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SUPPLEMENTARY INFORMATION

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Cardiac recovery via extended cell-free delivery of extracellular vesicles secreted by cardiomyocytes derived from induced pluripotent stem cells

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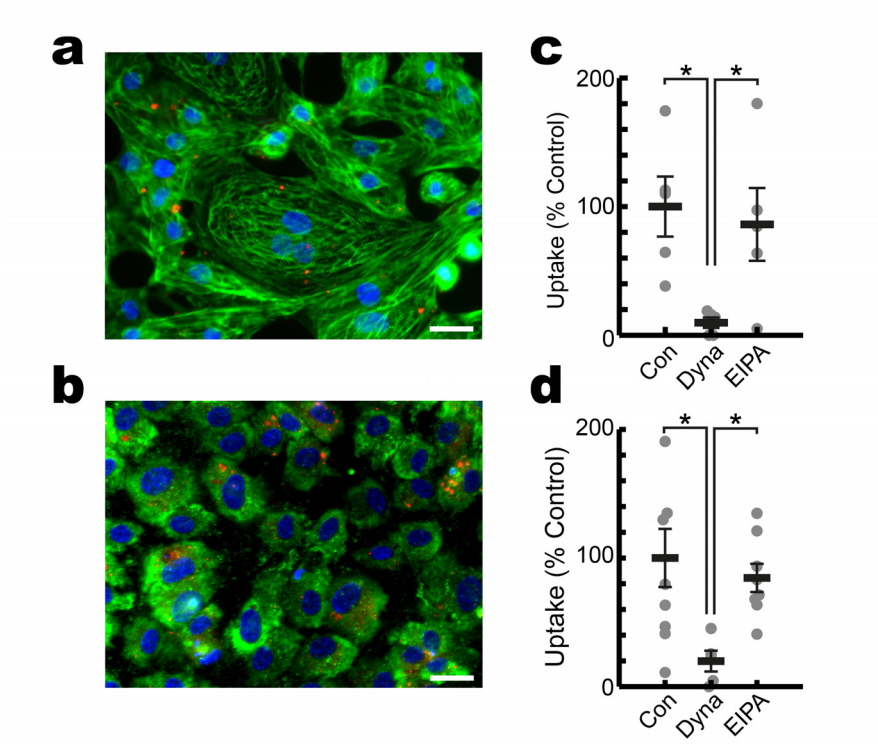
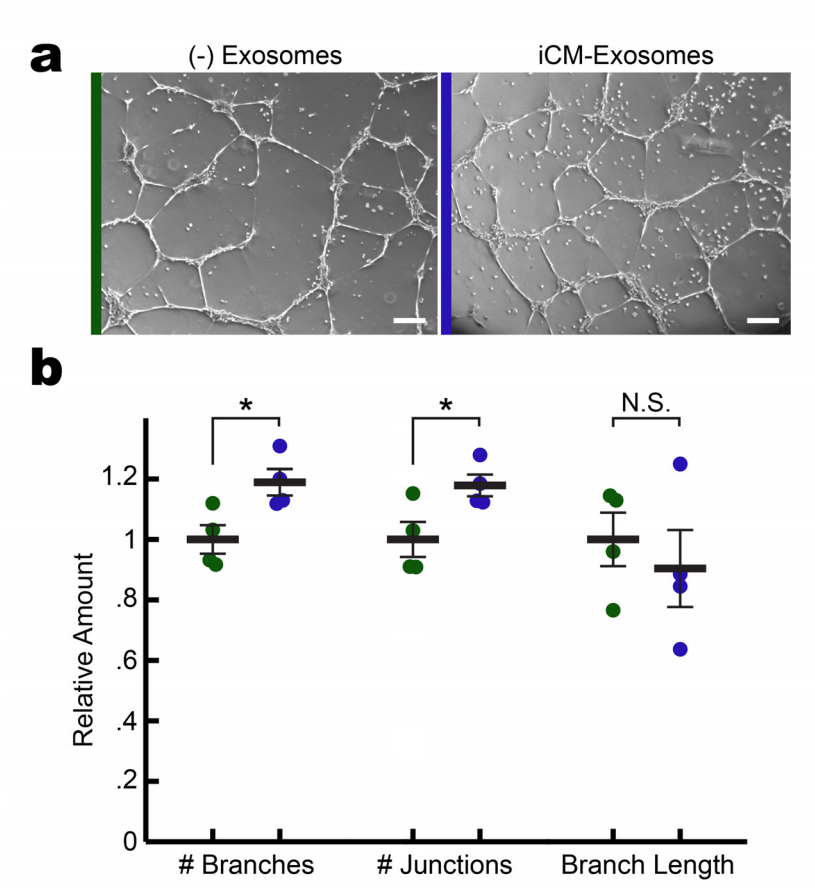
Supplementary figures



***Supp. Figure 1**: **iCMs and HUVECs uptake iCM-EVs via dynamin-mediated endocytosis.** **a)** Representative image of DiI (red) stained iCM-EVs found inside troponin (green) and DAPI (blue) stained iPS-CMs. Scale = 25 µm. **b)** Representative image of DiI (red) stained iCM-EVs found inside von Willebrand factor (green) and DAPI (blue) stained HUVECs. Scale = 25 µm. **c**) Uptake of EVs by iCMs in the presence of dynamin mediated endocytosis inhibitor dynasore or micropinocytosis inhibitor EIPA (Average ± SEM, n≥5). **d)** Uptake of EVs by HUVECs in the presence of dynasore or EIPA (Average ± SEM, n≥5)*



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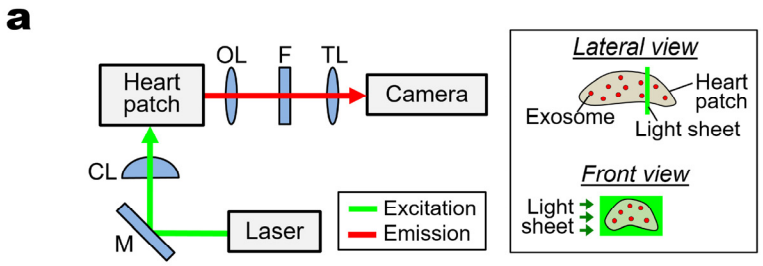
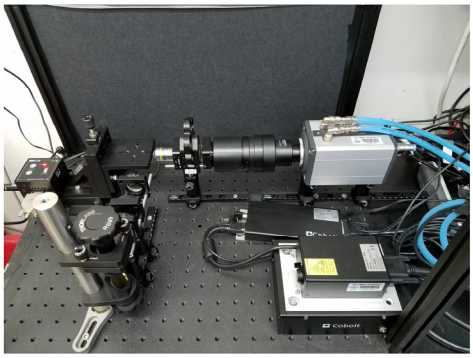
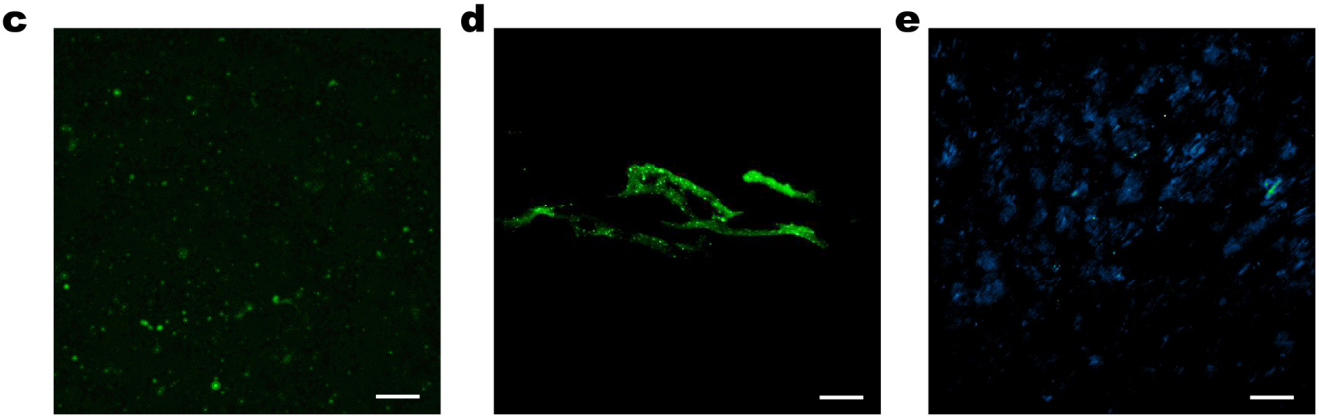
Supp. Figure 2: iCM-EVs protect HUVECs from hypoxic injury. ***a)*** *Representative phase contrast images of networks formed by HUVECs on Matrigel without (left) or with (right) EVs. Scale = 200 µm.* ***b)*** *The number of branches and junctions, and cumulative network branch length of HUVECs without (green) and with (blue) EVs (Average ± SEM, n=4). (* denotes p<0.05 N.S. denotes not significant).*

	miRNA-seq			miRNA-RTPCR		
	iCMex(TPM)	iPSCex(TPM)	log fold change	iCMex(dCT)	iPSCex(dCT)	log fold change
miR-1	137765	590	**7.38**	0.78	4.01	**3.24**
let-7a	178	561	**-1.66**	7.41	5.37	**-2.05**

Supp. Figure 3: RT-PCR Confirmation of miRNA differential expression. *Fold change of iCM-EV miRNA levels over iPS-EVs miRNA levels (Average, N = 3)*







f

	WT(dCT)	iCMex(dCT)	iPSex(dCT)	log fold change
miR-148a	12.12	4.08		**8.04**
	12.12		4.27	**7.85**
miR-302a	18.76	16.16		**2.61**
	18.76		13.65	**5.12**

Supp. Figure 4: In vivo EV imaging and quantification a) *Custom laser light sheet fluorescence microscopy platform schematic and* ***b)*** *image.* ***c)*** *iCM EVs (Green) stained with PKH-67 prior to patch incorporation,* ***d)*** *within the patch 24 hours after implantation, and* ***e)*** *within the myocardium (scale = 50um) (blue represents background)* ***f)*** *RT-PCR quantification of miR-148a and miR-302a from RNA isolated from the rat left ventricle directly beneath the patch 24 hours after application of iCMex patch or iPSex patch compared to no patch control. (Average, n=4 WT rats, n = 3 iCMex and iPSex patch rats).*

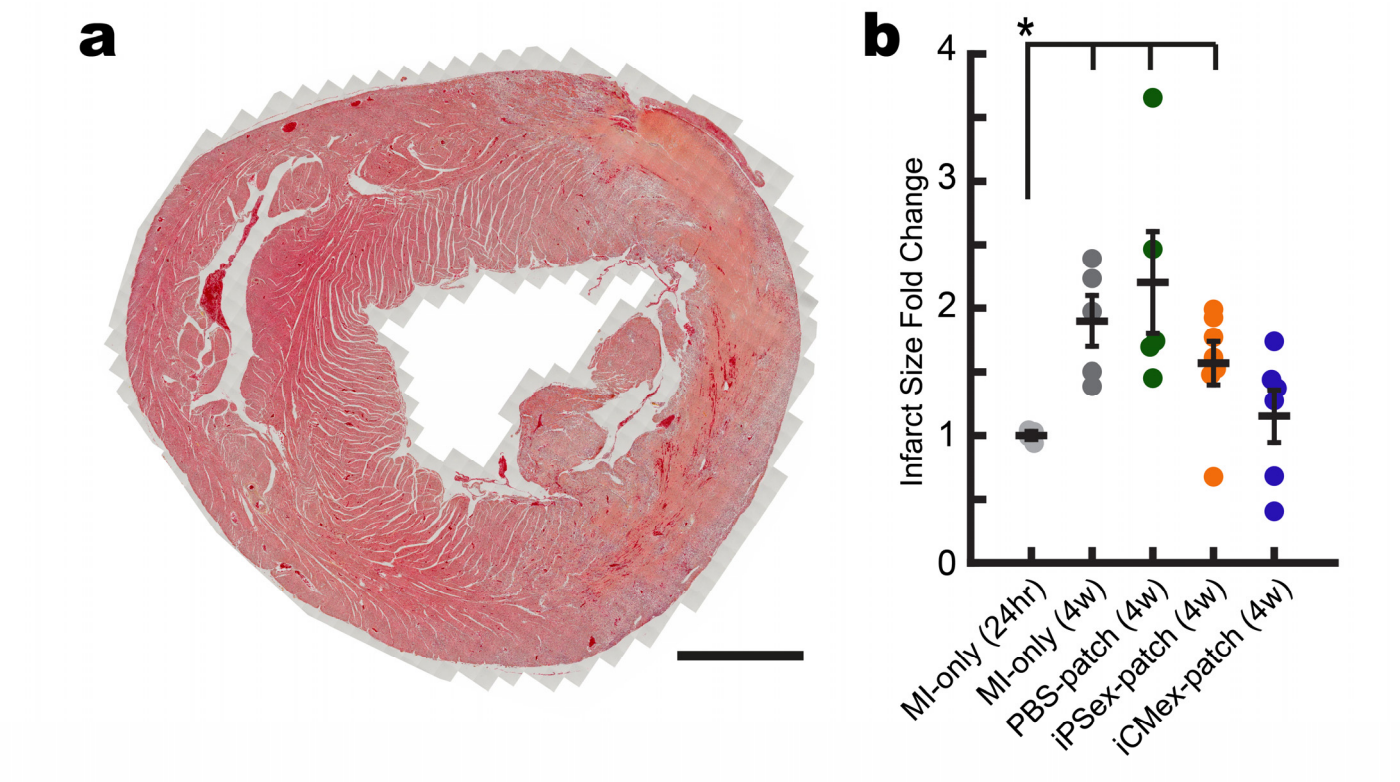
a

	Sham		MI only		PBS		iPS-Exosomes		iCM-Exosomes	
	Number	Avg ± SEM	Number	Avg ± SEM	Number	Avg ± SEM	Number	Avg ± SEM	Number	Avg ± SEM
AVB	3/3	4 ± 1.2	4/4	11.4 ± 2.2	5/5	12.8 ± 4.5	7/7	7.0 ± 1.3	5/5	4.6 ± 1.4
PVC	0/3	0 ± 0	4/4	67.2 ± 14.6	5/5	62.8 ± 10.5	7/7	76.6 ± 23.2	5/5	51.6 ± 12.9
VT	0/3	0 ± 0	0/4	0 ± 0	2/5	0.6 ± 0.4	2/7	1.9 ± 1.3	1/5	1.0 ± 1.0
VF	0/3	0 ± 0	0/4	0 ± 0	0/5	0 ± 0	0/7	0 ± 0	0/5	0 ± 0
Total	3/3	4 ± 1.2	4/4	78.8 ± 16.4	5/5	76.2 ± 8.9	7/7	85.4 ± 24.7	5/5	57.2 ± 13.7

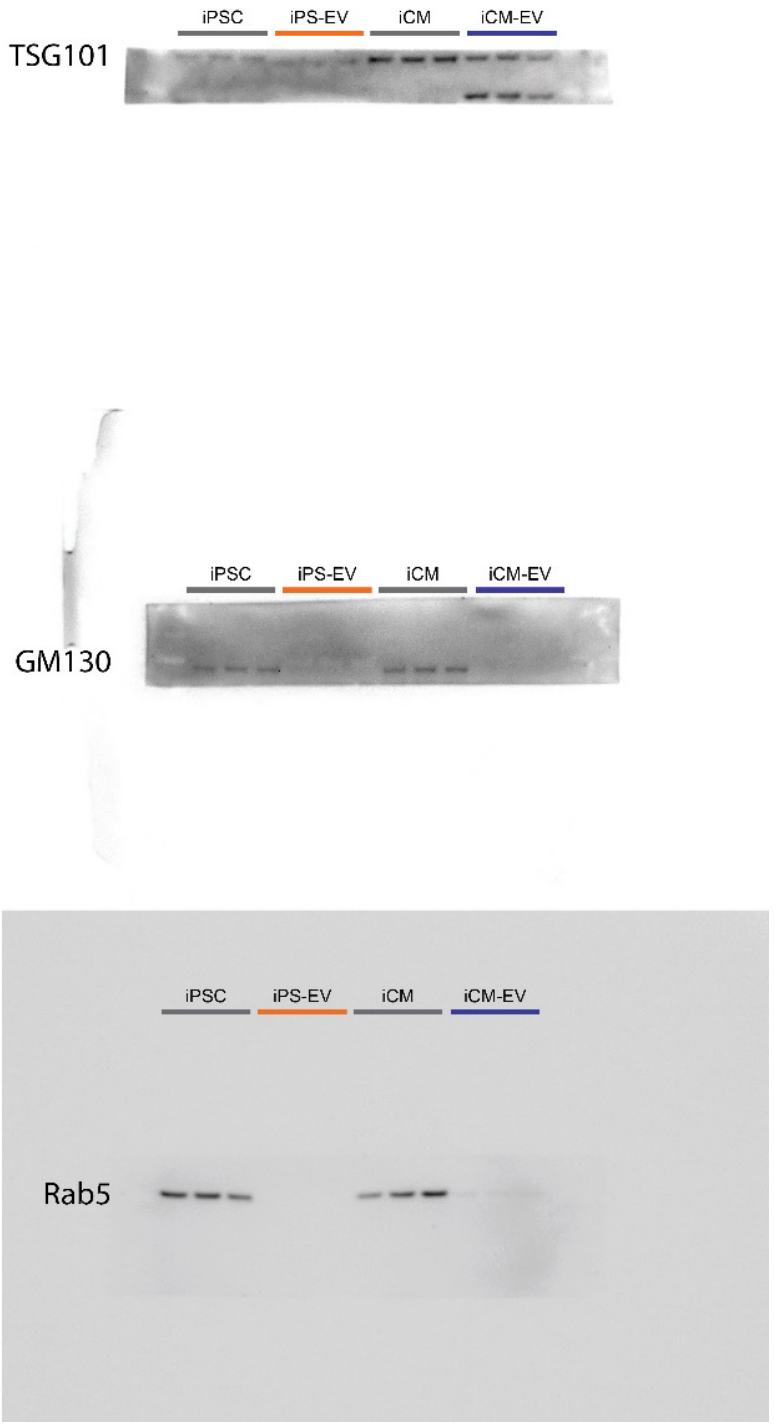
b

	Sham		MI only		PBS		iPS-Exosomes		iCM-Exosomes	
	Number	Avg ± SEM	Number	Avg ± SEM	Number	Avg ± SEM	Number	Avg ± SEM	Number	Avg ± SEM
AVB	2/3	3.7 ± 1.9	0/5	0 ± 0	5/5	12.8 ± 6.5	4/7	7.9 ± 3.9	3/5	1.0 ± 0.5
PVC	2/3	6.0 ± 4.2	5/5	31.2 ± 9.4	5/5	70.6 ± 21.3	7/7	22.9 ± 7.0	3/5	13.6 ± 10.1
VT	0/3	0 ± 0	2/5	1.6 ± 0.4	3/5	2 ± 1.5	1/7	0.14 ± 0.13	1/5	0.4 ± 0.4
VF	0/3	0 ± 0	0/5	0 ± 0	0/5	0 ± 0	1/7	0.14 ± 0.13	0/5	0 ± 0
Total	2/3	10.7 ± 5.8	5/5	32.8 ± 9.3	5/5	85.4 ± 18.5	7/7	31.1 ± 8.7	4/5	15.0 ± 10.5

Supp. Figure 5 iCM-EV treatment did not cause increased arrhythmias after MI. ***a)*** *Incidence and number of arrhythmias during the first 5 days after LAD ligation.* ***b)*** *Incidence and number of arrhythmias during the first 3 hours after isoproterenol challenge 7 days after LAD ligation.*



Supp. Figure 6 iCM-EVs prevent enlargement of infarct. a*) Representative transverse cardiac section 24 hours after LAD-ligation stained with Movat's pentachrome stain (scale = 2 mm)* ***b)*** *Infarct size as a percentage of total left ventricle area fold change compared to 24 hour infarct size. (Average ± SEM, * denotes p<0.05).*



Supp. Figure 7 Full western blot scans. Full scans of immunoblots of cell lysates and microvesicle fractions for the exosome marker TSG101, and intracellular proteins (GM130, Rab5) (n = 3 biologically independent samples per group).