Figure S1

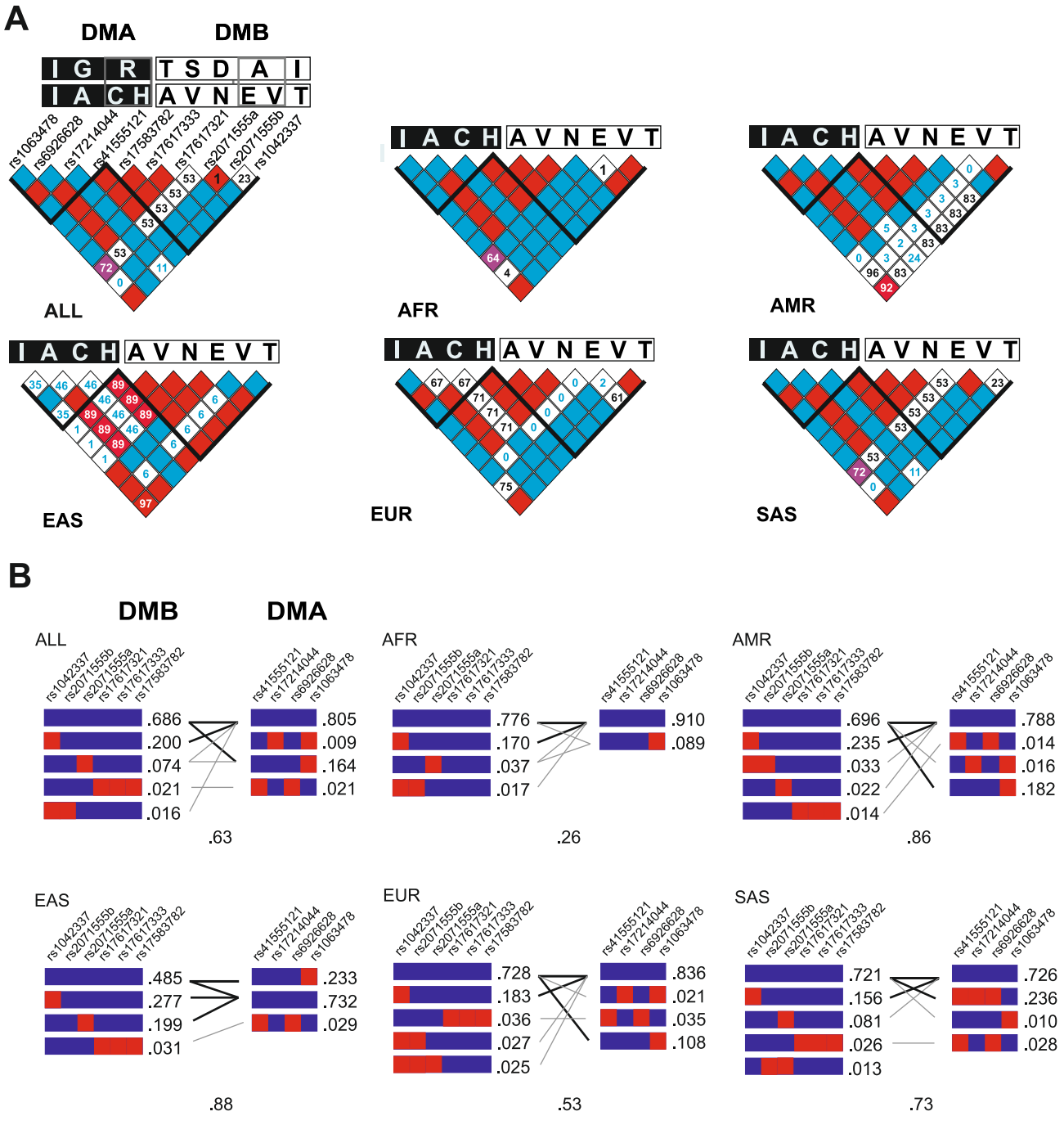

Figure S1. Analysis of Linkage Disequilibrium and haplotypes for DMA and DMB genes. A. Haploview-generated graphical representation of LD in HLA-DM gene region. The rsSNP described in different allelic variants of DMA and DMB spanning from position 32934628 to 32953121 according to GRCh37.p13 assembly were grouped into two blocks as indicated by black lines in the picture. Pairwise LD (D') is given for each SNP combination (showed in box). The color code in the haploview plot follows the standard color scheme for Haploview: white, |D'| < 1, LOD < 2; shades of pink/red, |D'| < 1, LOD = 2; blue, | D'| = 1, LOD < 2; red, |D'| = 1, LOD = 2. **B** Haplotype analysis for the gene markers used. The rsSNPs found for each allelic variant were investigated in the whole dataset and allowed us to group them in blocks depending whether they map to DMA or DMB. This grouping directly yield the association into the blocks as shown in the figure. For each of those, a red square represents the substitution taking place for each block. The frequencies for each DMA and DMB allele is shown in the right of the blocks and the overall measure of the LD between DMA and DMB genes is shown below.

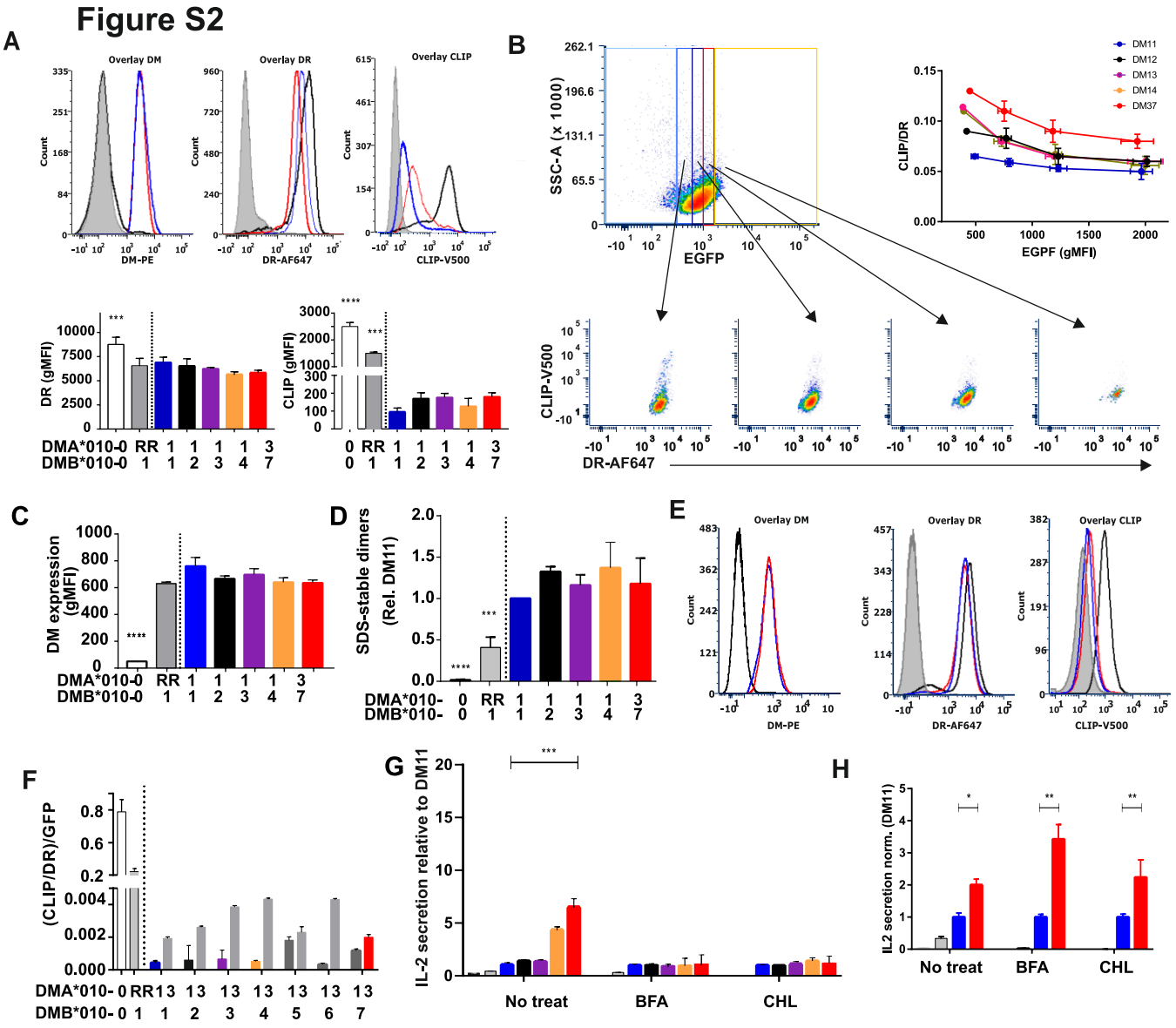

Figure S2. Cellular function of HLA-DM allotypes in T2.DR cell lines. A. Overlaid histograms of three exemplary samples of T2.DR3 cell lines stained with L243-Alexa-647 (HLA-DR; DR-A647) and CerCLIP.1-VB500 (CLIP; CLIP-V500) extracellularly, fixed and subsequently stained intracellularly with MaP.DM1-PE (HLA-DM; DM-PE). $5x10^4$ cells were measured per sample and GFP, DM-PE, DR-A647 and CLIP-V500 signals were recorded and are shown in black (DR3-NoDM), blue (DR3-DM11), red (DR3-DM37) and gray (isotype control). The lower panels show DR and CLIP surface expression of representative cell lines. Mean and SD of n = 3 independent experiments measured in duplicates. **B.** Parsing GFP into bins with different intensities allows defining the influence of DM in the CLIP/DR ratios. Representative examples of cell lines expressing five of the main haplotypes (DM11, 12, 13, 15 and 37) were used to measure the CLIP/DR ratios and reference them to the gMFI of GFP as a surrogate marker of DM. Mean and SD are shown for all cell lines measured in triplicates in n = 3 independent measurements. **C.** Bar-chart showing the average and SD values for the expression levels of DM in single-clone derived cell lines from the indicated DM allotypes. The gMFI of each cell line were determined by flow cytometry. Mean and SD of n = 3 independent experiments measured in duplicates. **D.** Quantitative analysis of the SDS-stable dimers using the five main DM haplotypes. **E.** Overlaid histograms as in (A) for representative T2.DR1 transfectants. **F.** Quantification of (CLIP/DR)/GFP ratios for all cell lines generated on the T2.DR1 background. Mean and SD values are shown for n = 3 independent experiments measured in duplicates. **G.** Antigen presentation assays using T2.DR3 cell lines stably expressing the indicated DM allotypes and human reactive $CD4^+$ T cells for an immunodominant MTB-A85B epitope (MTB-$A85B_{53-67}$: VPSPSMGRDIKVQFQ). T2 APCs were fed with 50 µg/ml MTB-85B antigen (protein) for 16 h (No treat); or treated either with 25 µM brefeldin A (BFA), or 100 µM Chloroquine (CHL). Mean and SD values from n = 3 independent experiments measured in duplicates (Average of IL-2 secretion of DM11 NoTreat= 75 ± 8 pg/ml; BFA= 2 ± 1 pg/ml; CHL= 3 ± 2 pg/ml) and then fixed. 10^5 of these APCs were incubated together with 10^5 responder cells for 48 h. IL-2 was measured by ELISA. **F.** Same as (A) using representative T2.DR1 cell lines. **H.** Antigen presentation assays using two T2.DR1 cell lines stably expressing no DM (white), DMmut (gray), DM11 (blue) and DM37 (red). A hybridoma cell line (9AF6) reactive to a MTB-A85B epitope (MTB-$A85B_{97-112}$: FEWYYQSGLSIVMPVG) presented by DR1 was used as reporter. T2.DR1 cell lines were used as APCs. They were fed with 20 µg/ml MTB-85B peptide for 6 h and then fixed. $4x10^4$ of these APCs were incubated together with 10^5 responder cells for 24 h. IL-2 was measured by ELISA. The bar chart shows the Mean and SD of n = 2 independent experiments measured in duplicates. All values were made relative to the most abundant DM allotype. APCs were either non-treated (No treat); or treated either with 25 µM brefeldin A (BFA), or 100 µM Chloroquine (CHL). (Average of IL-2 secretion of DM11 NoTreat= 115 ± 12 pg/ml; BFA= 38 ± 6 pg/ml; CHL= 46 ± 5 pg/ml).

Figure S3

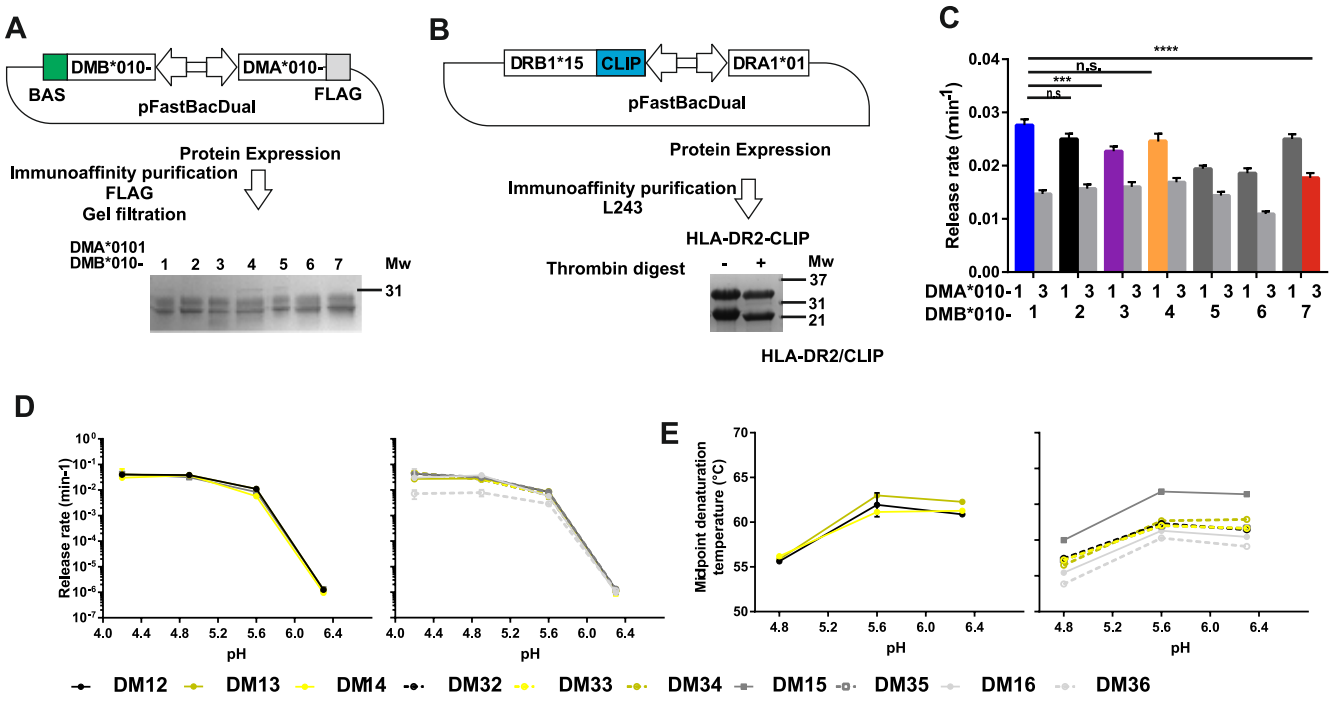

Figure S3. Mechanistic insights derived from the biochemical characterization of HLA-DM allotypes. A. Schematic representation of the constructs encoding for HLA-DM as well as their purification process. DMA*0101-DMB*010- allotypes purified are shown as example. **B**. Expression and purification of HLA-DR2 (DRA1*01-DRB1*15) fused to CLIP. Thrombin cleavage and subsequent gel filtration yield the final DR2/CLIP molecules used to load with MBP-FITC. **C.** Dissociation rates of MBP-FITC catalyzed by HLA-DM and measured by fluorescence polarization. All potential allotypes of DMA*0101 and DMA*0103 in combination with DMB* alleles were produced as described in (A). DR2/MBP-FITC (250 nM) complexes were incubated in the presence of different HLA-DM allotypes (300 nM) with an excess of unlabeled MBP (50 μM). Citrate phosphate buffer at pH 5.3 at 25 °C was used for the assay and FP was determined over 200 mins. The results shown here are the mean and SD of n = 3 independent experiments **D.** Release rates determined as in (B) under different pH conditions. Results show the mean and SD of n = 3 independent experiments measured in triplicates. **E.** Thermal stability of DMA-DMB allotypes at different pH values. The midpoint denaturation temperature of each protein is represented over the pH range of the assay. Results show the mean and SD of n = 6 independent experiments. Legend for (D) and (E) is shown on the right.

Figure S4

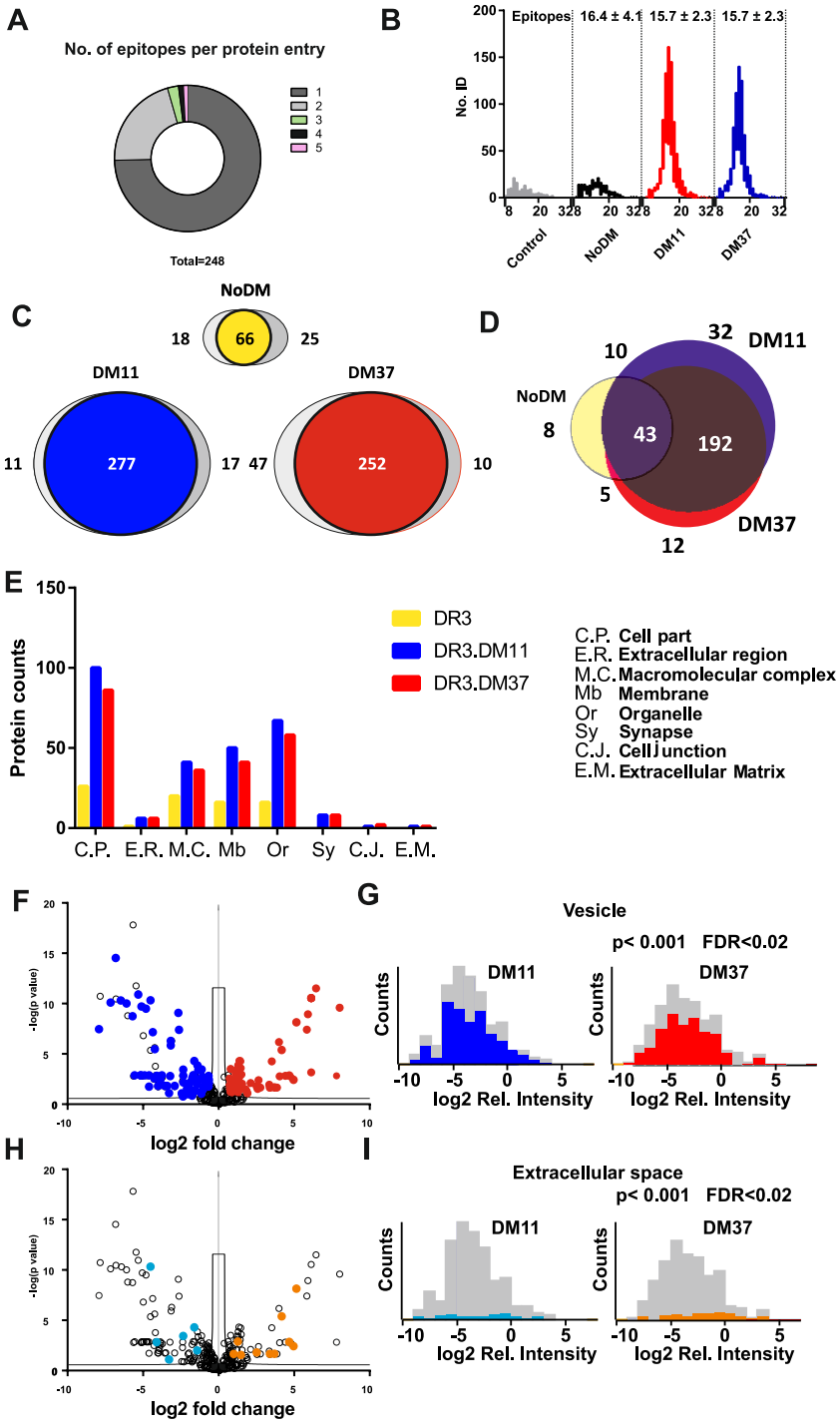

Figure S4. Analysis of the immunopeptidome presented by T2.DR3 cell lines expressing DM11 or DM37 allotypes. A. Number of epitopes identified per protein entry in the whole dataset. **B.** Length properties of the epitopes identified for each condition. Control refers to the immunoprecipitation control using T2 cells; noDM cells expressing only DR3; and the corresponding two DM expressing cell lines (DM11 and DM37). **C.** Extent of overlap between biological replicates of the analyzed conditions. **D.** Overlap between consensus epitopes found in both biological replicates of each condition. **E.** GO analysis of the cellular compartment of protein sources from which the consensus epitopes were identified. **F.** Volcano plot highlighting epitopes from sources annotated with the GO term vesicles and enriched in DM11 samples (blue) or DM37 (red). **G.** 1-D annotation enrichment for the protein sources identified in DM11 samples and. DM37. Total counts of protein sources *vs.* the log2 relative intensity these proteins sources is shown in light blue in the background. Overlaid are shown the counts of protein sources annotated as resident in vesicles for each condition according to the summed intensity of the corresponding epitopes (color-code as in (F)) **H.** Same as (F) but for epitopes annotated with the term extracellular space. DM11-enriched epitopes are shown in cyan and DM37-enriched epitopes are shown in orange. **I.** Same as (G) for protein sources annotated with the term extracellular space. For (G) and (I), from all annotations, only those two GO terms (vesicle and extracellular space) were found enriched for the DM37-samples when applying the Benjamin-Hochberg FDR, with truncation on both sides, and a threshold value of 0.02.