

Mitochondrial Transfer through Pericardium Contributes to Functional Recovery in Ischemic Cardiomyopathy

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Supplemental Materials

Table S1. Composition of the hADSC graft

	Composition	Content	Total
Solution A	Fibrinogen	60 μ L (4.8 mg)	200 μ L
	HBSS	140 μ L	
Solution B	Thrombin	30 μ L (9 units)	200 μ L
	ADSC/HBSS	5.0×10^5 - 5.0×10^6 cells in 170 μ L	

Solutions A and B were prepared immediately prior to transplantation; the number of ADSCs was adjusted in solution B. Both solutions were dropped onto the hearts' surface, forming the graft on site.

Table S2. Primer sequences for semi-quantitative PCR and sequencing of the mitochondrial genome

Species	Target gene	Forward primer (5'–3')	Reverse primer (5'–3')	Amplicon (bps)
Rat	mt	CCT CCC ATT CAT TAT CGC CCT TGC	GTC TGG GTC TCC TAG GTC TGG GAA	211
Human	mt	CCC CAC AAA CCC CAT TAC TAA ACC CA	TTT CAT GCG GAG ATG TTG GAT GG	221
Rat	Actin	GGG ATG TTT GCT CCA ACC AA	GCG CTT TTG ACT CAA GGA TTT AA	-

Bps, base pairs, mt, mitochondria

Table S3. Thermal cycler settings for DNA amplification and sequencing

	DNA amplification		Sequencing	
Initial denaturation	94 °C, 2 min		96 °C, 1 min	
Denaturation	94 °C, 30 s	40 cycles	96 °C, 10 s	25 cycles
Annealing	60 °C, 30 s		50 °C, 5 s	
Elongation	72 °C, 1 min		60 °C, 4 min	
Final extension	72 °C, 5 min		-	
Hold	4 °C, ∞		4 °C, ∞	

Table S4. Reaction mixture for DNA amplification and sequencing

DNA amplification		Sequencing	
TAKARA polymerase/Mix	10 μ L	BigDye Terminator Ready Reaction Mix	8 μ L
Template	60 ng	Template	10 ng
Primer FWD	1 μ L	Primer FWD	0.5 μ L
Primer REV	1 μ L	Primer REV	0.5 μ L
Nuclease-free water	Adjusted depending on template volume	Deionized water	Adjusted depending on template volume
Total volume	20 μ L	Total volume	20 μ L

Table S5. Culture conditions of each group

	Rat cardiomyocytes	Human ADSCs	α GA (final concentration 10 μ M)
A	1×10^6	1×10^6	-
B	1×10^6	1×10^6	+
C	-	1×10^6	-
D	-	1×10^6	+
E	1×10^6	-	-
F	1×10^6	-	+
G	-	-	-

Table S6. Composition of modified Tyrode's solution

Modified Tyrode's Solution	g/L
NaCl	8
KCl	0.2
NaH ₂ PO ₄ • 2H ₂ O	0.05
MgCl ₂ • 6H ₂ O	0.2
Glucose	1

Reagents were dissolved in distilled water and pH was adjusted to 7.4 using NaOH.

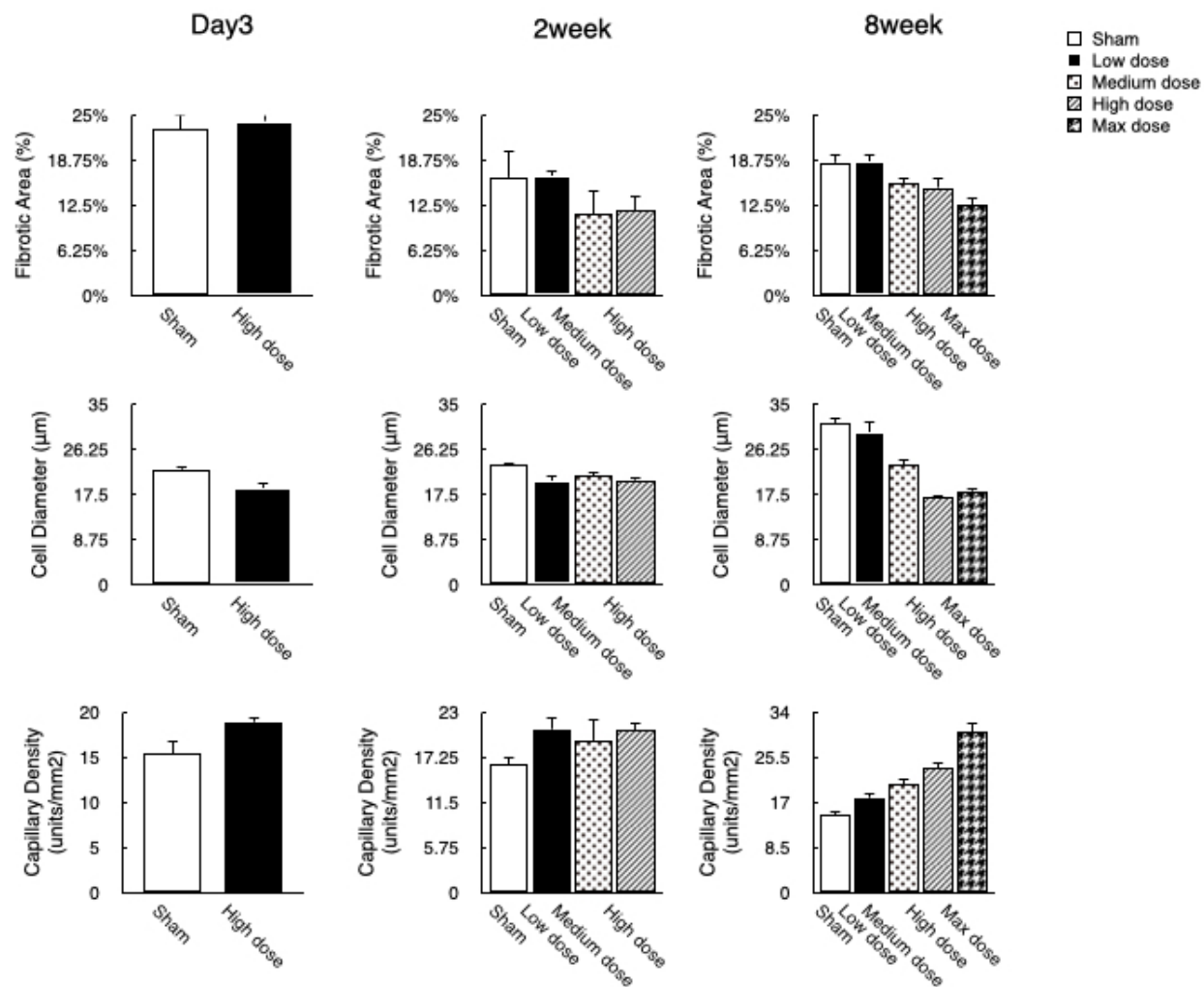


Figure S1

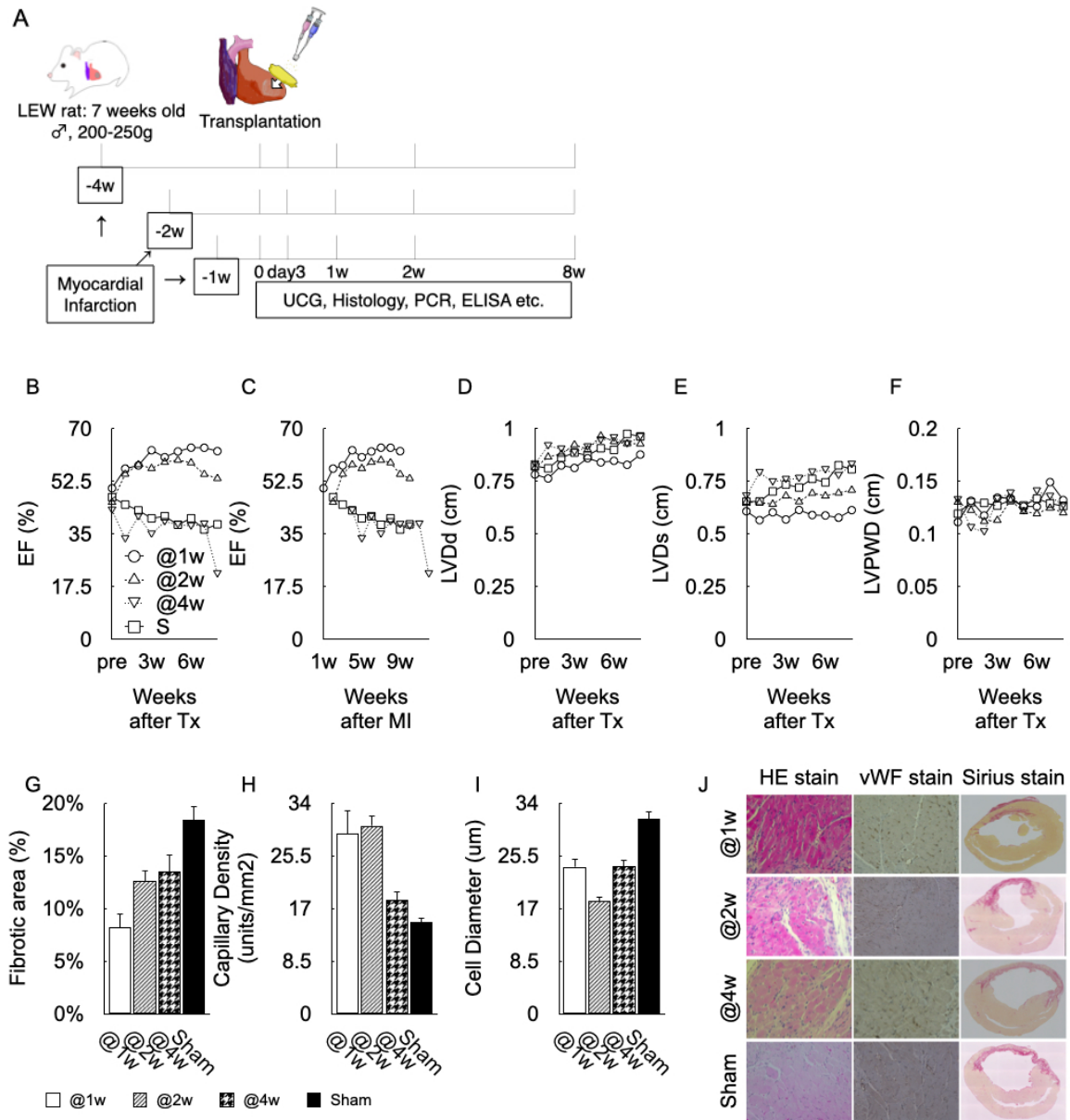


Figure S2