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Supplemental Information

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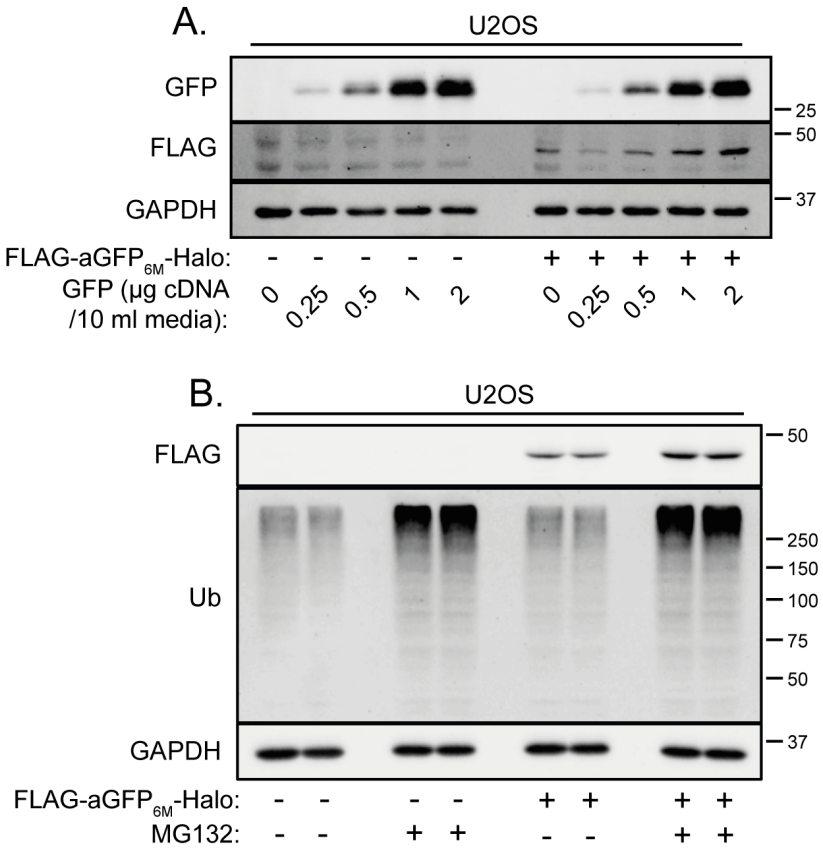
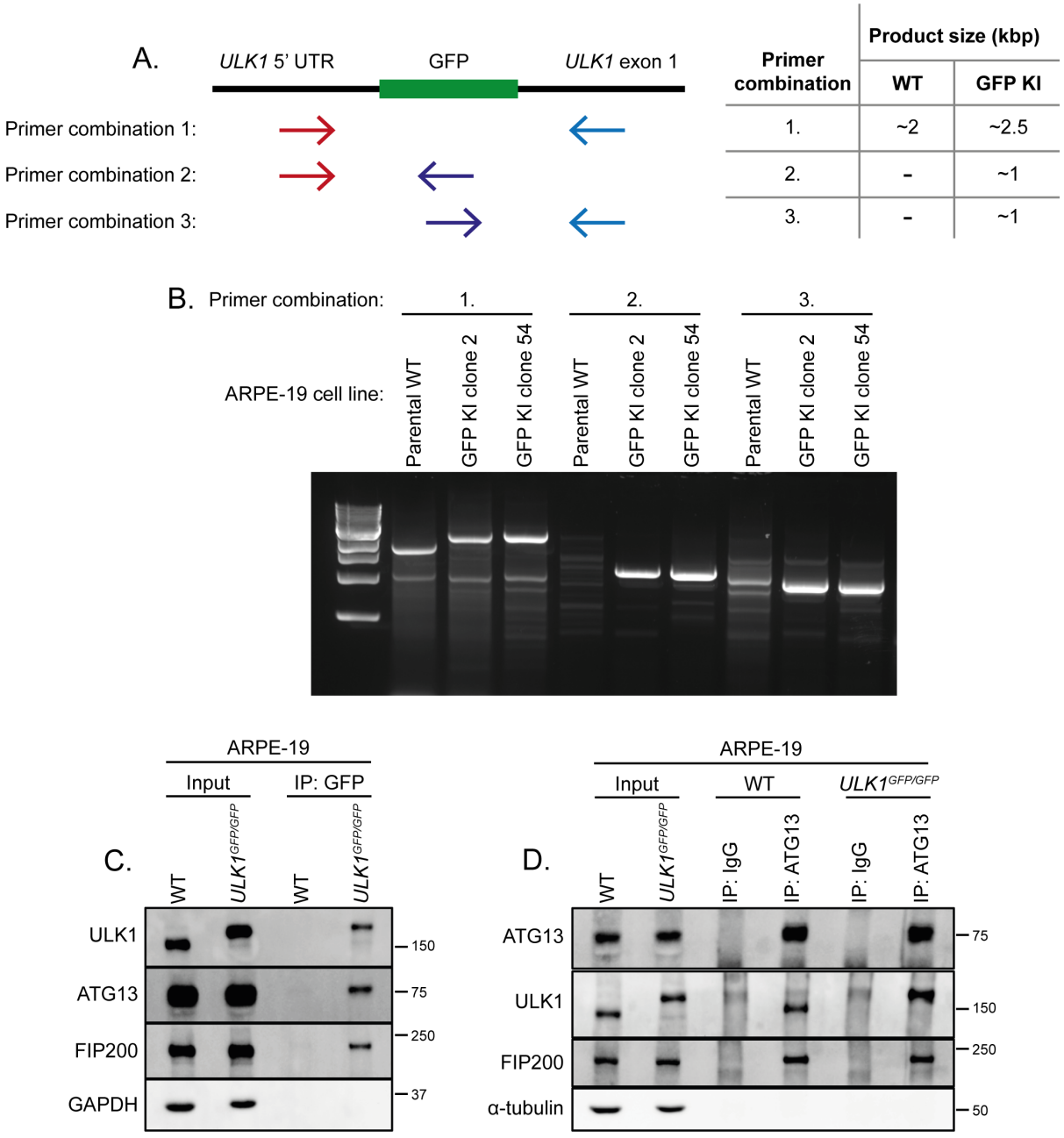


Figure S1, related to Figure 1. FLAG-aGFP$_{6M}$-Halo levels are controlled by GFP protein abundance. (**A**) GFP was transiently expressed with increasing cDNA concentrations (0-2 µg per 10 ml media) in U2OS FLAG-empty and FLAG-aGFP$_{6M}$-Halo expressing cells. (**B**) U2OS FLAG-empty and FLAG-aGFP$_{6M}$-Halo expressing cells were treated with 20 µM MG132 proteasome inhibitor for 6 hr as indicated. For both (**A**) and (**B**), extracts were resolved by SDS-PAGE and transferred on to PVDF membranes, which were subjected to immunoblotting with indicated antibodies.



Primer combination	Product size (kbp)	
	WT	GFP KI
1.	~2	~2.5
2.	-	~1
3.	-	~1

Figure S2, related to Figure 1. Characterisation of ARPE-19 ULK1 GFP knockin (KI) (*ULK1*$^{GFP/GFP}$) cells. (**A**) Primers were designed for analysis of the endogenous *ULK1* locus (primer combination 1) and internal primers to recognise the incorporated GFP-tag (primer combination 2 and 3). (**B**) Agarose gel analysis of ARPE-19 parental WT and ULK1 GFP KI cell line clones 2 and 54. In both clones, an electrophoretic mobility shift is observed in the PCR products of primer combination 1. With both primer combination 2 and 3, no PCR product was observed in parental WT cells, but can be detected for both clone 2 and 54. Clone 54 was used for subsequent experiments. (**C**) ARPE-19 WT and *ULK1*$^{GFP/GFP}$ cells were lysed and subjected to GFP TRAP immunoprecipitation (IP). ULK1 complex components including GFP-ULK1, ATG13 and FIP200 co-precipitated with GFP-ULK1 from *ULK1*$^{GFP/GFP}$ cell extracts. (**D**) ARPE-19 WT and *ULK1*$^{GFP/GFP}$ cells were lysed and subjected to ATG13 or IgG IP as indicated. ULK1 complex components including ATG13, ULK1 and FIP200 co-precipitated with ATG13 from WT and *ULK1*$^{GFP/GFP}$ cell extracts. For both (**C**) and (**D**), extracts and IPs were resolved by SDS-PAGE and transferred on to PVDF membranes, which were subjected to immunoblotting with indicated antibodies.

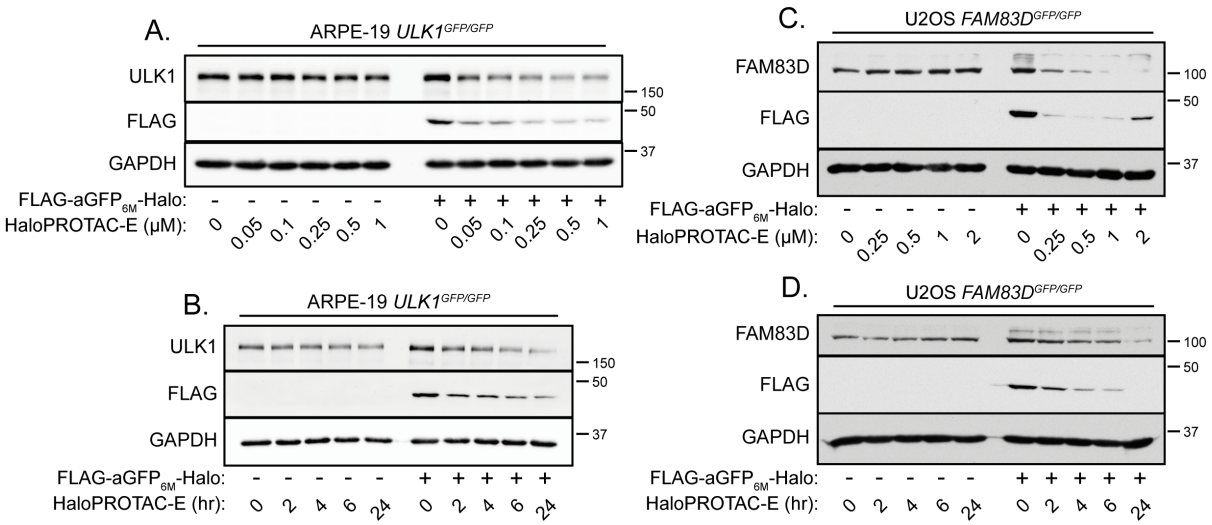


Figure S3, related to Figure 1. GFP-ULK1 and FAM83D-GFP are degraded with HaloPROTAC-E in cells expressing FLAG-aGFP$_{6M}$-Halo. (**A**) ARPE-19 *ULK1*$^{GFP/GFP}$ FLAG-empty and FLAG-aGFP$_{6M}$-Halo expressing cells were treated with increasing concentrations of HaloPROTAC-E (0-1 μM) for 24 hr as indicated. (**B**) ARPE-19 *ULK1*$^{GFP/GFP}$ FLAG-empty and FLAG-aGFP$_{6M}$-Halo expressing cells were treated with 250 nM HaloPROTAC-E for indicated times (0-24 hr). (**C**) U2OS *FAM83D*$^{GFP/GFP}$ FLAG-empty and FLAG-aGFP$_{6M}$-Halo expressing cells were treated with increasing concentrations of HaloPROTAC-E (0-2 μM) for 24 hr. (**D**) U2OS *FAM83D*$^{GFP/GFP}$ FLAG-empty and FLAG-aGFP$_{6M}$-Halo expressing cells were treated with 1 μM HaloPROTAC-E for indicated times (0-24 hr). For all experiments, extracts were resolved by SDS-PAGE and transferred on to PVDF membranes, which were subjected to immunoblotting with indicated antibodies.

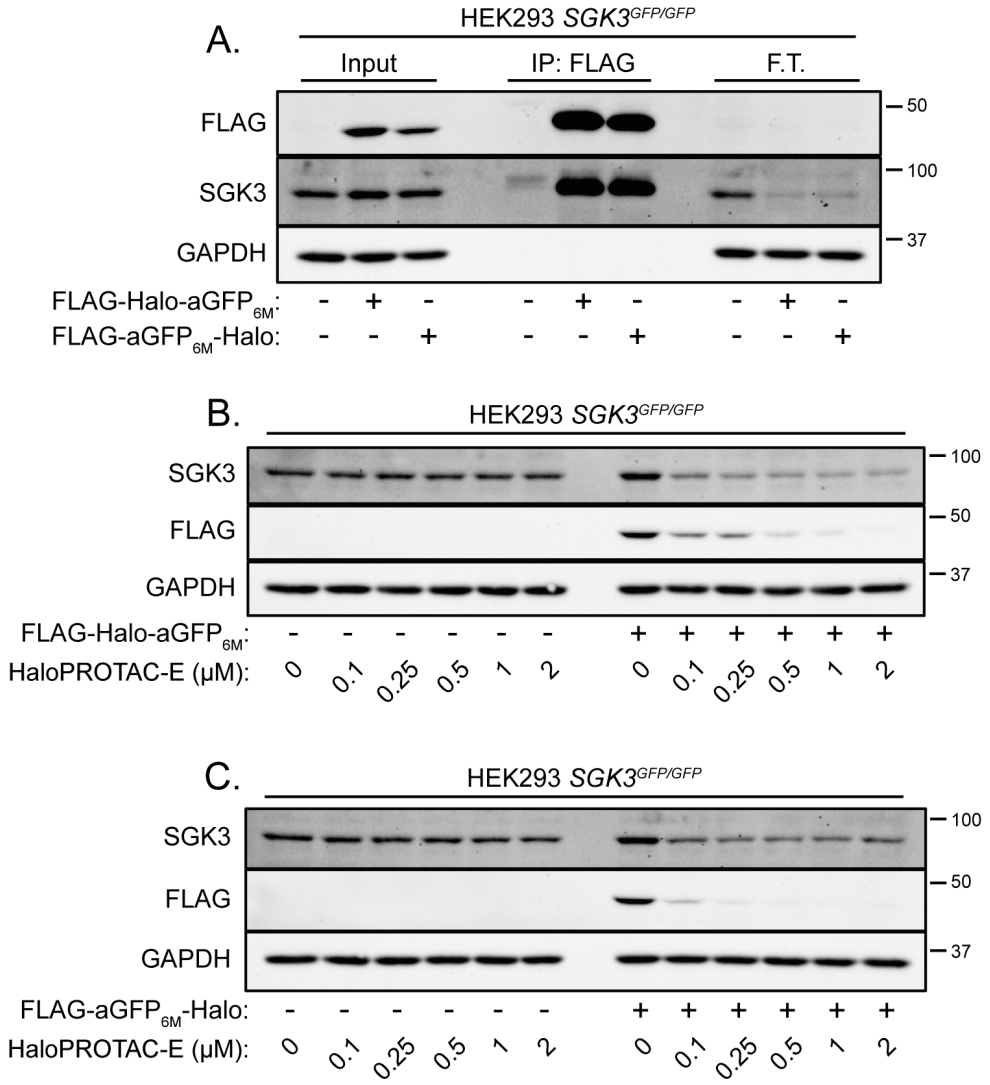


Figure S4, related to Figure 3. SGK3-GFP is degraded with HaloPROTAC-E in FLAG-Halo-aGFP$_{6M}$ and FLAG-aGFP$_{6M}$-Halo expressing cells. (**A**) HEK293 *SGK3*$^{GFP/GFP}$ FLAG-empty, FLAG-Halo-aGFP$_{6M}$ and FLAG-aGFP$_{6M}$-Halo expressing cells were lysed and subjected to immunoprecipitation (IP) with anti-FLAG M2 resin. F.T. = post-IP flow-through extract. HEK293 *SGK3*$^{GFP/GFP}$ FLAG-empty, FLAG-Halo-aGFP$_{6M}$ (**B**) and FLAG-aGFP$_{6M}$-Halo (**C**) expressing cells were treated with increasing concentrations of HaloPROTAC-E (0-2 μM) for 24 hr as indicated. In all cases, extracts and IPs were resolved by SDS-PAGE and transferred on to PVDF membranes, which were subjected to immunoblotting with indicated antibodies.