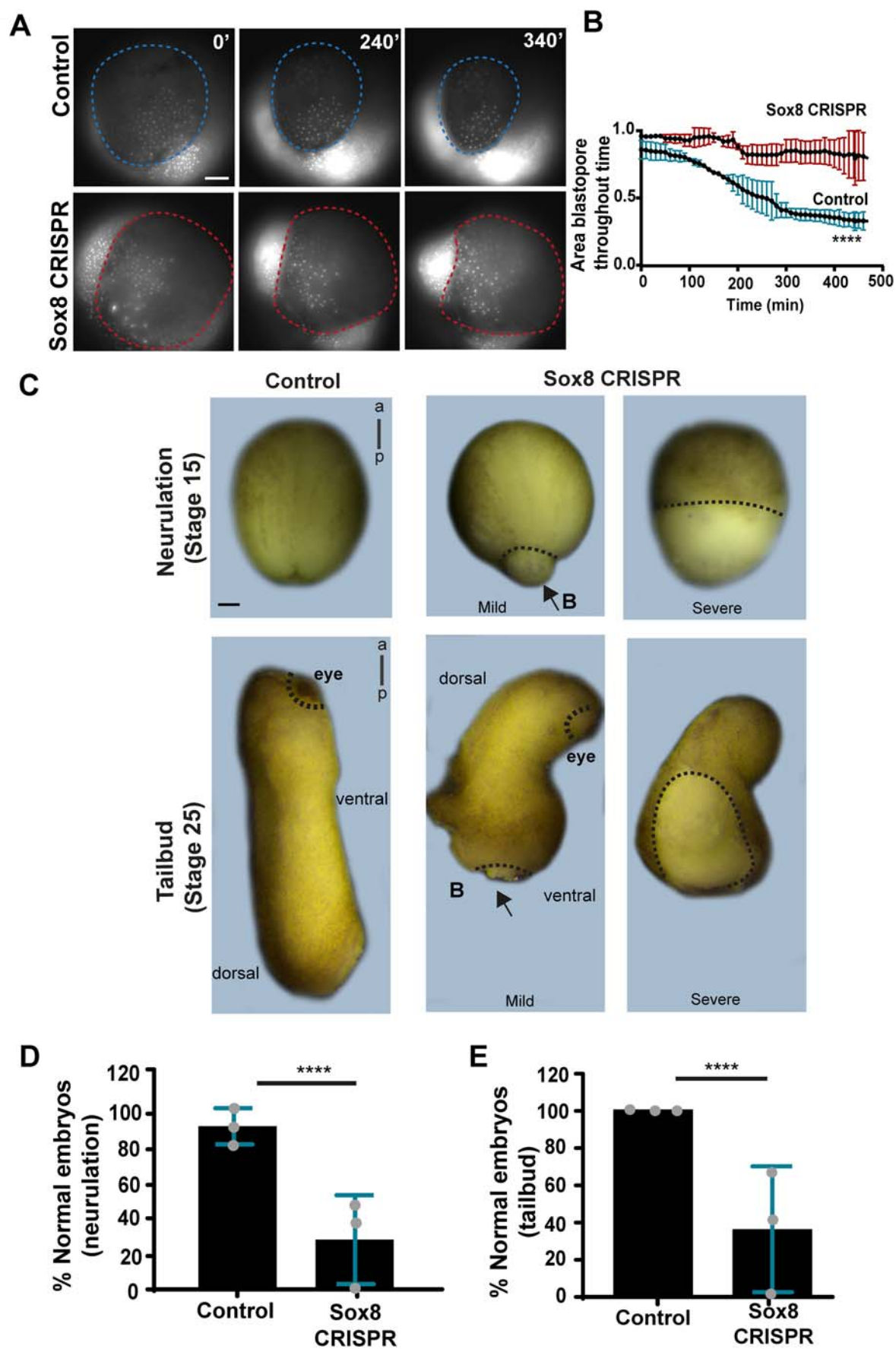
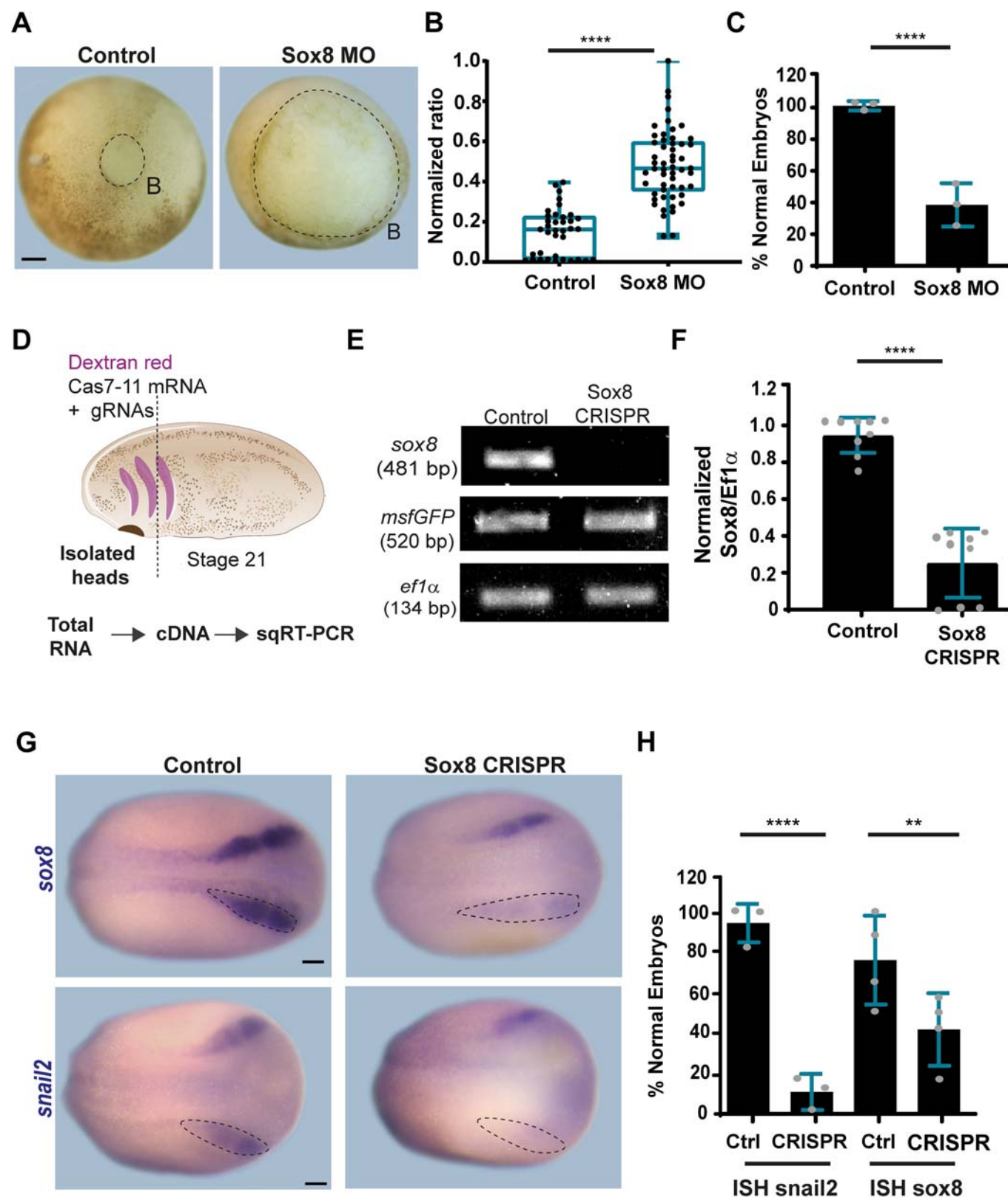


Expanded View Figures

Figure EV1. *sox8* knockdown by Cas7-11 in the ventrolateral mesoderm impairs embryonic development.

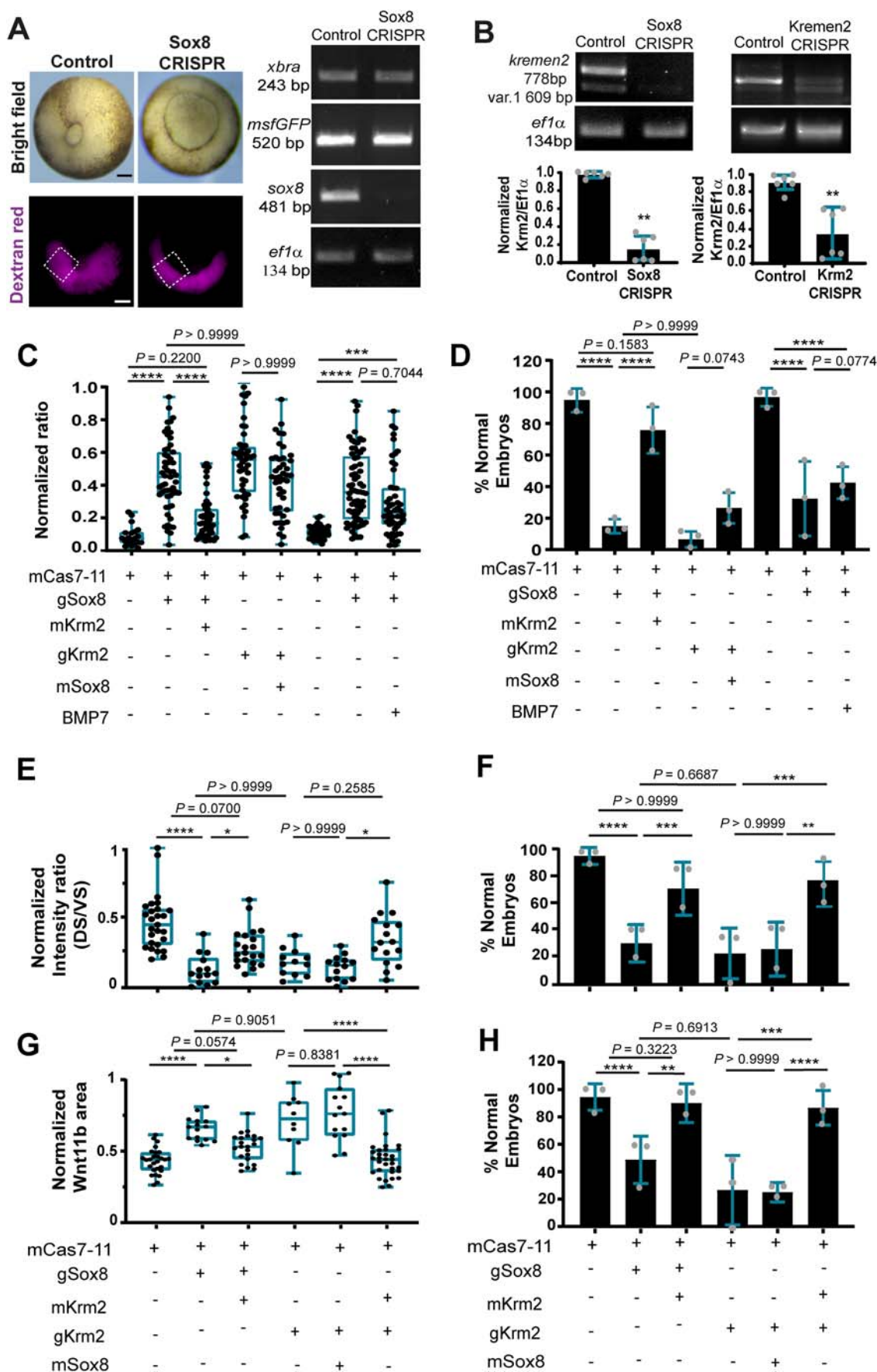
(A, B) Time-course analysis of the blastopore area shows delayed closure in *sox8* CRISPR embryos compared with controls. Dotted lines delineate the blastopore. Statistical significance was assessed using the two-tailed Mann–Whitney test, **** $P < 0.0001$. Error bars: mean $\pm$ SD. (C) Representative images of *sox8* CRISPR embryos at the neurula and tailbud stages showing open blastopores (arrow) and shortened anteroposterior axis. Dotted lines delineate the blastopore. (D) Percentage of embryos with normal morphology at the neurulation stage. Statistical significance was assessed using a two-sided Fisher's exact test, **** $P < 0.0001$. Error bars represent mean $\pm$ SD. $N = 3$ independent experiments ($n = 32$ control, $n = 56$ *sox8* CRISPR Cas7-11). (E) Quantification of embryos with normal morphology at the tailbud stage, showing a significant reduction in normal morphology following *sox8* knockdown. Two-sided Fisher's exact test, **** $P < 0.0001$. Error bars represent mean $\pm$ SD; $N = 3$ independent experiments ($n = 33$ control, $n = 55$ *sox8* CRISPR). Scale bars: 250 μm .





◀ **Figure EV2. *sox8* CRISPR recapitulates Sox8 MO phenotypes during the gastrulation and neurula stages.**

(A–C) Injection of a validated Sox8 morpholino (MO) into the ventrolateral mesoderm induces defects in blastopore closure, as illustrated by representative examples. Dotted lines delineate the blastopore (B). (B) Box plot showing the normalized ratio of blastopore area to embryo area. Boxes represent the interquartile range (IQR; 25th–75th percentile), with the center line indicating the median and whiskers extending to the minimum and maximum values. Statistical significance was assessed using the two-tailed Mann–Whitney test, **** $P < 0.0001$; $N = 3$ independent experiments ($n = 37$ control embryos; $n = 54$ *sox8* MO). Scale bars: 250 μm . (C) Percentage of embryos exhibiting normal blastopore size per condition (embryos displaying values below the maximum control ratio value are classified as normal). Two-sided Fisher's exact test, **** $P < 0.0001$; Error bars: mean $\pm$ SD. $N = 3$ independent experiments ($n = 37$ control; $n = 52$ *sox8* MO). (D–F) Semi-quantitative RT–PCR of stage 21 heads (early neurulation), confirming the depletion of *sox8*. The purple stripes represent target injections in the neural crest. Statistical significance was assessed using a two-tailed Mann–Whitney test, **** $P < 0.0001$; Error bars: mean $\pm$ SD. $N = 3$ independent experiments. (G) CRISPR Cas7-11-mediated depletion of *sox8* in the neural crest phenocopies previously reported *sox8* MO phenotypes in the neural crest, such as a delay in neural crest induction (as detected by *ISH* for *snail2*). Dotted lines delineate the neural crest region. (H) Quantification of the percentage of embryos with normal signals for *sox8* or *snail2*. Two-sided Fisher's exact test, **** $P < 0.0001$; ** $P = 0.0033$. Error bars: mean $\pm$ SD. $N = 3$ independent experiments; $n = 17$ control embryos (*ISH snail2*); $n = 18$ *sox8* CRISPR Cas7-11 (*ISH snail2*); $n = 38$ control embryos (*ISH sox8*); $n = 41$ *sox8* CRISPR (*ISH Sox8*). Scale bars: 250 μm .



◀ **Figure EV3. *sox8* and *kremen2* downregulation impairs blastopore closure and alters the *wnt11b* expression pattern.**

(A) SqRT–PCR was performed as a quality control for samples sent for RNA-seq. (B) SqRT–PCR and quantification of samples sent for RNA-seq revealed that *kremen2* is downregulated. Statistical significance was assessed by a two-tailed Mann–Whitney test, $^{**}P = 0.0022$. Control for *kremen2* depletion in the context of *kremen2* CRISPR is also shown. Mann–Whitney test, $^{**}P = 0.0022$; Error bars: mean $\pm$ SD. (C) Box plot showing the normalized ratio of blastopore area to embryos. Statistical significance was assessed by Kruskal–Wallis multiple comparisons test, $^{****}P < 0.0001$; $^{***}P = 0.0002$ (control vs *sox8* CRISPR + *bmp7* mRNA); ns non-significant: $P = 0.2200$ (control vs *sox8* CRISPR + *kremen2* mRNA); $P > 0.9999$ (*sox8* CRISPR vs *kremen2* CRISPR); $P > 0.9999$ (*kremen2* CRISPR vs *kremen2* CRISPR + *sox8* mRNA); $P = 0.7044$ (*sox8* CRISPR vs *sox8* CRISPR + *bmp7* mRNA). (D) Percentage of embryos exhibiting normal blastopore size per condition (embryos displaying values below the maximum control ratio value are classified as normal). Two-sided Fisher's exact test, $^{****}P < 0.0001$; ns non-significant: $P = 0.0774$ (*sox8* CRISPR vs *sox8* CRISPR + *bmp7* mRNA); $P = 0.1583$ (control vs *sox8* CRISPR + *kremen2*); $P > 0.9999$ (*sox8* CRISPR vs *kremen2* CRISPR); $P = 0.0774$ (*sox8* CRISPR vs *sox8* CRISPR + *bmp7* mRNA); $P = 0.0743$ (*kremen2* CRISPR vs *kremen2* CRISPR + *sox8* mRNA). Error bars: mean $\pm$ SD; For (C, D): $n = 20$ control Cas7-11; $n = 55$ *sox8* CRISPR; $n = 51$ *sox8* CRISPR + *kremen2* mRNA; $n = 45$ *kremen2* CRISPR; $n = 45$ *kremen2* CRISPR + *sox8* mRNA; $n = 39$ control (for rescue with *bmp7* mRNA); $n = 68$ *sox8* CRISPR (for rescue with *bmp7* mRNA); $n = 51$ *sox8* CRISPR + *bmp7* mRNA. $N = 3$ independent experiments. (E) Box plot showing the *wnt11b* intensity ratio between dorsal and ventral signals in different conditions. Statistical significance was assessed by Kruskal–Wallis multiple comparisons test, $^{****}P < 0.0001$; $^{*}P = 0.0184$ (*kremen2* CRISPR + *sox8* mRNA vs *kremen2* CRISPR + *kremen2* mRNA); $^{*}P = 0.0472$ (*sox8* CRISPR vs *sox8* CRISPR + *kremen2* mRNA); ns non-significant: $P = 0.0700$ (control vs *sox8* CRISPR + *kremen2* mRNA); $P > 0.9999$ (*kremen2* CRISPR + *kremen2* *sox8* mRNA); $P > 0.9999$ (*sox8* CRISPR vs *kremen2* CRISPR); $P = 0.2585$ (*kremen2* CRISPR vs *kremen2* CRISPR + *kremen2* mRNA). (F) Percentage of embryos displaying a normal *wnt11b* expression pattern is shown; percentage of normal and abnormal embryos in each condition (embryos displaying higher values than the baseline control intensity ratio value are classified as normal). Two-sided Fisher's exact test, $^{****}P < 0.0001$ (control vs *sox8* CRISPR); $^{***}P = 0.0001$ (*sox8* CRISPR vs *sox8* CRISPR + *kremen2* mRNA); $^{**}P = 0.0092$ (*kremen2* CRISPR + *sox8* mRNA vs *kremen2* CRISPR); $P > 0.9999$ (Control vs *sox8* CRISPR + *kremen2* mRNA); $P = 0.6687$ (*sox8* CRISPR vs *kremen2* CRISPR); $P > 0.9999$ (*kremen2* CRISPR vs *kremen2* CRISPR + *sox8* mRNA). Error bars: mean $\pm$ SD. $N = 3$ independent experiments; $n = 27$ control embryos; $n = 14$ *sox8* CRISPR; $n = 21$ *sox8* CRISPR + *kremen2* mRNA; $n = 8$ *kremen2* CRISPR; $n = 14$ *kremen2* CRISPR + *sox8* mRNA and $n = 15$ *kremen2* CRISPR + *kremen2* mRNA; (G) Box plot showing area of the *wnt11b* intensity in different conditions. Statistical significance was assessed by ordinary one-way ANOVA, $^{****}P < 0.0001$; $^{*}P = 0.0147$ (*sox8* CRISPR vs *sox8* CRISPR + *kremen2* mRNA); $P = 0.0574$ (control vs *sox8* CRISPR + *kremen2* mRNA); $P = 0.9051$ (*sox8* CRISPR vs *kremen2* CRISPR); $P = 0.8381$ (*kremen2* CRISPR vs *kremen2* CRISPR + *sox8* mRNA); (H) Percentage of embryos displaying a normal *wnt11b* expression pattern is shown; percentage of normal embryos in each condition (embryos displaying lower values than the maximum control area value are classified as normal). Two-sided Fisher's exact test, $^{****}P < 0.0001$; $^{***}P = 0.0004$ (*kremen2* CRISPR + *sox8* mRNA); $^{**}P = 0.0041$ (*sox8* CRISPR vs *sox8* CRISPR + *kremen2* mRNA); $P = 0.3223$ (control vs *sox8* CRISPR + *kremen2* mRNA); $P = 0.6913$ (*sox8* CRISPR vs *kremen2* CRISPR); $P > 0.9999$ (*kremen2* CRISPR + *kremen2* CRISPR + *sox8* mRNA). Error bars: mean $\pm$ SD. $n = 27$ control embryos; $n = 15$ *sox8* CRISPR; $n = 23$ *sox8* CRISPR + *kremen2* mRNA; $n = 10$ *kremen2* CRISPR; $n = 15$ *kremen2* CRISPR + *sox8* mRNA and $n = 32$ *kremen2* CRISPR + *kremen2* mRNA (E–H) $N = 3$ independent experiments; Scale bars: 250 μ m. For box plots in (C, E, G), boxes represent the interquartile range (IQR; 25th–75th percentile), with the center line indicating the median and whiskers extending to the minimum and maximum values.

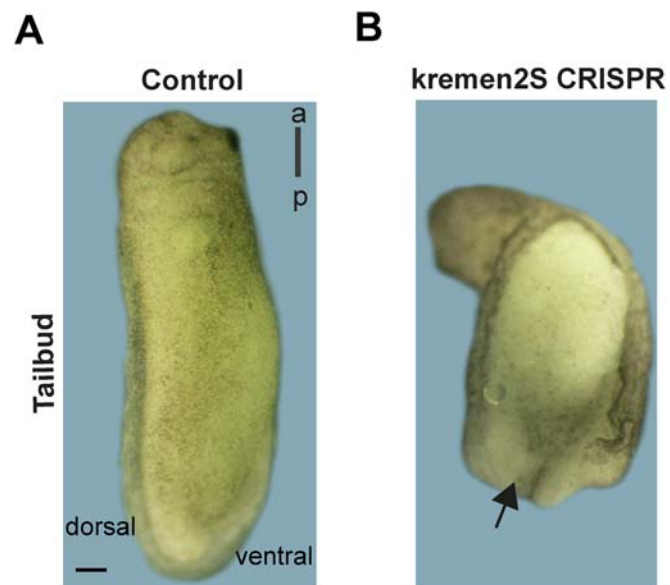
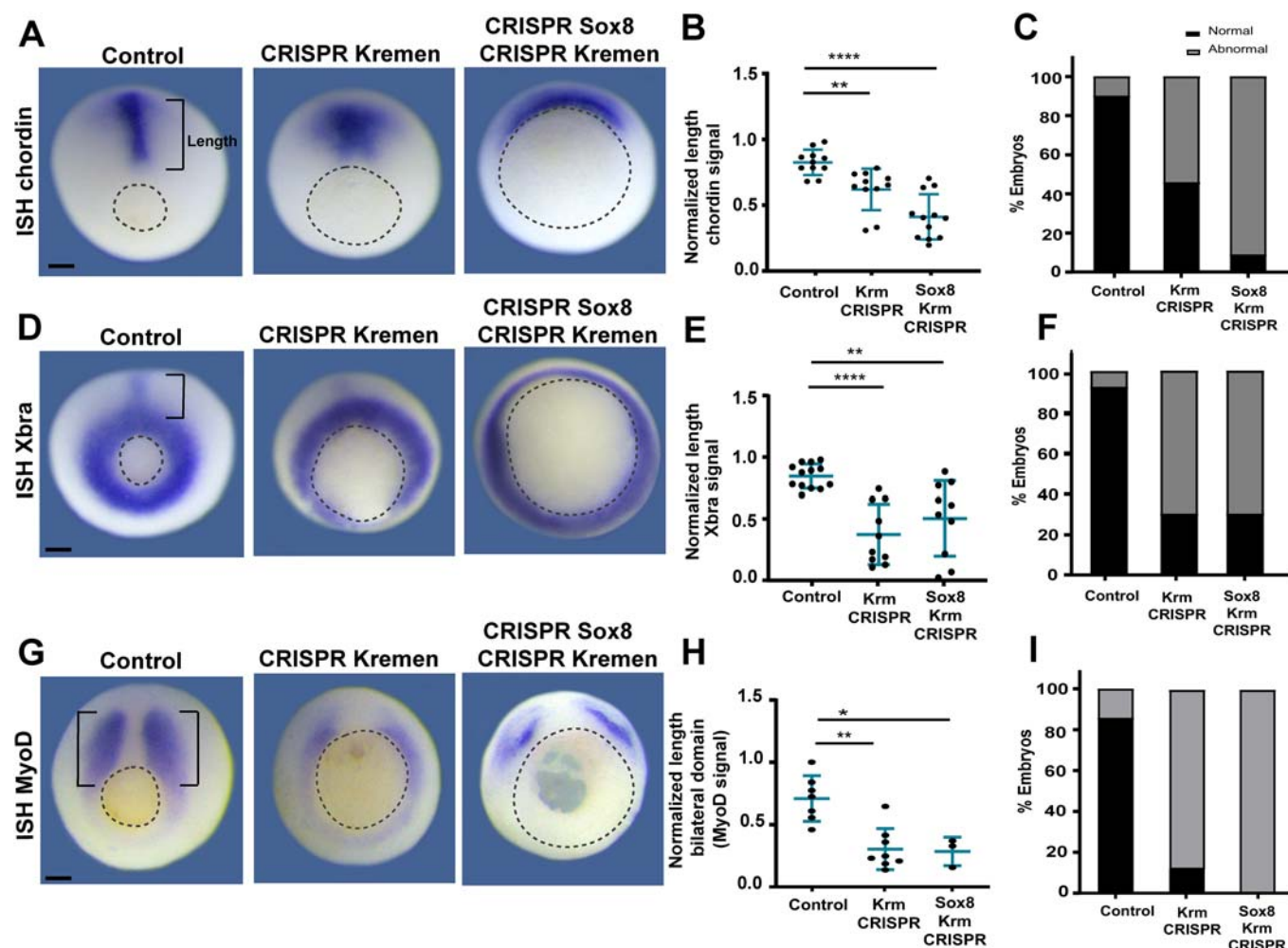


Figure EV4. *kremen2* knockdown by Cas7-11 in the ventrolateral mesoderm impairs embryonic development.

Representative images of (A) control embryos and (B) *kremen2* CRISPR embryos at the tailbud stage, showing open blastopores (arrows), with shortened embryos on the anteroposterior axis. Scale bars: 250 μ m.



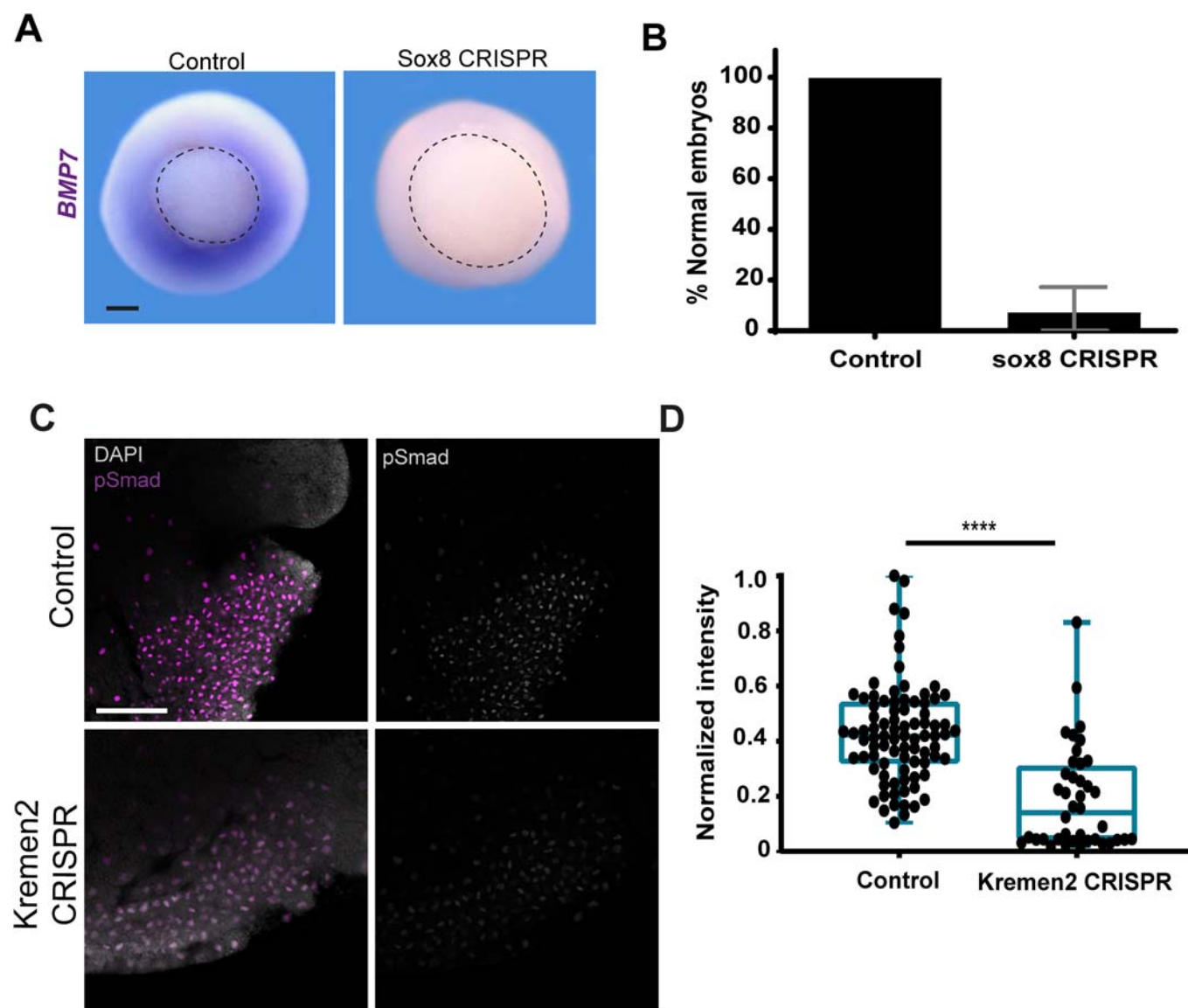


Figure EV6. BMP signaling is perturbed under CRISPR conditions.

(A) Whole-mount ISH for *bmp7* revealed reduced expression in *sox8* CRISPR embryos. Dotted lines delineate the blastopore. Scale bar: 250 μ m. (B) Quantification of the percentage of embryos with a normal *bmp7* ISH signal. $N = 2$ independent experiments ($n = 5$ embryos control; $n = 7$ embryos *sox8* CRISPR). (C) pSmad staining levels are decreased in *kremen2* CRISPR conditions compared with controls. (D) Box plot showing the quantification of the nuclear signal in the ventral regions. Boxes represent the interquartile range (IQR; 25th–75th percentile), with the center line indicating the median and whiskers extending to the minimum and maximum values. Statistical significance was assessed by a two-tailed Mann–Whitney test, **** $P < 0.0001$. $n = 9$ control embryos (85 cells); $n = 4$ *kremen2* CRISPR (40 cells). Scale bar: 100 μ m.