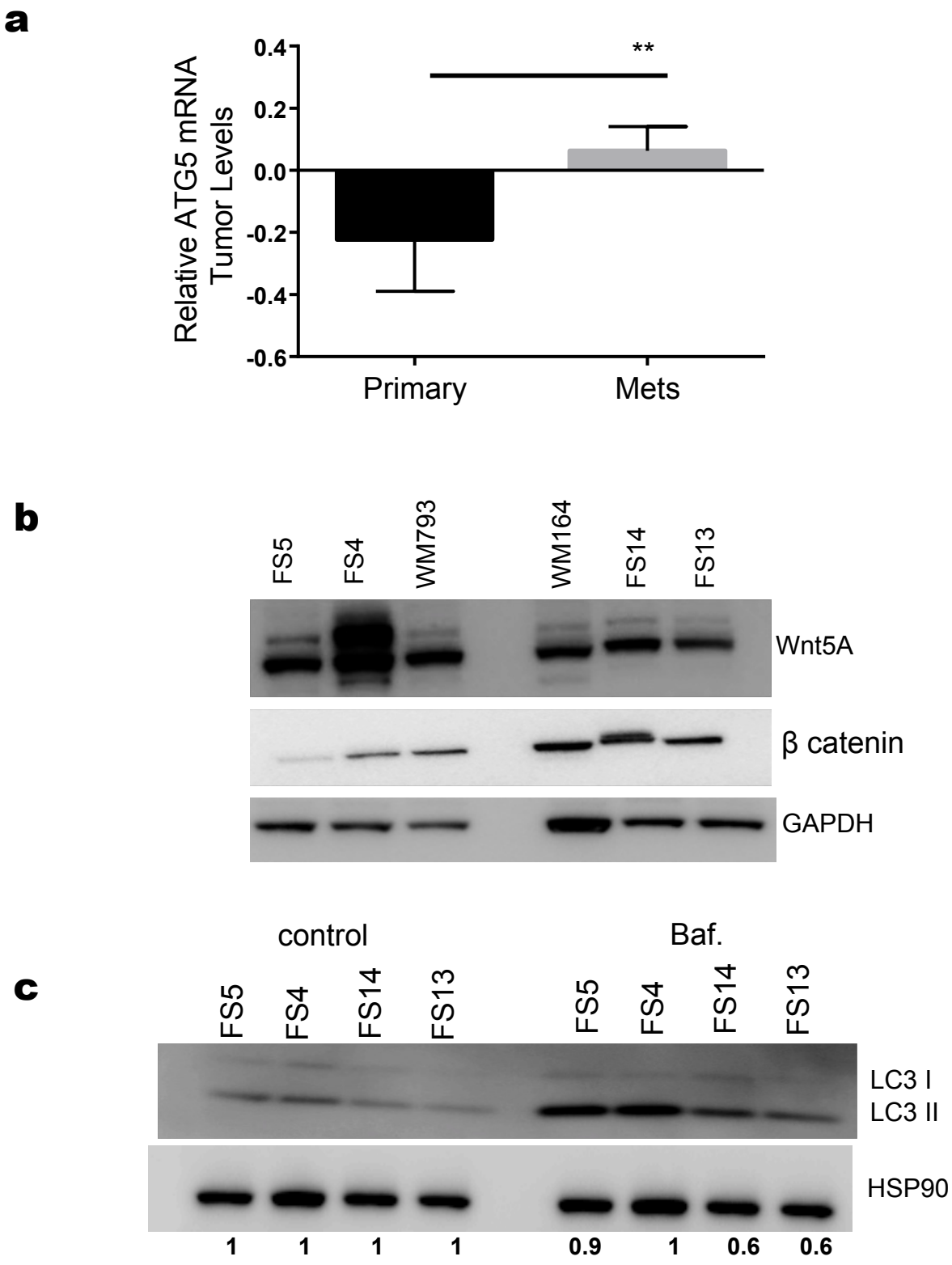
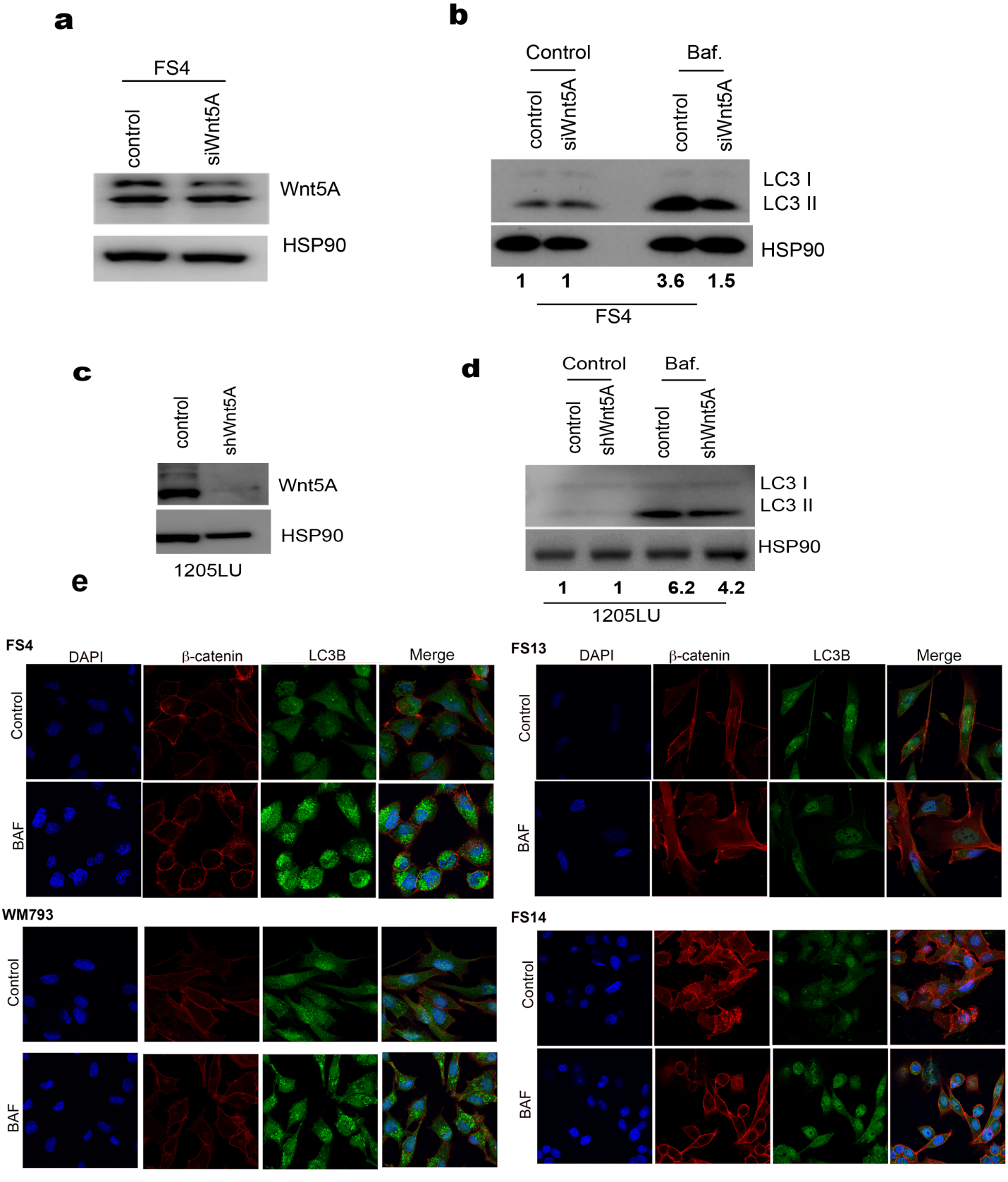
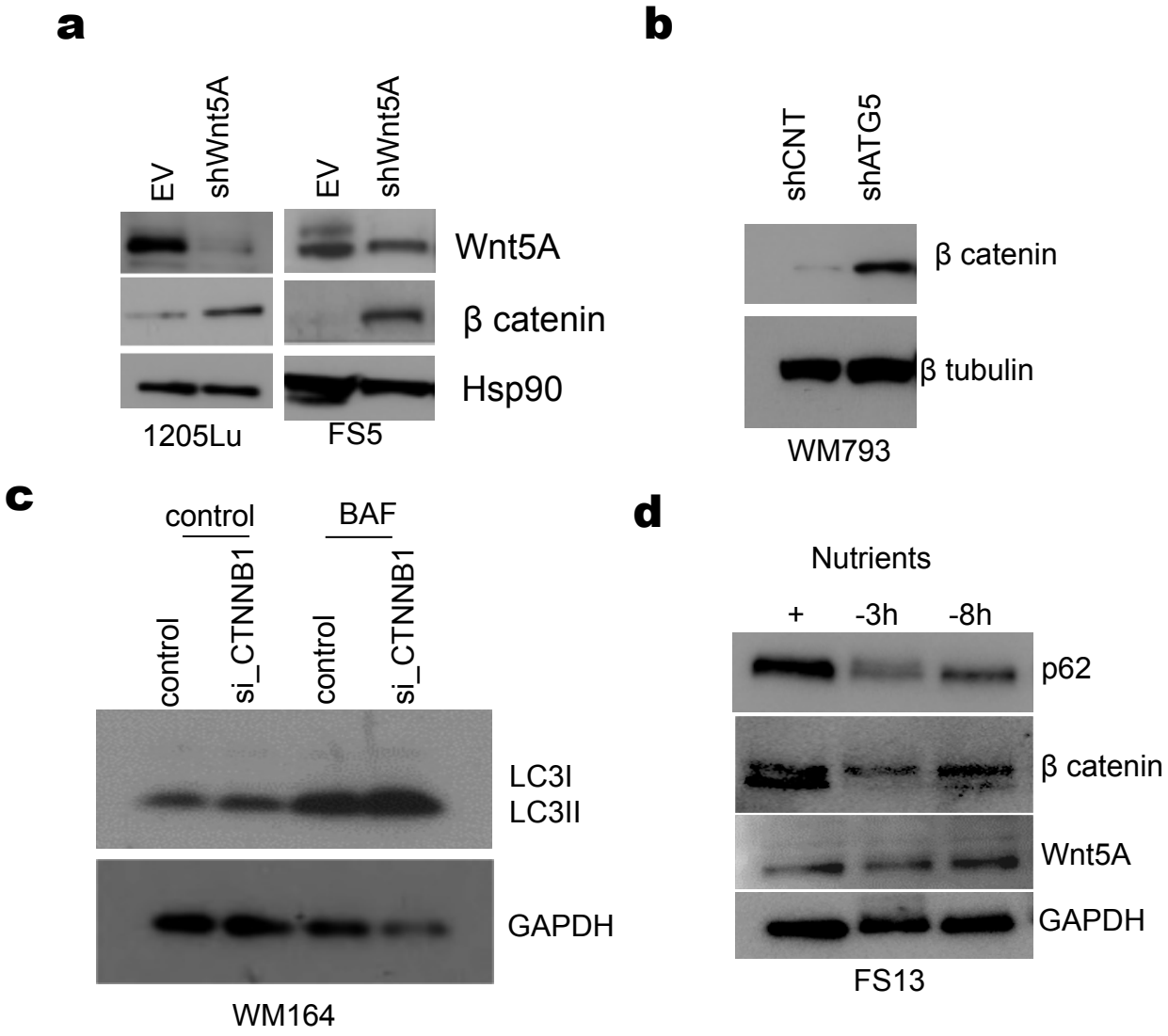


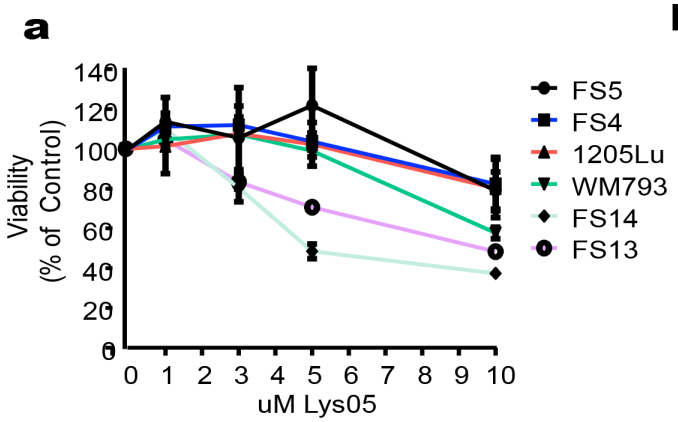
Supplementary Figure 1

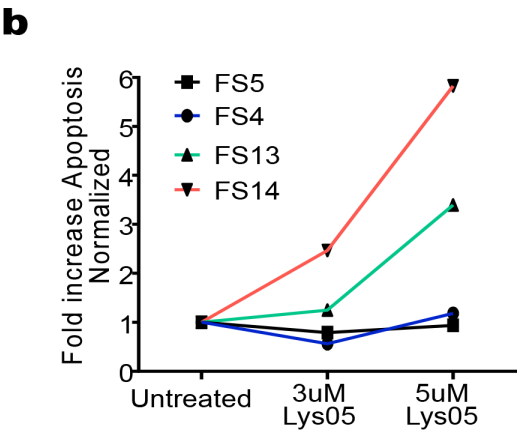


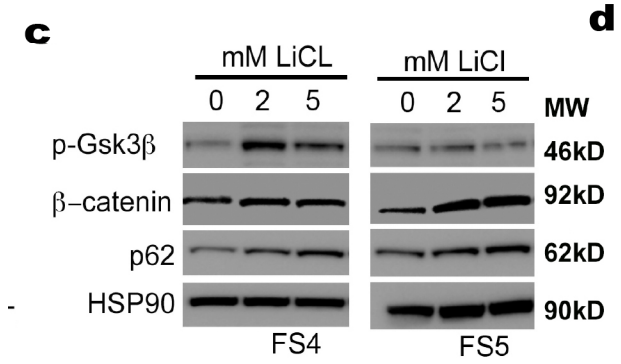
Supplementary Figure 2

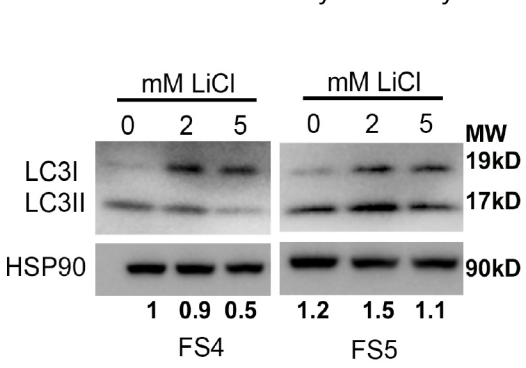


Supplementary Figure 3









e

BLISS NUMBER

WM35 Lys05

LY2090314	10	6.67	4.44	2.96	1.98	1.32	0.88
20	5	9	-9	-16	-8	-7	4
10	4	17	-8	-12	-17	-27	-23
5	2	10	-3	2	-8	-5	1
2.5	-5	-16	-12	-30	2	-9	-2
1.25	-8	-23	-16	-20	-5	-5	-12

WM164 Lys05

LY2090314	10	6.67	4.44	2.96	1.98	1.32	0.88
20	1	2	10	-27	-24	-22	-16
10	2	3	9	-13	-20	-18	-11
5	1	2	-7	-2	-11	-12	-7
2.5	2	2	-5	-13	-11	-11	2
1.25	0	1	-1	-7	-2	-3	-3

INHIBITION

Lys05

LY2090314	10	6.67	4.44	2.96	1.98	1.32	0.88	0
20	94	20	-25	-32	-33	-28	-22	-29
10	94	40	-7	-12	-24	-31	-32	-11
5	91	31	-6	-2	-20	-12	-11	-14
2.5	86	10	-7	-25	-1	-7	-6	-6
1.25	83	6	-7	-11	-3	1	-11	-1
0	91	31	10	10	3	6	2	12

Lys05

LY2090314	10	6.67	4.44	2.96	1.98	1.32	0.88	0
20	110	110	32	-7	-4	2	0	12
10	110	110	37	13	5	11	11	19
5	110	109	19	23	13	15	14	17
2.5	110	109	21	11	12	16	21	16
1.25	110	109	15	7	11	14	6	5
0	110	108	12	9	9	13	5	8

Supplementary Figure 4

Supplemental Figure Legends.

Supplementary Figure 1. Wnt5A expression correlates with high autophagy in melanoma. A. *ATG5* expression is increased in metastatic melanoma (p=0.003). **B.** Western blot of Wnt5A and β-catenin in Wnt5A-high (FS5, FS4, WM793) and Wnt5A-low (WM164, FS14, FS13) melanoma cells; loading control: GAPDH. **C**. Western blot of LC3II/I levels in Wnt5A-high (FS5, FS4) and Wnt5A-low (FS14, FS13) melanoma cells upon bafilomycin treatment for the measurement of autophagy flux; loading control: HSP90. Numbers represent ratio of Baf. (LC3II/HSP90) to control (LC3II/HSP90).

Supplementary Figure 2. Wnt5A increases autophagy in melanoma. A. Western blot of Wnt5A levels in FS4 melanoma cells upon siRNA-mediated knockdown of Wnt5A; loading control: HSP90. **B**. Western blot of LC3II/I levels in FS4 control and FS4 siWnt5A melanoma cells upon bafilomycin treatment for the measurement of autophagy flux; loading control: HSP90. Numbers represent ratio of Baf. (LC3II /HSP90) to control (LC3II/ HSP90). **C.** Western blot of Wnt5A in 1205LU upon shRNA-mediated knockdown of Wnt5A; loading control: HSP90. **D.** Western blot of LC3II/I levels in 1205LU control and 1205LU shWnt5A melanoma cells upon bafilomycin treatment for the measurement of autophagy flux; loading control: HSP90. Numbers represent ratio of Baf. (LC3II/HSP90) to control (LC3II/HSP90). **E**. Immunofluorescence analysis of β-catenin (CTNNb1) (red) and LC3 (green) in FS4, WM793 (CTNNb1-low melanoma cells) and FS13,

FS14 (CTNNB1-high melanoma cells) upon treatment with bafilomycin showing a negative correlation between β-catenin levels and autophagy; DAPI, 4,6-diamidino-2-phenylindole (blue).

Supplementary Figure 3. ATG5 affects Wnt signaling in melanoma. A. Western blot of Wnt5A and β-catenin in 1205Lu and FS5 upon shRNA-mediated knockdown of Wnt5A confirming that Wnt5A downregulates β-catenin; loading control: HSP90. **B.** Western blot of β-catenin levels in WM793 upon shRNA-mediated knockdown of ATG5; loading control: β-tubulin**. C.** Western blot of LC3II/I levels in WM164 control and WM164 si-CTNNB1 melanoma cells upon bafilomycin treatment for the measurement of autophagy flux; loading control: GAPDH. **D**. Western blot of p62, β-catenin and Wnt5A in FS13 melanoma cells after serum starvation for 3 and 8 hours; loading control: GAPDH.

Supplementary Figure 4. β-catenin increases the sensitivity of melanoma cells to autophagy inhibition. **A.** Measurement of cell viability by MTS assay in low β catenin/high Wnt5A melanoma cells (FS5, FS4, WM793 and 1205LU) and high β catenin/low Wnt5A melanoma cells (FS14, FS13) upon treatment with Lys05 at the indicated doses; graph represents viability as a percent of control. **B.** Annexin V/PI staining for the measurement of apoptosis in β-catenin low (FS5, FS4) and β-catenin high (FS13, FS14) cells. Data presented as fold increase in apoptosis normalized to control for each cell line. **C**. Western blot of phospho-GSK3β, β-catenin, and p62 in FS4 and FS5 melanoma cells treated with the GSK3 inhibitor (LiCl: lithium chloride)) at the indicated doses for 8 hours; loading control: HSP90. **D.** Western blot of LC3II/I levels in FS4 and FS5 cells upon

treatment with the GSK3 inhibitor LiCl (Lithium Chloride) for 8 hours at the indicated doses; loading control HSP90. Numbers represent ration of LC3II normalized to control. **E**. Melanoma cells with high β-catenin (WM35 and WM164) were treated with the indicated doses of the GSK3 inhibitor LY2090314 for 7 hours followed by the addition of Lys05 for 72 hours; cell growth was assessed by Alamar Blue. Synergy was calculated using the Bliss formula; the bliss numbers for each combination are color coded from red to green. Red represents no synergy (additive) effects of the two compounds. Green values are combinations with synergy greater than 20. The inhibition seen with each drug combination is color coded as a gradient from red (0%) through white (50%) to blue (100%) inhibition.

SUPPLEMENTAL TABLE 1: DENSITOMETRY FOR ALL WESTERNS

Figure 1F	WM35	WM793	1205LU
Wnt5A/HSP90	0.3	1	0.9
p62/HSP90	0.7	0.1	0.3
ATG5/HSP90	0.1	0.6	0.7
Figure 2D	0 (control)	100ng/ml	200 ng/ml
Wnt5A (top bands)/HSP90	0.04	0.2	1
Wnt5A (both bands)/HSP90	0.4	0.5	1.6
p62/HSP90	1.1	0.6	0.4
Figure 2F	C	sh1	sh2
Wnt5A top band/HSP90	1	0.3	0.2
Wnt5A (both bands)/HSP90	2.2	1.1	0.8

Figure 3B	WM793 ctrl	WM793 shATG5-1	FS4 ctrl	FS4 shATG5-1	FS4 shATG5-2
ATG5/HSP90	0.9	0.05	0.4	0.07	0.05
Wnt5A/HSP90	0.3	0.01	0.8	0.3	0.7

Figure 3G	FS4 CTRL	FS4 shATG5
ATG5/HSP90	0.9	0.3
WNT5A (both bands)/HSP90	0.8	0.4
WNT5A (upper band)/HSP90	0.5	0.09
P62/HSP90	0.09	0.4
B-catenin/HSP90	0.2	0.4
Figure 3H	WM164 CTRL	WM164 si-CTNNB1
B-catenin/HSP90	0.8	0.4
p62/HSP90	1.2	0.6
WNT5A /HSP90	0.2	0.6

Figure 3i	+	-1 h	-2 h	-3 h

Wnt5A/HSP90	0.4	0.7	0.8	0.8		
Beta catenin/HSP90	1.2	0.8	0.5	0.4		
Figure 4A	EV_1	EV_2	EV_3	WNT5A_1	WNT5A_2	WNT5A_3
Wnt5A/HSP90	0.07	0.08	0.04	0.6	0.8	0.5
B-catenin/HSP90	0.2	0.6	1.5	0.1	0.2	0.2
Figure 4C: Nuclear	1.7EV	2.1 CTNNB1	1.7_WNT5A			
B-catenin/Histone H3	0.2	0.7	0.2			
Figure 4C: Cytoplasmic	1.7EV	2.1 CTNNB1	1.7_WNT5A			
B-catenin/b-tubulin	0.7	0.6	0.1			
Figure 5C	control	shATG5				
Wnt5A/GAPDH	1.3	0.5				
P-PKC/GAPDH	0.5	0.2				
Total PKC/GAPDH	0.4	0.5				
P-CAMKII/GAPDH	0.7	0.3				
Total CAMKII/GAPDH	0.5	0.4				

Figure 6A	FS5	FS4	FS13	FS14
B-catenin/HSP90	0.2	0.5	1.7	2.2
FS5 Figure 6E	Ctrl (0)	2nM	5nM	10nM
B-catenin/HSP90	0.4	0.7	0.7	1.6
p62/HSP90	0.2	0.8	0.7	0.8
FS4 Figure 6E	Ctrl (0)	2nM	5nM	10nM
B-catenin/HSP90	0.4	0.7	0.3	1.5
p62/HSP90	0.4	0.9	0.8	1

FS4-supp Fig 4C	Ctrl (0)	2mM	5mM
P-GSK3 beta/HSP90	0.2	0.7	0.5
Beta catenin/HSP90	0.3	0.5	0.4
P62/HSP90	0.2	0.4	0.5
FS5-supp Fig 4C	Ctrl (0)	2mM	5mM
P-GSK3 beta/HSP90	0.4	0.5	0.4
Beta catenin/HSP90	0.3	0.6	0.7
P62/HSP90	0.4	0.5	0.6

Supplemental Table 2. Tissue microarrays (TMA) H scores. Table showing score and diagnosis for the tissue microarray. Non-melanoma samples are highlighted in gray.
MM: Malignant melanoma; Met: Metastatic; DN: Dysplastic Nevi; LN: Lymph Node; RCC: Renal cell cancer; SCC: Squamous cell cancer; CA: Cancer;

Diagnosis	Wnt5A H score	LC3 H score	b-catenin H score
Skin SCC	50	110	30
Met MM (metastatic malignant melanoma)	100	110	180
DN (nevi)	100	160	180
DN	80	160	110
Met MM	10	50	50
Met MM	20	40	60
Met MM	100	200	290
Met MM	100	100	290
Met MM	100	220	90
Met MM	190	230	80
Pleomorphic Lobular CA	120	80	0
Met MM (to skin)	30	200	270
Met MM	30	200	260
DN	100	210	250
Met MM	30	80	0
Invasive MM (invasive melanoma)	130	200	30
Invasive MM	120	200	90
DN	120	140	170
Met MM	50	140	30
Met MM	90	30	0
Skin Normal	0	0	0
Met Lobular CA	110	0	0
Met MM	270	280	0
Met MM Skin Met	250	280	10
DN	70	30	50
Invasive MM	150	140	10
Invasive MM (Stage N1)	140	200	0
Met MM	90	90	170
Met MM (Skin Met)	130	140	30
(Lung Adeno Ca)	100	200	0
Invasive MM	20	80	0
Invasive MM (Desmoplastic deeper)	0	40	0
Invasive MM (center)	30	70	0
Met MM	200	300	0

Met MM (1st S005-2863, #2 IV, 4.3 mm)	160	280	0
DN	30	60	60
Met MM (cut. Satellite)	200	210	140
Met MM	120	60	0
Met MM	60	50	0
Met RCC	100	200	60
DN	50	150	200
Met MM (cut. Satellite)	120	200	60
Met MM Epidermotropic	100	220	0
Met MM LN (lymph node)	120	230	0
Met MM (LN Met)	150	200	0
DN	50	20	110
Met MM	200	200	20
Met MM (LN MET)	160	180	10
Met MM	180	120	0
Met Sarcoma to skin	120	280	0
Met MM	100	200	200
Met MM (Skin Met)	120	200	100
DN	30	50	60
Met MM (Skin)	160	90	50
Invasive MM (Upper)	230	180	40
Invasive MM (SLN neg, with lymphocytic inv satellite	220	200	30
Met MM	110	80	70
Met MM (to lung)	140	100	100
DN	60	10	30
Met Adeno CA to Skin	20	30	0
Met MM	20	100	0
Met MM (To Lung)	100	100	70
Invasive MM	70	10	60
DN	100	70	70
Met MM	100	60	140
Met MM (to Lung)	120	100	100
Invasive MM (IV, 3.25 mm, acral lenti, ulcerated, 4/mm, 2H, 2I)	100	120	120
Invasive MM	80	180	0
Desmoplastic, LM type, V, 10mm, SLN neg	60	180	0
Met MM	110	10	70
Met MM	100	0	50
Met MM	100	0	30
Met MM	50	0	120

Met MM	100	100	220
Met MM	100	100	190
Met MM	30	30	100
Met MM	140	120	120
Met MM	70	30	70
Met MM	130	20	30
Met MM	0	0	0
Met MM	10	0	0
Met MM	150	80	70
Met MM	110	120	50
DN	30	20	110
Met Mm	10	20	110
Met MM	10	30	100
Met MM	100	120	300
Met MM	20	120	300
Met MM	80	50	0
Met MM	30	0	0
Met MM	130	20	0
Met MM	120	20	0
Met MM	90	40	0
Met MM	100	30	10
Met MM	130	100	70
Met MM	120	200	80
DN	50	30	90
Met MM	130	200	0
Met MM	100	50	0
Met MM	40	30	0
Invasive MM	100	0	0
Invasive MM	100	0	0
Met MM	120	80	0
Met MM	0	60	0
Met MM	110	100	0
Met MM	100	200	0
Met MM	90	150	0
DN	50	60	100
1st MM	30	90	10
1st MM	40	0	0
Met Mm	140	100	90
Met MM	10	10	0
Met MM	110	130	0
DN	30	0	90

Met MM	110	100	0
Met MM	110	100	30
Met MM	90	120	40
Met MM	20	100	60
Met MM	100	250	300
Met MM	90	110	300
Met MM	0	0	0
Met MM	0	0	0
Met MM	0	0	0
Met MM	20	0	0
Met MM	100	20	0
Met MM	100	20	0
Met colon Ca to liver	100	30	110
Met MM	120	200	10
Met MM	110	200	0
Met MM	120	70	300
Small bowel	150	10	80
Met MM	100	90	90
Met MM	120	90	80
Invasive MM	0	0	100
DN	30		40
DN	20	0	50
Serous Ca (peritoneal ca)	30	0	90
Invasive ductal Ca	70	70	10
Met MM to Bowel	70	0	0
LN Met MM	30	0	0
Met MM to lung (FS)	0	0	80
Met MM to lung (FS)	50	0	80
LN Met MM	70	100	200
Invasive MM	100	100	100
Invasive MM	100	50	40
Subcut met MM	30	90	0
Subcut met MM	40	120	0
LN Met MM	40	0	70
DN	0	0	70
LN Met MM	0	0	0
MM nasal sinus	0	80	0
MM nasal sinus	100	70	0
Invasive MM	100	20	100
Invasive MM	130	80	100
LN Met MM	0	140	90
LN Met MM	70	70	70
Lung control	100	100	30
Met MM LN TB05-632	70	0	30

Met MM LN TB05-632	100	0	30
LN Met MM	140	10	0
LN Met MM	120	20	0
Invasive MM	110	150	120
LN Met MM	100	160	0
LN Met mM	100	170	0
Lung Met mm	90	180	0
Lung Met mm	20	20	0
LN Met mm	70	0	200
Invasive MM	170	110	70
Invasive MM	30	0	0
Invasive Mm	10	100	0
Lung Met MM TB05-209	50	100	150
Lung Met MM TB05-209	50	70	150
LN Met MM TB05-206	0	100	30
LN Met MM TB05-206	0	70	0
Invasive MM	40	0	30
LN Met MM TB05-268	70	50	200
LN Met MM TB05-268	60	90	200
Met MM	80	120	0
Invasive MM	150	130	145
Bone met MM	50	100	10
Parotid Met MM TB05-638	100	0	100
LN met mm	80	0	0
Bowel Met MM	200	0	15
LN Met MM TB05-788	10	0	100
Invasive MM	10	0	20
LN met MM	100	0	90
LN met MM	100	0	90
Lung Met MM	90	110	0
Lung Met MM	90	100	0
LN Met MM	0	50	0
LN Met MM	0	0	
DN	90	0	100
DN upper	90	0	90
DN lower	100	70	90
DN	0	0	100
Lung ca	80	120	0