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A KRAB/KAP1-miRNA Cascade Regulates Erythropoiesis Through Stage-Specific Control of Mitophagy

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Abstract

During hematopoiesis, lineage- and stage-specific transcription factors work in concert with chromatin modifiers to direct the differentiation of all blood cells. Here, we explored the role of KRAB-containing zinc finger proteins (KRAB-ZFPs) and their cofactor KAP1 in this process. Hematopoietic-restricted deletion of Kap1 in the mouse resulted in severe hypoproliferative anemia. Kap1-deleted erythroblasts failed to induce mitophagy-associated genes and retained mitochondria. This was due to persistent expression of microRNAs targeting mitophagy transcripts, itself secondary to a lack of repression by stage-specific KRAB-ZFPs. The KRAB/KAP1-miRNA regulatory cascade is evolutionary conserved, as it also controls mitophagy during human erythropoiesis. Thus, a multilayered transcription regulatory system is present, where protein- and RNA-based repressors are super-imposed in combinatorial fashion to govern the timely triggering of an important differentiation event.

Through the process of erythropoiesis, about one hundred billion new red cells are generated every day in the human adult bone marrow. This process is initiated by the differentiation of hematopoietic stem cells (HSC) into the earliest erythroid progenitor, which was identified ex vivo as a slowly growing burst-forming unit-erythroid (BFU-E). This erythroid progenitor morphs into the rapidly dividing CFU-E (colony-forming unit-erythroid), the proliferation of which is stimulated by the hypoxia-induced hormone erythropoietin. Further differentiation occurs through a highly sophisticated program orchestrated by lineage- and stage-specific combinations of protein- and RNA-based transcription regulators (1–3). It culminates in the elimination of intracellular organelles including mitochondria and the nucleus to yield the fully mature erythrocyte, containing on the order of 250 million molecules of hemoglobin as almost sole cargo. Much is still to be learned about the molecular mechanisms of these events, not only to understand the cause of red cell disorders, but also to aid the in vitro manufacturing of the large supplies of oxygen-carrying cells for transfusion.

Higher vertebrate genomes encode hundreds of KRAB-ZFPs that can bind DNA in a sequence-specific fashion through a C-terminal array of C2H2 zinc fingers and recruit the

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corepressor KAP1 via their N-terminal KRAB domain (4–7). KAP1, also known as TRIM28 (tripartite motif protein 28), TIF1β (transcription intermediary factor 1 beta) or KRIP-1 (KRAB-interacting protein 1), acts as a scaffold for a multimolecular complex that silences transcription through the formation of heterochromatin (8–11). The KRAB/KAP1 system probably evolved initially to minimize retroelement-induced genome perturbations (12–14), but recent data indicate that it also regulates multiple aspects of mammalian physiology (15–24). The present study was undertaken to explore its role in hematopoiesis.

The hemato-specific knockout of Kap1 in the mouse, whereby the hematopoietic system of otherwise wild type animals is reconstituted from Kap1-deleted hematopoietic stem cells and progenitors (fig. S1), resulted in a series of hematological abnormalities (table S1). Mutant mice displayed fatal hyporegenerative anemia, characterized by the accumulation of transferrin receptor/CD71+ glycophorin-A-associated/Ter119− early erythroblasts and an almost complete absence of mature CD71−Ter119+ cells in the bone marrow (Fig. 1A). Electron microscopy and Mitotracker staining revealed that KO erythroblasts contain more mitochondria than their wild type counterparts (Fig. 1B), correlating with decreased expression of mitophagy genes such as Nix/Bnip3L, Ulk1, GABARAPl2, Sh3glb1, Atg12, Becn1 and Bcl2l1 (Fig. 2A). Since the KRAB/KAP1 pathway is mostly known to induce transcriptional repression (10, 11), it seemed likely that this effect was indirect. An examination of the miRNA expression profile of control and Kap1 KO CD71+Ter119+ cells revealed that, among 455 miRNAs tested, 5 were downregulated and 11 upregulated more than two-fold in KO cells (data are presented in the Gene Expression Omnibus dataset GSE44061). A recently described in silico approach (25, 26) suggested that six of these upregulated miRNAs had mitophagy-associated deregulated transcripts as their targets, notably miR-351, predicted to act on Bnip3L (Fig. 2A). Consistent with this hypothesis, levels of miR-351 abruptly dropped in CD71+Ter119+ cells, compared to their CD71+Ter119− precursors, mirroring Bnip3L induction (Fig. 2B). Furthermore, transduction of mouse erythroleukemia (MEL) cells with a GFP-expressing lentiviral vector harboring, 3′ of GFP, the Bnip3L 3′UTR sequence predicted to be targeted by miR-351 resulted in miR-351-dependent downregulation of the reporter (Fig. 2C). Finally, similar to their KAP1-depleted counterparts, miR-351-overexpressing MEL cells were blocked in differentiation and accumulated mitochondria, and this phenotype was reversed by expression of a Bnip3L transcript devoid of this 3′UTR sequence (fig. S2).

MiR-503 and miR-322*, which are located next to miR-351 on chromosome X, were also upregulated (2.46 and 2.17 fold, respectively) in Kap1 KO erythroblasts. Consistent with a role for KRAB/KAP1 in regulating this miRNA gene cluster, chromatin immunoprecipitation coupled to DNA sequencing (ChIPSeq) detected a strong KAP1 peak less than 4kb away (Fig. 3A). Because KAP1 is not a DNA binding protein, we postulated that it might be tethered to this and other relevant loci by stage-specific KRAB-ZFPs. Nine KRAB-ZFP genes were identified, which had human orthologs and were expressed exclusively in CD71+Ter119− and/or CD71+Ter119+ erythroblasts, but not in other hematopoietic cells. Six of these genes could be efficiently knocked down in MEL cells by lentivector-mediated RNA interference, and two of them, ZFP689 and ZFP13, emerged as potential Bnip3L regulators (fig. S3). Interestingly, ZFP689 is expressed in CD71+Ter119+ erythroblasts, whereas ZFP13 is expressed only in their CD71−Ter119+ counterparts (Fig. 3B). Both could repress reporter expression in MEL cells transduced with a lentiviral vector harboring the miR-351-close KAP1-binding site upstream of a human phosphoglycerate kinase promoter murine secreted alkaline phosphatase (mSEAP) cassette (Fig. 3C). We then validated these two candidates in vivo by transplanting CD45.2 hematopoietic stem cells (lineage−, Sca1+ and cKit+, or LSK) transduced with lentiviral vectors producing GFP and shRNAs against Zfp689, Zfp13, or Kap1 as a control, into irradiated CD45.1 mice, allowing the dual discrimination of donor vs. recipient and transduced vs. untransduced cells.

Analyses of the red cell compartment in bone marrow harvested eight weeks after the graft revealed that knockdown of either Zfp689 or Zfp13 led to a decrease in CD71+Ter119− cells as pronounced as that observed with the Kap1 knockdown (Fig. 3D). Furthermore, RNA analyses of sorted transduced CD71+Ter119+ cells demonstrated that ZFP689−, ZFP13− and KAP1-depleted cells all exhibited an upregulation of miR-351 (Fig. 3E) and a marked downregulation of Bnip3L (Fig. 3F).

In a last series of experiments, we asked whether this erythropoiesis-regulating system has its equivalent in humans. We first found that Kap1 knockdown impaired the differentiation of human erythroleukemia (HEL) cells and increased their mitochondrial content (Fig. 4ABC), blocking several mitophagy effectors including Nix/Bnip3L (Fig. 4D). We further verified that KAP1-depleted HEL cells had increased levels of hsa-miR-125a-5p (Fig. 4D), which has the same seed as murine miR-351, and that overexpressing this miRNA triggered a downregulation of Nix and a rise in the mitochondrial content of these cells (Fig. 4E). Finally, when we knocked down Kap1 in human cord blood CD34+ cells, it resulted in decreasing their ability to undergo cytokine-induced ex vivo erythroid differentiation, which correlated with reduced Nix expression and elevated mitochondrial content (Fig. 4F), a phenotype that could be reproduced by hsa-miR-125a overexpression (Fig. 4G).

These results unveil a multilayered transcription regulatory system, where protein- and RNA-based repressors are super-imposed in combinatorial fashion to govern the timely triggering of a necessary step of erythropoiesis. miR-351 and several other microRNAs with predicted targets in the mitophagy pathway were upregulated in Kap1-deleted murine erythroblasts (Fig. 2). This apparent redundancy, or rather addition of parallel effects aimed at a same physiological process, is commonly observed with RNA interference (27). Our discovery that it can be further modulated by KRAB-ZFP-mediated repression, and that the latter can itself be multifactorial, adds a remarkable level of modularity to this type of regulation. In human erythroblasts, although KAP1 represses the Nix-targeting hsa-miR-125a-5p, downregulation of several other miRNAs, including hsa-miR-24, -221, -222, and -223, was previously found important for erythroid differentiation, which conversely requires the upregulation of hsa-miR-144/451 cluster (2, 3). Whether stage-specific KRAB-ZFPs are involved in controlling some of these other miRNAs remains to be determined. Even though KAP1 likely influences erythropoiesis by more than just allowing mitophagy, it is interesting to note that Znf205 and Znf689, the respective human orthologs of murine Zfp13 and Zfp689, are expressed in HEL cells and induced upon erythroid differentiation of CD34+ cells (fig. S4). Therefore, polymorphism or mutations in any genetic component of the pathway unveiled here, whether Znf205, Znf689, the genomic binding sites of their products, hsa-miR-125a-5p and other KAP1-regulated miRNA genes, or the sequences targeted by these RNA regulators, could underlie red cell-related pathologies such as anemia, polycythemia, or erythroleukemia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and Notes

1. Xu J, et al. Combinatorial assembly of developmental stage-specific enhancers controls gene expression programs during human erythropoiesis. Dev. Cell. 2012; 23:796. doi:10.1016/j.devcel.2012.09.003. [PubMed: 23041383]
2. Hattangadi SM, Wong P, Zhang L, Flygare J, Lodish HF. From stem cell to red cell: Regulation of erythropoiesis at multiple levels by multiple proteins, RNAs, and chromatin modifications. Blood. 2011; 118:6258. doi:10.1182/blood-2011-07-356006 Medline.
3. Lawrie CH. microRNA expression in erythropoiesis and erythroid disorders. Br. J. Haematol. 2010; 150:144. Medline. [PubMed: 19912217]
4. Bellefroid EJ, Poncelet DA, Lecocq PJ, Revelant O, Martial JA. The evolutionarily conserved Krüppel-associated box domain defines a subfamily of eukaryotic multifingered proteins. Proc. Natl. Acad. Sci. U.S.A. 1991; 88:3608. doi:10.1073/pnas.88.9.3608 Medline. [PubMed: 2023909]
5. Vaquerizas JM, Kummerfeld SK, Teichmann SA, Luscombe NM. A census of human transcription factors: Function, expression and evolution. Nat. Rev. Genet. 2009; 10:252. doi:10.1038/nrg2538 Medline. [PubMed: 19274049]
6. Venter JC, et al. The sequence of the human genome. Science. 2001; 291:1304. doi:10.1126/science.1058040 Medline. [PubMed: 11181995]
7. Chinwalla AT, et al. Initial sequencing and comparative analysis of the mouse genome. Nature. 2002; 420:520. doi:10.1038/nature01262 Medline.
8. Nielsen AL, et al. Interaction with members of the heterochromatin protein 1 (HP1) family and histone deacetylation are differentially involved in transcriptional silencing by members of the TIF1 family. EMBO J. 1999; 18:6385. doi:10.1093/emboj/18.22.6385 Medline. [PubMed: 10562550]
9. Quenneville S, et al. The KRAB-ZFP/KAP1 system contributes to the early embryonic establishment of site-specific DNA methylation patterns maintained during development. Cell Rep. 2012; 2:766. doi:10.1016/j.celrep.2012.08.043.
10. Schultz DC, Ayyanathan K, Negorev D, Maul GG, Rauscher FJ 3rd. SETDB1: A novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. Genes Dev. 2002; 16:919. doi:10.1101/gad.973302 Medline. [PubMed: 11959841]
11. Schultz DC, Friedman JR, Rauscher FJ 3rd. Targeting histone deacetylase complexes via KRAB-zinc finger proteins: The PHD and bromodomains of KAP-1 form a cooperative unit that recruits a novel isoform of the Mi-2α subunit of NuRD. Genes Dev. 2001; 15:428. doi:10.1101/gad.869501 Medline.
12. Rowe HM, et al. KAP1 controls endogenous retroviruses in embryonic stem cells. Nature. 2010; 463:237. doi:10.1038/nature08674 Medline. [PubMed: 20075919]
13. Rowe HM, et al. TRIM28 repression of retrotransposon-based enhancers is necessary to preserve transcriptional dynamics in embryonic stem cells. Genome Res. 2012; 23:452. doi:10.1101/gr.147678.112.
14. Wolf D, Goff SP. TRIM28 mediates primer binding site-targeted silencing of murine leukemia virus in embryonic cells. Cell. 2007; 131:46. doi:10.1016/j.cell.2007.07.026 Medline. [PubMed: 17923087]
15. Bojkowska K, et al. Liver-specific ablation of Krüppel-associated box-associated protein 1 in mice leads to male-predominant hepatosteatosis and development of liver adenoma. Hepatology. 2012; 56:1279. doi:10.1002/hep.25767 Medline.
16. Chikuma S, Suita N, Okazaki IM, Shibayama S, Honjo T. TRIM28 prevents autoinflammatory T cell development in vivo. Nat. Immunol. 2012; 13:596. doi:10.1038/ni.2293 Medline. [PubMed: 22544392]
17. Jakobsson J, et al. KAP1-mediated epigenetic repression in the forebrain modulates behavioral vulnerability to stress. Neuron. 2008; 60:818. doi:10.1016/j.neuron.2008.09.036 Medline.
18. Li X, et al. A maternal-zygotic effect gene, Zfp57, maintains both maternal and paternal imprints. Dev. Cell. 2008; 15:547. doi:10.1016/j.devcel.2008.08.014 Medline. [PubMed: 18854139]

19. Mackay DJ, et al. Hypomethylation of multiple imprinted loci in individuals with transient neonatal diabetes is associated with mutations in ZFP57. Nat. Genet. 2008; 40:949. doi:10.1038/ng.187 Medline.
20. Santoni de Sio FR, et al. KAP1 regulates gene networks controlling T-cell development and responsiveness. FASEB J. 2012 10.1096/fj.12-206177. doi:10.1096/fj.12-206177.
21. Santoni de Sio FR, et al. KAP1 regulates gene networks controlling mouse B-lymphoid cell differentiation and function. Blood. 2012; 119:4675. doi:10.1182/blood-2011-12-401117 Medline. [PubMed: 22452978]
22. Shin JH, et al. PARIS (ZNF746) repression of PGC-1α contributes to neurodegeneration in Parkinson's disease. Cell. 2011; 144:689. doi:10.1016/j.cell.2011.02.010 Medline. [PubMed: 21376232]
23. Zheng L, et al. Sequence-specific transcriptional corepressor function for BRCA1 through a novel zinc finger protein, ZBRK1. Mol. Cell. 2000; 6:757. doi:10.1016/S1097-2765(00)00075-7 Medline. [PubMed: 11090615]
24. Ziv Y, et al. Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. Nat. Cell Biol. 2006; 8:870. doi:10.1038/ncb1446 Medline.
25. Marín RM, Vanícek J. Efficient use of accessibility in microRNA target prediction. Nucleic Acids Res. 2011; 39:19. doi:10.1093/nar/gkq768 Medline. [PubMed: 20805242]
26. Marín RM, Vaníček J. Optimal use of conservation and accessibility filters in microRNA target prediction. PLoS ONE. 2012; 7:e32208. doi:10.1371/journal.pone.0032208 Medline. [PubMed: 22384176]
27. Ebert MS, Sharp PA. Roles for microRNAs in conferring robustness to biological processes. Cell. 2012; 149:515. doi:10.1016/j.cell.2012.04.005 Medline.

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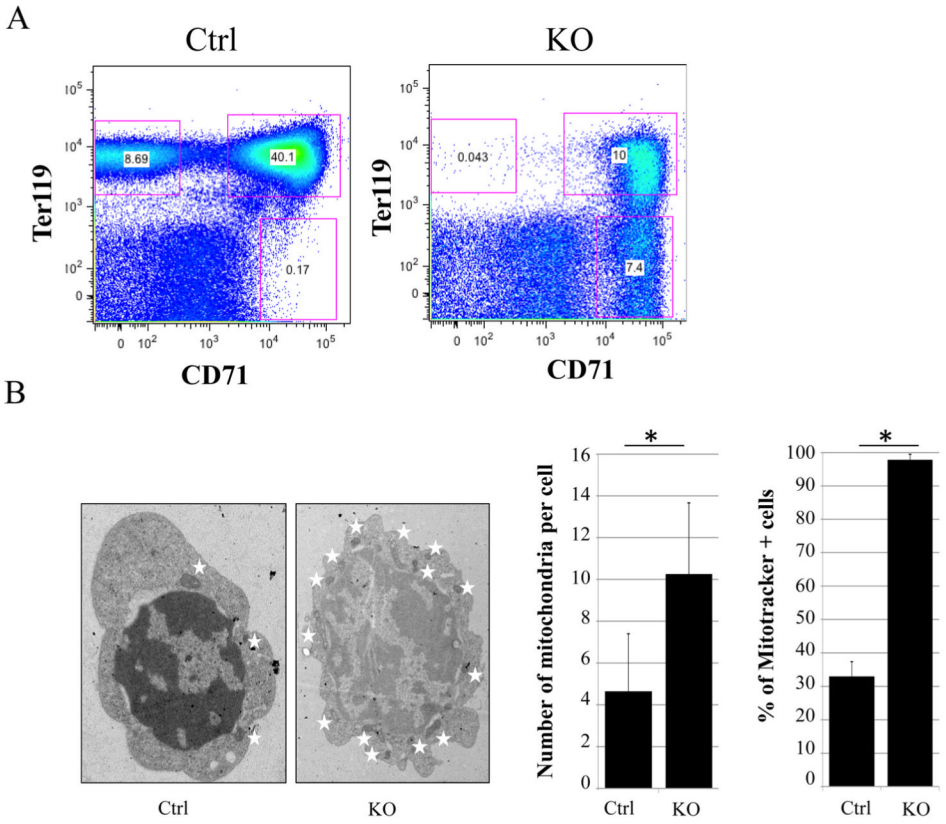

Fig. 1.

Blocked erythrocyte maturation and accumulation of mitochondria in Kap1-deleted erythroblasts. (**A**) FACS analysis of CD71 and Ter119 in bone marrow from control (Ctrl) and Kap1 KO mice 7 weeks after pIC injection. Percentage of each population from the total bone marrow is indicated. (**B**) Electron microscopy (left, stars indicate mitochondria; middle, average number of mitochondria visualized per cell; n = 10, *p < 0.05) and Mitotracker staining (right, n = 4, *p < 0.05). Decreased nuclear density was frequent in Kap1 KO cells, perhaps reflecting altered chromatin condensation.

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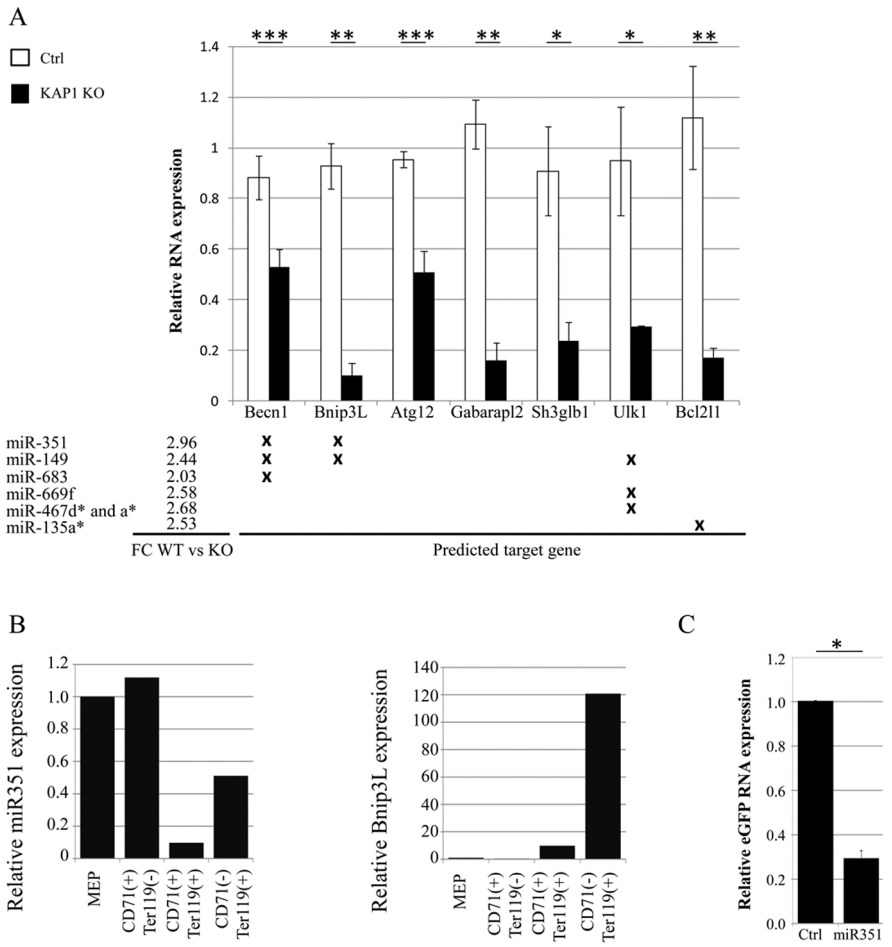

Fig. 2.

A KAP1-miRNA cascade controls red cell mitophagy. (**A**) Top, mitophagy-related transcripts in erythroblasts from control (Ctrl) and Kap1 KO mice (n = 4, *p < 0.05, **p < 0.01, ***p < 0.001). Bottom, indicated miRNAs expression in same samples; predicted miRNA-target pairs are indicated by X. (**B**) miR351 and Bnip3L expression in megakaryocyte/erythroid progenitors (MEP: Lin–Sca1–CD117+ CD34–CD16.32–, in which expression was set at 1) and indicated erythroblast subsets. (**C**) MiR-351 targets the Bnip3L 3′UTR. Ctrl, for which the normalized value was set at 1, was a combination of MEL cells not overexpressing miR-351 and transduced with a GFP-expressing lentiviral vector with the Bnip3L 3′UTR, and cells overexpressing miR-351 but transduced with the same vector without this sequence (n = 3, *p < 0.05).

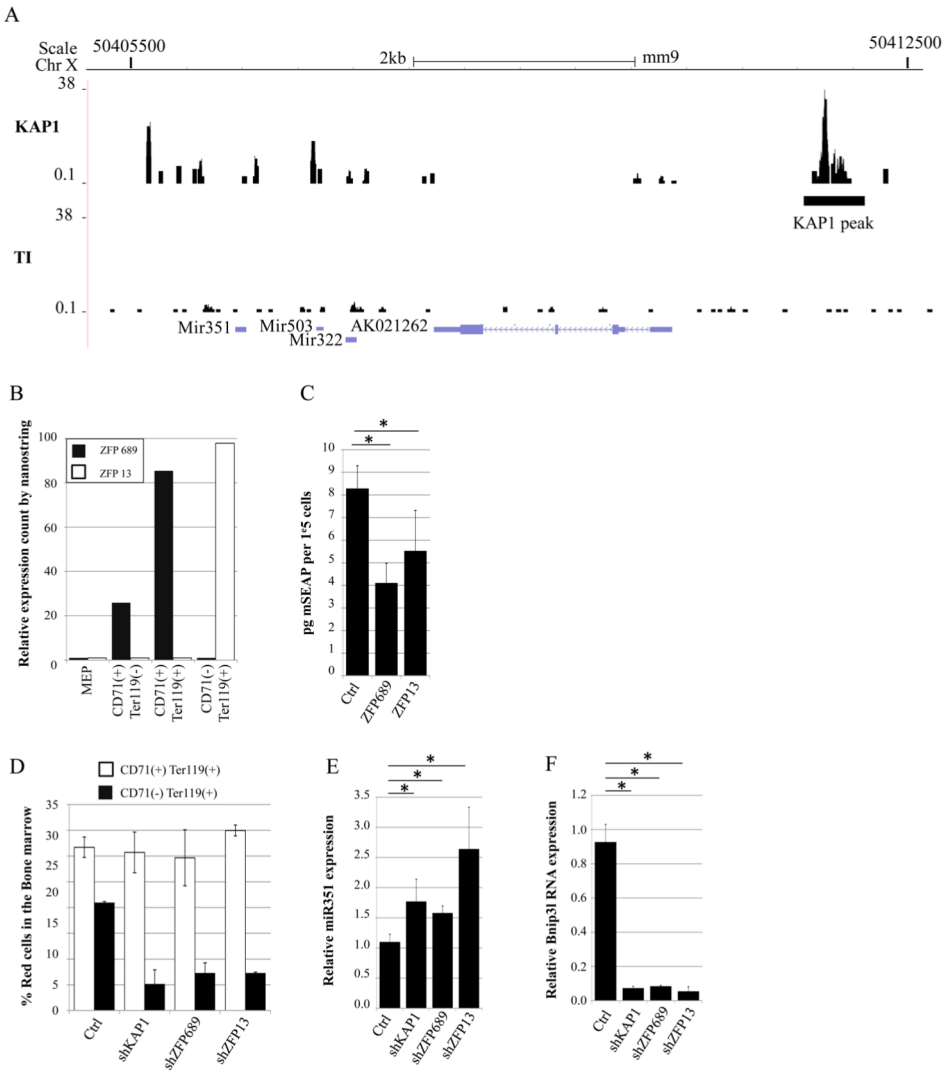

Fig. 3.

Erythroblast-specific KRAB-ZFPs control the miR-351/Bnip3L/mitophagy axis. **A** Screen shots from the UCSC Genome Browser, with results of a KAP1 ChIPSeq analysis performed on CD71+Ter119+ bone marrow cells. (**B**) Zfp689 and Zfp13 are induced during erythroid differentiation. (**C**) ZFP689 and ZFP13 repress a lentiviral vector carrying a miR-351-close KAP1-binding site in transduced MEL cells (Ctrl is a combination of ZFP-overexpressing cells transduced with a vector without the KAP1-binding site and cells LacZ-overexpressing cells transduced with a vector carrying the KAP1-binding site; n = 3, *p < 0.05). (**D**) CD45.2+ LSK cells were transduced with GFP-expressing, empty or scramble (Ctrl), Kap1-, Zfp689- or Zfp13-directed shRNA lentiviral vectors, engrafted into irradiated CD45.1+ mice, and erythroid differentiation was evaluated by FACS 8 wks later. (**E** and **F**) The CD71+Ter119+, CD45.2+, eGFP+ population was then sorted and analyzed by RT-QPCR for miR351 (E) and Bnip3L (F) expression (n = 6, *p < 0.05).

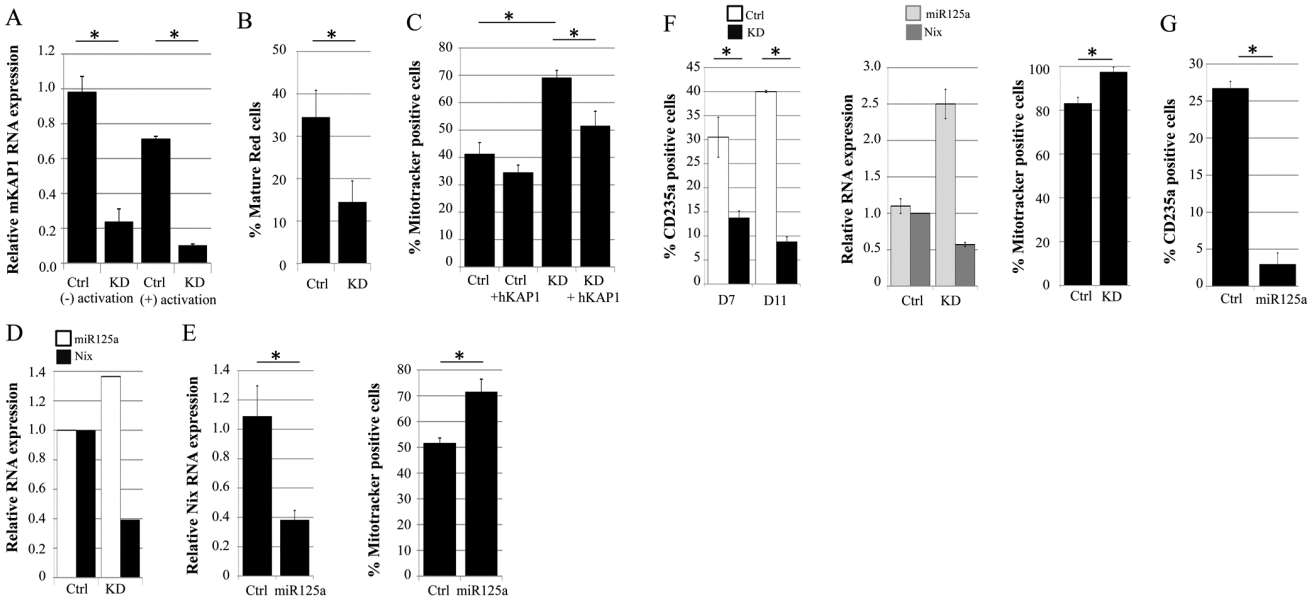

Fig. 4.

KAP1-regulated RNA interference controls human red cell mitophagy. (**A** to **C**) HEL transduced with scramble or Kap1-specific shRNA-expressing lentiviral vectors and induced or not to differentiate were evaluated for Kap1 mRNA expression (A), and by benzidine (B) (n = 3, counting 100 cells for each condition) or Mitotracker (C) (n = 3) staining (*p < 0.05). (**D**) hsa-miR-125a-5p (miR125a) and Nix expression measured respectively by NanoString nCounter direct RNA quantification and RNA sequencing in HEL cells transduced with empty or Kap1 knockdown vectors. (**E**) Nix expression in Ctrl (setting normalized value at 1) or hsa-miR-125a-5p-overexpressing HEL cells, measuring their mitochondrial content by Mitotracker staining (n = 4, *p < 0.05). (**F**) Decreased erythroid differentiation of Kap1 knockdown human cord blood CD34+ cells, assessed by CD235a surface expression at seven (D7) and eleven (D11) days. At D7, sorted CD235a+eGFP+ cells were analyzed by RT-QPCR for Nix and hsa-miR125a expression, and for mitochondrial content by Mitotracker staining (n = 3, *p < 0.05). (**G**) Percentage of CD235a-expressing cells 7 days after inducing the differentiation of CD34+ cells transduced with empty or unrelated-miRNA- (Ctrl) or hsa-miR-125a-5p-overexpressing lentiviral vectors (n = 3, *p < 0.05).

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