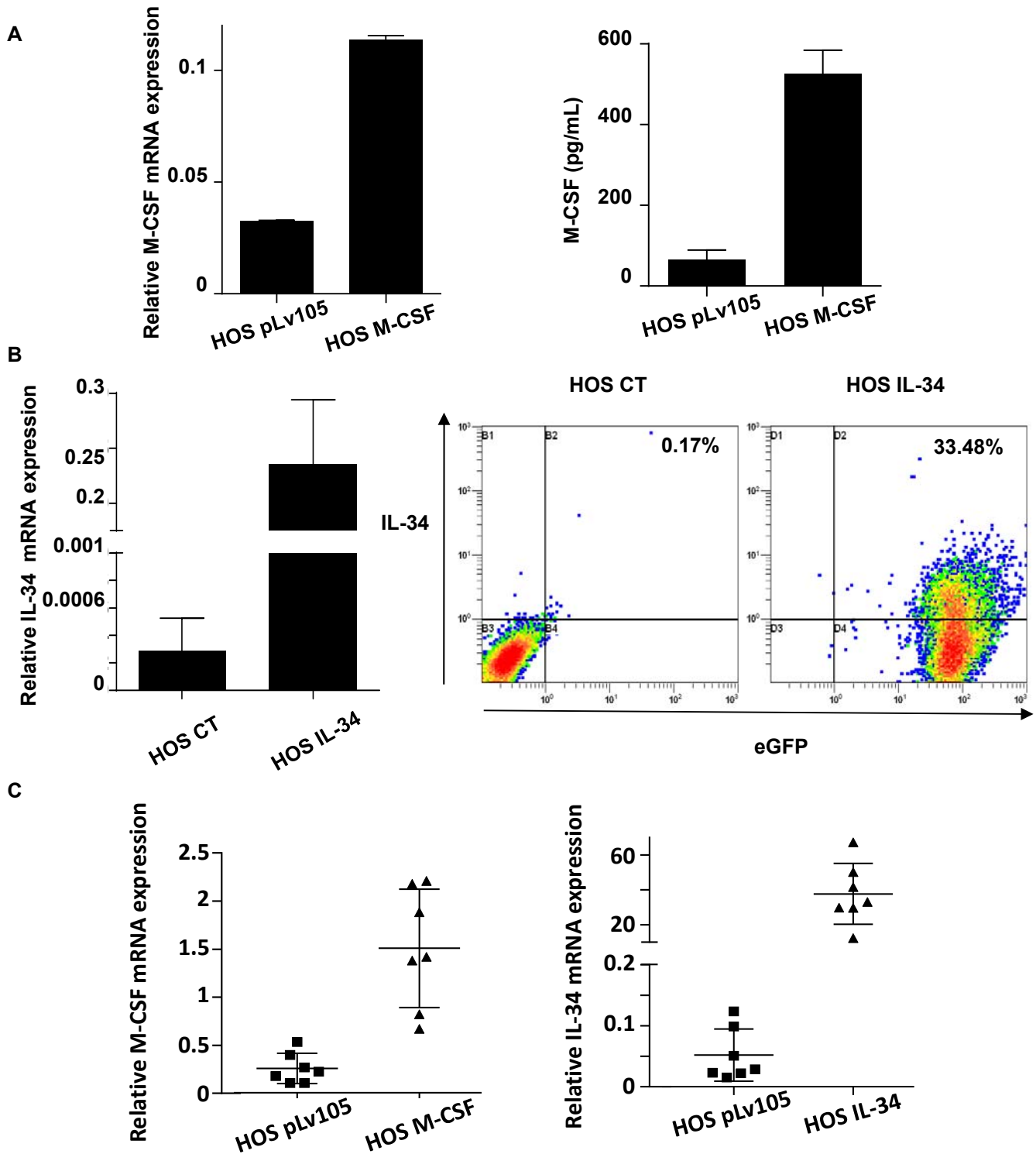
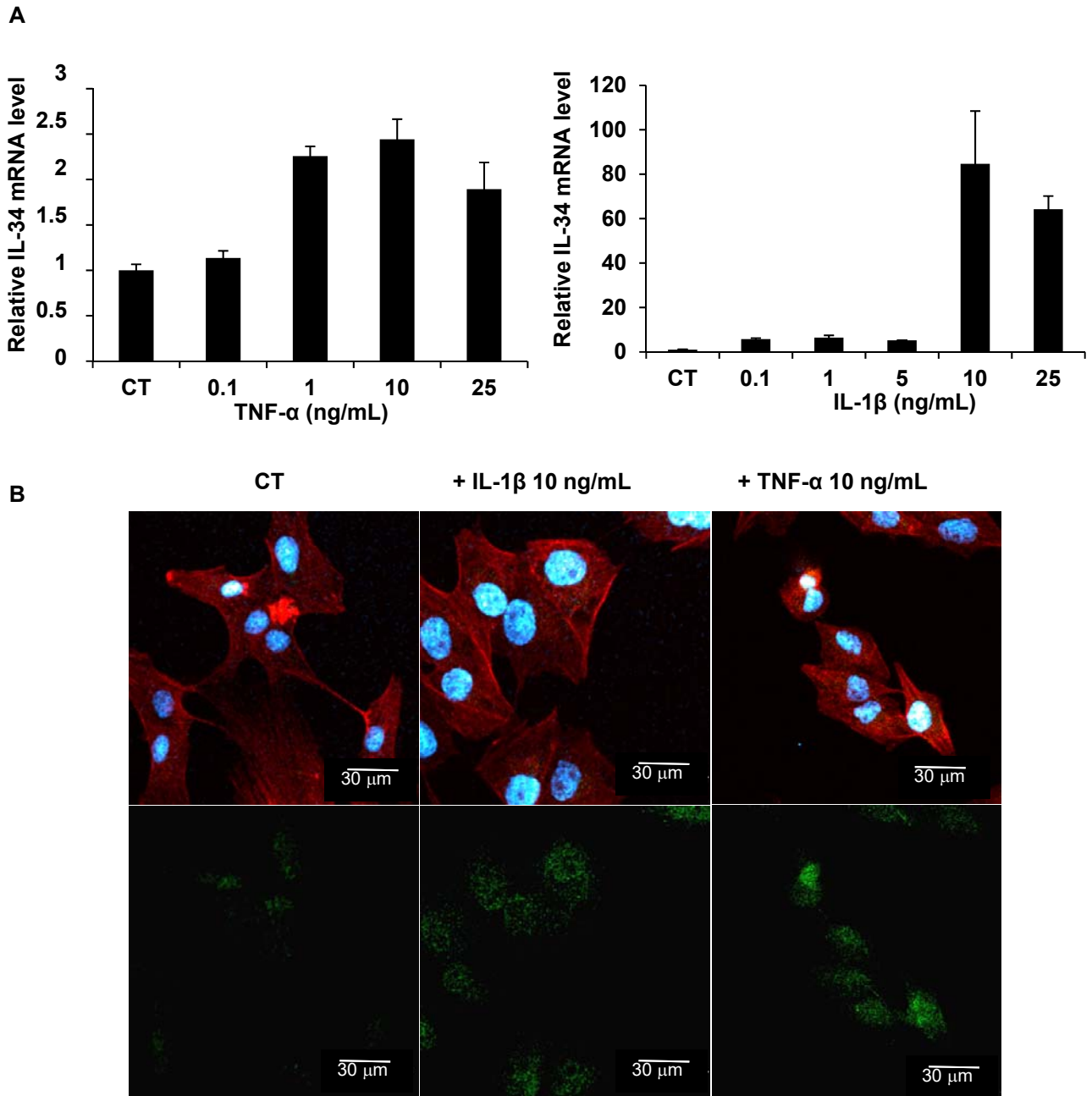


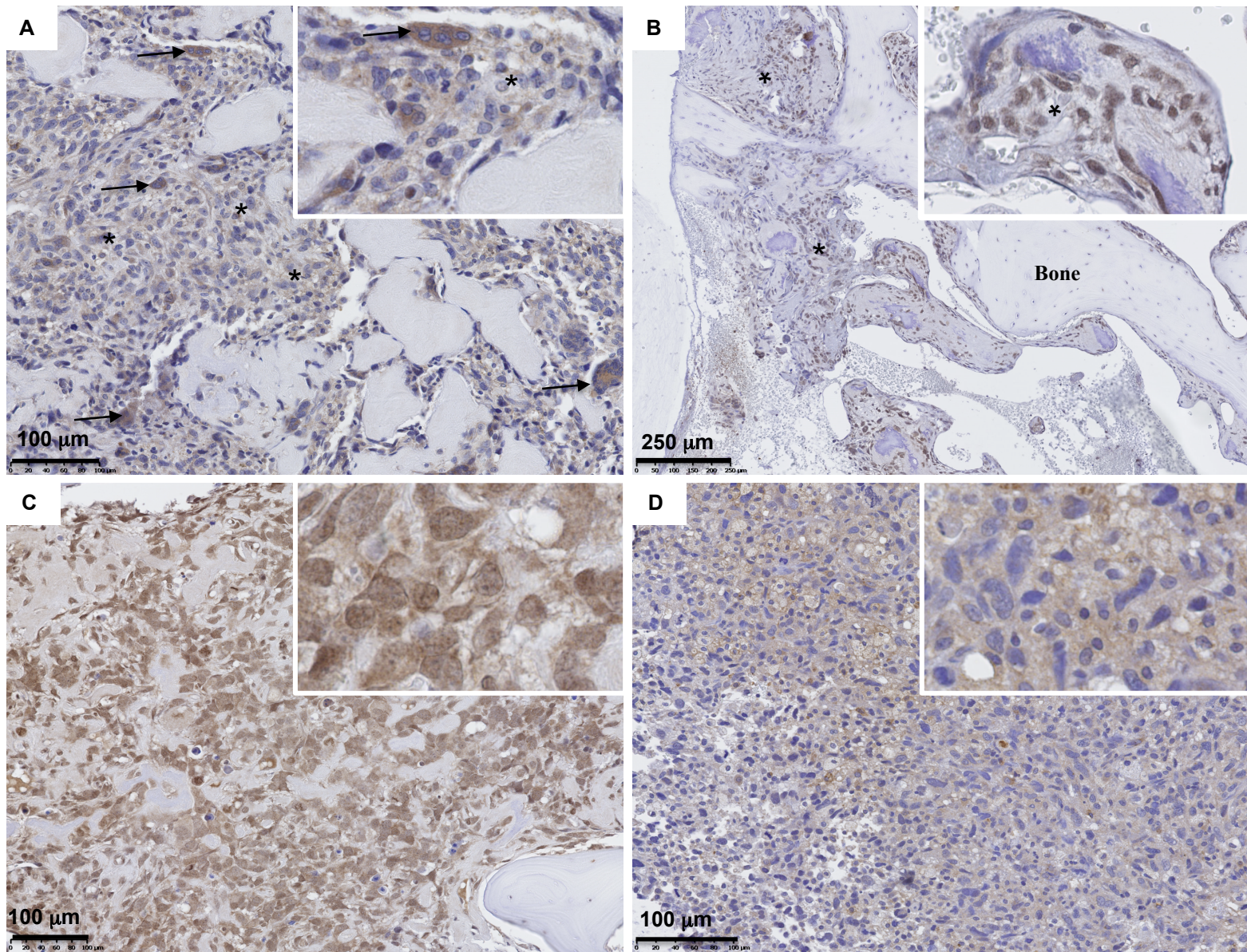
Supplementary Data 1: Human osteosarcoma expressed IL-34. (A) Clinical features of 12 osteosarcoma treated at the University Hospital of Nantes (Department of Orthopaedic Surgery, France). (B) IL-34 expression by quantitative PCR performed in human osteosarcoma biopsies (patients 1-12) and in human HOS, U2OS, Saos2 and MG63 osteosarcoma cell lines. IL-34 mRNA expression was normalized to the HPRT expression. (C) Clinical characteristics of 27 osteosarcoma patients and (D) relative IL-34 expression in the corresponding biospie specimens from GEO bank. OS: osteosarcoma.



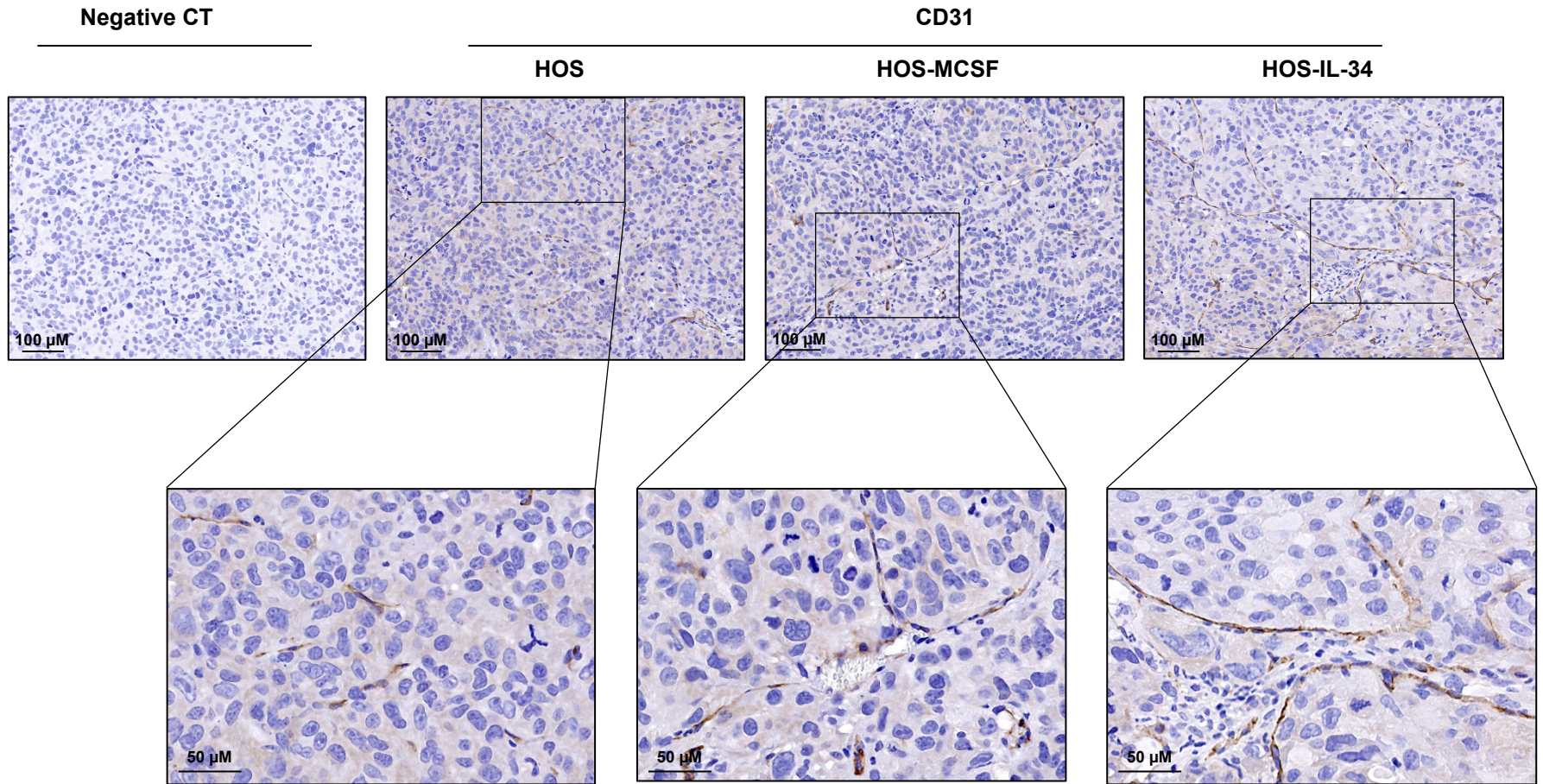
Supplementary Data 2: Validation data of the IL-34 and M-CSF expression by transduced HOS osteosarcoma cells. HOS osteosarcoma cells transduced by M-CSF (A) and IL-34 (B) overexpressed *in vitro* the corresponding cytokines compared to the control cells (empty vector, CT) at the transcriptional level [as demonstrated by quantitative PCR (A, B)] and at the protein level [as demonstrated by ELISA assay (A) and flow cytometry (B)]. Similarly, M-CSF and IL-34 were also overexpressed compared to control group (pLv105 vector) in explanted tumour tissues established from inoculation of M-CSF- or IL-34-transduced HOS osteosarcoma cells (C). For quantitative PCR, HPRT gene expression was used as the invariant control.



Supplementary Data 3: TNF- α and IL-1 β increase IL-34 mRNA expression in various osteosarcoma cells. (A) U2OS osteosarcoma cells were stimulated with increased dose (1–25 ng/ml) of TNF- α or IL-1 β for 8 h. After incubation, IL-34 mRNA levels were determined by RTqPCR, normalised to GAPDH. Error bars represent the standard deviation between 3 independent experiments (B) MG63 osteosarcoma cells cultured on plastic chamber on microscope glass slides were treated or not with 10 ng/mL of TNF- α or IL-1 β for 24 h. IL-34 expression detected by Alexa Fluor 488-conjugated antibody (green), actin filaments detected by alexa fluor 546-conjugated phalloidin (red), and nuclei stained by DAPI (blue) were observed by confocal microscopy. A representative experiment is shown.

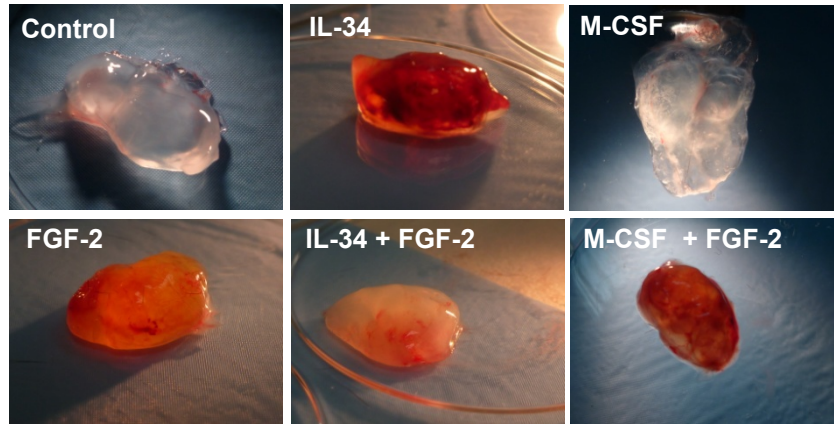


Supplementary Data 4: IL-34 is expressed by osteosarcoma cells. IL-34 expression was analyzed by immunohistochemistry in human osteosarcoma biopsies. In all samples studied, most of osteosarcoma cells showed a positive immunostaining for IL-34 (A-D). (A) Multinucleated cells (arrow) closely located to tumour cells (asterisk) also expressed IL-34 staining. (B) In contrast to osteosarcoma cells (asterisk), no IL-34 expression was observed in osteocytes. Osteosarcoma cells exhibited a nuclear (C) and a cytoplasmic immunostaining (D).

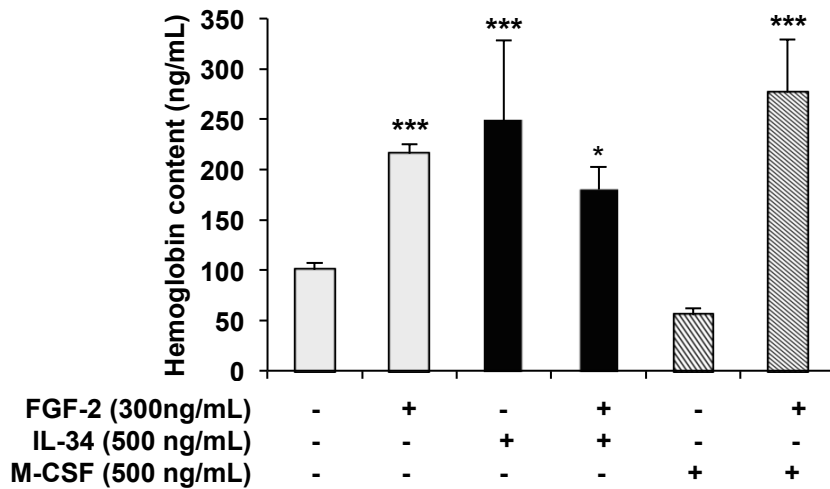


Supplementary Data 5: IL-34 and M-CSF increase the neo-formation of CD31⁺ vessels in a mouse osteosarcoma model. Human HOS osteosarcoma cells over-expressing IL-34 (HOS-IL34), M-CSF (HOS-MCSF) or transduced by the empty vector (HOS) were inoculated in close proximity to the tibia of female Rj:NMRI-nude mice. All mice were sacrificed by cervical dislocation at an equivalent volume of 1500 mm³ and immunohistochemical assessment of CD31 was carried out, as previously done for the CD146 staining.

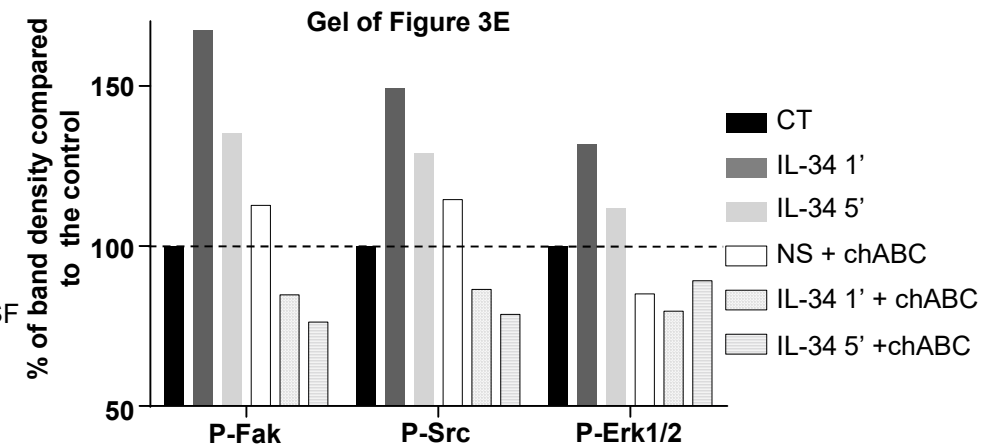
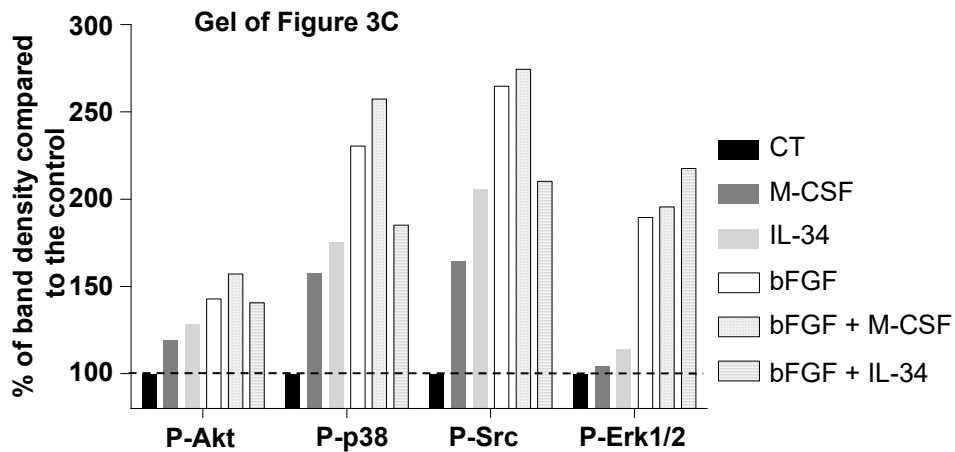
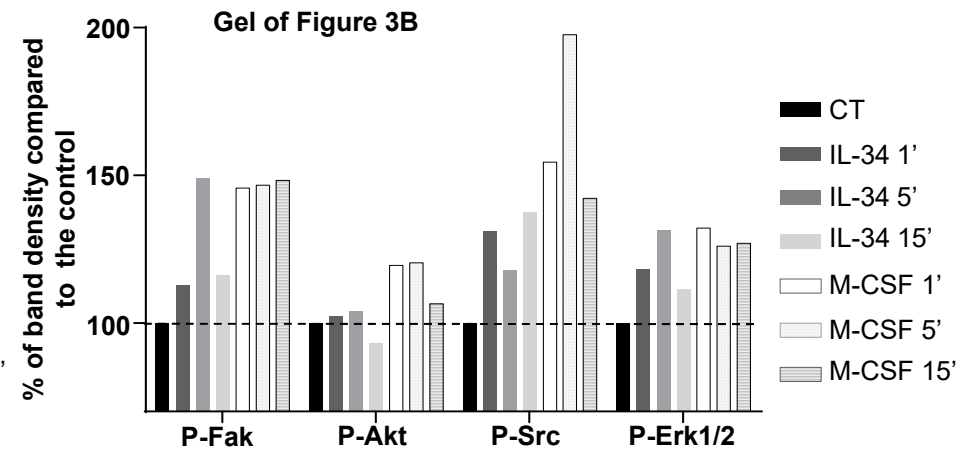
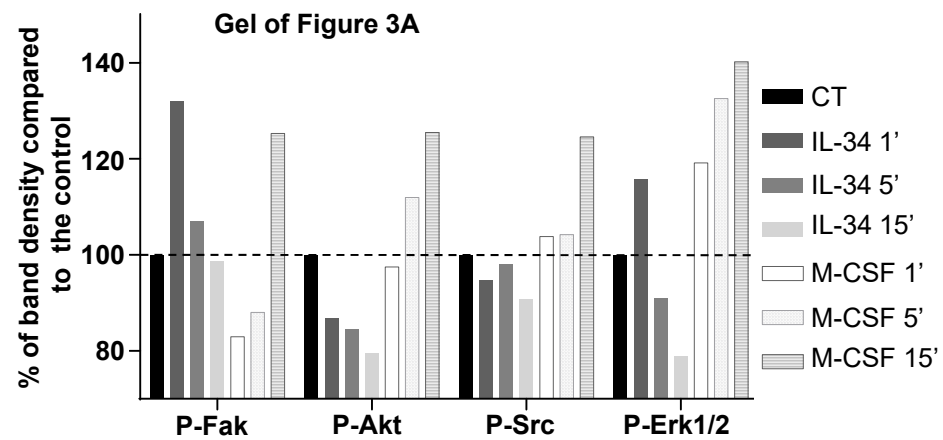
A



B



Supplementary Data 6: IL-34 and M-CSF modulate angiogenesis *in vivo*, in a mouse model of Matrigel® plug Assay. (A) Representative photographs of plugs excised on day 14 containing PBS (negative control); FGF-2 (350 ng/mL) used as a positive control, alone or with IL-34 (500 ng/mL), M-CSF (500 ng/mL). (B) Haemoglobin quantification: values represent mean \pm SEM of the haemoglobin content in Matrigel® plugs excised from C57Bl/6 mice (n=6 to 7 per experimental group). * p<0.05, ** p<0.01 and *** p<0.001 compared to the FGF-2 control group.



Supplementary Data 7: Western blot quantification. Bar graphs represent the relative densitometric values of phosphoproteins detected in Figure 3. The luminescence detected with a Charge Couple Device camera was quantified using the GeneTools programme (Syngene, Cambridge, United Kingdom).