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Supplementary Materials for

Inositol Polyphosphate Multikinase Is a Coactivator of p53-Mediated Transcription and Cell Death

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The PDF file includes:

Supplementary Figure 1

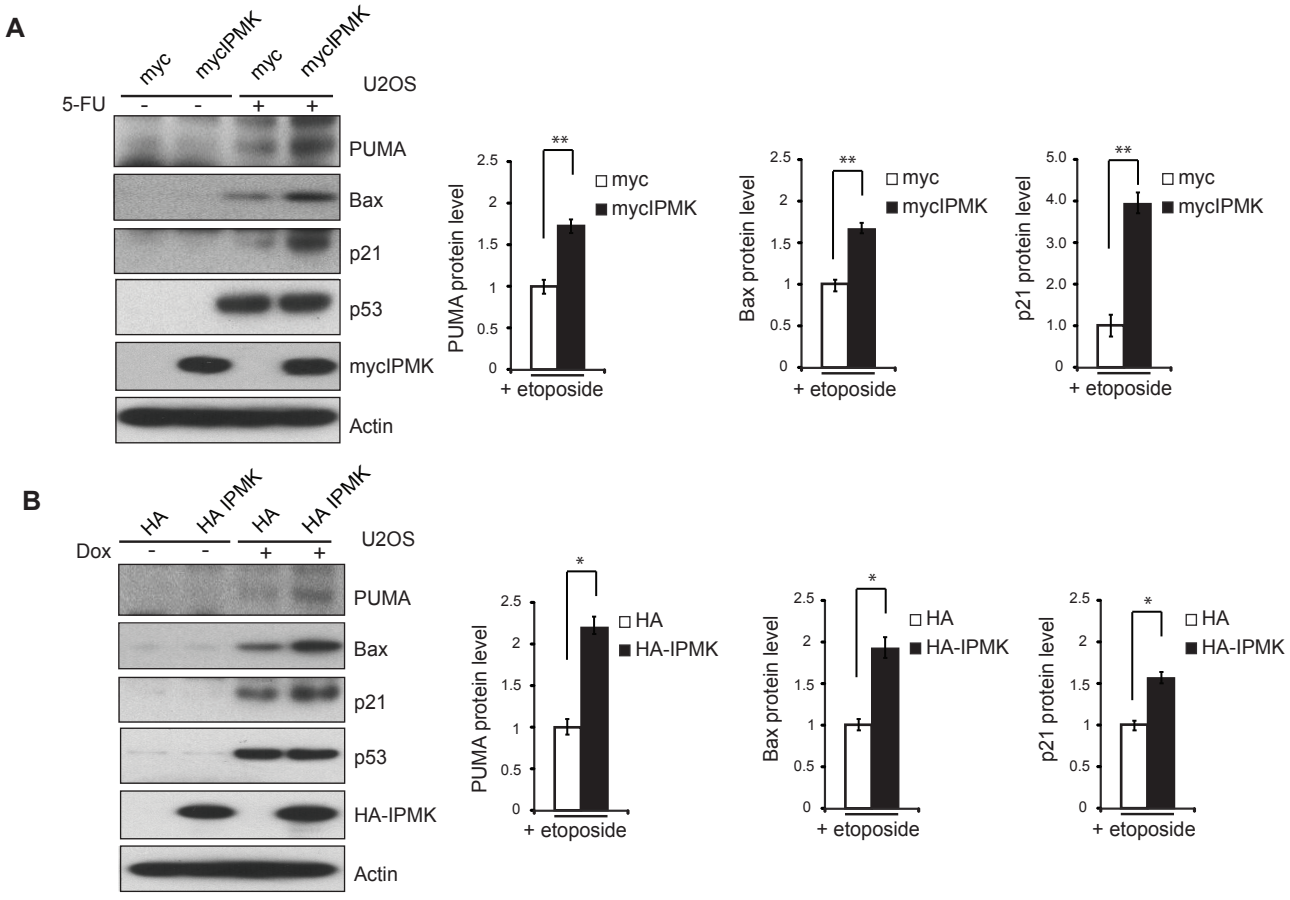


Fig. S1. IPMK regulates expression of p53 downstream targets. (**A**) Western blotting analysis and quantification of PUMA, Bax, and p21 protein abundance in U2OS cells transfected with plasmids encoding myc or mycIPMK and treated with 400 μM 5-fluorouracil (5-FU). (**B**) Western blotting analysis and quantification of PUMA, Bax, and p21 protein abundance in U2OS cells transfected with plasmids encoding HA or HA-IPMK and treated with 1 μM doxorubicin (Dox). Data are means ± SEM of three experiments. *P<0.05, Student's t-test.

Supplementary Figure 2

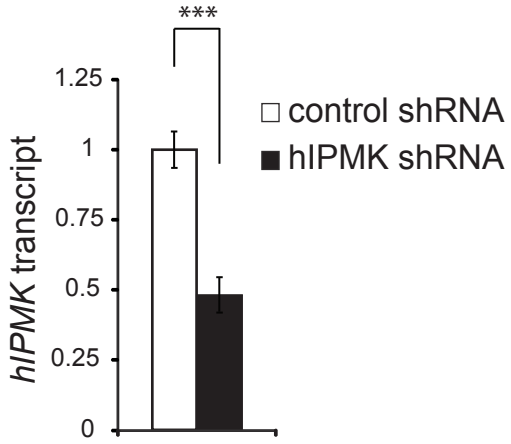
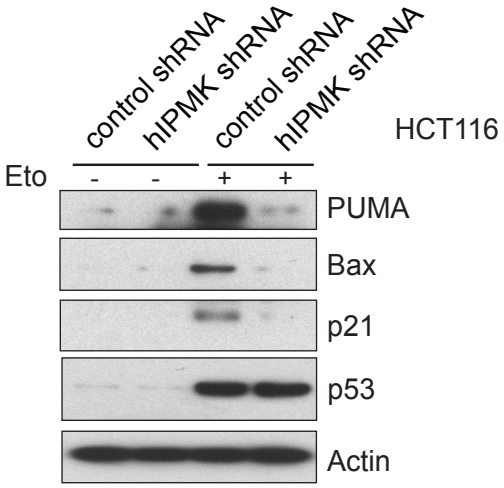
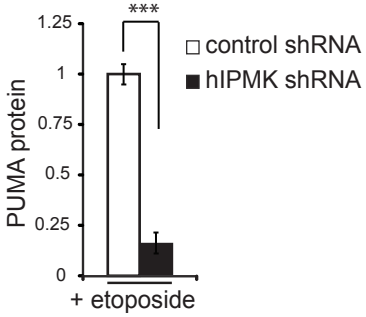
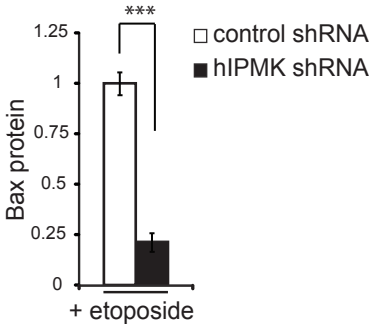


Fig. S2. IPMK is depleted with shRNA. qRT-PCR analysis of IPMK mRNA in HCT116 cells transfected with control or IPMK-specific shRNAs.Data are means ± SEM of four experiments. ***P<0.001, Student's t-test.

Supplementary Figure 3







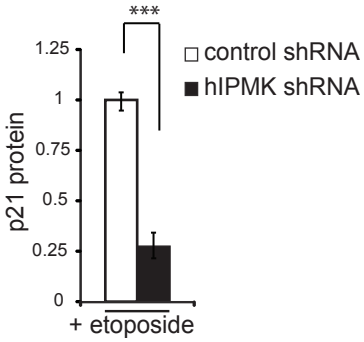


Fig. S3. Protein abundance of p53 targets is decreased by IPMK depletion. Western blotting analysis and quantification of PUMA, Bax, and p21 protein abundance in HCT116 cells transfected with control or IPMK-specific shRNA and treated with 10 μM etoposide. Data are means ± SEM of four experiments. ***P<0.001, Student's t-test.

Supplementary Figure 4

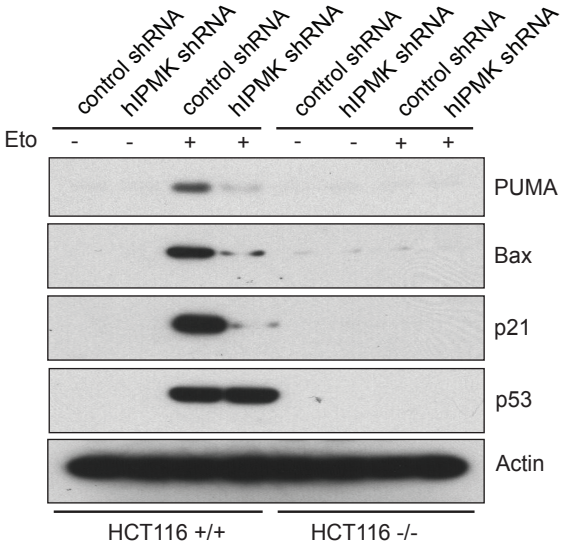


Fig. S4. Abundance of p53 target proteins increased by IPMK is p53-dependent. Western blotting analysis of PUMA, Bax, and p21 abundance in wild-type (+/+) and *p53*-null (-/-) HCT116 cells transfected with hIPMK-specific shRNA and treated with 10 μM etoposide. Blots are representative of 3 experiments.

Supplementary Figure 5

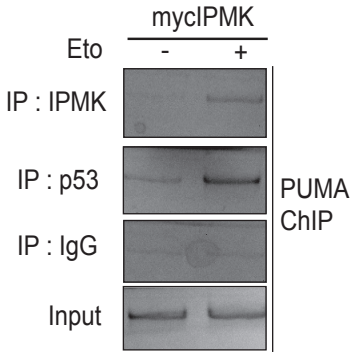


Fig. S5. IPMK binds the PUMA promoter. ChIP analysis of IPMK binding to the promoter of *PUMA* in U2OS cells transfected with plasmids encoding myc or mycIPMK and treated with 10 μM etoposide. Blots are representative of 4 experiments.

Supplementary Figure 6

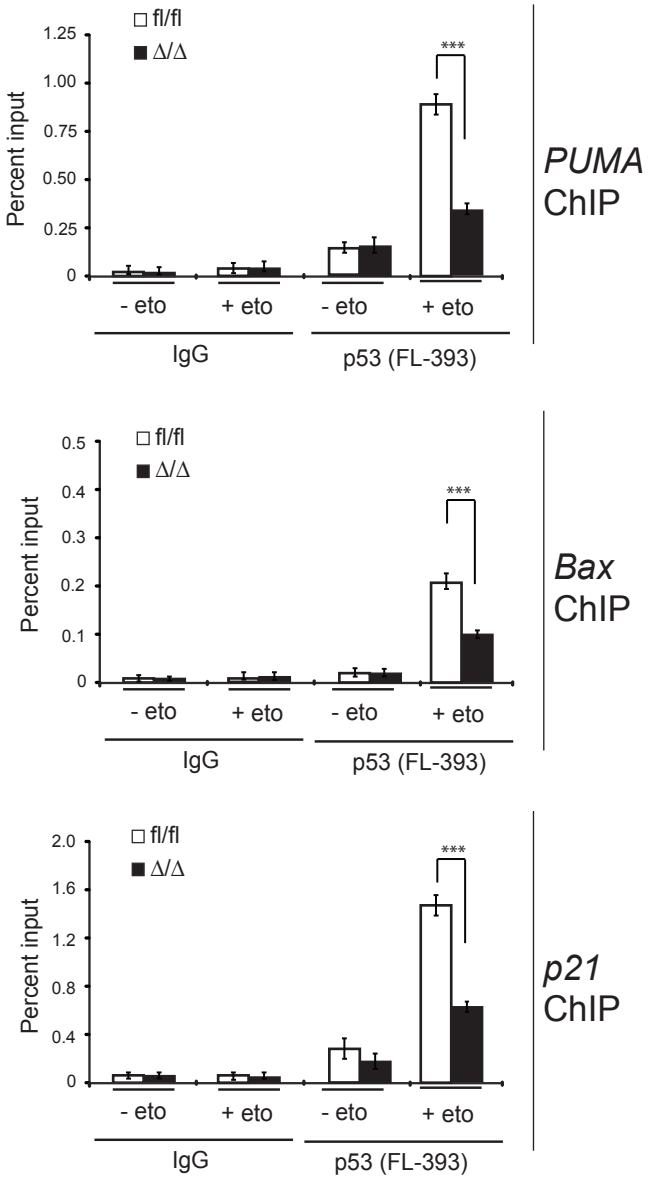


Fig. S6. Genetic deletion of IPMK decreases p53 binding to its target promoters. ChIP analysis of p53 binding to the promoters of *PUMA*, *Bax*, and *p21* in IPMK-deficient (Δ/Δ) MEFs treated with 20 μM etoposide. The FL-393 epitope antibody was used for p53. Data are means ± SEM of three experiments. ***P<0.001, Student's t-test.

Supplementary Figure 7

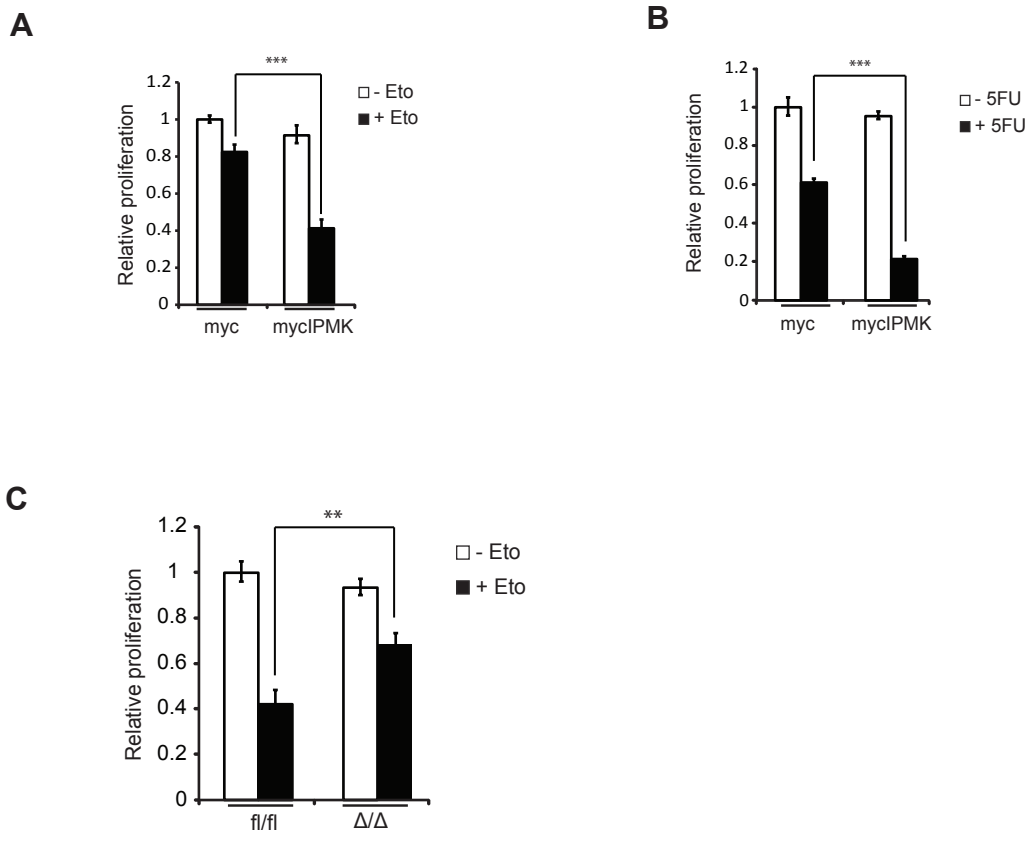


Fig. S7. IPMK inhibits cell proliferation. Proliferation of U2OS cells transfected with plasmid encoding myc or mycIPMK and treated with either 15 μM etoposide (**A**) or 400 μM 5-FU (**B**). ***P<0.001, n=4, mean ± s.e.m., Student's t-test. (**C**) Proliferation of IPMK-deficient (Δ/Δ) MEFs treated with 20 μM etoposide was measured with the MTT assay. Data are means ± SEM of three experiments. **P<0.01, Student's t-test.