T-Cell Receptor- and CD28-induced Vav1 activity is required for the accumulation of primed T cells into antigenic tissue.

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Supplementary Data

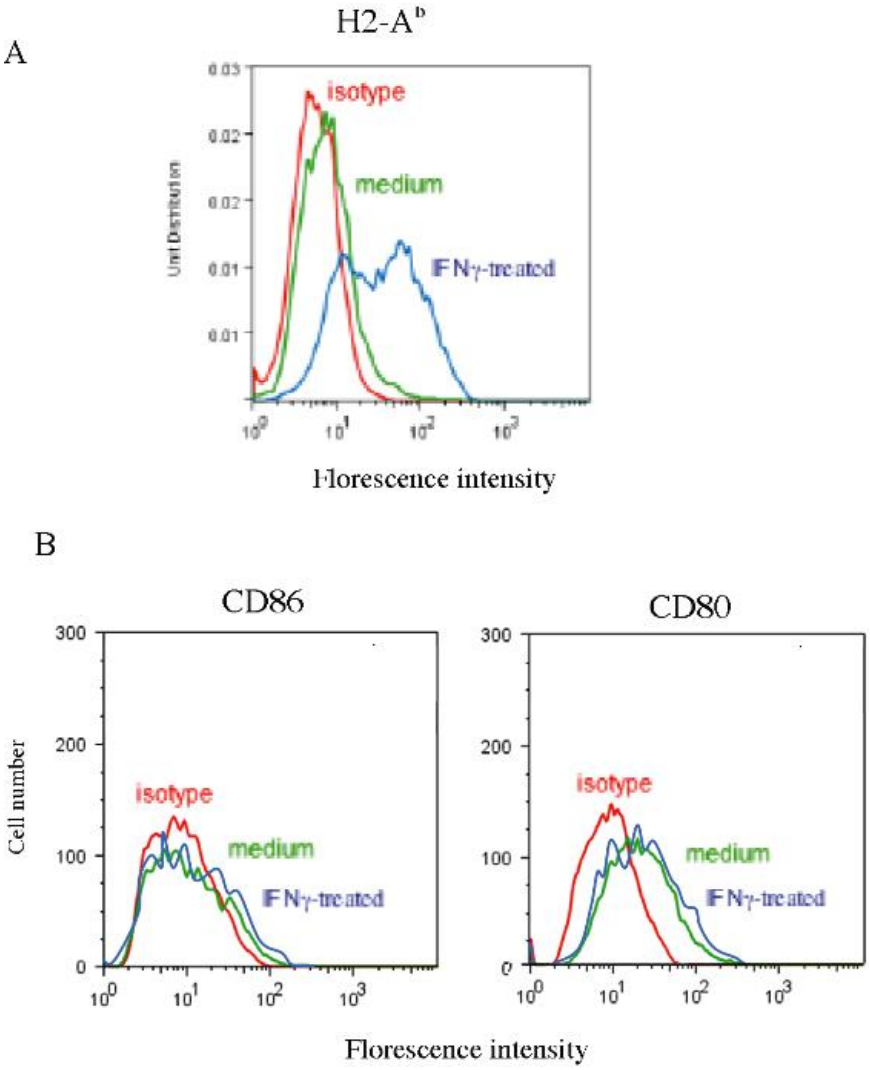

Figure S1. MHC class II, CD80, and CD86 expression by endothelial cells following IFNγ treatment in vitro and in vivo (JPG, 64.7 KB).

Treatment of murine lung-derived ECs with 300U/ml IFNγ for 48 to 72 hours results in MHC class II expression induction (panel A), as detected by flow cytometry. Expression of CD80 and CD86 molecules is not modified by IFNγ treatment (B,C). Panels A-C were obtained from cells treated for 48 hours. Similar expression was observed at 72 hours.

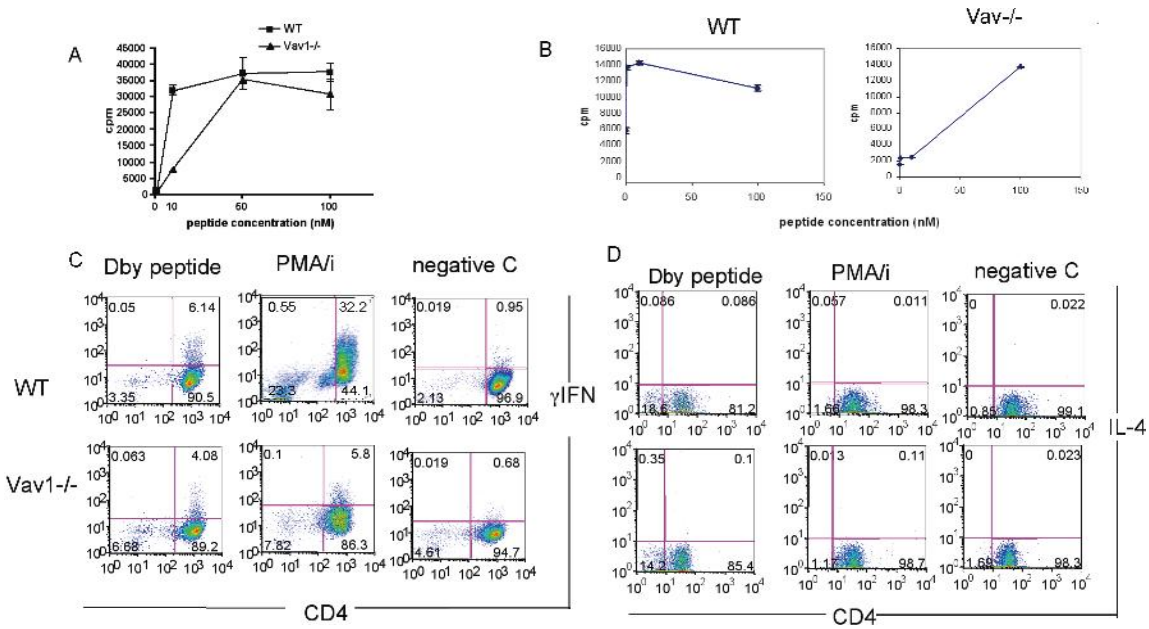

Figure S2. Specificity and cytokine secretion by WT and Vav1$^{-/-}$ T cells (JPG, 114 KB)

(A,B) Dby-specific, H2-Db-restricted CD4^{+} WT and Vav1$^{-/-}$ T cells (10^5/well) used between days seven and ten post-stimulation were incubated with irradiated syngeneic female splenocytes (10^5/well) pulsed with different concentrations of HY Dby peptide (panel A) or 10^4 irradiated syngeneic female endothelial cells pre-exposed to different concentrations of Dby peptide plus 0.5 U/ml rIL-2 (panel B) for 48 hours. To induce MHC class II expression, endothelial cells were pre-treated with IFNγ (300U/ml, Peprotech) for 48 hours prior to use in experiments. Tritiated thymidine (1µCi/well) incorporation was measured 48 hours later. The proliferative response is expressed as counts per minute (cpm). (C,D) WT and Vav1$^{-/-}$ T cells used between days seven and ten post-stimulation were stimulated for four hours with irradiated female splenocytes and Dby peptide (50 nM), PMA/ionomycin (5µg/ml and 2.5µg/ml respectively) Golgi plug alone as a negative control. IFNγ (B) and IL-4 (C) secretion by CD4^{+} T cells was measured by intracellular staining (R&D Systems, Abingdon, UK).

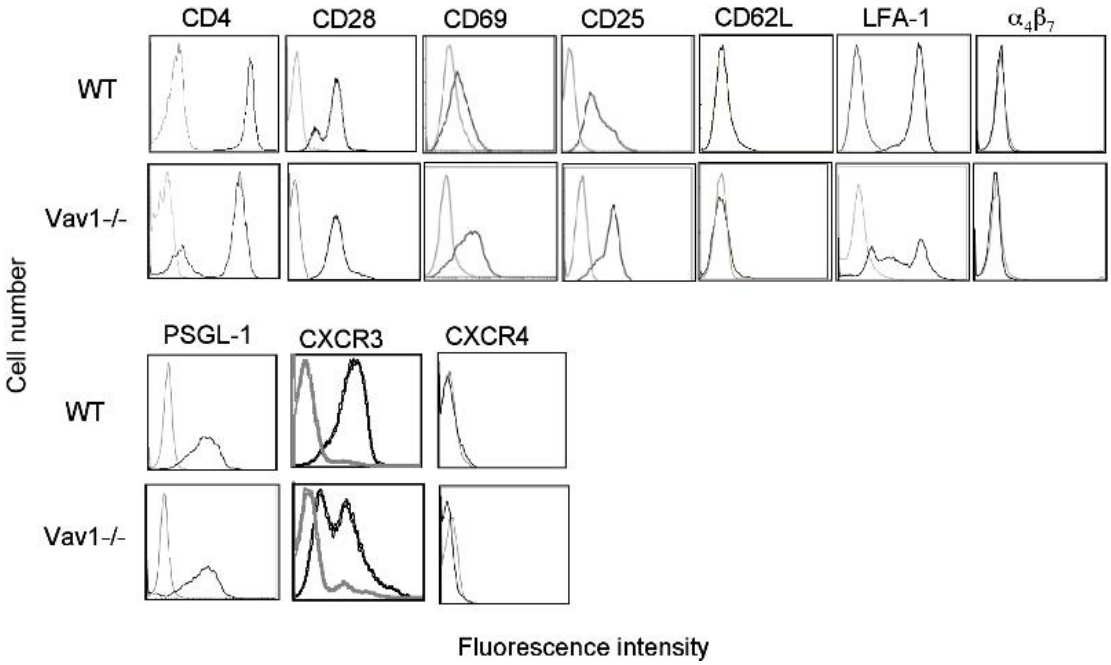

Figure S3. Phenotype of HY-specific T cells at the time of injection (JPG, 71.5 KB)

Expression of the molecules indicated above each set of panels by wild type (WT) and Vav1$^{-/-}$ HY-specific T cells was assessed at the time of injection (*i.e.*, 7 days following stimulation *in vitro*) by flow cytometry. Histograms are representative of a number of routinely performed analyses carried out prior to injection. The comparison of individual molecules was performed on cells injected in the same experiment. Staining with an isotype-matched control antibody is indicated by the grey profiles. Although variations in the expression of some of the surface molecules indicated was occasionally observed, these were not found to be statistically significant, with the exception of LFA-1 (Fig. 1A), when data from all the analyses performed were averaged (as differential MFI between the experimental sample and the isotype-control).

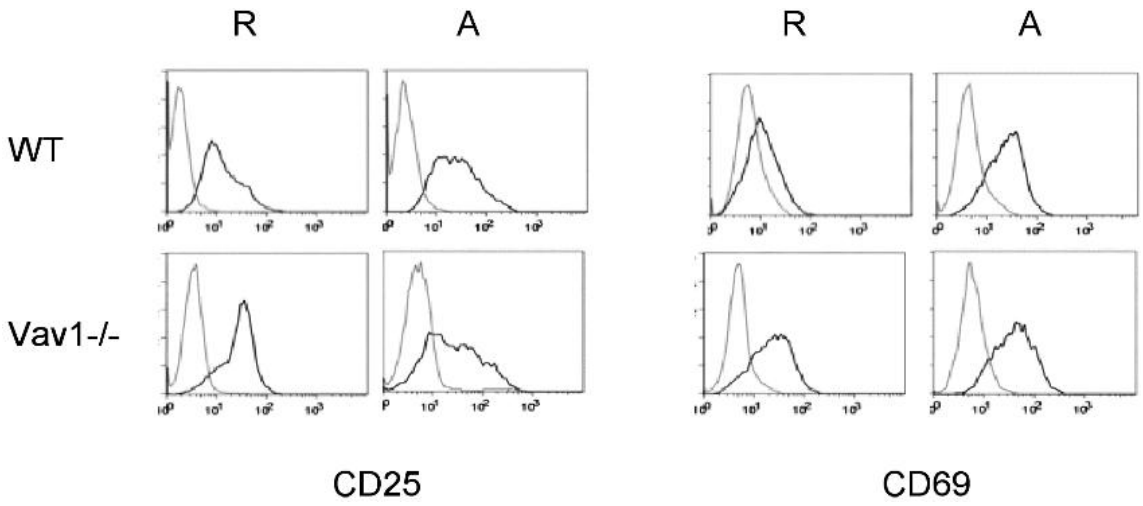

Figure S4. Phenotypic changes induced by antibody-activation in WT and Vav1$^{-/-}$ T cells (JPG, 57.3 KB).

HY-specific WT and Vav1$^{-/-}$ CD4^{+} T cells were isolated by density centrifugation 7 days after the last stimulation *in vitro* (indicated as R). To induce activation (indicated as A), T cells (10^6/well) were then incubated for 24 hours at 37°C with plastic bound anti-CD3 (1µg/ml, clone 2C11, BD Biosciences, Oxford, UK) and anti-CD28 (5µg/ml, clone 37.51, BD Biosciences) in 24 well plates. Expression of the surface molecules indicated in each panel was then assessed by flow cytometry. Both WT and Vav1$^{-/-}$ T cells upregulated CD25 (A), CD69 (B).

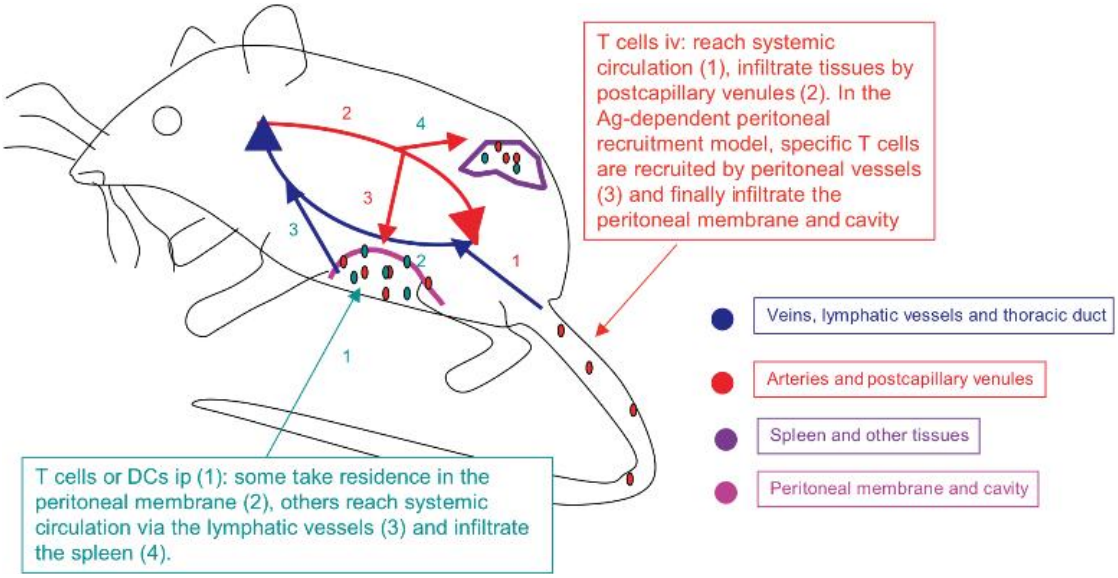

Figure S5. Trafficking of adoptively transferred T cells and DCs (JPG, 84.6 KB).

Based on the anatomy of recirculation we envisage the following scenario. Intravenously injected cells are directly recruited by antigen presenting post-capillary venules in the peritoneum, infiltrate the tissue and likely reversely transmigrate through the mesothelium of the peritoneal cavity, where they can be detected. Cells injected in the peritoneal cavity (static conditions) infiltrate the peritoneal tissue through the mesothelium, some taking residence in the peritoneal tissue and the majority entering the lymphatic system and then joining the blood stream via the thoracic duct, thus being able to disseminate systemically in both lymphoid and non-lymphoid tissues. Please note that the T cells used in this study did not reach lymph nodes due to a lack of appropriate homing receptors (CCR7, CD62L).

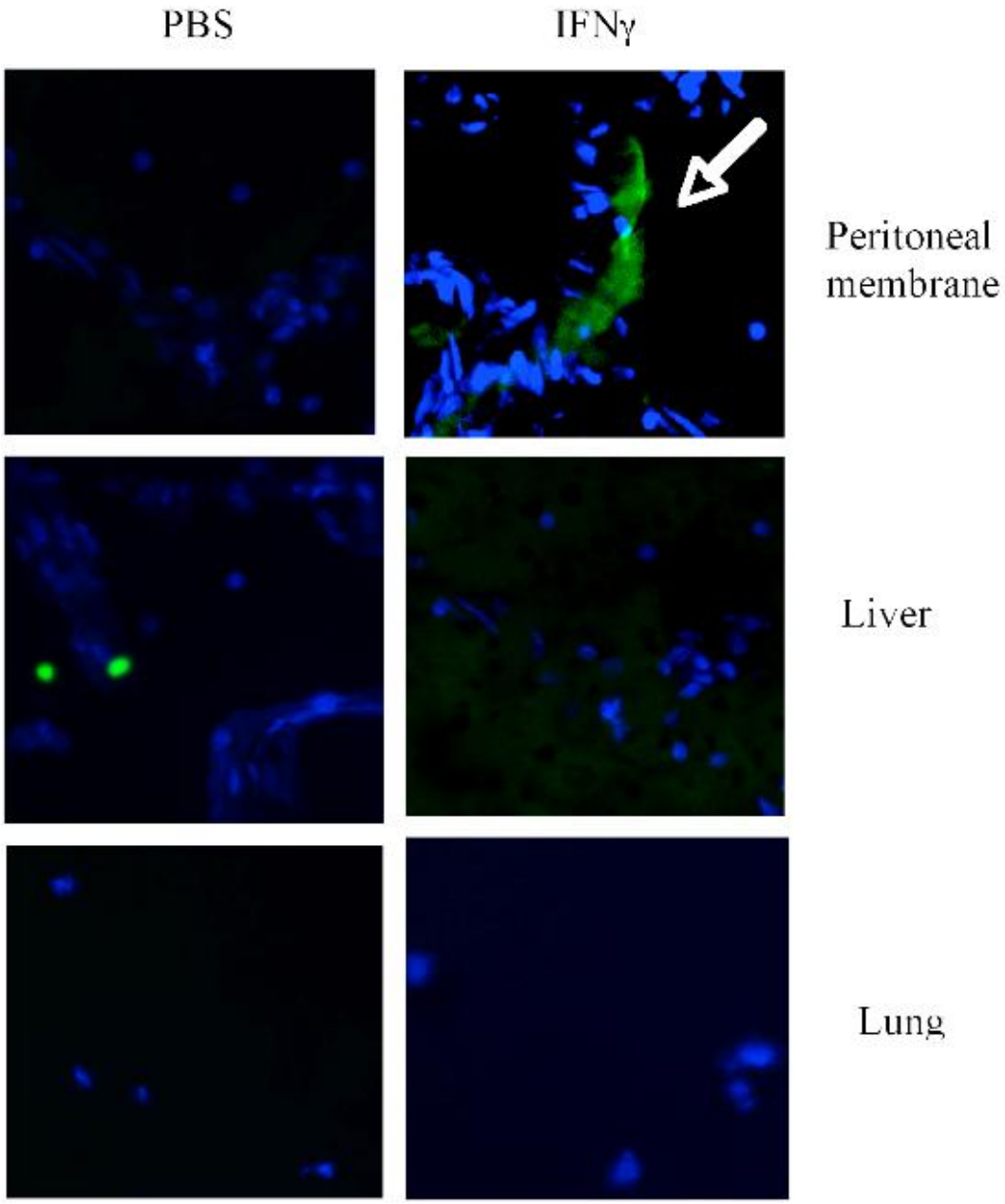

Figure S6. Expression of MHC class II molecules following IFNγ administration in vivo (JPG, 58.6 KB)

Female WT mice were injected ip with either PBS (left-hand panels) or 600U IFNγ (right-hand panels). This treatment resulted in the upregulation of MHC class II in the peritoneal cavity by a number of cells, including the mesothelium and endothelial cells lining blood vessels (indicated by the arrow), as assessed by staining of various tissue samples (obtained 48 hours after γ-IFN injection, 40× magnification) with a FITC-conjugated anti-H2-A^b mAb (0.5 μg/ml). In the liver, small-sized scattered cells expressing H2-A^b molecules could be detected, irrespective of IFNγ treatment.

Video 1. Migration of WT T cells to ICAM-1 (MP4, 465 KB)
Note: You should be able to use QuickTime to play this video.
WT HY-specific T-cell migration to ICAM-1 (2µg/ml) immobilized on plastic was analysed by time lapse microscopy as described in Materials and Methods.

Video 2. Migration of Vav1$^{-/-}$ T cells to ICAM-1 (MP4, 458 KB)
Note: You should be able to use QuickTime to play this video.
Vav 1$^{-/-}$ HY-specific T-cell migration to ICAM-1 (2µg/ml) immobilized on plastic was analysed by time lapse microscopy as described in Materials and Methods.