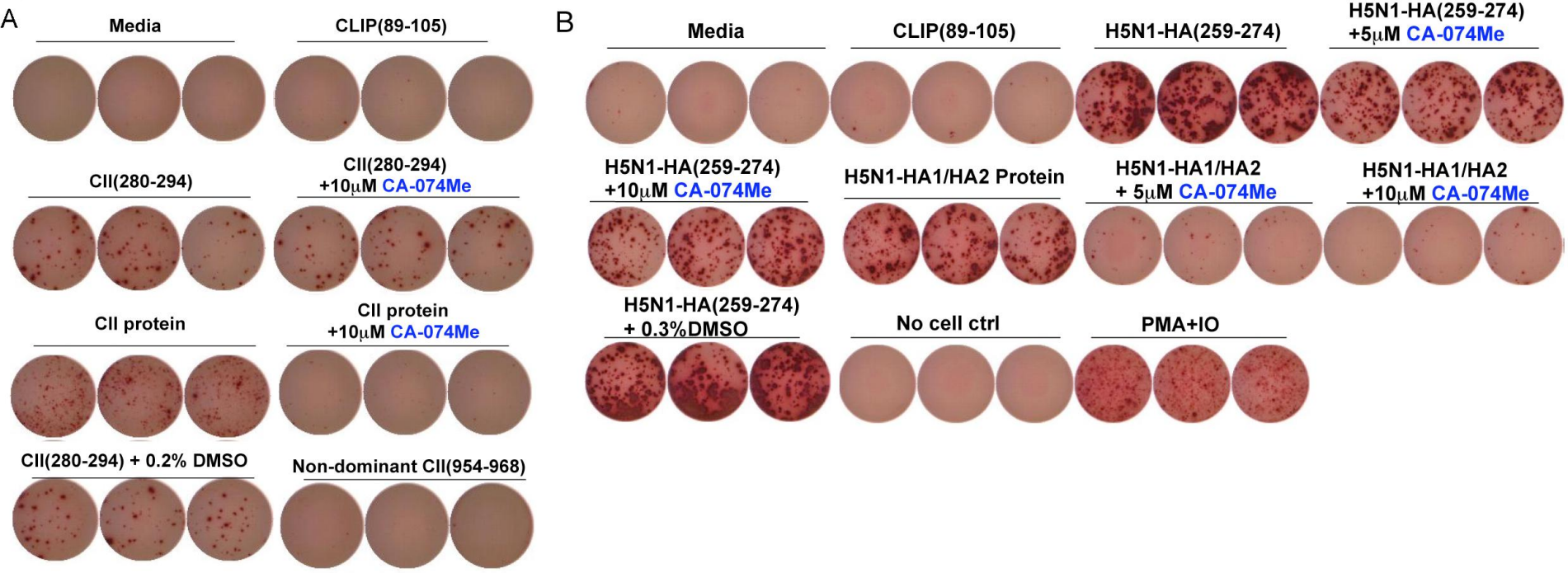
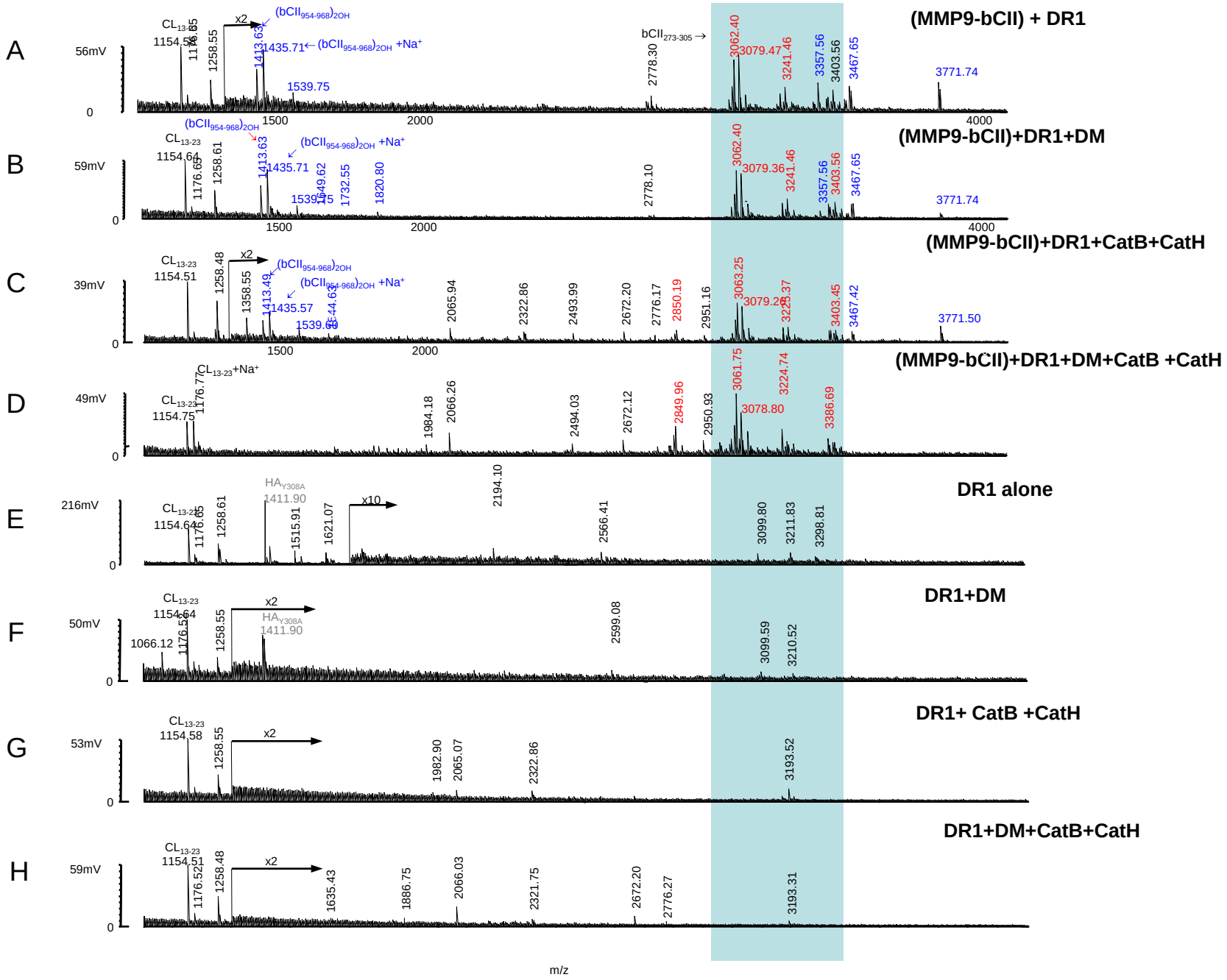
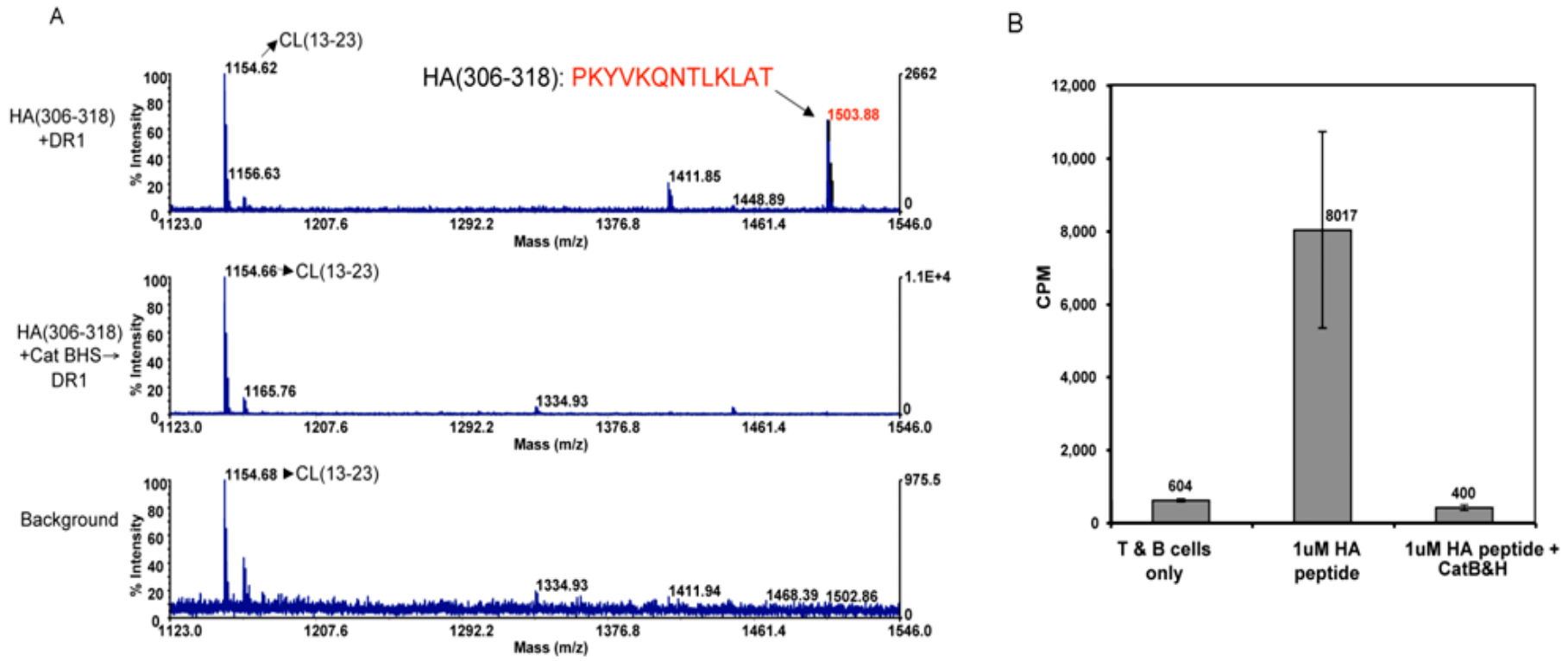


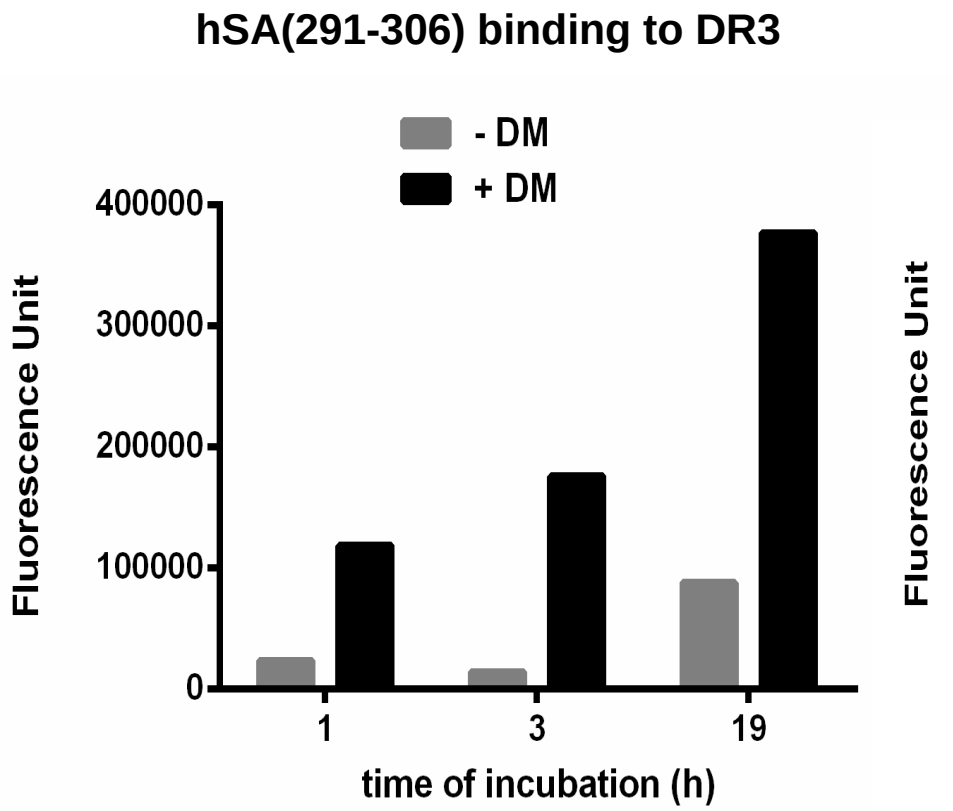
Supplementary Figure 1. Cathepsin B inhibitor, CA-074ME, blocks the presentation of type II collagen and H5N1 HA protein derived immunodominant epitopes. (**A-B**) shows IFN-γ production detected by the ELISPOT assay. HLA-DR1 mice were immunized with either CII(280-294) (**A**) and HA(259-274) peptide (**B**). At day 8, LN cells were harvested and stimulated with peptides, or proteins in the absence of presence of CA-074ME. Representative of three independent experiments.

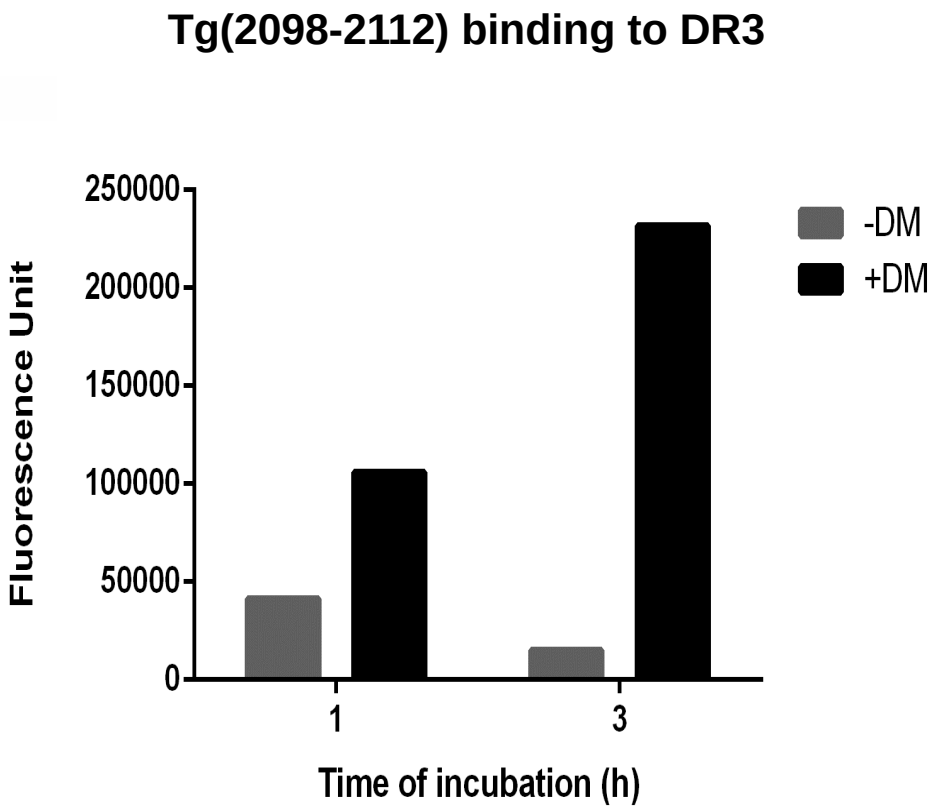

A
(MMP9-bCII) + DR1
B
(MMP9-bCII)+DR1+DM
C
(MMP9-bCII)+DR1+CatB+CatH
D
(MMP9-bCII)+DR1+DM+CatB +CatH
E
DR1 alone
F
DR1+DM
G
DR1+ CatB +CatH
H
DR1+DM+CatB+CatH
m/z

Supplementary Figure 2. HLA-DR1 selectively captures the immunodominant epitope of type II collagen. (**A-H**) The mass spectra of peptides eluted from DR1 under these following conditions. DR1 is incubated with the following components: with or without MMP9-fragmented bCII (bCII+/–), with or without DM (DM+/–), and with or without Cathepsins B and H (CatB+CatH +/–). All eight permutations are tested. These spectra are presented in an unabridged form of Fig. 1 including more background controls. (**A-D**) show the reactions containing MMP9-fragmented bCII. (**E-H**) show the negative control reactions carried out without MMP9-fragmented bCII. With the exception of (**D**), DR1 used in all experiments shown here was pre-incubated with HA(Y308A) (2-3 days at 37° C) prior to use. CL(13-23) is residues 13-23 of the Autographica Californica nucleopolyhedrovirus conotoxin-like peptide (NCBI accession number NP_054032), and is present as a background peptide in nearly all of our DR1 preparations. $(bCII_{954\text{-}968})_{2OH}$ is residues 954-968 of bovine type II collagen (bCII) with two hydroxylated residues. $(bCII_{273\text{-}305})$4OH is residues 273-305 of bCII with four hydroxylated residues, which contains $CII_{282\text{-}289}$, the core DR1-restricted immunodominant epitope of CII.

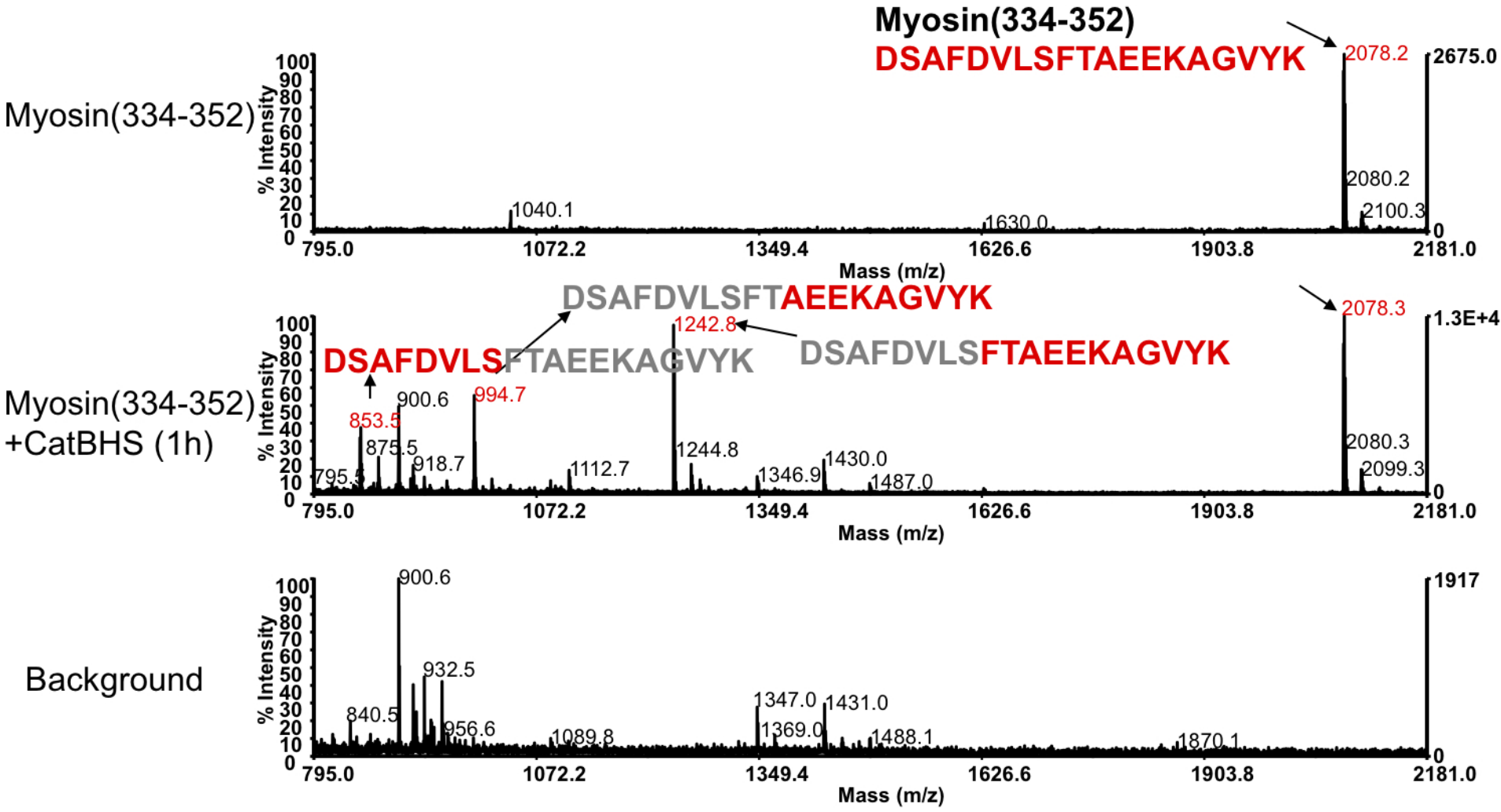


Supplementary Figure 3. HLA-DR1 restricted immunodominant epitope HA(306-318) is sensitive to cathepsin digestion. (**A**) Mass spectra of detecting HA(306-318) peptide, after digestion with CatB, CatH, and CatS. DR1 used in all experiments shown here was pre-incubated with HA(Y308A) (2-3 days at 37° C) prior to use. Synthetic peptide, HA(306-318) incubated with HA(Y308A)/DR1 for 3h is shown in top spectrum. Synthetic peptide, HA(306-318), digested first with the cathepsins for 1h, and then incubated with HA(Y308A)/DR1 for 3h at 37 ° C is shown in the middle spectrum. Incubation of cathepsins and HA(Y308A)/DR1 in the absence of HA(306-318) is shown in bottom spectrum. The peptides were eluted from DR1 and they were run on MALDI. The experiments were repeated three times. (**B**) The HA(306-318) peptide is incubated with cathepsins B and H in citrate phosphate buffer pH 4.0 at 37° C. A control reaction without cathepsins is assembled in parallel. After the incubation, the pH is adjusted to pH 7.4 and the cathepsins are heat inactivated by incubating at 80° C for 3 hrs. Both samples are then added to DR1+ EBV transformed B cells to yield a final HA(306-318) concentration of 1μM. After a 4 hr. incubation, the B cells are irradiated and then incubated with Clone 1 cells, a cultured human $CD4^+$ T cell population specific for HA(306-318)/DR1. Proliferation of Clone 1 cells is measured by H^3-Thymidine incorporation.

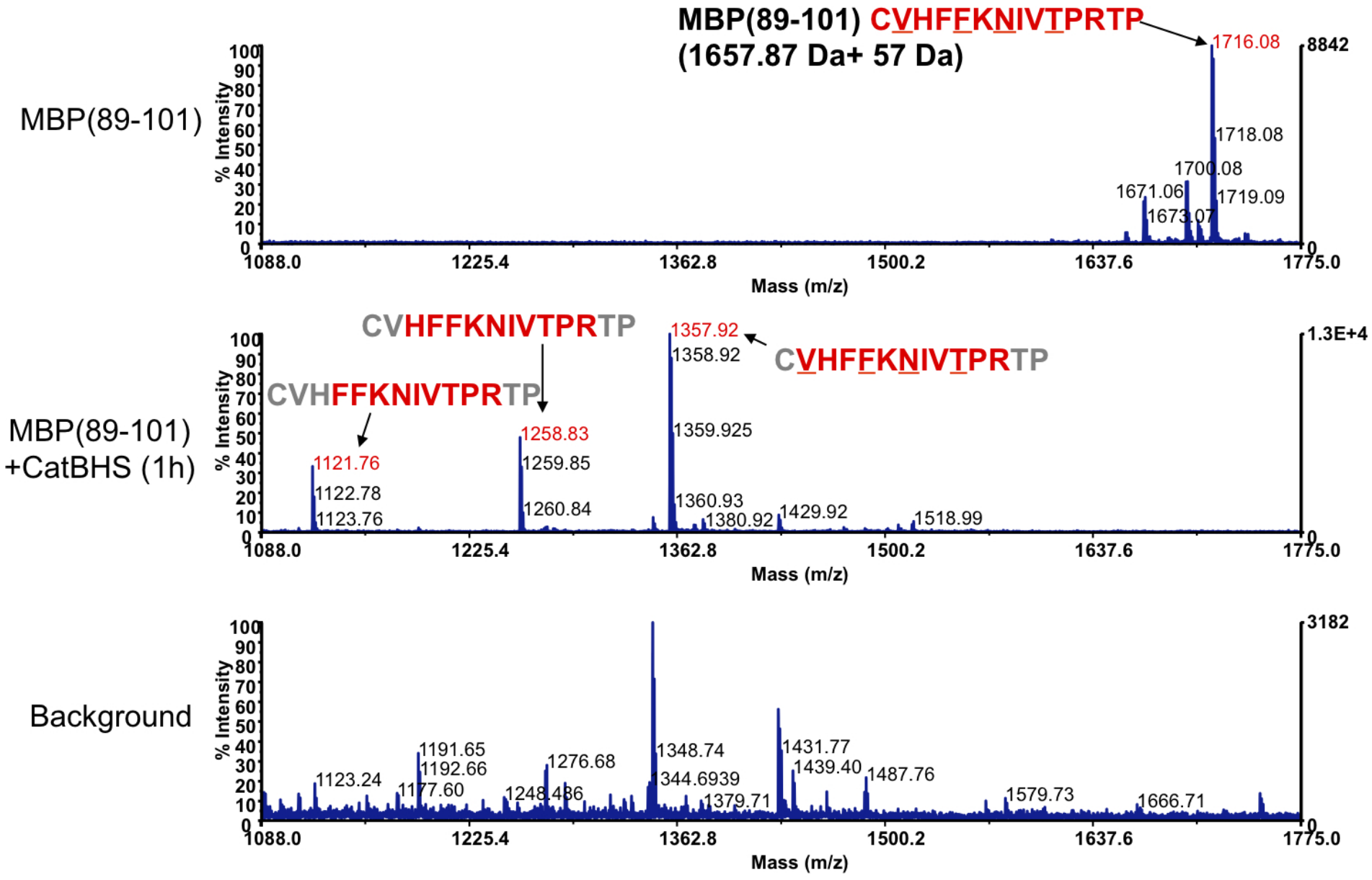




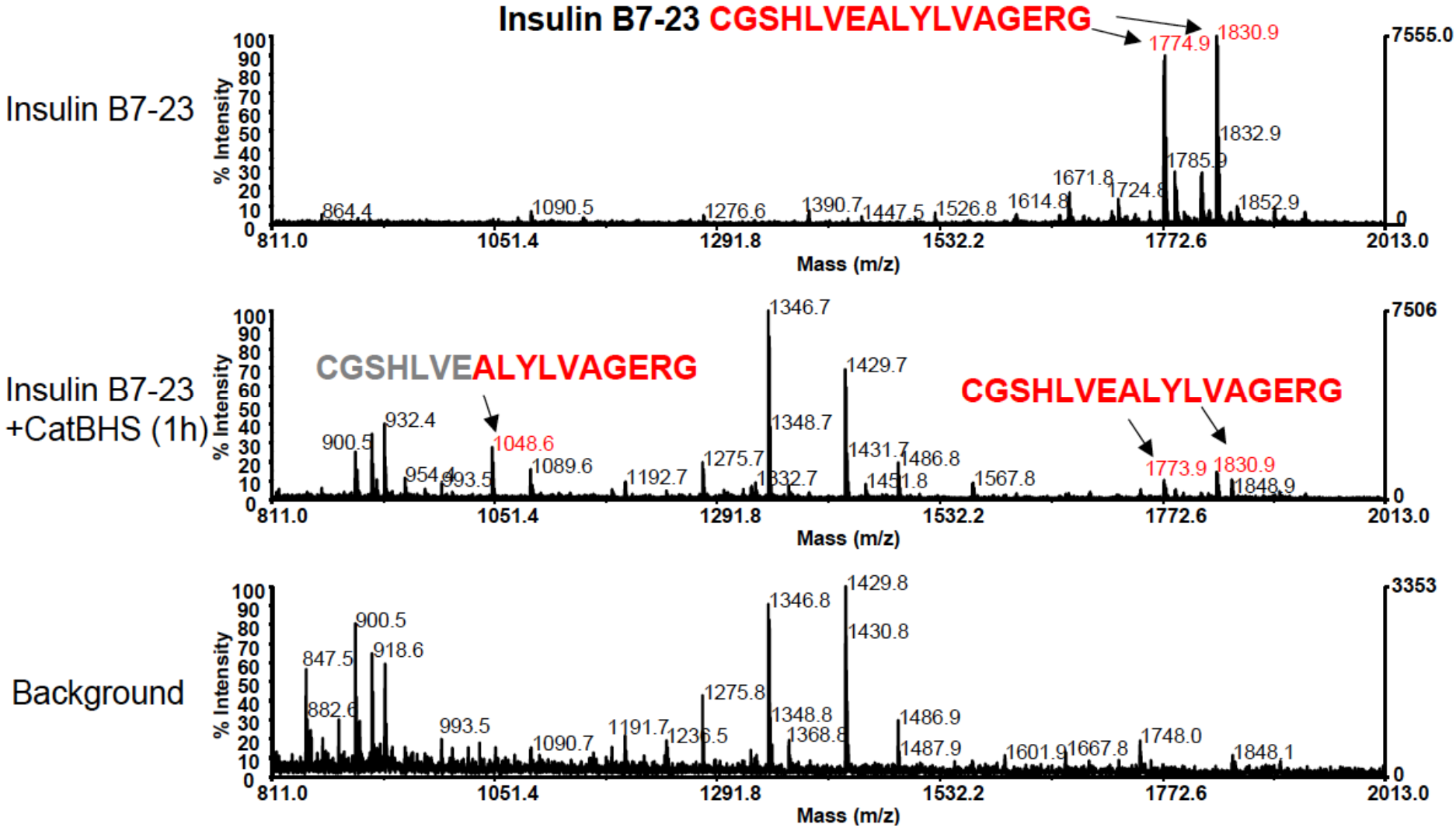
Supplementary Figure 4. hSA(291-306) and Tg(2098-2112) binding to soluble HLA-DR3 are enhanced by HLA-DM. Binding of fluorescently labeled hSA(291-306) (left) and Tg(2098-2122) (right) to DR3 in the presence or absence of DM for indicated times at 37° C. Experiments were repeated twice.



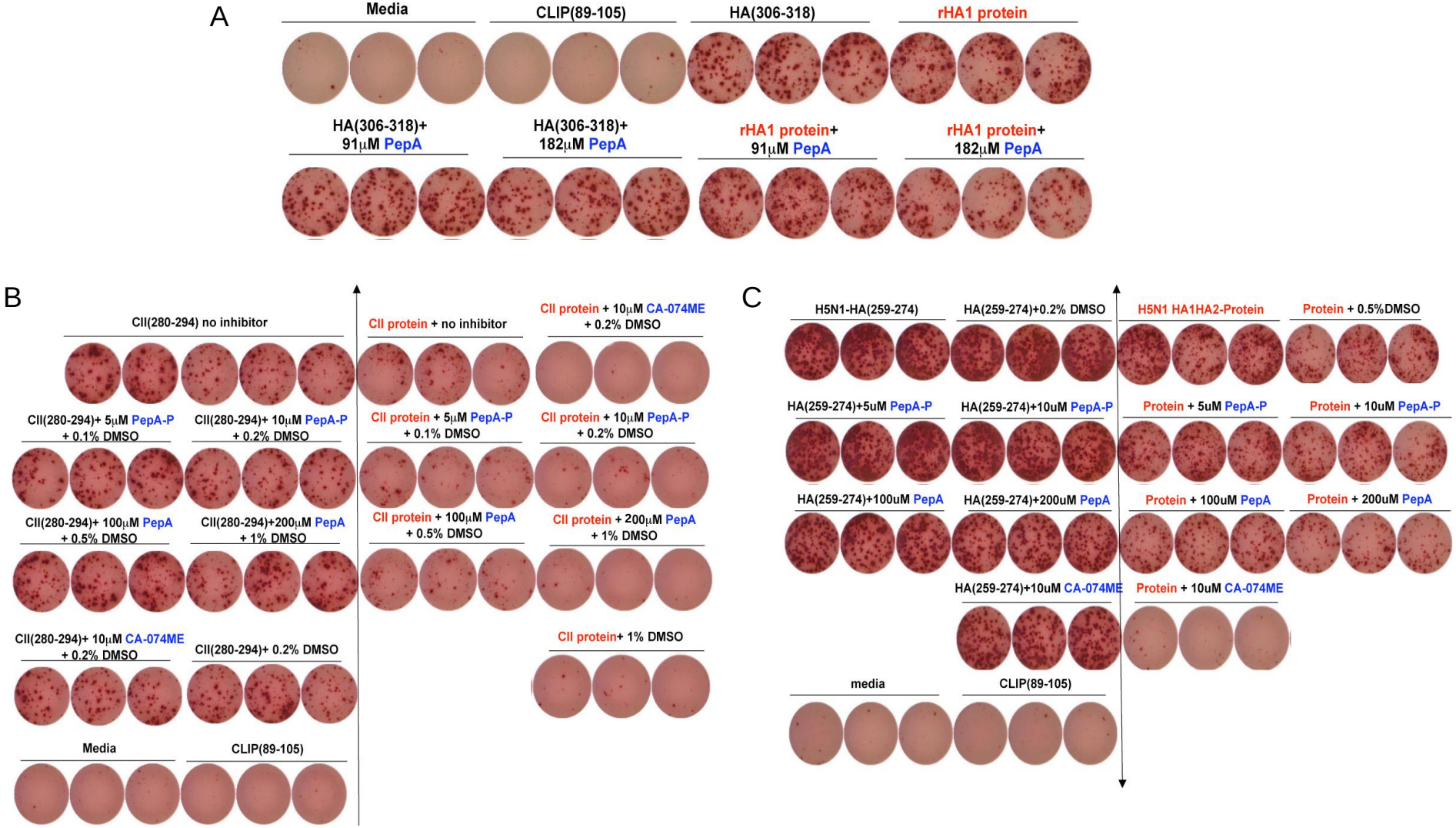
Supplementary Figure 5. Myosin(334-352) survives cathepsin digestion. Mass spectra of detecting Myosin(334-352) peptide, after digestion with CatB, CatH, and CatS. Undigested synthetic peptide, Myosin(334-352), is shown in top spectrum. Myosin(334-352) digested with the cathepsins for 1h, is shown in the middle spectrum. The bottom spectrum represents sample containing cathepsins only. Samples were run on MALDI.



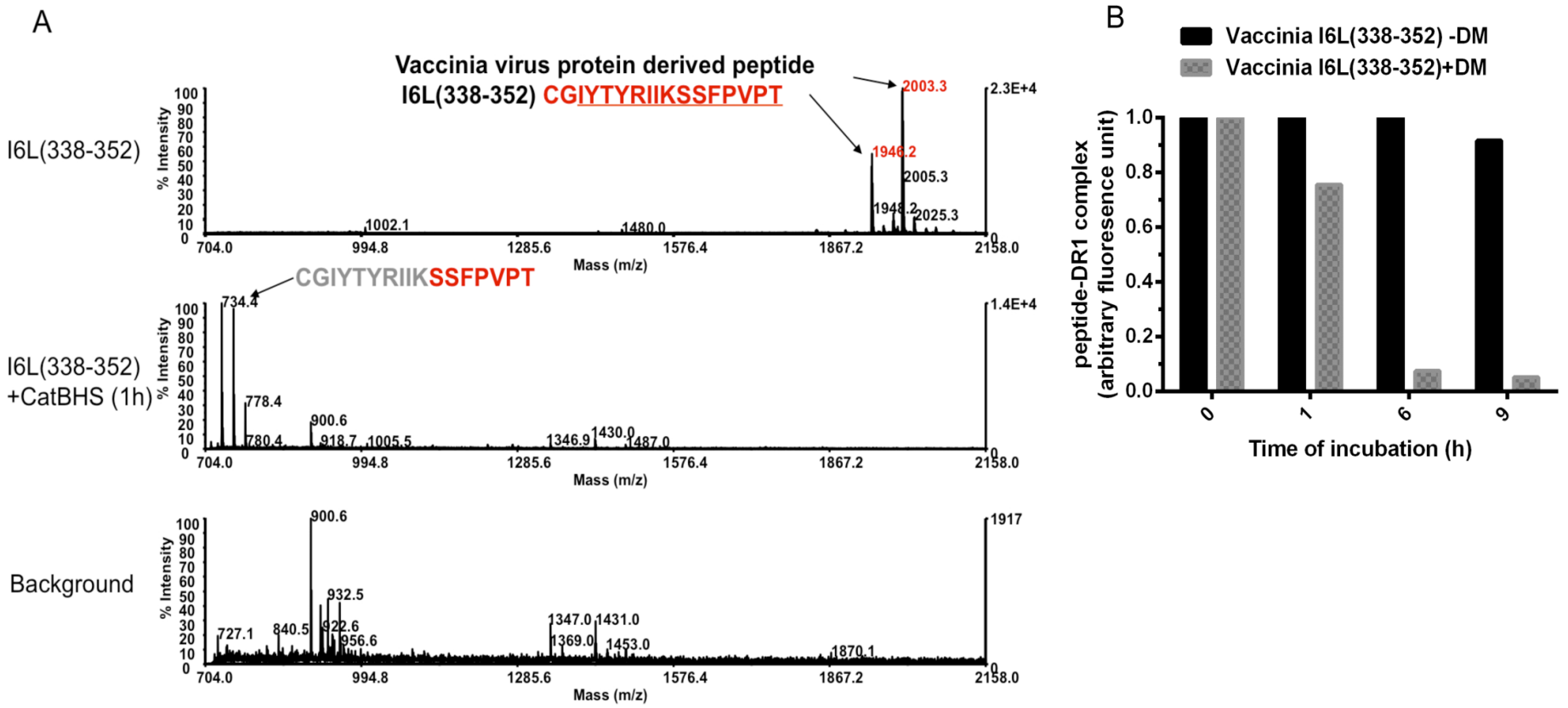
Supplementary Figure 6. MBP(89-101) survives cathepsin digestion. Mass spectra of detecting MBP(89-101) peptide, after digestion with CatB, CatH, and CatS. Undigested synthetic peptide, MBP(89-101), is shown in top spectrum. MBP(89-101) digested with the cathepsins for 1h, is shown in the middle spectrum. The bottom spectrum represents sample containing cathepsins only. Samples were run on MALDI. Anchor residues that bind to HLA-DR2b are underlined.



Supplementary Figure 7. Insulin B7-23 survives cathepsin digestion. Mass spectra detecting synthetic insulin B7-23, after digestion with CatB, CatH, and CatS for 1h at 37° C. Untreated synthetic peptide, insulin B7-23, is shown in top spectrum. Synthetic insulin B7-23 digested with the cathepsins for 1h, is shown in the middle spectrum. Background control that include cathepsins are shown in the bottom spectrum. Samples were run on MALDI. The experiments were done three times.



Supplementary Figure 8. Cathepsin D/E inhibitors do not inhibit processing and generation of immunodominant epitopes from three protein antigens. (**A**-**C**) show IFN-γ production as detected by the ELISPOT assay. HLA-DR1 mice were immunized with peptides HA(306-318) (**A**), CII(280-294) (**B**), or H5N1-HA(259-275) (**C**) in CFA. On day 8, cells from the draining LNs were cultured and stimulated with peptides, or proteins in the presence, or absence of CatD/E inhibitors, Pepstatin A (PepA) and Pepstatin A-Penetratin (PepA-P), or CatB inhibitor CA-074ME. Results show raw data of IFN-γ ELISPOT plates. Peptide presentation data are on the left and protein processing and presentation are to the right of the central axes.



Supplementary Figure S9. Vaccinia I6L(338-352) is both sensitive to cathepsin digestion and HLA-DM. (**A**) Mass spectra of detecting I6L(338-352) peptide, after digestion with CatB, CatH, and CatS for 1h at 37° C. Top spectrum shows untreated synthetic I6L(338-352). I6L(338-352) peptide digested with the cathepsins for 1h at 37 ° C is shown in the middle spectrum, and background is shown in the bottom spectrum. The samples were analyzed by MALDI. The experiments were repeated three times. When exposed to the cathepsins I6L(338-352) peptide was completely lost and the cleavage product, SSFPVPT, detected at m/z 734Da was much too short to bind DR1 stably. (**B**) Dissociation of fluorescently labeled I6L(338-352) from DR1 in the presence or absence of DM for indicated times at 37° C. Dissociation experiment was repeated three times.