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Supplemental Data

Poly(ADP-Ribosyl)ation of High Mobility Group Box 1 (HMGB1) Protein Enhances Inhibition of Efferocytosis

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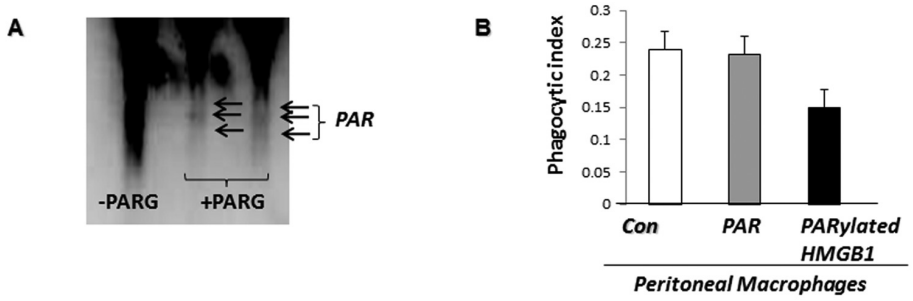

Figure S1. Production of free PAR by PARG digestion. (S1-A). PARP-1 protein was incubated in a poly(ADP-ribosyl)ation reaction for 30 min at 37°C. PARG was then added to the reaction for 30 min at 37°C. Separation of free PAR from PARP-1 was performed using Amicon Ultra 0.5 filters. A portion (10%) of the reaction mixture was used for immunoblot analysis with antibodies to PAR. (S1-B). Free PAR or PARylated HMGB1 was incubated with peritoneal macrophages for 2 h before the addition of apoptotic thymocytes. Representative gels are shown. A second independent experiment provided similar results.

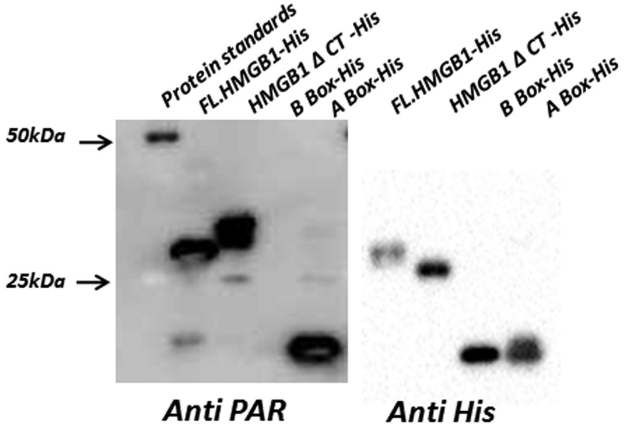

Figure S2. PARylation of full length HMGB1 and HMGB1 domains by PARP-1. Full length HMGB1-His, ΔC-HMGB1-His, HMGB1 A Box-His, or HMGB1 B Box-His (500 ng/ml) were incubated with recombinant PARP-1 in poly(ADP-ribosyl)ation reactions. The reactions were terminated with sample buffer and subjected to immunoblot analysis with antibodies to poly(ADP-ribose) (PAR) or His. Results from a representative experiment are shown. A second experiment provided similar findings.