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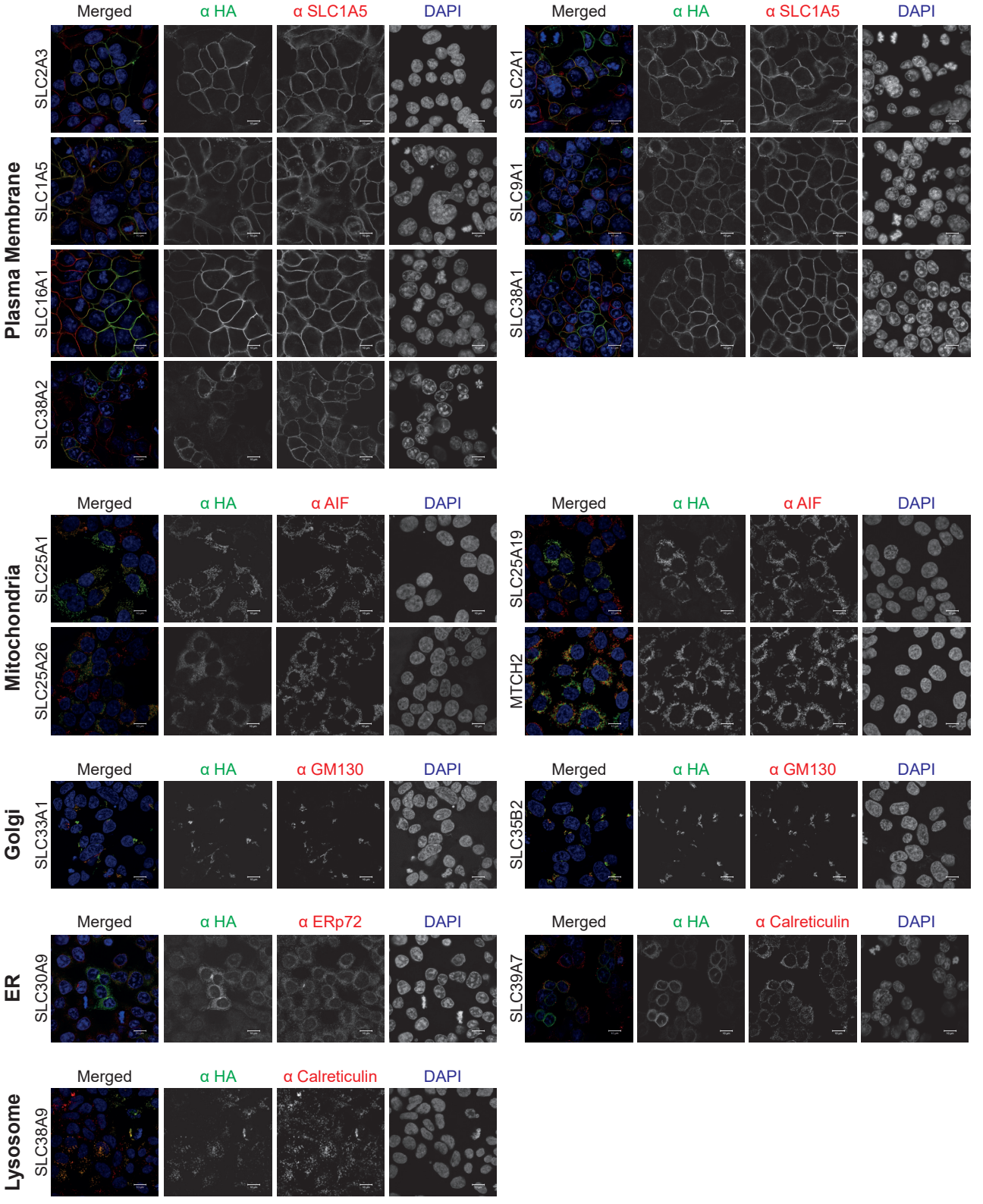
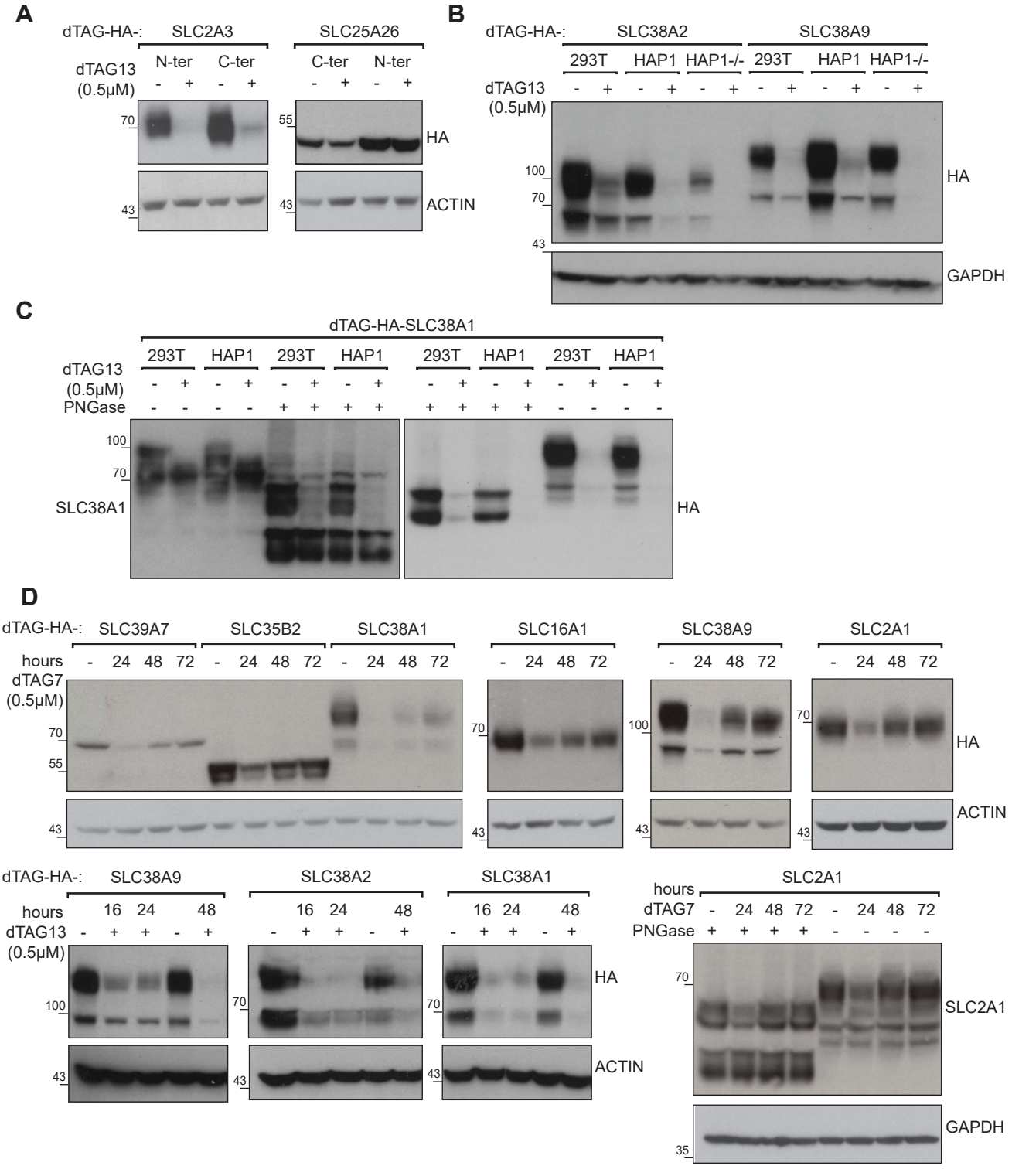
Supplemental Information

Targeted Degradation of SLC Transporters Reveals Amenability of Multi-Pass Transmembrane Proteins to Ligand-Induced Proteolysis

Ariel Bensimon, Mattia D. Pizzagalli, Felix Kartnig, Vojtech Dvorak, Patrick Essletzbichler, Georg E. Winter, and Giulio Superti-Furga



Supplementary Figure 1: Subcellular localization of exogenously expressed dTAG-HA SLC proteins, related to Figure 1: Subcellular localization of dTAG-HA SLC proteins was assessed by co-localization with the respective endogenous compartment marker. SLC1A5, AIF, GM130, LAMP2, and ERp72 or Calreticulin were used to identify the plasma membrane, mitochondria, Golgi, lysosome and endoplasmic reticulum (ER), respectively. PM: SLC2A3, SLC2A1, SLC1A5, SLC9A1, SLC16A1, SLC38A1 and SLC38A2; Mitochondria: SLC25A1, SLC25A19, SLC25A26, MTCH2; Golgi: SLC33A1, SLC35B2; Lysosome: SLC38A9; ER: SLC30A9, SLC39A7. Scale bar 10µm.



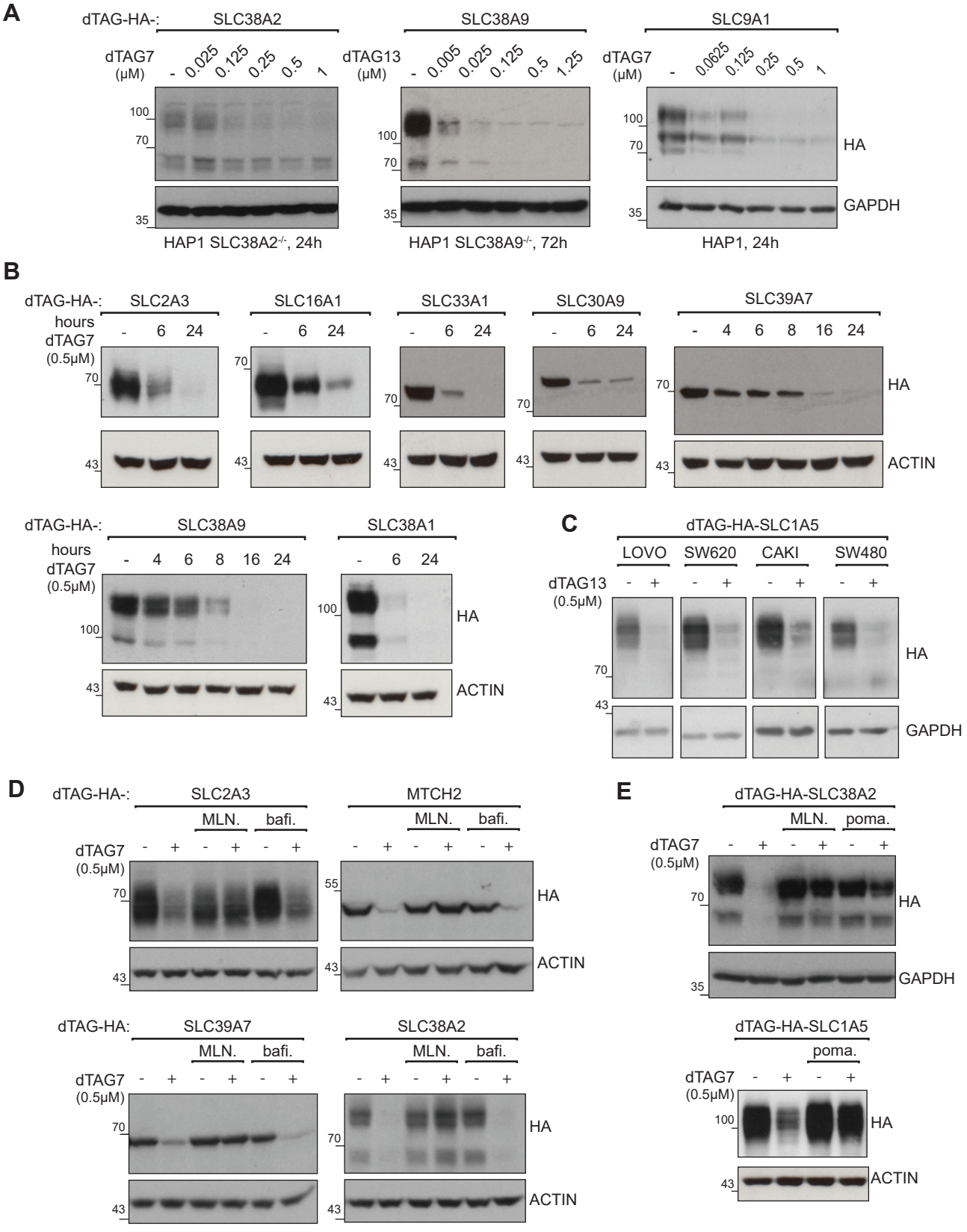
Supplementary Figure 2: Amenability of dTAG-HA SLC proteins to targeted degradation, related to Figure 1:

(A) HAP1 Cell lines expressing N- or C-terminus dTAG-HA-SLC2A3 were treated for 24 hours with 0.5 μM dTAG13 and SLC2A3 was degraded in both. Cell lines expressing N- or C-terminus dTAG-SLC25A26 did not demonstrate significant degradation after 24 hours of treatment with 0.5 μM dTAG13.

(B) Expression of dTAG-HA SLC38A2 or SLC38A9 in either 293T or HAP1 WT or a HAP1 clone in which the respective SLC was genetically ablated. All cells were treated with 0.5 μM dTAG13 for 72 hours.The variance in expression levels of the exogenous protein indicated that degradation is achieved irrespective of the initial stable expression level, nor dependent on the presence of the endogenous SLC.

(C) dTAG-HA SLC38A1, expressed in HAP1 or 293T, was degraded by treatment with 0.5 μM dTAG13 for 72 hours. Indicated protein extracts were de-glycosylated by treatment with PNGase. Western blotting with antibodies against the HA-tag and the total protein confirmed that the tagged protein as a whole is completely degraded.

(D) Temporal control with dTAG7 vs. dTAG13: HAP1 cells expressing dTAG-HA SLC39A7, SLC38A1, SLC35B2, SLC16A1, SLC38A9 or SLC2A1 were treated with 0.5 μM of dTAG7 for 24, 48 or 72 hours. For SLC2A1, Western blotting with antibodies against the HA-tag and the total protein mirrored the observed pattern. dTAG7 leads to reversible degradation of the target SLC, allowing accurate temporal control over protein levels. HAP1 cells expressing dTAG-HA SLC38A9, SLC38A2, or SLC38A1 were treated with 0.5 μM dTAG13 for 16, 24 or 48 hours. Near-complete degradation of the target SLC was observed and maintained, suggesting dTAG13 is favourable for long-term treatments.



Supplementary Figure 3: Characterization of dTAG-HA SLC protein degradation, related to Figure 2:

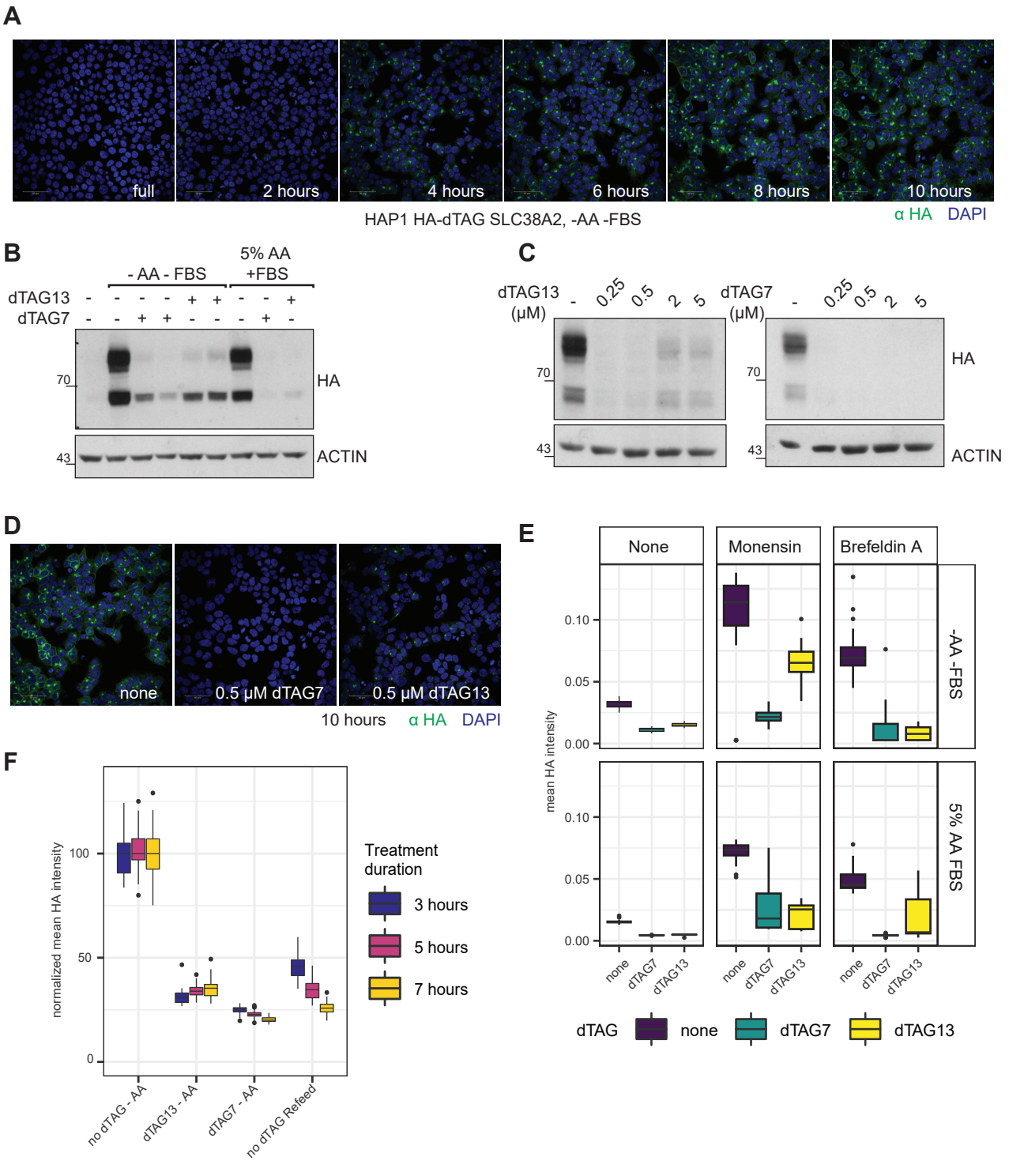
(A) A range of dTAG7 and dTAG13 concentrations was tested in the indicated HAP1 cell lines expressing dTAG-HA SLC38A2, SLC38A9 and SLC9A1. The concentration required to achieve complete or near to complete degradation depends on the SLC.

(B) A time course of dTAG mediated SLC degradation demonstrates initiation of degradation within six hours. HAP1 cell lines expressing dTAG-HA SLC2A3, SLC16A1, SLC33A1, SLC30A9, SLC38A9, SLC38A1 or SLC39A7 were treated with 0.5 μM dTAG7.

(C) dTAG-HA SLC1A5 was expressed in LOVO, SW620, CAKI and SW480 cell lines. Cells were treated with 0.5 μM dTAG13 and degradation was assessed after 48 hours.

(D) Chemical "rescue" of dTAG driven degradation by MLN4924 but not bafilomycin A1. HAP1 cell lines expressing dTAG-HA SLC2A3, SLC39A7, SLC38A2, and MTCH2 were treated with 0.5 μM dTAG7 for 12 hours. These cells were treated with MLN4924 (MLN, 1μM), or bafilomycin A1 (bafi., 2.5μM) to monitor the inhibition of degradation.

(E) Chemical "rescue" of dTAG driven degradation by pomalidomide (poma., 10μM). Cell lines expressing dTAG-HA SLC38A2 or dTAG-HA SLC1A5 were treated with 0.5 μM dTAG7 for 18 hours.



Supplementary Figure 4: Characterization of endogenous HA-dTAG-SLC38A2 degradation, related to Figure 3:

(A) Representative images of the induction of HA-dTAG-SLC38A2 expression at different time points after addition of media depleted of amino acids (AAs) and FBS, compared to normal full media. Scale bar 50μm.

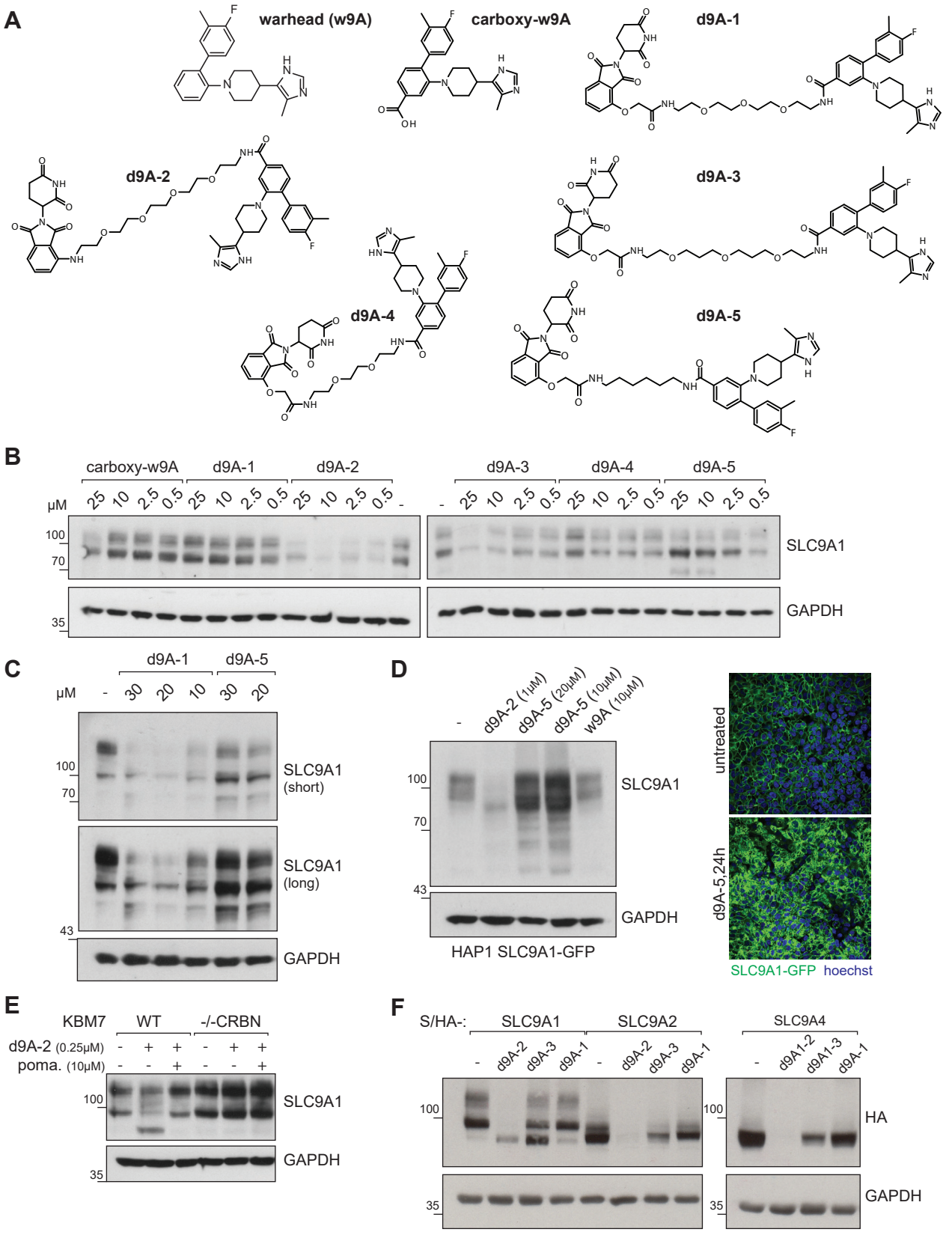
(B) Degradation of HA-dTAG-SLC38A2 by co-treatment with dTAG13 or dTAG7 (0.5 μM) for eight hours during induction of SLC38A2 expression in one of the two media: media deprived of AAs and FBS; media with 5% non essential AAs and with FBS. Complete AA depreviation leads to a slight increase in a fraction of SLC38A2 that is not completely degraded by dTAG13.

(C) A dose curve of HA-dTAG-SLC38A2 degradation following a 16-hour cotreatment with dTAG7 and dTAG13, in media depleted of amino acids and FBS.

(D) Representative images of the differences in degradation of HA-dTAG-SLC38A2 in HAP1 cells following cotreatment with dTAG7 and dTAG13 for ten hours. Scale bar 50μm.

(E) The effects of halting HA-dTAG-SLC38A2 during maturation by co-treatment with brefeldin A(5 μg/ml) or monensin (2 μM) were assayed by imaging (α-HA). Co-treatments with dTAG7 or dTAG13 (0.5 μM) under two different media conditions demonstrate that SLC38A2 is amenable to degradation throughout its maturation. While dTAG7 appears to effectively degrade SLC38A2 under all pertubations, dTAG13 treatment demonstrates more variability in effectiveness.

(F) Time course of degradation of HA-dTAG-SLC38A2 by targeted degradation, compared to natural removal of the protein following refeeding with full media. Expression of the endogenous SLC was monitored by imaging (α-HA), quantified and normalized per time point. As control, the SLC was induced by media depleted of amino acids and FBS for a total of 21 hours ("no dTAG –AA"). At the indicated time points, the media was supplemented with dTAG7/dTAG13 (0.5 μM) or replaced with full media.



Supplementary Figure 5: Characterization of d9A series, related to Figure 4:

(A) Chemical structures of the the warhead (w9A), the carboxy-warhead (carboxy-w9A) and the derived d9A series.

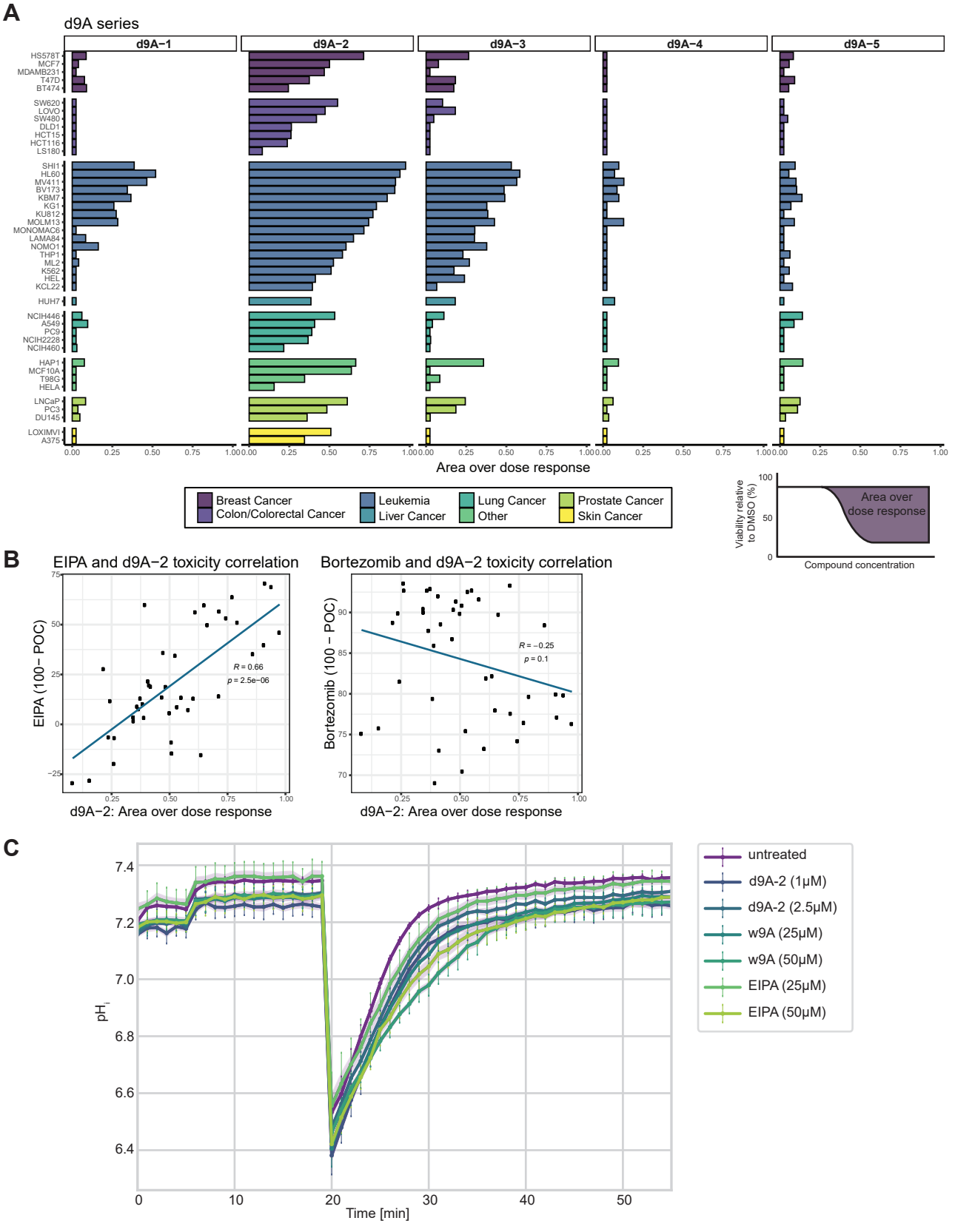
(B) HAP1 cells were treated with a dose curve of carboxy-w9A, d9A-1, d9A-2, d9A-3, d9A-4, d9A-5 for 24 hours. In HAP1, SLC9A1 degradation was most effective with d9A-2 but could also be achieved with d9A-3.

(C) KBM7 cells were treated with varying concentrations of d9A-1, d9A-5 for 18 hours. Reduction of SLC9A1 expression was observed with d9A-1, while d9A-5 led to a difference in the immunoblot migratory pattern.

(D) SLC9A1-GFP, overexpressed in HAP1, is monitored by immunoblot and immunofluorescence following treatment for 24 hours. d9A-2 led to degrdation, while d9A-5 led to a difference in the immunoblot migratory pattern, and w9A had no effect on protein levels. Representative images of SLC9A1-GFP modulation by d9A-5 are presented (scale bar 50μm).

(E) Rescue of SLC9A1 degradation in KBM7 -/- CRBN cell lines after eight hours of d9A-2 treatment. In WT KBM7, SLC9A1 degradation is chemically rescued with pomalidomide.

(F) HAP1 cell lines expressing Strep/HA- SLC9A1, SLC9A2, and SLC9A4 were treated with d9A-2 (1 μM), d9A-3 (10 μM) and d9A-1 (10 μM) for 18 hours.



Supplementary Figure 6: Cell viability and pH_i measurements after treatment with d9A series, related to Figure 5:
(A) Viability of various cells lines after treatment with the d9A series. Cells were treated with a 3-point dose curve of d9A-5, and d9A-4, a 5-point dose curve of d9A-1, and d9A-3, and a 9-12 point dose curve for d9A-2. Viability was assayed using the CTG assay, 72 hours post treatment. The activity of each molecule in each cell line was estimated as depicted on the right, based on the area above the dose response, relative to DMSO as control.
(B) Correlation between d9A-2 toxicity and EIPA or bortezomib toxicity. Toxicity of EIPA/bortezomib, was assayed at a single point, along with DMSO, as an internal control for each cell line. Toxicity was assessed by subtracting the percentage of DMSO control (100-POC). A significant correlation is observed between toxicity of EIPA and toxicity of d9A-2, while no significant correlation is observed with bortezomib.
(C) Raw data of the acid load study comparing different concentrations of d9A-2, the warhead w9A and EIPA. Time points indicated in Figure 5A relate to time after the recorded pH_i-minimum of each sample. Data are represented as mean ± SD.