Supplementary documents

Experimental procedures

Materials

GenomePlex Complete Whole Genome Amplification (WGA) Kit was purchased from Sigma.

Antibodies

Anti-FLAG M2 affinity gel was purchased from Sigma. Anti-Histone H2B (07-371), histone H3 acetyl K9 and K14 (06-599) antibodies were purchased from Millipore. Histone H3 trimethyl K4 (ab1012), histone H3 mono- (ab9045), di- (ab1220), trimethyl K9 (ab8898) and histone H3 trimethyl K27 (ab6002) antibodies were purchased from Abcam.

ChIP-on-Chip analysis

hMSC-hTERT-E6/E7 cells ($1x10^8$ cells), stably-expressing Flag-tagged SF-1, were used for ChIP. Preparation of samples for ChIP is described in the text, except for immunoprecipitation. Immunoprecipitation of ChIP samples with anti-FLAG M2 affinity gel was performed overnight at 4°C with rotation. The whole-genome amplification (WGA) method was used to amplify sufficient amounts of DNA for hybridization to the microarray. Labeling of the ChIP sample and the Input DNA, hybridization, and scanning of the arrays were performed by NimbleGen, Inc (Madison, WI). A microarray experiment was performed with a Whole Genome Tiling Array from NimbleGen (designated number: C4524-19-01; 146,115,199 to 158,821,243 on chr 7 and 1 to 64,734,981 on chr 8). Log_2 ratios were computed for a sample pair and after Tukey bi-weight normalization, visualized by SignalMap software (Roche NimbleGen).

Figure legends

Supplementary Fig. 1

ChIP-on-Chip analysis of SF-1 binding sites near the human *StAR* gene on chromosome 8. ChIP experiments were followed by amplification and labeling of the input and the IP DNA using anti Flag-antibody. The labeled input and IP DNAs were then applied to high density oligonucleotide microarray slides, and the data were analyzed and validated as described in the Materials and methods. ChIP-enriched regions were found at the *StAR* promoter (-149/+174) and at 3 kb (-3,521/-2,872) and 15 kb (-15,970/-15,621) upstream sites relative to the *StAR* transcription start site (TSS).

Supplementary Fig. 2

Transcriptional activities of DNA fragments from the region upstream of the *StAR* gene either with or without 8Br-cAMP treatment. Each DNA fragment was cloned into a luciferase vector, and the reporter constructs, along with a negative control vector pGL4.24, were co-transfected with control vector (pCMV) or an SF-1 expression vector (pCMV/mycSF-1) into HEK293 cells. Luciferase activities were measured 48 h after transfection. Only the -3,494/-3,009 DNA fragment showed strong transcriptional activity after expression of SF-1. 8Br-cAMP treatment further strengthened transcriptional activity of the -3,494/-3,009 fragment. Note that DNA from -15 kb showed no transcriptional activity, in spite of its ability to bind SF-1.

Supplementary Fig. 3

Luciferase activities of *StAR* upstream region deletion constructs either with or without 8Br-cAMP treatment. Luciferase activities were measured in the presence or absence of 8Br-cAMP. Deletion constructs were co-transfected with pCMV or pCMV/mycSF-1 into HEK293 cells. Cells were or were not treated with 1 mM 8Br-cAMP, 12 h after transfection. Luciferase activities were then measured 48 h after transfection.

Supplementary Fig. 4

Effects of mutations in the SF-1 sites within the distal control region on luciferase activity either with or without 8Br-cAMP treatment. Mutant constructs were co-transfected with pCMV or pCMV/mycSF-1 into HEK293 cells. Cells were or were not treated with 1 mM 8Br-cAMP, 12 h after transfection. Luciferase activities were then measured 48 h after transfection. The 8Br-cAMP treatment further increased luciferase activities in all constructs.

Supplementary Fig. 5

(A) Expression of mycLRH-1 and mycSF-1 proteins after adenovirus-mediated introduction of mycLRH-1 or mycSF-1 genes into MSCs (UE7T-13).

(B) Induction of expression of steroidogenesis related genes after adenovirus-mediated introduction of mycLRH-1 or mycSF-1 genes into MSCs.

Supplementary Fig. 6

Recruitment of modified histone molecules to the promoter of the human *StAR* gene. Acetylated histone H3 (H3Ac), mono- (H3K9(me1), di- (H3K9(me2), tri- (H3K9(me3) methylated lysine 9 residue of histone H3 molecules as well as native H3 molecules were present at the promoter site in LacZ-expressing MSC cells. Differentiation induced by adenovirus-mediated expression of SF-1 resulted in decreased binding of the molecules, whereas binding of SF-1 and RNAP II were increased after SF-1 induced differentiation.

Supplementary Fig. 7

Transcriptional activities of DNA fragments from the upstream region of *StAR* in KGN cells, which express endogenous SF-1. Each DNA fragment was cloned into a luciferase vector and the reporter constructs, along with the negative control vector pGL4.24, were transfected into KGN cells. Luciferase activities were measured 48 h after transfection. Only the -3,494/-3,009 DNA fragment showed strong transcriptional activity in KGN cells. 8Br-cAMP treatment further strengthened transcriptional activity of the -3,494/-3,009 fragment. Note that DNA from -15 kb showed no transcriptional activity, in spite of its ability to bind SF-1.

Supplementary Fig. 8

Luciferase activities of deletion constructs of the *StAR* upstream region in KGN cells either with or without 8Br-cAMP treatment. Luciferase activities were measured in the presence or absence of 8Br-cAMP. Deletion constructs were transfected into KGN cells. Cells were or were not treated with 1 mM 8Br-cAMP, 12 h after transfection. Luciferase activities were then measured 48 h after transfection.

Supplementary Fig. 9

Effects of mutations in the SF-1 sites within the distal control region on luciferase activity in KGN cells either with or without 8Br-cAMP treatment. Mutant constructs were transfected to KGN cells. Cells were or were not treated with 1 mM 8Br-cAMP, 12 h after transfection. Luciferase activities were then measured 48 h after transfection. The 8Br-cAMP treatment further increased luciferase activities in all constructs.

Supplementary Table 1

Nucleotide sequences of oligonucleotides used in plasmid constructions.

Mutated bases are underlined.

Plasmid constructs	Nucleotide sequences (5'-3')
pGL4.24/hStAR(-15 k) F	GAAGCAGCAACAGCAGCATGAATTGCTTA
pGL4.24/hStAR(-15 k) R	TACAGGTATTATTTGGTGTTTTTGTAAGA
pGL4.24/hStAR(-5,966/-5,521) F	GAGTGGTGTCACGACCCAAGT
pGL4.24/hStAR(-5,966/-5,521) R	CATTTGGTCTTTTGTAATCACCTA
pGL4.24/hStAR(-3,494/-3,009) F	TTTTTTGCTCCAAAGCCCTG
pGL4.24/hStAR(-3,494/-3,009) R	TATTATGGGACAGGCCA
pTA2/hStAR(-5,957/-95) F	AACCCAGAGAACTGAGTGGTGTCACGACCCAA
pTA2/hStAR(-5,957/-95) R	GGTCAAGGATAGAGCGATTGCCTCACACTG
pBS/hStAR(-2,459/+45) F	TCCACAGAAGAGACAAGCAAACAC
pBS/hStAR(-2,459/+45) R	CCTGAGCCCCTCAAGCTTCGCCTCTGAG
pGL4.11/hStAR(-3,402/+33) F	CTCCTCCCCCATGGCCT
pGL4.11/hStAR(-2,958/+33) F	GTGCAGGAGAGGCACATGC
pGL4.11/hStAR(-244/+33) F	TGTGTGCCTTCATCTAAGCT
Mut 1 (For nucleotide substitutions)	CTCCTCCCCCATGGCCTGCCATTTTCATCTGGCCTCCTTTC

For site-directed mutagenesis

Mut 2 F	TTGGCCCTTGCAGATTTCACCCAGCACCA
Mut 2 R	TGGTGCTGGGTGAAATCTGCAAGGGCCAA
Mut 3 F	GGCAGCAGCAGGGAATTGATCATCATC
Mut 3 R	AGATGATGATCAATTCCCTGCTGCTGC

Supplementary Table 2

Nucleotide sequences of oligonucleotides used in EMSAs.

Mutated bases are underlined.

Oligonucleotides	Nucleotide sequences (5'-3')
-113/-86 F	gAATCGCTCTATCCTTGACCCCTTCCTTTG
-113/-86 R	aCAAAGGAAGGGGTCAAGGATAGAGCGATT
-113/-86 mutant F	gAATCGCTCTATCCTCGACCCCTTCCTTTG
-113/-86 mutant R	aCAAAGGAAGGGGTCGAGGATAGAGCGATT
-924/-896 F	aCTGGCCAGCTGTTTGACCTTGAACAAGTC
-924/-896 R	tGACTTGTTCAAGGTCAAACAGCTGGCCAG
-924/-896 mutant F	aCTGGCCAGCTGTTTGAAATTGAACAAGTC
-924/-896 mutant R	tGACTTGTTCAATTTCAAACAGCTGGCCAG
-3,397/-3,363 F	tCCCCCATGGCCTGCCATGGTCATCTGGCCTCCTTT
-3,397/-3,363 R	gAAAGGAGGCCAGATGACCATGGCAGGCCATGGGGG
-3,397/-3,363 mutant1 F	tCCCCCATGGAATGCCATGGTCATCTGGCCTCCTTT
-3,397/-3,363 mutant1 R	gAAAGGAGGCCAGATGACCATGGCATTCCATGGGGG
-3,397/-3,363 mutant2 F	tCCCCCATGGCCTGCCATTTTCATCTGGCCTCCTTT
-3,397/-3,363 mutant2 R	gAAAGGAGGCCAGATGAAAATGGCAGGCCATGGGGG
-3,397/-3,363 mutant3 F	tCCCCCATGGCCTGCCATGGTCATCTGGAATCCTTT
-3,397/-3,363 mutant3 R	gAAAGGATTCCAGATGACCATGGCAGGCCATGGGGG
-3,397/-3,363 mutant13 F	tCCCCCATGGAATGCCATGGTCATCTGGAATCCTTT
-3,397/-3,363 mutant13 R	gAAAGGATTCCAGATGACCATGGCATTCCATGGGGG
-3,204/-3,187 F	cTTGCAGAGGTCACCCAGC
-3,204/-3,187 R	tGCTGGGTGACCTCTGCAA
-3,204/-3,187 mutant F	cTTGCAGATTTCACCCAGC
-3,204/-3,187 mutant R	tGCTGGGTGAAATCTGCAA
-3,056/-3,031 F	gGCAGCAGCAGGGCCTTGATCATCATC
-3,056/-3,031 R	aGATGATGATCAAGGCCCTGCTGCTGC

-3,056/-3,031 mutant F	gGCAGCAGCAGGGAATTGATCATCATC
-3,056/-3,031 mutant R	aGATGATGATCAATTCCCTGCTGCTGC

Supplementary Table 3

Nucleotide sequences of oligonucleotides used in real-time RT-PCR and ChIP-qPCR.

Plasmid constructs	Nucleotide sequences (5’-3’)
qRTPCR StAR F	CCACCCCTAGCACGTGGA
qRTPCR StAR R	TCCTGGTCACTGTAGAGAGTCTCTTC
qRTPCR GAPDH F	AATCCCATCACCATCTTCCA
qRTPCR GAPDH R	TGGACTCCACGACGTACTCA
ChIP-15 K F	GGCTTTCATTGTAACTTCTGTGGTG
ChIP-15 K R	TCACAGGCAGTGGCATTATCAG
ChIP-5,966 F	GAGTGGTGTCACGACCCAAGT
ChIP-5,904 R	CCAGCAGTGAGCCCTCTCTCT
ChIP-4,207 F	TATGCACAGGATGGATGGCA
ChIP-4,153 R	ACCCAGCAGTGCTCAGCTCT
ChIP-3,673 F	CTTTAGAGCCACATAGAGACATTCAGG
ChIP-3,619 R	CCTCCATTCATTCAACAGTCCTTT
ChIP-3,495 F	TTTTTTGCTCCAAAGCCCTG
ChIP-3,437 R	CATGCTGGAGAGGAAGGCC
ChIP-3,237 F	GCCTGGAAAGGATGAGGGA
ChIP-3,186 R	TGCTGGGTGACCTCTGCAA
ChIP-3,099 F	CACCTCCACCCCTTTCAGAA
ChIP-3,032 R	ATGATGATCAAGGCCCTGCT
ChIP-2,459 F	TCCACAGAAGAGACAAGCAAACAC
ChIP-2,411 R	TGGTCCTGATTTGTTGAATGGTT
ChIP-1,987 F	CCCAATATTCTTGCCCAGTGA
ChIP-1,929 R	CCCTTTACAGTGACCTTGAGGC
ChIP-902 F	ACAAGTCAGTTCCACTCTGTGGAC
ChIP-851 R	TGCTCTTCTTCTGGGTGAGGAC

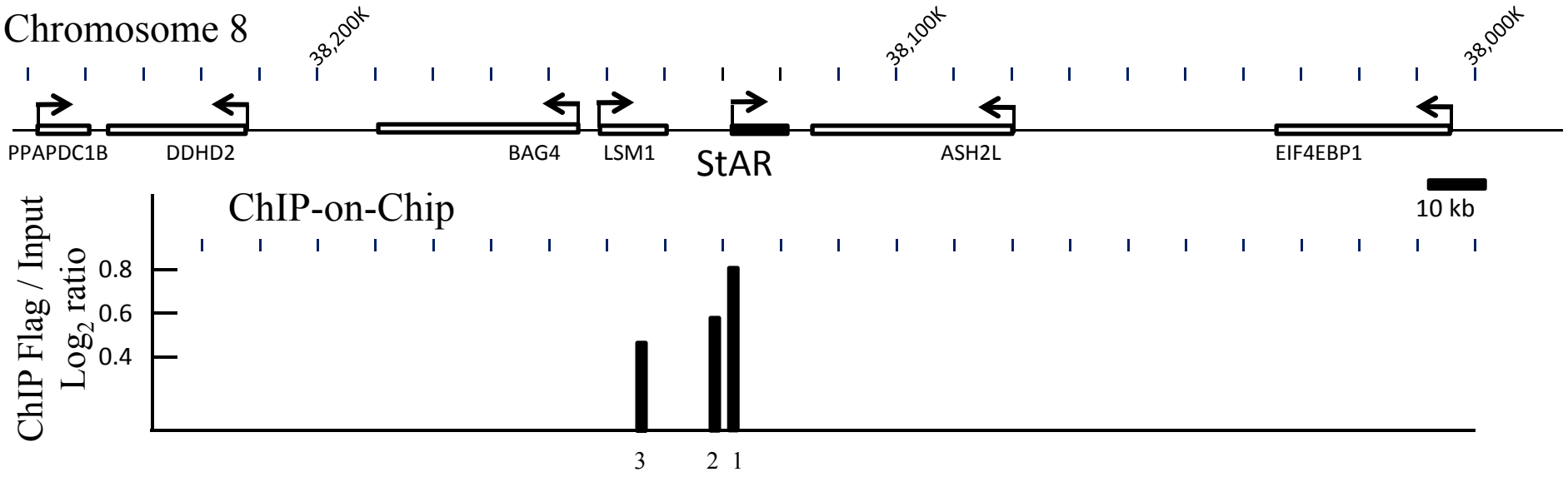
ChIP-118 F	GAGGCAATCGCTCTATCCTTGA
ChIP-68 R	CGCCATCACTCACTGTGCA
ChIP+263 F	GGCCACCCAATTCTGATCC
ChIP+313 R	TGCCCAGGTTCACCCAGTAA
ChIP+6,691 F	AGCCTGCTGTTCCCAACTGT
ChIP+6,751 R	AGGGATTCTCCTGATGAGCGT

Supplementary Table 4

Nucleotide sequences of oligonucleotides used in 3C.

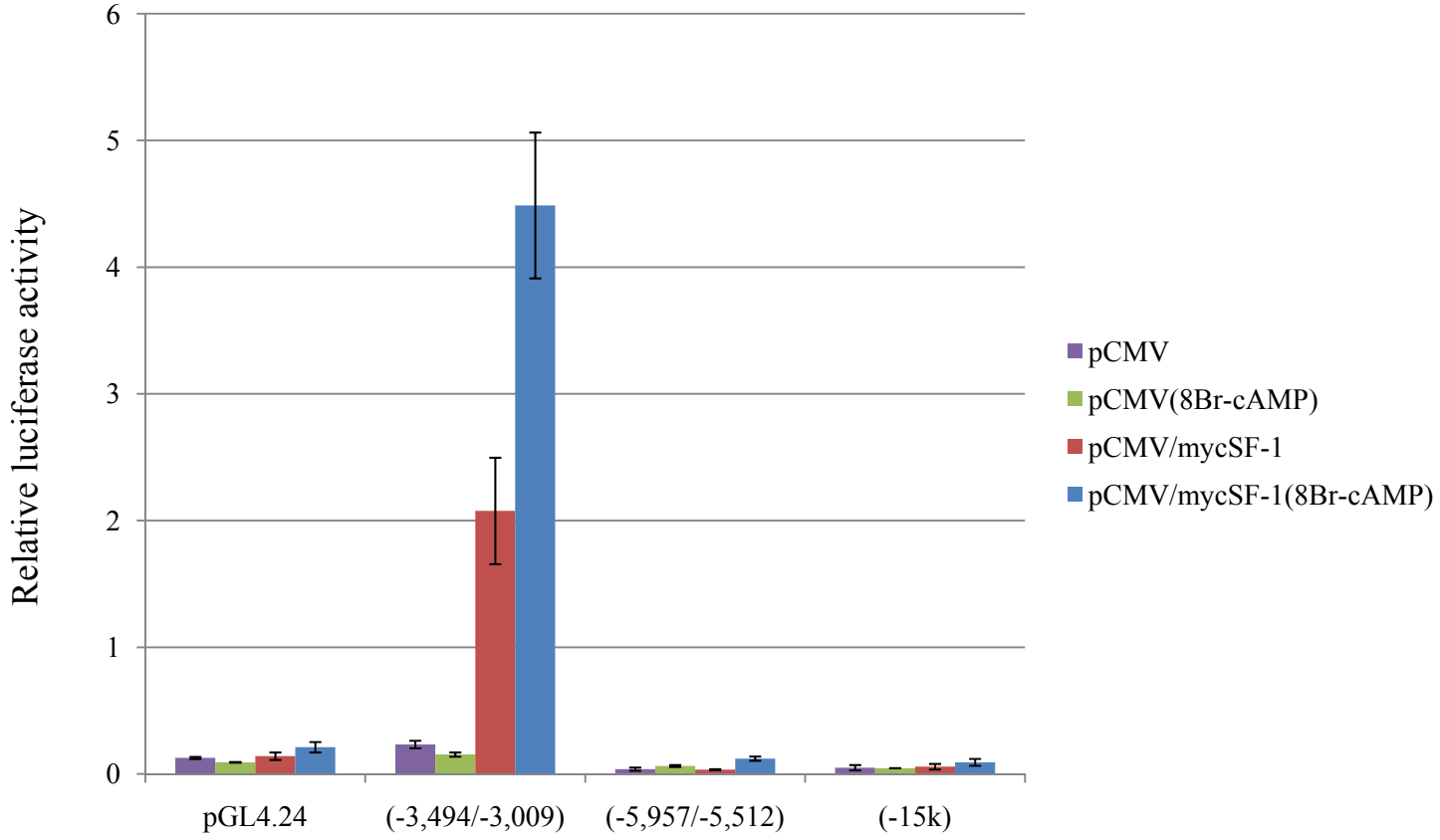
Plasmid constructs	Nucleotide sequences (5’-3’)
3C Common Primer	GGTCAAGGATAGAGCGATTGCCTCACACTG
3C -3 k primer	AGGCTCTATCCTCCGTCTCCCACACCACCT
3C -8 k primer	AGATGGCAGCCAGGCATATGCTAAGATGCA
3C -12 k primer	GTAAGTATGTATCCTATAGTAGTGTTGTCAGGCA
Loading Control Primer F	GCCTGGAAAGGATGAGGGA
Loading Control Primer R	GGCCATGCAGATGATGATCA

Supplementary Fig. 1

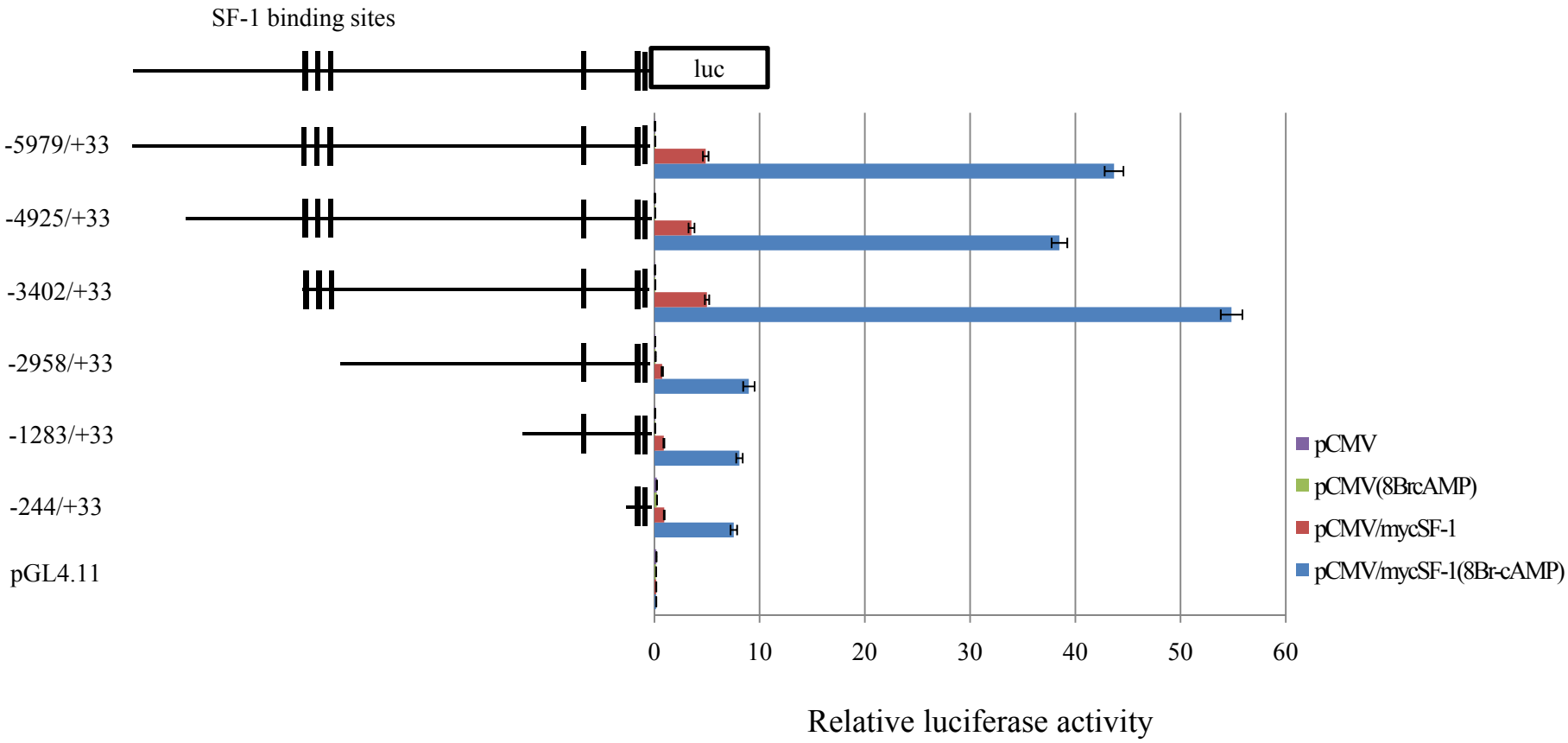


1, -149/+174
2, -3,521/-2,872
3, -15,970/-15,621 (base)

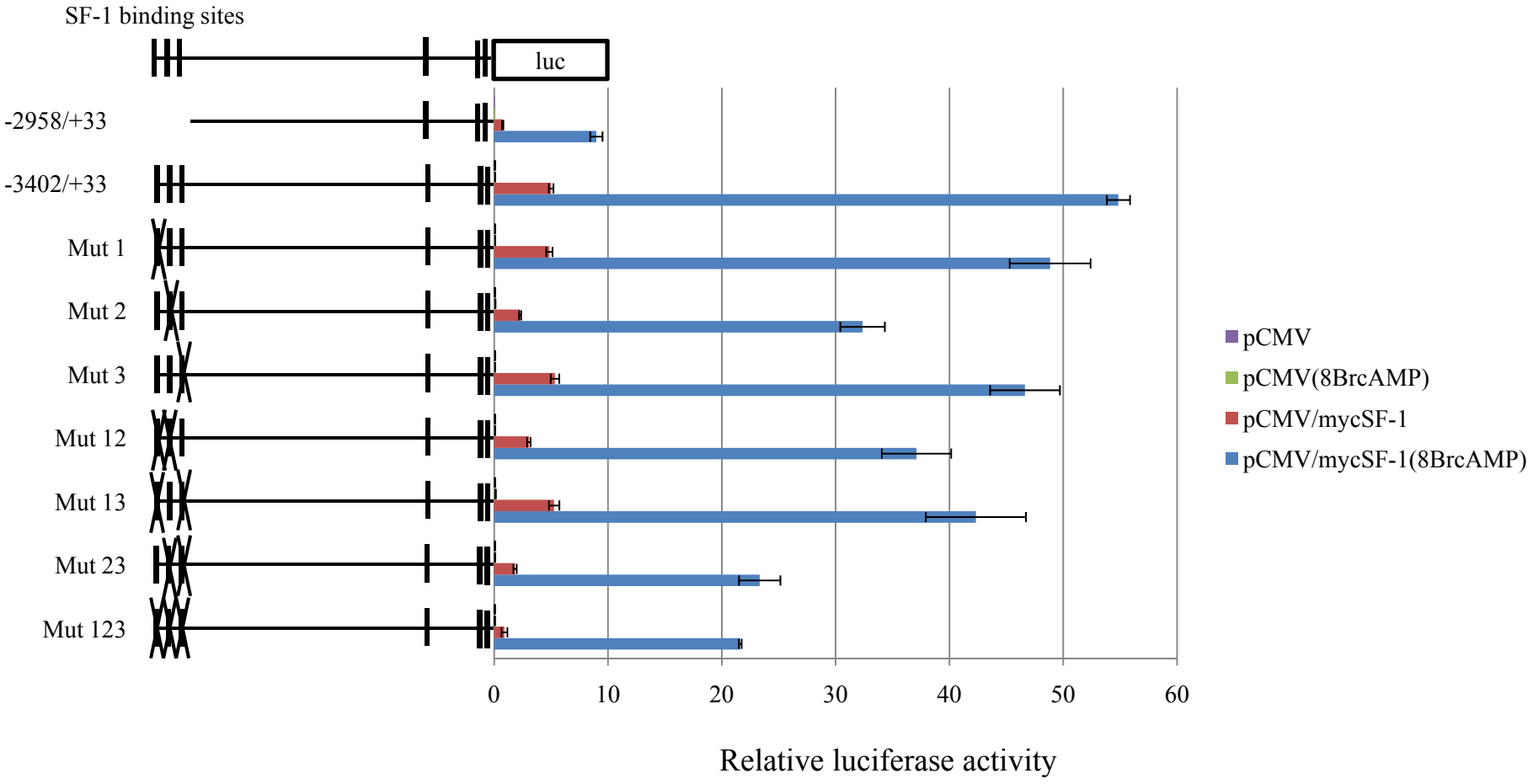
Supplementary Fig. 2



Supplementary Fig. 3

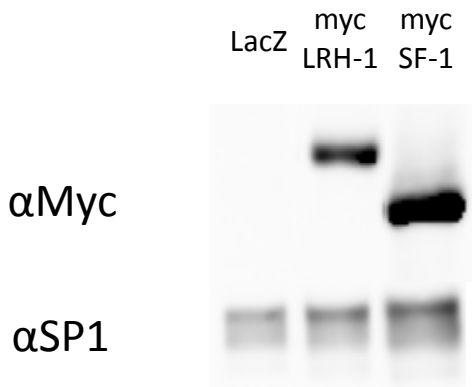


Supplementary Fig. 4

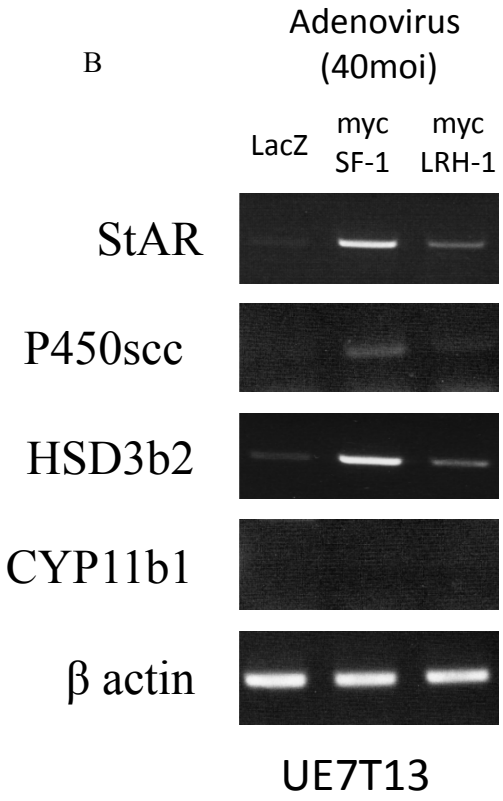


Supplementary Fig. 5

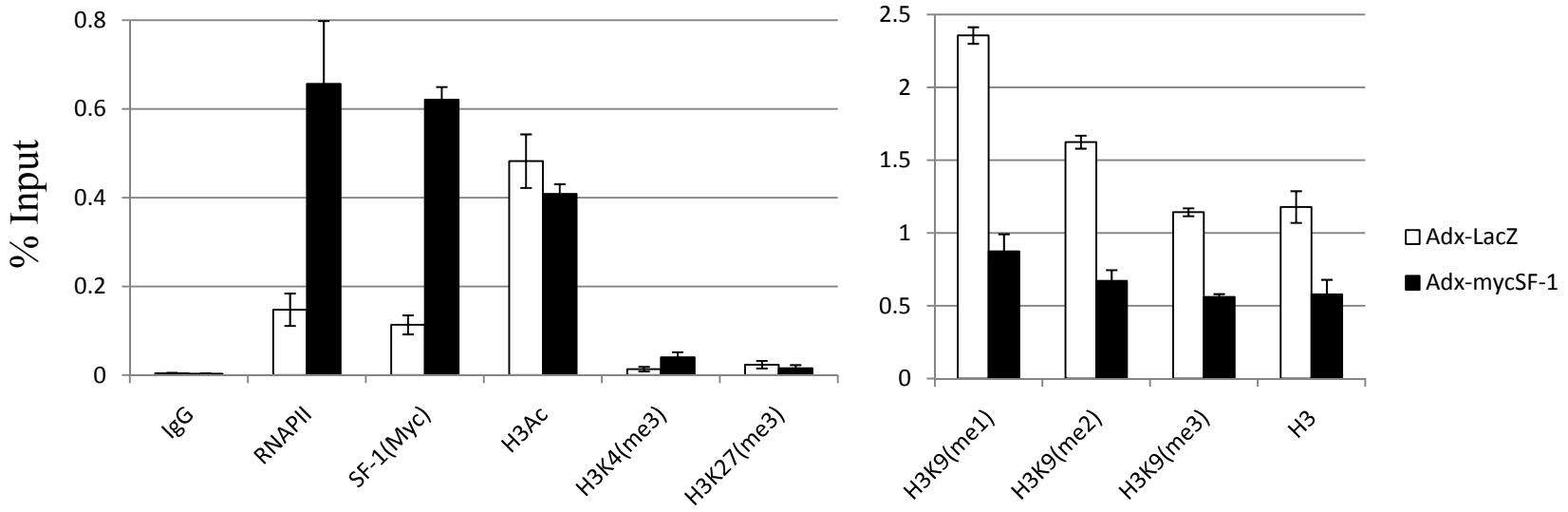
A



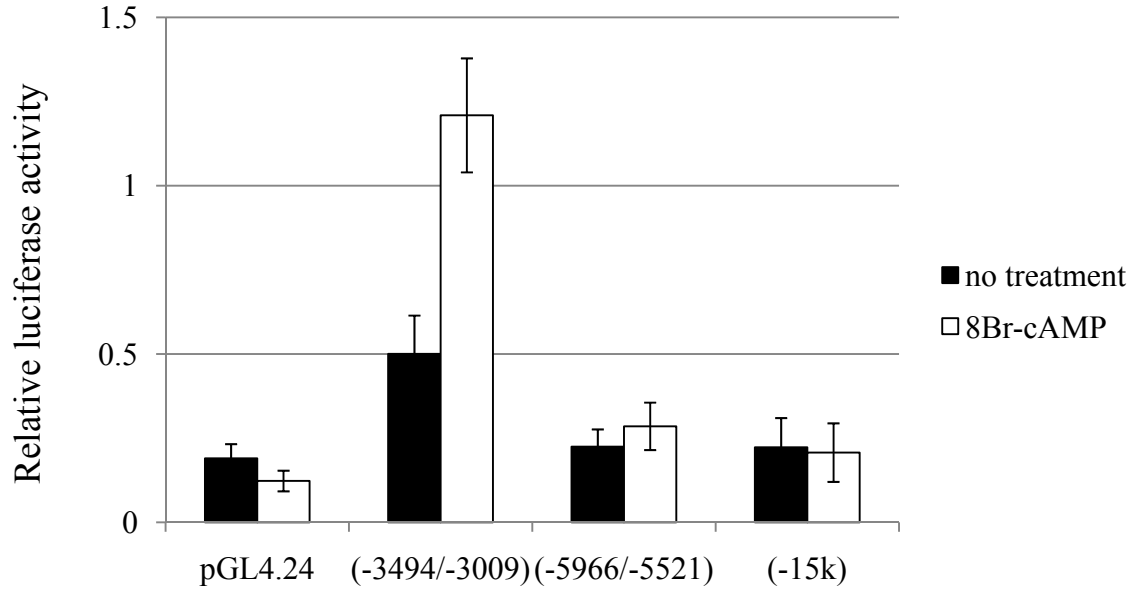
B



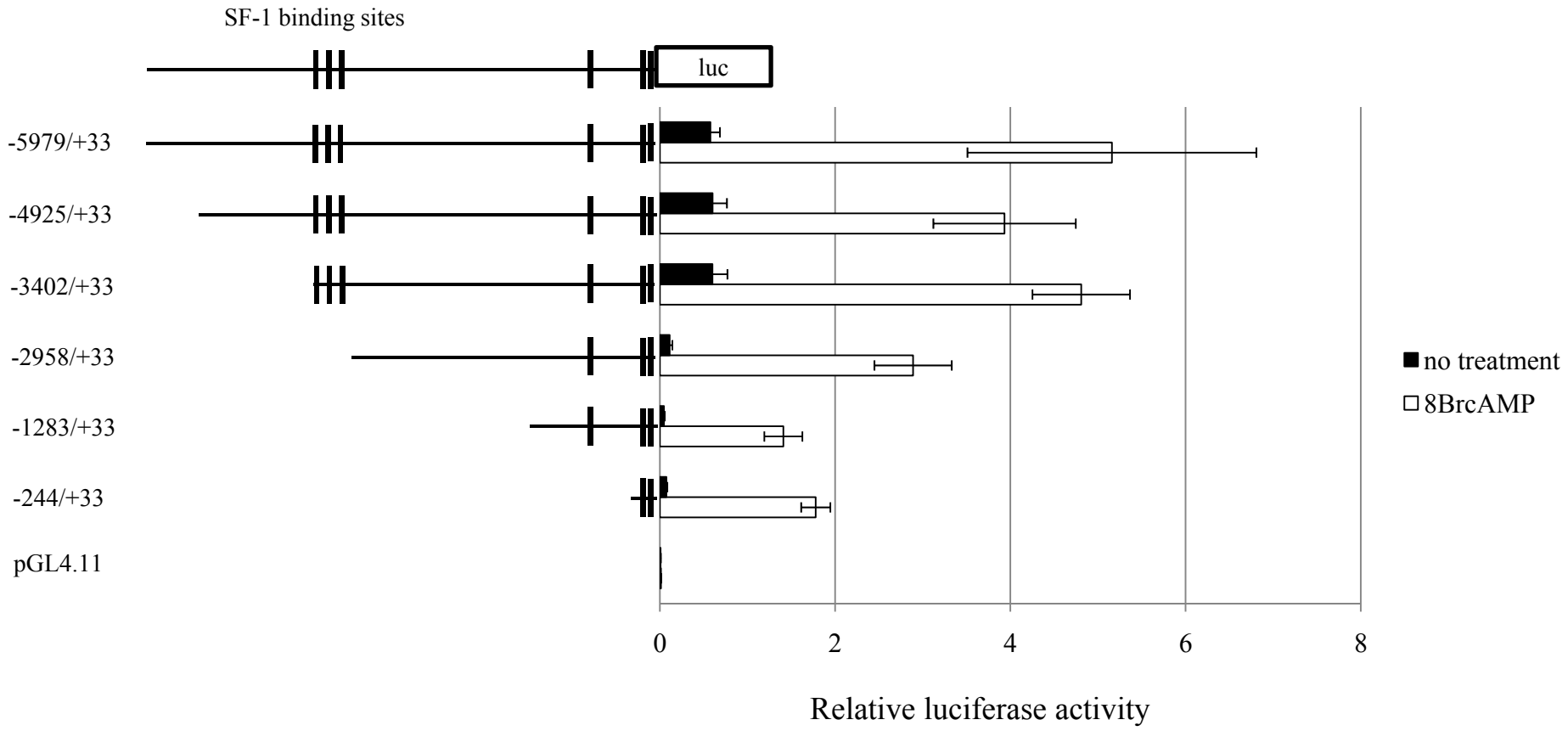
Supplementary Fig. 6



Supplementary Fig. 7



Supplementary Fig. 8



Supplementary Fig. 9