

Supplementary Figure 1: The combination of M-CSF and IL-34 induces an additive effect by increasing the viability of human CD14<sup>+</sup>. CD14<sup>+</sup> cells were magnetically isolated from the peripheral blood of 4 healthy donors and cultured in the presence of M-CSF or IL-34 for three days. Cell viability was assessed by measuring metabolic cell activity (Alamar Blue assay). (A) Viablity of CD14<sup>+</sup> cultured in the presence of increasing doses of M-CSF or IL-34 (0.1 to 200 ng/mL). The dose-response shown is representative of all donors assessed. (B) Mean of CD14<sup>+</sup> cell viability from 4 healthy donors. Viability of CD14<sup>+</sup> isolated from 4 independent donors and cultured with 5 or 10 ng/mL of MCSF or IL-34 or with both 5 ng/mL M-CSF and 5ng/mL IL-34 for 4 donors. Histograms represent the percentage of cell viability obtained with 5 ng/mL of M-CSF. Error bars represent the standard error of mean (SEM) between each measurement of the different experiments for each donor. \*\* p < 0.01, n.s.: not significant.



Supplementary Figure 2 : Effect of M-CSF and IL-34 combination on human osteoclastogenesis.  $CD14^+$  cells isolated from healthy donor were cultured with 10 or 25 ng/mL of M-CSF, or 12 or 25 ng/mL of IL-34, or with 10 ng/mL of M-CSF and 12 ng/mL of IL-34. Media were changed every 3 days and 100 ng/mL of RANKL was added at D3 to induce osteoclastic differentiation. After 10 days of culture, TRAP<sup>+</sup> multinucleated cells were considered as osteoclasts and manually counted (\* p < 0.05; \*\* p < 0.001 compared to the control without M-CSF or IL-34, NS: non significant compared to M-CSF or IL-34 alone).



Supplementary Figure 3 : Validation of the siM-CSF efficiency in HOS M-CSFR cells. (A) HOS M-CSFR cells were transiently transfected with 2 siRNAs directed against the human M-CSF gene (siM-CSF<sub>621</sub> and siM-CSF<sub>952</sub>). A siRNA directed against the luciferase (SiLucF) gene was used as a negative control. Forty eight hours after the transfection, relative M-CSF mRNA expression was determined by RT-qPCR, normalized to HPRT gene expression. Experiment was done with the same cells used in figure 4 to study the M-CSF knockdown efficiency. (B) Forty eight hours after transfection, cells were cultured in serum free medium for 12 hours and then stimulated with 200 ng/mL of M-CSF or IL-34 for 5 min. Phosphorylations of M-CSFR (Tyr<sub>708</sub> and Tyr<sub>723</sub>) were analyzed by Western blot. β-actin was used as a loading control.



Supplementary Figure 4: IL-34 and M-CSF specifically and differentially regulate the M–CSFR localization. (A) M-CSF and IL-34 protein overexpression were measured by ELISA assay or flow-cytometry in HEK M-CSF and HOS M-CSFR cells. (B) M-CSFR expression was assessed by Western blot in HOS M-CSFR 48 hours after transfection with a vector encoding *M-CSF*, *IL-34* or an empty vector (Mock) upon 5 min stimulation with 50 ng/mL M-CSF or IL-34.



Supplementary Figure 5: M-CSF and IL-34 control M-CSFR location in HOS M-CSFR cells. HOS M-CSFR were transfected with an empty vector (Mock), a vector coding for M-CSF (M-CSF) or IL-34 (IL-34) or co-transfected with both vectors encoding M-CSF and IL-34 (M-CSF/IL-34). Forty-eight hours after transfection, the cells were fixed and expression of the M-CSFR was assessed by confocal microscopy (green staining). Actin is stained in red, and nuclei are shown in blue.



Supplementary Figure 6: Membrane and intracellular localization of the M-CSFR in murine K7M2 cells spontaneously expressing M-CSF and IL-34. The localization of the M-CSFR (green) was analyzed by confocal microscopy in the murine K7M2 osteosarcoma cells that express high levels of M-CSF and IL-34. Actin is stained in red, and nuclei are in blue (right panel). M-CSFR expression was also analyzed by Western blot. Vimentin and  $\beta$ -actin were used as the loading controls (left panel).