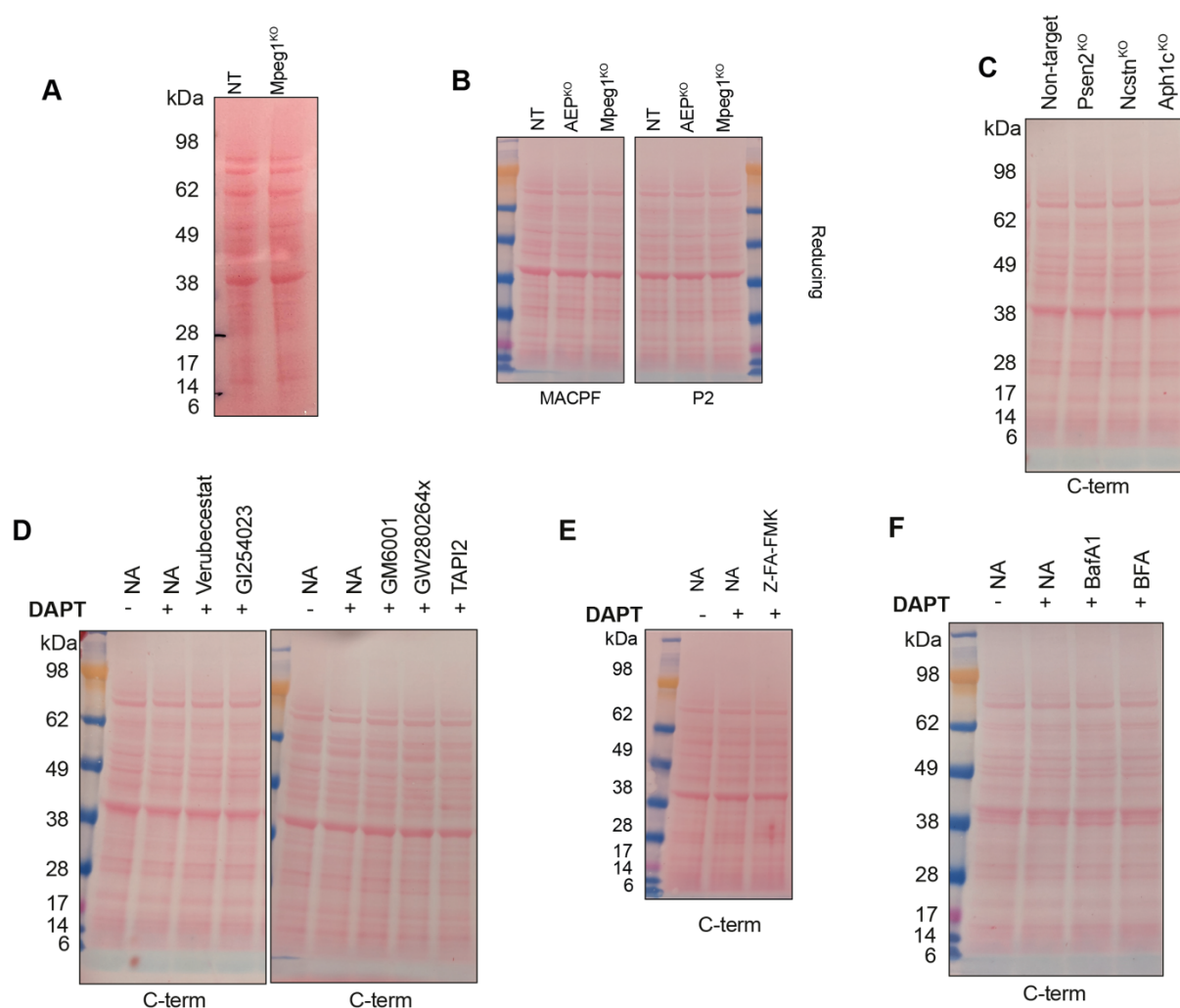


Appendix for Anchoring of perforin-2 via the transmembrane domain is required for antigen escape into the cytosol

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Appendix Figure S1



Appendix Figure S1. Ponceau-stained blots for Western Blots included in the manuscript.

A Loading control for Figure 2B

B Loading control for Figure 2C

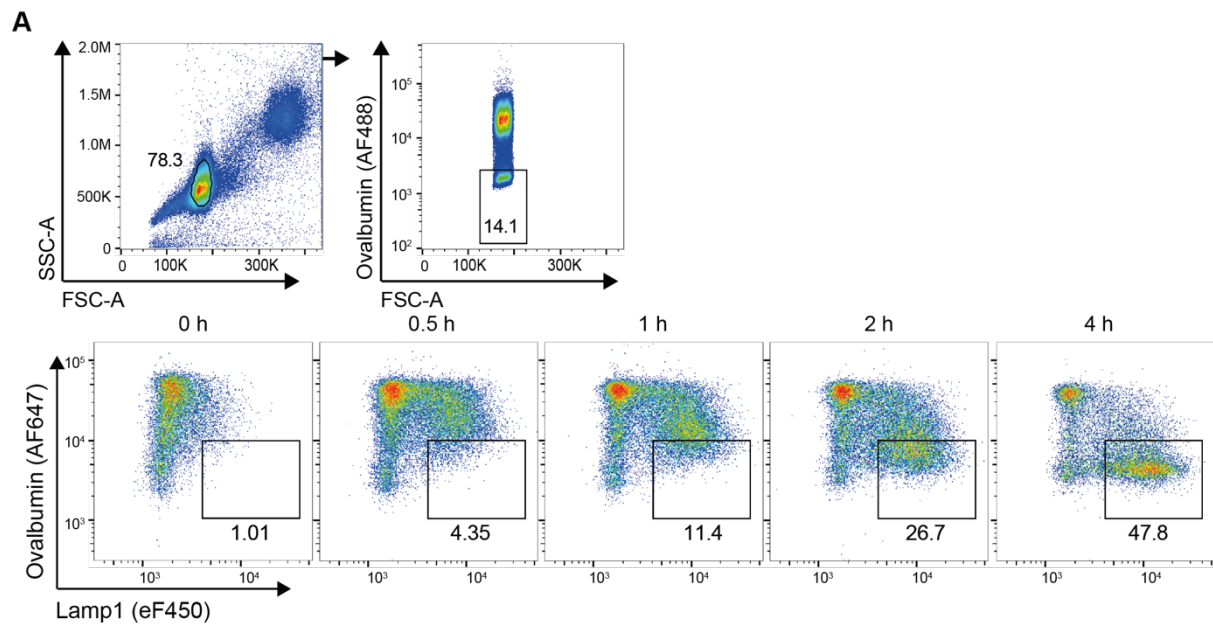
C Loading control for Figure 3B

D Loading control for Figure 4C

E Loading control for Figure 4E

F Loading control for Figure 4D

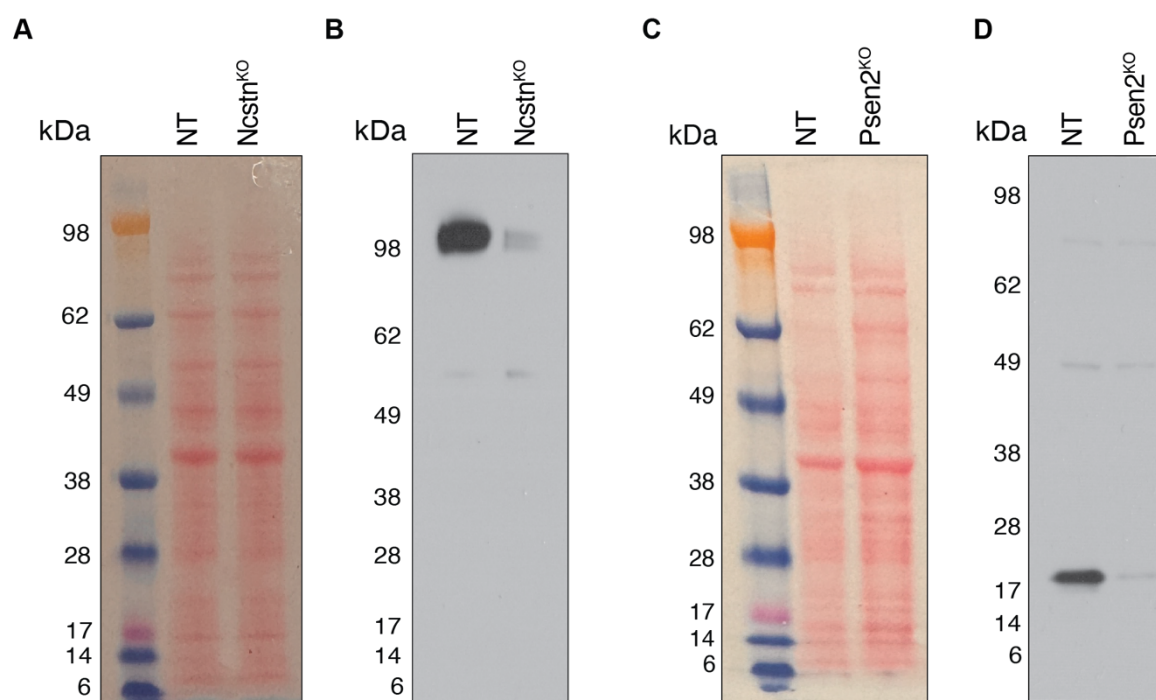
Appendix Figure S2



Appendix Figure S2. Gating strategy for the phagoFACS assays.

A Gating strategy for analysis of phagosomes isolated from MutuDCs. The cells were pulsed with OVA beads for 25 min at 16°C followed by 5 min at 37°C and either immediately placed on ice (0 h) or chased for the indicated time. Isolated phagosomes were stained with antibodies against ovalbumin and Lamp1. The gate in the bottom panel indicates mature phagosomes. n = 5 independent experiments.

Appendix Figure S3

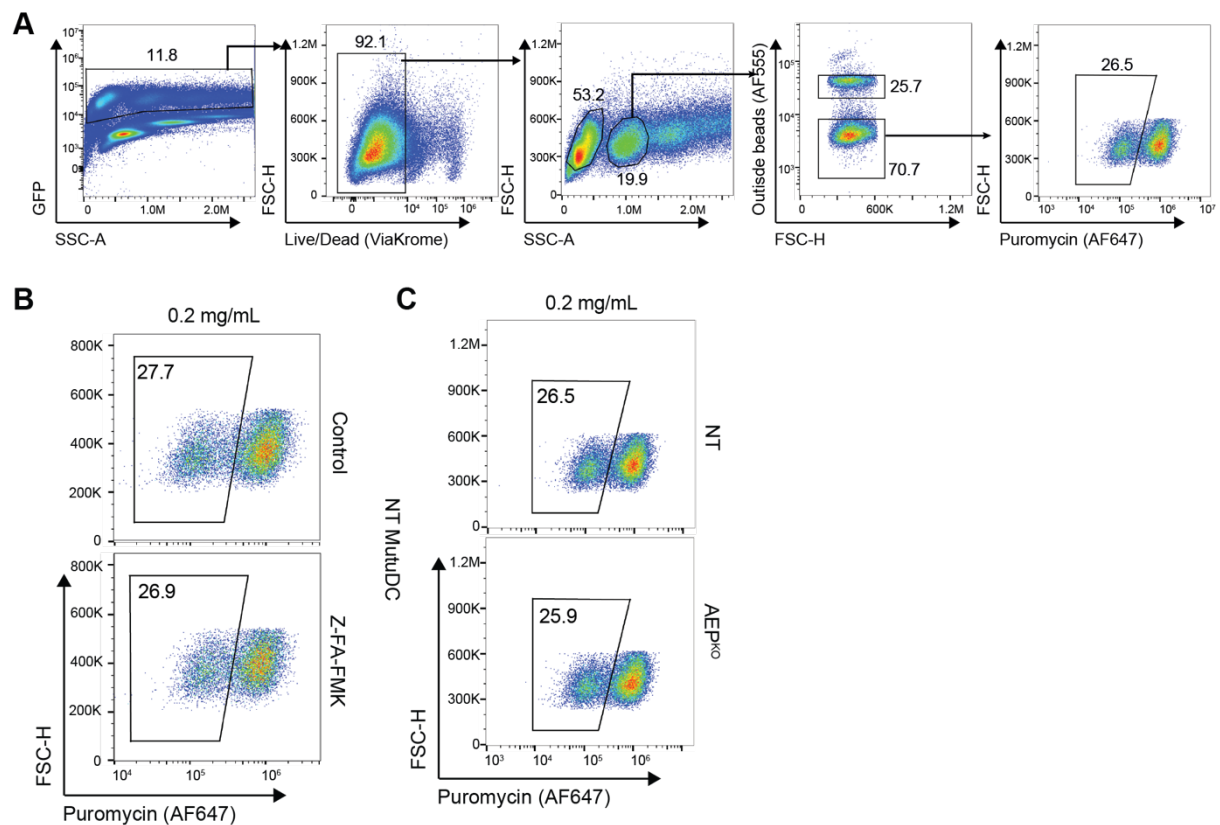


Appendix Figure S3. Validation of the knockout cell lines.

A,B Loading control (A) and immunoblot of control and Ncstn^{KO} MutuDCs with the α Ncstn antibody.

C,D Loading control (C) and immunoblot (D) of control and Psen2^{KO} MutuDCs with the α Psen2 antibody.

Appendix Figure S4

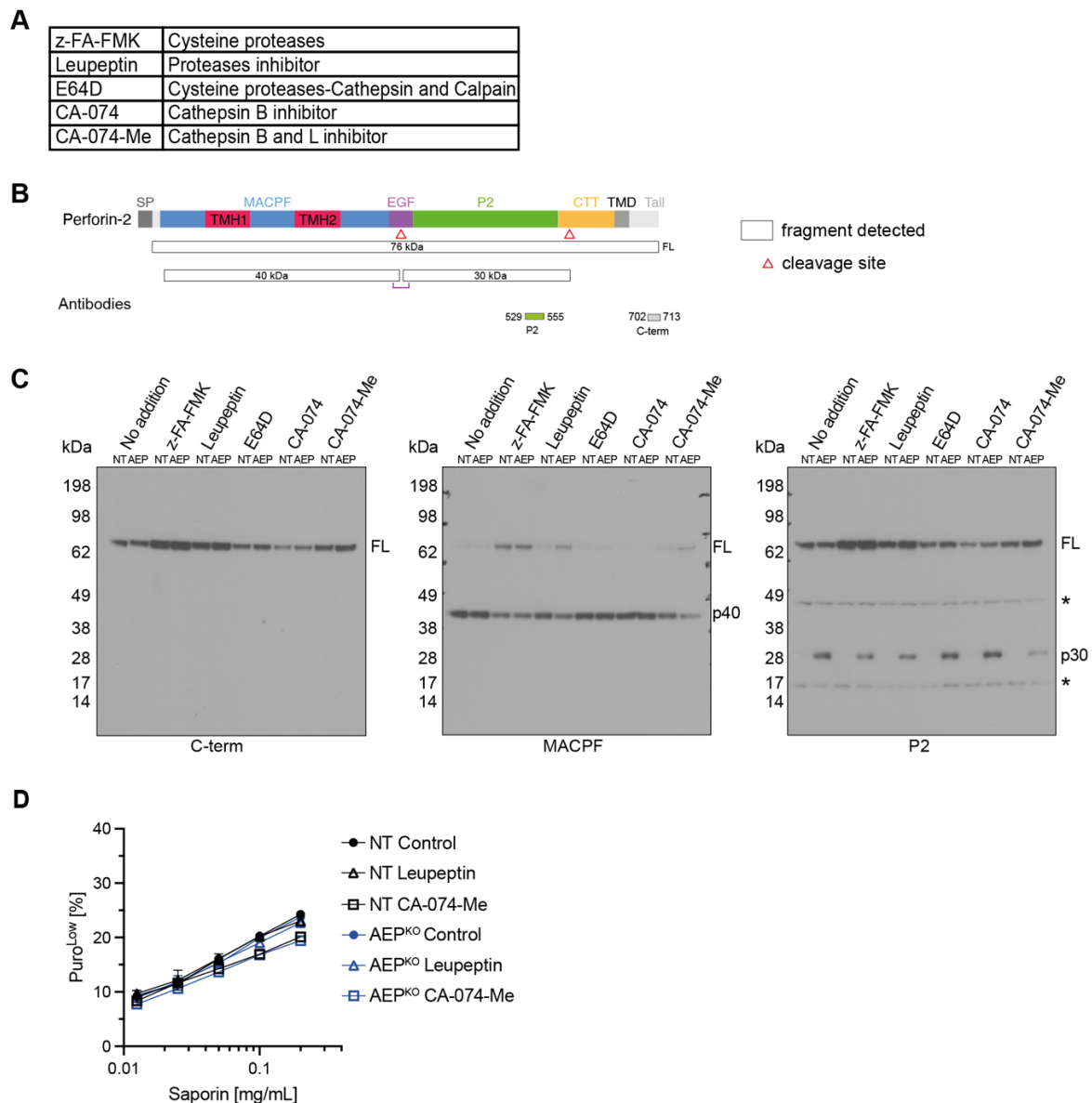


Appendix Figure S4. Saporin-puromycin phagosomal escape assay.

A Gating strategy for the saporin-puromycin assay to monitor antigen escape from phagosomes (NT sample from panel C is used as an example).

B,C Representative flow plots from the saporin-puromycin assay performed in the presence and absence of Z-FA-FMK (with 1 h pre-incubation) (B) or in NT and AEP^{KO} MutuDCs (C).

Appendix Figure S5



Appendix Figure S5. Promiscuous cleavage in the EGF domain is not required for saporin escape.

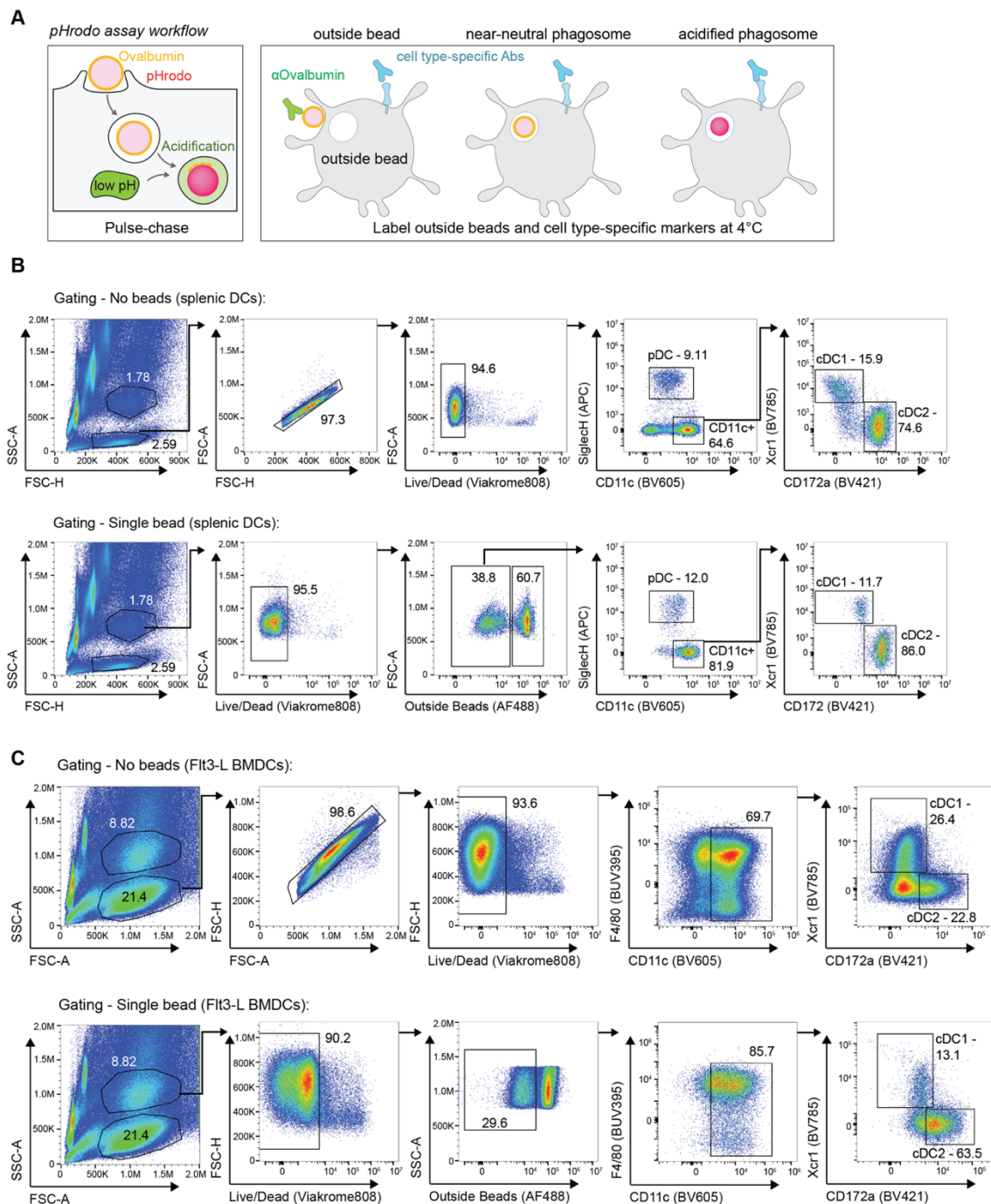
A Small-molecule inhibitors and their targets.

B Diagram of perforin-2 fragments relevant for the immunoblots in this Figure.

C Immunoblot of NT or AEP^{KO} MutuDCs untreated or incubated with Z-FA-FMK (10 μ M), Leupeptin (100 μ M), E64D (10 μ M), CA-074 (50 μ M) or CA-0740-Me (50 μ M) for 6 hours. Immunoblot under reducing conditions using the α C-term, α MACPF or α P2 antibodies. Leupeptin and CA-074-Me have a synergistic effect with AEP depletion, suggesting that cleavage in the EGF linker is promiscuous and can be executed by either AEP or cathepsins. n = 2 independent experiments.

D Quantification of PuroLow cells in saporin-puromycin escape assay in NT and AEP^{KO} MutuDCs with and without treatment with the leupeptin or CA-074-Me. These data support the notion that cleavage in the EGF domain is not required for endocytic escape. Mean and SEM of n = 2 independent experiments.

Appendix Figure S6



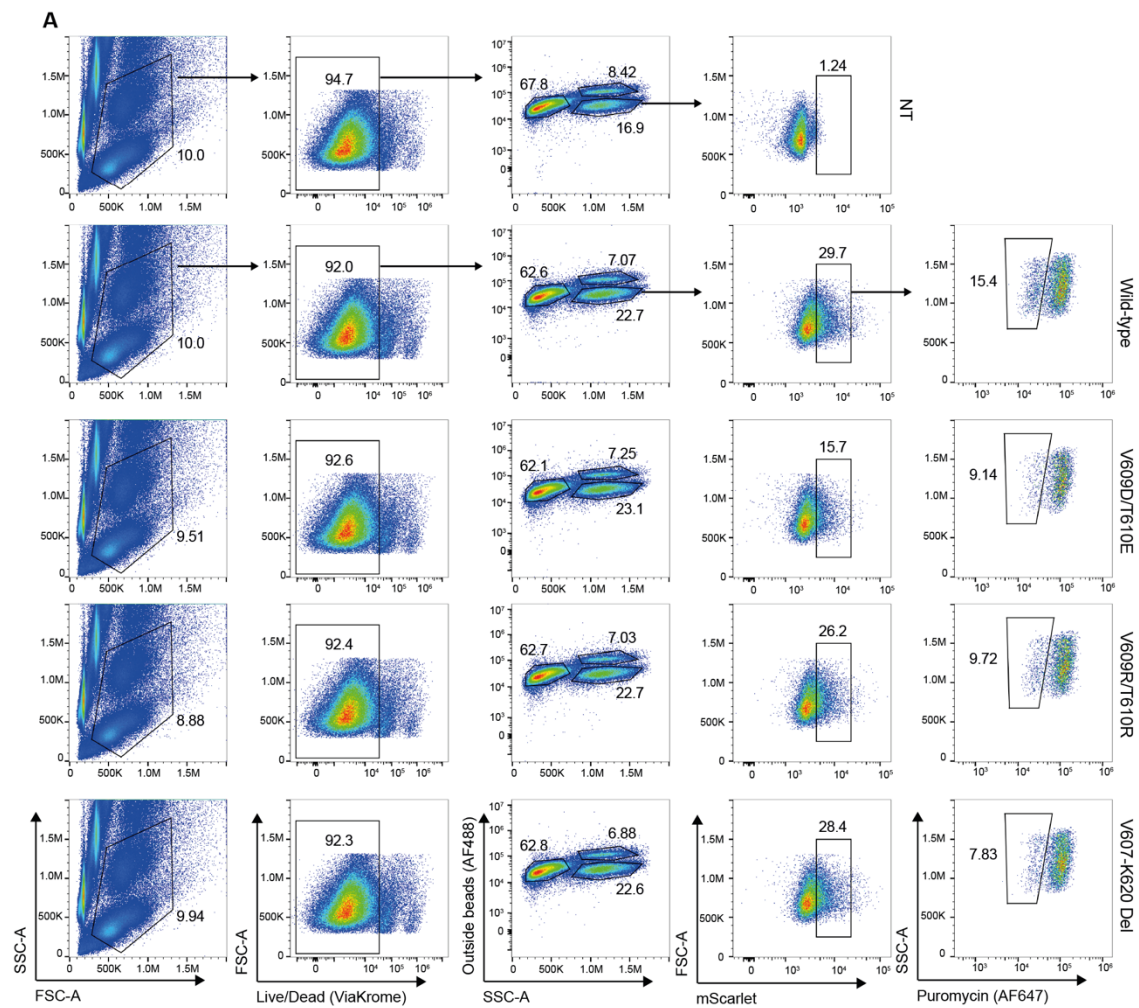
Appendix Figure S6. pHrodo assay in primary and Flt3L-derived DCs.

A Schematic representation of the pHrodo bead assay.

B Gating strategy for the pHrodo bead assay in primary splenic DCs. Two different gating strategies of the same sample are shown, which were used to identify cells with and without beads.

C Gating strategy for the pHrodo bead assay in primary Flt3L-bone marrow derived dendritic cells (Flt3-BMDCs). Two different gating strategies of the same sample are shown, which were used to identify cells with and without beads.

Appendix Figure S7



Appendix Figure S7. Gating strategy for saporin assay with the perforin-2 CTT mutants.

A Gating strategy for the saporin-puromycin assay in the indicated MutuDC lines: NT (control), and Mpeg1^{KO} MutuDC reconstituted with WT, V609D/T610E, V609R/T610R and V607-K620 Del perforin-2 mutants. mScarlet levels were used to normalise for transduction efficiency. Mean and SEM of n = 2 independent experiments.