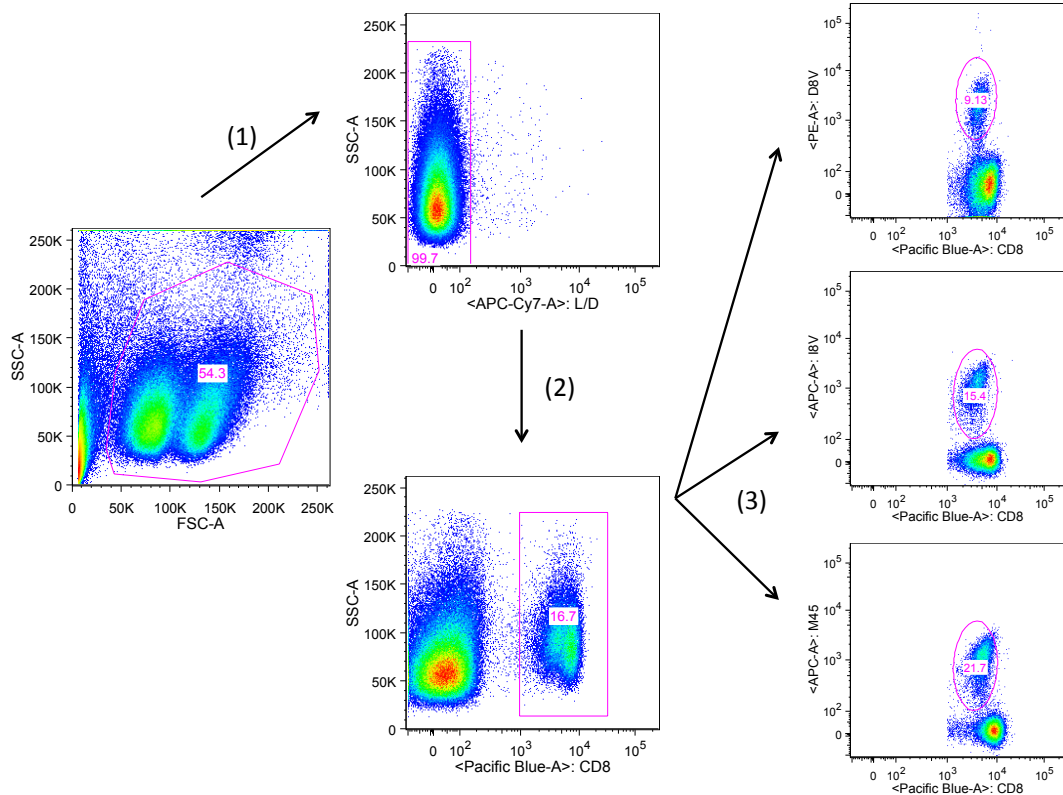1   **Supplementary Figure 1:**

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4   All flow cytometry was performed on the BD LSR II machine. Events were initially

5   collected for lymphocytes on FSC/SSC. **(1)** A live dead marker was then used to gate

6   onto the live lymphocytes. **(2)** A CD8a-PB (eFluro450) stain was then used to gate on

7   total CD8$^+$ T cells. Events recorded on each experiment were collected on the CD8$^+$ T

8   cells gate (30,000 events). **(3)** Gates could then be applied to count the individual

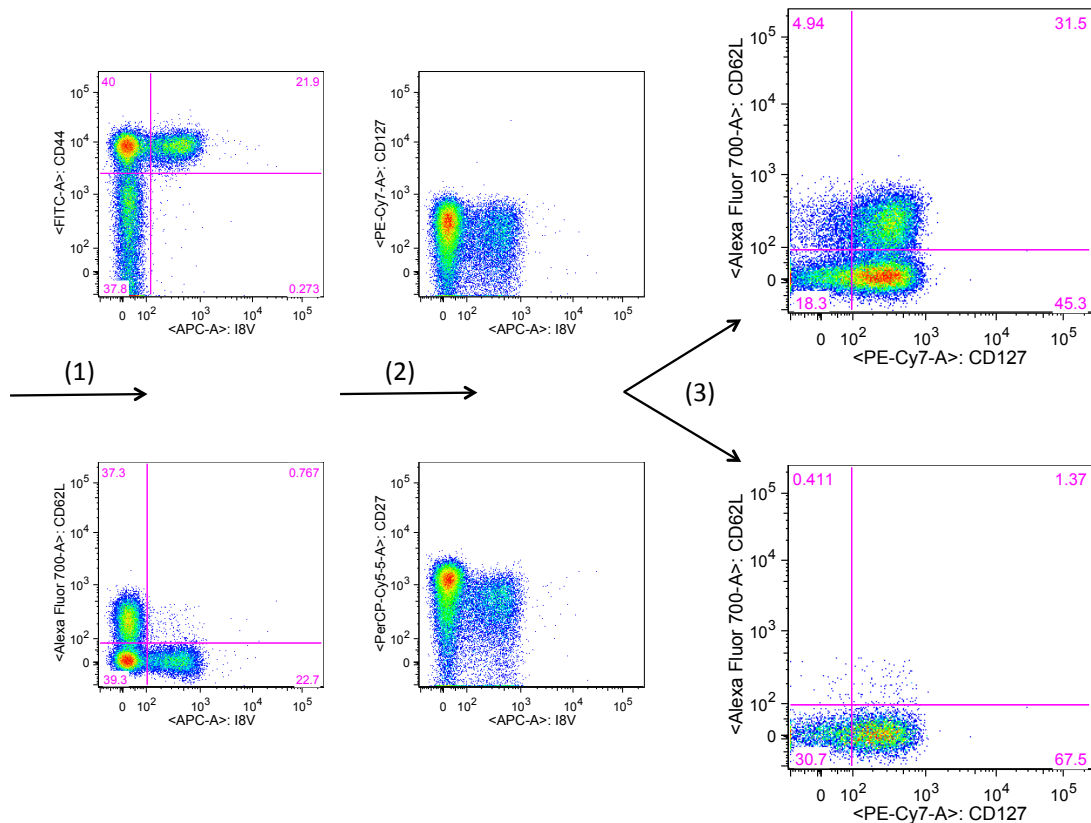9   tetramer-specific populations, with I8V-APC, D8V-PE and M45-APC shown.

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19   Starting from total CD8^{+} T cells, individual antibody stains could then be assessed on

20   whole CD8^{+} T cells and / or tetramer-specific populations of CD8^{+} T cells. **(1)** Total

21   CD8^{+} T cells are shown with the antibody stain on the y-axis and the applicable

22   tetramer stain (I8V-specific responses following Ad-I8V immunisation in this case)

23   on the x-axis. CD44 and CD62L were analysed as the percentage of total CD8^{+} T

24   cells / total tetramer-specific CD8^{+} T cells that were CD44 or CD62L positive. **(2)**
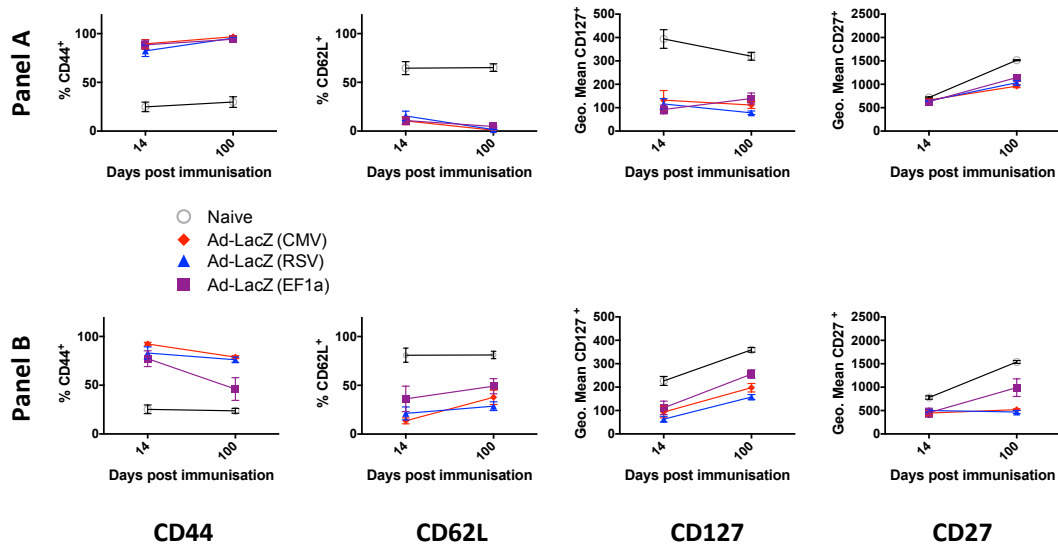
25   CD27 and CD127 were analysed according to the geometric mean of total CD8^{+} T

26   cells. **(3)** On a plot of total CD8^{+} T cells (top right) or tetramer-specific CD8^{+} T cells

27   (bottom right – I8V in this case) a comparison of CD127 vs. CD62L could be made

28   and the phenotype of these CD8^{+} T cells inferred from that stain.

29 **Supplementary Figure 3:**

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31   Phenotypic markers, in blood, for the D8V and I8V-specific responses following

32   immunisation with the Ad-LacZ constructs with differing promoters is shown. The

33   CD44 and CD62L results are shown as a percentage of the tetramer-positive $CD8^+$ T

34   cells and the CD27 and CD127 as the geometric mean. Panel A represents results for

35   the D8V (inflationary) specific responses and panel B the I8V (non-inflationary)

36   responses. An early (day 14) and later (day 100) time-point is shown for each

37   antibody marker. Groups of 4 mice were used for each vector, with the mean ± SEM

38   shown. These results are representative of 3 separate experiments.

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