Supplementary Methods

Cloning of DNM2 constructs and lentiviral vectors

A vector that contains full-length human DNM2 was obtained from Fisher Scientific and primers with AscI and PacI overhangers/extensions were used for amplification of the full-length DNM2 (forward primer: 5’-atcgggcgcgccatgggcaaccgcggg-3’; reverse primer: 5’-atcgttaataattagtcg agcagggatggc-3’) and DNM2 PRDΔ (forward primer: 5’-atcgggcgcgccatgggcaaccgcggg-3’; reverse primer: 5’-atcgttaattaattacgtggacacagtgctgg-3’). PCR products and the KA391 vector were sequentially digested by AscI and Pac1 restriction enzymes, followed by the ligation reaction using T4 ligase (NEB). Finally, the ligation products were transformed into One Shot® MAX efficiency® DH5α™-T1^R competent cells (Invitrogen).

The pGFP-C-lenti vector containing the non-targeting sequence or DNM2 shRNA constructs was used as templates to amplify the U6-promoter-shDNM2s (forward primer: 5’-taggctagccccagtggaaagacgcgcaggca-3’; reverse primer: 5’-catgccttgcaaaatggcgttactgcaaccc taactgacacacattccacagg-3’), while the pRRL-PPT-SF-GFP-pre vector was used as a template to amplify the SFFV promoter (forward primer: 5’-tgcagtaacgccattttgcaaggcatg-3’; reverse primer: 5’-ccaccggtctcgacggtatcgataagcttgattcg-3’). The two PCR products were then mixed and used as the template for the third overlapping PCR (forward primer: 5’-taggctagccccagtggaaagacgcgca ggca-3’; reverse primer: 5’-ccaccggtctcgacggtatcgataagcttgattcg-3’). The TOPO vector (Invitrogen) that the final PCR product was cloned into, and the pRRL-PPT-SF-GFP-pre vector, were digested with NdeI (NEB) and AgeI (NEB) restriction enzymes, followed by the ligation reaction and finally transformation into One Shot® MAX efficiency® DH5α™-T1^R competent cells. All constructs were verified and confirmed by sequencing.

293T cell transfection using PEI

For use in co-IP assays, 16-24 hours before transfection, 7-8x10^6 293T cells were plated in a 10 cm Falcon® tissue culture dish in DMEM media, supplemented with 10% FBS and L-glutamine. 20 µg of DNA plasmids and 60 µg of polyethylenimine (PEI, Polysciences Inc) were mixed in 1 mL DMEM without phenol red (Life Technologies) and incubated at room temperature for 20 minutes. Then the mixture was added to the cells, followed by incubation at 37°C for 48 hours. Subsequently, cells were washed with cold PBS, scraped off the culture dish and pelleted for cell lysis. For use in immunostaining, 0.9x10^6 293T cells were added into a 6-well plate (Corning Inc.) containing small coverslips (Fisher Scientific) coated with poly-L-lysine (R&D SYSTEMS). A mixture of 3 µg of plasmids and 9 µg of PEI was used for 24-hour transfection.

Immunostaining and confocal analysis

For immunostaining in 293T cells, coverslips with adherent cells were transferred into the wells of a 6-well plate where all procedures were performed. Cells were washed three times with PBS, then fixed with 4% paraformaldehyde in PBS for 10 minutes at room temperature, followed by permeabilization with 0.1% Triton-100 (Sigma Aldrich) in PBS for 5 minutes at room temperature. Cells were then blocked with 3% BSA in PBS at room temperature for 1 hour, followed by incubation overnight with primary antibodies at 4°C. On the second day, the cells were incubated with secondary antibodies at room temperature in the dark for one hour. Finally, the slides were mounted in Prolong Gold Antifade Reagent (Life Technologies) with DAPI. Cells were washed three times with PBS between each step. Imaging was performed with a Nikon C1 confocal microscope.

Proximity ligation assay

Cells co-transfected with HA-Ahi-1 and Myc-DNM2 constructs were fixed with 3.7% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA) in PBS for 15 minutes at 23°C, followed by permeabilization with PBS containing 0.1% Triton-100 (Sigma Aldrich) for 10 minutes at 23°C. The cells were then blocked with Blocking Solution (Sigma-Aldrich) in a 37°C incubator for 30 minutes, and incubated overnight with primary antibodies diluted with Antibody Diluent (Sigma-Aldrich) at 4°C. One the second day, cells were washed twice with Wash Buffer A (Sigma-Aldrich) for 5 minutes and incubated with PLA probe PLUS and MINUS (Sigma-Aldrich), which were diluted 5X with Antibody Diluent at 37°C for 1 hour. Cells were washed with Wash Buffer A for 2 x 5 minutes under gentle agitation, followed by incubation with the Ligation-Ligase solution at 37°C for 30 minutes. Cells were then washed with Wash Buffer A for 2 x 2 minutes under gentle agitation, followed by incubation with the Amplification-Polymerase solution at 37°C for 100 minutes. Subsequently, cells were washed with Wash Buffer B for 2 x 10 minutes under gentle agitation, followed by a 1-minute wash with 0.01x Wash Buffer B. Finally, the slides were mounted in Prolong Gold Antifade Reagent with DAPI, and the fluorescent signals were detected using a confocal Nikon X1 microscope. Primary antibodies used in this study were anti-HA and anti-Myc antibodies with 1:750 dilution.

cDNA synthesis and Q-RT-PCR

After RNA was extracted using TRIzol reagent, the concentration was measured using a Nanodrop ND-100 spectrophotometer (Life Technologies). Subsequently, cDNA was synthesized from 100 ng RNA with SuperScript® VILO™ Master Mix (Life Technologies). The PCR mixture was made up of 1 μL of 20 μM primer pairs, 6 μL SYBR® Green PCR Master Mix (Life Technologies), 1 μL of cDNA and 4 μL of RNase-free water, and quantitative real-time

PCR was performed on the 7500 Real Time PCR System (Applied Biosystems). Data analysis was performed with the ΔΔCt method with *GAPDH* or *β2-microglobulin* (*β2M*) as controls. The primers for human *DNM2* used in this study are 5'-ggtgctggagaacttcgtg-3' (forward primer) and 5'-tcggcatgttctgtttttga-3' (reverse primer).

Production of lentiviruses and infection of BCR-ABL^{+} cells and primary CD34^{+} cells

18-20 hours before transfection, $6x10^6$ 293T cells were plated in a 10-cm Falcon® tissue culture dish in 10 mL DMEM supplemented with 10% FBS and L-glutamine, and incubated in a 37°C, 5% CO_2 incubator. Medium was replaced and reduced to 4.5 mL 4 hours prior to transfection. A 250 µL DNA mixture containing 6 µg of shRNA plasmids, packaging vectors (1.5 µg of REV and 3.9 µg of ΔR), 2.1 µg of vesicular stomatitis virus glycoprotein (VSV-G) envelope and Opti-MEM® medium (Thermo Fisher Scientific), and a 250 µL PEI solution containing 40 µL of PEI and 210 µL Opti-MEM® medium (Life Technologies), were prepared in two separate tubes, and then mixed and incubated for 20 mins at room temperature. Subsequently, the 500 µL mixture of transfection reagents was added to 293T cells in one culture dish in a dropwise fashion. After a 48-hour incubation, the viral supernatant was collected and passed through a 0.45 µm filter (Pall Corporation, New York, NY) and then subjected to 1.5-hour ultracentrifugation. The resultant virus pellet was resuspended in serum-free medium supplemented with 5% DNase, and then aliquoted and stored at -80℃ for future use.

For stable knockdown of DNM2 in K562 and BV173 cells, 10 µL of concentrated viruses generated from the pGFP-C-shLenti viral vectors, containing a non-targeting sequence or shRNA constructs targeting human DNM2, were added to $4x10^5$ cells in a 24-well plate in 400 µL RPMI 1640 in the presence of protamine sulfate for 18 hours. After an 18-hour incubation, infected cells were washed with PBS and cultured in complete RPMI medium. Finally, the sorted GFP^{+}

K562 or BV173 cells were maintained in complete RPMI medium containing puromycin. For $CD34^+$ CML cells, cells were thawed and pre-stimulated with the growth factor cocktail for 16 hours, followed by 6-hour infection with 5 ul of concentrated virus in the presence of protamine sulfate. Cells were then washed and cultured for three days before other assays were performed.

Autophagy Flux assay with Cyto-ID green detection dye

The Cyto-ID kit (Enzo Life Sciences) was used according to the manufacturer's instructions. In short, $CD34^+$ treated CML cells were harvested, washed with Hank's buffer, resuspended in staining buffer containing Cyto-ID green detection dye and incubated for 30 minutes in the dark on ice before cells were washed one more time and flow cytometry was performed.