

Fig. S1. NEK2 regulates Beclin-1 at protein level but not affects its mRNA expression and phosphorylation. (A) Proteins binding to NEK2 were pulled down by NEK2 antibodies and stained with coomassie brilliant blue (CBB) R250 prior to MS. (B) The expressions of NEK2 and Beclin-1 relative to β -actin were quantified, the expression correlation between NEK2 and Beclin-1 was analyzed by pearson correlation coefficient. (C) Relative mRNA levels of NEK2 and BECN1 in KMS11 EV, KMS11 NEK2 OE, KMS11 Scr, KMS11 NEK2 sh, RPMI 8226 EV, RPMI 8226 NEK2 OE, RPMI 8226 Scr, and RPMI 8226 NEK2 sh. (D) Western blots of NEK2, p-mTOR (S2448), p-Beclin-1 (S90/93/96) and β -actin in KMS11 EV, KMS11 NEK2 OE, KMS11 Scr, and KMS11 NEK2 sh. Error bars indicate SD.

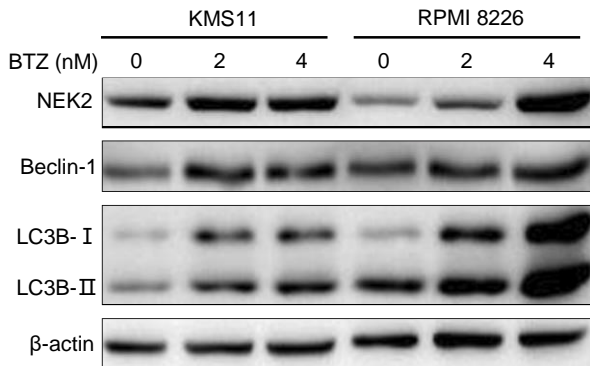
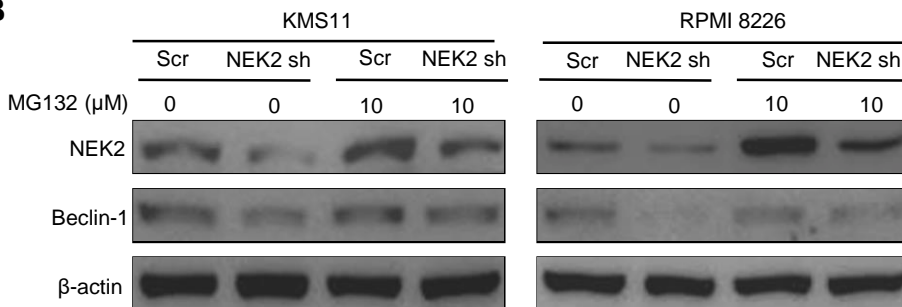
A**B**

Fig. S2. Beclin-1 is regulated by proteasome inhibitors. (A) KMS11 and RPMI 8226 were treated with BTZ (0, 2 nM, 4 nM) for 24 hours, cells were lysed, and then the levels of NEK2, Beclin-1, LC3B and β -actin were analyzed by western blotting. (B) KMS11 Scr, KMS11 NEK2 sh, RPMI 8226 Scr, and RPMI 8226 NEK2 sh were treated with MG132 (0, 10 μ M) for 10 hours, cells were lysed, the levels of NEK2, Beclin-1 and β -actin were analyzed by western blotting.

Table S1. Clinical characteristics of healthy donors and MM patients

| Subject number | Disease | Gender | Age | M-component type | Stage (ISS) | Plasma Cell (%) | Cytogenetics | Last treatment |
|----------------|---------|--------|-----|-------------------------|-------------|-----------------|---|----------------|
| 1 | HD | 60 | M | | | | | |
| 2 | HD | 44 | M | | | | | |
| 3 | HD | 45 | M | | | | | |
| 4 | HD | 50 | M | | | | | |
| 5 | HD | 25 | F | | | | | |
| 6 | HD | 24 | M | | | | | |
| 7 | NDMM | 67 | M | IgA Lambda | III | 12 | Normal Fish | |
| 8 | NDMM | 44 | M | IgA Kappa | III | 1 | 1q21 amplification | |
| 9 | NDMM | 49 | F | Kappa light chain only | I | 3.5 | Normal Fish | |
| 10 | NDMM | 56 | F | Lambda light chain only | I | 3.5 | Normal Fish | |
| 11 | NDMM | 76 | F | IgM Lambda | III | 57 | Normal Fish | |
| 12 | NDMM | 70 | M | IgA Kappa | III | 35 | Normal Fish | |
| 13 | NDMM | 66 | M | Lambda Light chain only | II | 68 | 1q21 amplification, RB deletion | |
| 14 | NDMM | 48 | F | IgG Lambda | I | 20 | RB1 deletion, D13S319 deletion | |
| 15 | NDMM | 52 | F | IgG Kappa | III | 8.9 | Normal Fish | |
| 16 | RMM | 67 | M | IgG Lambda | III | 3.5 | T (11;14) (q13; q32) | VD |
| 17 | RMM | 49 | F | Lambda light chain only | I | 3.5 | Normal Fish | VD |
| 18 | RMM | 46 | M | Kappa light chain only | I | 15.5 | 1q21 amplification, RB deletion | VTD |
| 19 | RMM | 64 | M | Kappa light chain only | III | 5.3 | Normal Fish | VCD |
| 20 | RMM | 48 | M | IgA Kappa | II | 45.5 | Normal Fish | VD |
| 21 | RMM | 66 | F | IgG Lambda | II | 48 | 1q21 amplification, IgH translocation, 13q deletion, t (14;16) (q32; q23) | VAD |
| 22 | RMM | 69 | F | IgG Lambda | III | 13.5 | Normal Fish | VAD |

HD, healthy donors; NDMM, Newly diagnosed MM patients; RMM, Relapsed MM patients; M, Male; F, Female; VD, Bortezomib + Dexamethasone; VAD, Bortezomib + Adriamycin + Dexamethasone; VCD, Bortezomib + Cyclophosphamide + Dexamethasone; VTD, Bortezomib + Thalidomide + Dexamethasone.

Table S2. The list of primer sequences

| Primer name | Sequence |
|-------------------------------|---|
| NEK2 Xba I sense | TGGTCTAGAGCCACCATGCCTTCCC GGGCTGAG |
| NEK2 BamH I antisense | TCTGGATCCCTAGCGCATGCCAGGAT |
| NEK2 shRNA sense | CCGGCGT TACTCTGATGAATTGAATCTCGAGATTCAATTCATCAGAGTAACGTTTTTG |
| NEK2 shRNA antisense | AATTCAAAAACGTTACTCTGATGAATTGAATCTCGAGATTCAATTCATCAGAGTAACG |
| BECN1 shRNA sense | CCGGCCCGT GGAATGGAATGAGATTCTCGAGAATCTCATTCCATTCCACGGGTTTTTG |
| BECN1 shRNA antisense | AATTCAAAAACCCGTGGAATGGAATGAGATTCTCGAGAATCTCATTCCATTCCACGGG |
| Scramble shRNA sense | CCGGGCGCGATAGCGCTAATAATTTCTCGAGAAATTATTAGCGCTATCGCGCTTTTT |
| Scramble shRNA antisense | AATTA AAAAGCGCGATAGCGCTAATAATTTCTCGAGAAATTATTAGCGCTATCGCGC |
| NEK2 QPCR sense | AGGAATGCCACAGACGAA |
| NEK2 QPCR antisense | TACAGCAAGCAGCCCAAT |
| BECN1 QPCR sense | CTGGACACTCAGCTCAACGTCA |
| BECN1 QPCR antisense | CTCTAGTGCCAGCTCCTTTAGC |
| β -actin QPCR sense | GTCTTCCCCTCCATCGTG |
| β -actin QPCR antisense | TTCTCCATGTCGTCCCAG |