Supplemental Data

Journal: Molecular Pharmacology

Title: Mis-trafficking of Endosomal Urokinase Proteins Triggers Drug-Induced Glioma Non-Apoptotic Cell Death.

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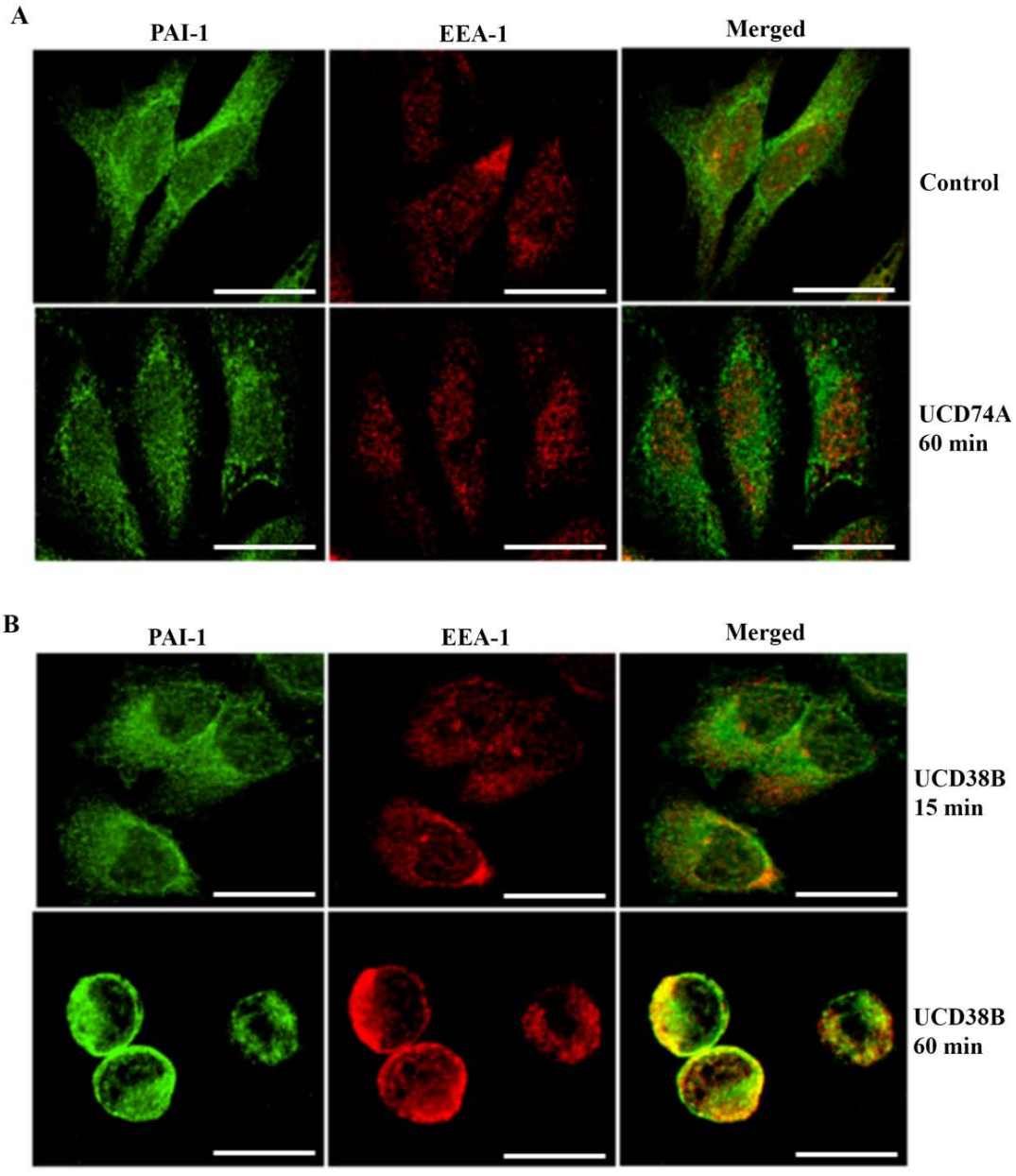
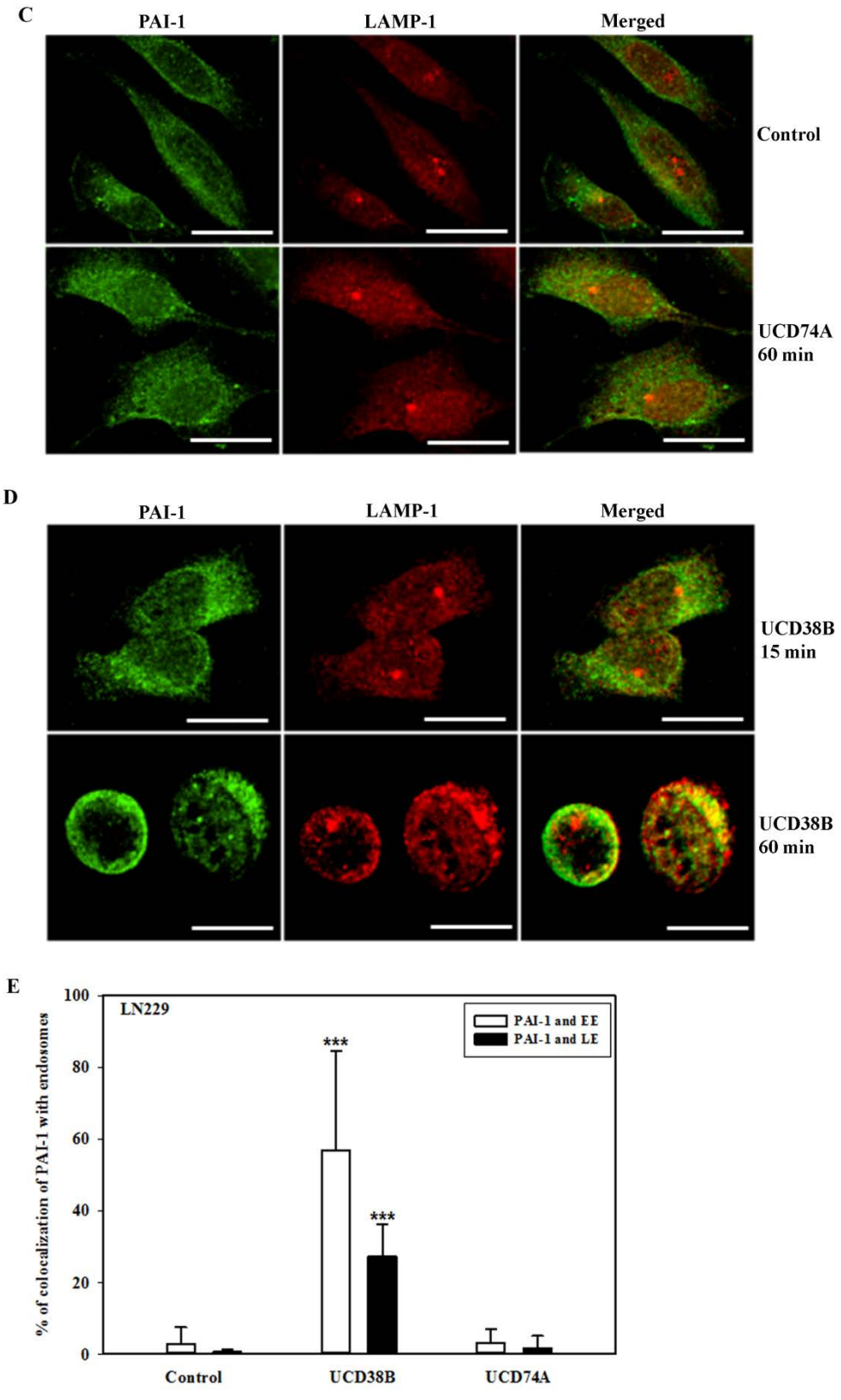
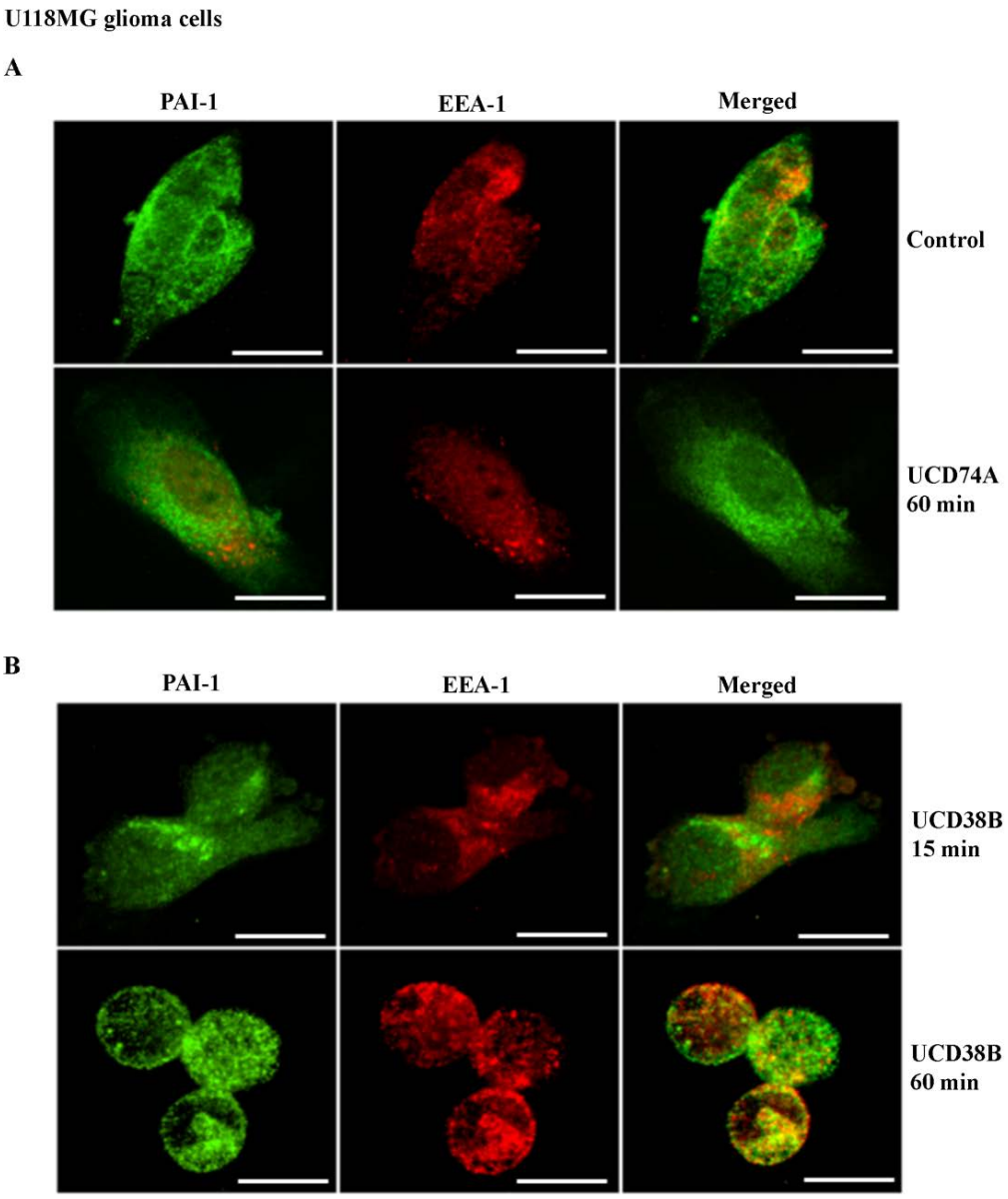
LN229 glioma cells

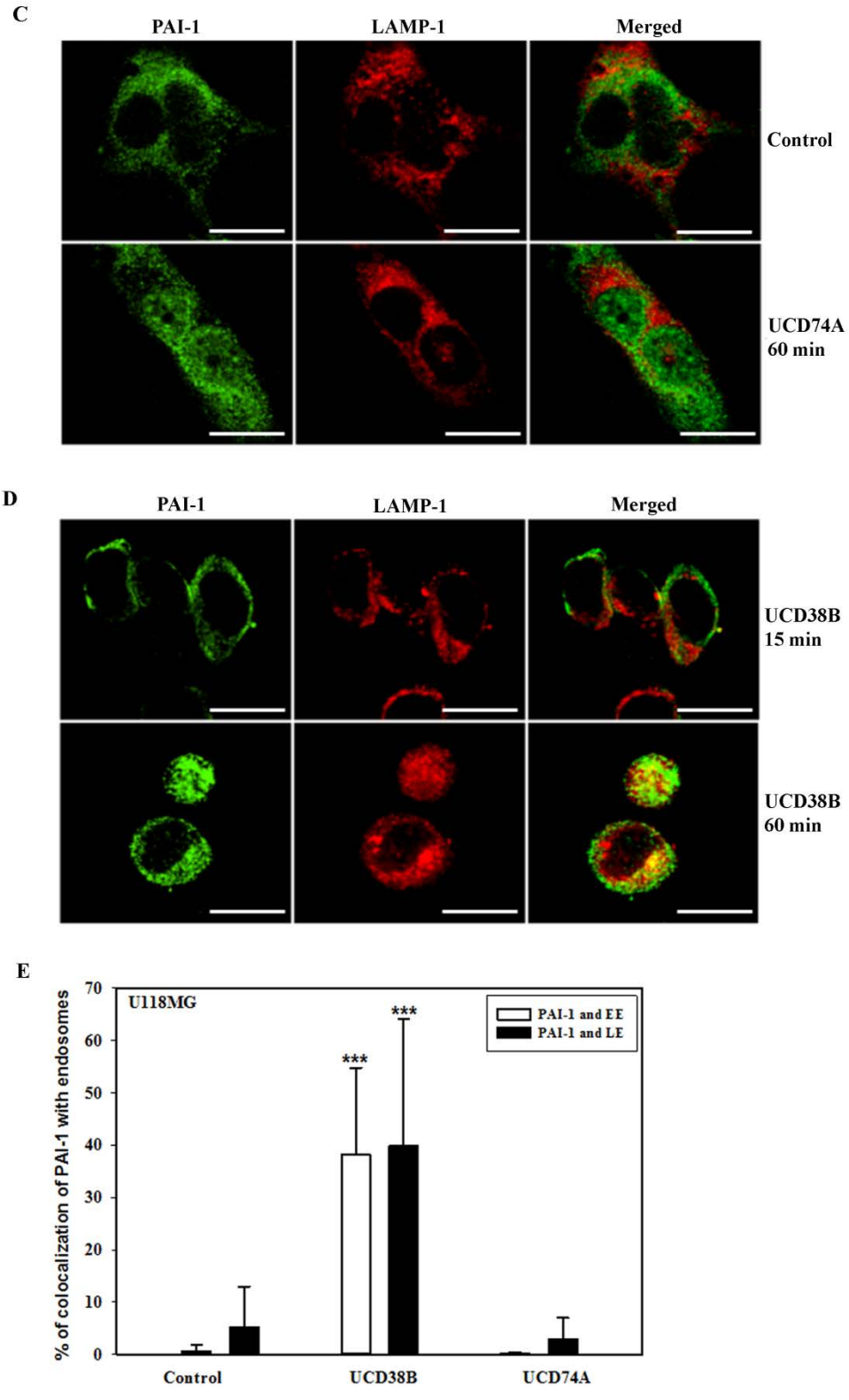




Supplemental figure1. Co-localization of PAI-1 with early and late endosomes in LN229 glioma cells: Co-localization of PAI-1 with early and late endosomes was detected by

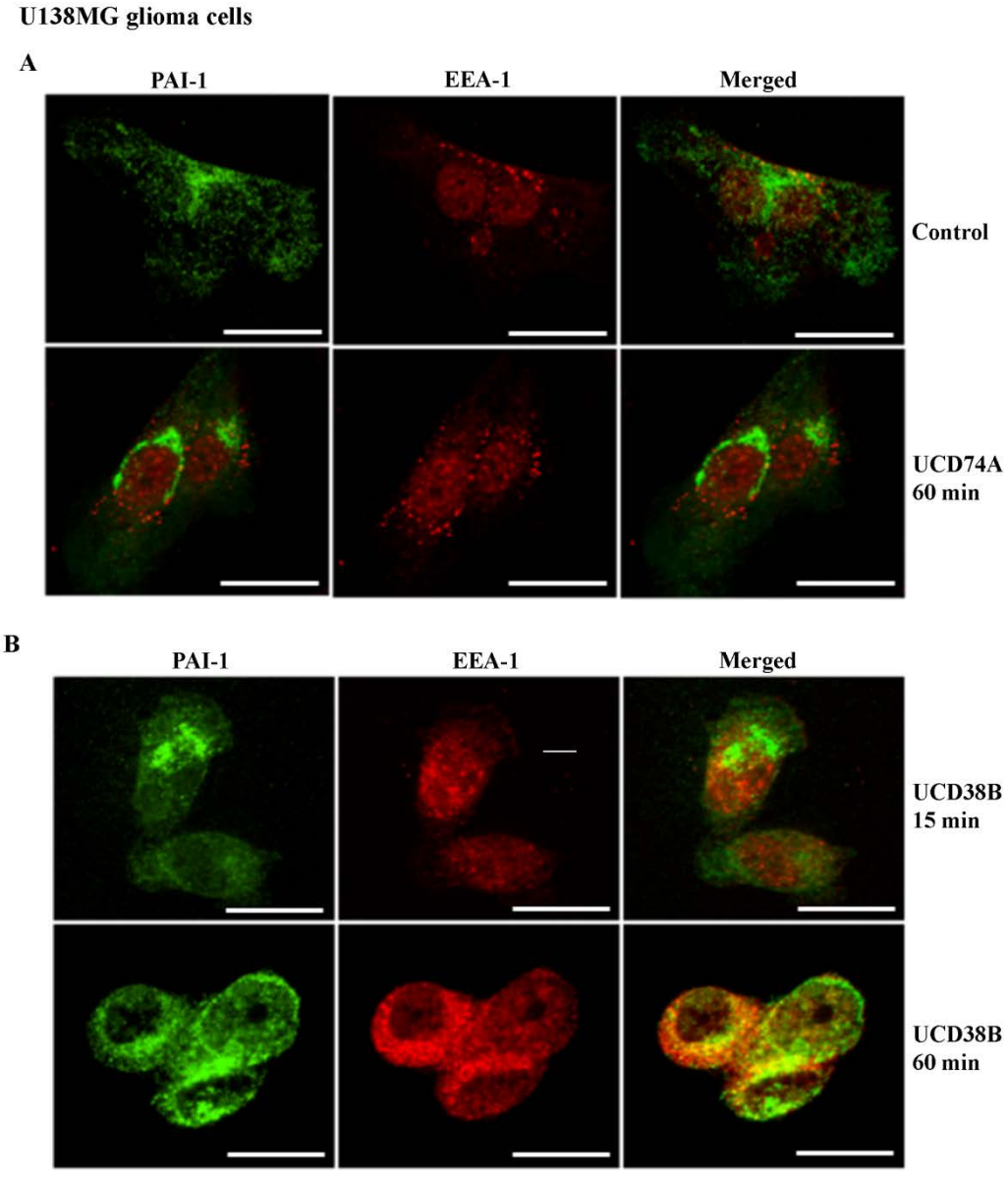
immunofluorescent confocal microscopy. EEA-1 and LAMP-1 were used as early and late endosomal marker proteins, respectively. LN229 glioma cells were treated with either vehicle control or 250 μM of UCD74A for 60 min **(A)** and **(C)** or with 250 μM of UCD3B for 60 min **(B)** and **(D)**. Scale bar is 50 μM. Quantification of the colocalization of PAI-1 with endosomes following 60 min of treatment was determined by imaging LN229 glioma cells using the high content imaging system ImageXpress and analyzed by a custom module using MetaXpress 5.0 software **(E)**. Results are presented as the mean ± S.D. of $n = 15$ with $^{***}P < 0.001$.

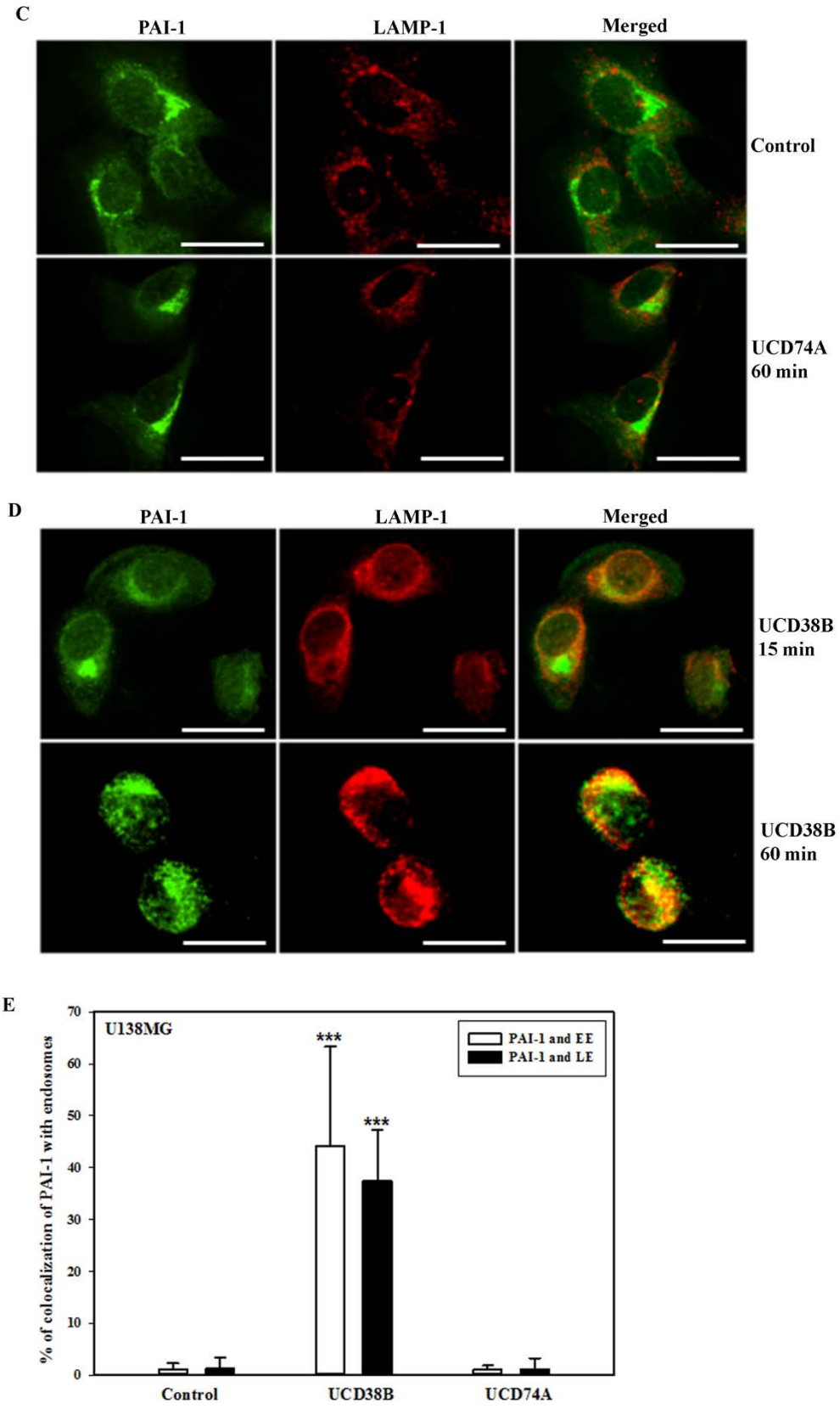




Supplemental figure 2. Co-localization of PAI-1 with early and late endosomes in U118MG glioma cells: Association of PAI-1 with early and late endosomes was detected by confocal

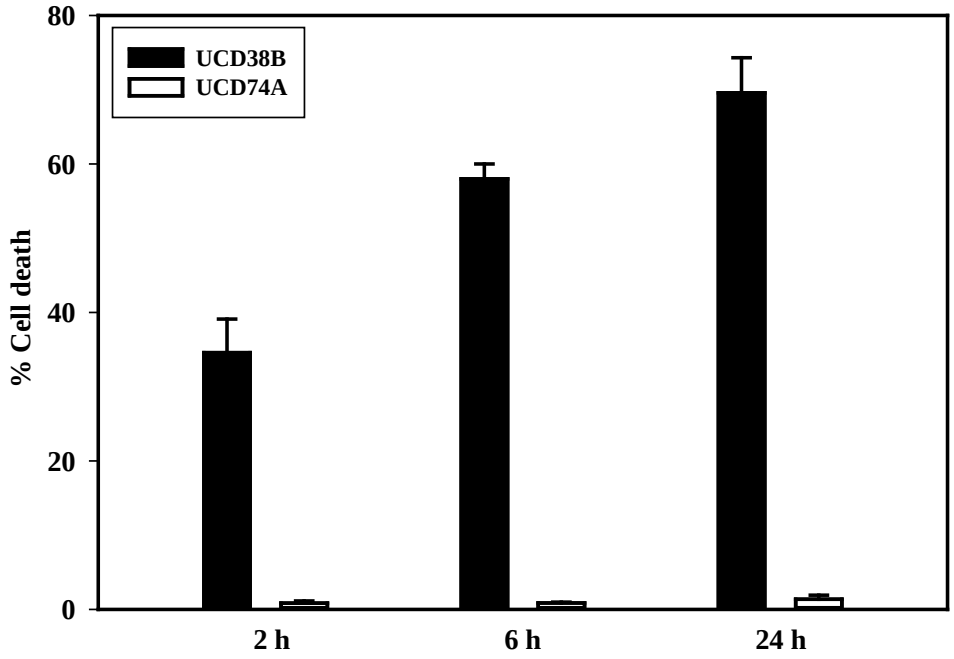
microscopy. EEA-1 and LAMP-1 were used as early and late endosomal marker proteins, respectively. U118MG glioma cells were treated with either vehicle control or 250 μM of UCD74A for 60 min **(A)** and **(C)** or with 250 μM of UCD38B for 60 min **(B)** and **(D)**. Scale bar is 50 μM. Quantification of the colocalization of PAI-1 with endosomes was determined by imaging U118MG glioma cells using the high content imaging system ImageXpress and analyzed by a custom module using MetaXpress 5.0 software **(E)**. Results are presented as the mean ± S.D. of $n = 15$ with $^{***}P < 0.001$.





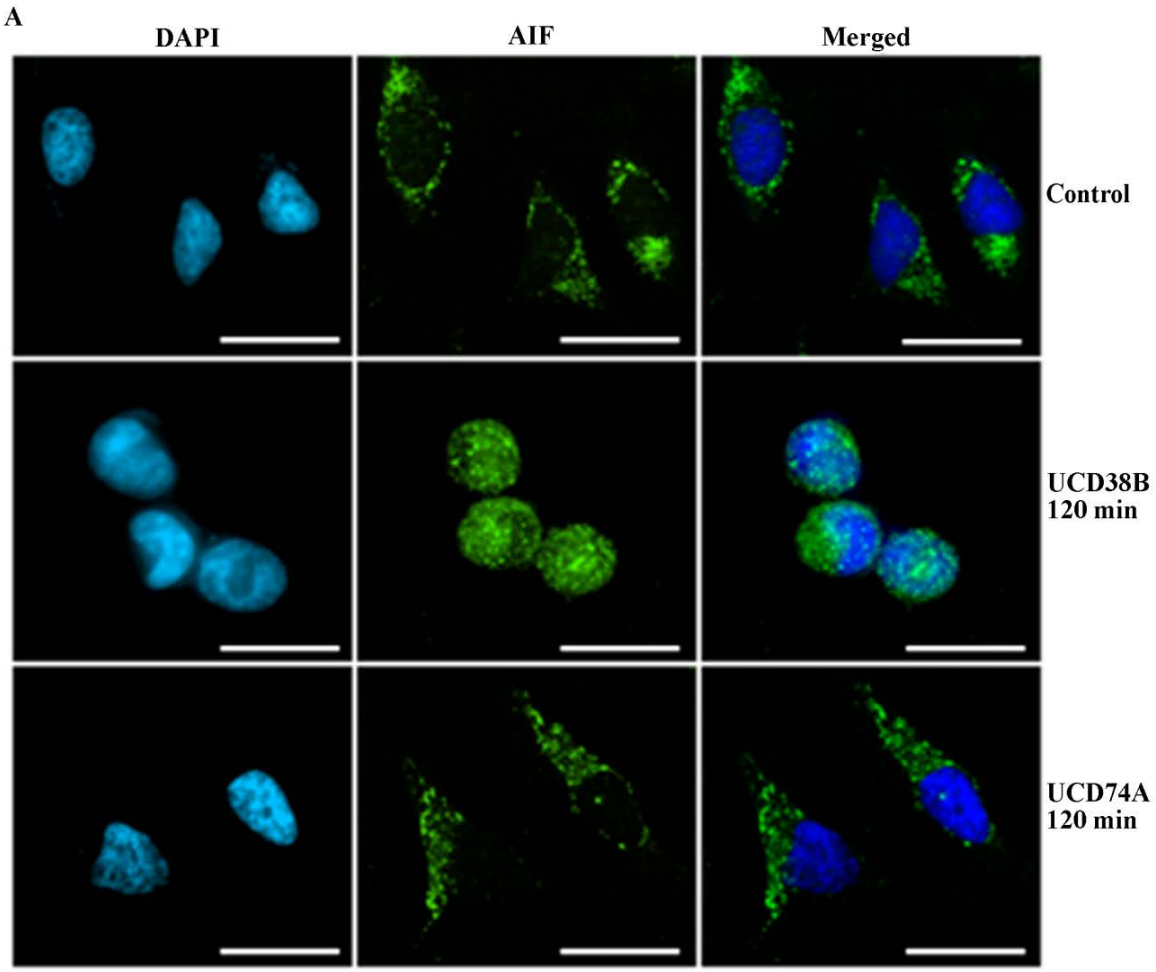
Supplemental figure 3. Co-localization of PAI-1 with early and late endosomes in U138MG glioma cells: Immunofluorescent confocal microscopy was used to detect the colocalization of

PAI-1 with early and late endosomes using EEA-1 and LAMP-1 as early and late endosomal marker proteins, respectively. U138MG glioma cells were treated with either vehicle control or 250 μM of UCD74A for 60 min **(A)** and **(C)** or 250 μM of UCD38B for 60 min **(B)** and **(D)**. Scale bar is 50 μM. Colocalization of PAI-1 with endosomes following 60 min of treatment was quantified by imaging U138MG glioma cells using the high content imaging system ImageXpress and analyzed by a custom module using MetaXpress 5.0 software **(E)**. Results are presented as the mean ± S.D. of $n = 15$ with ***$P < 0.001$.



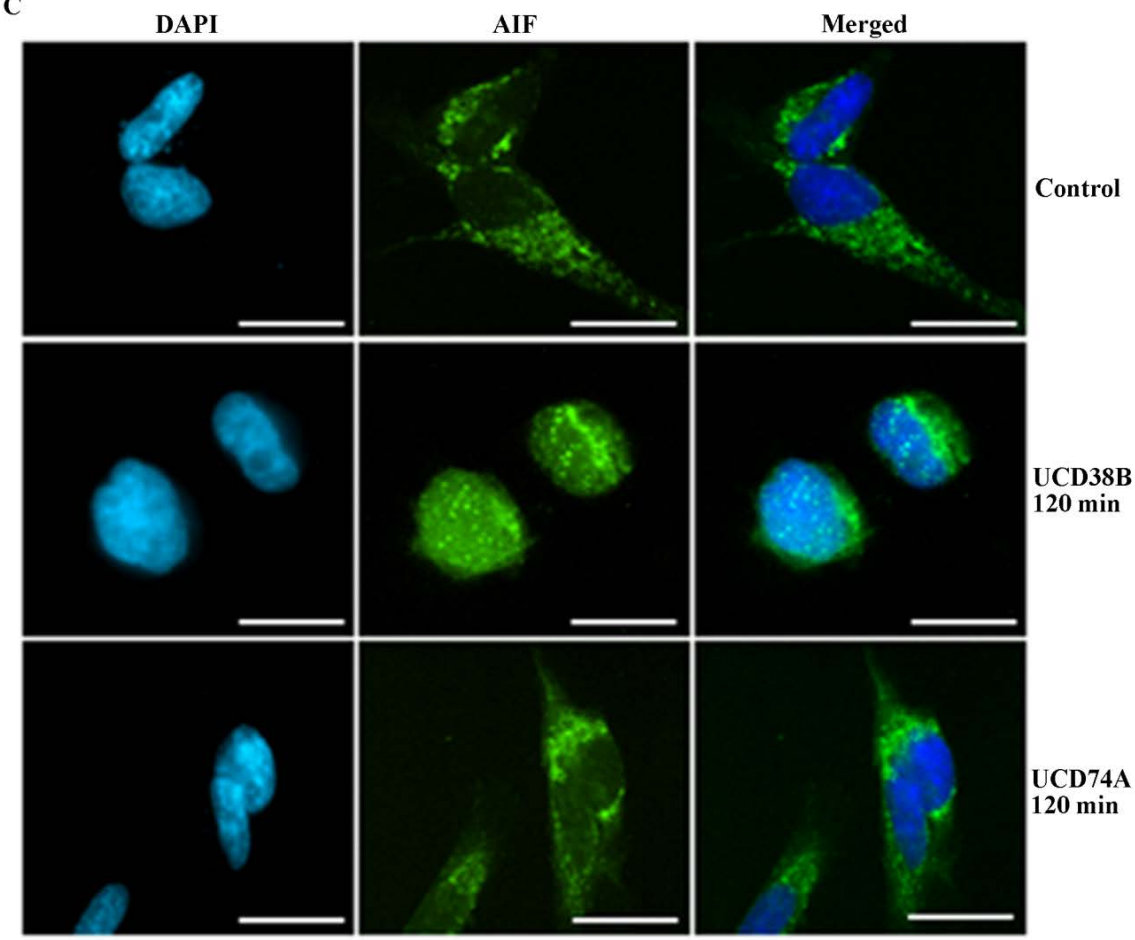
Supplemental figure 4. Drug cytotoxicity by trypan blue exclusion assay: U87MG glioma cells were incubated with UCD38B or UCD74A for 2, 6 and 24 h at 4 ^{0}C. Glioma cells were washed with 1X PBS, replaced with fresh media at 4^{o}C without drug and then transferred to 37^{0}C for 24 h. Cytotoxicity was determined by manual cell counts using the trypan blue exclusion assay. The manual dead cell counts correspond with the LDH cytotoxicity data shown in Fig. 10B.

LN229 glioma cells

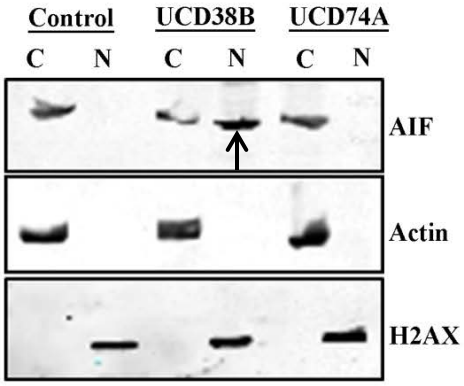


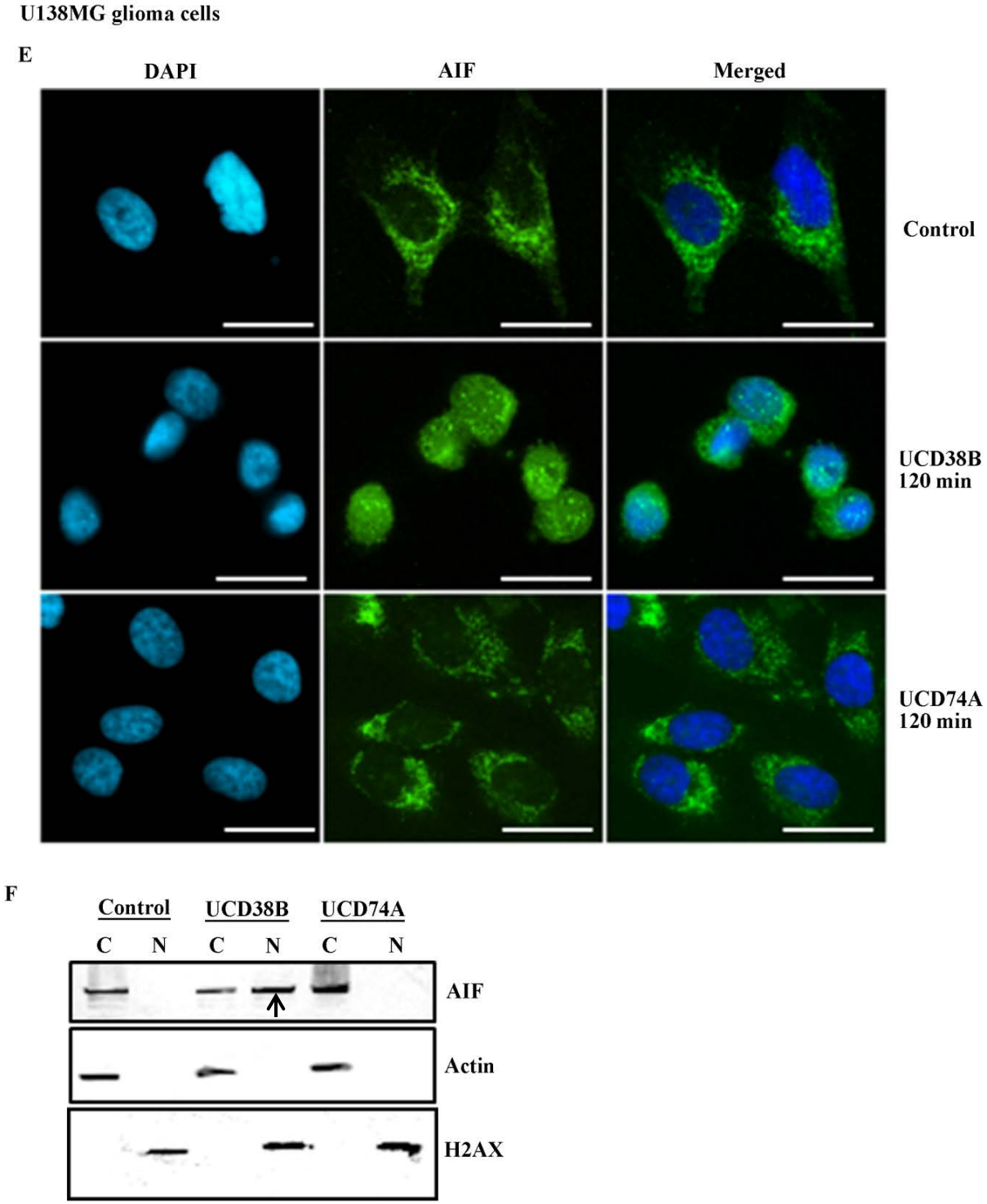
U118MG glioma cells

C



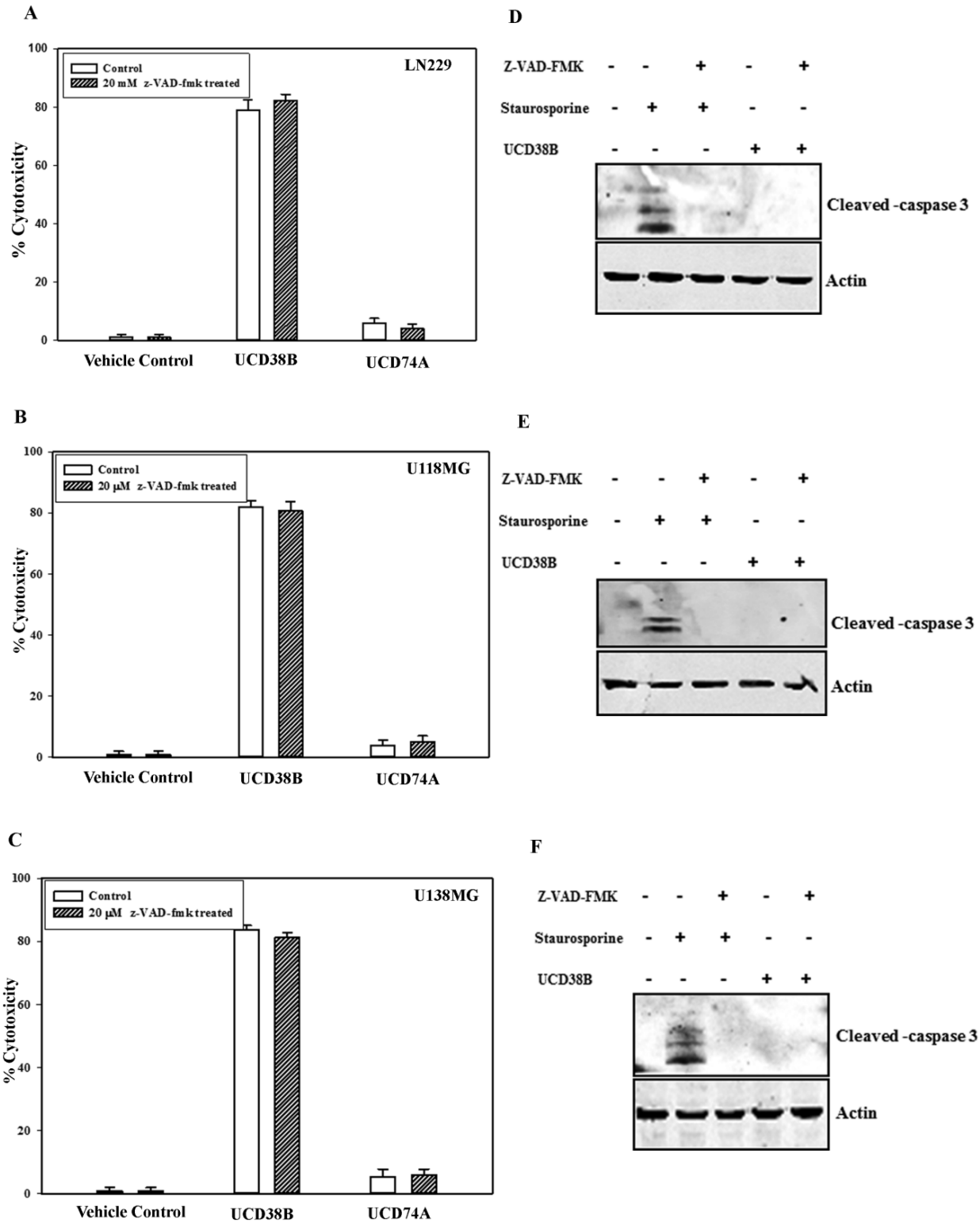
D





Supplemental figure 5. Nuclear translocation of AIF: Glioma cell lines LN229 **(A)**, U118MG **(C)**, and U138MG **(E)** were treated with UCD38B or UCD74A at 250 μM, or vehicle for 120 min and then immunostained for apoptosis inducing factor (AIF). Nuclei were stained with

DAPI. Confocal microscopy demonstrated the nuclear presence of AIF (green) following 120 min after exposure to UCD38B, but not following treatment with UCD74A or vehicle (control). Scale bar is 50 μm. Cytosolic, C, and nuclear, N, enriched cell fractions were separated on SDS-PAGE, AIF expression was visualized on immunoblots with Actin and H2AX as cytosolic and nuclear protein biomarkers, respectively. AIF levels were increased in the nuclear enriched fractions following treatment with UCD38B, but not with UCD74A, or vehicle in the following human glioma cell lines: LN229 **(B)**, U118MG **(D)**, and U138MG **(F)** glioma cells.



Supplemental figure 6. UCD38B does not activate caspase-3: Caspases were inactivated in three glioma cell lines by pretreatment with or without (control) 20 μM z-VAD-fmk for 1 h

followed by 24 h treatments with UCD38B or UCD74A at 250 μM, or vehicle (0.1% v/v DMSO) in LN229, U118MG and U138MG human glioma cells. LDH cytotoxic assays were performed and no significant differences in drug cytotoxicities were observed between control and caspase-inactivated gliomas cells LN229 **(A)**, U118MG **(B)** and U138MG **(C)**. Immunoblots demonstrate that caspase -3 cleavage by staurosporine (1 μM) was appropriately prevented by pretreatment with Z-VAD-fmk (20 μM), and that no caspase-3 activation was detected on the immunoblot following UCD38B treatment of LN229 **(D)**, U118MG **(E)** and U138MG **(F)** human glioma cells .