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Supplemental Material to:

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In vitro affinity maturation of a natural human antibody overcomes a barrier to in vivo affinity maturation

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In vitro affinity maturation of a natural human antibody overcomes a barrier to *in vivo* affinity maturation

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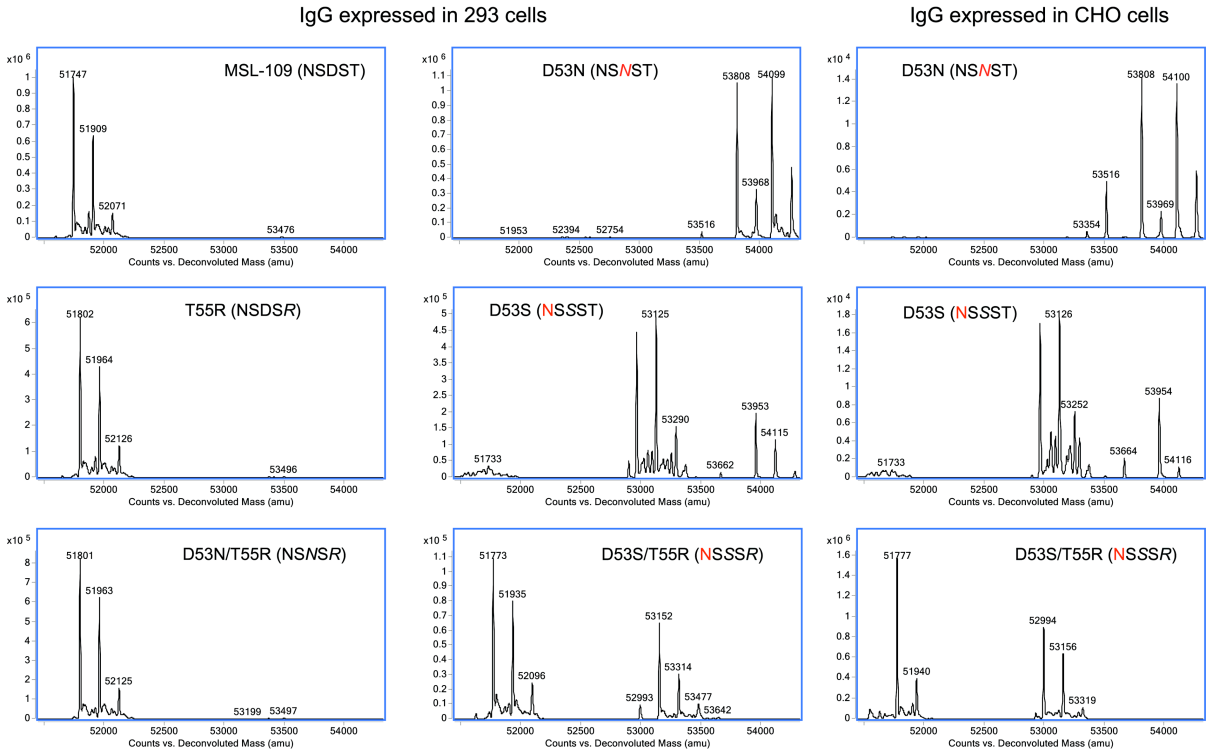
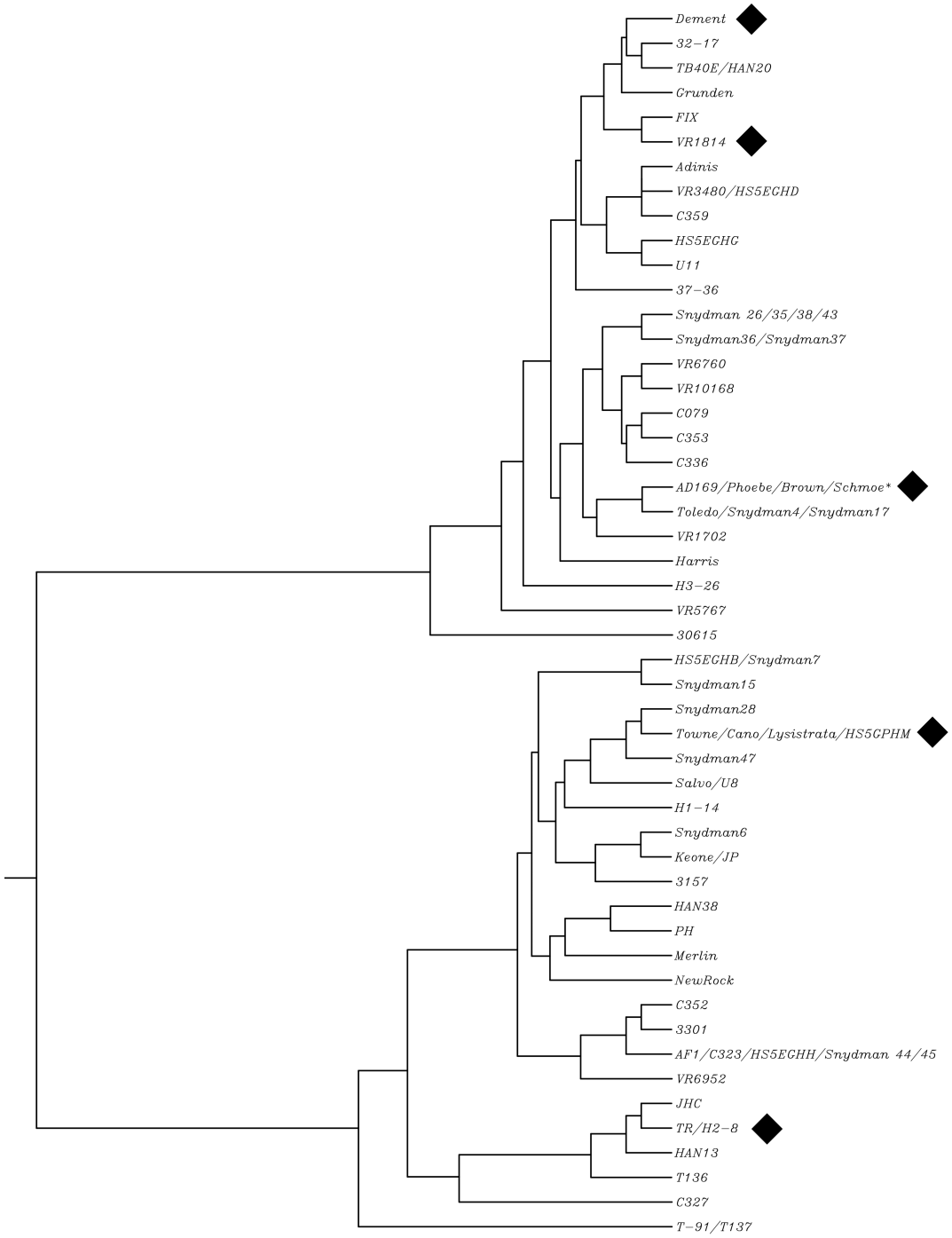
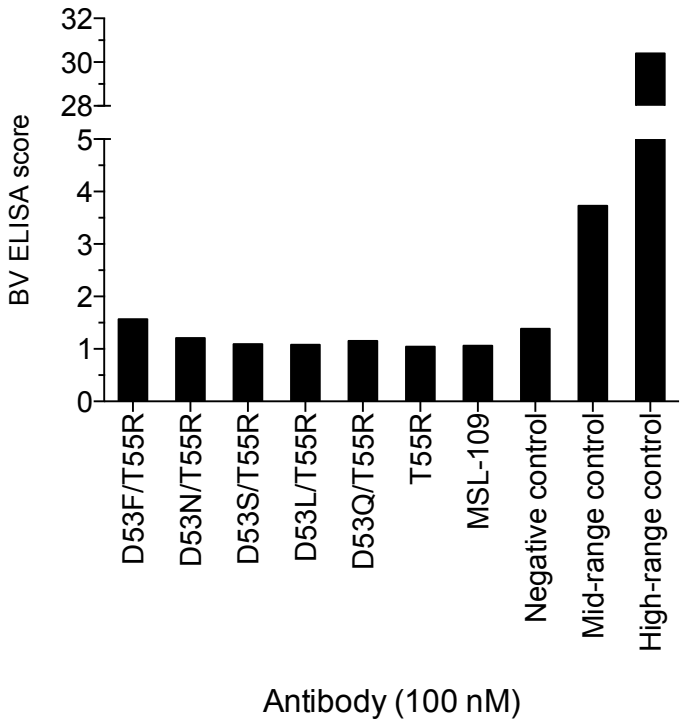
Supplementary Figures



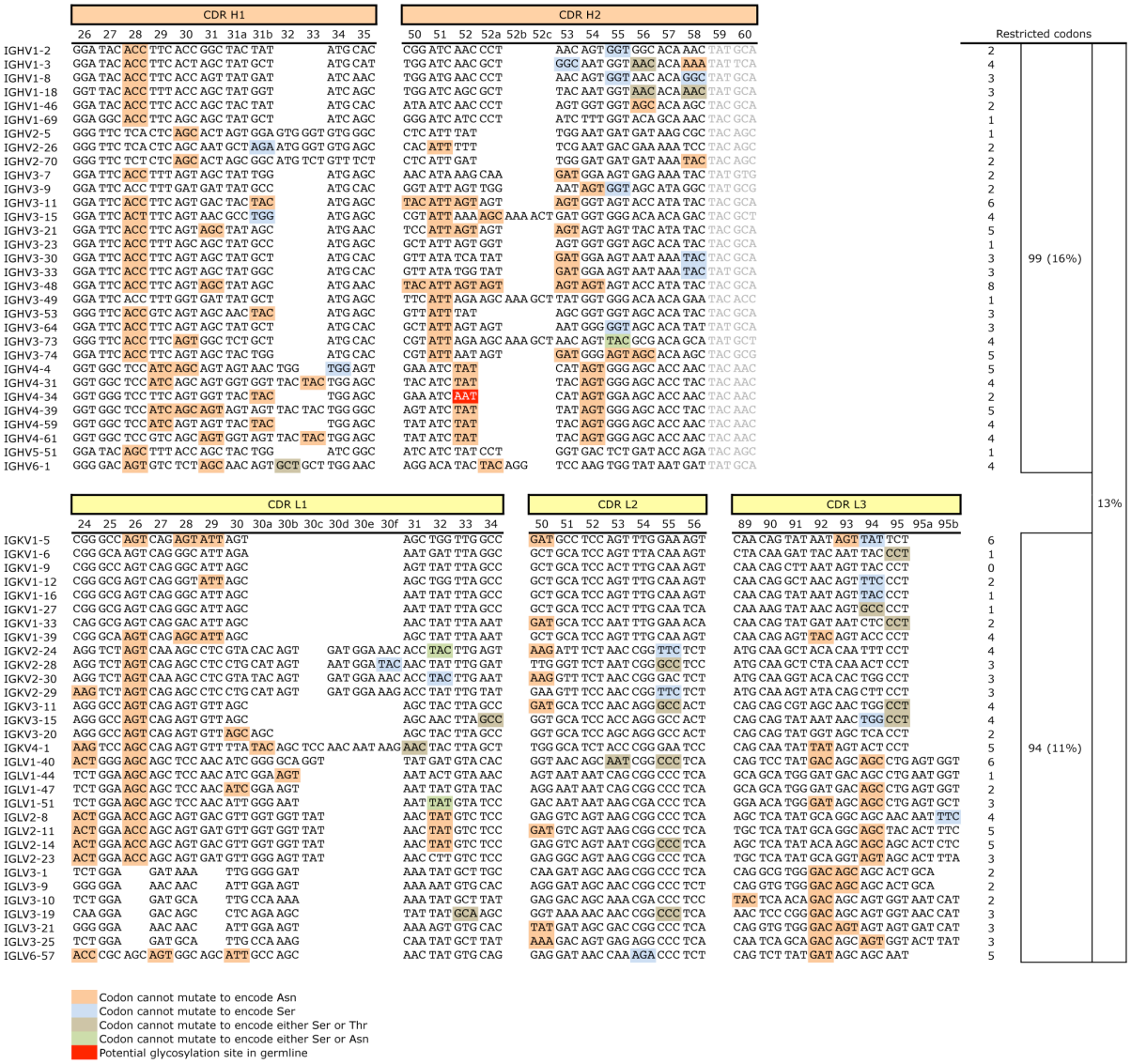
Supplementary Figure 1 – Mass spectrometry analysis of IgG heavy chains from different MSL-109 variants expressed in 293 and CHO cells. The sequence between heavy chain residues 52 and 55 of each variant is shown in parenthesis with mutation shown in italics and potential glycosylation sites in red. The light chains of all IgG variants had a mass of 23,981 ± 1 Da (calculated reduced mass = 23,980), omitted for clarity.



Supplementary Figure 2 – Dendogram of CMV strains based on gH glycoprotein sequences. The six strains used in neutralization assays are indicated with diamonds. The asterisk indicates three additional strains, Watkins, Powers and HS5GLYH, that have gH sequences identical to AD169, Phoebe, Brown and Schmoe. The overall minimum amino acid identity, between strains 37-36 and VR6952, is 96.1%. The strain used in neutralization assays with gH most distantly related to VR1814 is Cano, with 96.8% identity.



Supplementary Figure 3 – Non-specific baculovirus (BV) particle ELISA of MSL-109 mutant clones with baculovirus particle antigen. The BV ELISA score is the absorbance with the test IgG divided by the absorbance of blank wells (no IgG).



Supplementary Figure 4 – CDR codons in human heavy and light variable region germlines that cannot mutate to Ser, Thr or Asn codons without introducing potential N-linked glycosylation sites in the germline sequence context. Only positions that can access Ser, Thr or Asn codons with 1 base-pair mutations are indicated. The *01 alleles of each of the top 95% most used human germlines (usage data obtained from the IMGT database at http://www.imgt.org/) are shown. The Chothia numbering system is used to allow structurally correct alignment of germlines. The germline CDR boundaries shown are a combination of Kabat and Chothia definitions except for CDR H2 where only the region more likely to be in contact with antigen is shown, with codons 59 and 60 shown in grey only to put codons in position 58 in context. CDR L3 junctional diversity was not considered when defining restricted codons. The number and percentage of restricted codons is shown for each germline, counting codons restricted in two different ways (e.g. neither Ser nor Asn allowed) as one.