

Backgrounds

- Classical monocytes (CM) are critical mediators of ischemia-reperfusion injury(IRI), contributing to organ transplant failure, myocardial infarction, and stroke.
- Circulating CM originate from the bone marrow (BM).
- Most BM-derived CM are sequestered in the spleen, specifically in the red pulp.
- BM-derived CM are insufficient to drive lung IRI.

Science 2009, *Nat Med* 2011, *J Clin Invest* 2018

Research questions

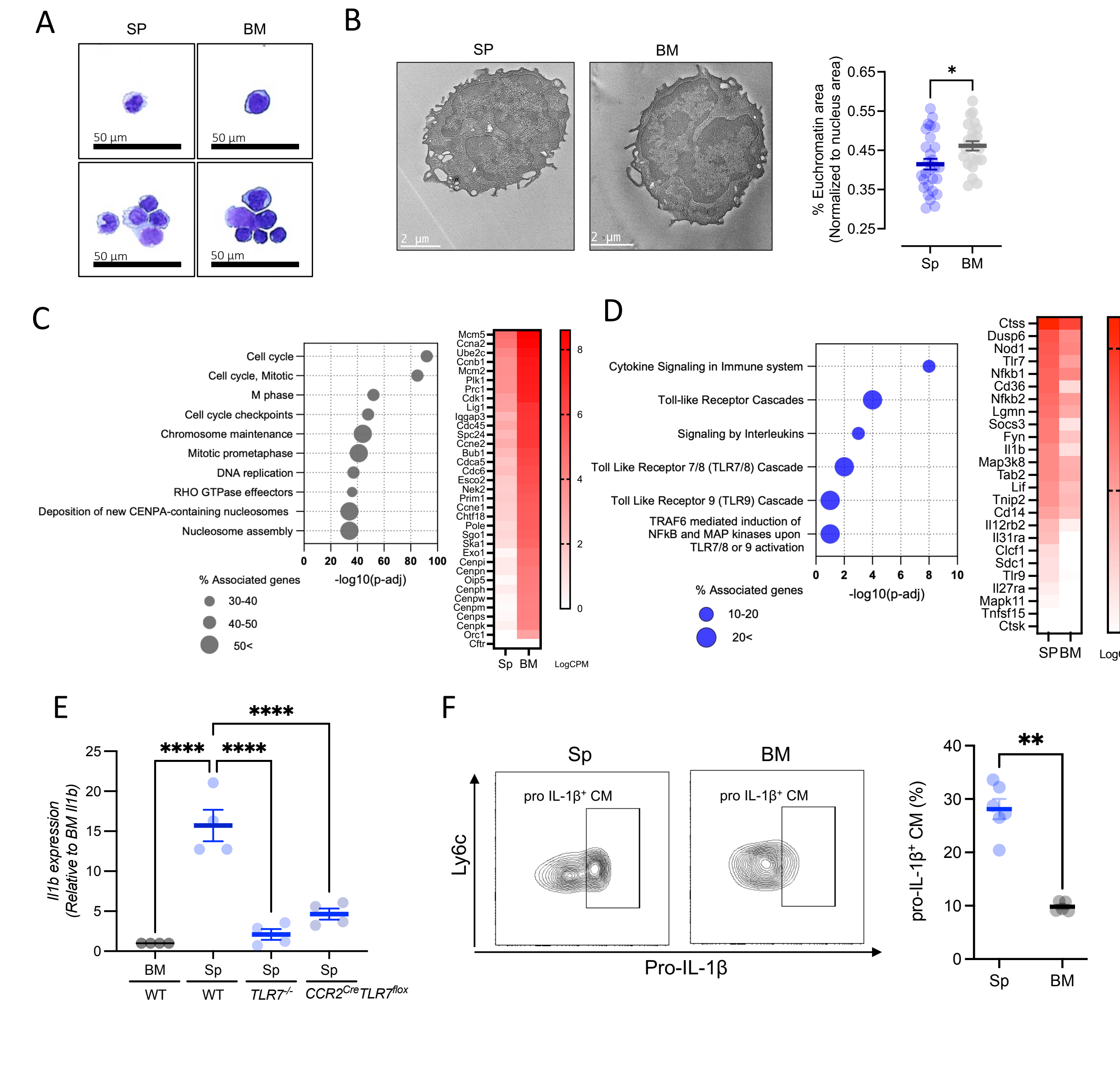
- How do BM-derived CM acquire a proinflammatory state?
- Could proinflammatory-primed CM be a therapeutic target to mitigate multi-organ IRI?

Hypothesis and aim

- BM-derived CM acquire a proinflammatory phenotype through bystander priming mechanisms, contributing to multi-organ IRI**
- Aim 1.** Determine the differential characteristics of CM derived from BM and spleen
- Aim 2.** Elucidate how BM-derived CM acquire a proinflammatory phenotype
- Aim 3.** Assess bystander-primed CM as a therapeutic target in multi-organ IRI

Results

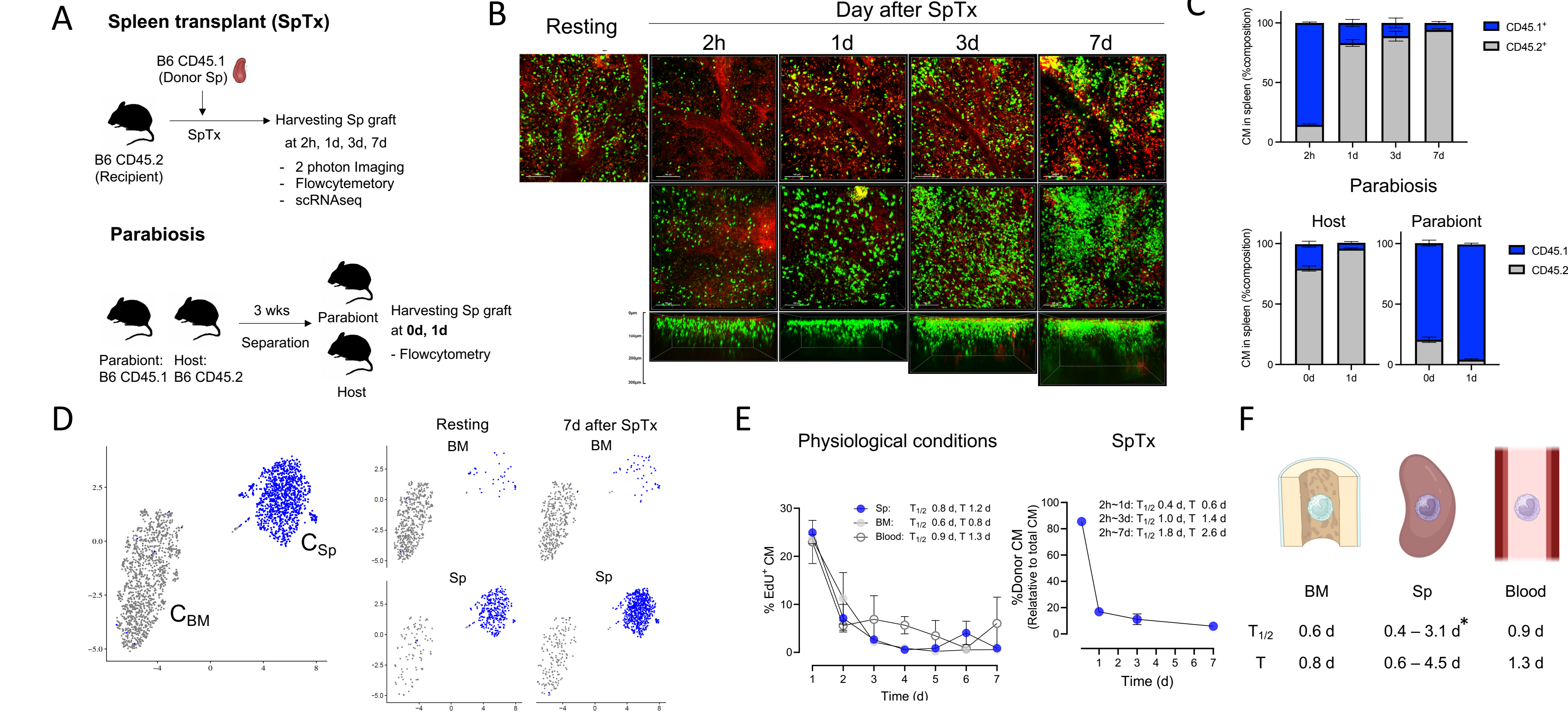
Figure 1. BM-derived CM undergo phenotypical transition



CM were sorted by the panel with Ly6C^{hi}MHCII-CD11b⁺ in mouse BM and spleen (Sp) tissues in the resting state. **(A)** Diff-Quick staining showing cell size and the nuclear-to-cytoplasmic (N/C) ratio (n=3). **(B)** Transmission electron microscopy (TEM) illustrating the euchromatin area in the nucleus (n > 6 cells/replicate, 3 replicates). **(C)** Top REACTOME pathways identified by enrichment analysis in BM-CM, with a heatmap of the top 35 genes in this pathway group (n=3). **(D)** Top REACTOME pathways related to "Signaling by Interleukin" in splenic CM, with a heatmap of the top 25 genes in this pathway group (n=3). **(E)** qPCR analysis of *Il1b* expression in sorted CM from BM and spleen in B6 wild-type (WT) mice, and spleen from toll-like receptor 7 knockout (TLR7^{-/-}) and CCR2^{Cre}TLR7^{flx} mice at the resting (n=4). **(F)** Flow cytometry analysis using an anti-pro-IL-18 antibody in BM-CM and splenic CM at the resting (n=5). Data presented as Mean ± SEM. *, p<0.05; **, p<0.01; ***, p<0.001, ****, p<0.0001

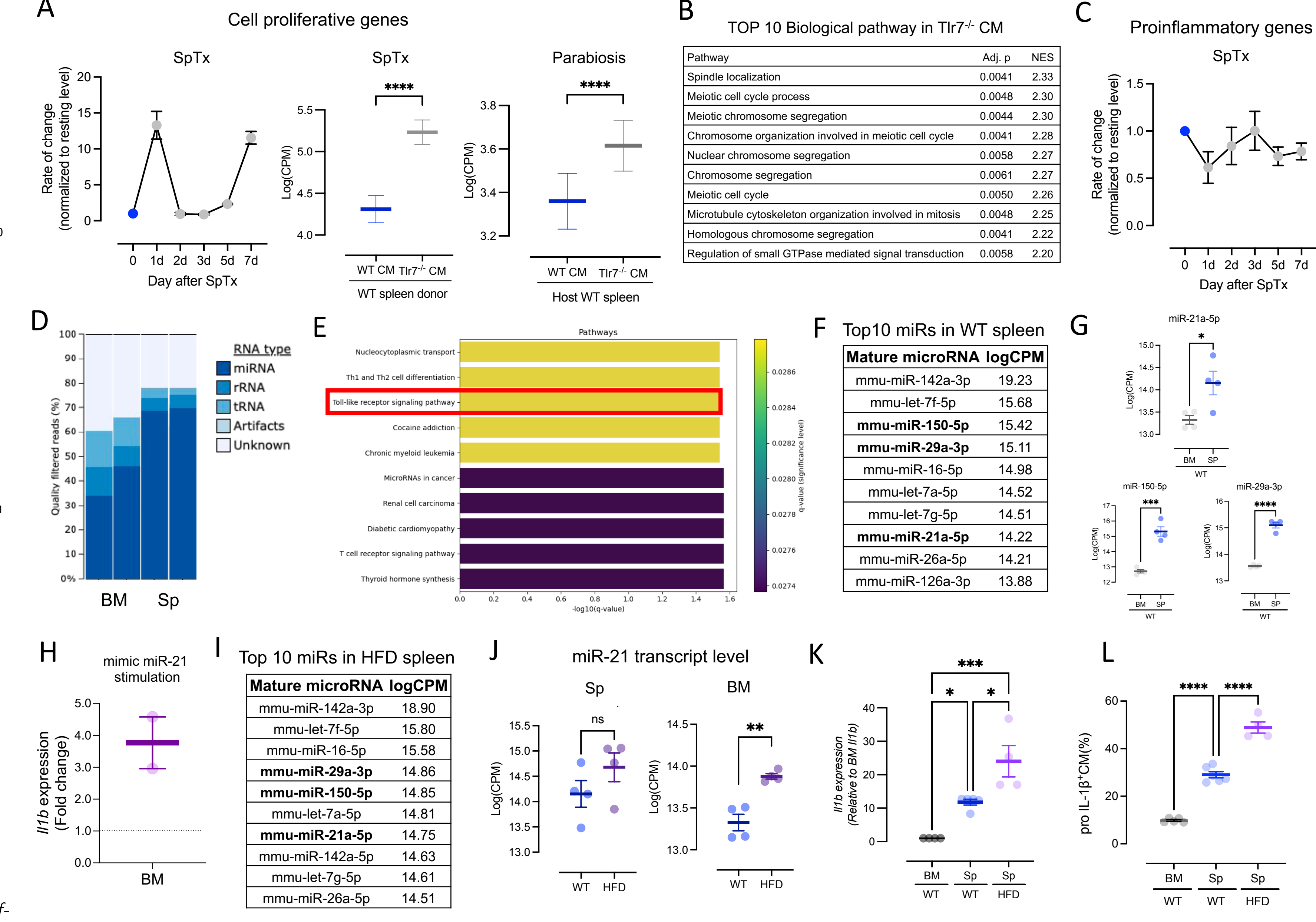
Results (continued)

Figure 2. Dynamics and fate of BM-derived CM



(A) Experimental design of heterochronic spleen transplantation (SpTx) and parabiosis in mice. **(B)** Representative two-photon image of the transplanted spleen. Ccr2-GFP B6 mice were used as recipients (n=3). **(C)** Quantification of CM counts from donor/parabiont (CD45.1⁺) and recipient/host (CD45.2⁺) spleens by flow cytometry (n=4-6). **(D)** Single-cell RNA sequence (scRNAseq) of CM in BM and spleen (Sp) tissues at the resting or 7 days post SpTx (n=2-3). Cluster BM (C_{BM}) and Cluster Spleen (C_{Sp}). **(E)** Half-life (T_{1/2}) and average lifespan (T) of CM in BM, spleen, and blood, determined by an EdU-based tracking system under physiological conditions and SpTx model under inflammatory conditions. **(F)** Summary of T_{1/2} and T of CM. * data calculated from C_{Sp} and C_{BM} in scRNAseq. T_{1/2} = (100 * ln(2)) / (100 - y), T = T_{1/2} / ln(2) [y : percentage of C_{Sp}]

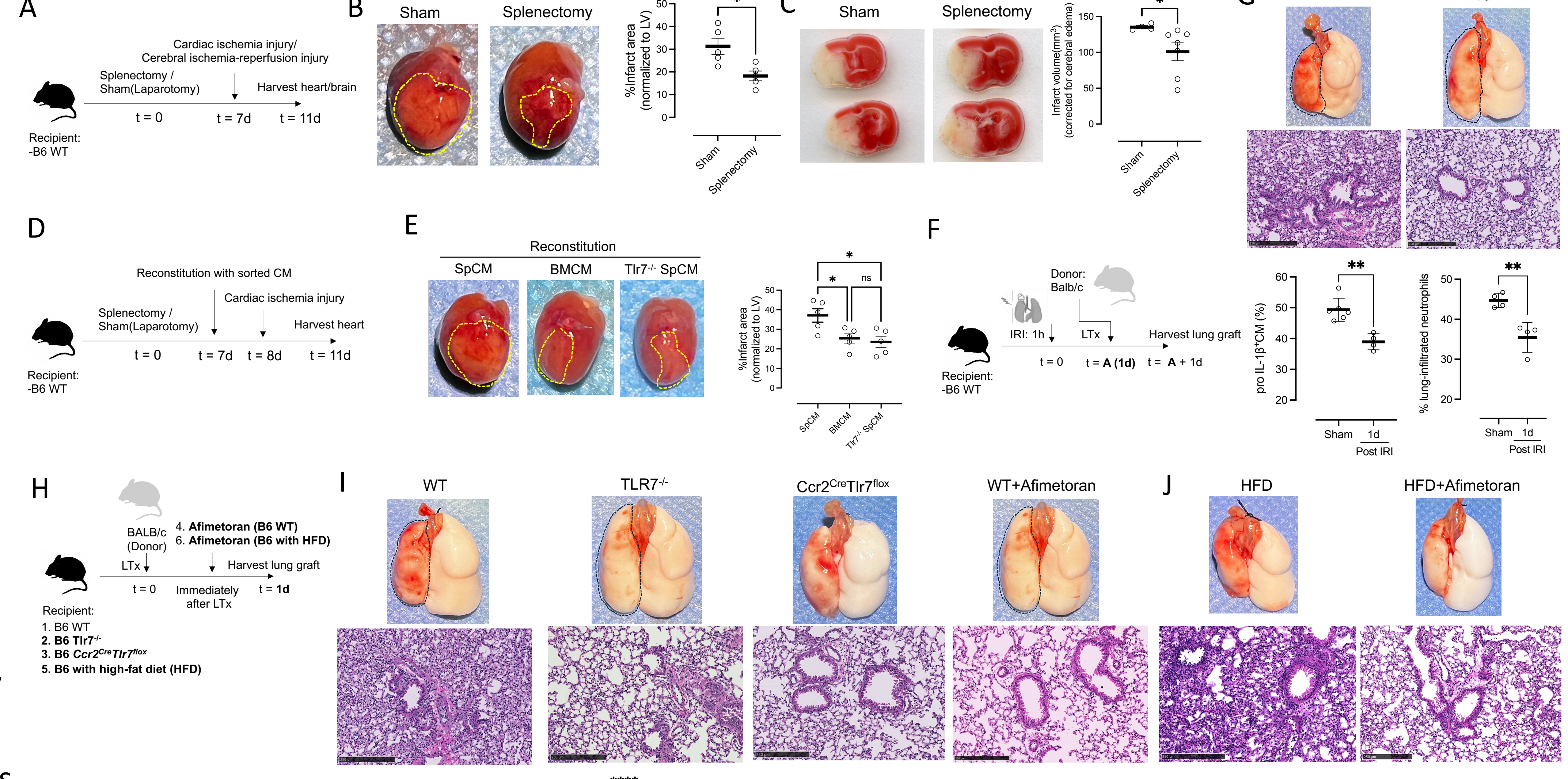
Figure 3. BM-derived CM shift from proliferative to proinflammatory gene expression profiles via the miR-21/TLR7 signaling axis



(A) Average expression level of cell proliferative genes normalized to resting level, as defined by REACTOME pathway selection in SpTx model (n=3-4). Expression of cell proliferative genes in WT and Tlr7^{-/-} CM in SpTx and parabiosis model (n=3-5). **(B)** Top 10 biological pathways identified by enrichment analysis in Tlr7^{-/-} CM from the parabiosis model. **(C)** Average expression level of proinflammatory genes in SpTx model. **(D)** RNA type composition in WT BM and spleen tissues by small RNA deep sequencing (n = 2). **(E)** Top 10 enriched pathways in WT spleen. Bold indicates TLR7-binding miRs predicted by deep learning approach. **(G)** Expression levels of top 3 TLR7-binding miRs (n=4). **(H)** qPCR of *Il1b* expression in BM-CM stimulated with an miR-21 mimic in vitro (n=2). **(I)** Top 10 miRs in high fat diet (HFD)-treated spleen. Bold indicates TLR7-binding miRs predicted. **(J)** Expression levels of miR-21a-5p in HFD spleen and BM (n=4). **(K)** qPCR of *Il1b* expression and **(L)** Flow cytometry analysis using anti-pro-IL-18 antibody in sorted CM: BM and spleen of B6 WT mice, and spleen of B6 WT mice with HFD at the resting (n=4-7). Data presented as Mean ± SEM. *, p<0.05; **, p<0.01; ***, p<0.001, ****, p<0.0001

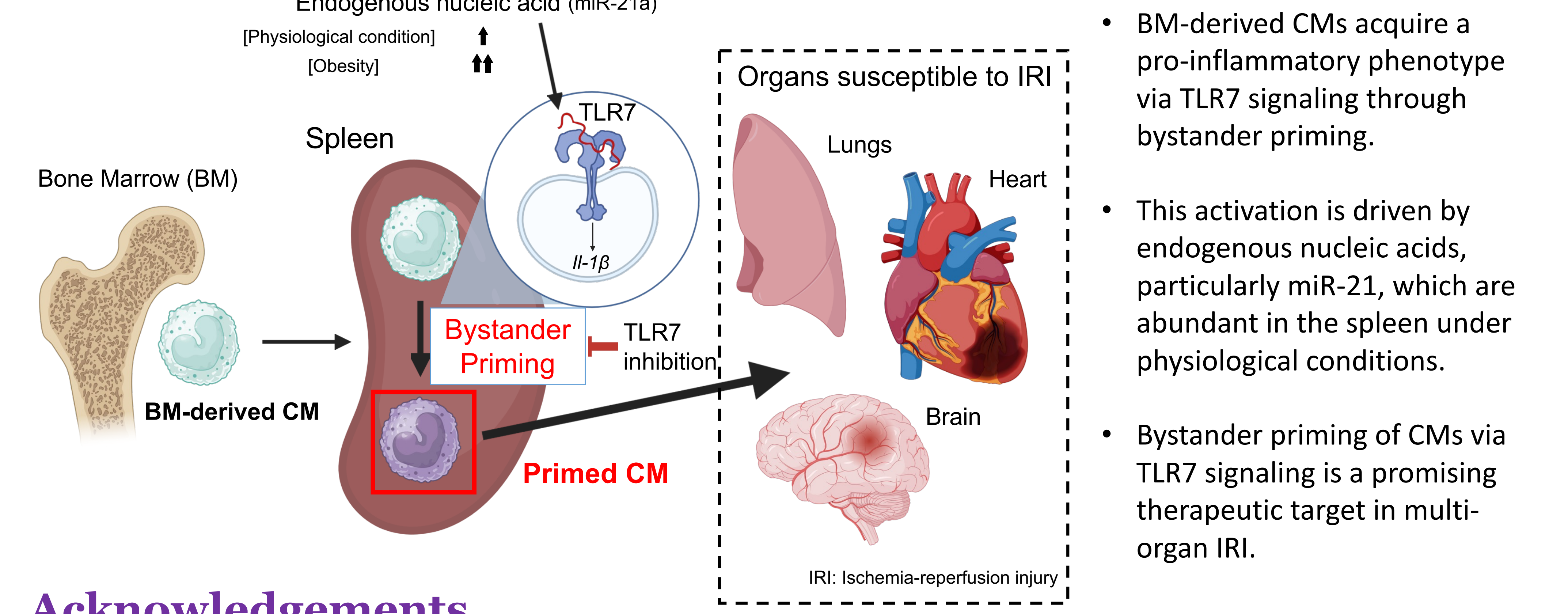
Results (continued)

Figure 4. Bystander-primed CM is a therapeutic target for mitigating IRI



(A-E) Effect of splenectomy in models of cardiac ischemic injury (B, n=5) and stroke (C, n=5) and reconstitution of CM in cardiac ischemic injury model (D-E, n=4-5). Infarct area or volume was evaluated by 2,3,5-triphenyltetrazolium chloride (TTC) staining. **(F-I)** Ischemic preconditioning (F-G, n=4-6) and genetic or pharmacological interventions (H-I, WT lean n=5-6; HFD obese n=4-5) in lung transplantation (LTx) model. The number of CM expressing pro-IL-18 and the percentage of lung-infiltrated neutrophils were quantified by flow cytometry. Data presented as Mean ± SEM. *, p<0.05; **, p<0.01; ***, p<0.001, ****, p<0.0001

Summary



Acknowledgements

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- BM-derived CMs acquire a pro-inflammatory phenotype via TLR7 signaling through bystander priming.
- This activation is driven by endogenous nucleic acids, particularly miR-21, which are abundant in the spleen under physiological conditions.
- Bystander priming of CMs via TLR7 signaling is a promising therapeutic target in multi-organ IRI.