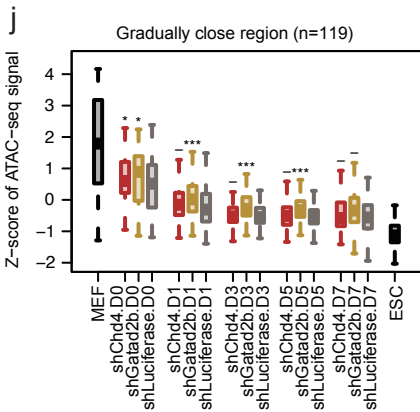
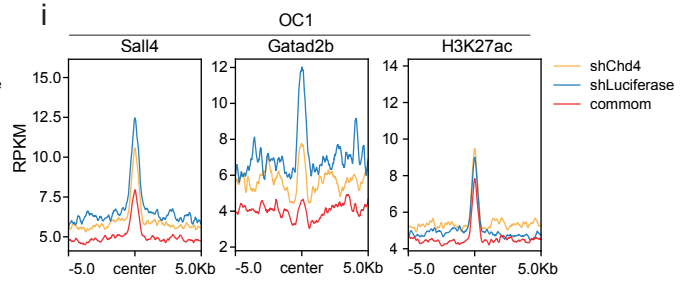
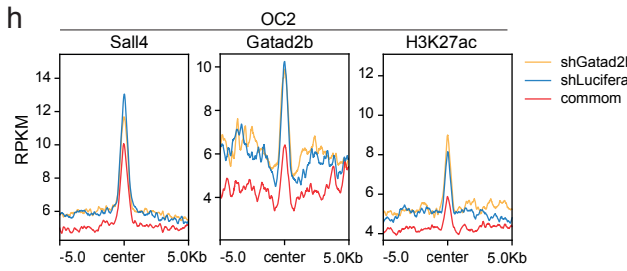
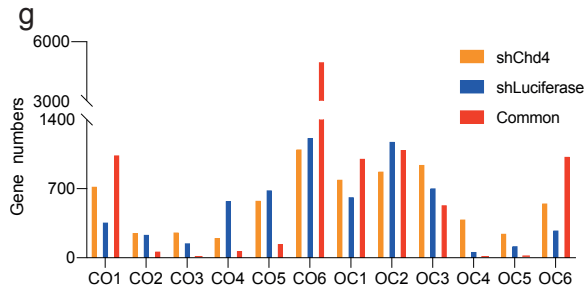
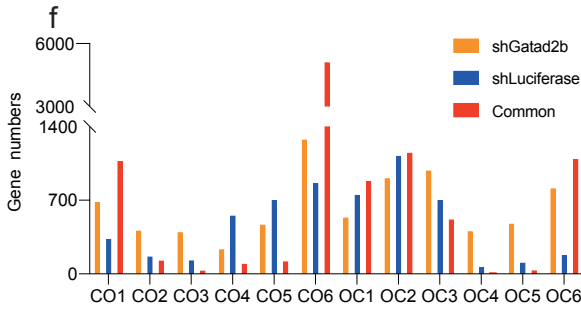
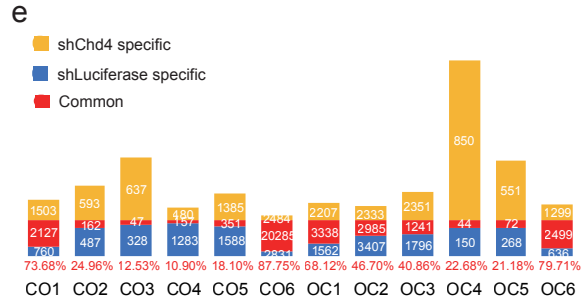
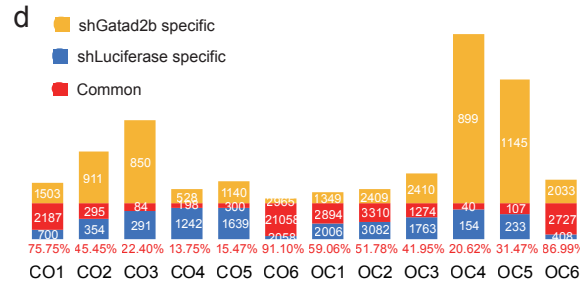
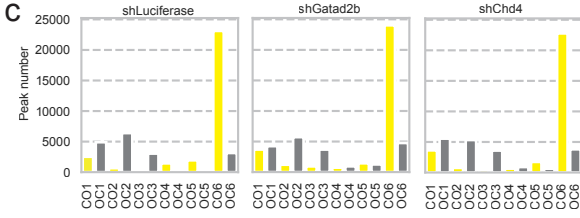
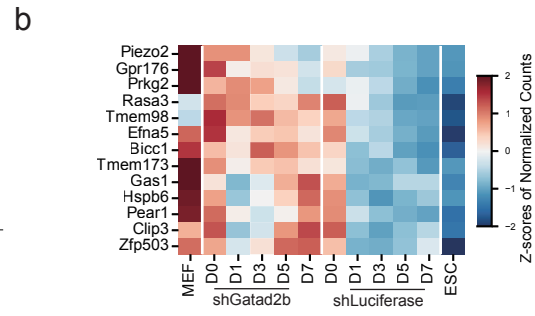
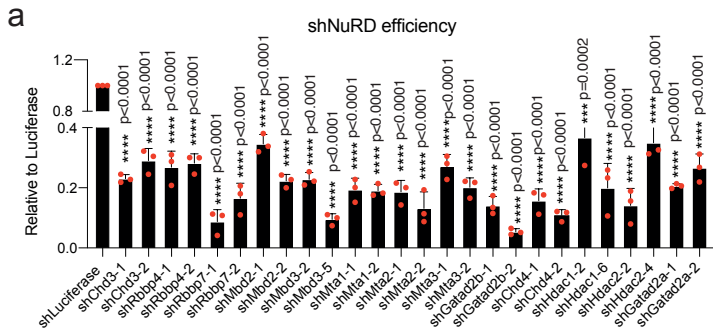


Supplementary Fig. 1 Establishment of JGES reprogramming

a, Schematic diagram of establishment of JGES(Jdp2-Glis1-Esrrb-Sall4)-iCD3 reprogramming system. **b**, Single chemical dropout based on 7F-iCD1 system. Data are mean \pm s.d., two-sided, unpaired t test; n = 3 independent experiments, *p < 0.05, **p < 0.01 ****p < 0.0001. **c**, GSK-LSD1 2HCl and SGC0946 hits at 1 μ M and 5 μ M concentration among the chemical screening library. Two-sided, unpaired t test; n = 3 independent experiments, FC(Fold Change) = 1.5, p-value < 0.05. **d**, Components and differences between iCD1 and iCD3. **e, f**, GFP+ clones number and photograph of 7F-iCD1 and 7F-iCD3 system at day7 per 1.5×10^4 MEF cells. Scale bars, 2.5mm. Data are mean \pm s.d., two-sided, unpaired t test; n = 3 independent experiments, ***p < 0.001. **g**, Oct4-GFP+ colonies number of single factor dropout based on 7F-iCD3 at day7 per 1.5×10^4 MEF cells. Data are mean \pm s.d., two-sided, unpaired t test; n = 3 independent experiments, *p < 0.05, ***p < 0.001, ****p < 0.0001. **h,i**, Comparison between JGES and 7F GFP+ clones number at day7 in iCD3 medium. Scale bars, 2.5mm. Data are mean \pm s.d., two-sided, unpaired t test; n = 3 independent experiments, ns > 0.05. **j**, PCR for genomic insertion identification of OKSM-iPSCs, 7F-iPSCs, JGES-iPSCs-1, JGES-iPSCs-2. **k**, Morphology of JGES-iPSCs at passage 2 and 5. Scale bars, 250 μ m. **l**, Karyotype of JGES-iPSCs. **m**, Offspring of JGES-iPSCs cell line and JGES-iPSCs clones picked at day7 Chimeric and germline transmission mice. **n**, Table to show living pups of Chimaeras and germline transmission of JGES-iPSCs cell line and JGES-iPSCs clones picked at day7. Source data related to Fig 1b, e,g,h are provided as a Source Data file.

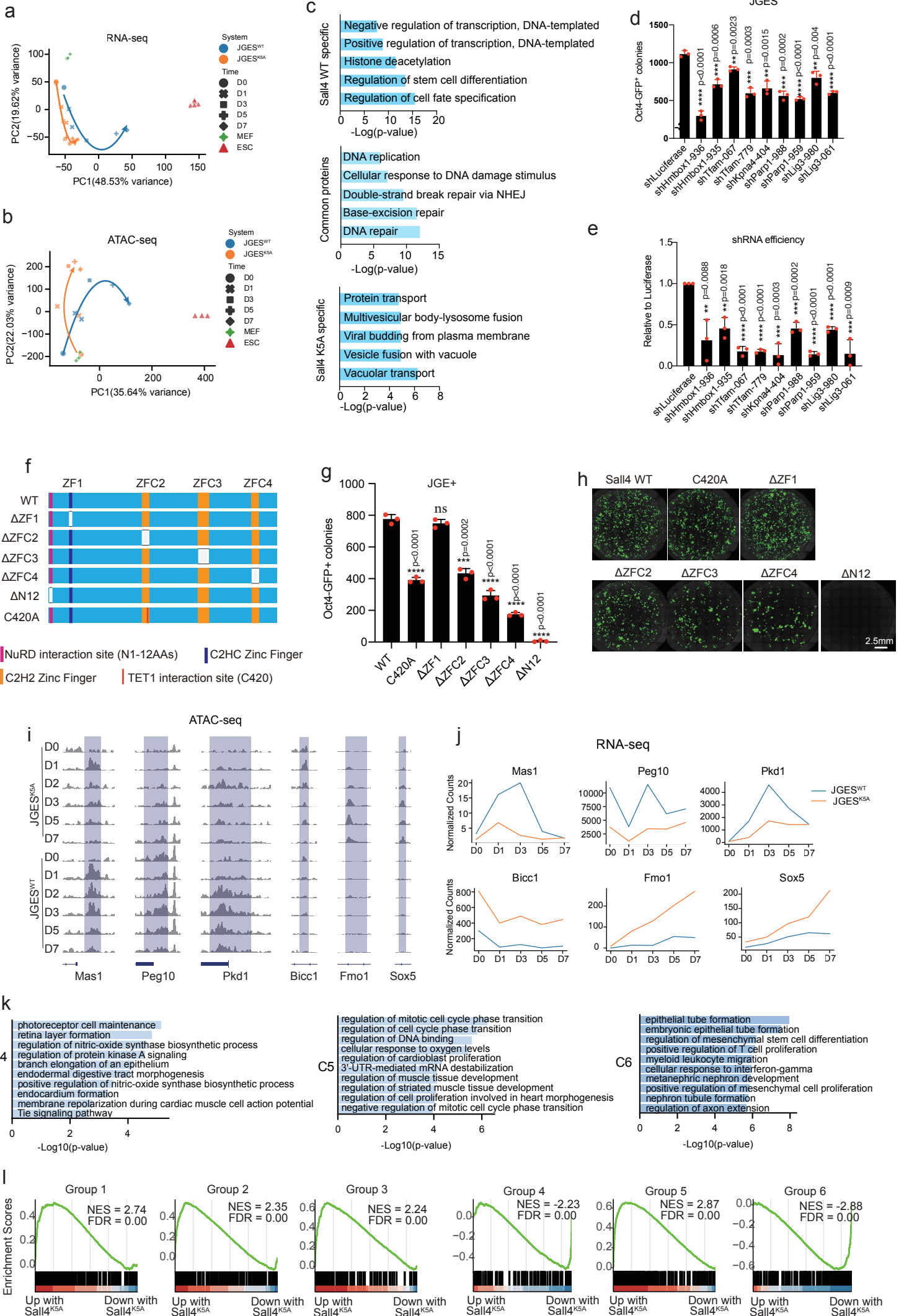


k

Rank	Motif	p-Value	Matches to Known Motif
1		1e-22	Fos(bZIP), Fra1(bZIP), JunB(bZIP), Fosl2(bZIP), FOSB:JunB, BATF(bZIP), Atf3(bZIP), FOSL2:JUNB, FOS:JUNB, FOSL2:JUND
2		1e-10	TEAD1(TEAD), TEAD3, TEAD4(TEA), SPIC, IKZF1, TEAD(TEA), TEAD2, TEAD2(TEA), ELF3(ETS), PB0058.1
3		1e-10	EWS:ERG, EWS:FLI1, ERG(ETS), Eln2(Ets), ELF3, ELF1, ETV1, IKZF1, GABPA, ETV4,
4		1e-9	PB0208.1, ZFX(Zf), ZNF711(Zf), PB0120.1, KLF11, RUNX2(Runt), PB0201.1, ZNF692(Zf), Egr2(Zf), PLAGL2
5		1e-9	Brm2(POU), Pit1(Homeobox), PB0170.1_Sox17, PB0178.1, MEF2B, MEF2D, PH0148.1, OCT:OCT, Mef2a(MADS), Hoxa13(Homeobox)

Supplementary Fig. 2 Knockdown NuRD subunits compromise JGES reprogramming

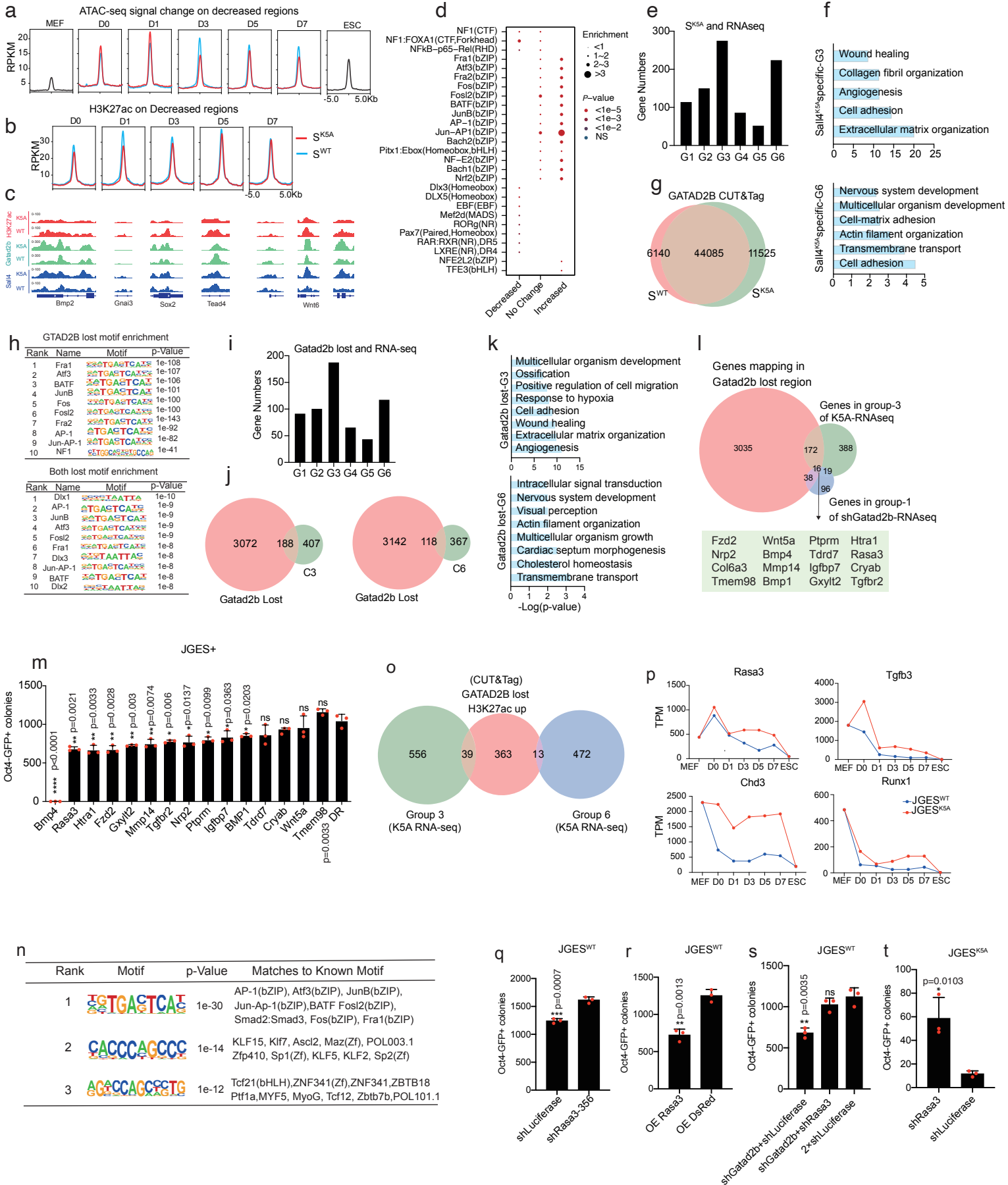
a, knocking down efficiency of NuRD subunits by shRNAs compared to shLuciferase, n=3 independent replicates. Data are mean \pm s.d., two-sided, unpaired t test; n = 3 independent experiments, ***p < 0.01 ****p < 0.0001. Source data is provided as a Source Data file. **b**, Heatmap showing the transcription of represented genes that failed to silence with knocking down Gatad2b. **c**, Bar plot showing the number of peaks defined in each OC/CO categories. Loci of open chromatin were arranged into groups depending upon the day of reprogramming they changed from closed to open (CO) or open to closed (OC) in shLuciferase. **d**, **e**, Bar plot showing the number of common and specific peaks in each OC/CO category when comparing shGatad2b (**d**) or shChd4 (**e**) to shLuciferase. Colors indicated peaks found in shGatad2b or shChd4 (yellow) only, shLuciferase (blue) only and both (red), respectively. Data under each column are presented as the percentage of common peaks that found in shGatad2b or shChd4 relative to shLuciferase. **f**, **g**, Bar plot showing the number of genes located from peaks in fig **d** and **e**, respectively. **h**, **i**, Pileup of Sall4, Gatad2b and H3K27ac CUT&Tag signal for specific and common regions in OC2 (shGatad2b) and OC1 (shChd4), respectively. **j**, Box plot showing the ATAC-seq signal of the gradually closing regions with knockdown Gatad2b and Chd4. The box plots indicate the medians (centerlines), first and third quartiles (bounds of boxes) and 1.5 multiply by interquartile range (whiskers). A two-sided Student's t-test was performed for comparisons between classes (***: p-value < 0.001; **: p-value < 0.01; *: p-value < 0.05; -: p-value > 0.05). **k**, Enriched motifs of the regions in **j**. Source data related to Fig s2a is provided as a Source Data file.



Supplementary Fig. 3 Sall4 mutation and deletion inhibits JGES reprogramming

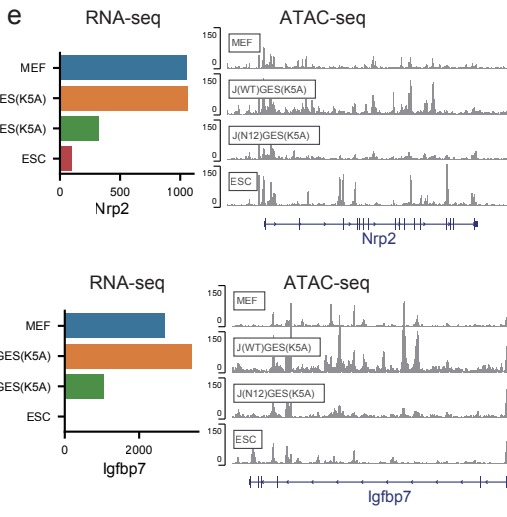
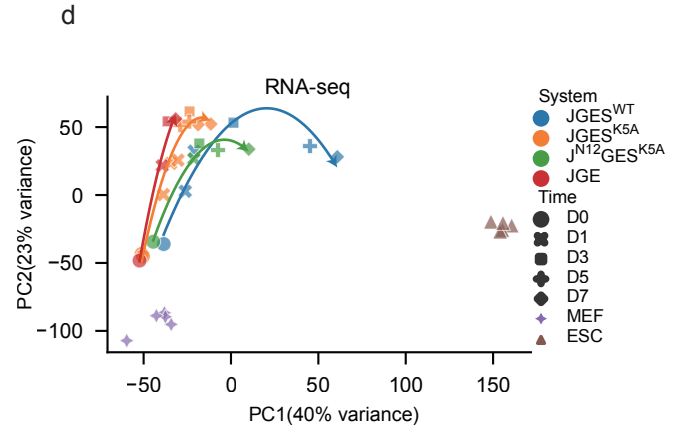
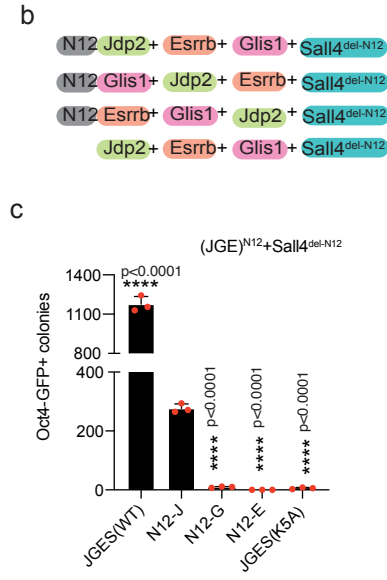
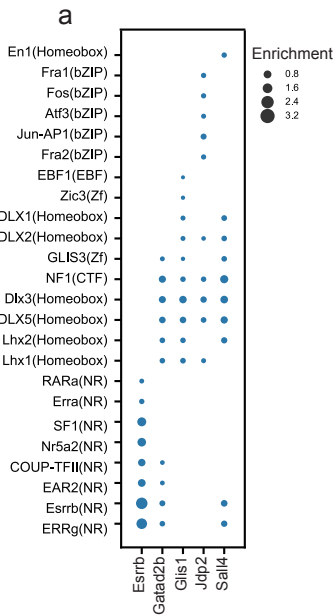
a, PCA of transcriptome dynamics during JGES reprogramming by WT or K5A mut Sall4 in combination with JGE. Blue and yellow solid line with arrows represent WT and K5A mut Sall4 respectively. **b**, PCA of chromatin accessibility during reprogramming by WT or K5A mut Sall4 in combination with JGE. Blue and yellow solid line with arrows represent WT and K5A mut Sall4 respectively. **c**, Gene ontology analysis of IP-MS enriched proteins. **d**, Oct4-GFP positive colonies number of depletion the genes of Sall4^{WT} and Sall4^{K5A} common enriched proteins by shRNA during JGES reprogramming. Data are mean \pm s.d., two-sided, unpaired t test; n = 3 independent experiments, **p < 0.01, ***p < 0.001, ****p < 0.0001. **e**, Knockdown efficiency of the genes of Sall4^{WT} and Sall4^{K5A} common enriched proteins by shRNA; Data are mean \pm s.d., two-sided, unpaired t test; n = 3 independent experiments, **p < 0.01, ***p < 0.001, ****p < 0.0001. **f**, Diagram of mutation and truncated SALL4 protein. **g**, **h**, OCT4-GFP positive colonies number and photograph of mutation and truncated Sall4 co-transfected with Jdp2-Glis1-Esrrb at day7. Scale bars, 2.5mm. Data are mean \pm s.d., two-sided, unpaired t test; n = 3 independent experiments, ns > 0.05. ***p < 0.001, ****p < 0.0001. **i**, Selected genomic views of the ATAC-seq data: Mas1 (chr17:12,838,069-12,851,859, scale: 0-50 RPKM), Peg10 (chr6:4,747,306-4,760,517, scale: 0-50 RPKM), Pkd1 (chr17:24,549,950-24,596,514, scale: 0-50 RPKM), Bicc1 (chr10:70,922,837-71,159,690, scale: 0-70 RPKM), Fmo1 (chr1:162,829,788-162,860,002, scale: 0-150 RPKM), Sox5 (chr6:143,828,425-143,947,108, scale: 0-100 RPKM). Identified regions are marked with a gray box. **j**, Line plot showing the expression for the respective genes is shown in i. **k**, gene ontology (GO) analysis of the genes located in the region cluster in 2f. **l**, Gene-set enrichment analysis of the gene clusters in Fig 1f to the samples in reprogramming of WT/K5A Sall4 with JGE on day3. NES, normalized enrichment score; FDR, false discovery rate. Source data related to Fig s3d,e,g are provided as a Source Data file.

Fig S4



Supplementary Fig. 4 SALL4 K5A mutation fail to close the somatic chromatin

a, b, Pileup of ATAC-seq and H3K27ac signal of decreased regions in 3c for MEF, day0-7 (JGE+Sall4^{WT}/Sall4^{K5A} reprogramming), and ESC. **c**, selected genomic views of H3K27ac (day1) for the indicated genes. **d**, Enriched motifs of regions in 3c. Hypergeometric test. **e**, Histogram showing the overlap of genes between Sall4^{K5A} specific annotated nearest genes (Fig.3a) and RNA-seq data (Fig.2i). **f**, Gene ontology (GO) analysis of the genes in cluster 3 and 6 (Fig S4e). The p-value in GO results were calculated by hypergeometric test. **g**, overlapping peaks on day 1 in JGES^{WT} or JGES^{K5A} reprogramming. **h**, Enriched motifs of both lost regions and Gatad2b lost regions in 3k. Hypergeometric test. **i, j**, overlap of genes between Gatad2b lost annotated nearest genes (Fig 3i) and RNA-seq data (Fig 2i). **k**, Gene ontology (GO) analysis of the genes (Fig S3j), **l**, overlapping genes between Gatad2b lost regions (Fig 3i), gene cluster 1 in Fig 1f and gene cluster3 in Fig 2i. The common genes in those three groups were listed in the box below venn map. **m**, Oct4-GFP positive colonies of overexpression factors in Fig S4l during JGES reprogramming at day7. Data are mean \pm s.d., two-sided, unpaired t test; n = 3 independent experiments, ns > 0.05. *p < 0.05, **p < 0.01, ****p < 0.0001. **n**, Enriched motifs of the regions in Fig 3m. **o**, Venn diagram showing the overlapping genes between annotated nearest genes in Fig 3l and genes in cluster3/6 in Fig 2i. **p**, Line plot showing the expression changes of genes in Fig S4nl. **q, r**, Oct4-GFP positive colonies of knocking down and overexpressing of Rasa3 in JGES reprogramming system at day7. Data are mean \pm s.d., two-sided, unpaired t test; n = 3 independent experiments, ***p < 0.001. **s, t**, knocking down Rasa3 could rescue knocking down of Gatad2b in JGES reprogramming. Data are mean \pm s.d., two-sided, unpaired t test; n = 3 independent experiments, ***p < 0.001. Source data related to Fig s1m, q-t are provided as a Source Data file.



g

Rank	Name	Motif	p-Value
1	Fra2		1e-132
2	Atf3		1e-125
3	Fos		1e-123
4	JunB		1e-121
5	BATF		1e-120
6	Fra2		1e-117
7	AP-1		1e-114
8	Fosl2		1e-109
9	Jun-AP1		1e-98
10	Bach2		1e-59

Supplementary Fig. 5 Rescue of Sall4 N12 deletion by grafting N12 onto Jdp2

a, Dot plot showing the motifs enriched by Esrrb, Gatad2b, Glis1, Jdp2 and Sall4 on day 2 of reprogramming. **b**, Diagram showing grafting N12 individually to Jdp2, Esrrb, and Glis1 during reprogramming with Sall4^{delN12}. Jdp2^{N12}: (Jdp2^{N12}+Glis1+Esrrb+Sall4^{delN12}), Glis1^{N12}:(Jdp2+Glis1^{N12}+Esrrb+Sall4^{delN12}), Esrrb^{N12}:(Jdp2+Glis1+Esrrb^{N12}+Sall4^{delN12}). **c**, Numbers of Oct4 GFP positive iPS colonies induced by Jdp2^{N12}, Glis1^{N12}, and Esrrb^{N12}; n = 3 independent experiments, ****p < 0.0001. **d**, PCA of RNA-seq for MEF, JGES, JGE, JGE with K5A mut Sall4, GE with N12 delete Jdp2 and K5A mut Sall4 and ESC samples. Solid line with arrows represents one reprogramming sample at sequential time. **e**, selected genomic views of ATAC-seq data (right) are shown for the indicated genes in Jdp2^{N12/WT} and Sall4^{K5A} reprogramming on day 1, MEF, and ESC with corresponding RNA expression (left). **f**, Venn diagram showing the overlap of GATAD2B binding sites on JDP2 binding sites. JDP2 CUT&Tag and GATAD2B CUT&Tag were performed in Jdp2^{WT} and Jdp2^{N12} condition. JDP2 binding sites were divided into three distinct categories: JDP2^{WT}-spec, JDP2-common, JDP2^{N12}-spec. **g**, Motif enrichment of overlap binding sites for GATAD2B in Jdp2^{N12} condition (406). Source data related to Fig s5c is provided as a Source Data file.