

S5 Table Analysis of cell clones randomly collected from Cas9B-3-BD.

| Clone NO. | Gene-sgRNA | sgRNA-Sequence | Mutation Status |
|---------------------|-------------------|-----------------------|-----------------|
| 1 | <i>Clec11a-1</i> | TCAGCCCAATGGCGGCGTCC | Wild-type |
| 2 | <i>Edil3-3</i> | TCCACAACACTAGAGCAGTT | Mutation |
| 3 | <i>Dapk2-2</i> | CTCAAGGATGAGCACCACGT | Mutation |
| 4 | <i>Dpt-2</i> | ATCCATCATCACCGTAGTCC | Mutation |
| 5 | <i>Fhl2-3</i> | TGAATACTCGTCCAAGTGCC | Mutation |
| 6 | <i>Glis2-1</i> | AAGCGTCCGTCCACCTTCTC | Wild-type |
| 7 | <i>Gulp1-3</i> | AAGTTGTGAGAGATGCTGTC | Wild-type |
| 8 | <i>Fmod-2</i> | AGGGTCATAGGGGTCGTAGT | Mutation |
| 9 | <i>Irx3-2</i> | CCTCCTCGTCCGTGCGACTA | Mutation |
| 10 | <i>Lum-1</i> | GTGGACCGACGGACTCGGTC | Mutation |
| 11 | <i>Maf-1</i> | GCACTTCGACGACCGCTTCT | Mutation |
| 12 | <i>Greb1-1</i> | CAGCAGCTGTCATCGTTAGA | Mutation |
| 13 | <i>Ms4a7-2</i> | GGTACTTACCCCAGGTTTGT | Mutation |
| 14 | <i>Sema7a-2</i> | CAGCCCGGATGAGAACTCCC | Mutation |
| 15 | <i>Omd-3</i> | CCATTTAACATACCTTCGTG | Mutation |
| 16 | <i>Plagl1-2</i> | GCTCCCGAGAACGGGCTTGA | Mutation |
| 17 | <i>Podn-2</i> | CGGGGTAGATCTTCTCCAGC | Wild-type |
| 18 | <i>Sepp1-1</i> | GGCCGTCTTGTGTATCACCT | Mutation |
| 19 | <i>Zbtb32-1</i> | TCGGCCTCGAGGAAGAGTAG | Mutation |
| 20 | <i>Srpx-3</i> | GACTCACCACTAGAAGACGA | Mutation |
| 21 | <i>Steap4-2</i> | CTATCCGTTACTATGTTCTGA | Mutation |
| 22 | <i>Tbx15-3</i> | GGTCCGCTCGGTGAGCACCT | Mutation |
| 23 | <i>Tead2-3</i> | GACGATGGCCACCATGTCTT | Wild-type |
| 24 | <i>Tnfrsf19-1</i> | ACCAGCGCACAGTCCGCACA | Mutation |
| 25 | <i>Gulp1-3</i> | AAGTTGTGAGAGATGCTGTC | Mutation |
| 26 | <i>Srpx-3</i> | GACTCACCACTAGAAGACGA | Wild-type |
| 27 | Blank | GGGTCTTCGAGAAGAC | - |
| 28 | <i>Fhl2-3</i> | TGAATACTCGTCCAAGTGCC | Mutation |
| 29 | <i>Dio2-3</i> | ACCTACAAGAAGTCCGAAGT | Mutation |
| 30 | <i>Aebp1-1</i> | TAACCGATCCAGCGTTTCCA | Mutation |
| 31 | <i>Tmtc1-3</i> | CCATGTGCGCCGAGAGCTATG | Mutation |
| 32 | <i>Clec11a-3</i> | CCACCAAGAACCGTCGTCTG | Mutation |
| 33 | <i>Foxs1-3</i> | CCTCGTGATGACCGCAAGCC | Mutation |
| 34 | <i>Edil3-3</i> | TCCACAACACTAGAGCAGTT | Wild-type |
| 35 | <i>Trim35-2</i> | TGGTGTAGGTGCAAACGACT | Mutation |
| Mutation Efficiency | | | 77.1% (27/35) |

SgRNAs targeted by 1 time: 26

SgRNAs targeted by 2 times: 4

SgRNAs totally targeted: 30

Genes totally targeted: 29