# Wermeling et al., http://www.jem.org/cgi/content/full/jem.20070600/DC1

## SUPPLEMENTAL MATERIALS AND METHODS

**Clearance evaluation in KO mice.** Two different approaches were used to evaluate if the KO mice had deficiencies in clearing apoptotic cells. First, WT and KO mice ($n$ = 6 per genotype) were bled without prior treatment from the tail artery into tubes containing heparin (Leo Pharma) and kept on ice. Erythrocytes were lysed by two rounds of ACK treatment. The cells were stained with annexin V–FITC and analyzed with flow cytometry. Second, syngeneic thymocytes were labeled with 0.1 μM CFSE (Invitrogen) as described by the manufacturer before induction of apoptosis as described above. $10^8$ cells were injected i.v. in age-, sex-, and weight-matched WT and KO mice ($n$ = 6–8 per genotype). Blood was collected from the tail vein after 30 min and 3 h. After lysis of erythrocytes, the $CFSE^+$ population was analyzed with flow cytometry.

**Anti-C1q ELISA.** Anti-C1q antibodies were measured in serum samples from (NZB x NZW)F1 and $Fc\gamma RIIB^{-/-}$ mice as described previously (Hogarth, M.B., P.J. Norsworthy, P.J. Allen, P.K. Trinder, M. Loos, B.J. Morley, M.J. Walport, and K.A. Davies. 1996. *Clin. Exp. Immunol.* 104:241–246) using 0.8 μg/ml human C1q (Sigma-Aldrich) and the reagents used in the ELISAs above. As suggested in the protocol, samples were diluted as suggested in a block buffer with a final concentration of 1 M NaCl to reduce the unspecific binding of the Fc part of immunoglobulins to C1q. All samples were run in duplicates and corrected for background binding.

**Evaluation of sMARCO purity.** To verify that the reactivity seen against sMARCO is not due to DNA contaminations, sMARCO was treated with DNase I (grade II; Roche) before coating. Thus, sMARCO and DNase I or buffer was mixed at a final concentration of 1 and 50 μg/ml in HBSS, respectively, and incubated at 37°C for 15 min (HBSS contains both magnesium and calcium ions necessary for the DNase I efficiency). After this, EDTA was added to a final concentration of 5 mM to inactivate the DNase I, and the samples were coated on ELISA plates (Nunc) overnight in 4°C. (NZB x NZW)F1 sera ($n$ = 5) was assayed as described above under "anti-scavenger receptor responses" against sMARCO with or without DNase I (the sMARCO control were treated the same way as the sample with DNase I, except for the addition of DNase I). To verify the functionality of DNase I, a (NZB x NZW)F1 serum sample was also tested for the presence of anti-DNA in wells coated with DNA either treated or non with DNase I. All samples were run in duplicates and corrected for background binding and are presented as mean ± SEM.