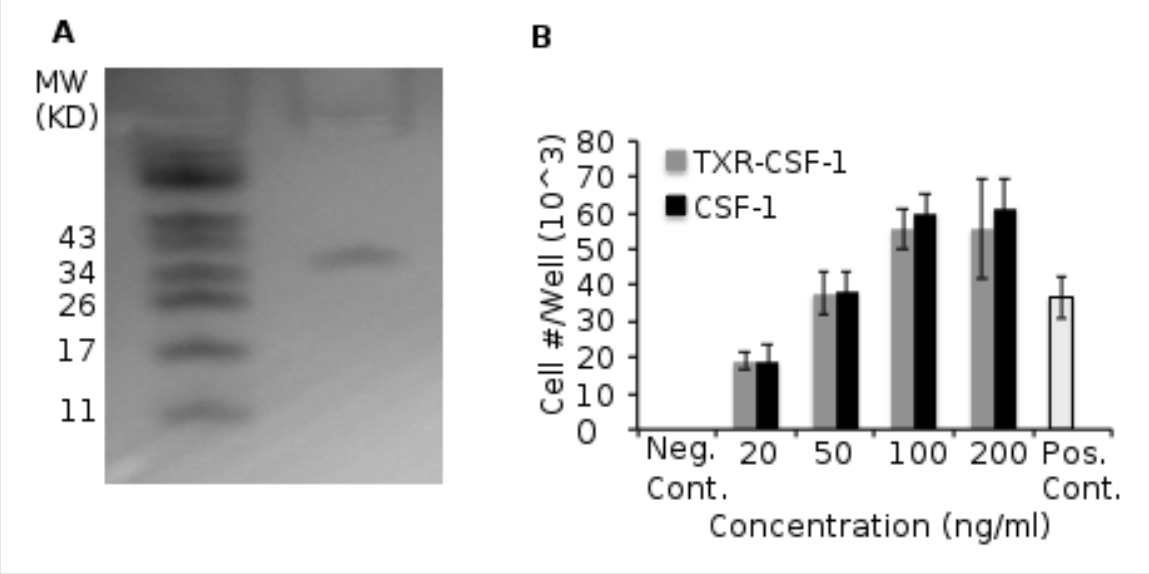
SUPPLEMENT



S Figure1. **Texas red labeled recombinant human CSF-1 (TXR-CSF-1) has the same biological activity as non-labeled CSF-1. a.** SDS-PAGE showed pure CSF-1 dimer (MW 34 KDa). **b.** Both CSF-1 and TXR-CSF-1 are both competent for bone marrow macrophage differentiation. Positive control is the number of bone marrow macrophage cultured by L cell supernatant added bone marrow medium.

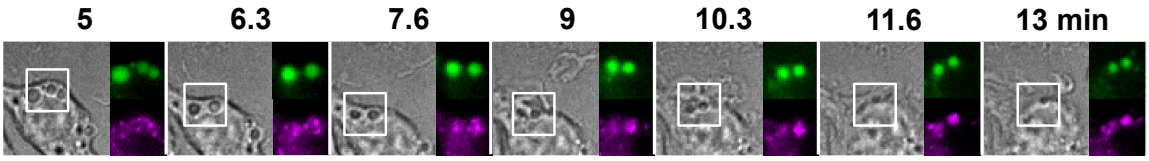


Figure S2. Traffic to the CSF-1R to macropinosomes labeled with the fluid phase marker Lucifer Yellow. Live BMM were exposed to Dylight 594-CSF-1 and Lucifer Yellow (LY) 5 minutes and imaged. Dylight 594-CSF-1 associated with LY positive macropinosomes at 5 minutes and was delivered into the lumen around ~8 min.

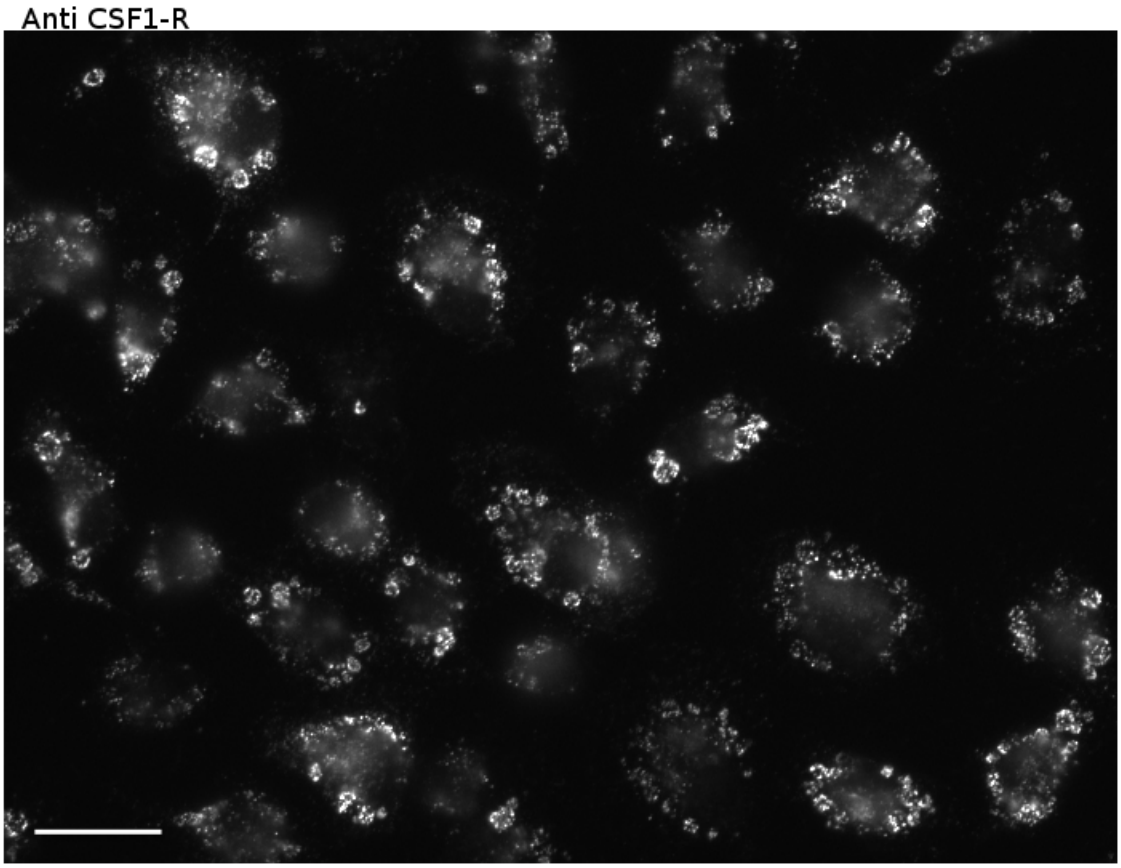


Figure S3. Generality of the observation that the CSF-1R is delivered to macropinosomes after exposure to CSF-1 for 15 min. The localization and distribution of CSF-1R was followed by immunofluorescence after stimulating BMM with 100 ng/ml CSF-1 for 15 min. Most CSF-1R clustered and appeared as large circular structures (macropinosomes) that contained many small puncta (scale bar = 10 μm).

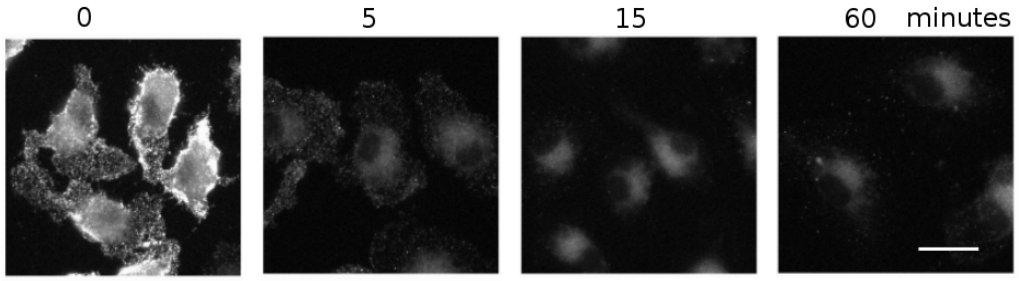
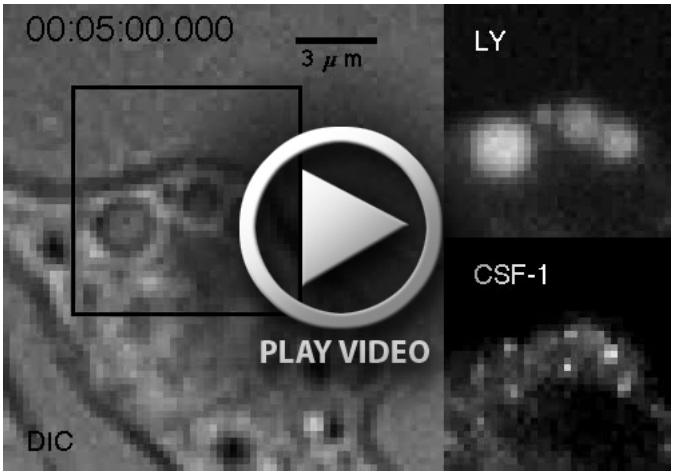


Figure S4. No detectable CSF-1R is recycled back to cell surface. Immunofluorescence against the cell surface CSF-1R in BMM showed that the CSF-1R is internalized by 15 min. following exposure to 100 ng/ml MCSF for 15 minutes and there is minimum amount of receptors been recycled. (scale bar = 5 μm)

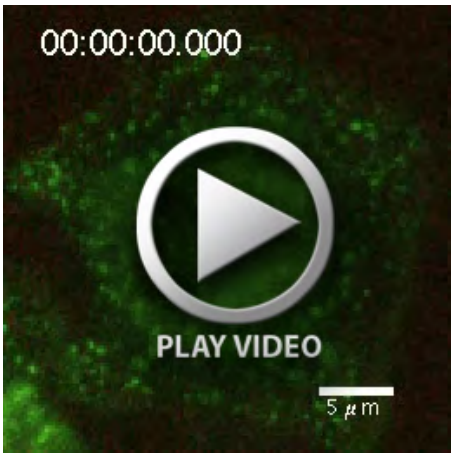
Movie 1. CSF-1 induced ruffling and macropinocytosis in BMM. CSF-1 stimulated ruffling and macropinocytosis in BMM. BMM starved of CSF-1 overnight were exposed to 100 ng/mL for 1 minute (3rd frame) after the start of the acquisition. Note the formation of circular ruffles that close to form macropinosomes. Images were acquired at a rate of 1 frame every 20 seconds for a total of 15 minutes (playback is 150× real time).

Movie 2. TXR-CSF-1 traffic to macropinosomes. BMM were exposed to CSF- to 100 ng/mL TXR-CSF-1 1 minute after the start of the acquisition (white frames). TXR-CSF-1 is internalized by small vesicles that undergo homotypic fusion and then traffic to macropinosomes (large ring like structures), whereupon the TXR-CSF-1 is delivered to the lumen (large circles of fluorescence). The cell in white box is also shown in Fig. 1. Duration was 17 minutes at a frame rate of 1 frame every 20 seconds, playback speed is 100 times real time.



Movie 3. CSF-1 traffics to nascent macropinosomes marked by Lucifer Yellow. BMM stimulated with 100 ng/mL DiLight594-CSF-1, in the presence of Lucifer Yellow (LY), for 5 minutes prior to imaging. Macropinosomes, visible in DIC, were tracked in the black boxed region in DIC image. These macropinosomes contained LY when formed and accumulated DiLight594-CSF-1 over the time. Images were acquired at a rate of 1 frame every 20 seconds (playback is 45× real time).

Movie 4. Selective permeabilization of BMM plasma membrane. Macrophages expressing cytosolic mCerulean were stimulated with 100 ng/mL CSF-1, in the presence of 0.5 mg/mL TXR-Dex (70,000 MW), for 5 minutes prior to imaging. Digitonin (40 μg/mL) and 0.1% TritonX-100 were added at the times indicated, resulting in the loss of mCerulean from the cytosol (Digitonin) and subsequent loss of TXR-Dex from the macropinosome (TritonX-100). Images were acquired at a rate of 1 frame every 2 seconds (playback is 7× real time).



Movie 5. CSF-1-containing vesicles fuse with Rab5-positive macropinosomes. BMM expressing mCit-Rab5a (green) were exposed to TXR-CSF-1 (red) 1 minute after the start of the acquisition (yellow frames). Initially, small endosomes bearing TXR-CSF-1 localized with mCit-Rab5a. Newly formed macropinosomes acquire mCit-Rab5a (large green rings) and subsequently TXR-CSF-1 laden vesicles. Images from this sequence are shown in Fig. 3. Duration is 17 minutes, 1 frame every 20 s, playback is 100× real time.