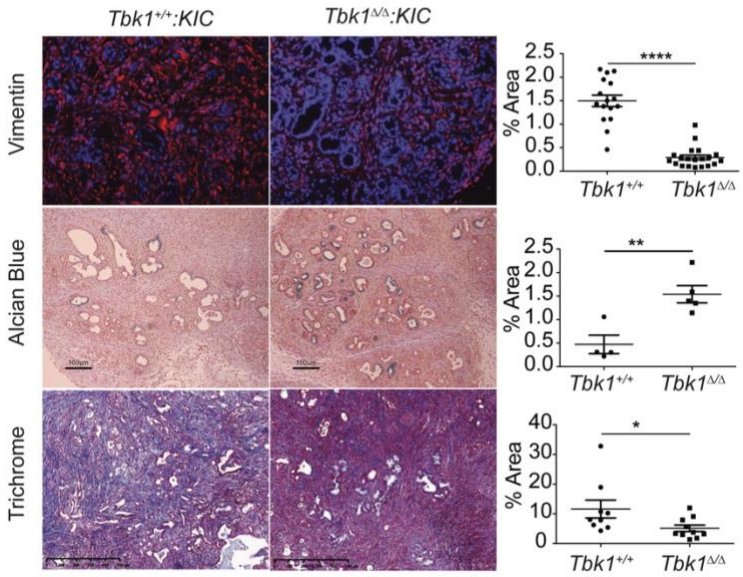
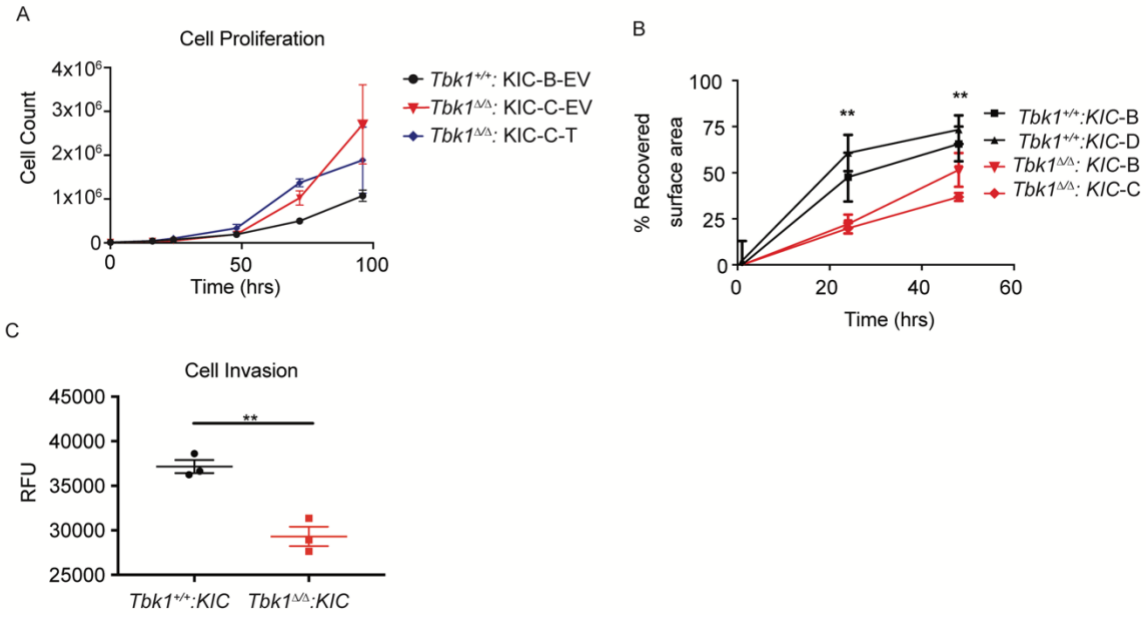
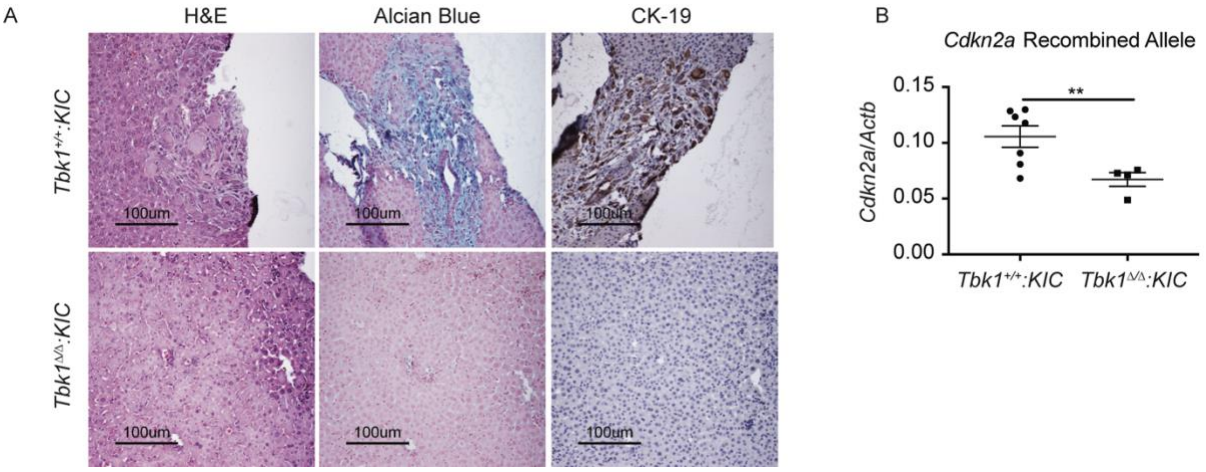
Cruz & Arner et al.,
126117-INS-RG-TR-2
Supplemental Figures



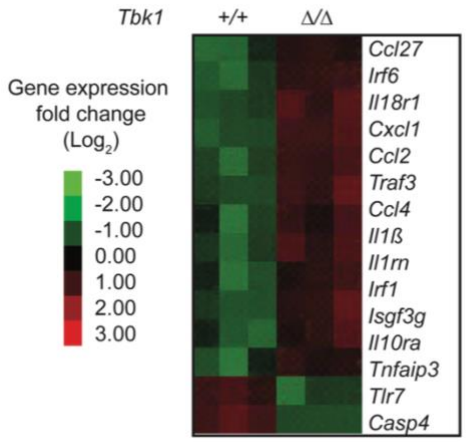
Supplemental Figure 1. Tumors in *Tbk1$^{\Delta/\Delta}$: KIC* mice display enhanced epithelial differentiation. Representative images of vimentin, alcian blue, and trichrome stained tumors from 8-week-old *Tbk1$^{+/+}$*: *KIC* and *Tbk1$^{\Delta/\Delta}$*: *KIC* mice. Scale bars indicate 100μm for alcian blue images and 500μm for trichrome images; n ≥ 4 mice/group, 4 images/mouse.



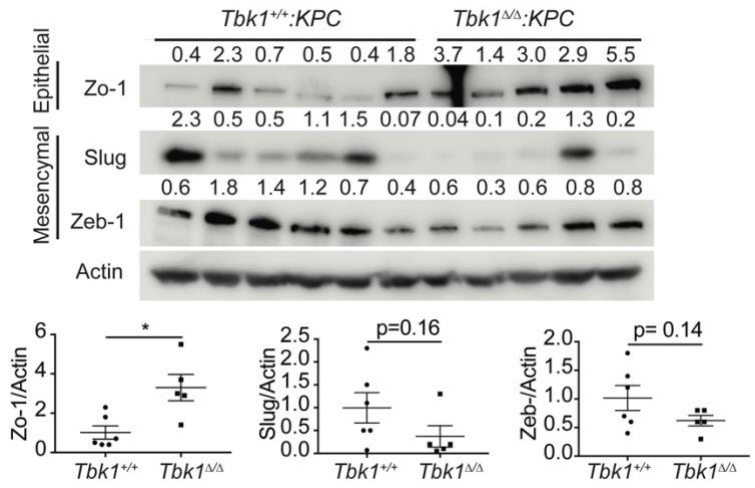
Supplemental Figure 2. *Tbk1*$^{\Delta/\Delta}$*: KIC* tumor cells are less migratory and invasive than *Tbk1*$^{+/+}$*: KIC* cells. A) Cell proliferation of *Tbk1: KIC* cell lines infected with EV or T. Cells were counted over 96 hours, n = 3 plates/cell line. The migratory capacity of cell lines derived from *Tbk1: KIC* tumors was investigated using scratch **(B)** and transwell migration **(C)** assays. **B)** Recovered surface area of *Tbk1*: *KIC* cell lines at 0, 24, and 48 hours post. **C)** Quantification (expressed as relative fluorescent units) of *Tbk1*: *KIC* cells after migration through ECM-coated transwell membranes 24 hours after plating in serum-free media. The lower chamber of the transwell were filled with 10% FBS containing media as the chemoattractant. Assays are representative of n> 3 repeats. Results are representative of mean +/- SEM. Results are representative of mean +/- SEM. Unpaired two-tailed *t* test, **p* < 0.05, ***p* < 0.01.



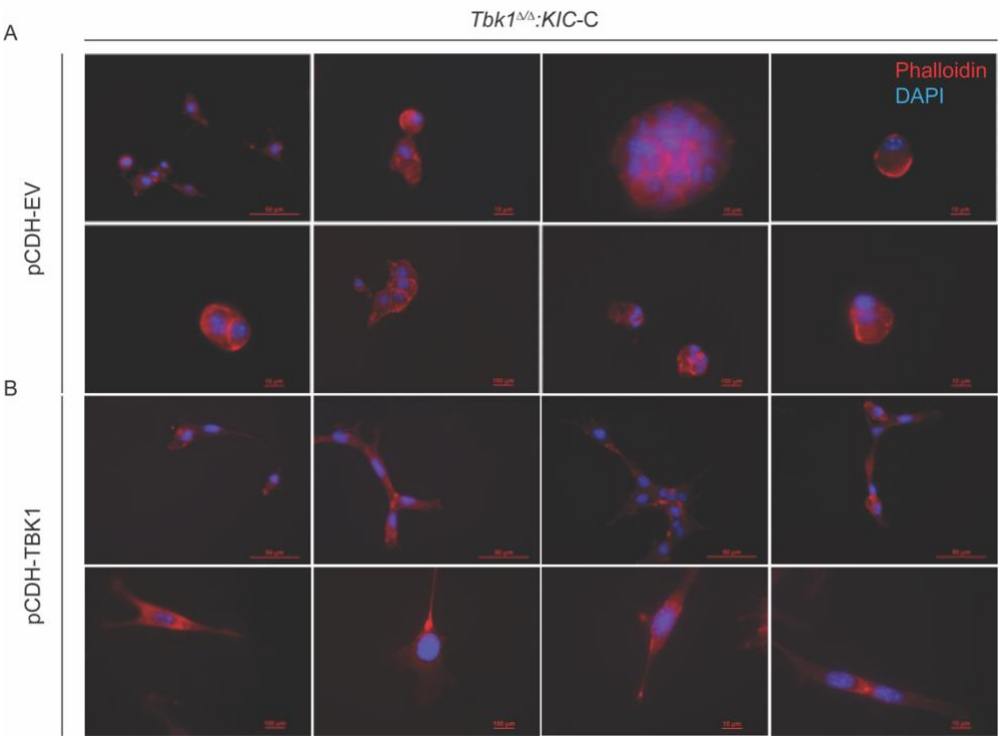
Supplemental Figure 3. *Tbk1$^{\Delta/\Delta}$: KIC* tumors are less metastatic than *Tbk1$^{+/+}$*: *KIC* tumors. A) Representative liver histology in mice from 8-week-old *Tbk1$^{+/+}$*: *KIC* and *Tbk1$^{\Delta/\Delta}$*: *KIC* mice, including H&E, alcian blue, and CK19 immunohistochemical staining. Scale bars indicate 100 μm. **B)** Metastasis to the liver was quantified by qPCR for recombined *Cdkn2a* allele (n = 4-7 mice/group). Results are representative of mean +/- SEM. Unpaired two-tailed *t* test, *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$.



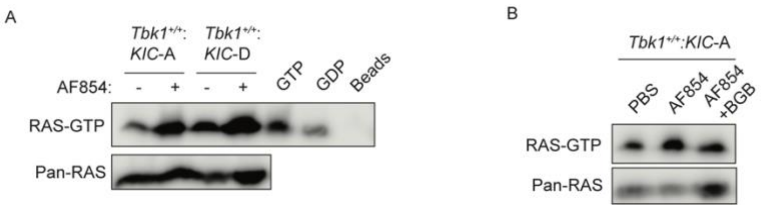
Supplemental Figure 4. *Tbk1$^{\Delta/\Delta}$: KIC* tumors contain higher pro-inflammatory gene expression than *Tbk1$^{+/+}$: KIC* tumors. Heatmap representing gene expression fold change (log_2) of inflammatory genes from *Tbk1$^{\Delta/\Delta}$*: *KIC* and *Tbk1$^{+/+}$*: *KIC* tumors. Color key indicates gene expression fold change; n = 3 tumors/genotype, $p < 0.05$ for all genes between *Tbk1$^{\Delta/\Delta}$*: *KIC* and *Tbk1$^{+/+}$*: *KIC* tumors.



Supplemental Figure 5. *Tbk1* promotes mesenchymal protein expression in KPC cell lines. Protein lysates isolated from *Tbk1*$^{+/+}$: *KPC* and *Tbk1*$^{\Delta/\Delta}$: *KPC* cell lines were immunoblotted for indicated epithelial and mesenchymal markers. Actin was used as a loading control. Signal intensity of each sample was quantified and normalized to the mean *Tbk1*$^{+/+}$: *KPC* signal. Results are representative of mean +/- SEM. Unpaired two-tailed *t* test, **p* < 0.05.



Supplemental Figure 6. TBK1 re-expression drives a mesenchymal morphology in *Tbk1$^{\Delta/\Delta}$: KIC* cell lines. Representative images of *Tbk1$^{\Delta/\Delta}$*: *KIC-C* cells infected with **(A)** lentiviral empty vector (EV) or **(B)** full-length TBK1 (T) plated on a mixed layer of collagen and Matrigel. Cells are fixed and nuclei are labeled with DAPI (blue) and F-actin is labeled with phalloidin (red).



Supplemental Figure 7. Axl-activating antibody AF854 stimulates Ras activity. **A)** Protein lysates from *Tbk1*$^{+/+}$: *KIC-A* and *Tbk1*$^{+/+}$: *KIC-D* cells treated with PBS or AF854 (6nM) for 30 min were assayed for active Ras and Pan-Ras. GTP and GDP protein loading were used as positive and negative controls, respectively. **B)** Protein lysates isolated from *Tbk1*$^{+/+}$: *KIC-A* cells treated with either PBS, AF854 (6nM), or AF854 (6nM) + BGB324 (BGB, 2 µM) for 30 min were assayed for active Ras and Pan-Ras.