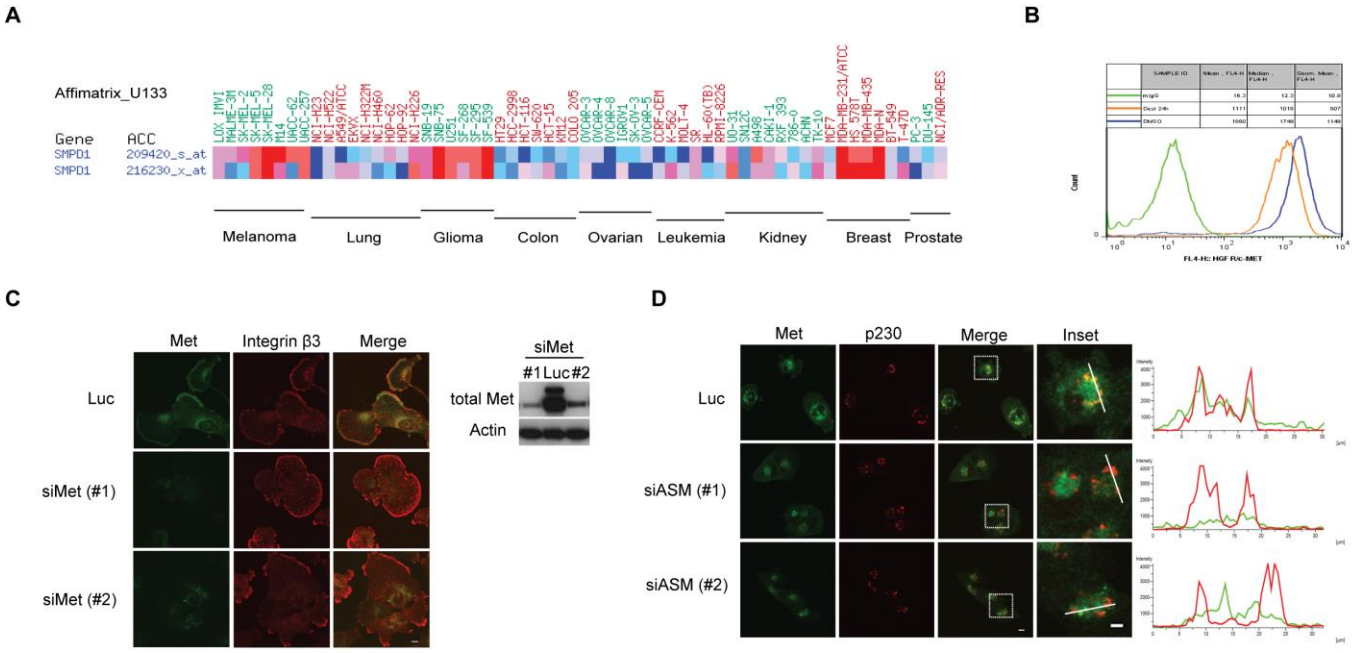

Fig. S1. Expression of ASM in various cancer cells and regulation of Met distribution by ASM. (A) The expression of ASM (SMPD1) in various cancer cell lines was analyzed using the microarray mRNA data of the Cancer Genome Anatomy Project database (https://discover.nci.nih.gov/cellminer/) in 60 cancer cell lines collected by National Cancer Institute. ASM/SMPD1 was highly expressed in several cancer cells including glioma/glioblastoma cells as indicated. (B) Histograms of FACS to detect cell surface Met. U373-MG cells were treated with DMSO and 30 M desipramine as in Fig. 2E. Live cells were detached using the Accutase Cell Detachment Solution and processed for surface staining of live cells with a monoclonal IgG control and an anti-Met monoclonal APC-conjugated antibody (orange and blue fluorescence intensities). Cell surface fluorescence of labeled Met was measured using Flow Cytometry (FACS). Green fluorescence: monoclonal IgG control cells, orange fluorescence: desipramine treated cells, and blue fluorescence: DMSO treated control cells. (C) The immunofluorescence stainings of Met reveal the specific presence of Met on the plasma membrane and intracellular compartments. U373-MG cells were transfected with 50 nM luciferase (control) siRNA and two independent siRNAs against Met. After 48 hours transfection, cells were fixed, stained for Met and integrin 3 as in Fig. 2D, or harvested for examining Met ablation by western blot as indicated. Scale bar, 10 m. (D) Ablation of ASM by siRNAs and desipramine produce the same intracellular trapping of Met in the TGN marked by p230. U373-MG cells were transfected with 50 nM luciferase siRNA and two independent siRNAs against ASM. 72 hours post-transfection, both control and ASM siRNA treated cells were exposed to 35 M desipramine for 2 hours as in Fig. 2A. Cells were fixed and stained with specific antibodies for Met and p230. Scale bar, 10 m. No additive effects of desipramine after siRNA-mediated ASM ablation were found.

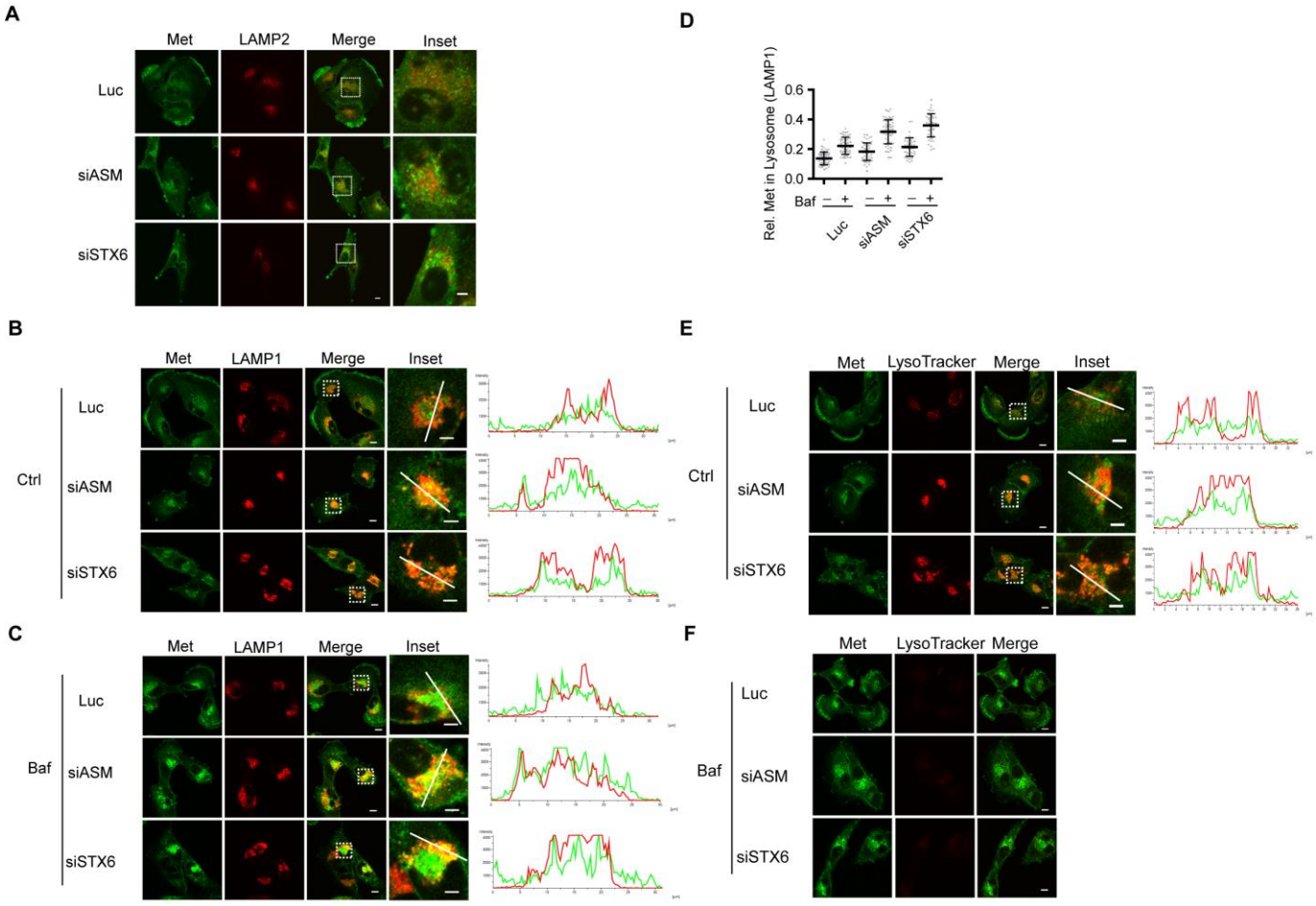

Figure S2. Loss of ASM or STX6 leads to aberrant traffic of Met to lysosomes. (A) U373 cells were transfected with siRNAs against luciferase, ASM, and STX6 for 24 hours, and processed as in Fig. 5F. Cells were fixed and stained with specific antibodies against Met and LAMP2. Scale bar, 10 m. (B-F) Cells were treated with control, ASM or STX6 siRNAs for 52 hours and then treated with bafilomycin A1 (Baf, 100 nM) for additional 7 hours where indicated. Cells were fixed and co-stained with anti-Met and LAMP1 antibodies (B and C) or anti-Met antibody and LysoTracker (E, F), and processed as in Fig. 3. Quantification of relative Met staining in the lysosome region (marked by LAMP1 staining) was carried out as described in Fig. 3F in 3G.

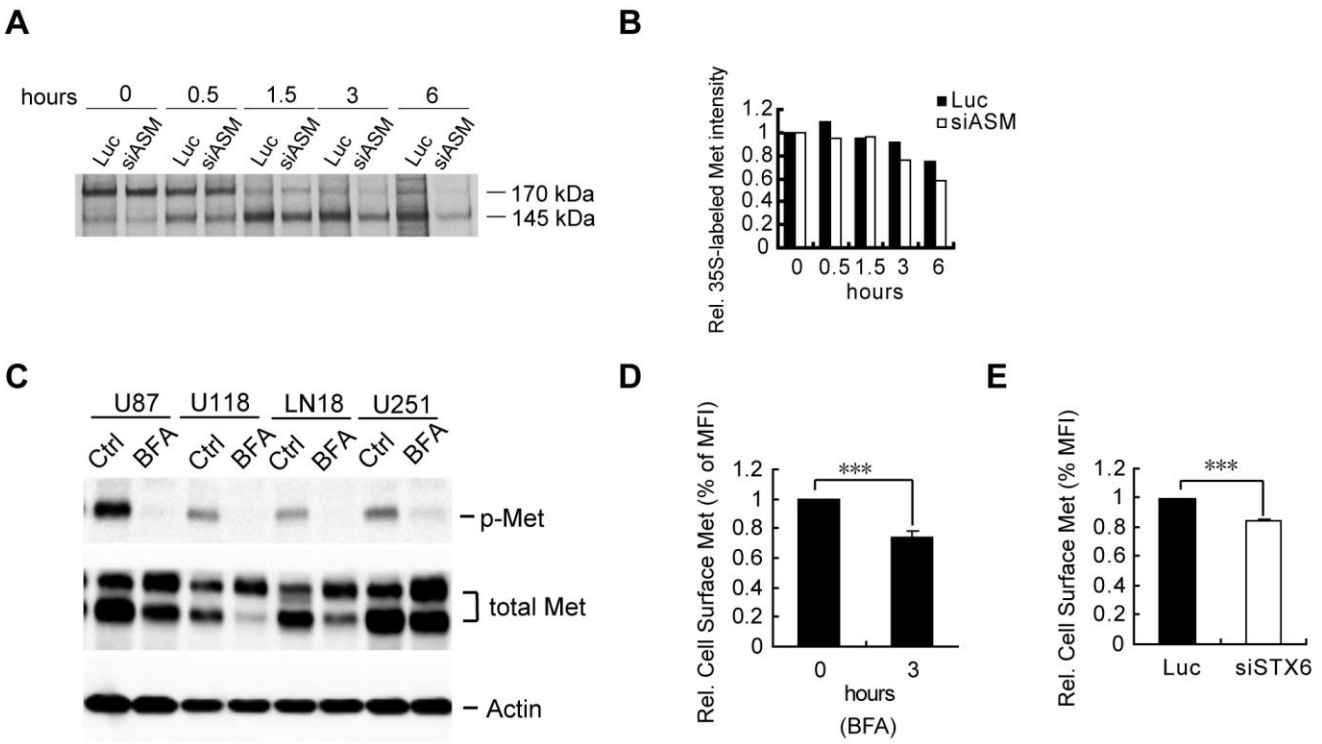

Fig. S3. Effects of Brefeldin A (BFA) and ablation of ASM or STX6 on phosphorylation and intracellular localization of Met. (A) U373-MG cells were transfected with control (Luc) and ASM siRNAs for 72 hours. They were pulse-labeled with ^{35}S-methionine for 1 hour, washed, and chased for various time points in fresh medium without the isotope as indicated. Cells were lysed and Met proteins were immunoprecipitated by anti-MET antibodies, resolved in protein gel, and visualized by fluorography (top panel) and quantified (B). (C) Human glioblastoma U87-MG, U118-MG, LN18, U251-MG cells were treated with or without 3 g/ml BFA for 3 hours. Whole cell lysates were immunoblotted with indicated antibodies, as in Fig. 4F. (D and E) U373-MG cells were treated with or without BFA for 3 hours (D) or transfected with control or STX6 siRNAs for 72 hours (E). Live cells were detached from plates as described in Fig. 2E for FACS analysis. The cells were immunostained with an APC-conjugated anti-Met monoclonal antibody or an isotype mouse IgG control antibody. Cells were then analyzed by FACS method for the surface expression of Met. Results were analyzed by the Student's t test, ***$p<0.001$. Error bars, SD.

Table S1. Antibodies used for immunostaining, immunoprecipitation (IP) and immunoblotting (WB) studies.

Antibodies	Supplier, Catalog Number	Dilution	Uses
SMPD1 (ASM) (mouse)	R&D Systems, MAB5348	1:400	IP
SMPD1 (ASM) (goat)	R&D Systems, AF5348	1:500	WB
Met (mouse, APC-conjugated)	R&D Systems, FAB3582A	1:15	FACS
Control IgG (mouse, APC-conjugated)	R&D Systems, IC002A	1:15	FACS
Syntaxin 6 (sheep, biotinylated)	R&D Systems, BAF5664	1:150	Immunostaining
Met (goat)	R&D Systems, AF276	1:500	IP
Met (rabbit)	Cell Signaling, #8198	1:2000	WB
Met (rabbit, AF488-conjugated)	Cell Signaling, #8494S	1:70	Immunostaining
phospho-Met (Tyr1234/1235)	Cell Signaling, #3077	1:600	WB
phospho-p70 S6K (T389)	Cell Signaling, #9205S	1:1000	WB
phospho-AKT (S473)	Cell Signaling, #4060S	1:1000	WB
p70 S6K (total)	Cell Signaling, #2708S	1:1000	WB
AKT (total)	Cell Signaling, #4691S	1:2000	WB
Syntaxin 6 (rabbit)	Cell Signaling, #2869	1:2000	WB
IGF1R (rabbit)	Cell Signaling, #9750	1:1000	WB
-Actin (mouse)	Cell Signaling, #3700S	1:7000	WB
CD61/integrin 3 (mouse)	BD Biosciences, 555752	1:300	Immunostaining
p230 (mouse)	BD Biosciences, 611280	1:300	Immunostaining
GM130 (mouse)	BD Biosciences, 610822	1:300	Immunostaining
Syntaxin 6 (mouse)	BD Biosciences, 610635	1:300	Immunostaining
LAMP1 (mouse, AF647-conjugated)	BD Biosciences, 562622	1:300	Immunostaining
LAMP2 (mouse, AF647-conjugated)	BD Biosciences, 565305	1:300	Immunostaining

CD63 (mouse, AF647-conjugated)	BD Biosciences, 561983	1:300	Immunostaining
VSVG (rabbit)	Bethyl Laboratories, A190-131A	1:500	IP
GFP (mouse)	Santa Cruz Biotechnology, Sc-9996	1:500	WB
Goat anti-mouse IgG (AF488-conjugated)	Jackson Immunologicals, 115-546-146	1:150	Immunostaining
Goat anti-rabbit IgG (AF488-conjugated)	Jackson Immunologicals 111-546-144	1:150	Immunostaining
Goat anti-mouse IgG (AF647-conjugated)	Jackson Immunologicals 115-606-146	1:300	Immunostaining
Goat anti-rabbit IgG (AF647-conjugated)	Jackson Immunologicals, 115-606-146	1:300	Immunostaining
Streptavidin (Brilliant Violet 421-conjugated)	BioLegend, 405225	1:1000	Immunostaining