

**Supplemental Figure 1:** DNA and protein sequence of (A) ROSA26 eZFNs L6 and (B) R4. Both proteins contain one SV40 Nuclear Localization Signal (purple) and FokI domains with the obligate heterodimer mutations (Q486E, I499L, N496D in L6 and I538K, E490K, and H538R for R4) and Sharkey mutations (K441E, S418P) represented in red. The nomenclature used for the mutations corresponds to that used in the original publications and does not correlate with the amino acid numbers in the ROSA26 eZFNs. The DNA binding modules of ROSA26 eZFN L6 and R4 are represented in pink.

### A. ROSA26 eZFN L6

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ATG TAC CCA TAC GAT GTC CCA GAC TAC GCG AAT TCC CCT GGG ATC CCA GGC ATG GGG CCC AAA AAG AAA CGC AAA GTT GGG CGC CTC GAG CCC GGG GAA A < 100
M Y P Y D V P D Y A N S P G I P G M G P K K K R K V G R L E P G E K
10 20 30 40 50 60 70 80 90

>HA tag                                >SV40 NLS1
AA CCC TAT AAA TGC CCC GAG TGT GGT AAG TCA TTC TCT CAA AGC GGG GAT TTA AGA AGA CAC CAG AGA ACC CAC ACC GGG GAA AAG CCC TAC AAG TGT CC < 200
P Y K C P E C G K S F S Q S G D L R R H Q CAG R T H T G G E K P E C G G K P E C G K P
110 120 130 140 150 160 170 180 190

>Module L6: GCA
T GAG TGC GGA AAG TCT TTC TCC ACT AGC GGT TCA TTA GTA AGA CAC CAG AGG ACA CAC ACC GGG GAG AAA CCT TAT AAA TGC CCA GAA TGC GGG AAA TCG < 300
E C G K S F S C T S G S L V R H Q T H T G E K P Y K T A A T C C A G A A T G G K S
210 220 230 240 250 260 270 280 290

>Module L5: GTT
TTC AGT CAA AGA GCA CAT TTA GAA AGA CAT CAA CGG ACC CAC ACC GGG GAA AAA CCA TAC AAG TGC CCC GAG TGT GGC AAG AGC TTT AGT ACC CAC CTC G < 400
F S Q R A H L E R H Q C R T H T G E K P Y K C P E C G K S F S T H L D
310 320 330 340 350 360 370 380 390

>Module L4: GGA
AC CTG ATT AGA CAC CAG CGC ACC CAC ACC GGG GAA AAG CCA TAT AAG TGC CCT GAG TGT GGA AAG AGC TTC AGT AGG AAG GAT AAC CTT AAA AAC CAC CA < 500
L I R H Q R T H T G E K P Y K C P E C G K S F S R K D N L K N H Q
410 420 430 440 450 460 470 480 490

>Module L3: ACT                                >Module L2: AAG
A AGA ACC CAC ACC GGG GAA AAG CCC TAT AAG TGC CCA GAG TGC GGG AAA TCA TTC TCA CAG CTG GCA CAT CTT AGA GCC CAC CAG CGG ACC CAC ACC GGT < 600
R T H T G E K P Y K C P E C G K S F S Q L A H L R A H Q R T H T G
510 520 530 540 550 560 570 580 590

>Module L1: AGA
GCG GCC GCC GGC CTG GTG AAG AGC GAG CTG GAG GAG AAG AAG TCC GAG CTG CGG CAC AAG CTG AAG TAC GTG CCC CAC GAG TAC ATC GAG CTG ATC G < 700
A A A R A L V K S E L E E K K S E L R H K L K Y V P H E Y I E L I E
610 620 630 640 650 660 670 680 690

>S418P Sharkey Mutation                                >K441E Sharkey Mutation
AG ATC GCC AGG AAC CCC ACC CAG GAC CGC ATC CTG GAG ATG AAG GTG ATG GAG TTC TTC ATG AAG GTG TAC GGC TAC AGG GGA GAG CAC CTG GGC GGA AG < 800
I A R N P T Q D R I L E M K V M E F F M K V Y G Y R G E H L G G S
710 720 730 740 750 760 770 780 790

C AGA AAG CCT GAC GGC GCC ATC TAT ACA GTG GGC AGC CCC ATC GAT TAC GGC GTG ATC GTG GAC ACA AAG GCC TAC AGC GGC GGC TAC AAT CTG CCT ATC < 900
R K P D G A I Y T V G S P I D Y G V I V D T K A Y S G G Y N L P I
810 820 830 840 850 860 870 880 890

>Q486E Mutation                                >N496D Mutation                                >I499L Mutation
GGC CAG GCC GAC GAG ATG GAG AGA TAC GTG GAG GAG AAC CAG ACA CGG GAT AAG CAC CTC AAC CCC AAC GAG TGG TGG AAG GTG TAC CCT AGC AGC GTG A < 1000
G Q A D E M E R Y V E E N Q T R D K H L N P N E W W K V Y P S S V T
910 920 930 940 950 960 970 980 990

CC GAG TTC AAG TTC CTG TTC GTG AGC GGC CAC TTC AAG GGC AAC TAC AAG GCC CAG CTG ACC AGG CTG AAC CAC ATC ACC AAC TGC AAT GGC GCC GTG CT < 1100
E F K F L F V S G H F K G N Y K A Q L T R L N H I T N C N G A V L
1010 1020 1030 1040 1050 1060 1070 1080 1090

G AGC GTG GAG GAG CTG CTG ATC GGC GGC GAG ATG ATC AAA GCC GGC ACC CTG ACA CTG GAG GAG GTG CGG CGC AAG TTC AAC AAC GGC GAG ATC AAC TTC < 1200
S V E E L L I G G E M I K A G T L T L E E V R R K F N N G E I N F
1110 1120 1130 1140 1150 1160 1170 1180 1190

TGA < 1203
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## B. ROSA26 eZFN R4

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      >HA tag
ATG TAC CCA TAC GAT GTC CCA GAC TAC GCG AAT TCC CCT GGG ATC CCA GGC ATG GGG CCC AAA AAG AAA CGC AAA GTT GGG CGC CTC GAG CCC GGG GAG A < 100
M Y P Y D V P D Y A N S P G I P G M G P K K K R K V G R L E P G E K
      10 20 30 40 50 60 70 80 90

      >SV40 NLS1
      70 80 90

      >Module 4: GTC
AA CCA TAC AAA TGC CCC GAG TGT GGA AAG TCA TTT AGT GAT CCA GGC GCA TTA GTA AGA CAT CAG CGG ACA CAT ACC GGG GAG AAA CCT TAT AAA TGC CC < 200
P Y K C P E C G K S T A F S D P G A L V R H Q R T H T G E K P Y K C P E C G K S
      110 120 130 140 150 160 170 180 190

      >Module 3: GGA
A GAA TGC GGG AAA TCG TTC AGT CAA AGA GCA CAT TTA GAA AGA CAT CAA CGG ACC CAC ACC GGG GAG AAG CCA TAC AAG TGT CCC GAA TGC GGG AAG TCA < 300
E C G K S F S Q R A H L E R H Q R T H T G E K P Y K C P E C G K S
      210 220 230 240 250 260 270 280 290

      >Module 2: GCG
TTC TCC AGA AGT GAC GAT TTA GTA AGA CAT CAG CGC ACG CAC ACC GGG GAA AAG CCA TAT AAA TGC CCC GAG TGC GGC AAA TCA TTC AGC AGA AGC GAC C < 400
F S R S D D L V R H Q R T H T G A A A A R A L V K S E L E E K K S E L R H K L K
      310 320 330 340 350 360 370 380 390

      >Module 1: TGG
AC CTG ACC ACC CAC CAG CGC ACC CAT ACC GGT GCG GCC GCC CGC GCC CTG GTG AAG AGC GAG CTG GAG GAG AAG AAG TCC GAG CTG CGG CAC AAG CTG AA < 500
L T T H Q R T H T G A A A A R A L V K S E L E E K K S E L R H K L K
      410 420 430 440 450 460 470 480 490

      >S418P Sharkey Mutation
G TAC GTG CCC CAC GAG TAC ATC GAG CTG ATC GAG ATC GCC AGG AAC CCC ACC CAG GAC CGC ATC CTG GAG ATG AAG GTG ATG GAG TTC TTC ATG AAG GTG < 600
Y V P H E Y I E L I E I A R N P T Q D R I L E M K V M E F F M K V
      510 520 530 540 550 560 570 580 590

      >K441E Sharkey Mutation
TAC GGC TAC AGG GGA GAG CAC CTG GGC GGA AGC AGA AAG CCT GAC GGC GCC ATC TAT ACA GTG GGC AGC CCC ATC GAT TAC GGC GTG ATC CTG GAC ACA A < 700
Y G Y R G E H L G G S R K P D G A I Y T V G S P I D Y G V I V D T K
      610 620 630 640 650 660 670 680 690

      >E490K mutation
AG GCC TAC AGC GGC GGC TAC AAT CTG CCT ATC GGC CAG GCC GAC GAG ATG CAG AGA TAC CTG ANG GAG AAC CAG ACC CGG AAT AAG CAC ATC AAC CCC AA < 800
A Y S G G Y N L P I G Q A D E M Q R Y V V K E N Q T R N N K H I N P N
      710 720 730 740 750 760 770 780 790

C GAG TGG TGG AAG CTG TAC CCT AGC AGC GTG ACC GAG TTC AAG TTC CTG TTC GTG AGC GGC CAC TTC AAG GGC AAC TAC AAG GCC CAG CTG ACC AGG CTG < 900
E W W K V Y P S S V T E F K F L F V S G H F K G N Y K A Q L T R L
      810 820 830 840 850 860 870 880 890

      >I538K Mutation
      >H537R Mutation
AAC CGC AAA ACC AAC TGC AAT GGC GCC GTG CTG AGC GTG GAG GAG CTG CTG ATC GGC GCC GAG ATG ATC AAA GCC GGC ACC CTG ACA CTG GAG GAG GTG C < 1000
N R K T N C N G A V L S V E E L L I G G E M I K A G T L T L E E V R
      910 920 930 940 950 960 970 980 990

GG CGC AAG TTC AAC AAC GGC GAG ATC AAC TTC TGA < 1035
R K F N N G E I N F *
      1010 1020 1030

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**Supplemental Figure 2:** DNA sequence of the ROSA26 eZFNs L6 and R4 in FASTA format.

**>ROSA26-L6-eZFN**

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ATGTACCCATACGATGTCCCAGACTACGCGAATTCCCCTGGGATCCCAGGCATGGGGCCC
AAAAAGAAACGCAAAGTTGGGCGCCTCGAGCCCGGGGAAAAACCCTATAAATGCCCGGAG
TGTGGTAAGTCATTTCTCTCAAAGCGGGGATTTAAGAAGACACCAGAGAACCCACACCGGG
GAAAAGCCCTACAAGTGTCTGAGTGCAGAAAGTCTTTCTCCACTAGCGGTTTCATTAGTA
AGACACCAGAGGACACACACCCGGGAGAAACCTTATAAATGCCCAGAATGCGGGAAATCG
TTCAGTCAAAGAGCACATTTAGAAAGACATCAACGGACCCACACCCGGGAAAAACCATAC
AAGTGCCCCGAGTGTGGCAAGAGCTTTAGTACCCACCTCGACCTGATTAGACACCAGCGC
ACCCACACCCGGGAAAAAGCCATATAAGTGCCCTGAGTGTGGAAAGAGCTTCAGTAGGAAG
GATAACCTTAAAAACCACCAAGAACCACACCCGGGAAAAAGCCCTATAAAGTGCCCAGAG
TGCGGGAAATCATTTCTCACAGCTGGCACATCTTAGAGCCCACCAGCGGACCCACACCGGT
GCGGCCGCCCGCGCCCTGGTGAAGAGCGAGCTGGAGGAGAAGAAGTCCGAGCTGCGGCAC
AAGCTGAAGTACGTGCCCCACGAGTACATCGAGCTGATCGAGATCGCCAGGAACCCACC
CAGGACCGCATCCTGGAGATGAAGGTGATGGAGTTCTTCATGAAGGTGTACGGCTACAGG
GGAGAGCACCTGGGCGGAAGCAGAAAGCCTGACGGCGCCATCTATACAGTGGGCAGCCCC
ATCGATTACGGCGTGATCGTGGACACAAAGGCCACAGCGGCGGCTACAATCTGCCTATC
GGCCAGGCCGACGAGATGGAGAGATACGTGGAGGAGAACCAGACACGGGATAAGCACCTC
AACCCCAACGAGTGGTGGAAAGGTGTACCCTAGCAGCGTGACCGAGTTCAAGTTCCTGTTC
GTGAGCGGCCACTTCAAGGGCAACTACAAGGCCAGCTGACCAGGCTGAACCACATCACC
AACTGCAATGGCGCCGTGCTGAGCGTGGAGGAGCTGCTGATCGGCGGCAGATGATCAA
GCCGGCACCTGACACTGGAGGAGGTGCGGCGCAAGTTCAACAACGGCGAGATCAACTTC
TGA
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**>ROSA26-R4-eZFN**

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ATGTACCCATACGATGTCCCAGACTACGCGAATTCCCCTGGGATCCCAGGCATGGGGCCC
AAAAAGAAACGCAAAGTTGGGCGCCTCGAGCCCGGGGAGAAACCATACAAATGCCCGGAG
TGTGGAAAAGTCATTTAGTGATCCAGGCGCATTAGTAAGACATCAGCGGACACATACCGGG
GAGAAACCTTATAAATGCCCAGAATGCGGGAAATCGTTTCAGTCAAAGAGCACATTTAGAA
AGACATCAACGGACCCACACCCGGGAGAAAGCCATACAAGTGTCCCGAATGCGGGAAAGTCA
TTCTCCAGAAGTGACGATTTAGTAAGACATCAGCGCACGCACACCCGGGAAAAAGCCATAT
AAATGCCCGGAGTGCGGCAAATCATTCAGCAGAAGCGACCACCTGACCACCCACCAGCGC
ACCCATACCGGTGCGGCCGCCCGCGCCCTGGTGAAGAGCGAGCTGGAGGAGAAGAAGTCC
GAGCTGCGGCACAAGCTGAAGTACGTGCCCCACGAGTACATCGAGCTGATCGAGATCGCC
AGGAACCCACCCAGGACCGCATCCTGGAGATGAAGGTGATGGAGTTCTTCATGAAGGTG
TACGGCTACAGGGGAGAGCACCTGGGCGGAAGCAGAAAGCCTGACGGCGCCATCTATACA
GTGGGCAGCCCCATCGATTACGGCGTGATCGTGGACACAAAGGCCACAGCGGCGGCTAC
AATCTGCCTATCGGCCAGGCCGACGAGATGCAGAGATACGTGAAGGAGAACCAGACCCGG
AATAAGCACATCAACCCCAACGAGTGGTGGAAAGGTGTACCCTAGCAGCGTGACCGAGTTC
AAGTTCCTGTTCTGTGAGCGGCCACTTCAAGGGCAACTACAAGGCCAGCTGACCAGGCTG
AACCGCAAAACCAACTGCAATGGCGCCGTGCTGAGCGTGGAGGAGCTGCTGATCGGCGGC
GAGATGATCAAAGCCGGCACCCCTGACACTGGAGGAGGTGCGGCGCAAGTTCAACAACGGC
GAGATCAACTTCTGA
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