

Inventory of Supplementary Material

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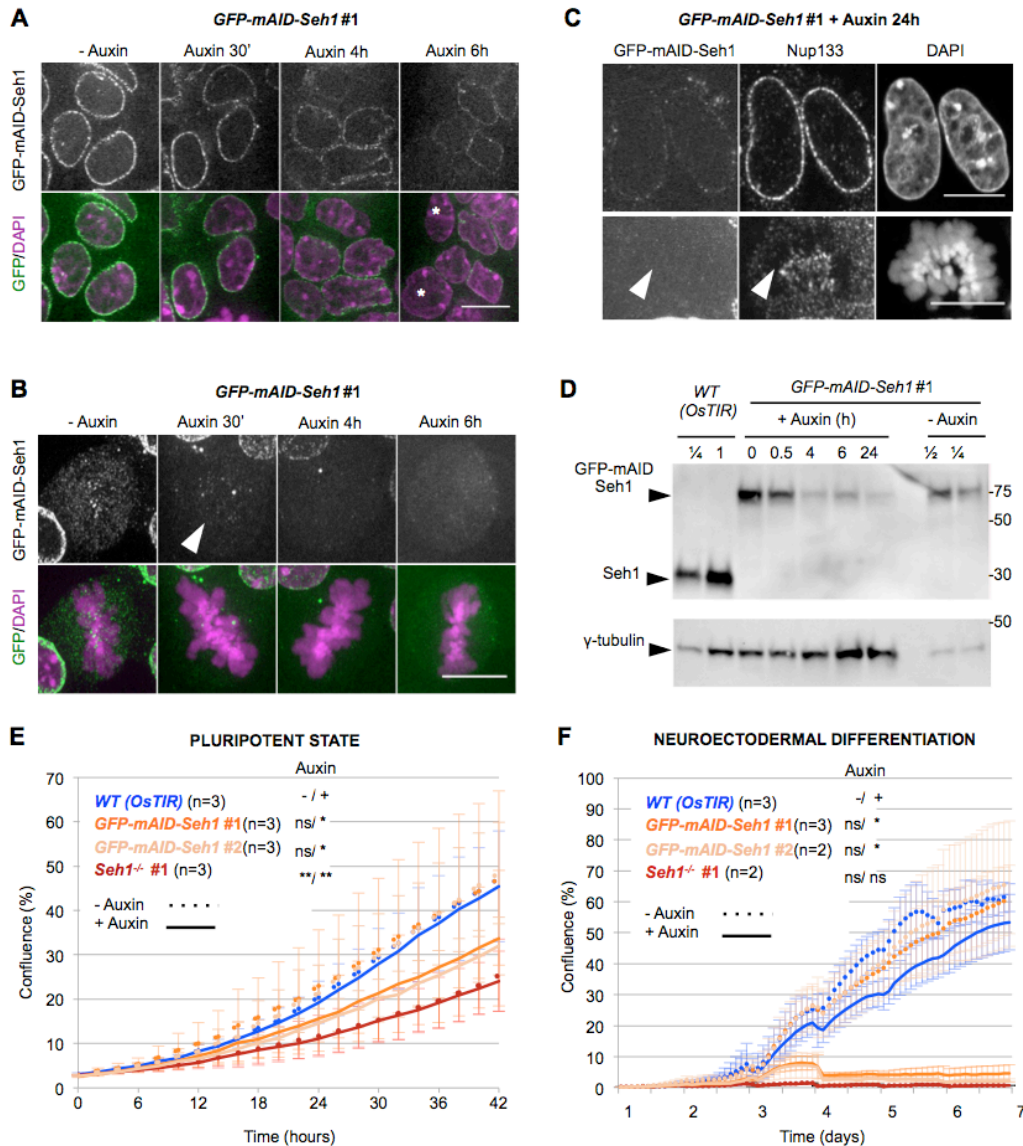


Figure S1, related to Fig. 1. Auxin-induced GFP-mAID-Seh1 degradation in mESCs recapitulates *Seh1*^{-/-} phenotypes. A-B. GFP-mAID-Seh1 localizes at NE in interphase cells (**A**; a single confocal plane is presented) and is enriched at kinetochores in mitotic cells (**B**; a projection of 9 optical sections is presented). After 30 minutes of auxin treatment, the GFP signal is only slightly decreased at the NE in interphase cells but no longer detectable in mitotic cells (arrowhead). Longer treatments (4 to 6 hours) with auxin lead to a progressive decrease of the NE signal, and to the appearance of GFP negative cells (indicated by white stars on the Auxin 6h - GFP/DAPI panel, and likely corresponding to cells that went through mitosis). Scale bars, 10 μ m. **C.** Localization of Nup133 in interphase (upper panels) and mitotic (lower panels) *GFP-mAID-Seh1* #1 mESCs after 24h of auxin treatment. The arrowheads point to the kinetochores that are labelled by Nup133 despite the lack of GFP-mAID-Seh1. Scale bar, 10 μ m. **D.** Whole cell extracts from *WT-OsTIR* and *GFP-mAID-Seh1* #1 mESCs, treated or not with auxin for the indicated time, were analyzed by western blot using anti-Seh1 (top) and an anti-gamma-tubulin antibodies (bottom, used as loading control). 1/2 and 1/4 dilutions of *WT (OsTIR)* and non-treated *GFP-mAID-Seh1* #1 cell extracts were also loaded. Molecular weights are indicated (kilodaltons). **E.** Cell growth at pluripotent stage of the indicated cell lines was analyzed using cell confluence measurements with the IncuCyte® system. Cells were treated with Auxin for 18 to 26 h prior to time 0. Error bars correspond to standard deviations from 3 independent experiments. **F.** The % of cell confluence upon neuroectodermal differentiation was quantified with the IncuCyte® system. Cells were exposed to Auxin at the beginning of differentiation (day 0). Error bars correspond to standard deviations of 4 (*GFP-mAID-Seh1* #1 ad *OsTIR* WT) or 3 (*GFP-mAID-Seh1* #2 and *Seh1*^{-/-} #1) distinct wells acquired in 3 and 2 independent experiments, respectively. Statistical analyses were performed using the two-tailed Paired Student's t-test (See Materials and Methods), by comparing all control (-Auxin) or treated (+Auxin) mutant cell lines to *Wt (OsTIR)* -Auxin or +Auxin, respectively.

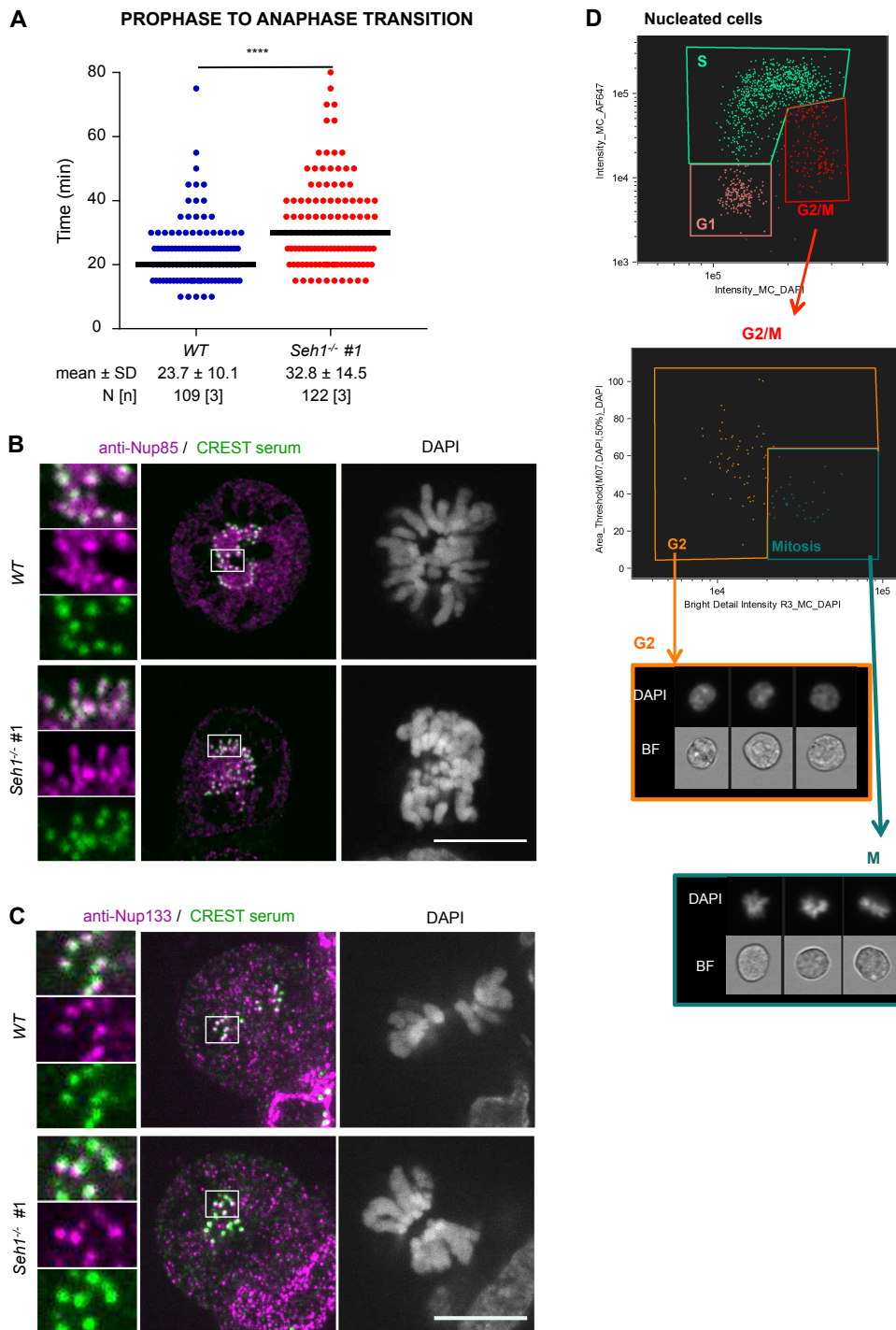


Figure S2, related to Fig. 2: *Seh1*^{-/-} mESCs have prolonged mitosis despite proper localization of the Y-complex at kinetochores

A. Quantification of the time spent from prometaphase to anaphase in *WT* and *Seh1*^{-/-} mESCs. Each dot represents one cell and the median time is represented with a black bar. The number (N) of cells, quantified in 3 independent experiments is indicated. **B, C.** Representative spinning-disk images of mitotic *WT* and *Seh1*^{-/-} mESCs, immunolabeled with **(C)** anti-Nup85 or **(D)** anti-Nup133, along with CREST serum (centromere marker) and stained with DAPI. A projection of three Z sections is presented. 3-fold enlargements of the boxed areas are also presented. Scale bars, 10 μ m. **D.** Representative plots of the last steps of our ImageStream® cell cycle analyses (*WT* mESCs). Top: the G1, S and G2/M phases of the cell cycle were defined based on DNA content (DAPI intensity) and EdU incorporation (AF647 channel). Middle: the G2 and M (Mitotic) population were then discriminated based on DAPI intensity and area. Bottom: bright Field (BF) and corresponding DAPI images of representative cells defined by these gating as G2 (orange) or mitotic (blue) are presented.

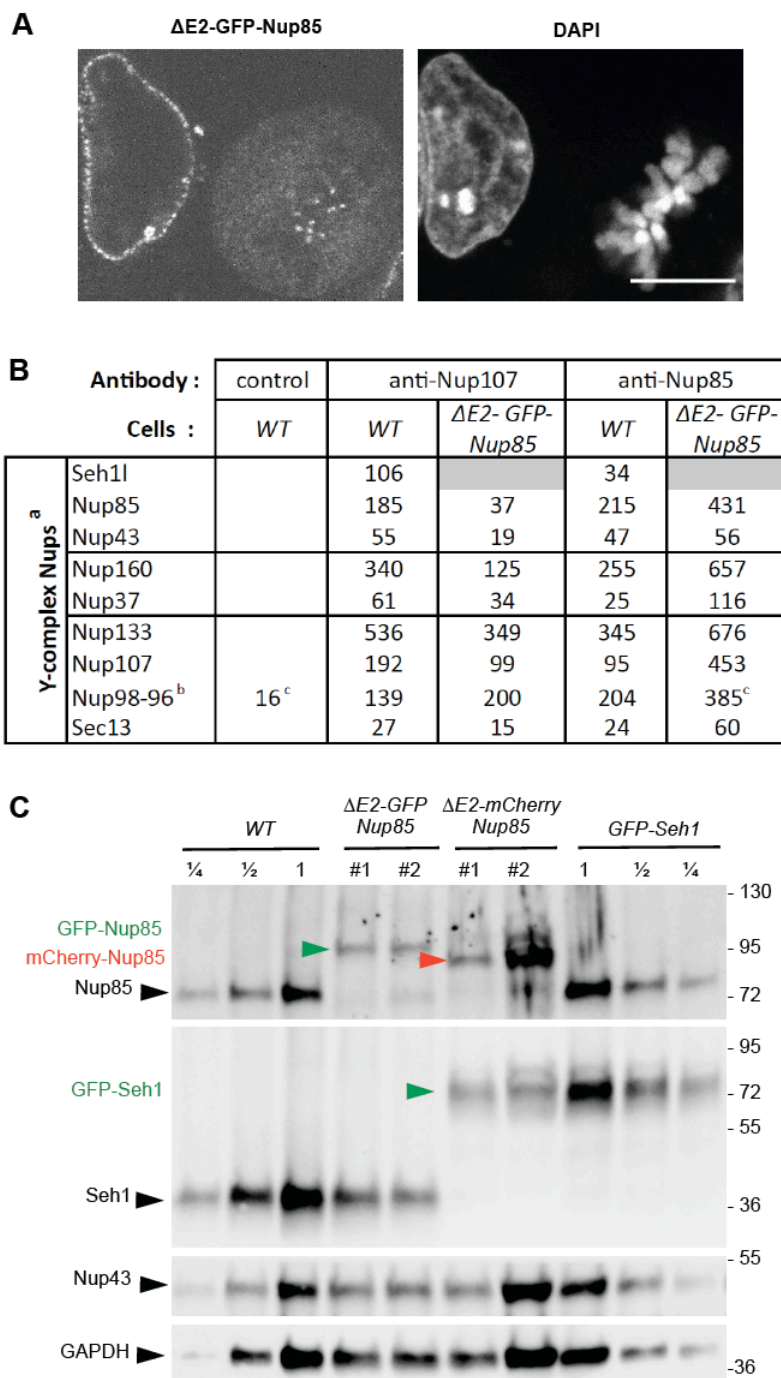


Figure S3, related to Figs 6-8. Characterization of the $\Delta E2$ -GFP-Nup85 and $\Delta E2$ -mCherry-Nup85 cell lines. (A) Representative spinning disk images (single plane) of interphase (left) and mitotic (right) $\Delta E2$ -GFP-Nup85 mESCs fixed and stained with DAPI. Scale bar, 10 μ m. **B.** The table provides the Mascot score for an immunoprecipitation experiment (see Materials and Methods) using either a pre-immune serum (control) or anti-Nup107 or -Nup85 antibodies, to pull down the Y-complex from WT or $\Delta E2$ -GFP-Nup85 mESC protein extracts. ^aElys/AHCTF1 was not identified in this immunoprecipitation experiment. ^bThe autoproteolytic cleavage of the Nup98-Nup96 precursor generates Nup98 and Nup96 (Fontoura et al., 1999); only the latter is a stable component of the Y-complex. Nearly all (35 out of 36) peptides identified in these immune pellets originates from Nup96. ^cSamples in which the Nup98 peptide was identified. **C.** Whole cell extracts of the indicated cell lines were analyzed by western-blot using anti-Nup85, -Seh1, -Nup43, and -GAPDH antibodies. Two- and four fold dilution (1/2, 1/4) of the WT and GFP-Seh1 mESC extracts were also loaded. Molecular markers are indicated on the right (kDa).

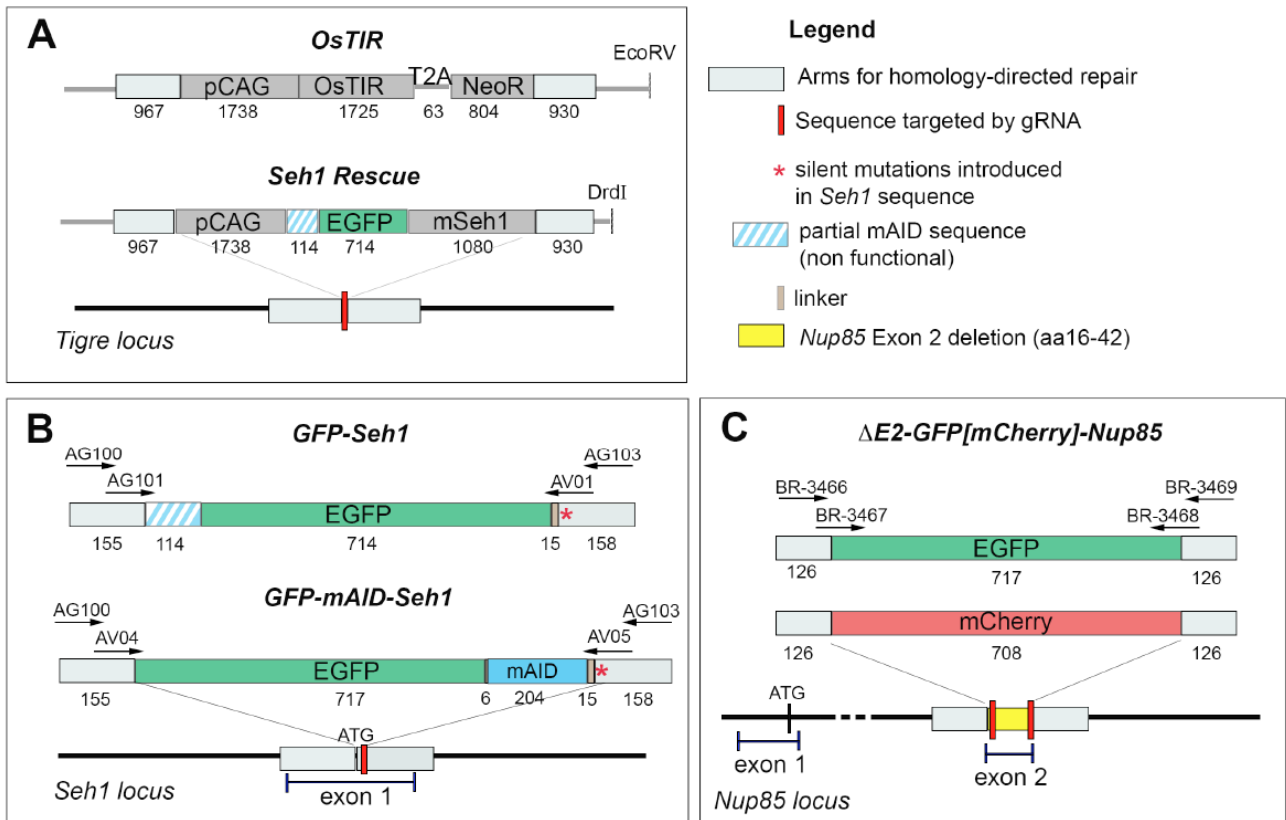


Figure S4, related to Materials and Methods and Tables S2 and S3: Strategies for CRISPR-Cas9-mediated cell line establishment via homologous recombination.

The constructs used for homologous recombination (**A**, plasmid digested with the indicated restriction enzyme, and **B-C**, PCR products: the sequences of the oligonucleotides (arrows) used to amplify the template are listed in **Table S3**) leading to the indicated cell lines are represented above each targeted genomic locus (**A**: *Tigre*, **B**: *Seh1*, and **C**: *Nup85*). The sequences targeted by the gRNAs (red boxes), the sequences used for homology-directed repair (light grey boxes), and when relevant, the position of the exons and the first ATG are displayed. The size of the various segments is indicated in bp. Note that the construct used for GFP-*Seh1* expression (**A**. *Seh1 rescue* and **B**. *GFP-Seh1* lines) further contains a non-functional 38 amino-acid long fragment of mAID. In **B**., the red star indicates the position of silent CRISPR/Cas9 blocking mutations introduced in one *Seh1* HR-arm.

Table S1: Plasmids.

Plasmids used (sequence upon request)	Source	Identifier
pX-280_Cas9WT-EGFP-Seh1gRNA1 - Seh1gRNA 2	This paper	#1808
pU6-sgTigre_CBh-Cas9-T2A-mCherry-3UTR	From P. Navarro Gil and N. Festuccia. (Festuccia et al., 2019)	#2061
<i>Tigre</i> HR-pCAG-EGFP-mSeh1- <i>Tigre</i> HR	This paper	#2077
<i>Tigre</i> HR-pCAG-OsTir-T2A-NeoR- <i>Tigre</i> HR	From P. Navarro Gil and N. Festuccia. (Festuccia et al., 2019)	#2064
pX-853_Cas9WTmCherry Seh1-gRNA1	This paper	#2098
pCMV-EGFP-mAID-hSeh1	This paper	#2100
GeneArt CRISPR Nuclease Vector (OFP_Cas9)	Invitrogen, Carlsbad, CA	A21174
GeneArt CRISPR pOFP-Cas9-Mios gRNA2	This paper	#2110
GeneArt CRISPR pOFP-Cas9-Mios gRNA4	This paper	#2111
GeneArt CRISPR pOFP-Cas9-Nup43 gRNA1	This paper	#2104
pX-672-Cas9HF-mCherry-gRNANup85-1_ gRNANup85-2	This paper	#2005
pX-864-Cas9HF-GFP-gRNANup85-1_ gRNANup85-2	This paper	#2101
pBOS-H2B-Cherry-IRES-Neo	From Doye lab (Bolhy et al., 2011)	#824
pBOS-H2B-GFP-IRES-Neo	From Doye lab (Bolhy et al., 2011)	#805

Table S2, related to Experimental Procedures. Cell lines used in this study

NAME	SOURCE (IF COMMERCIAL) OR DESIGN FOR CELL LINES GENERATED IN THIS STUDY	IDENTIFIER	Plate ref.	MUTATIONS (genomic sequences set at 1 for the first <u>A</u> TG)	Chromosome count
MEF (DR4)	Applied StemCell	ASF-1001	-	-	-
WT mESCs (HM1)	ThermoFisher Scientific (Selfridge et al., 1992)	MES4303	-	-	40
Seh1^{-/-}	<p>Parental cell line: HM1</p> <p>Cas9 & gRNA plasmid: pX-280_Cas9WT-EGFP-Seh1gRNA1 - Seh1gRNA 2</p> <p>HR template: (200 bp synthetic oligonucleotide initially designed to facilitate screening)</p>	<i>Seh1^{-/-}</i> #1	A18	Only one PCR band observed suggesting that both alleles bear the same mutation. <u>Allele 1 (and 2)</u> : Deletion of 351bp from -317 to +33. Insertion of 32bp (from HR template) at +71.	40 Chr (>60%)
		<i>Seh1^{-/-}</i> #2	B42	<u>Allele 1</u> : 1bp indel (c>a) at -2 plus Deletion of 77bp from +4 to +77. Insertion of 31 bp (from HR template) at +4. <u>Allele 2</u> : Deletion of 30bp from -2 to +27; one bp deletion at +70.	40 Chr (>60%)
		<i>Seh1^{-/-}</i> #3	B37	<u>Allele 1</u> : Deletion of 52 bp from +19 to +71 <u>Allele 2</u> : Deletion of 15bp from +13 to +27; one bp deletion at +70.	40 Chr (>60%)
Seh1-Rescue	<p>Parental cell line: <i>Seh1^{-/-}</i> #1 mESCs</p> <p>Cas9 & gRNA plasmid: pU6-sgTIGRE_CBh-Cas9-T2A-mCherry-3UTR</p> <p>HR template: linearized (with DrdI) TIGRE HR-pCAG-EGFP-mSeh1-TIGRE HR (#2077)(Figure S4)</p>	<i>Seh1-Rescue</i> #1	R11G	Rescue of <i>Seh1^{-/-}</i> #1 (A18). Insertion of pCAG-GFP-Seh1 in only one of the <i>TIGRE</i> alleles	40 Chr (55%) 39 Chr (45%)
		<i>Seh1-Rescue</i> #2	R11F	Rescue of <i>Seh1</i> KO A18 (#1). Insertion of pCAG-GFP-Seh1 in only one of the <i>TIGRE</i> alleles	40 Chr (>60%)
OsTIR	<p>Parental cell line: HM1</p> <p>Cas9 & gRNA plasmid (s): pU6-sgTIGRE_CBh-Cas9-T2A-mCherry-3UTR</p> <p>HR template: linearized (with EcoRV) TIGRE HR-pCAG-OsTir-T2A-NeoR-TIGRE HR (#2064) (Figure S4)</p>	<i>OsTIR</i>	11B	Insertion of pCAG-OsTir-T2A-NeoR in only one of the <i>TIGRE</i> alleles.	42Chr (>60%)

Table S2- continued

NAME	DESIGN OF THE CELL LINES	IDENTIFIER	Plate ref.	MUTATIONS (genomic sequences set at 1 for the first <u>ATG</u>)	Chromosome count
GFP-mAID- Seh1	<p>Parental cell line: <i>OsTIR</i></p> <p>Cas9 & gRNA plasmid (s): pX-853 _Cas9WTmCherry Seh1-gRNA1</p> <p>HR template: PCR product: HR-EGFP-mAID-mSeh1 (gRNA resistant)-HR; amplification of plasmid #2100 with primers indicated in Figure S4 and Table S3</p>	<i>GFP-mAID- Seh1</i> #1	F9	<u>Alleles 1 and 2</u> : GFP-mAID-Seh1	42Chr (>60%)
		<i>GFP-mAID- Seh1</i> #2	E6	<u>Allele 1</u> : GFP-mAID Seh1 <u>Allele 2</u> : <i>Seh1</i> KO; 1 bp insertion at + 18	42Chr (>60%)
Mios^{-/-}	<p>Parental cell line: HM1/</p> <p>Cas9 & gRNA plasmid (s): GeneArt CRISPR pOFP-Cas9-Mios gRNA2 GeneArt CRISPR pOFP-Cas9-Mios gRNA4</p> <p>HR template: none</p>	<i>Mios^{-/-}</i> #1	5C	Only one PCR band observed suggesting that both alleles bear the same mutation. <u>Allele 1 (and 2)</u> : Deletion of 593 bp from - 310 to 282.	40 Chr (>60%)
		<i>Mios^{-/-}</i> #2	7C	<u>Allele 1</u> : Deletion of 33bp from +74 to 106. <u>Allele 2</u> : Deletion of 3 bp from +84 to +86	40 Chr (>60%)
		<i>Mios^{-/-}</i> #3	7D	<u>Allele 1</u> : Deletion of 3bp from +83 to 85 and of 7 bp from 195 to 201. <u>Allele 2</u> : Replacement from bp 85 C by GT (=1bp) and insertion of a T after bp 196.	40 Chr (>60%)
ΔE2-GFP- Nup85	<p>Parental cell line: HM1</p> <p>Cas9 & gRNA plasmid (s): pX-672-Cas9HF-mCherry-gRNANup85-1_gRNANup85-2</p> <p>HR template: PCR product: HR-EGFP-HR (Figure S4); amplification of EGFP with primers indicated in Figure S4 and Table S3</p>	<i>ΔE2-GFP- Nup85</i> #1	B2	<u>Allele 1</u> : In frame integration of GFP in exon 2 and deletion from bp 639 to 716. <u>Allele 2</u> : <i>Nup85</i> KO; deletion of 2bp at +633-634 leading to premature stop codon.	41 Chr (56%) 40 Chr (25%)
		<i>ΔE2-GFP- Nup85</i> #2	B10	<u>Allele 1</u> : In frame integration of GFP in exon 2 and deletion from bp 639 to 716. <u>Allele 2</u> : <i>Nup85</i> KO; Deletion of 20 bp from 622 to 641.	42 Chr (57%) 41 Chr (29%) 40 Chr (14%)
		<i>ΔE2-GFP- Nup85</i> #3	B3	<u>Allele 1</u> : In frame integration of GFP in exon 2 and deletion from bp 639 to 716. <u>Allele 2</u> : <i>Nup85</i> KO; deletion of 13 bp from 627 to 647	42 Chr (>60%)

Table S2- continued

NAME	DESIGN OF THE CELL LINES	IDENTIFIER	Plate ref.	MUTATIONS (genomic sequences set at 1 for the first <u>ATG</u>)	Chromosome count
<i>GFP-Seh1</i>	<p>Parental cell line: HM1</p> <p>Cas9 & gRNA plasmid (s): pX-853 _Cas9WTmCherry Seh1-gRNA1</p> <p>HR template: PCR product HR_EGFP-mSeh1 (gRNA resistant)-HR. Amplification of plasmid #2077 with primers indicated in Figure S4 and Table S3</p>	<i>GFP-Seh1</i>	B10	<u>Allele 1</u> : <i>GFP-Seh1</i> . In frame N-term integration of GFP at +4 in <i>Seh1</i> sequence. <u>Allele 2</u> : <i>Seh1</i> KO. Deletion from -2 to +27.	40 Chr (>60%)
<i>ΔE2-mCherry-Nup85</i>	<p>Parental cell line: <i>GFP-Seh1</i></p> <p>Cas9 & gRNA plasmid (s): pX-864-Cas9HF-GFP-gRNANup85-1_gRNANup85-2</p> <p>HR template: PCR product HR-mCherry-HR (Figure S4); amplification of mCherry with primers indicated in Figure S4 and Table S3</p>	<i>ΔE2-mCherry-Nup85</i> #1	B6	<u>Allele 1</u> : in frame mCherry integration in exon2 and deletion from bp 639 to 716. <u>Allele 2</u> : <i>Nup85</i> KO. Deletion of 8bp (+630 -- +638) and of 10 bp (+706 to +716) in exon 2.	41 Chr (>60%)
		<i>ΔE2-mCherry-Nup85</i> #3	C5	Same genotype as above (#1 =B6)	41 Chr (>60%)
		<i>ΔE2-mCherry-Nup85</i> #2	G4	<u>Allele 1</u> : in frame mCherry integration in exon2 and deletion from bp +639 to +716. <u>Allele 2</u> : <i>Nup85</i> KO. Deletions of 42 (+635 -- + 677; 12 bp also inserted) and 33 bp (+683 -- +716; 9 bp also inserted) in exon 2.	41 Chr (>60%)
<i>Nup43^{-/-}</i>	<p>Parental cell line: HM1</p> <p>Cas9 & gRNA plasmid (s): GeneArt CRISPR pOPF-Cas9-Nup43 gRNA1</p> <p>HR template: none</p>	<i>Nup43^{-/-}</i> #1	E4	<u>Allele 1</u> : <i>Nup43</i> KO. Deletion of 40 bp from +22 to +62. <u>Allele 2</u> : <i>Nup43</i> KO. Deletion of 32 bp from +38 to +70.	40 Chr (>60%)
		<i>Nup43^{-/-}</i> #2	B5	<u>Allele 1</u> : <i>Nup43</i> KO. Insertion of 1 bp at +52. <u>Allele 2</u> : <i>Nup43</i> KO. Insertion of 2 bp at +52.	40 Chr (>60%)

Table S3: Sequences of gRNAs and PCR primers used to generate the template for homology-directed repair (HDR)

Sequences of gRNAs used in this study		
IDENTIFIER	SEQUENCE	SOURCE
Seh1-gRNA1 [Exon 1 Fw]	TTGTGGCGCGCAGCATCG	This paper
Seh1-gRNA2 [Exon 1 Rv]	TGGCCATCCGGCGCCCG	This paper
sgTigre	ACTGCCATAACACCTAACTT	This paper
Mios-gRNA2 [Exon 3 Fw]	CCTATATGAAATGTGTTGCG	This paper
Mios-gRNA4 [Exon 3 Fw]	AGAATTGAGCCTTTATCATG	This paper
Δ E2-GFP-Nup85-gRNA1 [Exon 2 Rv]	TTCTTCTTGAATTCACACC	This paper
Δ E2-GFP-Nup85-gRNA2 [Exon 2 Fw]	CTTCCTTCAACCAAACAGGT	This paper
Nup43-gRNA1 [Exon 1 Fw]	AAATTAGCAAAAACCGCTGG	This paper

PCR primers used to generate the template for homology-directed repair (HDR)		
IDENTIFIER	SEQUENCE	SOURCE
AG100 [Fw, HDR left arm] <i>GFP-Seh1</i> , <i>GFP-mAID-Seh1</i>	CCCGCGCAGGGCGTGTCCCTCGGGGCGTGGCGCGCCGGGGCC GACGCGGGCGGGCAGGCGAGGGCCGACGTGCCGTACGTGCCT CCGCGGTGG	This paper
AG101 [Fw, HDR left arm] <i>GFP-Seh1</i>	TACGTGCCTCCGCGGTGGTCCGGGGGCTGCGGGCCGCACCGC CGCCCTCTCGGAGGCGGGCCCGGCAGACGGCGGGCCATGG TTTCTGCCAAAAATCAAGC	This paper
AG103 [Rv, HDR right arm] <i>GFP-Seh1</i> , <i>GFP-mAID-Seh1</i>	GCGCCGCCTCCCTCCCCTCCCGCCGGGCGAGCGGCGAGTGCCG CGCGCACCTTGACGCTCTGATCGCTGGAGCAGGTGGCCATCCG GCGCCCGTGAAGTGC	This paper
AV01 [Rv, HDR right arm] <i>GFP-Seh1</i>	CGGCGCCCGTGAAGTCGAAAGACACATCGTGGATGAGGTCC TTGTGGTCCGCCGATAGACGGGCGACGAAGGATCTGAGT CCGGACTTGTACAGC	This paper
AV04 [Fw, HDR left arm] <i>GFP-mAID-Seh1</i>	CGGCGCCCGTGAAGTCGAAAGACACATCGTGGATGAGGTCC TTGTGGTCCGCCGATAGACGGGCGACGAAGGATCTGAGT CCGGATTATA	This paper
AV05 [Rv, HDR right arm] <i>GFP-mAID-Seh1</i>	TACGTGCCTCCGCGGTGGTCCGGGGGCTGCGGGCCGCACCGC CGCCCTCTCGGAGGCGGGCCCGGCAGACGGCGGGCCATGG TGAGCAAGGGCGAGGAG	This paper
BR3466 [Fw2, HDR left arm] <i>ΔE2-GFP[mCherry]-Nup85</i>	TCATTGGATACGACCTAGGCTGTTGCGCTGGTGTATGGTGTTCG CAGTTACACTTGAAGTTGAATGGACAGTTGTAGGGCTAAC	This paper
BR3467 [Fw1, HDR left arm] <i>ΔE2-GFP[mCherry]-Nup85</i>	GAATGGACAGTTGTAGGGCTAACAGCTCCTGTGCCTCCTTATG TCTTACTAGTGGATTCCAGGTATGGTGGAGCAAGGGCGAGGAG	This paper
BR3468 [Rv1, HDR right arm] <i>ΔE2-GFP[mCherry]-Nup85</i>	CAGTTTCTGCCTCAGAGAGTCAACCAATGTGCCAAGACAGAT GTGAAAAAGAACTGCCTACCCTTGACAGCTCGTCCATGCCG	This paper
BR3469 [Rv, HDR right arm] <i>ΔE2-GFP[mCherry]-Nup85</i>	GCAACTTGTCTCAAATAGCAAAAAACAAAGTTTCAAAAAATA AAAATATTGCCAAATAACTCCAGTTTCTGCCTCAGAGAGTC	This paper

Table S4: Antibodies

Primary antibodies	Dilution	Source	Identifier
Human autoantibody against-centromeres (CREST serum)	IF: 1/500	ImmunoVision, Springdale, AR	HCT-0100
Rabbit polyclonal anti-GAPDH	WB: 1/2000	Trevigen, Minneapolis, MN	2275-PC100
Mouse anti-gamma-tubulin	WB: 1/50000	Abcam, San Francisco, CA	Ab11316
Rabbit polyclonal antibody anti-Mios	WB: 1/1000	Cell signalling, Danvers, MA	#13557
Affinity-purified rabbit polyclonal anti-Nup43 antibody	WB: 1/1000	From Doye lab (Zuccolo et al., 2007)	# 571/08-111-C1
Rabbit polyclonal anti-mNup85 serum	WB: 1/2000 IF: 1:1000 IP: 1/10	From D. Forbes (Harel et al., 2003)	N/A
Rabbit monoclonal antibody anti-Nup98 C39A3	IF: 1/20	Cell signalling	#2598
Rabbit polyclonal anti-Nup107	WB: 1/1000 IP: 1/10	From Doye lab (Belgareh et al., 2001)	#520-82
Rat monoclonal anti-mouse Nup133 antibody #74; clone 9C2H8)	IF: 1/100	From Doye lab (Berto et al., 2018)	N/A
Rabbit polyclonal antibody anti-Seh1	WB: 1/1000 IP: 1/500	Abcam, San Francisco, CA	ab218531
Rabbit polyclonal antibody anti-Tpr	IF: 1/200	Abcam, San Francisco, CA	ab84516
Rabbit polyclonal antibody anti Ostir	WB: 1/1000	From M.T. Kanemaki (Natsume et al., 2016)	N/A
Rabbit polyclonal antibody anti- Wdr24	WB: 1/1000	Proteintech, Manchester, UK	20778-1-AP

Secondary antibodies	Dilution	Source	Identifier
Cy™3 AffiniPure Donkey anti-rabbit	IF: 1/1000	Jackson ImmunoRes, West Grove, PA	711-165-152
Cy™3 AffiniPure Donkey anti-rat	IF: 1/1000	Jackson ImmunoRes	712-165-153
Cy™3 AffiniPure Goat anti-rabbit	IF: 1/1000	Jackson ImmunoRes	111-165-144
AlexaFluor 568 Donkey anti-rabbit	IF: 1/1000	ThermoFisher Scientific	A10042
Alexa 647 Donkey anti-human	IF: 1/500	Jackson ImmunoRes	709-605-149
Cy™5 AffiniPure Donkey anti-rat	IF: 1/1000	Jackson ImmunoRes	712-175-153
Peroxidase AffiniPure Donkey anti-rabbit	WB: 1/5000	Jackson ImmunoRes.	711-035-152
Peroxidase AffiniPure Goat anti-mouse	WB: 1/5000	Jackson ImmunoRes.	115-035-068
Peroxidase AffiniPure Goat anti-ratCy™5 AffiniPure Donkey Anti-Rat	WB: 1/5000	Jackson ImmunoRes.	112-035-167
Rabbit TrueBlot ®: Anti-Rabbit IgG HRP	WB: 1/1000	Rockland, Limerick, PA	18-8816-33

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