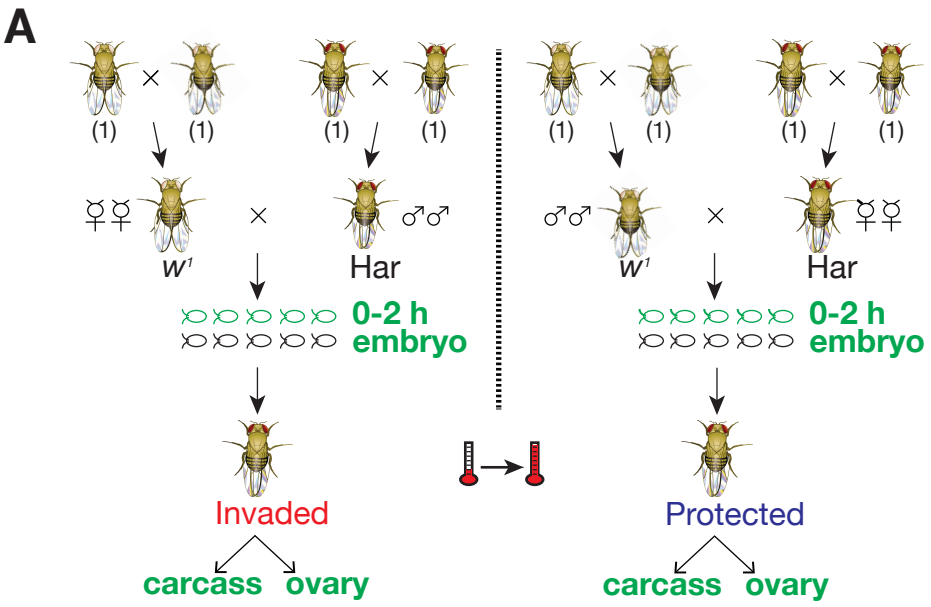
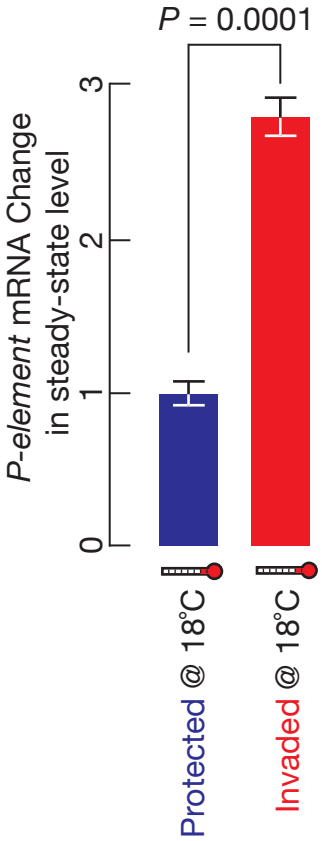
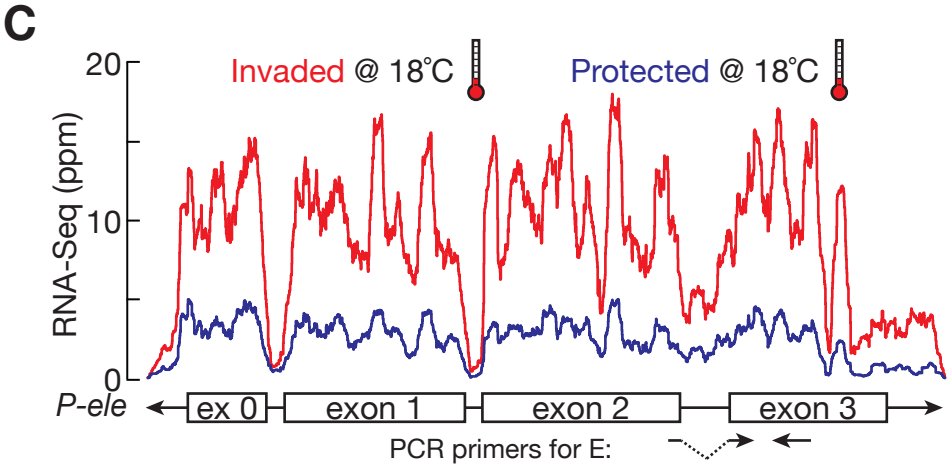
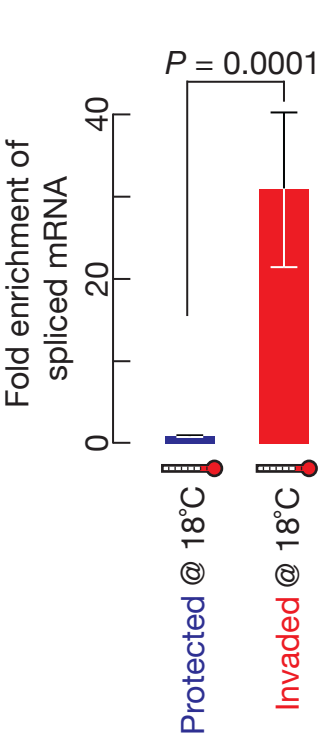

A
(1)
(1)
(1)
(1)
(1)
(1)
(1)
(1)
w¹
Har
w¹
Har
0-2 h embryo
0-2 h embryo
Invaded
Protected
carcass
ovary
carcass
ovary
• Samples sequenced
• Pair-wise (single fly) cross was used to minimize background variation
• Embryo DNA is used as reference to define new insertions
• Carcass (in which P-element is not active) DNA is used to estimate the false-positive insertion rate


B
P = 0.0001
P-element mRNA Change in steady-state level
0
1
2
3
Protected @ 18°C
Invaded @ 18°C


C
Invaded @ 18°C
Protected @ 18°C
RNA-Seq (ppm)
0
10
20
P-ele
ex 0
exon 1
exon 2
exon 3
PCR primers for E:


E
P = 0.0001
Fold enrichment of spliced mRNA
0
20
40
Protected @ 18°C
Invaded @ 18°C

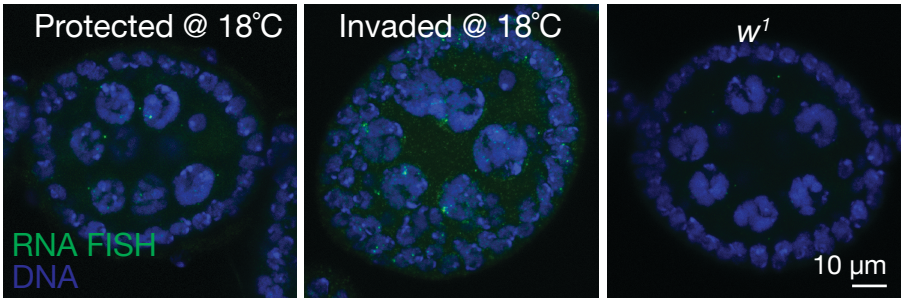

D
Protected @ 18°C
Invaded @ 18°C
w¹
RNA FISH
DNA
10 µm

Figure S1. *P-elements* are active in invaded ovaries at 18°C, Related to Figure 1.
(A) Experimental design of fly cross to measure *P-element* transposition events. Single-fly crosses were set up to minimize genetic variation in progeny that are sequenced in this study. Carcass samples, in which the splicing of the last intron of *P-element* is suppressed and therefore P-element is not active, serve as negative control to define the potential false-positive rate of the assay. (B) Quantitative real time PCR to measure the steady-state level of *P-element* transcript at 18°C. Data are represented as mean ± SD. (C) RNA-Seq profiles for *P-element* from invaded and protected ovaries are shown. (D) Confocal images of stage 5 egg chambers co-stained for DNA and *P-element* transcripts (FISH). Egg chamber from w^1 flies serves as negative control to test the specificity of FISH probes. (E) qRT-PCR assay to quantify the *P-element* mRNAs without the last intron (spliced). Data are mean ± SD for three independent biological replicates. Primer design is depicted in panel C.

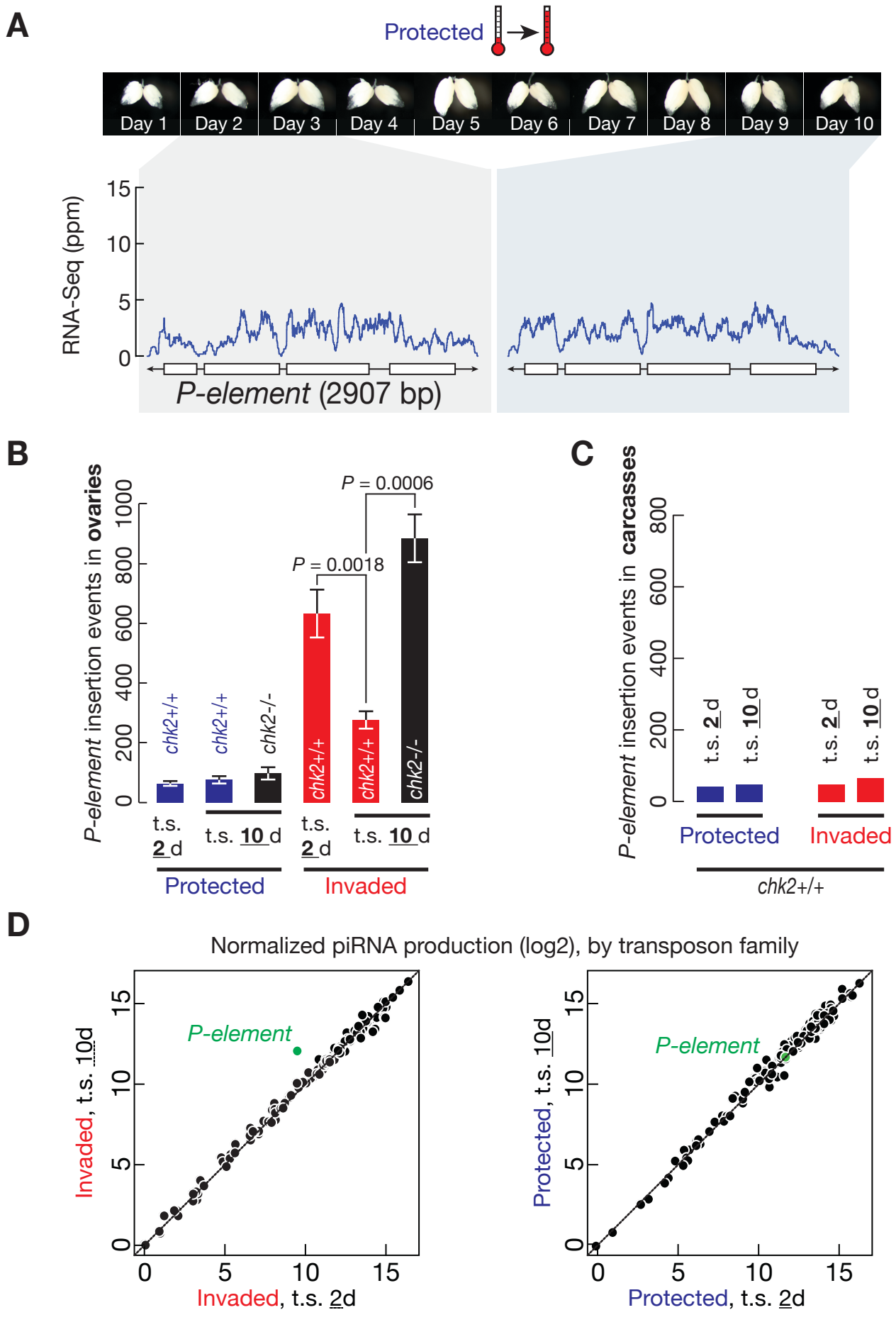

A
Protected
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
RNA-Seq (ppm)
15
10
5
0
P-element (2907 bp)
B
P-element insertion events in ovaries
0
200
400
600
800
1000
P = 0.0006
P = 0.0018
chk2+/+
chk2+/+
chk2-/-
chk2+/+
chk2+/+
chk2-/-
t.s. 2 d
t.s. 10 d
t.s. 2 d
t.s. 10 d
Protected
Invaded
C
P-element insertion events in carcasses
0
200
400
600
800
t.s. 2 d
t.s. 10 d
t.s. 2 d
t.s. 10 d
Protected
Invaded
chk2+/+
D
Normalized piRNA production (log2), by transposon family
P-element
Invaded, t.s. 10d
Invaded, t.s. 2d
0
5
10
15
P-element
Protected, t.s. 10d
Protected, t.s. 2d
0
5
10
15

Figure S2. *P-elements* are silenced in recovered ovaries by piRNAs, Related to Figure 3.
(A) *P-element* transcript in protected ovaries, measured by RNA-Seq. Ovary morphology pictures are re-used from Figure 2C to serve as time scale. (B) Genome sequencing to measure new *P-element* insertion events from ovaries. Data are mean ± SD for three independent biological samples. (C) Transposition events of *P-elements* from gonad-ectomised carcasses. (D) Small RNA sequencing to detect the production of piRNAs mapped to transposon. Each dot represents one transposon family. Only piRNAs targeting *P-elements* (green dots) increased in recovered progeny.

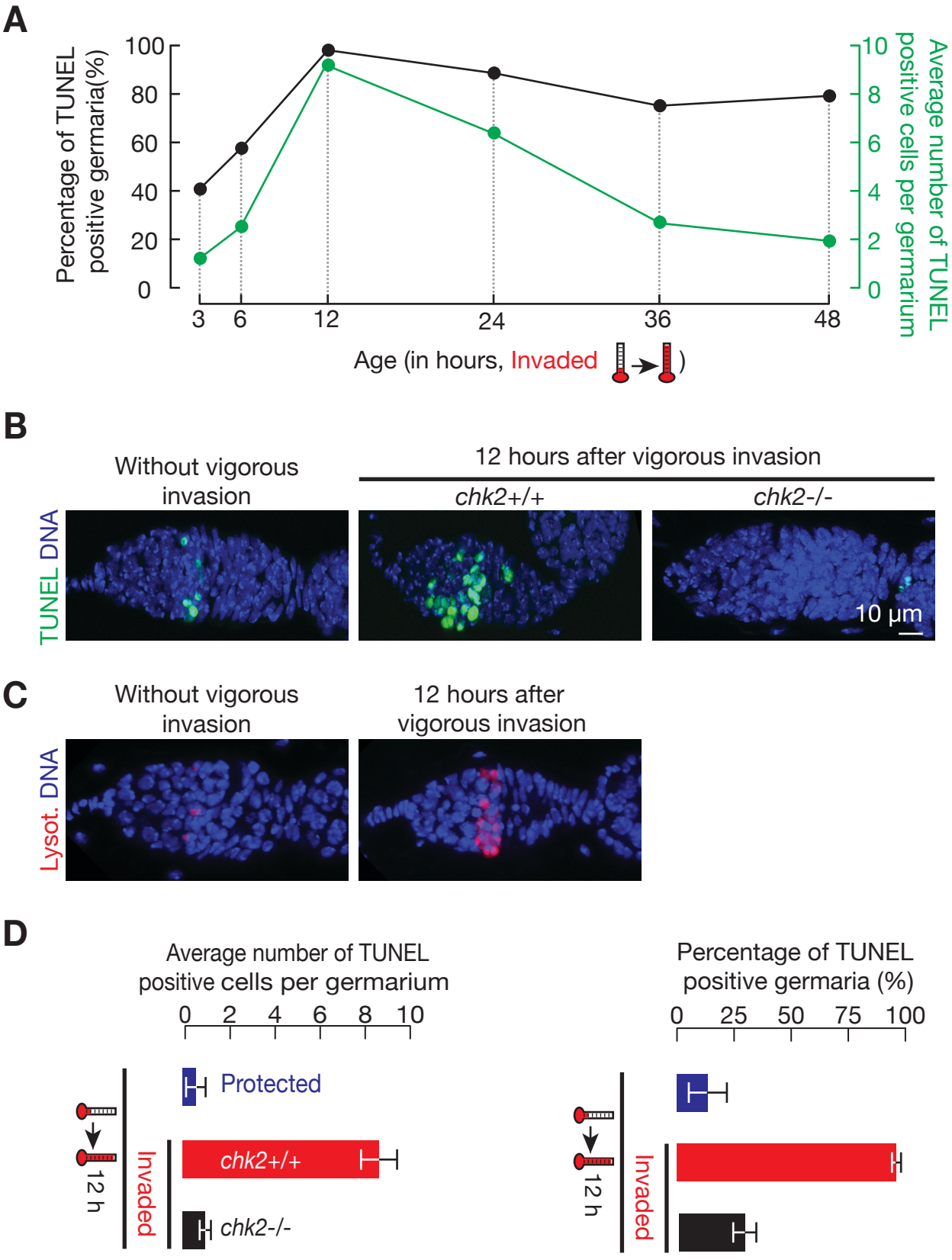

A
Percentage of TUNEL positive germaria(%)
100
80
60
40
20
0
3
6
12
24
36
48
Age (in hours, Invaded)
Average number of TUNEL positive cells per germarium
10
8
6
4
2
0
B
Without vigorous invasion
12 hours after vigorous invasion
chk2+/+
chk2-/-
TUNEL DNA
10 μm
C
Without vigorous invasion
12 hours after vigorous invasion
Lysot. DNA
D
Average number of TUNEL positive cells per germarium
0 2 4 6 8 10
Protected
chk2+/+
chk2-/-
12 h
Invaded
Percentage of TUNEL positive germaria (%)
0 25 50 75 100
12 h
Invaded

Figure S3. Intensive *P-element* invasion causes selective cell death, Related to Figure 4.
(A) Quantification of TUNEL positive cells in invaded ovaries at 6 time points after the temperature-shift. (B) Representative images of TUNEL staining to quantify the number of apoptotic cells. (C) Representative images of LysoTracker staining to detect the apoptotic cells. (D) Quantification for average number of TUNEL positive cells per germarium (left) and percentage of TUNEL positive germaria (right). Data are represented as mean ± SD for three independent biological replicates.

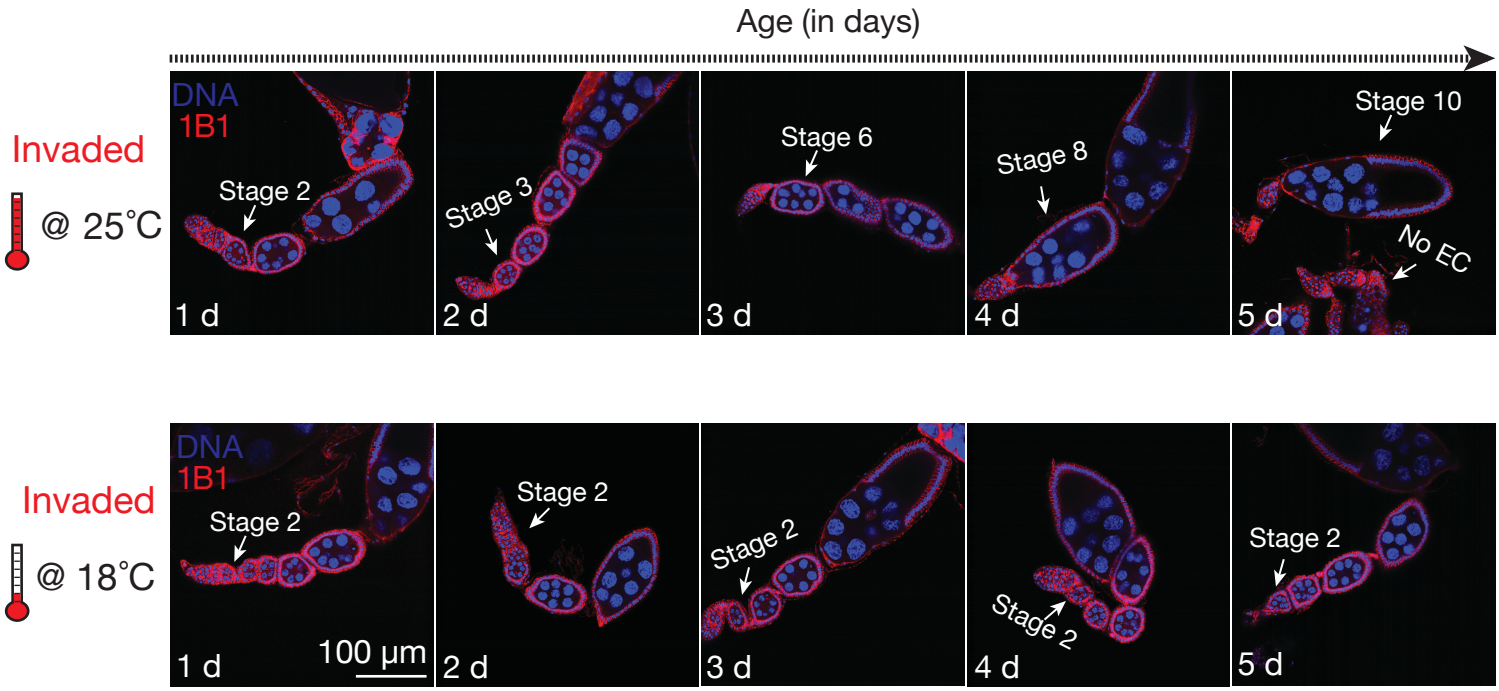


Figure S4. Transposon invasion leads to germarial arrest at 25˚C, but not 18˚C, Related to Figure 4. Morphology of ovarioles is visualized by 1B1 immuno-staining (Red) from invaded progeny. DNA is visualized by DAPI (Blue). At 18˚C, all ovarioles are normal, as germaria are connected with early stage egg chambers and then followed by later stage egg chambers. However, *P-element* invasion at 25˚C leads to germarial arrest, as evidenced by no new egg chamber is forming but the previous ones keep developing into later stages. For example, at day 5, the germaria are either directly connected with stage 10 egg chambers or no egg chamber (EC).

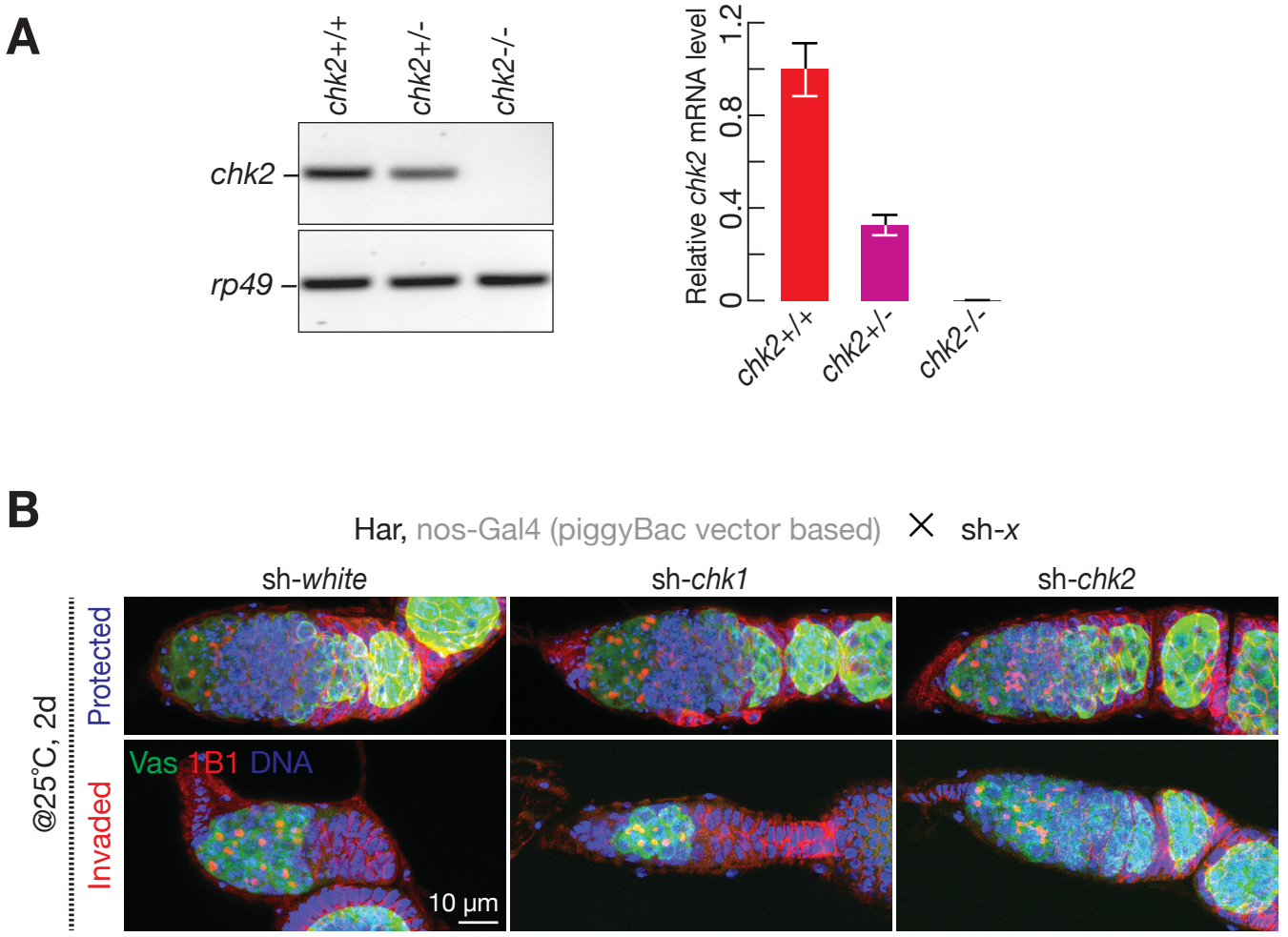


Figure S5. Depleting Chk2 in germ cells by RNAi rescues stem cell arrest phenotype, Related to Figure 4.

(A) Quantitative real time PCR to measure the steady state level of *chk2* transcripts in wild-type, *chk2* heterozygotes and *chk2* homozygotes. Data are represented as mean ± SD for three independent biological replicates. (B) Germarial structure from invaded and protected progeny that have either Chk1, or Chk2, or White (control) silenced in germ cells by using a nanos-Gal4 driver. This Gal4 driver is generated using piggyBac vector, therefore is stable in invaded ovaries. Suppressing Chk2 activity in germ cells completely rescues differentiation arrest phenotypes in invaded progeny.

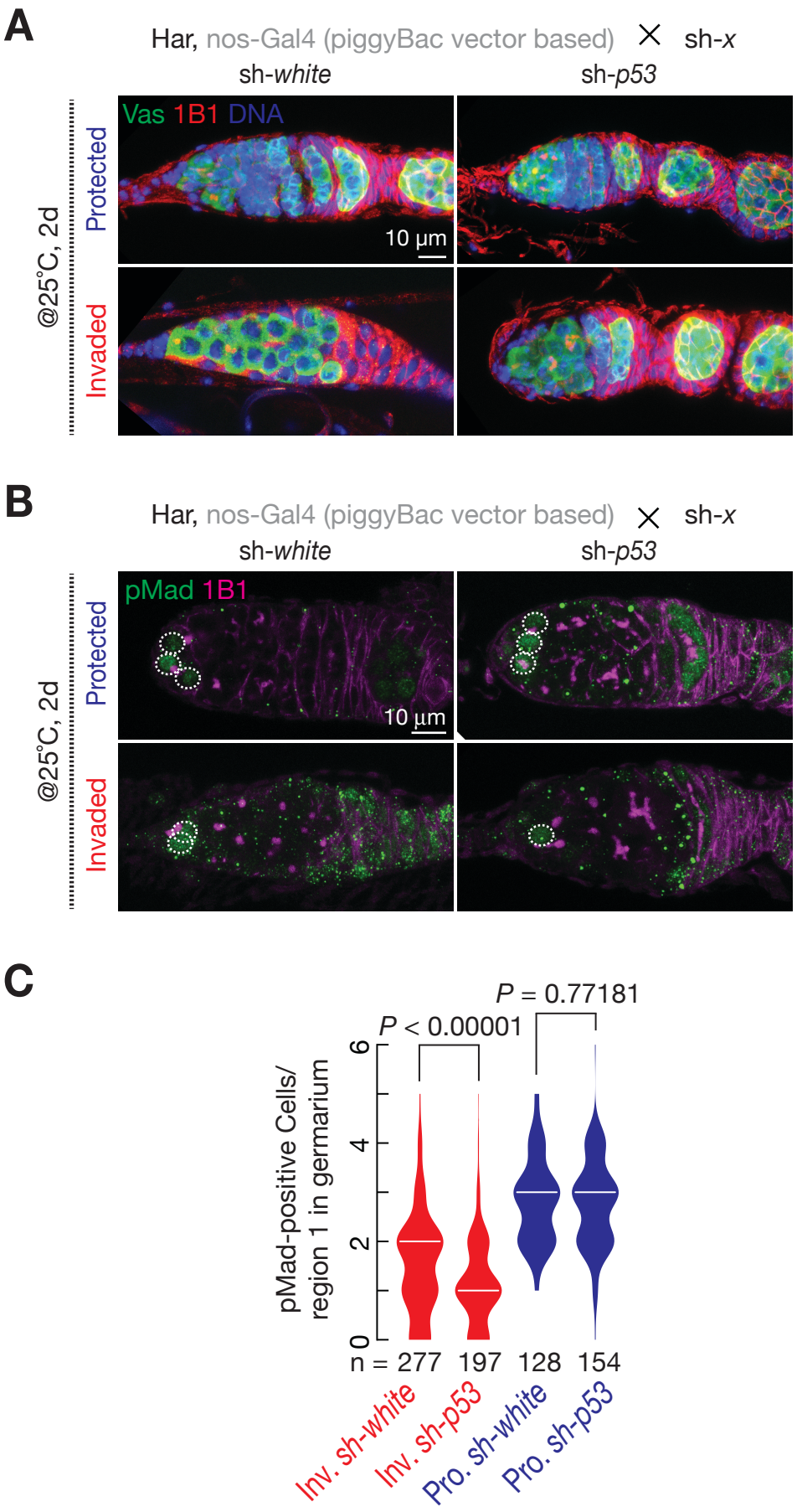

A
Har, nos-Gal4 (piggyBac vector based) × sh-x
sh-white
sh-p53
@25°C, 2d
Protected
Invaded
Vas 1B1 DNA
10 μm
B
Har, nos-Gal4 (piggyBac vector based) × sh-x
sh-white
sh-p53
@25°C, 2d
Protected
Invaded
pMad 1B1
10 μm
C
P = 0.77181
P < 0.00001
pMad-positive Cells/ region 1 in germarium
0
2
4
6
n = 277 197 128 154
Inv. sh-white
Inv. sh-p53
Pro. sh-white
Pro. sh-p53

Figure S6. Depleting p53 in germ cells by RNAi rescues stem cell arrest phenotype, Related to Figure 4.

(A) Germarial structure from invaded and proteced progeny that have either p53 or White (control) silenced in germ cells by using a nanos-Gal4 driver. Suppressing p53 activity in germ cells also rescues differentiation arrest phenotype in invaded progeny. (B) Germarial structure from the same genotype indicated in (A). pMad-positive germ cells mark germline stem cells (dotted circles) in region 1 of germarium. (C) Quantification of pMad-positive germ cells in region 1 of germarium. Pro., Protected progeny; Inv., Invaded progeny. *p-value* by two-tailed Mann-Whitney test. White bars indicate median values.

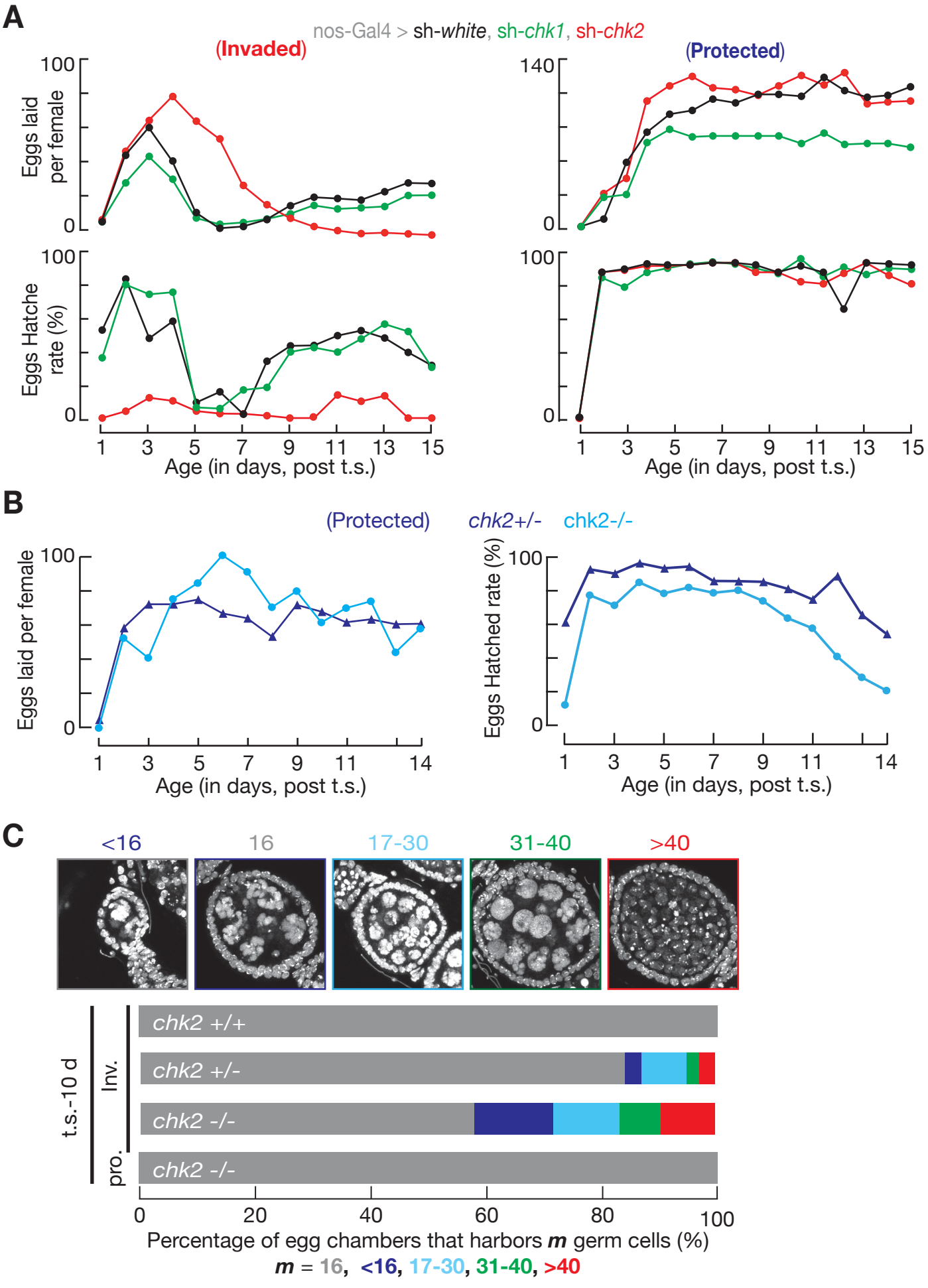

A
nos-Gal4 > sh-white, sh-chk1, sh-chk2
(Invaded)
(Protected)
Eggs laid per female
Eggs Hatche rate (%)
100
0
140
1 3 5 7 9 11 13 15
Age (in days, post t.s.)
B
(Protected)
chk2+/-
chk2-/-
Eggs laid per female
Eggs Hatched rate (%)
1 3 5 7 9 11 14
Age (in days, post t.s.)
C
<16
16
17-30
31-40
>40
t.s.-10 d
Inv.
pro.
chk2 +/+
chk2 +/-
chk2 -/-
chk2 -/-
0 20 40 60 80 100
Percentage of egg chambers that harbors m germ cells (%)
m = 16, <16, 17-30, 31-40, >40

Figure S7. Chk2 is required for *P-element* endogenization, Related to Figure 6.
(A) Fertility of protected and invaded progeny that have either Chk1, or Chk2, or White depleted by RNAi. Silencing Chk2 in germ cells leads to fertility decline without recovery. (B) Fertility of protected progeny that are in either *chk2* heterozygous or homozygous background. (C) Loss of Chk2-mediated arrest leads to cell proliferation defects upon transposon invasion. Quantification of germ cell number per egg chamber for each genotype. Five representative images for each category are shown. Pro., Protected progeny; Inv., Invaded progeny.