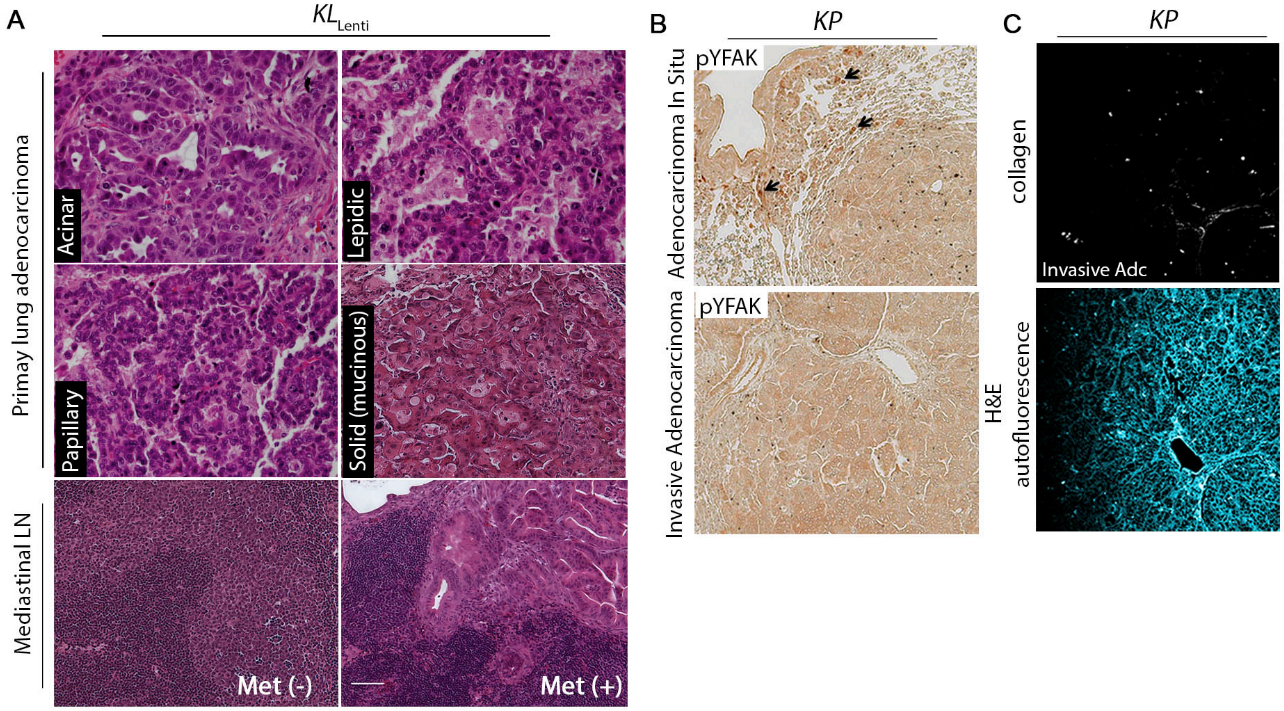
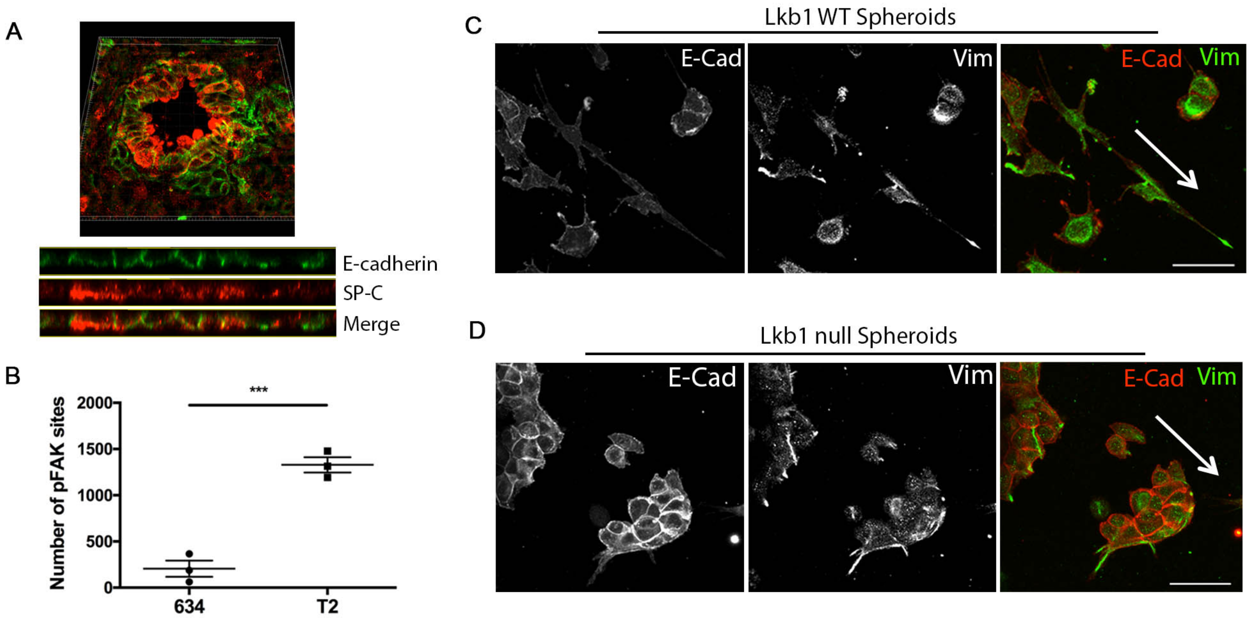
Supplemental Figure 1



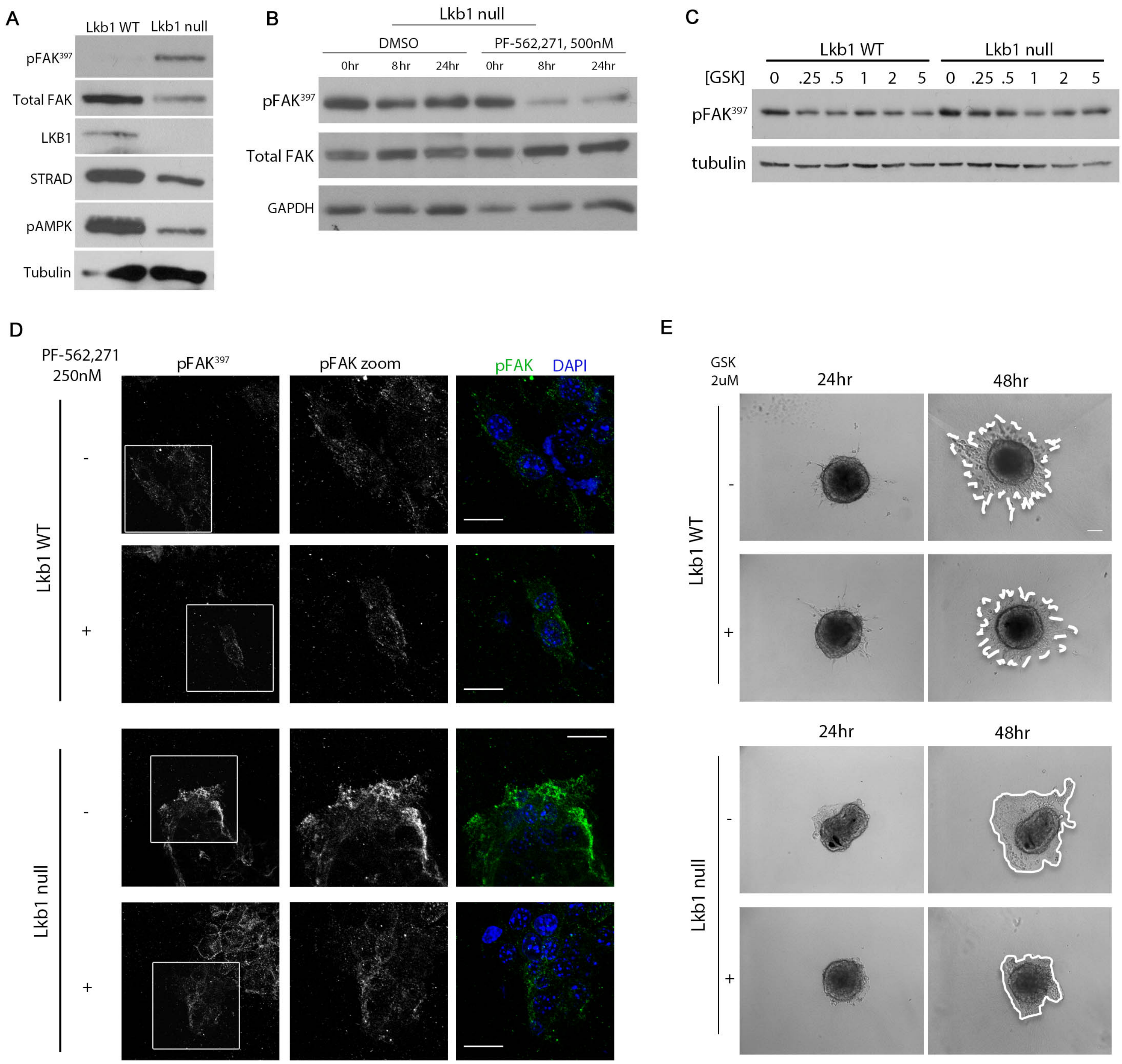
Supplemental Figure 1. *KL_{Lenti}* mice develop adenocarcinoma, metastasis to the mediastinal lymph nodes and *KP* mice lack high pYFAK staining and collagen levels in high-grade lung tumors.
(**A**) Representative images of H&E stained primary mouse lung adenocarcinoma in the LV-Cre induced *LSL-Kras*^{G12D}; *Lkb1*$^{fl/fl}$ (*KL_{Lenti}*) model (n = 96 mice) of the indicated histologic subtypes (400X), and H&E stained normal and metastatic mediastinal lymph nodes (Scale bar = 100um) (n = 33 mice). (**B**) Representative images of immunohistochemistry (IHC) of pYFAK^{397} in *Kras*^{G12D}, *p53*$^{fl/fl}$ (*KP*) adenocarcinoma in situ (AIS) (top) and invasive adenocarcinoma (Inv Adc) (bottom) (200X). Black arrows mark pYFAK positive immune cells (n = 5 mice). (**C**) Representative images of SHG detection of collagen (top) in *KP* Inv Adc. Cell structure is visualized by H&E autofluorescence (bottom; pseudo-colored blue) (200X).

Supplemental Figure 2



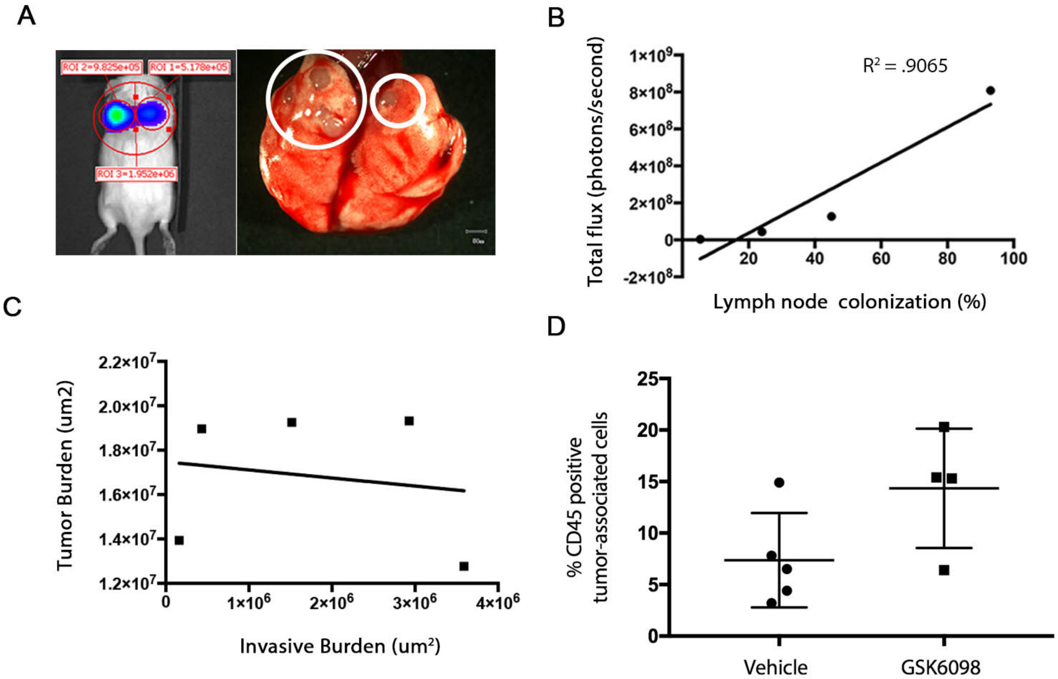
Supplemental Figure 2. Kras, Lkb1-mutant collective invasion packs (CIPs) maintain junctional E-Cadherin in vivo and in vitro. (**A**) (Top) 3D reconstruction of z-stack confocal images of a CIP expressing both SP-C (red) and E-Cadherin (green). (Bottom) A YZ cross-section was generated to highlight junctional E-Cadherin. **(B)** Number of pYFAK^{397}sites at the invasive front in Lkb1 WT and Lkb1 null spheroids after 48 hours of invasion into a collagen matrix (each box represents an independent spheroid). Data are represented as mean (SD). *P* values were calculated using a 2-tailed Student's *t*-test. ***P<.001. Confocal images of the invasive front of Lkb1 WT (**C**) or Lkb1 null (**D**) spheroids embedded in a collagen matrix and stained for E-Cad (red in merge) and Vimentin (Vim, green in merge).White arrows mark the direction of invasion. Scale bar = 50μm. Data are representative images from 3 independent experiments.

Supplemental Figure 3



Supplemental Figure 3. Efficacy of FAK inhibitors on 3D mouse tumor spheroids (**A**) Western analysis of Lkb1 pathway signaling in Lkb1 WT and Lkb1 null mouse tumor cells. (**B**) Western showing efficacy of the FAK inhibitor PF-562,271 over time on Lkb1 null mouse tumor cells **(C)** Western showing efficacy of GSK2256098 in Lkb1 WT and Lkb1 null tumor cells. (**D**) Immunofluorescence for pYFAK^{397} on Lkb1 WT and Lkb1 null spheroids embedded in collagen in the presence of either DMSO control or 250nM of PF-562,271. Immunofluorescence analysis was performed on pYFAK^{397} 48 hours post-embedding. Scale bar = 50um. (**E**) Representative images of Lkb1 WT (top) and Lkb1 null (bottom) tumor spheroids treated with either DMSO or GSK6098 (GSK) and embedded into collagen. Invasion was measured after 48hrs. Scale bar = 50um.

Supplemental Figure 4



Supplemental Fig. 4. Bioluminescent output is proportional to tumor burden and LN colonization in *KLL*$_{Lenti}$ mice, and GSK6098 treatment increases the tumor-associated leukocytes. (A) Region of interest (ROI) analysis of total flux and corresponding lungs in *KLL*$_{Lenti}$ mice. Tumors are outlined by white circles. Scale bar = 50mm **(B)** Regression plot of % LN colonization vs. total flux (p/s) in *KLL*$_{Lenti}$ mice. Linear regression, R^2 = .9065, *P* = .0479. (**C**) Regression plot of tumor burden (um^2) vs. invasive burden (um^2). Linear regression, R^2 = .02886 *P* = ns. (**D**) % Tumor associated , CD45-positive leukocytes in Vehicle vs. GSK6098 short-term treated mice. Each dot represents an individual mouse. 2-tailed *t* test; *P* = .0819.

Supplemental Table 1.

Tumor Grade	Vehicle (%)	GSK6098 (%)
Grade I	7 (9.1)	13 (25.0)
Grade II	62 (80.5)	37 (71.2)
Grade III	3 (3.9)	0 (0.0)
Grade IV	5 (6.5)	2 (3.9)

Supplemental Table 1.
Total and percent tumor grade in short-term Vehicle vs. GSK6098-treated mice. Fisher's exact test; *P* = .053.

Supplemental Table 2. Mice used in pre-clinical studies

Mouse ID	DOB	Sex	Treatment	Length of Treatment
2404	8/22/14	M	Vehicle	5 weeks
2410	8/22/14	M	Vehicle	5 weeks
2383	8/12/14	F	Vehicle	5 weeks
2468	9/17/14	F	Vehicle	5 weeks
2517	11/30/14	F	Vehicle	5 weeks
2529	12/10/14	M	Vehicle	5 weeks
2535	12/10/14	F	Vehicle	5 weeks
2541	12/9/14	F	Vehicle	5 weeks
2549	12/14/14	F	Vehicle	5 weeks
2550	12/14/14	F	Vehicle	5 weeks
2451	9/8/14	M	GSK (75mg/kg)	5 weeks
2465	9/17/14	M	GSK (75mg/kg)	5 weeks
2472	9/22/14	M	GSK (75mg/kg)	5 weeks
2474	9/22/14	F	GSK (75mg/kg)	5 weeks
2478	9/23/14	F	GSK (75mg/kg)	5 weeks
2479	9/23/14	F	GSK (75mg/kg)	5 weeks
2484	9/30/14	F	GSK (75mg/kg)	5 weeks
2394	8/13/14	F	GSK (75mg/kg)	5 weeks
2515	11/30/14	F	GSK (75mg/kg)	5 weeks

Mouse ID	DOB	Sex	Treatment	Days to onset of clinical symptoms
2671	4/30/15	F	Vehicle	65
2703	5/20/15	M	Vehicle	8
2711	5/26/15	F	Vehicle	9
2733	6/12/15	F	Vehicle	34
2736	6/12/15	M	Vehicle	48
2741	6/12/15	F	Vehicle	16
2702	5/26/15	F	Vehicle	70
2684	4/30/15	F	Vehicle	64

2709	5/26/15	F	Vehicle	73
2750	6/22/15	F	Vehicle	77
2739	6/12/15	M	Vehicle	77
2746	6/22/15	M	Vehicle	72
2715*	5/29/15	M	GSK (75mg/kg)	45
2716	5/29/15	M	GSK (75mg/kg)	58
2688	5/19/15	M	GSK (75mg/kg)	67
2670	4/30/15	F	GSK (75mg/kg)	71
2673	4/30/15	F	GSK (75mg/kg)	70
2748	6/22/15	M	GSK (75mg/kg)	76
2730	6/1/15	F	GSK (75mg/kg)	74
2725	6/1/15	F	GSK (75mg/kg)	72
2695	5/26/15	M	GSK (75mg/kg)	78
2691	5/19/15	F	GSK (75mg/kg)	75
2719*	5/29/15	F	GSK (75mg/kg)	19

* censored (2715 died from severe fight wounds and 2719 died from systemic infection).

Supplemental Table 3

	pYFAK	
Genotype	**Focal**	**Diffuse**
KRAS	1	6
KRAS, LKB1	5	1

Supplemental Table 3
Contingency table of *KRAS* and *KRAS, LKB1* lung adenocarcinoma patient tumors that exhibited focally upregulated (Focal) vs. majority diffuse (Diffuse) $pYFAK^{397}$ staining pattern. Fisher's exact test $P = 0.0291$.