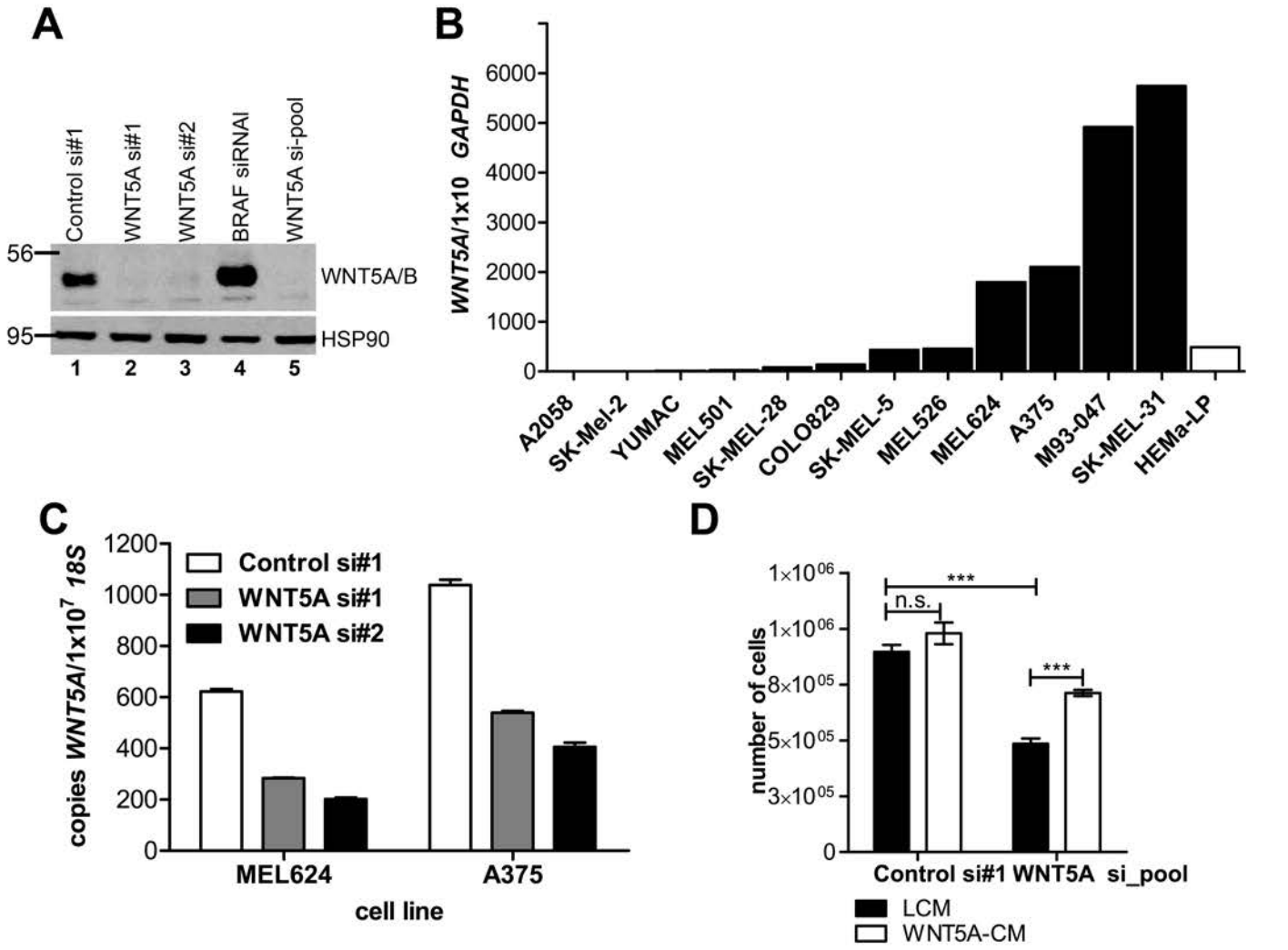
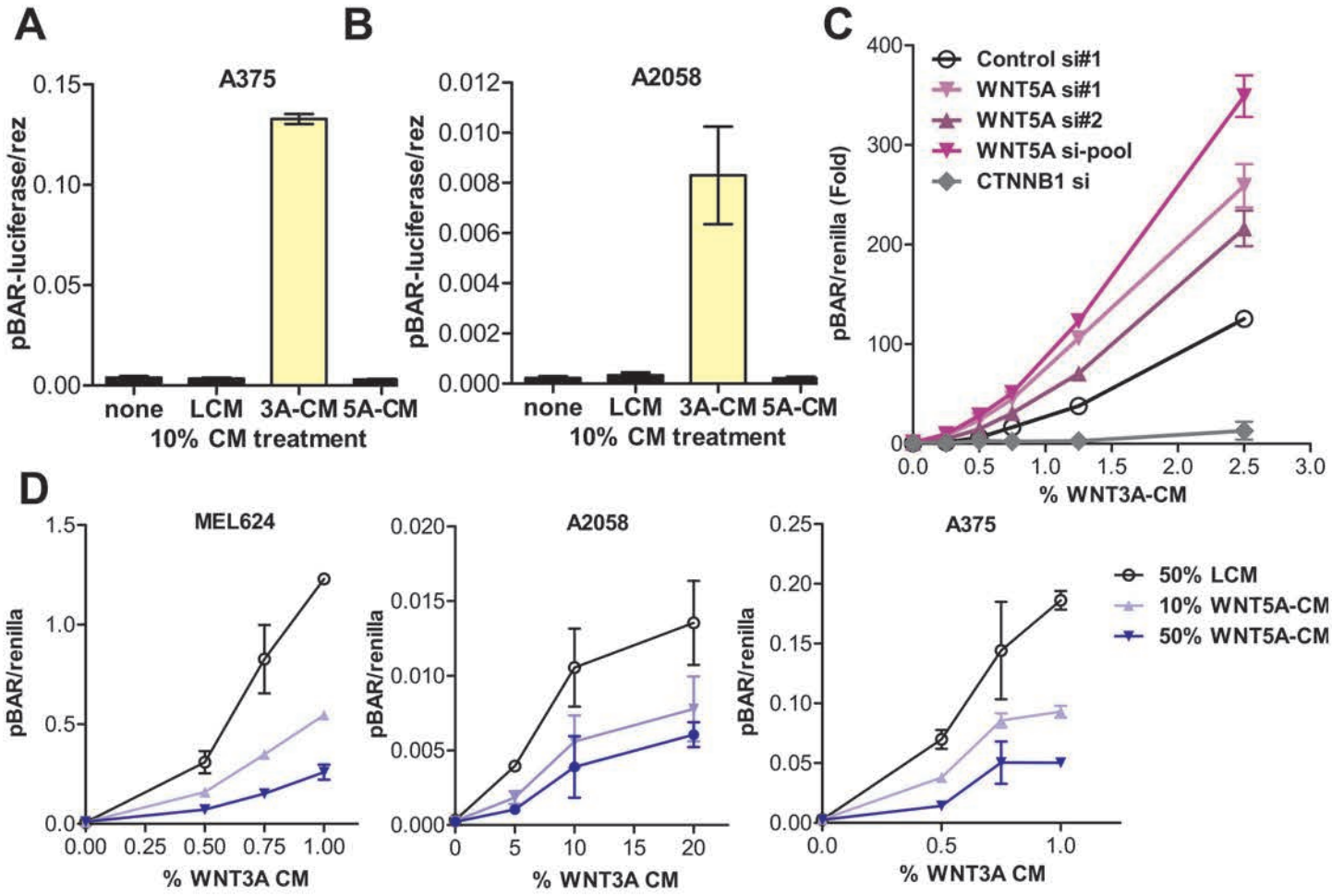


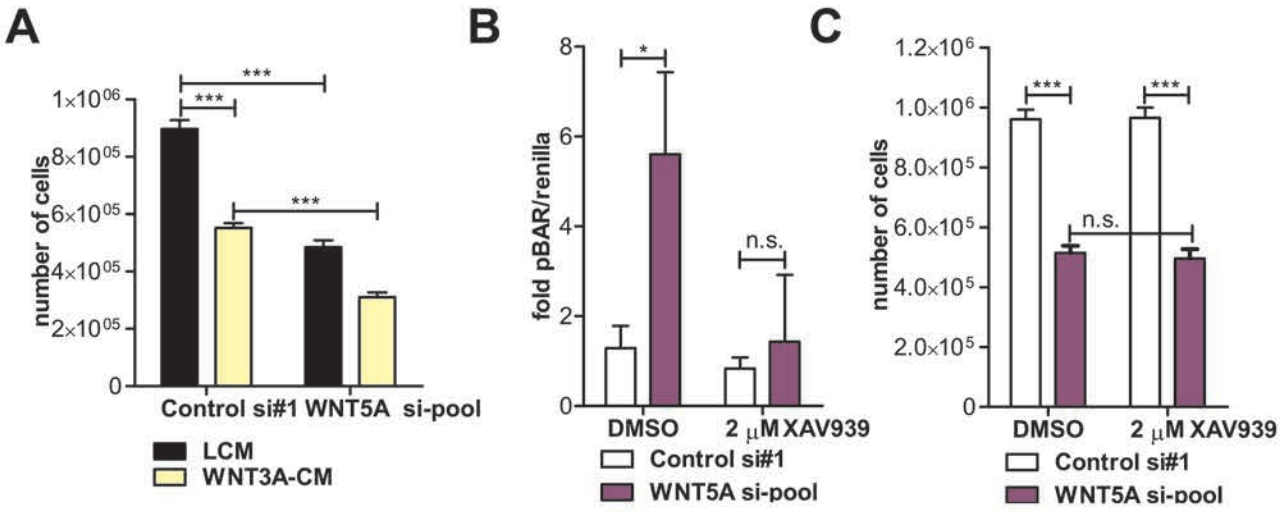
Supplemental Figure 1

Analysis and manipulation of WNT5A expression in melanoma cell lines. (**A**) Immunoblot analysis of whole cell lysates collected three days following transfection with control siRNA, WNT5A siRNAs, BRAF siRNA, or a pool of WNT5A siRNAs. (**B**) *WNT5A* transcript expression normalized to *GAPDH* in various melanoma cell lines (black bars) and HEMa-LP (melanocytes, white bar) determined by RT-PCR. (**C**) *WNT5A* transcript expression normalized to *18S* rRNA in A375 and MEL624 as determined by RT-PCR following transfection with the indicated siRNAs. (**D**) A375 cell number following siRNA transfection and treatment with L-cell conditioned media (-CM). Two days following transfection with siRNAs A375 cells were re-plated in the presence of control L-cell conditioned media (LCM), or in the presence of conditioned media from L-cells overexpressing WNT5A (WNT5A-CM) and allowed to grow for a period of three days before counting. (***P>0.001, ANOVA).



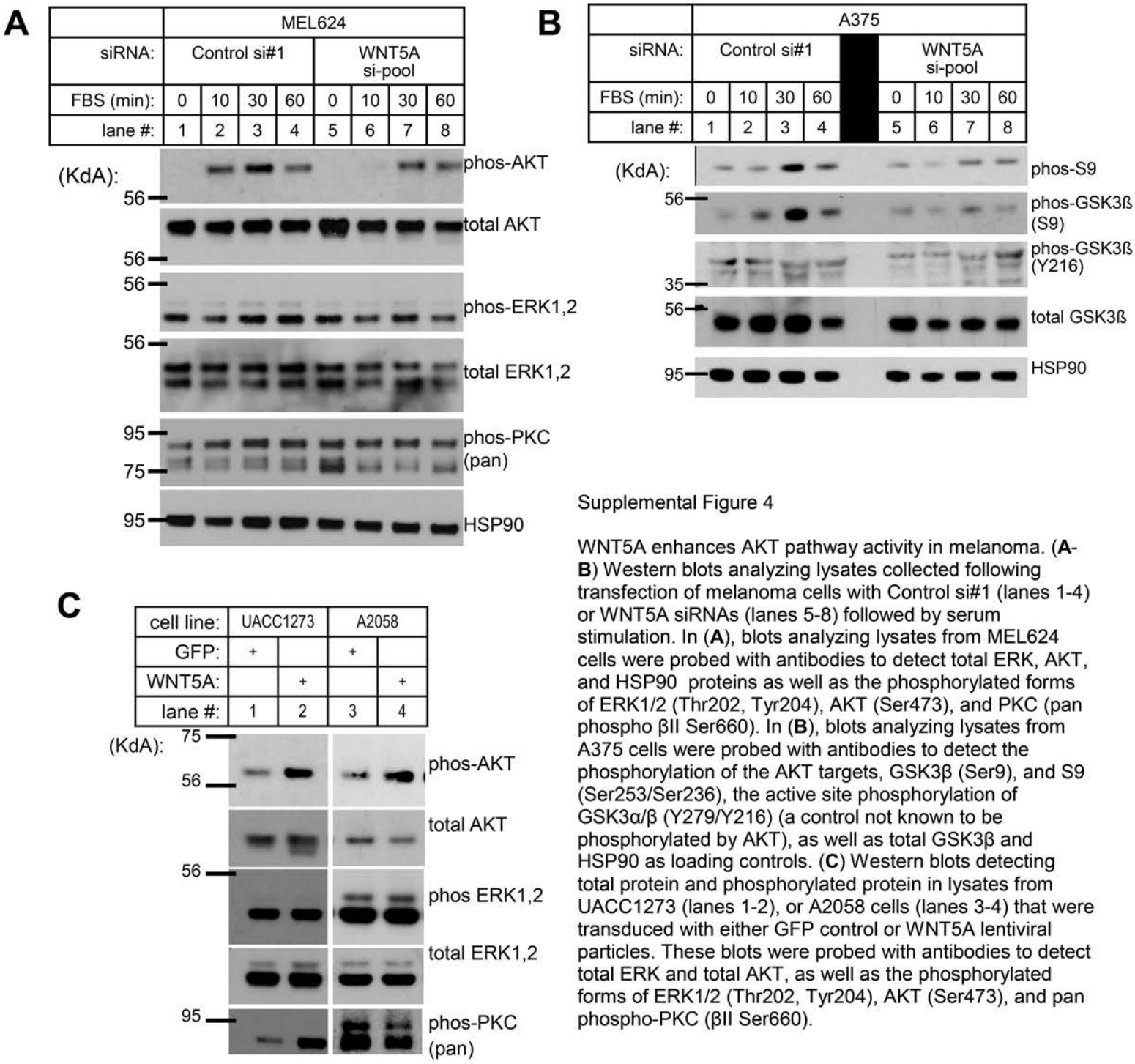
Supplemental Figure 2

WNT5A negatively regulates WNT/β-catenin signaling in melanoma. (**A**-**B**) Average normalized activity of a WNT/β-catenin-activated luciferase reporter (pBAR) following treatment of (**A**), A375 cells or (**B**), A2058 cells, with either control LCM, WNT5A-CM, or WNT3A-CM. (**C**) Average fold increase in pBAR luciferase reporter activity induced in response to increasing doses of WNT3A-CM relative to LCM treatment following transfection with either negative Control si#1, CTNNB1 siRNA (positive control targeting β-catenin), or WNT5A siRNAs. (**D**) Average normalized activity of pBAR luciferase following treatment with increasing doses of WNT3A-CM as indicated on the X-axis, combined with either LCM control media or two concentrations of WNT5A-CM (10% and 50%). Data were obtained from MEL624 (left), A2058 (center), and A375 (right) cells. Transcriptional reporter data in panels, (**A**-**D**) are representative of at least three independent experiments conducted using each cell line, with error bars indicating the standard deviation calculated from biological triplicates.



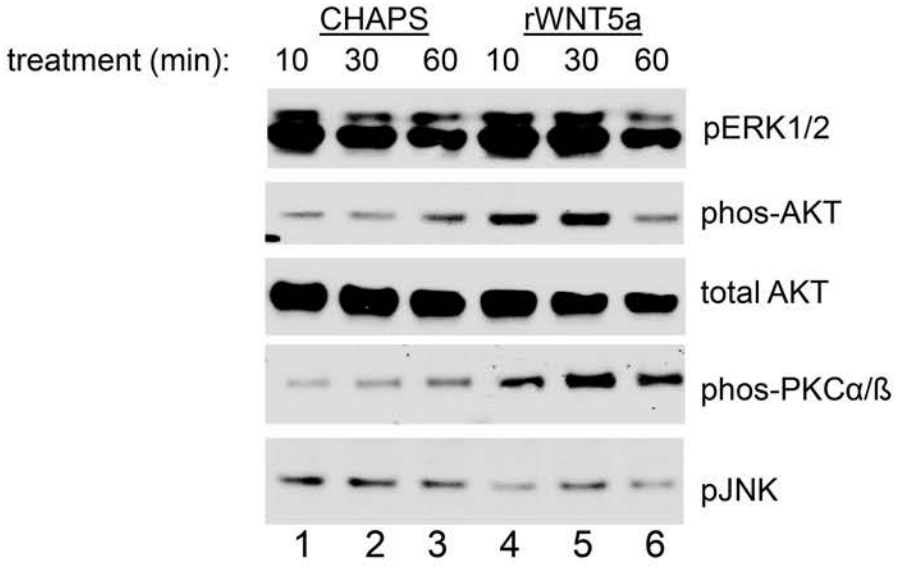
Supplemental Figure 3

WNT5A inhibition of WNT/β-catenin signaling does not account for growth defects observed due to WNT5A depletion. (**A**) Average number of cells counted following transfection with either Control siRNA #1 or pooled WNT5A siRNAs (2 days) and subsequent treatment with either LCM or WNT3A-CM for three days. Error bars indicate standard deviation from the mean from three experiments. (**B**) Average normalized activity of pBAR luciferase following transfection with the indicated siRNAs followed by treatment with 2 μM XAV939 for three days (*$p<0.05$). Data are representative of three independent experiments with error bars indicating the standard deviation calculated from biological triplicates. (**C**) Average number of cells following transfection with the indicated siRNAs and treatment with 2 μM XAV939 for three days (***$p<0.001$). Error bars indicate standard deviation from the mean from three experiments.



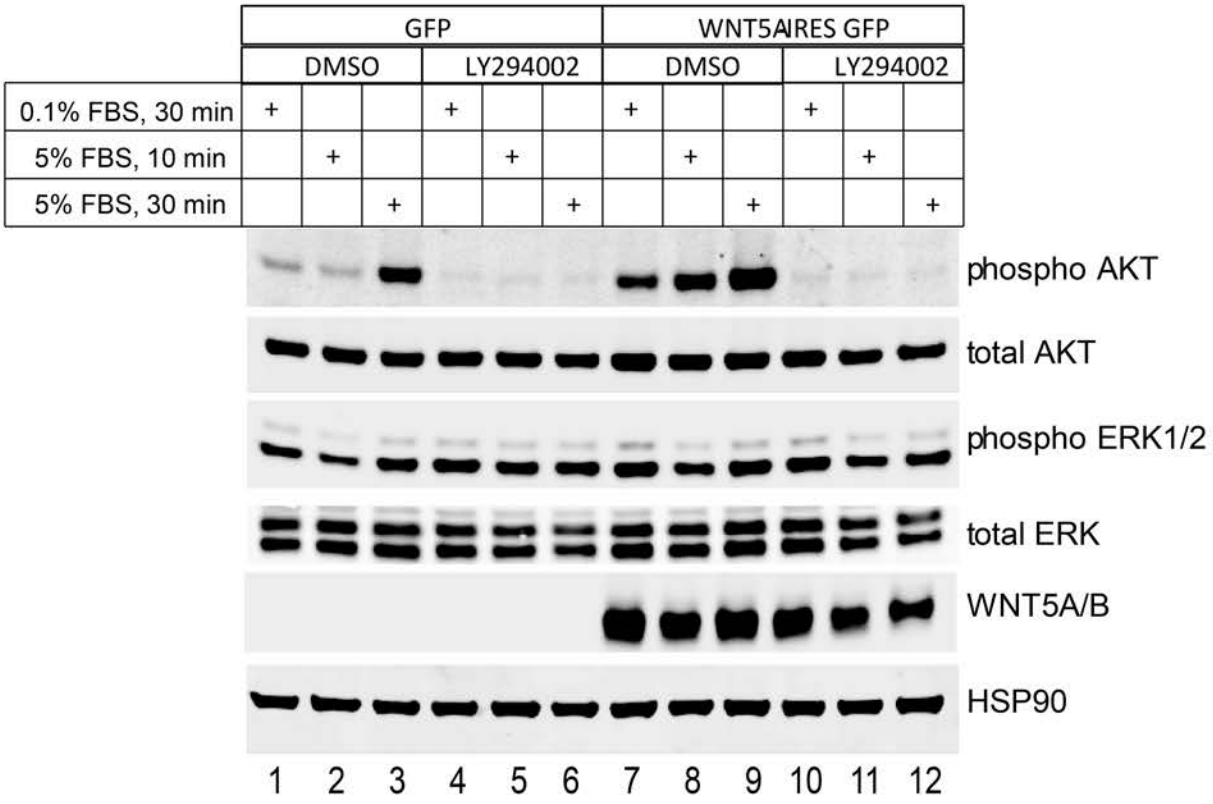
Supplemental Figure 4

WNT5A enhances AKT pathway activity in melanoma. (**A-B**) Western blots analyzing lysates collected following transfection of melanoma cells with Control si#1 (lanes 1-4) or WNT5A siRNAs (lanes 5-8) followed by serum stimulation. In (**A**), blots analyzing lysates from MEL624 cells were probed with antibodies to detect total ERK, AKT, and HSP90 proteins as well as the phosphorylated forms of ERK1/2 (Thr202, Tyr204), AKT (Ser473), and PKC (pan phospho βII Ser660). In (**B**), blots analyzing lysates from A375 cells were probed with antibodies to detect the phosphorylation of the AKT targets, GSK3β (Ser9), and S9 (Ser253/Ser236), the active site phosphorylation of GSK3α/β (Y279/Y216) (a control not known to be phosphorylated by AKT), as well as total GSK3β and HSP90 as loading controls. (**C**) Western blots detecting total protein and phosphorylated protein in lysates from UACC1273 (lanes 1-2), or A2058 cells (lanes 3-4) that were transduced with either GFP control or WNT5A lentiviral particles. These blots were probed with antibodies to detect total ERK and total AKT, as well as the phosphorylated forms of ERK1/2 (Thr202, Tyr204), AKT (Ser473), and pan phospho-PKC (βII Ser660).



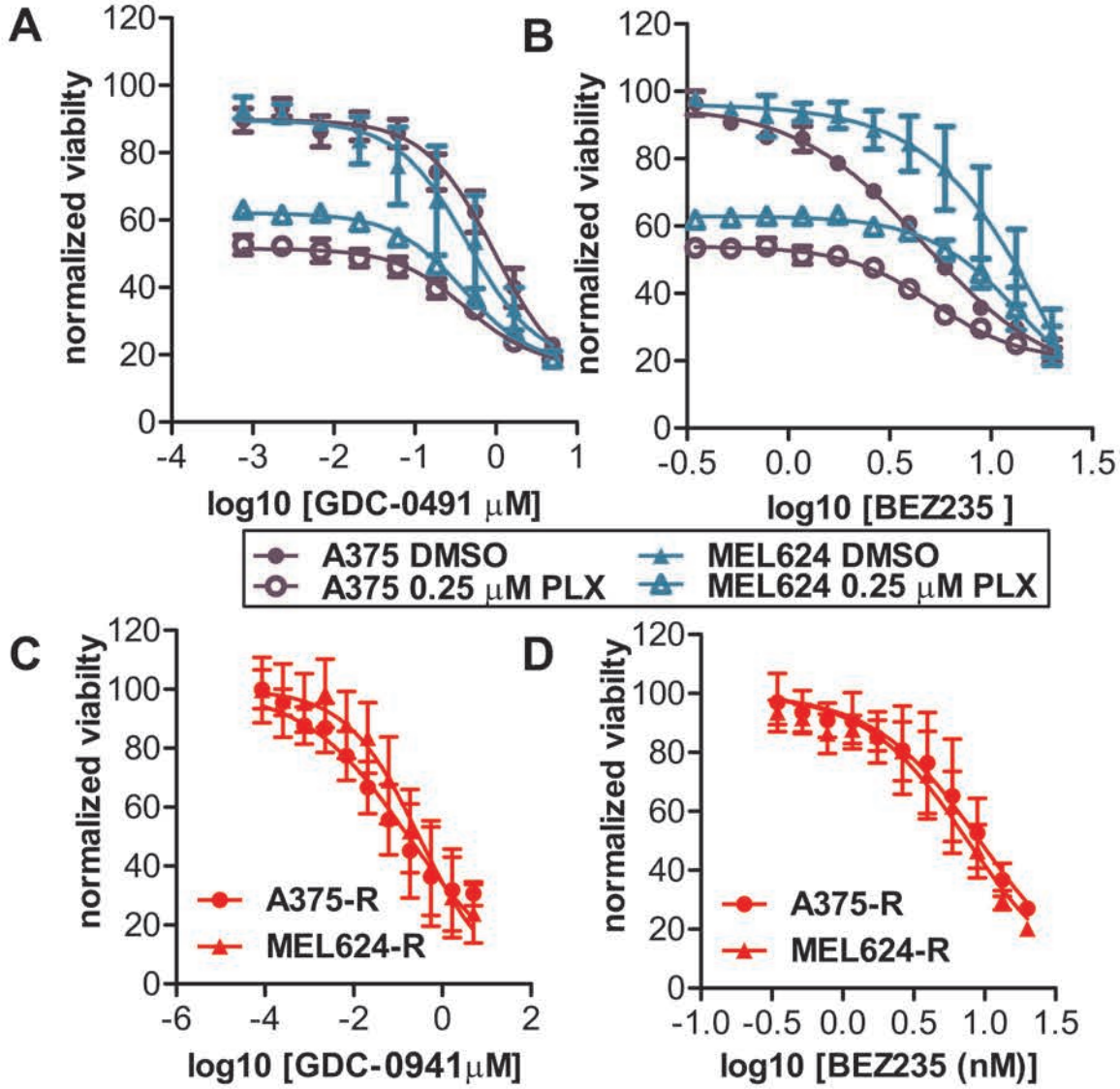
Supplemental Figure 5

Recombinant WNT5A can induce AKT phosphorylation in melanoma. Western blots of cell lysates from A2058 cells following treatment with either CHAPS vehicle control or 100 ng/mL recombinant human WNT5A (Stem Cell Technologies) for 10, 30 or 60 minutes.



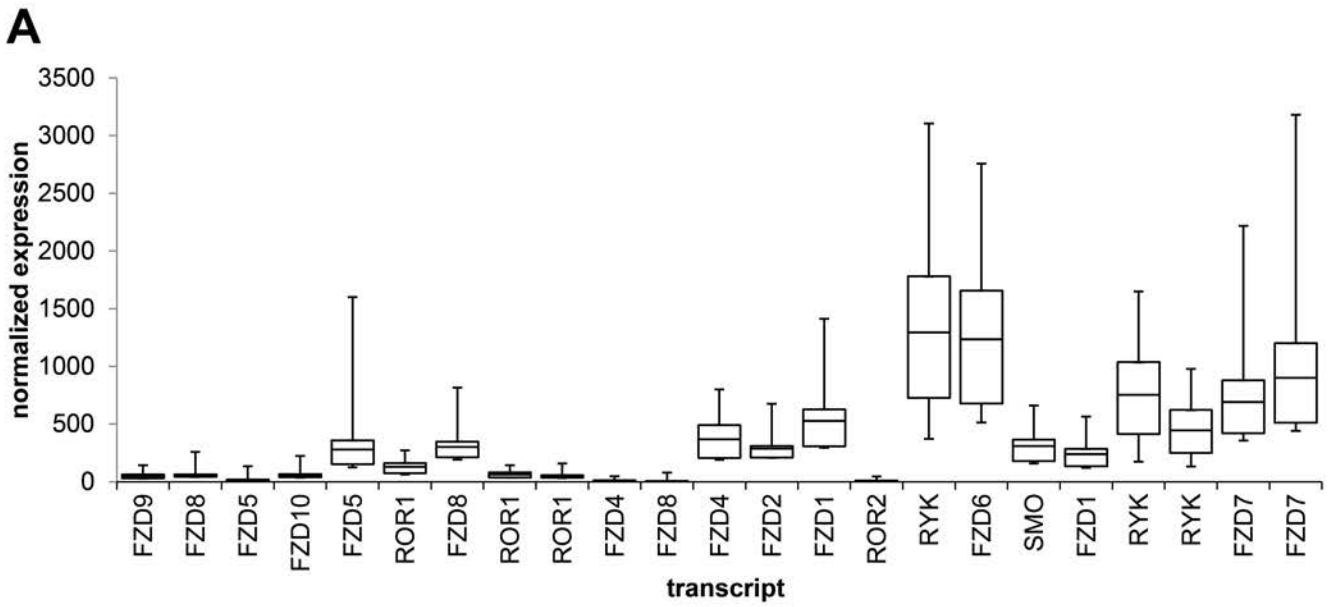
Supplemental Figure 6

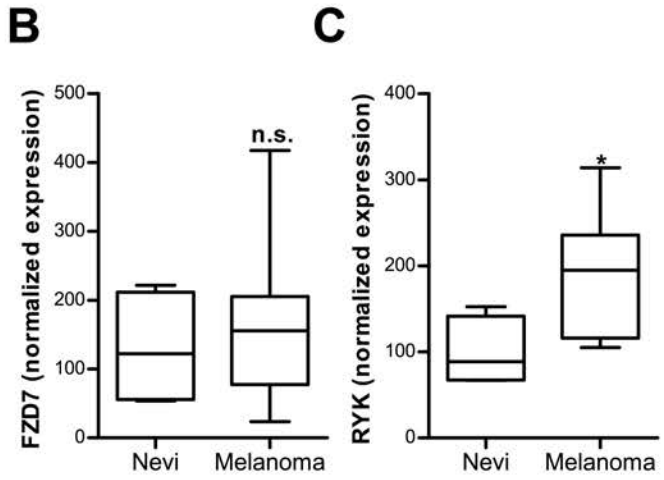
WNT5A promotes AKT phosphorylation in a PI3K-dependent manner. UACC1273 melanoma cells expressing either GFP control, or WNT5A were serum starved for twenty hours, pre-treated with either vehicle or LY294002 for 60 minutes to inhibit PI3K activity and then stimulated with 5% FBS for 10 or 30 minutes to activate PI3K signaling. After these combinations of treatments lysates were collected and Western blots were analyzed using antibodies to detect total ERK and total AKT, as well as the phosphorylated forms of ERK1/2 (Thr202, Tyr204), AKT (Ser473), HSP90, and WNT5A/B.



Supplemental Figure 7

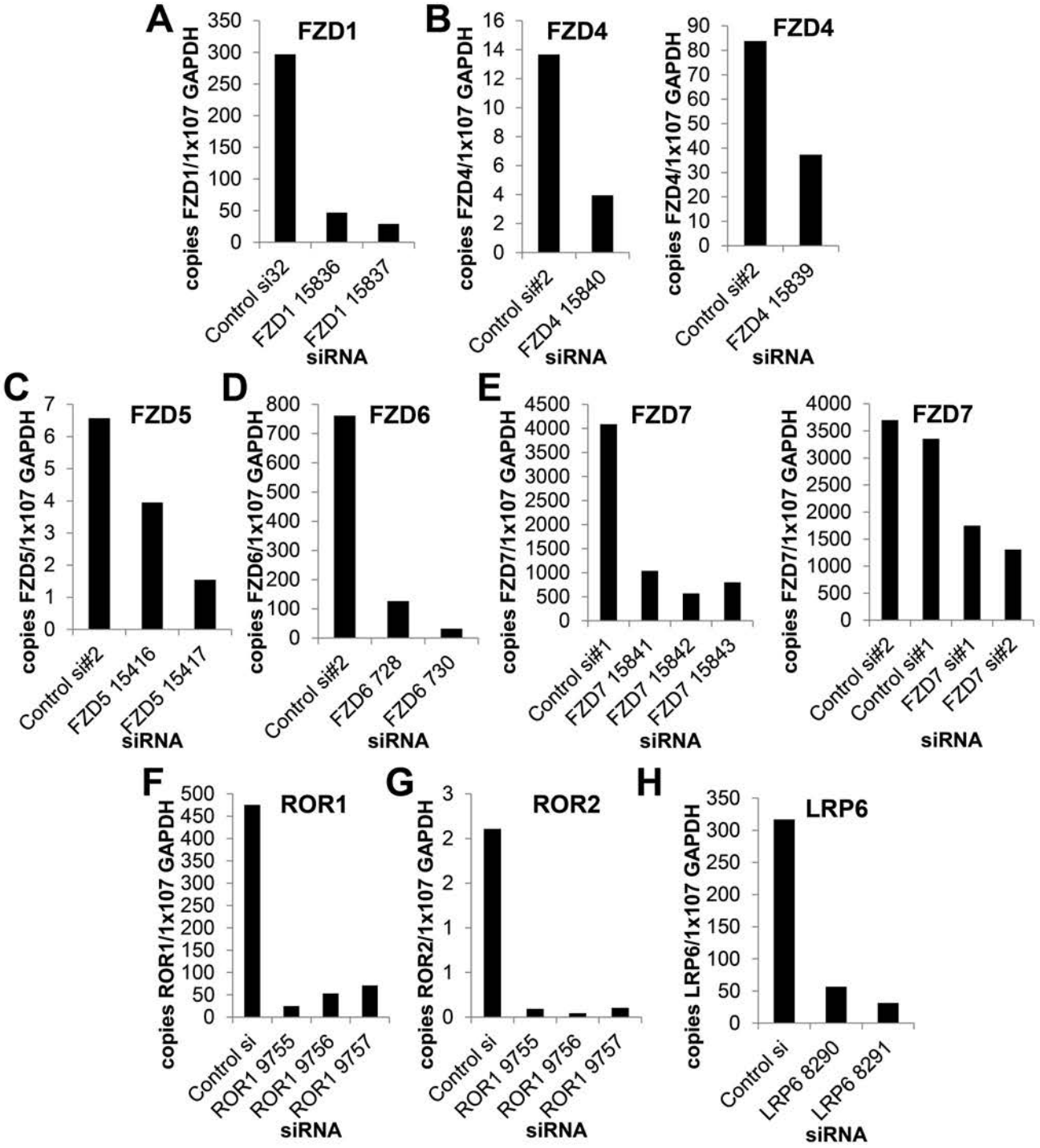
Both parental and BRAFi-resistant cells require AKT pathway activity for optimum growth. (**A**-**B**) Normalized viability of A375 cells (blue lines) and MEL624 cells (purple lines) treated with either DMSO control, or 0.25 μM PLX4720 (open circles and triangles). In (**A**), cells were treated with increasing concentrations of GDC-0941 in combination with PLX4720 or DMSO. In (**B**), cells were treated with increasing concentrations of BEZ235 in addition to PLX4720 or DMSO. (**C**-**D**) Normalized viability of A375-R (circles) and MEL624 (triangles) grown in the presence of 2 μM PLX4720 and increasing concentrations of (**C**), GDC-0941 or increasing concentrations of (**D**), BEZ235. Error barns indicate the standard deviation calculated from four experiments. Best fit curves were modeled using GraphPad software and a nonlinear regression model.





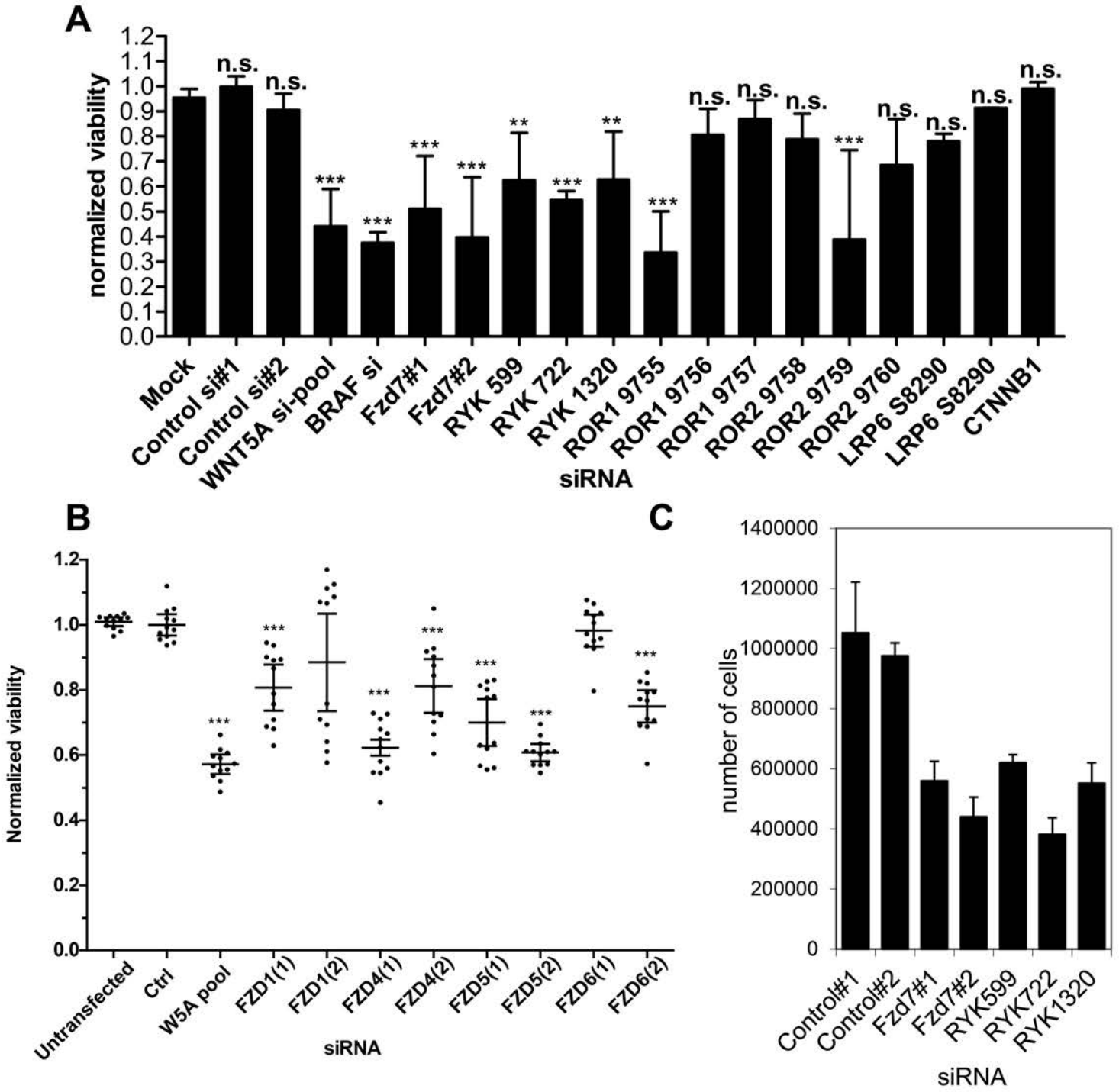
Supplemental Figure 8

Expression of WNT receptors in melanoma and normal human skin. (**A**) Normalized expression of WNT receptors in melanoma relative to other normal and cancerous tissues from a meta-analysis of gene expression patterns in 950 samples (Array express, E-MTAB-37).*FZD6* ($p=4.93\times10^{-5}$), *FZD7* (probe 1, $p= 3.84\times10^{-9}$, and probe 2, $p= 4.94\times10^{-10}$) and RYK (probe 1, $p= 4.76\times10^{-4}$, probe 2, $p= 2.80\times10^{-6}$ and probe 3, $p= 1.45\times10^{-6}$) were significantly up-regulated in melanoma. (**B**-**D**) Normalized expression of (**B**), *FZD7*, and (**D**), *RYK* in skin and in melanoma as determined by analysis of publically available microarray dataset (54).



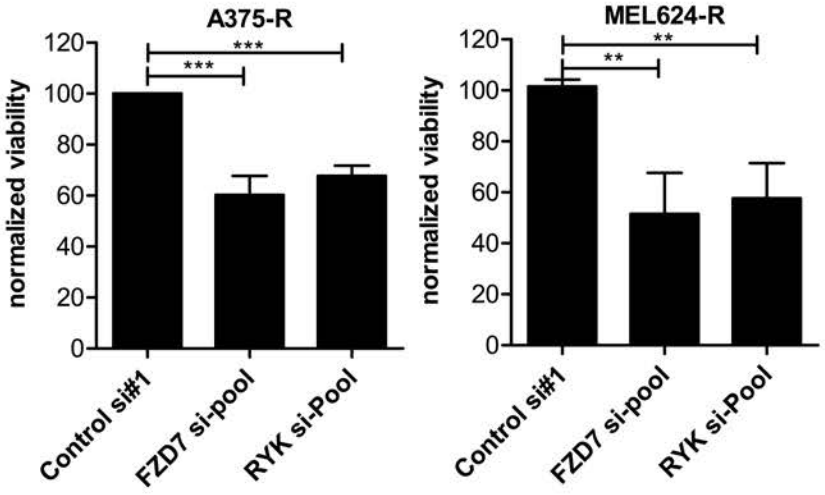
Supplemental Figure 9

Validation of siRNAs targeting various WNT receptors used in this study. (**A**)-(**H**) A375 cells were transfected with various siRNAs and then RNA was purified and quantified by RTPCR. Graphs show average copies of each of the indicated receptor mRNAs normalized to *GAPDH* as follows: (**A**) *FZD1*, (**B**) *FZD4*, (**C**) *FZD5*, (**D**) *FZD6*, (**E**) *FZD7*, (**F**) *ROR1*, (**G**) *ROR2*, and (**H**) *LRP6.*



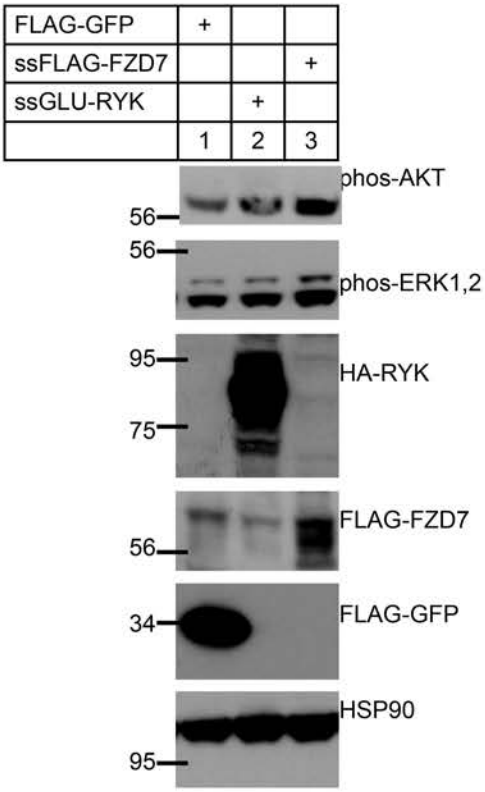
Supplemental Figure 10

The WNT receptors FZD7, FZD4, FZD5 and RYK enhance melanoma cell viability and growth. (**A-B**) Average cell viability of A375 cells plated at a density of 5,000 cells per well in 96-well plates two days following transfection with various siRNAs targeting WNT receptors. Cells were allowed to grow for three days following plating in 96-well plates and cell viability was determined using resazurine as a metabolic indicator. In (**A**), error bars show standard deviation of at least three experiments with quadruplicate wells for each siRNA. siRNAs resulting in a reduction in cell viability compared were determined by One-Way ANOVA. (** $P<0.01$, *** $P<0.001$). In (**B**), error bars show standard deviation of two experiments with 6 biological replicates. *** $P<0.0001$ in one-way ANOVA with Dunnett's post-test comparison of columns to control. (**C**) Average number of A375 cells following transfection with Control siRNAs (#1 and #2) and siRNAs targeting *FZD7* and *RYK*. In these experiments A375 cells were transfected with siRNAs and allowed to grow for 48 hours, and then split to a lower cell density, allowed to grow for three additional days and then counted.



Supplemental Figure 11

BRAFi-resistant melanoma cells require FZD7 and RYK for optimal growth and viability. Normalized viability of A375-R cells (left) and MEL624-R cells following transfection with either Control si#1 or pooled siRNAs targeting FZD7 and RYK. Data are normalized to Control si#1 set to 100% and error bars indicate standard deviation calculated from three independent experiments. ***P<0.01* and ****P<0.001* by two-tailed Student's T-test.



Supplemental Figure 12

Overexpression of FZD7, but not RYK is sufficient to induce AKT phosphorylation in melanoma Western blots of lysates from A375 cells transfected with either (1) FLAG-GFP, (2) (2) ssFLAG-or (3) ssGLU-RYK. Blots were probed with antibodies to detect HA-tagged RYK, FLAG, the phosphorylated forms of ERK1/2 (Thr202, Tyr204), AKT (Ser473), and total AKT.

Gene	Product	cat #
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	Silencer Select	15837
h Fzd4	Silencer Select	15840
	Silencer Select	15839
h Fzd5	Silencer Select	15417
	Silencer Select	15415
h Fzd6	Silencer Select	728
	Silencer Select	730
h Fzd7	Silencer Select	15841
	Silencer Select	15842
	Silencer Select	15843
h LRP6	Silencer Select	8290
	Silencer Select	8291
h ROR1	Silencer Select	9755
	Silencer Select	9756
	Silencer Select	9757
h ROR2	Silencer Select	9758
	Silencer Select	9759
	Silencer Select	9760
h RYK	Stealth	599
	Stealth	722
	Stealth	1320

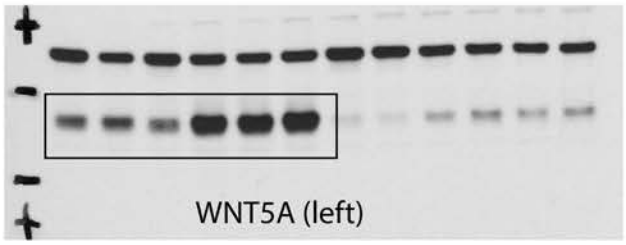
Gene	Product	cat #
h Wnt5a	Silencer Select	4546
		4642

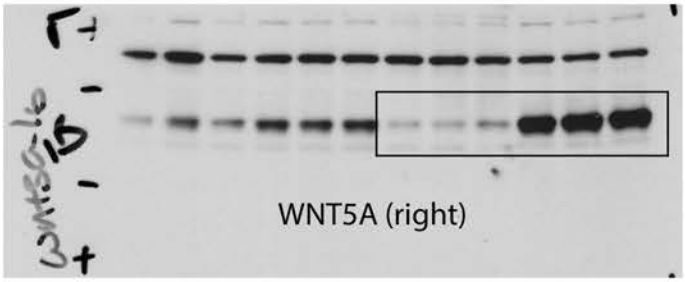
Supplementary Table 1: List of product numbers associated with the siRNAs used in this study. All siRNAs were purchased from Life Technologies.

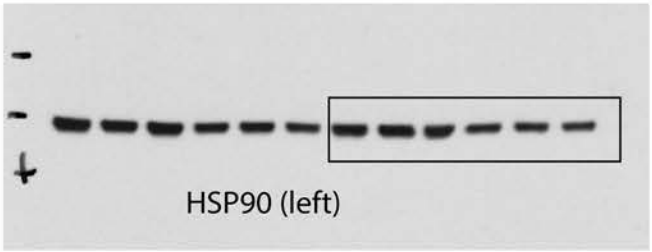
Gene	Forward Primer	Reverse Primer
h Fzd1	TGAGTGAGATAACCAATGCC	TCCCAAATCTCAGTAACCAC
h Fzd4	CCTCTCCTTTCACATTCCAG	ACAGTAAAGTTCAGCAGACC
h Fzd5	ATTCTCACTCCTAACAGCAC	TAAGAACCACCCACTACCTC
h Fzd6	GAGTGAAGGAAGGATTAGTCCA	TTGCTTCTGAGTAACGAGAG
h Fzd7	TACCATAGTGAACGAAGAGGA	TGTCAAAGGTGGGATAAAGG
h LRP6	ATCACTCAAGACCATCAGCA	CCATCCAACTTTGACACCTC
h ROR1	CAAGGAGGTGGTTTCTTCCA	ATTTCACATTCATCGCGACA
h ROR2	GACGTACCCTCGTGTAGTCC	CTTTGAGTTTGGCCTGTTTGTG
h RYK	TCTACCTGAGCGAGGACGAG	CCACTTGGAATCCCAGCTTA
h Wnt5a	GCCATGAAGAAGTCCATTGG	CTTCAATTACAACCTGGGCG
hGAPDH	TGAAGGTCGGAGTCAACGGA	CCATTGATGACAAGCTTCCCG
β-Actin	AGAGCAAGAGAGGCATCCTC	CTCAAACATGATCTGGGTCA

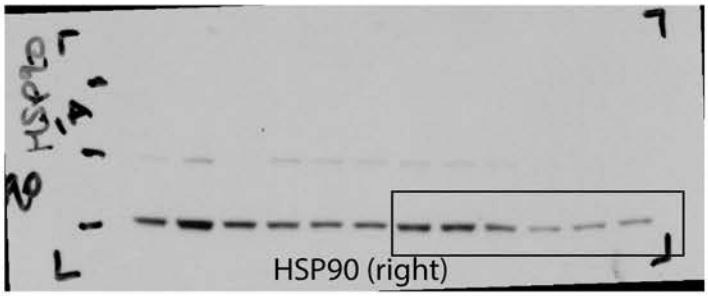
Supplementary Table 2: RT-PCR primer sequences.

Anastas et al. Full unedited gels for Figure 1C

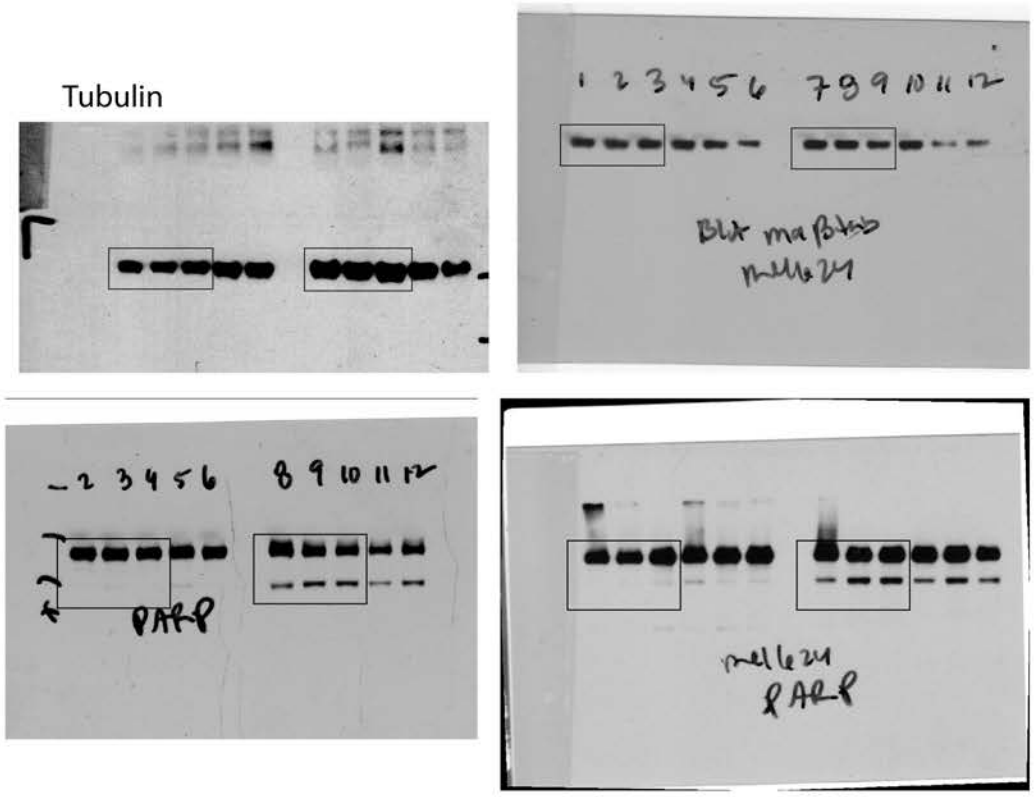




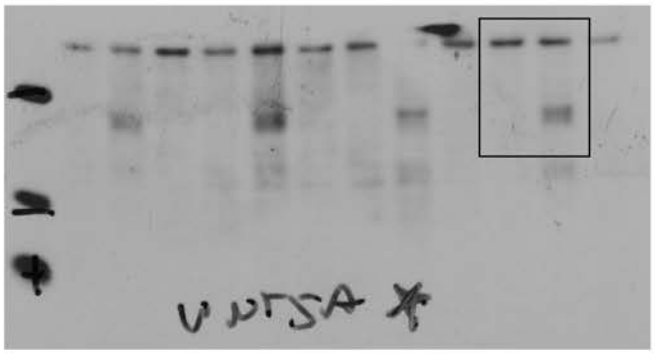


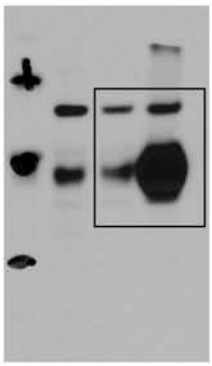


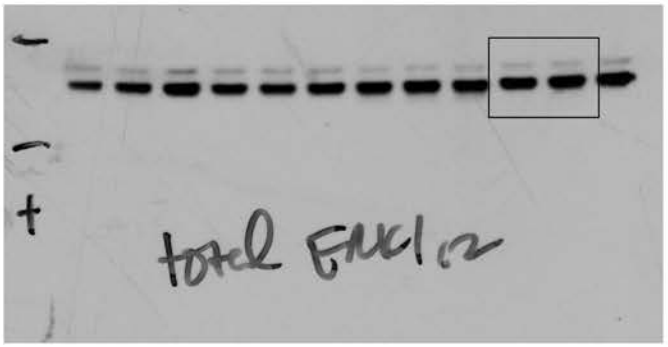
Anastas et al. Full unedited gels for Figure 3B

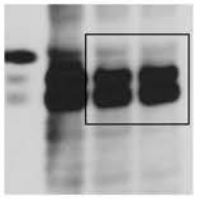


Anastas et al. Full unedited gels for Figure 4A

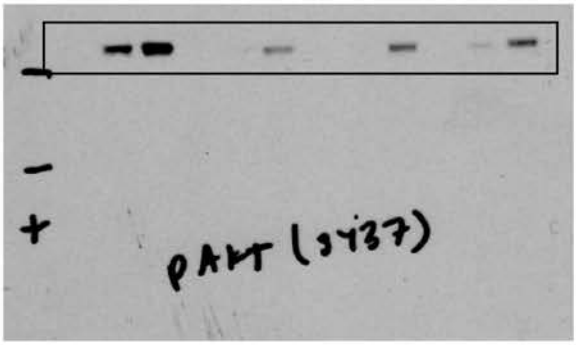








Anastas et al. Full unedited gels for Figure 5A



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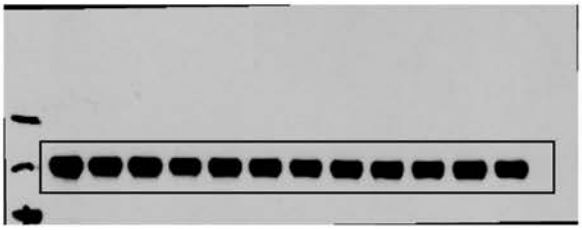
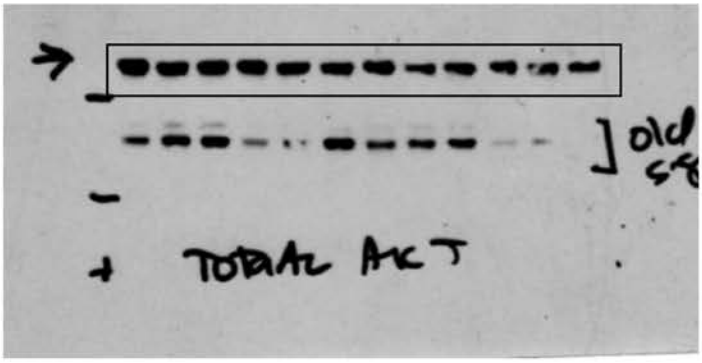
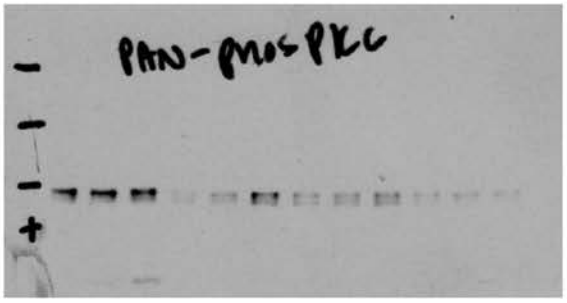
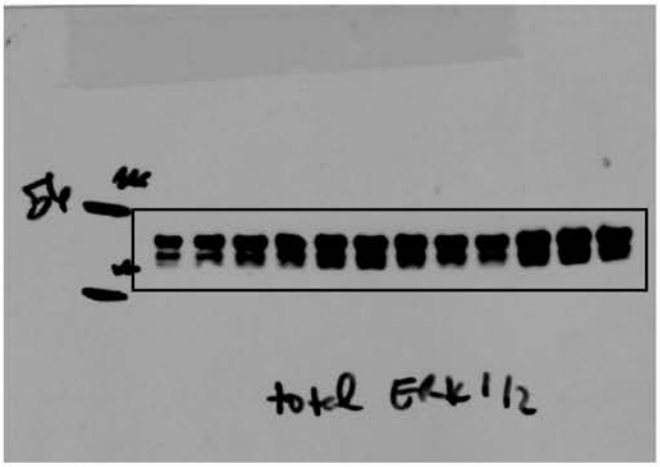


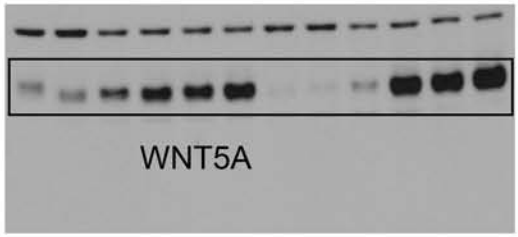


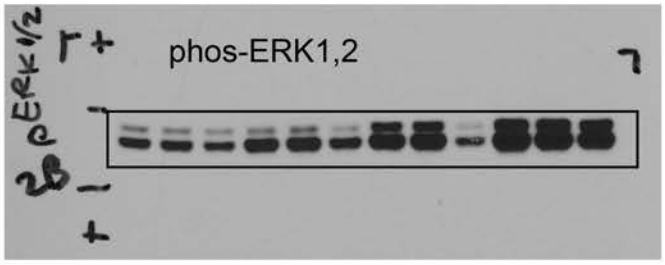


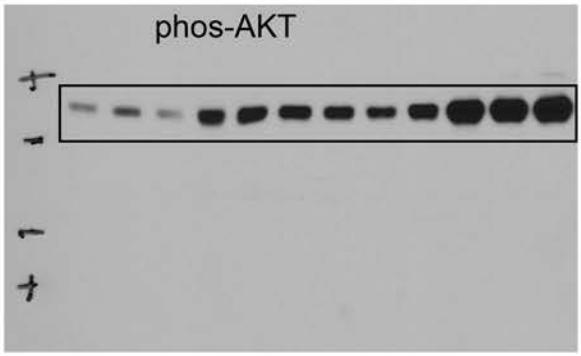


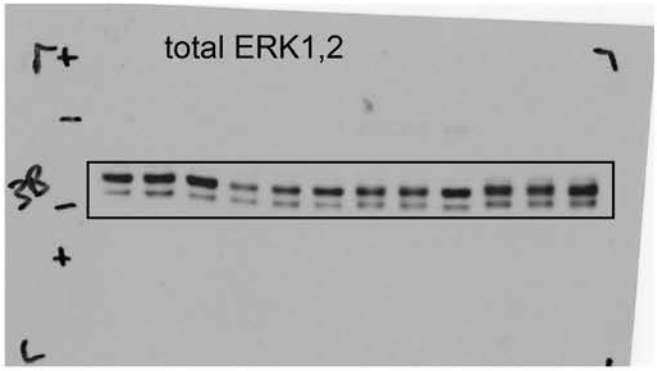
phospho ERK1/2

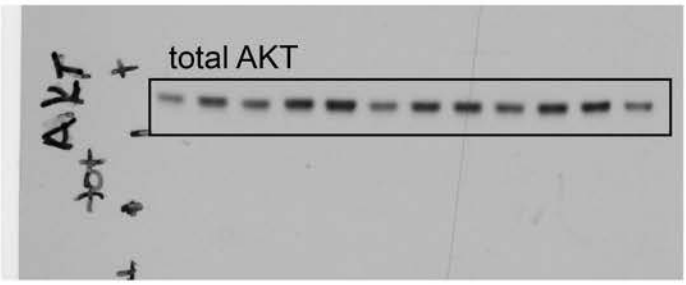
Anastas et al. Full unedited gels for Figure 5B

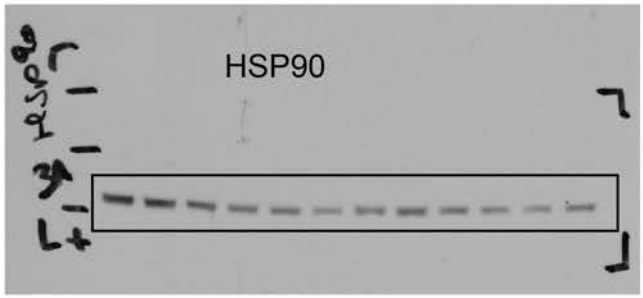




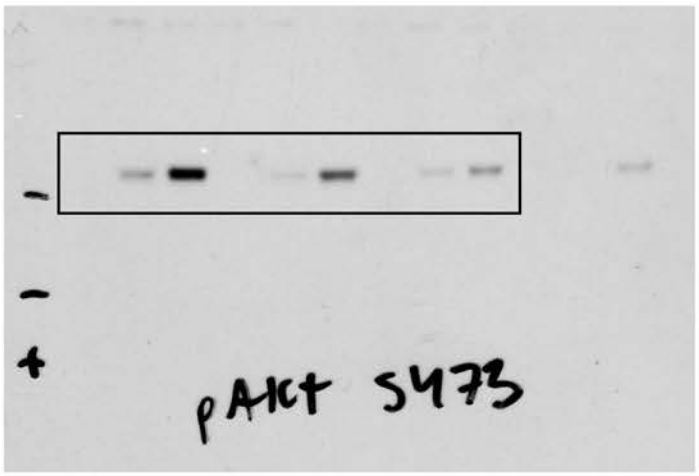


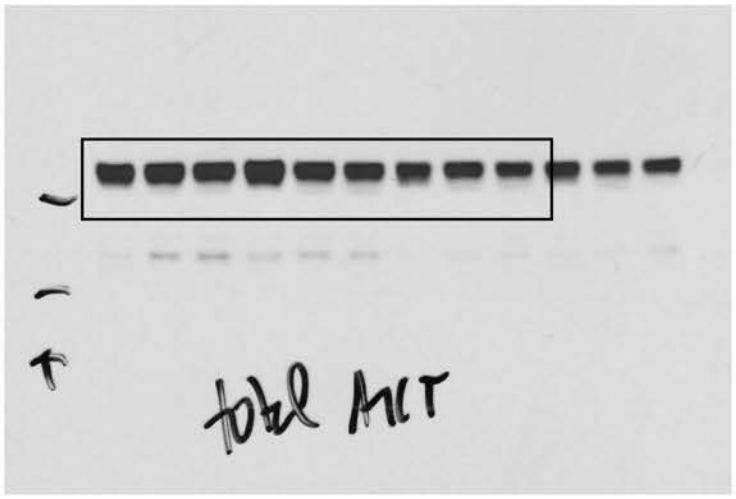


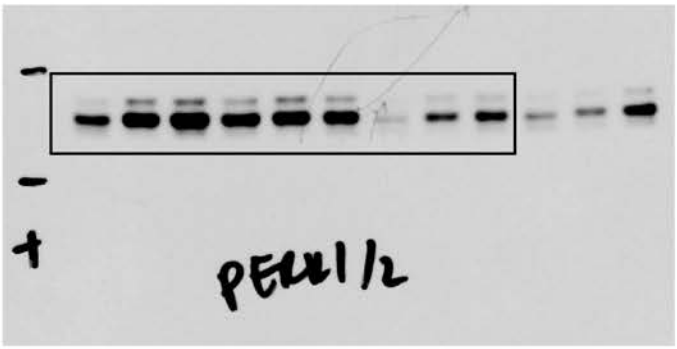


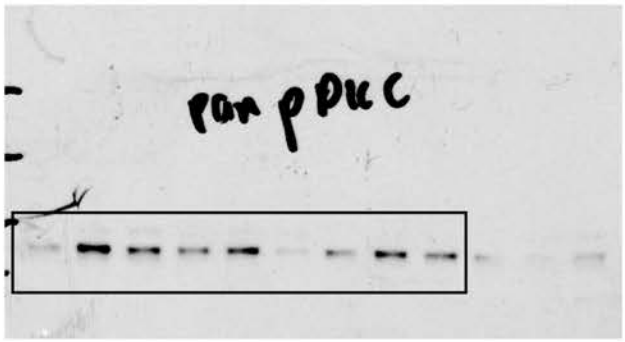


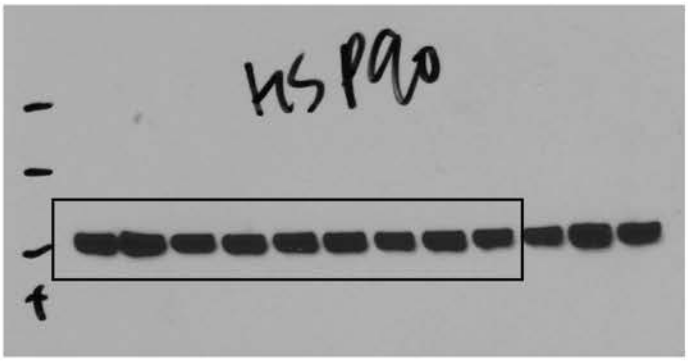
Anastas et al. Full unedited gel for Figure 6E

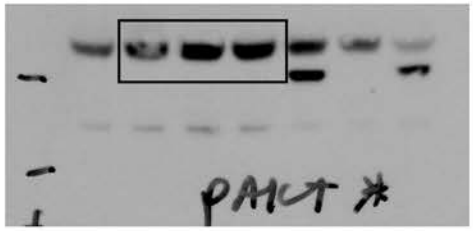




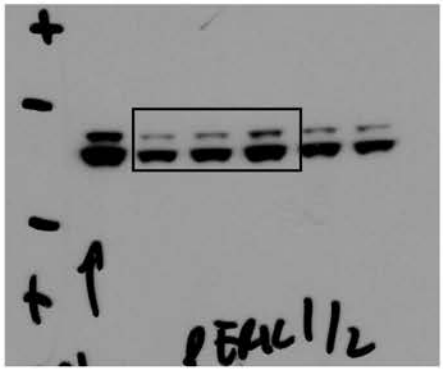








phos-AKT



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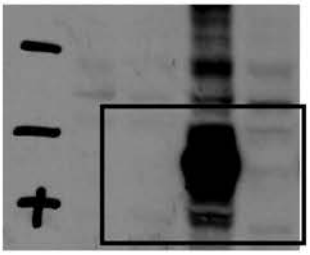

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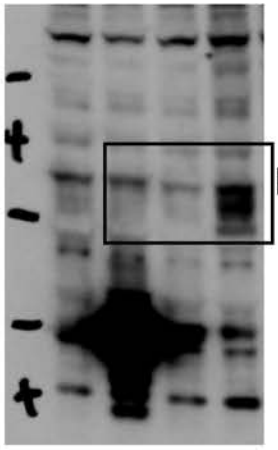

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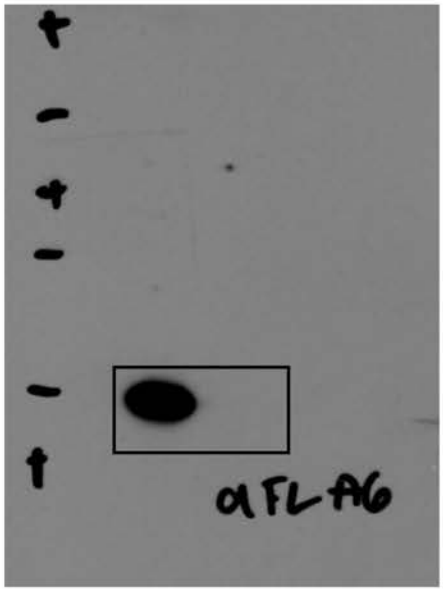

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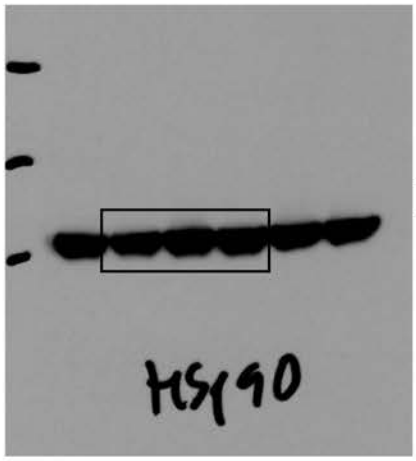
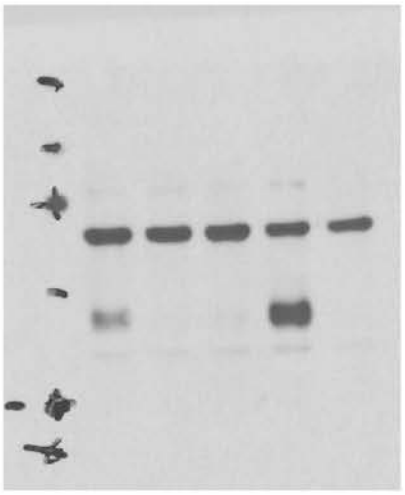
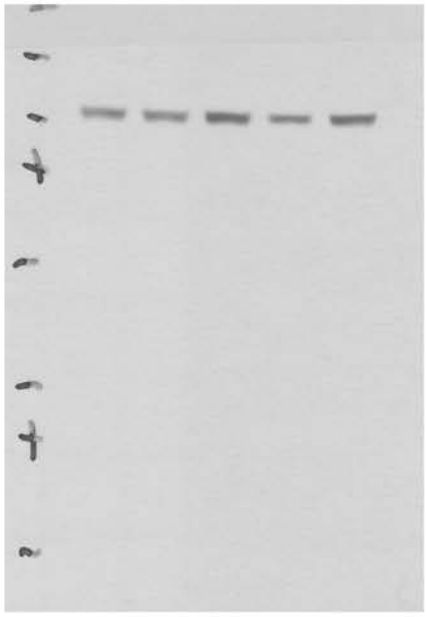


HSP90

Anastas et al. Full unedited gels for Supplemental Figure 1A



WNT5A



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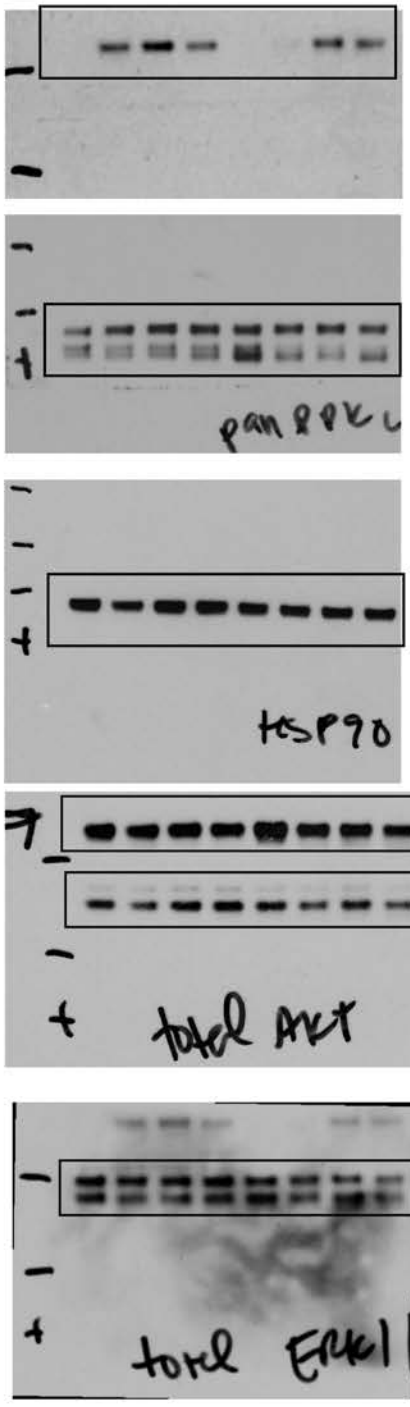
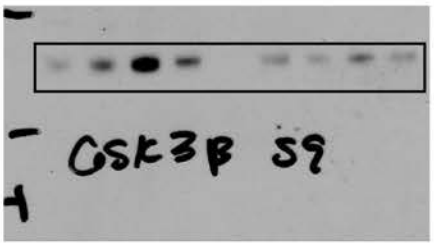
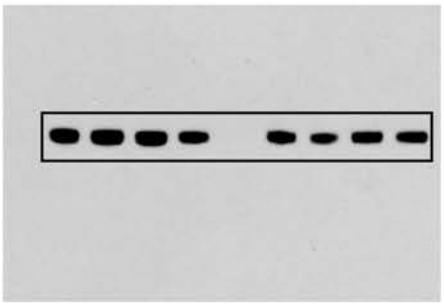
Anastas et al. Full unedited gels for Supplemental Figure 4A



Anastas et al. Full unedited gels for Supplemental Figure 4B



GSK3beta phospho-s9



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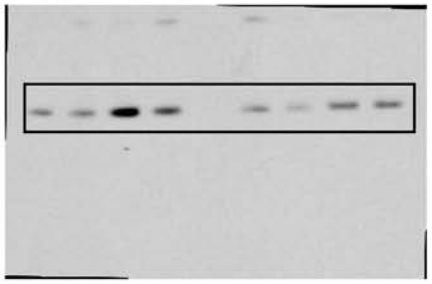
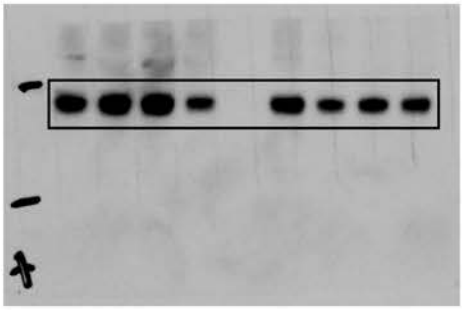
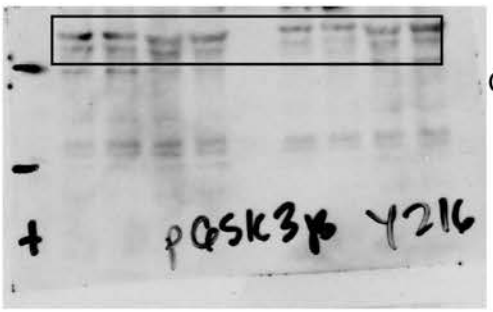


phospho-S9

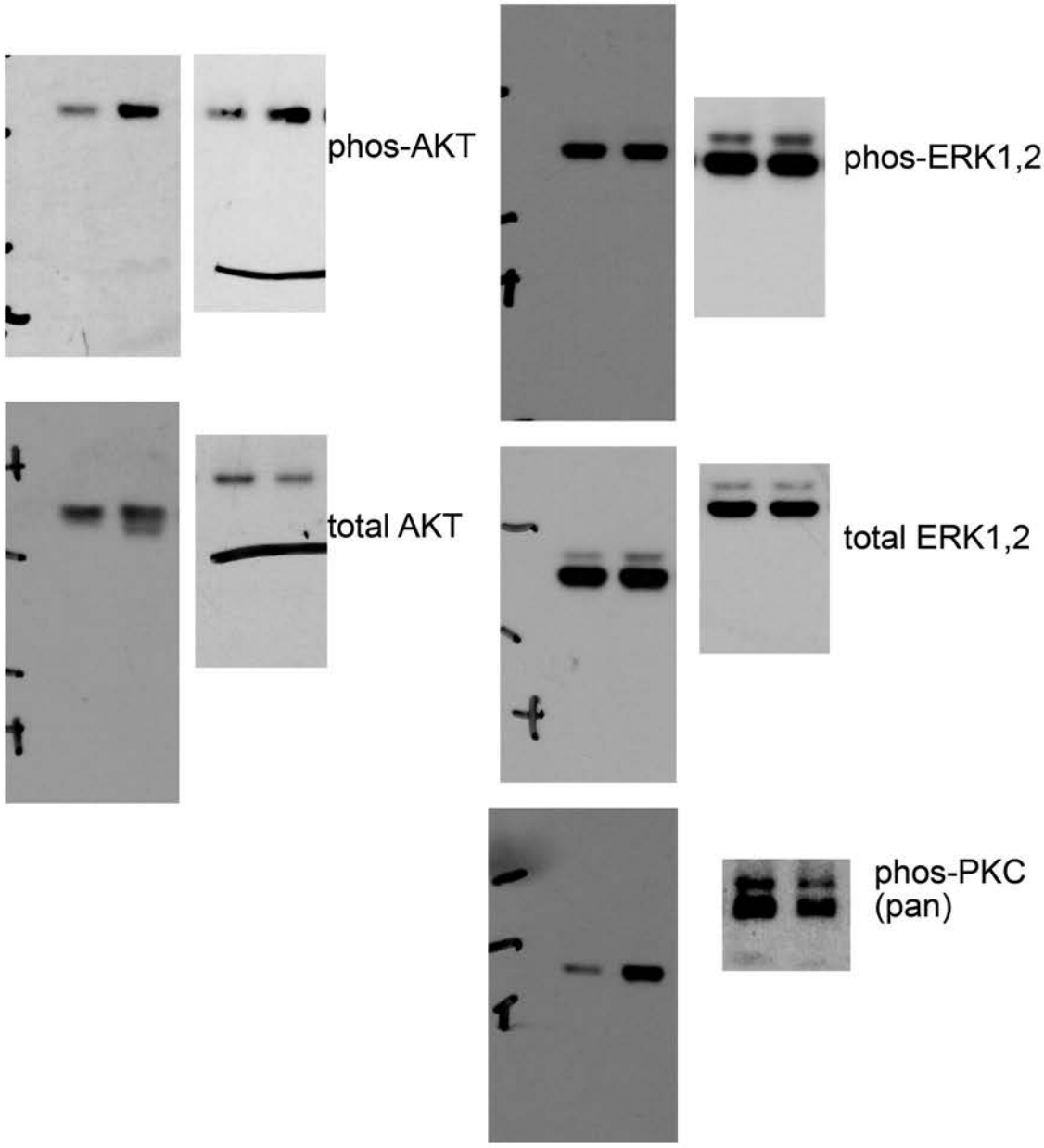


total GSK3beta

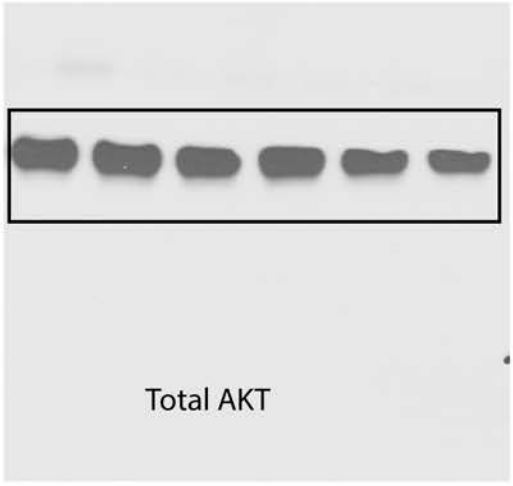


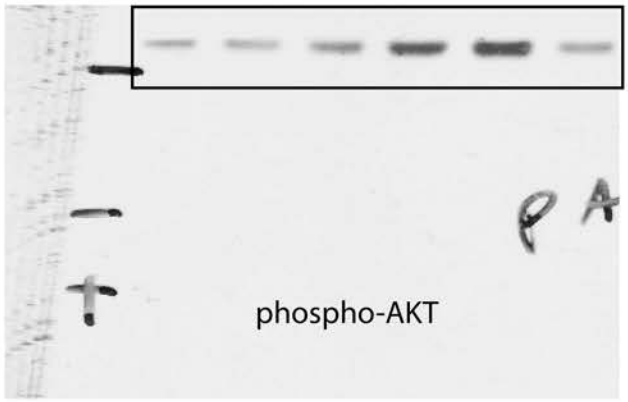
GSK3beta phospho-Y216

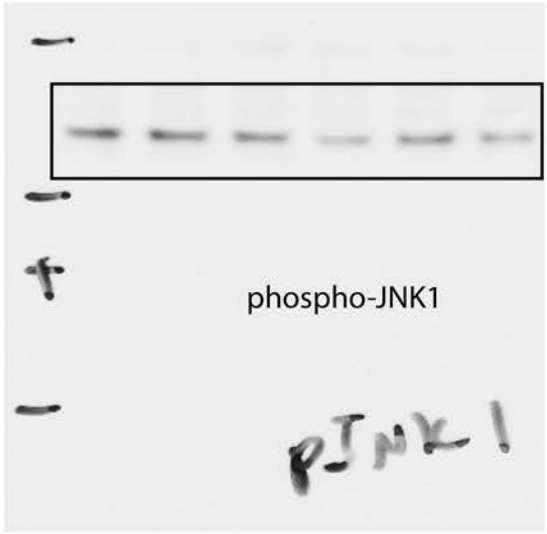
Anastas et al. Full unedited gels for Supplemental Figure 4C

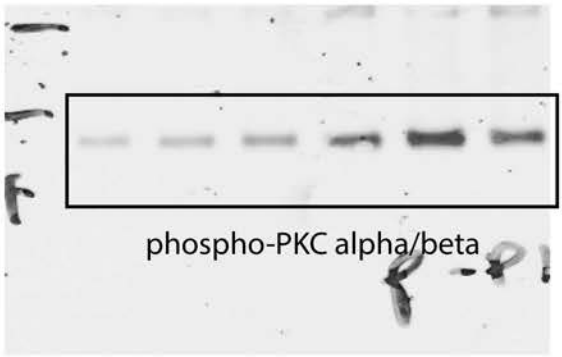


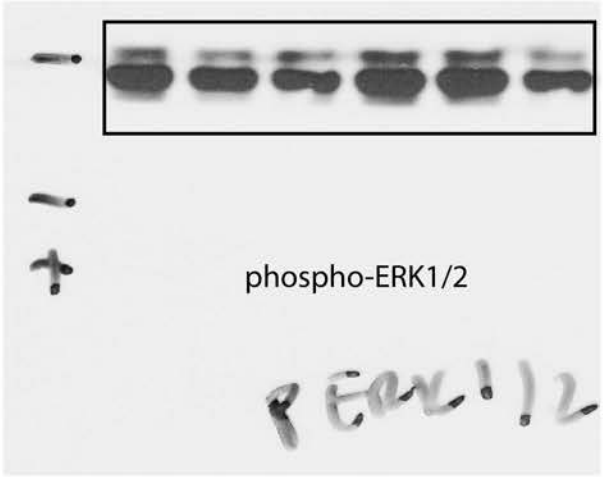
Anastas et al. Full unedited gels for Supplementary Figure S5

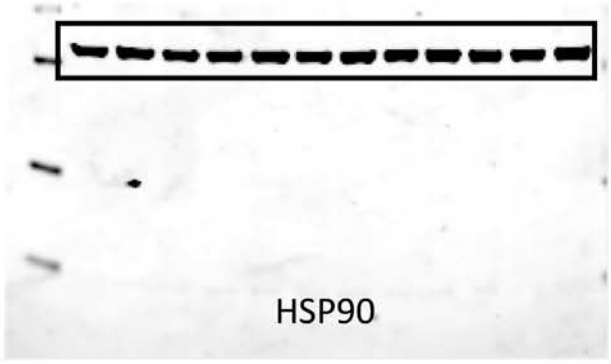








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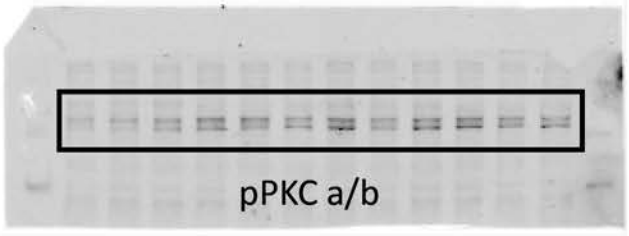
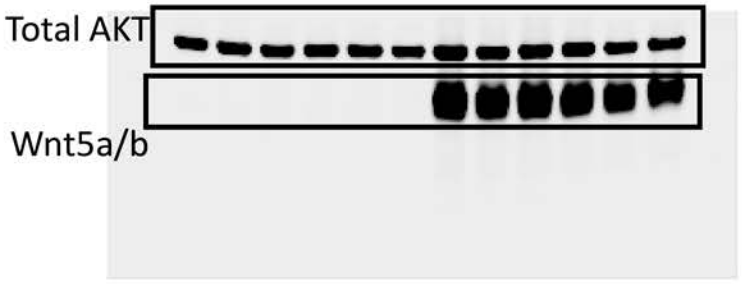
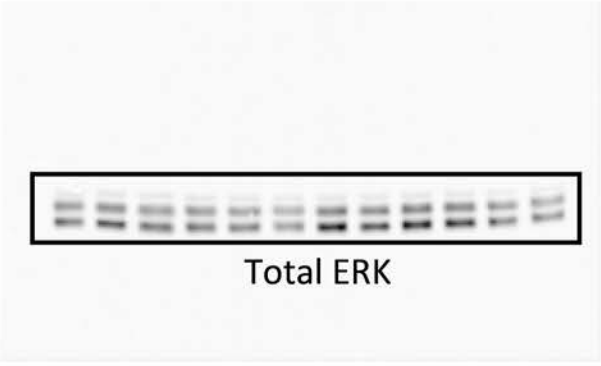

pPKC a/b


Total AKT
Wnt5a/b


Total ERK


phospho-AKT
phospho-ERK