Cell Reports, Volume *28*

Supplemental Information

The Cardiac Microenvironment Instructs Divergent Monocyte Fates and Functions in Myocarditis

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Figure S1

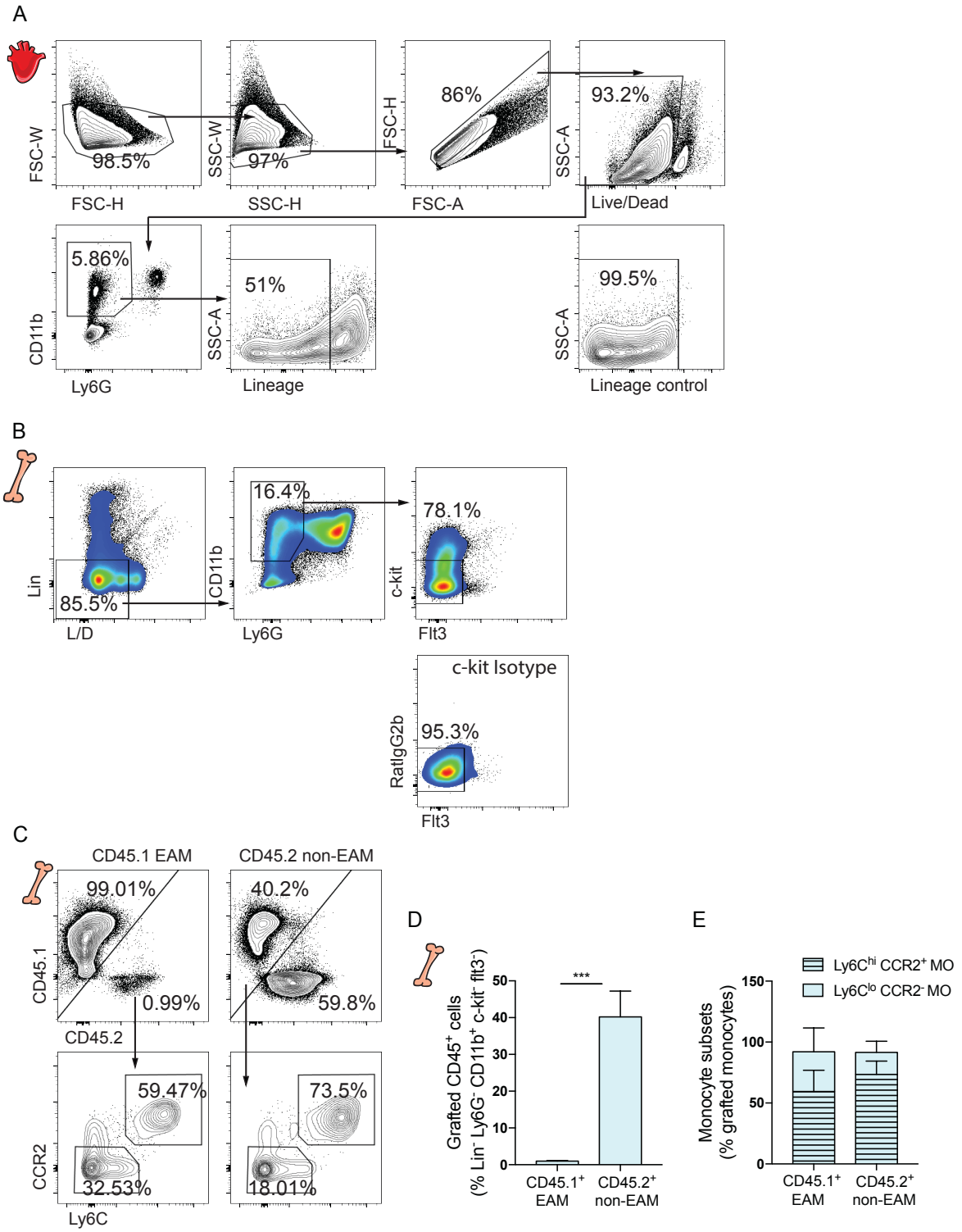

Figure S1. Characterization of monocytes and/or macrophages in murine hearts and bone marrows during EAM development. Related to Figure 1. (A) Representative gating strategy showing identification of $CD11b^{+}Ly6G^{-}Lin^{-}$ myeloid cells in the hearts and (B) mature $Lin^{-}CD11b^{+}Ly6G^{-}c\text{-}kit^{-}Flt3^{-}$ monocytes in the BM. (C) Flow cytometry plots showing grafted $Lin^{-}CD11b^{+}Ly6G^{-}c\text{-}kit^{-}Flt3^{-}$ monocytes that consist of both $Ly6C^{hi}CCR2^{+}$ and $Ly6C^{lo}CCR2^{-}$ populations in the BM. (D) Percentages of grafted $Lin^{-}CD11b^{+}Ly6G^{-}c\text{-}kit^{-}Flt3^{-}$ monocytes in the BM. (E) Percentages of grafted $Ly6C^{hi}$ and $Ly6C^{lo}$ monocytes out of total grafted monocytes in the BM. (A) (B) Lineage includes CD3e, B220, NKp46, CD90.2 and Ter119. Data are representative of two independent experiments with biological triplicates. Groups were compared using Student's *t* test. ***, $P < 0.001$. (D, E) All data were presented as mean ± SD.

Figure S2

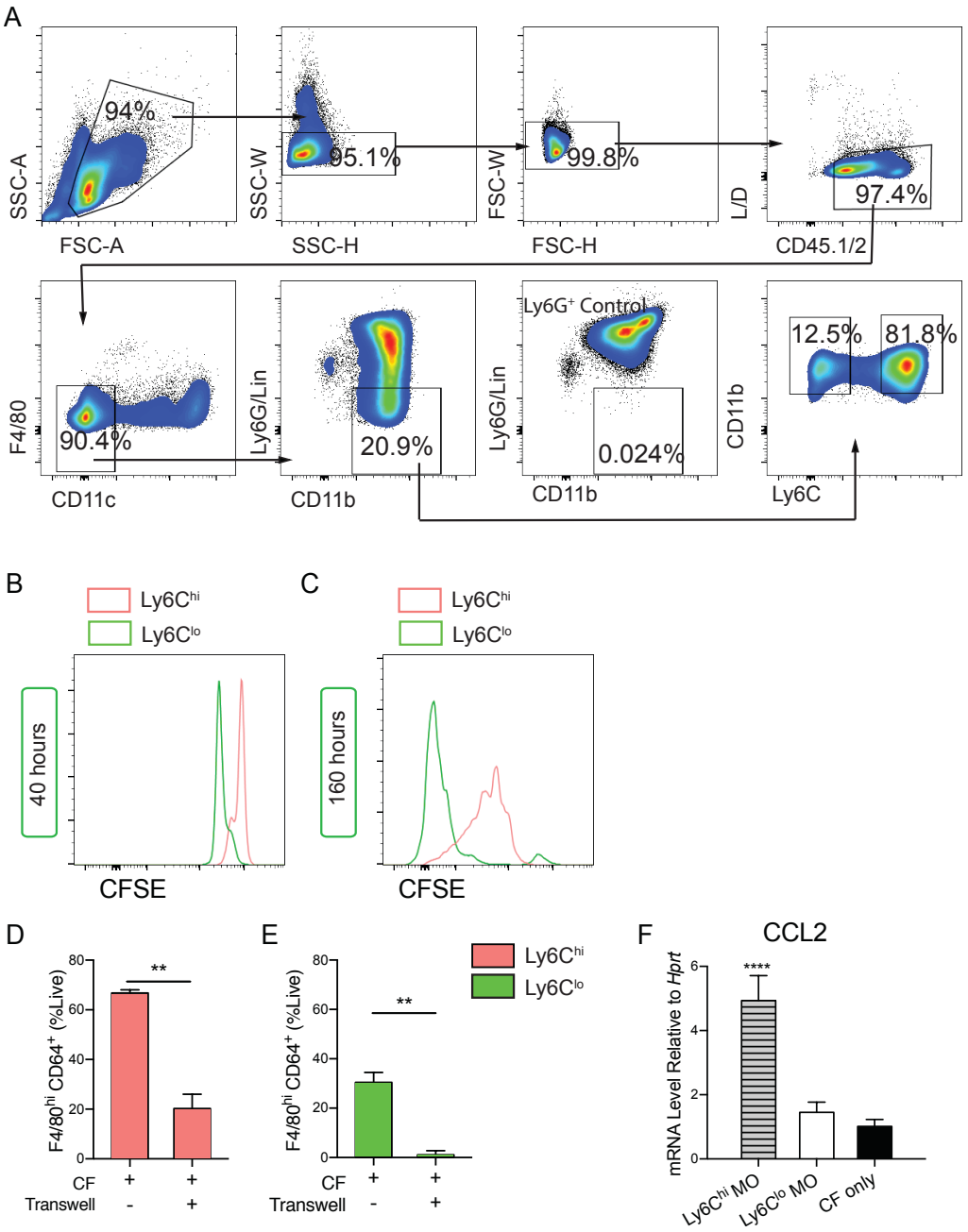

Figure S2. *In vitro* co-culture of splenic Ly6C^{hi} or Ly6C^{lo} monocytes with cardiac fibroblasts. Related to Figure 2. (A) Representative gating strategy of FACS sorted splenic Ly6C^{hi} and Ly6C^{lo} monocytes from WT (or IL-17Ra$^{-/-}$) EAM mice. Cells were gated stringently to obtain pure populations. Lineage marks include CD3e, CD19, NKp46, CD49b. (B) Histograms of MFI showing CFSE staining of viable Ly6C^{hi} and Ly6C^{lo} monocytes at 40 hours and (C) at 160 hours. (D) A transwell co-culture was established with cardiac fibroblasts in the lower chamber and either Ly6C^{hi} or Ly6C^{lo} monocytes on the upper chamber. Cells were separated with a 0.4-micron pore size membrane. We examined Ly6C^{hi} and (E) Ly6C^{lo} monocyte-to-macrophage differentiation at 40 hours and 160 hours respectively. (F) Separately, cardiac fibroblasts were harvested in 40 hours and *Ccl2* mRNA levels were assessed. Data are representative of two independent experiments with technical triplicates. (C, D) Groups were compared using Student's *t* test. **, $P < 0.01$. (E) Groups were compared using one-way ANOVA followed by Tukey test. ****, $P < 0.0001$. (D – F) All data were presented as mean ± SD.

Figure S3

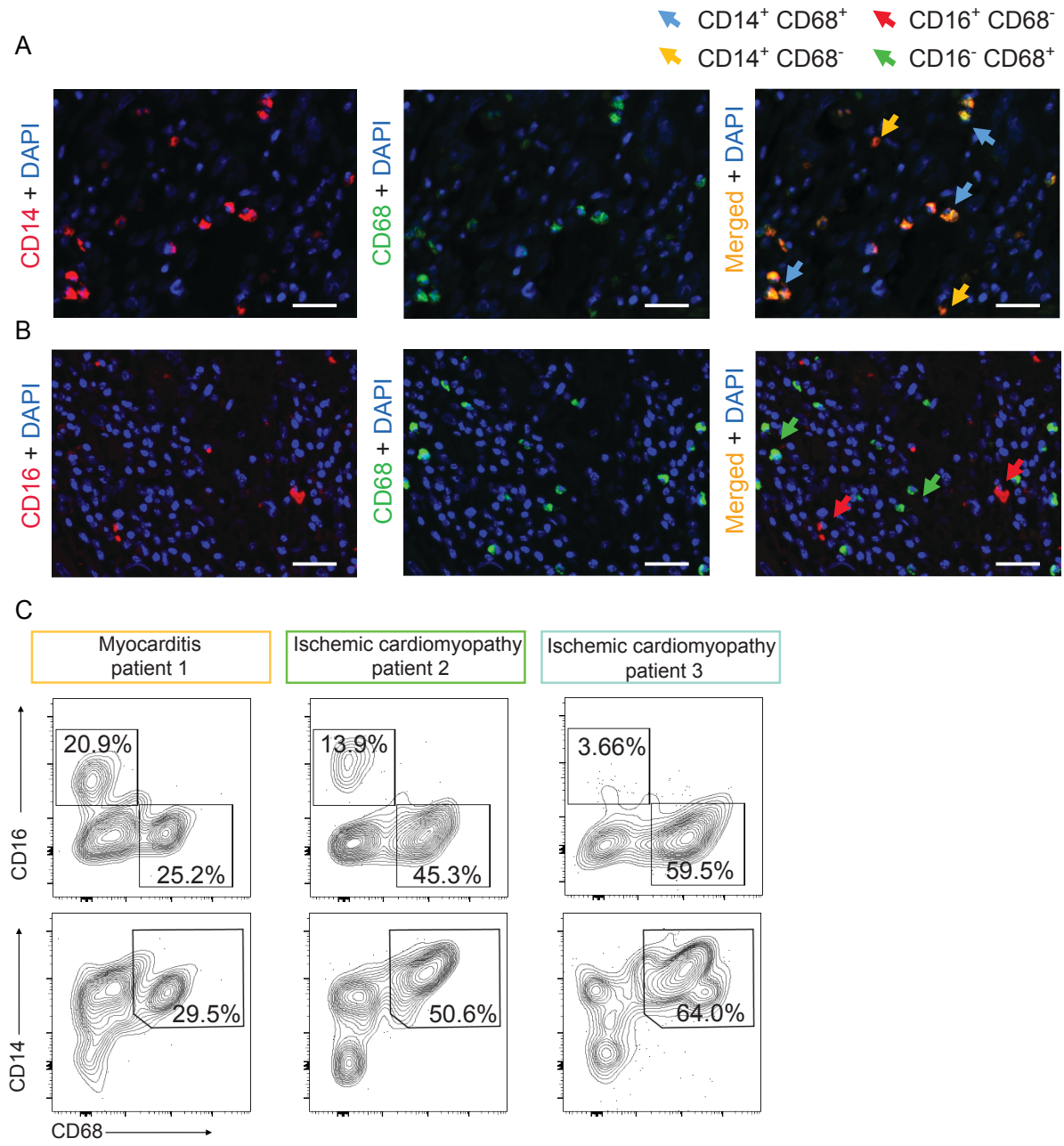

Figure S3. Examination of human macrophage ontogeny. Related to Figure 3 and Table S1. (A) Immunofluorescent (IF) staining in a human giant cell myocarditis biopsy sample showing numerous $CD14^{+}CD68^{+}$ double positive cells (blue arrows) and some $CD14^{+}CD68^{-}$ single positive cells (yellow arrows). (B) The same biopsy sample showing numerous $CD16^{-}CD68^{+}$ single positive cells (green arrows) and few $CD16^{+}CD68^{-}$ single positive cells (red arrows). Bars: 50 µm. (C) Gating of $CD16^{+}$ or $CD14^{+}$ and $CD68^{+}$ from viable $CD45^{+}CD11b^{+}$ myeloid cells in the hearts of three HF patients with ventricular assist device explant.

Figure S4

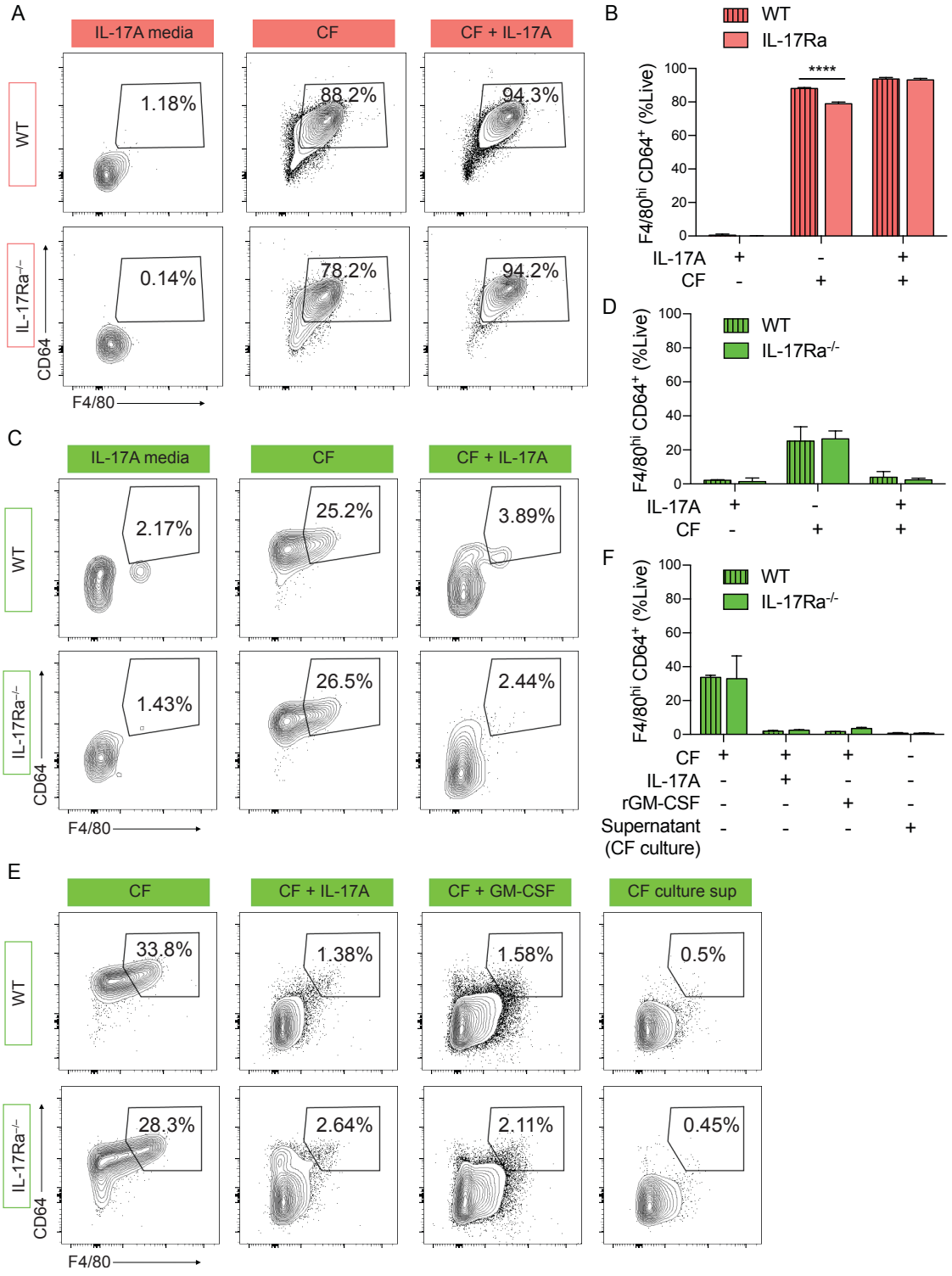

Figure S4. Validation of WT monocyte differentiation *in vitro*. Related to Figure 4. Cardiac fibroblasts were harvested from WT naïve mice, whereas monocytes were sorted from either EAM WT or EAM IL-17Ra$^{-/-}$ mice. (A) Flow cytometry plots showing differentiation status of splenic Ly6C^{hi} monocyte from either WT or IL-17Ra$^{-/-}$ mice. (B) Percentages of Ly6C^{hi} MDMs. (C) Flow cytometry plots showing differentiation status of splenic Ly6C^{lo} monocyte from either WT or IL-17Ra$^{-/-}$ mice. (D) Percentages of Ly6C^{lo} MDMs. (E) Flow cytometry plots showing the inhibitory effects of IL-17A and rGM-CSF on Ly6C^{lo} monocyte-to-macrophage differentiation, regardless of monocyte sources. Cardiac fibroblasts supernatant alone was insufficient in promoting WT and IL-17Ra$^{-/-}$Ly6C^{lo} monocyte-to-macrophage differentiation. (F) Percentages of Ly6C^{lo} MDMs. Groups were compared using two-way ANOVA followed by Sidak test. ****, $P < 0.0001$. (B, D, F) All data were presented as mean ± SD.

Figure S5

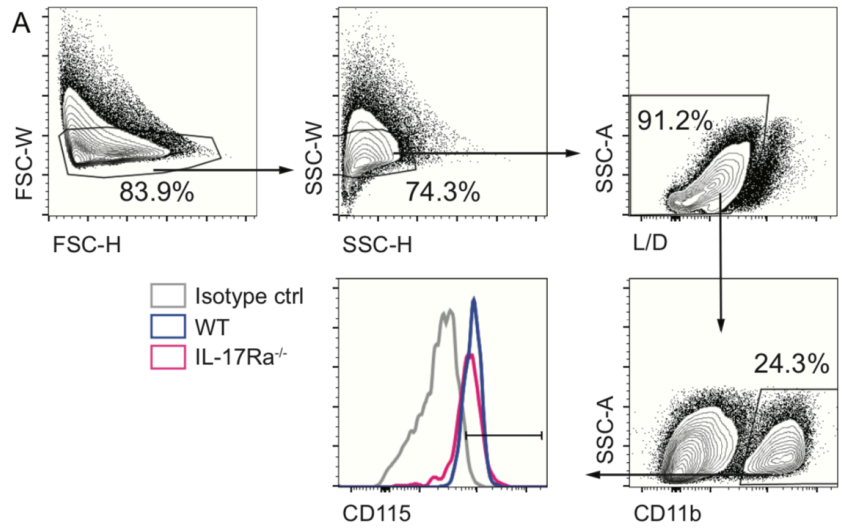

Figure S5. Monocyte gating strategy in the heart. Related to Figure 5. (A) Representative gating strategy to identify monocytes in the hearts. Appropriate CD115 isotype control was included.

Figure S6

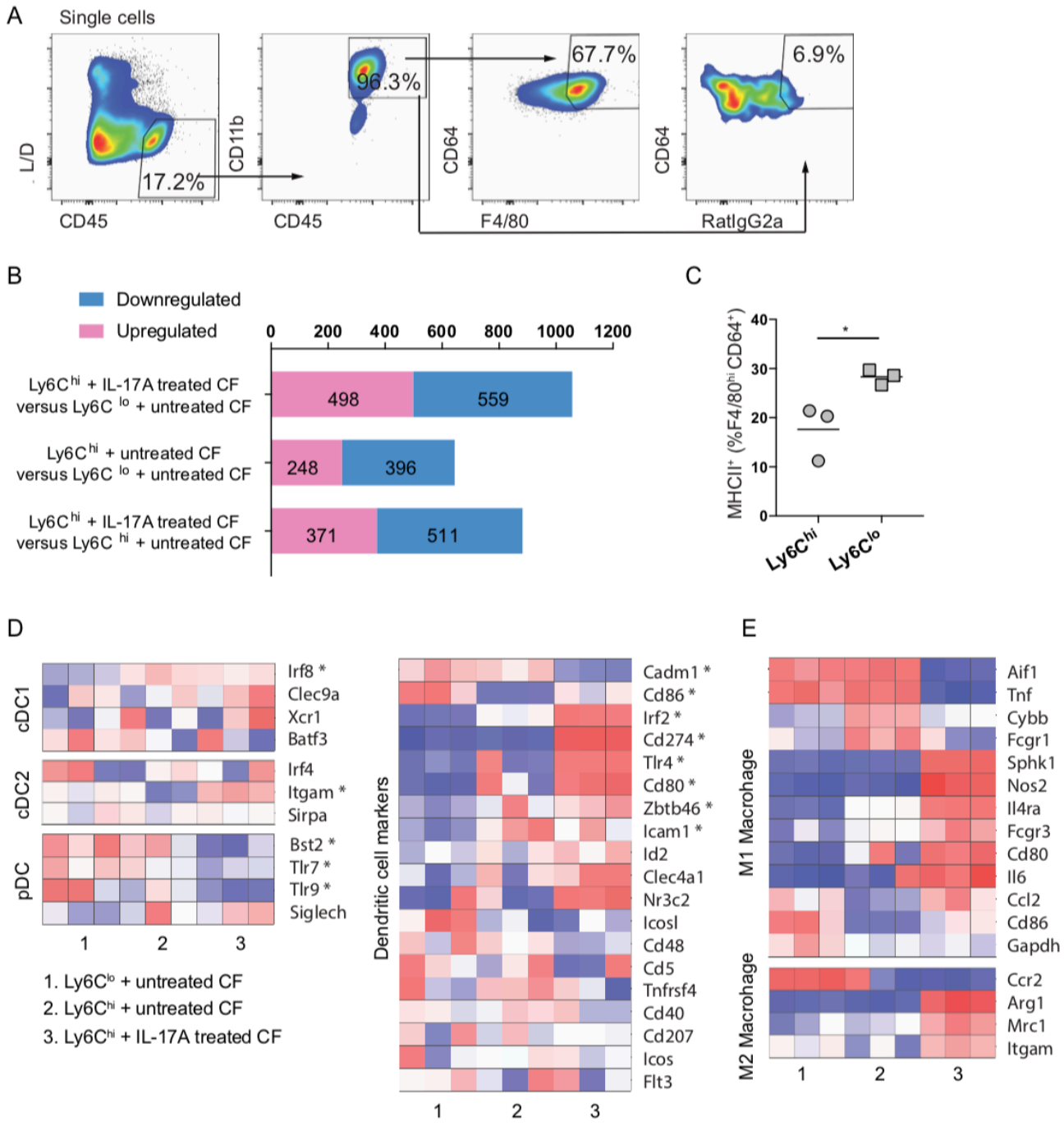

Figure S6. Validating the identities of *in vitro* derived macrophage subsets. Related to Figure 6. (A) Representative gating strategy used to FACS sort CD45^{+}CD11b^{+}CD64^{+}F4/80^{hi} macrophages derived from the *in vitro* monocyte-fibroblast co-culture. F4/80 isotype control RatIgG2a was included. (B) Bar graph displaying the number of differentially regulated genes, using a threshold of 2× fold change and p value<0.05. (C) Frequencies of MHCII^{+} subset out of total intracardially injected MDMs in IL-17Ra$^{-/-}$ recipient mice. (D) Heat maps showing relative fold changes in genes associated with dendritic cells and (E) M1/M2 dichotomy of macrophages. (C) Data are representative of two independent experiments with biological triplicates. Groups were compared using Student's *t* test. *, P < 0.05. All data were presented as mean ± SD. (D) * Represent selected genes displayed on the heat maps have a one-way ANOVA p value<0.05 among groups compared.

Figure S7

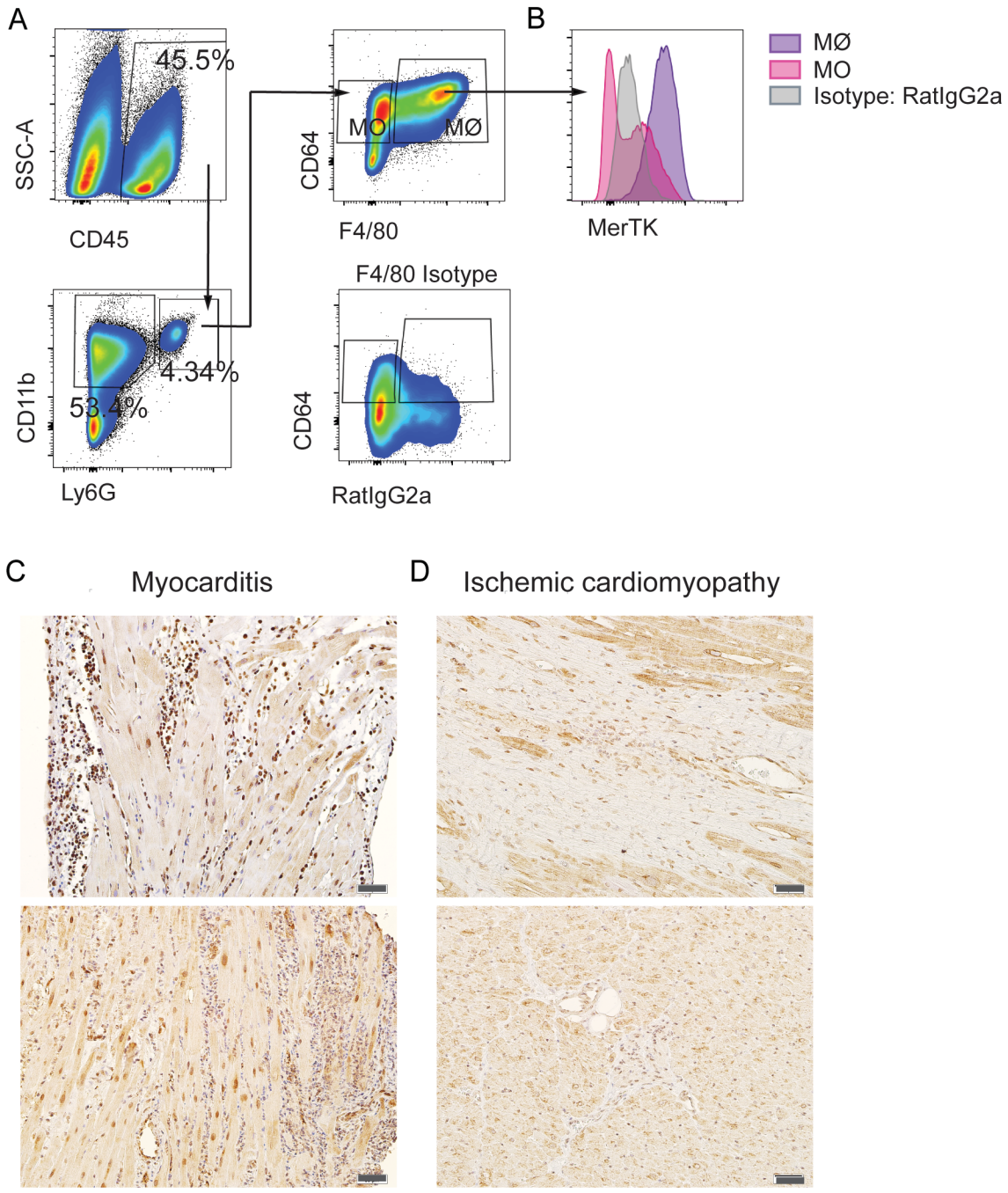

Figure S7. Murine MerTK expression levels by monocytes/macrophages and IL-17A levels in human endomyocardial biopsies. Related to Figure 7 and Table S2, 3. (A) Representative gating strategy of concatenated $CD45^{+}Ly6G^{-}CD11b^{+}F4/80^{hi}CD64^{+}$ macrophages and $CD45^{+}Ly6G^{-}CD11b^{+}F4/80^{-}CD64^{+}$ monocytes in the hearts. Appropriate F4/80 isotype control was shown. (B) Macrophages and monocytes' MerTK expression level as compared to isotype control for MerTK. (C) Immunohistochemistry staining for human IL-17A in the hearts of representative median implant samples of myocarditis patients and (D) ischemic cardiomyopathy patients.

Sample	Myocarditis patient 1	Ischemic cardiomyopathy patient 2	Ischemic cardiomyopathy patient 3
Age	32	61	57
Race/Ethnicity	Caucasian	Caucasian	Caucasian
Gender	Male	Male	Male
Etiology	Viral myocarditis	Ischemic	Ischemic
Type of VAD	HM II 10600	3000 rpm	8000 rpm
Diabetes	No	No	Yes
Height	175 cm	170 cm	180 cm
Weight	86.63 Kg	86.63 Kg	100.69 Kg
LVIDd	3.9 cm	6.6 cm	6.5 cm
LVPWd	1.3 cm	0.9 cm	1.0 cm
IVSd	1.6 cm	1.1 cm	1.0 cm
EF			
Troponin level		<0.15	
Days on VAD	1167	270	432
Explant reason	OHT	OHT	OHT
Dead	No	No	No
BNP		185	376
CKMB		1.7	0.5
Tissue Amount (mg)	307.5 (LV)	294.1 (LV)	339.6 (LV)
Path explant		cardiomegaly, concentric LVH, posterior wall fibrosis, fatty infiltration of IVS, mild fibrous epicarditis CAD	cardiomegaly with concentric biventricular hypertrophy, CAD, fibrosis

Table S1. Patient information part 1. Related to Figure 3 and S3. Patient information from individuals with end stage HF who underwent implantation of left ventricular assist device at the Texas Heart Institute. Tissues were collected for flow cytometry assessments. OHT: Orthotopic Heart Transplant.

Sample	Myocarditis patient I	Myocarditis patient II	Myocarditis patient III	Myocarditis patient IV
Age	43	25	15	63
Race/Ethnicity		Hispanic	African American	Caucasian
Gender	Male	Male	Male	Male
Etiology	Viral	Viral	Viral	Viral
Type of VAD	HM XVE	HM II	HM II	Jarvik 2
Diabetes	No	No	No	Yes
Height	188 cm	172 cm	185 cm	183 cm
Weight	100.01 Kg	100.69 Kg	113.39 Kg	87.54 Kg
LVIDd		7.0 cm	7.2 cm	6.7 cm
LVPWd		1.1 cm	0.8 cm	0.7 cm
IVSd		1.1 cm	0.9 cm	0.7 cm
EF	10 – 15%	<20%	<15%	<15%
Troponin level		<0.15		
Days on VAD	50	502	1121	600
Explant reason	OHT	OHT	To Jarvik, died 2d after	OHT
Dead	Yes	No	Yes	No
BNP		423	1163	
CKMB		0.2		2.2
Tissue Amount (mg)	44	130	231.4	245.5
Path Implant		focal interstitial edema, minimal hypertrophy	myocyte hypertrophy	myocyte hypertrophy and lysis

Table S2. Patient information part 2. Related to Figure 7 and S7. Viral myocarditis patient information from individuals with end stage HF who underwent explant of left ventricular assist device and orthotopic heart transplant at the Texas Heart Institute. Tissues were collected for IHC and flow cytometry assessments. OHT: Orthotopic Heart Transplant.

Sample	Ischemic cardiomyopathy patient I	Ischemic cardiomyopathy patient II	Ischemic cardiomyopathy patient III	Ischemic cardiomyopathy patient IV	Ischemic cardiomyopathy patient V
Age	61	59	64	56	72
Race/Ethnicity	Caucasian	Caucasian	Hispanic	Caucasian	Caucasian
Gender	Male	Male	Male	Male	Male
Etiology	Ischemic	Ischemic	Ischemic	Ischemic	Ischemic
Type of VAD	HM II	HW	HM II	HM II	HW
Diabetes	No	No	Yes	Yes	Yes
Height	170 cm	170 cm	170 cm	180 cm	178 cm
Weight	77.11 Kg	73.48 Kg	90.71 Kg	97.97 Kg	69.39 Kg
LVIDd	6.92 cm	7.2 cm	7.0 cm	8.0 cm	6.9 cm
LVPWd	1.1 cm	1.1 cm	1.0 cm	1.1 cm	1.0 cm
IVSd	0.63 cm	0.9 cm	1.1 cm	1.0 cm	1.1 cm
EF	20%	30%	<15%	20%	<20%
Troponin level	0.06	<0.15	<0.15		<0.15
Days on VAD	270	452	363	432	182
Explant reason	OHT	OHT	OHT	OHT	OHT
Dead	No	No	yes	No	No
BNP	2983	222	1130	283	240
CKMB	1.6	1.2	5.9		0.2
Tissue Amount (mg)	273	251	240.6	271.9	218
Path Implant	myocyte hypertrophy	Myocyte hypertrophy, interstitial and replacement-type myocardial fibrosis	severe myocyte hypertrophy with extensive fibrosis	myocyte hypertrophy, patchy interstitial fibrosis	Replacement type myocardial fibrosis

Table S3. Patient information part 3. Related to Figure 7 and S7. Ischemic cardiomyopathy patient information from individuals with end stage HF who underwent explant of left ventricular assist device and orthotopic heart transplant at the Texas Heart Institute. Tissues were collected for IHC and flow cytometry assessments. OHT: Orthotopic Heart Transplant.