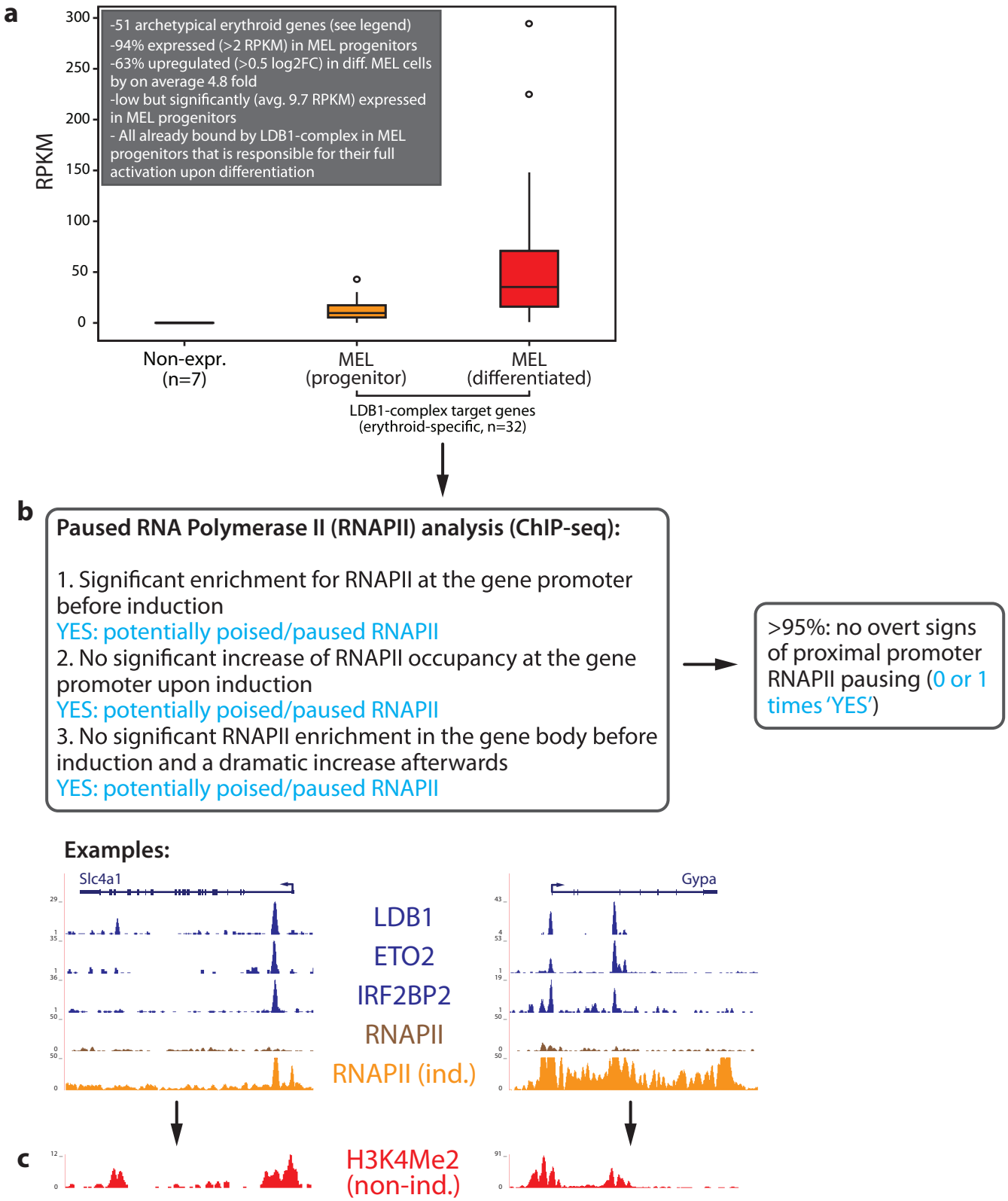
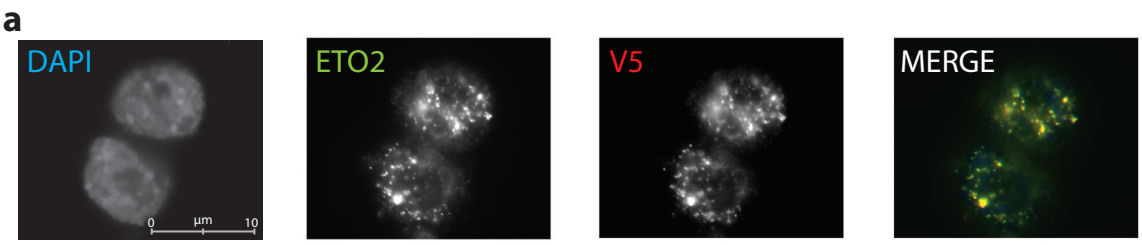
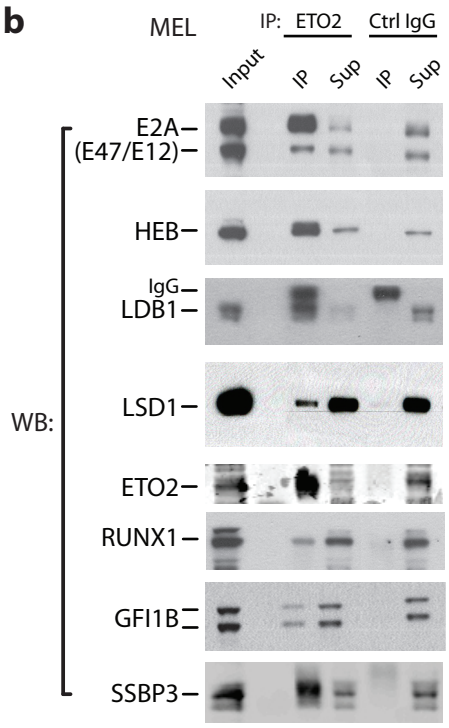
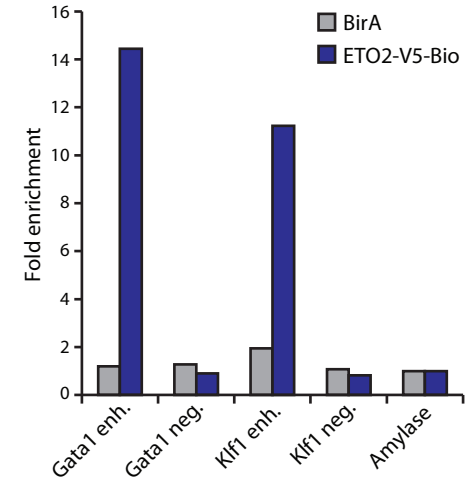


Supplementary Figure 1. Transcriptional and epigenetic definition of primed LBD1-complex target genes. (a) Quantification box plot depicting the average expression levels (RPKM) of non-expressed genes ('Non-expr.) and 51 archetypical erythroid genes (involved in heme biosynthesis and erythrocyte membrane function[1]) in MEL cells before ('progenitor') and after ('differentiated') induction of erythroid maturation. Various characteristics of this gene set during MEL cell differentiation are outlined in the gray box. (b) LDB1-complex target genes (n=32, as defined by ChIP-Seq) from the erythroid gene set used in panel a were inspected for RNA Polymerase II (RNAPII) enrichment (measured by ChIP-Seq) during MEL cell differentiation. Three criteria were defined that indicate the presence of paused RNAPII at the proximal promoter of these genes. Genes scoring a positive response for 2 or 3 criteria were defined as 'paused'. Examples of two of the examined archetypical erythroid genes (*Slc4a1* and *Gypa*) are shown, both showing no overt signs of RNAPII pausing. (c) H3K4Me2 ChIP-Seq profiles from non-induced MEL progenitor cells for the *Slc4a1* and *Gypa* loci shown in panel b. ind.: induced MEL, non-ind.: non-induced MEL



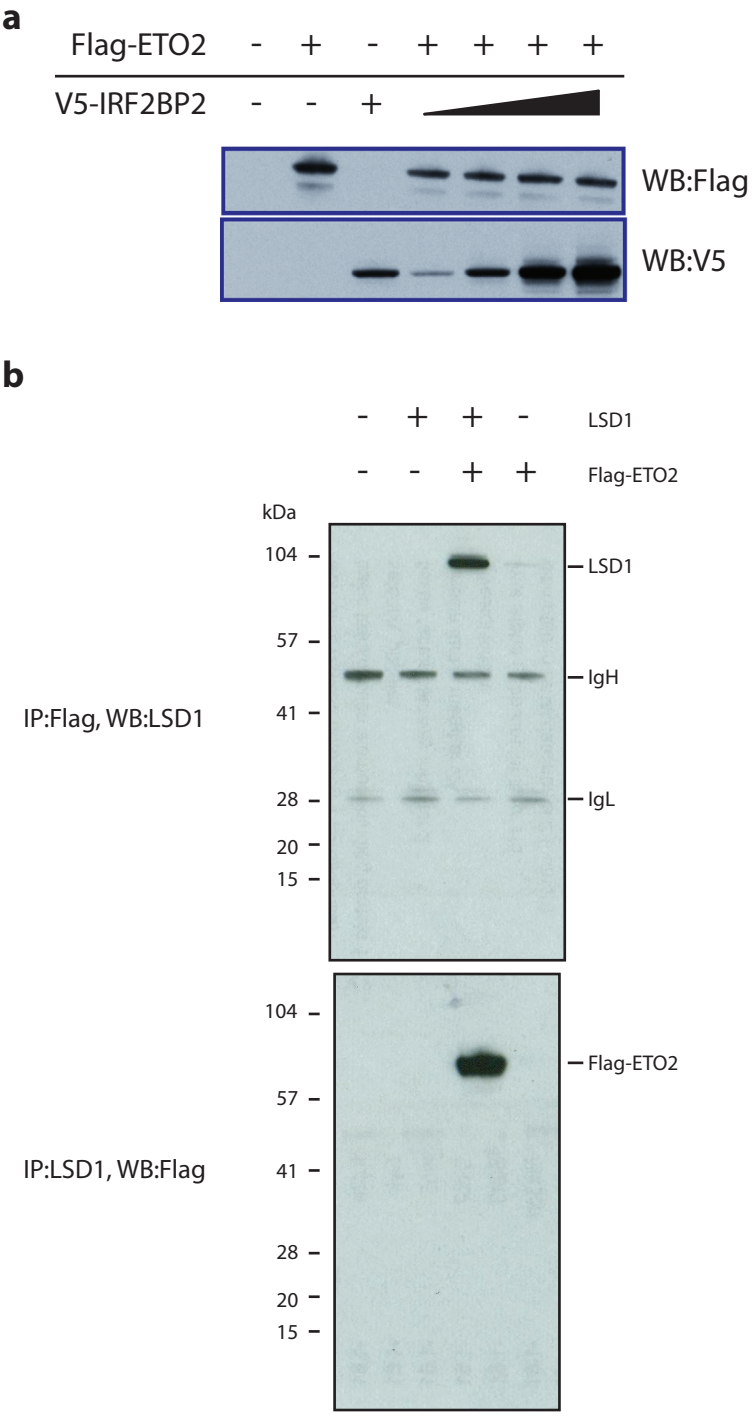




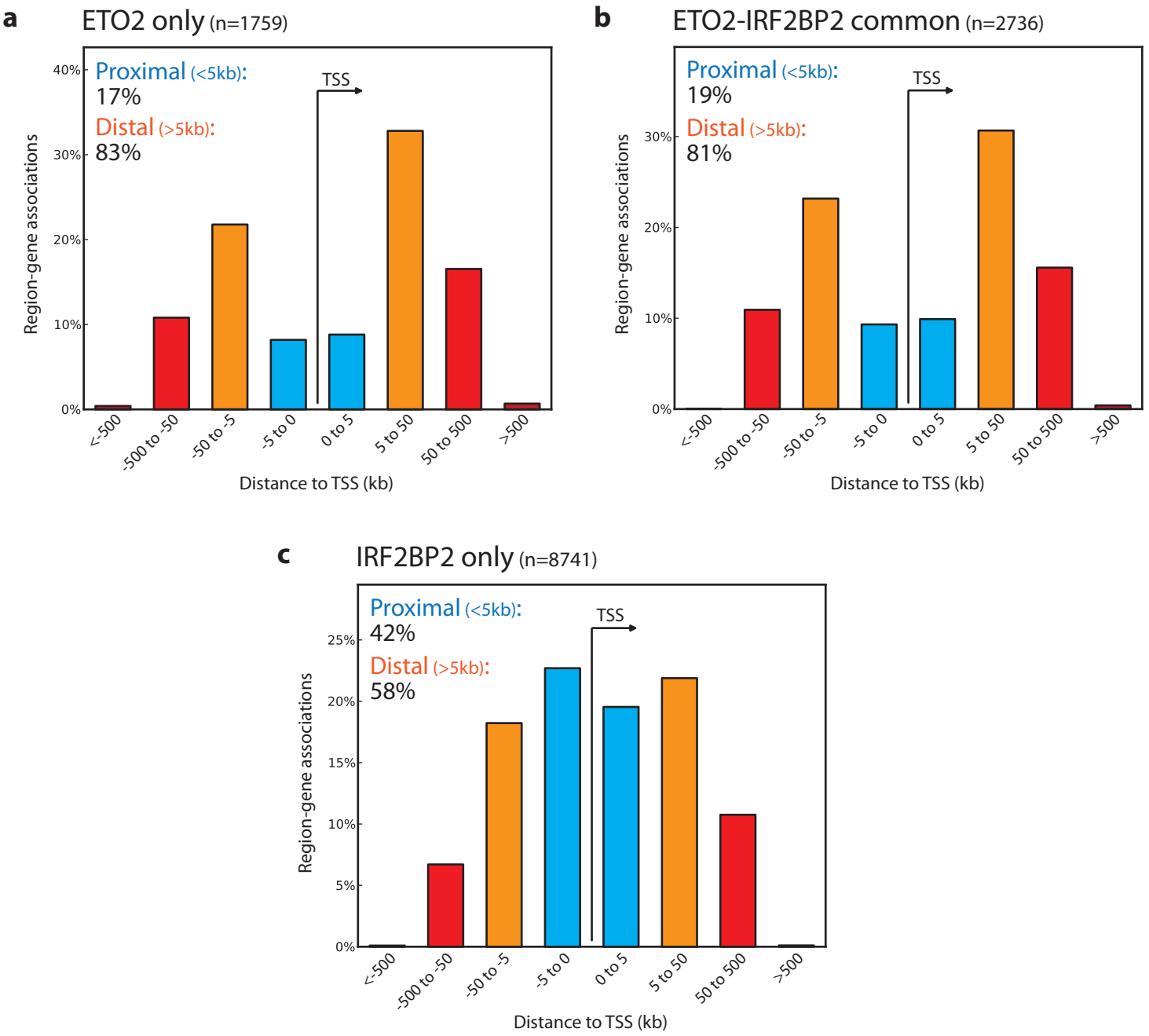
d

	Proteins identified by LC-MS/MS in non-induced MEL cells			
	ETO2-V5-Bio Streptavidin pulldown		Endogenous ETO2 pulldown	
Precipitated proteins	nb unique peptides ETO2-V5-Bio	nb unique peptides BirA	nb unique peptides ETO2 IP	nb unique peptides IgG IP
LIM binding protein				
LDB1	6	0	12	2
ETO family				
ETO2	7	0	29	0
MTGR1	6	0	21	0
Basic helix-loop-helix				
LYL1	4	0	1	0
SCL/TAL1	7	0	15	3
E2.2	4	0	14	0
E2A (E12/E47)	13	0	21	0
HEB	19	0	19	0
SSBP family				
SSBP2	5	0	5	0
SSBP3	4	0	5	0
SSBP4	3	0	3	0
Transcriptional activator				
FUS/TLS	2	0	nd	
Repressor / co-repressor				
IRF2BP2	6	0	4	0
GFI1B	2	0	nd	
Runt related				
Runx1	4	0	8	4
CBF-beta	1	0	2	0
BHC repressor complex				
LSD1	13	2	10	3
GSE1	9	1	5	1
REST co-repressor 3	4	0	nd	
HMG20a	2	0	nd	
HDAC2	3	0	nd	
Jumonji-domain demethylase 1C/2C	1	0	nd	

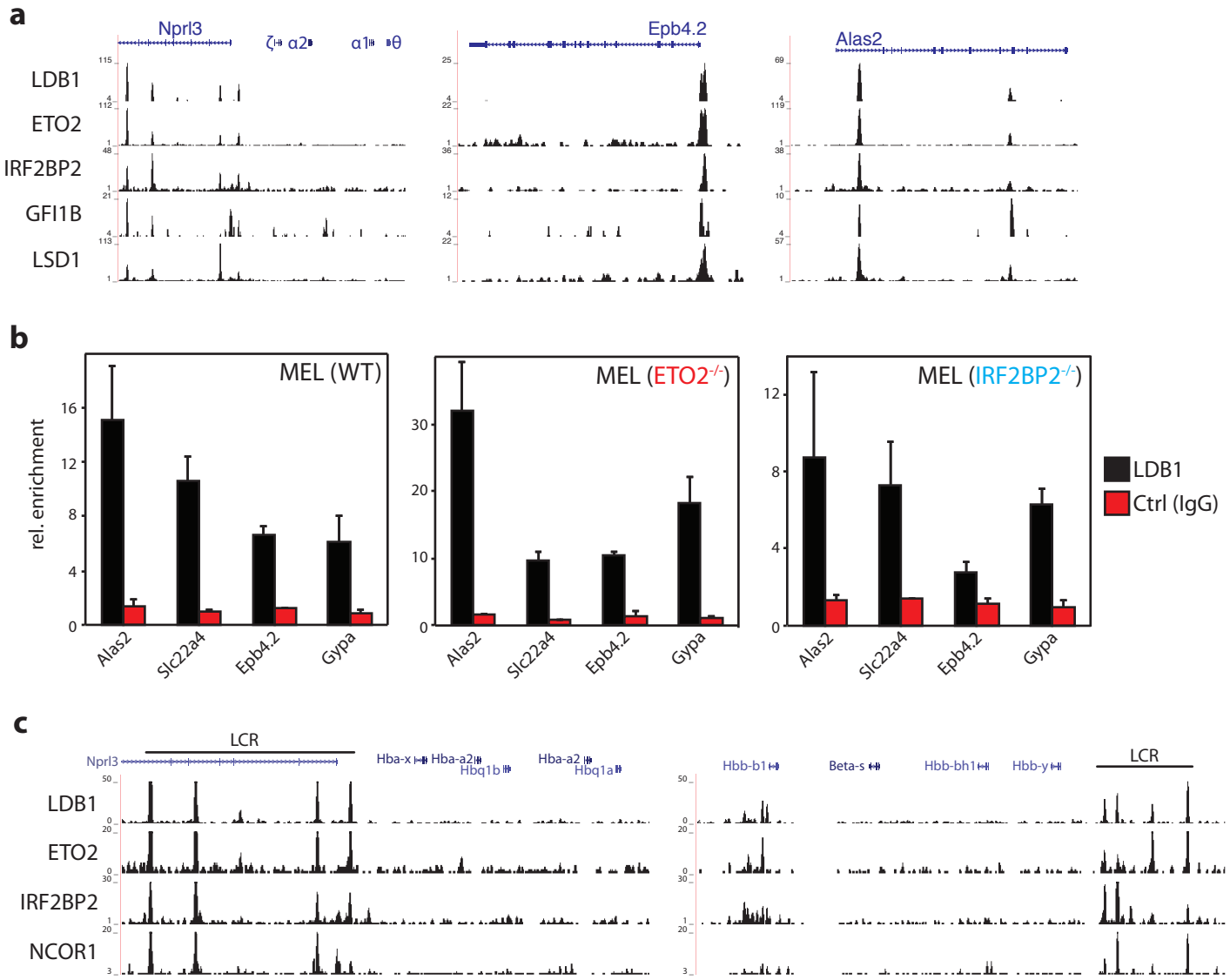
Supplementary Figure 2. Validation of ETO2-V5-Bio functionality in MEL cells. (a) MEL cells stably expressing BirA and ETO2-V5-Bio cells were fixed and stained for endogenous ETO2 (in green) or for ETO2-V5-Bio (using a V5 antibody, in red). Note the nuclear localization (as compared to the DAPI nuclear stain) of ETO2-V5-Bio and co-localization with endogenous ETO2. (b) Endogenous co-immunoprecipitation validations of ETO2-V5-Bio interacting proteins in MEL cells identified by LC-MS/MS. Species-matched IgG was used to control for non-specific binding. Full-size blot images can be found in Supplementary Fig.10. (c) Bio-ChIP qPCR experiments showing recruitment of ETO2-V5-Bio to known endogenous ETO2 genomic binding sites (*Gata1* -3.5 HS enhancer and the *Klf1* upstream enhancer). Regions immediately up- or downstream (+ or – 1kb) of the enhancer (enh.) sites were used as negative (neg.) controls. Enrichments were normalized to Amylase promoter values. A representative of two independent experiments is shown. (d) A comparison between proteins identified by mass spectrometry using the ETO2-V5-Bio streptavidin pulldown and the endogenous ETO2 pulldown. See Methods section for further details on methodology and analysis. Full-size images of all Western Blots shown can be found in Supplementary Fig.10. WB, Western Blot; IP, immunoprecipitation



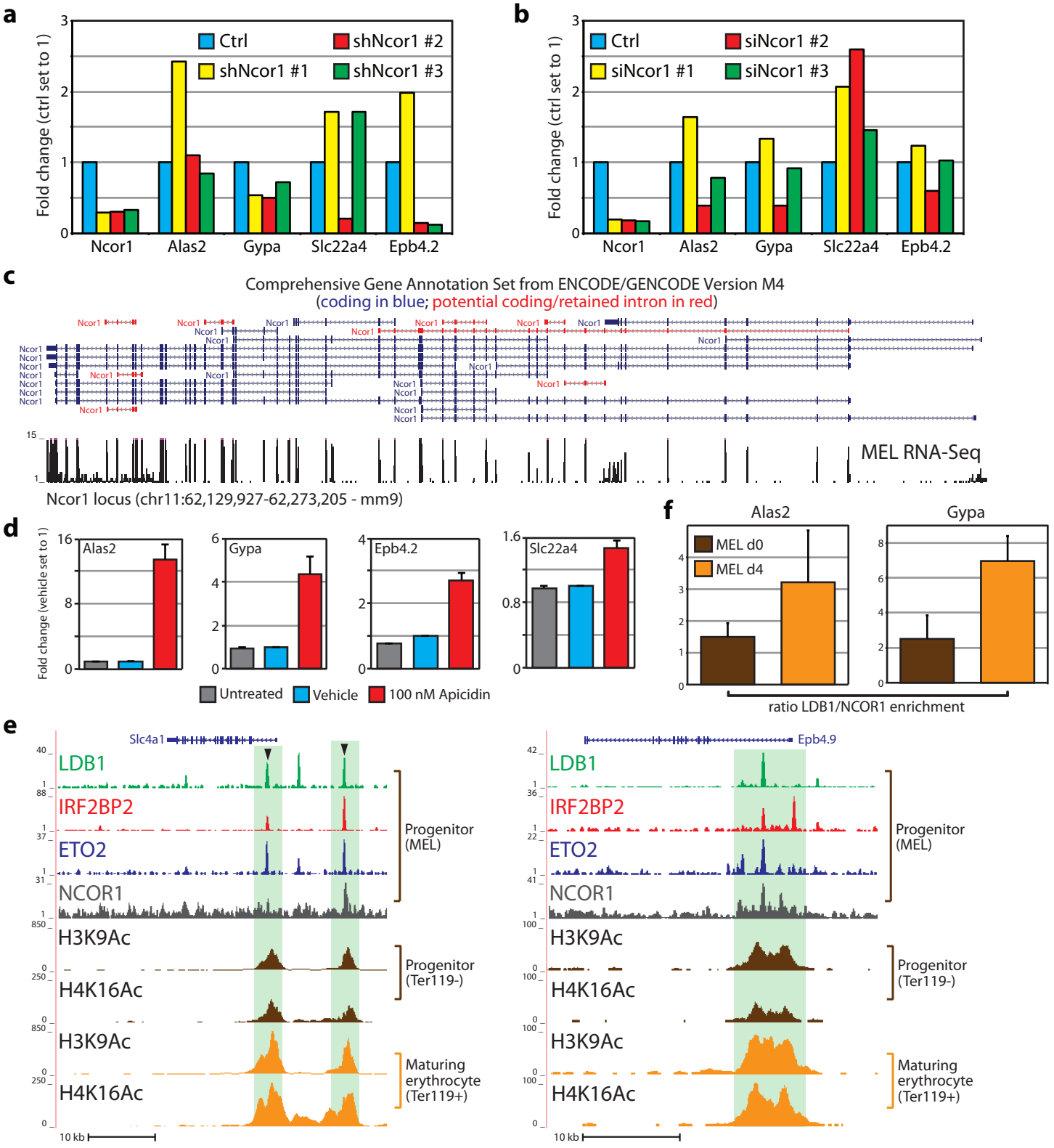
Supplementary Figure 3. Effect of increasing IRF2BP2 levels on ETO2 protein stability and ETO2-LSD1 co-immunoprecipitation. (a) Equal amounts of Flag-ETO2 and variable amounts of V5-IRF2BP2 expression constructs were co-transfected into HEK 293T cells. Protein extracts were prepared 48h post-transfection and Flag-ETO2 and V5-IRF2BP2 protein levels were visualized using Western Blot (WB) analysis. Full-size blot images can be found in Supplementary Fig.11. (b) Co-immunoprecipitation experiment showing the interaction of Flag-ETO2 with LSD1 in HEK 293T cells transfected with pcDNA plasmids encoding the two proteins.



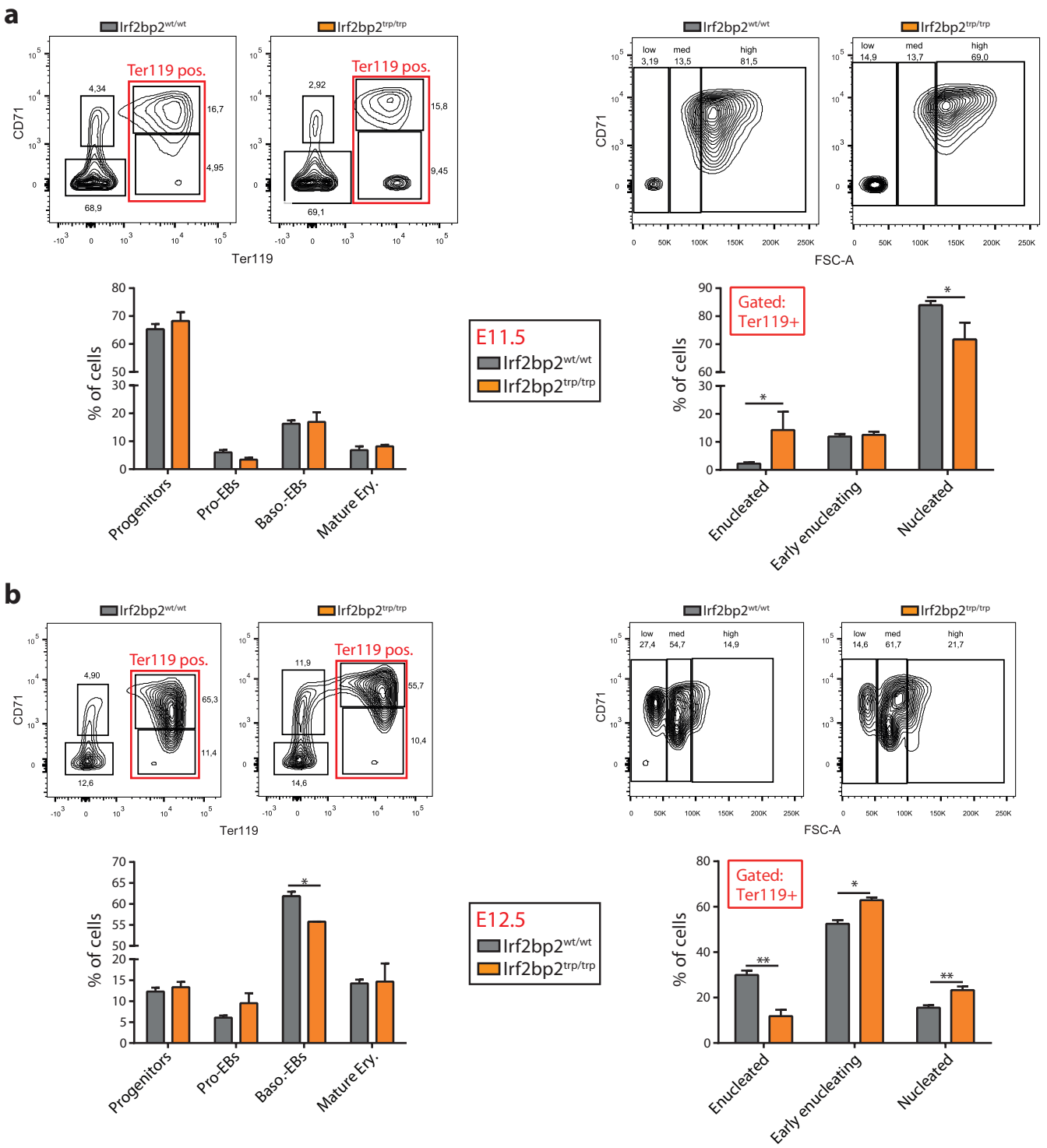
Supplementary Figure 4. ETO2 and IRF2BP2 ChIP-Seq peak positioning relative to transcription start sites. The GREAT analysis tool (see Methods) was used to determine the distance of ETO2 only (a), ETO2-IRF2BP2 (b) and IRF2BP2 only (c) binding sites to the nearest transcription start site (TSS). Note the predominantly distal (>80%) binding profile of ETO2 only and ETO2-IRF2BP2 common sites, in accordance with the binding profile of the LDB1-complex (Soler et al. 2010 [ref.6]). In contrast, many genomic sites occupied only by IRF2BP2 (42%) are located in the proximal promoter region.



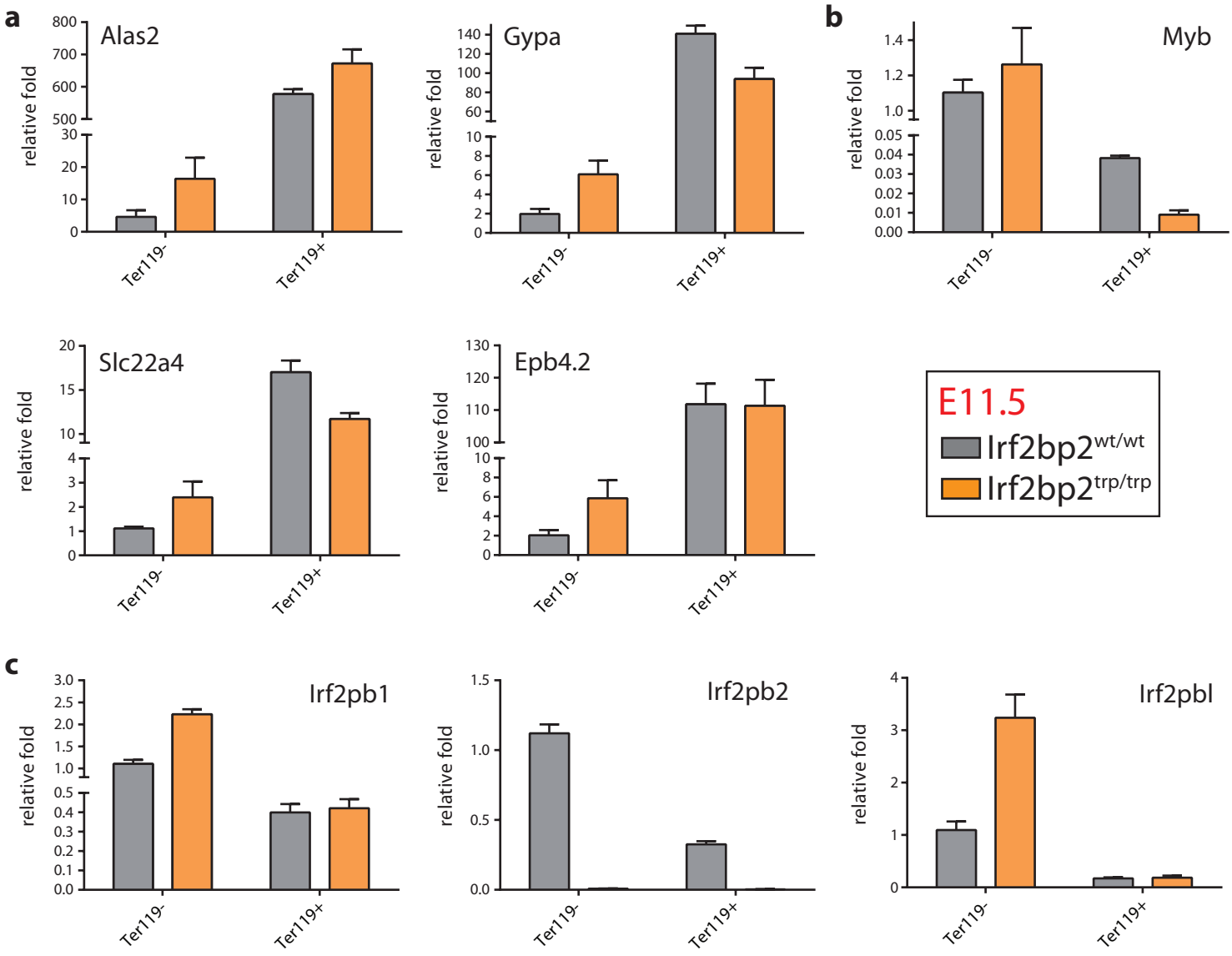
Supplementary Figure 5. Transcription factor binding profiles and LDB1 ChIP-qPCR in ETO2/IRF2BP2 knockout MEL cell lines. (a) ChIP-Seq data for LDB1, ETO2, IRF2BP2, GFI1B and LSD1 (from MEL erythroid progenitor cells) is shown for the α-globin (left), *Epb4.2* (middle) and *Alas2* (right) loci. Note the high degree of co-occupancy of the ETO2-interacting corepressor proteins (IRF2BP2, GFI1B and LSD1) on known LDB1/ETO2-complex target genes. (b) ChIP-qPCR experiments showing that LDB1 protein binding in MEL cells is maintained at erythroid genes in the absence of ETO2 or IRF2BP2. Enrichments are shown as relative values over background signal at the amylase gene promoter (n=2-3, error bars denote s.e.m.). (c) ChIP-Seq data for LDB1, ETO2, IRF2BP2, and NCOR1 (from MEL erythroid progenitor cells) is shown for the α-globin (left) and β-globin (right) loci. Note the high degree of co-occupancy of the LDB1 complex (represented here by LDB1, ETO2 and IRF2BP2) and NCOR1 at the locus control region (LCR) that regulates globin gene expression at these loci.



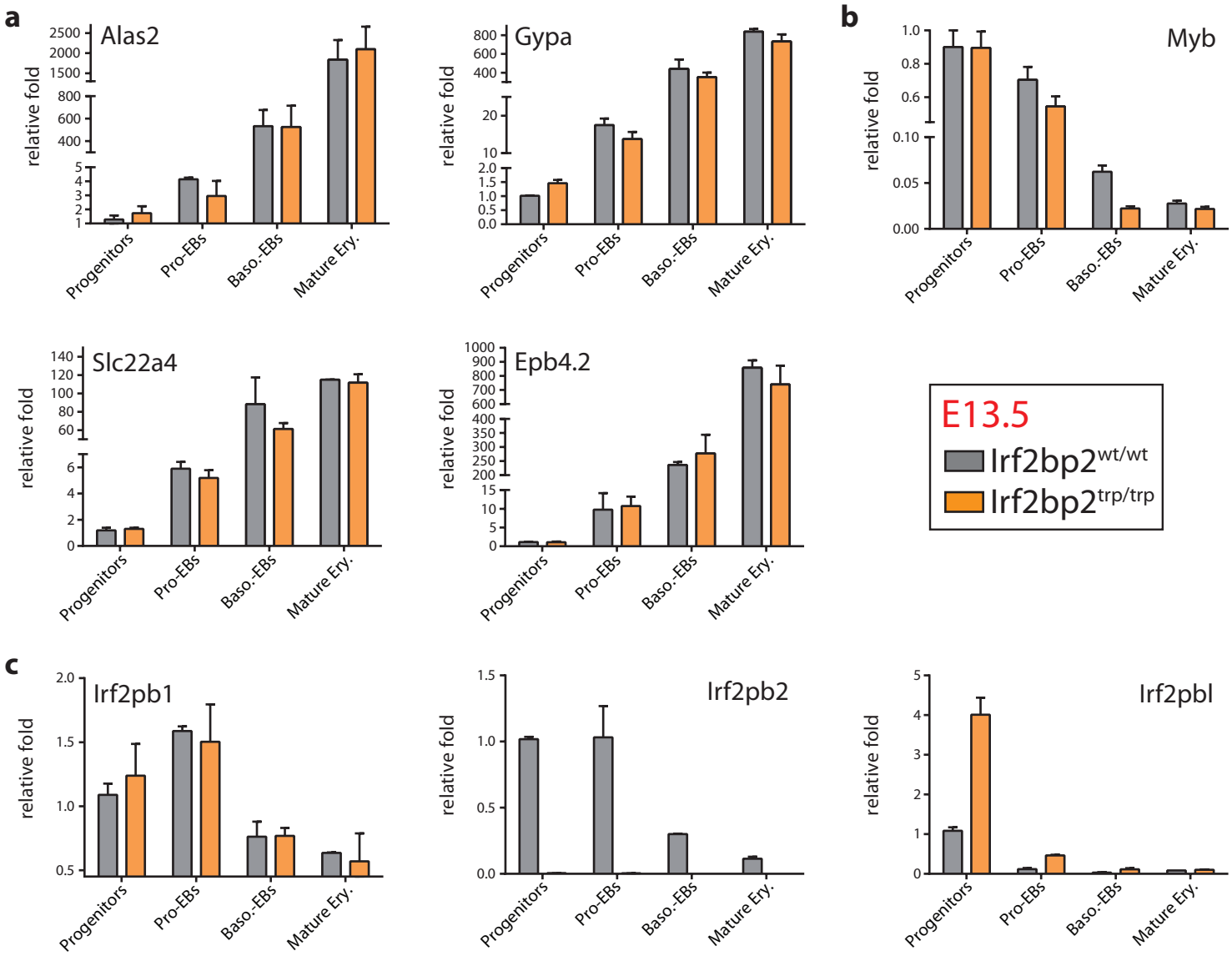
Supplementary Figure 6. NCOR1 knockdown and HDAC3 inhibition in erythroid progenitors and histone acetylation dynamics at selected primed erythroid genes during development. (a,b) shRNA (panel a) and siRNA (panel b) mediated knockdown of *Ncor1* mRNA in MEL cells. qPCR gene expression analysis was performed 48-72h after transduction/transfection (normalized versus *Rnh1* levels). 3-4 different shRNAs/siRNAs were used; shown is a representative experiment. (c) UCSC browser screenshot depicting RNA-Seq data from MEL cells for the *Ncor1* locus. Note the large amount of protein-coding isoforms potentially expressed in MEL cells. (d) MEL cells were treated with either vehicle (DMSO) or 100 nM of Apicidin and harvested after 24h for erythroid gene expression analysis by qPCR (normalized versus *Rnh1* levels). Untreated cells were analyzed in parallel as an additional control. Bar graphs depict the average of two independent experiments. Error bars denote s.d. (e) ChIP-Seq profiles for the *Slc4a1* and *Epb4.9* loci showing LDB1, IRF2BP2, ETO2 and NCOR1 co-occupying regulatory elements controlling these two archetypical late erythroid genes in MEL cells. Histone acetylation profiles (H3K9Ac and H4K16Ac, taken from Wong et al. 2010 [ref.21]) from primary erythroid progenitors (Ter119-) and maturing erythrocytes (Ter119+) are shown below. (f) Ratios of LDB1 over NCOR1 ChIP enrichments (n=2, error bars denote s.d.) at selected primed erythroid genes during MEL cell differentiation.



Supplementary Figure 7. Flowcytometry analysis of E11.5 and E12.5 fetal liver erythropoiesis in IRF2BP2-deficient mice. Flowcytometry analysis (CD71-Ter119 double staining) of fetal livers from E11.5 (a) or E12.5 (b) embryos with the indicated genotypes (n=3-4 embryos per genotype). Representative flowcytometry plots are shown on top; average values are plotted as bar graphs underneath. The right side of the figure shows Ter119^{+} FL cells separated into three populations based on FSC profile (as in Figure 7). Differences between wildtype and Irf2bp2$^{trp/trp}$ embryos were tested for statistical significance (Mann Whitney U test; *P<0.05, **P<0.01). Error bars denote s.d.

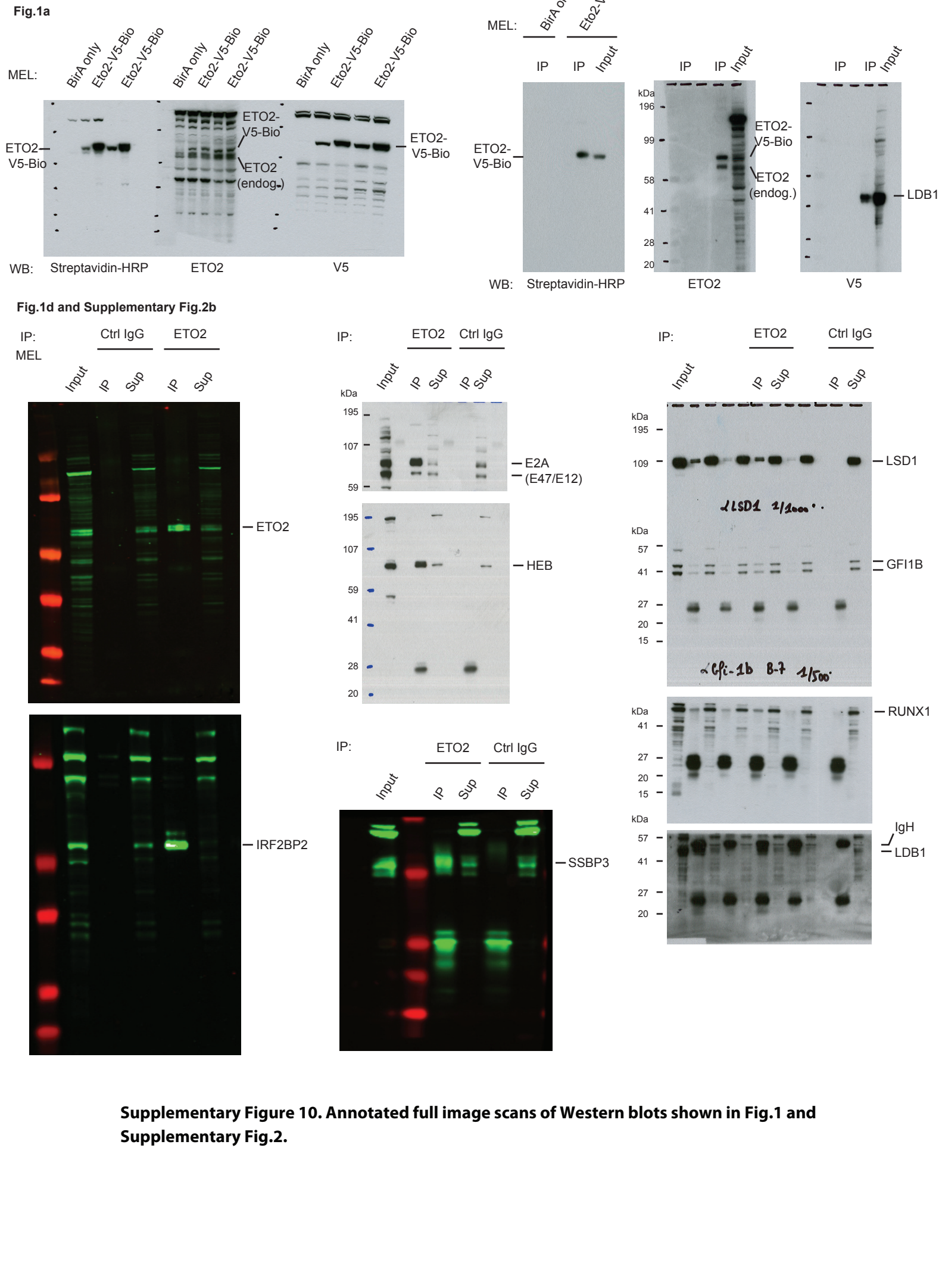


Supplementary Figure 8. Gene expression analysis on IRF2BP2-deficient erythroid cell populations purified from E11.5 fetal liver. Gene expression comparison between wildtype and Irf2bp2$^{trp/trp}$ E11.5 FACS-sorted erythroid populations separated into Ter119- erythroid progenitors and Ter119+ maturing erythrocytes. mRNA levels of late erythroid genes (a), the immature marker *Myb* (b) and the three *Irf2bp* family members (c) were measured using qPCR (normalized versus *Rnh1* levels). Bar graphs depict the average of at least two independent experiments. Error bars denote s.d.



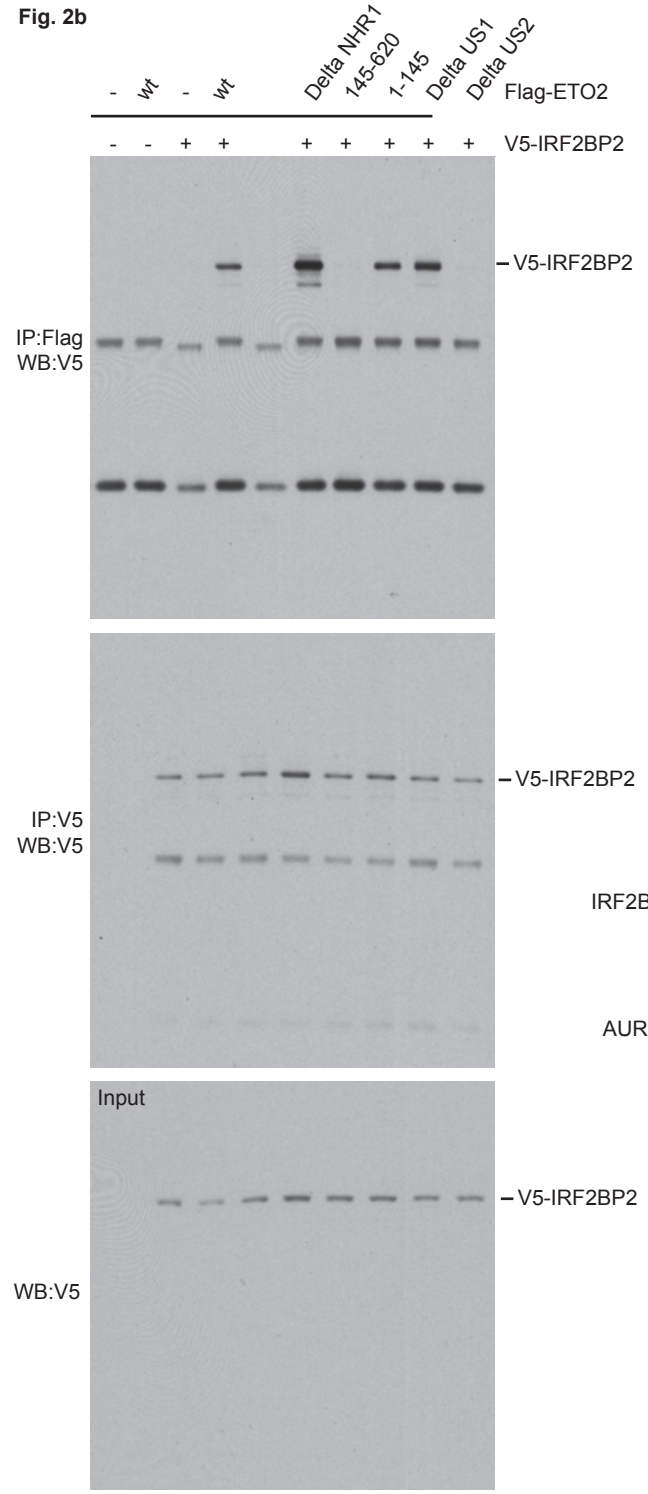
Supplementary Figure 9. Gene expression analysis on IRF2BP2-deficient erythroid cell populations purified from E13.5 fetal liver. Gene expression comparison between wildtype and Irf2bp2$^{trp/trp}$ E13.5 FACS-sorted erythroid populations separated into CD71-/Ter119- progenitors, CD71+/Ter119- pro-erythroblasts (pro-EBs), CD71+/Ter119+ basophilic erythroblasts (Baso.-EBs) and CD71-/Ter119+ mature erythrocytes (Mature Ery.). mRNA levels of late erythroid genes (a), the immature marker *Myb* (b) and the three *Irf2bp* family members (c) were measured using qPCR (normalized versus *Rnh1* levels). Bar graphs depict the average of at least two independent experiments. Error bars denote s.d.

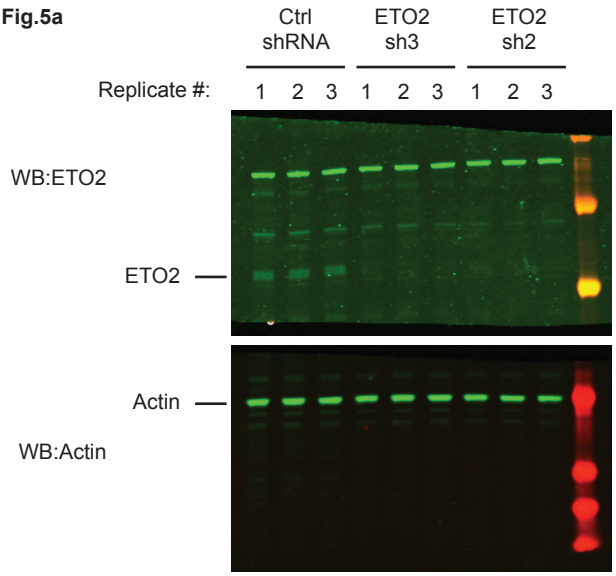
Full scans of blots corresponding to:

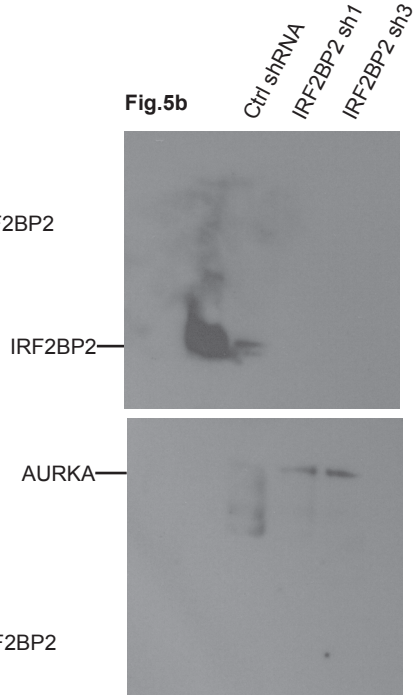


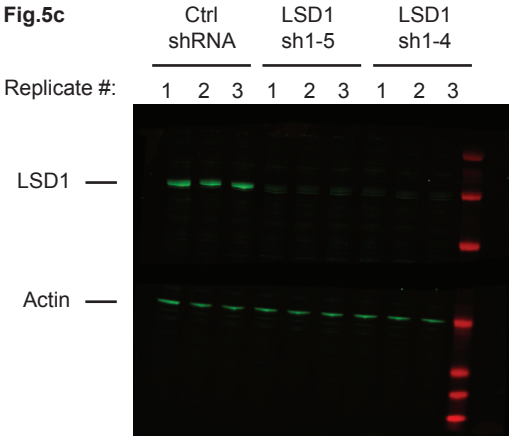
Supplementary Figure 10. Annotated full image scans of Western blots shown in Fig.1 and Supplementary Fig.2.

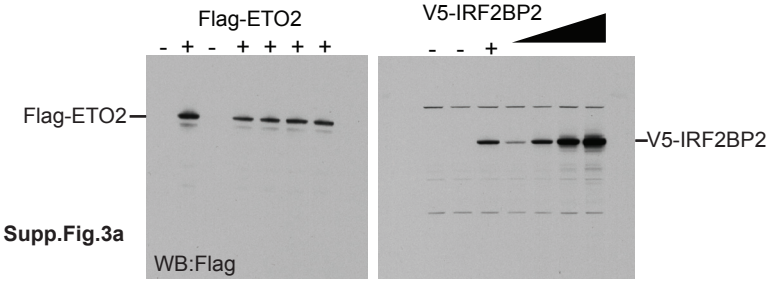
Full scans of blots corresponding to:

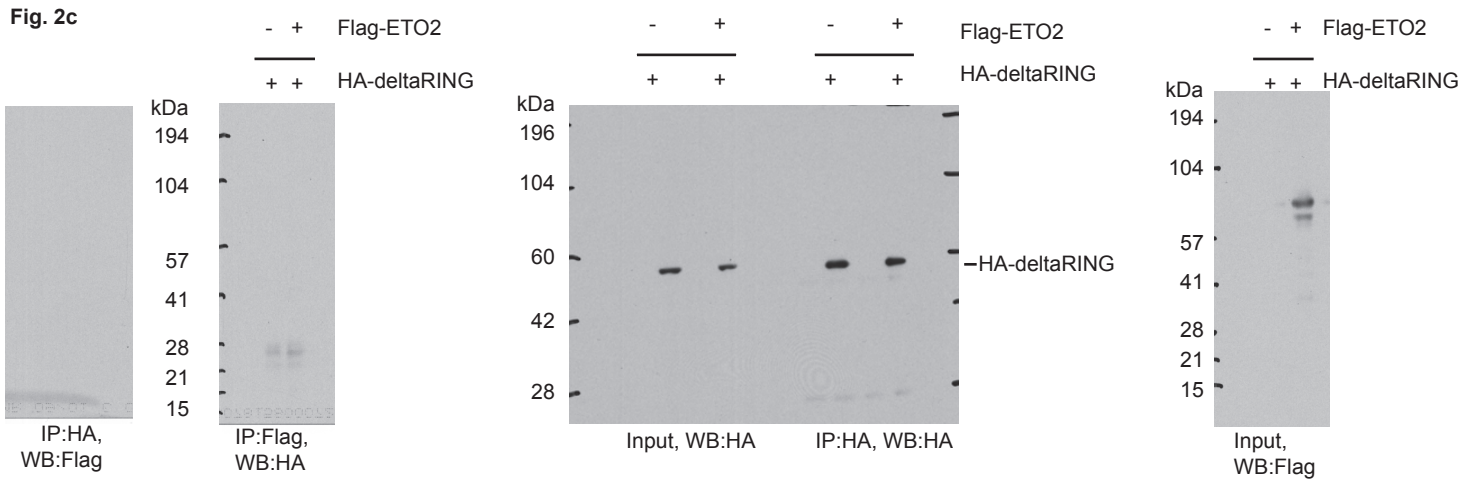












Supplementary Figure 11. Annotated full image scans of Western blots shown in Fig.2, Fig.5 and Supplementary Fig.3.

Supplementary Table 1. Oligonucleotide sequences used in this study.

Expression Primers

Primer name	*Target mRNA*	*Sequence (5'-3')*
Alas2-78F	Alas2	TTAGCCACTTTGCCCAGGAG
Alas2-78R	Alas2	CCGTCTTTGGTTCGTCCTCA
Cbfa2t3-F	ETO2	GTGAACTCAACTCTGACGAT
Cbfa2t3-R	ETO2	GCAGCAGTGGAAGATTAGC
Epb4.2-F	Epb4.2	GCTTTATCTACCTGGGCAC
Epb4.2-R	Epb4.2	TCACTTGCTTGTCCATACTC
Gypa-F	Gypa	TGAAGTGTCTGCTGCGTT
Gypa-R	Gypa	CCGATAATCCCTGCCATCA
Irf2bp1-F	Irf2bp1	TACCCGGACACAAGTTCTGC
Irf2bp1-R	Irf2bp1	GACAGAGGAACCCACCAGTG
Irf2bp2-F	Irf2bp2	GGCAGGTTGTTGGGTTTC
Irf2bp2-R	Irf2bp2	CAAGGCTGTGTCTCACCA
Irf2bpL-F	Irf2bpl	GCGGTTCAAGGGTCTCTCTC
Irf2bpL-R	Irf2bpl	TACCTTGGGCCTTCATGCAG
Myb-1121-1235-F	Myb	CGACGAAGACCCTGAGAAGG
Myb-1121-1235-R	Myb	GCTGCAAGTGTGGTTCTGTG
Ncor1-F	Ncor1	ACTGTGCTGTCTGGTTCC
Ncor1-R	Ncor1	CACCTTCAAATGCTCGGA
Slc22a4-F	Slc22a4	TCACCACCTCCCTGTTCTT
Slc22a4-R	Slc22a4	TTTCTTCCTGCCAAACCTG
Slc4a1-F1	Slc4a1	GCTCTTCCCACAGAGCAAAC
Slc4a1-R1	Slc4a1	CTGCCTCCACCCATTGTAGT
Rnh1-F	Rnh1	TGCAGGCACTGAAGCACCA
Rnh1-R	Rnh1	TCCAGTGTGAGCAGCTGAG

ChIP Primers

Primer name	*Target genomic region*	*Sequence (5'-3')*
Alas2-F	*Alas2* intron1	GGAACTGGGACATCTTGAC
Alas2-R	*Alas2* intron1	ACCATTAGAGTCTGGCTACT
Amy-F	Amylase promoter	CTCCTTGTACGGGTTGGT
Amy-R	Amylase promoter	AATGATGTGCACAGCTGAA
Epb4.2-F	*Epb4.2* promoter	TGAGAAGACTTGCTGGCT
Epb4.2-R	*Epb4.2* promoter	GTTCAGAGTTGGGTTCGGA
Gata1-enh-F	*Gata1* -3.5kb enhancer	TCAGGGAAGGATCCAAGGAA
Gata1-enh-R	*Gata1* -3.5kb enhancer	CCGGGTTGAAGCGTCTTCT
Gata1-neg-F	*Gata1* upstream region	CACTAGCAGCTGGGTGGGTTA
Gata1-neg-R	*Gata1* upstream region	TGCCGCTTGCCTTTGTAAG
Gypa-F	*Gypa* intron3	TCCTCTCCTTACACCCTGTCT
Gypa-R	*Gypa* intron3	CTCTTACCAACAAGCCAGCC
Klf1-enh-F	*Klf1* promoter/enhancer	CTGGCCCCCCTACCTGAT
Klf1-enh-R	*Klf1* promoter/enhancer	GGCTCCCTTTCAGGCATTATC

Klf1-neg-F	*Klf1* upstream region	TGCTCCCCACTATGATAATGGA
Klf1-neg-R	*Klf1* upstream region	GCCACAACCAAAGAAGACATTTT
Slc22a4-F	*Slc22a4* intron1	TCTGTTGACTGCTCTGTAGT
Slc22a4-R	*Slc22a4* intron1	TAGGTTCCTCCCAATGAGAT

Mouse Genotyping Primers

Primer name	*Target genomic region*	*Sequence (5'-3')*
V76-F	Gene-trap vector	CTTGCAAAATGGCGTTACTTAAGC
Irf2bp2-F2	*Irf2bp2* gene	GGGTGGGTGGTAGGGATCC
Irf2bp2-R2	*Irf2bp2* gene	GGGCTCTGAGCCTGCACA

Crispr gRNA cloning

Primer name	*Target genomic region*	*Sequence (5'-3')*
Eto2-N3-F	*Cbfa2t3* intron 5	CACCGGACTGGGGCCTCACAAACGA
Eto2-N3-R	*Cbfa2t3* intron 5	AAACTCGTTTGTGAGGCCCCAGTCC
Eto2-C3-F	*Cbfa2t3* intron 3	CACCGGAACGGTTGCAGGGACAGAG
Eto2-C3-R	*Cbfa2t3* intron 3	AAACCTCTGTCCCTGCAACCGTTCC
IRF2BP2-N3-F	*Irf2bp2* exon 1	CACCGGGTCAACGGTTCTGCCGCGC
IRF2BP2-N3-R	*Irf2bp2* exon 1	AAACGCGCGGCAGAACCGTTGACCC
IRF2BP2-C2-F	*Irf2bp2* exon 2 (3'UTR)	CACCGGGCTTTCCTGCTGACCAGCC
IRF2BP2-C2-R	*Irf2bp2* exon 2 (3'UTR)	AAACGGCTGGTCAGCAGGAAAGCCC

(for guide RNA primer sequences, the target genomic sequence is underlined)

Crispr-deleted MEL KO clones genotyping

Primer name	*Target genomic region*	*Sequence (5'-3')*
Eto2-Del-F1	*Cbfa2t3* intron 5	AAAGGGCGAGAGCCACAGCTCA
Eto2-Del-R	*Cbfa2t3* intron 3	CAGTCTGGGAGTTTGAACTGGGCAT
IRF2BP2-Del-F	*Irf2bp2* exon 2 (3'UTR)	TGCAGGCTTCCTCTACCCACACT
IRF2BP2-Del-R	*Irf2bp2* exon 1	CGCTCAGGCTATGGAGCGCTA

Supplementary References

1 Kassouf, M. T. *et al.* Genome-wide identification of TAL1's functional targets: insights into its mechanisms of action in primary erythroid cells. *Genome research* **20**, 1064-1083, doi:10.1101/gr.104935.110 (2010).

2 Soler, E. *et al.* The genome-wide dynamics of the binding of Ldb1 complexes during erythroid differentiation. *Genes & development* **24**, 277-289, doi:10.1101/gad.551810 (2010).

3 Wong, P. *et al.* Gene induction and repression during terminal erythropoiesis are mediated by distinct epigenetic changes. *Blood* **118**, e128-138, doi:10.1182/blood-2011-03-341404 (2011).