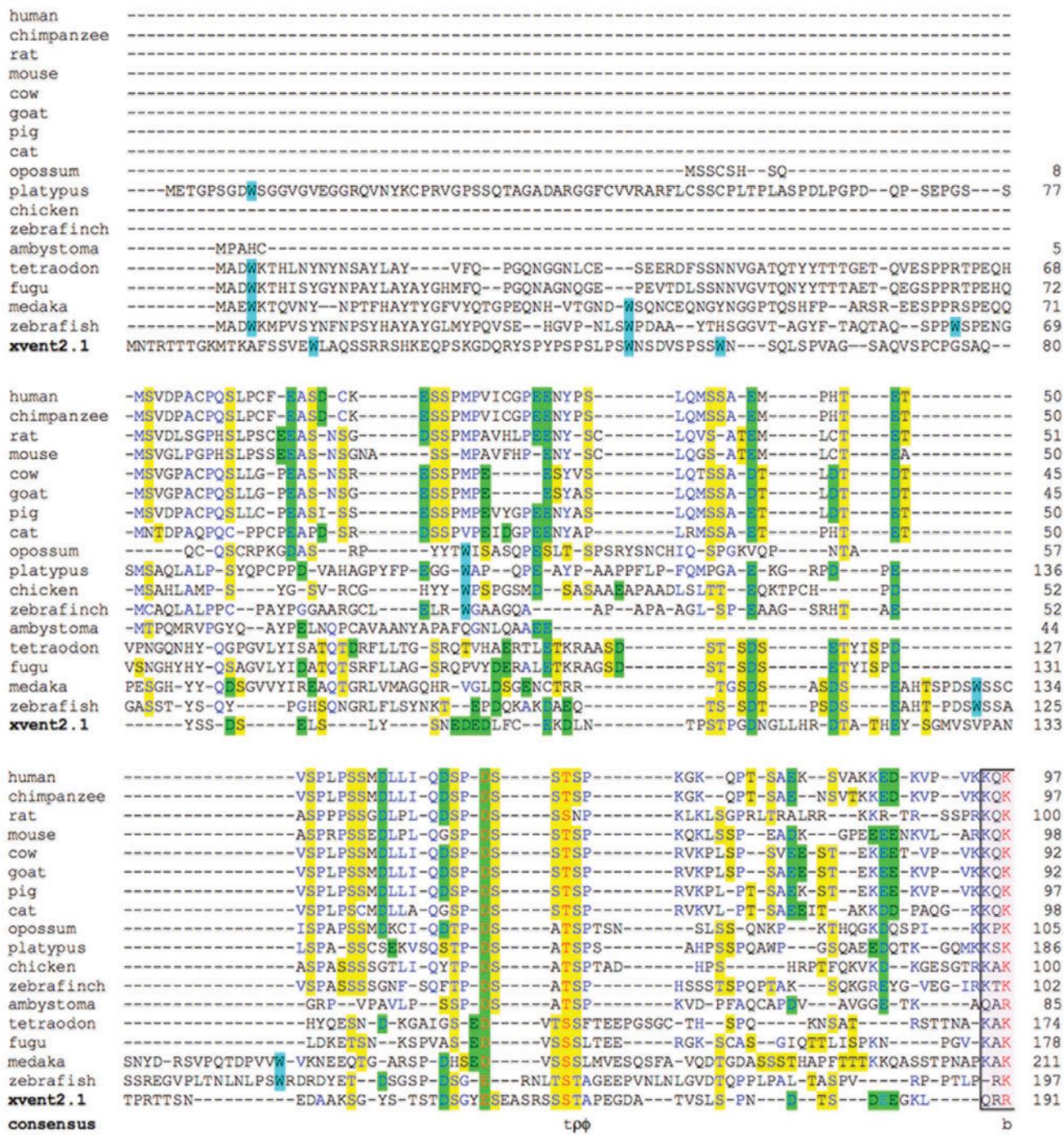


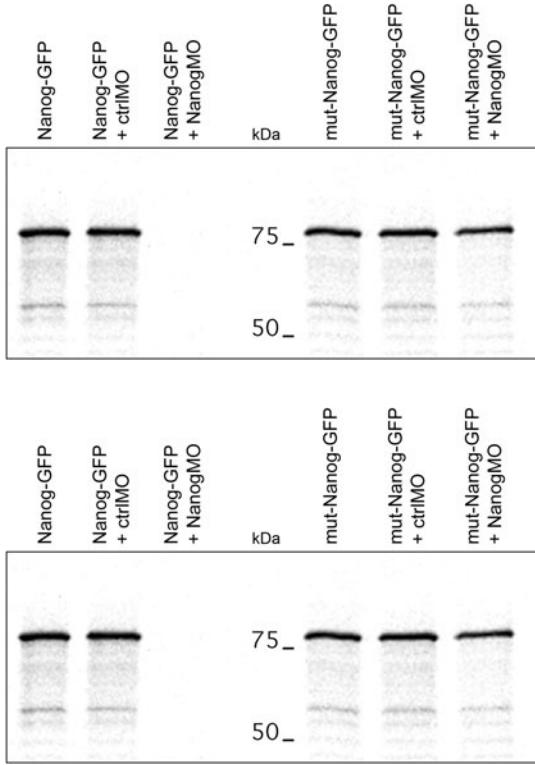
Supplementary Data



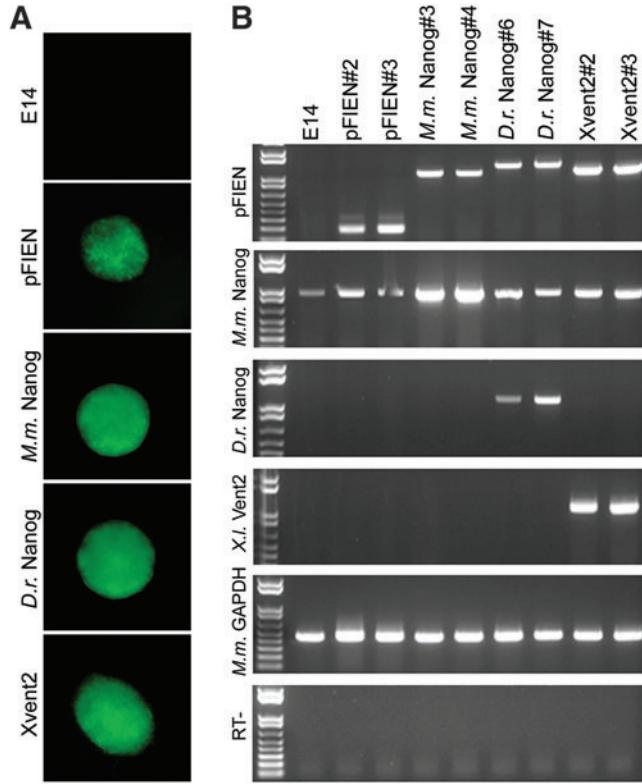
SUPPLEMENTARY FIG. S1. Alignment of Nanog proteins from human, chimpanzee, rat, mouse, cow, goat, pig, and cat (eutheria), opossum (metatheria), platypus (protheria), chicken and zebrafinch (birds), axolotl (amphibians), tetraodon, fugu, medaka, zebrafish (bony fishes), and Xvent2.1 of *Xenopus tropicalis*. Amino acids (PAM homology) are shown in blue (at least 8 of 17). Invariant amino acids in Nanog are given in red. The homeodomain (HD) is boxed, and its α -helical structure is schematically shown. Characteristic amino acid exchanges found in the Nanog HD are shaded in gray. Conserved acidic stretches are shaded in green, conserved serine and threonine residues are shaded in yellow, and tryptophan residues are shaded in bright blue, respectively. A triplet of amino acids (tpφ), which is invariant in all Nanog proteins, is partially conserved in Xvent2.1 (S at pos. 71 in murine Nanog), but not in other BarH proteins or in the related Dll proteins (data not shown). Several S/T residues exist in the N-terminus of Xvent2.1 as described for Nanog. However, the 2 invariant amino acids flanking a polar residue adjacent to the HD domain of Nanog at the C-terminus, a basic and a tryptophan residue (b-p_W), are not present in Xvent2.1. A common consensus sequence for all proteins is given next. b, basic aa; p, polar aa; φ, hydrophobic aa; s, serine or threonine; t, threonine or serine.

	α1	α2	α3	
human	TRIVFSSTQLCVLNDRFQRQKYLSQLQMQUELSNLNL	SYKQVKTWFWQNQRMKSKRWQK	NNWP--KNSNGVTQKASAP-TYPSLYSS	180
chimpanzee	TRTVFSSSTQLCVLNDRFQRQKYLSQLQMQUELSNLNL	SYKQVKTWFWQNQRMKSKRWQK	NNWP--KNSNGVTQKASAP-TYPSLYSS	180
rat	MRTVFSQAQLCALKDRFQRQKYLSQLQMQUELSNLNL	SYKQVKTWFWQNQRMKCKRWQK	NNWP--KTSNGLTO-GSAPVEYPSIHCS	183
mouse	MRTVFSQAQLCALKDRFQRQKYLSQLQMQUELSNLNL	SYKQVKTWFWQNQRMKCKRWQK	NNWP--KTSNGLIQKGSAPVEYPSIHCS	182
cow	IRTVFSQTQLCVLNDRFQRQKYLSQLQMQUELSNLNL	SYKQVKTWFWQNQRMKCKRWQK	NNWP--RNSNGMPQGP-AMAEPYGFYS-	174
goat	IRTVFSQTQLCVLNDRFQRQKYLSQLQMQUELSNLNL	SYKQVKTWFWQNQRMKCKRWQK	NNWP--RNSNGVPQGP-ATAEYGFYS-	174
pig	IRTVFSQTQLCVLNDRFQRQKYLSQLQMQUELSNLNL	SYKQVKTWFWQNQRMKCKRWQK	NNWP--RNSNSVIOG-SASTEYGFYS-	179
cat	IRTVFSQTQLYVLNDRFQRQKYLSQLQMQUELSNLNL	SYKQVKTWFWQNQRMKCKRWQK	NNWP--KNNNTVSQNSSANPEYGFYS-	181
opossum	MRTVFSQAQLNVLSRVEQKYLSPQQIRNVAENLN	TYKQVKTWFWQNQRMKSKRWDKDLMW	--KNGNRNVQNGSALGEYISLYSP	190
platypus	IRTAFTQTQLNTLNRRFQTQKYLSPQQIRLAMSL	TYKQVKTWFWQNQRMKSKRDRKDNLWIG	--R-SVMVQNGSLPATYMSLYSA	271
chicken	SRTAFSQEQLTQLHQRFQSOKYLSPHQIRELAALGL	TQVKTWFQKRCQKESQWV-DK	-GIYLQNGFHQAAYLDMPT	185
zebrafinch	SRTAFSQEQLTQLHQRFQSOKYLSPHQIRELAALGL	TYKQVKTWFWQNQRMKCKRWQK	SQWV-DK-GIYLQNGFHQAAYLDMPT	187
ambystoma	KRTCFSQEQLVALHRMFQKQHYMNPMQAAQQLAADLN	TYKQVKRWQK	-SER-AQCLTQSGFQSGTYLDVHPK	170
tetraodon	ARTAFSESQMNVLVHKFSQLPAAEMKNAEVITGLTYK	--QNRMRMKLRRHQKDTSWVSE	--YIINNGTSVQGTIVYNI-PP	252
fugu	ARTAFSESQMNVLVHKFSIQRYLPSEMKNLAEVITGLTYK	--QNRMRMKLRRHQKDSSWISER	--YAINNGAPVNGTVYNI-PP	256
medaka	VRAAFSESQMSTLVQRFQSVQRYLAPAEMKNAEVITGL	TGYKQVKTWFWQNRRMKLRRHQKDTSWVSE	--YI-NKDNTAADTV-FSNV	294
zebrafish	TRAFAFSEEQMNALVNRFNVQRYLTPAEMKTLAGATI	GTYKQVKTWFWQNRRMKLRRHQDSSWMT	--YVNV--AVPNP-----	273
xvent2.1	<u>LRTAFTSDQISTLEKTFQKHYLGA</u> SERRKLAALKLQ	<u>SEVQIKT</u> WFWQNRRMKYKREI	<u>ODGRPDSYH</u> --PAQF--FGV	264
consensus	R F s Q φ L F Q b y φ φ φ	L Ty K Q V K p W F Q N R M K b b p W b		
human	YHQGCLVNP-TGNL-PMWS---NQWTNNSTT-SNQQTQNIQSNSN-HSNW	-----TQIACCTQSNNNQAVN-SPFYNCGEESLQS	250	
chimpanzee	YHQGCLVNP-TGNL-PMWS---NQWTNNSTT-SNQQTQNIQSNSN-HSNW	-----TQIACCTQSNNNQAVN-SPFYNCGEESLQS	250	
rat	YQQGYLMLNA-SGNL-PVWG---SQTWTNPWTNNQWTNPWTNSQ-TWTNPWTNSQWTQACNSQTWNAAPLHNFGEDSLQP	-----TQIACCTQSNNNQAVN-SPFYNCGEESLQS	264	
mouse	YQQGYLMLNA-SGSLSL-MWGG---SQTWTNPWTNSQWTNSQWTNSQ-TWTNPWTNSQWTQACNSQTWNAAPLHNFGEDSLQP	-----TQIACCTQSNNNQAVN-SPFYNCGEESLQS	258	
cow	YHQGCLVN-SPGNL-PMWG---NQWTNNPTT-SNQSWNSNSNSN-HSNW	-----SQACPQAWNNQPNH-NQFNNYMEESFLQP	244	
goat	YHQGCLVN-SPGNL-PMWG---NQWTNNPTT-SNQSWNSNSNSN-HSNW	-----SQACPQAWNNQPNH-NQFNNYMEESFLQP	244	
pig	YHQGCLVN-SGNV-PVWG---NQSWNNPSNSQWTNSQNSNSN-SQW-TWN	-----SQACPQAWNNQPNH-NQCNNYMEESFLQP	249	
cat	YHQGYLMN-TSGNL-PIWG---NQTSNSQWTNSQWTNSQNSQ-TWNNSQS	-----SQACPQAWNNQPNH-NQCNNYMEESFLQP	261	
opossum	FHQDYMVSS-SGTL-PVWS---NQWTNNQ-FQW-SGE-----SYQHQIF	-----QHSYPAIDLGATFGNTGG-AYSMKS-QT	255	
platypus	LQHSLVLPNATGPNL-PPWA---GQAW---AS-----EPQGF-VJ-GVGGQQQHQAEEA-SQPF-AKAMVG-----YPPQ-----QA	-----QHSYPAIDLGATFGNTGG-AYSMKS-QT	331	
chicken	FHQGFPV-VANRNLQAVTSQHAYSSGQTYGNGQGLYPFMADVDEGF	-----EGFFGKGTC-NTQQAMGLLS-QQMFYHGYSTNVYISSLQA	269	
zebrafinch	FHQGYPIATA-GN1QTVPA---PCQHYYGAGQNAYTIVTSEEDGGVFGK	-----SVQQTGVFIAQHVDFYHYPGSVEYPGSKT	262	
ambystoma	CPESSSHLPQRYTVHH-SA-LAQRVTSHPYQKYSQGQPH-QKVLSEDAATVQHREAAPQ-CM-GPQQYMNRH-QNY-PTIY-AG	-----SVQQTGVFIAQHVDFYHYPGSVEYPGSKT	248	
tetraodon	YSTSIVYFKIV---VYTMMFGQVFS-GTLYYRLEDNLKHGM-IFIFFFQYQG-----SQRHMVASFKNP-QNVAFYMATMA--PG	-----SVQQTGVFIAQHVDFYHYPGSVEYPGSKT	328	
fugu	YSPSVSIHLKST---IYTNVLCGIF-FSTSMV-LMASNLGNEMK-NVFQSHQYQGEGRPQHMVDTAKNTAPQNVALYMATVG--PG	-----SVQQTGVFIAQHVDFYHYPGSVEYPGSKT	334	
medaka	APHVP-----PY-QG-CGMHSLRHH-YN-----QHMMGAFAKNTIPHNLAFYLAAMGNPPG	-----SVQQTGVFIAQHVDFYHYPGSVEYPGSKT	341	
zebrafish	ASQS-----QFQ-----SEPPGA-NQDH-YN-----PQVRSPV-FKRSPPKT	-----PFYPSYPQPRSPT	321	
xvent2.1	YG-----YSQQPTPVFH-----AVQQ-PY--PGY-SPLM	-----TLPG	295	
human	CMQFQP-NSPASDLEAALS-A-AGEGLNVIQQTRYFSTP-QTMEL-FLNYSMNM-QPESDV	-----	305	
chimpanzee	CMQFQP-NSPASDLEAALS-A-AGEGLNVIQQTRYFSTP-QTMEL-FLNYSMNM-QPESDV	-----	305	
rat	YVPLQQ-NFSASCLEANLEATR-ESQ-----AH-FSTP-QAEL-FLNYSVTP-PGEI	-----	311	
mouse	YVQLQQ-NFSASDLEVNLEATR-ESH-----AH-FSTP-QAEL-FLNYSVTP-PGEI	-----	305	
cow	GIQLQQ-NSPVCVDEAATL-GTAGENYNVIQQTVKYFNSSQQQITDL-FPNYPLNI-QPESDL	-----	300	
goat	GIQLQQ-NSPVCVDEAATL-GTAGENYNVIQQAVKYFSSQQQITDL-FPNYPLNI-QPESDL	-----	300	
pig	QLQFOQ-NSPISDLEAVL-TAGENHNVIQQTSKYCSTQQQIMDL-FPNYSMNI-QPESDM	-----	305	
cat	QIQLQQ-NSV-SQDQSIL-TTGESHSVIIQQTAKYFSAQ-QIMEL-FPNY-----PEHTA	-----	311	
opossum	SLSFNTPYPMELPLPSYSMMQLTHSKSDEEDYDYR---QAS-AQTQFLDPSVVVFQOS	-----	309	
platypus	ALTFSPPGAACCLPAFPTILPLA-HIKAE-SYSQPTFLAA-HSQFPDTSGLHLYQ-----	-----	385	
chicken	ECTYS-FQS-TS-----ITQFSSSPVRHQYQAPWHTLG-TQ-----NGYET	-----	309	
zebrafinch	VQLH-GSWQCQISKKNNSILILSPIRVG-SQDQVGQH-LGK-OKAFNMVSHSVAQPPPSLAT-IKWLRLMGKAVLGTSGGVGNH	-----	342	
ambystoma	ARPVEGYNLKTPQYPSMAPYPNYYY---QQPPPYIH-QQGRPDIFR-QST-----QGM	-----	297	
tetraodon	STGYP---SNTTVPCTAVFIRTQQLWPTN-PHFVFNAGFANDQCTSFEKNGA-IDRS	-----	384	
fugu	SAGYP---SNTSVPQQTAVPIRTQHROWPTN-PNTGHY-YNPHVFNVGFSSQGCTS-LESKN-G-SVONs	-----	398	
medaka	TAGYPP-WSSSPPQAAVPSRPQVPGWPLPPGRSQFGFCPIPYDPSAASLN--NFGRNAIPSKDG-SAGGANAAILHNAVQ	-----	420	
zebrafish	QATSRRPGTNPPLPPAVHYEFPNPISYMPARGSNAVNESSPSPLAT-SP-----TAGLWATKGITLL	-----	384	
xvent2.1	TMPYAMHPPAM-LS-----NH-FNSPP-FQ-MFYMPQQHLG-----QPLAY	-----	333	

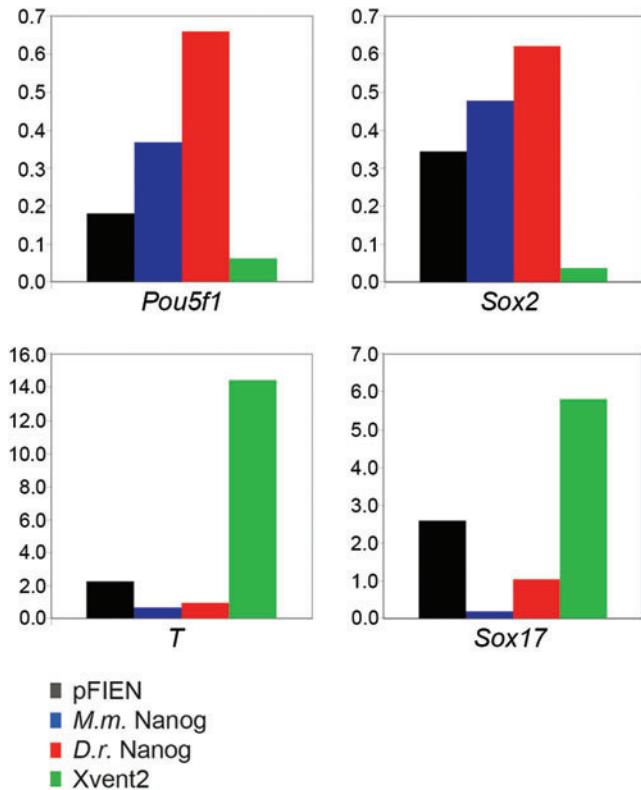
SUPPLEMENTARY FIG. S1. (Continued)



SUPPLEMENTARY FIG. S2. In vitro MO-binding test. The binding efficiency of NanogMO was tested in vitro in a coupled transcription/translation system (TNT®T7/SP6 Coupled Reticulocyte Lysate System; Promega) in the presence of ^{35}S labeled methionine. The translation of a *Nanog-GFP* fusion RNA is inhibited by NanogMO but not by ctrlMO. The inhibitory effect of NanogMO is specific, because transcription/translation of 0.5 ng *mut-Nanog-GFP* plasmid is inhibited by neither 10 ng ctrlMO nor 10 ng NanogMO. MO, morpholino oligonucleotides; ctrl MO, control MO.



SUPPLEMENTARY FIG. S3. Transfection control of mouse E14 ES cells. *M.m. Nanog*, *D.r. Nanog*, and *Xvent2* sequences were integrated into the eukaryotic expression vector pFIEN (provided by P.R. Tata, Ulm), which harbors a neomycin/kanamycin resistance cassette, a pCAGGS derived promoter, the integrated sequence, an IRES, and the EGFP/SV40 polyA part of the pIRE2-EGFP vector (Clontech). **(A)** Stably transfected E14 mouse ES cell lines with *M.m. Nanog*, *D.r. Nanog*, *Xvent2*, and an empty control (pFIEN) vector after 7 passages. EGFP (Enhanced Green Fluorescence Protein) signal indicates the stable expression of each construct. **(B)** Single GFP-positive ES cell clones were picked, expanded, and tested for proper stable expression of the constructs by RT-PCR with indicated primer pairs (Supplementary Table S1C). Two independent cell clones of each construct were used. RT-: cDNA synthesis without reverse transcriptase, analyzed with GAPDH primers used as an internal control. Numbers represent different cell lines. ES, embryonic stem; RT-PCR, reverse transcriptase–polymerase chain reaction.



SUPPLEMENTARY FIG. S4. Analysis of marker gene expression of embryoid bodies after 4 days of culturing in the absence of leukemia inhibitory factor. RNA was extracted and submitted to RT-PCR by using primers for *Pou5f1*, *Sox2*, *T*, and *Sox17* (see Supplementary Table S1C). Although *M.m.* Nanog and *D.r.* Nanog transfected cells show higher expression of pluripotency genes (*Pou5f1*, *Sox2*), Xvent2 and pFIEN transfected cells show higher expression of differentiation markers (*T*, *Sox17*).

SUPPLEMENTARY TABLE S1. PRIMERS AND CONDITIONS USED FOR REAL-TIME REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION
 (A) PRIMERS USED FOR *XENOPUS LAEVIS*

Primer pairs	Sequence forward primer (5'→3)	Sequence reverse primer (5'→3)	Annealing temperature (°C)/time(s)
BMP4	TTTATGTGGATTTCAGCGATGTG	AGCCAAGGGAAATGGACAATCT	53/5
Cdx2	TACCCCTCTGCCCCCCA	GGAAGGTGCCCATGGTCT	53/5
Chordin	AACTGCCAGGACTGGATGGT	GGCAGGATTAGAGTTGCTTC	52/5
Cxcr4b	CCTCACTACCACACATTCAATGAT	CCTTGTGTAGGGTGTCTCCCA	54/5
E-cadherin	GACTCACTCCCTGTGTTGATTACG	TGGCGAGTCAGAGAGCTG	54/5
Ectodermin	TGTGTAAGCTCAGCCTGCAAA	GACAGCGGCAGACAGAATGA	54/5
Epidermal	GCTCATCTAGCAAAGGTGGCTT	AGCCAGAACCCACACCACGT	54/5
Keratin			
Fut1	GAGGGTGGGATATGGACTGTTAAA	GGACAGAGCATACAAGGTGGC	54/5
Gata4	GCTACCTCACCTGTCTATGTCC	TTTGTGAGATGAGCCGCTG	53/5
Gata6	ACAATCACATCAGCGCAAGGA	GAUTGGAGAGCTGGCAGCG	53/5
Geminin H	GGATATGGAGAAGCCTACCATGTC	GATGCAGATGGCTGGATTACTTT	53/5
Grhl1	CATCGCTACCCCATCAAA	CAAAGCGACAATCCGTTCT	53/5
Gsc	GATGCCGCCAGTGCCTC	TGCAGCTCAGTCGTGACAAA	54/5
H4	GGCAAAGGAGGAAAAGGACTG	GGTGATGCCCTGGATGTTGT	55/5
Klf4	ATGAACCGACCCGCCAC	AAGCTCGATCACATCGCTGA	53/5
Ncam	AAGTCCAACAGAGGCAACCAA	GTCCCCCATCAATTGAAA	54/5
Oct60	TGACGGAAATTGCCAAAGAAC	CCTTGGACATTCTGAATTGCTC	54/5
PepCK	CCAACGTGGCAGAGACCACT	TGACCCCAGGAGGAAGTGG	54/5
Sall4	CAGTATGCCACCGCGCTT	TACTGATAACAGGAGCACCAGCC	54/5
Siamois	AAGGAACCCCACCAAGGATAAT	TGTTGTTCTGGTCTTCAAACCTCA	54/5
Sod2	CAGGCTCACTGTTGCGCA	TCATAAGGCAAGTCAGGAGCAG	52/5
Tert	CAGGTGCTTGGGATTGCGA	CCTGAACCTCTCAGAGTCTCCCTCT	54/5
Tsg1	CAGCAAATGCCATAACAGGAGCT	GTGCCCCAGACACAGCATACACTC	55/5
Wee1b	AGCACCCCTCAGGTCAACATC	CAAAGCCCAGGAGAAGACT	54/5
Xbra	AGCTCACCAACGAGATGATCG	AACCGTATAACATTGCATTGGGAT	54/5
Xema	TCCCGAGAAAGAGGAAAGAAA	ACTTAGCGGGCTCCCTTCAG	54/5
XSox2	CCAGTCCACCTGTAGTCACCTCT	CACTTCTGCCAGGTAGGTAC	54/5
XSox3	GTTTATGGTTTGGTCCCCGG	AGCGCCAATCTTGTCTG	55/5
XSox17 α	TGGCAAGTCGTGGAAGTCTCT	GTGGCTCTGCATGTGCTGC	54/5

(B) PRIMERS USED FOR *DANIO RERIO*

Primer pairs	Sequence forward primer (5'→3)	Sequence reverse primer (5'→3)	Annealing temperature (°C)/time(s)
BMP4	CCCGAGGAAGGGAAAGAAGAA	TCAAAGTCCCGCAGCAGC	54/5
Cxcr4b	CCTGGAACAGGGTCTGGAGA	TAGGGTTGAGACAGCAGTGGAA	54/5
Gata4	CCTCGTCTCTCGCCATG	GGTTTGCTGATGTTTTGGG	54/5
Gata6	GAAGCCCGCATACCTCAA	TATCCCTCGATGTGCCGTTAT	55/5
Gsc	CGCTGGGATGTTAGTATCGACA	GGTGGAGCAGAACCGAGTCTT	53/5
H4	CTCCCCGCTATAAGTGTATGTTT	GCGTAAGTGGGCTGTGAGGTT	54/5
Nanog	ACAGCCAGTACCCGGGACAC	TTGGTCGGGCTCAGTCTTG	54/5
Pax2a	TGTTTGAACGGGGCGTCA	CCTGCTCTGGCTGATGTGTT	54/5
Pou2	CCGCTTCATCCCCATCT	TCCGCTAAAAAATCCTGGC	54/5
Shh	CACCTCTTTTGTCTCGACA	TCCGGCTCTGACACTGCTG	54/5
Ved	TATCCCCTCAGCCCGC	GGATGTACAGGGATGCTCG	54/5
Vent	GCGGATGAAGCTGAAGCG	GAAAAACAGCGGGATAGAGGAA	55/5
Vox	CCGTGGACTGGCTTGC	TTGTCTTTCTCCGGCTCCT	54/5

(C) PRIMERS USED FOR CELL CULTURE (*M.M.* ES CELL LINE E14)

Primer pairs	Sequence forward primer (5'→3)	Sequence reverse primer (5'→3)	Annealing temperature (°C)/time(s)
pFIEN	TGGGCAACGTGCTGGTTGTTGT	TTCCAAGCGGCTTCGGCCAG	60/70
M.m. Na- nog	GGCAATTGATGAGTGTGGGTCTCCTG	CCGCTCGAGTCATATTTCACCTGGTGGAGTC	60/70
D.r.	GGAATTCATGGCGACTGGAAGATGC	ACCGCTCGACTCACAGCAAAGTTATTCCCT	60/70
Nanog Xvent2	GGAATTCATGACTAAAGCTTCTCCTC	CCGCTCGAGCTAATAGGCCAGAGGTTGCCA	60/70

(continued)

SUPPLEMENTARY TABLE S1. (CONTINUED)

Primer pairs	Sequence forward primer (5'→3)	Sequence reverse primer (5'→3)	Annealing temperature (°C)/time(s)
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCTGTTGCTGTA	57/70
M.m. H4	AAGCGCAAGACGGTTACGG	TACAGACTGCCGCCCTGG	54/5
M.m. T	CCGGTGCTGAAGTAAATGTG	GGTGTCAAGCCGTACGAAGTC	55/5
M.m. Sox17	CCCTTGTGTATAAGCCCGAGA	CGTGGCTGTCTGAGAGGTTCA	52/5
M.m. Pou5f1	GTTGGCGTGGAGACTTGCA	GAGGTTCCCTCTGAGTTGCTTC	54/5
M.m. Sox2	TGGCCCAGGAGAACCCCC	GACAAAAGTTCCACTCCGCG	54/5

Denaturation temperature was set to 95°C and extension was done by 72°C for 14 s.

SUPPLEMENTARY TABLE S2. FREQUENCIES OF OBSERVED PHENOTYPES AFTER GAIN AND LOSS OF FUNCTION AND CHANGES IN MARKER GENE EXPRESSION

(A) In Situ HYBRIDIZATION: DELOCALIZED EXPRESSION AFTER NANOG GAIN OF FUNCTION IN *DANIO RERIO* EMBRYOS

	Control injected % (a)	D.r. Nanog RNA (400 pg) % (a)
Patched-1 at tailbud stage	0 (0/17)	72 (13/18) (lower expression, expression domain quite different)
Sox17 at 80% epiboly	6 (2/31)	46 (20/44) (dorsal forerunner cells dispersed along margin)
Shh at tailbud stage	0 (0/33)	82 (32/39) (expression domain shorter and wider)

(B) SOUTHPAW IN SITU HYBRIDIZATION AFTER NANOG GAIN OF FUNCTION IN *DANIO RERIO* EMBRYOS

At 20 hpf	Wild type % (a)	Control injected % (a)	D.r. Nanog RNA (400 pg) % (a)
Left side	76 (57/75)	73 (35/48)	50 (35/70)
Right side	4 (3/75)	2 (1/48)	7 (5/70)
Both sides	19 (14/75)	23 (11/48)	43 (30/70)

(C) LOSS OF FUNCTION, GAIN OF FUNCTION, AND RESCUE: *DANIO RERIO* EMBRYOS

Analyzed at Phenotype	70%–80% epiboly Disturbed epiboly % (a)	1 dpf Lethality % (a)
Wild type	1 (1/85)	9 (20/212)
ctrlMO	2 (2/83)	11 (13/116)
NanogMO	99 (102/103)	86 (120/139)
NanogMO+D.r. mut-Nanog RNA	33 (9/27)	21 (18/86)
NanogMO+M.m. Nanog RNA	8 (3/31)	13 (4/31)

(D) IN SITU HYBRIDIZATION: DECREASED EXPRESSION AFTER NANOG LOSS OF FUNCTION IN *DANIO RERIO* EMBRYOS

At 70%–80% epiboly	controlMO % (a)	NanogMO % (a)
Ved	0 (0/40)	78 (28/36)
BMP4	0 (0/53)	100 (33/33)

(E) GAIN OF FUNCTION: *XENOPUS LAEVIS* EMBRYOS

Analyzed at Phenotype	Stage 11.5 Failure in blastopore formation % (a)	Stage 29		
		No head, no cement gland % (a)	Head, but no cement gland % (a)	Head, but reduced cement gland % (a)
Wild type	5 (2/37)	2 (1/54)	0 (0/54)	4 (2/54)
D.r. Nanog RNA (600 pg)	43 (84/196)	84 (76/91)	2 (2/91)	3 (3/91)
M.m. Nanog RNA (800 pg)	42 (58/139)	12 (14/119)	7 (8/119)	26 (31/119)
Xvent2 RNA (800 pg)	42 (79/187)	77 (97/126)	9 (11/126)	3 (4/126)

^aObserved phenotype/total number of embryos.
MO, morpholino oligonucleotides; hpf, hours postfertilization.

SUPPLEMENTARY TABLE S3. CHANGES IN THE RATE OF TRANSCRIPTION (NUMBERS IN BOLD) AS MONITORED BY REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION INCLUDING STANDARD DEVIATIONS (REGULAR NUMBERS)

(A) NANOG LOSS OF FUNCTION IN ZEBRAFISH EMBRYOS

	<i>Pou2</i>	<i>Cxcr4b</i>	<i>Gsc</i>	<i>Shh</i>	<i>BMP4</i>	<i>Vent</i>	<i>Vox</i>	<i>Ved</i>	<i>Pax2a</i>	<i>Gata6</i>	<i>Gata4</i>
+ 1.5ng NanogMO	0.39 0.02	3.75 0.47	0.16 0.03	1.16 0.42	1.04 0.59	0.41 0.18	0.42 0.10	0.38 0.24	6.35 4.22	0.10 0.03	0.14 0.00
+ 15 pg Nanog RNA	1.65 0.13	0.74 0.21	1.56 0.09	0.83 0.10	1.37 0.28	1.19 0.31	1.12 0.20	1.33 0.20	0.88 0.04	1.21 0.14	0.99 0.27
+ 60 pg Nanog RNA	2.57 1.29	0.70 0.05	2.09 0.53	0.53 0.01	1.69 0.18	0.79 0.04	0.96 0.28	1.89 0.31	0.53 0.19	0.91 0.12	0.80 0.03
(B) NANOG GAIN OF FUNCTION IN ZEBRAFISH EMBRYOS											
	<i>Pou2</i>	<i>Cxcr4b</i>	<i>Gsc</i>	<i>Shh</i>	<i>BMP4</i>	<i>Vent</i>	<i>Vox</i>	<i>Ved</i>	<i>Pax2a</i>	<i>Gata6</i>	<i>Gata4</i>
+ 15 pg Nanog RNA	1.65 0.13	0.74 0.21	1.56 0.09	0.83 0.10	1.37 0.28	1.19 0.31	1.12 0.20	1.33 0.20	0.88 0.04	1.21 0.14	0.99 0.27
+ 60 pg Nanog RNA	2.57 1.29	0.70 0.05	2.09 0.53	0.53 0.01	1.69 0.18	0.79 0.04	0.96 0.28	1.89 0.31	0.53 0.19	0.91 0.12	0.80 0.03

(C) GAIN OF FUNCTION OF *M.m. Nanog*, *D.r. Nanog*, *Xvent1*, AND *Xvent2* IN *XENOPUS* EMBRYOS AT DIFFERENT DEVELOPMENTAL STAGES

Stage	<i>M.m. Na-</i> <i>nog</i>	0.84 0.04	0.88 0.30	0.73 0.29	0.52 0.33	1.04 0.54	1.99 0.56	1.70 0.42	2.13 0.14	0.73 0.25	0.48 0.26	2.35 1.89	2.76 0.36	0.90 0.04	7.25 4.27	0.77 0.05	0.89 0.12	0.86 0.01	0.87 0.09	0.44 0.45	1.25 0.03	0.90 0.07	0.93 0.16	2.71 0.41	0.97 0.07		
10	<i>D.r. Nanog</i>	0.75 0.55	1.62 0.28	0.33 0.02	2.44 0.86	0.29 0.03	0.44 0.01	2.90 2.18	0.91 0.13	0.46 0.16	1.30 0.16	0.41 0.02	0.33 0.04	4.25 0.12	1.02 0.06	3.88 0.94	0.54 1.65	1.02 1.02	0.10 0.10	0.65 0.65	0.38 0.38	0.27 0.27	4.94 4.94	0.48 0.48			
	<i>Xvent1</i>	0.74 0.18	0.85 0.13	0.82 0.06	0.51 0.22	0.58 0.20	0.93 0.16	0.44 0.13	1.11 0.08	0.73 0.16	0.32 0.16	0.68 0.52	5.16 1.59	0.22 0.08	1.00 0.02	0.71 0.24	0.71 0.02	0.71 0.03	1.64 1.26	0.98 0.11	0.71 0.11	0.87 0.06	0.53 0.06	2.78 0.09	2.46 1.57		
	<i>Xvent2</i>	0.79 0.04	0.87 0.09	0.65 0.06	0.91 0.26	1.44 0.28	2.44 0.16	0.19 1.24	1.19 1.18	0.81 0.15	0.09 0.01	0.95 0.45	1.19 0.03	0.57 0.42	1.11 0.46	0.25 0.46	0.63 0.46	1.02 0.04	1.44 0.12	1.27 0.15	1.10 0.09	2.22 0.08	0.48 1.99	0.10 0.10			
11	<i>M.m. Na-</i> <i>nog</i>	0.86 0.01	0.41 0.36	0.73 0.10	0.20 0.34	1.03 0.06	2.57 0.01	0.96 1.12	1.48 0.11	0.85 0.02	2.03 0.54	2.66 1.52	2.97 1.52	1.68 0.25	1.63 0.60	1.00 0.18	0.84 0.71	0.90 0.36	1.47 0.35	1.26 0.29	0.27 0.24	0.32 0.24	0.71 0.21	1.18 0.60	4.72 0.46		
	<i>D.r. Nanog</i>	0.70 0.01	0.54 0.18	0.34 0.06	0.61 0.22	0.57 0.25	1.26 0.35	0.53 0.40	0.99 0.13	0.44 0.16	0.39 0.16	0.51 0.16	0.76 0.52	1.32 1.59	0.56 0.08	1.32 0.24	0.99 0.02	0.57 0.03	1.07 0.03	1.16 0.11	1.01 0.11	0.18 0.11	0.31 0.11	0.70 0.11	0.33 1.11	2.40 0.65	
12	<i>M.m. Na-</i> <i>nog</i>	2.26 0.87	1.26 0.25	1.09 0.33	1.30 1.32	0.99 0.62	1.74 0.18	0.16 0.06	0.99 0.50	0.46 0.04	1.36 0.21	1.72 0.35	3.25 1.22	0.41 0.01	1.41 0.84	4.10 0.10	1.64 0.48	1.31 0.48	0.44 0.37	0.04 0.04	0.37 0.37	0.04 0.04	0.11 0.11	0.49 0.11	0.16 0.11	0.70 1.11	2.25 0.65
	<i>Xvent2</i>	0.67 0.15	0.54 0.21	0.97 0.06	1.72 0.63	0.74 0.08	0.69 0.08	0.24 0.06	1.03 0.01	1.59 0.01	0.12 0.01	0.65 0.03	1.33 0.03	1.05 1.16	0.31 0.05	0.93 0.16	0.45 0.23	0.64 0.08	0.64 0.19	0.42 0.21	1.10 0.54	1.93 0.21	1.09 0.54	1.76 0.20	3.92 0.10	0.50 0.16	
	<i>Xvent1</i>	1.18 0.42	2.55 1.61	0.49 0.27	3.58 0.11	1.34 0.51	2.73 0.32	1.43 0.06	0.88 0.28	2.39 0.21	0.90 0.15	2.14 0.03	3.61 0.53	0.12 0.01	3.64 0.21	1.71 0.13	0.79 0.06	1.79 0.68	1.02 0.44	0.44 0.44	0.74 0.74	0.23 0.23	0.97 0.97	2.45 2.45	1.22 1.22		
	<i>D.r. Nanog</i>	1.14 0.11	3.79 1.85	0.44 1.35	4.35 2.42	3.13 1.85	2.56 2.40	0.94 0.08	0.71 0.12	2.40 0.50	0.40 0.17	4.46 1.56	3.72 1.17	6.49 0.86	1.31 0.37	2.03 0.14	0.81 0.17	1.62 1.18	0.64 0.90	0.56 0.56	0.64 0.64	0.45 0.45	0.33 0.33	0.59 0.13	0.47 0.21	3.30 0.13	2.39 1.37
	<i>Xvent1</i>	0.62 0.01	0.79 0.30	0.55 0.06	1.10 0.68	1.75 1.04	0.69 0.16	0.71 0.08	0.31 0.26	0.42 0.34	0.05 0.04	0.28 0.13	0.93 0.07	0.85 0.08	1.07 0.08	0.28 0.08	0.26 0.13	0.63 0.08	1.69 0.53	0.63 0.01	0.39 0.11	0.45 0.15	1.87 0.35	1.35 0.15	0.45 0.15	1.87 0.15	1.35 0.15
	<i>Xvent2</i>	0.92 0.25	3.28 3.77	1.21 0.08	1.33 0.13	0.68 0.06	2.62 0.25	1.64 0.15	1.73 0.32	0.21 0.19	0.96 0.11	0.11 0.68	2.81 1.61	2.07 0.73	3.35 1.41	1.18 0.04	0.93 0.06	0.66 0.06	0.73 0.11	1.26 0.01	2.72 0.02	1.71 0.01	1.44 0.70	1.00 0.21	1.42 0.57	0.92 0.03	