SUPPLEMENTARY APPENDIX

Next-generation sequencing and recombinant expression characterized aberrant splicing mechanisms and provided correction strategies in factor VII deficiency

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Figure S1

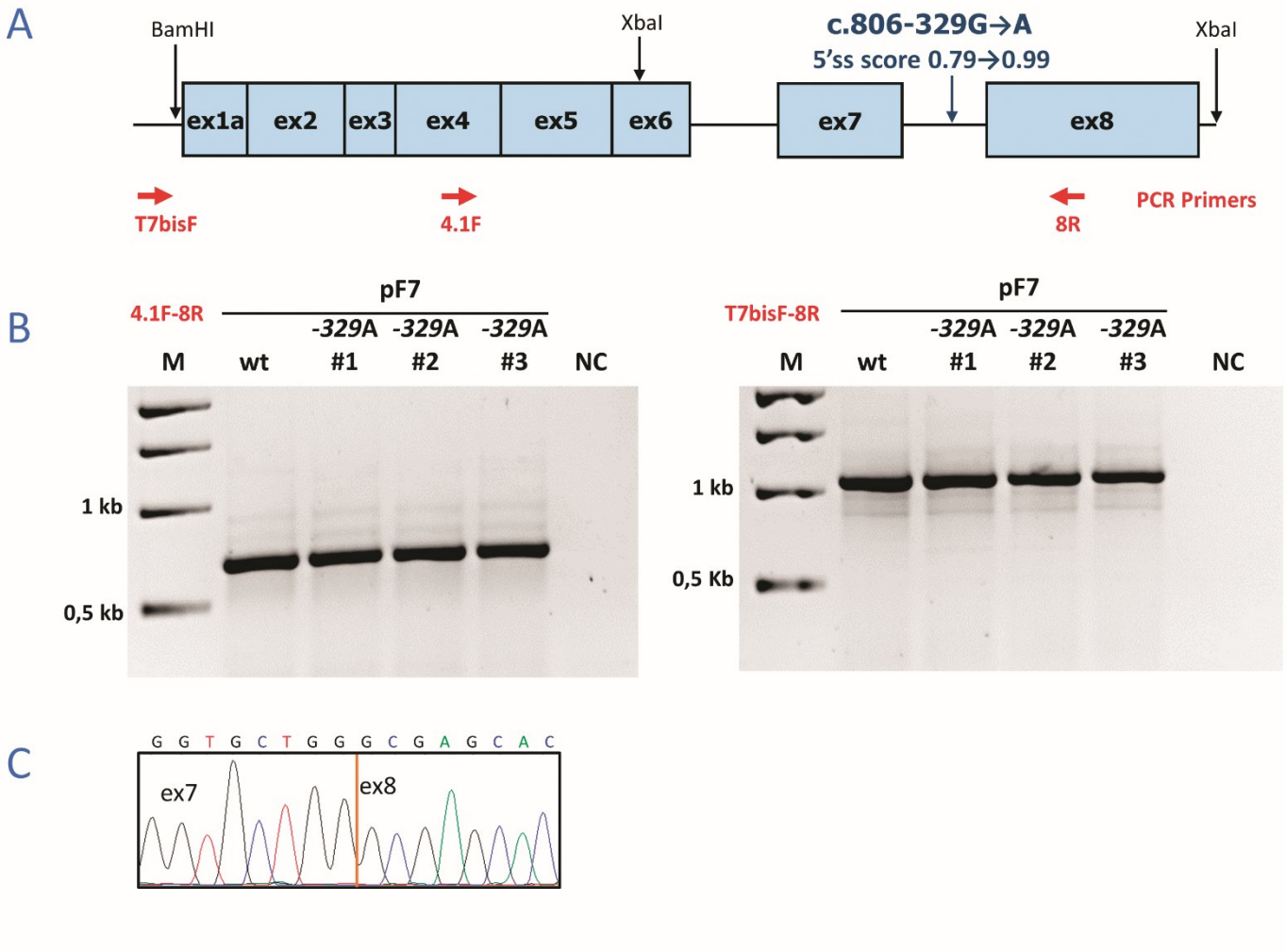
A
BamHI
XbaI
c.806-329G→A
5'ss score 0.79→0.99
XbaI
ex1a
ex2
ex3
ex4
ex5
ex6
ex7
ex8
T7bisF
4.1F
8R
PCR Primers
B
4.1F-8R
pF7
-329A
-329A
-329A
M
wt
#1
#2
#3
NC
1 kb
0,5 kb
T7bisF-8R
pF7
-329A
-329A
-329A
M
wt
#1
#2
#3
NC
1 kb
0,5 Kb
C
G G T G C T G G G C G A G C A C
ex7
ex8

Figure S1. **Minigene expression studies did not reveal a splicing effect for the c.806-329A mutation.**

(A) Scheme of the *F7* minigene (pF7) cloned in the pCDNA3 expression vector used to evaluate the impact of the c.806-329A variant. The mutation, the change of the cryptic 5'ss score, the restriction sites used for cloning and the primers exploited for PCR (red arrows) are indicated.

(B) Splicing patterns in HEK293T cells transiently transfected with the *F7* wild type (pF7-wt) or mutated (pF7-329A) minigenes (three independent mutated minigene constructs were tested; #1, #2 and #3). PCRs were performed with the reverse oligonucleotide 8R ($^{5'}$GAAGGCCAGCGTCCTC TCA$^{3'}$) and the forward oligonucleotide 4.1F ($^{5'}$GTGCCTCAAGTCCATGCCAGAA$^{3'}$; 677bp product, left panel) or T7bis ($^{5'}$CACTGCTTACTGGCTTATCGAAAT$^{3'}$; 1059bp product, right panel).

M, 1 kB ladder.

(C) Sequencing of the amplicon obtained with oligonucleotides 4.1F and 8R using the RNA isolated from HEK293T cells transiently transfected with the pF7-329A construct, and demonstrating the presence of the correct exon 7-8 junction.