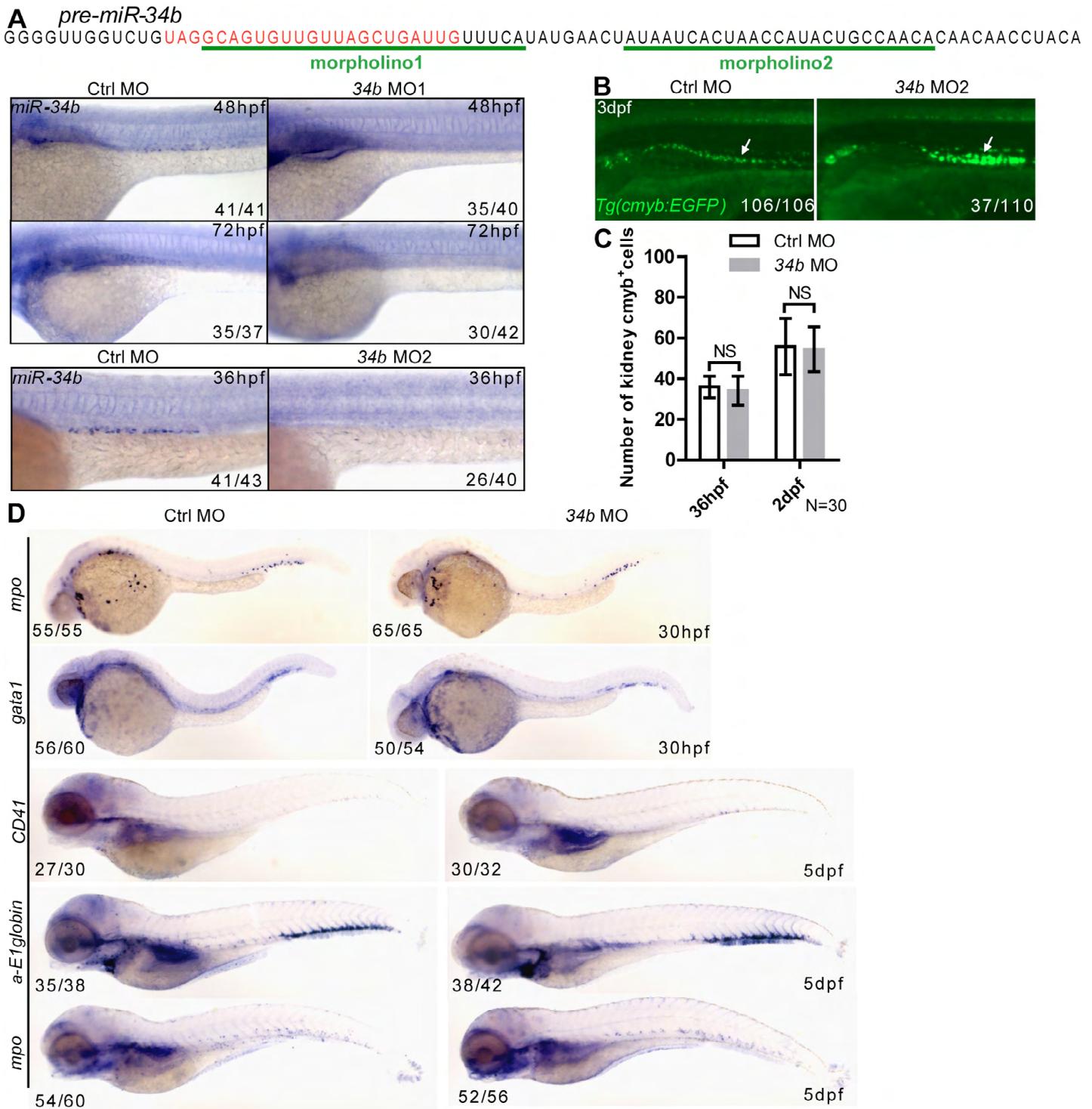
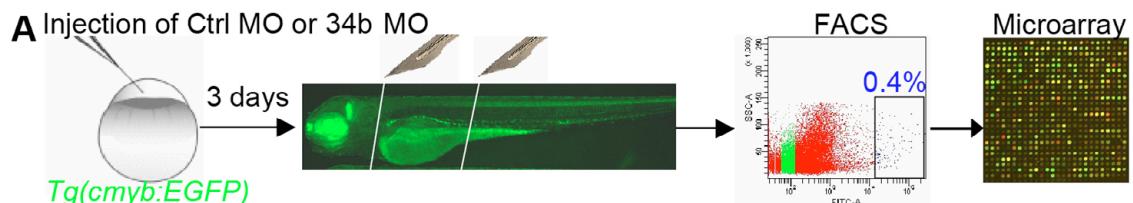


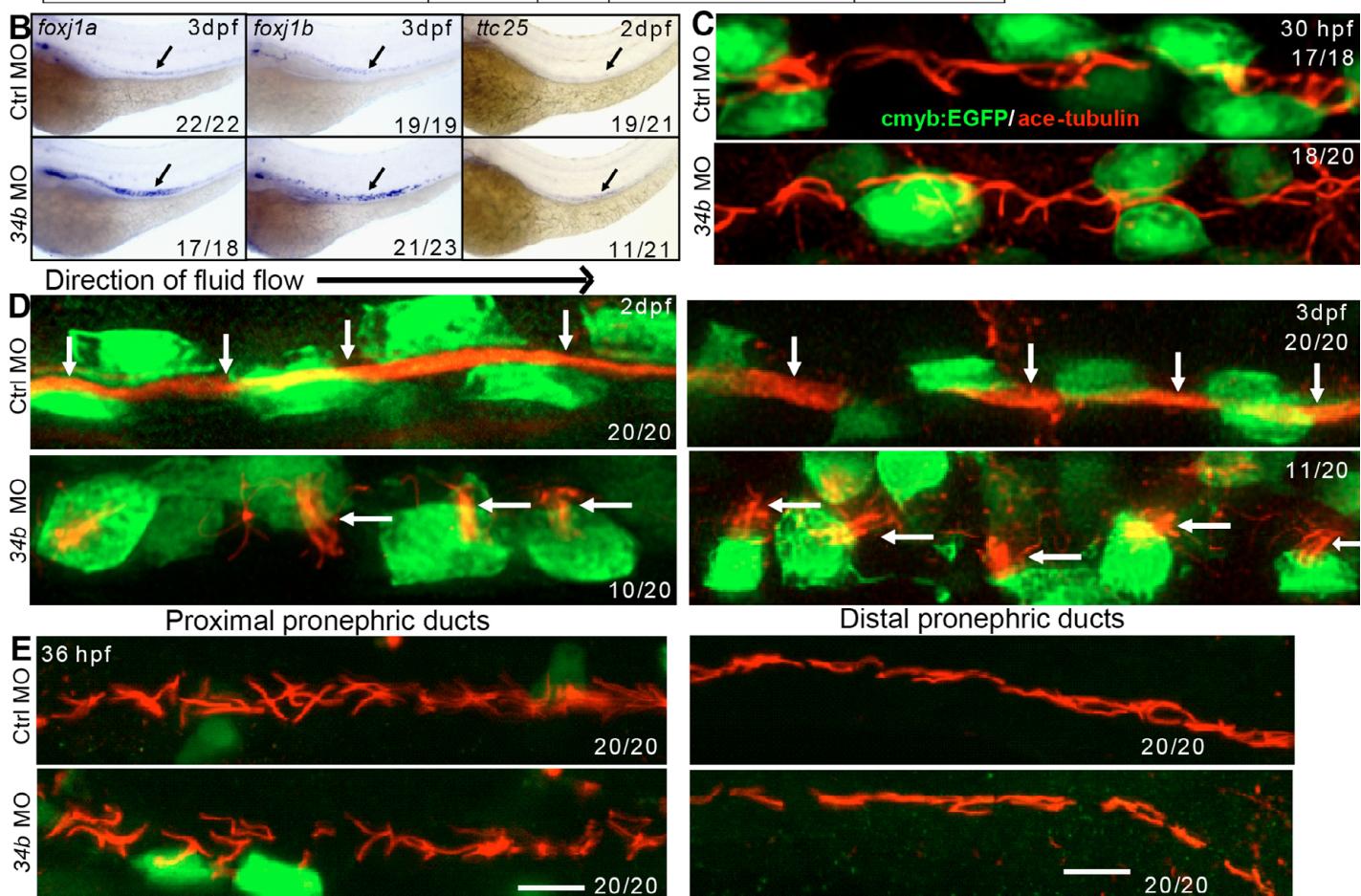
**Fig. S1. Expression pattern of miR-34b (WISH) during development.** (A-F) Expression of miR-34b is first observed at 24 hpf (C) at the kidney region and persists until at least 5 dpf (F). Magnified lateral view with a small angle to dorsal shows that the labeled cells reside in both pronephric ducts (C,D, insets). Magnified dorsal view (E,F) of the kidney region is shown in the inset. Olfactory placode is indicated by arrows.



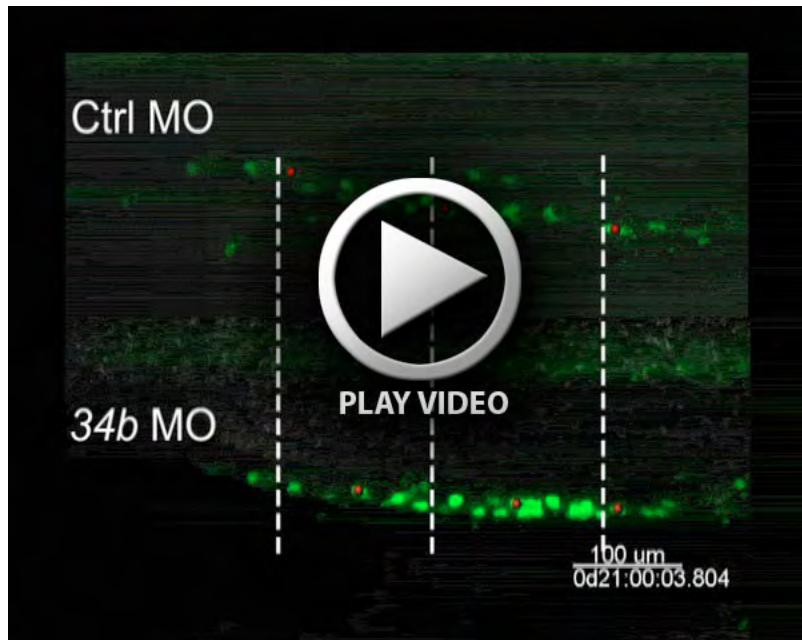
**Fig. S2. Successful knockdown of miR-34b by morpholinos.** (A) Two morpholinos were designed and shown to block the biogenesis of mature miR-34b (WISH). (B) Image of *Tg(cmyb:EGFP)* embryo shows that MCCs accumulate in the middle part of pronephric duct at 3 dpf after miR-34b MO2 injection. (C) Manual count of MCCs labeled by *Tg(cmyb:EGFP)* at 36 and 48 hpf.  $n=30$  for Ctrl MO and miR-34b MO (mean  $\pm$  s.d.). (D) Representative results show hematopoiesis after miR-34b knockdown.



| Go Term                      | Count | %   | Fold Enrichment | P-value  |
|------------------------------|-------|-----|-----------------|----------|
| cilium assembly              | 8     | 2.1 | 19.8            | 7.9E-08  |
| microtubule-based process    | 13    | 3.4 | 7.6             | 10.0E-08 |
| cell projection assembly     | 9     | 2.3 | 14.0            | 1.6E-07  |
| cilium morphogenesis         | 8     | 2.1 | 167             | 2.9E-07  |
| cell projection organization | 11    | 2.8 | 5.2             | 4.3E-05  |



**Fig. S3. Analysis of MCC gene expression profile and multiciliogenesis.** (A) Work flow of microarray analysis. (B) Expression analysis of upregulated ciliary genes in the microarray data by WISH. (C,D) The ciliary morphology (anti-acetylated tubulin staining, red, arrows) of MCCs (anti-GFP staining, green) at 30 hpf (C) 2 dpf and 3 dpf (D) in normal embryos and miR-34b morphants. (E) The ciliary morphology (red) at proximal and distal pronephric ducts of 36-hpf normal embryos and miR-34b morphants.



**Movie 1. The migration of MCCs labeled by *Tg(cmyb:EGFP*) from 51 to 72 hpf.** Three representative MCCs were labeled (red spots) in embryos injected with Ctrl MO (up) or miR-34b MO (down) using image analysis software; each embryo was captured independently and combined (see Materials and methods).

**Table S1. Morpholino and duplex sequences**

| Name          | Sequence                    | Dose                     | Reference              |
|---------------|-----------------------------|--------------------------|------------------------|
| 34b MO1       | TGAAACAATCAGCTAACACACTGC    | 1 mM                     | This paper             |
| 34b MO2       | TGTTGGCAGTATGGTTAGTGATTAT   | 0.25 mM                  | This paper             |
| Ctrl MO       | CCTCTTACCTCAGTTACAATTATA    | 1 mM                     | This paper             |
| rfx2 MO       | GGGTGTAGTCTGACCTGGTAC       | 0.25 mM                  | Liu et al., 2007       |
| rfx2 MO       | GGGTGTAGTCTGACCTGGTAC       | 0.0625/0.125 mM (rescue) | Liu et al., 2007       |
| hoxb8a MO     | AGCTCATTTTACTGCTGTTGGTG     | 0.5 mM (knockdown)       | This paper             |
| hoxb8a MO     | AGCTCATTTTACTGCTGTTGGTG     | 0.375 mM (rescue)        | This paper             |
| cmyb MO       | TCGCCATCCCGCTGTCGAGAGGAA    | 0.125 mM (knockdown)     | Soza-Ried et al., 2010 |
| cmyb MO       | TCGCCATCCCGCTGTCGAGAGGAA    | 0.075/0.125 mM (rescue)  | Soza-Ried et al., 2010 |
| 34b duplex F  | UAGGCAGUGUUGUUAGCUGAUUGdTdT | 10 µM                    | This paper             |
| 34b duplex R  | CAAUCAGCUAACACACUGCCAAdTdT  | 10 µM                    | This paper             |
| Ctrl duplex F | CCAGGGAUUUCAGUCCAUGUAdTdT   | 10 µM                    | This paper             |
| Ctrl duplex R | UACAUCGACUGAAAUCCCUCGdTdT   | 10 µM                    | This paper             |

**Table S2. QRT-PCR primer sequences**

| Gene             | Primer sequence (forward/reverse)            |
|------------------|--|
| <i>rfx2</i>      | CCGGAGATCATCAGCACTAAGG/GGTCGCTGAGCATCTGATTGA |
| <i>foxj1a</i>    | TACTTCCGCCACGCAGAT/GGGTCGATTTCCAGAAGC        |
| <i>foxj1b</i>    | CGTGAAGCCACCCTATTAT/GGATTGAGTTCTGCCAGCTC     |
| <i>ttc25</i>     | ACGCCGAGAGCTCACTAAA/AACCTAAAATGCCCATAGTGT    |
| <i>ift57</i>     | GATGCCAAGGGTTGTCTGTT/GCTCCTCACCTTGTGAGC      |
| <i>bbs2</i>      | GAACCCACTGGAAAAGTCACA/ACGTCTGGACTGTCGATGC    |
| <i>rsph9</i>     | TGATGAAGAACAGGAAGTC/CATCTGCAATCATGGACTCG     |
| <i>lrrc50</i>    | CGTATCCACATCGATGAACG/AGCAGCTGCACCTCTGAGAAC   |
| <i>cmyb</i>      | TGATGCTTCCAACACAGAG/TTCAGAGGAATCGTCTGCT      |
| <i>β-actin 1</i> | CGAGCAGGAGATGGGAACC/CAACGGAAACGCTCATTGC      |

**Table S3.** Target identification by 2xMRE and UTR reporter assay

| Name         | Sequence                         | Response to miR-34b mimic |
|--------------|----------------------------------|---------------------------|
| cdk6 MRE     | UGUUCAGUUCCACACACUGCCAG          | No                        |
| taf5 MRE     | AUACAAGCUUUUAUACUACUGCCUA        | Yes                       |
| strap MRE    | UAAUGCUALAUCCUCCAGCUGCCUG        | No                        |
| mycb MRE     | CAAUUCACACUGCCUC                 | Yes                       |
| skap2 MRE    | UAAAUCGUAAAGCAAACUGCCUG          | Yes                       |
| znf395 MRE   | CCUUUUAUUCUAAAACAAACUGCCUA       | Yes                       |
| mycl1b MRE   | ACUUUAUGCUGUUUUCACUGCCUA         | Yes                       |
| hells MRE    | UGAUCAGUUUUGGCAAAGUCCACUGCCUG    | No                        |
| rarab MRE    | CAGUCAACAACAACACUGCCU            | Yes                       |
| taxbp3 MRE   | UAAAUAUUAACACAUUUGCCU            | No                        |
| Ikbkg MRE    | AAAAAAGCUCAUCUGUACUGCCUU         | Yes                       |
| tdrd7 MRE    | UGAACAUCAUCCCUUACUGUCU           | No                        |
| gab1 MRE     | AGAUCAGCUAGUUUACACUGCUUC         | No                        |
| thbs4b MRE   | CAAAUAUCAGCUUAACUGCCUA           | Yes                       |
| ptprg MRE    | UAUGAAAAAUACAGGUUACUGCCUA        | Yes                       |
| mta3 MRE     | CCAUUCCAAUAAAAGACUGCCUG          | No                        |
| hoxb8a MRE   | UUCCAACAACAUGAAACUGCCUA          | Yes                       |
| hoxa9a MRE   | UAGUCACUUCACUGCCUU               | No                        |
| creb1 MRE    | CAGUCGCUGAUGGGCUCUGCCUG          | No                        |
| gadd45ba MRE | ACAUGAGCUGUGAAAGCUGCCUG          | Yes                       |
| cmyb MRE1    | UAACUUUUUGUUAUUCGCUGCCUU         | No                        |
| cmyb MRE2    | CAUACGUUUUUUAUACAAACUGCCUU       | No                        |
| ttc25 MRE    | GUCUUUAGUUUAGGUUACUUUAUGACUGCCUU | Yes                       |
| mpp7 MRE     | UAUUUUUUUAUUAUCGGCUGCCUG         | No                        |
| jag2a MRE    | UCAUCUGUUCUCCUUCCGCGCCUGCCUU     | No                        |
| mycb UTR     | NM200172.1 1364-1645             | No                        |
| creb1a UTR   | NM200909.1 1075-1897             | No                        |
| cmyb UTR     | BC059803 1978-3131               | No                        |
| ptprg UTR    | Based on EST assembly            | No                        |
| foxj1a UTR   | NM001076706.2 1625-3442          | No                        |
| foxj1b UTR   | NM001008648.1 1549-2212          | No                        |
| ttc25 UTR    | NM200316.1 1561-1771             | No                        |
| jag2a UTR    | NM131862 3941-5269               | No                        |
| hoxb8a UTR   | NM131120.1 1116-1963             | Yes                       |
| mpp7 UTR     | NM130976.1 1786-4032             | No                        |
| rfx2 UTR     | NM001013278.1 2364-3607          | No                        |

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