

SUPPLEMENTARY MATERIAL

Interleukin-10–engineered mesenchymal stem/stromal cells exhibit robust immunomodulatory effects *in vitro* and *in vivo*

Carneiro, D. C. et al. Stem Cell Research & Therapy, 2026.

Figure S1. Isolation of mesenchymal stem/stromal cells from mouse bone marrow.

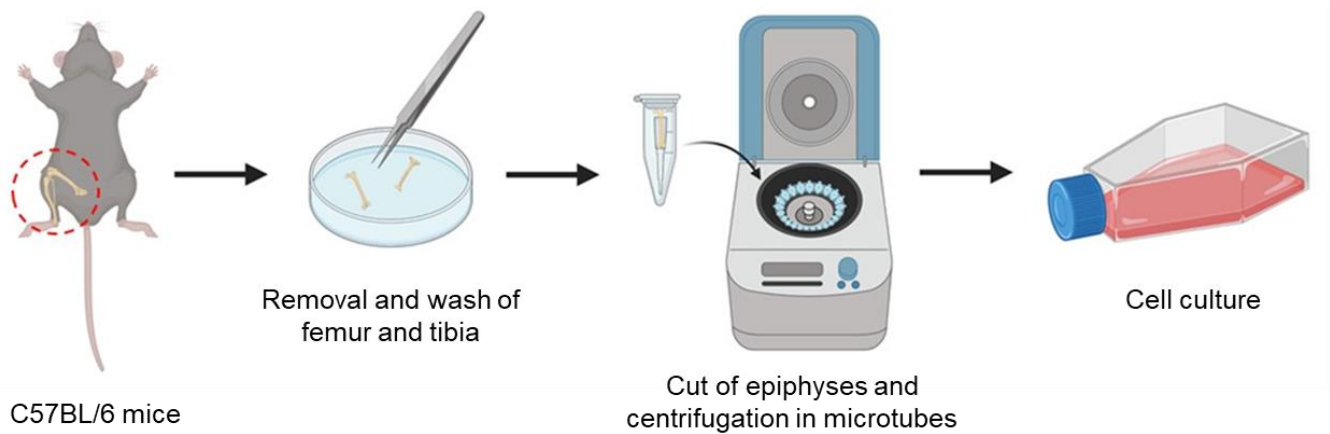


Figure S2. Digestion with BamHI and BsrGI of pEGIP and pMA-RQ with synthetic IL-10.

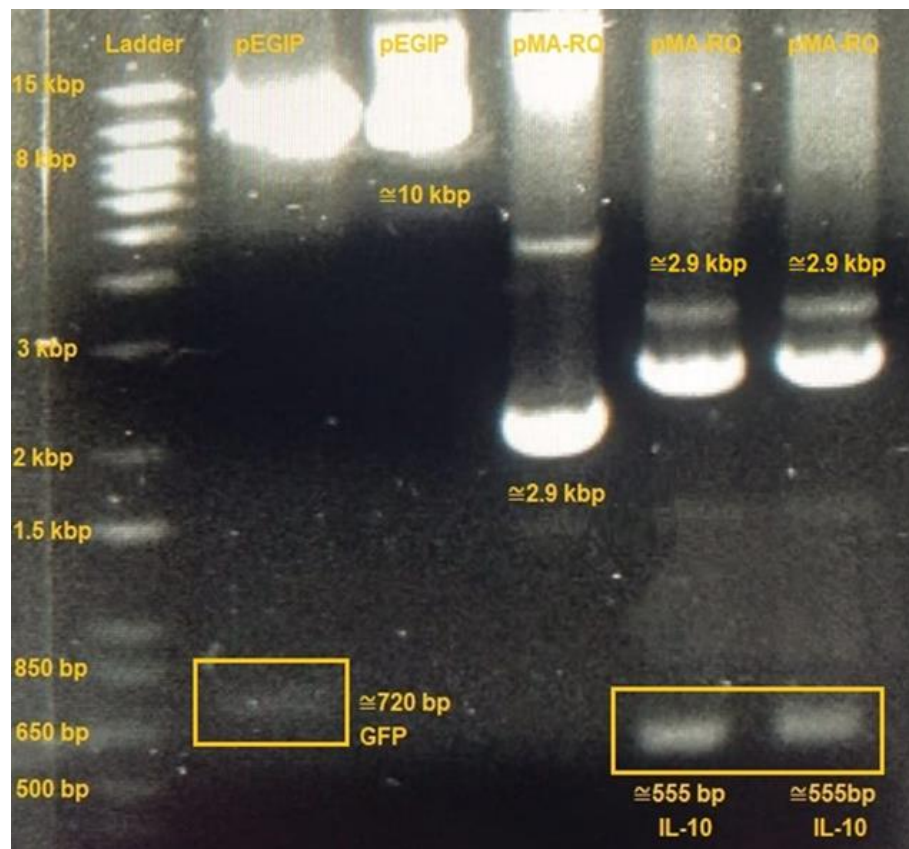


Figure S3. Digestion with BamHI and BsrGI of pEGIP-IL-10 to confirm IL-10 insert.

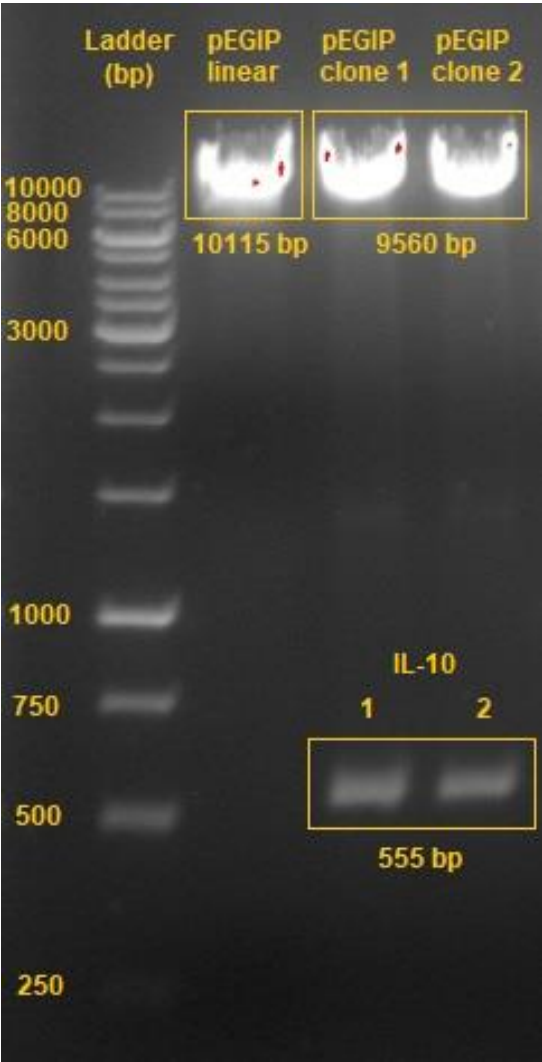


Figure S4. ClustalW multiple alignment between the sequence obtained from sequencing and the predicted sequence for pEGIP-IL-10 at the beginning and end.

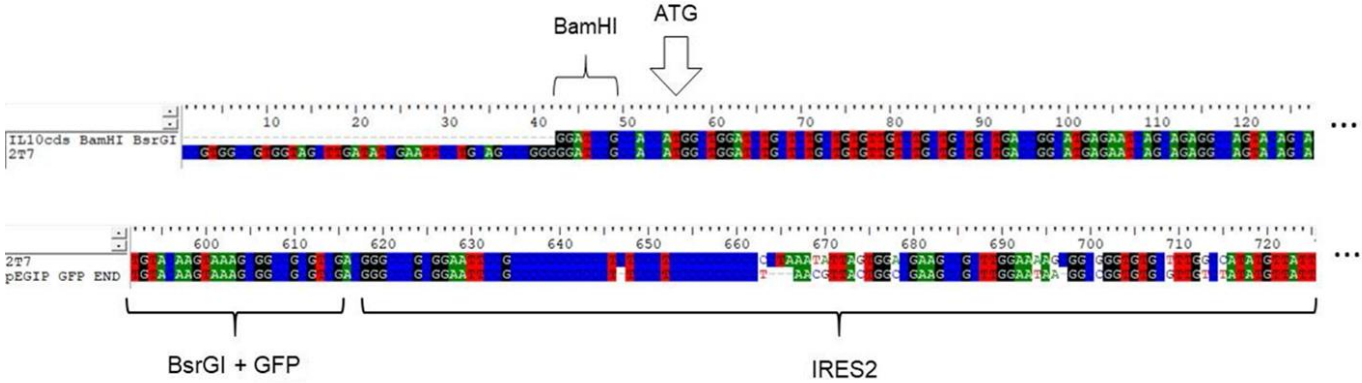


Figure S5. GFP expression by pEGIP and pEGIP-IL-10 transfected HEK293T cells and transduced mesenchymal stem/stromal cells.

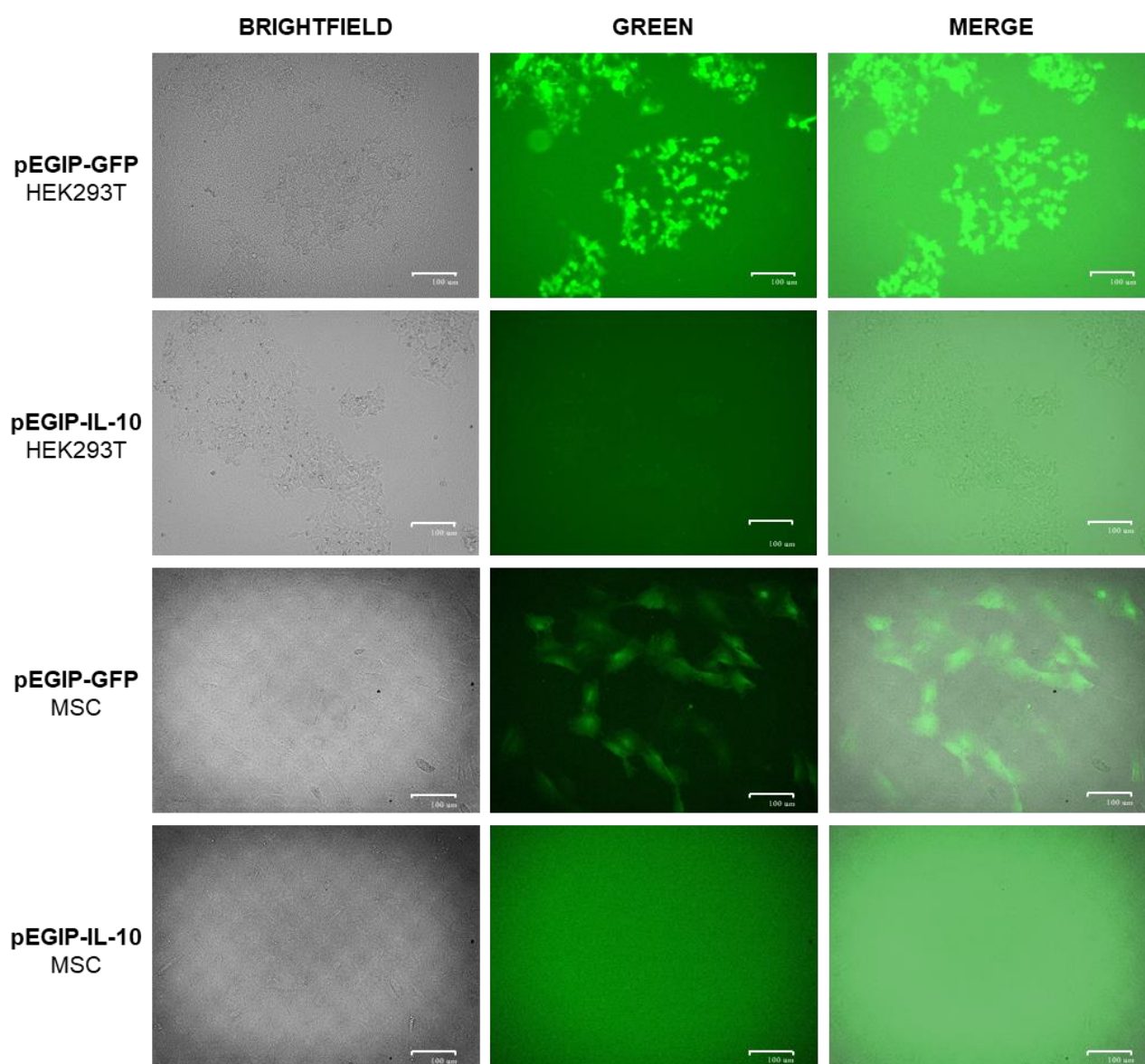


Figure S6. Genetic modification of the mesenchymal stem/stromal cells.

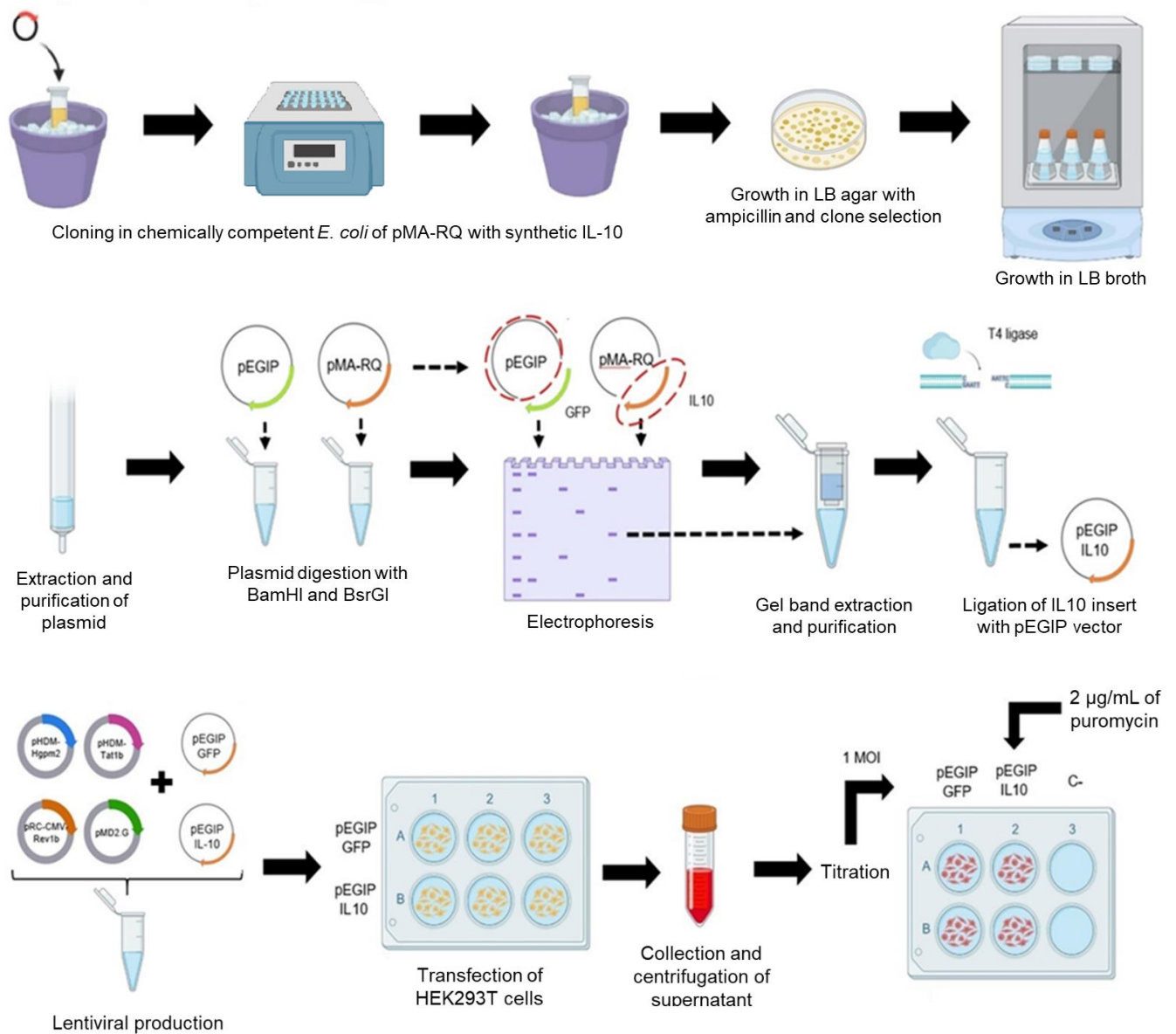


Figure S7. Histological analysis of organs from naïve mice.

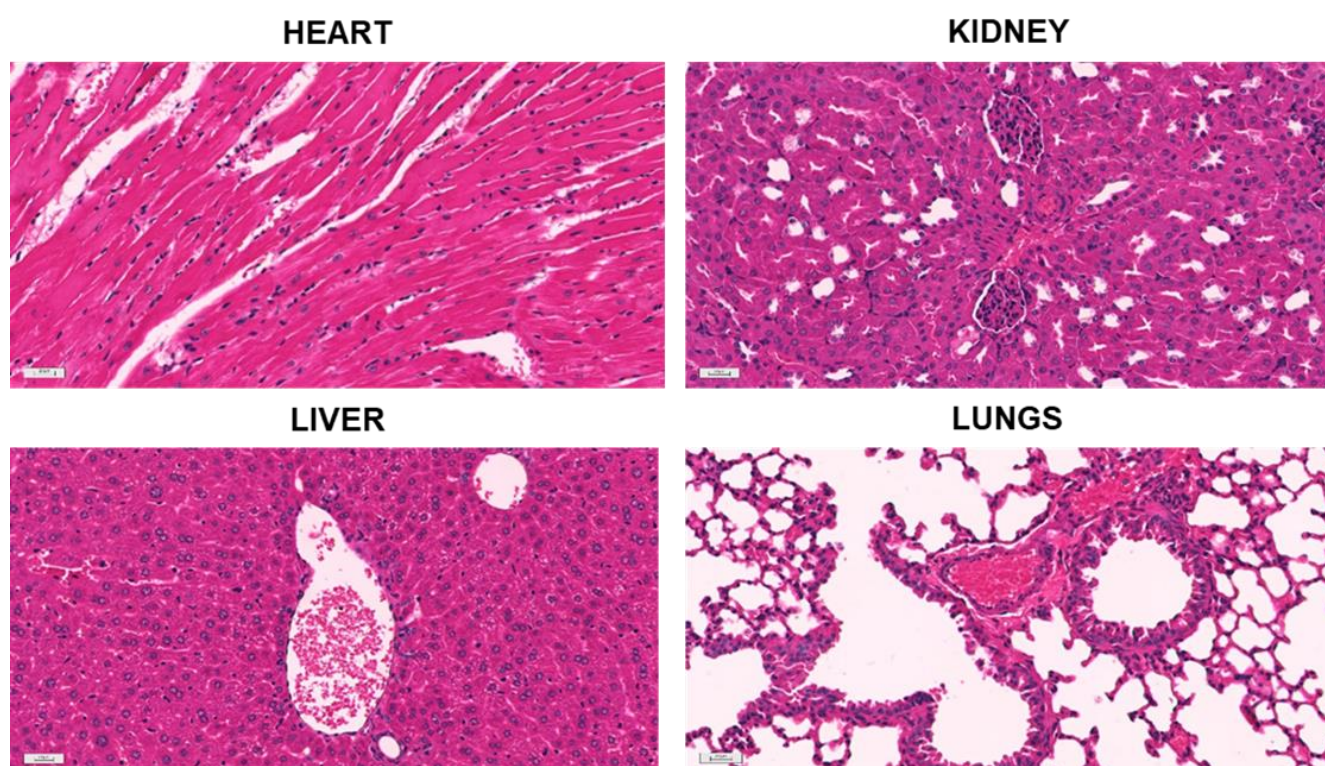


Table S1. Plasmids used in the lentiviral system.

Plasmid	Addgene	Quantity per well
pHDM-Hgpm2	#164441	0,22 µg
pHDM-Tat1b	#164442	0,22 µg
pRC-CMV-Rev1b	#164443	0,22 µg
pMD2.G	#12259	0,34 µg
pEGIP (GFP/IL-10)	#26777	1 µg

Table S2. Target genes and primers used for RT-qPCR.

Target genes	5' to 3' primer sequence	
	Forward	Reverse
<i>Ipo8</i>	CTGAAGGGCACTATCGACCC	TAGGACTGGTTAAGCTCCGTC
<i>Gapdh</i>	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
<i>Oct4</i>	AGAGGATCACCTTGGGGTACA	CGAAGCGACAGATGGTGGTC
<i>Nanog</i>	TCTTCCTGGTCCCCACAGTTT	GCAAGAATAGTTCTCGGGATGAA
<i>Sox2</i>	GCGGAGTGGAACTTTTGTCC	CGGGAAGCGTGTACTTATCCTT
<i>Tgfb1</i>	ACTGGAGTTGTACGGCAGTG	GGGGCTGATCCCGTTGATTT
<i>Ifng</i>	ATGAACGCTACACACTGCATC	CCATCCTTTTGCCAGTTCCTC
<i>Cox2</i>	TTCAACACACTCTATCACTGGC	AGAAGCGTTTGCGGTACTCAT
<i>Ccl2</i>	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
<i>Ido1</i>	GCTTTGCTCTACCACATCCAC	CAGGCGCTGTAACTGTGT
<i>Ido2</i>	TGGGGAGATAACCACATTTCTGA	TGAGGAAGTCTGAGGGCAATTT
<i>Ho1</i>	AAGCCGAGAATGCTGAGTTCA	GCCGTGTAGATATGGTACAAGGA
<i>Il4</i>	CCCCCAGCTAGTTGTCATCC	AGGACGTTTGGCACATCCAT
<i>Il10</i> (non-optimized)	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
<i>Il10</i> (optimized)	CAGATTTCTGCCCTGCGAGA	GTCGAACTCGTTCATGGCCT
<i>Tnfa</i>	GTAGCCACGTCGTAGCAA	ACAAGGTACAACCCATCGGC
<i>Il1b</i>	TGCCACCTTTTGACAGTGATG	AAGGTCCACGGGAAAGACAC
<i>Il6</i>	CCCCAATTTCCAATGCTCTCC	CGCACTAGGTTTGCCGAGTA
<i>Il12</i>	AGACATCACACGGGACCAAAC	CCAGGCAACTCTCGTTCTTGT

Table S3. Percentage of cells expressing positive and negative mesenchymal markers from the cultured wild type MSCs (MSC-WT) and IL-10 engineered MSCs (MSC-IL-10).

Marker	Type	MSC-WT (%)	MSC-IL-10 (%)
CD105	Positive	92.3 ± 4.0	94.3 ± 1.1
CD73		81.3 ± 1.7	95.0 ± 2.5
CD29		95.4 ± 0.2	93.2 ± 0.5
CD45	Negative	4.6 ± 2.1	1.95 ± 1.5
CD34		27.7 ± 2.9	17.1 ± 1.5
CD11b		1.4 ± 0.4	0.81 ± 0.9