## General information

As it appears to be difficult to gate Marginal Zone B-cells (and where they come frome),

we decided to take a step back and make the gating more simple.

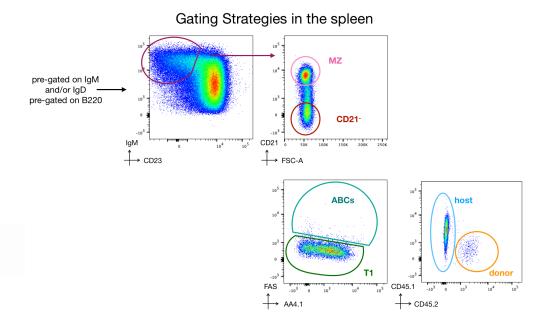
We are no longer using the AA4.1 marker to distinguish between transitional and mature

B-cells. It seems that some transitional 1 B-cells have lost their AA4.1 (this means that some T1s are older cells).

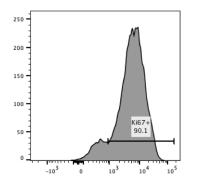
Instead, we gate on B220<sup>+</sup> IgM<sup>hi</sup> CD23<sup>-</sup> cells.

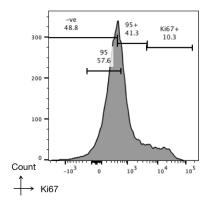
We further discriminate MZ B-cells based on their high CD21 expression.

T1s are by definition CD21<sup>-</sup>. As mice get older, age-associated B-cells start to appear in the B220<sup>+</sup> IgM<sup>hi</sup> CD23<sup>-</sup> CD21<sup>-</sup> gate. In order to gate these ones out, we excluded FAS<sup>+</sup> cells.



## Ki67 gating strategies





As Ki67 profiles look different, Ki67 gates are set differently between T1 and MZ

## **Busulfan Chimeras: Transitional 1 -> Marginal Zone**

