

SUPPLEMENTARY MATERIAL

corresponding to:

**Expression of NANOG and NANOGP8 in a variety of
undifferentiated and differentiated human cells**

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eNanog_ORF	ATGAGTGTGGATCCAGCTTGTCCCAAAGCTTGCCTTGCTTTGAAGCATCCGACTGTAAA
NanogP8_ORF	ATGAGTGTGGATCCAGCTTGTCCCAAAGCTTGCCTTGCTTTGAAGAAATCCGACTGTAAA
eNanog_ORF	GAATCTTCACCTATGCCTGTGATTTGTGGGCCTGAAGAAAACATCCATCCTTGCAAATG
NanogP8_ORF	GAATCTTCACCTATGCCTGTGATTTGTGGGCCTGAAGAAAACATCCATCCTTGCAAATG
eNanog_ORF	TCTTCTGCTGAGATGCCTCACACGGAGACTGTCTCTCCTTCTCCTCCATGGATCTG
NanogP8_ORF	TCTTCTGCTGAGATGCCTCACACAGAGACTGTCTCTCCTTCTCCTCCATGGATCTG
eNanog_ORF	CTTATTCAGGACAGCCCTGATTCTTCCACCAGTCCCAAAGGCAAACAACCCACTTCTGCA
NanogP8_ORF	CTTATTCAGGACAGCCCTGATTCTTCCACCAGTCCCAAAGGCAAACAACCCACTTCTGCA
eNanog_ORF	GAGAAAGTGTGCGCAAAAAAGGAAAGACAAGGTCCCGGTCAAGAAACAGAAGACCAGAACT
NanogP8_ORF	GAGAAATAGTGTGCGCAAAAAAGGAAAGACAAGGTCCCGGTCAAGAAACAGAAGACCAGAACT
eNanog_ORF	GTGTTCTCTTCCACCCAGCTGTGTGACTCAATGATAGATTTCCAGAGACAGAAATACCTC
NanogP8_ORF	GTGTTCTCTTCCACCCAGCTGTGTGACTCAATGATAGATTTCCAGAGACAGAAATACCTC
eNanog_ORF	AGCCTCCAGCAGATGCAAGAATCTCCAACATCCTGAACCTCAGCTACAAACAGGTGAAG
NanogP8_ORF	AGCCTCCAGCAGATGCAAGAATCTCCAACATCCTGAACCTCAGCTACAAACAGGTGAAG
eNanog_ORF	ACCTGGTTCAGAACCCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACAACCTGGCCGAAG
NanogP8_ORF	ACCTGGTTCAGAACCCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACAACCTGGCCGAAG
eNanog_ORF	AATAGCAATGGTGTGACGCAGAAGGCCTCAGCACCTACCTACCCAGCCTTTACTCTTCC
NanogP8_ORF	AATAGCAATGGTGTGACGCAGAAGGCCTCAGCACCTACCTACCCAGCCTTTACTCTTCC
eNanog_ORF	TACCACCAGGGATGCCTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACC
NanogP8_ORF	TACCACCAGGGATGCCTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACC
eNanog_ORF	TGGAACAATTCACCTGGAGCAACCAGACCCAGAACATCCAGTCTGGAGCAACCCTCC
NanogP8_ORF	TGGAACAATTCACCTGGAGCAACCAGACCCAGAACATCCAGTCTGGAGCAACCCTCC
eNanog_ORF	TGGAACACTCAGACCTGGTGCACCCAATCCTGGAACAATCAGGCCTGGAACAGTCCCTTC
NanogP8_ORF	TGGAACACTCAGACCTGGTGCACCCAATCCTGGAACAATCAGGCCTGGAACAGTCCCTTC
eNanog_ORF	TATAACTGTGGAGAGGAATCTCTGCAGTCTGCATGCACTTCCAGCCAAATTCTCCTGCC
NanogP8_ORF	TATAACTGTGGAGAGGAATCTCTGCAGTCTGCATGCACTTCCAGCCAAATTCTCCTGCC
eNanog_ORF	AGTGACTTGGAGGCTGCCTTGGAAAGCTGCTGGGGAAGGCCTTAATGTAATACAGCAGACC
NanogP8_ORF	AGTGACTTGGAGGCTGCCTTGGAAAGCTGCTGGGGAAGGCCTTAATGTAATACAGCAGACC
eNanog_ORF	ACTAGGTATTTTAGTACTCCACAAACCATGGATTTATTCTAAACTACTCCATGAACATG
NanogP8_ORF	ACTAGGTATTTTAGTACTCCACAAACCATGGATTTATTCTAAACTACTCCATGAACATG
eNanog_ORF	CAACCTGAAGACGTGTGA
NanogP8_ORF	CAACCTGAAGACGTGTGA

Supplementary Fig. S1. Sequence alignment of the open reading frames for published eNANOG and NANOGP8 sequences. They differ at 5 positions - P47, P144, P246, P531 and P759 relative to the ATG start codon (shaded regions).

B5_ORF -TGAGTGTGGATCCAGCTTGTCCCAAAGCTTGCCTTGCTTTGAAGCATCCGACTGTAAA
B12_ORF -TGAGTGTGGATCCAGCTTGTCCCAAAGCTTGCCTTGCTTTGAAGCATCCGACTGTAAA
NanogP8_ORF ATGAGTGTGGATCCAGCTTGTCCCAAAGCTTGCCTTGCTTTGAAGATCCGACTGTAAA
eNanog_ORF ATGAGTGTGGATCCAGCTTGTCCCAAAGCTTGCCTTGCTTTGAAGCATCCGACTGTAAA

B5_ORF GAATCTTCACCTATGCCTGTGATTTGTGGGCTGAAGAAAACATCCATCCTTGCAAATG
B12_ORF GAATCTTCACCTATGCCTGTGATTTGTGGGCTGAAGAAAACATCCATCCTTGCAAATG
NanogP8_ORF GAATCTTCACCTATGCCTGTGATTTGTGGGCTGAAGAAAACATCCATCCTTGCAAATG
eNanog_ORF GAATCTTCACCTATGCCTGTGATTTGTGGGCTGAAGAAAACATCCATCCTTGCAAATG

B5_ORF TCTTCTGCTGAGATGCCTCACACGAGACTGTCTCTCTCTTCTTCTTCCATGGATCTG
B12_ORF TCTTCTGCTGAGATGCCTCACACGAGACTGTCTCTCTCTTCTTCTTCCATGGATCTG
NanogP8_ORF TCTTCTGCTGAGATGCCTCACACGAGACTGTCTCTCTCTTCTTCTTCCATGGATCTG
eNanog_ORF TCTTCTGCTGAGATGCCTCACACGAGACTGTCTCTCTCTTCTTCTTCCATGGATCTG

B5_ORF CTTATTTCAGGACAGCCCTGATTTCTCCACCAGTCCCAAAGGCAAAACACCCACTTCTGCA
B12_ORF CTTATTTCAGGACAGCCCTGATTTCTCCACCAGTCCCAAAGGCAAAACACCCACTTCTGCA
NanogP8_ORF CTTATTTCAGGACAGCCCTGATTTCTCCACCAGTCCCAAAGGCAAAACACCCACTTCTGCA
eNanog_ORF CTTATTTCAGGACAGCCCTGATTTCTCCACCAGTCCCAAAGGCAAAACACCCACTTCTGCA

B5_ORF GAGAAAGTGTGCAGAAAAAGGAGACAAGGTCCTCCGTCAGAAAACAGAAAGCCAGAACT
B12_ORF GAGAAAGTGTGCAGAAAAAGGAGACAAGGTCCTCCGTCAGAAAACAGAAAGCCAGAACT
NanogP8_ORF GAGAAAGTGTGCAGAAAAAGGAGACAAGGTCCTCCGTCAGAAAACAGAAAGCCAGAACT
eNanog_ORF GAGAAAGTGTGCAGAAAAAGGAGACAAGGTCCTCCGTCAGAAAACAGAAAGCCAGAACT

B5_ORF GTGTTCTCTTCCACCAGCTGTGTGTAAGTCAATGATAGATTTTCAGAGACAGAAATACCTC
B12_ORF GTGTTCTCTTCCACCAGCTGTGTGTAAGTCAATGATAGATTTTCAGAGACAGAAATACCTC
NanogP8_ORF GTGTTCTCTTCCACCAGCTGTGTGTAAGTCAATGATAGATTTTCAGAGACAGAAATACCTC
eNanog_ORF GTGTTCTCTTCCACCAGCTGTGTGTAAGTCAATGATAGATTTTCAGAGACAGAAATACCTC

B5_ORF AGCCTCCGCGAGATGCAAGAAGCTTCCAACATCCTGAACCTCAGCTACAACAGGTGAAG
B12_ORF AGCCTCCGCGAGATGCAAGAAGCTTCCAACATCCTGAACCTCAGCTACAACAGGTGAAG
NanogP8_ORF AGCCTCCGCGAGATGCAAGAAGCTTCCAACATCCTGAACCTCAGCTACAACAGGTGAAG
eNanog_ORF AGCCTCCGCGAGATGCAAGAAGCTTCCAACATCCTGAACCTCAGCTACAACAGGTGAAG

B5_ORF ACCTGGTTCAGAACCCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACAACCTGGCCGAAG
B12_ORF ACCTGGTTCAGAACCCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACAACCTGGCCGAAG
NanogP8_ORF ACCTGGTTCAGAACCCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACAACCTGGCCGAAG
eNanog_ORF ACCTGGTTCAGAACCCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACAACCTGGCCGAAG

B5_ORF AATAGCAATGGTGTGACGCAGAAGGCTCAGCACCTACCTACCCAGCCTTACTCTTCC
B12_ORF AATAGCAATGGTGTGACGCAGAAGGCTCAGCACCTACCTACCCAGCCTTACTCTTCC
NanogP8_ORF AATAGCAATGGTGTGACGCAGAAGGCTCAGCACCTACCTACCCAGCCTTACTCTTCC
eNanog_ORF AATAGCAATGGTGTGACGCAGAAGGCTCAGCACCTACCTACCCAGCCTTACTCTTCC

B5_ORF TACCACCAGGGATGCTTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACC
B12_ORF TACCACCAGGGATGCTTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACC
NanogP8_ORF TACCACCAGGGATGCTTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACC
eNanog_ORF TACCACCAGGGATGCTTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACC

B5_ORF TGGAACAATTCACCTGGAGCAACCAGACCCAGAACATCCAGTCTGGAGCAACCACCTCC
B12_ORF TGGAACAATTCACCTGGAGCAACCAGACCCAGAACATCCAGTCTGGAGCAACCACCTCC
NanogP8_ORF TGGAACAATTCACCTGGAGCAACCAGACCCAGAACATCCAGTCTGGAGCAACCACCTCC
eNanog_ORF TGGAACAATTCACCTGGAGCAACCAGACCCAGAACATCCAGTCTGGAGCAACCACCTCC

B5_ORF TGGAACACTCAGACCTGGTGCACCAATCCTGGAACAATCAGGCTGGAAACAGTCCCTTC
B12_ORF TGGAACACTCAGACCTGGTGCACCAATCCTGGAACAATCAGGCTGGAAACAGTCCCTTC
NanogP8_ORF TGGAACACTCAGACCTGGTGCACCAATCCTGGAACAATCAGGCTGGAAACAGTCCCTTC
eNanog_ORF TGGAACACTCAGACCTGGTGCACCAATCCTGGAACAATCAGGCTGGAAACAGTCCCTTC

B5_ORF TATAACTGTGGAGAGGAATCTCTGCAGTCTGCATGCACCTCCAGCCAAATTCCTCTGCC
B12_ORF TATAACTGTGGAGAGGAATCTCTGCAGTCTGCATGCACCTCCAGCCAAATTCCTCTGCC
NanogP8_ORF TATAACTGTGGAGAGGAATCTCTGCAGTCTGCATGCACCTCCAGCCAAATTCCTCTGCC
eNanog_ORF TATAACTGTGGAGAGGAATCTCTGCAGTCTGCATGCACCTCCAGCCAAATTCCTCTGCC

B5_ORF AGTGACTTGGAGGCTGCCTTGGAAAGCTGCTGGGGAAGGCTTAATGTAATACAGCAGACC
B12_ORF AGTGACTTGGAGGCTGCCTTGGAAAGCTGCTGGGGAAGGCTTAATGTAATACAGCAGACC
NanogP8_ORF AGTGACTTGGAGGCTGCCTTGGAAAGCTGCTGGGGAAGGCTTAATGTAATACAGCAGACC
eNanog_ORF AGTGACTTGGAGGCTGCCTTGGAAAGCTGCTGGGGAAGGCTTAATGTAATACAGCAGACC

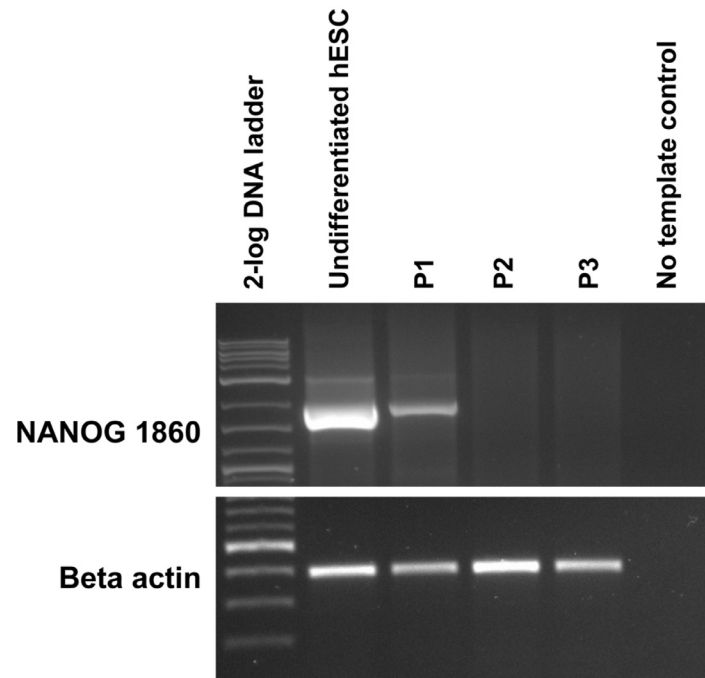
B5_ORF ACTAGGTATTTTAGTACTCCACAACCATGGATTTATTCCTAAACTACTCCATGAACATG
B12_ORF ACTAGGTATTTTAGTACTCCACAACCATGGATTTATTCCTAAACTACTCCATGAACATG
NanogP8_ORF ACTAGGTATTTTAGTACTCCACAACCATGGATTTATTCCTAAACTACTCCATGAACATG
eNanog_ORF ACTAGGTATTTTAGTACTCCACAACCATGGATTTATTCCTAAACTACTCCATGAACATG

B5_ORF CAACCTGAAGACGTGTGA
B12_ORF CAACCTGAAGACGTGTGA
NanogP8_ORF CAACCTGAAGACGTGTGA
eNanog_ORF CAACCTGAAGACGTGTGA

Supplementary Fig. S2. Sequence alignment of two representative sequences (clones B5 and B12) from adult human fibroblasts (CRL-2352, ATCC) against eNANOG and NANOGP8. Among the five positions that differ between eNANOG and NANOGP8, clones B5 and B12 show match with eNANOG at P47 (yellow) and with NANOGP8 at P144, P246, P531 and P759 (green). Base changes at positions 368 and 488 (pink) are unique to this cell line.

NanogP8_ORF	TGAGTGTGGATCCAGCTTGTCCCAAAGCTTGCCTTGCTTTGAAGCAATCCGACTGTAAG
hSMC_ORF	TGAGTGTGGATCCAGCTTGTCCCAAAGCTTGCCTTGCTTTGAAGCATCCGACTGTAAG
NanogP8_ORF	AATCTTCACCTATGCCTGTGATTTGTGGGCTGAAGAAAACATCCATCCTTGCAAATGT
hSMC_ORF	AATCTTCACCTATGCCTGTGATTTGTGGGCTGAAGAAAACATCCATCCTTGCAAATGT
NanogP8_ORF	CTTCTGCTGAGATGCCTCACACAGAGACTGTCTCTCTCTTCTTCTCCATGGATCTGC
hSMC_ORF	CTTCTGCTGAGATGCCTCACACAGAGACTGTCTCTCTCTTCTTCTCCATGGATCTGC
NanogP8_ORF	TTATTCAGGACAGCCCTGATTCTTCCACCAGTCCCAAAGGCAAAACAACCCACTTCTGCAG
hSMC_ORF	TTATTCAGGACAGCCCTGATTCTTCCACCAGTCCCAAAGGCAAAACAACCCACTTCTGCAG
NanogP8_ORF	AGAATAGTGTGCAAAAAAGGAAGACAAGGTCCCGGTCAAGAAACAGAAGACCAGAAGT
hSMC_ORF	AGAATAGTGTGCAAAAAAGGAAGACAAGGTCCCGGTCAAGAAACAGAAGACCAGAAGT
NanogP8_ORF	TGTTCTCTTCCACCCAGCTGTGTGACTCAATGATAGATTTTCCAGAGACAGAAATACCTCA
hSMC_ORF	TGTTCTCTTCCACCCAGCTGTGTGACTCAATGATAGATTTTCCAGAGACAGAAATACCTCA
NanogP8_ORF	GCCTCCAGCAGATGCAAGAACTCTCCAACATCCTGAACCTCAGTACAAACAGGTGAAGA
hSMC_ORF	GCCTCCAGCAGATGCAAGAACTCTCCAACATCCTGAACCTCAGTACAAACAGGTGAAGA
NanogP8_ORF	CCTGGTTCCAGAACCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACAACCTGGCCGAAGA
hSMC_ORF	CCTGGTTCCAGAACCAGAGAATGAAATCTAAGAGGTGGCAGAAAAACAACCTGGCCGAAGA
NanogP8_ORF	ATAGCAATGGTGTGACGCAGAAGGCCCTCAGCACCTACCTACCCAGCCTCTACTCTTCTCT
hSMC_ORF	ATAGCAATGGTGTGACGCAGAAGGCCCTCAGCACCTACCTACCCAGCCTCTACTCTTCTCT
NanogP8_ORF	ACCACCAGGGATGCCTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACCT
hSMC_ORF	ACCACCAGGGATGCCTGGTGAACCCGACTGGGAACCTTCCAATGTGGAGCAACCAGACCT
NanogP8_ORF	GGAACAATTCAACCTGGAGCAACCAGACCCAGAACATCCAGTCTGGAGCAACCACCTCT
hSMC_ORF	GGAACAATTCAACCTGGAGCAACCAGACCCAGAACATCCAGTCTGGAGCAACCACCTCT
NanogP8_ORF	GGAACACTCAGACCTGGTGCACCCAATCCTGGAACAATCAGGCCTGGAACAGTCCCTTCT
hSMC_ORF	GGAACACTCAGACCTGGTGCACCCAATCCTGGAACAATCAGGCCTGGAACAGTCCCTTCT
NanogP8_ORF	ATAACTGTGGAGAGGAATCTCTGCAGTCTGCATGCACCTCCAGCCAAATTTCTCTGCCA
hSMC_ORF	ATAACTGTGGAGAGGAATCTCTGCAGTCTGCATGCACCTCCAGCCAAATTTCTCTGCCA
NanogP8_ORF	GTGACTTGGAGGCTGCCTTGAAGCTGCTGGGAAGGCCTTAATGTAATACAGCAGACCA
hSMC_ORF	GTGACTTGGAGGCTGCCTTGAAGCTGCTGGGAAGGCCTTAATGTAATACAGCAGACCA
NanogP8_ORF	CTAGGTATTTTAGTACTCCACAACCATGGATTTATTCCTAAACTACTCCATGAACATGC
hSMC_ORF	CTAGGTATTTTAGTACTCCACAACCATGGATTTATTCCTAAACTACTCCATGAACATGC
NanogP8_ORF	AACCTGAAGACGTGTGA
hSMC_ORF	AACCTGAAGACGTGTGA

Supplementary Fig. S3. Sequence alignment of NANOG clone derived from hSMCs and NANOGP8, which differ by only one base at P47 relative to the ATG start codon.



Supplementary Fig. S4. Down regulation of NANOG transcript in differentiated hESCs. *The level of NANOG transcript is reduced significantly after one passage (P1 = 7 days) and disappears altogether after second passage (P2 = 14 days) and remains off out to the third passage (P3 = 25 days). Beta actin control PCR product is shown in the bottom panel. 2-log DNA ladder (New England Biolabs) was used as size marker.*