

# Supplementary Materials: Random Splicing of Several Exons Caused by a Single Base Change in the Target Exon of CRISPR/Cas9 Mediated Gene Knockout

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(a) WT AGA sequence (coding region, exons 1-7 only)

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ATGGCGCGGAAGTCGAACTTGCCGTGCTTCTCGTGCCGTTTCTGCTCTGCCAGGCCCTAGTGCCTGCTCCAGC
CCTCTGCCCTGGTGTCAACACTTGGCCCTTAAAGAAATGCAACCGAAGCAGCGTGGAGGGCATTAGCATCTGGA
GGCTCTGCCCTGGATGCAGTGGAGAGCGGCTGTGCCATGTGTGAGAGAGAGCAGTGTGACGGCTCTGTAGGCTTT
GGAGGAAGTCCTGATGAACTTGGAGAAACCACACTAGATGCCATGATCATGGATGGCACTACTATGGATGTAGGA
GCAGTAGGAGATCTCAGACGAATTAATAATGCTATTGGTGTGGCAGCGAAAGTACTGGAACATACAACACACACA
CTTTTAGTAGGAGAGTCCAGCCACCACATTTGCTCAAAGTATGGGGTTTATCAATGAAGACTTATCTACCAGTCT
TCTCAAGCTCTTCATTCAGATTGGCTTGCTCGGAATTGCCAGCCAAATTATTGGAGGAATGTTATACCAGATCCC
TCAAATACTGCGGACCCACAAAGCACCTGGTATCTTAAAGCAGGATATTCCTATCCATAAAGAAACAGAAGAT
GATCGTGGTCATGACACTATTGCATGGTTGTAATCCATAAGACAGGACATATTGCTGCTGGTACATCTACAAAT
GGTATAAAATTCAAAATACATGGCCGTGTAGGAGACTCACCAATACCTGGAGCTGGAGCCTATGCTGACGATACT
GCAGGGGCAGCCGCAGCCACTGGGAATGGTGATATATTGATGCGCTTCTGCCAAG ...

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(b) AGA CRISPR/Cas9: exon 2, 3, 4, 5 and 6 spliced out

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ATGGCGCGGAAGTCGAACTTGCCGTGCTTCTCGTGCCGTTTCTGCTCTGCCAGGCCCTAGTGCCTGCTCCAGC
CCTCTGCCCTGGTGTCAACACTTGGCCCTTAAAGAAATGCAACCGAAGCAGCGTGTAGGAGACTCACCAATAC
CTGGAGCTGGAGCCTATGCTGACGATACTGCAGGGGCAGCCGAGCCACTGGGAATGGTGATATATTGATGCGCT
TCCTGCCAAG ...

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(c) AGA CRISPR/Cas9: exon 3, 4, 5 and 6 spliced out

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ATGGCGCGGAAGTCGAACTTGCCGTGCTTCTCGTGCCGTTTCTGCTCTGCCAGGCCCTAGTGCCTGCTCCAGC
CCTCTGCCCTGGTGTCAACACTTGGCCCTTAAAGAAATGCAACCGAAGCAGCGTGGAGGGCATTAGCATCTGGA
GGCTCTGCCCTGGATGCAGTGGAGAGCGGCTGTGCCATGTGTGAGAGAGAGCAGTGTGACGGCTCTGTAGGCTTT
GGAGGAAGTCCTGATGAACTTGGAGAAACCACACTAGATGCCATGATCATGGATGGCCGTGTAGGAGACTCACCA
ATACCTGGAGCTGGAGCCTATGCTGACGATACTGCAGGGGCAGCCGAGCCACTGGGAATGGTGATATATTGATG
CGCTTCTGCCAAG ...

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**Figure S1.** Consequences of the CRISPR/Cas9-mediated AGA gene knockout in HEK293T cells at mRNA level. Total RNA was isolated from the knockout clones and reverse-transcribed. The coding region of AGA was amplified with specific primers, cloned into a vector, and four plasmid clones were sequenced. (a) WT human AGA coding region (exons 1–7 only) with color-coded exons (pink: exon 1 beginning from the ATG start codon). (b) Clone with exons 2-6 spliced out; (c) Clone with exons 3–6 spliced out.

(a) WT AGA protein sequence

MARKSNLPVLLVPFLLCQALVRCSSPLPLVVNTWPFKNATEA<sup>AW</sup>RALASGGSSALDAVESGCAMCEREQCD  
 GSVGFGGSPDELGETTLDAMIMD<sup>GT</sup>TMDVGA<sup>VDL</sup>RRIKNAIGVARKVLEHTHTLLVGES<sup>AT</sup>TFAQSMG  
 FINEDLSTTASQALHSDWLARNCPNYWRNVI<sup>PD</sup>PSKYCGPYKPPGILKQDIPIHKETEDDRGHDTI<sup>GMV</sup>  
 VIHKTGHIAAGTSTNGIKFKIH<sup>GR</sup>VGDSPIPGAGAYADDTAGAAAATGNGDILMRFL<sup>ES</sup>YQAVEYMRERGE  
 DPTIACQKVISRIQKHFFPEFFGA<sup>VICAN</sup>VTGSY<sup>G</sup>AACNKLSTFTQFSFMVYNSEKNQPTTEEKVDCI

(b) AGA CRISPR/Cas9: exon 2, 3, 4, 5 and 6 spliced out: frame-shift and early stop

atggcgcggaagtgcgaacttgccctgtgcttctctgtgccgtttctgctctgcccaggccctagtgcgctgctccagc  
 M A R K S N L P V L L V P F L L C Q A L V R C S S  
 cctctgcccctggctgctcaacacttgccctttaagaatgcaaccgaagca<sup>g</sup>ccgtgtaggagactcaccaatac  
 P L P L V V N T W P F K N A T E A A V E T H Q Y

(c) AGA CRISPR/Cas9: exons 3, 4, 5 and 6 spliced out: first 94 residues AGA, amino acids 95-232 are missing, then again AGA sequence

atggcgcggaagtgcgaacttgccctgtgcttctctgtgccgtttctgctctgcccaggccctagtgcgctgctccagc  
 M A R K S N L P V L L V P F L L C Q A L V R C S S  
 cctctgcccctggctgctcaacacttgccctttaagaatgcaaccgaagca<sup>g</sup>ctggaggccattagcatctgga  
 P L P L V V N T W P F K N A T E A A W R A L A S G  
 ggctctgcccctggatgcagtgagagcggctgtgccatgtgtgagagagagcagtggtgacggctctgtaggcttt  
 G S A L D A V E S G C A M C E R E Q C D G S V G F  
 ggaggaagtcctgatgaacttgagaaaccacactagatgccatgatcatggatgg<sup>g</sup>ccgtgtaggagactcacca  
 G G S P D E L G E T T L D A M I M D G R V G D S P  
 atacctggagctggagcctatgctgacgatactgcaggggagccgcagccactgggaatggatgatattgatg  
 I P G A G A Y A D D T A G A A A A T G N G D I L M  
 egcttctgccaag...

**Figure S2.** Predicted consequences of the changes in AGA mRNA sequences at the protein level. ExPasy Translate tool was used to convert the sequencing data to protein sequences. (a) WT AGA protein sequence with the same exon color coding as in Suppl. Figure S1. The codons for the residues marked in red are compiled from the flanking exons. (b) Removal of exons 2–6 results in a frame shift and early stop, whereas (c) removal of exons 3–6 produces a putative protein containing the first 94 residues of AGA, then residues 95–232 are deleted, after which the AGA sequence continues from residue 233.