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SUPPLEMENTARY MATERIAL

corresponding to:

Apolipoprotein C-I mediates Wnt/Ctnnb1 signaling during neural border formation and is required for neural crest development

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Supplementary figures for Materials and Methods

TOPFlash luciferase assay

14XTOPFlash reporter plasmid was created by inserting 14 tandem repeats of TCF binding sites into pGL4.26(luc2/minP/Hygro) (Promega). 100pg of 14XTOPFlash and 25pg of renilla (pRL Renilla; Promega) DNA were co-injected with mRNAs into two animal cells at 4 to 8cell-stage embryos, and firefly and renilla luciferase were measured at st10 *Xenopus* embryos with Dual-Glo Luciferase Assay System (Promega). Triplicate samples (Five embryos/each) were used for an individual condition, and three independent experiments were examined.

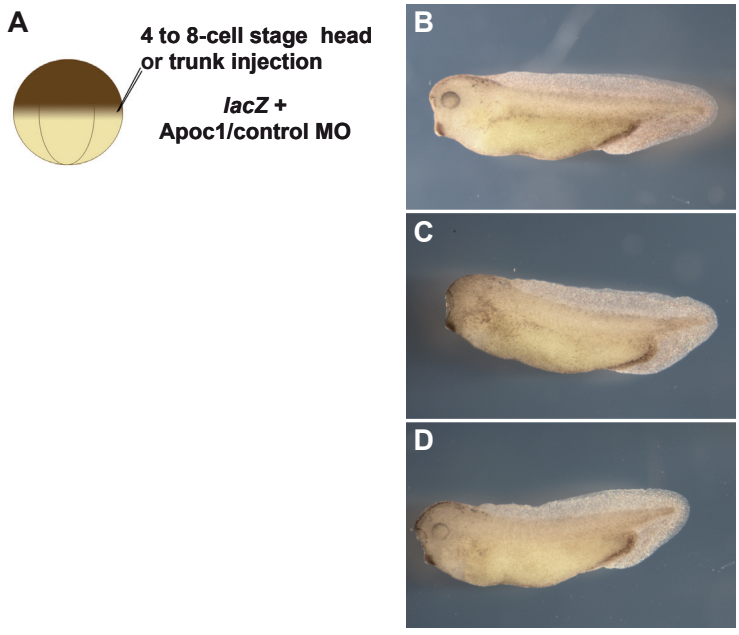

Fig. S1.The apoc1 spMO morphants resulted in identical phenotypes as tbMO morphants. (A) *Schematic drawing of the experiment. 40 ng Apoc1 spMO was injected into either the presumptive head (C) or trunk/tail (D).* **(B)** *Embryos injected with 40 ng control MO were normal.* **(C)** *Injections targeted to the head region resulted in 56.4% (n=55) of the embryos displaying small or missing eyes and head deformation.* **(D)** *Injections targeted to the trunk region resulted in perturbed dorsal fin development in 45.2% (n=31) of the embryos.*

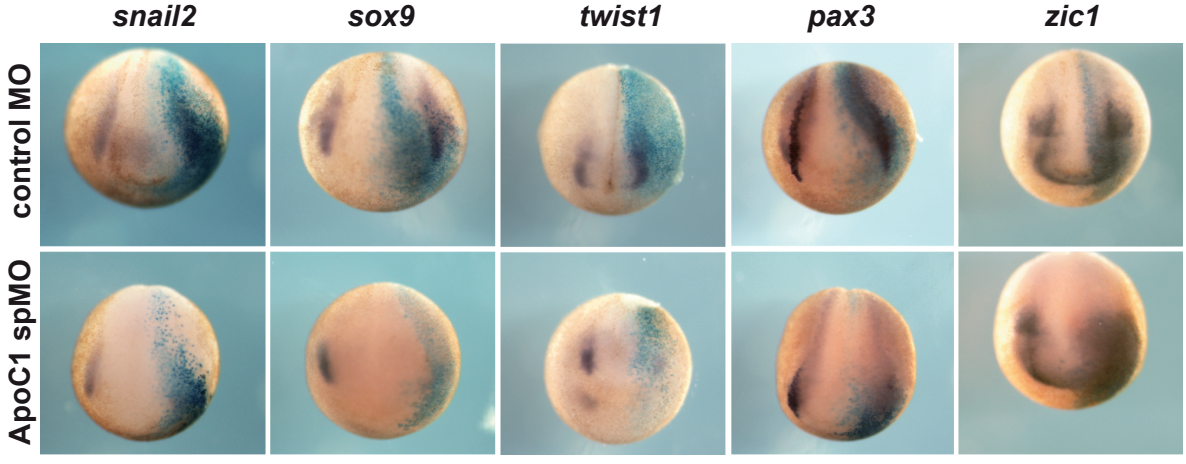

Fig. S2. *Apoc1* is required for neural border formation and neural crest emergence. *Whole mount* in situ *hybridization for the neural crest (NC) markers* snai2, sox9 *and* twist1 *and the neural plate border specifiers* pax3 *and* zic1. *Control MO (40ng) injected embryos are shown in upper panel and Apoc1 spMO (40ng) injected ones are shown in lower panel. Injection of Apoc1 spMO also results in a reduction of the expression of the NC markers as well as the neural plate border specifiers on the injected side.*

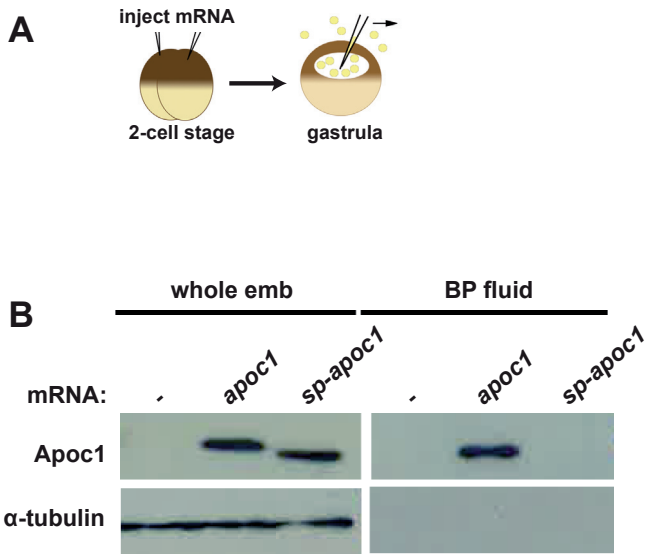

Fig. S3. *Apoc1* mutant lacking signal sequence cannot be secreted. (A) *Schematic drawing of the Apoc1 secretion experiment. mRNA was injected at the two-cell stage. The blastopore fluid was collected at the gastrula stage and subjected to western blot (WB) analysis to determine whether mutant flag-tagged Apoc1 protein (lacking signal sequence) is secreted from cells during embryonic development.* **(B)** *Apoc1, but not mutant protein was detected by WB in the blastocoel fluid after injection of 500pg of flag-tagged* apoc1 *or flag-tagged mutant* apoc1 *mRNA. The tubulin blot shows that there is no cellular contamination in the blastopore fluid.*

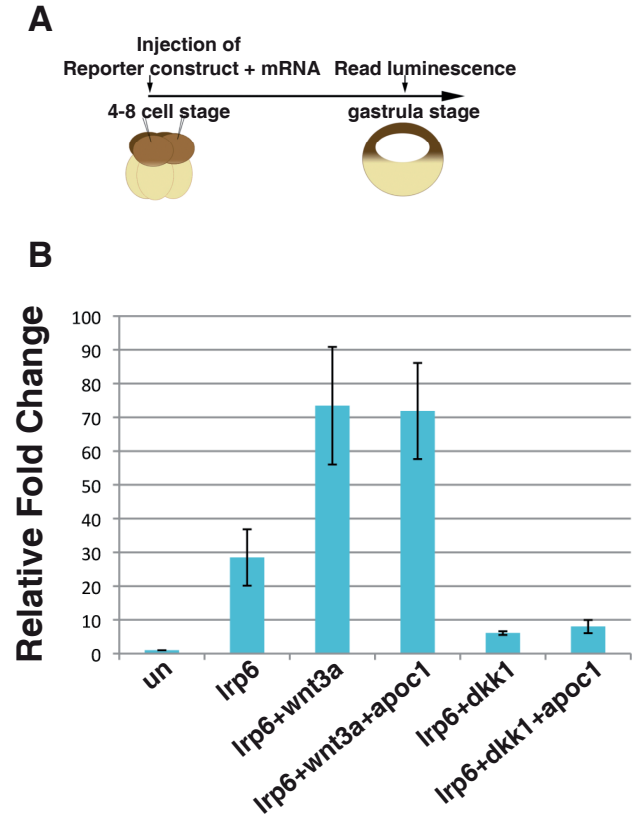

Fig. S4. *Apoc1* does not cooperates with extracellular Wnt components to modulates Wnt signaling in TOPFlash assay. (A) *Schematic drawing of the experiment.* **(B)** *300 pg of* lrp6 *mRNA and either 20 pg of* wnt3a *or 50 pg* dkk1 *with or without 500 pg of* apoc1 *mRNA were co-injected with 100 pg of 14XTOPFlash and 25 pg Renilla reporter DNA into 4 to 8cell-stage* Xenuous *embryos. Firefly and Renilla luciferase were measured at st.10.*

SUPPLEMENTARY TABLE S1

STATISTICAL DATA FOR FIG. 1 AND FIG. S1

Apoc1 tbMO

phenotypes	head injection (%)	trunk injection (%)
normal	13 (9.70%)	5 (13.2%)
head developmental defects	102 (76.1%)	0 (0%)
defects in dorsal fin	21 (15.7%)	21 (55.3%)
others	11 (8.21%)	10 (26.3%)
total	n=134	n=38

Apoc1 spMO

phenotypes	head injection (%)	trunk injection (%)
normal	7 (12.7%)	3 (9.68%)
head developmental defects	31 (56.4%)	0 (0%)
defects in dorsal fin	7 (12.7%)	14 (45.2%)
others	13 (23.6%)	14 (45.2%)
total	n=55	n=31

control MO

phenotypes	head injection (%)	trunk injection (%)
normal	29 (90.6%)	14 (77.8%)
head developmental defects	2 (6.25%)	2 (11.1%)
defects in dorsal fin	0 (0%)	0 (0%)
others	1 (3.13%)	2 (11.1%)
total	n=32	n=18

SUPPLEMENTARY TABLE S2

STATISTICAL DATA FOR FIG. 4 AND FIG. S2

	Apoc1 tbMO	cont MO	Apoc1 spMO	Apoc1 tbMO+apoc1 mRNA
c-myc	decreased 55.9% (n=68)	decreased 8.6% (n=58)		
sox9	decreased 68.3% (n=82)	decreased 4.3% (n=47)	decreased 61.4% (n=44)	decreased 21.1% (n=38)
snai2	decreased 66.7% (n=69)	decreased 12.5% (n=48)	decreased 62.2% (n=37)	decreased 23.8% (n=42)
twist1	decreased 72.2% (n=54)	decreased 7.4% (n=54)		
id3	decreased 60.7% (n=28)	decreased 18.2% (n=22)		
msx1	decreased 70.9% (n=55)	decreased 9.6% (n=83)		
pax3	decreased 68.8% (n=48)	decreased 8.1% (n=37)		
zic1	increased 54.9% (n=51)	increased 5.9% (n=36)		
sox2	expanded 70.3% (n=37)	expanded 3.7% (n=27)		
xk81a1	expanded 76.3% (n=38)	expanded 0% (n=21)		

SUPPLEMENTARY TABLE S3

STATISTICAL DATA FOR FIG. 5C

	sox9	snai2
zic1 inj	ectopic expression 16.7% (n=30)	ectopic expression 0% (n=27)
zic1+apoc1 i	ectopic expression 73.1% (n=26)	ectopic expression 45.8% (n=24)

SUPPLEMENTARY TABLE S4

STATISTICAL DATA FOR FIG. 6

	Gbx2.2 MO	cont MO
apoc1	decreased 17.69% (n=34)	decreased 12.5% (n=24)

	Apoc1 tbMO	cont MO
gbx2.2	decreased 13.2% (n=38)	decreased 11.1% (n=27)

	Apoc1 tbMO + gbx2.2 mRNA	Gbx2.2 MO + apoc1 mRNA
sox9	decreased 64.5% (n=31)	decreased 69.2% (n=39)
snai2	decreased 70.4% (n=27)	decreased 67.4% (n=43)

	GR Ctnnb1
apoc1	ectopic expression 94.9% (n=39)
gbx2.2	ectopic expression 91.4% (n=35)
msx1	ectopic expression 77.3% (n=22)
pax3	ectopic expression 88.9% (n=27)
snai2	anterior expression75.8% (n=33)
sox9	anterior expression 82.1% (n=28)

	GR Ctnnb1+Apoc1 tbMO
gbx2.2	ectopic expression 87.9% (n=33)
snai2	anterior expression 22.6% (n=31)

	GR Ctnnb1+Gbx2.2 MO
apoc1	ectopic expression 91.7% (n=36)
snai2	anterior expression 16% (n=25)

SUPPLEMENTARY TABLE S5

STATISTICAL DATA FOR FIG. 7

	apoc1 inj
snai2	symmetric 83.3% (n=48)
pax3	symmetric 74.3% (n=74)
foxi4.1	symmetric 87.0% (n=46)

	Apoc1 tbMO	cont MO
foxi4.1	decreased 42.9% (n=49)	decreased 17.8% (n=45)