

Appendix

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Appendix Figure S1

CCGGCATGGCTGTTAGCTTGGTACC**ATG**GTGCGGGTACAGCGACCACGGCAAGAGATCATTG
AAGACTTGTCTACATGGTGCGTGAGCTCCTCATCCAATTCTACAAGTCCACCCGTTTCAAG
CCTACCCGCATCATCTTCTACCGAGATGGGGTGCCTGAAGGCCAGCTACCCAGATACTCCA
CTATGAGCTACTGGCCATTTCGTGATGCCTGCATCAAACCTGGAAAAGGACTACCAGCCTGGGA
TCACTTATATTGTGGTGCAGAAACGCCATCACACCCGCCTTTTCTGTGCTGACAAGAATGAG
CGAATTGGGAAGAGTGGTAACATCCCAGCTGGGACCACAGTGGACACCAACATCACCCACCC
ATTTGAGTTTGACTTCTATCTGTGCAGCCACGCAGGCATCCAGGGCACCAGCCGACCATCCC
ATTACTATGTTCTTTGGGATGACAACCGTTTCACAGCAGATGAGCTCCAGATCCTGACGTAC
CAGCTGTGCCACACTTACGTACGATGCACACGCTCTGTCTCTATCCCAGCACCTGCCTACTA
TGCCCGCCTGGTGGCTTTCCGGGCACGATACCACCTGGTGGACAAGGAGCATGACAGTGGAG
AGGGGAGCCACATATCGGGGCAGAGCAATGGGCGGGACCCCCAGGCCCTGGCCAAAGCCGTG
CAGGTTTACCAGGATACTCTGCGCACCATGTACTTCGCT**TGA**AGGCAGAACGCTGTTACCTC
ACTGGATAGAAGAAAGCTTTCCAAGCCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAA
GGAGGAGGGAGGTGGGGGGATCC**GACTACAAGGACGACGATGACAAGTACCCTTATGACGTG**
CCCGATTACGCTAGCGGCCGCTCGAGTCTAGAGGGCCCTTCGAACAAAACTCATCTCAGAA
GAGGATC**TGA**ATATGCATACCGGTCATCATCACCATCACCATTGAGTTTAAACCCGCTGATC
ACCTCAGT

Appendix Figure S1. Result of sequencing of *AGO1*-UGA-3'UTR-*FLAG*-*HA* construct (see Fig 1B, C and EV2B). T7 forward primer (5' TAATACGACTCACTATAGGG 3') was used for sequencing. Start codon – bold and green; *AGO1* coding sequence – blue; Canonical stop codon and the stop codon after *FLAG*-*HA* tag – bold and red; proximal 3'UTR of *AGO1* – purple; *FLAG* tag – dark orange; *HA* tag – orange; pCDNA vector backbone – black.

Appendix Figure S2

ATGGTGCGGGTACAGCGACCACGGCAAGAGATCATTGAAGACTTGTCTACATGGTGCCTGA
 GCTCCTCATCCAATTCTACAAGTCCACCCGTTTCAAGCCTACCCGCATCATCTTCTACCGAG
 ATGGGGTGCCTGAAGGCCAGCTACCCAGATACTCCACTATGAGCTACTGGCCATTCGTGAT
 GCCTGCATCAAACCTGGAAAAGGACTACCAGCCTGGGATCACTTATATTGTGGTGCAGAAACG
 CCATCACACCCGCCTTTTCTGTGCTGACAAGAATGAGCGAATTGGGAAGAGTGGTAACATCC
 CAGCTGGGACCACAGTGGACACCAACATCACCCACCCATTTGAGTTTGAAGTTCTATCTGTGC
 AGCCACGCAGGCATCCAGGGCACCAGCCGACCATCCCATTACTATGTTCTTTGGGATGACAA
 CCGTTTTCACAGCAGATGAGCTCCAGATCCTGACGTACCAGCTGTGCCACACTTACGTACGAT
 GCACACGCTCTGTCTCTATCCCAGCACCTGCCTACTATGCCCGCCTGGTGGCTTTCCGGGCA
 CGATACCACCTGGTGGACAAGGAGCATGACAGTGGAGAGGGGAGCCACATATCGGGGCAGAG
 CAATGGGCGGGACCCCCAGGCCCTGGCCAAAGCCGTGCAGGTTACCAGGATACTCTGCGCA
 CCATGTACTTCGCT**TGA**AGGCAGAACGCTGTTACCTCACTGGATAGAAGAAAGCTTTCCAAG
 CCCCAGGAGCTGTGCCACCCAAATCCAGAGGAAGCAAGGAGGAGGGAGGTGGGGGGGGCTC
 CGGGCGCTCCCTCGTGCTCGAGGAAGACGCCAAAAACATAAAGAAAGGCCCGGCCATTCT
 ATCCTCTAGAGGATGGAACCGCTGGAGAGCAACTGCATAAGGCTATGAAGAGATACGCCCTG
 GTTCTTGGAACAATTGCTTTTACAGATGCACATATCGAGGTGAACATCACGTACGCGGAATA
 CTTCGAAATGTCCGTTTCGGTTGGCAGAAGCTATGAAACGATATGGGCTGAATACAAATCACA
 GAATCGTCGTATGCAGTGAACACTCTCTTCAATTCTTTATGCCGGTGTGGGCGCGTTATTT
 ATCGGAGTTGCAGTTGCGCCCGCGAACGACATTTATAATGAACGTGAATTGCTCAACAGTAT
 GAACATTTTCGCAGCCTACCGTAGTGTGTTTCCAAAAAGGGGTGCAAAAAATTTTGAACG
 TGCAAAAAAATTACCAATAATCCAGAAAATATTATCATGGATTCTAAAACGGATTACCAG
 GGATTTTCAGTCGATGTACACGTTTCGTACATCTCATCTACCTCCCGGTTTTAATGAATACGA
 TTTTGTACCAGAGTCCTTTGATCGTGACAAAACAATTGCACTGATAATGAATTCCTCTGGAT
 CTACTGGGTACCTAAGGGTGTGGCCCTTCCGCATAGAACTGCCTGCGTCAGATTCTCGCAT
 GCCAGAGATCCTATTTTTGGCAATCAAATCATTCGGGATACTGCGATTTTAAAGTGTGTTCC
 ATTCCATCACGTTTTTGAATGTTTACTACACTCGGATATTTGATATGTGGATTTTCAGTCG
 TCTTAATGTATAGATTTGAAGAAGAGCTGTTTTTACGATCCCTTCAGGATTACAAAATTCAA
 AGTGCGTTGCTAGTACCAACCTATTTTCATTCTTCGCCAAAAGCACTCTGATTGACAAATA
 CGATTTATCTAATTTACACGAAATTGCTTCTGGGGGCGCACCTCTTTTCGAAAGAAGTCGGGG
 AAGCGGTTGCAAAACGCTTCCATCTTCCAGGGATACGACAAGGATATGGGCTCACTGAGACT
 ACATCAGCTATTCTGATTACACCCGAGGGGGATGATAAACCAGGGGCGCGGTTCGGTAAAGTTGT
 TCCATTTTTTTGAAGCGAAGGTGTGGATCTGGATACCGGGAAAACGCTGGGCGTTAATCAGA
 GAGGCGAATTATGTGTCAGAGGACCTATGATTATGTCCGGTTATGTAAACAATCCGGAAGCG
 ACCAACGCCTTGATTGACAAGGATGGATGGCTACATTCTGGAGACATAGCTTACTGGGACGA
 AGACGAACACTTCTTCATAGTTGACCGCTTGAAGTCTTTAATTAAATACAAAGGATATCAGG
 TGGCCCCCGCTGAATTGGAATCGATATTGTTACAACACCCCAACATCTTCGACGCGGGCGTG
 GCAGGTCTTCCCGACGATGACGCCGGTGAACCTCCCGCCGCGGTTGTTGTTTTGGAGCACGG
 AAAGACGATGACGGAAAAAGAGATCGTGGATTACGTCGCCAGTCAAGTAACAACCGCGGAAAA
 AGTTGCGCGGAGGAGTTGTGTTTGTGGACGAAGTACCGAAAGGTCTTACCGGAAAACCTCGAC
 GCAAGAAAAATCAGAGAGATCCTCATAAAGGCCAAGAAGGGCGGAAAGTCCAAATTG**TAA**

Appendix Figure S2. Result of sequencing of AGO1-UGA-3'UTR-*FLuc* construct (see Fig 1E, EV1E, and EV2C). Multiple primers were used for sequencing. Start codon – bold and green; AGO1 coding sequence – blue; Canonical stop codon and the stop codon after firefly luciferase (*FLuc*) – bold and red; proximal 3'UTR of AGO1 – purple; linker sequence – orange; *FLuc* – brown. Following primers (5' to 3') were used for sequencing: AGO1 F1- GTGCGGG TACA GCGACCACGGCAAGAG; AGO1 F2- GGGAGCCACATATCGGGGCAG; *FLuc* forward - GAAGACGCCAAAAACATA; *FLuc* reverse - TTACAATTTGGACTTTCCG; BGH Reverse - TAGAAGGCACAGTCGAGG.

Appendix Figure S3

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WT  CAAAG CCGTGCAGGT TCACCAGGAT
Δ   CCAAG CCGTGCAGGT TCACCAGGAT
    *  ***  *****

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WT  ACTCTGCGCA CCATGTACTT CGCTTGAAGG CAGAACGCTG TTACCTCACT GGATAGAAGA
Δ   ACTCTGCGCA CCATGTACCT TGCTTGAAGG CAGAACGCTG TTACCTC--- -----
    *****  *****  *   *****  *****  *****

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WT  AAGCTTTCCA AGCCCCAGGA GCTGTGCCAC CCAAATCCAG AGGAAGCAAG GAGGAG-GGA
Δ   -----AG AGGAAGCAAG GAGGAGAGGA
    ** ***** *****

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WT  GGTGGGGTAG GG...
Δ   GGGGGGGTAG GG...
    ** *****

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Appendix Figure S3. Alignment of wild-type sequence (WT, blue) of *AGO1* region near its canonical stop codon, and the same region in HEK293 cells transfected with Cas9 and sgRNAs (Δ, brown). *, perfect match.

Appendix Figure S4

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WT  ACCCC AGCCTTTGTT TTCCATTTC
Δ   ACCCC AGCCTTTGTT TTCCATTTC
    *****

WT  CTCAGATGTG ACAAGCCGAG GCGGTGAGCC GGCAGGAGG AAGGAGCCTC CCTCAGGGTT
Δ   CTCAGATGTG ACAAGCCGAG GCGGTGAGCC GGCAGGAGG AAGGAGCCTC C-----
    *****

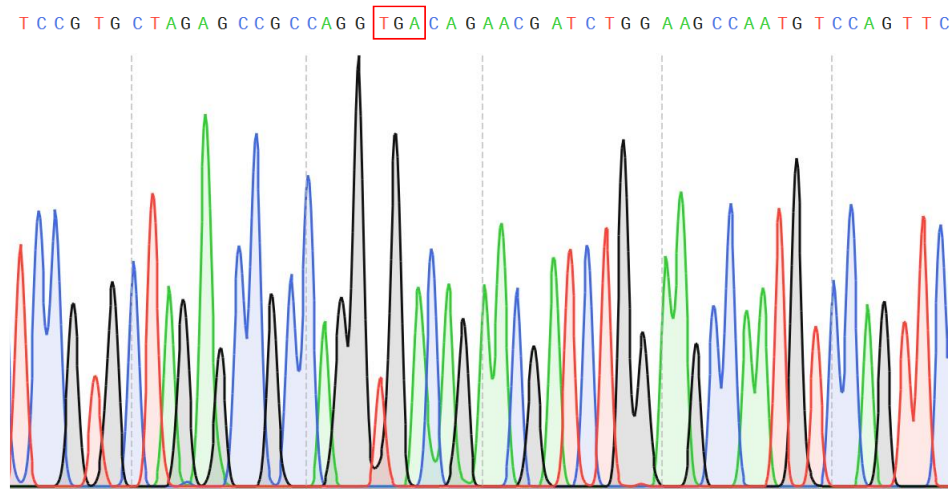
WT  TCGGGAACCA GATCTCTCAC CAGGAAAGAC TGATACAGAA CGATCGATAC AGAAACCACG
Δ   -----

WT  CTGCCGCCAC CACACCATCA CCATCGACAG AACAGTCCTT AATCCAGAAA CC...
Δ   -----ACAG AACAGTCCTT AATCCAGAAA CC...
    *****

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Appendix Figure S4. Alignment of wild-type sequence (WT, blue) of *VEGFA* region near its canonical stop codon, and the same region in HEK293 cells transfected with Cas9 and sgRNAs(Δ, brown). *, perfect match.

Appendix Figure S5



Appendix Figure S5. Electropherogram showing the PTC (TGA, red box) generated using CRISPR-Cas9 system at 891st codon of *SPTA1* gene in K562 cells.