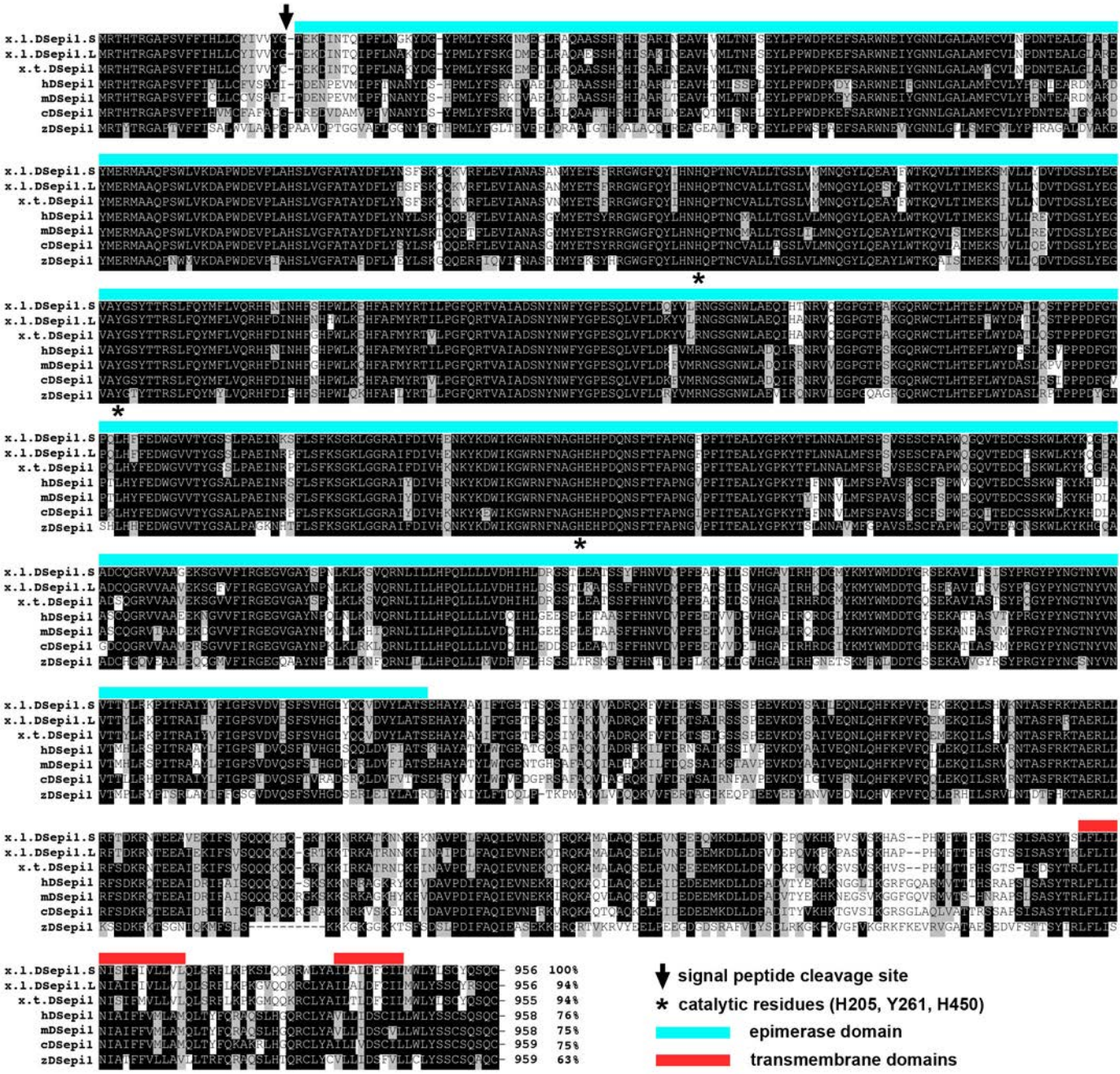

Figure S1. Comparison of DS-epi1 sequences in *Xenopus* and other vertebrates. DS-epi1 contains a cleavable signal peptide (arrow), an epimerase domain, and two putative transmembrane domains. Three amino acids underlaid with stars are catalytic residues of the epimerase domain: histidine 205, tyrosine 261, and histidine 450, which are required for epimerase activity (Pacheco et al., 2009a). The alignment was performed using Bioedit software and the ClustalW algorithm. Identical amino acids are indicated in black, and similar residues are indicated in grey. Gaps are introduced as dots. At the end of each sequence, the number indicates the total protein length and the percentage the amino acid identity to the x.l.DS-epi1.S. GenBank accession numbers of the DS-epi1 sequences are: x.l., *Xenopus laevis* (S, KU877109; L, Xenbase); x.t., *Xenopus tropicalis* (NP_001120268.1); z, *Danio rerio* (NP_001025396.1); c, *Gallus gallus* (XP_419777.3); m, *Mus musculus* (NP_766096.1); and h, *Homo sapiens* (NP_037484.1).

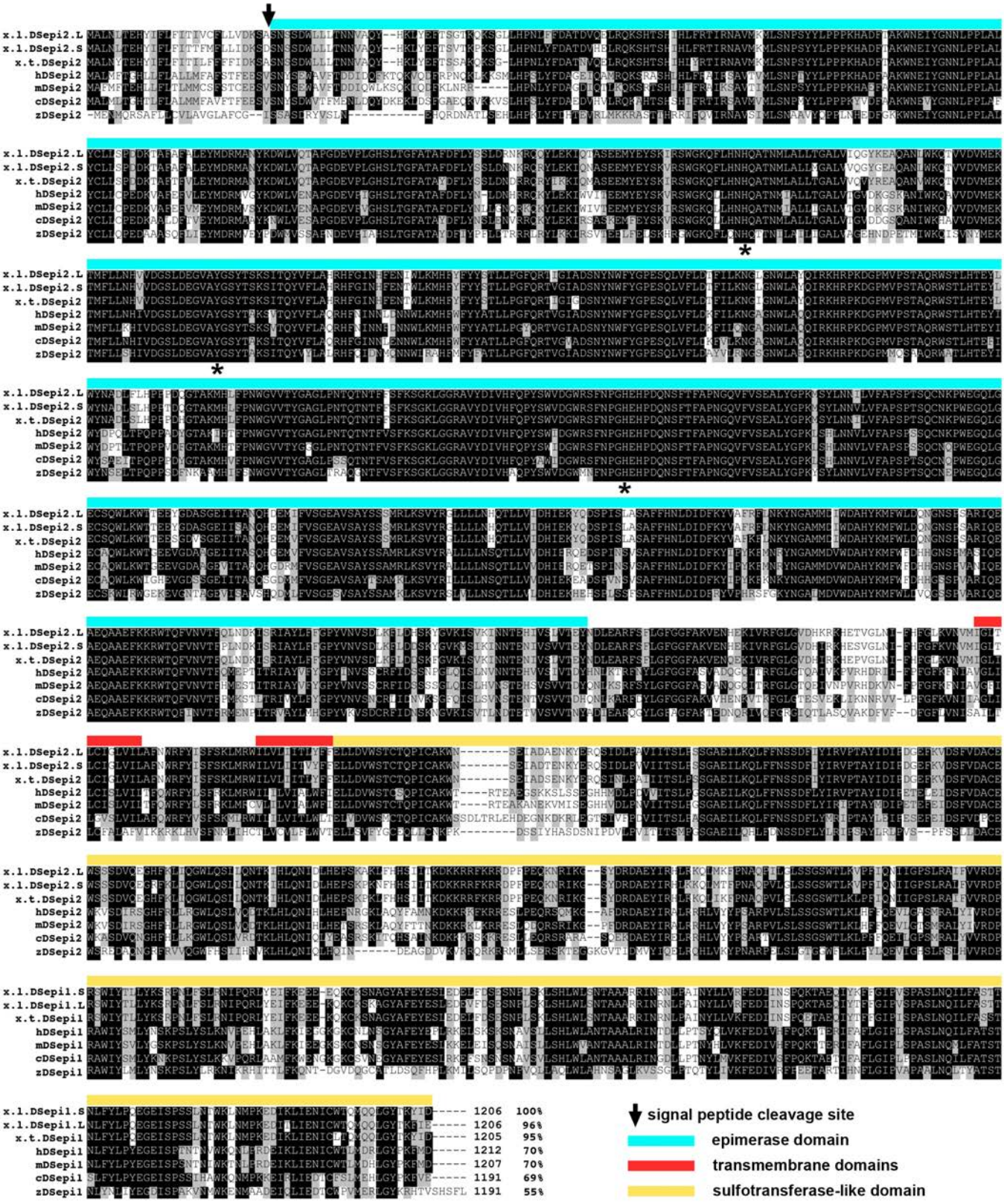

Figure S2. Comparison of DS-epi2 sequences in *Xenopus* and other vertebrates. DS-epi2 contains a cleavable signal peptide (arrow), an epimerase domain, two putative transmembrane domains, and a sulfotransferase-like domain. The 3 amino acids underlaid with stars represent putative catalytic residues of the epimerase domain. x.l., *Xenopus laevis* (L, KU877110; S, Xenbase); x.t., *Xenopus tropicalis* (XP_002939660.1); z, *Danio rerio* (XP_696502.2); c, *Gallus gallus* (XP_004935280.1); m, *Mus musculus* (AAI37797.1); and h, *Homo sapiens* (AAI17326.1).

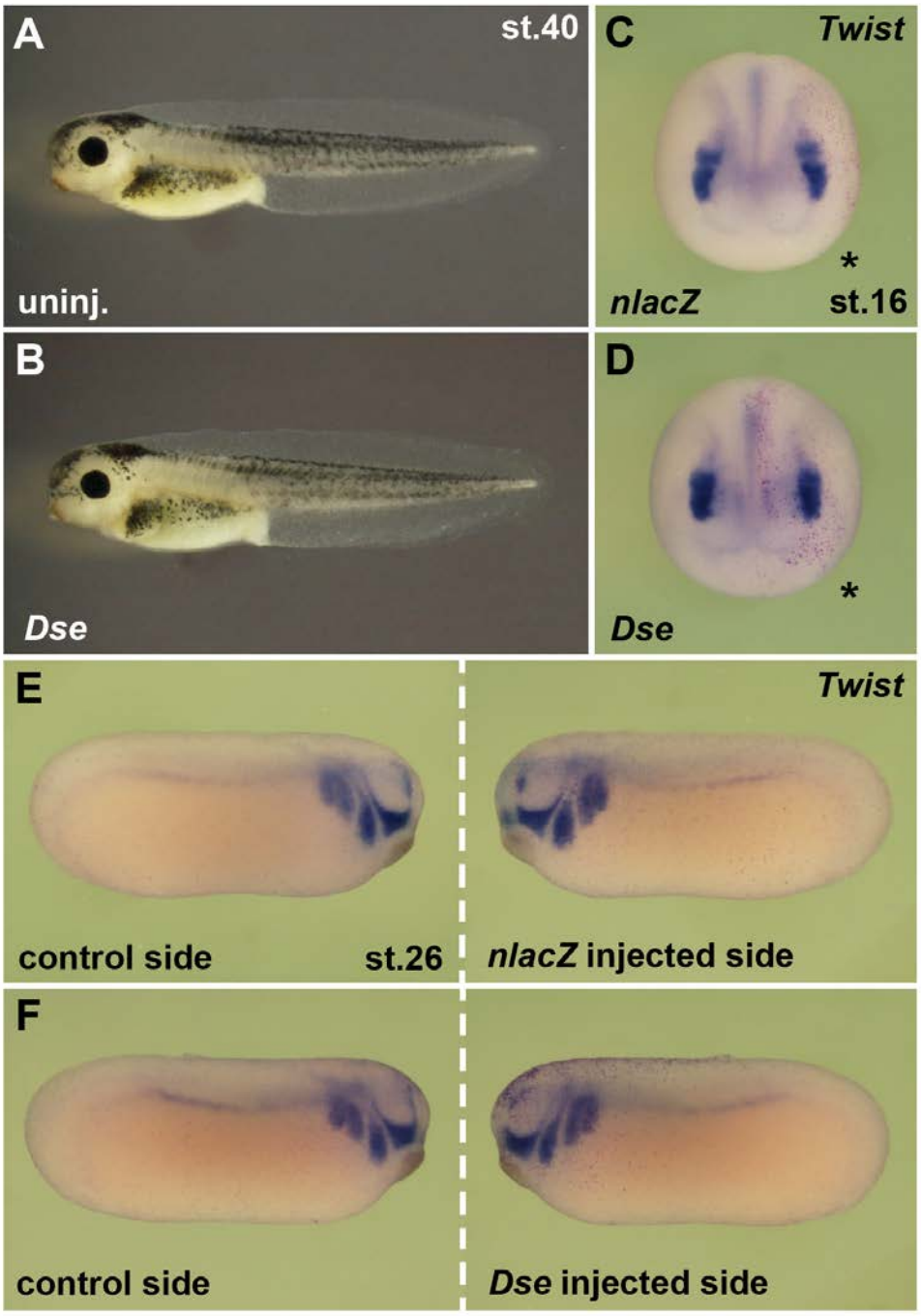

Figure S3. Overexpression of DS-epi1 does not affect embryonic and cranial neural crest development.

(A) Uninjected tadpole embryo.

(B) Embryo following injection of 4 ng *Dse* mRNA.

(C,D) Anterior view of neurula embryos following injection into a single blastomere with *nlacZ* mRNA as a lineage tracer (red nuclei) and whole-mount *in situ* hybridization. A single dose of 1 ng *Dse* mRNA has no effect on *Twist* expression in pre-migratory cranial neural crest cells on the injected side (labeled with star).

(E,F) Lateral view of tailbud stage embryos. A single injection of 1 ng *Dse* mRNA does not affect *Twist* expression in migrating cranial neural crest cells.

Embryos were animally injected at the 4-cell stage. Indicated phenotypes were identified in B, 165/174; C, 14/19; D, 17/23; E, 14/15; and F, 16/18.

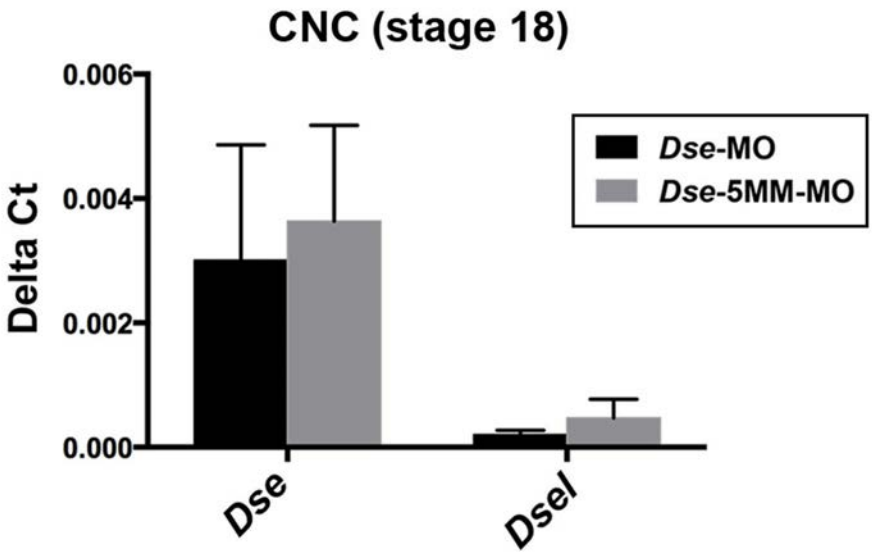

Figure S4. Expression of *Dse* and *Dsel* is not feedback-regulated.

qPCR analysis of CNC explants at stage 18. *Dse*-MO does not differentially affect the mRNA levels of *Dse* and *Dsel* compared with *Dse*-5MM-MO. Mean ±SDEV from triplicates. Number of biological replicates n=4.

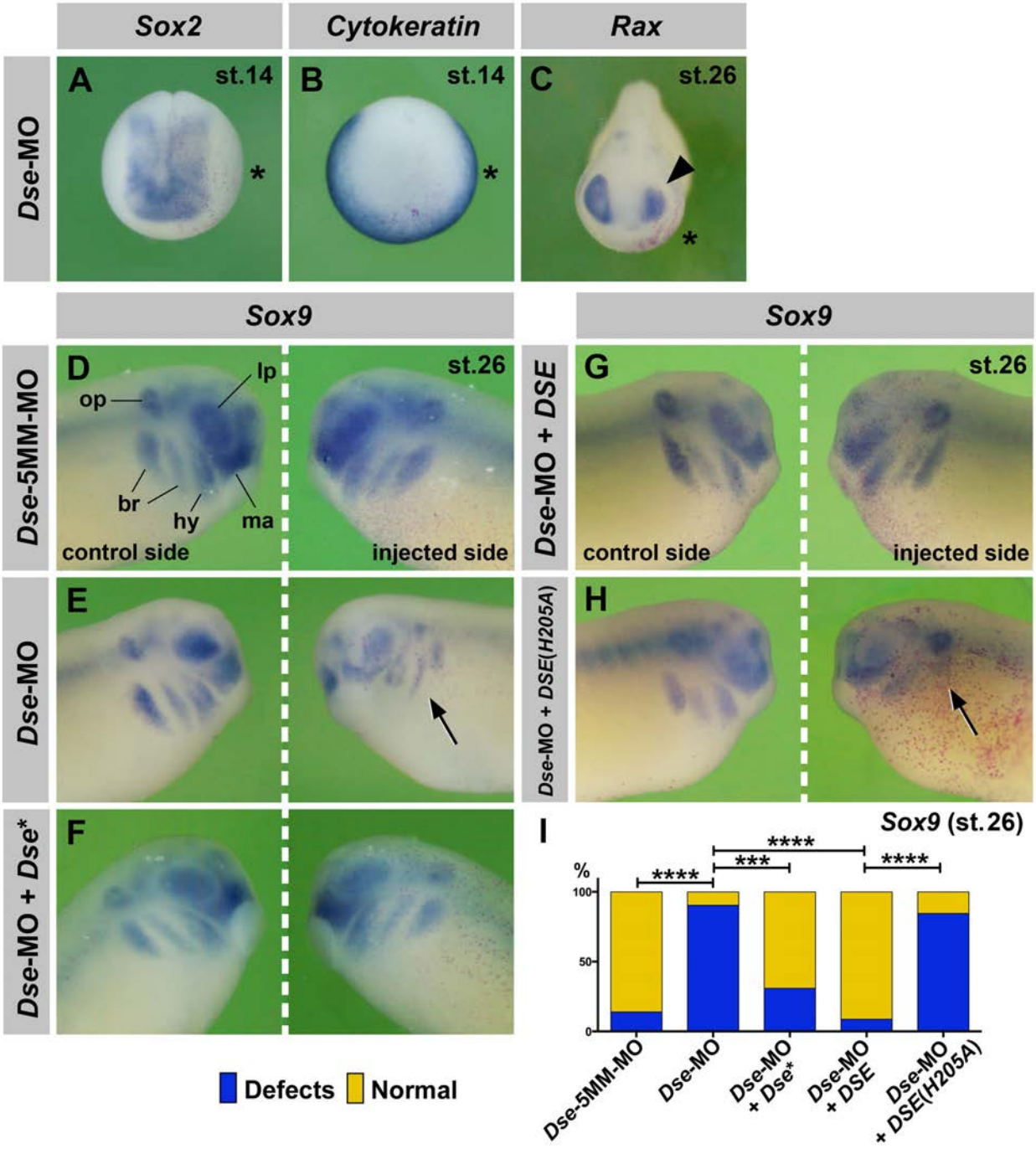

Figure S5. DS-epi1 regulates eye development and neural crest cell migration. Whole-mount *in situ* hybridization of embryos in anterior (A-C) and lateral (D-H) views. *nlacZ* mRNA was injected as a lineage tracer (red nuclei).

(A,B) A single animal injection of *Dse*-MO does not affect *Sox2* and *Cytokeratin* expression on the targeted side (stars) in early neurula embryos.

(C) *Dse*-MO reduces *Rax* expression (arrowhead) at the tailbud stage.

(D-I) *Dse*-MO causes impaired migration of *Sox9*$^{+}$ neural crest cells (arrow) on the injected side, which is rescued by the co-injection of 250 pg *Dse** mRNA and 25 pg pcDNA3/CTAP-*DSE* plasmid, but not by 25 pg pcDNA3/CTAP-*DSE* (*H205A*) plasmid DNA.

br, branchial segment; hy, hyoid segment; lp, lens placode; ma, mandibular segment; op, otic placode. Indicated phenotypes are shown as follows: A, 13/13; B, 13/13; C, 13/18; D, 19/22; E, 37/41; F, 14/20; G, 24/26; and H, 16/19. Statistical significance was determined using Fisher's exact test and two-tailed P values, ***, $p<0.005$; ****, $p<0.0001$.

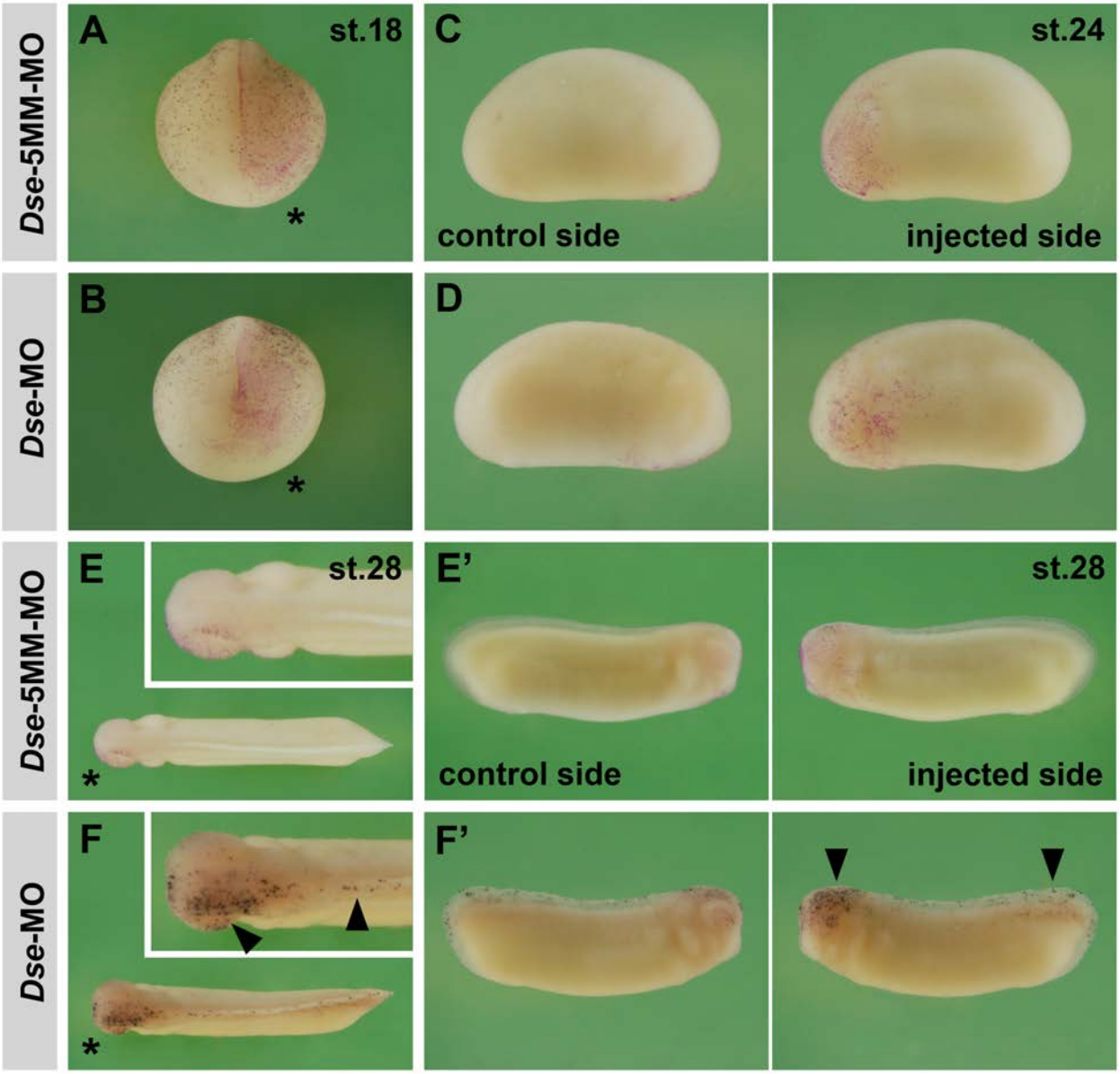

Figure S6. DS-epi1 regulates cell survival at the advanced tailbud stage but not at earlier stages. TUNEL staining labels apoptotic cells (black dots). *nlacZ* mRNA was co-injected as a lineage tracer (red nuclei).

(A,B) Anterior view of late neurulae. A single animal injection of *Dse*-5MM-MO and *Dse*-MO does not affect the number of TUNEL^{+} cells on the injected side (star).

(C,D) Early tailbud embryos in the lateral view. Non-injected control and injected sides of the same embryo are facing each other. *Dse*-5MM-MO and *Dse*-MO do not enhance apoptosis.

(E-F') Tailbud embryos at stage 28 in the dorsal view (E,F; insets are magnifications) and lateral view (E',F'). Note that *Dse*-MO- but not *Dse*-5MM-MO-injected embryos exhibit an increase in the number of apoptotic cells in the neural crest area (arrowheads).

An increase in apoptotic cells was identified in: A, 0/35; B, 3/35; C, 0/40; D, 0/40; E, 0/16; and F, 16/16.

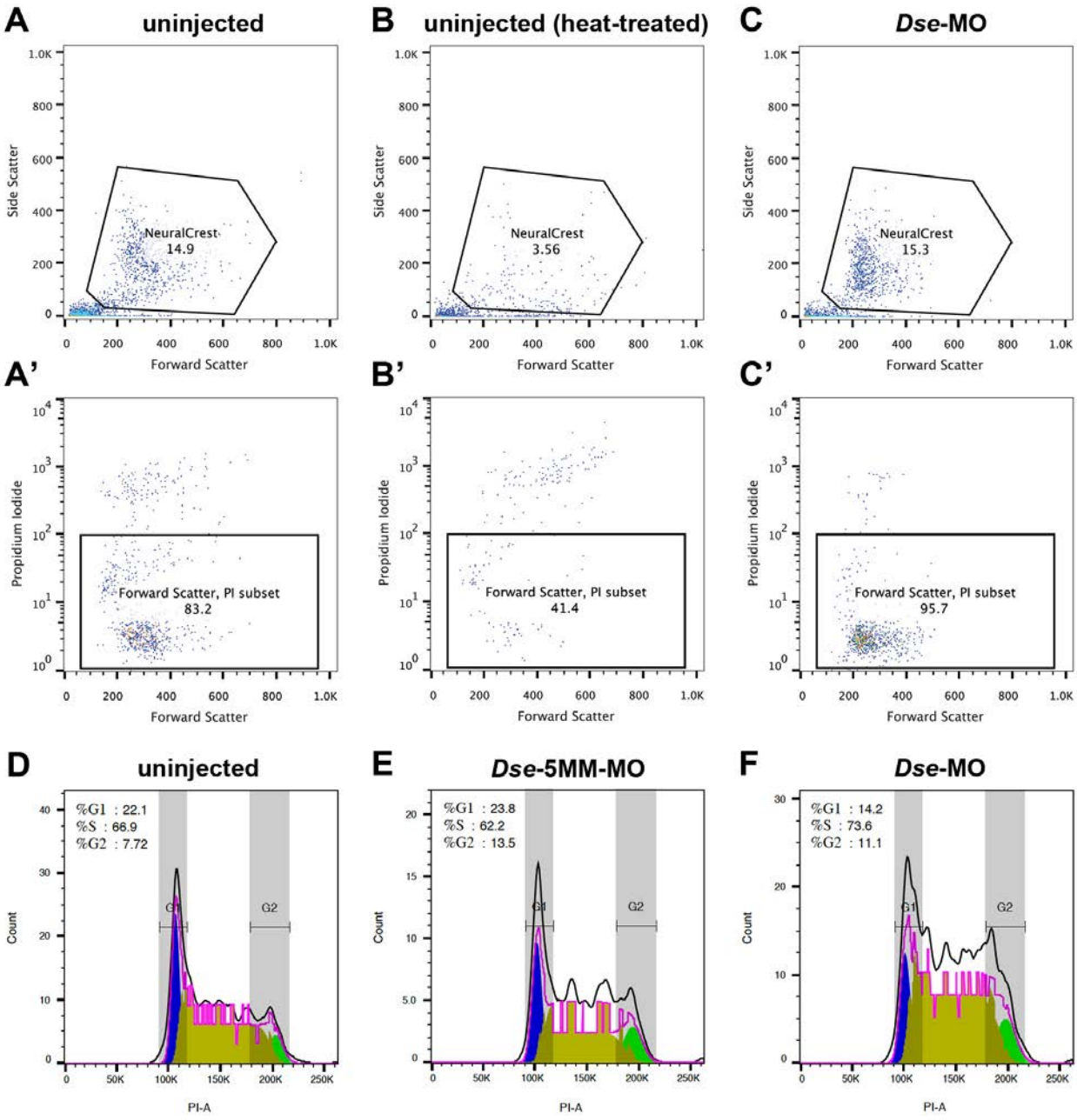

Figure S7. Knockdown of DS-epi1 does not affect apoptosis or cell cycle progression in CNC cells *in vitro*. CNC explants were dissected from stage 17 embryos; cells were dissociated, DNA-stained with PI and analyzed via FACS ~2 hours after extraction.

(A,A') Living CNC cells of uninjected control embryos were selected (framed area in A), and their PI incorporation was determined (A'), which indicated ~83% survival.

(B,B') Following treatment at 50°C for 30 min, only ~41% of the selected cells are scored as survivors, which validated the effective PI labeling of dead cells.

(C,C') Selected CNC cells of *Dse*-morphant embryos have a cell viability of >95%.

(D-F) Cell cycle analysis indicates a normal distribution of PI-stained DNA in fixed CNC cells of *Dse*-MO-injected embryos (F) compared with uninjected (D) and *Dse*-5MM-MO-injected siblings (E).

Two independent experiments (n=2). G1, gap1 phase; G2, gap2 phase; PI, propidium iodide; S, DNA-synthesis phase.

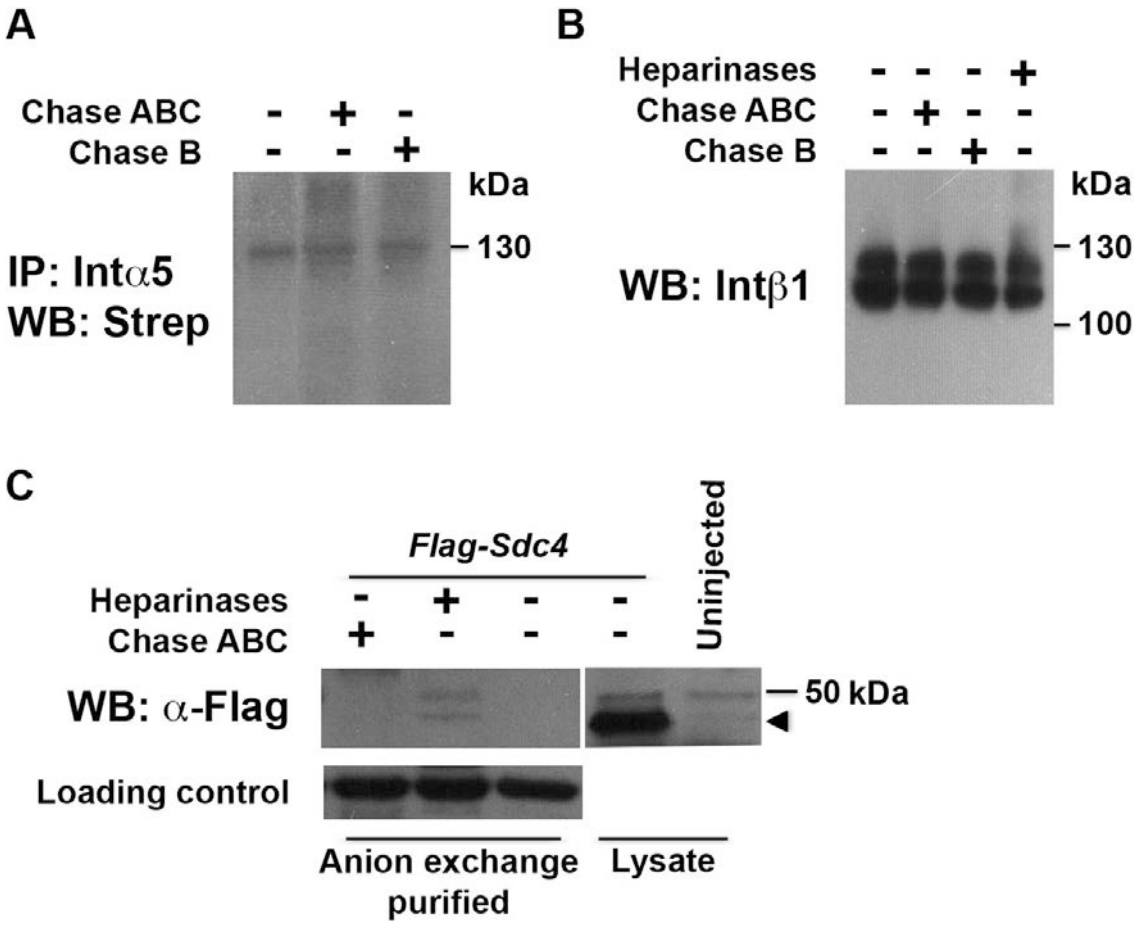

Figure S8. Integrin α5β1 and Syndecan-4 do not appear to be CS/DS-PGs in *Xenopus* embryos. Western blot analyses at stage 18 of lysates following treatment with Chondroitinase ABC (ChABC, specific for CS/DS), Chondroitinase B (ChB, specific for DS) or Heparinases (specific for HS).

(A) CNC explants from uninjected embryos. Biotinylated cell surface endogenous integrin α5 was immunoprecipitated with a monoclonal anti-Intα5 antibody and probed following lyase treatment with streptavidin.

(B) Explants enriched in neural crest and epidermis from uninjected embryos. Endogenous integrin β1 was probed following lyase treatments using a monoclonal antibody against Intβ1.

(C) Explants enriched in neural crest and epidermis from embryos injected with 600 pg *Flag-Sdc4* mRNA. Overexpressed Flag-tagged Sdc4 is detected as a dimeric core protein at ~45 kDa (arrowhead), and no PG smear was identified. PGs were enriched by anion exchange purification prior to lyase treatments. Note that only treatment with Heparinases releases the Flag-Sdc4 core protein, which demonstrates that *Xenopus* Sdc4 is a HS-PG. BSA serves as a loading control.

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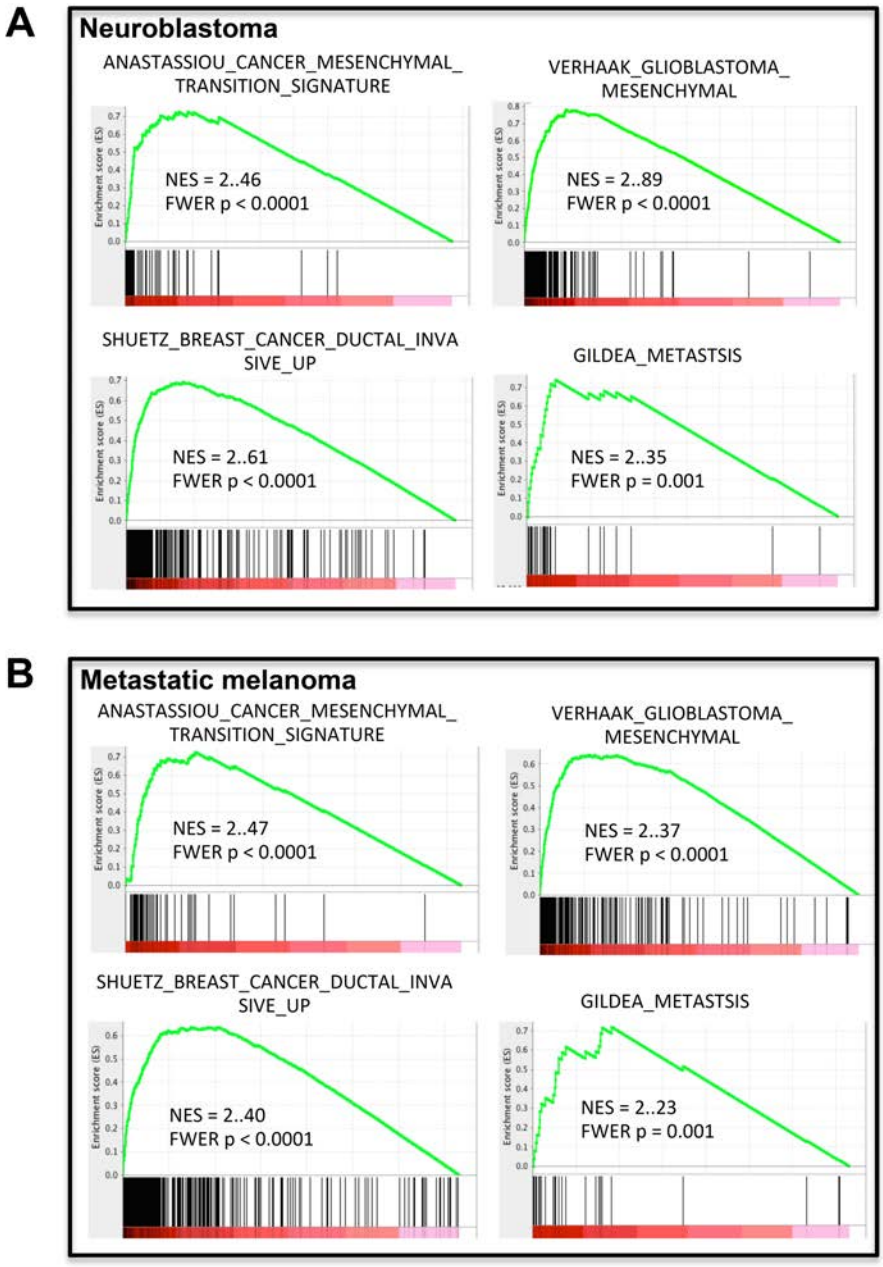

Figure S9. *DSE* expression in neural crest-derived tumors

Publically available datasets that consist of expression data from 498 neuroblastomas (GSE62564) and 44 metastatic melanomas (GSE19234) were used to examine *DSE* expression in NC-derived tumors.

(A,B) Gene set enrichment analyses ranked gene lists according to the correlation with *DSE* expression in neuroblastoma (A) and metastatic melanoma (B). Significant enrichments of the gene sets associated with the mesenchymal transition (top left, Anastassiou et al., 2011), aggressive mesenchymal phenotype in glioblastoma (top right, Verhaak et al., 2010), invasive phenotype in ductal breast cancer (bottom left, Schuetz et al., 2006) and metastasis (bottom right, Gildea et al., 2002) were identified in both cancer types. Normalized enrichment scores (NES) and FWER corrected p-values are provided.

Table S1. PCR primers used for RT-PCR analysis

Gene	Primer pair	Cycles	Annealing Temp.	Reference
Dse	5'- GGCAGTTCATGTAATGCTGACC -3' 5'- GGTGAGGTTTCTAGAGGTAATCGC -3'	30	60	This study
Dsel	5'- GTGGAACAGTGAAATTGCAGACGC -3' 5'- CATTGATTTGCATGAGCCTAGC -3'	30	60	This study
Histone H4	5'- CGGGATAACATTCAGGGTATCACT -3' 5'- CATGGCGGTAACTGTCTTCCT -3'	27	50	Hou et al., 2007

Table S2. PCR primers used for qPCR analysis. All sequences were newly designed in this study.

Gene	Primer pair
Dse	5'- CTGCGTCCTGAATCCAGATA -3' 5'- ACTTCATCCCAAGGAGCATC -3'
Dsel	5'- ATCTGGAAAGCTTGGTGGTC -3' 5'- TCCATTGGGAGCAAATGTAA -3'
c-Myc	5'- GCGACCAAAGGAATATTGGA -3' 5'- GGGCTGCAAGTCATAATCGT -3'
Itga5	5'- CCTCCAATCACCCAGCTAAT -3' 5'- CTGAGATGAGTCGGGCAGTA -3'
Itgb1	5'-GGATAGTCGGGAAGAGTTGC-3´ 5'-GGTTCCTTCACAACATGCAC-3
Bgn	5'- GCCACCTATGGATTTATGCC -3' 5'- GTGGTGTCTTTCGGAAGGTT -3'
Vcan	5'- CTCCAGGACAACTGAAAGCA -3' 5'- GAATCCCTTCCTTTCCCATT -3'
CD44	5'- TACACCCTTGGCAATAACGA -3' 5'- CGTTGAGGAGGAGAACAGGT -3'
Sdc1	5'- GAAGCATCAGGAGACGATGA -3' 5'- GCAACGCAGATGTAGAAGGA -3'
Sdc3	5'- CATTTGGCCTTTGGGATTAT -3' 5'- TCAACAGGTCGCTCATCTTC -3'
Sdc4	5'- CTGATGTTTGTGCTGCTCCT -3' 5'- TCGTCGTCCTCATCAATCTC -3'
Eef1A1	5'- ATTGATGCTCCAGGACACAG -3' 5'- CACGGGTTTGTCCATTCTTT -3'