doi: 10.1387/ijdb.103233sg

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

SUPPLEMENTARY MATERIAL

corresponding to:

Regulation of programmed cell death during neural induction in the chick embryo

ANNA GIBSON, NEIL ROBINSON, ANDREA STREIT, GUOJUN SHENG and CLAUDIO D. STERN

Supplementary Information

Dad1 *does not affect neural, epidermal or neural plate border fates*

To establish whether expression of *Dad1* might influence cell fate choices at early stages of neural development, it was misexpressed using *in vivo* electroporation. Ectopic expression of DAD1 does not induce the neural markers *Sox3* (0/21) or *Sox2* (0/19; Suppl. Fig. 1), the neural crest and neural plate border markers *Dlx5* (0/15) or *Pax7* (0/9; Suppl. Fig. 2) or of *Gata2* (0/8; not shown). There was also no detectable effect of *Dad1* misexpression on the other two genes (*Ubll* and *fth1*) studied in this paper (0/8 and 0/13, respectively; not shown). Control electroporation of the pCAβ vector does not alter the expression of any of the genes tested (some shown in Suppl. Figs. 1-2).

To assess whether Dad1 is required for neural plate or neural plate border formation, we used two different loss-of-function approaches. *Dad1* lacking the last 6 C-terminal amino acids acts as a dominant-negative (Makishima *et al.*, 2000), while morpholinos targeting the translation start site prevents its translation. Misexpression of dominant negative Dad1 or morpholino mediated Dad1 knockdown do not affect the expression of *Sox3*, *Sox2*, *Dlx5*, *Gata2* or *fth1* (Suppl. Figs. 1-2 and not shown). In conclusion, neither gain- nor loss of DAD1 function affects the expression of markers for epidermis, neural plate border or neural plate.

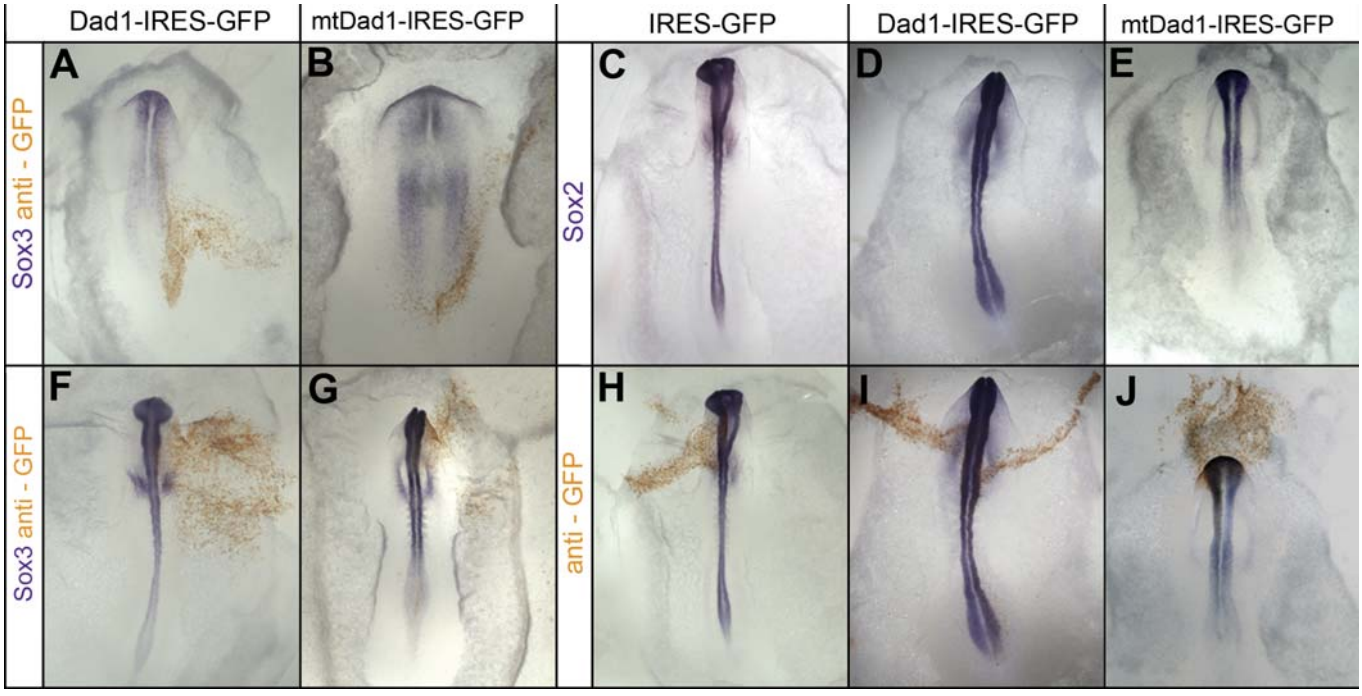
Ubll *does not affect cell fate at the neural plate*

To explore a possible role of *Ubll* in early neural development, *Ubll* was misexpressed *in vivo*. *Ubll* electroporation does not alter expression of the early neural markers *Sox3* (0/9; not shown), or *Sox2* (0/16; Suppl. Fig. 3), of the pre-neural marker *ERNI* (Suppl. Fig. 4) or of the neural plate border marker *Pax7* (0/7; Suppl. Fig. 4). Likewise, misexpression of GFP or of a mutated version of *Ubll* lacking the last two ubiquitin monomers does not change expression of any marker tested (Suppl. Figs. 3-4). In conclusion, ectopic expression of *Ubll* is not sufficient to induce early neural or neural crest markers.

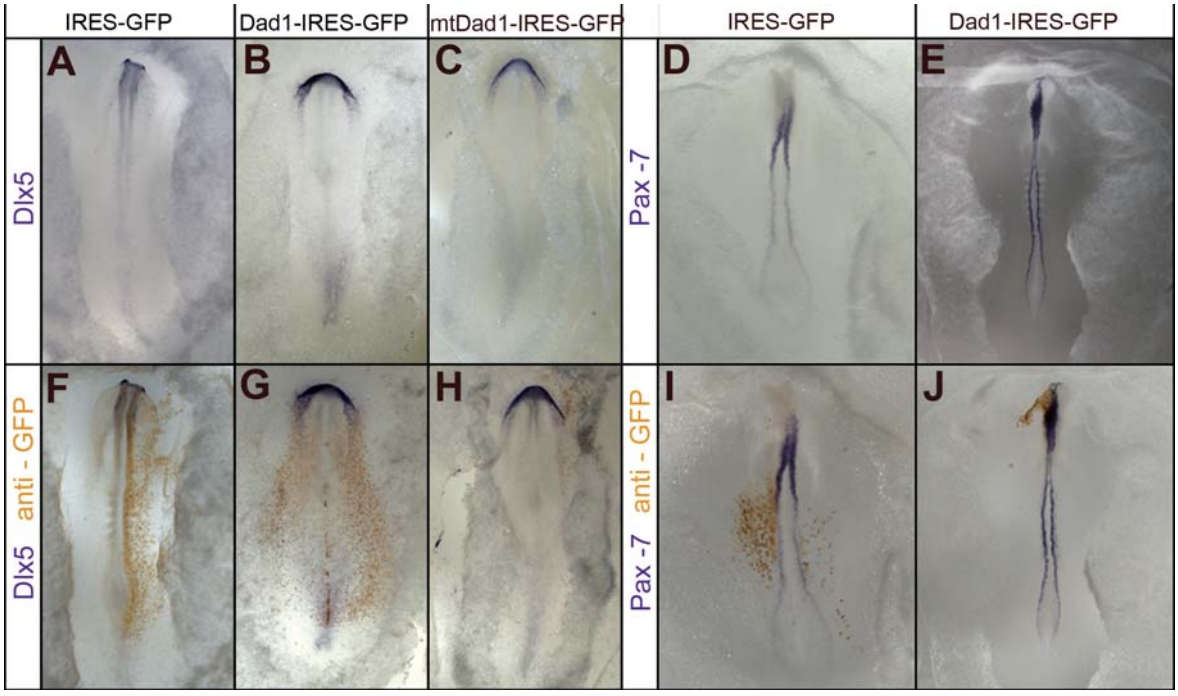
fth1 *does not affect neural fates*

To establish a possible role of *fth1* during neural plate development, it was misexpressed by electroporation. Two different constructs were tested: a cDNA containing the entire ORF as well as the 5' untranslated sequence that encodes the iron regulatory sequence (IRE), and the ORF alone, lacking the 5' IRE. Neither construct induces the neural markers *Sox3* (0/15 and 0/14; not shown) or *Sox2* (0/9 and 0/11; Suppl. Fig. 5) nor the neural plate border marker *Dlx5* (0/9 and 7/7; not shown) after 6 hours (for *Sox3*), 9 hours (for *Sox2*) or overnight (for *Sox2* and *Dlx5*) culture.

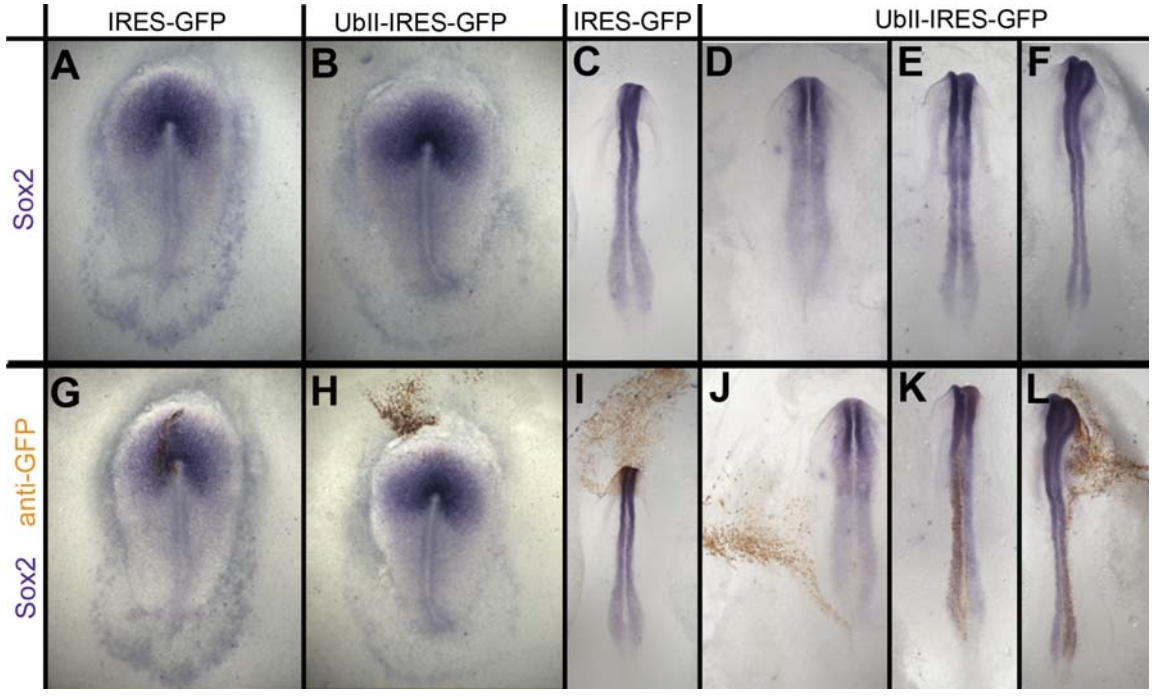
It has been reported that the iron regulatory protein IRP2 is a main regulator of FTH1 synthesis in the nervous system, and that IRP2 levels are regulated by ubiquitin-mediated degradation (LaVaute *et al.*, 2001). To assess the possibility of a feedback loop by which FTH1 and ubiquitin-related genes might regulate each other's expression, we analysed Ubll expression after misexpression of *fth1*: no effect was observed (not shown). In conclusion, we were unable to demonstrate any effect of fth1 misexpression on early neural markers or on *Ubll*.



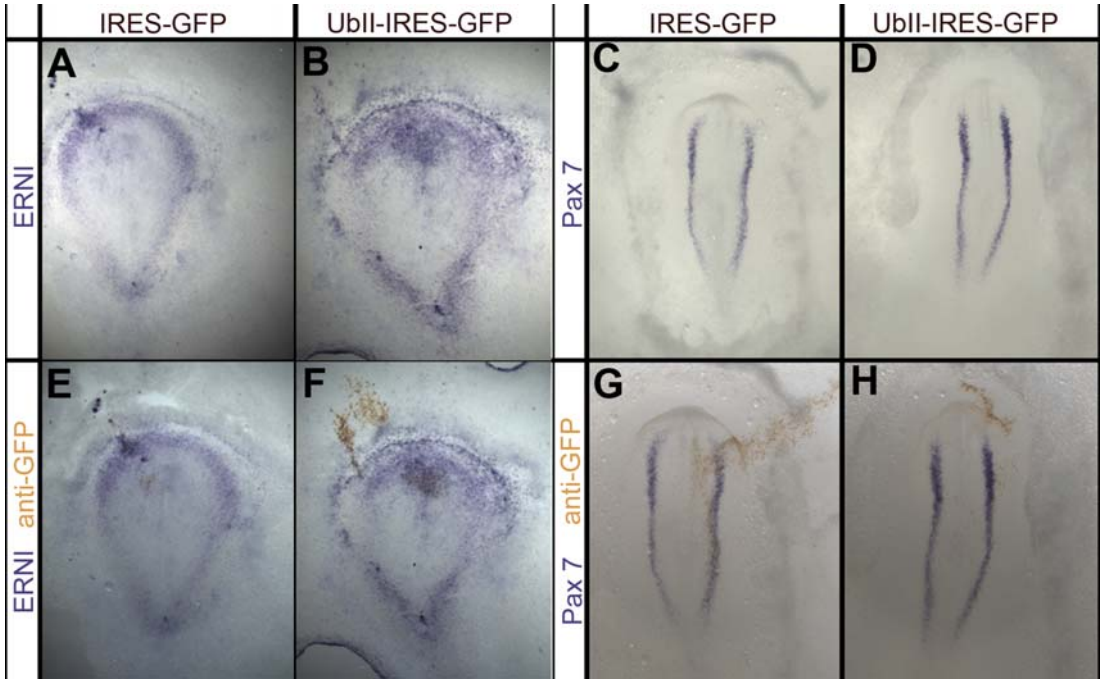
Supplementary Fig. 1. Dad1 does not affect expression of neural markers. *Electroporation of an expression construct encoding wild-type* Dad1 *has no effect on* Sox3 **(A,F)** *or* Sox2 **(D,I)** *expression. Electroporation of a mutated, dominant-negative* Dad1 *construct* **(B,G,E,J)** *similarly has no effect, nor does empty vector IRES-GFP* **(C,H)**. *(***C-E)** *Embryos after* in situ *hybridization for* Sox2 *(blue).* **(H-J)** *The same embryos after further staining with anti-GFP antibody (brown) to reveal the electroporated cells. The remaining panels show the double staining for the marker (blue) and GFP (brown) as indicated.*



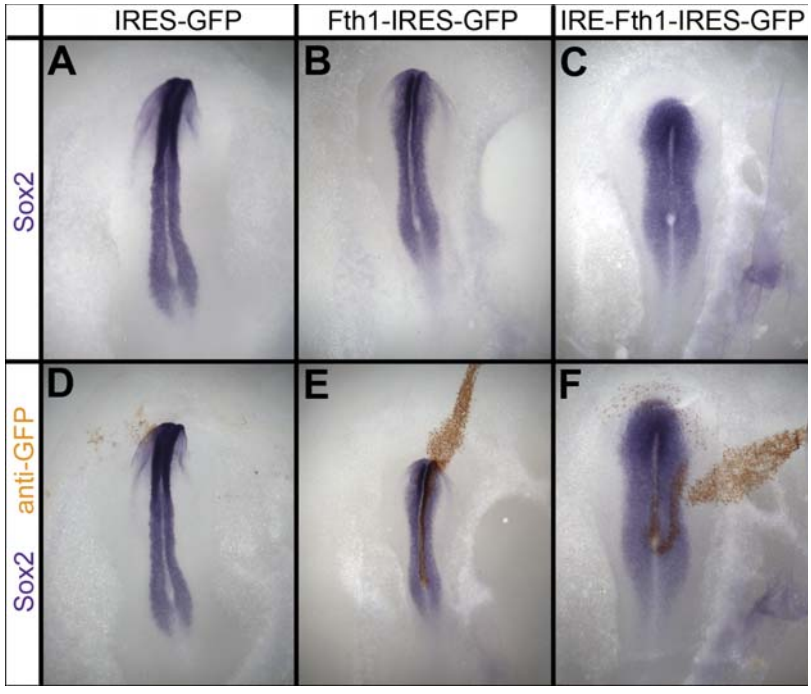
Supplementary Fig. 2. Dad1 does not affect expression of border markers. *Misexpression of* Dad1 *does not affect expression of the border markers* Dlx5 **(B,G)** *or* Pax7 **(E,J)**. *A dominant-negative* Dad1 *likewise has no effect on* Dlx5 **(C,H)**. *The IRES-GFP vector similarly has no effect on either marker* **(A,D,F,I)**.



Supplementary Fig. 3. UbII does not affect expression of Sox2. *Misexpression of UbII does not alter expression of neural plate marker* Sox2 *either at an early stage (stage 5⁻; B, H) or when examined at a later stage (stage 7-9; D-F, J-L). Electroporation of control vector likewise does not affect Sox2 expression at either stage* **(A,G,C,I)**. *The embryos are shown before* **(A-F)** *and after* **(G-L)** *staining with anti-GFP (brown).*



Supplementary Fig. 4. UbII does not affect expression of pre-neural or border markers. *Misexpression of UbII does not affect expression of the early pre-neural marker* ERNI **(B,F)** *or of the neural plate border marker* Pax7 **(D,H)**. *Comparable embryos electroporated with control vector are shown* **(A,E,C,G)**. **(A-D)** *The embryos after* in situ *hybridization (blue).* **(E-H)** *The same embryos after staining with anti-GFP (brown).*



Supplementary Fig. 5. Fth1 does not affect neural fate. *Misexpression of Fth1 either containing* **(C,F)** *or lacking* **(B,E)** *the Iron Regulatory Element IRE does not alter expression of the neural plate marker* Sox2 *either within or outside the neural plate. Controls electroporated with the vector are also shown* **(A,D)**.