**Supplemental Figure S1: S1A and S1B** Graphical representation of shRNA counts obtained by deep sequencing in both cells lines (H322C and HCC4006). Only shRNAs showing differences between untreated and treated groups (14,000 to 17,000 shRNAs) are included, with the requirement that the median of one group is greater than the maximum of the other. Unsupervised hierarchical clustering of results are shown. The intensity of red corresponds to the number of shRNA reads in each replicate, and the top 10 'hits' enriched and depleted in both control and treated samples are shown. Note that additional criteria (statistical and based on the number of shRNAs/gene) are used to determine synthetic lethality.

**Supplemental Figure S2: S2A** Expression of tankyrase-targeting shRNAs resulted in ~70% knockdown of tankyrase gene expression as measured by real-time RT-PCR and western blotting. The expression of AXIN2 mRNA was measured by real-time RT-PCR in the same cells. **S2B** H322C cells expressing either negative control shRNA (shScrbl) or shRNAs targeting TNKS1 (TNKS1-2; in triplicate) were treated with vehicle (DMSO) or 500 nM gefitinib (GEF) for 4 days, followed by replating without drug for 4 more days. Viable cells were counted by flow cytometry using PI-exclusion. **S2C** HCC4006 cells were treated in triplicate with DMSO or gefitinib (GEF, 20 nM) and/or the TNKS inhibitor XAV939 (5 µM) for 4 days, and then replated without drugs for 3 additional days. Viable cells were counted by flow cytometry using PI-exclusion. **S2D** The indicated NSCLC cell lines were treated in triplicate with DMSO, gefitinib and/or XAV939 at the indicated concentrations for 5 days, and then cell viability was assessed by MTT assays. Cell viability relative to vehicle treated cultures (set at 100%) is graphed. Graphs at bottom show the effects of gefitinib in the context of each concentration of XAV939 (normalized to the cultures treated with XAV939 alone); cells were treated as above for 5 days, replated without drugs for 3 days and cell viability analyzed by MTT assays. **S2E** As in S2C, with data either plotted in for Combination Indices (CI) to determine additivity (CI=1), synergism (CI<1) and antagonism (CI>1) using Calcusyn software (top row) or graphed for cell viability relative to vehicle treated cultures (bottom row). **S2F** The H3122 NSCLC cell line expressing the oncogenic driver EML4-ALK fusion protein was treated in sextuplicate with DMSO, gefitinib and/or XAV949 at the indicated concentrations for 5 days, and cell viability was assessed by MTT assays. The graph on the left shows the percent control for each drug as a single agent and at each combination. The right graph was plotted for CI values. Note that the combinations of 0.25 µM gefitinib + 3 µM XAV, 0.5 µM gefitinib + 3 µM XAV and 1 µM gefitinib + 1µM XAV resulted in CI > 2 and the combination of 1 µM gefitinib + 3 µM XAV resulted in a CI > 4, but the graph is truncated at 2. **S2G** Primary Human Foreskin Fibroblast (HFF) cells were treated in triplicate with DMSO or 500 nM gefitinib and/or the TNKS inhibitor XAV939 (3 µM) for 4 days, and then replated without drugs for 3
additional days. Viable cells were counted. **S2H** The H322 NSCLC cell line was treated in sextuplicate with DMSO, gefitinib and/or XAV939 at the indicated concentrations for 5 days, and cell viability was assessed by MTT assays. The graphs were plotted for CI values.

**Supplemental Figure S3: S3A** H322C cells were treated as in Fig 2B for 72h and stained with FITC-Annexin V (left) or for propidium iodide exclusion (right). * indicates p<0.05 **S3B** H322C cells were treated with DMSO (D) or gefitinib (GEF; 500 nM for H322C) and/or the TNKS inhibitors XAV939 (XAV; 5 µM) for 5 days, and then stained for senescence-associated β-galactosidase (sa-β-gal) activity (blue). **S3C** Proposed model.

**Supplemental Figure S4: S4A** H322C cells expressing either a negative control shRNA (shScrbl) or shRNAs targeting CSNK1A1 (CK1-2) were treated with DMSO or 500 nM gefitinib for 4 days, followed by replating without drug for 3 days. Viable cells were counted. **S4B** Expression of CK1α-targeting shRNAs resulted in ~80% knockdown of CK1α gene expression as measured by real-time RT-PCR and western blotting. **S4C** As in S4A, except H322C cells expressed either shScrbl or shRNAs targeting CSNK2A1 (CK2-3). **S4D** Expression of CK2α-targeting shRNAs resulted in 80-90% knockdown of CK2α gene expression as measured by real-time RT-PCR and western blotting. **S4E** H322C cells expressing either a negative control shRNA (C) or shRNAs targeting CSNK1A1 (CK1-2) were treated with vehicle (DMSO) or 500 nM gefitinib (Gef) for 4 days and then replated without drugs for colony forming assays. Colonies were counted after 8 days. **S4F** H322C and HCC4006 cells were treated in triplicate with vehicle (DMSO, D) or gefitinib (GEF, 500 nM for H322C or 20 nM for HCC4006) and/or the pyrvinium pamoate (Pyr, 10 nM for H322C and 2 nM for HCC4006) for 4 days, and then replated without drugs for 3 additional days. Viable cells were counted by flow cytometry using PI-exclusion. **S4G** H322C and HCC4006 cells were treated in triplicate with DMSO, gefitinib and/or pyrvinium pamoate at the indicated concentrations for 5 days, and then cell viability was assessed by MTT assays. Graph was plotted in for CI values using CalcuSyn software (top row), or for viability relative to control (vehicle) cultures (bottom row). **S4H** The H322 NSCLC cell line was treated in sextuplet with DMSO, gefitinib and/or pyrvinium at the indicated concentrations for 5 days, and cell viability was assessed by MTT assays. The graphs were plotted for CI values. **S4I** HFF cells were treated in triplicate with DMSO or 500 nM gefitinib and/or pyrvinium (20 nM) for 4 days, and then replated without drugs for 3 additional days. Viable cells were counted.
**Supplemental Figure S5: S5A)** H322C cells expressing either shScrbl or shRNAs targeting β-catenin (shBCat-2) were treated with DMSO or gefitinib (GEF; 500 nM) for 4 days, followed by replating without drug for 3 more days. **S5B** Expression of β-catenin-targeted shRNAs resulted in ~60 or 80% knockdown of gene expression as measured by real-time RT-PCR and western blotting. **S5C** HCC4006 and H322C cells (in triplicate) expressing either negative control shRNA (shScrbl, CON) or shRNAs targeting TCF4 (TCF4-35 and TCF4-36) were treated with vehicle (DMSO) or gefitinib (GEF; 30 nM for HCC4006, 500 nM for H322C) for 4 days, followed by replating without drug for 3 more days. Viable cells were counted by flow cytometry using PI-exclusion. Expression of TCF4-targeting shRNAs resulted in knockdown of the TCF4 gene expression as measured by western blotting. **S5D** Correlations between the expression of canonical Wnt target genes and gefitinib sensitivity for a panel of NSCLC cell lines. We carried out a targeted evaluation of global gene expression analyses which have been performed on 28 NSCLC lines with variable sensitivity to gefitinib [1]. We asked whether the expression of Wnt/β-catenin target genes correlates with gefitinib sensitivity. Gene expression levels for Wnt/β-catenin canonical pathway target genes (the Wnt homepage [2]) were determined using data from Coldren et al [1] for 28 NSCLC lines.

The 6 “Down” genes are described as being downregulated by Wnt/β-catenin signaling [2]. 66 genes are described as being upregulated by Wnt in mammalian systems, and a correlation with gefitinib sensitivity was not evident for all 66 positively regulated canonical Wnt target genes. However, a subset of 14 genes were found to be overexpressed >1.25 fold in the gefitinib resistant lines (IC50>1.0), with a weak positive correlation with resistance. The expression of the 6 genes shown to be negatively regulated by Wnt/β-catenin signaling exhibited a weak inverse correlation with gefitinib sensitivity.

While these analyses indicate that a subset of β-catenin target genes may exhibit a trend towards higher canonical Wnt signaling in the more resistant lines, a clear correlation was absent, highlighting the importance of functional screens for the identification of compensatory pathways for cancer cells. The heatmap is generated by standardizing the expressions of a gene across the cell lines (mean = 0, standard deviation = 1) where red and green colors represent over- and under-expression of genes, respectively. Columns and rows represent lung cancer cell lines and Wnt target genes, respectively. IC50 values for gefitinib were as determined by [1]. **Correlation of SLuGs with gefitinib induced genes.** To investigate whether SLuGs were enriched in gefitinib induced genes, we compared our 104 gene SLuG2 and 1237 gene SLuG\(^{H322C}\) lists to genome-wide gene expression profiling of H322C cells before and after treatment with gefitinib [3]. After performing robust multi-array average (RMA) analyses on these arrays, we found 1443 genes up-regulated at least 2-fold after gefitinib treatment in H322C cells. Conversely, 618 genes were down-regulated at least 2-fold after gefitinib treatment in H322C cells. 9 up-
regulated (*NFIB, FBXO9, FAR1P1, ADD3, CASQ2, CEBPA, ACSL1, BCL11A, PLCE1*) and 2 down-regulated (*MDM2, SMOX*) genes after gefitinib treatment were overlapping with the 104 SLuG2 genes. 91 up-regulated and 44 down-regulated genes after gefitinib treatment overlapped with the 1237 SLuGH322C genes. Using the hypergeometric test, none of these differentially genes were statistically significantly enriched in either SLuG2 or SLuGH322C. SLuGs are not enriched in lung cancer specific genes. We next asked whether SLuG2s are enriched in NSCLC specific genes (genes mutated or with altered copy numbers in NSCLC). We cross-checked the 21 lung cancer specific genes (*AKT1, ALK, BAP1, BRAF, CD74, EGFR, EML4, ERBB2, FGFR2, KDR, KRAS, MYCL1, NFE2L2, RB1, ROS1, SMARCA4, SOX2, STK11, TFG, TP53*) obtained from COSMIC database (Cancer Gene Census, version 3/22/2011), and none of the SLuG2s are found in this list.

**Supplemental Figure S6:**  
**S6A)** HCC4006 and H322C cells (left and right panels, respectively) were treated as in Figure 2B, 2C and 5B with the indicated drugs for 24 hours, and then analyzed by western blotting for levels of phosphorylated (Y1068) EGFR, total EGFR, pERK and total ERK.  
**S6B)** HCC4006 were treated for 5 days with the following drugs and concentrations: XAV939 (XAV), at 3 µM, pyrvinium pamoate (Pyr) at 20 nM and PNU74654 (PNU) at 20 µM. Cells were harvested at 24, 72 and 120h of treatment and AXIN2 mRNA was assayed by qPCR as a query of Wnt signaling. mRNA levels were normalized to GAPDH levels in all samples[4].  
**S6C)** HCC4006 cells expressing vector or activated β-catenin (4XmtBCat) were analyzed by western blotting for the expression of active β-catenin, total β-catenin, or Actin.  
**S6D)** The HCC4006 cells expressing vector or activated β-catenin were treated in sextuplet with DMSO, gefitinib and/or XAV949 at the indicated concentrations for 5 days, and cell viability was assessed by MTT assays.

**Supplemental Figure S7:** Second xenograft experiment utilizing a different shRNA for TNKS1.  
**S7A.** The same data shown in Figure 7A is regraphed for the first 35 days only, to provide a closeup of these early timepoints.  
**S7B.** Nude mice with H322C xenografts expressing shRNAs targeting TNKS or control shRNAs (Scrb1) were treated with vehicle (Veh) or gefitinib (50 mg/kg/d) as indicated, and tumors measured at least weekly (n=8/group). Arrows indicate when treatment was started and stopped. The tumor volumes at the start of treatment are shown in the graph below.

**Supplemental Table S1:** Lists of SLuGs. Excel file contains the lists of SLuG2, SLuG\textsuperscript{H322C} and SLuG\textsuperscript{HCC4006}, as defined in the Results. Columns for DMSO and Gefitinib show the actual shRNA counts from Illumina sequencing. The adj.p column is the p-value adjusted for multiple comparisons, and the weightZ_P column shows the p-value [P(wZ)] from the weighted Z-transformation method (which combines
individual p-values for shRNAs targeting the same gene). The Sequence column shows the shRNA sequence detected by Illumina sequencing.

To determine whether the 104 SLuG²s are enriched in any cancer-related genes, we queried the 457 cancer-related genes list of COSMIC (Cancer Gene Census, version 3/22/2011). Eight genes were found to overlap between the SLuG² and COSMIC cancer gene lists. Assuming the human genome contains 20,000 genes and 457 validated cancer-related genes, SLuGs are enriched with cancer-related genes (p = 0.0007, hyper-geometric test). The eight overlapping SLuG genes are: ARID1A, BCL11A, CEBPA, MDM2, MYH11, NFIB, PTPN11 and SSX2.

**Supplemental Table S2: Primers, shRNAs and antibodies.** Primers and probes for real-time RT-PCR and TRC shRNA sequences are shown. Antibodies used are also shown.