Supplemental document to:

Identification and analysis of a novel NR0B1 mutation in late-onset adrenal hypoplasia congenita and hypogonadism

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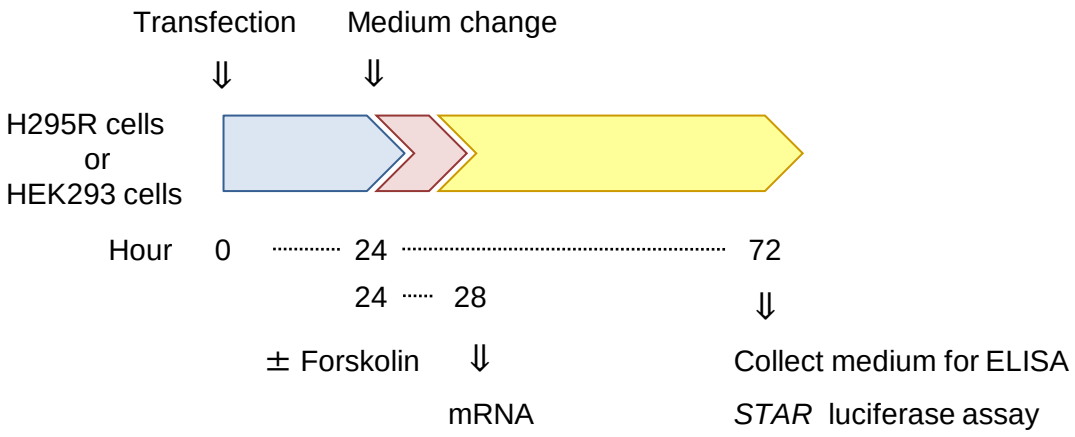
Mutation Taster (http://www.mutationtaster.org/)

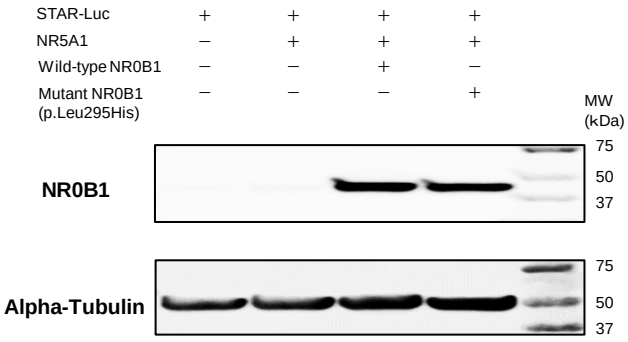
Nucleotide Change	Amino Acid Change	Mutation Taster
c.884 T>A	p.Leu295His	Disease causing

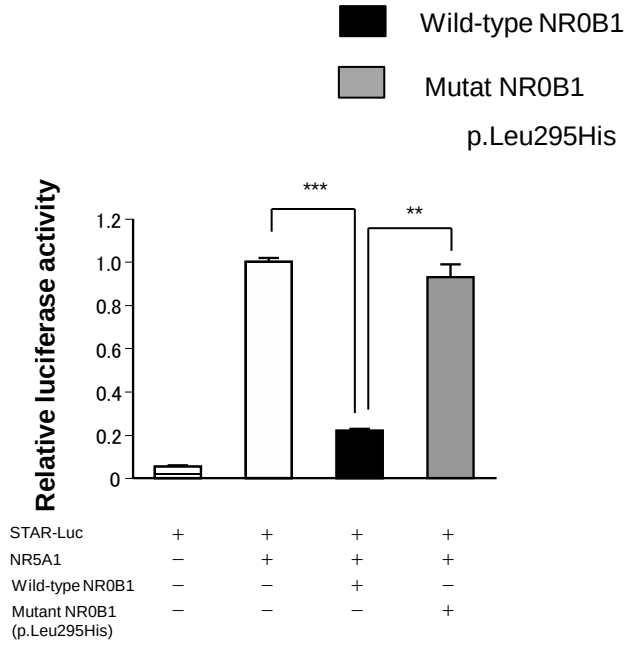
Supplementary Figure. 1



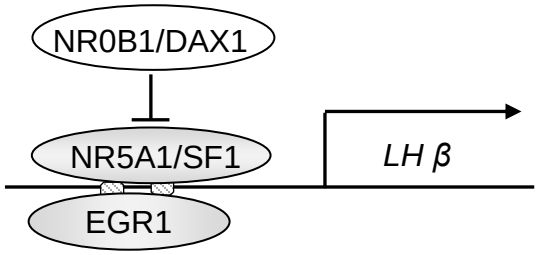
Supplementary Figure. 2

HEK293 Cells





Supplementary Figure. 3



Supplementary Figure. 4

Supplementary Figure Legends

Supplementary Figure 1. In silico analysis of the novel *NR0B1/DAX1* missense mutation

The p.Leu295His mutation in the NR0B1/DAX1 gene was predicted "Disease causing" by Mutation Taster.

Supplementary Figure 2. Scheme of experimental procedures

Supplementary Figure 3. The functional analysis of mutant p.Leu295His NR0B1/DAX1

Immunoblotting of HEK293 cells with indicated transfections. α-Tubulin was used as loading control (left). Luciferase assay for NR5A1/SF1-mediated STAR transcriptional activity. STAR-luc, NR5A1/SF1 and NR0B1/DAX1 (wild type and mutant p.Leu295His) expression vectors were co-transfected into HEK293 cells, n=4 (right). ** $P<0.01$, *** $P<0.001$.

Supplementary Figure 4. The scheme of NR5A1/EGR1-mediated synergistic activation of LHβ

The gene expression of LHβ was measured in H295R cells with indicated transfections.

Supplementary Table 1. Primer sequences used in the study.

Primer sequences used for quantitative RT-PCR.

Gene	Forward	Reverse
AKR1B1	GTGACACCAGCACGCATTG	GCATTGAAGGGATAGTCTTCCAA
CYP11A1	GAGGCCCAGCGATTCATTGAT	TCCTGAACAGACGGAACAGGT
CYP11B1	TCATGTTCAAATCCACCGTCC	GCTGGTGTACTGTTGAGGGC
CYP11B2	TTCAACCGCCCTCAACACTAC	GGAAACGCTGTCGTGTCCA
CYP17A1	CCGTAAGGGTATCGCCTTCG	CCATCCTTGAACAGGGCAAAG
CYP19A1/Aromatase	ACTACAACCGGGTATATGGAGAA	TCGAGAGCTGTAATGATTGTGC
CYP21A2	CATCCAAATTGTGGACGTGATTC	CCACGATGTGATCCCTCTTCTC
HPRT1	ACCAGTCAACAGGGGACATAA	CTTCGTGGGGTCCTTTTCACC
HSD3B1	CACATGGCCCGCTCCATAC	GTGCCGCCGTTTTTCAGATTC
HSD3B2	CTTGTGCGTTAAGACCCACAT	GGGTTGACTGTAGAGAACTTTCC
LHB*	GCTACTGCCCCACCATGATG	ATGGACTCGAAGCGCACATC
		AGAGCCACAGGGAAGGAGAC
		AGCTGAGAGCCACAGGGAAG
TBP	CACGAACCACGGCACTGATT	TTTTCTTGCTGCCAGTCTGGAC

Primer sequences used for *NR0B1/DAX1* sequence.

	Forward	Reverse
NR0B1 Exon1-1	TGAGACAGGGAAAGGGGTAAT	CCGGGCTCATCGCCGCACGAA
NR0B1 Exon1-2	TGGTGGATCAGTGTTGGGGC	CCGGGATCAGAGCCGCACGAA
NR0B1 Exon1-3	AAGCAAACGTACGCGGCAC	CCTCTGCGCGAAGTAGGAGC
NR0B1 Exon1-4	TAGCTCAAAGCAAACGCACGTG	GACGCCCAGCAGTTGCGCAC
NR0B1 Exon1-5	GCCTCAGCGGGCCTGTTGAAG	CCCGATGCTTTTGTGAGCTGGGAA
NR0B1 Exon2-1	GCTAGCAAAGGACTCTGTGGT	TGTGTGGCCCACATGACTTTA

Primer sequences used for mutagenesis of *NR0B1/DAX1* .

Gene	Forward	Reverse
NR0B1 mutagenesis primer	CACATGCTTGAGCTGGCCCAGGACCGCT	CAGGGACGCCCAGCAGTTGCGCACC

*For *LHB* , one forward primer was combined with different reverse primers