Supplemental Experimental Procedures

Immunofluorescent staining of cells

On day 0, 1×10^5 cells were seeded on glass coverslips per well in 24-well plates. On day 2, cells were fixed in 4% paraformaldehyde in PBS, permeabilized in 0.25% Triton x-100 in PBS, blocked in 10% goat serum and incubated with primary antibody, phospho-Histone H3 (Ser 10) antibody (9701, Cell Signaling), at 4℃ overnight. Secondary antibody, goat anti-rabbit IgG (H+L)-HRP conjugate (Bio-Rad) was used for incubation at room temperature for 1 hour in dark. DAPI was used for nuclear staining. Fluoro Gel (Electron Microscopy Sciences, Hatfield, PA) was applied to mounting. Duplicate cultures were examined, and the results were representative of two independent experiments. The percentage of M phase cells was counted from 5 different fields (200X) of the microscope.

Primer sequences

Primers for the *Ccnb2* promoter in ChIP assay were synthesized by Integrated DNA Technologies (IDT, Coralville, IOWA).
Primers for the amplicon 1:
5'-GAAATGTCAGATTTGGGCGAAGGG-3' and 5'-AGTGCCAGCAGAACGACTTGAGAT-3'
Primers for the amplicon 2:
5'-AGCCAGCCAATCAACGTGCAGAAA-3' and 5'-TGACGCACTATTGGGTAGACGCAC-3'
Primers for the amplicon 3:
5'-TCTACCCAATAGTGCGTCAGC-3' and 5'-AAGTGCGGACGAGGCACA-3'
Primers for the amplicon 4:
5'-ATTCCTGTTACCACTCAGGGCTGT-3' and 5'-TTGGCCAGGAAGGCAGTATGAAGA-3'

Primers for the *HoxA9* promoter in ChIP assay were synthesized by Integrated DNA Technologies (IDT, Coralville, IOWA) and the sequences were:
5'-CGGGTACTGGGCTTATTTCA-3' and 5'-CCAAAAGGGGGAAAATTCAT-3'

The sequence of shRNAs targeting cyclin B2

The 4 shRNA that silence cyclin B2 were purchased from Open Biosystems (Thermo Fisher Scientific, Inc), the Catalog Number is RMM4534, and the sequences were provided as following:

sh1-CCGG<u>GCTTCTCAGATCCTGTATGTA</u>CTCGAG<u>TACATACAGGATCTGAGAAGC</u>TTTTTG;
Sense Antisense
sh2-CCGG<u>CTCTGCAAGATCGAGGACATA</u>CTCGAG<u>TATGTCCTCGATCTTGCAGAG</u>TTTTTG;
Sense Antisense
sh3-CCGG<u>GCAGCAGTATTACACAGGCTA</u>CTCGAG<u>TAGCCTGTGTAATACTGCTG</u>TTTTTG;
Sense Antisense
sh4-CCGG<u>GATGTTGAACAGCACACTTTA</u>CTCGAG<u>TAAAGTGTGCTGTTCAACATC</u>TTTTTG.
Sense Antisense

Supplemental Figure Legends

Figure S1. Increased percentage of M phase cells in *Men1*$^{-/-}$ MEFs as compared with *Men1*$^{+/+}$ MEFs. A, DAPI staining for nucleus in *Men1*$^{-/-}$ MEFs. B, Immunofluorescent staining with phospho-H3S10 antibody in *Men1*$^{-/-}$ MEFs. C, Merge of DAPI staining and phospho-H3S10 antibody staining in *Men1*$^{-/-}$ MEFs. D, DAPI staining for nuclear in *Men1*$^{+/+}$ MEFs. E, Immunofluorescent staining with phospho-H3S10 antibody in *Men1*$^{+/+}$ MEFs. F, Merge of DAPI staining and phospho-H3S10 antibody staining in *Men1*$^{+/+}$ MEFs. Scale bar was 250μm.

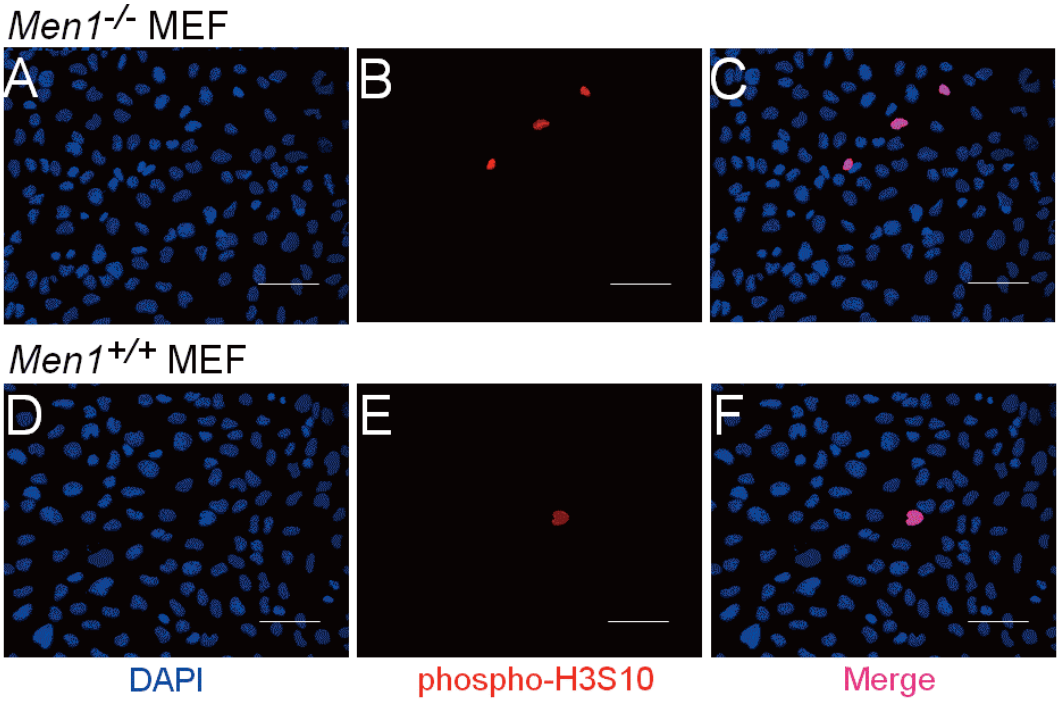
Figure S2. Menin mutants, A242V or L22R, fail to reduce the number of M phase cells as compared with wild type menin. A, DAPI staining for the nuclear DNA in the indicated cells. B, Immunofluorescent staining with phospho-H3S10 antibody in the indicated cells. C, Merge of DAPI staining and phospho-H3S10 antibody staining in control cells. D, G, J, DAPI staining for the nuclear DNA in the indicated cells.. E, H, K, Immunofluorescent staining with phospho-H3S10 antibody in the indicated cells. F, I, L, Merge of DAPI staining and phospho-H3S10 antibody staining in the indicated cells. Scale bar was 250μm.

Figure S3. Reduced percentage of M phase cells in cyclin B2 knockdown cells. A, DAPI staining for the nucleus in *Men1*$^{-/-}$ MEFs expressing control shRNA vector. B, Immunofluorescent staining with phospho-H3S10 antibody in *Men1*$^{-/-}$ MEFs expressing control shRNA vector. C, Merge of DAPI staining and phospho-H3S10 antibody staining in *Men1*$^{-/-}$ MEFs expressing control shRNA vector. D, G, J, M, DAPI staining for the nucleus in *Men1*$^{-/-}$ MEFs expressing one of the four different cyclin B2 shRNAs. E, H, K, N, Immunofluorescent staining with phospho-H3S10 antibody in *Men1*$^{-/-}$ MEFs expressing one of the four different cyclin B2 shRNAs. F, I, L, O, Merge of DAPI staining and phospho-H3S10 antibody staining in the indicated cells. Scale bar was 250μm.

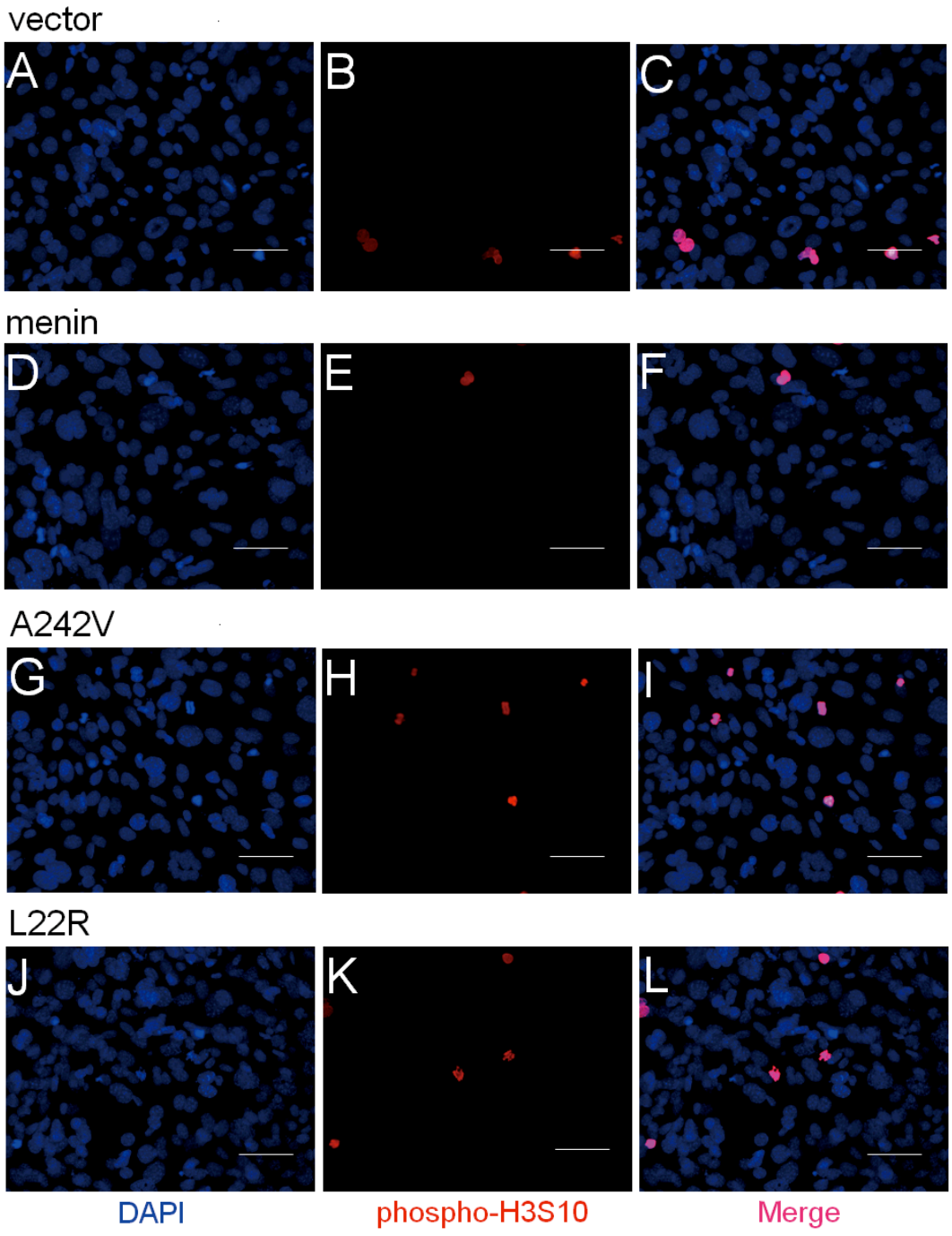
Figure S4. Menin increases the H3K4me3 level at the Hoxa9 promoter. ChIP assay for detection of H3K4m3 at the *Ccnb2* promoter in *Men1*$^{-/-}$ and *Men1*$^{+/+}$ MEFs. Real-time PCR was performed using the primers amplifying the *Hoxa9* promoter; the products of real-time PCR were analyzed in 1% agarose gel electrophoresis.

Figure S5. Menin expression does not change the levels of tested proteins that are involved in regulating cyclin B2 transcription in *Men1*$^{-/-}$ and *Men1*$^{+/+}$ MEFs. A, Western Blotting analysis of *Men1*$^{-/-}$ and *Men1*$^{+/+}$ MEFs using the antibodies against RNA Pol Ⅱ, E2F2, E2F3, H3K4me3. Ponceau S red staining was used to show equal loading of cell lysates. B, Western blotting analysis of the indicated cells using the antibodies of CBP, HDAC-3, NF-Y, acetylated histone H3 in the indicated cells. The antibodies used were as described under "Chromatin Immunoprecipitation" under Experimental Procedures.

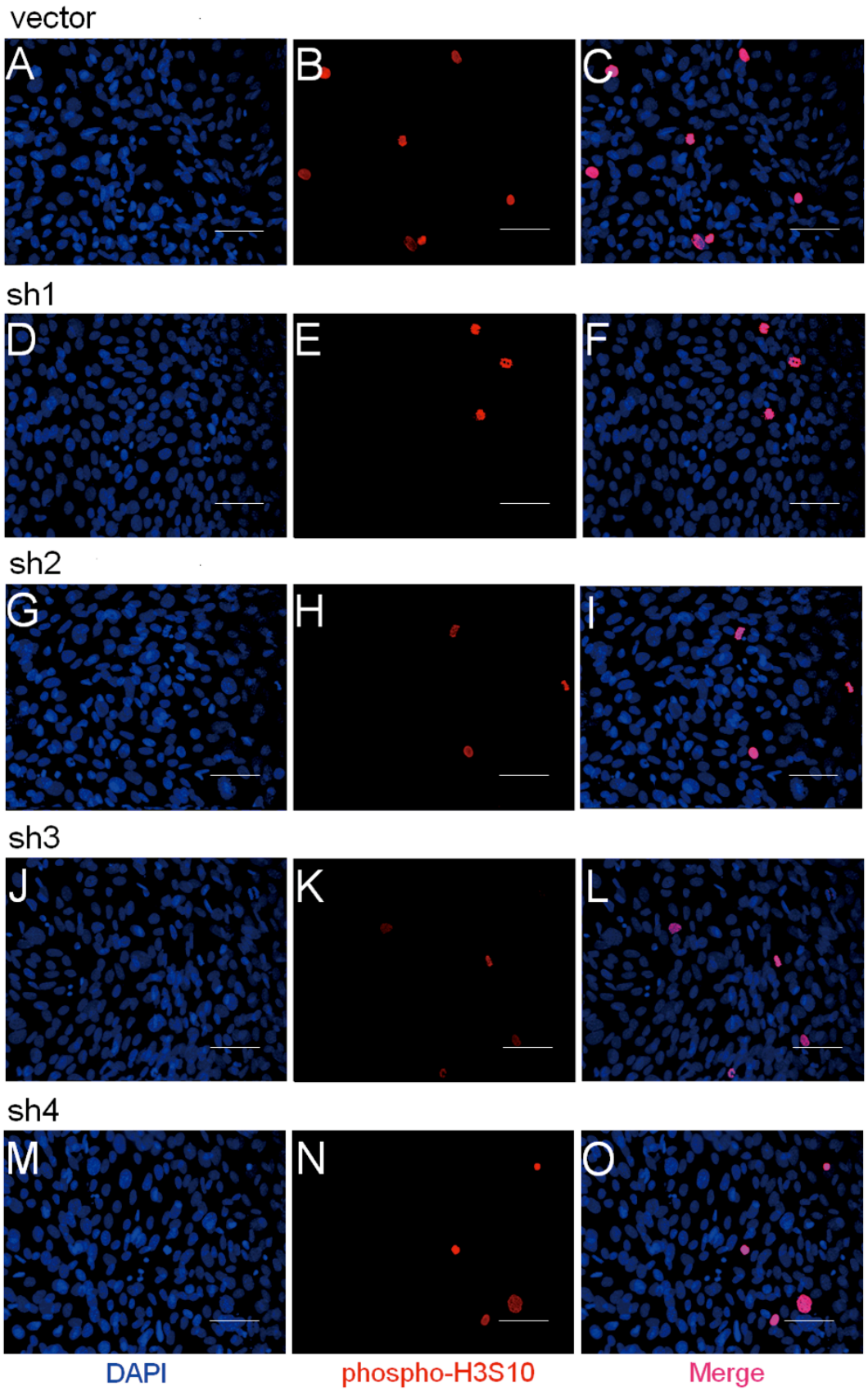
Ting Wu Fig.S1



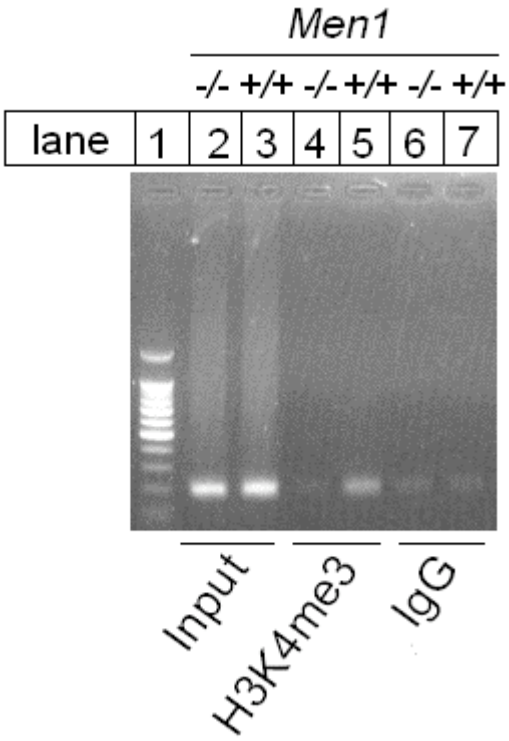
Ting Wu Fig.S2



Ting Wu Fig.S3



Ting Wu Fig.S4



Ting Wu Fig.S5