SUPPORTING INFORMATION

Therapeutic Efficacy of a Family of pHLIP-MMAF Conjugates in Cancer Cells & Mouse Models

Kelly E. Burns[1], Harvey H. Hensley[2], Matthew K. Robinson[2] and Damien Thévenin[1]*

[1]Department of Chemistry, Lehigh University, 6 East Packer Ave, Bethlehem PA 18015

[2]Molecular Therapeutics Program, Fox Chase Cancer Center, 333 Cottman Ave, Philadelphia, PA 19111

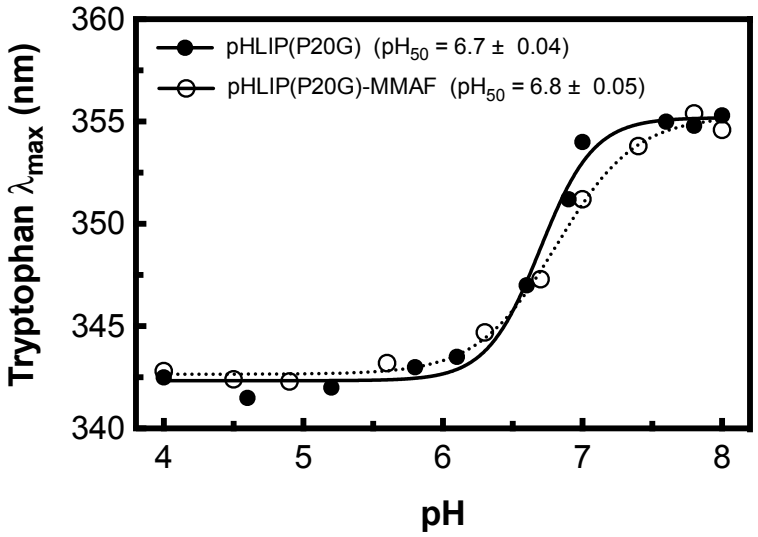

Figure S1: Fluorescence spectral maximum changes upon pH titration of pHLIP(P20G) (close circles) and pHLIP(P20G)-MMAF (open circles) in the presence of liposomes.

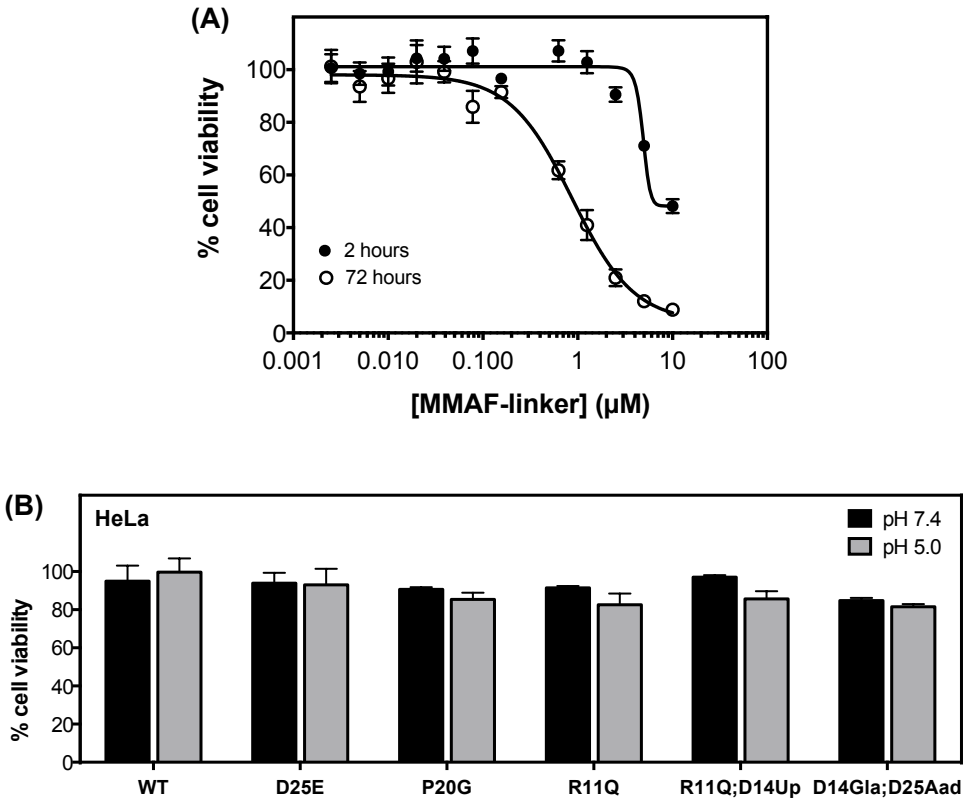

Figure S2: Effects of Free MMAF-linker and pHLIP Variants on HeLa Cell Proliferation. For each cell assay, 3,000 cells/well (96-well plate) were seeded, allowed to adhere overnight, treated for either 2 or 72 hours with MMAF (A) or for 2 hours with 10 μM pHLIP variants (B) at pH 7.4 (black bars) and a low pH (grey bars). After the 2-hour treatment, cells were washed once with media and cultured for 72 hours in complete medium at physiologic pH. Cell viability was assessed with the MTT assay. MMAF treated cells were normalized to the 1% DMSO controls and pHLIP variants were normalized to the media control (0 μM, pH 7.4), as 100% cell viability. 2-hour and 72-hour MMAF-linker treatments resulted in IC_{50} of 5 μM and 834 nM, respectively. Results are shown as mean ± SEM (n=9)

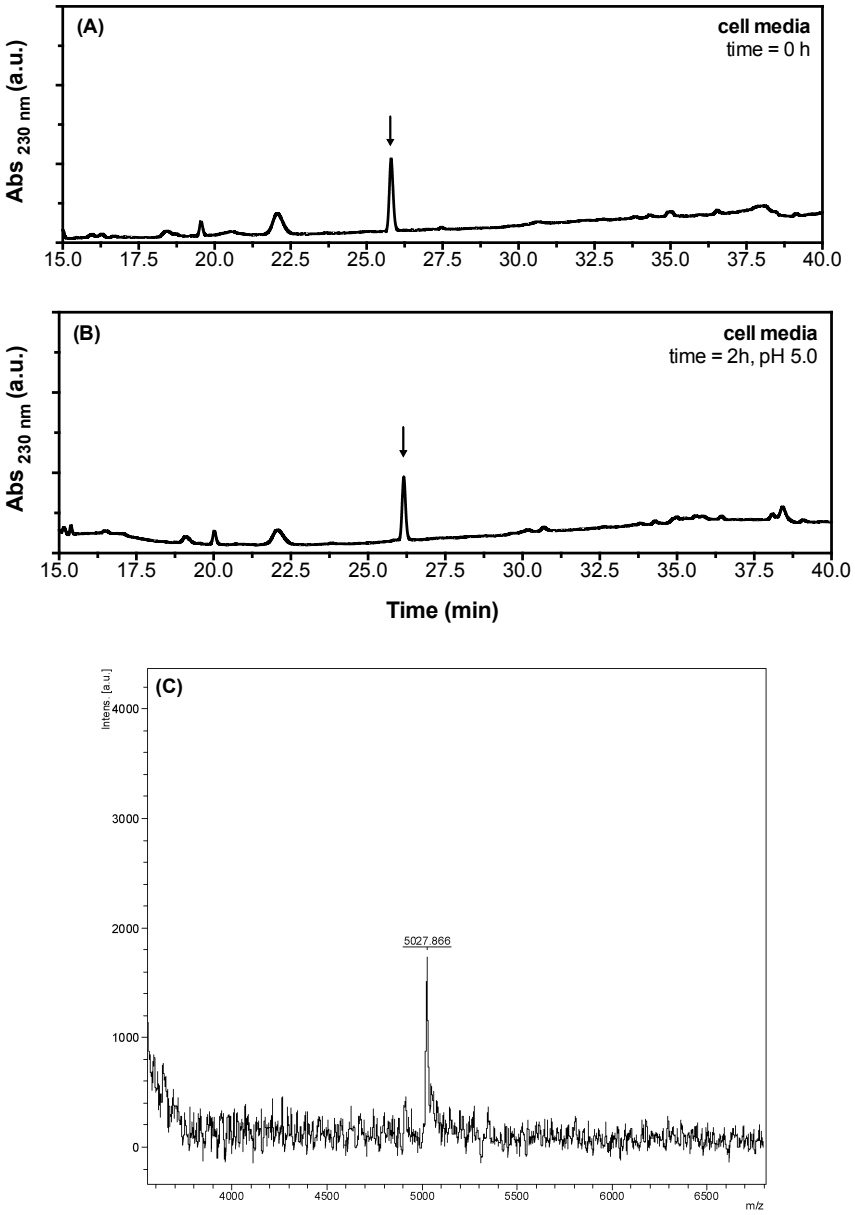

Figure S3: Chemical Stability of pHLIP(WT)-MMAF in cell media at pH 5.0. HPLC traces of 10 μM pHLIP(WT)-MMAF before (A) and after (B) incubation in cell media for 2h at pH 5.0 and 37 ºC. Flow rate 5 mL/min, phase A: water, 0.01% TFA; phase B: acetonitrile, 0.01% TFA; gradient 45 min from 95/5 A/B to 0/100 A/B. The arrow indicates the peak corresponding to pHLIP(WT)-MMAF. The conjugate appears to be stable in these conditions, as no degradation products are observed by HPLC and mass spectroscopy. The conjugate was found intact as checked by mass spectroscopy (C). Calculated ($M+H^+$) = 5030; found ($M+H^+$) = 5028.

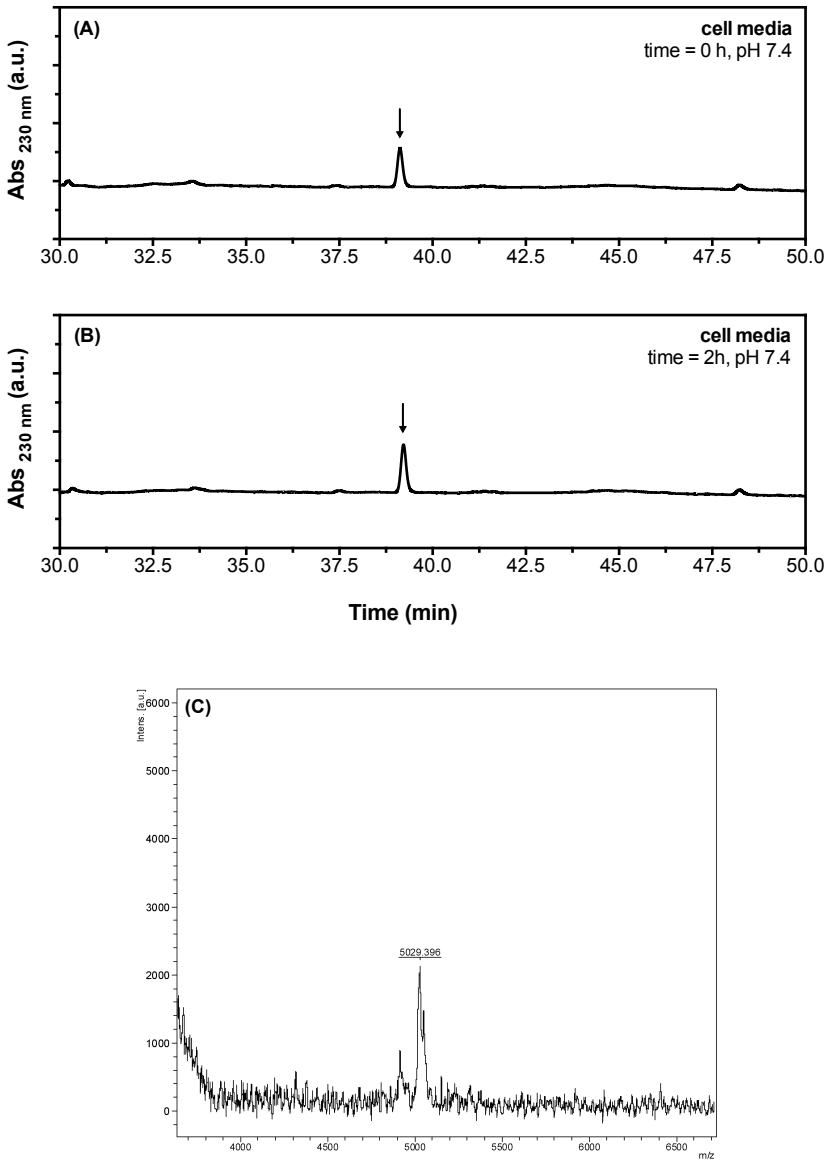

Figure S4: Chemical stability of pHLIP(WT)-MMAF in cell media at pH 7.4. HPLC traces of 10 μM pHLIP(WT)-MMAF before (A) and after (B) incubation in cell media for 2h at pH 7.4 and 37 ºC. Flow rate 3 mL/min, phase A: water, 0.01% TFA; phase B: acetonitrile. 0.01% TFA; gradient 60 min from 95/5 A/B to 0/100 A/B. The arrow indicates the peak corresponding to pHLIP(WT)-MMAF. The conjugate appears to be stable in these conditions, as no degradation products are observed by HPLC and mass spectroscopy. The conjugate was found intact as checked by mass spectroscopy (C). Calculated ($M+H^+$) = 5030; found ($M+H^+$) = 5029.

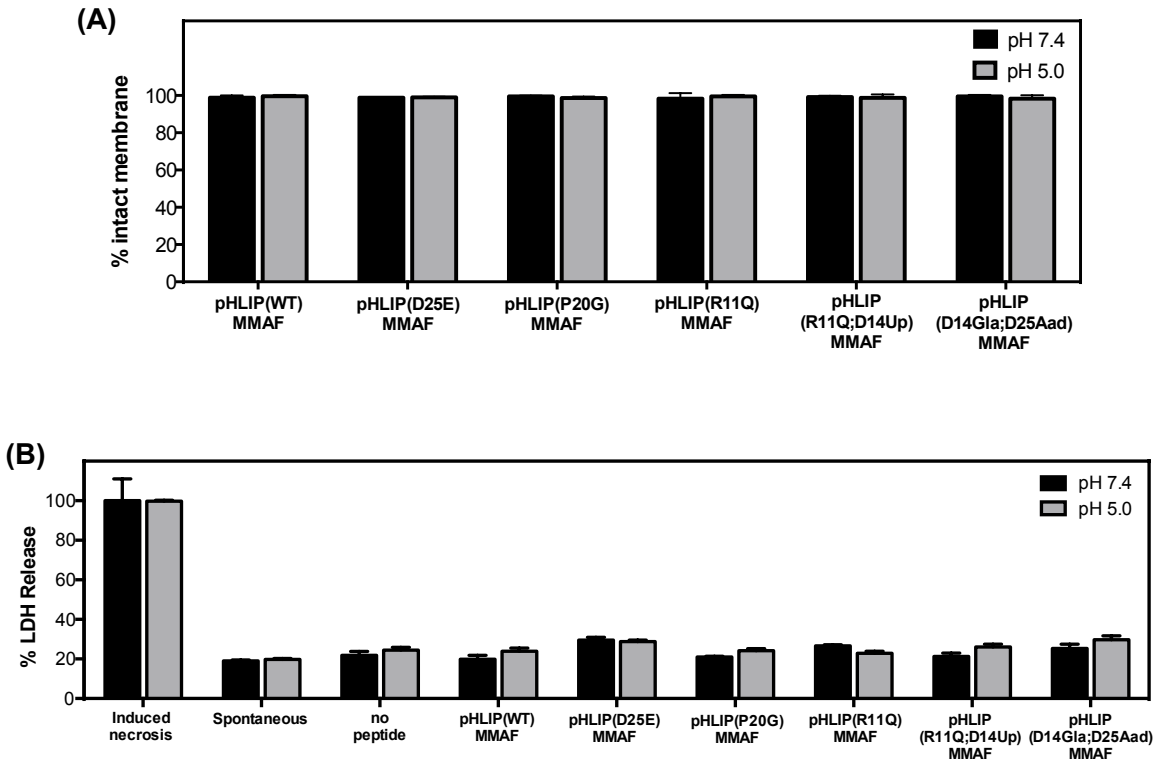

Figure S5. Effect of pHLIP-MMAF Variants on Plasma Membrane Integrity and Lactate Dehydrogenase (LDH) Release. (A) The integrity of the plasma membrane is assessed by the uptake of trypan blue dye. 3,000 cells/well of HeLa cells were seeded, incubated until confluent (~72 hours) and treated with 10 μM for 2 hours at pH 7.4 (black bars) or pH 5.0 (grey bars). Cells were detached and counted based on trypan blue uptake with an hemacytometer: % of intact cells corresponds to the number of cells not showing any dye uptake over the total number of cells. Results are shown as mean ± SD (n=3). (B) LDH release assay. Cells were seeded at a density of 200,000 cells/well in a 24 well plate, allowed to adhere overnight and treated with 10 μM for 2 hours at pH 7.4 (black bars) or pH 5.0 (grey bars). Cell media supernatant was monitored for the presence of LDH following treatment. Results are shown as mean ± SD (n=3).

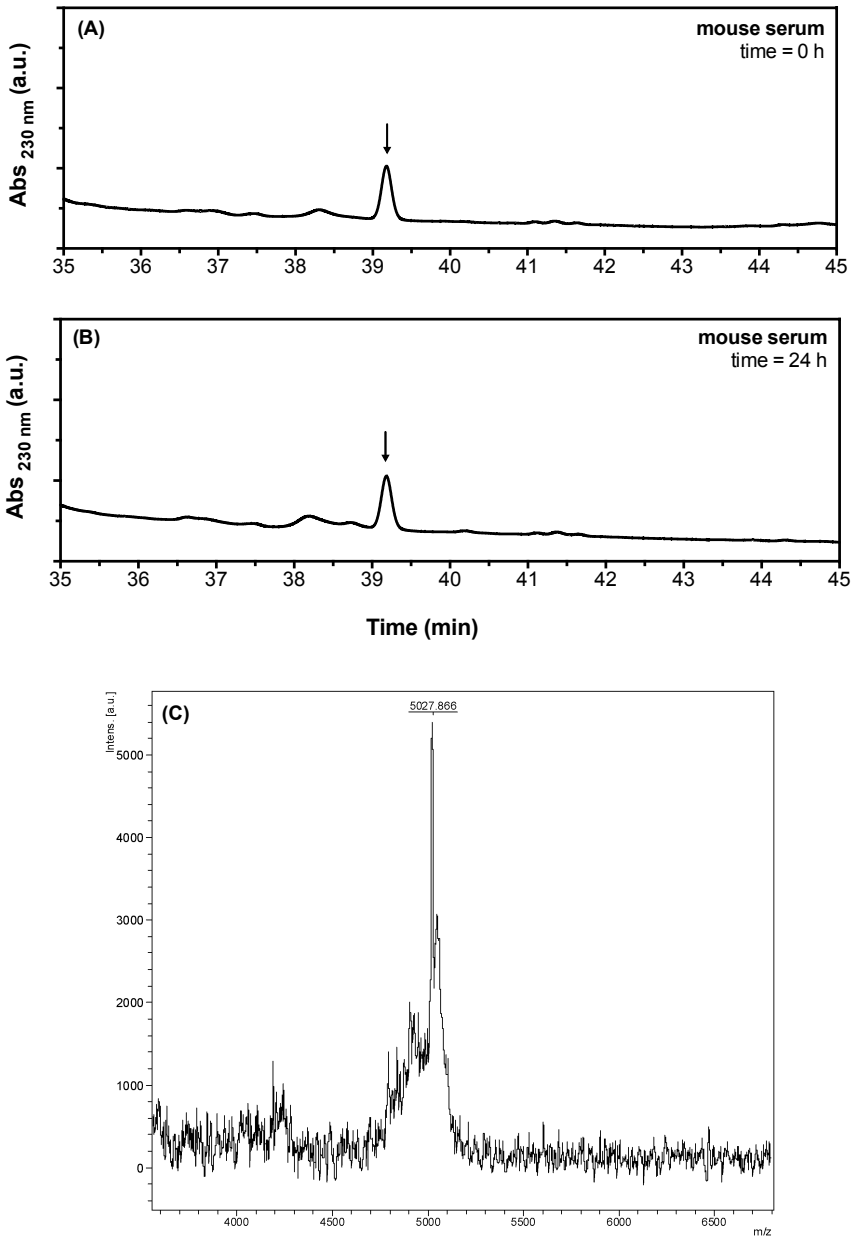

Figure 65: Chemical Stability of pHLIP(WT)-MMAF in mouse serum. HPLC traces of 20 μM pHLIP(WT)-MMAF before (A) and after (B) incubation in mouse serum for 24h at 37 ºC. The arrow indicates the peak corresponding to pHLIP(WT)-MMAF. The conjugate appears to be stable in these conditions, as no degradation products are observed by HPLC and mass spectroscopy. The conjugate was found intact as checked by mass spectroscopy (C). Calculated ($M+H^+$) = 5030; found ($M+H^+$) = 5028.

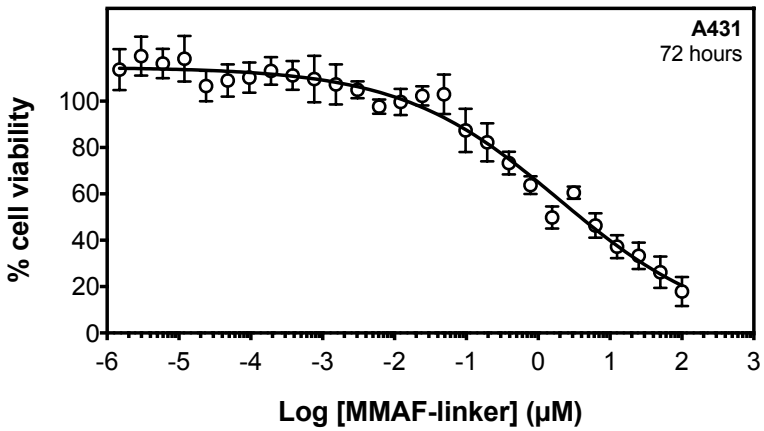

Figure S7: Effects of Free MMAF-linker on A431 Cell Proliferation. For each cell assay, 3,000 cells/well (96-well plate) were seeded, allowed to adhere overnight, treated 72 hours with MMAF pH 7.4. Cell viability was assessed with the MTT assay. MMAF treated cells were normalized to the 1% DMSO controls, as 100% cell viability. IC_{50} = 872 ± 120 nM. Results are shown as mean ± SEM (n=9).