

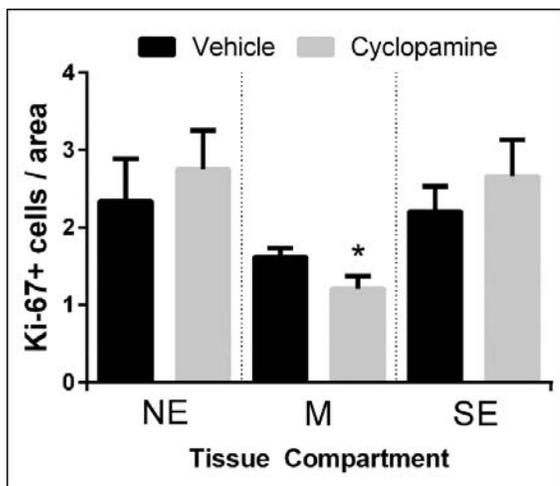
EVERSON *ET AL.* 2017 SUPPLEMENTAL MATERIAL

Figure S1. Quantification of Ki-67 staining. Ki-67 positive cells were quantified in medial nasal processes (MNP) of $n=20$ vehicle- and $n=24$ cyclophamide-treated embryos. A significant reduction of proliferating cells was observed in the MNP mesenchyme of cyclophamide-exposed embryos compared to control but not in the neuroectoderm (NE) or surface ectoderm (SE).

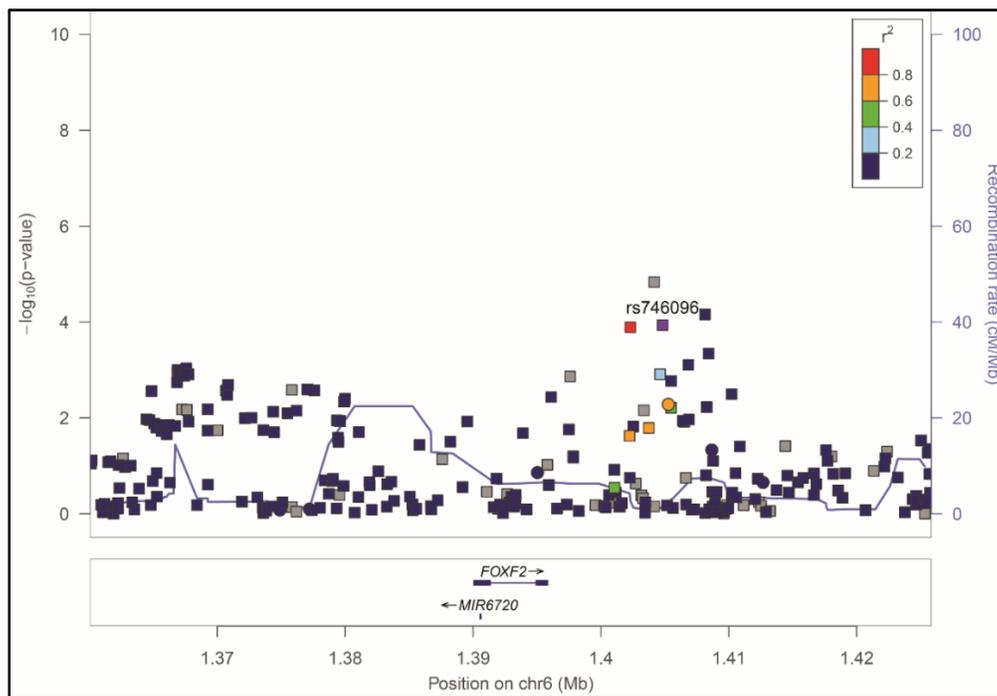


Figure S2. SNPs at *FOXF2* locus are associated with cleft lip in humans. Regional association plots showing $-\log_{10}(P\text{values})$ for genotyped (circles) and imputed (squares) SNPs for the *FOXF2* region in CL/P. Plot was generated using LocusZoom. The recombination overlay (blue line, right y-axis) indicates the boundaries of the linkage disequilibrium-block. Points are color coded according to pairwise linkage disequilibrium (r^2) with the index SNP in a European population. Genes within the region of interest are annotated at the bottom, with arrows indicating the direction of transcription.

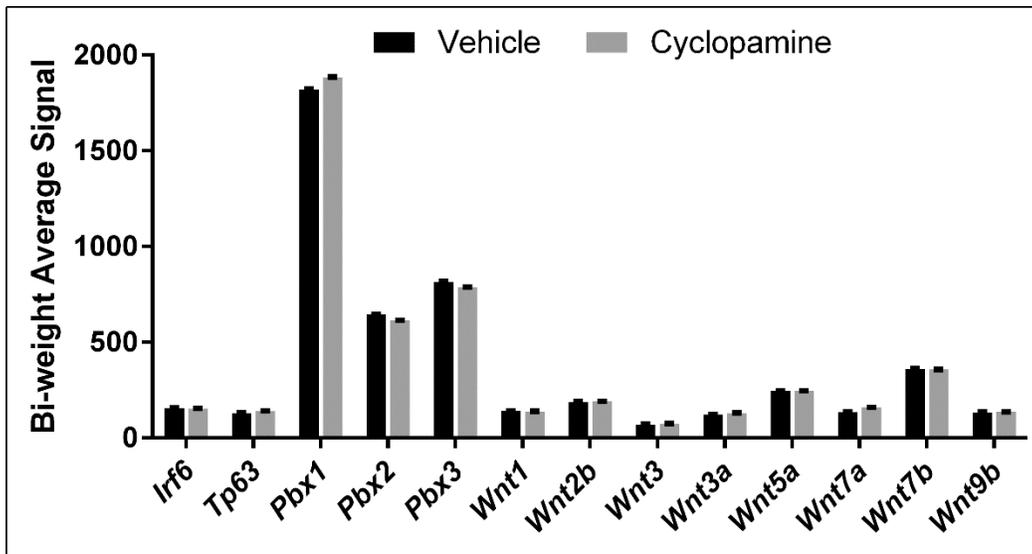


Figure S3. The Pbx-Wnt-p63-Irf6 regulatory module is not dysregulated in the FNP during the initial pathogenesis of cyclopamine-induced cleft lip. Expression of several Wnt and Pbx genes as well as *Irf6*, and *Tp63* was not significantly changed during the initial pathogenesis of cleft lip. Bi-weight average microarray signal \pm SD is shown for n=6 pooled litters per group.

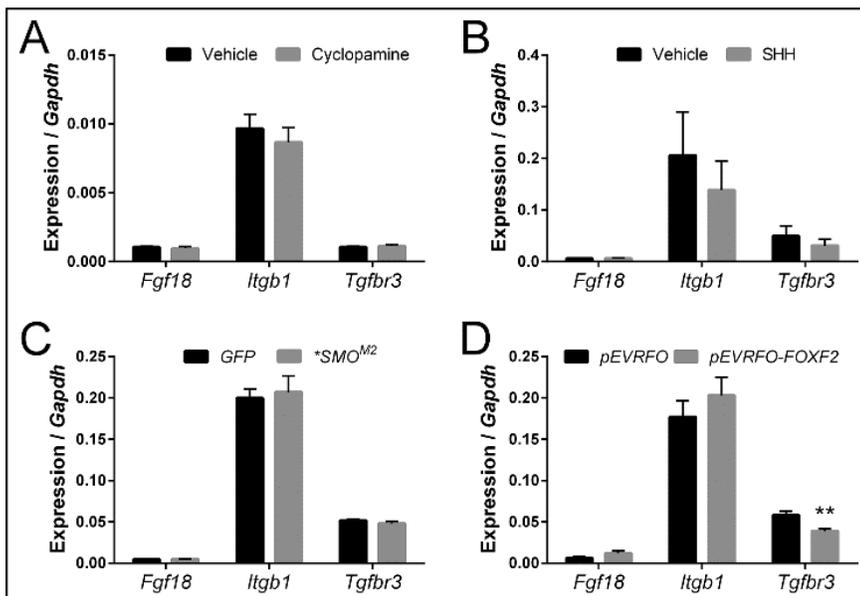


Figure S4. *Fgf18* and targets of Tgf β signaling are not regulated by Shh-Foxf2 signaling in cNCC *in vitro* or in cleft lip pathogenesis. (A) *Fgf18*, *Itgb1*, and *Tgfr3* expression was not significantly changed during the initial pathogenesis of cleft lip (n=6 pooled litters per group). (B) *Fgf18*, *Itgb1*, and *Tgfr3* expression was not significantly changed in cranial neural crest cells (cNCCs) \pm SHH (n=5). (C) *Fgf18*, *Itgb1*, and *Tgfr3* expression was not significantly changed in cNCCs overexpressing constitutively active *SMO*, compared to cells overexpressing *GFP* only (n=5). (D) *Fgf18* and *Itgb1* expression was not significantly changed, while *Tgfr3* expression is significantly reduced in cNCCs overexpressing *FOXF2*, compared to cells overexpressing pEVRFO empty vector only (n=5). Mean expression per *Gapdh* \pm SEM is shown. Significance: ** = $p < 0.01$

SUPPLEMENTAL TABLES

Table S1. RT-PCR Primer Sequences	
Gene/Direction	Sequence
<i>Shh</i> -fwd	AATGCCTTGGCCATCTCTGT
<i>Shh</i> -fwd	GCTCGACCCCTCATAGTGTAGAGACT
<i>Gli1</i> -fwd	GGAAGTCTTATTTCACGCCTTGA
<i>Gli1</i> -rev	CAACCTTCTTGCTCACACATGTAAG
<i>Ptch1</i> -fwd	CTCTGGAGCAGATTTCCAAGG
<i>Ptch1</i> -rev	TGCCGCAGTTCTTTTGAATG
<i>Foxb2</i> -fwd	AAGTGAAGAGCCTGGGAAGA
<i>Foxb2</i> -rev	TGGCGGTCAGTGAGATGTAAGA
<i>Foxc1</i> -fwd	TTCTTGCGTTCAGAGACTCG
<i>Foxc1</i> -rev	TCTTACAGGTGAGAGGCAAGG
<i>Foxc2</i> -fwd	ACGAGTGCGGATTTGTAACC
<i>Foxc2</i> -rev	ACAGTTGGGCAAGACGAAAC
<i>Foxd1</i> -fwd	CGTTTCTAGATTCTCACTCCTC
<i>Foxd1</i> -rev	TCCACTGTGGTCCCTTTA
<i>Foxf1</i> -fwd	GGAGCAGCCATACCTTCACCAAA
<i>Foxf1</i> -rev	ATGCTGGGCGACTGTGAGTGATA
<i>Foxf2</i> -fwd	TTCTCTAGTTCCTGGCTCAGTAG
<i>Foxf2</i> -rev	TGTTCTTTGGCACCTGTATCCG
<i>Foxl1</i> -fwd	GAGGGCAGGACTTAGAGATA
<i>Foxl1</i> -rev	CCCACATCCAATGAGATCAA
<i>Foxm1</i> -fwd	GCCCTAGACCAGAGAGTAAA
<i>Foxm1</i> -rev	GAGACCTGGAAGCTGTATTG
<i>Foxo1</i> -fwd	TACGGAGGATTGAACCAGTA
<i>Foxo1</i> -rev	CTGGCATGACCGAATTAGGG
<i>SMO</i> -fwd	ACCTATGCCTGGCACACTTC
<i>SMO</i> -rev	GTGAGGACAAAGGGGAGTGA
dn <i>GLI2</i> (<i>mutB</i>)-fwd	ATGACTGTAAGCAGGAGGCTGA
dn <i>GLI2</i> (<i>mutB</i>)-rev	CGTGGATGTGCTCGTTGTTGAT
Full length <i>FOXF2</i> -fwd	ACCAGAGCGTCTGTGATGATATT
Full length <i>FOXF2</i> -rev	GTGACTTGAATCCGTCCCAGTTTC
dn <i>FOXF2</i> -fwd	AACTCGGTGCGCCACAATC
dn <i>FOXF2</i> -rev	CGGGTCGATGGTCCAGTAGT
<i>Fgf18</i> -fwd	AGTGGAGACAGATACCTTCGG
<i>Fgf18</i> -rev	GTACTTGGCAGACATCAGGG
<i>Itgb1</i> -fwd	TCAGACTTCCGCATTGGCTTTG
<i>Itgb1</i> -rev	GCTAAATGGGCTGGTGCAGTTT
<i>Tgfb3</i> -fwd	TTGACTCCAACCATCAGCCTTTC
<i>Tgfb3</i> -rev	ACCTGAAACTCCCTGTCTCTGT

Table S2. *In situ* Hybridization Riboprobe Primer Sequences

Gene/Direction	Sequence
<i>Gli1</i> -fwd	CCCTCCTCCTCTCATTCAC
<i>Gli1</i> -rev + T7 leader	CGATGTTAATACGACTCACTATAGGGTCCAGCTGAGTGTGTGCCAG
<i>Ptch1</i> -fwd	GACGTGAGGACAGAAGATTG
<i>Ptch1</i> -rev + T7 leader	CGATGTTAATACGACTCACTATAGGGAACGGGCAGCTATGAAG
<i>Foxb2</i> -fwd	GGCTTTCCGAGAGGCGTTAAG
<i>Foxb2</i> -rev + T7 leader	CGATGTTAATACGACTCACTATAGGGCCGGCTGCTGGTGGAAATAG
<i>Foxc1</i> -fwd	ACTCACCTCGTGGTACCTGAA
<i>Foxc1</i> -rev + T7 leader	CGATGTTAATACGACTCACTATAGGGGGCAAGACGAATGAGCAGGAAAG
<i>Foxc2</i> -fwd	TTCTAGCACTCGGAAGGG
<i>Foxc2</i> -rev + T7 leader	CGATGTTAATACGACTCACTATAGGGCGTTCAGAGTGATCTTCTTCTC
<i>Foxd1</i> -fwd	AGCTCCGTTTCTAGATTCTCACTCC
<i>Foxd1</i> -rev + T7 leader	CGATGTTAATACGACTCACTATAGGGCAGGTCATCGTCGCTCCCTCCTC
<i>Foxf1</i> -fwd	CGCGGATCCTGACCCTCAGCGAGATCTACC
<i>Foxf1</i> -rev + T7 leader	CGATGTTAATACGACTCACTATAGGGCCCAAGCTTATGGCTGCTCCAGGGAGTGCC
<i>Foxf2</i> -fwd	GGTTATGGTGGCCTCGACAT
<i>Foxf2</i> -rev + T7 leader	CGATGTTAATACGACTCACTATAGGGCACACACACCTCCCTTTTCA
<i>Foxl1</i> -fwd	CCGGAATTCGAGCAGAGGGTCACACTGAACG
<i>Foxl1</i> -rev + T7 leader	CGATGTTAATACGACTCACTATAGGGTGCTCTAGAGCGTTGAAGGGAGGCGCTAGG

Table S3. Electrophoretic Mobility Shift Assay Primer Sequences

Gene/Direction	Sequence
<i>Foxf2</i> -fwd	5' - ACTGACGTGATGAGGTCTCTGTTTTGA - 3'
<i>Foxf2</i> -rev	5' - TCAAAACAGAGACCTCACATCACGTCAGT - 3'
mut <i>Foxf2</i> -fwd	5' - ACTGACGTGAACTCGTCTCTGTTTTGA - 3'
mut <i>Foxf2</i> -rev	5' - TCAAAACAGAGACGAGTGTTCACGTCAGT - 3'
<i>Foxl1</i> -fwd	5' - TGATTCAACTTGGGTGGGCGGACTTATCA - 3'
<i>Foxl1</i> -rev	5' - TGATAAGTCCGCCACCCAAGTTGAATCA - 3'
mut <i>Foxl1</i> -fwd	5' - TGATTCAACTACCCACGGCGGACTTATCA - 3'
mut <i>Foxl1</i> -rev	5' - TGATAAGTCCGCGGTGGGTAGTTGAATCA - 3'
<i>Foxb2</i> -fwd	5' - GTGGGCAGCGTGGGGCGTCTGTCTCAGTT - 3'
<i>Foxb2</i> -rev	5' - AACTGAGACAGACGCCCCACGCTGCCCCAC - 3'
mut <i>Foxb2</i> -fwd	5' - GTGGGCAGCGACCCCGGTCTGTCTCAGTT - 3'
mut <i>Foxb2</i> -rev	5' - AACTGAGACAGACCGGGTTCGCTGCCCCAC - 3'
Non-specific competitor-fwd	5' - CGGGGGACAACCTTGGAGCAGT - 3'
Non-specific competitor-rev	5' - ACTGCTCCAAGTTGTCCCCCG - 3'