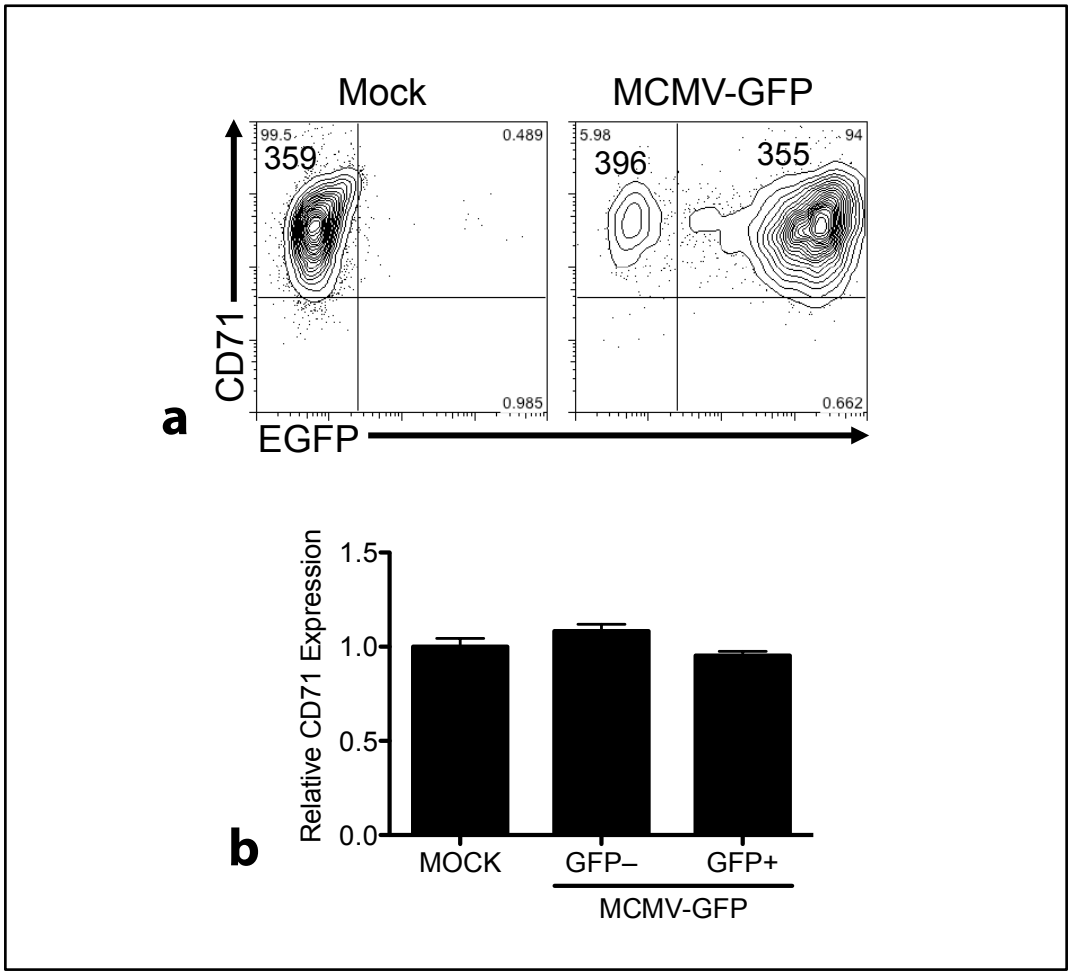
Online Supplemental Data

Materials and Methods

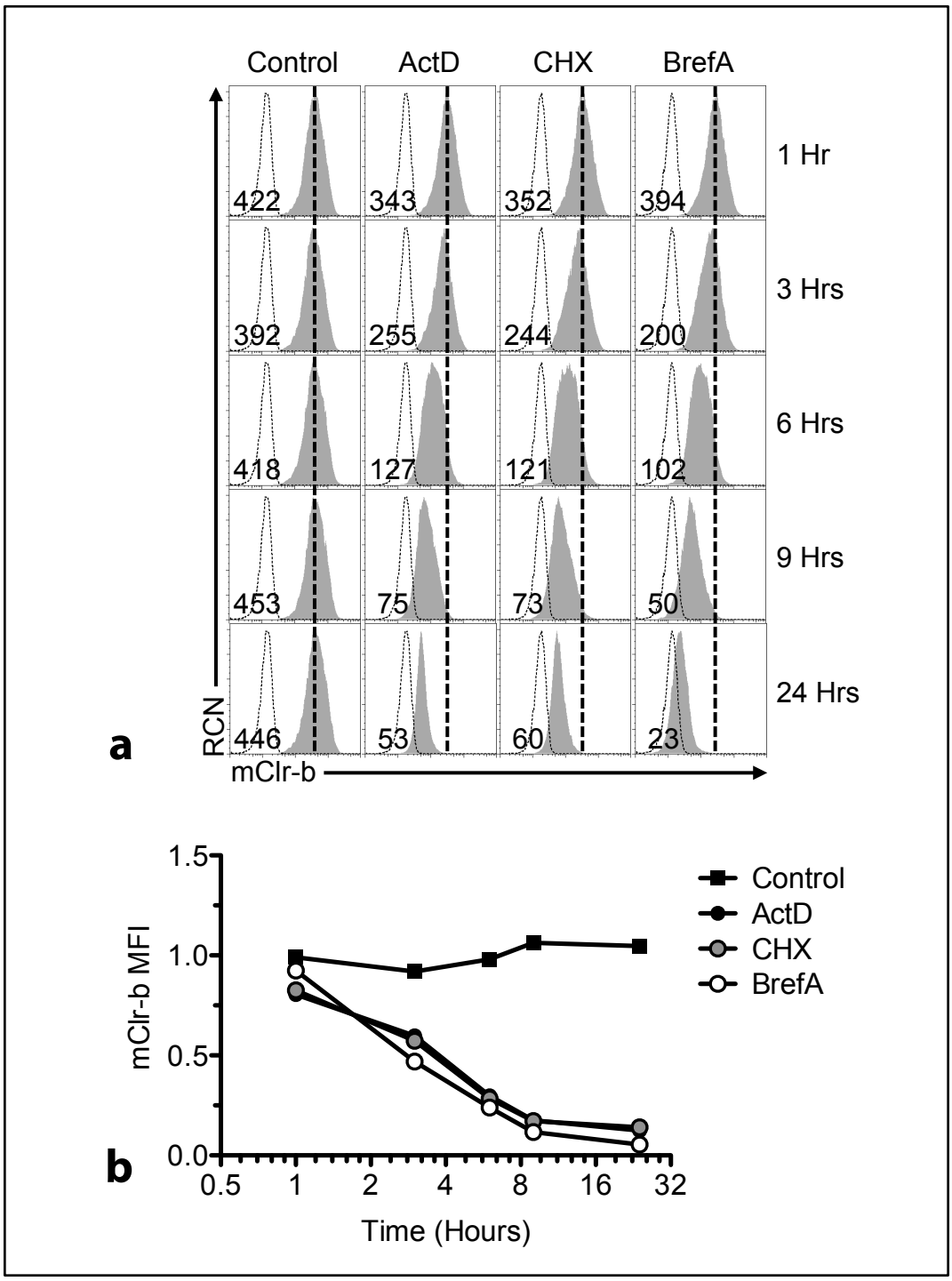
Animals and cells

WT, *Ifnar*$^{-/-}$, and *Irf3*$^{-/-}$*Irf7*$^{-/-}$ MEF were obtained from Dr. K. Mossman (McMaster University, Canada). WT and *Aim2*$^{-/-}$ MEF were obtained from Dr. K. Fitzgerald (University of Massachusetts, Amherst, USA). WT, *Asc*$^{-/-}$, and *Caspase-1*$^{-/-}$ fibroblasts were obtained from Dr. M. Saleh (McGill University, Canada). WT and *Mavs*$^{-/-}$ MEFs were obtained via Dr. A. Iwasaki (Yale University, USA) from Dr. Z.J. Chen (University of Texas Southwestern Medical Center, USA). WT, *Tbk1*$^{-/-}$*Ikke*$^{-/-}$, and *Zbp1*$^{-/-}$ fibroblasts were obtained from Dr. S. Gasser (National University of Singapore, Singapore). WT, *Rnasel*$^{-/-}$, *Pkr*$^{-/-}$, and *Pkr*$^{-/-}$*Rnasel*$^{-/-}$ were obtained via Dr. E. Fish (University of Toronto) from Dr. R. Silverman (Lerner Research Institute, Cleveland, USA).

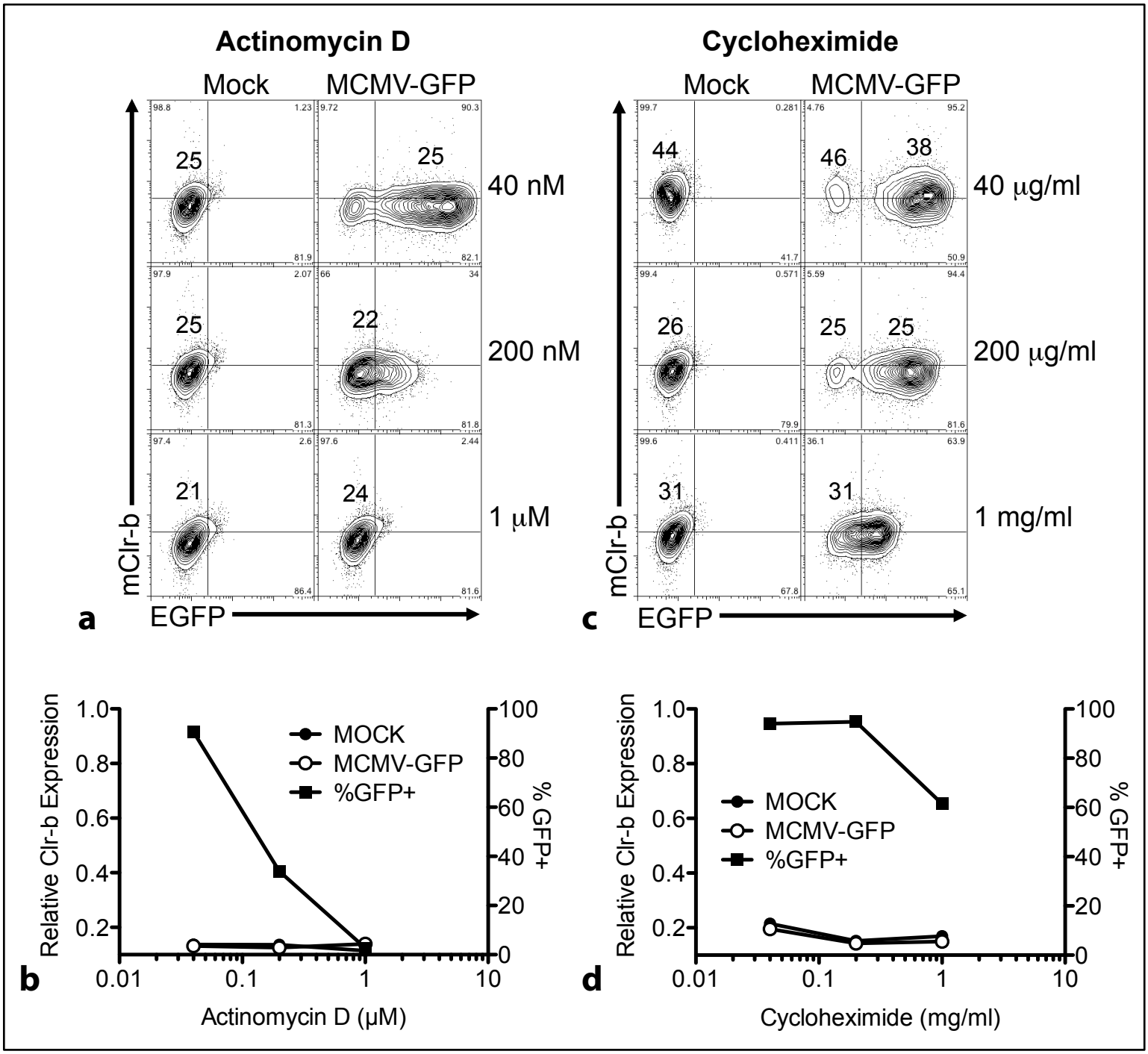
Supplemental Figures



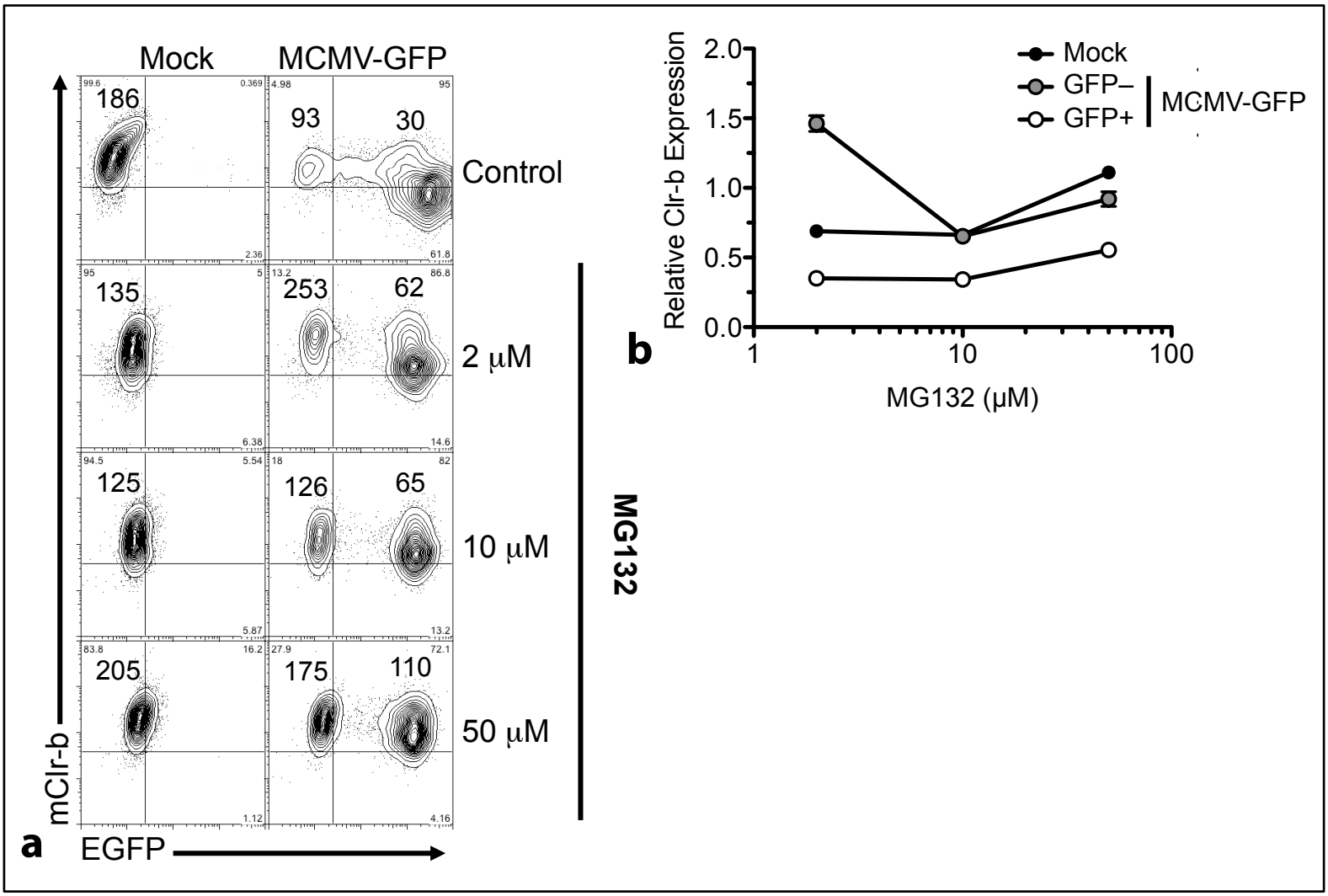
Suppl. Fig. 1. Cell surface expression of CD71 (Transferrin Receptor) is unaltered during MCMV Infection. NIH3T3 cells were infected with MCMV-GFP (MOI of 0.5 PFU/cell) and analyzed by flow cytometry 24 h.p.i. using APC-conjugated anti-mouse CD71 mAb. **a** Representative flow plots of uninfected and infected NIH3T3. The numbers in the gates corresponds to the CD71 MFI. **b** Quantitation of CD71 expression in mock-infected and infected populations normalized to mock levels. Graph shows mean±SEM of normalized MFI values in **a**. Data are representative of at least three independent experiments.



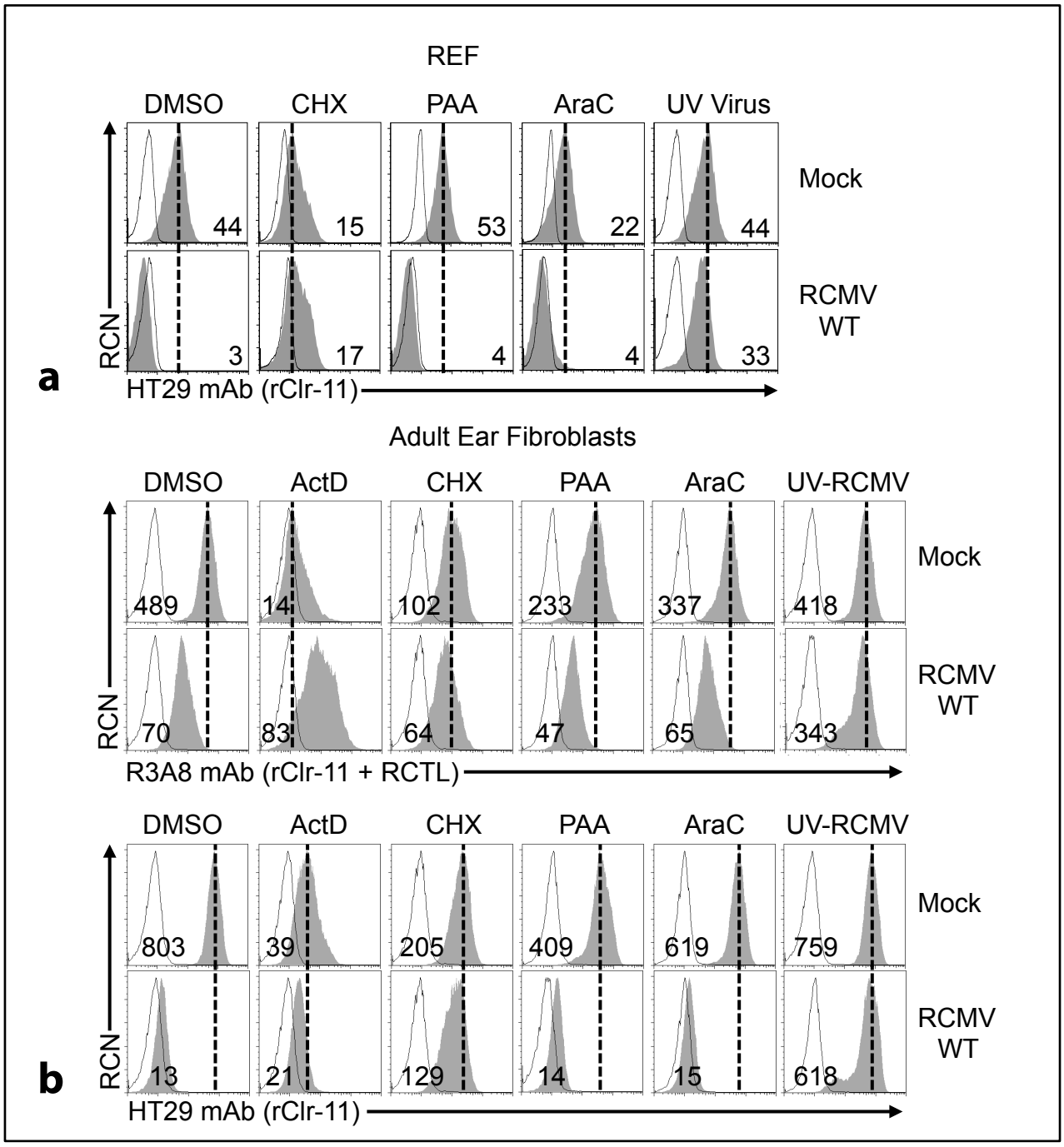
Suppl. Fig. 2. Kinetic studies reveal that mClr-b on mouse fibroblasts has a short half-life. **a** NIH3T3 cells were treated with inhibitors of transcription, translation, and golgi maturation; actinomycin D (ActD, 10 nM), cycloheximide (CHX, 10 μg/ml), and brefaldin A (BrefA, 10 μg/ml) respectively, at different timepoints and stained by flow cytometry using 4A6 mAb. Shaded histograms represents 4A6 staining; black line, secondary reagent alone; dotted vertical line, reference for control MFI level; numbers indicate MFI values. **b** Quantitation are mClr-b MFI levels in **a** normalized to mock control levels (mean ± SEM).



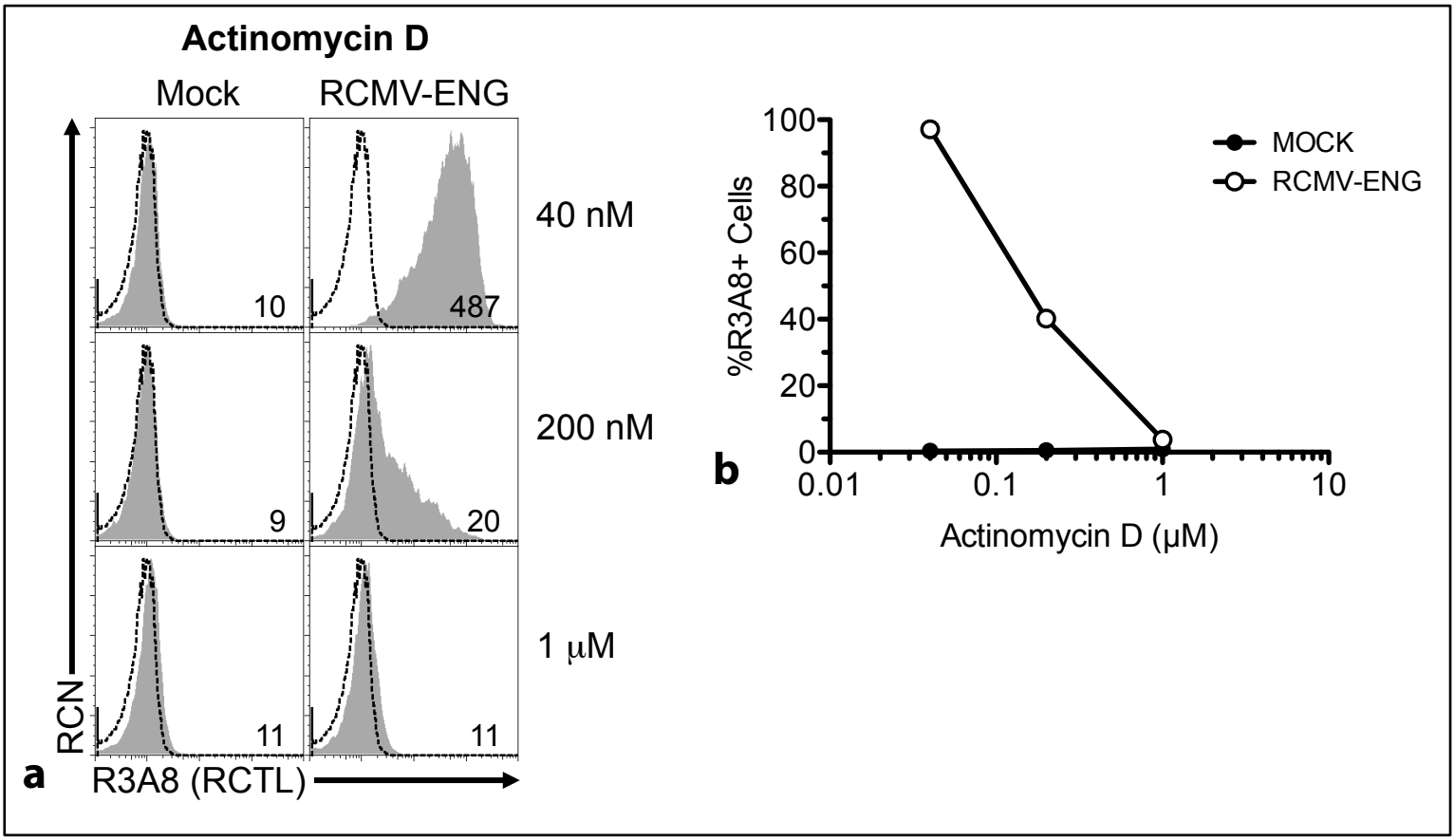
Suppl. Fig. 3. Immediate early gene expression is abolished with high doses of ActD or CHX and during MCMV infection. NIH3T3 cells were treated with titrated doses of **a** ActD or **c** CHX and either mock or MCMV-GFP infected and analyzed for Clr-b expression 24 h.p.i by flow cytometry. Numbers correspond to Clr-b MFI of population. **b** and **d** Quantitation of mClr-b MFI levels normalized to mock DMSO control levels for **a** and **c**, respectively (mean ± SEM). The left Y-axis corresponds to the mClr-b exression (mock and MCMV-GFP infected) whereas the right Y-axis measures the % of GFP+ cells in infected samples. Data are representative of at least three independent experiments.



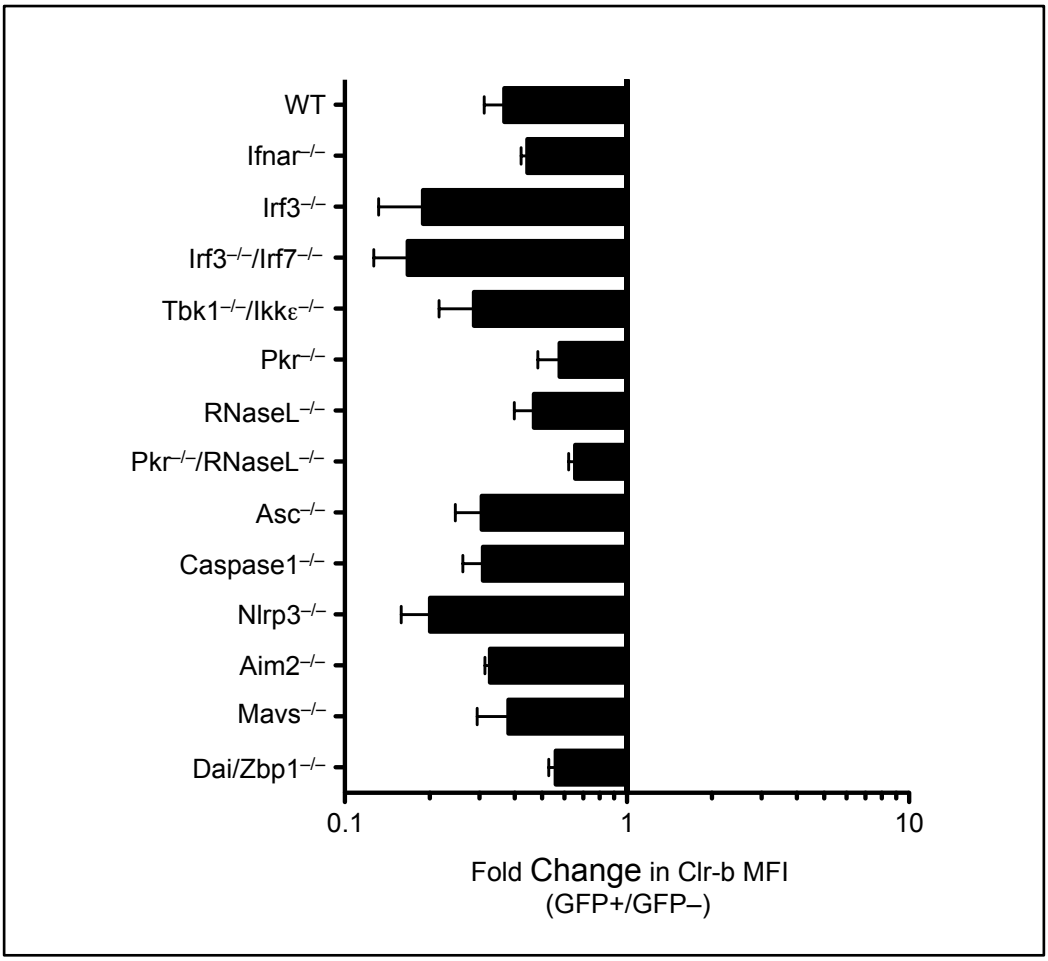
Suppl. Fig. 4. Inhibition of ubiquitin-proteosomal degradation pathway partially blocks mClr-b downregulation during MCMV infection. NIH3T3 cells were treated with titrated doses of the proteosomal inhibitor, MG132, and either mock-infected or MCMV-infected and analyzed by flow cytometry 24 h.p.i. **a** Representative flow plots of uninfected and infected NIH3T3 stained with 4A6 mAb. The numbers in the gates corresponds to the mClr-b MFI. **b** Quantitation of mClr-b expression in mock-infected and MCMV-infected populations normalized to mock DMSO levels. Graph shows mean±SEM of normalized MFI values in **a**. Data are representative of at least three independent experiments.



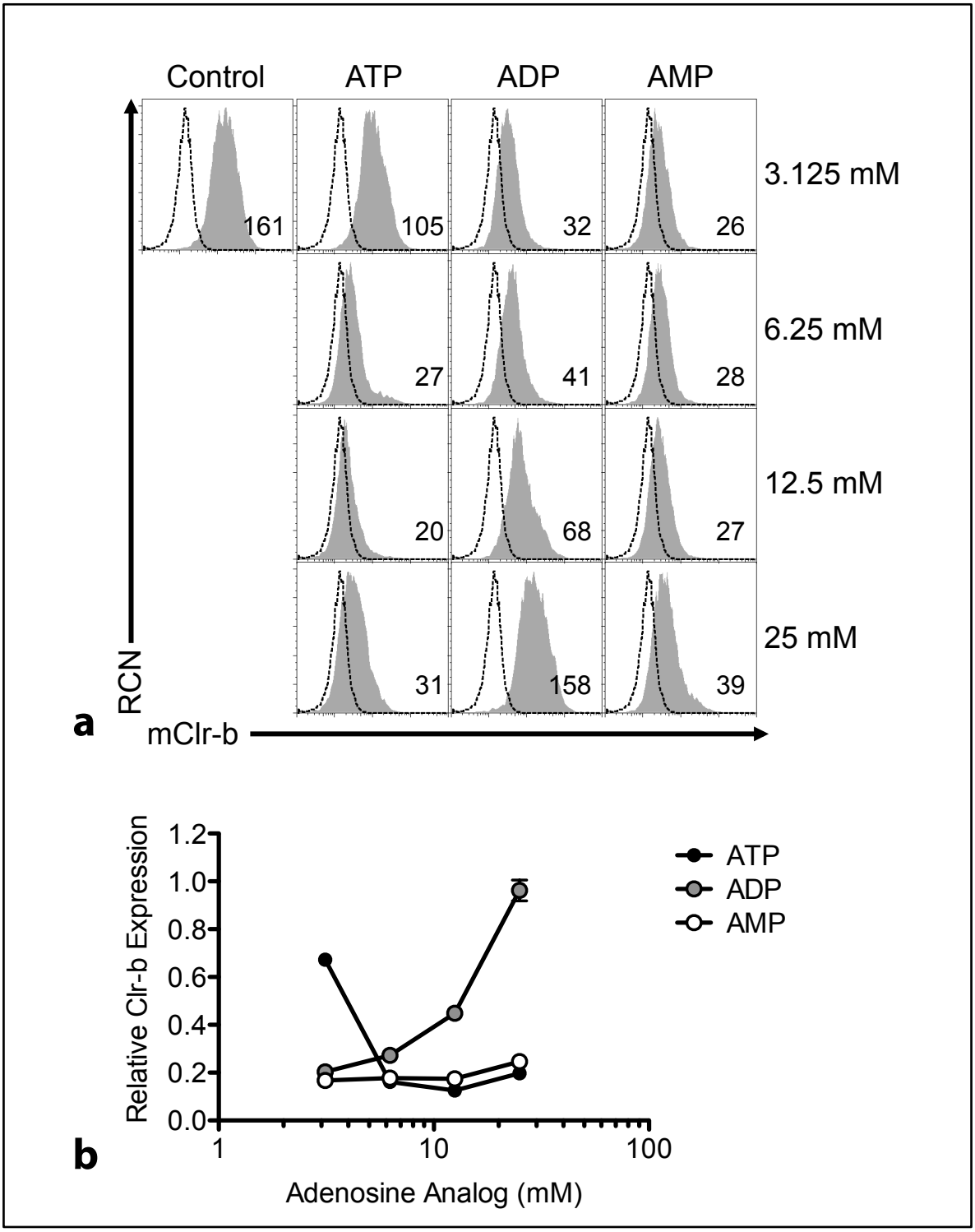
Suppl. Fig. 5. Extended analysis of RCMV-mediated rat Clr-11 downregulation and RCTL immunoevasin induction using RCMV-E WT virus and inhibitor treatments. **a** Characterization of rClr-11 (HT29 mAb) expression upon RCMV-E WT virus infection of REF cells in the presence of various chemical inhibitors or using UV-inactivated virus. REF cells were pre-treated with DMSO (control), CHX (10 μg/ml), PAA (400 μg/ml), or AraC (50 μg/ml), then infected with RCMV-E WT virus, or treated with UV-inactivated RCMV-E virus, then analyzed by flow cytometry at 24 h.p.i. Shaded histograms represent HT29 (rClr-11) mAb; black line, secondary reagent alone; dotted vertical line, reference for Mock control MFI level; numbers indicate MFI values. **b** Characterization of rClr-11/RCTL (R3A8 mAb, top) or rClr-11 (HT29 mAb, bottom) expression upon RCMV-E WT virus infection of primary rat adult ear fibroblast (AEF) cells in the presence of chemical inhibitors or using UV-inactivated virus. Rat AEF cells were pre-treated with treatments as in **a**, then infected with RCMV-E WT virus, or treated with UV-inactivated RCMV-E virus, then analyzed by flow cytometry at 24 h.p.i. Shaded histograms represent R3A8 (rClr-11/RCTL) or HT29 (rClr-11) mAb; black line, secondary reagent alone; dotted vertical line, reference for Mock control MFI level; numbers indicate MFI values.



Suppl. Fig. 6. High dose of ActD blocks expression of RCTL during RCMV-E infection. **a** REFs were treated with titrated doses of ActD and either mock or RCMV-E infected and analyzed for R3A8 (RCTL) expression 24 h.p.i by flow cytometry. Numbers correspond to R3A8 MFI of population. **b** Quantitation of %R3A8+ cells in both mock-infected and RCMV-infected treatments for **a** (mean ± SEM). Data are representative of at least three independent experiments.



Suppl. Fig. 7. Mouse Clr-b downregulation upon MCMV-GFP infection of primary fibroblasts from mice deficient in select genes involved in innate immune recognition. Primary mouse MEF or AEF cells from various mutant mouse strains were infected with MCMV-GFP and analyzed for Clr-b expression 24 h.p.i. by flow cytometry. Fold changes were calculated by ratios of Clr-b MFI levels between infected (GFP^+) versus uninfected (GFP^-) cells. Data are representative of at least three independent experiments.



Suppl. Fig. 8. Adenosine nucleotide analogs affect mClr-b cell surface expression in mouse fibroblasts. NIH3T3 cells were treated with titrated doses of AMP, ADP, or ATP and analyzed by flow cytometry 24 hrs post-treatment by flow cytometry. **a** Representative flow plots of treated NIH3T3. The numbers in the gates corresponds to the mClr-b MFI. **b** Quantitation of mClr-b expression in treated populations normalized to untreated controls values. Graph shows mean±SEM of normalized MFI values in **a**. Data are representative of at least three independent experiments.