An exosomal-carried short periostin isoform induces cardiomyocyte proliferation

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Supplementary Figures:

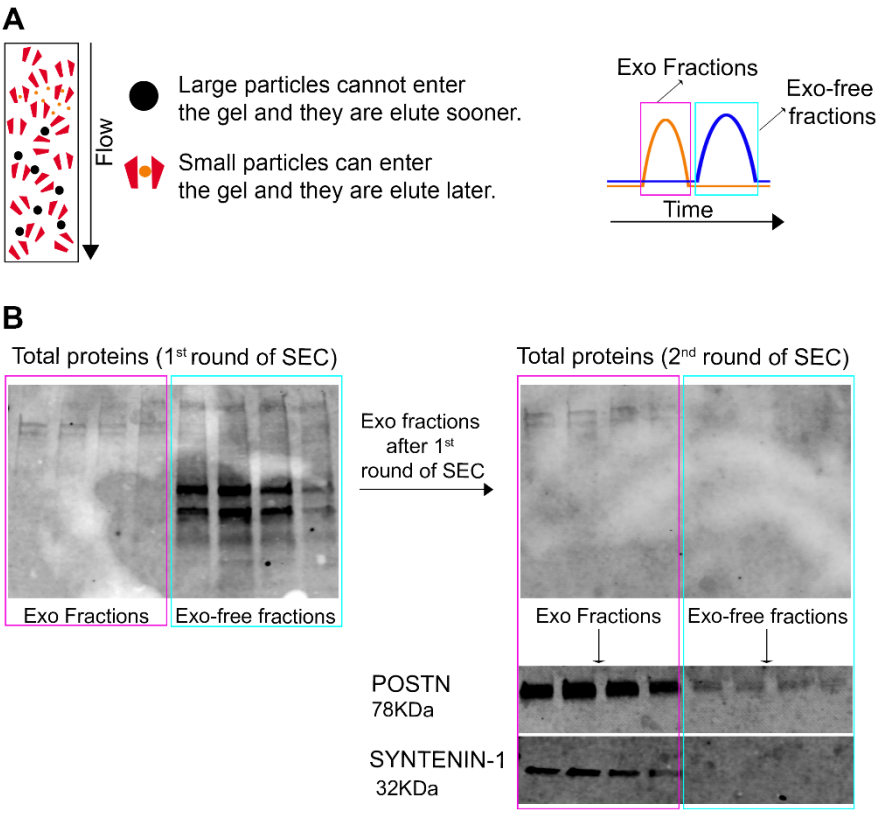

Figure S1: Periostin enrichment in exosome-containing SEC fractions of CPC conditioned medium

A Schematic of size exclusion chromatography (SEC) isolation principle. **B** Western blot analysis of total protein, periostin (POSTN) and exosome marker SYNTENIN-1. Left panel: Total protein staining after the first round of SEC purification. Right panel: Total protein, POSTN and SYNTENIN-1 after the second round of Exo SEC isolation. POSTN was enriched in Exo fractions, identified by high SYNTENIN-1 levels, vs. non-vesicular fractions.

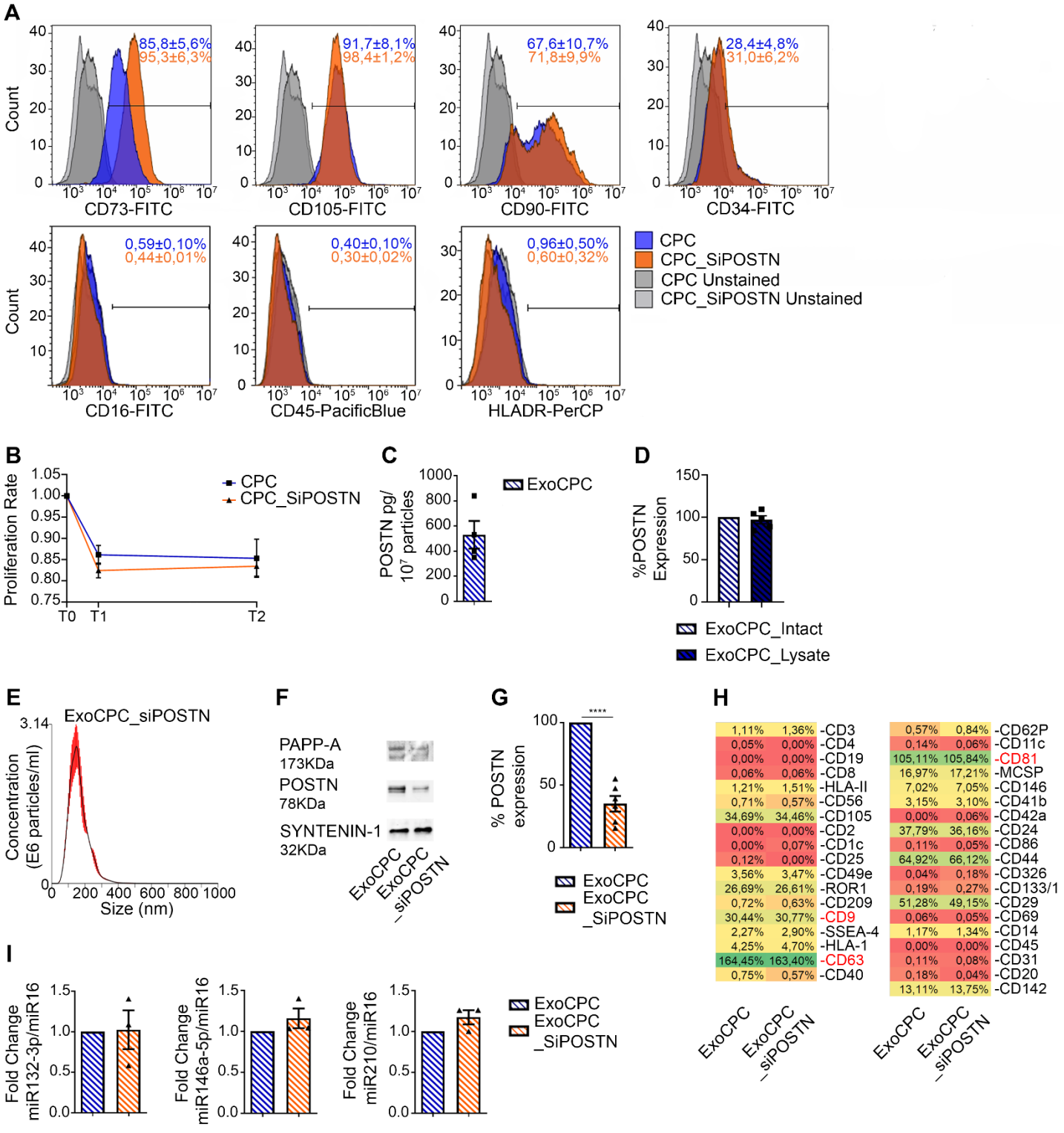

Figure S2: Characterization of periostin-silenced CPC and CPC-derived exosomes

A Flow cytometry analysis of surface antigen expression in naïve CPC and CPC transfected with a siRNA against periostin (CPC_SiPOSTN; *n* = 3). **B** Proliferation rates of naïve CPC and CPC_SiPOSTN at T0 (pre-transfection); T1 (24 hrs post-transfection) and T2 (5 days post-

transfection; *n* = 3). **C** POSTN quantification (pg/10^7 Exo particles) on intact ExoCPC by ELISA (*n* = 4). **D** POSTN levels in ExoCPC lysate vs. intact ExoCPC, measured by ELISA (% of levels in intact ExoCPC; *n* = 5). **E** Nanoparticle tracking analysis of Exo from CPC_SiPOSTN (ExoCPC_SiPOSTN). **F** Western analysis of pregnancy-associated plasma protein-A (PAPP-A)/pappalysin-1, POSTN, and SYNTENIN-1 in ExoCPC and ExoCPC_SiPOSTN. **G** POSTN levels, normalized for SYNTENIN-1 levels, in ExoCPC_SiPOSTN vs. naïve ExoCPC, as assessed by Western blotting (% of levels in naïve ExoCPC; *n* = 6; **** *p<0.0001*). **H** MACSPlex analysis of surface marker expression in ExoCPC and ExoCPC_SiPOSTN (green, yellow, and red colours indicate high, moderate, and low expression levels, respectively). **I** Real-time RT-PCR analysis of miR132-3p, miR146a-5p, and miR210 expression, in ExoCPC_SiPOSTN vs. ExoCPC (*n* = 3).

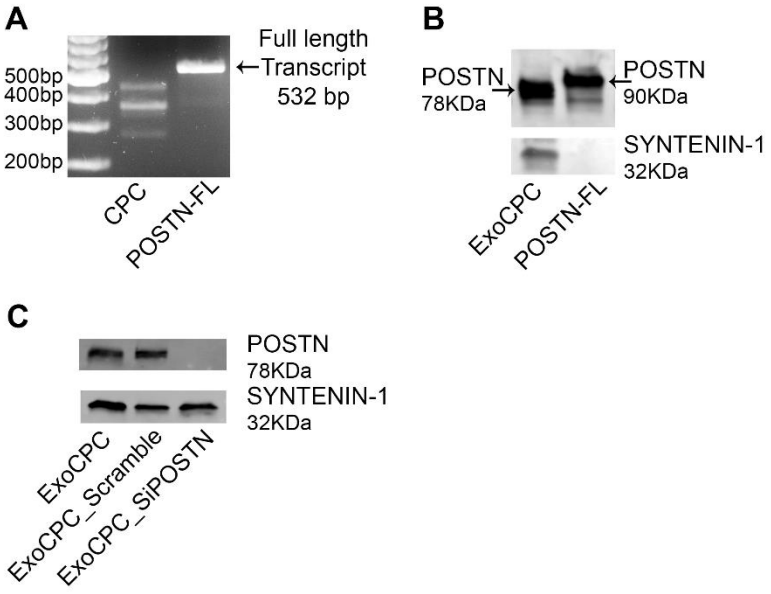

Figure S3: Assessment of POSTN transcripts in CPC and POSTN protein levels in exosomes from naïve, scramble-siRNA transfected, and periostin-silenced transfected CPC

A PCR analysis of human periostin (POSTN) on CPC cDNA. cDNA from a full-length human periostin (POSTN-FL) expression plasmid was used for comparison. **B** Western analysis of POSTN and SYNTENIN-1 (exosome marker) expression in ExoCPC. Recombinant human POSTN-FL was loaded for size comparison. **C** Western analysis of POSTN and SYNTENIN-1 expression in ExoCPC and in Exo from CPC transfected with scamble-siRNA (ExoCPC_Scramble), or with siRNA against periostin (ExoCPC_SiPOSTN).

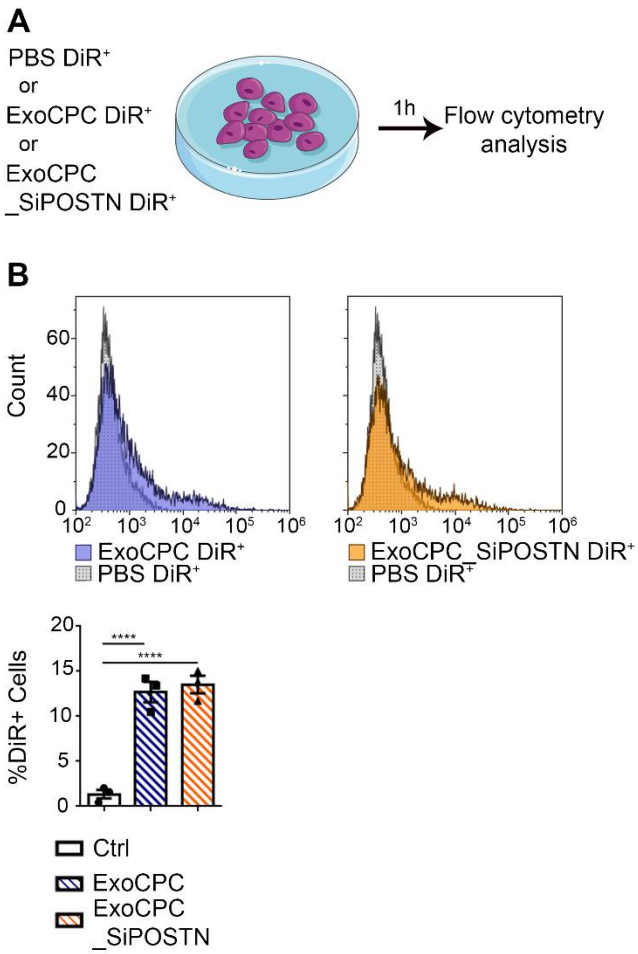

Figure S4: Periostin silencing does not affect exosome cellular uptake

A Schematic of the experimental protocol. CPC-derived Exo (ExoCPC) were labelled with DiR fluorescent dye (ExoCPC DiR$^+$) and re-purified by SEC to remove dye excess. DiR diluted in PBS at the same concentration used in ExoCPC, and processed with a SEC column, was used as a control (PBS DiR$^+$). ExoCPC DiR$^+$ or PBS DiR$^+$ were added to cultured neonatal rat cardiomyocytes, which were analysed by flow cytometry 1 hour later. **B** Quantification of DiR$^+$ cells after treatment with naïve ExoCPC or Exo from CPC transfected with a siRNA against periostin (ExoCPC_SiPOSTN; *n* = 3; **** *p<0.0001*).

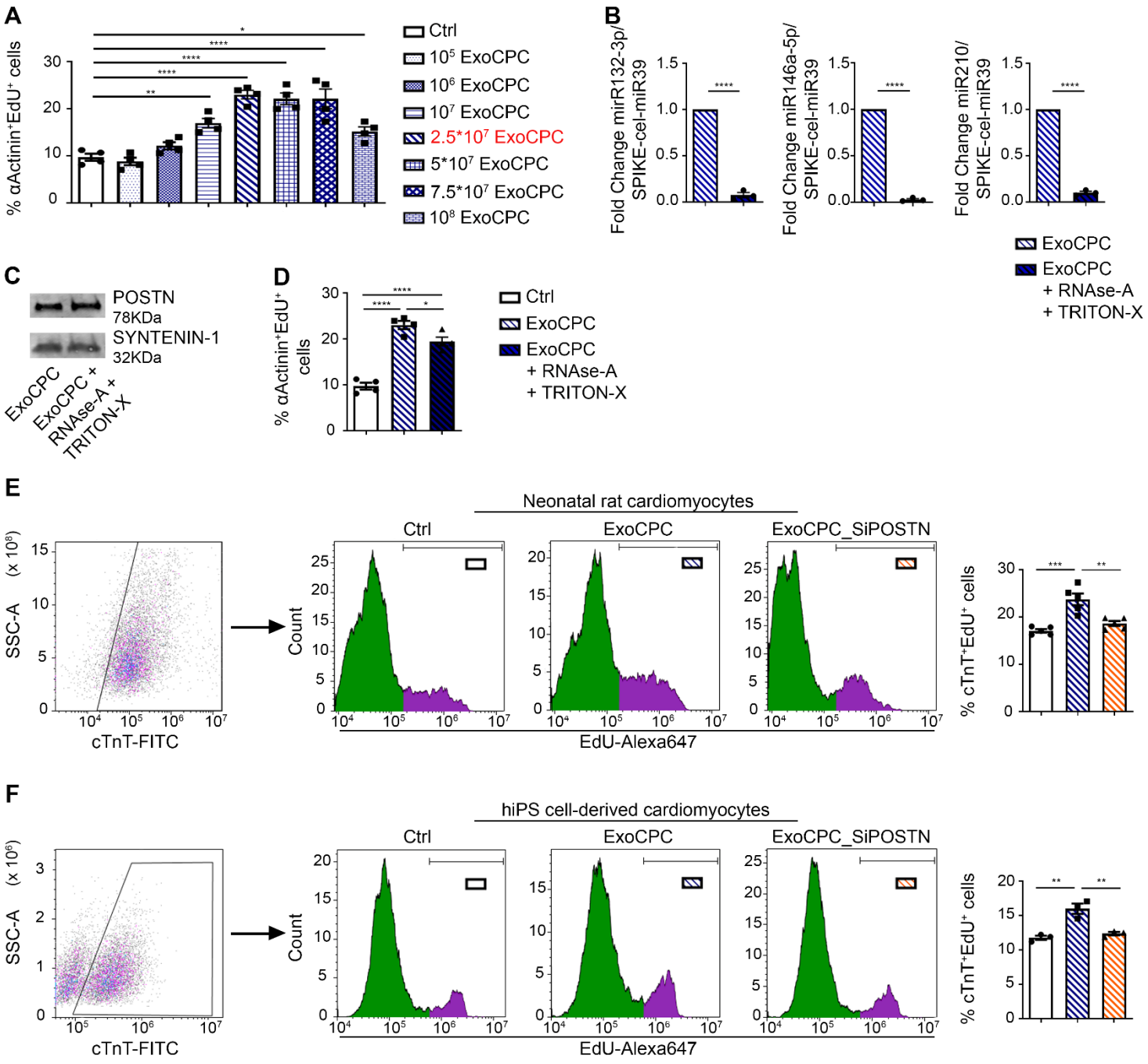

Figure S5: Exosome dose-response study, effect of miRNAs on cell cycle activity, and periostin-dependent effect of exosomes on rat neonatal cardiomyocyte and hiPS cell-derived cardiomyocyte proliferation

A Dose-response study of CPC-secreted Exo (ExoCPC) and neonatal rat cardiomyocyte cell cycle activity. Quantitative analysis of EdU-positive cardiomyocytes (% of cardiac α-actinin–positive cells; *n* = 4; * *p=0.0413;* ** *p=0.0033;* **** *p<0.0001*). A concentration of 2,5*10^7 particles/well was found to be most effective. **B** Real-time RT-PCR analysis of miR132-3p, miR146a-5p, and miR210 levels

in both untreated and RNAse-A–treated ExoCPC (fold-changes vs. ExoCPC; *n* = 3; **** *p<0.0001*). **C** Western analysis of POSTN and SYNTENIN-1 levels in both untreated and RNAse-A–treated ExoCPC. **D** Quantitative analysis of EdU-positive rat cardiomyocytes (% of αActinin-positive cells; *n* = 4; * *p=0.0417;* **** *p<0.0001*) treated with naïve CPC-derived Exo (ExoCPC), RNAse-A treated ExoCPC or PBS (Ctrl). **E** Flow cytometric analysis of EdU^+ cardiomyocytes (% of cardiac-specific troponin T positive cells (TnT^+)) treated with naïve ExoCPC, Exo from CPC transfected with a siRNA against POSTN (ExoCPC_SiPOSTN), or PBS (Ctrl; *n* = 3; ** *p=0.0019;* *** *p=0.0002*). **F** Flow cytometry analysis of EdU^+ hiPS cell-derived cardiomyocytes (% of $cTnT^+$ cells) treated with naïve ExoCPC, ExoCPC_SiPOSTN, or PBS (Ctrl; *n* = 3; ** *p=0.0021* and *p=0.0045*).

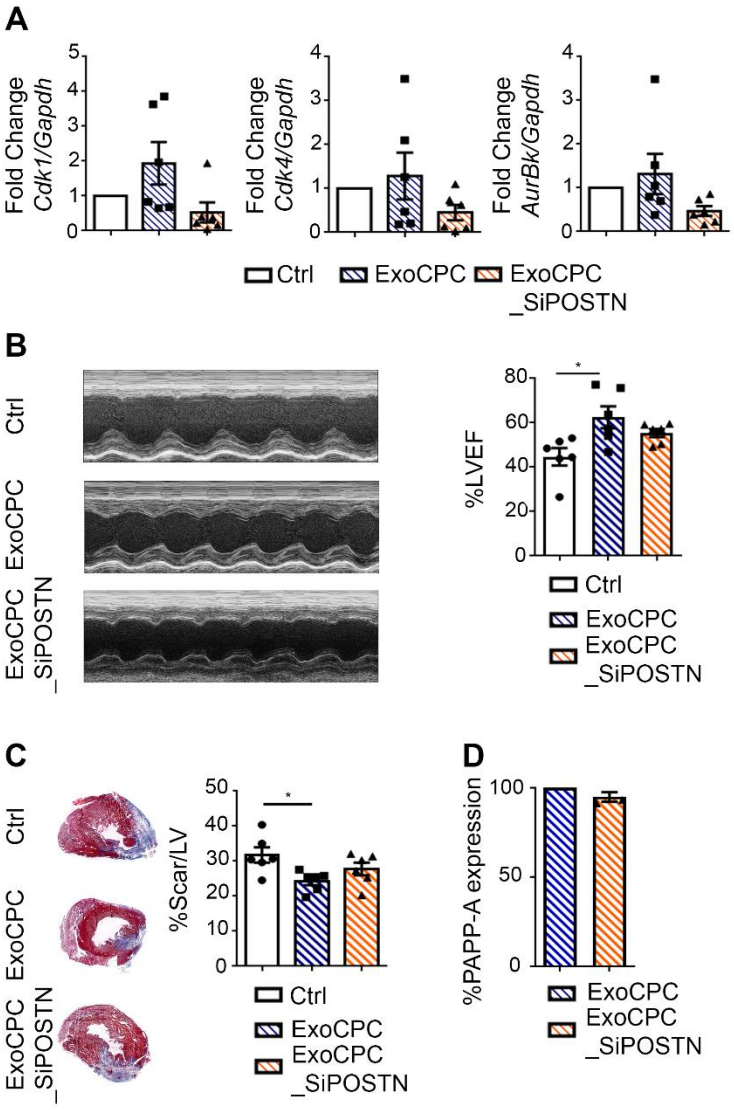

Figure S6: Effects of CPC-derived exosomes in an *in vivo* model of adult rat myocardial infarction (MI).

A Real-time RT-PCR analysis of *Cdk1*, *Cdk4*, and *AurBk* mRNA expression in dispersed isolated cardiomyocytes from rat hearts at day 14 post-MI. Infarcted rat hearts were injected intramyocardially with naïve ExoCPC, Exo from CPC transfected with a siRNA against periostin (ExoCPC_SiPOSTN), or PBS (Ctrl; 6 animals/group). **B** Left panel: Representative M-mode echocardiographs at day 14 post-MI in the different groups. Right panel: Quantitative analysis of left ventricular ejection fraction (LVEF; %) at day 14 post-MI (n = 6; * $p=0.0124$). **C** Representative heart sections at day 14 post-MI stained with Masson-trichrome. Quantitative analysis of infarct scar size (% of LV area; n = 6; * $p=0.0251$). **D** Western blot analysis of pregnancy-associated plasma protein-A (PAPP-A) expression, normalized for SYNTENIN-1 expression, in naïve ExoCPC and ExoCPC_SiPOSTN. (% ExoCPC; n = 3).

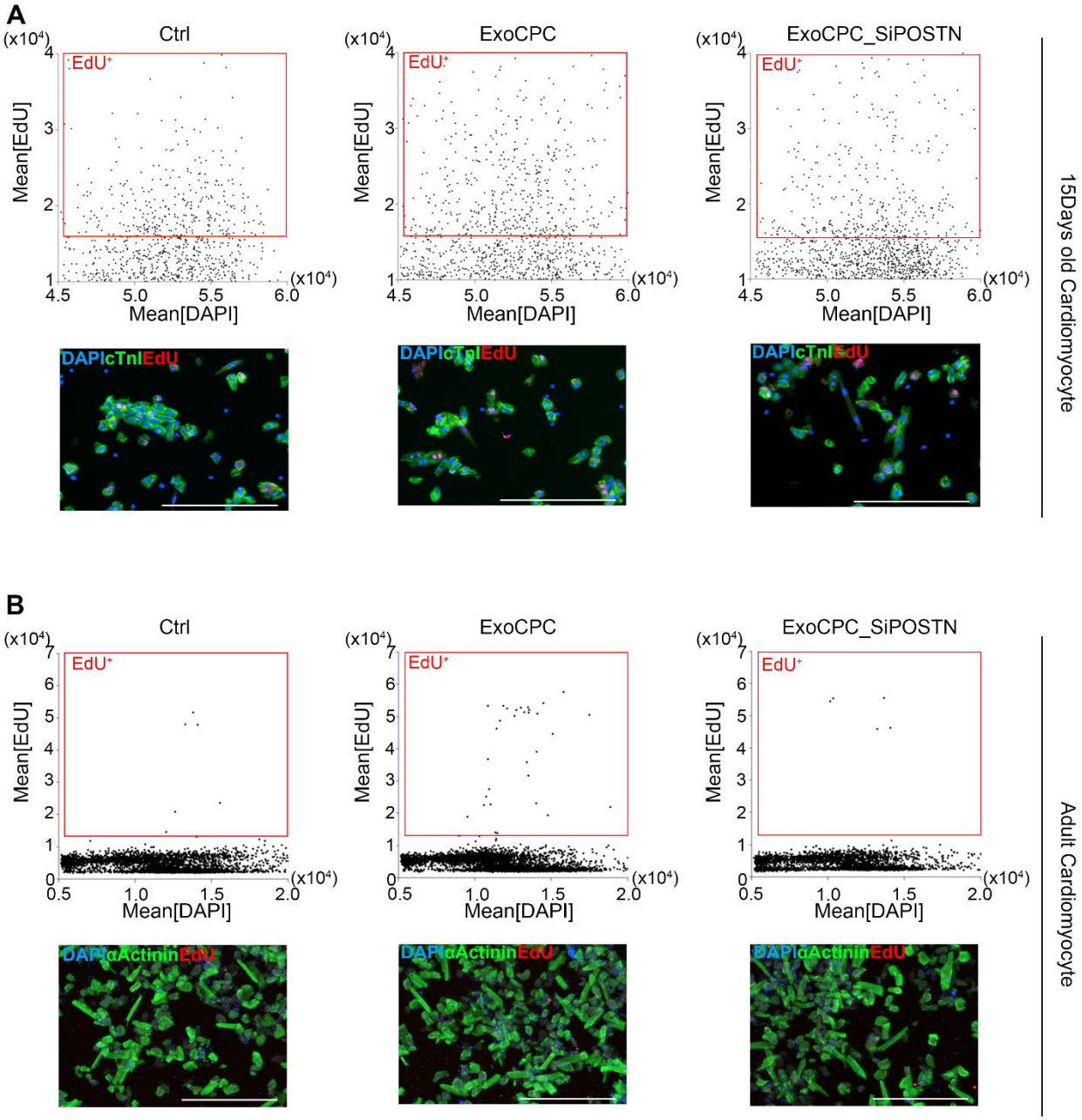

Figure S7: EdU nuclear incorporation analysis in dispersed isolated cardiomyocytes

A Dispersed isolated neonatal rat cardiomyocyte (see Figure 5E for the schematic protocol of the treatment) immunostained for EdU (red) and cardiac-specific troponin I (cTnI; green). Scale bar: 200µm. Representative scatter plot of EdU+ nuclei on DAPI stained nuclei are shown. Quantitative analysis is shown in Figure 5E. **B** Dispersed isolated adult rat cardiomyocyte from hearts explanted at day 14 post-MI immunostained for EdU (red) and cardiac-specific sarcomeric α-actinin (green).

Scale bar: 200µm. Representative scatter plot of EdU+ nuclei on DAPI stained nuclei are shown. Quantitative analysis is shown in Figure 6C.

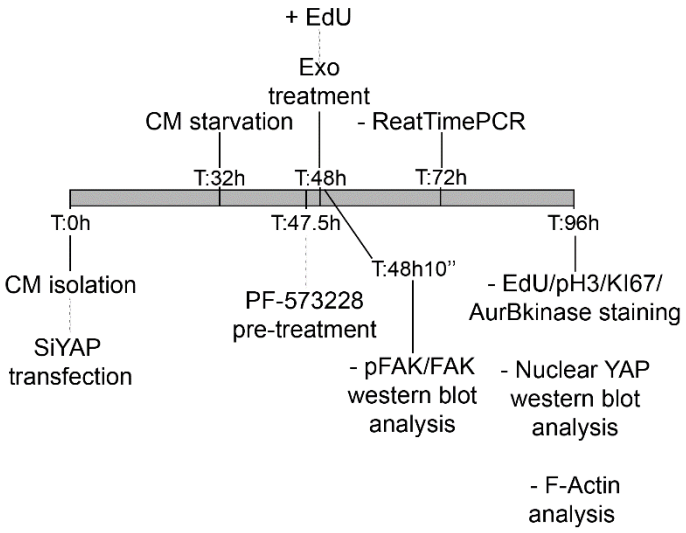

Figure S8: *In vitro* experimental timeline

Schematic of the experimental protocol of *in vitro* assays on neonatal rat cardiomyocytes. The relevant time points (T) are indicated.

Supplementary Tables:

Supplementary Table 1

Accession	Description	nPSM
30102	type I collagen [Homo sapiens]	185,6
119590947	fibronectin 1, isoform CRA_l [Homo sapiens]	184,2
762938	unnamed protein product [Homo sapiens]	130,3
211904152	glia-derived nexin isoform b precursor [Homo sapiens]	116,0
378404908	glyceraldehyde-3-phosphate dehydrogenase isoform 2 [Homo sapiens]	114,2
557786190	**periostin isoform 7 precursor [Homo sapiens]**	98,4

119623487	histone 1, H2bj, isoform CRA_b [Homo sapiens]	57,8
194388798	unnamed protein product [Homo sapiens]	44,6
109150416	peroxidasin homolog precursor [Homo sapiens]	44,2
530366841	PREDICTED: peroxidasin homolog isoform X1 [Homo sapiens]	42,5
86651742	tyrosine 3-monooxygenasea/tryptophan 5-monooxygenase activation protein zeta [Homo sapiens]	42,4
119591511	collagen, type VI, alpha 3, isoform CRA_c [Homo sapiens]	40,2
8101724	AF258350_1 canstatin, partial [Homo sapiens]	40,0
133778299	TUBB2B protein [Homo sapiens]	38,9
158256710	unnamed protein product [Homo sapiens]	37,9
89243632	ITGB1 protein [Homo sapiens]	35,7
530400269	PREDICTED: myosin light polypeptide 6 isoform X6 [Homo sapiens]	34,4
87196339	collagen alpha-1(VI) chain precursor [Homo sapiens]	32,3
40226101	ACTG1 protein, partial [Homo sapiens]	30,6
386997	prebeta-migrating plasminogen activator inhibitor, partial [Homo sapiens]	30,2
193787599	unnamed protein product [Homo sapiens]	28,7
16741721	Procollagen-lysine 1, 2-oxoglutarate 5-dioxygenase 1 [Homo sapiens]	27,5
119625664	annexin A5, isoform CRA_c [Homo sapiens]	24,3
340234	vimentin, partial [Homo sapiens]	22,8
11935049	AF304164_1 keratin 1 [Homo sapiens]	21,2
221042224	unnamed protein product [Homo sapiens]	21,2
34069	unnamed protein product [Homo sapiens]	20,4
12667788	myosin-9 [Homo sapiens]	19,0

34782802	PKM2 protein, partial [Homo sapiens]	18,8
194376948	unnamed protein product [Homo sapiens]	18,3
148922238	Thrombospondin 2 [Homo sapiens]	17,7
119615401	heparan sulfate proteoglycan 2 (perlecan), isoform CRA_b [Homo sapiens]	16,0
282721077	sushi repeat-containing protein SRPX isoform 3 precursor [Homo sapiens]	15,5
119615036	collagen, type I, alpha 1, isoform CRA_a [Homo sapiens]	15,4
530425079	PREDICTED: tubulin beta-4A chain isoform X2 [Homo sapiens]	14,8
115527062	collagen alpha-2(VI) chain isoform 2C2 precursor [Homo sapiens]	12,9
84708830	TUBB6 protein [Homo sapiens]	12,0
119616320	hyaluronan and proteoglycan link protein 1, isoform CRA_b [Homo sapiens]	11,8
197692249	annexin I [Homo sapiens]	10,3
49257389	CCDC80 protein [Homo sapiens]	10,3
194384606	unnamed protein product [Homo sapiens]	10,0
38197240	LAMB1 protein, partial [Homo sapiens]	8,4
157653329	procollagen C-endopeptidase enhancer 1 precursor [Homo sapiens]	8,3
3777617	serine protease [Homo sapiens]	8,2
119616322	EGF-like repeats and discoidin I-like domains 3, isoform CRA_a [Homo sapiens]	7,6
12653033	MYH10 protein [Homo sapiens]	7,3
62089314	Pro-alpha-1 type V collagen variant [Homo sapiens]	7,3
189054446	unnamed protein product [Homo sapiens]	6,3

194097352	alpha-actinin-1 isoform c [Homo sapiens]	5,8
12653633	Lysyl oxidase-like 2 [Homo sapiens]	5,8
119582898	MAM domain containing 2, isoform CRA_b [Homo sapiens]	5,6
119590446	nidogen 1, isoform CRA_b [Homo sapiens]	4,6
189054446	unnamed protein product [Homo sapiens]	3,8
119607847	pregnancy-associated plasma protein A, pappalysin 1, isoform CRA_c [Homo sapiens]	3,6
119568019	fibronectin type III domain containing 1 [Homo sapiens]	3,5
30353925	CLTC protein [Homo sapiens]	3,2
9309503	AC013451_1 LTBP-2 [Homo sapiens]	2,9
553348	hexabrachion, partial [Homo sapiens]	2,8
119593150	filamin A, alpha (actin binding protein 280), isoform CRA_a [Homo sapiens]	2,4
118572606	hemicentin-1 precursor [Homo sapiens]	1,0

Table S1: **Proteomic Analysis.**

Supplementary Table 2

		Ctrl		**ExoCPC**		**ExoCPC_SiPOSTN**	
		Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
CO	**mL/min**	75,80	9,21	112,61	19,59	90,7	10,89
EF	**%**	44,69	3,92	62,50	4,95	55,41	1,842
FAC	**%**	35,20	7,08	54,93	3,87	47,85	4,69
FS	**%**	10,91	1,97	19,13	2,06	16,39	2,44
SV	**µL**	242,41	22,35	310,81	49,52	258,48	27,50
Vd	**µL**	560,32	62,15	484,93	40,87	462,79	38,66
Vs	**µL**	317,91	58,30	174,12	16,00	207,31	12,84

Table S2: Echocardiographic Data at Day 14 post-MI

CO: Cardiac Output; EF: Ejection Fraction; FAC: Fractional Area Change; FS: Fractional Shortening; SV: Stroke Volume; Vd: Diastolic Volume; Vs: Systolic Volume.

Supplementary Table 3

Antibody	Application	Dilution	Company	# Ref
TSG101	WB	1:1000	Abcam	ab125011
SYNTENIN-1	WB	1:1000	Abcam	ab133267
CALNEXIN	WB	1:300	SantaCruz Biotechnology	sc-70481
POSTN	WB	1:300	SantaCruz Biotechnology	sc-398631
cTnI	IF	1:200	Abcam	ab47003
αSarcomeric	IF	1:300	Abcam	ab9465
pH3	IF	1:300	Abcam	ab14955
pAKT	WB	1:1000	Cell Signaling	4060
AKT	WB	1:1000	Cell Signaling	9272
GAPDH	WB	1:5000	Abcam	ab181602
CYCLIN D1	WB	1:30000	Abcam	ab134175
pFAK	WB	1:1000	Abcam	ab81298
FAK	WB	1:1000	Abcam	ab76496
YAP1	WB	1:500	Invitrogen	PA5-87568
H3	WB	1:5000	Abcam	ab176842
AuroraBKinase	IF	1:250	BD	611083
cTnT	IF	1:100	Miltenyi Biotec	130-119-674

CD63	IF	1:100	SantaCruz Biotechnology	sc-365604
PAPP-A	WB	1:300	HyTest Ltd	4P41

Table S3: **Detailed information on antibodies used in the study.**