Figure 4

(A) Establish lab SOP for MHC peptide multimer staining:

A1 Count at least 100,000 CD8 T cells per staining.

A2 Establish adequate measured to quantify non-specific binding of MULTIMER to CD8-postive cells (e.g. irrelevant MULTIMER or autofluorescence).

A3 Establish adequate measures to reduce the amount of non-specific binding of MULTIMERS in the CD8-positive population to allow accurate quantification (e.g. DUMP channel or DEAD cell dyes).

(B) Establish SOP for software analyses of stained samples, including:

B1 Gating strategy.

B2 Rules to set the gates.

(C) Establish a human auditing process of all final results:

C1 Are all dot plots correctly compensated?

C2 Have the gates been set correctly?

C3 Are the reported frequencies of multimer-positive cells plausible?

(D) Lab environment

D1 Only let experienced personnel (per lab SOP) conduct assay.

(E) *Implement a structured framework to report data from MULTIMER experiments that makes sure that essential pieces of information are not missed (e.g. MIATA or other MI projects).*

E*1 Showing at least one representative data set that provides information on the gating style applied and the amount of MULTIMER binding to CD8-negative cells.*