Factor	Information	DNA sequence used for mRNA synthesis
PSGL-1	Human P-selectin glycoprotein ligand 1 (24-26, 35).	ATGCCTCTGCAACTCCTCCTGTTGCTGATCCTACTGGGCCCTGGCAACAGCTTGCAGCTGTGGGACACCTGGGCAGATGAAGCCGAGAAAGCCTTGGGTCCCCTGCTTGCCCGGG ACCGGAGACAGGCCACCGAATATGAGTACCTAGATTATGATTTCCTGCCAGAAACGGAGCCTCCAGAAATGCTGAGGAACAGCACTGACACCACTCCTCTGACTGGGCCTGGAACC CCTGAGTCTACCACTGTGGAGCCTGCTGCAAGGCGTTCTACTGGCCTGGATGCAGGAGGGGCAGTCACAGAGCTGACCACGGAGCTGGCCAACATGGGGAACCTGTCCACGGAT TCAGCAGCTATGGAGATACAGACCACTCAACCAGCAGCCACGGAGGCACAGACCACTCCACTGGCAGCCACAGAGGCACAGACAACTCGACTGACGGCCACGGAGGCACAGACC ACTCCACTGGCAGCCACAGAGGCACAGACCACTCCACCAGCAGCCACGGAAGCACAGACCACTCAACCCACAGGCCTGGAGGCACAGACCACTGCACCAGCAGCCATGGAGGCA CAGACCACTGCACCAGCAGCCATGGAAGCACAGACCACTCCACCAGCAGCCATGGAGGCACAGACCACTCAAACCACAGCCATGGAGGCACAGACCACTGCACCAGAAGCCACG GAGGCACAGACCACTCAACCCACAGCCACGGAGGCACAGACCACTCCACTGGCAGCCATGGAGGCCCTGTCCACAGAACCCAGTGCCACAGAGGCCCTGTCCATGGAACCTACT ACCAAAAGAGGTCTGTTCATACCCTTTTCTGTGTCCTCTGTTACTCACAAGGGCATTCCCATGGCAGCCAGCAATTTGTCCGTCAACTACCCAGTGGGGGCCCCAGACCACATCTCT GTGAAGCAGTGCCTGCTGGCCATCCTAATCTTGGCGCTGGTGGCCACTATCTTCTTCGTGTGCACTGTGGTGCTGGCGGTCCGCCTCTCCCGCAAGGGCCACATGTACCCCGTGC GTAATTACTCCCCCACCGAGATGGTCTGCATCTCATCCCTGTTGCCTGATGGGGGTGAGGGGCCCTCTGCCACAGCCAATGGGGGCCTGTCCAAGGCCAAGAGCCCGGGCCTGAC GCCAGAGCCCAGGGAGGACCGTGAGGGGGATGACCTCACCCTGCACAGCTTCCTCCCTTAG
SLEX (FUT-7)	Human Alpha-(1,3)-fucosyltransferase which adds Sialyl-Lewis X oligosaccharides to PSGL-1 scaffold (24-26, 35).	ATGAATAATGCTGGGCACGGCCCCACCCGGAGGCTGCGAGGCTTGGGGGTCCTGGCCGGGGTGGCTCTGCTCGCTGCCCTCTGGCTCCTGTGGCTGCTGGGGTCAGCCCCTCG GGGTACCCCGGCACCCCAGCCCACGATCACCATCCTTGTCTGGCACTGGCCCTTCACTGACCAGCCCCCAGAGCTGCCCAGCGACACCTGCACCCGCTACGGCATCGCCCGCTG CCACCTGAGTGCCAACCGAAGCCTGCTGGCCAGCGCCGACGCCGTGGTCTTCCACCACCGCGAGCTGCAGACCCGGCGGTCCCACCTGCCCCTGGCCCAGCGGCCGCGAGGGC AGCCCTGGGTGTGGGCCTCCATGGAGTCTCCTAGCCACACCCACGGCCTCAGCCACCTCCGAGGCATCTTCAACTGGGTGCTGAGCTACCGGCGCGACTCGGACATCTTTGTGCC CTATGGCCGCCTGGAGCCCCACTGGGGGCCCTCGCCACCGCTGCCAGCCAAGAGCAGGGTGGCCGCCTGGGTGGTCAGCAACTTCCAGGAGCGGCAGCTGCGTGCCAGGCTGT ACCGGCAGCTGGCGCCTCATCTGCGGGTGGATGTCTTTGGCCGTGCCAATGGACGGCCACTGTGCGCCAGCTGCCTGGTGCCCACCGTGGCCCAGTACCGCTTCTACCTGTCCTT TGAGAACTCTCAGCACCGCGACTACATTACGGAGAAATTCTGGCGCAACGCACTGGTGGCTGGCACTGTGCCAGTGGTGCTGGGGCCCCCACGGGCCACCTATGAGGCCTTCGTG CCGGCTGACGCCTTCGTGCATGTGGATGACTTTGGCTCAGCCCGAGAGCTGGCGGCTTTCCTCACTGGCATGAATGAGAGCCGATACCAACGCTTCTTTGCCTGGCGTGACAGGC TCCGCGTGCGACTGTTCACCGACTGGCGGGAACGTTTCTGTGCCATCTGTGACCGCTACCCACACCTACCCCGCAGCCAAGTCTATGAGGACCTTGAGGGTTGGTTTCAGGCCTGA
CD	Yeast cytosine deaminase fused to the UPRT to increase enzyme efficiency (27).	ATGGTCACAGGAGGCATGGCTTCAAAGTGGGACCAGAAGGGCATGGACATTGCCTATGAGGAGGCTGCTCTGGGCTACAAGGAGGGAGGGGTCCCAATTGGTGGCTGCCTCATCA ACAACAAGGATGGCAGTGTCCTGGGCAGGGGCCACAACATGAGGTTCCAGAAGGGCAGTGCCACCCTGCATGGGGAGATCAGCACCCTGGAGAACTGTGGCAGGCTGGAGGGCA AGGTCTACAAGGACACCACTCTGTACACCACCCTCAGCCCTTGTGACATGTGCACAGGGGCCATCATCATGTATGGCATTCCCAGGTGTGTGGTGGGAGAGAATGTCAACTTCAAG TCAAAAGGAGAGAAGTACCTCCAGACCAGGGGCCATGAGGTGGTTGTGGTGGATGATGAGAGGTGCAAGAAGATTATGAAGCAGTTCATTGATGAGAGACCCCAGGACTGGTTTGA GGACATTGGGGAGGCCTCTGAGCCCTTCAAGAATGTGTACCTCCTCCCCCAGACCAACCAACTCCTGGGACTCTACACCATCATCAGGAACAAGAACACCACCAGGCCAGACTTCA TCTTCTACAGTGACAGGATCATCAGGCTCCTGGTGGAGGAGGGCCTCAACCACCTCCCTGTGCAGAAGCAGATTGTGGAGACTGACACCAATGAGAACTTTGAGGGAGTGTCTTTC ATGGGCAAGATTTGTGGGGTGTCCATTGTGAGGGCTGGGGAGAGCATGGAGCAGGGCCTGAGGGACTGTTGCAGGAGTGTGAGGATTGGCAAGATCCTGATCCAGAGGGATGAG GAGACTGCCCTGCCCAAGCTGTTCTATGAGAAGCTCCCTGAAGACATCTCTGAGAGGTATGTCTTCCTCCTGGACCCCATGCTGGCAACTGGAGGCTCTGCAATCATGGCCACTGA GGTGCTCATCAAGAGGGGAGTCAAGCCTGAGAGGATCTACTTCCTCAACCTCATCTGCTCAAAGGAGGGCATTGAGAAGTACCATGCTGCCTTCCCTGAAGTGAGGATTGTCACTG GGGCTCTGGACAGGGGCCTGGATGAGAACAAGTACCTGGTCCCTGGCCTGGGAGACTTTGGGGACAGATACTACTGTGTCTAA
OPG	Truncated human osteoprotegerin (aa 1-194) containing a Y49R mutation to prevent binding to TRAIL and fused to the Fc fragment of human IgG1 (28, 29).	ATGAACAAGTTGCTGTGCTGCGCGCTCGTGTTTCTGGACATCTCCATTAAGTGGACCACCCAGGAAACGTTTCCTCCAAAGTACCTTCATTATGACGAAGAAACCTCTCATCAGCTGT TGTGTGACAAATGTCCTCCTGGTACCTACCTAAAACAACACTGTACAGCAAAGTGGAAGACCGTGTGCGCCCCTTGCCCTGACCACTACCGCACAGACAGCTGGCACACCAGTGAC GAGTGTCTATACTGCAGCCCCGTGTGCAAGGAGCTGCAGTACGTCAAGCAGGAGTGCAATCGCACCCACAACCGCGTGTGCGAATGCAAGGAAGGGCGCTACCTTGAGATAGAGT TCTGCTTGAAACATAGGAGCTGCCCTCCTGGATTTGGAGTGGTGCAAGCTGGAACCCCAGAGCGAAATACAGTTTGCAAAAGATGTCCAGATGGGTTCTTCTCAAATGAGACGTCAT CTAAAGCACCCTGTAGAAAACACACAAATTGCAGTGTCTTTGGTCTCCTGCTAACTCAGAAAGGAAATGCAACACACGACAACATATGTTCCGGAAACAGTGAATCAACTCAAAAAGA CAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCA CATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACA GCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCAT CTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCC AGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG ACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA

<u>Supplementary Table 1</u>: DNA coding sequences used for mRNA synthesis of homing and therapeutic factors.

PSGL-1 bearing SLEX post-translational serves as a counterreceptor for P- and E-selectin, thus mediating the rolling/tethering of engineered MSC towards tumour site. The CD expressed by PSGL-1/SLEX/CD/OPG MSC is fused to the uracil phosphoribosyltransferase (UPRT), which increases its ability to convert the 5-FC pro-drug into the 5-FU chemotherapeutic drug. The OPG secreted by engineered MSC inhibits osteoclastic activity without preventing TRAIL-induced apoptosis thanks to an Y49R mutation in the binding site for TRAIL. The modified OPG is a truncated form of the native protein (no binding to glycosaminoglycans) fused to the Fc fragment of human IgG1 (for increased half-life).

Time-point	Comparison	Model-based Estimate	Raw P	Adjusted P
Right after treatment	CD/OPG MSC - CD MSC	-0.64 (-1.80, 0.52)	0.2830	0.4043
	CD/OPG MSC - OPG MSC	-1.88 (-3.04, -0.72)	0.0025	0.0123
	CD MSC - PBS	-1.81 (-3.09, -0.54)	0.0073	0.0244
	OPG MSC - PBS	-0.58 (-1.85, 0.70)	0.3796	0.4745
	CD/OPG MSC - PBS	-2.45 (-3.73, -1.18)	0.0004	0.0039
End-point	CD/OPG MSC - CD MSC	-0.24 (-2.25, 1.76)	0.8127	0.8127
	CD/OPG MSC - OPG MSC	-0.72 (-2.73, 1.28)	0.4829	0.5366
	CD MSC - PBS	-2.45 (-4.66, -0.25)	0.0332	0.0664
	OPG MSC - PBS	-1.98 (-4.18, 0.23)	0.0843	0.1405
	CD/OPG MSC - PBS	-2.70 (-4.90, -0.49)	0.0197	0.0494

Supplementary Table 2: CD/OPG MSC treatment inhibits tumour growth of MDA-MB231 tumours implanted in the tibias of Nude mice. A pilot experiment was done to assess the efficacy of CD and OPG therapeutic factors *in vivo.* The following treatments were done locally after inducing intratibial tumours: PBS, CD MSC (PSGL-1/SLEX/CD MSC), OPG MSC (PSGL-1/SLEX/OPG MSC) and CD/OPG MSC (PSGL-1/SLEX/CD/OPG MSC), n=4 per group. From the results of that experiment, a power analysis was done to determine the minimal number of mice to include for the final study. This study, presented in the main figures, contains the following groups: PBS, Mock MSC (Mock transfected MSC), CD MSC (PSGL-1/SLEX/CD MSC), OPG MSC (PSGL-1/SLEX/OPG MSC) and CD/OPG MSC (PSGL-1/SLEX/CD/OPG MSC), n=10 per group. A linear mixed model (LMM) was used to compare the tumour growth ratio (bioluminescence values at different time-points normalised to value before treatment) among different treatment groups with a random effect to adjust for the potential mice correlation within each experiment. As the first experiment did not include Mock MSC, we used PBS as our control group here for comparisons. A method of false discovery rate (FDR) was used to correct for multiple comparisons. Table shows the pairwise group comparisons of interest. First column contains the model-based average of natural log of tumour growth ratio for each treatment group at two different time points (right after the treatment, and at the end-point). The 95% confidence interval was provided in the parenthesis. Adjusted P is the *p* value after FDR correction.

Outcome	Comparison	Model-based Estimate	Raw P	Adjusted P
Trabecular bone volume (End-point)	CD/OPG MSC - CD MSC	0.06 (-0.19, 0.32)	0.6447	0.6447
	CD/OPG MSC - OPG MSC	-0.12 (-0.38, 0.14)	0.3606	0.4507
	CD MSC - PBS	0.30 (0.01, 0.59)	0.0447	0.0745
	OPG MSC - PBS	0.48 (0.19, 0.77)	0.0018	0.0090
	CD/OPG MSC - PBS	0.36 (0.08, 0.64)	0.0152	0.0379

Supplementary Table 3: OPG MSC and CD/OPG MSC treatments prevent bone loss induced by MDA-MB231 tumours in the tibias of Nude mice. A pilot experiment was done to assess the efficacy of CD and OPG therapeutic factors *in vivo.* The following treatments were done locally after inducing intratibial tumours: PBS, CD MSC (PSGL-1/SLEX/CD MSC), OPG MSC (PSGL-1/SLEX/OPG MSC) and CD/OPG MSC (PSGL-1/SLEX/CD/OPG MSC), n=4 per group. From the results of that experiment, a power analysis was done to determine the minimal number of mice to include for the final study. This study, presented in the main figures, contains the following groups: PBS, Mock MSC (Mock transfected MSC), CD MSC (PSGL-1/SLEX/CD MSC), OPG MSC (PSGL-1/SLEX/OPG MSC), and CD/OPG MSC (PSGL-1/SLEX/CD/OPG MSC), n=10 per group. A linear mixed model (LMM) was used to compare the tumour growth among different treatment groups with a random effect to adjust for the potential mice correlation within each experiment. As the first experiment did not include Mock MSC, we used PBS as our control group here for comparisons. A method of false discovery rate (FDR) was used to correct for multiple comparisons. Table shows the pairwise group comparisons of interest. First column contains the model-based average of natural log of tumour growth ratio for each treatment group at two different time points (right after the treatment, and at the end-point). The 95% confidence interval was provided in the parenthesis. Adjusted P is the *p* value after FDR correction.

<u>Supplementary Table 4</u>: PSGL-1/SLEX/CD/OPG MSC treatment preserves trabecular bone integrity.

	Trabecular volume (mm^3)	
	HEALTHY LEG	**TUMOUR LEGS**
CT	0.52001	0
	0.40861	0
Native MSC	0.39211	0
	0.40878	0
Engineered MSC	0.34741	0.36487
	0.58204	0.4962

Trabecular bone volume values are shown for each animal (in mm^3) at the end-point of the experiment for tumour bearing legs and their corresponding healthy legs (2 animals per group). Trabecular bone volume was measured over 1.4mm (100 slices), starting from the growth plate of the tibia.

<u>Supplementary Table 5:</u> List of antibodies used in the study.

Type	Use	Target	Clone/Specie	Conjugate	Manufacturer	Dilution used
Primary antibody	WB	Human IgG (Fc specific)	Polyclonal Goat	N/A	Sigma (I2136)	1:500
Primary antibody	WB	Human/murine GAPDH	Monoclonal (14C10) Rabbit	N/A	Fisher Scientific (50190704)	1:1,000
Primary antibody	WB	Yeast cytosine deaminase	Polyclonal Sheep	N/A	Fisher Scientific (PA185365)	1:500
Primary antibody	IF	Human PSGL-1	Monoclonal (PL1) Mouse	N/A	Santa Cruz Biotechnology (sc-18855)	1:100
Primary antibody	IF	Murine CD62P	Polyclonal Goat	N/A	R&D systems (AF737)	1:50
Primary antibody	IF	Murine CD41	Monoclonal (MWReg30) Rat	N/A	Abcam (ab206636)	1:50
Primary antibody	IF	Murine endomucin	Monoclonal (V.7C7.1) Rat	N/A	Abcam (ab106100)	1:100
Primary antibody	FC	Murine CD45	Monoclonal (30-F11) Rat	FITC	TONBO (35-0451)	1:50
Primary antibody	FC	Human/murine CD45R (B220)	Monoclonal (RA3-6B2) Rat	APC	TONBO (20-0452)	1:50
Primary antibody	FC	Human/murine CD11b	Monoclonal (M1/70) Rat	PE	TONBO (50-0112)	1:50
Primary antibody	FC	Murine Gr-1	Monoclonal (RB6-8C5) Rat	PE-Cy7	TONBO (60-5931)	1:50

Secondary antibody	IF	Murine IgG	Polyclonal Donkey	Alexa Fluor 488	Jackson ImmunoResearch (715-545-150)	1:1,000
Secondary antibody	IF	Goat IgG	Polyclonal Donkey	Alexa Fluor 647	ThermoFisher (A21447)	1:1,000
Secondary antibody	IF	Sheep IgG	Polyclonal Donkey	Alexa Fluor 488	Jackson ImmunoResearch (713-545-003)	1:1,000
Secondary antibody	IF	Rat IgG	Polyclonal Donkey	Alexa Fluor 647	Abcam (ab150155)	1:1,000
Secondary antibody	IF	Goat IgG	Polyclonal Donkey	DyLight 755	Fisher Scientific (SA510091)	1:500
Secondary antibody	IF	Rat IgG	Polyclonal Donkey	DyLight 755	Fisher Scientific (SA510031)	1:500
Secondary antibody	WB	Goat IgG	Polyclonal Donkey	HRP	Santa Cruz Biotechnology (sc-2020)	1:10,000
Secondary antibody	WB	Rabbit IgG	Polyclonal Donkey	HRP	Santa Cruz Biotechnology (sc-2313)	1:10,000
Secondary antibody	WB	Sheep IgG	Polyclonal Donkey	HRP	Santa Cruz Biotechnology (sc-2473)	1:5,000

WB: Western Blotting, IF: Immunofluorescence, FC: Flow cytometry, HRP: Horseradish Peroxidase, FITC: Fluorescein isothiocyanate, APC: Allophycocyanin, PE: Phycoerythrin, PE-Cy7: Phycoerythrin-Cyanine 7, N/A: non-applicable.