

FIGURE SUPPLEMENTAL 1

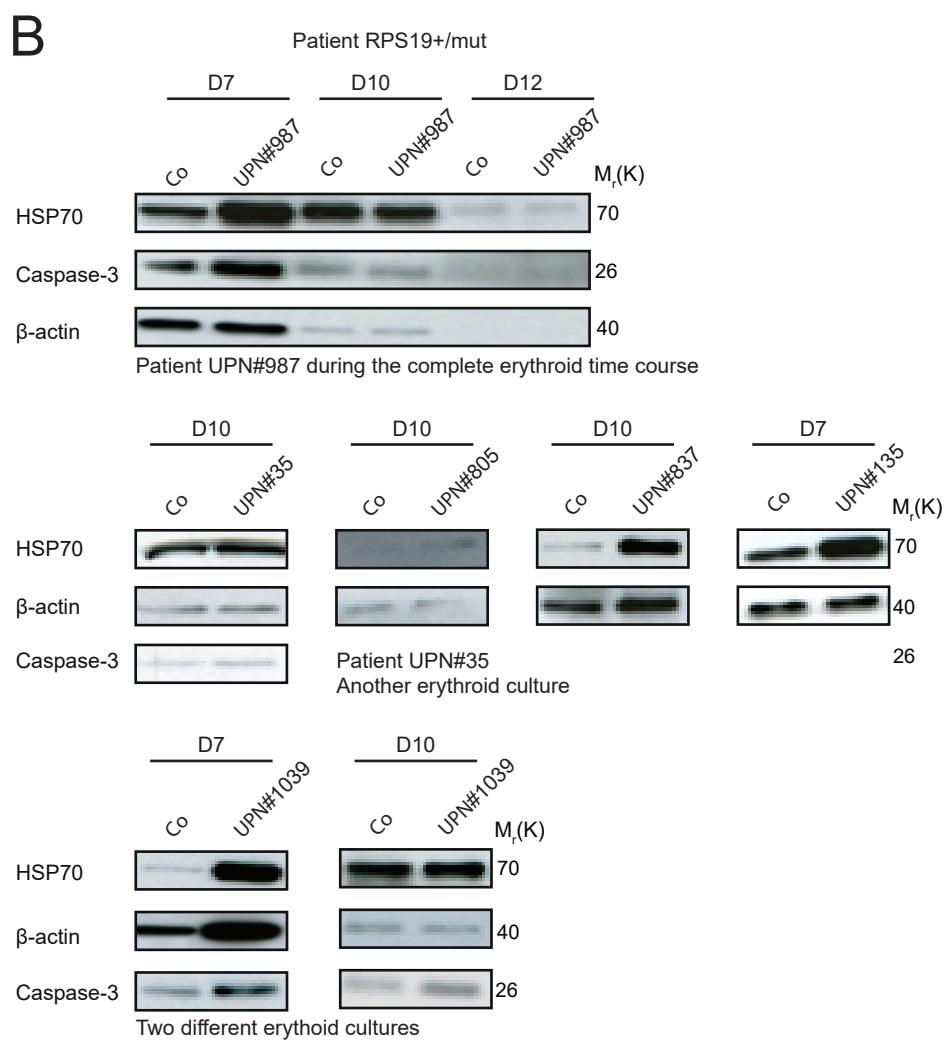
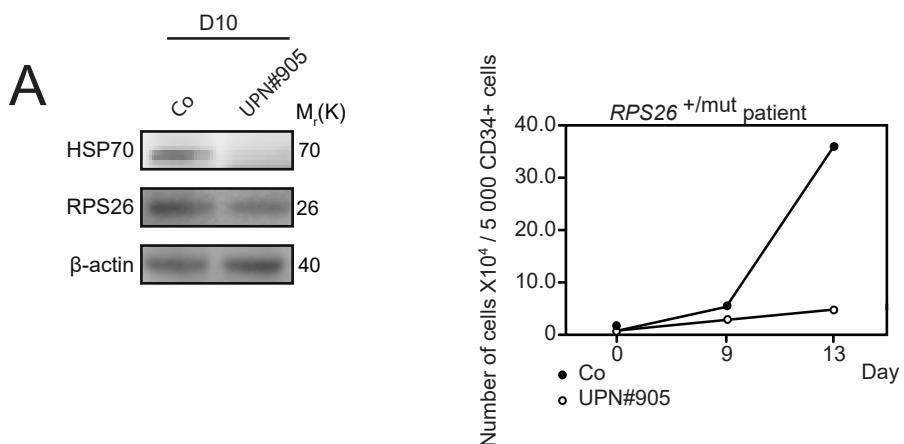


FIGURE SUPPLEMENTAL 2

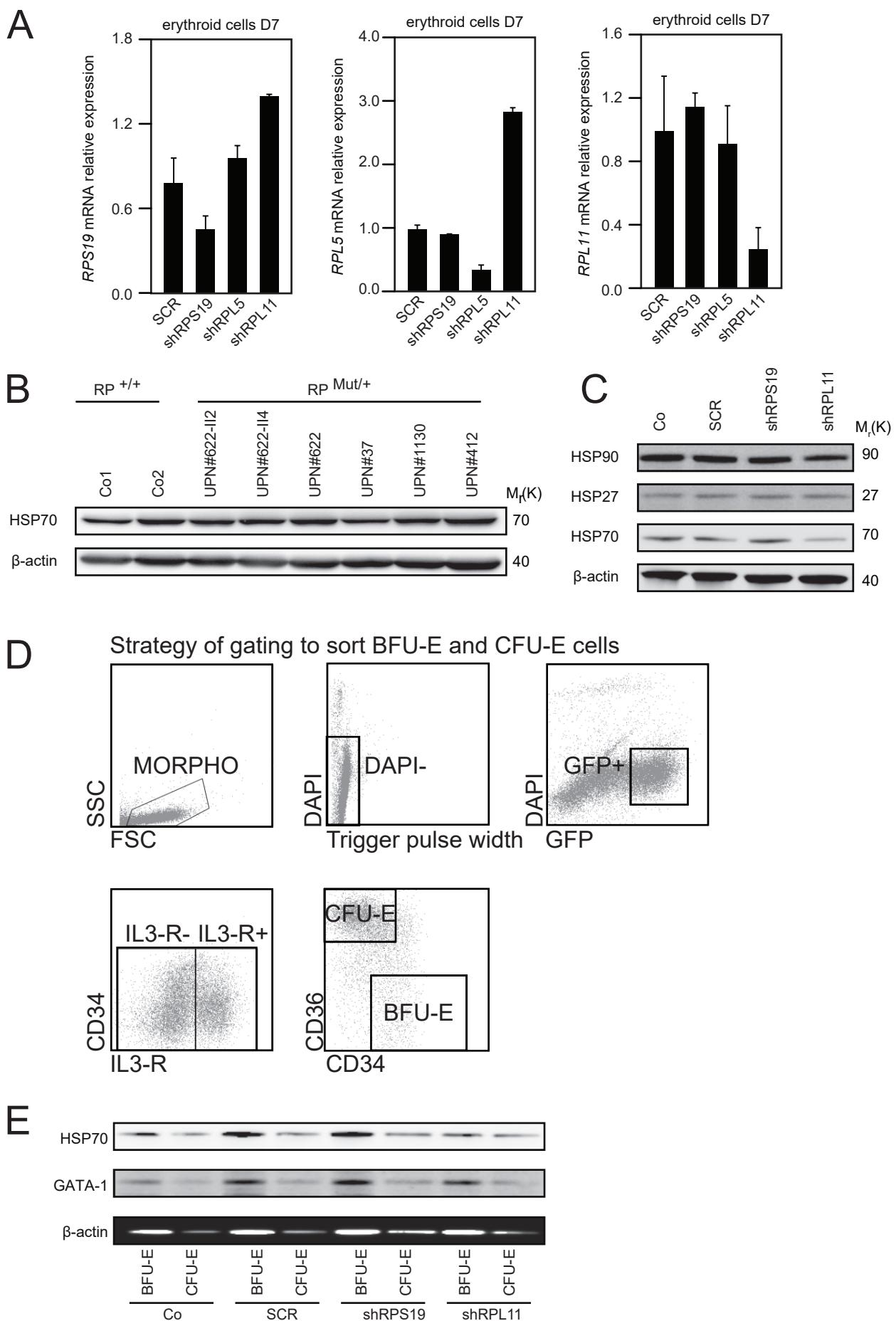


FIGURE SUPPLEMENTAL 3

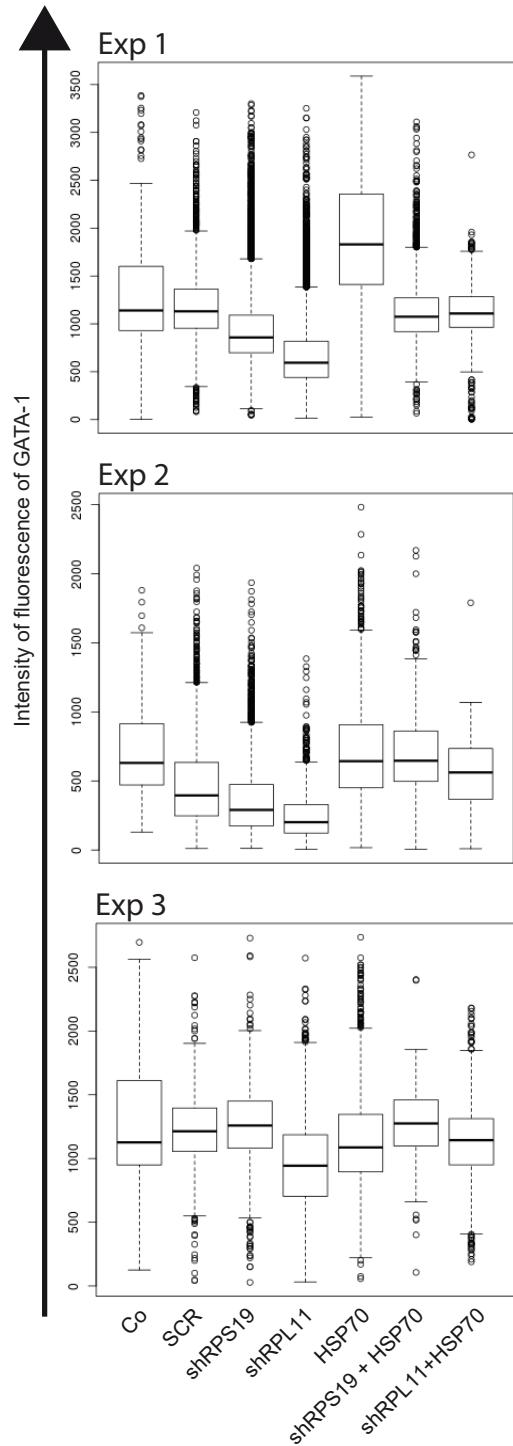


FIGURE SUPPLEMENTAL 4

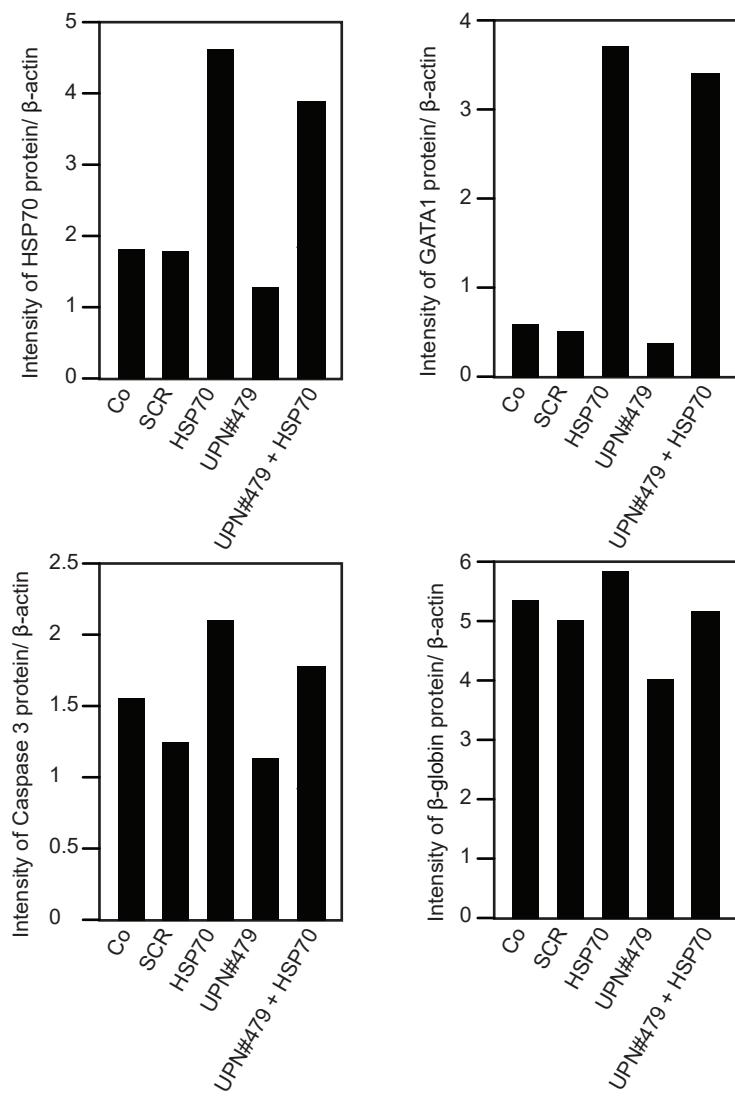
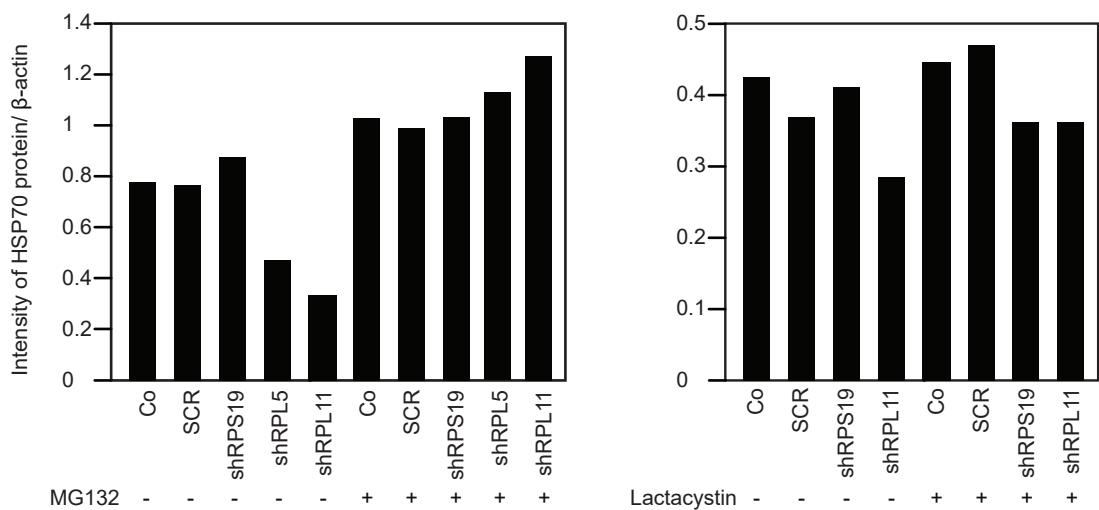
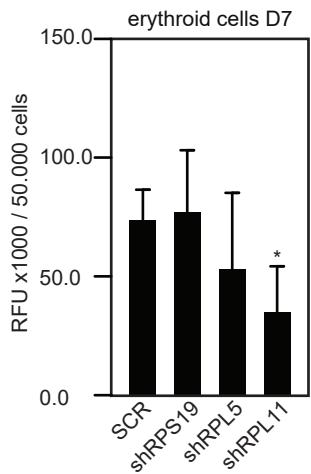


FIGURE SUPPLEMENTAL 5

A



B



Supplemental Figure 6: Putative sites of ubiquitination in Human HSP70 protein sequence.

➤ Analysis Tool#1 UbPred (The Rockefeller University)

<http://www.ubpred.org/cgi-bin/ubpred/ubpred.cgi>

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>sp|P08107|HSP71_HUMAN Heat shock 70 kDa protein 1A/1B OS=Homo sapiens
GN=HSPA1A                                         PE=1
MAKAAAIGIDLGTTYSCGVFQHGKVEIIANDQGNRTTPSYVAFTDTERLIGDAA
KNQVALNPQNTVFDAKRLIGRKFGDPVVQSDMKHWPfqVINDGDKPKVQVSYK
GETKAFYPEEISSMVLTKMKEIAEA YLGYPVTNA VITVPAYFNDSQRQATKDAG
VIAGLNVLRIINEPTAAAIA YGLDRTGKGERNVLIFDLGGGTFDVSILTIDDGIFEV
KATAGDTHLGGEDFDNRLVNHFVEEFKRKHKKDISQNKR AVRRLRTACERAKR
TLSSTQASLEIDSLFEGIDFYTSITRARFEELCSDLFRSTLEPVEKALRDAKLDKA
QIHDLVLVGGSTRIPKVQKLLQDFNGRDLNK SINPDEAVAYGAAVQAAILMDG
KSENVQDLLLLDVAPLSLGLETAGGVMTALKRNSTIPTKQTQIFTTYSDNQPGV
LIQVYEGERAMTKDNNNLLGRFELSGIPPAPRGVPQIEVTFDIDANGILNVTATDKS
TGKANKITITNDKGRLSKEEIERMVQEAEKYKA EDEVQRERVSAKNALESYAFN
MKSAVEDEGLKGKISEADKKVLDK CQEVISWLDANTLAEKDEFEHKRKELEQ
VCNPIISGLYQGAGGPGPGFFGAQGPKGGSGSGPTIEEV
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The lysine K labeled in gray has been rule out as a putative ubiquitylation site. The table below describes only the sites from a low to high confidence in the HSP70 ubiquitination site prediction.

Residue	Score	Ubiquitinated
3	0.72	Yes Medium confidence
108	0.68	Yes Low confidence
112	0.63	Yes Low confidence
451	0.62	Yes Low confidence
512	0.65	Yes Low confidence
524	0.86	Yes High confidence
526	0.86	Yes High confidence
539	0.80	Yes Medium confidence
550	0.84	Yes High confidence
559	0.68	Yes Low confidence
561	0.69	Yes Medium confidence
589	0.75	Yes Medium confidence
595	0.69	Yes Medium confidence
597	0.68	Yes Low confidence

628	0.75	Yes Medium confidence
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Legend:

Label	Score range	Sensitivity	Specificity
Low confidence	$0.62 \leq s \leq 0.69$	0.464	0.903
Medium confidence	$0.69 \leq s \leq 0.84$	0.346	0.950
High confidence	$0.84 \leq s \leq 1.00$	0.197	0.989

➤ Analysis Tool#2 Gbase (Harvard University)

<https://gbase.hms.harvard.edu/protein/view/IPI:IPI00304925.5>

IPI:IPI00304925.5

Protein Information

Protein	IPI:IPI00304925.5
Gene Symbol	HSPA1A;HSPA1B
Protein Description	Heat shock 70 kDa protein 1
Sequence Length	641
Mwt	70009.037 Da
Sequence	MAKAAAIGID LGTTYSCVGV FQHGKVEIIA NDQGNRTTPS YVAFTDTERL IGDAAKNQVA LNPQNTVFDA KRLIGRKFGD PVVQSDM K HW PFQVINDGDK K PKVQVSY K GE TKA F YPEEIS SMVLT K MKEI AEAYLGYPVT NAVITVPAYF NDSQRQAT K D AGVIAGLNVL RIINEPTAAA IAYGLDRTGK GERNVLIFDL GGGTFDVSIL TIDDGIFEVK ATAGDTHLGG EDFDNRLVNH FVEEF K RKH K KDISQNKRAV RRLRTACERA KRTLSSSTQA SLEIDS L FEG IDFYTSITRA R FEELCSSDLF RSTLEPVE K A LRDAKLD K AQ IHDLVLVGGS TRIPKV Q K LL QDFFNGRDLN K SINPDEAVA YGAAVQAAIL MGDKSENVQD LLLL D VAPLS LGLETAGGVM TALIKRNSTI PT K QTQIFTT YSDNQPGVLI QVYGERAMT K DNNLLGRFE LSGIPPAPRG VPQIEVTFDI DANGILNVTA TDKSTGK K ITITND K GRL SKEIERMVQ EAE K Y K AEDE VQRERVSA K N ALESYAFNM K SAVEDEGL K G KISEADKKKV LD K CQEVISW LDANTLAEKD EFEHKR K ELE QVCNPIISGL YQGAGGPGPG GFGAQGP K GG SGSGPTIEEV D

The predict ubiquitination sites 108, 112, 451, 512, 524, 526, 539, 550, 559, 561, 597, and 628 are redundant in both analyses.