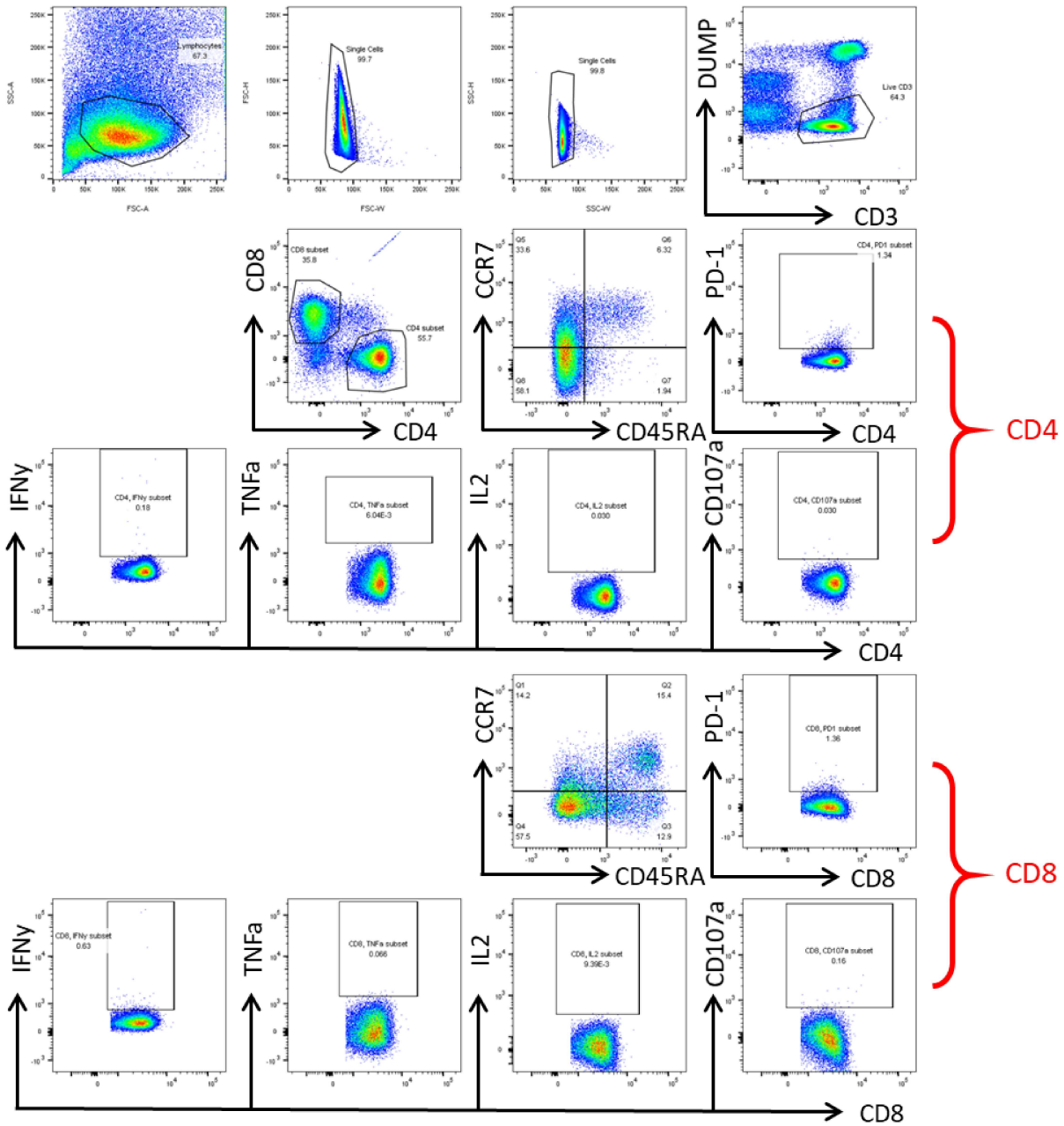
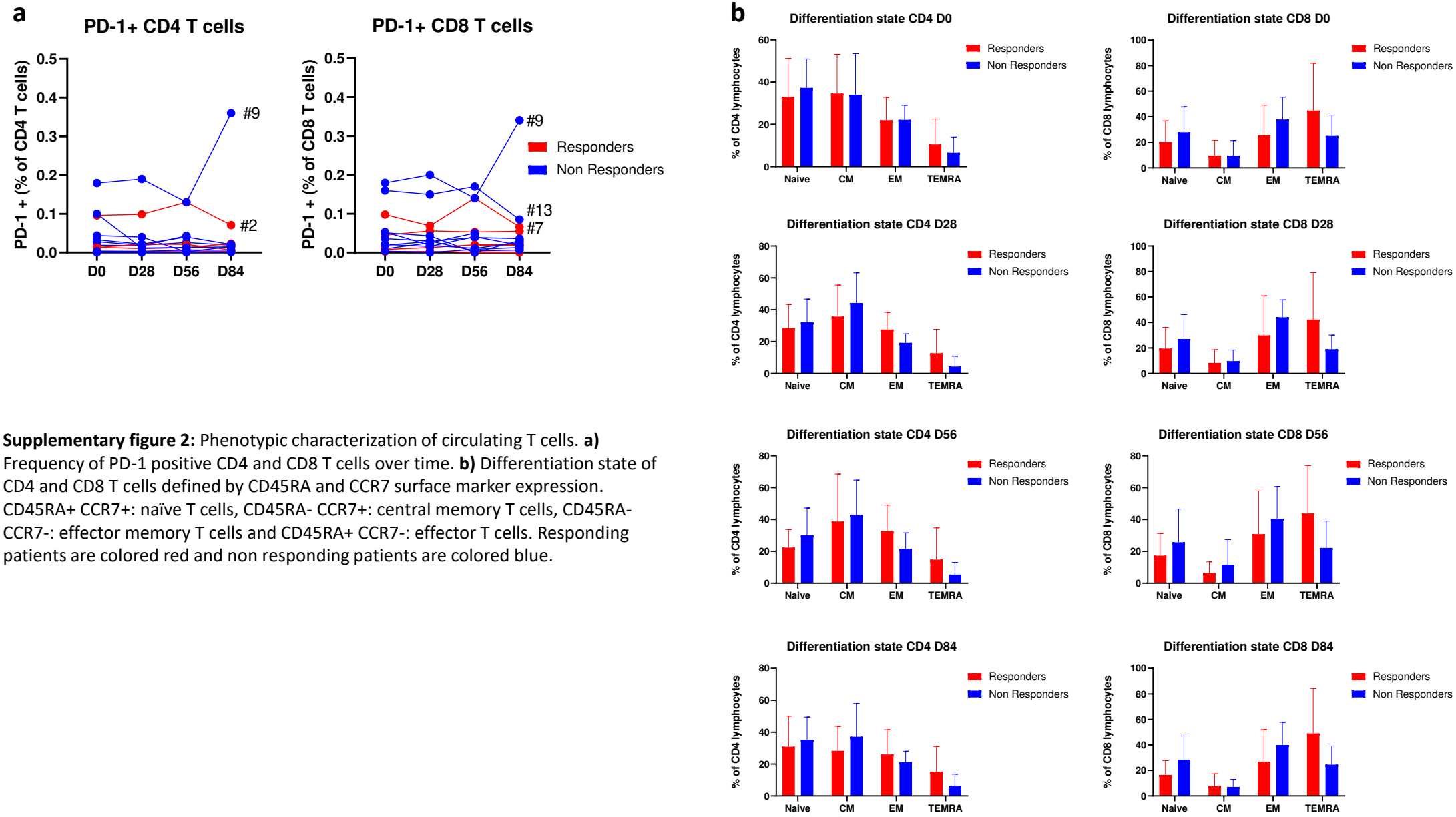
Supplementary figures

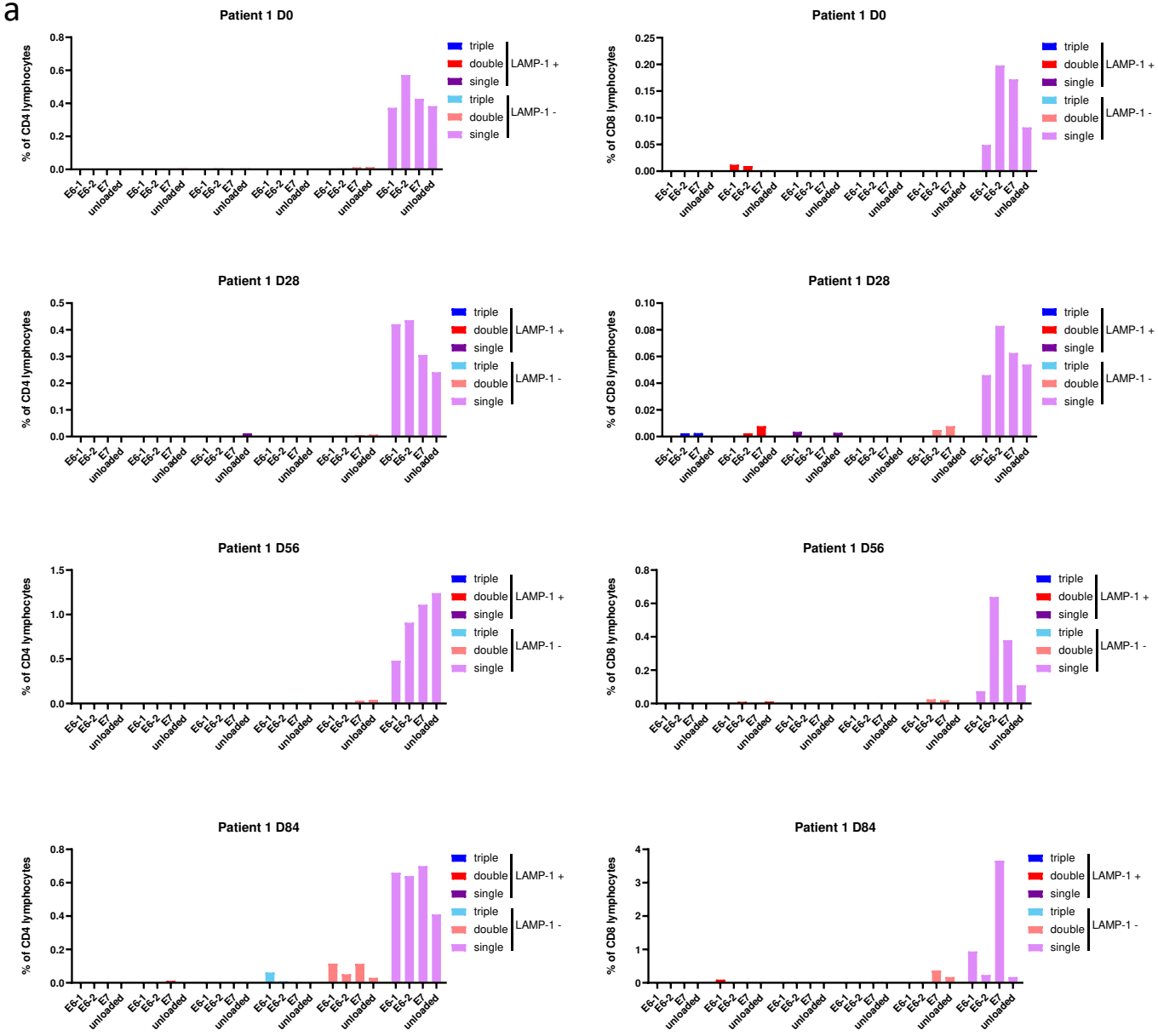


Supplementary figure 1: Gating strategy example of phenotypic characterization and cytokine production of CD4 and CD8 T cells.



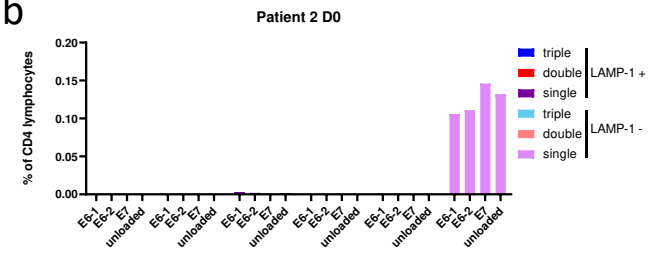
Supplementary figure 2: Phenotypic characterization of circulating T cells. **a)** Frequency of PD-1 positive CD4 and CD8 T cells over time. **b)** Differentiation state of CD4 and CD8 T cells defined by CD45RA and CCR7 surface marker expression. CD45RA+ CCR7+: naïve T cells, CD45RA- CCR7+: central memory T cells, CD45RA- CCR7-: effector memory T cells and CD45RA+ CCR7-: effector T cells. Responding patients are colored red and non responding patients are colored blue.

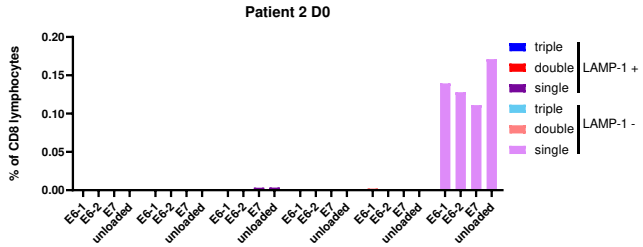
a

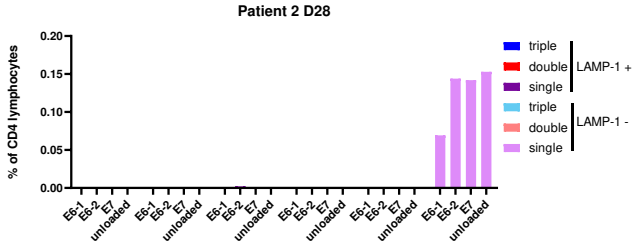


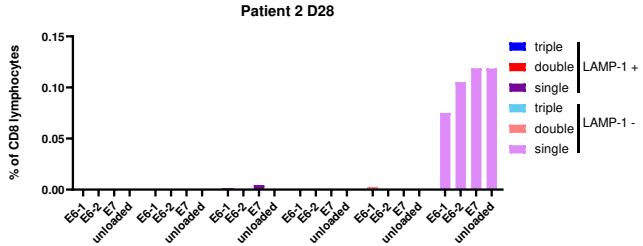
Supplementary figure 3: Depicted are the frequencies of single, double and triple cytokine producing CD4 and CD8 T cells, with and without co-expression of degranulation marker CD107a (LAMP-1), determined using Boolean gating. Depicted are immunologically responding patients **a** patient 1, **b** patient 2, **c** patient 4, **d** patient 7 and **e** patient 8.

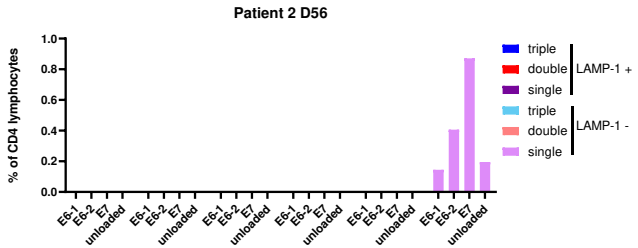
b

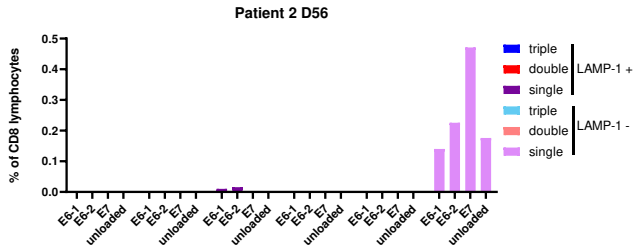

Patient 2 D0
% of CD4 lymphocytes
triple
double
single
triple
double
single
LAMP-1 +
LAMP-1 -
E6-1
E6-2
E7
unloaded

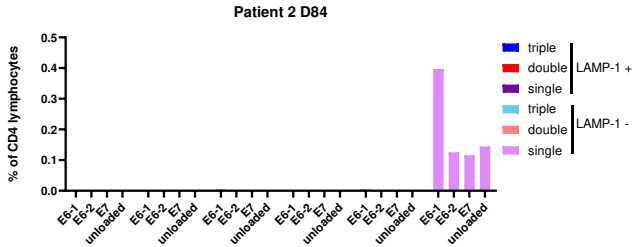

Patient 2 D0
% of CD8 lymphocytes
triple
double
single
triple
double
single
LAMP-1 +
LAMP-1 -
E6-1
E6-2
E7
unloaded

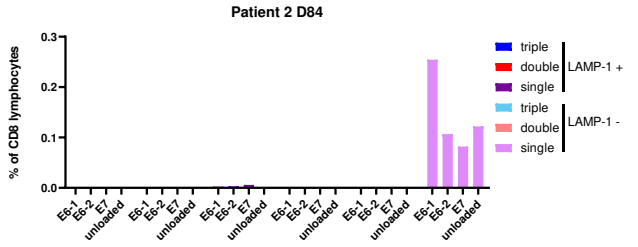

Patient 2 D28
% of CD4 lymphocytes
triple
double
single
triple
double
single
LAMP-1 +
LAMP-1 -
E6-1
E6-2
E7
unloaded


Patient 2 D28
% of CD8 lymphocytes
triple
double
single
triple
double
single
LAMP-1 +
LAMP-1 -
E6-1
E6-2
E7
unloaded

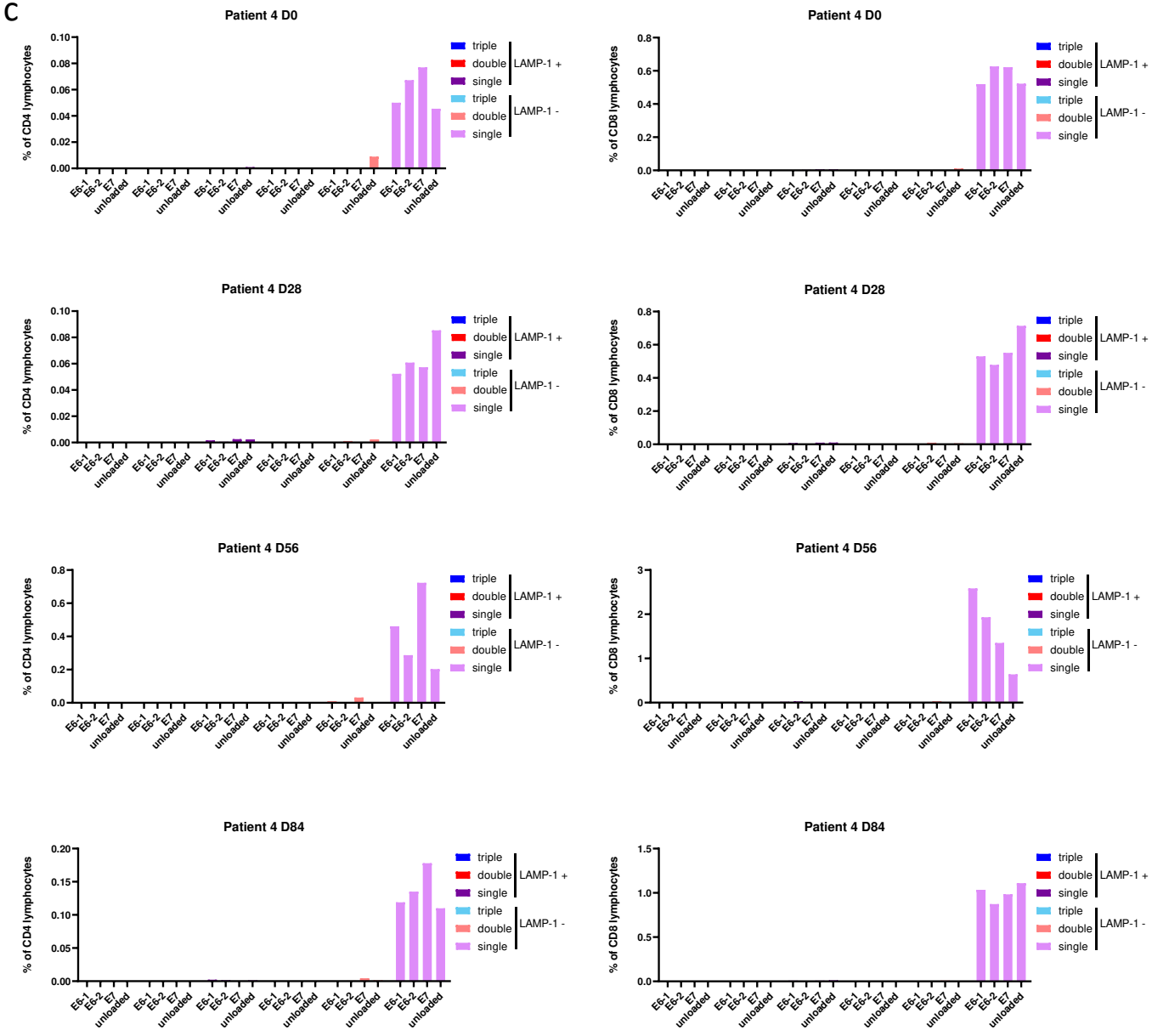

Patient 2 D56
% of CD4 lymphocytes
triple
double
single
triple
double
single
LAMP-1 +
LAMP-1 -
E6-1
E6-2
E7
unloaded


Patient 2 D56
% of CD8 lymphocytes
triple
double
single
triple
double
single
LAMP-1 +
LAMP-1 -
E6-1
E6-2
E7
unloaded

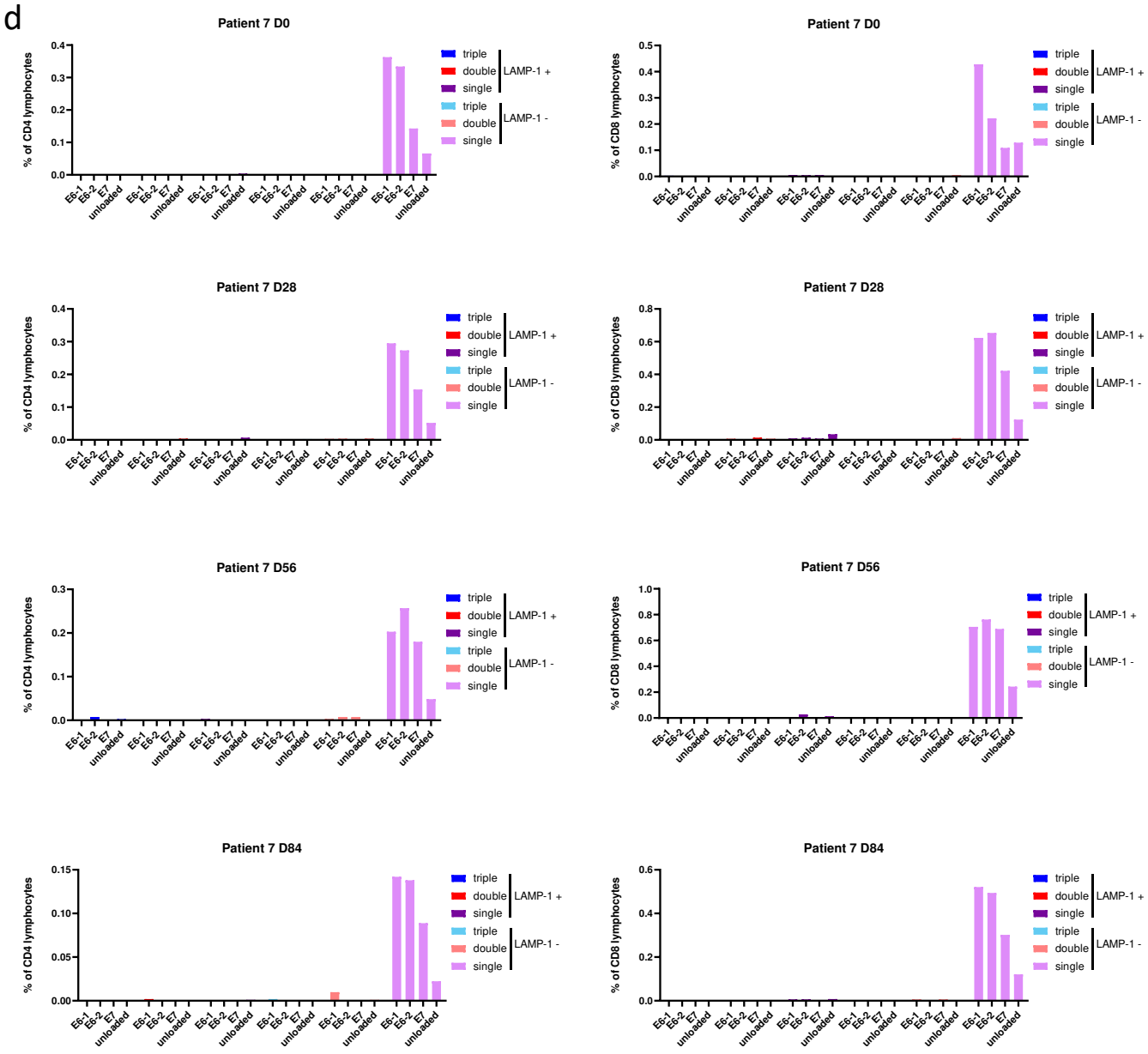

Patient 2 D84
% of CD4 lymphocytes
triple
double
single
triple
double
single
LAMP-1 +
LAMP-1 -
E6-1
E6-2
E7
unloaded


Patient 2 D84
% of CD8 lymphocytes
triple
double
single
triple
double
single
LAMP-1 +
LAMP-1 -
E6-1
E6-2
E7
unloaded

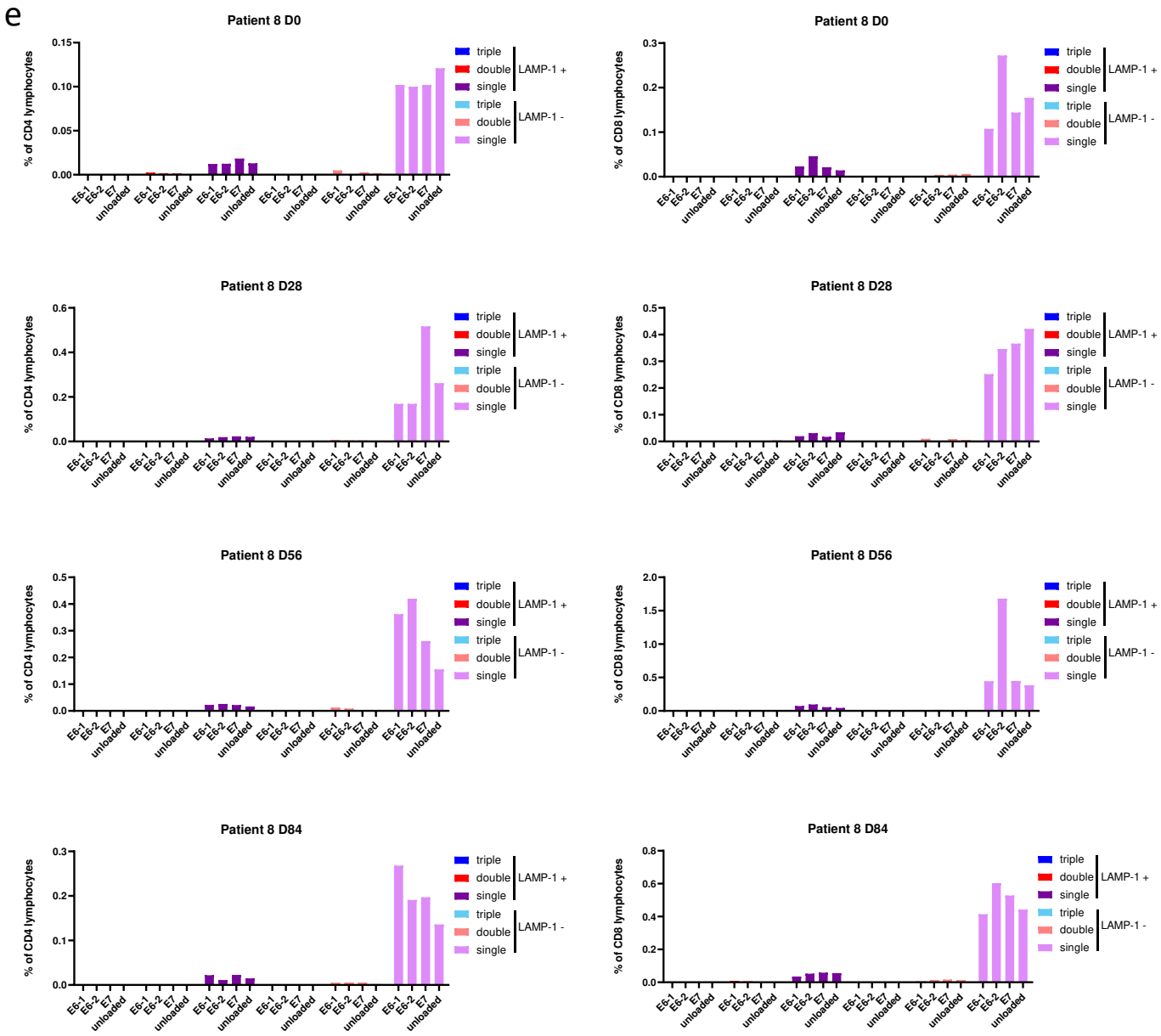
C

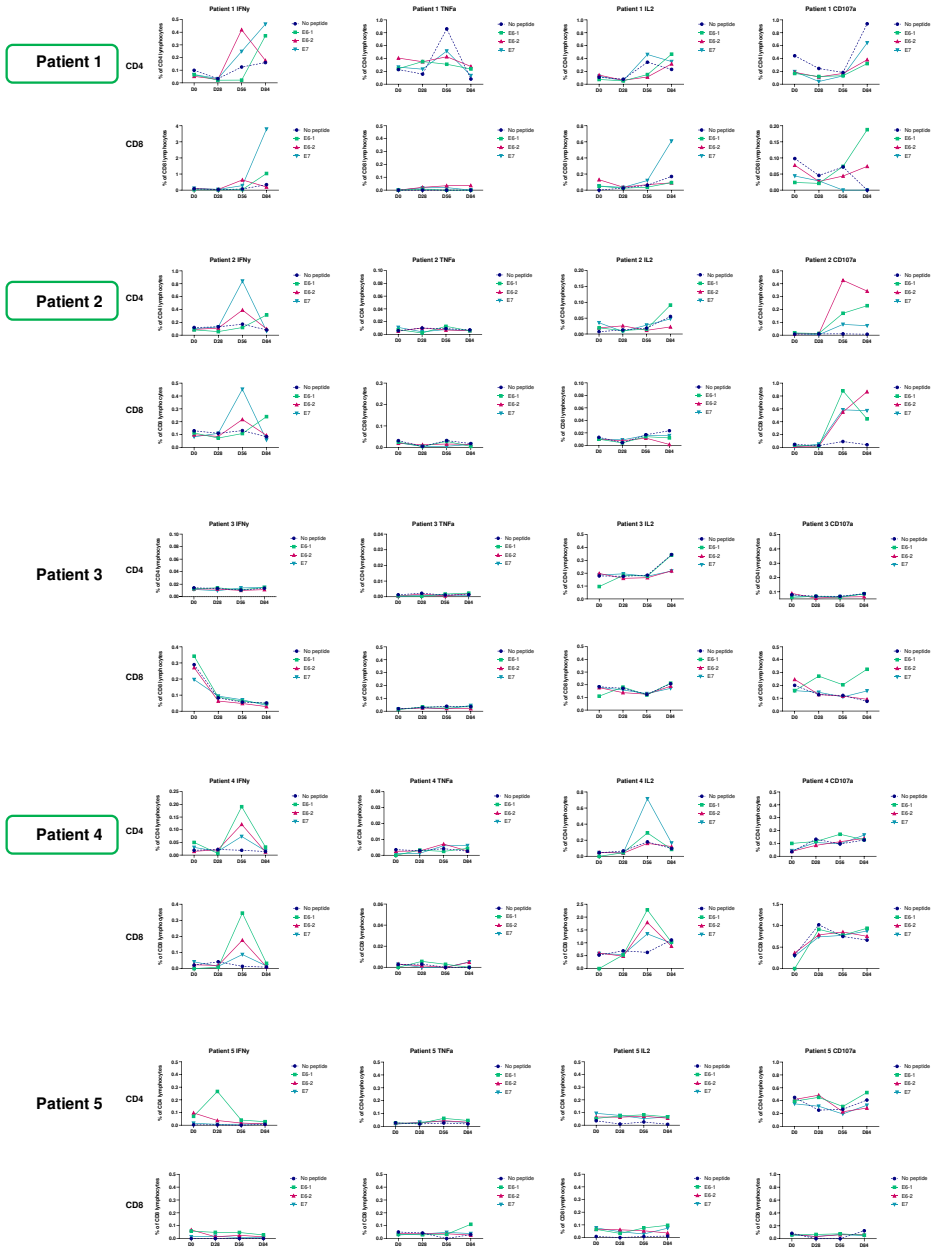

Patient 4 D0
% of CD4 lymphocytes
Patient 4 D0
% of CD8 lymphocytes
Patient 4 D28
% of CD4 lymphocytes
Patient 4 D28
% of CD8 lymphocytes
Patient 4 D56
% of CD4 lymphocytes
Patient 4 D56
% of CD8 lymphocytes
Patient 4 D84
% of CD4 lymphocytes
Patient 4 D84
% of CD8 lymphocytes
triple
double
single
LAMP-1 +
LAMP-1 -
E6-1
E6-2
E7
unloaded

d


Patient 7 D0
Patient 7 D0
Patient 7 D28
Patient 7 D28
Patient 7 D56
Patient 7 D56
Patient 7 D84
Patient 7 D84
% of CD4 lymphocytes
% of CD8 lymphocytes
triple
double
single
LAMP-1 +
LAMP-1 -
E6-1
E6-2
E7
unloaded

e

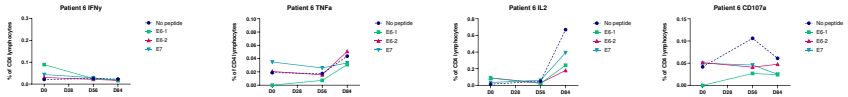

Patient 8 D0
% of CD4 lymphocytes
Patient 8 D0
% of CD8 lymphocytes
Patient 8 D28
% of CD4 lymphocytes
Patient 8 D28
% of CD8 lymphocytes
Patient 8 D56
% of CD4 lymphocytes
Patient 8 D56
% of CD8 lymphocytes
Patient 8 D84
% of CD4 lymphocytes
Patient 8 D84
% of CD8 lymphocytes
triple
double
single
LAMP-1 +
LAMP-1 -
E6-1
E6-2
E7
unloaded



Supplementary figure 4: Time course graphs showing the IFNγ, TNFα, IL-2 and CD107a responses against E6-1, E6-2 and E7 peptide pool for each patient. No peptides indicate that T cells were co-cultured with unloaded APCs to access background or aspecific reactivity. The green boxes highlight the patients with an immunological response.

Patient 6

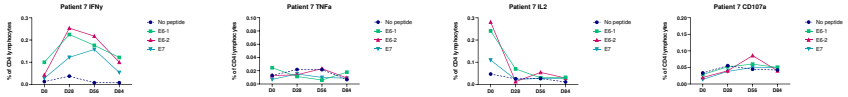
CD4



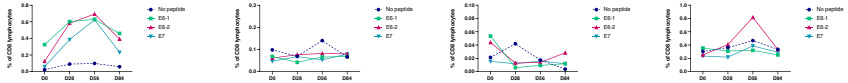
CD8

Patient 7

CD4

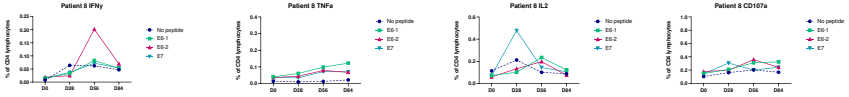


CD8



Patient 8

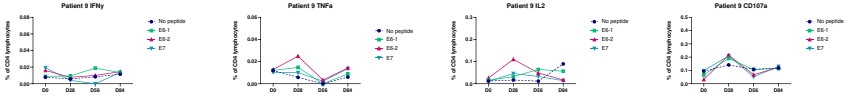
CD4



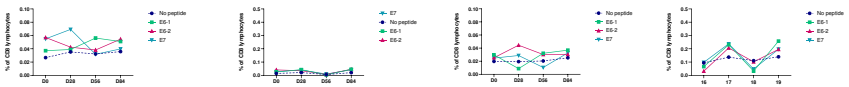
CD8

Patient 9

CD4

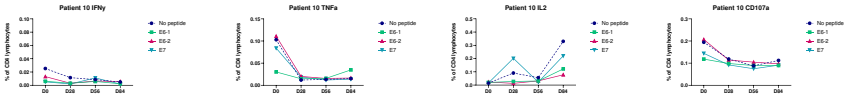


CD8

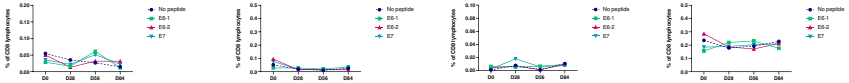


Patient 10

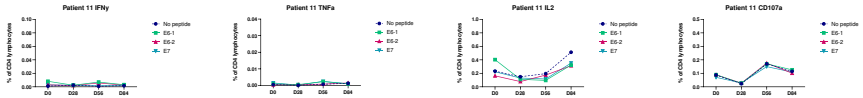
CD4



CD8

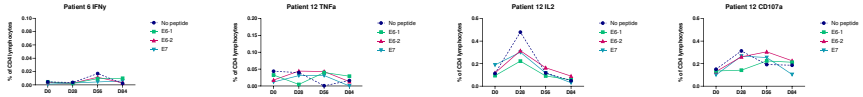


Patient 11 CD4

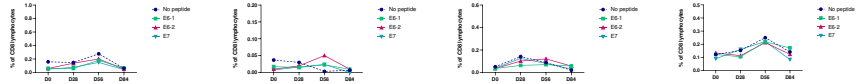


CD8

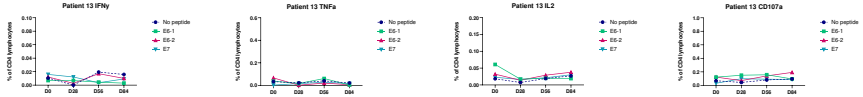
Patient 12 CD4



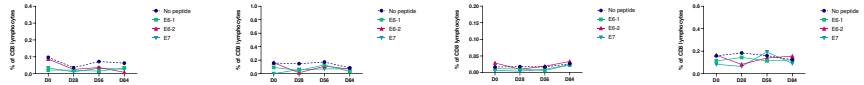
CD8



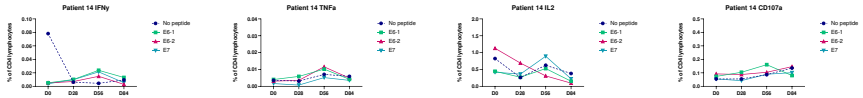
Patient 13 CD4



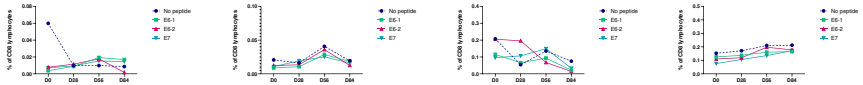
CD8

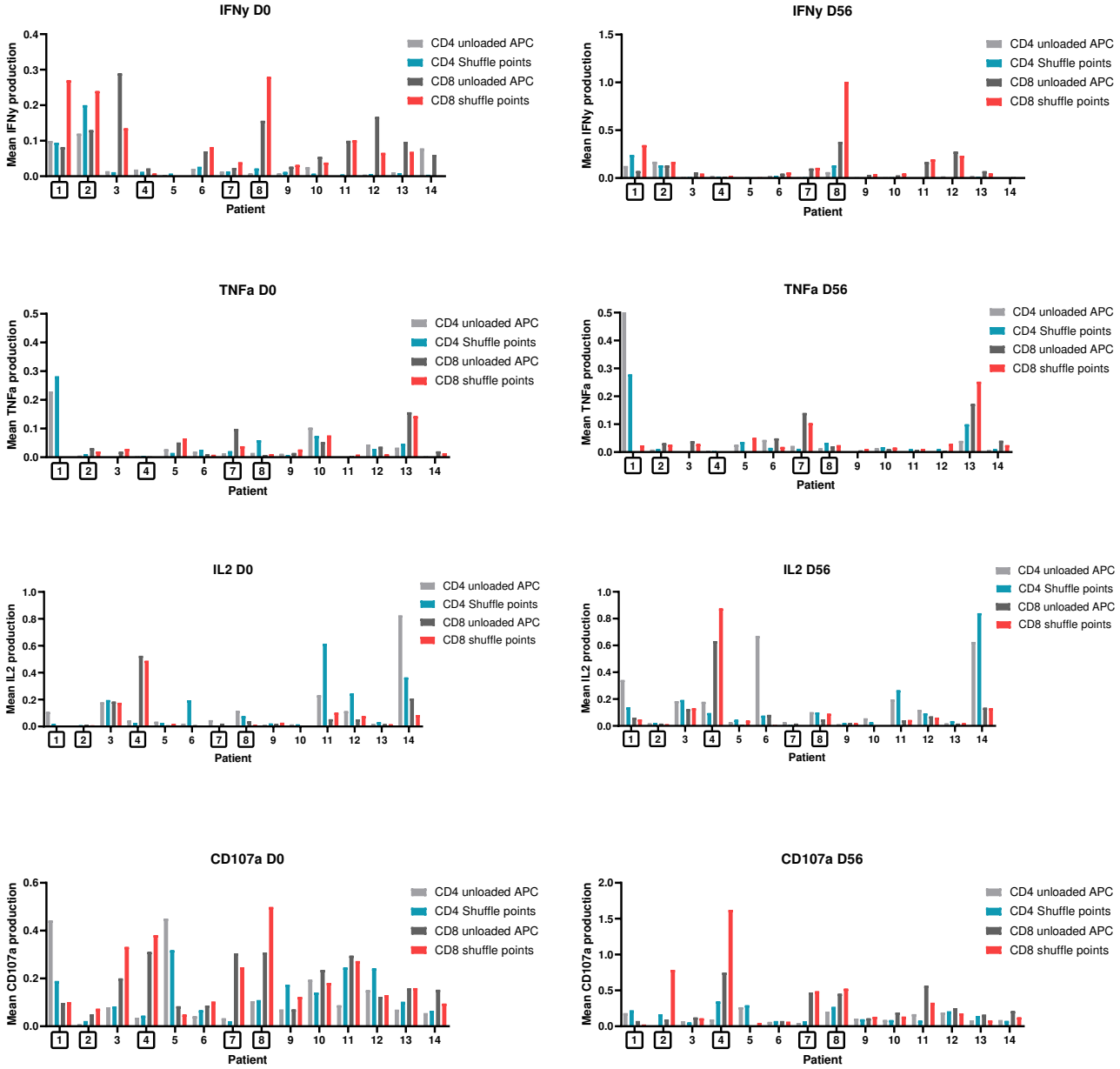


Patient 14 CD4

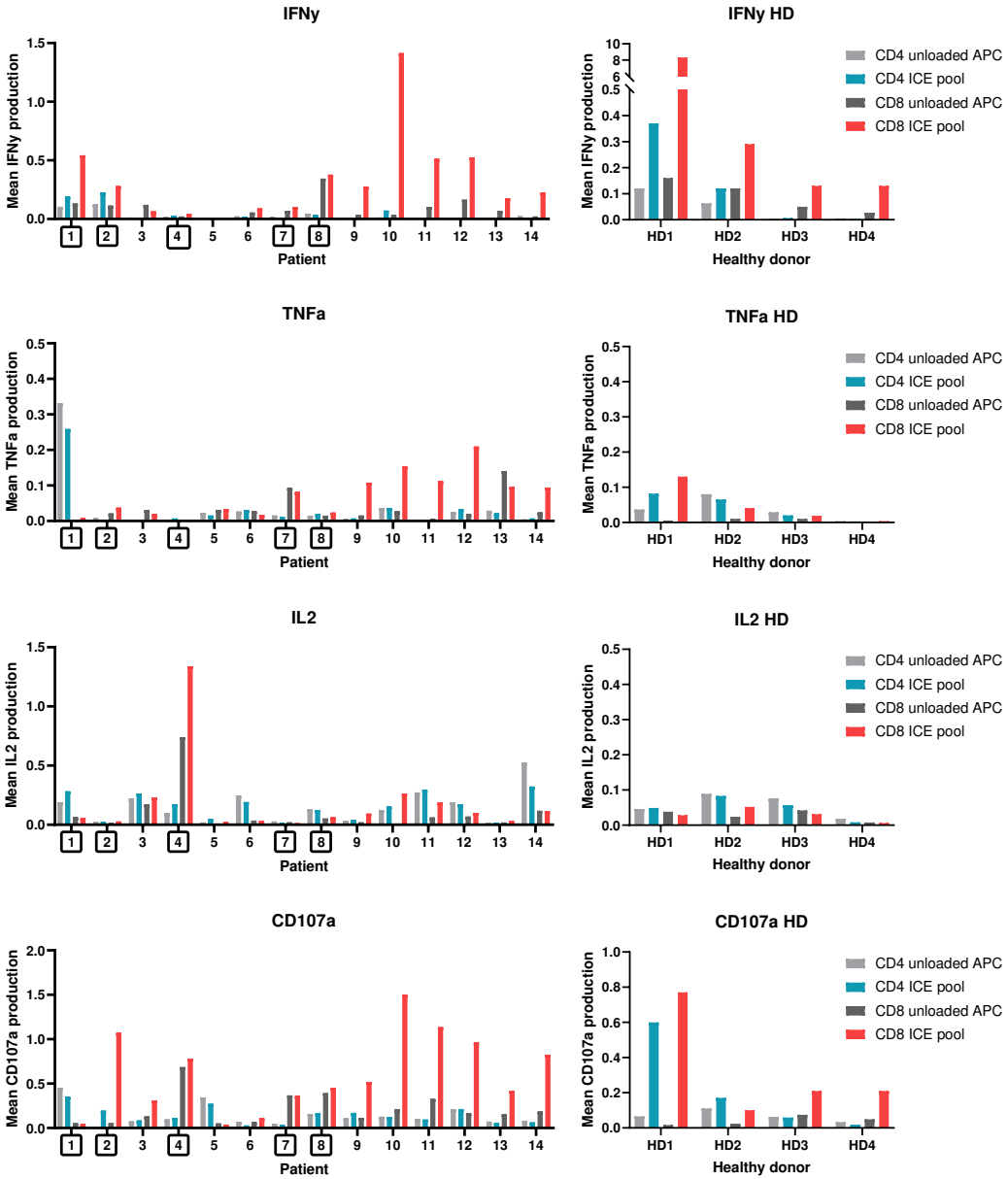


CD8





Supplementary figure 5: Reactivity against the peptides that arose as a consequence of shuffling the E6 and E7 coding sequences. Boxes highlight responding patients.



Supplementary figure 6: Reactivity against ICE peptide pool at day 0. On the left, patient responses are depicted and on the right four healthy controls are depicted for the matched cytokines or LAMP-1. The black boxes on the x-axis highlight responding patients.