Figure S1: Splice variants of the *NANOG1* and *NANOG2* gene

1. NANOG1 variants

NANOG1A (E3-E4-E5-E6):
ATGAGTGTGGATCCAGCTTGTCCCCAAAGCTTGCCTTGCTTTGAAGCATCCGACTGTAAAGAATCTTC
ACCTATGCCTGTGATTTGTGGGCCTGAAGAAAACTATCCATCCTTGCAAATGTCTTCTGCTGAGATGC
CTCACACGGAGACTGTCTCTCCTCTTCCTTCCTCCATGGATCTGCTTATTCAGGACAGCCCTGATTCT
TCCACCAGTCCCAAAGGCAAACAACCCACTTCTGCAGAGAAGAGTGTCGCAAAAAAGGAAGACAAGGT
CCCGGTCAAGAAACAGAAGACCAGAACTGTGTTCTCTTCCACCCAGCTGTGTGTACTCAATGATAGAT
TTCAGAGACAGAAATACCTCAGCCTCCAGCAGATGCAAGAACTCTCCAACATCCTGAACCTCAGCTAC
AAACAGGTGAAGACCTGGTTCCAGAACCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACAACTGGCC
GAAGAATAGCAATGGTGTGACGCAGAAGGCCTCAGCACCTACCTACCCCAGCCTTTACTCTTCCTACC
ACCAGGGATGCCTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACCTGGAACAATTCA
ACCTGGAGCAACCAGACCCAGAACATCCAGTCCTGGAGCAACCACTCCTGGAACACTCAGACCTGGTG
CACCCAATCCTGGAACAATCAGGCCTGGAACAGTCCCTTCTATAACTGTGGAGAGGAATCTCTGCAGT
CCTGCATGCAGTTCCAGCCAAATTCTCCTGCCAGTGACTTGGAGGCTGCCTTGGAAGCTGCTGGGGAA
GGCCTTAATGTAATACAGCAGACCACTAGGTATTTTAGTACTCCACAAACCATGGATTTATTCCTAAA
CTACTCCATGAACATGCAACCTGAAGACGTGTGA

NANOG1Ba (E1-E2-E3(+3)-E4-E5-E6):
GACACAATGGGACAGGGAGCGGGGGATGGGGGAATTCAGCTCAGGCTTTTATGCAAAGACCCCCTTCT
GCAAAGAACAAAGCTTCTGGTACCTGCCCTTTGGAGAGCTGCGGGCAAGCTCAGCCTCGAAACACACA
CACCCACACGAGATGGGCACGGAGTAGTCTTGAAAGACATGACAAATCACCAGACCTGGGAAGAAGCT
AAAGAGCCAGAGGGAAAAAGCCAGAAGTCGACTACCTGGGAGGAGGGATAGACAAGAAACCAAACTAA
AGGAAACTAAGGAGTGTGGATCCAGCTTGTCCCCAAAGCTTGCCTTGCTTTGAAGCATCCGACTGTAA
AGAATCTTCACCTATGCCTGTGATTTGTGGGCCTGAAGAAAACTATCCATCCTTGCAAATGTCTTCTG
CTGAGATGCCTCACACGGAGACTGTCTCTCCTCTTCCTTCCTCCATGGATCTGCTTATTCAGGACAGC
CCTGATTCTTCCACCAGTCCCAAAGGCAAACAACCCACTTCTGCAGAGAAGAGTGTCGCAAAAAAGGA
AGACAAGGTCCCGGTCAAGAAACAGAAGACCAGAACTGTGTTCTCTTCCACCCAGCTGTGTGTACTCA
ATGATAGATTTCAGAGACAGAAATACCTCAGCCTCCAGCAGATGCAAGAACTCTCCAACATCCTGAAC
CTCAGCTACAAACAGGTGAAGACCTGGTTCCAGAACCAGAGAATGAAATCTAAGAGGTGGCAGAAAAA
CAACTGGCCGAAGAATAGCAATGGTGTGACGCAGAAGGCCTCAGCACCTACCTACCCCAGCCTTTACT
CTTCCTACCACCAGGGATGCCTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACCTGG
AACAATTCAACCTGGAGCAACCAGACCCAGAACATCCAGTCCTGGAGCAACCACTCCTGGAACACTCA
GACCTGGTGCACCCAATCCTGGAACAATCAGGCCTGGAACAGTCCCTTCTATAACTGTGGAGAGGAAT
CTCTGCAGTCCTGCATGCAGTTCCAGCCAAATTCTCCTGCCAGTGACTTGGAGGCTGCCTTGGAAGCT
GCTGGGGAAGGCCTTAATGTAATACAGCAGACCACTAGGTATTTTAGTACTCCACAAACCATGGATTT
ATTCCTAAACTACTCCATGAACATGCAACCTGAAGACGTGTGA

NANOG1Bb (E1-E2-E3(+6)-E4-E5-E6):
GACACAATGGGACAGGGAGCGGGGGATGGGGGAATTCAGCTCAGGCTTTTATGCAAAGACCCCCTTCT
GCAAAGAACAAAGCTTCTGGTACCTGCCCTTTGGAGAGCTGCGGGCAAGCTCAGCCTCGAAACACACA
CACCCACACGAGATGGGCACGGAGTAGTCTTGAAAGACATGACAAATCACCAGACCTGGGAAGAAGCT
AAAGAGCCAGAGGGAAAAAGCCAGAAGTCGACTACCTGGGAGGAGGGATAGACAAGAAACCAAACTAA
AGGAAACTAAGTGTGGATCCAGCTTGTCCCCAAAGCTTGCCTTGCTTTGAAGCATCCGACTGTAAAGA
ATCTTCACCTATGCCTGTGATTTGTGGGCCTGAAGAAAACTATCCATCCTTGCAAATGTCTTCTGCTG
AGATGCCTCACACGGAGACTGTCTCTCCTCTTCCTTCCTCCATGGATCTGCTTATTCAGGACAGCCCT
GATTCTTCCACCAGTCCCAAAGGCAAACAACCCACTTCTGCAGAGAAGAGTGTCGCAAAAAAGGAAGA
CAAGGTCCCGGTCAAGAAACAGAAGACCAGAACTGTGTTCTCTTCCACCCAGCTGTGTGTACTCAATG
ATAGATTTCAGAGACAGAAATACCTCAGCCTCCAGCAGATGCAAGAACTCTCCAACATCCTGAACCTA
GCTACAAACAGGTGAAGACCTGGTTCCAGAACCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACACT
GGCCGAAGAATAGCAATGGTGTGACGCAGAAGGCCTCAGCACCTACCTACCCCAGCCTTTACTCTTCC
TACCACCAGGGATGCCTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACCTGGAACAA
TTCAACCTGGAGCAACCAGACCCAGAACATCCAGTCCTGGAGCAACCACTCCTGGAACACTCAGACCT
GGTGCACCCAATCCTGGAACAATCAGGCCTGGAACAGTCCCTTCTATAACTGTGGAGAGGAATCTCTG
CAGTCCTGCATGCAGTTCCAGCCAAATTCTCCTGCCAGTGACTTGGAGGCTGCCTTGGAAGCTGCTGG

GGAAGGCCTTAATGTAATACAGCAGACCACTAGGTATTTTAGTACTCCACAAACCATGGATTTATTCC
TAAACTACTCCATGAACATGCAACCTGAAGACGTGTGA

NANOG1Bc (E1-E2-E3(+17)-E4-E5-E6):
GACACAATGGGACAGGGAGCGGGGGATGGGGGAATTCAGCTCAGGCTTTTATGCAAAGACCCCCTTCT
GCAAAGAACAAAGCTTCTGGTACCTGCCCTTTGGAGAGCTGCGGGCAAGCTCAGCCTCGAAACACACA
CACCCACACGAGATGGGCACGGAGTAGTCTTGAAAGACATGACAAATCACCAGACCTGGGAAGAAGCT
AAAGAGCCAGAGGGAAAAAGCCAGAAGTCGACTACCTGGGAGGAGGGATAGACAAGAAACCAAACTAA
AGGAAACTAAGCTTGTCCCCAAAGCTTGCCTTGCTTTGAAGCATCCGACTGTAAAGAATCTTCACCTA
TGCCTGTGATTTGTGGGCCTGAAGAAAACTATCCATCCTTGCAAATGTCTTCTGCTGAGATGCCTCAC
ACGGAGACTGTCTCTCCTCTTCCTTCCTCCATGGATCTGCTTATTCAGGACAGCCCTGATTCTTCCAC
CAGTCCCAAAGGCAAACAACCCACTTCTGCAGAGAAGAGTGTCGCAAAAAAGGAAGACAAGGTCCCGG
TCAAGAAACAGAAGACCAGAACTGTGTTCTCTTCCACCCAGCTGTGTGTACTCAATGATAGATTTCAG
AGACAGAAATACCTCAGCCTCCAGCAGATGCAAGAACTCTCCAACATCCTGAACCTCAGCTACAAACA
GGTGAAGACCTGGTTCCAGAACCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACAACTGGCCGAAGA
ATAGCAATGGTGTGACGCAGAAGGCCTCAGCACCTACCTACCCCAGCCTTTACTCTTCCTACCACCAG
GGATGCCTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACCTGGAACAATTCAACCTG
GAGCAACCAGACCCAGAACATCCAGTCCTGGAGCAACCACTCCTGGAACACTCAGACCTGGTGCACCC
AATCCTGGAACAATCAGGCCTGGAACAGTCCCTTCTATAACTGTGGAGAGGAATCTCTGCAGTCCTGC
ATGCAGTTCCAGCCAAATTCTCCTGCCAGTGACTTGGAGGCTGCCTTGGAAGCTGCTGGGGAAGGCCT
TAATGTAATACAGCAGACCACTAGGTATTTTAGTACTCCACAAACCATGGATTTATTCCTAAACTACT
CCATGAACATGCAACCTGAAGACGTGTGA

2. NANOG2 variants

NANOG2D1: (E3-E4-E5-E6)
ATGAGTGTGGATCCAGCTTGTCCATAAAGCCTGCCTTGCTCCAAAGCATCTGACTGTAAAGACTGGTC
ACCTATACCTGTGATTTGTGGGCCTGAAGAAAACCATCCATCCTTGCAAATGTCTTCTGCTGAGATGC
CTCACACAGAGACTGTCTCTCCTCTTCCTTCCTCCATGGATCTGCCTATTCAGGACAGCCATGATTCT
TCCACCAGTCCCAAAGGCAAACAACCCACTACTGCAGAGAAGAGTGCCACAAAAAAGGAAGACAAGGT
CCCGGTCAAGAAACAGAAGACCAGAACTGTGTTCTCTTCCACCCAGCTGTGTGTACTCAATGATAGAT
TTCAGAGACAGAAATACCTCAGCCTCCAGCAGATGCAAGAACTTTCCAACATCCTGAACCTCAGCTAC
AAACAGGTGAAGACCTGGTTCCAGAACCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACAACTGGCT
GAAGAATAGCAATGGTGTGACGCAGGGATGCCTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCA
ACCAGACCTGGAACAATTCAACCTGGAGCAACCAGACCCAGAACATCCAGTCCTGGAGCAACCACTCC
TGGAACACTCAGACCTGGTGCACCCAATCCTGGAACAATCAGGCCTGGAACAGTCCCTTCTATAACTG
TGGAGAGGAATCTCTGCAGTCCTGCATGCAGTTCCAGCCAAATTCTCCTGCCAGTGACTTGGAGGCTG
CCTTGGAAGCTGCTGGGGAAGGCCTTAATGTAATACAGCAGACCACTAGGTATTTTAATACTCCACAA
ACCATGGATTTATTCCTAAACTACTCCATGAACATGCAACCTGAAGACGTGTGA

NANOG2D2a (E1-E3(+3)-E4-E5-E6(+48))
GACGCAATGGGACAGGGAGCGGGGGATGGGGGAATTCAGCTCAGGCTTTTATGCAAAGTCCCCCTTCA
GCAAAGAACAAAGCTTCTGGTACCTGCCCTTTGGACAGCTGCGGGCAAGCTCAGCCTCGGAGTGTGGA
TCCAGCTTGTCCATAAAGCCTGCCTTGCTCCAAAGCATCTGACTGTAAAGACTGGTCACCTATACCTG
TGATTTGTGGGCCTGAAGAAAACCATCCATCCTTGCAAATGTCTTCTGCTGAGATGCCTCACACAGAG
ACTGTCTCTCCTCTTCCTTCCTCCATGGATCTGCCTATTCAGGACAGCCATGATTCTTCCACCAGTCC
CAAAGGCAAACAACCCACTACTGCAGAGAAGAGTGCCACAAAAAAGGAAGACAAGGTCCCGGTCAAGA
AACAGAAGACCAGAACTGTGTTCTCTTCCACCCAGCTGTGTGTACTCAATGATAGATTTCAGAGACAG
AAATACCTCAGCCTCCAGCAGATGCAAGAACTTTCCAACATCCTGAACCTCAGCTACAAACAGGTGAA
GACCTGGTTCCAGAACCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACAACTGGCTGAAGAATAGCA
ATGGTGTGACGCAGGGATGCCTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACCTGG
AACAATTCAACCTGGAGCAACCAGACCCAGAACATCCAGTCCTGGAGCAACCACTCCTGGAACACTCA
GACCTGGTGCACCCAATCCTGGAACAATCAGGCCTGGAACAGTCCCTTCTATAACTGTGGAGAGGAAT
CTCTGCAGTCCTGCATGCAGTTCCAGCCAAATTCTCCTGCCAGTGACTTGGAGGCTGCCTTGGAAGCT
GCTGGGGAAGGCCTTAATGTAATACAGCAGACCACTAGGTATTTTAATACTCCACAAACCATGGATTT
ATTCCTAAACTACTCCATGAACATGCAACCTGAAGACGTGTGA

NANOG2D2b (E1-E3(+6)-E4-E5-E6(+48))

GACGCAATGGGACAGGGAGCGGGGGATGGGGGAATTCAGCTCAGGCTTTTATGCAAAGTCCCCCTTCA
GCAAAGAACAAAGCTTCTGGTACCTGCCCTTTGGACAGCTGCGGGCAAGCTCAGCCTCGTGTGGATCC
AGCTTGTCCATAAAGCCTGCCTTGCTCCAAAGCATCTGACTGTAAAGACTGGTCACCTATACCTGTGA
TTTGTGGGCCTGAAGAAAACCATCCATCCTTGCAAATGTCTTCTGCTGAGATGCCTCACACAGAGACT
GTCTCTCCTCTTCCTTCCTCCATGGATCTGCCTATTCAGGACAGCCATGATTCTTCCACCAGTCCCAA
AGGCAAACAACCCACTACTGCAGAGAAGAGTGCCACAAAAAAGGAAGACAAGGTCCCGGTCAAGAAAC
AGAAGACCAGAACTGTGTTCTCTTCCACCCAGCTGTGTGTACTCAATGATAGATTTCAGAGACAGAAA
TACCTCAGCCTCCAGCAGATGCAAGAACTTTCCAACATCCTGAACCTCAGCTACAAACAGGTGAAGAC
CTGGTTCCAGAACCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACAACTGGCTGAAGAATAGCAATG
GTGTGACGCAGGGATGCCTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACCTGGAAC
AATTCAACCTGGAGCAACCAGACCCAGAACATCCAGTCCTGGAGCAACCACTCCTGGAACACTCAGAC
CTGGTGCACCCAATCCTGGAACAATCAGGCCTGGAACAGTCCCTTCTATAACTGTGGAGAGGAATCTC
TGCAGTCCTGCATGCAGTTCCAGCCAAATTCTCCTGCCAGTGACTTGGAGGCTGCCTTGGAAGCTGCT
GGGGAAGGCCTTAATGTAATACAGCAGACCACTAGGTATTTTAATACTCCACAAACCATGGATTTATT
CCTAAACTACTCCATGAACATGCAACCTGAAGACGTGTGA

NANOG2D2c (E1-E3(+17)-E4-E5-E6(+48))

GACGCAATGGGACAGGGAGCGGGGGATGGGGGAATTCAGCTCAGGCTTTTATGCAAAGTCCCCCTTCA
GCAAAGAACAAAGCTTCTGGTACCTGCCCTTTGGACAGCTGCGGGCAAGCTCAGCCTCGCTTGTCCAT
AAAGCCTGCCTTGCTCCAAAGCATCTGACTGTAAAGACTGGTCACCTATACCTGTGATTTGTGGGCCT
GAAGAAAACCATCCATCCTTGCAAATGTCTTCTGCTGAGATGCCTCACACAGAGACTGTCTCTCCTCT
TCCTTCCTCCATGGATCTGCCTATTCAGGACAGCCATGATTCTTCCACCAGTCCCAAAGGCAAACAAC
CCACTACTGCAGAGAAGAGTGCCACAAAAAAGGAAGACAAGGTCCCGGTCAAGAAACAGAAGACCAGA
ACTGTGTTCTCTTCCACCCAGCTGTGTGTACTCAATGATAGATTTCAGAGACAGAAATACCTCAGCCT
CCAGCAGATGCAAGAACTTTCCAACATCCTGAACCTCAGCTACAAACAGGTGAAGACCTGGTTCCAGA
ACCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACAACTGGCTGAAGAATAGCAATGGTGTGACGCAG
GGATGCCTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACCTGGAACAATTCAACCTG
GAGCAACCAGACCCAGAACATCCAGTCCTGGAGCAACCACTCCTGGAACACTCAGACCTGGTGCACCC
AATCCTGGAACAATCAGGCCTGGAACAGTCCCTTCTATAACTGTGGAGAGGAATCTCTGCAGTCCTGC
ATGCAGTTCCAGCCAAATTCTCCTGCCAGTGACTTGGAGGCTGCCTTGGAAGCTGCTGGGGAAGGCCT
TAATGTAATACAGCAGACCACTAGGTATTTTAATACTCCACAAACCATGGATTTATTCCTAAACTACT
CCATGAACATGCAACCTGAAGACGTGTGA

NANOG2D2* (E1*-E3(+77)-E4-E5-E6(+48))

GACGCAATGGGACAGGGAGCGGGGGATGGGGGAATTCAGCTCAGGCTTTTATGCAAAGTCCCCCTTCA
GCAAAGAACAAAGCTTCTGGTACCTGCCCTTTGGACAGCTGCGGGCAAGCTCAGCCTCGGTGAGTCTT
GGTGGCCTTGACAGCCCCCACTTAACACACTGTGCTGATTAAGAGAGACAGGAGGGCAAGTTTTTCCC
TTTTTTAAAGAAATCATCCTCGACATGGACTACCTGCCTTGAAGCATGATCTATCTAGTTCCACTTAC
CTCCTCCCCGACGCCCCCATTCTGACTCTTCTCCAGAGTGGAGGTCTGTGATTTGTGGGCCTGAAGAA
AACCATCCATCCTTGCAAATGTCTTCTGCTGAGATGCCTCACACAGAGACTGTCTCTCCTCTTCCTTC
CTCCATGGATCTGCCTATTCAGGACAGCCATGATTCTTCCACCAGTCCCAAAGGCAAACAACCCACTA
CTGCAGAGAAGAGTGCCACAAAAAAGGAAGACAAGGTCCCGGTCAAGAAACAGAAGACCAGAACTGTG
TTCTCTTCCACCCAGCTGTGTGTACTCAATGATAGATTTCAGAGACAGAAATACCTCAGCCTCCAGCA
GATGCAAGAACTTTCCAACATCCTGAACCTCAGCTACAAACAGGTGAAGACCTGGTTCCAGAACCAGA
GAATGAAATCTAAGAGGTGGCAGAAAAACAACTGGCTGAAGAATAGCAATGGTGTGACGCAGGGATGC
CTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACCTGGAACAATTCAACCTGGAGCAA
CCAGACCCAGAACATCCAGTCCTGGAGCAACCACTCCTGGAACACTCAGACCTGGTGCACCCAATCCT
GGAACAATCAGGCCTGGAACAGTCCCTTCTATAACTGTGGAGAGGAATCTCTGCAGTCCTGCATGCAG
TTCCAGCCAAATTCTCCTGCCAGTGACTTGGAGGCTGCCTTGGAAGCTGCTGGGGAAGGCCTTAATGT
AATACAGCAGACCACTAGGTATTTTAATACTCCACAAACCATGGATTTATTCCTAAACTACTCCATGA
ACATGCAACCTGAAGACGTGTGA

NANOG2E (E1-E4-E5-E6(+48))

GACGCAATGGGACAGGGAGCGGGGGATGGGGGAATTCAGCTCAGGCTTTTATGCAAAGTCCCCCTTCA
GCAAAGAACAAAGCTTCTGGTACCTGCCCTTTGGACAGCTGCGGGCAAGCTCAGCCTCGTCTCTCCTC
TTCCTTCCTCCATGGATCTGCCTATTCAGGACAGCCATGATTCTTCCACCAGTCCCAAAGGCAAACAA

CCCACTACTGCAGAGAAGAGTGCCACAAAAAAGGAAGACAAGGTCCCGGTCAAGAAACAGAAGACCAG
AACTGTGTTCTCTTCCACCCAGCTGTGTGTACTCAATGATAGATTTCAGAGACAGAAATACCTCAGCC
TCCAGCAGATGCAAGAACTTTCCAACATCCTGAACCTCAGCTACAAACAGGTGAAGACCTGGTTCCAG
AACCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACAACTGGCTGAAGAATAGCAATGGTGTGACGCA
GGGATGCCTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACCTGGAACAATTCAACCT
GGAGCAACCAGACCCAGAACATCCAGTCCTGGAGCAACCACTCCTGGAACACTCAGACCTGGTGCACC
CAATCCTGGAACAATCAGGCCTGGAACAGTCCCTTCTATAACTGTGGAGAGGAATCTCTGCAGTCCTG
CATGCAGTTCCAGCCAAATTCTCCTGCCAGTGACTTGGAGGCTGCCTTGGAAGCTGCTGGGGAAGGCC
TTAATGTAATACAGCAGACCACTAGGTATTTTAATACTCCACAAACCATGGATTTATTCCTAAACTAC
TCCATGAACATGCAACCTGAAGACGTGTGA

NANOG2 F (E1-E3-E4(+39)-E5-E6(+48))
GACGCAATGGGACAGGGAGCGGGGGATGGGGGAATTCAGCTCAGGCTTTTATGCAAAGTCCCCCTTCA
GCAAAGAACAAAGCTTCTGGTACCTGCCCTTTGGACAGCTGCGGGCAAGCTCAGCCTCGATGAGTGTG
GATCCAGCTTGTCCATAAAGCCTGCCTTGCTCCAAAGCATCTGACTGTAAAGACTGGTCACCTATACC
TGTGATTTGTGGGCCTGAAGAAAACCATCCATCCTTGCAAATGTCTTCTGCTGAGATGCCTCACACAG
AGACTGGACAGCCATGATTCTTCCACCAGTCCCAAAGGCAAACAACCCACTACTGCAGAGAAGAGTGC
CACAAAAAAGGAAGACAAGGTCCCGGTCAAGAAACAGAAGACCAGAACTGTGTTCTCTTCCACCCAGC
TGTGTGTACTCAATGATAGATTTCAGAGACAGAAATACCTCAGCCTCCAGCAGATGCAAGAACTTTCC
AACATCCTGAACCTCAGCTACAAACAGGTGAAGACCTGGTTCCAGAACCAGAGAATGAAATCTAAGAG
GTGGCAGAAAAACAACTGGCTGAAGAATAGCAATGGTGTGACGCAGGGATGCCTGGTGAACCCGACTG
GGAACCTTCCAATGTGGAGCAACCAGACCTGGAACAATTCAACCTGGAGCAACCAGACCCAGAACATC
CAGTCCTGGAGCAACCACTCCTGGAACACTCAGACCTGGTGCACCCAATCCTGGAACAATCAGGCCTG
GAACAGTCCCTTCTATAACTGTGGAGAGGAATCTCTGCAGTCCTGCATGCAGTTCCAGCCAAATTCTC
CTGCCAGTGACTTGGAGGCTGCCTTGGAAGCTGCTGGGGAAGGCCTTAATGTAATACAGCAGACCACT
AGGTATTTTAATACTCCACAAACCATGGATTTATTCCTAAACTACTCCATGAACATGCAACCTGAAGA
CGTGTGA

All cloned and sequenced NANOG1 and NANOG2 transcript variants are summarized above. Exons are alternatively marked in red and in blue. Translation start and stop codons are indicated (underlined).

Figure S2: Identified splice sites in human *NANOG1* and *NANOG2*

1. NANOG1

Exon 1a intron Exon 2
GCGAAGAATGTA·gtaagtcggcct – cccttcccccag·**AAACACACACAC**

Exon 1b intron Exon 2
AGCTCAGCCTCG·gtgagtcttgtt – cccttcccccag·**AAACACACACAC**

Exon 1b* intron Exon 2
GTTTACTTTTCG·gtatggaagact – cccttcccccag·**AAACACACACAC**

Exon 2 intron Exon 3
AAGGAAACTAAG·gtaggtgctgaa – ctatactaac**AT**·**GAGTGTGGATCC (+3)**
– tactaac**ATGAG**·**TGTGGATCCAGC (+6)**
– **GTGTGGATCCAG**·**CTTGTCCCCAAA (+17)**

Exon 3 intron Exon 4
ACACGGAGACTG·gtaagaaagaaa – tgttcccaacag·**TCTCTCCTCTTC**

Exon 4 intron Exon 5
AGCTACAAACAG·gtaggcttgttt – tttttcctgcag·**GTGAAGACCTGG**

Exon 5 intron Exon 6
GGTGTGACGCAG·gtaacaggaaac – cttctctttcag·**AAGGCCTCAGCA**
– **TCCTACCACCAG**·**GGATGCCTGGTG (+48)**

2. NANOG2

Exon 1b intron Exon 3
AGCTCAGCCTCG·gtgagtcttggt – taatgac**ATGAG**·**TGTGGATCCAGC (+6)**
– **GTGTGGATCCAG**·**CTTGTCCATAAA (+17)**
Exon 1b*
CAGAGTGGAGGT·ctgagaagaaaa – **GGTCACCTATAC**·**CTGTGATTTGTG (+77)**

Exon 3 intron Exon 4
ACACAGAGACTG·gtaagaaagaaa – tgtttccaacag·**TCTCTCCTCTTC**
– **CTGCCTATTCAG**·**GACAGCCATGAT (+39)**

Exon 4 intron Exon 5
AGCTACAAACAG·gtaggcttattt – tttttcctgcag·**GTGAAGACCTGG**

Exon 5 intron Exon 6
GGTGTGACGCAG·gtaacaggaaac – cttctctttgag·**AAGGCCTCAGCA**
– **TCCTACCACCAG**·**GGATGCCTGGTG (+48)**

Exon sequences are marked in bold letters and are shown for both the *NANOG1* and *NANOG2* gene. Intronic sequences are in lower case letters. Exonic and intronic sequences were separated by a dot. All splice sites of all identified splice varinats were indicated.

Figure S3: Mass spectrometry data from NANOG1A protein

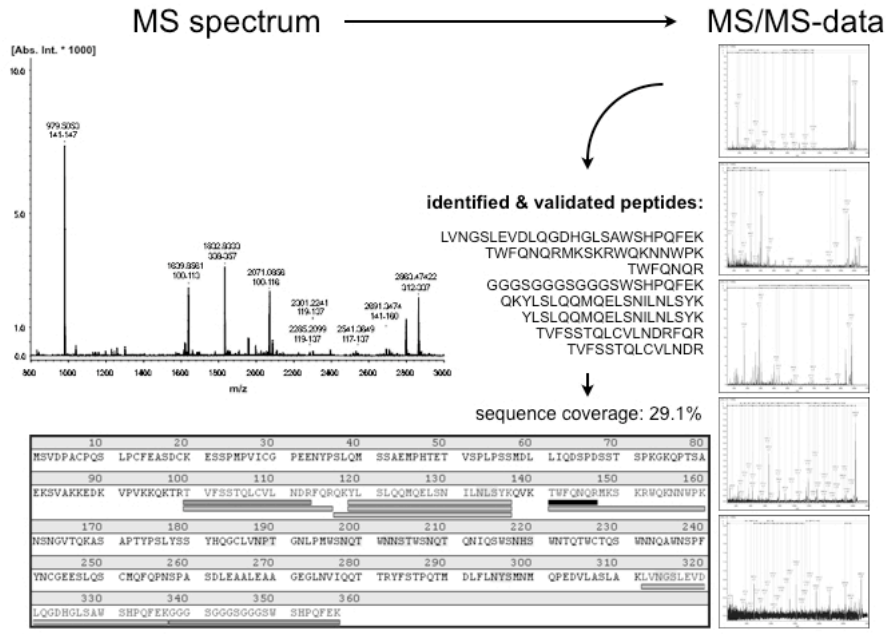
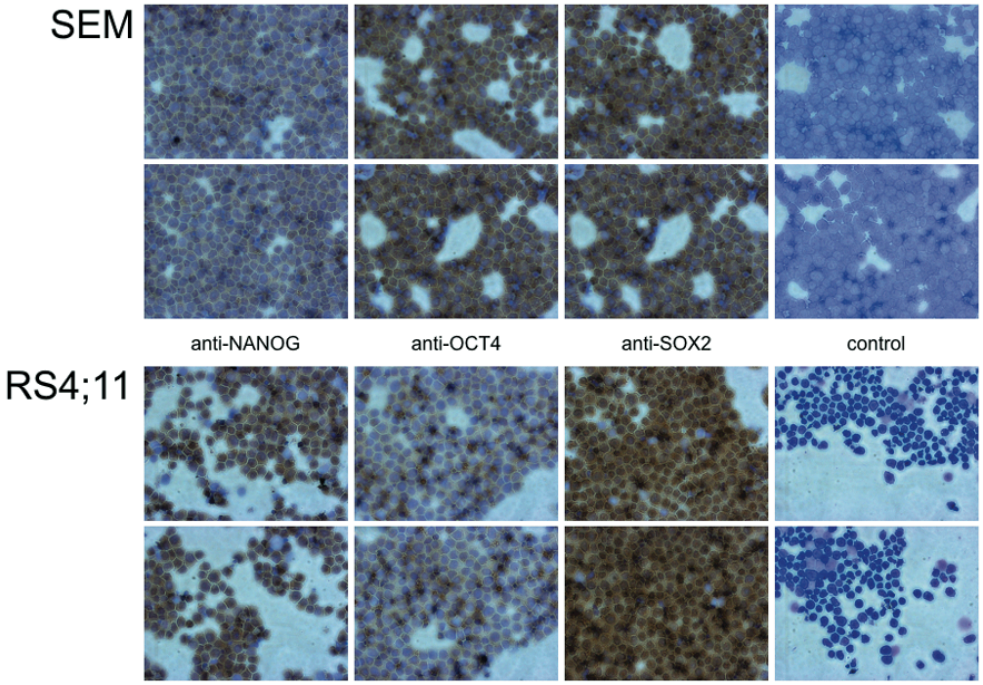
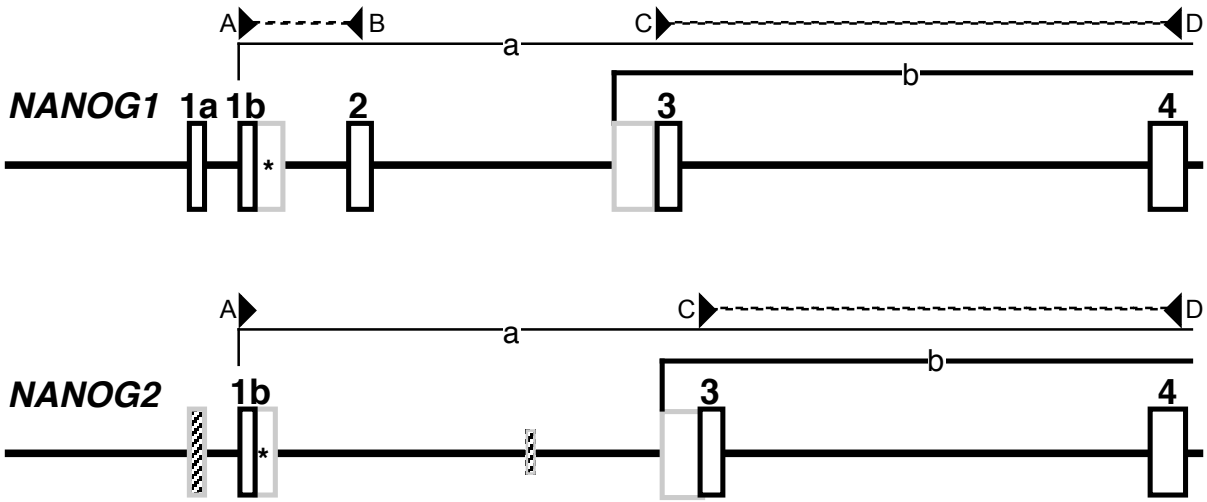


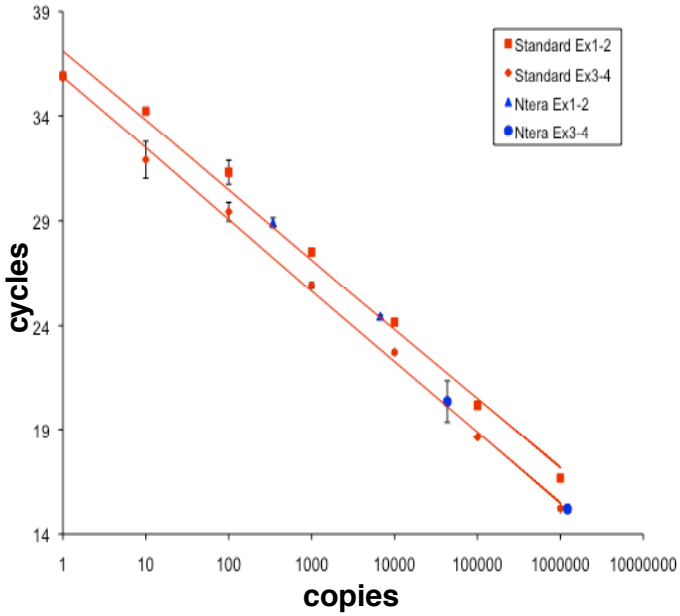
Figure S4: Immunohistology



Cytospinned SEM and RS4;11 cells were treated with HRP-conjugated antisera raised against NANOG, OCT4 and SOX2. Counterstainings were performed with a DAPI solution. Controls were treated with all solutions except the antisera. A few SEM cells seem to express NANOG, while OCT4 and SOX2 seem to be expressed in nearly all cells. RS4;11 cells seems to express NANOG and SOX2, while only few cells express OCT4.

Figure S5: QPCR-experiments to estimate the amount of *NANOG1* variants





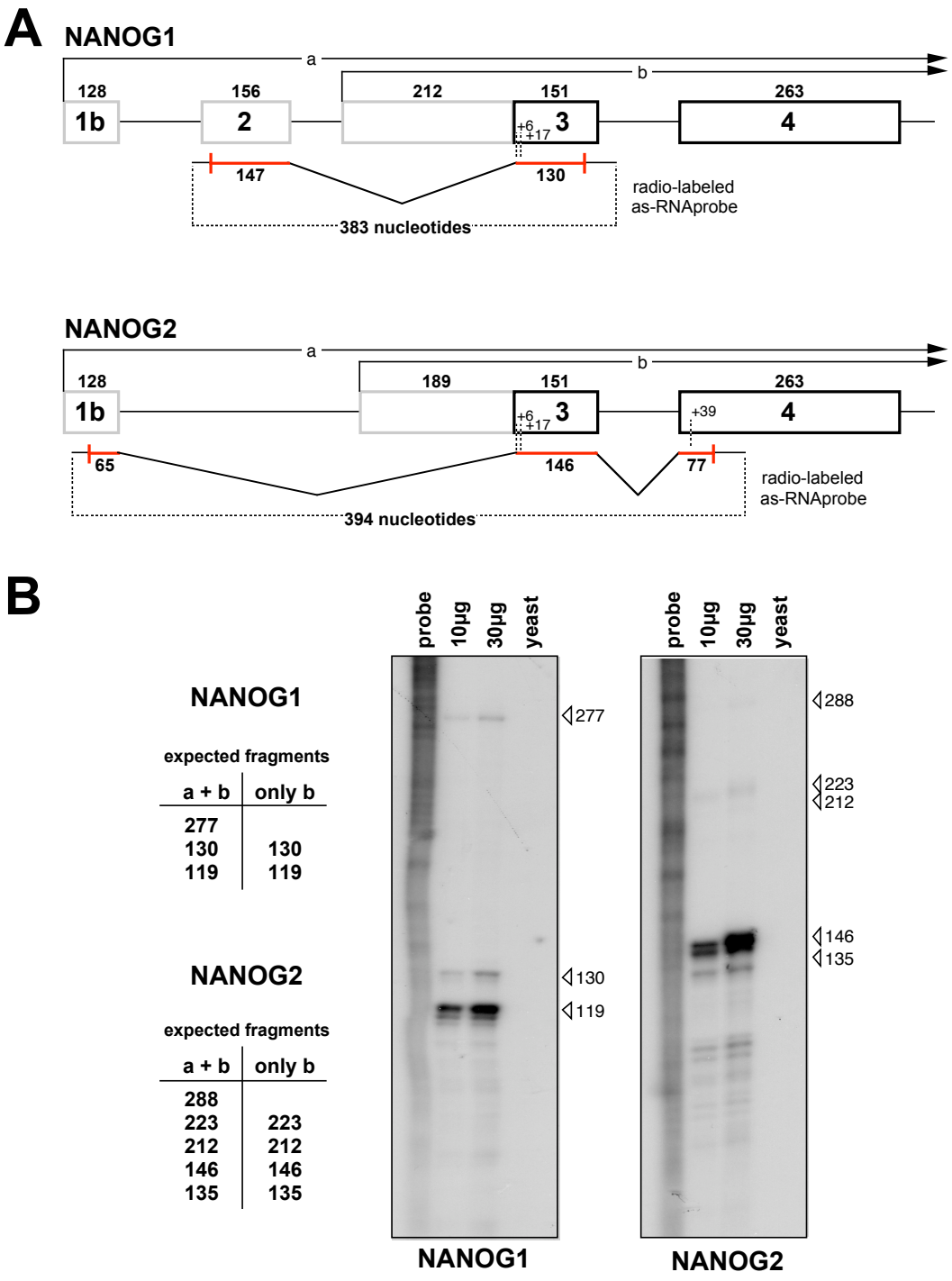
Q-PCR result:
Primer A-B: ~10.000 copies
Primer C-D: ~1.000.000 copies

Formula:
A-B: transcripts starting from NANOG1 ex 1b = a
C-D: transcripts starting NANOG1 ex 1b, NANOG1 ex 3, NANOG2 ex 1b and NANOG2 ex 3 = 2a + 2b

Calculation:
if a = 10.000
and 2a + 2b = 1.000.000
then b = 490.000

Top: partial gene structure of NANOG1 and NANOG2 (exons 1-4). Nomenclature as in Figure 2 of the manuscript. QPCR-primer design: primer A binds to NANOG1/2 exon 1b, primer B to NANOG1 exon 2, primer C to NANOG1/2 exon 3 and primer D to NANOG1/2 exon 4. Lower left panel: Results of independent QPCR experiments made with reverse transcribed total RNA isolated from NTERA2 cells. The experiments were carried out as described in Material and methods. All experiments were carried out in parallel with log-diluted plasmid copies (1 – 10^6 copies) that encode the NANOG1Bb splice variant. Lower right panel: Primers AB create an amplimer specific for the NANOG1 gene, while primers CD are able to identify transcripts starting from both NANOG genes (4 different transcripts), we estimated roughly the relative amount of transcripts starting from NANOG1 ex1b in relation to NANOG1 ex3 about 1/50.

Figure S6: RNase protection experiments to validate the existence of novel NANOG1/2 transcripts



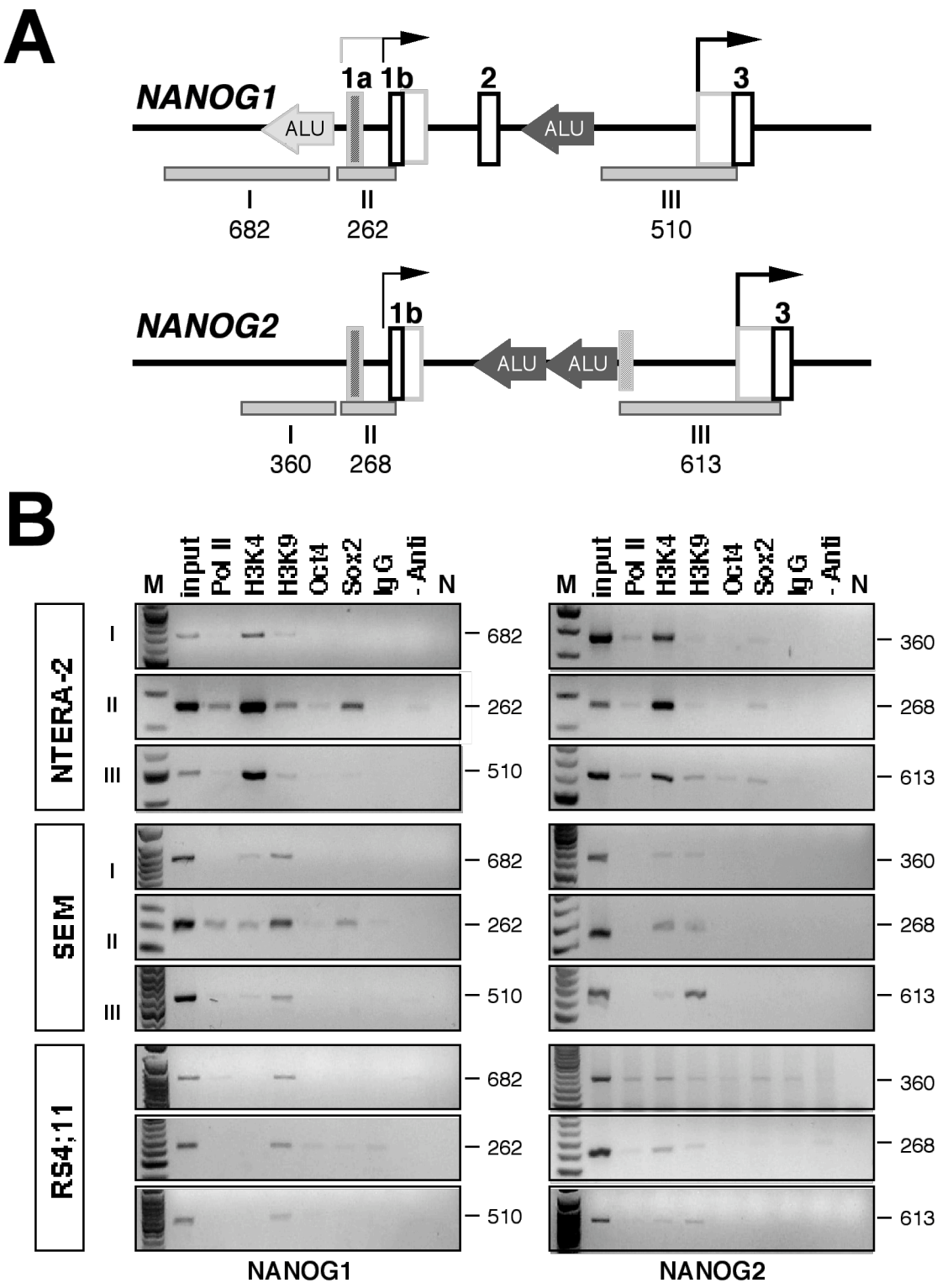
A. Outline of the performed RNase protection experiments. Radiolabeled antisense RNA probes were generated with the MAXIscript Kit (AMBION). For NANOG1 we used a cloned exon 2-3 fragment (Δ5 splice variant); for NANOG2 we used a cloned exon 1-3-4 fragment (Δ5 splice variant). Both fragments were cloned in pGEM-T plasmid (Promega); plasmid were digest and subjected to SP6 polymerase transcription in the presence of ^{32}P-UTP nucleotides. Radioactive labeled probes were separated on a 5% denaturating PAA gel. Gel slices were cut out and eluated over night at 37°C. Eluated RNA probes were then precipitated and dissolved into 40.000 cpm/µl.

B. RNase protection experimente were carried out as recommendend by the manufacturer (AMBION). Briefly, we used 10 and 30 µg total RNA from NTERA2 cells. About 80.000 cpm were co-precipitated with these RNAs. Appropriate controls were performed by suing yeast RNA.

B. NTERA cells express both the NANOG1 and NANOG2 gene. Due to our NANOG1-probe, 3 different protected species were expected. A 277 nucleotide-long species proves that transcripts spanned exons 2 and 3, while the shorter 130 nucleotide-long species represents transcripts starting upstream of NANOG exon 3. The shortest 119 nucleotide-long species is indicative for a splice product from exon 2 to nucleotide +17 of exon 3. Thus, NANOG1 transcripts in NTERA2 cells are starting upstream of the 5' terminal nucleotide of the 277 protected fragment in exon 2 and predominantly splice to nucleotide +17 of exon 3.

Due to our NANOG2-probe, 5 different protected species were expected. The longest protected fragment is indicative for the presence of NANOG2 transcripts coding for exons 1b, 3 and 4. The 223 nucleotide-long fragment represents NANOG2 transcripts starting upstream of NANOG exon 3 and containing exon 4 sequences. The 212 nucleotide-long species represents again a splice variant from NANOG2 exon 1b to nucleotide +17 of exon 3. The 146 nucleotide-long species are again transcripts starting upstream of exon 3, but are alternatively splice to nucleotide +39 of exon 4. The shortest protected fragment with 135 nucleotides represents transcripts starting from exon 1b that alternative splice to nucleotide +17 of exon 3 and alternatively to nucleotide +39 of exon 4. Thus, transcripts starting upstream of NANOG exon 3 are predominantly used in NTERA2 cells.
In summary, this experiment validated independently the existence of NANOG1 exon 1b and 2 and NANOG2 exon 1b in transcripts deriving from both genes.

Figure S7: ChIP-experiments

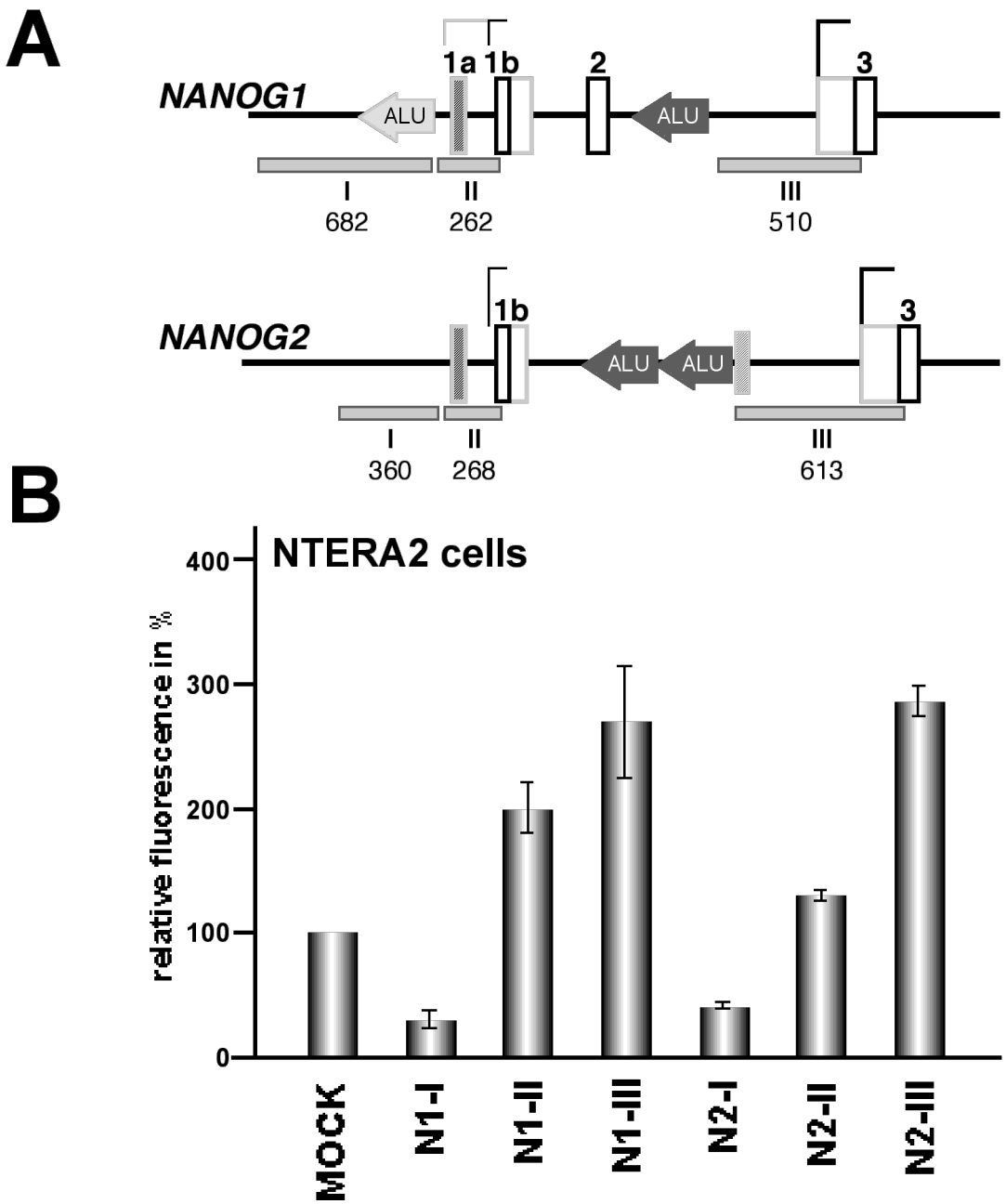


A. Tested fragments I-III for NANOG1 and NANOG2 in ChIP experiments.

B. ChIP experiments. Chromatin was cross-linked with 1% formaldehyde at room temperature for 10 min in PBS. Cells were then washed twice with PBS, collected in SDS Buffer (100 mM NACl, 50 mM Tris pH 8.1, 5 mM EDTA pH 8.0, 0,5% SDS) and centrifuged for 6 min at 1.200 rpm. For sonication, cells were collected in IP Buffer (100 mM NaCl, 67 mM Tris pH 8.3, 5 mM EDTA pH 8.0, 1,7% Triton X-100, 0,3% SDS) and sonicated 4 x 20

sec with an amplitude of 30% (Branson Digital Sonifier W-250 D, Danbury, CT**)** followed by centrifugation for 50 min at 20.000 x g at 4°C. The chromatin was then pre-cleared with protein A/G-sepharose (25 µl of 50% slurry in TE buffer (Santa Cruz, CA), containing 2 µg of salmon sperm DNA (Trevigen USA), and 50 µg BSA**)** for 2-4 h at 4°C. Immunoprecipitations were performed overnight at 4°C with specific antibodies (Abcam, UK: α RNA polymerase II, α Histone H3-trimethyl K4, α Histone H3-trimethyl K9, α Sox2; Santa Cruz, USA: α Oct-3/4 C-20, α IgG). After immunoprecipitation, 20 µl protein A-Sepharose with 1,6 µg of salmon sperm DNA and 40 µg BSA were added and the incubation was continued for another 2-3 h. Precipitates were washed sequentially three times with wash buffer (WB) 1 (150 mM NaCl, 200 mM Tris pH 8.1, 50 mM EDTA pH 8.0, 5,2% sucrose, 1% Triton X-100, 0,2% SDS), two times with WB 2 (0,1% deoxycholic acid, 1 mM EDTA, 50 mM HEPES pH7,5, 500 mM NaCl, 1% Triton X-100), two times with WB 3 (0,5% deoxycholic acid, 1 mM EDTA, 250 mM LiCl, 0,5% NP-40, 10 mM Tris pH 8.0) and one time with TE Buffer. DNA was eluted by incubating the protein A-sepharose in 300 µl of 1% SDS, 0,1 M $NaHCO_3$ overnight at 65°C, shaking. Proteins were removed by incubation with 60 µg of Proteinase K for 60 min. DNA fragments were purified with a QIAquick Spin Kit (Qiagen, Germany). The recovered DNA was then analyzed by PCR. All PCR experiments were performed in 50 µl reactions with the following setting: initial denaturation with 2 min at 94°C, followed by 35 cycles with 30 sec at 94°C, 30 sec at 60°C and 30 sec at 72°C. A final elongation step (3 min at 72°C) was used for all reactions. The following oligonucleotides were used: NANOG1 fragment I (5'-cagggtaccatctgctcactaagtgttag-3'; 5'-cagaagcttgttaaaatgagctaacggct-3'); NANOG1 fragment II (5'-cagggtacccgtgcccagccgttag-3'; 5'-cagaagcttctttgcataaaagcctgag-3'); NANOG1 fragment III (5'-cagggtaccatcccattcctgttga-3'; 5'-cagaagcttctggatccacactcatgt-3'); NANOG2 fragment I (5'-cagggtaccatctgctcactaagtgttag-3'; 5'-cagaagcttgttaaaatgagctaacaatttag-3'); NANOG2 fragment II (5'-cagggtacctcaactctactaaattgttag-3'; 5'-cagaagcttctttgcataaaagcctgag-3'); NANOG2 fragment III (5'-cagggtaccgtgctggaacccaactct-3'; 5'-cagaagcttctggatccacactcatgt-3').

Figure S8: Luciferase reporter gene assays

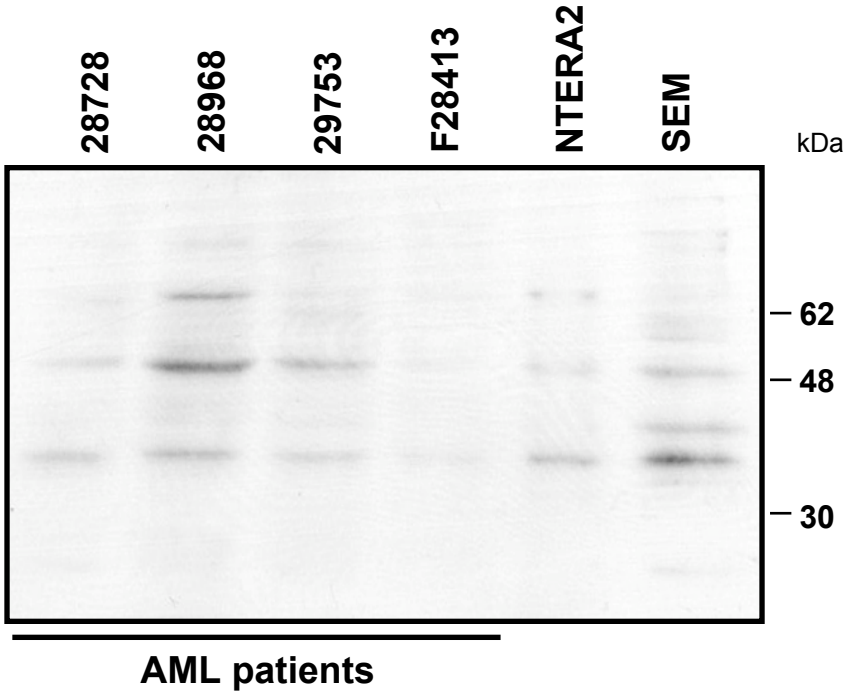


A. Tested NANOG1/2 DNA fragments. Different reporter plasmids containing the NANOG1-I to III and NANOG2-I to III regions were cloned into the pGL3-Luciferase plasmid. The promoter-less pGL3-Basic vector was used as negative control.

B. Luciferase reporter assays. All constructs were transiently co-transfected in 1 x 10^5 NTERA2 cells by using Lipofectamin-transfection. NTERA-2 cells were electroporated with 1 µg of each of the pGL-3 constructs together with 25 ng pGL3-Renilla plasmid (internal control). All experiments were performed independently 3 times in triplicates and all measurements were made 24 h after transfection.

These experiments revealed that the presence of an upstream promoter element for both tested NANOG genes.

Figure S9: Western blot experiments performed with leukemia patient material



blot: C-terminal antibody @ NANOG

Western blot experiment using patient biopsy samples from individual AML patients (2-5 x 10^6 cells), along with soluble lysates prepared from NTERA2 and SEM cells. Blots were stained with the C-terminal antibody against NANOG. Only 4 out of 10 investigated leukemia samples had enough cells to perform this experiment. The displayed patients all express NANOG protein, most likely the NANOG2 protein.