List of Supplementary Materials

HLA-DO modulates the diversity of the MHC-II self-peptidome

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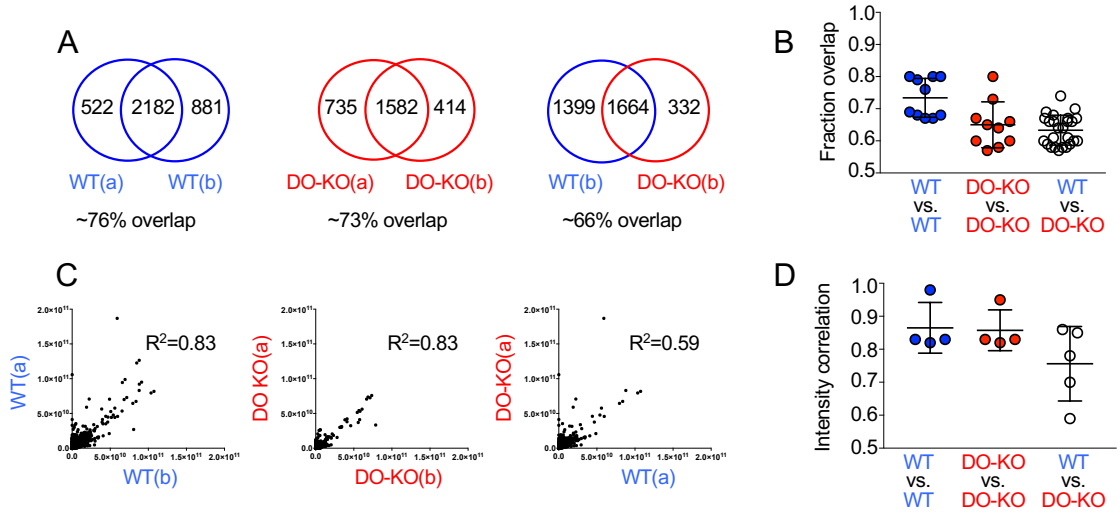
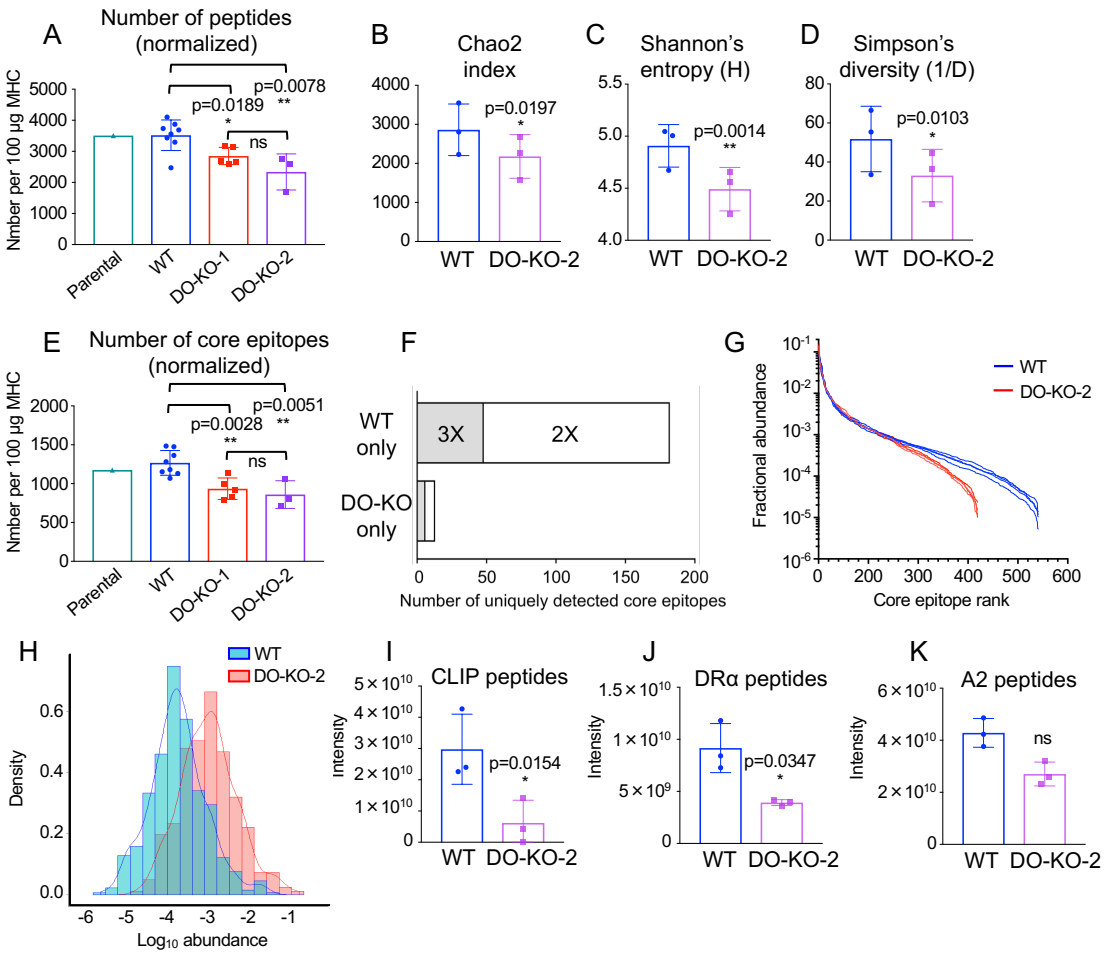
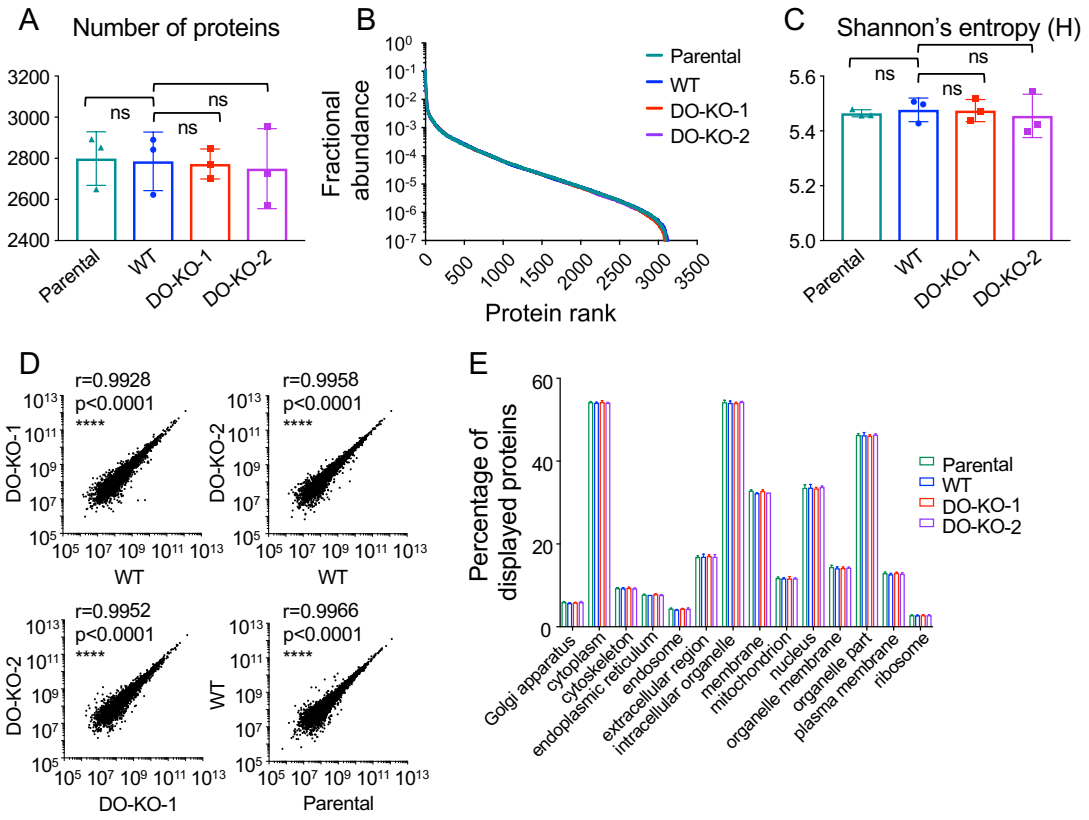


Supplementary Figure 1. Qualitative and quantitative mass spectrometry analysis. (A,B) Data-dependent acquisition analysis, comparing peptide sequences identified in fragmentation spectra (MS/MS) of WT and DO-KO samples. (A) Overlap between replicate WT samples (WT[a]) and WT[b]), replicate DO-KO-1 samples (DO-KO[a] and DO-KO[b]), or between WT and DO-KO samples. Each sample was analyzed in triplicate with peptide lists combined. (B) Peptide identification overlap for all pairwise combinations of 5 WT samples and 5 DO-KO-1 samples. (C,D) Data-independent acquisition analysis, comparing intensities in parent ion (MS1) spectra of ion signatures (frames) identified in both WT and DO-KO-1 samples. (C) Correlation of intensities in various samples analyzed in panel B. (D) MS1 intensity correlation for all pairwise combinations of 5 WT and 5 DO-KO-1 samples.

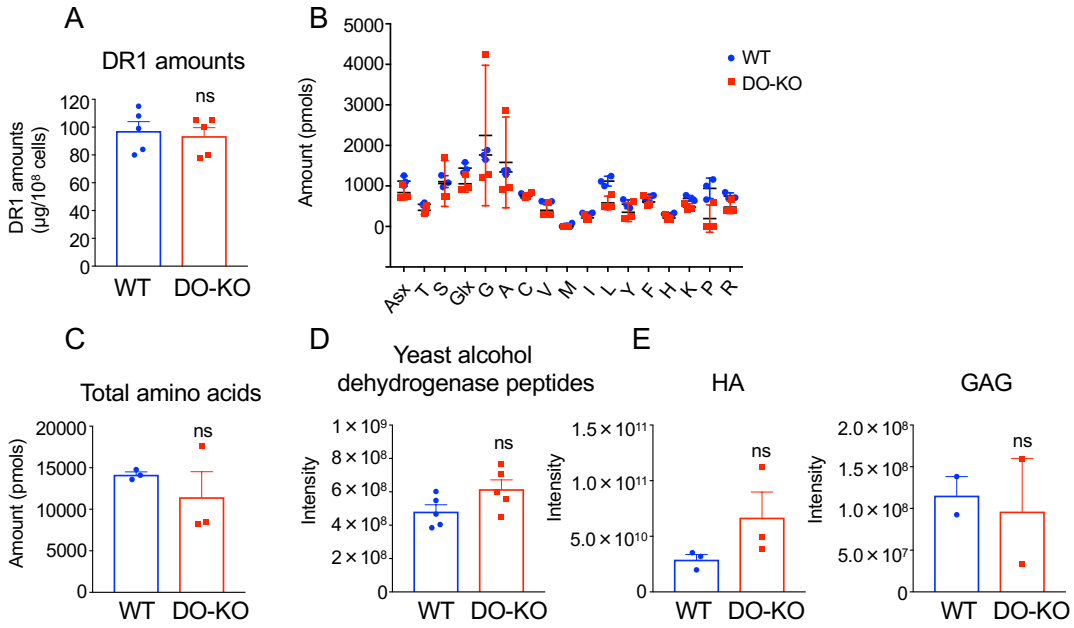


Supplementary Figure 2. Identification and characterization of peptides from DO-KO-2. (A) Peptides were eluted from WT and DO-KO Clone 2 (DO-KO-2) independently in 3 different experiments, peptides from WT and DO-KO Clone 1 (DO-KO-1) were eluted independently in 5 different experiments, and peptides were eluted once from the parental LG2 line as a control. The number of peptides eluted in each biological sample is represented as the normalized number of peptides/100µg HLA-DR1. The number of peptides eluted from all WT replicates is significantly greater than for DO-KO-1 and DO-KO-2. Mean ± SD for all biological replicates is shown, and an unpaired nonparametric Mann-Whitney test was used to calculate p-values. (B,C,D) Chao2 index (B), Shannon's entropy (C) and Simpson's diversity (D) indices indicate the WT peptidome is more diverse than the DO-KO-2 peptidome. Mean ± SD is shown, and a paired parametric t-test was used to calculate p-values. (E) The normalized number of core epitopes/100µg of HLA-DR1 was analyzed as described in the main text. The WT peptidome is comprised of a greater number of epitopes as compared to the peptidomes of DO-KO-1 and DO-KO-2. Mean ± SD for all biological replicates is shown, and an unpaired nonparametric Mann-Whitney test was used to

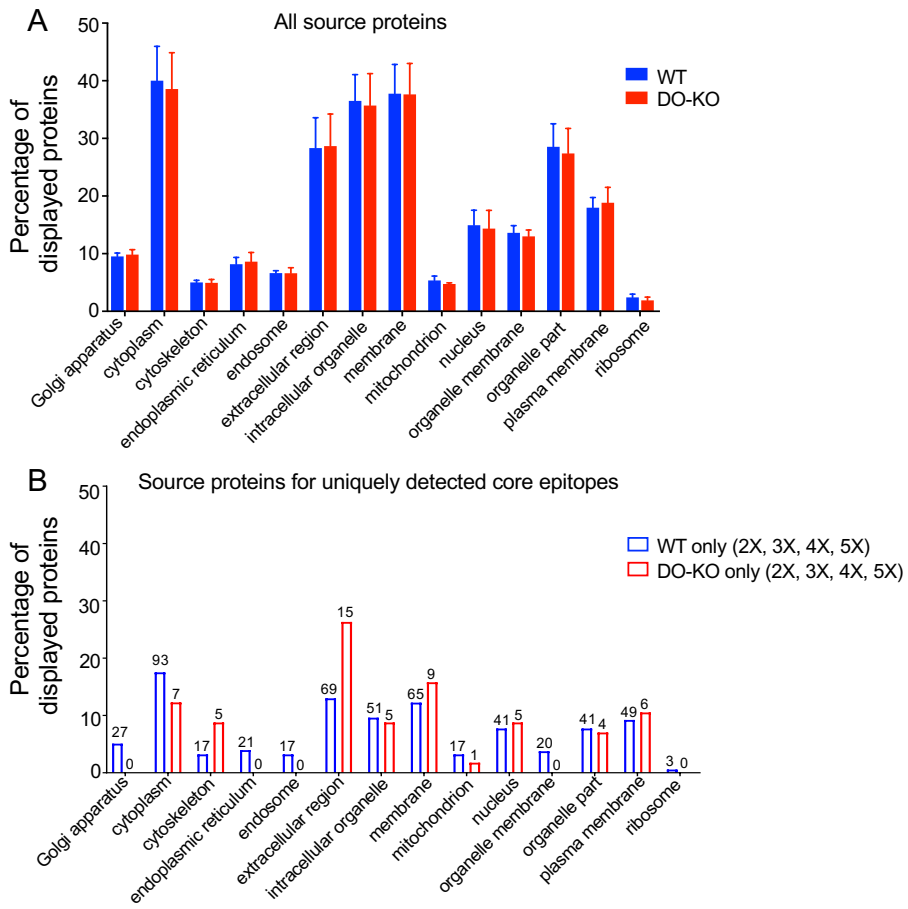
calculate p-values. (F) Greater numbers of unique core epitopes are identified in WT as compared to DO-KO-2 samples. Bar shading indicates number of replicate samples for which the core epitope was identified. For example, the gray bar labeled “3X” indicates epitopes identified in each of 3 WT samples and none of the 3 DO-KO-2 samples, the light bar labeled “2X” indicates samples identified in 2/3 WT samples and no DO-KO-2 samples, etc. (G) Rank abundance plot. Fractional intensity of core epitopes from WT (blue) or DO-KO-2 (red) in each biological sample is represented as an individual line. (H) Histogram of fractional intensities of core epitopes, overlaid with kernel density plot. (I,J,K) A set of peptides with known DM sensitivity was analyzed as described in the main text. Amounts of the DM-sensitive peptides CLIP (I) and DRα (J) were greater in WT cells as compared to DO-KO-2 cells. Amounts of the DM-resistant A2 peptides (K) were unaffected. Mean ± SD is shown, and a paired parametric t-test was used to calculate the p-values.



Supplementary Figure 3. Whole-cell proteomic analysis of WT and DO-KO clones. (A) Whole proteomes from parental, WT, DO-KO-1 and DO-KO-2 were analyzed in 3 independent experiments. Numbers of proteins identified were not significantly different between parental, WT and DO-KO clones. (B) Rank abundance plot. Average fractional intensity of proteins from parental (green), WT (blue), DO-KO-1 (red) and DO-KO-2 (purple) are represented as an individual line. (C) Shannon's entropy did not show any significant differences between different sample types. Mean ± SD is shown, and a paired parametric t-test was used to calculate p-values. (D) Protein intensity correlation plot between different sample types showed a strong Pearson's correlation coefficient as indicated. (E) Source protein cellular component analysis using GO terms shows similar overall subcellular localization distribution of proteins in parental, WT, DO-KO-1 and DO-KO-2 cells. No significant differences were observed between WT and DO-KO samples using elution data from 3 independent experiments (a multiple t-test correction using the Benjamini, Krieger and Yekutieli method was performed to adjust the p-value.)



Supplementary Figure 4. Equal amounts of DR1, eluted amino acids and mass spectrometry controls were detected in WT and DO-KO-1 samples. **(**A) To ensure that equal amounts of DR1-peptide complexes were used for peptide elution from WT and DO-KO cells, amounts of DR1 from the extracted membrane fraction was determined by ELISA, for all independent experiments. In every experiment, equal amounts of DR1 were present in the WT and DO-KO membrane fractions. **(**B,C) Amino acid analysis of peptides from WT and DO-KO was performed to ensure that equal amounts of peptides were eluted. Similar amounts of individual amino acids (B) were found in WT (blue) and DO-KO (red) in 3 independent experiments. Analysis of the total number of amino acids (C) in WT and DO-KO indicates similar total amounts of amino acids were eluted from both sets of cells. Mean ± SD is shown, and a paired parametric t-test was used to calculate p-values. (D) Yeast alcohol dehydrogenase peptides were spiked into the pools of eluted WT and DO-KO peptides as controls and were detected at equal levels in WT and DO-KO. (E) DR1-GAG (3 experimental replicates) or DR1-HA (2 experimental replicates) peptide complexes were spiked into the membrane fractions of WT and DO-KO clones as controls to ensure equal recovery of total peptides in WT and DO-KO. GAG and HA peptides were detected without any significant differences between WT and DO-KO.



Supplementary Figure 5. Source protein analysis for peptides eluted from WT and DO-KO-1 cells. (A) Source protein cellular component analysis using GO terms shows similar overall subcellular localization distribution of peptides in WT and DO-KO cells. No significant differences were observed between WT and DO-KO samples using elution data from 5 independent experiments (a multiple t-test correction using the Benjamini, Krieger and Yekutieli method was performed to adjust the p-value.) (B) Source protein cellular component analysis for uniquely detected core epitopes in at least 2, 3, 4 or 5 WT or DO-KO samples as defined in Fig 3B. Due to the small number of core epitopes detected uniquely in DO-KO samples, no statistical analysis was performed. Numbers above bars correspond to numbers of unique core epitopes in each compartment.