Figure S1. Dye injection into the lateral ventricles of wild-type and Gldc-deficient mice. Gldc<sup>+/+</sup> and Gldc<sup>GT1/GT1</sup> mice were injected with 0.4% trypan blue at post-natal day 1. The injection sites are visible in each pup (after removal of skin). After removal of the skull the distribution of dye in the brain is visible (paired images A-B, C-D, E-F, G-H, I-J, K-L are corresponding samples). Dye distribution encompassed the posterior region, including the fourth ventricle, in Gldc<sup>+/+</sup> mice but not in Gldc-deficient pups with ventriculomegaly. Scale bars represent 5 mm.

The Gldc<sup>+/+</sup> panel in Figure 1C is reshown in Figure S1H. The Gldc<sup>GT1/GT1</sup> panel in Figure 1S is reshown in Figure S1L.
Figure S2. Ependymal cell lining is intact in the lateral ventricles and aqueduct of wild-type and \textit{Gldc}-deficient foetuses at E18.5. Representative images of haematoxylin and eosin stained sections of \textit{Gldc}^{+/+} (A-E'), unaffected \textit{Gldc}^{GT1/GT1} (F-J) and \textit{Gldc}^{GT1/GT1} fetuses with ventriculomegaly (K-T). Where visible, the lining of the aqueduct (B, G and enlarged in C, H) was intact, although the aqueduct was occluded or very small in \textit{Gldc} mutants with ventriculomegaly (L-M, Q-R). The appearance of the lining of the lateral ventricles did not differ between genotypes (D, I, N, S and enlarged in E, J, O, T) irrespective of ventriculomegaly. Scale represents 1 mm in A, F, K, P and 100 µm in all other panels.
Figure S3. Ependymal cell layer is intact in the in third ventricle of a Gluc GT1/GT1 mutant with ventriculomegaly and aqueduct stenosis at E18.5. In contrast to other Gluc GT1/GT1 mutants (4 of 5), sections through the third ventricle (III) of 1 mutant showed that the ependymal cell layer was intact, with possible exception of a small region at the dorsal aspect (arrow in C). Sections of the lateral ventricle and aqueduct of the same fetus are shown in Supplementary Fig. 2G-I. Scale bar represents 1 mm in A and 100 µm in B-C.
Figure S4. Phenotypes among Glcd-deficient fetuses at E18.5 (untreated or exposed to maternal formate supplementation). Typical appearance of coronal sections through the lateral ventricles around the level of the pineal gland (pi). Sections are in anterior to posterior sequence within samples (e.g. A-C) and matched for axial level in columns (e.g. A, D, G, I, K, N, Q). The typical appearance of unaffected Glcd<sup>GT1/GT1</sup> fetuses (G-M) does not differ from wild-types (A-F), whereas affected foetuses show enlarged lateral ventricles and absent gland (Q-S). An intermediate phenotype, comprising moderately enlarged ventricles and absent or malformed pineal gland (N-P) was observed among a subset of Glcd<sup>GT2/GT</sup> foetuses. The Glcd<sup>+/+</sup> panel in Figure 1C is reshow in Figure S4B.