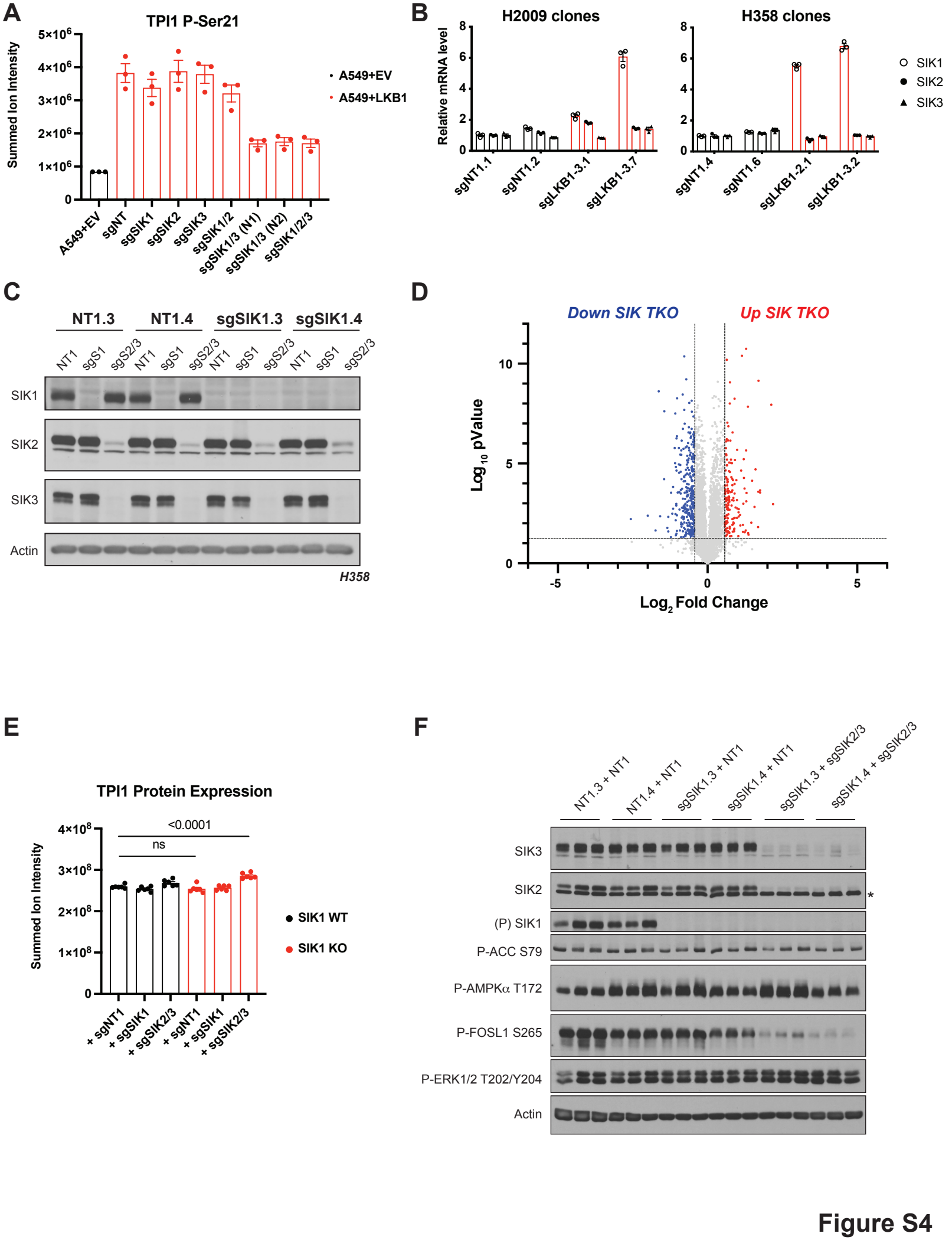
A
TPI1 P-Ser21
Summed Ion Intensity
5×10^6
4×10^6
3×10^6
2×10^6
1×10^6
0
A549+EV
sgNT
sgSIK1
sgSIK2
sgSIK3
sgSIK1/2
sgSIK1/3 (N1)
sgSIK1/3 (N2)
sgSIK1/2/3
A549+EV
A549+LKB1
B
H2009 clones
H358 clones
Relative mRNA level
sgNT1.1
sgNT1.2
sgLKB1-3.1
sgLKB1-3.7
sgNT1.4
sgNT1.6
sgLKB1-2.1
sgLKB1-3.2
SIK1
SIK2
SIK3
C
NT1.3
NT1.4
sgSIK1.3
sgSIK1.4
NT1
sgS1
sgS2/3
SIK1
SIK2
SIK3
Actin
H358
D
Down SIK TKO
Up SIK TKO
Log10 pValue
Log2 Fold Change
E
TPI1 Protein Expression
Summed Ion Intensity
<0.0001
ns
SIK1 WT
SIK1 KO
+ sgNT1
+ sgSIK1
+ sgSIK2/3
F
NT1.3 + NT1
NT1.4 + NT1
sgSIK1.3 + NT1
sgSIK1.4 + NT1
sgSIK1.3 + sgSIK2/3
sgSIK1.4 + sgSIK2/3
SIK3
SIK2
(P) SIK1
P-ACC S79
P-AMPKα T172
P-FOSL1 S265
P-ERK1/2 T202/Y204
Actin

Figure S4

Figure S4. Members of the salt Inducible kinase family phosphorylate TPI1.
(A) Bar graph of summed ion intensity for the TPI1-derived Ser-21 phospho-peptide in extracts of A549 cell-lines with transgenic expression of an empty vector or wild type LKB1 and guide RNAs specifically targeting combinations of members of the Salt Inducible Kinase family selectively in LKB1 transgenic expressing cells. Cell lines were cultured in 11.1 mM glucose. Cell lines were cultured in 11.1 mM glucose prior to analysis. Ion intensities were normalized to identified non-phosphorylated peptides across conditions to control for protein expression and reported as the mean (-/+ s.e.m.). **(B)** Bar graphs depicting the mean mRNA level (+ s.e.m) in biological triplicate of the Salt Inducible Kinases in the indicated isogenic clones of H2009 (left) and H358 (right) with sgLKB1 clones represented with red bars for each. **(C)** Western blots showing abundance of SIK1, SIK2 and SIK3 in H358 isogenic clones after exposure to the indicated guide RNAs for members of the SIK family. H358 isogenic clones expressing Cas9 and containing non-targeting control (sgNT1.3 and sgNT1.4) or SIK1 specific (sgSIK1.3 and sgSIK1.4) guide RNA and additional non-targeting (NT1), SIK1 (sgSIK1) or dual SIK2 and SIK3 (sgSIK2/3) guide RNAs. **(D)** Volcano plot of quantitative unenriched total proteomic data used to compare protein expression in H358 clones (2 KP clones and 2 KP SIK TKO clones, with 3 biological replicates of each) and serving as a companion to figure 2C. Cells were cultured in 0.5 mM glucose for 6 hours before lysis. Proteins that pass statistical criteria (p-value <0.05) are highlighted in black, red and blue; those that do not satisfy this criterion are colored grey. Proteins highlighted in red satisfy the fold change threshold (>1.5) after triple deletion of SIK1,2,3. Phospho-peptides highlighted in blue satisfy the fold change threshold of < -1.5) for a decrease after SIK1,2,3 triple deletion. **(E)** Bar graph of summed ion intensities for the TPI1 protein expression in extracts of isogenic H358 cell-lines containing a non-targeting control (sgNT1.3 and sgNT1.4) or SIK1 specific (sgSIK1.3 and sgSIK1.4) guide RNA and additional control (NT1) SIK1 (sgSIK1) or dual SIK2 and SIK3 (sgSIK2/3) guide RNAs in a polyclonal population. To remove bias all peptides containing Ser21 were removed prior to summation within each replicate. Cell lines were cultured in 0.5 mM glucose prior to lysis, analyzed in biological triplicate per clone, N=6 per genotype and reported as the mean (-/+ s.e.m.). Statistical significance determined by two-tailed paired t-test. **(F)** Western blots of proteomic companion lysates in biological triplicate showing abundance of SIK1, SIK2 and SIK3 in H358 isogenic clones after exposure to the indicated guide RNAs for members of the SIK family and treated with 0.5 mM glucose for 6 hr. H358 isogenic clones expressing Cas9 and containing non-targeting control (sgNT1.3 and sgNT1.4) or SIK1 specific (sgSIK1.3 and sgSIK1.4) guide RNA and additional non-targeting (NT1), SIK1 (sgSIK1) or dual SIK2 and SIK3 (sgSIK2/3) guide RNAs. Signaling including AMPK substrates; P-ACC S79 and P-AMPKα T172 included to show consistent activity across lysates and replicates in response to 0.5 mM glucose treatment. Additional cross validation of P-FOSL1 T202/Y204 identified in phosphor-proteomic analysis confirmed and P-MAPK p42/p44 included to show P-FOSL1 differences are independent of this signaling axis.