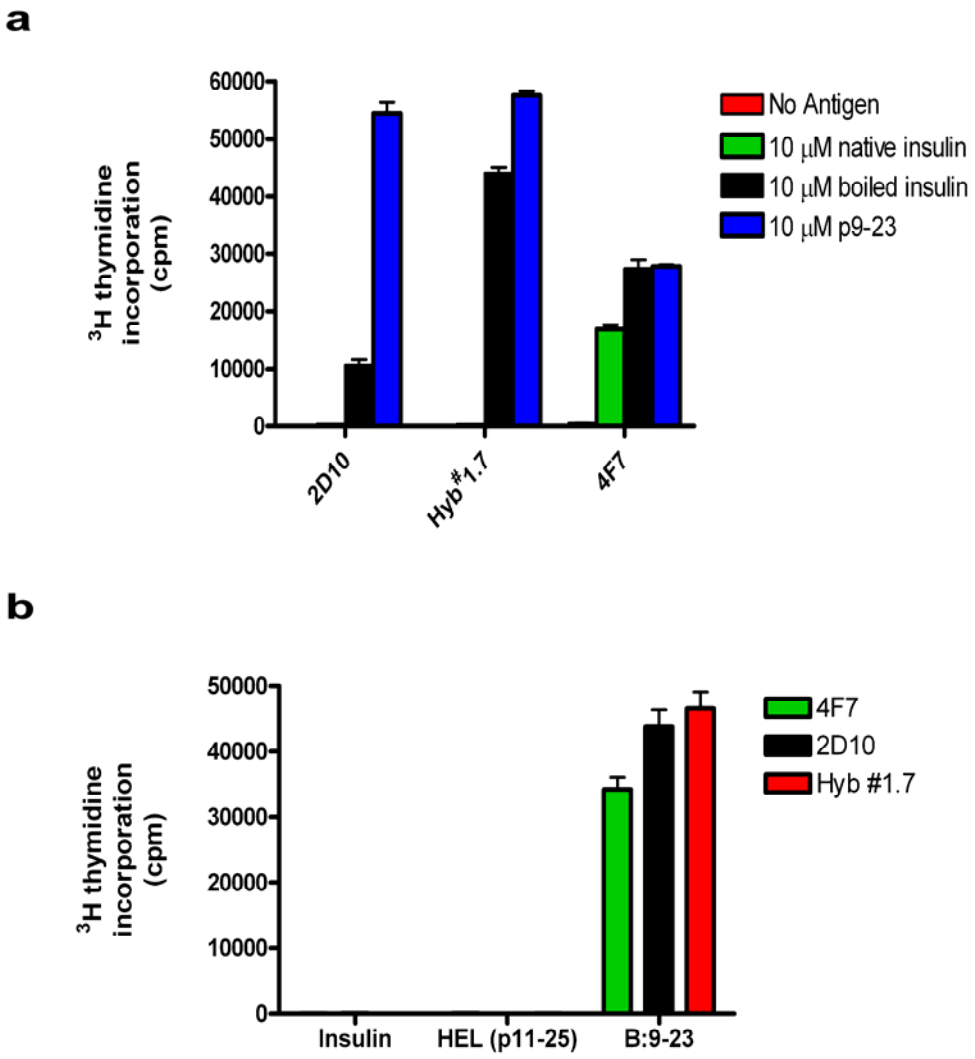
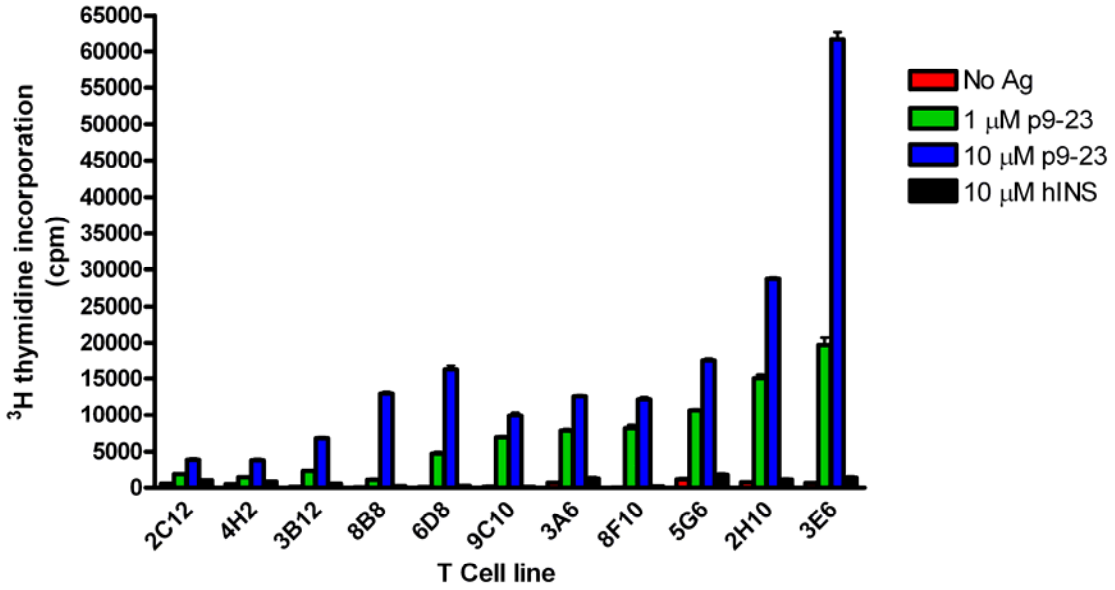
Hybridomas and lines	T cells examined (numbers)	C3.G7		Primary APC		
		B:9-23	INS	B:9-23	INS	M12.C3.G7B:9-23
Type A hybs (immunization)	2	+	+	+	+	+
Type A hybs (islet infiltrating)	16	+	+	+	+	+
Type B hybs (immunization)	10	+	-	+	-	-
Type B hybs (islet infiltrating)	16	+	-	+	-	-
Primary Type B T cell lines (immunization)	11	+	-	+	-	-

Supplementary Table 1. NOD insulin T cell hybridomas used in this study and the location of their origin. All hybridomas examined regardless of their place of origin fell into distinct subsets, 'type A' and 'type B'. 'Type A' hybridomas always recognized protein or peptide regardless of APC used in the assay. All of the 'type A' hybridomas recognized the covalently linked peptide-MHC complex on M12.C3G7B9-23 cells. 'Type B' hybridomas always recognized peptide but never recognized processed protein regardless of APC. 'Type B' hybridomas never recognized the covalent peptide-MHC complex.



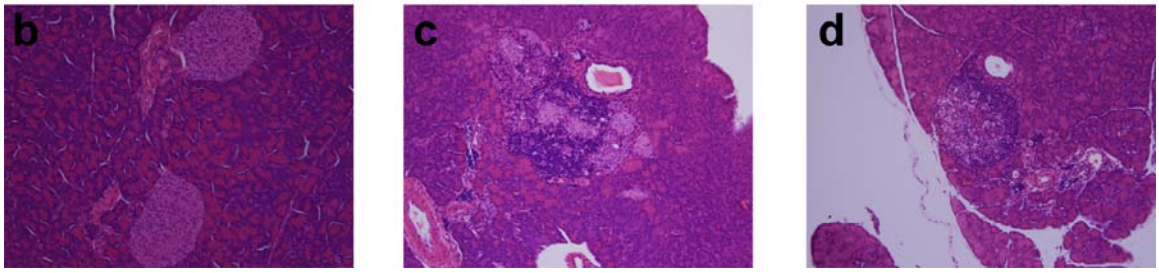
Supplementary Figure 1. Type A and B hybridomas recognize denatured insulin and soluble I-A^{g7} peptide-MHC complexes. (**a**) IL-2 production in type A (4F7) and type B (2D10 & Hyb #1.7) hybridomas stimulated by C3.G7 APCs incubated with native insulin denatured by a 10 minute incubation at 95° C. (**b)** IL-2 production in type A (4F7) and type B (2D10 & Hyb #1.7) hybridomas stimulated with soluble I-A^{g7} class II molecules loaded with B:9-23 peptide.



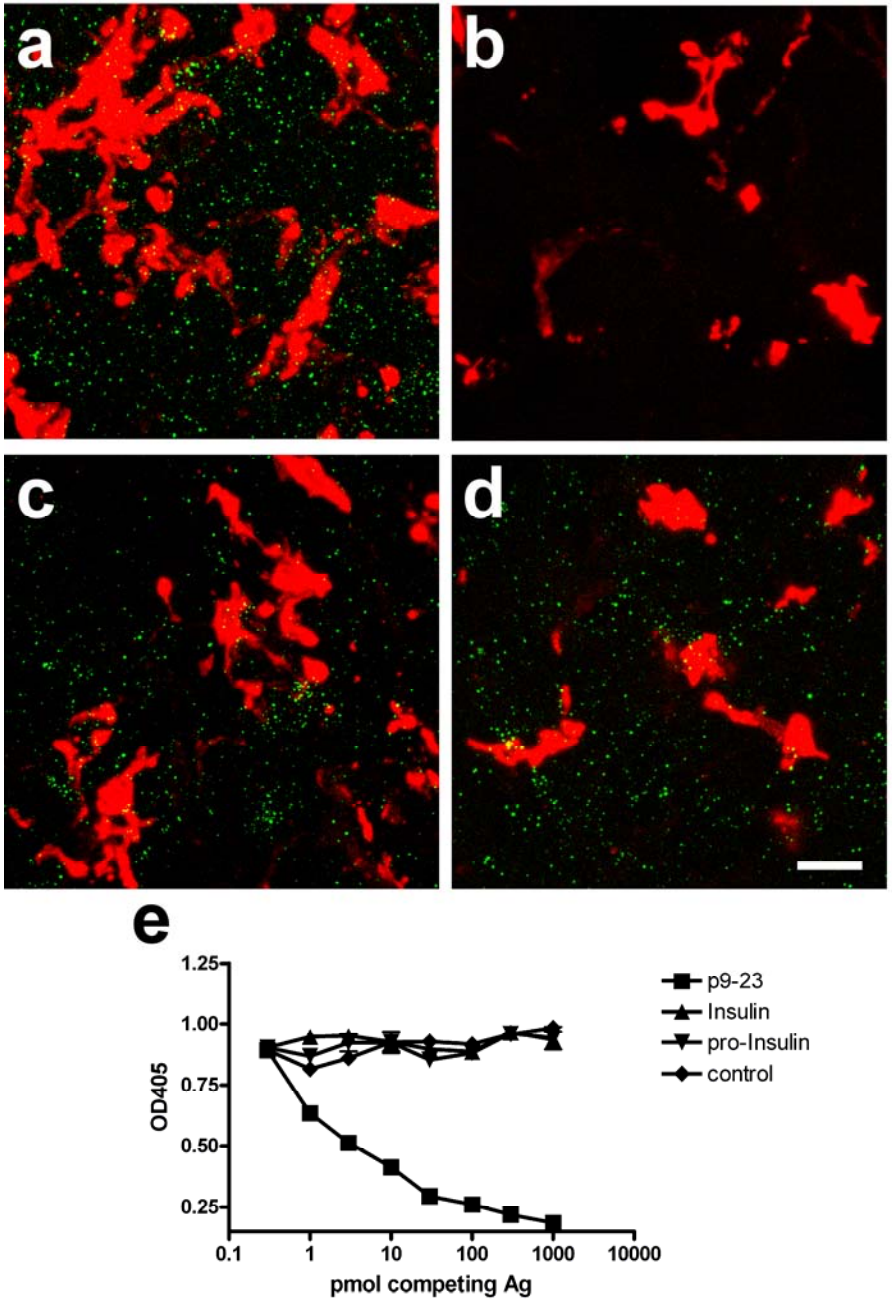
Supplementary Figure 2. Reactivity of primary 'Type B' CD4$^+$ T cell lines. T cell activation measured by ^{3}H thymidine incorporation 72 h post stimulation with either B:9-23 or insulin and 5 x 10^4 bone marrow derived DC

a

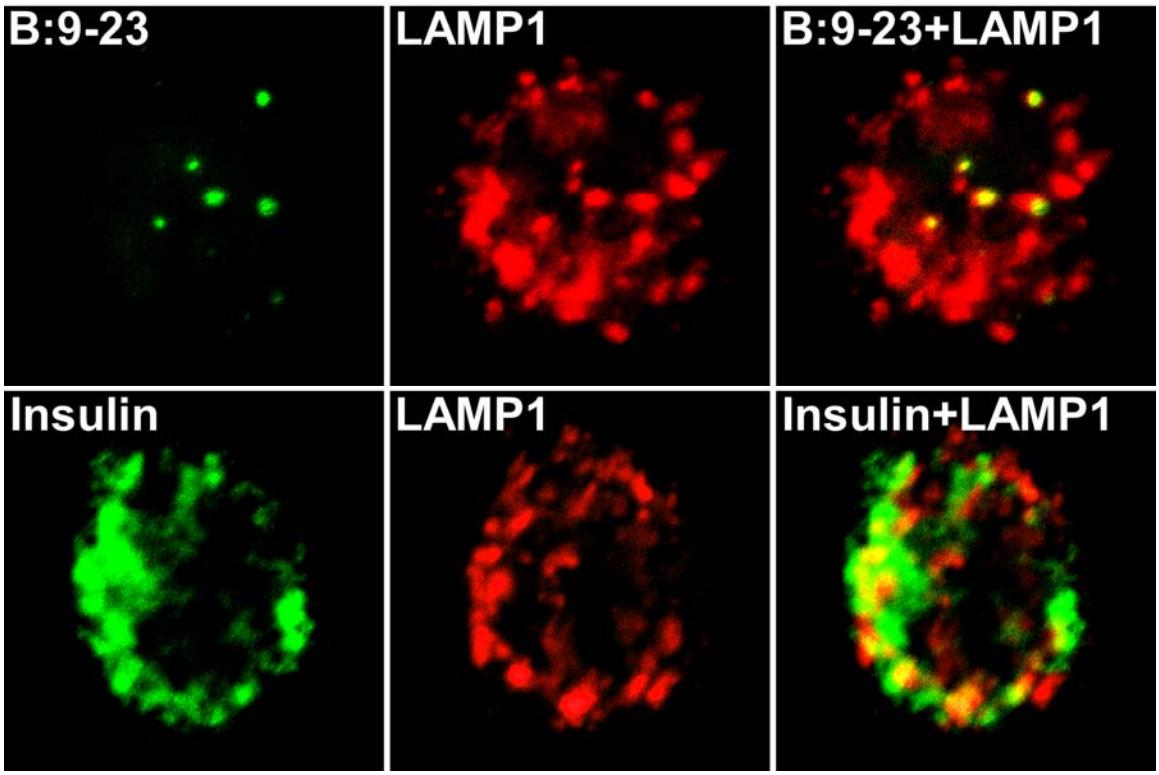
T Cell Line	Young NOD tx	Adult NOD.Scid tx	Cytokine production (ng/ml)			
			IL-2	TNFα	IFNγ	IL-4
2H10		0/2	2	18.2	27.9	8.8
3A6	3/4	1/3	0.1	12.4	26.5	6.8
5G6	2/4	1/3	3.7	15.3	19.2	7.7
6D8	1/4	0/3	1.6	21.8	14.1	3.9
3E6	0/3	3/3	8.4	28.5	5.1	3
3B12	2/4	1/3	nd	2.3	0.6	12.2
8F10	1/4	3/3	nd	13.5	2.6	34.6
4H2	-	0/3	nd	3.2	4.1	54.6
2C12	-	1/3	nd	4.8	4.3	83.6
9C10	1/4	2/3	-	-	-	-
8B8	-	0/3	-	-	-	-



Supplementary Figure 3. Primary T cell lines induced diabetes in NOD.SCID recipients and accelerated diabetes in young NOD mice **(a)** Efficiency of disease transfer in young NOD and adult NOD.SCID mice of independent T cell lines. Corresponding cytokine profiles are shown. Haematoxylin and eosin stained pancreatic sections of 4 week old NOD mice: control unmanipulated (b), and diabetic recipients that received either 5G6 T cells **(c)** or 3A6 T cells **(d)**.



Supplementary Figure 4. Confocal microscopy reconstruction of islets stained for intracellular B:9-23 of NOD.rag 1 -/- islets. $CD11c^{+}$ (red) and B:9-23 staining (green) was performed either alone (**a)**, with competing B:9-23 free peptide (**b**), competing insulin (**c)** or competing pro-insulin (**d**). Scale bar, 20μm. (**e)** Competition ELISA for plate bound B:9-23 in the presence of soluble antigen as indicated.

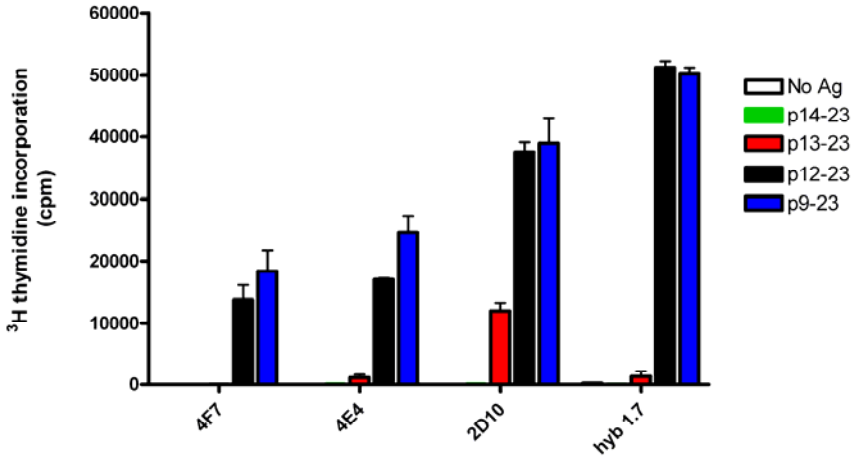


Supplemental Figure 5. Localization of Insulin and proteolytic fragments of the insulin beta chain (B:9-23) in lysosomal compartments in beta cells of NOD.rag 1-/- islets**.** Confocal microscopy reconstruction of a beta cell from dispersed islets stained for B:9-23, insulin and LAMP-1. Note the absolute co-localization of B:9-23 with LAMP-1 and a less co-localization of insulin with LAMP-1. Images represent a single stack with thickness of 1.5μm.

a

INS B Chain peptides identified by MS	residues	Relative Abundance
VEALYLVCGERG	12-23	1.00
SHLVEALY	9-16	0.81
GSHLVEALY	8-16	0.75
LVEALYLVCGERG	11-23	0.42
VEALYLVCGERGF	12-24	0.22
LVCGERGF	17-24	0.23
EALYLVCGERG	13-23	0.14
ALYLVCGERG	14-23	0.10
LVEALYLVCGERGF	11-24	0.02
ALYLVCGERGF	14-24	0.02

b



Supplementary Figure 6. Mass Spectrometry of Nit-1 insulin granules reveals the presence of peptide fragments derived from the insulin beta chain**.** (**a)** Ten most abundant beta chain peptides identified by MS analysis in insulin granules purified from Nit-1 cells. (**b)** IL-2 production by 'type A' and 'type B' hybridomas incubated with the most abundant beta chain peptide (B:12-23) found in the Nit-1 insulin granules.