Supplementary data figure legends

SI 1: Transgenic expression of Paerg corrects the hydrocephalus in qk\(^v\) mutant mice

Coronal brain sections from 3 week old mice were stained with H&E and the cross sectional area of the LV was measured at Bregma 0.14mm. In comparison to wt, qk\(^v\) mutant mice demonstrated a 2.7-fold increase in LV area, wt: 100±13.5\% Vs qk\(^v\): 268 ±14.7\%, mean±SEM, p=7.1x10\(^{-8}\), n≥5 per genotype. Transgenic expression of Paerg restored the LV area to wt levels, wt: 100±13.5\% Vs qk\(^v\)–Tg: 102±11.3\%, Mean±SEM, n≥4 per genotype, *** p<0.001.

SI 2: Ventricular expansion in qk\(^v\) mice

Histological examination showed evidence of expansion of the third ventricle (Bregma -1.15mm) in qk\(^v\) (C) compared to wildtype (A). Compression of the medial habenular nucleus is indicated by the * in panel C. There was no evidence of alteration to the fourth ventricle (Bregma -6.06mm) in qk\(^v\) (D) compared to wildtype (B). Quantitation of the ventricles using Scion Image software demonstrated a two-fold increase in the qk\(^v\) mutant 3V (wt: 100±5.5\% Vs qk\(^v\): 201±30\%, mean±SEM, p=0.04, n=3 per genotype) but no significant difference in the 4V (wt: 100±12.9\% Vs qk\(^v\): 94.3±7.4\%, mean±SEM, p=0.72, n=3 per genotype). * p<0.05.

SI 3: Hydrocephalus in qk\(^v\) is acquired after birth

Coronal brain sections of 1 day old mice were stained with H&E. Neuroanatomical assessment of ventricular volume in wildtype (A) and qk\(^v\) mutant mice (B) revealed no evidence of hydrocephalus. This was confirmed by quantitation of ventricular volume.
using Scion image software. No significant difference between wildtype and $qk^v$ mice was observed ($C$). Error bars represent standard deviation. Calibration bar = 1mm.

**SI 4: $Qk^v$ is affected by communicating hydrocephalus.**

Neuroanatomical analysis of H&E stained brain coronal sections at bregma points -3.08, -3.28, -3.68, -4.08 and -4.65mm respectively, showed a patent aqueduct of Sylvius in both wildtype ($A$-$E$) and $qk^v$ mice ($F$-$J$). $n = 3$. Scale bar = 20µm.

**SI 5: $Qk^v$ is affected by communicating hydrocephalus.**

Ink tracer was injected into the left LV of mice and ten minutes later the brain was sectioned at approximately bregma -4.84mm and -7mm to visualise the aqueduct and 4V, respectively. Ink tracer was visible in the aqueduct and 4V of both the wildtype ($A,C$) and $qk^v$ mutant ($B,D$) mice. $n = 3$.

**SI 6: Fertility is restored in $qk^v$–Tg mice**

($A$) Robust expression of the *Pacrg* transgene was observed in the testis of four independent $qk^v$–Tg founders (1-4). An unrelated primer set for *RanBPM* was used to confirm the integrity of the cDNA template. ($B$) The transgene was translated into protein; a representative image shows Pacrg (red) was readily detected in testis extracts of wt and $qk^v$–Tg mice but not in the $qk^v$ mutant (lanes5-6). ($C$) H&E staining was used to visualise the morphology of the testis; wt and $qk^v$–Tg testis showed spermatids with flagella extending into the tubule lumen, which were absent in the $qk^v$ testis. Consistent with testis transgene expression, all four $qk^v$–Tg lines
showed a recovery in daily sperm production \( (D) \) and ability to sire litters \( (E) \). Mean±SEM, \( n=16 \) per genotype, *** \( p<0.001 \).

**SI 7: Normal structure and function of respiratory cilia in the \( qk^v \) mutant mouse**

Pacrg is localised to the axoneme of the respiratory cilia. Intense Pacrg \( (A) \) and acetylated \( \alpha \)-tubulin \( (B) \) immunoreactivity co-localised to the cilia tufts in isolated tracheal epithelial cells derived from wt mice \( (C) \). Cell nuclei were stained with Dapi (blue). Scale bar = 10\( \mu \)m. Decalcified nasal cavities were sectioned in the coronal plane and PAS stained to investigate mucociliary clearance. Nasal cavities \( (NC) \) of both wt \( (D) \) and \( qk^v \) mutant \( (E) \) mice showed no evidence of mucus accumulation. Scale bar = 20 \( \mu \)m, \( n=3 \) per genotype. Tracheal sections stained with the axonemal marker, acetylated \( \alpha \)-tubulin were used for unbiased stereological analysis to determine cilial length and cilial density relative to epithelial surface. No significant difference was observed in cilial length \( (F) \) or density \( (G) \) in \( qk^v \) mutant compared to wt mice. Trachea samples were assessed for CBF using high-speed videomicroscopy. No significant difference in CBF was observed between wt and \( qk^v \) mice \( (H) \). Mean±SEM, \( n\geq3 \) per genotype.

**SI 8: Transgenic expression of Pacrg rescues ependymal cilial mediated flow**

Latex polystyrene beads were added to coronal brain sections of the lateral ventricle (Bregma 0.2-1.2) and the time taken for beads to travel 5\( \mu \)m recorded. Movement of the beads (arrows) in wt, \( qk^v \), \( qk^v \)-Tg mice is shown in the boxed region \( (5\mu m) \) at starting position \( (T1, 0.00 \text{ seconds}) \), halfway point \( (T2, 0.11 \text{ seconds}) \) and the end point \( (T3, 0.22 \text{ seconds}) \). A rapid cilial mediated flow was observed in wt mice, with the bead travelling the 5\( \mu \)m distance of the boxed region by T3. In contrast, the cilial
mediated flow in $qk''$ mutant mice was significantly reduced, with the bead travelling approximately 1/3 of the boxed region. A restoration in ciliary mediated flow was observed in $qk''$-Tg mice, where the bead travelled significantly further by T3 than in the $qk''$ mutant mice.

**SI Movies:**

Coronal sections of the lateral ventricle (Bregma 0.2-1.2) were assessed for CBF using high-speed videomicroscopy. Wildtype mice showed a rapid synchronous ciliary beat (movie A). In contrast, a significant reduction in CBF was observed $qk''$ (movie B) which was restored to wildtype levels in the $qk''$-Tg (movie C).