Defective silencing of retrotransposable elements has been linked to inflammageing, cancer and autoimmune diseases. However, the underlying mechanisms are only partially understood. Here we implicate the histone H3.3 chaperone Daxx, a retrotransposable element repressor inactivated in myeloid leukaemia and other neoplasms, in protection from inflammatory disease. Loss of Daxx alters the chromatin landscape, H3.3 distribution and histone marks of haematopoietic progenitors, leading to engagement of a Pu.1-dependent transcriptional programme for myelopoesis at the expense of B-cell differentiation. This causes neutrophilia and inflammation, predisposing mice to develop an autoinflammatory skin disease. While these molecular and phenotypic perturbations are in part reverted in animals lacking both Pu.1 and Daxx, haematopoietic progenitors in these mice show unique chromatin and transcriptome alterations, suggesting an interaction between these two pathways. Overall, our findings implicate retrotransposable element silencing in haematopoiesis and suggest a cross-talk between the H3.3 loading machinery and the pioneer transcription factor Pu.1.
While the number of distal regions opening or closing in CMPs or GMPs compared with LT-HSCs were similar in the WT mice, substantially fewer distal regions were open in the Daxx-KO animals (Fig. 1b); most remained closed, suggesting Daxx-KO CMPs and GMPs became more restricted in their gene expression.

By overlapping accessible regions with known haematopoietic enhancers, we found more enhancers opening in KO LT-HSCs (2,509) compared with CMPs and GMPs (1,040 and 716, respectively; Fig. 1c). Based on unsupervised clustering (Fig. 1d), one set of enhancers showed increased read counts in KO LT-HSCs compared with WT LT-HSCs but reduced counts in CMPs and GMPs. Another set displayed similar counts in WT CMPs and GMPs as well as KO LT-HSCs, suggesting that chromatin features of Daxx-KO LT-HSCs may resemble myeloid-committed progenitors. The KO LT-HSCs displayed increased general opening of enhancers, including those overlapping with ERVs, in accordance with a Daxx-repressive role (Fig. 1e). ERV-overlapping enhancers were enriched in transcription factor motifs for the master regulators of haematopoiesis Pu.1 and Ets1 (Fig. 1f).

Neighbouring genes displayed enrichment in immune-cell function and pathways associated with myeloid-committed progenitors (Extended Data Fig. 1d,e).

More acute effects of Daxx loss were assessed by collecting mice at 3 d post induction (d.p.i.). Like at 3 w.p.i., most changes occurred at distal regions. However, more sites displayed decreased (8,421) than increased accessibility (3,247). Despite a similar number of known haematopoietic enhancers being open or closed (1,020 versus 1,022), more enhancers overlapping with ERVs opened (818 versus 617), with more pronounced differences at open ERV-overlapping enhancers (Fig. 1g). RNA sequencing (RNA-seq) (Extended Data Fig. 1f and Supplementary Table 1) showed increased expression of genes close to open enhancers and ERV-overlapping enhancers (Fig. 1h). However, genes proximal to enhancers with reduced accessibility also showed increased expression, an effect potentially due to enhancer occupancy by transcription factors (Fig. 1i).

Inagenuity pathway analysis (IPA) and Gene Ontology suggested engagement of the double-stranded RNA (dsRNA)-recognition machinery and activation of interferon (IFN)-stimulated genes (ISGs; Fig. 1j–m and Supplementary Table 2). Key components of these pathways were indeed upregulated (for example, Irf3, Irf7, Mavs and Ddx58; Fig. 1l,m) along with selected ERV subtypes, including the Daxx targets ERV1 and IAPs, but not non-ERV RETEs (Fig. 1n and Extended Data Fig. 1g). Enriched cell cycle-related pathways, along with upregulation of the proliferation marker Mki67 and downregulation of the quiescence gene Egr1, pointed at increased cell-cycle entry (Extended Data Fig. 1h and Supplementary Tables 1,2). Notably, upregulated genes such as Mxi1, the Ift7 cluster, Irf7 and Mki67 showed increased accessibility at ERV-overlapping enhancers and at Pu.1-binding sites (based on 16; Fig. 1o,p and Extended Data Fig. 1h), suggesting a role for Pu.1 in their regulation. Spil (coding for Pu.1) regulatory elements showed increased opening but its expression was unaltered (Extended Data Fig. 1h).

In agreement with the reported role for Atrx in the regulation of the telomeric repeat-containing RNA (TERRA)21–23, TERRA-binding sites (TERRA-BS)24 were more open across all chromosomes in Daxx-KO LT-HSCs, including those at Mid1 and Erdr1, a potential HSC regulator and repressor of inflammatory skin disease25–27 (Extended Data Fig. 1i,j). Chromatin opening correlated with their downregulation, as was also observed at the autosomal Wls locus (Extended Data Fig. 1j). TERRA knockdown also reduced expression of these loci, whereas Atrx loss increased their expression1, suggesting that Daxx and Atrx may antagonize each other for TERRA regulation.

Overall, our data suggest that Daxx loss in LT-HSCs alters the chromatin landscape at ERV-overlapping enhancers and TERRA-BS, with potential implications for induction of an IFN type I response and cell-cycle entry.

**Daxx loss skews haematopoiesis towards myeloid differentiation**

We next investigated the effect of Daxx loss on haematopoiesis. The overall cell numbers in the KO bone marrow (BM) were significantly reduced at 3 d.p.i. (Fig. 2a), whereas the frequencies and numbers of LT-HSCs, c-Kit+Lineage (Lin)+Sca-1+ (KLS) cells and myeloid-restricted multipotent progenitor (MPP) 3 cells (Fig. 2b) were increased. Both GMPs and lymphoid-biased MPP4 cells showed increased frequencies. The neutrophil frequency in the BM was augmented, whereas the frequency and number of B220+ cells were reduced (Fig. 2c). The B-cell defect encompassed pre-B, pre-B, and immature and mature B cells (Fig. 2d). The neutrophil frequency and number in the spleen were increased, whereas the monocye number was reduced. B cells were unaffected and remained viable (Fig. 2e,f). At 2 w.p.i., the frequency and number of neutrophils were higher, whereas those of B cells were lower (Fig. 2g). Thus, Daxx loss causes early expansion of stem and progenitor cells, followed by increased BM and peripheral accumulation of neutrophils at the expense of B cells.
To study the long-term effects of Daxx loss on haematopoiesis, we employed haematopoiesis-specific Cre lines. Daxx deletion during prenatal haematopoiesis using the Csf1rCre line failed to produce viable Daxx-KO pups (not shown), similar to a germline Daxx-KO line. Haematopoiesis-specific and polyinosinic:polycytidylic acid (pI:pC)-inducible Daxx deletion in adult mice (using the Mx1Cre line)
Fig. 2 | Acute and chronic Daxx loss lead to perturbations of haematopoiesis. a, Total number of cells (n=5 WT and 7 Daxx-KO mice). b, Frequency and total cell number of mature lymphoid and myeloid cells (n=6 WT and 7 Daxx-KO mice). c, Frequency and total cell number of mature lymphoid and myeloid cells (n=6 WT and 7 Daxx-KO mice). d, Frequency and total cell numbers of B-cell progenitor populations (n=4 WT and 5 Daxx-KO mice). e, Frequency and total counts of mature lymphoid and myeloid cells (n=4 mice per genotype). f, Frequencies of apoptotic B cells (n=4 WT and 5 Daxx-KO mice). g, Frequency and total cell numbers of B cells and neutrophils at 2 w.p.i. (n=3 mice per genotype). h, Representative images of haematopoietin and eosin (H&E) staining of bones (n=2 independent experiments). Scale bars, 100 µm (left) and 20 µm (right; higher-magnification images). i, Frequencies of immature and mature myeloid progenitors (Gr1+CD11b+ cells; n=3 mice per genotype). j, Examples of the flow cytometry analysis of myeloid progenitor cells. k,l, Frequency (k) and total count (l) of haematopoietic progenitors (n=3 mice per genotype). m, Concentration of different cytokines in plasma (n=6 mice per genotype; analysis of variance and corresponding non-parametric Conover’s test). TPO, thrombopoietin. n, Frequencies of neutrophils (n=6 mice per genotype; non-parametric Mann–Whitney test). o, Number of neutrophils (n=5 mice per genotype; non-parametric Mann–Whitney test). p, Cxcr2 levels measured in mature neutrophils (n=3 mice per genotype). MFU, mean fluorescent units. q, Examples of flow cytometry analysis of B-cell progenitors and frequencies of B cells and progenitors (right; n=6 mice per genotype; non-parametric Mann–Whitney test). a-g, RosAceER72 mice. h-q, Mx1Cre mice. a-d, i-n, q. BM. e-g, Spleen. a-g, i-k, p. Student’s t-test. b, g-i, k-o. Boxplots show the minimum and maximum values (box boundaries) and the mean (horizontal line). a-p. Data are the mean±s.d. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 and NS, not significant. Daxx f/F, Daxx KO and Daxx +/-, Daxx WT. Exact P values and numerical source data are provided.
Haematopoietic defects of Daxx deficiency are cell-intrinsic

To determine the nature of the phenotypic changes described earlier, we performed colony-forming unit (CFU) assays using either Lin− progenitors (HPCs) or LT-HSCs from untreated Daxx+/−Mx1Cre−/− and Daxx−/−Mx1Cre−/− mice, which underwent spontaneous recombination in vitro, probably due to activation of IFN signalling (not shown). An increase in colonies was observed following Daxx loss in HPCs (Extended Data Fig. 6a) at passage 0 (P0). Conversely, at P1 Daxx-deficient cells gave rise to fewer colonies, suggesting that their colony-forming potential was reduced. The CFU assays with LT-HSCs showed a similar phenotype following Daxx loss (Extended Data Fig. 6b,c), with an increased number of KLS cells at P0 and reduction at P1 (Extended Data Fig. 6d). Similar changes were observed in the number of CD11b+ cells and neutrophils (Extended Data Fig. 6e).

Finally, transplantation of BM cells isolated from pI:pC-treated mice into congenic recipients (Extended Data Fig. 6f) suggested a reduced reconstitution capacity of Daxx-KO stem/progenitor cells, resulting in a low frequency of CD45.2+ cells in the periphery (Extended Data Fig. 6g). Despite similar white-blood-cell counts (Extended Data Fig. 6h), KO transplants displayed altered frequencies of myeloid and B cells in the BM, PB and spleen, and of KLS cells and GMPs in the BM, as observed in the donor mice (Extended Data Fig. 6i–l).

Overall, these data suggest that the aberrant blood-cell composition observed following Daxx loss is due to a cell-intrinsic defect.

Pu.1-driven myeloid signature following Daxx loss

We performed transcriptome analyses of KLS and GMP subpopulations isolated from BM chimaeras (see the ‘Haematopoietic defects of Daxx deficiency are cell-intrinsic’ section; stress haematopoiesis) as well as from pI:pC-treated mice collected at 3 and 24 w.p.i. (Fig. 5a and Supplementary Tables 3–8; steady-state haematopoiesis). A PCA analysis revealed clustering according to genotype (Fig. 5a and Extended Data Fig. 7a) and that differences increased with time and following stress. When plotting the samples along pseudotime, Daxx-KO KLS cells followed a different trajectory, whereas KO GMPs did not (Fig. 5b and Extended Data Fig. 7b,c). Unsupervised clustering of selected master regulators and haematopoiesis markers revealed clustering according to genotype and condition as well as increased expression with time of myeloid-associated genes such as Mpo, Elane, Spi1 and Cebpa. This was accomplished by a reduction in the expression levels of lymphoid genes (Flt3, Ebf1, Irf4, Pax5 and Gfi1b) in the KO KLS cells (Fig. 5c) but not in GMPs (Extended Data Fig. 7d). Accordingly, by comparing cells at 3 and 24 w.p.i. we found that Daxx-KO KLS cells switch from pathways compatible with increased lymphopoiesis to increased myelopoiesis (Extended Data Fig. 7d). Accordingly, by comparing cells at 3 and 24 w.p.i. we found that Daxx-KO KLS cells switch from pathways compatible with increased lymphopoiesis to increased myelopoiesis and reduced B-cell generation (Fig. 5d,e and Extended Data Fig. 7e,f). This effect was even more pronounced in transplantation settings (Fig. 5f) and Extended Data Fig. 7g).

Furthermore, an increasing number of Pu.1-target genes driving myeloid fate were upregulated over time and following stress, accompanied by reduced expression of lymphoid Pu.1-target genes (Fig. 5g–i). These data are in accordance with neutrophilia becoming more pronounced with time (Fig. 4h), which was also confirmed by pathway analysis for Daxx-KO GMP cells (Extended Data Fig. 7h).

Transcriptomics of lymphoid- and myeloid-primed multipotent progenitors (MPP4 and MPP3, respectively) combined with unsupervised clustering of myeloid marker gene and transcription factor expression showed that Daxx-KO MPP4 cells cluster together with WT MPP3 (Fig. 5j, top). A similar analysis based on lymphoid genes showed Daxx-KO MPP4 clustering with MPP3 cells, suggesting that Daxx-KO lymphoid-biased MPP4 have shifted towards a more myeloid-biased gene expression (Fig. 5j, bottom).

Deregulation of ERVs/RTEs in Daxx-KO KLS cells and GMPs became more pronounced with time and following stress, especially that of ERV1 and IAPs (Fig. 5k and Extended Data Fig. 7i). These changes correlated with induction of ISGs and RNA-sensing factors in both Daxx-KO KLS and GMPs, mostly at 24 w.p.i., and in transplanted cells (Fig. 5l and Extended Data Fig. 7j). Spleen staining showed increased levels of the retinoic acid-inducible gene I-pathway (RIG-I) component melanoma differentiation-associated gene 5 (Mda5) and double-stranded RNA (dsRNA; Extended Data Fig. 8a). Furthermore, TERRA long-noncoding RNA (lncRNA) was upregulated in Daxx-KO BM cells (Extended Data Fig. 8b) and there was significant overlap between differentially expressed genes (DEGs) from TERRA knockdown in embryonic stem cells50 with DEGs in Daxx-KO versus WT GMP datasets (Extended Data Fig. 8c).

Finally, ERV-overlapping enhancers in proximity of both up- and downregulated genes were more accessible in Daxx-KO KLS cells at 3 w.p.i. (Fig. 5m,n). For instance, Spli and Gfi1 (which together drive neutrophilic differentiation51) displayed increased enhancer accessibility at 3 w.p.i. but were upregulated only in transplantation settings. In contrast, Irf4 expression (which cooperates with Pu.1 for B-cell differentiation52) was higher at 3 w.p.i. but lower at 24 w.p.i. in transplantation settings.

Daxx loss alters H3.3, Pu.1 and histone-mark distribution

We next performed low-input chromatin immunoprecipitation combined with sequencing using Cleave Under Targets and Tagmentation (CUT&Tag) for H3.3, Pu.1, H3 lysine 27 acetylation (H3K27ac) and H3K9 trimethylation (H3K9me3) on WT and Daxx-KO HSPCs at 3 w.p.i. Daxx loss led to significant changes in H3.3 distribution and Pu.1 binding (Fig. 6a). Reduction in both H3.3 and Pu.1 at distal regions, particularly at ERVs, was accompanied by enrichment of both proteins at promoters (Fig. 6a), suggesting that their respective chromatin associations may be linked. H3.3-depleted enhancers were characterized by increased chromatin accessibility and H3K27ac but decreased Pu.1 levels (Fig. 6b,c). In turn, nearby genes showed increased Pu.1 binding around and downstream of the transcription start site (TSS), coinciding with increased chromatin accessibility and H3.3 enrichment (Fig. 6c). Pathway analysis for genes close to distal regions with altered Pu.1 expression showed that Daxx-KO MPP4 cells cluster together with WT MPP3 (Fig. 5j, top). A similar analysis based on lymphoid genes showed Daxx-KO MPP4 clustering with MPP3 cells, suggesting that Daxx-KO lymphoid-biased MPP4 have shifted towards a more myeloid-biased gene expression (Fig. 5j, bottom).

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**Overview image**

- **a**
  - Daxx +/+ vs Daxx F/F
  - Overview image

- **b**
  - Mean percentage of CD45+ cells
  - Daxx +/+ vs Daxx F/F

- **c**
  - Mean percentage of CD45+ cells
  - CD1b, CD11c, Ly6G, CD41, B220, CD19, CD138

- **d**
  - Total number of spleen cells
  - Mean percentage of CD45+ cells

- **e**
  - Mean percentage of CD45+ cells
  - Neutrophils, Eosinophils

- **f**
  - Cxcr2 level

- **g**
  - Total number of FOB cells

- **h**
  - Mean percentage of CD45+ cells
  - F4/80, MZB cells

- **i**
  - Percentage of Hoechst + area
  - CD11b + area

- **j**
  - Hoechst, F4/80, CD11b

- **k**
  - Hoechst, F4/80, CD11b
In addition, altered Pu.1 binding at Pu.1-regulated transcription factors (Fig. 6c) corresponded to changes in their gene expression (Fig. 5g).

Enhancers overlapping with ERVs/RTEs showed stronger depletion of H3K9me3 and increase in H3K27ac than other enhancers (Fig. 6f), despite a similar increase in chromatin...
accessibility. Generally, ERVs/RTEs showing increased accessibility were enriched in H3K27ac but displayed minor changes in H3K9me3 (Fig. 6g). Finally, among enhancers (Fig. 6h) and ERVs/RTEs gaining H3K27ac (Fig. 6i), one subset displayed clear reorganization in H3.3 following Daxx loss.

Together, these findings suggest that Daxx loss alters chromatin features and Pu.1 distribution at enhancers, including those overlapping with ERVs.

Phenotypic rescue and unique changes after Daxx and Pu.1 DKO

We next investigated whether the phenotypic alterations caused by Daxx loss were dependent on Pu.1 by using a Sp1+/− line[7]. The BM cellularity was not significantly different in Daxx−/− mice compared with Daxx and Pu.1 double-KO (DKO) mice, although the latter displayed a tendency towards higher cellularity (Fig. 7a). At 8 w.p.i., the BM of the DKO mice displayed significant reduction in the GMP and neutrophil frequencies and numbers compared with the Daxx−/−KO (Fig. 7b,c), whereas the B220+ cells in the BM were still compromised (Fig. 7c). Conversely, in DKO spleens, the B-cell and neutrophil frequencies and numbers were normalized (Fig. 7d–g) along with spleen architecture and selected cytokines (at 3 and 8 w.p.i., Fig. 7h–j). The B-cell frequency was also partially rescued in the DKO PB (Fig. 7k,l). Neutrophil and B-cell numbers and frequencies in the BM, spleen and PB were similar to WT following the loss of Pu.1 alone (Extended Data Fig. 8d,e).

To link the phenotypic rescue to normalization of molecular perturbations, we studied changes in transcription, chromatin landscape and histone marks in single-KO and DKO mice (Supplementary Tables 9 and 10). The PCA analysis of RNA-seq data showed that each genotype clustered separately (Fig. 8a,b). Although DEGs from different comparisons (Daxx−/−KO versus WT, DKO versus WT and Pu.1-KO versus WT) partially overlapped, many genotype-specific changes also occurred, particularly in KLS cells (Extended Data Fig. 9a,b). Pathway analysis based on DKO-specific and Pu.1-KO-specific DEGs in KLS cells and GMPs identified distinct biological functions and diseases, including some associated with lymphoid neoplasms (Fig. 8c,d and Extended Data Fig. 9c,d). Clustering of expression for haematopoietic transcription factors and markers, Pu.1-target genes, ISGs and the dsRNA-sensing machinery revealed both common and genotype-specific changes between Pu.1-KO and DKO cells (Extended Data Fig. 9e–g). Altered expression of selected ERV/RTE subtypes in Daxx−/−KO cells and GMPs were in part reverted in the DKO cells (Fig. 8e,f). Finally, Pu.1-KO KLS cells and GMPs showed upregulation of some ERV/RTE subtypes and satellite repeats, suggesting a role for Pu.1 in their regulation.

On investigation of the chromatin changes, we found that 90.8% of distal regions that opened following Daxx loss became closed in DKO cells (Fig. 8g). Conversely, the vast majority of distal regions found closed in the Daxx−/−KO KLS cells opened in DKO KLS cells. In addition, we found DKO-specific chromatin changes: 15,587 distal regions that were open in WT and Daxx−/−KO KLS cells became closed in DKO cells, while 3,922 distal sites that opened in DKO cells were closed in the other genotypes. When we compared open regions in WT, Daxx−/−KO and DKO KLS cells, we found that DKO cells generally displayed the lowest accessibility (Fig. 8h). Many of the genes that were significantly upregulated in the Daxx−/−KO KLS cells at 3 w.p.i. and resided near enhancers with increased accessibility were downregulated in the DKO cells (Fig. 8i). Finally, opening of TERRA-Bs at sex chromosomes in Daxx−/−KO KLS cells was reverted in DKO cells (Extended Data Fig. 10a). Closing of chromatin at the TERRA-Bs in the vicinity of the Mid1 and Erd1 genes in the DKO cells correlated with rescued gene expression compared with the Daxx−/−KO cells (Extended Data Fig. 10b).

H3K9me3 alterations found in Daxx−/−KO HSPCs were reverted to some extent in DKO cells (Fig. 8i). In addition, we found that there were more distal regions in DKO HSPCs that gained H3K9me3.
(7,862) than those with reduced H3K9me3 (1,147) compared with WT or Daxx-KO HSPCs. We also observed that the overall levels of H3.3 were markedly increased in DKO cells at enhancers and ERVs compared with both the WT and Daxx-KO cells (Fig. 8k).

Finally, we zoomed in on the locus encoding Fbp1 and Fbp2 (Fig. 8l), two critical regulators of gluconeogenesis that affect HSPC repopulation capacity via inhibition of glycolysis under the control of Setdb1 (ref. 43). Fbp1 was upregulated in Daxx-KO KLS
cells, whereas Fbp2 remained silenced apart from transplantation settings. Fbp1 silencing was restored in DKO cells, suggesting that Pu1 is required for its induction. This is in accordance with three main regions bound by Pu1 at this locus (as reported in43), two of which coincide with haematopoietic enhancers44. H3K27ac enrichment at these regions in Daxx-KO cells was reverted in DKO cells. Although H3.3 was reduced at the proximal enhancer in Daxx-KO cells, its levels were restored in DKO cells, suggesting involvement of another chaperone, such as Hira. A similar trend applied to H3K9me3 at this enhancer. The large block of H3K9me3 over the Fbp2 gene and its regulatory regions, which was previously linked to Fbp2 upregulation in HSPCs25, following Setdb1 loss, was further enriched in Daxx-KO cells but not DKO cells. Finally, H3K27me3 was substantially reduced across the entire locus in the Daxx-KO and DKO cells, especially at the most proximal enhancer of Fbp1, suggesting that this mark may cooperate with Pu1 loss for Fbp1 silencing.

Discussion

Together, our findings implicate the H3.3 chaperone and ERV/RTE repressor Daxx in the regulation of haematopoiesis and protection from inflammation. We propose that Daxx acts as an epigenetic barrier controlling cell plasticity in HSPCs in part via RTE control, in turn ensuring balanced cell differentiation (Extended Data Fig. 10c). Recent work in zebrafish implicates RTE-driven engagement of RNA-sensing in HSPC emergence in haematopoiesis45. In this respect, we showed that ERV/RTE induction correlates with the IFN type I-like response in Daxx-deficient LT-HSCs. IFN type I signalling is normally repressed in HSCs, as it may promote cell death45, but when activated in quiescent HSCs it causes cell-cycle entry45. Following acute Daxx loss, the number of LT-HSCs and other progenitor types did indeed increase. As it is unlikely that stem/progenitor cells are able to produce IFNs, ISG induction could be IFN-independent as reported in viral-infection models47,48. This could explain the moderate ISG induction in Daxx-KO LT-HSCs.

Furthermore, it is possible that ERV/RTE derepression following Daxx loss may impair B-cell differentiation, as observed in mice lacking the ERV/RTE-silencing Setdb1–Kap1 complex, a Daxx interactor49–50.

Our study points to a link between Daxx and the pioneer transcription factor Pu1. Pu1 engagement does not seem to be an immediate effect of Daxx loss in HSCs. We instead observed progressive activation of a Pu1 myeloid programme in haematopoietic progenitors with time and following stress, along with aggravation of neutrophilia. This mirrors the response to viral infections, where HSC activation is followed by a return to quiescence and an accumulation of myeloid-biased HSCs33, a phenomenon that has also been observed in ageing7. Thus, Pu1 may contribute to the myeloid bias within a subset of Daxx-deficient HSCs that have returned to quiescence.

Concomitant Daxx and Pu1 loss reverts some phenotypic perturbations found in Daxx-KO mice, including a partial recovery of B-cell differentiation and spleen architecture. This would be in agreement with the reported role of Pu1 in inhibition of terminal B-cell and plasma-cell differentiation44. At the molecular level, the increase in enhancer accessibility that typifies Daxx-KO KLS cells is reverted by over 90% in DKO cells. Closing of chromatin in DKO cells at TERRA-BS correlated with rescued gene expression of neighbouring genes, suggesting that perturbations at TERRA-BS and its target genes, such as Erdr1, may contribute to the phenotypes caused by Daxx loss56. However, DKO progenitors also show unique chromatin and transcriptome alterations that are not found in single Pu1- or Daxx-KO mice, suggesting a genetic interaction between the two pathways. In support of this hypothesis, Daxx loss causes reduction in the levels of both Pu1 and H3.3 at enhancers, correlating with their increased enrichment at neighbouring genes. These findings suggest that Pu1 and H3.3 may influence their reciprocal genome distribution.

Finally, dysfunction of intrinsic immunity mechanisms devoted to ERV/RTE silencing may predispose to inflammation and neoplastic...
transformation during ageing\(^2^{342}\). While transposition-driven mutagenesis could lead to the accumulation of potentially leukaemogenic mutations in HSCs and promote clonal haematopoiesis\(^2\), the resulting anti-viral response may suppress the expansion of mutated HSCs\(^2\). In this respect, neutrophilia and inflammation triggered by Daxx loss might be tumour-suppressive. Further genetic alterations (de novo or pre-existing) such as downregulation of Pu.1 may allow Daxx-deficient myeloid or lymphoid progenitors to escape differentiation and undergo neoplastic transformation, in accordance with the reported leukaemogenic effect of Pu.1 downregulation\(^2\). It is therefore possible that DKO mice may become leukaemic at old age.

Overall, this work implicates mechanisms governing accessibility to repeat elements in homeostasis of the haematopoietic system via cross-talk with pioneer transcription factors such as Pu.1. Perturbations of these mechanisms may contribute to inflammation and predispose to leukaemogenesis.

### 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/s41556-021-00774-y](https://doi.org/10.1038/s41556-021-00774-y).

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### References

1. Kunarso, G. et al. Transposable elements have rewired the core regulatory network of human embryonic stem cells. *Nat. Genet.* **42**, 631–634 (2010).

2. Chuong, E. B., Rumi, M. A., Soares, M. J. & Baker, J. C. Endogenous retrovirus function as species-specific enhancer elements in the placent. *Nat. Genet.* **45**, 325–329 (2013).

3. Mita, P. & Boeke, J. D. How retrotransposons shape genome regulation. *Curr. Opin. Genet. Dev.* **37**, 90–100 (2016).

4. Volkman, H. E. & Setson, D. B. The enemy within: endogenous retroelements and autoimmune disease. *Nat. Immunol.* **15**, 415–422 (2014).

5. Tugnet, N., Ryalance, P., Roden, D., Trela, M. & Nelson, P. Human endogenous retroviruses (HERVs) and autoimmune rheumatic disease: is there a link? *Open Rheumatol. J.* **7**, 13–21 (2013).

6. Gonzalez-Cao, M. et al. Human endogenous retroviruses and cancer. *Cancer Biol. Med.* **13**, 483–488 (2016).

7. Downey, R. F. et al. Human endogenous retrovirus K and cancer: innocent bystander or tumorigenic accomplice? *Int. J. Cancer* **137**, 1249–1257 (2015).

8. Manghera, M., Ferguson-Parry, J., Lin, R. & Douville, R. N. NF-κB and IRF1 induce endogenous retrovirus K expression via interferon-stimulated response elements in its 5’ long terminal repeat. *J. Virol.* **90**, 9338–9349 (2016).

9. Grosh, S. & Schotta, G. Silencing of endogenous retroviruses by heterochromatin. *Cell. Mol. Life Sci.* **74**, 2055–2065 (2017).

10. He, Q. et al. The Daxx/Atrx complex protects tandem repetitive elements during DNA hypomethylation by promoting H3K9 trimethylation. *Cell Stem Cell* **19**, 273–286 (2016).

11. Elsasser, S. J., Noh, K. M., Diaz, N., Allis, C. D. & Banaszynski, L. A. Histone H3.3 is required for endogenous retroviral element silencing in embryonic stem cells. *Nature* **522**, 240–244 (2015).

12. Qadeer, Z. A. et al. ATRX in-frame fusion neuroblastoma is sensitive to EZH2 inhibition via modulation of neuronal gene signatures. *Cancer Cell* **36**, 512–527 (2019).

13. Lewis, P. W., Elsasser, S. J., Noh, K. M., Stadler, S. C. & Allis, C. D. A Daxx is an H3.3-specific histone chaperone and cooperates with ATRX in replication-independent chromatin assembly at telomeres. *Proc. Natl Acad. Sci. USA* **107**, 14075–14080 (2010).

14. Goldberg, A. D. et al. Distinct factors control histone variant H3.3 localization at specific genomic regions. *Cell* **140**, 678–691 (2010).

15. Dyer, M. A., Qadeer, Z. A., Valle-Garcia, D. & Bernstein, E. ATRX and DAXX: mechanisms and mutations. *Cold Spring Harb. Perspect. Med.* [https://doi.org/10.1101/cshperspect.a026567] (2017).

16. Drane, P., Ouararhni, K., Depaux, A., Shaub, M. & Hamiche, A. The death-associated protein DAXX is a novel histone chaperone involved in the replication-indendent deposition of H3.3. *Genes Dev.* **24**, 1253–1265 (2010).

17. Ray-Gallet, D. et al. Functional activity of the H3.3 histone chaperone complex HIRA requires trimerization of the HIRA subunit. *Nat. Commun.* **9**, 3103 (2018).

18. Lara-Astiaso, D. et al. Immunogenetics. Chromatin state dynamics during blood formation. *Science* **345**, 943–949 (2014).

19. Pundhir, S. et al. Enhancer and transcription factor dynamics during myeloid differentiation reveal an early differentiation block in *Cebpa* null progenitors. *Cell Rep.* **23**, 2744–2757 (2018).

20. Nguyen, D. T. et al. The chromatin remodelling factor ATRX suppresses R-loops in transcribed telomeric repeats. *EMBO Rep.* **18**, 914–928 (2017).

21. Redon, S., Reichenbach, P. & Linger, J. The non-coding RNA TERRA is a natural ligand and direct inhibitor of human telomerase. *Nucleic Acids Res.* **38**, 5797–5806 (2010).

22. Pfeiffer, V. & Linger, J. TERRA promotes telomere shortening through exomucle1-mediated resection of chromosome ends. *PLoS Gen.* **8**, e1002747 (2012).

23. Chu, H. P. et al. TERRA RNA antagonizes ATRX and protects telomeres. *Cell* **170**, 86–101 (2017).

24. Zhang, L. F. et al. Telomeric RNAs mark sex chromosomes in stem cells. *Genetics* **182**, 685–698 (2009).

25. Lee, H. R. et al. ERDR1 enhances human NK cell cytotoxicity through an actin-regulated degranulation-dependent pathway. *Cell. Immunol.* **292**, 78–84 (2014).

26. Houth, Y. K., Kim, K. E., Park, H. J. & Cho, D. Roles of erythroid differentiation regulator 1 (Erdr1) on inflammatory skin diseases. *Cell. Immunol.* **273**, 273–286 (2015).

27. Deneault, E. et al. A functional screen to identify novel effectors of erythroid differentiation. *Blood* **117**, 2733–2742 (2011).

28. Michaelson, J. S., Bader, D., Kuo, F., Kozak, C. & Leder, P. Loss of Daxx, a death-associated protein DAXX is a novel histone chaperone involved in the replication-independent deposition of H3.3. *Genes Dev.* **24**, 1253–1265 (2010).

29. Velasco-Hernandez, T. A., Macrì, P., Byrder, D. & Camomenga, J. Potential pitfalls of the Mx1–Cre system: implications for experimental modeling of normal and malignant hematopoiesis. *Stem Cell Rep.* **7**, 11–18 (2016).

30. Marzano, A. V. et al. Mechanisms of inflammation in neutrophil-mediated skin diseases. *Front Immunol.* **10**, 1059 (2019).
31. Mutua, V. & Gershwin, L. J. A review of neutrophil extracellular traps (NETs) in disease: potential anti-NETs therapeutics. *Clin. Rev. Allergy Immunol.* https://doi.org/10.1007/s12016-020-08804-7 (2020).

32. Castanheira, F. V. S. & Kubes, P. Neutrophils and NETs in modulating acute and chronic inflammation. *Blood* **133**, 2178–2185 (2019).

33. Kaplan, M. J. & Radic, M. Neutrophil extracellular traps: double-edged swords of innate immunity. *J. Immunol.* **189**, 2689–2695 (2012).

34. Regan-Komito, D. et al. GM-CSF drives dysregulated hematopoietic stem cell activity and pathogenic extramedullary myelopoesis in experimental spondyloarthitis. *Nat. Commun.* **11**, 1355 (2020).

35. Hamilton, J. A. Colony-stimulating factors in inflammation and autoimmunity. *Nat. Rev. Immunol.* **8**, 533–544 (2008).

36. Manz, M. G. & Boettcher, S. Emergency granulopoiesis. *Nat. Rev. Immunol.* **14**, 302–314 (2014).

37. Wollina, U. & Haroske, G. Pyoderma gangraenosum. *Curr. Opin. Rheumatol.* **23**, 50–56 (2011).

38. Nye, J., Melters, D. P. & Dalal, Y. The art of war: harnessing the epigenome to fight cancer. *Nat. Rev. Cancer* **14**, 191–205 (2014).

39. Chen, C. et al. HIRA, a DfiGeorge syndrome candidate gene, confers proper chromatin accessibility on HSCs and supports all stages of hematopoiesis. *Cell Rep.* **30**, 2136–2149 (2020).

40. Hock, H. & Orkin, S. H. Zinc-finger transcription factor Gfi-1: versatile regulator of lymphocytes, neutrophils and hematopoietic stem cells. *Curr. Opin. Hematol.* **13**, 1–6 (2006).

41. Pang, S. H. et al. PU.1 cooperates with IRF4 and IRF8 to suppress pre-B-cell leukemia. *Leukemia* **30**, 1375–1387 (2016).

42. Iwasaki, H. et al. Distinctive and indispensable roles of PU.1 in maintenance and function of hematopoietic stem cells. *Cell Rep.* **19**, 2345–2356 (2017).

43. Koide, S. et al. Setdb1 maintains hematopoietic stem and progenitor cells by restricting the ectopic activation of nonhematopoietic genes. *Blood* **128**, 638–649 (2016).

44. LeFkopoulos, S. et al. Repetitive elements trigger RIG-I-like receptor signaling that regulates the emergence of hematopoietic stem and progenitor cells. *Immunity* **53**, 934–951 (2020).

45. Pieters, E. M. et al. Re-entry into quiescence protects hematopoietic stem cells from the killing effect of chronic exposure to type I interferons. *J. Exp. Med.* **211**, 245–262 (2014).

46. Sato, T. et al. Interferon regulatory factor-2 protects quiescent hematopoietic stem cells from type I interferon-dependent exhaustion. *Nat. Med.* **15**, 1590–1600 (2009).

47. Hertzog, P. I., O’Neill, L. A. & Hamilton, J. I. The interferon in TLR signaling: more than just antiviral. *Trends Immunol.* **24**, 534–539 (2003).

48. Dixit, E. et al. Peroxisomes are signaling platforms for antiviral innate immunity. *Cell* **141**, 668–681 (2010).

49. Ashley, C. L., Abendroth, A., McSharry, R. P. & Slopedam, B. Interferon-independent innate responses to cytomegalovirus. *Front. Immunol.* **10**, 2751 (2019).

50. Ashley, C. L., Abendroth, A., McSharry, R. P. & Slopedam, B. Interferon-independent upregulation of interferon-stimulated genes during human cytomegalovirus infection is dependent on IRF3 expression. *Viruses* https://doi.org/10.3390/v11030246 (2019).

51. Djezghoul, D. et al. Age-associated decrease of the histone methyltransferase SUV39H1 in HSC perturbs heterochromatin and B lymphoid differentiation. *Stem Cell Rep.* **6**, 970–984 (2016).

52. Pasquarella, A. et al. Retrotransposon derepression leads to activation of the unfolded protein response and apoptosis in pro-B cells. *Development* **143**, 1788–1799 (2016).

53. Collins, P. L., Kyle, K. E., Egawa, T., Shinkai, Y. & Oltz, E. M. The histone methyltransferase SETDB1 represses endogenous and exogenous retroviruses in B lymphocytes. *Proc. Natl Acad. Sci. USA* **112**, 8367–8372 (2015).

54. Peters, A. H. et al. Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell* **107**, 323–337 (2001).

55. Hoelder, D., Huang, H., Jain, A. Y., Patel, D. J. & Lewis, P. W. Structural and mechanistic insights into ATRX-dependent and -independent functions of the histone chaperone DAXX. *Nat. Commun.* **8**, 1193 (2017).

56. Hirche, C. et al. Systemic virus infections differentially modulate cell cycle state and functionality of long-term hematopoietic stem cells in vivo. *Cell Rep.* **19**, 2345–2356 (2017).

57. Beerman, I. et al. Functionally distinct hematopoietic stem cells modulate hematopoietic lineage potential during aging by a mechanism of clonal expansion. *Proc. Natl Acad. Sci. USA* **107**, 5465–5470 (2010).

58. Carotta, S., Wu, L. & Nutt, S. L. Surprising new roles for PU.1 in the adaptive immune response. *Immunological Rev.* **238**, 63–75 (2010).

59. Woods, B. A. & Levine, R. L. The role of mutations in epigenetic regulators in myeloid malignancies. *Immunological Rev.* **263**, 22–35 (2015).

60. Kramer, A. & Challen, G. A. The epigenetic basis of hematopoietic stem cell aging. *Semin. Hematol.* **54**, 19–24 (2017).

61. Hu, D. & Shilatifard, A. Epigenetics of hematopoiesis and hematological malignancies. *Genes Dev.* **30**, 2021–2041 (2016).

62. Herquel, B. et al. Trim24-repressed VL30 retrotansomos regulete gene expression by producing noncoding RNA. *Nat. Struct. Mol. Biol.* **20**, 339–346 (2013).

63. Biechonski, S., Yassin, M. & Milyavsky, M. DNA-damage response in hematopoietic stem cells: an evolutionary trade-off between blood regeneration and leukemia suppression. *Carcinogenesis* **38**, 367–377 (2017).

64. Barbieri, D. et al. Thrombopoietin protects hematopoietic stem cells from retrotansomon-mediated damage by promoting an antiviral response. *J. Exp. Med.* **215**, 1463–1480 (2018).

65. Basova, P. et al. Aggressive acute myeloid leukemia in PU.1/p53 double-mutant mice. *Oncogene* **33**, 4735–4745 (2014).

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Methods

Patient sample information. We included H&E staining of skin from patients with pyoderma gangrenosum in our study, which were performed by J.W. at the University of Bonn. The histological picture of the pyoderma gangrenosum lesions was taken from a skin sample of a patient with pyoderma gangrenosum during a normal diagnostic procedure. This routine procedure includes the preparation of H&E samples. Based on German law, it is possible to take pictures from these skin samples (the patient identity is unknown and no additional investigations were performed during these samples). As a result, a ‘patient consent’ is not needed. However, the patient did give informed consent to perform the skin biopsy during the diagnostic procedure. This is also in accordance with the Helsinki Ethical guidelines. J.W. has ethical approval from the University of Bonn, which in principle allows the use of skin material taken for diagnostic proposes in later research (BN090/04).

Experimental model details. Mouse models and treatments. The C57BL/6N Daxx conditional-KO (DaxxΔ174) mice were generated by Taconic Artemis. The targeting vector contained a neoycin (NeoR) gene flanked by flipase sequences (FRT) and a puromycin (PuroR) gene flanked by F3 sites, which were removed by in vivo Flp-mediated recombination. Daxx exons 2-7 were flanked by LoxP sites. C57BL/6N Daxx mice were crossed with C57BL/6J CbyJcre (JAX, 029206), C57BL/6 Mx1Cre (JAX, 035536) or C57BL/6 Rosa26CreERT2 (JAX, 008463) mice. The C57BL/6N Hira conditional-KO (HiraΔ10) mice were provided by P. Adams and crossed with C57BL/6 Mx1Cre mice. The C57BL/6/P1 conditional-KO (P1,Δ10) mice were provided by E. Mass (JAX, 006922) and crossed with C57BL/6 Mx1Cre mice. C57BL/6 Mx1Cre mice were used at 8–12 weeks old (JAX, 002014) for the BM chimaera experiments. Genotyping of the C57BL/6N Daxx mice was performed using an Extract-N-amp tissue PCR kit (Sigma-Aldrich) with the primers DAXX_33 (5′-AGATCTGGTCTCTGTGCTTCC-3′) and DA_27 (5′-CCTGCTACTTATTCTCCAGTCC-3′) and DAXX_recombined (5′-GCTCAGCCTTATGCTGGA-3′). C57BL/6 Nira mice were genotyped using the primers 2292_27 (5′-AATGTTGTCTGTTTGTGG-3′) and 2292_28 (5′-CTTCTGACTCCTTTATCGTGATCC-3′), and the primer 2293_30 (5′-GATGACTATAGGCCAGGCGT-3′) was added for the analysis of recombination efficiency. Genotyping of the C57BL/6/P1.1 mice was performed with the primers P11_Flox_FW (5′-CTTGACTCTGCTTATTGGCTGACT-3′) and P11_Flox_Rev (5′-CTTGGCCGGACAGGTTGGTTAAGGGA-3′) and the primer P11_MUT (5′-CAAAGCGTGATCTAGCTGAGGA-3′) was added to determine the recombination efficiency. Cre mice were genotyped according to protocols released by The Jackson Laboratory. The mice were housed under specific pathogen-free conditions at the central animal facility of the University College London and German Center for Neurodegenerative Diseases. The mice were bred and subjected to listed procedures according to protocols approved by the English (Home Office) and German authorities (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen). Activation of Cre in Daxx; Rosa26RΔ mice was induced by administration of 80 mg kg-1 tamoxifen in corn oil (Sigma-Aldrich) via oral gavage on five consecutive days (ATAC-seq was performed at 3 weeks) or by administration of 100 mg kg-1 tamoxifen in corn oil via intraperitoneal injection (five consecutive days, break of two days, two consecutive days; genomic and phenotypic analyses were performed at 3 and 7 weeks). For the induction of Cre activation in Daxx-KO, Hira-KO and Daxx and P1.1 DKO, Mx1Cre mice received three intraperitoneal injections of 300 µl plpSC (Sigma-Aldrich) administered every other day. The mice were usually treated at 5–11 weeks of age. Mice of both sexes were used in this study. The n value in the figure legends reflects the number of mice analysed in each experiment.

BM chimaera mice. For BM transplantation studies, C57BL/6/CD45.1 mice were received a lethal irradiation dose of 10 Gray before transplantation of 2.5×105 CD45.1 DaxxΔ174;Mx1Cre or DaxxΔ174;Mx1Cre BM cells together with 1×106 CD45.1 BM support cells. Peripheral blood samples were collected from the tail vein or by heart puncture. For the isolation of haematopoietic organs, the mice were euthanized by CO2 exposure. Unless otherwise indicated, the mice were killed two months post treatment. Bone marrow was isolated from the femur and tibia of the hind legs. All isolated organs were directly processed for analysis, frozen for cryopreservation or fixed in neutral buffered formalin (Sigma-Aldrich) for subsequent paraffin embedding. HPCs were isolated from BM using an EasySep mouse haematopoietic progenitor cell isolation kit (STEMCELL Technologies) according to the manufacturer’s instructions. If required, RBCs were lysed for 10 min in ammonium chloride (STEMCELL Technologies) and washed twice before downstream analysis.

Analysis of BM cellularity. Mice were killed two months after induction, except when indicated otherwise. BM cells were isolated from all four hind-leg bones and collected in equal volumes of PBS. The number of cells was determined using a hemocytometer cell counter (Bürker). The RBCs were washed for 10 min in ammonium chloride (STEMCELL Technologies), washed twice and collected in equal volumes of PBS. The number of cells in the samples was calculated again after RBC lysis. If indicated, analyses were performed separately for male and female mice to control for differences in body size and weight.

Flow cytometry and cell sorting. For flow cytometry analysis, cells were isolated from the specified organs. Dead cells were stained by propidium iodide (Sigma-Aldrich) or Aqua Zombie fixable viability dye (BioLegend) according to the manufacturer's protocol. Fluorochrome-labelled antibodies, as indicated in the Supplementary Table 6, were used according to the manufacturer’s instructions. For cell sorting, the Lin- antibody panel included antibodies to B220, CD19, Gr-1, CD11b, NK1.1, TER-119 and CD3e. For RNA-seq, the Lin- antibody panel included antibodies to B220, Gr-1, CD11b, TER-119, CD8, CD4 and CD3e. For staining with multiple brilliant violet dyes, the BD Horizon brilliant stain buffer was used according to the manufacturer’s instructions. For BM and spleen samples, cells isolated from the spleen were first stained with antibodies to CD45, CD11b and CD20, followed by staining with a CellEvent caspase-3/7 green flow cytometry assay kit (Invitrogen) according to the manufacturer’s instructions. The cells were washed and filtered through a 70 µm filter before flow cytometric analysis using a BD LSRFortessa, BD FACSymphony, BD FACSVerse, BD FACS aria III or Beckman Coulter Guava machine. Cell sorting was performed by BD FACS Aria Fusion machine using a 70 µm or 100 µm nozzle. Data were analysed using the FlowJo software. Neutrophils were gated as CD11b+Ly6G−Ly6c+ and eosinophils as CD11b+Ly6c−SSC+cLy6G Gr-1.

CFU assay with re-plating. For the CFU assays, HPCs were isolated from BM as described in the ‘Haematopoietic-cell and organ preparation’ section. The HPCs were plated (15,000 cells per 35 mm dish) in MethoCult M3231 medium supplemented with 100 ng ml-1 SCF, 10 ng ml-1 GM-CSF, 10 ng ml-1 IL-3 and 10 ng ml-1 IL-6 (all STEMCELL Technologies) to promote myeloid-cell formation. The colonies were cultured for 1 week at 37°C. Next, the colonies were counted under a microscope and 15,000 cells were re-plated under the same conditions. After another week of cultivation at 37°C, the colonies were again counted under a microscope.

For the CFU assays with LT-HSCs, Lin- cells were enriched using a MojoSort mouse hematopoietic progenitor cell isolation kit (BioLegend). LT-HSCs were then sorted using a BD FACSaria III cell sorter with antibodies to Lin (B220, CD19, Gr-1, CD11b, CD11c, NK1.1, TER-119 and CD3e), Sca-1, c-Kit, CD150 and c-Kit. The purified Lin− cells were cultured with the following growth factors: SCF (10 ng ml-1), GM-CSF, 10 ng ml-1 IL-3 and 10 ng ml-1 IL-6 (all STEMCELL Technologies) to promote myeloid-cell formation. The cells were cultured for 1 week at 37°C. Then, the colonies were counted under a microscope and 15,000 cells were re-plated under the same conditions. After another week of cultivation at 37°C, the colonies were again counted under a microscope.

RNA isolation and quantitative PCR with reverse transcription. Total RNA was isolated from BM cells using a NucleoSpin kit (Qiagen) according to the manufacturer's instructions. The RNA concentration was determined using a ND1000 Spectrophotometer (NanoDrop), followed by reverse transcription using a High-capacity cDNA reverse transcription kit (Applied Biosystems). Quantitative real-time PCR was performed on a 7500 Fast real-time PCR system (Applied Biosystems) using the Fast SYBR Green master mix (Applied Biosystems) with the primers Mdxass sense (5′-GATGACTATAGGCCAGGCGT-3′), Mdxass antisense (5′-TGCTGCTCCTCTGCTGCTCGG-3′), Mdxara sense (5′-GGTGTGTTTGGGAATCCTGGCTAG-3′), Mdxara antisense (5′-CCGCGCGCGTGCTCC-3′), and Mtx1 sense (5′-AGGCTTCTCTTATTGGCTGACT-3′) and Mtx1 antisense (5′-CCGCTTGAGATGACGCCA-3′). The relative abundance of the specific transcript was normalized to TATA box binding protein (TBP) messenger RNA and calculated using the 2−ΔΔCT method.

Western blotting. Cells were washed in PBS and lysed in RIPA buffer with cComplete protease inhibitor cocktail (Roche). The lysed samples were sonicated for 10 s and the protein concentrations were determined using a Pierce protein assay.
Immunofluorescence and H&E staining. For immunofluorescence imaging, cells were incubated with secondary antibodies for 90 min at room temperature. The cells and tissue sections were mounted with Roti-Mount Aqua (ROTH) medium. Images were obtained on a Zeiss Epi-Scope microscope.

Cytokine analysis. Blood samples were obtained from mice and spun at 1,000g for 10 min at 4 °C to collect the plasma. Cytokine concentrations were determined using a LEGENDplex mouse inflammation panel (BioLegend, 740146) and subsequently processed for paraffin embedding. Tissue sections were cut to 5–15 μm using a CUT5062 microtome (SLEE medical). The sections were washed twice for 5 min in xylene, followed by a 5 min incubation in a 90:10 xylene:ethanol mixture, and subsequently processed for paraffin embedding. Tissue sections were cut to 7–15 μm using a CryoStar NX70 (Thermo Fisher Scientific) cryostat. The cells and tissue sections were fixed in 4% paraformaldehyde for 20 min, washed three times in PBS-T (PBS with 0.1% Triton X-100) and permeabilized for 15 min using 0.3% Triton X-100. Blocking was performed for 1 h in 5% goat serum and the samples were incubated with primary antibodies overnight at 4 °C. The antibodies are listed in Supplementary Table 12. After washing in PBS-T, the sections were incubated with secondary antibodies for 90 min at room temperature. The sections were mounted for 10 min in 1 μg/ml Hoechst 33342 (Thermo Fisher Scientific), followed by additional wash steps. The cells and tissue sections were mounted using Roti-Mount Aqua (ROTH) medium. Images were obtained on a Zeiss Epi-Scope microscope.

Northern blotting. RNA was isolated from BM cells using a Direct-zol RNA miniprep plus kit (Zymo Research) according to the manufacturer’s protocol. Subsequently, DNA was digested using a Turbo DNA-free kit (Thermo Fisher) following the manufacturer’s protocol. The RNA was recovered and concentrated using an RNA clean and concentrator-5 kit (Zymo Research). Northern blot analysis was performed according to standard procedures. Briefly, 5 μg of total RNA was run on a 1% formaldehyde agarose gel, transferred to a Hybond-N+ membrane (GE Healthcare) by capillary transfer and fixed by ultraviolet-light crosslinking. The membrane was pre-hybridized at 42 °C for 20 min and then hybridized in PerfectHyb plus hybridization buffer (Sigma) containing 1 × 106 cpm ml−1 of [32P]-labelled TERRA DNA probe 5′-(TAACCC)5-3′ and 0.1 mg ml−1 herring sperm DNA (Thermo Fisher). Hybridization was carried out overnight at 42 °C. The following day, the membrane was washed once in low stringency buffer (2xSSC and 0.1% SDS) at room temperature for 5 min and twice in high stringency buffer (0.5xSSC and 0.1% SDS) at 42 °C for 20 min. The membranes were exposed to mullatoradiography film at −80 °C. After exposure, the membrane was stripped in boiling stripping buffer. The membrane was sequentially washed in 0.1 M NaCl, 0.1% Tween-20 and water before autoradiography.

RNA-seq of cells collected at steady state. LT-HSCs (Lin−c-Kit+Src-1−Il-7Ra−Flk2 CD48 CD150), GMPPs, and MPMPs (Lin−c-Kit+Src-1−Il-7Ra−Flk2 CD48 CD150) and KLS cells were sorted from BM 3 d.p.i. (RosaCreER mice: LT-HSCs), 3 w.p.i. (Mx1Cre mice: GMPPs and KLS; MMP3 and MMP4 cells) or 24 w.p.i. (Mx1Cre mice: KLS cells and GMPPs). Total RNA was isolated from 2,000–5,000 cells using a Nucleospin RNA XS plus kit (Machery-Nagel) according to the manufacturer’s instructions. The RNA quantity and quality (RINe) were assessed using the HS RNA analysis screen tape assay on a 4200 TapeStation system (Agilent Technologies). Total RNA was converted into double-stranded cDNA libraries as a template for high-throughput sequencing using the SMARTer stranded total RNA-seq kit v.2-pico input mammalian kit (TaKaRa Bio) according to the manufacturer’s instructions. Briefly, after first-strand cDNA synthesis with SMARTer oligonucleotides, barcoded adapters for Illumina sequencing were added via limited-cycle PCR. The PCR products were purified and resuspended in ribosomal cDNA was then denatured. The remaining cDNA fragments were further amplified by PCR (15 cycles) with primers universal to all libraries. After purifying the resulting PCR products, the size distribution of the cDNA library was measured using the Litespeed DNA assay on a Tapestation system (Agilent Technologies) and the cDNA concentrations were determined using a Qubit dsDNA HS assay kit (Thermo Fisher Scientific). After cluster generation, the samples were sequenced either as single-end 1 × 100 bp or paired-end 2 × 100 bp on a NovaSeq 6000 system (Illumina). The experiments were run in duplicate or triplicate.

ATAC-seq of RosaCreER mice at 3 w.p.i. For ATAC-seq, RosaCreER Daxx mice were killed 3 weeks after tamoxifen treatment. The BM was isolated from the femur and tibia, followed by RBC lysis and sorting of CMP (Lin−c-Kit+Src-1−Il-7Ra−Flk2 CD48 CD150) and GMP populations following antibody staining. Enrichment of c-Kit+ cells using CD117 MicroBeads (Milteny Biotec) was performed before sorting LT-HSCs (Lin−c-Kit+Src-1−Il-7Ra−Flk2 CD48 CD150). Between 8,000 and 50,000 flow-sorted cells were collected in flow buffer (PBS, 2% FBS and 2 mM EDTA) and immediately processed following previously published protocols. Briefly, the sorted cells were spun down at 500g for 5 min at 4 °C, washed once in cold PBS and spun down in 50 μl cold lysis buffer (10 mM Tris-Cl (pH 7.4), 10 mM NaCl, 3 mM MgCl2, and 0.1% IGEPAL CA-630) for 500g at 10 min for 4 °C. Immediately thereafter the tagmentation reaction was started by adding Nextra’s Tn5 transposase (TDE1) in reaction buffer. The transposition reaction mix was diluted 1:100 for 30 min at 37 °C, followed by DNA purification using a MinElute PCR purification kit (Qiagen). ATAC-seq libraries were sequenced as a single-read 75 bp, rapid run on the HiSeq 1500 system using HiSeq Rapid v2 chemistry. The resulting data were de-multiplexed using CASAVA version 1.8.4. In addition, ATAC-seq libraries from CMP and GMP cells were sequenced as a single-read 75 bp, rapid run on the HiSeq 1500 system using HiSeq Rapid v2 chemistry. The resulting data were de-multiplexed using CASAVA version 1.8.4. The experiment was run in duplicates.

NTAT-seq of 3 d.p.i. LT-HSCs and 3 w.p.i. KLS cells. LT-HSCs (Lin−c-Kit+Src-1−Il-7Ra−Flk2 CD48 CD150) and KLS cells were sorted from BM 3 d.p.i. (LT-HSCs) or 3 w.p.i. (KLS cells). The LT-HSCs (2,000) and KLS cells (5,000) were directly sequenced as a single-read 75 bp, rapid run on the 4200 TapeStation system (Agilent). The library fragments were sequenced as a single-read 75 bp, rapid run on the HiSeq 1500 system using HiSeq Rapid v2 chemistry. The resulting data were de-multiplexed using CASAVA version 1.8.4. The experiment was run in duplicates.
The libraries were sequenced in a paired-end 2×100 bp run on a NovaSeq 6000 system (Illumina). The experiments were run in duplicate or triplicate.

**Materials and Methods**

**CUT&Tag** HSPCs were isolated by magnetic cell sorting by first enriching Lin− cells using an EasySep mouse hematopoietic progenitor cell isolation kit (STEMCELL Technologies) according to the manufacturer's instructions and then isolatingLin− cells from the resulting cell suspension using mouse CD117 MicroBeads (Miltenyi Biotech). The nuclei were isolated, washed and frozen in wash buffer according to the 'Bench top CUT&Tag V2.0' protocol (https://www.protocols.io/view/bench-top-cut-amp-tag-bcuihews). For the actual CUT&Tag experiment, we followed the CUT&Tag-direct protocol described by Henikoff et al.14,29 using the CUTANA pAG-Tn5 enzyme (Epicypcr). Briefly, aliquots of 30,000–45,000 native nuclei per reaction were bound to activated Concanavalin A beads. After successive incubations with primary (overnight at 4°C) and secondary (0.5–1 h) antibodies in wash buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM spermidine and 1mM protease inhibitor), the beads were washed and resuspended in pAG-Tn5 (1:20 dilution) in 300-wash buffer (wash buffer containing 300 mM NaCl) for 1 h. The incubations were performed at room temperature, except when otherwise stated, in volumes of 25–50 μl in low-retention PCR tubes. Tagmentation was performed for 1 h in 300-wash buffer supplemented with 10 mM MgCl2. Following tagmentation, the beads were washed in 50 μl TAPS buffer (10 mM TAPS pH 8.5 and 0.2 mM EDTA), resuspended in 5 μl TBS release buffer (0.1% SDS and 10 mM TAPS pH 8.5) and incubated for 1 h at 58°C. The TBS was neutralized with 15 μl of 6.7% Triton X-100, and 4 μl of dual-indexed primers from the Illumina Nextera DNA UD Indexes Set A (Illumina) as well as 25 μl of NEBNext high-fidelity 2×PCR master mix (NEB) were added. Gap filling and 18 cycles of PCR were performed, followed by clean-up with 65 μl of SPrIselect beads (Beckman Coulter). We used antibodies, at a 1:20 dilution, to H3K9me3 (Active Motif), H3K27ac (Epicypcr), H3K27me3 (Cell Signaling Technologies), histone H3.3 (Merck Millipore) and Pu.1 (Abcam). The size distribution and concentration of the CUT&Tag libraries were measured using the HS 5000 DNA assay on a 4200 TapeStation system (Agilent Technologies). After cluster generation, the samples were sequenced either as paired-end 2×75 bp on a NextSeq 500 system (Illumina) or as paired-end 2×50 bp on a NovaSeq 6000 system (Illumina). The experiments were run in duplicate or triplicate.

**Biogenetics analysis.** A detailed description of the bioinformatics analyses for the ATAC-seq, RNA-seq and CUT&Tag data can be found in Supplementary Note 1.

**Statistics and reproducibility.** Statistical analyses were performed using GraphPad Prism (GraphPad Software) or the R program. Summarized views on data that underlie the statistical tests as well as the exact P values are available in the source data. The statistical details for each experiment are also provided in the figure legends. Statistical tests were run as two-sided tests, when appropriate. Most experiments were performed independently at least twice; details are provided in the figure legends. The genomic assay data (Figs. 1, 5, 6, 8 and Extended Data Figs. 1–7, 10) are based on two or three biological replicates. The experimental data in Figs. 2g, 3k–p, 3b, 4c–f, 7a, d1 and Extended Data Figs. 2d, 4b, c, 8b,d,e are based on biological replicates (n is given in the figure legends) that were not repeated independently on a different day.

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

**Data availability**

All sequencing data that support the findings of this study can be found at Gene Expression Omnibus under the accession number GSE119309. An overview of the genomics studies run as part of this study can be found in Supplementary Table 1. Previously published sequencing data that were re-analysed here are available under the accession codes GSE60101 and GSE79180 (SRR2062971 and SRR2062968). The GTF and FASTA files used for Bioinformatics analysis (mm10, GENCODE release M14) can be downloaded from GENCODE (https://www.gencodegenes.org/mouse/release_M14.html). All other data supporting the findings of this study are available from the corresponding author on reasonable request. Source data are provided with this paper.

**Code availability**

Rscripts exemplifying the counting of paired-end (Supplementary Code 1) and single-end RNA-seq reads (Supplementary Code 1) are available online in Supplementary Note 1. The custom Rscript to generate non-overlapping mouse gene annotations as well as the created gene annotations used for RNA-seq read counting are available from the corresponding author on request.

**References**

66. Rai, T. S. et al. HIRA orchestrates a dynamic chromatin landscape in senescence and is required for suppression of neoplasia. *Genes Dev.* 28, 2712–2725 (2014).

67. Buenrostro, J. D., Giresi, P. G., Zaba, L. C., Chang, H. Y. & Greenleaf, W. J. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nat. Methods* 10, 1213–1218 (2013).

68. Kaya-Okur, H. S. et al. CUT&Tag for efficient epigenomic profiling of small samples and single cells. *Nat. Commun.* 10, 1930 (2019).

69. Henikoff, S., Henikoff, J. G., Kaya-Okur, H. S. & Ahmad, K. Efficient chromatin accessibility mapping in situ by nucleosome-tethered tagmentation. *eLife* https://doi.org/10.7554/eLife.63274 (2020).

70. Shen, L. et al. diffleps: Detecting differential chromatin modification sites from ChiP-seq data with biological replicates. *PLoS ONE* 8, e55598 (2013).

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**Author contributions**

J.P.G., J.R. and P.S. conceived the original idea, experimental design and overall strategy. V.C. contributed to the experimental design. J.P.G., J.R., V.C., N.O., S.S., N.G., N.I., S.M., K.H., P.D., A.H.K., H.-M.L., S.P., T.S., R.Z. and J.K. conducted and analysed experiments. J.R. and P.S. wrote the manuscript. J.R. led the revisions of the manuscript. P.D.A., T.E., W.M., C.B., J.L.S., M.C., R.N., E.M. and P.S. acquired funding and provided supervision.

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**Competing interests**

The authors declare no competing interests.

**Additional information**

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**Correspondence and requests for materials**

should be addressed to Paolo Salomoni.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Expression of H3.3 chaperones in haematopoietic cells, creating Daxx F/F mice and analysis of LT-HSC ATAC-seq and RNA-seq data. 

a, Heatmap of Daxx, Atrx and Hira expression levels (data from18). 
b, Daxx genomic locus, Daxx floxed locus following homologous recombination, conditional KO allele following deletion of the FRT-flanked or F3-flanked Neomycin (NeoR) and Puromycin (PuroR) resistance gene; Cre-mediated deletion of Daxx exons 2-7. 
c, PCA for ATAC-seq data (3 w.p.i.; top-500 most variable peaks): displayed principal components (PC) 1 versus 2. 
d, Enriched IPA immune system-related canonical pathways for ERV-overlapping enhancer peaks that open upon Daxx KO in LT-HSCs (3 w.p.i.). 
e, Enriched IPA immune system-related biofunctions for ERV-overlapping enhancer peaks that open upon Daxx KO in LT-HSCs (3 w.p.i.). 
f, PCA plot for LT-HSC RNA-seq data (3 d.p.i.). 
g, Counts per million of LINE and SINE elements in LT-HSCs (3 d.p.i.). 
h, Genome browser tracks of the regulatory region of Spi1, Mki67 and Egr7 (3 d.p.i.). 
i, ATAC-seq coverage around TERRA-B5 across all chromosomes or across sex chromosomes at 3 w.p.i. and 3 d.p.i. 
j, Genome browser tracks for wild-type (blue) and Daxx KO (red) LT-HSCs (ATAC-seq: 3 w.p.i. and 3 d.p.i.; RNA-seq: 3 d.p.i.) around Endr1, Mid1, Gm1976 and Wls; TERRA CHIRT-seq based on published data23. Light-blue regions are called TERRA binding sites and yellow region highlights the Gm1976 gene. For Gm1976 and Wls boxes show ATAC-seq coverage at the major TERRA binding site. 

LT.HSC, long-term haematopoietic stem cells; ST.HSC, short-term haematopoietic stem cells; MPP, multipotent progenitor; CMP, common myeloid progenitor; GMP, granulocyte-monocyte progenitor; CLP, common lymphoid progenitor; MEP, megakaryocyte erythroid progenitor. 
n = 2 independent biological samples analysed 3 weeks post induction with tamoxifen (c–e, i, j). Analysis of gene expression and chromatin accessibility of LT-HSCs from n = 2 Daxx +/+ and Daxx +/+F as well as n = 3 Daxx F/F samples collected 3 days post final induction with tamoxifen (f–j). Data are Activation Z-score from IPA Fisher’s exact tests with multiple testing adjusted p-values < 0.05 (d, e). Activation Z-score > 2 suggests increased activation of shown biofunctions. Numerical source data provided in Source data.
Extended Data Fig. 2 | Daxx-deficient BM displays increased proliferation and altered stem/progenitor cell frequencies and numbers. a, Genotyping of Daxx<sup>−/−</sup>;Mx1Cre<sup>−/−</sup> and Daxx<sup>−/−</sup>;Mx1Cre<sup>+/−</sup> mice untreated (−) or treated with pI:pC (+). Arrows indicate the wild-type, floxed and recombined alleles (n = 5 independent experiments). b, Daxx mRNA levels upon pI:pC treatment in bone marrow. Shown is relative expression of Daxx mRNA over Tbp mRNA levels (n = 3 mice per genotype). c, IF of Daxx in bone marrow, scale bar = 10 μm (n = 4 mice per genotype, two independent experiments). Inlay image zoomed on representative nuclei. d, Western blot of Daxx, alpha-tubulin and H3.3 in Lineage-negative and Lineage-positive bone marrow cells (n = 2 mice per genotype). e, Bone marrow cellularity counts before and after RBC lysis in male mice (n = 5 mice per gender, non-parametric Mann–Whitney test, box-and-whiskers plot: min to max (whiskers), 25<sup>th</sup> and 75<sup>th</sup> percentile and median). f, Bone marrow cellularity counts before and after RBC lysis in female mice (n = 5 mice per gender, non-parametric Mann–Whitney test, box-and-whiskers plot: min to max (whiskers), 25<sup>th</sup> and 75<sup>th</sup> percentile and median). g, Flow cytometry analysis of Ki-67<sup>+</sup> cells in BM and HPCs (n = 5 mice per genotype, repeated in two independent experiments, non-parametric Mann–Whitney test). h,i, Flow cytometry analysis of HSC and MPP populations (n = 3 mice per genotype, repeated in two independent experiments, Student’s t-test). BM, bone marrow; HPC, haematopoietic progenitor cells; HSC, haematopoietic stem cell; MPP, multipotent progenitors. Data in box plots are mean and min to max. ns, not significant; * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001. Exact p-values and numerical source data can be found in the accompanying source data. Unprocessed blots provided in Source data.
Extended Data Fig. 3 | Daxx deficiency results in myeloid/lymphoid imbalance in the bone marrow. a, Flow cytometry analysis of myeloid cell surface markers (n = 6 mice per genotype, repeated in three independent experiments). b, Flow cytometry analysis of lymphoid cell surface markers (n = 6 mice per genotype, repeated in three independent experiments). c, Frequencies of B cell progenitors (n = 6 mice per genotype, repeated in three independent experiments). d, Western blot of Daxx, H3.3 and corresponding β-actin control in isolated B220+ cells from bone marrow (n = 3 mice per genotype, repeated in two independent experiments). e, Bones isolated from two Daxx+/+;Mx1Cre and Daxx-/-;Mx1Cre mice. f, Frequencies of erythroblast populations (n = 6 mice per genotype, repeated in three independent experiments). Data in box plots are mean and min to max. ns, not significant; * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001. Wilcoxon rank test. Exact p-values and numerical source data can be found in the accompanying source data. Source image file provided in Source data.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Loss of Daxx leads to neutrophilia and B-cell lymphopenia in the periphery. a, Flow cytometry analysis of peripheral blood collected 4 weeks post induction (n=6 mice per genotype, repeated in three independent experiments). b, Cxcr2 levels in mature neutrophils of the blood (n=3 mice per genotype, Student’s t-test). c, Quantification of WBC, RBC and platelets using Sysmex (n=3 mice per genotype). d, IF images of spleen stained for Daxx and B220 (n=2 mice per genotype, repeated in two independent experiments). Scale bar indicates 10 µm. e, Western Blot of Daxx protein in spleen (n=3 mice per genotype, repeated in three independent experiments). f, H&E stain of spleen sections in Cre-negative animals, scale bar = 100 µm (n=2 mice per genotype). g, Representative flow cytometry plots showing Gr-1+/Ly6C− populations in bone marrow upon CD11b+, CD11c−, Ly6G−, SSC− gating. h, Frequencies of Ki67+ cells in spleen (n=5 mice per genotype, repeated in two independent experiments). i, Frequencies of erythroblast populations in spleen (n=6 mice per genotype, repeated in three independent experiments). Data in box plots are mean and min to max. ns, not significant; * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001, Wilcoxon rank test if not otherwise noted. Exact p-values and numerical source data can be found in the accompanying source data. Source image file provided in Source data.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Loss of the H3.3 chaperone Hira has marginal effects on haematopoiesis. a, Representative images of genotyping results using primers to detect WT, floxed and recombined bands of Hira in bone marrow cells (n = 3 independent experiments). b, Hira mRNA levels upon pl:pC treatment in bone marrow cells. Shown is relative expression of Hira mRNA over Tbp mRNA levels (n = 3 mice per genotype). c, Representative Western blots for Daxx and β-Actin in total bone marrow of Daxx^+/−, Hira^fl/fl^ and Daxx/Hira double KO Mx1Cre mice (n = 3 mice per genotype, repeated in two independent experiments). d, Bones of WT, Daxx KO and Hira KO mice. e, Frequencies of erythroblast populations in bone marrow (WT mice n = 9; Daxx KO n = 3; Hira KO n = 6). f, Flow cytometry analysis of mature cell markers in bone marrow (WT mice n = 9; Daxx KO n = 3; Hira KO n = 6). g, Flow cytometry analysis of B cells in bone marrow (WT mice n = 9; Daxx KO n = 3; Hira KO n = 6). h, Frequencies of neutrophils in bone marrow (WT mice n = 9; DKO n = 3; HKO n = 6). i, Frequencies of neutrophils in spleen (WT mice n = 9; Daxx KO n = 3; Hira KO n = 6). j, Spleens isolated from WT, Daxx KO and Hira KO mice. k, Flow cytometry analysis of mature cells in spleen (WT mice n = 9; Daxx KO n = 3; Hira KO n = 6). l, Flow cytometry plots gated on monocyte- and macrophage-like populations in spleen. m, H&E stain of spleen sections (n = 3 mice per genotype), scale bar = 100 µm. Higher magnification, scale bar = 20 µm. Data produced 3 weeks post pl:pC treatment. Data in box plots are mean and min to max. ns, not significant; * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001, Wilcoxon rank test. Exact p-values and numerical source data can be found in the accompanying source data. Source image file provided in Source data.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | The haematopoietic phenotype of Daxx deletion is due to a cell-intrinsic defect. a, Number of colonies after first plating (P0) and first passage (P1) using haematopoietic progenitor cells (n = 5 mice per genotype, repeated in three independent experiments, box-and-whiskers plot: min to max, 25th to 75th percentile and median). b, Number of colonies after first plating (P0) and first passage (P1) using LT-HSCs (n = 2 mice per genotype). c, Number of cells after first plating (P0) and first passage (P1) using LT-HSCs (n = 2 mice per genotype). d, Number of KLS cells and e, number of CD11b+ cells and neutrophil-like cells (Ly6C+ ,Ly6G+) after first plating (P0) of LT-HSCs and first passage (P1; n = 2 mice per genotype). f, Schematic representation of the experimental set-up to generate bone marrow chimeras. g, Frequencies of CD45.1+ and CD45.2+ cells in transplanted mice (n = 8 mice per genotype). h, WBC counts in total bone marrow (n = 8 mice per genotype, box-and-whiskers plot: min to max, 25th to 75th percentile and median). i, Flow cytometry analysis in peripheral blood of transplanted mice at two different time points: 1st: 4-5 weeks post transplantation; 2nd pooled data from two independent experiments collected at 8 or 12 weeks post transplantation (n = 8 mice per genotype). j, Frequencies of mature CD45.2+ blood cells in bone marrow of recipient mice (n = 8 mice per genotype, repeated in three independent experiments). k, Frequencies of CD45.2+ KLS and GMPs in bone marrow (n = 4 mice per genotype, repeated in two independent experiments). l, Flow cytometry analysis of CD45.2+ mature blood cells in spleen of recipient mice (n = 8 mice per genotype, repeated in three independent experiments). BM, bone marrow; PB, peripheral blood; KLS, c-Kit+, lineage− , Sca-1+; GMP, granulocyte-monocyte progenitor; P0, first plating; P1, first passage. Data in box plots are mean and min to max. ns, not significant; * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001, Wilcoxon rank test. Exact p-values and numerical source data can be found in the accompanying source data.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Gene expression changes in Daxx-deficient haematopoietic progenitors.  

**a**, PCA of the top-500 most variable genes for GMPs.  

**b**, Trajectory of gene expression of KLS collected at 3 w.p.i., 24 w.p.i. or from transplanted animals.  

**c**, Boxplot showing mean pseudotime for each sample group for GMPs (n=2 mice per genotype).  

**d**, Heatmap showing scaled expression of selected transcription factors and regulators involved in blood cell differentiation (n=2 mice per genotype).  

**e**, Top 5 haematologic biofunctions and diseases altered at 3 w.p.i. in KLS Daxx KO cells.  

**f**, Top 5 haematologic biofunctions and diseases altered at 24 w.p.i. in KLS Daxx KO cells.  

**g**, Top 5 haematologic biofunctions and diseases altered in KLS Daxx KO cells isolated from transplanted animals.  

**h**, Top 5 haematologic biofunctions and diseases altered in GMP Daxx KO cells collected at 3 w.p.i., 24 w.p.i. and from transplanted animals.  

**i**, Boxplots of mean normalized read counts of ERV subtypes and satellite repeats (n=2 mice per genotype) in GMP cells.  

**j**, Heatmap showing scaled expression of interferon response genes and the dsRNA recognition machinery for GMPs. Data in box plots are min to max and mean. Numerical source data can be found in the accompanying source data.
Extended Data Fig. 8 | Changes in the RNA-sensing machinery and TERRA expression following Daxx loss. 

**a**, IF images of spleens stained for B220, Mda5 and dsRNA (n = 2 independent experiments), scale bar = 20 µm. 

**b**, Northern blot autoradiograph showing TERRA levels and quantification analysis (n = 3 mice per genotype, unpaired t-test with Welch's correction).

**c**, Barplot showing the overlap of differentially expressed genes in TERRA knockdown cells with DEG in our KLS and GMP Daxx KO datasets (transplantation) compared to random genes (Fisher's Exact Test; **p-value < 0.01, ns, not significant). 

**d**, Quantification of B cells and neutrophils in bone marrow, spleen and peripheral blood at 3 weeks post induction (n = 2 mice per genotype).

**e**, Quantification of B cells and neutrophils in bone marrow, spleen and peripheral blood at 24 weeks post induction (n = 2 mice per genotype). Data in box plots are min to max and mean. Exact p-values and numerical source data can be found in the accompanying source data.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | RNA-seq data from wild-type, Daxx single KO, Daxx Pu.1 double KO and Pu.1 single KO KLS and GMP cells collected at 3 w.p.i.

a, Venn diagrams of differentially upregulated and downregulated genes in comparison to wild-type KLS. b, Venn diagrams of differentially upregulated and downregulated genes in comparison to wild-type GMP cells. c, IPA graphical summary for KLS Daxx Pu.1 double knock-out specific and Pu.1 single KO specific gene expression changes. d, IPA graphical summary for GMP Daxx Pu.1 double knock-out specific and Pu.1 single KO specific gene expression changes. e, Heatmap showing scaled expression of selected transcription factors and regulators involved in blood cell differentiation (n=2 mice per genotype). f, Heatmap showing scaled expression of transcription factors regulated by Pu.1 (n=2 mice per genotype). g, Heatmap showing scaled expression of interferon response genes and the dsRNA recognition machinery (n=2 mice per genotype). Numerical source data provided in Source data.
Extended Data Fig. 10 | Changes in gene expression are associated with increased accessibility at TERRA-BS in sex chromosomes. a, ATAC-seq coverage around TERRA-BS across all chromosomes or across sex chromosomes at 3 w.p.i. b, Genome browser tracks for wild-type (blue), Daxx KO (red) and Daxx/Pu.1 KO (green) KLS cells at 3 w.p.i. around Erdr1 and Mid1; TERRA CHIRT-seq based on published data. Light-blue regions are called TERRA-BS. c, Proposed model: Daxx contributes to epigenetic barriers restricting fate/identity in haematopoietic stem cells and progenitors thus contributing to balanced differentiation output. Upon Daxx loss, stem cells enter differentiation and produce both myeloid and lymphoid-biased progenitors. However, while myeloid differentiation towards neutrophils is enhanced leading to inflammation, generation of mature B cells is strongly impaired. By inhibiting the pioneer TF Pu.1, the block of B-cell differentiation is partly relieved, while peripheral accumulation of neutrophils is hampered. Given that Pu.1/Daxx DKO progenitors display unique chromatin and transcriptome features and that both Daxx and Pu.1 inactivation/repression are linked to myeloid leukaemia, we hypothesize that loss of both genes may make progenitor cells susceptible to neoplastic transformation.
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Software and code

Policy information about availability of computer code

Data collection

Primary processing of ATAC-seq data was performed with CASAVA [1.8.4], FastQC [0.11.5], Trimmomatic [0.36 with options -phred33, seedMismatch=2, pairedEndClip=0.3, simpleClipThreshold=30, simpleClipThreshold=10 and option MINLEN=36], STAR [2.5.3 with option -aAlignIntronMax 1 and -aAlignMatesGapMax 1800], samtools [1.4.1], MarkDuplicates (option REMOVE_DUPLICATES=true) from Picard tools [2.9.2] and bedtools [2.25.0].

Primary processing of RNA-seq data was performed with CASAVA [1.8.4], FastQC [0.11.5], Cutadapt [1.13], STAR [2.5.3], samtools [1.4.1] and the PORT pipeline (https://github.com/mtmas/Normalisation).

CUT&Tag was performed on HSPCs (Lineage-negative, c-Kit-positive) isolated by MACS. Primary processing was done with CASAVA [1.8.4], FastQC [0.11.5], bowtie2 [4.8.5], samtools [1.4.1] and bedtools [2.25.0].

A detailed description can be found in the Methods section and all tools and datasets used are listed in the Supplementary Information.

Data analysis

ATAC-seq data was analyzed using the ENCODE ATAC-seq analysis pipeline [0.3.4]. Resulting peak files were annotated with the HOMER software package [4.9.1] as described in the Methods section. Genome Browser coverage plots and profile plots were created with deepTools [3.0.2] and Pathway analysis was performed with the Ingenuity Pathway analysis pipeline from Qiagen.

Statistical analysis was run in R [3.4.0] and GraphPad Prism [7.03], which were also used for plotting. Gene set enrichment analysis (GSEA) was performed with the GSEA program provided by the Broad Institute as described in the Methods section. Differential transposable element RNA expression was determined by applying the TEToolkit [2.0.2] as described in the Methods section. Differential expression analysis employed the R or Bioconductor packages GenomicFeatures [1.28.3], biomaRt [2.32.1], Rsamtools [1.28.0], GenomicAlignments [1.12.2] and DESeq2 [1.16.1]. Genomic regions differentially enriched in H3.3, H3K27ac, H3K9me3, H3K27me3 or Puf1 were called by using diffrexps. Differential regions were annotated using the region_analysis package from diffrexps.

FACS data was analyzed with FlowJo [10.6.1].

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All sequencing data related to the manuscript that support the findings of this study can be found at Gene Expression Omnibus under the accession number GSE119390. An overview of the genomics studies run as part of this study can be found in Supplementary Table 11. Previously published sequencing data that were reanalyzed here are available under the accession codes GSE603101 and GSE79180 (SRR2062971 and SRR2062968). GTF and FASTA files used for Bioinformatics analysis (mm10, GENCODE release M14) can be downloaded from GENCODE (https://www.genecodegenes.org/mouse/release_M14.html). Source Data for Figs. 1-8 and Extended Data Figs. 1-8 are available online. All other data supporting the findings of this study are available from the corresponding author on 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 | Preliminary experiments were run to test for biological outcome. On the basis of the statistical analysis of this data, the sample size required to achieve statistically significant results was calculated using Power Analysis. Sample sizes are noted in each case. |
| Data exclusions | Data was partially excluded from the analysis of cytokine concentrations using Legendplex Mouse Inflammation Panel (Biolegend, 740446) and Mouse HSC Panel (Biolegend, 740677). The kits include a standard that is used for reference in the LEGENDplexTM Data Analysis Software. For several cytokines the analyzed concentrations of our samples were either not detectable or outside the analyzable range of the standard curve. In this case, single samples were excluded. Statistical analysis were run only if at least three biological replicates were available. The cytokines included in the kit that did not fall into the analyzable range of the standard curve are not shown as data in the manuscript. |
| Replication | Experiments run on the same day always included wild-type and knock-out mice. For most experiments (except for ATAC-seq, RNA-seq and CUT&Tag samples) included at least one replicate experiment run on a different date. The attempts of replication were successful and no experiment was excluded. All samples and mice were handled following the same protocols. Due to limitations in animals (numbers approved by authorities and COVID pandemic related restrictions for animal breeding) and complexity of required cell extraction, cell sorting and genomics library preparation, genomics-based experiments were run with biological replicates but without repetition at different dates. |
| Randomization | Sample allocation was random and mice for experiments were randomly chosen based on availability of the right genotypes. |
| Blinding | The investigators were not blinded to the mouse group allocation nor when assessing the outcome of experiments to ensure monitoring of mice for occurrence of skin lesions or signs of leukemia (in this case mice needed to be sacrificed). Blinding for analysis was not necessary as total cells are isolated from the different organs and cells are randomly assessed within the flow cytometer. To avoid bias during cell sorting (which are randomly assessed) the same sorting gates are used for all samples run on the same date. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
| ☐ | Antibodies |
| ☒ | Eukaryotic cell lines |
| ☒ | Palaeontology |
| ☐ | Animals and other organisms |
| ☐ | Human research participants |
| ☒ | Clinical data |

Methods

| n/a | Involved in the study |
| ☐ | ChIP-seq |
| ☒ | Flow cytometry |
| ☒ | MRI-based neuroimaging |
Antibodies

Antibodies used
Antibodies used are described in detail in Supplementary Table 12.

Validation
All antibodies used are commercially available antibodies and detailed validation information of each antibody can be found from the manufacturer, whose information can be found in Supplementary Table 12.

Animals and other organisms

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

Laboratory animals
CS7BL/6N Daxx F/F mice were crossed with C57BL/6J Csflr-Cre mice [JAX 029206], C57BL/6J Mx1Cre mice [JAX 003556] or C57BL/6J Rosa26CreERT2 mice [JAX 008463]. The C57BL/6J Hira conditional KO (HiraF/F) mice were crossed with C57BL/6J Mx1Cre mice [JAX 003556]. The C57BL/6J Pu.1 conditional KO [Pu.1F/F; JAX 006922] were crossed with C57BL/6 DaxxF/F;Mx1Cre mice. For BM chimera experiments C57BL/6J CD45.1 mice were used [JAX 002014]. Activation of Cre in Daxx;RosaCreER mice was induced by administration of 80 mg/kg tamoxifen in corn oil (Sigma-Aldrich) via oral gavage on five consecutive days or by administration of 100 mg/kg tamoxifen in corn oil via i.p. injection on five consecutive days, two days break, followed by 2 more consecutive days. For induction of Cre activation Daxx KO, Hira KO and Daxx Pu.1 double KO Mx1Cre mice received intraperitoneal injection with 300 µg polyinosinic-polycytidylic acid (plC, Sigma-Aldrich) three times every other day. Mice were usually treated between 5-11 weeks of age. Mice of both genders were used in this study. The n value in Figure legends reflects the number of mice analyzed in each experiment.

Wild animals
The study did not involve wild animals.

Field-collected samples
The study did not involved field-collected samples.

Ethics oversight
All mouse experiments were performed in compliance with the UK and German Law for Welfare of Laboratory Animals and were approved by the Home Office in the UK [Project license 70-8240] as well as by the Landesamt fuer Natur, Umwelt und Verbraucherschutz [LANUV] of Nordrhein-Westfalen, Germany [Project license 84-004.04.2016.A486].

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
We included H&E stainings of skin from patients with Pyoderma gangraenosum in our study.

Recruitment
The text below is adapted from an email from Prof Wenzel that I have forwarded to the Editorial office.

The histological picture of the PG lesions has been taken from a skin sample that had been taken from a PG patient within the normal diagnostic procedure. This routine procedure includes the preparation of H&E samples. Following German law is it fine to make pictures from these skin samples (as it is not possible to recognize the patient and no additional investigations have been done with this sample), so a "patient consent" is not needed. This is also, as far as I know, in accordance with the Helsinki Ethical guidelines. However, the patient has given his informed consent to the skin biopsy within the diagnostic procedure.

Ethics oversight
Prof Wenzel has ethical Votum from the University of Bonn that allows to use skin material that had been taken for diagnostic proposes for research afterwards (BDN090/04) in principle, but Prof Wenzel doesn’t have a specific consent of this patient to make a picture of his H&E slide, because it wasn’t needed to do this.

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

ChIP-seq

Data deposition
✓ Confirm that both raw and final processed data have been deposited in a public database such as GEO.
✓ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
Raw sequencing data was deposited in GEO under accession number GSE119309.

Files in database submission
See Supplementary Table 11

Genome browser session
[e.g. UCSC]
Methodology

Replicates
2-3 biological replicates

Sequencing depth
about 10 million paired-end reads per sample

Antibodies
Antibodies against H3.3, Pu.1, H3K9me3, H3K27me3 and H3K27ac. Details can be found in Supplementary Table 12.

Peak calling parameters
Online Methods

Data quality
Online Methods

Software
Online Methods

Flow Cytometry

Plots
Confirm that:
- The axis 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
Peripheral blood samples were collected from the tail vein of living mice. Bone marrow (BM) was isolated from femur and tibia of the hind legs and spleen cells from spleen of euthanized mice. Spleens were homogenized into single cells suspensions through a 70 μm cell strainer. Unless red blood cells (RBC) were the cell population to be analyzed, RBC lysis was performed for 10 min in ammonium chloride and washed twice prior to antibody staining. Haematopoietic progenitor cells (HPC) were isolated from BM using the EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Kit (STEMCELL Technologies) according to manufacturer’s instructions.

Instrument
Flow cytometry analysis was performed on a BD LSR Fortessa, BD FACS Symphony, BD FACS Celesta, BD FACS Aria III or Beckman Coulter Gallios machine.
Cell sorting was performed on a BD FACS Aria III or BD FACS Aria Fusion machine using a 70 μm or 100 μm nozzle.

Software
Collection of data on BD FACS machines was performed by using BD FACSDIVA software. Collection of data on Beckman Coulter Gallios machines was performed by using Beckman Coulter KALUZA software. Data was analyzed using FlowJo (FlowJo, LLC) software.

Cell population abundance
For RNA sequencing CD45.2+ KLS (Lin−, CD45.2+, c-kit+, Sca1+) and GMP (Lin−, CD45.2+, c-kit+, Sca1−, CD16/32−, CD34+) populations were sorted from the bone marrow of transplanted mice. Haematopoietic stem cells (KLS) are of very low and GMP cells of low abundance in the bone marrow. Minimum 4700 cells were used for the RNA isolation. For ATAC seq sorting of CMP (Lin−, CD45.2+, c-kit+, Sca1−, CD16/32−, CD34+) and long-term HSCs (Lin−, CD45.2+, c-kit+, Sca1+, CD48−, CD150+) was performed. HSCs are of very low and GMP/CMP cells of low abundance in the bone marrow. Minimum 8000 cells were processed for ATACseq. To enrich frequency of haematopoietic stem cells in the sorting population, c-kit enrichment was performed prior to sorting of long term HSCs.
To minimize the isolation of other cell populations during sorting, strict gating strategies were set based on FMO controls and unstained samples. Purity check of samples after sorting was performed by running small amounts of the sorted samples through the sorter.

Gating strategy
The typical gating strategy was exclusion of cell debris on FSC/SSC plot, followed by single cell gating on FSC-H/FSC-A plot, followed by exclusion of dead cells using live/dead cells stains propidium iodide or Aqua Zombie fixable viability dye, followed by gating on the interesting cell populations based on the fluorochrome.
For setting positive and negative gates, unstained controls, single stained controls or FMO controls were used.

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