Supplemental Material

The following supplemental sections are provided as additional information:

Supplement 1: Study Timeline

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Supplement 5: Iron Overloading within Lysosomes

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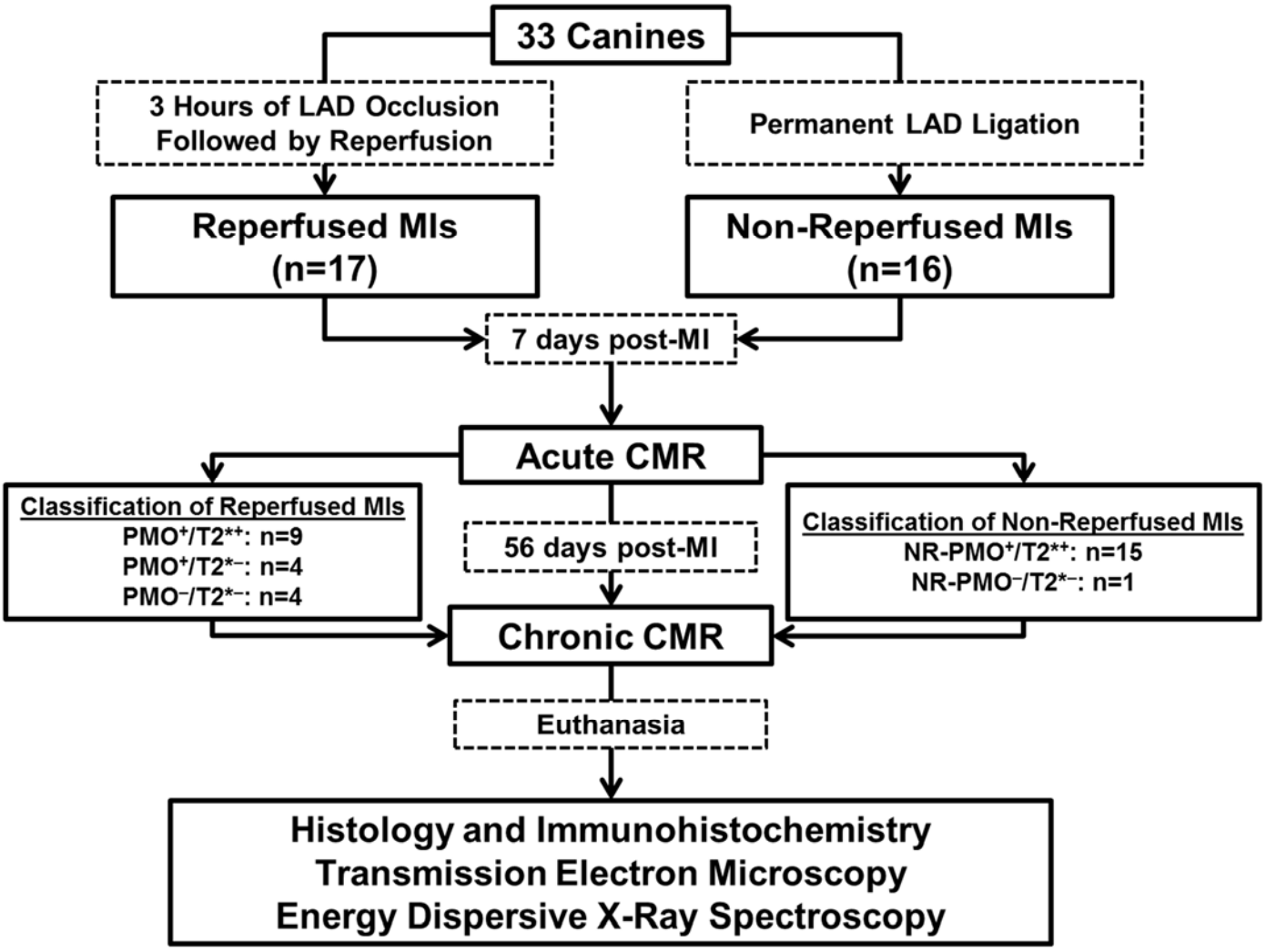
Supplement 7: Summary on Structural Remodeling

Supplement 8: Details on Functional Remodeling

Supplement 1: Study Timeline



Supplement 2: Cardiac MR Imaging Parameters

Table S1. *Cardiac MR imaging parameters for studying acute and chronic reperfused and non-reperfused myocardial infarctions.*

Imaging Method	Cine	T2*-weighted	LGE
Sequence	Balanced SSFP	Multiple GRE	IR – prepared GRE
TR (ms)	3.1	12.0	3.0
TE (ms)	1.6	2.0 – 9.5 (ΔTE = 1.5 ms)	1.5
Flip Angle	40°	10°	25°
Bandwidth (Hz/pixel)	930	930	586
In-plane Resolution	1.4 x 1.4 mm^2		
Slice Thickness	6mm		
Other Parameters	25-30 cardiac phases	6 TEs	Optimal TI to null the remote myocardium

Supplement 3: CMR Image Analysis

All cardiac MRI (CMR) image analyses were performed on cvi[42] image processing software (Circle Cardiovascular Imaging Inc., Calgary, AB). Endocardial and epicardial contours were manually drawn on all images. Remote myocardium was identified on LGE images as the region showing no hyperintensity and a reference region-of-interest (ROI) was drawn in it. Infarcted myocardium was then defined on LGE images using the Mean + 5 Standard Deviations (SD) technique relative to the reference ROI. Persistent microvascular obstruction (PMO) was defined as the hypointense core within the hyperintense infracted myocardium identified on LGE images using the Mean+5SD criterion. Infarct size was calculated by summing the volumes of the hyperintense regions on LGE images identified using the Mean+5SD criterion and the hypointense PMO cores.

The presence of iron arising from blood degradation within infarcted myocardium were identified as hypointense regions on T2*-weighted images confined to the hyperintense LGE territory. The reference ROIs drawn on LGE images were copied on to the corresponding T2*-weighted images. Spatial extent of the hypointense regions were identified on the T2*-weighted image acquired at TE=6.5ms using the Mean-2SD criterion relative to the reference ROI. Off-resonance artifacts arising due to susceptibility differences at the heart-lung interface were manually excluded.

Supplement 4: Antibodies Used for Histopathology

Table S2. *Antibodies used for studying different histopathological markers*

Histopathological Marker	Antibody Used
CD163	Bioss, bs-2527R
Mac387	Abcam, ab22506
IL-1β	Abcam, ab34837
TNF-α	Abcam, ab6671
MMP-9	Abcam, ab38898

Supplement 5: Iron Overloading within Lysosomes

Figure S1 below shows potential overloading of lysosomes within macrophages.

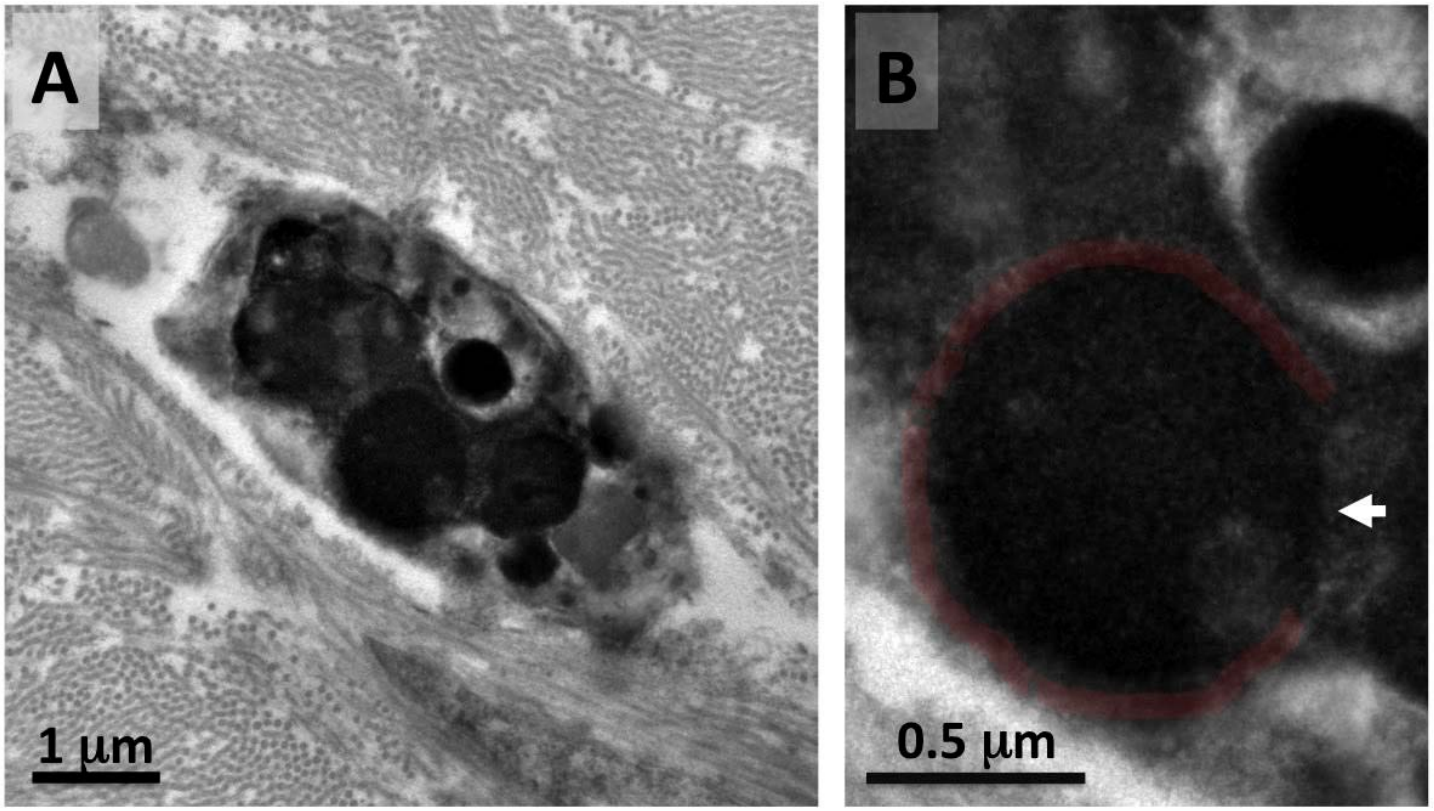


Figure S1. *TEM of Iron Overloaded Lysosomes within a Macrophage*. Panel **A** shows several membrane enclosed spherical organelles, which are consistent with previous description of lysosomes. Panel **B** shows an enlarged version of a potential lysosome (with membrane highlighted in red), which contains electro dense material (iron) whose diameter is well in excess of 1 μm with potential membrane disruption indicated by white arrow.

Supplement 6: Summary on Infarct Characteristics

Table S3 below provides details on infarct volume, PMO/NR-PMO volume, and iron volume in acute and chronic phases of reperfused and non-reperfused myocardial infarctions.

Table S3. *Summary of infarct characteristics between reperfused and non-reperfused MI*

INFARCT CHARACTERISTICS: REPERFUSED VS. NON-REPERFUSED MI				
Phase of MI	**Group**	**Infarct Volume (%LV)**	**PMO/NR-PMO Volume (%LV)**	**Iron Volume (%LV)**
Acute	Reperfused (n=17) (Median (Q1-Q3))	20.7 (18.1-33.6)	1.7 (1.2-7.1)	0.45 (0.0-8.3)
	Non-Reperfused (n=16) (Median (Q1-Q3))	16.2 (7.7-20.0)	2.2 (1.1-4.2)	1.4 (1.0-3.7)
	p-value (Reperfused vs. Non-Reperfused)	0.052	0.86	0.86
Chronic	Reperfused (n=17) (Median (Q1-Q3))	11.3 (6.2-15)	0 (–)	2.9 (0.7-4.3)
	Non-Reperfused (n=16) (Median (Q1-Q3))	7.0 (2.7-9.8)	0 (–)	1.6 (0.8-3.3)
	p-value (Reperfused vs. Non-Reperfused)	0.38	–	0.38

*Statistically significant difference ($p<0.05$)

Supplement 7: Summary on Structural Remodeling

Table S4 below provides details on structural LV remodeling in acute and chronic phases of reperfused and non-reperfused myocardial infarctions.

Table S4. *Summary of structural LV remodeling between reperfused and non-reperfused MI*

STRUCTURAL LV REMODELING REPERFUSED VS. NON-REPERFUSED MI		
Phase of MI	**Group**	**End-Diastolic Sphericity Index**
Acute	Reperfused (n=17) (Median (Q1-Q3))	0.43 (0.38-0.53)
	Non-Reperfused (n=16) (Median (Q1-Q3))	0.42 (0.39-0.47)
	p-value (Reperfused vs. Non-Reperfused)	0.46
Chronic	Reperfused (n=17) (Median (Q1-Q3))	0.49 (0.41-0.58)
	Non-Reperfused (n=16) (Median (Q1-Q3))	0.49 (0.44-0.56)
	p-value (Reperfused vs. Non-Reperfused)	0.79

*Statistically significant difference ($p<0.05$)

Supplement 8: Summary on Functional Remodeling

Tables S5 through S8 below provide details on functional LV remodeling in acute and chronic phases of reperfused and non-reperfused myocardial infarctions. **Table S9** compares reperfused and non-reperfused myocardial infarctions in terms of functional LV remodeling.

Table S5. *Functional LV remodeling in reperfused myocardial infarctions*

REPERFUSED MYOCARDIAL INFARCTIONS				
Group	**Parameter**	**End-Diastolic Volume (mL/m^2)**	**End-Systolic Volume (mL/m^2)**	**Ejection Fraction (%)**
PMO$^+$/T$_2$*$^+$ (n = 9)	Acute (Median (Q1-Q3))	67.4 (58.4-76.3)	47.5 (42.4-56.5)	28.5 (25.1-36.8)
	Chronic (Median (Q1-Q3))	75.5 (69.4-85.1)	58.4 (51.3-68.9)	21.5 (14.6-24.2)
	% Change (Acute to Chronic)	16.0 (8.7-22.2)	19.3 (11.8-29.3)	-23.5 (-32.0-13.7)
	p-value (Acute vs. Chronic)	0.008*	0.01*	0.03*
PMO$^+$/T$_2$*$^-$ (n = 4)	Acute (Median (Q1-Q3))	56.4 (54.1-58.3)	33.7 (27.8-46.4)	40.2 (36.3-46.3)
	Chronic (Median (Q1-Q3))	60.0 (54.1-65.1)	35.2 (29.2-40.0)	42.1 (36.2-49.6)
	% Change (Acute to Chronic)	5.8 (-1.0-12.3)	0.7 (-6.7-8.4)	2.2 (-7.1-9.4)
	p-value (Acute vs. Chronic)	0.30	0.43	0.81
PMO$^-$/T$_2$*$^-$ (n = 4)	Acute (Median (Q1-Q3))	48.2 (46.1-49.1)	29.3 (24.6-31.9)	44.6 (41.4-53.1)
	Chronic (Median (Q1-Q3))	45.0 (41.0-48.3)	30.4 (20.8-39.1)	46.3 (39.7-53.0)
	% Change (Acute to Chronic)	2.5 (-8.2-3.5)	-3.4 (-9.0-0.5)	1.4 (-6.9-7.3)
	p-value (Acute vs. Chronic)	0.32	0.81	0.88

*Statistically significant difference ($p<0.05$)

Table S6. *Comparison of functional LV remodeling across groups in reperfused myocardial infarctions*

REPERFUSED MYOCARDIAL INFARCTIONS				
Parameter	**Phase of MI**	**$PMO^{+}/T_2{*}^{+}$ vs. $PMO^{+}/T_2{*}^{-}$**	**$PMO^{+}/T_2{*}^{+}$ vs. $PMO^{-}/T_2{*}^{-}$**	**$PMO^{+}/T_2{*}^{-}$ vs. $PMO^{-}/T_2{*}^{-}$**
EDV	p-value (Acute)	0.01*	0.02*	0.13
	p-value (Chronic)	0.02*	0.003*	0.04*
	p-value (ΔEDV between Acute and Chronic)	0.03*	0.03*	0.21
ESV	p-value (Acute)	0.001*	<0.001*	0.22
	p-value (Chronic)	0.007*	0.002*	0.17
	p-value (ΔESV between Acute and Chronic)	0.01*	0.008*	0.65
EF	p-value (Acute)	0.01*	0.01*	0.65
	p-value (Chronic)	0.03*	0.01*	0.08
	p-value (ΔEF between Acute and Chronic)	0.04*	0.02*	0.51

*Statistically significant difference ($p<0.05$)

Table S7. *Functional LV remodeling in non-reperfused myocardial infarctions*

NON-REPERFUSED MYOCARDIAL INFARCTIONS				
Group	**Parameter**	**End-Diastolic Volume (mL/m^2)**	**End-Systolic Volume (mL/m^2)**	**Ejection Fraction (%)**
NR-PMO$^+$/T$_2$*$^+$ (n = 15)	Acute (Median (Q1-Q3))	58.5 (55.2-69.3)	37.8 (34.6-47.9)	34.6 (30.5-29.2)
	Chronic (Median (Q1-Q3))	74.1 (70.5-79.2)	44.2 (39.3-54.6)	38.1 (32.3-44.7)
	% Change (Acute to Chronic)	19.1 (11.6-29.6)	14.3 (8.2-28.4)	8.2 (-2.0-14.6)
	p-value (Acute vs. Chronic)	0.001*	0.003*	0.14
NR-PMO$^-$/T$_2$*$^-$ (n = 1)	Acute (Median (Q1-Q3))	56.1	32.0	43.0
	Chronic (Median (Q1-Q3))	67.4	37.8	43.9
	% Change (Acute to Chronic)	20.1	18.1	7.6
	p-value (Acute vs. Chronic)	–	–	–

*Statistically significant difference ($p<0.05$)

Table S8. *Comparison of functional LV remodeling across groups in non-reperfused myocardial infarctions*

NON-REPERFUSED MYOCARDIAL INFARCTIONS		
Parameter	**Phase of MI**	**NR-PMO$^+$/T$_2$*$^+$ vs. NR-PMO$^+$/T$_2$*$^-$**
EDV	p-value (Acute)	0.04*
	p-value (Chronic)	0.01*
	p-value (ΔEDV between Acute and Chronic)	0.65
ESV	p-value (Acute)	0.005*
	p-value (Chronic)	0.003*
	p-value (ΔESV between Acute and Chronic)	0.06
EF	p-value (Acute)	0.005*
	p-value (Chronic)	0.01*
	p-value (ΔEF between Acute and Chronic)	0.91

*Statistically significant difference ($p<0.05$)

Table S9. *Summary of functional LV remodeling between reperfused and non-reperfused MI*

FUNCTIONAL LV REMODELING: REPERFUSED VS. NON-REPERFUSED MI				
Phase of MI	**Parameter**	**Ejection Fraction (%)**	**End-Diastolic Volume (mL/m^2)**	**End-Systolic Volume (mL/m^2)**
Acute	Reperfused (n=17) (Median (Q1-Q3))	39.1 (30.4-48.1)	55.3 (49.8-62.9)	33.9 (27.8-44.6)
	Non-Reperfused (n=16) (Median (Q1-Q3))	36.0 (30.7-40.3)	57.5 (55.6-69.6)	37.2 (32.1-46.0)
	p-value (Reperfused vs. Non-Reperfused)	0.21	0.33	0.27
Chronic	Reperfused (n=17) (Median (Q1-Q3))	39.4 (25.8-48.6)	68.1 (57.4-79.2)	35.5 (26.2-49.2)
	Non-Reperfused (n=16) (Median (Q1-Q3))	38.6 (32.4-45.0)	73.3 (69.1-78.0)	42.1 (39.1-53.2)
	p-value (Reperfused vs. Non-Reperfused)	0.12	0.65	0.76

*Statistically significant difference ($p<0.05$)