Howitt et al., http://www.jcb.org/cgi/content/full/jcb.201105009/DC1

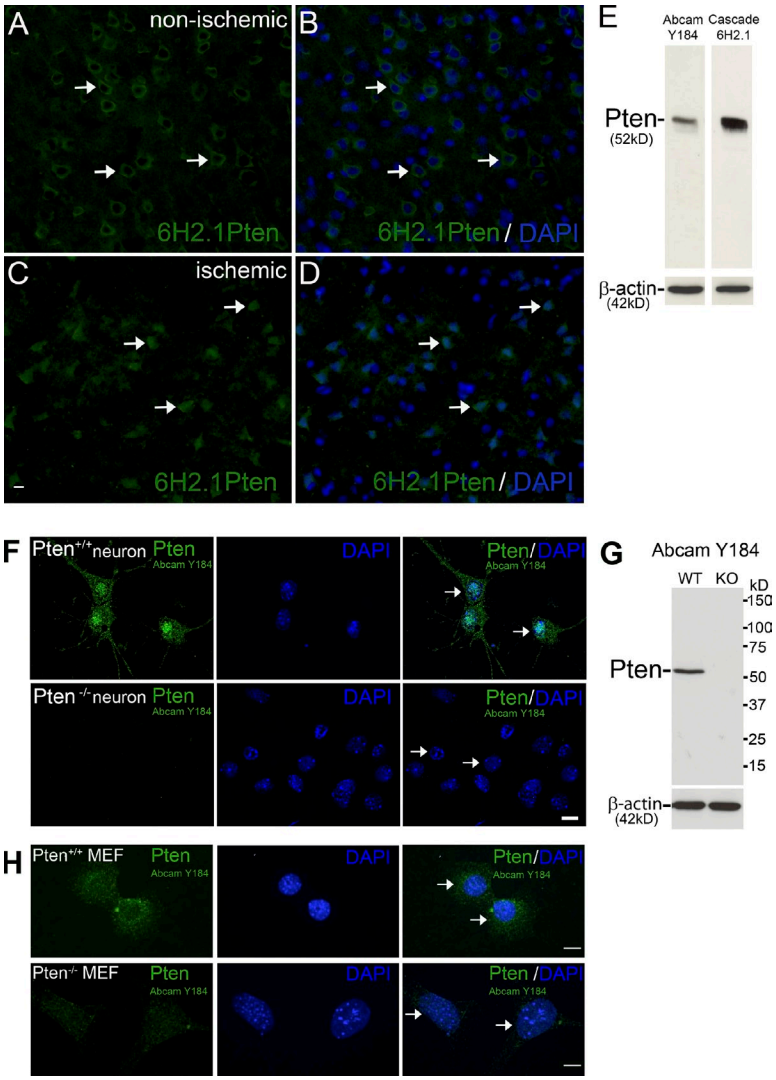

Figure S1. **Abcam and Cascade BioScience antibodies are specific for Pten in mouse brain tissue and fibroblasts.** (A and B) In the nonischemic mouse cortex, PTEN 6H2.1 antibody shows predominantly cytoplasmic Pten staining (arrows), similar to staining observed using PTEN Y184 antibody in Fig. 1 (A and C). (C and D) In ischemic neurons, PTEN 6H2.1 antibody shows predominantly nuclear staining (arrows), similar to staining observed using PTEN Y184 antibody in Fig. 1 (D and F). (E) In Western blots, both Y184 and 6H2.1 recognize a Pten-specific band (~52 kD) in mouse cortex. (F and G) Mouse primary neurons (E15 and 5 d in vitro) from *Pten*$^{loxp/loxp}$*;Nestin-Cre* mice were used to test the specificity of PTEN Y184 antibody. In *Pten*$^{+/+}$ neurons, Pten staining was observed in the cytoplasm and nucleus of the cell, but no staining was observed in *Pten*$^{-/-}$ neurons (arrows). Western blotting of primary cultures recognized a Pten-specific band (~52 kD) in *Pten*$^{+/+}$ neurons but not in *Pten*$^{-/-}$ neurons. (H) Similar results were observed after staining with Y184 antibody in WT (*Pten*$^{+/+}$) MEFs and Pten-deficient MEFs (arrows), the generation of which has been described previously (images are in extended focus; Chen et al., 2005). Bars, 10 µm.

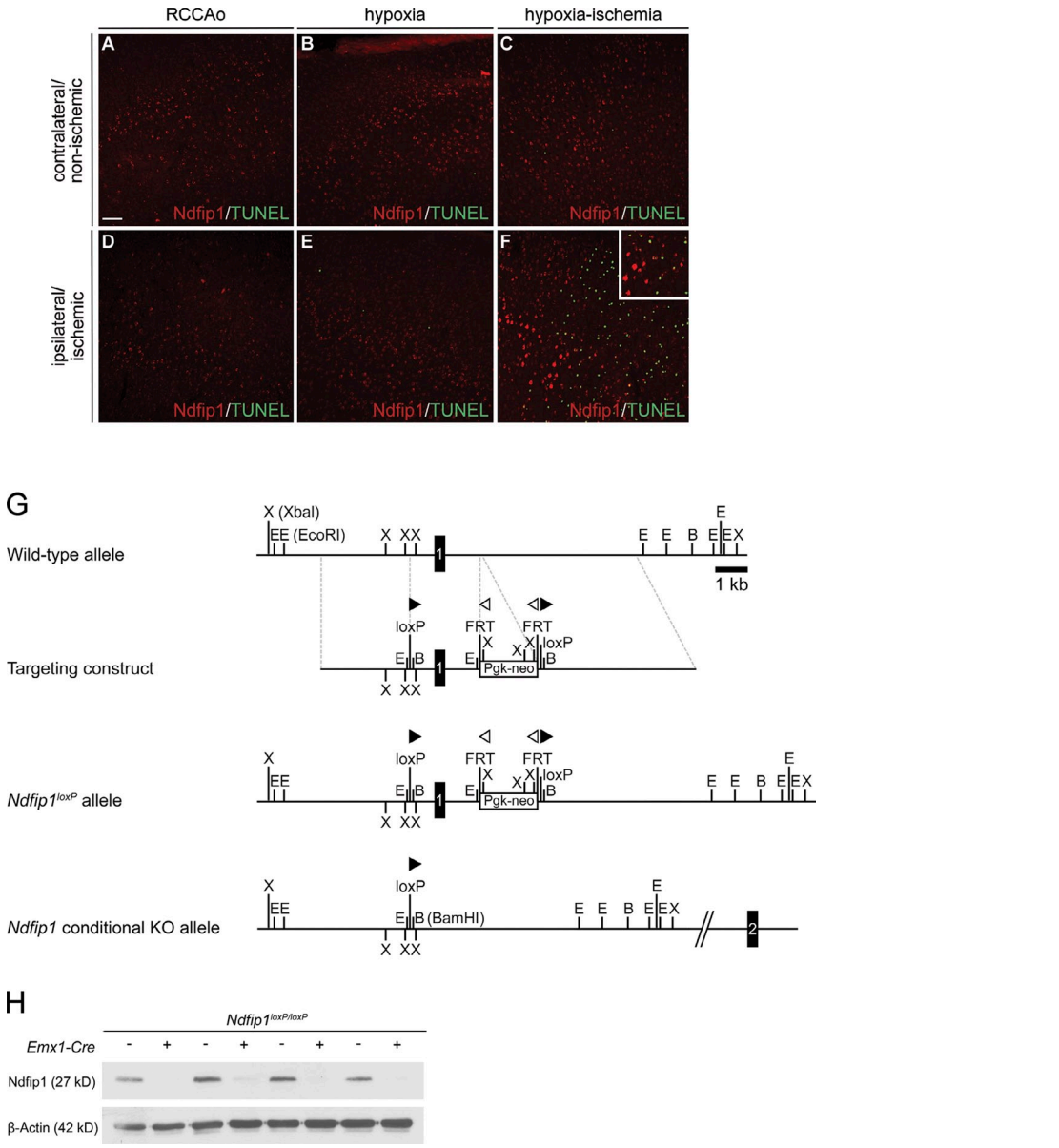

Figure S2. **Ndfip1 in hypoxia and gene deletion in mice.** (A–C) In nonischemic cortex (contralateral hemisphere), Ndfip1 shows low-level expression in neurons 24 h after right carotid artery occlusion (RCCAo), hypoxia, or HI (RCCAo + hypoxia). (D–F) In the ischemic cortex (ipsilateral hemisphere), neither carotid artery occlusion nor hypoxia alone results in Ndfip1 up-regulation or apoptosis, as detected by TUNEL. Instead, a combination of RCCAo + hypoxia is required before Ndfip1 up-regulation, and apoptosis is observed in neurons (inset in F). Bar, 50 µm. (G) A schematic diagram of the *Ndfip1* WT locus, targeting construct, floxed allele, and excised allele. The targeting construct was generated by flanking exon 1 with *loxP* sites and insertion of a neomycin phosphotransferase selection cassette (*Pgk-Neo*) flanked by *FRT* sites into intron 1. *Emx1-Cre*–mediated excision of the floxed allele results in conditional deletion of exon 1 (that codes for the start codon) and the *Pgk-Neo* cassette. Black boxes indicate exon number. (H) Western analysis of adult cortex reveals Emx1-specific loss (pyramidal neurons only) of Ndfip1 protein in conditional KO mice ($Ndfip1^{loxP/loxP}$;*Emx1-Cre*$^{+}$) compared with control littermates ($Ndfip1^{loxP/loxP}$;*Emx1-Cre*$^{-}$; $n = 4$ animals per genotype). β-actin is shown for a loading control.

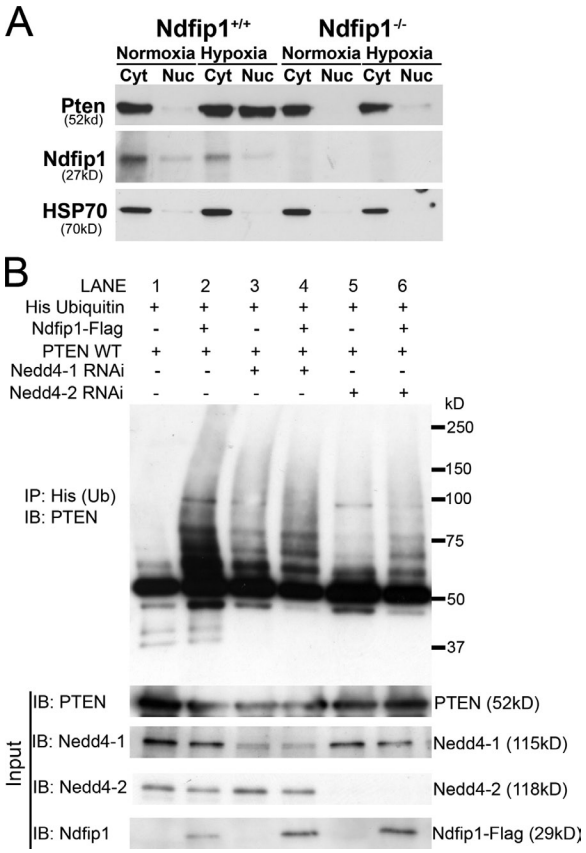

Figure S3. **Pten accumulates in the nucleus of neurons after hypoxia in vitro, and RNAi knockdown of either Nedd4-1 or Nedd4-2 reduces ubiquitination of PTEN.** (A) Cytoplasmic (Cyt) and nuclear (Nuc) fractionation of Ndfip1$^{+/+}$ neurons under normoxic conditions shows Pten to be predominantly in the cytoplasmic fraction (using HSP70 as a cytoplasmic marker). Under hypoxic conditions (<1% O_2), an increase in the level of Pten in the nuclear fraction is observed. In Ndfip1$^{-/-}$ neurons under normoxic conditions, Pten is found in the cytoplasmic fraction. These neurons do not elevate Pten in the nuclear fraction under hypoxic conditions. (B) PTEN ubiquitination (Ub) assay shows increased ubiquitination of PTEN with coexpression of Ndfip1 (compare lanes 1 and 2). When Nedd4-1 was reduced in the presence of Ndfip1 (compare lanes 2 and 4), a reduction in the ubiquitin profile of PTEN was observed. A similar effect was also observed with Nedd4-2 knockdown (compare lanes 2 and 6). IB, immunoblotted; IP, immunoprecipitated.

Reference

Chen, Z., L.C. Trotman, D. Shaffer, H.K. Lin, Z.A. Dotan, M. Niki, J.A. Koutcher, H.I. Scher, T. Ludwig, W. Gerald, et al. 2005. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature*. 436:725–730. http://dx.doi.org/10.1038/nature03918