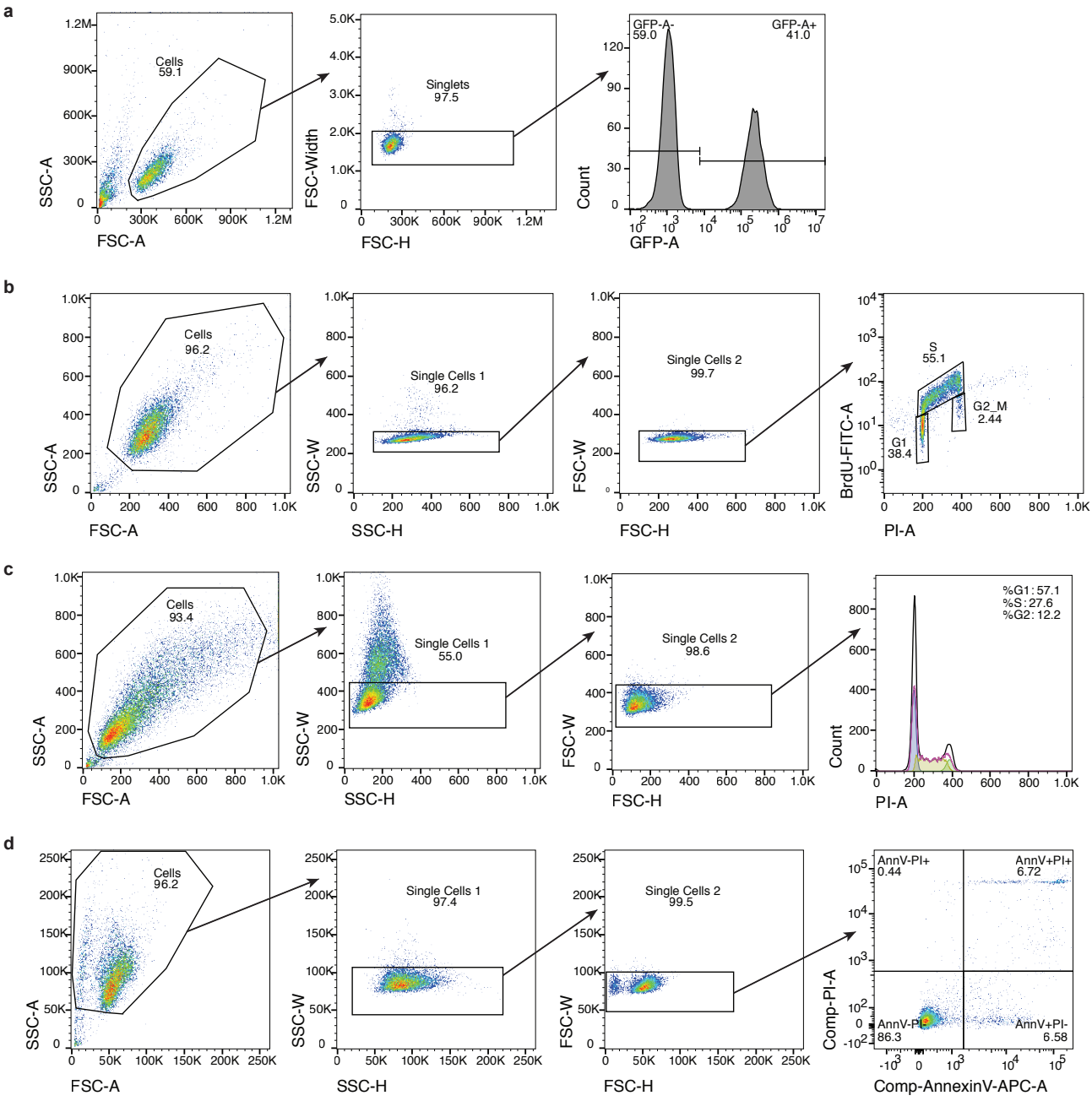
Supplementary Figure 1



Supplementary Figure 1. Flow cytometry gating stragies.
a, Gating strategy to quantify % of GFP+ cells in competition assays in Extended Data Fig 1h,i. **b**, Gating strategy to quantify the % of cycling S-phase cells by BrdU/PI staining in Fig. 1d,i,j, Extended Data Figs 1l, 4d,e, 5b-d and for U2OS, NCI-H1792, and NCI-H460 cells in Extended Data Fig 1o. **c**, Gating strategy to quantify % of cycling S-phase cells by PI staining for MCF7 cells in Extended Data Fig 1o. Gating of G1, S, and G2 populations were done using the cell cycle analysis function in FlowJo. **d**, Gating strategy to quantify % of apoptotic (AnnexinV+PI+) cells in Extended Data Fig. 1j,k.

Supplementary Methods

sg-ID barcodes for Tuba-seq lentiviral vectors

sgRNA	sgID	20N Barcoding Reverse Primer Sequence (5' -> 3')	sgRNA sequence
Ambra1#1	CGATTAGG	AGCTAGTCCGGACGATTAGGGCNNNNNTANNNNNGCNNNNNTANNNNNGCATGCCCAAGAAGAAGAGGAAGGTGTC	TGACACACCATGGAGTACGG
Ambra1#2	CTTACGGT	AGCTAGTCCGGACTTACGGTGCNNNNNTANNNNNGCNNNNNTANNNNNGCATGCCCAAGAAGAAGAGGAAGGTGTC	GGGTATAAATGCGTTCCCAG
Ambra1#3	CGTCTATG	AGCTAGTCCGGACGTCTATGGCNNNNNTANNNNNGCNNNNNTANNNNNGCATGCCCAAGAAGAAGAGGAAGGTGTC	GCCAGTAAGAAGGTCGAGCG
Apc#1	AGGAGTCC	AGCTAGTCCGGAAGGAGTCCGCNNNNNTANNNNNGCNNNNNTANNNNNGCATGCCCAAGAAGAAGAGGAAGGTGTC	TTGAGCGTAGTTTCACTCCG
Rb1#1	GAGAACTC	AGCTAGTCCGGAGAGAACTCGCNNNNNTANNNNNGCNNNNNTANNNNNGCATGCCCAAGAAGAAGAGGAAGGTGTC	TACGTTCAGAATCCACGGGA
Rb1#2	TCATGAGC	AGCTAGTCCGGATCATGAGCGCNNNNNTANNNNNGCNNNNNTANNNNNGCATGCCCAAGAAGAAGAGGAAGGTGTC	TCTTACCAGGATTCCATCCA
Neo#1	CTAGGCTA	AGCTAGTCCGGACTAGGCTAGCNNNNNTANNNNNGCNNNNNTANNNNNGCATGCCCAAGAAGAAGAGGAAGGTGTC	TCATGGCTGATGCAATGCGG
Neo#2	AATGCTGG	AGCTAGTCCGGAAATGCTGGGCNNNNNTANNNNNGCNNNNNTANNNNNGCATGCCCAAGAAGAAGAGGAAGGTGTC	GATATTGCTGAAGAGCTTGG
Neo#3	ACGCATAC	AGCTAGTCCGGAACGCATACGCNNNNNTANNNNNGCNNNNNTANNNNNGCATGCCCAAGAAGAAGAGGAAGGTGTC	GAATAGCCTCTCCACCCAAG
NT#1	AGTTGCTC	AGCTAGTCCGGAAGTTGCTCGCNNNNNTANNNNNGCNNNNNTANNNNNGCATGCCCAAGAAGAAGAGGAAGGTGTC	GCGAGGTATTCGGCTCCGCG
NT#2	ACGTCGAA	AGCTAGTCCGGAACGTCGAAGCNNNNNTANNNNNGCNNNNNTANNNNNGCATGCCCAAGAAGAAGAGGAAGGTGTC	GACGTAGCCTTCCGAAATAT
NT#3	ACGCTAGA	AGCTAGTCCGGAACGCTAGAGCNNNNNTANNNNNGCNNNNNTANNNNNGCATGCCCAAGAAGAAGAGGAAGGTGTC	GACTGAAATCCAAGGACTGT
Rbm10#1	ACCTTAGG	AGCTAGTCCGGAACCTTAGGGCNNNNNTANNNNNGCNNNNNTANNNNNGCATGCCCAAGAAGAAGAGGAAGGTGTC	TGGGAACGAAAGCTCACCTG
Rbm10#2	GCTCACTT	AGCTAGTCCGGAGCTCACTTGCNNNNNTANNNNNGCNNNNNTANNNNNGCATGCCCAAGAAGAAGAGGAAGGTGTC	GTATTTCCTGAACAGATCCG

Short-interfering RNA (siRNA) sequences

Dharmacon siGENOME SMARTpool siRNAs

Catalog Number	Target	Sequence
D-001206-13-05	Non-Targeting siRNA Pool #1	UAGCGACUAAACACAUCAA UAAGGCUAUGAAGAGAUAC AUGUAUUGGCCUGUAUUAG AUGAACGUGAAUUGCUCAA
M-003210-05-0005	*CCND1*	GUUCGUGGCCUCUAAGAUG CCGAGAAGCUGUGCAUCUA GAACAGAAGUGCGAGGAGG ACAACUUCCUGUCCUACUA
M-029987-01-0005	*AMBRA1*	GAGUAGAACUGCCGGAUAG CCACCCAUGUGAACCAUAA GCGGAGACAUGUCAGUAUC CUGAAUCGCUGUCGUGCUU
M-012610-01-0005	*CUL4A*	GGAAGAGACUAAUUGCUUA GAACAGCGAUCGUAAUCAA GCAUGUGGAUUCAAAGUUA GAACCCAUAUUAUUAGUGA
M-017965-01-0005	*CUL4B*	GCUAUUGGCCGACAUAUGU CAGAAGUCAUUAAUUGCUA CAAACGGCCUAGCCAAAUC CGGAAAGAGUGCAUCUGUA
M-017673-00-0005	*CUL7*	AGAAGCAGGUGAACAAUUU GAACUCAACUCGGUGAAUG GAACCUGCCUGCCCUCCUA GAACUCCGCUACAGGGAAU

Supplementary Methods

HPLC methods for ubiquitylated protein analysis

Sample pickup:
Volume [µl] : 2.00
Flow [µl / min] : 20.00

Buffer A: 0.1% formic acid
Buffer B: 90% acetonitrile in 0.1% formic acid

Sample loading:
Volume [µl] : 5.00
Flow [µl / min] : (unspecified)
Max. pressure [Bar] : 850.00

Gradient:

Time [mm:ss]	Duration [mm:ss]	Flow [nl/min]	Mixture [%B]
00:00	00:00	300	5
01:00	01:00	300	5
113:00	112:00	300	30
114:00	01:00	300	95
120:00	06:00	300	95

Pre - column equilibration:
Volume [µl] : 0.00
Flow [µl / min] : (unspecified)
Max. pressure [Bar] : 250.00

Analytical column equilibration:
Volume [µl] : 8.00
Flow [µl / min] : (unspecified)
Max. pressure [Bar] : 850.00

Auto - sampler wash:
Flush volume [µl] : 100.00

Supplementary Methods

Mass spectrometry methods for ubiquitylated proteins analysis

MS Run Time (min): 120.00

Sequence override of method parameters not enabled.

Divert Valve: not used during run

Contact Closure: not used during run

Syringe Pump: not used during run

MS Detector Settings:

Real-time modifications to method disabled

Stepped collision energy not enabled

Additional Microscans:

MS2	0	0
MS3	0	0
MS4	0	0
MS5	0	0
MS6	0	0
MS7	0	0
MS8	0	0
MS9	0	0
MS10	0	0

Experiment Type: Nth Order Double Play

Tune Method: 092613-NSI-VE-injection-times

Scan Event Details:
1: FTMS + p norm o(200.0-2000.0)
CV = 0.0V
2: ITMS + c norm Dep Rapid MS/MS Most intense ion from (1)
Activation Type: CID
Min. Signal Required: 500.0
Isolation Width: 2.00
Normalized Coll. Energy: 35.0
Default Charge State: 2
Activation Q: 0.250
Activation Time: 10.000
CV = 0.0V
Scan Event 2 repeated for top 20 peaks.

Lock Masses:
Pos List Name: N/A
Source: API Source
Mass List: (none)
Neg List Name: N/A
Source: API Source
Mass List: (none)

Data Dependent Settings:
Use separate polarity settings disabled
Parent Mass List: (none)
Reject Mass List: (none)
Neutral Loss Mass List: (none)
Product Mass List: (none)
Neutral loss in top: 3
Product in top: 3
Most intense if no parent masses found not enabled
Add/subtract mass not enabled
FT master scan preview mode enabled
Charge state screening enabled
Charge state dependent ETD time not enabled
Monoisotopic precursor selection enabled
Charge state rejection enabled
Unassigned charge states : rejected
Charge state 1 : rejected
Charge state 2 : not rejected
Charge state 3 : not rejected
Charge states 4+ : not rejected
Chromatography mode is disabled

Global Data Dependent Settings:
Predict ion injection time enabled
Use global parent and reject mass lists not enabled
Exclude parent mass from data dependent selection not enabled
Exclusion mass width relative to mass
Exclusion mass width relative to low (ppm): 10.00
Exclusion mass width relative to high (ppm): 10.00
Parent mass width by mass
Parent mass width low: 0.5000
Parent mass width high: 0.5000
Reject mass width relative to mass
Reject mass width relative to low (ppm): 10.00
Reject mass width relative to high (ppm): 10.00
Zoom/UltraZoom scan mass width by mass
Zoom/UltraZoom scan mass width low: 5.00
Zoom/UltraZoom scan mass width high: 5.00
FT SIM scan mass width low: 5.00
FT SIM scan mass width high: 5.00
Neutral Loss candidates processed by decreasing intensity
Neutral Loss mass width by mass
Neutral Loss mass width low: 0.5000
Neutral Loss mass width high: 0.5000
Product candidates processed by decreasing intensity
Product mass width by mass
Product mass width low: 0.5000
Product mass width high: 0.5000
MS mass range: 0.00-1000000.00
MSn mass range by mass
MSn mass range: 0.00-1000000.00
Use m/z values as masses not enabled
Analog UV data dep. not enabled
Dynamic exclusion enabled
Repeat Count: 1
Repeat Duration: 20.00
Exclusion List Size: 500
Exclusion Duration: 20.00
Exclusion mass width relative to mass
Exclusion mass width relative to low (ppm): 10.00
Exclusion mass width relative to high (ppm): 10.00
Expiration: disabled
Isotopic data dependence not enabled

Custom Data Dependent Settings:
Not enabled