iScience, Volume *25*

Supplemental information

Structurally-discovered KLF4 variants accelerate and stabilize reprogramming to pluripotency

Evgeniia Borisova, Ken Nishimura, Yuri An, Miho Takami, Jingyue Li, Dan Song, Mami Matsuo-Takasaki, Dorian Luijkx, Shiho Aizawa, Akihiro Kuno, Eiji Sugihara, Taka-aki Sato, Fumiaki Yumoto, Tohru Terada, Koji Hisatake, and Yohei Hayashi

Figure S1 (related to Figure 1)

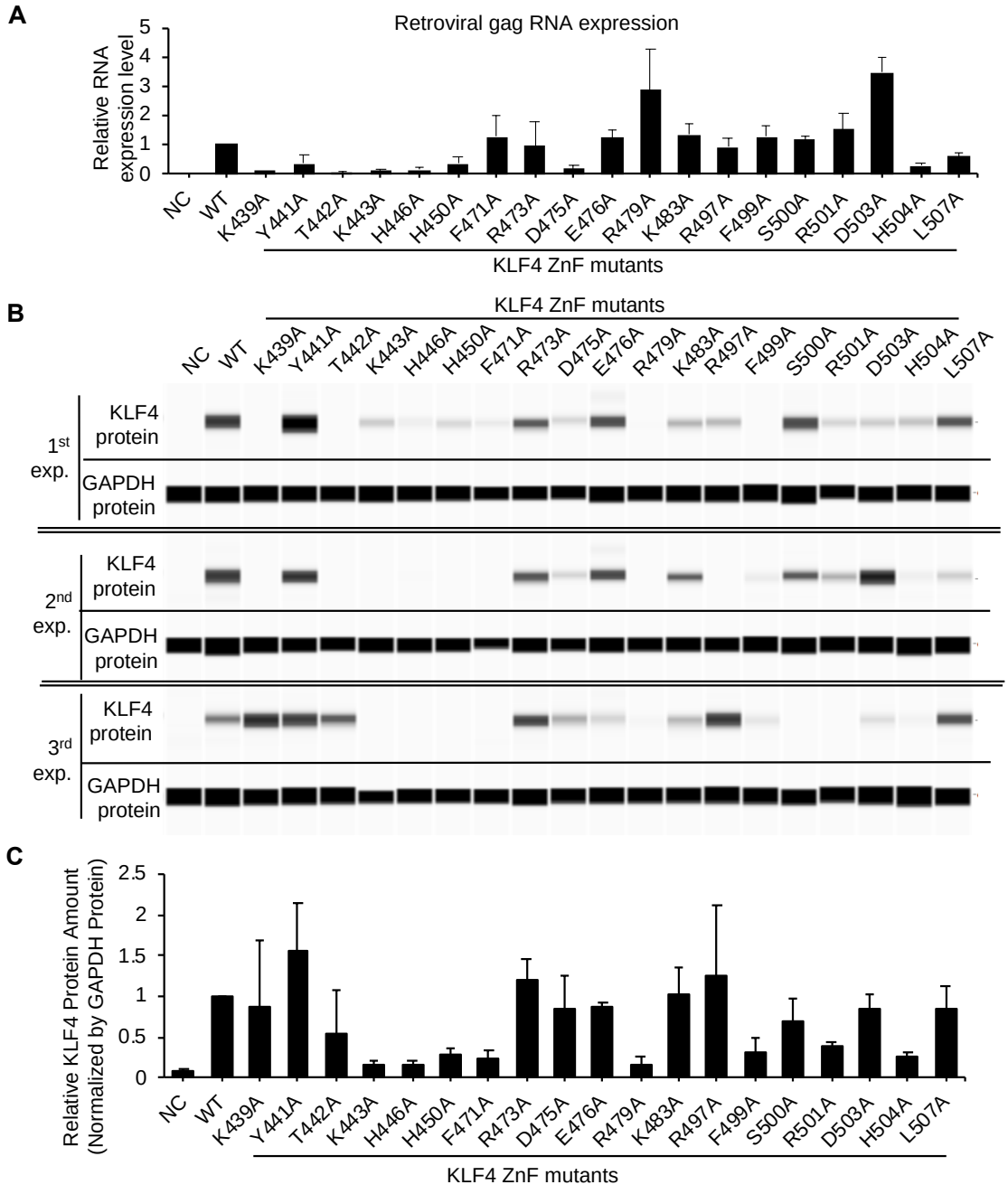


Figure S1. The protein expression levels of KLF4 ZnF alanine-substituted mutants transduced by retrovirus vectors.

(A) Relative retroviral gag RNA expression levels in the retroviral infected MEF after 3 days of infection. Expression levels of gag RNA were normalized by GAPDH mRNA amounts calculated from RT-qPCR. KLF4 WT RNA amount of each experiment was set as 1. Results are mean and SE, n=5.

(B) Capillary western blot data of KLF4 and GAPDH proteins in human neonatal fibroblasts 4 days after transduction of KLF4 alanine-substitute mutants. Blot images of three biological replicates are shown.

(C) Relative KLF4 protein expression levels calculated from capillary western blot data of KLF4 protein amounts normalized by GAPDH protein amounts. KLF4 WT protein amount of each experiment was set as 1. Results are mean and SE, n=3.

Figure S2 (related to Figure 4)

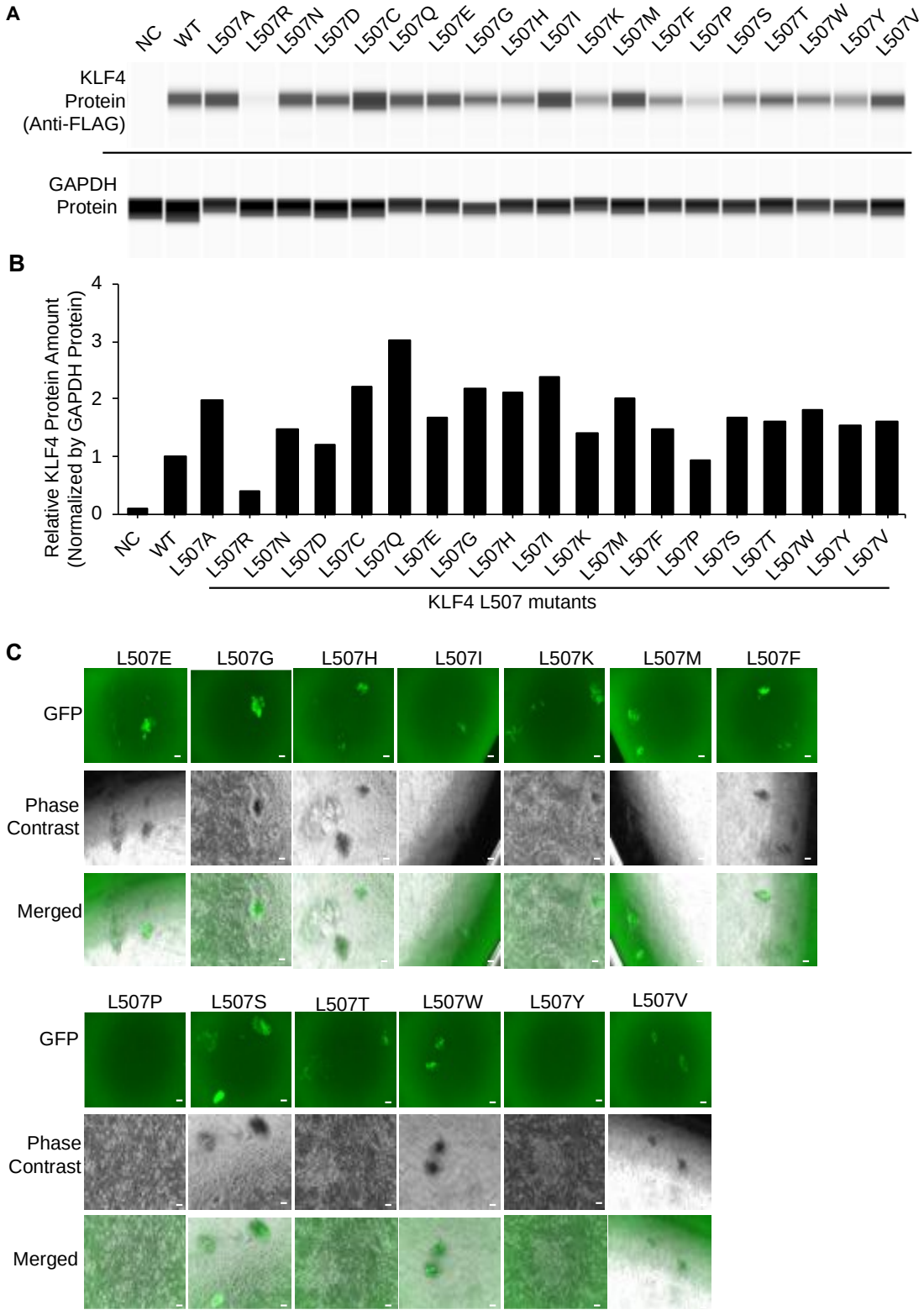


Figure S2. The protein expression levels of KLF4 L507 mutants and iPSC colonies generated with these mutants.

(A) A capillary western blot data of KLF4 and GAPDH proteins in human neonatal fibroblasts 4 days after transduction of KLF4 alanine-substitute mutants.

(B) Relative KLF4 protein expression levels calculated from capillary western blot data of KLF4 protein amounts normalized by GAPDH protein amounts. KLF4 WT protein amount of each experiment was set as 1.

(C) Typical colony morphologies reprogrammed with KLF4 L507 variants (continued from Figure 4A). Scale bars, 200 μm.

Figure S3 (Related to Figure 4 and 5)

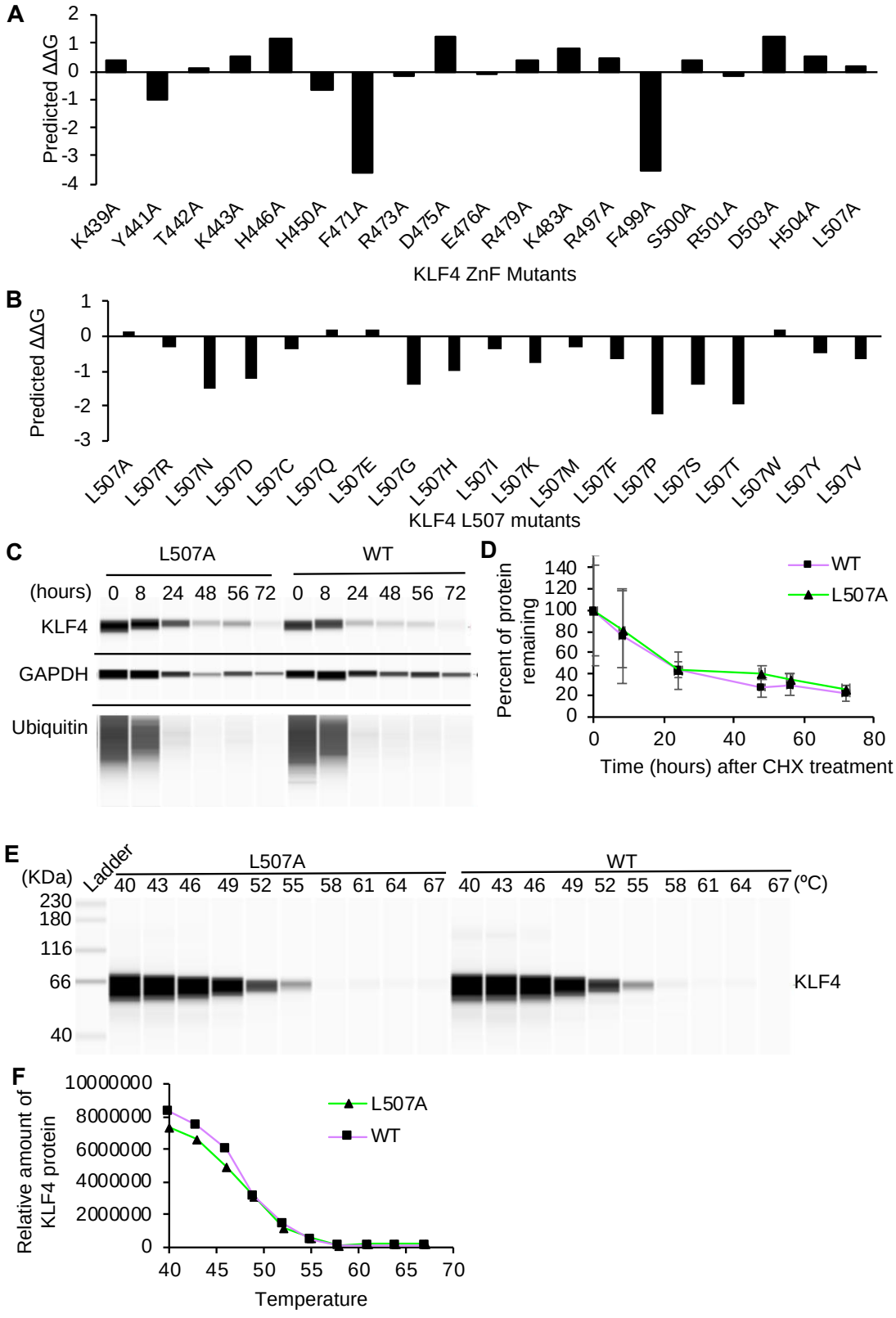


Figure S3. The protein stability of KLF4 WT and L507A variant.

(A and B) Computational prediction of the effect of KLF4 ZnF alanine mutants (A) and KLF4 L507 variants (B) on protein stability using X-ray crystal structure of KLF4 ZnF and DNA complex (PDB: 2WBU) by SDM (Site Directed Mutator) program.

(C) A typical capillary western blot data of KLF4, GAPDH, and ubiquitinated proteins in human neonatal fibroblasts overexpressing KLF4 WT or L507A in the time course after cycloheximide (CHX) treatment.

(D) The ratio of KLF4 protein abundance calculated from the data of capillary western blot on human neonatal fibroblasts overexpressing KLF4 WT or L507A in the time course after CHX treatment. Results are mean and SE, n=3.

(E) A capillary western blot data of KLF4 protein through after cellular thermal shit assay performed on human neonatal fibroblasts overexpressing KLF4 WT or L507A.

(F) The quantification of KLF4 protein abundance through after cellular thermal shit assay performed on human neonatal fibroblasts overexpressing KLF4 WT or L507A.

Figure S4 (Related to Figure 5A)

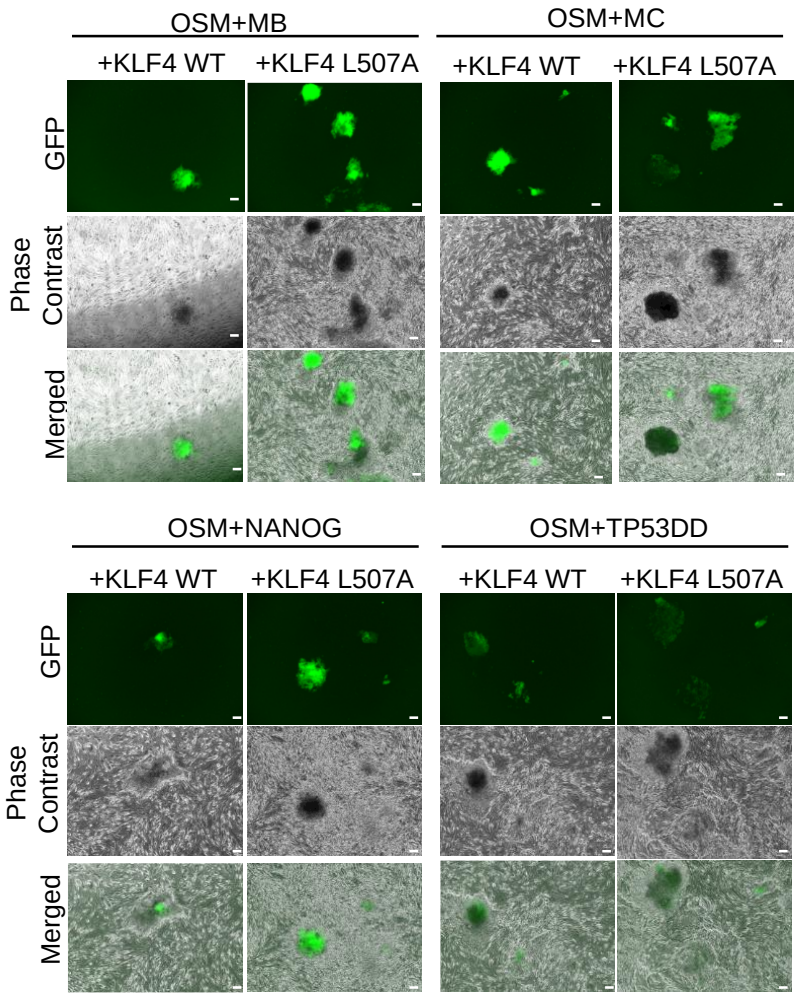


Figure S4. Nanog-GFP colonies in reprogramming Nanog-GFP MEF transduced with KLF4 WT or L507A with pro-programming factors.

Scale bars, 200 μm. (continued from Figure 4A).

Figure S5 (Related to Figure 6)

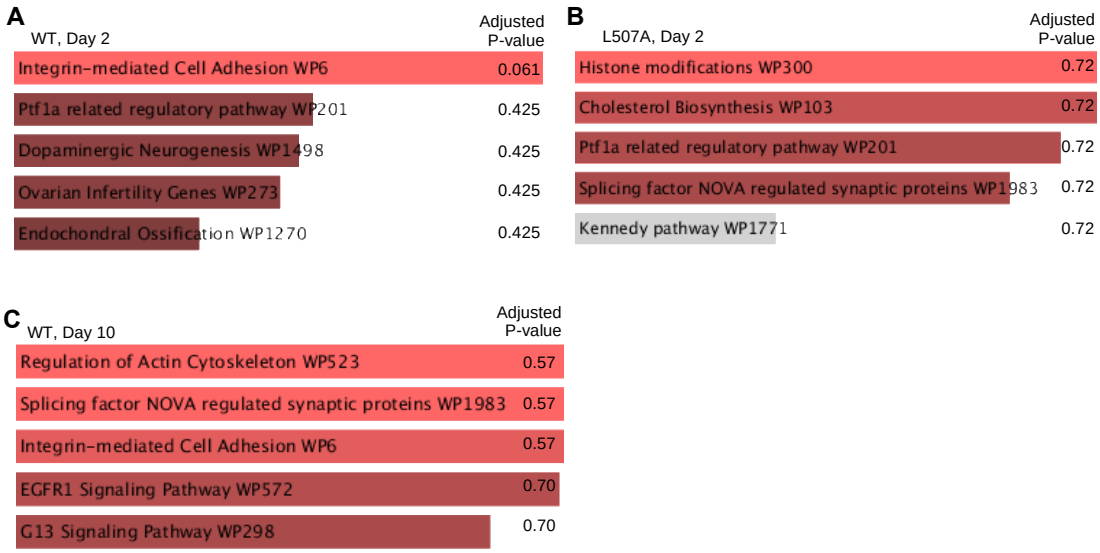
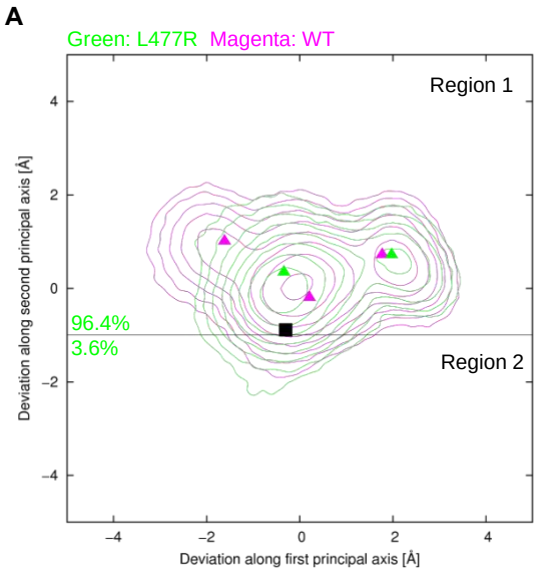
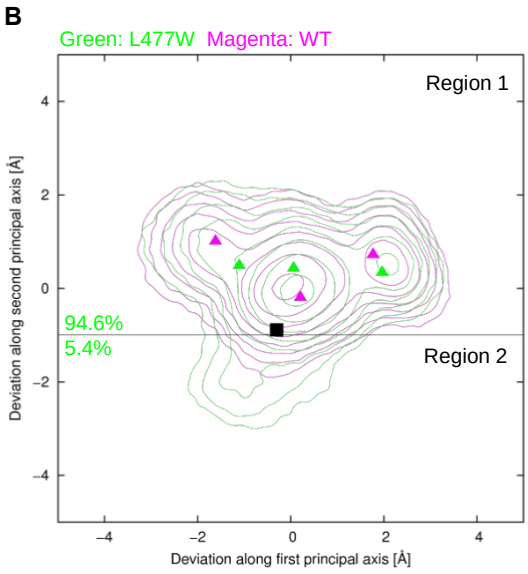


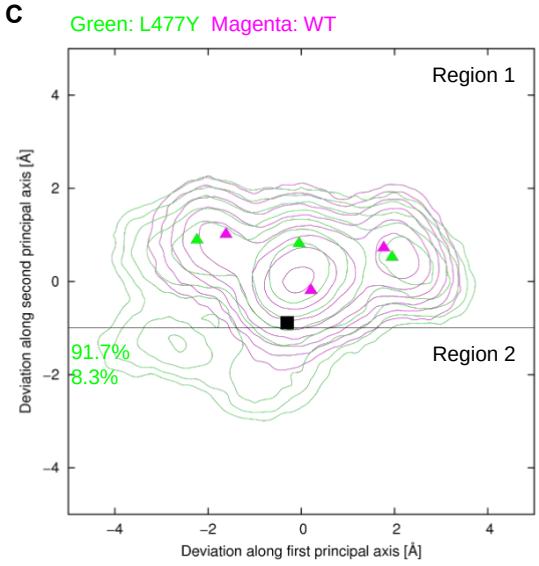
Figure S5. Wikipathway enrichment analysis from ChIP-seq data.

Wikipathway enrichment analysis was performed on the gene set derived from the sample of WT on day 2 (A), L507A on day 2 (B) and WT on day 10 (C). Top 5 pathways that are enriched with statistically significant are illustrated with the adjusted P-values.

Figure S6 (Related to Figure 8)







D Ratio of hydrogen bond formation in each structural region

All	WT	L477A	L477R	L477W	L477Y
Arg467@NE,NH1,NH2 ⇔ B2@OP1,OP2	0.001	0.113	0.001	0.016	0.027
Arg452@NE,NH1,NH2 ⇔ C4@OP1,OP2	0.232	0.250	0.124	0.256	0.120
Ser472@OG ⇔ C5@OP2	0.243	0.329	0.206	0.182	0.143

Region 1 (above the line)	WT	L477A	L477R	L477W	L477Y
Arg467@NE,NH1,NH2 ⇔ B2@OP1,OP2	0.000	0.000	0.000	0.000	0.003
Arg452@NE,NH1,NH2 ⇔ C4@OP1,OP2	0.233	0.123	0.122	0.258	0.116
Ser472@OG ⇔ C5@OP2	0.247	0.227	0.211	0.186	0.154

Region 2 (below the line)	WT	L477A	L477R	L477W	L477Y
Arg467@NE,NH1,NH2 ⇔ B2@OP1,OP2	0.040	0.332	0.014	0.286	0.292
Arg452@NE,NH1,NH2 ⇔ C4@OP1,OP2	0.200	0.498	0.158	0.212	0.165
Ser472@OG ⇔ C5@OP2	0.078	0.526	0.048	0.110	0.028

Figure S6. MD simulation analysis of the effect of the mouse Klf4 L477 mutations (identical to human KLF4 L507) on the structure of the Klf4 ZnF-DNA complex (A-C) Conformational distribution of the mouse Klf4 (WT)-DNA complex (magenta contours) and the Klf4(L477R)-DNA complex (green contours)

(A), the Klf4(L477W)-DNA complex (green contours) (B), or the Klf4(L477Y)-DNA complex (green contours) (C). The level of n-th contour is $2^{n-1} \times 10^{-4}$. The positions of the initial structure and the representative structures obtained from the cluster analysis of the MD trajectories of the Klf4(WT)-DNA and the Klf4(L477A)-DNA complexes are indicated by a black rectangle, magenta triangles, green triangles, respectively. The black line separates conventional conformation distribution, Region 1, and unique conformation distribution of the Klf4(L477A)-DNA complex as Region 2.

(D) Ratio of hydrogen bond formation in each structural region of MD simulation on Klf4 (WT)-DNA complex, Klf4 (L477A)-DNA complex, Klf4(L477R)-DNA complex (green contours), Klf4(L477W)-DNA complex (green contours), and the Klf4(L477Y)-DNA complex.

Table S1. The list of DNA-interacting amino acid residues in KLF4 ZnF domain, related to Figure 1. The amino acid residues highlighted in gray are omitted in site mutagenesis study.

Position within ZnF	Polypeptide numbering		DNA interaction determined by PDBePISA		Conservation among KLF2, KLF4, and KLF5
	Mouse Klf4	Human KLF4	PDB ID: 2WBU	PDB ID: 4M9E	
ZnF 1	Lys409	Lys439	Yes	Yes	Yes
	Thr410	Thr440	Yes	Yes	No
	Tyr411	Tyr441	Yes	Yes	Yes
	Thr412	Thr442	Yes	Yes	Yes
	Lys413	Lys443	Yes	Yes	Yes
	Ser415	Ser445	No	Yes	Yes
	His416	His446	Yes	Yes	Yes
	Ala419	Ala449	Yes	Yes	Yes
	His420	His450	Yes	Yes	Yes
ZnF 2	Thr423	Thr453	Yes	No	Yes
	Phe441	Phe471	Yes	Yes	Yes
	Arg443	Arg473	Yes	Yes	Yes
	Asp445	Asp475	Yes	Yes	Yes
	Glu446	Glu476	Yes	Yes	Yes
	Arg449	Arg479	Yes	Yes	Yes
	Lys453	Lys483	Yes	Yes	Yes
ZnF 3	Arg458	Arg488	Yes	No	No
	Arg467	Arg497	Yes	Yes	Yes
	Phe469	Phe499	Yes	Yes	Yes
	Ser470	Ser500	Yes	Yes	Yes
	Arg471	Arg501	Yes	Yes	Yes
	Asp473	Asp503	Yes	Yes	Yes
	His474	His504	Yes	Yes	Yes
	Leu477	Leu507	Yes	Yes	Yes

Table S2. The list of primer sequence for making KLF4 alanine mutants, related to STAR Methods.

No.	Name	Sequence (5 - 3)
1-1 (FW)	439	TGCGGCGCAACCTACACAAAGAGTTC
1-2 (RV)		GTAGGTTGCGCCGCAGCCCGCGTAATC
2-1	441	AAAACCGCCACAAAGAGTTCCCATCTC
2-2		CTTTGTGGCGGTTTTGCCGCAG
3-1	442	ACCTACGCAAAGAGTTCCCATCTCAAGGCACACC
3-2		CTTTGCGTAGGTTTTGCCGCAGCCCG
4-1	443	TACACAGCGAGTTCCCATCTCAAGGCACACCTGCG
4-2		TACTCGCTGTGTAGGTTTTGCCGCAGCCCG
5-1	446	AGTTCCGCTCTCAAGGCACACCTGCGAAC
5-2		GAGAGCGGAACTCTTTGTGTAGGTTTTGCCGCAGC
6-1	450	AAGGCAGCCCTGCGAACCCACACAGGTGAGAAACC
6-2		CAGGGCTGCCTTGAGATGGGAACTCTTTGTGTAGG
7-1	471	TGGAAAGCCGCCCGCTCAGATGAACT
7-2		GCGGGCGGCTTTCCATCCACAGCCGTC
8-1	473	TTCGCCGCCTCAGATGAACTGACCAG
8-2		ATCTGAGGCGGCGAATTTCCATCCACA
9-1	475	CGCTCAGCTGAACTGACCAGGCACTA
9-2		CAGTTCAGCTGAGCGGGCGAATTTCC
10-1	476	TCAGATGCACTGACCAGGCACTACCG
10-2		GGTCAGTGCATCTGAGCGGGCGAATT
11-1	479	CTGACCGCGCACTACCGTAAACACAC
11-2		GTAGTGCGCGGTCAGTTCATCTGAGCG
12-1	483	TACCGTGCACACACGGGGCACCGCCCG
12-2		CGTGTGTGCACGGTAGTGCCTGGTCAGTTCATCTG
13-1	497	TGCGACGCAGCATTTTCCAGGTCGGA
13-2		AAATGCTGCGTCGCATTTTTGGCACTG
14-1	499	CGAGCAGCTTCCAGGTCGGACCACCT
14-2		CCTGGAAGCTGCTCGGTCGCATTTTTG
15-1	500	AGCATTTGCCAGGTCGGACCACCTCG
15-2		GACCTGGCAAATGCTCGGTCGCATTT
16-1	501	TTTTCCGCGTCGGACCACCTCGCCTT
16-2		GTCCGACGCGGAAAATGCTCGGTCGCA
17-1	503	AGGTCGGCCCACCTCGCCTTACACAT
17-2		GAGGTGGGCCGACCTGGAAAATGCTC
18-1	504	TCGGACGCCCTCGCCTTACACATGAA
18-2		GGCGAGGGCGTCCGACCTGGAAAATGC
19-1	507	CTCGCCGCACACATGAAGAGGCATTT
19-2		CATGTGTGCGGCGAGGTGGTCCGACC

Table S3. The list of plasmids made in this study, related to STAR Methods.

Plasmid Name	RIKEN DNA Bank RDB No.
pMXs-3xFLAG-KLF4	19004
pMXs-3xFLAG-KLF4(K439A)	19005
pMXs-3xFLAG-KLF4(Y441A)	19006
pMXs-3xFLAG-KLF4(T442A)	19007
pMXs-3xFLAG-KLF4(K443A)	19008
pMXs-3xFLAG-KLF4(H446A)	19009
pMXs-3xFLAG-KLF4(H450A)	19010
pMXs-3xFLAG-KLF4(F471A)	19011
pMXs-3xFLAG-KLF4(R473A)	19012
pMXs-3xFLAG-KLF4(D475A)	19013
pMXs-3xFLAG-KLF4(E476A)	19014
pMXs-3xFLAG-KLF4(R479A)	19015
pMXs-3xFLAG-KLF4(K483A)	19016
pMXs-3xFLAG-KLF4(R497A)	19017
pMXs-3xFLAG-KLF4(F499A)	19018
pMXs-3xFLAG-KLF4(S500A)	19019
pMXs-3xFLAG-KLF4(R501A)	19020
pMXs-3xFLAG-KLF4(D503A)	19021
pMXs-3xFLAG-KLF4(H504A)	19022
pMXs-3xFLAG-KLF4(L507A)	19023
pMXs-3xFLAG-KLF4(L507R)	19024
pMXs-3xFLAG-KLF4(L507N)	19025
pMXs-3xFLAG-KLF4(L507D)	19026
pMXs-3xFLAG-KLF4(L507C)	19027
pMXs-3xFLAG-KLF4(L507Q)	19028
pMXs-3xFLAG-KLF4(L507E)	19029
pMXs-3xFLAG-KLF4(L507G)	19030
pMXs-3xFLAG-KLF4(L507H)	19031
pMXs-3xFLAG-KLF4(L507I)	19032
pMXs-3xFLAG-KLF4(L507K)	19033
pMXs-3xFLAG-KLF4(L507M)	19034
pMXs-3xFLAG-KLF4(L507F)	19035
pMXs-3xFLAG-KLF4(L507P)	19036
pMXs-3xFLAG-KLF4(L507S)	19037
pMXs-3xFLAG-KLF4(L507T)	19038
pMXs-3xFLAG-KLF4(L507W)	19039
pMXs-3xFLAG-KLF4(L507Y)	19040
pMXs-3xFLAG-KLF4(L507V)	19041