Supporting Information:

Enhancing the specificity of recombinase-mediated genome engineering through dimer interface redesign

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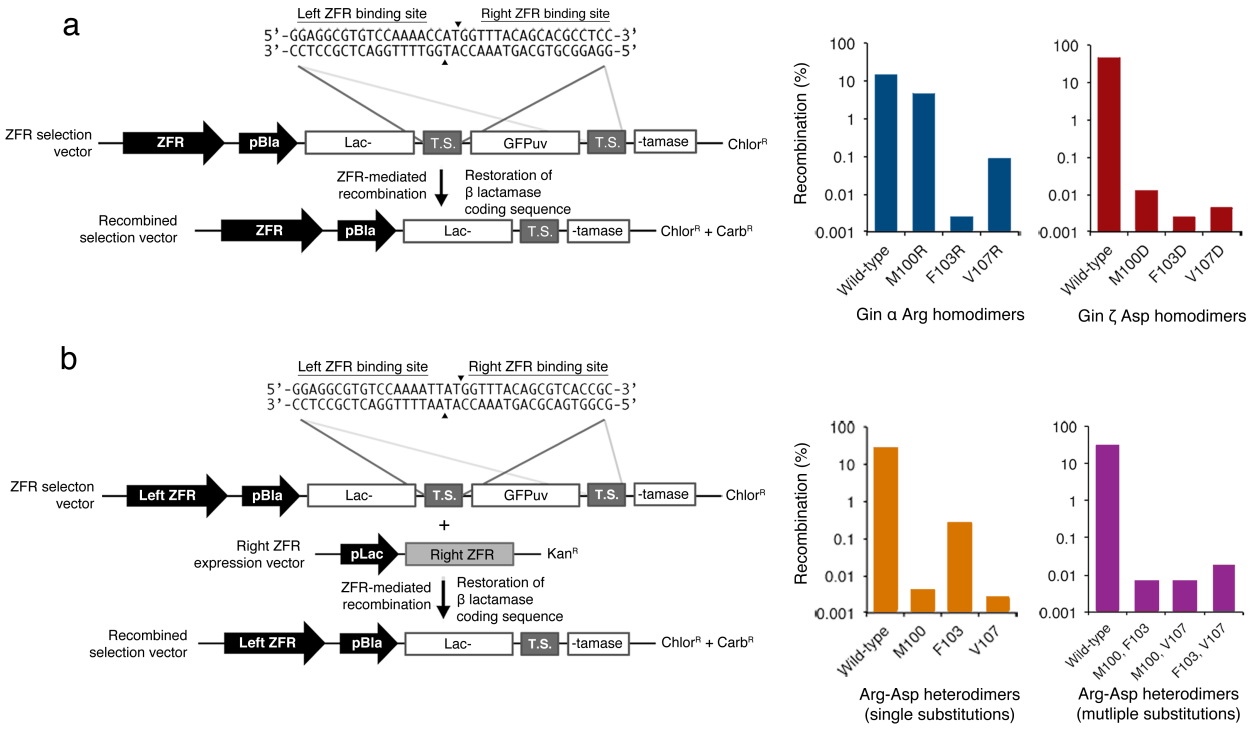

Figure S1. Impact of charged substitutions within the dimer interface of the Gin recombinase. (a, Left) Schematic representation of the split gene reporter system used to evaluate homodimer-mediated recombination. Black triangles indicate cleavage site within the DNA target. T.S. indicates target site **(Right)** Homodimer-mediated recombination by Gin α and ζ catalytic domains containing either Arg (Gin α) or Asp (Gin ζ) substitutions at positions 100, 103 and 107. **(b, Left)** Schematic representation of the split gene reporter system used to evaluate heterodimer-mediated recombination. Assays were performed in cells that harbored a Gin α mutant expression plasmid. **(Right)** Heterodimer-mediated recombination by Gin α and ζ catalytic domains containing single or multiple charge-complementary mutations (i.e. Arg-Asp) at positions 100, 103 and 107.

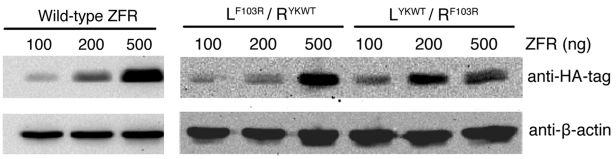

Figure S2. Expression of wild-type and obligate ZFR heterodimers. Western blot of lysate from HEK293 cells transfected with increasing amounts of expression vector of standard or obligate ZFR heterodimers. Samples were taken 48 hr after transfection and probed with horseradish peroxidase-conjugated anti-HA and anti-β-actin (loading control) antibodies.

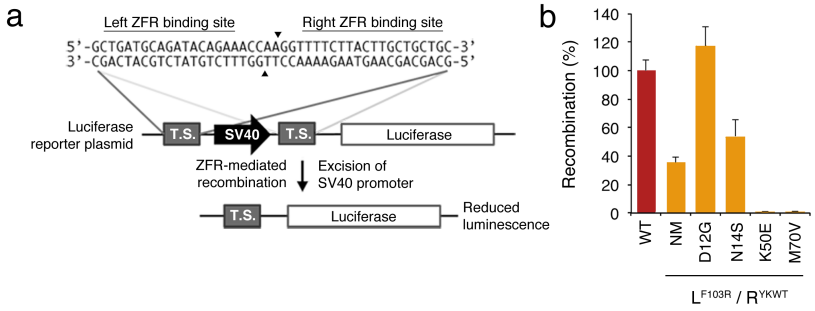

Figure S3. Recombination by low-activity ZFR heterodimers containing potential hyperactivating mutations. (a) Schematic representation of the luciferase reporter system used to evaluate ZFR activity. ZFR-mediated recombination leads to excision of the SV40 promoter and reduced luciferase expression in mammalian cells. Black triangles indicate cleavage site within the DNA target. **(b)** Recombination by a ZFR pair designed to target human chromosome 4, composed of the L^{F103R} / R^{YKWT} dimeric framework and containing one of four substitutions predicted to enhance obligate ZFR heterodimer activity. Recombination normalized to the unmodified wild-type ZFR pair. "NM" indicates the obligate ZFR heterodimeric variant with no additional hyperactivating mutations. *Renilla* luciferase expression was used to normalize for transfection efficiency and cell number. Error bars indicate standard deviation (n = 3).

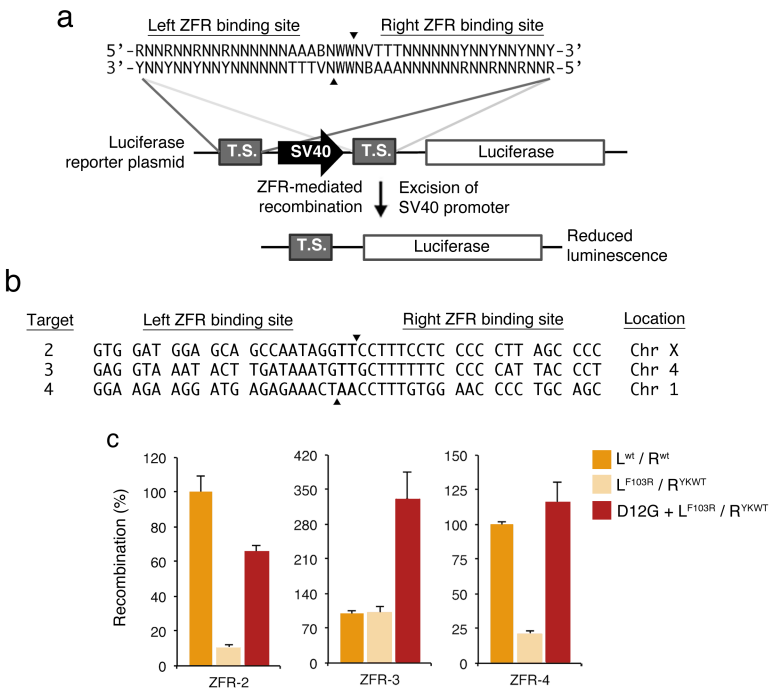

Figure S4. Portability of the eZFR architecture. (a) Schematic representation of the luciferase reporter system used to evaluate ZFR activity. Same cartoon as Figure S3A. **(b)** Target sites used to evaluate eZFR activity. **(c)** Recombination efficiency of standard and eZFR pairs 2, 3 and 4, which are designed to target sites from human chromosomes X, 4 and 1, respectively. Recombination efficiency was normalized to the wild-type ZFR pair. *Renilla* luciferase expression was used to normalize for transfection efficiency and cell number. Error bars indicate standard deviation (n = 3).

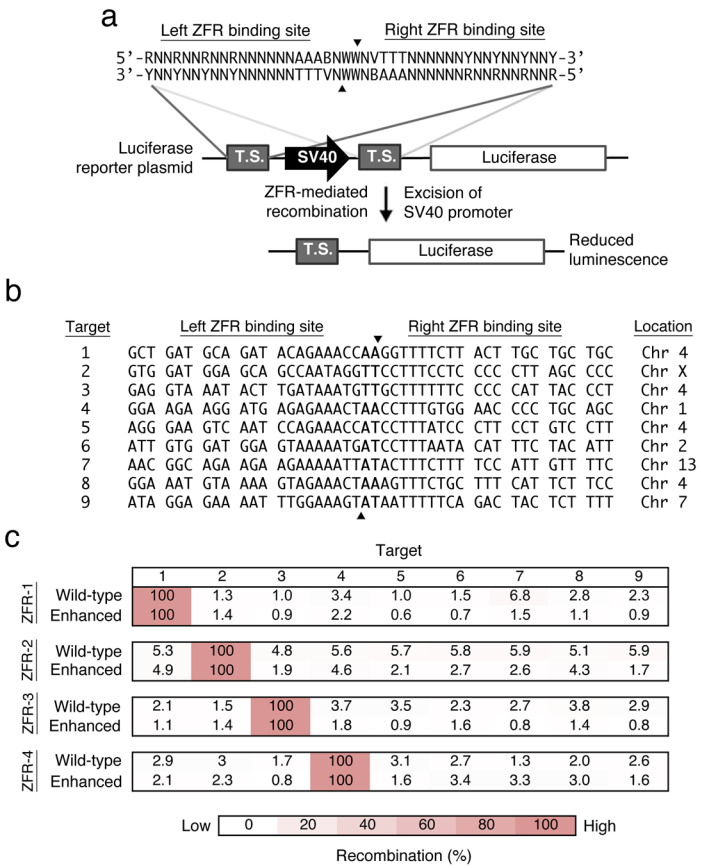

Figure S5. Recombination specificity of enhanced ZFRs. (a) Schematic representation of the luciferase reporter system used to evaluate ZFR activity. Same cartoon as Figure S3A. **(b)** Target sites used to evaluate eZFR specificity. **(c)** Recombination specificity of standard and eZFR 1, 2, 3 and 4, which are designed to target sites from human chromosomes 4, X, 4 and 1, respectively. Recombination was normalized to the activity of each ZFR pair on its intended DNA target. The eZFRs in this study contained the L^{F103R} / R^{YKWT} dimeric configuration. *Renilla* luciferase expression was used to normalize for transfection efficiency and cell number.

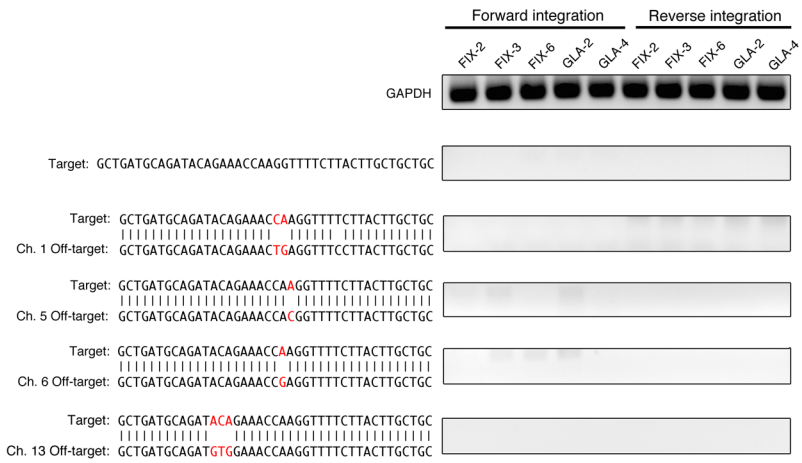

Figure S6. Analysis of off-target eZFR-mediated integration. (Left) Potential off-target integration sites identified by BLAST using the human chromosome 4 ZFR target site as reference. Mismatches between the intended and pseudo-target sites are highlighted red. **(Right)** PCR analysis of off-target integration within individual puromycin-resistant HEK293 clones transfected with eZFRs and donor plasmids containing either the human FIX or GLA cDNA. Integration was evaluated in the forward and reverse orientations. GAPDH indicates PCR control. Faint bands are non-specific PCR products.

>Left ZFR-1 Standard (Target: 1; Location: Ch. 4)

MLIGYVRVSTNDQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLD
RLGRSMKHLISLVGELRERGINFRSLTDSIDTSSPMGRFFFYVMGALAEMERELIIERTMAGLA
AARNKGRIGGRPPKSGTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSQSGD
LRRHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTH
TGKKTSGQAGQ

>Left ZFR-1 D12G/F103R (Target: 1; Location: Ch. 4)

MLIGYVRVSTNGQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLD
RLGRSMKHLISLVGELRERGINFRSLTDSIDTSSPMGRRFFYVMGALAEMERELIIERTMAGLA
AARNKGRIGGRPPKSGTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSQSGD
LRRHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTH
TGKKTSGQAGQ

>Left ZFR-1 D12G/YKWT (Target: 1; Location: Ch. 4)

MLIGYVRVSTNGQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLD
RLGRSMKHLISLVGELRERGINFRSLTDSIDTSSPYGRFKFYWTGALAEMERELIIERTMAGLA
AARNKGRIGGRPPKSGTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSQSGD
LRRHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTH
TGKKTSGQAGQ

>Right ZFR-1 Standard (Target: 1; Location: Ch. 4)

MLIGYVRVSTNDQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLD
RLGRSMKHLISLVGELRERGINFRSLTDSIDTSSPMGRFFFYVMGALAEMERELIIERTMAGLA
AARNKGRIGGRPPKSGTGEKPYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSQSGD
LRRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTH
TGKKTSGQAGQ

>Right ZFR-1 D12G/F103R (Target: 1; Location: Ch. 4)

MLIGYVRVSTNGQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLD
RLGRSMKHLISLVGELRERGINFRSLTDSIDTSSPMGRRFFYVMGALAEMERELIIERTMAGLA
AARNKGRIGGRPPKSGTGEKPYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSQSGD
LRRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTH
TGKKTSGQAGQ

>Right ZFR-1 D12G/YKWT (Target: Location: Ch. 4)

MLIGYVRVSTNGQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLD
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AARNKGRIGGRPPKSGTGEKPYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSQSGD
LRRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTH
TGKKTSGQAGQ

>Left ZFR-2 Standard (Target: 2; Location: X)
MLIGYVRVSTNDQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLD
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LERHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTH
TGKKTSGQAGQ

>Left ZFR-2 D12G/F103R (Target: 2; Location: X)
MLIGYVRVSTNGQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLD
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LERHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTH
TGKKTSGQAGQ

>Left ZFR-2 D12G/YKWT (Target: 2; Location: X)
MLIGYVRVSTNGQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLD
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LERHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTH
TGKKTSGQAGQ

>Right ZFR-2 Standard (Target: 2; Location: X)
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LKNHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTH
TGKKTSGQAGQ

>Right ZFR-2 D12G/F103R (Target: 2; Location: X)
MLIGYVRVSTNGQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLD
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LKNHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTH
TGKKTSGQAGQ

>Right ZFR-2 D12G/YKWT (Target: 2; Location: X)
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TGKKTSGQAGQ

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LTVHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTH
TGKKTSGQAGQ

>Left ZFR-3 D12G/F103R (Target: 3; Location: Ch. 4)
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LTVHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTH
TGKKTSGQAGQ

>Left ZFR-3 D12G/YKWT (Target: 3; Location: Ch. 4)
MLIGYVRVSTNGQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLD
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AARNKGRRFGRPPKSGTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSTTGN
LTVHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTH
TGKKTSGQAGQ

>Right ZFR-3 Standard (Target: 3; Location: Ch. 4)
MLIGYVRVSTNDQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLD
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TGKKTSGQAGQ

>Right ZFR-3 D12G/F103R (Target: 3; Location: Ch. 4)
MLIGYVRVSTNGQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLD
RLGRSMKHLISLVGELRERGINFRSLTDSIDTSSPMGRRFFYVMGALAEMERELIIERTMAGIA
AARNKGRRFGRPPKSGTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRRDE
LNVHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTH
TGKKTSGQAGQ

>Right ZFR-3 D12G/YKWT (Target: 3; Location: Ch. 4)
MLIGYVRVSTNGQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQKGDTLVVWKLD
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>Left ZFR-4 Standard (Target: 4; Location: Ch. 1)
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TGKKTSGQAGQ

>Left ZFR-4 D12G/F103R (Target: 4; Location: Ch. 1)
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>Left ZFR-4 D12G/YKWT (Target: 4; Location: Ch. 1)
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>Right ZFR-4 Standard (Target: 4; Location: Ch. 1)
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TGKKTSGQAGQ

>Right ZFR-4 D12G/F103R (Target: 4; Location: Ch. 1)
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LVRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTH
TGKKTSGQAGQ

>Right ZFR-4 D12G/YKWT (Target: 4; Location: Ch. 1)
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LVRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTH
TGKKTSGQAGQ

Table S1. ZFR amino acid sequences. Selected heterodimer mutations are highlighted orange. The D12G substitution is highlighted blue. Arm region mutations that endow target specificity within the catalytic domain are highlighted red. Specificity-determining α-helical zinc-finger residues are highlighted green.

>pDonor (empty)

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GCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGG
AAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAA
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ATGACCTGGTGCGCCACCAGCGTACCCACACGGGTGAAAAACCGTATAAATGCCCAGAGTGCGG
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TACAAATGTCCAGAATGTGGCAAGTCTTTCAGCCAGAGCGGCGATCTGCGCCGCCACCAACGTA
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TGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAA
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TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCG
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CCTAGGCTTTTGCAAAAAGCTTACCATGACCGAGTACAAGCCCACGGTGCGCCTCGCCACCCGC
GACGACGTCCCCAGGGCCGTACGCACCCTCGCCGCCGCGTTCGCCGACTACCCCGCCACGCGCC
ACACCGTCGATCCGGACCGCCACATCGAGCGGGTCACCGAGCTGCAAGAACTCTTCCTCACGCG
CGTCGGGCTCGACATCGGCAAGGTGTGGGTCGCGGACGACGGCGCCGCGGTGGCGGTCTGGACC

ACGCCGGAGAGCGTCGAAGCGGGGGCGGTGTTCGCCGAGATCGGCCCGCGCATGGCCGAGTTGA
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GCCCGCGTGGTTCCTGGCCACCGTCGGCGTCTCGCCCGACCACCAGGGCAAGGGTCTGGGCAGC
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CCGCGCCCCGCAACCTCCCCTTCTACGAGCGGCTCGGCTTCACCGTCACCGCCGACGTCGAGGT
GCCCGAAGGACCGCGCACCTGGTGCATGACCCGCAAGCCCGGTGCCTGACGCCCGCCCCACGAC
CCGCAGCGCCCGACCGAAAGGAGCGCACGACCCCATGCATCGATAAAATAAAAGATTTTATTTA
GTCTCCAGAAAAAGGGGGGAATGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAAC
GCCATTTTGCAAGGCATGGAAAAATACATAACTGAGAATAGAGAAGTTCAGATCAAGGTCAGGA
ACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCT
CACGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTC
CTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTA
GAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAAC
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CCCACAACCCCTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCCGGGTACCCGTGTAT
CCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTC
TGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCACATGCAGCATGTATCAAAATTAATTTGGT
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>pDonor-FIX-ZFR
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GCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACA
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TGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTC
ACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCC
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CCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATC
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A

>pDonor-GLA-ZFR

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GATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCG
GCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAA
CTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGT
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ATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGC
CCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATT
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TGACATTGATGATTCCTGGAAAAGTATAAAGAGTATCTTGGACTGGACATCTTTTAACCAGGAG
AGAATTGTTGATGTTGCTGGACCAGGGGGTTGGAATGACCCAGATATGTTAGTGATTGGCAACT
TTGGCCTCAGCTGGAATCAGCAAGTAACTCAGATGGCCCTCTGGGCTATCATGGCTGCTCCTTT
ATTCATGTCTAATGACCTCCGACACATCAGCCCTCAAGCCAAAGCTCTCCTTCAGGATAAGGAC
GTAATTGCCATCAATCAGGACCCCTTGGGCAAGCAAGGGTACCAGCTTAGACAGGGAGACAACT
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GATTGGTGGACCTCGCTCTTATACCATCGCAGTTGCTTCCCTGGGTAAAGGAGTGGCCTGTAAT
CCTGCCTGCTTCATCACACAGCTCCTCCCTGTGAAAAGGAAGCTAGGGTTCTATGAATGGACTT
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GATGTCATTAAAAGACTTACTTTTGGACCCAGCTTTCTTGTACAAAGTGGTTCGATCTAGAATG
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CATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCT
TTCCTAATAAAATGAGGAAATTGCATCGCATTGCTGATGCAGATACAGAAACCAAGGTTTTCTT
ACTTGCTGCTGCGGATCCGGTGAGATGCCTTATAAGTGTCCGGAATGTGGTAAGTCCTTCAGCC
GCAGCGATGACCTGGTGCGCCACCAGCGTACCCACACGGGTGAAAAACCGTATAAATGCCCAGA
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AAGCCATACAAATGTCCAGAATGTGGCAAGTCTTTCAGCCAGAGCGGCGATCTGCGCCGCCACC
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ACCCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTA
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ATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTA
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ACCCGCGACGACGTCCCCAGGGCCGTACGCACCCTCGCCGCCGCGTTCGCCGACTACCCCGCCA
CGCGCCACACCGTCGATCCGGACCGCCACATCGAGCGGGTCACCGAGCTGCAAGAACTCTTCCT
CACGCGCGTCGGGCTCGACATCGGCAAGGTGTGGGTCGCGGACGACGGCGCCGCGGTGGCGGTC
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CGAGGTGCCCGAAGGACCGCGCACCTGGTGCATGACCCGCAAGCCCGGTGCCTGACGCCCGCCC
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AAAGAGCCCACAACCCCTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCCGGGTACCC
GTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGT
CTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCACATGCAGCATGTATCAAAATTAA
TTTGGTTTTTTTTCTTAAGTATTTACATTA

Table S2. Donor plasmids used in this study. The PstI and BamH1 restriction sites are highlighted red and orange, respectively (empty donor only). The CMV promoter is highlighted green. The human coagulation factor IX and α-galactosidase genes are highlighted blue and purple, respectively. ZFR target sites are highlighted yellow.

>GFP-ZFR-ζ-H1-α-P2-XbaI-Fwd
TTAATTAAGAGTCTAGAGGAGGCGTGTCCAAAATTATGGTTTACAGCGCCACTGCAGATCTAGG
AGGAATTTAAAATGAG

>GFP-ZFR-ζ-H1-α-P2-HindIII-Rev
ACTGACCTAGAGAAGCTTGCAGTGGCGCTGTAAACCATAATTTTGGACACGCCTCCCTGCAGTT
ATTTGTACAGTTCATC

>SV40-ZFR-BglII-Fwd
TTAATTAAGAGAGATCTGCTGATGCAGATACAGAAACCAAGGTTTTCTTACTTGCTGCTGCGCG
ATCTGCATCTCAATTAGTCAGC

>SV40-ZFR-HindIII-Rev
ACTGACCTAGAGAAGCTTGCAGCAGCAAGTAAGAAAACCTTGGTTTCTGTATCTGCATCAGCTT
TGCAAAAGCCTAGGCCTCCAAA

>PstI-CMV-Donor-Fwd
CACCACCACCTGCAGGTTGACATTGATTATTGACTAGTTATTAAT

>BamH1-ZFR-Donor
CACCACCACGGATCCGCAGCAGCAAGTAAGAAAACCTTGGTTTCTGTATCTGCATCAGCAATGC
GATGCAATTTCCTCATTTTATTAGGAAAGGACAGTGGGAGTGGCA

>Gin-Dimer-Lib-Rev
CCATTTCAGCCAGGGCACCMNHMNHGTAGAAMNHMNHACGCCCMNHTGGAGATGACGTA

>Gin-Dimer-Fwd
GCCCTGGCTGAAATGGAACGAGAACTAATTA

>Gin-HBS-D12G-Koz
CACCACCACGCGCGCAAGCTTAGATCTGGCCCAGGCGGCCACCATGCTGATTGGCTATGTAAGG
GTATCAACAAATGGCCAGAATACAGACC

>Gin-YKWT-Rev
ACTGACAGTATTGATACGTCATCTCCATATGGGCGTTTTAAGTTCTACTGGACGGGTGCCCTGG
CTGAAATGG

>Gin-F103R-Rev
GTAGAAGAAACGACGCCCCATTGGAGATGACGT

>Gin-YKWT-Fwd
ATACGTCATCTCCATATGGGCGTTTTAAGTTCTACTGGACGGGTGCCCTGGCTGAAATGGAACG
AGA

>Gin-F103R-Fwd
CCAATGGGGCGTCGTTTCTTCTACGTTATGGG

>Gin-AgeI-Rev
CACCACCACACCGGTTCCCGATTTAGGTGGGCGAC

>pUC18-Prim-1
TGCGGGCCTCTTCGCTATTAC

>pUC18-Prim-2
AATGTGAGTTAGCTCACTCATTAG

>GAPDH-External-Fwd
AACTGCTTAGCACCCCTGGCCAA

>GAPDH-External-Rev
CTTCAAGGGGTCTACATGGCAAC

>GAPDH-Internal-Fwd
GACCACAGTCCATGCCATCAC

>GAPDH-Internal-Rev
TCCACCACCCTGTTGCTGTAG

>ZFR-1-External-Fwd
GGAAGGACTCAGTCATCACAGATGCT

>ZFR-1-External-Rev
GGGTGTGTAATTCACTGTAGCCCTTGACAG

>ZFR-1-Internal-Fwd
GTTCCTGCCAGGATCCACTAG

>ZFR-1-Internal-Rev
GCATGTGTCCAGATGCATAGG

>CMV-External
GGTCATTAGTTCATAGCCCATATATGGAGTTC

>CMV-Internal
TGACGTCAATGACGGTAAATGG

>FIX-Internal
TTTCACGAAGGCAAGATTGGCATATCATTG

>GLA-Internal
GCCCTCAAGCCAAAGCTCTCCTTCAGGATA

>Off-target-Ch-1-External-Fwd
TTGACCTAACCTTGGTATTTGTGTGACACA

>Off-targe-Ch-1-External-Rev
CATGGCCTTCATGACTGCTGGCTTCC

>Off-target-Ch-1-Internal-Fwd
AAGCCCTCCGTTAGAATTAAATCCCCT

>Off-target-Ch-1-Internal-Rev
TGGAGAAATACCTTGCTCTTAAGTCTT

>Off-target-Ch-5 External-Fwd
TGGGACACCTCGCAGGATCTCG

>Off-target-Ch-5 External-Rev
GTTGCTTATCAGCTTAAGGAGAA

>Off-target-Ch-5-Internal-Fwd
TAGAATTAAATCCCCTCTGAAGA

>Off-target-Ch-5- Internal-Rev
AGAAGCACAGAGTACTCAATTTC

>Off-target-Ch-6-External-Fwd
CCTAGTATTTCTTCTGATGCTTTGAA

>Off-target-Ch-6-External-Rev
AACTTGATGGAGATCTTTCTCATGCGC

>Off-target-Ch-6-Internal-Fwd
GAGGAAGGACTCAGTCATCACAGAT

>Off-target-Ch-6-Internal-Rev
TTTCAAAATGGAGAAATACCTTGCTCTTA

>Off-target-Ch-13-External-Fwd
TGAGCTCTCCAAGACACCTCACAGGA

>Off-target-Ch-13-External-Rev
ACATCTTGCTGGAGGTCATTGTGTGT

>Off-target-Ch-13-Internal-Fwd
AGGCAAACGCATCTCCAGTCTTGCAT

>Off-target-Ch-13-Internal-Rev
GGCACAGACTTCTCAATTTCATTACA

Table S3. Primers used in this study. Where applicable, ZFR target sites are underlined.