Polarization dictates iron handling by inflammatory and alternatively activated macrophages

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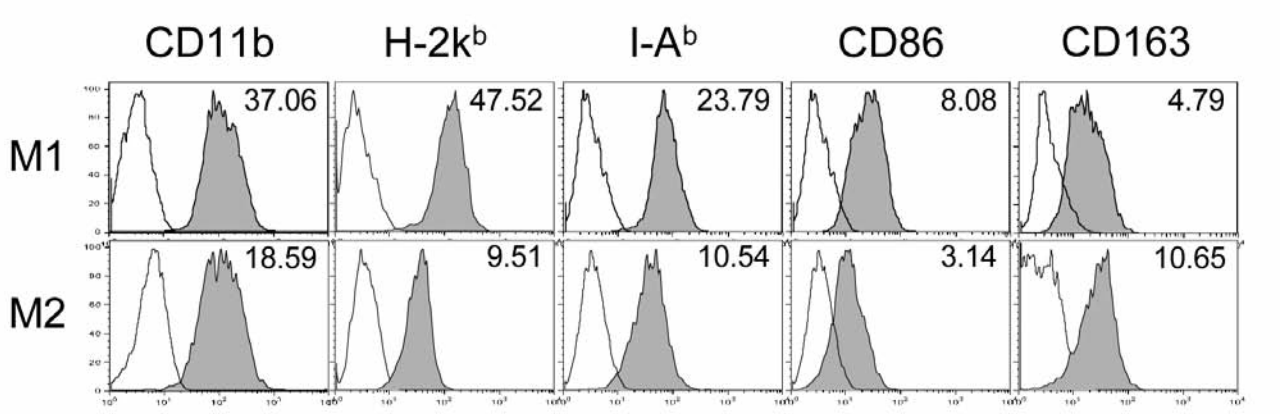
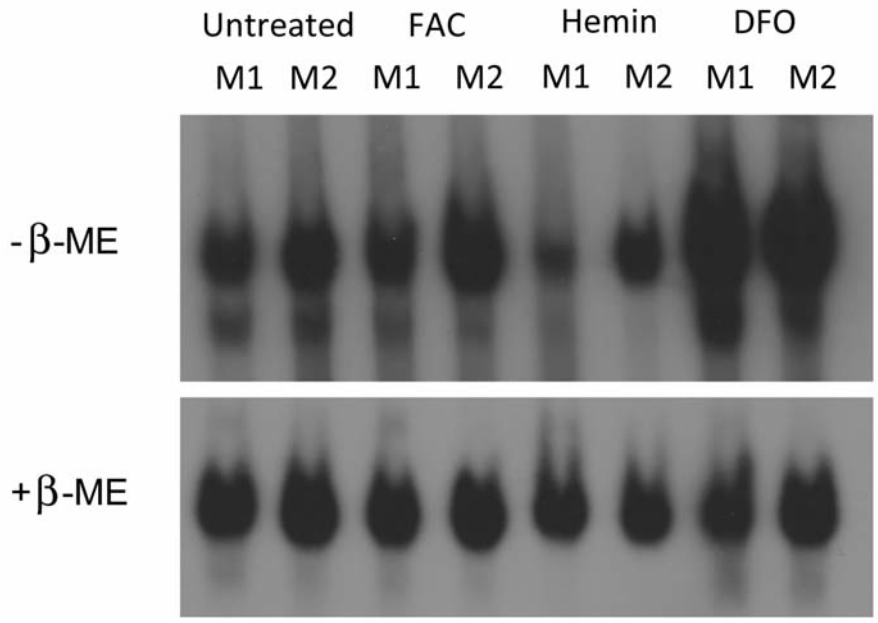
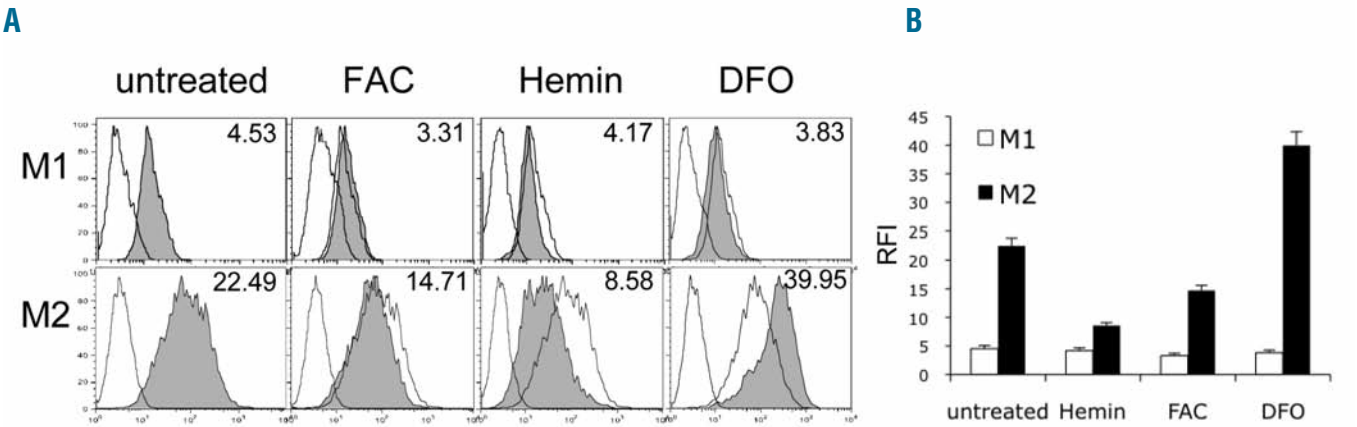


Online Supplementary Figure S1. Surface markers of polarized macrophages. M1 and M2 macrophage populations were analyzed by flow cytometry after staining with labeled antibodies directed against CD11b, a β_2 integrin chain expressed by all macrophages, against molecules involved in T-cell activation and co-stimulation (H-2k^b, I-A^b and CD86) or against the hemoglobin/haptoglobin scavenger receptor CD163. Filled histograms indicate binding of specific antibodies, whereas open histograms indicate the fluorescence background obtained in the presence of isotype-matched control antibodies. Numbers indicate the relative fluorescence intensity (RFI) values, calculated by dividing the mean fluorescence intensity obtained in the experimental sample by the one obtained with the relevant control. The results shown are representative of routine experiments (n>12).



Online Supplementary Figure S2. Effect of β-mercaptoethanol (β-ME) on IRP1 binding activity. Cytoplasmic extracts of M1 and M2 macrophages treated or not with FAC, hemin, and DFO were incubated with an excess of a ^{32}P-labeled iron responsive element probe in the presence or in the absence of 2% β-ME. RNA-protein complexes were resolved on non-denaturing polyacrylamide gels and revealed by autoradiography. The results shown are representative of three independent experiments.



Online Supplementary Figure S3. Membrane expression of TfR1 in M1 and M2 macrophages. (A) FACS analysis was performed using an anti-TfR1 FITC-conjugated monoclonal antibody. M1 and M2 macrophages were analyzed either untreated or incubated overnight in the presence of FAC, hemin and DFO. Filled histograms from representative experiments show binding of specific antibodies, whereas open histograms represent the fluorescent background obtained in the presence of isotype-matched control antibodies. Open overlaid histograms represent the expression level in basal condition (*i.e.* without any overnight treatment). Numbers in the upper right corner indicate the relative fluorescente intensity (RFI) values, calculated by dividing the mean fluorescence intensity obtained in the experimental sample by the one obtained with the relevant control. Results are representative of three independent experiments. (B) Bars represent the mean ±s.e.m. of RFI obtained in three independent experiments performed as described above.