The Calcium-Mediated Repression of β-Catenin and Its Transcriptional Signaling Mediates Neural Crest Cell Death in an Avian Model of Fetal Alcohol Syndrome

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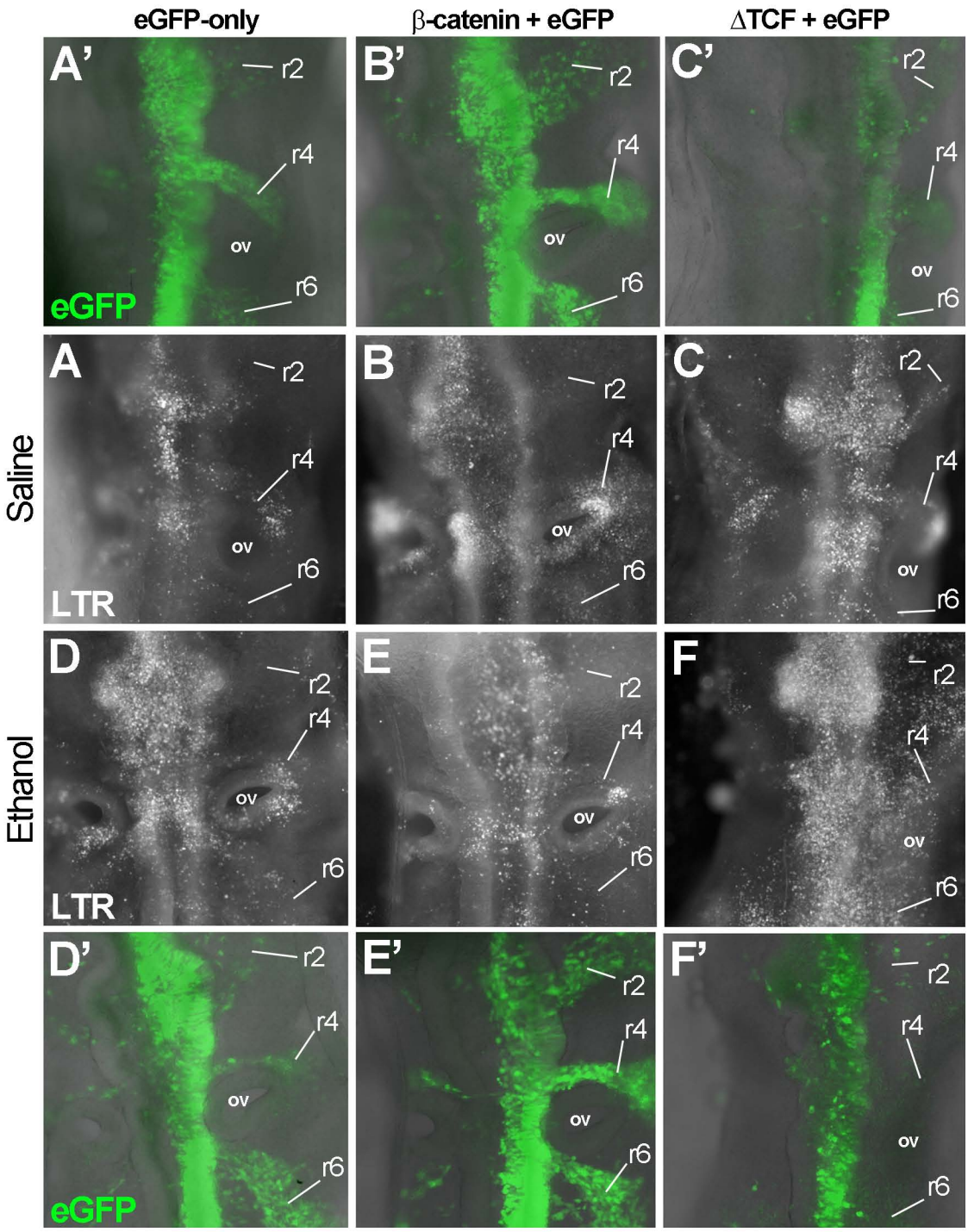
Supplemental Figure 1. Ethanol-induced apoptosis and neural crest numbers are altered by β-catenin and TCF transfection in hindbrain. Shown are dorsal views of the intact HH12+ (17s) hindbrain, transfected as indicated and challenged 3hr thereafter with saline or 52 mM ethanol. **Panels A-F** depict apoptotic cells visualized with LysoTracker Red (LTR, white dots). **Panels A'-F'** show green fluorescent signals overlaid on bright-field images of the same embryos, showing expression of the eGFP internal control in the right lateral hindbrain and migrating neural crest. Lines indicate the neural crest migrating from rhombomeres 2 (r2), r4 and r6. **(A-C)** Saline-treated embryos transfected with **(A)** eGFP, **(B)** β-catenin + eGFP, or **(C)** ΔTCF + eGFP. **(D-F)** Embryos challenged with 52 mM ethanol and transfected with **(D)** eGFP, **(E)** β-catenin + eGFP, or **(F)** ΔTCF + eGFP. Ethanol treatment significantly enhanced the apoptosis level in eGFP-treated hindbrain (compare abundance of white dots in **A vs. D**). In contrast, β-catenin overexpression strongly reduced the apoptosis levels in both saline and ethanol-treated hindbrain (compare abundance of white dots, **B vs. A, E vs. D**). ΔTCF overexpression elevated apoptosis levels in both saline-treated and ethanol-treated hindbrain (compare abundance of white dots, **C vs. A, F vs. D**). In embryos transfected with ΔTCF + eGFP, although eGFP expression extended along the hindbrain's rostrocaudal length, the eGFP signal per unit area was consistently lower than for embryos receiving eGFP-only and β-catenin + eGFP. This may reflect the increased apoptosis of the ΔTCF-transfected cells. Abbreviations: ov, otic vesicle; r, rhombomere.

Supplemental Figure 1



Supplemental Table 1

Real-Time PCR Primer Sequences

Transcript	Accession #	Forward Primer	Reverse Primer
APC	XM_001233410.1	GAGCACCTCAAGGCAAGCATG	TTCGAGCAGCAGAACCCTGACCA
Bmp4	NM_205237	TTCGTCTTCAACCTCAGCAG	GGCTCACATC(A)AAAGGTCTCC
β-catenin	NM_205081	AATTGTGCGTACCATGCAAA	ACACAGAATCCACCGGAGAC
FoxD3	U37274	TCTGCGAGTTCATCAGCAAC	CGAACATGTCCTCAGACTGC
SFRBP	AF218056.1	TGGCCCGACATGCTGGACTGCA	AGGCATCGCAGACTTTGGGTGCTTC
Gapdh	M11213	CGTGTTGTGGACTTGATGGT	TGGAGGAAGAAATTGGAGGA
GSK3β	XM_416557.2	TCCTTCGCGGAGAGCTGCAAGC	GGACCTTGTCCAGGAGTTGCCA
Slug	X77572	CATTACTGTGTGGACTAC	TGTAGTCCACACAGTAATG
Snail	Y09905	CCTTTCCCGTGCAGATACAT	AAAGATGTCCAGATGGGTGC
Tgfβ2	NM_001031045	GGAATGTGCAGGATAATGC	ATGGTGAGGGGCTCTAAAT
Wnt1	AY655699.1	AAGGGCCGAGATCTGCGCTTCCTG	ACATGCCGTGGCATTTGCACTCCTG
Wnt5a	AB006014	ACATCGAGTACGGATACCGC	CAGAGACACCGTGGCACTTA
Wnt6	AF378331	ACATCAAACCTCCCGACAAG	CACAGGCAGTTCTCCTCCAG
Wnt9a	AY753293.1	ACCAGTTTCGCTTTGAGCGTTGGAA	CGCATGTGTCAGCCCTGCAGAA
Wnt11	D31901	TTCATCTTTGGCCCTGAATC	AGCTCGATGGATGAGCAGTT