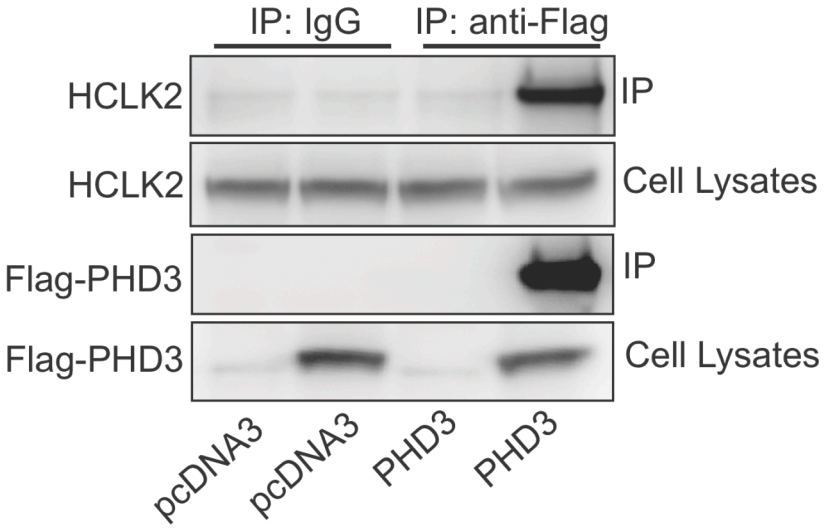

Figure S1.

PHD3 interacts with HCLK2. Flag-PHD3 or empty vector was transfected into Hela cells. Cell lysates were immunoprecipitated with anti-Flag antibody or mouse IgG. Western blots were then performed with the indicated antibodies.

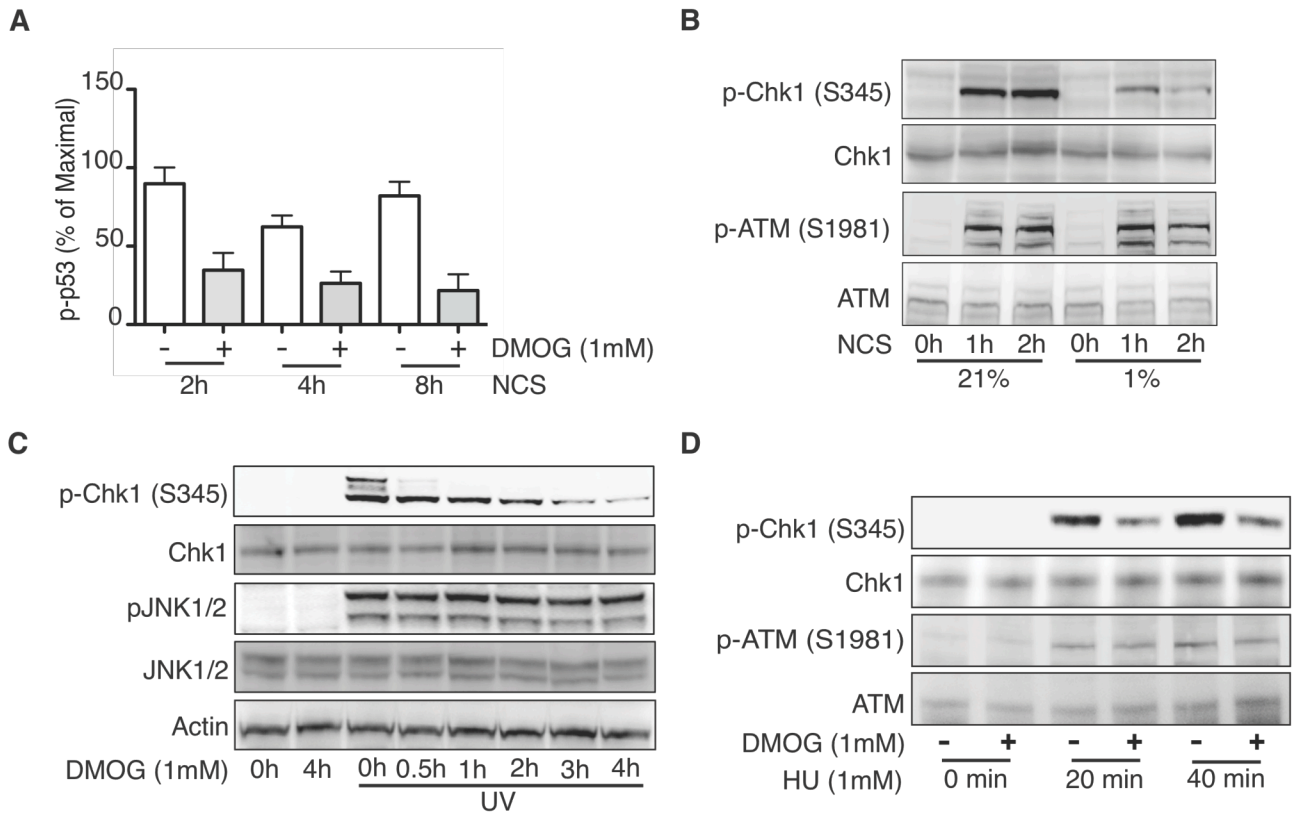

Figure S2.

DMOG or hypoxia specifically inhibits the activation of Chk-1 upon DNA damage. **(A)** Hela cells were pre-treated with DMOG for 4h followed by NCS treatment as indicated. Western blots were performed with anti-phospho-p53 (S15) or p53 antibodies. Densitometry analyses of p-p53 levels that were normalized with p53 levels were performed from three experiments. **(B)** Hela cells cultured at 21% or 1% O_2 tension (for 15 hours) were treated with NCS (200 ng/ml) for one or two hours. Cells were then harvested and western blots were performed with the indicated antibodies. (**C**) After treatment with DMOG (1mM) for the indicated times, Hela cells were exposed to UV light (250 j/m^2) and harvested 2 hours later. Western blots were performed with an anti-phospho-Chk1 or anti-phospho-JNK1/2 antibody. (**D**) After treatment with DMOG (1mM) for 4 hours, Hela cells were treated with 1M hydroxyurea (HU) for the indicated times and western blots were then performed with indicated antibodies.

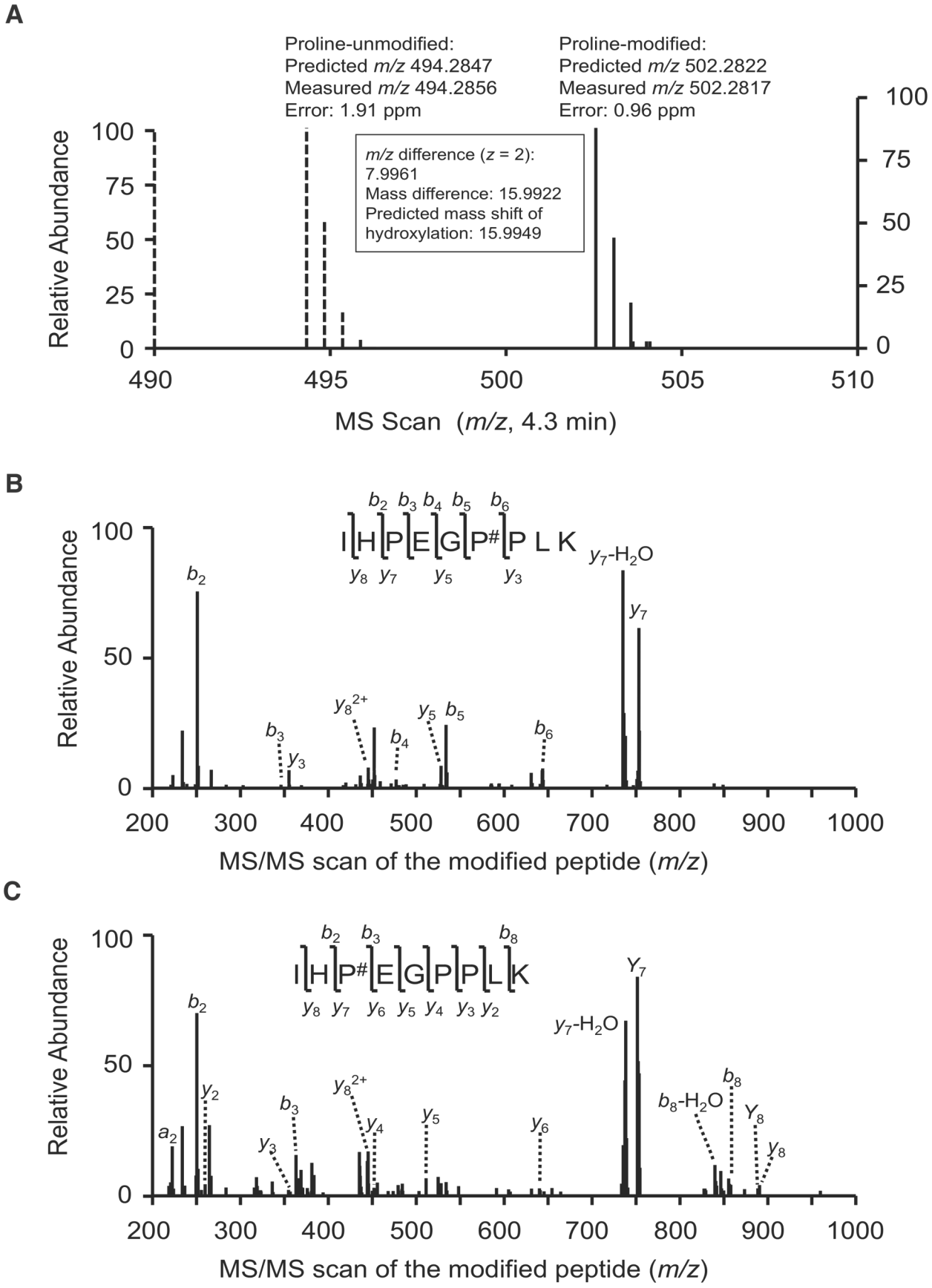
A
Proline-unmodified:
Predicted m/z 494.2847
Measured m/z 494.2856
Error: 1.91 ppm
Proline-modified:
Predicted m/z 502.2822
Measured m/z 502.2817
Error: 0.96 ppm
m/z difference (z = 2):
7.9961
Mass difference: 15.9922
Predicted mass shift of
hydroxylation: 15.9949
Relative Abundance
MS Scan (m/z, 4.3 min)
B
IHPEGP#PLK
Relative Abundance
MS/MS scan of the modified peptide (m/z)
C
IHP#EGPPLK
Relative Abundance
MS/MS scan of the modified peptide (m/z)

Figure S3

Proline hydroxylation of Pro419 and 422 of HCLK2 was identified by the comparison of the unmodified peptide (dashed lines) and the modified forms (solid lines), and the MS/MS spectra of the modified forms. **(A)** The high resolution mass difference between the unmodified and modified forms indicated hydroxylation modification. **(B)** The MS/MS spectrum by collision-induced dissociation showed the modification on Pro422. Characteristic b_6 and y_3 ions could distinguish Pro422 from Pro423 modification, while other ions (e.g. b_3, b_4, b_5, and y_5) could distinguish Pro422 from Pro419 modification. **(C)** During a number of sample analysis, we also found the MS/MS spectrum by higher-energy collisional dissociation indicating possible modification on Pro419. The hydroxylation site was proposed based on the assignment of multiple characteristic weak ions, including b_3, y_4, y_5, and y_6 ions. Under this dissociation condition, we also observed "Y_i" ions (e.g. Y_7 and Y_8 ions, $m/z = y_i - 2$, as previously described by Burlingame et al., http://dx.doi.org/10.1006/meth.1994.1030).

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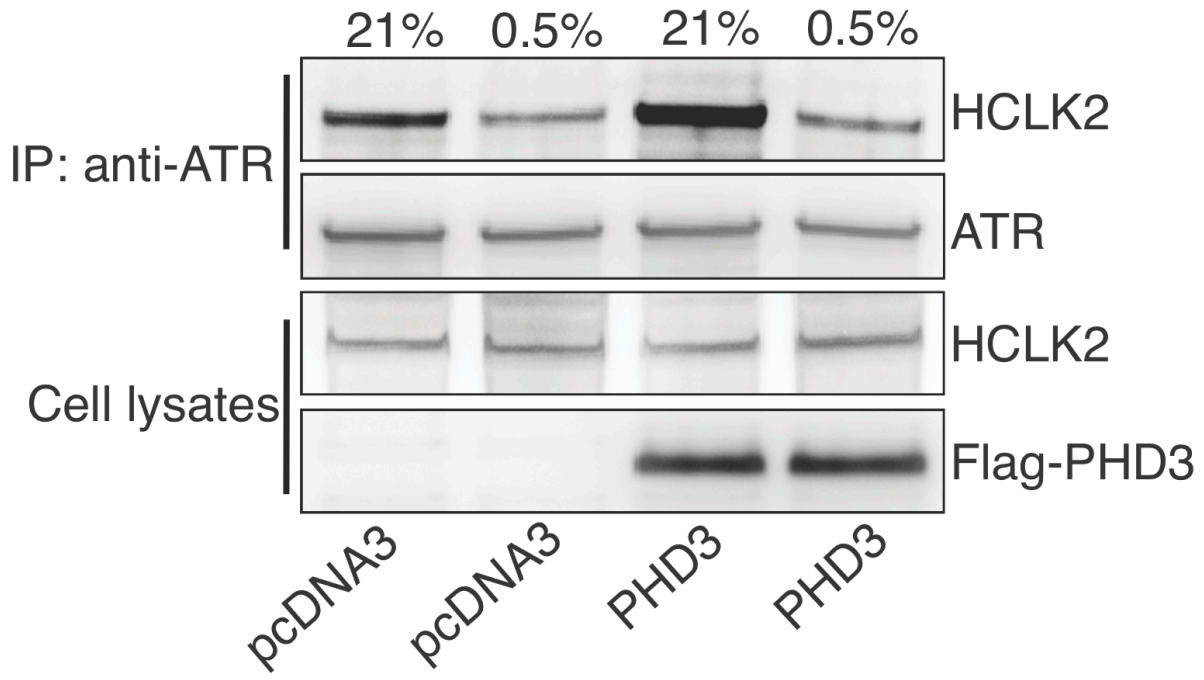

Figure S4

Hypoxia inhibits the interaction between HCLK2 and ATR. Hela cells were co-transfected with ATR and HCLK2 together with pcDNA3 or Flag-PHD3 as indicated. One day later, cells were treated with hypoxia (0.5% O_2) for another 8 hours and then harvested for immunoprecipitation using anti-ATR antibody. Western blots were then performed with the indicated antibodies.

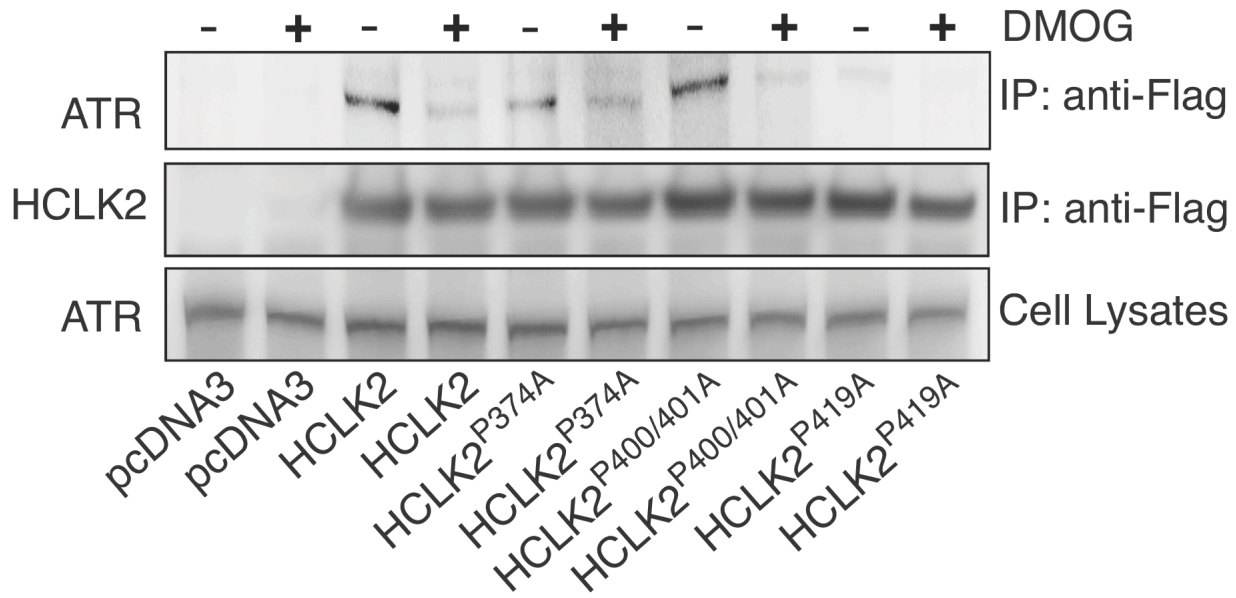

Figure S5

Mutation of Pro374 or Pro419 of HCLK2 impairs the interaction between HCLK2 and ATR. Hela cells transfected with pcDNA3, Flag-HCLK2, Flag-HCLK2^{P374A}, Flag-HCLK2$^{P400/401A}$ or Flag-HCLK2^{P419A} were treated with DMOG for 6 hours. Cells were then harvested and immunoprecipitated with anti-Flag beads. Western blots were performed with the indicated antibodies.

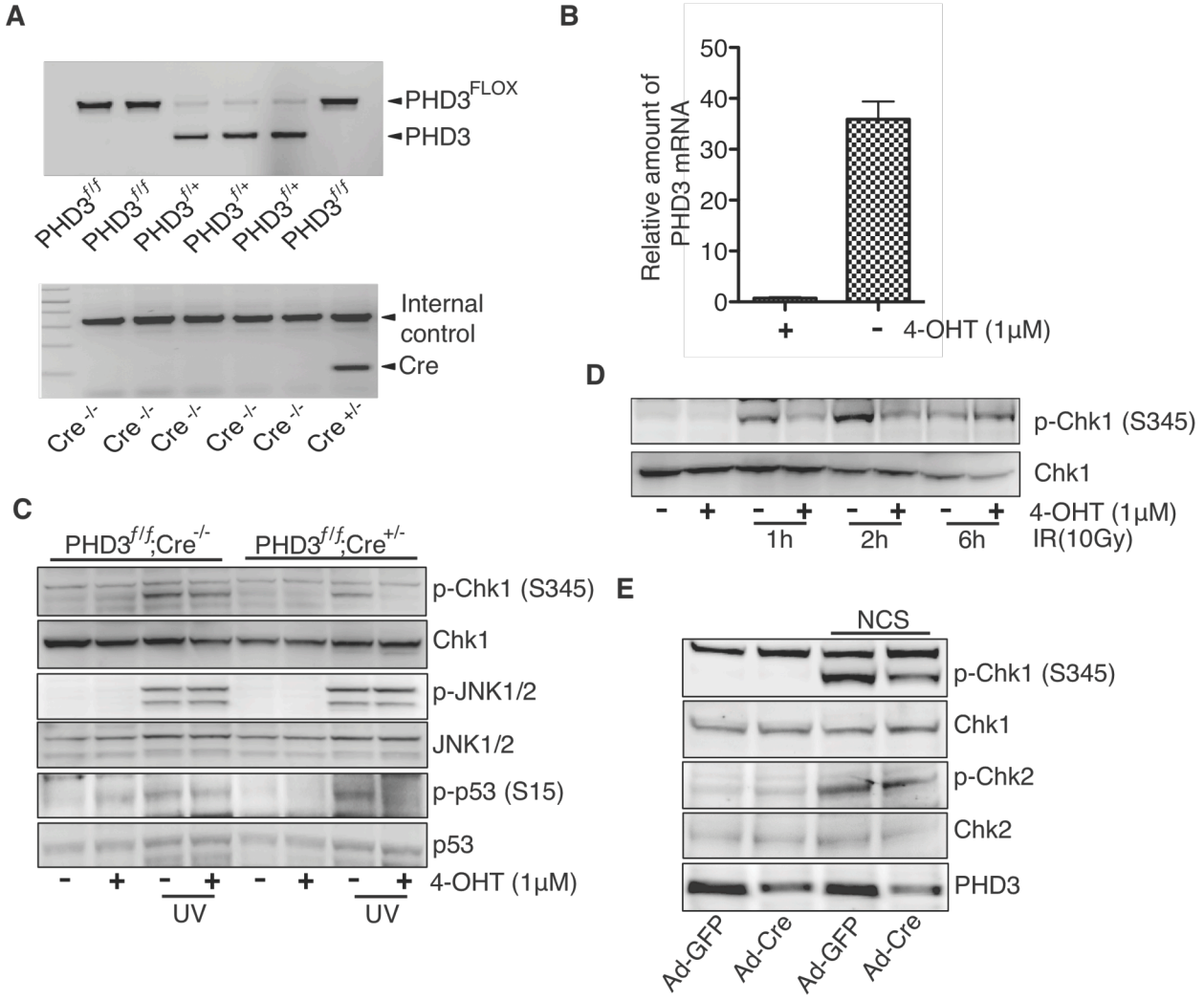

Figure S6

Depletion of PHD3 from PHD3$^{f/f}$; Cre$^{+/-}$ MEFs specifically inhibits the activation of Chk1 induced by UV or IR. **(A)** Genotyping of 6 primary MEF clones isolated at day 13.5 from 6 embryos in the same uterus using primers for PHD3 (upper panel) or Cre (lower panel). **(B)** PHD3$^{f/f}$; Cre$^{+/-}$ MEFs were treated with or without 4-OHT for three days. Total RNA was then isolated and real time PCRs were performed using primers for PHD3 or 18S RNA. The relative mRNA level of PHD3 was plotted from three independent samples. **(C and D)** After treatment with 4-OHT for 3 days, PHD3$^{f/f}$;Cre$^{+/-}$ and PHD3$^{f/f}$;Cre$^{-/-}$ MEFs were exposed to UV light (250 j/m^2) (C) or IR (10 Gy) (D) as indicated. Two hours later, cells were harvested and cell lysates were analyzed by Western blot with the indicated antibodies. **(E)** PHD3$^{f/f}$;Cre$^{-/-}$ MEFs were infected with Ad-Cre or Ad-GFP. After three days, cells were treated with NCS (200 ng/ml) and harvested 2 hours later. Western blots were performed with the indicated antibodies.

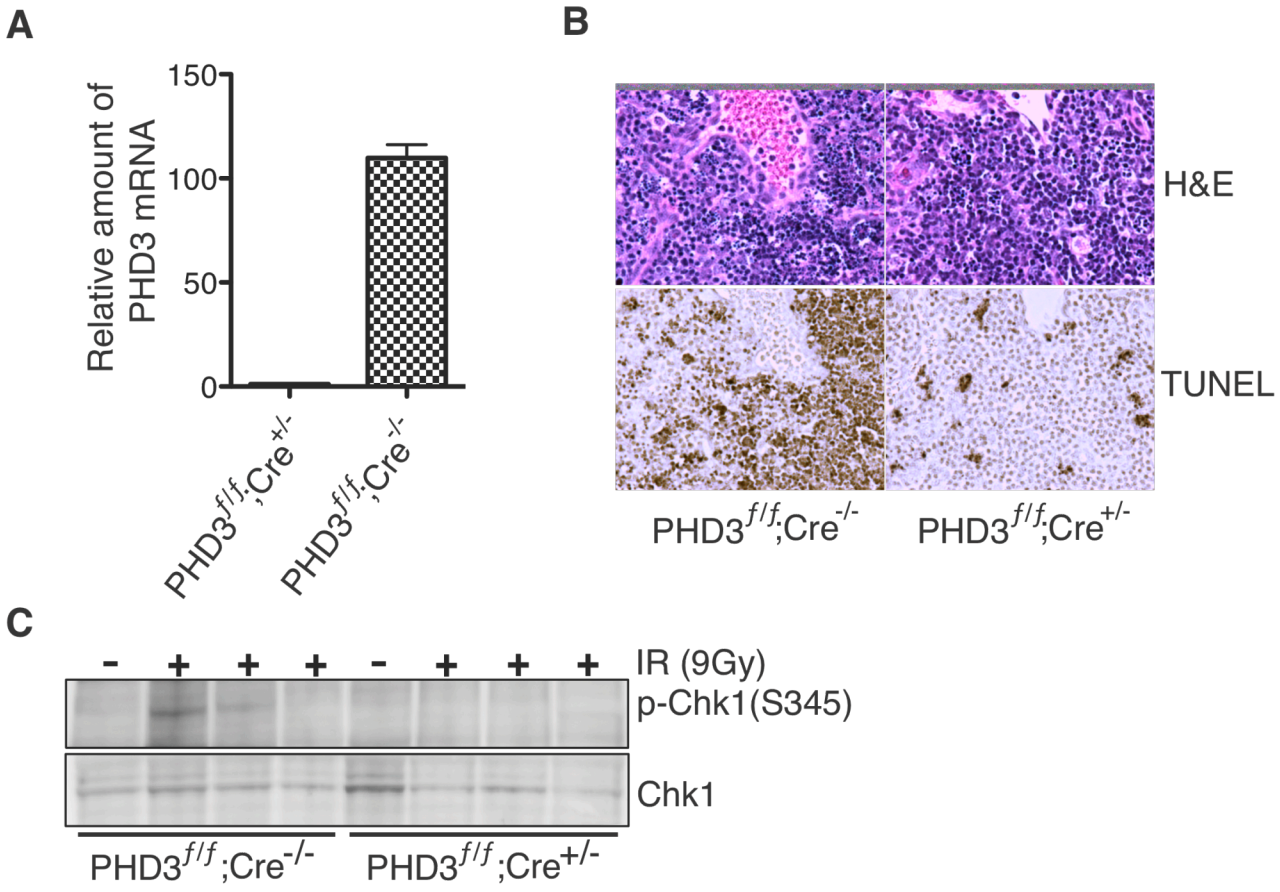

Figure S7

Depletion of PHD3 inhibits Chk1 activation and apoptosis induced by IR in thymuses *in vivo*. Age- and sex-matched 6-8 week-old PHD3$^{f/f}$;Cre$^{+/-}$ and PHD3$^{f/f}$;Cre$^{-/-}$ mice were injected intraperitonealy with tamoxifen (2 mg/20 g body weight/day) for five consecutive days. A week later, **(A)** total RNA was isolated from thymuses and real time PCRs were performed using primers for PHD3 or 18S RNA. The relative mRNA level of PHD3 was plotted (n = 3). **(B)** Mice from the indicated genotypes were treated with IR (9 Gy). After 8 hours, thymuses were harvested and fixed. Hematoxylin/eosin and TUNEL staining were performed as indicated. Representative images are shown. **(C)** Mice from the indicated genotypes were treated with IR (9 Gy, 3 mice/genotype). After 2 hours, thymuses were harvested and Western blots were performed with an anti-Chk1 or anti-phospho-Chk1 antibody.

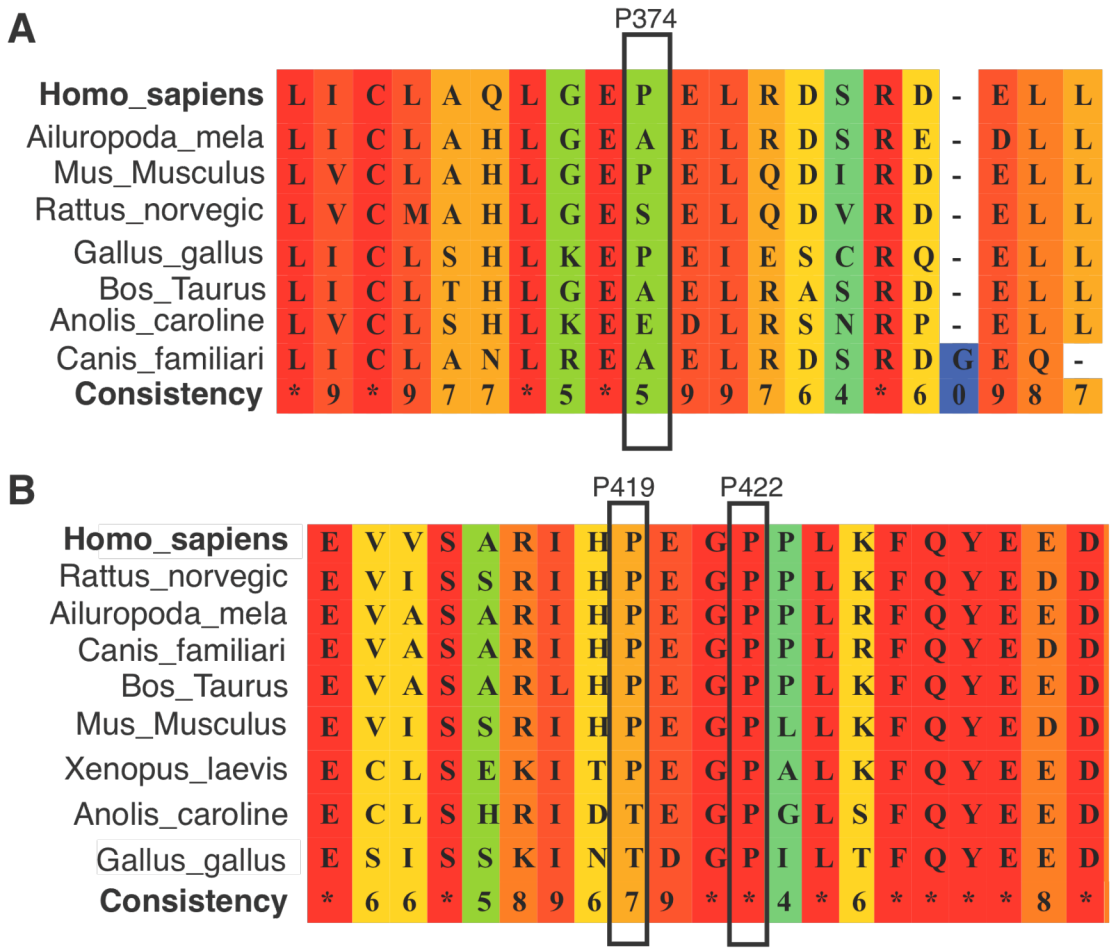

Figure S8

Sequence alignment of vertebrate HCLK2 protein. **(A)** Amino acid sequence alignment between the region of HCLK2 containing Pro374 and the corresponding regions of vertebrate homologs. **(B)** Amino acid sequence alignment between the region of HCLK2 containing Pro419 and P422 and the corresponding regions of vertebrate homologs.