SUPPLEMENTAL FIGURE LEGENDS.

Figure S1. Pearson correlation of the identified peptides between individual runs. Pairwise comparisons of the ion peak intensities corresponding to the assigned peptides from each individual preparation of HLA-B*40:02 ligands. Pearson correlation values are indicated. Red squares show comparisons between triplicates from the same cell line.

Figure S2. Assignment of HLA-B*40:02 ligands on the basis of their theoretical affinity. (A) Theoretical affinity of identified peptides lacking the canonical motifs of B*35:03 (Pro2) and C*04:01 (Phe2 or Tyr2). Peptides above the horizontal dashed line (IC50: 10000 nM) were filtered out and those below the line were considered as B*40:02 ligands. Their numbers are indicated. (B) Theoretical affinity of peptides with IC50<10000 nM for HLA-B*40:02 towards HLA-B*35:02 and C*04:01. The medians are indicated in all cases as continuous red lines.

Figure S3. Venn diagram for the identified B*40:02 ligands. Numbers of peptides identified in the WT line (blue), WT1 (red), WT2 (green), KO1 (Yellow) and KO3 (brown). Overlapping sections show the peptides identified in more than one cell line.

Figure S4. Volcano plots from pairwise comparisons. Student's t-test between shared peptides for each pairwise comparison between the indicated cell lines was performed and p-values were plotted as a function of the normalized intensity ratio (IR). Vertical red lines represent IR=1.5, peptides outside the lines are overrepresented in one of the compared cell lines (IR>1.5) while peptides between the lines are similarly expressed (IR>1.0-1.5). Horizontal red lines represent a p-value of 0.05.

Figure S5. Global effects of ERAP2 on the length of HLA-B*40:02 ligands. Ion peak intensities relative to the total intensity of all the peptides from the indicated cell lines were calculated and their values were grouped according to peptide length. The data are means and standard deviation of three independent experiments. Statistically significant differences were calculated using the Student's t-test and their p-values are shown.

Figure S6. Comparison of the length of B*40:02 ligands with IR>1.0-1.5 upon ERAP2 depletion. The changes in the % frequency of peptides according to their length between the polyclonal and monoclonal WT lines (upper panels) and between WT and the ERAP2 KO lines

(lower panels) are shown. Statistically significant differences were calculated using the χ^2 test with Bonferroni correction and their p-values are indicated. Other differences are not significant. The numbers of peptides compared are given in **Table 1**.

Figure S7. Quantitative effects of ERAP2 depletion on N-terminal residue frequencies among HLA-B*40:02 ligands: comparisons involving clonal lines. Comparison of the P1 residue frequencies among peptides with IR>1.5 (left panels) or IR>1.0-1.5 (right panels) from the indicated cell lines. The numbers of peptides compared are given in **Table 1**. Statistically significant differences were calculated using the χ^2 test with Bonferroni correction. Residues statistically increased in the absence of ERAP2 are labeled with (*). Other differences were not significant or were increased in the presence of ERAP2.

Figure S8. N-terminal residue frequencies of HLA-B*40:02 ligands in the IR>1.0-1.5 subsets as a function of peptide length. Comparison of the P1 residue frequencies among 8-mers (upper panels), 9-mers (intermediate panels) and 10-mers (lower panels) with IR>1.0-1.5 from the indicated cell lines. The numbers of peptides compared were as follows. WT/KO1: 107/109 (8-mers), 750/674 (9-mers), and 191/182 (10-mers); WT/KO3: 111/122 (8-mers), 776/699 (9-mers), and 182/172 (10-mers). Statistically significant differences were calculated using the χ^2 test with Bonferroni correction. A single residue statistically increased in the absence of ERAP2 in one comparison is indicated by its p-value. Other differences were not significant.

Figure S9. Quantitative effects of ERAP2 depletion on residue frequencies at P3 among HLA-B*40:02 ligands: comparisons involving clonal lines. Comparison of the P3 residue frequencies among peptides with IR>1.5 (left panels) or IR>1.0-1.5 (right panels) from the indicated cell lines. The numbers of peptides compared are given in **Table 1**. Statistically significant differences were calculated using the χ^2 test with Bonferroni correction. Residues statistically increased in the absence or in the presence of ERAP2 are labeled with red or black asterisks, respectively. Other differences were not significant.

Figure S10. Length-dependent effects of ERAP2 depletion on P3 residue frequencies among HLA-B*40:02 ligands. Comparison of the P3 residue frequencies among 8-mers (upper

panels), 9-mers (intermediate panels) and 10-mers (lower panels) with IR>1.5 from the indicated cell lines. The numbers of peptides compared were as follows. WT/KO1: 376/585 (8-mers), 2038/2136 (9-mers), and 701/389 (10-mers); WT/KO3: 344/613 (8-mers), 2100/2028 (9-mers), and 735/371 (10-mers). Statistically significant differences were calculated using the χ^2 test with Bonferroni correction. Residues statistically increased in the presence or absence of ERAP2 in each comparison are labeled with black and red asterisks, respectively and their p-values are shown in the table at the bottom.

Figure S11. P3 residue frequencies of HLA-B*40:02 ligands in the IR>1.0-1.5 subsets as a function of peptide length. Comparison of the P3 residue frequencies among 8-mers (upper panels), 9-mers (intermediate panels) and 10-mers (lower panels) with IR>1.0-1.5 from the indicated cell lines. The numbers of peptides compared were as follows. WT/KO1: 107/109 (8-mers), 750/674 (9-mers), and 191/182 (10-mers); WT/KO3: 111/122 (8-mers), 776/699 (9-mers), and 182/172 (10-mers). No Statistically significant differences were observed using the χ^2 test with Bonferroni correction.

Figure S12. Hydrophobicity and affinity of HLA-B*40:02 ligands. (A) Comparison of the mean hydropathy of residues at the indicated positions among B*40:02 ligands of all sizes in the IR>1.5 (left panels) and IR>1.0-1.5 subsets (right panels) from WT and KO1 and WT and KO3 cells. The numbers of peptides compared are given in **Table 1.** (B) The same comparisons as in panel A were carried out for P1 to P9 among nonamers of the same subsets (The N. of 9-mers compared were: IR>1.5, WT/KO1, 2038/2136; WT/KO3, 2100/2028; IR>1.0-1.5, WT/KO1, 750/674; WT/KO3, 776/699. (C) Theoretical affinity of the B*40:02 ligands from the indicated subsets in the WT/KO1 (left) and WT/KO3 (right) comparisons. red lines indicate the median values. No statistical differences were observed.