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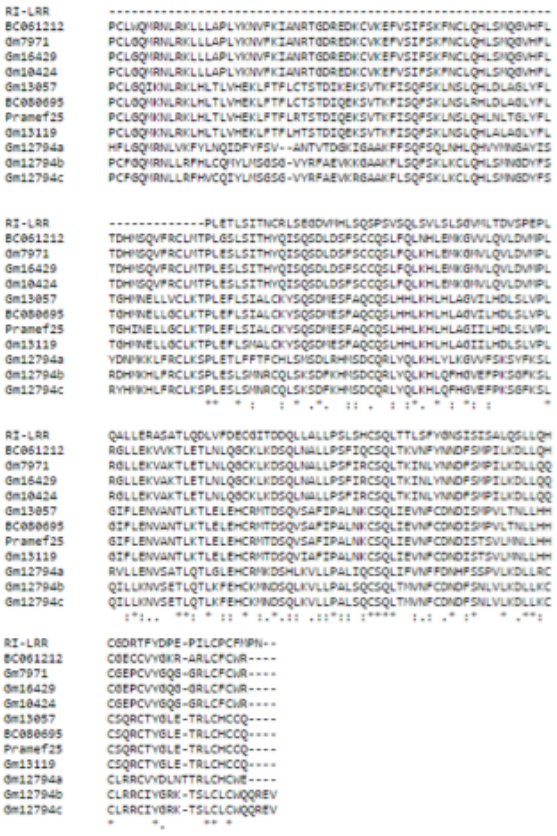
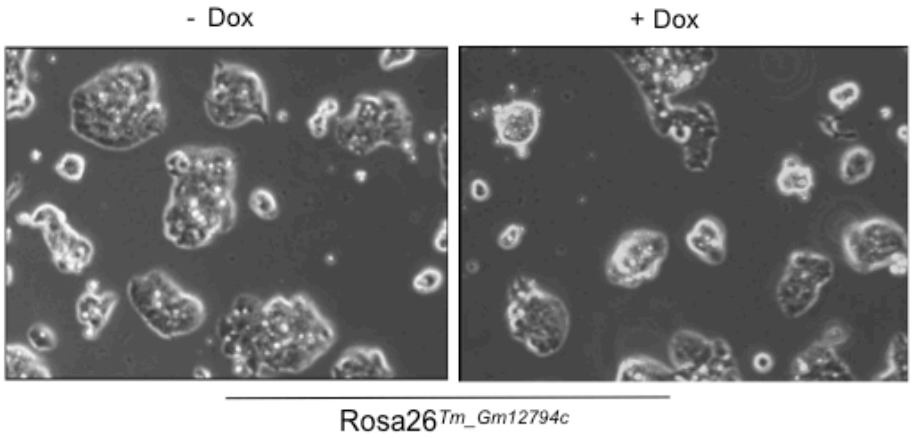
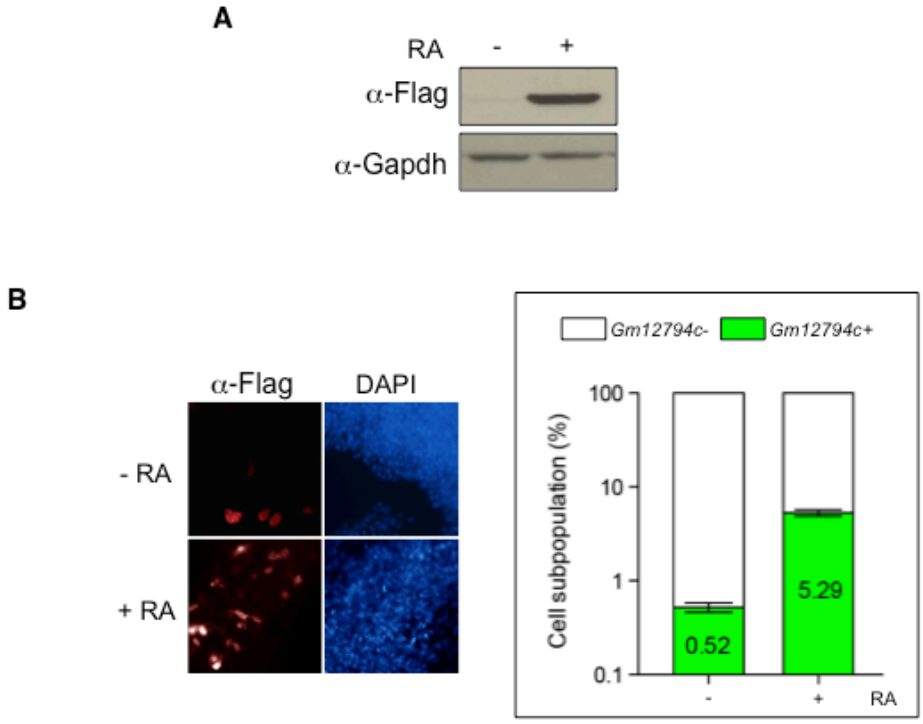


Supplementary Figure 1

1



Supplementary Figure 2



Supplementary Figure 3

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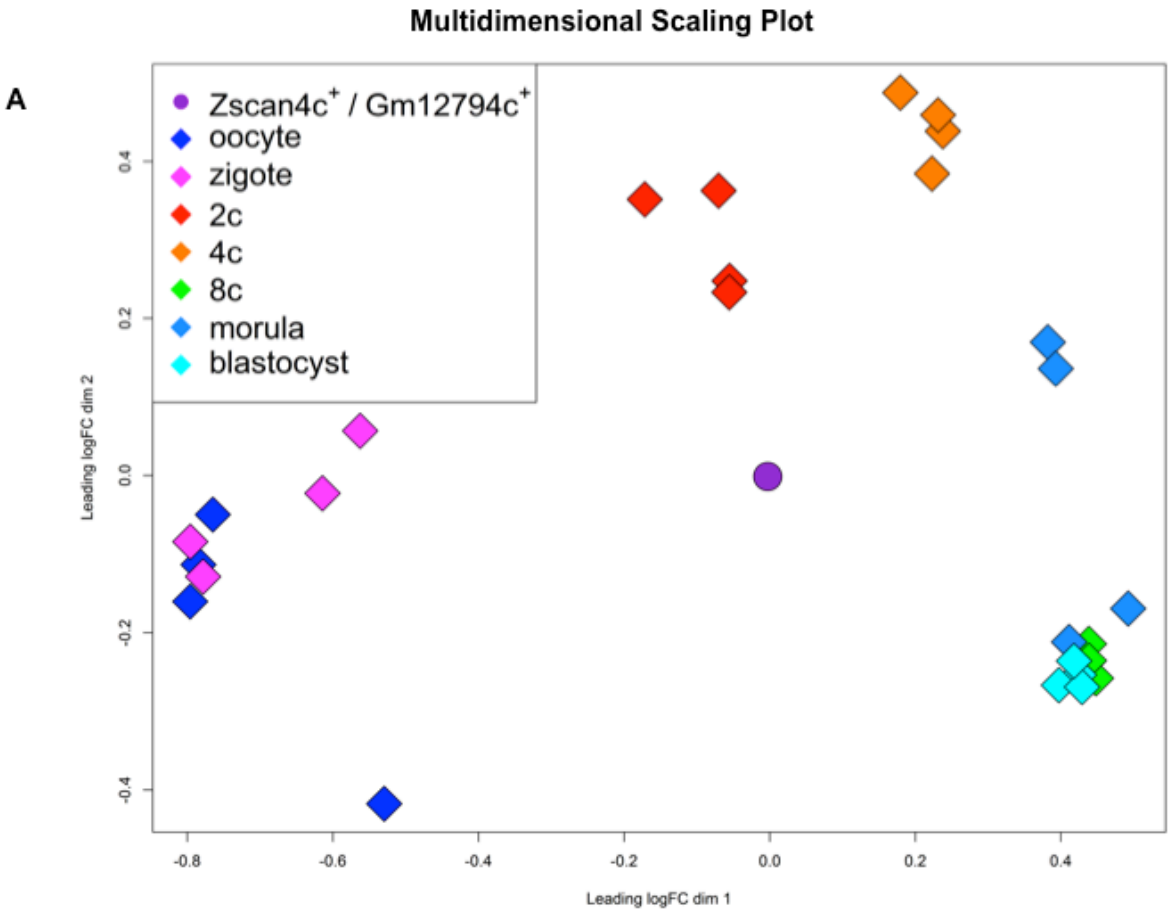
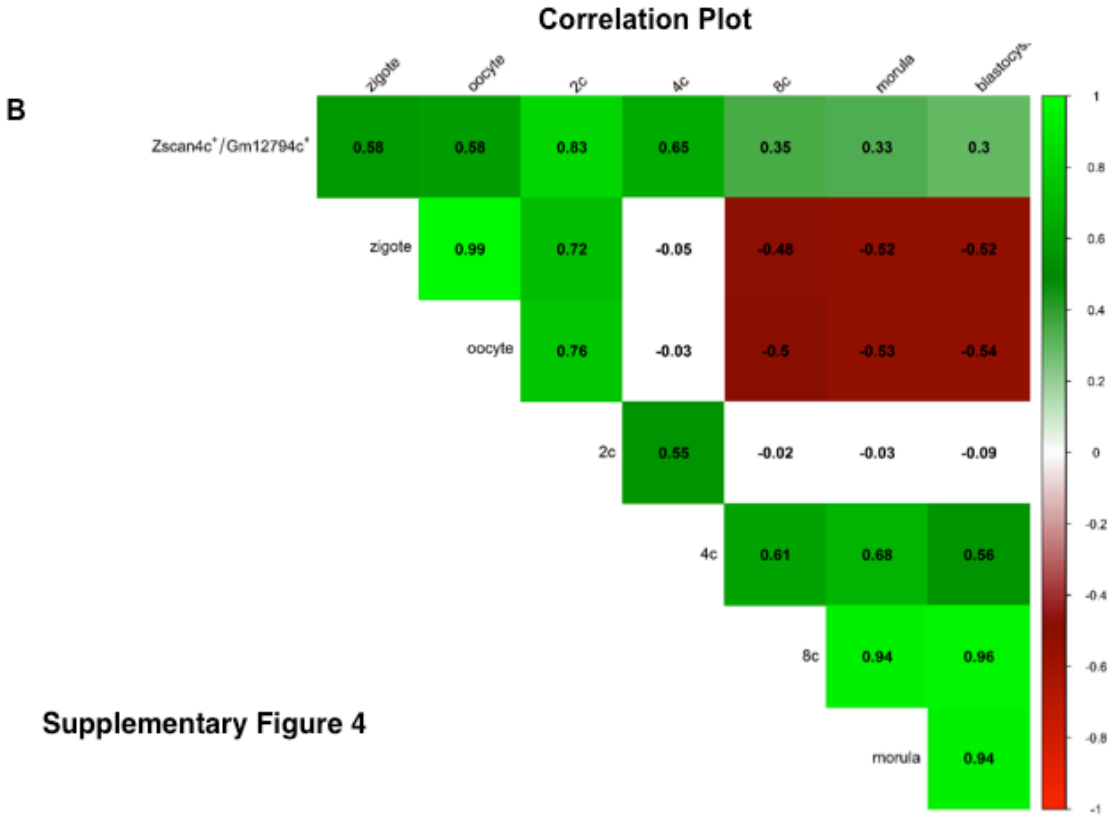
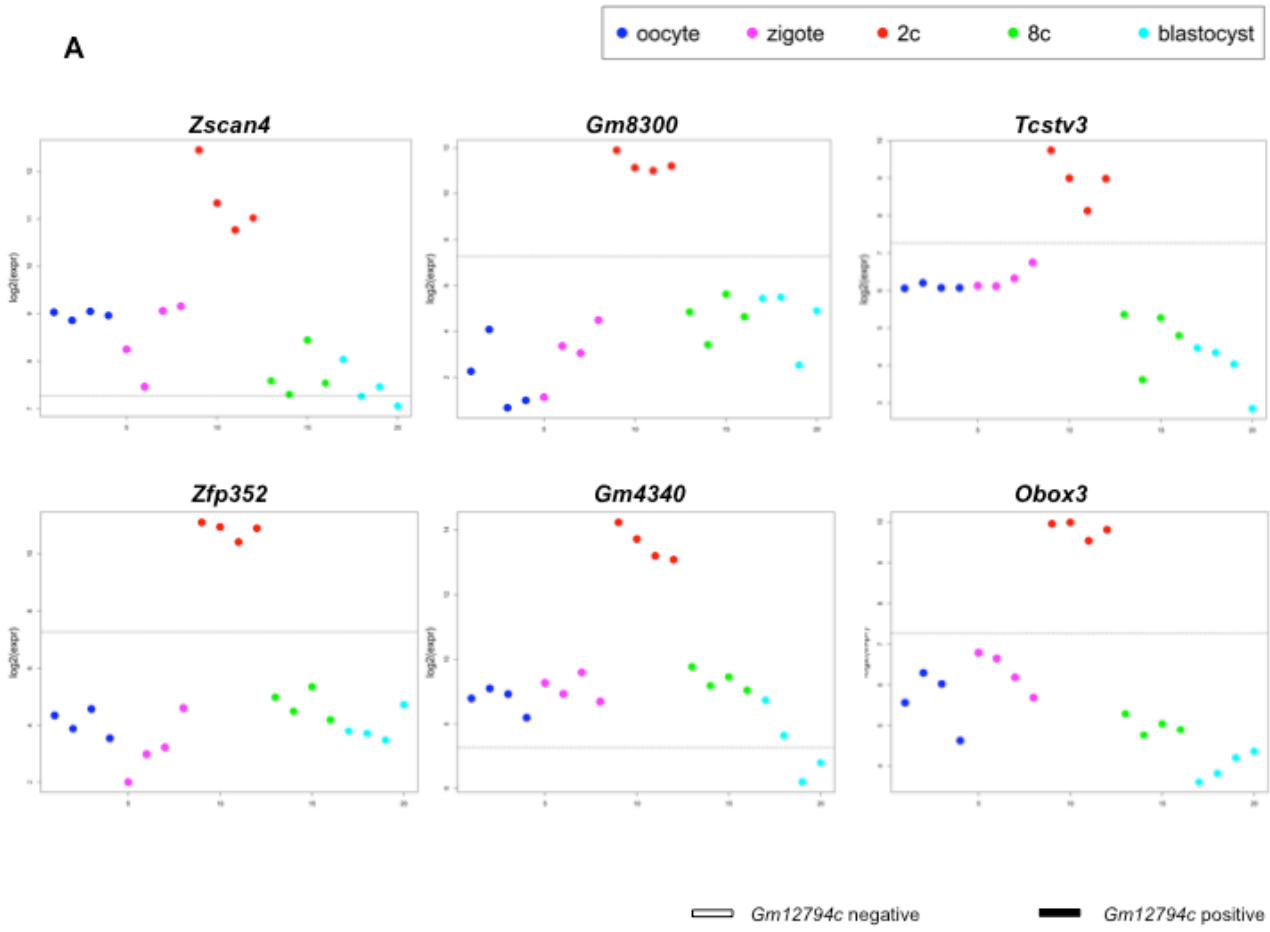
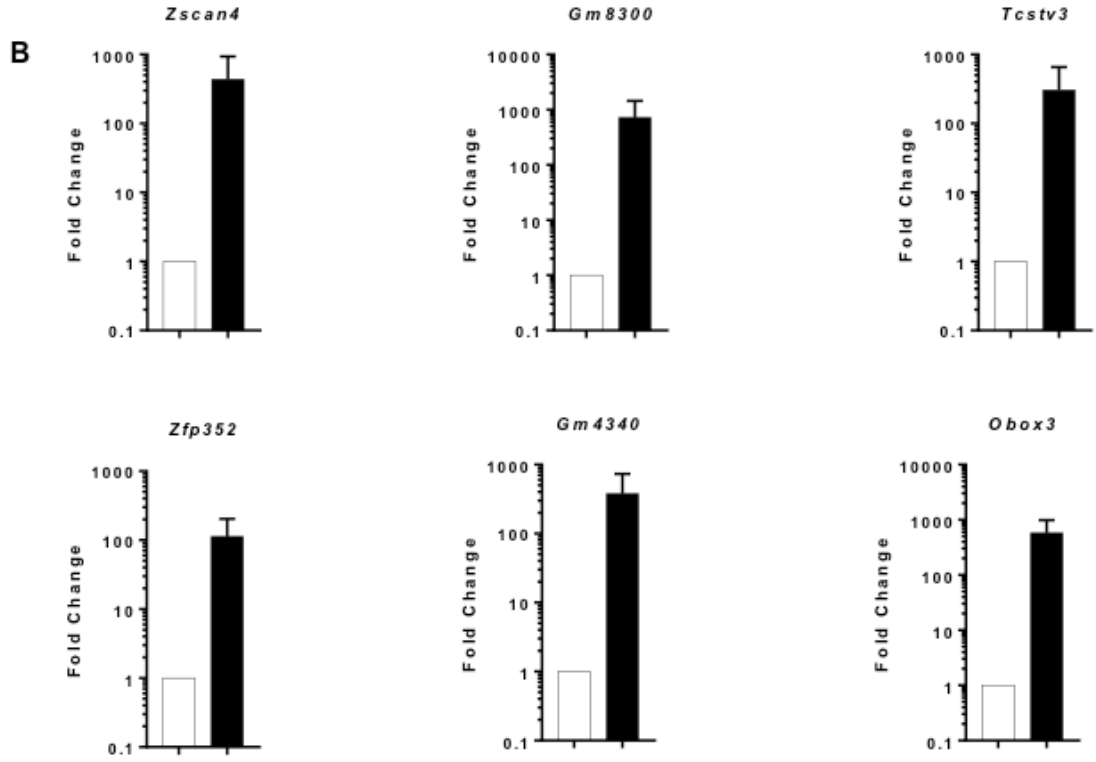




Supplementary Figure 4





Supplementary Figure 5

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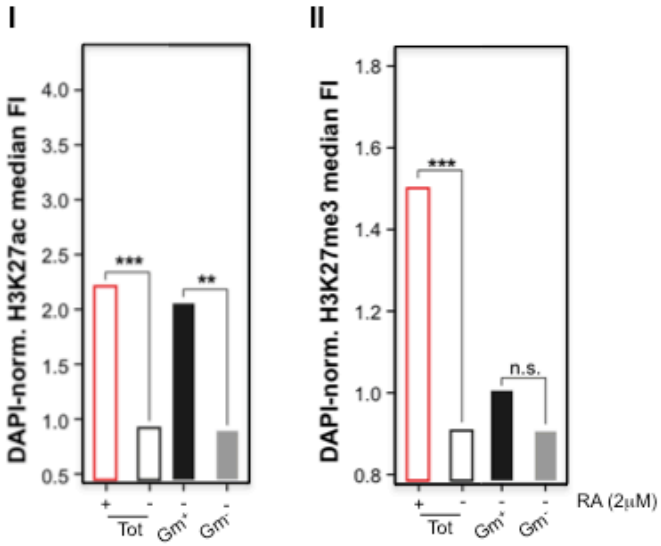

Supplementary Figure 6

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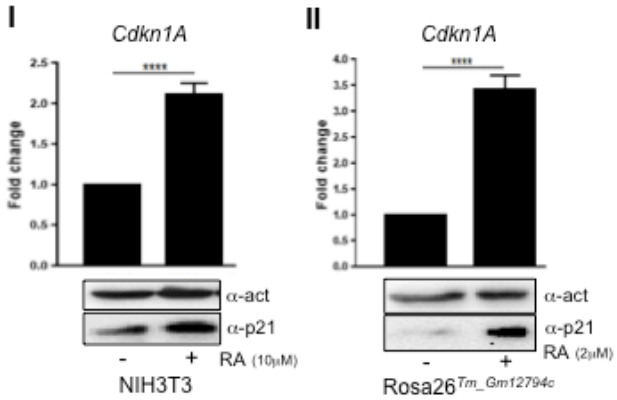

Supplementary Figure 7

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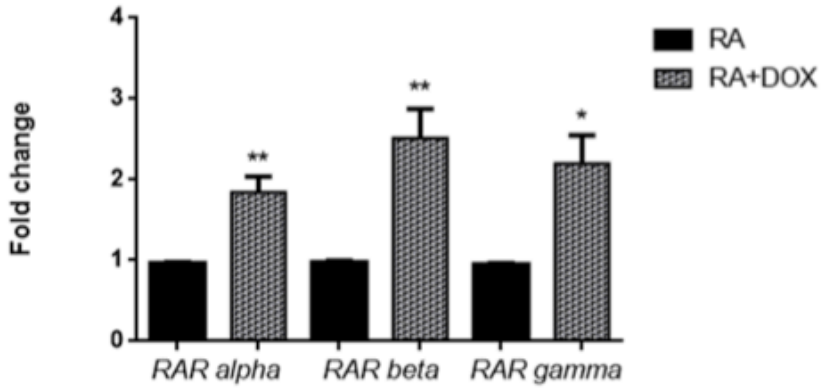

Supplementary Figure 8

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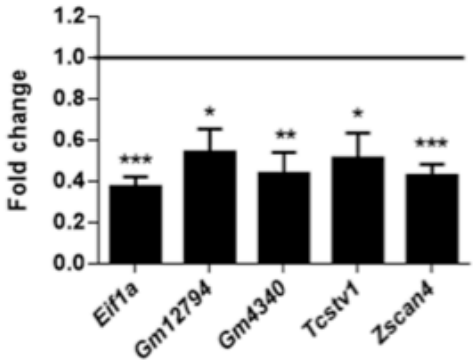
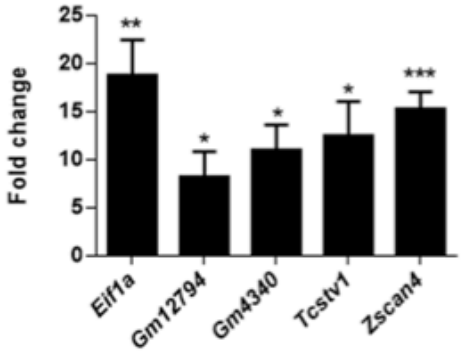
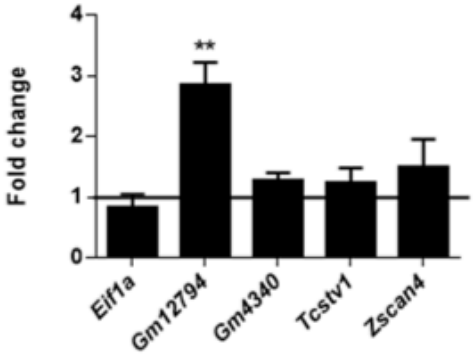


A. 2i culture condition effect on 2C-like gene signature



B. Effect of 2i on the expression of 2C-like genes in the presence of RA.



C. Gm12794 effects on 2C-like genes in 2i culture condition

Supplementary Figure 9

1

Supplementary Figure 1

Peptide sequence alignment of human RNI-like LRR protein with the murine RA-responsive Pamef25 and its 10 paralogs.

Asterisks (*) indicate identical aminoacids; single dots (.) indicate aminoacids with similarities comprised between 35% and 75%: double dots (:) indicate aminoacids showing a degree of similarity higher than 70%; dashes (-) are used to indicate gaps among the analyzed sequences inserted by the software to allow a more precise alignment.

Supplementary Figure 2

Rosa26^{Tm_Gm12794c} cells in standard culture conditions and treated with 1,5µg/ml Dox for three days were captured by contrast phase light microscopy using the Nikon TE Eclipse 2000 microscope. Representative images are shown. Experiments have been performed at least four times and each sample has been prepared in duplicate.

Supplementary Figure 3

RA-induced secondary colonies are enriched with Gm12794c-expressing cells

Murine E14 cells stably expressing a FLAG-*Gm12794c* chimera under the regulation of the endogenous *Gm12794c* promoter were treated on not with 1.5µM RA (4 days) and then processed for WB and immunofluorescence using anti-FLAG antibody. ***(A)*** Total cellular extracts were probed with anti-FLAG antibody. Anti-GAPDH has been used as a loading control. ***(B)*** Cells have been treated as in (A), fixed and probed with anti-FLAG antibody. Representative images captured with the Nikon TE Eclipse 2000 microscope are shown. Cells have been counted and *Gm12794c*-expressing ones (green bars) have been reported as percentage over the total cells analyzed (number of nuclei analyzed n≥100). Error bars indicate standard deviations for at least three independent experiments.

Supplementary Figure 4

A*) *Multidimensional scaling plot of the merged datasets.

The mouse preimplantation stages and *Zscan4c*$^{+}$/*Gm12794c*$^{+}$ samples are plotted on a two-dimensional scatterplot. The first dimension separates the 1-cell state from the 8-cells and late cleavage states. The second dimension highlights the peculiar activation status of the 2-cell and the 4-cell stage embryos.

B) Correlation Plot scheme

It is reported the correlation plot of the Top 100 genes more variable in the merged datasets. Significant (p-value <0.01) correlations depicted in green or in red when positive or negative, accordingly.

Supplementary Figure 5

A) Preimplantation samples are plotted on a two-dimensional scatterplot. On the x-axis, the sequence of the samples grouped by preimplant stage; on the y-axis the log2 expression of the gene. The dashed line defines presence or absence threshold.

B) pr$_{Gm12794c}$Strawberry cells were treated with 2µM RA for 4 days. *Gm12794c*-expressing cells (black bars, *Gm12794c*$^{+}$) were separated from *Gm12794c*-negative (empty bars, *Gm12794c*$^{-}$) by FACS then relative mRNA levels of 2-cell stage genes were analyzed by qRT-PCR.

Experiments have been performed at least three times and each sample has been prepared in duplicate. Data are presented as the mean of independent experiments and standard deviations are shown. Statistical analysis was performed using Student's t test: * p≤0.05, ** p≤0.01.

Supplementary Figure 6

Gm12794c favors acetylation of lysine 27 of histone H3 (H3K27ac)

pr$_{Gm12794c}$Strawberry cells were treated (or not) with 2µM retinoic acid (RA) for 96 hours before fixation. H3K27ac or H3K27me3 were then probed and revealed by immunofluorescence. Confocal images (individual planes; *z* = 700-800 nm) were analyzed by FIJI image analysis software. The integrated (total) nuclear fluorescence intensity (FI) signal from H3K27ac/me3 was normalized over the DNA (DAPI) or the histone content (H3), to take into account the cell cycle, the chromatin compaction and the substrate histone abundance. ***(I)*** median H3K27ac FI for all (empty bars) and *Gm12794c*-positive and negative cells (black and grey, respectively). Statistical significance was assessed by Wilcoxon rank sum test. ***: $p < 10^{-6}$; **: $p < 0.01$. ***(II)*** median H3K27me3 FI for all (empty bars) and *Gm12794c*-positive and negative cells (black and grey, respectively). Description and statistics are as in (**I**). n.s.: non significant. Independent RA treatments: *n*=3.

Supplementary Figure 7

NIH3T3 ***(I)*** and Rosa26^{Tm_Gm12794c} cells ***(II)*** were grown for 24 hours with 10μM RA (NIH3T3) or 96 hours with 2μM RA (Rosa26^{Tm_Gm12794c}) and mRNAs and protein levels of p21/*Cdkn1A* were assessed by qRT-PCR and WB respectively. Statistical analysis was performed using Student's *t* test: ****, p<0.0001. Error bars indicate standerd deviation. Experiments have been performed at least three times in duplicate (WB) or triplicate (qRT-PCR). Representative WB images are shown.

Supplementary Figure 8

Gm12794c affects transcription of RARs

Rosa26^{Tm_Gm12794c} cells were treated with 2μM retinoic acid (RA) alone for 96 hours or grown in the presence of 1,5μg/ml doxycycline (Dox) for 72 hours and then supplemented with 2μM RA and cultured for 96 more hours. Relative mRNA levels of 2C-like genes and RAR-alpha, RAR-beta and RAR-gamma were analyzed by qRT-PCR.

Experiments have been performed at least three times and each sample has been prepared in duplicate. Data are presented as the mean of independent experiments and standard deviations are shown. Statistical analysis was performed using Student's t test: * p≤0.05, ** p≤0.01.

Supplementary figure 9

A) 2i culture condition effect on 2C-like gene signature

Mouse E14Tg2a.4 cells were cultured for two passages on gelatin-coated feeder-free plates in N2B27 medium +*2i* and in ES serum-based medium for 72h. The expression of 2-cell stage genes was quantitatively measured by qRT-PCR. The reported data were obtained comparing 2C-like gene expressions in N2B27 medium +*2i* normalized to ES serum-based medium.

B) Effect of 2i culture condition on the expression of 2C-like genes in the presence of RA

Mouse E14Tg2a.4 cells were cultured for two passages on gelatin-coated feeder-free plates in N2B27 medium + *2i*, and subsequently maintained in N2B27 medium + *2i*, with RA for 72h. Relative mRNA levels of 2-cell stage genes were analyzed by qRT-PCR and normalized to cells grown in N2B27+*2i* without RA.

C*) *Gm12794c effect on 2C-like genes in 2i culture condition

Rosa26^{Tm_Gm12794c} cells were cultured in N2B27 +*2i* + RA for 72h or in N2B27 +*2i* + RA/doxycycline for 72h. Relative mRNA levels of 2C-like genes were analyzed by qRT-PCR. Values were normalized to Rosa26^{Tm_Gm12794c} cells cultured in N2B27 +*2i* + RA. Experiments have been performed at least three times and each sample has been prepared in duplicate. Data are presented as the mean of independent experiments and standard deviations are shown. Statistical analysis was performed using Student's *t* test: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Electronic supplementary material

Southern Blot

Southern blot analyses have been performed as previously described [**5**]. Sequences of probes used are available upon request.

Supplementary Table 1

Oligos used for qRT-PCR

Name	Sequence (5'-3')
Cdkn1A forw	GAACATCTCAGGGCCGAAAA
Cdkn1A rev	CAATCTGCGCTTGGAGTGAT
Gapdh forw	TCTTCTGGGTGGCAGTGATG
Gapdh rev	TGCACCACCAACTGCTTAGC
Gm12794c forw	GTTAGTCTCAGAAGGAAGCTTCAAGTC
Gm12794c rev	TCGTTTTTCCCAGTCCATACATC
Eif1a forw	TGGGAGACATAGGCAAGAGG
Eif1a rev	CTTTGCAGTTCCTGCATTGA
Gm4340 forw	TGGCTGCCGACTGTACCTTG
Gm4340 rev	GTCATGACGTCTTTGCTGGA
Tcstv1 forw	ATCCTCAGGAACTGAGAACTTCTGG
Tcstv1 rev	ATCCCATTCGGCAATCCAGC
Zscan4 forw	AGTCTGACTGATGAGTGCTTGAAGCC
Zscan4 rev	GGCCTTGTTGCAGATTGCTGTTG
Oct3/4 forw	CCGTGTGAGGTGGAGTCTGGA
Oct3/4 rev	CGCCGGTTACAGAACCATACTCG
Nanog forw	AACCAGTGGTTGAAGACTAGCAATGGTC
Nanog rev	TTCCAGATGCGTTCACCAGATAGC
Rex1 forw	CAGAAGAAAGCAGGATCGCCTCAC
rex1 rev	GCCACTTGTCTTTGCCGTTTTC

Oligos used for ChIP

Name	Sequence (5'-3')
pr_*Cdkn1A* forw TSS	GGCCTTCAGGAACATGTCTTG
pr_*Cdkn1A* rev TSS	ACCACCCTGCACTGAAGCA
pr_*Cdkn1A* forw -3000	ATATGCTCCCTGCCTGTCGTA
pr_*Cdkn1A* rev -3000	GTAACGAAGGCAAGCATGCA
pr_*Cdkn1A* forw -10000	CCACAGAGGATGGTCAGTCATC
pr_*Cdkn1A* rev -10000	TGGTGGTAGCCCCACTATGG
pr_*Cdkn1A* forw -1000	AAAAGCAAGCCAGTAACCAATGTT
pr_*Cdkn1A* rev -1000	AAGCCAGGGCAGGAACCT
pr_*Cdkn1A* forw +3000	CCCGAGTGCTGTGTTTTGG
pr_*Cdkn1A* rev +3000	GGAACGTGGCCAGCATTG

Oligos used for construction of the mutant FLAG-*Gm12794c*LKDVV expression plasmid

Name	Sequence (5'-3')	Tm (°C)
5' 3XFlag-*Gm12794c* EcoRI	AAAAGAATTCAATGAGCACCTACAACCCTCCCACA	68,3
3' mut2xVal	TAGATTGGCCATGCATTTCACAACGTCTTTCAGTAC AAGATT	69,4
5' mut2xVal	CTTGTACTGAAAGACGTTGTGAAATGCATGGCCAAT	68,3
3' 3xFlag-*Gm12794c* BamhI	AAAAGGATCCCTAATGATGATGATGATGAACTTCTC TTTG	68,5

Oligos used for construction of the p21-Luc plasmid vector

5’ Cdkn1A promoter	AAA AAG AGC TCA GCA GGC CTG GGT CTG TTC AG	77°C
3’ Cdkn1A promoter	AAA AAA AGC TTT CCA CCA CCC TGC ACT GAA GCA GC	77°C

Supplementary Table 2

List of the antibodies used for western blot

Epitope	Name	Supplier	Dilution
FLAG	F3165	SIGMA-ALDRICH	1:1000
p21	sc-397	Santa Cruz Biotechnology	1:1000
p21	sc-6246	Santa Cruz Biotechnology	1.1000
actin	sc-1616	Santa Cruz Biotechnology	1:2000

List of the antibodies used for Immunofluorescence

Epitope	Name	Supplier	Dilution
5mC	C15200081	Diagenode	1:100
p21	sc-397	Santa Cruz Biotechnology	1:100
FLAG	F3165	SIGMA-ALDRICH	1:100
H3K27ac	ab4729	Abcam	1:100
H3K27me3	ab6002	Abcam	1:100

List of the antibodies used for ChIP

Epitope	Name	Supplier
FLAG	F3165	SIGMA-ALDRICH
H3K27ac	ab4729	Abcam
H3K27me3	ab6002	Abcam
H3K4me3	07-449	Upstate