Supplementary information

Peptide-independent stabilization of MHC class I molecules breaches cellular quality control

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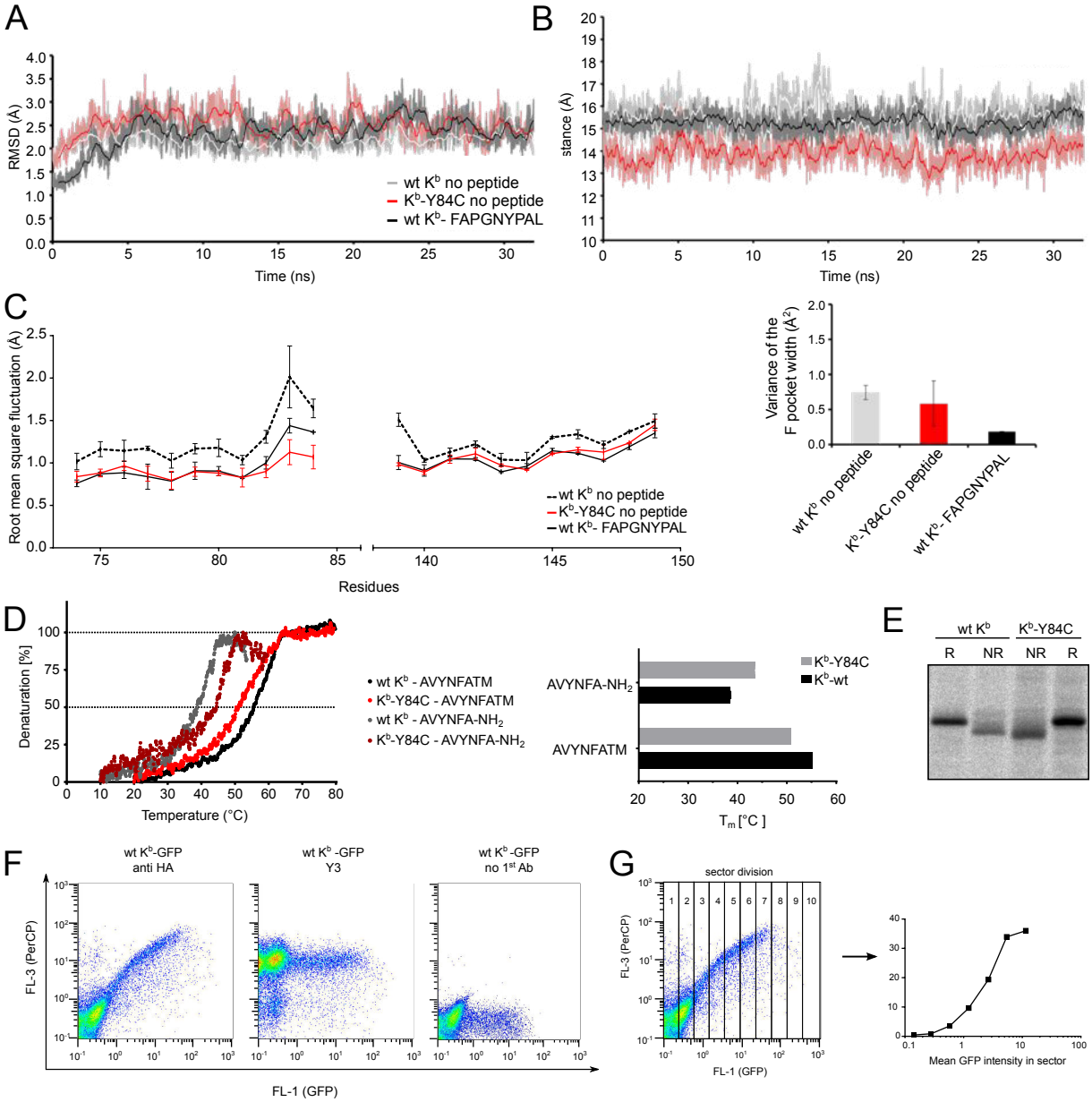
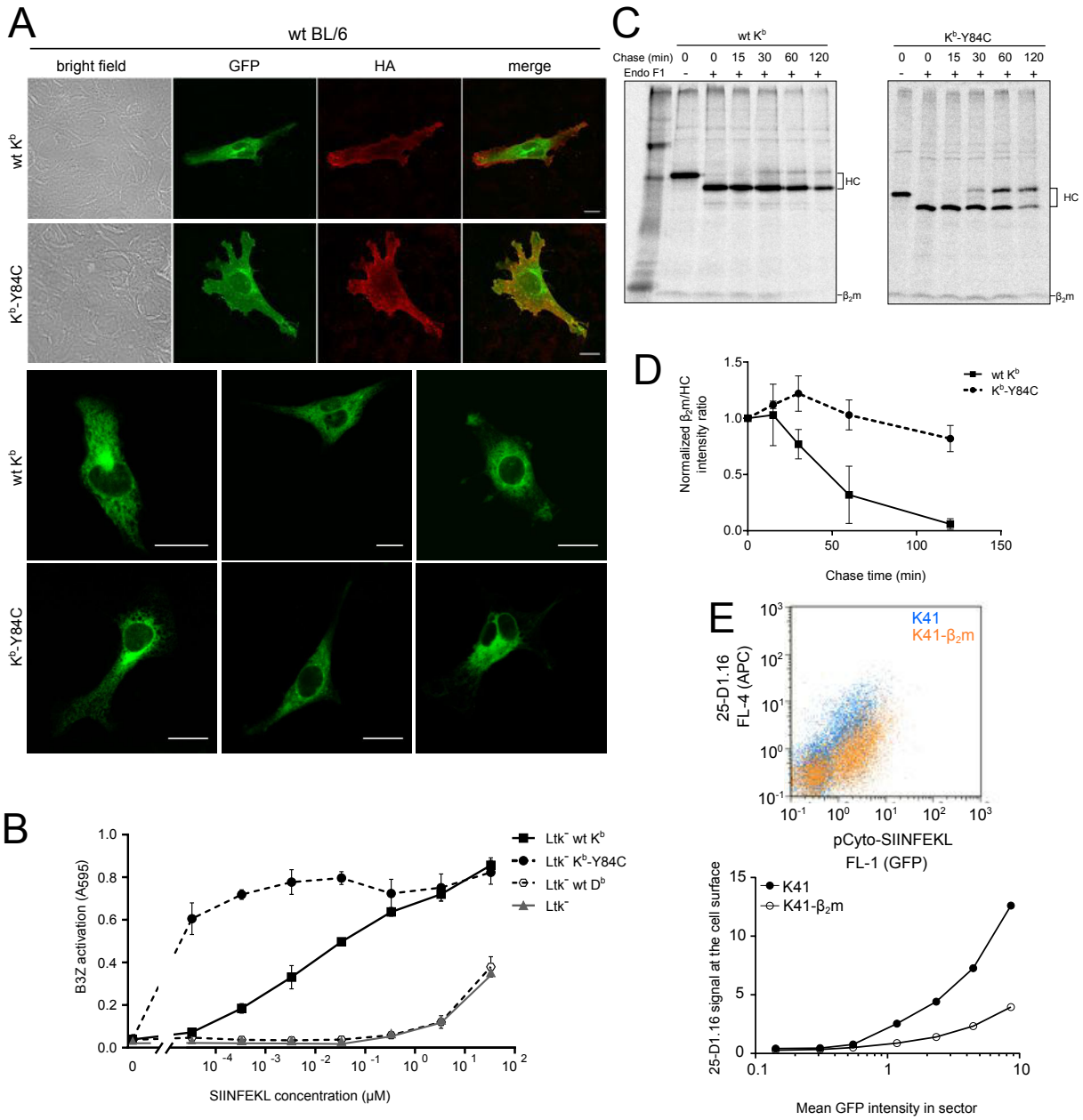


SUPPLEMENTARY FIGURE S1. A-C. In K^b-Y84C, the mobility of the polypeptide backbone in the F pocket region is restrained, whereas the overall backbone mobilities of wild type K^b and K^b-Y84C are very similar. Data from a 32 ns molecular dynamics simulation following 8 ns equilibration; see also Figure 1B, C, D. **A**. The mobilities of the polypeptide backbones of the three K^b proteins (wild type K^b without peptide, wild type K^b in complex with FAPGNYPAL, and K^b-Y84C without peptide) are similar. Root mean square deviations (RMSD, calculated relative to the starting crystal structure) of the α carbons of the entire polypeptide backbone of the K^b proteins during the simulations. **B**. The width of the F pocket is restrained in K^b-Y84C. Top: F pocket width (measured as the distance between the centers of mass of the α carbons of residues 73 to 84 and the α carbons of residues 139 to 150) plotted against simulation time. Bottom: Variance of the F pocket widths over the course of the simulation. **C**. Root mean square fluctuations (RMSF) of the α carbons of the residues in the direct vicinity of residues 84 (left) and 139 (right), the site of the C84-C139 disulfide bond in K^b-Y84C. **D**. Stability of K^b-peptide complexes determined by circular dichroism. Complexes of K^b with murine β_2m and with the indicated peptides were prepared by in vitro folding, subjected to heat denaturation, and circular dichroism was recorded at 218 nm. The transition midpoint (melting temperature, Tm) is the 50% line intersection of the curves and depicted as a bar chart on the right panel. AVYNFA-NH_2 has a C-terminal amide to prevent repulsion of the truncated carboxyl terminus in the binding groove. **E**. The additional disulfide bond of K^b-Y84C forms in vivo. COS cells were transiently transfected with K^b heavy chains containing an N-terminal HA tag, radioactively labeled with [^{35}S] methionine, and lysed. 20 mM N-ethylmaleimide was added to the lysates to block free thiol groups. K^b molecules were immunoprecipitated with anti-HA MAb, and immunoisolates were denatured (lanes NR) or denatured and reduced (lanes R) and resolved on a 6-16% SDS-polyacrylamide gel. **F**. Quantification of K^b expression at the cell surface. Flow cytometry plots. K^b GFP was expressed in BL/6 fibroblasts. Cells were stained with monoclonal antibodies to the N-terminal HA (influenza hemagglutinin) tag and subsequently with PerCP-conjugated anti-mouse secondary antibody, and then the cells were analyzed by flow cytometry. Plots show for each cell the surface levels of K^b molecules (y axis) vs. the GFP signal, i.e., the total cellular amount of K^b GFP (x axis). **G**. Quantification of flow cytometry data. Flow cytometry plots were divided into ten numbered sectors (left panel). For sectors 1 to 8 of each sample, the mean intensity of surface staining (mean y) was calculated and plotted against the GFP mean fluorescence intensity (mean x) (right panel). This way of quantification was used in Figures 3 ABC,4A, and S2E.



SUPPLEMENTARY FIGURE S2. A.K^b-GFP constructs are expressed at the cell surface in wild type cells. Wild type BL/6 fibroblasts were transfected with HA-K^b-GFP constructs (green; upper and lower panel), and surface K^b molecules were detected by staining with an anti-HA MAb (red; upper panel only). **B**. K^b-Y84C is highly efficient in presenting peptide to T cells. Ltk- cells (H-2k) were transiently transfected with MHC-I heavy chains as indicated, incubated with indicated amounts of SIINFEKL peptide, and cocultured with B3Z cells. T cell activity was measured by a β galactosidase assay. The mean fluorescence intensity levels of transiently transfected cells were: untransfected, 112 and 125 (with K10-56 (K^b) and B22.249 (D^b) antibodies, respectively; see materials and methods); wild type D^b, 2682 (B22.249); wild type K^b, 703 (K10-56); K^b-Y84C, 650 (K10-56). **C**. K^b-Y84C has an increased affinity to β_2m.Image of the entire SDS-PAGE gel of a pulse chase experiment performed in TAP deficient BL/6 fibroblasts, similar to that shown in Figure 5 (center panel), showing the band for co-immunoprecipitated β_2m at the bottom. **D**. Quantification of the β_2m and K^b heavy chain bands. The errors are ± SEM, n=2. **E**. Overexpression of β_2m impairs high affinity peptide selection in wild type cells. K41 fibroblasts were stably transduced with human β_2m. Wild type and β_2m-overexpressing cells were infected with lentiviruses coding for MSIINFEKL-IRES-GFP gene product (pCyto-SIINFEKL). Surface K^b-SIINFEKL complexes were detected by staining non-permeabilized cells by 25-D1.16 antibody. Dot plots (top) were quantified as explained in Fig.S1G and depicted in a linear graph (bottom).

Supplementary Table 1. Statistics of diffraction data and structure refinement

	K[b]-Y84C/AVYNFATM
Statistics of diffraction data	
PDB ID	4HS3
Wavelength (Å)[a]	0.87260
Resolution (Å)	40.9-2.1 (2.2-2.1)
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	$a = 45.6$ $b = 90.6$, $c = 137.4$
No. of observed reflections	111070 (14465)
No. of unique reflections	32975 (4754)
Redundancy	3.4 (3.0)
Completeness (%)	97 (98)
R_{merge}[b] (%)	13.0 (60.0)
‹I/σ(I)›	7.8 (2.6)
Statistics of refinement and structure model	
No. of molecules in ASU	1
R_{cryst}[c] (%)	18.0
R_{free}[d] (%)	22.9
Number of atoms	
protein	3154
water	418
ligand	13
RMSD from ideal geometry	
bond lengths (Å)	0.008
bond angles (°)	1.07
Average B factor (Å^2)[e]	
Protein	24.20
Solvent	33.10
Ramachandran plot (%)	
Most favored regions	92.1
Allowed regions	7.6
Generously allowed regions	0.3
Disallowed regions	0.0

[a] Number in parentheses indicate the outer-resolution shell.

[b] $R_{merge} = \sum_{hkl} \sum_i \left| I_i(hkl) - \langle I(hkl) \rangle \right| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and ‹I (hkl)› is the weighted average intensity for all observations i of reflection hkl.

[c] $R_{cryst} = \sum_{hkl} |F_{obs} - F_{calc}| / \Sigma_{hkl} |F_{obs}|$, where $|F_{obs}|$ and $|F_{calc}|$ are the observed and calculated structure factor amplitudes of a particular reflection and the summation is over 95% of the reflections in the specified resolution range. The remaining 5% of the reflections were randomly selected (test set) before the structure refinement and not included in the structure refinement.

[d] R_{free} was calculated for the test set using the same equation as for R_{cryst}.

Supplementary Table 2: Comparison of wild type K_b/gp34 and K^b-Y84C/gp34 structures.
Calculated root mean square deviation (RMSD) of the Cα atoms between the indicated portions of the structures of wild type K^b/gp34 (PDB 1S7S, Fig. 2) and K^b-Y84C/gp34 (PDB 3ROO, Fig. 2) or wild type K^b/gp33 (PDB 1S7Q).
The RMSD values between the two wild-type structures (wild type K^b/gp34 and wild type K^b/gp33) are included as a comparison for two structures that are essentially identical.
Resolutions are: K^b-Y84C/gp34 = 2.1 Å; wild type K^b/gp34 = 2.0 Å; wild type Kb/gp33 = 2.0 Å.

	RMSD (Å) between wild type K^b and :	
	K^b-Y84C/gp34	wild type K^b/gp33
entire complex	0.45	0.43
heavy chain only	0.47	0.44
β_2m only	0.24	0.22
peptide only	0.12	0.15
peptide binding cleft (2-176) only	0.32	0.26