Electronic Supplementary Information

Stimulation of bone formation by monocyte-activator functionalized graphene oxide *in vivo*

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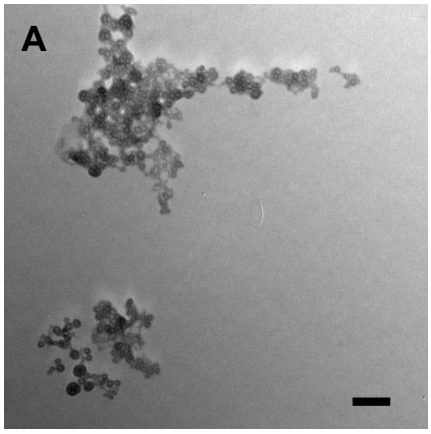
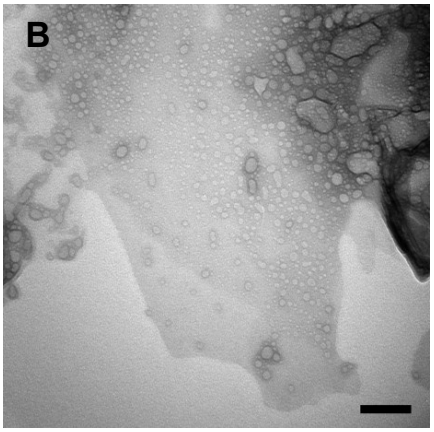
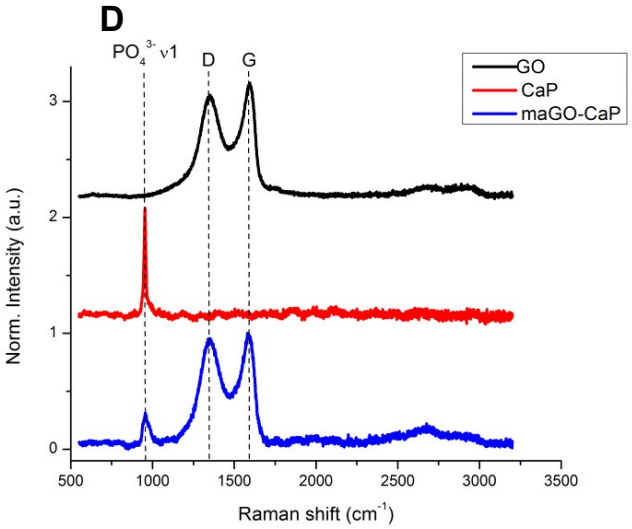




C

	Before maturation	After maturation
CaP	1.11±0.09	1.75±0.01
maGO-CaP	1.34±0.04	2.2±0.2



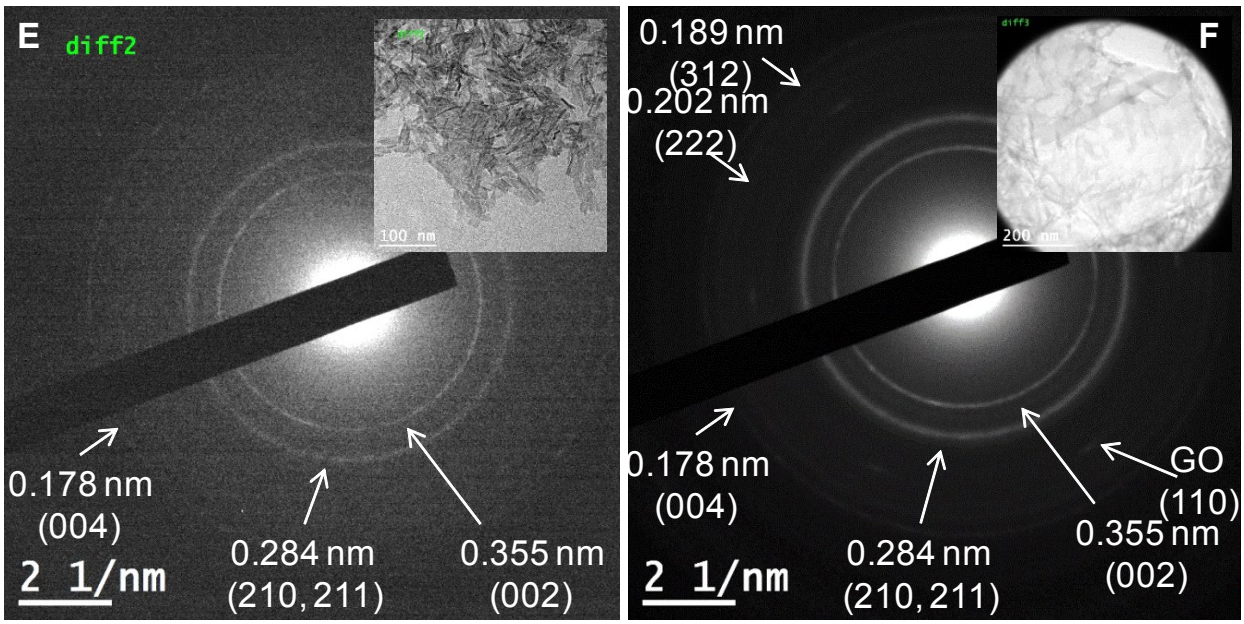


Figure S1. A) TEM of freshly synthetized CaP and B) maGO-CaP (scale bar: 100 nm), C) Ca/P ratio obtained from XPS of CaP and maGO-CaP, D) Raman of GO, CaP and maGO-CaP, E) SAED of CaP and F) maGO-CaP with diffraction assignations (see inset for image analyzed). Interplanar spacing (d) was calculated from d = 2/distance between two bright spots.

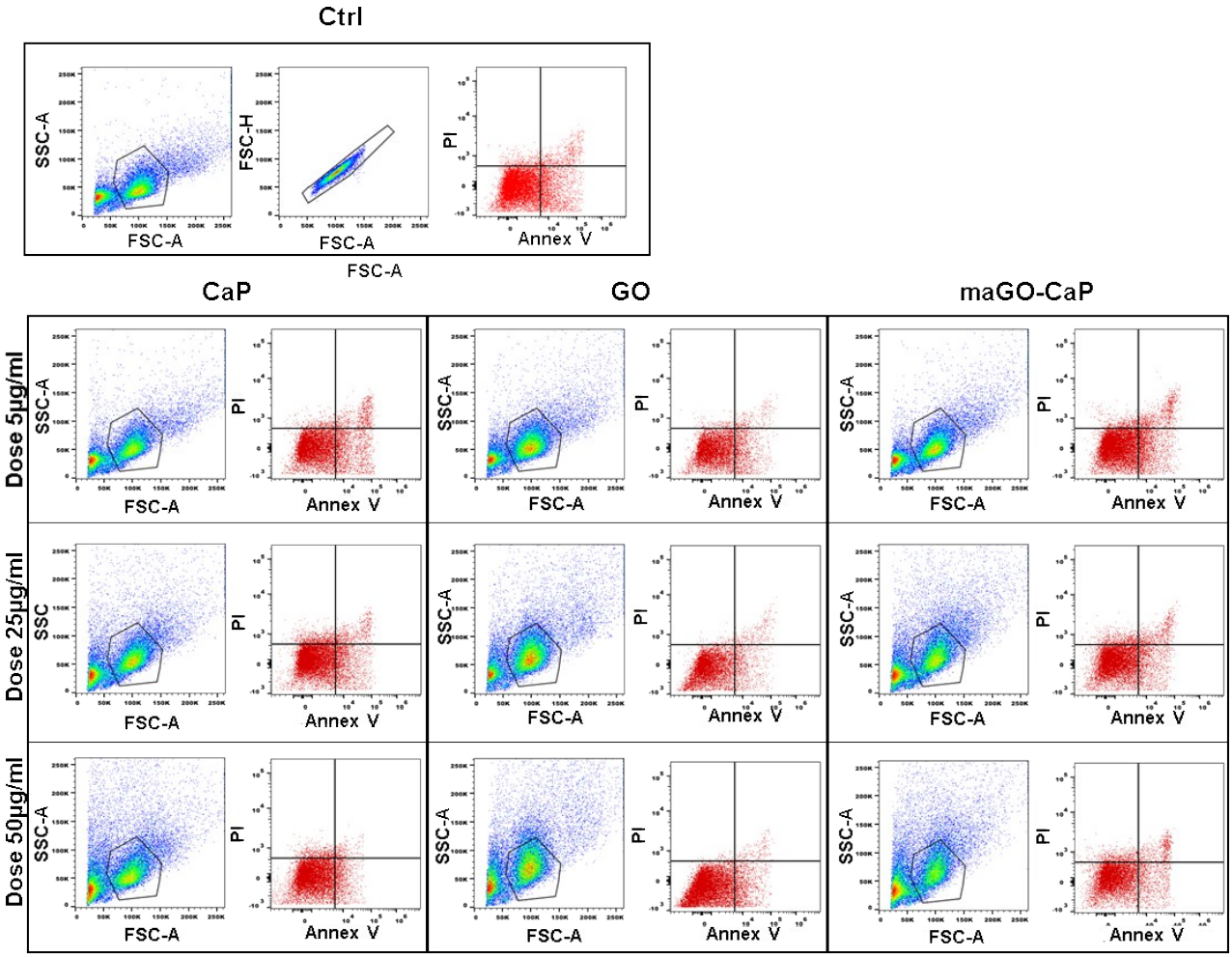


Figure S2. FACS profile. The dot plots represent the flow cytometry profiles of monocytes after 24 h of treatment with GO, CaP and maGO-CaP, stained with Annexin V/PI.

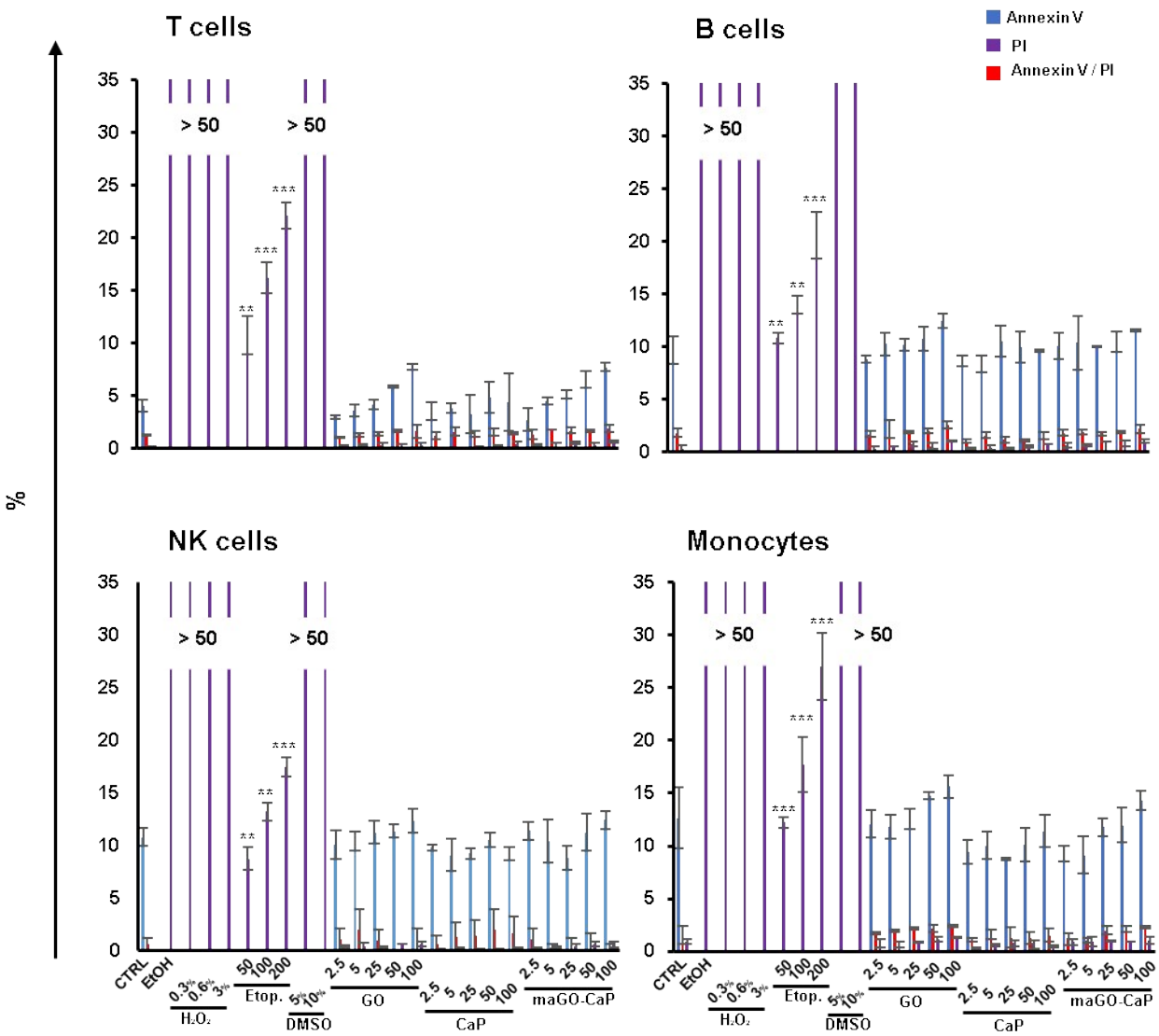


Figure S3. PBMC viability. PBMCs were isolated from buffy coat of healthy donors and cultured in presence or absence of increasing doses of GO, maGO-CaP and CaP (2.5, 5, 25, 50 and 100 µg/ml) for 24 h or left untreated. Viability was assessed by Annexin V/PI staining. Ethanol at 70%, etoposide at different concentrations (50, 100, and 200 µM), H_2O_2 (0.3%, 0.6% and 3%) and DMSO (5% and 10%) were used as positive controls.

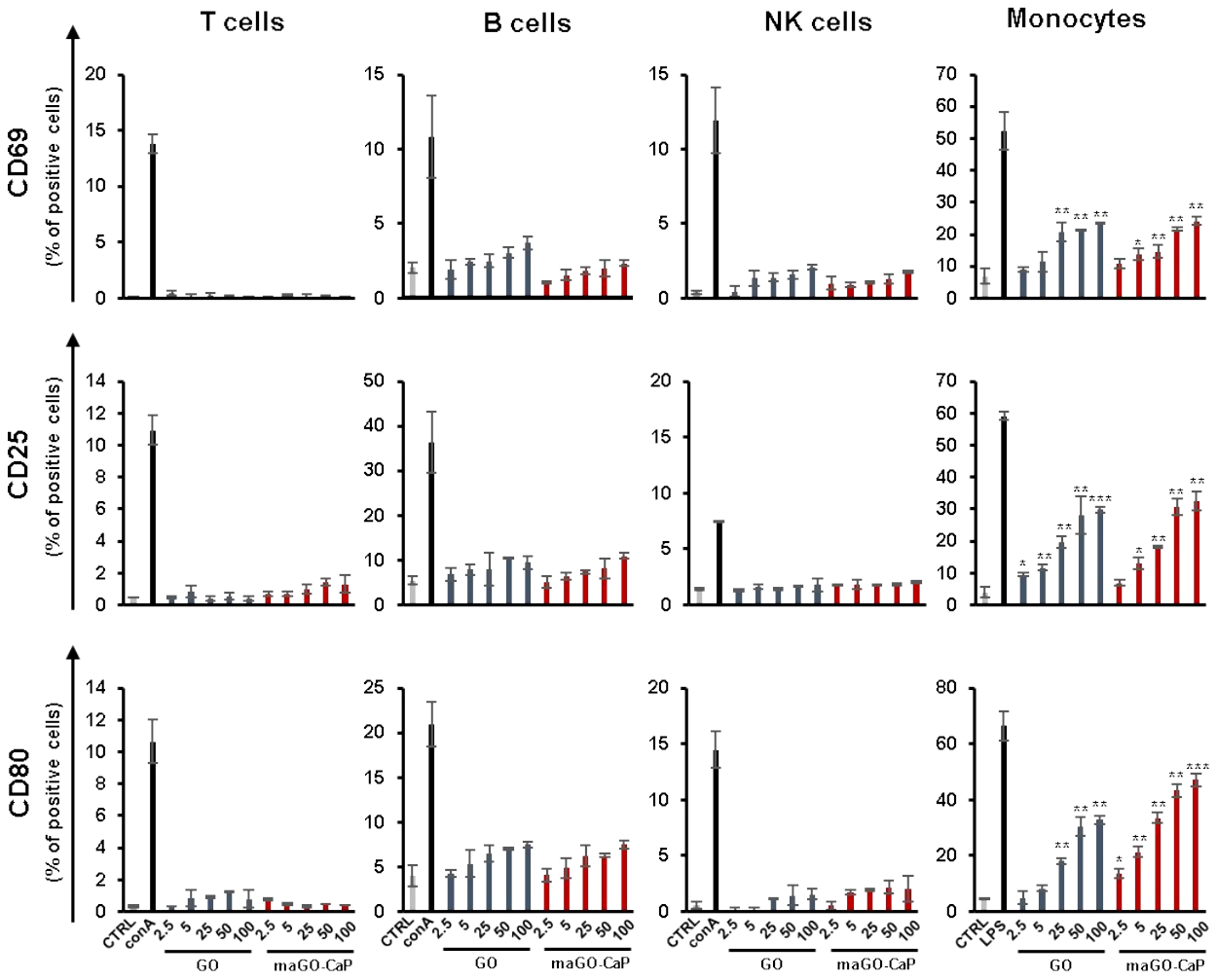


Figure S4. PBMC activation. PBMCs were isolated from buffy coat of healthy donors and treated with increasing doses of GO, maGO-CaP and CaP (2.5, 5, 25, 50 and 100 µg/ml) for 24 h or left untreated. LPS and conA were used as positive controls. The activation status was evaluated analyzing the expression of CD69, CD25 and CD80 by flow cytometry.

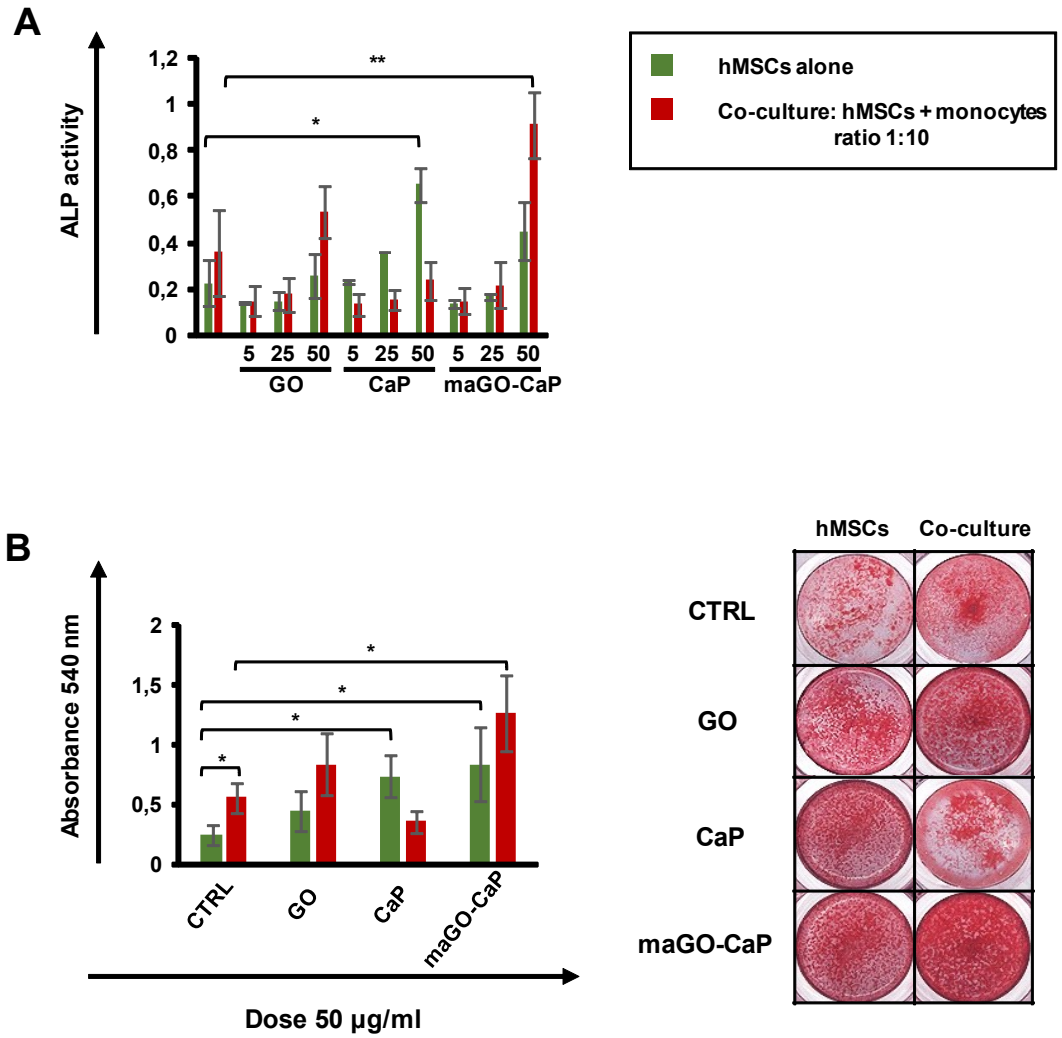


Figure S5. Osteogenic differentiation of hMSCs in presence or absence of monocytes. hMSCs and monocytes co-culture and only hMSCs without monocytes were incubated with GO, CaP and maGO-CaP at 5, 25 and 50 µg/ml or left untreated. A) ALP activity was quantified after 7 days of incubation. B) Alizarin red assay was performed to visualize the bone matrix formation at day 14.

Layout	01	02	03	04	05	06	07	08	09	10	11	12
A	ACVR1 **1.09**	AHSG **-2.16**	ALPL **-2.68**	ANXA5 **1.53**	BGLAP **1.02**	BGN **2.18**	BMP1 **1.43**	BMP2 **4.71**	BMP3 **4.31**	BMP4 **2.12**	BMP5 **1.89**	BMP6 **2.26**
B	BMP7 **2.38**	BMPR1A **1.18**	BMPR1B **1.14**	BMPR2 **1.56**	CALCR **2.51**	CD36 **-1.53**	CDH11 **3.27**	CHRD **2.20**	COL10A1 **4.36**	COL14A1 **3.22**	COL15A1 **-1.11**	COL1A1 **1.83**
C	COL1A2 **2.85**	COL2A1 **1.16**	COL3A1 **1.39**	COL5A1 **1.82**	COMP **1.35**	CSF1 **1.56**	CSF2 **1.07**	CSF3 **-1.47**	CTSK **1.06**	DLX5 **1.82**	EGF **-1.35**	EGFR **1.41**
D	FGF1 **-1.58**	FGF2 **-1.00**	FGFR1 **1.53**	FGFR2 **1.79**	FLT1 **4.21**	FN1 **2.32**	GDF10 **-1.63**	GLI1 **-1.68**	ICAM1 **1.68**	IGF1 **-2.61**	IGF1R **1.96**	IGF2 **1.83**
E	IHH **-2.25**	ITGA1 **1.69**	ITGA2 **1.56**	ITGA3 **2.08**	ITGAM **-1.73**	ITGB1 **1.89**	MMP10 **1.24**	MMP2 **2.19**	MMP8 **-1.09**	MMP9 **2.14**	NFKB1 **1.42**	NOG **-1.97**
F	PDGFA **1.38**	PHEX **1.31**	RUNX2 **1.95**	SERPINH1 **1.33**	SMAD1 **2.22**	SMAD2 **1.06**	SMAD3 **-1.27**	SMAD4 **1.44**	SMAD5 **1.22**	SOX9 **1.85**	SP7 **2.27**	SPP1 **-1.85**
G	TGFB1 **1.05**	TGFB2 **1.05**	TGFB3 **2.13**	TGFBR1 **-1.44**	TGFBR2 **1.28**	TNF **1.41**	TNFSF11 **2.00**	TWIST1 **2.16**	VCAM1 **-1.19**	VDR **1.38**	VEGFA **1.53**	VEGFB **1.62**

Figure S6. Heat map table. The table describes the value of log 2-fold change for the all genes examined in the gene expression analysis reported in Figure 4.

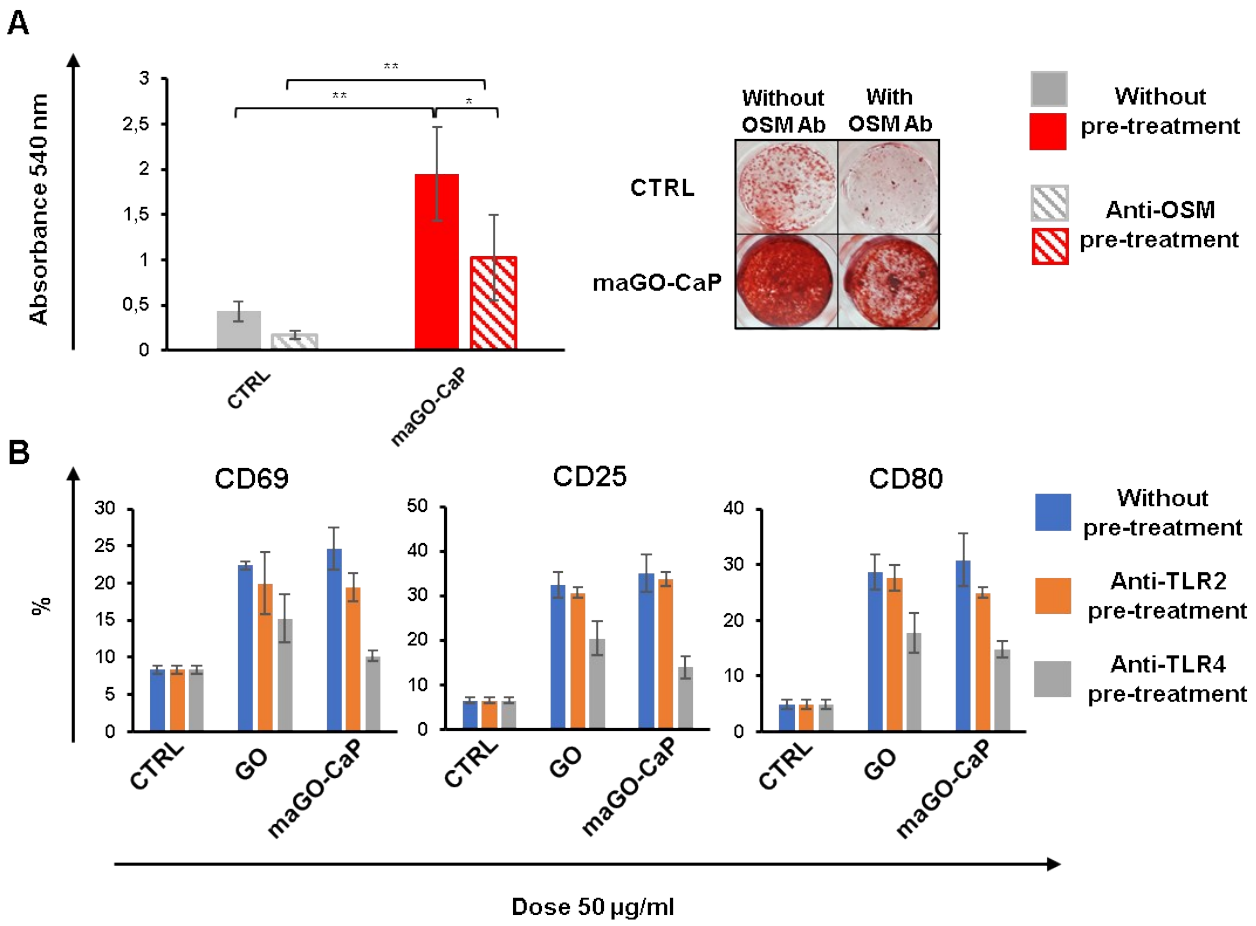


Figure S7. Mechanism of activation of monocytes and hMSCs differentiation. A) hMSC-monocyte co-culture were treated with 50 µg/ml of maGO-CaP. OSM neutralizing antibody was added or not to co-cultures at the concentration of 100 ng/ml in osteogenic media. After 14 days, the formation of the bone nodules was evaluated with alizarin red staining. B) Monocytes were pre-treated with anti-TLR2 and anti-TLR4 antibodies. After 30 min of incubation, cells were treated with 50 µg/ml of GO and maGO-CaP. The expression of CD69, CD25 and CD80 were evaluated by flow cytometry.

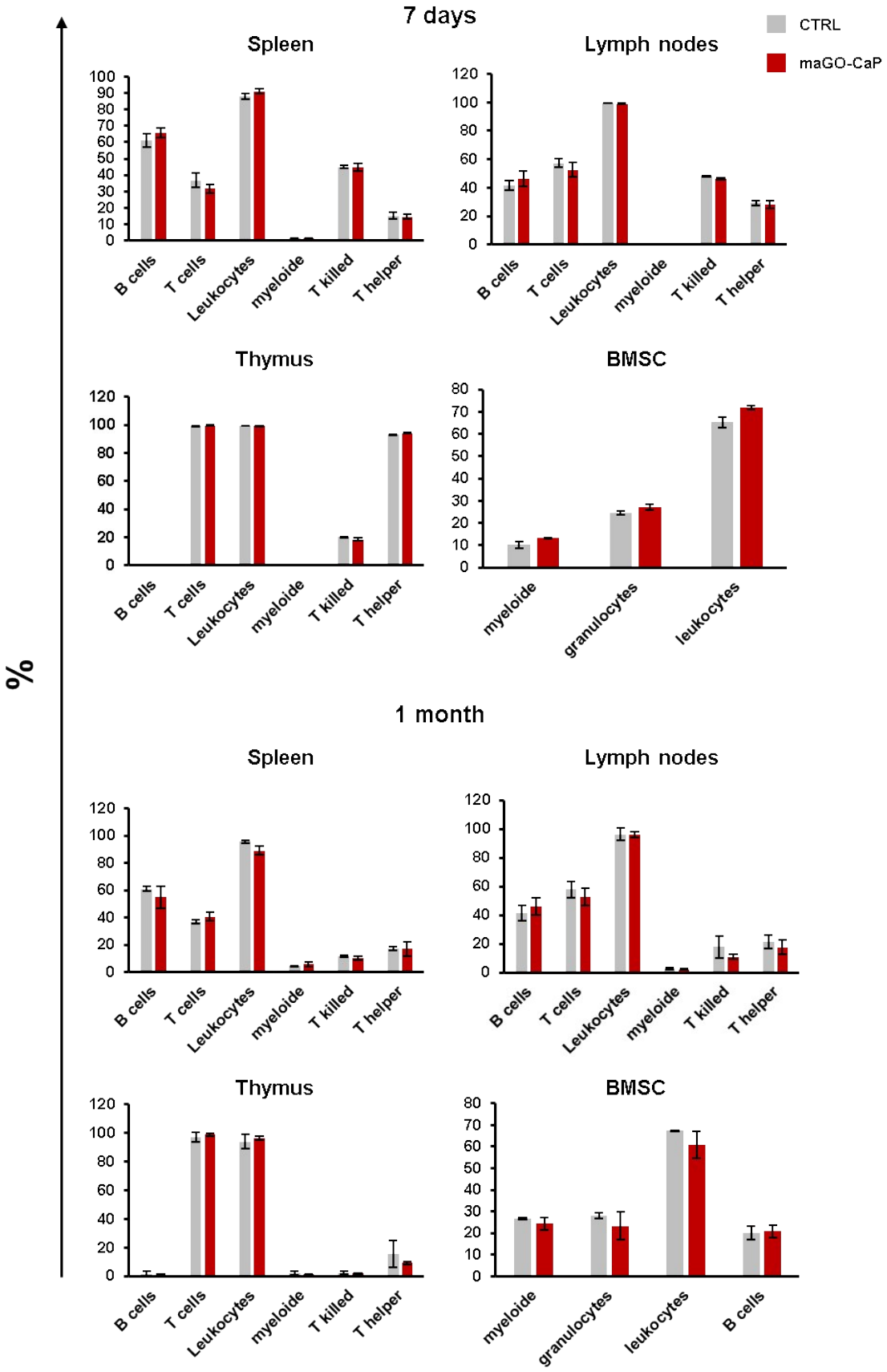


Figure S8. Systemic inflammation *in vivo*. The systemic inflammation of maGO-CaP *in vivo* was analyzed by flow cytometry. After one week and one month, the mice were sacrificed and spleen, the lymph nodes, the thymus and the bone marrow were harvested. The percentage of cells involved in inflammatory reaction were quantified.

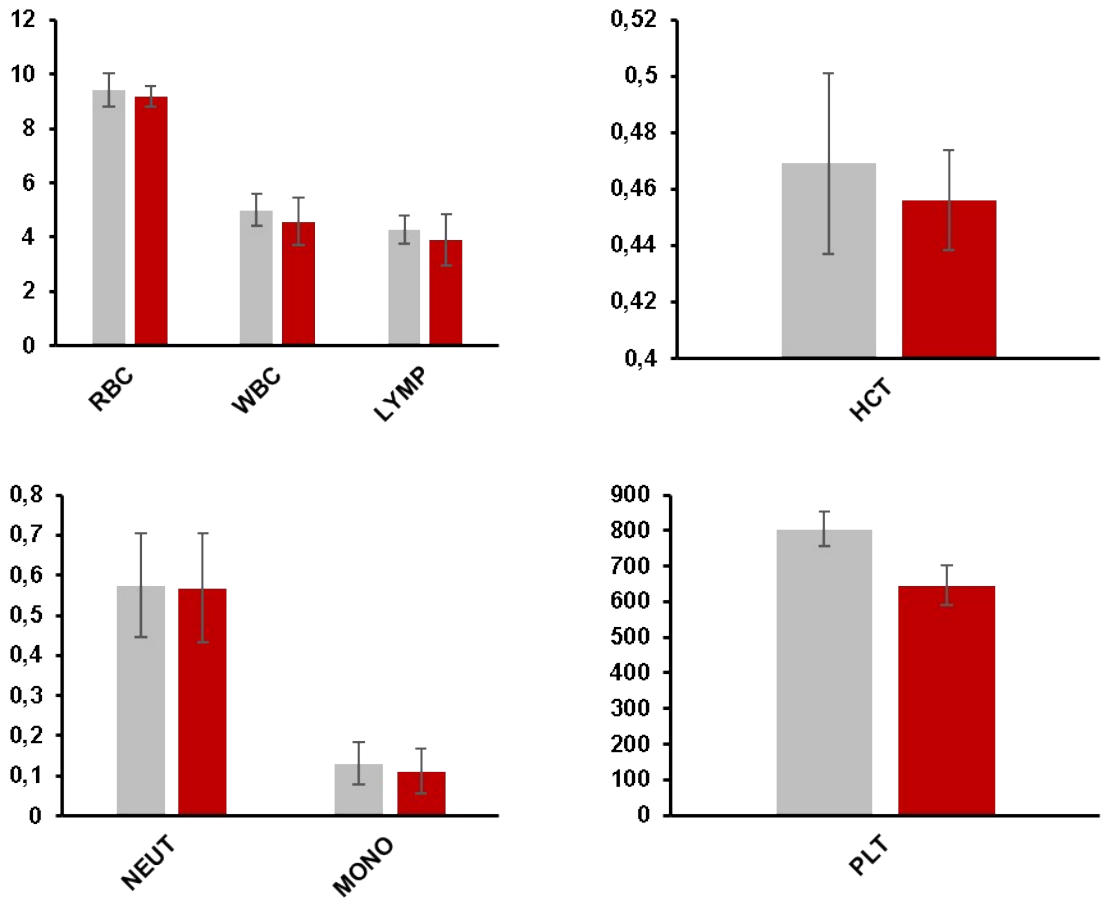


Figure S9. The histogram illustrates the values reported in Table S1, on the blood analysis, with the respective standard deviations. RBC, red blood cells; WBC, white blood cells; LYMP, lymphocytes; HCT, haematocrit; NEUT, neutrophils; MONO, monocytes; PLT, platelets.

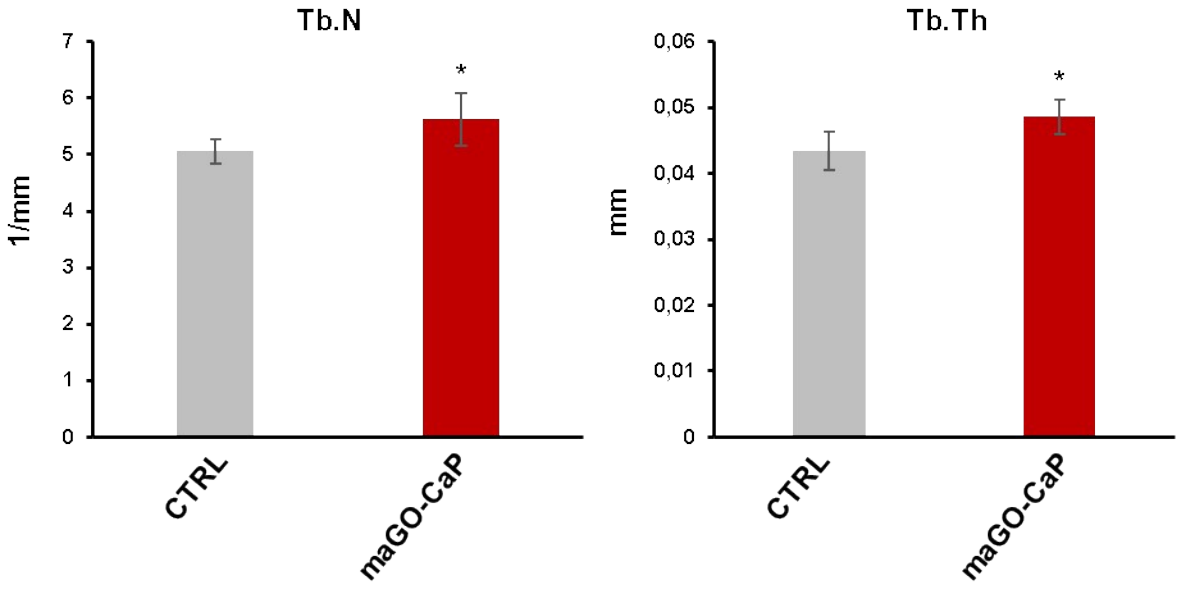


Figure S10. *In vivo* bone formation. A) The tibias of mice were used for µCT analysis. Trabecular number (TB.N) and trabecular thickness (TB.Th) were evaluated after one month of maGO-CaP treatment.

Layout	01	02	03	04	05	06	07	08	09	10	11	12
A	Acvr1 **1.57**	Ahsg **1.93**	Alpl **1.97**	Anxa5 **-1.36**	Bglap **2.20**	Bgn **2.66**	Bmp1 **1.32**	Bmp2 **-1.10**	Bmp3 **2.04**	Bmp4 **1.74**	Bmp5 **-1.12**	Bmp6 **1.23**
B	Bmp7 **1.21**	Bmpr1a **1.19**	Bmpr1b **1.63**	Bmpr2 **1.48**	Cd36 **1.12**	Cdh11 **2.98**	Chrd **1.42**	Col10a1 **1.54**	Col14a1 **-1.08**	Col1a1 **2.85**	Col1a2 **4.16**	Col2a1 **1.13**
C	Col3a1 **-1.02**	Col4a1 **1.98**	Col5a1 **1.31**	Comp **-1.24**	Csf1 **1.58**	Csf2 **1.97**	Csf3 **1.69**	Ctsk **-1.71**	Dlx5 **1.25**	Egf **-1.02**	Fgf1 **1.25**	Fgf2 **1.39**
D	Fgfr1 **1.26**	Fgfr2 **1.40**	Flt1 **1.58**	Fn1 **2.66**	Gdf10 **-1.05**	Gli1 **1.00**	Icam1 **1.58**	Igf1 **-1.40**	Igf1r **2.38**	Ihh **1.14**	Itga2 **2.10**	Itga2b **1.31**
E	Itga3 **-1.31**	Itgam **1.21**	Itgav **1.74**	Itgb1 **1.20**	Mmp10 **1.38**	Mmp2 **1.22**	Mmp8 **-1.02**	Mmp9 **1.22**	Nfkb1 **1.42**	Nog **-1.23**	Pdgfa **-1.09**	Phex **1.04**
F	Runx2 **1.59**	Serpinh1 **1.32**	Smad1 **1.25**	Smad2 **1.13**	Smad3 **1.52**	Smad4 **1.30**	Smad5 **1.51**	Sost **-1.17**	Sox9 **-1.36**	Sp7 **1.24**	Spp1 **1.07**	Tgfb1 **2.42**
G	Tgfb2 **-2.29**	Tgfb3 **-1.06**	Tgfbr1 **1.14**	Tgfbr2 **1.17**	Tgfbr3 **-1.39**	Tnf **-1.09**	Tnfsf11 **3.61**	Twist1 **-1.08**	Vcam1 **-1.55**	Vdr **-1.03**	Vegfa **1.34**	Vegfb **1.08**

Figure S11. Heat map table. The table describes the value of log 2-fold change for the all genes examined reported in Figure 6D.

Table S1. Blood analysis indicates the value of red blood cells, haematocrit, platelet, white blood cells, neutrophil, lymphocytes and monocytes, of treated and untreated mice. In the table it is also indicates the p value and the standard deviations between the two groups of mice.

	PBS mean value	maGO-CaP mean value	P value	St Dev
Red Blood Cells $[10^{12/L}]$	9.43	9.164	0.448	0.188
Haematocrit [Ratio]	0.469	0.456	0.459	0.009
Platelet $[10^{9/L}]$	804.5	807	0.420	112.297
White Blood Cells $[10^{9/L}]$	4.99	4.584	0.446	0.287
Neutrophil $[10^{9/L}]$	0.575	0.568	0.939	0.004
Lymphocytes $[10^{9/L}]$	4.28	3.904	0.504	0.265
Monocytes $[10^{9/L}]$	0.13	0.112	0.638	0.012