Schiffmacher et al., https://doi.org/10.1083/jcb.201604006

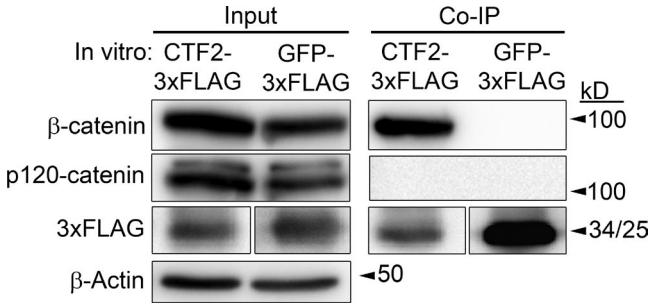

Figure S1. **Cad6B CTF2 physically associates with β-catenin, but not p120-catenin, in vitro.** LMH cell transfection (as in Fig. 4 G) reveals β-catenin coimmunoprecipitation (co-IP) after CTF2-3xFLAG, but not GFP-3xFLAG, pull-down. CTF2-3xFLAG does not coimmunoprecipitate with p120-catenin, as observed in vivo (Fig. 1 C).

A

***β-catenin* siRNA sequence**

siRNA	Sequence (5'-3')	Length (nt)
β-catenin siRNA #1	GGUUUGACACUGACCUGUAAAUCGA GACCAAACUGUGACUGGACAUUUAGCU	27
β-catenin siRNA #2	GUGAAAUUCUUGGCUAUUACAACAG UGCACUUUAAGAACCGAUAAUGUUGUC	27
β-catenin siRNA #3	CUGGAAUUCUUUCUAACCUCACUTG UCGACCUUAAGAAAGAUUGGAGUGAAC	27

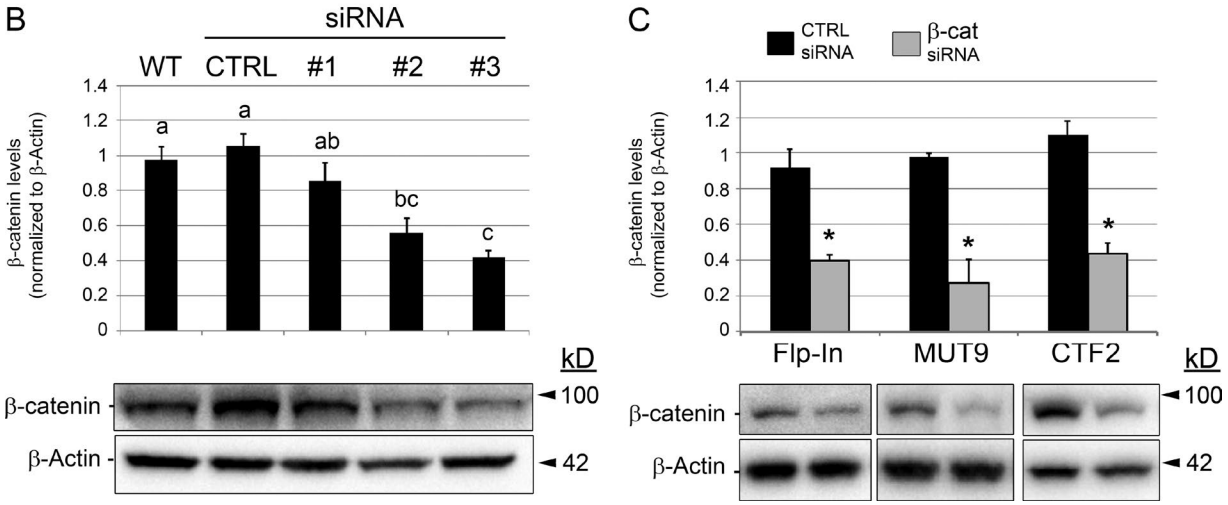

Figure S2. **Depletion of β-catenin from CHO cells using DsiRNAs.** (A) Sequence of the β-catenin DsiRNAs designed for knockdown assays. (B) Three β-catenin DsiRNAs were tested for knockdown efficacy in Flp-In-CHO cells. DsiRNA #1 did not produce appreciable knockdown (15%), whereas DsiRNAs #2 and #3 significantly decreased β-catenin protein levels compared with untransfected and negative control (CTRL) DsiRNA-treated cells (44% and 59%, respectively; $n = 3$, $P < 0.05$). Means that share letter superscripts are not significantly different ($P < 0.05$). DsiRNA #3 was used in subsequent experiments. (C) DsiRNA #3 decreases β-catenin protein levels within the parental Flp-In (58%), Flp-In-Cad6B-CTF2-HA (56%), and Flp-In-Cad6B-MUT9-HA CHO cells (72%; *, $P < 0.05$; $n = 3$). The degree of knockdown is not significant between cell lines ($P < 0.22$). WT, wild type. Error bars show SEMs.

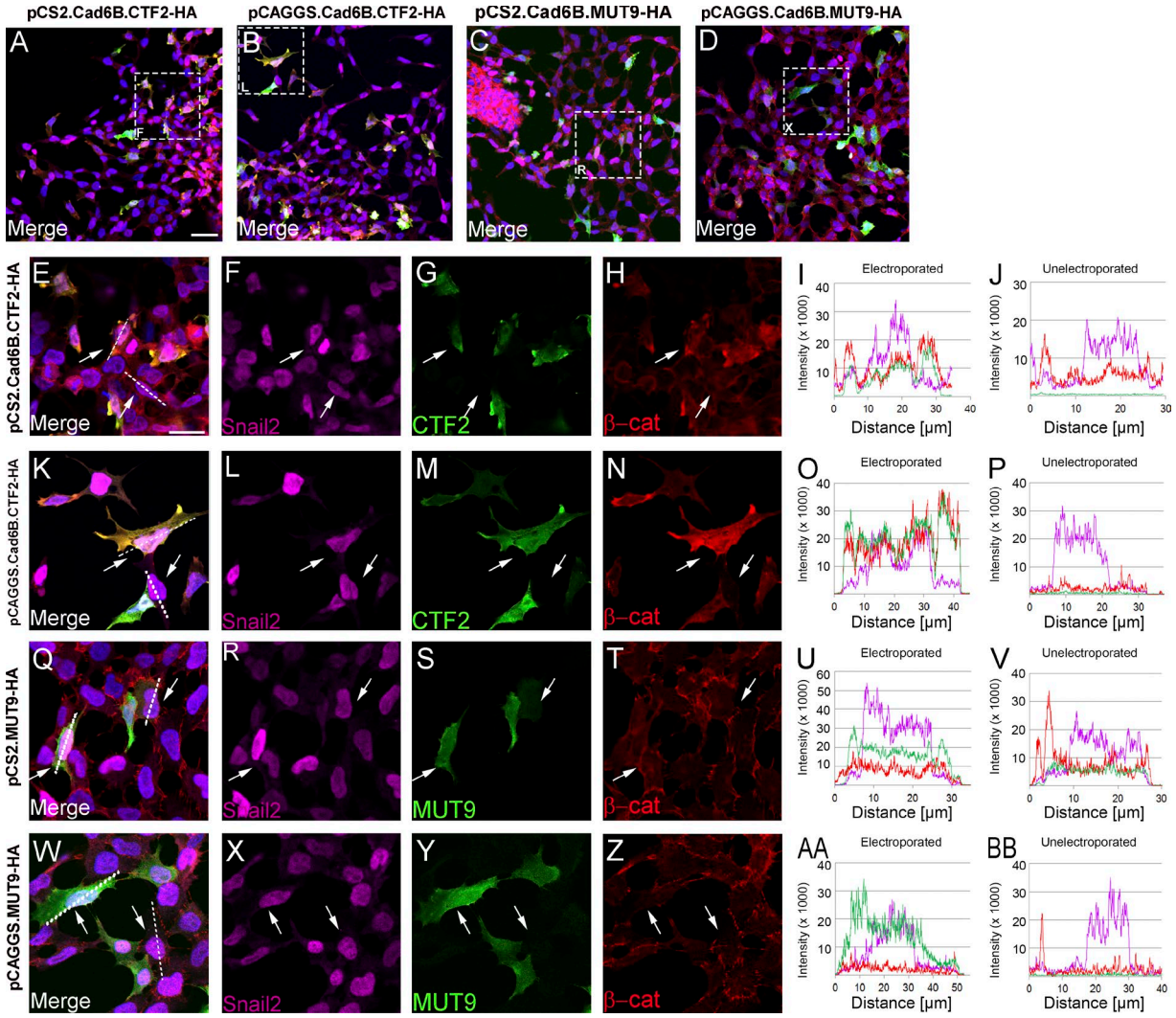

Figure S3. **Cad6B CTF2 and β-catenin colocalize in the cytosol and nucleus in cranial neural crest cells treated with LMB ex vivo.** Representative explants of neural crest cells overexpressing CTF2-HA (pCS2-CTF2-HA, A and E–H; line scans, I and J; pCAGGs-CTF2-HA, B and K–N; line scans, O and P), MUT9-HA (pCS2-MUT9-HA, C and Q–T; line scans, U and V; or pCAGGs-MUT9-HA, D and W–Z; line scans, AA and BB), followed by immunostaining for Snail2 (purple), HA (green), and β-catenin (red). Boxes in lower-magnification 20× merge images (A–D) mark respective magnified (63×) areas (E–H, K–N, Q–T, and W–Z). Bars: (A–D) 20 µm; (E–Z) 10 µm. Observed phenotypes are comparable to that described in Fig. 4.

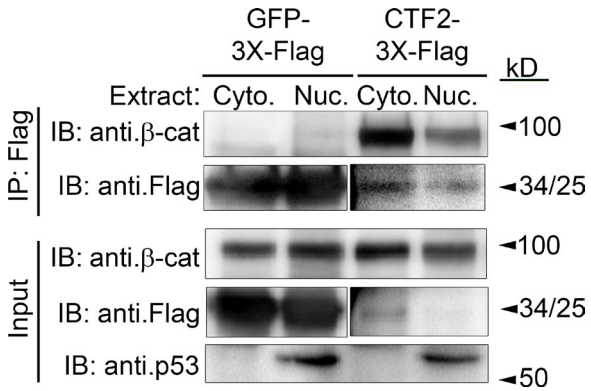

Figure S4. **β-Catenin is coimmunoprecipitated with CTF2-3xFLAG, but not GFP-3xFLAG, in both cytosolic and nuclear protein fractions of transfected LMH cells in vitro.** p53 levels (53 kD) were assessed to determine degree of nuclear protein enrichment after fractionation. IB, immunoblot; IP, immunoprecipitation.

Table S1. **Primer sequences for *Snail2* promoter ChIP-QPCR (Fig. 5 C)**

***Snail2* ChIP-QPCR primer**	**Sequence (5′–3′)**	**Amplicon size**
		nt
Set #1		122
Sense	CACCACTCAGTCTGTAATTTGGG	
Antisense	CTGTGTCACTCTTTTGCCTGTC	
Set #2		137
Sense	GACAGGCAAAAGAGTGACACAG	
Antisense	GCATACTGGTTCAGACAAGCTG	
Set #3		87
Sense	ACAGCTTGTCTGAACCAGTATGC	
Antisense	GCTGAGGGAGTAGTCTGTGG	
Set #4		118
Sense	GACACACTGCCCGCTAAG	
Antisense	GCAGATTTCAAAGGCAGCTCC	
Set #5		129
Sense	GGGCTTCCCGAATAAGTCACG	
Antisense	CCTTAGCGGGCAGTGTGTC	
Set #6		104
Sense	ACGTGACTTATTCGGGAAGCC	
Antisense	GCTCCTCGGAGTTTCAGTCTAG	
Set #7		129
Sense	CTAGACTGAAACTCCGAGGAGC	
Antisense	GGAGGCTTTGCTTCAGGTTTC	
Set #8		117
Sense	GAAACCTGAAGCAAAGCCTCC	
Antisense	CTGCTCCTCCAATCACTTCTGG	
Set #9		97
Sense	CCAGAAGTGATTGGAGGAGCAG	
Antisense	GTGTAGTGGCACTGCAGAGG	
Set #10		117
Sense	CCTGACTTGCGGGTATTTACG	
Antisense	CGCACACTATCACTGCGAG	
Set #11		105
Sense	CAATGCGATAGGGACCGATG	
Antisense	TGAAGGCAGGCTTTCTCCTTC	