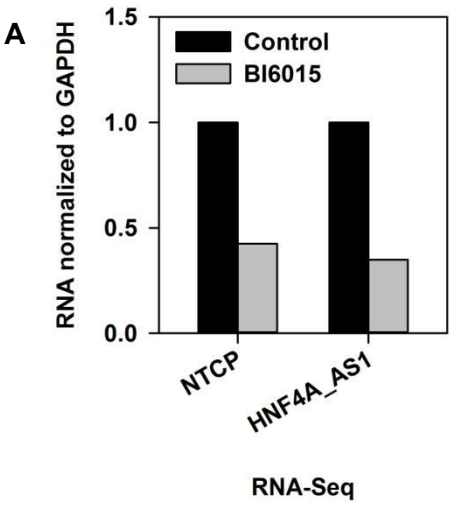
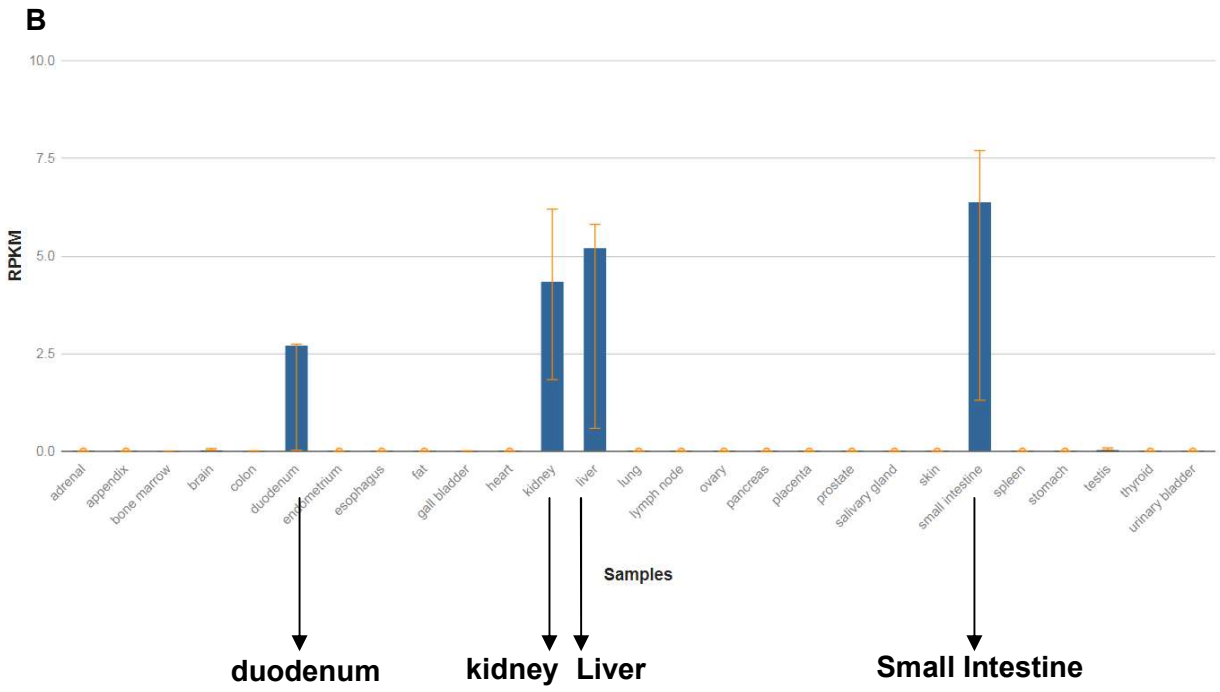


Fig. S1. **(A)** RNA-seq data comparing the mRNA changes of NTCP and *HNF4A-AS1* in primary human hepatocytes treated with an HNF4α inhibitor BI6015 (10 μM) for 24 h. **(B)** Tissue distribution of *HNF4A-AS1 in humans (*from NCBI Gene database).



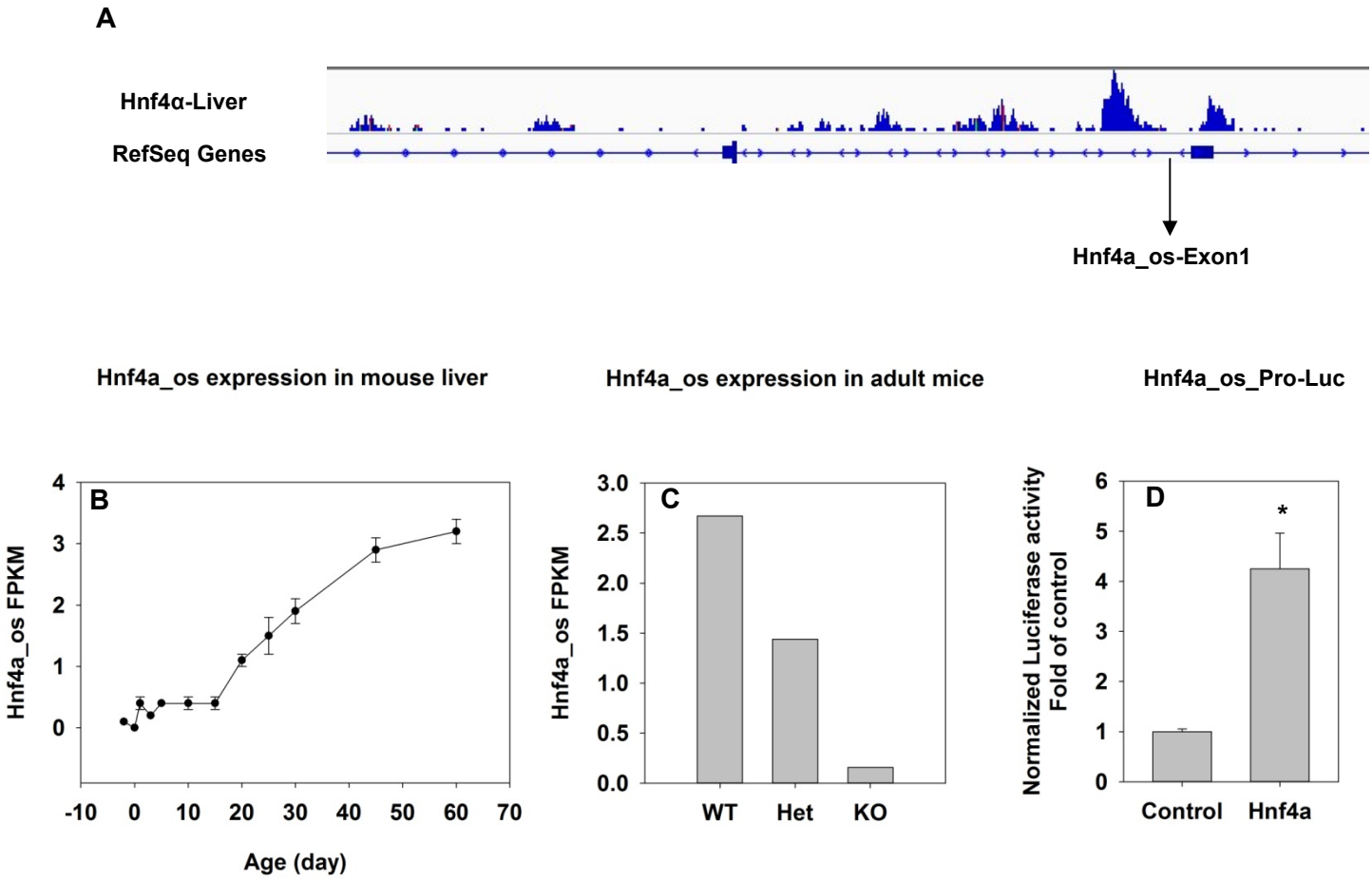


Fig. S2. **(A)** ChIP-seq data of the binding of Hnf4α to the promoter and exons of Hnf4a_os in adult wildtype mouse liver. **(B)** RNA-seq data of expression of Hnf4a_os in the developing wildtype mouse liver (GSE58827). N=3, mean ± SE. **(C)** RNA-seq data of pooled liver total RNAs that indicate the difference in Hnf4a_os expression in livers from adult male WT mice and mice with liver-specific heterozygous (Het) and knockout (KO) of Hnf4α. **(D)** Dual-luciferase reporter activities of Hnf4a_os-Pro-Luc induced by Hnf4α in HEK293 cells. N=4, mean ± SE. * $p<0.05$ vs control.

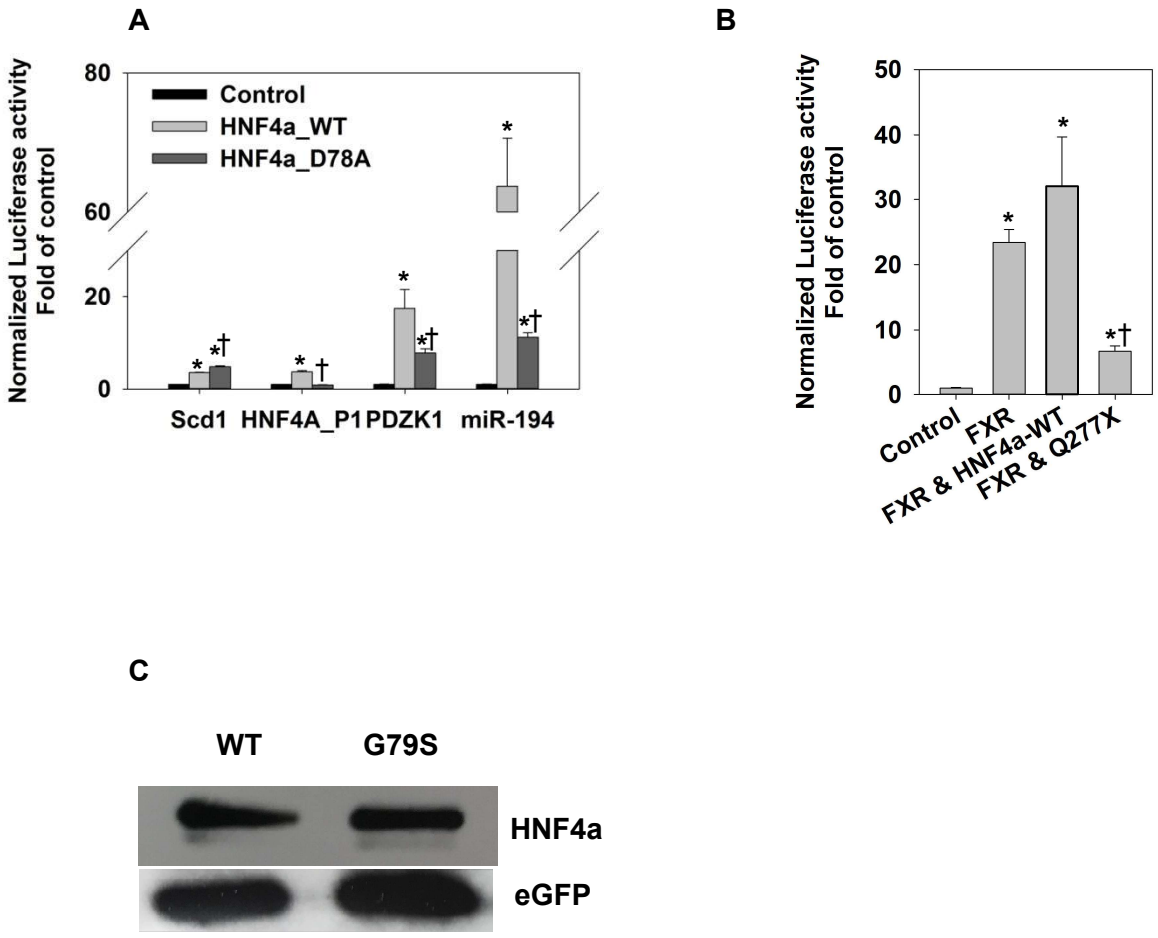


Fig. S3. (A) Effects of wildtype (WT) and D78A mutant of HNF4α2 on luciferase activities of promoters of Scd1, HNF4A P1, PDZK1, and miR-194 in HEK293 cells. N=4, mean ± SE. *p<0.05 vs control. † p<0.05 vs HNF4α_WT. **(B)** Effects of HNF4α-Q277X mutant on the FXR-transactivated BSEP promoter in HEK293 cells. N=4, mean ± SE. *p<0.05 vs control. † p<0.05 vs HNF4α_WT. **(C)** Western blot determination of the protein expression of the transiently transfected HNF4α_WT, HNF4α_G79S, and the co-transfected eGFP protein in HEK293 cells.

Supplemental Table 1. Sequence information of vector constructions

Name of Plasmids	Engineered motifs	Sequence
HNF4A-P1-Luc	985 bp Proximal promoter of HNF4A-P1 Promoter (-985 ~ +1 bp)	agaaacaggggatggcaaggggatacgaaacagggagagggaggagggg gaagaggatggacgtctaccaggccccacttggtgcttgatttatgccatctcatttcct tctcaaaccacccttgaagttgattgtacattttacagaaaaggaaactgaggctcgg agaggagaatcatttacccaaggtccagttagtagacggtaggtgcctgaatgtaa atccaggtctctgcctgctccgggaggggtgggggtgagggaaacaggagaatg tgatgggaaaatccgagatggagccagcctgggccagaaacactgggagctgtg ggagacggagaggggcagggtgggatcacagggagcaggagcggggaattgg aggtgaatctggccctcccaaacttccagtccattctgctcccaggggaaccgggaa actgcgggggaactggaagggagctcccagaacaaggatccagaagattggcat ctggggcctgggatttaggtttctaaatcgtgggccatggggcagccttatctctgcaa aagcattgagggtagaagtcaatgatttgggaagttattgaattagggatctcggag gtaggctgtcagtgcctgatagtatcagttagaatgcctgacttggggtgacaatggct tggaggggtgggtgagtcaagggtcaaatgagtgcccgtgagtcatgatgcctgcct tgtacaattgataactgaacatcggtgagttagggccccagcagttgtaattagcacc ccgggtgtcacagccagaaaccaacaaacagccaaatccctgcagccccgccca gcctatccaccggcgggggaccgattaaccattaaccccacccctccccggcag agcctccacccttcacagaggctaggccaagactcccagcagatcttcccagagg acggtttgaaaggaaggcagagagggcacT
pGL3T7-5`UTR	89 bp 5`UTR of P1-HNF4A	GGGAGGAGGCAGTGGGAGGGCGGAGGGCGGGGGCCTT CGGGGTGGGCGCCCAGGGTAGGGCAGGTGGCCGCGGC GTGGAGGCAGGGAGA
HNF4A-P1-5`UTR-Luc	985 bp Proximal promoter of HNF4A-P1 Promoter	Same as above
	89 bp 5`UTR of P1-HNF4A	Same as above
HNF4A-AS1-Pro-Luc	313 bp Proximal promoter and exon 1 of HNF4A-AS1 (-168 ~ +145 bp)	ttttcttccccacccaccactccgggagaggtgcagagaaaactgggacttatcaag acaaagaacaaaagtcgtggaggaaagaagccaagagccatctctactctgggg tagggcccttcagttttgccccttgaaatttcaaattccagtttgtggacaaagtcCTA ACTATCTCACAATATAGGTCCCCAACCACTGACCAAACTC CAGTCCAGGCAGCCACCAGCTGGCCTGGTCTTGCTGCTT CCTTTAGCGGCTTCCAAGGTCCAGGGACAGGGGGTCTG GGCCACCAAGAGGCTCTGCTAGGCT
HNF4A-AS1-Dis-P1-5`UTR-Luc	313 bp The reverse complementary strand of the proximal promoter of HNF4A-AS1	agcctagcagagcctcttggtggcccagacccccctgtccctggaccttggaagccgc taaaggaagcagcaagaccaggccagctggtggctgcctggactggagtttggtca gtggttggggacctatattgtgagatagttaggactttgtccacaaactggaatttgaaa tttcaaaggggcaaaactgaagggccctaccccagagtagagatggctcttggcttc tttcctccacgacttttgttctttgtcttgataagtcccagttttctctgcacctctcccggagt ggtgggtggggaagaaaa
	985 bp HNF4A-P1 proximal promoter	Same as above
	89 bp 5`UTR of P1-HNF4A	Same as above
HNF1A-Pro	135 bp Proximal promoter of HNF1A (-105 ~ +30bp)	tgtccctctccgctgccatgaggcctgcactttgcaggctgaagtccaaagttcagtc ccttcgctaagcacacggataaatatgaaccttggagaatttccccAGCTCCAA TGTAAACAGAACAGGCAGGGGC
PDZK1-Pro-Luc	224 bp human PDZK1 proximal promoter and 5' UTR (-141 ~ +83 bp)	gagcttttggtttgctgaggtttgtcagattttccagctcagggcccagccagctggcag gaagcaggacagaggtcacttgaattcagaccacatgtccctgttaaatacattagct ttaaatcaatctttgttcaaagTCCAGTGAGTTGCAAGCTTAATGCT CACCTGCAGAGACAGAATTCCTGAGTGAACGAACAGAGC AGCTCCTCTTCCATCTCCA

miR-194-Pro-Luc	405 bp human miR-194-2 promoter (-405 ~ +1 bp)	tctctgccccatggctgcagcagggagggtgggcaaagggcagaggtgacagtgg ccaaacctgaaactgaggcccagctctggttgggaaagaaacaaggaaacaaaa taacttggggacagagggcgtggggtcatcagagcgggcaaaggctgcccagga gttgtaaatccgagccgctcccggctttatgaggccgatttggggttgagcctggactttt gcccaggaaggagtgctggcagcacacggggcgccaggtgtgggccccggatg ataagaagcctcggtgaaaagaccatggacctggggccacgaagactggggagc ccaggtgagtgggcatgggacactctccagagccctgccgaaggcggacgccag gcagggccccggtctccaatT
Scd1-Pro-Luc	728 bp Mouse Scd1 promoter and 5' UTR (-708 ~ + 20 bp)	cagaggcggtccccggcagacggagcccaatgagtgagtgcagttgtaactcagc gtgtgctgtcaagaacgcagcagcaaccggatgctggctgaagggatacactattgt tttcccgggtcagagccctgggcgcacctcatatcccagtctcccggggtttctctttg ctggtgcctggaagtgggggtagatgtgaagttagaccgagttgtgagtggcggtag ccagtgtcttcctcacttctttcgatgcgatttccccagtgaaccatttgctaagcgccag accaaagtcctaggcttgcacacaattcctacttggaatcacgttatcctgctcttaaag aaaagtcacccatcagcccacagcaaagaggataaggagaaaaagaggggag gagagagcgagaagctagaggcagagggaacagcagattgcgcctagccaatg gaaaaggcaggacaaggtggcaccaaattctctttggccaatgacaagacgggctt cacaggaggcacattagcatttatccccaggcaggggttggagcagcgcgccct gttgatgccttcagcatcccggcgcctccaaggtctactctggaatctacttggctttctttt cccgttcttggtcccgccctctctctctccctccctccctccctccttcctccctccc tccctccctccctccctCACCTCCACGCCTGGCTTCC
BSEP 1445-Luc	1531 bp human BSEP proximal promoter and 5' UTR (-1445 ~ +86 bp)	ttgactgctgtcaataacttcagcacagcaaaggtagcaaaattctattgggaatctttt cccaatcaaagctacagccccatagctgtgttgccttttggttttgtgtgaagcaaaatt tttttcctgggtcctaattggtgccaaatccaatattactacatttgcgtcaactcagaagt caacccattcagtttgcatagaggaaacatctagaaatcttgcttttctttgctgaccttg atatatttgagatttgaacatataataacatataaaattatatgttataattttgggttttatg ggctaagtcataaaccatcttatacataaattccaatagagaaaaaatggtggatgct gaattttaataaaaaatttatgacagagaaacctaaaattgagaaaatttgatcttaca gtttaatttctgcaaattaagaagcactggcccatcaattgcatttcagagcacagagt ggaagaaggttagcacagactggcatgtggcttcacatctactagttgttacaccttag gaggattatttaacctctctgtgcttcagtttccccaactataaaatagaaatgacatgat agcacccaactcctagggctgttgagaggcccaaatgaggtgatacaaataacattt ttgaagtgggccaggcacccaaccaagtcttgaccaatgttgcctattattattcattgc tgaaggctggagtgagaggcatttagggaaaagtaagctcaggcaaaggagaaa aaataagaacattgtaggaaaaatggaaagattcacaagaagggagaggaaga ggcagcacaaatatattggaggagctccacatgcttatttgactcaagacctgttcattt gaaccttagaaaatcgttcatctttgcttatacagagcttcatctggtgtgtccatgcca gggtgcaagagttgtctgtgcactcagacttttgagcaaggctgtttcaaatgttctttta gggtatttgtctccacaaaactctatagctgggccaggagcatctggatcctgcaacc agggattttccaagagcaatcttttatattgaggggaaagtttaaaggtatttttttttttg tcttgttatgtttttaagtaacttttcacaactacaggcctgtaaaaaataagggttgggat agcctgaattccagggctcttgctgggcccactctgctcaatttgcctctcgttccaagg tgaatcagcaatttccaaggcctgttgacaccctcagagggtttcccaagcacactct gtgtttggggttattgctctgagtatgtttctcgtatgtcactgaactgtgcttgggctgccct tagggacattgatcctaggcaaatagataatgttcttgaaaaagtttgaattctgttcag tgctttagAATGATGAAAACCGAGGTTGGAAAAGGTTGTGAAA CCTTTTAACTCTCCACAGTGGAGTCCATTATTTCCTCTGG CTTCCTCAAATTCATA

Note. Lowercase sequences denote promoters and uppercase sequences denote exons.

Putative DNA-binding sites of transcription factors predicted by PROMO and HNF4 binding site scanner: HNF4α: green-shaded; HNF1α: yellow-shaded; HNF6: pink-shaded; SP1: grey-shaded; FXR: blue-shaded.

Supplemental Table 2. Numerical changes induced by HNF4α mutants on 3 HNF4α target promoters

	HNF4A-AS1-Pro-Luc		HNF1A-Pro-Luc		miR194-Pro-Luc	
HNF4α	*VS* control	*VS* WT	*VS* control	*VS* WT	*VS* control	*VS* WT
Wild Type	↑ 27 x *	-	↑ 50 x *	-	↑ 15 x *	-
D78A	↑ 19 x *	↓30.6% †	↑ 42 x *	N/S	↑ 3 x *	↓80.0% †
G79S	N/S	↓93.5% †	↑ 10 x *	↓81.0% †	N/S	↓92.2% †
R163X	N/S	↓95.7% †	↑ 4 x *	↓92.7% †	N/S	↓92.2% †
D215Y	↑ 26 x *	N/S	↑ 45 x *	N/S	↑ 8 x *	↓49.3% †
Q277X	N/S	↓96.5% †	N/S	↓96.8% †	N/S	↓90.8% †
E285Q	N/S	↓96.4% †	N/S	↓98.0% †	N/S	↓94.0% †
L341P	↑ 28 x *	N/S	↑ 53 x *	N/S	↑ 10 x *	↓38.1% †
W349X	N/S	↓96.9% †	N/S	↓95.0% †	N/S	↓95.0% †
V402I	↑ 24 x *	N/S	↑ 85 x *	↑70% †	↑ 12 x *	N/S
I463V	↑ 44 x *	N/S	↑ 83 x *	↑66% †	↑ 17 x *	N/S

Supplemental Table 3. Numerical changes induced by HNF4α mutants/HNF1α on AS1-Dis-P1-5`UTR-Luc

	AS1-Dis-P1-5`UTR-Luc		AS1-Dis-P1-5`UTR-Luc with HNF1α	
HNF4α	*VS* control	*VS* WT	*VS* control	*VS* WT
Wild Type	↑ 3.7 x *	-	N/S	N/S
D78A	N/S	↓76.5% †		
G79S	N/S	↓65.7% †		
R163X	N/S	↓75.0% †		
D215Y	↑ 2.1 x *	↓42.1% †		
Q277X	N/S	↓76.3% †		Q277X ↓42.1% †
E285Q	N/S	↓82.7% †		
L341P	↑ 1.5 x *	↓59% †		
W349X	N/S	↓80.1% †		
V402I	↑ 2.8 x *	N/S		
I463V	↑ 2.8 x *	N/S		

Supplemental Table 4. Numerical changes induced by HNF4α mutants/HNF1α/HNF6 on PDZK1-Pro-Luc

	PDZK1-Pro-Luc		PDZK1-Pro with HNF1a		PDZK1-Pro with HNF6	
HNF4α	*VS* control	*VS* WT	*VS* control	*VS* WT	*VS* control	*VS* WT
Wild Type	↑ 17 x *	-	↑ 10 x *	-	↑ 21 x *	-
D78A	↑ 8 x *	↓55.5% †	↑ 6 x *	N/S	↑ 17 x *	N/S
G79S	↑ 2 x *	↓87.8% †	↑ 3 x *	↓75.1% †	↑ 3 x *	↓84.0% †
R163X	N/S	↓95.1% †	N/S	↓92.7% †	N/S	↓94.5% †
D215Y	↑ 12 x *	N/S	↑ 9 x *	N/S	↑ 16 x *	N/S
Q277X	N/S	↓95.7% †	N/S	↓95.6% †	N/S	↓95.8% †
E285Q	N/S	↓95.8% †	N/S	↓93.0% †	N/S	↓94.9% †
L341P	↑10 x *	↓41.5% †	↑ 6 x *	↓44.7% †	↑ 9 x *	↓56.2% †
W349X	N/S	↓95.1% †	N/S	↓90.2% †	N/S	↓95.8% †
V402I	↑ 14 x *	N/S	↑ 8 x *	N/S	↑ 17 x *	N/S
I463V	↑ 13 x *	N/S	↑ 11 x *	N/S	↑ 19 x *	N/S

Symbols & Notes "*VS* control": The luciferase activity versus control (No HNF4α added) "*VS* WT": The Promoter activity regulated by mutant HNF4α versus wild-type HNF4α"↑" & "↓": increase and decrease; "x" : fold; "*" & "†" : A statistical difference, $p < 0.05$"N/S": Not a statistical difference