## SUPPLEMENTAL MATERIAL

**Yang et al., http://www.jem.org/cgi/content/full/jem.20101354/DC1**

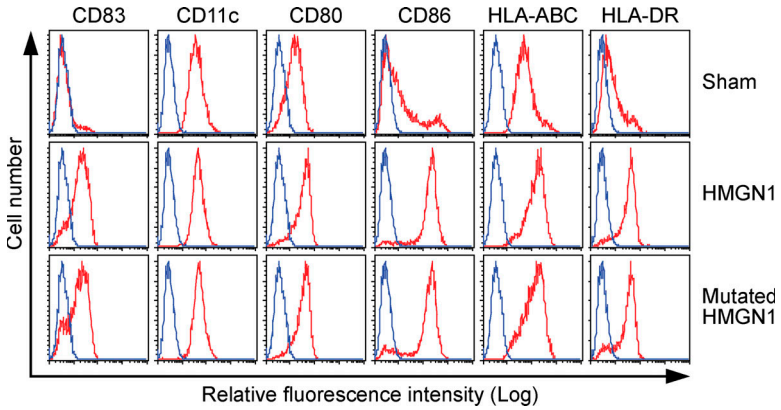


**Figure S1. Mutated HMGN1 incapable of chromatin binding was similarly effective at up-regulating the expression of DC surface marker molecules.** Human DCs were incubated at 5 × $10^5$/ml in medium in the absence (sham) or presence of 1 µg/ml HMGN1 or mutated HMGN1 for 48 h before being analyzed for their expression of the indicated surface markers (red lines). Blue lines indicate isotype-matched control. Shown are the results of one experiment representative of three.

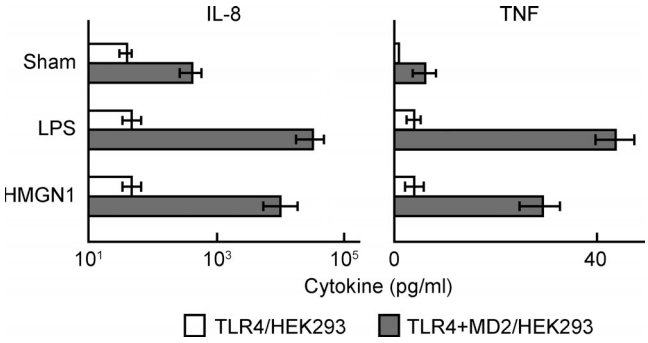


**Figure S2. HMGN1 induction of cytokines depends on the TLR4–MD2 signaling complex.** HEK2903 cells transfected to express TLR4 or a combination of TLR4 and MD2 were incubated with LPS or HMGN1 (both at 1 µg/ml) for 24 h before their supernatants were harvested for the quantitation of cytokines. Shown is the mean (±SD) of three experiments.

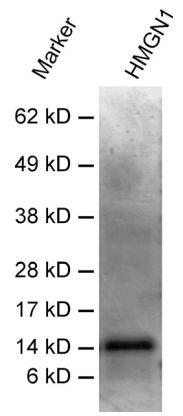


**Figure S3. Preparation and purity of recombinant HMGN1 protein.** HMGN1 generated in *E. coli* was purified sequentially by perchlorate precipitation, reverse phase high-performance liquid chromatography on C4 Butyl Aquapore, and cation exchange fast protein liquid chromatography on Mono S. The purity of the HMGN1 preparation was analyzed by Coomassie brilliant blue staining of a 4–12% SDS-NuPAGE gel.

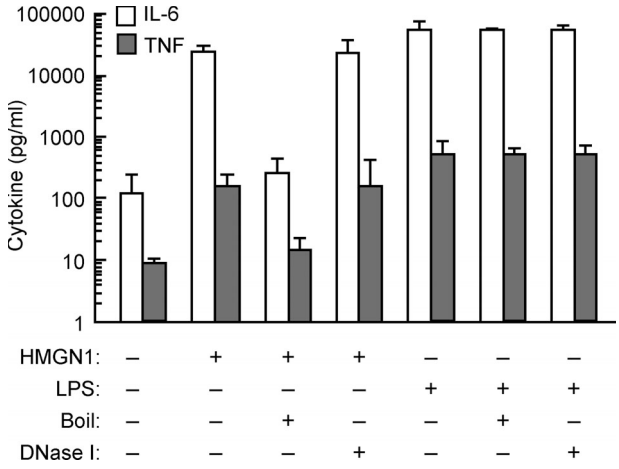


**Figure S4. The capacity of HMGN1 to induce DC production of IL-6 and TNF was destroyed by heating but not by DNase I digestion.** Human MoDCs were cultured with 1 µg/ml HMGN1 or LPS that was either untreated or pretreated by boiling (100°C for 5 min) or DNase I digestion (37°C for 30 min) for 48 h before the supernatants were harvested for quantitation of IL-6 and TNF. Shown is the mean (±SD) of triplicate wells of one experiment representative of two.

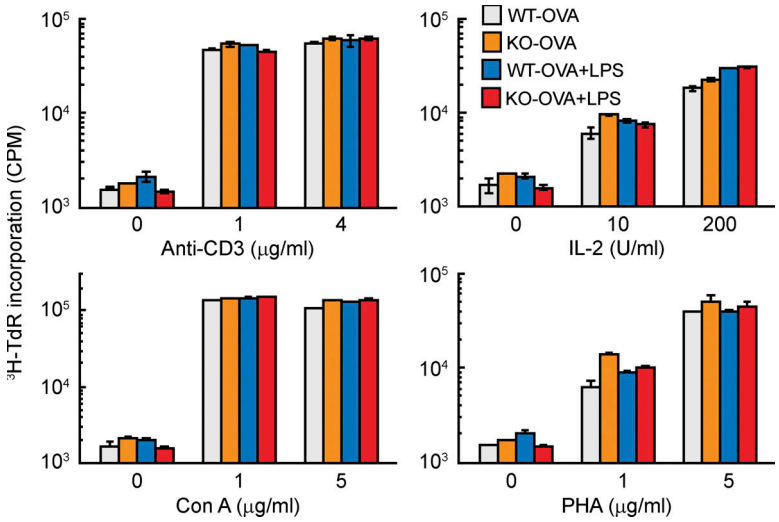


**Figure S5. The splenocytes of immunized WT and HMGN1 KO mice proliferated similarly in response to various polyclonal stimulators.** Splenocytes were cultured in triplicate in a 96-well plate ($10^5$/0.2 ml/well) in the presence of coated anti-CD3, IL-2, Con A, or PHA at specified concentrations for 3 d at 37°C in humidified air containing 5% $CO_2$. The culture was pulsed with 1 μCi/well [$^3$H]TdR for the last 18 h before harvest for determination of [$^3$H]TdR incorporation. Shown is the mean cpm (±SD) of triplicate wells of one experiment representative of three.

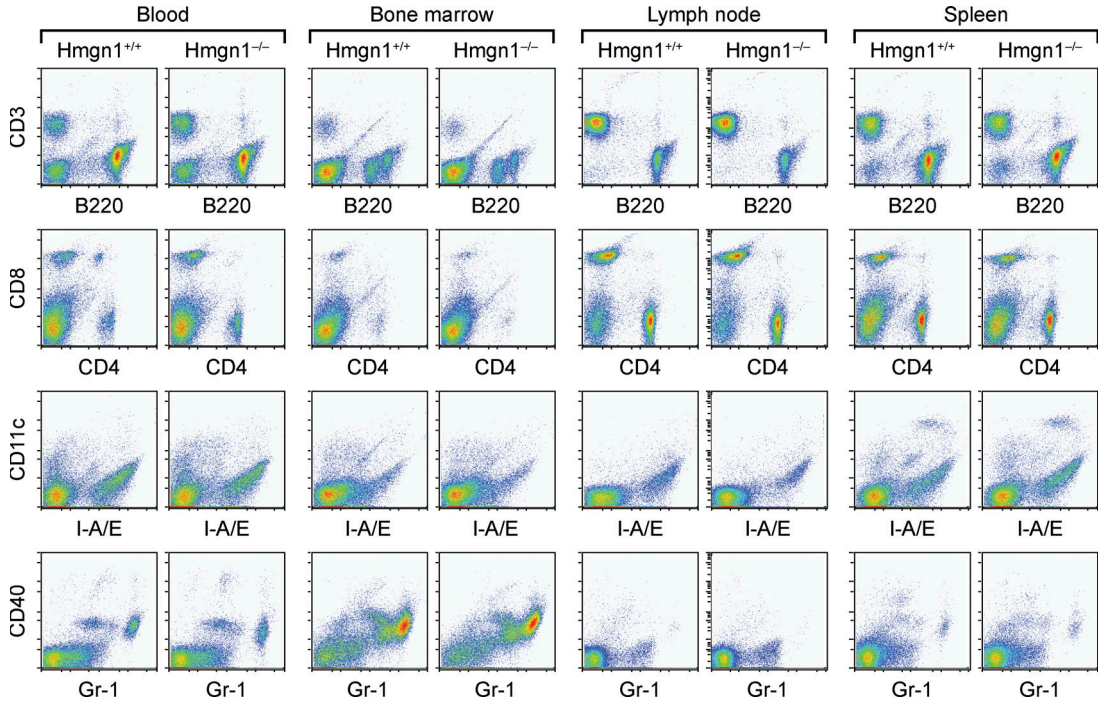


**Figure S6. Hmgn1$^{-/-}$ and littermate-matched Hmgn1$^{+/+}$ mice have similar distribution of various subsets of leukocytes.** The leukocytes of Hmgn1$^{-/-}$ and littermate-matched Hmgn1$^{+/+}$ mice isolated from peripheral blood, bone marrow, lymph node, and spleen were immunostained with FITC- or PE-conjugated antibodies specific for various mouse cell surface markers and analyzed by flow cytometry. Shown are the dot-plots of parallel analysis of Hmgn1$^{-/-}$ and littermate-matched Hmgn1$^{+/+}$ mice in terms of the distribution of various subsets of leukocytes in the indicated organs. Similar results were obtained in three separate experiments.

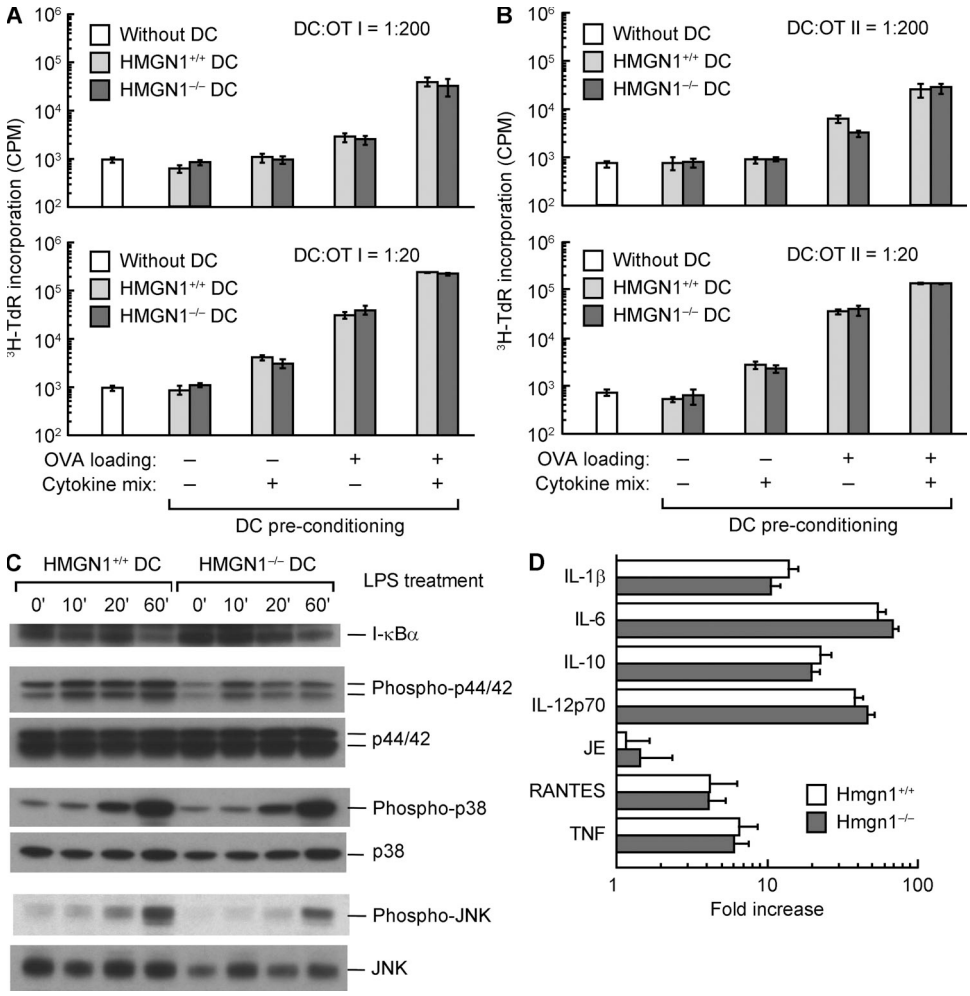


**Figure S7. DCs of Hmgn1$^{-/-}$ and littermate-matched Hmgn1$^{+/+}$ mice had comparable antigen-uptake and antigen-presenting capacity as well as signaling.** (A and B) OT I and OT II lymphocytes were isolated from the spleens and lymph nodes of OT I and OT II mice. DCs generated from the bone marrow progenitors of Hmgn1$^{-/-}$ and littermate-matched Hmgn1$^{+/+}$ mice were preconditioned by incubating without or with 100 µg/ml OVA for 24 h and subsequently cultured at 2 × $10^5$/ml in the presence or absence of a cytokine cocktails consisting of 20 ng/ml IL-1β, 50 ng/ml TNF, and 1 µg/ml of soluble CD40 ligand for another 24 h. Preconditioned DCs were washed, γ-irradiated (3,000 rad), and cultured in 96-well plates with OT I or OT II lymphocytes ($10^5$/well) at a DC/lymphocyte ratio of 1:20 or 1:200 for 48 h at 37°C in humidified air containing 5% $CO_2$. The culture was pulsed with 1 µCi/well [$^3$H]TdR for the last 18 h before harvest for measurement of [$^3$H]TdR incorporation. The proliferation of splenocytes was shown as the mean cpm (±SD) of triplicate wells. (C) Mouse bone marrow–derived DCs were treated with 1 µg/ml LPS for 10–60 min before they were solubilized in lysis buffer ($10^6$ DCs/0.1 ml). The cell lysates were separated on a gel and transferred onto a polyvinylidene fluoride membrane, and the levels of I-κBα and phosphorylated MAPKs (p44/42, p38, and JNK) were detected by Western blot. After stripping, the membranes were reprobed with antibodies specific for unphosphorylated MAPKs antibodies. Comparable levels of I-κBα degradation and phosphorylation of MAPKs (p44/42, p38, and JNK) were detected between Hmgn1$^{+/+}$ and Hmgn1$^{-/-}$ DCs. (D) Bone marrow–derived Hmgn1$^{+/+}$ and Hmgn1$^{-/-}$ DCs were cultured at 5 × $10^5$/ml in the absence or presence of 1 µg/ml LPS for 24 h before measurement of the indicated cytokines or chemokines in the supernatants. The results of one experiment representative of two, shown as the mean (±SD) fold increase (LPS treated over sham treated) of individual mediator of triplicate wells, are shown.

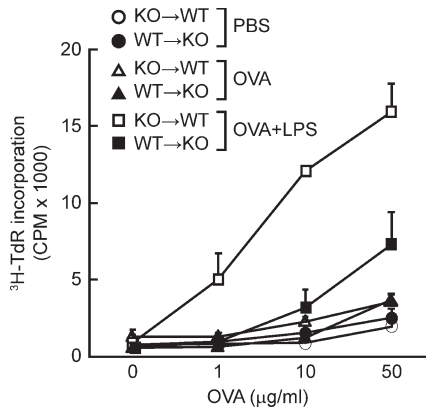


**Figure S8. OVA-specific splenocyte proliferation of immunized chimeric mice.** Bone marrow chimeric mice were generated by reconstituting lethally irradiated Hmgn1$^{-/-}$ mice with Hmgn1$^{+/+}$ bone marrow mononuclear cells (WT→KO) or vice versa (KO→WT). The chimeric mice ($n$ = 3) were i.p. immunized with PBS containing OVA (50 μg/mouse) or OVA in the presence of LPS (1 μg/mouse) on day 1 and boosted on day 14. On day 21, splenocytes isolated from euthanized mice were cultured in a 96-well plate ($5 \times 10^5$/0.2 ml/well) in the presence of specified concentrations of OVA for 4 d. The culture was pulsed with 1 μCi/well [$^3$H]TdR for the last 18 h before harvest for measurement of [$^3$H]TdR incorporation. The results are shown as the mean cpm (±SD) of triplicate wells of one experiment representative of two.