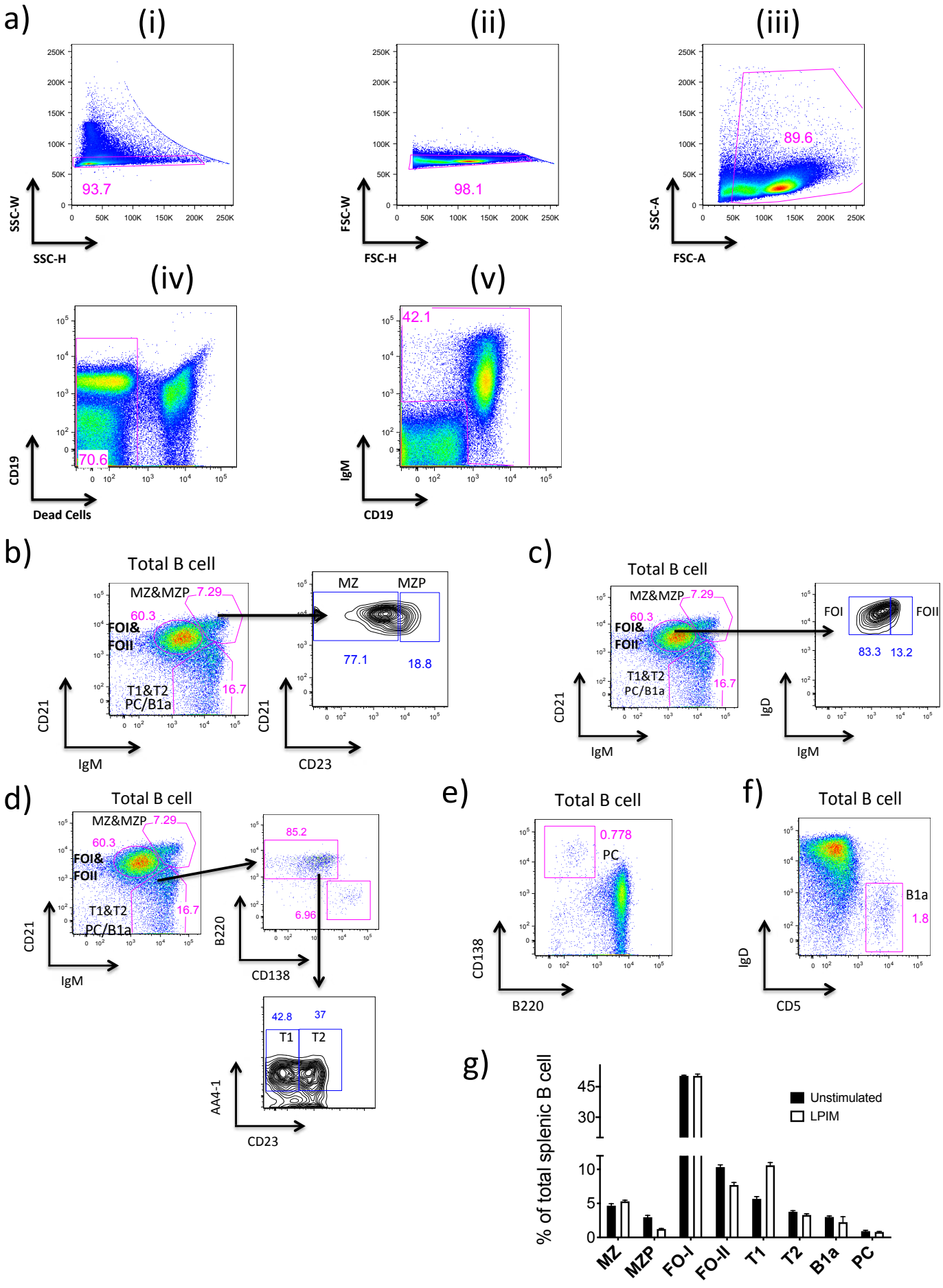
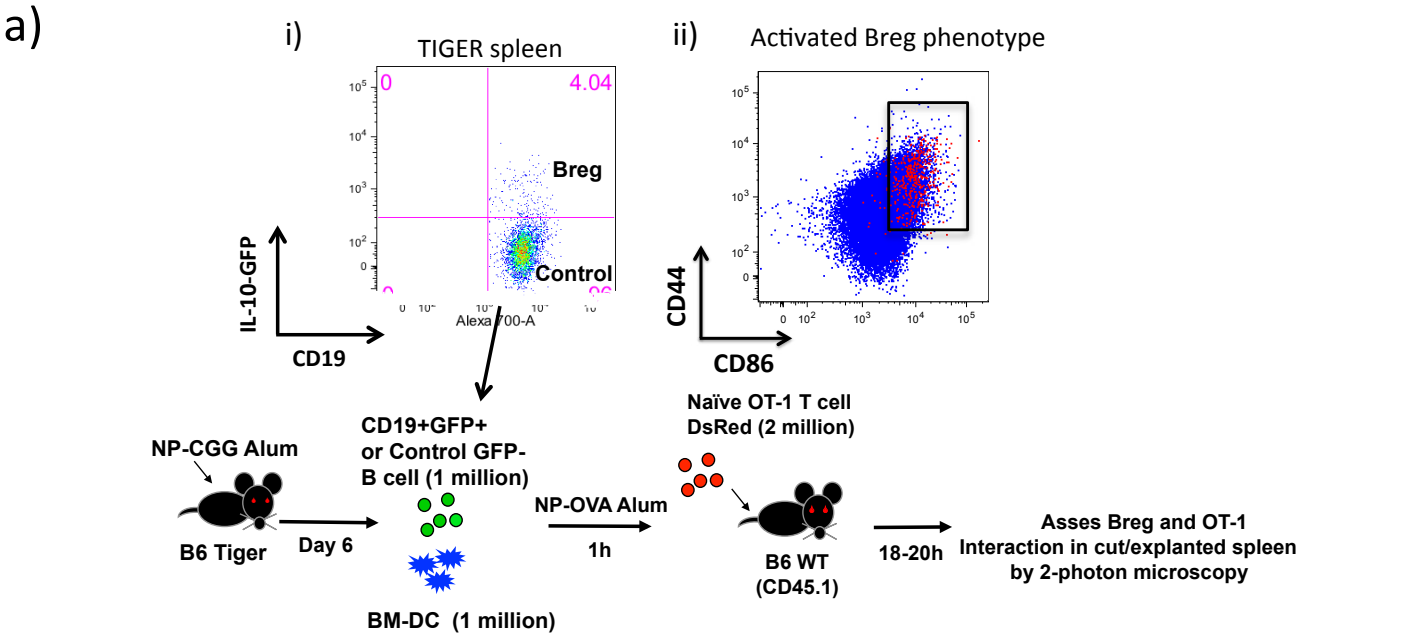
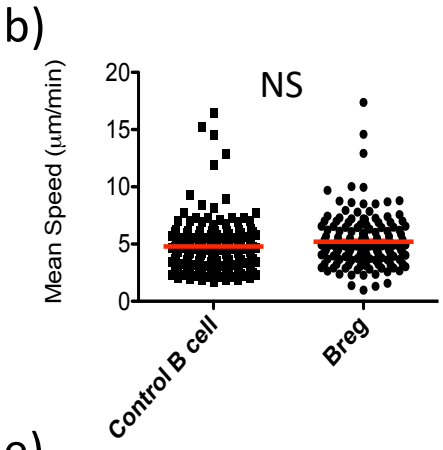
Supplemental Fig. 1

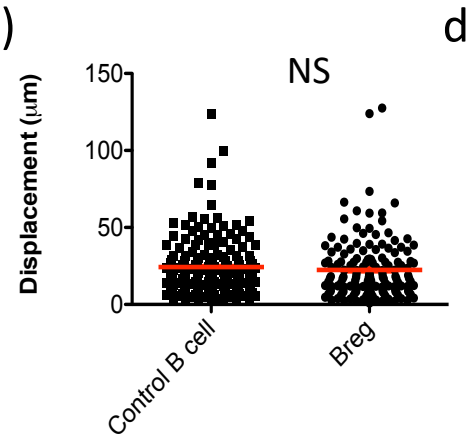


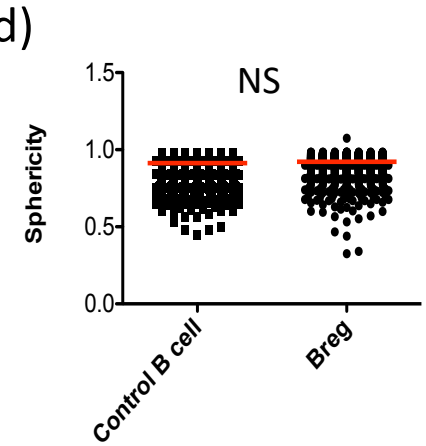
Supplemental Figure 1: Gating strategy to examine IL-10 expression in various B cell subsets. a) Gating strategy for total B cells. (i) SSC-W/SSC-H and (ii) FSC-W/FSC-H doublet exclusion, (iii) splenocyte gating, (iv) dead cell exclusion gate, (v) gating onB cells using CD19 and IgM. **b)** Gating shown to examine marginal zone (MZ: IgMhi, CD21hi, CD23lo/-) and marginal zone precursor (MZP: IgMhi, CD21hi, CD23hi) B cell subsets. **c)** Gating shown to examine follicular I (FOI: IgDhi, IgMlo, CD23hi) and follicular II (FOII: IgDhi, IgMInt, CD23hi) B cell subsets. **d)** Gating shown to examine transitional 1 (T1: IgMhi, IgD-, CD21-, CD23-, B220+, AA4.1+) and Transitional 2(T2: IgMhi, IgD-, CD21-, CD23+, B220+, AA4.1+) B cell subsets. **e)** Gating shown to examine plasma cell/plasmablast (PC: IgMhi, IgD-, CD21-, B220-, CD138+) B cell subset. **f)** Gating shown to examine B1a B cell subset. g) Summary graphing showing the frequency of each subset from total splenic B cells before and after stimulation with LPIM.

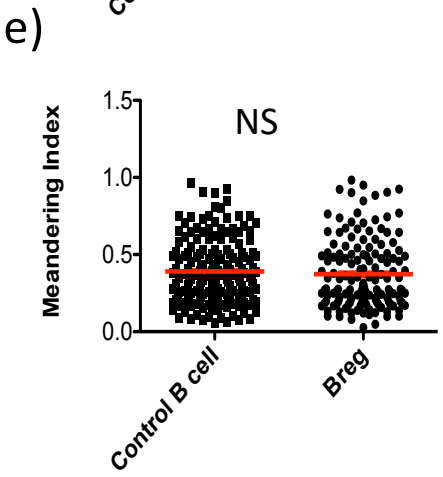
Supplemental Fig. 2

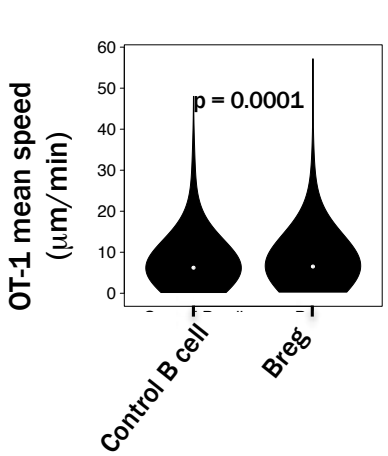


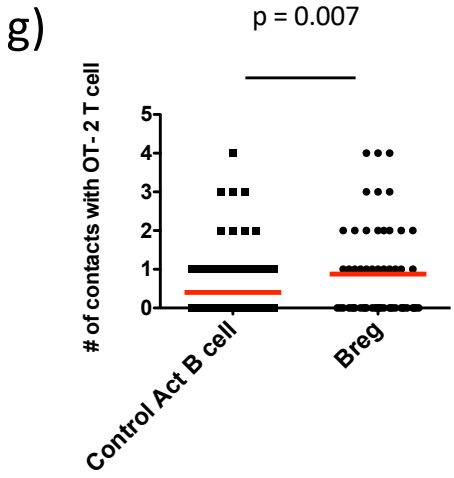


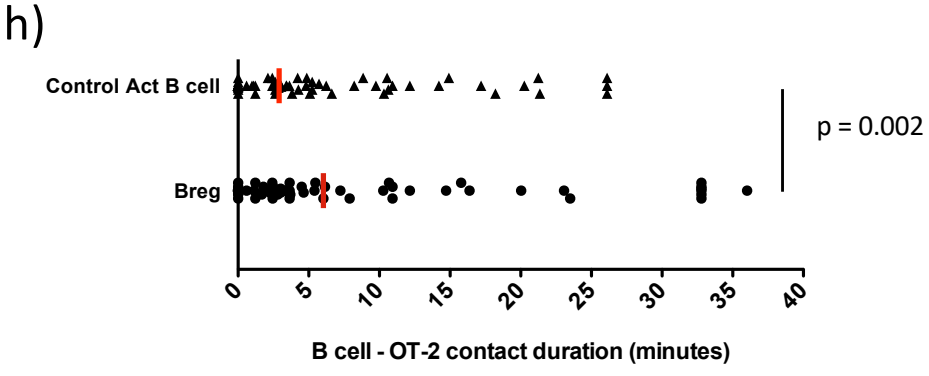






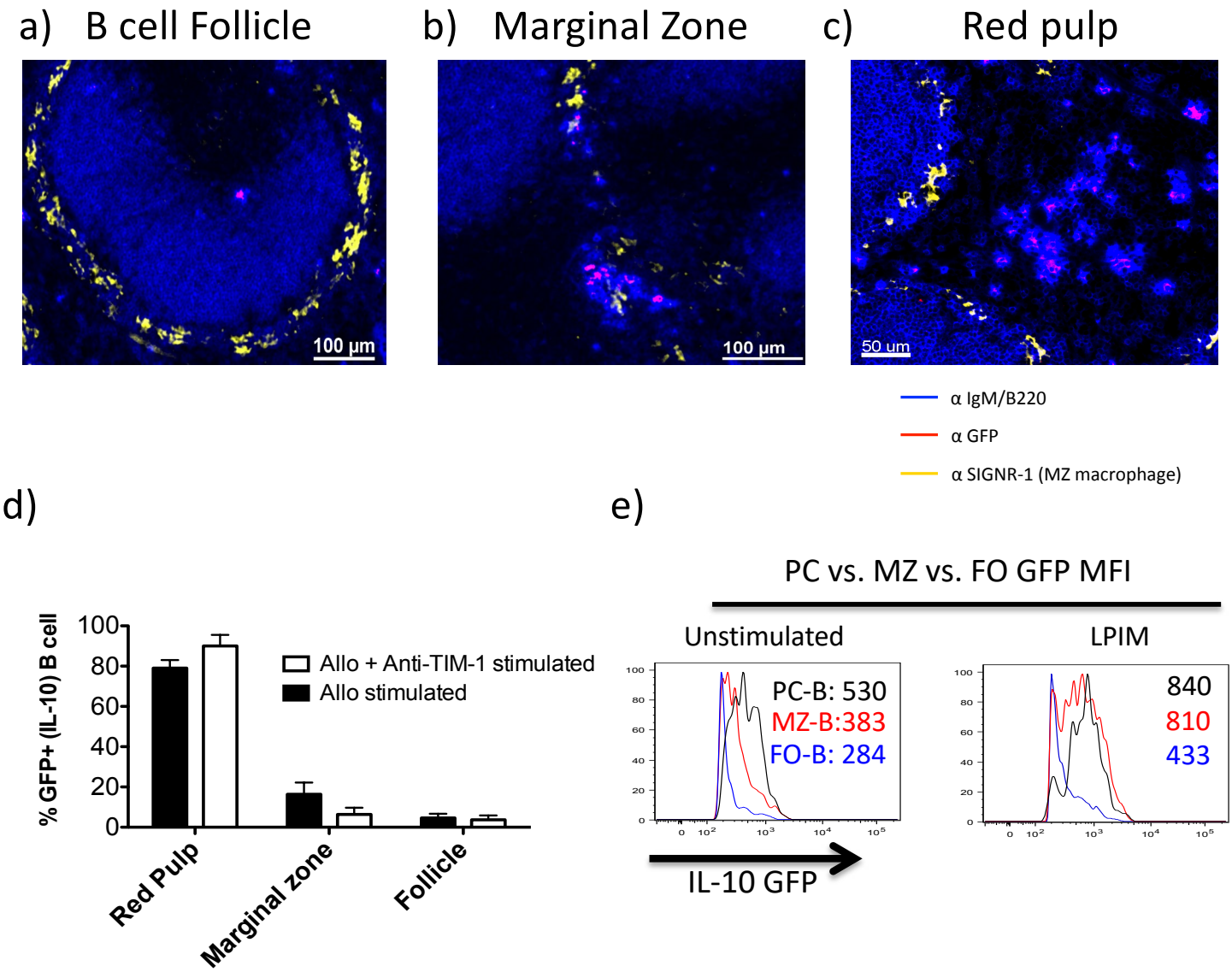






Supplemental Figure 2: Experimental procedure and motility parameters derived from 2 photon imaging of Bregs vs. Control B cells. a) Breg (GFP+) (i), Control B cells (GFP-) (i) or Control Activated B cells (ii) were sorted from NP-CGG immunized Tiger mice with BD FACSARIA on day 6-7 after immunization. Gate shows CD44 and CD86 expression level of Breg (pink) over-layed on total B cells (blue). Control activated B cells (GFP-) were sorted from within this gate. The sorted cells were stained briefly with 1.5mM CFSE and pulsed with 10μg/ml of NP-OVA for one hour. Bone marrow derived DC's (BMDC), generated using GM-CSF and IL-4 using a standard 7 day culture system, were treated with 100ng/ml of LPS for 5-6 hours and pulsed with 10μg/ml of NP-OVA for 1 hour. One million of both B cells and DCs were adoptively transferred into WT CD45.1 mice that had received 2 million naïve DsRed OT-1 the day before. After 18-20h, spleens from the recipient mice were removed and cut with a vibratome in order to facilitate imaging of the white pulp. Unused cut pieces of the spleen were snap frozen in OCT for further analysis by immunofluorescence staining. **b-g:** Graphs comparing motility parameters of Control B cells vs. Bregs as analyzed using Bitplane Imaris software. (Each dot represents one cell and red horizontal lines indicate mean for each graph). **b)** Mean speed of Control B cell vs. Bregs. **c)** Displacement of Control B cell vs. Bregs. **d)** Sphericity of Control B cell vs. Bregs. **e**) Meandering index of Control B cell vs. Bregs. **f**) Violin plot showing mean speed of OT-1 T cells obtained from all movies where recipient mice received either Control B cell or Bregs. **g)** The number of contacts individual (NP-OVA pulsed) Control Activated B cells or Bregs made with OT-2 T cells. **h)** Duration (minutes) of each contact made by Control Activated B cell or Bregs with OT-2 T cells. n = 2-5 mice for each group.

Supplemental Fig. 3



Supplemental Figure 3: Immuno-fluorescent images showing Breg localization in immunized Tiger spleens. a) Immuno-fluorescent image of a IL-10-GFP reporter mouse spleen immunized with allo-antigen for 72 hours and stained for B cells (anti-IgM/B220 - blue), marginal zone macrophage (anti- SIGNR-1- yellow) and IL-10-GFP (anti-GFP – red) showing a B cell follicle with Breg localized to T:B border, **b)** Breg localized to MZ, **c)** Breg localized to to red pulp . **d)** Graph showing cumulative frequency of Breg in immunized IL-10-GFP reporter mice spleens with and without anti-TIM-1 treatment. n = 3-5 mice per group.