Supplementary Figures and tables

Local administration of stem cell-derived extracellular vesicles in a thermoresponsive hydrogel promotes a pro-healing effect in a rat model of colo-cutaneous post-surgical fistula

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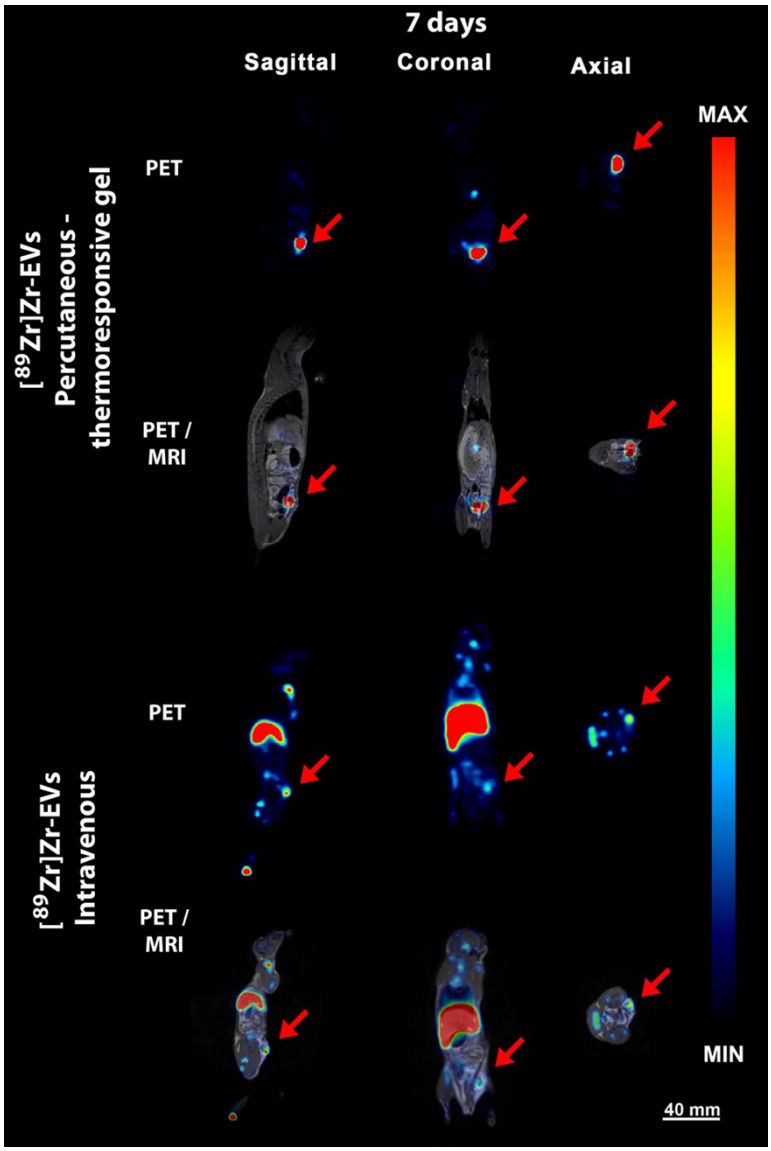

Figure S1: PET and PET/MRI images illustrate the biodistribution and local uptake of [^{89}Zr]Zr-EVs 7 days after administration. The top images illustrate the treatment with [^{89}Zr]Zr-EVs in the thermoresponsive gel and bottom images illustrate the intravenous injection in saline. Red arrows indicate the radiotracer uptake in the colo-cutaneous fistula. Note that 7 days after [^{89}Zr]Zr-EVs administration in the thermoresponsive gel, the tracer signal was still at the fistula site, with SUV decrease of just 15% compared to the SUV at 24 hours. On the other hand, intravenous injection revealed a decrease of 380% of [^{89}Zr]Zr-EVs uptake when comparing 7 days to 24 hours.

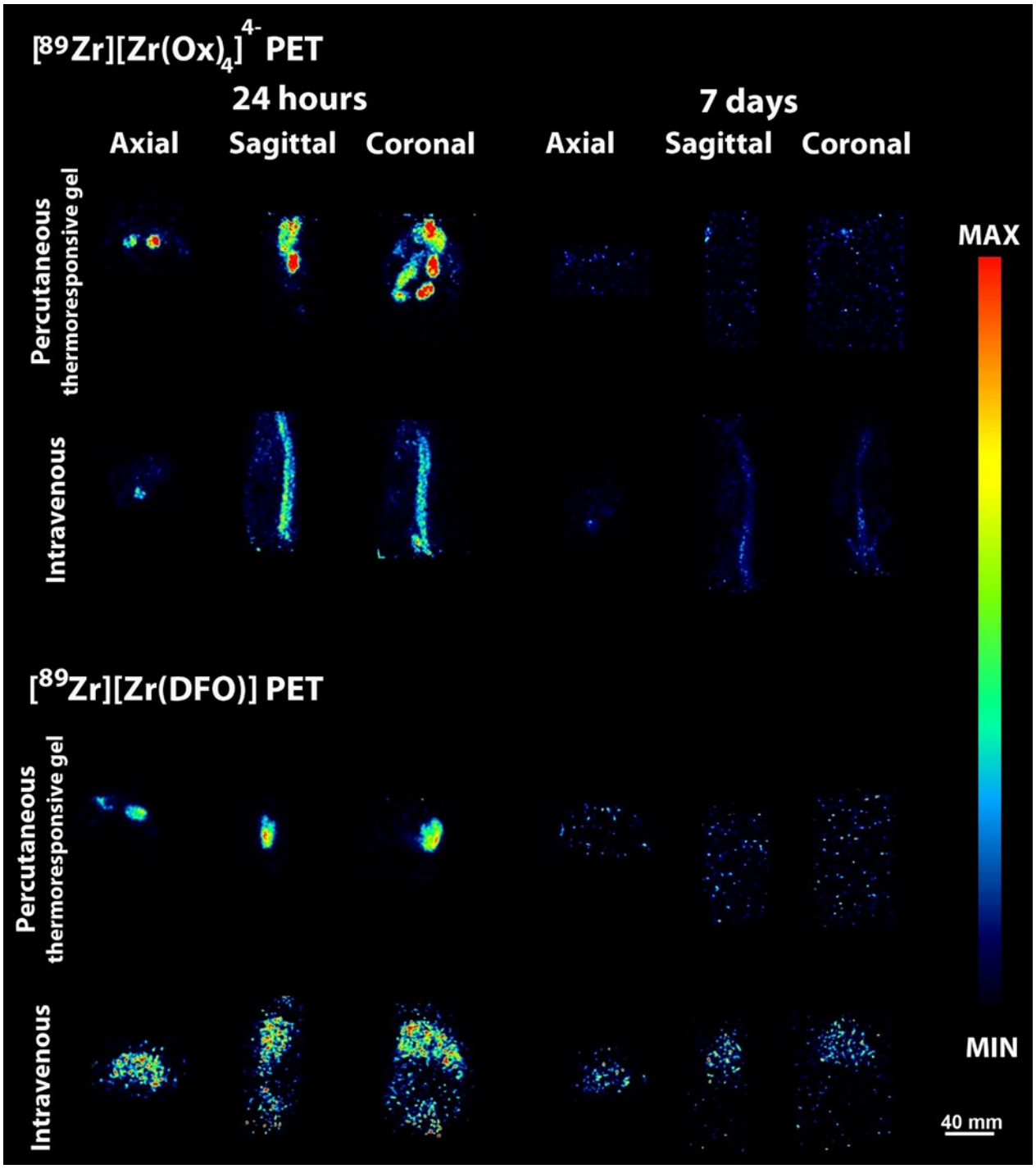

Figure S2: PET acquisition was performed to illustrate the biodistribution of $[^{89}Zr][Zr(ox)_4]^{4-}$ or $[^{89}Zr][Zr(DFO)]$ after percutaneous intra-fistula or intravenous administration after 24 hours and 7 days, as a conjugation quality control. Note all these images of the $[^{89}Zr]$zirconium tracer (with or without the DFO conjugation molecule) displayed a biodistribution pattern quite distinct from the ones observed when the $[^{89}Zr]$zirconium tracer was coupled to EVs.

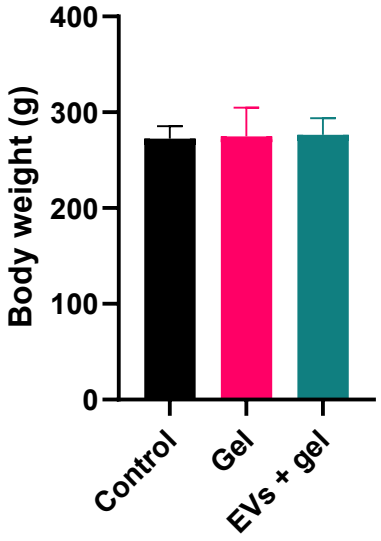

Figure S3: Mean body weight for animals in the control, gel and EVs + gel group at day 60th after the begin of therapy.

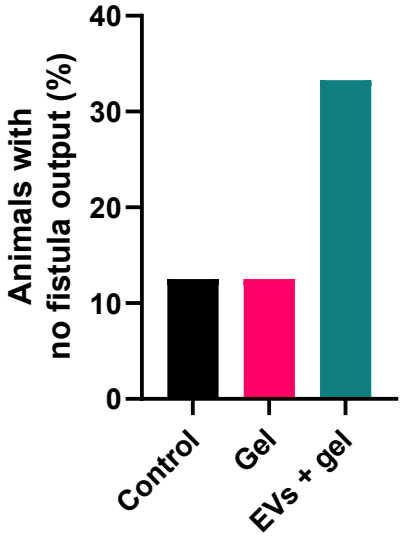

Figure S4: Preclinical evaluation of fistulas at day 30 indicating the percentage of animals per group featuring the absence of faeces (no output) at the external fistula orifice.

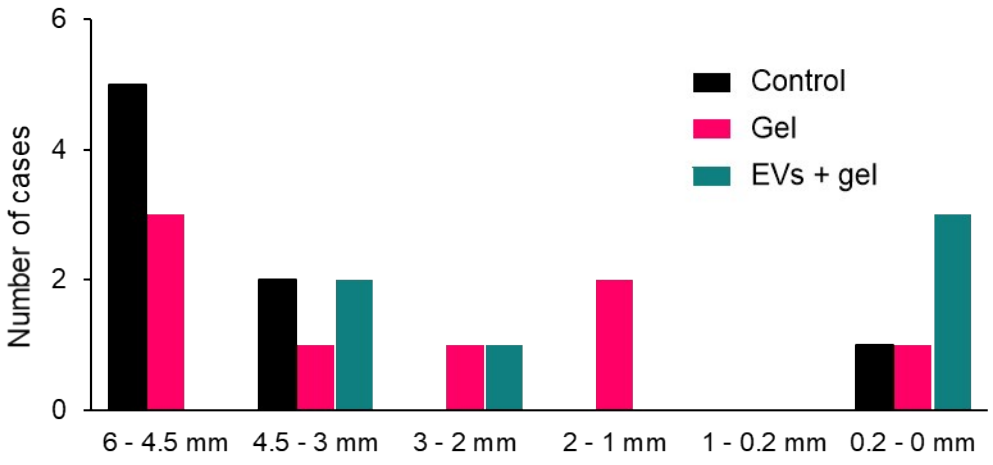

Figure S5: External orifice diameter distribution for the control, gel and EVs + gel groups. The number of cases for each diameter range was plotted for the control, gel and EVs + gel group at day 60th after the begin of therapy.

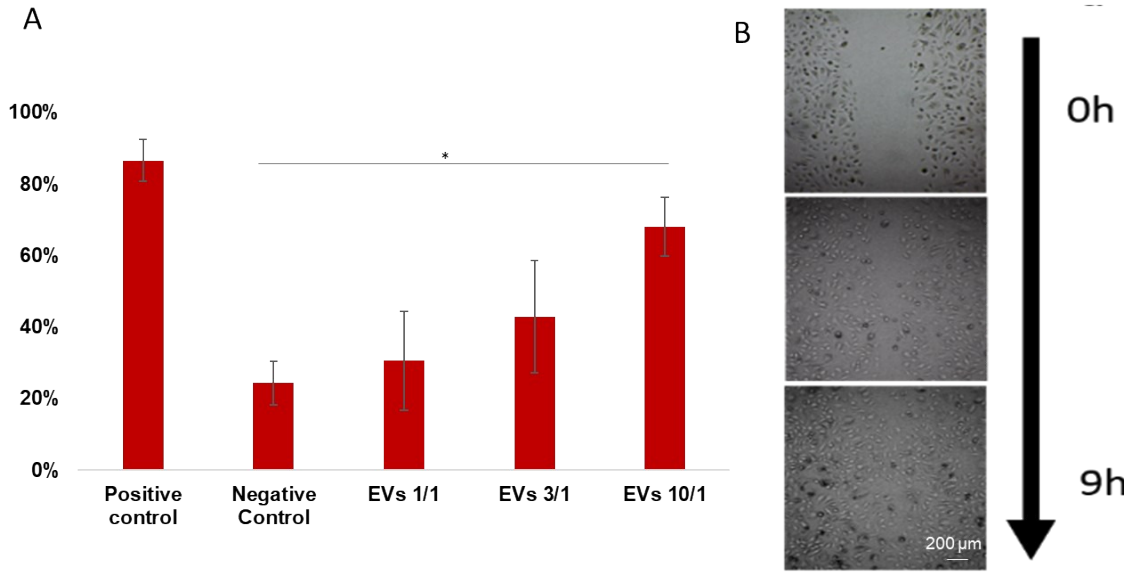

Figure S6: Scratch test assay to evaluate the pro-angiogenic (pro-migratory) potential of EVs. Complete medium and serum-free medium were used as positive and negative control (respectively). HUVEC recipient cells were incubated with EVs setting the EV dose by the number of EV producer cells per recipient cell at 1/1, 3/1 and 10/1 (A). Optical microscopy image of scratches for the EV 10/1 condition as a function of the time (B).

Table S1: Probe-based confocal laser endomicroscopy evaluation for animals from control, gel and EVs + gel groups.

	Control	**Gel**	**EVs + gel**	**p**			
				All	**Control vs Gel**	**Control vs EVs + gel**	***Gel vs EVs + gel***
Mean of crypts	7.8	7.1	9.1	*0.773*	*0.402*	*0.754*	*0.917*
Mean diameter of crypts (µm)	5.8	9.1	9.1	*0.403*	*0.834*	*0.347*	*0.175*
Inter crypts space (µm)	6.8	9.4	7.8	*0.651*	*0.602*	*0.754*	*0.347*
Mean diameter of central crypts (µm)	6.0	9.6	8.4	*0.429*	*0.530*	*0.295*	*0.295*
Score of inflammation (0 to 10)	5.9	10.7	7.4	*0.200*	*0.340*	*0.738*	***0.052***
Vessel diameter (µm)	8.6	7.6	7.8	*0.932*	*0.917*	*0.754*	*0.754*
Total Vessel Length (µm)	5057	4985	5192	*0.852*	*0.754*	*0.754*	*0.602*
Total Vessel area (µm^2)	82814	90671	92742	*0.432*	*0.251*	*0.347*	*0.465*
Mean vessel diameter (µm)	6.3	9.6	8.1	*0.505*	*0.465*	*0.402*	*0.347*
Diameter_Standard_Deviation	7.3	9.2	7.5	*0.761*	*0.675*	*0.917*	*0.402*
Functional Capillary Density Length (µm)	9.0	7.5	7.5	*0.368*	*1.000*	*0.317*	*0.317*
Functional Capillary Density Area	5.9	8.5	9.6	*0.404*	*0.530*	*0.293*	*0.249*

Table S2: MRI analysis for animals from control, gel and EVs + gel groups.

	Control	**Gel**	**EVs + gel**	**p**			
				All	**Control vs Gel**	**Control vs EVs + gel**	***Gel vs EVs + gel***
Small diametre (mm)	0.977	0.840	1.650	*0.874*	*0.724*	*0.663*	*0.724*
External diameter (mm)	4.195	3.553	3.770	*0.717*	*0.289*	*0.773*	*0.724*
Internal diameter (mm)	2.290	2.810	2.902	*0.741*	*0.724*	*0.564*	*0.480*
Fistula length (mm)	6.007	6.210	4.405	*0.640*	*1.000*	*0.386*	*0.480*
Thickness of fistula (mm)	1.037	0.690	1.205	*0.045*	*0.034*	*0.564*	*0.034*

Table S3: Parameters considered for calculating the histology score.

Parameter	0	1	2	3	Total
Epithelial damage	Normal	Hyperproliferative	< 50% crypts loss	> 50% crypts loss, and/or ulceration	0-3
Mucosal inflammation	None	Mild	Moderate	Severe	0-3
Submucosal inflammation	None	Mild	Moderate	Severe	0-3

Table S4: Histologycal analysis for animals from control, gel and EVs + gel groups. PNN: polynuclear neutrophils and PNE: polynuclear eosinophil.

	Control	**Gel**	**EVs + gel**	**p**			
				Control vs Gel	**Control vs EVs + gel**	***Gel vs EVs + gel***	**Control vs Gel**
Density of lymphocyte, (From 0 to 5)	1.43	1.33	1.25	*0.775*	*0.735*	*0.480*	*0.742*
Density of plasmocyte score, (From 0 to 5)	0.0	1.17	0.38	*0.101*	*0.042*	*0.080*	*0.384*
Density of macrophage score, (From 0 to 5)	2.86	2.5	1.88	*0.189*	*0.696*	*0.063*	*0.293*
Density of PNN score, (From 0 to 5)	4.14	2.83	1.88	*0.019*	*0.120*	*0.006*	*0.215*
Density of PNE score, (From 0 to 5)	0.00	0.50	0.00	*0.016*	*0.040*	*1.000*	*0.030*
Density of granulome score, (From 0 to 5)	0.71	3.0	1.13	*0.033*	*0.026*	*0.422*	*0.029*
Density of abscess score, (From 0 to 5)	0.43	0.17	0.13	*0.972*	*1.000*	*0.845*	*0.832*
Density of fibrosis score, (From 0 to 5)	3.57	3.33	2.5	*0.073*	*0.756*	*0.027*	*0.084*
Density of fibroblasts score, (From 0 to 5)	3.29	3.17	2.6	*0.155*	*0.804*	*0.065*	*0.189*
Density of neo-vessels score, (From 0 to 5)	1.29	1.83	2.0	*0.036*	*0.058*	*0.022*	*0.529*
Fibrosis score, (From 0 to 25)	11.7	10.83	6.6	*0.013*	*0.774*	*0.006*	*0.027*
Histology scoring, (from 0 to 9)	5.86	6.0	8.0	*0.009*	*0.306*	*0.004*	*0.041*