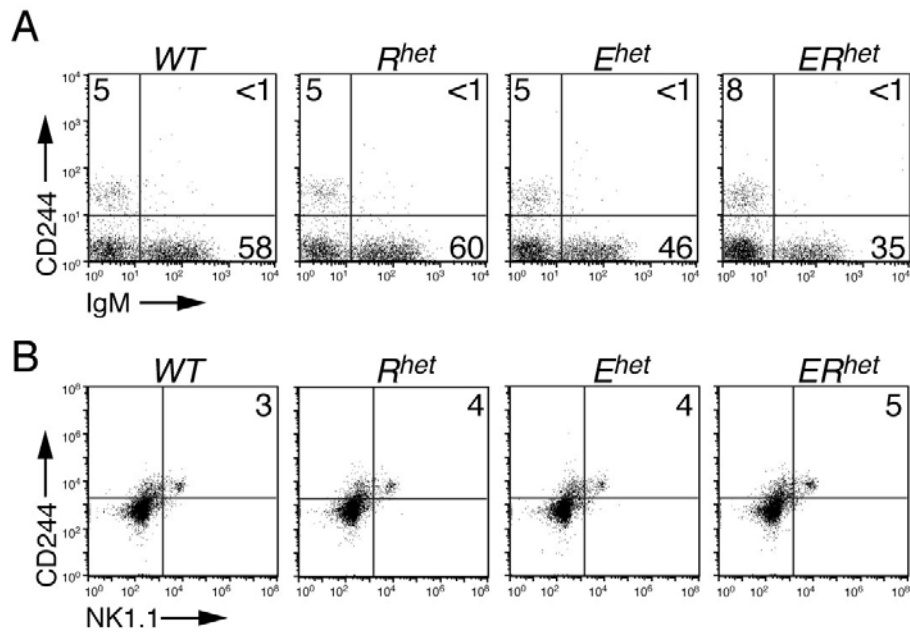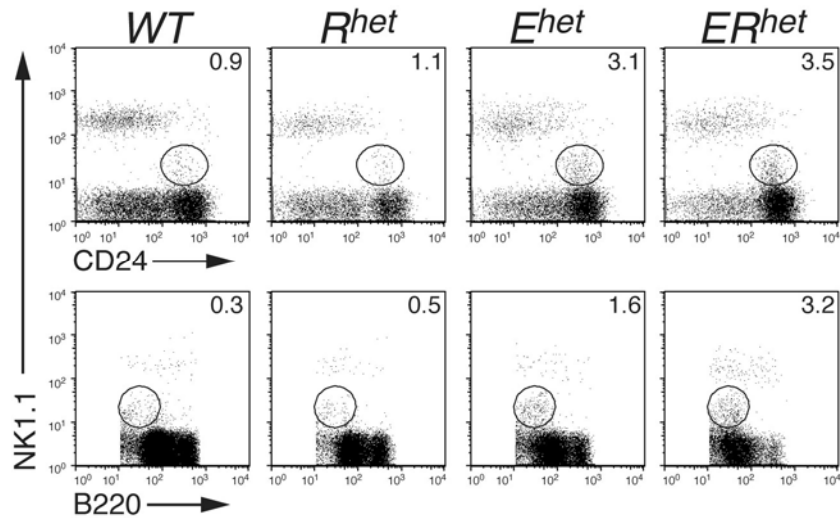**Supplementary Figure 1: Lack of CD244 expression on CD19$^+$-gated CD25$^+$ cells from the bone marrow.** All data are representative of greater than five independent experiments. Cell staining and analysis were performed using CD19-Pacific Blue, CD25-Alexa Fluor 488 and CD244-Alexa Fluor 647 (all from eBioscience, San Diego, CA, USA).

**Supplementary Figure 2: Expression of CD244 on splenocyes of *WT* and mutant mice.** A. Splenocytes were stained with CD244- and IgM-specific antibodies for detection by flow cytometry. B. Splenocytes were stained with CD244- and NK1.1-specific antibodies for detection by flow cytometry. Cell staining and analysis were performed with the following antibodies: CD244-Alexa Fluor 647 (eBioscience, San Diego, CA), IgM-FITC (BD Pharmingen, San Diego, CA) and NK1.1-PE (eBioscience, San Diego, CA).

**Supplementary Figure 3: NK1.1 expression on bone marrow cells of *WT* and mutant mice.**
Expression of NK1.1 on B220$^+$CD43$^+$–gated CD24$^+$ bone marrow cells (upper panel);
expression of NK1.1 on B220$^+$CD43$^-$–gated B220$^{lo}$ bone marrow cells (lower panel).
B220$^+$CD43$^+$-gated NK1.1$^{hi}$CD24$^-$ cells are NK cell progenitors [1]. The data are representative
of three independent experiments. Cell staining and analysis were performed with the following
antibodies: B220-FITC (eBioscience, San Diego, CA), CD43-APC (BD Pharmingen, San Diego,
CA), CD24-biotin (BD Pharmingen, San Diego, CA) with SA-APC-Cy7 (Biolegend, San Diego,
CA) and NK1.1-PE (eBioscience, San Diego, CA).

1       **Rolink, A., ten Boekel, E., Melchers, F., Fearon, D. T., Krop, I. and Andersson, J.,** A
subpopulation of B220$^+$ cells in murine bone marrow does not express CD19 and contains
natural killer cell progenitors. *J Exp Med* 1996. **183**: 187-194.

**Supplementary Table 1**. **NK cell-specific transcripts are enriched in RNA from pre-B cells of *ER^het* mice.[a]**

| Gene | Symbol | *WT* pre-B cells | | *ER^het* pre-B cells | |
|---|---|---|---|---|---|
| Granzyme B | Gzmb | 36.07 | 51.19 | 120.56 | 176.48 |
| Interferon gamma | Ifng | 43.12 | 106.55 | 390.37 | 499.12 |
| CD244 (2B4) | Cd244 | 43.42 | 62.77 | 192.21 | 256.56 |
| Killer cell lectin-like receptor family E member 1 | Klre1 | 45.71 | 64.91 | 179.37 | 226.54 |
| Killer cell lectin-like receptor, subfamily D, member 1 | Klrk1 | 155.16 | 158.52 | 629.23 | 1213.95 |
| Natural killer cell group 7 sequence | Nkg7 | 127.75 | 192.66 | 642.77 | 912.29 |
| Killer cell lectin-like receptor, subfamily A, member 7 | Klrb1a | 67.21 | 45.42 | 288.32 | 467.51 |
| Granzyme A | Gzma | 817.15 | 1024.72 | 2407.22 | 2786.82 |
| Killer cell lectin-like receptor, subfamily A, member 3 | Klrc3 | 274.79 | 533.61 | 1498.28 | 1714.65 |
| Killer cell lectin-like receptor, subfamily A, member 18 | Klra18 | 48.71 | 118.88 | 333.29 | 370.79 |
| Fas ligand (TNF superfamily, member 6) | Fasl | 38.13 | 25.36 | 259.03 | 143.53 |
| Eomesodermin homolog (Xenopus laevis) | Eomes | 68.60 | 50.84 | 264.57 | 298.40 |
| Klra8 / Klra21 / LOC545879 | | 107.14 | 145.59 | 818.81 | 642.14 |

[a]RNA was extracted from purified adult B220$^+$CD2$^+$IgM$^-$ bone marrow pre-B cells as described for qRT-PCR using a QIAGEN RNeasy Micro Kit [1]. RNA was labeled and amplified according to the Affymetrix^TM GeneChip Expression Analysis Technical Manual and hybridized against MOE430 2.0 Affymetrix gene expression arrays chips. Chips were scanned using a GeneChip^TM Scanner 3000. Probe level expression values were calculated using RMAExpress [2] and further analysis was done using dChip (www.dchip.org)[3]. Cluster analyses have been done with a list of genes with at least 2 times differentially expression in either of cell populations.

1     **Zandi, S., Månsson, R., Tsapogas, P., Zetterblad, J., Bryder, D., Sigvardsson, M.,** **EBF1 is essential for B-lineage priming and      establishment of a tran**scription factor network in common lymphoid  progenitors. *J Immunol* 2008. **181**:3364-3372.
2     **Irizarry, R.A., Hobbs, B., Collin, F., et al.,** Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 2003. **4**:249-264.
3     **Li, C., Wong, W.H.,** Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. *Proc Natl Acad Sci U S A* 2001. **98**:31-36.

**Supplementary Table 2: PCR primers used in these studies.**

| Gene | Forward Primer | Reverse Primer |
|------|----------------|----------------|
| **qRT-PCR** | | |
| *Hprt* | 5'-GGGGGCTATAAGTTCTTTGCTGACC-3' | 5'-CCTGTATCCAACACTTCGAGAGGTCC-3' |
| *Cd244* | 5'-TGAGGTAGTCTCGCTGTGTCCTGC-3' | 5'-GCCCTGTTCTGTCTTCTTCCATTG-3' |
| *Cd160* | 5'-CCTGGCCAAAGCTGCTGTGC-3' | 5'-GCCACAAAGTACAGGTGAGGTCCAG-3' |
| *Klrb1c* | 5'-TGGACACAGCAAGTATCTACCTCG-3' | 5'-GACTCGCACTAAGACACTCATCCC-3' |
| *Ly6a* | 5'-GGAGTCCCATTTGAGACTTCTTGCC-3' | 5'-GCTACATTGCAGAGGTCTTCCTGGC-3' |
| *Igll1* | 5'-TTGGTATGTCTTTGGTGGTGGGAC-3' | 5'-TAAGGAAGGCAGGAACAGAGTGAC-3' |
| | | |
| **Single cell PCR** | | |
| *Hprt* | 5'-GGGGGCTATAAGTTCTTTGC-3' | 5'-TCCAACACTTCGAGAGGTCC-3' |
| | 5'-GTTCTTTGCTGACCTGCTGG-3' | 5'-TGGGGCTGTACTGCTTAACC-3' |
| *Pax5* | 5'-CTACAGGCTCCGTGACGCAG-3' | 5'-TCTCGGCCTGTGACAATAGG-3' |
| | 5'-ATGGCCACTCACTTCCGGGC-3' | 5'-GTCATCCAGGCCTCCAGCCA-3' |
| *Cd79a* | 5'-CCTCCTCTTCTTGTCATACG-3' | 5'-GAACAGTCATCAAGGTTCAGG-3' |
| | 5'-AAACAATGGCAGGAACCC-3' | 5'-TGATGATGCGGTTCTTGG-3' |
| *Cd244* | 5'-TTAGCCTTCTGATTCCACATCAC-3' | 5'-ATGCTGCATGACACAGGATGAGG-3' |
| | 5'-ATATTCAGTAGTCCAGCCTTCC-3' | 5'-ATCAAAGTTCTCCAGCTCTCTGC-3' |
| *Cd160* | 5'-GACCAACTTAGAACAGCTTAGG-3' | 5'-CAGGAAGCCTGAACTGAGAGTGC-3' |
| | 5'-TTGGTGTTCACCATAGAACAAGC-3' | 5'-CGTTGATATGGCTGAAGTCAGG-3' |
| *Klrb1c* | 5'-CAGGTTGGCTCTGAAGCTCAGC-3' | 5'-ACAGTCAGCTTGACCTTCCTCC-3' |
| | 5'-GCTCATCCTCCTTGTCCTGACC-3' | 5'-GAAACGTGAAAGCACTTATCTCG-3' |