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Supplemental Material to:

Alexander V Brechalov, Sofia G Georgieva, and Nataliya V Soshnikova

Mammalian cells contain two functionally distinct PBAF complexes incorporating different isoforms of PHF10 signature subunit

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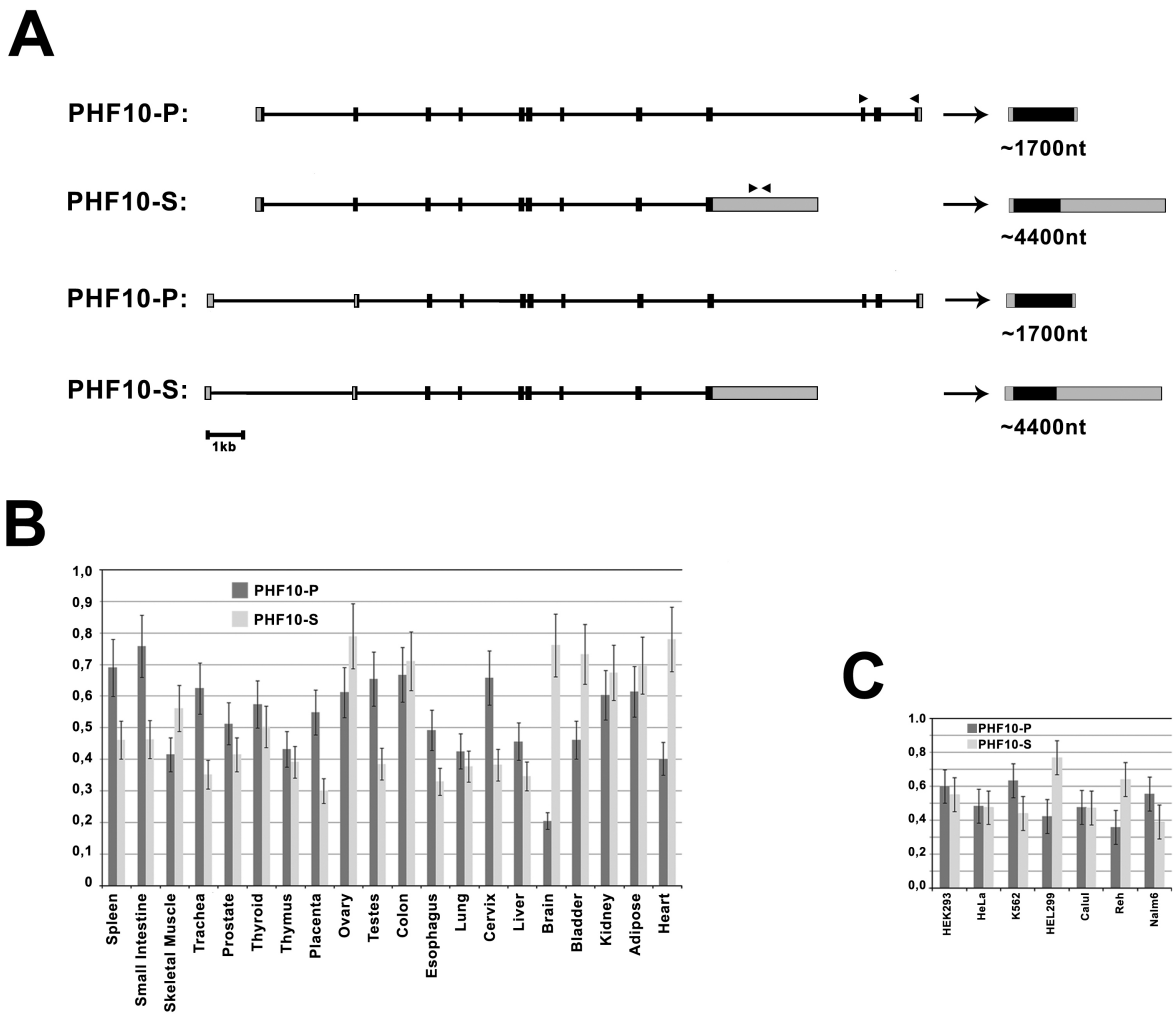
Supplementary_Fig.1



Supplementary_Fig.2

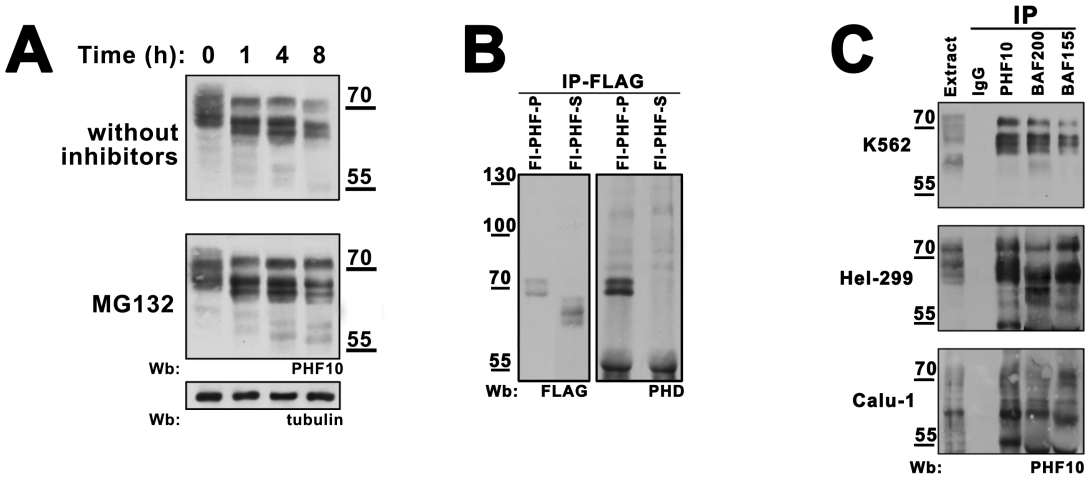
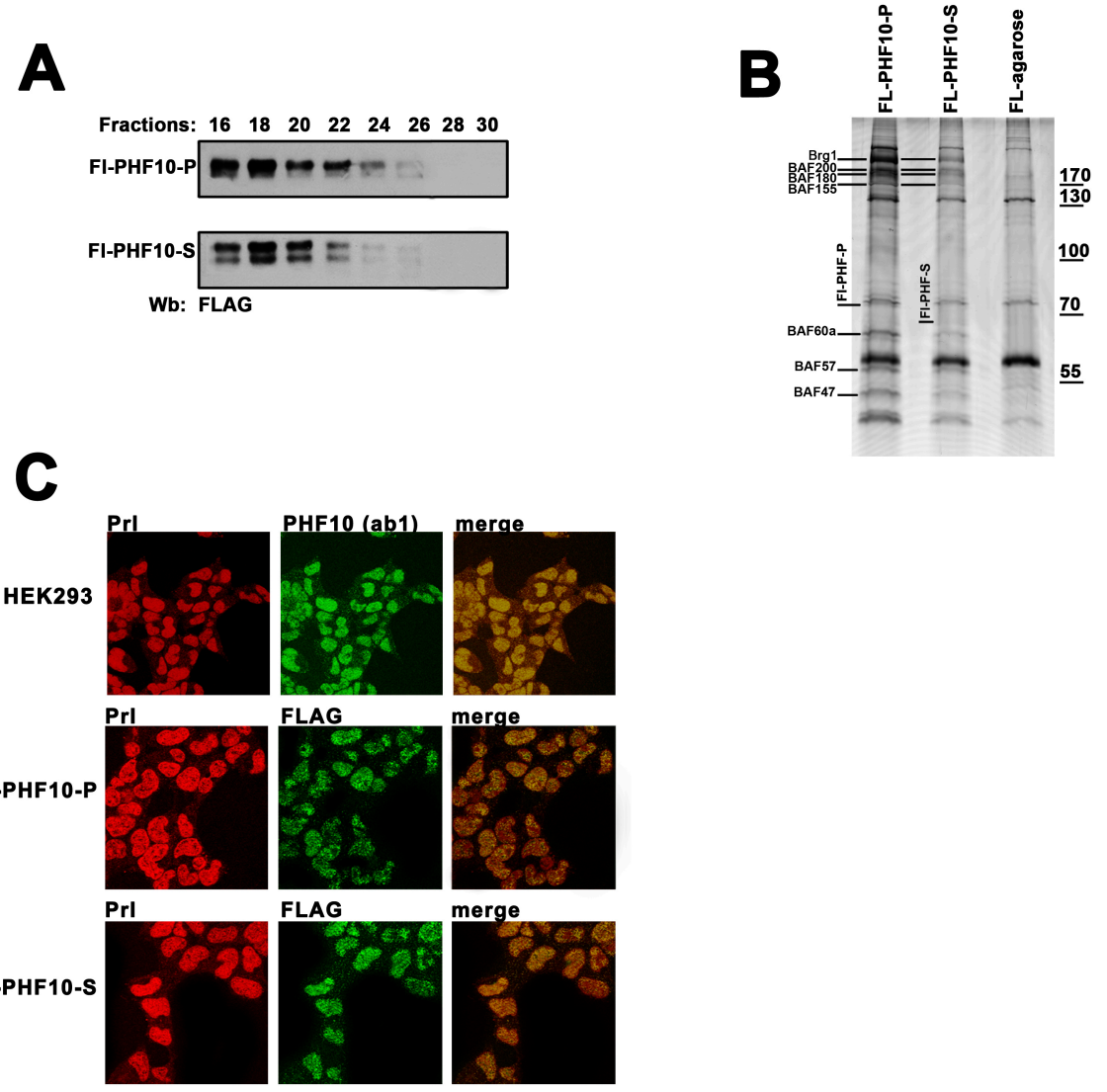


Table1

process	P _DOWN	P _UP	S _DOWN	S _UP
Proliferation(%)	16(15,09%)	21(23,59%)	8(7,02%)	7(4,14%)
Cell Cycle(%)	8(7,54 %)	19(21,34%)	4(3.6%)	13(7,69%)
Apoptosis(%)	4(3,77%)	8(8,99%)	13(11,71%)	19(11,24%)
Total	106(100%)	89(100%)	111(100%)	169(100%)

Supplementary_Fig.3



Supplemental Material

Isolation of PHF10 transcripts

Several new cDNA forms were isolated from the HEK293 cell line, and three new transcripts with alternative 3' and 5' ends were obtained using specific primers (Fig. 1A). One of them had the same transcription start as the NM_018288.3 transcript from the NCBI database, and its 3' end was the same as in the sequence ENST 00000480008 and contained a retained intron. Its length was about 4200 bp. The protein form (PHF10-Slong) translated from this transcript was 376 amino acids long and did not contain PHD fingers. The second novel transcript (about 2200 bp long) was raised from a primer annealed on the second exon of ENST 00000413088 (the third exon of this EST was not present in the new PHF10 transcript) and had the same N-end as the NM_018288.3 transcript from the NCBI database. Structural alteration of its 5' end gave rise to the open reading frame encoding the protein that lacked 50 N-terminal amino acids, compared to the protein encoded by NP_060758.2. Its length was 451 amino acids. The third transcript had a new alternative 5' end as in PHF10-Ps and a long 3' end as in PHF10-Sl. It encoded the isoform consisting of 329 amino acids. In addition to the three novel transcripts, we also found their variants that encoded PHF10 isoforms lacking two amino acids in the SAY domain, as described for the PHF10-Pl transcript (NP_579866.2).

Figures

Supplementary Fig. 1

(A) Scheme of the exon-intron structure of alternative transcripts encoded by the PHF10 gene. Transcripts encoding the PHD- and PDSM-containing isoforms are designated PHF10-P and PHF10-S; their coding and noncoding regions are shown in dark and light gray, respectively. The approximate length of corresponding PHF10 mRNAs is indicated. Arrowheads indicate the positions of the primers used in qPCR for measuring the transcription levels of PHD- and PDSM-containing PHF10 isoforms. **(B)** The levels of PHF10 transcripts encoding PHF10 isoforms with PHD fingers (dark gray) or PDSM motif (light gray) in the total RNA from human tissue samples (FirstChoice Human Total RNA survey panel, Applied Biosystems) as measured by qPCR. **(C)** The levels of PHF10 transcripts relative to the total PHF10 mRNA level (taken as 1) in human cell cultures of different origin as measured by qPCR with the primers indicated in (A).

Supplementary Fig. 2

(A) The molecular weight of PHF10 isoforms decreased rapidly during incubation of the cell lysate was at room temperature (upper panel). The addition MG132, an inhibitor of proteasomal degradation, had no effect on this process (lower panel). **(B)** The specificity of the anti-PHD antibody was confirmed in experiments with recombinant PHF10. FLAG-PHF10-Pl and FLAG-PHF-Sl were overexpressed in

HEK293 cells, purified on FLAG-agarose, and resolved by SDS PAGE. The Western blot was stained with anti-FLAG or anti-PHD antibodies, the latter detecting only the FLAG-PHF10-Pl form. **(C)** PHF10 was associated with the PBAF complex in K562, Hel-299, and Calu-1 cell lines. Endogenous PHF10 forms were immunoprecipitated from cell extract with antibodies against BAF200 and BAF155.

Supplementary Fig. 3

(A) The nuclear extract from HEK293 stably transformed with FLAG-PHF10-P or FLAG-PHF10-S was fractionated on Superose 6 gel filtration column. The collected fractions were analyzed for the presence of FLAG-PHF10 using antibodies against FLAG. Fraction numbers are indicated. Both recombinant isoforms migrated in the same fractions as endogenous PHF10, indicating that they were incorporated in PBAF. **(B)** Purification and subunit composition of FLAG-PHF10-P- or FLAG-PHF10-S- associated PBAF complexes. Superose 6 fractions 16–24 (see above) after each fractionation were collected to precipitate the PBAF complex with antibodies against FLAG. The precipitate was washed, and the proteins were identified by MALDI-TOF analysis. **(C)** Recombinant PHF10-Pl and PHF10-Sl have nuclear localization, similar to endogenous PHF10. Wild-type HEK293 cells were stained with antibodies against total PHF10 (ab1, green) and cells stably transformed with FLAG-PHF10-Pl or FLAG- PHF10-Sl were stained with anti-FLAG antibodies (green). The nuclei were stained with propidium iodide (PrI, red).

Supplementary Table

Total numbers of genes involved in proliferation, cell cycle, and apoptosis that were up- or downregulated in PHF10-P or PHF10-S stably transformed cells.

Methods:

PHF10 transcripts

To raise PHF10 transcripts, mRNA from HEK293 was treated by DNaseI and then reverse transcription (RT) was performed with oligo dT primer and RevertAid Premium reverse transcriptase (ThermoScientific). We used Phusion High Fidelity DNA Polymerase (New England Biolabs) for PCR and the following primers for appropriate PHF10 forms:

PHF10-Ps: 5’-CAGACAAGAAGTGCGGCGGAAG (forward),
5’-GTTGTAAATGGCACCAAATATTCC) (reverse);
PHF10-Ss: 5’-CAGACAAGAAGTGCGGCGGAAG (forward),
5’-GCTCCCCCCAACAAAAGGATAGTG (reverse);
PHF10-Sl: 5’-TCGGGCTGATGGCGGCGGC (forward),
5’-GCTCCCCCCAACAAAAGGATAGTG (reverse);

PHF10-Pl: 5’-TCGGGCTGATGGCGGCGGC (forward),
5’-GTTGTAAATGGCACCAAATATTCC (reverse).

To measure the level of PHF10 transcripts, total RNA from cell lines was extracted with Trizol (Ambion) and treated with DNase I. A FirstChoice Human Total RNA Survey Panel (Ambion, Invitrogen) was used for human tissue-specific RNA. RT was performed from random hexanucleotide primers and measured by quantitative PCR. Measurements at each point were made in at least three replications, and the mean value was calculated. Samples for RT contained equal amounts of RNA, and GAPDH transcription was measured in all experiments to evaluate the total RNA level that remained unchanged in stable and control lines and under knockdown conditions. Primers used for transcription level measurements were as follows:

PHF10, all forms: 5’-CCGGGAACGCATGGAAGAAAG (forward),
5’-CACCATCACTGTCTAGAGCAGGGAGC (reverse);

PHF10-Sl form: 5’-AAGCTTAATTCAGCAAGGGTTGG (forward),
5’-CGGCCACTTCCATTTAAAGCTCT (reverse);

PHF10-Pl form: 5’-TCCCAATGTGAGAATAGTGGCCA (forward),
5’- CAGCGACCTGATGGAATAGCACC (reverse);

BAF200: 5’-AAGCAACAGCATCCACCAACAT (forward),
5’-GAAAACCGCTGTCGCTGAAAA (reverse);

BAF180: 5’-GTGTGATGAACCAAGGAGTGGC (forward),
5’-TTGGCTGCTGTATGACAGGGG (reverse);

BRD7: 5’-CCAGATTGCTCAGGGAACTCCA (forward),
5’-AAGTTGTTTTCCATGACGGGGG (reverse);

BAF155: 5’-CACCCCAGCCAGGTCAGATACC (forward),
5’-GGAGGTTCCCTGCATCTTCCAG (reverse);

BRG1: 5’-CACGCTGGAGGAGATCGAAGA (forward),
5’-TGTACTTGATCACGGCATCCACA (reverse);

BAF250: 5’-CATGATTCGGCGGGATATCAC (forward),
5’-TAATGCCCATGTGCTCTCTGCC (reverse);

GAPDH: 5’-GACATCAAGAAGGTGGTGAAGCAG (forward),
5’-GCGTCAAAGGTGGAGGAGTGG (reverse).

Cell lines and transfection

Human Embryonic Kidney (HEK293, ATCC CRL-1573) and HeLa cells (ATCC CCL-2.2) were grown in Dulbecco’s modified Eagles medium (Invitrogen) with 10% fetal calf serum (Invitrogen), 100 μg/mL penicillin, and 100 U/mL streptomycin (Invitrogen). K562 (ATCC CCL 243), HEL 299 (ATCC CCL-137), Reh (ATCC CRL-8286), and NALM-6 cells (DSMZ ACC-128) were grown in RPMI 1640 medium (Invitrogen) with 2 mM L-glutamine and 10% fetal bovine serum (Invitrogen). Calu-1 cells (ATCC HTB-

54) were grown in EMEM medium with L-glutamine, 1% NEAA, 1 mM sodium-pyruvate, and 10% fetal bovine serum (Invitrogen). Transfection of HEK293 cells was performed with polyethylenimine (PEI) (Sigma-Aldrich). HEK293 stable lines were generated by transfection with plasmids Fl-PHF10-Pl-pcDNA or Fl-PHF10-Sl-pcDNA. Clones were selected with 50 μg/mL geneticin (G418) and tested for recombinant protein expression.