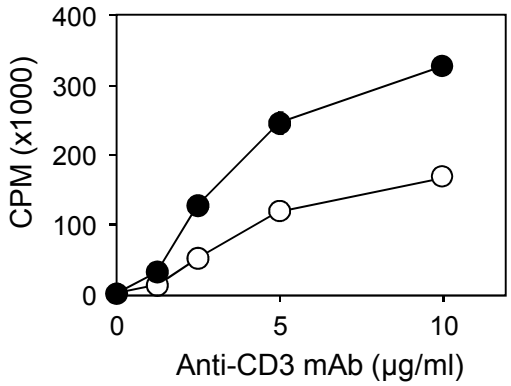
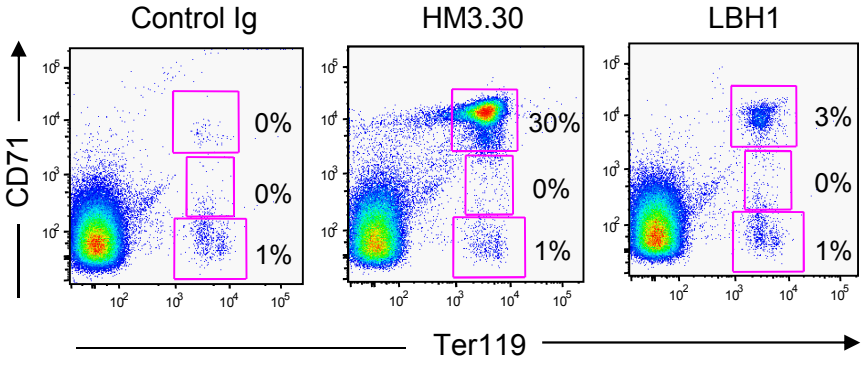
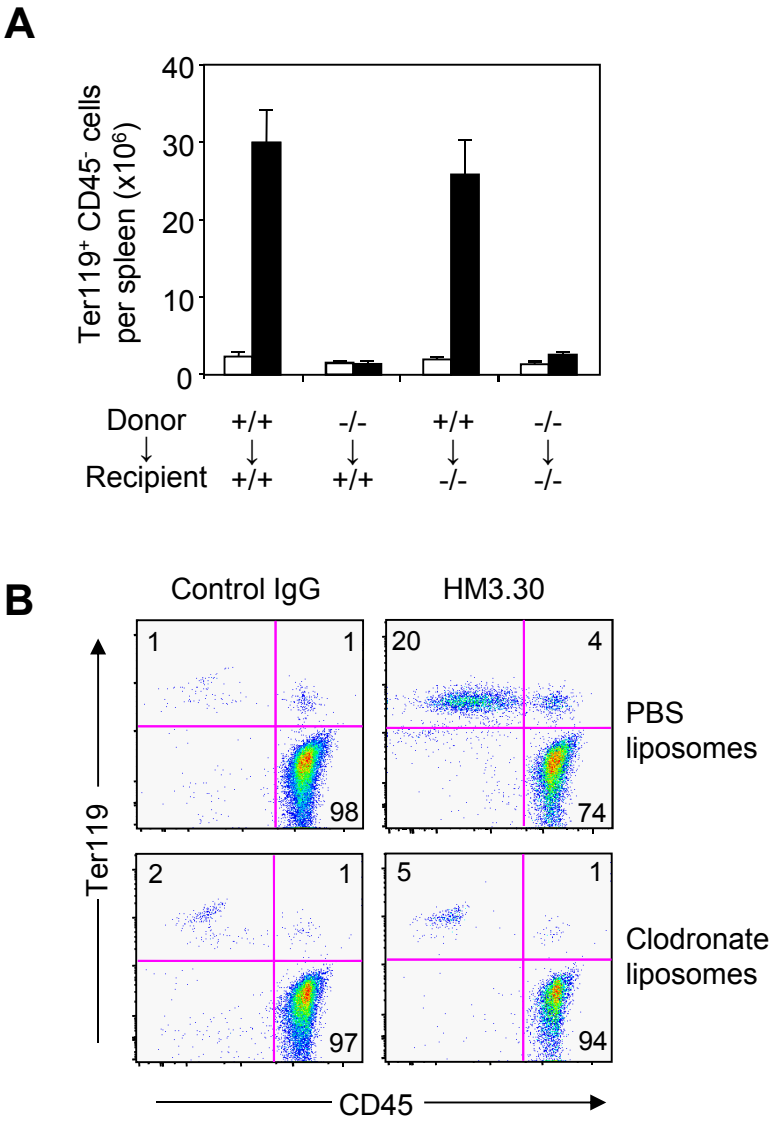


Supplemental Figure 1. Generation of agonistic anti-HVEM mAb HM3.30
T cells purified from wild-type B6 mice were cultured in 96-well plates coated with 10 μg/ml HM3.30 (filled circles) or control hamster Ig (open circles) in the presence of titrated doses of immobilized anti-mouse CD3 mAb. Proliferation was measured by ^{3}H-thymidine incorporation during the last 12 hours of 3-days culture.



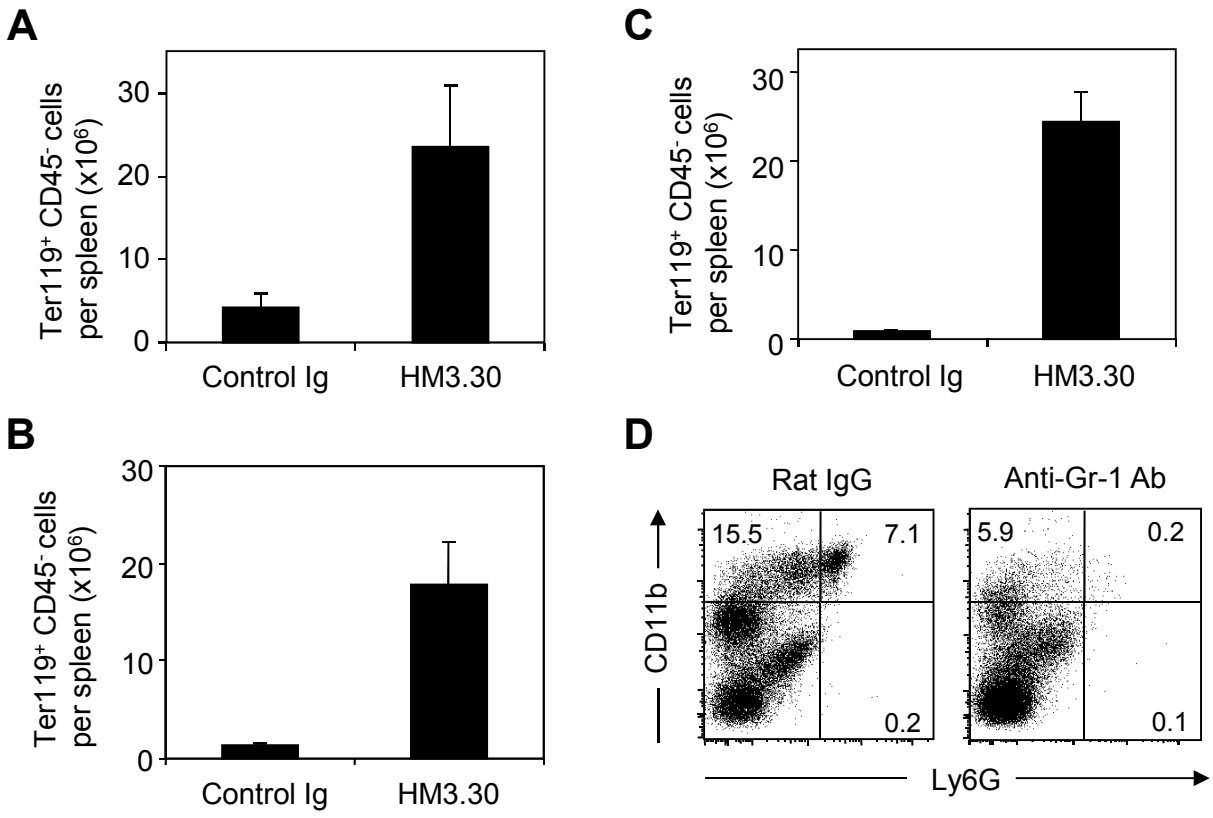
Supplemental Figure 2. HM3.30-mediated erythropoiesis is associated with its agonistic, but not blocking, features against HVEM

WT B6 mice were injected i.p. with 150 μg control Ig, HM3.30, or LBH1. After 4 days, expressions of Ter119 and CD71 on spleen cells were assessed by flow cytometry. The numbers indicates percentages of basophilic (Ter119+ $CD71^{high}$), polychromatophilic (Ter119+ $CD71^{medium}$), and orthochromatic (Ter119+ $CD71^{low}$) erythroblasts.



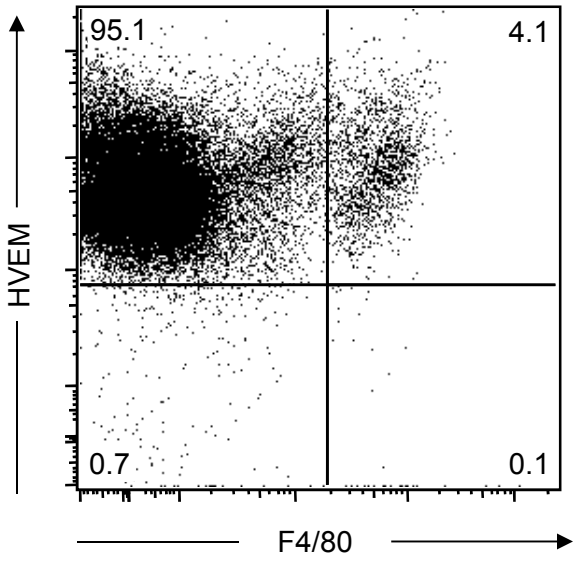
Supplemental Figure 3. Macrophage plays an important role in HM3.30-mediated erythropoiesis

(**A**) BM chimeric mice were established as shown in Figure 5A. Spleens were harvested from the chimeric mice (n=3 per group) to measure the numbers of Ter119+ CD45- cells 4 days after i.p. injection of 150 μg control Ig (white bar) or HM3.30 (black bar). Data represent mean ±SD. (**B**) Naïve B6 mice were injected i.v. with 200 μl clodronate-liposomes or control PBS-liposomes. One day later, the mice were treated i.p. with 150 μg HM3.30 or control Ig, as described in Figure 5B. After 4 days, percentages of Ter119+ CD45- cells in spleen were examined by flow cytometry. The numbers indicate percentages of cells in each quadrant. Data representative of 3 independently repeated experiments are shown.

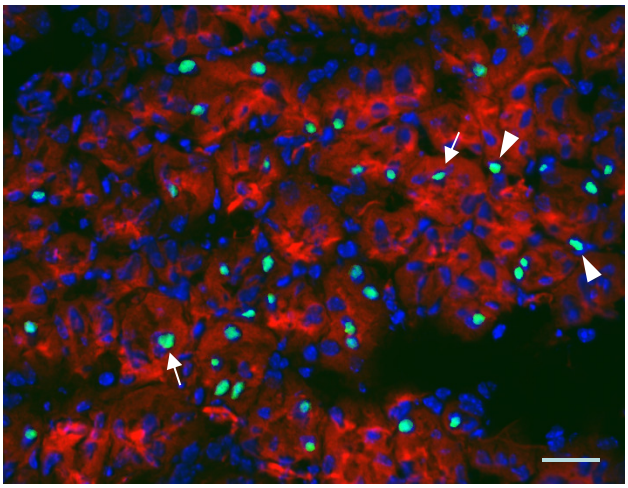


Supplemental Figure 4. HVEM-mediated erythropoiesis is independent of T, B, NK cells and granulocytes

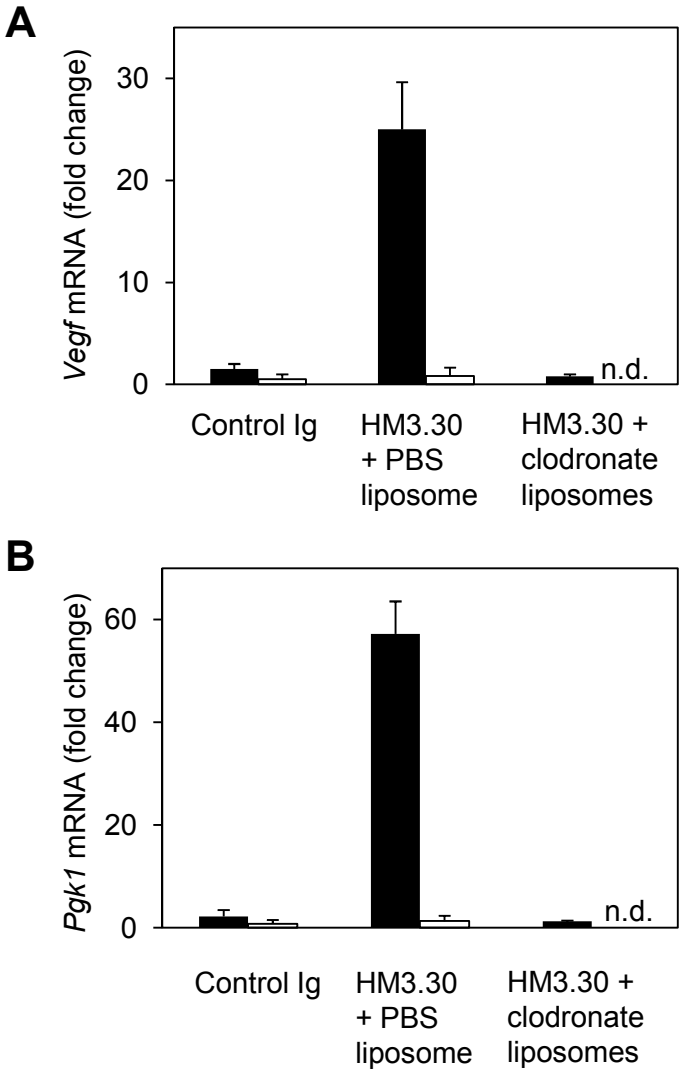
(**A**) B6 *Rag*-KO mice were injected i.p. with 150 μg control Ig or HM3.30. After 4 days, absolute number of Ter119+ CD45- cells per spleen was assessed by flow cytometry. (**B** and **C**) *Rag*-KO mice received i.p. injections of 500 μg anti-NK1.1 mAb (**B**) or anti-Gr-1 mAb (**C**) on days 0 and 2. On day 1, the mice were treated i.p. with 150 μg control Ig or HM3.30. On day 5, absolute number of $Ter119^+$ $CD45^-$ cells per spleen was assessed by flow cytometry. (**D**) B6 *Rag*-KO mice received i.p. injections of 500 μg rat Ig or anti-Gr-1 mAb on day 0 and 2. On day 5, the presence of $Ly6G^+CD11b^+$ granulocytes in spleens was examined by flow cytometry. The numbers indicate percentages of cells in each quadrant. Data represent mean ± SD.



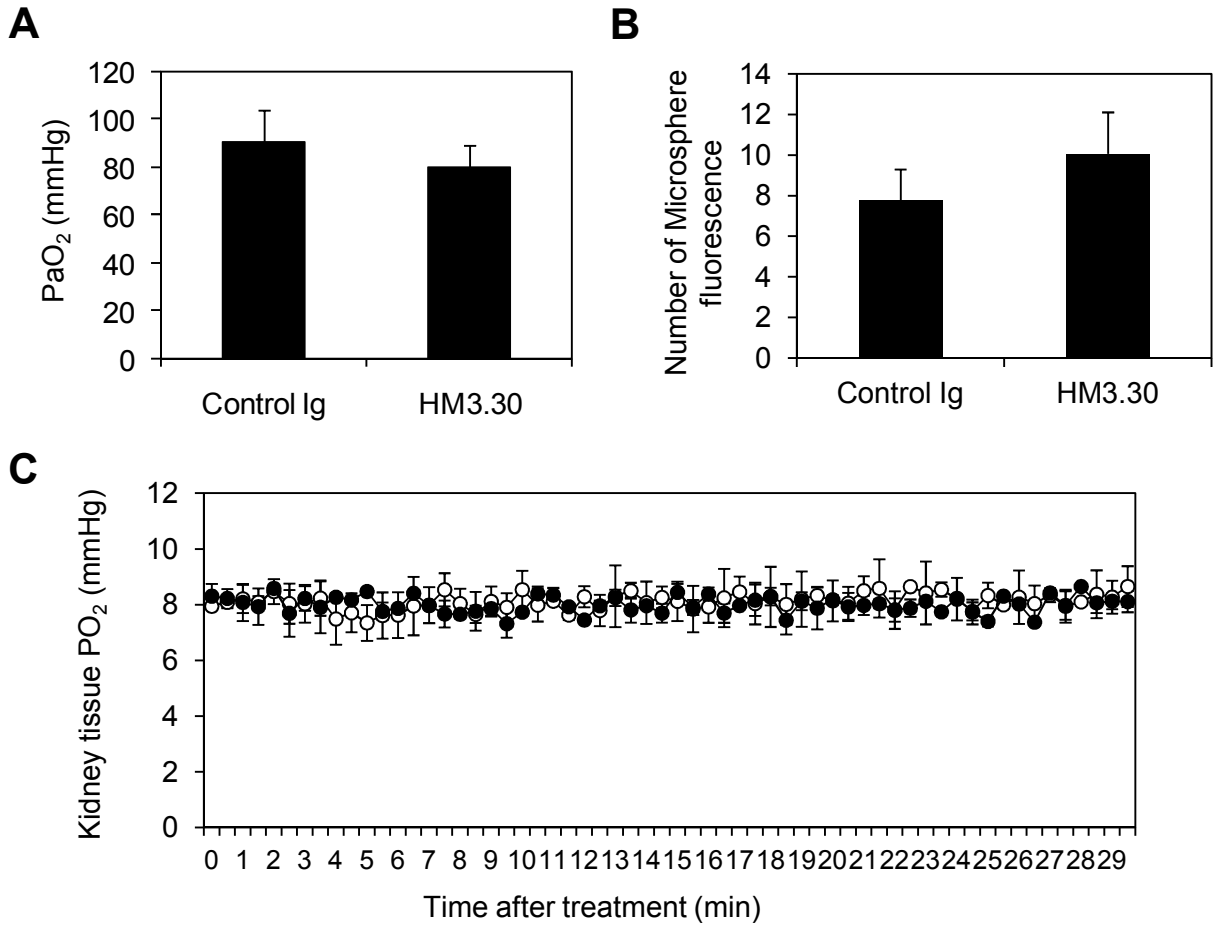
Supplemental Figure 5. Expression of HVEM on F4/80-positive macrophages
Spleen cells from wild-type B6 mice were stained with PE-conjugated anti-F4/80 mAb and biotin-conjugated HM3.30 followed by APC-conjugated streptavidin. Expression of HVEM on F4/80-positive macrophages was analyzed by a flow cytometry. The numbers indicate percentages of cells in each quadrant. Data are representative of 2 repeated experiments.



Supplemental Figure 6. Expression of HIF-1α in renal tubular and peritubular interstitial cells in HM3.30-treated mice
WT B6 mice were injected i.p. with 150 μg HM3.30. After 2 hrs, the kidneys were harvested and stained with anti-HIF-1α Ab followed by detection with Alexa Fluor 488-conjugated secondary Ab (green). The nuclei and cytoplasm were counter-stained with DAPI (blue) and eosin (red), respectively. Arrows and arrowheads indicate HIF-1α staining in tubular cells and peritubular interstitial cells, respectively. Original magnification: x400. Scale bar indicates 20 μm.



Supplemental Figure 7. HM3.30-mediated upregulation of *VEGF* and *PGK1* genes in the kidney but not in the liver.
WT B6 mice were injected i.v. with 200 µl clodronate-liposomes or control PBS-liposomes. One day later, the mice were treated i.p. with 150 µg HM3.30 or control Ig. After 8 hrs, RNA was isolated from the kidney (black bar) and liver (white bar), and the expressions of *Vegf* (**A**) and *Pgk1* (**B**) were analyzed by real-time PCR (n=3 per group). n.d.: not determined. Data represent mean ± SEM.



Supplemental Figure 8. HM3.30 treatment dose not induce systemic and renal hypoxia. (**A** and **B**) Naïve B6 mice were injected i.p. with 200 µg control Ig or HM3.30 (n=3 per group). (**A**) After 2 hrs, arterial blood samples were collected in heparinized syringe with 25-gauge needle by percutaneous left ventricular sampling of modestly anesthetized mice spontaneously breathing room air. Then, blood PaO_2 was immediately analyzed by Nova Biomedical STAT Profile pHOX Plus L Blood Gas Analyzer (Nova Biomedical). (**B**) After 4 hours, 5 x 10^4 red fluorescent microspheres (15 µm size FluoSpheres; Molecular Probes, Invitrogen) suspended in 50 µl PBS was injected into the left ventricle of anesthetized mice. One minute later, the kidneys were removed and snap-frozen in OCT compound. Twenty five-micron sections were cut and the number of red fluorescent microspheres per section was counted in at least 4 sections per mouse. (**C**) PO_2 in the kidney tissue after HM3.30 treatment was monitored by NeoFox oxygen sensing system (Ocean Optics). Naïve B6 mice were anesthetized with isoflurane and maintained on 37°C heat pad without exogenous oxygen supply. The left kidney was exposed through a mid-abdominal incision and the fiber-optic probe (Foxy-AL300 probe; Ocean Optics) was inserted perpendicular 2 mm deep into the renal capsule. The positioning and depth of insertion was determined in the same way between groups (n ≥ 3 mice per group). After PO_2 level became stabilized, mice were injected i.v. with 200 µg control Ig (o) or HM3.30 (•) and PO_2 was monitored for 30 min using NeoFox Viewer acquisition software V2.3 (Ocean Optics). Data represent mean ± SD.