SUPPORTING ONLINE MATERIAL

FIGURE LEGENDS

Fig. 1. *Erk2* $^{-/-}$ mutant ES cell line. (A) Southern blot analysis of wild-type (+/+), heterozygous (+/-), and homozygous (-/-) ES cell DNA. Genomic DNA from the different ES cell lines was digested with Kpn I and hybridized with the 3'-external probe. The wild type and mutant bands correspond to the 9.2 kb and 10.3 kb hybridizing fragments respectively. (B) Immunoblot analysis of whole lysate from ES cell lines using α1cp44 antibody which recognizes both ERK1 and ERK2 isoforms (Meloche *et al.*, 1995).

Fig. 2. Immunohistochemistry analysis of ERK1/2 activating phosphorylation in wild-type E5.5 mouse embryos. (A) Negative control using only the secondary antibody and amplification step. (B) ERK1/2 is activated in the deciduum surrounding the implantation site (white arrowhead) and in extraembryonic tissues, namely the ectoplacental cone (black arrow), the extraembryonic ectoderm (black arrowhead) and trophectoderm giant cells (white arrow).

Fig. 3. Proliferation rates of E6.5 *Erk2*$^{-/-}$ and wild-type embryos as assessed by immununohistochemistry. Sections from E6.5 mutant and wild-type embryos were treated with anti-phospho histone H3 antibody and revealed by fluorescent secondary antibody. *Erk2*$^{-/-}$ mutant and wild-type embryos did not differ significantly in proliferation rate.

METHODS

Genotyping of *Erk2* mutants by PCR

Genotypes were determined by PCR using the following primers: K2A: 5'CAGGAGAGTGTGC CGTGTTC-3', K2C 5'GGTGTTCAGCAGGAGGTTGG-3', and K2N 5'CGGCCGGAGAA CCTGCGTGCAA3'. Cycling conditions were: 95ºC for 30 sec, 63ºC for 30 sec, 72ºC for 30 sec, for a total of 30 cycles. Primers K2A and K2N yield a 510-bp product diagnostic of the targeted allele, and primers K2A and K2C give rise to a 340-bp wild-type band.

Histology

For histological analysis, embryos were fixed in 2.5% glutaraldehyde in PBS overnight, dehydrated and embedded in epon. 2µm sections were counterstained with 1% toluidine blue. Embryos previously processed for whole-mount in situ hybridisation, were postfixed overnight in 2.5% glutaraldehyde in PBS, rinsed in PBS and embedded using the JB-4 embedding Kit (Polysciences, Inc.). 5 µm sections were mounted directly.

Whole-mount *in situ* hybridisation

Mouse embryos were staged according to their morphology as described previously (Downs & Davies, 1993). Whole-mount in situ hybridisation was performed as previously described (Gradwohl *et al.,* 1996). For section in situ hybridisation, decidua were fixed for two hours in 4% paraformaldehyde in PBS, equilibrated in 20% sucrose overnight and embedded in OCT (Tissue-Tek, Miles). In situ hybridisation on 8 µm frozen sections was performed as described (Conlon *et al.,* 1993), and sections were counterstained with hematoxylin.

Immunohistochemistry

Wild-type decidua were collected at E5.5, fixed in 8% PFA overnight and embedded in wax. Immunohistochemistry for activated ERK1/2 was performed on 7-µm sections using a polyclonal phospho-specific antibody to ERK1/2 (Cell Signaling Technology, 1:50 dilution), a biotinylated secondary antibody (Vector, 1:100 dilution) and an amplification step with avidin-conjugated peroxydase (Vector Elite ABC kit). Sections were stained using diaminobenzidine (Vector) and counterstained with hematoxylin.

Proliferation assays

Proliferation rates of E6.5 embryos were determined by immunohistochemistry with anti-phospho-histone H3 (PH3) antibody (Upstate Biotechnology, dilution 1:1000). Fluorescent labeling was subsequently carried out using a goat anti-rabbit secondary antibody conjugated to Cy3 (Jackson Immunoresearch, dilution 1:200).

REFERENCES

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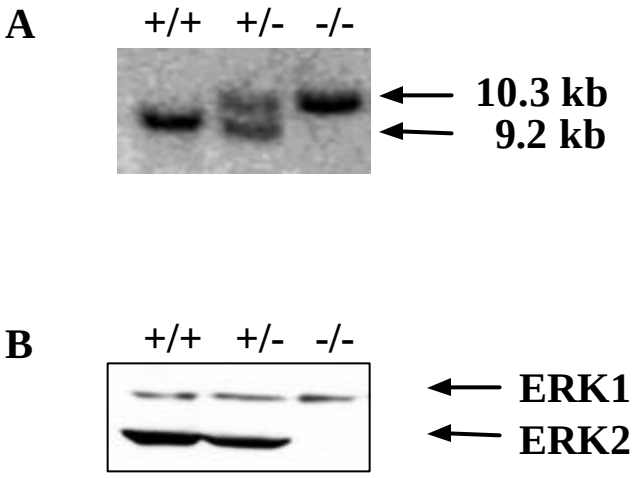
Gradwohl, G., Fode, C. & Guillemot, F. (1996) Restricted expression of a novel murine atonal-related bHLH protein in undifferentiated neural precursors. *Dev. Biol.*, **180**, 227-241.

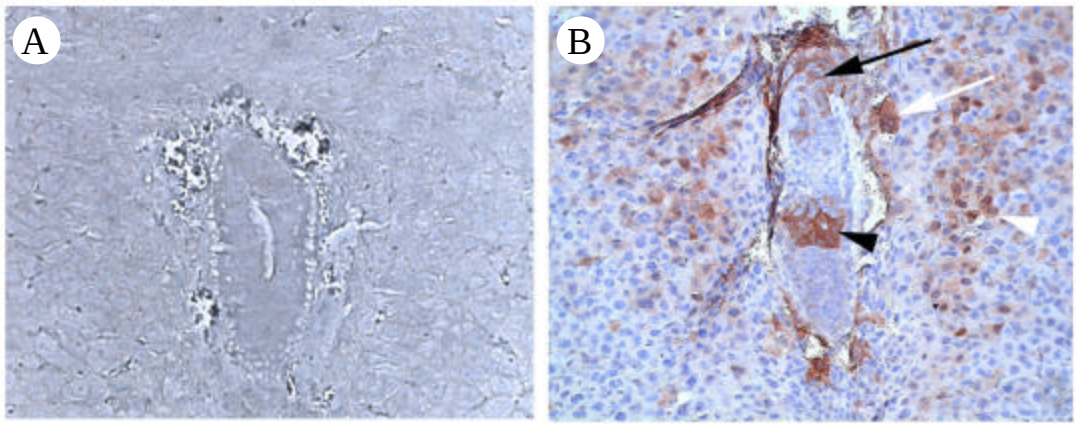
Supporting online material

Figure S1



Supporting online material

Figure S2



Supporting online material

Figure S3