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Supplementary Materials for

Controlled ploidy reduction of pluripotent 4n cells generates 2n cells during mouse embryo development

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Published 16 October 2019, *Sci. Adv.* **5**, eaax4199 (2019)
DOI: 10.1126/sciadv.aax4199

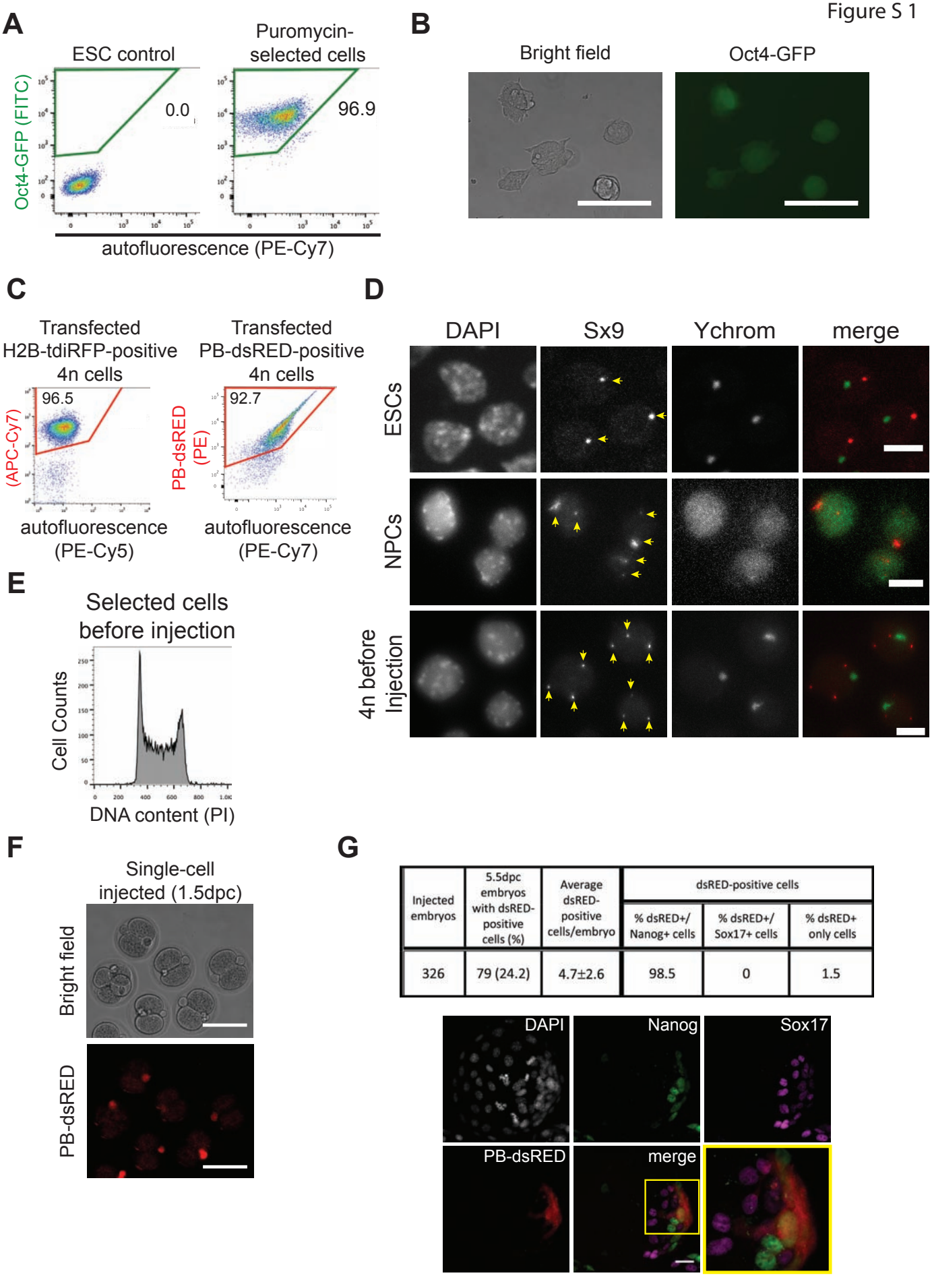
The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/5/10/eaax4199/DC1)

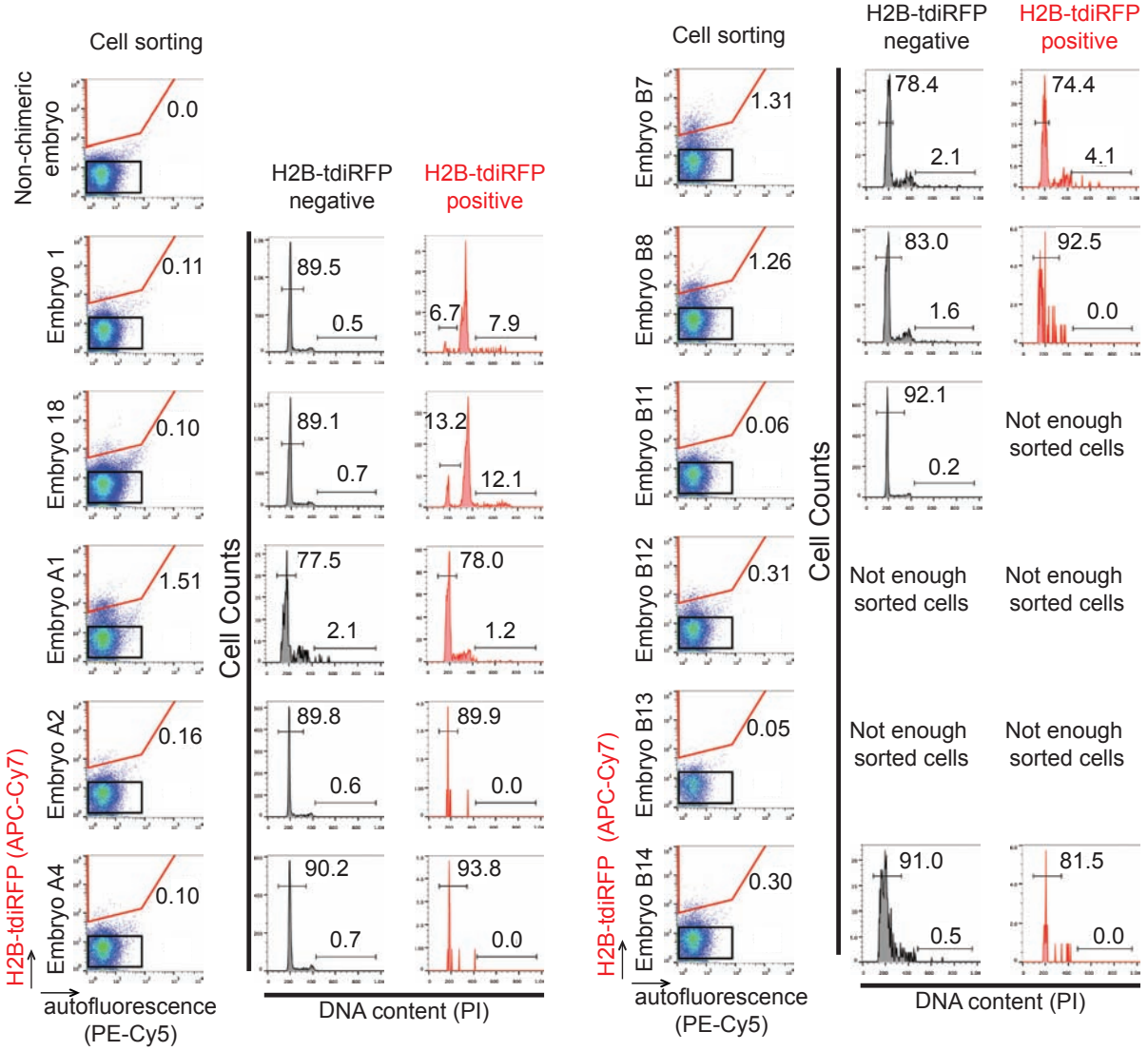
Movie S8 (.avi format). Fly-through images of CT studies performed in 8-week-old chimeric mouse. Mouse 6.
Movie S9 (.avi format). Fly-through images of CT studies performed in 8-week-old chimeric mouse. Mouse 7.
Movie S10 (.avi format). Fly-through images of CT studies performed in 8-week-old chimeric mouse. Mouse 8.
Movie S11 (.avi format). Fly-through images of CT studies performed in 8-week-old chimeric mouse. Mouse 9.
Movie S12 (.avi format). Time-lapse images of a synkaryon 4n cell carrying mRFP-tagged histone H2B (H2B-RFP) undergoing bipolar division.
Movie S13 (.avi format). Time-lapse images of a synkaryon 4n cell carrying H2B-RFP undergoing tripolar division.
Movie S14 (.avi format). Time-lapse images of a synkaryon 4n cell carrying H2B-RFP undergoing tripolar division without mitotic catastrophe.
Movie S15 (.avi format). Time-lapse images of a sorted hybrid cell generated after fusion between ESC-H2B-eGFP and NPC-H2B-mRFP that does not undergo mitosis.
Movie S16 (.avi format). Time-lapse images of a sorted hybrid cell generated after fusion between ESC-H2B-eGFP and NPC-H2B-mRFP that undergoes bipolar mitosis.
Movie S17 (.avi format). Time-lapse images of a sorted hybrid cell generated after fusion between ESC-H2B-eGFP and NPC-H2B-mRFP that undergoes tripolar mitosis with random segregation.
Movie S18 (.avi format). Time-lapse images of a sorted hybrid cell generated after fusion between ESC-H2B-eGFP and NPC-H2B-mRFP that undergoes tripolar mitosis with non-random segregation.
Movie S19 (.avi format). Time-lapse images of a sorted hybrid cell generated after fusion between ESC-H2B-eGFP and NPC-H2B-mRFP with parental chromosomes showing different spatial occupancy after bipolar mitosis.
Movie S20 (.avi format). Time-lapse images of a sorted hybrid cell generated after fusion between ESC-H2B-eGFP and NPC-H2B-mRFP with parental chromosomes showing different spatial occupancy after tripolar mitosis.
Movie S21 (.avi format). Time-lapse images of a long-time tracking a sorted hybrid cell generated after fusion between ESC-H2B-eGFP and NPC-H2B-mRFP.
Table S1 (Microsoft Excel format). SNP genotyping raw data.

Figure S 1



Injected embryos	5.5dpc embryos with dsRED-positive cells (%)	Average dsRED-positive cells/embryo	dsRED-positive cells		
			% dsRED+/ Nanog+ cells	% dsRED+/ Sox17+ cells	% dsRED+ only cells
326	79 (24.2)	4.7±2.6	98.5	0	1.5

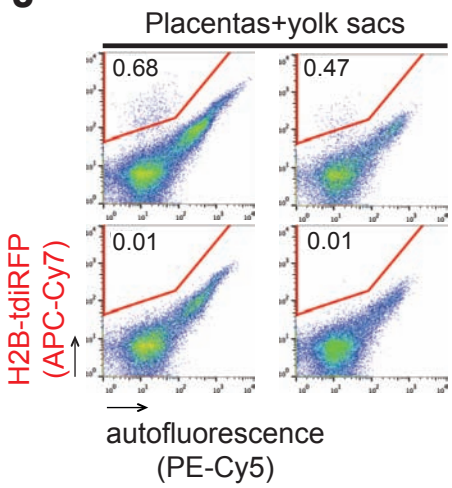
H



I

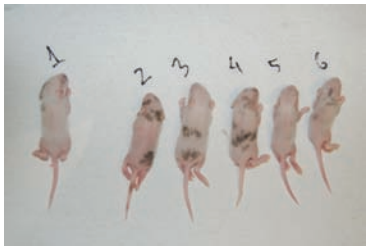
Embryo ID (12.5dpc)	% of tdiRFP-positive cells	dsRED-negative		dsRED-positive	
		% cells 2n	% cells >2n-G2	% cells 2n	% cells >2n-G2
#1	0.11	89.5	0.5	6.67	7.92
#18	0.10	89.1	0.7	13.2	12.1
#A1	1.51	77.5	2.1	78.0	1.2
#A2	0.16	89.8	0.6	88.9	0
#A4	0.10	90.2	0.7	93.8	0
#B7	1.31	78.4	2.1	74.4	4.1
#B8	1.26	83.0	1.6	92.5	0
#B11	0.06	92.1	0.2	n.d.	n.d.
#B12	0.31	n.d.	n.d.	n.d.	n.d.
#B13	0.05	n.d.	n.d.	n.d.	n.d.
#B14	0.30	91.0	0.5	81.5	0

J



K

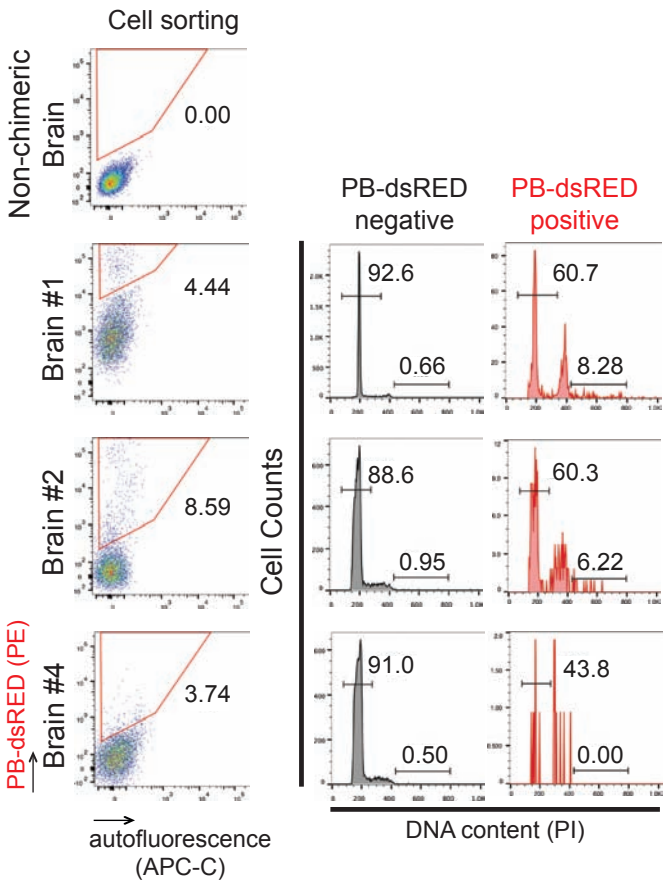
4n-injected chimeras
(8 days old)

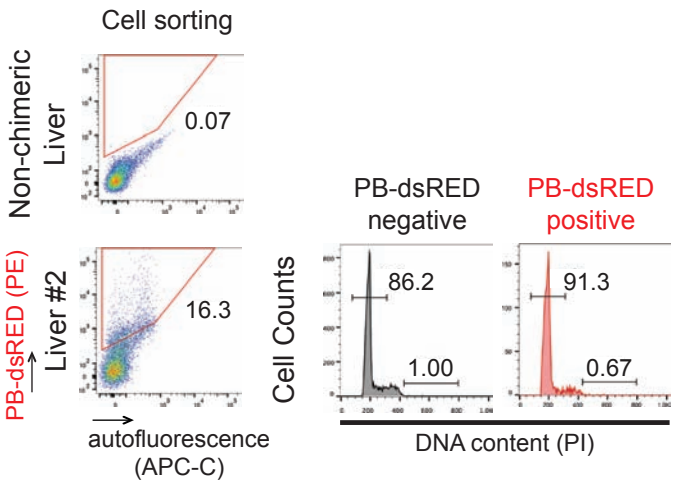


L

Animal #	Brain	Heart	Kidneys	Liver	Lungs	Spleen
1	4.44	0.00	0.16	0.89	0.68	0.10
2	8.59	15.4	1.21	16.3	1.69	0.04
3	4.01	1.73	0.11	2.19	1.01	0.03
4	3.74	0.68	0.15	0.63	0.16	0.02
5	1.96	0.00	0.00	0.41	0.45	0.05
6	3.76	1.30	1.53	1.18	0.99	0.37

M

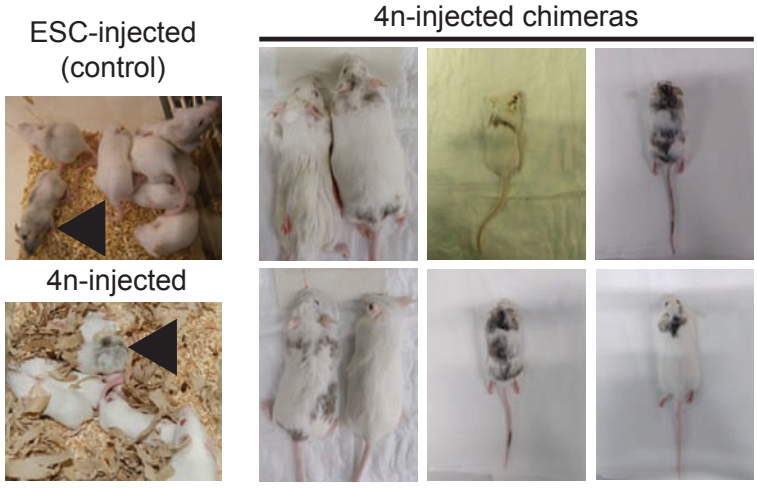




N

Organs	dsRed-negative		dsRed-positive	
	% cells 2n	% cells >2n-G2	% cells 2n	% cells >2n-G2
Brain 1	92.6	0.66	60.7	8.28
Brain 2	88.6	0.95	60.3	6.22
Brain 4	91.0	0.50	43.8	0.00
Liver 2	86.2	1.00	91.3	0.67

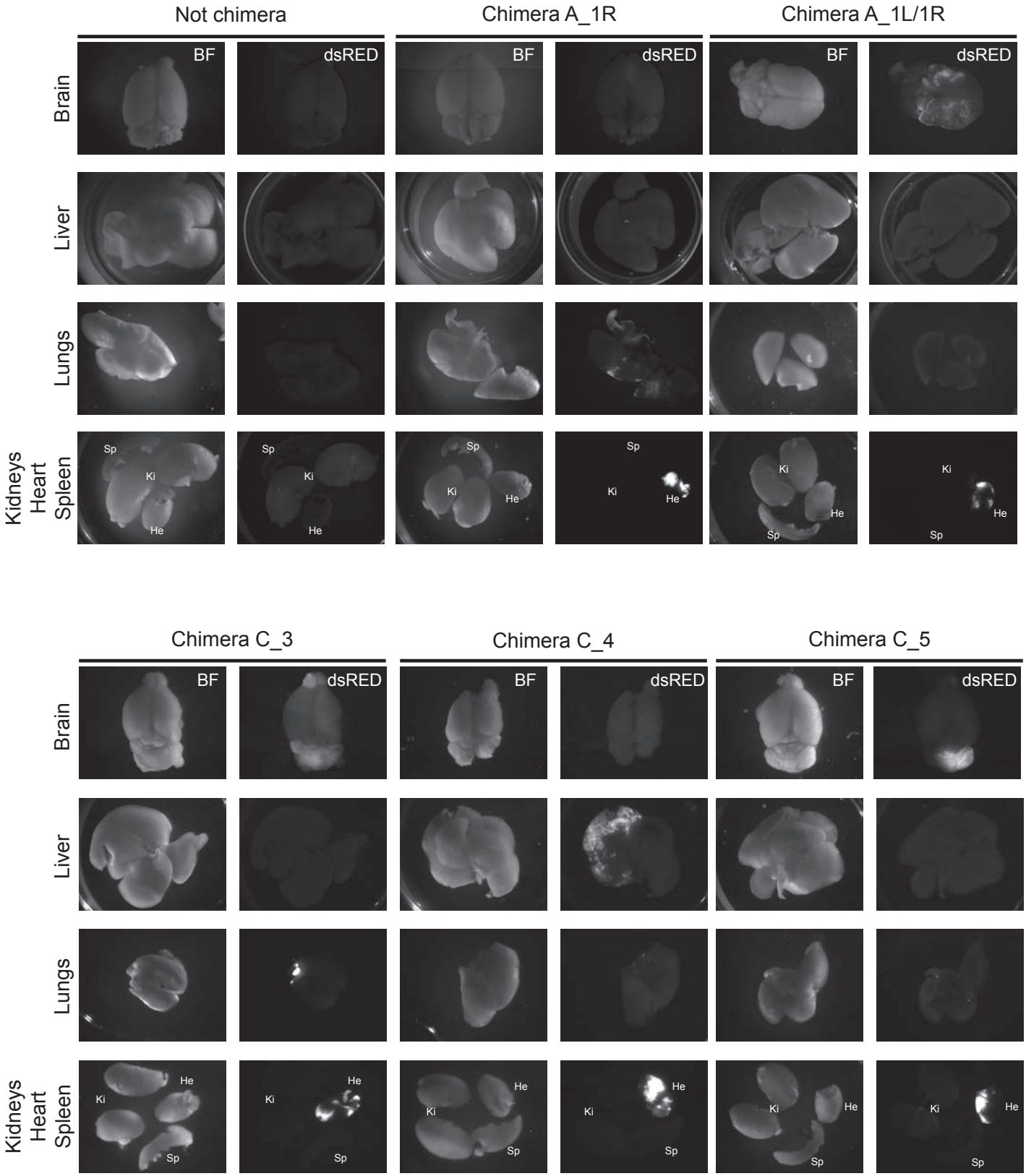
O



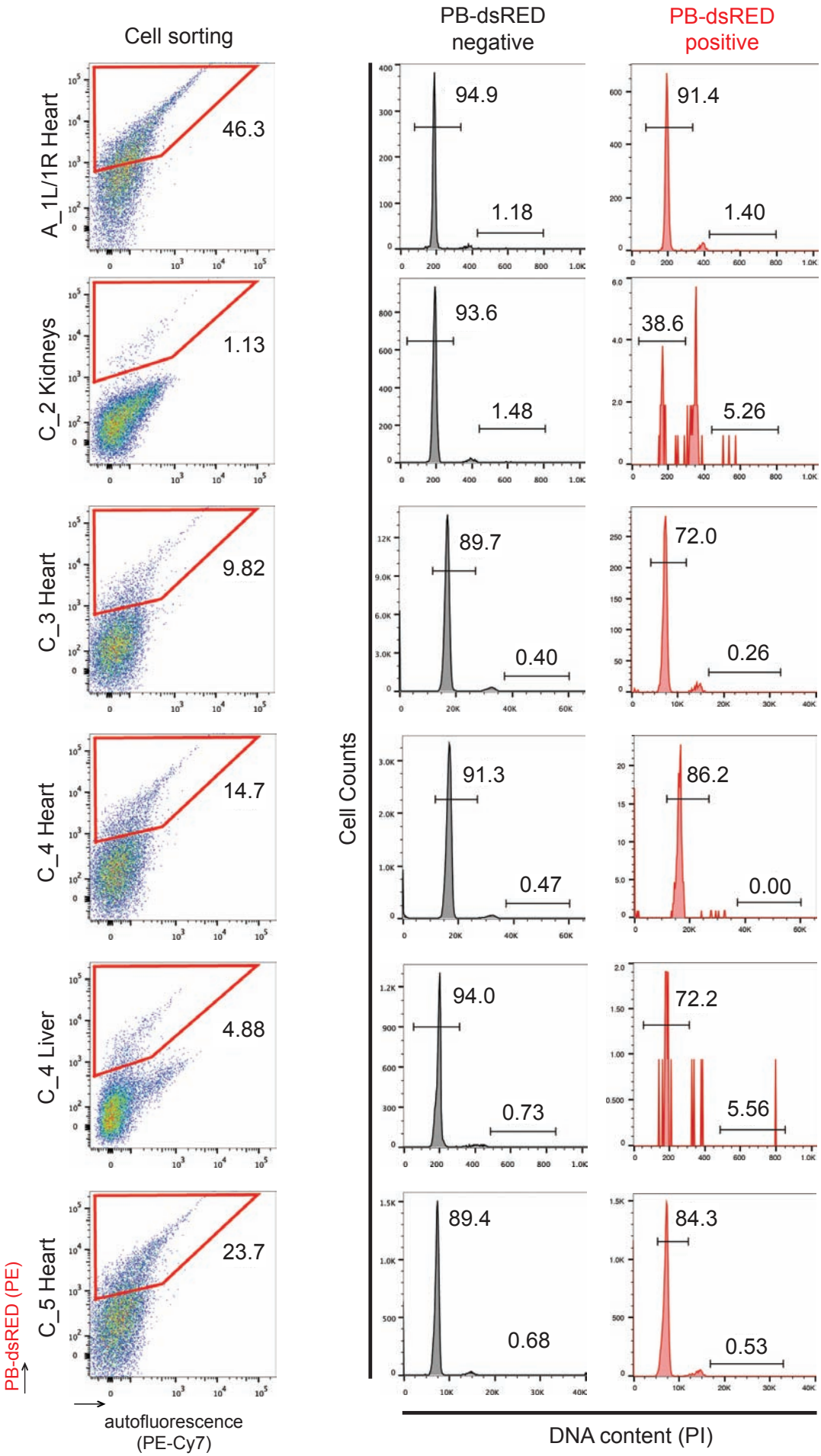
P

Animal #	Brain	Heart	Kidneys	Liver	Lungs	Spleen
A_1L	0.63	0.00	0.45	0.00	0.10	n.a.
A_1R	0.50	0.53	0.41	0.02	0.00	n.a.
A_1L/1R	0.24	46.3	0.02	0.00	0.00	0.00
C_1L/1R	0.02	0.00	0.03	0.00	0.41	n.a.
C_2	1.96	13.7	1.13	0.02	0.15	0.47
C_3	9.37	9.82	0.31	0.06	0.00	0.02
C_4	0.48	14.7	0.11	4.88	0.05	0.00
C_5	23.3	23.7	1.41	0.31	0.01	0.01

Q


Not chimera
Chimera A_1R
Chimera A_1L/1R
Chimera C_3
Chimera C_4
Chimera C_5
BF
dsRED
Brain
Liver
Lungs
Kidneys
Heart
Spleen
Sp
Ki
He

R

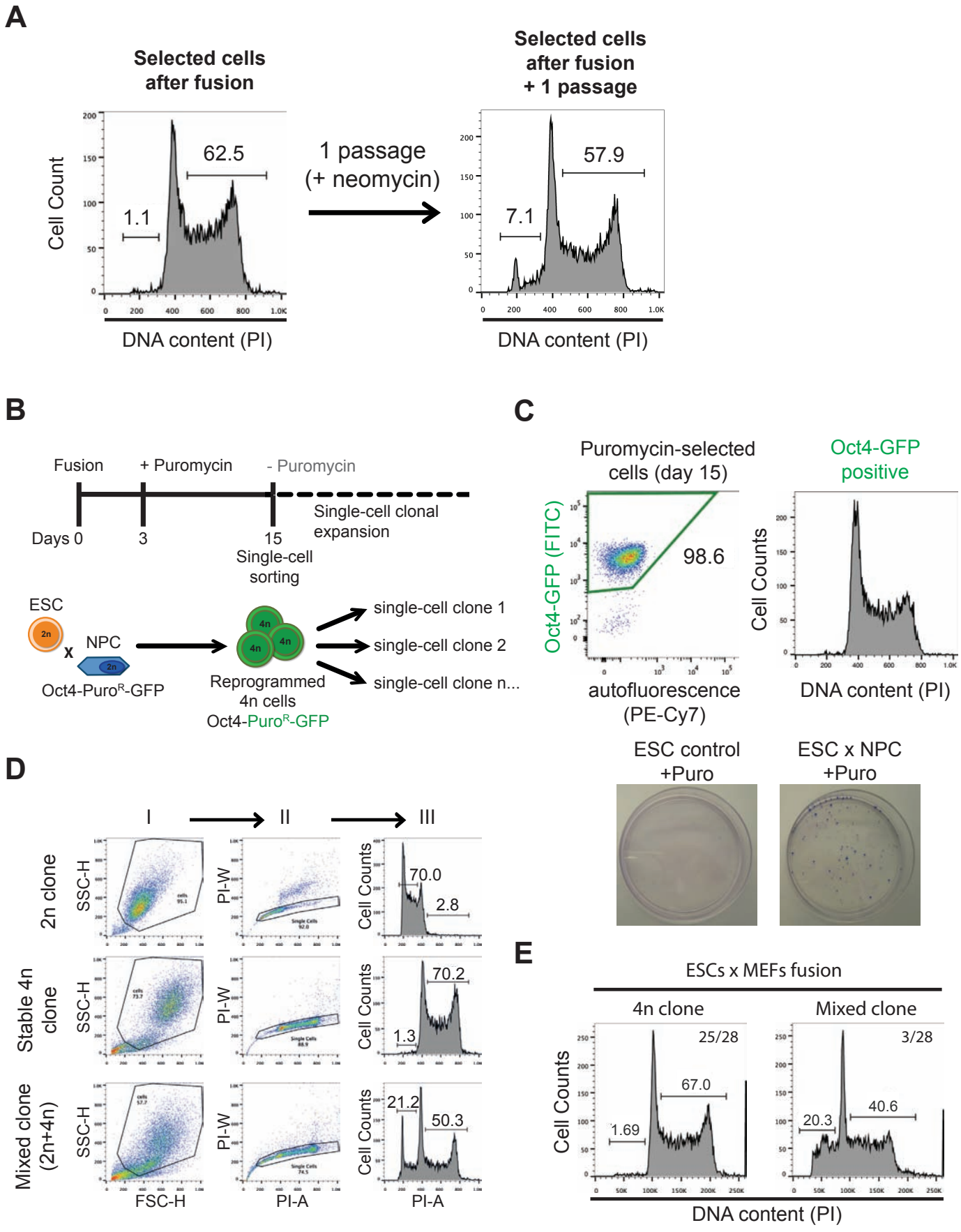

Cell sorting
PB-dsRED negative
PB-dsRED positive
A_1L/1R Heart
46.3
94.9
1.18
91.4
1.40
C_2 Kidneys
1.13
93.6
1.48
38.6
5.26
C_3 Heart
9.82
89.7
0.40
72.0
0.26
C_4 Heart
14.7
91.3
0.47
86.2
0.00
C_4 Liver
4.88
94.0
0.73
72.2
5.56
C_5 Heart
23.7
89.4
0.68
84.3
0.53
Cell Counts
PB-dsRED (PE)
autofluorescence (PE-Cy7)
DNA content (PI)

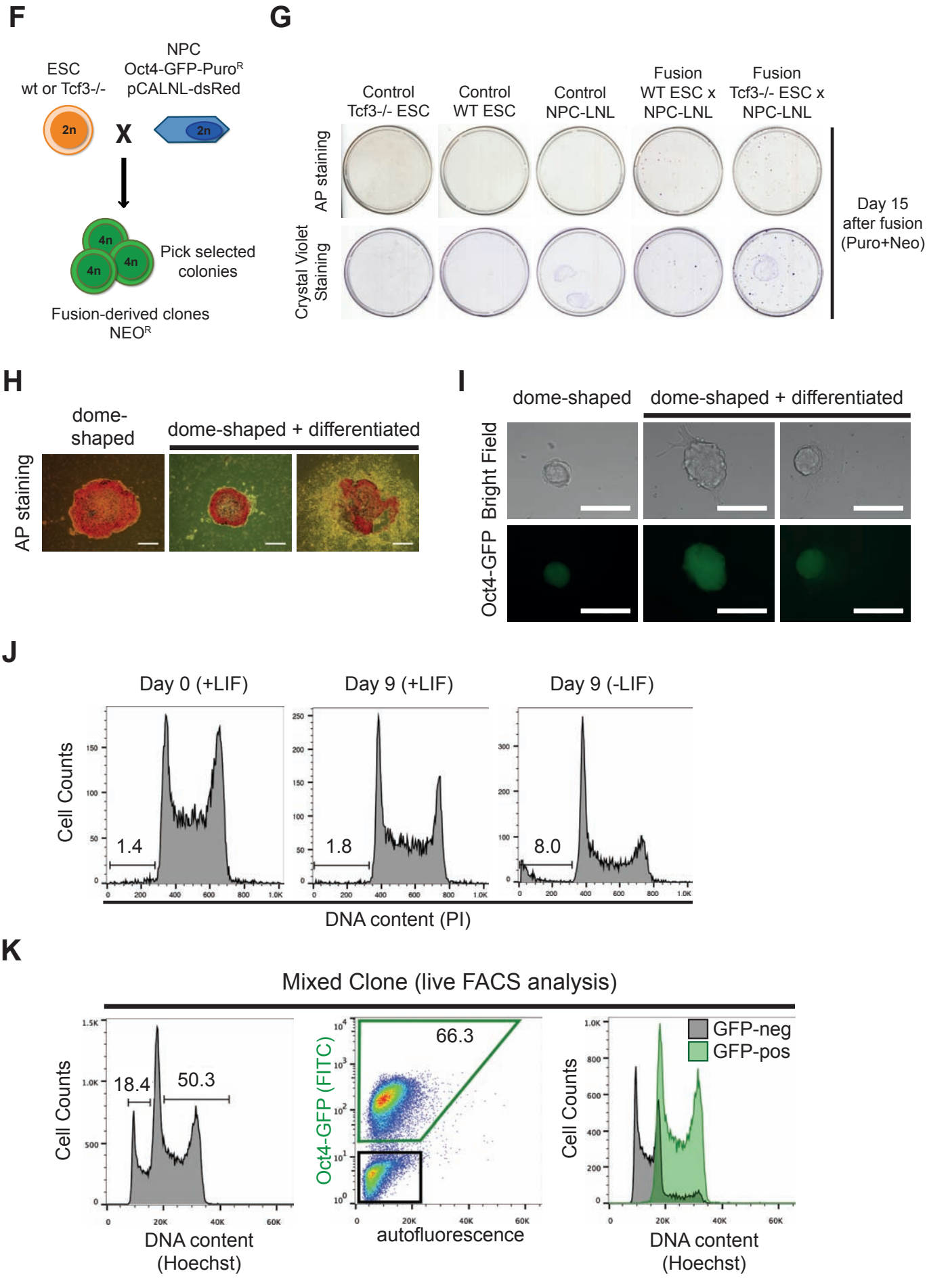
S

Organs	dsRed-negative		dsRed-positive	
	% cells 2n	% cells >2n-G2	% cells 2n	% cells >2n-G2
A_1L/1R Heart	94.9	1.18	91.4	1.40
C_1L/1R Lungs	91.7	2.27	90.8	0.96
C_2 Heart	96.1	0.45	92.4	0.86
C_2 Kidneys	93.6	1.48	38.6	5.26
C_3 Heart	89.7	0.40	72.0	0.26
C_4 Heart	91.3	0.47	86.2	0.00
C_4 Liver	94.0	0.73	72.2	5.56
C_5 Heart	89.4	0.68	84.3	0.53

Fig. S1. Tetraploid Oct4-GFP–positive cells reduce ploidy during embryo development. (**A**) FACS plots showing the expression of the Oct4-GFP transgene in control ESCs (left panel) and in puromycin-selected cells (right panel). (**B**) Microscope images (bright field and GFP) of puromycin-selected cells (scale bar=200 μm). (**C**) Representative FACS plots showing the expression of H2B-tdiRFP (left) and PB-dsRED (right) in transfected puromycin-selected 4n cells. (**D**) Representative microscope images of FISH/chromosome painting for the chromosome X (Sx9 region) and for the Y chromosome in male ESCs (XY), female NPCs (XX) and fusion-derived synkaryon 4n cells used for embryo injections (XXXY). Yellow arrows indicate position of Sx9 locus (scale bar ESC=5 μm; scale bars NPC and 4n cells=10um). (**E**) Representative cell cycle analysis of tetraploid cells used for embryo injections. (**F**) 2-cell stage (1.5dpc) embryos injected with dsRED-positive single 4n cells (scale bar=100 μm). (**G**) Quantification of the contribution to 5.5dpc embryos of single 4n dsRed-positive cells injected into 1.5dpc embryos. A representative immunofluorescence staining of a 5.5dpc *in vitro* embryo where a single 4n PB-dsRED cell was injected at 1.5dpc is shown below. Co-staining with Nanog (epiblast) and Sox17 (primitive endoderm) markers is shown (scale bar=20 μm). (**H**) FACS plots showing the expression of H2B-tdiRFP in 12.5dpc injected embryos (left columns; sorting gates are shown in black and red for negative and positive cells, respectively). The cell cycle profiles

of sorted H2B-tdiRFP-negative and -positive cells are shown for each embryo. (**I**) Summary table of 12.5dpc injected embryos where H2B-tdiRFP-positive cells were identified. Samples in which the number of sorted cells was insufficient for the cell cycle analysis are indicated with n.d. (no data). (**J**) Representative FACS plots showing the expression of H2B-tdiRFP in chimeric placenta and yolk sac. (**K**) Picture of representative 8-day-old chimeras generated by 4n cell injection in 2n blastocysts. (**L**) Percentage of PB-dsRED-positive cells identified in the six 8-day-old chimeras shown in (K). Samples from which we could sort enough cells for cell cycle analysis are highlighted in orange. (**M**) FACS plots showing the expression of PB-dsRED in organs of 8-day-old chimeric mice (left: sorting gates of positive cells are shown). The cell cycle profiles of sorted PB-dsRED-negative and -positive cells are shown for each sample. (**N**) Percentage of cells with a diploid (2n) and more than diploid (>2n-G2) DNA content found in dsRED-negative and -positive cells of 8-day-old chimeras. (**O**) Pictures of representative 8-week-old chimeras generated by 4n cell injection in 2n blastocysts. (**P**) Percentage of PB-dsRED-positive cells identified in 8-week-old chimeras. Samples from which we could sort enough cells for cell cycle analysis are highlighted in orange. (**Q**) Bright field (BF) and dsRED pictures of organs isolated from 8-week-old representative chimeras (Sp-spleen; Ki-kidneys; He-heart). (**R**) FACS plots showing the expression of PB-dsRED in organs of 8-week-old chimeric mice (left: sorting gates of positive cells are shown). The cell cycle profiles of sorted PB-dsRED-negative and -positive cells are shown for each sample. (**S**) Percentage of cells with a diploid (2n) and more than diploid (>2n-G2) DNA content found in dsRED-negative and -positive cells of organs of 8-week-old chimeras. (Photo credit: João Frade, Center for Genomic Regulation)


A
Selected cells after fusion
Selected cells after fusion + 1 passage
Cell Count
62.5
1.1
57.9
7.1
1 passage (+ neomycin)
DNA content (PI)
DNA content (PI)
B
Fusion
+ Puromycin
- Puromycin
Days 0
3
15
Single-cell sorting
Single-cell clonal expansion
ESC
NPC
Oct4-PuroR-GFP
Reprogrammed 4n cells
Oct4-PuroR-GFP
single-cell clone 1
single-cell clone 2
single-cell clone n...
C
Puromycin-selected cells (day 15)
Oct4-GFP positive
Oct4-GFP (FITC)
98.6
autofluorescence (PE-Cy7)
Cell Counts
DNA content (PI)
ESC control +Puro
ESC x NPC +Puro
D
I
II
III
2n clone
Stable 4n clone
Mixed clone (2n+4n)
SSC-H
PI-W
Cell Counts
70.0
2.8
70.2
1.3
21.2
50.3
FSC-H
PI-A
PI-A
E
ESCs x MEFs fusion
4n clone
Mixed clone
25/28
3/28
67.0
1.69
20.3
40.6
Cell Counts
DNA content (PI)


F
ESC
wt or Tcf3-/-
NPC
Oct4-GFP-Puro^R
pCALNL-dsRed
2n
X
2n
4n
4n
4n
Pick selected colonies
Fusion-derived clones
NEO^R
G
Control
Tcf3-/- ESC
Control
WT ESC
Control
NPC-LNL
Fusion
WT ESC x
NPC-LNL
Fusion
Tcf3-/- ESC x
NPC-LNL
AP staining
Crystal Violet
Staining
Day 15
after fusion
(Puro+Neo)
H
dome-
shaped
dome-shaped + differentiated
AP staining
I
dome-shaped
dome-shaped + differentiated
Bright Field
Oct4-GFP
J
Day 0 (+LIF)
Day 9 (+LIF)
Day 9 (-LIF)
Cell Counts
1.4
1.8
8.0
DNA content (PI)
K
Mixed Clone (live FACS analysis)
Cell Counts
18.4
50.3
DNA content
(Hoechst)
Oct4-GFP (FITC)
66.3
autofluorescence
Cell Counts
GFP-neg
GFP-pos
DNA content
(Hoechst)

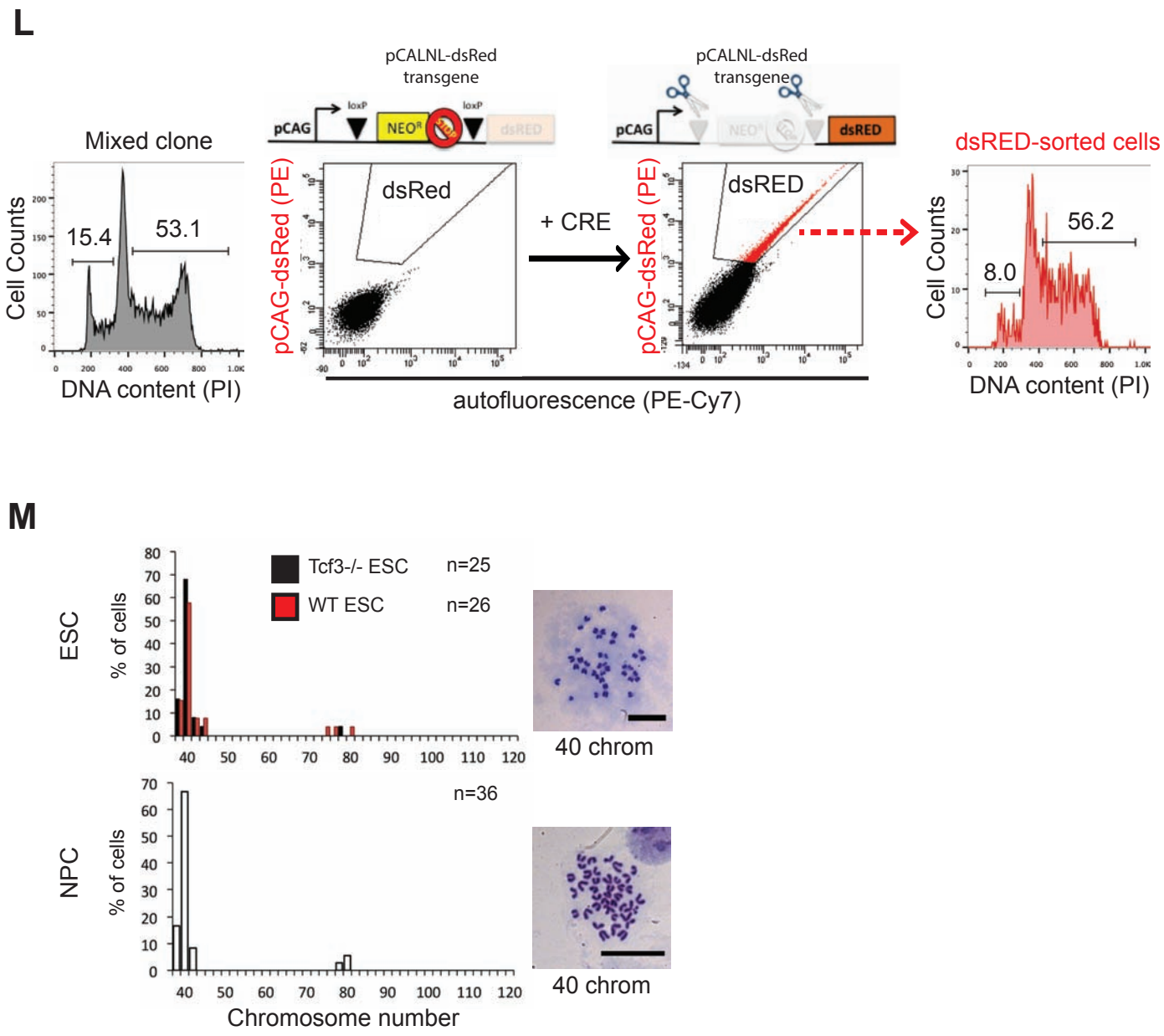
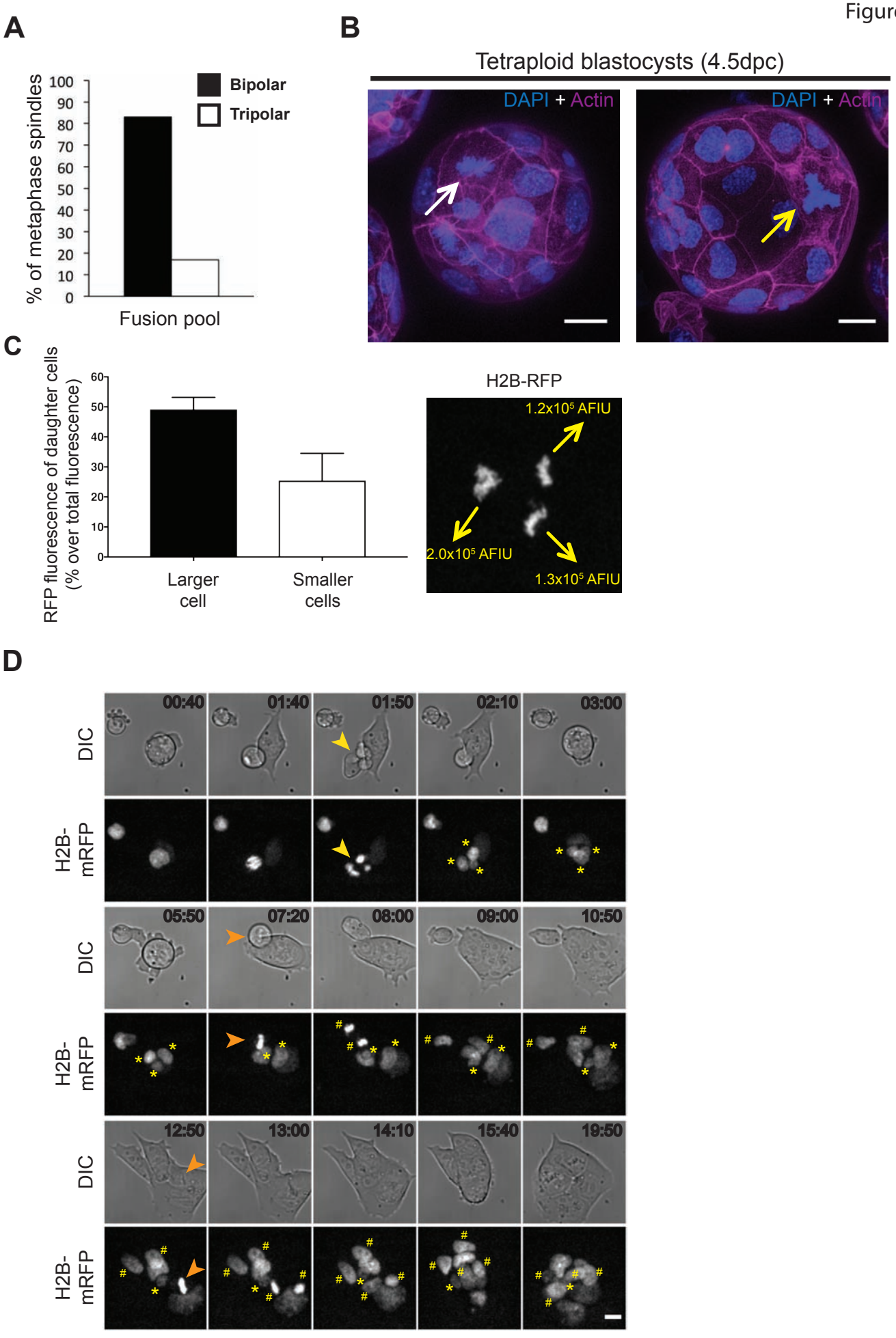


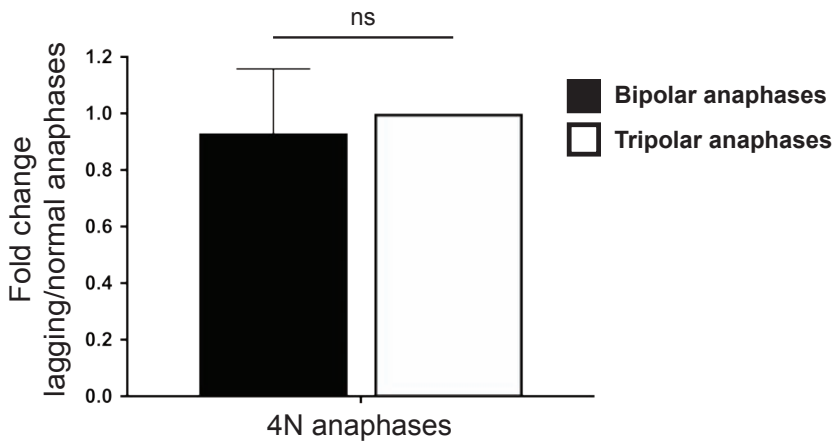
Fig. S2. Fusion-derived cells proliferate in self-renewal conditions and originate Oct4-GFP–positive 4n and Oct4-GFP–negative 2n cells. (**A**) Cell cycle profile of 4n cells (from ESC wt x NPC fusion - used for blastocyst injections) kept in self-renewal culturing conditions for one passage without selection. A clear 2n-G1 peak is visible on the right profile. (**B**) Experimental plan for selection and single-cell sorting of reprogrammed hybrids (Oct4-Puro^{R}-GFP positive) after fusion. (**C**) Oct4-GFP FACS plot of puromycin-selected cells at day 15 after fusion (left panel) and cell cycle profile of Oct4-GFP-positive cells at the time of single-cell sorting (right panel). Representative crystal violet

staining of control and fusion plates selected with puromycin, at day 15 after fusion, are shown below. (**D**) Sequential FACS gating strategy used for cell cycle analysis of 2n, stable 4n and mixed clones. I – cells were gated according to size (FSC-H) and granularity (SSC-H). II – cell aggregates were excluded from the analysis. The gate delimitates the single cells. III – cell cycle profiles showing the relative DNA content according to the PI staining. SSC-side scatter; FSC-forward scatter; PI-propidium iodide. (**E**) Representative cell cycle profiles of a 4n and a mixed clone originated after fusion of ESCs and MEFs and selection of fusion-derived clones. Numbers indicate frequencies (total number = 28 clones). (**F**) Fusion scheme between ESCs and NPCs carrying Oct4-Puro^{R}-GFP and pCALNL-dsRED transgenes. See Materials and Methods section for details on selection strategy. (**G**) Representative AP (alkaline phosphatase) and crystal violet staining of control and fusion plates at day 15 after fusion (see Figure 2B for experimental scheme of selection with puromycin and neomycin). (**H**) AP staining of drug-selected colonies from Tcf3-/- ESCs x NPCs-LNL (pCALNL-dsRED) fusions. (**I**) Bright field (upper panel) and Oct4-GFP (lower panel) images of drug-selected colonies from Tcf3-/- ESC x NPC-LNL fusions. (J) Cell cycle profile of a stable 4n clone cultured in self-renewal conditions (Day 9 + LIF) and in differentiation conditions, upon Embryoid Body (EB) assay (Day 9 - LIF). Note that there is no 2n-G1 peak, while a percentage of apoptotic cells normally generated during EB differentiation can be observed. This clone is representative of 8 stable 4n clones. (**K**) Cell cycle profile and Oct4-GFP expression in live cells of a mixed clone. Merged cell cycle profiles of GFP-negative (grey) and GFP-positive (green) cells is shown on the right. (**L**) Cell cycle profile of a mixed clone (left) and FACS plots showing the expression of dsRED protein before and after induction of CRE recombinase. CRE cuts the NEO^{R}-STOP cassette and allows the expression of dsRED. Cell cycle profile of sorted dsRED-positive cells is shown on the right. (**M**) Quantification of karyotypes from ESCs (upper panel) and NPCs (lower panel). Most cells have 40 +/- 2 chromosomes, the variation might be due to the karyotype preparation. N = number of counted metaphase spreads/cell

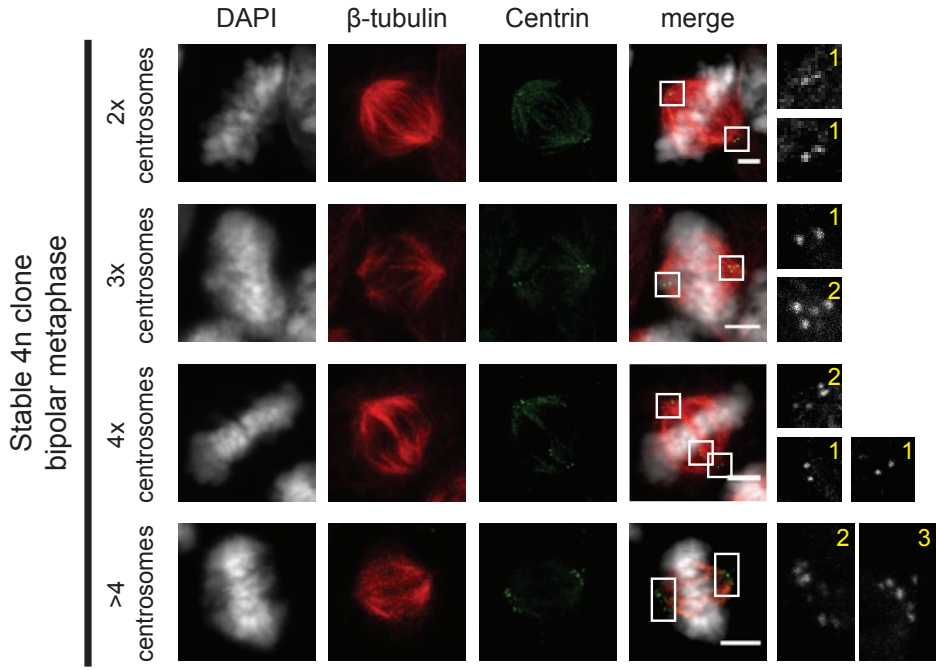
line. Representative metaphase spreads are shown on the right. Scale bars=20 μm. (Photo Credit: Frederic LLuis, KU Leuven)


A
% of metaphase spindles
100
90
80
70
60
50
40
30
20
10
0
Bipolar
Tripolar
Fusion pool
B
Tetraploid blastocysts (4.5dpc)
DAPI + Actin
DAPI + Actin
C
RFP fluorescence of daughter cells
(% over total fluorescence)
60
50
40
30
20
10
0
Larger cell
Smaller cells
H2B-RFP
1.2x10⁵ AFIU
2.0x10⁵ AFIU
1.3x10⁵ AFIU
D
DIC
H2B-mRFP
00:40
01:40
01:50
02:10
03:00
05:50
07:20
08:00
09:00
10:50
12:50
13:00
14:10
15:40
19:50

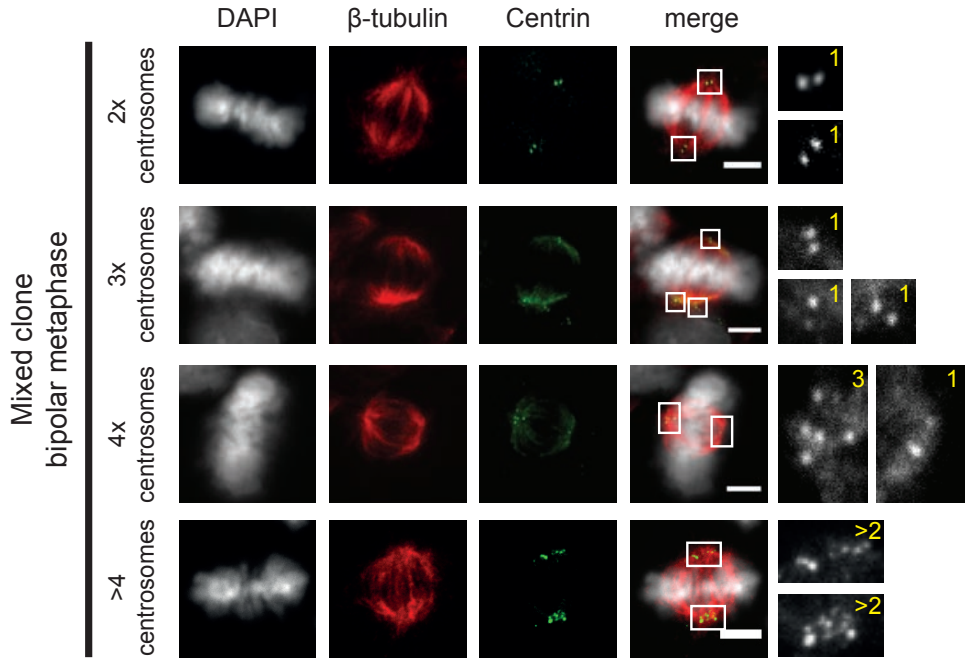
E


ns
Fold change
lagging/normal anaphases
1.2
1.0
0.8
0.6
0.4
0.2
0.0
4N anaphases
Bipolar anaphases
Tripolar anaphases

F


DAPI
β-tubulin
Centrin
merge
Stable 4n clone
bipolar metaphase
2x centrosomes
3x centrosomes
4x centrosomes
>4 centrosomes

G


DAPI
β-tubulin
Centrin
merge
Mixed clone
bipolar metaphase
2x centrosomes
3x centrosomes
4x centrosomes
>4 centrosomes

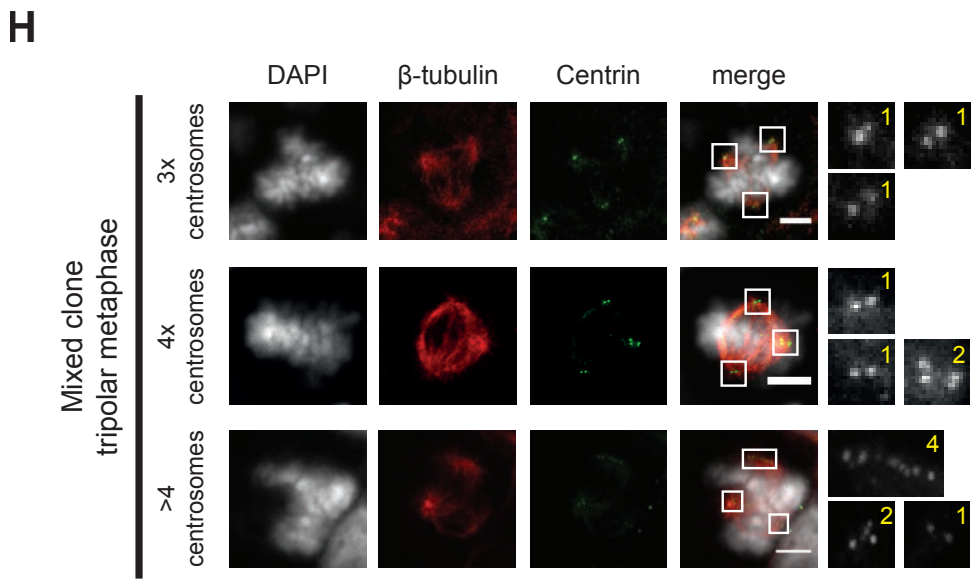
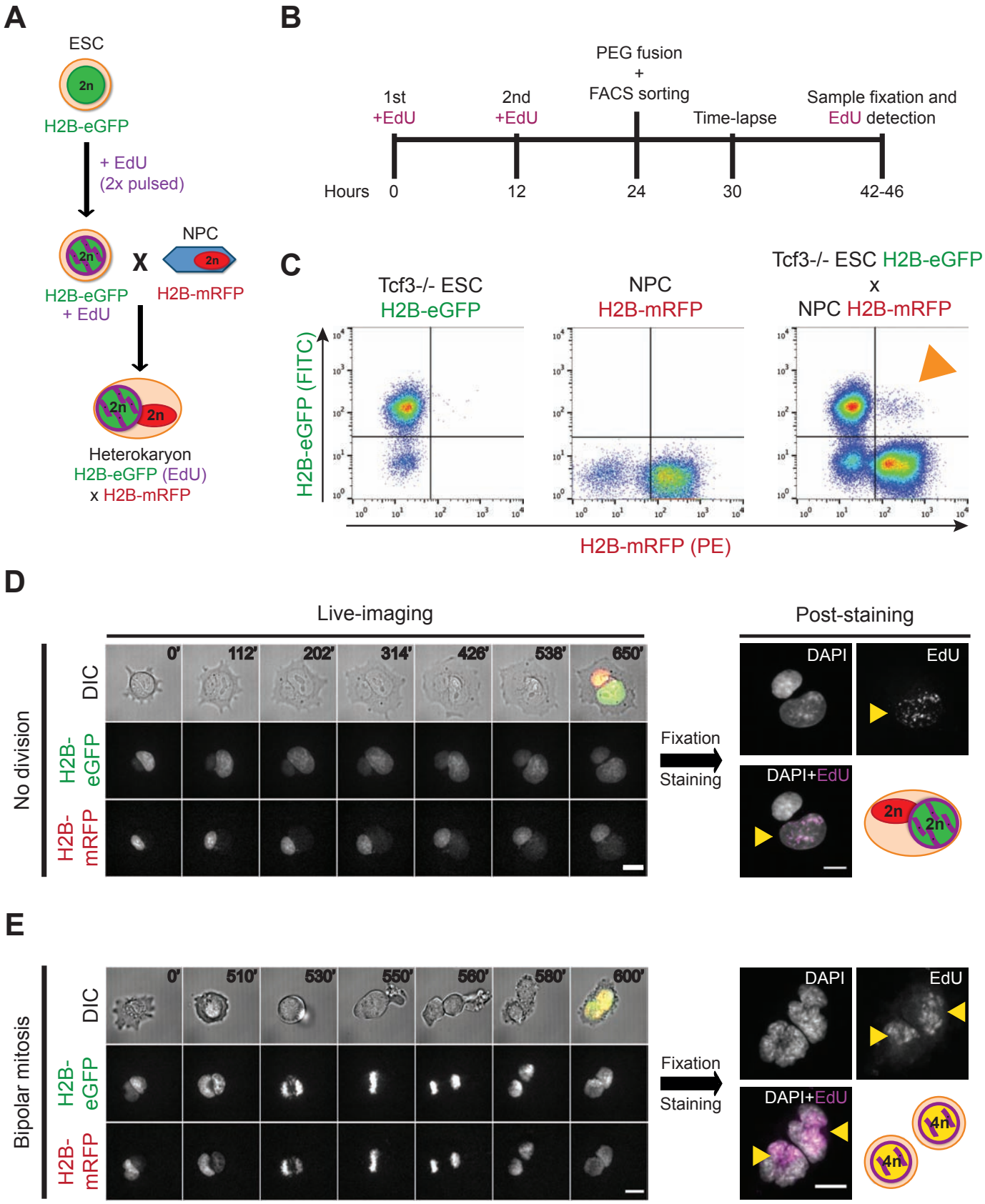
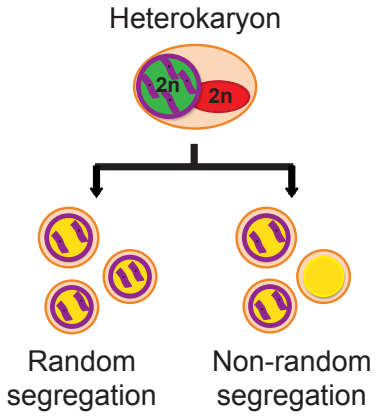


Fig. S3. Tetraploid cells undergo tripolar mitosis and form viable daughter cells. (**A**) Quantification of bipolar and tripolar metaphase spindles in a pool of cells at passage 3 after fusion (under neomycin selection). (**B**) Immunofluorescence staining of 4n 4.5dpc blastocysts generated from 2-cell stage fusion. White arrow - bipolar metaphase; yellow arrow - tripolar metaphase (observed in 1 out of 9 metaphases of 4n blastomeres). Scale bars=20 μm. (**C**) Quantification of H2B-RFP fluorescence of the daughter cells of a tripolar mitosis (N=4 tripolar telophases; mean +/- standard deviation). A representative image (tripolar mitosis of Figure 3C) used for quantification is show on the right. AFIU=artificial fluorescence intensity units. (**D**) Time-lapse images of a synkaryon 4n cell undergoing tripolar mitosis (yellow arrowhead). Of the three daughter cells (marked with an asterisk *), two cells divide again (orange arrowheads). The daughter cells of the second division are marked with a hash (#). Time progression is shown as hours:minutes (hh:mm). See Movie S14 for cell tracking. Scale bar=10 μm. (**E**) Fold change (f.c.) differences between anaphases containing lagging chromosomes and normal anaphases in bipolar and tripolar mitosis of 4n cells from mixed clones (mean +/- standard deviation; f.c. bipolar=0.93+/-0.23; f.c. tripolar=1+/-0.00; *p-value*=0.706136; total of 46 bipolar mitosis and 14 tripolar metaphases of 2 independent clones; ns = non-significant

difference). (**F-H**) Representative immunofluorescence images of metaphase spindles of tetraploid cells. Tetraploid cells in stable 4n clones can form bipolar spindles with different number of centrosomes (**F**). Tetraploid cells in mixed clones can form bipolar spindles with two or more centrosomes (**G**), or tripolar spindles always with more than 2 centrosomes (**H**). DNA (DAPI-grey), β-tubulin (red) and centrioles (centrin-green) staining are shown. Insets (on the right) show zooms on spindle poles. The number of centrosomes is indicated inside each inset. Scale bars=5 μm.

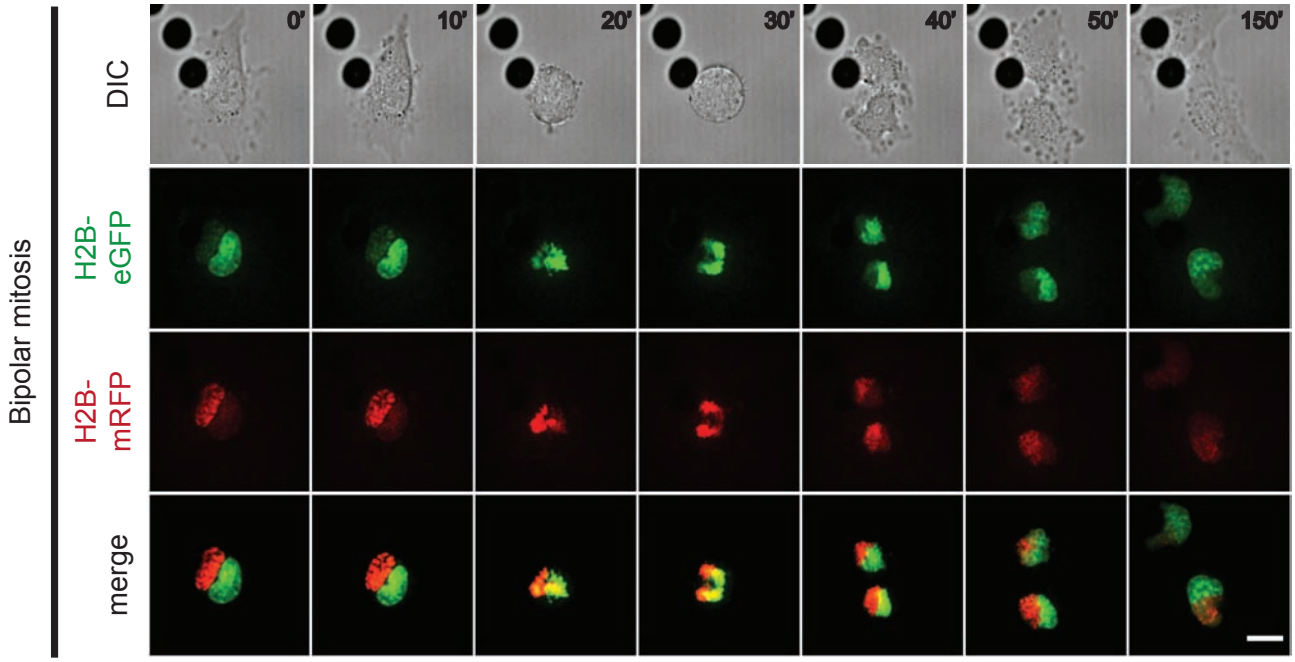

A
ESC
2n
H2B-eGFP
+ EdU
(2x pulsed)
2n
H2B-eGFP
+ EdU
X
NPC
2n
H2B-mRFP
2n
2n
Heterokaryon
H2B-eGFP (EdU)
x H2B-mRFP
B
PEG fusion
+
FACS sorting
1st
+EdU
2nd
+EdU
Time-lapse
Sample fixation and
EdU detection
Hours
0
12
24
30
42-46
C
Tcf3-/- ESC
H2B-eGFP
NPC
H2B-mRFP
Tcf3-/- ESC H2B-eGFP
x
NPC H2B-mRFP
H2B-eGFP (FITC)
H2B-mRFP (PE)
D
Live-imaging
Post-staining
No division
DIC
H2B-eGFP
H2B-mRFP
0'
112'
202'
314'
426'
538'
650'
Fixation
Staining
DAPI
EdU
DAPI+EdU
2n
2n
E
Bipolar mitosis
DIC
H2B-eGFP
H2B-mRFP
0'
510'
530'
550'
560'
580'
600'
Fixation
Staining
DAPI
EdU
DAPI+EdU
4n
4n

F

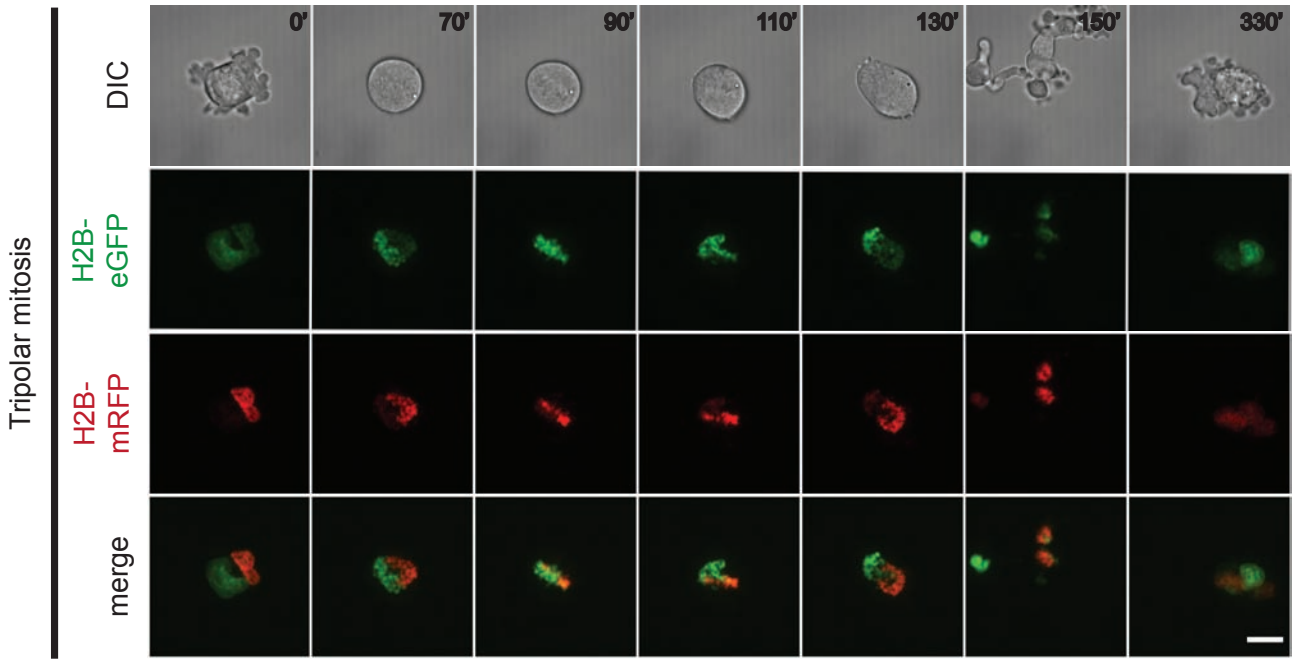


	Number of events
Random segregation	10
Non-random segregation	6
Total	16

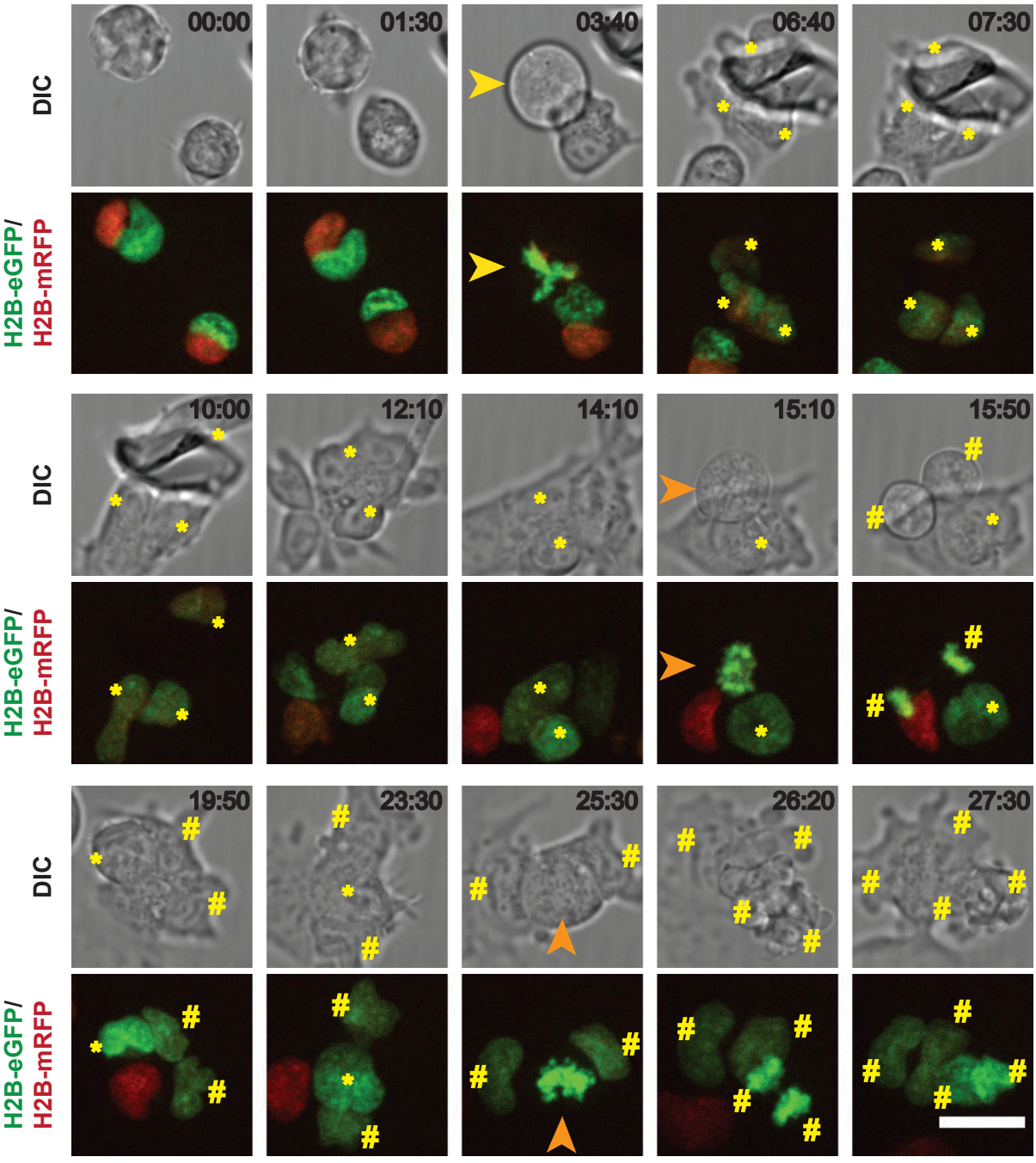
G



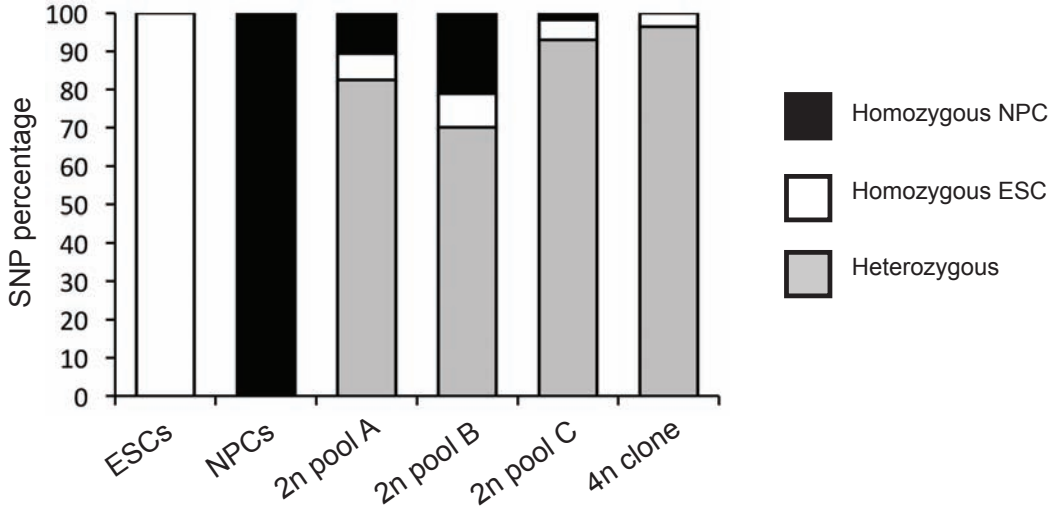
H



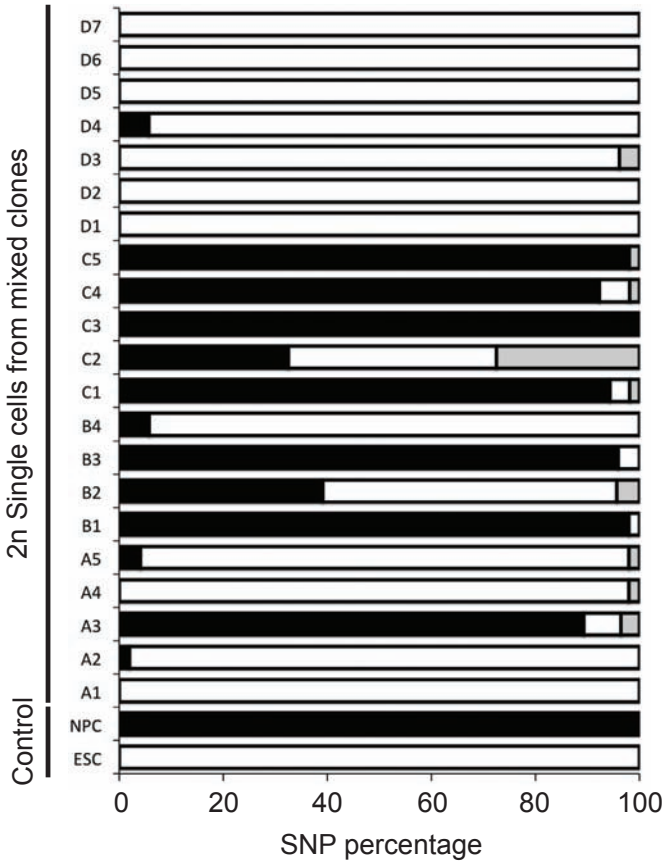
I


DIC
H2B-eGFP/
H2B-mRFP
00:00
01:30
03:40
06:40
07:30
10:00
12:10
14:10
15:10
15:50
19:50
23:30
25:30
26:20
27:30

J


SNP percentage
100
90
80
70
60
50
40
30
20
10
0
ESCs
NPCs
2n pool A
2n pool B
2n pool C
4n clone
Homozygous NPC
Homozygous ESC
Heterozygous

K


2n Single cells from mixed clones
Control
D7
D6
D5
D4
D3
D2
D1
C5
C4
C3
C2
C1
B4
B3
B2
B1
A5
A4
A3
A2
A1
NPC
ESC
0
20
40
60
80
100
SNP percentage

L

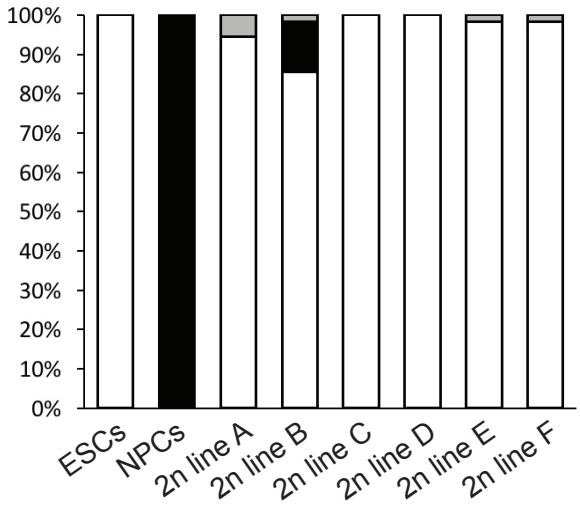

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
ESCs
NPCs
2n line A
2n line B
2n line C
2n line D
2n line E
2n line F

Fig. S4. Parental chromosome segregation during hybrids' division can be nonrandom. (**A**) Schematic representation of fusion between EdU-pulsed ESCs and NPCs, constitutively expressing H2B-eGFP (green nucleus) and H2B-mRFP (red nucleus) fusion proteins, respectively. (**B**) Experimental plan for ESC chromosome tagging with EdU during the hybrids' first division. (**C**) Representative FACS plots showing Tcf3-/- ESCs H2B-eGFP, NPCs H2B-mRFP and fusion between these two lines. Hybrid cells express both fluorescent proteins (orange arrowhead). (**D**, **E**) Representative time-lapse images (left panels) and post-staining images (right panels) of hybrid cells that did not divide (**D**) and divided in two daughter cells (**E**). Merged images correspond to the last frame of the respective time-lapse (time progression in minutes is shown). Yellow arrowheads indicate EdU-positive cells (pseudocolored magenta) after staining. Scale bars=10 μm. (**F**) Schematic representation of the two observed outcomes after tripolar mitosis of the ESC/NPC hybrids (left); summary of quantified random and non-random segregation events in tripolar mitosis (right). ESC nucleus-green; NPC nucleus-red; daughter cells' nuclei-yellow; EdU-labelled chromosomes-magenta. (**G**, **H**) H2B-eGFP- and H2B-mRFP-tagged chromosomes are spatially separated during and after hybrid's bipolar (**G**) and tripolar (**H**) divisions (scale bars=10 μm). (**I**) Time-lapse images of hybrid cells after fusion between ESC-H2B-eGFP and NPC-H2B-mRFP (DIC – upper panels; merged H2B-eGFP and H2B-mRFP – lower panels). One hybrid undergoes tripolar mitosis (yellow arrowhead). Of the three daughter cells (marked with an asterisk *), two cells are followed until they divide again (orange arrowheads), while the third one moves out of the frame. The daughter cells of the second division are marked with a hash (#). Time progression is shown as hours:minutes (hh:mm). See Movie S21 for cell tracking. Scale bar=10 μm. (**J**) Percentage of homozygous NPC (black), homozygous ESCs (white) and heterozygous SNPs (grey bars) present in control ESCs, NPCs and pools of G1-2n cells sorted from three different mixed clones (2n pools A, B and C). The SNP percentage of a pool of 4n cells from a stable 4n clone is also shown. (**K**) SNP percentage of 21 single G1-2n cells sorted from four different mixed clones (A-D, Y axis). SNP percentage of NPC and ESC single cells are shown as

control. **(L**) Percentage of homozygous NPC (black), homozygous ESCs (white) and heterozygous SNPs (grey bars) in six 2n clonal lines generated after expansion of 4n single cells (from Figure 2A).

Figure S 5

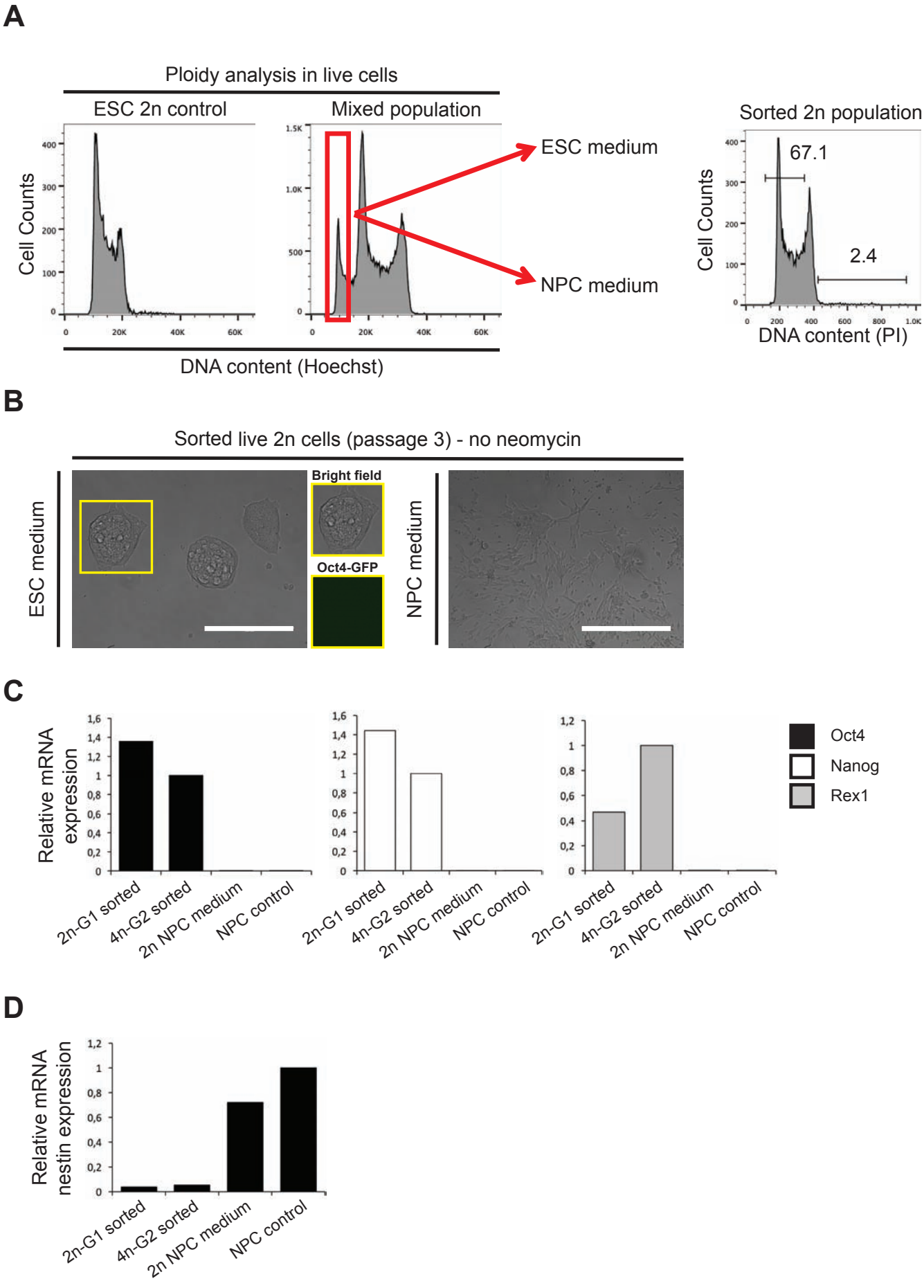

A
Ploidy analysis in live cells
ESC 2n control
Mixed population
Cell Counts
DNA content (Hoechst)
ESC medium
NPC medium
Sorted 2n population
67.1
2.4
DNA content (PI)
B
Sorted live 2n cells (passage 3) - no neomycin
ESC medium
Bright field
Oct4-GFP
NPC medium
C
Relative mRNA expression
2n-G1 sorted
4n-G2 sorted
2n NPC medium
NPC control
Oct4
Nanog
Rex1
D
Relative mRNA nestin expression

Fig. S5. 4n-derived 2n cells show NPC and ESC phenotype if cultured in the respective culture medium. (**A**) Ploidy analysis of live ESCs (ESC 2n control) and a fusion-derived mixed population (4n+2n) stained with Hoechst33342. 2n-G1 cells (red box) were sorted and maintained in ESC or NPC medium. Cell cycle profile of 2n-G1 sorted cells in ESC medium is shown on the right. (**B**) Morphology of 2n sorted cells in ESC and NPC medium at passage 3 after sorting. Note that the 2n cells sorted in ESC medium do not express the Oct4-GFP transgene, suggesting the absence of the NPC genome (scale bars=400 μm). (**C**, **D**) mRNA expression levels of pluripotency (**C**) and neural marker Nestin (**D**) in 2n (2n-G1 sorted) and 4n (4n-G2 sorted) cells isolated immediately after sorting and in sorted 2n cells cultured in NPC medium for 3 passages after sorting (2n NPC medium).

Movie S1. Time-lapse imaging of an embryo injected with one single PB-dsRED 4n cell. Example 1. Note that the dsRED cell proliferates and multiple cells contribute to the inner cell mass of the embryo. Time progression is shown as hours:minutes (hh:m).

Movie S2. Time-lapse imaging of an embryo injected with one single PB-dsRED 4n cell. Example 2. Note that the dsRED cell proliferates and multiple cells contribute to the inner cell mass of the embryo. Time progression is shown as hours:minutes (hh:m).

Movies S3–S11. Fly-through images of CT studies performed in nine 8-week-old chimeric mice. A total of 2048 sections are shown for the whole body of each mouse. Images were analysed using ImageJ and Imaris.

Movie S12. Time-lapse images of a synkaryon 4n cell carrying mRFP-tagged histone H2B (H2B-RFP) undergoing bipolar division. DIC and RFP images were taken every 7 minutes. Time progression is shown as hours:minutes (hh:mm).

Movie S13. Time-lapse images of a synkaryon 4n cell carrying H2B-RFP undergoing tripolar division. DIC and RFP images were taken every 10 minutes. Time progression is shown as hours:minutes (hh:mm).

Movie S14. Time-lapse images of a synkaryon 4n cell carrying H2B-RFP undergoing tripolar division without mitotic catastrophe. Tracking marks (red circles) follow a cell that undergoes tripolar division (first division) and three daughter cells (pink, light blue and yellow circles). Two daughter cells (pink and light blue circles) undergo bipolar divisions (second division). Tracking is shown as an overlay on the RFP channel. DIC and RFP images were taken every 10 minutes. Time progression is shown as hours:minutes (hh:mm). Scale bar=10 μm.

Movie S15. Time-lapse images of a sorted hybrid cell generated after fusion between ESC-H2B-eGFP and NPC-H2B-mRFP that does not undergo mitosis. The cell does not undergo mitosis during the time-lapse. DIC, GFP and RFP images were taken every 16 minutes. Time progression is shown as hours:minutes (hh:mm).

Movie S16. Time-lapse images of a sorted hybrid cell generated after fusion between ESC-H2B-eGFP and NPC-H2B-mRFP that undergoes bipolar mitosis. The cell undergoes bipolar mitosis during the time-lapse. DIC, GFP and RFP images were taken every 10 minutes. Time progression is shown as hours:minutes (hh:mm).

Movie S17. Time-lapse images of a sorted hybrid cell generated after fusion between ESC-H2B-eGFP and NPC-H2B-mRFP that undergoes tripolar mitosis with random segregation. The cell undergoes tripolar mitosis during the time-lapse. DIC, GFP and RFP images were taken every 10 minutes. Time progression is shown as hours:minutes (hh:mm).

Movie S18. Time-lapse images of a sorted hybrid cell generated after fusion between ESC-H2B-eGFP and NPC-H2B-mRFP that undergoes tripolar mitosis with non-random segregation. The cell undergoes tripolar mitosis during the time-lapse. DIC, GFP and RFP images were taken every 10 minutes. Time progression is shown as hours:minutes (hh:mm).

Movie S19. Time-lapse images of a sorted hybrid cell generated after fusion between ESC-H2B-eGFP and NPC-H2B-mRFP with parental chromosomes showing different spatial occupancy after bipolar mitosis. The cell undergoes bipolar mitosis with different spatial occupancy of H2B-

eGFP and H2B-mRFP fusion proteins. GFP and RFP images were taken every 16 minutes. Time progression is shown as hours:minutes (hh:mm).

Movie S20. Time-lapse images of a sorted hybrid cell generated after fusion between ESC-H2B-eGFP and NPC-H2B-mRFP with parental chromosomes showing different spatial occupancy after tripolar mitosis. The cell undergoes tripolar mitosis with different spatial occupancy of H2B-eGFP and H2B-mRFP fusion proteins. GFP and RFP images were taken every 10 minutes. Time progression is shown as hours:minutes (hh:mm).

Movie S21. Time-lapse images of a long-time tracking a sorted hybrid cell generated after fusion between ESC-H2B-eGFP and NPC-H2B-mRFP. Tracking marks (blue circles) follows an heterokaryon cell that undergoes tripolar division (first division) and two of the daughter cells (red and yellow circles) that undergo bipolar divisions (second division). The third daughter cell (light blue circles) moves out of the frame. Tracking is shown as an overlay on the GFP channel. DIC, GFP and RFP images were taken every 10 minutes. Time progression is shown as hours:minutes (hh:mm).

Table S1. SNP genotyping raw data. Includes SNP genotyping of control ESC and NPC, of known mouse genetic backgrounds, of 2n cell pools, single cells from fusion-derived clones and of 2n pure lines derived from 4n cells.