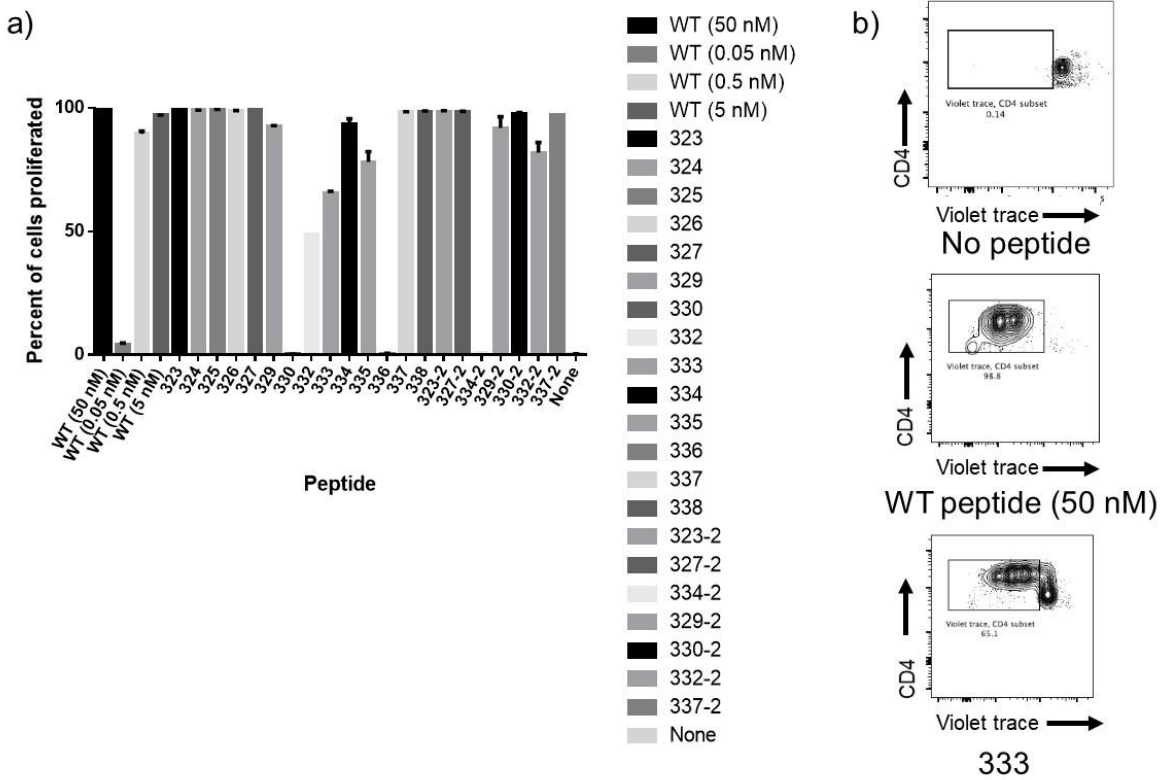


Peptide name	Peptide mass calculated [M+H]	Peptide mass observed	Purity (%)	OT-II (I-A ^b), IFN γ response 10 μ M peptide, ELISpot		DO11.10 (I-A ^d), IL2 response 10 μ M peptide, ELISA			
				Average	SD	Mean	SD		
WT	1773.9	1773.7	>95						
323	1787.9	1787.9	>95	WT	362	45	WT	1.23	0.082
323-2	1787.9	1788.2	>95	323	351	35	323	1.12	0.064
324	1787.9	1788.1	>95	323-2	357	72	323-2	1.22	0.004
325	1787.9	1788.3	88	324	295	107	324	1.26	0.077
326	1787.9	1788.5	92	325	368	51	325	1.31	0.043
326-2	1787.9	1787.6	>95	326	383	42	326	1.23	0.217
327	1787.9	1789.3	>95	326-2	ND		326-2	ND	
327-2	1787.9	1788.2	>95	327	381	80	327	1.18	0.066
329	1787.9	1788.7	>95	327-2	343	105	327-2	0.77	0.073
329-2	1787.9	1788.1	>95	329	106	41	329	0.29	0.070
330	1787.9	1788.6	>95	329-2	93	14	329-2	0.28	0.048
330-2	1787.9	1787.9	>95	330	ND		330	ND	
332	1787.9	1788.2	92	330-2	177	29	330-2	0.17	0.000
332-2	1787.9	1787.8	>95	332	22	10	332	1.16	0.064
333	1787.9	1788.2	>95	332-2	198	64	332-2	0.20	0.006
334	1787.9	1788.2	>95	333	7	1	333	0.13	0.014
334-2	1787.9	1787.6	77	334	11	12	334	0.19	0.009
335	1787.9	1788.3	>95	334-2	13	6	334-2	0.15	0.006
336	1787.9	1788.2	>95	335	53	16	335	0.50	0.037
337	1787.9	1788.1	>95	336	8	7	336	0.92	0.060
337-2	1787.9	1787.9	>95	337	339	56	337	1.18	0.012
338	1787.9	1788	>95	337-2	320	33	337-2	0.97	0.050
G ₃ -WT	1944	1943.2	>95	338	399	80	338	1.19	0.046
2 β	1799.9	1800.1	93				Background	0.14	0.003
G ₃ -3 β	1986	1985.2	87				+++ is < 0.9		

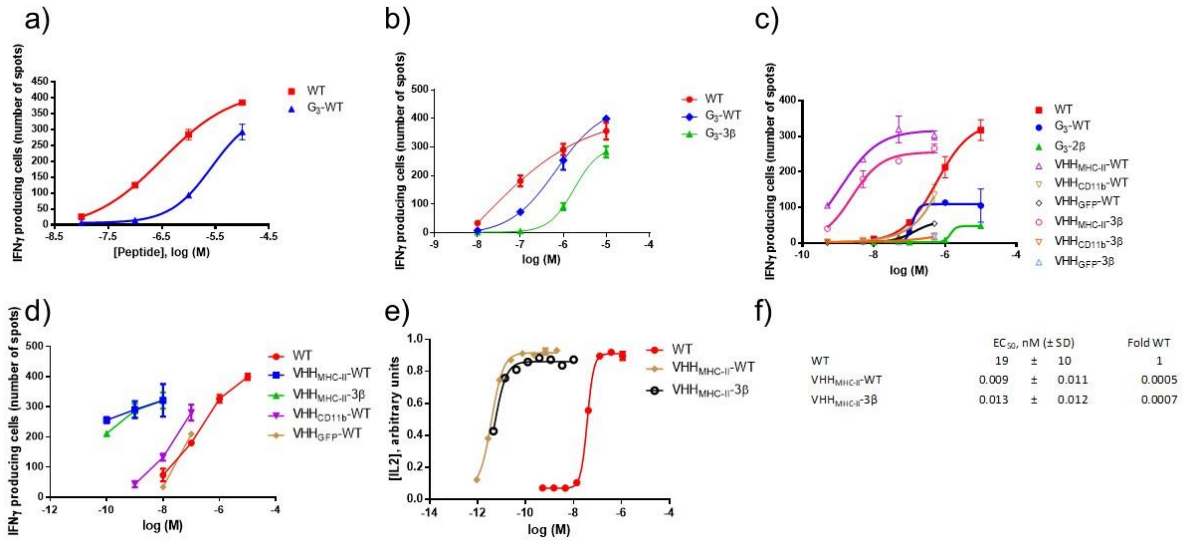
+++ is < 250 spots
 ++ is 100<x<250 spots
 + is 50<x<100 spots
 - is <50 spots

+++ is < 0.9
 ++ is 0.3<x<0.9
 + is 0.2<x<0.3
 - is <0.2

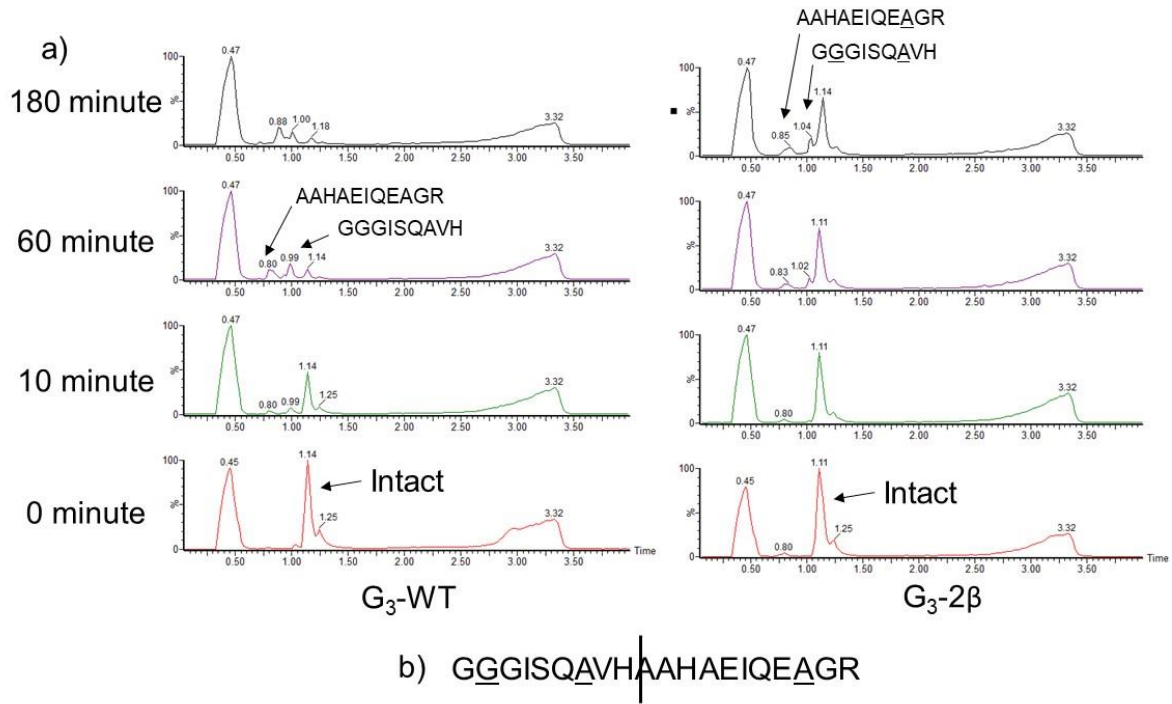
Supporting Figure 1: Tabulation of peptide analytical data (left) and T cell stimulation data (right). (Left) Observed peptide masses were recorded using mass spectrometry as described in the methods section. Peptide purities were quantified using C18 analytical HPLC. (Right) Averages represent the mean values from 4 independent experiments. SD indicates standard deviation. OT-II data represent the number of spots recorded in ELISpot assays. DO11.10 data represent the absorption recorded in an ELISA to measure media levels of IL2.



Supporting Figure 2: OT-II T cell proliferation induced by peptides. **(a)** Summary of OT-II proliferation in the presence of peptides at a dose indicated (50 nM, unless otherwise indicated). Proliferation was measured by flow cytometry as described in the methods section. Error bars represent standard deviation from replicate 2 or 3 experiments. **(b)** Representative flow cytometry plots measuring dye dilution of OT-II T cells in response to the peptide listed below the plot.



Supporting Figure 3. Effective stimulation of T cells by VHH_{MHC-II}-peptide conjugates. **(a-d)** Dose-response curves for stimulation of IFN γ release by peptides or VHH-peptide conjugates. Note that each of these panels have data from independent experiments. OT-II splenocytes were stimulated overnight and IFN γ production was measured using ELISpot as described in the methods section. **(e)** Representative dose-response curve for stimulation of IL2 release from DO11.10 cells. Data points are mean \pm SD and curves result from the fitting of four parameter dose-response model. **(f)** Tabulation of dose-response values for peptides and active VHH-peptide conjugates. Data are from ≥ 2 independent experiments. Data in this table for WT are identical to those presented in Figure 3.



Supporting Figure 4: Characterization of peptide degradation by cathepsin S. **(a)** LC/MS spectra total ion chromatographs of peptides at indicated durations of incubation with protease. The peaks corresponding to the intact peptide and the fragments observed, as determined by mass spectra, are indicated in specific panels. Positions of β -residue incorporation are underlined. Retention times of peaks (m) are listed above the corresponding peaks. **(b)** Position of site of cleavage for each of the peptides tested. The bond cleavage by cathepsin S is indicated by the vertical line. Sites of β -residue incorporation in G_3 -3 β are underlined.