TITLE

Hydra-elastin-like polypeptides increase rapamycin potency when targeted to cell-surface GRP78

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Supplemental Table S1: Full amino acid sequences of A24, FKBP, 5FA, and X-5FA

A24 ($VPGAG_{24}$):

G VPGA

FKBP:

G VQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTI SPDYAYGATGHPGIIPPHATLVFDVELLKLE

5FA:

MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKL TI SPDYAYGATGHPGIIPPHATLVFDVELLKLEGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAG VPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAG VPGAGVPGAGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQM SVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLEGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAG VPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVP GAGVPGAGVPGAGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVA QMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLEGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPG AGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAG VPGAGVPGAGVPGAGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEG VAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLEGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGV PGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPGAGVPG AGVPGAGVPGAGVPGAGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWE EGVAQMSVGQRAKLTI SPDYAYGATGHPGIIPPHATLVFDVELLKLE

W-5FA:

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L-5FA:

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P6-5FA:

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GKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLEGVPGAGVPGAGVPGAGVPGA
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FKFMLGKQEVIRGWEEGVAQMSVGQRAKLTI SPDYAYGATGHPGIIPPHATLVFDVELLKLE

P13-5FA:

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GVPGAGVPGAGVPGAGVPGAGVPGAGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKPFKFML
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GVPGAGVPGAGVPGAGVPGAGVPGAGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKP
FKFMLGKQEVIRGWEEGVAQMSVGQRAKLTI SPDYAYGATGHPGIIPPHATLVFDVELLKLE

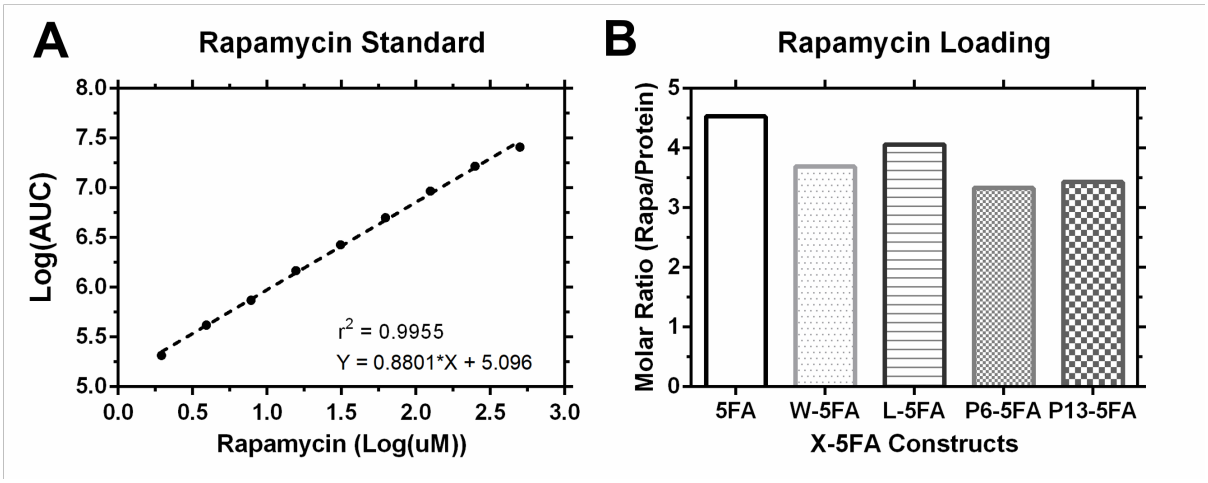
Supplemental Table S2: MALDI-TOF (M+XH)/X charge states

Charge state	5FA (Da)	W (Da)	L (Da)	P6 (Da)	P13 (Da)
(M+1H)/1	95527.9	97486.5	97657.5	97549.2	97852.3
(M+2H)/2	47684.5	48845.3	48976	48899	48904.7
(M+3H)/3	32178.1	32558.2	32606.2	32617	32485.6

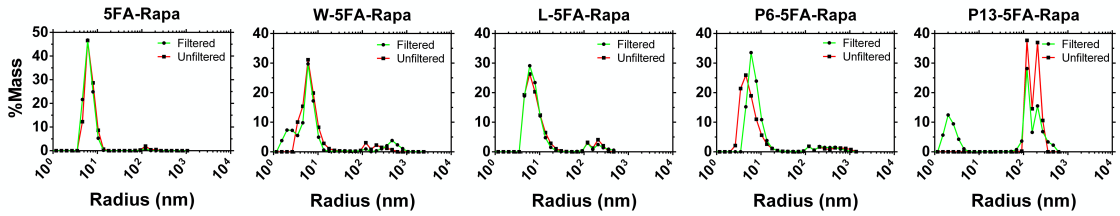
Supplemental Table S3: Molar Extinction Coefficients for ELPs as Evaluated by UV-Vis 280 nm

Sample	5FA	W-5FA	L-5FA	P6-5FA	P13-5FA
Extinction coefficient ($A_{280\ nm}$ $M^{-1}cm^{-1}$)[1]	50,100	61,100	51,590	51,590	51,590

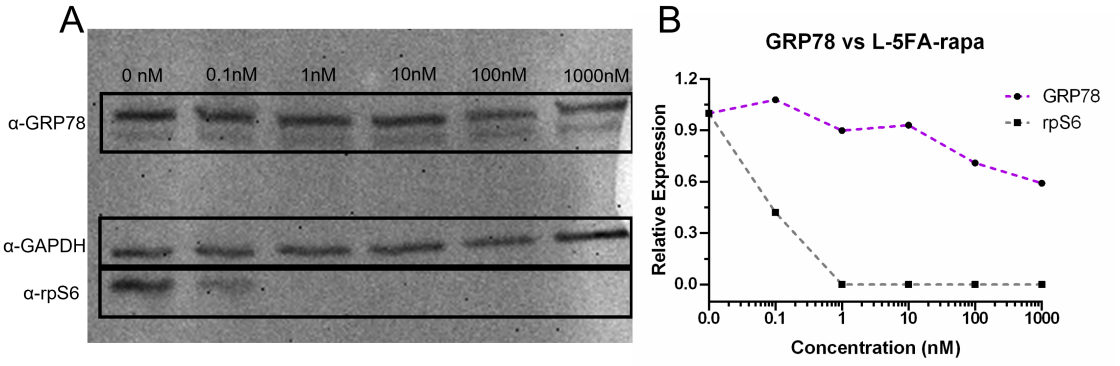
[1] Estimated using Pace et al. (1995)



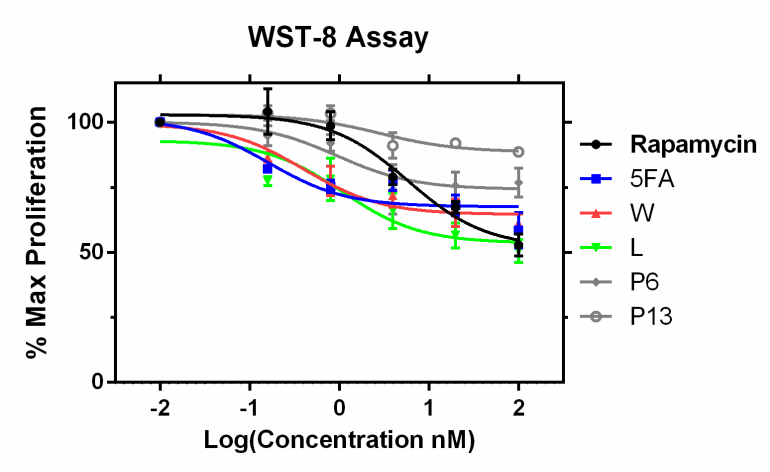
Supplemental Figures S1. Analytical Reverse Phase High Performance Liquid Chromatography was used to quantify rapamycin. RP-HPLC was performed as described in the methods. **A)** Using the AUCs observed after injection of standards, a linear calibration curve was developed. This curve was used to estimate the rapamycin concentrations within unknown samples, which were 725, 58, 211, 150, 266 uM for 5FA, W-5FA, L-5FA, P6-5FA, and P16-5FA respectively. The protein concentrations were estimated independently using UV-Vis spectroscopy at 280 nm using the molar extinction coefficients for each Hydra-ELP (**Supplemental Fig S3**), which were 160, 16, 52, 45, and 78 uM for 5FA, W-5FA, L-5FA, P6-5FA, and P16-5FA respectively. **B)** Using these, the loading ratio of Rapamycin per molecule was estimated at 4.5, 3.7, 4.1, 3.3, and 3.4 for 5FA, W-5FA, L-5FA, P6-5FA, and P16-5FA, respectively.



Supplemental Figure S2. Distribution of Hydrodynamic Radii of Hydra-ELPs complexed with Rapamycin. Rapamycin loaded Hydra-ELPs were characterized by dynamic light scattering with and without filtration to analyze the distribution of particle diameters. Rapamycin does not appear to significantly affect the radius of loaded Hydra-ELPs except for P13-5FA-Rapamycin, which shows a significant distribution between 10^2 nm and 10^3 nm. Filtration through a 0.02 µm filter does not appear to affect distribution except for P13-5FA-Rapa, which is split into two populations, one of which overlaps with the unfiltered population (X > 100 nm). A slightly smaller proportion of the unfiltered P13-5FA-Rapa population has a radius similar to filtered P13-5FA without rapamycin (**Fig. 4B**). For the remaining, there is a slight increase in radius to about 10 nm for all Hydra-ELPs, except for P13-5FA-Rapa, which remains consistent with filtered P13-5FA without rapamycin.



Supplemental Figure S3: GRP78 expression is attenuated after treatment with L-5FA-rapa in a concentration dependent manner. After samples from a concentration-based assay were imaged for rpS6 and GAPDH (**Fig. 6A**), the membrane was blocked with 5% BSA and incubated with anti-BiP/GRP78 rabbit mAb (Cell Signaling Technologies Inc., Boston, MA, Part #3177) overnight at 4°C. **A)** Images were acquired after incubation with anti-rabbit horse radish peroxidase and imaged on an iBright system. The relative expression of GRP78 and rpS6 at each concentration were normalized to GAPDH and quantified as a ratio to 0 nM. While rpS6 phosphorylation appears to significantly decrease with increasing L-5FA-rapa concentration (~1 nM), GRP78 is marginally affected beginning at (~100 nM). **B)** GRP78 appears to slightly decrease relative to GAPDH, but not directly proportional to a decrease in rpS6 phosphorylation. The data confirms reports in literature that suggest exposure to rapamycin affects mTORC1-dependent expression of GRP78. This effect appears to be at a range beyond the relevant therapeutic concentration for rpS6 phosphorylation.



Supplemental Figure S4: BT474 cells were treated with Hydra-ELPs loaded with rapamycin and assayed using a cell-viability assay. After reaching 30% confluence, cells were treated with Hydra-ELPs (5FA, W-5FA, L-5FA, P6-5FA, or P13-5FA) loaded with rapamycin at the following concentrations: 100, 20, 4, 0.8, or 0.16 nM. Cells were incubated for 48-hrs and assayed using the WST-8 reagent (Abcam, #228554) following the manufacturer protocol and read for absorbance 450 nm. Data is displayed as Log(concentration) vs. the percent of maximum possible proliferation. The absorbance of each sample was normalized to the difference between the untreated cells and cell-free blank wells. After fitting to a dose-response curve in Graphpad Prism, IC50 values were calculated for Rapamycin, 5FA-Rapa, W-5FA-Rapa, L-5FA-Rapa, P6-Rapa, and P13-5FA-Rapa at 5.8 (±13.8) nM, 0.17 (±0.70) nM, 0.49 (±1.15) nM, 0.80 (±4.7) nM, 0.94 (±3.9) nM, and 2.8 (±22.6) nM, respectively. Values indicate mean (±SD) for triplicates.