

Supplementary Fig. S1. *A.* Comparison of amino acid sequences of mammalian TCR α and TCR β chains. *B.* Cell lysates from HeLa cells transfected with plasmids encoding WT or SS-AA mutant TCR α -HA were left untreated or digested with Endo H or PNGase F before immunoblotting with anti-HA monoclonal antibody. Molecular mass markers (in kilodaltons) are indicated on the right. *C-H.* HeLa cells transfected with plasmids encoding WT or SS-AA mutant TCR α -HA were fixed, permeabilized and immunostained with rabbit polyclonal anti-HA (green channel; C & F) and mouse monoclonal anti-calnexin antibodies (red channel; D & G), followed by Alexa Fluor 488 goat anti-rabbit IgG and Alexa Fluor 555 goat anti-mouse IgG antibodies. Cells were visualized by confocal fluorescence microscopy. Bars, 10 μ m. *I.* A postnuclear supernatant (PS) of HeLa cells transfected with WT or SS-AA mutant TCR α -HA was separated into cytosolic (Cyt) and membrane (Memb) fractions by ultracentrifugation at 105,000 $\times g$ for 1 h. The membrane fraction was treated with 0.1 M Na₂CO₃, pH 11.3 (Na₂CO₃) or 0.5% (v/v) Triton X-100 (Tx100) for 1 h at 4 °C. After ultracentrifugation at 105,000 $\times g$ for 1 h, supernatants were collected. All fractions were analyzed by SDS-PAGE and immunoblotting with anti-HA monoclonal antibody.

Supplemental Figure 1

A

	Transmembrane →	← Cytosolic		Transmembrane →	← Cytosolic
TCR α	NLSVIGFRILLLKVAGFNLLMTLRWSS		TCR β	ILLGKATLYAVLVSALVLMAMVKKKDSRG	
Human	NLSVIGFRILLLKVAGFNLLMTLRWSS		Human	ILLGKATLYAVLVSALVLMAMVKKKDSRG	
Mouse	NLSVMGLRILLLKVAGFNLLMTLRWSS		Mouse	ILLGKATLYAVLVSGLVLMAMVKKKNS--	
Rat	NLSVMGLRILLLKVAGFNLLMTLRWSS		Rat	ILIGKATLYAVLVSTLVVMAMVKKKSS--	
Rabbit	NLSVIGLRILLLKVAGFNLLMTLRWSS		Rabbit	ILLGKATLYAVLVSALVLMAMVKKKNP--	
Pig	NLSVMGFRILLKMGFNLLMTLRWSS		Pig	ILLGKATLYAVLVSALVLMATVKKKDS--	
Sheep	NLSVTVFRILLLKVGFNLLMTLRWSS		Sheep	ILLGKATLYAVLVSALVLMAMVKKKDS--	

