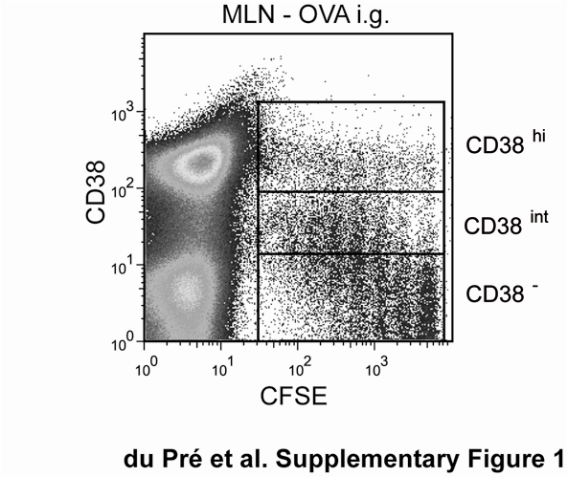
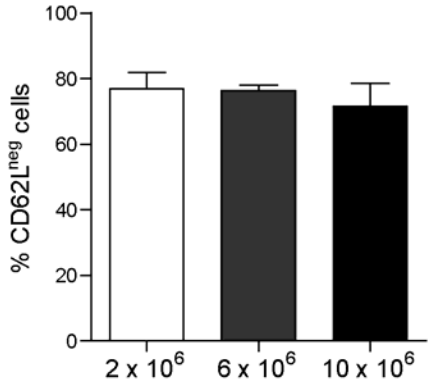
Supplementary Figures



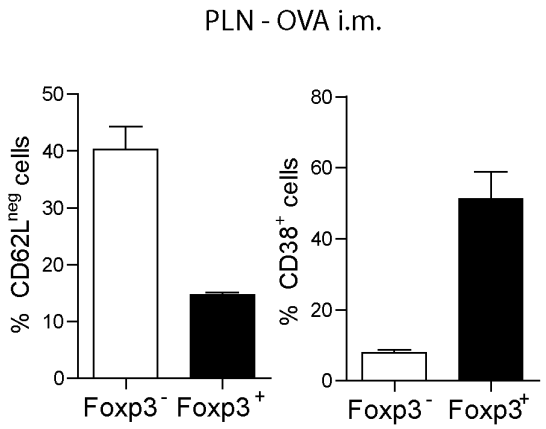
Supplementary Figure 1: Gating of CD38^{int} and CD38^{hi} T cells.

6x10^6 CFSE-labelled CD4$^+$KJ1.26$^+$ cells were transferred to naive BALB/c mice. One day after transfer, acceptor mice received 70mg OVA i.g. At 72h post OVA, single cell suspensions of draining MLN were prepared and stained for CD38. Gating of CD38^{int} and CD38^{hi} on CFSE$^+$ CD4$^+$KJ1.26$^+$ cells was determined based on CD38 expression on non-transgenic lymphocytes (mainly B cells) within the same LN.



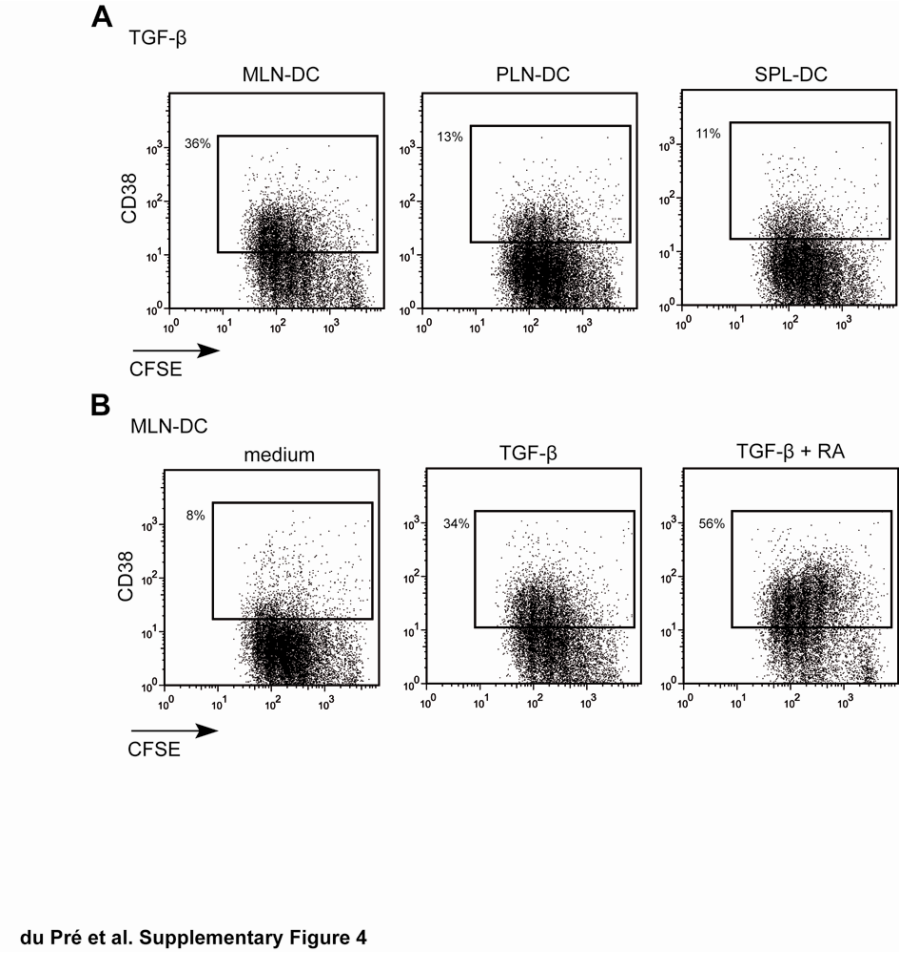
Supplementary Figure 2: Expression of CD62L is not dependent on DC – T-cell ratio.

2-10x10^6 CFSE-labelled $CD4^+KJ1.26^+$ cells were transferred to naive BALB/c mice. One day after transfer, acceptor mice received 70mg OVA i.g. At 72h post OVA, single cell suspensions of draining MLN were prepared and the percentage of $CD62L^{neg}$ cells within $CFSE^+CD4^+$ $KJ1.26^+$ cells was determined (n=3, mean ± SD).

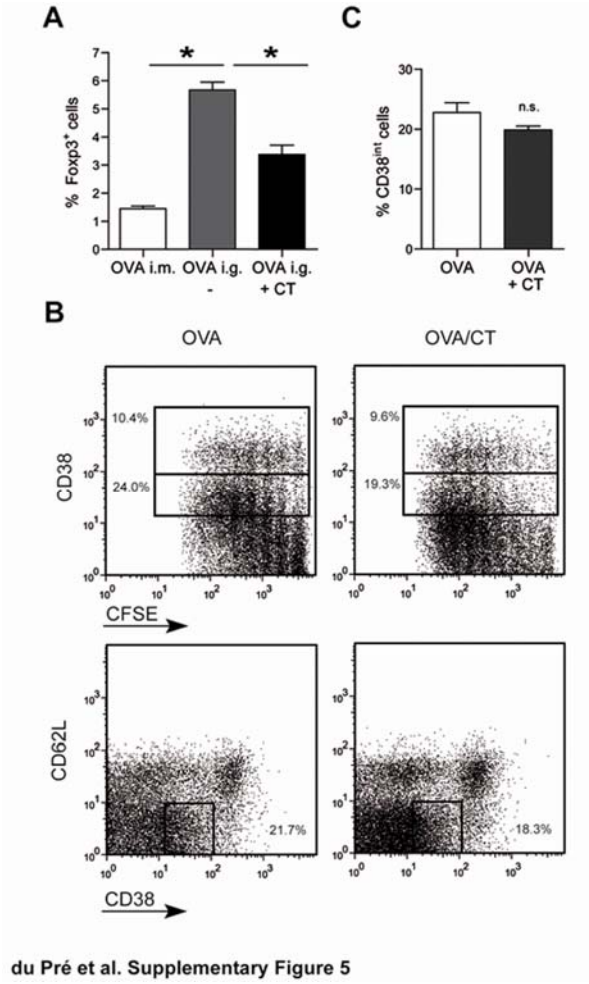


Supplementary Figure 3: Expression of CD38 is preferentially induced by mucosal DC in a TGF-β and RA-dependent manner.

$5x10^6$ CFSE-labelled $CD4^+KJ1.26^+$ cells were transferred to naive BALB/c mice. One day after transfer, acceptor mice received 400μg OVA i.m. in each hind limb. At 72h post OVA, single cell suspensions of draining PLN were prepared and analyzed for expression of Foxp3, CD62L and CD38. Quantitative analysis of $CD62L^{neg}$ and $CD38^+$ cells within $Foxp3^+CD4^+KJ1.26^+$ and $Foxp3^-CD4^+KJ1.26^+$ gated cells is shown (n=3, mean ± SD).



Supplementary Figure 4: Expression of CD38 is induced by mucosal DC and dependent on RA. (**A**) OVA-peptide loaded DC ($2x10^4$) from MLN, PLN and the spleen were incubated with $5x10^5$ CFSE-labelled $CD4^+KJ1.26^+$ T cells in the presence of TGF-β (20ng/ml) for 96h. $CD4^+KJ1.26^+$ T cells were analyzed for CD38 expression by flow cytometry. One representative dot plot is shown. (**B**) $CD4^+KJ1.26^+$ T cells were stimulated with OVA-peptide loaded MLN-DC in the presence and absence of TGF-β or TGF-β + RA (10nM). At 96h, expression of CD38 was determined by flow cytometry and Q-PCR. One representative dot plot is shown.



Supplementary Figure 5. Expression of CD38 is maintained upon abrogation of tolerance. BALB/c mice enriched with CFSE-labelled $CD4^+KJ1.26^+$ cells were tolerized with OVA i.g. or orally primed by giving OVA together with CT (20μg) i.g.. 72h after gavage, single cell suspensions were obtained from MLN and $CD4^+KJ1.26^+$ T cells were analyzed for CFSE content and expression of CD62L, CD38 and Foxp3 by flow cytometry. (**A**) Foxp3 positive cells as a percentage of total $CFSE^+CD4^+KJ1.26^+$ T cells in PLN after OVA i.m. and in MLN after OVA i.g. and OVA/CT i.g. (n=3, mean ± SD). (**B**) Representative dot plots showing CFSE dilution and expression of CD38 and CD62L on $CFSE^+CD4^+KJ1.26^+$ T cells in the MLN 72h after OVA and OVA/CT i.g. (**C**) Quantitative data of $CD38^{int}$ T cells as percentage of $CD4^+KJ1.26^+$ T cells in the MLN 72h after OVA and OVA/CT i.g. (n=3, mean ± SD). * Statistically significant ($p<0.05$)

Supplementary Methods

Antibodies

Murine cells:
CD4 (GK1.5),CD38 (90), CD62L (MEL-14, all BD-Pharmingen, Woerden, The Netherlands), Foxp3 (JFK-16S, EMELCA Bioscience, Bergen op Zoom, The Netherlands) and DO11.10 Tg TCR (KJ1.26, Invitrogen, Breda, The Netherlands)

Human Peripheral blood:
CD4 (RPA-T4), CD38 (HIT2), CD62L (DREG-56, all BD), CD45RA (MEM-56, Invitrogen), Foxp3 (PCH101, EMELCA), CCR9 (FAB1791A) and appropriate isotype control (R&D Systems, Abingdon, UK).

Human biopsies:
CD45 (HI30, eBioscience), CD62L (DREG-56), CD4 (SK3), CD38 (HB7) and TCRαβ (T10B9.1A-31, all BD)

Tetramer stainings PBMC:
CD62L (DREG-56), CD38 (HIT2), CD11c (3.9), CD45RA (HI100, all eBioscience), CD4 (SK3, BD) and CCR9 (FAB1791A, R&D).

Primers:

CYCLO: Fw: 5'-AACCCCACCGTGTTCT-3' Rv: 5'-CATTATGGCGTGTAAAGTCA-3'

FOXP3: Fw: 5'-ACCTGGGATCAATGTGG-3' Rv: 5'-TGGCAGTGCTTGAGAAA-3'

CD38: Fw: 5'-GCTGCCTCATCTACACTCA-3' Rv: 5'-TTTGCTCCAAAAGAGAGTCT-3'