Supplementary Figure and Table legends

Supplementary Figure 1. Comparison of the relative binding of the hybridoma derived NCD1.2 murine IgG and recombinant canine-murine chimeric IgG. An ELISA with recombinant antibody expressed into the supernatant of HEK293 cells was used to define activity to the CD20 epitope peptide in relation to the NCD1.2 IgG. A titration in quadruplicate (with bars showing the error as a mean) of the light-chain-7/heavy chain antibody and murine NCD1.2 After adding the murine or canine IgG, either anti-mouse Fc HRP conjugated secondary antibody was added to the NCD1.2 reaction or anti-canine Fc HRP conjugated secondary antibody was added to the recombinant canine-murine chimeric antibody. The data are plotted as binding activity (in relative light units) a function of the amount of antibody titrated (in nanograms).

Supplementary Figure 2. Calculation of peptide antibody saturation. Fluorescence polarization of CD20 peptide (FAM)-NCDPANPSEK incubated with purified monoclonal antibody NCD1.2. Antibody concentration is reported as the molar concentration of immunoglobulin binding sites. The binding equilibrium and the dissociation constant between the antibody and the peptide were determined by fluorescence polarization using FAM labelled CD20 peptide as a tracer. The equilibrium dissociation constant (Kd) was calculated by fitting the sigmoidal dose-dependent FP increases as a function of protein concentrations using Graphpad Prism. The following formulas were used to calculate the antibody saturation of the peptide in the HDX experiment. Saturation (S) is expressed as the ratio between the concentrations of peptide-bound antibody and total antibody concentration after addition of deuterated water. In the HDX experiment, after the addition of deuterium, the antibody concentration was 2 000 nM with ten times molar excess of peptide (20 000 nM). In this setup, the peptide occupancy of the antibody (S) was calculated to be 98.77%.

$$\mathrm{Kd} = \frac{[\mathrm{A}].[\mathrm{P}]}{[\mathrm{AP}]} \;\; ; \;\; [\mathrm{A}] = \mathrm{A} - [\mathrm{AP}] \;\; ; \;\; [\mathrm{P}] = \mathrm{P} - [\mathrm{AP}]$$

$$[\mathrm{AB}] = \frac{\mathrm{A} + \mathrm{P} + \mathrm{Kd} - \sqrt{\mathrm{A}^2 + \mathrm{P}^2 + \mathrm{Kd}^2 + 2.\mathrm{A}.\mathrm{P} + 2.\mathrm{A}.\mathrm{Kd} + 2.\mathrm{P}.\mathrm{Kd} - 4.\mathrm{A}.\mathrm{P}}}{2}$$

$$\mathrm{S} = \frac{[\mathrm{AP}]}{\mathrm{A}}$$

A - total antibody concentration
P - total peptide concentration
[AP] - concentration of antibody peptide complex
[P] - concentration of unbound peptide
[A] -concentration of unbound antibody
Kd - dissociation constant
S - saturation of antibody with peptide

Supplementary Figure 3. Interaction of the NCD1.2 monoclonal antibody with the CD20 peptide suppresses deuteration at specific CDRs. Deuteration levels (exchanged deuterons, #D) of ligand-free NCD1.2 and in complex with the CD20 peptide in molar ratios of 1 : 1 or 1 : 10 (NCD1.2 : CD20), alternatively incubated in H_2O buffer for 60 min at room temperature before the time course of deuteration (10 sec, 1 min, and 10 min). The reactions at antibody : peptide ratio of 1:1 were pre-incubated for 60 min at room temperature and the reactions at antibody : peptide ratio of 1:10 mixed and were immediately subjected to deuteration. Numbers at the left indicate the NCD1.2 peptide fragments belonging to light and heavy chains; schematic representation at the right shows NCD1.2 antibody CDR and FR regions. Dashed lines in the graphs indicate S.D. of the measurement (see Experimental procedures and Supplementary Table 1). The green and red lines represent data obtained from reactions at a 1 : 1 or a 1 : 10 (NCD1.2 : CD20) ratio with preincubation in H_2O buffer for 60 min and the light brown line represents data obtained from reactions at a 1 : 10 (NCD1.2 : CD20) ratio without preincubation in H_2O buffer for 60 min.

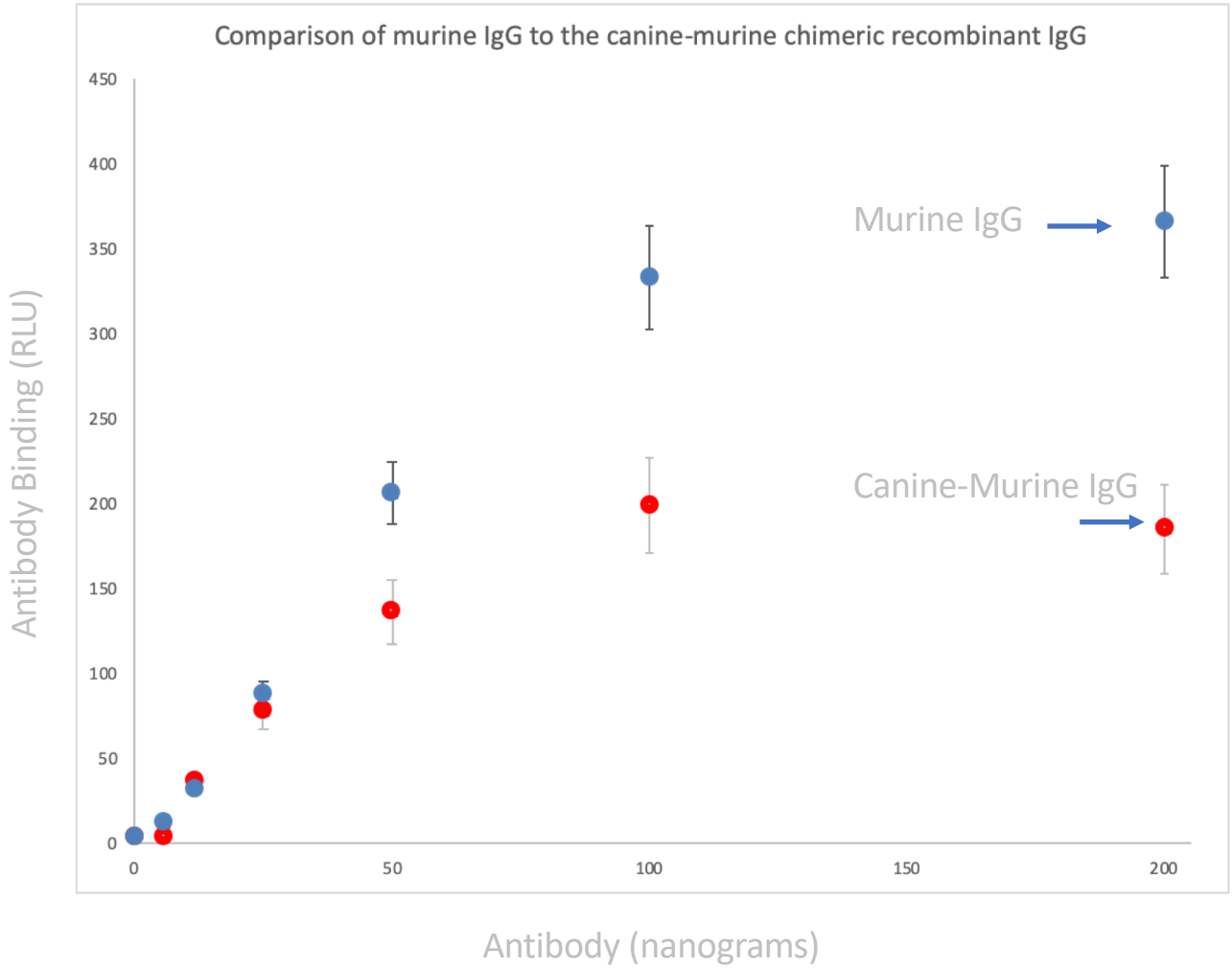
Supplementary Table 1. Summary of NCD1.2 deuteration after interaction with the CD20 peptide at different molar ratios (NCD1.2 : CD20 peptide). The table contains data exported from HDExaminer and Proteome Discoverer 1.4 programs. The start and end residues of the peptides with amino acid sequence of NCD1.2 peptides are listed in columns **A-C**. The time of deuteration and theoretical maximally exchanged deuterons in each peptide (maxD) are in columns **D** and **E**. Experimentally measured exchanged deuterons, their number (#D, column **F**) or percentage (%D, column **G**) with calculated S.D. from triplicates (column **H**). These values (#D, %D, and S.D.) are introduced for all states of NCD1.2 antibody (free and in complex with the CD20 peptide).

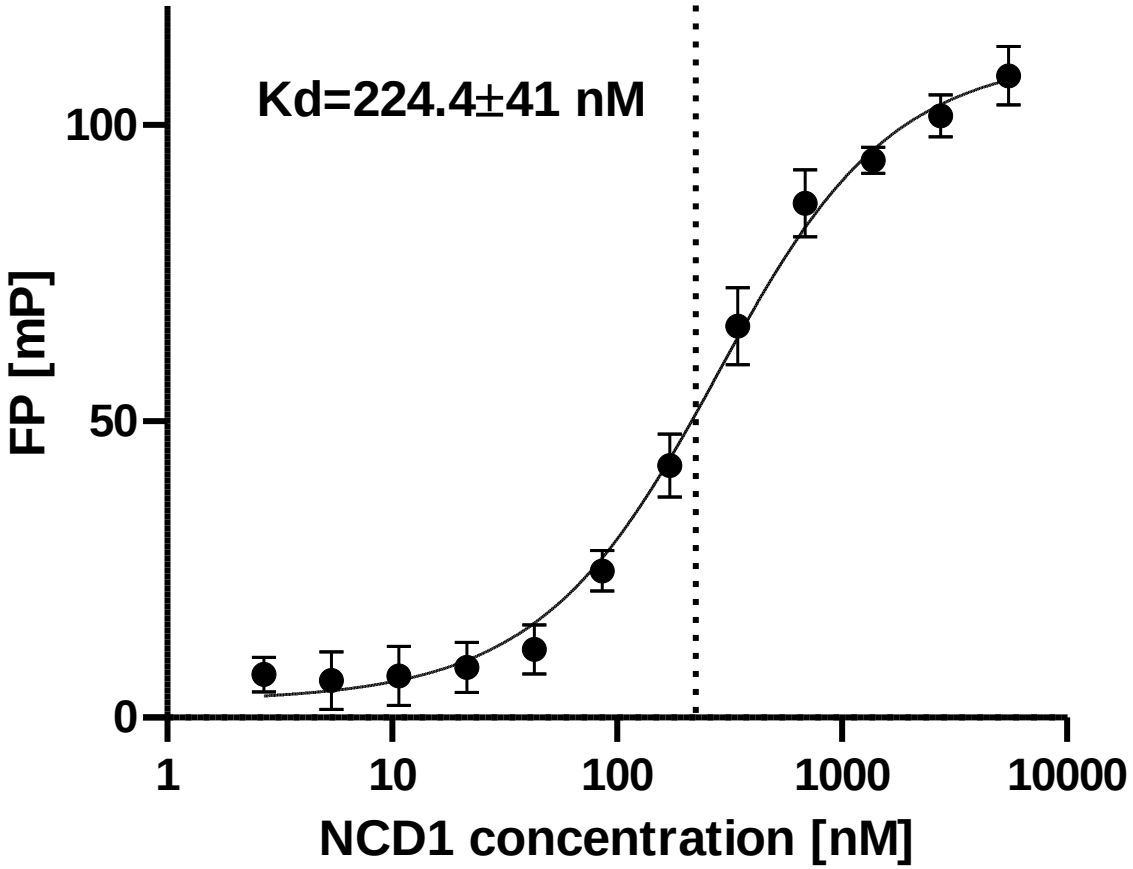
Supplementary Table 2. HDX-MS summary results of the standard myoglobin protein. The table contains data exported from HDExaminer. The start and end residues of the peptides with amino acid sequence of myoglobin peptides are listed in columns **A-C**. Time of deuteration was 30h for preparation of fully deuterated protein (column **D**). Columns **E-H** were described in Supplementary Table 1. Percentage of back exchange was calculated as 100 minus %D (column **F**).

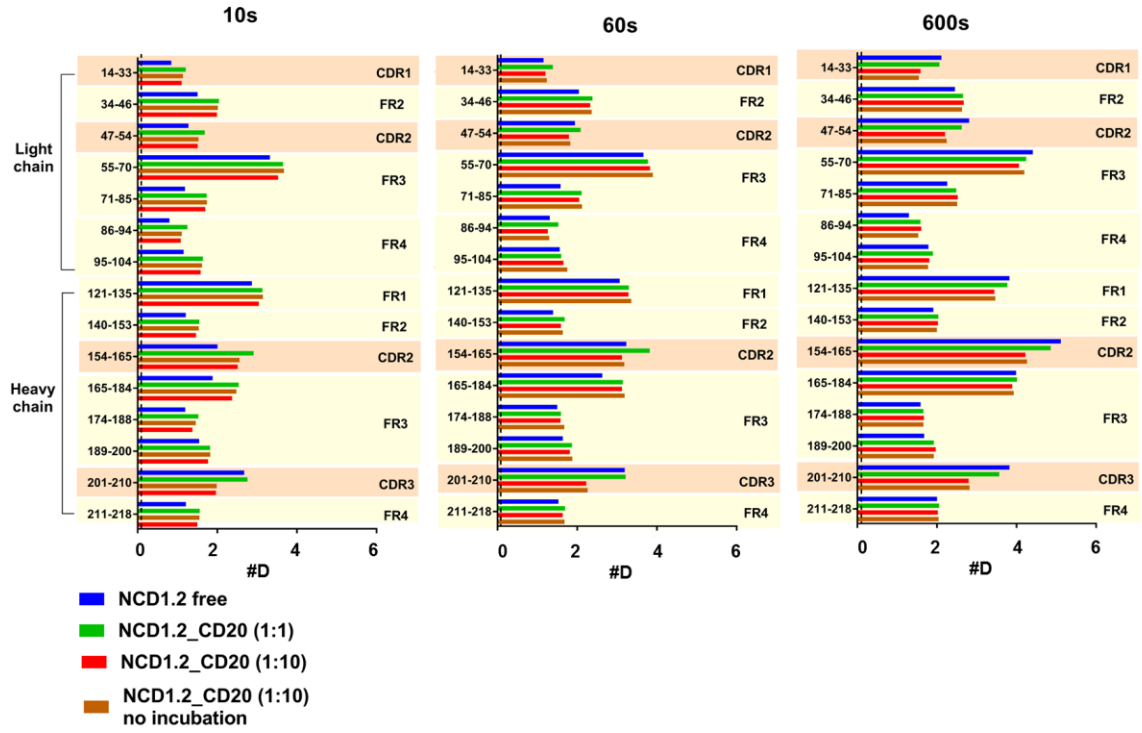
Supplementary Table 3. List of the raw files from mass spectrometry analysis submitted to ProteomeXchange Consortium via the PRIDE partner repository. The raw files were deposited as two datasets.

Supplementary Table 4. Summary of NCD1.2 mass spectrometry characterization and peptide deuteration after interaction with the CD20 peptide at a molar ratio of 1 : 10 (NCD1.2 : CD20 peptide). The table contains data exported from HDExaminer and Proteome Discoverer 1.4 programs. The start and end residues of the peptides with amino acid sequence of the NCD1.2 peptides are listed in columns **A-C**. Mass spectrometry characterization of the peptides is performed by: high confidence threshold (Xcorr, column **D**), charge of peptide (column **E**), mass of single-charged peptic peptide MH^+ in Da (column **F**), and retention time

in min (column **G**). Time of deuteration (10 sec, 60 sec, 600 sec and MAX that was 24h for preparation of fully deuterated protein, column **H**). Columns **I-O** were described in Supplementary Table 1.


Comparison of murine IgG to the canine-murine chimeric recombinant IgG
Antibody Binding (RLU)
450
400
350
300
250
200
150
100
50
0
0
50
100
150
200
Antibody (nanograms)
Murine IgG
Canine-Murine IgG


Kd=224.4±41 nM
100
50
0
FP [mP]
1
10
100
1000
10000
NCD1 concentration [nM]


10s
60s
600s
Light chain
Heavy chain
14-33
34-46
47-54
55-70
71-85
86-94
95-104
121-135
140-153
154-165
165-184
174-188
189-200
201-210
211-218
CDR1
FR2
CDR2
FR3
FR4
FR1
CDR3
0
2
4
6
#D
NCD1.2 free
NCD1.2_CD20 (1:1)
NCD1.2_CD20 (1:10)
NCD1.2_CD20 (1:10) no incubation

1) The first dataset: MS data from mapping the antibody peptide fragments (PXD018510; Username: reviewer38806@ebi.ac.uk, Password: eOgipxVu)

IDA_120min_TripleTOF_NCD1_Pepsin_digestion

IDA_120min_TripleTOF_NCD1_Pepsin_digestion__FDR

IDA_120min_TripleTOF_NCD1_Pepsin_digestion_1

IDA_120min_TripleTOF_NCD1_Pepsin_digestion_1.wiff

IDA_120min_TripleTOF_NCD1_Pepsin_digestion_2

IDA_120min_TripleTOF_NCD1_Pepsin_digestion_2.wiff

IDA_120min_TripleTOF_NCD1_Pepsin_digestion_3

IDA_120min_TripleTOF_NCD1_Pepsin_digestion_3.wiff

IDA_120min_TripleTOF_NCD1_Pepsin_digestion_PeptideSummary

LC-MSMS_NCD_1.2_In-sol_digestion_pepsin.msf

LC-MSMS_NCD_1.2_In-sol_digestion_pepsin.raw

LC-MSMS_NCD_1.2_In-sol_digestion_trypsin.msf

LC-MSMS_NCD_1.2_In-sol_digestion_trypsin.raw

LC-MSMS_NCD_1.2_In-sol_digestion_trypsin_LysC.msf

LC-MSMS_NCD_1.2_In-sol_digestion_trypsin_LysC.raw

(2) The second dataset: MS data from HDX-MS analysis (PXD018550; Username: reviewer76109@ebi.ac.uk, Password: IiYmwPfe).

HDX_myoglobin_MAP.msf

HDX_myoglobin_MAP.raw

HDX_myoglobin_ND_replicate1.raw

HDX_myoglobin_ND_replicate2.raw

HDX_myoglobin_ND_replicate3.raw

HDX_myoglobin_replicate1.raw

HDX_myoglobin_replicate2.raw

HDX_myoglobin_replicate3.raw

HDX_NCD_1min_replicate1.raw

HDX_NCD_1min_replicate2.raw

HDX_NCD_1min_replicate3.raw

HDX_NCD_10min_replicate1.raw

HDX_NCD_10min_replicate2.raw

HDX_NCD_10min_replicate3.raw

HDX_NCD_10s_replicate1.raw

HDX_NCD_10s_replicate2.raw

HDX_NCD_10s_replicate3.raw

HDX_NCD_FD_replicate1.raw

HDX_NCD_FD_replicate2.raw

HDX_NCD_FD_replicate3.raw

HDX_NCD_MAP.msf

HDX_NCD_MAP.raw

HDX_NCD_ND_replicate1.raw

HDX_NCD_ND_replicate2.raw

HDX_NCD_ND_replicate3.raw

HDX_NCD_peptide_1_1_1min_replicate1.raw

HDX_NCD_peptide_1_1_1min_replicate2.raw

HDX_NCD_peptide_1_1_1min_replicate3.raw

HDX_NCD_peptide_1_1_10min_replicate1.raw

HDX_NCD_peptide_1_1_10min_replicate2.raw

HDX_NCD_peptide_1_1_10min_replicate3.raw

HDX_NCD_peptide_1_1_10s_replicate1.raw

HDX_NCD_peptide_1_1_10s_replicate2.raw

HDX_NCD_peptide_1_1_10s_replicate3.raw

HDX_NCD_peptide_1_10_1min_replicate1.raw

HDX_NCD_peptide_1_10_1min_replicate2.raw

HDX_NCD_peptide_1_10_1min_replicate3.raw

HDX_NCD_peptide_1_10_10min_replicate1.raw

HDX_NCD_peptide_1_10_10min_replicate2.raw

HDX_NCD_peptide_1_10_10min_replicate3.raw

HDX_NCD_peptide_1_10_10s_replicate1.raw

HDX_NCD_peptide_1_10_10s_replicate2.raw

HDX_NCD_peptide_1_10_10s_replicate3.raw

HDX_NCD_peptide_1_10_no_preincubation_10min_replicate1.raw

HDX_NCD_peptide_1_10_no_preincubation_10min_replicate2.raw

HDX_NCD_peptide_1_10_no_preincubation_10min_replicate3.raw

HDX_NCD_peptide_1_10_no_preincubation_1min_replicate1.raw

HDX_NCD_peptide_1_10_no_preincubation_1min_replicate2.raw

HDX_NCD_peptide_1_10_no_preincubation_1min_replicate3.raw

HDX_NCD_peptide_1_10_no_preincubation_10s_replicate1.raw

HDX_NCD_peptide_1_10_no_preincubation_10s_replicate2.raw

HDX_NCD_peptide_1_10_no_preincubation_10s_replicate3.raw