**CLN3 Q352X 561 off-target analysis Labbook**

Method extracted from Xiaobing’s paper:

We did an unbiased detection of 8 potential off-target sites with highest scores based on COD (cas9.wicp.net). For each site, we designed a pair of primers to amplify around 1000bps of the predicted region. Sanger sequencing analysis showed that no detectable off-target mutations or indels were induced.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Chromosome | start | end | strand | MM | target\_seq | PAM | alignment | distance | position | gene name | gene id |
| chr16 | 28482128 | 28482150 | + | 0 | TGAAACGGATGCGACAGCAG | CGG | ||||||||[||||||||||||]PAM | 0 | Exonic | CLN3 | ENSG00000188603 |
| chr17 | 45768365 | 45768387 | - | 4 | TATTAGGGATGCGACAGCAG | CGG | |---|-||[||||||||||||]PAM | 15893 | Intronic | CRHR1 | ENSG00000120088 |
| chr1 | 1.62E+08 | 1.62E+08 | - | 3 | TGGAAGGGAAGCGACAGCAG | AGG | ||-||-||[|-||||||||||]PAM | 1440 | Intronic | RP11-456P18.2 | ENSG00000229808 |
| chrX | 31281990 | 31282012 | + | 4 | TGAGAGTCATGCGACAGCAG | TGG | |||-|---[||||||||||||]PAM | 15038 | Intronic | DMD | ENSG00000198947 |
| chr12 | 1.11E+08 | 1.11E+08 | + | 4 | TGACGGGGAGGCGACAGCAG | TGG | |||---||[|-||||||||||]PAM | 7340 | Intronic | SH2B3 | ENSG00000111252 |
| chr2 | 2E+08 | 2E+08 | - | 3 | TGATAGGGATGAGACAGCAG | TGG | |||-|-||[|||-||||||||]PAM | 17117 | Intronic | C2orf47 | ENSG00000162972 |
| chr11 | 1.24E+08 | 1.24E+08 | - | 4 | GGACAGGGATGAGACAGCAG | TGG | -||-|-||[|||-||||||||]PAM | 4075 | Intronic | GRAMD1B | ENSG00000023171 |
| chr2 | 2.23E+08 | 2.23E+08 | - | 4 | TCATAAGGATGAGACAGCAG | AGG | |-|-|-||[|||-||||||||]PAM | 23413 | Intergenic | AC016712.3 | ENSG00000264133 |
| chr5 | 1.24E+08 | 1.24E+08 | + | 4 | TGCATCGGTGGCGACAGCAG | AGG | ||-|-|||[--||||||||||]PAM | 0 | Exonic | CSNK1G3 | ENSG00000151292 |

**Off-target 1 Chr17 🡪 CRHR1 (antisense) 908bp (only an amplicon). New sequencing primer!**

CAACCCCCCTCGCAGAGCAGTGGTCGCAAACGAAGGATTCAATAAAGCACGTACAGAGAAGCAGCCCAGCCCTATGTCAGACCCTCTGCCTTAGAACCACTTGCCAATAATAAATCACCCACCACCCCTATGAAACAGGTGGTTCATGAAGTCTTGTCAATCAGTCATACCTTAGTCTAGAAGTCCTTTCCTGGGAGACAGGGGGAAGCATGGAGTCTGGGGTTCCTCCTGGGGCTGTTGGGATATCAAAAGCCTTACCTGGCTTCCCACCATCCCTGTGGAAATCCCCCAAGTGGAAGCCATGGGCCGCCACAAGACGTGGCCCCAGCCCAGCTTTCCAATCTCAGAACGTCATCCCTGCTGTCGCATCCCTAATACCTGTCCTGGGGTCCTAGGACTCCTCGCTGTTCCCCAAACAGACGGAGCACAGTCCCACCTCCCGGCCTTTGCTCACAATGGTGCCTCCACCTGGAATGCTGTTGCCCCTTCTCCCCTCTGTCTCTCTCCCCTCTGTCCCCTACATTTCTATGTTGGTAGAAATCCTGCTCATCTTTTAAAGTACAAATGGACCGGGTGCAGTGGCTCACACCTGTAATCTCAGCACTCTGGGAGGCCGAGGCAGGCGGATCGCAAGGTCAGGAGATTGAGACCATCCTGGCTAACACGGTGAAACCCCATTTCTACTAAAAATACAAAAAATTAGCCAGGTGTGGTGGCGGGTGCCTGTAGTCCCAGCTACTCCGGAGGCTGAGGCAGGAGAATGGCGTGAACCCGGTAGGTGGAGCTTGCAGTGAGCCGAGATGGCGCCACTGCCCTCCAGCCTGGGCGACAGAGCGAGACTCCGTCTCAAAAAAAAAAAAAAGAAAAAAGAAAAAAAAGAAAGTACAAGTGCCACCAACTCTTTGATGAAGTTTTCCCTATCCCGTGGCTAGAATGAGCCCCTCAGTGTCCCTCTTCTAACAGCATTTGAAA

There are two products flanking this region: at the 5’ site, the pleckstrin homology domain-containing family M member 1 and at the 3’ site, the corticotropin-releasing factor receptor 1 isoform 1 (CRHR1). The 5’ sequence does not contain the off-target, so the sequencing was correct, we were checking the wrong gene.

**NEW Off-target 1 Chr17 🡪 CRHR1 (antisense) 291bp**

CTTGACAGTCACATTAGTGAAGGCTGGGTTAACCCTTGATGCTCTGCCCTCCAGCACCAGGGCAGGACCTGGACACCAGGGTCATTCCCTCCCTGGCACCTATGGAGACTGGATGCTGAGGAGGGAAAGGTGAAGCCACAGCCAACTATTTTTAGTTCAAGAATTGGTTGCCAGGAACACTGAGCCGACTCCCTGCTGTATATTAGGGATGCGACAGCAGCGGTGTTGTTTGCAGGGTCTAATTGTGGGGATTTATTGTAACATGCAACTTGTCAGCCACTGCTCTGCCTAGGGCCCAGTCTCCTTGTCATCGGAATCTGAGCTATTTCCCAACTCCCTGGGTGCAGGGGAAGGAAATGGGGCAGAGAAGGAGCAGGAGTGTGCCCAGGGACCCTGGACAGCTCACAGCTGACCTTGCACCAA

**Off-target 2 Chr1 🡪 RP11-456P18.2 (antisense) 797bp (might have mismatch amplification)**

AACAGGGGCCAGCTTCCACGAGCTGGTCCATACCTCTCCTGCCTTATTTCCTGACACTTTCCCTTATACCTTGAGATCCAGCTTGCCAGTATTTCCCCAATACCTCCACCGCATATACAACCACATACATATACACCCTTATCCCCTCCCTCATGTACCACCCATATATAATTCTCTTTACCGCATCCTTTATCACACTCCTACAAGCCTTGAAGCAATCTGAACATAGAAACCATCTTTGATTTTCAACTCCTAATTGGGACTGAACATACTTGTCTTGAACACAGATTGCTGCCTTGTTAGTATAGGTATCCTAATATGGCACATGTGTATAGTTGAAGCTATAATGCATTCCCCAAGTTCCCTTTCAGGACTAAAGGGTTATTTCCCAACAGCTGGAAGTCCTGCTGCCAGACAGCCTTCTTCAGGAATTGCCTTGTCCCCAACTCAAGACAACCCTGAAGGACCTTTCCCAGTTTTCCATGGGGTCCACTGAGGCCTTGAGATTGTACTGAAGTCAACTTCTCCCTCTGCTGTCGCTTCCCTTCCACAGGTGTTTATCACAAGAACAATCCTTAATAAACTTCCTGTAAGCACACTCTCAGAGTCAGCTTGCTGGGGAACCCAAGCTATAACAACATGTTTATCTTGTTCACACTTTCTTCTGAGTTCTAAATCATGGGAAGTTACAGTTATCCAAAATCTTACTAATTGGAAGATCATTTCATCAGACGTGTATTGAGCACCTGCTTCATGTCAGGCATTGTGCCAGATACAATGGAAACAAATCAAGACTTGGCCTCTGTCCTTGAGTTTACAGTCTGAGGTGACAGTTACATATGAACCTAACTGTATTAAAGCATCTTGAGAATCTTTTTGATTCAAATGTCAATAATTTTAGTATAAAATTCAAAAGCATGGATTTTGTGGTGTTACAAAGAACAAAAGGAAAATATTTCAGGGGAAGGAAAATGAATATAAATAAGTAGA

**Off-target 3 ChrX 🡪 DMD (sense) 750bp (might have mismatch amplification)**

TCATGGGAGGAGGAGTGACTTTTAGTGGATACTGGATAAGAAGAATGCACTCCTGTTGAGTAGAATGAGATCCCATGGCAGCACTTCTAACAAATTAGACCTAGTCTCTGAACAAAAACAATATGAGTTAAGATCGTCCCTAAGATTATCTAGAAAAGAATCCCGTTCCCATAAAGCTGAGTAACATTTTGGTCAGATTTGTGTCATTTTAAAAGCCAAAATATTTACCTAAAAAGCAAGTGATTTGAATGTCTCAGACTAAGTTATTTTTTACTGATCTATGGCCCTCAAATGAGTTGATGACCTTCAGTAGAGAAATGGTTCTTTATTTCTGATTTGTGAGAGTCATGCGACAGCAGTGGATGGGTTAAGAAGGTTAGAGGAAATTTGTTACATTGCTTTTTAGCTGTTGTTTTTTAGTTTGGTGAACAAACGAGATAACATAGCTAGTGCCTTTTAGTCATAAAGCAAAAATAAAACTACAGTGTTTTCTAATATTGTACCCTAAACCTATGCAAATCTCCAATTTACATCACCCACAAGCCTTCTATATTTTTGCAGATTTTAACTAGAGCTAAATAATAGGCATATATTTATACTTAATTGTAATCAAATGTAATGACGTGTGCAAAACAGGCTTTGCTCAAAGTGCTCTTTATAGATGAAATATGATTATTGAACTTGACAGTAGATCATTCATTGGAGGGAAAAGTGATTAATCACTTCTCTGTGCCTCTACCTTCCTACCCT

**Off-target 4 Chr12 🡪 SH2B3 (sense) 659bp (only one amplicon)**

GAGGATCTGGCGGTGCCAGGCCGGCATTGGAGGGGGTCCCAGCCTGGCCTGTGCTGCCACTGGTTCAGCAGTAGAGCCTGACGGGGAGGCGACAGCAGTGGGGGCCCTGGGGAAGGAGAAAGATTCCAGAAGCAGCTGCTAGGCTGGAGTAGCTGGCCTTGGGCCCCAGACCTTCCCGGGGAGGTGGGGCTCCCATTTCAAATGGAGCATGCCCAGCCTGCCGGGCCTCAGTTTCCACATATGCCCAGGCATGGATTAGCTGAGATGGTCTTGAAGTCCTGCCAGCCCTGGTGGTTCTGGAGGTTGAGCCTCAGTTTCCCTCATCTGTACAGTGGAGGACATGATGGAGTTCTAGCTGTTGATAAATGCCCTTTAAACCACCATTTATTGGGCTTTCCTTATACAGCAGGCACTTTACTGACCTGGCTTCATTTTATCTTCACAGCAGCCCTGTGAGGGTAGCTGCGCCATTAGGCCCATTTCACAGATGGGGAGACTAGGTCTGAGAGAGCTTAGGTGGCTTTCCCAAGGTCTCACAGCCCCTGTGGCTGAGAGGGGCTTGGAACCTGGGCAGTTGGCTGCCCCTGTGAGCAGTGAGTCCTTAGAAGGACTTACTGTCAGTATTATTGATGGCACTGTTAACTAGGGCCCTGCATGGG

**Off-target 5 Chr2 🡪 C2orf47 (antisense) 695bp (only one amplicon)**

GAGAAGAAGCCTTCGCAGCATCTGTCTTCTTCAGCTGTTGCCGCTGATAATTTCAGACATATTCACAAACCCTGCTCTGCATCAGACAGCTGTTACGTGGTTACCGAGTGTTTGTGTGAGAGTGTGTGGCTCTGTGTCTCCTTTCAAGTTTCCACTTGCATGTAAGATTGTCTGAGAAAGGTTTTGTGCTATTTCCCCAGTGCAGTGTGTTTCCTGTTTTCCTTGGGAGGGCTCTGTATTGTAATCATTTTTCCACTCCAAGATCCTTCTAGGCTGATTTCCTTAACTGTTTTGACTCCTTCACGGTGTCTAAATTTGCATGTTGGCAATACAATGTTGGAGTTTATTTCACAGGGGCAGAGCACATTCCACTGCTGTCTCATCCCTATCATTTCTCTTTACTCCATTTATCTAAGCACTTGCCACATTCTTCTAAACCTAAGATCCGAAACCTCAGTTTGGGTTTTCTAGTGCTTGGATGGATGTCAAGATGATTTATAAAGCACTCTCATCCCAGTGCCATGGGCCAGTGATACCATCAGTTTAGCAACTCCTGAACTGCTTACCTAGAGTTGCCTATTGATTAAGGCTGCTACCAGATGCAGCTTGGTCCACATGCTCCAACCAGCAAGCCCTAGTTTATGAGCCAGTCATTTATGATGAGCATCATCTCAGTCATCATTGCGCGATGTGCT

**Off-target 6 Chr11 🡪 GRAMD1B (antisense) 595bp (might have mismatch amplification)**

CAGGCTGGTCTCACACTCCTGACCTAAAGTGATCCGTCCGAATTGGCTTCCCAAAGTGCTGGGATTACAAGCACGAGCCACTGTGCCCAGCCTGGAATCTACGTTTTTACATGTGTTCACTGGGCCATTTTGAAGTACAGTAAAGTTTGGGGGCTGCTGTATTAAATTACCAAGACTCCTAAGACAAAAGCACCATTCCTAATCCTATGCGGGCCAAGACCCTCCTGGCAAGGTGGCAGAGCTGGGGAGAGTGAGGGAGCAGCCCCATTTGTTTTAAGGGTCTGACTTTTCCTGGGAATCCACATCTTCCCCACCCCACTGCTGTCTCATCCCTGTCCCTAGCTTCTTGGTCTGGAGGCTTTCTTGCCCCATTGGCCTGGCTTTCATTTCCCTGGATTTTGCTGCTTTGAGGACCAAAATCTGTGCTTTTAGTATTGCTACTTTGACCTGAAGATCTGACGGTCTTTTTGGGCTTGGTCCCCCTCTTGCTAATGCTGAAGGTTGTATAGGGTCCATGGGAAGGAAGAGGGTGCCTCAGACAGATCCCGAAGCTGTTGATCTCAGCGTCTCCACCACCAGCGTGTCTGAAACTGGA

**Off-target 7 Chr2 🡪 AC016712.3 (antisense) 364bp (might have mismatch amplification)**

AGCATGTACAGCTACTGCACATGTGCACCCAGAGGACCAACCAGAACATGCTTACTAGCAACACCTCTTCCCACCTCCTTATGAATGATCGTGTAAGGGAGCCTCCTTCGTGCCAGCCTCTGCTGTCTCATCCTTATGAGCAGCCGCCCTGAATCCTCTCCCTCTTAGGGTGTACTGTCTATTCTGTGCTTTCAAAATATTCTTTCTCCTTTACGATAAGTTGTTCTATGCTGCATCTCCTTTGCTGTGTGTCTCCTGTTTAAAATATTTTAAACTAGGAAGACAAGAACTGAGGTATCACAGCAGCCGGTCAGATCCTGAGAGGACCTGAGACTGTCACTTTCACCACCTGCAGGTCACCATT

**Off-target 8 Chr5 🡪 CSNK1G3 (sense) 758bp (might have one mismatch amplification). With new primer amplification 401bp (only amplicon)**

TCCATGTGTTCACTTCCGGGTCCGGCGTCGATCCGGATGCCCGAGGCAGAAGGATGTTTGACCTCCGGATAAGCGAGGCGCTGCTGTGCATTCATTCCGGGCTGCATCGGTGGCGACAGCAGAGGCTCGGGCGGCGACTCTCCGGCCAGCGGCGGCGGTAGGAGGCACCAGCGGCAGTGCAGGGACCGAATCCGAGCAGCGCTGCGTTACCTCTCTCTCTCGCTCCTTCCCCCCTACCTCGCTCGCTCGCTCGCTCGCTCCCTGCGTGGCTCGCTTTCCTCCTCCGGCCGCCGGCGGGTGTGATGTGCCGCCGCCGCTGCCCCCGCCGGCGCTGACGGACTGGGGGCGCCGCCCGCGCCCGGGACCGACCCCTCTGCTCGCGGCCGCGCCTTTGAGGTAAAGCCCCCTGCGCGTAGGTGCTCCGCTGCCAGCCCCGGCCCGGCGGCTGCCTCGGCGCGGCCGCGGCGCCCTCCCTCCCGGGTGGGGCTGGGGTCCCGGGCGGGTGGGGTGGGGTGCGGCGGGGGCGGCGCCGCAGGCGCGGGTGTCGGGGGAGGGGTGGGCGAGGACCATGGCGGGTGGCCCGGAGGCGTGGGCAGGAGGGGGCCGCGGTCGGAGGCTGAGGGCGCGGAGGGCTAGGGGGCAGCGGGGGCCGCGGGTGGCGGGTCGGGCTGGGTGCAAAAGTTGCCGGAGTGGTGCGGCGCGACTGGGGCGGCGAGGGAGGTGGGCCAACTGGGCCGGCGGGGGCTTCCTTAGGAGAG