Figure 4.3.1 - Western blot analysis of p70 S6 kinase and LC3 in SH-SY5Y cells overexpressing hBCATm. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 2.5 µg of hBCATm overexpression vector for 48 hours. Cell lysates were collected using RIPA extraction and 10 µg of protein was separated on a 12% SDS-PAGE gel and transferred to PVDF membrane for western blot analysis. The membrane was probed with anti-p70 S6 kinase (1:1000) overnight followed by a 1 hour incubation with anti-rabbit HRP (1:2000). The bands were visualised using chemiluminescent HRP substrate and exposed to Hyperfilm. The membrane was stripped with 1 M NaOH for 7 minutes and re-probed with anti-LC3-II (1:500), anti-hBCATm (1:3000) and then anti-GAPDH (1:10,000). N=3.
**Figure 4.3.2 - Western blot analysis of SH-SY5Y cells overexpressing a range of hBCATm plasmid concentrations.** SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATm overexpression vector for 48 hours. Cell lysates were collected using RIPA extraction and 10 µg of protein was separated on a 12% SDS-PAGE gel and transferred to PVDF membrane for western blot analysis. The membranes were probed with anti-p70 S6 kinase (1:1000) overnight followed by a 1 hour incubation with anti-rabbit HRP (1:2000). The bands were visualised using BM chemiluminescent HRP substrate and exposed to Hyperfilm. The membrane was stripped with 1 M NaOH for 7 minutes and re-probed with anti-LC3-II (1:500), anti-hBCATm (1:3000) and anti-GAPDH (1:10,000). N=3.
Figure 4.3.3 - Western blot analysis of SH-SY5Y cells overexpressing a range of hBCATc plasmid concentrations. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATc overexpression vector for 48 hours. Cell lysates were collected using RIPA extraction and 10 µg of protein was separated on a 12% SDS-PAGE gel and transferred to PVDF membrane for western blot analysis. The membranes were probed with anti-p70 S6 kinase (1:1000) overnight followed by a 1 hour incubation with anti-rabbit HRP (1:2000). The bands were visualised using BM chemiluminescent HRP substrate and exposed to Hyperfilm. The membrane was stripped with 1 M NaOH for 7 minutes and re-probed with anti-LC3-II (1:500), anti-hBCATc (1:3000) and anti-GAPDH (1:10,000). N=3.
Figure 4.3.4 - Densitometry analysis of SH-SY5Y cells overexpressing a range of hBCATm plasmid concentrations. SH-SY5Y cells transfected with 0.5 - 2.5 µg of hBCATm overexpression vector for 48 hours were subject to western blot analysis. 10 µg of protein was separated on a 12% SDS-PAGE gel and transferred to PVDF membrane. The membrane was probed with anti-p70 S6 kinase (1:1000) overnight followed by a 1 hour incubation with anti-rabbit HRP (1:2000) (B). The bands were visualised using chemiluminescent HRP substrate and exposed to Hyperfilm. The membrane was stripped with 1 M NaOH for 7 minutes and re-probed with anti-LC3-II (1:500) (A). Densitometry analysis relative to the transfection control was carried out using ImageJ™ software (Wayne Rasband, National Institute of Health, USA). Data are the mean ±SEM, N=3. Statistical significance using a one-way ANOVA with Dunnett’s post hoc test (*) p
Figure 4.3.5 - Densitometry analysis of SH-SY5Y cells overexpressing a range of hBCATc plasmid concentrations. SH-SY5Y cells transfected with 0.5 - 2.5 µg of hBCATc overexpression vector for 48 hours were subject to western blot analysis. 10 µg of protein was separated on a 12% SDS-PAGE gel and transferred to PVDF membrane. The membrane was probed with anti-p70 S6 kinase (1:1000) overnight followed by a 1 hour incubation with anti-rabbit HRP (1:2000) (B). The bands were visualised using chemiluminescent HRP substrate and exposed to Hyperfilm. The membrane was stripped with 1 M NaOH for 7 minutes and re-probed with anti-LC3-II (1:500) (A). Densitometry analysis relative to the transfection control was carried out using ImageJ™ software (Wayne Rasband, National Institute of Health, USA). Data are the mean ±SEM, N=3. Statistical significance using a one-way ANOVA with Dunnett’s post hoc test (*) p ≤ 0.05, (**) p ≤ 0.01, (***) p ≤ 0.001
Figure 4.3.6 - Confocal microscopy of SH-SY5Y cells overexpressing 1.0 µg of hBCATm plasmid.

SH-SY5Y cells were grown on glass coverslips and transfected using JetPRIME® transfection reagent with 1.0 µg of hBCATm overexpression vector for 48 hours. The cells were fixed in 0.25% glutaraldehyde and subject to multiple wash steps (Section 2.20). Cells were probed with anti-hBCATm (1:250) and anti-LC3 (1:100) for 1.5 hours, and then anti-mouse Alexa Fluor® 488 (1:250) and anti-rabbit Alexa Fluor® 568 (1:250) for 1.5 hours. The coverslips were then mounted in VECTASHIELD HardSet antifade mounting medium with DAPI and imaged using a Zeiss Axiovert 200 confocal microscope.

N=1. Blue - DAPI; Green - hBCATm; Red - LC3
Figure 4.3.7 - Confocal microscopy of SH-SY5Y cells overexpressing 1.0 µg of hBCATc plasmid.

SH-SY5Y cells were grown on glass coverslips and transfected using JetPRIME® transfection reagent with 1.0 µg of hBCATc overexpression vector for 48 hours. The cells were fixed in 0.25% glutaraldehyde and subject to multiple wash steps (Section 2.20). Cells were probed with anti-hBCATc (1:250) and anti-LC3 (1:100) for 1.5 hours, and then anti-mouse Alexa Fluor® 488 (1:250) and anti-rabbit Alexa Fluor® 568 (1:250) for 1.5 hours. The coverslips were then mounted in VECTASHIELD HardSet antifade mounting medium with DAPI and imaged using a Zeiss Axiovert 200 confocal microscope.

N=1. Blue - DAPI; Green - hBCATc; Red - LC3
Figure 4.3.8 - Confocal microscopy of SH-SY5Y cells under autophagy-inducing conditions. SH-SY5Y cells were grown on glass coverslips and treated with 100 nM rapamycin for 30 minutes or starved in EBSS for 3 hours. The cells were fixed in 0.25% glutaraldehyde and subject to multiple wash steps (See Methods 2.20). Cells were probed with anti-hBCATc (1:500) and anti-Vps34 (1:100) for 1.5 hours, and then anti-mouse Alexa Fluor® 488 (1:250) and anti-rabbit Alexa Fluor® 568 (1:250) for 1.5 hours. The coverslips were then mounted and imaged using a Zeiss Axiovert 200 confocal microscope. The Mander’s co-localisation coefficients (Mx) were derived using Volocity (Perkin-Elmer). N=3. Blue - DAPI; Green - hBCATc; Red - Vps34
Figure 4.3.9 - Confocal microscopy of SH-SY5Y cells under mTOR-inducing conditions. SH-SY5Y cells were grown on glass coverslips and starved in EBSS for 3 hours or kept in media. The cells were fixed in 0.25% glutaraldehyde and subjected to multiple wash steps (See Methods 2.20). Cells were probed with anti-hBCATc (1:500) and anti-4EBP1 (1:200) for 1.5 hours, and then anti-mouse Alexa Fluor® 488 (1:250) and anti-rabbit Alexa Fluor® 568 (1:250) for 1.5 hours. The coverslips were then mounted and imaged using a Zeiss Axiovert 200 confocal microscope. N=3. Blue - DAPI; Green - hBCATc; Red - 4EBP1
Figure 4.3.10 - Analysis of insulin treated SH-SY5Y cells overexpressing a range of hBCATc plasmid concentrations. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATc overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 100 nM insulin. Cell lysates were collected using RIPA extraction and subject to western blot analysis for hBCATc (A). Densitometry analysis was carried out using ImageJ™ software (Wayne Rasband, National Institute of Health, USA) (B). SH-SY5Y cells were either starved in EBSS or treated with 100 nM insulin for 3 hours before being fixed in 0.25% glutaraldehyde for immunocytochemistry (See Methods 2.20). Cells were probed with anti-hBCATc (1:250) and anti-rabbit Alexa Fluor® 568 (1:250) (C). N=1.
Figure 4.3.11 - LC3 analysis of insulin treated SH-SY5Y cells overexpressing a range of hBCATc plasmid concentrations. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATc overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 100 nM insulin. Cell lysates were collected using RIPA extraction and subject to western blot analysis for LC3 (A). Densitometry analysis was carried out using ImageJ™ software (Wayne Rasband, National Institute of Health, USA) for LC3-I (B) and LC3-II (C). N=1.
Figure 4.3.12 - *p70 S6 kinase analysis of insulin treated SH-SY5Y cells overexpressing a range of hBCATc plasmid concentrations.* SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATc overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 100 nM insulin. Cell lysates were collected using RIPA extraction and subject to western blot analysis for p70 S6 kinase (A). Densitometry analysis was carried out using ImageJ™ software (Wayne Rasband, National Institute of Health, USA) for p70 S6 kinase (B). N=1.
Figure 4.3.13 - Analysis of insulin treated SH-SY5Y cells overexpressing a range of hBCATm plasmid concentrations. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATm overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 100 nM insulin. Cell lysates were collected using RIPA extraction and subject to western blot analysis for hBCATm (A). Densitometry analysis was carried out using ImageJ™ software (Wayne Rasband, National Institute of Health, USA) (B). SH-SY5Y cells were either starved in EBSS or treated with 100 nM insulin for 3 hours before being fixed in 0.25% glutaraldehyde for immunocytochemistry (See Methods 2.20). Cells were probed with anti-hBCATm (1:250) and anti-rabbit Alexa Fluor® 568 (1:250) (C). N=1.
Figure 4.3.14 - LC3 analysis of insulin treated SH-SY5Y cells overexpressing a range of hBCATm plasmid concentrations. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATm overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 100 nM insulin. Cell lysates were collected using RIPA extraction and subject to western blot analysis for LC3 (A). Densitometry analysis was carried out using ImageJ™ software (Wayne Rasband, National Institute of Health, USA) for LC3-I (B) and LC3-II (C). N=1.
Figure 4.3.15 - p70 S6 kinase analysis of insulin treated SH-SY5Y cells overexpressing a range of hBCATm plasmid concentrations. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATm overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 100 nM insulin. Cell lysates were collected using RIPA extraction and subject to western blot analysis for p70 S6 kinase (A). Densitometry analysis was carried out using ImageJ™ software (Wayne Rasband, National Institute of Health, USA) for p70 S6 kinase (B). N=1.
Figure 4.3.16 - Western blot analysis of leucine treated SH-SY5Y cells overexpressing a range of hBCATc plasmid concentrations. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATc overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 5 mM leucine. Cell lysates were collected using RIPA extraction and subject to western blot analysis for hBCATc (A). Densitometry analysis was carried out using ImageJ™ software (Wayne Rasband, National Institute of Health, USA) (B). SH-SY5Y cells were either starved in EBSS or treated with 5 mM leucine for 3 hours before being fixed in 0.25% glutaraldehyde for immunocytochemistry (See Methods 2.20). Cells were probed with anti-hBCATc (1:250) and anti-rabbit Alexa Fluor® 568 (1:250) (C). N=1.
Figure 4.3.17 - LC3 analysis of leucine treated SH-SY5Y cells overexpressing a range of hBCATc plasmid concentrations. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATc overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 5 mM leucine. Cell lysates were collected using RI-PA extraction and subject to western blot analysis for LC3 (A). Densitometry analysis was carried out using ImageJ™ software (Wayne Rasband, National Institute of Health, USA) for LC3-I (B) and LC3-II (C). N=1.
Figure 4.3.18 - p70 S6 kinase analysis of leucine treated SH-SY5Y cells overexpressing a range of hBCATc plasmid concentrations. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATc overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 5 mM leucine. Cell lysates were collected using RIPA extraction and subject to western blot analysis for p70 S6 kinase (A). Densitometry analysis was carried out using ImageJ™ software (Wayne Rasband, National Institute of Health, USA) for p70 S6 kinase (B). N=1.
**Figure 4.3.19 - Western blot analysis of leucine treated SH-SY5Y cells overexpressing a range of hBCATm plasmid concentrations.** SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATc overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 5 mM leucine. Cell lysates were collected using RIPA extraction and subject to western blot analysis for hBCATm (A). Densitometry analysis was carried out using ImageJ™ software (Wayne Rasband, National Institute of Health, USA) (B). SH-SY5Y cells were either starved in EBSS or treated with 5 mM leucine for 3 hours before being fixed in 0.25% glutaraldehyde for immunocytochemistry (See Methods 2.20). Cells were probed with anti-hBCATm (1:250) and anti-rabbit Alexa Fluor® 568 (1:250) (C). N=1.
Figure 4.3.20 - LC3 analysis of leucine treated SH-SY5Y cells overexpressing a range of hBCATm plasmid concentrations. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATm overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 5 mM leucine. Cell lysates were collected using RIPA extraction and subject to western blot analysis for LC3 (A). Densitometry analysis was carried out using ImageJ™ software (Wayne Rasband, National Institute of Health, USA) for LC3-I (B) and LC3-II (C). N=1.
Figure 4.3.21 - p70 S6 kinase analysis of leucine treated SH-SY5Y cells overexpressing a range of hBCATm plasmid concentrations. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATm overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 5 mM leucine. Cell lysates were collected using RIPA extraction and subject to western blot analysis for p70 S6 kinase (A). Densitometry analysis was carried out using ImageJ™ software (Wayne Rasband, National Institute of Health, USA) for p70 S6 kinase (B). N=1.
Figure 4.3.22 - Confocal microscopy of SH-SY5Y cells overexpressing a range of hBCATc plasmid concentrations probed for amyloid β and phospho-tau. SH-SY5Y cells were grown on glass coverslips and transfected using JetPRIME® transfection reagent with 0.2 - 1.0 µg of hBCATc overexpression vector for 48 hours. The cells were fixed in 0.25% glutaraldehyde and subject to multiple wash steps (See Methods 2.20). Cells were probed with anti-amyloid β (1:150) and anti-phospho-tau (1:100) for 1.5 hours, and then anti-mouse Alexa Fluor® 488 (1:250) and anti-rabbit Alexa Fluor® 568 (1:250) for 1.5 hours. The coverslips were then mounted in VECTASHIELD Hard-Set antifade mounting medium with DAPI and imaged using a Zeiss Axiovert 200 confocal microscope. N=1. Blue - DAPI; Green - phospho-tau; Red - amyloid β
Figure 4.3.23 - Confocal microscopy of SH-SY5Y cells overexpressing a range of hBCATm plasmid concentrations probed for amyloid β and phospho-tau. SH-SY5Y cells were grown on glass coverslips and transfected using JetPRIME® transfection reagent with 0.2 - 1.0 µg of hBCATm overexpression vector for 48 hours. The cells were fixed in 0.25% glutaraldehyde and subject to multiple wash steps (See Methods 2.20). Cells were probed with anti-amyloid β (1:150) and anti-phosphor-tau (1:100) for 1.5 hours, and then anti-mouse Alexa Fluor® 488 (1:250) and anti-rabbit Alexa Fluor® 568 (1:250) for 1.5 hours. The coverslips were then mounted in VECTASHIELD Hard-Set antifade mounting medium with DAPI and imaged using a Zeiss Axiovert 200 confocal microscope. N=1. Blue - DAPI; Green - phospho-tau; Red - amyloid β
Figure 4.3.24 - Western blot analysis of insulin treated SH-SY5Y cells overexpressing a range of hBCATc plasmid concentrations. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATc overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 100 nM insulin. Cell lysates were collected using RIPA extraction and 10 µg of protein was separated on a 12% SDS-PAGE gel and transferred to PVDF membrane for western blot analysis. The membranes were probed with anti-β-amyloid (1:1000) (A) and anti-phospho-tau (1:10,000) (B). N=1.
Figure 4.3.25 - Western blot analysis of insulin treated SH-SY5Y cells overexpressing a range of hBCATm plasmid concentrations. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATm overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 100 nM insulin. Cell lysates were collected using RIPA extraction and 10 µg of protein was separated on a 12% SDS-PAGE gel and transferred to PVDF membrane for Western blot analysis. The membranes were probed with anti-β-amyloid (1:1000) (A) and anti-phospho-tau (1:10,000) (B). N=1.
Figure 4.3.26 - Western blot analysis of leucine treated SH-SY5Y cells overexpressing a range of hBCATc plasmid concentrations. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATc overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 5 mM leucine. Cell lysates were collected using RIPA extraction and 10 µg of protein was separated on a 12% SDS-PAGE gel and transferred to PVDF membrane for Western blot analysis. The membranes were probed with anti-β-amyloid (1:1000) (A) and anti-phospho-tau (1:10,000) (B). N=1.
Figure 4.3.27 - Western blot analysis of leucine treated SH-SY5Y cells overexpressing a range of hBCATm plasmid concentrations. SH-SY5Y cells were transfected using JetPRIME® transfection reagent with 0.5 - 2.5 µg of hBCATm overexpression vector for 48 hours. The cells were starved in EBSS for 1 hour before a 3 hour treatment with 5 mM leucine. Cell lysates were collected using RIPA extraction and 10 µg of protein was separated on a 12% SDS-PAGE gel and transferred to PVDF membrane for Western blot analysis. The membranes were probed with anti-β-amyloid (1:1000) (A) and anti-phospho-tau (1:10,000) (B). N=1.
Figure 4.3.28 - Confocal microscopy of SH-SY5Y cells overexpressing GFP control plasmid probed for hBCAT, LC3, amyloid β and phospho-tau. SH-SY5Y cells were grown on glass coverslips and transfected using JetPRIME® transfection reagent with 0.6 µg of GFP overexpression vector for 48 hours. The cells were fixed in 0.25% glutaraldehyde and subject to multiple wash steps (See Methods 2.20). Cells were probed with anti-hBCATm/hBCATc (1:250), anti-LC3 (1:100), anti-amyloid β (1:150) and anti-phospho-tau (1:100) for 1.5 hours, and then anti-mouse Alexa Fluor® 488 (1:250) and anti-rabbit Alexa Fluor® 568 (1:250) for 1.5 hours. The coverslips were then mounted in VECTASHIELD HardSet antifade mounting medium with DAPI and imaged using a Zeiss Axiovert 200 confocal microscope. N=1. Blue - DAPI; Green - hBCAT/phospho-tau; Red - LC3/amyloid β