Experimental Procedures

Bacterial Strains

S. Typhimurium expressing green fluorescent protein (GFP) under the control of the curli promoter was described (Rapsinski et al., 2014). The *csgA* deletion in Nissle 1917 was constructed by transduction with $P1_{vir}$ from JW2105, an *E. coli* K-12 Δ*csgA::kan* strain in the Keio knock-out collection (Baba et al., 2006). For selection, kanamycin was used at a concentration of 35 μg/ml. Bacterial strains were grown in LB media with nalidixic acid (50 μg/ml), or carbenicillin (100 μg/ml) where necessary. LB-NoSalt Media was used to promote biofilm formation.

Biofilms and Confocal Laser Scanning Microscopy (CLSM)

Bacterial strains were grown in LB no salt media at 30° C to promote biofilm formation. DNA was visualized in biofilms by staining with 1 μg/ml of propidium iodide (Molecular Probes), 1 μg/ml of Hoescht 33258 or 1 μg/ml of TOTO-1 (Life Technologies). Curli fibers were stained with 10 μM Thioflavin T or 10 μg/ml of Congo Red and visualized with a Leica SP5 microscope with a TCS confocal system.

For CLSM imaging of dendritic cells on biofilms, biofilms were grown in 8-chamber microscope coverslip (Nunc) at an angle to allow for the formation of pellicle biofilm on the coverslip. After 72 hours, media was removed and GFP expressing bone marrow-derived dendritic cells generated from C57BL/6-Tg(CAG-EGFP)131Osb/LeySopJ mice, kindly provided by Dr. Doina Ganea (Temple University, Philadelphia, US) were placed on the biofilms. CLSM images of biofilm of wild type *S.* Typhimurium containing a plasmid vector for constitutive expression the red fluorescent

protein, d-Tomato, kindly provided by Dr. Yao-hui Sun at UC Davis. Crystal Violet Assay was performed as described previously (Crawford et al., 2008).

Purification of Curli Fibers

Curli was purified as previously described from *S.* Typhimurium *msbB* mutant (RPW3) (Collinson et al., 1991). After purification, all curli samples were sonicated for 15 sec to break up any large clumps of fibers.

Amyloid Polymerization Assays

Synthetic peptide corresponding to the 4^{th} and 5^{th} repeat regions of CsgA, CsgA R4-5 was previously described (Tukel et al., 2009) and was purchased from Biosynthesis Inc. Reconstitution of lyophilized synthetic peptide, CsgA R4-5, was conducted as previously described (Rapsinski et al. 2013). Lag times were calculated as described previously (Hartman *et al.*, 2013).

Dendritic Cells studies

Mouse bone marrow-derived DCs (cDCs) were generated as previously described (Sriram et al., 2012). To assess the production of pro-inflammatory cytokines by cDC exposed to biofilms, biofilms of *S.* Typhimurium wild type, *msbB*, and *ΔfliCfljB* strains were grown in 48 well tissue culture plates at an angle to allow for attachment of pellicle biofilms to the bottom of the wells. 10^6 cDCs were placed on top of attached biofilms. cDCs were also stimulated *in vitro* with dose titrations of curli/DNA composites purified from *S.* Typhimurium *msbB* mutant biofilms. IL-12, IL-6 and TNF-α was measured by

ELISA (eBioscience). *Irf7* and *Isg15* expression was analysed in cDCs quantitative real-time Reverse Transcriptase-PCR (qRT-PCR) using Taqman probes (Sriram et al., 2014).

***In vivo* stimulation**

NZBxW/F1 and C57BL/6 mice were injected i.p. with 50 ug of curli-DNA composite. Flow cytometry analysis was performed to measure the activation of T-cells, B-Cells and DCs from spleens. mRNA expression of two interferon-stimulated genes (ISGs), *Irf7* and *Isg15* was analysed by qRT-PCR in splenic CD11c+ DCs isolated with magnetic beads.

Autoantibody ELISA

Anti-dsDNA and anti-chromatin were measured by ELISA as previously described (Sriram et al., 2012). Sera from five naïve B6 mice were used as negative controls. Autoantibody IgG subclasses were measured using isotype specific biotinylated goat anti-mouse antibodies (SouthernBiotech) and following the same protocol.

Antinuclear Antibody Assay

Pre-fixed Hep-2 seeded slides were purchased from MBL-Bion. Serum diluted 1:40 was incubated on Hep-2 cells for 30 minutes at room temperature, allowing autoreactive antibodies to bind Hep-2 cells. Cells were then rinsed with PBS and washed in Coplin jars for 5 minutes. Cells were stained with anti-mouse IgG-FITC (SouthernBiotech, Cat. No. 1030-04). After washing as above, cells were mounted and visualized using fluorescence microscopy (Olympus BX60 microscope with Diagnostics Instruments Camera and Spot Advanced Software).

Statistical analysis

Data were analyzed using Prism software (GraphPad, San Diego). One-sample t-test or two-tailed Student's t-test were used as appropriate. A p value of <0.05 was considered significant.

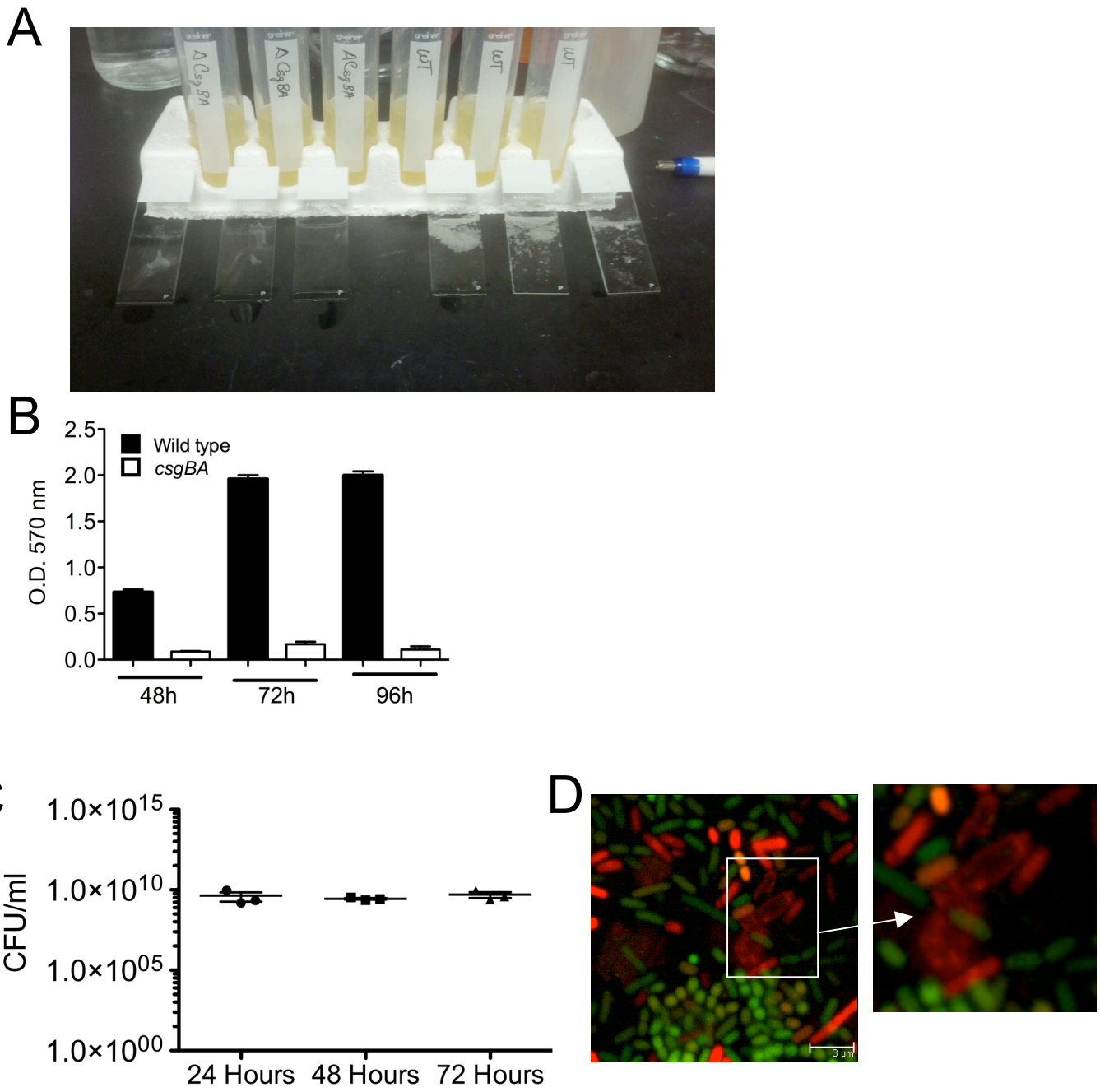

Fig S1: *S.* Typhimurium *csgBA* mutant fails to form pellicle biofilms under static conditions. (A) *S.* Typhimurium *csgBA* mutant formed no pellicle biofilms attached to microscope slide when grown at 30° C for 72 hours as compared to the robust biofilm attached to microscope slides seen in the wild type *S*. Typhimurium. (B) Crystal violet biofilm assay of wild type and *csgBA* mutant *S.* Typhimurium confirming no biofilm production by *csgBA* mutant. (C) Bacterial colony counts from WT *S.* Typhimurium biofilms over a time course of 72 hours. (B) CLSM images of *S.* Typhimurium biofilm (green) stained with PI (red) shows DNA extrusion from dead bacterial cells.

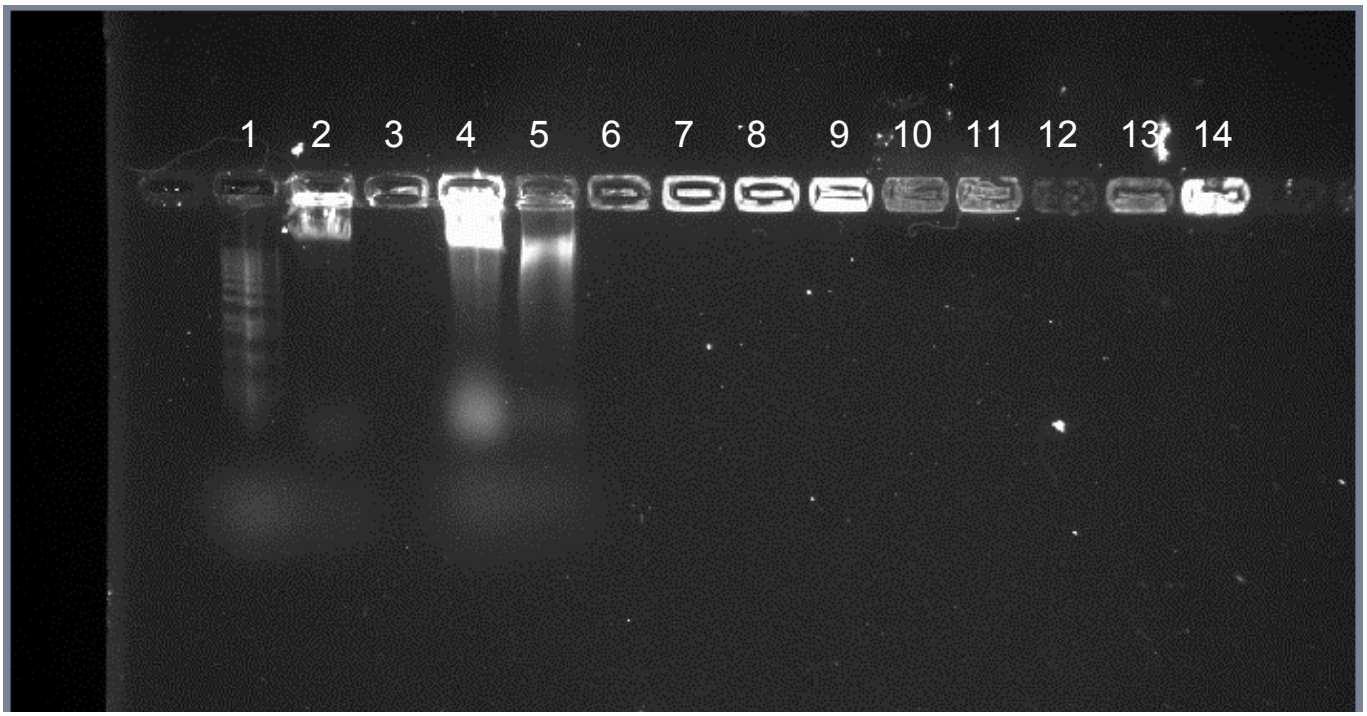

Fig S2. DNA within amyloid/DNA complexes is detectable by ethidium bromide (EtBr) staining in agarose gels. 1% agarose gel containing EtBr was loaded with DNA Ladder (Lane 1), *Salmonella* genomic DNA (Lane 2), genomic DNA treated with DNAse (Lane 3), hexafluoroisopropanol (HFIP) (Lane 4), dimethylsulfoxide (DMSO) (Lane 5), or genomic DNA treated with both HFIP and DMSO (Lane 6). Other wells contained purified curli (Lane 7), purified curli treated with DNAse (Lane 8), RNAse (Lane 9), HFIP (Lane 10), DMSO (Lane 11), or curli treated with both HFIP and DMSO (Lane 12). Lane 13 contained polymerized synthetic CsgA R4-5 peptides and Well 14 contained CsgA R4-5 peptides polymerized in the presence of 20 µg/ml *Salmonella* genomic DNA. The gel was visualized using the BioRad GelDoc System.

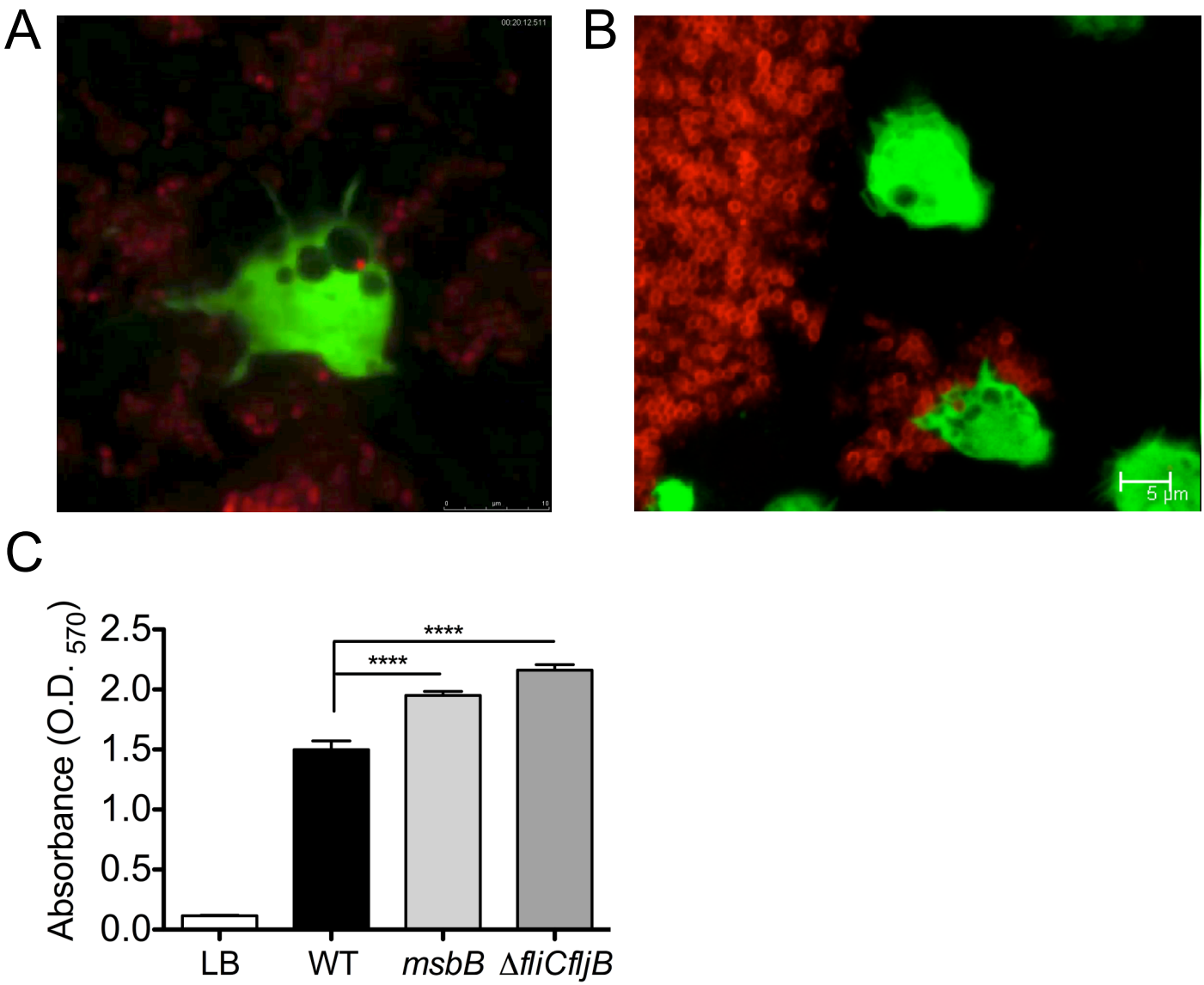

Fig S3. Interactions of dendritic cells with *S.* Typhimurium biofilms. (A) Still image of a time-lapse video showing a GFP expressing BMDC phagocytosing S. Typhimurium expressing α-tomato (red) from the biofilm. (B) GFP expressing BMDC interacting with *S.* Typhimurium biofilm stained with Congo Red (CR). (C) Crystal violet staining of WT, *msbB,* and Δ*fliCfljB* biofilms grown statically also demonstrate similar biofilm development in these strains. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$,**** , $p < 0.0001$

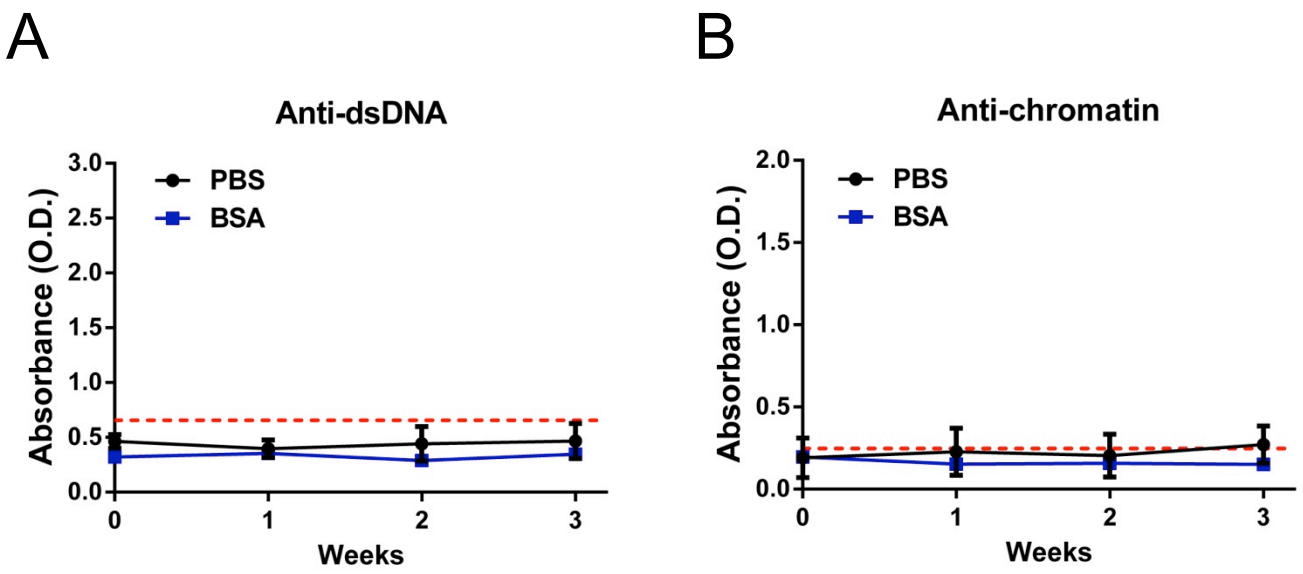

Fig S4. BSA does not induce autoantibodies in lupus-prone mice. (A) Anti-dsDNA and (B) anti-chromatin autoantibody ELISAs from young NZBxW/F1 mice injected with 50ug of BSA per mouse three times a week. Optical density (O.D.) indicates ELISA color change and the presence of serum anti-dsDNA or anti-chromatin. Error bars indicate SEM, n=5 per group. Horizontal dashed line indicates the cutoff for positivity, two standard deviations above the B6 average (n=5).

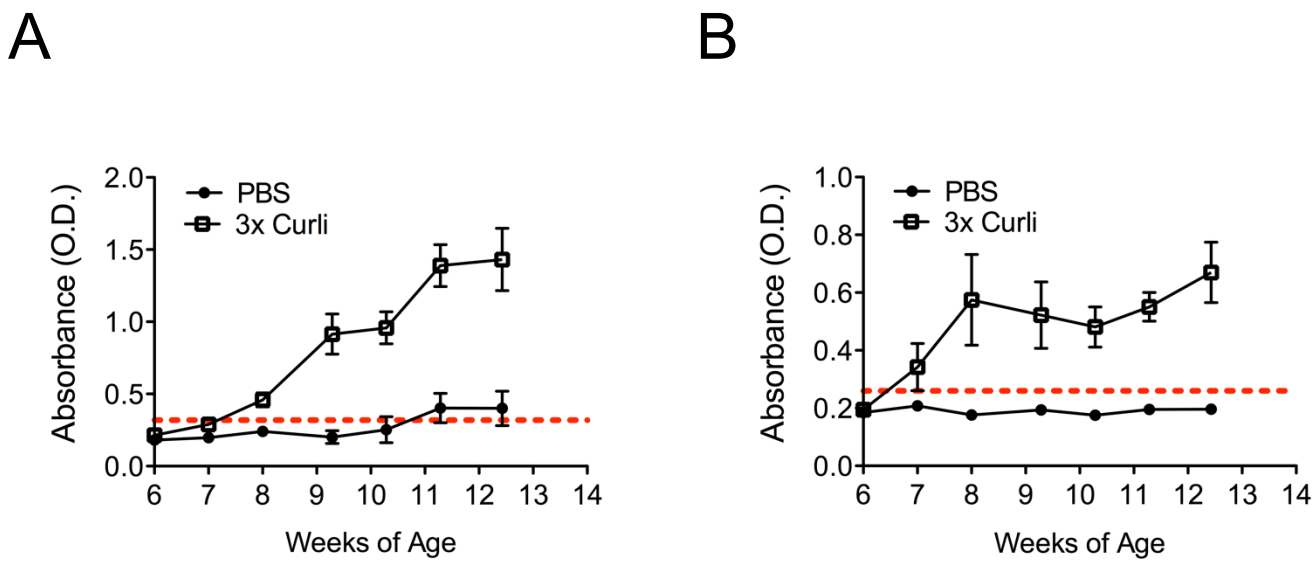

Fig S5. Curli/DNA composites induce autoantibodies in SvJ-129 mice. (A) Anti-dsDNA and (B) anti-chromatin autoantibody ELISAs from SvJ-129 mice injected continuously with PBS or curli three times a week. Optical density (O.D.) indicates ELISA color change and the presence of serum anti-dsDNA or anti-chromatin. Error bars indicate SEM, n=7 per group. Horizontal dashed line indicates the cutoff for positivity, two standard deviations above the B6 average (n=5).

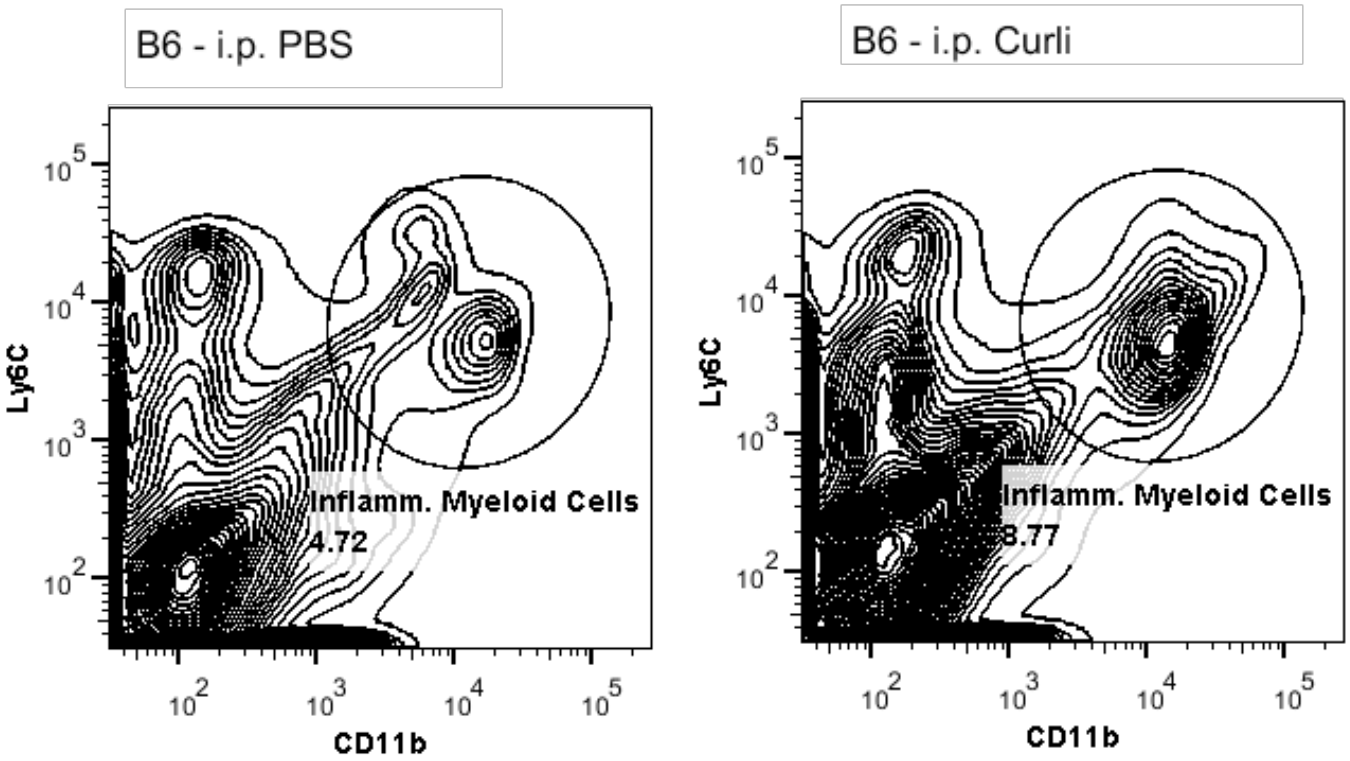

Fig S6. Curli/DNA composites cause influx of Ly6C+ CD11b+ myeloid cells into the spleen. C57BL/6 mice were injected i.p. with PBS or curli. Spleens were harvested after 24 hrs and stained for Ly6C and CD11b. Curli injection caused an increase in inflammatory myeloid cells (Ly6C+ CD11b+) within the spleen. Figures are representative of 3 mice per treatment group.