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Supplementary data

mRNA transcripts of minigene constructs expressed in HEK293T cells

Sequences in red are PCR primers, PI12-132F and PI12-132R. Sequences marked by green color are adenovirus exonic Sequence of vector.

pIN12-In45-56 vector

TAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTTAGCT
TGGTACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTGAGCTCACTCTCTCCGCA
TCGCTGTCTGCGAGGTACCCTACCAGGGATTAAAGACAATGTCTATCGACAGAGAAGAAAGTATTTG
TGGATGTGGCCATGGGTTATAAATATGGTCAGCCCATCCCAGGGTGGAGTATACTGAAGAAGAACTA
AAACTTGGGGTGTGTATTCGGGAGCTCTCCAACTCTATCCCCTCATGCTTGCCGAGAGTATTTGA
AAAACCTCCCTCTGCTGACTAAATACTGTGGCTACAGAGAGGACAATGTGCCTCAACTCGAAGATGTCT
CCATGTTTCTGAAAGTTCGCGGTGAGCTGCAGGACAACTCTTCGCGGTCTCTATGCATCTCCGAA
TCCAGAGCTAAGCTTACTAGAGCTAGAGGGCCCTATTCTATAGTGTACCTAAAT

c.1978-2A>C

Exon16+Exon17 373 bp

TAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTTAGCT
TGGTACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTGAGCTCACTCTCTCCGCA
TCGCTGTCTGCGAGGTACCCTACCAGTTACTACCAGTACTTTAGCTATGGGAGTAAATTTGCCTGCTCA
CCTAGTAGTTATAAAATCTACAATGCATTATGCTGGAGGACTGTTTGAAGAGTACAGTAAACAGATAT
TCTACAGATGATTGGTAGAGCTGGTCGACCTCAATTTGACACTACAGCTACTGCAGTTATCATGACTCG
ATTAAGCACAAAGGACAAGTACATTCAGATGTTAGCTTGTAGAGACTGTAGAAAGCAGTTCGCGGT
GAGCTGCAGGACAACTCTTCGCGGTCTCTATGCATCTCCGAACTCCAGAGCTAAGCTTACTAGAG
CTAGAGGGCCCTATTCTATAGTGTACCTAAAT

Alternative splicing only Exon16 retained 278 bp

TAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTTAGCT
TGGTACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTGAGCTCACTCTCTCCGCA
TCGCTGTCTGCGAGGTACCCTACCAGTTACTACCAGTACTTTAGCTATGGGAGTAAATTTGCCTGCTCACCTAG
TAGTTATAAAATCTACAATGCATTATGCTGGAGGACTGTTTGAAGAGTACAGTAAACAGATATTCACAGATGAT
TGGTAGAGCTGGTCGACCTCAAGTTCGCGGTGAGCTGCAGGACAACTCTTCGCGGTCTCTATGCATCTC
TCAACGCCAACAGCTAAGCTTACTAGAGCTAGAGGGCCCTATTCTATAGTGTACCTAAAT

1 **c.2680+3_2680+4delAT**

2 **Splicing in wildtype construct**

3 Exon24+Exon25 363 bp

4 TAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTTAGCT
5 TGGTACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTGAGCTCACTCTCTCCGCA
6 FCGTGTCTGCGAGGTACCCTACCAGTCTTATTCAGGCTCAACTAGGATGCATCCCATAACAAGATTTTGCTTT
7 GACACAAGATACCGCAAAGATTTTCAGACATGGCTCCCGAATTACAAGATGGTTGTCAGATTTTGTAGCTGCTCAA
8 GAAAAGAAGTTTGTGTACTATTGAATAGTTTATTTAGCTAAATGTTTATAGGTGTAACCTTTGGGAAAACCTCTC
9 TGCATGTATCCAAACAACcTGGAAAAAATTGTCGCGGTTGAGCTGCAGGACAAACTCTTCGCGGTCTCTAT
10 GCATCCTCCGAACGCCAAGGCTAAGCTTACTAGAGCTAGAGGGCCCTATTCTATAGTGTACCTAAA
11 T

12 Exon24 230 bp

13 TAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTTAGCT
14 TGGTACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTGAGCTCACTCTCTCCGCA
15 FCGTGTCTGCGAGGTACCCTACCAGTCTTATTCAGGCTCAACTAGGATGCATCCCATAACAAGATTTTGCTTT
16 GACACAAGATACCGCAAAGATTTTCAGACATGGCTCCCGAATTACAAGATGTCGCGGTTGAGCTGCAGGACAAA
17 CTCTTCGCGGTCTCTATGCATCCTCCGAACGCCAAGGCTAAGCTTACTAGAGCTAGAGGGCCCTATT
18 CTATAGTGTACCTAAAAT

19 **Splicing in variant construct**

20 Exon24+Intron24+Exon25 446 bp

21 TAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTTAGCT
22 TGGTACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTGAGCTCACTCTCTCCGCA
23 FCGTGTCTGCGAGGTACCCTACCAGTCTTATTCAGGCTCAACTAGGATGCATCCCATAACAAGATTTT
24 GCTTTGACACAAGATACCGCAAAGATTTTCAGACATGGCTCCCGAATTACAAGATGtatgttacaggat
25 tagtggatttttaaatatTTTTTccaataaaactggatatttagatttaataactggaactTTTTTTTTtag
26 GGTTGTCAGATTTTGTAGCTGCTCAAGAAAAGAAGTTTGTGTACTATTGAATAGTTTGTATTTAGCTA
27 AATGTTTTAGGTGTAACCTTTGGGAAAACCTCTGTCATGTATCCAAACAACcTGGAAAAAATTGTCG
28 GGTGAGCTGCAGGACAAACTCTTCGCGGTCTCTATGCATCCTCCGAACGCCAAGGCTAAGCTTACT
29 AGAGCTAGAGGGCCCTATTCTATAGTGTACCTAAAAT

30 Exon25 265 bp

31 TAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTTAGCT
32 TGGTACCGAGCTCGGATCCACTAGTAACGGCCGCCAGTGTGCTGGAATTGAGCTCACTCTCTCCGCA
33 FCGTGTCTGCGAGGTACCCTACCAGGTTGTCAGATTTTGTAGCTGCTCAAGAAAAGAAGTTTGTGTACTAT
34 TGAATAGTTTATTTAGCTAAATGTTTATAGGTGTAACCTTTGGGAAAACCTCTGTCATGTATCCAAACAACcTGG
35 AAAAAATTGTCGCGGTTGAGCTGCAGGACAAACTCTTCGCGGTCTCTATGCATCCTCCGAACGCCAAGG
36 CTAAAGCTTACTAGAGCTAGAGGGCCCTATTCTATAGTGTACCTAAAAT

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1 Supplementary table 1 Semen Characteristics of II-2 and II-3's spouses

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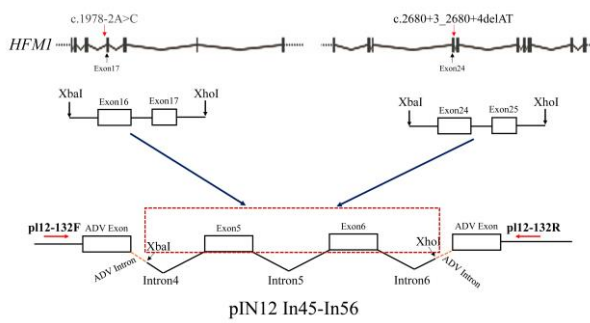
Semen Characteristics

| | pH | Volume (ml) | Sperm density (10 ⁶ /ml) | Total progressive motility (10 ⁶) | sperm fragmentation (%) | DNA index |
|---------------|-----|-------------|-------------------------------------|---|-------------------------|-----------|
| II-2's spouse | 7.4 | 4.8 | 71.5 | 234 | 17.5 | |
| II-3's spouse | 7.4 | 6.0 | 31 | 82.8 | 14 | |

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7 Supplemental figure S1. Schematic representation of hybrid minigene construction to test the effect of
8 the candidate mutations.

9 Mutant and wildtype amplicons covering *HFM1* variant sites, c.1978-2A>C and
10 c.2680+3_2680+4delAT, respectively, were inserted into pIN12 In45-In56 vectors between restriction
11 sites Xba I and Xho I. Primer pI12132F and pI12-132R were used to test the effects of splice site
12 mutations.

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HFM1; NM_001017975.4: c.1978-2A>C



HFM1; NM_001017975.4: c.2680+3_2680+4delAT



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- 2 Supplemental figure S2. Amino acid sequence changes of splicing variants, c.1978-2A>C (upper part)
- 3 and c.2680+3_2680+4delAT (lower part), in *HFM1* gene. Changes in the amino acid sequence of the
- 4 *HFM1* variants are marked by in red font.