

Supplemental Material to:
**Identification of secreted proteins that reflect autophagy dynamics within
tumor cells**

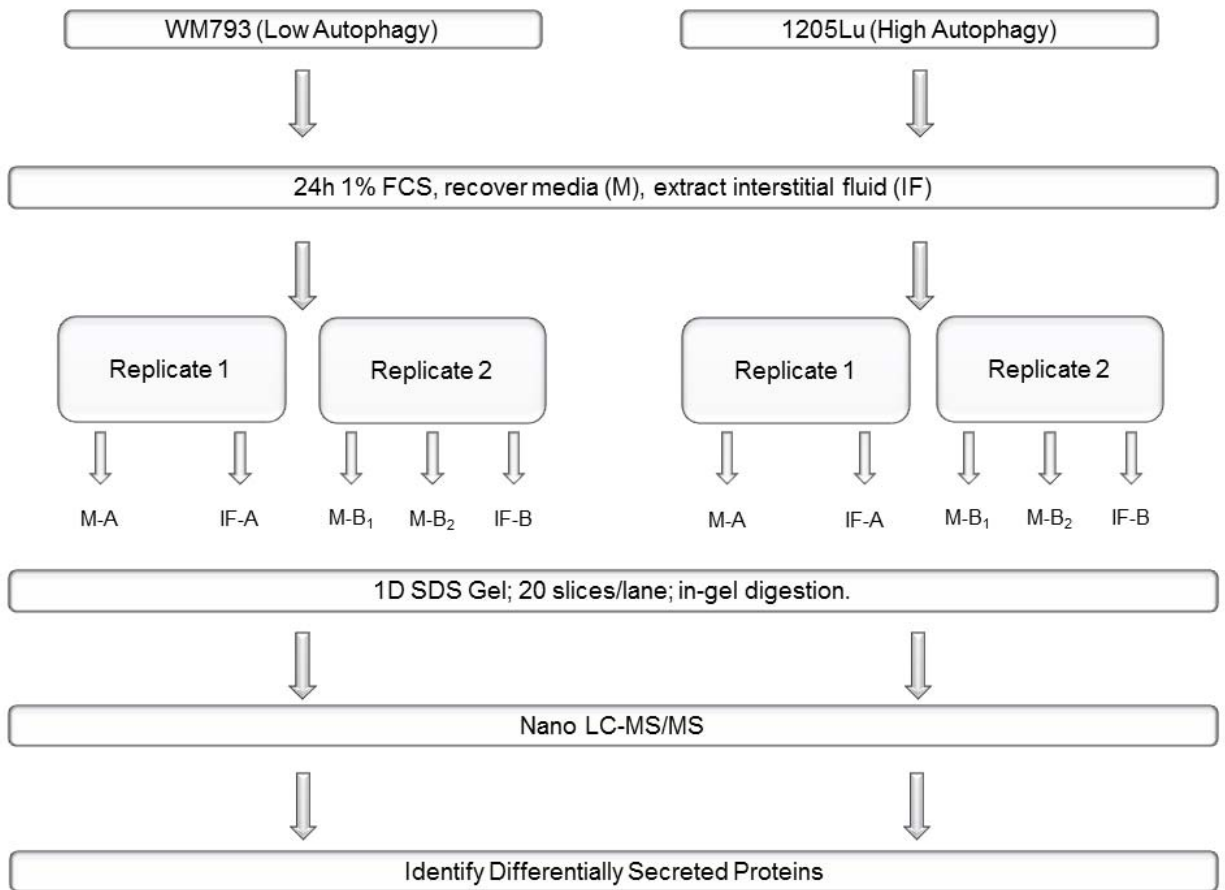


Figure S1. Workflow for the comparative secretome analysis of high- and low-autophagy cells. Schematic of the label-free approach used to identify differentially secreted proteins in the conditioned media (M) and the interstitial fluid (IF) of WM793 and 1205Lu cells.

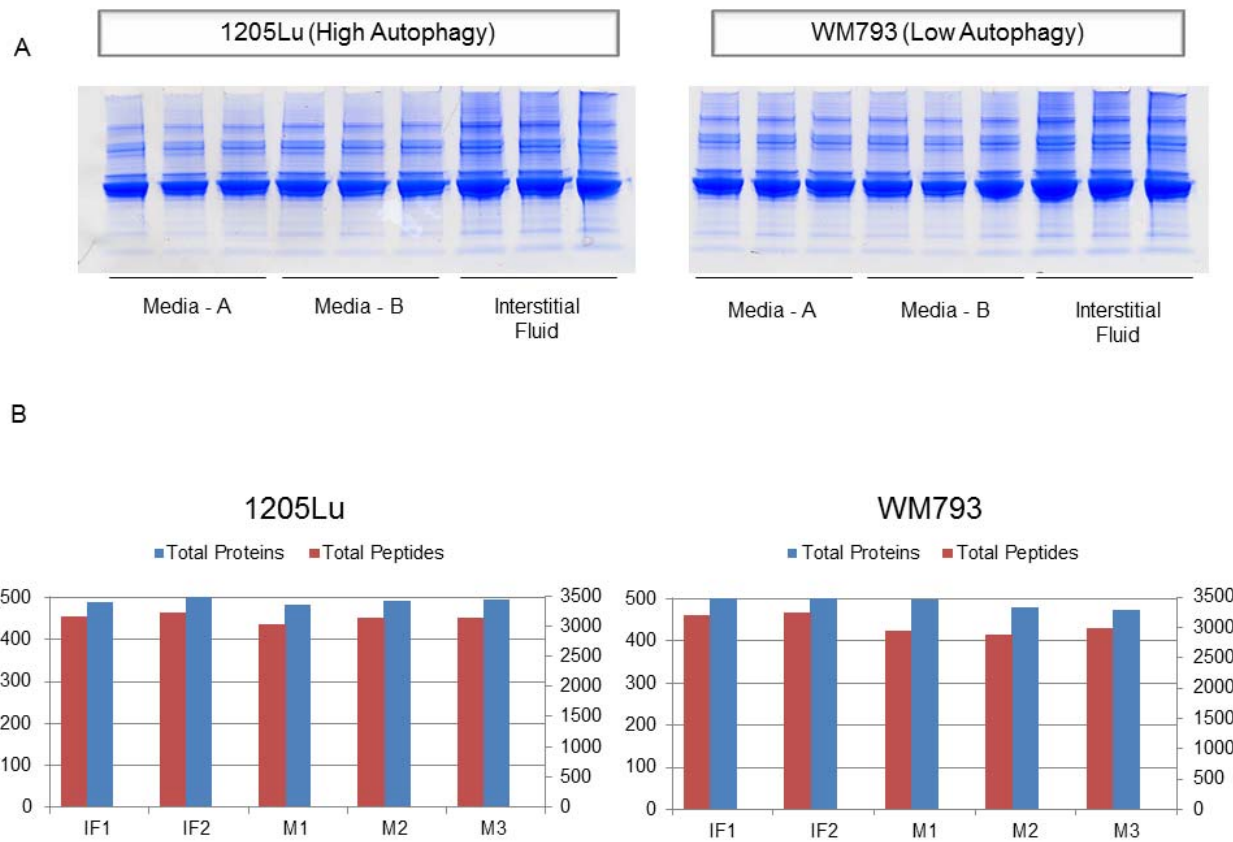


Figure S2. SDS-PAGE separation of melanoma conditioned medium and secretome reproducibility. **(A)** “Preparative” minigels of conditioned medium of low- and high- autophagy melanoma cell lines. Gels were run to 2-cm length and excised into 20 1-mm fractions. **(B)** Total protein (left axis) and peptide identifications (right axis) by cell culture compartment and by biological and technical replicates in 1205Lu and WM793 cell lines.

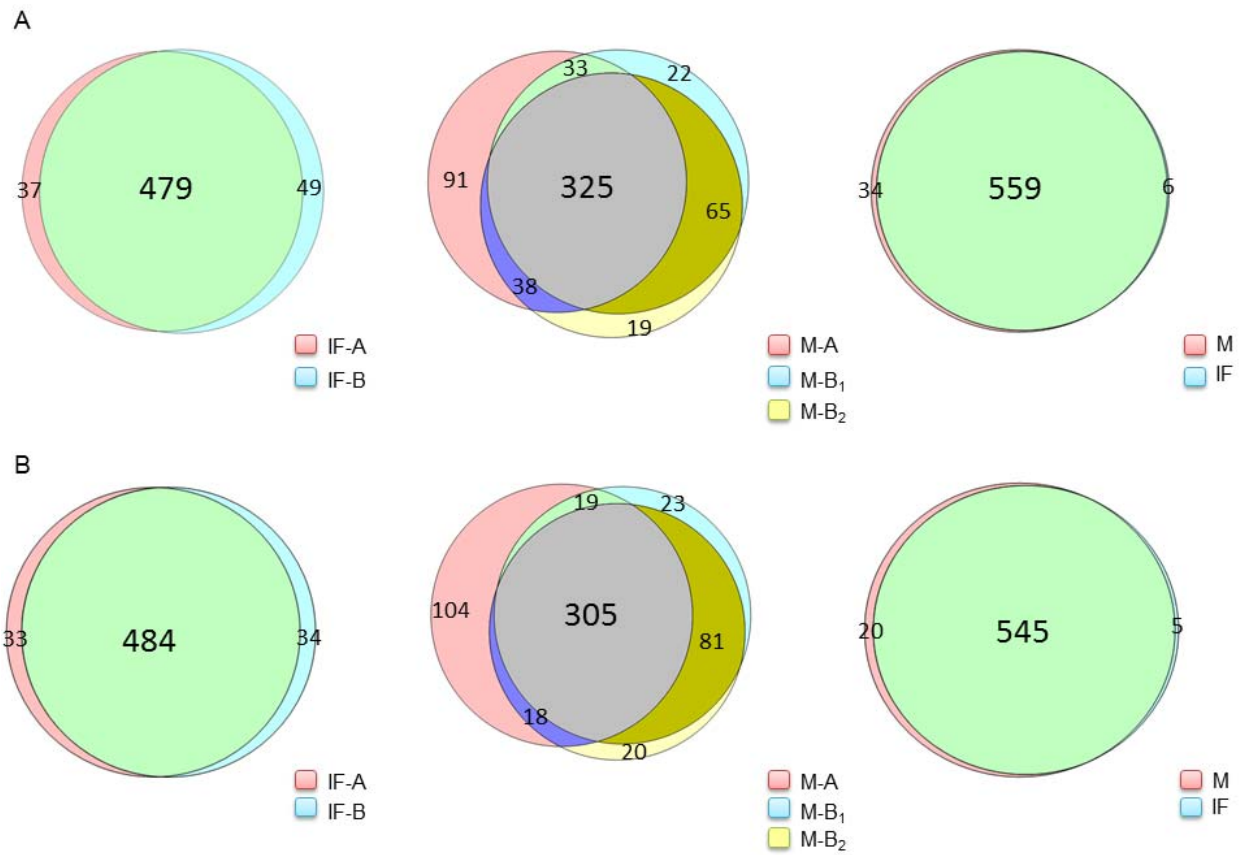
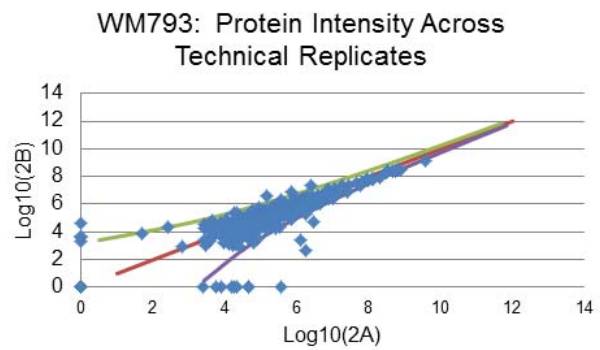
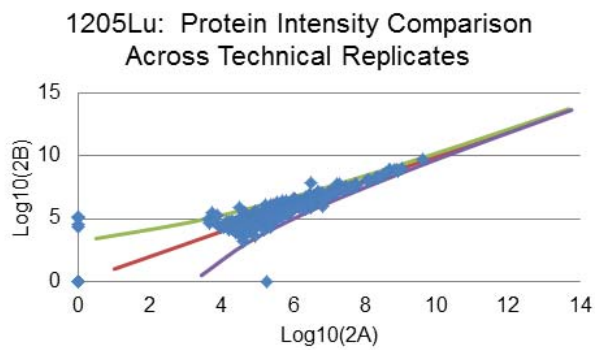


Figure S3. Venn diagrams illustrating total numbers of shared and unique proteins identified across: left panels - biological replicates (**A and B**) of interstitial fluid (IF); middle panels – biological replicates (M-A, M-B) and technical replicates (M-B1, M-B₂) of media; right panels – combined media replicates vs. combined interstitial fluid replicates. (**A**) 1205Lu and (**B**) WM793.

A



B

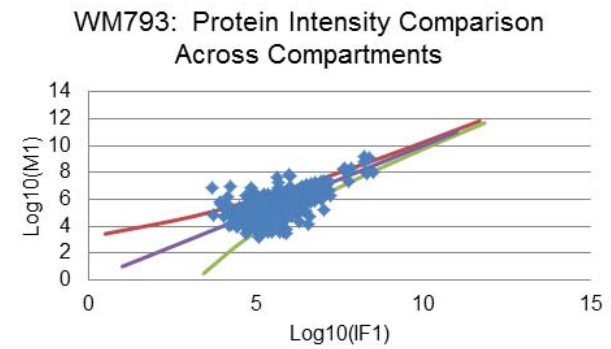
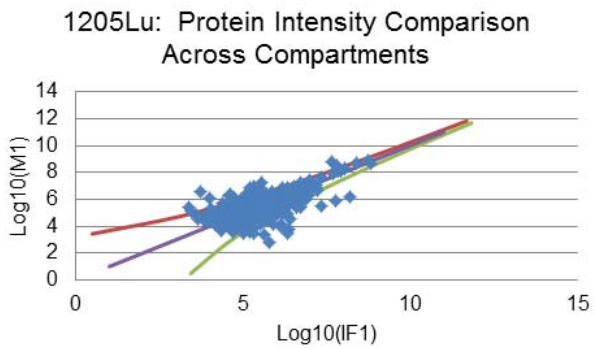


Figure S4. Statistical analysis of protein intensity variation across replicates. **(A)** Protein intensity comparison of technical replicates, with associated 95% confidence intervals for 1205Lu and WM793. **(B)** Protein intensity comparison of supernatant and interstitial fluid compartments by cell line with overlaid 95% confidence intervals.

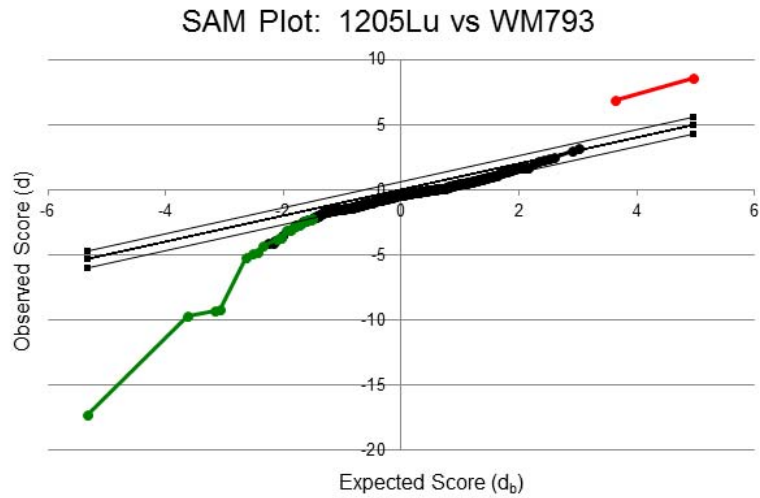


Figure S5. SAM analysis and data filtering. SAM plot illustrating observed scores plotted as a function of permuted/expected scores. False discovery rate was set based on the Δ tuning parameter. Data points in green represent proteins elevated in 1205Lu, whereas data points in red are those elevated in WM793 (FDR = 3.52%).

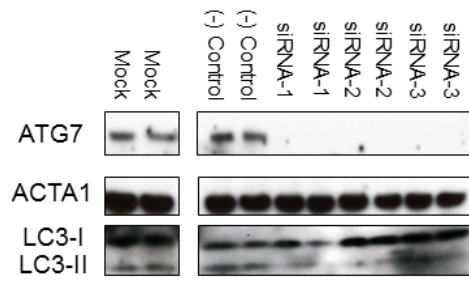


Figure S6. ATG7 silencing in WM1346 melanoma cell line. ATG7 and LC3 expression in WM1346 cells 96 h post-transfection with 20 nM siRNA.

Table S1. Differentially secreted proteins across high- and low-autophagy melanoma cells.

Accession ID	Gene Name	Protein Name	Mean Intensity (1205Lu)	Mean Intensity (WM793)	Fold Change (1205Lu/WM793)
Q16769	QPCT	Glutamyl-peptide cyclotransferase	4.30E+06	2.79E+05	15
P10145-2	CXCL8	Chemokine (C-X-C motif) ligand 8 (Interleukin 8)	1.64E+06	1.19E+05	14
P15018	LIF	Leukemia inhibitory factor	6.90E+06	5.20E+05	13
O00560	SDCBP	Syndecan binding protein (syntenin)	6.00E+05	4.75E+04	13
P01584	IL1B	Interleukin 1, beta	1.19E+05	9.62E+03	12
C9JF17	APOD	Apolipoprotein D	6.72E+06	5.50E+05	12
Q92520	FAM3C	Family with sequence similarity 3, member C	4.77E+06	4.76E+05	10
Q16610-4	ECM1	Extracellular matrix protein 1	1.14E+07	1.25E+06	9.1
UPI0001AE6B78	DKK3	Dickkopf WNT signaling pathway inhibitor 3	1.92E+06	2.19E+05	8.8
P04083	ANXA1	Annexin A1	1.20E+05	2.11E+04	5.7
B2R699	GM2A	GM2 ganglioside activator	8.82E+04	1.58E+04	5.6
O00468	AGRN	Agrin	1.06E+06	2.11E+05	5.0
P16035	TIMP2	TIMP metalloproteinase inhibitor 2	2.90E+06	6.40E+05	4.5
P15121	AKR1B1	Aldo-keto reductase family 1, member B1 (aldose reductase)	1.54E+07	3.77E+06	4.1
B3KQF4	TIMP1	TIMP metalloproteinase inhibitor 1	5.04E+07	1.25E+07	4.0
P22314	UBA1	Ubiquitin-like modifier activating enzyme 1	2.85E+06	7.42E+05	3.8
UPI0000445E06	ACTG1P2	Actin, gamma 1 pseudogene 2	8.12E+04	2.22E+04	3.7
P07942	LAMB1	Laminin, beta 1	2.66E+07	7.82E+06	3.4
P01034	CST3	Cystatin C	1.21E+06	3.68E+05	3.3
Q2TU84	GLCC1	Glucocorticoid induced transcript 1	3.75E+05	1.19E+05	3.2
P45877	PPIC	Peptidylprolyl isomerase C (cyclophilin C)	7.46E+05	2.44E+05	3.1
Q14914	PTGR1	Prostaglandin reductase 1	4.02E+05	1.43E+05	2.8
A8K061	ANGPTL3	Angiopoietin-like 3	1.24E+06	4.65E+05	2.7
P08670	VIM	Vimentin	7.09E+06	2.64E+06	2.7
Q59GM9	GOT1	Glutamic-oxaloacetic transaminase 1, soluble	3.11E+05	1.15E+05	2.7
Q16674	MIA	Melanoma inhibitory activity	1.44E+06	6.20E+05	2.3
A6NII8	LCN2	Lipocalin 2	5.33E+06	1.85E+07	-3.5
Q9NRR1	CYTL1	Cytokine-like 1	4.12E+04	2.68E+05	-6.5