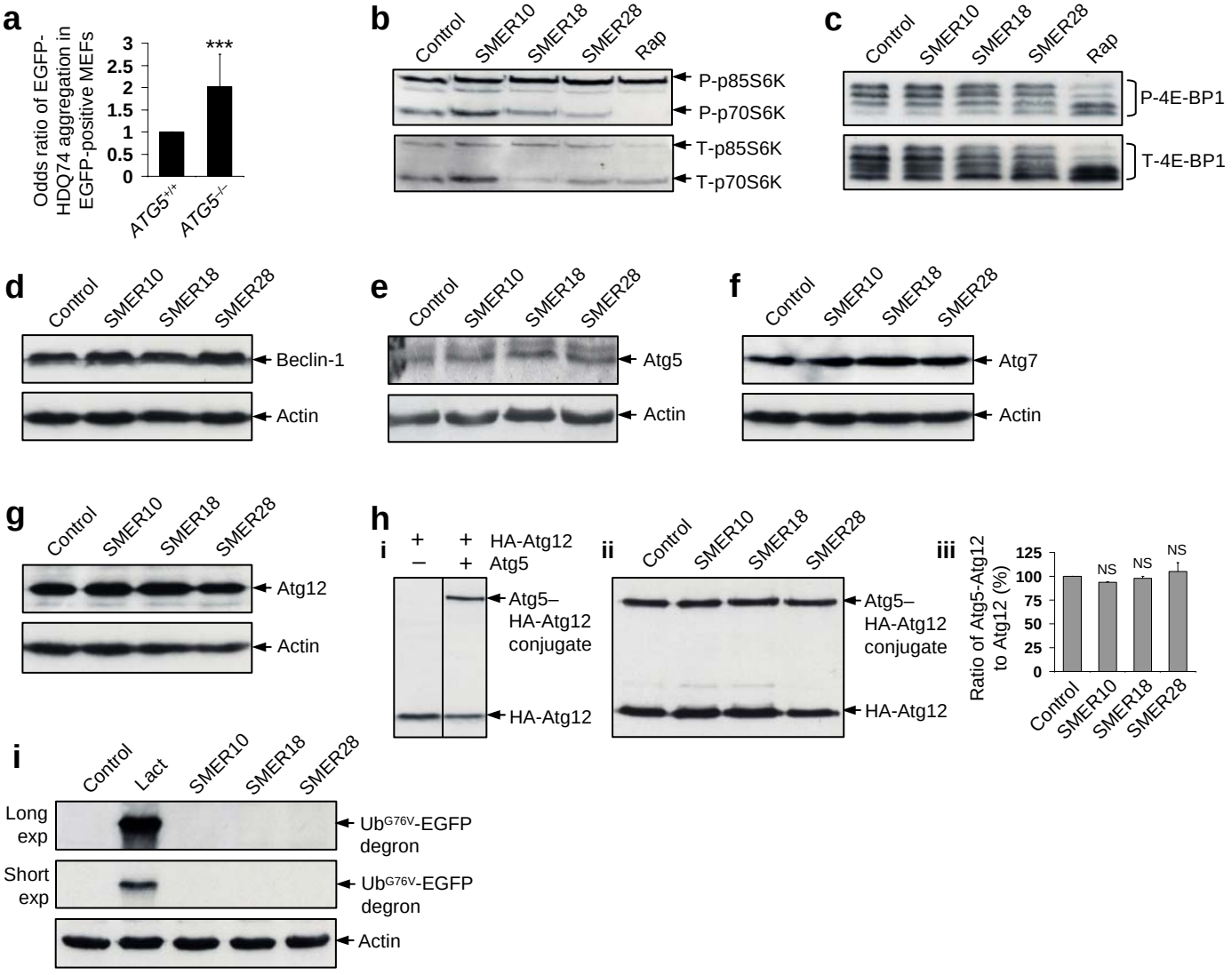
Supplementary Figure 3



Supplementary Figure 3. The effect of SMERs 10, 18 and 28 on mTOR activity, Beclin-1/Atg6, Atg5, Atg7, Atg12, Atg5-Atg12 conjugation and proteasome activity.

(**a**) Wild-type (*ATG5*$^{+/+}$) and knock-out (*ATG5*$^{-/-}$) Atg5 mouse embryonic fibroblasts (MEFs) were transfected with *EGFP-HDQ74* construct for 4 h and fixed at 48 h post-transfection. The percentage of EGFP-positive cells with EGFP-HDQ74 aggregates were assessed and expressed as odds ratio. The control (EGFP-HDQ74 aggregation in *ATG5*$^{+/+}$ cells) was taken as 1. Error bars: 95 % confidence interval. $p<0.0001$.

(**b,c**) COS-7 cells treated with DMSO (control), 47 μM SMER10, 43 μM SMER18, 47 μM SMER28 or 0.2 μM rapamycin (rap) for 24 h, were analysed for mTOR activity by immunoblotting for levels of phospho- and total p70S6K (**b**) and 4E-BP1 (**c**). Note that 4E-BP1 runs as a set of bands on gels, as phosphorylation slows its mobility – the bands with the slowest mobility are decreased with rapamycin.

(**d**) COS-7 cells treated with DMSO (control) or with 47 μM SMER10, 43 μM SMER18 or 47 μM SMER28 for 24 h, were analysed for Beclin-1 levels by immunoblotting with anti-Beclin-1 antibody.

(**e–g**) HeLa cells treated with DMSO (control) or with 47 μM SMER10, 43 μM SMER18 or 47 μM SMER28 for 24 h, were analysed for Atg5 (**e**), Atg7 (**f**) or Atg12 (**g**) levels by immunoblotting with anti-Atg5 (**e**), anti-Atg7 (**f**) or anti-Atg12 (**g**) antibodies.

(**h**) COS-7 cells transfected with HA-Atg12 and either Atg5 or empty vector (1:2 ratio) for 4 h were analysed for Atg5–HA-Atg12 conjugation levels at 24 h post-transfection by immunoblotting with anti-HA antibody (i). Atg5–HA-Atg12 conjugate is only seen when Atg5 is co-transfected with HA-Atg12, compatible with data reported previously[14]. Note that the gel strips are from non-adjacent lanes of the same immunoblot (i). COS-7 cells transfected with HA-Atg12 and Atg5 (1:2 ratio) for 4 h and then treated with DMSO (control) or with 47 μM SMER10, 43 μM SMER18 or 47 μM SMER28 for 24 h, were analysed for Atg5–HA-Atg12 conjugation levels by immunoblotting with anti-HA antibody (ii) and densitometry analysis of Atg5–HA-Atg12 conjugate to Atg12 (iii). Error bars denote standard error of mean. $p=0.3638$ (SMER10), $p=0.742$ (SMER18), $p=0.4547$ (SMER28).

(**i**) HeLa cells stably expressing Ub^{G76V}-EGFP reporter, treated with or without 10 μM lactacystin (lact), 47 μM SMER10, 43 μM SMER18 or 47 μM SMER28 for 24 h, were analysed for inhibition of proteasome activity by immunoblotting with antibody against EGFP.

***, $p<0.0001$; NS, Non-significant.