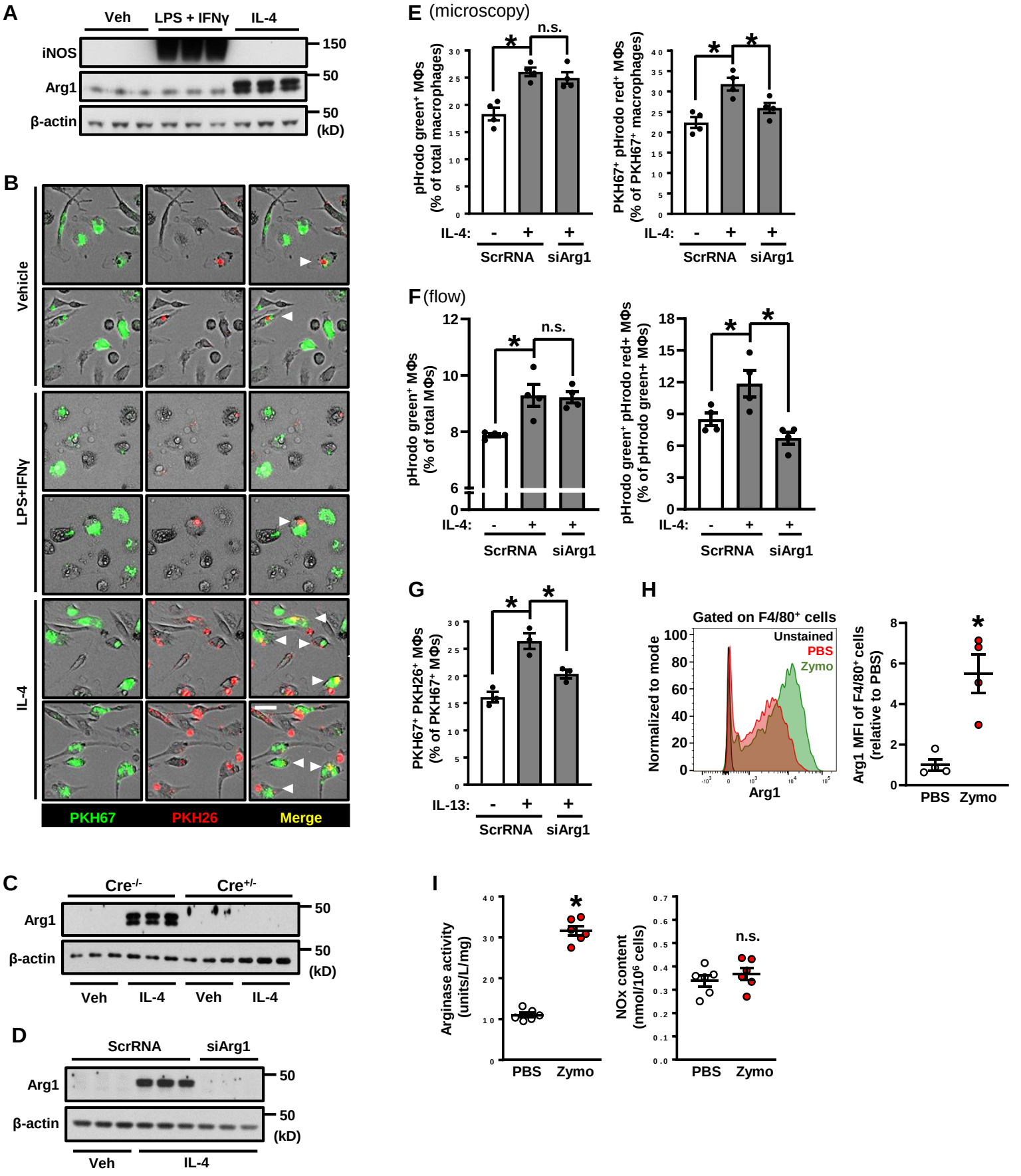
Supplemental Table 1. Amino Acid and Acylcarnitine Levels in AC$^-$ and AC$^+$ Macrophages, Related to Figure 1. Metabolite values from Figure 1A. Amino acid and acylcarnitine levels in AC$^-$ macrophages, AC$^+$ macrophages, ACs alone, and macrophages alone (*i.e.*, macrophages not incubated with ACs) are shown. Leu/Ile are reported as isobaric species and Glx and Asx are the totals of Glu/Gln and Asp/Asn, respectively. Values are means ± S.E.M.; n = 3 biological replicates.

nmol/mg of cell protein	ACs alone		MΦs alone		Cells sorted into AC$^-$ and AC+ MΦs						
					AC$^-$ MΦs		AC$^+$ MΦs		Fold Change (AC$^+$/AC$^-$ MΦs)		*P* value
	Average	± SEM	Average	± SEM	Average	± SEM	Average	± SEM	Average	± SEM	
Arginine	6.12	0.07	2.36	0.20	1.79	0.12	6.07	0.98	3.4	0.55	0.01
Ornithine	1.27	0.03	0.26	0.03	0.21	0.01	0.62	0.09	2.9	0.42	0.01
Proline	4.57	0.27	0.90	0.14	0.69	0.07	1.80	0.45	2.6	0.65	0.07
Histidine	1.97	0.15	1.71	0.22	1.24	0.01	2.63	0.64	2.1	0.52	0.09
Phenylalanine	4.60	0.17	4.66	0.42	3.71	0.45	7.55	1.53	2.0	0.41	0.07
Valine	5.09	0.14	4.98	0.57	3.72	0.11	7.61	1.39	2.0	0.37	0.05
Citrulline	0.13	0.003	0.12	0.04	0.12	0.028	0.24	0.03	2.0	0.24	0.04
Tyrosine	4.95	0.39	3.96	0.34	3.44	0.18	7.00	1.52	2.0	0.44	0.08
Methionine	2.16	0.14	2.42	0.25	1.99	0.25	3.70	0.71	1.9	0.36	0.09
Leu/Ile	8.70	0.29	11.7	0.72	8.95	0.88	16.7	4.01	1.9	0.45	0.13
Alanine	10.2	0.45	6.31	0.54	4.11	0.25	7.31	1.76	1.8	0.43	0.15
Glycine	20.4	0.14	14.8	1.21	12.1	1.59	17.9	5.25	1.5	0.43	0.35
Glx	73.5	4.85	49.6	4.90	42.6	1.87	60.3	13.3	1.4	0.31	0.26
Asx	21.8	0.67	20.1	2.11	16.1	0.69	21.4	4.4	1.3	0.27	0.30
Serine	18.9	1.28	22.9	2.75	20.5	1.65	22.9	3.87	1.1	0.19	0.60
FC	2.45	0.05	12.9	1.08	11.2	0.37	14.9	3.43	1.3	0.31	0.34
C2	2.16	0.24	5.82	0.56	4.34	0.18	4.61	1.04	1.1	0.24	0.81
C3	0.17	0.01	1.11	0.13	0.71	0.07	0.52	0.11	0.7	0.16	0.22
C4	0.040	0.0005	0.063	0.011	0.054	0.008	0.043	0.011	0.8	0.2	0.46
C5:1	0.006	0.0004	0.011	0.003	0.009	0.002	0.008	0.002	0.9	0.18	0.8
C5	0.035	0.0066	0.106	0.008	0.080	0.004	0.060	0.007	0.8	0.09	0.08
C4-OH	0.019	0.0010	0.027	0.003	0.018	0.001	0.017	0.003	1.0	0.18	0.81
C6	0.0033	0.00056	0.014	0.001	0.010	0.002	0.013	0.004	1.3	0.34	0.5
C5-OH	0.0035	0.00040	0.013	0.001	0.013	0.0004	0.010	0.0017	0.7	0.13	0.12
C3-DC	0.0019	0.00044	0.011	0.0001	0.009	0.0009	0.007	0.0009	33	0.1	0.17
C4-DC	0.0015	0.00022	0.017	0.0025	0.014	0.0022	0.014	0.0032	1.0	0.23	0.95
C8:1	0.0010	0.00032	0.001	0.0006	0.002	0.0004	0.0014	0.0004	0.8	0.21	0.63
C8	0.0023	0.00033	0.0099	0.0017	0.0042	0.0011	0.0060	0.0007	1.4	0.17	0.26
C5-DC	0.0022	0.00027	0.0043	0.0015	0.0033	0.0007	0.0043	0.0009	1.3	0.28	0.48
C8:1-OH/C6:1-DC	0.0018	0.00027	0.0015	0.0006	0.0025	0.0009	0.0015	0.0003	0.6	0.13	0.33
C8-OH/C6-DC	0.0025	0.00044	0.0066	0.0012	0.0059	0.0002	0.0045	0.0002	0.8	0.04	0.01
C10:3	0.00093	0.00036	0.0084	0.0013	0.0056	0.0006	0.0076	0.0015	1.4	0.27	0.29
C10:2	0.00050	0.00026	0.0008	0.0003	0.0005	0.0002	0.0006	0.0004	1.3	0.86	0.81
C10:1	0.00132	0.00017	0.0024	0.0001	0.0021	0.0004	0.0028	0.0003	1.4	0.16	0.2
C10	0.00083	0.00056	0.0034	0.0019	0.0025	0.0015	0.0006	0.0006	0.2	0.25	0.32
C8:1-DC	0.00190	0.00045	0.0022	0.0006	0.0018	0.0005	0.0021	0.0005	1.2	0.3	0.7
C8-DC	0.00121	0.00024	0.0027	0.0007	0.0024	0.0003	0.0028	0.0001	1.2	0.06	0.32
C12:1	0.00091	0.00020	0.0035	0.0007	0.0019	0.0005	0.0029	0.0010	1.5	0.51	0.43
C12	0.00174	0.00031	0.0080	0.0018	0.0064	0.0006	0.0063	0.0011	1.0	0.18	0.93
C12-OH/C10-DC	0.00050	0.00013	0.0014	0.0003	0.0013	0.00016	0.0009	0.00012	0.7	0.09	0.08
C14:2	0.00150	0.00074	0.0018	0.0003	0.0023	0.00057	0.0024	0.00040	1.1	0.17	0.86
C14:1	0.00403	0.00039	0.0137	0.0022	0.0150	0.00185	0.0130	0.00153	0.9	0.1	0.43
C14	0.00679	0.00046	0.0257	0.0019	0.0184	0.00078	0.0176	0.00382	1.0	0.21	0.84
C14:1-OH	0.00197	0.00059	0.0071	0.0002	0.0062	0.00044	0.0053	0.00109	0.9	0.18	0.49
C14-OH/C12-DC	0.00089	0.00002	0.0024	0.0006	0.0034	0.00032	0.0026	0.00032	0.8	0.09	0.15
C16:2	0.00106	0.00024	0.0033	0.0005	0.0044	0.00164	0.0032	0.00149	0.7	0.34	0.64
C16:1	0.00697	0.00133	0.0143	0.0029	0.0109	0.00202	0.0123	0.00221	1.1	0.2	0.67
C16	0.02709	0.00128	0.0670	0.0066	0.0399	0.00334	0.0587	0.01134	1.5	0.28	0.19
C16:1-OH/C14:1-DC	0.00193	0.00106	0.0056	0.0004	0.0040	0.00012	0.0038	0.00049	0.9	0.12	0.64
C16-OH	0.00232	0.00009	0.0018	0.0010	0.0029	0.00025	0.0021	0.00027	0.7	0.09	0.1
C18:2	0.00232	0.00054	0.0067	0.0006	0.0042	0.00058	0.0057	0.00229	1.3	0.54	0.57
C18:1	0.02881	0.00209	0.0479	0.0069	0.0355	0.00257	0.0475	0.00944	1.3	0.27	0.29
C18	0.01076	0.00107	0.0237	0.0028	0.0187	0.00061	0.0238	0.00553	1.3	0.3	0.42
C18:2-OH	0.00138	0.00056	0.0028	0.0015	0.0020	0.00058	0.0038	0.00214	1.9	1.05	0.48
C18:1-OH/C16:1-DC	0.00177	0.00039	0.0032	0.0007	0.0029	0.00026	0.0025	0.00073	0.9	0.25	0.66
C18-OH/C16-DC	0.00189	0.00023	0.0032	0.0004	0.0029	0.00058	0.0038	0.00104	1.3	0.35	0.5
C20:4	0.00242	0.00027	0.0231	0.0025	0.0163	0.00179	0.0124	0.00317	0.8	0.19	0.34
C20	0.00116	0.000448	0.0020	0.0006	0.0015	0.00049	0.0023	0.00079	1.5	0.52	0.46
C18:1-DC	0.00157	0.000238	0.0011	0.0004	0.0001	0.00025	0.0008	0.00027	0.8	0.27	0.62
C18-DC/C20-OH	0.00106	0.000095	0.0014	0.0006	0.0015	0.00049	0.0013	0.00038	0.8	0.25	0.69
C22	0.00079	0.000419	0.0016	0.0002	0.0020	0.00019	0.0022	0.00060	1.1	0.3	0.75

Supplemental Table 2. Systemic Parameters in Myeloid-Arg1 WT and KO Mice During Atherosclerosis Progression and Regression, Related to Figure 2. Plasma cholesterol, fasting blood glucose, body weight, complete blood counts, and circulating Ly6C^{high} and Ly6C^{low} monocytes profiles from atherosclerosis progression and regression mice. Values are means ± S.E.M.; n.s., not significant; n = 15-20 mice per group.

Systemic Parameter	Arg1$^{fl/fl}$ Cre$^{-/-}$ (Progr)	Arg1$^{fl/fl}$ Cre$^{+/-}$ (Progr)	*P value*	Arg1$^{fl/fl}$ Cre$^{-/-}$ (Regr)	Arg1$^{fl/fl}$ Cre$^{+/-}$ (Regr)	*P value*
Total cholesterol (mg/dL)	**1104 ± 50.7**	**1124 ± 61.7**	**n.s.**	**110.1 ± 12.0**	**120.7 ± 11.5**	**n.s.**
Blood glucose (mg/dL)	**137.4 ± 5.4**	**138.3 ± 5.5**	**n.s.**	**122.2 ± 3.4**	**120.5 ± 3.6**	**n.s.**
Body weight (grams)	**32.8 ± 1.4**	**34.6 ± 1.5**	**n.s.**	**28.6 ± 0.8**	**27.4 ± 0.7**	**n.s.**
Leukocytes (x10^3/μL)	**9.33 ± 0.8**	**7.28 ± 0.8**	**n.s.**	**8.66 ± 1.0**	**9.63 ± 0.7**	**n.s.**
Neutrophils (x10^3/μL)	**3.16 ± 0.29**	**2.56 ± 0.29**	**n.s.**	**1.80 ± 0.23**	**2..21 ± 0.15**	**n.s.**
Lymphocytes (x10^3/μL)	**5.51 ± 0.57**	**4.24 ± 0.57**	**n.s.**	**7.74 ± 1.20**	**8.01 ± 0.79**	**n.s.**
Monocytes (x10^3/μL)	**0.50 ± 0.03**	**0.40 ± 0.04**	**n.s.**	**0.67 ± 0.08**	**0.67 ± 0.06**	**n.s.**
Eosinophils (x10^3/μL)	**0.06 ± 0.009**	**0.06 ± 0.013**	**n.s.**	**0.04 ± 0.010**	**0.05 ± 0.008**	**n.s.**
Basophils (x10^3/μL)	**0.020 ± 0.004**	**0.021 ± 0.005**	**n.s.**	**0.016 ± 0.007**	**0.012 ± 0.002**	**n.s.**
Ly6C^{high} monocytes (% of CD115$^+$ monocytes)	**46.4 ± 2.9**	**49.8 ± 3.5**	**n.s.**	**41.3 ± 2.5**	**41.2 ± 2.7**	**n.s.**
Ly6C^{low} monocytes (% of CD115$^+$ monocytes)	**11.7 ± 0.84**	**12.2 ± 0.77**	**n.s.**	**17.5 ± 0.87**	**17.3 ± 1.2**	**n.s.**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Veh
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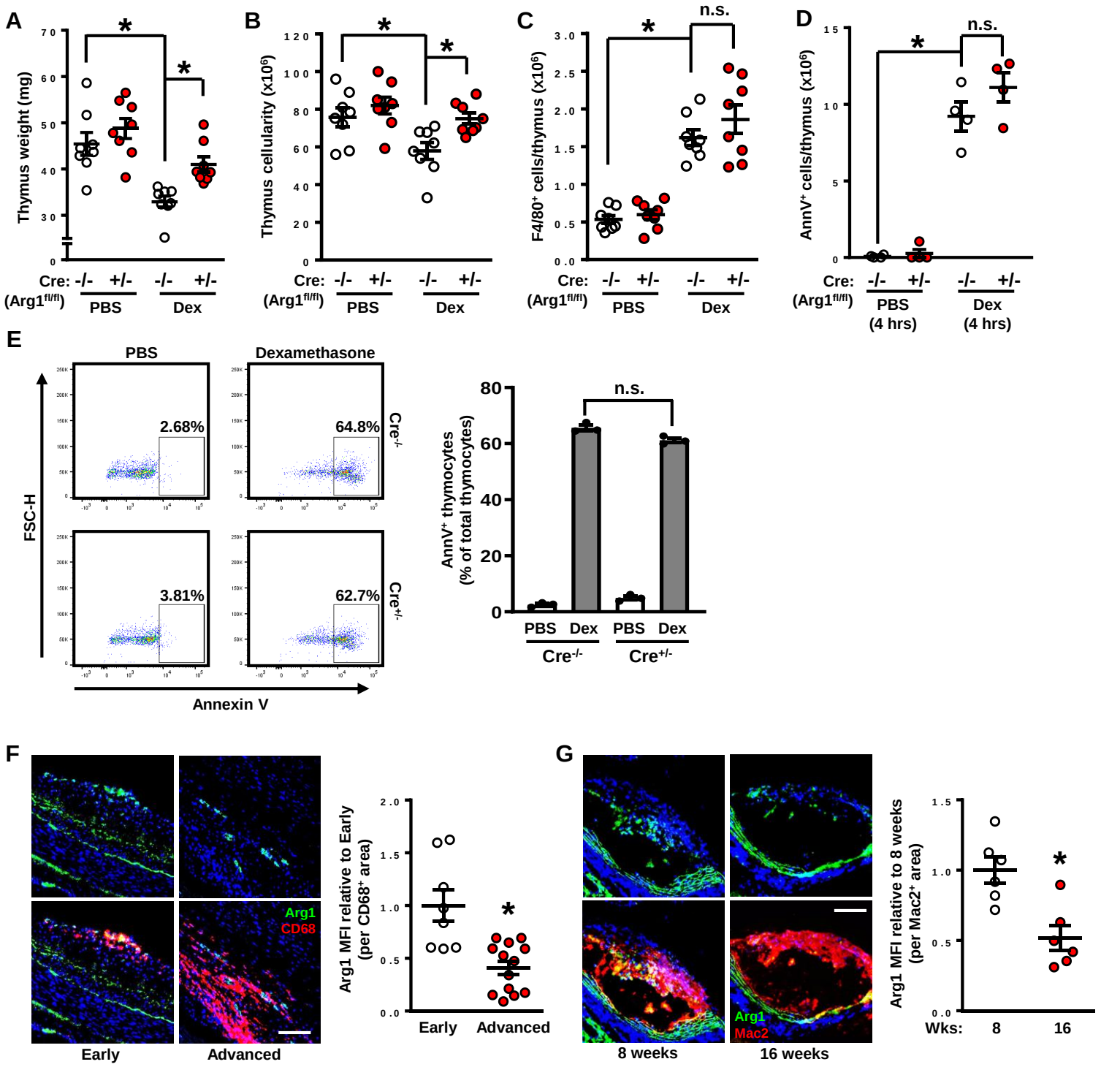
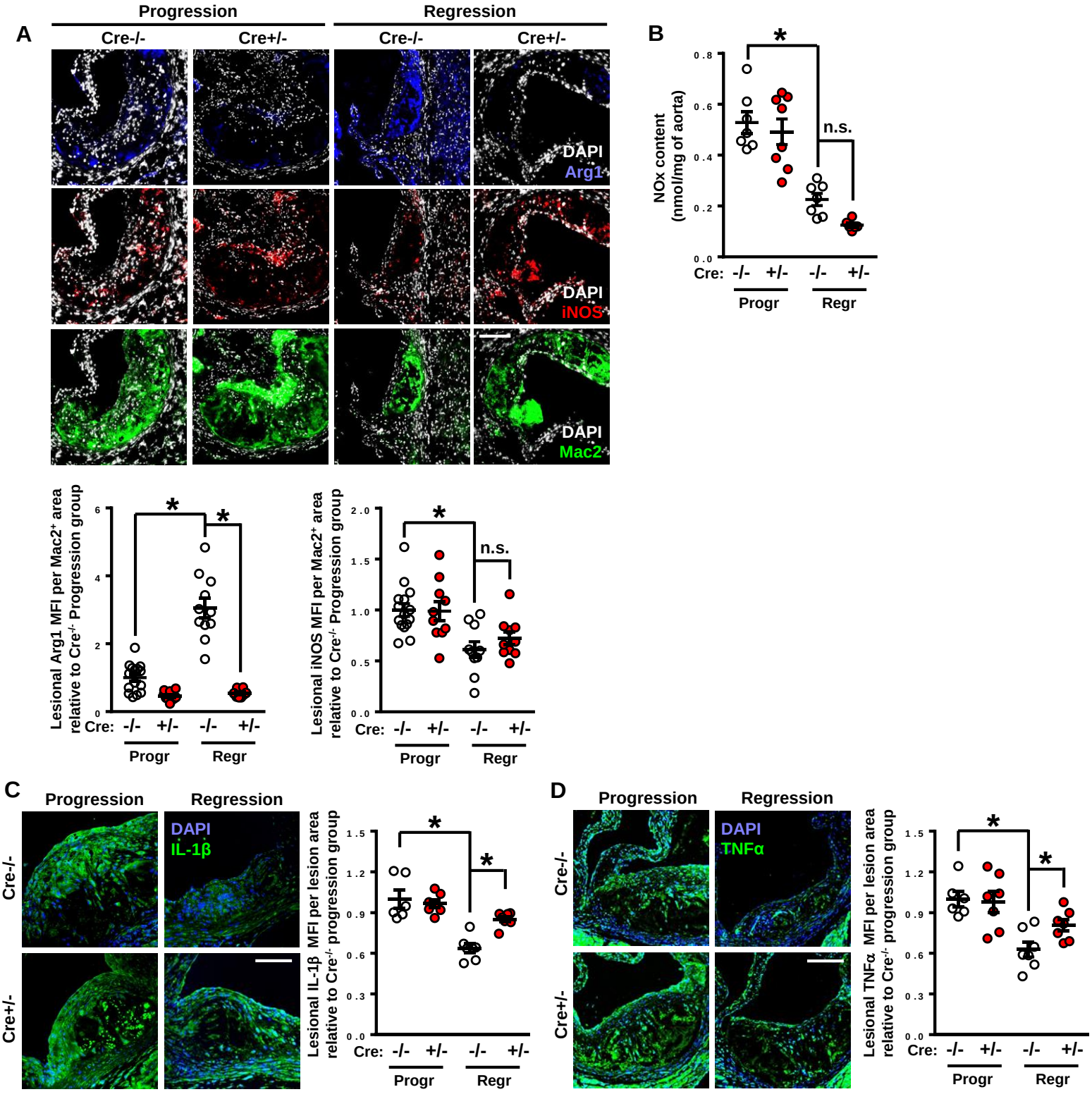
IL-4
iNOS
Arg1
β-actin
150
50
50
(kD)
B
Vehicle
LPS+IFNγ
IL-4
PKH67
PKH26
Merge
C
Cre-/-
Cre+/-
Arg1
β-actin
50
50
(kD)
Veh
IL-4
Veh
IL-4
D
ScrRNA
siArg1
Arg1
β-actin
50
50
(kD)
Veh
IL-4
E (microscopy)
pHrodo green+ MΦs (% of total macrophages)
n.s.
IL-4: - + +
ScrRNA
siArg1
PKH67+ pHrodo red+ MΦs (% of PKH67+ macrophages)
IL-4: - + +
ScrRNA
siArg1
F (flow)
pHrodo green+ MΦs (% of total MΦs)
n.s.
IL-4: - + +
ScrRNA
siArg1
pHrodo green+ pHrodo red+ MΦs (% of pHrodo green+ MΦs)
IL-4: - + +
ScrRNA
siArg1
G
PKH67+ PKH26+ MΦs (% of PKH67+ MΦs)
IL-13: - + +
ScrRNA
siArg1
H
Gated on F4/80+ cells
Unstained
PBS
Zymo
Normalized to mode
Arg1
Arg1 MFI of F4/80+ cells (relative to PBS)
PBS
Zymo
I
Arginase activity (units/L/mg)
PBS
Zymo
NOx content (nmol/10^6 cells)
n.s.
PBS
Zymo

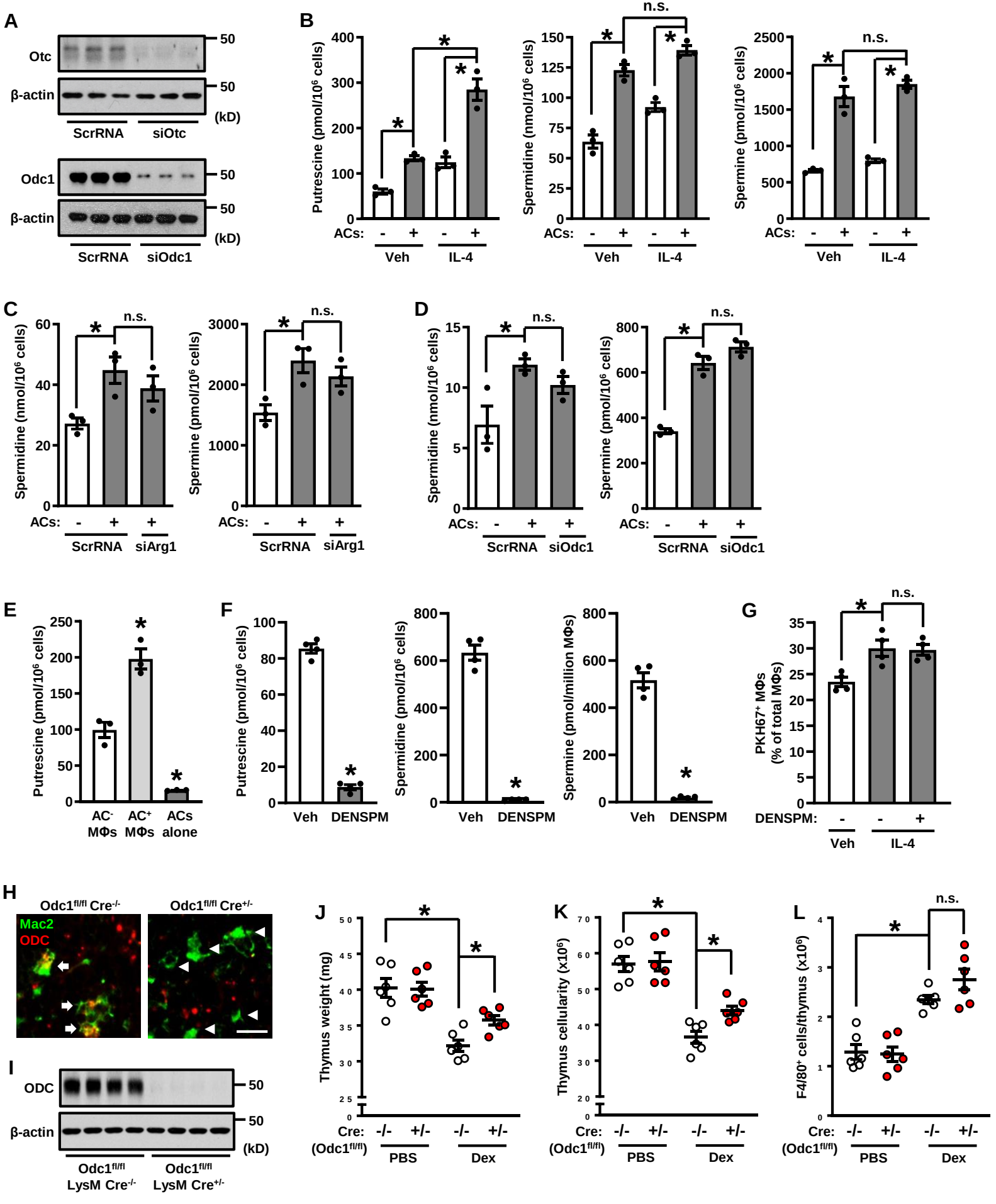
Supplemental Figure 1. Representative Images of Continual Efferocytosis, Cre-Mediated Arg1 Deletion, Arg1 Silencing Efficiency *In Vitro*, and Arg1 Activity and Nitric Oxide Content in Post-Zymosan Peritoneal Exudate Cells, Related to Figure 1. (**A**) Immunoblots of iNOS, Arg1, and β-actin from lysates of bone marrow-derived macrophages treated for 24 hr with vehicle (Veh), 50 ng/mL LPS + 25 ng/mL IFNγ, or 10 ng/mL IL-4 (n = 3 biological replicates). (**B**) Representative images from the efferocytosis experiment in Figure 1B. Bar, 15 μm. (**C**) Immunoblots of Arg1 and β-actin from lysates of macrophages from $Arg1^{fl/fl}$ $LysMCre^{-/-}$ and $Arg1^{fl/fl}$ $LysMCre^{+/-}$ mice treated with either vehicle or IL-4 (n = 3 biological replicates). (**D**) Immunoblots of Arg1 and β-actin from lysates of macrophages transfected with either ScrRNA or siArg1 then treated with either vehicle or IL-4 (n = 3 biological replicates). (**E**) ScrRNA or siArg1-transfected macrophages were treated with IL-4 then first incubated with pHrodo Green-labeled ACs at a 5:1 ratio for 45 min. The percentage of pHrodo $Green^{+}$ macrophages of total macrophages was quantified by epifluorescence microscopy (n = 3 biological replicates). To assess continual efferocytosis, PKH67-labeled ACs at a 5:1 ratio were added to macrophages for 45 min. The ACs were removed by rinsing, and then, after a 120-min interval, the macrophages were incubated with a second round of pHrodo Red-labeled ACs at a ratio of 5:1 for 45 min. The unengulfed ACs were removed, and the percentages $PKH67^{+}$ pHrodo Red^{+} macrophages of $PKH67^{+}$ macrophages was quantified by epifluorescence microscopy (n = 3 biological replicates). (**F**) Continual efferocytosis was assayed as in panel E, except cells were detached and assayed by flow cytometry (n = 4 biological replicates). (**G**) Macrophages were treated with 10 ng/mL IL-13 instead of IL-4, and efferocytosis was assessed as in Figure 1B (n = 3 biological replicates). (**H**) Mice were injected with PBS or 0.1 mg of zymosan A (i.p.). Six days later, peritoneal exudates were collected, and the cells were stained for F4/80 and Arg1 and then subjected to flow cytometric analysis. A representative flow cytometry histogram and quantification of $F4/80^{+}$ cells for Arg1 mean fluorescence intensity (MFI) are shown. Data are presented relative to the average value obtained for the vehicle specimens (n = 4 biological replicates). (**I**) Mice were injected i.p. with either PBS or 0.1 mg of zymosan A. After 6 days, cell pellets from peritoneal lavages were assayed for Arg1 activity (left panel) and nitric oxide species (right panel). Values are means $\pm$ S.E.M.; *$p < 0.05$; n.s., not significant; n = 6 mice per group.



Supplemental Figure 2. Deletion of Arg1 in Myeloid Cells Increases Thymus Weight and Cellularity in Dexamethasone-Treated Mice, and Arg1 Immunostaining in Lesions from Mouse and Human Atherosclerosis, Related to Figure 2. (**A-C**) Thymi from myeloid-Arg1 WT (Cre -/-) and KO (Cre +/-) mice were harvested 18 hours after the mice were injected i.p. with PBS or dexamethasone. The thymi were weighed (A) and assayed for total cells (B) and F4/80$^+$ macrophages (C) by flow cytometry (n=8 mice for per group). (**D**) Thymi from myeloid-Arg1 WT (Cre -/-) and KO (Cre +/-) mice were harvested 4 hours after the mice were injected with PBS or dexamethasone and then assayed for annexin V$^+$ cells (n = 4 biological replicates). (**E**) Thymocytes from myeloid-Arg1 WT (Cre -/-) and KO (Cre +/-) mice were treated in vitro with PBS or 100 μM dexamethasone for 6 hr then stained with annexin V and analyzed for the percentage of annexin V+ cells by flow cytometry (n = 3 biological replicates per group). (**F**) Early- and advanced-stage human atherosclerotic lesions were immunostained for Arg1 and CD68. Using the Stary grading system, early-stage atherosclerosis was defined as stage 0-2 and advanced-stage atherosclerosis was defined as stage 3-5. Arg1 staining was quantified as MFI within CD68$^+$ cells. Data are presented relative to the average value obtained for the early-stage specimens. Representative images are shown, and the data were quantified for the entire cohort (n = 8-13 different human specimens per group). Bar, 100 μm. (**G**) Arg1 staining was quantified as MFI within Mac2$^+$ cells. Data are presented relative to the average value obtained for the 8-week WD-fed specimens. Representative images are shown, and the data were quantified for the entire cohort (n = 6 mice per group). Bar, 100 μm.

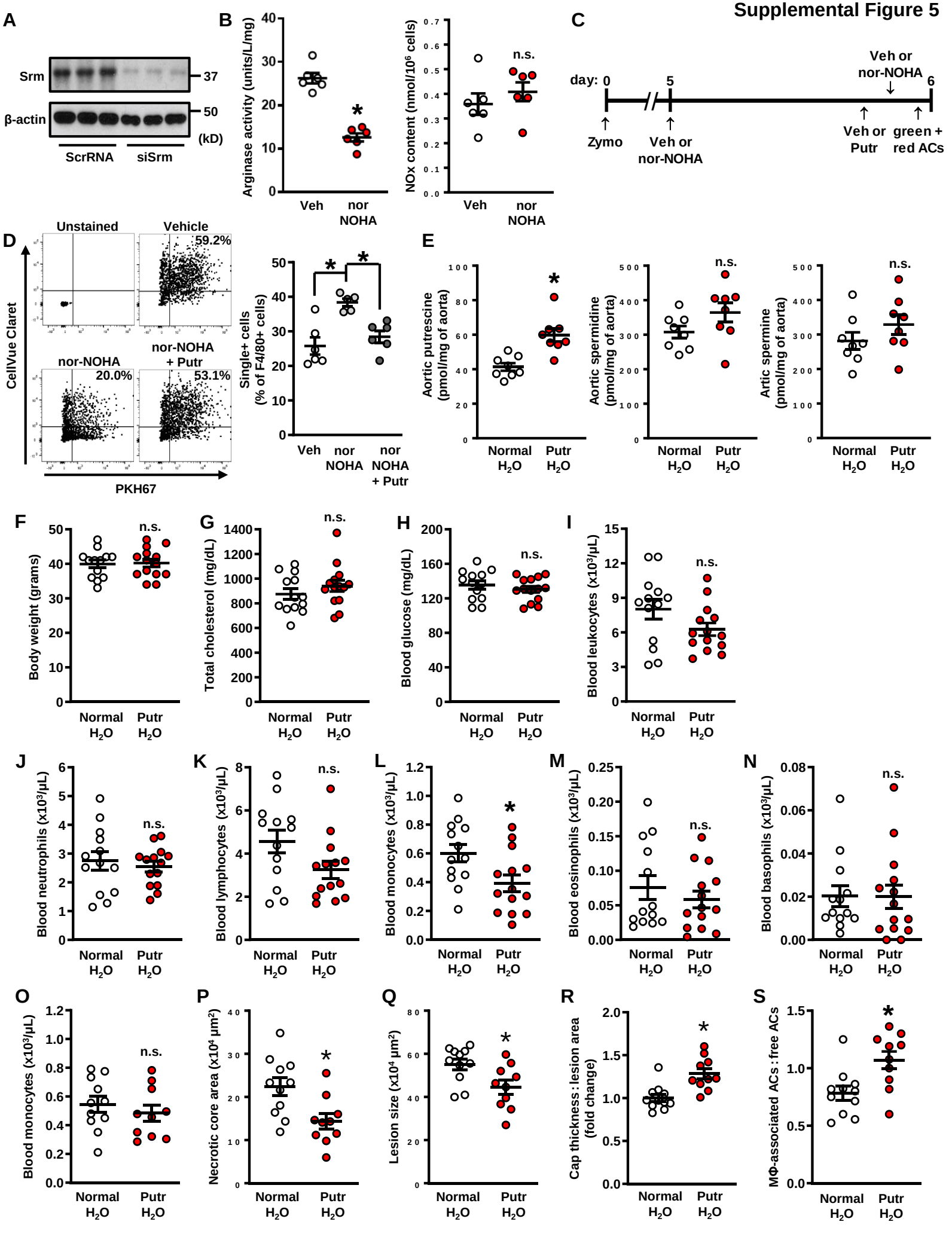


Supplemental Figure 3. Arg1, iNOS, TNFα, and IL-1β Immunostaining, and Nitric Oxide Detection in Lesions from Myeloid-Arg1 WT and KO Mice During Atherosclerosis Progression and Regression, Related to Figure 2. (**A**) Aortic root cross-sections were immunostained for Arg1, iNOS, and Mac2 and quantified as in Figure 2A. Data are presented relative to the average value obtained for the Cre-/- progression specimens. Representative images are shown (n = 10-15 mice per group). Bar, 200 μm. (**B**) Aortas from myeloid-Arg1 WT (Cre-/-) and KO (Cre+/-) *Ldlr*$^{-/-}$ mice on progression or regression protocols were measured for nitric oxide species content. (**C and D**) Aortic root cross-sections were immunostained for IL-1β and TNFα, respectively, and quantified as in Figure 2B. Data are presented relative to the average value obtained for the Cre-/- progression specimens. Representative images are shown (n = 6-7 mice per group). For all graphs, values are means ± S.E.M.; *p < 0.05; n.s., not significant. Bar, 200 μm.


A
Otc
β-actin
50
50
(kD)
ScrRNA
siOtc
Odc1
β-actin
50
50
(kD)
ScrRNA
siOdc1
B
Putrescine (pmol/10⁶ cells)
Spermidine (nmol/10⁶ cells)
Spermine (pmol/10⁶ cells)
n.s.
ACs: - + - +
Veh
IL-4
C
Spermidine (nmol/10⁶ cells)
Spermine (pmol/10⁶ cells)
n.s.
ACs: - + +
ScrRNA
siArg1
D
Spermidine (nmol/10⁶ cells)
Spermine (pmol/10⁶ cells)
n.s.
ACs: - + +
ScrRNA
siOdc1
E
Putrescine (pmol/10⁶ cells)
AC⁻ MΦs
AC⁺ MΦs
ACs alone
F
Putrescine (pmol/10⁶ cells)
Spermidine (pmol/10⁶ cells)
Spermine (pmol/million MΦs)
Veh
DENSPM
G
PKH67⁺ MΦs (% of total MΦs)
n.s.
DENSPM: - - +
Veh
IL-4
H
Odc1fl/fl Cre-/-
Odc1fl/fl Cre+/-
Mac2
ODC
I
ODC
β-actin
50
50
(kD)
Odc1fl/fl LysM Cre-/-
Odc1fl/fl LysM Cre+/-
J
Thymus weight (mg)
Cre: -/- +/- -/- +/-
(Odc1fl/fl)
PBS
Dex
K
Thymus cellularity (x10⁶)
Cre: -/- +/- -/- +/-
(Odc1fl/fl)
PBS
Dex
L
F4/80⁺ cells/thymus (x10⁶)
n.s.
Cre: -/- +/- -/- +/-
(Odc1fl/fl)
PBS
Dex

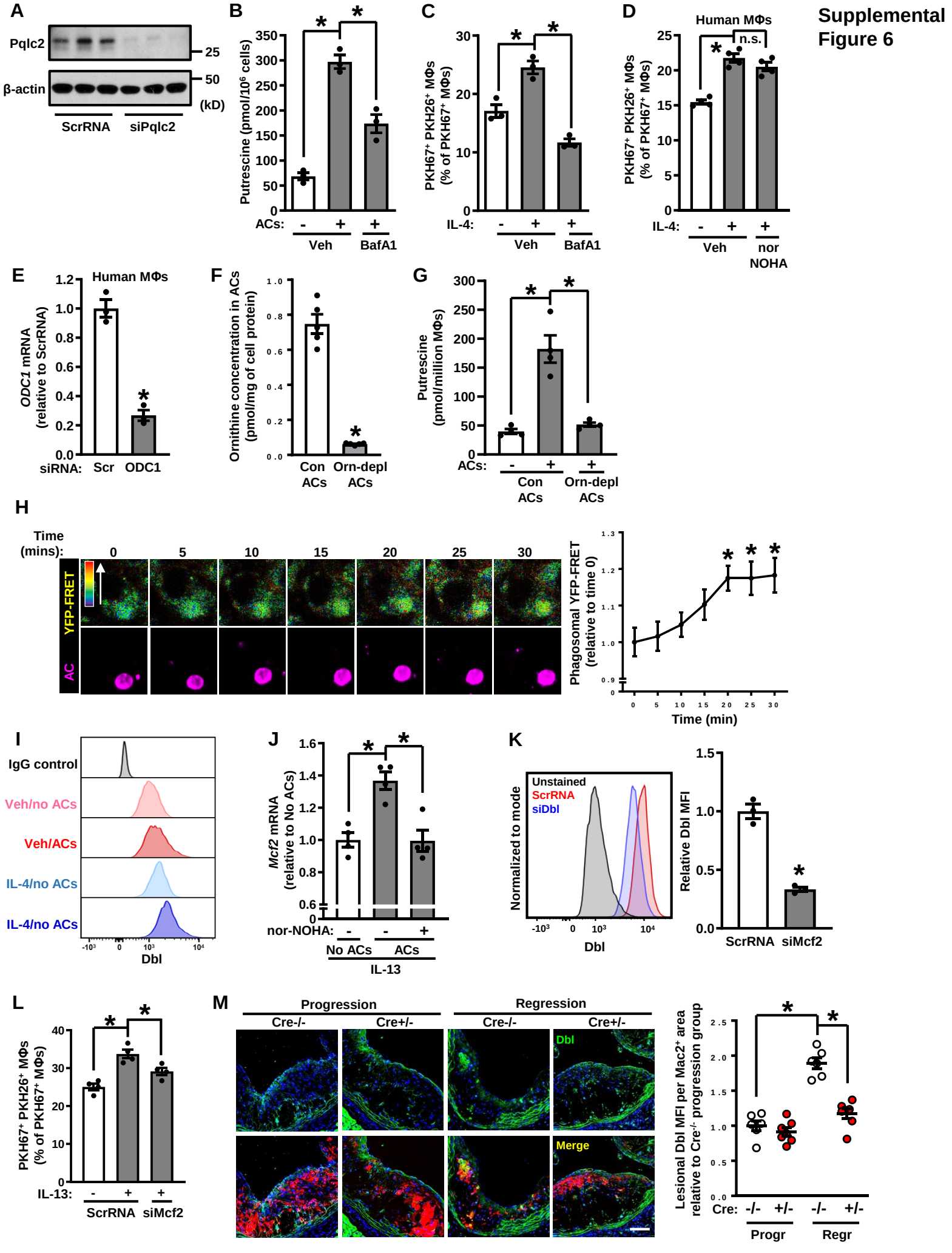
Supplemental Figure 4. siRNA Validation, Polyamine Measurements, Odc1 Immunostaining in Odc1$^{fl/fl}$ Mice, and Thymus Parameters from Myeloid Odc1-WT and KO Mice Injected with Dexamethasone, Related to Figure 3. (**A**) IL-4-treated macrophages were transfected with siRNA targeting Otc (top panel) or Odc1 (bottom panel) and then analyzed for knockdown efficiency by immunoblotting (n = 3 biological replicates). (**B**) Macrophages were treated with either vehicle or IL-4 and then incubated with ACs for 45 min. The macrophages were then rinsed with PBS, incubated for another 2 hr, and assayed for polyamines by HPLC (n = 3 biological replicates). (**C-D**) Macrophages transfected with the ScrRNA or the indicated siRNAs were treated with IL-4 and then incubated with ACs and assayed for polyamines as in panel B (n = 3 biological replicates). (**E**) Putrescine content of IL-4-treated macrophages, IL-4-treated macrophages that had ingested an AC, and ACs alone (n = 3 biological replicates). (**F**) Macrophages were treated with 10 μM DENSPM for 24 hr then treated with IL-4 for another 24 hr. Polyamines were measured by HPLC (n = 4 biological replicates). (**G**) Macrophages were treated with 10 μM DENSPM for 24 hr, then treated with IL-4 for another 24 hr. Macrophages were then incubated with PKH67-labeled ACs for 45 min, rinsed with PBS to wash off unbound ACs, and assayed for efferocytosis (n = 4 biological replicates). (**H**) Representative images of thymus cross-sections from mice in Figure 3E immunostained for Mac2 and Odc1 are shown. Arrows indicate Mac2^{+} macrophages that express Odc1 whereas arrowheads indicate Mac2^{+} macrophages that lack Odc1. Bar = 40 μms. (**I**) Immunoblots of ODC and β-actin from lysates of bone marrow-derived macrophages from *Odc1$^{fl/fl}$ LysMCre$^{-/-}$* and *Odc1$^{fl/fl}$ LysMCre$^{+/-}$* mice (n = 4 biological replicates). (**J-L**) Thymi harvested from myeloid-Odc1 WT (Cre-/-) and KO (Cre+/-) mice 18 hours after i.p. injection with PBS or dexamethasone were weighed (J) and then assayed for total cells (K) and F4/80^{+} macrophages (L) by flow cytometry (n = 6 mice for per group). Values are means $\pm$ S.E.M.; *$p < 0.05$; n.s., not significant.

Supplemental Figure 5

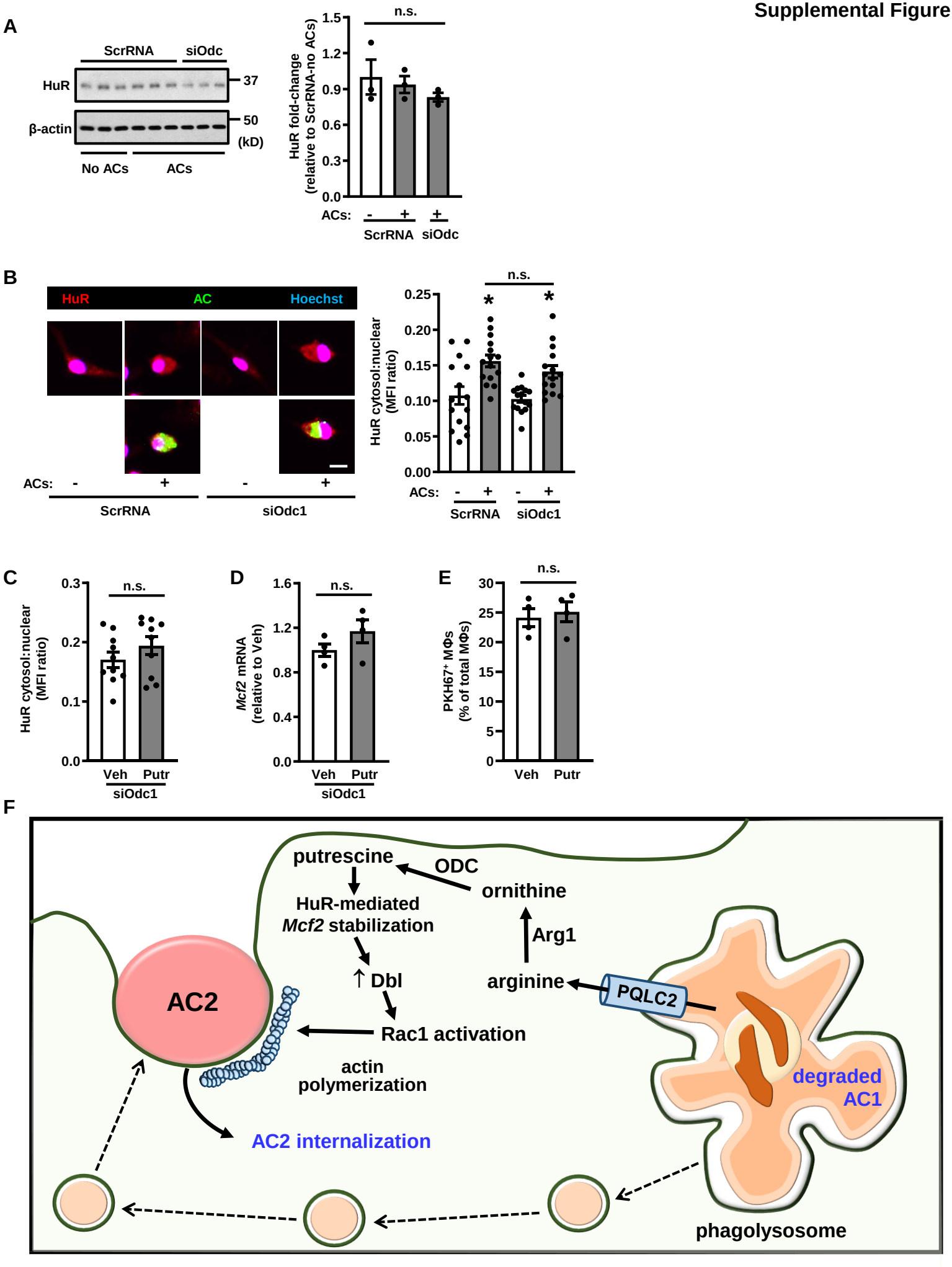


Supplemental Figure 5. siRNA Validation and Systemic Parameters in Mice Given Putrescine-Supplemented Water During Atherosclerosis Progression, Related to Figures 4 and 5. (**A**) IL-4-treated mouse bone marrow-derived macrophages were transfected with ScrRNA or siSrm and then lysed and analyzed for knockdown efficiency by quantifying *Srm* mRNA (n = 3 biological replicates). (**B**) Mice were injected with 0.1 mg of zymosan A (i.p.) and analyzed 6 days later. 24 hours and 2 hours before sacrifice, the mice were injected i.p. with either vehicle or 200 μg/mouse nor-NOHA. Peritoneal exudates were assayed for arginase activity (left panel) and nitric oxide species content (right panel). (**C**) Scheme of experimental plan for experiment for Figure 4C and this figure, panel D. (**D**) Selected flow cytometry contour plots from Figure 4C are shown. Values for all graphs are means ± S.E.M.; *p < 0.05; n.s., not significant. (**E**) Aortas from *Ldlr*$^{-/-}$ mice fed a Western diet for 10 weeks with putrescine intervention at 8 weeks (2 weeks of putrescine treatment) were assayed for polyamine content (n = 8 mice per group). (**F-N**) Plasma cholesterol, fasting blood glucose, body weight, and complete blood counts from mice given 8 weeks of putrescine-enriched water during atherosclerosis progression (n = 13-14 mice per group). (**O-S**) The aortic root lesions of a subgroup of putrescine-supplemented and control mice with similar monocyte counts (O) were assayed for necrotic core area (P), lesion size (Q), and fibrous cap:lesion area ratio (R), and ratio of macrophage-associated ACs:free ACs (S) (n = 10-11 mice in each group). Values are mean ± S.E.M., *P<0.05, n.s., not significant.

Supplemental Figure 6



Supplemental Figure 6. siRNA Validation, Putrescine Measurements, Ornithine Depletion Verification, Rac1 Activation in Macrophages Isolated From Raichu Rac-FRET Transgenic Mice, Dbl Immunostaining in Treated Macrophages *In Vitro*, Dbl Immunostaining of Macrophages from Zymosan-Treated Mice, and Immunostaining in Lesions of Myeloid-Arg1 WT and KO Mice During Atherosclerosis Progression and Regression; Related to Figure 5 and 6. (**A**) IL-4-treated mouse macrophages were transfected with ScrRNA or siPqlc2 and then lysed and analyzed for knockdown efficiency by quantifying *Pqlc2* mRNA (n = 3 biological replicates). (**B**) IL-4-treated macrophages were pre-treated for 30 min with 100 nM bafilomycin A1 or vehicle control (Veh) and then incubated with ACs and assayed for putrescine content (n = 3 biological replicates). (**C**) Macrophages were treated as in panel B and then assayed for double-AC efferocytosis as in Figure 1B (n = 3 biological replicates). (**D**) Human monocyte-derived macrophages were treated with IL-4 for 24 hours. The cells were then treated for 1 hour with vehicle control or 500 μM nor-NOHA. Double-cell efferocytosis was assayed as in Figure 1B (n = 4 biological replicates). (**E**) IL-4-treated human monocyte-derived macrophages were transfected with ScrRNA or siOdc1 and then lysed and analyzed for knockdown efficiency by quantifying *Odc1* mRNA (n = 3 biological replicates). (**F**) Prior to inducing apoptosis, Jurkat cells were cultured for 3 days under normal conditions (Con ACs) or in arginine-free media containing 10% dialyzed FBS and nor-NOHA to deplete ornithine (Orn-depl ACs). The cells were assayed for ornithine as in Table 1 (n = 5 biological replicates). (**G**) IL-4-treated macrophages were incubated with Con ACs or ornithine-depleted ACs and assayed for putrescine content (n = 4 biological replicates). (**H**) Macrophages isolated from Raichu-Rac1 FRET transgenic mice were incubated with CellVue Claret-labeled ACs, and Rac1 activity was quantified for YFP:CFP fluorescence ratio (YFP-FRET) at 5 min intervals for 30 min (n = 8-15 cells per group, with two plates of macrophages examined for each condition). Representative ratiometric images are shown. (**I**) Representative histogram plots for Figure 5E are shown. (**J**) Bone marrow-derived macrophages treated with vehicle or IL-13 were assayed for *Mcf2* mRNA as in Figure 5D (n = 4 biological replicates). (**K**) Macrophages were transfected with either ScrRNA or siRNA targeting Dbl (siMcf2), then detached, permeabilized, immunostained for Dbl, and analyzed by flow cytometry. Representative histograms and relative Dbl MFI (relative to ScrRNA controls) are shown (n = 3 biological replicates). (**L**) ScrRNA- and siMcf2-transfected bone marrow-derived macrophages treated with vehicle or IL-13 were assayed for efferocytosis as in Figure 1B (n = 4 biological replicates). (**M**) Aortic root cross-sections were immunostained for Dbl (green) and Mac2 (red), and analyzed as in Figure 5I. Data are presented relative to the average value obtained for the $Cre^{-/-}$ progression group. Illustrative images are shown, bar = 100 μm (n = 6-7 mice per group). Values are mean $\pm$ S.E.M., *P<0.05, n.s.=not significant.


A
ScrRNA
siOdc
HuR
37
β-actin
50
(kD)
No ACs
ACs
HuR fold-change (relative to ScrRNA-no ACs)
n.s.
ACs:
ScrRNA
siOdc
B
HuR
AC
Hoechst
ACs:
ScrRNA
siOdc1
HuR cytosol:nuclear (MFI ratio)
n.s.
C
HuR cytosol:nuclear (MFI ratio)
n.s.
Veh
Putr
siOdc1
D
Mcf2 mRNA (relative to Veh)
n.s.
E
PKH67+ MΦs (% of total MΦs)
n.s.
F
putrescine
ODC
ornithine
HuR-mediated Mcf2 stabilization
Arg1
Dbl
arginine
PQLC2
AC2
Rac1 activation
actin polymerization
degraded AC1
AC2 internalization
phagolysosome

Supplemental Figure 7. HuR Localization and Expression After Efferocytosis, Related to Figure 6, and Summary Scheme of How Arg1^{+} Macrophages Link Degradation of an Initial AC With Rac1-Mediated Internalization of a Subsequent Apoptotic Cell (Continual Efferocytosis). (**A**) ScrRNA or siOdc-transfected macrophages were treated with IL-4 for 24 hours and then incubated with ACs for 45 mins. Unbound ACs were rinsed away and two hours later macrophages were lysed and immunoblotted for HuR and β-actin (n = 3 biological replicates). (**B**) Macrophages were treated as in A except PKH67-labeled ACs were used, and after the two hr incubation cells were fixed, permeabilized, and immunostained for HuR. Macrophages were analyzed for cytosolic: nuclear HuR intensity (n = 15 cells/group from 3 biological replicates). (**C**) siOdc-transfected macrophages were treated with putrescine (100 μM) for 3 hrs. Cells were then fixed, permeabilized, immunostained for HuR, and analyzed for cytosolic: nuclear HuR intensity (n = 10 cells/group from two biological replicates). (**D**) Macrophages were treated as in panel C, except cells were harvested and analyzed for *Mcf2* mRNA by qRT-PCR (n = 4 biological replicates). (**E**) Macrophages were treated with putrescine for 3 hr, incubated with PKH67-labeled ACs for 45 min, and assayed for single-AC uptake (n = 4 biological replicates). (**F**) Phagolysosomal degradation of the first-encountered AC (AC1), in addition to enabling recycling of membranes to the cell surface for subsequent phagosome formation (dotted line arrows; Wang et al., 2017), generates arginine from the proteolysis of AC proteins. The arginine is transported into the cytoplasm via the PQLC2 transporter and is then metabolized to putrescine through the sequential actions of Arg1 and ODC. Putrescine promotes HuR-mediated stabilization of *Mcf2*, leading to an increase in the Rac1 GEF Dbl. Dbl activates Rac1, which is a necessary process in actin-mediated internalization of the subsequent AC (AC2). Values are mean ± S.E.M., *P<0.05, n.s.=not significant.