% buffer B at 150 minutes to 100% buffer B from 155 minutes to 165 minutes. A cycle consisted of one full scan mass spectrum (300-1600 m/z) followed by 20 data-dependent collision induced dissociation (CID) MS/MS spectra. MS/MS spectra were extracted using RawXtract (version 1.9.9) (McDonald et al., 2004). MS/MS spectra were searched with the SEQUEST algorithm (Eng, 1994) against a *Saccharomyces cerevisiae* and *Escherichia coli* database concatenated to a decoy database in which the sequence for each entry in the original database was reversed (Peng et al., 2003) supplemented with UniProt sequences for human Mapk5, Mk14, Mp2k6 and Kat5. The SEQUEST search was performed using full enzyme specificity, static modification of cysteine due to carboxyamidomethylation (57.02146) and differential modification of serine and threonine due to phosphorylation (79.9663) and lysine due to acetylation (42.0106). Up to three modified residues and two missed cleavages were considered.

For both methods, search results were assembled and filtered using the DTASelect (version 2.0) algorithm (Tabb et al., 2002). The peptide identification false positive rate was kept below one percent and all peptide-spectra matches had less than 10ppm mass error.