**Supplemental Figure Legends**

**Figure S1.** Screening mouse embryonic stem cells for positive recombination at the *Rosa26* locus. **A.** Schematic illustrating PCR screening strategy for identifying mouse ES cell clones positive for homologous recombination. Primers A (5’-CGCCTAAAGAAGAGGCTGTG-3’) and B (5’-gtctcacagaacggctccac-3’) generate an amplicon of ~150 bp with the uncombined, WT *Rosa26* locus. Primers A and C (5’-gagatcctgccccggcactt-3’) generate an amplicon of ~2200 bp unique to the targeted (recombined) *Rosa26* locus. Primer A hybridizes to the *Rosa26* locus outside and exclusive of the 5’ targeting arm on the pROSA26PAm1 targeting vector. Primer C hybridizes to the PKG-Neo resistance marker. Therefore, a ~2200 bp amplicon will only be produced with successful targeting of the LSL-Kif2b construct specifically to the *Rosa26* locus and not elsewhere in the mouse genome. **B.** Results from PCR screening of several ES cell colonies electroporated with the linearized Rosa26-LSL-Kif2b-6xHis-IRES-eGFP targeting vector (see materials and methods). 13 out of 192 ES cell clones screened are shown.

**Figure S2.** Expression of transgenic human Kif2b in mice. **A.** Genotyping PCR using genomic DNA extracted from 3 F1 progeny resulting from crossing *R26LSL-Kif2b/Kif2b* mice to mice carrying the ubiquitously expressed CMV-Cre transgene. PCR reactions with primers 1 & 4 (left) reveal efficient excision of the 3X STOP cassette in all mice. See main text Figure 1 for hybridization site details on primers 1, 2, 3 & 4. Cre primers (right) produce an amplicon of 100 bp in the when transgenic Cre is present (CreF: 5’-GCGGTCTGGCAGTAAAAACTATC-3’, CreR: 5’-GTGAAACAGCATTGCTGTCACTT-3’). **B.** Immunoblots of homogenized lung and spleen tissue from control (*R26*+/+) mice and mice expressing transgenic human Kif2b (*R26*Kif2b/+) using an anti-Kif2b antibody. Anti-Tubulin (DM1α) antibody was used as a loading control. Each lane represents an individual mouse from the indicated genotype.

**Figure S3.** Characterization of *R26*Kif2b; *K-Ras*G12D MEFs. **A.** Genotyping PCR using genomic DNA from 11 individual embryos used to generate MEF lines expressing transgenic human Kif2b in combination with mutant G12D K-Ras. **B.**  Immunoblots of MEFs with the indicated genotypes using an anti-Kif2b antibody. Anti-Tubulin (DM1α) antibody was used as a loading control. Each lane represents an individual MEF line.