Supplemental Materials and Methods

Survival analysis in TRAMP mice. The *Hip1*$^{null/null}$ and *Hip1*$^{\Delta 3\text{-}5/\Delta 3\text{-}5}$ mice were maintained on a mixed C57BL/6;129svJ background, and the TRAMP mice were maintained on a pure C57BL/6 background. Intercross matings of TRAMP and *Hip1*$^{null/null}$ and *Hip1*$^{\Delta 3\text{-}5/\Delta 3\text{-}5}$ mice were performed as previously described (1). Fourteen TRAMP;*Hip1*$^{\Delta 3\text{-}5/\Delta 3\text{-}5}$, 13 TRAMP;*Hip1*$^{+/\Delta 3\text{-}5}$, 18 TRAMP;*Hip1*$^{+/null}$, and 15 TRAMP/*Hip1*$^{null/null}$ littermates were analyzed for tumors at 6.5 months. Prior to these necropsies, a group of mice from these cohorts either unexpectedly died or became moribund and required euthanasia. This survival data for each of these observation groups was compared.

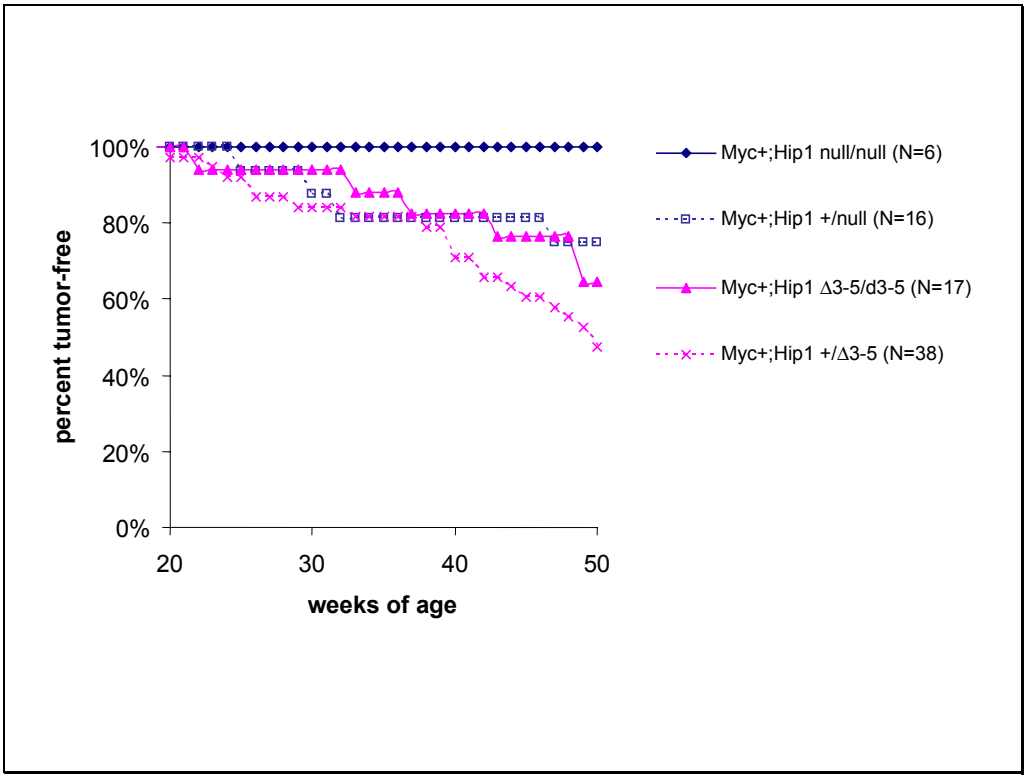
RNA isolation and northern blot. Total RNA was isolated from cultured tumor cells, tissues, and early passage mouse embryonic fibroblast (MEF) extracts using the TRIzol reagent (Invitrogen). Poly (A) RNA was isolated from total RNA using the Poly(A) Purist MAG protocol (Ambion). Then, 5ug of this poly(A) RNA was separated on a 1% agarose gel with 6% formaldehyde, stained with ethidium bromide, transferred to Nytran membrane (Schleicher & Schuell), and cross-linked. The membrane was prehybridized in a buffer containing 5X SSC, 5X Denhardt solution, 1% sodium dodecyl sulfate (SDS) (wt/vol), and 100 µg of denatured salmon sperm DNA/ml for 3 h at 65°C. For the mouse *Hip1* Northern probe, an 850-bp *Eco*R1 and *Not*1 digested fragment encoded by m*Hip1* exons 10-14 was used. The probe was ^{32}P labeled using a random-primed labeling kit according to manufacturer's directions (Roche). The blot was hybridized overnight at 65°C, washed twice in 2X SSC for 20 min, once in 1X SSC for 10 min, and twice in 0.1X

SSC for 10 min. The blot was exposed for 4 to 5 days on Kodak Biomax film. The mRNA abundance was normalized with the signal for glyceraldehydes-3-phosphate dehydrogenase (GAPDH).

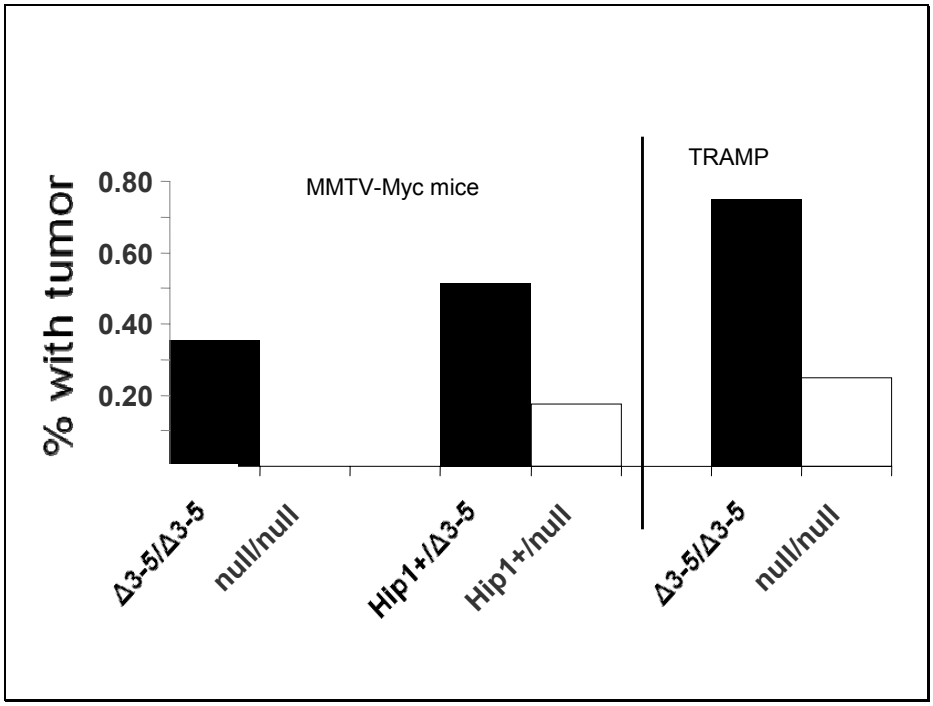
Tissue preparation and western blot analysis. Tissue harvesting, preparation, and immunoblotting of tissues was performed as previously described (2). A polyclonal anti-HIP1 (1:5,000, UM354) antibody was used to detect HIP1 expression in MMTV-myc, TRAMP and Mx1-Cre mice, and polyclonal anti-actin (1:1000, Sigma) antibody was used as a control.

Co-immunoprecipitation of HIP1 and EGFR and endocytic factors. Full-length and mutant EGFR and HIP1 cDNA constructs in pcDNA3 have previously been described (3). A 15-cm dish of 70% confluent 293T cells was transfected with 20 μg of HIP1 cDNA and 20 μg of EGFR cDNA using Superfect reagent (Qiagen). Twenty-four hours post-transfection, the cells were lysed using an all-purpose lysis buffer [50 mmol/L Tris (pH7.4), 150 mmol/L NaCl, 1% Triton X-100, 1.5 mmol/L MgCl2, 5 mmol/LEGTA, 10% glycerol, Complete EDTA-free protease inhibitor tablets (Roche), 30 mmol/L sodium pyrophosphate, 50 mmol/L sodium fluoride, and 100μmol/L sodium orthovanadate]. Five milligrams of protein were incubated with pre-immune serum, polyclonal anti-HIP1 serum (UM323), or sheep polyclonal anti-EGFR (Upstate). One hundred microliters of a 3:1 slurry of protein G-sepharose beads (GE Healthcare) in lysis buffer were then incubated with the lysate-antibody mixture at 4ºC for 60 min with rotation. The protein G pellets were washed four times with 1 mL of lysis buffer. The

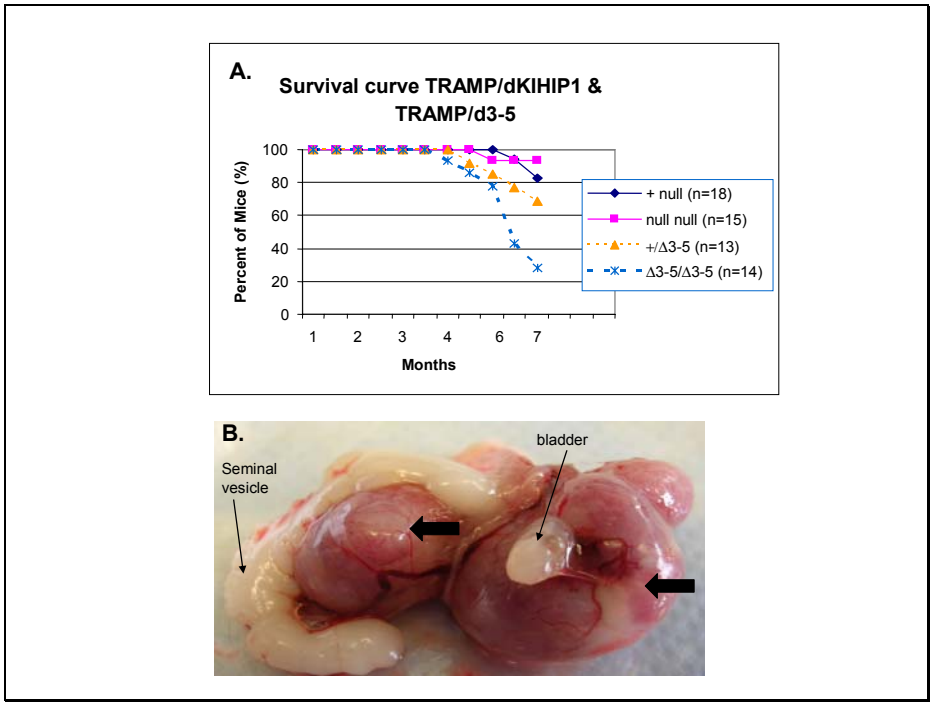
entire pellet was dissolved in SDS sample buffer, boiled for 5 min, separated on 7% SDS-PAGE, and transferred to nitrocellulose membranes. Antibodies used for western blot analysis were the anti-HIP1/4B10 antibody (mouse monoclonal, human anti-HIP1 immunoglobulin G1, 400 ng/mL), anti-adaptin-α antibody (rabbit polyclonal, BD Biosciences), anti-clathrin heavy chain TD-1 antibody (kind gift of Linton Traub, University of Pittsburgh), and an anti-EGFR antibody (sheep polyclonal antibody, Upstate Biotechnology, Charlottesville, VA).



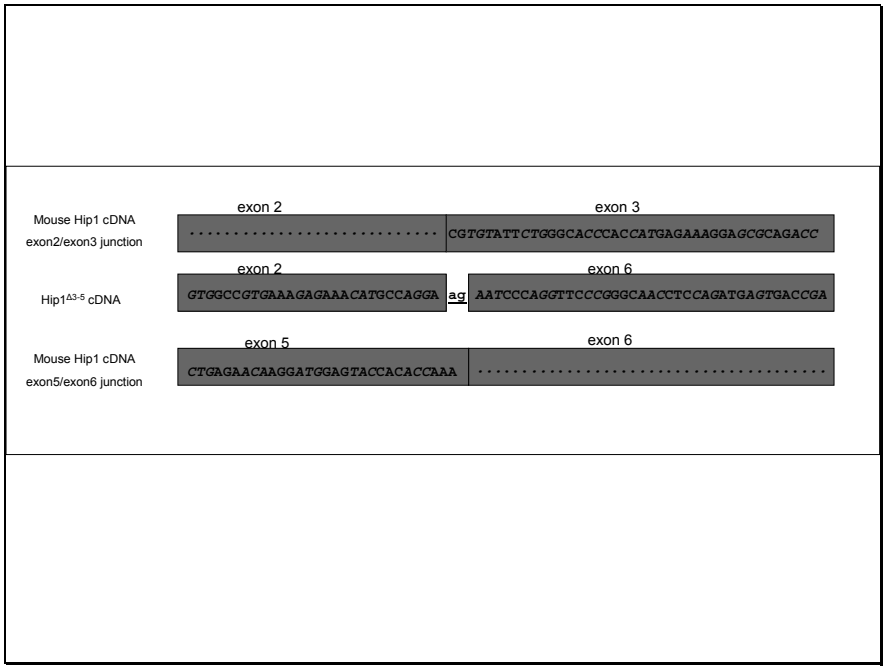
Supplemental Figure 1. Tumorigenesis in MMTV-myc mice in the absence of HIP1. Percent tumor-free survival out to 50 weeks of age is shown for MMTV-Myc+ mice on various HIP1 knockout backgrounds. Mice were sacrificed when the tumor ulcerated or impeded movement.



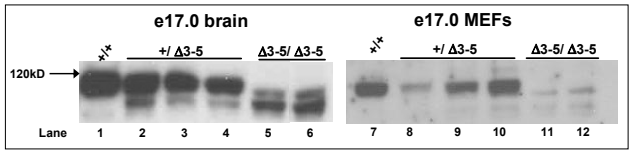
Supplemental Figure 2. Tumor incidence in mice with Hip1D3-5 and Hip1Null alleles. MMTV-myc transgenic and TRAMP mice were mated with mated on to $HIP1^{null/null}$ and $HIP1^{\Delta 3\text{-}5/\Delta 3\text{-}5}$ backgrounds and analyzed for tumor incidence. TRAMP mice that survived to 6 months of age were scored for gross prostate tumors. In each case the mice with the D3-5 allele were afflicted with more tumors.



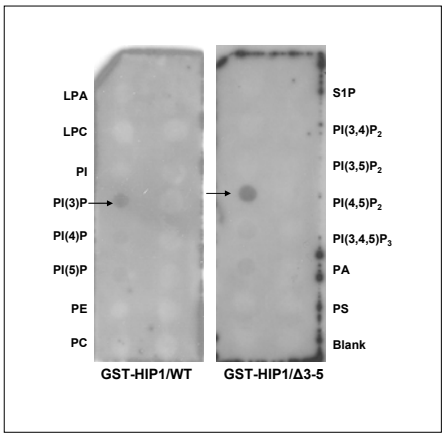
Supplemental Figure 3. A. Survival curve of TRAMP mice in different Hip1 mutant backgrounds. **B.** Example of bilateral synchronous prostate tumors. Arrows indicate tumors arising from distinct lobes of the prostate (not visible).



Supplemental Figure 4. Partial Hip1Δ3-5 cDNA sequence alignment with wild type mouse Hip1 cDNA. Shown is an alignment of cDNA sequence from the nested PCR reaction with the exon 2/3 and exon 5/6 junctions of mouse *Hip1* mRNA reference sequence (NM_146001). Dots in the moue *Hip1* mRNA reference sequence denote identity with the *Hip1* D3-5 cDNA sequence. Codons are represented by alternating bold/nonbold trinucleotide sequences. Note the AG dinucleotide insertion between exons 2 and 6 of the Hip1D3-5 cDNA sequence, which maintains the open reading frame of the transcript.



Supplemental Figure 5. HIP1Δ3-5/insAG 106 kDa protein is present in mouse embryonic brain and fibroblasts. Western blot analysis for HIP1 expression in the brains (left panel) and fibroblasts (right panel) of 17-day old mouse embryos of different genotypes demonstrated the presence of a slightly truncated HIP1 product. MEF cells of different genotypes were cultured from eleven individual embryos from the same mother. Only 5 samples are shown here but all demonstrated similar banding patterns as related to the distinct genotypes.



Supplemental Figure 6. Comparison of lipid binding specificity and relative affinity GST-5'HIP1/Δ3-5 and GST-5'HIP1 using PIP strips. Protein solutions containing 10μg of either purified GST-5'HIP1 or GST-5'HIP1/Δ3-5 protein in TBST with 1% milk were incubated with PIP strips (Echelon) containing 15 different lipids at 100 pmol/spot. Lipid-protein interactions were detected using a polyclonal antibody (UM354) that recognizes both GST-5'HIP1 and GST-5'HIP1/Δ3-5 proteins. Both proteins bound preferentially to PI(3)P on the PIP strips.

Supplemental Data References

1. Bradley SV, Oravecz-Wilson KI, Bougeard G*, et al.* Serum antibodies to huntingtin interacting protein-1: a new blood test for prostate cancer. Cancer Res 2005;65(10):4126-33.
2. Hyun TS, Li L, Oravecz-Wilson KI*, et al.* Hip1-related mutant mice grow and develop normally but have accelerated spinal abnormalities and dwarfism in the absence of HIP1. Mol Cell Biol 2004;24(10):4329-40.
3. Bradley SV, Holland EC, Liu GY, Thomas D, Hyun TS, Ross TS. Huntingtin interacting protein 1 is a novel brain tumor marker that associates with epidermal growth factor receptor. Cancer Res 2007;67(8):3609-15.