Zhang et al., http://www.jcb.org/cgi/content/full/jcb.200801009/DC1



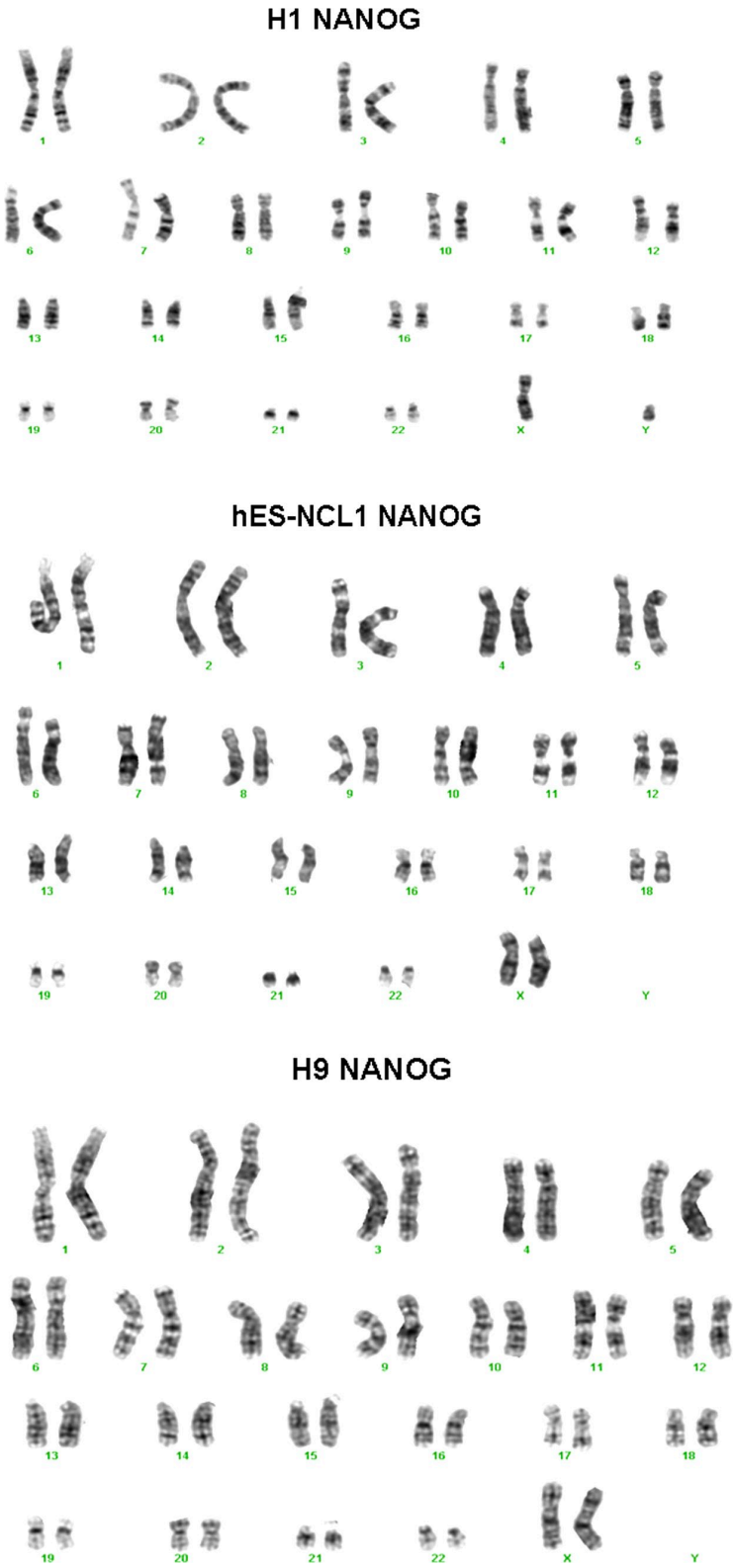


Figure S1. **Karyotype analysis of H1 NANOG and hES-NCL1 NANOG clones after 20 passages in culture.**

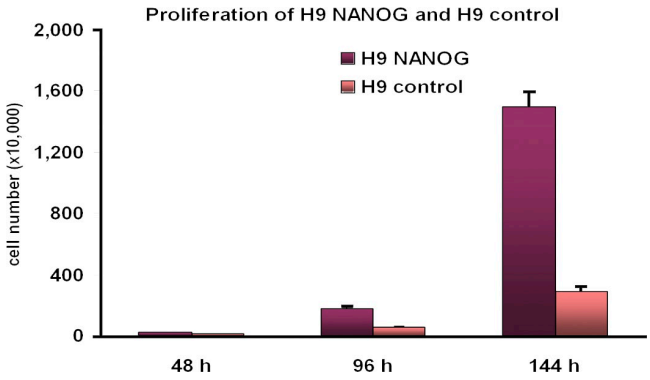


Figure S2. **Cell proliferation assessed by cell counting over three time points.** 25,000 cells from the H9 NANOG and control sublines were plated at day 0, and cell counts were assessed after 2, 4, and 6 d. The data are presented as mean ± SEM (error bars; *n* = 4).

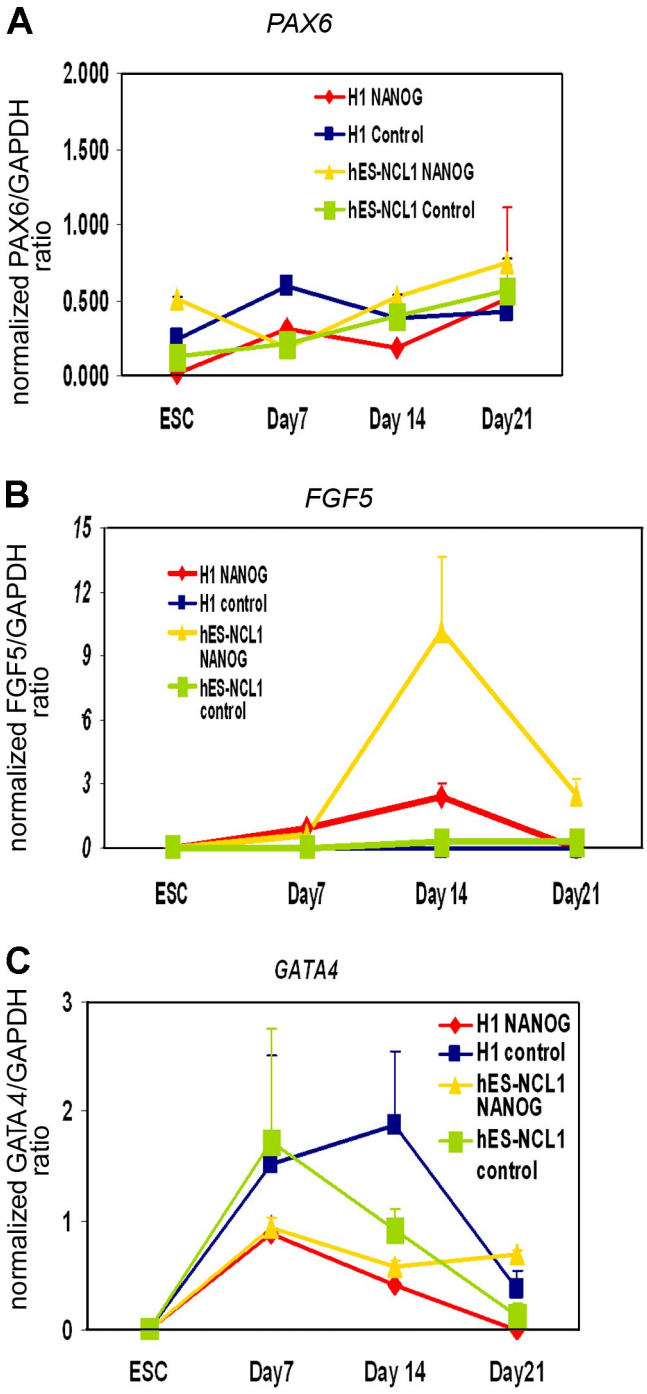


Figure S3. **Maintenance of pluripotency and differentiation capability of NANOG-overexpressing hESC clones.** (A–C) Quantitative RT-PCR analysis for the expression of *PAX6* (A), *FGF5* (B), and *GATA4* (C) during the differentiation of NANOG-overexpressing and control clones for 21 d in suspension culture. The data represent the mean ± SEM (error bars) from two independent experiments. Statistical analysis was performed using two-factor analysis of variance with replication.

**A**

***CDK6* intron 1 fragment (corresponding to position 92106071 to 92106651 on chromosome 7)**

ACATGCTCCTCAGACCACTAATATGCAAAAGAACCTAAGGATCTGGTCACCAAGTTACGTCAAAAGAGTT
TACCCCATAGTCCTTTTTACTGCCTTTTGCAGAACAAACGTTTAATAATGTCGCTATTGTTCATTGGTTA
TCAAGAAATAACTCATTGTAACTCAGAAGTGCTTAGCAAAGAATCTATCTCATATGTACTTTCTTTATTT
CAAGAACTTTAAGGAGGCAGGTTTTCTACGTGTGTTCACCAAAGCGCCAAGATTTTTTTTAATATTTTGA
GAAAATATTTTCAGTAAATCTGTATTCATTGAATGAAATATTAAGTGGATCCATAAAGAAGCAGATGATA
TAAGTCCCAGGATAACATCAGGAGAATCTGAACACAGTGTAGCTGAAGCATTTCATCACCCCCCAACAAG
AAAATTGCTATTTTGCCAACTATTCTACCACATTGCAGTGTTTCTAAGATCTACATTTTTTTCACATTTA
TTTCTAAAAATCAAAATGTGAATTGCAGTCAACTGGTGACATCTTAGATCTGATGAGATATGGTGGTTCT
CCTGGAAAAGCAAAAGTTAGT

***CDC25A* promoter fragment (corresponding to position 48204409 to 48205364 on chromosome 3)**

AGGCCGAGCCAAGGCTGGATCCGGCCAGACCTCCACAGGTCTTCCTTAGCCTCCACATTGCCTCAGAGTG
TGGGGCGCCCGGCTGGGGGCGAGGTAGCGGAGGCCCAAAGGGGGCCGAAGCTAACTGGACGGCAGCTCGC
GATGGGAACTACGCTTCCCAGCATGCGACGGGGCAAAGGGGCCTTTCAGCCGCGAGCAGCGCCTCGCAGG
TTCTGCTGGGAGTTTTCATTGACCTCTGCTCCCCCTCTCATTTTGATCCCCGCTCTTCTGCTCTGGGCTC
CGCCCCCTTCTGAGAGCCGATGACCTGGCAGAGTCCCGCGAGCCGCTTTCTTCTTCCCCTCTCATTGGCC
CAGCCTAGCTGCCATTCGGTTGAGAGGAGGAGAAGTTGCTTACTGATTGGTGGATTCCGTTTGGCGCCAA
CTAGGAAAGGGGGGCGGGGCAGCAGCTGGCCCCACTGAGCCGCTATTACCGCGAAAGGCCGGCCTGGCTG
CGACAGCCTGGGTAAGAGGTGTAGGTCGGCTTGGTTTTCTGCTACCCGGAGCTGGGCAAGCGGGTGGGAG
AACAGCGAAGACAGCGTGAGCCTGGGCCGTTGCCTCGAGGCTCTCGCCCGGCTTCTCTTGCCGACCCGCC
ACGTTTGTTTGGATTTAATCTTCAGGTTGCCGGCGCCCGCCCGCCCGCTGGCCTCGCGGTGTGAGAGGGA
AGCACCCGTGCCTGTGGCTGGTGGCTGGCGCCTGGAGGGTCCGCACACCCGCCCGGCCGCGCCGCTTGCC
CGCGGCAGCCGCGTCCCTGAACCGCGGAGTCGTGTTTGTGTTTGACCCGCGGGCGCCGGTGGCGCGCGGC
CGAGGCCGGTGTCGGCGGGGCGGGGCGGTCGCGGCGGAGGCAGAGGAAGAGGGAGCGGGAGCTCTGCGAG
GCCGGGCGCCGCCATGGAACTGGGCCCGGAGCCCCCGCACCGCCGC

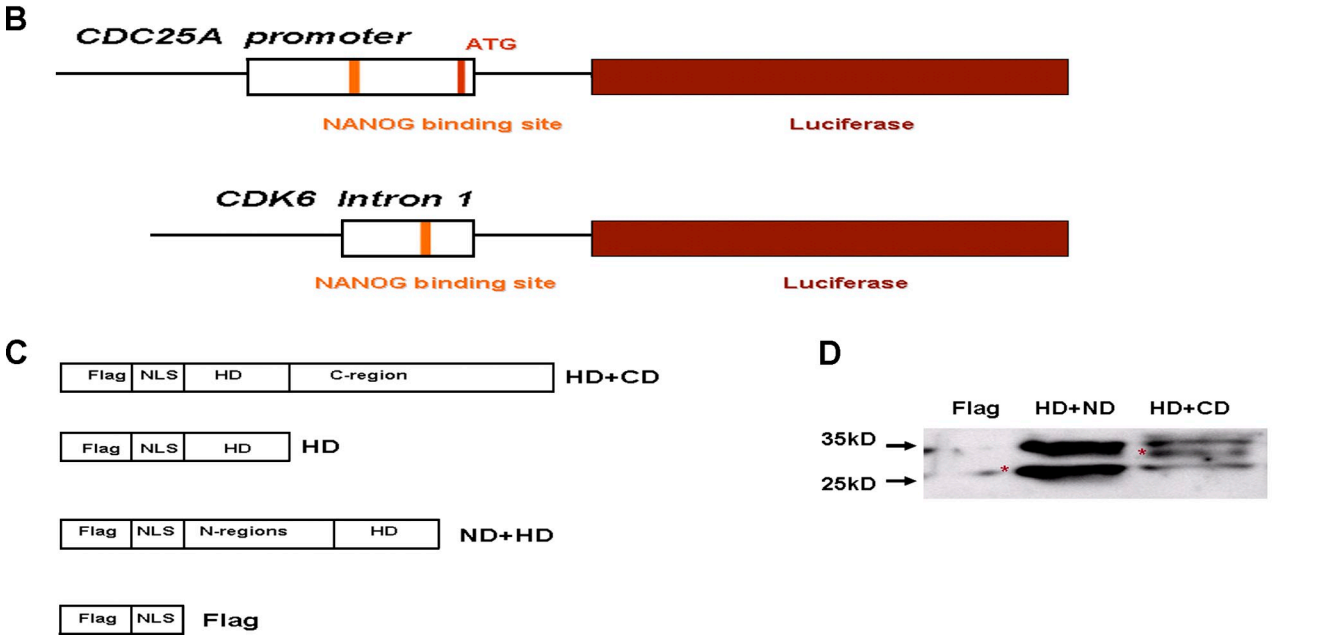


Figure S4. **The C-terminal domain of NANOG is responsible for transactivation of *CDK6* and *CDC25A*.** (A) DNA fragments of *CDK6* (intron 1) and *CDC25A* (promoter) containing the NANOG consensus–binding motif (shown in orange) showing the most important region ATTA and other matching nucleotides (underlined). The transcription start site in the *CDC25A* gene is indicated by blue, and the translational start codon ATG is shown in red. (B) Schematic representation of CDC25A- and CDK6-luciferase constructs. NANOG-binding sites are indicated in orange, and the translational start codon ATG is shown in red. (C) Schematic representation of constructs containing different regions of human NANOG (gift from J.-H. Kim). HD + CD, DNA construct containing the homeodomain and C-terminal region of *NANOG*; HD, DNA construct containing the homeodomain region of *NANOG*; ND + HD, DNA construct containing the homeodomain and the N-terminal region of *NANOG*; Flag, DNA construct without *NANOG* cDNA. (D) Western blot with Flag antibody of hESC transfected with NANOG constructs highlighted in C. The asterisks indicate the predicted positions based on the calculated molecular weight. This analysis also revealed the additional fragment with larger molecular mass than predicted, corroborating data also reported by Oh et al. (Oh, J.H., H.J. Do, H.M. Yang, S.Y. Moon, K.Y. Cha, H.M. Chung, and J.H. Kim. 2005. *Exp. Mol. Med.* 37:250–254) and suggesting the formation of higher molecular weight complexes.

Table S1. **Sequences of siRNAs used for the down-regulation of *CDK6*, *CDC25A*, and NANOG**

| Gene name | Supplier | Sequence of the corresponding sense strand for the siRNA |
|---|---|---|
| *NANOG* | Sana Cruz Biotechnology, Inc. | 5′-AAGGGUUAAGCUGUAACAUAC-3′ |
| *NANOG* | Invitrogen | 5′-GGGUUCACGCCAUUCUCCUGCCUCA-3′<br>5′-CAGUGACUUGGAGGCUGCUUUGGAA-3′<br>5′-UCUUUGUAGAAAGAGGUCUUGUAUU-3′ |
| *CDK6* | Santa Cruz Biotechnology, Inc. | 5′-GAGUAGUUCUCUCUAACUA-3′<br>5′-GCAGAAAUGUUUCGUAGAA-3′<br>5′-GACUCAAGGUGGUCAGUAA-3′<br>5′-GGAGAGUAGUUCUCUCUAA-3′ |
| *CDK6* | Invitrogen | 5′-GGCAAAGACCUACUUCUGAAGUGUU-3′<br>5′-GACCACUUACUUGGAUAAAGUUCCA-3′<br>5′-ACCGAGUAGUGCAUCGCGAUCUAAA-3′ |
| *CDC25A* | Santa Cruz Biotechnology, Inc. | 5′-GGCGCUAUUUGGCGCUUCA-3′ |
| *CDC25A* | Invitrogen | 5′-GGACAAUGACCCAAGGGACCUUAUA-3′<br>5′-GCCAACCUCAUUAAAGAGUUUGUUA-3′<br>5′-GGGAGAUGUACAGUCGUCUGAAGAA-3′ |

Table S2. **Sequences of oligonucleotides used for the quantitative RT-PCR analysis**

| Gene name | Forward primer | Reverse primer |
|---|---|---|
| *CDK6* (total expression) | 5′-AGGATAAGCCAACCTGAGAC-3′ | 5′-ACTGAGAGTATGACTGGCAA-3′ |
| *CDK6* (endogenous) | 5′-GTAATCGTGTCTGTGTTGAG-3′ | 5′-TCTGCACCCGCACGCGCTTC-3′ |
| *NANOG* (total expression) | 5′-AGAAGGCCTCAGCACCTAC-3′ | 5′-GGCCTGATTGTTCCAGGATT-3′ |
| *NANOG* (endogenous) | 5′-GGACTGAGCTGGTTGCCTCAT-3′ | 5′-CGTGTGAGGCATCTCAGCAG-3′ |
| *CDK4* | 5′-ACTGGCCTCGAGATGTATCC-3′ | 5′-TGCTGCAGAGCTCGAAAGGC-3′ |
| *CDC25A* (total expression) | 5′-CGGTATGTGAGAGAGAGAGA-3′ | 5′-GACTGTACATCTCCCTCTTG-3′ |
| *CDC25A* (endogenous) | 5′-CGCGTCCCTGAACCGCGGAG-3′ | 5′-CGGCGGCTGAAGCGCCAAATA-3′ |
| *SUV39H1* | 5′-CATAGACAACCTTGACGAGC-3′ | 5′-CCACACTTGCATTCAATACG-3′ |
| *p16* | 5′-CGCACCGAATAGTTACGGTC-3′ | 5′-ACCACCAGCGTGTCCAGGAA-3′ |
| *c-MYC* | 5′-TCGCAAGACTCCAGCGCCTT-3′ | 5′-GCAGAAGGTGATCCAGACTC-3′ |
| *CYCLIN D1* | 5′-TGGTGAACAAGCTCAAGTGG-3′ | 5′-TGAGGCGGTAGTAGGACAGG-3′ |
| *CYCLIN D2* | 5′-TGAGGCGGTGTAGGACAGG-3′ | 5′-ATATCCCGCACGTCTGTAGG-3′ |
| *CYCLIN D3* | 5′-CATGTACCCGCCATCCAT-3′ | 5′-AGCTTCGATCTGCTCCTGAC-3′ |
| *p19* | 5′-ATGCTGCTGGAGGAGGTTC-3′ | 5′-CAGCAGTGTGACCCTCTTGA-3′ |
| *p15* | 5′-CGGGGACTAGTGGAGAAGGT-3′ | 5′-GGTGAGAGTGGCAGGGTCT-3′ |
| *p18* | 5′-ACGTCAATGCACAAAATGGA-3′ | 5′-CGAAACCAGTTCGGTCTTTC-3′ |
| *E2F1* | 5′-CCTCATCCCCTCACCACAGAT-3′ | 5′-CCCCAAAGTCACAGTCGAAG-3′ |
| *CYCLIN E1* | 5′-CCCCAAAGTCACAGTCGAAG-3′ | 5′-GGGGAGAGGAGAAGCCCTAT-3′ |
| *CYCLIN A* | 5′-GGGTATCAGTGGTGCGACAT-3′ | 5′-GGGGAGAGGAGAAGCCCTAT-3′ |
| *CDK2* | 5′-TGTACCTCCCCTGGATGAAG-3′ | 5′-CATCCTGGAAGAAAGGGTGA-3′ |
| *RB* | 5′-TGAATCTCTTTTTGGATTTTATGTCA-3′ | 5′-TTTCTGCTTTGCATTCGTG-3′ |
| *c-ABL* | 5′-GCTGCAGAGCACAGAGACAC-3′ | 5′-CTCTTTTCGAGGGAGCAATG-3′ |

RB, Retinoblastoma.

Table S3. **Sequences of oligonucleotides used for the quantitative PCR after ChIP experiments**

| Gene name | Forward primer | Reverse primer |
|---|---|---|
| *CDK6* | 5′-ACATGCTCCTCAGACCACTAAT-3′ | 5′-ACTAACTTTTGCTTTTCCAGG-3′ |
| *CDK4* | 5′-CCTCTGCTCCTCAGAGCAAT-3′ | 5′-GACAGGAGGTGCTTCGACTG-3′ |
| *CDK2* | 5′-GAAACTTGGACCCAAAGCAG-3′ | 5′-CCAGGCCTTTCTATTGGTCA-3′ |
| *CDC25A* | 5′-CGGTATGTGAGAGAGAGAGA-3′ | 5′-TTGCCCAGCTCCGGGTAGCA-3′ |
| *c-MYC* | 5′-CCGCCTGCGATGATTTATAC-3′ | 5′-CAGCCGAGCACTCTAGCTCT-3′ |