Supplementary Material for: "INPP4B Is a PtdIns(3,4,5)P_3 Phosphatase that can Act as a Tumor Suppressor"

This Supplementary Data file includes supplementary figure legends.

Supplementary Figure Legends

Figure S1. *In vitro* PtdIns(3,4,5)P_3–phosphatase assay. (A) FLAG-tagged recombinant INPP4A as well as INPP4B dephosphorylated PtdIns(3,4,5)P_3. Phosphatase activity was assessed by measuring released product/phosphate by the malachite green assay. When the conserved cysteine residue which carries out a nucleophilic attack on phosphorylated substrates was replaced with a serine (INPP4A C895S and INPP4B C842S), the hydrolytic activities were abolished, demonstrating that the PtdIns(3,4,5)P_3–phosphatase activities were intrinsic to these INPP4 isozymes. INPP4B dephosphorylated PtdIns(3,4)P_2 but not PtdIns(4,5)P_2. Note that there was no difference between wild type and the CS mutant in PtdIns(4,5)P_2 phosphatase activity. Phosphatase activity was assessed by mea (B) ^{32}P-labeled PtdIns(3,4,5)P_3 was synthesized from PtdIns(4,5)P_2 by the action of recombinant class IA PI3-kinases. The resultant PtdIns(3,4,5)P_3 was incubated with recombinant INPP4A, INPP4B, PTEN and

SHIP1. PtdIns(3,4,5)P_3 phosphatase activities were assessed by measuring radioactive intensities of the substrate/PtdIns(3,4,5)P_3 spot on TLC plates.

Figure S2. H&E staining of thyroid and lung from a 40-week-old *Inpp4B*$^{+/\Delta}$;*Pten*$^{+/-}$ mice. Thyroid histopathologies and pulmonary metastasis were observed in *Inpp4B*$^{+/\Delta}$;*Pten*$^{+/-}$ mice just as observed in *Inpp4B*$^{\Delta/\Delta}$;*Pten*$^{+/-}$ mice. Asterisk, trachea. Arrows, thyroid follicular cells stuck at pulmonary alveoli.

Figure S3. More frequent INPP4B protein reduction in FV-PTCs comparing to non FV-PTCs. The "OncoPrint" results were analyzed based on the International Classification of Disease for Oncology histology code, and the percentage of samples with decreased INPP4B protein in FV-PTCs or non-FV-PTCs were calculated.

Figure S4. Representative fluorescent immunohistochemical analyses of sections of human follicular thyroid carcinomas classified in the Low, Medium and High groups of INPP4B and PTEN protein expression (see METHODS). Control (goiter) staining fitted into the Medium fluorescence group. Bars:

Figure S5. Coincident mutations in *PTEN* and *INPP4B* genes in thyroid carcinomas and endometrial carcinomas. An “OncoPrint” (upper panels) and a summary table of “mutually exclusivity and co-occurrence analysis” (lower panels) of the 511 cases of papillary thyroid cancers (A) and 240 cases of uterine corpus endometrial carcinomas (B) listed in The Cancer Genome Atlas (TCGA) database (cBioPortal). In the upper panels, individual genes are represented as rows, and individual patients are represented as columns.

Figure S6. Quantitation of HPLC analyses of phosphoinositides prepared from *Pten*$^{+/-}$ and *Inpp4B*$^{\Delta/\Delta}$*;Pten*$^{+/-}$ thyroid glands from mice that were metabolically labeled *in vivo* with [^{32}H]-phosphorus (see METHODS). PtdIns(5)P, PtdIns(3,5)P_2 and PtdIns(3,4,5)P_3 were undetectable under the experimental conditions used. PtdIns(3,4)P_2 levels are expressed as a percentage relative to the sum of PtdIns(4,5)P_2 and PtdIns(4)P and are the mean ± SEM (n = 3). A representative of three independent experiments with similar results is shown.