

# **Marine-derived natural product HDYL-GQQ-495 targets P62 to inhibit autophagy**

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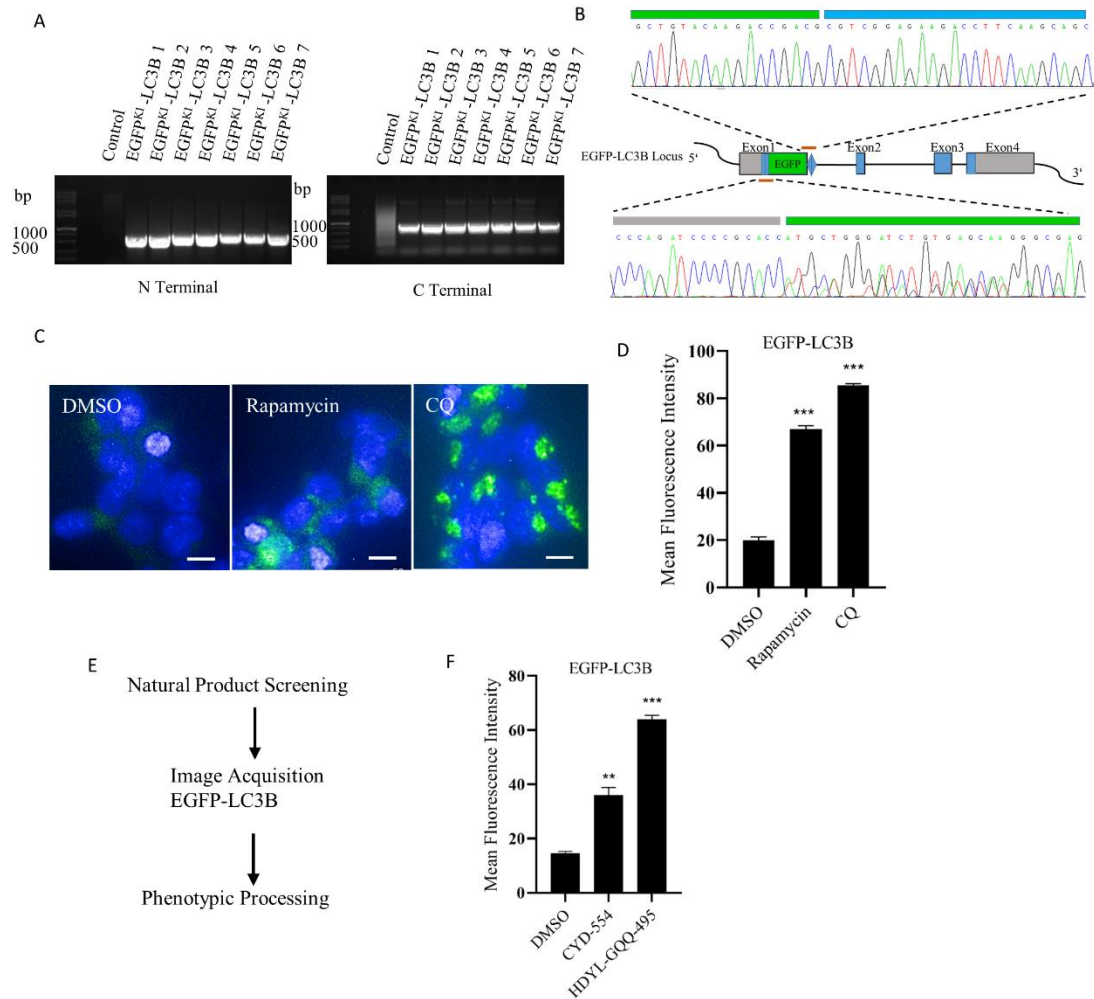
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## Supplementary Figures



**Figure S1. Identification of EGFP-LC3B knock-in 293T cell lines**

(A) Genotyping PCR analysis of the integration of GFP in the indicated cells. Genomic DNA was amplified by PCR and subsequently sequenced.

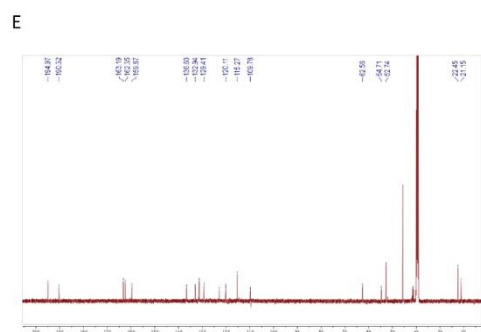
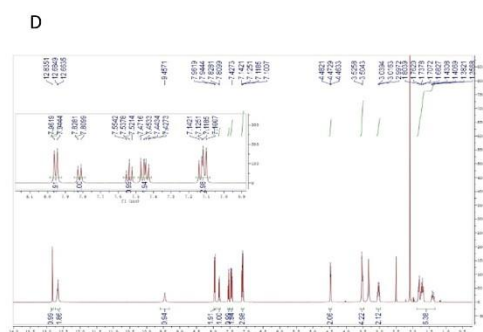
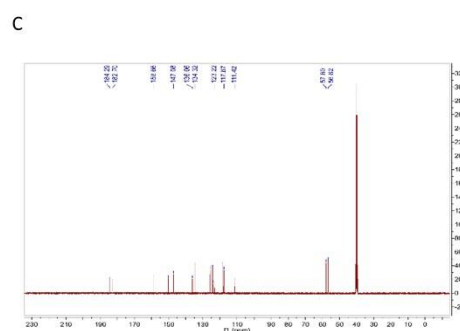
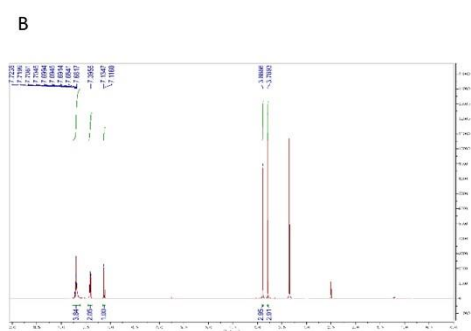
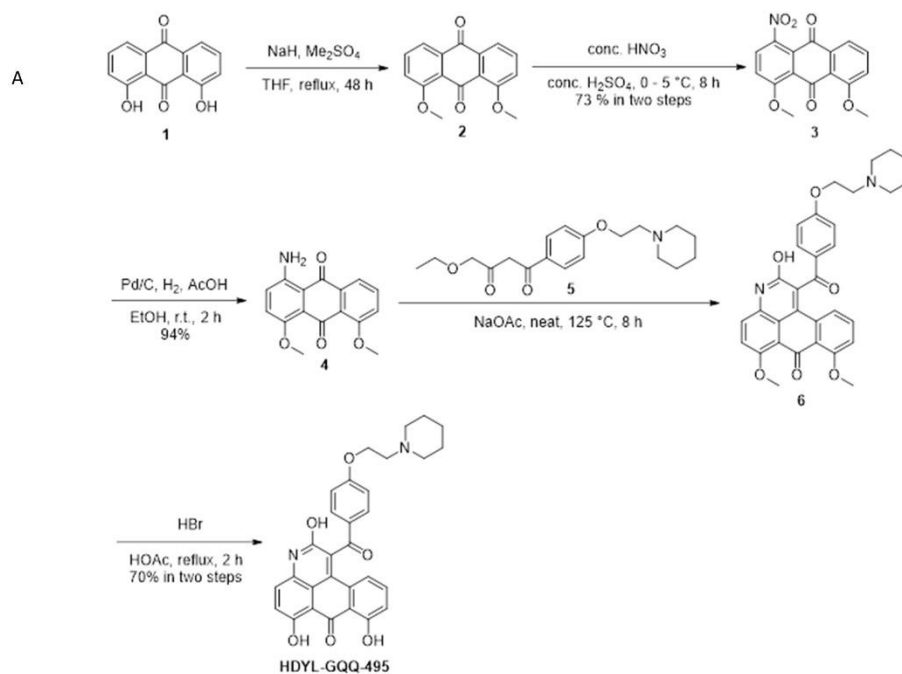
(B) Representative graphic of genomic sequencing results around breakage end of the incorporated EGFP amplified from the EGFP-LC3-KI5 cell lines. The meaning of bars with different colors are listed below.

(C) Representative pictures of EGFP puncta merged with DAPI staining in the indicated cell lines with the indicated treatment. Scale bars, 10 $\mu$ m.

(D) Statistical analysis of the relative EGFP-LC3 expression in 293T EGFP<sup>KI</sup>-LC3B with the indicated treatment. Data are shown as the mean  $\pm$  SEM (n=3). \*\*\*P < 0.001, compared with DMSO.

(E) Schematic representation of the strategy of High-throughput screening in 293T EGFP<sup>KI</sup>-LC3B 5 cells.

(F) Statistical analysis of the relative EGFP-LC3 expression in 293T EGFP<sup>K1</sup>-LC3B with the indicated treatment. Data are shown as the mean  $\pm$  SEM (n=3). \*\*P < 0.01 \*\*\*P < 0.001, compared with DMSO.



## Figure S2. Synthesis HDYL-GQQ-495

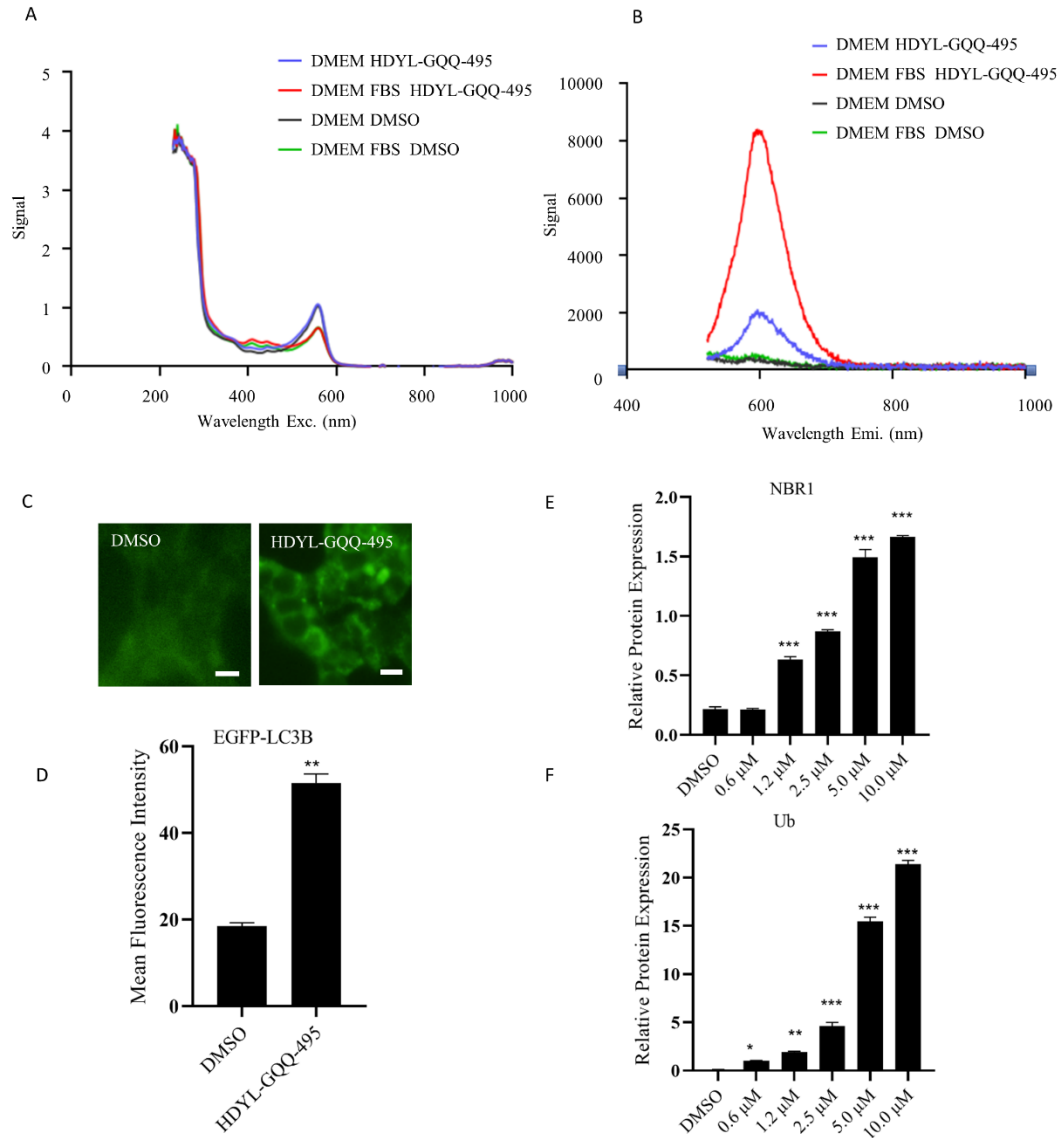
(A) Designed procedure to synthesize HDYL-GQQ-495.

(B)  $^1\text{H}$  NMR of **4**,  $d_6$ -DMSO.

(C)  $^{13}\text{C}$  NMR of **4**,  $d_6$ -DMSO.

(D)  $^1\text{H}$  NMR of **495**,  $d_6$ -DMSO.

(E)  $^{13}\text{C}$  NMR of **495**,  $d_6$ -DMSO.



**Figure S3. HDYL-GQQ-495 is a fluorescent reagent with excitation at 500 nm and emission at 590 nm**

(A-B) The emission and excitation spectra spectra of GQQ-495 scanned by EnSpire.

(C) Representative pictures of EGFP puncta in the 293T EGFP<sup>KL</sup>-LC3B cells with HDYL-GQQ-495 and chloroquine. Scale bars, 10 $\mu\text{m}$ .

(D) Statistical analysis of the relative EGFP-LC3 expression in 293T EGFP<sup>K1</sup>-LC3B with the indicated treatment. Data are shown as the mean  $\pm$  SEM (n=3). \*\*P < 0.01, compared with DMSO.

(E) Statistical analysis of the relative NBR1 expression in HeLa cells with the indicated treatment. Data are shown as the mean  $\pm$  SEM (n=3). \*\*\*P < 0.001, compared with DMSO.

(F) Statistical analysis of the relative Ub expression in HeLa cells with the indicated treatment. Data are shown as the mean  $\pm$  SEM (n=3). \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, compared with DMSO.

**Supplementary Table S1. Relative mean GFP fluorescence**

<b>Compounds</b>		<b>Relative Mean GFP</b>
	DMSO	100.00
	CYD-019	81.23
	CYD-008	109.21
	CYD-003	96.16
	CYD-049	79.94
	CYD-050	80.98
	CYD-061	94.37
	CYD-007	112.37
	CYD-018	102.46
	CYD-109	111.27
	CYD-101	105.45
CYD-111	(HDYL-GQQ-495)	143.36
	CYD-112	86.90
	CYD-113	80.45
	CYD-114	118.88
	CYD-115	109.90
	CYD-116	115.56
	CYD-117	96.59
	CYD-118	86.52
	CYD-119	88.88
	CYD-220	87.93
	CYD-221	99.56
	CYD-222	102.34
	CYD-223	89.94
	CYD-224	105.78
	CYD-225	80.90
	CYD-226	118.88
	CYD-227	83.45
	CYD-228	89.93
	CYD-229	92.40
	CYD-230	95.69
	CYD-331	90.80
	CYD-332	100.34
	CYD-333	118.98
	CYD-334	105.24

CYD-335	109.98
CYD-336	88.79
CYD-337	80.80
CYD-338	83.59
CYD-339	86.39
CYD-440	98.84
CYD-441	104.87
CYD-442	110.34
CYD-443	119.43
CYD-444	89.99
CYD-445	80.34
CYD-446	90.36
CYD-447	83.24
CYD-448	97.24
CYD-449	92.46
CYD-450	100.35
CYD-451	118.40
CYD-452	83.33
CYD-553	136.45
CYD-554	102.93
CYD-555	114.34
CYD-556	108.46
CYD-557	89.42
CYD-558	93.35
CYD-559	112.39
CYD-560	94.89
CYD-161	86.39
CYD-262	90.38
CYD-263	108.35
CYD-264	119.44
CYD-265	87.77
CYD-246	83.59
CYD-267	109.24
CYD-268	113.24
CYD-569	112.49
CYD-270	100.03
CYD-371	89.94
CYD-022	84.30
CYD-173	94.49

CYD-074	99.98
CYD-275	108.09
CYD-076	117.77
CYD-177	109.56
CYD-554	129.50
CYD-345	116.38
CYD-137	119.34
CYD-121	108.79
CYD-502	99.38
H-002	90.78
H-003	94.38
H-004	106.89
H-005	110.38
H-006	117.39
H-007	113.21
H-008	115.89
H-009	116.47
H-010	102.53
H-011	87.95
H-012	117.74
H-013	117.51
H-014	109.09
H-015	91.34
H-016	92.16
H-017	94.41
H-018	90.00
H-019	103.16
H-020	97.99
H-021	115.54
H-022	117.43
H-023	107.04
H-024	89.54
H-025	85.99
H-026	90.47
H-027	91.83
H-028	91.68
H-029	113.26
H-030	111.04
H-031	90.70



H-032	113.93
H-033	112.82
H-034	85.42
H-035	88.66
H-036	113.67
H-037	130.48
H-038	96.36
H-039	93.28
H-040	83.15
H-041	110.90
H-042	88.60
H-043	98.99
H-044	91.94
H-045	85.55
H-046	90.45
H-047	106.86
H-048	105.78
H-049	113.71
H-050	115.72
H-051	109.65
H-052	82.00
H-053	98.22
H-054	95.45
H-055	116.60
H-056	107.40
H-057	113.79
H-058	114.00
H-059	107.62
H-060	88.22
H-061	108.68
H-062	93.83
H-063	105.42
H-064	88.77
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B-103	96.99
B-104	117.45
B-105	98.20
B-106	108.83
B-107	103.60

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B-109	101.17
B-110	91.52
B-111	103.18
B-112	93.30
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B-116	90.43
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B-121	116.82
B-122	118.70
B-123	91.09
B-124	119.14
B-125	94.64
B-126	106.84
B-127	100.32
B-128	108.96
B-129	115.95
B-130	108.88
B-131	102.37
B-132	115.55
B-133	105.42
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B-135	100.65
B-136	107.97
B-137	116.60
B-138	105.47
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B-140	106.23
B-141	96.78
B-142	112.80
B-143	82.71
B-144	83.60
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B-146	103.16

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B-153	119.71
B-154	113.86
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B-156	96.61
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I-103	98.28
I-104	91.30
I-105	94.96
I-106	83.32
I-107	103.14
I-108	102.34
I-109	106.13
I-110	96.25
I-111	105.08
I-112	81.97
I-113	126.40
I-114	99.53
I-115	105.03
I-116	92.10
I-117	119.52
I-118	108.37
I-119	107.24
I-120	101.38
I-121	119.57
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I-123	91.69
I-124	84.08
I-125	98.82
I-126	88.03
I-127	95.98
I-128	119.73

I-129	81.64
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I-132	103.88
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I-145	85.57
I-146	118.18
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G-009	86.63
G-010	87.51
G-011	124.35
G-012	106.90
G-013	102.08
G-014	89.21
G-015	106.38
G-016	84.42
G-017	90.32
G-018	85.98
G-019	81.00
G-020	82.01
G-021	119.96

G-022	110.98
G-023	84.83
G-024	91.42
G-025	107.91
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G-027	88.17
G-028	91.14
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G-039	107.14
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G-045	101.17
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