Supplementary materials and methods

Generation of antigen-specific CD4+ T-cell lines and clones

CD4+ T-cells were purified from peripheral blood mononuclear cells (PBMCs) by positive selection with magnetic microbeads (Miltenyi Biotec, Auburn, CA, USA) (1). Monocyte-derived dendritic cells (DCs) were generated from CD14+ cells by in vitro culture, as described previously (2), and used as antigen-presenting cells (APCs) to induce antigen-specific CD4+ T-cells. Dendritic cells (1 × 10^4/well) were pulsed with 10 µg/mL long peptide (LP) for 3 h and irradiated (45 Gy), and subsequently mixed with CD4+ T-cells (3 × 10^4/well) in 200 µL AIM-V supplemented with 5% human decomplemented plasma in each well of a 96-well, flat-bottomed culture plate. After 7 days, half of the medium was removed from each culture, and fresh medium (100 µL/well) containing irradiated (50 Gy) autologous PBMCs (1 × 10^5) pulsed with peptide (10 µg/mL) and 5 ng/mL recombinant human interleukin 7 (rhIL-7) was added. Two days after the second stimulation with peptide, rhIL-2 was added to each well (10 IU/mL). A week later, the stimulated CD4+ T-cells in each well were analyzed for specificity in IFN-γ ELISPOT assays. The T-cells showing a specific response to the cognate peptide were transferred to 24-well plates and re-stimulated at weekly intervals.
with irradiated autologous PBMCs (1 × 10^6/well) pulsed with the peptide in medium supplemented with rhIL-2 (20 IU/mL) and rhIL-7 (5 ng/mL).

**CD107a mobilization assay**

To identify degranulating CD4+ and CD8+ T lymphocytes stimulated by the peptides, CD107a on the cell surface was analyzed by flow cytometry (3, 4). In brief, a CD107a mobilization assay was performed as described previously (5). The cognate-LP, SP, or control LP (1 µg/mL) were added as stimulants, and FITC-labeled anti-human CD107a mAb or FITC-labeled isotype control mouse IgG1 and monensin were added to each well. Cells were cultured for 5 h at 37°C. After culture, the peptide-stimulated Th cells CTLs were stained with PE-conjugated anti-human CD4 antibody (eBioscience, San Diego, CA), or PerCP-labeled anti-human CD8 mAb (BioLegend) and PE-labeled tetramer of the HLA-A*24:02/KIF20A-A2466-74 complex (MBL, Nagoya, Japan). Cells were analyzed on a FACScan (BD Bioscience, Bedford, MA) flow cytometer.

**In vitro induction of KIF20A-A2466-75 SP-specific CTLs by stimulation of PBMCs with KIF20A60-84-LP**

To assess induction of KIF20A-A2466-75 SP-specific CTLs from an HLA-A24+ donors (HD4 and HD8) by simulation with KIF20A60-78-LP in vitro, PBMCs (2 ×
10⁶/well of 24-well plates) were incubated with KIF20A₆₀₋₈₄-LP (30 µg/mL) for 2 weeks. On day 0 and day 7, KIF20A₆₀₋₈₄-LP (30 µg/mL) was added, and then rhIL-2 (20 U/mL) was added on day 9 and day 11. On day 14 of in vitro stimulation with KIF20A₆₀₋₈₄-LP, the cells were harvested and the number of IFN-γ producing T-cells (1 × 10⁵/well) in response to stimulation with KIF20A-A₂₄₆₆₋₇₅ SP-pulsed C₁R-A₂₄₀₂ cells (2 × 10⁴/well) was counted by ELISPOT assay.

**CD107a expression of KIF20A-specific CD₈⁺ T-cells expanded by activated KIF20A-specific Th cells**

CD107a expression of KIF20A-A₂₄₆₆₋₇₅ SP-specific CTLs cultured in the presence of activated KIF20A₈₀₉₋₈₃₃-LP-specific Th cells for 1 week was examined. KIF20A₈₀₉₋₈₃₃-LP-specific bulk CD₄⁺ T-cells (1 × 10⁵ cells/well, 48-well plates) and KIF20A-A₂₄₆₆₋₇₅ SP-specific bulk CD₈⁺ T-cells (1 × 10⁵ cells/well) derived from HLA-A₂₄⁺/DR₁₅⁺ HD4 were cultured with autologous DCs (2 × 10⁴ cells/well) in the presence of KIF20A-A₂₄₆₆₋₇₅ SP (10 µg/mL; SP alone), KIF20A-A₂₄₆₆₋₇₅ SP + Control LP (10 µg/mL; Control LP + SP), or KIF20A-A₂₄₆₆₋₇₅ SP + KIF20A₈₀₉₋₈₃₃-LP (10 µg/mL; KIF20A₈₀₉₋₈₃₃-LP + SP) without addition of any cytokine. Induction of KIF20A-A₂₄₆₆₋₇₅ SP-reactive bulk CTLs from an HLA-A₂₄⁺/DR₁₅⁺ donor (HD4) by stimulation with KIF20A-A₂₄₆₆₋₇₅ SP was performed as described previously (5, 6).
After 1-week *in vitro* culture with peptides, the cultured cells were stained with PE-labeled tetramer of the HLA-A*24:02/KIF20A-A24<sub>67-75</sub> complex (MBL, Nagoya, Japan) (7), FITC-labeled anti-human CD107a mAb (MBL, Nagoya, Japan), and PerCP-labeled anti-human CD8 mAb (BioLegend).

**In vivo cross-priming assay**

HLA-A24 (HHH) transgenic mice (Tgm) were kindly provided by Dr. F. A. Lemonnier (8). Mice were immunized by intravenous transfer of KIF20A<sub>60-84</sub>-LP-pulsed (50 µg/mouse, 3h) bone marrow-derived DCs (BM-DCs, 5 × 10<sup>5</sup> cells/mouse), and then intradermally injected at the base of the tail with KIF20A<sub>60-84</sub>-LP solution (100 µg/mouse) emulsified in incomplete Freund adjuvant (IFA; used to stimulate the immune system), twice at 7-day intervals. Seven days after the third vaccination with KIF20A<sub>60-84</sub>-LP, CD8<sup>+</sup> T cells were isolated from inguinal lymph nodes by positive selection with magnetic microbeads (Miltenyi Biotec, Auburn, CA, USA). The number of IFN-γ producing CD8<sup>+</sup> T cells (2.5 × 10<sup>5</sup>/well) in response to stimulation with KIF20A-A24<sub>67-75</sub> SP-pulsed BM-DCs (2 × 10<sup>4</sup>/well) was counted by *ex vivo* ELISPOT assay (2, 9).
References


Supplementary figure legends

Supplementary figure 1. KIF20A-derived and promiscuous HLA class II-binding peptides predicted by a recently developed computer algorithm. A, the amino acid sequence of the human KIF20A protein was analyzed using an algorithm (IEDB analysis resource, consensus method), http://tools.immuneepitope.org/analyze/html/mhc_II_binding.html. Numbers on the horizontal axis indicate amino acid positions at the N-terminus of KIF20A-derived 15-mer peptides. Higher consensus percentile rank indicates stronger binding affinity to HLA class II molecules. B, The 25-mer LPs, KIF20A_{60-84}-LP and KIF20A_{809-833}-LP with high consensus percentile ranks for 3 HLA-class II allelic (DRB1*04:05, DRB1*15:02, and DRB4*01:03) products and bearing 9- or 10-mer CTL-epitopes recognized by HLA-A2- or HLA-A24-restricted CTLs were synthesized (A, right and left black bar). The 24-mer LP KIF20A_{494-517}-LP with high consensus percentile ranks for multiple HLA-class II allelic products, which does not include a known CTL-epitope, was also synthesized (A, middle black bar).

Supplementary figure 2. Induction of KIF20A-LPs-specific Th cells from healthy donors. A, Th cells generated by stimulation of purified CD4$^+$ T-cells with
KIF20A$_{60-84}$-LP were restimulated with autologous PBMCs pulsed with KIF20A$_{60-84}$-LP. The number of IFN-$\gamma$-producing Th cells was analyzed by ELISPOT assay. The HLA class-II genotype of HLA-DR15- and DP2-negative donor (HD3) is indicated at the top of the panels. This result suggests that KIF20A$_{60-84}$-LP-specific Th cells from HD3 are restricted by HLA-DR4 or DR53. Data are presented as the mean ± SD of triplicate assays. Representative data from at least 5 independent experiments with similar results are shown. B, The Th cells generated from HLA-DR15$^+$ donor (HD4) by stimulation with KIF20A$_{809-833}$-LP were restimulated with autologous PBMCs pulsed with KIF20A$_{809-833}$-LP. The HLA class-II genotype of HD4 is indicated at the top of the panels. The Th cells were suggested to be restricted by HLA-DR. C, induction of HLA-DR4-restricted KIF20A$_{494-517}$-LP-specific Th cells from an HLA-DR4$^+$ healthy donor (HD2). KIF20A-specific Th cells were generated from HD2 by stimulation of purified CD4$^+$ T-cells with KIF20A$_{494-517}$-LP-pulsed autologous DCs or PBMCs. The generated Th cells were restimulated with autologous PBMCs or L-cells pulsed with KIF20A$_{494-517}$-LP. The number of IFN-$\gamma$-producing Th cells was analyzed by ELISPOT assay. Data are presented as the mean ± SD of triplicate assays. Representative data from at least 3 independent experiments with similar results obtained from HD2 are shown. The HLA class-II genotype of HD2 is indicated above
the panels. The underlined HLA-class II alleles (\textit{HLA-DRB1*04:05}) encode HLA-class II-molecule presenting the peptides to Th cells.

**Supplementary figure 3.** Enhanced induction of KIF20A-SP-specific CTLs by KIF20A-LP-specific CD4\(^+\) T-cells. A, PBMCs from an HLA-A2\(^+\)/DR53\(^+\) healthy donor (HD2), from which an HLA-DR53-restricted KIF20A\(_{809–833}\)-LP-specific Th-clone was generated, were cultured for 11 days with KIF20A-A2\(_{809–817}\) SP (SP), KIF20A\(_{809–833}\)-LP (LP), KIF20A-A2\(_{809–817}\) SP + KIF20A\(_{809–833}\)-LP (SP + LP), KIF20A\(_{809–833}\)-LP + KIF20A\(_{809–833}\)-LP-specific Th clone (LP + Th-clone) or SP + LP + KIF20A\(_{809–833}\)-LP-specific Th-clone (SP + LP + Th-clone). On day 11, the cells were stained with KIF20A-A2\(_{809–817}\) SP-specific tetramer with an anti-human CD8 mAb and were analyzed by flow cytometry. B, Representative KIF20A-A2\(_{809–817}\) SP-specific tetramer staining (gated on CD8\(^+\) T-cells) obtained from 3 independent experiments with similar results is shown. C, CD107a expression of KIF20A-A2\(_{466–75}\) SP-specific CD8\(^+\) T-cells expanded by activated KIF20A\(_{809–833}\)-LP-specific Th cells. KIF20A\(_{809–833}\)-LP-specific bulk CD4\(^+\) T-cells and KIF20A-A2\(_{466–75}\) SP-specific bulk CD8\(^+\) T-cells derived from HLA-A24\(^+\)/DR15\(^+\) HD4 were cultured with autologous DCs in the presence of KIF20A-A2\(_{466–75}\) SP (SP alone), KIF20A-A2\(_{466–75}\) SP + Control LP.
(Control LP + SP), or KIF20A-A24<sub>66-75</sub> SP + KIF20A<sub>809-833</sub>-LP (KIF20A<sub>809-833</sub>-LP + SP) without addition of any cytokine. After 1-week in vitro culture with peptides, the cultured cells were stained with PE-labeled tetramer of the HLA-A*24:02/KIF20A-A24<sub>67-75</sub> complex and PerCP-labeled anti-human CD8 mAb. Data are presented as the mean ± SD of triplicate assays. Representative data from 3 independent experiments with similar results are shown. D, After 1-week in vitro culture with peptides, the cultured cells were re-stimulated with KIF20A-A24<sub>66-75</sub> SP and stained with PE-labeled tetramer of the HLA-A*24:02/KIF20A-A24<sub>67-75</sub> complex, FITC-labeled anti-human CD107a mAb, and PerCP-labeled anti-human CD8 mAb. The absolute number of KIF20A-A24<sub>66-75</sub> SP-specific CTLs expressing CD107a on the cell surface after re-stimulation with KIF20A-A24<sub>66-75</sub> SP was shown. Data are presented as the mean ± SD of triplicate assays. Representative data from 3 independent experiments with similar results are shown.

**Supplementary figure 4.** Induction of KIF20A-A24<sub>66-75</sub> SP-specific CTLs in mice immunized with KIF20A<sub>60-84</sub>-LP. HLA-A24 Tgm were immunized with KIF20A<sub>60-84</sub>-LP. After the third vaccination with KIF20A<sub>60-84</sub>-LP, mouse CD8<sup>+</sup> T-cells in the inguinal lymph nodes were stimulated with BM-DC pulsed with
KIF20A-A24<sub>66-75</sub> SP. The number of IFN-γ-producing murine CD8<sup>+</sup> T-cells was analyzed by *ex vivo* ELISPOT assay. Representative data from 5 independent experiments with similar results are shown.