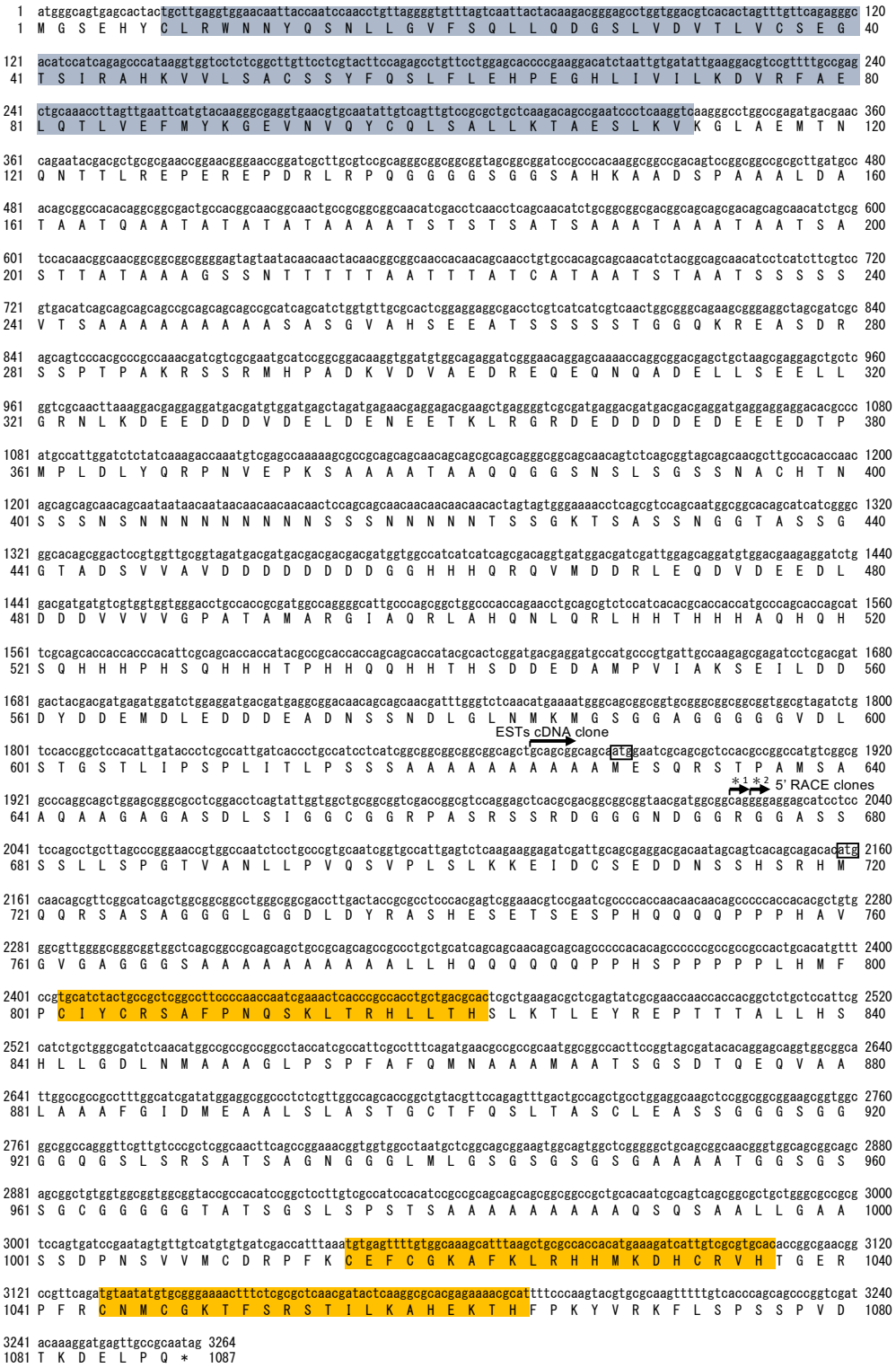
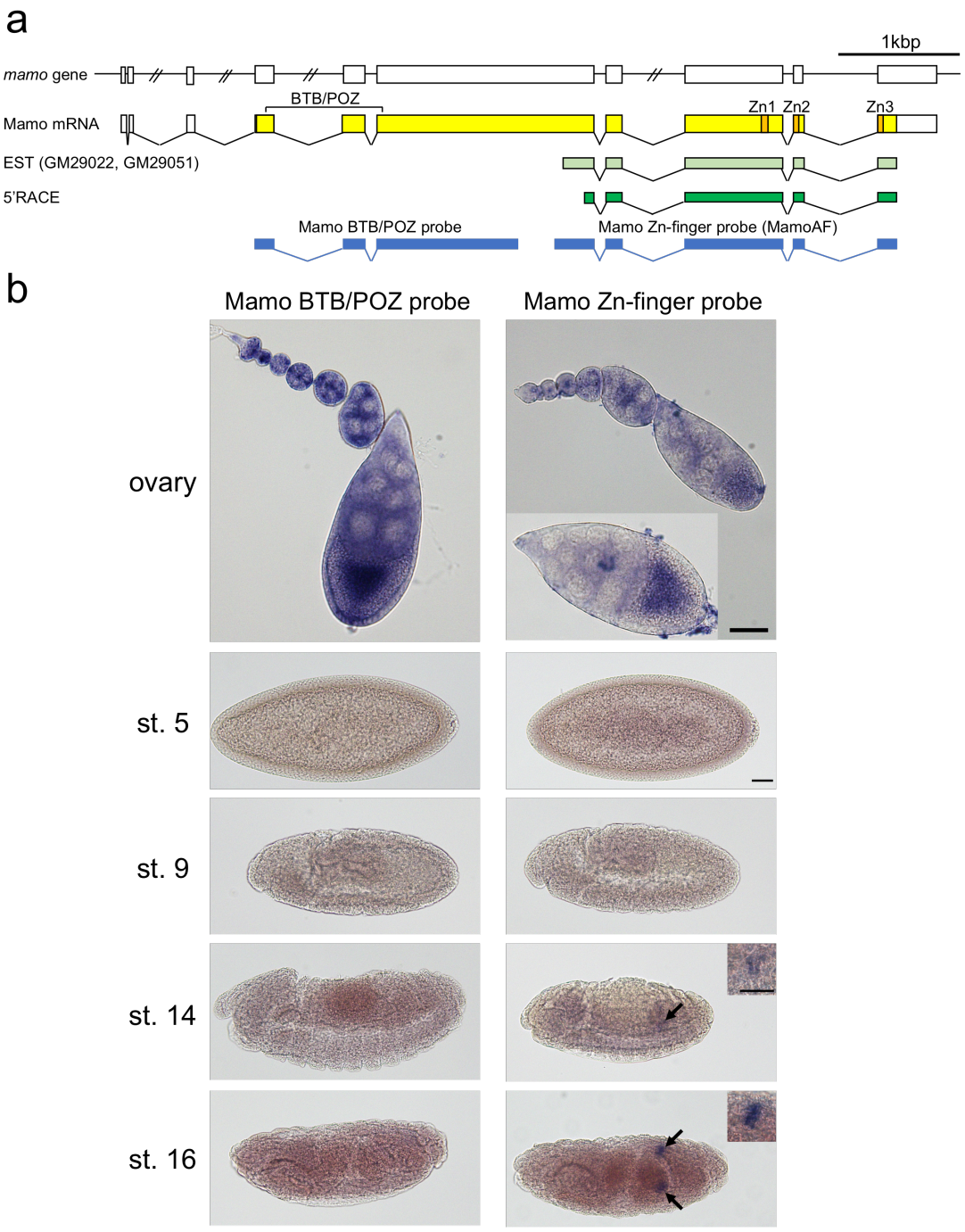
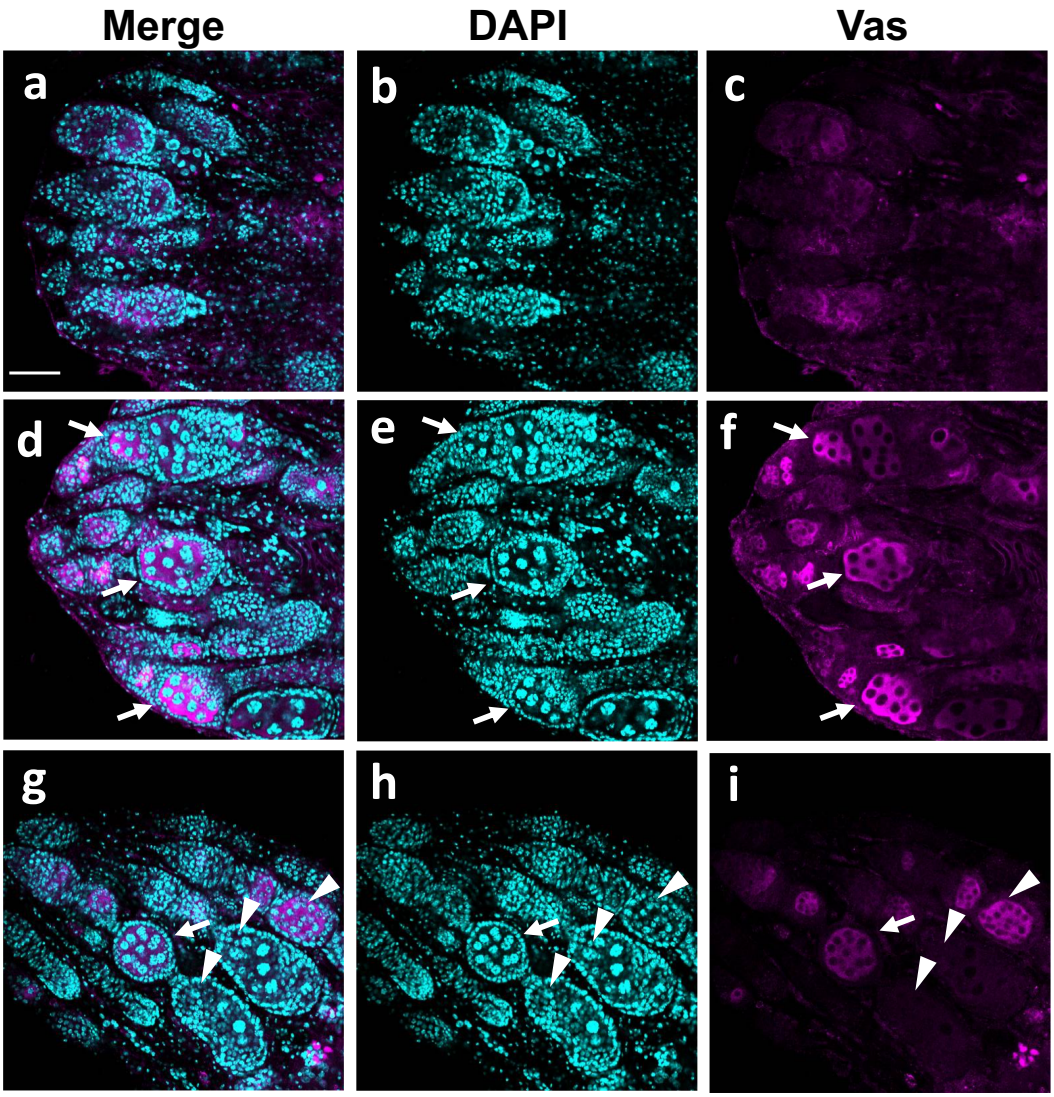

ESTs cDNA clone
*1 *2 5' RACE clones

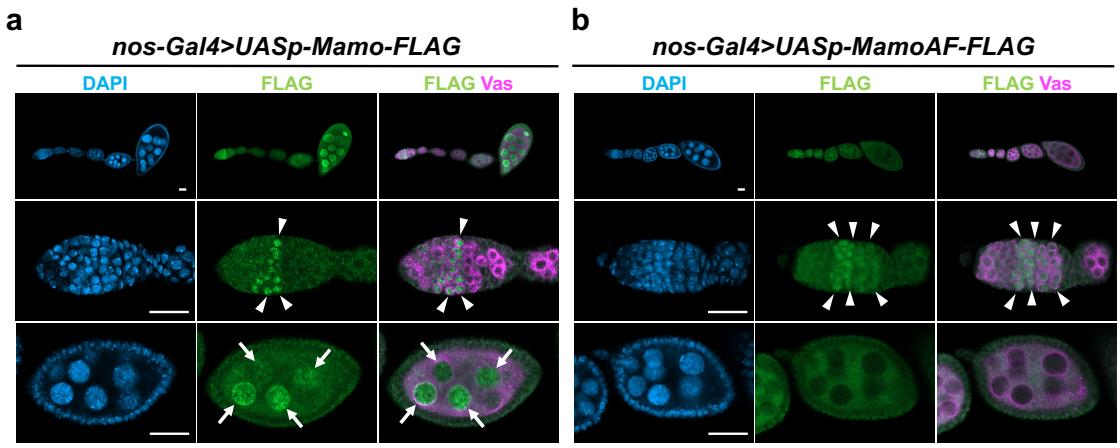
**Supplementary Figure 1**
Nucleotide and predicted amino acid sequences of full-length and short-form Mamo cDNAs. Arrows indicate the transcription initiation site of EST clones (GM29022 and GM29051), and the transcription initiation sites revealed by 5'-RACE, respectively. The alternative transcriptional start sites indicated by the arrows labeled with #1 and #2. The number of clones isolated with each transcription initiation sites are 5 and 2, respectively. Boxes indicates translational start codon of short-isoforms of Mamo, respectively. Grey indicates the BTB/POZ-domain. Yellow indicate the Zn-finger domains.


a
1kbp
mamo gene
BTB/POZ
Mamo mRNA
Zn1 Zn2
Zn3
EST (GM29022, GM29051)
5'RACE
Mamo BTB/POZ probe
Mamo Zn-finger probe (MamoAF)
b
Mamo BTB/POZ probe
Mamo Zn-finger probe
ovary
st. 5
st. 9
st. 14
st. 16

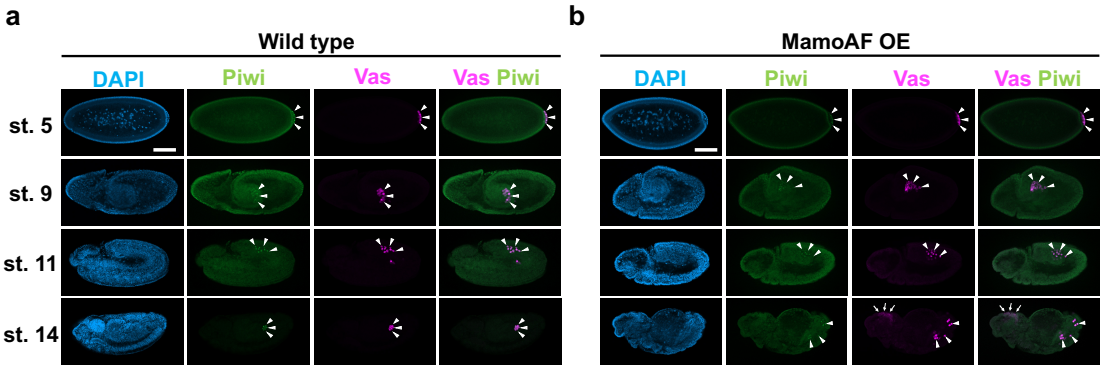
**Supplementary Figure 2**
The Mamo mRNA encoding Mamo short-isoform is expressed in PGCs. **a** A schematic diagram of *mamo* locus. **b** *in situ* hybridisation of wild type ovaries and embryos using a Mamo BTB/POZ probe (left panels) or a Mamo Zn-finger probe (right panels). Arrows indicate the embryonic gonads containing PGCs, in which Mamo mRNA encoding Mamo short-isoform is expressed. Insets indicate close-up views of the embryonic gonads. Scale bar: 30 μm.


Merge
DAPI
Vas
a
b
c
d
e
f
g
h
i

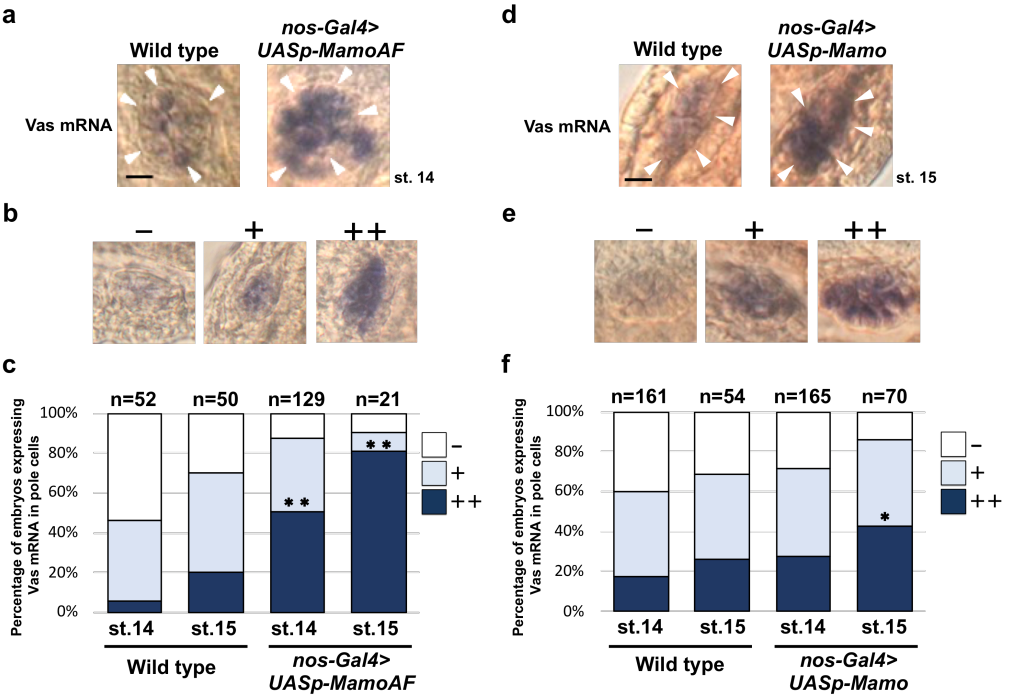
**Supplementary Figure 3** Differentiation of egg chambers containing *mamo* mutant germline clones expressing full-length Mamo-FLAG or MamoAF-FLAG. Immunostaining of the ovarioles containing *mamo* mutant germline (**a**-**c**) and the ovarioles containing *mamo* mutant germline clones expressing full-length Mamo-FLAG (**d**-**f**) and MamoAF-FLAG (**g**-**i**). Arrows indicate differentiating egg chambers after oogenic stage 6. Arrowheads indicate degenerating egg chambers. Scale bar: 50 μm.


a
nos-Gal4>UASp-Mamo-FLAG
DAPI
FLAG
FLAG Vas
b
nos-Gal4>UASp-MamoAF-FLAG
DAPI
FLAG
FLAG Vas

**Supplementary Figure 4** Expression patterns of full-length Mamo-FLAG and MamoAF-FLAG in ovaries. Immunostaining of the ovarioles expressing full-length Mamo-FLAG (**a**) or MamoAF-FLAG (**b**) under the control of *nos-Gal4* driver. Arrowheads indicate the signals of full-length Mamo-FLAG or MamoAF-FLAG in the nuclei of germline cysts in the germarium. Arrows indicate the full-length Mamo-FLAG signals in the nuclei of nurse cells in the egg chamber at stage 6. Scale bar: 20 μm.

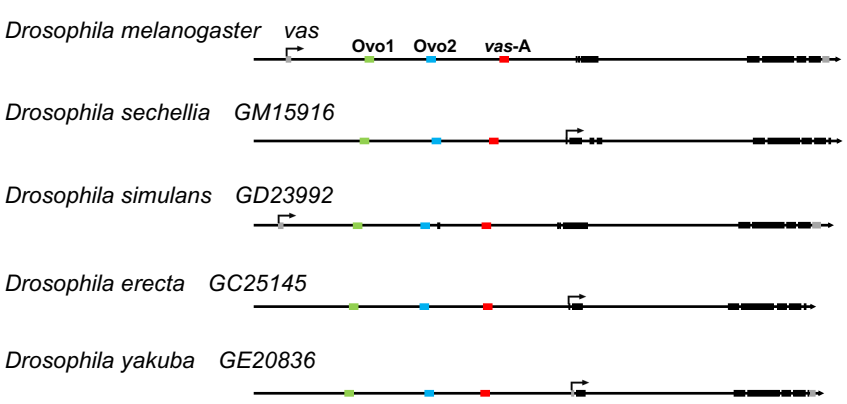

a
Wild type
DAPI
Piwi
Vas
Vas Piwi
st. 5
st. 9
st. 11
st. 14
b
MamoAF OE
DAPI
Piwi
Vas
Vas Piwi

**Supplementary Figure 5** PGC formation and migration in MamoAF-OE embryos. Immunostaining of wild type (**a**) and MamoAF OE embryos (**b**). Arrowheads indicate PGCs. Arrows indicate ectopic Vas signals. Piwi was used as a PGC marker. Maternal Piwi is enriched and maintained in PGCs during embryogenesis. Scale bar: 100 μm.


a
Wild type
nos-Gal4>
UASp-MamoAF
Vas mRNA
st. 14
d
Wild type
nos-Gal4>
UASp-Mamo
Vas mRNA
st. 15
b
–
+
++
e
–
+
++
c
f
Percentage of embryos expressing Vas mRNA in pole cells
100%
80%
60%
40%
20%
0%
n=52
n=50
n=129
n=21
n=161
n=54
n=165
n=70
st.14
st.15
st.14
st.15
Wild type
nos-Gal4>
UASp-MamoAF
nos-Gal4>
UASp-Mamo
–
+
++

**Supplementary Figure 6** MamoAF and Mamo overexpression promote Vas mRNA expression in PGCs. **a** Vas mRNA *in situ* hybridisation of wild type and the embryos overexpressing MamoAF under the control of maternal *nos-Gal4* driver. Arrowheads indicate Vas mRNA expression in PGCs at stage 14. Scale bar: 10 μm. **b** Embryos were classified into three groups depending on their strong (++), middle (+), and low (-) signal intensities of Vas mRNA in PGCs. **c** Percentages of the embryos carrying PGCs with strong, middle, and low signals in wild type and the embryos overexpressing MamoAF under the control of maternal *nos-Gal4* driver. $^{**}P<0.01$ (Significances are calculated with wild type by Fisher's exact test). **d** Vas mRNA *in situ* hybridisation of wild type and the embryos overexpressing Mamo under the control of maternal *nos-Gal4* driver. Arrowheads indicate Vas mRNA expression in PGCs at stage 15. **e** Embryos were classified into three groups depending on their strong (++), middle (+), and low (-) signal intensities of Vas mRNA in PGCs. **f** Percentages of the embryos carrying PGCs with strong, middle, and low signals in wild type and the embryos overexpressing Mamo under the control of maternal *nos-Gal4* driver. $^{*}P<0.05$ (Significances are calculated with wild type by Fisher's exact test).

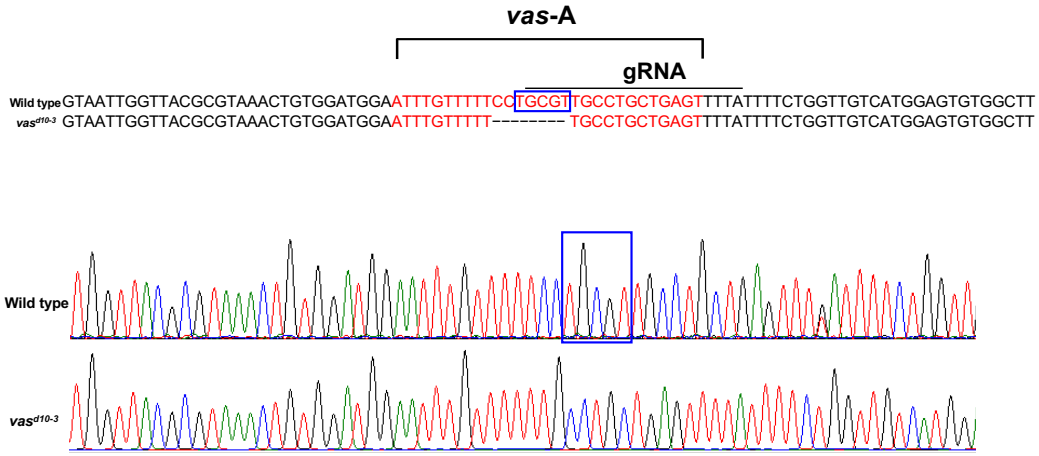
**a**



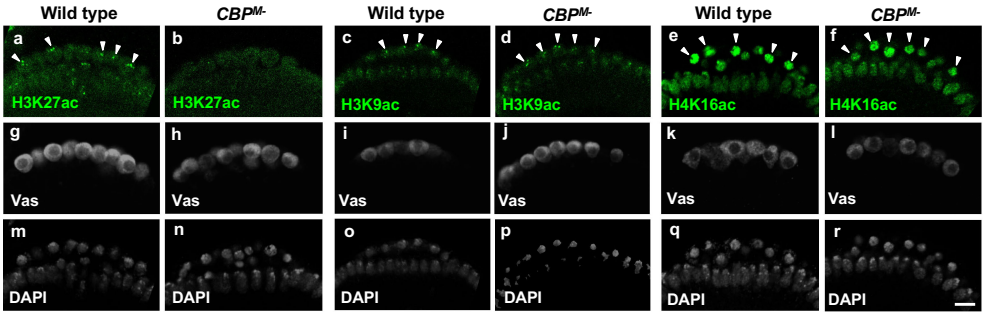

**b**

| | ***vas*-A** |
|---|---|
| *Drosophila melanogaster* | ATTTGTTTTTCCTGCGTTGCCTGCTGAGT |
| *Drosophila sechellia* | TGTTTTTCCTGCGTTGCCTGCTGAGT |
| *Drosophila simulans* | TGTTTTTCCTGCGTTGCCTGCTGAGT |
| *Drosophila erecta* | TTTTCCTGCGTTGCCCTGCTGAGT |
| *Drosophila yakuba* | TGTTTTTCCTGCGTCGCCTGCTGAGT |

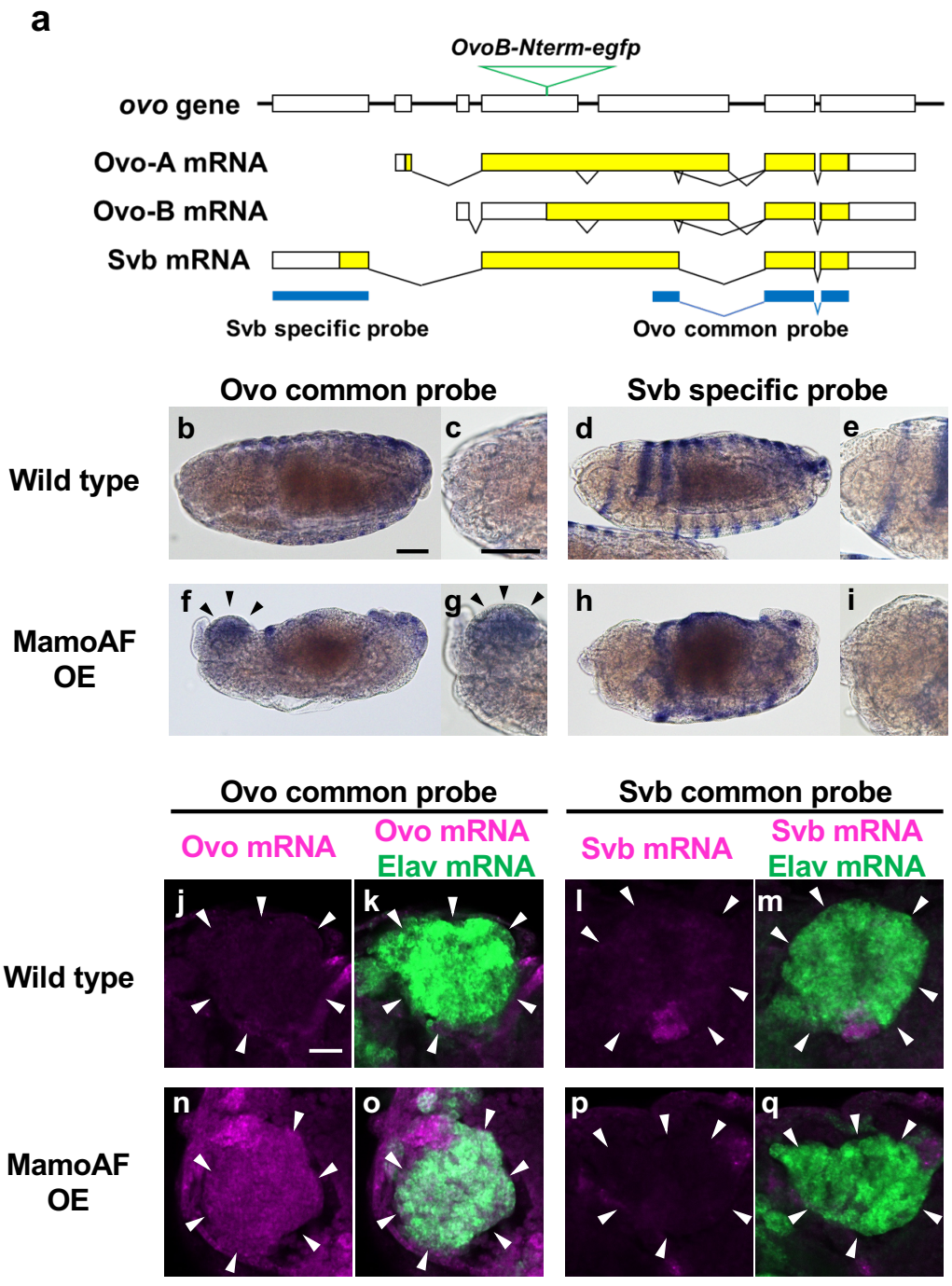
**Supplementary Figure 7** *vas*-A element and Ovo binding consensus sequences in *vas* locus are conserved in several *Drosophila* species**. a** A schematic diagram of *vas* loci in *Drosophila melanogaster*, *Drosophila sechellia*, *Drosophila simulans*, *Drosophila erecta* and *Drosophila yakuba*. **b** *vas*-A element in *Drosophila melanogaster*, *Drosophila sechellia*, *Drosophila simulans*, *Drosophila erecta* and *Drosophila yakuba*. Red indicates Mamo binding consensus sequences.


vas-A
gRNA
Wild type GTAATTGGTTACGCGTAAACTGTGGATGGAATTTGTTTTTCCTGCGTTGCCTGCTGAGTTTTATTTTCTGGTTGTCATGGAGTGTGGCTT
vasd10-3 GTAATTGGTTACGCGTAAACTGTGGATGGAATTTGTTTTT-------- TGCCTGCTGAGTTTTATTTTCTGGTTGTCATGGAGTGTGGCTT
Wild type
vasd10-3

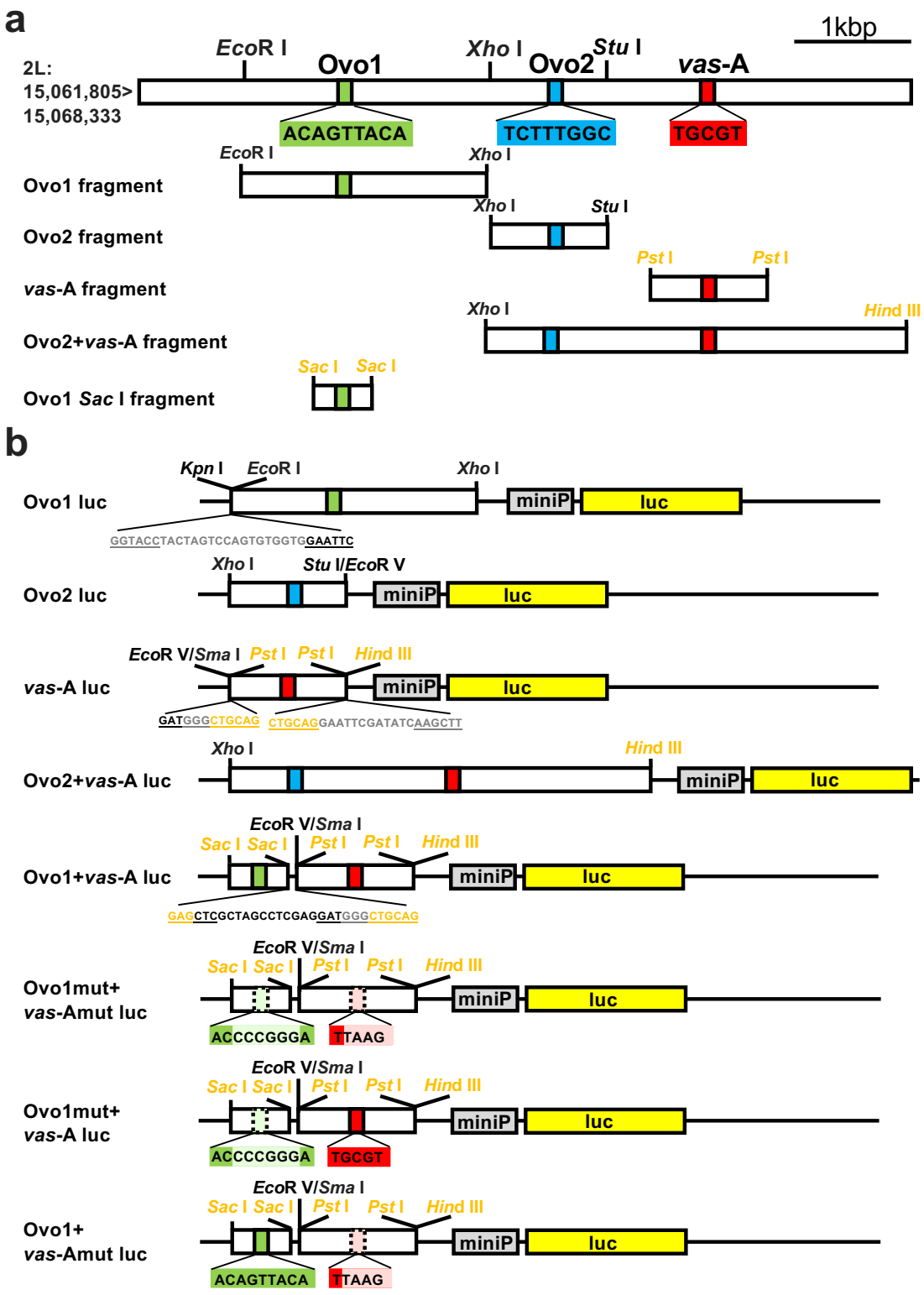
**Supplementary Figure 8** *vas*-A element is deleted in $vas^{d10\text{-}3}$ mutant. Sequeneces of the intronic region encompassing *vas*-A element of wild type and $vas^{d10\text{-}3}$. Red indicates *vas*-A element. A blue box indicates a Mamo binding consensus sequence. $vas^{d10\text{-}3}$ mutation was generated by using CRISPER-Cas9 system. A line indicates the sequence corresponding guide RNA used for the mutagenesis.


Wild type
CBPM-
Wild type
CBPM-
Wild type
CBPM-
a
H3K27ac
b
H3K27ac
c
H3K9ac
d
H3K9ac
e
H4K16ac
f
H4K16ac
g
Vas
h
Vas
i
Vas
j
Vas
k
Vas
l
Vas
m
DAPI
n
DAPI
o
DAPI
p
DAPI
q
DAPI
r
DAPI

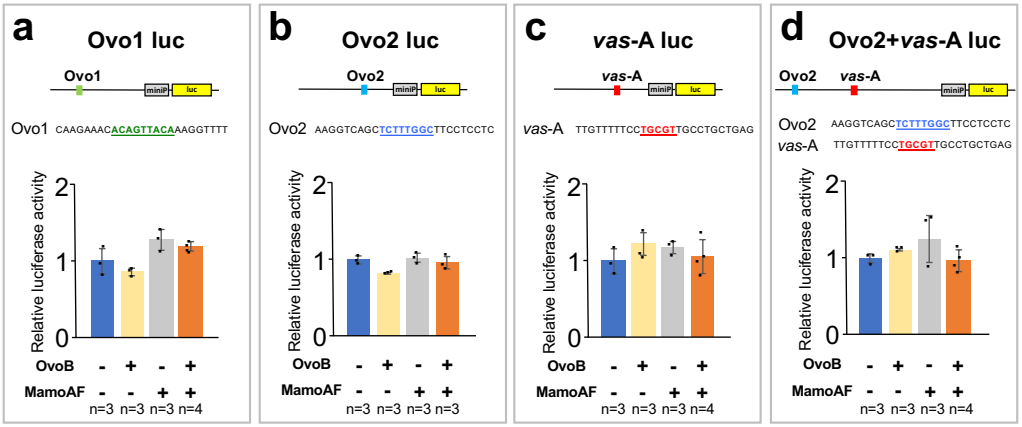
**Supplementary Figure 9** Maternal CBP activity is required for H3K27ac accumulation in PGCs. **a**-**r** Wild type embryos and $CBP^{M-}$ embryos derived from germline clones homozygous for $nej^{0.3}$ mutation at stage 5 are stained for histone modifications (**a**-**f**), Vas (**g**-**l**) and DAPI (**m**-**r**). Embryos are stained for H3K27ac (**a**, **b**), H3K9ac (**c**, **d**) and H4K16ac (**e**, **f**), respectively. Arrowheads indicate signals of the histone modification in PGCs. H3K27ac preferentially marks active enhancers. H3K9ac and H4K16ac, both of which are active histone markers. Scale bar: 10 μm.


a
OvoB-Nterm-egfp
ovo gene
Ovo-A mRNA
Ovo-B mRNA
Svb mRNA
Svb specific probe
Ovo common probe
Ovo common probe
Svb specific probe
Wild type
MamoAF OE
b
c
d
e
f
g
h
i
Ovo common probe
Svb common probe
Ovo mRNA
Ovo mRNA Elav mRNA
Svb mRNA
Svb mRNA Elav mRNA
Wild type
MamoAF OE
j
k
l
m
n
o
p
q

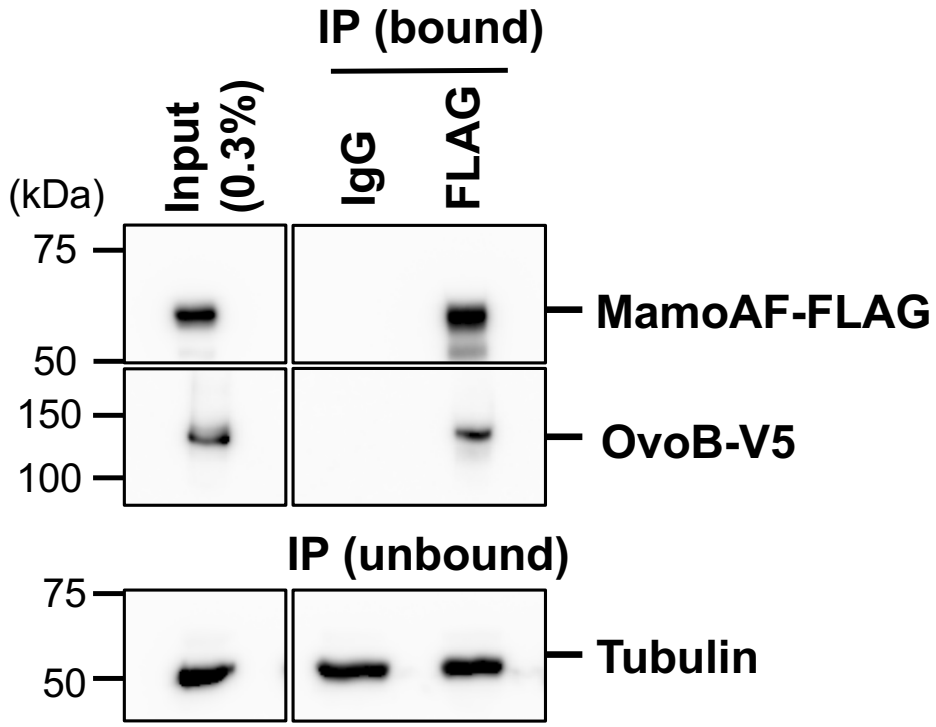
**Supplementary Figure 10** MamoAF induces ectopic Ovo mRNA expression in brain. **a** A schematic diagram of *ovo* locus. **b**-**i** *in situ* hybridisation of wild type embryos and MamoAF OE using an Ovo common probe (**b**, **c**, **f** and **g**) and a Svb specific probe (**d**, **e**, **h** and **i**). Right panels (**c**, **e**, **g** and **i**) are close-up views of brain of left panels (**b**, **d**, **f** and **h**), respectively. Arrowheads indicate ectopic Ovo signals. Scale bar: 50 μm. **j**-**q** Double FISH of wild type embryos and MamoAF OE. Confocal sections of the brain of the embryos hybridised with Ovo and Elav probes (**j**, **k**, **n** and **o**) and with Svb and Elav probes (**l**, **m**, **p** and **q**). Elav is a neural marker. Scale bar: 20 μm.


a
1kbp
2L:
15,061,805>
15,068,333
EcoR I
Ovo1
Xho I
Ovo2
Stu I
vas-A
ACAGTTACA
TCTTTGGC
TGCGT
Ovo1 fragment
Ovo2 fragment
vas-A fragment
Ovo2+vas-A fragment
Ovo1 Sac I fragment
Pst I
Hind III
Sac I
b
Ovo1 luc
Kpn I
GGTACCTACTAGTCCAGTGTGGTGGAATTC
Ovo2 luc
Stu I/EcoR V
vas-A luc
EcoR V/Sma I
GATGGGCTGCAG
CTGCAGGAATTCGATATCAAGCTT
Ovo2+vas-A luc
Ovo1+vas-A luc
GAGCTCGCTAGCCTCGAGGATGGGCTGCAG
Ovo1mut+
vas-Amut luc
ACCCCGGGA
TTAAG
Ovo1mut+
vas-A luc
Ovo1+
vas-Amut luc
miniP
luc

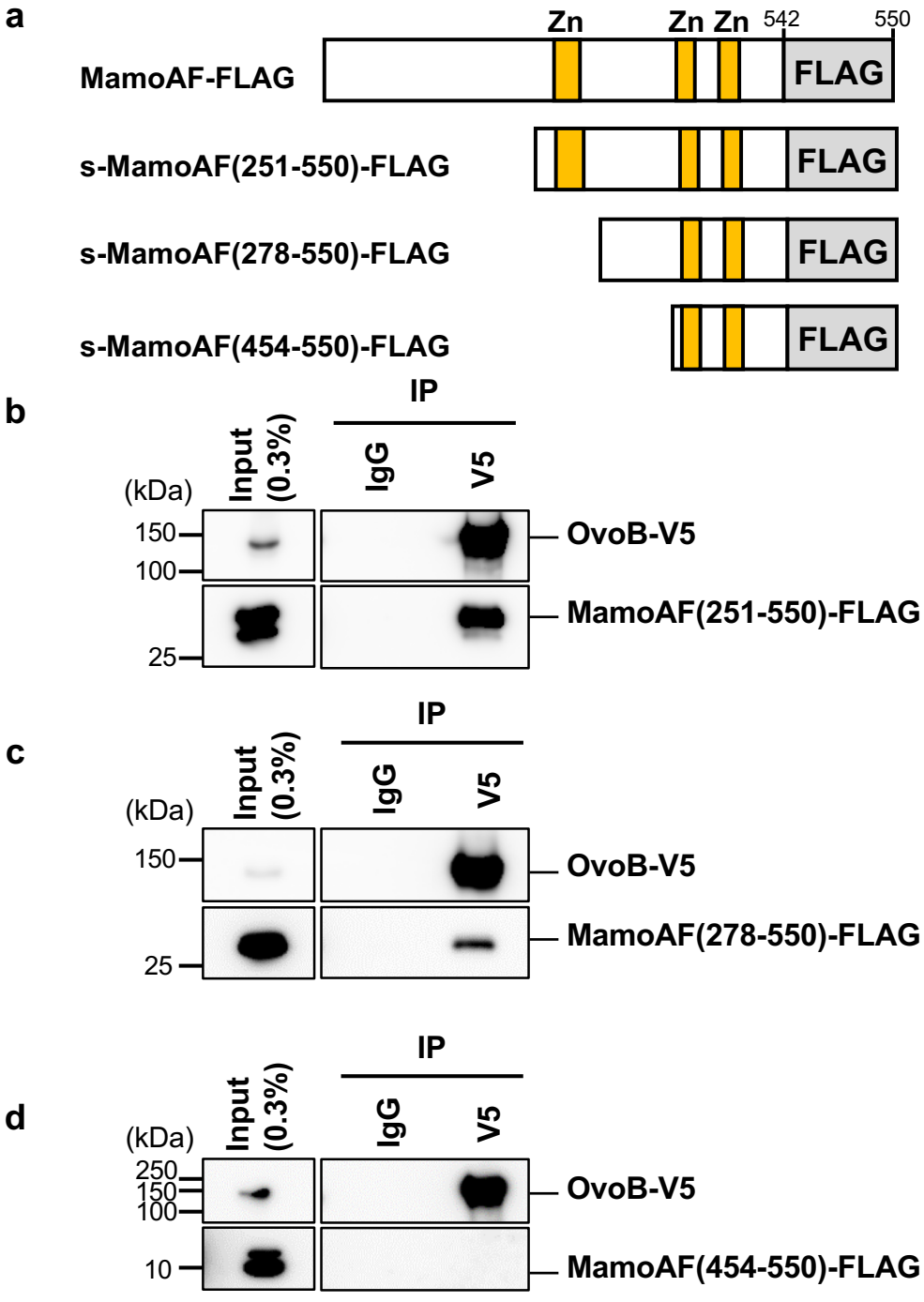
**Supplementary Figure 11** The luciferase reporter constructs containing cis-elements in the first intron of *vas*. **a** Schematic representation of the first intron of *vas* (2L: 15,061,805–15,068,333) and the genomic fragments containing the cis-elements used to construct the luciferase reporters. **b** Schematic representation of the luc reporter constructs used in this study. Restriction enzymes and linker sequences are shown. Restriction sites indicated by black are endogenous sites. Yellow indicates the primer-induced restriction sites. Gray box, mini promoter. Yellow box, luciferase reporter gene.


a
Ovo1 luc
Ovo1
miniP
luc
Ovo1 CAAGAAACACAGTTACAAAGGTTTT
Relative luciferase activity
2
1
0
OvoB - + - +
MamoAF - - + +
n=3 n=3 n=3 n=4
b
Ovo2 luc
Ovo2
miniP
luc
Ovo2 AAGGTCAGCTCTTTGGCTTCCTCCTC
Relative luciferase activity
2
1
0
OvoB - + - +
MamoAF - - + +
n=3 n=3 n=3 n=3
c
vas-A luc
vas-A
miniP
luc
vas-A TTGTTTTTCCTGCGTTGCCTGCTGAG
Relative luciferase activity
2
1
0
OvoB - + - +
MamoAF - - + +
n=3 n=3 n=3 n=4
d
Ovo2+vas-A luc
Ovo2
vas-A
miniP
luc
Ovo2 AAGGTCAGCTCTTTGGCTTCCTCCTC
vas-A TTGTTTTTCCTGCGTTGCCTGCTGAG
Relative luciferase activity
2
1
0
OvoB - + - +
MamoAF - - + +
n=3 n=3 n=3 n=4

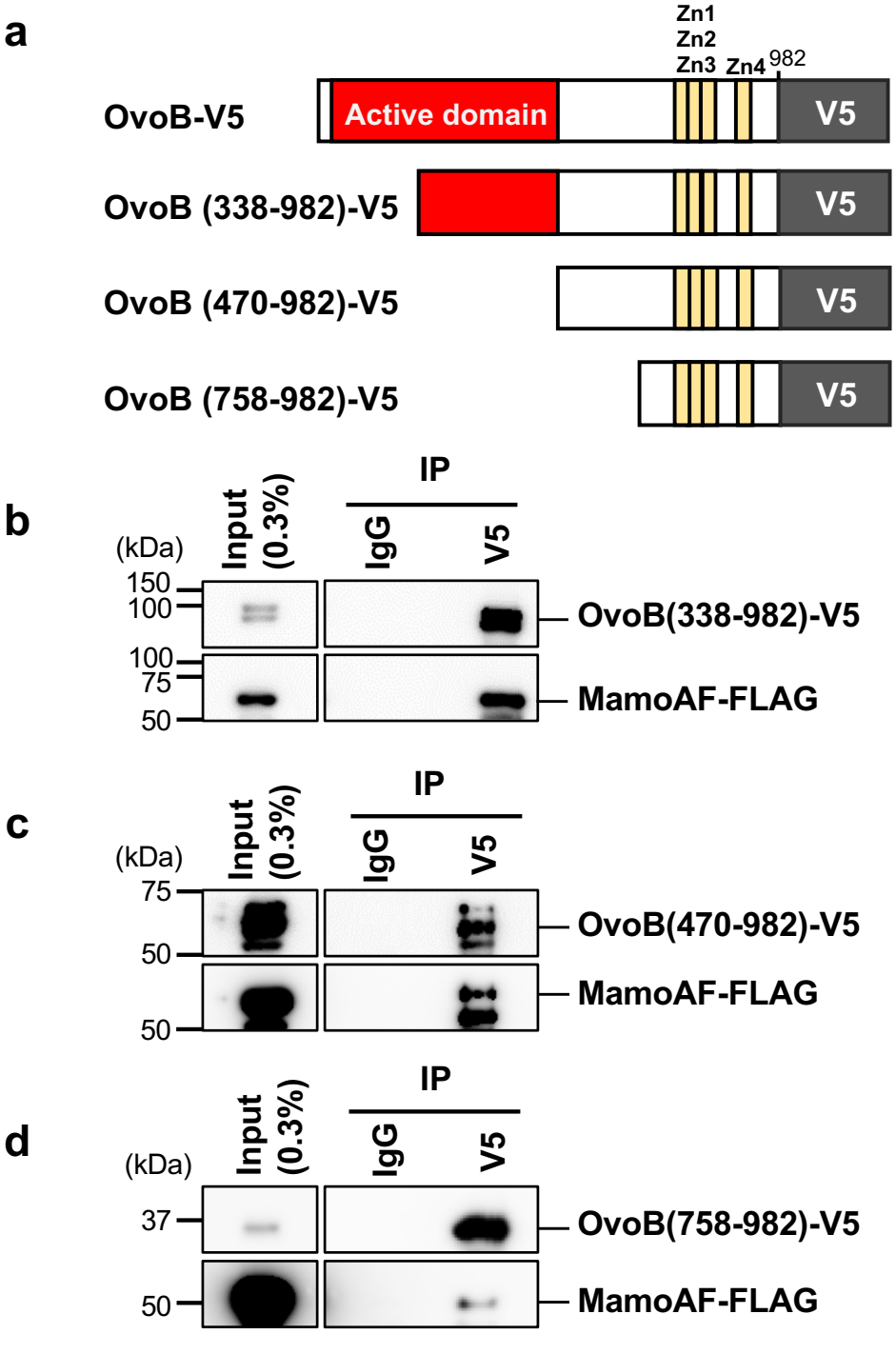
**Supplementary Figure 12** The individual cis-elements in the first intron of *vas* does not response to MamoAF and OvoB. The luciferase reporter constructs containing the *vas* intronic fragments, Ovo1 luc, Ovo2 luc, *vas*-A luc and Ovo2+*vas*-A luc were used for reporter assays. The mean of relative luciferase activities and SD are shown.


IP (bound)
Input (0.3%)
IgG
FLAG
(kDa)
75
50
150
100
MamoAF-FLAG
OvoB-V5
IP (unbound)
75
50
Tubulin

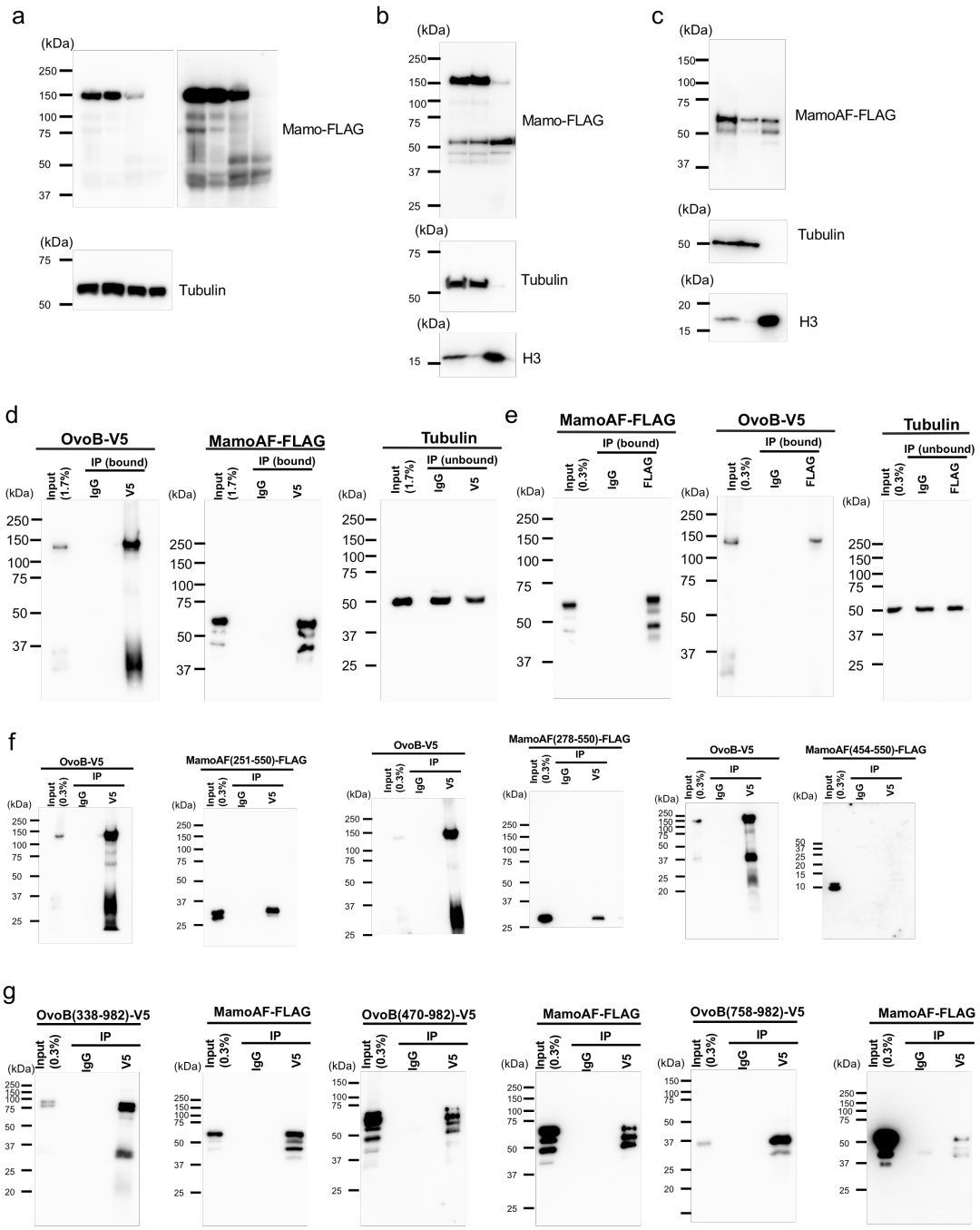
**Supplementary Figure 13** MamoAF interacts with OvoB. Immunoprecipitation assays with nuclear extracts of S2 cells that had been transfected with both FLAG-tagged MamoAF and V5-tagged OvoB. The precipitate with a FLAG antibody were analysed by FLAG and V5 antibodies.


a
Zn
Zn Zn
542
550
MamoAF-FLAG
FLAG
s-MamoAF(251-550)-FLAG
FLAG
s-MamoAF(278-550)-FLAG
FLAG
s-MamoAF(454-550)-FLAG
FLAG
b
IP
Input (0.3%)
IgG
V5
(kDa)
150
100
25
OvoB-V5
MamoAF(251-550)-FLAG
c
IP
Input (0.3%)
IgG
V5
(kDa)
150
25
OvoB-V5
MamoAF(278-550)-FLAG
d
IP
Input (0.3%)
IgG
V5
(kDa)
250
150
100
10
OvoB-V5
MamoAF(454-550)-FLAG

**Supplementary Figure 14** N-terminal region of MamoAF is required for the interaction with OvoB. **a** A schematic diagram of FLAG-tagged MamoAF fragments used for immunoprecipitation assays. **b**-**d** Immunoprecipitation assays with nuclear extracts of S2 cells that had been transfected with both V5-tagged OvoB and FLAG-tagged MamoAF fragments: MamoAF(251-550)-FLAG (**b**), MamoAF(278-550)-FLAG (**c**) and MamoAF(454-550)-FLAG (**d**).


a
Zn1
Zn2
Zn3
Zn4
982
OvoB-V5
Active domain
V5
OvoB (338-982)-V5
V5
OvoB (470-982)-V5
V5
OvoB (758-982)-V5
V5
b
IP
Input (0.3%)
IgG
V5
(kDa)
150
100
100
75
50
OvoB(338-982)-V5
MamoAF-FLAG
c
IP
Input (0.3%)
IgG
V5
(kDa)
75
50
50
OvoB(470-982)-V5
MamoAF-FLAG
d
IP
Input (0.3%)
IgG
V5
(kDa)
37
50
OvoB(758-982)-V5
MamoAF-FLAG

**Supplementary Figure 15** N-terminal region of OvoB enhances the interaction between MamoAF and OvoB. **a** A schematic diagram of V5-tagged OvoB fragments used for immunoprecipitation assays. **b**-**d** Immunoprecipitation assays with nuclear extracts of S2 cells that had been transfected with both MamoAF-FLAG and V5-tagged OvoB fragments: OvoB(338-982)-V5 (**b**), OvoB(470-982)-V5 (**c**) and OvoB(758-982)-V5 (**d**).


a
(kDa)
250
150
100
75
50
37
Mamo-FLAG
(kDa)
75
50
Tubulin
b
(kDa)
250
150
100
75
50
37
25
Mamo-FLAG
(kDa)
75
50
Tubulin
(kDa)
15
H3
c
(kDa)
150
100
75
50
37
MamoAF-FLAG
(kDa)
50
Tubulin
(kDa)
20
15
H3
d
OvoB-V5
Input (1.7%)
IP (bound)
IgG
V5
(kDa)
250
150
100
75
50
37
MamoAF-FLAG
Input (1.7%)
IP (bound)
IgG
V5
(kDa)
250
150
100
75
50
37
Tubulin
Input (1.7%)
IP (unbound)
IgG
V5
(kDa)
250
150
100
75
50
37
25
e
MamoAF-FLAG
Input (0.3%)
IP (bound)
IgG
FLAG
(kDa)
250
150
100
75
50
37
OvoB-V5
Input (0.3%)
IP (bound)
IgG
FLAG
(kDa)
250
150
100
75
50
37
Tubulin
Input (0.3%)
IP (unbound)
IgG
FLAG
(kDa)
250
150
100
75
50
37
25
f
OvoB-V5
Input (0.3%)
IP
IgG
V5
(kDa)
250
150
100
75
50
37
25
MamoAF(251-550)-FLAG
Input (0.3%)
IP
IgG
V5
(kDa)
250
150
100
75
50
37
25
OvoB-V5
Input (0.3%)
IP
IgG
V5
(kDa)
250
150
100
75
50
37
25
MamoAF(278-550)-FLAG
Input (0.3%)
IP
IgG
V5
(kDa)
250
150
100
75
50
37
25
OvoB-V5
Input (0.3%)
IP
IgG
V5
(kDa)
250
150
100
75
50
37
25
20
MamoAF(454-550)-FLAG
Input (0.3%)
IP
IgG
V5
(kDa)
50
37
25
20
15
10
g
OvoB(338-982)-V5
Input (0.3%)
IP
IgG
V5
(kDa)
250
150
100
75
50
37
25
20
MamoAF-FLAG
Input (0.3%)
IP
IgG
V5
(kDa)
250
150
100
75
50
37
25
OvoB(470-982)-V5
Input (0.3%)
IP
IgG
V5
(kDa)
150
100
75
50
37
25
MamoAF-FLAG
Input (0.3%)
IP
IgG
V5
(kDa)
250
150
100
75
50
37
25
OvoB(758-982)-V5
Input (0.3%)
IP
IgG
V5
(kDa)
150
100
75
50
37
25
20
MamoAF-FLAG
Input (0.3%)
IP
IgG
V5
(kDa)
250
150
100
75
50
37
25

**Supplementary Figure 16 Original blots.**
**a** Original blots of Figure 1a. Membranes were cut before incubation with the antibody.
**b** Original blots of Figure 1b. Membranes were cut before incubation with the antibody.
**c** Original blots of Figure 1c. Membranes were cut before incubation with the antibody.
**d** Original blots of Figure 6g.
**e** Original blots of Supplementary Figure 13.
**f** Original blots of Supplementary Figure 14.
**g** Original blots of Supplementary Figure 15.

**Supplementary Table 1**
Differentiation of egg chambers containing *mamo* germline clones can be rescued by overexpressing full-length Mamo-FLAG and MamoAF-FLAG

| Genotype[a] | Percentages of ovarioles with differentiating egg chambers (n) [e] |
|---|---|
| Wild type | 97.7 (128) |
| *mamo$^{SVA53}$/mamo$^{SVA53}$, nos-Gal4*[b] | 1.4 (74) |
| *mamo$^{SVA53}$/mamo$^{SVA53}$, UASp-Mamo-FLAG, nos-Gal4*[c] | 28.8 (73) |
| *mamo$^{SVA53}$/mamo$^{SVA53}$, UASp-MamoAF-FLAG, nos-Gal4*[d] | 13.2 (197) |

[a] Genotypes of females.
[b] Females with germline clones homozygous for the *mamo* mutation and carrying *nos-Gal4* driver were dissected and the ovaries were examined.
[c] Females with germline clones homozygous for the *mamo* mutation and carrying both *UASp-Mamo-FLAG* and *nos-Gal4* driver were dissected and the ovaries were examined.
[d] Females with germline clones homozygous for the *mamo* mutation and carrying both *UASp-MamoAF-FLAG* and *nos-Gal4* driver were dissected and the ovaries were examined.
[e] Ovarioles containing egg chambers after oogenic stage 6 were scored.

**Supplementary Table 2**
Differentiation of mature eggs derived from *mamo* germline clones can be rescued by overexpressing full-length Mamo-FLAG but not MamoAF-FLAG

| Maternal genotype[a] | Eggs/h/female[e] | n | Hatching (%)[f] | n |
|---|---|---|---|---|
| Wild type | 1.07 ± 0.37 | 8 | 92.1 ± 1.5 | 8 |
| *mamo$^{SVA53}$/mamo$^{SVA53}$, nos-Gal4*[b] | 0 | 4 | - | - |
| *mamo$^{SVA53}$/mamo$^{SVA53}$, UASp-Mamo-FLAG, nos-Gal4*[c] | 0.29 ± 0.1 | 4 | 36.9 ± 9.1 | 4 |
| *mamo$^{SVA53}$/mamo$^{SVA53}$, UASp-MamoAF-FLAG, nos-Gal4*[d] | 0 | 4 | - | - |

[a] Genotypes of females.
[b] Females with germline clones homozygous for the *mamo* mutation and carrying *nos-Gal4* driver were used.
[c] Females with germline clones homozygous for the *mamo* mutation and carrying both *UASp-Mamo-FLAG* and *nos-Gal4* driver were used.
[d] Females with germline clones homozygous for the *mamo* mutation and carrying both *UASp-MamoAF-FLAG* and *nos-Gal4* driver were used.
[e] Females with each genotype were mated with *y w* males. 30–77 females were used. Eggs produced by the females were scored. Standard deviation is given.
[f] 30–151 embryos were collected, and incubated at 25 °C for 1 day. Hatching rate of embryos was scored. Standard deviation is given.