Supporting Material for Li et al.

Supplementary Figure Legends

Figure S1 (A) An outline of steps taken to differentiate ESCs into myeloid progenitors and subsequently neutrophils. Phase-contrast images and Wright-Giemsa staining of cells at various stages are shown. (B) The experimental condition for immortalizing ESC-derived progenitors. (C) mEB8-ER or mBB8-ER cells ($2x10^5$) were cultured in the presence or absence of β-estradiol for 2, 4, 6, and 8 d. Cells were counted with a hemocytometer. Quantification of four separate experiments is shown. Each represents the mean±SEM (error bars).

Figure S2. Wright-Giemsa staining of mEB8-ER (top) or mBB8-ER cells (bottom) induced to differentiate with 2 ng/ml G-CSF for indicated times. Bar, 10 μm.

Figure S3. (A) Surface expression of B220 and TER119 in mEB8-ER or mBB8-ER cells measured with flow cytometry. Cells were induced to differentiate in the presence of G-CSF (2 ng/ml, 6 days). Cells prior to induction of differentiation were used as a negative control ("Control"). (B) Wright-Giemsa staining (left) and surface expression of Gr-1 and CD11b (right) in mEB8-ER (top) and mBB8-ER cells (bottom) assessed with flow cytometry. Cells were induced to differentiate for 6 days after the addition of 2 ng/ml GM-CSF. Cells prior to induction of differentiation were used as a negative control ("Control").

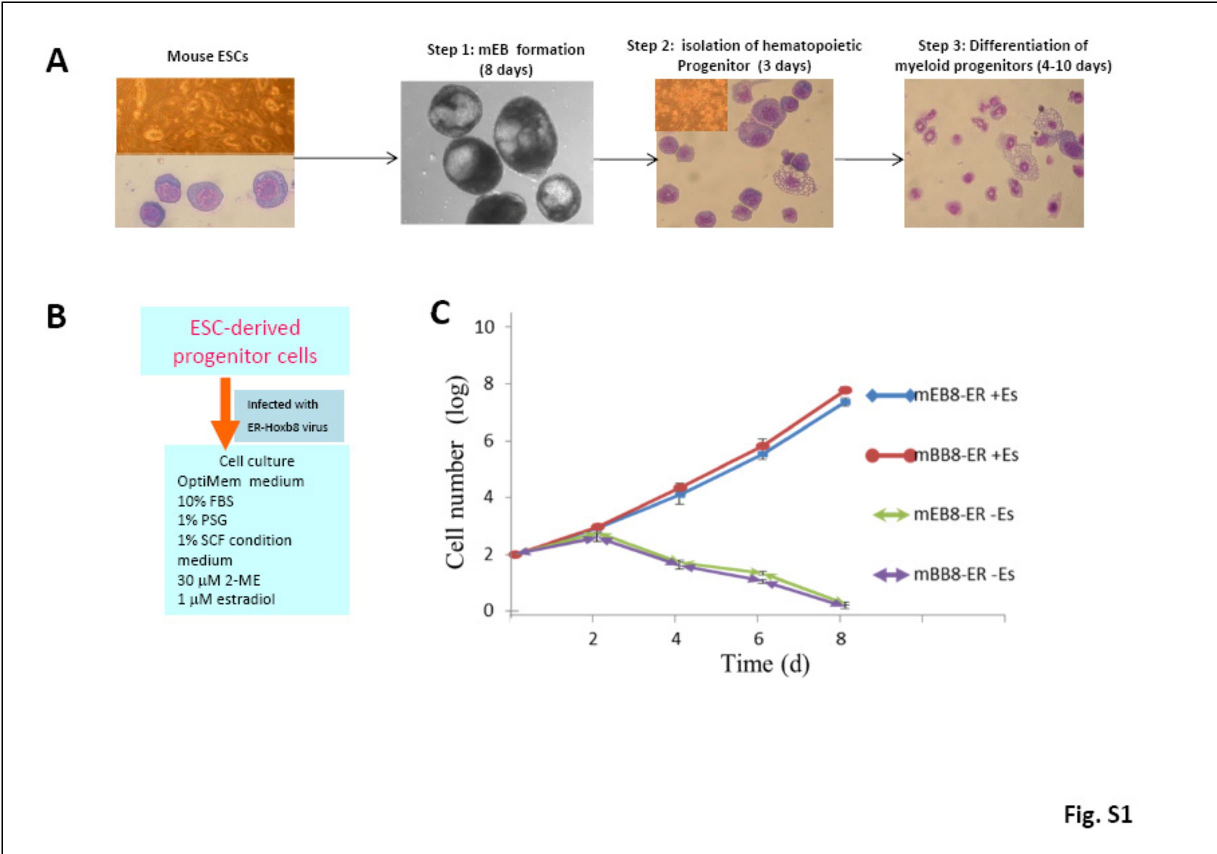
Figure S4. Histograms of surface expression of Gr-1(left) and CD11b (right) in mEB8-ER cells induced to differentiate with G-CSF (2 ng, 6 days) with (black lines) or without inhibitor treatments (red lines). AG1296 exerted little effects while bisindolylmaleimide I, Syk inhibitor and

rapamycin reduced the level of Gr-1 and CD11b. Numbers denote the level of expression of the markers.

Figure S5. (A) Rate of apoptosis in differentiated mEB8-ER cells with or without rapamycin treatment. mEB8-ER cells were induced to differentiate in the presence of G-CSF and treated with rapamycin (100 nM, 6 days). Apoptotic cells were analyzed using flow cytometry. Cells without any treatment were used as a control. The ratios of annexin V positive cells for the "control", the "G-CSF" or the "G-CSF+Rapamycin" group are 6.8%, 9.5% and 9.7%, respectively. No significant statistical differences were observed. (B) Relative number of mEB8-ER cells with (Rap) or without (Control) rapamycin treatment. mEB8-ER cells were cultured as a monolayer in the presence of β-estradiol for 5 d and counted with a hemocytometer. Quantification of four separate experiments is shown. Each represents the mean±SEM (error bars). All values were normalized to the level (=1) in cells without rapmycin treatment (Control). (C) Relative size of colonies of mEB8-ER cells with (Rap) or without (Control) rapamycin treatment. Cells were cultivated in MethoCult® GF M3434 medium for 10 days. Quantification of four separate experiments is shown. Each represents the mean±SEM (error bars). All values were normalized to the level (=1) in cells without rapmycin treatment (Control). (D) Relative number of mEB8-ER cells cultured with (G+Rap) or without (G-CSF) rapamycin treatment under differentiation conditions (i.e., in the presence of G-CSF for 6 days). Quantification of four separate experiments is shown. Each represents the mean±SEM (error bars). All values were normalized to the level (=1) in cells without rapmycin treatment.

Figure S6. (A) Relative percentage of B220 and TER-119-positive cells in mEB8-ER cells with or without rapamycin treatment, measured with flow cytometry. Cells were induced to differentiate in the presence of G-CSF (2 ng/ml, 6 days) with ("G+Rap") or without ("G-CSF") rapamycin treatment (100 nM). Four separate experiments were conducted, and quantification of three replicates of a typical experiment is shown. Each bar represents the mean ± SEM (error bars). All values were normalized to the level (=1) in cells without depletion. (B) Western blot of Rictor in mEB8-ER cells with or without shRNAs targeting Rictor. α-Tubulin was a loading control. A typical blot from 4 independent experiments is shown. Bottom panel: Quantification of blots from four separate experiments is shown. Each bar represents the mean ± SEM. *, $p<0.05$. LMP: the empty retrovector. (C) Relative percentage of CD11b and Gr-1-positive cells with or without Rictor depletion, measured with flow cytometry. Four separate experiments were conducted, and quantification of three replicates of a typical experiment is shown. Each bar represents the mean ± SEM (error bars). All values were normalized to the level (=1) in cells without depletion.

Supplementary Figures



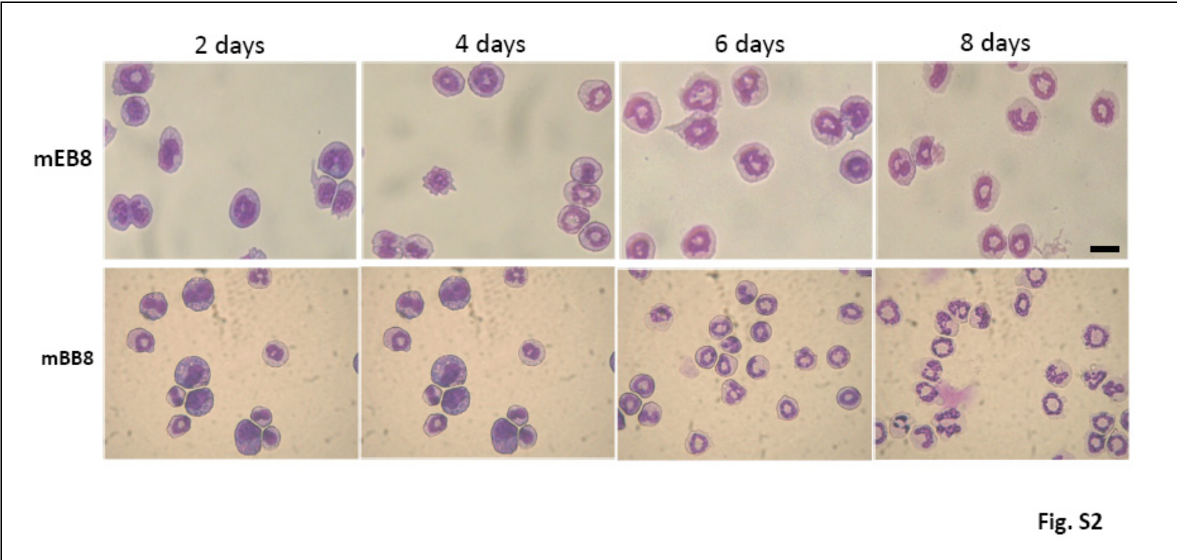
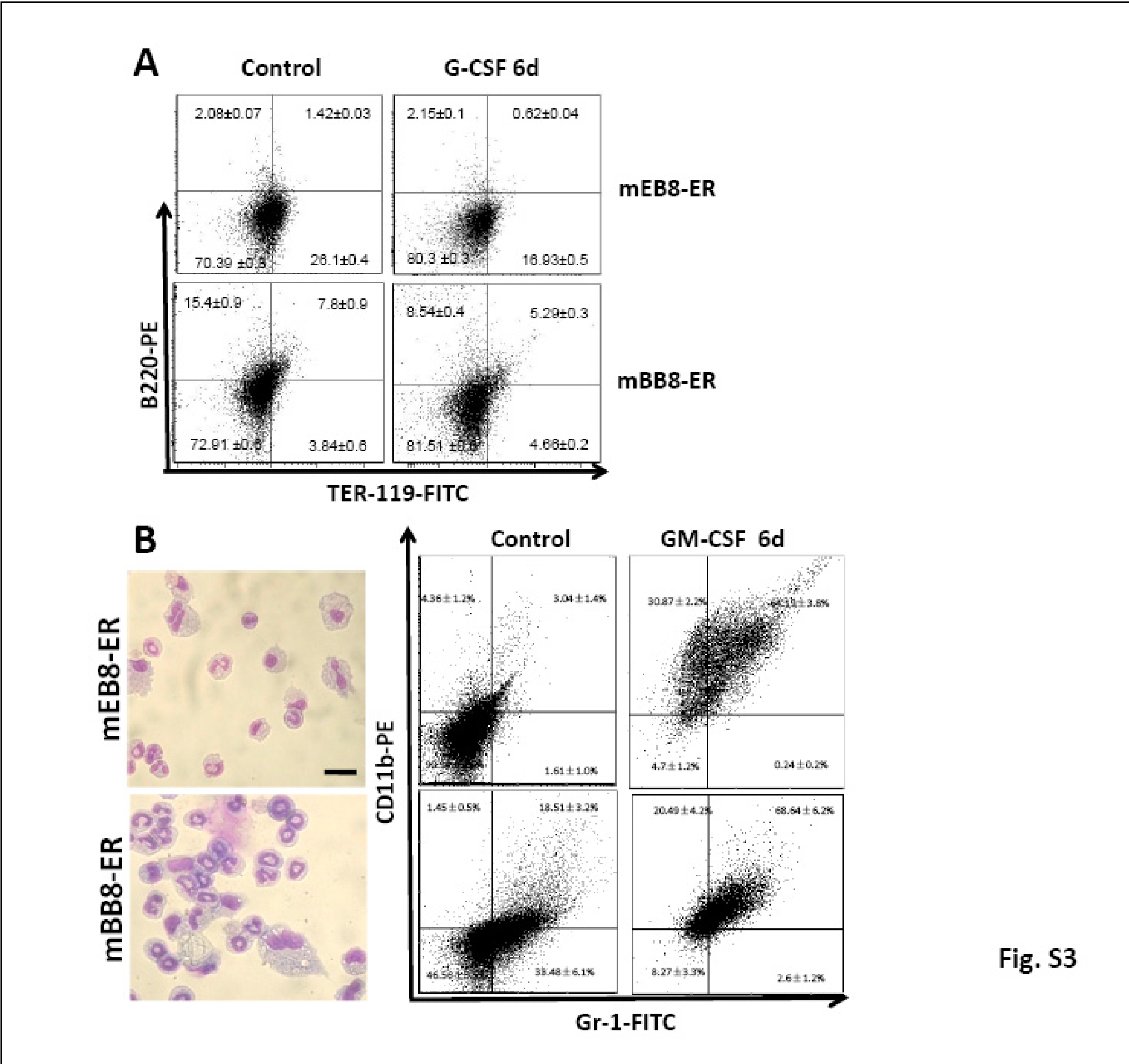


Fig. S2


A
Control
G-CSF 6d
2.08±0.07
1.42±0.03
2.15±0.1
0.62±0.04
mEB8-ER
26.1±0.4
16.93±0.5
15.4±0.9
7.8±0.9
8.54±0.4
5.29±0.3
mBB8-ER
3.84±0.6
4.66±0.2
B220-PE
TER-119-FITC
B
mEB8-ER
mBB8-ER
Control
GM-CSF 6d
4.36±1.2%
3.04±1.4%
30.87±2.2%
1.61±1.0%
4.7±1.2%
0.24±0.2%
1.45±0.5%
18.51±3.2%
20.49±4.2%
68.64±6.2%
33.48±6.1%
8.27±3.3%
2.6±1.2%
CD11b-PE
Gr-1-FITC
Fig. S3

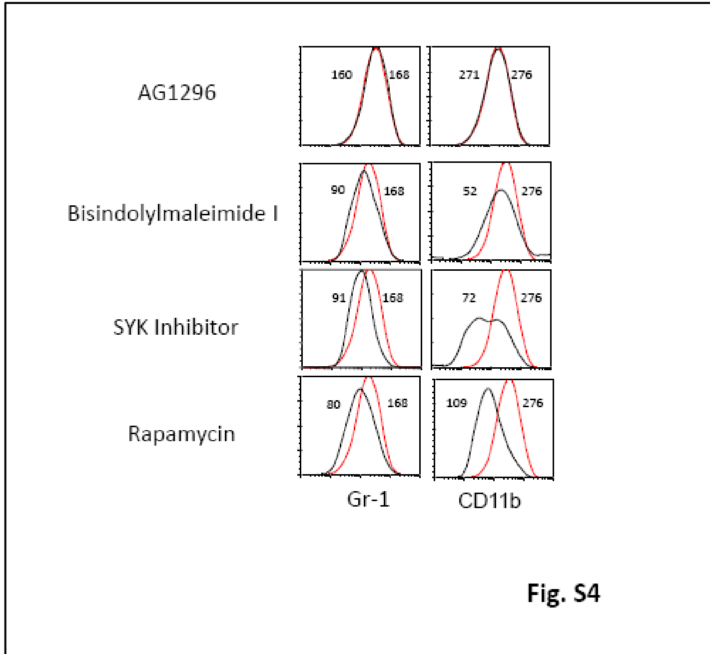
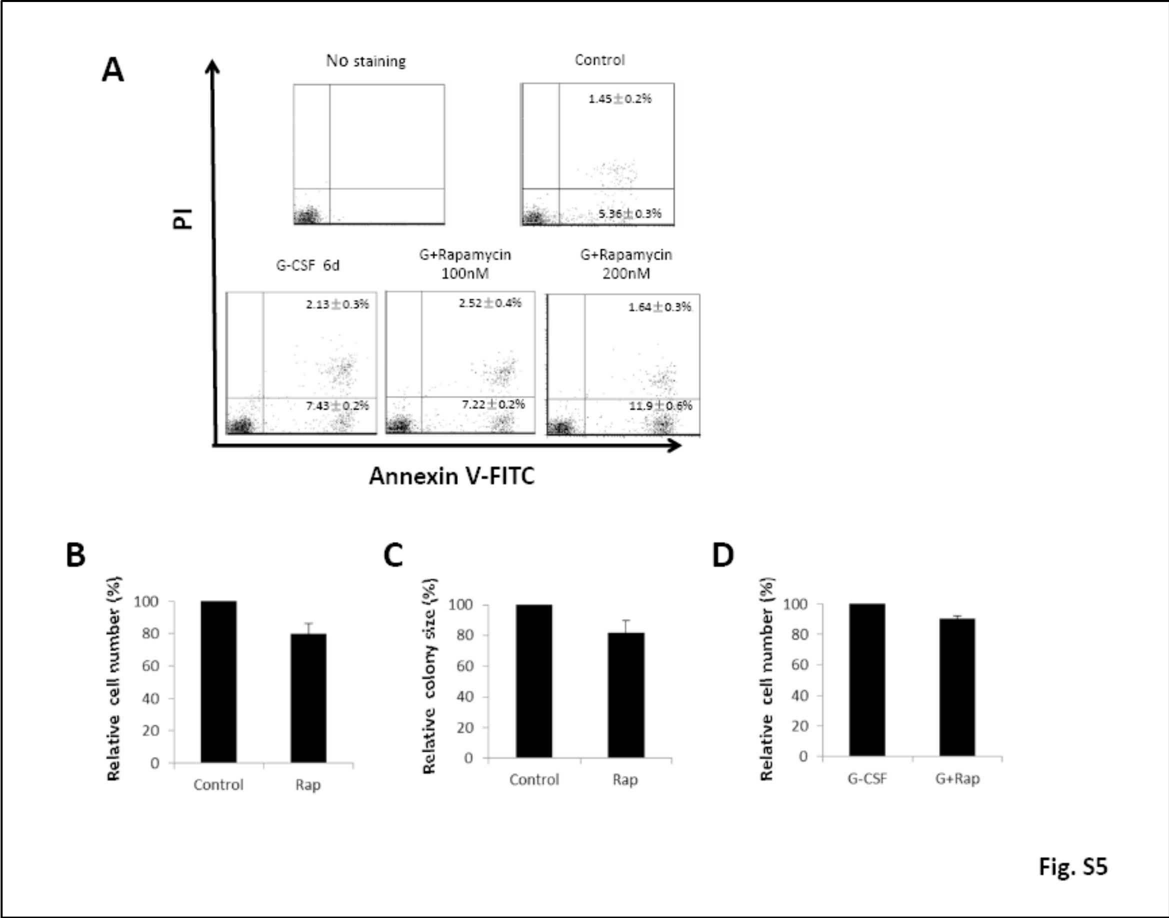
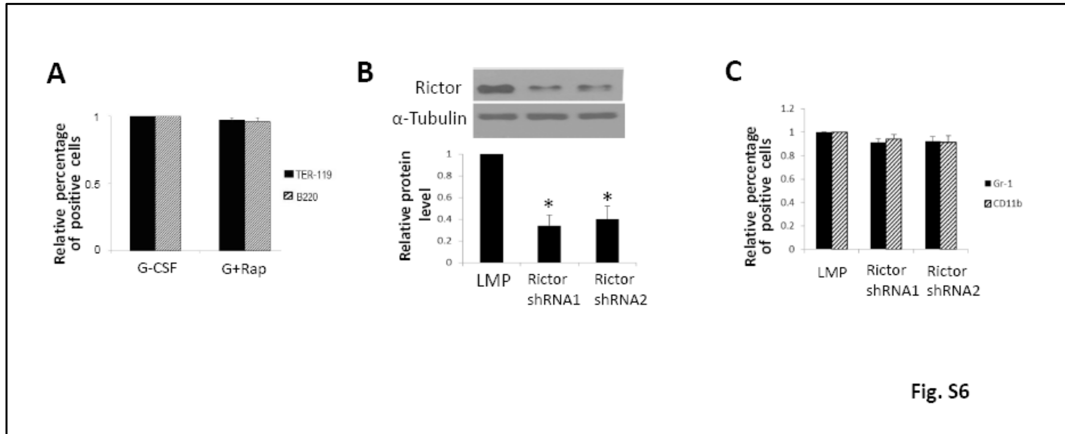


Fig. S4


A
No staining
Control
1.45±0.2%
5.36±0.3%
PI
G-CSF 6d
G+Rapamycin 100nM
G+Rapamycin 200nM
2.13±0.3%
7.43±0.2%
2.52±0.4%
7.22±0.2%
1.64±0.3%
11.9±0.6%
Annexin V-FITC
B
Relative cell number (%)
100
80
60
40
20
0
Control
Rap
C
Relative colony size (%)
100
80
60
40
20
0
Control
Rap
D
Relative cell number (%)
100
80
60
40
20
0
G-CSF
G+Rap
Fig. S5


A
Relative percentage of positive cells
1
0.5
0
TER-119
B220
G-CSF
G+Rap
B
Rictor
α-Tubulin
Relative protein level
1
0.8
0.6
0.4
0.2
0
*
*
LMP
Rictor shRNA1
Rictor shRNA2
C
Relative percentage of positive cells
1.2
1
0.8
0.6
0.4
0.2
0
Gr-1
CD11b
LMP
Rictor shRNA1
Rictor shRNA2
Fig. S6