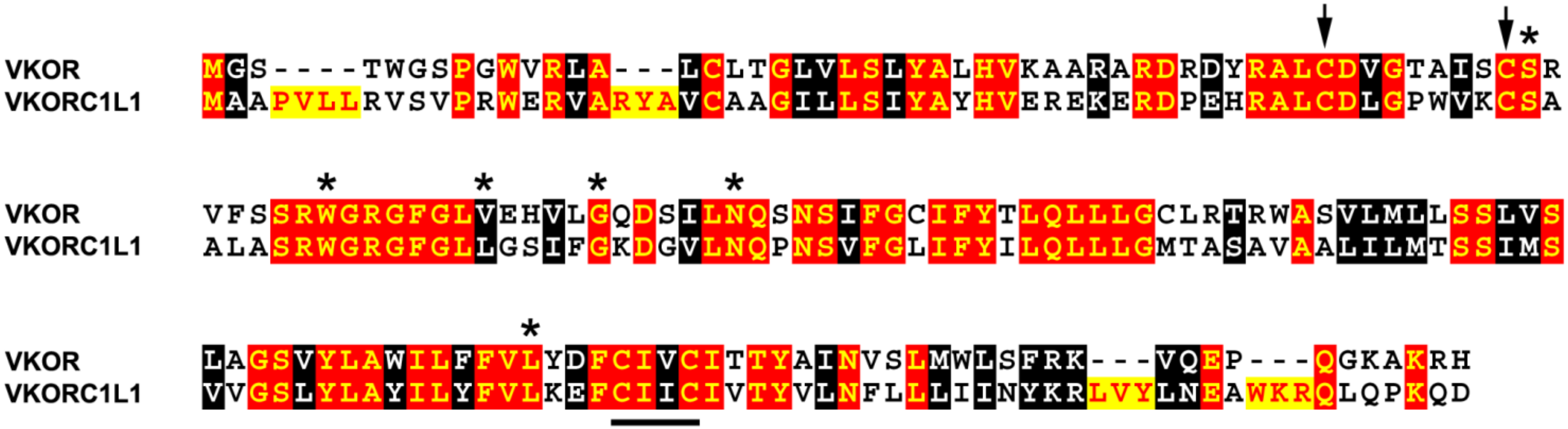
Supplementary Figure Legends:

Figure S1. Sequence alignment of human VKOR and VKORC1L1. The amino acid sequences of human VKOR and VKORC1L1 were aligned by CLUSTAL W. Completely conserved residues are shown in yellow with red background. Similar residues are shown in white with black background. Conserved loop cysteines are indicated by arrows. The active site CXXC redox motif is indicated by a bold line. Residues reported to be associated with warfarin resistance in patients and studied in this work are indicated by asterisks.

Figure S2. TALENs-mediated VKORC1L1 knockout in HEK 293 cells. (A) Functional screening of VKORC1L1 knockout HEK293 cells. The black and gray bars represent the concentrations of carboxylated FIXgla-PC in the cell culture medium when cells were fed with KO and vitamin K, respectively. Non-transfected HEK293 cells produce similar level of carboxylated FIXgla-PC when fed with either vitamin K or KO. **(B)** TALENs-mediated gene editing of VKORC1L1 locus in HEK293 cells. Wild-type VKORC1L1 target sequence is shown on the top with the TALENs binding site indicated. The ATG start codon is underlined. Deletions are indicated by dashes. Insertions are indicated by lowercase letters in italic or an “i” and the number of base pairs inserted against a gray background. The sizes of deletions or insertions are indicated on the left. The sequence of the 343-base pair insertion is shown in lowercase letters in italic. In-frame stop-codon is indicated by a bold line.

Figure S3. Large sequence insertion in TALENs-mediated VKORC1L1 genome editing. (A) A 343-bp nucleotide sequence insertion was identified in one of the TALENs-edited VKORC1L1 loci. The inserted nucleotide sequences are shown in lower case letters and the VKORC1L1 cDNA sequences are shown in bold capital letters. The ATG start codon is shown in red. The translated amino acid sequence is shown under the nucleotide sequence with VKORC1L1 sequence in bold. The first methionine is shown in blue and translation stop codons are indicated by red asterisks. **(B)** A BLAST search reveals that the 343-bp insert is from chromosome 5.

Figure S4. Distribution of naturally occurring VKOR mutations in the three-transmembrane domain topology model. Amino acid residues that have been found to be mutated in patients requiring high-dose anticoagulants were highlighted in red. Conserved loop cysteines and the active site cysteines are highlighted in green.



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VKOR       VFSSRWGRGFGLVEHVLGQDSILNQSNSIFGCIFYTLQLLLGCLRTRWASVLMLLSSLVS
VKORC1L1   ALASRWGRGFGLLGSIFGKDGVLNQPNSVFGLIFYILQLLLGMTASAVAALILMTSSIMS

VKOR       LAGSVYLAWILFFVLYDFCIVCITTYAINVSLMWLSFRK---VQEP---QGKAKRH
VKORC1L1   VVGSLYLAYILYFVLKEFCIICIVTYVLNFLLLIINYKRLVYLNEAWKRQLQPKQD
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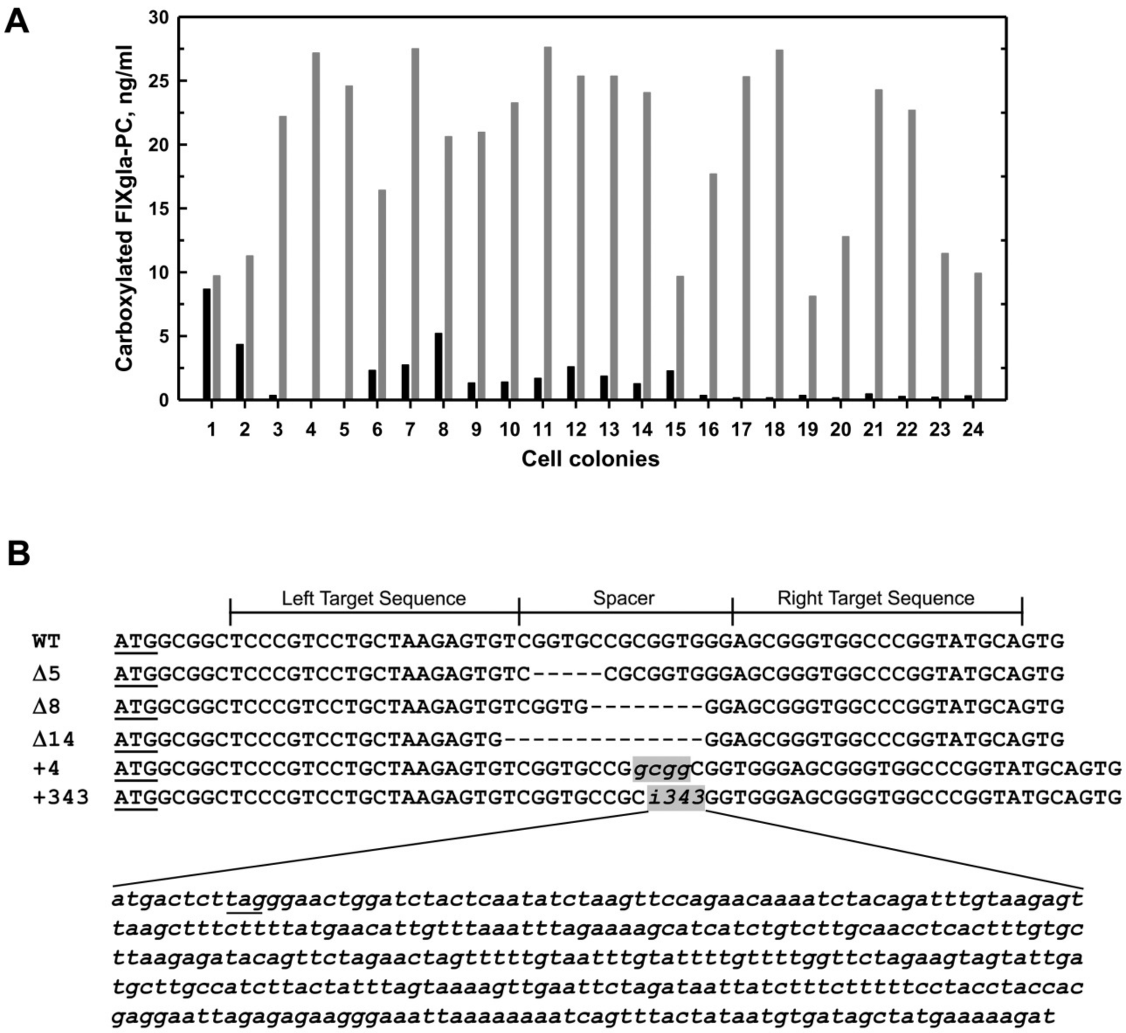
Figure S1


A
Carboxylated FIXgla-PC, ng/ml
0
5
10
15
20
25
30
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Cell colonies
B
Left Target Sequence
Spacer
Right Target Sequence
WT ATGGCGGCTCCCGTCCTGCTAAGAGTGTCGGTGCCGCGGTGGGAGCGGGTGGCCCGGTATGCAGTG
Δ5 ATGGCGGCTCCCGTCCTGCTAAGAGTGTC-----CGCGGTGGGAGCGGGTGGCCCGGTATGCAGTG
Δ8 ATGGCGGCTCCCGTCCTGCTAAGAGTGTCGGTG--------GGAGCGGGTGGCCCGGTATGCAGTG
Δ14 ATGGCGGCTCCCGTCCTGCTAAGAGTG--------------GGAGCGGGTGGCCCGGTATGCAGTG
+4 ATGGCGGCTCCCGTCCTGCTAAGAGTGTCGGTGCCGgcggCGGTGGGAGCGGGTGGCCCGGTATGCAGTG
+343 ATGGCGGCTCCCGTCCTGCTAAGAGTGTCGGTGCCGCi343GGTGGGAGCGGGTGGCCCGGTATGCAGTG
atgactcttagggaactggatctactcaatatctaagttccagaacaaaatctacagatttgtaagagt
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ttaagagatacagttctagaactagtttttgtaatttgtattttgttttggttctagaagtagtattga
tgcttgccatcttactatttagtaaaagttgaattctagataattatctttctttttcctacctaccac
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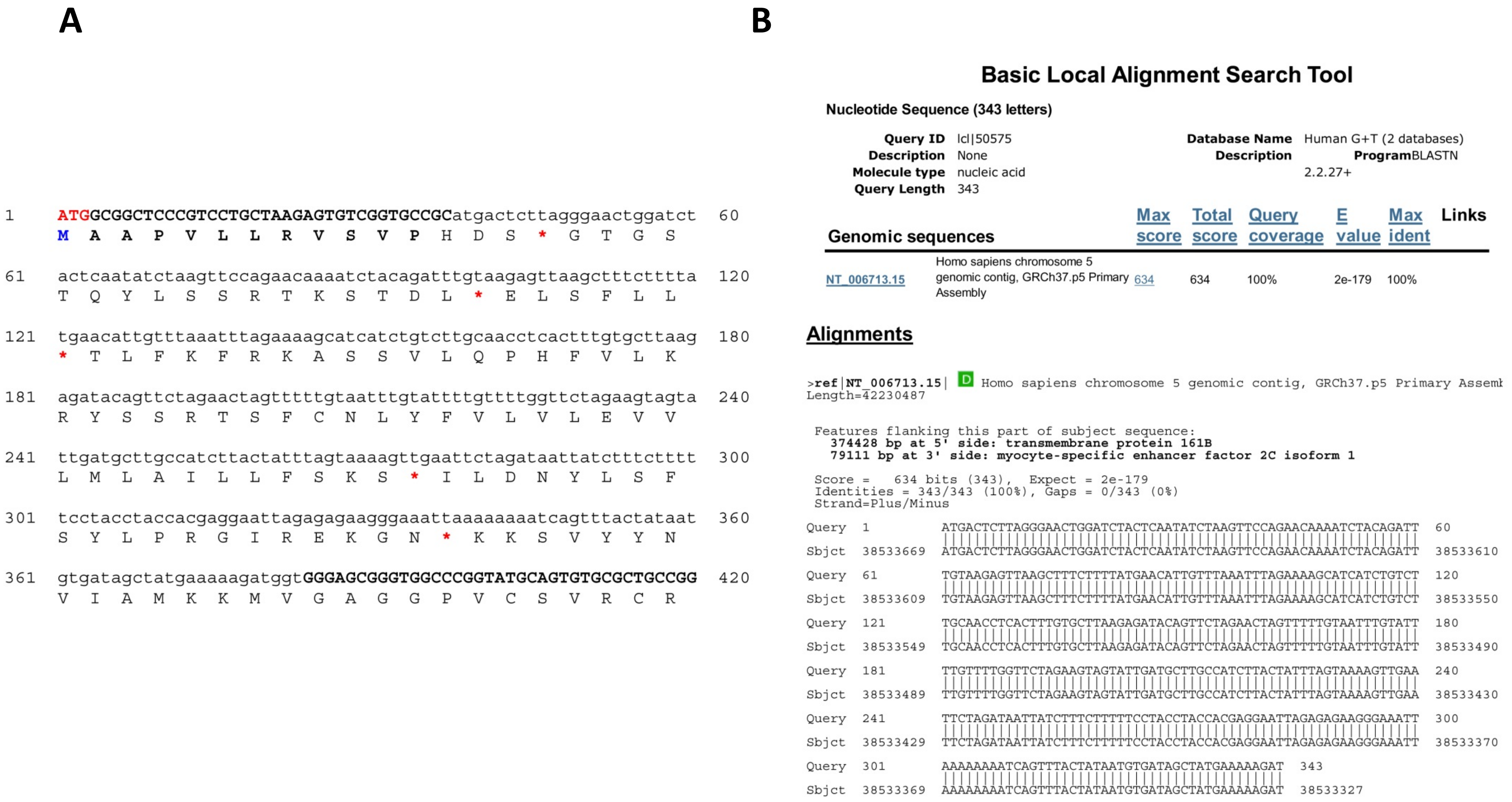
Figure S2



A

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1    ATGGCGGCTCCCGTCCTGCTAAGAGTGTCGGTGCCGCatgactcttagggaactggatct  60
     M  A  A  P  V  L  L  R  V  S  V  P  H  D  S  *  G  T  G  S

61   actcaatatctaagttccagaacaaaatctacagatttgtaagagttaagctttctttta  120
     T  Q  Y  L  S  S  R  T  K  S  T  D  L  *  E  L  S  F  L  L

121  tgaacattgtttaaatttagaaaagcatcatctgtcttgcaacctcactttgtgcttaag  180
     *  T  L  F  K  F  R  K  A  S  S  V  L  Q  P  H  F  V  L  K

181  agatacagttctagaactagtttttgtaatttgtattttgttttggttctagaagtagta  240
     R  Y  S  S  R  T  S  F  C  N  L  Y  F  V  L  V  L  E  V  V

241  ttgatgcttgccatcttactatttagtaaaagttgaattctagataattatctttctttt  300
     L  M  L  A  I  L  L  F  S  K  S  *  I  L  D  N  Y  L  S  F

301  tcctacctaccacgaggaattagagagaagggaaattaaaaaaaatcagtttactataat  360
     S  Y  L  P  R  G  I  R  E  K  G  N  *  K  K  S  V  Y  Y  N

361  gtgatagctatgaaaaagatggtGGGAGCGGGTGGCCCGGTATGCAGTGTGCGCTGCCGG  420
     V  I  A  M  K  K  M  V  G  A  G  G  P  V  C  S  V  R  C  R
```

B

Basic Local Alignment Search Tool

Nucleotide Sequence (343 letters)

Query ID lcl|50575
Description None
Molecule type nucleic acid
Query Length 343

Database Name Human G+T (2 databases)
Description
Program BLASTN 2.2.27+

Genomic sequences		Max score	Total score	Query coverage	E value	Max ident	Links
NT_006713.15	Homo sapiens chromosome 5 genomic contig, GRCh37.p5 Primary Assembly	634	634	100%	2e-179	100%	

Alignments

```
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Length=42230487

 Features flanking this part of subject sequence:
   374428 bp at 5' side: transmembrane protein 161B
   79111 bp at 3' side: myocyte-specific enhancer factor 2C isoform 1

 Score =   634 bits (343),  Expect = 2e-179
 Identities = 343/343 (100%), Gaps = 0/343 (0%)
 Strand=Plus/Minus

Query  1         ATGACTCTTAGGGAACTGGATCTACTCAATATCTAAGTTCCAGAACAAAATCTACAGATT  60
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Query  61        TGTAAGAGTTAAGCTTTCTTTTATGAACATTGTTTAAATTTAGAAAAGCATCATCTGTCT  120
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Sbjct  38533549  TGCAACCTCACTTTGTGCTTAAGAGATACAGTTCTAGAACTAGTTTTTGTAATTTGTATT  38533490

Query  181       TTGTTTTGGTTCTAGAAGTAGTATTGATGCTTGCCATCTTACTATTTAGTAAAAGTTGAA  240
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Figure S3

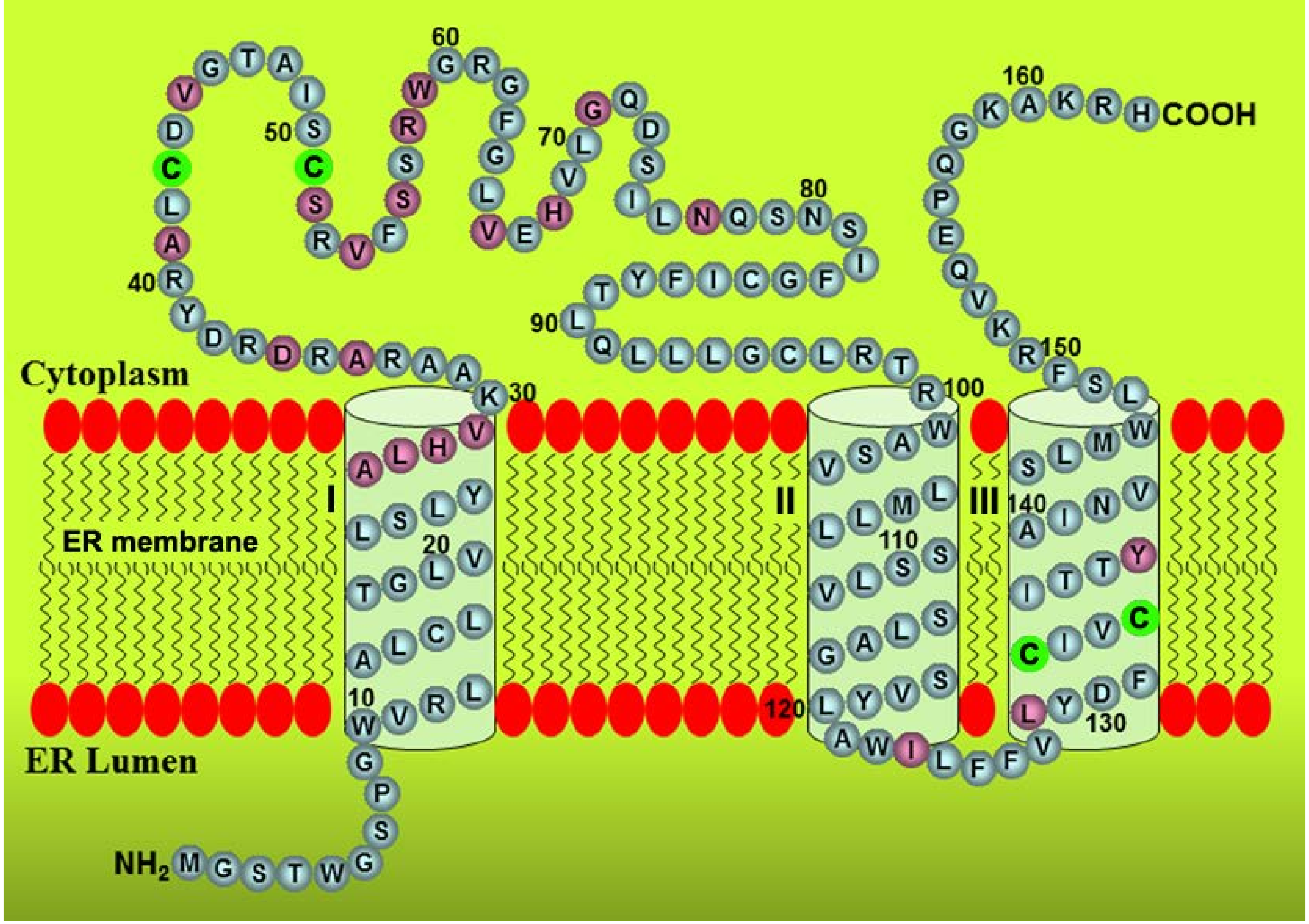
Cytoplasm
ER membrane
ER Lumen
NH$_2$
COOH
I
II
III
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160

Figure S4