ONLINE METHODS

Subjects. Experiments on humans were approved by Institutional Review Boards at all participating universities. Written informed consent was obtained from all subjects. Our criteria for classic DWM include (i) CVH affecting the posterior more severely than the anterior vermis, (ii) upward rotation of the vermis producing cystic enlargement of the fourth ventricle and (iii) enlarged posterior fossa. Criteria for mild DWM are the same, but the vermis is only mildly rotated so that the inferior roof of the fourth ventricle is parallel to the brainstem (rather than being rotated even further up) and the posterior fossa is only mildly enlarged. Our criteria for mega-cisterna magna as a true malformation include (i) CVH affecting the posterior more severely than the anterior vermis, (ii) normal position of the vermis with no rotation and (iii) enlarged posterior fossa. Isolated CVH as expected consists of vermis hypoplasia affecting the posterior more severely than the anterior vermis with normal vermis position and posterior fossa size. Thus, the malformations in order of severity are: DWM > mild DWM > MCM > isolated CVH. We recognized two forms of meningeal deficiency in these individuals. The first consists of inappropriate interdigitation and contact of gyri from the mesial left and right cerebral hemispheres, indicating deficiency of the falx cerebri. The second consists of medial and anterior displacement of mesial parietal and occipital gyri into the upper posterior fossa, between the splenium of the corpus callosum and the top of the vermis.

Detection of 6p deletion. Array comparative genomic hybridization (aCGH) using a chromosome 6 oligonucleotide array on DNA from LR08-198 was performed at Roche NimbleGen as previously described[37].

Candidate gene expression analysis. *In situ* hybridization (ISH) was performed on wild-type CD-1 E10.5 and E12.5 whole embryos and E17.5 cerebella as previously described[38] using mouse *Dusp22* (ref. 39), *Irf4* (ref. 40), *Exoc2* (IMAGE 5702581), *Hus1b*[41], *Foxq1* (IMAGE 6489152), *Foxf2* (ref. 42), *Foxc1* (ref. 43) and *Gmds* probes. Brains from wild-type and mutant littermates of adult mice or E18.5 embryos were obtained for *Irf4* (ref. 44; H. Sing), *Hus1b* (H. Hang, unpublished data), *Foxq1* (ref. 33; R. Arkell), *Foxf2* (ref. 42; N. Miura) and *Foxc1* (ref. 10; T. Kume). Histology was performed as previously described[45].

***Gmds* probe.** IMAGE clones 4165509 and 6329829 gave unreliable sequence confirmation for *Gmds*. Total RNA was isolated from hindbrain of an E12.5 wild-type Black Swiss embryo using the RNeasy Mini Kit (Qiagen). cDNA was synthesized using the First Strand cDNA Synthesis Kit (Invitrogen). Amplification of *Gmds* cDNA (AK086078) was performed according to the Invitrogen protocol using primers (**Supplementary Table 3**) designed using Primer3 (http://frodo.wi.mit.edu/primer3/input.htm). A *Gmds* cDNA plasmid was generated using the TOPO TA Cloning Kit (Invitrogen) according to the manufacturer's instructions. Six clones were sequence verified. Plasmid was linearized and transcribed to generate digoxigenin RNA–labeled (Roche) sense and antisense probes using KpnI (NEB) and T7 (Promega) and NotI (NEB) and Sp6 (Promega), respectively.

Mouse husbandry and genotyping. *Foxc1*$^{-}$ (*Mf1*^{LacZ}) mice[10] obtained from T. Kume were maintained by crossing to Black Swiss mice (Taconic). *Foxc1*$^{-}$ genotyping was carried out as previously described[12]. *Foxc1*^{hith} mice[15] obtained from S. Pleasure were initially crossed to Black Swiss mice (Taconic). Primer3 was used to design PCR primers (**Supplementary Table 3**) to amplify the *Foxc1*^{hith} locus. PCR reactions were performed in 50-μl volumes that contained 1 μg tail DNA; 0.75 U AmpliTaq Gold DNA polymerase (Applied Biosystems (ABI)); 1× PCR buffer, minus Mg; 1.5 mM $MgCl_2$; 5% betaine (Sigma); 250 μM primers; and 100 μM dNTPs. Amplification was performed using the following PCR conditions (96 °C for 10 min, 35 cycles of 96 °C for 30 s, 59 °C for 2 min, 72 °C for 2 min, followed by a final extension at 72 °C for 5 min). PCR products were digested using BsrI (NEB) and separated on a 2% agarose gel stained with ethidium bromide to distinguish *Foxc1*$^{+}$ (255, 142 and 87 bp) and *Foxc1*^{hith} (397 and 87 bp) alleles. All experiments were carried out on F_2 mice.

***Foxc1* mutant mice tissue analysis.** X-Gal staining and histology were performed as previously described[10]. *In situ* hybridization was performed as previously described[46] using mouse *Gdf7* (T. Jessell), *Ttr* (W. Duan), *Atoh1* (J. Johnson), *Bmp2, Bmp4* (B. Hogan), *Cxcl12* (M.E. Hatten), *Cxcr4* (R. Miller), *Fgf15* (E. Grove) and *Tgfb1* (IMAGE 3586216) probes. Immunohistochemistry was performed as previously described[38], using the following primary antibodies: anti-Atoh1, anti-Lhx1/5 (DSHB, The University of Iowa, Department of Biological Sciences), anti-Lmx1a (M. German, unpublished data), anti-Lhx2/9[47], anti-Ptf1a (H. Edlund, unpublished data), anti-Zic1 (Rockland Immunochemicals), anti-laminin (Sigma) and anti-Tbr1 (ref. 48) together with species-appropriate secondary antibodies (Jackson Immunological). Brains from embryos were collected and fixed overnight in 4% paraformaldehyde, cryoprotected in 30% sucrose, frozen in Tissue-Tek OCT Compound (Sakura Finetek) and cryosectioned in 20-μm increments. Before anti-Tbr2 or anti-Pax6 application, sections were steamed in 1× Target Retrieval Solution (Dako) for 10 min and then cooled for 30 min at room temperature. Sections were blocked in 5% goat serum, 2% BSA and 0.1% Triton for 60 min at room temperature then incubated with anti-Tbr2 (R. Hevner) or anti-Pax6 primary antibody together with species-appropriate Alexa Fluor secondary antibody (Invitrogen). Sections were counterstained with the nuclear stain DAPI.

Mouse quantitative real-time RT-PCR. Total RNA was isolated from head or hindbrain region of E12.5 embryos using the RNeasy Mini Kit (Qiagen). cDNA was synthesized using the First Strand cDNA Synthesis Kit (Invitrogen). *Foxc1* and *Cxcl12* primers for quantitative real-time RT-PCR (qRT-PCR) were obtained from a previous report[23]. All other qRT-PCR primers (**Supplementary Table 3**) were designed using Mouse qPrimerDepot (http://mouseprimerdepot.nci.nih.gov/), which designs intron-spanning primer pairs whenever possible. qRT-PCR was carried out using the Power SYBR Green PCR Master Mix (ABI) in 20-μl volumes and amplified using standard conditions for absolute quantification on an ABI Prism 7900HT Fast Real-Time PCR machine and SDSv2.2.1 software (ABI). Relative transcript levels were normalized to *Gapdh* using the following equation: $2^{(\mathrm{target_{WT}}-\mathrm{target_{MUT}})}/2^{(\mathrm{Gapdh_{WT}}-\mathrm{Gapdh_{MUT}})}$, where 'target' and 'Gapdh' are the average cycle thresholds of 3–6 replicates for target and *Gapdh* transcripts, respectively, in wild-type (WT) and *Foxc1*$^{-/-}$ (MUT) embryos.

Human RT-PCR. Total RNA was isolated from human lymphoblastoid cell lines for two controls using a standard Trizol (Invitrogen) RNA extraction protocol. cDNA was synthesized using the First Strand cDNA Synthesis Kit (Invitrogen) and targets were amplified according to the manufacturer's instructions. Primers (**Supplementary Table 3**) used for both target amplification and qRT-PCR were designed using qPrimerDepot (http://primerdepot.nci.nih.gov/), which designs intron-spanning primer pairs whenever possible. qRT-PCR was carried out using the Power SYBR Green PCR Master Mix (ABI) in 10-μl volumes and amplified using standard conditions for absolute quantification on an ABI Prism 7900HT Fast Real-Time PCR machine and SDSv2.2.1 software (ABI). Relative transcript levels were normalized to *GAPDH* using the average cycle thresholds of four replicates for target and *GAPDH* transcripts.

Statistical analysis. One-tailed Student's *t*-test for two samples with unequal variance was used as implemented in Excel to assess the significance of the roof plate size, *Foxc1*-target transcript level and adult cerebellar size differences observed between wild-type and mutant littermates.

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