

SUPPLEMENTAL MATERIAL

supplemental Methods

Cloning

The WHIM mutation R334X was created using QuikChange Site-directed Mutagenesis Kit (Stratagene). Human wild-type (WT) and mutant CXCR4 were cloned into the pEGFPC3 vector (Clontech), to create N-terminal fusion proteins with EGFP (Enhanced Green Fluorescent Protein), enabling us to ensure that the cloned receptor was expressed in the membrane. The choice of N-terminal as opposed to C-terminal fusion was made to avoid any steric hindrance by the EGFP of signaling mediated by the C-terminal domain of CXCR4, which may be important in conferring the WHIM-associated phenotype. A membrane targeting sequence from lactase phlorizin hydrolase was included at the N-terminus of the fusion protein (gift from Dr S. Manes, CSIC, Spain). The constructs were subsequently cloned into the m5p retroviral system.

Turnover assay

CXCR4 Turnover assay was performed as in^{5,32}. In brief, primary CD4 T cells expressing WT or WHIM-mutant CXCR4 were incubated at 37°C with 200nM CXCL12 for varying amounts of time. Following an acidic glycine buffer wash [pH 2.7], CXCR4 was detected by using APC-conjugated anti-CXCR4 (12G5; BD). Background fluorescence was determined by using matching immunoglobulin isotype control antibody on replicate samples. Reduction in CXCR4 MFI was calculated by normalizing to the MFI for unstimulated cells.

supplemental Figure Legends

supplemental Figure 1. Ectopically expressed WHIM-mutant CXCR4 displays defective ligand-induced recycling. CD4⁺ T cells expressing WT or WHIM-mutant EGFP-CXCR4 were incubated with CXCL12 at 37°C for varying amounts of time. The expression of CXCR4 among EGFP-expressing cells was assayed by FACS after staining with an anti-CXCR4 APC-conjugated monoclonal antibody. Mean Fluorescence Intensity is shown. Experiment repeated four times, representative experiment shown. A 2-way ANOVA followed by Bonferroni post tests was performed. **, P < 0.01; ***, P < 0.001.

supplemental Figure 2. T-DC interaction duration distribution in OVA-specific control (non-retrogenic) CD4⁺ T cells within imaged lymph node slices. The distribution of T-DC interactions of control CD4⁺ T cells and DC that were (A) pulsed with 10µg/ml OVA peptide or (B) unpulsed are shown, distributed in “bins” of 2-minute width.

supplemental Figure 3. WHIM-mutant CXCR4 recruitment to the T-APC synapse is impaired by competing external CXCL12 and restored by AMD3100.

Primary CD4⁺ T cells from healthy donor peripheral blood transfected with EGFP-CXCR4 (WT) or EGFP-CXCR4 (WHIM) formed conjugates for 15' with unpulsed or superantigen (sAg)-pulsed EBV-B cells, in the presence or absence of 5nM to 100nM CXCL12 and 12.6µM AMD3100. The cells were fixed, imaged in confocal microscopy, and the Relative Recruitment Index to the T-APC synapse (RRI) for EGFP-CXCR4 was calculated. The graph shows the RRI for all analyzed cells (from left to right, n=83, 97, 92, 90, 45, 45, 45, 44, 86, 95, 94, 88, 44, 43, 40, 45). Grey bars indicate the mean of each condition. The dotted black line indicates the mean of the control condition (WT T with unpulsed EBV-B).

Supplemental Figure 4. The tissue distribution of CD4⁺EGFP⁺ rgWT or rgWHIM T cells transferred into RAG1^{-/-} mice along with T-depleted splenocytes (as described in Figure 6), at the end of the timecourse, 6 months after transfer. Relative numbers (normalized to spleen) of CD4⁺EGFP⁺ T cells shown for spleen, bone marrow (BM), blood, pooled inguinal, paraaortic and axillary lymph nodes (LN) or mesenteric lymph nodes (mesLN).

Videos: Representative examples of cropped volumes from 30 min timelapse 2-photon microscopy videos from imaged lymph node slices, with a frame acquired every 30 sec. Video running time accelerated to 15 sec. Videos were compressed using the H264 codec to 520x340 pixel resolution. The grid squares shown are 10 µm in length. The tracks of traced T cells are shown.

Video 1. Interactions between non-retrogenic, control OVA-specific T cells (blue) and unpulsed DC (red).

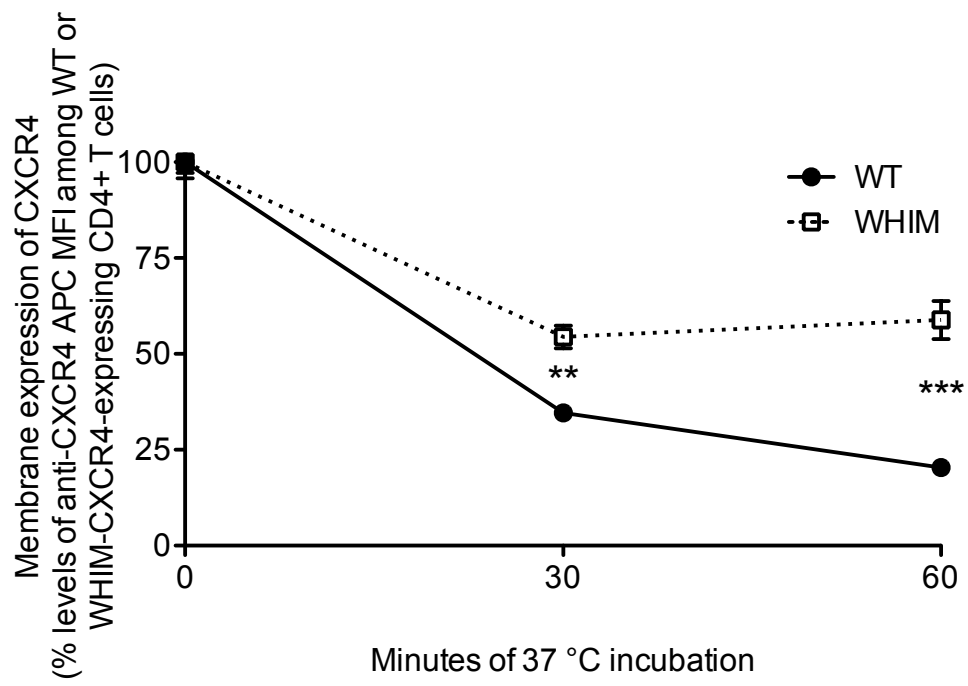
Video 2. Interactions between rgWT T cells (green) and unpulsed DC (red).

Video 3. Interactions between rgWT T cells (green) and OVA-pulsed DC (red).

Video 4. Interactions between rgWHIM T cells (green) and unpulsed DC (red).

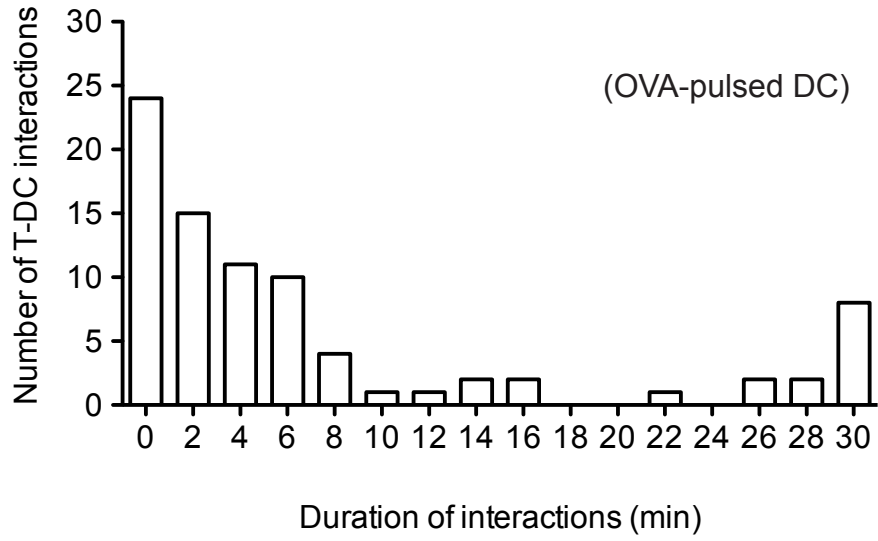
Video 5. Interactions between rgWHIM T cells (green) and OVA-pulsed DC (red).

Video 6. Interactions between rgWHIM T cells (green) and OVA-pulsed DC (red) in the presence of AMD3100.

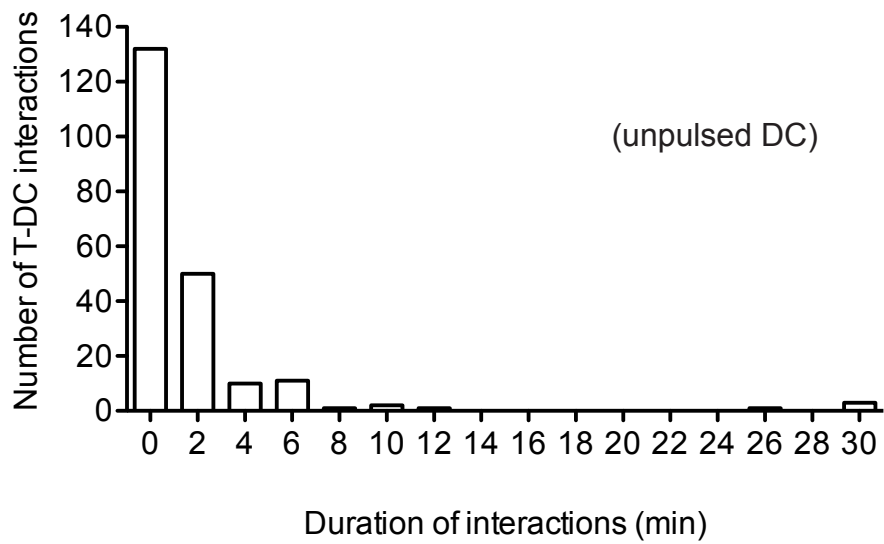


supplemental Figure 1

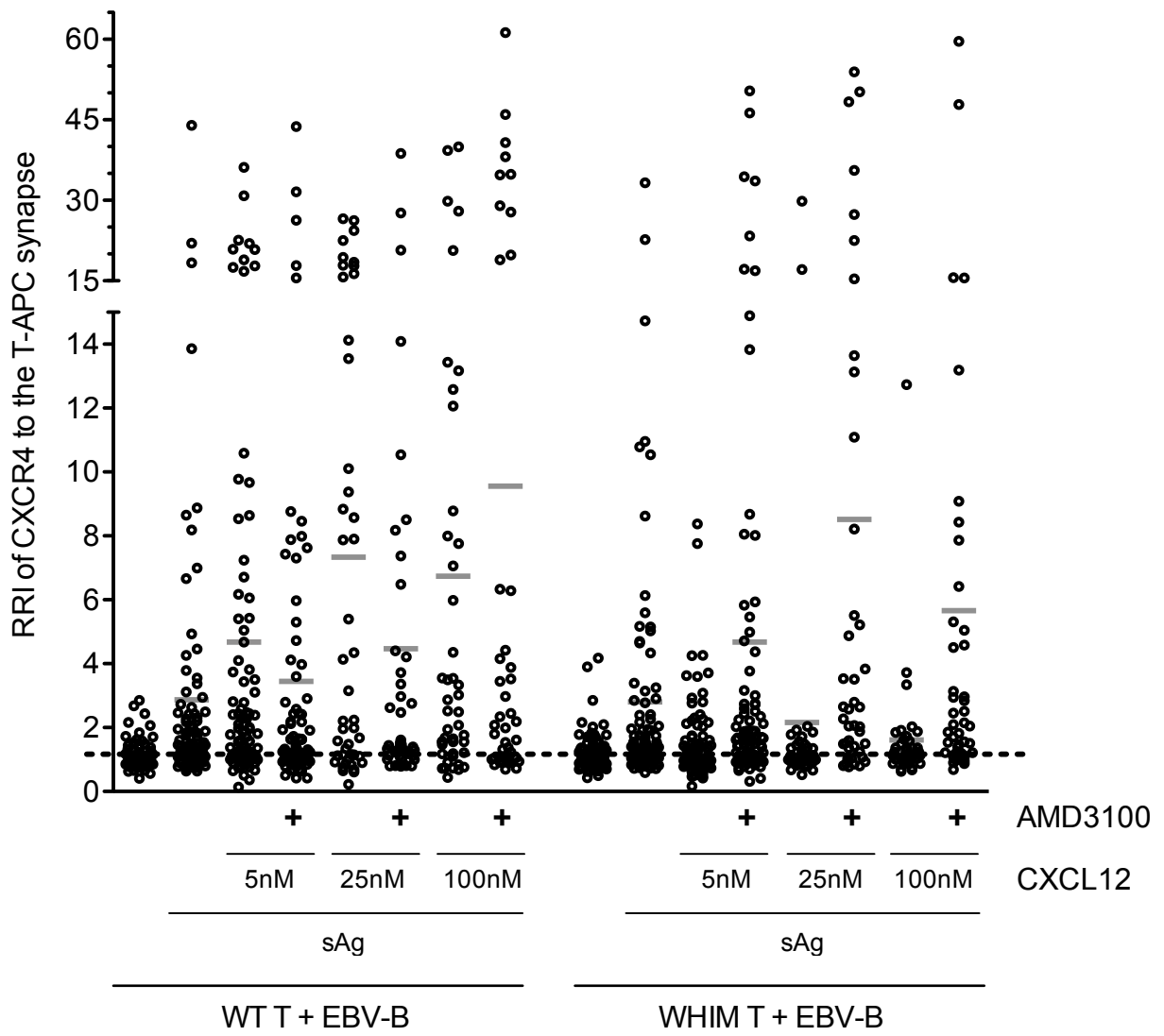
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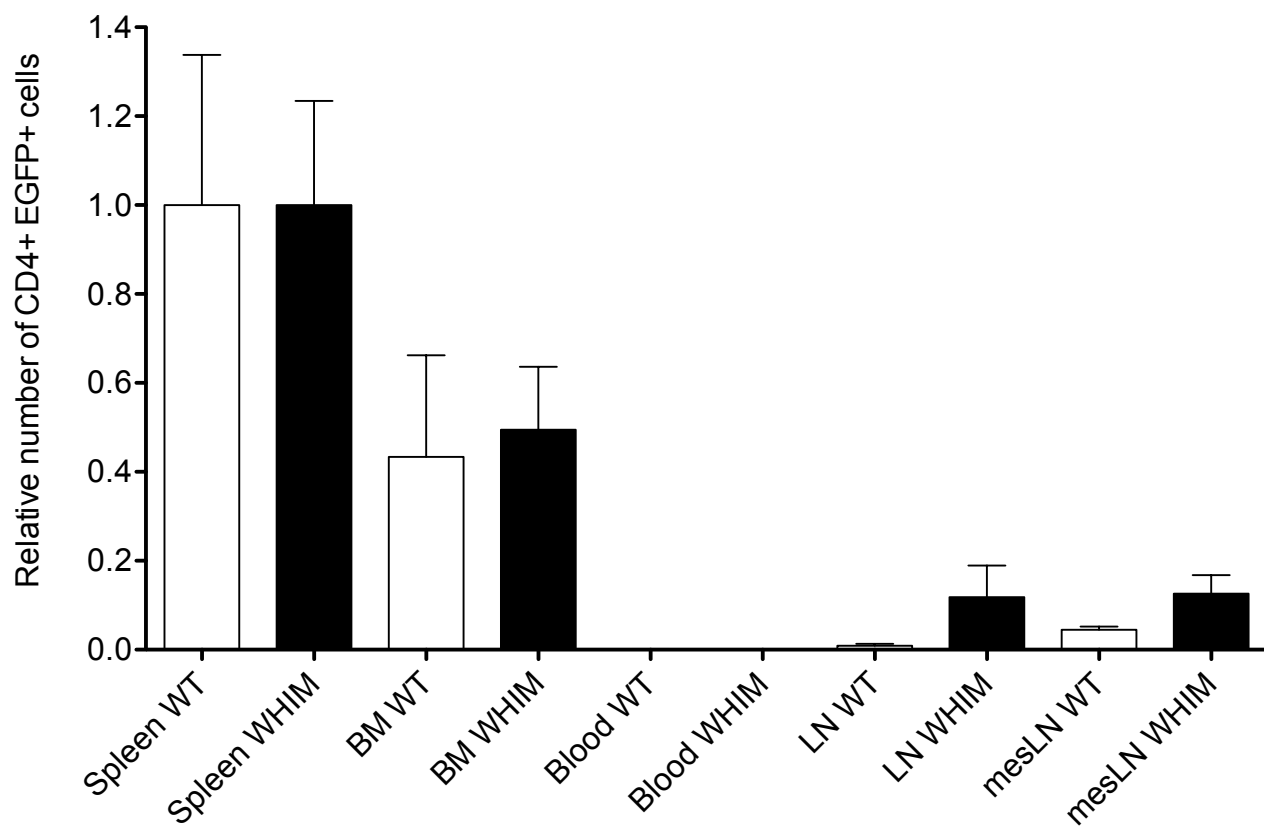
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supplemental Figure 2



supplemental Figure 3



supplemental Figure 4