Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/5/8/eaaw1822/DC1

Supplementary Materials for

Functional diversification of hybridoma-produced antibodies by CRISPR/HDR genomic engineering

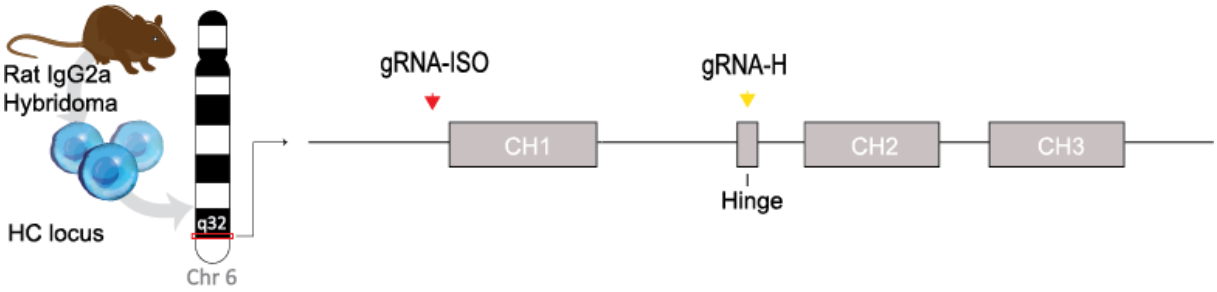
Johan M. S. van der Schoot, Felix L. Fennemann, Michael Valente, Yusuf Dolen, Iris M. Hagemans, Anouk M. D. Becker, Camille M. Le Gall, Duco van Dalen, Alper Cevirgel, Jaco A. C. van Bruggen, Melanie Engelfriet, Tomislav Caval, Arthur E. H. Bentlage, Marieke F. Fransen, Maaike Nederend, Jeanette H. W. Leusen, Albert J. R. Heck, Gestur Vidarsson, Carl G. Figdor, Martijn Verdoes*, Ferenc A. Scheeren*

*Corresponding author. Email: martijn.verdoes@radboudumc.nl (M.Ve.); f.a.scheeren@lumc.nl (F.A.S.)

Published 28 August 2019, *Sci. Adv.* **5**, eaaw1822 (2019)
DOI: 10.1126/sciadv.aaw1822

This PDF file includes:

A



B

Rattus norvegicus: Rn Celera
Chr.6 (AC_0000741)
129,955,150 - 129,957,604

AGAAAGATCTGAGTAGAACCAAGGTAAAAAGTGTGGGTAAAAACACATGTTCACAGGCCTGGCTGACATGATGCTGGGCACGTATGGAGGCAAA
GTCAAGAGGGCAGTGTAAGGGCCAGAAGTGAATCCTGACCCAAGAATAGAGAGTGCTAAACCTACGTAGATCGAAGCCAACTAAAAAGACAAGC
TACAAAACGAAGCTAAGGCCAGAGATCTTGGACTGTGAAGAGTTCAGAGAACCTAGGATCAGGAACCATTAGTAACAGGCCAAGGAAGATAGAA
GCTGCCTAGGACTTGGCAAGAGCCAACATGGTTGGACTGGAAAAGAAAGGAGGAGACAGAAGACAGGAGAGATGTGCCAACTTGATTTTGGGCT
TCACTGTTGTCCATACTGTGTGCAGCCATATGGCCCACAGATAACAGGTTTAGCCGAGGAACACAGATACCCACATTGGACAATGGTGGGGGAA
CACAGATACCCATACTACAGGGCTCTTTAGGGCATTTCCTGAAAGTGTACTAGGAGTGGGACTGGGCTCAAAGGGATTAGGTGTGATCTGGCCT
GGTGAGGCTGACATTGGCAAGCCCAATGGTTGGGTGTTGCCTCCTCCATGTAGACAGCCACAGACTTGGGGAGGGTACAAAATGGAGGACTTGT
AGGAGCTTGGGTCCAGACCTGTCAGACAAAATGATCACGCATACTTATTCTTGTAGCTGAAACAACAGCCCCATCTGTCTATCCACTGGCTCCT
GGAACTGCTCTCAAAAGTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTCACCGTGACCTGGAACTCTGGAG
CCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGGACTCTACACTCTCACCAGCTCAGTGACTGTACCCTCCAGCACCTGGTC
CAGCCAGGCCGTCACCTGCAACGTAGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGGTGAGAGAACAACCAGGGGATGAGGGGCT
CACTAGAGGTGAGGATAAGGCATTAGATTGCCTACACCAACCAGGGTGGGCAGACATCACCAGGGAGGGGGCCTCAGCCCAGGAGACCAAAAAT
TCTCCTTTGTCTCCCTTCTGGAGATTTCTATGTCCTTTACACCCATTTATTAATATTCTGGGTAAGATGCCCTTGCATCATGACATACAGAGGC
AGACTAGAGTATCAACCTGCAAAAGGTCATACCCAGGAAGAGCCTGCCATGATCCCACACCAGAACCAACCTGGGGCCTTCTCACCTATAGACC
ATACTAACACACAGCCTTCTCTCTGCAGTGCCAAGGGAATGCAATCCTTGTGGATGTACAGGTAAGTCACTAGGACTATTACTCCAGCCCCAGA
TTCAAAAAATATCCTCAGAGGCCCATGTTAGAGGATGACACAGCTATTGACCTATTTCTACCTTTCTTCTTCATCTACAGGCTCAGAAGTATCA
TCTGTCTTCATCTTCCCCCCAAAGACCAAAGATGTGCTCACCATCACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATTAGCCAGAATG
ATCCCGAGGTCCGGTTCAGCTGGTTTATAGATGACGTGGAAGTCCACACAGCTCAGACTCATGCCCCGGAGAAGCAGTCCAACAGCACTTTACG
CTCAGTCAGTGAACTCCCCATCGTGCACCGGGACTGGCTCAATGGCAAGACGTTCAAATGCAAAGTCAACAGTGGAGCATTCCCTGCCCCCATC
GAGAAAAGCATCTCCAAACCCGAAGGTGGGAGCAGCAGGGTGTGTGGTGTAGAAGCTGCAGTAGGCCATAGACAGAGCTTGACTTAACTAGACT
TCTGCCCTCTTACTGACCTCCATGCTGACCACTCTCTGTATCCACAGGCACACCACGAGGTCCACAGGTATACACCATGGCGCCTCCCAAGGAA
GAGATGACCCAGAGTCAAGTCAGTATCACCTGCATGGTAAAAGGCTTCTATCCCCCAGACATTTATACGGAGTGGAAGATGAACGGGCAGCCAC
AGGAAAACTACAAGAACACTCCACCTACGATGGACACAGATGGGAGTTACTTCCTCTACAGCAAGCTCAATGTAAAGAAAGAAACATGGCAGCA
GGGAAACACTTTCACGTGTTCTGTGCTGCATGAGGGCCTGCACAACCACCATACTGAGAAGAGTCTCTCCCACTCTCCTGGTAAATGATCCCAG
AGTCCAGTGGCCCCTCTTGGCCTAAAGGATGCCAACACCTACCTCTACCACCTTTCTCTGTGTAAATAAAGCACCCAGCTCTGCCTTGGGACCC
TGCAAAAATGTCCTGGTTCTTTCTGAGATACAGAGTCCAGTGAGGTCATGGGCTGAGGGGCATCCAGGGTTTGAGGCCTGAGGTTTGACTAAGG
AAAAAGGGTGG

Fig. S1. Genomic map and annotated base pair sequence of rIgG2a constant domains. The genomic location (**A**) and annotated basepair sequence (**B**) and of the IgH locus of rat IgG2a located on chromosome 6 (Rn celera, AC_0000741) are given. The exons of CH1, Hinge, CH2 and CH3 are indicated (grey highlight) with splice acceptor and donor sites (underlined, cursive). The targeted protospacer adjacent motifs (PAMs) for gRNA-H (yellow, underlined) and gRNA-ISO (red, underlined) are indicated.

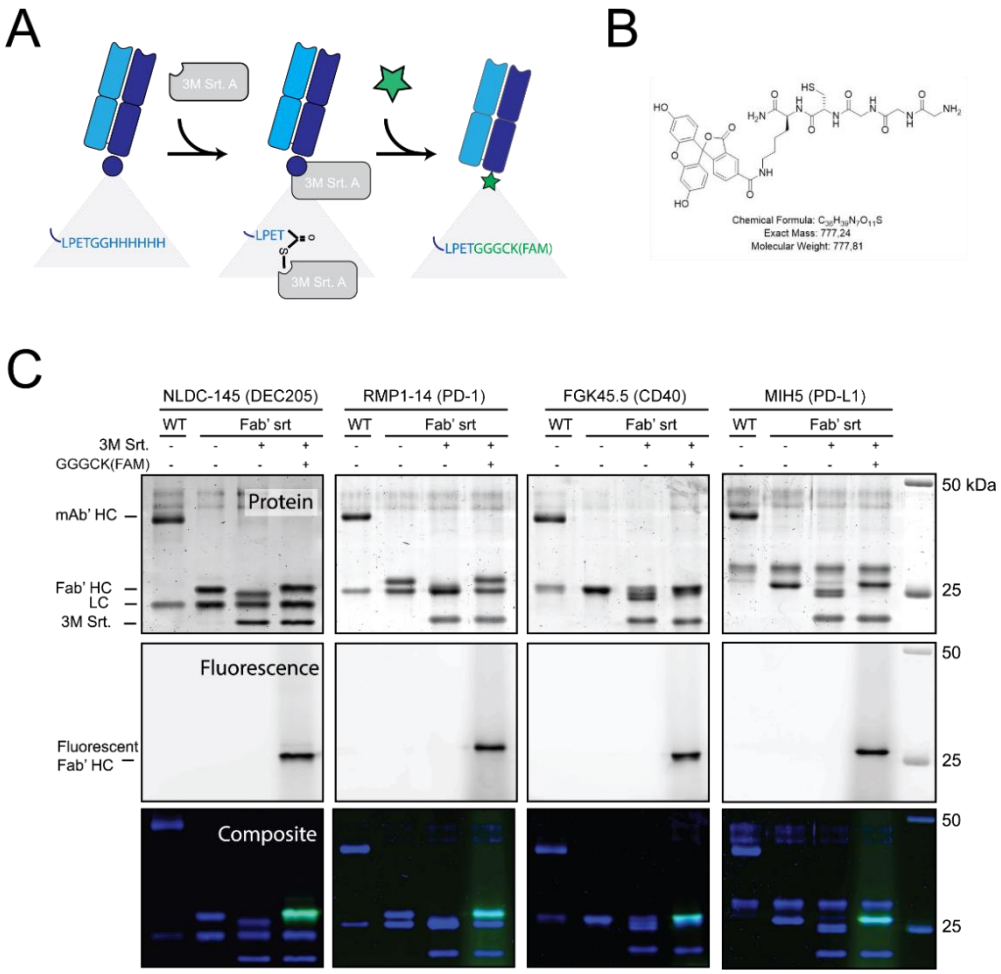


Fig. S2. Sortagging of Fab′ fragments derived from CRISPR/HDR-engineered hybridomas. The CRISPR/HDR strategy as outlined in Fig. 1 was also performed on hybridomas RMP1-14 (PD-1), FGK45.5 (CD40) and MIH5 (PD-L1). The resulting Fab' hybridomas all secreted the designed Fab' fragments which could be easily isolated from the supernatant, demonstrating universal applicability of this approach. (**A**) All Fab' fragments were equipped with a c-terminal sortag motif to perform sortase mediated ligation. (**B**) We synthesized the fluorophore GGGCK(FAM) to functionalize the c-terminus of the Fab' fragment heavy chain. (**C**) To this purpose, we incubated 5µg of Fab' fragment (10µM) with equimolar amounts of an evolved sortase (3M Srt., 10µM) and with or without 50 molar equivalents of GGGCK(FAM) for one hour at 37ºC. From each reaction mix we loaded 5µg on reducing SDS-page together with the parental mAb and unmodified Fab and acquired the protein and fluorescent scans. The left panel displays the protein and fluorescent scans of Fab'DEC205-srt, of which the composite is shown in Fig. 1G.

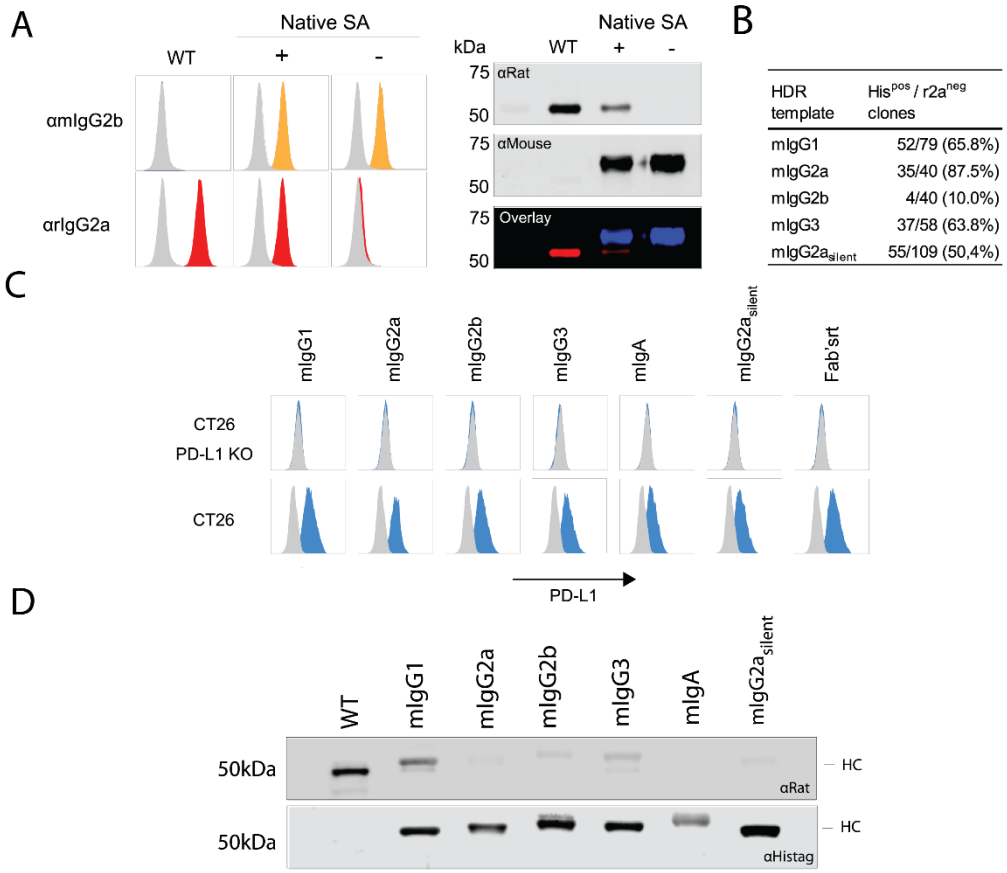


Fig. S3. Characterization of MIH5 Fc variants hybridomas. (**A**) Presence of the original splice acceptor adjacent to the rat CH1 exon in the donor construct results in background secretion of the native rIgG2a MIH5 in the supernatant. In the supernatant of engineered hybridomas where the splice acceptor is removed, production of the native isotype is abrogated as determined by flow cytometry (left panels) and western blot (right panels). The data shown is from two MIH5 mIgG2b clones, which were generated using donor constructs with (+) or without (-) the native splice acceptor in the 3' homology arm of the donor construct. (**B**) Table displaying the number of clones after CRISPR/HDR employing HDR templates in which the native splice acceptor has been removed for all murine isotypes, followed by selection, that secrete the designed MIH5 isotype variant ($His^{pos}/r2a^{neg}$). Clones were analyzed by flow cytometry with secondary antibodies against his-tag and rat IgG2a isotype on CT26 stained with supernatant from monoclonal cell suspensions. (**C**) Flow cytometry plots of CT26 and $CT26^{PDL1\ KO}$ stained with MIH5 Fc variants in combination with a secondary antibody against his-tag, indicating that specificity for PD-L1 is retained. (**D**) Western blot analysis demonstrates substitution of the rat heavy chain for murine heavy chains.

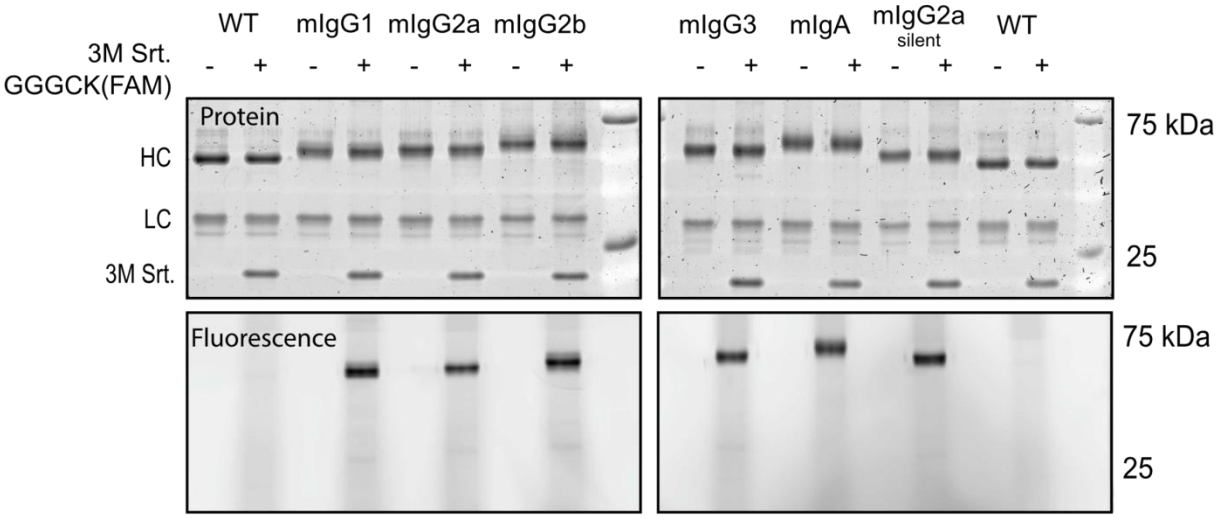


Fig. S4. Raw images of sortagging of MIH5 isotype variants. Raw images of protein and fluorescent SDS-page scans of sortagging of MIH5 isotype variants as displayed in Fig. 2E. Of each antibody 5µg was incubated with 1 molar equivalent of 3m Sortase (3m Srt) and 100 molar equivalent GGGCK(FAM) for one hour. From each reaction 500ng was run on reducing SDS-PAGE. Subsequently, protein and fluorescent images of the SDS-page were acquired.

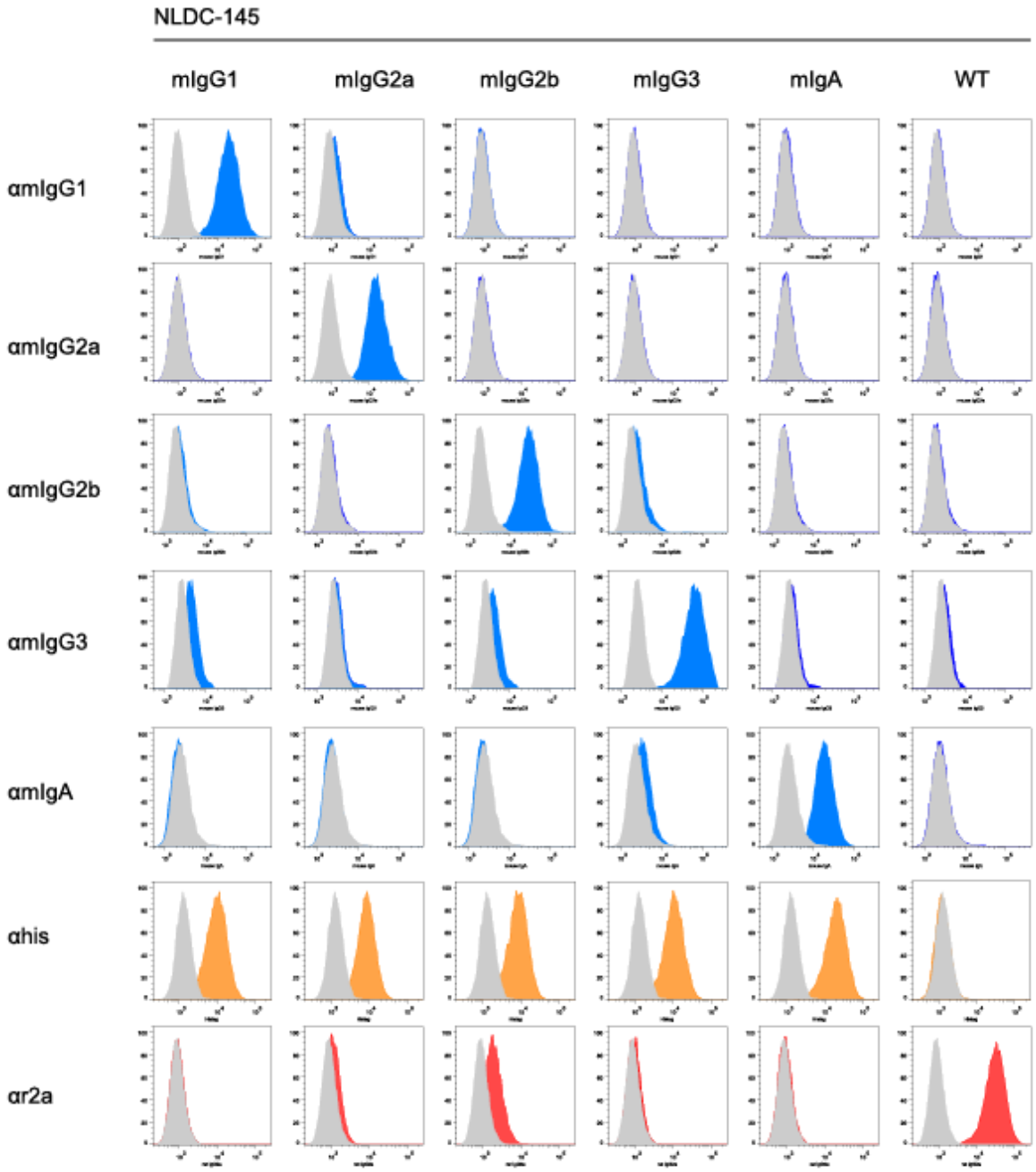


Fig. S5. Murine isotype panel generation of NLDC-145 via CRISPR/HDR. The CRISPR/HDR strategy as outlined in Fig. 2 was applied to the NLDC-145 to obtain recombinant hybridomas secreting murine IgG1, IgG2a, IgG2b, IgG3 and IgA. For each isotype, hybridoma supernatant was used to stain DEC205 expressing cell line JAWS II in combination with a panel of secondary antibodies against rat IgG2a, his-tag and murine isotypes.

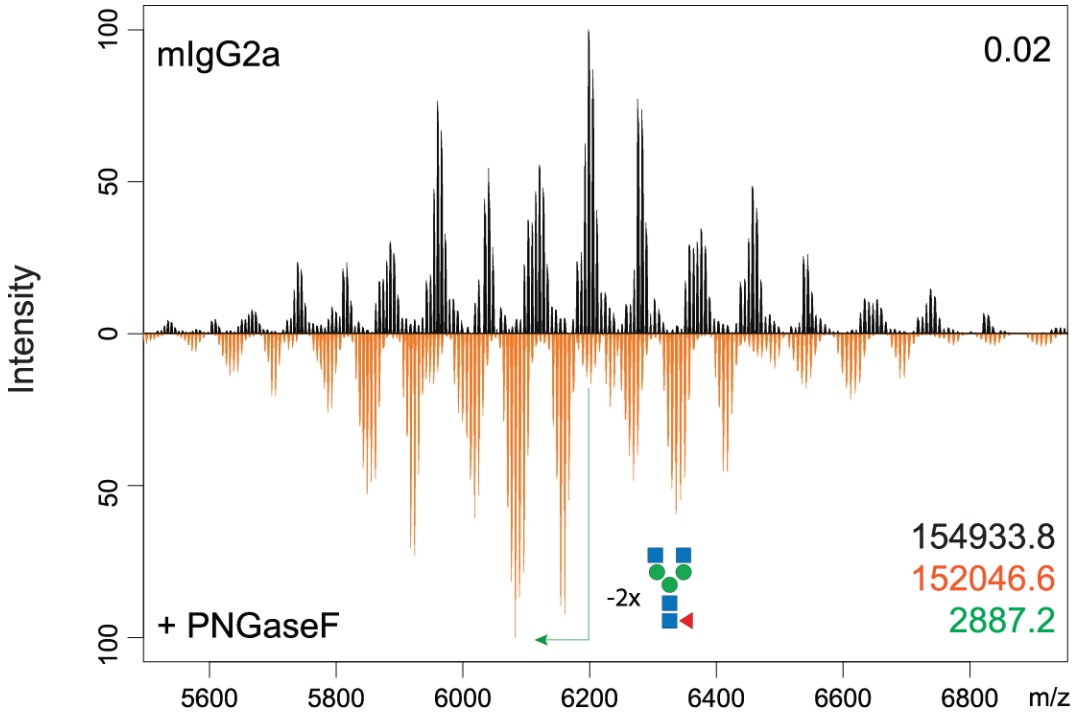


Fig. S6. Glycosylation profile of MIH5 mIgG2a via native mass spectrometry. Purified MIH5 mIgG2a was treated overnight with PNGAseF (orange) and compared to an untreated sample (black) via high resolution native mass spectrometry. The Pearson correlation coefficient between the two spectra over all ion signals is given in the upper right corner. The molecular mass belonging to the base peak of the untreated (black font) and treated (orange font) sample and the leading difference in mass (green) is depicted in the lower right corner. The difference in mass between treated and untreated samples indicates the loss of two glycans.

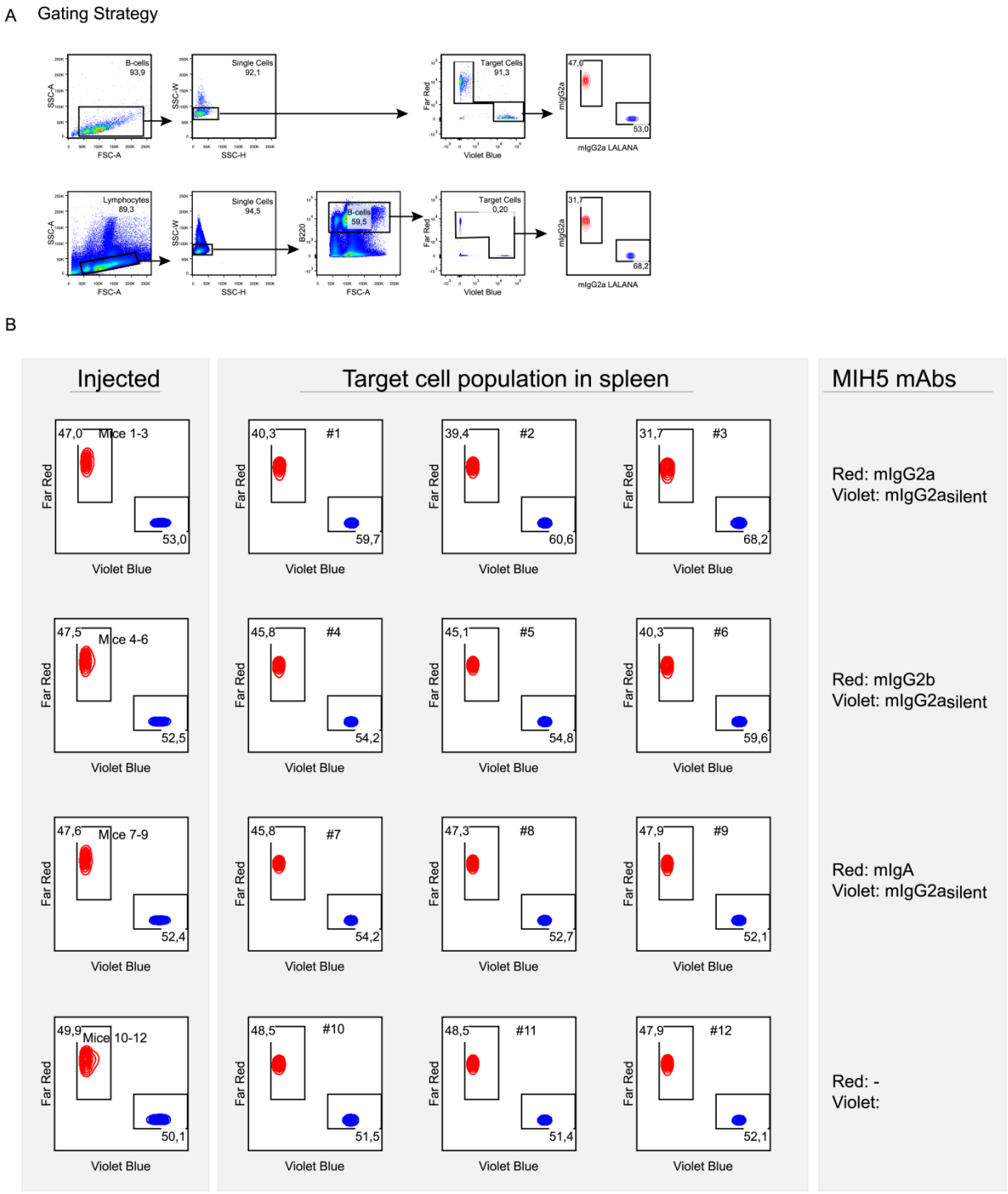


Fig. S7. Gating and FACS plots of isotype-dependent depletion in vivo. Isolated B-cells were stimulated overnight with IFN-y and labeled with either red or violet tracer dyes. Subsequently, the violet B-cells were opsonized with MIH5 mIgG2a$_{silent}$, while the red B-cells were opsonized with MIH5 mIgG2a, mIgG2b or mIgA. The populations were mixed and intravenously injected into LPS stimulated BL/6 mice. After 24 hours the mice were sacrificed and spleens isolated to determine the

ratio of violet blue and far red target cells. These were used to determine the isotype dependent target cell depletion. The gating strategy (**A**) and the plots (**B**) depicting the labeled B-cell ratios used for determing the specific target depletion in Fig 5C are given.

Table S1. Fab′ donor construct for HDR. Table displays sequences of each feature of the donor construct used to convert rat IgG2a hybridomas to sortagable Fab'fragment secreting cell lines.

Isotype	Sequence
5' HA	CCTGGAACTCTGGAGCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGGACTCTACACTCTCACCAGCTCAG TGACTGTACCCTCCAGCACCTGGTCCAGCCAGGCCGTCACCTGCAACGTAGCCCACCCGGCCAGCAGCACCAAGGTGGACAA GAAAATTGGTGAGAGAACAACCAGGGGATGAGGGGCTCACTAGAGGTGAGGATAAGGCATTAGATTGCCTACACCAACCAGGG TGGGCAGACATCACCAGGGAGGGGGCCTCAGCCCAGGAGACCAAAAATTCTCCTTTGTCTCCCTTCTGGAGATTTCTATGTCCT TTACACCCATTTATTAATATTCTGGGTAAGATGCCCTTGCATCATGACATACAGAGGCAGACTAGAGTATCAACCTGCAAAAGGTC ATACCCAGGAAGAGCCTGCCATGATCCCACACCAGAACCAACCTGGGGCCTTCTCACCTATAGACCATACTAACACACAGCCTT CTCTCTGCAGTGCCAAG
Sortag, his-tag, Stop	GGAAGGAGGCGGAGGCAGCCTGCCGGAAACCGGCGGCCATCATCATCATCATCATTGA
IRES	GGATCCCAATTGCTCGAGGCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCG TTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCAT TCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTG AAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCAC GTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCT CCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACA TGCTTTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGAT GATAATATGGCCACAGAATTCGCCACC
Bsr	TGGCCAAGCCTTTGTCTCAAGAAGAATCCACCCTCATTGAAAGAGCAACGGCTACAATCAACAGCATCCCCATCTCTGAAGACTA CAGCGTCGCCAGCGCAGCTCTCTCTAGCGACGGCCGCATCTTCACTGGTGTCAATGTATATCATTTTACTGGGGGACCTTGTGC AGAACTCGTGGTGCTGGGCACTGCTGCTGCTGCGGCAGCTGGCAACCTGACTTGTATCGTCGCGATCGGAAATGAGAACAGGG GCATCTTGAGCCCCTGCGGACGGTGCCGACAGGTGCTTCTCGATCTGCATCCTGGGATCAAAGCCATAGTGAAGGACAGTGAT GGACAGCCGACGGCAGTTGGGATTCGTGAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCTAAGTACTAGTCGA
SV40 polyA terminati on	GTACTAGTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCAC TCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGG CAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAGATCTTGTACA
3'HA	GGTAAGTCACTAGGACTATTACTCCAGCCCCAGATTCAAAAAATATCCTCAGAGGCCCATGTTAGAGGATGACACAGCTATTGAC CTATTTCTACCTTTCTTCTTCATCTACAGGCTCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGACCAAAGATGTGCTCACCAT CACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATTAGCCAGAATGATCCCGAGGTCCGGTTCAGCTGGTTTATAGATGA CGTGGAAGTCCACACAGCTCAGACTCATGCCCCGGAGAAGCAGTCCAACAGCACTTTACGCTCAGTCAGTGAACTCCCCATCGT GCACCGGGACTGGCTCAATGGCAAGACGTTCAAATGCAAAGTCAACAGTGGAGCATTCCCTGCCCCCATCGAGAAAAGCATCTC CAAACCCGAAGGTGGGAGCAGCAGGGTGTGTGGTGTAGAAGCTGCAGTAGGCCATAGACAGAGCTTGACTTAACTAGACTT

Table S2. Isotype donor constructs for HDR. Table displays sequences of each feature of donor constructs used to change the isotype of rat IgG2a hybridomas. The 5' HA, IRES-*Bsr*-PolyA and 3' HA are constant in each HDR construct, while the isotype features are interchangeable between HDR constructs. The native splice acceptor adjacent to the rat IgG2 CH1 underlined in 3' HA and removed in certain donor constructs.

	Sequence
5' HA	AGAAAGATCTGAGTAGAACCAAGGTAAAAAGTGTGGGTAAAAACACATGTTCACAGGCCTGGCTGACATGATGCTGGGCACGTATGGAGGCAAAGTCAAGAGGGCAGTGTAAG GGCCAGAAGTGAATCCTGACCCAAGAATAGAGAGTGCTAAACCTACGTAGATCGAAGCCAACTAAAAAGACAAGCTACAAAACGAAGCTAAGGCCAGAGATCTTGGACTGTGAA GAGTTCAGAGAACCTAGGATCAGGAACCATTAGTAACAGGCCAAGGAAGATAGAAGCTGCCTAGGACTTGGCAAGAGCCAACATGGTTGGACTGGAAAAGAAAGGAGGAGACA GAAGACAGGAGAGATGTGCCAACTTGATTTTGGGCTTCACTGTTGTCCATACTGTGTGCAGCCATATGGCCCACAGATAACAGGTTTAGCCGAGGAACACAGATACCCACATTG GACAATGGTGGGGGAACACAGATACCCATACTACAGGGCTCTTTAGGGCATTTCCTGAAAGTGTACTAGGAGTGGGACTGGGCTCAAAGGGATTAGGTGTGATCTGGCCTGGT GAGGCTGACATTGGCAAGCCCAATGGTTGGGTGTTGCCTCCTCCATGT
mIgG1	GCTAGCGATCGCAGGCGCAATCTTCGCATTTCTTTTTTCCAGCAAAGACCACACCACCTTCTGTGTACCCACTCGCACCAGGCAGCGCCGCTCAAACCAACAGTATGGTGACCT TGGGGTGTCTTGTGAAGGGCTACTTTCCCGAGCCCGTTACCGTCACCTGGAACTCCGGGTCTCTCTCAAGCGGCGTTCACACCTTCCCCGCCGTACTGCAATCAGACCTCTAT ACCCTGTCTTCCAGCGTCACTGTACCCAGTTCCACCTGGCCCAGTGAAACTGTCACATGTAATGTCGCACACCCAGCTTCCTCCACCAAAGTGGACAAAAAGATCGTGCCTAGG GACTGTGGATGCAAGCCATGTATCTGCACTGTCCCCGAGGTTTCAAGTGTATTTATCTTCCCCCAAAGCCCAAAGATGTCCTCACCATAACACTCACACCCAAGGTGACCTGC GTGGTGGTCGATATCAGTAAAGATGACCCCGAGGTCCAATTCAGCTGGTTTGTGGATGATGTAGAGGTCCACACTGCTCAGACTCAGCCACGGGAAGAGCAGTTTAACTCAAC ATTTCGGAGTGTGTCCGAACTGCCAATCATGCACCAAGATTGGCTCAATGGGAAAGAATTCAAATGCAGGGTGAATAGTGCCGCCTTTCCTGCACCAATAGAGAAAACCATATC AAAGACAAAGGGCAGGCCCAAGGCCCCTCAAGTCTATACTATACCTCCACCCAAGGAGCAAATGGCTAAAGATAAGGTAAGCCTCACCTGCATGATCACAGATTTTTTCCCAGA AGACATAACTGTCGAATGGCAATGGAACGGCCAACCTGCAGAAAATTACAAGAATACTCAGCCTATTATGGACACCGATGGCAGCTACTTCGTGTATTCAAAGATTGAACGTACA GAAATCTAATTGGGAAGCAGGGAATACATTCACATGCTCTGTGTTGCATGAAGGGCTCCATAACCACCACACTGAAAAGAGCTTGAGCCACTCTCCCGGAAAGCGTACGGGAG GCGGAGGCAGCCTGCCGGAAACCGGCGGCCATCATCATCATCATTGA
mIgG2a	GCTAGCGATCGCAGGCGCAATCTTCGCATTTCTTTTTTCCAGCTAAGACAACAGCCCCATCTGTCTATCCACTGGCTCCTGTATGTGGTGACACAACTGGCTCCTCGGTGACCC TGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTCACCTTGACCTGGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGATCTCTAC ACTCTCAGTTCCTCAGTGACTGTAACTTCCAGCACCTGGCCCAGCCAGAGCATAACCTGCAACGTAGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGAGCCAAG GGGACCCACTATTAAGCCATGTCCACCATGCAAATGCCCAGCACCAAATCTCCTCGGTGGTCCTTCTGTCTTCATCTTCCCCCCAAAGATCAAAGATGTGCTCATGATCTCTTTG AGTCCTATTGTCACGTGTGTTGTGGTAGACGTTAGCGAGGATGATCCCGACGTCCAGATCAGCTGGTTTGTAAATAACGTGGAAGTCCACACAGCTCAGACTCAGACCCACCG GGAGGACTACAACAGCACTTTAAGGGTAGTCTCTGCACTCCCCATCCAGCACCAGGACTGGATGTCTGGCAAGGAGTTCAAATGCAAAGTCAACAACAAGGATTTACCTGCCC CCATCGAGCGAACCATCTCCAAACCCAAAGGCAGTGTACGAGCCCCACAGGTATACGTGCTGCCCCCTCCCGAGGAAGAGATGACCAAGAAGCAAGTCACTCTCACCTGCATG GTAACAGATTTCATGCCCGAAGACATTTATGTGGAGTGGACGAACAACGGGAAGACCGAGCTAAACTACAAGAACACTGAACCTGTGTTGGACTCAGATGGGAGTTACTTCATG TACTCCAAGCTCCGTGTAGAGAAAAAGAACTGGGTTGAGAGAAACAGCTACTCGTGTAGCGTGGTGCATGAGGGCCTGCACAACCACCATACTACGAAGTCTTTCTCCCGGAC ACCTGGTAAACGTACGGGAGGCGGAGGCAGCCTGCCGGAAACCGGCGGCCATCACCATCATCACCATTGA
mIgG2b	GCTAGCGATCGCAGGCGCAATCTTCGCATTTCTTTTTTCCAGCTAAAACAACACCCCCATCAGTCTATCCACTGGCCCCTGGGTGTGGAGATACAACTGGTTCCTCTGTGACTC TGGGATGCCTGGTCAAGGGCTACTTCCCTGAGTCAGTGACTGTGACTTGGAACTCTGGATCCCTGTCCAGCAGTGTGCACACCTTCCCAGCTCTCCTGCAGTCTGGACTCTAC ACTATGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCAAGTCAGACCGTCACCTGCAGCGTTGCTCACCCAGCCAGCAGCACCACGGTGGACAAAAAACTTGAGCCCA GCGGGCCCATTTCAACAATCAACCCCTGTCCTCCATGCAAGGAGTGTCACAAATGCCCAGCTCCTAACCTGGAGGGTGGACCATCCGTCTTCATCTTCCCTCCAAATATCAAGG ATGTACTCATGATCTCCCTGACACCCAAGGTCACGTGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGACGTCCGGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACA GCTCAGACACAAACCCATAGAGAGGATTACAACAGTACTATCCGGGTGGTCAGTGCCCTCCCCATCCAGGACCAGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAA CAACAAAGACCTCCCATCACCCATCGAGAGAACCATCTCAAAAATTAAAGGGCTAGTCAGAGCTCCACAAGTATACATCTTGCCGCCACCAGCAGAGCAGTTGTCCAGGAAAGA TGTCAGTCTCACTTGCCTGGTCGTGGGCTTCAACCCTGGAGACATCAGTGTGGAGTGGACCAGCAATGGGCAATCACAAGGACACCGCACCAGTCCTGGACT CTGACGGTTCTTACTTCATATACAGCAAGCTCGATATAAAAACAAGCAAGTGGGAGAAAACAGATTCCTTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAA GAAGACCATCTCCCGGTCTCCGGGTAAACGTACGGGAGGCGGAGGCAGCCTGCCGGAAACCGGCGGCCATCATCATCATCATCATTGA
mIgG3	GCTAGCGATCGCAGGCGCAATCTTCGCATTTCTTTTTTCCAGCTACAACAACAGCCCCATCTGTCTATCCCTTGGTCCCTGGCTGCAGTGACACATCTGGATCCTCGGTGACAC TGGGATGCCTTGTCAAAGGCTACTTCCCTGAGCCGGTAACTGTAAAATGGAACTATGGAGCCCTGTCCAGCGGTGTGCGCACAGTCTCATCTGTCCTGCAGTCTGGGTTCTATT CCCTCAGCAGCTTGGTGACTGTACCCTCCAGCACCTGGCCCAGCCAGACTGTCATCTGCAACGTAGCCCACCCAGCCAGCAAGACTGAGTTGATCAAGAGAATCGAGCCTAGA ATACCCAAGCCCAGTACCCCCCCAGGTTCTTCATGCCCACCTGGTAACATCTTGGGTGGACCATCCGTCTTCATCTTCCCCCCAAAGCCCAAGGATGCACTCATGATCTCCCTA ACCCCCAAGGTTACGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCATGTCAGCTGGTTTGTGGACAACAAAGAAGTACACACAGCCTGGACACAGCCCCGTG AAGCTCAGTACAACAGTACCTTCCGAGTGGTCAGTGCCCTCCCCATCCAGCACCAGGACTGGATGAGGGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGCCCTCCCAGCC CCCATCGAGAGAACCATCTCAAAACCCAAAGGAAGAGCCCAGACACCTCAAGTATACACCATACCCCCACCCCGTGAACAAATGTCCAAGAAGAAGGTTAGTCTGACCTGCCT GGTCACCAACTTCTTCTCTGAAGCCATCAGTGTGGAGTGGGAAAGGAACGGAGAACTGGAGCAGGATTACAAGAACACTCCACCCATCCTGGACTCAGATGGGACCTACTTCC TCTACAGCAAGCTCACTGTGGATACAGACAGTTGGTTGCAAGGAGAAATTTTTACCTGCTCCGTGGTGCATGAGGCTCTCCATAACCACCACACACAGAAGAACCTGTCTCGCT CCCCTGGTAAACGTACGGGAGGCGGAGGCAGCCTGCCGGAAACCGGCGGCCATCATCATCATCATCATTGA
mIgA	GCTAGCGATCGCAGGCGCAATCTTCGCATTTCTTTTTTCCAGGCTCAGAGTCTGCGAGAAATCCCACCATCTACCCACTGACACTCCCACCAGCTCTGTCAAGTGACCCAGTGA TAATCGGCTGCCTGATTCACGATTACTTCCCTTCCGGCACGATGAATGTGACCTGGGGAAAGAGTGGGAAGGATATAACCACCGTAAACTTCCCACCTGCCCTGGCCTCTGGG GGACGGTACACCATGAGCAGCCAGTTGACCCTGCCAGCTGTCGAGTGCCCAGAAGGAGAATCCGTGAAATGTTCCGTGCAACATGACTCTAACCCCGTCCAAGAATTGGATGT GAATTGCTCTGGTCCTACTCCTCCTCCTCCTATTACTATTCCTTCCTGCCAGCCCAGCCTGTCACTGCAGCGGCCAGCTCTTGAGGACCTGCTCCTGGGTTCAGATGCCAGCAT CACATGTACTCTGAATGGCCTGAGAAATCCTGAGGGAGCTGTCTTCACCTGGGAGCCCTCCACTGGGAAGGATGCAGTGCAGAAGAAAGCTGTGCAGAATTCCTGCGGCTGCT ACAGTGTGTCCAGCGTCCTGCCTGGCTGTGCTGAGCGCTGGAACAGTGGCGCATCATTCAAGTGCACAGTTACCCATCCTGAGTCTGGCACCTTAACTGGCACAATTGCCAAA GTCACAGTGAACACCTTCCCACCCCAGGTCCACCTGCTACCGCCGCCGTCGGAGGAGCTGGCCCTGAATGAGCTCTTGTCCCTGACATGCCTGGTGCGAGCTTTCAACCCTAA AGAAGTGCTGGTGCGATGGCTGCATGGAAATGAGGAGCTGTCCCCAGAAAGCTACCTAGTGTTTGAGCCCCTAAAGGAGCCAGGCGAGGGAGCCACCACCTACCTGGTGACA AGCGTGTTGCGTGTATCAGCTGAAACCTGGAAACAGGGTGACCAGTACTCCTGCATGGTGGGCCACGAGGCCTTGCCCATGAACTTCACCCAGAAGACCATCGACCGTCTGTC GGGTAAACCCACCAATGTCAGCGTGTCTGTGATCATGTCAGAGGGAGATGGCATCTGCTACCGTACGGGAGGCGGAGGCAGCCTGCCGGAAACCGGCGGCCATCATCATCAT CATCATTGA
mIgG2a silent	GCTAGCGATCGCAGGCGCAATCTTCGCATTTCTTTTTTCCAGCTAAGACAACAGCCCCATCTGTCTATCCACTGGCTCCTGTATGTGGTGACACAACTGGCTCCTCGGTGACCC TGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTCACCTTGACCTGGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGATCTCTAC ACTCTCAGTTCCTCAGTGACTGTAACTTCCAGCACCTGGCCCAGCCAGAGCATAACCTGCAACGTAGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGAGCCAAG GGGACCCACTATTAAGCCATGTCCACCATGCAAATGCCCAGCACCAAATGCCGCCGGTGGTCCTTCTGTCTTCATCTTCCCCCCAAAGATCAAAGATGTGCTCATGATCTCTTT GAGTCCTATTGTCACGTGTGTTGTGGTAGACGTTAGCGAGGATGATCCCGACGTCCAGATCAGCTGGTTTGTAAATAACGTGGAAGTCCACACAGCTCAGACTCAGACCCACC GGGAGGACTACGCCAGCACTTTAAGGGTAGTCTCTGCACTCCCCATCCAGCACCAGGACTGGATGTCTGGCAAGGAGTTCAAATGCAAAGTCAACAACAAGGATTTACCTGCC CCCATCGAGCGAACCATCTCCAAACCCAAAGGCAGTGTACGAGCCCCACAGGTATACGTGCTGCCCCCTCCCGAGGAAGAGATGACCAAGAAGCAAGTCACTCTCACCTGCAT GGTAACAGATTTCATGCCCGAAGACATTTATGTGGAGTGGACGAACAACGGGAAGACCGAGCTAAACTACAAGAACACTGAACCTGTGTTGGACTCAGATGGGAGTTACTTCAT GTACTCCAAGCTCCGTGTAGAGAAAAAGAACTGGGTTGAGAGAAACAGCTACTCGTGTAGCGTGGTGCATGAGGGCCTGCACAACCACCATACTACGAAGTCTTTCTCCCGGA CACCTGGTAAACGTACGGGAGGCGGAGGCAGCCTGCCGGAAACCGGCGGCCATCACCATCATTGA
IRES *Bsr* polyA	GTCGACGTCGAGGCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTT TTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCA GTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGA TACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGA AGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTC CTTTGAAAAACACGATGATAATATGGCCACAGAATTCGCCACCATGGCCAAGCCTTTGTCTCAAGAAGAATCCACCCTCATTGAAAGAGCAACGGCTACAATCAACAGCATCCC CATCTCTGAAGACTACAGCGTCGCCAGCGCAGCTCTCTCTAGCGACGGCCGCATCTTCACTGGTGTCAATGTATATCATTTTACTGGGGGACCTTGTGCAGAACTCGTGGTGCT GGGCACTGCTGCTGCTGCGGCAGCTGGCAACCTGACTTGTATCGTCGCGATCGGAAATGAGAACAGGGGCATCTTGAGCCCCTGCGGACGGTGCCGACAGGTGCTTCTCGAT CTGCATCCTGGGATCAAAGCCATAGTGAAGGACAGTGATGGACAGCCGACGGCAGTTGGGATTCGTGAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCTAAGTACTAGTCGA GTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGC ATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG AGATCTTGT
3' HA	TGTACAACTTGGGGAGGGTACAAAATGGAGGACTTGTAGGAGCTTGGGTCCAGACCTGTCAGACAAAATGATCACGCATACTTATTCTTGT***AGC***TGAAACAACAGCCCCATCTG TCTATCCACTGGCTCCTGGAACTGCTCTCAAAAGTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTCACCGTGACCTGGAACTCTGGAGCC CTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGGACTCTACACTCTCACCAGCTCAGTGACTGTACCCTCCAGCACCTGGTCCAGCCAGGCCGTCACCTGCAA CGTAGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGGTGAGAAGAACAACCAGGGGATGAGGGGCTCACTAGAGGTGAAGGCATTAGATTGCCTCACACCA ACCAGGGTGGGCAGACATCACCAGGGAGGGGGCCTCAGCCCAGGAGACCAAAAATTCTCCTTTGTCTCCCTTCTGGAGATTTCTATGTCCTTTACACCCATTTATTAATATTCT

Table S3. FcγR affinity values of MIH5 Fc variants and comparison to literature. Affinity quantification by K_D (μM) of CRISPR/HDR antibodies for mFcγRI, FcγRIIb and mFcγRIV (bold) comparison to described values in literature.

Fc variant	**mFcγRI**	**mFcγRIIb**	**mFcγRIV**
mIgG1	**-/-** -/- (*28–32*)	**0.26 ± 0.05** 0.15 (*28*); 0.30 (*32*); 0.83 (*29*); 0.17 (*30*);	**-/-** -/- (*28–32*)
mIgG2a	**0.017 ± 0.007** 0.012 (*28*); 0.006 (*32*) 0.026 (*29*); 0.018 (*30*); 0.033 (*31*); 0.013 - 0.022 (*33*)	**0.66 ± 0.20** 0.69 (*28*); 2.4 (*32*); 1.8 (*29*)	**0.13 ± 0.02** 0.060(*28*); 0.035(*32*); 0.071(*29*); 0.010(*30*)
mIgG2b	**-/-** -/- (*28*, *29*, *33*); 0.021 (*33*)	**1.24 ± 0.06** 0.83 (*28*); 0.45 (*32*); 0.91 (*29*);	**0.20 ± 0.02** 0.12 (*28*); 0.059 (*32*); 0.063 (*29*);
mIgA	**-/-** -/- (*31*)	**-/-** -/- (*31*)	**-/-** -/- (*31*)
mIgG2a$_{silent}$	**-/-** -/- (*26*)	**-/-** -/- (*26*)	**-/-** -/- (*26*)