

Figure S3. Sequence data demonstrating the conversion of Ser711 to Ala in the EPOR/M-CSFR chimeric receptor. The pMX vector was used as the backbone vector.



Figure S4. (A) M-CSF-evoked ERK and AKT activation are comparable in RAW264.7 cells expressing EPOR/M-CSFR or EPOR/M-CSFR Ser711A chimeras. The cell clones in Fig. 6B were stimulated with EPO (30ng/ml) for the indicated times (+) or left unstimulated (-). Cell lysates were resolved by SDS-PAGE and immunoblotted with the indicated antibodies. Note that, by contrast to EPO-stimulated cells, M-CSF stimulation results in a comparable magnitude and duration of AKT activation. (B) RAW264.7 cells expressing wild type EPOR/M-CSFR chimera were starved (-), and then treated DMSO or UO126 for 2 hours and stimulated with 5U/ml erythropoietin (EPO) for the indicated times (+). Cell lysates were immunoprecipitated with anti-FLAG antibody, and then immunoblotted with anti-pPKA substrate antibodies. Note that the blockade of ERK/RSK activation by UO126 had no effect on EPOR/M-CSFR phosphorylation. Data shown are a representative of the three (n=3) independent experiments.