

Supplementary Figure S1. Secreted BARF1 inhibits tyrosine phosphorylation of CSF-1R. 500 μ l of conditioned medium prepared from a wild-rEBV- or a BARF1-rEBV-infected cell clone was pre-incubated with 150 ng/ml of recombinant human CSF-1 (Peprotech, London, UK) for 30 min at 37°C. Mouse 3T3 cells expressing human CSF-1R (1.6×10^6 cells) were detached from culture plates by treatment with 2 mM EDTA-PBS, washed with serum-free medium three times, then incubated with the reaction mixture of CSF-1 and conditioned medium for various times up to 20 min at 37°C. The cells were washed with ice-cold PBS three times and lysed in NP-40 lysis buffer (20mM Tris-HCl [pH 7.5], 150mM NaCl, 1% NP40, 1mM PMSF, pepstatin [1 μ g/ml], leupeptin [1 μ g/ml], 100-fold diluted phosphatase inhibitor cocktail II [Sigma]). Cell lysates were resolved by SDS-PAGE on 8% gels and subjected to immunoblot analyses with rabbit anti-CSF-1R antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and rabbit anti-phospho-CSF-1R (Tyr723) antibody (Cell signaling Technology, Beverly, MA).