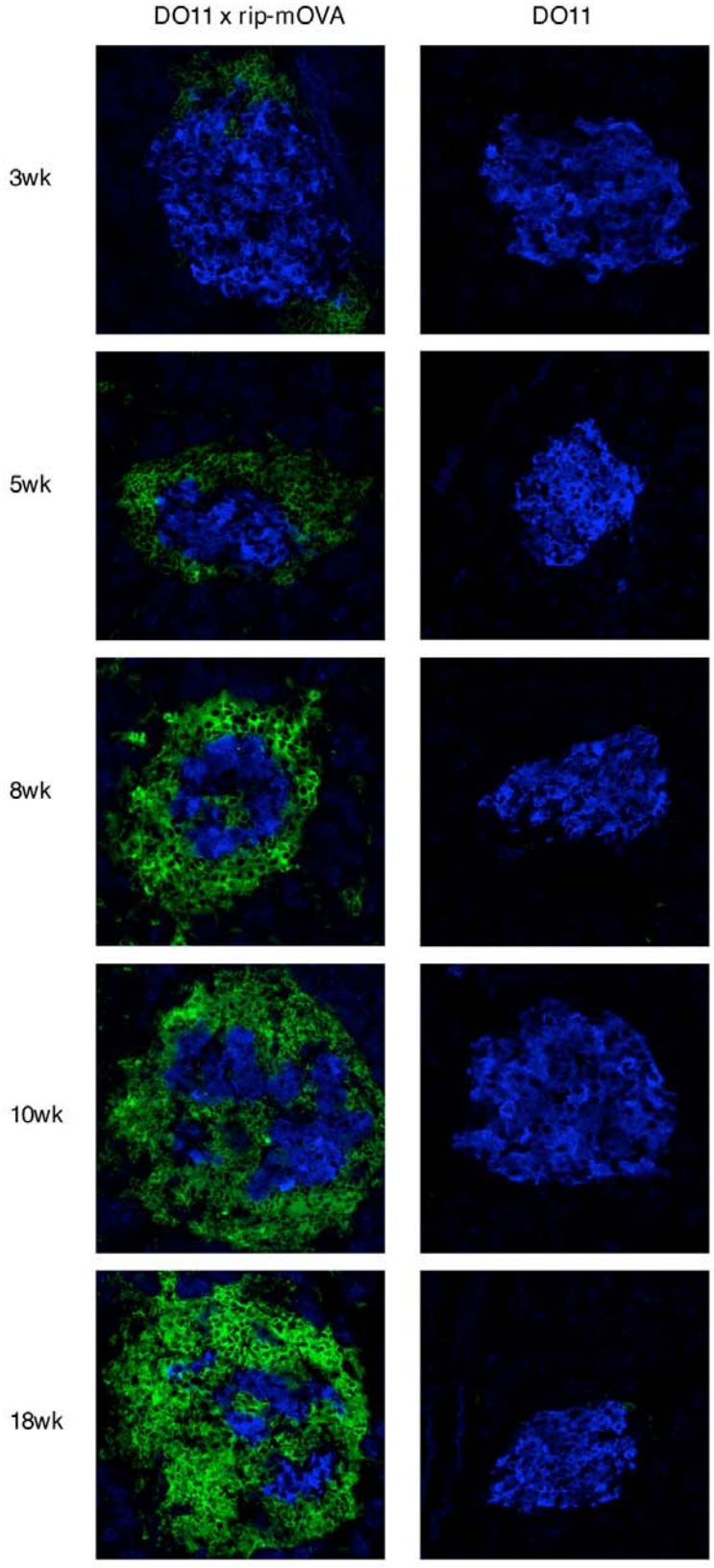
Supplemental Figures S1-S4

Figure S1. Timecourse of insulitis in the DO11 x rip-mOVA model. Pancreas sections were prepared from DO11 x rip-mOVA animals, or DO11 single transgenic controls, at the indicated age. Sections were stained for CD45 (green) and insulin (blue) and imaged by confocal microscopy. CD45 staining was visualised using FITC-conjugated anti-CD45 followed by Goat anti-FITC-Alexa-488. Insulin was detected using chicken anti-insulin followed by Goat anti-chicken Alexa-555. A single islet is shown for each timepoint but it should be noted that there is considerable heterogeneity between the appearance of individual islets within the same pancreas section.

Figure S2. Detection of adoptively transferred B1 cells within the pancreatic infiltrates of DO11 x rip-mOVA/rag^{-} recipients. Pancreas sections from DO11 x rip-mOVA/rag^{-} mice that had been reconstituted with 10^6 B1 cells 6 wk previously were stained for CD3 (blue) and CD19 (brown). CD3 staining was visualised using hamster anti-CD3 followed by goat anti-hamster AP. CD19 staining was visualised using CD19-FITC, rabbit anti-FITC, anti-rabbit Ig HRP. Images are shown from 3 individual mice.

Figure S3. Full insulitis scores for the experiments presented in Figure 4B and 5D. Pancreata from DO11 x rip-mOVA/rag^{-} mice subjected to the indicated treatment were sectioned and stained for KJ-126 and insulin (as described in the Materials and Methods) The proportion of pancreatic islets exhibiting light, medium, heavy or no infiltration was assessed. Each bar represents an individual mouse.

Figure S4. Effect of B1 cells on T cell proliferation in the pancreatic lymph node. RIP-mOVA+ or RIP-mOVA- rag-deficient mice were left untreated or injected i.p. with 10^6 B1 cells. 4d later mice received 2 x 10^6 CFSE-labelled CD4+ T cells from a DO11+Thy1.1+ donor. 3d later mice were killed and the CFSE profile of gated CD4+Thy1.1+ cells in PanLN and IngLN was assessed. The % cells in the CFSE low gate is indicated. Lower plot shows the CD4+Thy1.1+ gate used. Each plot shows combined LN from 2 recipient mice; results show one representative experiment of 3 performed.



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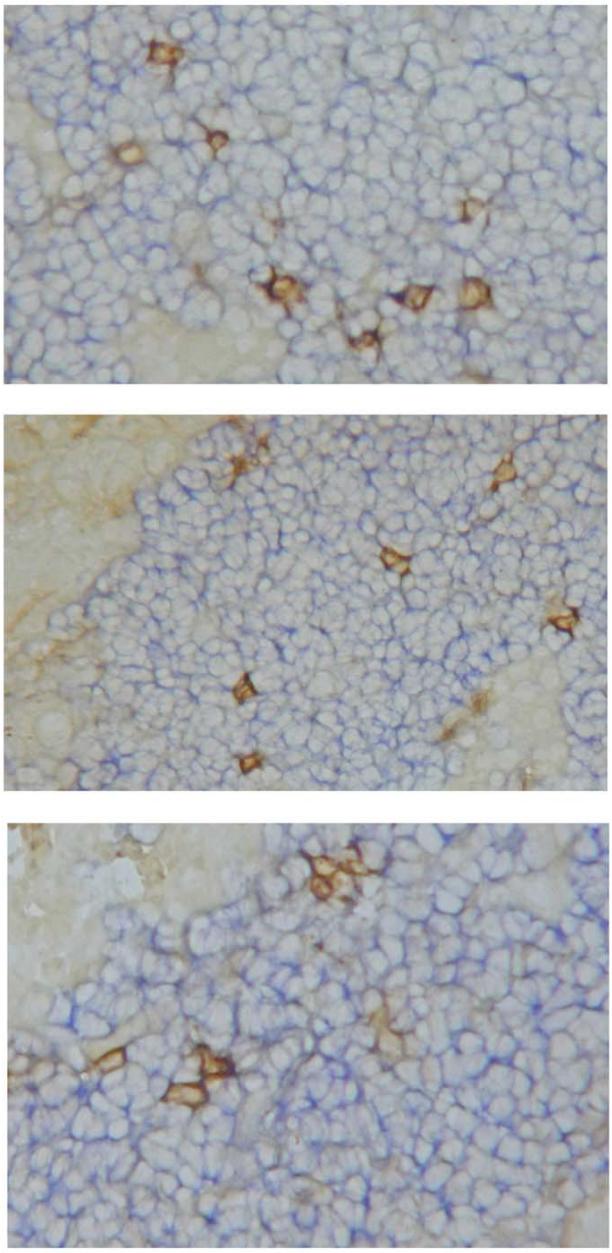
Supplementary Fig. 1



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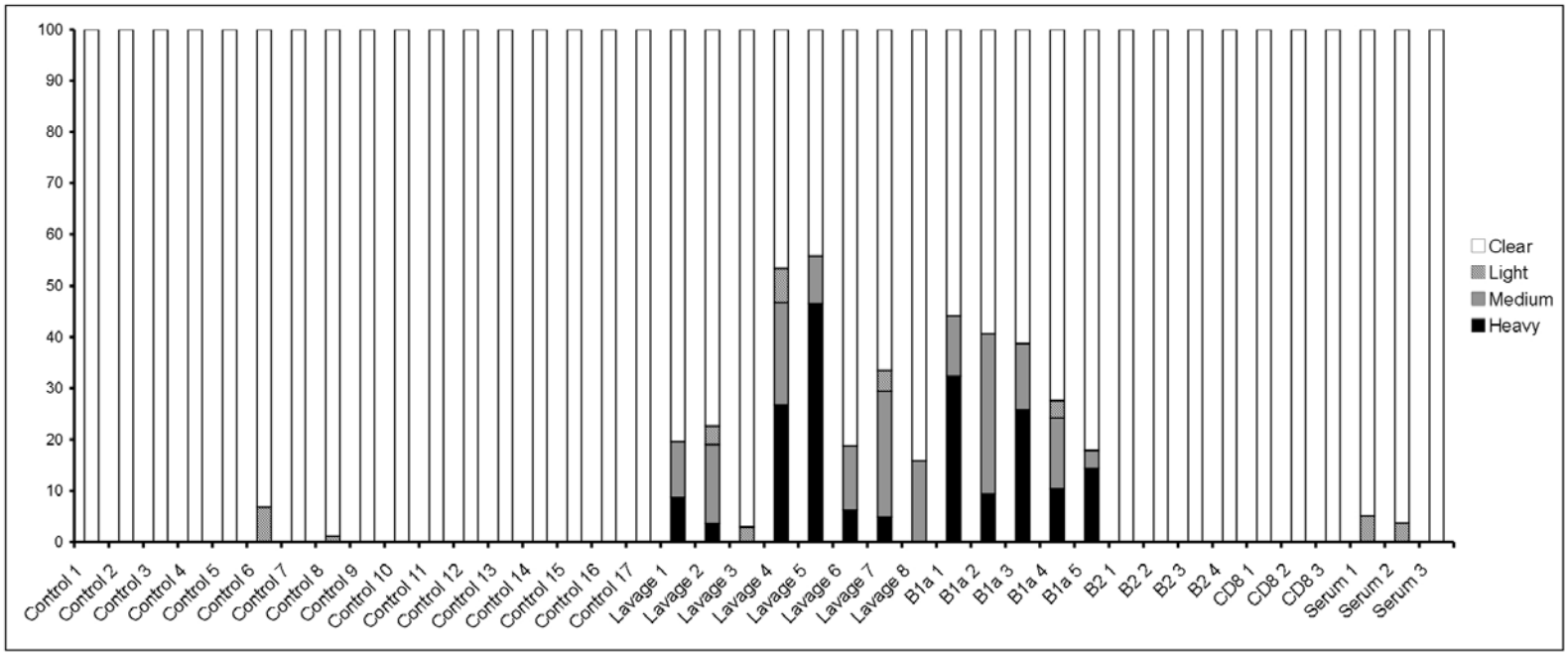
Supplementary Fig. 2

A



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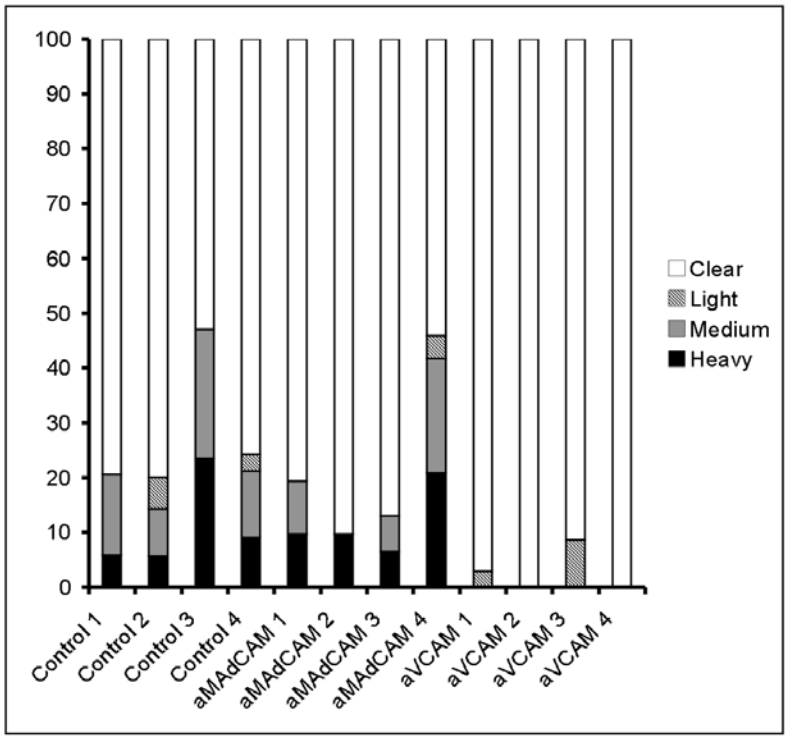

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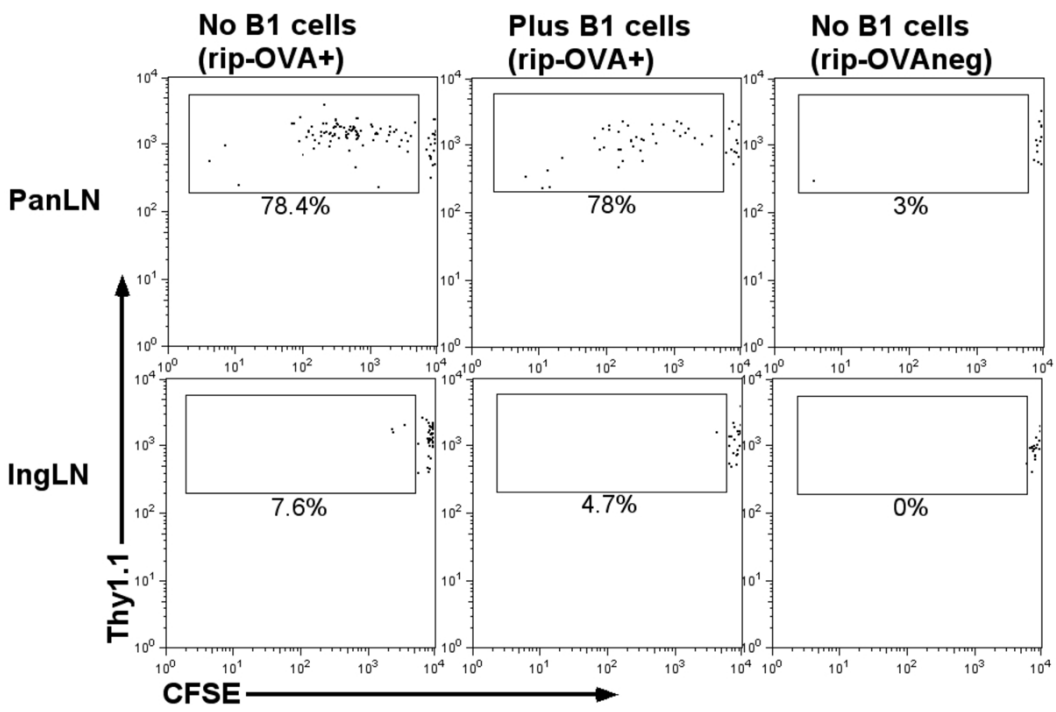
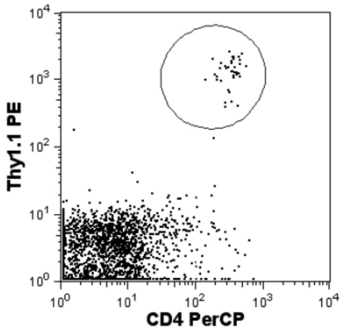
Supplementary Fig. 3





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Supplementary Fig. 4