Provided are modified FIX polypeptides. The modified FIX polypeptides provided have improved procoagulant therapeutic properties compared to an unmodified FIX polypeptide. For example, among the modified FIX polypeptides provided herein are those that exhibit increased coagulant activity, increased catalytic activity, increased resistance to AT-III, heparin and/or the AT-III/heparin complex, and/or improved pharmacokinetic properties, such as i) decreased clearance, ii) altered (e.g. increased or decreased) volume of distribution, iii) enhanced *in vivo* recovery, iv) enhanced total protein exposure *in vivo* (i.e., AUC), v) increased serum half-life (α-, β-, and/or γ-phase), and/or vi) increased mean resonance time (MRT). In some examples, the improved pharmacokinetic properties are a result of increased glycosylation and/or decreased binding to the low-density lipoprotein receptor-related protein (LRP). Also provided are nucleic acids encoding the modified FIX polypeptides and methods of using the modified FIX polypeptides, such as for treatment of bleeding disorders.

In one embodiment, there is provided a modified FIX polypeptide, comprising an amino acid replacement in an unmodified FIX polypeptide at residue R318 or at a residue corresponding to 318, wherein:

the amino acid replacement is selected from among Y, E, F and W , or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide:

corresponding amino acid residues are identified by alignment of the unmodified FIX polypeptide with the polypeptide of SEQ ID NO:3;the unmodified FIX polypeptide comprises a sequence of amino acids set forth in any of SEQ ID NOS: 2, 3, 20 or 325, or a sequence that is an active fragment of a FIX polypeptide set forth in any SEQ ID NOS: 2, 3, 20 or 325 , or a sequence having at least 90% sequence identity with the FIX polypeptide set forth in any of SEQ ID NOS: 2, 3, 20 or 325 or fragment thereof,

the modified FIX polypeptide exhibits at least one FIX activity or property that is improved compared to the unmodified FIX polypeptide that does not contain the modification; and

the activity is one or both of increased resistance to the inhibitory effects of AT-III and increased procoagulant activity.

In one embodiment, there is provided a nucleic acid molecule, comprising a sequence of nucleotides encoding a modified FIX polypeptide as defined above.

In one embodiment, there is provided a vector, comprising the nucleic acid molecule as defined above.

In one embodiment, there is provided a cell, comprising the vector as defined above.

In one embodiment, there is provided a method of producing a modified FIX polypeptide , comprising:

culturing a cell as defined above, whereby the cell expresses the modified FIX polypeptide; and

optionally isolating the modified FIX polypeptide.

In one embodiment, there is provided a pharmaceutical composition, comprising a modified FIX polypeptide as defined above in a pharmaceutically acceptable vehicle.

In one embodiment, there is provided a modified FIX polypeptide as defined above for use in treating a disease or condition that is treated by administration of FIX or a pro-coagulant.

In one embodiment, there is provided the use of a modified FIX polypeptide as defined above in the preparation of a medicament for treatment of a disease or condition that is treated by administration of FIX or a pro-coagulant.

Among the modified FIX polypeptides provided herein are those containing two amino acid replacements in unmodified FIX polypeptide, wherein the first amino acid replacement is at a position corresponding to a position selected from among 53, 61, 64, 85, 103, 104, 105, 106, 108, 155, 158, 159, 167, 169, 172, 179, 202, 203, 204, 205, 228, 239, 241, 243, 247, 249, 251, 257, 259, 260, 262, 265, 284, 293, 312, 314, 315, 316, 317, 318, 319, 321, 333, 338, 343, 346, 345, 392, 394, 400, 403, 410, 412 and 413 in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3, and the second amino acid replacement is at a position corresponding to a position selected from among 5, 53, 61, 64, 85, 155, 158, 159, 167, 239, 260, 284, 293, 312, 318, 333, 338, 346, 400, 403, 410, 412 and 413 in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3.

In some examples, the first or the second amino acid replacement is replacement with an amino acid residue selected from among alanine (Ala, A); arginine (Arg, R); asparagine (Asn, N); aspartic acid (Asp, D); cysteine (Cys, C); glutamic acid (Glu, E); glutamine (Gln, Q); glycine (Gly, G); histidine (His, H); isoleucine (Ile, I); leucine (Leu, L); lysine (Lys, K); methionine (Met, M); phenylalanine (Phe, F); proline (Pro, P); serine (Ser, S); threonine (Thr, T); tryptophan (Trp, W); tyrosine (Tyr, Y); and valine (Val, V), providing the replacing