LOSS OF THE METHYL-LYSINE EFFECTOR MOLECULE PHF20 IMPACTS THE EXPRESSION OF GENES REGULATED BY THE LYSINE ACETYLTRANSFERASE MOF

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Supplemental Information

Tissue ChIP primers

Ing1 P1(-780 bp tss) 194 bp	For GTTCTGCCGGAGGATGAAT Rev TGAGTCCCAGGTCCTCAATC
Ing 1 P2 (3'UTR end) 128 bp	For GTACATCTTGCCCCTGCTGT Rev TGGCCAAGTTTCATTTATTTCTT
Actb P1 (+100 bp TSS) 149 bp	For TTCGCTCTCTCGTGGCTAGT Rev TGTGGCTGCAAAGAGTCTACA
Actb P2 (end 3'UTR) 105 bp	For AATTTCTGAATGGCCCAGGT Rev GCTGCCTCAACACCTCAAC
Cdk4 P1 (intron1), 179 bp	For ATTATGGAAGGTGGCCCAAT Rev GTCCCGGAGGAAGAAAAAGA
Cdk4 P2 (intron 5), 200 bp	For TAGGCATGCCCTGGATTTAG Rev ACCACACGGTGGTTCACAA
Morf4L1 P1 (beginning of intron 1), 166 bp	For CTGCGGAGGGTAATCTCAGT Rev GGCACCTCCCTTCAATAGAC
Morf4L1 P2 (intron 9), 162 bp	For TGCCTTTAATCCCAGCACTC Rev CAGCGCATTTATGCTTTCAA

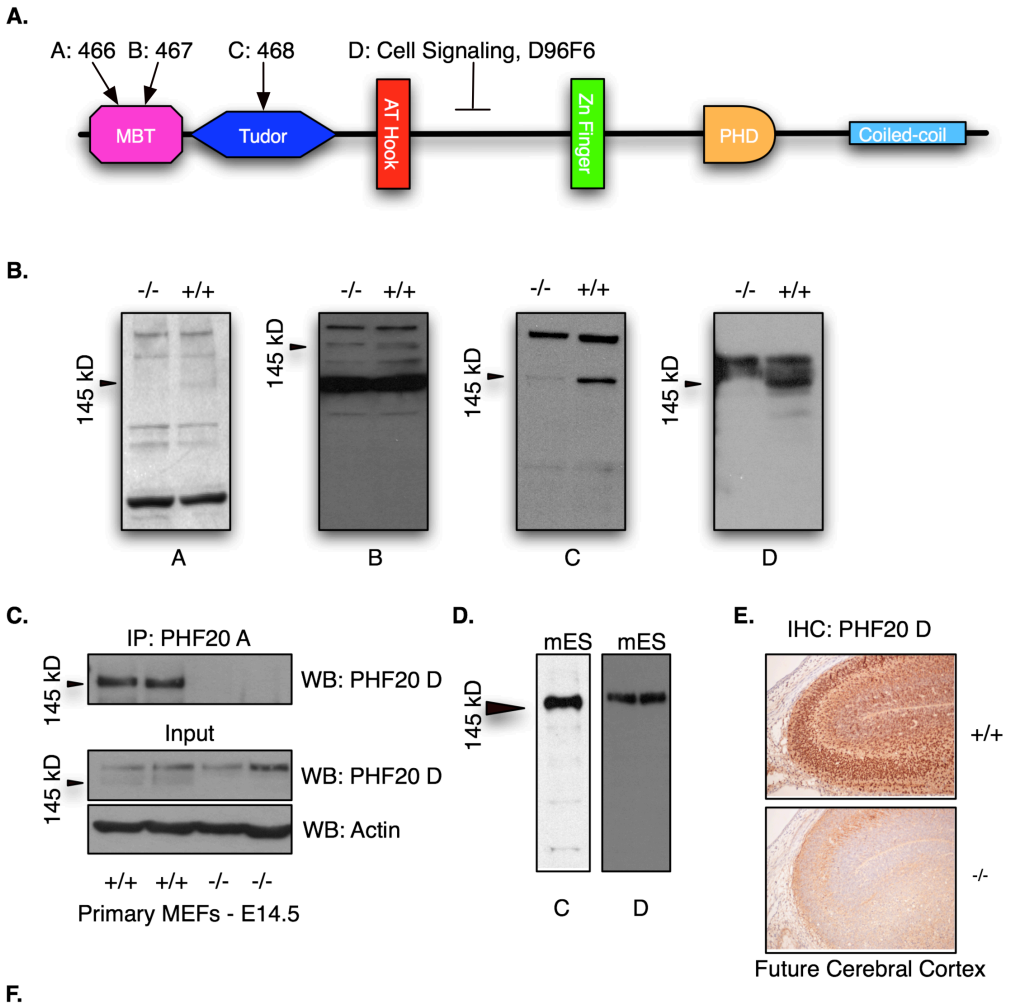
Real-time qPCR primers

Ing1 (exon1-exon2) 160 bp	For AATCGAGTCACTGCCTTTCG Rev CTGGATGCAGTGCAGTACCC
Actb (Exon1-exon2) 157 bp	For CAGCTTCTTTGCAGCTCCTT Rev CACGATGGAGGGGAATACAG
Cdk4 (exon 5-6), 161 bp	For CTGGTACCGAGCTCCTGAAG Rev GTCGGCTTCAGAGTTTCCAC
Morf4L1 (exon3-4), 144 bp	For GGTTGCCATAAAGGACAAACA Rev TTGGCCTTTTGAAGTTCTCG

Figure S1. Antibody characterization. (**A**) Shown are the antigen regions used to immunize rabbits for the antibody production. (**B**) WT and KO embryonic brain tissue lysate was probed with the four antibodies – A, B, C & D. Only antibodies C and D show a clear band at the correct size (145 kDa) that disappears in the KO lysate. (**C**) WT and KO primary MEF lysate was used to highlight the IP-western combination of A-D that is ideal for PHF20 detection. (**D**) PHF20 is expressed strongly in mES cells, and runs as a single band; shown here with antibodies C and D. (**E**) The best antibody for immunohistochemistry, D, was used to stain WT and KO brain sections and shows strong PHF20 expression in the developing cerebral cortex. (**F**) Summarized in these tables are the verified applications available for the four PHF20 antibodies.

Figure S2. PHF20 null phenotypes and expression patterns. (**A**) X-ray analysis of surviving Day 25 mice first demonstrated the loss of one lumbar vertebrae in the PHF20 null mice. The bone density of PHF20 null mice appeared reduced in the x-rays. Therefore the femurs of day 25 mice were submitted for micro-CT bone scans which revealed the KO mice to have a ~10% decrease in bone volume (**B**). (**C**) The loss of one lumbar vertebrae in PHF20 null mice suggests a developmental role for PHF20 in spine formation. Utilizing the PHF20 promoter-driven β-gal expression in heterozygous embryos, we could show that indeed PHF20 is normally expressed in the developing neural tube. (**D**) H&E analysis of vertebrae demonstrate a loss in bone marrow cellularity in the PHF20 KO mice. (**E**) PHF20 KO thymuses and spleens are smaller than wild-type counterparts, with the PHF20 KO spleens displaying disorganization between white and red pulp regions. (**F**) We chose to perform ChIP-seq analysis using embryonic brain tissue because both PHF20 and MOF are highly expressed in these tissues and this organ provides sufficient material for such analysis. (**G**) To confirm that loss of PHF20 does not affect global levels of H4K16Ac in an independent system, an inducible RNAi scheme was used to knockdown PHF20 in human U87 glioblastoma cells. Indeed, the levels of H4K16Ac remain the same after PHF20 KO.

Figure S1



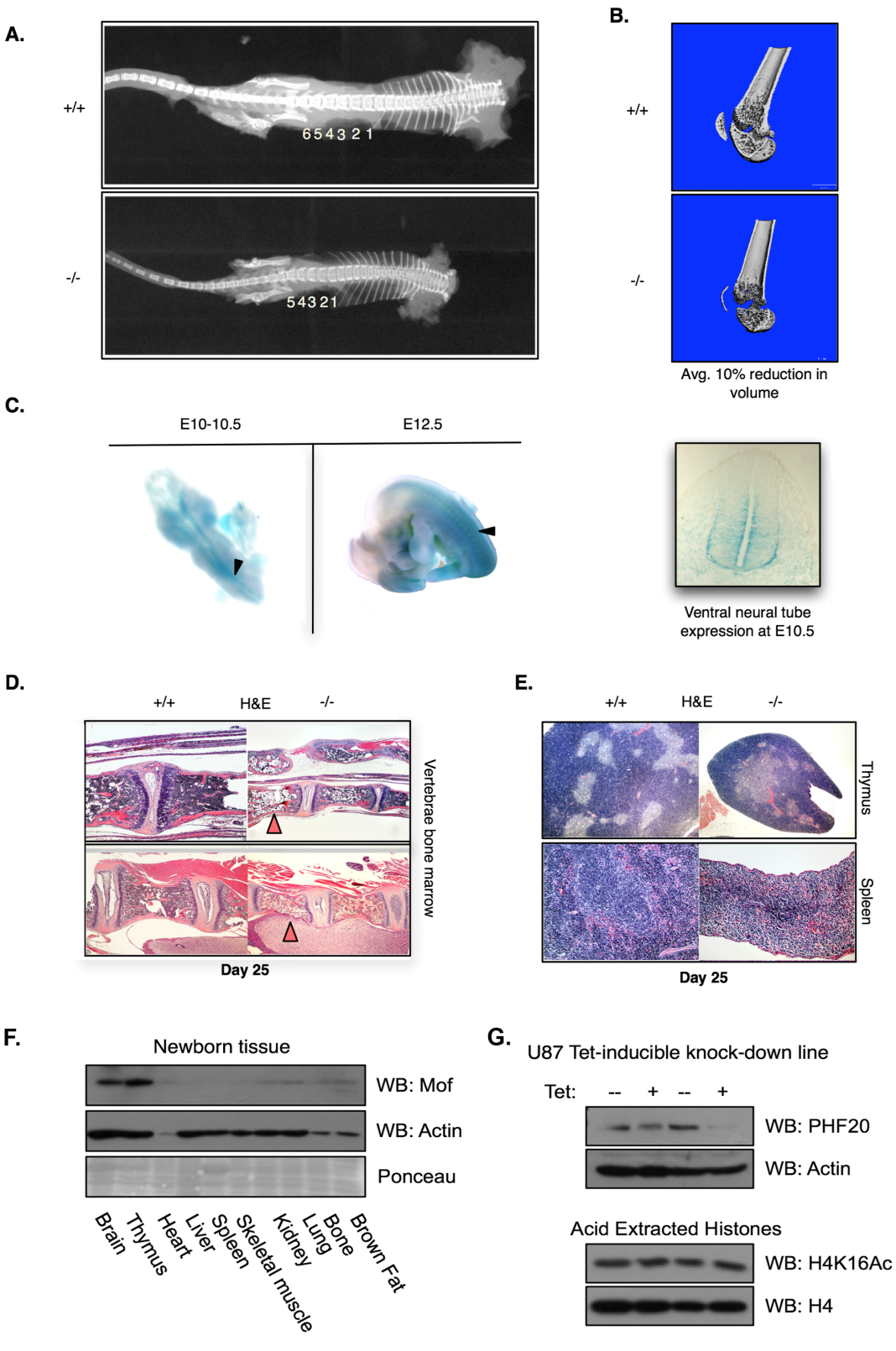
F.

Antibody	IP	Western
A	Yes	No
B	Yes	No
C	Yes	Yes
D	Yes	Yes

IP-Western Combinations
IP: A, Western: D
IP: D, Western: B

Antibody	IHC
A	Not tested
B	No
C	No
D	Yes

Figure S2


A.
+/+
6543 21
-/-
54321
B.
+/+
-/-
Avg. 10% reduction in volume
C.
E10-10.5
E12.5
Ventral neural tube expression at E10.5
D.
+/+
H&E
-/-
Vertebrae bone marrow
Day 25
E.
+/+
H&E
-/-
Thymus
Spleen
Day 25
F.
Newborn tissue
WB: Mof
WB: Actin
Ponceau
Brain
Thymus
Heart
Liver
Spleen
Skeletal muscle
Kidney
Lung
Bone
Brown Fat
G.
U87 Tet-inducible knock-down line
Tet: -- + -- +
WB: PHF20
WB: Actin
Acid Extracted Histones
WB: H4K16Ac
WB: H4