SUPPLEMENTAL MATERIAL

Rasmussen et al., http://www.jem.org/cgi/content/full/jem.20100458/DC1
Figure S1. Generation of miR–144/451 and miR–451 null alleles. (A) Overview of the miR–144/451 locus at high resolution (top). The organization of the miR144/451 locus and adjacent Era1 gene that lies on the opposite strand is depicted (bottom). The EcoRI (E) and AflII (A) restriction sites are indicated as the well as the respective Southern fragments detected by probe A. Position of the DNA encoding the premiR–144 and premiR–451 are indicated in red and blue, respectively. The filled rectangles represent exons and the unfilled rectangle represents the 3’UTR of Era1. (B) Targeting strategy and validation of the miR–144/451 null allele. The targeting vector used for introduction of loxP sites into the miR144/451 locus and the schematic map of the targeted miR144/451 before and after, Flp and Cre-mediated-recombination are shown (left). Shaded triangles represent loxP sites and frt sites as indicated. Shaded rectangles indicate the position of Neomycin (Neo) and Diptheria toxin A (DTA) selection marker genes. The EcoRI (E) restriction sites are indicated in the miR–144/451 null allele as well as the respective Southern fragment detected by probe A. Deletion diagnosed by Southern blotting of tail derived EcoRI-digested DNA is presented (right panel). The absence of miR–451 and miR–144 in bone marrow determined by Northern blotting is shown. The miRNA Let–7a expression is shown as a loading control. The data are representative of two independent experiments. (C) Targeting strategy and validation of the miR–451 null allele. The targeting strategy for the inactivation of miR–451 is depicted (left panel) as in (B). The AflII (A) restriction sites are indicated in the miR–451 null allele as the well as the respective Southern fragment detected by probe A. Deletion diagnosed by Southern blotting of tail derived AflII-digested DNA is presented (right panel). The selective absence of miR–451 in bone marrow determined by Northern blotting is shown. U6 expression is shown as a loading control. The data are representative of two independent experiments.
Figure S2. Characterization of miR–144/451−/− erythroid cells. (A) Modified Wright-stained peripheral blood smears from wild-type and miR–144/451−/− mice. Mutant erythrocytes show variation in size and shape (anisocytosis) as well as increased levels of young erythrocytes (blue shade) in circulation. Representative images from five independent experiments are shown. (B) Representative FACS analysis is shown for the c-kit-positive erythroid cell populations in the bone marrow and spleen of wild-type and miR–144/451−/− mice. The gates illustrate the following developmentally defined subpopulations: pre–colony-forming unit erythroid precursor (Pre-CFU-E), colony forming unit erythroid (CFU-E), pre–megakaryocyte/erythroid precursor (Pre-MegE), granulo monocyte precursor (GMP), and pregranulo monocyte precursor (Pre-GM). (C) Comparative enumeration of lineage-negative erythroid precursors in the bone marrow and spleen of wild-type and miR–144/451−/− mice. Numbers are expressed relative to the wild-type and plotted for the indicated subpopulations. The data are from biological quadruplicates and representative of two independent experiments. A single asterisk represents significance of P < 0.01. (D) Histological analysis of wildtype (upper panel) and miR–144/451−/− (lower panel) erythropoietic tissues by H&E staining. Architecture and morphology of bone marrow (left) and spleen (right) are shown. miR–144/451-deficient spleen shows a pronounced increase in the density of clusters of maturing erythroblasts, whereas no marked morphological differences are observed in bone marrow. Images are representative of 4 independent experiments. (E) Diff-Quick-Benzidine–stained cytospins of in vitro erythroid cultures derived from wildtype (left) and miR–144/451−/− (right) mice at the indicated time points during differentiation. Brown staining indicates presence of hemoglobin. Thin arrows indicate nascent reticulocytes and thick arrows indicate nucleated erythroblast. Images are representative of four independent experiments. Bars: (A) 10 μm; (D) 40 μm; (D, inset) 20 μm; (E) 10 μm.
Figure S3. The erythroid defects in miR-144/451^−/− mice are hematopoietic autonomous. (A) Representative FACS analysis is shown of erythroid cell populations in the bone marrow and spleen of lethally irradiated mice reconstituted with wild-type or miR-144/451^−/− bone marrow, as indicated. Mice were analyzed 12 wk after reconstitution. Numbers indicate the percentages of cells of the developmentally defined subpopulations. The roman numerals indicate the identity of the following defined erythroid subpopulations (Socolovsky et al., 2001): I, proerythroblasts; II, basophilic erythroblasts; III, polychromatophilic erythroblasts; and IV, orthochromatophilic erythroblasts. (B) Comparative enumeration of erythroid precursors in the bone marrow and spleen of reconstituted mice. Genotype of donor bone marrow is indicated. Numbers are expressed relative to the wild-type and plotted for the developmentally defined erythroid subpopulations indicated in (A) by roman numerals. (C) MiR-144/451 deficiency alters several erythrocyte parameters in the peripheral blood. Bar charts are shown expressing values for RBC, hemoglobin (Hb), hematocrit (HCT); and red cell distribution width (RDW). Bars represent mean values (n = 4), error bars indicate standard deviation. The data in all panels are from biological quadruplicates and representative of two independent experiments. *P < 0.05; **, P < 0.01.