

Appendix Figures

(Page 2) Appendix Figure S1. Genotypes of novel mutant CH12 cells generated for this study.

WT CH12 cells were used to knockout the indicated genes through CRISPR/Cas9 using the gRNAs listed in Table EV2. Sequencing results for each of these mutants are shown.

(Page 3) Appendix Figure S2. Flow Cytometry analysis of Iglo WT, 53bp1^{-/-} and Shld2^{-/-} CH12

subclones. Ig-lo cells from WT, 53bp1^{-/-} and Shld2^{-/-} CH12 cells were sorted and subcloned and reanalyzed for expression of IgM and IgA by flow cytometry. As positive controls, expression of IgA and IgM are shown for two specific subclones that are IgA or IgM positive.

Xlf #1 (586/A05)

1
WT V S Q H L I H P L M G V S L A L Q S H V R E L A A L L R M K D L E I Q A Y Q E S G A
GTCTCTCAGCATTGATTCACTCTCTGATGGGCTGTAGCCCTGGCACTGCAGAGTCATGTGAGGGAGCTAGCAGCATTGCTTCGGATGAAGGACCTTGAGATCCAGGCCCTACCAGGAGAGTGGGGCT
Allele 1 GTCTCTCAGCATTGATTCACTCTCTGATGGGCTGTAGCCCTGGCACTGCAGAGTCATGTGAGGGAGCTAGCAGCATTGCTTCGGATGAAGGACCTTGAGATCCAGGCCCTACCAGGAGAGTGGGGCT

V S Q H L I H P L M G V S L A L Q S H V R E L A A L L R M K D L E I Q A Y Q E S G A
WT GTCTCTCAGCATTGATTCACTCTCTGATGGGCTGTAGCCCTGGCACTGCAGAGTCATGTGAGGGAGCTAGCAGCATTGCTTCGGATGAAGGACCTTGAGATCCAGGCCCTACCAGGAGAGTGGGGCT
Allele 2 GTCTCTCAGCATTGATTCACTCTCTGATGGGCTGTAGCCCTGGCACTGCAGAGTCATGTGAGGGAGCTAGCAGCATTGCTTCGGATGAAGGACCTTGAGATCCAGGCCCTACCAGGAGAGTGGGGCT

Xlf #2 (586/B10)

1
WT V S Q H L I H P L M G V S L A L Q S H V R E L A A L L R M K D L E I Q A Y Q E S G A
GTCTCTCAGCATTGATTCACTCTCTGATGGGCTGTAGCCCTGGCACTGCAGAGTCATGTGAGGGAGCTAGCAGCATTGCTTCGGATGAAGGACCTTGAGATCCAGGCCCTACCAGGAGAGTGGGGCT
Allele 1 GTCTCTCAGCATTGATTCACTCTCTGATGGGCTGTAGCCCTGGCACTGCAGAGTCATGTGAGGGAGCTAGCAGCATTGCTTCGGATGAAGGACCTTGAGATCCAGGCCCTACCAGGAGAGTGGGGCT

V S Q H L I H P L M G V S L A L Q S H V R E L A A L L R M K D L E I Q A Y Q E S G A
WT GTCTCTCAGCATTGATTCACTCTCTGATGGGCTGTAGCCCTGGCACTGCAGAGTCATGTGAGGGAGCTAGCAGCATTGCTTCGGATGAAGGACCTTGAGATCCAGGCCCTACCAGGAGAGTGGGGCT
Allele 2 GTCTCTCAGCATTGATTCACTCTCTGATGGGCTGTAGCCCTGGCACTGCAGAGTCATGTGAGGGAGCTAGCAGCATTGCTTCGGATGAAGGACCTTGAGATCCAGGCCCTACCAGGAGAGTGGGGCT

Xrcc4 #1 (999/15)

1
WT M E R K V S R I Y L A S E P N V P Y F L Q V S W E R T I G S G F V I T L T D G H S A W
ATGGAAGGAAAGTAAGCAGAAATCTATCTTGCTCTGAACCCCAACGTACCTTATTTCTGCAAGTGTCTTGGGAGAGAACAAATAGGATCCGGCTTTGTTATTACACTTACTGACGGCCATTACAGCCTGG
Allele 1 ATGGAAGGAAAGTAAGCAGAAATCTATCTTGCTCTGAACCCCAACGTACCTTATTTCTGCAAGTGTCTTGGGAGAGAACAAATAG-ATCCGGCTTTGTTATTACACTTACTGACGGCCATTACAGCCTGG

M E R K V S R I Y L A S E P N V P Y F L Q V S W E R T I G S G F V I T L T D G H S A W
WT ATGGAAGGAAAGTAAGCAGAAATCTATCTTGCTCTGAACCCCAACGTACCTTATTTCTGCAAGTGTCTTGGGAGAGAACAAATAGGATCCGGCTTTGTTATTACACTTACTGACGGCCATTACAGCCTGG
Allele 2 ATGGAAGGAAAGTAAGCAGAAATCTATCTTGCTCTGAACCCCAACGTACCTTATTTCTGCAAGTGTCTTGGGAGAGAACAAAG-ATCCGGCTTTGTTATTACACTTACTGACGGCCATTACAGCCTGG

Xrcc4 #2 (999/31)

1
WT M E R K V S R I Y L A S E P N V P Y F L Q V S W E R T I G S G F V I T L T D G H S A W
ATGGAAGGAAAGTAAGCAGAAATCTATCTTGCTCTGAACCCCAACGTACCTTATTTCTGCAAGTGTCTTGGGAGAGAACAAATAGGATCCGGCTTTGTTATTACACTTACTGACGGCCATTACAGCCTGG
Allele 1 ATGGAAGGAAAGTAAGCAGAAATCTATCTTGCTCTGAACCCCAACGTACCTTATTTCTGCAAGTGTCTAG-ATCCGGCTTTGTTATTACACTTACTGACGGCCATTACAGCCTGG

M E R K V S R I Y L A S E P N V P Y F L Q V S W E R T I G S G F V I T L T D G H S A W
WT ATGGAAGGAAAGTAAGCAGAAATCTATCTTGCTCTGAACCCCAACGTACCTTATTTCTGCAAGTGTCTTGGGAGAGAACAAATAGGATCCGGCTTTGTTATTACACTTACTGACGGCCATTACAGCCTGG
Allele 2 ATGGAAGGAAAGTAAGCAGAAATCTATCTTGCTCTGAACCCCAACGTACCTTGAATTTCTTAGTGTATTA-----TTATTACACTTACTGACGGCCATTACAGCCTGG

Paxx #1 (275/8)

1
WT M A P P L L S L P L C I L P P G S G S P R L V C Y C E R D S G G D G D R D D F N L
ATGGCTCCTCCGTTGTTGCTGCTCCGCTTTGTTATTCTGCCCGGGTTTCGGGCTCCCCCGCCTGGTGTGC TACTGCGAGCGGGATAGTGGTGGAGACGGGGACCGGACGACTTCAACCTC
Allele 1 ATGGCTCCTCCGTTGTTGCTGCTCCGCTTTGTTATTCTGCCCGGGTTTCGGGCTCCCCCGCCTGGTGTGC TACTGCGAGCGGGATAGTGGTGGAGACGGGGACCGGACGACTTCAACCTC

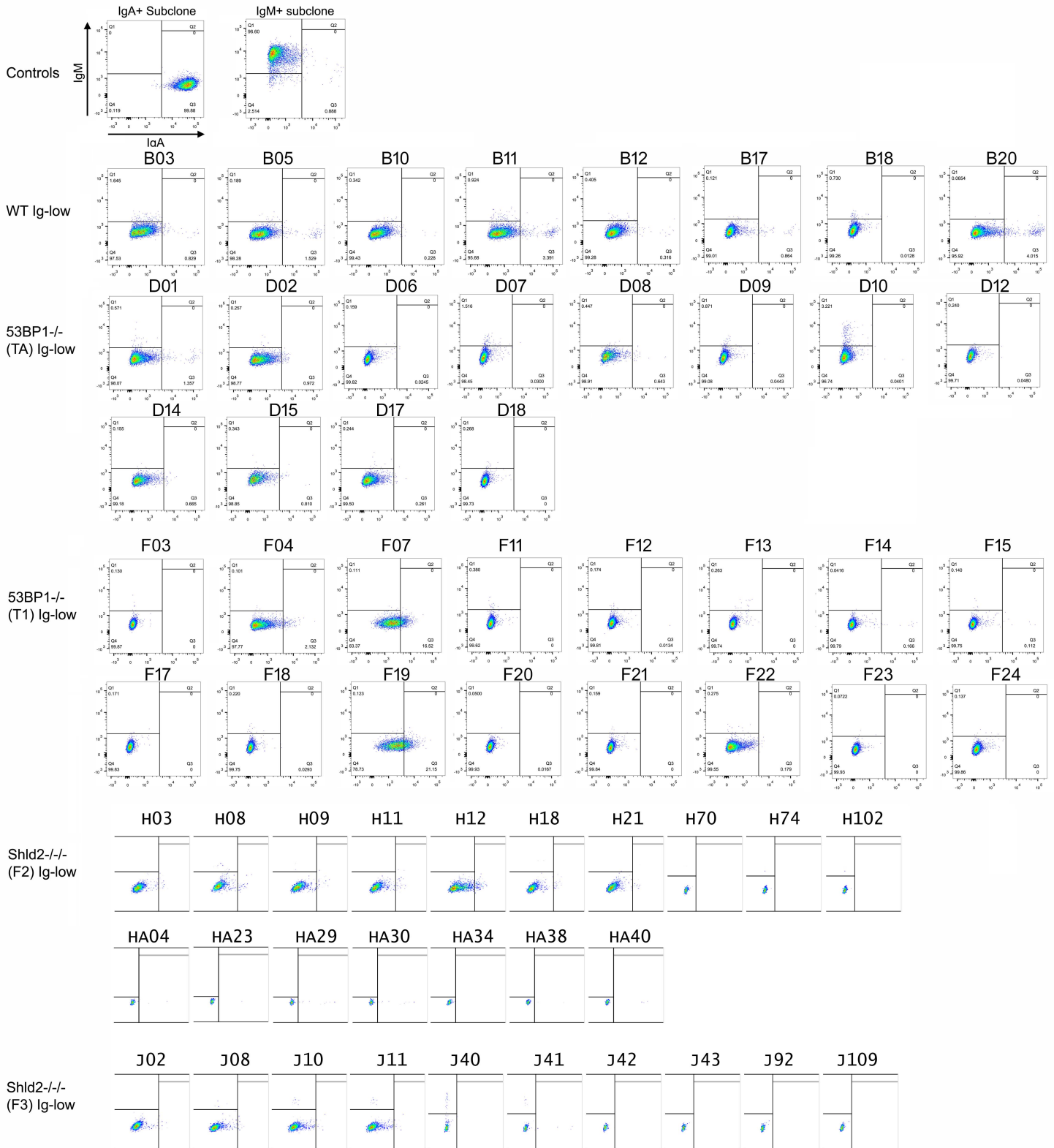
M A P P L L S L P L C I L P P G S G S P R L V C Y C E R D S G G D G D R D D F N L
WT ATGGCTCCTCCGTTGTTGCTGCTCCGCTTTGTTATTCTGCCCGGGTTTCGGGCTCCCCCGCCTGGTGTGC TACTGCGAGCGGGATAGTGGTGGAGACGGGGACCGGACGACTTCAACCTC
Allele 2 ATGGCTCCTCCGTTGTTGCTGCTCCGCTTTGTTATTCTGCCCGGGTTTCGGGCTCCCCCGCCTGGTGTGC AACAGGGGACGGCA-ACTGGTGGAGACGGGGACCGGACGACTTCAACCTC

Paxx #2 (275/14)

1
WT M A P P L L S L P L C I L P P G S G S P R L V C Y C E R D S G G D G D R D D F N L
ATGGCTCCTCCGTTGTTGCTGCTCCGCTTTGTTATTCTGCCCGGGTTTCGGGCTCCCCCGCCTGGTGTGC TACTGCGAGCGGGATAGTGGTGGAGACGGGGACCGGACGACTTCAACCTC
Allele 1 ATGGCTCCTCCGTTGTTGCTGCTCCGCTTTGTTATTCTGCCCGGGTTTCGGGCTCCCCCGCCTGGTGTGC TACTGCGAGCGGGATAGTGGTGGAGACGGGGACCGGACGACTTCAACCTC

M A P P L L S L P L C I L P P G S G S P R L V C Y C E R D S G G D G D R D D F N L
WT ATGGCTCCTCCGTTGTTGCTGCTCCGCTTTGTTATTCTGCCCGGGTTTCGGGCTCCCCCGCCTGGTGTGC TACTGCGAGCGGGATAGTGGTGGAGACGGGGACCGGACGACTTCAACCTC
Allele 2 ATGGCTCCTCCGTTGTTGCTGCTCCGCTTTGTTATTCTGCCCGGGTTTCGGGCTCCCCCGCCTGGTGTGC TACTGCGAGCGGGATAGTGGTGGAGACGGGGACCGGACGACTTCAACCTC

Appendix Figure S1. Genotypes of novel mutant CH12 cells generated for this study. WT CH12 cells were used to knockout the indicated genes through CRISPR/Cas9 using the sgRNAs listed in Table EV2. Sequencing results for each of these mutants are shown.



Appendix Figure S2. Flow Cytometry analysis of IgM WT, 53bp1^{-/-} and Shld2^{-/-} CH12 subclones. Ig-lo cells from WT, 53bp1^{-/-} and Shld2^{-/-} CH12 cells were sorted and subcloned and reanalyzed for expression of IgM and IgA by flow cytometry. As positive controls, expression of IgA and IgM are shown for two specific subclones that are IgA or IgM positive.