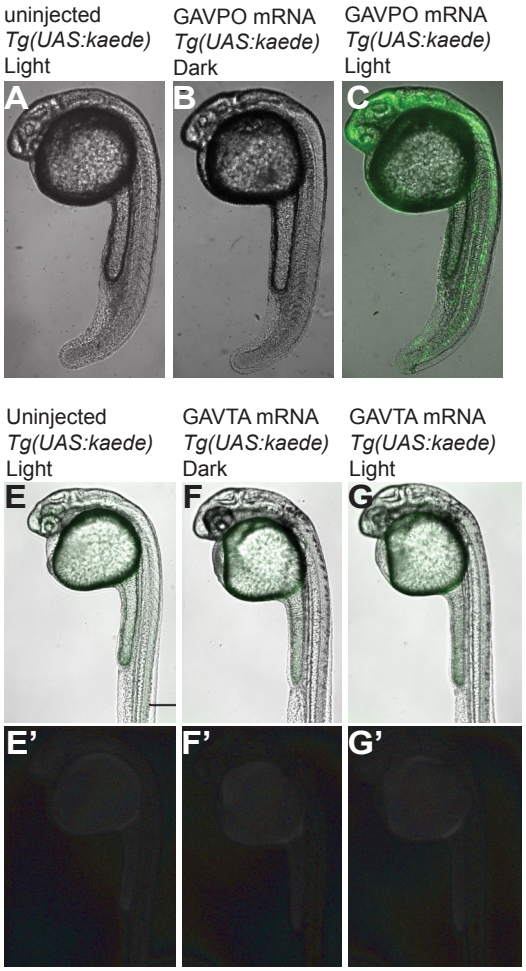


Figure S1: LightOn system induces kaede expres-sion from the UAS promoter in a light-gated manner. A-C. Tg(UAS:kaede) embryos were injected with GAVPO mRNA and then illuminated with con-stant, global blue light (465 nm) from approximately 4-24 hpf (hours post-fertilization). GAVPO injected embryos showed robust induction of Kaede fluores-cent protein at 24 hpf (C), with no visible induction of Kaede expression in dark (B) or uninjected controls (A). **D-F**. Following blue light illumination, GAVTA is unable to induce transcription of kaede from the UAS promoter (F, F').

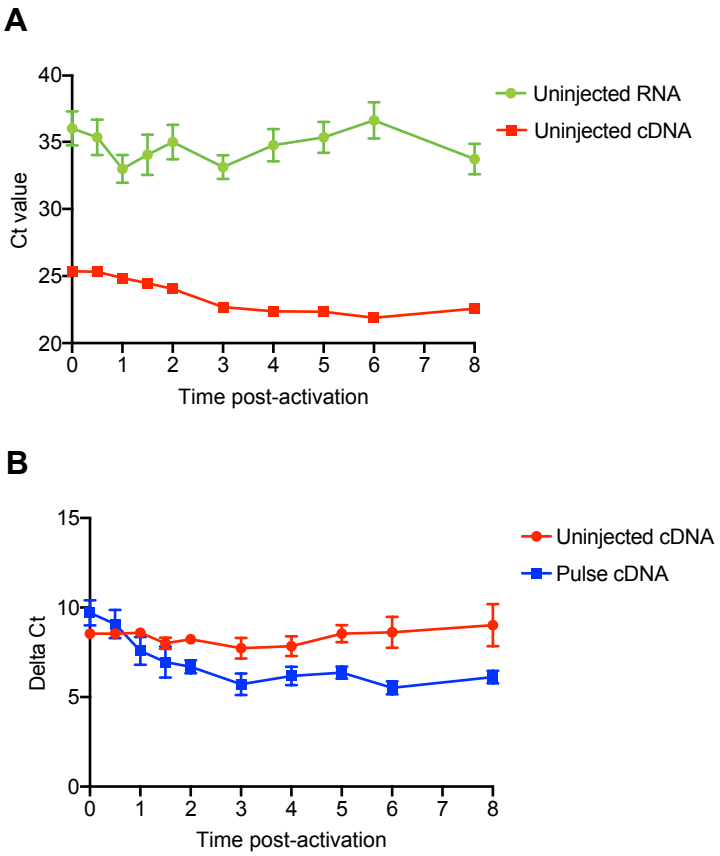


Figure S2. Assessing leakiness of the C120 promoter.
Uninjected *Tg(cryaa:Venus;C120:mcherry)* embryos were globally illuminated with pulsed 465 nm light (1 hour on, 1 hour off) starting at 3-3.5 hpf and mCherry mRNA levels were measured at the indicated time points by qPCR. **A**. Average uncorrected CT values suggest that low-level mCherry expression can be detected in uninjected embryos (red line). This signal is likely not due to contaminating genomic DNA as qPCR using total RNA rather than cDNA as template resulted in CT values > 10 cycles higher. **B**. Delta-CT measurements show that relative mCherry expression remains low and unchanged in uninjected embryos (red line) compared to embryos injected with 100 pg TAEL mRNA and illuminated with pulsed blue light (blue line). Delta-Ct = CT(mCherry)-CT(ef1a).

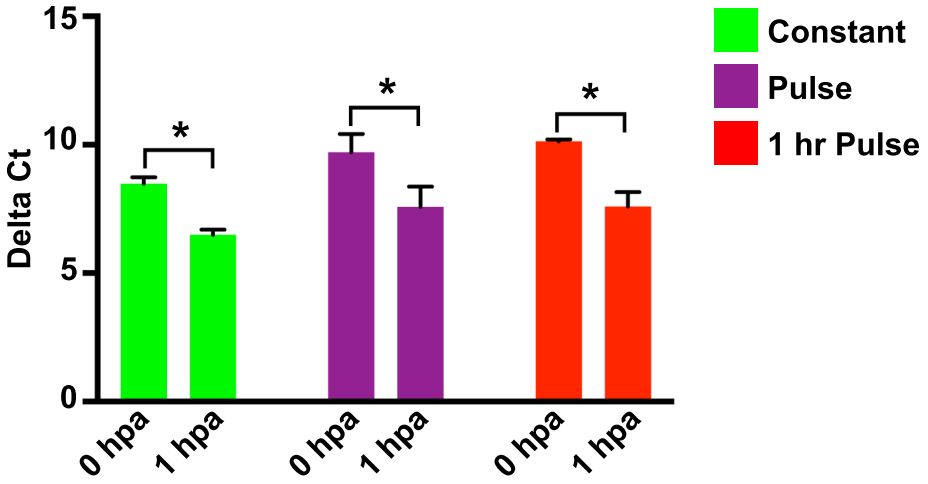
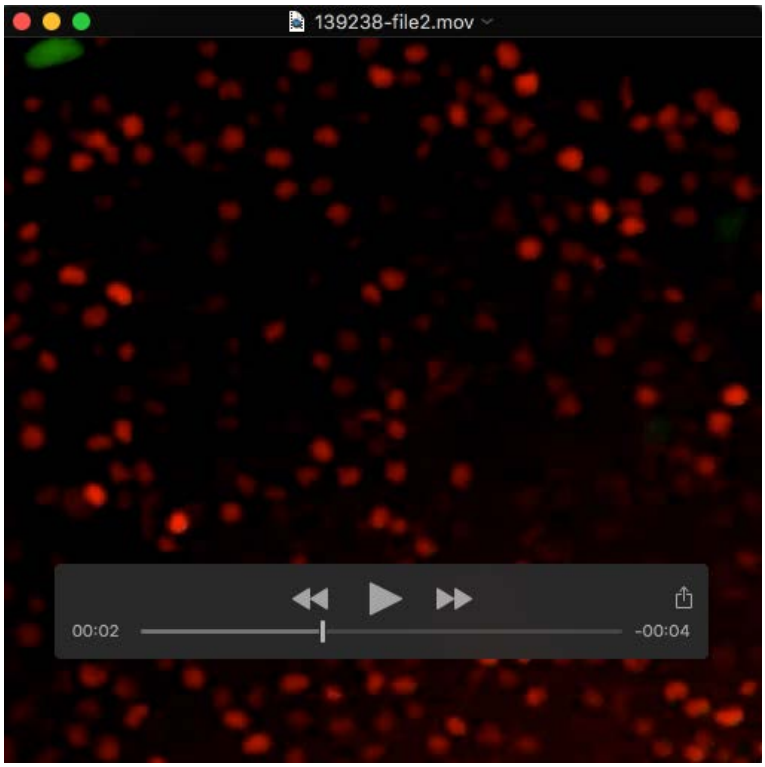
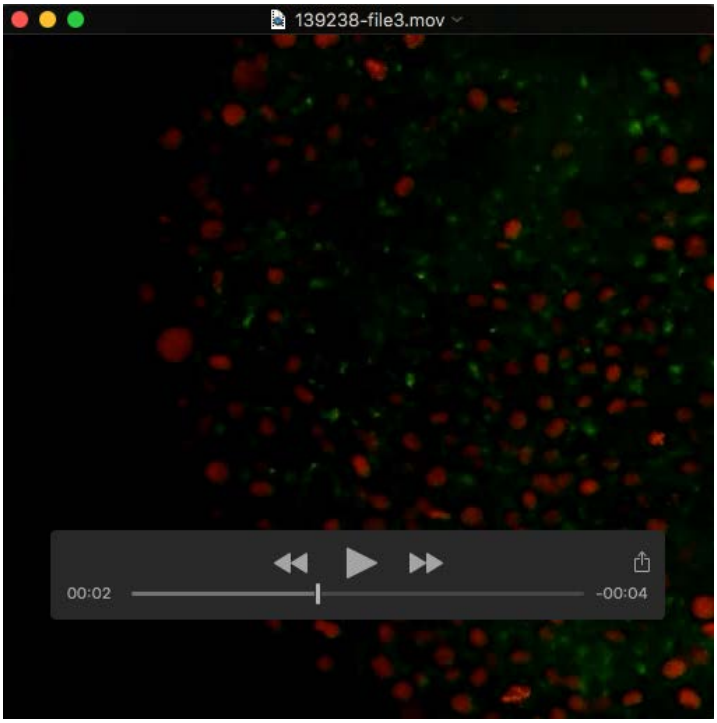


Figure S3. mCherry expression is significantly upregulated at 1 hour post-activation. Tg(cryaa:Venus; 5xC120:mCherry) embryos were injected with 100 pg TAEL mRNA and globally illuminated with 465 nm light. Levels of mCherry expression were measured by qPCR. Delta-Ct measurements show that mCherry expression is significantly upregulated at 1 hour post-activation for all illumination patterns tested. Delta Ct = Ct(mCherry) – Ct(ef1a). hpa, hours post-activation. *p<0.05 by paired t-test. Error bars, S.E.M.



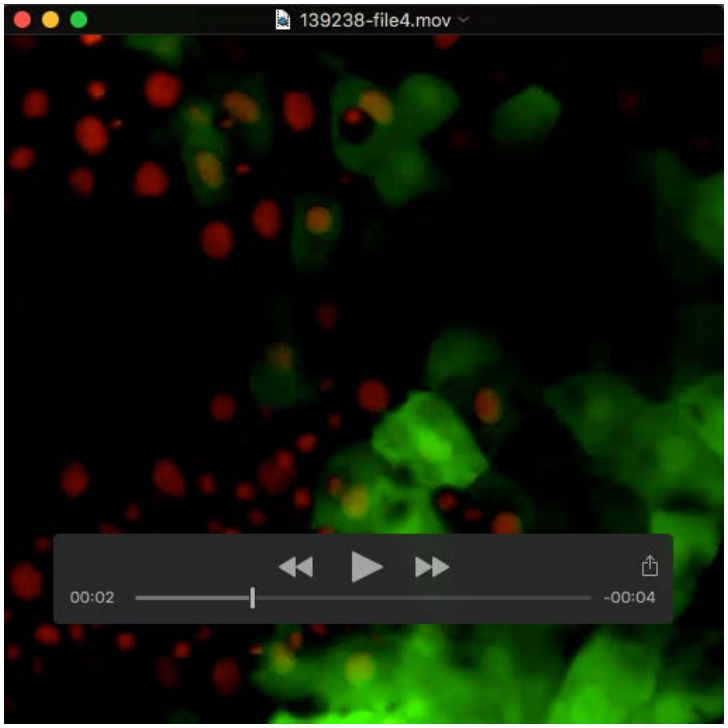
Movie 1: Canonical endoderm (*sox17)* expression profile.

Z-stack through a *Tg(cryaa:Venus; 5xC120:sox32); Tg(sox17:GFP)* embryo at approximately 70% epiboly. The embryo was injected with H2B-mCherry mRNA to label all nuclei (red). Images were acquired every 0.9 µm on a Nikon Ti-E microscope equipped with a Yokogawa CSU-22 spinning disk confocal unit. The z-stack starts at the embryo surface and goes through towards the yolk. *Tg(sox17:GFP)* expression (green) appears in only at the innermost layer closest to at the yolk, consistent with endogenous endoderm localization.



Movie 2: Ectopic endoderm is not induced when TAEL injected embryos are kept in the dark.

Z-stack through a *Tg(cryaa:Venus; 5xC120:sox32); Tg(sox17:GFP)* embryo at approximately 70% epiboly. The embryo was injected with H2B-mCherry mRNA to label all nuclei (red) and TAEL mRNA, then kept in the dark until image acquisition. Images were acquired every 0.9 µm on a Nikon Ti-E microscope equipped with a Yokogawa CSU-22 spinning disk confocal unit. The z-stack starts at the embryo surface and goes through towards the yolk. *Tg(sox17:GFP)* expression (green) appears in only at the innermost layer closest to at the yolk, consistent with endogenous endoderm localization.



Movie 3: Ectopic endoderm induction via over-expression of Sox32.

Z-stack through a *Tg(cryaa:Venus; 5xC120:sox32); Tg(sox17:GFP)* embryo at approximately 70% epiboly. The embryo was injected with H2B-mCherry mRNA to label all nuclei (red) and TAEL mRNA. At 3-4 hpf, the animal pole was illuminated with a 2 min pulse of blue light to activate TAEL-dependent *sox32* expression. Images were acquired every 0.9 µm on a Nikon Ti-E microscope equipped with a Yokogawa CSU-22 spinning disk confocal unit. The z-stack starts at the embryo surface and goes through towards the yolk. *Tg(sox17:GFP)* expression (green) is apparent in the outermost cell layers as well as within the endogenous endoderm layer.