CCR5 R30 OT-CCR2

dRNA3 dRNA2 CCR5-R30 sgRNA CCR5-R30 on-target

CCR5-R30 OT (CCR2) dRNA1 dRNA5 dRNA5s

dRNA4

GCAGCGAGTAGAGCGGAGG GCACCAGCGAGTAGAGCGG GTAGAGCGGAGGCAGGAGGCGGG

 ${\tt CCAAAGATGAACACCAGTGAGTAGAGCGGAGGCAGGAGGCGGGCTGCGATTTGCTTCACATT}$ **GGT**TTCTACTTGTGGTC**G**

GGTTAAACGAAGTG**CG**

GGTCGCTCATCTCGCG

HBB G10 OT1

dRNA7 HBB-G10 sqRNA HBB-G10 on-target

HBB-G10 OT1 dRNA4 dRNA5

dRNA6

GCCCCACAGGGCAGTAAGG CTTGCCCCACAGGGCAGTAACGG CATCCACGTTCACCTTGCCCCACAGGGCAGTAACGGCAGACTTCTCCTCAGGA TCAGAGCAGTGCTTCAGCCCCACAGGGCAGTAAGGGCAGCCTTCCTCTAAATA AGTCTCGTCACGAAGTCGGGGTGTCCCGTCATTCCCGTCGGAAGGAGATTTAT **GGGGTGTCCCGTCATTCCG** GGGTGTCCCGTCATTCCCG

GGTGTCCCGTCATTCCCG

GGGGCAA<mark>A</mark>GTGAACG<u>TGG</u>

TCTGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTTGGTGGTGA

ACTGCTGTCAATGCCCTGTGGGGCAAAGTGAACGTGGATGCAGTTGGTGGTGA

<mark>r</mark>gacg<mark>acagtt</mark>acgggacaccccgtt<mark>t</mark>cacttgcacctac<mark>g</mark>tcaaccaccact

CACGTTCACTTTGCCCCACAGGG

TCACCACCAACTTCATCCACGTTCACCTTGCCCCACAGGGCAGTAACGGCAGA

TCACCACCAACTGCATCCACGTTCACTTTGCCCCCACAGGGCATTGACAGCAGT

GGTGCAAGTGAAACGGGG

HBB R04 OT-HBD

CCACGTTCACTTTGCCCCACAGG

GGGACACCCCGTTTCACTG

GTGGATGCAGTTGG<u>TGG</u>

GAACGTGGATGCAGT<u>TGG</u>

GTGAACGTGGATGAAGTTGG<u>TGG</u>

GCCCACAGGGCAGTAACGG

GGTGTCCCGTAACTGTCG

GGTGTCCCGTGAATGCCG

GCCCACAGGGCAGTAACGG

HBB R01 OT-HBD

dRNA6 dRNA4 dRNA3 dRNA2 HBB-R01 sgRNA

HBB-R01 on-target HBB-R01 OT (HBD)

dRNA7

HBB R03 OT-HBD

GCCGTTACTGCCCTG<u>TGG</u>

GCTGTCAATGCCCTGTGG

GGCAATGACGGGACACCCG

GGTTGACGTAGGTGCAAG

GGTGGTTGACGTAGGTG

HBB-R03 sgRNA HBB-R03 on-target

HBB-R03 OT (HBD) dRNA1 dRNA2

dRNA3 dRNA4 dRNA6

HBB-R04 sgRNA HBB-R04 on-target HBB-R04 OT (HBD) dRNA1 dRNA2

dRNA5

TCACCACCAACTTCATCCACGTTCACCTTGCCCCACAGGGCAGTAACGGCAGA TCACCACCAACTGCATCCACGTTCACTTTGCCCCACAGGGCATTGACAGCAGT AGTGGTGGTTGA<mark>C</mark>GTAGGTGCAAGTG<mark>A</mark>AACGGGGTGTCCCGT<mark>A</mark>A<mark>C</mark>TG<mark>T</mark>CGTC<mark>A</mark> GGTTGACGTAGGTGCAAG <u>GGT</u>GGTTGACGTAGGTG **GGT**GCAAGTGAAACGGGG dRNA3 dRNA4 **GGT**GTCCCGTAACTGTCG dRNA6 **GGT**GTCCCGTGAATGCCG

GGATTTGTGGGATGGA<u>GGG</u>

VEGFA sgRNA1 OT1

dRNA2 dRNA2s dRNA7 dRNA7s dRNA9 VEGFA sqRNA1 VEGFA sgRNA1 on-target VEGFA sgRNA1 OT1

GATTTGTGGGATGGAGGG GGAGGGAGTTTGCTCC<u>TGG</u> GAGGGAGTTTGCTCC<u>TGG</u> GGGAGTTTGCTCCTGGGG ${\tt GGGTGGGGGGGGGTTTGCTCC\underline{TGG}}$ GGCTTTGGAAAGGGGGTGGGGGGGAGTTTGCTCCTGGACCCCCTATTTCTGACC rgggatggagggagtttgctcctgg<mark>ggtgtcagaatgtcc</mark>t

→ Best dRNA OT mismatch

PAM 5' G mismatch VEGFA sgRNA1 OT4

GCCCATTATGATAGGGAGG **GTTATGATAGGGAGGGTGG** GGGTGGAGTGGGCTCCTGG GGTGGAGTGGGCTCCTGG GTGGAGTGGGCTCCTGGG GGAGTGGGCTCCTGGGG GTGGGCTCCTGGGGATGG GGGTGGGGGGAGTTTGCTCCTGG ${\tt GAATGGGCTTTGGAAAGGGGGTGGGGGGGGGGTTTGCTCCTGGACCCCTATTTCTGACCT}$ GGCTAGCCCATTATGATAGGGAGGGTGGAGTTTGCTCCTGGGGATGGAAGGG GGACCCCTACCTTCCCGG

VEGFA sgRNA1 OT6

GAGGGAGTTTGCTCCTGG

GGGTGGGGGGAGTTTGCTCC<u>TGG</u>

GGGAGTTTGCTCCT<u>GGG</u>

GGAGTTTGCTCCTGGGG

GGACCCCTTGGACACTAG

GAGCTCCCATCACGGGGG dRNA4s GCTCCCATCACGGGGG GTCCCATCACGGGGGAGGG dRNA2 dRNA1 dRNA5 dRNA6 VEGFA sgRNA1 VEGFA sgRNA1 on-target GGAGAGAGGCTCCCATCACGGGGGAGGGAGTTTGCTCCTGGGGAACCTGTGATCCCCA VEGFA sgRNA1 0T6 dRNA8

VEGFA sgRNA1 OT11

GCTGTCACAACTCGGGG

dRNA7 dRNA6 dRNA5 dRNA4 dRNA1 dRNA2 dRNA3 VEGFA sgRNA1 VEGFA sgRNA1 on-target VEGFA sgRNA1 OT11 dRNA8

dRNA3

dRNA2

dRNA1

dRNA1s

dRNA4

dRNA5

dRNA6

dRNA8

VEGFA sgRNA1

VEGFA sgRNA1 0T4

VEGFA sgRNA1 on-target

GTCACAACTCGGGGAGG GTCACAACTCGGGGAGGG GTCACAACTCGGGGAGGGG GCTCCTGGCATTCAGTGG GGGTGGGGGGAGTTTGCTCCTGG GAATGGGCTTTGGAAAGGGGGTGGGGGGGAGTTTGCTCCTGGACCCCTATTTCTGACCT TTTTTGCTGTCACAACTCGGGGAGGGGAAGTTTGCTCCTGGCATTCAGTGGGTAGAGT <mark>AAAAACGACAGTGTTGAG</mark>CCC<mark>CT</mark>CCCCTTCAAACGAGGACC<mark>GTAAGTCACCCATCTC</mark>A GGACCGTAAGTCACCCATG

VEGFA sgRNA2 OT1

dRNA1 VEGFA sgRNA2 VEGFA sgRNA2 on-target VEGFA sgRNA2 OT1 dRNA2s dRNA2 dRNA4

GGCTGCTGTTGCAGAGG GCCCCCACCCCGCCCCCCGG GACCCCTCCACCCCGCCTCCGG ${\tt ACGCCGCGAGCCCCGACCCCTCCACCCCGCCTCCGGGCGCGGGCTCCGGCCCCTGCC}$ TTGCATTTCCAAAAGACCCCCCCCCCCCCCCCCCCCCCGCTGCTGTTGCAGAGGACAAG **GGTTTT**CTGGGGGGGGTG GGTTTTCTGGGGGGGGTGG GGGGGTGGGGCGGGG

VEGFA sgRNA2 OT2

VEGFA sgRNA2 VEGFA sqRNA2 on-target VEGFA sgRNA2 OT2 dRNA4 dRNA6 dRNA7 dRNA8

dRNA8

dRNA1

dRNA2

VEGFA sgRNA2

GCCAAGGCGCTCCTAGGGG GACCCCCTCCACCCCGCCTCCGG TTCAGTGCGACGCCGCGAGCCCCGACCCCTCCACCCCGCCTCCGGGCGCGGGCTCCGG CTCCTAGGGGCCCCTCCACCCCGCCTCTGGCCAAGTTTTGAGG BAGGATCCCCGGGGAGGTGGGGCGGAGACCGGTTCAAAACTCC GGATCCCCGGGGAGGTGG **GGA**GGTGGGGCGGAGACCG <u>GGT</u>GGGGCGGAGACC<mark>G</mark>G GGGGCGGAGACCGGTTCAG

VEGFA sgRNA2 OT17

GTCCAACAGGGTTG<u>TGG</u>

GGAGTCCTGCAGGTTG

GCCCCCACCCGCCTCCGG GACCCCCTCCACCCCGCCTCCGG VEGFA sgRNA2 on-target CGCCGCGAGCCCCTCCACCCCGCCTCCGGGCGCGCGCCCCCGGCCCCTGCCC AGAGTTCCCTTGCACACCCCCCCCCCCCCCCCGCCTCAGGACGTCCAACAGGGTTGTGGCTC VEGFA sgRNA2 OT17 SAACGTGTGGGGGGGGGGGGGGGGGTCCTGCAGGTTGTCCCAACACCGAG

VEGFA sgRNA2 OT19

dRNA1 GCCCCCACCCCGCCTCAGG VEGFA sgRNA2 GACCCCTCCACCCGCCTCCGG VEGFA sgRNA2 on-target GCGAGCCCCGACCCCTCCACCCCGCCTCCGGGCGCGCGCCCCTGCCCGCGG VEGFA sgRNA2 OT19 GGAGTCCTTTCTTATTTGG dRNA3 GGGGGGGTGGGGCGGAGTG dRNA3s **GGGGGGGGGGGGGAG** GGGGGGTGGGGCGGAGTCG dRNA4 GGGTGGGGCGGAGTCCTTG dRNA5

VEGFA sgRNA3 OT2

dRNA5 dRNA2 dRNA1 VEGFA sgRNA3 VEGFA sgRNA3 on-target VEGFA sgRNA3 OT2

GTGTGTGGGGGGGGACTCGG GAGTGAGTGTGTGTGGG GTGAGTGTGTGTGTGGGG GGTGAGTGAGTGTGCGTGTGG GACGTGTGTGTGTGTGGGTGAGTGAGTGTGTGCGTGTGGGGTTGAGGGCGTTGGAG TGAGAGAGCGAGTGAGTGAGTGAGTGTGTGTGTGTGGGGGGGACTCGGCTTGTTGT ACTCTCTCGCTCACTCACTCACTCACACACACACCCCCCCTGAGCCGAACAAC

VEGFA sgRNA3 OT4

GGCTTTAGCGGGAAGCAGG

dRNA4s dRNA4 dRNA3 dRNA2 GCGTGTGGCTTTAGCGG GTGAGTGTATGCGTGTGG dRNA1 GGTGAGTGAGTGTGTGCGTGTG VEGFA sgRNA3 VEGFA sgRNA3 on-target GTGTGTGTGTGTGGGTGAGTGAGTGTGTGCGTGTGGGGTTGAGGGCGTTGGAGCGG GAGCACGCACTTAACGCTGAGTGAGTGTATGCGTGTGGCTTTAGCGGGAAGCAGGGGG VEGFA sgRNA3 OT4 CTCGTGCGTGAATTGCGACTCACTCACATACGCACACCGAAATCGCCCTTCGTCCCCC

VEGFA sgRNA3 OT18

dRNA2 VEGFA sgRNA3 VEGFA sgRNA3 on-target VEGFA sgRNA3 OT18 dRNA4 dRNA5

GACCACCTTTTATGTGTGG GCCACCTTTTATGTGTGGG GGTGAGTGAGTGTGTGCGTG<u>TGG</u> GAGAGTGAGGACGTGTGTGTGTGTGGGTGAGTGAGTGTGCGTGTGGGGTTGAGG **GTCACCACACTTACCACCTTTTATGTGTGGGTGAGTGTGTGCGTGAGGACATTTAA** <mark>IGTCAATGGTGGAAAAT</mark>ACACACCCACTCACACACGCACTCCTGTAAATT <mark>GGA</mark>AAATACACACCCACTG **GGTGGAAAATACACACCCG**

ZSCAN2 sgRNA1 OT1

dRNA2 dRNA1s dRNA1 ZSCAN2 sgRNA1 ZSCAN2 sgRNA1 on-target ZSCAN2 sgRNA1 OT1

GGCCTCAAATCTTCTGG GCAAGGGCTTCAGCCAGG GCAAGGGCTTCAGCCAGG GTGCGGCAAGAGCTTCAGCC<u>GGG</u> ${\tt CTACAAATGCCTCATGTGCGGCAAGAGCTTCAGCCGGGGCTCCATTCTGGTCATGCAC}$ CTATAAATGTGAGGAGTGTGGCAAGGGCTTCAGCCAGGCCTCAAATCTTCTGGCCCAT GATATTTACACTCCTCACACCGTTCCCGAAGTCGGTCCGGAGTTTAGAAGACCGGGTA **GGTCCGGAGTTTAGAAG**

ZSCAN2 sgRNA1 OT2

GAGCTGTTCCCTGTGAGGG

dRNA4 dRNA1s dRNA1 ZSCAN2 sqRNA ZSCAN2 sgRNA1 on-target ZSCAN2 sgRNA1 OT2 dRNA2

dRNA3

GGAAGAGCTTCAGCAAGG GGGAAGAGCTTCAGCAAGG GTGCGGCAAGAGCTTCAGCCGGG AGCCCTACAAATGCCTCATGTGCGGCAAGAGCTTCAGCCGGGGCTCCATTCTGGTCAT TTCCAGAGAGCTGTTCCCTGTGAGGGAAGAGCTTCAGCAAGGTAGCCCAGATTCTATA AAGGTCTCTCGACAAGGGACACTCCCTTCTCGAAGTCGTTCCATCGGGTCTAAGATAT **GGA**CAC**T**CC**C**TTCTCGAAG GGGACACTCCCTTCTCG