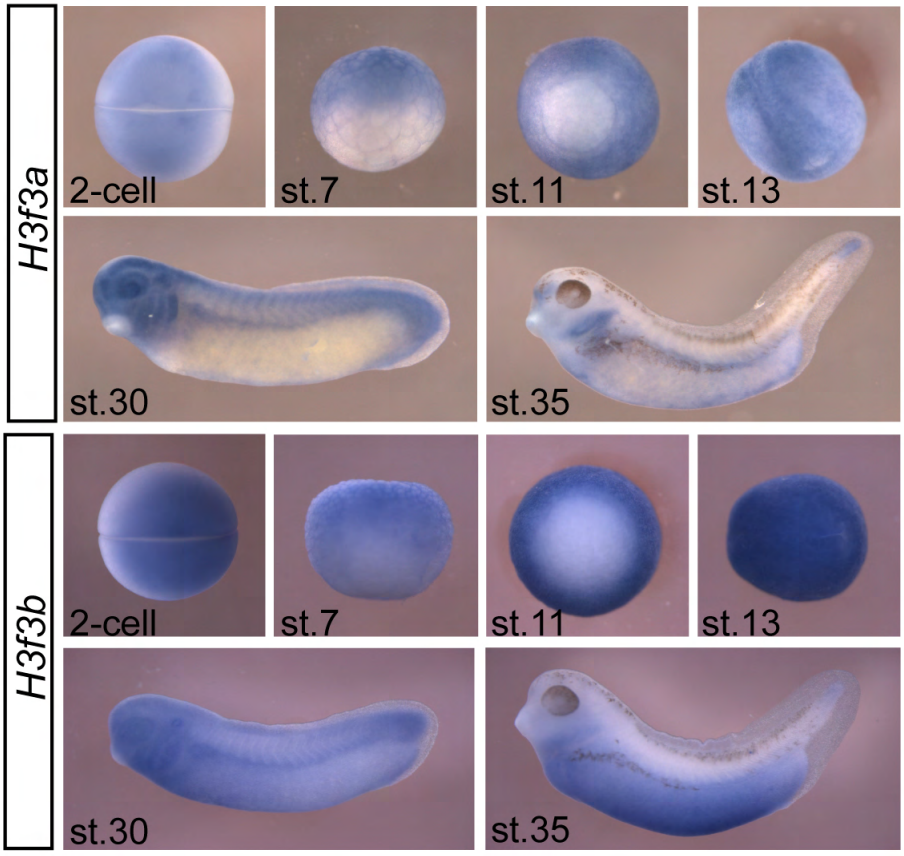

Fig. S1. *H3f3a* and *H3f3b* genes are co-expressed during early embryonic development. Expression patterns of *H3f3a* and *H3f3b* determined by whole-mount *in situ* hybridization.

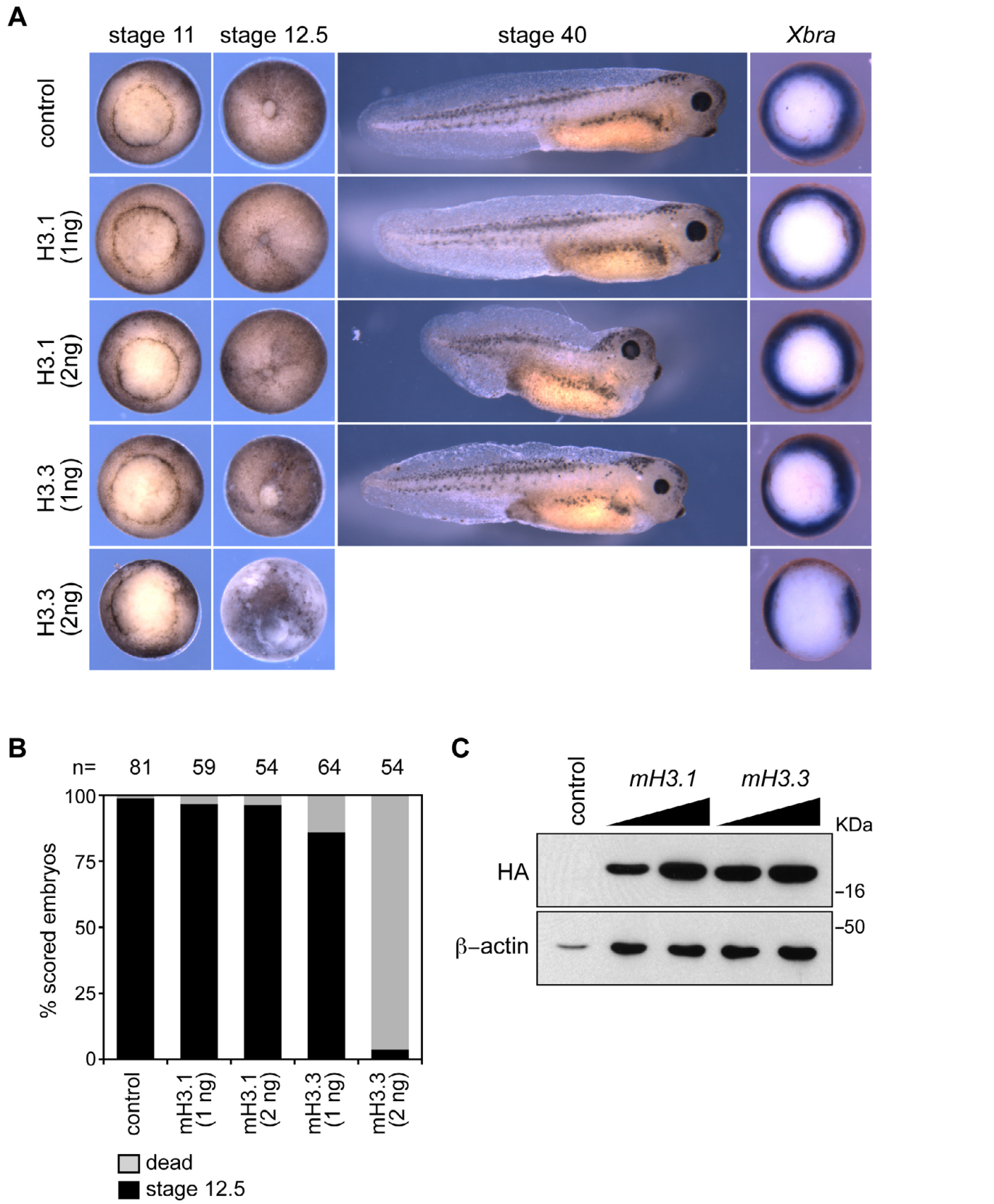

Fig. S2. Overexpression of H3.3 leads to early embryonic lethality. (**A**) Embryos at the two- to four-cell stages were injected with 1 or 2 ng of mRNA encoding HA-tagged H3.1 or H3.3. Injected embryos were either allowed to develop to late tailbud stage, or fixed at stage 10.5 and subjected to whole-mount *in situ* hybridization analysis of *Xbra* expression. (**B**) Percentage of injected embryos that developed normally to late gastrula stage 12.5. (**C**) Synthesis of exogenous H3.1-HA or H3.3-HA proteins in injected embryos detected by immunoblotting for the HA epitope.

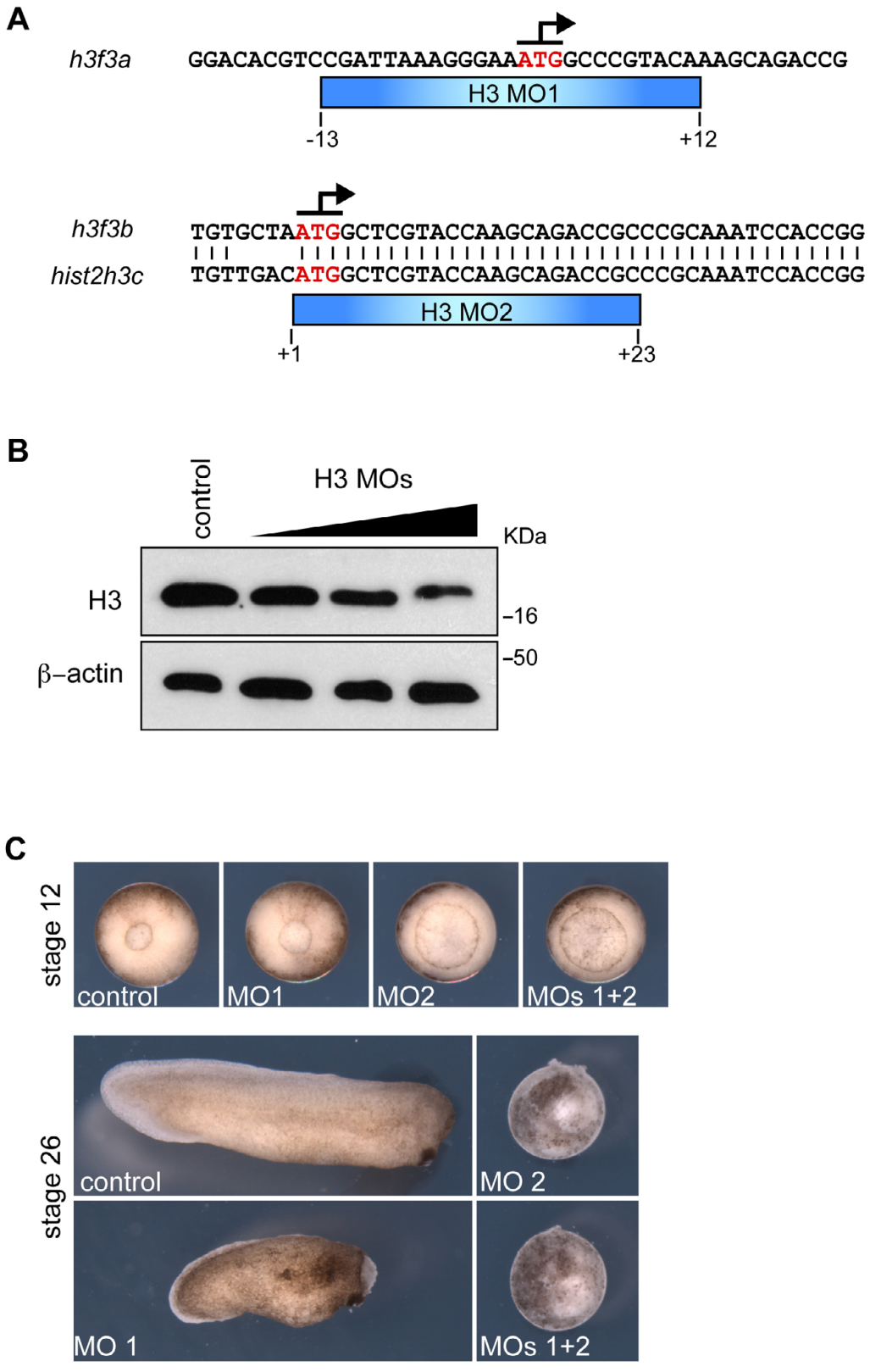

Fig. S3. Developmental defects in embryos partially or completely depleted of H3.3. (**A**) Target regions of H3 MO1 and MO2. (**B**) Western blot analysis showing dose-dependent depletion of endogenous H3 proteins in H3 MO-injected embryos. Doses of H3 MOs tested were 6.5 ng, 26 ng and 65 ng. (C) Development of control or embryos injected with H3 MO1 or MO2 alone or MO1+MO2 at stages 12 and 26.

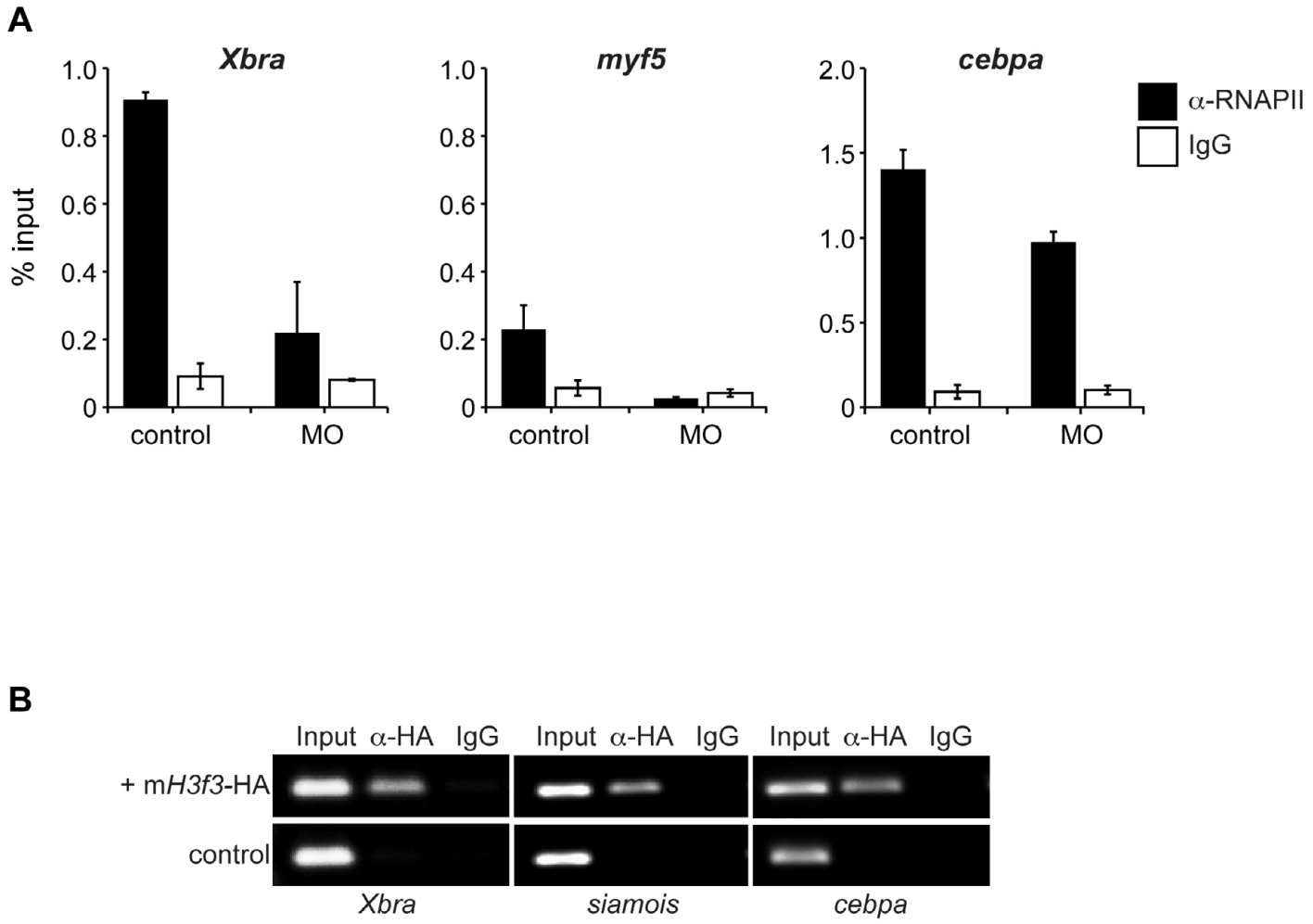

Fig. S4. Presence of RNA polymerase II and H3.3 at the promoters of active genes in gastrula embryos. (**A**) Presence of endogenous RNA polymerase II at the promoters of *Xbra*, *myf5* and *cebpa* were analyzed by ChIP-qPCR analysis of stage 10.5 control and H3 MOs-injected embryos. Level of enrichment is determined as a percentage of input. (**B**) Chromatin immunoprecipitation using anti-HA antibody was performed on stage 10.5 control and embryos injected with 750 pg of *mH3.3a*-HA mRNA. Presence of H3.3-HA at the promoters of *Xbra*, *siamois* and *cebpa* were analyzed by PCR using promoter-specific primers.

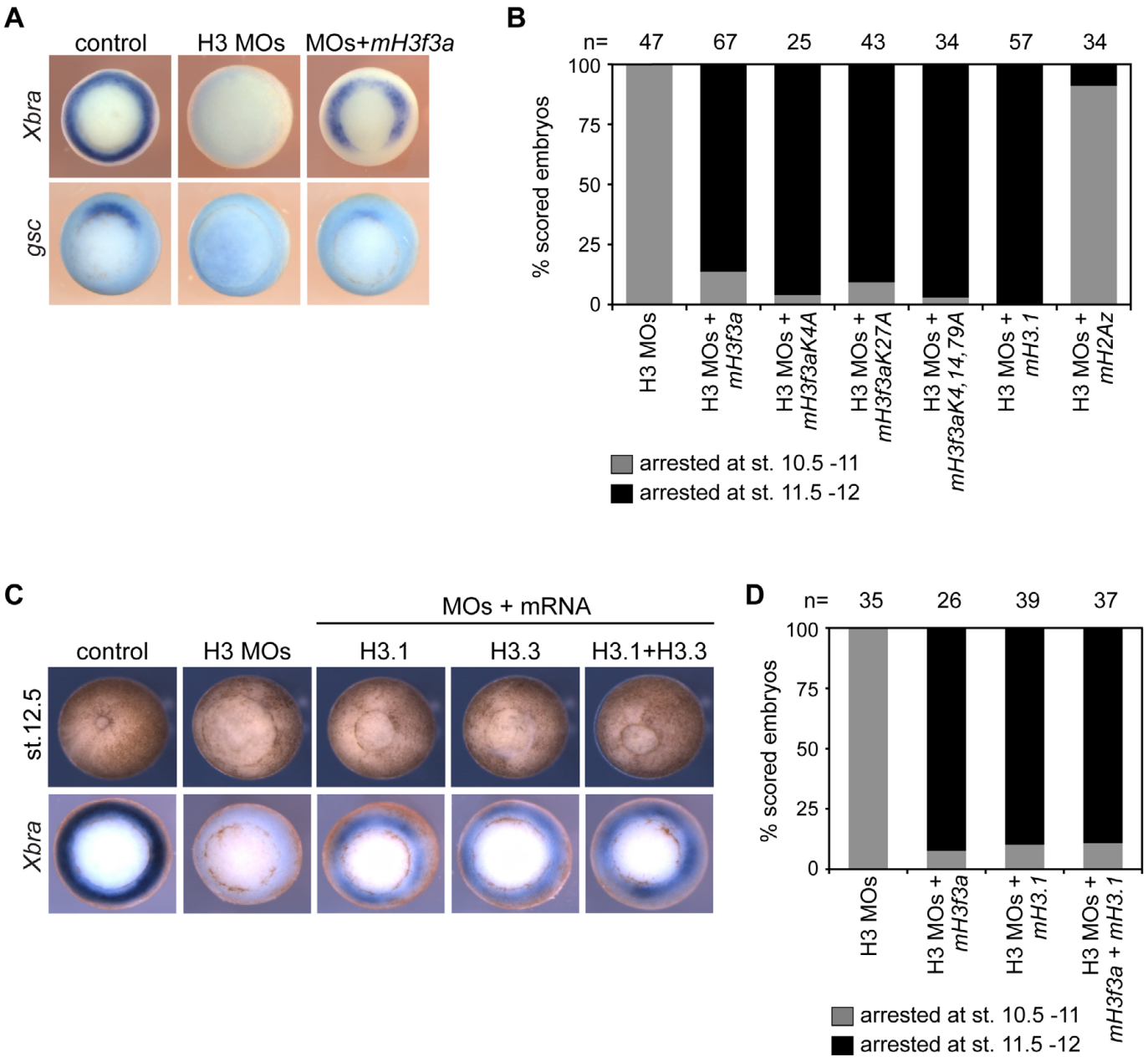

Fig. S5. Mesoderm formation and gene expression in H3 MOs morphants is rescued by co-injection of mouse *H3f3a* mRNA. (**A**) Control and injected embryos fixed at early gastrula were subjected to RNA *in situ* hybridization analysis of *Xbra* and *gsc* transcripts. (**B**) Percentage of embryos co-injected with mRNA encoding HA-tagged wild-type or mutant H3.3, or H3.1 that arrested at early (stage 10.5-11) or late (stage 11.5-12) gastrula stages. (**C**) Rescue experiments were performed by co-injecting H3.3 MO with a total amount of 750 pg of mRNA encoding HA-tagged H3.1 or H3.3, or both. Injected embryos were either allowed to develop to late gastrula stage 12.5 (top panels), or fixed at stage 10.5 and subjected to whole-mount *in situ* hybridization analysis of *Xbra* expression (bottom panels). (**D**) Percentage of injected embryos that arrested at early (stage 10.5-11) or late (stage 11.5-12) gastrula stages.

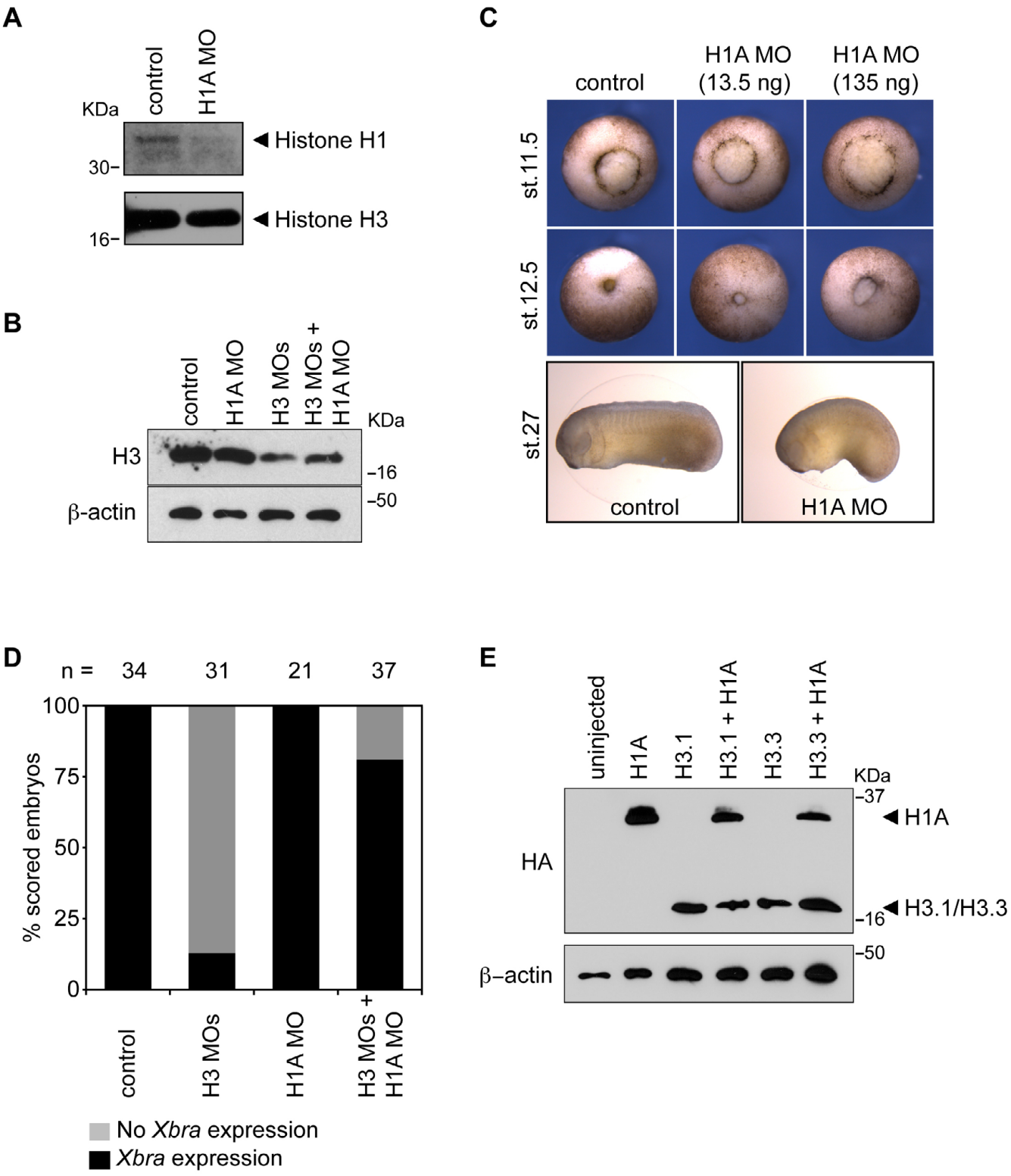

Fig. S6. Mesodermal competence is regulated by interplay between nucleosomal H3 and linker histone H1. (**A**) Synthesis of somatic linker histone H1A is inhibited by H1A antisense MO. Western blot analysis of acid nuclear extracts from gastrula stage control or H1A MO-injected embryos shows ~70% knockdown of histone H1 (arrow). (**B**) Western blot analysis of endogenous H3 protein levels in gastrula stage control and injected embryos. (**C**) Time-lapse images tracking development of control and H1A-depleted embryos. Development of H1A knockdown embryos was slightly delayed but otherwise normal. (**D**) Percentage of control and injected embryos that express *Xbra*. Embryos from two independent experiments were fixed at early gastrula and subjected to RNA *in situ* hybridization analysis of *Xbra* transcripts. (**E**) Protein synthesis of injected mRNAs shown by western blot analysis of cellular extracts from stage 10.5 embryos.

Table S1. The mean sizes of micrococcal nuclease digested DNA fragments from control or injected early gastrula stage 10.5 embryos

Nucleosome number	Control (bp)	H3.3 MO (bp)	H1A MO (bp)	H1A + H3.3 MO (bp)
1	182	196	171	187
2	351	377	334	350
3	521	546	499	512
4	691	720	657	688
*NRL (bp±s.d)	176±3.5	187±2.9‡	167±3.8‡	176±3.6§

*The nucleosomal repeat length (NRL) was calculated from the length of oligomeric DNA divided by the nucleosome number. Oligomeric DNA fragment sizes and standard deviation (s.d) of NRL were calculated from six independent experiments.

‡Differences in NRL of control versus injected embryos are statistically significant ($P<0.0001$, two-tailed paired t-test).

§NRL of control versus injected embryos are not significantly different ($P=0.78$).

Table S2. Primer sequences

Cloning of *Xenopus* H3f3a and H3f3b cDNA		
	Forward	Reverse
H3f3a	TTCGAATTCAGAGGACACGTCCGATTAAAG	AGGCTCGAGACTTACAGGAACAGCACAG
H3f3b	TTCGAATTCTGCAGGAGGCTAGTGAGGCT	AGGCTCGAGACACTCACCAACTATCTG
Cloning of mouse H3f3a-HA-Flag		
	Forward	Reverse
mH3f3a	GAATTCACCATGGCTCGTACAAAGCAGAC	GAATTCTTACTTATCGTCGTCATCCTTGTAATCAGCGTAATCTGGAACATCGTATGGGTAAGCACGTTCTCCGCGTATG

ChIP-qPCR primers		
	Forward	Reverse
Xbra promoter	TGAACAATCTATCCAGGCCACCT	AGAGAGCTCTATGATAATCCTGGGA
siamois promoter	GGGACTTTGAAGTCTTGCCA	TCTGATGACACGTGTTTCCC
cebpa promoter	ACAGGTGCAGAGATACATTCAGGCT	ACCTGCTTCAATGCAGCTACTTGCA
myf5 promoter	TGAGGCTGTGTTGAGATAAAGTATGCTAA	AATCAGGTTTCAGGAAATCTCTTAGGAGT

RT-PCR primers		
	Forward	Reverse
Xbra	TTCTGAAGGTGAGCATGTCG	GTTTGACTTTGCTAAAAGAGACAGG
siamois	TCTCCAGCCACCAGTACCAGATCTA	TGTATCCTGGGCTCAGGAATGCCAGT
cebpa	ACATCATCACCACCATCACCTGCA	CTGTACTCGTTGCTGTTCTTGTCCA
ODC	GATGTGAAACTGAAGTTTGAAGAGAT	CCAGAATCTGCTGGGAAGTATT
aldh1a2	TGTAGCAGATGATATGCGGATTGCC	ACAGTTCCTGCTTGCATTGCTGAAG
dlc	AGATGCCTGTAGTTCCAAACCCTGCA	AGCAGTCCCAGACAAACCACCATGGT
eomes	TCAGGTTTCTCAGAAGATCTCCTAC	TAGTCCTCAGTGCCAATGTCTTCAC
foxi1	AGATGAAGATGATCCAGGCAAGGGCA	AGCGGGCTCCCTTCAGGCTTGTCAGA
ina	ACCATCGGCCAGCTTGACAATGCTT	ATGGGTAGCCAATACCGAACATGCT
lhx1	CACCCATCTAGTGACGCTCAGAGGT	TGGTTGCCATAACCTCCATTGACTG
mespa	TCCGTGCCTTTCTCTGAAGCAAGAT	TGACTGACAGGCGTAGCTACTGTGCT
mix1	GCCCAACAGGAAAGAAGTCA	GACATTTTGGACTGGGCTCT

myf5	ATGGAGATGGTAGATAGCTGCCATT	ACCAATAGGTGCTCTGACATGCTCGT
nkx6	AGAGACACGCAGCAGAGATGGCCAC	CTGCAAGGGCTGAGAAGGTTCAAGT
tbx6	AGGAGTCGCCTCACATCAGCCACAGT	AGTTCATGGAGAAACCTCTGCCCAT
Xwnt8	GCTACCCACAATGGACTTCG	GAGCTAATGGCATGCACAAA
Xnr5-14	TGCTTATCGATGTGAAGGATCCTGC	CCTTCATACATGAGCATTGACAGA
znf521	GACTCGATGTTCTAGCTGCAATGTT	TCAGATTGAGGACTTATCCTGGCCA