Online supplemental material:

Myocardial infarction does not accelerate atherosclerosis in a mouse model of type 1 diabetes

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Supplemental table 1:Antibodies and key reagents

Antibody	Clone/product ID	Manufacturer	Dilution
CD45-FITC	30-F11	eBioscience	1:500 (0.1 μg/100 μl)
CD115-APC	AFS98	eBioscience	1:100 (0.2 μg/100 μl)
GR1-PE-Cy7	RB6-8C5	eBioscience	1:1000 (0.05μg/100 μl)
CD49D-PE (α4 integrin)	PS/2	Southern Biotech	1:1000 (0.01 μg/100 μl)
CD11B-PE	M1/70	eBioscience	1:500 (0.1 μg/100 μl)
F4/80-PE-Cy7	BM8	eBioscience	1:200 (0.2 μg/100 μl)
Viability dye e450	NA	eBioscience	1:1000
Mac-2	CL8942AP	Cedarlane	1 μg/ml
Fluoresbrite yellow green microspheres (latex particles)	1715210	Polysciences, Inc.	250 μl/mouse (diluted 1:4)
Alpha Smooth muscle actin	Ab5694	Abcam	0.2 μg/ml
IL1β	Ab9722	Abcam	1.25 μg/ml
TREM2	50-172-8776 (Fisher)	Proteintech (via Fisher)	3.7 μg/ml

Supplemental table 2: Primer sequences

Gene	Forward primer	Reverse primer
Rn18s	CATTAAATCAGTTATGGTTCCTTTGG	CCCGTCGGCATGTATTAGCT
Ccl2	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Il1b	GGGCTGCTTCCAAACCTTTG	TGATACTGCCTGCCTGAAGCTC
Tnfa	CCTGTAGCCCACGTCGTAG	GGGAGTAGACAAGGTACAACCC
Il6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC

Supplemental table 3: Leukocyte counts

Study	WBC ($x10^6$/ml)	Monocytes ($x10^6$/ml) %	Neutrophils ($x10^6$/ml) %
Sham vs MI, no BMT or prior fat feeding			
Sham, ND	5.8 ± 1.1 (3)	0.48 ± 0.1 (3) 8.8 ± 2.0	0.69 ± 0.07 (3) 12.7 ± 2.3
MI, ND	5.8 ± 0.5 (5)	0.44 ± 0.04 (5) 7.5 ± 0.12	0.78 ± 0.13 (5) 13.2 ± 1.8
Sham, D	7.4 ± 0.4 (4)	0.39 ± 0.06 (4) 4.4 ± 0.38*	1.5 ± 0.3 (4) 20.7 ± 4.9
MI, D	4.6 ± 0.5 (6)#	0.24 ± 0.03 (6)* 4.9 ± 0.56*	1.3 ± 0.12 (5) 30.1 ± 3.1*
Female (12 week HF+4 week diabetes), no BMT			
ND, female	6.2 ± 0.3 (13)	0.51 ± 0.07 (13) 8.1 ± 0.7	0.83 ± 0.08 (13) 13.6 ± 1.0
D, female	5.4 ± 0.5 (14)	0.36 ± 0.03 (14) 6.6 ± 0.5	1.22 ± 0.14 (14) 22.5 ± 1.5*
ND, male	6.7 ± 0.5 (12)	0.44 ± 0.04 (12) 6.5 ± 0.3	0.76 ± 0.3 (12) 11.4 ± 0.9
D, male	5.0 ± 0.5 (12)	0.32 ± 0.03 (12) 6.8 ± 0.2	1.18 ± 0.16 (12) 25.0 ± 2.2*
Female (LCMV control), no BMT			
ND, saline (blood glucose 7.8 mM)	4.6 ± 0.3 (5)	0.36 ± 0.05 (5) 7.8 ± 1.0	0.70 ± 0.05 (5) 16.3 ± 1.6
ND, LCMV (blood glucose 8.2 mM)	7.3 ± 0.7 (5)	0.36 ± 0.05 (5) 5.0 ± 0.4*	0.66 ± 0.08 (5) 9.0 ± 0.6*

*statistically significant ($p<0.05$) compared to ND (sham or saline), # statistically significant ($p<0.05$) compared to D (sham) (or opposite gender if applicable).

Figure S1. **Gating for blood monocytes/Ly6C^{hi} monocytes/Ly6C^{low} monocytes, labeled monocytes and neutrophils**. **A**. Blood cells were gated as shown. **B**. Example of monocytes and neutrophils on a pseudocolor plot.

Figure S2. Diabetes suppresses levels of white blood cells. A. Lymphocyte cell count at 3 weeks after induction of diabetes measuresed using a Hemavet® veterinary hematology system. **B**. Neutrophil count at 3 weeks using a Hemavet® veterinary hematology system. **C**. Total monocyte counts normalizing the data from the flow cytometry to total white blood cells counts. **D.** Spleen weights. N=12-19 (A-C) and N=9-12 (D). *p<0.05, **p<0.01, ***p<0.001 three-way ANOVA (WT vs KO, ND vs D, Sham vs MI as the parameters, or the interaction between these) and followed by Sidak's multiple comparison tests comparing groups that differ by one parameter (as indicated by lines with asterisks).

Figure S3. **Neutrophil, Ly6C^{hi} andLy6C^{low} monocyte integrin α4 expression. A.** Flow cytometric analysis of blood neutrophil integrin α4 expression at 7 days and at 3 weeks. **B**. Ly6C^{hi} monocyte integrin α4 expression at 7 days and at 3 weeks. **C**. Ly6C^{lo} monocyte integrin α4 expression at 7 days and at 3 weeks. N=14-22. *p<0.05, **p<0.01, ***p<0.001 three-way ANOVA (WT vs KO, ND vs D, Sham vs MI as the parameters, or the interaction between these) and followed by Sidak's multiple comparison tests comparing groups that differ by one parameter (as indicated by lines with asterisks). The differences between each WT and KO group are not indicated with lines in A (all **).

Figure S4. Neither diabetes nor MI alter lesion size. **A**. Representative images of aortic atherosclerosis stained *en face* with the lipophilic stain Sudan IV. **B**. Representative pictures of brachiocephalic artery (BCA) lesions stained with Movat's pentachrome stain. Arrows indicate necrotic cores. **C**. BCA lesion quantification. Data analyzed by three-way ANOVA (WT vs KO, ND vs D, Sham vs MI as the parameters, or the interaction between these) followed by Sidak's multiple comparison tests comparing groups that differ by one parameter. No statistical significance detected.

Figure S5. **Integrin α4-deficient reduces monocyte recruitment. A**. Monocytes were isolated from myeloid cell-selective (driven by LysM) deletion of integrin α4 and (KO; integrin $\alpha4^{MC-/-}$) bone marrow from the mice in the main study and allowed to adhere to unstimulated or TNFα-activated (20 ng/ml) mouse endothelium. N=11-12. **B**. Number and percentage of monocytes labeled with yellow-green (YG) latex beads, respectively. **C** LyC^{hi} and LyC^{lo} monocytes labeled with yellow-green (YG) latex beads, respectively, expressed as % of the population. **D**. YG-monocyte recruitment to the brachiocephalic artery (BCA) normalized to monocyte labeling efficiency. **E**. Mac-2 and alpha-smooth muscle (α-SM) actin immunohistochemistry with negative controls, rat IgG and rabbit IgG, respectively. **F**. Sinus α-SM, expressed as % of lesion. **G**. Picrosirius red staining, expressed as % of lesion. *p<0.05, **p<0.01, ***p<0.001 three-way ANOVA (WT vs KO, ND vs D, Sham vs MI as the parameters, or the interaction between these) followed by Sidak's multiple comparison tests comparing groups that differ by one parameter (as indicated by lines with asterisks).

Figure S6. Diabetes increases macrophage cell death. LCMV (lymphocytic choriomeningitis virus) was used to induce diabetes (D), or saline (non-diabetic; ND), in female *Ldlr*$^{-/-}$*;Gp*^{Tg} mice. 2 weeks post LCMV injection mice were subjected to permanent ligation of the left anterior descending coronary artery (experimental MI) or a sham surgery and maintained for an additional 3.5 weeks. Mice were maintained on a low-fat semipurified diet from the onset of diabetes. Thioglycolate-elicited macrophages were isolated 4 days after thioglycolate administration (n=3-6). **A**. Total cellular cholesterol. **B**. Cholesteryl esters. **C**. Viability dye-positive (dead) within the macrophage population (F4/80 and CD11B-positive) in the thioglycolate-elicited cells. **D**. Viability dye-positive (dead) within the splenic macrophage population (F4/80 and CD11B-positive). *p<0.05, **p<0.01, ***p<0.001, two-way ANOVA followed by Tukey's multiple comparison tests, indicated by lines with asterisks.

Figure S7. Diabetes increases necrotic core size. A. Examples of necrotic cores in aortic sinus (top) and brachiocephalic artery (BCA; bottom) with necrotic cores traced with black dashed lines. **B**. Aortic sinus necrotic cores expressed as μm^2. **C**. BCA necrotic cores expressed as μm^2. N=14-21, *p<0.05, **p<0.01, ***p<0.001 three-way ANOVA (WT vs KO, ND vs D, Sham vs MI as the parameters, or the interaction between these) and followed by Sidak's multiple comparison tests comparing groups that differ by one parameter (as indicated by lines with asterisks).

Figure S8. Macrophage gating strategy. Gating strategy for identifying viability dye-positive (dead) resident peritoneal macrophages within the macrophage population (F4/80 and CD11B-positive).