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 (UbII 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 the pCAβ vector does not alter the expression of any of the genes tested (some shown in Suppl. Figs. 1-2).

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) and 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).