```
WT      10857  CGGATCAGGCCTCTGCGCCTTCCCGGGGAGCCCCGCAAGGCCGCAGACAAGATGAACATT  10916
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mutant  206    CGGATCAGGCCTCTGCGCCTTCCCGGGGAGCCCCGCAAGGCCGCAGACAAGATGAACATT  147

                                  Exon 2                    |      Intron
WT      10917  CTGGCGCCCGTGCGGAGGGACCGCGTCCTGGCGGAGCTGCCCCAGGTAGGCACCAGGGCC  10976
               |||||                                       |||
Mutant  146    CTGGC-----------------------------------------|----------GCC  139

WT      10977  ACGTCGGGCCTCTGGCTGTCGCTGAGTGTGGGCGGGGAGAAGGTGGCCCAGCCCCGTCGC  11036
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mutant  138    ACGTCGGGCCTCTGGCTGTCGCTGAGTGTGGGCGGGGAGAAGGTGGCCCAGCCCCGTCGC  79

WT      11037  CCTGCAAGGCCCGCTGCTCCTGGGTGGTCCGCTTCCCACCCCACCCCCAGCCCAGGGCTG  11096
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Mutant  78     CCTGCAAGGCCCGCTGCTCCTGGGTGGTCCGCTTCCCACCCCACCCCCAGCCCAGGGCTG  19

WT      11097  CT  11098
               ||
Mutant  18     CT  17
```

Figure S1. A Rab34 CRISPR mutant allele.

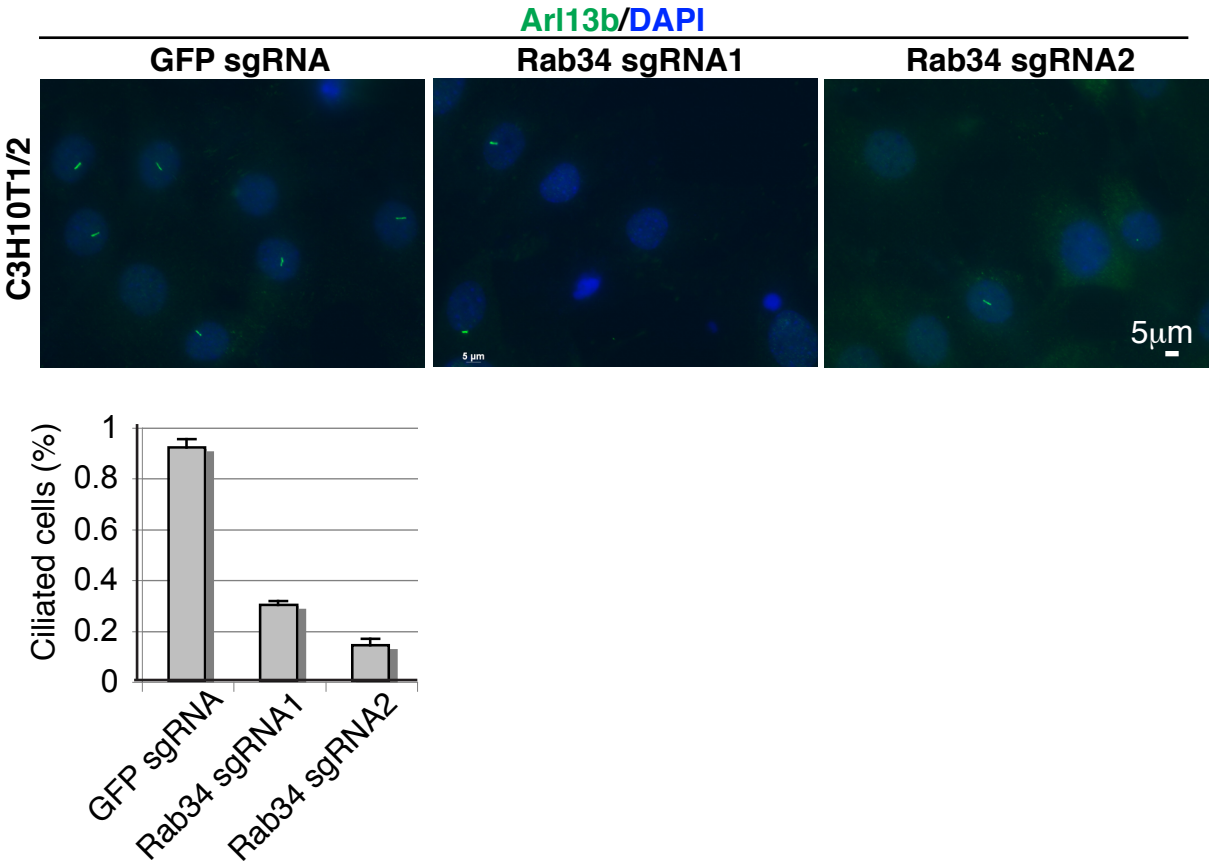

Figure S2. Loss of Rab34 in cultured cells results in a significant decrease in ciliogenesis. C3H10T1/2 cells were stably infected with Lentivirus that expressed sgRNAs as indicated. The cells were then immunostained for Arl13b, a ciliary marker, and counterstained for nuclei (DAPI). Two-tailed Student t-test P values ≤ 0.0001 (n = 3 independent experiments, ≥100 cells counted for each category).

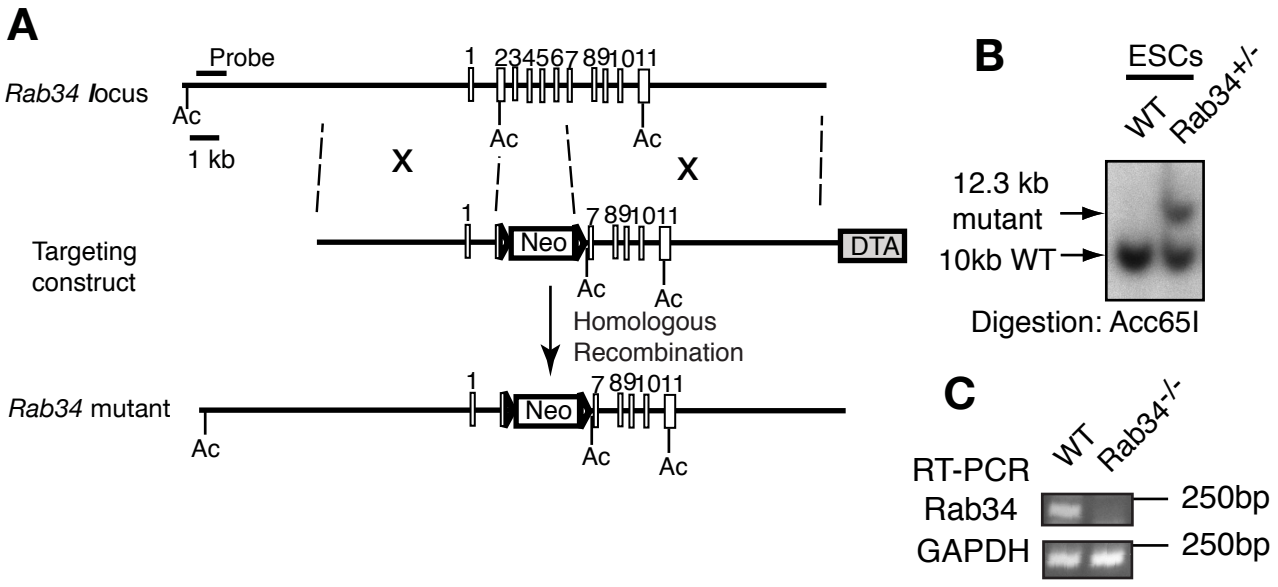

Figure S3. (A) The gene targeting strategy used to create a mouse Rab34 mutant allele. Open rectangles are referred to as exons and lines as introns. The probe used for Southern blot is shown. Triangle, loxP site; Neo, neomycin; DTA, diphtheria toxin A; number, exons; Ac, Acc65I. (B) Southern blot of representative mutant and wild type (wt) ES cell clones (n = 1 experiment). (C) RT-PCR shows that Rab34 transcript is undetectable in the mutant. GAPDH is a control.

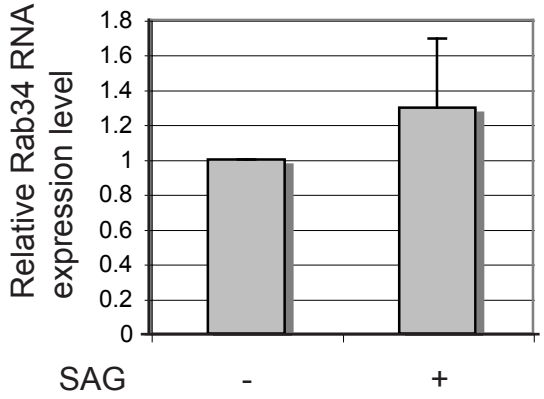

Figure S4. Activation of Hedgehog signaling does not significantly increase Rab34 RNA expression. RT-qPCR showing relative Rab34 RNA expression level in WT MEFs after stimulation with SAG. Two-tailed Student t-test p value is 0.176, not significant.

Table S1 Rab34 mutant E10.5 embryos

Phenotypes	Genotypes			
	+/+	+/-	-/-	Total
	11	24	10	45
Heart looping (left orientation)	11	24	10	45