Exogenous, TAP-independent lysosomal presentation of a respiratory syncytial virus CTL epitope

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*Immunology and Cell Biology* (2013) 91, 259–260; doi:10.1038/icb.2013.4; published online 22 January 2013

**Correction to:** *Immunology and Cell Biology* (2012) 90, 978–982; doi:10.1038/icb.2012.43; published online 28 August 2012

The correct Figure 1 appears below.

The typesetter would like to apologise for their error.

Regrettably, the name of the virus in Figure 1 corrupted on upload. It reads ‘vvFsig’ but should read ‘vvFsig’.

**Figure 1** See next page for figure legend.
Proteasome-independent and TAP-independent presentation of epitope F249-258 follows a lysosomal exogenous pathway. (a) rVACV encoding different forms of RSV F glycoprotein used in this study were based on the Western Reserve (WR) wild-type vaccinia strain. Virus vvF encodes the wild-type F protein of RSV. Hydrophobic regions are signal peptide (sp), fusion peptide (fp) and transmembrane region (tm). Processing by furin-like proteases in the ectodomain (arrows) yields the F1 and F2 chains. Virus vvFsig− encodes a cytosolic form of the F protein lacking the signal peptide. The CTL epitope (▲) presented by Kd assessed in this study is F249-258 (TYMLTNSELL) and is resistant to brefeldin A (BFA) and to lactacystin (LC). (b) CV-1 cells were infected for 24 h with the indicated virus at the indicated m.o.i., p.f.u. counted and cells stained with anti-RSV F antibody. For UVi, an estimated m.o.i.<10−7 was calculated. T2/Kd cells were infected for 5 h with indicated rVACV (m.o.i. 5) or with their UVi counterparts (m.o.i.<10−5). Background staining by cells infected with WR is shown in grey. (c–f) T2/Kd cells were infected with rVACV at a m.o.i. of 3–15 (light grey bars) or with the same volume of UVi non-infectious virus, equivalent to a m.o.i.<10−5 (dark grey bars). ICS assays were performed by stimulating CTL F/F249-258 monospecific for epitope F249-258 with the indicated fixed targets in the presence of brefeldin A to intracellularly accumulate IFNγ produced upon CTL activation. Represented data are the percentages of total CD8+ lymphocytes that expressed IFNγ or the percentage of inhibition of CTL activation. When the mean is represented, the s.d. is shown by error bars. Lack of side effects by inhibitors was controlled by measuring CTL activation by limiting concentrations of exogenous peptide in the presence of inhibitors, as well as their effect on rVACV protein expression. (c) Data are the mean of at least five independent experiments. DC, dendritic cells. (d) Cells were infected with the indicated virus in presence of protein-synthesis inhibitor cycloheximide (CH). Data in the left panel are the mean of one to five independent experiments. A representative experiment is shown in the right panel. (e) Cells were incubated with vvF, UVi vvF or parental strain WR at 37°C for the indicated times. As control, CTL activation induced by cells incubated with synthetic peptide just before fixation and washing is shown (x). Results from a representative experiment are shown. (f) Cells were infected with vvFsig− in the presence of inhibitors NH4Cl (lysosomotropic agent), Leup (inhibitor of lysosomal serine and cysteine proteases) or 3-methyladenine (3MA) (autophagy inhibitor). Percentage activated CTLs in the absence of inhibitors ranged from 11 to 38. Data are from one to two independent experiments.