Qeios

Peer Review

Review of: "CHD3 Regulates BMP Signalling Response During Cranial Neural Crest Cell Specification"

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1) Please tighten claims about "CNCC specification" vs "EMT + BMP response required for the protocol"

A key conceptual point: the data strongly support that **CHD3 is required for BMP-responsive enhancer accessibility and EMT**, which are essential for CNCC formation *in this differentiation protocol*. However, statements implying direct equivalence to in vivo CNCC biology should be toned down (or explicitly framed as "in vitro model of CNCC induction").

Suggestion: revise wording throughout Results/Discussion to emphasize:

"CHD3 is required for BMP response / enhancer accessibility during CNCC induction in vitro"

avoid definitive claims that CHD3 "regulates CNCC specification in vivo" without in vivo validation.

2) BMP rescue experiment: justify dose, units, and demonstrate ligand activity with a positive control

The BMP rescue section is currently vulnerable because:

The **BMP2 concentrations** (as written) appear unusually low compared with common in vitro usage, and there is potential confusion in **units** (pg/mL vs ng/mL vs µg/mL).

A negative result (no rescue) requires confidence that BMP2 was biologically active in the assay.

Required additions:

Clear unit reporting and rationale for the chosen BMP2 range.

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A **positive control readout** demonstrating BMP pathway activation under your conditions (e.g., pSMAD1/5/9, SMAD6/7, ID1/ID2 induction).

Ideally, include a short time-course (e.g., 1–6 h after BMP addition) for pathway activation.

3) qPCR interpretation for CHD3 residual signal: confirm specificity

CHD3 KO shows no protein by Western blot, but qPCR still reports residual CHD3 transcript. This can happen due to:

primer-dimer/background signal

amplification of partially processed transcripts

alternative isoforms not disrupted

Please add:

melt curves + gel validation (or at least a melt curve summary)

primer location relative to the CRISPR target

if possible, qPCR using an independent primer set (different exon junction)

align this with RNA-seq evidence (show CHD3 gene-level and transcript/isoform-level expression at D0/D14/D18).

Declarations

Potential competing interests: No potential competing interests to declare.