

Supplemental material S1.

A) FASTA FORMAT OF THE HUMAN EDA mRNA REFERENCE SEQUENCE AND REFERENCE NUCLEOTIDE CDS (CODING SEQUENCE). NOTE: THE COLOR CODE HIGHLIGHTS THE 8 CONSENSUS EXON SEQUENCES DERIVING FROM ALIGNMENT OF THE OUTPUT ELECTROPHERGRAMS FROM CONTROL AND SS PATIENTS 1 AND 2 GENOMIC SEQUENCING. THE ATG INITIATION CODON AND THE TAG TERMINATION CODON ARE REPORTED IN EXONS 1 AND 8. EXON 8 SEQUENCING REVERSE PRIMER (rev) COVERS THE EXONIC REGION COMPRISING THE TAG TERMINATION CODON; THE REMAINING UNSEQUENCED REGION OF EXON 8 IS INDICATED AS EXON 8 3' UTR.

>gi|54112099|ref|NM_001399.4| Homo sapiens ectodysplasin A (EDA), transcript variant 1, mRNA

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ATTCCTCCGCGGGCCGAGCCCTCCCTCTCTCCGCGCCCTCCCTCCCTTCCCAACCCCTCGGAGTAA
GCTGCACTATCCGCGCTGCTCCCTGCTCCGCTCCGCGCCGAGCCAGCTGTCGCGCAGGAAACGGTCCCTCGAGCC
CCCGCCGATGGCAGCAGTAGACCGCTCTGACAGAGCTGTAAACGGCTGAGGCAGACGACGCGCTCC
GGCCTCAAGAGAGTGGGTGTCTCCGAGGGCCATGCTCCCTCCGAGAGTGGAGCCGAGGAACTCCTGGC
TGCAGCAGCCCGGGAGCGAGGGAGCCAGGGCTGGGGGTGGCGGGGGCCCTGCCCGGGGGGGAA exon 1
GGAAACAGCTGCCTGCTCTCTCCGCTCTCTCCGCTCTCCGCTGGCCCTCCACCTGCTGACGTTGGCT
GCTACCTAGAGTTCGCTCGGAGTTCGCGCGGGAACGTGGAGCCGAGTCCCGCTTGGCGCTCGGGCAC
CCCTGCGACCTCTGGCACCTTAAGACGCTCGGTGGCTGCAACCTGACAGCCCATCCACAGTCACTT
GGCAGCCGCTCACCTAAGCAGCAGCCATTGGAACCGGAGAGCCGACCTCACTCTGACTCCAGGAGC
GGCACAGTGGCCCTATTGAATTCCTCTCCCTGATGAAAAGCCATACCTGAGAAGAAGTAGGGC exon 2
TGTCCGCGCAATAAAGAGCAAAAGCAATGAAAGGACAGATCCCGCAGTAAAGCAAGAAAGGGA exon 3
AAGAAGCAGGACCTCCCTGGACCCATGGCCCTCCAGGACCCGAGGACCTCCAGGACCCGAGGACCC
CAGAAATCCAGGATTCCTGGAATCCAGGAAACACTTTATGGGACCACTGGTCCCTCCAGCTCCCTCC
TGGTCTCAAGGACCCCTGGCTCCAGGACCTCTGCTGGCTGATAAAGCTGGAACTCGAGGAAAGC exon 4
CAGTCCAGGACCCCTGGCTCCAGGACCTCTGCTGGCTGATAAAGCTGGAACTCGAGGAAAGC exon 5
TGTCTAATGACTGGCTCCGATCACTATGAACCCAGGTTTAAAGTACATCCGCGCGGGAGT exon 6
GGAGTACTGGTGGACCGCACTAATTCATCTATGATCAGGTAGAGTATACACTCACTGAC
TTTGGCAGCTATGAGTGGTGGTGGATGAGAAGCCCTTCTCGAGCTGACACAGCAGCAGTCCGAGCGGGCA
AGCACACTCAACACTTGTATACCGCAGGCGTCTGCCTCTCAAGGCGCGGAGAAAGTCCCGCTCAA
GATGGTGCAGCCTGACATCTCCATCAACTGAGCAGCAGCAGCAGTCTTCTTGGGCCATCAGGCTGGT
GAAGCCCTGCACTCAGTATTCCTCCCTCTGCTCCGCTCCCTCCCTGGTGGTGGGAGCCAG
GACTCCAGAACCTAAGTCTGCTGTGGAGTGGAGTGTATGGTGTTCAGCCGAGAGAAATGCCCTC
AGTGTATTATTCCTCCAGTACTCCAGGTTGACAGGCTGCTTGGCTGACTTTCAGAAATGACCTTGAGTTA
ACAGGACAGTGTGAGGACCCAGGTTTACATGAGCAGAACTTCTTGGTCCATGTTGACTGACTT
ATGGCATGACTCTCAACCCGAGGTCCTGTTGACAGATCTATGTTTGGTGCATAAATGAGGATCC
AGGCGCAGCAGGCGAGAAAGCAAAAGTGCATCTCAGACTCTGGGGTGGACATCTGACCCGAGGGGGG
TSCCTCTCTCTCTTGGGTAGGTTAGTGGCTGGGGTGGAGTGGGAAGGAGCATTCGACCTAAGAAGA
GGCCAGAGAGGAAAAGGACGTTGCTTTTGGCAGAGCACAAGAGAACTGCCAAGGAGCATCCTGGG
CAGTGGGAATGTTCTTTCTGCTATACTATGTTGGCTGCAGGAGGTTGGAGTGTCTTCCCACTCCAGT
GACAGCCACCCGTTGGCAGCTTGTGGGCTTTGGGAAGTTTGGTGTGCTTTGGAACAATCACAGGAAATG
GCCAACAACCTGCCGCGCTAAGACCTGAAATCCGTAATTTGGTACATGACTCTATTATTTACAGCT
GTGCTCCACACTCAGAAAATTCCTGGGGTCACTTCTAGTGGCCCATCCAGGCTGACTAGAATC
CTGCTCTTCTTCTCCATGGAGCTACCTGCTGTGAGCAGGTGCTTAACCTGGGACCTGTGGTCAATGG
AGTCTGGGATATTCTTTAGCTTACCTGGGCACAACAGAAATTTCCATTTATTAAGCAGTACAATGTTT
TTCATCCATCTTAATCAAAATCTGCTGGGGACGAGGGTTGGACGGGATGACCTCCAGAAAGTCCCTTC
AATTTCTAGTACCTGACTCTTACGCTTACACAGCCCTTCAAAATCTCAAAATCTAGACTGCTCTCTG
GGCATTAGCAGGCGAGCCCTTTTACCTGGCTAGAAAGGGCAGAGGGTGGAGTGGAGCAGAGGGGATT
TTGTTCAAGTTTGTGCAACCAAGTGGACCTTAGGCGAGCCCTATCTGAAAGGCGCAGCAGCTGATGCT
GTACTAACCAGTCTTCTTCACTCTGGCTTCAAAAAGGCTCAGCAGCAGCATGTCACCCGAGTCTCA
TSCCTCTCCCTAAAGCCAGGCTCAGGAGAGCCAGCTTCCAAACAGATCATATGGTAGGACCTCGAGAGC
TCAGGTCAAAATTCACCTTCCCTAAACCCAGGTTCCCAAAGATCATATGGTAGGACCTCGAGAGC
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CTTACTTCAAAGTGCCTGGGCTCAGCCTGTTTTCTGGGTGCTAGATCCAGCCCAACCTGGGAAGCCAG
CCTTGTACAGTCTGCTCCTTGTTCCTGAAATGTTTCTTTCCAGGAGATGGGAATAATTTCTCCTC
AGGCAGCTGAAATCCCAAGAACAGCGGTACTTATTTCTCAGCTGCTCCTTCCCTTTAAGCAACCA
CACTGCTTGGCCCTTCAAGGGTCAGGGTGAAGCTGGTGGCTAGGCTCCGTTGTCTGGTTGCTAATGA
CAGCCTTGCACCCAAAGTGAAGTGAATCCAGGCTGTTGGTGGCCCTAACCTCATAAAGTGCCTCGG
ACAGTCCGCGAGTGTAGCAGAAACCAACAAGAACCTCCTTCAATGTTGGAAAAATTTCTCTTGTAT
TATCTCCTTTGAAGAGGCAAGCTGATAATATGACAAACATCATTTGTTAGATGAGGCTCAGAGAGGTA
GCACCTCAGAGTGTTTTGACCAAGTTTAAAGCCGACAGCTGGAGCTCAGCCAGGCTGACTCCAAAGCT
GTTCCATTACACACAGCATTTGTGGAAATTTGAGGCTTAGAGAGACCAATAAATGTTGTAATGGGAA
CTGAAATCTTAGAGTTCGGGGAGAAACCCAGAGATGCTGATTTTCACTTCCCTCGATGATAATCCCT
CCTCTGGCTGCCAGGGGCTCTGTGGCAAAAAGAGTCAAGCATTTCTTTGGAAAAACGCAACGCTTA
GAGCTTGTGTTTCAAGAAATCTTCCCTGGCAAAATTTGGAGCAGCAGGCTCTGGGACCCACAGAACT
TGTGGCTTTATGTTTCCACCATCTTAGGAACAGCAACCATCATGTGTAGAGCCCTACTGTGGG
CAAAGTCTCCTTTTACCTACAGACAGCTTACAGAGCCAGCCTGCTTCCCAACAATACTAGTGTG
ACTCTTATCTCTTCCACCATACCTTAGAGACTTTGATACACAGGCTCTCTCAGGATGGGAGGAG
ACCTGAAAGAGAGGACTGGTCTGAGGCGAGAAAGTGTGAGGAGAGAGGAGGAAAGTCTTCTAATTG
TGCCCTTAAAGAGCCTCTGATACCATCTTATTTCCAGACATGAGGAGGATGATAAAGAAATAGGATC
TCACTGGACCTTGTATCATTTGAACTTCCAAAGGAACTAGAGGCGGAGGATGATGAGGAGAGC
AATAGGTAGCTGGGAGCCCTATTGCTGCTAAGTCAATGGCAAGTGAACAAGCAATTTACTGATGAGG
AATGTGGAAATAGATGTGCAGTTTGGAAATATGTTGGTGTGAATTTGCCAGAGGACCAATGCTGATGG
AGATGGGACGAGGACATTTGTGGCAAGCAGATGACAGAGGTTTGAAGAGAAATGCTATGGCAGGAGT
TCTGCCATTTACTTGGGCTTCAAGCCAGCTGGCCAAAAGACAGCTGGCGAGGCTGCTCGCTACT
GGTTACCTGGAGAGTATGATTTGGCTATTTCCCTTCACTCACTGAGCCAAATTTCTTTTGTGAA
CAGAAAGAGCTAGGAACCTGGAGTAAACAAGACTTTGATCCATGATGAGTGTATGTTTATGTA
ACTTCTGTGGTGCATAATAGATTCAGAGAAATTTAGAGCTAAAAGGCCCTTAGAGGAAATAGGCCA
ACCTACATTCACCCCTGTACTTATGTAGAACTGAGGCCAGAGGAGGAAATGACTGGCAAGGAGG
TGAGAAAGCACAACCTCCAGACTCAGCAGAGTGGGAGGTTAAAGCAGTCTCTGCTCCACATGGCCATCT
TCTTTCTCCACCCACAACCTCCAGGCTGGAAGTACTTTGGCCCTCAGGAGCCTCCAGGACCCAGGAG
AGAGTACTGACGCTTCACTCAGAACTTCTCTCTCCCAAGGCAATCTCCAGCTCTAGCTCTGACT
GGAAGCACAAGACTGGCCAGTGCAGCAAGTCTTAGGCTACTGATGATGCTCTCAGGACCCATCC
TGCTTGGAGCCTCTTAGGCTCTGAGCACAAGAGAAAGTGTATTTTGTCTTTAATCCATTTCA
GGACTCTCCAGGAGGCTCGGGTGTGTCAITTAATTTCCCTCAGCTGGATTTGGGGTGGGCTT
TGTGTGAAATGGCTGGAGGCGCAATGCTGCTTTTGGGGTCACTCCAGTGTGAGTACTGTG
TATATAAATATATAATGATATAAATGGAGTAAAGTTGTGTAATTAATGGTTTATTTCTTCCCA
AATAAACCGTTTCCCGCTGTTCTTGAATAAAAAAAAAAAAAA
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Reference CDS Nucleotide Sequence (1176 nt from ATG to STOP codon)

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ATGCGCTACCCGAGGTGGAGCGCAGGAACTCCTGCTGACGAGCCCGCGGGAGCGAGGAGCCAGG
GCTGGGGTGTGGGGGGCCCTGCCCGGGCGGCAAGGAAACAGCTGCTGCTCTCTCTGGGTTCTT
TGCCCTCTGCTGGCCCTCCACTGCTGACGTTGGTGTCTGCTACTAGATGTGGCTCGGATTTGGCGCG
GAACCTGGAGCCGACTCCGCTTTGGCGCTCCGGCACCTTGGCACTTCTGGACCTTAAGCACCTCG
GTGGCTCGACCTGACAGCCCATCACCAGTCACTTGGGAGCCGCTCACTAAGCAGCAGCCATTGGA
ACCGGAGAGAGCCCACTCCACTTGAATCCAGGACGGGCAACAGATGGCCCTATTGAAATTTCTTTC
CCTGATGAAAAGCCATCTCTGAAGAAGAAATGAGCGTGTTCGCCCAATAAAGAAAGCAAAAGCAATG
AAGGAGCAGATGGCCAGTTAAAACAAGAAAAGGAAAGAAAGCAGGACCTCTCGGACCAATGGCCCT
TCCAGGACCCCAAGGACTCCAGGACCCCAAGGACCCCAAGAAATCCAGGATTTCCGAAATTCAGGA
ACAATCTTATGGGACCACTGCTCCTCAGGCTCCTGCTGCTCAAGGACCCCTGGCCTCCAGGAGC
CTCTGTGTGCTGCTGATAAAGTGAAGTCCGAGAAACAGCCAGCTGTGGTGCATCTACAGGCGCAAG
GTCAGCAATCAAGTCAAGAAATGATCTTTCAGTGGAGTCTCAATGACTGGTCTCGCATCACTATGAC
CCCAAGGTGTTAAGTACATCCCGCAGCGGGAGCTGGAGTACTGTGGACGCGCACTACTTCACT
ATAGTCAAGTGAAGTACTACATCACTTCACTGACTTTCCAGCTATGAGTGGTGGTGGATGAGAA
GCCCTTCTGCAAGTGCACAGCAGCTCAGAGCGGCAAGCACTCAACACCTTCTATACCCGAGCG
GCTGCTCTCAAGCCCGCAGAAAGTCCGCTCAAGTGTGACAGCTGACTTCCATCAACATGA
GCAAGCACACCAGTCTTGGGGCCATCAGGCTGGTGGAGCCCTGCATCCAG
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Multiple sequence alignment of exon 3 electropherograms - forward sequencing

ctrl_ex3_for
pat1_ex3_for
pat2_ex3_for
----- Intron 2 Exon 3
-----ATTTTCTGTTTCATTTCCAATGACTTAATTATCTATTTTATTTTCTTATAGGCC 96
-----ATTTTCTGTTTCATTTCCAATGACTTAATTATCTATTTTATTTTCTTATAGGCC 99
-----ATTTTCTGTTTCATTTCCAATGACTTAATTATCTATTTTATTTTCTTATAGGCC 100

Intron 3
ctrl_ex3_for CAGTAAAAACAAGAAAAGGTAAGTTCCTGACTTTATAAAATGCTGCTTGT---- 156
pat1_ex3_for CAGTAAAAACAAGAAAAGGTAAGTTCCTGACTTTATAAAATGCTGCTTGT---- 159
pat2_ex3_for CAGTAAAAACAAGAAAAGGTAAGTTCCTGACTTTATAAAATGCTGCTTGT---- 160

ctrl_ex3_for
pat1_ex3_for
pat2_ex3_for

Local alignment: CAP3 contig of exon 3 Forw/Rev electropherograms vs human EDA RefSeq mRNA NM_001399.4

gi|54112099|ref|NM_001399.4|
Contig3

-----GCCGCAATAAAGAAAGCAAAAGCAAGTGAAGGAGCA 740
----- intron 2 --
Exon 2 Exon 3 Exon 4
GATGCCCCAGTTAAAAAACAGAAAAGGAAAGAAAGCAGGACCTCCTGG 790
-----GCCCCAGTTAAARACAGAAAAGGTAAGT -- intron 3 --

gi|54112099|ref|NM_001399.4|
Contig3

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Multiple sequence alignment of exon 4 electropherograms - forward sequencing

pat1_ex4_for
pat2_ex4_for
ctrl_ex4_for
----- Intron 3 Exon 4

pat1_ex4_for TCCTWTTTTTCAGGAAAGACAGGACCTCCTGGACCCAAATGGCCCTCCAGGACCCCA 175
pat2_ex4_for TCYTWTTTTTCAGGAAAGACAGGACCTCCTGGACCCAAATGGCCCTCCAGGACCCCA 176
ctrl_ex4_for TCCTATTTTTTCAGGAAAGACAGGACCTCCTGGACCCAAATGGCCCTCCAGGACCCCA 180

pat1_ex4_for GGACCTCCAGGACCCAGGACCCCAAGGAATCCAGGGATTCTCGAATTCAGGAACA 235
pat2_ex4_for GGACCTCCAGGACCCAGGACCCCAAGGAATCCAGGGATTCTCGAATTCAGGAACA 240
ctrl_ex4_for GGACCTCCAGGACCCAGGACCCCAAGGAATCCAGGGATTCTCGAATTCAGGAACA 236

pat1_ex4_for ACTGTTATGGGACCACCTGGTCTCCTCAGGTCCTCTGCTCCTCAAGGACCCCTGGCCTC 295
pat2_ex4_for ACTGTTATGGGACCACCTGGTCTCCTCAGGTCCTCTGCTCCTCAAGGACCCCTGGCCTC 300
ctrl_ex4_for ACTGTTATGGGACCACCTGGTCTCCTCAGGTCCTCTGCTCCTCAAGGACCCCTGGCCTC 296

Intron 4
pat1_ex4_for CAGGACCTCTCAGGTTCCCTSTCTCYMCCMCMMSWKGKRYRYTWWRW 355
pat2_ex4_for CAGGACCTCTCAGGTTCCCTSTCTCYMCCMCMMSWKGKRYRYTWWRW 360
ctrl_ex4_for CAGGACCTCTCAGGTTCCCTSTCTCYMCCMCMMSWKGKRYRYTWWRW 356

pat1_ex4_for
pat2_ex4_for
ctrl_ex4_for

Local alignment: CAP3 contig of exon 4 Forw/Rev electropherograms vs human EDA RefSeq mRNA NM_001399.4

gi|54112099|ref|NM_001399.4|
Contig4

Exon 3 Exon 4
PTAAAAACAAGAAAAGGAAAGAAAGCAGGACCTCCTGGACCCAAATGGC 800
----- intron 3 ---AGGAAAGAAAGCAGGACCTCCTGGACCCAAATGGC 196

gi|54112099|ref|NM_001399.4|
Contig4

CCTCCAGGACCCCAAGGACCTCCAGGACCCCAAGGACCCCAAGGAAATTC 850
CCTCCAGGACCCCAAGGACCTCCAGGACCCCAAGGACCCCAAGGAAATTC 246

gi|54112099|ref|NM_001399.4|
Contig4

AGGGATTCTGGAATTCAGGAACAACCTGTTATGGGACCCCTGGTCTCT 900
AGGGATTCTGGAATTCAGGAACAACCTGTTATGGGACCCCTGGTCTCT 296

gi|54112099|ref|NM_001399.4|
Contig4

CAGGTCCTCCTGGTCTCAAGGACCCCTGGCCCTCCAGGACCTCTCTGT 950
CAGGTCCTCCTGGTCTCAAGGACCCCTGGCCCTCCAGGACCTCTCTGT 346

gi|54112099|ref|NM_001399.4|
Contig4

GCTGCTGATAAAGCTGGAAGCTCGAGAAACACGCTGCTGATGATCT 1000
G----- intron 4 -----

gi|54112099|ref|NM_001399.4|
Contig4

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Multiple sequence alignment of exon 5 electropherograms - forward sequencing

ctrl_ex5_for
pat2_ex5_for
pat1_ex5_for
----- Intron 4 Exon 5 Intron 5
-----CCTATTTTTCAGGCTGCTGATAAAGCTGGAAGCTCGAGAAAACAG 149
-----CCTATTTTTCAGGCTGCTGATAAAGCTGGAAGCTCGAGAAAACCA 150
-----CCTATTTTTCAGGCTGCTGATAAAGCTGGAAGCTCGAGAAAAC 151

ctrl_ex5_for
pat2_ex5_for
pat1_ex5_for

Local alignment: CAP3 contig of exon 5 Forw/Rev electropherograms vs human EDA RefSeq mRNA NM_001399.4

Contig5
gi|54112099|ref|NM_001399.4|

----- intron 4 -----AG 135
TCCAGGTCCTCCTGGTCTCAAGGACCCCTGGCCCTCCAGGACCTCTG 948
** * * * * * Exon 4
Exon 5
GTGCTGCTGATAAAGCTGGAAGCTCGAGAAAACAGCT intron 5 -- 173
GTGCTGCTGATAAAGCTGGAAGCTCGAGAAAACAGCCAGCTGGGTGG 998
***** Exon 6
Contig5
gi|54112099|ref|NM_001399.4|

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Multiple sequence alignment of exon 6 electropherograms - forward sequencing

pat2_ex6_for
pat1_ex6_for
ctrl_ex6_for
----- Intron 5 Exon 6

pat1_ex6_for CTTCTATCCTGCCAGCTAGCTGTGGTGCATCTACAGGGCCAAAGGTCAGCAATTCAGT 162
pat2_ex6_for CTTCTATCCTGCCAGCTAGCTGTGGTGCATCTACAGGGCCAAAGGTCAGCAATTCAGT 162
ctrl_ex6_for CTTCTATCCTGCCAGCTAGCTGTGGTGCATCTACAGGGCCAAAGGTCAGCAATTCAGT 161

Intron 6
pat1_ex6_for CAAGAAGTAAAGAAATCAAATAGGCTCTCTCCCAAGAGGAGCTTCTCCCTCGCC 222
pat2_ex6_for CAAGAAGTAAAGAAATCAAATAGGCTCTCTCCCAAGAGGAGCTTCTCCCTCGCC 222
ctrl_ex6_for CAAGAAGTAAAGAAATCAAATAGGCTCTCTCCCAAGAGGAGCTTCTCCCTCGCC 221

pat2_ex6_for
pat1_ex6_for
ctrl_ex6_for

Local alignment: CAP3 contig of exon 6 Forw/Rev electropherograms vs human EDA RefSeq mRNA NM_001399.4

Contig6
gi|54112099|ref|NM_001399.4|

----- intron 5 -----CAGAGCTGGTGCAT 153
CCTGCTGATAAAGCTGGAAGCTCGAGAAAACAGCCAGCTGGGTGG 1000
***** Exon 5
Contig6
gi|54112099|ref|NM_001399.4|

CAGGCCAAAGGTCAGCAATCAAGTCAAGAACTG intron 6 - 190
CAGGCCAAAGGTCAGCAATCAAGTCAAGAACTG intron 6 - 1050
***** Exon 6
Contig6
gi|54112099|ref|NM_001399.4|

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Multiple sequence alignment of exon 7 electropherograms - forward sequencing

pat1_ex7_for
pat2_ex7_for
ctrl_ex7_for
----- Intron 6 Exon 7

pat1_ex7_for -----TGAGTGACTGCCCTTCTCTACTACTGAGTTTCAGGTGGAGTGTCAATGAC 223
pat2_ex7_for -----TGAGTGACTGCCCTTCTCTACTACTGAGTTTCAGGTGGAGTGTCAATGAC 223
ctrl_ex7_for -----TGAGTGACTGCCCTTCTCTACTACTGAGTTTCAGGTGGAGTGTCAATGAC 221

pat1_ex7_for TGGTCTCGCATCACTATGAACCCCAAGGTGTTTAAAGTACATCCCCGAGCGGGGAGCTG 283
pat2_ex7_for TGGTCTCGCATCACTATGAACCCCAAGGTGTTTAAAGTACATCCCCGAGCGGGGAGCTG 283
ctrl_ex7_for TGGTCTCGCATCACTATGAACCCCAAGGTGTTTAAAGTACATCCCCGAGCGGGGAGCTG 281

Intron 7
pat1_ex7_for GAGGTACTGGTGGACGGCACCCTACTTCATCTATAGTCAGGTAAGT GAGTACGGTCTTA 343
pat2_ex7_for GAGGTACTGGTGGACGGCACCCTACTTCATCTATAGTCAGGTAAGT GAGTACGGTCTTA 343
ctrl_ex7_for GAGGTACTGGTGGACGGCACCCTACTTCATCTATAGTCAGGTAAGT GAGTACGGTCTTA 341

pat1_ex7_for
pat2_ex7_for
ctrl_ex7_for

Local alignment: CAP3 contig of exon 7 Forw/Rev
electropherograms vs human EDA RefSeq mRNA NM_001399.4

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Contig7          ----- intron 6 ----- Exon 7 229
gi|54112099|ref|NM_001399.4| ACAGGGCCAAAGGCTCAGCAATTCAGTCAAGTAATCTCTTCAGGTGGAG 1050
ACAGGGCCAAAGGCTCAGCAATTCAGTCAAGTAATCTCTTCAGGTGGAG
Exon 7 *****

Contig7          TGCTCAATGACTGGGCTGGCACTATGAACTCCAGGCTGATGAGCTA 279
gi|54112099|ref|NM_001399.4| TGCTCAATGACTGGGCTGGCACTATGAACTCCAGGCTGATGAGCTA 1100
TGCTCAATGACTGGGCTGGCACTATGAACTCCAGGCTGATGAGCTA
*****

Contig7          TATCCCGGAGCGGGAGCTGGAGTACTGGTGGAGGCACTACTTCAAT 329
gi|54112099|ref|NM_001399.4| TATCCCGGAGCGGGAGCTGGAGTACTGGTGGAGGCACTACTTCAAT 1150
TATCCCGGAGCGGGAGCTGGAGTACTGGTGGAGGCACTACTTCAAT
*****

Contig7          CTATAGTCAGGTAGAACTG- intron 7 ----- 353
gi|54112099|ref|NM_001399.4| CTATAGTCAGGTAGAACTGATACTACATCAACTTCACTGACTTTGCCAGCT 1200
CTATAGTCAGGTAGAACTGATACTACATCAACTTCACTGACTTTGCCAGCT
*****
Exon 8

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Multiple sequence alignment of exon 8 electropherograms -
forward sequencing

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ctrl_ex8_for    -----
pat1_ex8_for    -----
pat2_ex8_for    -----

ctrl_ex8_for    CAGGGAGGGCCCCCACCCTCTCTTCCCTCTCTTCCCAATCCCTTCTTGTTCCTCTCA 148
pat1_ex8_for    CAGGGAGGGCCCCCACCCTCTCTTCCCTCTCTTCCCAATCCCTTCTTGTTCCTCTCA 148
pat2_ex8_for    CAGGGAGGGCCCCCACCCTCTCTTCCCTCTCTTCCCAATCCCTTCTTGTTCCTCTCA 150
*****

- Intron 7 Exon 8 -
ctrl_ex8_for    CTCAGCTATACTACATCAACTTCACTGACTTTGCCAGCTATGAGTGGTGGTGGATGAGA 208
pat1_ex8_for    CTCAGCTATACTACATCAACTTCACTGACTTTGCCAGCTATGAGTGGTGGTGGATGAGA 208
pat2_ex8_for    CTCAGCTATACTACATCAACTTCACTGACTTTGCCAGCTATGAGTGGTGGTGGATGAGA 210
*****

ctrl_ex8_for    AGCCCTTCTCGAGTGCACACGACATCGAGACGGGCAAGCAACTACAACACTTGTCT 268
pat1_ex8_for    AGCCCTTCTCGAGTGCACACGACATCGAGACGGGCAAGCAACTACAACACTTGTCT 268
pat2_ex8_for    AGCCCTTCTCGAGTGCACACGACATCGAGACGGGCAAGCAACTACAACACTTGTCT 270
*****

ctrl_ex8_for    ATACCCGAGCGCTCTGCCTCCTCAAGGCCCGGCAAGATCGCCGTCAGATGGTGCACG 328
pat1_ex8_for    ATACCCGAGCGCTCTGCCTCCTCAAGGCCCGGCAAGATCGCCGTCAGATGGTGCACG 328
pat2_ex8_for    ATACCCGAGCGCTCTGCCTCCTCAAGGCCCGGCAAGATCGCCGTCAGATGGTGCACG 330
*****

ctrl_ex8_for    CTGACATCCCATCAACATGAGCAAGCACACCAGTCTTTGGGGCCATCAGGCTGGGTG 388
pat1_ex8_for    CTGACATCCCATCAACATGAGCAAGCACACCAGTCTTTGGGGCCATCAGGCTGGGTG 388
pat2_ex8_for    CTGACATCCCATCAACATGAGCAAGCACACCAGTCTTTGGGGCCATCAGGCTGGGTG 390
*****

ctrl_ex8_for    AAGCCCTGCATCCTAGATTCCCCCAATTTGCCTCTGTCCGTGCCCTTCCCTGGGTTT 448
pat1_ex8_for    AAGCCCTGCATCCTAGATTCCCCCAATTTGCCTCTGTCCGTGCCCTTCCCTGGGTTT 448
pat2_ex8_for    AAGCCCTGCATCCTAGATTCCCCCAATTTGCCTCTGTCCGTGCCCTTCCCTGGGTTT 450
*****

ctrl_ex8_for    GGGAGCCAGGACTCCCAGAACCCTAAGTGTCTGTGGAGTGGGTGATTGGTGTTC 508
pat1_ex8_for    GGGAGCCAGGACTCCCAGAACCCTAAGTGTCTGTGGAGTGGGTGATTGGTGTTC 508
pat2_ex8_for    GGGAGCCAGGACTCCCAGAACCCTAAGTGTCTGTGGAGTGGGTGATTGGTGTTC 510
*****

- Reverse primer Exon 8 3' UTR -
ctrl_ex8_for    -----
pat1_ex8_for    -----
pat2_ex8_for    -----

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Local alignment: CAP3 contig of exon 8 Forw/Rev
electropherograms vs human EDA RefSeq mRNA NM_001399.4

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Contig8          -----
gi|54112099|ref|NM_001399.4| -----

Contig8          -- intron 7 -- AGGTATACTACATCAACTTCACTGACTTTGCCAGCT 219
gi|54112099|ref|NM_001399.4| CTATAGTCAGGTAGAACTGATACTACATCAACTTCACTGACTTTGCCAGCT 1200
CTATAGTCAGGTAGAACTGATACTACATCAACTTCACTGACTTTGCCAGCT
Exon 7 *****

Contig8          ATGAGGTGGTGGTGGATGAGAAGCCCTTCTCGAGTGCACACGACGATC 269
gi|54112099|ref|NM_001399.4| ATGAGGTGGTGGTGGATGAGAAGCCCTTCTCGAGTGCACACGACGATC 1250
ATGAGGTGGTGGTGGATGAGAAGCCCTTCTCGAGTGCACACGACGATC
*****

Contig8          GAGACGGGCAAGCAACTACAACACTTGTATACCGCAGCGCTCTGCCT 319
gi|54112099|ref|NM_001399.4| GAGACGGGCAAGCAACTACAACACTTGTATACCGCAGCGCTCTGCCT 1300
GAGACGGGCAAGCAACTACAACACTTGTATACCGCAGCGCTCTGCCT
*****

Contig8          CCTCAAGGCCCGGCAAGATCGCCGTCAGATGGTGCACGCTGACATCT 369
gi|54112099|ref|NM_001399.4| CCTCAAGGCCCGGCAAGATCGCCGTCAGATGGTGCACGCTGACATCT 1350
CCTCAAGGCCCGGCAAGATCGCCGTCAGATGGTGCACGCTGACATCT
*****

Contig8          CCATCAACATGAGCAAGCACACCAGTCTTTGGGGCCATCAGGCTGGGT 419
gi|54112099|ref|NM_001399.4| CCATCAACATGAGCAAGCACACCAGTCTTTGGGGCCATCAGGCTGGGT 1400
CCATCAACATGAGCAAGCACACCAGTCTTTGGGGCCATCAGGCTGGGT
*****

Contig8          GAAGCCCTGCATCCTAGATTCCCCCAATTTGCCTCTGTCCGTGCCCT 469
gi|54112099|ref|NM_001399.4| GAAGCCCTGCATCCTAGATTCCCCCAATTTGCCTCTGTCCGTGCCCT 1450
GAAGCCCTGCATCCTAGATTCCCCCAATTTGCCTCTGTCCGTGCCCT
*****

Contig8          TCCTTGGGTTTGGGAGCCAGGACTCCCAGAACCCTAAGTGTCTGTGG 519
gi|54112099|ref|NM_001399.4| TCCTTGGGTTTGGGAGCCAGGACTCCCAGAACCCTAAGTGTCTGTGG 1500
TCCTTGGGTTTGGGAGCCAGGACTCCCAGAACCCTAAGTGTCTGTGG
*****

Exon 8 reverse primer seq
Contig8          AGTGAGGTGATTTGGTGTTCAGCCCGCAGAGAAATGCC----- 562
gi|54112099|ref|NM_001399.4| AGTGAGGTGATTTGGTGTTCAGCCCGCAGAGAAATGCC----- 1550
AGTGAGGTGATTTGGTGTTCAGCCCGCAGAGAAATGCC-----
*****
Exon 8 3' UTR
Contig8          ATTCCCCAGTGACTCCAGGGTGACAAGGCTGCTTGACTTTCCAGAAATGA 1600
gi|54112099|ref|NM_001399.4| -----

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Supplemental material S1.

***In silico* analysis of exon sequencing in gDNA samples from control and SS patients 1 and 2.**

In A), the FASTA FORMAT of the human EDA mRNA reference nucleotide sequence and reference CDS (CODING SEQUENCE) are reported. The output electropherograms from control and SS patients 1 and 2 were processed by alignment (see “materials and methods” section for details), and the color code highlights the 8 consensus exon sequences obtained by CAP3 assembly. The ATG initiation codon and the TAG termination codon are annotated in exons 1 and 8. The Exon 8 reverse primer (rev) adopted for sequencing covers the exonic region comprising the TAG termination codon; the remaining unsequenced region exon 8 is indicated as Exon 8 3' UTR.

In B), alignments of edited electropherograms are reported to show complete sequencing of exon regions. For brevity, forward sequencing alignment is reported in graph to display 100% nucleotide identity of exon sequences, except the exon 1 downstream region, for which reverse sequencing is shown for displaying resolved alignment related to two single nucleotides (pointed out in red). For each exon, alignment of the reference mRNA (NM_001399.4) nucleotide sequence versus the consensus sequence (contig) deriving from the six electropherograms (forward and reverse sequencing for each of the three gDNA sample analysed), processed by the CAP3 *in silico* program suite is reported. Exon-intron boundaries (exon-exon boundaries on NM_001399.4) and canonical splice sites (GT/AG donor/acceptor, green/blue respectively) are indicated.