Supplementary Materials

The Human Fetal and Adult Stem Cell Secretome Can Exert Cardioprotective Paracrine Effects against Cardiotoxicity and Oxidative Stress from Cancer Treatment

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Table S1. Biochemical analyses on mNVCM and mNVFib exposed to Dox, with or without hAFS-CM and hMSC-CM priming. Values are expressed as mean ± s.e.m. of at least *n* = 3 independent experiments and refers to data shown in Figure 2 and Figure 3.

Biochemical Analyses	**mNVCM**						**mNVFib**					
	Ctrl	**Dox**	**hAFS-CM + Dox**	**hMSC-CM + Dox**	**hAFS-CM**	**hMSC-CM**	**Ctrl**	**Dox**	**hAFS-CM + Dox**	**hMSC-CM + Dox**	**hAFS-CM**	**hMSC-CM**
Oxygen consumption (nmol/min/ 10^6 cells)	25.75 + 1.39 (*n* = 6)	8.51 + 0.62 (*n* = 3)	17.8 + 0.43 (*n* = 3)	15.03 + 0.66 (*n* = 3)	31.84 + 0.52 (*n* = 3)	30.5 + 1.42 (*n* = 3)	5.28 + 0.15 (*n* = 4)	1.1 + 0.15 (*n* = 4)	3.99 + 0.24 (*n* = 3)	3.35 + 0.21 (*n* = 3)	6.16 + 0.41 (*n* = 3)	6.29 + 0.22 (*n* = 3)
ATP production (nmol/min/ 10^6 cells)	62.42 + 2.56 (*n* = 6)	14.22 + 1.23 (*n* = 3)	42.79 + 0.81 (*n* = 3)	36.13 + 0.70 (*n* = 3)	78.30 + 1.63 (*n* = 3)	74.17 + 2.28 (*n* = 3)	12.76 + 0.26 (*n* = 4)	1.42 +0.19 (*n* = 4)	10.07 + 0.46 (*n* = 3)	7.87 + 0.80 (*n* = 3)	14.83 + 0.75 (*n* = 3)	15.13 + 1.01 (*n* = 3)
OxPhos efficiency (P/O P/M ratio)	2.43 + 0.09 (*n* = 6)	1.67 + 0.04 (*n* = 3)	2.40 + 0.03 (*n* = 3)	2.41 + 0.13 (*n* = 3)	2.46 + 0.06 (*n* = 3)	2.43 + 0.63 (*n* = 3)	2.41 + 0.03 (*n* = 4)	1.32 + 0.20 (*n* = 4)	2.52 + 0.14 (*n* = 3)	2.34 + 0.09 (*n* = 3)	2.41 + 0.04 (*n* = 3)	2.41 + 0.07 (*n* = 3)
Glucose consumption (mM /10^6 cells)	9.05 + 0.17 (*n* = 6)	11.45 + 0.21 (*n* = 3)	10.06 + 0.15 (*n* = 3)	10.05 + 0.20 (*n* = 3)	12.53 + 0.14 (*n* = 3)	12.36 + 0.30 (*n* = 3)	14.80 + 0.49 (*n* = 4)	17.74 + 0.49 (*n* = 4)	16.65 + 0.40 (*n* = 3)	16.49 + 0.38 (*n* = 3)	16.97 + 0.56 (*n* = 3)	15.78 + 0.23 (*n* = 3)
Lactate release (mM /10^6 cells)	0.62 + 0.09 (*n* = 6)	3.44 + 0.29 (*n* = 3)	1.43 + 0.18 (*n* = 3)	1.27 + 0.08 (*n* = 3)	0.43 + 0.02 (*n* = 3)	0.45 + 0.30 (*n* = 3)	4.28 + 0.18 (*n* = 4)	8.67 + 0.57 (*n* = 4)	5.95 + 0.24 (*n* = 3)	6.30 + 0.27 (*n* = 3)	2.65 + 0.22 (*n* = 3)	3.09 + 0.16 (*n* = 3)
% Lactate Fermentation	3.43 + 0.48 (*n* = 6)	15.07 + 1.49 (*n* = 3)	6.33 + 0.47 (*n* = 3)	7.16 + 0.99 (*n* = 3)	1.74 + 0.07 (*n* = 3)	1.81 + 0.17 (*n* = 3)	14.46 + 0.43 (*n* = 4)	24.41 + 0.94 (*n* = 4)	17.88 + 1.11 (*n* = 3)	19.11 + 1.11 (*n* = 3)	7.80 + 0.57 (*n* = 3)	9.80 + 0.62 (*n* = 3)

Table S2. Left ventricle functional parameters in mice exposed to Dox, with or without hAFS-CM and hMSC-CM priming. Values are expressed as mean ± s.e.m. from echocardiography evaluation and are shown in Figure 5.

Cardiac Function Parameters	Dox (*n* = 6)	hAFS-CM + Dox (*n* = 5)	hMSC-CM + Dox (*n* = 6)	Dox (*n* = 6)	hAFS-CM + Dox (*n* = 5)	hMSC-CM + Dox (*n* = 6)
Day	0	0	0	42	42	42
LVEDd (mm)	3.6 ± 0.08	3.8 ± 0.03	3.6 ± 0.03	3.7 ± 0.12	4.1 ± 0.09	3.8 ± 0.12
LVEDs (mm)	2.3 ± 0.08	2.4 ± 0.03	2.2 ± 0.07	2.7 ± 0.04$^{\#\#}$	2.7 ± 0.14	2.5 ± 0.11
IVSd (mm)	0.50 ± 0.01	0.53 ± 0.03	0.51 ± 0.03	0.50 ± 0.02	0.60 ± 0.02	0.54 ± 0.02
IVSs (mm)	0.90 ± 0.02	1.0 ± 0.03	0.92 ± 0.03	0.85 ± 0.02	0.99 ± 0.04	0.97 ± 0.05
LVPWd (mm)	0.50 ± 0.03	0.55 ± 0.03	0.51 ± 0.03	0.46 ± 0.02	0.55 ± 0.03	0.54 ± 0.02
LVPWs (mm)	0.89 ± 0.05	0.94 ± 0.05	0.84 ± 0.04	0.68 ± 0.06	0.87 ± 0.07	0.83 ± 0.05
FS (%)	37.0 ± 1.3	37.8 ± 1.0	37.5 ± 1.4	26.8 ± 1.3$^{\#\#\#\#}$	34.0 ± 2.2**	34.3 ± 1.2**
EF (%)	66.1 ± 2.1	68.6 ± 1.3	68.4 ± 1.8	52.1 ± 2.8$^{\#\#\#}$	63.1 ± 3.0	63.9 ± 1.6*

Dox day 0 vs day 42: $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$, $^{\#\#\#\#}$ $p < 0.0001$; hAFS-CM + Dox and hMSC-CM +Dox vs Dox at day 42: * $p < 0.05$, ** $p < 0.01$ by two-way repeated-measures ANOVA with Tukey's post-hoc test. LVEDd: left ventricular end-diastolic diameter; LVEDs: left ventricular end-systolic diameter; IVSd: interventricular septal thickness at end-diastole; IVSs: interventricular septal thickness at end-systole; LVPWd: left ventricular posterior wall thickness at end-diastole: LVPWs, left ventricular posterior wall thickness at end-systole; FS: fractional shortening; EF: ejection fraction; mm: millimeter.

Table S3. Biochemical analyses on hearts of mice treated with Dox after hAFS-CM and hMSC-CM priming. Values are expressed as mean ± s.e.m. of at least *n* = 3 independent experiments and refer to data shown in Figure 5.

Biochemical Analyses	Ctrl	Dox	hAFS-CM + Dox	hMSC-CM + Dox
Glucose-6 phosphate dehydrogenase (G6PD) activity	7.66 ± 0.11	11.59 ± 0.12	8.68 ± 0.05	8.88 ± 0.09
(mU/mg)	(*n* = 3)	(*n* = 4)	(*n* = 5)	(*n* = 3)
Glutathione reductase (GRx) activity	66.54 ± 2.74	36.90 ± 0.51	44.74 ± 1.44	46.46 ± 0.51
(mU/mg)	(*n* = 3)	(*n* = 4)	(*n* = 5)	(*n* = 3)
Catalase activity	153.84 ± 13.37	86.66 ± 3.64	124.49 ± 10.79	127.48 ± 6.48
(mU/mg)	(*n* = 3)	(*n* = 4)	(*n* = 5)	(*n* = 3)
Malondialdheyde (MDA) intracellular concentration	10.39 ± 0.21	42.30 ± 0.42	25.17 ± 0.37	25.28 ± 0.58
(μM MDA/mg)	(*n* = 3)	(*n* = 4)	(*n* = 5)	(*n* = 3)
Complex I (MRC I) activity	1.1 ± 0.03	0.44 ± 0.02	0.76 ± 0.02	0.73 ± 0.02
(mU/mg)	(*n* = 3)	(*n* = 4)	(*n* = 5)	(*n* = 3)

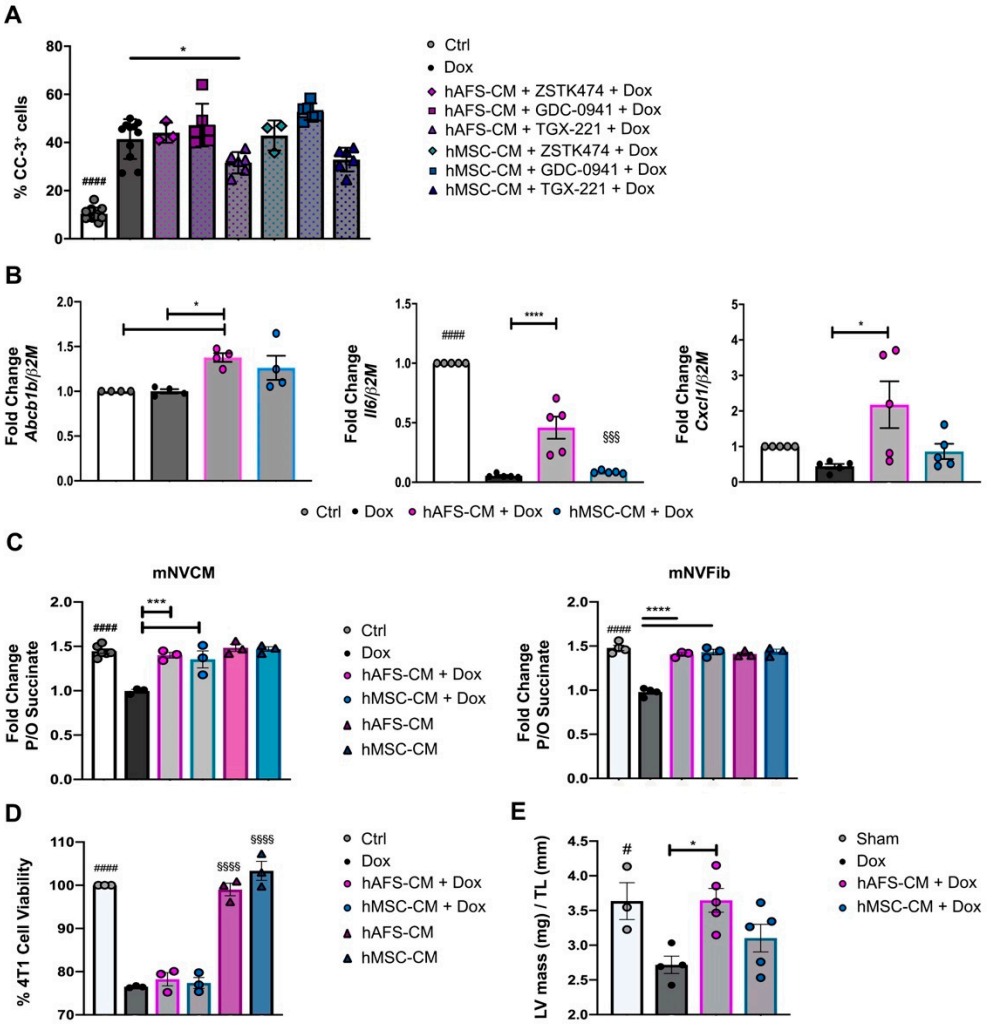

Figure S1. A. Percentage of mNVCM expressing cleaved-caspase-3 (*%CC-3⁺ cells*) in control condition (*n* = 11, *Ctrl: 10.35 ± 0.80%*), after exposure to 1 µM Dox with or without (*n* = 11, *Dox: 41.43 ± 2.50%*) pre-incubation with hAFS-CM or hMSC-CM in combination with the pan class I PI3K inhibitor ZSTK474, the PI3Kα and PI3Kδ inhibitor GDC-0941 and the PI3Kβ inhibitor TGX-221 (*n* = 3, *hAFS-CM + ZSTK474 + Dox: 44.06 ± 2.39%; n* = 6, *hAFS-CM + GDC-0941 + Dox: 47.24±3.63%; n* = 6, *hAFS-CM + TGX-221 + Dox: 31.60 ± 1.80%; n* = 3, *hMSC-CM + ZSTK474 + Dox: 42.93 ± 3.58%; n* = 6, *hMSC-CM + GDC-0941 + Dox: 52.78 ± 1.40%; n* = 6, *hMSC-CM + TGX-221 + Dox: 32.90 ± 2.00%*). Values are expressed as mean ± s.e.m. of independent experiments; *Ctrl* vs. *Dox* $^{\#\#\#\#}p < 0.0001$; *hAFS-CM + TGX-221 + Dox* vs. *Dox* * $p = 0.0393$. **B.** Real time qRT-PCR analysis on mNVCM after exposure to 1 µM Dox with or without (*Dox*) pre-incubation with hAFS-CM (*hAFS-CM + Dox*) or hMSC-CM (*hMSC-CM+ Dox*) versus mNVCM in control condition (*Ctrl,* considered as calibrator*)* for the fold-change expression of *Abcb1b* (*n* = 4, *Dox: 1.00±0.02; hAFS-CM + Dox: 1.40± 0.05*; *hMSC-CM + Dox: 1.26 ± 0.13; hAFS-CM + Dox* vs. *Dox and hAFS-CM + Dox* vs. *Ctrl, *p = 0.0146); Il6* (*n* = 5, *Dox: 0.05±0.01; hAFS-CM + Dox: 0.46 ± 0.10*; *hMSC-CM + Dox: 0.08±0.005; Ctrl* vs. *Dox,* $^{\#\#\#\#}p < 0.0001$; *hAFS-CM + Dox* vs *Dox,* ****$p < 0.0001$ *and hAFS-CM + Dox vs. hMSC-CM + Dox,* $^{§§§}p = 0.0002$*)* and *Cxcl1* (*n* = 5, *Dox: 0.44 ± 0.07; hAFS-CM + Dox: 2.17 ± 0.66*; *hMSC-CM + Dox: 0.86 ± 0.21; hAFS-CM + Dox* vs. *Dox, *p* < 0.0136*) over the housekeeping gene *Beta-2 Microglobulin* (*β2M*). **C.** From the top left corner: mNVCM oxygen consumption rate stimulated with succinate in control condition (*n* = 6, *Ctrl: 18.91± 0.95*), after exposure to 1 µM Dox (*n* = 3, *Dox: 4.98± 0.58),* pretreated with hAFS-CM (*n* = 3 *hAFS-CM +Dox: 13.06 ± 0.86*) or hMSC-CM (*n* = 3, *hMSC-CM + Dox: 11.73± 1.05*) before the Dox stimulation, or only pretreated with hAFS-CM (*n*= 3, *hAFS-CM: 27.42 ± 0.60*) or hMSC-CM (*n* = 3, *hMSC-CM: 26.10± 0.90*). mNVFib oxygen consumption rate stimulated with succinate in control condition (*n*= 4, *Ctrl: 4.25± 0.17*), after exposure to 1 µM Dox (*n* = 4, *Dox: 0.98 ± 0.10),* pretreated with hAFS-CM (*n* = 3 *hAFS-CM +Dox: 3.74± 0.25*) or hMSC-CM (*n* = 3, *hMSC-CM + Dox: 3.06 ± 0.19*) before the Dox stimulation, or only pretreated with hAFS-CM (*n*= 3, *hAFS-CM: 5.44± 0.35*) or hMSC-CM (*n* = 3, *hMSC-CM: 5.45 ± 0.46*). In each panel, values are expressed as mean ± s.e.m. of independent experiments; *Ctrl* vs. *Dox* $^{\#\#\#\#}p < 0.0001$; *hAFS-CM + Dox* vs. *Dox and hMSC-CM +Dox* vs. *Dox* ***$p < 0.001$ (mNVCM, $p = 0.0002$ and $p = 0.0008$, respectively) or ****$p < 0.0001$ (mNVFib). **D.** 4T1 cell viability percentage by MTT assay normalized to untreated control cells (*n* = 3, *Ctrl*) considered as 100% viable, after exposure to 1 µM Dox with or without (*n* = 3, *Dox: 76.51 ± 0.15%*) pre-incubation with hAFS-CM (*n* = 3, *hAFS-CM + Dox*: *78.25±1.53%*) or hMSC-CM (*n* = 3, *hMSC-CM + Dox*: *77.37 ± 1.30%*) or tretaed with hAFS-CM (*n* = 3, *hAFS-CM*: *99.03±1.44%*) or hMSC-CM (*n* = 3, *hMSC-*

CM + Dox: *103.35 ± 2.20%*) only. Values are expressed as mean ± s.e.m. of independent experiments; *Ctrl* vs. *Dox* $^{\#\#\#\#}p <$ 0.0001; *hAFS-CM* vs. *Dox* and *hMSC-CM* vs. *Dox* $^{\S\S\S\S}p <$ 0.0001. **E.** Left ventricle (LV) mass (milligram, *mg*) over tibial length (TL, in millimeter, *mm*) ratio in sham healthy mice ($n = 3$, *Sham: 3.64 ± 0.27*) treated with a cumulative dose of 12 mg/Kg Dox ($n = 4$, *Dox: 2.72 ± 0.18)* with or without either hAFS-CM ($n = 5$, *hAFS-CM + Dox: 3.65 ± 0.11)* or hMSC-CM ($n = 5$, *hAFS-CM + Dox: 3.10 ± 0.20)* after 42 days. Values are expressed as mean ± s.e.m. of independent animals; *Sham* vs. *Dox* $^{\#}p = 0.0383$; *hAFS-CM + Dox* vs. *Dox* * $p = 0.0163$.