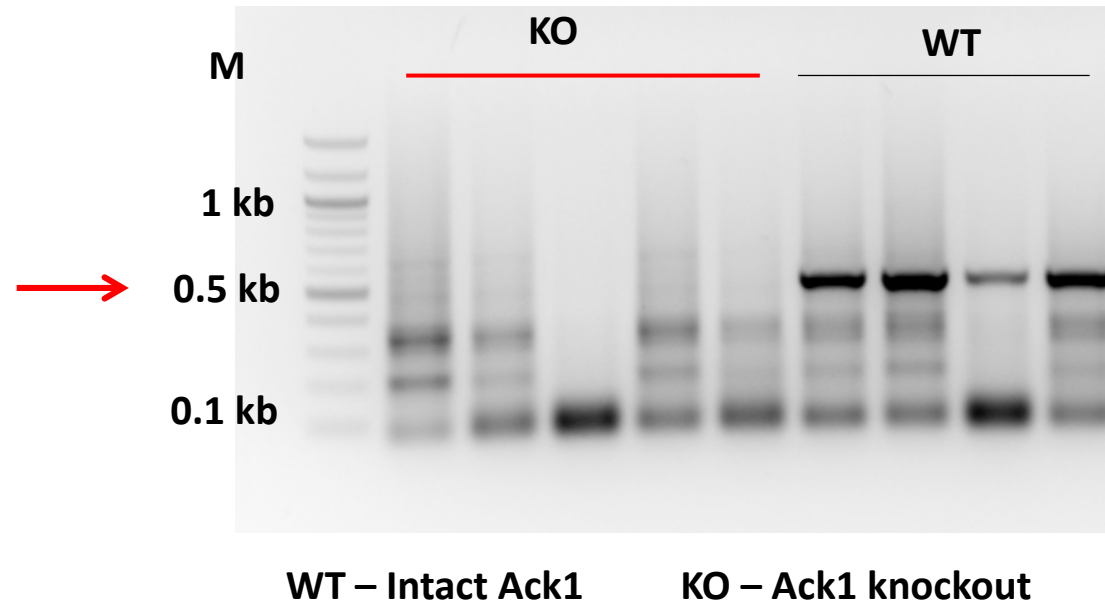


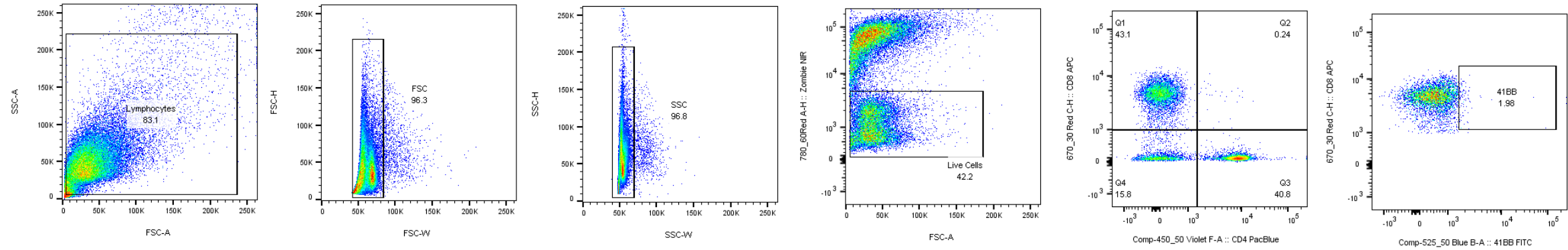
Supplementary Fig. 1c

Genotype confirmed by tail PCR

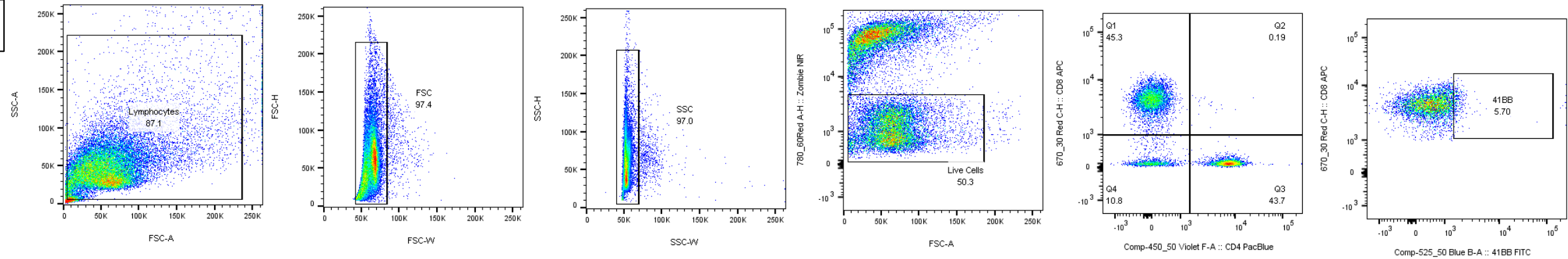


Supplementary Fig. 2

WT

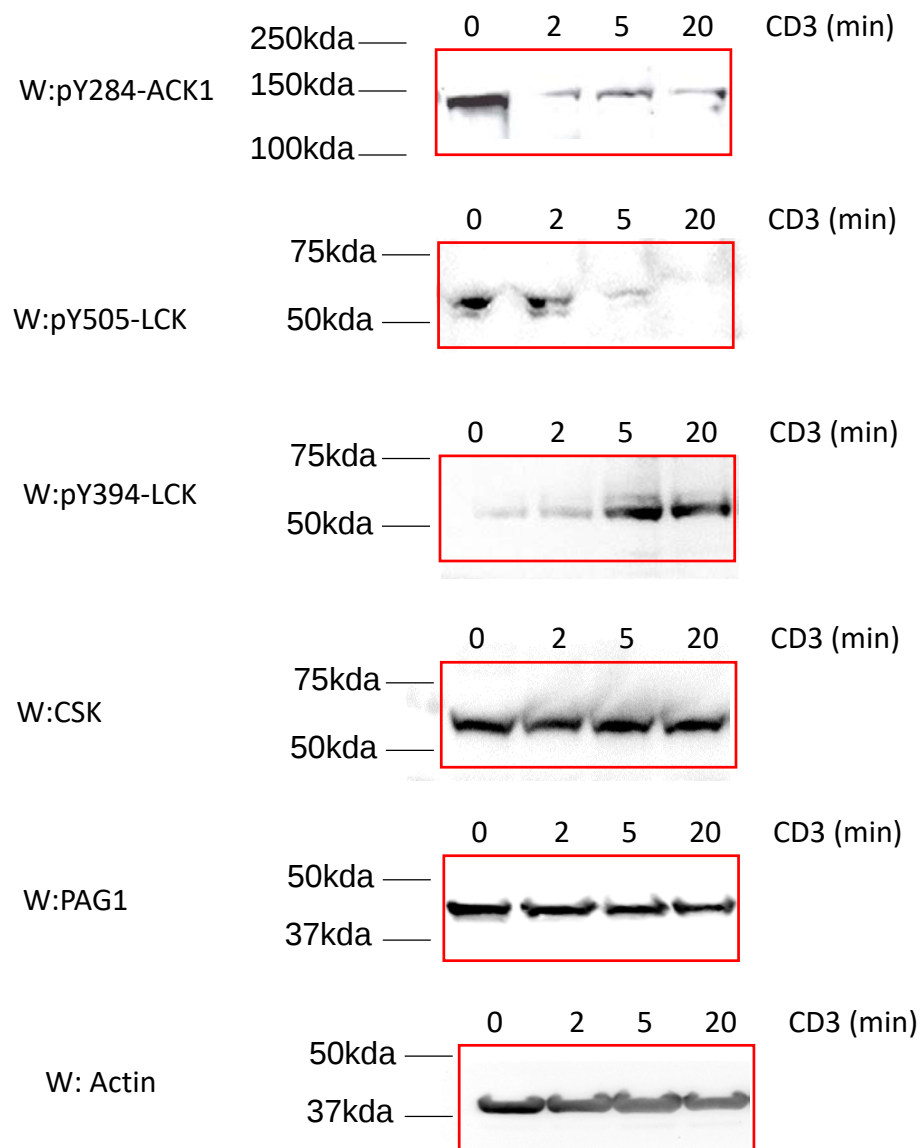


KO

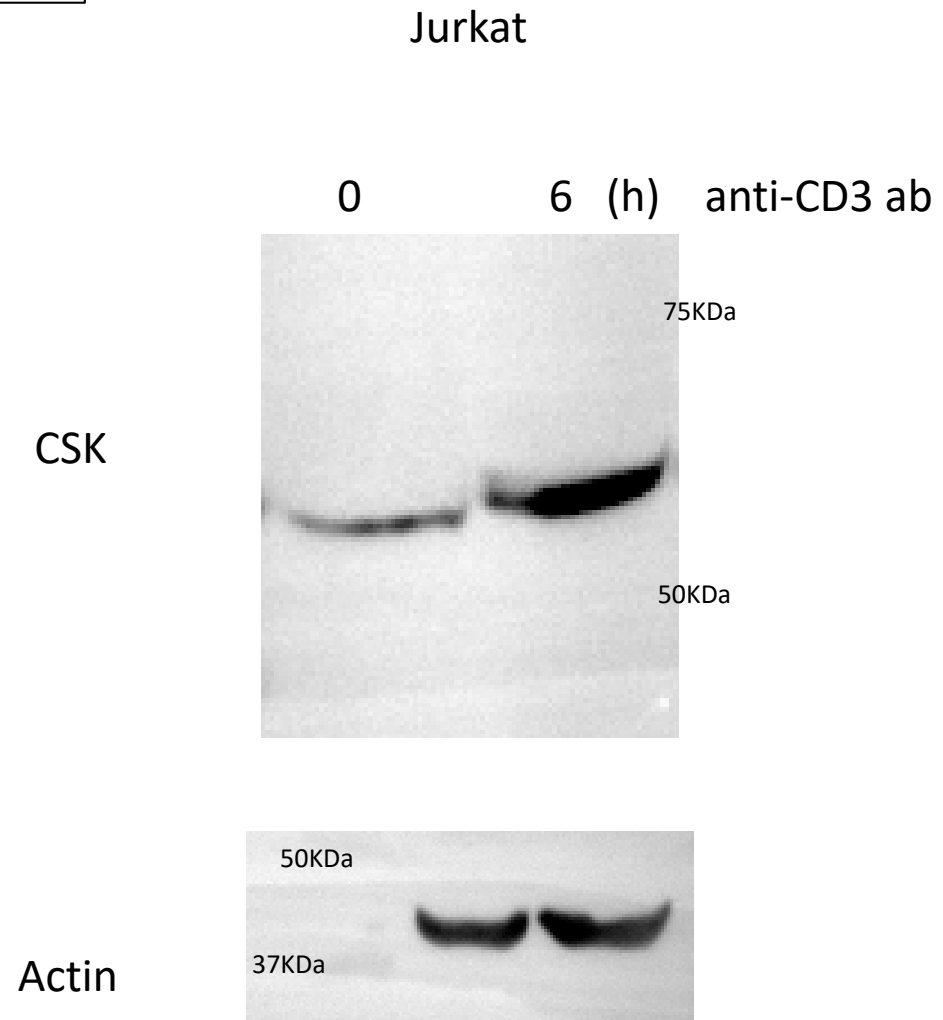


Flow cytometric analysis of CD137 on CD8 gated population isolated from splenocytes of WT and *Ack1* KO mice injected with TRAMP-C2 cells.

Supplementary Fig. 4a

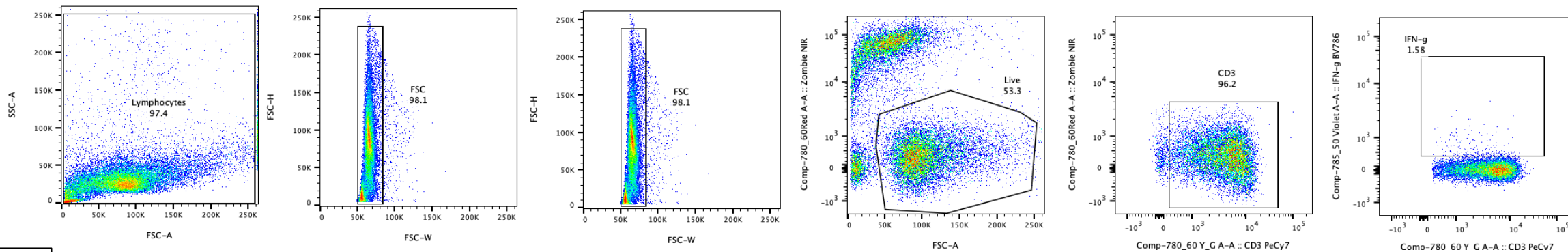


Supplementary Fig. 4b

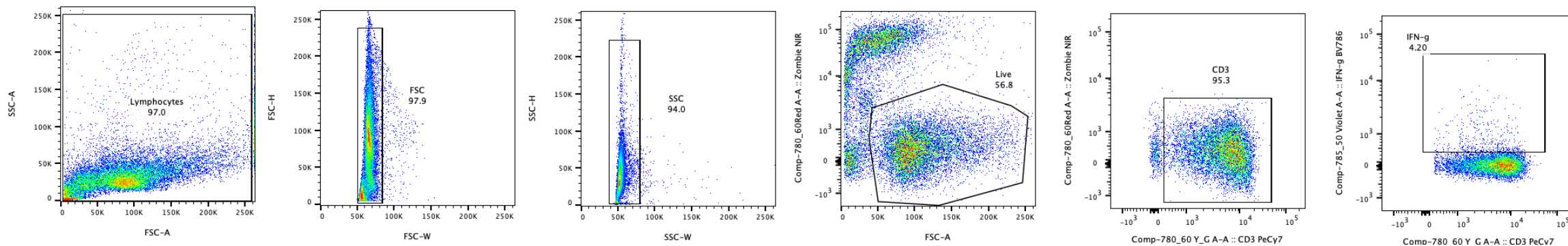


Supplementary Fig. 4c

WT



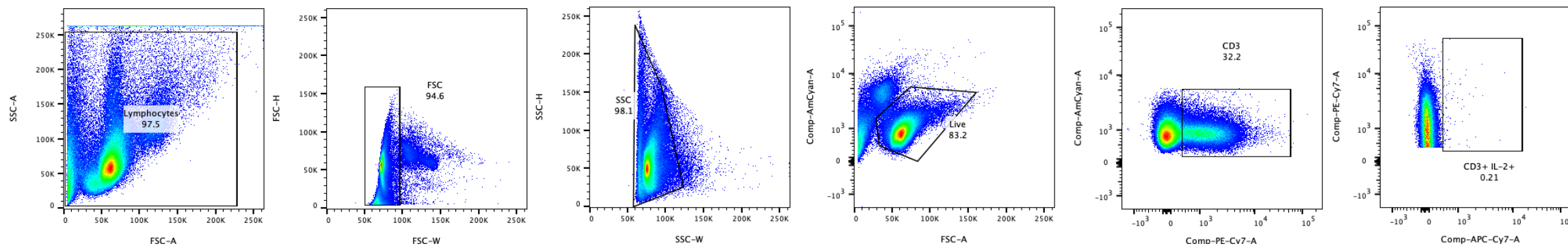
KO



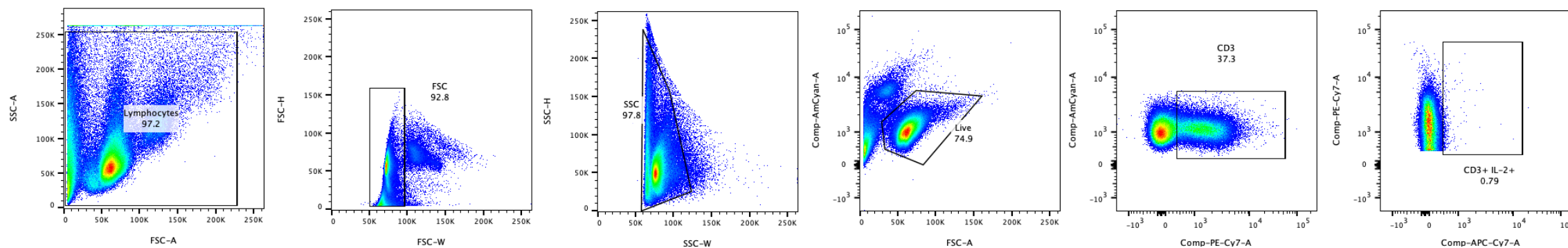
Flow cytometric analysis of IL-2 levels on CD8 gated population isolated from splenocytes of WT and *Ack1* KO mice

Supplementary Fig. 4d

WT



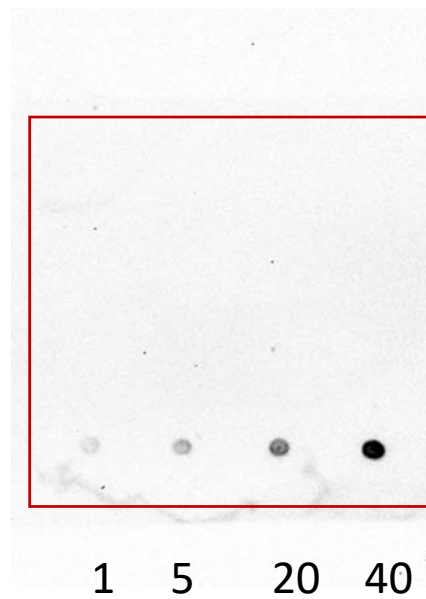
KO



Flow cytometric analysis of IL-2 levels on CD8 gated population isolated from splenocytes of WT and *Ack1* KO mice

Supplementary Fig. 6a

W: pY18-CSK



pY37-H2B peptide

non-phosphopeptide to CSK

pY18-CSK phosphopeptide

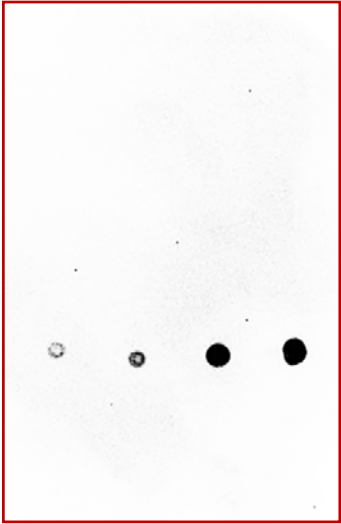
nM of peptide

Ponceau staining



Supplementary Fig. 6c

W: pY18-CSK



pY-Akt
pY-ATP5F1a
pY-H2a
non-phosphopeptide to CSK
pY18-CSK phosphopeptide
pY-H4
pY-H3

1 10 50 100 nM of peptide

Ponceau staining

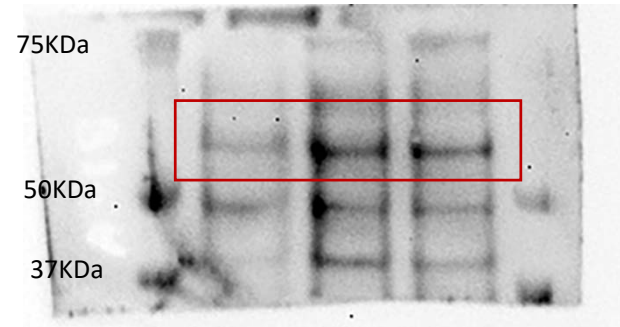


pY-Akt
pY-ATP5F1a
pY-H2a
non-phosphopeptide to CSK
pY18-CSK phosphopeptide
pY-H4
pY-H3

1 10 50 100 nM of peptide

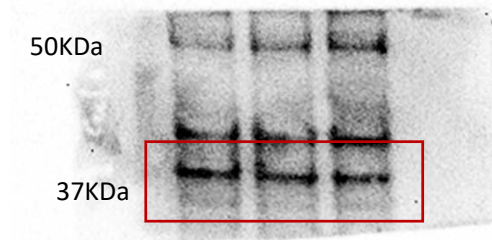
Supplementary Fig. 6d

pY18-CSK



1 2.5 3.5 Protease inhibitor cocktail (μ l)

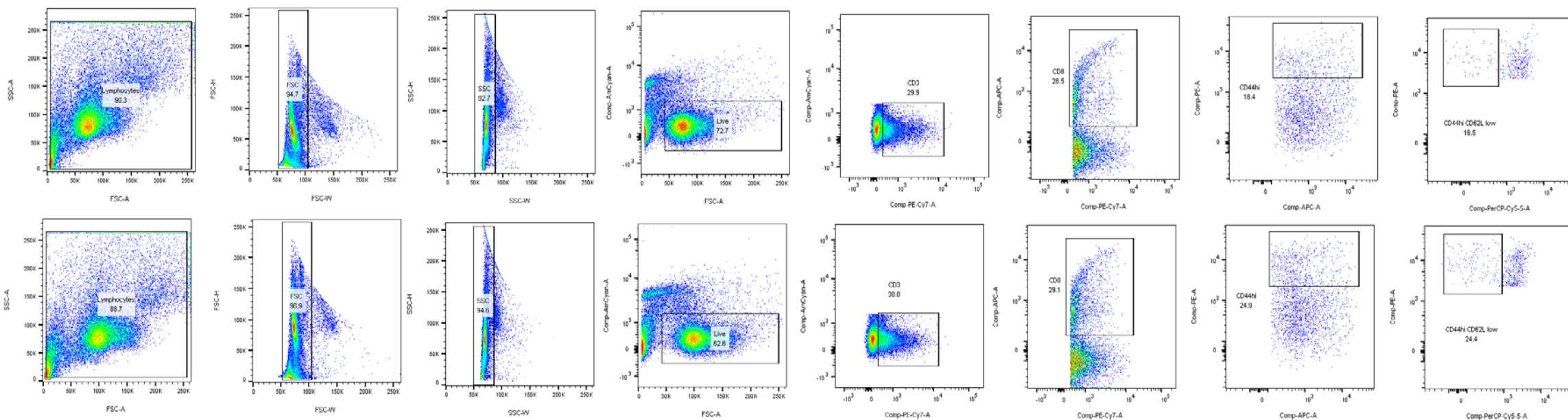
Actin



1 2.5 3.5 Protease inhibitor cocktail (μ l)

Supplementary Fig. 7c

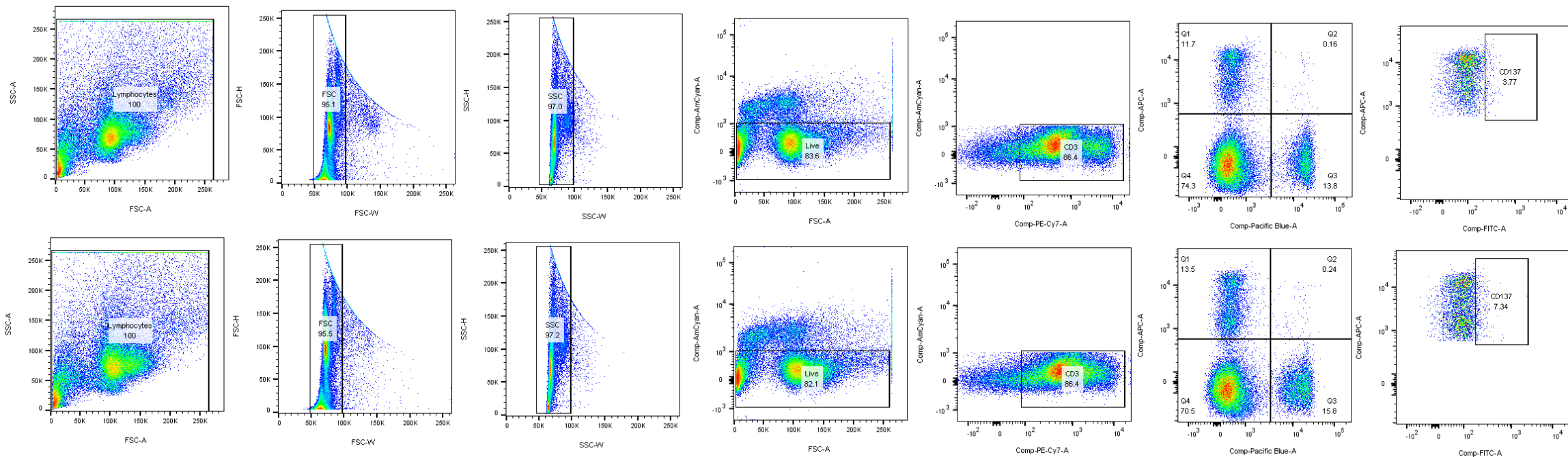
WT



Flow cytometric analysis of CD44^{hi}/CD62L^{low} on CD8 gated population isolated from splenocytes of WT and *Ack1* KO mice injected with TRAMP-C2 tumors

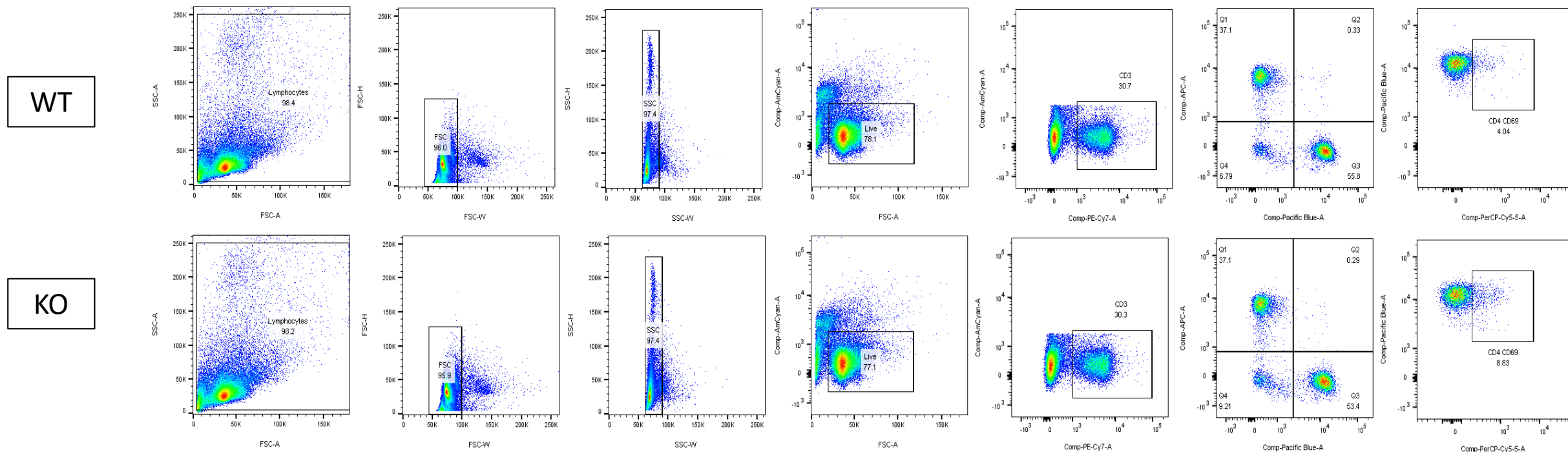
Supplementary Fig. 7d

WT



Flow cytometric analysis of CD137 on CD8 gated population isolated from splenocytes of WT and *Ack1* KO mice injected with TRAMP-C2 tumors.

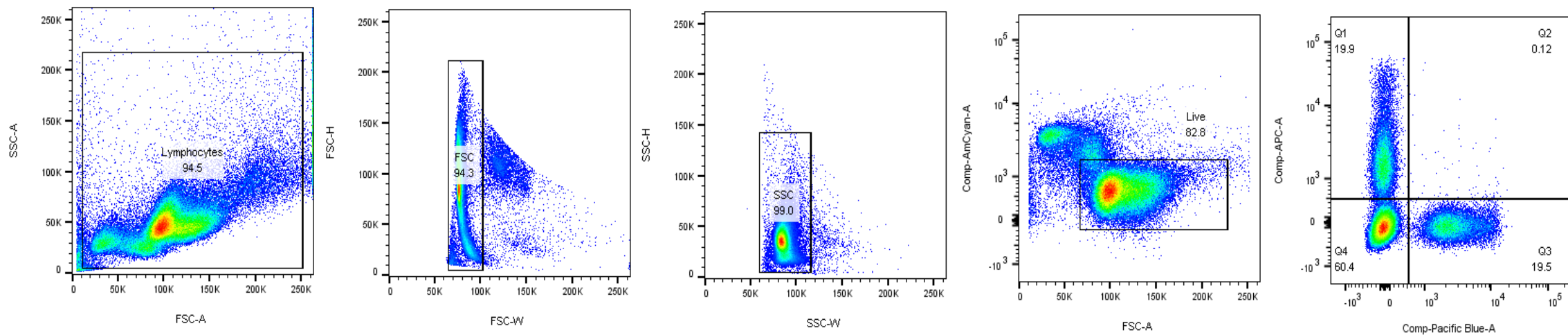
Supplementary Fig. 7e



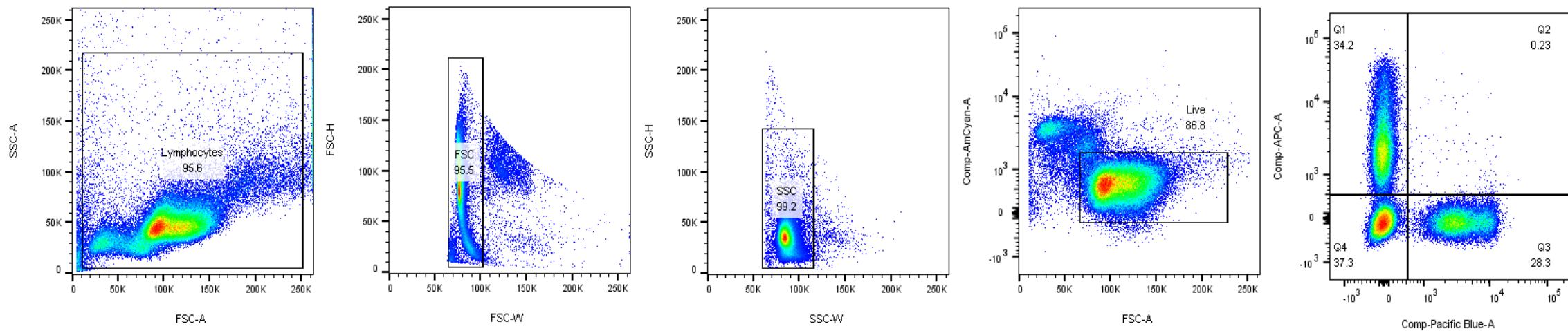
Flow cytometric analysis of CD69 on CD4 gated population isolated from splenocytes of WT and *Ack1* KO mice injected with TRAMP-C2 tumors.

Supplementary Fig. 7f

WT



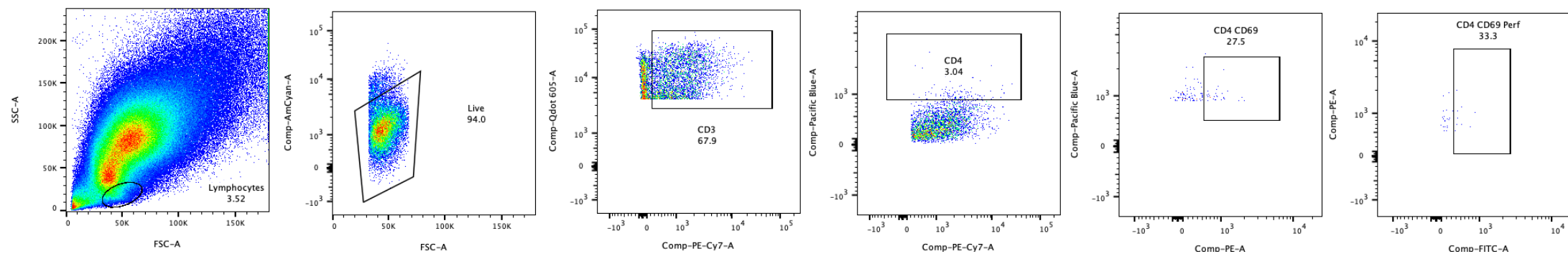
KO



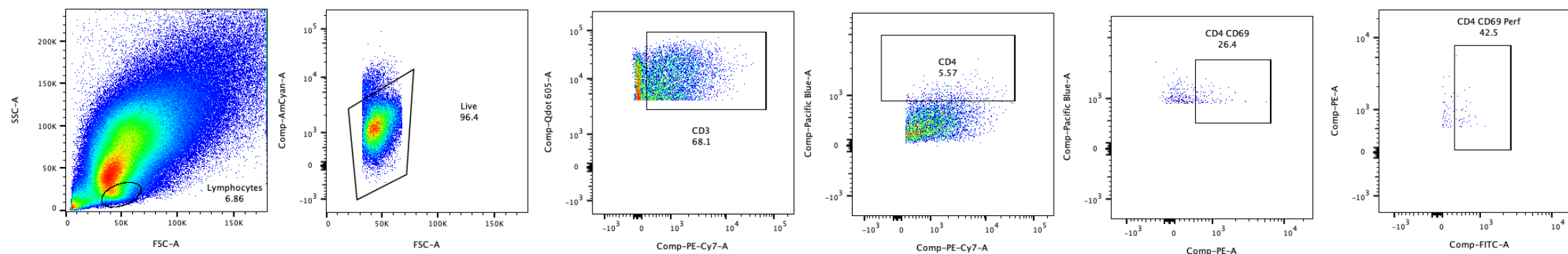
Flow cytometric analysis of CD4 and CD8 population in lymph nodes drained from WT and *Ack1* KO mice implanted with TRAMP-C2 tumors.

Supplementary Fig. 8a

WT



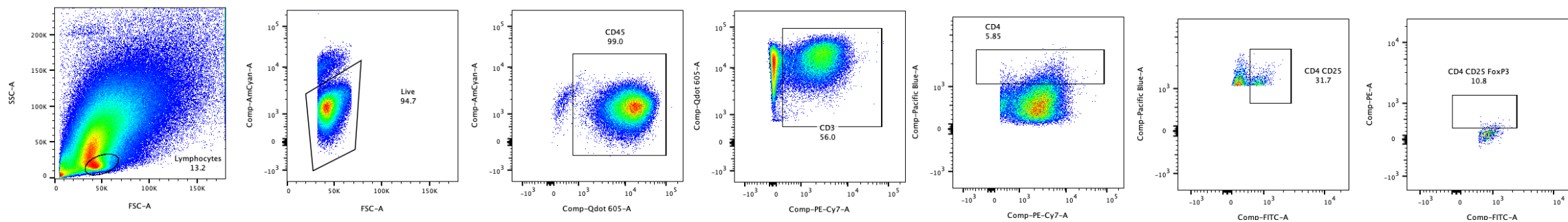
KO



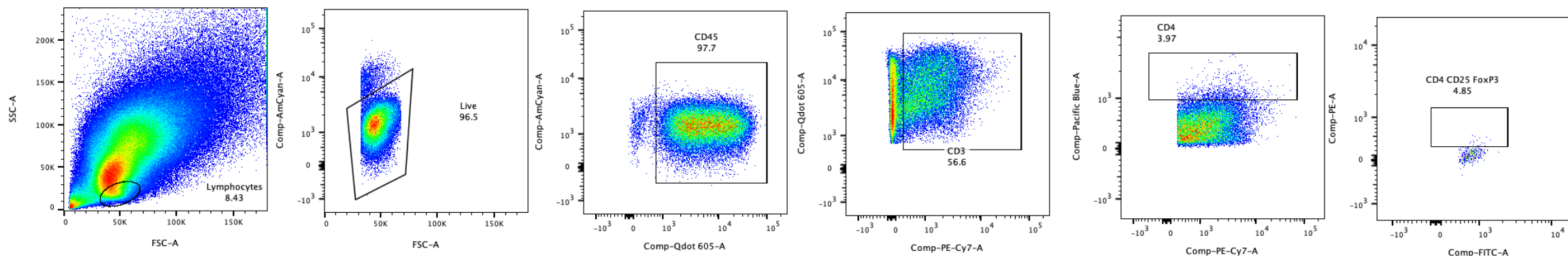
Flow cytometric analysis of Perforin expressing CD4+CD69+ cells in TILs from TRAMP-C2 tumors injected in WT and *Ack1* KO mice.

Supplementary Fig. 8b

WT



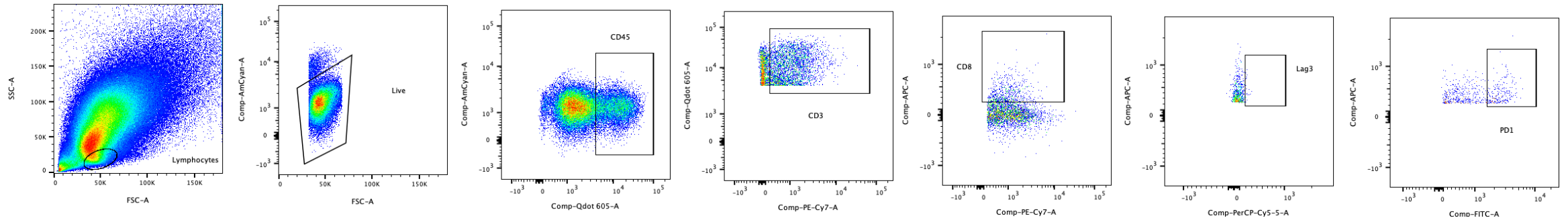
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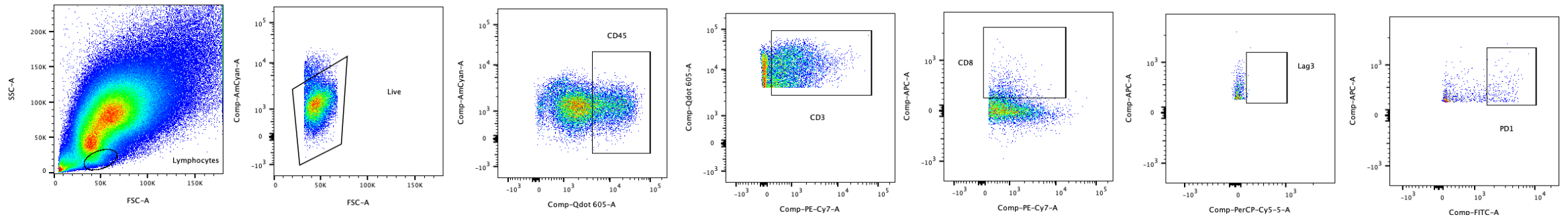
Flow cytometric analysis of Tregs in TILs from TRAMP-C2 tumors injected in WT and *Ack1* KO mice.

Supplementary Fig. 8c

WT



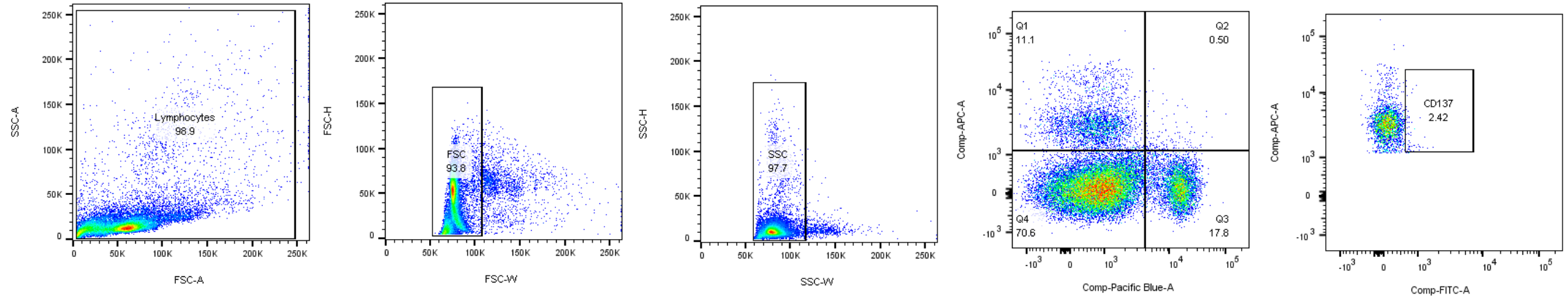
KO



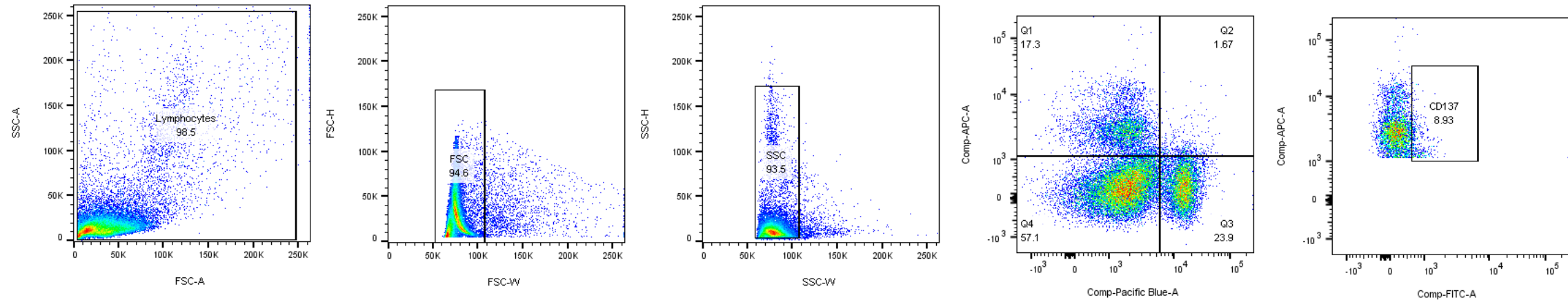
Flow cytometric analysis of exhaustion markers on CD8 gated population in TILs from TRAMP-C2 tumors injected in WT and *Ack1* KO mice.

Supplementary Fig. 9c

Vehicle



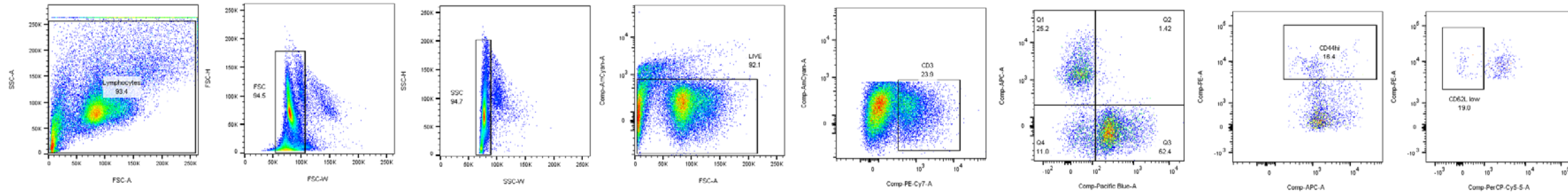
(R)-9b



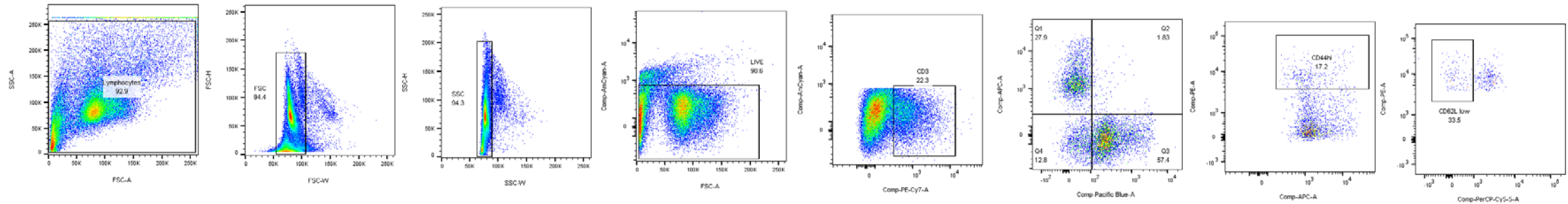
Flow cytometric analysis of CD137 on CD8 gated population isolated from splenocytes of vehicle and (R)-9b injected mice with TRAMP-C2 tumors.

Supplementary Fig. 9d

Vehicle



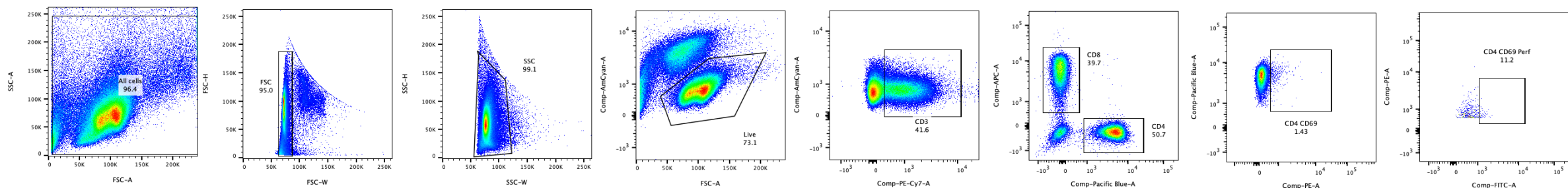
(R)-9b



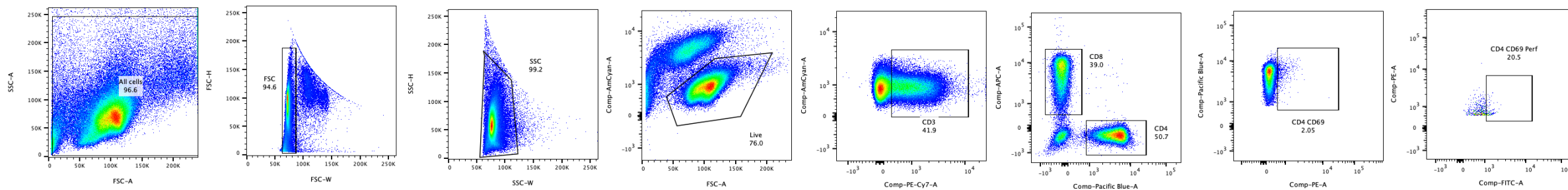
Flow cytometric analysis of CD44^{hi}/CD62L^{low} on CD8 gated population isolated from splenocytes of vehicle and (R)-9b injected mice with TRAMP-C2 tumors.

Supplementary Fig. 10a

Vehicle



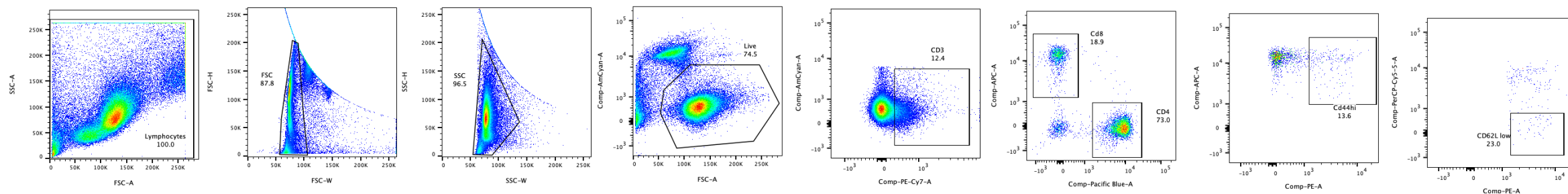
(R)-9b



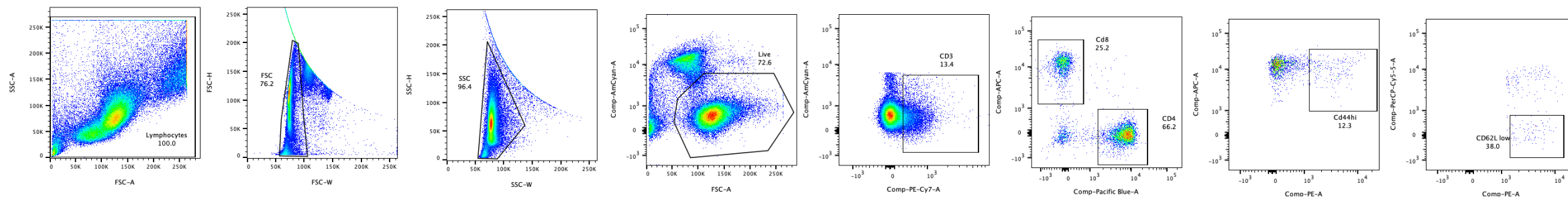
Flow cytometric analysis of Perforin expressing CD4+CD69+ cells from splenocytes of vehicle and (R)-9b injected mice with TRAMP-C2 tumors.

Supplementary Fig. 10b

Vehicle



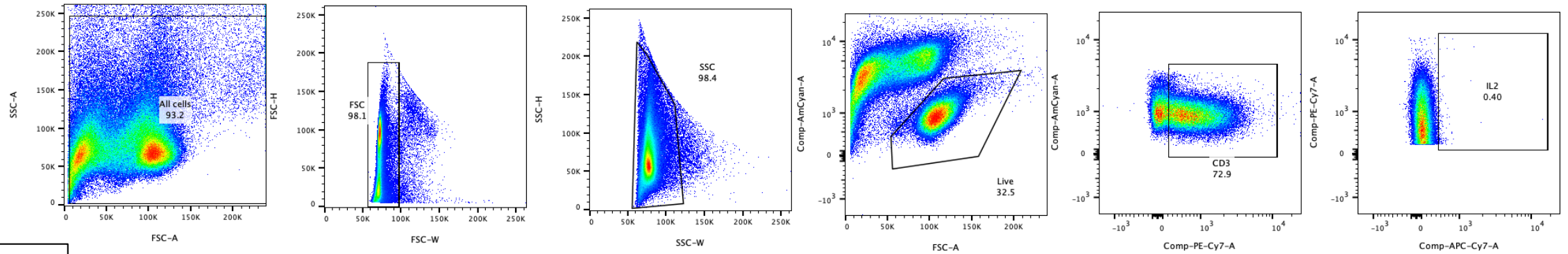
(R)-9b



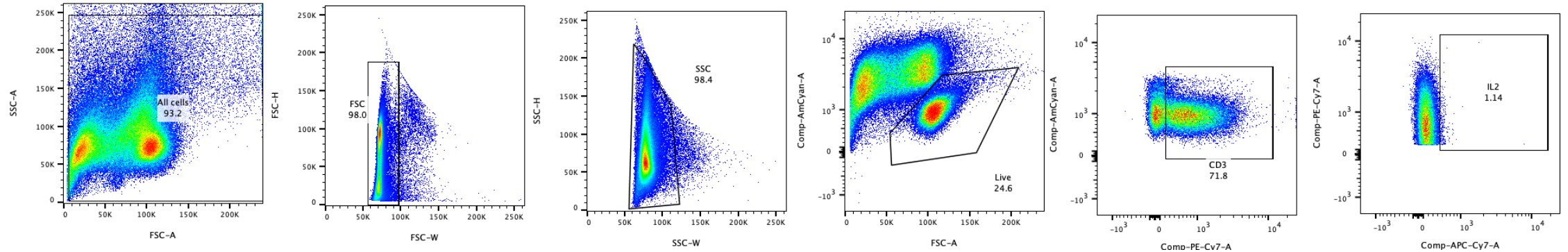
Flow cytometric analysis of CD44^{hi}/CD62L^{low} on CD8 gated population isolated from lymph node of vehicle and (R)-9b injected mice with TRAMP-C2 tumors.

Supplementary Fig. 10c

Vehicle



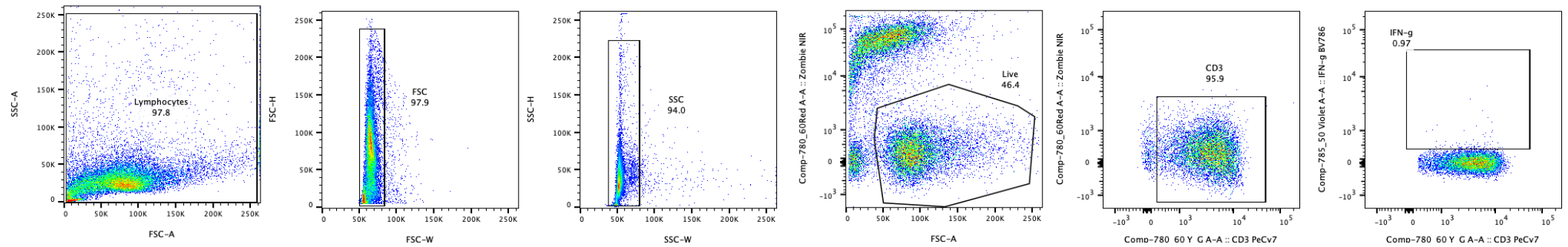
(R)-9b



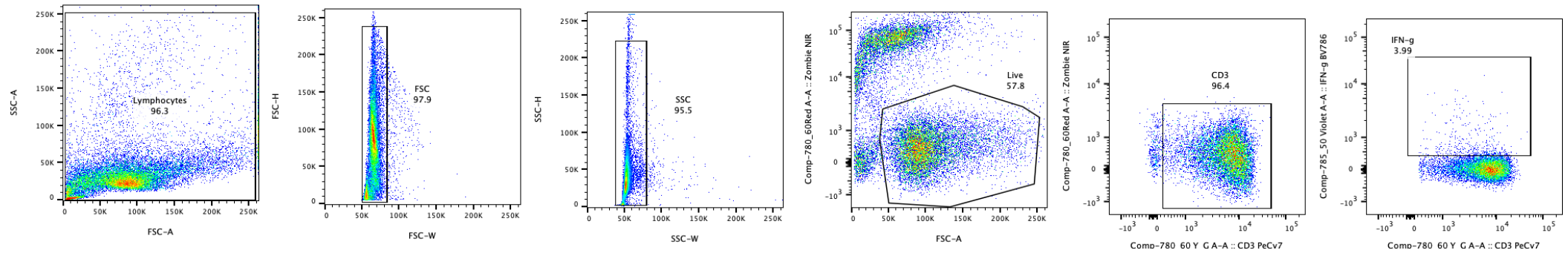
Flow cytometric analysis of IL-2 expressing CD3 population from isolated splenocytes of vehicle and (R)-**9b** injected mice with TRAMP-C2 tumors.

Supplementary Fig. 10d

Vehicle



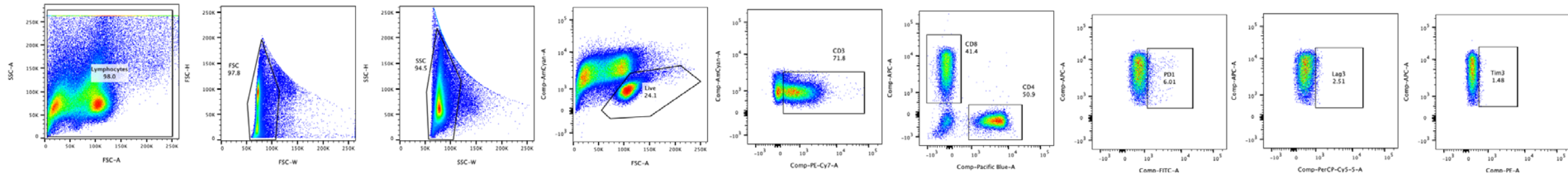
(R)-9b



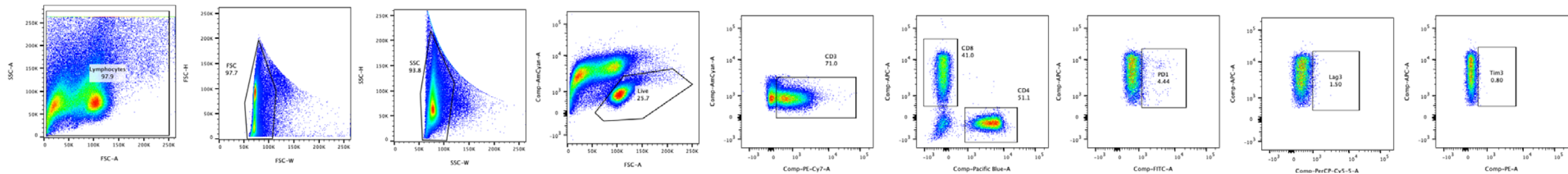
Flow cytometric analysis of IFN- γ expressing CD3 population from isolated splenocytes of vehicle and (R)-9b injected mice with TRAMP-C2 tumors.

Supplementary Fig. 11a

Vehicle



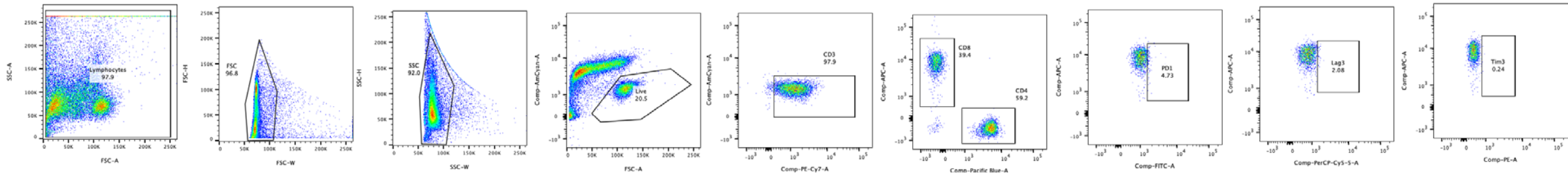
(R)-9b



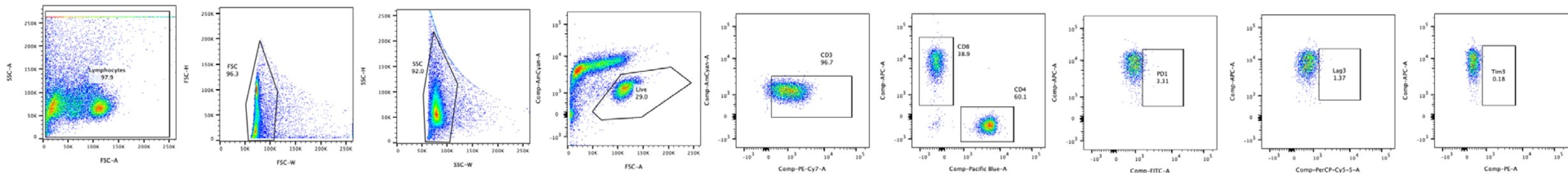
Flow cytometric analysis of exhaustion markers on CD8 gated population from isolated splenocytes of vehicle and (R)-9b injected mice with TRAMP-C2 tumors.

Supplementary Fig. 11b

Vehicle



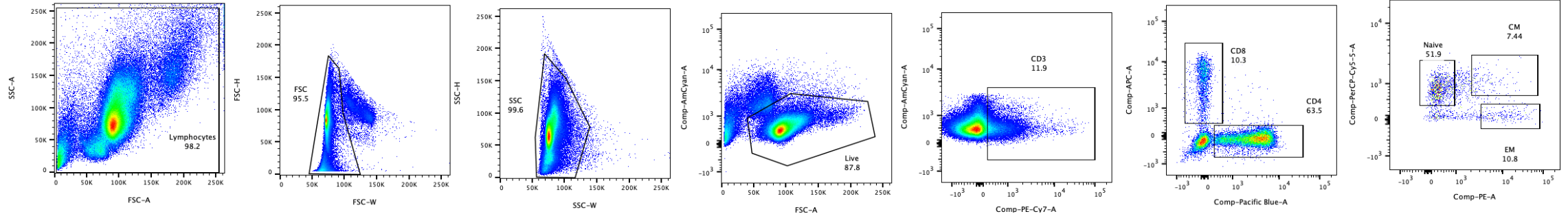
(R)-9b



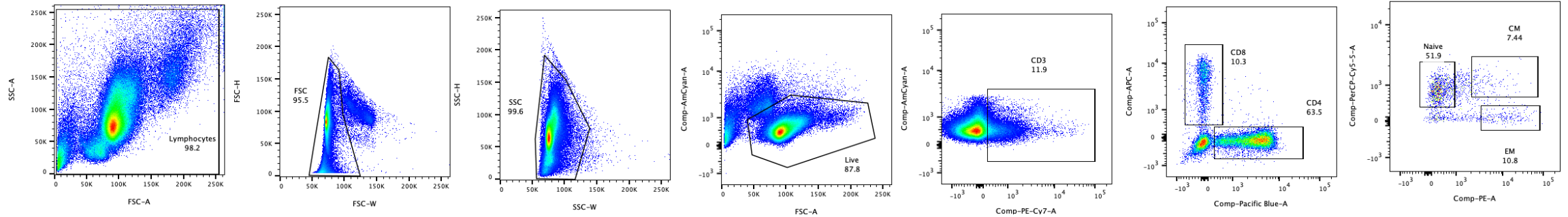
Flow cytometric analysis of exhaustion markers on CD8 gated population from isolated draining lymph nodes of vehicle and (R)-9b injected mice with TRAMP-C2 tumors.

Supplementary Fig. 12a

WT



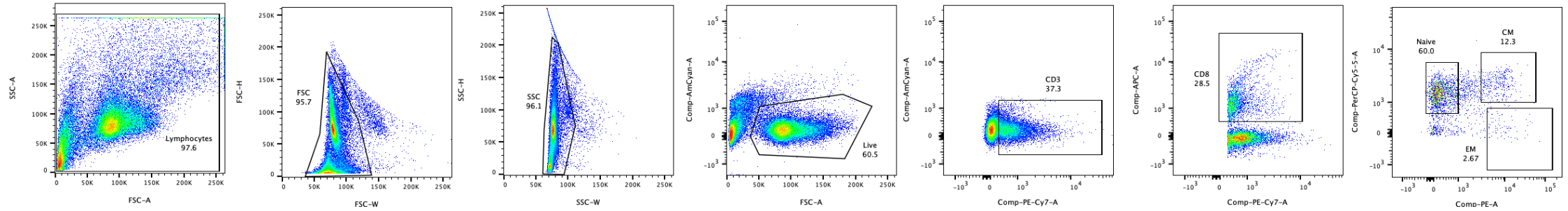
KO



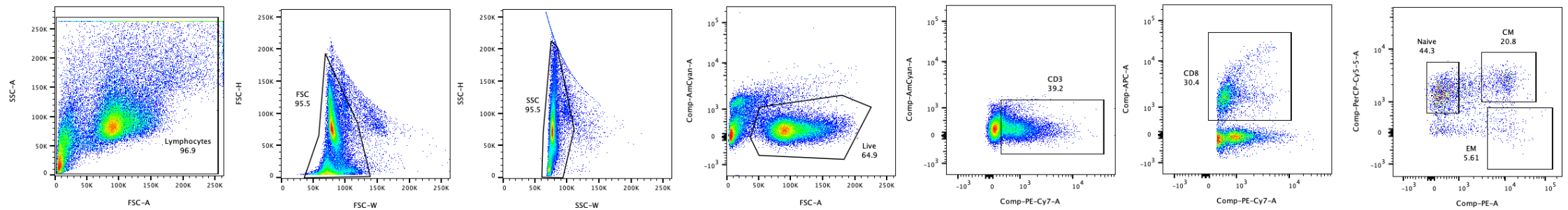
Flow cytometric analysis of naïve, central memory (CM), effector memory (EM) CD8 T cells in splenocytes isolated from TRAMP-C2 tumors injected in WT and *Ack1* KO mice.

Supplementary Fig. 12b

Vehicle



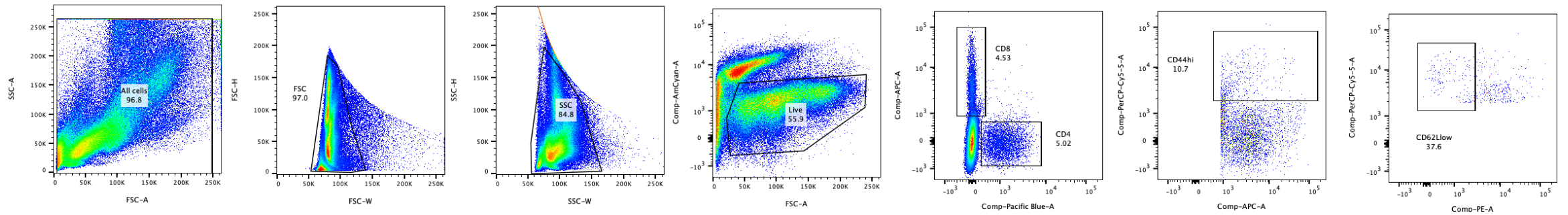
(R)-9b



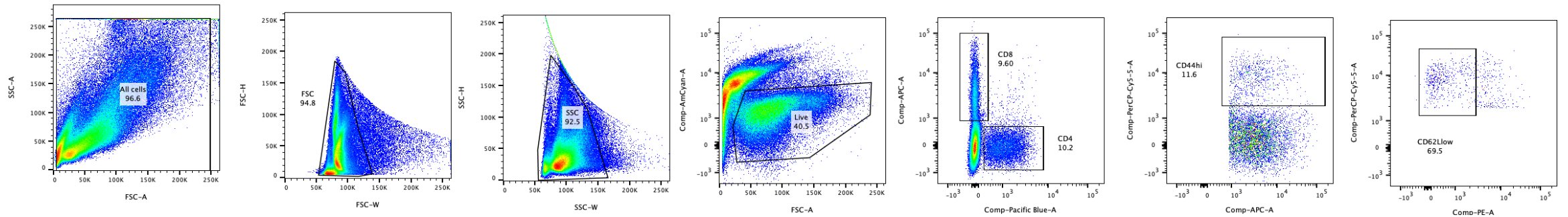
Flow cytometric analysis of naïve, central memory (CM), effector memory (EM) CD8 T cells in splenocytes of vehicle and (R)-9b injected mice with TRAMP-C2 tumors.

Supplementary Fig. 13e

WT



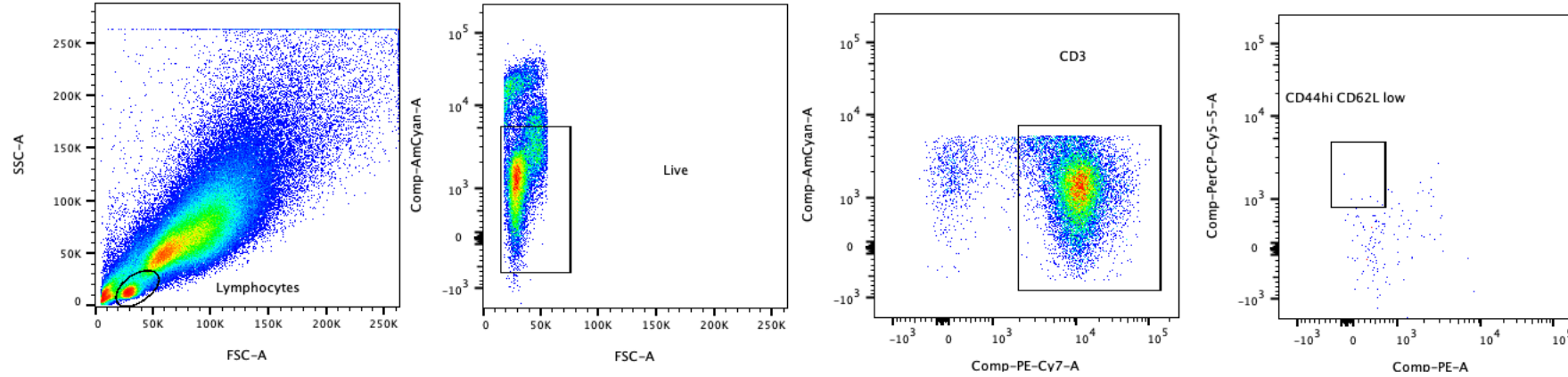
KO



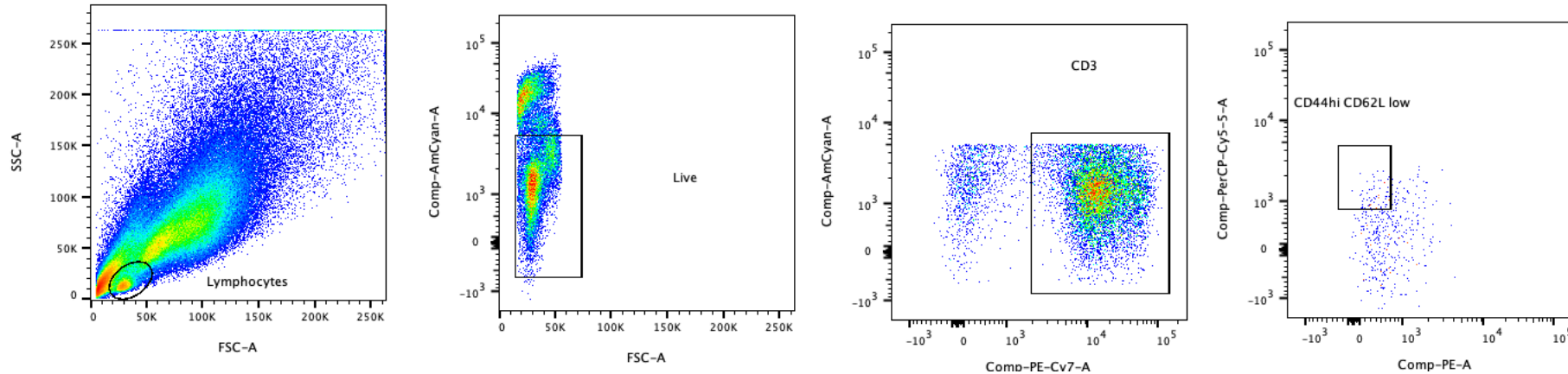
Flow cytometric analysis of CD44^{hi}/CD62L^{low} on CD8 gated population after adoptive transfer T cells from WT and *Ack1* KO mice into TRAMP-C2 tumor bearing NSG mice

Supplementary Fig. 13e

WT



KO

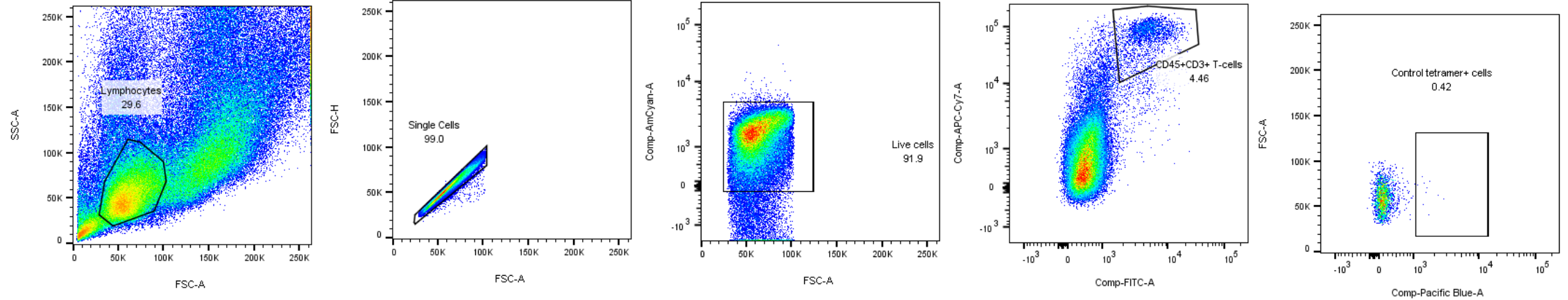


Flow cytometric analysis of CD44^{hi}/CD62L^{low} on CD8 gated population in the TILs after adoptive transfer T cells from WT and *Ack1* KO mice into TRAMP-C2 tumor bearing NSG mice

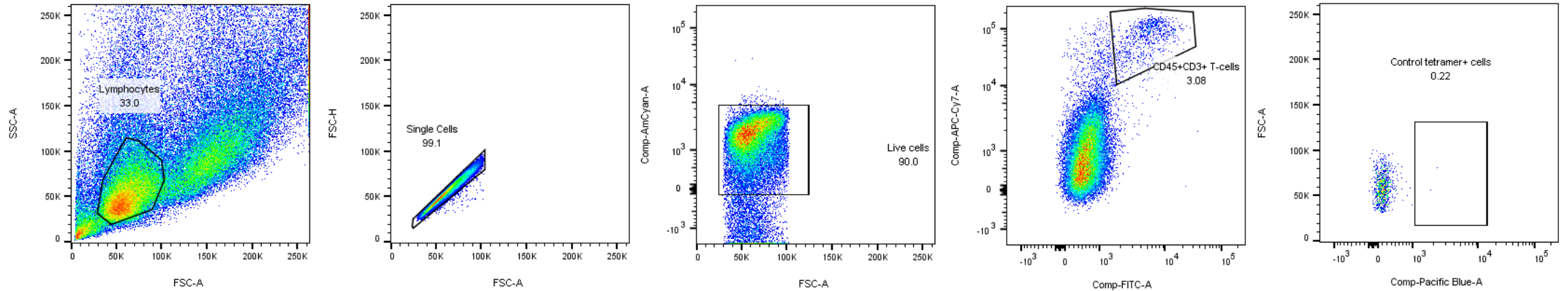
Supplementary Fig. 13f

Control tetramer

WT



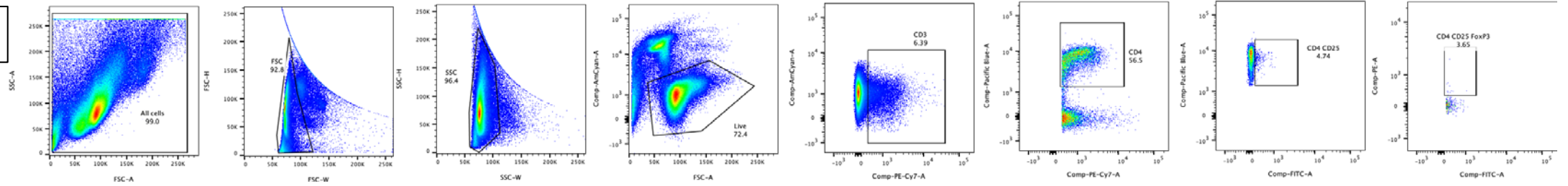
KO



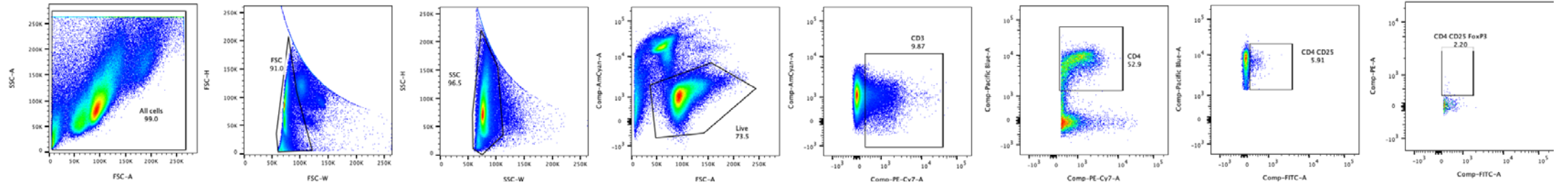
Scatter plot of the control tetramer stained splenocytes assessed by flow cytometry.

Supplementary Fig. 15

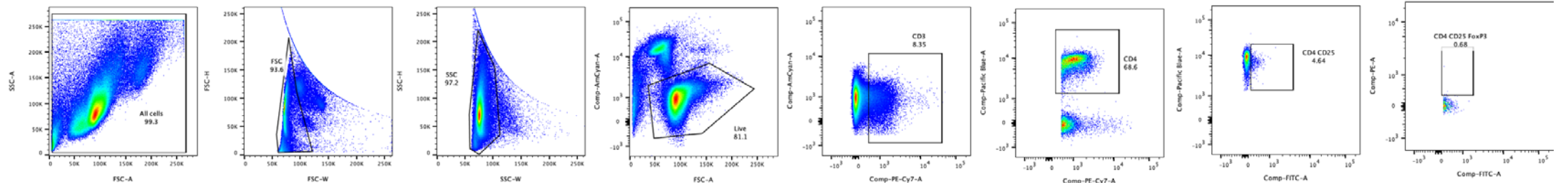
Vehicle



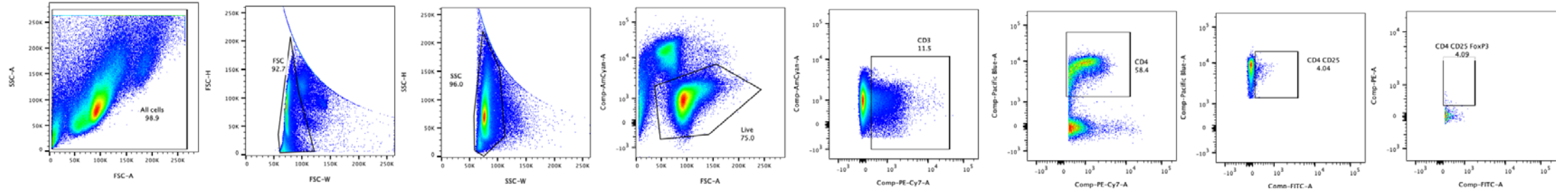
ICB



(*R*)-9b



ICB+
(*R*)-9b

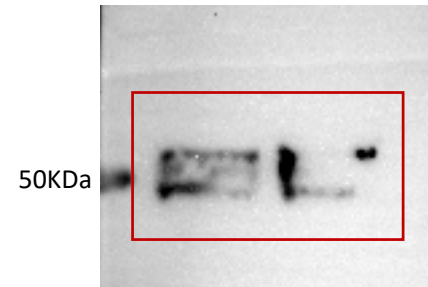


Flow cytometric analysis of Tregs in TILs from TRAMP-C2 tumors injected in C57BL/6 mice treated with vehicle, ICB antibodies, (*R*)-9b and combination.

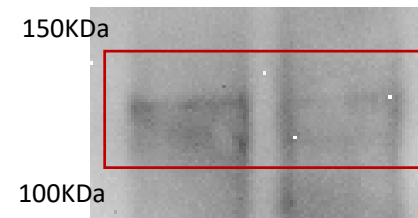
Supplementary Fig. 17c

PBMC

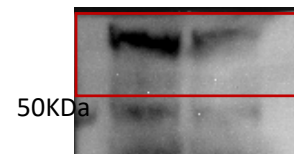
pY505-LCK



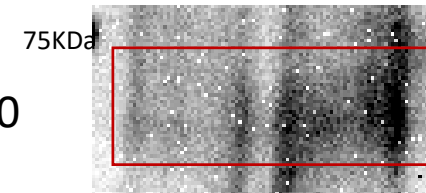
pY284-ACT1



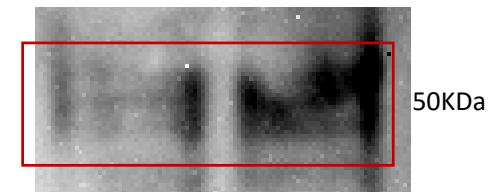
pY18-CSK



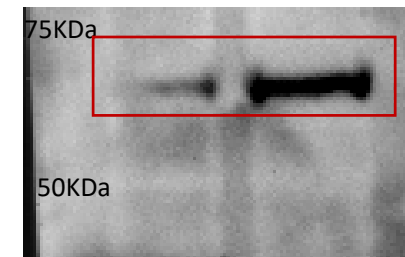
pY319-ZAP70



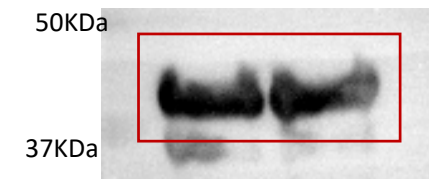
pY394-LCK



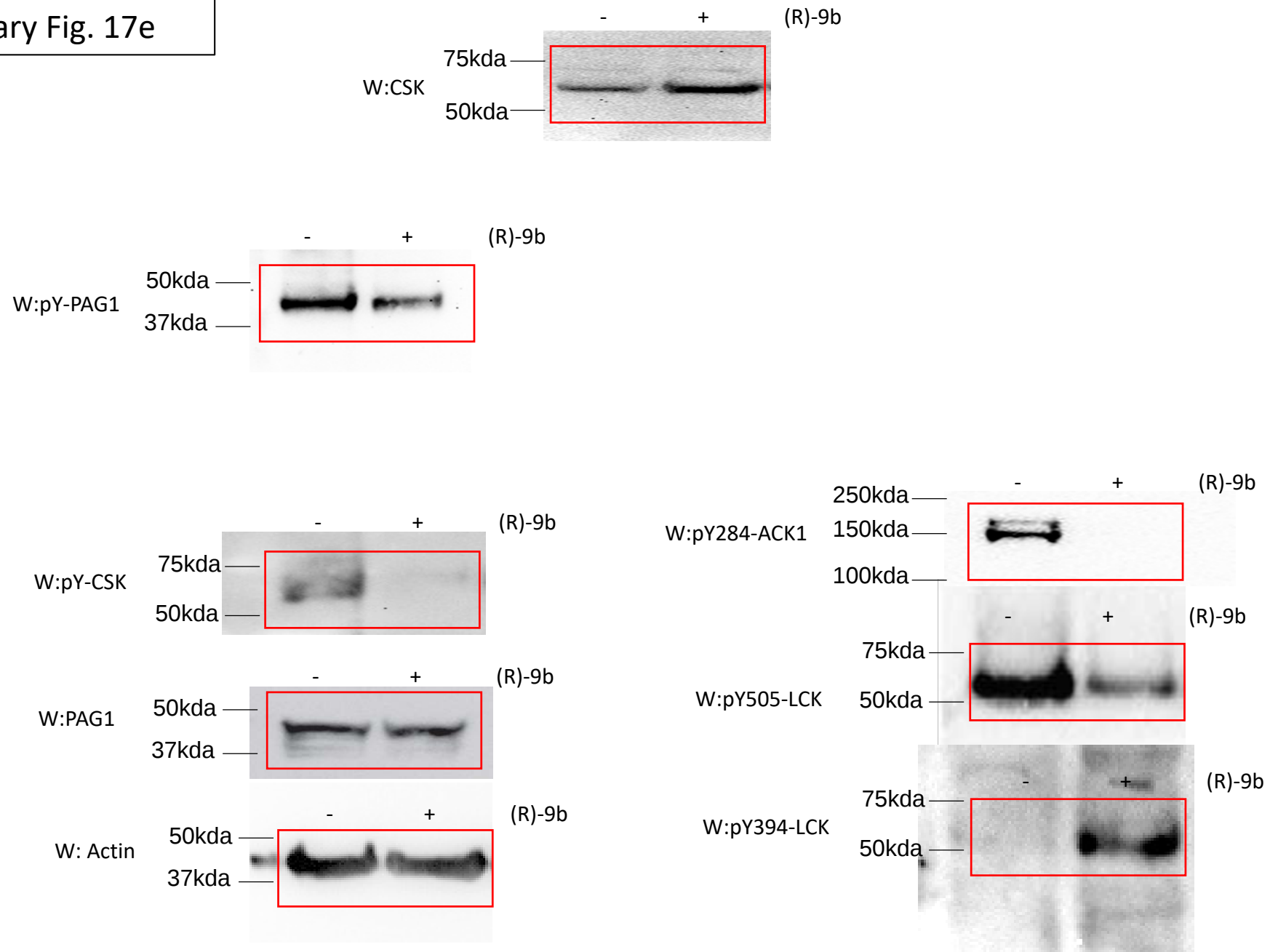
CSK



ACTIN



Supplementary Fig. 17e



Supplementary Fig. 17f

