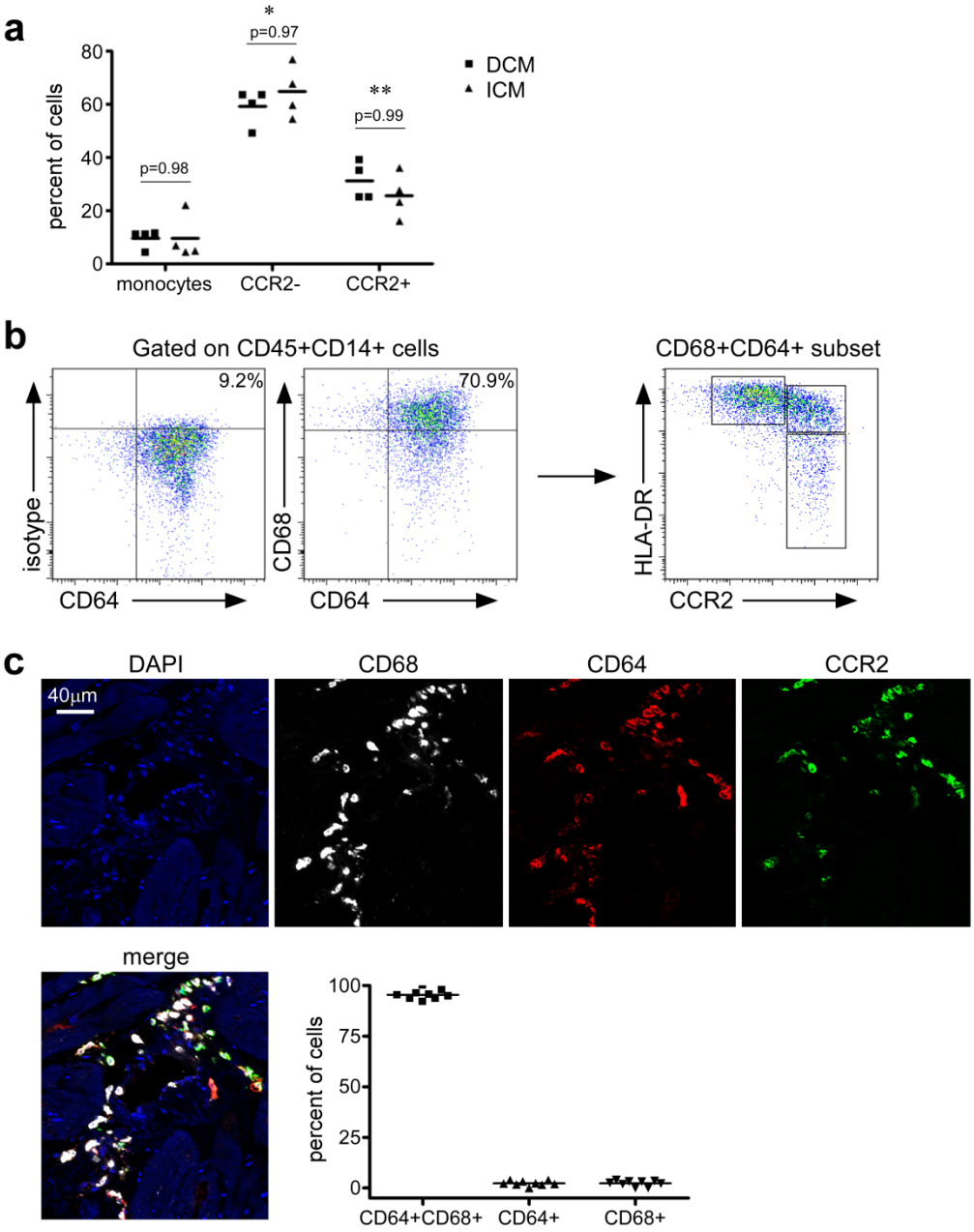
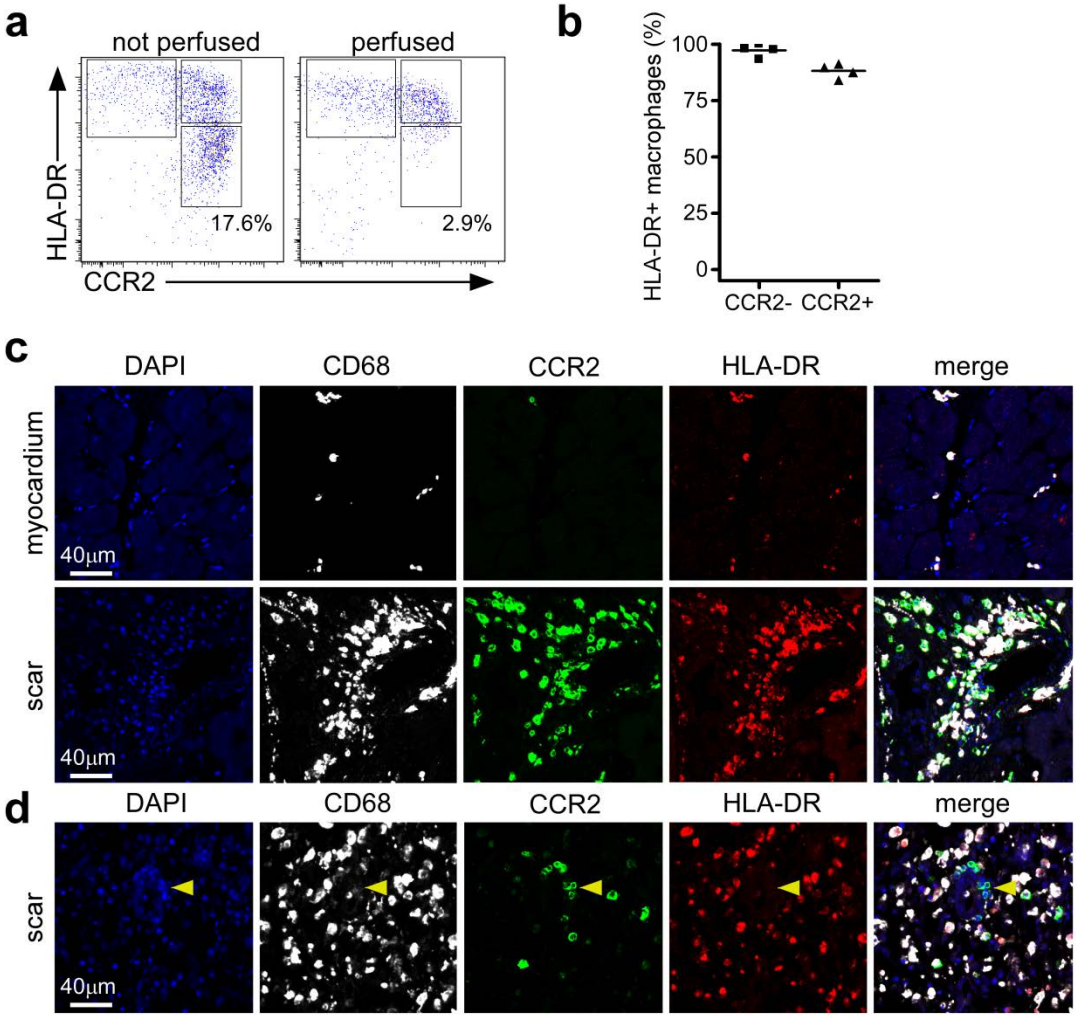
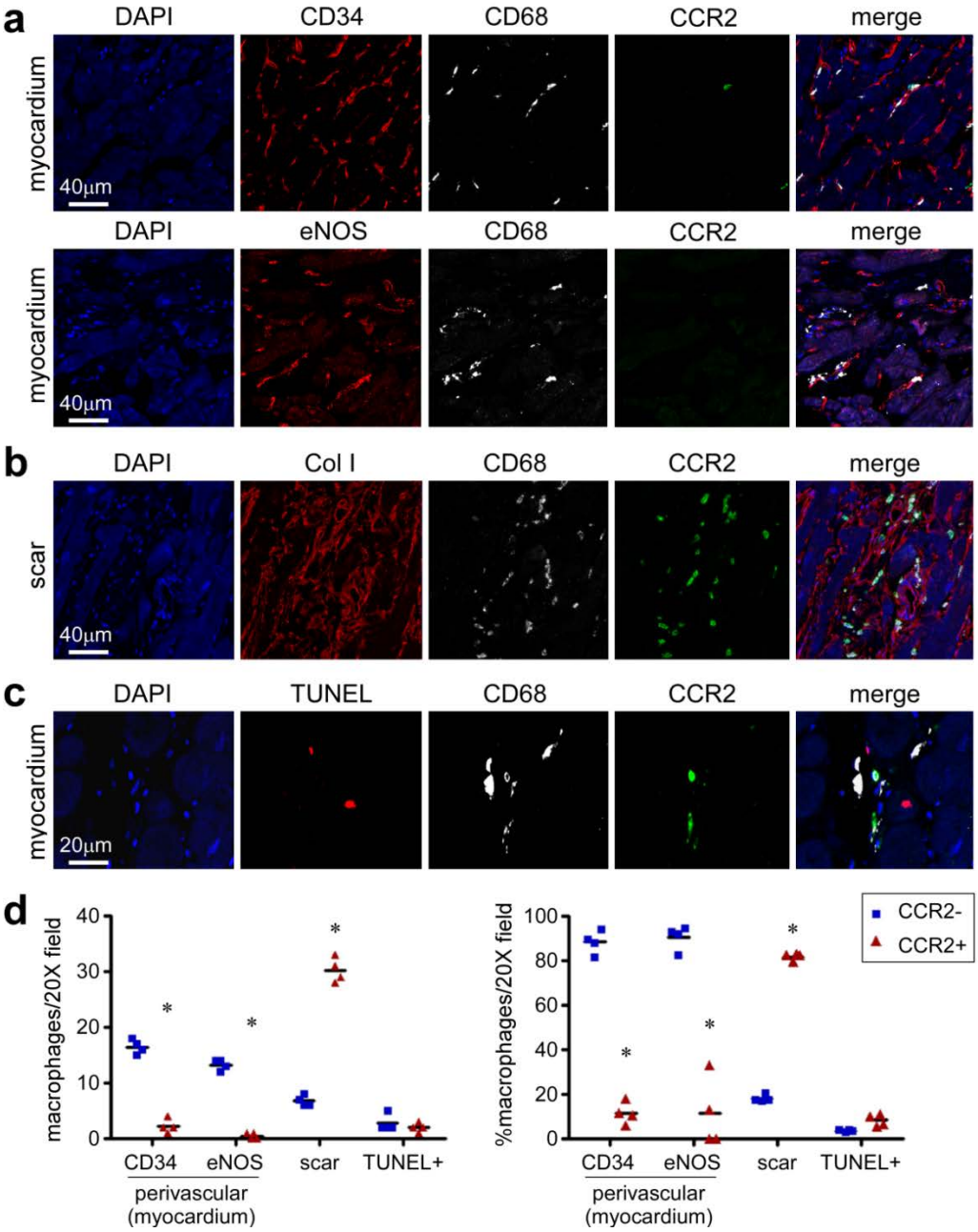


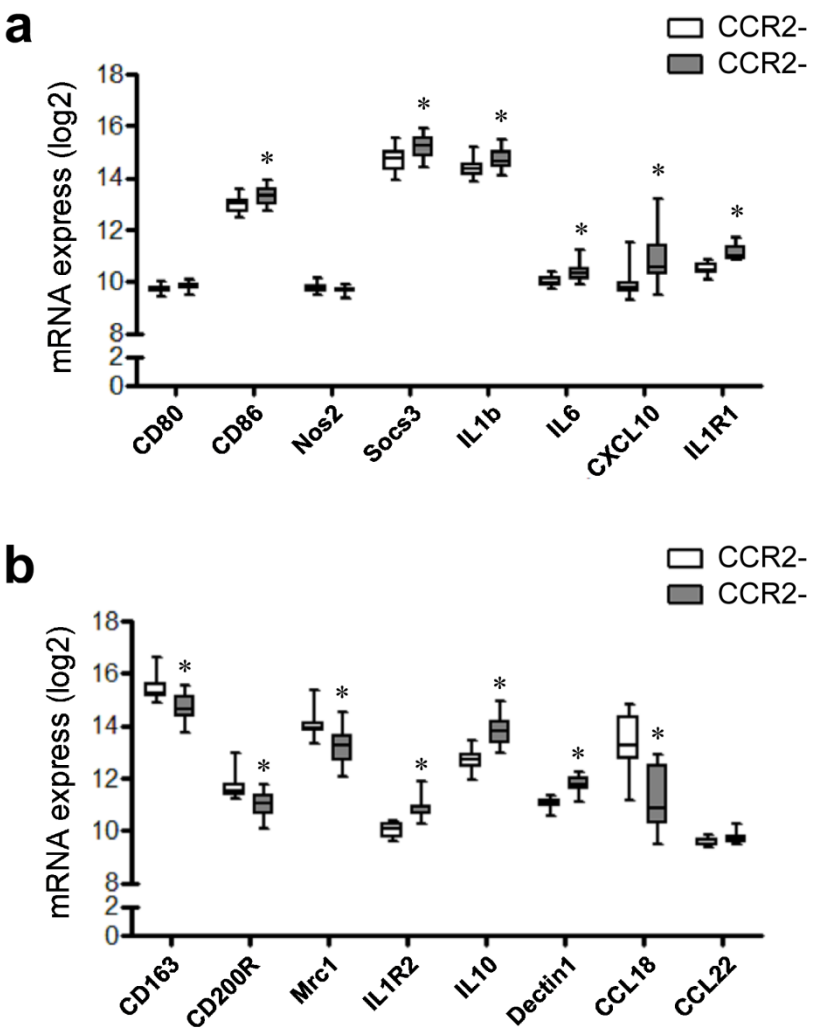
Supplementary Figure 1. Flow cytometry and immunostaining of human cardiac macrophages. a, Quantification of monocyte and macrophage composition measured in the hearts of patients with ischemic (ICM) and dilated (DCM) cardiomyopathy using flow cytometry. Asterisks denotes p<0.001 (Mann Whitney test, two-sided) compared to monocytes. Double asterisks denotes p<0.01 compared to monocytes and CCR2- macrophages (Mann Whitney test, two-sided). No significant differences were evident between patients with ICM and DCM (absolute p-values shown). Each data point represents a biologically independent sample (n=4) and lines indicate mean values. **b**, Intracellular flow cytometry showing that the majority of CD45+CD14+CD64+ cells express CD68 (left) and, that monocytes, CCR2- macrophages, and CCR2+ macrophages are contained within the CD45+CD14+CD64+CD68+ gate (right). Experiment replicated 3 times with similar results. **c**, Immunostaining for CD68 (white), CD64 (red), and CCR2 (green) showing that both CCR2- and CCR2+ cardiac macrophages express CD64 and CD68. Quantification of the percent of cells that are CD64+CD68+, CD64+, and CD68+. Each data point (n=9) represents a biologically independent heart failure specimen. c: 200X magnification. Blue: DAPI.



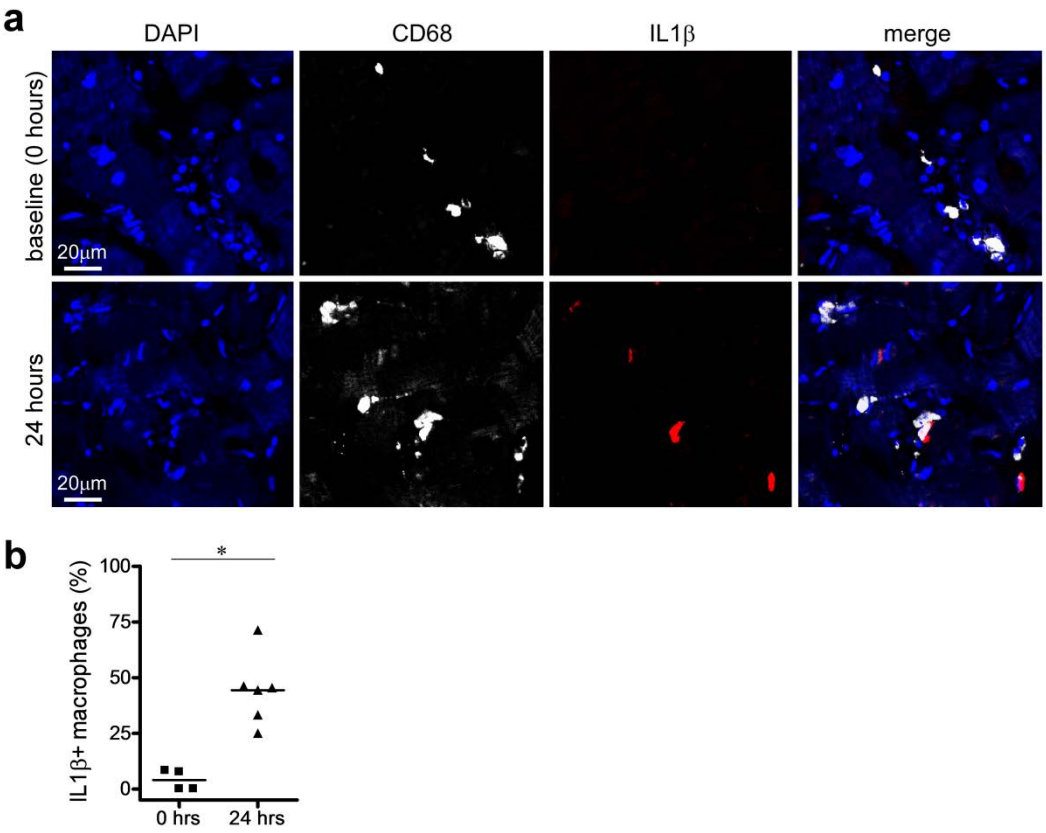
Supplementary Figure 2. Tissue perfusion and monocyte localization. a, Flow cytometry of cardiac tissue preparations highlighting removal of the majority of monocytes. **b**, Quantification of immunostaining analysis of perfused cardiac tissue preparations showing the majority of CCR2-CD68+ and CCR2+CD68+ cells express the macrophage marker HLA-DR. Each data point (n=4) represents a biologically independent human heart specimen **c**, Immunostaining for CD68 (white), CCR2 (green), and HLA-DR (white). **d**, Rare monocytes (yellow arrowhead) defined as CD68+CCR2+HLA-DR^{neg} are located in perivascular regions. Flow cytometry and immunostaining experiments were repeated 4 times with similar results. **c,d**: 200X magnification. Blue: DAPI.



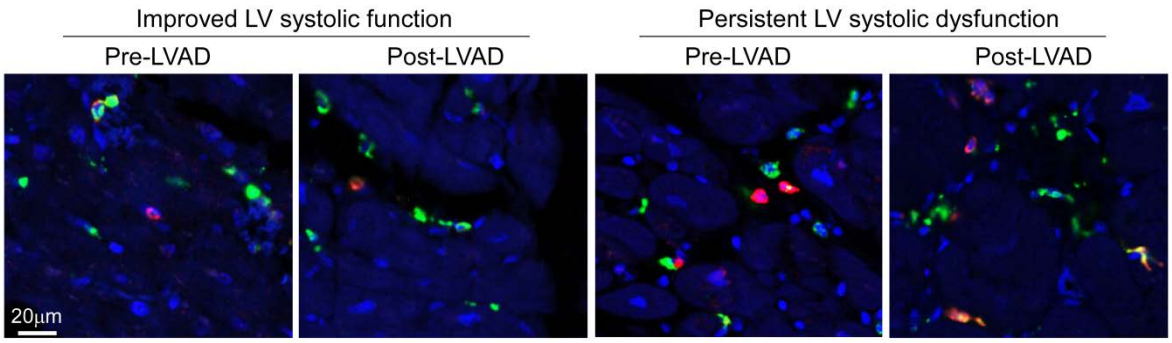
Supplementary Figure 3. Localization of cardiac macrophage subsets in the human heart. a, Immunostaining for CD34 (red, top), eNOS (red, bottom), CD68 (white), and CCR2 (green) indicates that CCR2- macrophages are localized within viable portions of the myocardium in a perivascular distribution. **b**, Immunostaining for Col I (red), CD68 (white), and CCR2 (green) indicates that CCR2+ macrophages preferentially populate myocardial areas containing scar or fibrosis. **c**, Staining for TUNEL (red) and immunostaining for, CD68 (white) and CCR2 (green) indicates that both CCR2- and CCR2+ macrophages surround dying cardiomyocytes. Blue: DAPI. **d**, Absolute cell numbers (left) and percentages (right) of CCR2- and CCR2+ macrophages with the indicated locations. Shown are the number or percentage of CCR2- and CCR2+ macrophages that co-stain for CD34, eNOS or TUNEL, or that are present in scar area. Perivascular (myocardium) refers to the percent of macrophages adjacent to CD34 and eNOS positive endothelial cells located within viable myocardial regions. Each data point (n=4) represents an individual heart failure sample. Asterisks denotes $p<0.001$ (Mann Whitney test, two-tailed). The line represents the mean value. a-b: 200X magnification, c: 400X magnification.



Supplementary Figure 4. Expression of M1 and M2 markers in human cardiac macrophages. Normalized mRNA expression of classic human M1 (**a**) and M2 (**b**) markers in CCR2- macrophages (n=19) and CCR2+ macrophages (n=19) isolated from biologically independent hearts of patients with ICM and DCM. Expression data are derived from microarray analyses and displayed as box and whisker plots on a log2 scale. The box denotes the 25th and 75th percentiles, the line indicates the median value, and the whiskers reflect the minimum and maximum values. Asterisks denotes $p<0.05$ (false discovery rate) compared to CCR2- macrophages.



Supplementary Figure 5. IL1β expression in human cardiac slice culture. a, High magnification images of cardiac tissue slice preparations immunostained for CD68 (white) and IL1β (red). 400X magnification. Blue: DAPI. **b**, Quantification of the percent of IL1β+ macrophages. Asterisks denotes p=0.0095 (Mann Whitney test, two-sided) compared to baseline (0 hours). Each data point (0 hrs n=4 and 24 hrs n=6) represents a biologically independent heart specimen.



Supplementary Figure 6. **Cardiac macrophages composition following LVAD implantation**. High magnification images of immunostaining for CD68 (green) and CCR2 (red) in myocardial tissue specimens obtained from heart failure patients at the time of left ventricular assist device placement and at the time of transplant. Patients were stratified into those who displayed persistent LV systolic dysfunction (n=17) and individuals who displayed improved LV systolic function (n=18). Blue: DAPI, 400X magnification.

Supplemental Table 1. Demographic data of heart failure and transplant specimens.

	heart failure samples	transplant samples
Sample size	19	9
Age, yrs	51.0	50.6
Male	15 (78.9)	9/9 (100)
African american	9 (47.4)	1 (11.1)
Heart failure etiology		
-ICM	8 (42.1)	4 (44.4)
-DCM	11 (57.9)	5 (55.6)
LVAD	16 (84.2)	0 (0)
Heart explant	3 (15.8)	0 (0)
Heart failure duration, yrs	5.7 ± 5.1	N/A
Endomyocardial biopsy	0 (0)	9 (100)
Time from transplant, yrs	N/A	8.8 ± 3.3
Ejection Fraction	20.2 ± 11.7	57.7 ± 8.6

Parentheses indicate percentage. yrs: years, ICM: ischemic cardiomyopathy, DCM: dilated cardiomyopathy, LVAD: left ventricular assist device. Displayed values are mean ± standard deviation.

Supplemental Table 2. LVAD cohort clinic data

	improved	persistant	p-value
Sample size	18	18	
Age (yrs)	50.1	53.3	0.59
Male	14 (78)	17 (94)	0.34
Heart failure etiology			0.27
-ICM	8 (44)	6 (33)	
-DCM	7 (40)	11 (61)	
-Valvular	0 (0)	1 (6)	
-Peripartum	1 (6)	0 (0)	
-Chemo	2 (11)	0 (0)	
HTN	8 (44)	4 (22)	0.29
Diabetes	4 (22)	5 (27.8)	0.72
Smoker	9 (50)	6 (33)	0.34
NYHA class	3.6 ± 0.5	3.7 ± 0.5	0.49
EF, pre-LVAD (%)	20.8 ± 8.6	16.4 ± 6.5	0.11
EF, 6 months (%)	48.2 ± 8.3	16.5 ± 6.4	<0.01
Δ EF (%)	28.3 ± 13.3	-1.6 ± 6.3	<0.01
LVDD, pre-LVAD (cm)	6.1 ± 1.2	7.3 ± 1.1	<0.01
LVDD, 6 months (cm)	4.4 ± 0.9	7.0 ± 1.2	<0.01
Δ LVDD (cm)	-1.7 ± 1.2	-0.3 ± 0.6	<0.01
LVSD, pre-LVAD (cm)	5.5 ± 1.2	6.5 ± 1.1	0.02
LVSD, 6 months (cm)	3.7 ± 1.0	6.6 ± 1.1	<0.01
Δ LVSD (cm)	-1.8 ± 1.2	-0.4 ± 1.8	<0.01

Parentheses indicate percentage. yrs: years, ICM: ischemic cardiomyopathy, DCM: dilated cardiomyopathy, Chemo: chemotherapy associated cardiomyopathy, HTN: hypertension, NYHA: New York heart failure association, EF: ejection fraction, LVAD: left ventricular assist device, LVDD: left ventricular diastolic dimension, LVSD: left ventricular systolic dimension. Displayed values are mean ± standard deviation. P-values were calculated using either Fisher exact or Mann Whitney tests (two-sided).

Supplemental Table 3. Antibodies for Flow Cytometry

Antigen	Fluorophore	Clone	Manufacturer
CD45	PercCP/Cy5.5	2D1	Biolegend
CD14	PE and PE/Cy7	M5E2	Biolegend
CD64	FITC and BV421	10.1	Biolegend
CCR2	APC	K036C2	Biolegend
HLA-DR	APC/Cy7	L243	Biolegend
Mer/MertK	PE	125518	R&D
CD33	PE	WM53	Biolegend
CD163	PE	GHI/61	Biolegend
CD3e	PE	APA1/1	Biolegend
CD19	PE	HIB19	Biolegend
CD56	PE	5.1H11	Biolegend
CD68	APC	Y1/82A	Biolegend