

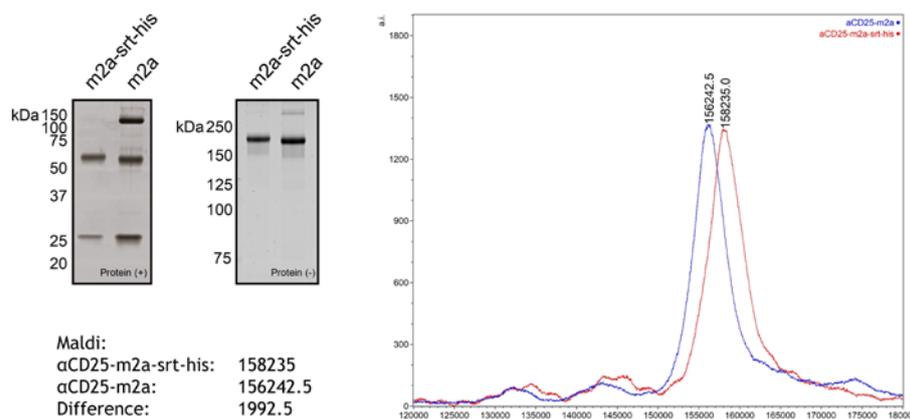
Rattus norvegicus: Rn Celera

Chr.6 (AC_0000741)

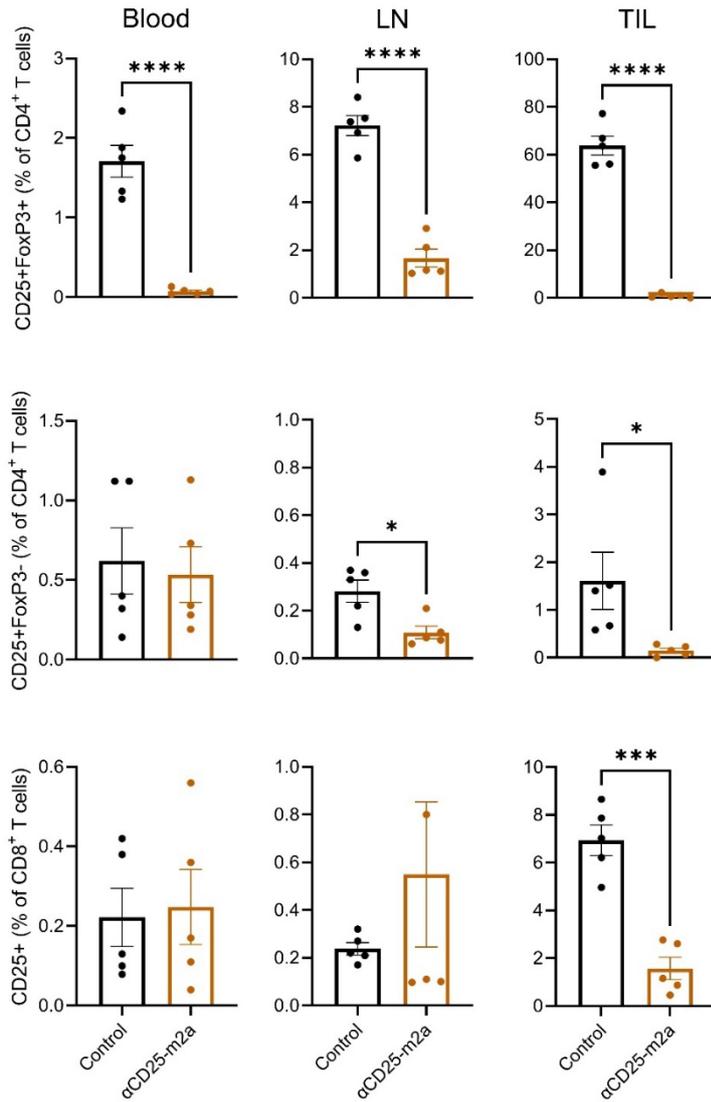
139,140,597 - 139,140,001

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TCCACTGGCTCCTGGAAC TGCTCTCAAAGTAAC TCCATGGT GACCTGGGATGCCTGGTCAAGGGCTATTTCCCTGAGCCAGTCACCGTGA
CCTGGAAC TCTGGAGCCCTGTCCAGCGGTGTGCACACCTTCCAGCTGTCTGCAGTCTGGGCTCTACACTCTCACCAGCTCAGTGACTGTA
CCCTCCAGCACCTGGCCCAGCCAGACCGTCACTGCAACGTAGCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGGTGAGAGAAC
AACCAGGGGACGAGGGGCTCACTAGAGGTGAGGATAAGGCATTAGACTGCCTACACCAACCAGGGTGGGCAGACATCACCAGGGAGGGGGCC
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CCTGGTGCCTTCTAACCTATAGACACCAATAACACACAGCCTTCTCTCTGCAGTGCAGAACTGTGGAGGTGATTGCAAGCCTTGTATAT
GTACAGGTAAGTTACTAGCCTTAAATTCCAGCCCAGGTCCAACAAATGTCTCTGAGGCCATGTTGGAGGATATTGGCATATTTCCACC
TTTCTTCTCATCTACAGGCTCAGAAGTATCATCTGTCTTCATCTTCCCCCAAAGCCAAAGATGTGCTCACCATCACTCTGACTCCTAAG

Supplementary Figure S1. Genomic sequence of rat IgG1 heavy chain locus. Genomic sequence of rat IgG1 heavy chain immunoglobulin locus with the exons for the CH1, Hinge, CH2 and CH3 highlighted in grey. The PAM sequence for gRNA-R1 (AGG) is underlined and highlighted in yellow.



Supplementary Figure S2. Sortase mediated his-tag removal leads to covalent inter heavy chain linkage in αCD25-m2a. Reducing SDS-PAGE (left gel) of αCD25-m2a suggests generation of side product from sortase-mediated reaction (Fig 1E). Non-reducing SDS-page (right gel) and mass spectrometry measurements indicate monomeric antibody product, suggesting sortase mediated reaction results in covalent bond forming between heavy chains of single molecules.



Supplementary Figure S3. CD25⁺ T-cell subsets in blood, lymph nodes and tumor from mice treated with α CD25-m2a or an isotype control. Figure is supplementary to figure 2C. The percentage CD25⁺FOXP3⁺ of CD4⁺ T-cells, percentage CD25⁺FOXP3⁻ of CD4⁺ T cells and the percentage of CD25⁺ of CD8⁺ T-cells are shown. Data is represented as the mean \pm SEM. Statistical significance was determined using an unpaired two-tailed Student's T test and is shown as * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$.

Supplementary Table S1: HDR Donor construct

Element	Sequence
5' HA	<p>AGGGCTGGGTATGAGCCACAGTGAATCTGACCCAGAATAGAGAGTGTAAACCTACTTACATCAAGGCCAAGTGAAGGACAGGGCCAGCAAAAGCAAGCTAAGGCCAGAGATCTTGGACTATGAAAGTTGAGAGAACCTAGGATCAGGAACDATTAGTGAACA GACAAAGGGCAGGTAAGCAGCCTAGGAGTGGACAAAGACACAGGAATAACAGAAAGACGGCAGGGATGACCCGACTTCAAGTTGGGCTTCACTGTGTGCCAACTGTGTGCAGATTATGGCCATGGGTAAAGGTTTAGCATTAGAACAGATACCCACATTGGAACAAT GGTGGGGAAACAGATACCCACTACTGCAAGGCTCTTCGGGCCCTTTCCAAAAGTGTACTAGGAGTGGGACTGGGCTCAAAAGGATTAGGTGTGACTGGCCCTGGTGAAGGCTGACACTGACAAGCCAAATGGTTGGGTGTTCATCTCCATTTATACAGCC</p>
mlg2a CH1-CH3	<p>GCTAGCGATCGCAGGGCAATCTTCGCAATTTCTTTTCCAGCTAAGACTACCGCTCTAGCGTTTATCCTTTGGCCCTGTATGTGGGGACACCACTGGCTCTTCTGTAACCTCTGGGCTGTTTGGTGAAGGGTACTTTCCAGAACCCTGTAACCTTTGACATGGAAATTCAGG GTCTCTCTCTCAGGAGTACATACTTTCCCGAGTTTTGCAAGGGATCTTTACACACTTAGTTCCTCCGTCACCGTTACTTCTTACCTGGCCAGTCAGTCCATAACATGTAACGTGGCCATCCTGCCAGCAGCAGCAAAAGTAGACAAAAATCGAACCTAGAGG CCCTACTATAAGCCCTGCCACCATTGCAATGCCAGCCCCAATCTCCTCGGTGGTCTTAGGCTTCTATCTTCCGCCCAAGATTAAAGGATGTGCTGATGATTTTCATTGAGCCCAATTTGCACATGTGTGGTCTGGTGTGTGAGGATGTGTCAGAGGATGACCCCTGACCTGCAAT ATCTTTGGTTTGAATAACGTAGAGGTGCATACCGCTCAGACTCAGACTCACCAGGGAGGACTATAATAGCACTCTCAGGGTGGTCTCCGCACTTCCAAATTCAGCACCAGGACTGGATGTCGGCAAAAGTTCAAGTGTAAAGTCAATAACAAGGATTTGCCGCAACA ATAGAACGGACCTCTCAAACTAAAGGGAGTGTACGGCCCCACAGGTTTACGTGCTGCCCCACCCGAGGAGGAAATGACCCAAAAGCAGGTGACACTACCTGCAATGGTTACGATTTTATGCCCAGAGACATATATGTTGAGTGGACTAACACGGGGAGACC GAGCTGAATTATAAAAATACCGAACCCGTTTTGGACTCAGATGGCTACTACTGATGTACTCCAACTCCGGGTAGAGAAAAAGAACTGGTTGAAAGAAACAGCTACTATGACAGGTGTGGTGTGATGAGGGGCTCCACAATCATCATAACCAAGTCTTTCTCACGGA CACCTGGAAAGTACGGGAA</p>
G4S,LPETGG,H6	<p>CGTACGGGAGGCGGAGGCAAGCTGCCGGAACCGGCGCATCATCATCATCATTTA</p>
IRES	<p>GTCCAGCTCGAGGCCCTCTCCCTCCGCCCCCTAACGTTACTGGCCGAAAGCCGCTTGAATAAGGCGGTGTGCGTTTGTCTATATGTTATTTCCACATATTGCCGCTTTTGGCAATGTAGGGCCCGGAAACCTGGCCCTGTCTTCTGACGAGCATTCCTAGGG GTCTTTCCCTCTCGCCAAAGGAATGCAAGGTTCTGTGAATGTCGGAAGGAAGCAGTCCCTCTGGAAGCTTCTTGAAGACAACAACGCTCTGTAGGGACCCCTTTCAGGACGCGGAACCCCACTGGGACAGGTGCTCTGGGCCAAAAGCCACGTGTATAAGA TACACCTGAAAAGGCGCACAAACCCAGTGCACGTTGTGAGTTGGATAGTTGTGAAAGATCAAAATGGCTCTCCCTCAAGCGTATTCAACAGGGGCTGAAAGGATGCCAGAAAGTACCCATTGTATGGATCTGATCTGGGCCCTCGGTGCACATGCTTACATGTG TTTAGTGGAGTTAAAAAACGCTCAGGCCCCCGAACACGGGGAGCTGGTTTTCTTTGAAAACACGATGATAATATGGCCACA</p>
Bsr	<p>GCCACCATGGCCAAAGCTTTGTCTCAAGAAGATCCACCTCATTGAAAGAGCAACGGCTACAATCAACAGCATCCCCTCTGTAAGACTACAGCGTCCGACGCGAGCTCTCTAGGACGGCCCATCTTCACTGAGTCAATGTATATCATTTTACTGGGGAC CTTGTGCAGAACTCGTGTCTGGGCACTGCTGCTGCTGCGGCACTGGCAACTGACTTGTATCTGCGGATCGGAAATGAGAACAGGGCATCTTGAAGCCCTGCGGACGGTCCGACAGGTGCTCTCGATCTGCATCTGGGATCAAGCCATAGTGAAGGACAC TGATGGACAGCCGACGGGATGGGATTCGTGAATTGCTGCCCTCTGGTTATGTGTGGGAGGCTAAGT</p>
SV40 polyA	<p>ACTAGTCSAGTGTGCCCTTCTAGTTCAGCAGCCTCTGTGTTTGGCCCTCCCGCTGCTCTTCCAGCCCTGGAAGTGCCACTCCACTGCTCTTCCATAAATAGSAGAAATGCATCGCATTTGTGAGTGTGATCTTCTATCTGGGGGGTGGGTGGGCGGACGA CAGCAAGGGGGAGGATTGGGAAGACAATAGCAGCATGCTGGGATGCGGTGGGCTCTATGGA GATCTTGTACA</p>
3'HA	<p>CAAAATGGAGACTGTAGGAGCTTGGTCCAGCCTGTCCAGACAAATGATCAGCATACTTTTCTGTACTGAAACAACAGCCCATCTGTCTATCCACTGGCTCCTGGAAGTACTCTCAAAAGTAACCTGATGGCTGGATGCCCTGGTCAAGGGCTATTTCC CCTGAGCCAGTCACCGTGAACCTGGAGCCCTGTCCAGGGGTGTGCACACCTTCCAGCTGTCTGCACTGGGCTCTACACTCTACCAAGCTCAGTACTGACCTCCAGCACCTGGCCAGCCAGCCGTCACCTGCAACGTAGCCACCCGGCCAGG AGCACCAGGTGGACAGAAATTTGGTGAAGAACCAACAGGGGACAGGGGCTCACTAGAGTGTAGGATAAGGCATTAGACTGCTACACCACCAAGGTTGGGACAGATCACCAAGGAGGGGGCTCAGCCCGGAGACCAACATTCTCTTTGTCTCCCTT</p>