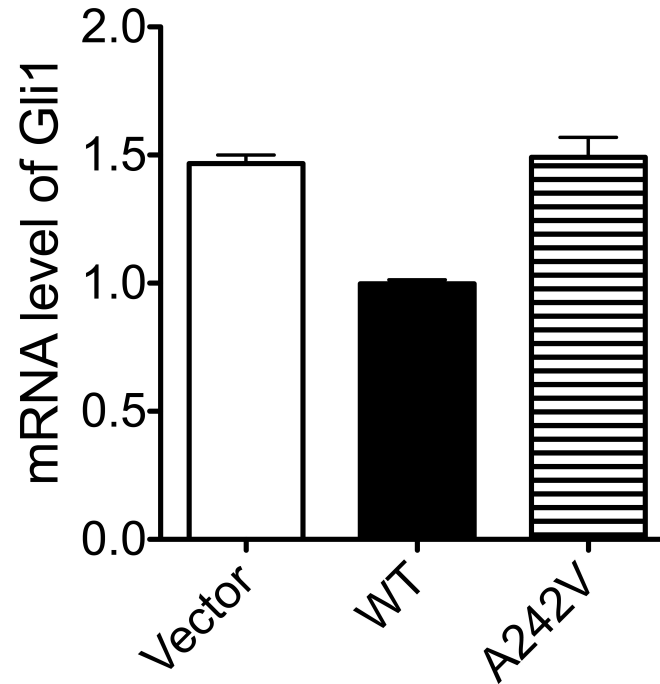


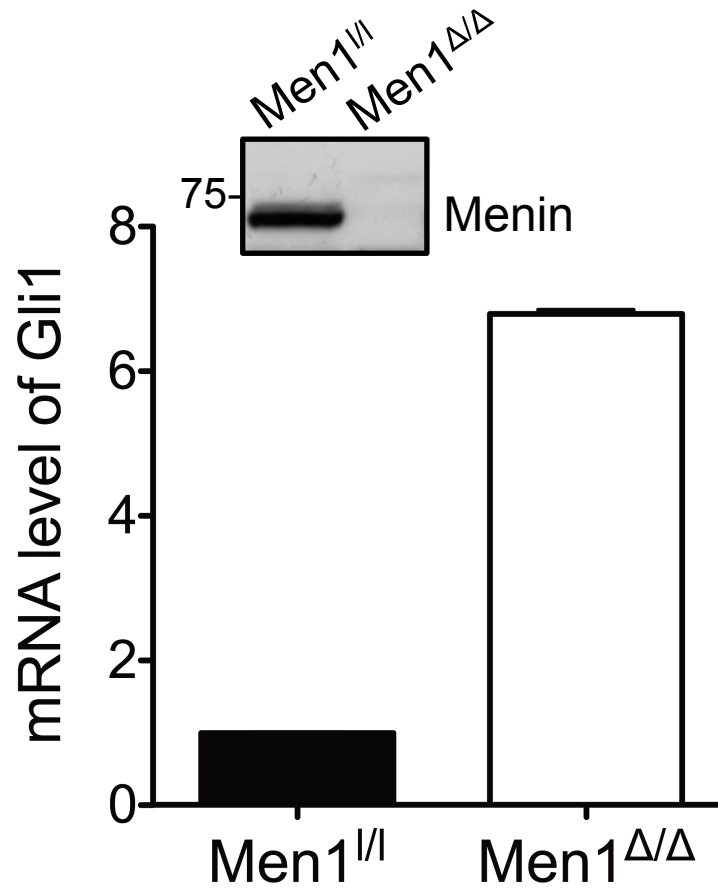
Menin Directly Represses expression of Gli1 Independent of the Canonical Hedgehog Signaling Pathway

Buddha Gurung¹, Zijie Feng², Xianxin Hua¹

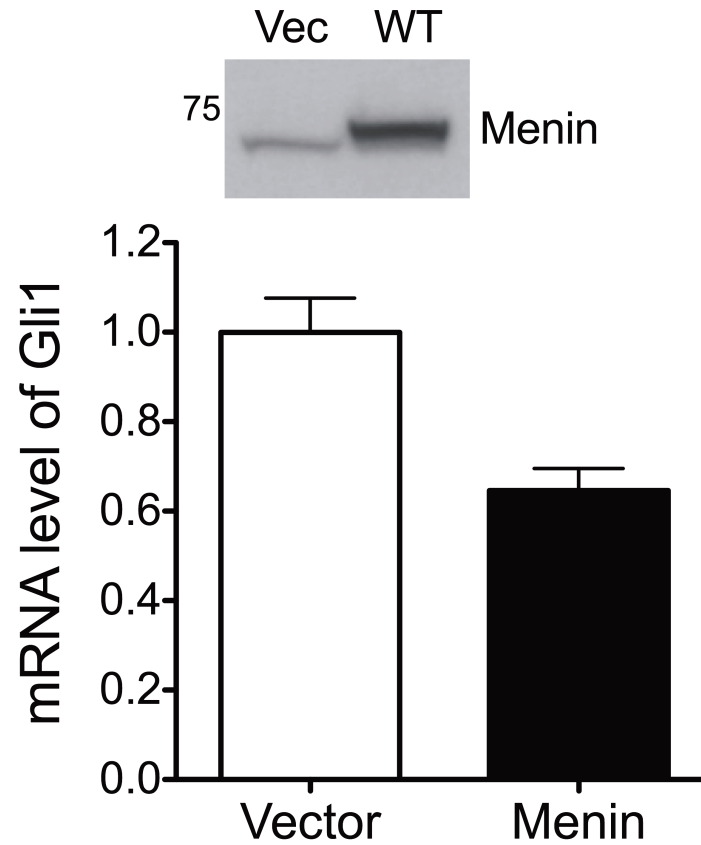
Supplementary Figures and Tables



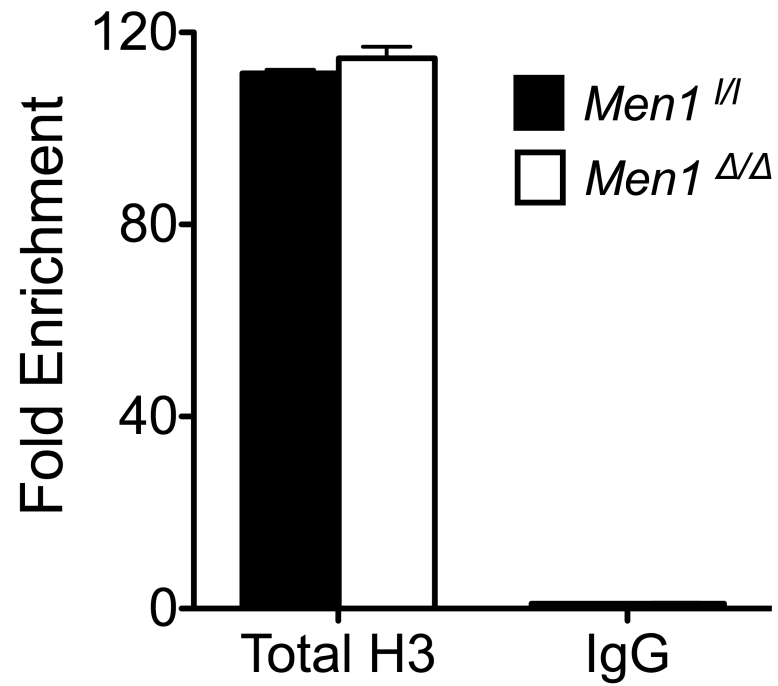
Supplementary Figure S1. *Gli1* mRNA levels in menin-null cells complemented with physiologically relevant *Men1* mutants. *Men1*-null MEF's were complemented with either vector, WT, or the mutant A242V and the levels of *Gli1* were determined by qRT-PCR. Data was normalized to *Men1*-null cells complemented with WT menin.



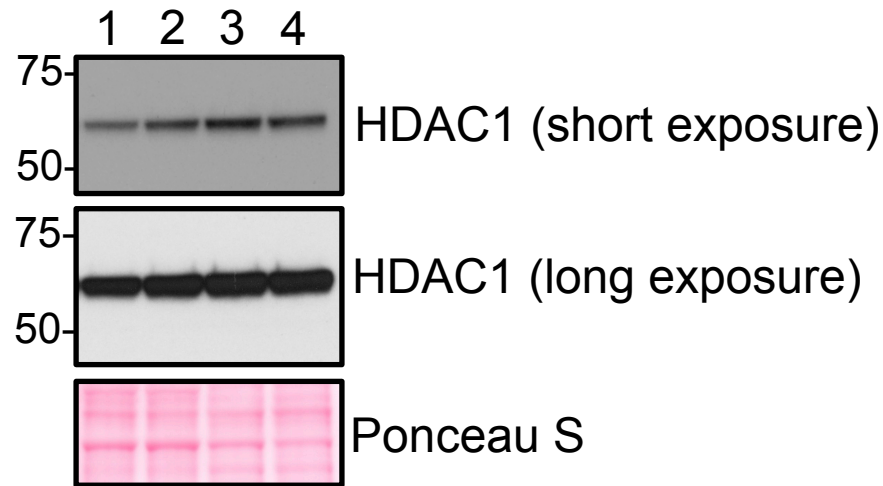
Supplementary Figure S2. *Gli1* mRNA levels are elevated upon *Men1* excision in MEFs. *Men1*^{+/+}; *Ubc9* CreER cells were treated with either DMSO or 4-hydroxy tamoxifen (4-OHT) and the levels of *Gli1* were determined by qRT-PCR. Inset, Western Blotting showing complete excision of menin upon 4-OHT treatment. Data were normalized to *Gli1* values in DMSO-treated cells.



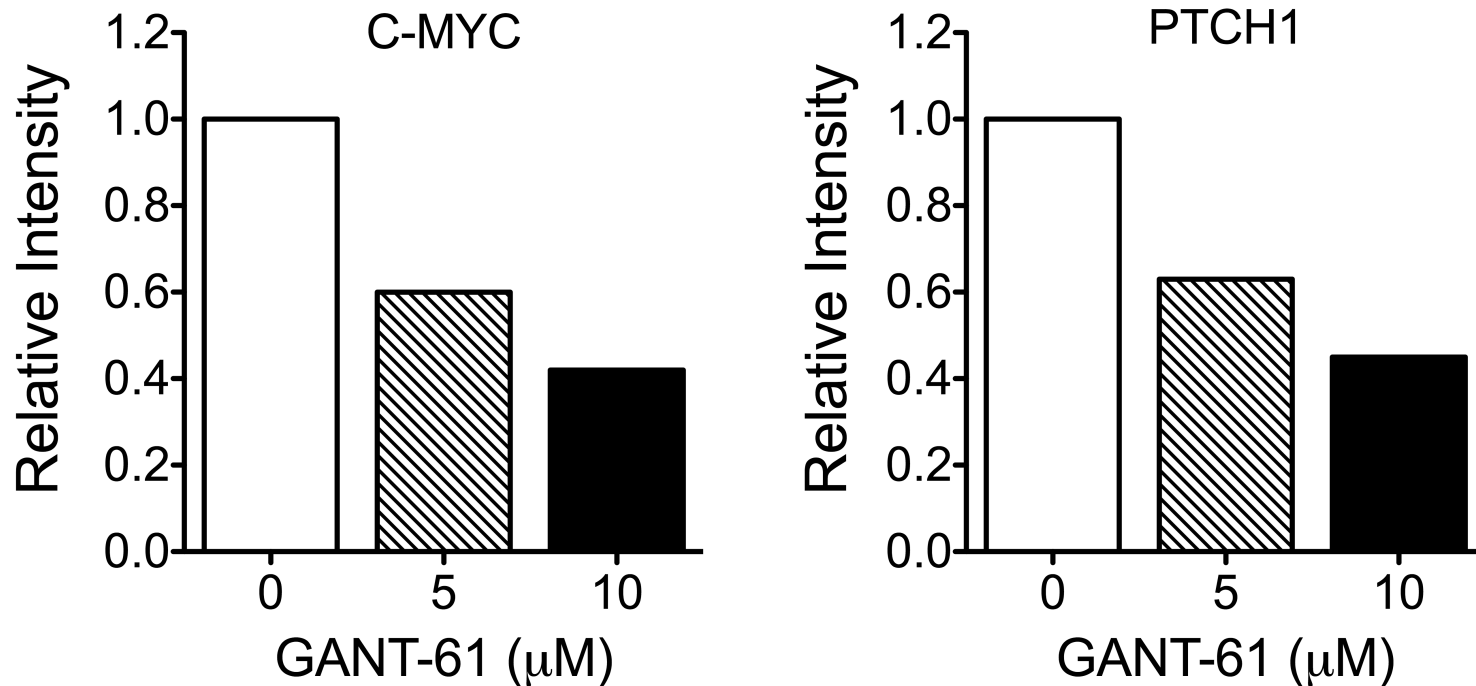
Supplementary Figure S3. Ectopic expression of menin results in reduction of *Gli1* in BON cells, a human carcinoid cell line. qRT-PCR for *Gli1* mRNA in BON cells ectopically expressing menin or control vector. Inset, Western blotting of menin in BON cells ectopically expressing menin or control vector.



Supplementary Figure S4. Chromatin IP (ChIP) for total histone H3 in PIME1 cells treated with either DMSO (*Men1*^{l/l}) or tamoxifen (*Men1*^{Δ/Δ}).



Supplementary Figure S5. Total HDAC1 level are unaffected upon *Men1* excision. Cell lysates from *Men1^{fl/fl};CreER* MEFs treated with either DMSO (lane 1) or tamoxifen (lane 2), and PIME cells treated with either DMSO (lane 3) or tamoxifen (lane 4) were subjected to SDS-PAGE and immunoblotted for HDAC1. Ponceau S is included as a loading control.



Supplementary Figure S6. Quantitation of C-MYC and PTCH1 protein levels in menin-null PIME1 cells treated with GANT-61. Cell lysates from menin-null PIME1 cells treated with either DMSO or GANT-61 were immunoblotted with anti-MYC and anti-PTCH1 antibodies, and the band intensities were quantitated by densitometric analysis using Image J software and normalized to the intensity of their respective Ponceau S stains. Data are normalized to DMSO-treated cells.

Table I: qRT-PCR primers

Gli1-Forward	GATGAAGGACCTTGTGTCTCG
Gli1-Reverse	GGCTGACTGTGTAAGCAGAGC

Table II: ChIP primers

Gli1 (-1203 bp / -925 bp) <i>Forward</i>	GAAGACTCTCTCTCCCCACC
Gli1 (-1203 bp / -925 bp) <i>Reverse</i>	CCTCGGAGTGGA AATGATTG