## SUPPLEMENTARY FIGURE LEGENDS

**Figure S1.** Amino-acid sequence of the minimal mitotic module of HNF1beta. Amino-acid sequence of the N-terminal part of HNF1beta representing the minimal mitotic-module. The amino-acid sequence deleted in  $\Delta$ Hom mutant is underlined, the amino-acid sequence deleted in the  $\Delta$ H3 mutant is in blue. The MODY mutations used in this study are represented in red.

**Figure S2.** Nter-HNF1alpha-GFP has an impaired nuclear localization and binds to mitotic chromosomes. Time-lapse microscopy of mIMCD3 cells transiently transfected with Nter-HNF1alpha-GFP. NEB indicates the moment of the nuclear envelope breakdown. Time, indicated at the bottom right corner of each frame, is measured in minutes. White arrows indicate the cells undergoing mitosis. Scale bars, 10µm.

**Figure S3. Temperature shifts do not affect the mitotic localization of wild-type Nter-HNF1beta-GFP.** A-C. MDCK cells stably expressing Nter-HNF1beta-GFP WT (green signal, GFP fusion) stained with Hoechst 33342 (red signal, DNA). The temperature in the medium was measured by three independent probes, as shown by the temperature curves. Black arrows indicate the moment in which the picture was taken. Temperature was suddenly dropped in panel A whereas it was suddenly raised in panels B and C. White arrows indicate the position of the mitotic cells. Scale bars, 10μm.

**Figure S4. Temperature shifts do not affect the mitotic localization of G287S Nter-HNF1beta-GFP.** MDCK cells stably expressing a mutant version of Nter-HNF1beta-GFP (G287S, green signal) were subjected to a sudden decrease (panel A) or increase (panel B) in temperature and imaged. The temperature change was followed by three independent probes, as shown by the temperature curves. Black arrows indicate the moment at which the picture was taken. White arrows indicate the position of the mitotic cells. Scale bars, 10µm.

**Figure S5.** ChIP assay on WT and mutant Nter-HNF1beta-GFP. ChIP-qPCR analysis of WT and mutant Nter-HNF1beta-GFP proteins on *PKHD1* and *HNF1B* promoters with GFP antibodies or control IgG. A region in the beta-globin gene *HBB-BH1* with no known nor predicted HNF1beta binding site was used as a negative control. Results are expressed as percentage of immunoprecipitated DNA compared to the input DNA and shown as mean  $\pm$  SEM from 4 independent experiments. Statistical significance was determined by Mann-Whitney test. \*: p<0.05.

Figure S6. Importazole treatment does not affect the localization of Nter-HNF1beta-GFP. A. Representative images of MDCK cells stably expressing Nter-HNF1beta-GFP submitted to a cold shock, in presence or absence of importazole. B. Difference of calculated ICQ before and after cold shock, in cells treated or not with importazole. Histograms represent the average difference  $\pm$  SEM. Images after cold-shock were taken 1 minute after addition of cold medium. White arrows indicate mitotic cells. Scale bars, 10 µm.

**Movie S1. Mitotic chromatin binding of HNF1beta wild-type protein.** Time-lapse microscopy of MDCK cells expressing an HNF1beta Nter-HNF1beta-GFP fusion protein. NEB indicates the moment of the nuclear envelope breakdown.

Movie S2. Effect of the  $\Delta$ Hom deletion mutation on mitotic chromatin binding of HNF1beta. Time-lapse microscopy of mIMCD3 cells transiently transfected with  $\Delta$ HOM-GFP. NEB indicates the moment of the nuclear envelope breakdown.

Movie S3. Effect of the  $\Delta$ H3 deletion mutation on mitotic chromatin binding of HNF1beta. Time-lapse microscopy of mIMCD3 cells transiently transfected with  $\Delta$ H3-GFP. NEB indicates the moment of the nuclear envelope breakdown. **Movie S4. The switch in DNA binding is a reversible phenomenon.** Time-lapse microscopy of MDCK cells stably expressing Nter-HNF1beta-GFP P256S (green signal) submitted to a first cold shock (COLD SHOCK 1), left to heat up to 37°C, and submitted to a second cold shock (COLD SHOCK 2).

Table 31. I finite 3 used for clothing and matagenesis	Table S1.	Primers	used for	cloning	and	mutagenesis
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Oligonucleotide	Sequence (5'-3')
10i1	GGGTCCTGCTAGCCACCATGGTGTCCAAGCTCACGTCG
10i2	CCGCGGTACCAGCTTTTGCCGGAATGCCTCCTC
10i3	CCGCGGTACCCCGAACCGGTTGCGGCGCATCTTCTT
10i4	ACCTCAGGTACCAAGTTGGAGCCCAGGCCGTG
10i5	CTGGCCATGGACACGTACAGC
10i6	TGCTGGATCCTGGGAGGAAGAGGCCATCTG
11i122	ACGATCGGCAAAAGAACTCGAGCAAGGAAGAGAGAGAGAG
11i123	CCTCTCTCTTCCTTGCTCGAGTTCTTTTGCCGATCGT
13i94	GAGAGAGAGGCCTTACTCGAGGAATGCAACAGG
13i95	CCTGTTGCATTCCTCGAGTAAGGCCTCTCTCTC
12i64	TCCAAAGCCCACGGCCTGAGCTCCAACTTGGTCACTGAG
12i65	CTCAGTGACCAAGTTGGAGCTCAGGCCGTGGGCTTTGGA

## Table S2. Primers used for qRT-PCR

Oligonucleotide	Sequence (5'-3')
HNF1b forward	AAAAGCCAGTCCCTGCAAAC
HNF1b reverse	GCACACCTCAACGTTAGCAA
PKHD1 forward	TGTGGTTGGAGCTAGTTAATGA
PKHD1 reverse	GGCCATCTTCTCTGCTCTTT
Hbb-bh1 forward	TCTCTACCAGGCCACCATTT
Hbb-bh1 reverse	CGAGTGGAGTTTTGTTCTTGC

MVSKLTSLQQELLSALLSSGVTKEVLVQALEELLPSPNFGVKLETLPLSPGSGAEPDTKPVFHTLTNGHA KGRLSGDEGSEDGDDYDTPPILKELQALNTEEAAEQRAEVDRMLSEDPWRAAKMIKGYMQQHNIPQREVV DVTGLNQSHLSQHLNKGTPMKTQKRAALYTWYVRKQREILRQFNQTVQSSGNMTDKSSQDQLLFLFPEFS QQSHGPGQSDDACSEPTNKKMRRNRF**KWGPASQQILYQAYDRQKNP**SKEEREALVEECNRAECLQRGVSP SKAHGLGSNLV*TEVRVYNWFANRRKEEAFRQKL* 

## △HOM deletion

<u>AH3 deletion</u> P,V,G: MODY mutations



Figure S2. Nter-HNF1alpha-GFP has an impaired nuclear localization and binds to mitotic chromosomes





## Figure S3. Temperature shifts do not affect the mitotic localization of wild-type Nter-HNF1beta-GFP



Figure S4. Temperature shifts do not affect the mitotic localization of G287S Nter-HNF1beta-GFP



Figure S5. ChIP assay on WT and mutant Nter-HNF1beta-GFP





Figure S6. Importazole treatment does not affect the localization of Nter-HNF1beta-GFP