Supplemental Figure and Table Legends

Supplemental Figure 1. Quantiative real-time PCR analysis of caspase 9 splice variants in A549 and HBEC-3KT cells. A549 and HBEC-3KT cells were cultured without serum overnight and total RNA was isolated and reverse transcribed. The resulting cDNA was subjected to quantitative real-time PCR using primer pairs specific for caspase 9a, caspase 9b, and 18s rRNA. Data are expressed as quantity of caspase 9a and caspase 9b mRNA over quantity of 18s rRNA. Columns represent the mean of two independent experiments ± S.E.

Supplemental Figure 2. Akt phosphorylation levels in HBEC-3KTs expressing either wild-type or activating mutant EGFR. HBEC-3KT cells expressing either wild-type EGFR, vector control, del E746-A750 EGFR, L858R EGFR, or K-Ras^{V12} were cultured without EGF for 24 hours. Total proteins were subjected to western blot analysis to determine the expression levels of **(A)** phospho-Akt, total Akt, **(B)** phospho-ERK, and total ERK1/2.

Supplemental Figure 3. The alternative splicing of Casp9 does not regulate the anchorage-independent growth of HBEC-3KT cells expressing KRAS^{V12}. RT-PCR analysis of Casp9 splice variants and the corresponding Casp9a/9b mRNA ratios from K-Ras^{V12}-expressing HBEC-3KT cells(22) transiently transfected with Casp9b shRNA or control shRNA. Colony formation assay of the described cells in soft agar. Data are depicted as mean ± S.E. represented as percent control.

Supplemental Figure 4. A549 cells were cultured with DMSO or erlotinib (10µM) overnight and total RNA was isolated and reverse transcribed. The resulting cDNA was subjected to quantitative real-time PCR using primer pairs specific for caspase 9a, caspase 9b and 18s rRNA. **A)** Depicted is the quantity of caspase 9a mRNA and quantity of caspase 9b mRNA normalized

to 18s rRNA. **B)** Depicted is the quantity of caspase 9a mRNA over the quantity of caspase 9b mRNA normalized to 18s rRNA. Columns represent the mean of two independent experiments ± S.E. **C)** Western blot analysis of phospho-Akt and total Akt protein expression in the indicated cell lines treated with or without erlotinib for 24 hours. A549, H838, and H460 cells were treated with 10µM erlotinib and HCC827 cells were treated with 100nM erlotinib.

Supplemental Figure 5. Inhibition of Protein Kinase C (PKC) does not effect the alternative splicing of caspase 9. Quantitative/competitive RT-PCR analysis of caspase 9 splice variants and the corresponding caspase 9a/9b mRNA ratios from A459s treated with DMSO control (0.1%), **A)** Gö6983 [10 µM] or **B)** Gö6776 [10 µM]. The ratio of caspase 9a to caspase 9b mRNA was determined by densitometric analysis of RT-PCR fragments. Data are expressed as means ± S.E. *P*-values in pairwise comparisons to the control; *P*-values < 0.05 are considered significant. Data are representative of three separate determinations on two separate occasions.

Supplemental Figure 6. Effects on downstream signaling pathways after treatment with mitogenic pathway inhibitors. A) Western blot analysis of phospho-Akt and total Akt protein expression in the indicated cell lines treated with or without Akt inhibitor VIII (25µM) for 24 hours. **B)** Western blot analysis of phospho-Akt, total Akt, phospho-Erk1/2 and total Erk1/2 protein expression in A549s treated with or without PD98059 (10µM) for 24 hours. **C)** Western blot analysis of phospho-Akt and total Akt protein expression in A549s treated with or without OSU03012 (10µM) for 24 hours.

Supplemental Figure 7. The alternative splicing of caspase 9 is regulated by PI_3Kinase/Akt in a linear manner. Quantitative/competitive RT-PCR analysis of caspase 9 splice variants and the corresponding caspase 9a/9b mRNA ratios from A459s treated with DMSO control (0.1%), LY294002

[50 μM], Akt VIII [25 μM], or both LY294002 [50 μM] and Akt VIII [25 μM] in combination. The ratio of caspase 9a to caspase 9b mRNA was determined by densitometric analysis of RT-PCR fragments. Data are expressed as means ± S.E. *P*-values in pairwise comparisons to the control; p values < 0.05 are considered significant. Data are representative of three separate determinations on two separate occasions.

Supplemental Figure 8. The phosphorylation status of SRp30a regulates the alternative splicing of caspase 9 pre-mRNA. **A)** Quantitative/competitive RT-PCR analysis of caspase 9 minigene-derived transcripts and the corresponding caspase 9a/9b mRNA ratios from A549s co-transfected with the caspase 9 minigene and the indicated SRp30a single and double mutant constructs. **B)** Quantitative/competitive RT-PCR analysis of endogenous caspase 9 splice variants from A549 cells transfected with SRp30a-WT, SRp30a-RD, and the indicated SRp30a triple mutant construct.

Supplemental Figure 9. Cellular localization of SRp30a phospho-mutants. Western blot analysis of cytosolic and nuclear protein fractions from A549s transfected with GFP-con, SRp30a-WT, SRp30a-QD, SRp30a-QA, or SRp30a-RD. Fractions were analyzed for expression of the T7 tag (e.g. ectopically expressed SRp30a), lamin, or α-tubulin.

Supplemental Figure 10. The alternative splicing of caspase 9 is regulated by the EGFR/PI_3Kinase/Akt pathway in a phospho-SRp30a-dependent manner. Following EGFR stimulation, PI_3Kinase becomes activated and in turn, generates specific inositol-phospholipids that are recognized by Akt. Akt then translocates to the membrane and, through binding of its PH-domain, becomes activated and is released to perform both cytosolic and nuclear functions. It is unclear whether Akt modifies SRp30a in the nucleus or the cytoplasm, or if Akt phosphorylates SRp30a directly or indirectly. Phosphorylated SRp30a, specifically on serine$^{199,\ 201,\ 227,}$ and ^{234} then functions with phosphorylated hnRNP L to repress the inclusion of the exon 3,4,5,6, cassette of caspase 9, favoring the production of caspase 9b mRNA. Ceramide can act to antagonize this pathway by activating ceramide

activated protein phosphatases (CAPPs), which have been shown to dephosphorylate SRp30a. We hypothesize that when SRp30a is phosphorylated, steric hindrance prevents the association of key binding partners responsible for exon cassette inclusion. However, non-phosphorylated SRp30a is able to associate with key binding partners, allowing it to enhance inclusion of the exon 3,4,5,6, cassette of caspase 9, favoring the production of caspase 9a.

Supplemental Table I. Pathology-verified patient normal tissue and tumor tissue samples analyzed for their caspase 9a/9b mRNA ratio. Each sample is detailed with diagnosis, group, and ratio of caspase 9a/9b mRNA. Represented are the squamous cell and large cell carcinoma samples analyzed. Adenocarcinoma samples utilized in this study were previously described(16).

Supplemental Table II. Characterization of NSCLC cell lines. Characterization of the NSCLC cell lines utilized in this study. Represented for each cell line are their histology, K-Ras and p53 mutational status, the corresponding caspase 9a/9b mRNA ratio, and the degree of dysregulation of the caspase 9a/9b mRNA ratio.

Supplemental Table III. Mitogen signaling pathways demonstrating no involvement in the alternative splicing of caspase 9 as analyzed by small molecule inhibitors. The table depicts the inhibitors and their respective concentrations utilized in this study. Mitogenic pathways were examined for effects on the ratio of Casp9a/9b utilizing small-molecule inhibitors at doses well characterized in the scientific literature and previously utilized in studies on A549 cells(26-32).

Supplemental Table IV. Potential phosphorylation sites in SRp30a involved in regulating the alternative splicing of caspase 9. This table depicts the potential phosphorylation sites in the RS domain of SRp30a predicted by various phospho-site determination databases. Indicated are the specific residues,

how the residues were determined, and whether the residue demonstrated an effect on the caspase 9a/9b mRNA ratio.