Supplementary Figure Legends

Supplementary Figure 1. Structure of LOXO-101. LOXO-101 has the chemical name of (S)-N-(5-((R)-2-(2,5-difluorophenyl)pyrrolidin-1-yl)pyrazolo[1,5-a]pyrimidin-3-yl)-3-hydroxypyrrolidine-1-carboxamide hydrogen sulfate and has a molecular weight of 526.52. LOXO-101 is the hydrogen sulfate form of ARRY-470.

Supplementary Figure 2. Schematic for TRK-SHC1 proximity ligation assay (PLA). This cartoon demonstrates detection of proximal (<40nM) TRK and SHC1 proteins in tumor cells. The TRK antibody (rabbit) used can detect the c-terminus of TRKA (encoded by *NTRK1*), TRKB (*NTRK2*) or TRKC (*NTRK3*) proteins. SHC1 is detected by a SHC1 antibody (mouse). Binding of species-specific secondary antibodies with covalently attached complementary nucleotide sequences allows an *in situ* PCR reaction to generate DNA, which can be detected by fluorescence *in situ* hybridization visualized in this method as red dots. The assay has the potential to detect activated TRK regardless of mechanism of activation (gene fusion, mutation, or autocrine/paracrine activation of the wildtype) or TRK receptor family member (TRKA/B/C).

Supplementary Figure 3. Validation of the TRK-SHC1 PLA. (A) The CUTO-3 cell line, derived from a malignant pleural effusion from a patient with stage IV lung adenocarcinoma harboring the *MPRIP-NTRK1* gene fusion, was transfected with a non-targeting control (NTC) siRNA, *NTRK1*-directed siRNA or untreated (control) and assayed for TRKA protein expression. Western blot analysis demonstrates a marked decrease in the TRKA protein levels, and corresponds to the MPRIP-TRKA fusion protein that migrates with an apparent molecular weight of 170 kD (A). TRK-SHC1 PLA was performed in cells treated as in (A) demonstrating a robust positive signal in the

siRNA control (B), but proportional decrease in the *NTRK1* siRNA (C). CUTO-3 cells were treated with DMSO (D) or LOXO-101 at a concentration of 100nM (E) for 2 hours demonstrating disruption of TRKA-SHC1 complexes in the LOXO-101 treated sample compared to control. CULC001 is a patient-derived tumor xenograft (PDX) derived from the same tumor as the CUTO-3 cell line and harbors the *MPRIP-NTRK1* gene fusion (not shown). CULC002 is a PDX from a NSCLC patient without a known driver (*ALK*, *ROS1*, *EGFR*, *KRAS, BRAF* negative) and is negative for an *NTRK1* gene fusion by *NTRK1* break-apart FISH (not shown). TRK PLA analysis demonstrates a robust signal in CULC001 (F) but no signal associated with tumor cells in CULC002 (G). H&E and (H) and fluorescence microscopy following TRK-SHC PLA (I) show a nerve bundle (highlighted by dotted white line) from the CULC001 PDX.

Supplementary Figure 4. TRK and ALK PLA in an ALK+ tumor sample. FFPE tumor sample from an ALK+ tumor was assayed using the TRK-SHC1 PLA (A) demonstrating an absence of signal or ALK-GRB2 PLA (B) showing robust ALK signaling.

Supplementary Figure 5. Serum tumor marker response. Serum CA125 was found to be elevated in this patient prior to initiation of study drug, and subsequently followed as a potential indicator of activity. Serum CA125 was drawn at baseline (day -8) prior to dosing and at the indicated time point points following the initiation of dosing at day -3 through day 112 demonstrating a time-dependent decrease in this tumor marker. The dashed red line indicates the upper limit of normal (35 U/mL) of this laboratory test.