Supplemental Data

Macrophage and T Cell Dynamics during the Development and Disintegration of Mycobacterial Granulomas

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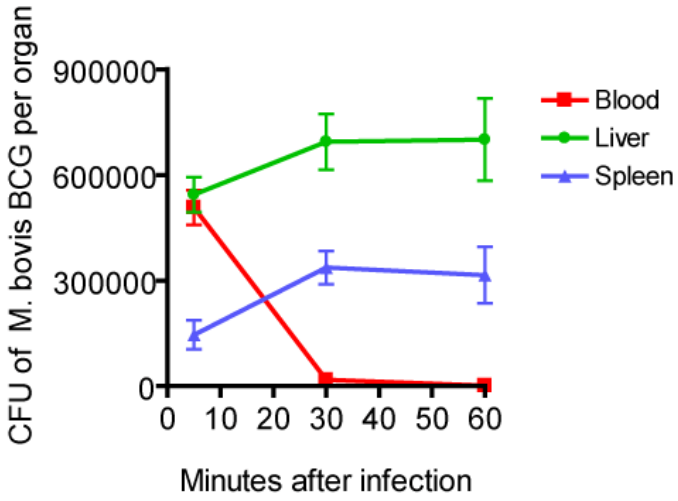

Figure S1. Rapid Clearance of Systemic BCG from the Blood and Association with the Liver

Blood, liver and spleen were collected from animals 5, 30 and 60 minutes after i.v. infection with BCG. The liver was perfused with PBS through the portal vein immediately prior to harvesting from the animals. Tissues were passed through a 70μm cell strainer prior to plating for colony forming unit (CFU) quantification.

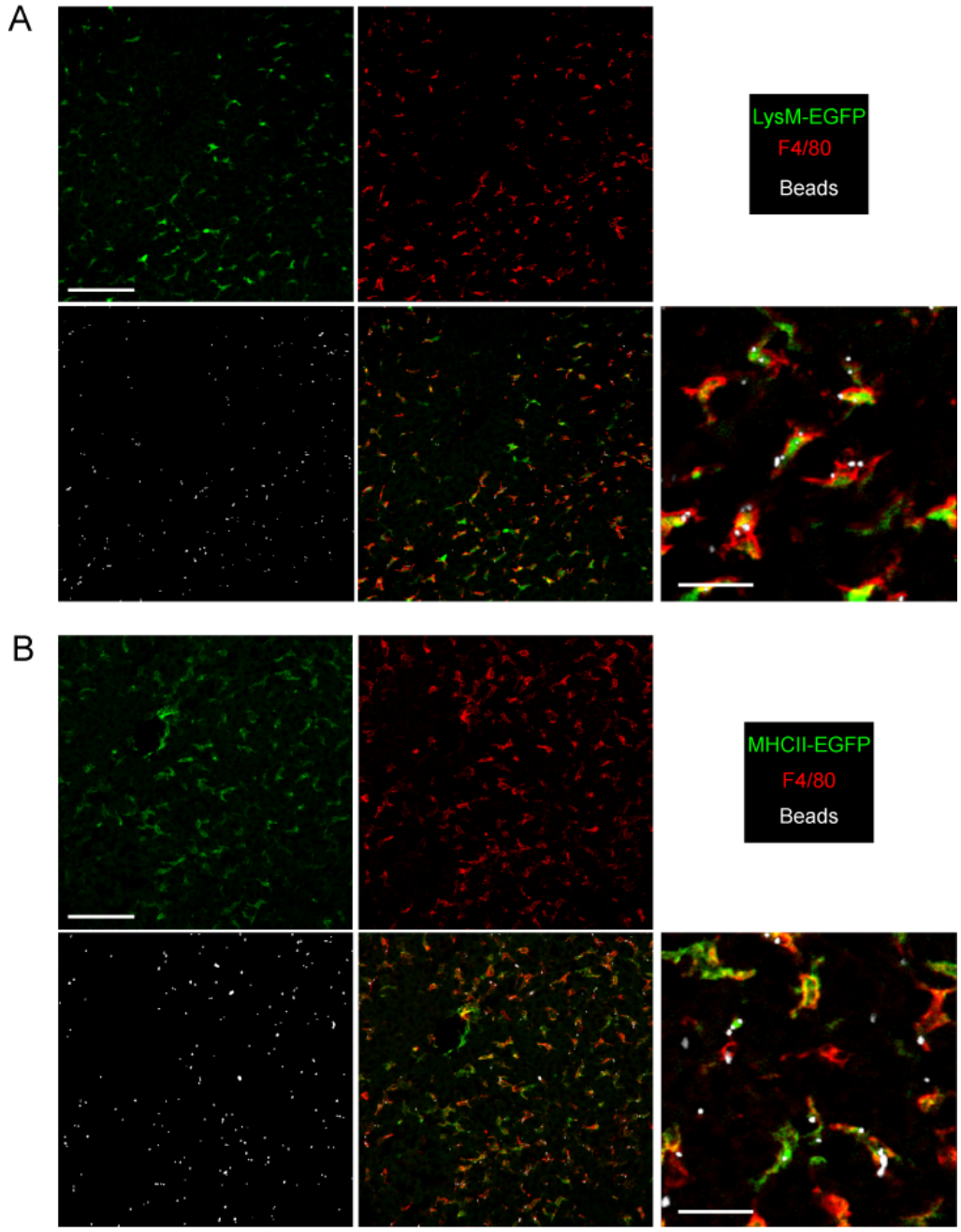

Figure S2. EGFP Expression by Kupffer Cells in LysM-EGFP and MHC Class II-EGFP Knockin Mice

LysM-EGFP (A) and MHCII-EGFP (B) mice were injected i.v. with 1μm fluorescent beads. 15 minutes later, livers were perfused with paraformaldehyde and harvested from the animals. Images from liver sections show the surface expression of the macrophage marker F4/80 (red) by EGFP-expressing cells (green) and the ability of these cells to associate with systemically introduced beads (white). Scale bar left panels= 100μm, scale bar right panels= 30μm.

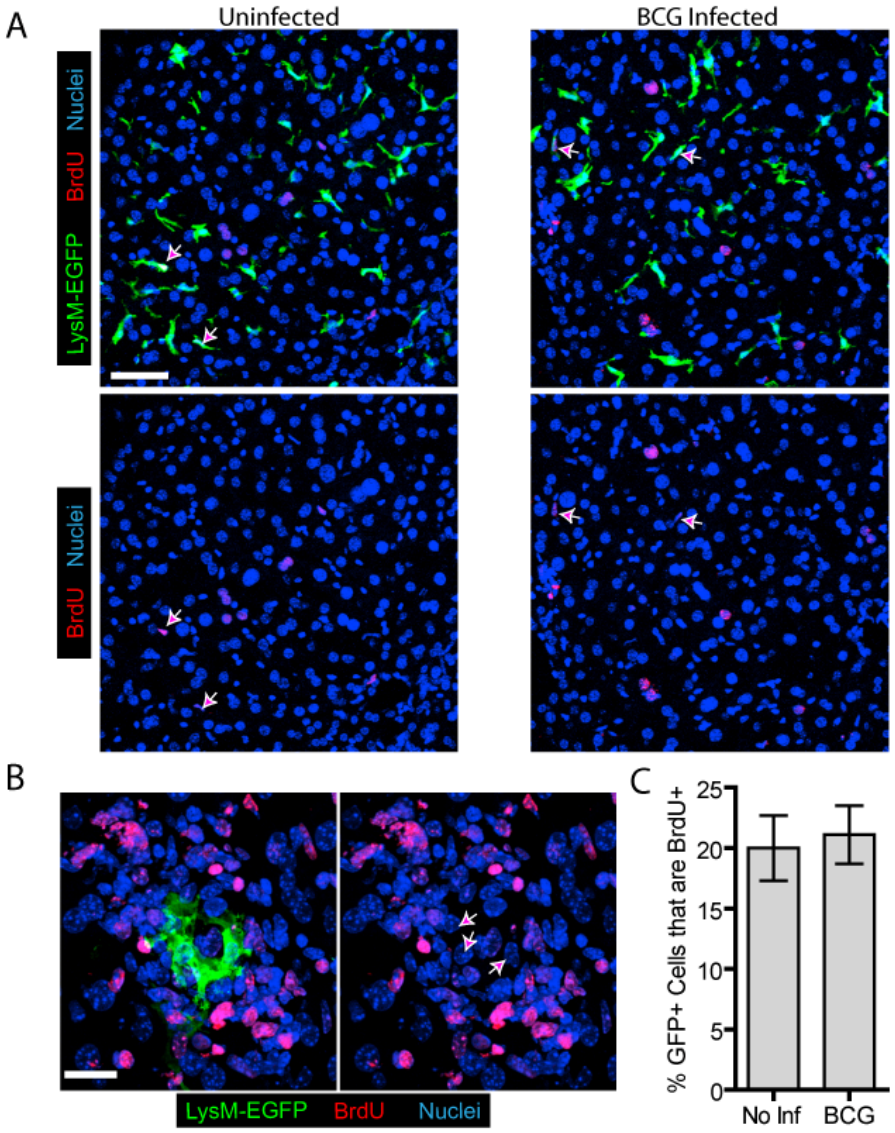

Figure S3. BCG Infection Does Not Induce Kupffer Cells to Proliferate

A-B) LysM-EGFP bone marrow chimeras were left uninfected or injected i.v. with BCG-RFP and immediately given BrdU in their drinking water (0.8mg/ml). At 1 (A) or 2 (B) weeks p.i. livers were sectioned and stained with rabbit anti-GFP [a gift from J. Ziskin (Johns Hopkins Medical Institute, Maryland)] followed by AlexaFluor 488-conjugated goat anti-rabbit IgG . Sections were re-fixed for 1 hour in 4% paraformaldehyde/PBS and treated with a 2M HCl solution. BrdU was detected using AlexaFluor 647-conjugated anti-BrdU (clone PRB-1) (Invitrogen). Arrows in part A show the sparse number of $EGFP^{+}BrdU^{+}$ nuclei in both BCG infected and uninfected livers. Arrows in part B indicate the nuclei of the $EGFP^{+}$ cells within the granuloma (notice the lack of BrdU staining). Scale bar for A, 50µm; Scale bar for B, 20µm.
C) For the 1 week time point, the number of EGFP-expressing cells whose nuclei were $BrdU^{+}$ was quantified from a total of 662 and 550 cells in the uninfected and infected animals, respectively. Graph shows mean +/- SEM from 4 mice obtained from 2 individual experiments.

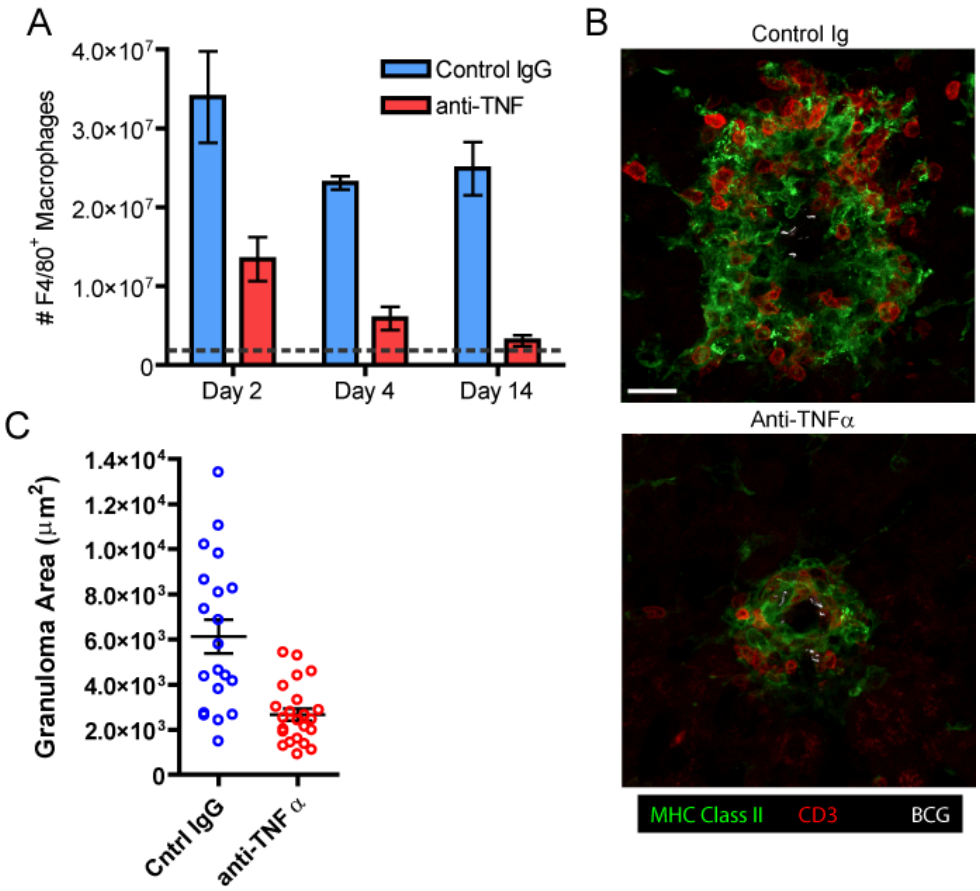

Figure S4. Anti-TNF-α Treatment Results in a Rapid Decrease in Granuloma Size and Macrophage Cellularity

A) C57BL/6 mice were infected with BCG-RFP bacteria to establish granulomas. 3 weeks following infection groups of animals were treated with anti-TNFα or control IgG every other day for 14 days. Livers were harvested on days 2, 4, and 14 and the absolute number of $F4/80^+$ cells was determined by flow cytometry. Graph shows mean +/- SEM from 3 mice/group. Dashed line represents average cell number in uninfected animals.

B) Livers from granuloma-bearing animals were harvested 4 days after the start of antibody treatment as in part A. Livers were sectioned and stained with antibodies specific to MHC Class II and CD3. Scale bar, 20μm.

C) Granuloma size was quantified on liver sections from part B by measuring the total area occupied by MHC class II staining. Each data point represents an individual granuloma. Mean +/- SEM is shown.

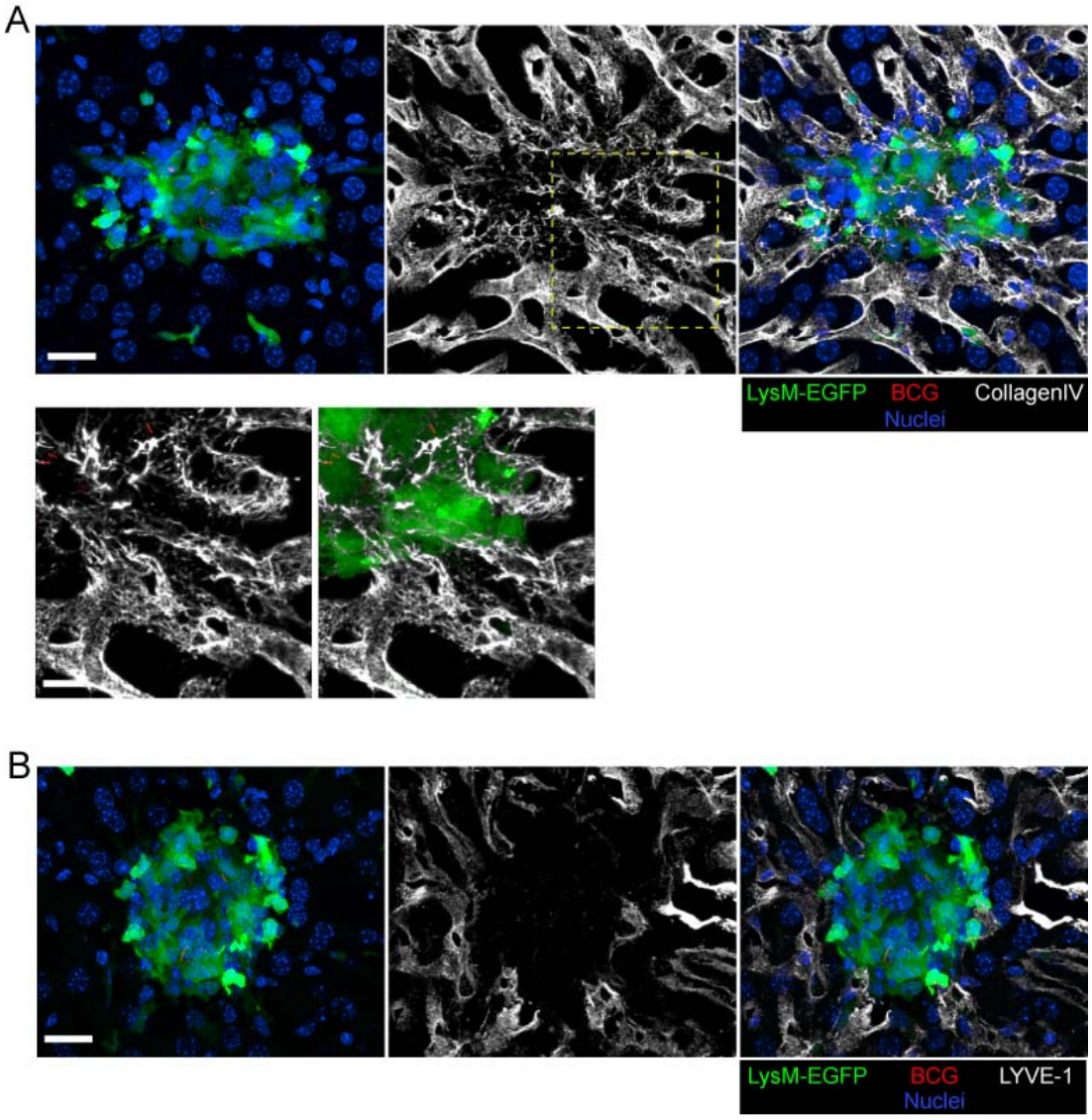

Figure S5. Granuloma Growth Is Associated with Disintegration of Sinusoidal Vessels, but Retention of the Collagen Backbone

Liver sections from LysM-EGFP mice that had been infected i.v. with BCG-RFP bacteria 3 weeks earlier were stained with antibodies specific for Collagen IV (A) or the sinusoidal marker LYVE-1 (B) and the nuclear dye DAPI. Lower panels in A show a magnified region of upper panel images (yellow box). Notice that the collagen matrix associated with the sinusoidal vessels continues beyond the macrophage border and into the core of the granuloma. Scale bar for A upper panel and B, 20μm; Scale bar for A lower panel, 10μm.

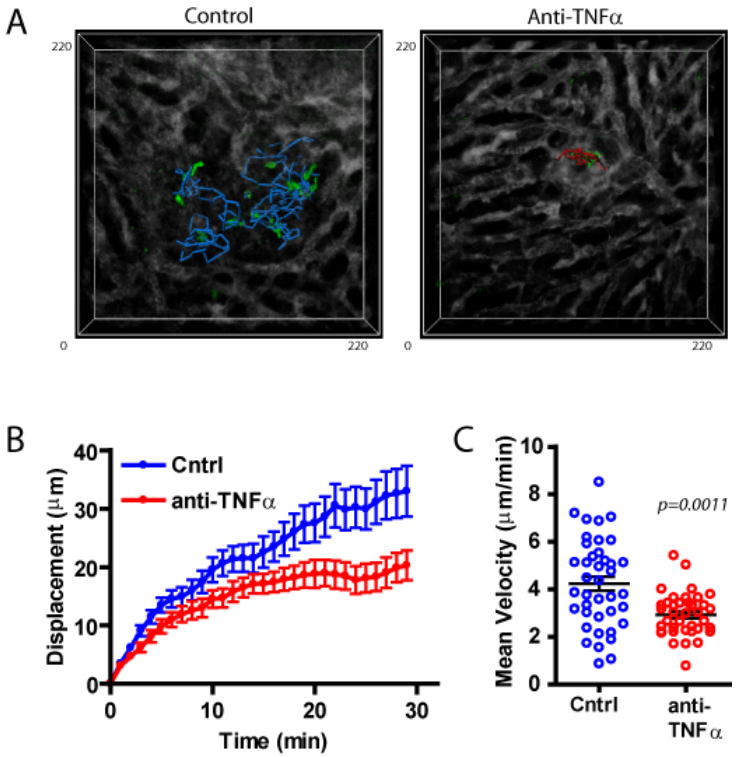

Figure S6. Anti-TNF-α Treatment Decreases T Cell Displacement and Velocity within the Granuloma

A) RAG1-deficient animals reconstituted with EGFP-expressing CD4 T cells were infected with BCG-RFP bacteria. 3 weeks following infection animals were treated with control IgG or anti-TNFα every other day for 4 days and then subject to hepatic IVM. A representative image from a 4D data set shows the migratory paths of several granuloma-associated T cells from control and anti-TNFα treated animals.
B-C) Quantification of displacement over time (B) and velocity (C) for granuloma-associated T cells from control IgG (blue) and anti-TNFα (red) treated animals. For C, data points represent individual cells compiled from 4 separate experiments. Graphs show mean +/- SEM. The p value from a Mann-Whitney test is shown.

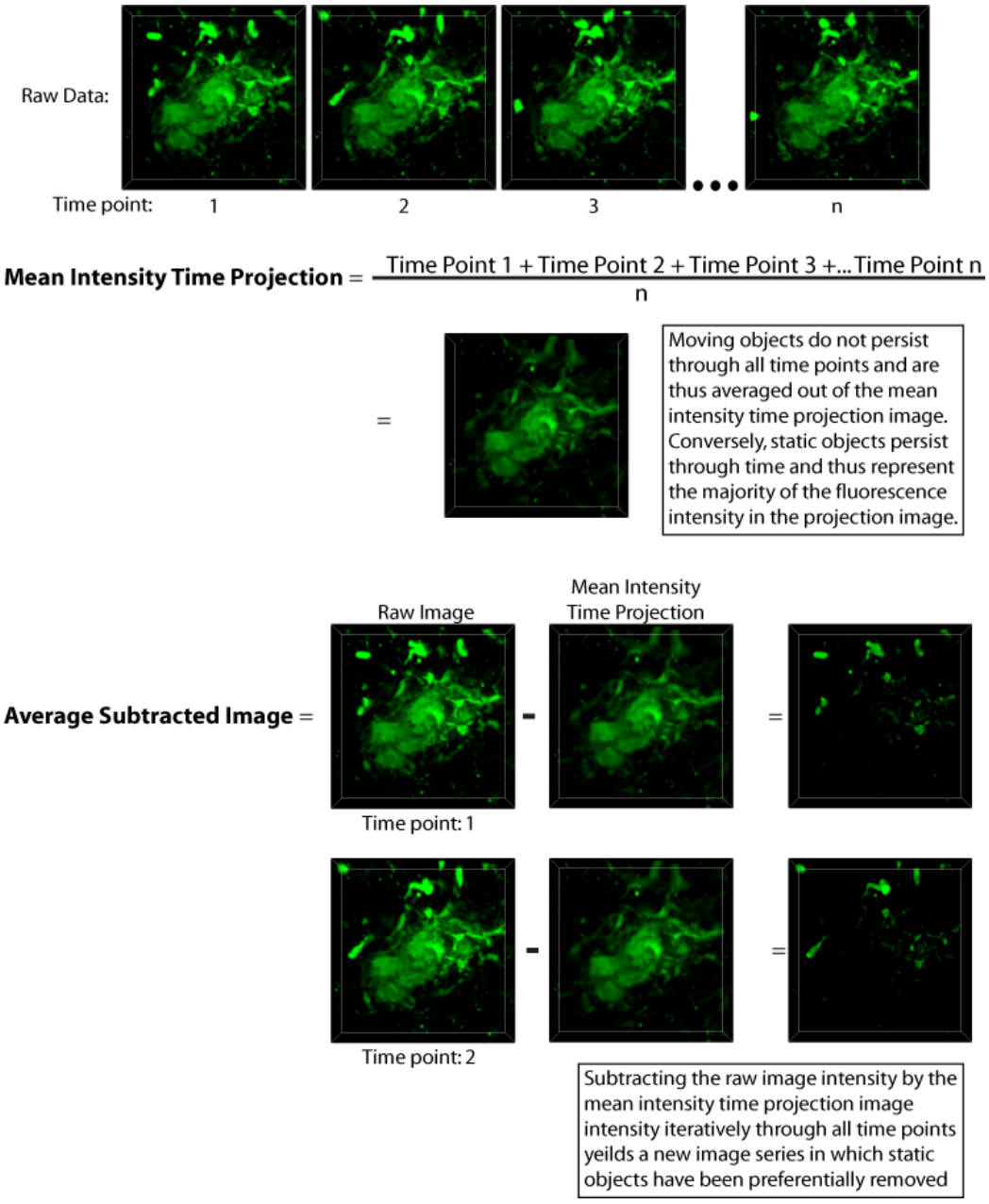

Figure S7. Description of Method Used to Generate Average Subtracted Images

Method used to generate Figure 4d and Movie S10.