Appendix-I

Materials and methods

Cell culture and treatment. K562 and K562G cells were provided by Professor Jie Jin (The First Affiliated Hospital of Zhejiang University). The cells were maintained in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) with 10% fetal bovine serum (FBS, Gibco; Thermo Fisher Scientific, Inc.) at 37˚C in 5% CO_2 atmosphere. HHT was purchased from Selleckchem (cat. no. s9015). Imatinib was purchased from Selleckchem (cat. no. s2475). Autophagic inhibitor Bafilomycin A1 (Baf-A1) was purchased from Abcam, and autophagic inhibitor chloroquine (CQ) was purchased from Sigma-Aldrich (Merck KGaA). Compounds were dissolved in dimethyl sulfoxide (DMSO) and added to culture media until it reached a final concentration of 0.1% DMSO. For the vehicle control, 0.1% DMSO alone was used.

Cell viability assay. K562 and K562G were seeded in 384-well plates at a density of 8,000 cells/well, and then treated with 0.1% DMSO (vehicle control) or imatinib at concentrations of 100, 50, 25, 12.5, 6.2, 3.1, 1.56, 0.78 and 0.39 ng/ml for 48 h. Cell proliferation was assayed using MTS assay (Promega Corp.) according to the manufacturer's instructions and the absorbance was measured at 490 nm using the EnVision microplate reader (PerkinElmer).

Western blotting and antibodies. Cells were lysed using RIPA buffer (pH 7.4) containing a protease inhibitor cocktail (Roche Diagnostics). Protein concentrations of the extracts were measured by BCA™ protein assay kit (Pierce; Thermo Fisher Scientific, Inc.) and equal amounts (20 μg) of total protein from each sample was separated by 8% or 12% SDS-PAGE and then were transferred onto NC (nitrocellulose) membrane (Millipore). NC membranes were blocked with 10% skim milk powder dissolved in TBST buffer at room temperature for 1 h. Then, NC membranes were washed with TBST, and then incubated with a primary antibody (dilution of 1:1,000) in TBST containing 5% bovine serum albumin (BSA) overnight at 4˚C. Next, membranes were washed with TBST three times and incubated with an HRP-conjugated anti-rabbit (dilution 1:10,000; cat. no. A16096) or anti-mouse secondary antibody (dilution 1:10,000; cat. no. 31430) (Thermo Fisher Scientific, Inc.) for 1 h at room temperature. The signals were detected using an ECL reagent (Pierce; Thermo Fisher Scientific, Inc.). All western blots were replicated in three times and the intensity of the western blot signals was analyzed using ImageJ 1.8.0 analysis software (NIH; National Institutes of Health, Bethesda, MD, USA). The primary antibodies were as follows: Antibody against C-Abl (cat. no. sc-887) and antibody against ubiquitin (Ub; cat. no. sc-166553; both from Santa Cruz Biotechnology, Inc.), antibodies against p62 (cat. no. PM045; MBL Beijing Biotech Co., Ltd.), antibodies against LC3B (cat. no. 2775), antibodies against ATG5 (cat. no. 2630) and antibodies against Beclin-1 (cat. no. 3495; all from Cell Signaling Technology, Inc.), antibodies against β-tubulin (cat. no. HC101-02) and antibodies against β-actin (cat. no. HC201-02; both from Beijing Transgen Biotech Co., Ltd.), antibodies against poly adenosine diphosphate (ADP)-ribose polymerase (PARP; cat. no. 9542) and antibodies against cleave caspase-3 (cat. no. 9664; both from Cell Signaling Technology, Inc.).

Figure S1. K562G is highly resistant to imatinib compared with K562 after incubation for 48 h in an MTS assay. K562 and K562G cells were treated with HHT at indicated concentrations for 48 h, respectively. Cell viability was determined by MTS assay. Values are presented as the means ± SD of three independent experiments. HTT, homoharringtonine.

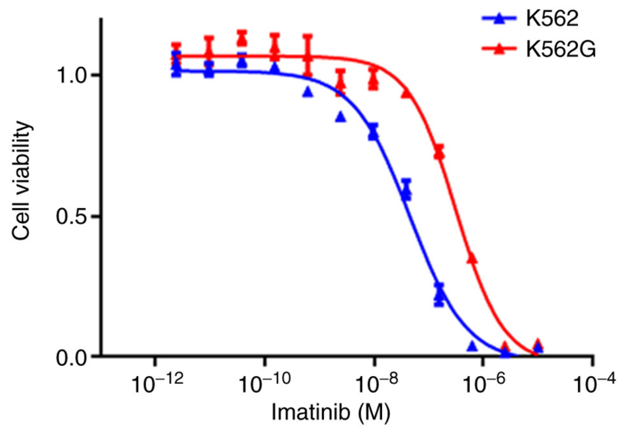

Figure S2. HHT downregulates BCR-ABL protein levels in K562 cells. K562 cells were treated with 0, 10, 25, 50 and 100 ng/ml HHT for 24 h, respectively. Cell lysates were analyzed to determine the protein levels of BCR-ABL by western blotting. HTT, homoharringtonine.

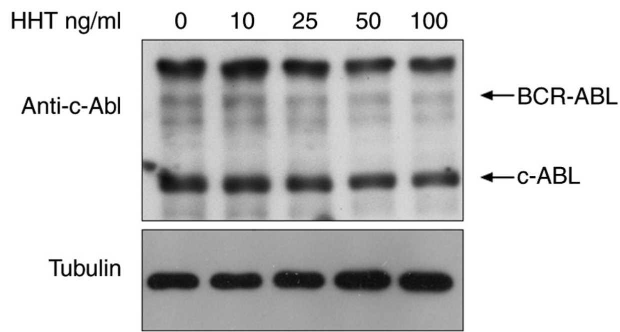

Figure S3. HHT promotes autophagic degradation of BCR-ABL in K562 cells. (A and B) The effects of autophagolysosome inhibitors CQ or Baf-A1 on HHT-induced BCR-ABL degradation. K562G cells were treated with HHT (20 ng/ml) for 1 h, and then co-treated with CQ (20 μM) or Baf-A1 (20 nM) for 6 h. Cell lysates were analyzed by immunoblotting with indicated antibodies. HTT, homoharringtonine; CQ, chloroquine; Baf-A1; Bafilomycin A1.