Glucocorticoid-receptor promotes the function of myeloid-derived suppressor cell by suppressing HIF1α-dependent glycolysis

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Supplementary Fig. 1

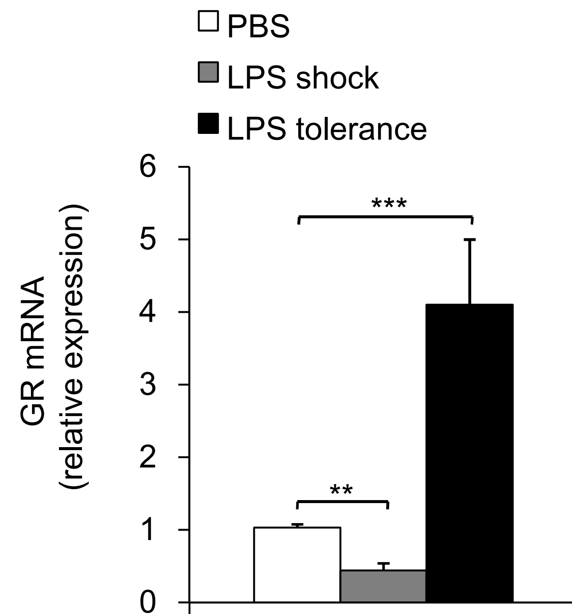

Fig. S1. GR expression of MDSCs in immune-mediated hepatic injuries. The experimental model for LPS tolerance and for LPS shock induction and GR mRNA expression was analyzed with qPCR in liver $CD11b^{+}Gr1^{+}$ cells. Data in Fig. S1 are representative of two (n=3-5). ***P*<0.01 and ****P*<0.001 compared with the indicated groups.

Supplementary Fig. 2

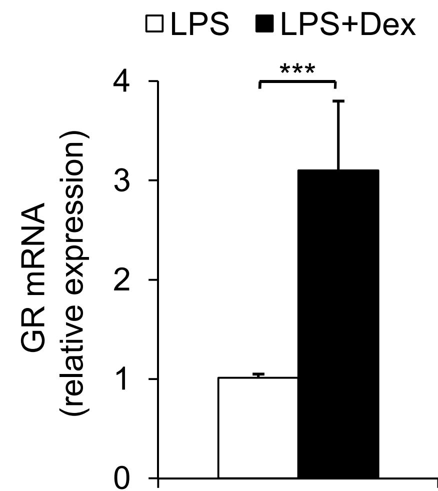

Fig. S2 Dex treatment upregulates GR expressions. Age-matched C57BL/6 mice were injected i.p. with PBS (solvent) or Dex (5 mg/kg body weight) daily starting at 6 h before LPS (5 mg/kg) injection. The GR mRNA expression on the $CD11b^{+}Gr1^{+}$ cells from liver 72 h following LPS injection was determined by qPCR. Data in Fig. S2 are representative of two (n=4). ****P*<0.001 compared with the indicated groups.

Supplementary Fig. 3

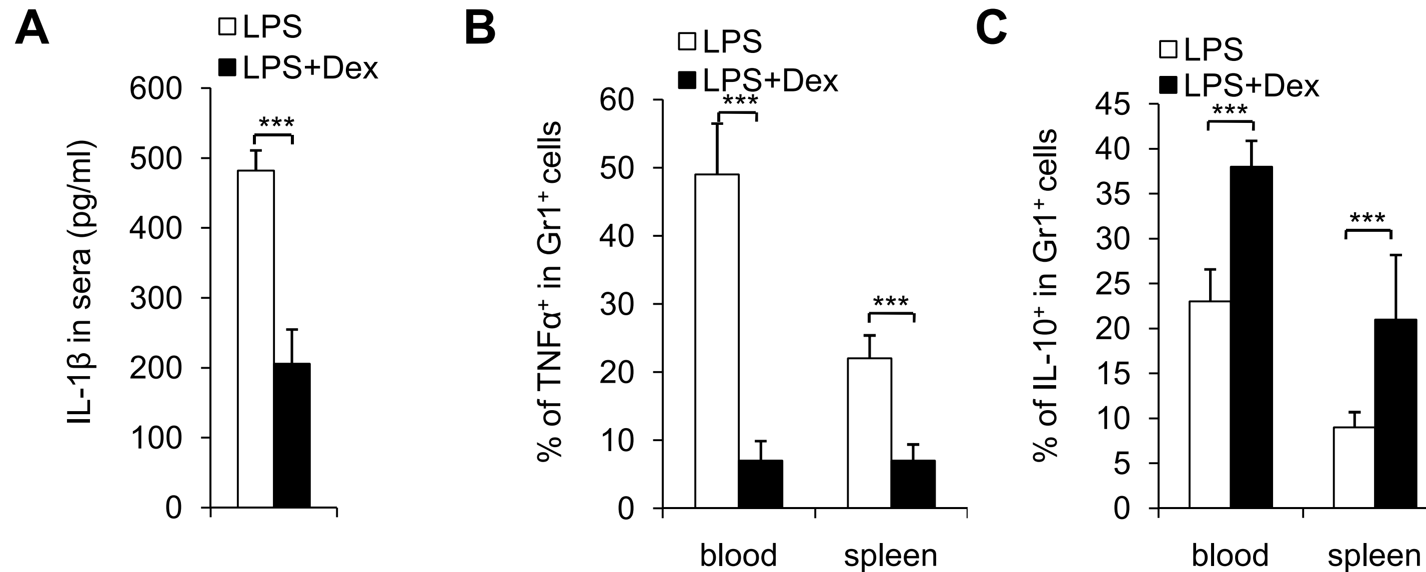

Fig. S3. Dex treatment potentiates MDSC activities in protecting against immune-hepatic injuries. Age-matched C57BL/6 mice were injected i.p. with PBS (solvent) or Dex (5 mg/kg body weight) daily starting at 6 h before LPS (5 mg/kg) injection, the mice were sacrificed at 72 h following LPS-injection. IL-1β level in serum was determined with ELISA (**A**). The TNFα (**B**) and IL-10 (**C**) production in $CD11b^{+}Gr1^{+}$ cells in blood and spleen were analyzed by FACS and results were summarized. Data in Fig. S3 are representative of three independent experiments (n=3-5). ****P*<0.001 compared with the indicated groups.

Supplementary Fig. 4

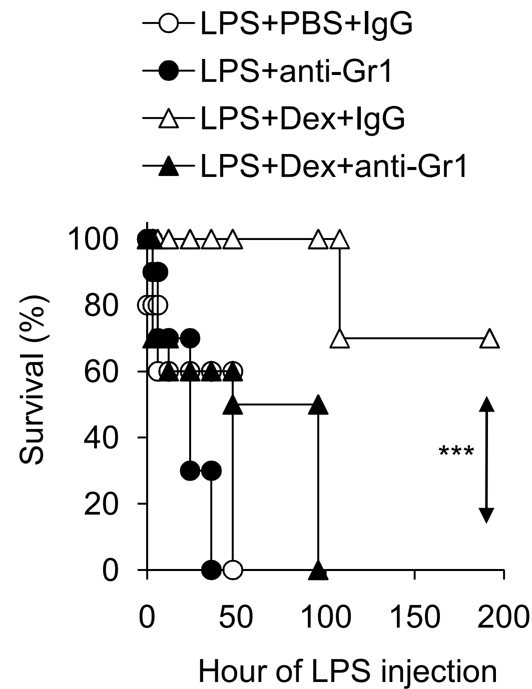

Fig. S4. Depleting Gr1$^+$ cells with mAb (RB6-8C5) abolishes Dex-mediated protection against immune-hepatic injuries. As described in Fig. 2, IMH was induced in mice (10 per gourp) with indicated treatment and survival curve was plotted. Data in Fig. S4 are representative of two (n=10). ***P<0.001 compared with the indicated groups.

Supplementary Fig. 5

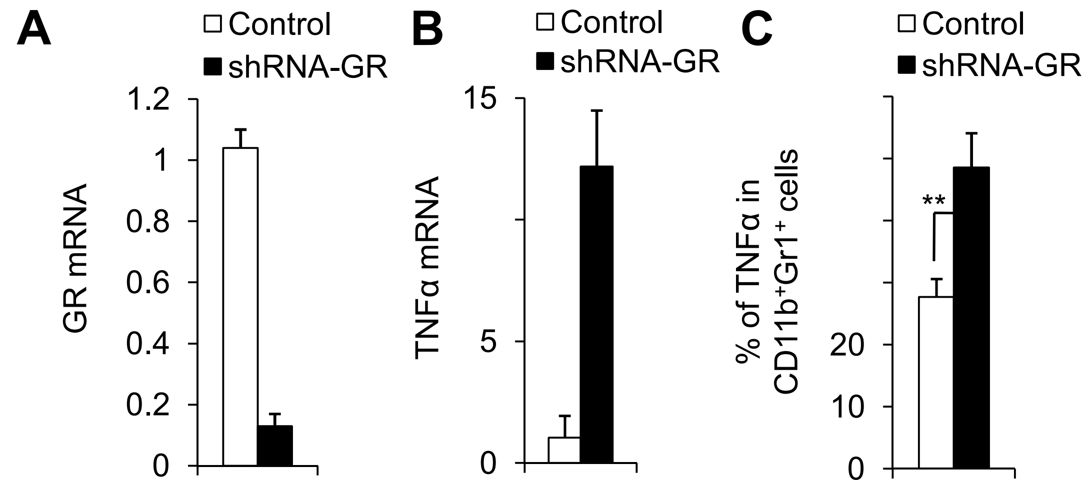

Fig. S5. GR signaling is required for establishing the function of MDSCs. Sorted liver $CD11b^{+}Gr1^{+}$ cells that were isolated from IMH mice were transduced with control or GR shRNA lentivirus, and GFP^{+}cells were sorted out for the subsequent experiments. The GR mRNA expressions (**A**) and TNFα mRNA expression (**B**) in the sorted GFP^{+} MDSCs were determined by qPCR. The intracellular TNFα protein in MDSCs was determined by FACS (**C**). Data in Fig. S5 are representative of two independent experiments (n=3-5). $^{**}P<0.01$ compared with the indicated groups.

Supplementary Fig. 6

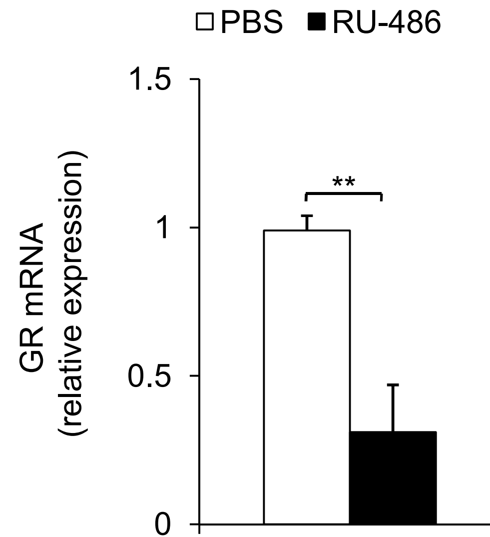

Fig. S6 The down-regulation of GR expression by RU-486. Two groups of aged matched C57BL/6 mice (n=5) were pretreated with low dose LPS (0.1 mg/kg) daily from day 4 prior to challenge with LPS (10 mg/kg) for LPS tolerance induction. Either PBS or RU-486 was administrated at 6 h before the last LPS-challenge. 72 h after the LPS challenge, GR mRNA expression of $CD11b^+Gr1^+$MDSC in liver was analyzed by qPCR. Data in Fig. S6 are representative of two (n=5). ***P*<0.01 compared with the indicated groups.

Supplementary Fig. 7

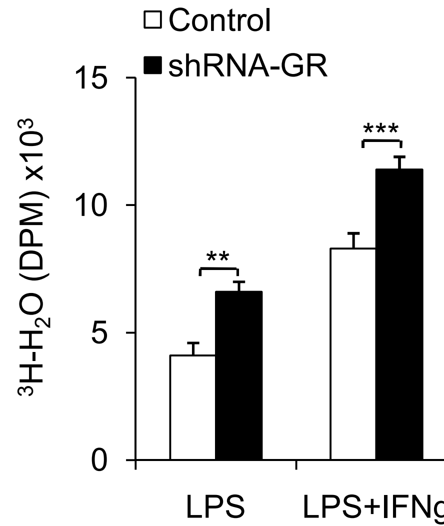

Fig. S7. Knockdown of GR via shRNA increased the glycolytic activity of MDSCs. Sorted liver $CD11b^+Gr1^+$ cells were transduced with control or GR shRNA lentivirus, and $GFP^+CD11b^+Gr1^+$ MDSCs were sorted out for the subsequent experiments. Sorted GFP^+MDSCs were stimulated with LPS or LPS + IFN-γ for 10 h. The glycolytic activity of these cells was measured by the generation of ^3H-labelled H_2O from [3-^3H]-glucose. Data in Fig. S7 are representative of three independent experiments (n=4). **P<0.01 and ***P<0.001 compared with the indicated groups.

Supplementary Fig. 8

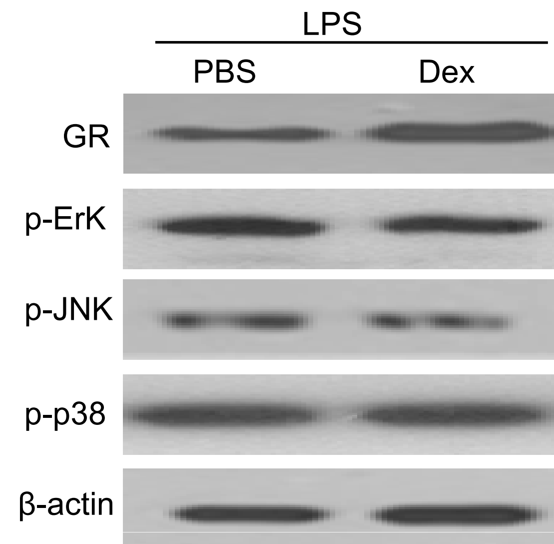

Fig. S8. The level of GR, p-ErK, p-JNK, p-p38 in MDSCs that were isolated from the Dex- and PBS-treated IMH mice were determined by western blot. Data in Fig. S8 are representative of two independent experiments.

Supplementary Fig. 9

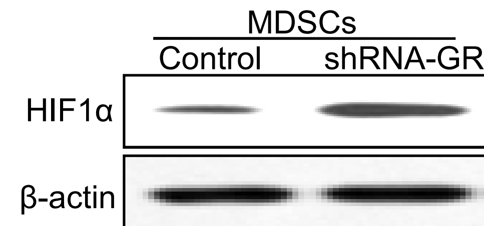

Fig. S9. Sorted liver $CD11b^{+}Gr1^{+}$ cells were transduced with control or GR shRNA lentivirus, and $GFP^{+}CD11b^{+}Gr1^{+}$ MDSCs were sorted out for the subsequent experiments. The protein level of HIF1α in MDSCs was determined by western blot.

Supplementary Fig. 10

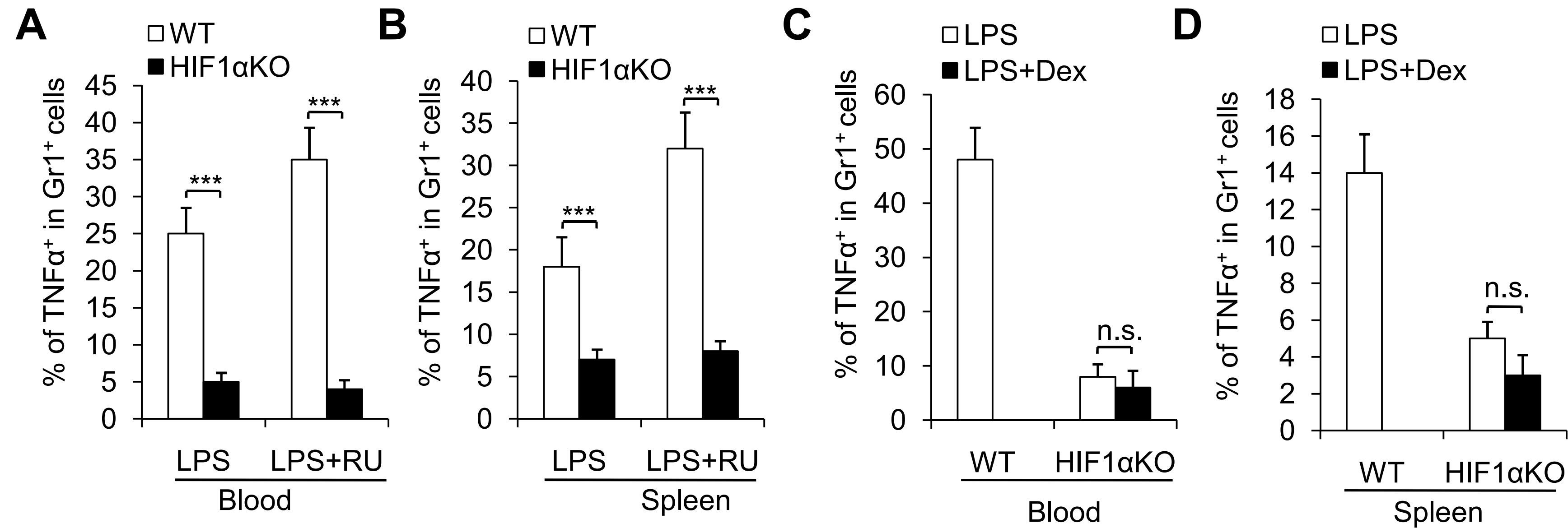

Fig. S10 HIF1α is a downstream effector of GR signaling in MDSCs in IMH. As described in Fig. 2, IMH was induced in WT or HIF1α$^{fl/fl}$, LysM Cre^{+} mice with indicated treatments. 72 h after LPS-injection, the TNFα production in CD11b^{+}Gr1^{+} cells in blood (A&C) and spleen (B&D) were analyzed by FACS and data was summarized. Data in Fig. S10 are representative of two or three independent experiments (n=3-5). ****P*<0.001 compared with the indicated groups. n.s., not significant.

Supplementary Fig. 11

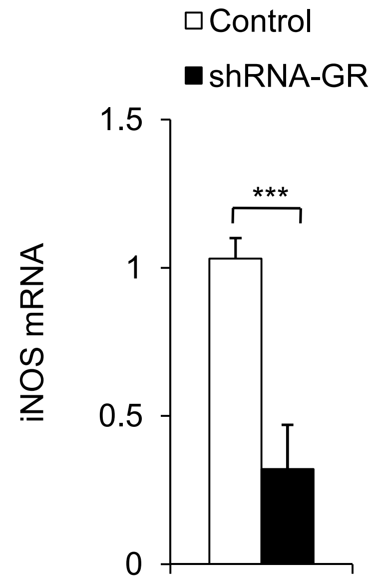

Fig. S11. Sorted liver $CD11b^+Gr1^+$ cells were transduced with control or GR shRNA lentivirus, and $GFP^+CD11b^+Gr1^+$ MDSCs were sorted out for the subsequent experiments. The level of iNOS mRNA in the sorted MDSCs was determined by qPCR. Data in Fig. S11 are representative of two independent experiments (n=3-5). ***$P<0.001$ compared with the indicated groups.

Supplementary Fig. 12

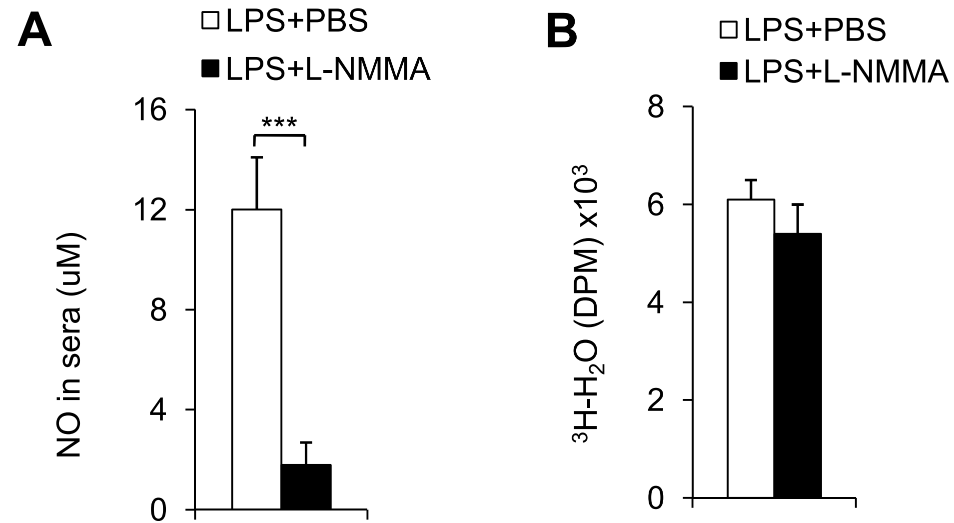

Fig. S12. Liver MDSCs were isolated from the LPS challenged mice that were pretreated with L-NMMA (80 mg/kg, gavage) and then stimulated with LPS for 24 h. The serum NO concentration was determined by Greiss assay (**A**). The glycolytic activity of indicated cells was measured by the generation of ^{3}H-labeled H_2O from [3-^{3}H]-glucose (**B**). Data in Fig. S12 are representative of two independent experiments (n=3). ****P*<0.001 compared with the indicated groups.

Supplementary Fig. 13

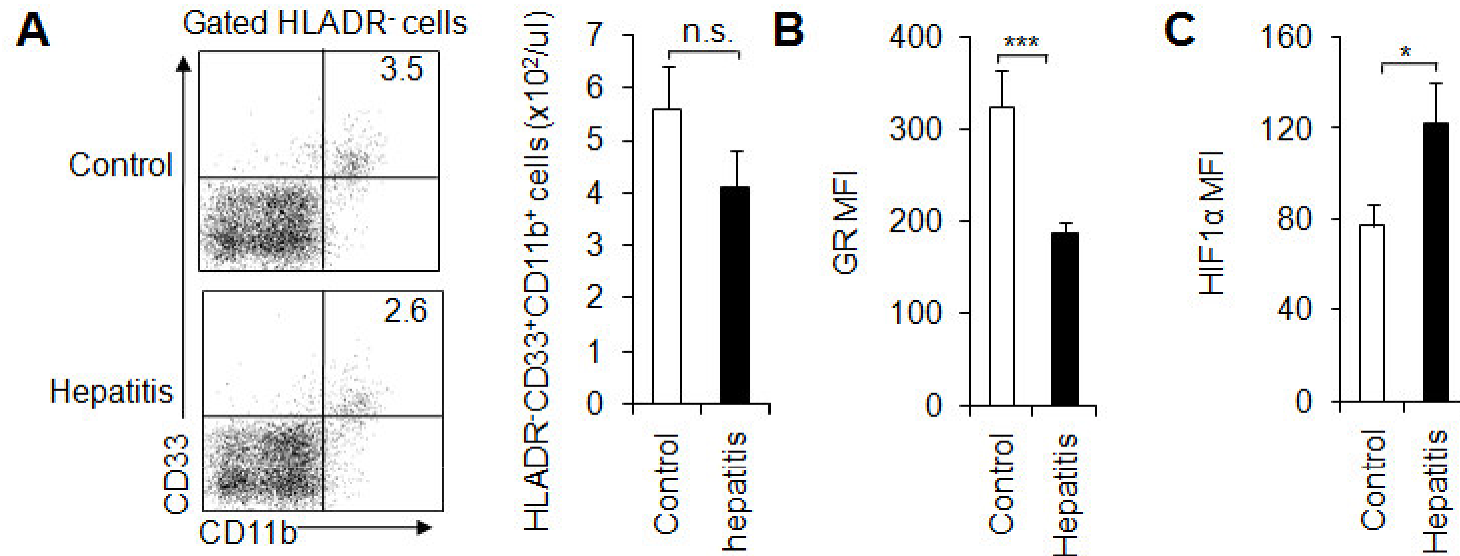

Fig. S13. GR expression in MDSCs is altered in human hepatitis patients.
Four patients with hepatitis from the Transplantation Center at the Ruijin Hospital and Medical School of Shanghai Jiao Tong University (Shanghai, China) were recruited in this study. All patients have been exhibited hepatitis characteristics prior to the surgical operation. The informed consent was obtained from all subjects and all experimental protocols were performed in accordance with the approval of the Ethics Committee of Shanghai Jiao Tong University and Fudan University, China. The peripheral blood of four hepatitis patients was collected and HLADR$^-$CD33$^+$CD11b$^+$ cell were analyzed with FACS. A representative flow-data was presented and the absolute cell numbers was graphed in **A**. The intracellular expression GR protein (**B**) and HIF1α (**C**) in purified HLADR$^-$CD33$^+$CD11b$^+$ cells was quantified with FACS, respectively. **P*<0.05 and ****P*<0.001 compared with the indicated groups. n.s.， not significant.