Legend to Supplemental Figure S1: (A) Representative Western blot analysis of phosphoserine 14-3-3 (pSer 14-3-3) and NHE1 in NHE1 IP eluate in amiloride-sensitive (Kasumi-1, MOLM-13 and MV4-11) and resistant (THP-1, OCI-AML3 and KG-1) AML cell lines. (B-E) Amiloride (10 µM) and HMA (10 µM) treatment significantly (B) suppressed growth (n=3), (C) reduced pHi (n=3), (D) induced apoptosis (n=4) and (E) suppressed proliferation (n=4) of Kasumi-1, MOLM-13 and MV4-11, but not other human AML cell lines. (F) Representative Western blot analysis of phosphoserine 14-3-3 (pSer 14-3-3) and NHE1 in NHE1 IP eluate in primary AML samples carrying either FLT3, RAS or KIT mutation controlled by AML samples without FLT3/RAS/KIT mutation. (G) Primary AML samples carrying different mutations showed no significant difference in cellular viability measured by Prestoblue™ after 3-day in vitro culture (n=29).

Legend to Supplemental Figure S2: (A) NHE1 expression was successfully knocked down by shRNA in THP-1 and OCI-AML3 examined by quantitative RT-PCR analysis (n=3). (B-C) In vitro NHE1 knockdown did not affect (B) pHi or (C) growth of THP-1 and OCI-AML3 (n=3). (D) NHE1 expression was successfully knocked down by shRNA in primary AML samples carrying FLT3 and RAS mutation examined by quantitative RT-PCR analysis (n=12).

Legend to Supplemental Figure S3: In (A-B) MV4-11 and (C-D) Kasumi-1, treatment with kinase inhibitors significantly reduced (A, C) the level of NHE1 phosphorylation and (B, D) pHi in vitro (n=3). (E-F) Only quizartinib (10 nM) and BRD7389 (10 µM), but not CGS 9343B (10 µM) and HA1100 (10 µM), (E) suppressed the growth and (F) reduced pHi of Kasumi-1, MOLM-13 and MV4-11 in vitro (n=3). (G) Effect of overexpression of BTK, FLT3-ITD or CDK4 and treatment with ibrutinib (10 µM), quizartinib (10 nM), ravoxertinib (100 nM) or ribociclib (10 µM) on pHi of HEK293 with wildtype and mutated NHE1 (n=3). (H) Representative Western blot analysis of FLT3, BTK, ERK and NHE1 in NHE1/FLT3 IP in MV4-11. (I) Treatment with crenolanib and BRD7389, but not quizartinib, reduced the pHi of Ba/F3 carrying FLT3-ITD and FLT3-ITD+D835Y mutation in vitro (n=3). (J) Primary AML samples carrying FLT3 mutation were more sensitive towards in vitro treatment with HMA (10 µM), crenolanib (10 µM) and BRD7389 (10 µM), compared to AML with wildtype FLT3 (n=32-55). (K) AUC of crenolanib, HMA and BRD7389 significantly correlated with each other in primary AML samples (n=87).

Legend to Supplemental Figure S4: (A-B) Treatment of amiloride (10 µM) enhanced (A) pHi acidification and (B) apoptosis induction in combination with kinase inhibitors (quizartinib 100 nM; BRD7389 10 µM; ibrutinib 10 µM) in Kasumi-1 in vitro (n=3). (C) NHE1-KD enhanced the growth inhibitory effect of kinase inhibitors in Kasumi-1 in vitro (n=3). (D) Leukemic burden analysis by bioluminescence in MV4-11 engrafting NSG mice upon in vivo
amiloride and quizartinib treatment (n=7-8). (E) Survival analysis of NSG mice engrafted with MV4-11 and MOLM-13 upon combined *in vivo* treatment with amiloride and quizartinib showed longer survival compared to those treated with vehicle control, amiloride or quizartinib only (n=10-12). The grey dash line indicated the start date of treatment. (F) Combined treatment with amiloride and quizartinib showed the greatest *in vivo* growth inhibitory effect in Kasumi-1 engrafted in NSG mice, compared to those treated with vehicle control, amiloride or quizartinib only (n=4-5). (G-H) The growth inhibition upon the treatment of amiloride (10 μM), quizartinib (10 nM) and ibrutinib (10 μM) were diminished in the presence of (G) NHE1 or (H) MCT4 overexpression *in vitro* (n=3).