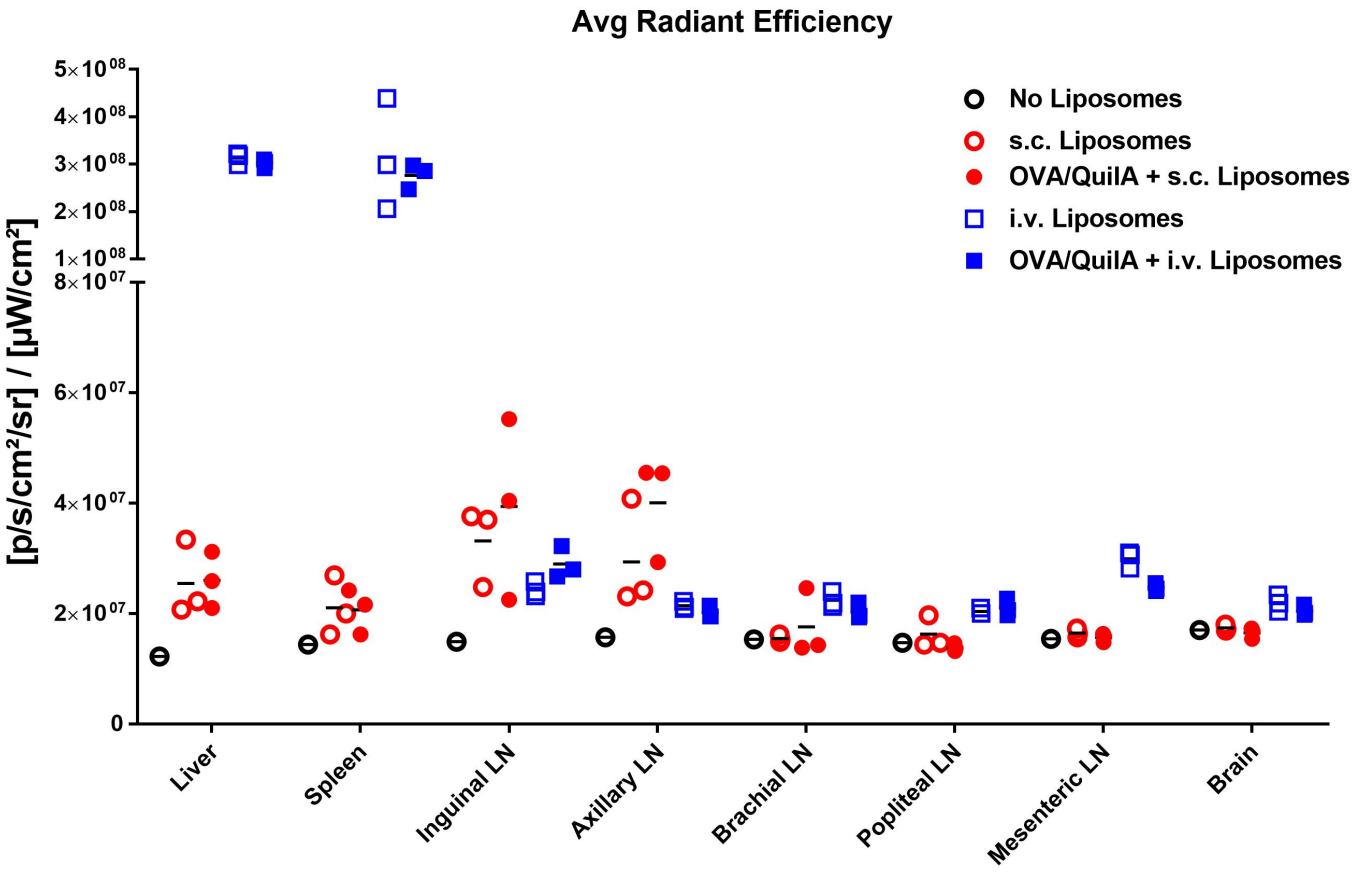
PD-L1 and calcitriol dependent liposomal antigen-specific regulation of systemic inflammatory autoimmune disease

Supplementary Information

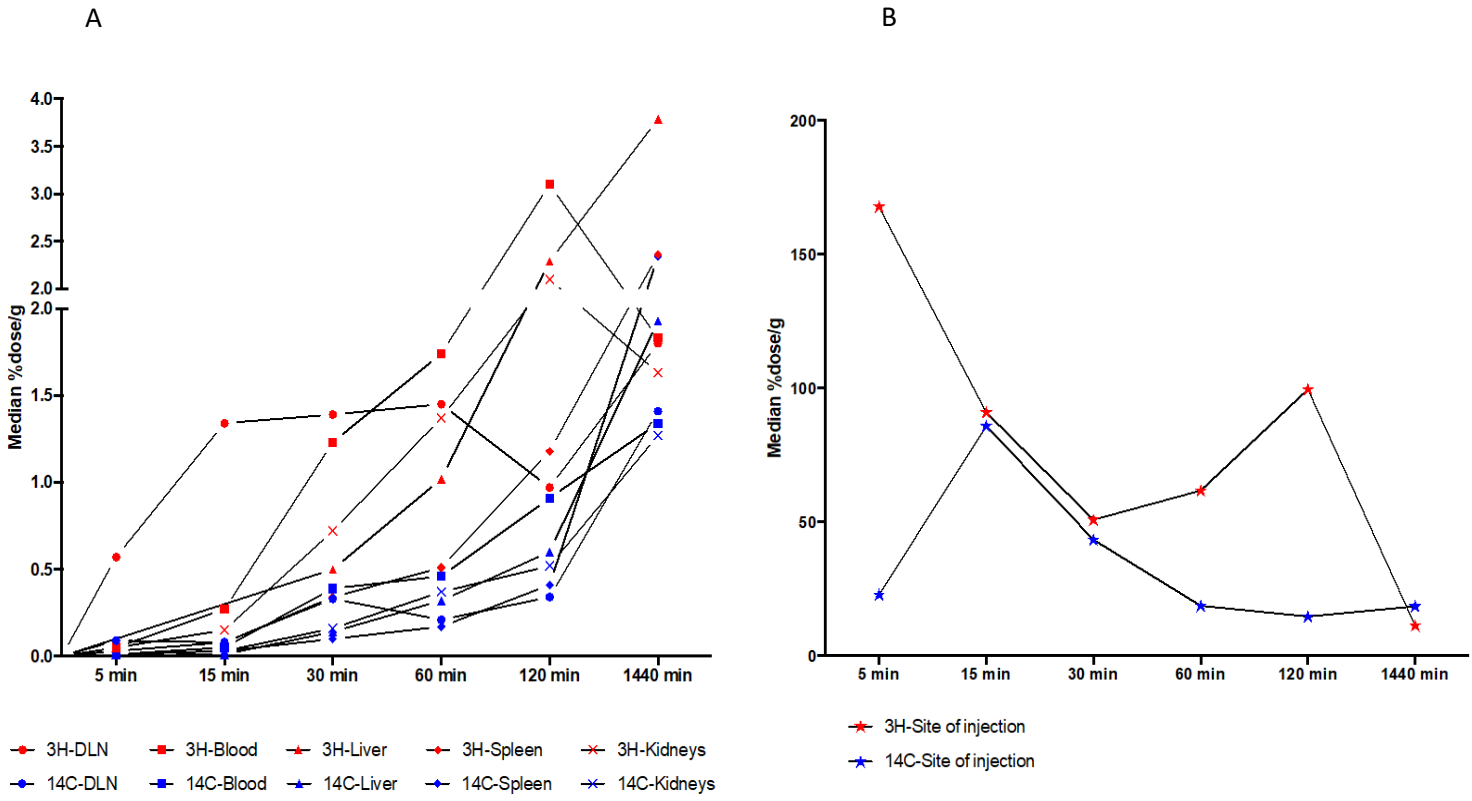
Supplementary Figure 1.

DiI-labelled calcitriol-$OVA_{323-339}$ liposomes were injected s.c. or i.v. to tailbase of naïve or mice primed in tailbase with OVA/QuilA 3 days previously. Biodistribution of liposomes in excised tissue from mice (n=3 per group) were assessed 24h after injection by IVIS in vivo imaging. Average tissue radiant efficiency was quantified as shown.



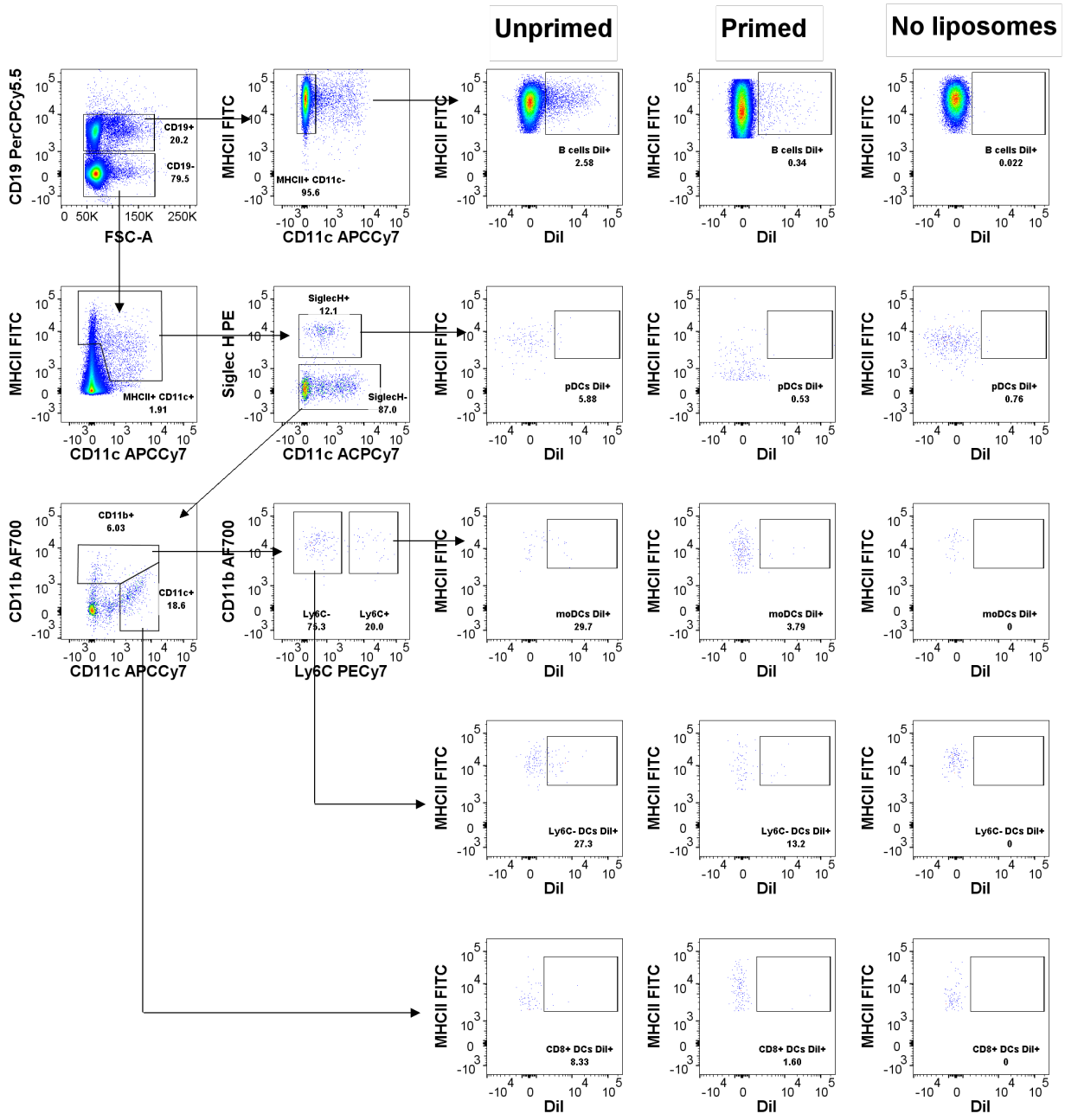
Supplementary Figure 2.

Liposomes were spiked with ^{3}H-calcitriol and ^{14}C phospholipids during preparation. Radiolabeled liposomes were injected s.c. (100 µl) and at pre-determined time intervals blood, liver, spleen, kidneys, draining lymph node (A) and injection site skin and subcutaneous tissue (B) were collected over 24 h (n=5).



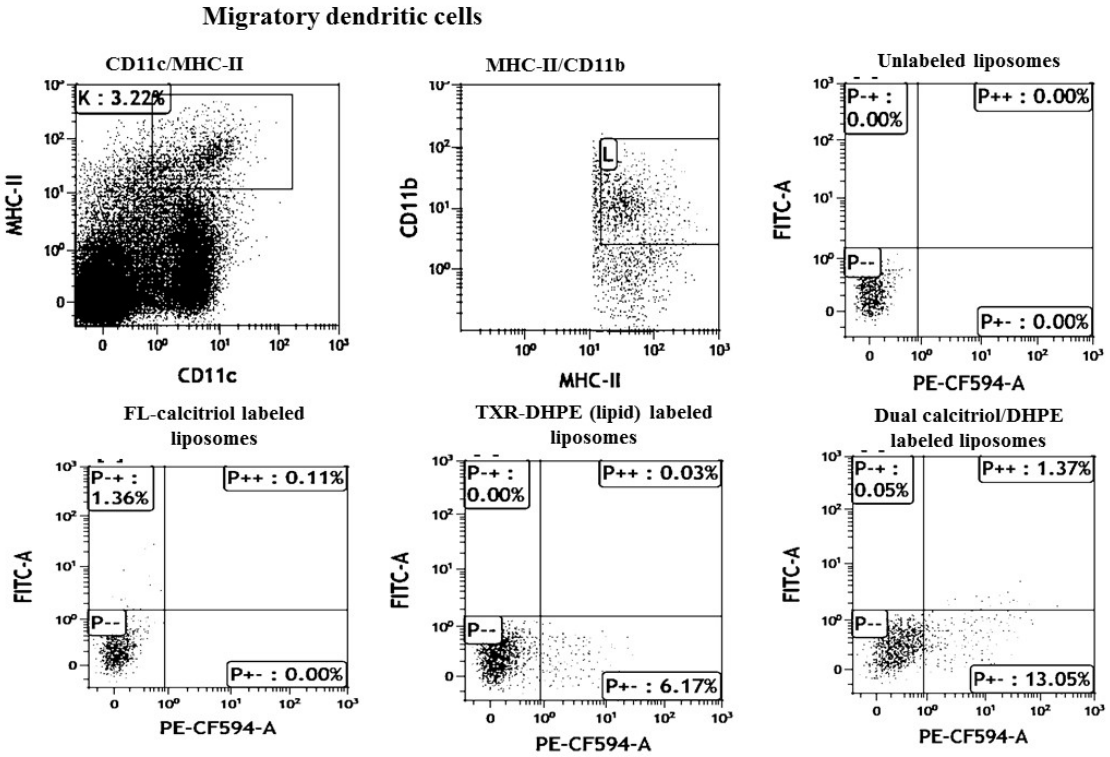
Supplementary Figure 3. Gating strategy for uptake studies and frequency of APC populations in naïve and primed ILN

DiI-labelled calcitriol-$OVA_{323-339}$ liposomes were injected s.c. to tailbase of naïve BALB/c mice or mice primed in tailbase with OVA/QuilA 3 days previously. dLN cells were stained with CD11c, CD11b, MHC class II, Siglec H, Ly6C and CD19. Unlabelled liposomes were injected and used to define DiI^+ populations. Doublets and dead cells were excluded. **A:** Gating strategy for antigen-presenting cell populations in draining lymph nodes. Cell populations were defined as: *B cells* $CD19^+$, MHC-II^+, $CD11c^-$; *moDCs* $CD19^-$, $Ly6gG^-$, MHC-$II^{lo/+}$, $CD11b^+$, $CD11c^{int}$, $Ly6C^+$; *cDCs* $CD19^-$, $Ly6G^-$, MHC-$II^{lo/+}$, $CD11b^{+/-}$, $CD11c^{hi}$, $Ly6C^-$; *$CD11b^+Ly6C^-$ DCs* $CD19^-$, $Ly6gG^-$, MHC-II^+, $CD11b^+$, $CD11c^{dim}$, $Ly6C^-$; *plasmacytoid DCs* $CD19^-$, MHC-II^+, Siglec H^+, $CD11c^{dim}$; and *$CD8^+$ DCs* $CD19^-$, $Ly6G^-$, MHC-$II^{lo/+}$, $CD11c^+$, $CD11b^-$. B**:** Numbers of cells taking up liposomes in naïve and primed lymph nodes. Flow cytometric data pooled from 5 mice/group.


Unprimed
Primed
No liposomes
CD19 PerCPCy5.5
FSC-A
CD19+ 20.2
CD19- 79.5
MHCII FITC
CD11c APCCy7
MHCII+ CD11c- 95.6
Dil
B cells Dil+ 2.58
B cells Dil+ 0.34
B cells Dil+ 0.022
MHCII+ CD11c+ 1.91
Siglec H PE
CD11c ACPCy7
SiglecH+ 12.1
SiglecH- 87.0
pDCs Dil+ 5.88
pDCs Dil+ 0.53
pDCs Dil+ 0.76
CD11b AF700
CD11b+ 6.03
CD11c+ 18.6
Ly6C PECy7
Ly6C- 75.3
Ly6C+ 20.0
moDCs Dil+ 29.7
moDCs Dil+ 3.79
moDCs Dil+ 0
Ly6C- DCs Dil+ 27.3
Ly6C- DCs Dil+ 13.2
Ly6C- DCs Dil+ 0
CD8+ DCs Dil+ 8.33
CD8+ DCs Dil+ 1.60
CD8+ DCs Dil+ 0

Supplementary Figure 4. Migratory dLN DCs take up calcitriol loaded liposomes

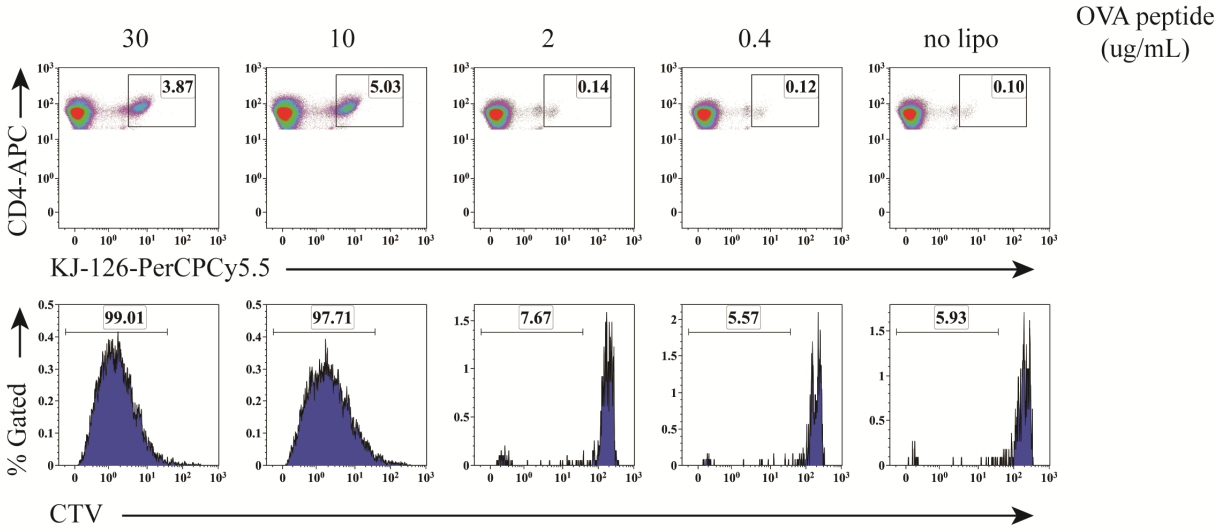
BALB/c female mice (6-8 weeks) were primed with OVA/Quil A 24 h before liposome injections. Primed mice were injected with 100µl of unlabeled liposomes, BODIPY FL-calcitriol liposomes, BODIPY TXR-labeled liposomes or dual labeled BODIPY FL-calcitriol + BODIPY TXR-labeled liposomes. After 5 h ILN were harvested. Single cell suspensions were stained with anti-CD11b-AF700, anti-CD11c-APC.Cy7, anti-I-A/I-E-pacific blue, anti-F4/80-PE.Cy7, anti-CD3-APC, anti-CD19-APC and then analyzed by flow cytometry. The uptake of FL-calcitriol and TXR-DHPE within gate L is shown in the bottom 3 panels.



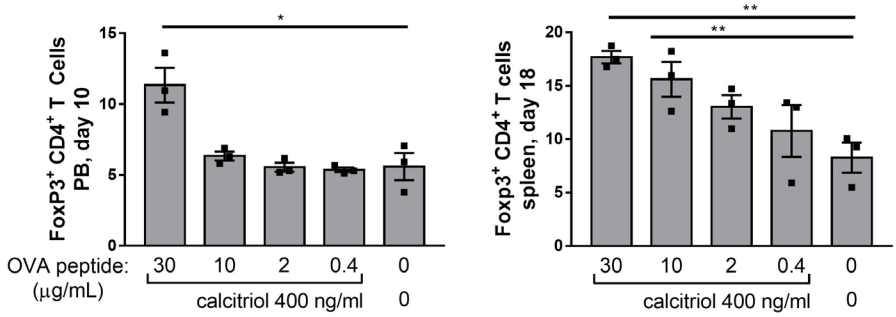
Supplementary Figure 5. Peptide concentration and volume-dependent induction of Treg by OVA/calcitriol liposomes

A, Liposomes (100 µL) encapsulating 400 ng/mL calcitriol and varying concentrations of $OVA_{323-339}$ were administered s.c. to tailbase of BALB/c mice adoptively transferred with 2 x 10^6 CTV-labelled DO11.10 T cells 24h previously. Three days later, CTV dilution of gated $CD4^+$KJ-126^+ T cells was analyzed in spleen by flow cytometry. **B,** The same liposome preparations (100µl) were administered s.c. twice to neck scruff of DO11.10 mice 7 days apart or mice remained naïve. $CD4^+Foxp3^+$ Treg were evaluated in PB on day 11. Mice were then primed with OVA/QuilA and $CD4^+Foxp3^+$ Treg were evaluated in spleen on day 18. **C,** Varying volumes of DiI-labeled liposomes encapsulating 400 ng/mL calcitriol and 30µg/ml $OVA_{323-339}$ were administered along with OVA/QuilA s.c. to tailbase of BALB/c mice. The proportion of DiI^+ $CD11b^+$ DCs was evaluated in iLN 24h later. Either 100 µl or a 1:3 dilution of calcitriol-OVA liposomes was administered s.c. into the neck scruff of DO11.10 mice on days 0 and 7. Splenic $CD4^+Foxp3^+$ T cells were analyzed on day 14.

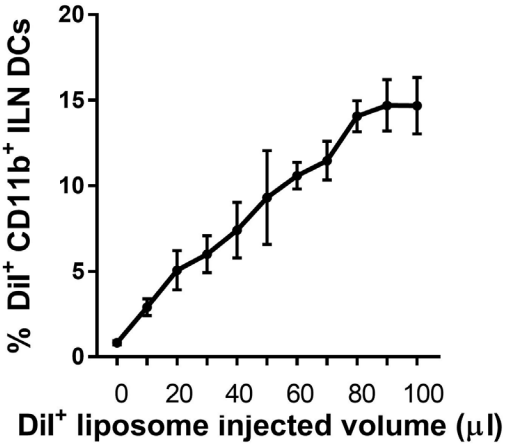
A

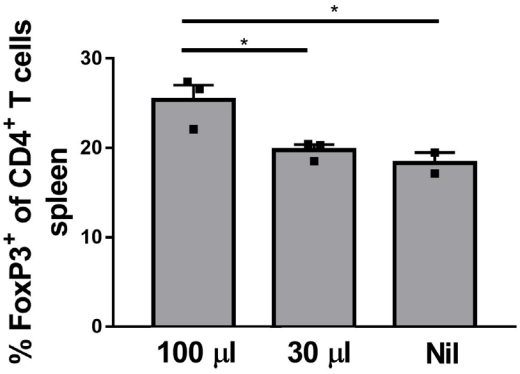


B



C

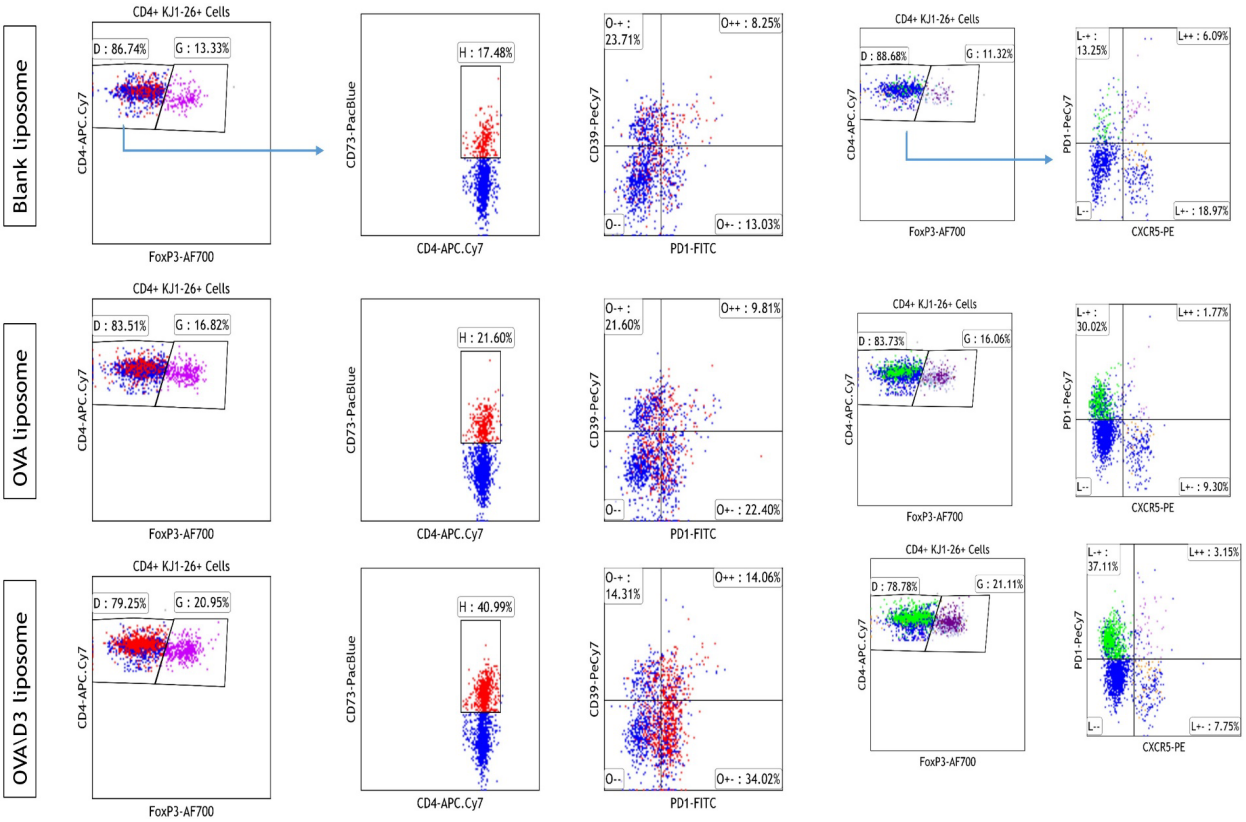

% DiI+ CD11b+ ILN DCs
20
15
10
5
0
0
20
40
60
80
100
DiI+ liposome injected volume (μl)


% FoxP3+ of CD4+ T cells spleen
*
*
30
20
10
0
100 μl
30 μl
Nil

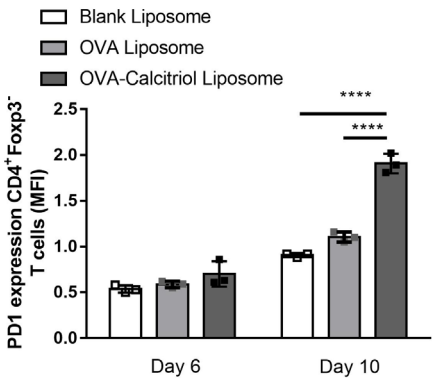
Supplementary Figure 6. Phenotype of OVA-specific T cells responding to liposomes

5 x 10^6 DO11.10 T cells were adoptively transferred to BALB/c hosts. Mice were treated with liposomes twice as shown, on days 0 and 6. A: Example of flow cytometric staining. $Foxp3^+$ T cells (pink) increase with OVA-calcitriol-liposomes. Of the $Foxp3^-$ T cells, CD73 (red) proportion and intensity increase with OVA-calcitriol liposomes. After OVA-calcitriol liposomes, these $CD73^+$ (red) cells co-express PD1, and some co-express CD39. $Foxp3^-PD1^+$ T cells (green) increase after OVA-calcitriol liposomes and all are $CXCR5^-$. B: PD1 expression (MFI) by $Foxp3^-$ T cells after treatment with blank, OVA or OVA-calcitriol liposomes.

A



B



Supplementary Figure 7. Phenotype of ex vivo-generated OVA-specific T_{EM} cells

Splenocytes and pooled LN cells isolated from DO11.10 mice were cultured for *ex vivo* memory T cell generation as described in Material and Methods. Representative phenotyping of cells at time of transfer is shown. CM= central memory T cells. EM= effector memory T cells.