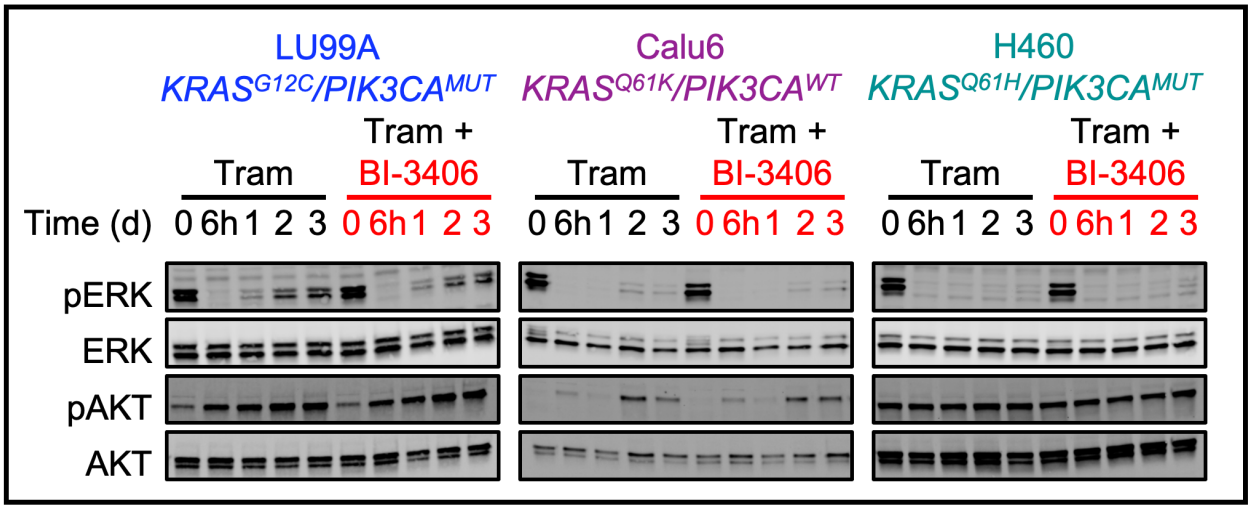
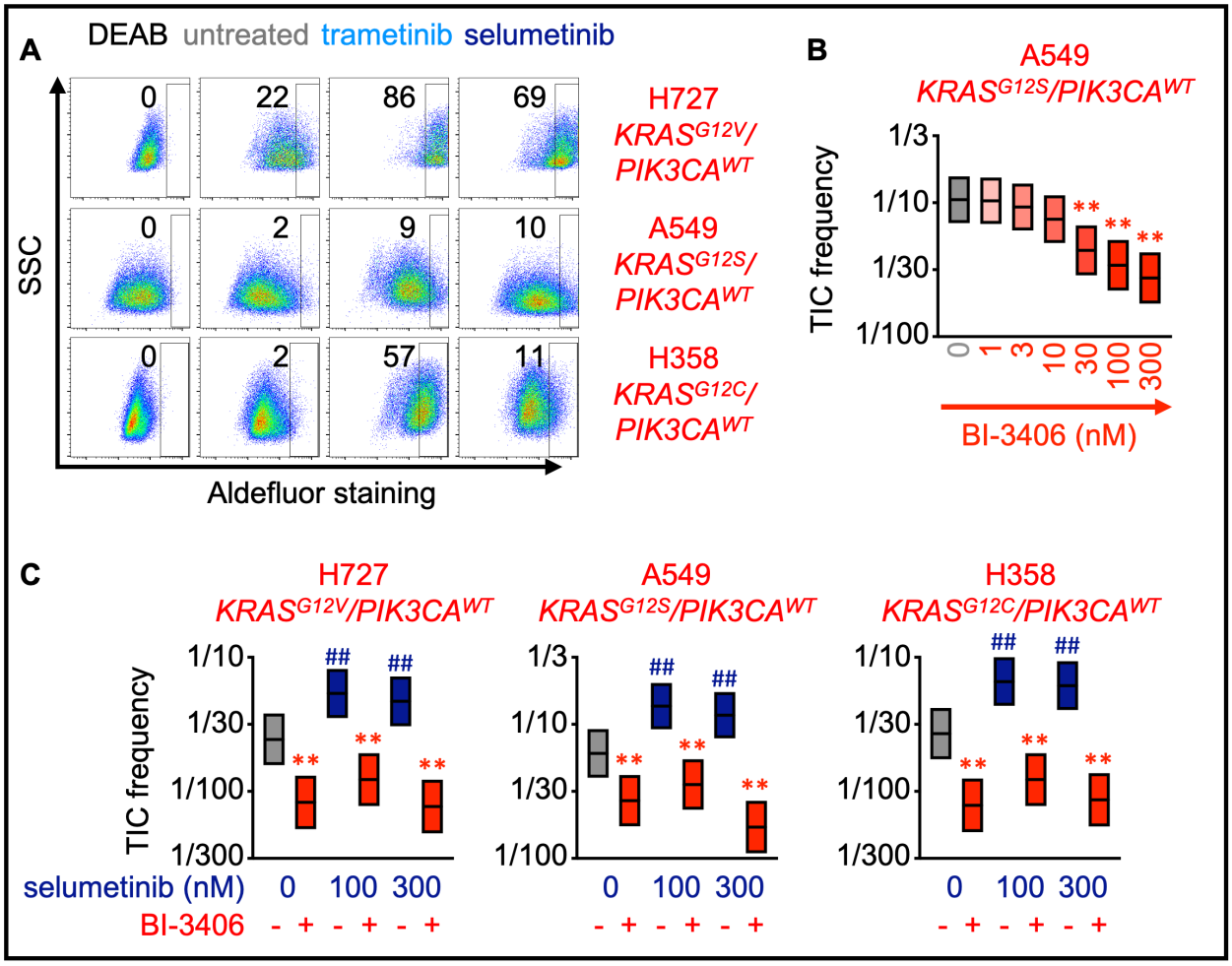


Supplemental Figure 1. SOS1 inhibition does not prevent RTK reactivation in *KRAS*^{Q61X}-mutated LUAD cells with or without a *PIK3CA* co-mutation.

Western blots of WCLs of 3D spheroid cultured LU99A, Calu6, or H460 cells treated with trametinib (10 nM) ± BI-3406 (300 nM) for the indicated times. Western blots are for pERK, ERK, pAKT (Ser 473), and AKT. The *KRAS* and *PIK3CA* mutational status of each cell line are indicated.



Supplemental Figure 2. SOS1 inhibition prevents MEK-I induced TIC outgrowth.

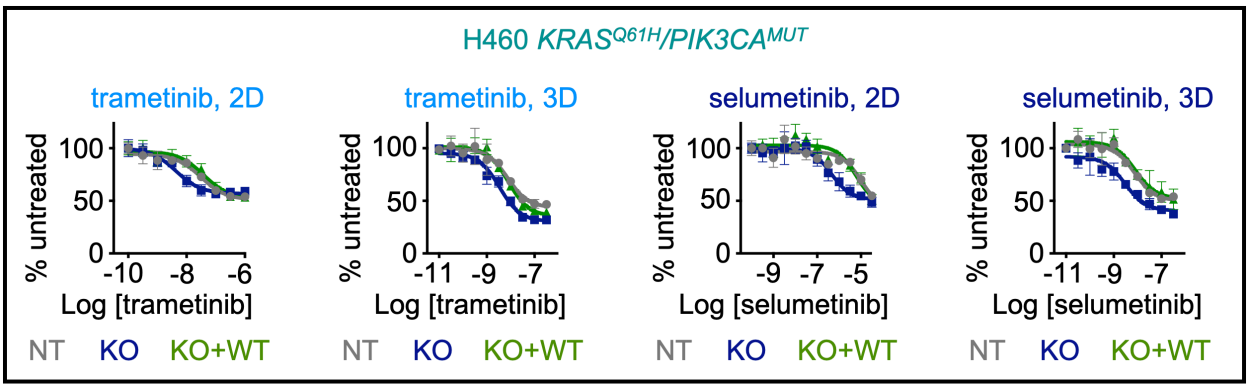
(A) Aldeflour staining for ALDH enzyme activity in DEAB negative control (DEAB), untreated cells, or cells treated with 100 nM trametinib or selumetinib for 72 hours for the indicated cell lines. H727 data are the same as in Fig. 2A and are repeated here for comparison purposes.

(B) TIC frequency from *in situ* ELDAs of A549 cells treated with the indicated doses of BI-3406.

(C) TIC frequency from *in situ* ELDAs of the indicated cell lines pre-treated with selumetinib for 72 hours to upregulate TICs, and then left untreated or treated with BI-3406.

# $p < 0.05$ vs untreated; ## $p<0.01$ vs. untreated for TIC upregulation by MEK inhibitor treatment vs. untreated controls. * $p < 0.05$ vs untreated; ** $p<0.01$ for TIC inhibition by BI-3406 treatment compared to untreated controls.

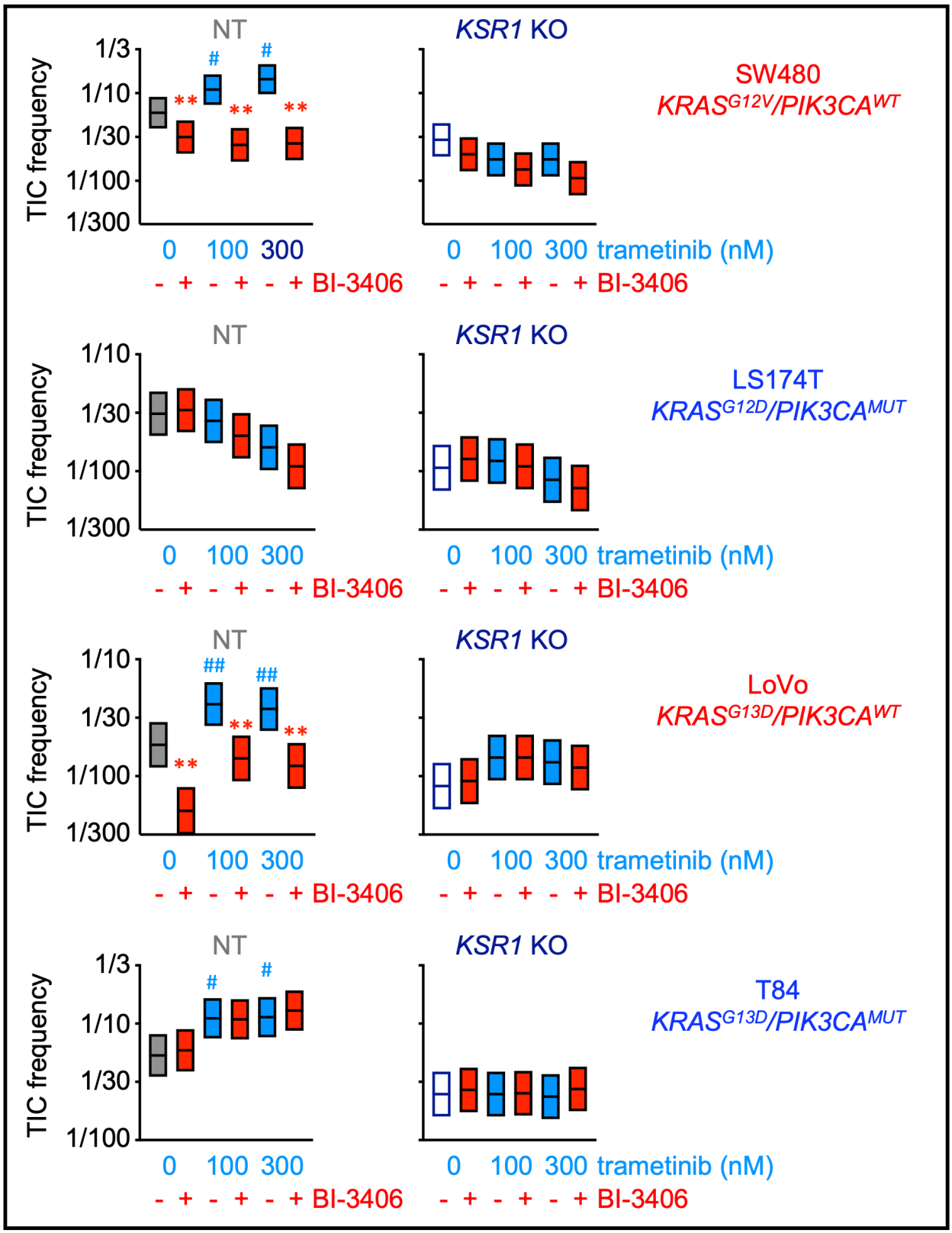
Data are representative from three independent experiments.



Supplemental Figure 3. *KSR1* KO sensitizes *KRAS*^{Q61H}-mutated LUAD cells to the killing effects of MEK inhibition.

Single-dose response curves for NT, *KSR1* KO, and *KSR1* KO+*KSR1* cells H460 (*KRAS*^{Q61H}/*PIK3CA*^{MUT}) LUAD cells treated with increasing doses of trametinib or selumetinib for 72 hours under 2D (anchorage-dependent) and 3D (anchorage-independent) conditions. Data are mean ± s.d. from three independent experiments.

*** p<0.01 vs non-targeting controls; ### p<0.001 vs. *KSR1* KO.


NT
KSR1 KO
SW480
KRASG12V/PIK3CAWT
LS174T
KRASG12D/PIK3CAMUT
LoVo
KRASG13D/PIK3CAWT
T84
KRASG13D/PIK3CAMUT
TIC frequency
1/3
1/10
1/30
1/100
1/300
0 100 300 trametinib (nM)
- + - + - + BI-3406

Supplemental Figure 4. SOS1 inhibition does not enhance the effect of *KSR1* KO on basal and trametinib-induced TICs in *KRAS*-mutated COAD cells.

TIC frequency from *in situ* ELDAs of the indicated NT and *KSR1* KO COAD cells pre-treated with trametinib for 72 hours to upregulate TICs, and then left untreated or treated with the SOS1 inhibitor BI-3406. The *KRAS* and *PIK3CA* mutational status for each cell line is indicated. NT cells in the left column are from Fig. 4 and are shown here for comparison purposes.

# $p < 0.05$ vs untreated; ## $p<0.01$ vs. untreated for TIC upregulation by MEK inhibitor treatment vs. untreated controls.

** $p<0.01$ for TIC inhibition by BI-3406 treatment compared to untreated controls.