Supplemental methods

Cell culture

No cell lines used in this study were listed in the database of cross-contaminated or misidentified cell lines suggested by International Cell Line Authentification Committee (ICLAC). Human breast cancer cell line (MDA-MB-231) and mouse TUBO cell line derived from BALB/c mice transgenic for the transforming rat HER2/neu oncogene (BALB-NeuT) [15] were gifted from Dr. Yang-Xin Fu at University of Texas Southwestern Medical Center.

Animals

8-weeks old female immune-deficient NOD-*scid* IL2Rgamma^{null} (NSG^{TM}, Jackson Laboratories, Bar Harbar, ME) were used to generate xenograft model by inoculating human breast cancer cell line. Syngeneic TUBO tumors were established in 8-weeks old female BALB/c mice (Charles River, Frederick, MD). 8-week old male C57BL/6 mice and C57BL/6 ly5.2 mice (Charles River) and Bak1 knock-out mouse (Jackson Laboratory) were used for bone marrow transplantation assay. All the mice were maintained in the Research Animal Facility at Children's National Institute. The Institutional Committee on the Use and Care of Animal approved all procedures involving experimental animals.

Flow cytometry and cell sorting

For aptamer binding assay, streptavidin-APC (Cat# 554067) (BD Bioscience, San Jose, CA, USA) was used for detecting the biotin-conjugated aptamers by flow cytometry. Other cell targeting aptamer specific for Ramos cells [20] was used for

negative control. CD3e (clone 145-2C11), B220 (clone RA3-6B2), CD11b (clone M1/70), Gr-1 (clone RB6-8C5), Ter-119 (clone Ter-119), Sca-1 (clone D7), c-Kit (clone 2B8), CD150 (clone TC15-12F12.2), and CD48 (clone HM48-1) antibodies were purchased from BD Bioscience. Annexin V staining was performed using AnnexinV apoptosis detection kit (BD Bioscience). Flow cytometry analysis was performed on FACS Canto II (BD Bioscience) and the data were analyzed with FlowJo (FLOWJO, Ashland, OR, USA). KIT/c-Kit$^+$ cells from tumors were isolated with CD117 micro beads kits (human or mouse) by autoMACS pro separator (Miltenyi biotec). LSK population (CD3e$^-$/B220$^-$/CD11b$^-$/Gr-1$^-$/Ter119$^-$/Sca-1$^+$/c-Kit$^+$) and HSCs (CD3e$^-$/B220$^-$/CD11b$^-$/Gr-1$^-$/Ter119$^-$/Sca-1$^+$/c-Kit$^+$/CD48$^-$/CD150$^+$) harvested from bone marrows were isolated by BD Influx cell sorter (BD Biosciences).

Real-time PCR

Total RNAs from cell lines and mouse tissues were extracted by RNeasy Plus Mini kit (Qiagen, Valencia, CA, USA). *miR-26a* levels were quantified by Taqman microRNA assay (assay ID; 000405) that covered both *has-miR-26a-5p* and *mmu-miR-26a-5p* (Thermo Fisher Scientific) according to manufacturer's protocol. U6 snRNA (Taqman microRNA assay, assay ID; 001973) was used as endogenous control. Real-time PCR was performed on 7500 Real Time PCR system (Thermo Fisher Scientific). Sequence primers for mouse *Bak1* were TCTCCACCAAGACCTGAAAAAT (forward) and CTTCGAAAGACCTCCTCTGTGT (reverse). For mouse *Ezh2*, TTGCTAAGAGGGCTATCCAGAC (forward) and TGTCAAGGGATTTCCATTTCTC (reverse). For mouse *Actb*, AGGAGTCCTGTTGATGTTGCCAGT (forward) and GGGACGCAGCAACTGACATTTCTA (reverse) were used as endogenous control.

Lentivirus production

For miR-26a overexpression, the sequence of mouse *miR-26a-1* stem loop structure and 400 base pairs of upstream and downstream flanking genomic sequence was cloned into a pGIPZ lentiviral vector (GE Dharmacon, Lafayette, CO, USA). For miR-26a inhibition, the miR-26 TuD (tough-decoy) inhibitor sequence (GACGGCGCTAGGATCATCAACAGCCTATCCTGGTCTCATTACTTGAACAAGTATTC TGGTCACAGAATACAACAGCCTATCCTGGTCTCATTACTTGAACAAGATGATCCTA GCGCCGTCTTTTTT) was cloned into pLL3.7 lentiviral vector (Addgene plasmid # 11795, Addgene, Cambridge, MA, USA) that expresses the RNAs under mouse U6 promoter. Viral production was performed in 293T cells following manufacture recommendation (Lenti-X Lentiviral expression system) (Clontech, Mountain View, CA).

Bone marrow transplantation

The c-Kit$^+$ cells harvested from bone marrow (BM) of 8-week old C57BL/6 donor mice (CD45.2) were transduced with miR-26 TuD or ctrl lentivirus in StemMACS HSC Expansion Medium (Miltenyi biotec) by spinoculation (800xg, 30min, 32^{o}C). Lethally irradiated (8.5Gy) mice were transplanted with the virus-transduced 5x10^5 BM cells. For competitive BM transplantation, the virus-transduced donor c-Kit$^+$ BM cells (CD45.2$^+$), mixed with same number (5x10^5 cells) of recipient-type competitive c-Kit$^+$ BM cells (CD45.1$^+$), were transplanted into recipients through the tail vein within 24 hours after irradiation.

Table S1.

TCGA BRCA patients used for correlation analysis (748 patients)

		(yrs.)	
Age		59 ± 13	
		(pts n.)	(%)
Stage	Stage I	75	10.0
	Stage IA	57	7.6
	Stage IB	4	0.5
	StageII	5	0.7
	StageIIA	243	32.5
	StageIIB	178	23.8
	StageIII	2	0.3
	StageIIIA	104	13.9
	StageIIIB	14	1.9
	StageIIIC	49	6.6
	StageIV	9	1.2
	StageX	2	0.3
	NA	6	0.8
PAM50	LumA	250	33.4
	LumB	115	15.4
	Her2	42	5.6
	Basal	83	11.1
	Normal	16	2.1
	NA	242	32.4

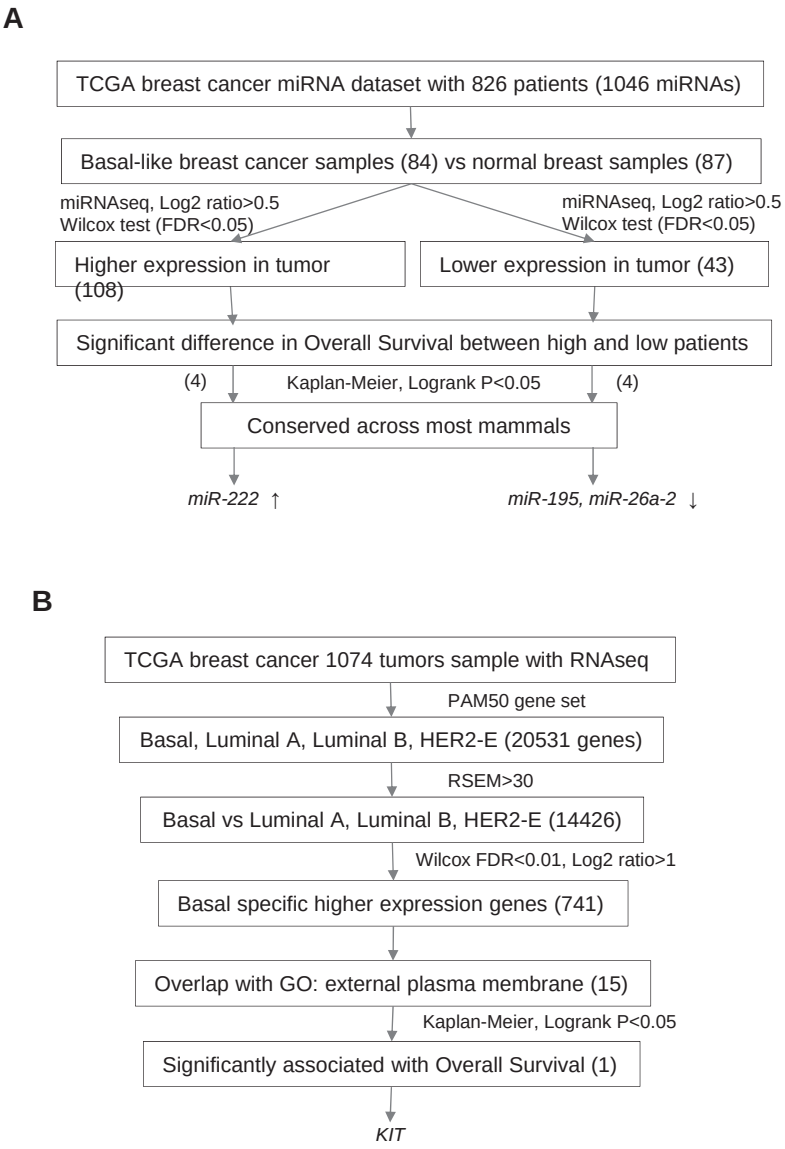

Figure S1

Oncogenomic screening of targeting genes in basal-like breast cancer. The TCGA data portal with downloaded level 3 RNA-seq profiles (N=1074), level 3 miRNA-seq profiles (N=826), and clinical data for breast cancer samples and matched normal samples (https://gdac.broadinstitute.org/, download in July 2016) were used for in silico analyses. (**A**) A workflow using TCGA database for screening target miRNAs that were significantly increased or decreased in basal-like breast cancer and also associated with overall survival of the patients. (**B**) A workflow using TCGA database for cell membrane protein-coding genes that were significantly increased in basal-like breast cancer and also associated with overall survival of the patients.

Figure S2

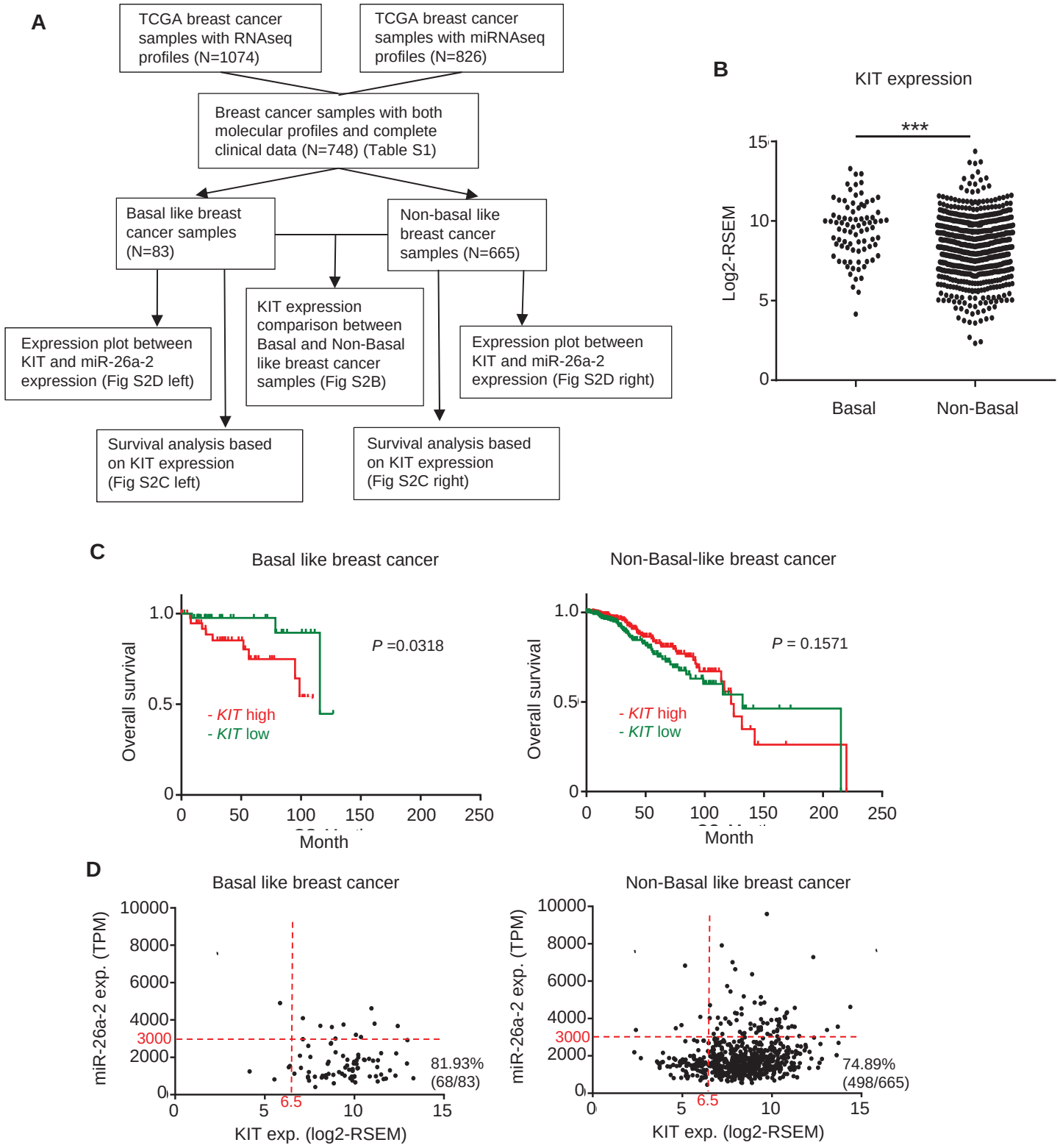

Figure S2

(**A**) A workflow using TCGA database for KIT and miR-26a-2 expression profile analysis and survival analysis based on KIT expression level in Basal like (N=83) and Non-Basal like (N=665) breast cancer samples. The 748 patients with both molecular data (RNAseq and miRNAseq) and complete clinical data (survival status and PAM50 status) in TCGA cohort (Table S1) were selected for the differential expression analysis of miR-26a and KIT. (**B**) The expression levels of *KIT* genes in basal-like breast cancer patients and non-basal like breast cancer patients. (**C**) The Kaplan-Meier curves for basal-like breast cancer patients and non-basal-like breast cancer patients with higher or lower expression level of *KIT*. Higher expression group (more than median), lower expression group (less than median). (**D**) The correlation of *miR-26a* and *KIT* genes in basal-like breast cancer patients and non-basal like breast cancer patients.

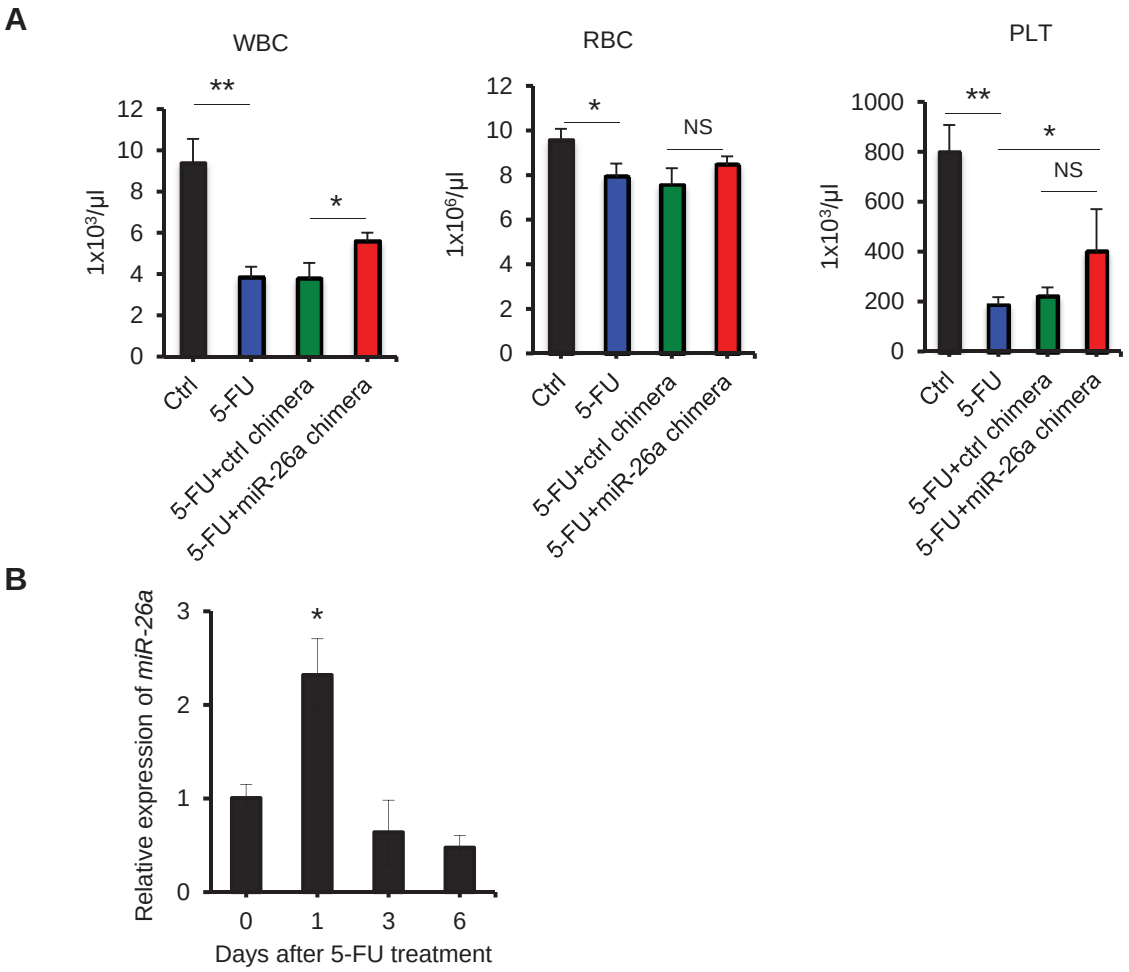

Figure S3

miR-26a chimera protects mice against 5-FU induced myelosuppression. (**A**) C57BL/6 mice were treated with miR-26a chimera intravenously (670 pmol/20g) daily for 3 days. At day2, 150 mg/kg 5-FU was injected intravenously. Peripheral blood was collected 5 days after the 5-FU treatment. The numbers of WBC, RBC, and PLT 5 days after 5-FU treatment. Data (mean+s.d.) were pooled from two experiments, involving a total of 6 mice. (**B**) Transient induction of miR-26a in HSPC during injury-induced hematopoiesis. c-Kit$^+$ cells were isolated at days 0- (before), 1-, 3- and 6days after 150 mg/kg 5-FU treatment, miR-26a were quantitated by qPCR. Data from each time points were derived from 3 mice, and obtained from 2 independent experiments. *, $P < 0.05$, **, $P < 0.01$.

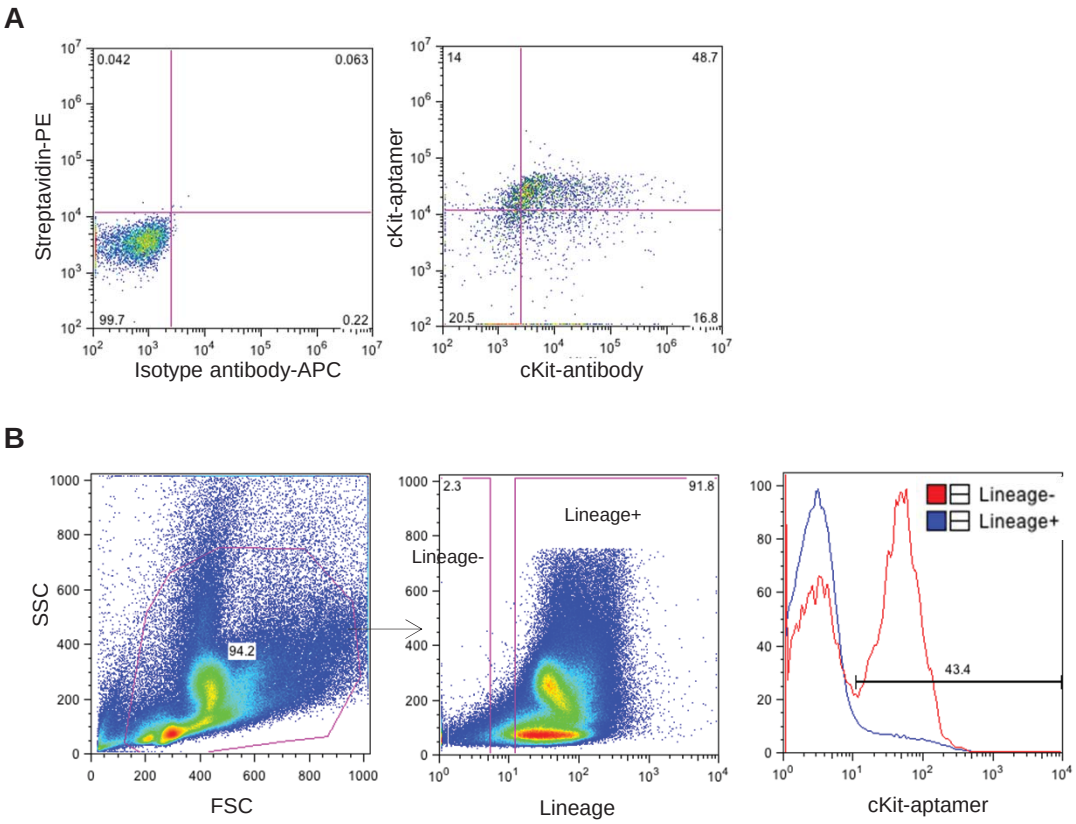

Figure S4

Mouse c-Kit aptamer bound to mouse c-Kit^{+} cells. (**A**) Our biotinylated c-Kit aptamer bound to exogenously expressed mouse c-Kit protein on mouse MEF cells as comparable to c-Kit antibody. (**B**) The c-Kit aptamer bound to Lineage- (CD3e^{-}/B220^{-}/CD11b^{-}/Gr-1^{-}/Ter119^{-}) BM cells harvested from C57BL/6 mice.

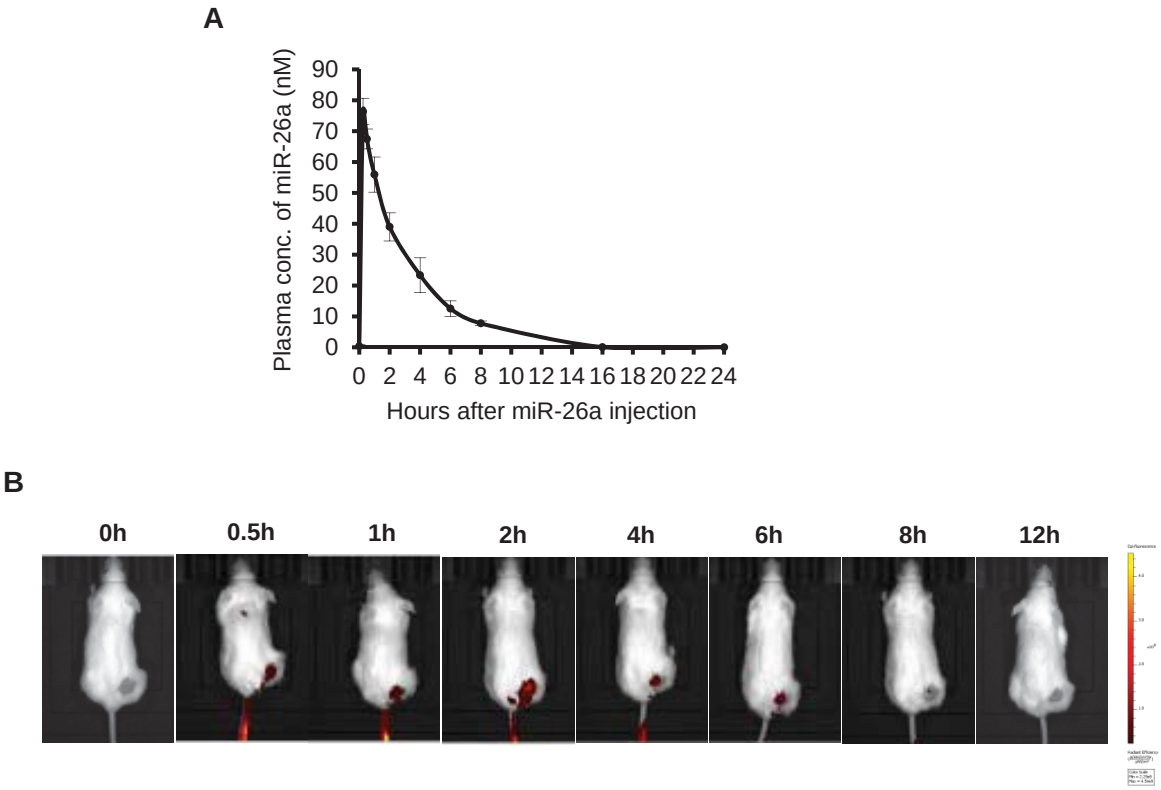

Figure S5

(**A**) Pharmacokinetics of plasma levels of miR-26a chimera and the single dose administration in BALB/c mice bearing mouse TUBO breast cancer cell line. miR-26a chimera (670 pmol/20g) was injected intravenously. Blood samples were collected at the indicated time points. The concentrations of miR-26a were determined by quantitative PCR using standard curve generated with dose-titrated miR-26a chimera. Data (mean±s.d.) were pooled from three experiments, involving a total of 3 mice. (**B**) *In vivo* imaging of miR-26a chimera conjugated with AF647 dye in BALB/c mice bearing tumor with TUBO cell line. A single dose of miR-26a chimera (670 pmol/20g) was intravenously injected and followed by serial fluorescence imaging at the indicated time points by IVIS spectrum (Caliper LifeSciences, Waltham, MA). A series of images in one experiment from two experiments, involving of a total 2 mice was represented.

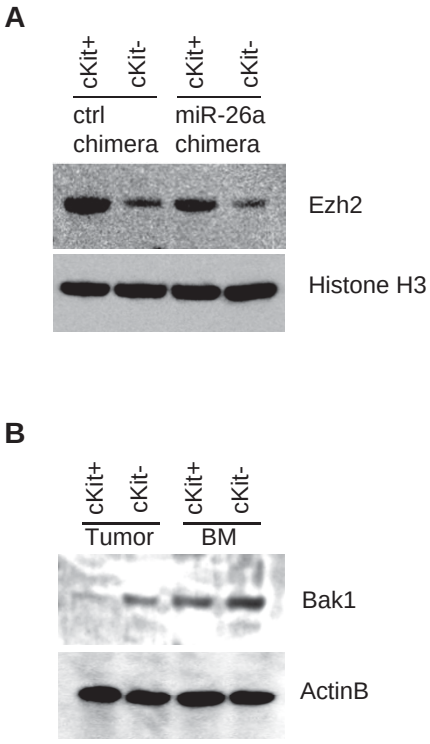

Figure S6

Immunoblot analyses. (**A**) Ezh2 expressions in c-Kit^{+} or c-Kit^{-} cells harvested from tumors in breast cancer (TUBO)-bearing BALB/c mice treated with ctrl chimera or miR-26a chimera (670 pmol/20g) for 3 days. (**B**) Bak1 expressions in c-Kit^{+} or c-Kit^{-} cells harvested from tumors and bone marrow (BM) cells in breast cancer (TUBO) bearing-BALB/c mice.

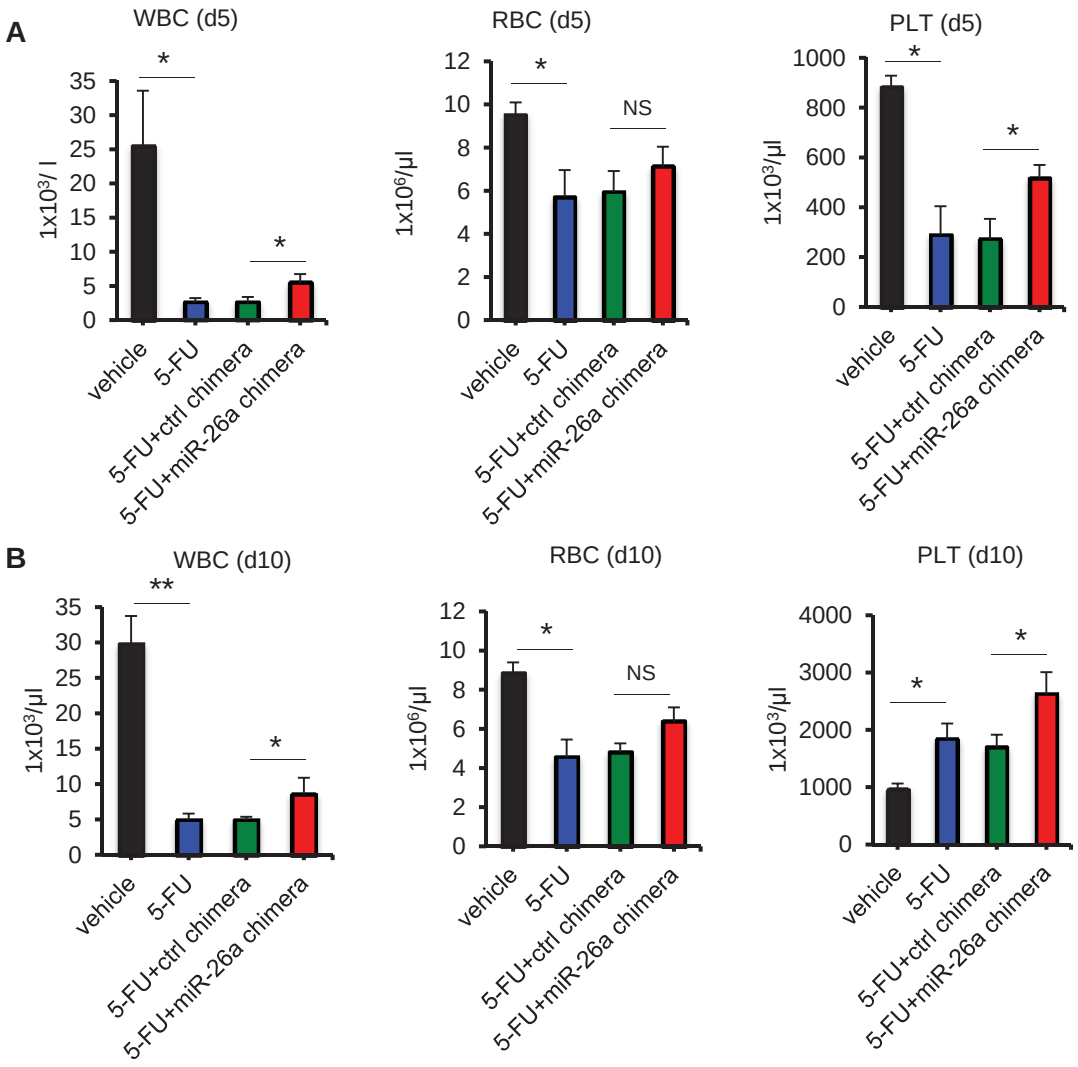

Figure S7

miR-26a ameliorates 5-FU induced myelosuppression in breast cancer-bearing mice. BALB/c mice bearing Tubo tumors were treated intravenously with miR-26a chimera (670 pmol/20g) daily for 5 days. At days 2, 3, and 4, 50 mg/kg 5-FU was intravenously injected. Peripheral blood was collected 5 and 10 days after the initial 5-FU treatment. (**A**) The numbers of WBC, RBC, and PLT 5 days after 5-FU treatment. (**B**) The numbers of WBC, RBC, and PLT 10 days after 5-FU treatment. Data (mean+s.d.) were pooled from two experiments, involving a total of 6 mice per group. *, $P < 0.05$, **, $P < 0.01$.