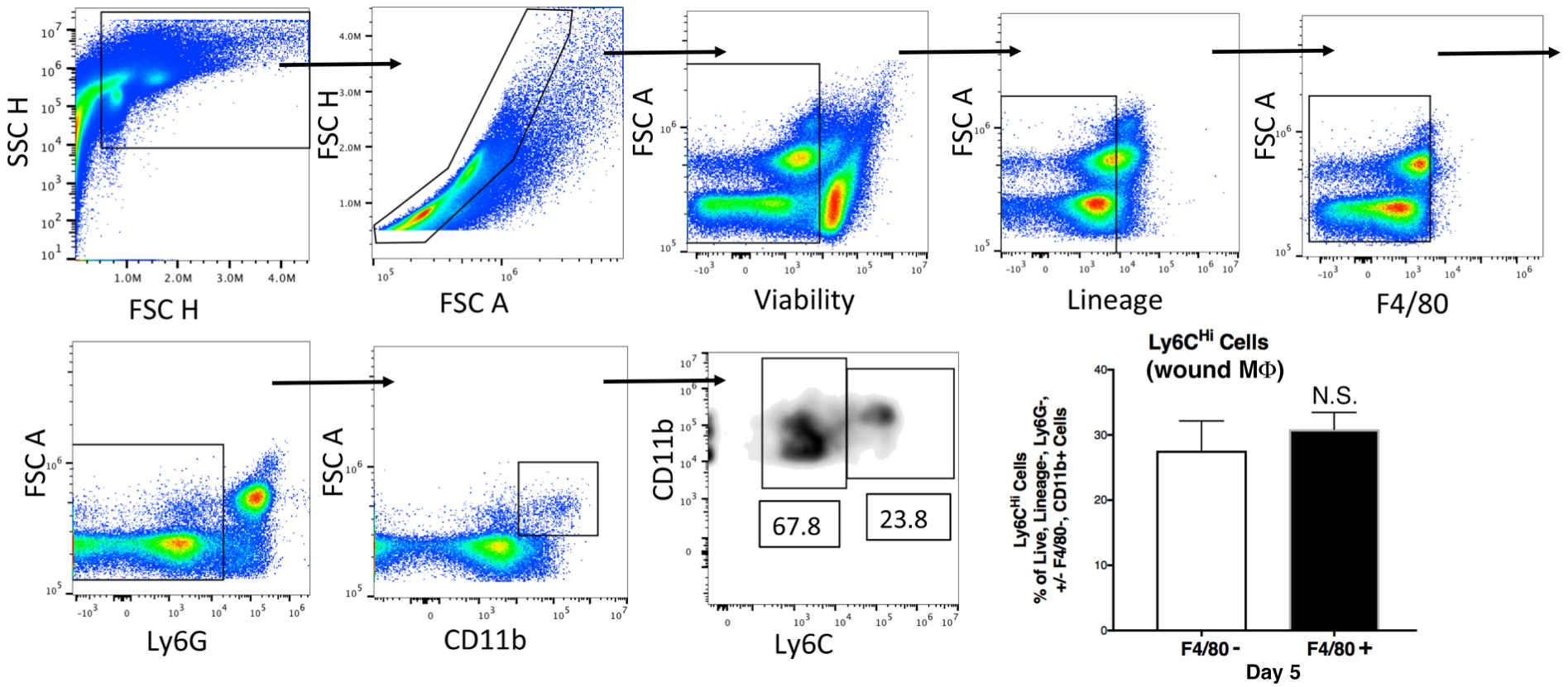
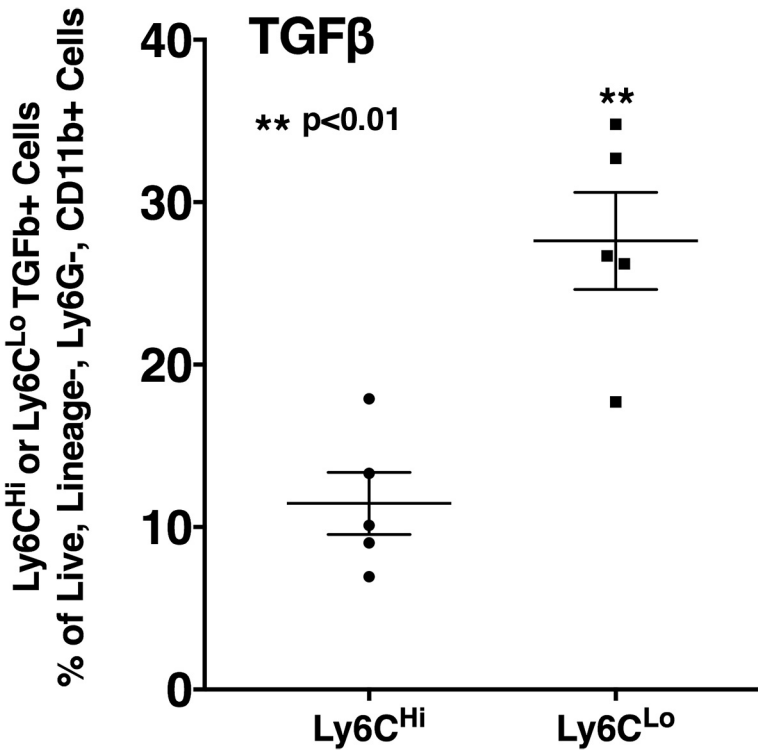
Supplemental Material

Supplemental Fig I



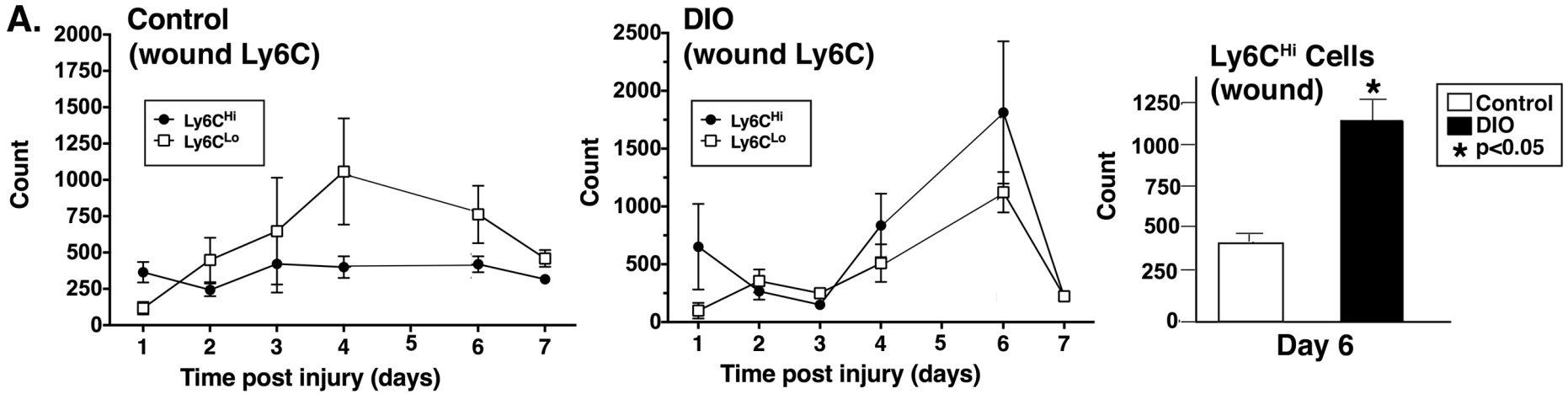
Supplemental Figure I. Gating with wound resident macrophage marker F/4/80^{+} does not impact infiltrating CD11b^{+}Ly6C^{Hi} cell percentages in wounds. Wounds were created using a 4mm punch biopsy on the back of male C57BL/6 mice. Wounds were harvested on day 5 post-injury and analyzed by flow cytometry. Gating strategy to select single, live, lin^{-},Ly6G^{-}, CD11b^{+}, Ly6C^{Hi} cells and stratify based on F4/80^{+} vs. F4/80^{-}. (n =10 mice; tissues of 2 wounds per mouse were pooled for a single biological replicate. Data is representative of 2 independent experiments.) All data are expressed as the mean +/- the standard error of the means (SEM).

Supplemental Fig II



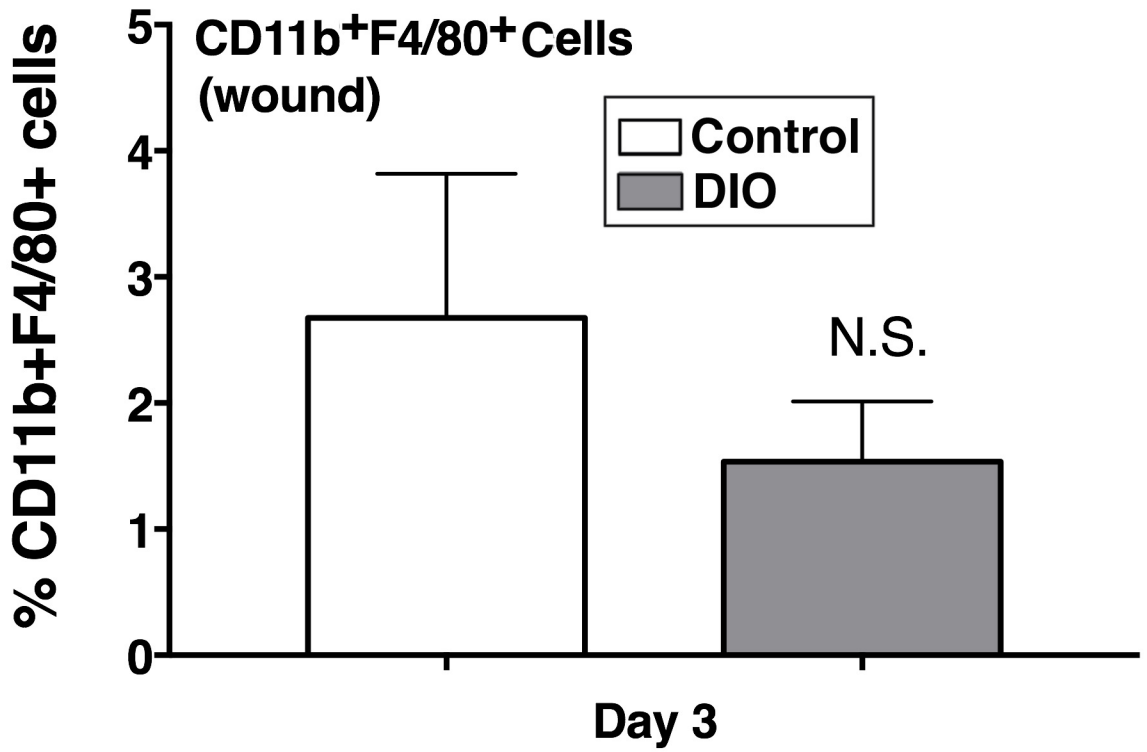
Supplemental Figure II. Wound CD11b^{+}Ly6C^{Lo} monocyte/macrophages produce more TGFβ than wound CD11b^{+}Ly6C^{Hi} cells. Wounds from C57BL/6 mice were collected on post-injury day 2 for cell isolation, *ex vivo* stimulation, and intra-cellular staining for flow cytometry. Percentage of single, live, lineage^{-} [CD3, CD19, NK1.1, Ter-119]$^{-}$, Ly6G^{-}, CD11b^{+}, Ly6C^{Hi} and Ly6C^{Lo} cells staining positive for TGFβ. (****P < 0.001;* n =15 mice; tissues of 2 wounds per mouse were pooled for a single biological replicate. Data is representative of 3 independent experiments.) All data are expressed as mean +/- the standard error of the means (SEM).

Supplemental Fig III

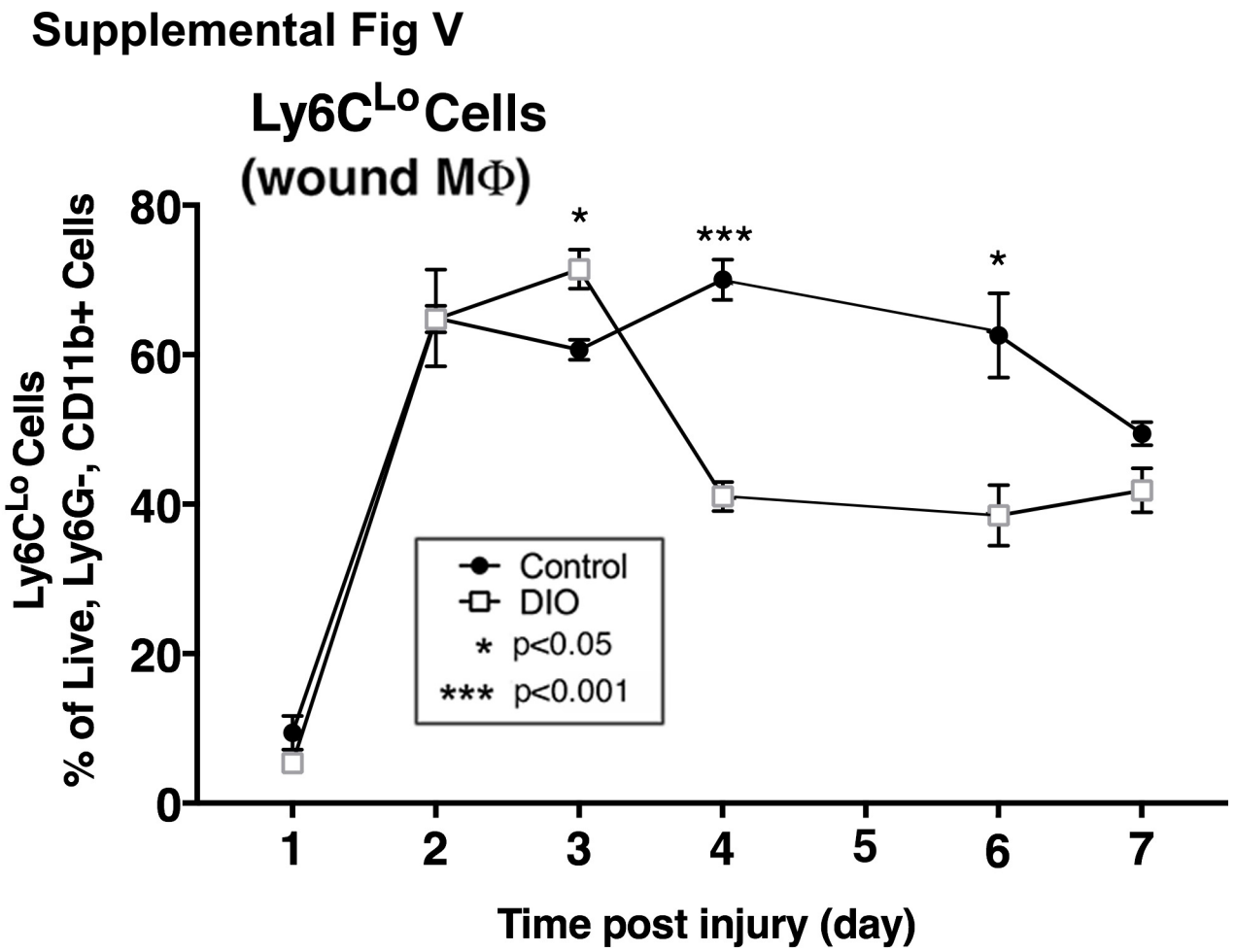


Supplemental Figure III. Diabetic and control wounds display similar numbers of CD11b$^+$ cells, but altered proportions of Ly6C^{Hi}[Live, Ly6G$^-$,CD11b$^+$] and Ly6C^{Lo}[Live, Ly6G$^-$,CD11b$^+$] cells at late time points. Wounds were created using a 4mm punch biopsy on the back of male C57BL/6 normal diet/control and high-fat diet (HFD)/DIO mice. Wounds were harvested on days 1-7 post-injury and analyzed by flow cytometry. (A) Live, lin$^-$, Ly6G, CD11b$^+$ cell counts in DIO and control wounds based on Ly6C^{Hi} or Ly6C^{Lo} plotted for 7 days post-wounding. (B) Representative plot of live, lin$^-$, Ly6G, CD11b$^+$ cell counts in DIO and control wounds. All days post-wounding demonstrate no change in live, lin$^-$, Ly6G, CD11b$^+$ cell counts (data not shown). (*$P < 0.05$;* n =30 mice; tissues of 2 wounds per mouse were pooled for a single biological replicate. Data is representative of 3 independent experiments.) All data are expressed as the mean +/- the standard error of the means (SEM).

Supplemental Fig IV

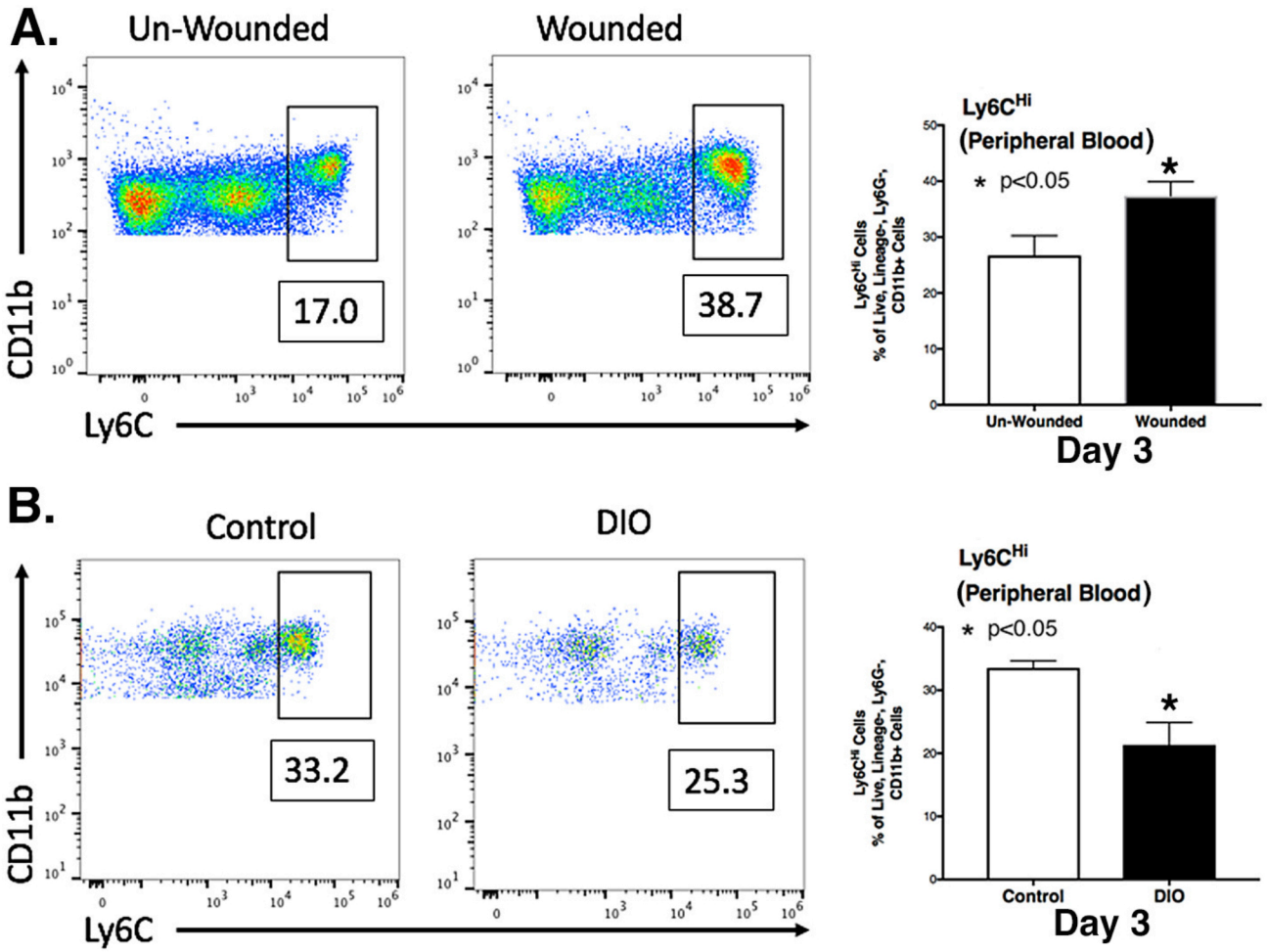


Supplemental Figure IV. Percentage of wound resident macrophages ($CD11b^+/F/4/80^+$) are similar between diabetic and control wounds. Wounds were created using a 4mm punch biopsy on the back of male C57BL/6 normal diet/control and high-fat diet (HFD)/DIO mice. Wounds were harvested on day 3 post-injury and analyzed by flow cytometry. Gating strategy to select single, live, lin^-,$Ly6G^-$, $CD11b^+$, $F4/80^+$ cells. (n =10 mice; tissues of 2 wounds per mouse were pooled for a single biological replicate. Data is representative of 3 independent experiments.) All data are expressed as the mean +/- the standard error of the means (SEM).



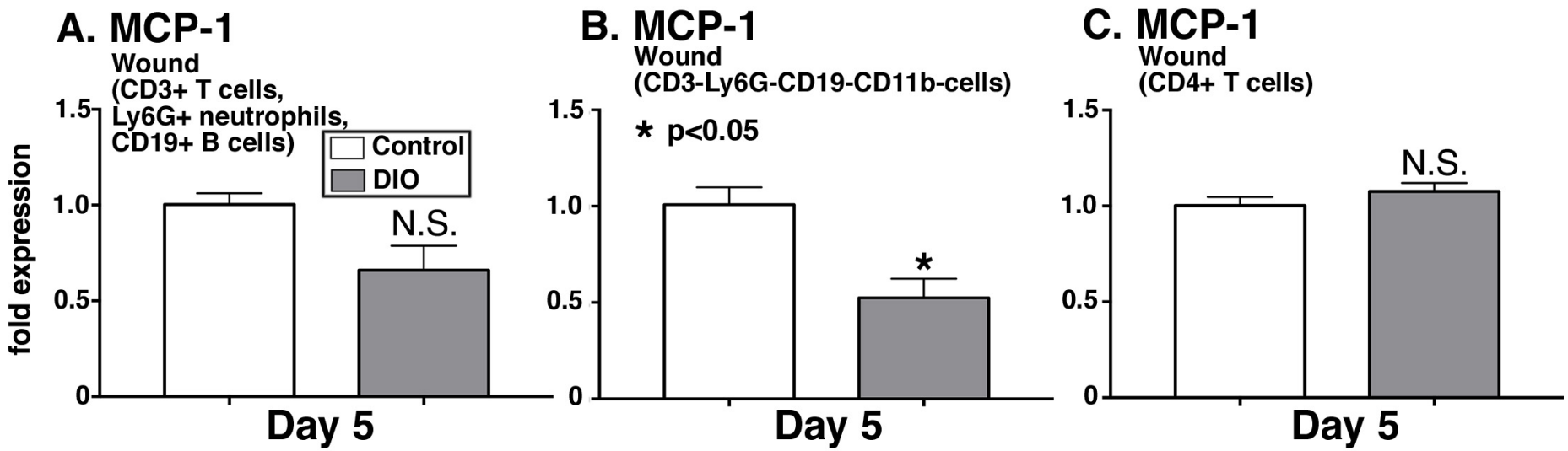
Supplemental Figure V. Ly6C^{Lo}[Live, Ly6G^{-},CD11b^{+}] cells are increased at late time points in control compared to DIO wounds. Wounds were created using a 4mm punch biopsy on the back of male C57BL/6 normal diet/control and high-fat diet (HFD)/DIO mice. Wounds were harvested on days 1-7 post-injury and analyzed by flow cytometry. The percentage of Ly6C^{Lo}[Live, Ly6G^{-},CD11b^{+}] cells in DIO and control wounds were plotted over time. (*P < 0.05, ****P < 0.001;* n =30 mice; tissues of 2 wounds per mouse were pooled for a single biological replicate. Data is representative of 2 independent experiments.) All data are expressed as the mean +/- the standard error of the means (SEM).

Supplemental Fig VI



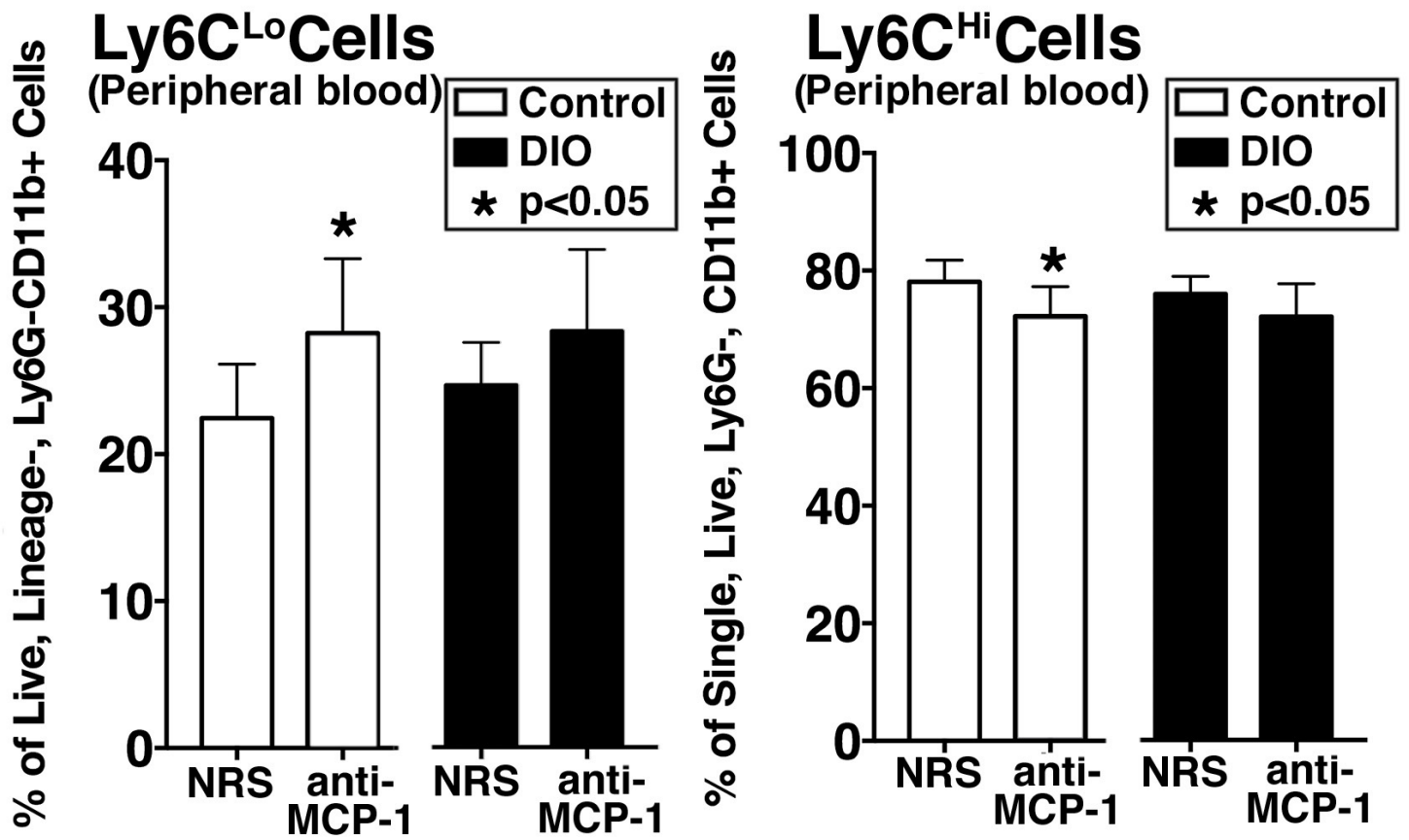
Supplemental Figure VI. Peripheral blood Ly6C^{Hi}[Lin$^-$ Ly6G$^-$CD11b$^+$] cells are decreased in diabetic mice during the inflammatory phase of healing. C57BL/6 mice were either anesthetized alone or anesthetized and wounded and peripheral blood was collected on post-injury day 3 for flow cytometry. (A) Representative flow plots of peripheral blood Ly6C^{Hi}[Lin$^-$Ly6G$^-$CD11b$^+$] cells in wounded and un-wounded control mice on post-injury day 3 and comparison of percentage of CD11b$^+$ cells. C57BL/6 mice were fed high fat chow (60%kCal) for 12-16 weeks to induce obesity and insulin resistance/glucose intolerance in the diet-induced obese model (DIO) of physiologic type 2 diabetes. Peripheral blood was collected on post-injury day 3, to correspond with the second influx of Ly6C^{Hi}[Lin$^-$ Ly6G$^-$CD11b$^+$] cells in DIO wounds, and analyzed by flow cytometry. (B) Representative flow plots and comparison of DIO and control Ly6C^{Hi}[Lin$^-$Ly6G$^-$ CD11b$^+$] peripheral blood cells on day 3 (*P < 0.05; n = 10 mice. Data is representative of 2 experiments). All data are expressed as mean +/- the standard error of the mean (SEM).

Supplemental Fig VII



Supplemental Figure VII. MCP-1 expression is similar in diabetic and control wound lymphocytes, neutrophils, T cells, B cells and structural cells. C57BL/6 mice were fed high fat chow (60%kCal) for 12-16 weeks to induce obesity and insulin resistance/glucose intolerance in the diet-induced obese model (DIO) of physiologic type 2 diabetes. Wounds were harvested on day 5 and neutrophils (Ly6G+), lymphocytes (CD3+), T cells (CD4+), B cells (CD19+) and structural cells CD11b-CD3-CD19-Ly6G- were isolated using magnetic-activated cell sorting (MACs). MCP-1 gene expression was compared between DIO and control wound cells (n = 5, repeated one time). All data are expressed as mean +/- the standard error of the means (SEM).

Supplemental Fig VIII



Supplemental Figure VIII. Peripheral blood Ly6C^{Hi}[Live, Ly6G^{-},CD11b^{+}] cells are decreased and Ly6C^{Lo}[Live, Ly6G^{-},CD11b^{+}] cells increased following anti-MCP1 injection. C57BL/6 mice were fed high fat chow (60%kCal) for 12-16 weeks to induce obesity and insulin resistance/glucose intolerance in the diet-induced obese model (DIO) of physiologic type 2 diabetes. Peripheral blood was collected on post-injury day 5, to correspond with the second influx of Ly6C^{Hi}[Live, Ly6G^{-},CD11b^{+}] cells in DIO wounds, and analyzed by flow cytometry. Ly6C^{Hi}[Live, Ly6G^{-},CD11b^{+}] and Ly6C^{Lo}[Live, Ly6G^{-},CD11b^{+}] peripheral blood cells were plotted in DIO and control mice on day 5 post-wounding. (*P <0.05; n = 10 mice; tissues of 2 wounds per mouse were pooled for a single biological replicate. Data is representative of 2 independent experiments.) All data are expressed as mean +/- the standard error of the means (SEM).

Supplemental Table I

Major Resources Tables

Animals

Species/Strain	Vendor or Source	Background Strain	Sex
Wild-type	Jackson Laboratory, Bar Harbor, ME	C57BL/6	Male
mT/mG mice (*Gt(ROSA)26Sor*$^{tm4(ACTB-tdTomato,-EGFP)Luo/J}$)	Jackson Laboratory, Bar Harbor, ME	C57BL/6	Male

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration
Anti-CD16/32	BioXCell	CUS-HB-197	1:200 dilution
Anti-CD3	Biolegend	100304	1:400 dilution
Anti-CD19	Biolegend	115504	1:400 dilution
Anti-Ter-119	Biolegend	116204	1:400 dilution
Anti-NK1.1	Biolegend	108704	1:400 dilution
Anti-Ly6G	Biolegend	127604	1:400 dilution
Anti-CD11b	Biolegend	101230	1:400 dilution
Anti-Ly6C	Biolegend	128035	1:400 dilution
Anti-F4/80	Biolegend	123121	1:400 dilution
Streptavidin	Biolegend	405208	1:1,000 dilution
Anti-IL1β	eBioscience	25-7114-82	1:200 dilution
Anti-TNFα	Biolegend	506308	1:200 dilution

Supplemental Table I.
List of major experimental resources and associated vendors.

Supplemental Table II

Ly6C^{Lo} down in HFD

	logFC	p value	FDR
Zfp963	3.3	0.003256	0.999133332
Hist1h2bb	3.6	0.020722	1
Col14a1	3.7	0.059306	1
8030453O22Rik	3.8	0.006858	1
Gm42879	3.8	0.003071	0.999133332
Xlr	4.0	0.004173	1
Gm10643	4.1	0.000839	0.498757072
Kif20b	5.2	0.000005	0.029665105

Ly6C^{Lo} up in HFD

	logFC	p value	FDR
Gm1821	-6.1	0.000496872	0.483046771
Gm13238	-4.5	0.000602217	0.493664509
Krt7	-4.5	0.000977739	0.530699893
Egfr	-3.6	0.003281357	0.999133332
Ugt1a2	-3.4	0.005386876	1
Gm11956	-3.4	0.00282438	0.985356972
Gm26415	-3.2	0.002590899	0.980145091
Lox	-3.2	0.008640524	1
Adm	-3.2	0.0391392	1
Rufy4	-3.0	0.019886325	1
Tvp23a	-3.0	0.01230331	1
Ska3	-2.9	0.007597023	1
Gm37490	-2.9	0.010837675	1
Gm45244	-2.8	0.051980692	1
Sycp2	-2.8	0.028037552	1
Col3a1	-2.8	0.009503922	1
Gm25613	-2.8	0.002781079	0.985356972
Gm43924	-2.8	0.00223108	0.928426824
Gm5483	-2.8	0.018366815	1
4933433G15Rik	-2.7	0.001209824	0.580901837
Chil1	-2.7	0.019059383	1
Gm22478	-2.7	0.056445751	1
A730017L22Rik	-2.6	0.003542641	1
Il23a	-2.5	2.92E-06	0.029665105

Ly6C^{Hi} down in HFD

	logFC	p value	FDR
Cd163	2.5	0.033831588	1
Cd5l	2.5	0.204814934	1
Hbb-bs	2.8	0.031606452	1
Prg4	3.0	0.000583966	0.560786763
Cd300ld2	3.2	0.004771237	1
Lyve1	3.6	0.061650162	1
Sparc	4.4	0.015034378	1
Col3a1	5.5	6.82E-06	0.028364945
Col1a1	5.6	1.37E-05	0.042774789
Gm1821	5.8	0.000777029	0.692888023

Ly6C^{Hi} up in HFD

	logFC	p value	FDR
Ltf	-5.6	3.10E-06	0.025536425
Krt7	-5.4	0.001084455	0.846146287
Aox3	-5.4	0.031953751	1
Gm37949	-5.3	0.00026188	0.412374168
Gm17233	-5.0	0.012716922	1
Gm43787	-4.5	0.006127952	1
Gbp4	-4.2	0.000270884	0.412374168
Cecr6	-4.0	0.010596233	1
Ngp	-4.0	4.14E-05	0.103386371
Grin3a	-3.9	0.017373863	1
Boc	-3.9	0.003593002	1
Gm10612	-3.7	0.013121808	1
Gm14212	-3.7	0.00017858	0.371565154
9130230N09Rik	-3.7	0.001776599	1
Zfp229	-3.6	0.000992756	0.826237643
Ms4a4b	-3.6	0.004683427	1
Tktl1	-3.6	0.006588717	1
Zfp760	-3.4	0.002508534	1
Gm37745	-3.3	0.021465555	1
Gm38020	-3.3	0.017924297	1
Gm37655	-3.3	0.039229686	1
Sgo1	-3.2	0.001601303	1
Gm13785	-3.2	0.013531473	1
Gm43961	-3.2	0.008867773	1
Angptl3	-3.2	0.004600298	1
Ugt1a6a	-3.1	0.006962539	1
Gm23479	-3.1	0.014062972	1
Capn11	-3.1	0.030575049	1
Gm37706	-3.1	0.064605987	1
AW146154	-3.0	0.004871876	1
Lrrc63	-3.0	0.00029729	0.412374168
A630072L19Rik	-3.0	0.034535499	1
Gm44237	-3.0	0.013400721	1
Gm43145	-3.0	0.010217434	1
Gm42701	-2.9	0.020805277	1
Prr33	-2.9	0.019196396	1
Gm13146	-2.9	0.013396669	1
Gm26530	-2.9	0.052921919	1
Hpgd	-2.8	0.019591682	1
Gm14984	-2.7	0.006645571	1
Gen1	-2.7	0.004783536	1
Ptgs2os	-2.7	0.026641216	1
Il12a	-2.6	0.033812844	1
Cep72	-2.6	0.016313818	1
Ppl	-2.6	0.009891927	1
Mast4	-2.6	0.014963024	1
Gm6542	-2.6	0.011599255	1
Mest	-2.5	0.044752346	1
BC035044	-2.5	0.09639372	1

Supplemental Table II.
Comparison was made between DIO and control wound Ly6C^{Hi}[Live,Ly6G,CD11b^{+}] and Ly6C^{Lo}[Live, Ly6G^{-},CD11b^{+}] cells. Differential gene expression was determined in edgeR using the common dispersion coefficient. Due to a low number of differentially expressed genes between groups, genes with a fold change of 2.5 or higher and p value of <0.05 are shown.