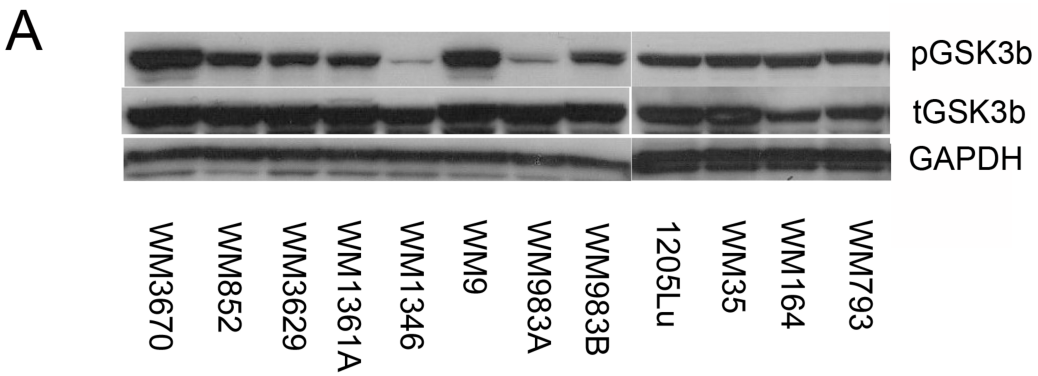
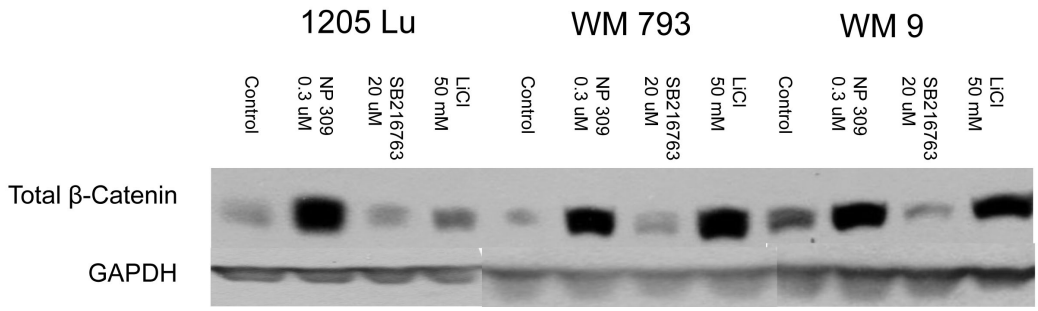
Cell line	BRAF	NRAS
WM3670	G469E	G12D
WM852	WT	61R
WM3629	D549G	G12D
WM1361A	WT	61L
WM1346	WT	61L
WM9	V600E	WT
WM983A	V600E	WT
WM983B	V600E	WT
1205Lu	V600E	WT
WM35	V600E	WT
WM164	V600E	WT
WM793	V600E	WT

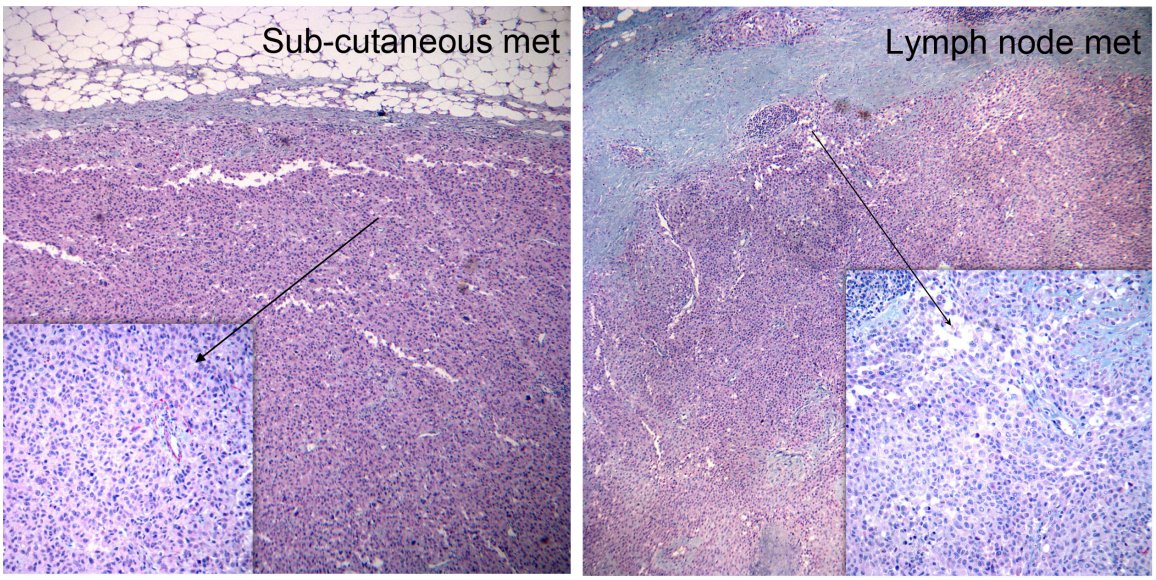
Supplemental Table 1: Mutational status of the melanoma cell line panel. Melanoma cells were genotyped as described in (Smalley KS, Xiao M, Villanueva J, Nguyen TK, Flaherty KT, Letrero R, et al. CRAF inhibition induces apoptosis in melanoma cells with non-V600E BRAF mutations. Oncogene. 2009;28:85-94).



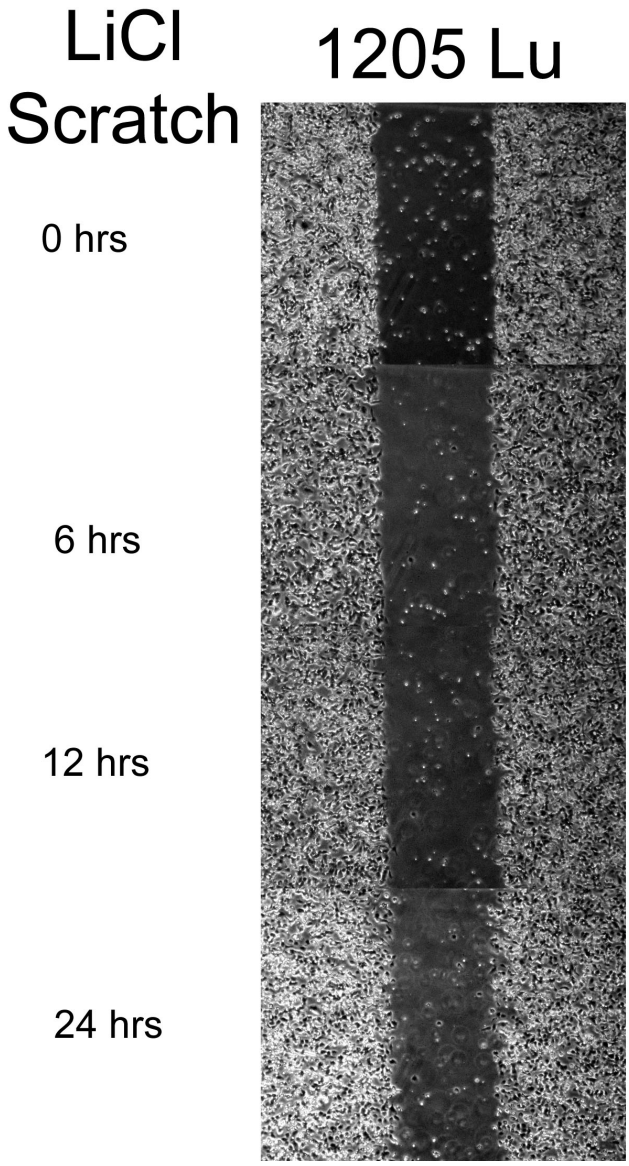
Supplemental Figure 1: Western blot of melanoma cell line panel showing the expression of phosphorylated GSK3β (Ser9) and total GSK3β. Blots were stripped once and reprobed for GAPDH to show even protein loading.



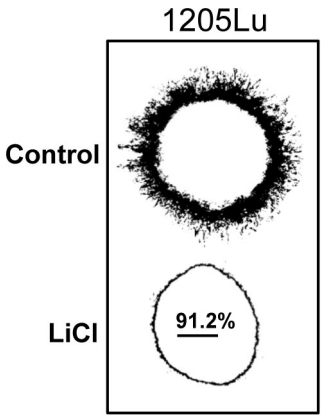
Supplemental Figure 2: Inhibition of GSK3β leads to upregulated β-catenin expression in 1205Lu, WM793 and WM9 melanoma cells. Cells were treated with either vehicle (control), NP-309 (0.3 μM), SB216763 (20 μM) or LiCl (50 mM) for 24 hrs. β-catenin expression was measured by Western Blotting. Protein loading was confirmed by stripping the blot and probing for GAPDH.



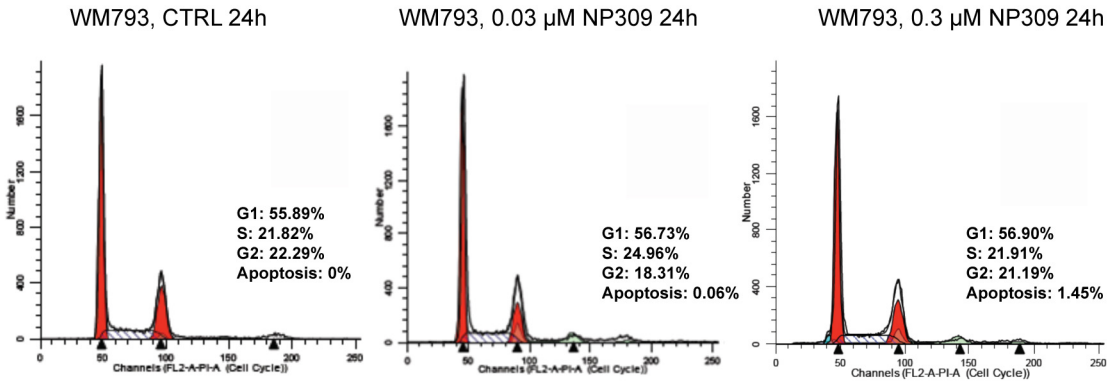
Supplemental Figure 3: Some melanoma specimens are negative for GSK3β staining. Immunohistochemical staining of representative melanoma metastases that are negative for GSK3β expression



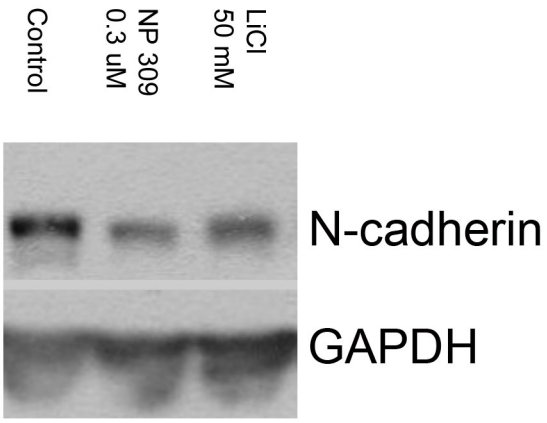
Supplemental Figure 4: Representative scratch experiment showing the anti-migratory effect of LiCl (50mM) on 1205Lu cells. Cells were scratched at time zero and photographed at 0, 6, 12 and 24 hrs.



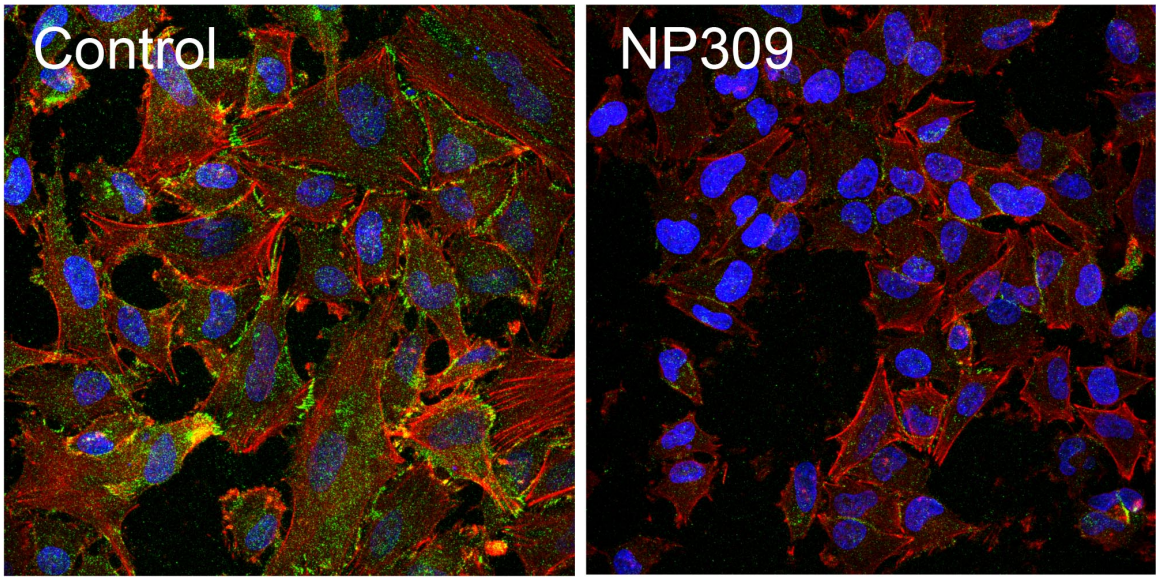
Supplemental Figure 5: LiCl treatment prevents the invasion of 1205Lu melanoma cells in a spheroid migration assay. Figures shows the % invasion from the original spheroid after 72 hrs treatment with either vehicle or LiCl (50 mM). Analysis was performed using ImageJ.



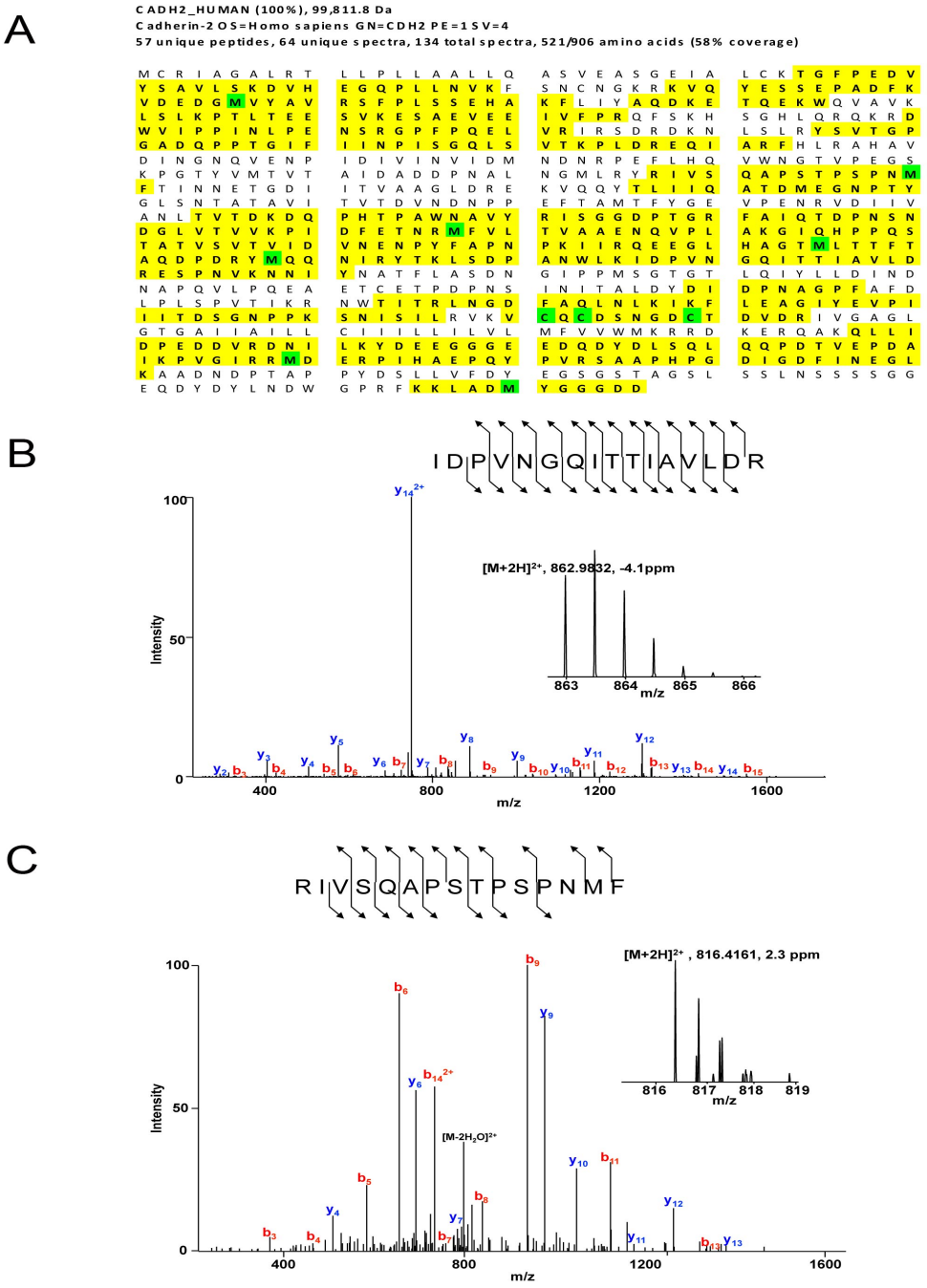
Supplemental Figure 6: NP-309 has minimal effects on cell cycle entry over 24 hrs. WM793 cells were treated with NP-309 (0.03 and 0.3 μM) for 24 hrs, before being fixed and stained with propidium iodide. Cells were then analyzed by flow cytometry.



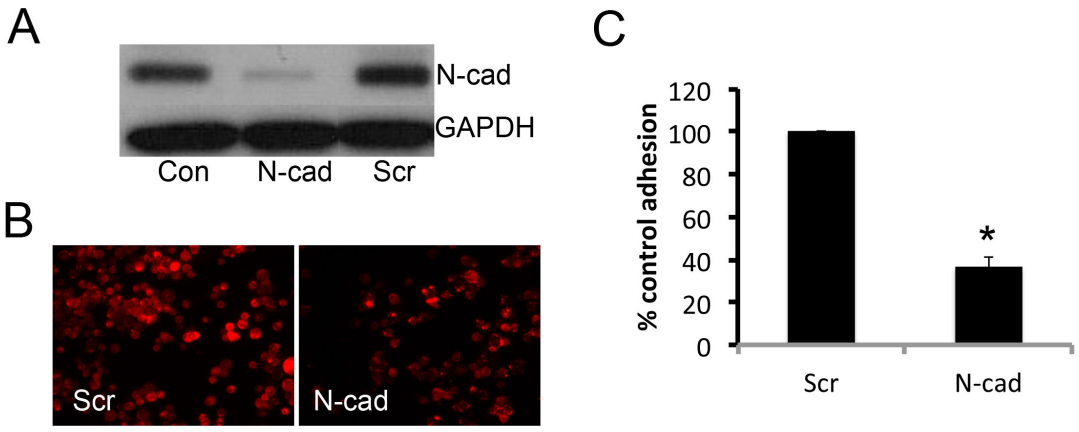
Supplemental Figure 7: Both NP-309 and LiCl reduce expression of N-cadherin in 1205Lu melanoma cells. Cultures were treated with vehicle, NP-309 (300 nM) or LiCl (50 mM) for 24 hrs. After this time, protein was extracted, resolved by Western blot and probed for expression of N-cadherin. Equal protein loading was confirmed by stripping the blot once and probing for GAPDH.



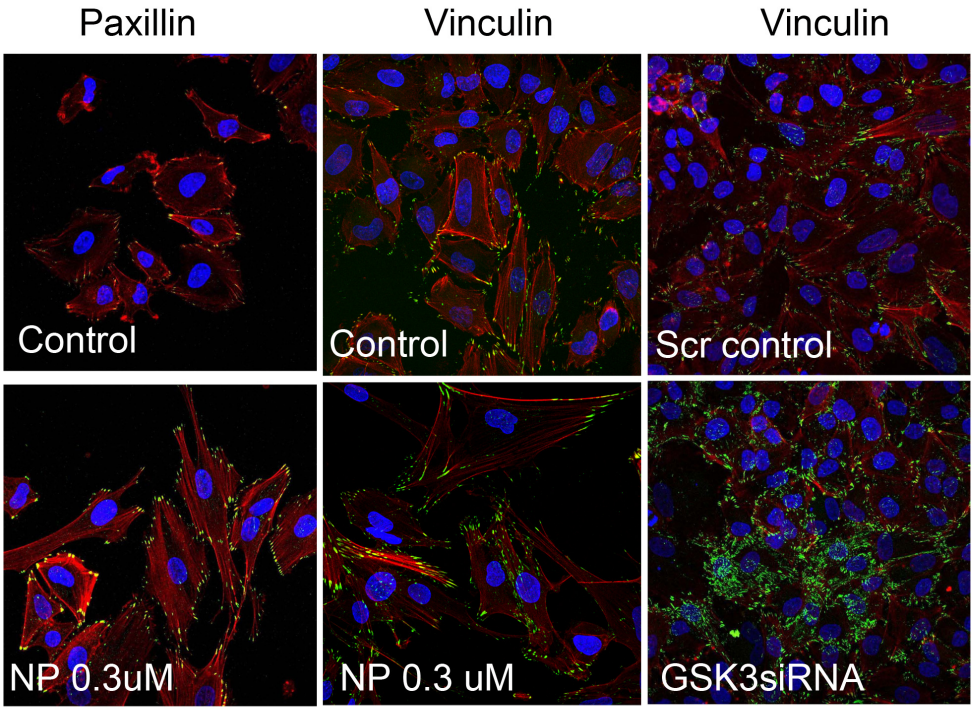
Supplemental Figure 8: Reduction of N-cadherin staining in WM793 cells following NP309 treatment. Cells were treated with NP309 (300nM, 24 hrs) before being fixed and stained for N-cadherin (green), actin (red) and DAPI (blue).



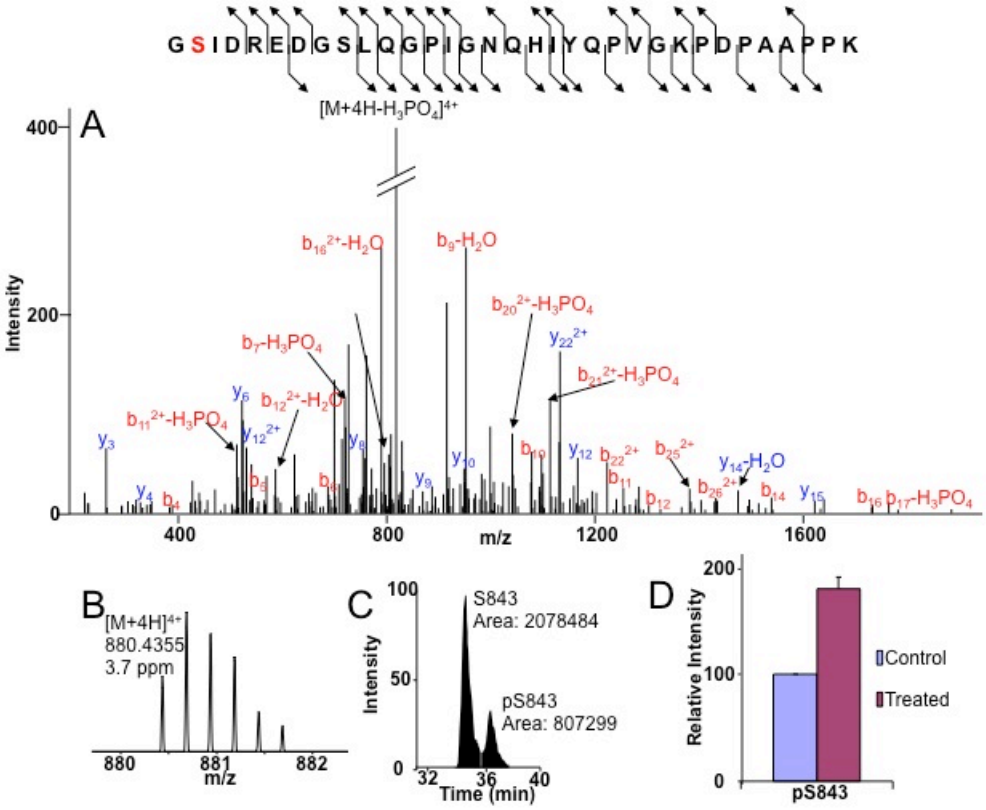
Supplemental Figure 9: Mass spectrometry analysis of N-cadherin immunoprecipitated from WM793 cells following NP309 treatment (300nM, 24 hrs). The immunoprecipitation elute was separated on SDS gel. The band containing Cadherin was excised and subjected to reduction/alkylation and proteolytic in-gel digestion (Trypsin and Chymotrypsin) were carried out and the purified peptides were analyzed with LC-MS/MS. A). Sequence coverage of N-cadherin was 58%. B) The identification of a tryptic peptide IDPVNGQITTIAVLDR. C). The identification of a chymotryptic peptide RIVSQAPSTPSPNMF. No changes in mass associated with post-translational modification were identified.



Supplemental Figure 10: siRNA knockdown of N-cadherin prevents the adhesion of WM793 to confluent monolayers of primary human skin fibroblasts. A: Western blot showing the knockdown of N-cadherin in WM793 cells. B: Representative experiment showing the reduction in cell numbers following siRNA knockdown of N-cadherin. C: Quantification of cell numbers after 15 mins of adhesion. Data shows mean, +/- SEM. *P<0.05



Supplemental Figure 11: Inhibition of GSK3 function using either NP-309 or by siRNA knockdown leads to increased paxillin and vinculin immunostaining. WM793 melanoma cells were either treated with NP-309 (0. 3μM, 24 hrs) or with siRNA to GSK3b for 48 hrs before being fixed and stained for paxillin or vinculin (both green) and phalloidin (red).



Supplemental Figure 12: Inhibition of GSK3β increases FAK phosphorylation at Ser843. The peptide containing phosphoserine 843 was identified from the MS/MS spectrum shown in **A** (Mascot score 43). The quadruply charged peptide GSIDREDGSLQGPIGNQHIYQPVGKPDPAAPPK was detected at m/z 880.4355, representing a mass error of 3.78 ppm (**B**). Extracted ion chromatograms (**C**) were used to calculate the integrated peak areas of both the phosphopeptide and the corresponding unmodified peptide (MS/MS data not shown). The percentage of phosphorylation was calculated by dividing the intensity of phosphopeptide by the sum of the intensities from both phospho- and unmodified peptides; data were normalized to the control samples (**D**).

Supplemental Methods.

Analysis by LC-MS/MS Mass Spectrometry

Following gel band excision, destaining, and trypsin digestion, peptides were concentrated to 20 μl using vacuum centrifugation. Each sample was analyzed in duplicate. A nanoflow liquid chromatograph (U3000, Dionex, Sunnyvale, CA) coupled to an electrospray ion trap mass spectrometer (LTQ-Orbitrap, Thermo, San Jose, CA) was used for tandem mass spectrometry peptide sequencing experiments. The sample was first loaded onto a pre-column (5mm x 300 μm ID packed with C18 reversed-phase resin, 5μm particles, 100Å pore size) and washed for 8 minutes with aqueous 2% acetonitrile and 0.04% trifluoroacetic acid. The trapped peptides were eluted onto the analytical column, (C18 Pepmap100, 75 mm ID x 15 cm, Dionex, Sunnyvale, CA). The following 120-minute gradient program was as applied using a 300 nl/min flow rate: 95% solvent A (2% acetonitrile + 0.1% formic acid) for 8 minutes, solvent B (90% acetonitrile + 0.1% formic acid) increasing from 5% to 50% in 90 minutes, then solvent B increasing from 50% to 90% in 7 minute and held at 90% for 5 minutes, followed by decreasing solvent B from 90% to 5% in 1 minute and re-equilibration for 10 minutes. Five tandem mass spectra were collected in a data-dependent manner following each survey scan. The MS^1 scans were performed in Orbitrap to obtain accurate peptide mass measurements at high resolution, and the MS/MS scans were performed in linear ion trap using 60 second exclusion for previously sampled peptide peaks. Sequest and Mascot searches were performed against the Uni-Prot human database downloaded on January 11, 2011.[i,ii] Two missed tryptic cleavages were allowed; precursor mass tolerance was set to 1.08 Da. MS/MS mass tolerance was set to 0.8 Da. Dynamic modifications included carbamidomethylation (Cys), oxidation (Met) and phosphorylation (Ser, Thr, Tyr). MASCOT and SEQUEST search results were summarized in Scaffold 3.0. The integrated

peak areas for phosphotyrosine peptide quantification were calculated from extracted ion chromatograms (EIC) using QuanBrowser from Xcalibur 2.0. These values were restricted by m/z (+/- 0.02) and retention time (120 seconds). The masses and isotopic peak patterns of the target peptides were manually inspected to insure proper sequence assignment and verify signal quality.

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[i] Jimmy K. Eng, Ashley L. McCormack, and John R. Yates, III. "An Approach to Correlate Tandem Mass Spectral Data of Peptides with Amino Acid Sequences in a Protein Database". J Am Soc Mass Spectrom 5: 976-989 (1994)

[ii] Perkins D.N., Pappin D.JC., Creasy D.M., Cottrell JS. "Probability-based protein identification by searching sequence databases using mass spectrometry data". Electrophoresis, 20(18) 3551-67 (1999)