Blockade of IL-22 signaling reverses erythroid dysfunction in stress-induced anemias

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Patients with myelodysplastic syndromes (MDSs) display severe anemia but the mechanisms underlying this phenotype are incompletely understood. Right open-reading-frame kinase 2 (RIOK2) encodes a protein kinase located at 5q15, a region frequently lost in patients with MDS del(5q). Here we show that hematopoietic cell-specific haploinsufficient deletion of RIOK2 (RIok2+/−Vav1cre) led to reduced erythroid precursor frequency leading to anemia. Proteomic analysis of Riok2+/−Vav1cre erythroid precursors suggested immune system activation, and transcriptomic analysis revealed an increase in p53-dependent interleukin (IL)-22 in Riok2+/−Vav1cre CD4+ T cells (T,22). Further, we discovered that the IL-22 receptor, IL-22RA1, was unexpectedly present on erythroid precursors. Blockade of IL-22 signaling alleviated anemia not only in Riok2+/−Vav1cre mice but also in wild-type mice. Serum concentrations of IL-22 were increased in the subset of patients with del(5q) MDS as well as patients with anemia secondary to chronic kidney disease. This work reveals a possible therapeutic opportunity for reversing many stress-induced anemias by targeting IL-22 signaling.

MDSs are a group of cancers characterized by failure of blood cells in the bone marrow to mature. About 7 out of 100,000 people are affected and the typical survival time following diagnosis is less than 3 years. While a sizable percentage of MDS cases progress to acute myelogenous leukemia (AML), most of the morbidity and mortality associated with MDS results not from transformation to AML but rather from hematological cytopenias.

Anemia is the most common hematologic manifestation of MDSs, particularly in the subset of patients with del(5q) MDS. Del(5q), either isolated or accompanied by additional cytogenetic abnormalities, is the most commonly detected chromosomal abnormality in MDSs, reported in 10–15% of patients1–4. The severe anemia in patients with del(5q) MDSs has been linked to haploinsufficiency of ribosomal proteins such as RPS14 and RPS19 (refs. 5,6). Previous studies using mice with haploinsufficient 5q gene deletions revealed diminished erythroid progenitor frequency7–10 but the mechanisms underlying this phenotype are incompletely understood. RIOK2 encodes an atypical serine–threonine protein kinase with an indispensable function as a component of the pre-40S ribosome subunit11. There is growing evidence for the role of activated innate immunity and inflammation as well as immune dysregulation in the pathogenesis of MDSs12–15. Abnormal expression of numerous cytokines has been reported in MDSs16–19. Chronic immune stimulation in both hematopoietic stem and progenitor cells and the bone marrow (BM) microenvironment20 was suggested to be central to the pathogenesis of MDSs. In patients with chronic inflammation, cytokines in the BM have been associated with inhibition of erythropoiesis21. Despite growing evidence for a link between the immune system and MDS pathogenesis, no study has identified the mechanism by which the immune microenvironment may initiate or contribute to the MDS phenotype. Further it remains unclear how ribosomal protein haploinsufficiency is connected with the immune system in MDSs.

Results

Riok2 haploinsufficiency leads to anemia. We previously noted that RIOK2 expression was reduced in T cells lacking the endoplasmic reticulum stress transcription factor Xbp1 (ref. 22). Riok2 is a little-studied atypical serine–threonine protein kinase23 encoded by RIOK2 at 5q15 in the human genome (Extended Data Fig. 1a), adjacent to the 5q commonly deleted regions in MDSs and frequently lost in MDSs and acute myeloid leukemia24–27. Gene expression commons (GEXC)23 analysis revealed that in mouse BM, Riok2 expression is highest in primitive colony-forming-unit erythroid (CFU-e) cells, suggesting that Riok2 may be involved in maintaining red blood cell (RBC) output (Extended Data Fig. 1d). To further study the role of Riok2 in hematopoiesis, we generated Vav1-Cre transgenic floxed Riok2 (Riok2fl/+Vav1cre) mice in which cre recombinase is under the control of the hematopoietic cell-specific Vav1 promoter. Riok2 floxed mice were generated with exons 5 and 6 flanked by loxP sites (Extended Data Fig. 1b,c). Notably, no Vav1-Cre floxed Riok2 homozygous-deficient mice (Riok2fl/flVav1cre) were recovered (Extended Data Fig. 1f), indicating embryonic lethality from complete hematopoietic deletion of Riok2. However, heterozygous Riok2fl/+Vav1cre mice were viable with approximately

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50% Riok2 mRNA expression in hematopoietic cells compared to that of Vav1+ controls (Extended Data Fig. 1e). As seen with other ribosomal protein haploinsufficiency mouse models, BM cells from Riok2−/− Vav1+/+ mice showed reduced nascent protein synthesis in vivo compared to Vav1+/+ controls (Extended Data Fig. 1g), consistent with a Riok2 role in maturation of the pre-40S ribosome. A recent study showed that ribosomal protein deficiency-mediated reduced protein synthesis predominantly affects erythropoiesis over myelopoiesis.

Consistent with the high expression of Riok2 in primitive CFU-e cells in the BM, aged (>60 weeks) mice with heterozygous deletion of Riok2 in hematopoietic cells (Riok2−/− Vav1+/+) displayed anemia with reduced peripheral blood (PB) RBC numbers, hemoglobin (Hb) and hematocrit (HCT) (Fig. 1a). We next determined whether Riok2 haploinsufficiency-mediated anemia was secondary to a defect in erythroid development in the BM, the major site of erythropoiesis. We characterized the stages (referred to here as RI, RII, RIII and RIV) of erythropoiesis by flow cytometry using the expression of Ter119 and CD71 (Extended Data Fig. 2a). Riok2−/− Vav1+/+ mice had impaired erythropoiesis in the BM (Fig. 1b and Extended Data Fig. 2b). Moreover, Riok2 haploinsufficiency led to increased apoptosis in erythroid precursors compared to controls (Fig. 1c). Additionally, Riok2−/− Vav1+/+ erythroid precursors showed a decrease in cell quiescence with cell cycle block at the G1 phase (Extended Data Fig. 2c). A block in cell cycle is driven by a group of proteins known as cyclin-dependent kinase inhibitors (CKIs). The expression of p21 (a CKI encoded by Cdkn1a) was increased in erythroid precursors from Riok2−/− Vav1+/+ mice compared to Riok2−/+ Vav1+/+ controls (Extended Data Fig. 2d).

We next examined the effect of Riok2 haploinsufficiency on stress-induced erythropoiesis using 8–12-week-old mice in which hemolysis was induced by nonlethal phenylhydrazine treatment (25 mg per kg body weight) on days 0 and 1. After acute hemolytic stress, Riok2−/− Vav1+/+ mice developed more severe anemia and had a delayed RBC recovery response compared to Riok2−/+ Vav1+/+ control mice (Fig. 1d) and succumbed faster to a lethal dose of phenylhydrazine (35 mg per kg on days 0 and 1) compared to Vav1+/+ controls (Extended Data Fig. 2e). The anemia in young phenylhydrazine-administered Riok2−/− Vav1+/+ mice seen on day 7 was preceded by a reduction in BM RIII and RIV erythroid precursor frequency on day 6, highlighting an erythropoiesis differentiation defect in Riok2-haploinsufficient mice (Fig. 1e and Extended Data Fig. 2f). In line with a role for Riok2 in driving erythroid differentiation, fewer CFU-e colonies were observed in erythropoietin-containing MethoCult cultures from Riok2−/− Vav1+/+ mice and Neutrophils (%)

Consequently, in Riok2−/− Vav1+/+ mice, we also observed an increased percentage of monocytes (ANOVA) with Tukey’s correction for multiple comparisons (d) was used to calculate statistical significance. *P < 0.05, **P < 0.01, ***P < 0.001. Data are shown as means±s.e.m and are representative of two (c,d,f-h) or three (a,b,e,i) independent experiments.

Riok2 haploinsufficiency increases myelopoiesis. In addition to the reduction in RBC numbers in PB from aged Riok2−/− Vav1+/+ mice, we also observed an increased percentage of monocytes.
Fig. 2 | Quantitative proteomics of Riok2-haploinsufficient erythroid precursors reveals immune activation signatures. a, Proteomic analysis of changes in protein expression in erythroid progenitors from Riok2-haploinsufficient mice and Vav1ffcre controls (n = 4–5 mice per group). b, Comparison of upregulated proteins with their respective P values and log fold-change values in erythroid precursors from Riok2-haploinsufficient mice and Rps14 haploinsufficient mice with their respective controls. c–d, GSEA performed on proteomics data shown in a to reveal similarity with Rps14 haploinsufficient data (c), activation of immune response (d) and enrichment of IL-22 signature genes (f). NES, normalized enrichment score; FDR, false discovery rate. e, MetaCore analysis of the Riok2 proteomics dataset shown in a. A two-sample moderated Student’s t-test with multiple hypothesis corrections was used to calculate statistical significance in b.

(monoctyosis) and decreased percentage of neutrophils (neutropenia) compared to controls (Fig. 1g and Extended Data Fig. 2i). Granulocyte–macrophage progenitors (GMPs) in the BM give rise to PB myeloid cells. The percentage of proliferating (Ki67+) GMPs in the BM was increased in Riok2ffcre mice compared to Riok2+/+Vav1ffcre controls (Fig. 1h and Extended Data Fig. 2j). To analyze the effect of Riok2 haploinsufficiency on myelopoiesis in the absence of in vivo compensatory mechanisms, we cultured LSK (lineage-c-kit+) cells from the BM of Riok2ff/ffcre mice and Riok2ff+/+cre controls (Fig. 2a). Notably, Riok2ff/ffcre mice gave rise to an increased percentage of CD11b+ myeloid cells (Fig. 1i and Extended Data Fig. 2k), suggesting a cell-intrinsic myeloproliferative effect due to Riok2 haploinsufficiency consistent with a myelodysplasia phenotype.

We also evaluated whether Riok2 haploinsufficiency affects early hematopoietic progenitors. Frequency and numbers of early hematopoietic progenitors were comparable between young Riok2ff/ffcre and Riok2ff+/+Vav1ffcre mice (Extended Data Fig. 3a); however, long-term hematopoietic stem cells (LT-HSCs) were increased in the BM of aged Riok2ff/ffcre mice (Extended Data Fig. 3a). To further corroborate these data, we analyzed the capacity of Riok2-haploinsufficient cells in a competitive transplantation assay. Starting at 8 weeks after tamoxifen treatment to induce Riok2 deletion, Riok2-haploinsufficient cells out-competed CD45.1+ competitor cells, whereas Ert2cre control cells had no competitive advantage (Extended Data Fig. 3b). Similarly to nontransplanted mice (Extended Data Fig. 3a), in competitive transplant experiments the frequency of Riok2-haploinsufficient LT-HSCs was significantly higher than Riok2-sufficient LT-HSCs in relation to competitor CD45.1+ cells (Extended Data Fig. 3c). Thus, in addition to its effect on erythroid differentiation, Riok2 haploinsufficiency increases myelopoiesis and affects early hematopoietic progenitor differentiation.

Reduced Riok2 induces alarmins in erythroid precursors. To elucidate a mechanism for the erythroid differentiation defect observed in Riok2ff/ffcre mice, we performed quantitative proteomic analysis of purified erythroid precursors using mass spectrometry. Riok2 haploinsufficiency led to upregulation of 564 distinct proteins (adjusted P value <0.05) in erythroid precursors compared to those from Vav1ffcre controls (Fig. 2a). Notably, Riok2 haploinsufficiency resulted in downregulation of other ribosomal proteins, the loss of some of which (RPS5 and RPL11) has been implicated in driving anemias (Extended Data Fig. 4a). The alarmins including S100A8, S100A9, CAMP, NGP and others were the most highly upregulated proteins in our dataset and notably, correlated significantly with those observed upon haploinsufficiency of Rps14 (ref. 19), another component of the 40S ribosomal complex (Fig. 2b). Using the 26 upregulated proteins in the Rps14-haploinsufficient dataset as an ‘Rps14 signature’ (Supplementary Table 1), gene set enrichment analysis (GSEA) revealed a marked enrichment for the Rps14 signature in the Riok2-haploinsufficient dataset, suggesting a shared proteomic signature upon deletion of distinct
riboosomal proteins (Fig. 2c). The increased expression of S100A8 and S100A9 in Riok2f+/− Vav1−/− mice was confirmed by flow cytometry and quantitative PCR with reverse transcription (qRT–PCR) (Extended Data Fig. 4b–e).

In Riok2f+/− Vav1−/− erythroid precursor cells, the upregulated proteins with the highest fold change (S100A8, S100A9, CAMP and NGP) are proteins with known immune functions such as antimicrobial defense. GSEA analysis of the proteomics data indicated a possible role for the immune system in driving the proteomic changes seen in Riok2-haploinsufficient erythroid precursors (Fig. 2d). An independent analysis of the Riok2 proteomics dataset using MetaCore pathway analysis software showed immune response as the top differentially regulated pathway in Riok2f+/− Vav1−/− mice (Fig. 2e).

To assess whether Riok2 haploinsufficiency leads to changes in immune cell function, we subjected naive T cells from Riok2f+/− Vav1−/− mice and Riok2f+/− Vav1−/− controls to in vitro polarization toward known CD4+ helper T cell lineages (T_{h}1, T_{h}2, T_{h}17, T_{h}22) and regulatory T (T_{reg}) cells. Secretion of interferon (IFN)-γ, IL-2, IL-4, IL-5, IL-13, IL-17A and the frequency of Foxp3+ T_{reg} cells was similar between Riok2f+/− Vav1−/− and Riok2f+/− Vav1−/− T cells (Extended Data Fig. 5a–g). Notably, however, we observed an exclusive increase in IL-22 secretion from Riok2f+/− Vav1−/− naive T cells polarized toward the T_{h}22 cell lineage (Fig. 3a). The frequency of IL-22+CD4+ T cells was higher in Riok2f+/− Vav1−/− T_{h}22 cell cultures compared to Vav1−/− control T_{h}22 cell cultures (Fig. 3b). The concentration of IL-22 in the serum and BM fluid (BMF) of aged Riok2f+/− Vav1−/− mice was also significantly higher compared to age-matched Vav1−/− controls (Fig. 3c).

Using known IL-22 target genes from the literature, we curated an "IL-22 signature" (Supplementary Table 1) gene set, which showed a statistically significant enrichment in the Riok2-haploinsufficient proteomics dataset using GSEA (Fig. 2f), further suggesting that IL-22-mediated inflammation is a contributing factor for Riok2 haploinsufficiency-mediated ineffective erythropoiesis and anemia. Increased numbers of splenic IL-22+CD4+ T cell, natural killer T (NKT) cell and innate lymphoid cells (ILCs) were observed in aged Riok2f+/− Vav1−/− mice compared to Riok2f+/− Vav1−/− mice (Fig. 3d and Extended Data Fig. 5h,i). Notably, mild anemia was observed in mice lacking Riok2 only in T cells (Extended Data Fig. 5k). Expression of IL-23, required for IL-22 production, was enhanced in Riok2-haploinsufficient dendritic cells (Extended Data Fig. 5j).
Rips14-haploinsufficient Tn122 cells also secreted elevated concentrations of IL-22 compared to Vav1+/+ control Tn122 cells (Extended Data Fig. 5l). Mutation(s) in the gene adenomatosis polyposis coli (Apc), also found on human chromosome 5q, lead to anemia in addition to adenomas21. In vitro generated Tn122 cells from Apch mice secreted elevated IL-22 levels compared to littermate controls (Extended Data Fig. 5m). In total, our analysis of three distinct heterozygous deletions of genes found on human chromosome 5q suggests that increased IL-22 is a generalized phenomenon observed upon heterozygous loss of genes found on chromosome 5q, leading to anemia.

p53 upregulation drives increased IL-22 secretion upon Riok2 loss. To identify cell-intrinsic molecular mechanism(s) driving the increase in IL-22 secretion upon Riok2 haploinsufficiency, we performed RNA-sequencing (RNA-seq) on in vitro polarized IL-22+ (Tn122) cells purified by flow cytometry from Riok2f/+/Il22+/+Vav1+/+ and Riok2f/−/Il22+/+Vav1+/+ mice (Fig. 3e). GSEA of the RNA-seq dataset identified activation of the p53 pathway in Riok2f/+/Vav1+/+ cells (Fig. 3f,g). p53 increase in Tn122 cells from Riok2f/+/Vav1+/+ mice was confirmed by flow cytometry (Fig. 3h,i). p53 upregulation was also observed in Riok2f/−/Vav1+/+ erythroid precursors (Extended Data Fig. 3f,g). The p53 pathway is activated by decreased expression of ribosomal protein genes34,35; however, its involvement in IL-22 regulation is not known.

p53 is a transcription factor with well-defined consensus binding sites. To assess whether p53 drives Il22 transcription, we analyzed the Il22 promoter for potential p53 binding sites using LASAGNA algorithm and found putative p53 consensus binding sequences in the Il22 promoter (Fig. 3j). Chromatin immunoprecipitation (ChIP) confirmed the presence of p53 on the Il22 promoter (Fig. 3k). In line with the ChIP data, p53 inhibition by pifithrin-α, p-nitro decreased IL-22 concentrations, whereas p53 activation by nutlin-3 increased IL-22 from in vitro polarized WT Tn122 cells (Fig. 3l,m). Treatment with either pifithrin-α, p-nitro or nutlin-3 did not decrease cell viability (Extended Data Fig. 5n). Accordingly, genetic deletion of Trp53 blunted the increase in IL-22 secretion observed upon Riok2 haploinsufficiency (Fig. 3n). A significant decrease in IL-22 secretion was also observed upon Trp53 deletion in Riok2-sufficient cells further suggesting a homeostatic role for p53 in controlling IL-22 production (Fig. 5n). Taken together, these data show that Riok2 haploinsufficiency-mediated p53 upregulation drives increased IL-22 secretion in Riok2f/−/Vav1+/+ mice.

IL-22 neutralization alleviates stress-induced anemia. Mice with compound genetic deletion of Il22 on the Riok2-haploinsufficient background (Riok2f/−/Il22+/+Vav1+/+) exhibited increased numbers of PB RBCs compared to IL-22-haploinsufficient mice on day 7 after two treatments with 25 mg per kg phenylhydrazine treatment (Fig. 4a). Interestingly, we also saw an increase in PB RBCs in Riok2-sufficient mice heterozygous for Il22 deletion (Riok2f/−/Il22+/+Vav1−/+), compared to RI22-sufficient IL-22 mice (Riok2f/−/Il22+/+Vav1−/+). PB Hb and HCT also were increased in Il22-haploinsufficient mice, regardless of Riok2

**Fig. 4** IL-22 neutralization alleviates stress-induced anemia in Riok2-sufficient and -haploinsufficient mice. a, PB RBC numbers, Hb and HCT in the indicated strains undergoing PhZ-induced stress erythropoiesis (n=6, 5, 5 and 5 mice for Riok2f/−/Il22+/+Vav1−/+ and Riok2f/−/Il22+/+Vav1+/+ mice, respectively). b, Frequency of erythroid progenitor/precursor populations among viable BM cells in the indicated strains undergoing PhZ-induced stress erythropoiesis (n=4–5 per group). c, PB RBC numbers, Hb and HCT in Riok2f/−/Vav1−/+ and Riok2f/−/Vav1+/+ mice undergoing PhZ-induced stress erythropoiesis treated with either an isotype control or anti-IL-22 antibody (n=4–5 per group). d, Frequency of apoptotic erythroid precursors among viable BM cells in Riok2f/−/Vav1−/+ and Riok2f/−/Vav1+/+ mice undergoing PhZ-induced stress erythropoiesis treated with either an isotype control or anti-IL-22 antibody (n=4, 5, 4 and 5 mice for isotype-treated Riok2f/−/Vav1−/+; anti-IL-22-treated Riok2f/−/Vav1−/+; iso-type-treated Riok2f/−/Vav1+/+ and anti-IL-22-treated Riok2f/−/Vav1+/+ mice, respectively). One-way ANOVA with Tukey’s correction for multiple comparison (a-d) was used to calculate statistical significance. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Data are shown as mean±s.e.m and are representative of two (c,d) or three (a,b) independent experiments.
Fig 5] Recombinant IL-22 exacerbates PhZ-induced anemia in WT mice. a, PB RBC numbers, Hb and HCT in WT C57BL/6J mice administered PBS (n = 5) or rIL-22 (n = 4) and subsequently treated with PhZ (n = 4–5 mice per group). b, PB reticulocytes in mice treated as in a (n = 4 mice per group). c, Percentage of RII–RIV erythroid precursors in the BM of PBS- or rIL-22-treated C57BL/6J mice 7 d after PhZ administration (n = 4 mice per group). d, Percentage of apoptotic RII erythroid precursors in mice treated as in c (n = 4 mice per group). e, f, Effect of recombinant IL-22 (500 ng ml⁻¹) on the frequency (left) and cell number (right) in an in vitro erythropoiesis assay (e) and dose-dependent effect of recombinant IL-22 (f) (n = 5 and 4 for PBS and IL-22 groups, respectively). g, p53 expression in in vitro erythropoiesis culture treated with rIL-22 or PBS (n = 5 and 4 mice for PBS and IL-22 groups, respectively). Data are shown as mean ± s.e.m and are representative of three (a,b) or two (c-g) independent experiments. Unpaired two-tailed Student’s t-test (a,d,g), multiple unpaired two-tailed Student’s t-tests with Holm–Sidak method (e) and one-way ANOVA with Tukey’s correction for multiple comparisons (f) were used to calculate statistical significance. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

background; however, this difference did not reach statistical significance (Fig. 4a). Next, we assessed whether the increase in PB RBCs in Rik2f⁻/⁻Il22f⁻/⁻Vav1f⁺ mice was due to increased erythropoiesis in the BM of these mice. We observed an increase in RII and RIV erythroid precursors in Rik2f⁻/⁻Il22f⁻/⁻Vav1f⁺ compared to Rik2f⁺/⁺Il22f⁺/⁺Vav1f⁻ mice (Fig. 4b and Extended Data Fig. 6a). Treatment of mice with a neutralizing IL-22 antibody in vivo, also reversed phenylhydrazine-induced anemia as evidenced by increase in PB RBCs, Hb and HCT in in vitro erythropoiesis culture treated with rIL-22 or PBS (n = 5 and 4 mice for PBS and IL-22 groups, respectively). Data are shown as mean ± s.e.m and are representative of three (a,b) or two (c-g) independent experiments. Unpaired two-tailed Student’s t-test (a,d,g), multiple unpaired two-tailed Student’s t-tests with Holm–Sidak method (e) and one-way ANOVA with Tukey’s correction for multiple comparisons (f) were used to calculate statistical significance. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Erythroid precursors express IL-22RA1 receptors. IL-22 signals through a cell surface heterodimeric receptor composed of IL-10Rβ and IL-22RA1 (encoded by Il22ra1)33. IL-22RA1 expression has been reported to be restricted to cells of nonhematopoietic origin (for example, epithelial cells and mesenchymal cells). We discovered, however, that erythroid precursors in the BM also express IL-22RA1 (Fig. 6a and Extended Data Fig. 8a). Moreover, we observed that among BM hematopoietic progenitors, IL-22RA1-expressing cells were exclusively of the erythroid lineage (Extended Data Fig. 8b). Using a second IL-22RA1-specific antibody (targeting a different epitope than the antibody used in Fig. 4a), we confirmed the presence of IL-22RA1 on erythroid precursors (Extended Data Fig. 8c). Il22ra1 mRNA expression was detected exclusively in erythroid precursors among all lineage-negative cells in the BM (Extended Data Fig. 8d).

Deletion of Il22ra1 in Rik2f⁻/⁻Il22ra1f⁻/⁻Vav1f⁺ mice led to improvement in PB RBCs and HCT compared to IL-22RA1 sufficient Rik2f⁻/⁻Il22ra1f⁺/⁺Vav1f⁻ mice (Fig. 6b). This improvement could be attributed to the increase in RII and RIV erythroid precursors in the BM of Rik2f⁻/⁻Il22ra1f⁻/⁻Vav1f⁺ mice (Fig. 6c and Extended Data Fig. 6c).

IL-22 worsens stress-induced anemia in wild-type mice. Phenylhydrazine administration to WT C57BL/6J mice treated intraperitoneally with recombinant IL-22 (rIL-22) led to decreased PB RBCs, Hb and HCT owing to decreased BM erythroid precursor cell frequency and number (Fig. 5a,c and Extended Data Fig. 6b). rIL-22 treatment led to increased apoptosis of erythroid precursors (Fig. 5d). This treatment also led to an increase in PB reticulocytes, an indication of increased erythropoiesis under stress (Fig. 5b). Recombinant IL-22 also dose-dependently decreased terminal erythropoiesis in an in vitro erythropoiesis assay (Fig. 5e,f). Notably, IL-22-mediated inhibition of in vitro erythropoiesis led to induction of p53 suggesting a feedback loop between IL-22 and p53 in driving dyserythropoiesis (Fig. 5g). Overall, these data show that exogenous recombinant IL-22 exacerbates stress-induced anemia in WT mice.

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In accordance with the upregulation of p53 upon in vitro IL-22 stimulation in an in vitro erythropoiesis assay (Fig. 5g) and p53 upregulation in erythroid precursors upon Rik2 haploinsufficiency (Extended Data Fig. 4f,g), we observed a synergistic effect of Rik2 haploinsufficiency in IL-22-responsive (IL-22RA1⁺)
IL-22-independent in other subtypes of MDSs. In a second cohort of patients with non-del(5q) MDS (Fig. 8d), a strong negative correlation between cellular \( \text{RIOK2} \) messenger RNA expression and BMF IL-22 concentration was evident in the del(5q) MDS cohort (Fig. 8b), indicating that a decrease in \( \text{RIOK2} \) expression is associated with increased IL-22 expression. In our MDS cohort, S100A8 concentrations were found to be higher than healthy controls regardless of del(5q) status (Fig. 8c). However, IL-22 positively correlated with S100A8 concentrations in the del(5q) MDS group (Fig. 8d). Of note, S100A8 concentrations were higher in BMF from patients with non-del(5q) MDS compared to those with del(5q) (Fig. 8c). These data suggest that the regulation of S100A8 expression may be IL-22-mediated in patients with del(5q) MDSs, but IL-22-independent in other subtypes of MDSs. In a second cohort of patients with MDSs, the frequency of CD4+ T cells producing IL-22 (T\( _i \)22 cells) among freshly isolated peripheral blood mononuclear cells (PBMCs) was significantly higher in patients with MDSs with 5q deletion compared to healthy controls (Fig. 8c; cumulative data of representative flow plots shown in Extended Data Fig. 9a). Our independent analysis of a large-scale microarray sequencing dataset of CD34+ cells from healthy controls and patients with del(5q) MDSs
and non-del(5q) MDSs showed that RIOK2 mRNA was significantly decreased in the del(5q) MDS cohort (78% (37/47)). Additionally, expression of known IL-22 target genes such as S100A10, S100A11, PTGS2, RAB7A and LCN2 was specifically increased in the del(5q) MDS cohort compared to both the healthy control and non-del(5q) groups (Extended Data Fig. 9b). Using differentially expressed proteins (adjusted P value <0.01) from the Rock2-haploinsufficient proteomics dataset as a reference set, GSEA of the CD34+ microarray dataset revealed significant enrichment scores (Extended Data Fig. 10a,b) further suggesting that the mouse model of Rock2 haploinsufficiency faithfully recapitulates the molecular changes seen in patients with del(5q) MDSs.

High IL-22 in anemic patients with chronic kidney disease. Anemia is frequently observed in patients with chronic kidney disease (CKD) and is associated with poor outcomes. Anemia of CKD is resistant to erythropoiesis-stimulating agents in 10–20% of patients, suggesting that pathogenic mechanisms other than erythropoietin deficiency are at play. We found a significant increase in IL-22 concentration in the plasma of patients with CKD compared to healthy controls and to patients with CKD without anemia (Fig. 8f). Plasma IL-22 concentration negatively correlated with hemoglobin in patients with CKD (Fig. 8g), suggesting a function for IL-22 in driving anemia in some patients with CKD.

Discussion

The overall conclusion of this study is that IL-22 signaling directly controls BM erythroid differentiation and that its neutralization is a potential therapeutic approach for anemias and MDSs. By exploring the function of a little-studied atypical kinase Rock2 in mammalian biology, we identified the erythroid precursors as a new target for IL-22 action via IL-22RA1. We further identified IL-22 as a disease biomarker for the del(5q) subtype of MDSs and last, we identified IL-22 signaling blockade as a potential therapeutic for stress-induced anemia in a much wider patient population.

Del(5q), either isolated or accompanied by additional cytogenetic abnormalities, is the most commonly detected chromosomal abnormality in MDSs, reported in 10–15% of patients and enriched in therapy-related MDSs. The severe anemia in patients with MDSs with isolated del(5q) has been linked to haptolinsufficiency of ribosomal proteins such as RPS14 (ref. 39) and RPS19 (ref. 40). While much research has focused on the effect of such gene deletions or mutations in hematopoietic stem cells and lineage-committed progenitors, the immunobiology underlying this MDS subtype has remained largely unexplored, thus impeding the development of immune-targeted therapies. With the exception of the tumor necrosis factor-α inhibitor etanercept, which proved to be ineffective, the only other therapy against immune cell-derived cytokines is luspatercept, a recombinant fusion protein derived from human activin receptor type Ib, which has only been approved for use in anemia in lower risk patients with MDSs. Here, we identify two critical and independent functions of an understudied atypical kinase, Rock2, that synergize to induce dyserythropoiesis and anemia. One effect of Rock2 loss in erythroid precursors is an intrinsic block in erythroid differentiation owing to its indispensable role in the maturation of the pre-40S ribosomal complex, leading to increased apoptosis and cell cycle arrest. The second effect of Rock2 loss is the induction of the erythropoiesis-suppressive cytokine IL-22 in T cells, which then directly acts on the IL-22RA1 on erythroid precursors (Extended Data Fig. 10c). While IL-22RA1 is known to be widely expressed on epithelial cells and hepatocytes, its expression has also been recently reported on specialized cells such as retinal Müller glial cells and now described here, on erythroid precursors. Our data reveal a new molecular link between haptolinsufficiency of a ribosomal protein and induction of erythropoiesis-suppressive cytokine IL-22. While IL-22 has been shown to modulate RBC production by controlling the expression of iron-chelating proteins such as hepcidin and haptoglobin, we have uncovered a new role for IL-22 in directly binding to the previously unknown IL-22R on erythroid precursors leading to their apoptosis. Diminished expression of ribosomal proteins has been shown to increase p53 levels. We show here that Rock2 haploinsufficiency leads to p53 upregulation in T cells, which drives the increase in IL-22 secretion. Additionally, we also show that IL-22-responsive erythroid precursors express elevated p53, further suggesting a role for p53 downstream of IL-22 signaling in driving dyserythropoiesis. Using banked and fresh del(5q) MDSs and samples from patients with CKD, we also show that IL-22 is elevated in these human diseases.

The role of inflammatory cytokines in directly regulating various aspects of BM erythropoiesis in steady-state and diseased conditions is increasingly being recognized. IL-22 is known to play a pathogenic role in some autoimmune diseases. Interestingly,
autoimmune diseases such as colitis, Behçet's disease and arthritis are common in patients with MDSs, with features of autoimmunity observed in up to 10% of patients\(^\text{9,10}\). It is intriguing to hypothesize that IL-22 may account both for the onset of MDSs and autoimmunity in this subset of patients. Studies have reported that in patients with coexistence of MDSs and autoimmunity, treatment for one can alleviate the symptoms of the other\(^\text{11,12}\). Low-level exposure to benzene, a hydrocarbon, has been associated with an increased risk of MDSs\(^\text{13}\). Hydrocarbons are known ligands for aryl hydrocarbon receptor (AHR), the transcription factor that controls IL-22 production in T cells. Stemregenin 1, an AHR antagonist, was shown to alleviate the symptoms of the other\(^\text{14,15}\). Low-level exposure to benzene, a hydrocarbon, has been associated with an increased risk of MDSs\(^\text{16}\).

Further, we provide evidence that neutralization of IL-22 signaling may be effective not only in the treatment of MDSs and other stress-induced anemias, but also in the anemia of chronic diseases such as CKD, which are very much in need of new therapeutic approaches. With currently approved MDS therapies (lenalidomide and other hypomethylating agents, erythropoiesis-stimulating agents), the survival time of patients with MDSs after diagnosis is only 2.5–3 years. Patients also develop resistance to these therapies, thus intensifying the need for additional therapeutic modalities. IL-22-based therapies could be used in conjunction with already existing therapeutics or after first-line therapies have failed due to acquisition of resistance.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at [https://doi.org/10.1038/s41590-021-00895-4](https://doi.org/10.1038/s41590-021-00895-4).

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Methods

Human samples and processing. Samples from patients with MDs and CKD were collected under institutional review board-approved protocols at Dana-Farber Cancer Institute (DFCI) and Brigham and Women’s Hospital, respectively. All samples were de-identified at the time of study. All individuals provided informed consent and data collection was performed in accordance with the Declaration of Helsinki.

PBMCs from EDTA-treated whole blood were isolated using density gradient centrifugation. PBMCs were then incubated in RPMI with 10% FBS and Cell Activation Cocktail (Tonbo Biosciences) for 4 h and then processed for flow cytometry as described below. Relevant clinical information of MDS samples is provided in Supplementary Tables. Adult CKD plasma samples were stored at −80°C until further use. Relevant clinical information of CKD samples is provided in Supplementary Table 3.

Generation of Rik2 floxed mice. Rik2flox mice were generated using Cre recombinase obtained from Mutant Mouse Resource and Research Centers (Rik2flox;KOMP10838) in C57BL/6J background. A conditional allele was engineered with Cre dependent lox-stop-lox cassette flanked by Cre recombinase recognition sites. A cre driver strain to create conditional Rik2-deleted mice (Extended Data Fig. 1a, b). Genotyping (Extended Data Fig. 1c) was carried out using the following primers:

Forward primer: 5′ CCATAGCTGATTACGACATAATGGC 3′
Reverse primer: 1′ GCCTTTCACTCAGTCTACTCAC 3′
Reverse primer 2: 5′ CCCAGACTCTTGTTAGGTCTGGC 3′

Mice. WT C57BL/6J mice (stock no. 000664), C57BL/6J Foxp3CreERT2 (stock no. 006463), C2C12-lux-cre (stock no. 031003), C57BL/6J mice (stock no. 000214), Trp53flox (stock no. 002101), Cd4Cre (stock no. 022071) and ApcFrt (stock no. 000220) were purchased from the Jackson Laboratory. I22+ mice were provided by R. Caspi (National Institutes of Health (NIH)) with permission from Genentech. EpoCre+ were a gift from U. Klingmuller (Deutsches Krebsforschungszentrum). I22+ idomato (Catch-22) mice were a gift from R. Lockley (University of California San Francisco). Rp14flox mice were a gift from B. Ebert (DFCI). Mice were housed in the Animal Research Facility at DFCI under ambient temperature and humidity with 12-h light/12-h dark cycle. Animal procedures and treatments were in compliance with the guidelines set forth by the Institutional Animal Care and Use Committee at DFCI. Age- and sex-matched mice were used within experiments.

Competitive BM transplantation. Overall, 2 × 10^6 freshly isolated BM cells from CD45.2+ Rik2flox or Rik2flox mice were transplanted in competition with 2 × 10^6 freshly isolated CD45.1+ WT BM cells via retro-orbital injection into lethally irradiated 8–10-week-old CD45.1+ WT recipient mice. The donor cell chimerism was determined in PB 4 weeks after transplantation before excision of Rik2 was induced by tamoxifen injection as well as every 4–8 weeks as indicated. Tamoxifen (75 mg per kg, i.p.) was administered as a single dose every 4 weeks before transplantation and tamoxifen was well tolerated with staining buffer to collect cells and then processed for flow cytometry as described above. Enumeration of colonies in MesoCult medium was performed with a StemVision instrument (StemCell Technologies).

Phenylhydrazine treatment. Ph2 was purchased from Sigma and injected intraperitoneally on 2 consecutive days (days 0 and 1) at a dose of 25 mg per kg (sublethal model) or 35 mg per kg (lethal model). PB was collected 3–4 days before the start of treatment and at day 4, 7 and 11. Ph2 treatment experiments were carried out in 8–12-week-old mice.

T cell polarization. Single-cell suspensions of mouse spleens were prepared by pressing tissue through a 70-μm filter and then dissociated in PBS with 3% FBS and 0.1% saponin. The 250–500 BM Lin–c-kit+ cells were sorted using a FACSAria flow cytometer (BD Biosciences). Phenotype of positive cells was verified by exclusion of dead cells using either DAPI or a fixable viability dye (Tonbo Bioscience). Gating for early and committed hematopoietic progenitors was performed as described elsewhere36. ELGs and NKT cells were identified as Lin–CD45+CD90+CD122+ and CD3ε+ NK1.1+, respectively.

Complete blood count. Mice were bled via the submandibular facial vein to collect blood in EDTA-coated tubes (BD Microtainer Capillary Blood Collector, BD 365974). Complete blood counts were obtained using the HemaVet CBC Analyzer (Drew Scientific) or Advia 120 (Siemens) instruments.

In vivo measurement of protein synthesis. A total of 100 μl of a 20 mM solution of O-Propargyl-Purinomycin (OP-Puro; BioMol) was injected intraperitoneally in mice and mice were then rested for 1 h. Mice injected with PBS were used as controls. BM was collected after 1 h and stained with antibodies against cell surface markers, washed to remove excess unbound antibodies, fixed in 1% paraformaldehyde and permeabilized in 0.1% saponin. The azide-alkyne cyclo-addition was performed using the Click-iT Cell Reaction Buffer kit (Thermo Fisher Scientific) and azide conjugated to Alexa Fluor 647 (Thermo Fisher Scientific) at 5 μM final concentration for 30 min. Cells were washed twice and analyzed by flow cytometry. ‘Relative rate of protein synthesis’ was calculated by normalizing OP-Puro signals to whole BM after subtracting autofluorescence.

Methylcellulose assay. The 250–500 BM Lin–c-kit+Sca-1− cells were flow sorted and plated in semi-solid methylcellulose culture medium (M5354, StemCell Technologies) and incubated at 37°C in a humidified atmosphere for 7–10 d. At the end of the incubation period, wells were trypsinized with staining buffer, collected cells and then processed for flow cytometry. ‘Relative rate of protein synthesis’ was calculated by normalizing OP-Puro signals to whole BM by subtracting autofluorescence.

IL-22 neutralization and reconstitution. Monoclonal anti-IL-22 (clone IL22JOP) blocking antibody and isotype control IgG2a (clone eB2R2a) were purchased from Thermo Fisher Scientific. Mice were administered anti-IL-22 (50 μg per mouse) or isotype intraperitoneally every 4 h until the conclusion of the experiment. For recombination IL-22−treatment, mice were injected with recombiant IL-22 (500 ng per mouse; PeproTech) intraperitoneally every 24 h until the conclusion of the experiment. Mice were administered these reagents at least five times before inducing Phz-mediated anemia.

Cytokine quantitation. IL-22 in human samples was quantified using either Human IL-22 ELISA (D2200) or Single Molecule Counting (SMC) Human IL-22 High Sensitivity Immunoassay kit (EMD Millipore, 03-0162-00) according to manufacturers’ instructions. The SMC assay was read on a SMC Pro (EMD Millipore) instrument. The lower limit of quantification of this immunoassay is 0.1 pg/ml. IL-22 in mouse samples was quantified using ELISA MAX Deluxe Set Mouse IL-22 (BioLegend, L36054). The lower limit of quantitation of this immunoassay is 3 pg/ml. S100A8 in human samples was quantified using Human S100A8 DuoSet ELISA (DY4570, R&D Systems).

Concentration of lineage-associated cytokines in cell culture supernatants of polarized T cells were quantified using a custom-made colorimetric assay from Hecipdin Murine-Compete ELISA kit from Intrinisic LifeSciences (HCMC-001).

mRNA quantitation. Cells were flow sorted directly into the lysing buffer provided with the Cells-to-CT 1-Step TaqMan kit (Thermo Fisher Scientific, A25605) and processed according to the manufacturer’s instructions. Pre-designed TaqMan
gene expression assays were used to quantify mRNA expression by qPCR using QuantStudio 6 (Thermo Fisher Scientific). Hprt was used as housekeeping control. Relative expression was calculated using the ΔΔct method. Supplementary Table 4 describes primer details.

Chromatin immunoprecipitation. ChIP was performed using EZ-ChIP kit (EMD Millipore) according to manufacturer’s instructions. Briefly, cells were fixed and cross-linked with 1% formaldehyde at 25 °C for 10 min and quenched with 125 mM glycine for an additional 10 min. Cell pellets were resuspended in lysis buffer and shearing was carried out using the Diagenode Bioruptor sonication system for a total of 40 cycles. Pre-cleared lysates were incubated with control Mouse IgG or anti-p53 (Santa Cruz Biotechnology) antibodies. The i22 promoter-specific primer pair was designed using Primer 3.0 Input as follows: Forward primer: 5’ CCAAACTTAACCTGACCTGGCC 3’ Reverse primer: 5’ TTCTTCAGACCTCCCA TTGC 3’

In vitro erythroid differentiation. Whole BM cells were labeled with biotin-conjugated lineage antibodies (cocktail of anti-CD3e, anti-CD11b, anti-CD45RB/B20, and anti-CD44 antibodies) and purified using anti-biotin beads and negative selection on the AutoMACS Pro (Miltenyi). Purified cells were then seeded in fibronectin-coated (2 μg/cm²) tissue-culture-treated polysyrene wells (Corning BioCoat Cellware) at a cell density of 10⁶/mL. Erythroid differentiation was carried out according to modified published protocols8. The erythropoietic medium was IMDM supplemented with erythropoietin at 10 U/mL, 10 ng/mL stem cell factor (PeproTech), 10 μM dexamethasone (Sigma-Aldrich), 15% FBS, 1% detoxified BSA (Stem Cell Technologies), 200 μg/mL holotransferrin (Sigma-Aldrich), 10 mg/mL human insulin (Sigma-Aldrich), 2 mM L-glutamine, 0.1 mM L-tryptophan and penicillin-streptomycin. After 48 h, the medium was replaced by IMDM containing 20% FBS, 1% L-glutamine, 0.1 mM L-tryptophan and penicillin-streptomycin. Then, 50% of the culture medium was replaced after 48 h and cell density was maintained at 0.5 × 10⁶/mL. The total culture period for the assay was 6 d. Recombinant mouse IL-22 (PeproTech/Cell Signaling) was used where indicated. R1I–R1V populations were gated as shown in Extended Data Fig. 2a.

Proteomic profiling. Proteomic profiling of sort-purified erythroid progenitors was performed as described elsewhere9,10. Briefly, cells were captured in collection microreactors and stored at ~80 °C. Cell lysis was performed by adding 10 μL of 8 M urea, 10 mM TCEP and 10 mM dodecylmaltoside in 50 mM ammonium bicarbonate to the cell pellet of 1 × 10⁶ erythroid progenitors and incubated at room temperature for 30 min, shaking in the dark. Then, 50 mM ammonium bicarbonate was used to dilute the urea to <2 M and the appropriate amount of trypsin for a 1:100 enzyme to substrate ratio was added and allowed to incubate at 37 °C overnight. Once digestion was completed, the lysate was spun through the glass mesh directly onto a C18 Stage tip (Empore)11 at 3,500 g until the entire digest passed through the C18 resin. Then, 75 μL 0.1% formic acid (FA) was used to ensure transfer of peptides to the C18 resin from the mesh while washing away buffer components. C18-bound peptides were immediately subjected to on-column tandem mass tag (TMT) labeling.

On-column TMT labeling. Resin was conditioned with 50 μL methanol, followed by 50 μL 50% acetonitrile (ACN)/0.1% FA and equilibrated with 75 μL 0.1% FA twice. The digest was loaded by spinning at 3,500 g until the entire digest passed through. One microliter of TMT reagent in 100% ACN was added to 100 μL freshly made HEPES (pH 8) and passed over the C18 resin at 350 g until the entire solution passed through. HEPES and residual TMT was washed away with two applications of 10 μL of 0.1% FA and peptides were eluted with 50 μL 50% ACN/0.1% FA followed by a second elution with 50% ACN/20 mM ammonium formate (NH₄HCO₃), pH 10. Peptide concentrations were estimated using an absorbance reading at 280 nm and checking of label efficiency was performed on 1/20th of the elution. After using 1/20th of the elution to test for labeling efficiency, the samples were mixed before fractionation and analysis.

Stage tip SDS-B fractionation. First, 200-μl pipette tips were packed with two punches of sulfonated divinylbenzene (SDB-RPS; Empore) with a 16-gauge needle. After loading ~20 μg peptides in total, a pH switch was performed using 25 μL 20 mM NH₄HCO₃, pH 10 (pH 10) and was considered part of fraction one. Then, step fractionation was performed using ACN concentrations of 5, 7.5, 10, 12.5, 15, 17.5, 20, 25, 42 and 50%. Each fraction was transferred to autosampler vials and dried via vacuum centrifugation and stored at 80 °C until analysis.

Data acquisition. Chromatography was performed using a Proxeon UHPLC at a flow rate of 200 μL/min. Peptides were separated at 50°C using a 75-μm i.d. PicoFrit (New Objective) column packed with 1.9 μm AQ-C18 material (Dr. Maisch, Germany) to 20 cm in length over a 110-min run. The on-line LC gradient started from 10% to 30% B in 85 min, followed by an increase to 60% B by 94 min, then to 90% by 95 min and finally to 100% B until the end of the run. Mass spectrometry (MS) was performed on a Thermo Scientific Lumos Trilobid mass spectrometer. After a precursor scan from 350 to 1,800 m/z at 60,000 resolution, the topmost intense multiply charged precursors in a 2-s window were selected for higher energy collisional dissociation at a resolution of 50,000. Precursor isolation width was set to 0.7 m/z and the maximum MS2 injection time was 10 ms for an automatic gain control of 6 × 10⁶. Dynamic exclusion was set to 45 s and only charge states two to six were selected for MS2. Half of each fraction was injected for each data acquisition run.

Data processing. Data were searched all together with Spectrum Mill (Agilent) using the Uniprot Mouse database (28 December 2017), containing common laboratory contaminants and 553 smtOFS. A fixed modification of carbamidomethylation of cysteine and variable modifications of N-terminal protein acetylation, oxidation of methionine and TMT-11plex labels were searched. The enzyme specificity was set to trypsin and a maximum of three missed cleavages was used for searching. The maximum precursor-ion charge state was set to six. The MS1 and MS2 mass tolerance were set to 20 ppm. Peptide and protein FDRs were calculated to be <1% using a reverse, decoy database. Proteins were only reported if they were identified with at least two distinct peptides and a Spectrum Mill score protein level score ≥ 20.

TMT11 reporter ion intensities in each MS/MS spectrum were corrected for isotopic impurities by the Spectrum Mill protein/peptide summary module using the aRICA correction method which implements deterministic calculations according to Cramer’s rule and general correction factors obtained from the reagent manufacturer’s certificate of analysis.

Differential protein abundance analysis. The median normalized, median absolute deviation-scale dataset was subjected to a moderated F-test, followed by Benjamini–Hochberg procedure correcting for multiple hypothesis testing. We drew an arbitrary cutoff at adjusted P < 0.05.

RNA sequencing. A total of 5,000 IL-22‘CD4HIIL-22‘(dihematopoietic) cells were FACs sorted directly into TLC Buffer (QIAGEN) with 1% β-mercaptoethanol. For the preparation of libraries, cell lysates were thawed and RNA was purified with 2.2× RNAClean SPRI beads (Beckman Coulter Genomics) without final elution12. The RNA captured beads were air-dried and processed immediately for RNA secondary structure denaturation (72°C for 3 min) and complementary DNA synthesis. SMART-seq2 was performed on the resultant samples following the published protocol13 with minor modifications in the reverse transcription step. A 15-μL reaction mix was used for subsequent PCR and performed ten cycles for cDNA amplification. The amplified cDNA from this reaction was purified with 0.8X Ampure SPRI beads (Beckman Coulter Genomics) and eluted in 21 μL TE buffer. We used 0.2 ng cDNA and one-eighth of the standard Illumina NexteraXT (Illumina FC-131-1096) reaction volume to perform both the tagmentation and PCR indexing steps. Uniquely indexed libraries were pooled and sequenced with NextSeq 500 high output V2 75 cycle kits (Illumina FC-404-2005) and 38×38 paired-end reads on the NextSeq 500 instrument. Reads were aligned to the mouse mm10 transcriptome using Bowtie14 and expression abundance transcripts per million million estimates were obtained using RSEM15.

Pathway analysis. GSEA was performed with Broad Institute’s GSEA Software16. The ‘IL-22_Signature’ and ‘Rps14_Increased’ gene sets were created from the literature17,18 (Fig. 2c,f). Full lists of genes in the individual gene sets can be found in Supplementary Table 1. Other complementary gene sets are available from MSigDB19. For GSEA analyses, mouse Uniprot IDs were converted to their orthologous human gene symbols using MSigDB 7.1 ChIP file mappings. Pathway enrichment (Fig. 2e) was performed using Clariavite Analytics’ MetaCore software20.

Microarray data analysis. Microarray data of CD34+ cells from healthy controls and patients with del(5q) and non-del(5q) MDSs were obtained from a previously published study21 submitted in Gene Expression Omnibus22 accessible under GSE19429.

Statistical tests. Data are presented as mean ± s.e.m. unless otherwise indicated. Comparison of two groups was performed using paired or unpaired two-tailed Student’s t-test. For multiple group comparisons, ANOVA with Tukey’s correction or Kruskal–Wallis test with Dunn’s correction was depending on data requirements. Statistical analyses were performed using GraphPad Prism v.8.0 (GraphPad Software). A p value <0.05 was considered significant.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The original mass spectra may be downloaded from MassIVE (http://massive.ucsd.edu), MSV000085287. The data are directly accessible via ftp://massive.ucsd.edu/MSV000085287. Raw RNA-seq data are accessible via Gene Expression Omnibus under accession code GSE165467. Source data for all applicable figures (main and extended) are provided with the paper. The remaining data supporting the findings of this study are available from the corresponding authors upon reasonable request. Materials will be provided with material transfer agreements as appropriate. Source data are provided with this paper.
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Author contributions

M.R. and L.H.G. conceived the study, designed experiments, analyzed the data and wrote the manuscript; M.R. performed the experiments; S.G. assisted with in vitro experiments; S.A.M. performed proteomic profiling and data analysis; M.S.C. performed RNA-seq; M.S. processed RNA-seq data; S.A.C. analyzed proteomic profiling data; S.S.W. and J.V.B. collected CKD patient samples and related clinical information and analyzed the data; J.R., R.M.S. and D.P.S. collected MDs patient samples and related clinical information and analyzed the data; and A.R. supervised RNA-seq experiments and analyzed data.

Competing interests

An invention disclosure has been filed based on the data generated in this study. From 4 August 2020, Meromit Singer has been an employee of Guardian Health. S.S.W. has served on the steering committee of a GSK trial on an oral hypoxia-inducible factor prolyl hydroxylase inhibitor, as a potential treatment for anemia associated with CKD; S.S.W. has also received consulting fees from Public Health Advocacy Institute, CVS, Roth Capital Partners, Kantum Pharma, Mallinckrodt, Wolters Kluwer, GE Health Care, Allena Pharmaceuticals, Mass Medical International, J.N., Venbbo, Strata, Takeda, Celus and Pfizer. D.P.S. has served on independent data safety monitoring committees for clinical trials supported by Takeda, Astex, Jansen and Onconova; has consulted for Celgene and Daiichi Sankyo; and has received research support (to the institution) for clinical trials sponsored by Aprea, H3 Biosciences, Sysco and Astra Zenea. J.R. reports research funding from Amgen, Equilibrium and Kite Pharma; and consulting income from Aleta Biotherapeutics, Arrboisio, Celgene, Falcon Therapeutics, LifeVault Bio, Rheo Medicines, Taliras Therapeutics and TScan Therapeutics. R.M.S. has served on independent data safety monitoring committees for trials supported by Celgene, Takeda and Argenx; has consulted for AbbVie, Actinimum, Agios, Amgen, Arog, Astellas, Astra Zenea, Biolnerx, Celgene, Daiichi Sankyo, Fujifilm, Janssen, Juno, Macrogenics, Novartis, Ono, Oresnis, Pfizer, Roche, Stemline, Sumitomo, Takeda and Trovagen; and has received research support (to the institution) for clinical trials sponsored by AbbVie, Agios, Arog and Novartis. S.A.C. is a member of the scientific advisory boards of Kymera, PTM BioLabs and Seer and is a scientific advisor to Pfizer and Biogen. A.R. is a Scientific Advisory Board member of Thermo Fisher Scientific, Neogene Therapeutics, Asimov and Syros Pharmaceuticals. A.R. is a cofounder of and equity holder in Celsius Therapeutics and an equity holder in Immunitas. From 1 August 2020, A.R. has been an employee of Genentech. L.H.G. is a former Director of Bristol-Myers Squibb and the Waters Corporation and is currently on the board of directors of and holds equity in GlaxoSmithKline Pharmaceuticals and Analog Devices. She also serves on the scientific advisory boards of Repare Therapeutics, Abpro Therapeutics and Kaleido Therapeutics. S.A.C. is a member of the scientific advisory boards of Kymera, PTM BioLabs, and Seer and is a scientific advisor to Pfizer and Biogen. All other authors declare no competing interests.

Additional information

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Extended Data Fig. 1 | Localization and expression of Riol2. **a**, Location of RIOK2 gene on human chromosome 5. **b**, Schematic representation of the Riol2<sup>tm1a(KOMP)Wtsi</sup> allele and generation of Riol2 floxed mice. **c**, Agarose gel showing genotyping of Riol2 floxed mice. Riol2<sup>Δ</sup> indicates deletion of Riol2. No band expected in the Riol2<sup>wt</sup> lane. **d**, Expression of Riol2 mRNA in mouse BM cells. Modified from Gene Expression Commons. Numbers next to bars indicate expression level. **e**, Riol2 mRNA expression by qRT-PCR in BM cells from Riol2 haploinsufficient mice and Vav1<sup>cre</sup> controls. n = 5 mice/group. **f**, Frequency of the genotypes indicated on the X-axis among 4 litters from 4 different breeding crosses of the genotypes mentioned. **g**, In vivo protein synthesis rates in the indicated cell types from Riol2 haploinsufficient mice (n = 2) and Vav1<sup>cre</sup> controls (n = 8). Unpaired two-tailed t-test (e), multiple unpaired two-tailed t-tests with Holm-Sidak method (g) used to calculate statistical significance. Data are shown as mean ± s.e.m (e,f) or mean ± s.d. (g) and are representative of two (e, g) or four (c, f) independent experiments. ** p < 0.01, *** p < 0.001, **** p < 0.0001.
Extended Data Fig. 2 | Riok2 haploinsufficient mice display anemia and myeloproliferation. a, Gating strategy used for the identification of erythroid progenitor/precursor cells in the BM. b, Number of erythroid progenitor populations among viable BM cells in Riok2\(^{-/-}\)Vav\(^+/+\) mice and Riok2\(^{-/-}\)Vav\(^+/+\) controls. n = 5/group. c, Cell cycle analysis of erythroid progenitor/precursor cells from Riok2 haploinsufficient mice in comparison to Vav\(^+/+\) controls. \(n = 5\) mice/group. d, 
Cdtn1a mRNA expression by qRT-PCR in erythroid progenitors from Riok2 haploinsufficient mice and Vav\(^+/+\) controls. \(n = 3\) mice/group. e, Kaplan-Meier survival curve for Riok2 haploinsufficient mice and Vav\(^+/+\) controls subjected to lethal dose of PhZ. f, Number of RIII and RIV erythroid precursor populations among viable BM cells in Riok2\(^{-/-}\)Vav\(^+/+\) mice and Riok2\(^{-/-}\)Vav\(^+/+\) controls day 6 after PhZ treatment. \(n = 4\) group. g, PB RBC numbers, Hb, and HCT in mice transplanted with either Riok2 haploinsufficient mice or Vav\(^+/+\) BM cells. n = 5 mice/group. h, PB RBC numbers, Hb, and HCT in mice with tamoxifen-inducible deletion of Riok2. Tamoxifen administered on days 3 – 7. n = 8 and 7 for Riok2\(^{-/-}\)Ert2\(^{+/+}\) and Riok2\(^{-/-}\)Ert2\(^{+/+}\) mice, respectively. i, Representative flow cytometry plots showing frequency of monocytes (CD11b\(^+\)Ly6G\(^-\)Ly6Chi) and neutrophils (CD11b\(^+\)Ly6G\(^+\)) in the PB of Riok2\(^{-/-}\)Vav\(^+/+\) and Riok2\(^{-/-}\)Vav\(^+/+\) mice. j, Representative flow cytometry plots showing Ki-67\(^+\)GMPs in the BM of Riok2\(^{-/-}\)Vav\(^+/+\) and Riok2\(^{-/-}\)Vav\(^+/+\) mice. k, Number of CFU-GM colonies in MethoCult from Lin\(^+\)Sca-1\(^+\)c-kit\(^+\) BM cells from Riok2\(^{-/-}\)Vav\(^+/+\) mice (n = 5) and Riok2\(^{-/-}\)Vav\(^+/+\) controls (n = 4) after a 7-day culture period. n = 4-5/group. Multiple unpaired two-tailed t-tests with Holm-Sidak method (b, c), unpaired two-tailed t-test (d, f, g, k), log-rank test (e), and 2-way ANOVA with Sidak’s correction for multiple comparisons (h) used to calculate statistical significance. Data are shown as mean ± s.e.m and are representative of two (b to k) independent experiments. * p<0.05, ** p<0.01, *** p<0.001.
Extended Data Fig. 3 | Riol2 haploinsufficiency alters early hematopoietic progenitors in an age-dependent fashion. a, Frequency and number of indicated cell types in the bone marrow of Riol2<sup>−/−</sup>Vav1<sup>tm</sup> and Riol2<sup>−/−</sup>Vav1<sup>tm</sup> mice. n = 4/group. LT-HSC = long term hematopoietic stem cells, ST-HSC = short term hematopoietic stem cells, MPP = multipotent progenitors, CLP = common lymphoid progenitors. b, % CD45.2 (donor) chimerism in PB from competitive BM transplant with CD45.1 recipient cells. Time point ‘-1’ reflects first bleeding 4 weeks after transplantation and one day before tamoxifen induced deletion of Riol2. Donor (CD45.2) chimerism of the HSC compartment in the BM of competitive transplantation experiments. n = 5/group. c, Frequency of donor (CD45.2<sup>+</sup>) early hematopoietic progenitors 24 weeks after tamoxifen treatment in a competitive transplantation assay as described in (b). n = 5 and 4 for Riol2<sup>−/−</sup>Ert2<sup>tm</sup> and Riol2<sup>−/−</sup>Ert2<sup>tm</sup> mice, respectively. Unpaired two-tailed t-test (a, c) and 2-way ANOVA with Sidak’s multiple comparison test (b) used to calculate statistical significance. Data are shown as mean ± s.e.m and are representative of two (a-c) independent experiments. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.
Extended Data Fig. 4 | Riok2 haploinsufficient erythroid precursors express increased S100 proteins. (a) Expression of ribosomal proteins quantified by proteomics in Riok2<sup>−/−</sup>-Vav<sup>+/+</sup> and Riok2<sup>+/−</sup>-Vav<sup>+/+</sup> erythroid precursors. S100A8 (b) and S100A9 (c) expression assessed by flow cytometry in BM erythroid precursors from Riok2<sup>−/−</sup>-Vav<sup>+/+</sup> and Riok2<sup>+/−</sup>-Vav<sup>+/+</sup> mice. n = 4 mice/group. d, e, S100a8 and S100a9 mRNA expression in erythroid precursors isolated from Riok2<sup>−/−</sup>-Vav<sup>+/+</sup> and Riok2<sup>+/−</sup>-Vav<sup>+/+</sup> mice. n = 4 mice/group. f, p53 expression assessed by flow cytometry in BM erythroid precursors from Riok2<sup>−/−</sup>-Vav<sup>+/+</sup> and Riok2<sup>+/−</sup>-Vav<sup>+/+</sup> mice. g, Graphical representation of data shown in (e). n = 5 mice/group. Data are shown as mean ± s.e.m and are representative of two (b to g) independent experiments. Unpaired two-tailed t-test (b to g) used to calculate statistical significance. ** p<0.01, *** p<0.001, **** p<0.0001.
Extended Data Fig. 5 | Expression of lineage-associated T cell cytokines is comparable between Riok2 haploinsufficient and sufficient T cells. a–g, Concentration of IL-2 (a), IFN-γ (b), IL-4 (c), IL-5 (d), IL-13 (e), IL-17A (f) and frequency of Foxp3+ cells (g) from in vitro polarized T cells of the indicated genotypes. n = 3 mice/group. h–i, Number of IL-22+ NKT cells (H) and ILCs (I) in the spleens of Riok2f/+ Vav1cre mice and Riok2+/+ Vav1cre controls (n = 4/group). j, Frequency of IL-23p19+ DCs in Riok2f/+ Vav1cre mice and Riok2+/+ Vav1cre controls. n = 4 mice/group. k, PB RBC numbers, Hb, and HCT in Riok2f/+ Cd4cre mice and Riok2+/+ Cd4cre controls. n = 4 mice/group. l, Secreted IL-22 from in vitro polarized TH22 cells from Rps14 haploinsufficient mice and DMSO controls. n = 4 mice/group. m, Secreted IL-22 from in vitro polarized TH22 cells from ApcMin mice and DMSO controls. n = 4 mice/group. n, Viable cells (expressed as percentage of total cells in culture) for the indicated treatments assessed by flow cytometry. n = 5 mice/group. Data are shown as mean ± s.e.m and are representative of two (a to n) independent experiments. Unpaired two-tailed t-test (a to n) used to calculate statistical significance. * p < 0.05, ** p < 0.01.
Extended Data Fig. 6 | Neutralization of IL-22 signaling increases number of erythroid precursors. a–d. Number of RI–RIV erythroid populations among viable BM cells in the indicated strains undergoing PhZ-induced stress erythropoiesis. For (a), n = 5, 5, 4, and 4 for Riok2+/+ Il22+/+ Vav1cre, Riok2+/+ Il22−/+ Vav1cre, Riok2f/f Il22+/+ Vav1cre, and Riok2f/f Il22−/+ Vav1cre, respectively. For (d), n = 5/group. Data are shown as mean ± s.e.m and are representative of three (a, c) or two (b, d) independent experiments. 1-way ANOVA with Tukey’s correction (a, c) or unpaired two-tailed t-test (b, d) used to calculate statistical significance. * p < 0.05, ** p < 0.01.
Extended Data Fig. 7 | IL-22 neutralization alleviates anemia in wt mice undergoing PhZ-induced stress erythropoiesis. a, PB RBC numbers, Hb, and HCT in naïve wt C57BL/6 J mice treated with isotype control (Rat IgG2ακ, 50 mg/mouse) or anti-IL-22 antibody (50 mg/mouse). n = 5 mice/group. b, PB RBC numbers, Hb, and HCT in wt C57BL/6 J mice undergoing PhZ-induced stress erythropoiesis treated with isotype control or anti-IL-22 antibody. n = 5 mice/group. c, Percentage of RI-RIV erythroid precursors in the BM of mice treated as in (b). n = 4 and 5 for Il22ra1+/Epor+ and Il22ra1Δ/Epor+ mice, respectively. Data are shown as mean ± s.e.m and are representative of three (a, b) or two (c) independent experiments. Unpaired two-tailed t-test (a to c) used to calculate statistical significance. * p<0.05, *** p<0.001.
Extended Data Fig. 8 | Erythroid precursors express IL-22RA1. a, Gating strategy employed for assessing IL-22RA1 expression on erythroid precursors. b, Gating strategy to show that majority of IL-22RA1+ cells in the mouse BM are erythroid precursors. c, IL-22RA1 expression on erythroid precursors assessed using flow cytometry and a second antibody targeting a different epitope of IL-22RA1. d, Il22ra1 mRNA expression in the indicated cell types assessed by qRT-PCR. T cells and liver represent negative and positive controls, respectively. n = 4 mice/group. Data are shown as mean ± s.e.m (d) and are representative of three (a to c) or two (d) independent experiments.
Extended Data Fig. 9 | Increased IL-22 and its signature genes in del(5q) MDS subjects. a, Representative flow cytometry plots showing frequency of CD4^+IL-22^+ cells among total PBMCs in the peripheral blood of MDS patients and healthy subjects. Pre-gated on viable CD3ε^+CD4^+ cells. Cumulative data shown in Fig. 4e. b, Expression of indicated IL-22 signature genes in CD34^+ cells from healthy controls and del(5q) and non-del(5q) MDS patients. n = 17, 47, and 136 for healthy, del(5q) MDS, and non-del(5q) MDS, respectively. Kruskal-Wallis test with Dunn’s correction for multiple comparisons (b) used to calculate statistical significance * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001. Solid lines represent median and dashed lines represent quartiles (b).
Extended Data Fig. 10 | Riok2 haploinsufficiency recapitulates del(5q) MDS transcriptional changes. a, b, GSEA enrichment plots comparing proteins up-regulated (a) and down-regulated (b) upon Riok2 haploinsufficiency to the transcriptional changes seen in del(5q) MDS. c, Schematic of mechanism underlying Riok2 haploinsufficiency-induced, IL-22-induced anemia.
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Software and code

Policy information about availability of computer code

Data collection: FACS Diva (BD Biosciences) for flow cytometry, QuantStudio 6 Flex for qRT-PCR. Details regarding data collection for proteomics and RNA-Seq are included in the Methods section of this manuscript.

Data analysis: FlowJo v9 (TreeStar) for flow cytometry; Graph Pad Prism v8 (GraphPad) for statistical analyses; Bowtie (Version 1.2.8) was used for analyzing RNA-Seq data. GSEA v4.0.2, MSigDB v7.1, and MetaCore v20.1 build 7000 (Clarivate Analytics) for in silico analyses of proteomics and RNA-Seq datasets. I22 promoter analysis for p53 binding sites was performed using Lasagna-Search 2.0. Other technical details regarding data analysis of the proteomics and RNA-Seq datasets are included in the Methods section of this manuscript.

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The original mass spectra may be downloaded from MassIVE [http://massive.ucsd.edu], M5Y000085387. Raw RNA-Seq data is accessible via Gene Expression Omnibus (GEO) under the accession code GSE165467. Source data for all applicable figures (Main and Extended) are provided with the paper. The remaining data supporting the findings of this study are available from the corresponding authors upon reasonable request. Materials will be provided with material transfer agreements (MTA) as appropriate. All other data that support the findings of this study are available from the corresponding authors upon reasonable request.
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
No statistical tests were used to predetermine sample size. Sample size was determined based on similar previous studies reported in the literature (Schneider RKM, et al. Nature Medicine 2016). 3-6 mice per group were used for the majority of the experiments. The exact numbers per experiment are stated in Figure Legends and are visible as individual data points.

Data exclusions
No data was excluded in this manuscript.

Replication
All reported data used at least 3-6 biological replicates within experiments. Each experiment was replicated independently 2-3 times. All attempts at replication were successful.

Randomization
Mice were randomly assigned for all experiments reported. Age- and gender-matched mice were used in individual experiments. Human samples were allocated to specific experimental groups dependent on their disease status as determined by collaborating medical practitioners.

Blinding
Investigators were not blinded to group allocations during the experiment and data analysis. Since treatment and experimental analysis could not be separated, blinding of the investigators was not feasible.

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- [ ] Animals and other organisms
- [ ] Human research participants
- [ ] Clinical data

Methods

- n/a
- [x] ChIP-seq
- [ ] Flow cytometry
- [ ] MRI-based neuroimaging

Antibodies

Antibodies used

Target, Clone, Cat
FLOW CYTOMETRY
BioLegend
PerCP/Cyanine5.5 anti-mouse CD3ε Antibody, 145-2C11, 100328
PerCP/Cyanine5.5 anti-mouse CD5 Antibody, 53-7-3, 100624
PerCP/Cyanine5.5 anti-mouse Ly-6G/Ly-6C (Gr-1) Antibody, RB6-8C5, 108428
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Brilliant Violet 510™ anti-mouse CD117 [c-Kit] Antibody, 105839
Brilliant Violet 650™ anti-mouse CD150 [SLAM] Antibody, 115932
Brilliant Violet 711™ anti-mouse Ly-6A/E [Sca-1] Antibody, 108131
Validation

All commercially available antibodies used in this study are routinely tested by the respective manufacturer. Related technical data sheets can be obtained from the manufacturer’s website using the catalog number provided above.

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Wild-type C57BL/6J mice (Stock no. 000664), Vav-ire mice (Stock no. 008610), R26-CreERT2 mice (Stock no. 008463), I22ra1-flxed (Stock no. 031003), CD45.1 C57BL/6J mice (Stock no. 020144), Trp53−/− (Stock no. 002101), Cd4-cre (Stock no. 022071), and ApcMin (Stock no. 002020) were purchased from The Jackson Laboratory (Bar Harbor, ME). R26ra1-flxed mice were generated by the authors of this study. I22ra1-flxed mice were provided by Dr. Rachel Caspi (National Institutes of Health, Bethesda, MD) with permission from Genentech (San Francisco, CA). E12ra1-cre mice were a gift from Dr. Ursula Klingmüller (Deutsches Krebsforschungszentrum (DKFZ), Germany). I22ra1-flxed mice were a gift from Dr. Richard Lockley (University of California at San Francisco, CA). Rps14-flxed mice were a gift from Dr. Benjamin Ebert (DFCI, Boston, MA).

Mice within each experiment were age- and sex-matched. Experiments involving young mice were performed 8-12 wk old mice. Experiments involving old mice were performed with mice 60 wks and older.

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve field-collected samples.

Ethics oversight

Animal procedures were approved and performed according to the institutional Animal Care and Use Committee (IACUC) at Dana-Farber Cancer Institute (DFCI).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Human research participants included in this study were aged 18 years and above with male and female representation.
Flow Cytometry

Plots
- Confirm that:
  - The axes labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
Whole bone marrow (BM) cells were isolated by crushing hind leg bones (lemur and tibia) with mortar and pestle in staining buffer (PBS [Corning] supplemented with 2% heat-inactivated fetal bovine serum [FBS, Atlanta Biologicals] and EDTA [GIBCO]). Whole BM was lysed with 1X PharmLyse (BD Biosciences) for 90 s, and the reaction was terminated by adding an excess of staining buffer. Cells were labeled with fluorochrome-conjugated antibodies in staining buffer for 30 min at 4°C. For flow cytometric analysis, cells were incubated with combinations of fluorochrome-conjugated antibodies to the following cell surface markers: CD3 (17A2), CD5 (53–7.3), CD11b (M1/70), Gr1 (RB6–8C5), B220 (RA3–6B2), Ter119 (TER119), CD71 (C2), c-kit (2B8), Sca-1 (D7), CD16/32 (93), CD150 (TC15–12F12.2), CD48 (HM48-1). For sorting of lineage-negative cells, lineage markers included CD3, CD6, CD11b, Gr1 and Ter119. For sorting erythroid progenitor cells, the lineage cocktail did not include Ter119. All reagents were acquired from BD Biosciences, Thermo Fisher Scientific, Novus Biologicals, Tocris Biosciences, or Biologend. Identification of apoptotic cells was carried out using the Annexin V Apoptosis Detection Kit (Biologend). Intracytoplasmic and intranuclear staining was performed using Foxp3/Transcription Factor Staining Kit (Thermo Fisher Scientific) or 0.1% saponin in PBS supplemented with 3% FBS. For staining performed with AF647 pS3 antibody (Cell Signaling Technology), cells were permeabilized with 90% ice-cold methanol. To increase the sorting efficiency, whole BM samples were lineage-depleted using magnetic microbeads (Miltenyi Biotech) and autoMACS Pro magnetic separator (Miltenyi Biotech). Cell sorting was performed on a FACS Aria flow cytometer (BD Biosciences), data acquisition was performed on a BD Fortessa X-20 instrument equipped with 5 lasers (BD Biosciences) employing FACS Diva software. Data were analyzed by FlowJo (Tree Star) version 9 software. Flow analyses were performed on viable cells by exclusion of dead cells using either DAPI or a viable viability dye (Tocris Biosciences). Gating for early and committed hematopoietic progenitors was performed as described elsewhere56. ILCs and NK cells were identified as Lin−/CD45+CD90+CD11b+ and CD3ε−/NK1.1+, respectively.

Instrument
- BD Fortessa for data collection, BD FACS ARIA II for cell sorting

Software
- FlowJo v9 (TreeStar), BD FACS Diva

Cell population abundance
Sorted populations were assessed for purity by post-sort analysis on BD FACS ARIA II. Purity of over 95% was routinely achieved.

Gating strategy
Erythroid progenitors/precursors were gated as viable, lineage−/low, CD71+/−, Ter119+ as shown in Extended Data Fig. 2a. Gating strategy for identification of IL-22RA1+ cells in the bone marrow is shown in Extended Data Fig. 8a and 8b. Briefly, viable singlet cells were gated to remove lineage-positive cells and then the erythroid progenitor gate (based on CD71 and Ter119 expression) was applied. IL-22RA1 expression on cells in the erythroid progenitor gate was assessed.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.