Table S1. Sequences of oligonucleotides used in this study

KT3	5'-ga tcc act agt ccc ggg ttg-3'
KT4	5'-a att caa ccc ggg act agt g-3'
dZfs	5'-ctg ctg gag gac ctg tgc tct ggt aat agc-3'
dZfa	5'-gct att acc aga gca cag gtc ctc cag cag-3'
TYmut1s	5'-gag gga att caa acc gcg gca gca gca cct aag aac ata aat-3'
TYmut1a	5'-att tat gtt ctt agg tgc tgc tgc cgc ggt ttg aat tcc ctc-3'
TYmut2s	5'-gca gca gca cct gcg aac ata aat aaa-3'
TYmut2a	5'-ttt att tat gtt cgc agg tgc tgc tgc-3'
GT1D1S	5′-c agc aat cac gcg ggc ggc-3′
GT1D1A	5′-gcc gcc cgc gtg att gct ggg cc-3′
pME primerF	5′-tcc tca gtg gat gtt gcc ttt act tc-3′
pME primerR	5'-att ata agc tgc aat aaa caa gtt aa-3'

The introduced restriction enzyme sites [(*Sac*II and *Bsp*MI for TYmut1(s/a) and TYmut2 (s/a), respectively)] are underlined.

Table S2. Potential NLS and NES sequences in hGATA6 predicted with various programs

Predicted Sequence		Program	Reference
(A) NLS			
^{345}**RKRK**P**K**^{350}		WoLF PSORT	(*56*)
^{338}**KK**EGIQT**RKRK**P**K**NI^{352}		NLStradamus	(*57*)
^{344}T**RKRK**P**K**N^{351}		ELM	(*58*)
^{345}**RKRK**P**K**^{350}		NLSdb	(*59*)
^{345}**RKRK**^{348}		NucPred	(*60*)
^{332}P**R**PLAM**KK**EGIQT**RKRK**P**K**NIN**K**S**K**TCSGNS^{362}		INSP	(*61*)
(B) NES			
^{328}**L**HG**V**PRP**L**A**M**^{337}	Class 1d*	NES Finder	(*62*)
^{019}**A**PGG**F**VHS**A**A**A**^{29}	Class 1c	Wregex	(*63*)
^{067}**A**NH**A**GG**A**G**A**^{75}	Class 1b		
^{106}**A**GS**A**AH**V**S**A**^{115}	Class 1a & 1d		
^{212}**V**LHS**L**QSR**A**G**A**^{222}	Class 1c & 1d		
^{318}**V**CN**A**CG**L**Y**M**^{326}	Class 1b & 2*		
^{041}**V**Y**V**PT**T**R**V**GSMLPGL^{55}	Class 2 & 3	LocNES	(*64*)
^{212}VLHS**L**QSR**A**GAP**L**P**V**^{226}	Class 1c & 1d		
^{263}YLCN**A**CG**L**YSK**M**NG**L**^{277}	Class 3*		
^{312}NAEGEP**V**CN**A**CG**L**Y**M**^{326}	Class 1b*		
^{317}P**V**CN**A**CG**L**Y**M**KLHGV^{331}	Class 1b & 3*		
^{323}GLYMK**L**HG**V**PRP**L**A**M**^{337}	Class 1d*		

Putative classical NLS and NES motifs in S-type hGATA6 (*14*) were searched for with the prediction methods widely used (*56-64*). The names of the methods and the relevant references are shown in the Table together

with the predicted amino acid sequences indicated by single letters. The residue numbers are based on the S-type sequence. **(A)** As for the predicted **NLS** sequences, basic residues (**K** and **R**) potentially recognized by importin α, which is involved in the classical nuclear import pathway (*24*), are indicated by bold letters. The clusters of basic residues in the sequences are classified as monopartite NLS, since the linker length between two clusters must be 10-12 residues in bipartite NLS (*37*). **(B)** In the predicted **NES**, hydrophobic residues in an NES are indicated by bold letters, double underlines or dots. The Cys residues (C) in the gray background coordinate zinc (*26*). The nomenclature for the pattern is also shown together with the predicted sequence since the predicted NES is further classified into six patterns (*65*). The hydrophobic residues which occupy four positions in Leucine-rich NES recognized by CRM-1 are L, I, V, M, F, C, W, A or T, where C, W, A or T is allowed to occur only once at hydrophobic positions in an NES (*65*). If we consider this positional rule, ^{318}**V**CN**A**CG**L**Y**M**^{326} alone seems to be a possible candidate among the sequences predicted with "Wregex". Furthermore, considering that at least two of the four hydrophobic residues are "**L**" or "**I**" for a more strictly defined pattern (*65*), the sequence ^{328}**L**HG**V**PRP**L**A**M**^{337} suggested by "NES Finder" could be one of the target sites for future study of cAMP-dependent nuclear export of GATA6. Although the class 1d sequence, which is rarely found, has extremely weak NES activity (*65*), several NES candidates overlapping with a real NES and/or the neighboring NES sequences may act in concert as a high affinity NES (*64*). The sequences located in the Zf are indicated by asterisks (*).

Figure S1

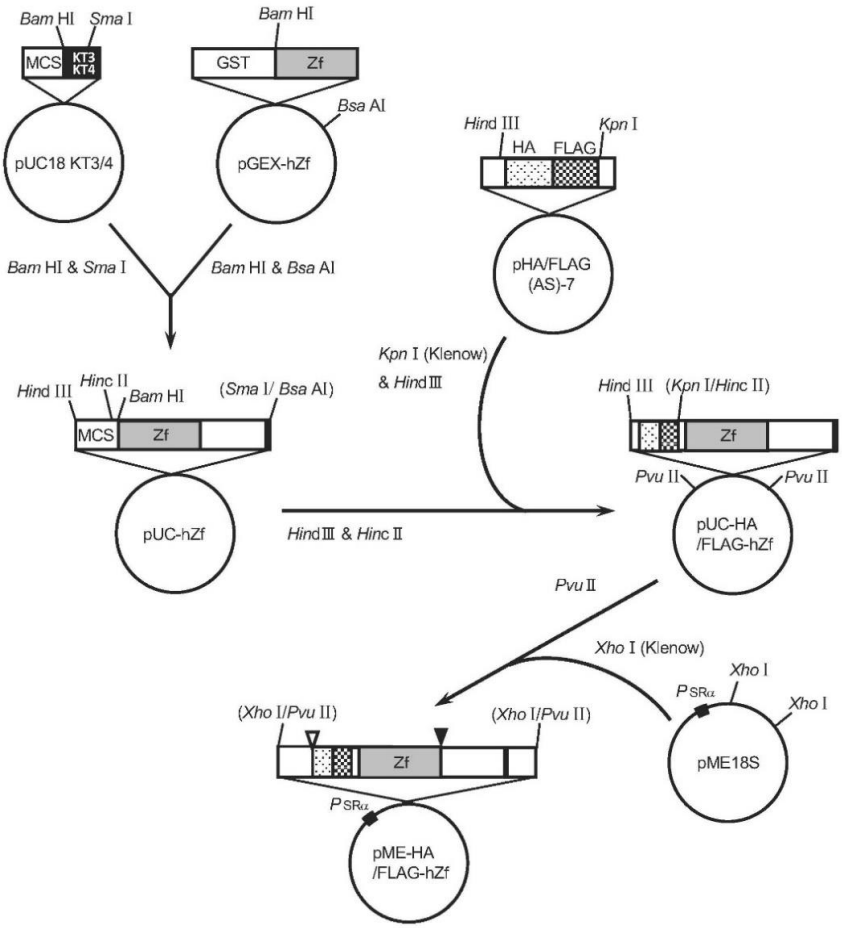

Figure S1. Construction of an expression plasmid for the Zf of hGATA6 with an N-terminal tandem HA/FLAG-tag. The Zf of hGATA6 (Glu^{240}-Thr^{357}) (*14*) with an N-terminal HA/Flag-tag was cloned into mammalian expression plasmid pME18S (*16*,*51*). A cassette, KT3/KT4 (see Table S1), was inserted between the *Bam*HI and *Eco*RI sites of the multi-cloning site (MCS) of pUC18 (*52*). Then the *Bam*HI and *Bsa*AI fragment of pGEX-hZf (*14*) was inserted between the *Bam*HI and *Sma*I sites of the cassette in pUC18 KT3/4. Into the *Hin*dIII and *Hin*cII sites of the resulting plasmid (pUC-hZf), a *Hin*dIII and *Kpn*I fragment, the *Kpn*I site being blunt-ended by Klenow treatment, from pHA/FLAG(AS)-7 (*53*) was introduced to connect the HA/FLAG tag to the amino-terminus of Zf. Then the *Pvu*II fragment of pUC-HA/FLAG-hZf was inserted into the *Xho*I sites of mammalian expression plasmid pME18S after Klenow treatment. This plasmid was named pME-HA/FLAG-hZf. The initiation and termination codons are indicated by open and closed triangles, respectively, in pME-HA/FLAG-hZf. Extra codons for GDSRGS and QFIVTD were inserted before and after the recombinant Zf, respectively. HA/FLAG-hZf was transcribed under the $P_{SR\alpha}$ promoter which is composed of the SV 40 early promoter and the R-U5 segment of the long terminal repeat of human T-cell leukemia virus type 1 (*51*).

Figure S2

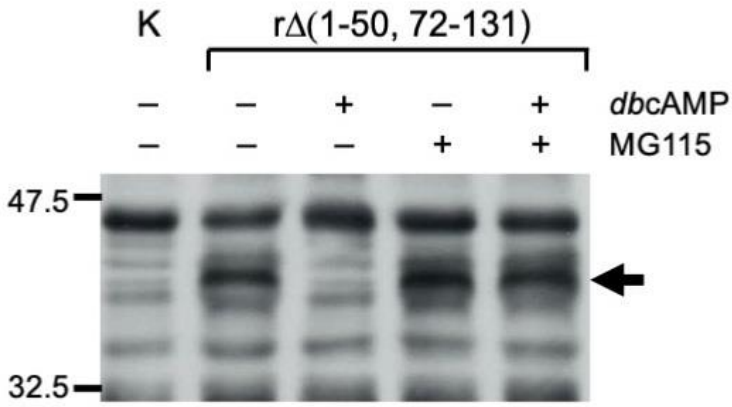

Figure S2. Detection of rGATA6Δ(1-50, 72-131) in the nucleus. The expression plasmid for rGATA6Δ(1-50, 72-131) was constructed as follows: pSRα-GT1' encoding rGATA6Δ50 (*11*) was digested with *Apa*I and *Sma*I, and then oligonucleodide cassette GT1D1S/GT1D1A (Table S1) was inserted into the large fragment. The resulting plasmid was named pSRα-GT1'(Δ72-131). This plasmid was stably expressed in CHO-K1 cells, and G418-resistant colonies were isolated similar for pME-HA/FLAG-hZf (see Materials and Methods). One positive clone was isolated from 28 colonies, and this clone was cultured in the presence (+) or absence (–) of *db*cAMP and MG115. rGATA6Δ(1-50, 72-131), denoted as rΔ(1-50, 72-131) in the figure, was detected by Western blotting after SDS-polyacrylamide gel-electrophoresis of the nuclear extract. The first antibodies were rabbit polyclonal antibodies (hGATA-6C) recognizing the Cys^{358}-Ala^{449} residues of hGATA6 (H-92, Santa Cruz Biotechnology, Dallas, Texas, USA) (× 2,000 diluted), and the second antibodies were horseradish peroxidase-linked donkey anti-rabbit Ig (× 4,000 diluted). Chemiluminescence was detected as described under Materials and Methods. Lane "K" is a negative control (nuclear extract of CHO-K1 cells). The values on the left side are molecular weights ($\times 10^{-3}$). The arrow on the right indicates the position of rGATA6Δ(1-50, 72-131).

Figure S3

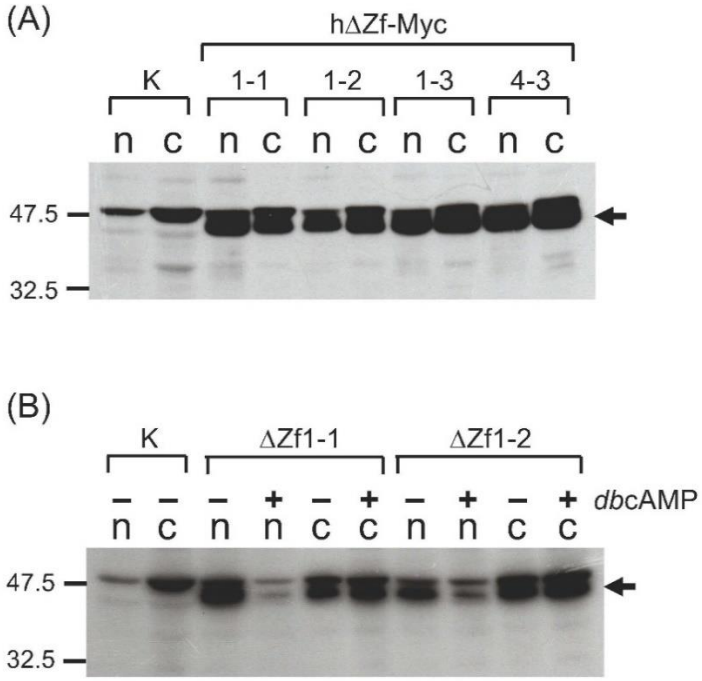

Figure S3. Detection of hΔZf-Myc expressed in stable clones. Expression plasmid pME-hGT1S(ΔZf)Myc was introduced into CHO-K1 cells, and hygromycin-resistant clones were isolated as described under Materials and Methods. Among 22 colonies isolated, four clones (1-1, 1-2, 1-3, 4-3) stably expressed hΔZf-Myc. **(A)** hΔZf-Myc was detected both in the cytoplasm (c) and nucleus (n) by Western blotting with anti-c-Myc after SDS-polyacrylamide gel-electrophoresis. Chemiluminescence was detected as described under Materials and Methods. Fractions of CHO-K1 cells (K) were also analyzed as negative controls. The values on the left side are molecular weights ($\times 10^{-3}$). The arrow on the right indicates the position of hΔZf-Myc. **(B)** Clones 1-1 (ΔZf1-1) and 1-2 (ΔZf1-2) were cultured in the presence (+) or absence (–) of *db*cAMP. hΔZf-Myc was similarly analyzed as in (A).

Figure S4

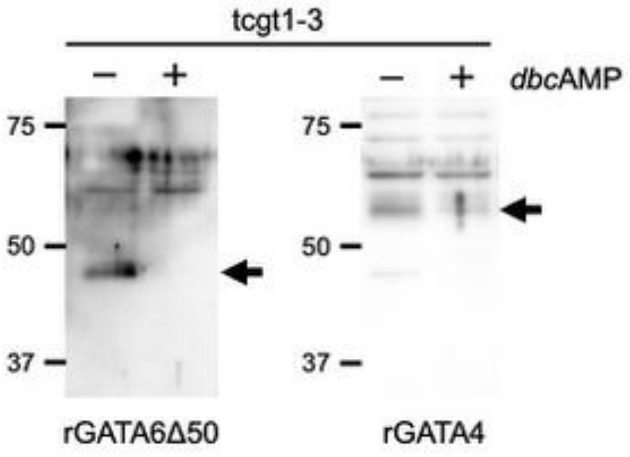

Figure S4. Response of rGATA4 to *db*cAMP treatment. pSRα-rGT2, an expression plasmid for rGATA4, was constructed by inserting the *Eco*RI fragment of cDNA into the *Eco*RI site of pSRαEco (*54*). This plasmid was introduced into tc1-17a cells, which were already expressing rGATA6Δ50 stably (*11*), together with pIREShyg2 (Clontech) in the ratio of 10:1 (w/w) by means of the calcium-phosphate method (*17*). The resistant colonies were isolated in the presence of 600 μg/ml hygromycin. Among 27 colonies isolated, four clones expressed detectable amounts of rGATA4 protein. One of the clones (tcgt1-3) was subjected to analysis. The cells were cultured in the presence (+) or absence (–) of *db*cAMP. rGATA4 and rGATA6Δ50 were detected by Western blotting after SDS-polyacrylamide gel-electrophoresis of the nuclear extract (5 μg protein). The rGATA4 was detected with rabbit polyclonal antibodies recognizing Leu^{54}-Ala^{130} (*54*) (× 500 diluted) of rGATA-4. The rGATA6Δ50 was detected with rabbit polyclonal antibodies recognizing the Thr^{383}-Ala^{441} residues of rGATA6 (*11*) (× 1,000 diluted). The second antibodies were horseradish peroxidase-linked goat anti-rabbit IgG (Cell Signaling Technology, Danvers, MA, USA) (× 5,000 diluted). rGATA4 and rGATA6Δ50 were visualized with a LAS-3000 (Fuji Film, Minato-ku, Tokyo, Japan). The values on the left side are molecular weights (× 10^{-3}). Arrows on the right indicate the positions of rGATA4 and rGATA6Δ50 (right and left panels, respectively). Protein was assayed by means of a Pierce™ Bicinchoninic Acid (BCA) Protein Assay (Thermo Fisher Scientific, Waltham, MA, USA) (*55*).

Supplementary references

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