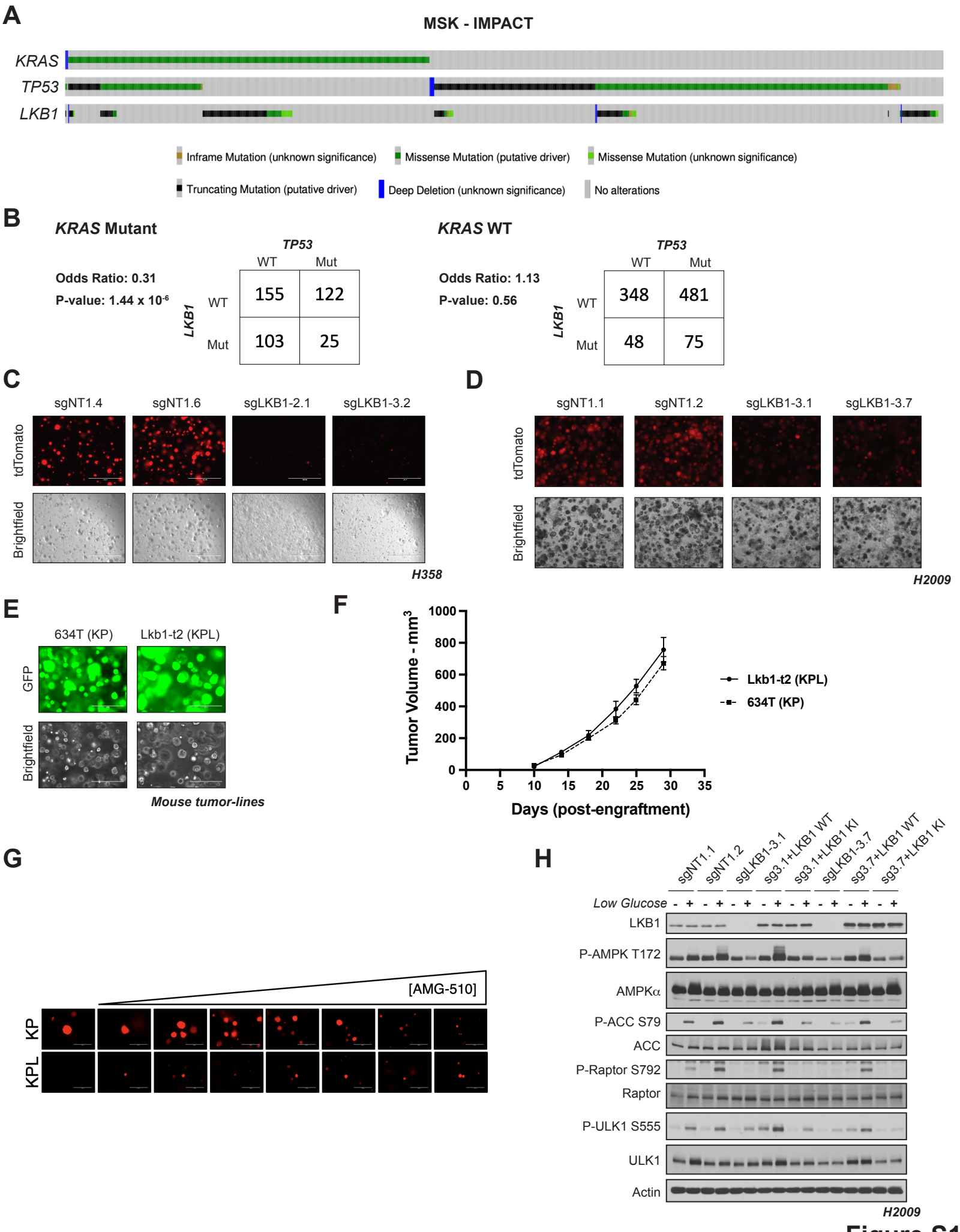
A
MSK - IMPACT
KRAS
TP53
LKB1
Inframe Mutation (unknown significance)
Missense Mutation (putative driver)
Missense Mutation (unknown significance)
Truncating Mutation (putative driver)
Deep Deletion (unknown significance)
No alterations
B
KRAS Mutant
Odds Ratio: 0.31
P-value: 1.44 x 10-6
TP53
WT
Mut
LKB1
WT
Mut
155
122
103
25
KRAS WT
Odds Ratio: 1.13
P-value: 0.56
TP53
WT
Mut
LKB1
WT
Mut
348
481
48
75
C
sgNT1.4
sgNT1.6
sgLKB1-2.1
sgLKB1-3.2
tdTomato
Brightfield
H358
D
sgNT1.1
sgNT1.2
sgLKB1-3.1
sgLKB1-3.7
tdTomato
Brightfield
H2009
E
634T (KP)
Lkb1-t2 (KPL)
GFP
Brightfield
Mouse tumor-lines
F
Tumor Volume - mm3
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
Days (post-engraftment)
Lkb1-t2 (KPL)
634T (KP)
G
[AMG-510]
KP
KPL
H
sgNT1.1
sgNT1.2
sgLKB1-3.1
sg3.1+LKB1 WT
sg3.1+LKB1 KI
sgLKB1-3.7
sg3.7+LKB1 WT
sg3.7+LKB1 KI
Low Glucose
- + - + - + - + - + - + - + - +
LKB1
P-AMPK T172
AMPKα
P-ACC S79
ACC
P-Raptor S792
Raptor
P-ULK1 S555
ULK1
Actin
H2009

Figure S1

Figure S1. Differential co-occurrence and effects of *KRAS*, *TP53* and *LKB1* mutations on human and mouse LUADs

(A) MSK Impact oncoprint of co-occurrence of *KRAS*, *TP53* and *LKB1* mutations in human lung adenocarcinomas , with Fisher's exact test of statistical likelihood of co-occurrence of *LKB1* and *TP53* mutations in a LUAD with a *KRAS* mutant or wildtype background. **(B)** Fisher's exact test of statistical likelihood of co-occurrence of *LKB1* and *TP53* mutations in a *KRAS* mutant or wildtype background respectively. **(C)** 3D spheroid growth in Matrigel of isogenic clones of the H358 cell line labeled with a tdTomato fluorescent reporter and expressing CAS9 and non-targeting controls (sgNT1.4 and sgNT1.6) or LKB1-specific (sgLKB1-2.1 and sgLKB1-3.2) guide RNAs. 5,000 cells were seeded into Matrigel and grown for 10 days in media changed every 24 hours. Images taken on EVOS fluorescence microscope under 4x magnification and filter to resolve tdTomato signal intensity and brightfield. **(D)** 3D spheroid growth in Matrigel of isogenic clones of the H2009 cell line labeled with a tdTomato fluorescent reporter and expressing CAS9 and non-targeting controls (sgNT1.1 and sgNT1.2) or LKB1-specific (sgLKB1-3.1 and sgLKB1-3.7) guide RNAs. 5,000 cells were seeded into Matrigel and grown for 10 days in media changed every 24 hours. Images taken on Nikon fluorescence microscope under 4x magnification and filter to resolve tdTomato signal intensity and brightfield. **(E)** 3D spheroid growth in Matrigel of GEMM-derived mLUAD cell lines containing transgenic lentiviral expression of GFP under control of a CMV promoter. 5,000 cells were seeded into Matrigel and assay was conducted for 10 days in culture media changed every 24 hours. Images taken on EVOS fluorescence microscope under 10x magnification and filter to resolve GFP signal intensity and brightfield. **(F)** Mean (-/+ s.e.m.) volumes of mouse 634T (KP) and Lkb1-t2 (KPL) lung adenocarcinoma allograft tumors. 1 x 10^4 cells implanted in right hind flank (n = 10 per cohort). **(G)** 3D spheroid growth in Matrigel of isogenic clones of the H358 cell line labeled with a tdTomato fluorescent reporter and expressing Cas9 and non-targeting controls (sgNT1.6) or LKB1-specific (sgLKB1-3.2) guide RNAs. 1,000 cells were seeded into Matrigel and grown for 14 days in media changed every 72 hours with addition of fresh AMG-510. Dose range for AMG-510 was 1 nM – 1 μM Images taken on EVOS fluorescence microscope under 4x magnification and filter to resolve tdTomato signal intensity. **(H)** Western blot analysis of H2009 (*KRAS*;*TP53*) isogenic clones (KP: sgNT1.1 and sgNT1.2; KPL: sgLKB1-3.1 and sgLKB1-3.7) and lines with additional transgenic expression of guide RNA resistant LKB1 wildtype (WT) (sgLKB1-3.1 + LKB1 WT and sgLKB1-3.7 + LKB1 WT) or LKB1 kinase inactive (KI) (sgLKB1-3.1 + LKB1 KI and sgLKB1-3.7 LKB1 KI) and treated with 11.1 mM or 0.5 mM glucose for 6 hours as indicated. Restoration of AMPK signaling in LKB1 WT lines in response to 0.5 mM glucose validated by blotting for P-AMPK Thr172 and downstream substrates (P-ACC S79, P-ULK1 S555, P-Raptor S792). Similar results observed in three independent experiments.