Supplemental Text

Gynecologists report:
A 30-year old G1P0 was referred to our tertiary centre for targeted ultrasonography at 13 weeks due to abnormal profile. We noted an abnormal retronasal triangle, absence of the palate in sagittal view and a protruding median part of the maxilla, indicating a severe bilateral cheilognathopalatoschisis. Forearms and hands were structurally normal but afunctional. Both feet were abnormally positioned. Termination of pregnancy on the couple's request was performed at 14 weeks. Consent was given to publish photographs and molecular data.
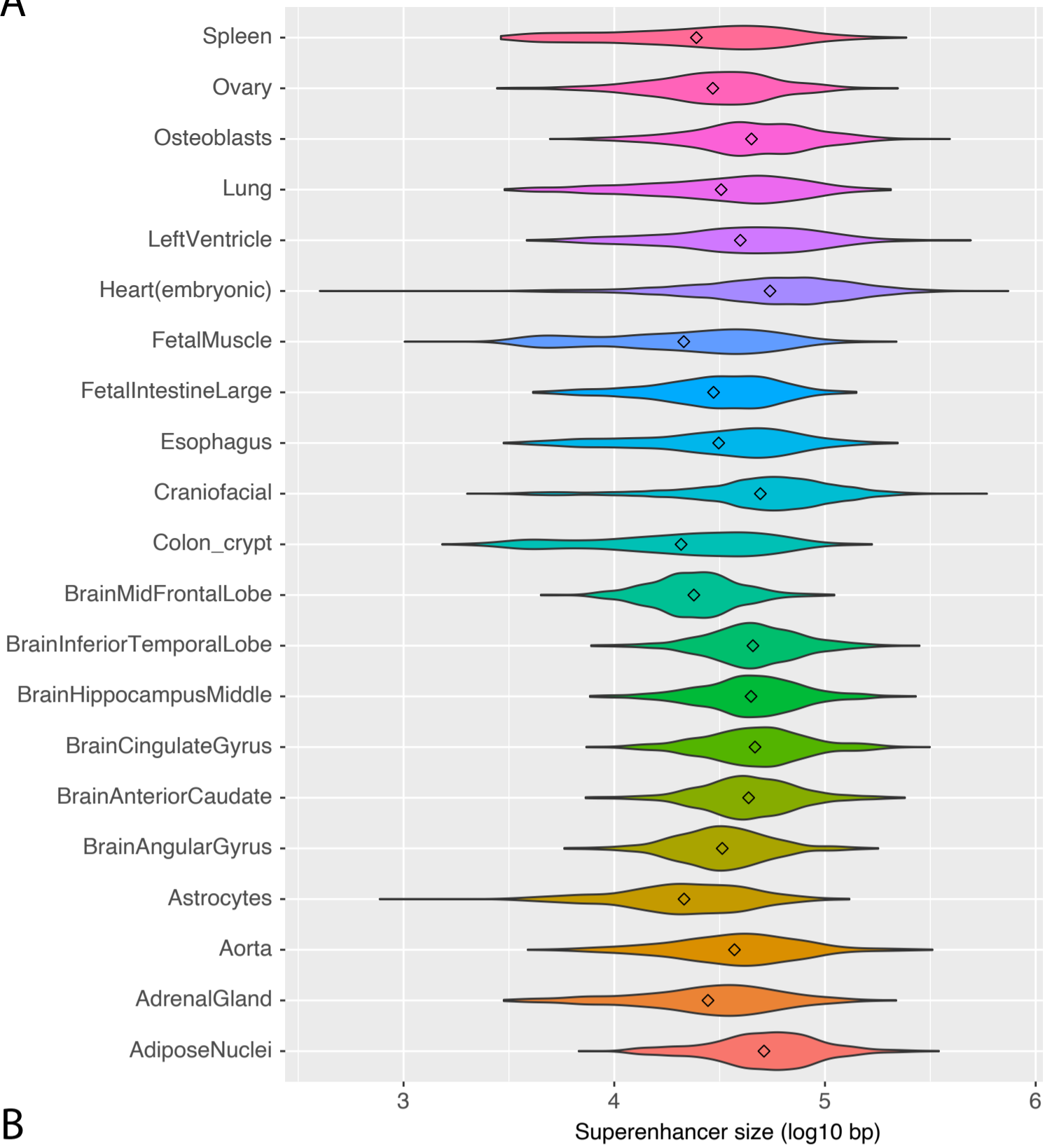
For the second case report consent was given to publish Magnetic Resonance Imaging (MRI), Computed Tomography (CT) Images, and molecular data.

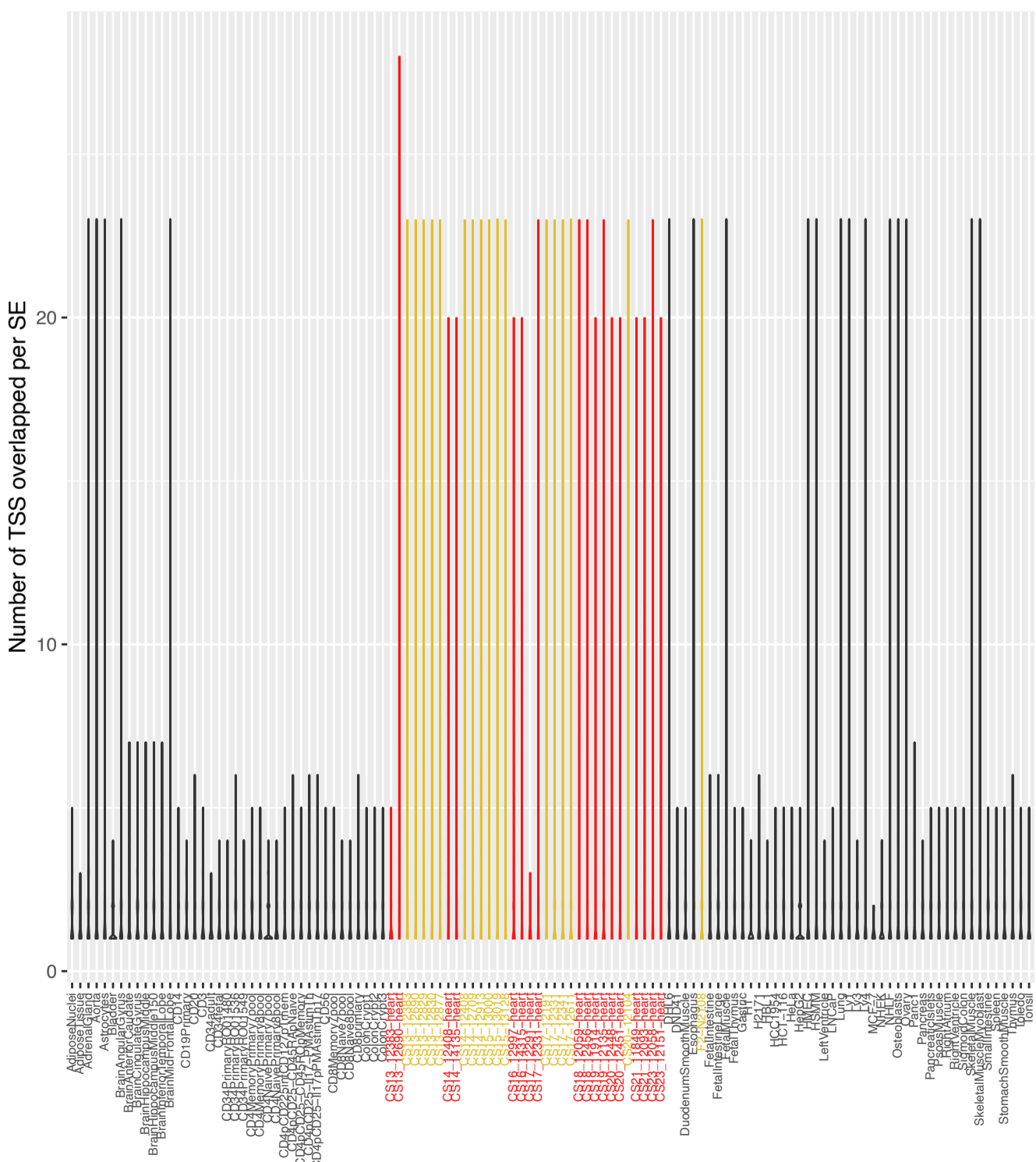16 Supplemental Tables and 29 Supplemental Figures

Figure S1- Jaccard similarity of superenhancer sequences in human embryonic craniofacial tissue samples.

Figure S2- Violin plots of number of transcription start sites (TSSs) overlapped by superenhancers in various tissues. Plots in black reference tissues and cell types in the dbSuper database. Plots in yellow are the results from human embryonic craniofacial tissue. Plots in red are the results from human embryonic heart. Superenhancers encompassing at minimum 1 and as many as 23 TSSs are found across multiple tissues. Median number of TSSs for protein-coding genes encompassed by superenhancers in dbSuper tissues and cell lines = 1. Median number of TSSs for protein coding genes encompassed by superenhancers in craniofacial and heart samples ranged from 1-3.

DisGeNET Disease Ontology Terms
from Genes Nearest Non-overlapping CF-specific SEs

Figure S3- Biological Process Gene Ontology terms enriched in genes assigned to superenhancers in non-coding regions. Assignment of the two nearest genes was done through the BedTools suite function "closest".

Biological Process Gene Ontology Terms
from Genes Nearest Non-overlapping CF-specific SEs

Figure S4- Disease Ontology terms from DisGeNet enriched in genes assigned to superenhancers in non-coding regions. Assignment of the two nearest genes was done through the BEDTools suite function "closest".

Figure S5- Expression of genes within 500kb of gene desert.
The $\log_2 + 1$ of counts from human primary craniofacial tissue, embryonic heart, fetal brain and 31 adult tissues from GTEx. These genes show ubiquitous expression across all tissues surveyed.

a

Adrenal Gland — scale 32 to 0

GM12878 — scale 50 to 0

Cortex — scale 93 to 1

Right Ventricle — scale 19 to 0

Liver — scale 50 to 0

IMR90 — scale 90 to 0

chr7 25,000,000 26,000,000 27,000,000 28,000,000

Gene track: NPY, MPP6, OSBPL3, NPVF, MIR148A, CBX3, SNX10, SKAP2, EVX1, HOXA Cluster, HIBADH, JAZF1, TAXBP1, CREB5, TRIL

b

H9 hESC — scale 2.24 / -1.78 – -1.77 / -4.48

H9-derived CNCCs — scale 2.82 / -1.46 / -4.32

chr7 25,000,000 26,000,000 27,000,000 28,000,000

Gene track: NPY, MPP6, OSBPL3, NPVF, MIR148A, CBX3, SNX10, SKAP2, EVX1, HOXA Cluster, HIBADH, JAZF1, TAXBP1, CREB5, TRIL

Figure S6- HiC from publicly available data of various human tissues and cell lines (a) and publicly available data from H9 (b- left) and H9-derived CNCCs (this study, b-right). Plots were generated through the HiC Browser hosted by Northwestern University. *HOXA* cluster and putative novel superenhancer region are highlighted with green shading.

a

500 kb
25,300,000   25,400,000   25,500,000   25,600,000   25,700,000   25,800,000   25,900,000   26,000,000   hg19

Craniofacial superenhancer
CNCC superenhancer
CNCC
25-state

|gRNA1   gRNA6|

chr7:25,295,587-25,921,144

NPVF   MIR148A

b

Heterozygous deletion

Breakpoint 1 spanning   Control Internal   Breakpoint 6 spanning

Deletion spanning

Hemizygous inversion

Combination 1F/6F   Control Internal   Combination 1R/6R

Deletion spanning

c

Geno3 (deletion-spanning)   1/6 Internal
Bulk #22 #31 #45 #47   Bulk #22 #31 #45 #47

Breakpoint 1   Breakpoint 6
Bulk #22 #31 #45 #47   Bulk #22 #31 #45 #47

d

| 1F/6F | | | | 1R/6R | | | |
| #22 | #31 | #45 | #47 | #22 | #31 | #45 | #47 |

Figure S7- a. Location of guide RNAs gRNA1 and gRNA6 relative to the WT orientation. b. Screening strategy for determining whether clones are heterozygous for the 1/6 deletion and determining if a clone contains an inversion of the targeted region. c-d. PCR results identifying heterozygous clones and the clone carrying the hemizygous inversion (#22, also referred to as INV).

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Clone 31 Breakpoint 1

+Clone_31_1F      AAAAATCATTCTGAAGCATATGGGAGGAGCTGNTTCTACAAACCTGATTTCCCAGCTGCA
                 ||||  ||||||||||||||||||||||||||| ||||||||||||||||||||||||||
+Reference_hg19  AAAAGTCATTCTGAAGCATATGGGAGGAGCTGCTTCTACAAACCTGATTTCCCAGCTGCA
-Reference_hg19  TTTTCAGTAAGACTTCGTATACCCTCCTCGACGAAGATGTTTGGACTAAAGGGTCGACGT
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_31_1R     TTTTCAGTAAGACTTCGTATACCCTCCTCGACGAAGATGTTTGGACTAAAGGGTCGACGT


+Clone_31_1F     CAGTGGGATCAGAGGAGCAGGGCCGGGG-----TGTTCCGCAGAGTCCACCCCCACTCCC
                 ||||||||||||||||||||||||||||      |||||||||||||||||||||||||||
+Reference_hg19  CAGTGGGATCAGAGGAGCAGGGCCGGGGACTTTTGTTCCGCAGAGTCCACCCCCACTCCC
-Reference_hg19  GTCACCCTAGTCTCCTCGTCCCGGCCCCTGAAAACAAGGCGTCTCAGGTGGGGGGTGAGGG
                 |||||||||||||||||||||||||||      |||||||||||||||||||||||||||
-Clone_31_1R     GTCACCCTAGTCTCCTCGTCCCGGCCC-----ACAAGGCGTCTCAGGTGGGGGGTGAGGG


+Clone_31_1F     TATAGAAGGAGAGCTTCCAGGTGGCCTAAGTCAGCAGAATCAAGCAGGGTGCCCCATCCT
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
+Reference_hg19  TATAGAAGGAGAGCTTCCAGGTGGCCTAAGTCAGCAGAATCAAGCAGGGTGCCCCATCCT
-Reference_hg19  ATATCTTCCTCTCGAAGGTCCACCGGATTCAGTCGTCTTAGTTCGTCCCACGGGGTAGGA
                 ||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||| |
-Clone_31_1R     ATATCTTCCTCTCGAAGGTCCACCGNATTCAGTCGTCTTAGTTCGTCCCACGGGGTAGCA


+Clone_31_1F     GGGCTCCTGGAGACAACAACAGATGGCGGATGGGCAAAGCAAATTAATCAAGCCACCAAA
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
+Reference_hg19  GGGCTCCTGGAGACAACAACAGATGGCGGATGGGCAAAGCAAATTAATCAAGCCACCAAA
-Reference_hg19  CCCGAGGACCTCTGTTGTTGTCTACCGCCTACCCGTTTCGTTTAATTAGTTCGGTGGTTT
                 |||||||||||||||||||||||||||| || ||||||||||||||||||||||||||||
-Clone_31_1R     CCCGAGGACCTCTGTTGTTGTCTACCGCCTANCCNTTTCGTTTAATTAGTTCGGTGGTTT


+Clone_31_1F     TGTT
                 ||||
+Reference_hg19  TGTT
-Reference_hg19  ACAA
                 ||||
-Clone_31_1R     ACAA


Clone 31 Breakpoint 6

+Clone_31_6F     GGGGACCCCCACCTTCCAAACCCCCTCCTGCTTTCCAGTAAGATTTGTANNAAGNNNAGA
                 |||||||||||||||||||||||||||||||||||||||||||||||||     |   |||
+Reference_hg19  GGGGACCCCCACCTTCCAAACCCCCTCCTGCTTTCCAGTAAGATTTGTAGTCTGGTTAGA
-Reference_hg19  CCCCTGGGGGTGGAAGGTTTGGGGGAGGACGAAAGGTCATTCTAAACATCAGACCAATCT
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_31_6R     CCCCTGGGGGTGGAAGGTTTGGGGGAGGACGAAAGGTCATTCTAAACATCAGACCAATCT

+Clone_31_6F     AGGACCATTTAGATGATGAAAGGAACCAGTGGNCAGCCCCTGATTTATAACCAGTGTGAt
                 |||||||||||||||||||||||||||||||| |||||||||||||||||||||||||||
+Reference_hg19  AGGACCATTTAGATGATGAAAGGAACCAGTGGACAGCCCCTGATTTATAACCAGTGTGAT
-Reference_hg19  TCCTGGTAAATCTACTACTTTCCTTGGTCACCTGTCGGGGACTAAATATTGGTCACACTA
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_31_6R     TCCTGGTAAATCTACTACTTTCCTTGGTCACCTGTCGGGGACTAAATATTGGTCACACTa
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+Clone_31_6F      tttttttAATATCTACCAAATTAATATTTTTATGTCTGTTCCCCCATTGAGTTGCTTCGG
                 |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
+Reference_hg19   TTTTTTTAATATCTACCAAATTAATATTTTTATGTCTGTTCCCCCATCGAGTTGCTTCGG
-Reference_hg19   AAAAAAATTATAGATGGTTTAATTATAAAAATACAGACAAGGGGGTAGCTCAACGAAGCC
                 |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
-Clone_31_6R      aaaaaaaTTATAGATGGTTTAATTATAAAAATACAGACAAGGGGGTAACTCAACGAAGCC


+Clone_31_6F      TTTGAATTTCAACGAGGGGAGCCTGCCAAGATCGTGTAGTAAAATACGAACTGGATCATA
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
+Reference_hg19   TTTGAATTTCAACGAGGGGAGCCTGCCAAGATCGTGTAGTAAAATACGAACTGGATCATA
-Reference_hg19
AAACTTAAAGTTGCTCCCCTCGGACGGTTCTAGCACATCATTTTATGCTTGACCTAGTAT
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_31_6R      AAACTTAAAGTTGCTCCCCTCGGACGGTTCTAGCACATCATTTTATGCTTGACCTAGTAT

+Clone_31_6F      AATCCCAGCAGGGGTGGGAGAACGTAAGACAGATTGCTAACACCTTaaaaaaaaaagttt
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
+Reference_hg19   AATCCCAGCAGGGGTGGGAGAACGTAAGACAGATTGCTAACACCTTAAAAAAAAAAGTTT
-Reference_hg19   TTAGGGTCGTCCCCACCCTCTTGCATTCTGTCTAACGATTGTGGAATTTTTTTTTCAAA
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_31_6R      TTAGGGTCGTCCCCACCCTCTTGCATTCTGTCTAACGATTGTGGAAtttttttttttcaaa


+Clone_31_6F      aaataaaaaaGGCAGGAATTTTCTTTAAAGTATACCAAGCCCAGCAGGGACTGGGAGATA
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
+Reference_hg19   AAATAAAAAAGGCAGGAATTTTCTTTAAAGTATACCAAGCCCAGCAGGGACTGGGAGATA
-Reference_hg19   TTTATTTTTTCCGTCCTTAAAAGAAATTTCATATGGTTCGGGTCGTCCCTGACCCTCTAT
                 ||||||||||||||||||||||||||||||||||| || || ||||||||||||||||||
-Clone_31_6R      tttattttttCCGTCCTTAAAAGAAATTTCATANGGNTCNGGTCGTCCCTGACCCTCTAT


+Clone_31_6F      CACAAGGCGGCTGAAGGAGGGAGCTA
                 ||||||||||||||||||||||||||
+Reference_hg19   CACAAGGCGGCTGAAGGAGGGAGCTA
-Reference_hg19   GTGTTCCGCCGACTTCCTCCCTCGAT
                 ||||||||||||||||||||||||||
-Clone_31_6R      GTGTTCCGCCGACTTCCTCCCTCGAT


Clone 31 across deletion (matching reference on bp1 side) (matching reference on bp6
side)

Clone_31_Geno3F   TGACTTCCCNGTATCTAGCTCACNTGTGAACACTCACTATGTTTCTATTGNAAAANGAAT
                 |||||||||  |||||||||||  ||||||||||||||||||||||||| ||||  ||||
Clone_31_Geno3R   TGACTTCCCAGTATCTAGCTCACATGTGAACACTCACTATGTTTCTATTGAAAAATGAAT


Clone_31_Geno3F   GGGGCACTTAAGTGTCTGGGTTGGGGGAGCAGGACAAGGGCAAAGATGAGCATCATTTTC
                  |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Clone_31_Geno3R  GGGGCACTTAAGTGTCTGGGTTGGGGGAGCAGGACAAGGGCAAAGATGAGCATCATTTTC


Clone_31_Geno3F   ATGACCTAGTTCAGAAAGCAAAGCTGAGCTTACTTATCAATGGCACTACACACCAAGTTT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Clone_31_Geno3R  ATGACCTAGTTCAGAAAGCAAAGCTGAGCTTACTTATCAATGGCACTACACACCAAGTTT


Clone_31_Geno3F   GTATAAGGCATTTGGTGAAAAGCTTTGCTCTGAAAAGAATGAGTTAACTCAGCCAAAAAG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Clone_31_Geno3R  GTATAAGGCATTTGGTGAAAAGCTTTGCTCTGAAAAGAATGAGTTAACTCAGCCAAAAAG
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Clone_31_Geno3F   GGTGGGGCGGGTAGCACAATTCAAATGTGACATTCTACAGTTCAGCTCAAAAGAAGCAAT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Clone_31_Geno3R   GGTGGGGCGGGTAGCACAATTCAAATGTGACATTCTACAGTTCAGCTCAAAAGAAGCAAT


Clone_31_Geno3F   TGGCTGATTGTCACCAAGTGACCTCACTTTGCTCTGCAGTTATCAGCATGGGAAGATATA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Clone_31_Geno3R   TGGCTGATTGTCACCAAGTGACCTCACTTTGCTCTGCAGTTATCAGCATGGGAAGATATA

Clone_31_Geno3F   AAACAGGTGAACCTGTGAATGTACACTGGTTTTAAACAGAGATAGAGGATGTAAAAGTCA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Clone_31_Geno3R   AAACAGGTGAACCTGTGAATGTACACTGGTTTTAAACAGAGATAGAGGATGTAAAAGTCA


Clone_31_Geno3F   TTCTGAAGCATATGGGAGGAGCTGCTTCTACAAACCTGATTTCCCAGCTGCACAGTGGGA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Clone_31_Geno3R   TTCTGAAGCATATGGGAGGAGCTGCTTCTACAAACCTGATTTCCCAGCTGCACAGTGGGA


Clone_31_Del_spanning_F   TCAGAGGAGCAGGG CCGGG GA ACTGG ATCATAAATCCCAGCAGGGGTGGGAGAACGTAAG
                          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Clone_31_Del_spanning_R   TCAGAGGAGCAGGG CCGGG GAACTGG ATCATAAATCCCAGCAGGGGTGGGAGAACGTAAG


Clone_31_Geno3F   ACAGATTGCTAACACCTTaaaaaaaaaaagtttaaataaaaaaGGCAGGAATTTTCTTTAA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Clone_31_Geno3R   ACAGATTGCTAACACCTTAAAAAAAAAAAGTTTAAATAAAAAAGGCAGGAATTTTCTTTAA


Clone_31_Geno3F   AGTATACCAAGCCCAGCAGGGACTGGGAGATACACAAGGCGGCTGAAGGAGGGAGCTACT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Clone_31_Geno3R   AGTATACCAAGCCCAGCAGGGACTGGGAGATACACAAGGCGGCTGAAGGAGGGAGCTACT


Clone_31_Geno3F   TTACCTTCCCAAGATCACTTTTCCGAGAAGCCCAAACCACAGAAAACCATTCTGGCAGGT
                  |||||||||||||||||||||||||||||||||||||| |||||||||||||||||||||
Clone_31_Geno3R   TTACCTTCCCAAGATCACTTTTCCGAGAAGCCCAAA**N**CACAGAAAACCATTCTGGCAGGT


Clone_31_Geno3F   AAATCGGGCCCCATGAGCTTTTCATCAAAAGCCAAGGATGTCTCTCTGGGATGTGTGTTA
                  ||||||||||||            ||||||||||||||||||||||||||||||||||||
Clone_31_Geno3R   AAATCGGGCCCCA**NNNNNN**TTTCATCAAAAGCCAAGGATGTCTCTCTGGGATGTGTGTTA


Clone_31_Geno3F   GAGATAAGGCAG
                  ||||||||||||
Clone_31_Geno3R   GAGATAAGGCAG
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Clone 22

Clone 22 across deletion

Clone 22 deletion-spanning PCR

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+Clone_22_Geno3F  GACTTCCCAGTATCTAGCTCACATGTGAACACTCACTATGTTTCTATNNNNAANNGAATG
                  |||||||||||||||||||||||||||||||||||||||||||||||    ||  |||||
-Clone_22_Geno3R  GACTTCCCAGTATCTAGCTCACATGTGAACACTCACTATGTTTCTATTGAAAAATGAATG

+Clone_22_Geno3F  GGGCACTTAAGTGTCTGGGTTGGGGGAGCAGGACAAGGGCAAAGATGAGCATCATTTTCA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_22_Geno3R  GGGCACTTAAGTGTCTGGGTTGGGGGAGCAGGACAAGGGCAAAGATGAGCATCATTTTCA

+Clone_22_Geno3F  TGACCTAGTTCAGAAAGCAAAGCTGAGCTTACTTATCAATGGCACTACACACCAAGTTTG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_22_Geno3R  TGACCTAGTTCAGAAAGCAAAGCTGAGCTTACTTATCAATGGCACTACACACCAAGTTTG

+Clone_22_Geno3F  TATAAGGCATTTGGTGAAAAGCTTTGCTCTGAAAAGAATGAGTTAACTCAGCCAAAAAGG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_22_Geno3R  TATAAGGCATTTGGTGAAAAGCTTTGCTCTGAAAAGAATGAGTTAACTCAGCCAAAAAGG

+Clone_22_Geno3F  GTGGGGCGGGTAGCACAATCCAAATGTGACATTCTACAGTTCAGCTCAAAAGAAGCAATT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_22_Geno3R  GTGGGGCGGGTAGCACAATCCAAATGTGACATTCTACAGTTCAGCTCAAAAGAAGCAATT

+Clone_22_Geno3F  GGCTGATTGTCACCAAGTGACCTCACTTTGCTCTGCAGTTATCAGCATGGGAAGATATAA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_22_Geno3R  GGCTGATTGTCACCAAGTGACCTCACTTTGCTCTGCAGTTATCAGCATGGGAAGATATAA

+Clone_22_Geno3F  AACAGGTGAACCTGTGAATGTACACTGGTTTTAAACAGAGATAGAGGATGTAAAAGTCAT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_22_Geno3R  AACAGGTGAACCTGTGAATGTACACTGGTTTTAAACAGAGATAGAGGATGTAAAAGTCAT

+Clone_22_Geno3F  TCTGAAGCATATGGGAGGAGCTGCTTCTACAAACCTGATTTCCCAGCTGCACAGTGGGAT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_22_Geno3R  TCTGAAGCATATGGGAGGAGCTGCTTCTACAAACCTGATTTCCCAGCTGCACAGTGGGAT

+Clone_22_Del_spanning_F  CAGAGGAGCAGGGCCGGGGANN-NNNATCATAAATCCCAGCAGGGGTGGGAGAACGTAAG
                          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_22_Del_spanning_R  CAGAGGAGCAGGGCCGGGGAAACTGGATCATAAATCCCAGCAGGGGTGGGAGAACGTAAG

+Clone_22_Geno3F  ACAGATTGCTAACACCTTaaaaaaaaaaagtttaaataaaaaaGGCAGGAATTTTCTTTAA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_22_Geno3R  ACAGATTGCTAACACCTTAAAAAAAAAAAGTTTAAATAAAAAAGGCAGGAATTTTCTTTAA

+Clone_22_Geno3F  AGTATACCAAGCCCAGCAGGGACTGGGAGATACACAAGGCGGCTGAAGGAGGGAGCTACT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_22_Geno3R  AGTATACCAAGCCCAGCAGGGACTGGGAGATACACAAGGCGGCTGAAGGAGGGAGCTACT

+Clone_22_Geno3F  TTACCTTCCCAAGATCACTTTTCCGAGAAGCCCAAACCACAGAAAACCATTCTGGCAGGT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
-Clone_22_Geno3R  TTACCTTCCCAAGATCACTTTTCCGAGAAGCCCAAACCACAGAAAACCATTCTGGCAGGT

+Clone_22_Geno3F  AAATCGGGCCCCATGAGCTTTTCATCAAAAGCCAAGGATGTCTCTCTGGGATGTGTGTTA
                  |||||||||||||||||     ||  |||||||||||||||||||||||||||||||  ||
-Clone_22_Geno3R  AAATCGGGCCCCATGANNNTTNNATCAAAAGCCAAGGATGTCTCTCTGGGATGTGTNNTA

+Clone_22_Geno3F  GAGATAAGGCAG
                  ||||||||||||
-Clone_22_Geno3R  GAGATAAGGCAG
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Clone 22 at inversion

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Clone_22_1F      nnnnnnnnnn nnnnnnnnnn nnnntggnnn nnnntnnnnc aggngnnnnt  50
+Reference_hg19                        25295459                        T

Clone_22_1F      gtgaatgtac actggtttta aacagagata gnnnnnnnaa aagtcattct  100
+Reference_hg19  GTGAATGTAC ACTGGTTTTA AACAGAGATA GaggatgtAA AAGTCATTCT


Clone_22_1F      gaagcatatg ggaggagctg cttnnncaaa cctgatttcc cagctgcaca  150
+Reference_hg19  GAAGCATATG GGAGGAGCTG CTTctaCAAA CCTGATTTCC CAGCTGCACA

Clone_22_1F      gtgggatcag aggagcaggg ccggggaGT ATTTTACTAC ACGATCTTGG
+Reference_hg19  GTGGGATCAG AGGAGCAGGG CCGGGGAC 25295587
-Reference_hg19                      25921143 GT ATTTTACTAC ACGATCTTGG

Clone_22_1F      CAGGCTCCCC TCGTTGAAAT TCAAACCGAA GCAACTCaAT GGGGGAACAG  250
-Reference_hg19  CAGGCTCCCC TCGTTGAAAT TCAAACCGAA GCAACTCgAT GGGGGAACAG

Clone_22_1F      ACATAAAAAT ATTAATTTGG TAGATATTAA AAAAAATCAC ACTGGTTATA  300
-Reference_hg19  ACATAAAAAT ATTAATTTGG TAGATATTAA AAAAAATCAC ACTGGTTATA

Clone_22_1F      AATCAGGGGC TGTCCACTGG TTCCTTTCAT CATCTAAATG GTCCTTCTAA  350
-Reference_hg19  AATCAGGGGC TGTCCACTGG TTCCTTTCAT CATCTAAATG GTCCTTCTAA

Clone_22_1F      CCAGACTACA AATCTTACTG GAAAGCAGGA GGGGGTTTGG AAGGTGGGGG  400
-Reference_hg19  CCAGACTACA AATCTTACTG GAAAGCAGGA GGGGGTTTGG AAGGTGGGGG

Clone_22_1F      TCCCCTTCCT GAGGCTTTGG AGACAGCTTA CATCCAACAC TTCCTCAAAC  450
-Reference_hg19  TCCCCTTCCT GAGGCTTTGG AGACAGCTTA CATCCAACAC TTCCTCAAAC

Clone_22_1F      CTGGCTTTGa
-Reference_hg19  CTGGCTTTG 25920862


Clone_22_6F nnnnnnnnnn nntgnnnnnn nnnnnnnnga nnnggncccc caaccttnna  50
            agcccctcct gctttccagt aagatttgta gnnnnnnnan aaggaccatt  100
+Ref_hg1925920937CCCCTCCT GCTTTCCAGT AAGATTTGTA Gtctggttag AAGGACCATT

Clone_22_6F tagatgatga aaggaaccag tggacagccc ctgatttata accagtgtga  150
+Ref_hg19   TAGATGATGA AAGGAACCAG TGGACAGCCC CTGATTTATA ACCAGTGTGA

Clone_22_6F ttttttttaa tatctaccaa attaatattt ttatgtctgt tcccccattg  200
+Ref_hg19   TTTTTTTTAA TATCTACCAA ATTAATATTT TTATGTCTGT TCCCCCATcG

Clone_22_6F agttgcttcg gtttgaattt caacgagggg agcctgccaa gatcgtgtag  250
+Ref_hg19   AGTTGCTTCG GTTTGAATTT CAACGAGGGG AGCCTGCCAA GATCGTGTAG


Clone_22_6F TAAAATACGT CCCCGGCCCT GCTCCTCTGA TCCCACTGTG CAGCTGGGAA  300
+Ref_hg19   TAAAATAC 25921143
-Ref_hg19 25295587 GT CCCCGGCCCT GCTCCTCTGA TCCCACTGTG CAGCTGGGAA

Clone_22_6F  ATCAGGTTTG TAGAAGCAGC TCCTCCCATA TGCTTCAGAA TGACTTTTAC  350
-Ref_hg19    ATCAGGTTTG TAGAAGCAGC TCCTCCCATA TGCTTCAGAA TGACTTTTAC

Clone_22_6F  ATCCTCTATC TCTGTTTAAA ACCAGTGTAC ATTCACAGGT TCACCTGTTT  400
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-Ref_hg19     ATCCTCTATC TCTGTTTAAA ACCAGTGTAC ATTCACAGGT TCACCTGTTT

Clone_22_6F   TATATCTTCC CATGCTGATA ACTGCAGAGC AAAGTGAGGT CACTTGGTGA  450
-Ref_hg19     TATATCTTCC CATGCTGATA ACTGCAGAGC AAAGTGAGGT CACTTGGTGA

Clone_22_6F   CAATCAGCCA A
-Ref_hg19     CAATCAGCCA A 25295385


Clone_22_1R   nnnnnnnnnn nnnnnnnnnn nntgnnnnnt aaatatgatg cacagnanca  50
-Ref_hg19     25295806                      T AAATATGATG CACAGtAaCA

Clone_22_1R   ntggcggtgt gtaactagta gcgaatttgn nnnnnnnnnn gagaacattt  100
-Ref_hg19     gTGGCGGTGT GTAACTAGTA GCGAATTTGa aaaccaaatct GAGAACATTT

Clone_22_1R   ggtggcttga ttaatttgct tcgccnatcc gccatctgtt gttgtctcca  150
-Ref_hg19     GGTGGCTTGA TTAATTTGCT TtGCCcATCC GCCATCTGTT GTTGTCTCCA

Clone_22_1R   ggagcccagg atggggcacc ctgcttgatt ctgctgactt aggccacctg  200
-Ref_hg19     GGAGCCCAGG ATGGGGCACC CTGCTTGATT CTGCTGACTT AGGCCACCTG

Clone_22_1R   gaagctctcc ttctataggg agtggggggtg gactctgcgg aacaaaagtA  250
-Ref_hg19     GAAGCTCT CCTTCTATAG GGAGTGGGGGTG GACTCTGCGG AACAAAAGT  25295586
+Ref_hg19                                  25921144              A

Clone_22_1R   ACTGGATCAT AAATCCCAGC AGGGGTGGGA GAACGTAAGA CAGATTGCTA  300
+Ref_hg19     ACTGGATCA TAAATCCCAG CAGGGGTGGG AGAACGTAAG ACAGATTGCTA

Clone_22_1R   ACACCTTAAA AAAAAAGTT TAAATAAAAA AGGCAGGAAT TTTCTTTAAA  350
+Ref_hg19     ACACCTTAA AAAAAAAGT TTAAATAAAA AAGGCAGGAA TTTTCTTTAAA

Clone_22_1R   GTATACCAAG CCCAGCAGGG ACTGGGAGAT ACACAAGGCG GCTGAAGGAG  400
+Ref_hg19     GTATACCAA GCCCAGCAGG GACTGGGAGA TACACAAGGC GGCTGAAGGAG

Clone_22_1R   GGAGCTACTT TACCTTCCCA AGATCACTTT TCCGAGtAGC CCAAACCACA  450
+Ref_hg19     GGAGCTACT TTACCTTCCC AAGATCACTT TTCCGAGaAG CCCAAACCACA

Clone_22_1R   GAAAACCATT CTGGCAa
+Ref_hg19     GAAAACCAT TCTGGCA 25921360



Clone_22_6R   nnnnnnnnnn nnnnnnnnnn nnntgngnnn gnnagtanct ccctccttca  50
-Ref_h19                         25921303 AGTAgCT CCCTCCTTCA

Clone_22_6R   gccgccttgt gtatctccca gtccctgctg gnnttggtat actttaaaga  100
-Ref_h19      GCCGCCTTGT GTATCTCCCA GTCCCTGCTG GgcTTGGTAT ACTTTAAAGA

Clone_22_6R   aaattcctgc cttttttatt taaacttttt tttttaaggt gttagcaatc  150
-Ref_h19      AAATTCCTGC CTTTTTTATT TAAACTTTTT TTTTTAAGGT GTTAGCAATC

Clone_22_6R   tgtcttacgt tctcccaccc ctgctgggat ttatgatcca gttACTTTTG  200
-Ref_h19      TGTCTTACGT TCTCCCACCC CTGCTGGGAT TTATGATCCA GTT         25921144
+Ref_h19                                     25295586    ACTTTTG

Clone_22_6R   TTCCGCAGAG TCCACCCCCA CTCCCTATAG AAGGAGAGCT TCCAGGTGGC  250
+Ref_h19      TTCCGCAGAG TCCACCCCCA CTCCCTATAG AAGGAGAGCT TCCAGGTGGC
```

```
Clone_22_6R  CTAAGTCAGC AGAATCAAGC AGGGTGCCCC ATCCTGGGCT CCTGGAGACA  300
+Ref_h19     CTAAGTCAGC AGAATCAAGC AGGGTGCCCC ATCCTGGGCT CCTGGAGACA


Clone_22_6R  ACAACAGATG GCGGATGGGC AAAGCAAATT AATCAAGCCA CCAAATGTTC  350
+Ref_h19     ACAACAGATG GCGGATGGGC AAAGCAAATT AATCAAGCCA CCAAATGTTC

Clone_22_6R  TCAGATTTGG TTTTCAAATT CGCTACTAGT TACACACCGC CACTGTTACT  400
+Ref_h19     TCAGATTTGG TTTTCAAATT CGCTACTAGT TACACACCGC CACTGTTACT

Clone_22_6R  GTGCATCATA TTTACTTGAC AGCATTGTCC AGTCCTTAGA TTTCTCTCAC  450
+Ref_h19     GTGCATCATA TTTACTTGAC AGCATTGTCC AGTCCTTAGA TTTCTCTCAC

Clone_22_6R  TGGCTCACGC TAAAGAAa
+Ref_h19     TGGCTCACGC TAAAGAA 25295859
```

Figure S8- (Word document) Sequence across breakpoints for clones #31 (heterozygous) and #22 (inversion, INV). Clone sequence based on Sanger sequencing results (see Methods) and compared against hg19 assembly.

Figure S9- Heatmap of differentially expressed genes between WT and INV CNCCs. Names followed by asterisks are HOXA2 targets as determined by HOXA2 ChIP-seq (Donaldson et al. 2012). Names in bold are HOXA2 associated genes as determined by RNA-seq from *Hoxa2*-/- mice (Donaldson et al., 2012).

a.

HOXA2 targets in permuted selection of 52 genes

p=0.135

HOXA2 targets in permuted selection of 44 genes

p=0.075

b.

HOXA2 related genes in permuted selection of 52 genes

p=0.013

HOXA2 related genes in permuted selection of 44 genes

p=0.805

Figure S10- Permutation tests for enrichment of Hoxa2 targets (a) in WT (left, number of *Hoxa2* targets shown as blue dotted line) and inversion (INV) (right, number of *Hoxa2* targets shown as red dotted line). Permutations tests for enrichment of Hoxa2-related genes (b) in WT (left, number of *Hoxa2*-related genes shown as blue solid line) and INV (right, number of *Hoxa2*-related genes shown as red solid line).

a.

Baseline expression
(d0)

b.

CNCC expression
(d5)

Figure S11- Relative expression of genes in and adjacent to the HOXA cluster at baseline (d0) (a) and after complete CNCC differentiation (d5) (b).

WT
INV
chr7:25,295,587-25,921,144

CYCS
OSBPL3
NPVF
MIR148A
HNRNPA2B1
CBX3
NFE2L3
SNX10
SKAP2
HOXA Cluster
EVX1
HIBADH
TAX1BP1
JAZF1

Figure S12- Subtraction HiC map generated by comparison of WT CNCC HiC data and INV CNCC HiC data on custom genome accounting for inversion. Representations of the WT and inverted superenhancer region are displayed below the triangular heatmap. Light blue boxes show areas of stronger interaction in the WT CNCCs, red triangles show novel interactions created by the superenhancer inversion.

a

Superenhancer subTAD

CTCF ChIP-seq

40 -
0
40 -
0

Predicted Loops

b

Bound CTCF orientation

4C-seq interactions

subTAD Center

TAD Boundary Viewpoints

HOXA Viewpoint

c

Inversion

Bound CTCF orientation

4C-seq interactions

subTAD Center

TAD Boundary Viewpoints

HOXA Viewpoint

CYCS

OSBPL3

NPVF

MIR148A   *SNX10*

HNRNPA2B1

CBX3

NFE2L3

SKAP2

HIBADH

EVX1

TAX1BP1

HOXA Cluster

JAZF1

Figure S13- Schematic showing consequences of superenhancer inversion on orientation of CTCF sites and boundary strength. Previously insulated 4C viewpoints are capable of interaction with *HOXA* cluster following creation of inversion and viewpoints at the TAD boundary capable of interacting with the *HOXA* cluster in the WT configuration retain contacts following inversion.

a.

rs2428431

HOXA2 (ENST00000222718.7)
HOXA2 (ENST00000612779.1)

b.

rs2428431 C/G (%) [counts]

WT GCR alleles

WT n=3: (50%/50%) [3/3]
         (50%/50%) [5/5]
         (45%/55%) [5/6]

Hemizygous inversion of GCR (INV)

INV n=3: (17%/83%) [2/10]
          (33%/67%) [2/4]
          (21%/79%) [4/15]

Figure S14- IGV browser shot showing C/G allele ratios in WT CNCCs, hemizygous inversion (INV) CNCCs and WT, INV and Heterozygous superenhancer deletion lines in which the TAD boundary of both alleles (WT) or the remaining allele was also deleted.

A

Scale | 1 Mb | hg19
chr7: | 25,000,000 | 25,500,000 | 26,000,000 | 26,500,000 | 27,000,000 | 27,500,000 | 28,000,000

Human Craniofacial Chromatin State Segmentation CS13-CS20, F2 (15-state model)

Mouse Craniofacial Chromatin State Segmentation E9.5-E15.5 (15-state model)

MPP6 DFNA5 CYCS C7orf31 RNU6-16P MIR148AI NFE2L3 HNRNPA2B1 SNX10 C7orf7 SKAP2 EVX1 HIBADH TAX1BP1 JAZF1

HOXA Cluster

Figure S15- Comparison of chromatin states in the 15-state model between human embryonic craniofacial tissue (hg19) and mouse embryonic craniofacial tissue (mm9 lifted to hg19).

CS13 craniofacial tissue and E11.5 craniofacial tissue

CS13 craniofacial tissue and E11.5 hindbrain

CS13_12383

1_TssA
2_TssFlnk
3_TssFlnkU
4_TssFlnkD
5_Tx
6_TxWk
7_EnhG1
8_EnhG2
9_EnhA1
10_EnhA2
11_EnhWk
12_ZNFRpts
13_Het
14_TssBiv
15_EnhBiv
16_ReprPC
17_ReprPCWk
18_Quies

Figure S16- Chromatin state composition comparison for 18-state model between CS13 craniofacial tissue and E11.5 craniofacial tissue or E11.5 hindbrain. Mouse chromatin states lifted from mm10 to hg19 using liftOver.

Scale
chr6:
50,500,000   51,000,000   51,500,000   52,000,000   52,500,000

1 Mb   mm10

E9.5
E10.5
E11.5
palate
u. palate
snout
CF meSE

Npvf   Nfe2l   Hoxa Cluster

Osbpl3   Snx10   Hibadh

Mpp6   Cycs   Hnrnpa2b1   Skap2

Mir6371   Evx1os   Tax1bp1

Gsdme   Mir148a   Halr1 Evx1   Jazf1
                      Cbx

Interior of subTAD

subTAD boundary

Skap2 promoter

Hoxa5/6 viewpoint

Figure S17- HiC data from WT E11.5 mouse craniofacial tissue, TADs or subTADs determined at 50Kb resolution. The superenhancer subTAD is marked throughout with dotted lines (Top). Chromatin states in 18-state model and mouse embryonic craniofacial superenhancers shown in orange bars above gene notations (Middle). Interactions identified though 4C-seq at all viewpoints tested (Bottom). Viewpoint1(red) and Viewpoint 2 (light green) are located within the interior of the superenhancer subTAD. Viewpoint 3 (magenta) and Viewpoint 4 (dark green) are located at the boundary of the subTAD. Viewpoint 5 (dark blue) is located near the *Skap2* promoter and Viewpoint 6 (purple) is located near the intergenic space between *Hoxa5* and *Hoxa6*.

2WT_Hoxa2 enh_E18.5_0S_CB-ventral.png

4HT_Hoxa2 enh_E18.5_0S_CB-ventral.png

11WT_Hoxa2 enh_E18.5_0S_CB-ventral.png

15HT_Hoxa2 enh_E18.5_0S_CB-ventral.png

1KO_Hoxa2 enh_E18.5_0S_CB-ventral.png

3KO_Hoxa2 enh_E18.5_0S_CB-ventral.png

7KO_Hoxa2 enh_E18.5_0S_CB-ventral.png

8KO_Hoxa2 enh_E18.5_0S_CB-ventral.png

9KO_Hoxa2 enh_E18.5_0S_CB-ventral.png

10KO_Hoxa2 enh_E18.5_0S_CB-ventral.png

12KO_Hoxa2 enh_E18.5_0S_CB-ventral.png

13KO_Hoxa2 enh_E18.5_0S_CB-ventral.png

14KO_Hoxa2 enh_E18.5_0S_CB-ventral.png

Figure S18- microCT renderings of skulls from all WT, Hoxa+/ΔGCR (HT), and Hoxa ΔGCR /ΔGCR (KO) E18.5 embryos.

Figure S19 4C-Seq data from E11.5 mouse heart and limb. The superenhancer subTAD is marked throughout with dotted lines. Same viewpoints depicted in Figure S17 were used for each tissue. Interactions identified though 4C-seq are indicated by loops for each viewpoint in heart (pink) and limb (green). Colored bars indicate superenhancer calls in respective tissues.

Craniofacial

PBX1 (p=1e-4)
Six4 (p=1e-4)
Pitx1 (p=1e-2)
GRE (p=1e-2)
OCT (p=1e-2)

Heart

Mef2d (p=1e-2)

Limb

ZNF415 (p=1e-4)
DMRT6 (p=1e-3
GRE (p=1e-3)
Foxh1 (p=1e-3)
PBX1 (p=1e-3)
DMRT1 (p=1e-2)
ZNF519 (p=1e-2)
GATA3 (p=1e-2)
Hoxc9 (p=1e-2)
MyoD (p=1e-2)
Pitx1 (p=1e-2)

Figure S20. Significantly enriched TF binding motifs in enhancer modules encompassed by superenhancer calls in the chromosome 7 gene desert.

a

E11.5 Face — Hoxa1 (p=0.001, padj=0.999), Hoxa2 (p=4.47×10⁻⁹, padj=4.31×10⁻⁵), Hoxaas2 (p=2.18×10⁻⁴, padj=4.2×10⁻¹⁰), Hoxa3 (p=9.82×10⁻⁶, padj=0.063), Hoxaas3 (p=0.54, padj=0.999), Hoxa4 (p=0.045, padj=0.999), Hoxa5 (p=0.012, padj=0.999), Hoxa6 (p=NA, padj=NA), Hoxa7 (p=NA, padj=NA), Hoxa9 (p=NA, padj=NA), Hoxa10 (p=NA, padj=NA), Hoxa11 (p=NA, padj=NA), Hoxa11os (p=NA, padj=NA), Hoxa13 (p=NA, padj=NA)

E11.5 Limb — Hoxa1 (p=0.12, padj=0.999), Hoxa2 (p=0.70, padj=0.999), Hoxaas2 (p=0.85, padj=NA), Hoxa3 (p=0.04, padj=0.705), Hoxaas3 (p=0.84, padj=0.999), Hoxa4 (p=0.72, padj=0.999), Hoxa5 (p=0.06, padj=0.815), Hoxa6 (p=0.005, padj=0.271), Hoxa7 (p=0.18, padj=0.999), Hoxa9 (p=0.0006, padj=0.086), Hoxa10 (p=0.10, padj=0.940), Hoxa11 (p=0.06, padj=0.836), Hoxa11os (p=0.38, padj=0.999), Hoxa13 (p=0.47, padj=0.999)

E11.5 Heart — Hoxa1 (p=0.71, padj=0.999), Hoxa2 (p=0.004, padj=0.610), Hoxaas2 (p=0.05, padj=0.999), Hoxa3 (p=0.012, padj=0.843), Hoxaas3 (p=0.29, padj=0.999), Hoxa4 (p=0.61, padj=0.999), Hoxa5 (p=0.17, padj=0.999), Hoxa6 (p=0.95, padj=NA), Hoxa7 (p=0.85, padj=0.999), Hoxa9 (p=0.60, padj=0.999), Hoxa10 (p=NA, padj=0.999), Hoxa11 (p=NA, padj=NA), Hoxa11os (p=NA, padj=NA), Hoxa13 (p=NA, padj=NA)

b

DE Genes ΔGCR/ΔGCR vs WT

Figure S21- Comparison of *Hoxa* gene expression in embryonic face, limb and heart (a). Gene expression changes in heart (b-left panel) and limb (b-right panel). Few significant genes change as a result of the deletion, and did not include the *Hoxa* genes in either heart or limb.

right forelimb: soft tissue morphology

$Hoxa^{+/+}$

#2 #11

$Hoxa^{+/\Delta GCR}$

#4 #15

$Hoxa^{\Delta GCR/\Delta GCR}$

#1 #3 #7 #8 #9

#10 #12 #13 #14

right forelimb: skeletal morphology

$Hoxa^{+/+}$

#2 #11

$Hoxa^{+/\Delta GCR}$

#4 #15

$Hoxa^{\Delta GCR/\Delta GCR}$

#1 #3 #7 #8 #9

#10 #12 #13 #14

Figure S22. Soft tissue and microCT renderings of limbs from all WT, *Hoxa*[+/ΔGCR,] and *Hoxa* [ΔGCR /][ΔGCR] E18.5 embryos. Genotypes are indicated in each dashed box and embryos are matched by number across rendering types.

a

b

GCR

CF meSE

Npvf
Osbpl3
Mpp6   Cycs
Gsdme
Mir6371

Nfe2l
Snx10
Hnrnpa2b1
Mir148a   Cbx

Skap2
Hoxa Cluster
Evx1os
Halr1   Evx1

Hibadh
Tax1bp1
Jazf1

c

Snx10   Cbx3
Nfe2l
Skap2
Halr1   Hoxa1-6?
Hoxa10-13?
Cycs
Npvf
Osbpl3
GCR subTAD

d

e

Δ GCR

CF meSE

Npy
Osbpl3
Mpp6
Gsdme
Mir6371

Npvf
Mir148a

Nfe2l
Snx10
Hnrnpa2b1
Cycs
Cbx

Hoxa Cluster
Skap2
Halr1   Evx1
Evx1os

Hibadh
Tax1bp1
Jazf1

f

Cbx3
Nfe2l
Snx10
Npvf
Skap2
Halr1   Cycs
Osbpl3
Hoxa Cluster

Figure S23- a. Deletion of GCR re-structures the *HoxA* regulatory landscape. The removal of ~625kb and almost complete removal of the superenhancers in the gene desert between *Npvf* and *Mir148a* disrupts the contact between the GCR and the *HoxA* cluster. Schematics based on HiC of E11.5 cranofacial tissue from WT (top panel) and ᐃGCR/ᐃGCR mice (bottom panel). The bottom panel was created by alignment to a custom genome based on mm10 with deletion of the GCR coordinates. Presentation of superenhancers in the bottom panel are predicted, based on the superenhancers as they appear in the WT. TADs predicted at 100Kb are represented with black outline, TADs predicted at 50Kb have no outline.

Figure S24. a. Copy number variants from DECIPHER database overlapping the putative novel craniofacial superenhancer region (black box with solid outline). CNVs represented by green bars have a noted phenotype but do not include a specifically described craniofacial phenotype, purple bars have a specifically described craniofacial phenotype and black bars have no phenotype reported. b. Enlargement of region in box with dotted outline. The orthologous positions removed in the GCR deletion mouse are indicated by a green bar. The orthologous positions to HIRE1 and HIRE2 deleted by Kessler et al are shown in shades of blue. Craniofacial superenhancers identified in this study are indicated by large orange bars. The track for gnomAD CNVs, filtered for CNVs >300bp appears below the DECIPHER CNV bars, blue represents gains and red losses. 300bp based on typical size range of CNVs identified in healthy human populations (Zarrei et al., 2015). A region with a notable lack of CNVs in gnomAD subjects is marked by a yellow triangle.
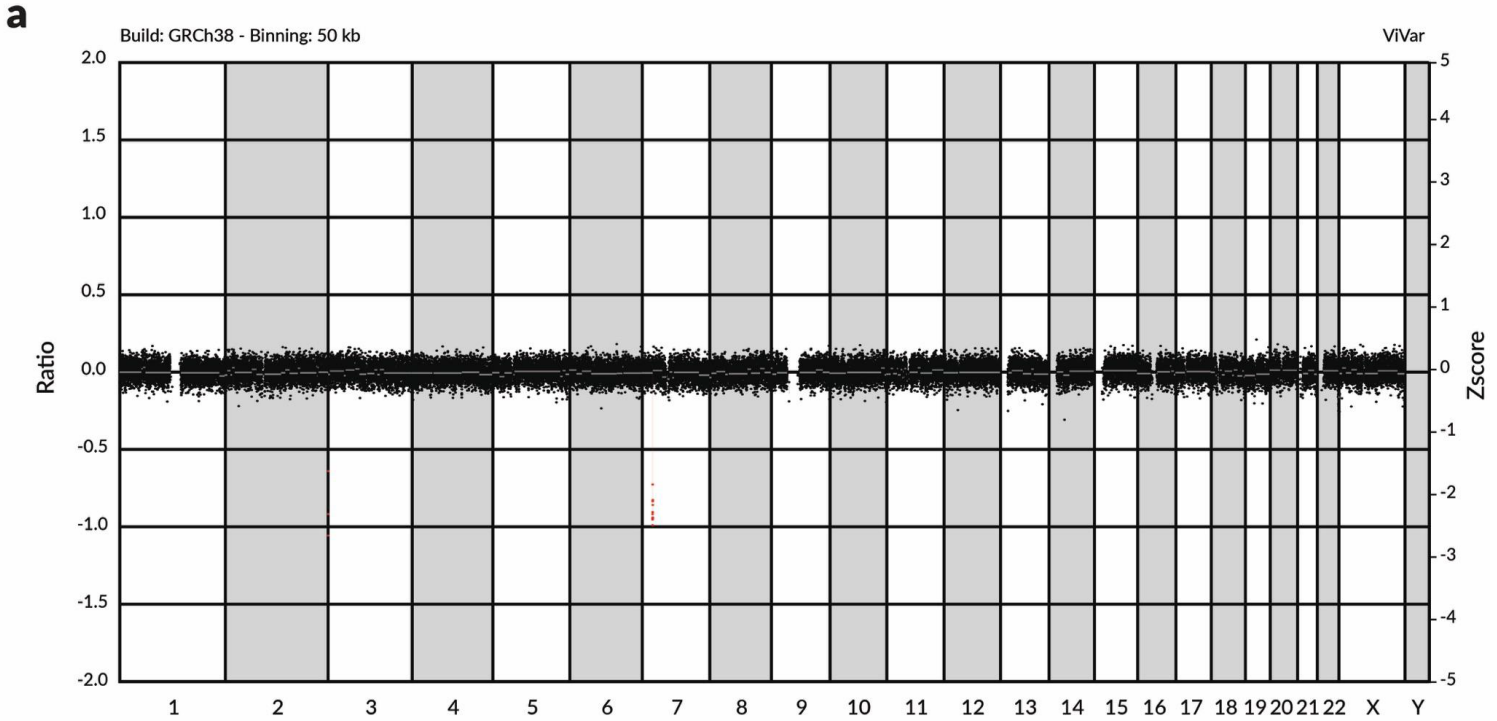
Figure S25. Frequency of phenotypes present in individuals within the DECIPHER Database with CNVs overlapping chr7: 25,580,400-25,849,400 compared to frequency of those phenotypes in the the DECIPHER Database for CNVs not overlapping the region. Statistical test is Fisher Exact Test. * p<0.05, ** p<0.01, *** p<0.001

Figure S26. Autopsy images of fetus with partial deletion of the HOXA superenhancer region. The fetus displayed bilateral cleft lip and palate, an underdeveloped nose and only one nostril, clubfeet and anal atresia.

a

Build: GRCh38 - Binning: 50 kb

ViVar

b

chromosome 7

2.55 Mb

ViVar

Build: GRCh38 - Binning: 50 kb

UCSC.refGene

NFE2L3 *SNX10*
HNRNPA2B1
CBX3

HOXA

c

Scale

chr7:

HIRE2   HIRE1
Mouse GCR Deletion
de novo deletion

ancer_regions_merge
CNCC-1_25state
CNCC-2_25state
CS13-12383_25state
CS13-12690_25state
CS13-12829_25state
CS13-12830_25state
CS13-12877_25state
13-combined_25state
CS14-12408_25state
CS14-12709_25state
CS14-12913_25state
14-combined_25state
CS15-13000_25state
CS15-13019_25state
CS15-13128_25state
15-combined_25state
CS17-12191_25state
CS17-12331_25state
CS17-12341_25state
CS17-12611_25state
17-combined_25state
CS20-12104_25state

Cons 100 Verts

Figure S27- Identification of a 550kb *de novo* deletion upstream of the *HOXA* cluster through shallow whole genome sequencing and copy-number variant (CNV) analysis using ViVar. (a) whole genome lineview. (b) zoomed in on deletion (binning: 50kb). ViVar analysis and visualization (Sante et al., 2014). (c). Genome browser view of same region as in (b). At top the de novo deletion is indicated in black. The orthologous positions removed in the GCR deletion mouse are indicated by a green bar. The orthologous positions to HIRE1 and HIRE2 deleted by Kessler et al are shown in shades of blue.
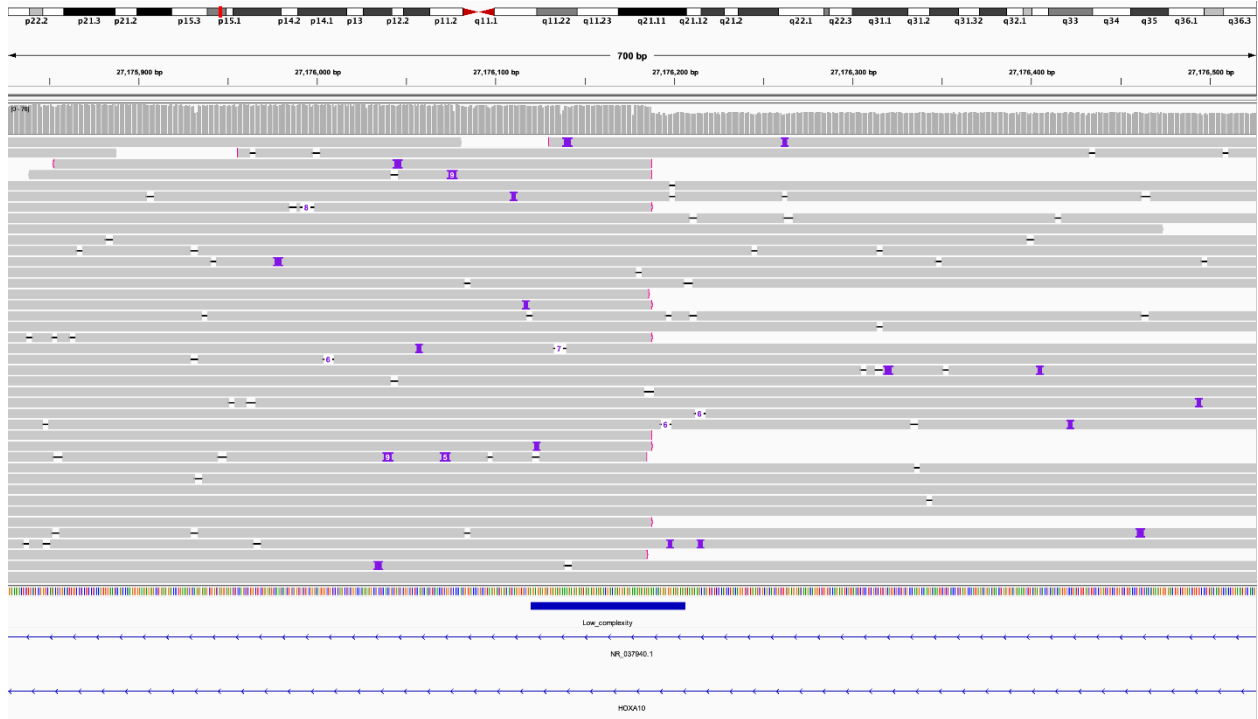
Figure S28. CT scan of patient with in Figure 9 at 18 months of age.

Fig S29: Targeted long-read sequencing identified the exact breakpoints of the duplication identified by clinical testing. In the IGV view shown depth of coverage (top track) shows a drop in coverage after the end of the duplication. Orientation of the mapped reads confirm that the duplication is tandem and that the breakpoint at chr7:27,176,187 lies within HOXA10 and a low-complexity GA-rich region. The breakpoint at chr7:25,220,918 is not shown.

Supplemental References

Sante, T., Vergult, S., Volders, P.-J., Kloosterman, W.P., Trooskens, G., De Preter, K., Dheedene, A., Speleman, F., De Meyer, T., and Menten, B. (2014). ViVar: a comprehensive platform for the analysis and visualization of structural genomic variation. PLoS One *9*, e113800.