Supplement

Revealing the DHR3-inducible *ftz-f1* transcript

At the beginning of the study, we mapped the DHR3-inducible transcript of the *ftz-f1* gene. Currently, three *ftz-f1* transcripts (A, B, and C) are annotated in the FlyBase (Supplementary Fig. 1A). Two of them, *ftz-f1-A* and *ftz-f1-B,* were identified more than 10 years ago (23,44). It has been considered that *ftz-f1-B* is expressed only in the early embryo (0–4 h) and probably independently of DHR3, while *ftz-f1-A* is expressed in a DHR3-dependent mode, i.e., immediately after the peak of DHR3 expression both in 16-h embryos and at the pupal stage. Lam et al. (1997) have pointed out that the pupal *ftz-f1* transcript starts upstream of the annotated transcription start site. Finally, the third transcript variant, *ftz-f1-C,* has appeared in the database a few months ago, its start site coinciding with that suggested by the above authors.

Using probes specific for different transcripts we found that only the *ftz-f1-C* transcript was strongly induced in S2 cells at the (+;–) stage (Supplementary Figs. 1B, 1C). The same transcript was also induced in pupae during metamorphosis (Supplement Fig. 1D). The precise start of the pupal inducible *ftz-f1-C* transcript was determined by 5'-RACE and mapped to the position about 1320 bp upstream of the start site previously annotated for *ftz-f1-A*. It proved to precisely coincide with the start indicated for the *ftz-f1-C* transcript in the FlyBase (Supplementary Fig. 1A). The inducible promoter and approximately 2 kb of the downstream region were located in the long intron, far from the constitutive promoter (Supplementary Fig. 1A).

In our opinion, *ftz-f1-C* is a DHR3-dependent pupal form of the *ftz-f1* gene, while previously annotated *ftz-f1-A* is a truncated form of *ftz-f1*-C.

Pol II distribution on the *hsp70* gene in transcriptionally repressed and active states

To be confident in the proper work of antibodies in ChIP, we first used them to study the distribution of Pol II on the *hsp70* gene in S2 cells before and after heat shock (Supplementary Fig 2). All antibodies confirmed their specificity by revealing Pol II distribution patterns similar to those described previously by other authors. Ser5-(P) Pol II was detected on the *hsp70* promoter in the repressed state (Supplementary Fig. 2B). The level of Ser2-(P) on the promoter was low in the repressed state but strongly increased in the active state (following heat shock), indicating Ser2 modification of Pol II close to the transcription start site. Both Ser5-(P) and Ser2-(P) levels increased in the coding region of the gene after heat shock, indicating transcription elongation.

Nuclear run-on analysis of the *hsp70* and *ftz-f1* genes

The nuclear run-on assay was used to find out whether Pol II paused on the ftz-f1 gene in the repressed state is transcriptionally engaged. The run-on analysis was first performed with the *hsp70* gene taken as a control (Supplementary Fig. 3A). The observed level of the *hsp70* activation was similar to that reported previously (3). Likewise, the transcription of the *ftz-f1* gene proved to be stimulated severalfold by sarcosyl or high salt buffer (Supplementary Fig. 3B). Thus, Pol II paused on the *ftz-f1* gene at a high ecdysone titer was found to be competent and engaged in transcription.

Supplementary Fig. 1

(A) Maps of *ftz-f1* transcripts annotated in FlyBase, with numerals indicating positions of probes used in different experiments.

(B, C) Levels of *ftz-f1-B* (B) and *ftz-f1-C* (C) transcripts in normal S2 cells upon ecdysone treatment and after ecdysone removal (relative to the transcription level in uninduced S2 cells taken as 1). The level of inducible pupal *ftz-f1-C* strongly increased after ecdysone removal (+;–), whereas the level of noninducible *ftz-f1-B* remained unchanged at this stage.

(D) Northern blot analysis of poly(A)+RNA from different stages of *D. melanogaster* development with probes recognizing specific regions of the *ftz-f1-C* transcript

Supplementary Fig. 2

Distribution of total Pol II, Pol II Ser5-(P) and Pol II Ser2-(P) on the *hsp70* gene before and after its activation by heat shock. Positions of probes used in RT-PCR (ChIP) are indicated on the X axis. Each distribution profile represents the average data of at least three experiments. ChIP data are presented as a percentage of the input.

(A) Distribution profile of total Pol II along the *hsp70* gene before (dark bars) and after heat shock (light bars) as determined by ChIP with anti-Pol II monoclonal antibodies 7C2.

(B) Distribution profile of Ser5-(P) along the *hsp70* gene before (dark bars) and after heat shock (light bars) as determined by ChIP with antibodies against CTD Ser5-phosphorylated Pol II.

(C) Distribution profile of Ser2-(P) along the *hsp70* gene before (dark bars) and after heat shock (light bars) as determined by ChIP with antibodies against CTD Ser2-phosphorylated Pol II.

Supplementary Fig. 3

Nuclear run-on analysis performed for (A) *hsp70* in the repressed state (without heat shock) and (B) *ftz-f1* at the (+) stage (high ecdysone titer). The level of corresponding RNA was measured by qPCR at 5, 30, and 90 minutes after the start of the reaction. Transcription was performed in

buffers containing 80 mM KCl without NTPs (80-NTPs, negative control), 80 mM KCl (80), 800 mM KCl (800), or 80 mM KCl and 0.6% sarcosyl (80 Sarcosyl). Ecdysone (1 μM) was added to transcription buffers for *ftz-f1*. The level of transcription at each point is shown relative to that at the low salt concentration (80). The transcription of *ftz-f1* was analyzed with the same primers that were used for activation analysis (Fig. 1, point 4).

Supplementary Fig. 4

A region of high nucleosome density at the 1.5-kb position of the *ftz-f1* gene is enriched with nucleosome-positioning sequences. The plot shows the probability of nucleosome positioning (Y axis) at different points along the *Drosophila* 3L chromosome (X axis) as calculated using the software described in [34]. The gray line indicates the *ftz-f1* gene region where the peak of chromatin barrier was observed at the (+) stage.

Supplementary Fig. 5

SAYP knockdown leads to *ftz-f1* transcription in the repressed (+) state. The levels of *ftz-f1* induction upon SAYP knockdown relative to control cells were measured at different points of the gene. The primer pairs for each point were the same as in experiments shown in Fig. 2D in the main text.

Supplementary Fig. 6

Transcription levels of *E75a* and *Usp* genes measured by qPCR in the presence of ecdysone (+) and 5 hours after its removal (+;–) in SAYP RNAi knockdown cells (light bars), compared to those in normal cells at the (–) stage or sham-treated (control) cells (dark bars). It is seen that the knockdown strongly reduced SAYP transcription but had no effect on the transcription of *E75a* and *Usp*.

Supplementary Fig.7

Distribution profiles of SAYP, Brahma (PB and Moira) and TFIID (TAF1 and TBP) subunits, and Pol II on the *ftz-f1* gene determined 30 min after ecdysone removal in SAYP RNAi knockdown cells (dotted line) compared to normal cells (solid line) (see Fig. 3 in the main text). Sham-treated cells were used as a control in RNAi experiments. It is seen that the knockdown reduced only the level of PB (in addition to SAYP), having no effect on the presence of other factors on the gene.

Supplementary Fig. 8

Transcription levels of *cdk9* and *cycT* genes (components of the pTEF complex) measured by qPCR 3 hours after ecdysone removal (+;–) in SAYP RNAi knockdown cells (light bars), compared to those in sham-treated (control) cells (dark bars). It is seen that the knockdown had no effect on the transcription of these genes.

Primers

Primers for qPCR.

ftz-f1 gene fragments:

Region	Forward primer	Reverse primer
–1	ACAAAAAACTGCTGAAGAAGAGACC	ACTGTGGGTATGGCATTATGAAAG
0	GAGGCAGAGGCAGCGACG	GCTTTGTCATCTATGTGTGTGTTGTTG
1	GTTCTCTTGCTGCGTTGCG	GAAAGTGGGTCACGAATTTATTGC
2	ACCGCAACCTATTTTACTACC	TTAGAAGACCGAAGAGTTATCC
3	ACAACAACAATAACAACGACAATGATGC	CTGATTGCCGCTGCCACTCC
4	CAGCAGCAACAGCAACAGAATATC	GCGAGTGTGAGGAGGTGGTG
5	CTCCTCACACTCGCAACAGAGC	AGCAGCATGTAGCCACCGC
6	CTCCGTAAGAGTCAGCTTTAAC	CAGGGACATCACACATACG
7	GAGGAGGAGGTGGCAATAATGC	GATCCTATTCCAGCCTCGTGG
8	AACATCTTACCGGAAATCCATGC	ATCTCCATGAGCAGCGTTTGG
0*	AGTCAATCGAGATACGTGGTTGATG	GTAACGCTTTGTCATCTATGTGTGT
FTZ-F1B	CCGCCAGCATTTGATCCTTGTG	ACCACCTGCAACATCAGCATCA

Int (Intron of *ftz-f1)*	GCAGCAACATGGTTCAAAGC	TTCAATGCACATTCTGCCG

hsp70 gene fragments:

Region	Forward primer	Reverse primer
–1	ATTGTGGTAGGTCATTTGTTTGGC	GTGACAGAGTGAGAGAGCATTAGTG
0	ACATACTGCTCTCGTTGGTTCG	TTGAATTGAATTGTCGCTCCGTAG
1	CTACTCCTGCGTGGGTGTCTAC	ATGAGGCGTTCCGAGTCTGTG
2	TTGGGCGGCGAGGACTTTG	GCTGTTCTGAGGCGTCGTAGG
3	GACGAGGCAGTGGCATACGG	CCTCCAGCGGTCTCAATTCCC
4	GTAAAGCAGTCCGTGGAGCAG	CTTCTCGGCGGTGGTGTTG
5	TATTGTCAGGGAGTGAGTTTGC	GCTGTTTAATAGGGATGCCAAC
6	GCCCCGCTAAGTGAGTCCTG	GTTGTTGAACTCCGTAACCATTCTG
Run-on detection	AACAAGCAAAGTGAACACG	GCAGTTGATTTACTTGGTTG

For transcription level detection:

Gene	Forward primer	Reverse primer
DHR3	TGCTGAAGACGGGCTCCTTTG	CGAGTCGGATGTGTAGAACGC
ras	GAGGGATTCCTGCTCGTCTTCG	GTCGCACTTGTTACCCACCATC
SAYP/ e(y)3	CATCGTCGTCGTCGTCCTCAC	TGGTATCTGCTGCTGCTGCTG
E75a	ATGACGCCCGAGCAGATGAAG	CTGATTCTGGACCGCCGATGG
Usp	CAGAGCCTCCAGCCGAATTG	CGAGAGTCCCGTGCCCTTC
cdk9	GCCTGCGTCCGTATGTCAAG	CCAGAAGAAGTCGTGATTCAGAGC
cycT	CAATGAGCCTCTGAAGATGGTTATC	GTGGTGGTAGCCGTAGTTGG

Primers for RNAi experiments.

Gene	Forward primer	Reverse primer
SAYP/ e(y)3 (#1)	CTAGCCGGTAGTAGCTGCATCAG	CGAGTCGAACAGCAGTAGTCAGG
SAYP/ e(y)3 (#2)	GACCTTCACGATAAATCAGCCGC	TGCAGCTCGATGTGCTGCTAAAG
BAP170	CCGCCCAATTACAGATCATG	CAGCACATCAAGCAGTTTGG
pSK	GTTACATGATCCCCCATG	TTTCGCCCCGAAGAACG

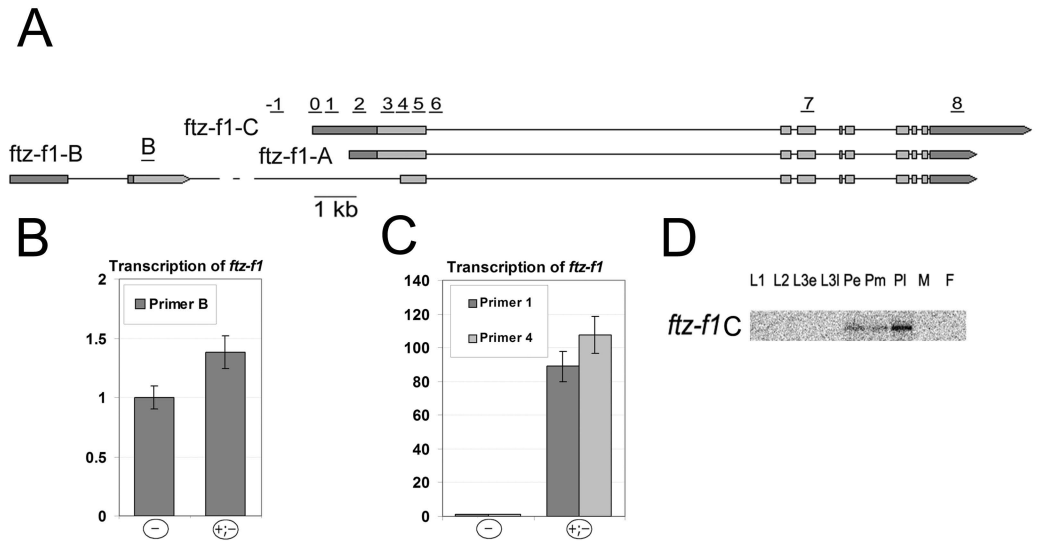
Primers for full-length transcripts detection by Northern blotting.

Gene	Forward primer	Reverse primer

DHR3	TCTTTCGAAGATCGCAGAGCTCC	CACATCGTTGATATCGCCATCC
ras	CGACGATGTGCCAGCCAAATTG	TCCTCTTGCCCTTCTTCTTGTAATCC
FTZ-F1 C	AGTCAATCGAGATACGTGGTTGATG	GAAAGTGGGTCACGAATTTATTGC

Supplementary reference

44. Koelle MR, Segraves WA, Hogness DS. (1992) DHR3: a Drosophila steroid receptor homolog. *Proc Natl Acad Sci U S A,* **89,** 6167-6171.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Transcription of ftz-f1
Primer B
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1
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+/−
C
Transcription of ftz-f1
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Primer 4
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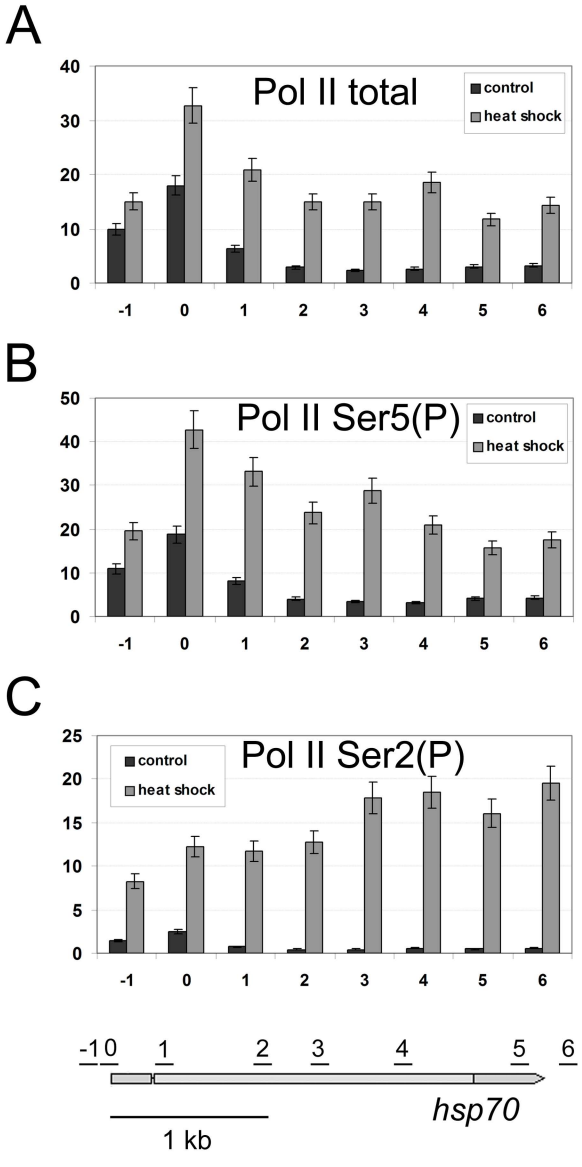
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control
heat shock
50
40
30
20
10
0
-1
0
1
2
3
4
5
6
C
Pol II Ser2(P)
control
heat shock
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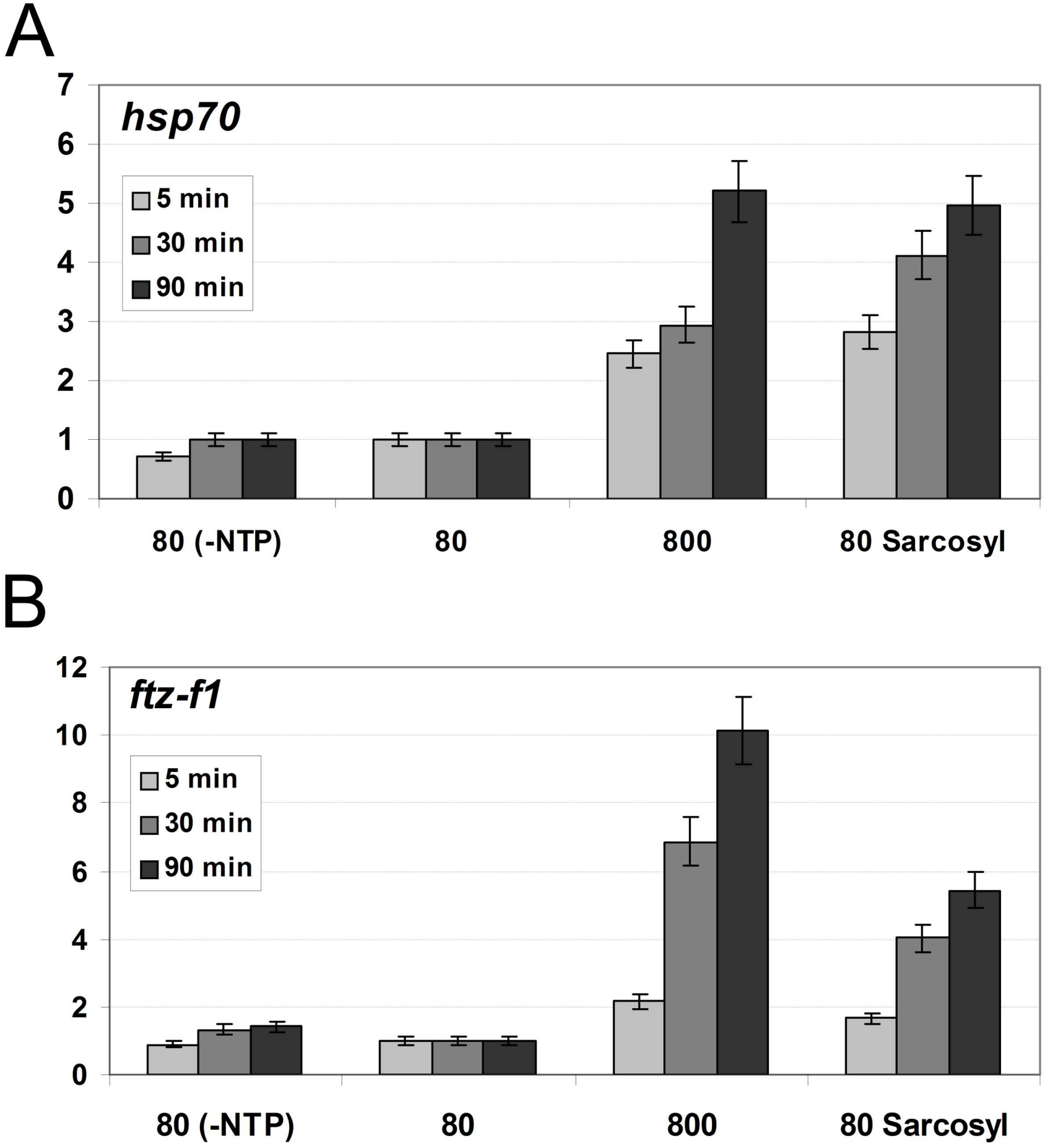
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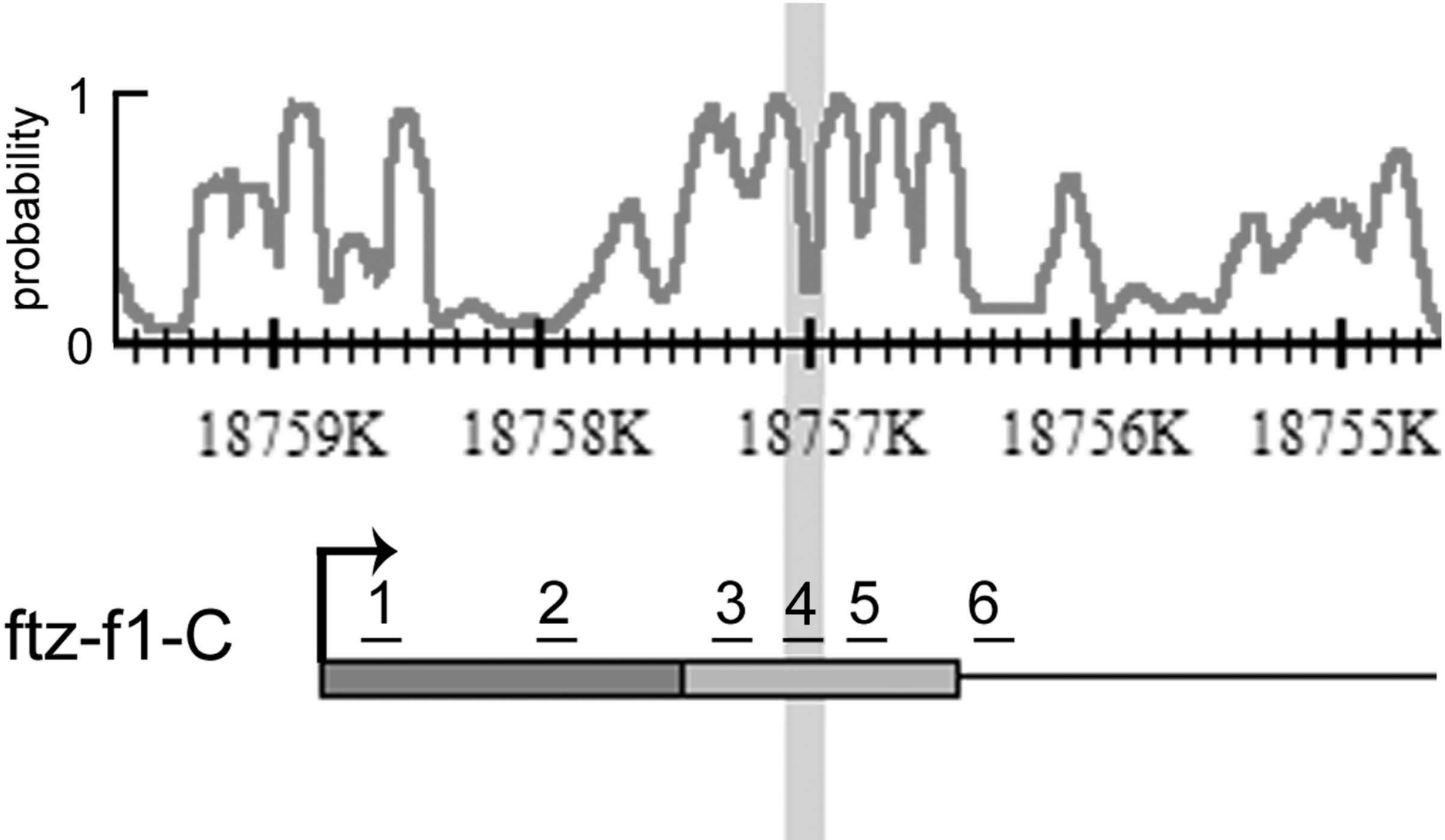
80 Sarcosyl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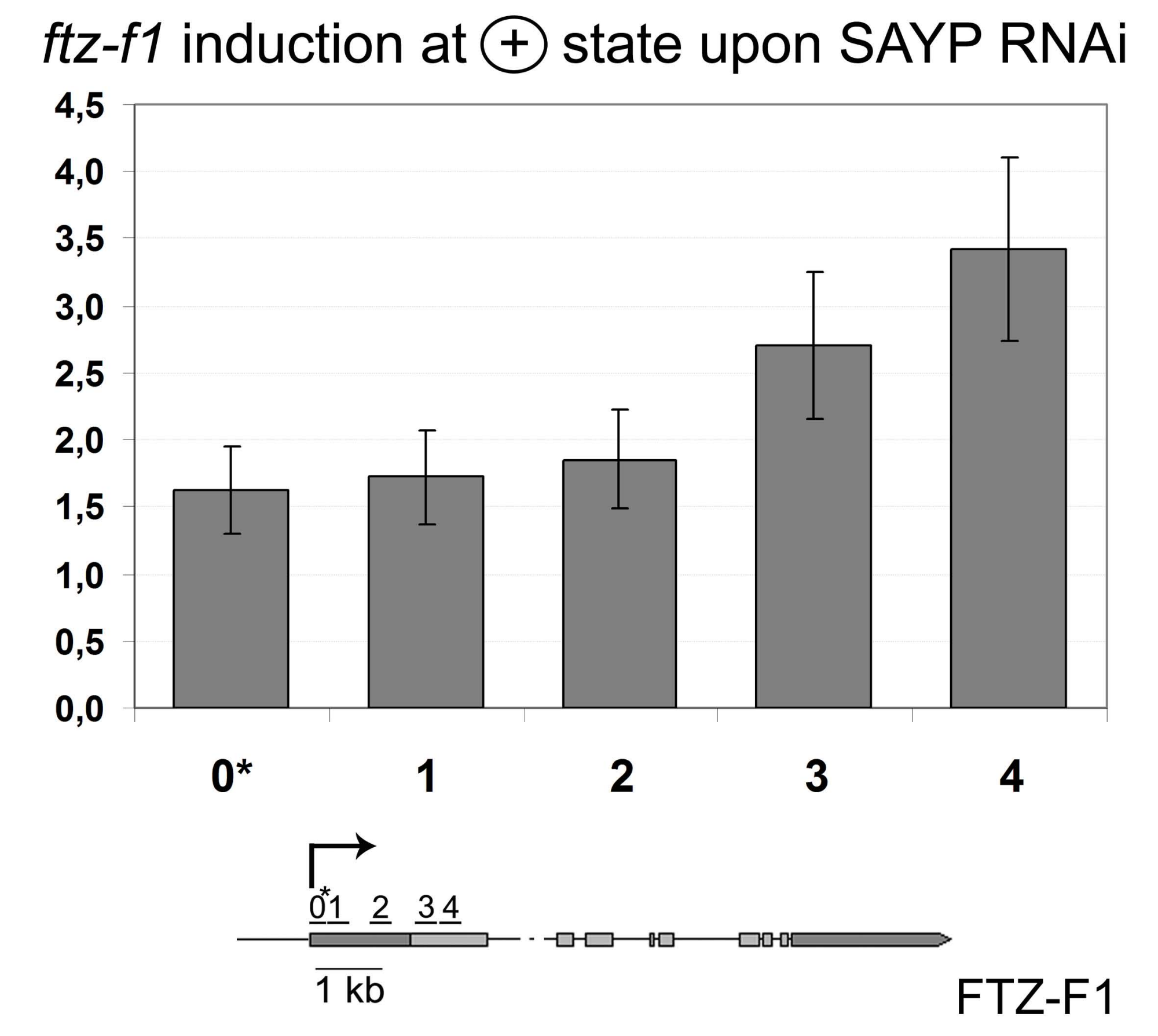
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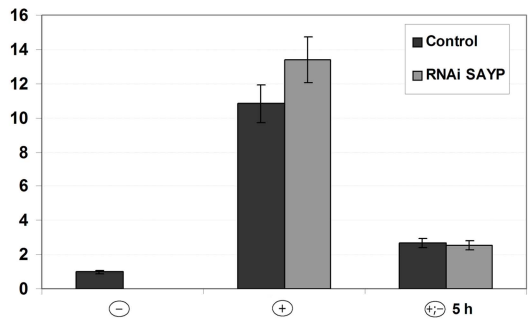
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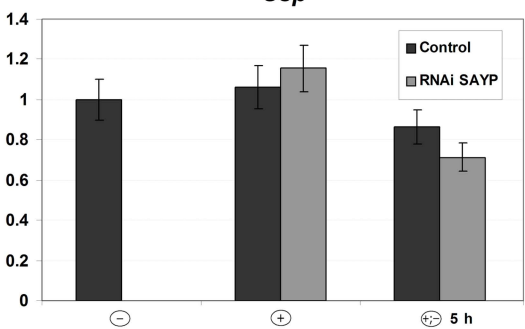
(+/−) 5 h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RNAi SAYP
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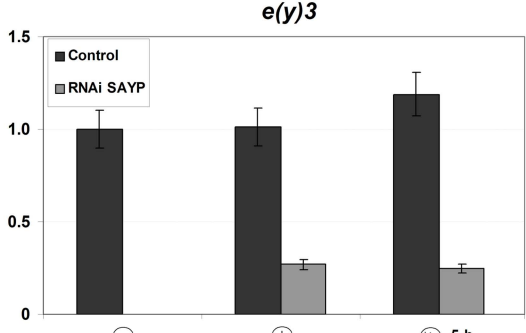
(+)
(+/−) 5 h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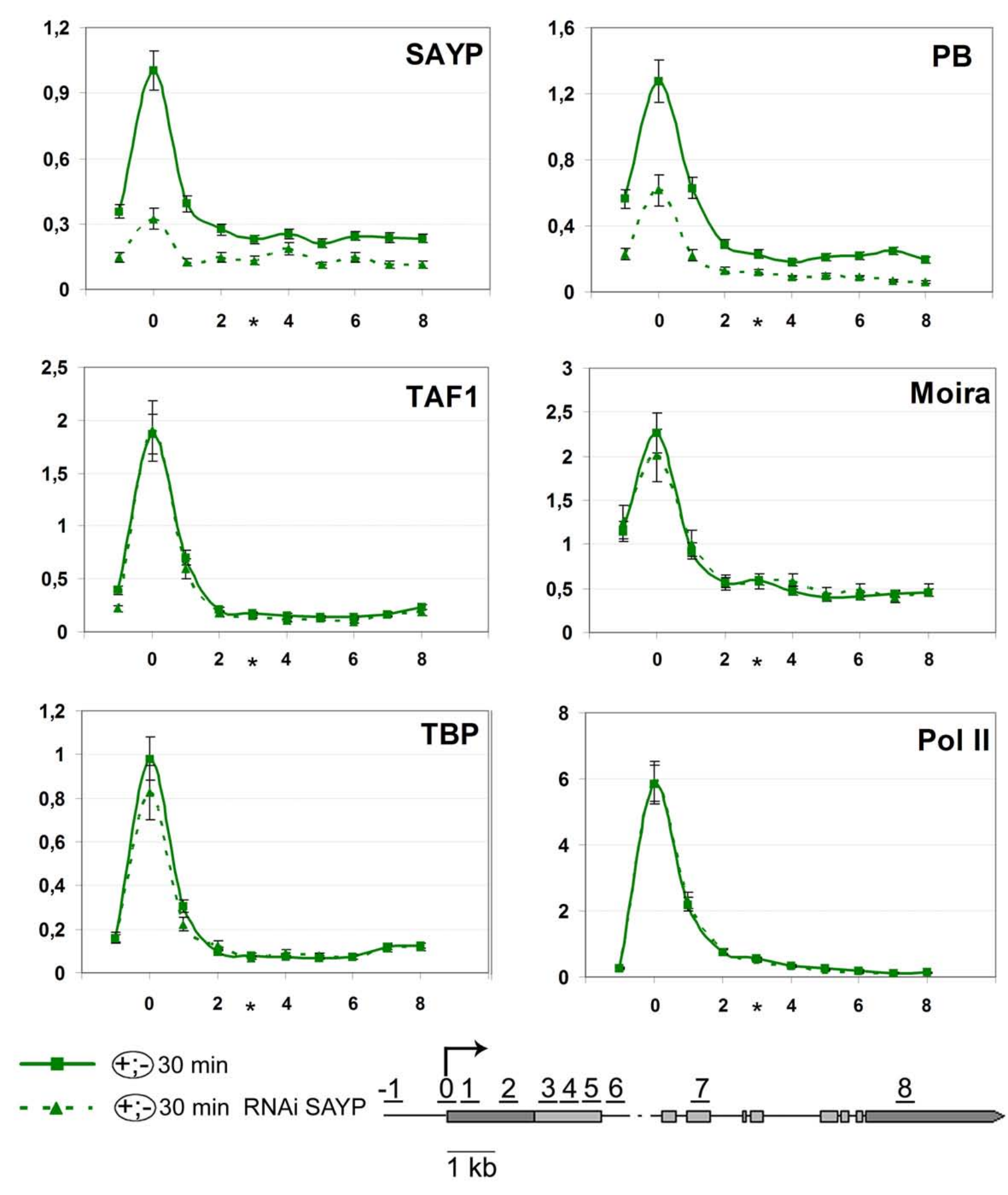
(+)
(+/−) 5 h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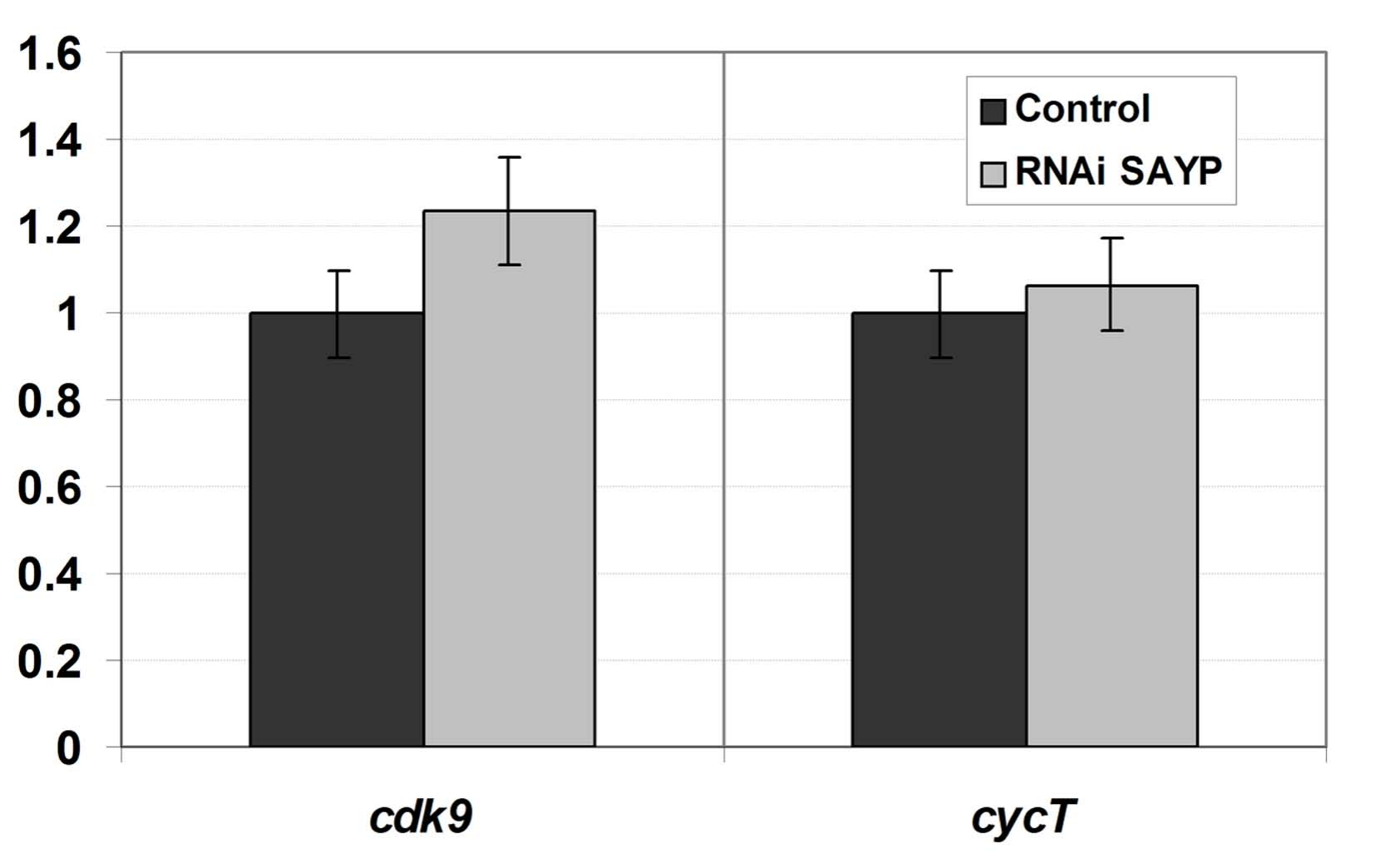
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