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SUPPLEMENTARY MATERIAL

corresponding to:

Expression analysis and essential role of the putative tyrosine phosphatase His-domain-containing protein tyrosine phosphatase (HD-PTP)

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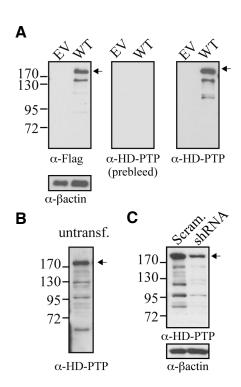


Figure S1. Characterization of the anti-HD-PTP antibody. (A-B) The efficiency of the anti-HD-PTP rabbit polyclonal antibody produced against a HIS-HD-PTP fragment (human amino acids 400-800) to recognize the overexpressed (A) or endogenous (B) HD-PTP protein was tested by western blot on total cell extracts from empty vector (EV) or Flag-HD-PTP wild-type (WT) transfected (A) or untransfected (B) 293T cells and compared to the prebleed. The anti-βactin antibody was used as a loading control. Unlike the prebleed, the anti-HDPTP antibody recognizes HD-PTP protein as a major band at 185 kDa (arrow) and minor bands corresponding to degradation products. (C) Renal carcinoma cell ACHN were stably downregulated for HD-PTP expression using the Mission shRNA lentiviral system (Sigma). Endogenous HD-PTP expression was assayed by western blot using the anti-HD-PTP antibody. Unlike a scrambled sequence (Sram.), shRNA 3047 which specifically targets the HD-PTP sequence leads to a severe decrease in HD-PTP full length expression (arrow) and its degradation products. Actin levels were monitored as a loading control.

f1	5'-TGG AAG TGC ATG AAA AGG CTT-3'
r1	5'-CCC GCA TCT CCT GCA CCT TGG CGA-3'
r2	5'-CCT TGA AGG ACT CCA ATA GGG TAC C-3'
GAPDH f	5'-CTC ATG ACC ACA GTC CAT GC-3'
GAPDH r	5'-CAC ATT GGG GGT AGG AAC AC-3'

Figure S2. Primer sequences. Forward (f) and reverse (r) primers used in figure 1C and 1D to genotype embryos and mice by PCR and RT-PCR. f1: forward primer located in the Ptpn23 exon 16, r1: reverse primer located in the Ptpn23 exon 17, r2: reverse primer located in the β -geo cassette. GAPDH, forward (f) and reverse (r) primers amplifying GAPDH RNA control.