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Disruption of Serine/threonine Protein Phosphatase 5 (PP5: PPP5c) in Mice Reveals a Novel Role for PP5 in the Regulation of UV Light-induced Phosphorylation of Serine/threonine Protein Kinase Chk1 (CHEK1).

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Experimental Procedures.

ES cell genomic DNA preparation and analysis- The genomic DNA from individual colonies was generated from cell lysates produced by lysing the cells overnight at 37°C in an ES cell lysis buffer (150 mM NaCl, 2 mM EDTA, 20 mM Tris-HCl [pH 8.0], 1.0% SDS, 50 µg/ml Proteinase K). Samples were transferred from the 24-well plates to 1.5 ml centrifuge tubes, 100 µl saturated NaCl was added, and the tube was vigorously shaken. Following a 15 minute, 3,000 X g centrifugation at room temperature, the supernatant was transferred to a 1.5 ml centrifuge tube, and DNA was precipitated with 100% ethanol. The DNA pellet was washed with 70% ethanol and then resuspended in 100 µl TE. Southern analysis was used to screen ES cell colonies. Genomic DNA from each colony was digested with SpeI and hybridized with a specific 3' external probe generated by PCR using the primer pair: 5'-CCT GCT GTA GTA TCC TGC TGT CC-3' and 5'-GGT GCC ATC CCT CCA CGT CCA C-3'. The 3' external probe was amplified using the following PCR conditions: 94°C 2:00min for 1 cycle; 94°C 1:00 min, 50°C 1:00 min, 72°C 1:00 min for 30 cycles; and a final cycle at 72°C for 1:30min. When genomic DNA is digested with SpeI, and hybridized with the 3' external probe, a wild-type PP5 allele produces an 18.5 kb band, and a correctly targeted PP5 allele produces a 6.2 kb band.

Supplemental Figure legends

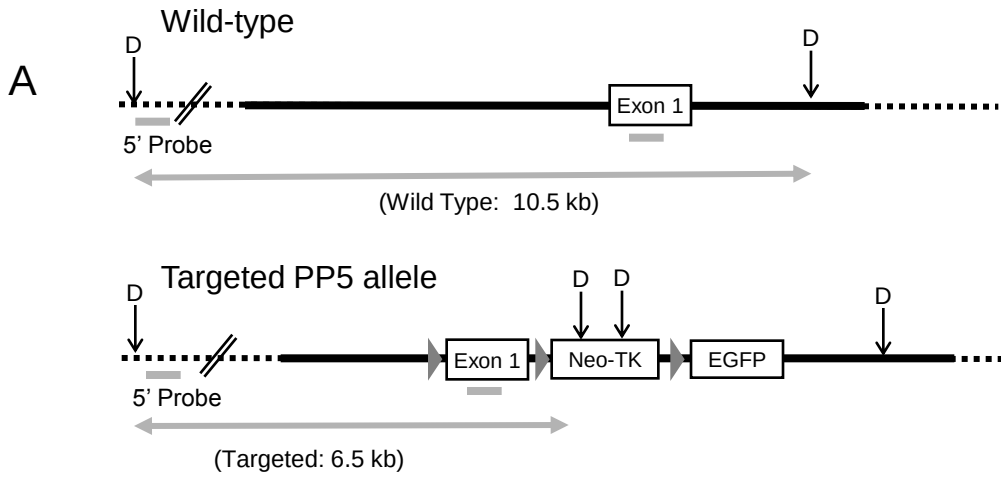
Figure 1. Validation of homologous recombination. A) Schematic diagram of the PP5 wild-type and targeted allele illustrating sites recognized by DrdI (D) and the expected size of DNA produced by digestion of genomic DNA with DrdI from ES cells containing wild-type or the targeted PP5 alleles. B) Autoradiogram showing the hybridization of a [^{32}P]labeled probe to genomic DNA from ES cells following digestion with Drd1. For Southern analysis, probes that target a region of the PP5 gene outside the targeting vector (indicted by dashed line) were used in combination with digestion sites outside and inside the targeting vector to ensure the identification of clones in which homologous recombination had occurred. Both wild-type (10.2 kb) and the 6.5 kb band indicating homologous recombination has occurred are indicated by arrows.

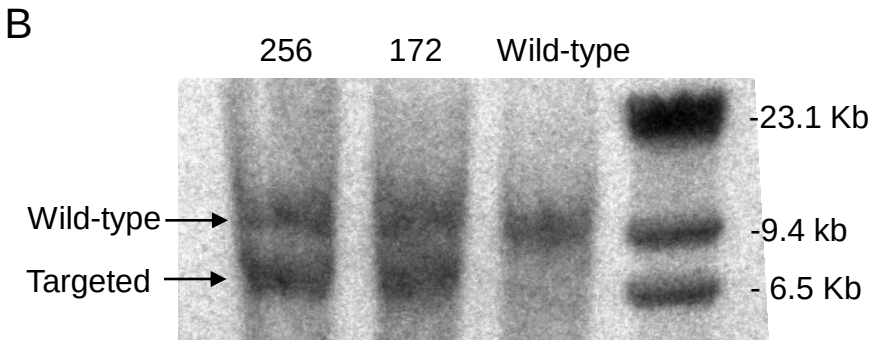
Figure 2. Analysis of PP5 floxed mice. A) Schematic diagram of PCR genotyping using primers that amplify the region between the promoter (P1) and intron 1 (P3) of PP5. Wild-type (WT, +), floxed exon 1 (F) and the knockout (-) alleles are illustrated. B) Image of ethidium bromide stained agarose gel after PCR products were separated by electrophoresis. C) Western blot showing PP5 levels in mouse liver lysates from homozygous floxed (F/F), heterozygous (F/+) and wild-type (+/+) PP5 mice.

Figure 3. Summary of *in vivo* selection marker removal using MeuCre40 mice. The breeding diagram illustrates how the floxed PP5 allele was obtained. Squares and circles indicate male and female mice, respectively. Mice containing the Cre allele are labeled with the letter C inside of the symbol. Diagonal line filled portions of the symbols indicate the presence of the conditional allele, horizontal line filled portions of the symbols indicate the presence of the Neo allele, and dotted portions of the symbols indicate the total recombination allele. C57BL/6J mice are indicated with a smaller black dot inside the symbol. Chimera mice were initially bred to C57BL/6J mice to verify transmission of the PP5-targeted allele (mouse # 1). This mouse was mated to a female MeuCre40 mouse (mouse # 212) and the progeny were screened for the recombination alleles. Many mice contained a combination of alleles. To isolate one allele, mice were mated to C57BL/6J mice. Mouse # 225-1 contained the conditional (PP5 flox) recombination allele. Mouse # 225-5 contained the total ($PP5^{-/-}$) recombination allele.

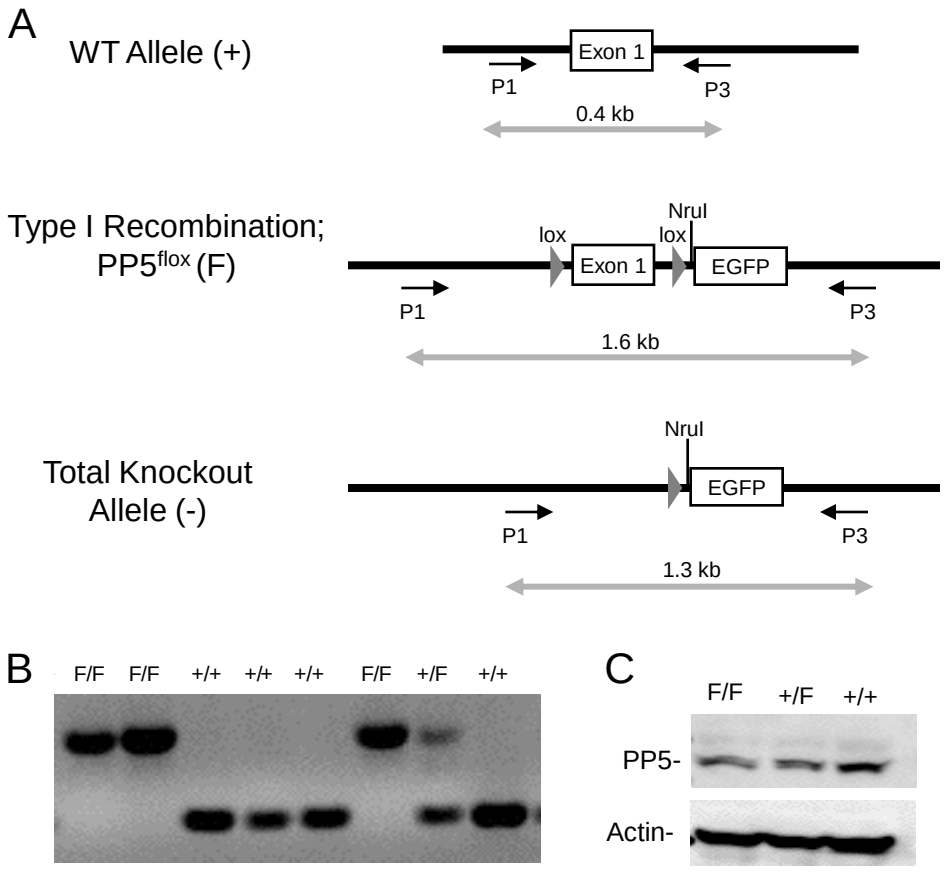
Figure 4. Sensitivity of MEFs to cisplatin. MEFs produced as in Figure 4 were treated with the indicated concentration of cisplatin. After 24 hours, cell viability was measured as described in Figure 4. The data represents the mean ± SEM from three separate experiments in which four replicates plates for each condition and cell type were processed simultaneously.

Figure 5. Phosphorylation of Chk1 (S317) and and Rad17 (S645) occurs in UV-treated $PP5^{-/-}$ MEFs. 13.5 day MEFs derived from $PP5^{+/+}$ (WT) or $PP5^{-/-}$ (KO) littermate embryos were exposed to 24 J/m^2 UV light as in Figure 5. Phosphorylation of Chk1 (S317), Rad17 (S545), H2AX (S139) or H2AX protein levels were detected by Western analysis. Representative images of three separate experiments are shown. * in our hands, the amount of Chk1 (S317) phosphorylation detected following exposure to UV light was highly variable and not highly reproducible.





Amable etal. Figure 1S



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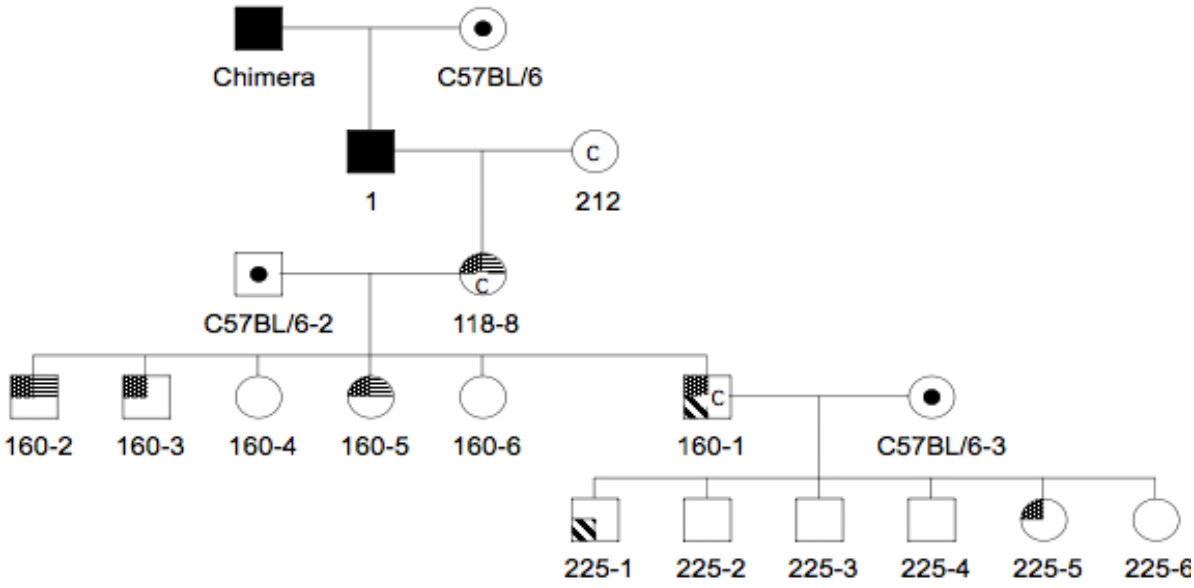

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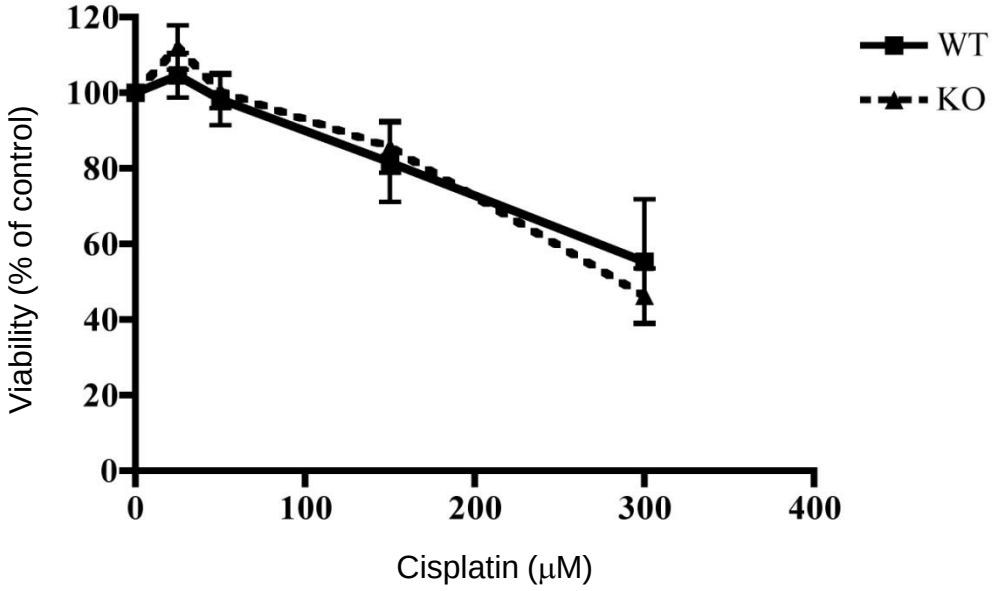

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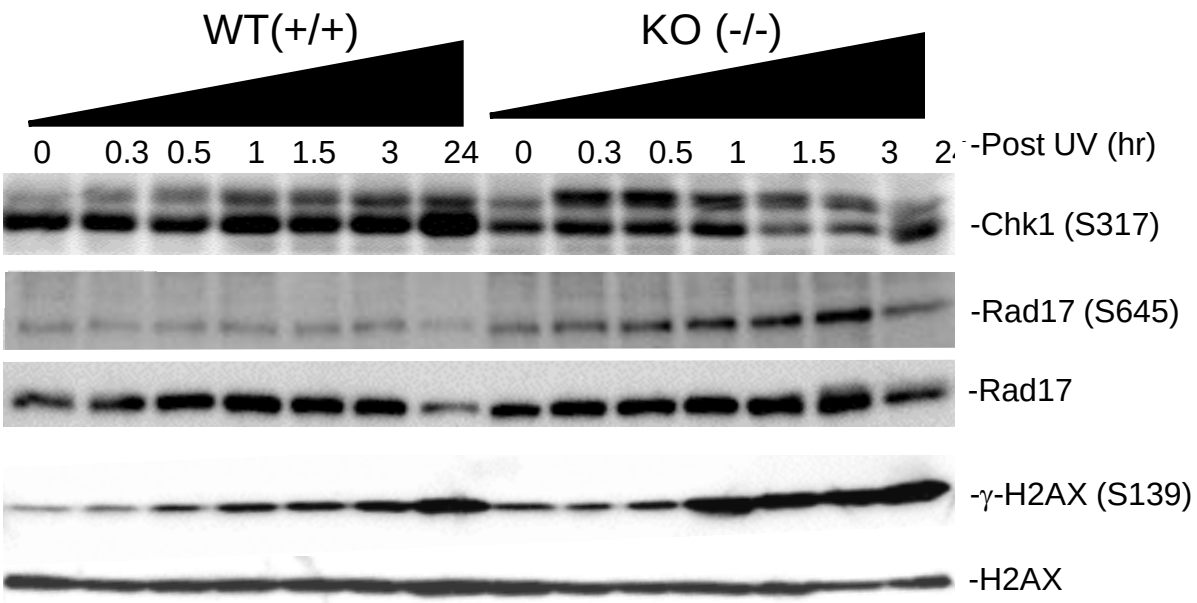

Amable et al. Figure 5S