***Figure S1***

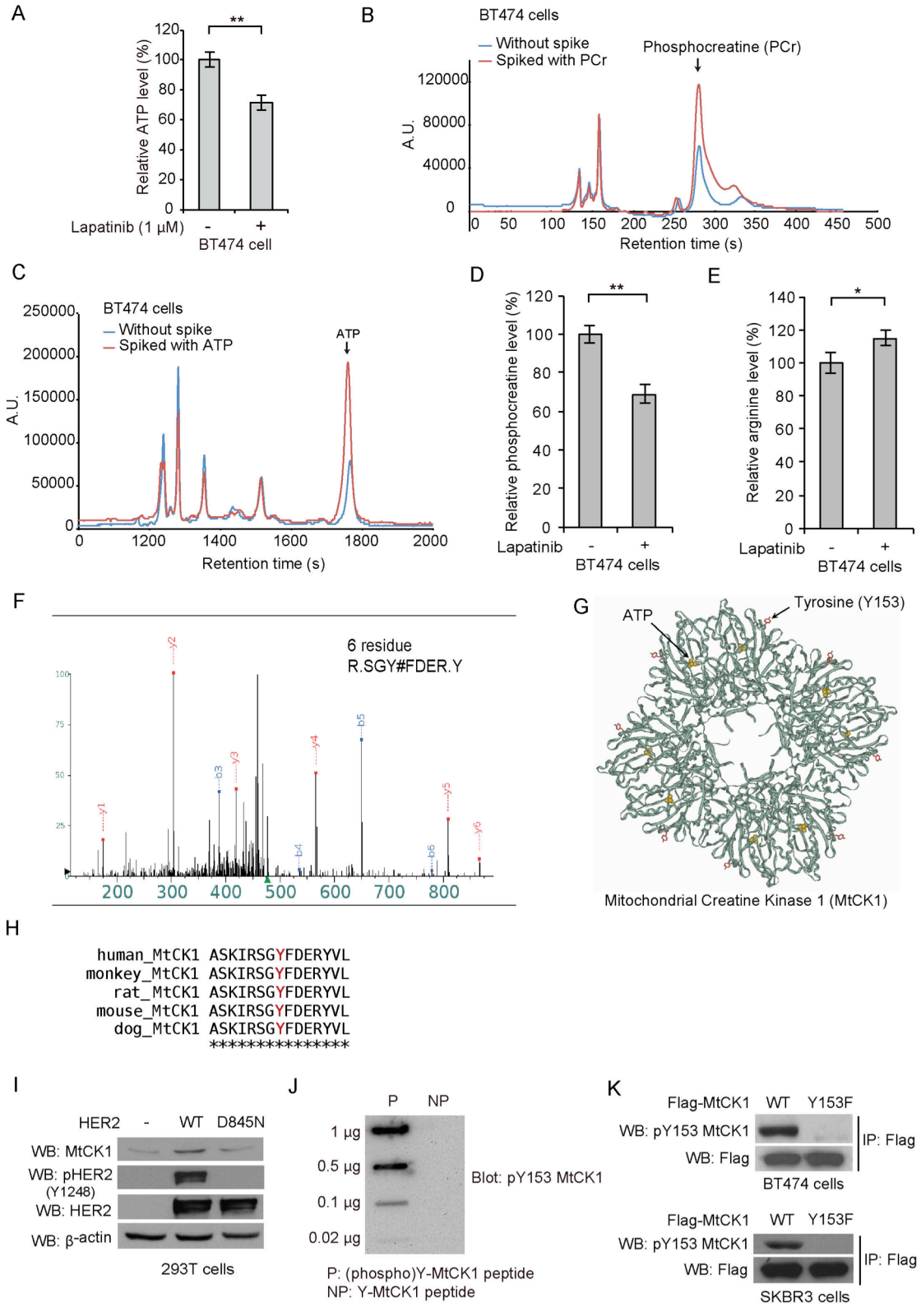

A
Relative ATP level (%)
**
Lapatinib (1 μM)
BT474 cell
B
BT474 cells
Without spike
Spiked with PCr
Phosphocreatine (PCr)
A.U.
Retention time (s)
C
BT474 cells
Without spike
Spiked with ATP
ATP
A.U.
Retention time (s)
D
Relative phosphocreatine level (%)
**
Lapatinib
BT474 cells
E
Relative arginine level (%)
*
Lapatinib
BT474 cells
F
6 residue
R.SGY#FDER.Y
G
ATP
Tyrosine (Y153)
Mitochondrial Creatine Kinase 1 (MtCK1)
H
human_MtCK1 ASKIRSGYFDERYVL
monkey_MtCK1 ASKIRSGYFDERYVL
rat_MtCK1 ASKIRSGYFDERYVL
mouse_MtCK1 ASKIRSGYFDERYVL
dog_MtCK1 ASKIRSGYFDERYVL
I
HER2 - WT D845N
WB: MtCK1
WB: pHER2 (Y1248)
WB: HER2
WB: β-actin
293T cells
J
P NP
1 μg
0.5 μg
0.1 μg
0.02 μg
Blot: pY153 MtCK1
P: (phospho)Y-MtCK1 peptide
NP: Y-MtCK1 peptide
K
Flag-MtCK1 WT Y153F
WB: pY153 MtCK1
WB: Flag
IP: Flag
BT474 cells
Flag-MtCK1 WT Y153F
WB: pY153 MtCK1
WB: Flag
IP: Flag
SKBR3 cells

**Figure S1. Phosphoproteomics analysis of HER2+ breast cancer cells identifies MtCK1 to be tyrosine phosphorylated at Y153, Related to Figure 1**

(A) ATP levels in BT474 cells treated with either control or 1 μM lapatinib. ATP levels were measured using BioVision ATP assay kit (#K255-200). (B and C) Chromatograms of BT474 cellular metabolite extracted with or without spiking of PCr (B) and ATP (C) standards. Metabolites were detected by HPLC with UV-detection at 210 nm for PCr and 260 nm for ATP and chromatograms shows a representative of three independent experiments, wh ich showed similar results. (D and E) Phosphocreatine and arginine levels in BT474 cells treated with either vehicle control or 1 μM lapatinib for 2 hours. Bar graphs were generated from the whole cell metabolomics analysis data presented in Supplemental Table S1. (F) Tandem mass spectrometry (MS/MS) spectrum of phosphorylated MtCK1 peptide RSG(pY)FDERY (residues 150-158). (G) Crystal structure of octameric MtCK1 showing the position of Y153 and the ATP binding site. Image of PDB ID 1QK1 (Eder, M., Fritz-Wolf, K., Kabsh, W., Wallimann, T., Schattner, U. 2000. Proteins:Struct., Funct., Genet. 39:216) was modified by using iMol software. (H) Sequence alignment of Y153 and surrounding amino acids in MtCK1 across mammalian species (I) Anti-MtCK1, anti-phospho-HER2 (Y1248), anti-HER2, and anti-β-actin immunoblots of lysates from 293T cells expressing vector control (ctrl), HER2 WT and HER2 D845N kinase-dead mutant. (J) Slot blot of indicated amounts of phosphorylated and non-phosphorylated short synthetic peptides corresponding to amino acids surrounding Y153 using anti-pY153 MtCK antibody (K) Anti-pY153 MtCK and anti-Flag immunoblots of anti-Flag immunoprecipitates from BT474 and SKBR3 cells expressing Flag-MtCK1 WT and Flag-MtCK1Y153F. All results represent experiments of three independent replicates and P values were determined by a two-tailed Student's t test (**P<0.01, *P<0.05).

***Figure S2***

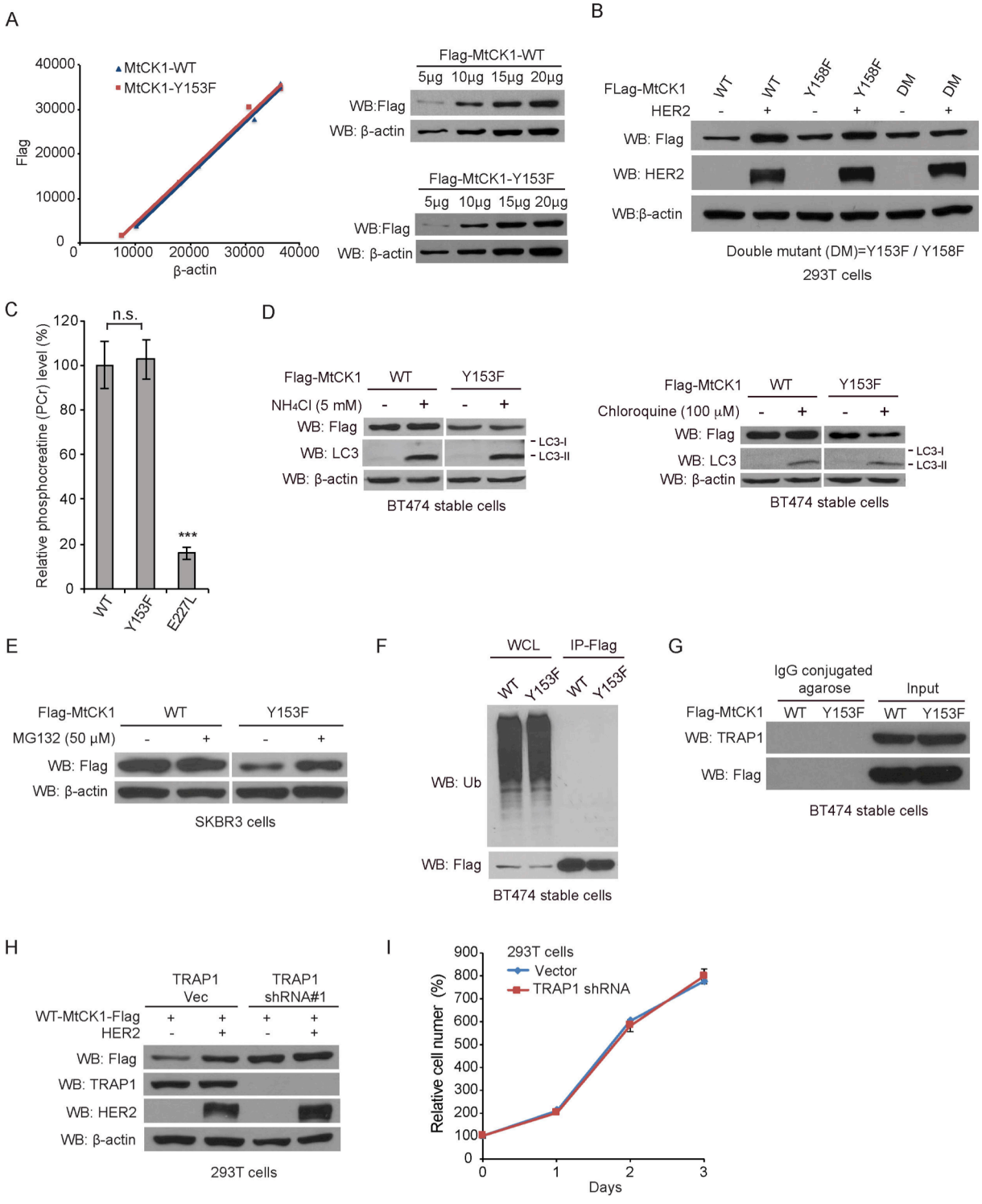

A
MtCK1-WT
MtCK1-Y153F
Flag
β-actin
Flag-MtCK1-WT
5μg 10μg 15μg 20μg
WB:Flag
WB: β-actin
Flag-MtCK1-Y153F
5μg 10μg 15μg 20μg
WB:Flag
WB: β-actin
B
FLag-MtCK1
HER2
WT WT Y158F Y158F DM DM
WB: Flag
WB: HER2
WB:β-actin
Double mutant (DM)=Y153F / Y158F
293T cells
C
Relative phosphocreatine (PCr) level (%)
n.s.
***
WT
Y153F
E227L
D
Flag-MtCK1 WT Y153F
NH4Cl (5 mM)
WB: Flag
WB: LC3
LC3-I
LC3-II
WB: β-actin
BT474 stable cells
Flag-MtCK1 WT Y153F
Chloroquine (100 μM)
WB: Flag
WB: LC3
LC3-I
LC3-II
WB: β-actin
BT474 stable cells
E
Flag-MtCK1 WT Y153F
MG132 (50 μM)
WB: Flag
WB: β-actin
SKBR3 cells
F
WCL IP-Flag
WT Y153F WT Y153F
WB: Ub
WB: Flag
BT474 stable cells
G
IgG conjugated agarose
Input
Flag-MtCK1 WT Y153F WT Y153F
WB: TRAP1
WB: Flag
BT474 stable cells
H
TRAP1 Vec
TRAP1 shRNA#1
WT-MtCK1-Flag
HER2
WB: Flag
WB: TRAP1
WB: HER2
WB: β-actin
293T cells
I
293T cells
Vector
TRAP1 shRNA
Relative cell numer (%)
Days

**Figure S2. Y153 is the key tyrosine site that regulates MtCK1 stability, Related to Figure 2 and 3**

(A) Densitometry analysis showing a linear correlation between anti-Flag and β-actin signals from immunoblots of lysates from 293T cells expressing MtCK1 WT and MtCK1 Y153F. Increasing amounts of cell lysates were blotted with anti-Flag and β-actin antibody and immunoblot densitometry were analyzed with ImageJ software. (B) Immunoblots of lysates from 293T cells co-expressing Flag-MtCK1 WT or Flag-MtCK1 Y158F or Flag-MtCK1 Y153F, Y158F along with vector control (ctrl) or HER2 WT. (C) HPLC analysis of PCr levels in the MtCK1 enzyme activity assay samples shown in Fig. 2C. (D) Anti-Flag, anti-LC3, and anti-β-actin immunoblots of lysates from MtCK1 WT and MtCK1 Y153F "rescue" BT474 cells treated with either 5 mM $NH_4Cl$ for 6 h (*left*) or 100μM chloroquine for 6h (*right*). (E) Immunoblots of lysates from $HER2^+$ SKBR3 breast cancer cell expressing Flag-MtCK1 WT and Flag-MtCK1 Y153F treated with 50 μM of proteasome inhibitor MG132 for 6 h. (F) Anti-Ub and anti-Flag immunoblots of whole cell lysate (WCL) and anti-Flag IP samples from MtCK1 WT and MtCK1 Y153F "rescue" BT474 cells pre-treated with proteasome inhibitor MG132. (G) Immunoblots of the IgG control immunorecipitated samples and the corresponding inputs from Flag-MtCK1 WT and Y153F "rescued" BT474 cell lysates. (H) Immunoblots of lysates from 293T cells co-expressing either vector control (ctrl) or HER2 and exogenous Flag-MtCK1 WT with or without stable knockdown of endogenous TRAP1. (I) Proliferation of 293T cells with or without stable knockdown of endogenous TRAP1. All results represent experiments of three independent replicates and P values were determined by a two-tailed Student's t test (***$p < 0.001$).

***Figure S3***

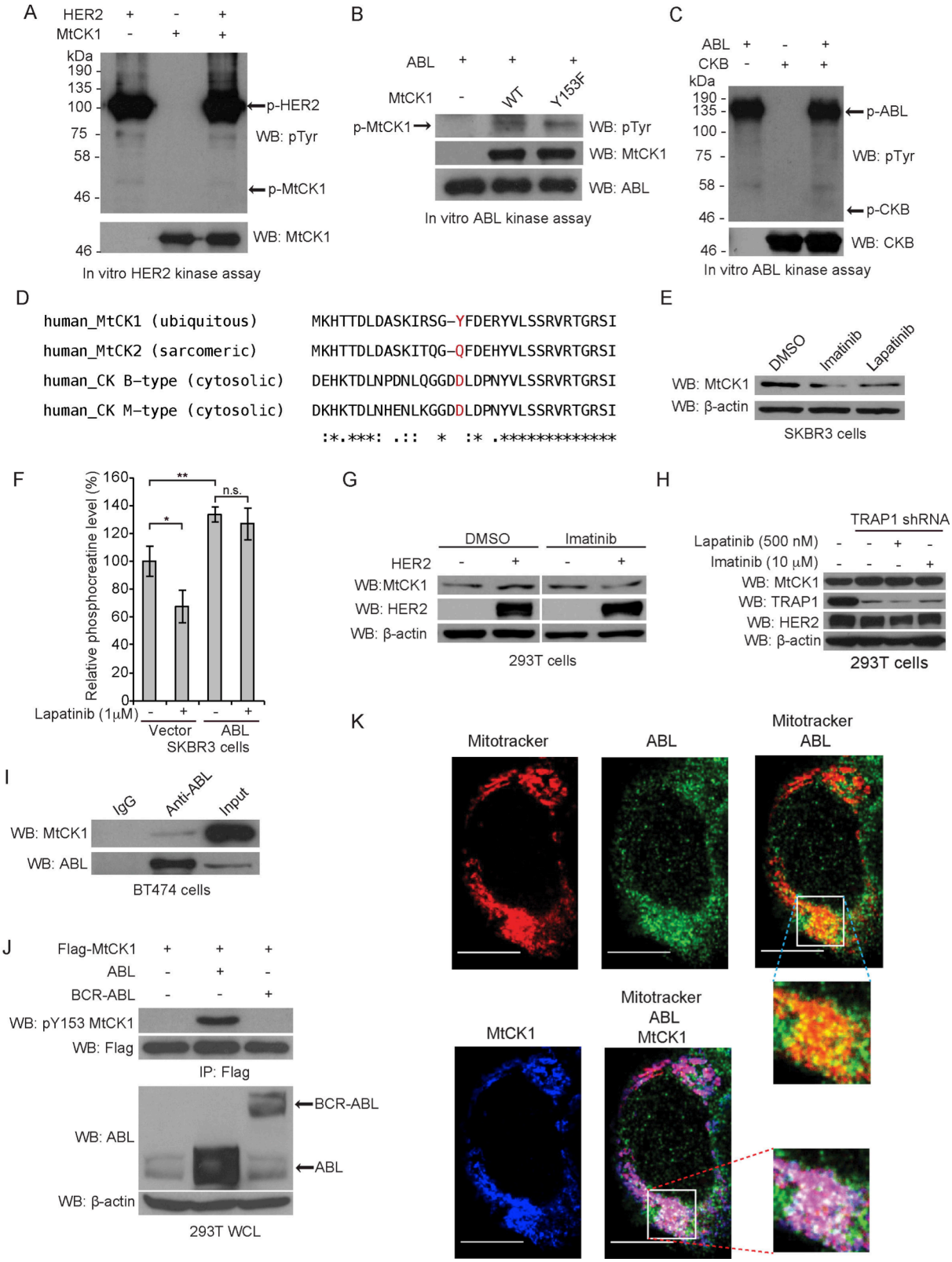

A
HER2 + - +
MtCK1 - + +
kDa
190
135
100
75
58
46
p-HER2
WB: pTyr
p-MtCK1
46
WB: MtCK1
In vitro HER2 kinase assay
B
ABL + + +
MtCK1 - WT Y153F
p-MtCK1
WB: pTyr
WB: MtCK1
WB: ABL
In vitro ABL kinase assay
C
ABL + - +
CKB - + +
kDa
190
135
100
75
58
46
p-ABL
WB: pTyr
p-CKB
46
WB: CKB
In vitro ABL kinase assay
D
human_MtCK1 (ubiquitous) MKHTTDLDASKIRSG-YFDERYVLSSRVRTGRSI
human_MtCK2 (sarcomeric) MKHTTDLDASKITQG-QFDEHYVLSSRVRTGRSI
human_CK B-type (cytosolic) DEHKTDLNPDNLQGGDDLDPNYVLSSRVRTGRSI
human_CK M-type (cytosolic) DKHKTDLNHENLKGGDDLDPNYVLSSRVRTGRSI
:*.***: .:: * :* .**********
E
DMSO
Imatinib
Lapatinib
WB: MtCK1
WB: β-actin
SKBR3 cells
F
Relative phosphocreatine level (%)
160
140
120
100
80
60
40
20
0
**
*
n.s.
Lapatinib (1μM) - + - +
Vector
ABL
SKBR3 cells
G
DMSO
Imatinib
HER2 - + - +
WB:MtCK1
WB: HER2
WB: β-actin
293T cells
H
TRAP1 shRNA
Lapatinib (500 nM) - - + -
Imatinib (10 μM) - - - +
WB: MtCK1
WB: TRAP1
WB: HER2
WB: β-actin
293T cells
I
IgG
Anti-ABL
Input
WB: MtCK1
WB: ABL
BT474 cells
J
Flag-MtCK1 + + +
ABL - + -
BCR-ABL - - +
WB: pY153 MtCK1
WB: Flag
IP: Flag
BCR-ABL
WB: ABL
ABL
WB: β-actin
293T WCL
K
Mitotracker
ABL
Mitotracker
ABL
MtCK1
Mitotracker
ABL
MtCK1

**Figure S3. HER signaling increases MtCK1 protein through Abl as an intermediate kinase, Related to Figure 4**

(A) Immunoblots of an in vitro tyrosine kinase assay using recombinant HER2 with purified, recombinant MtCK1 WT. (B) Immunoblots of an in vitro tyrosine kinase assay using recombinant ABL with purified, recombinant MtCK1 WT or Y153F protein. (C) Immunoblots of an in vitro tyrosine kinase assay using recombinant ABL with purified, recombinant CKB. (D) Protein sequence alignment of creatine kinase isoenzymes using MUltiple Sequence Comparison by Log-Expectation (MUSCLE) algorithm tool (EMBL-EBI). (E) Immunoblots of lysates from SKBR3 cells treated with DMSO, 20 μM imatinib or 500 nM lapatinib for 6 hour. (F) HPLC analysis of phosphocreatine levels in $HER2^+$ SKBR3 cells expressing either vector ctrl or ABL with or without treatment with lapatinib 1μM for 6 h. (G) Immunoblots of lysates from 293T cells expressing vector control (ctrl) and HER2 WT with or without 500 nM imatinib. (H) Immunoblots of lysates from 293T cells expressing HER2 with and without TRAP1 knockdown treated with either imatinib or lapatinib for 6 h. (I) Immunblots of anti-IgG control or anti-ABL IP samples and the corresponding input lysates from BT474 cells. (J) Immunoblots of anti-Flag IP samples and corresponding WCL from 293T cells co-expressing Flag-MtCK1 WT along with vector ctrl, ABL or BCR-ABL. (K) Confocal immunofluorescence images of BT474 cells stained with mitotracker (red), anti-ABL antibody (green) and anti-MtCK1 antibody (blue). *Top left*: Mitotracker image, *top middle*: ABL image, *top right*: merged mitotracker and ABL image, *bottom left*: MtCK1 image, *bottom middle*: merged mitotracker, ABL, and MtCK1 image. The zoomed merged images in the same region of interest are also shown in the bottom right panels. A white bar represents 15.2 μm. All results represent experiments of three independent replicates and P values were determined by a two-tailed Student's t test (*$P<0.05$, **$P<0.01$).

***Figure S4.***

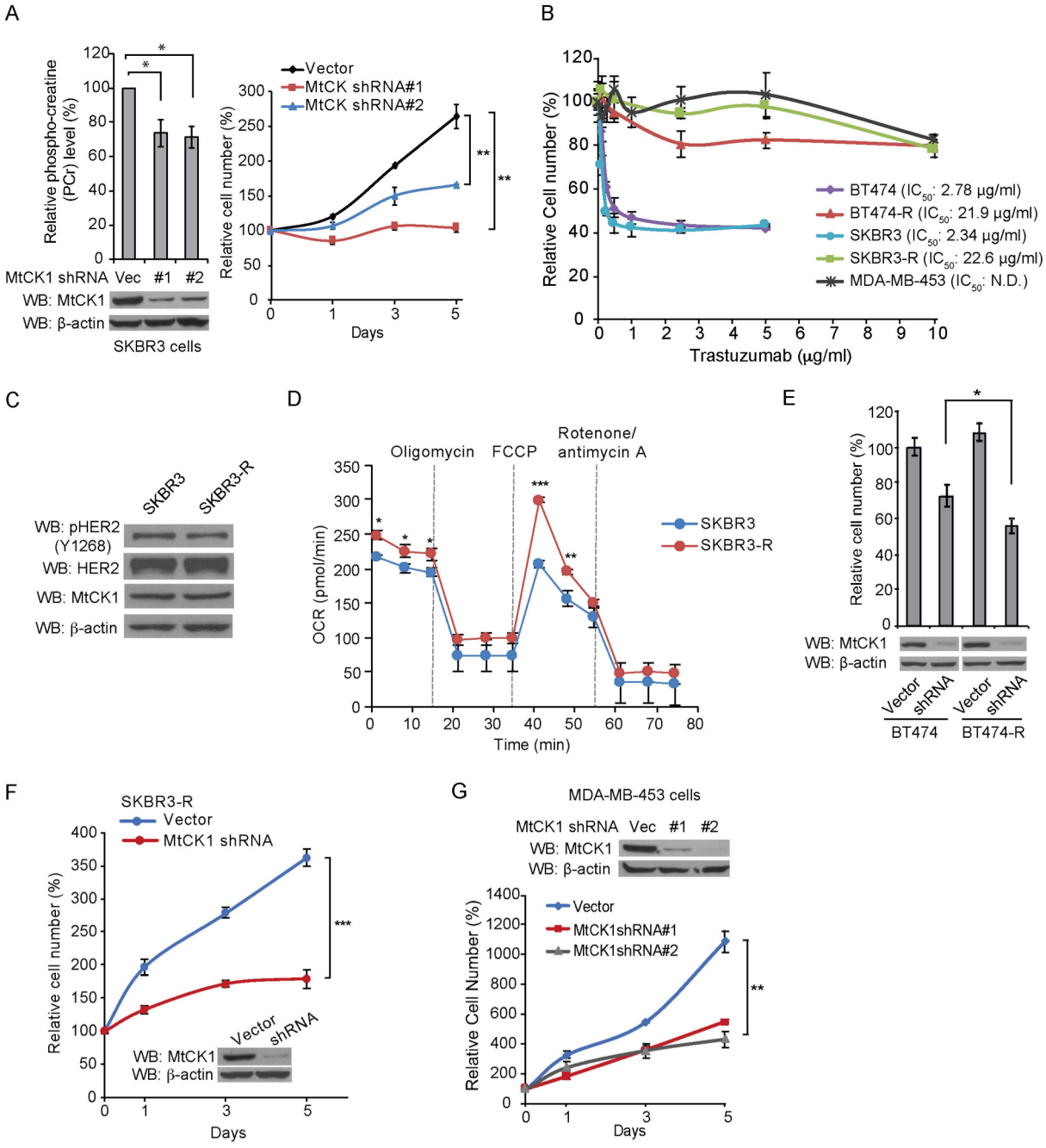

A
Relative phospho-creatine (PCr) level (%)
MtCK1 shRNA Vec #1 #2
WB: MtCK1
WB: β-actin
SKBR3 cells
Vector
MtCK shRNA#1
MtCK shRNA#2
Relative cell number (%)
Days
B
Relative Cell number (%)
Trastuzumab (μg/ml)
BT474 (IC50: 2.78 μg/ml)
BT474-R (IC50: 21.9 μg/ml)
SKBR3 (IC50: 2.34 μg/ml)
SKBR3-R (IC50: 22.6 μg/ml)
MDA-MB-453 (IC50: N.D.)
C
SKBR3
SKBR3-R
WB: pHER2 (Y1268)
WB: HER2
WB: MtCK1
WB: β-actin
D
Oligomycin
FCCP
Rotenone/ antimycin A
OCR (pmol/min)
Time (min)
SKBR3
SKBR3-R
E
Relative cell number (%)
WB: MtCK1
WB: β-actin
Vector
shRNA
BT474
BT474-R
F
SKBR3-R
Vector
MtCK1 shRNA
Relative cell number (%)
Days
WB: MtCK1
WB: β-actin
G
MDA-MB-453 cells
MtCK1 shRNA Vec #1 #2
WB: MtCK1
WB: β-actin
Vector
MtCK1shRNA#1
MtCK1shRNA#2
Relative Cell Number (%)
Days

**Figure S4. Expression of MtCK1 is increased in BT474-R cells compared to parental BT474 cells, Related to Figure 5**

(A) *Left:* HPLC analysis of PCr levels in SKBR3 cells with or without MtCK1 knockdown. Immunoblots of lysates from the corresponding cells are also shown below. *Right:* Proliferation assay results of SKBR3 cells with or without MtCK1 knockdown. (B) Proliferation assay results of breast cancer cell lines treated with indicated concentrations of trastuzumab. (C) Immunoblots of lysates from SKBR3 and SKBR3-R cells. (D) Mitochondrial oxygen consumption rate (OCR) of SKBR3 and SKBR3-R cells under basal conditions and in response to oligomycin (1μM), FCCP (1μM) and rotenone/antimycin A (1μM). (E) Proliferation results of trastuzumab-resistant BT474-R cells compared to parental BT474 cells with or without shRNA knockdown of MtCK1. Immunoblots of lysates from the corresponding cells are also shown. All the parental, vector control (ctrl) and MtCK1 shRNA expressing BT474R cells were cultured in the presence of 10 μg/ml trastuzumab during the assays. (F) Proliferation assay results of SKBR3 cells with or without MtCK1 knockdown. Immunoblots of the corresponding cell lysate are also shown. (G) Proliferation of $HER2^+$ MDA-MB-453 breast cancer cells with or without shRNA knockdown of MtCK1. Immunoblots of the corresponding cell lysate is also shown. All results represent experiments of three independent replicates with Error bars ± standard deviation of 3 independent measurements. P values were determined by a two-tailed Student's t test. *P<0.05, **P<0.01, ***P<0.001.

***Figure S5***

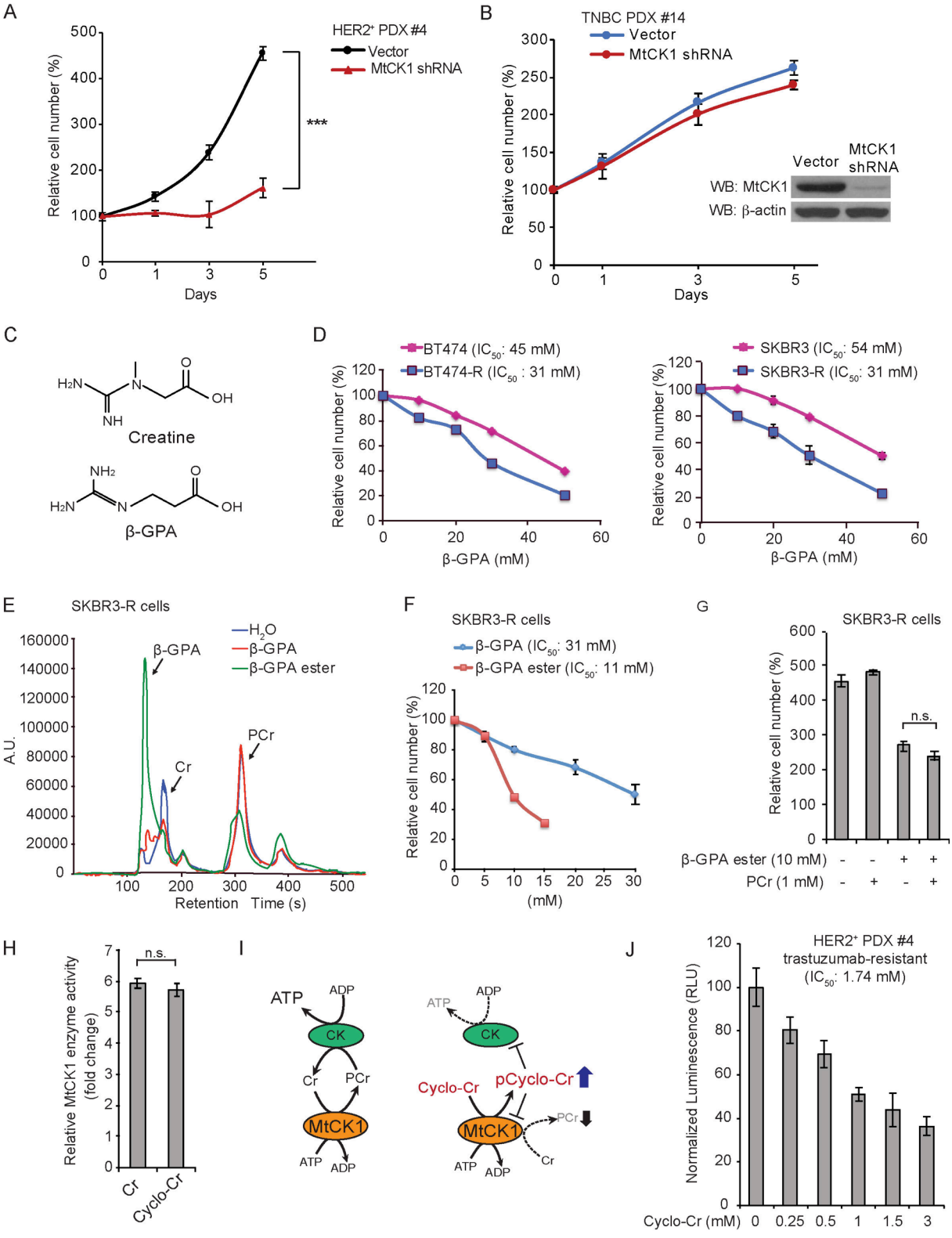

A
HER2+ PDX #4
Vector
MtCK1 shRNA
Relative cell number (%)
Days
***
B
TNBC PDX #14
Vector
MtCK1 shRNA
Relative cell number (%)
Days
Vector
MtCK1 shRNA
WB: MtCK1
WB: β-actin
C
Creatine
β-GPA
D
BT474 (IC50: 45 mM)
BT474-R (IC50 : 31 mM)
SKBR3 (IC50: 54 mM)
SKBR3-R (IC50: 31 mM)
Relative cell number (%)
β-GPA (mM)
E
SKBR3-R cells
H2O
β-GPA
β-GPA ester
A.U.
Cr
PCr
Retention Time (s)
F
SKBR3-R cells
β-GPA (IC50: 31 mM)
β-GPA ester (IC50: 11 mM)
Relative cell number (%)
(mM)
G
SKBR3-R cells
Relative cell number (%)
n.s.
β-GPA ester (10 mM)
PCr (1 mM)
H
Relative MtCK1 enzyme activity (fold change)
n.s.
Cr
Cyclo-Cr
I
ATP
ADP
CK
Cr
PCr
MtCK1
Cyclo-Cr
pCyclo-Cr
J
HER2+ PDX #4
trastuzumab-resistant
(IC50: 1.74 mM)
Normalized Luminescence (RLU)
Cyclo-Cr (mM)

**Figure S5. Among the creatine analogues, cyclocreatine specifically inhibits phosphocreatine energy shuttle in cancer cells, Related to Figure 6**

(A) Cell proliferation of $HER2^+$ PDX #4 with or without MtCK1 knockdown. Immunoblots of the corresponding cell lysates are shown in Figure 5H. (B) Cell proliferation of TNBC PDX #14 with or without shRNA knockdown of MtCK1. Immunoblots of the corresponding cell lysates are shown in Figure 5H. (C) Structures of creatine and its analog beta-guanidinopropionic acid (β-GPA). (D) Proliferation results of parental BT474 cells and SKBR3 compared to their trastuzumab-resistant cell lines in the presence of β -GPA. (E) HPLC analysis of β-GPA, creatine and phosphocreatine in BT474-R cells treated with water, 5 mM β-GPA, or 5 mM of the ethyl-ester derivative of β-GPA overnight. Chromatograms show a representative of three independent experiments which showed similar results. (F) Proliferation assay results of SKBR3-R cells treated with β-GPA and the ethyl-ester form of β-GPA (β-GPA ester). (G) Proliferation assay results of SKBR3-R treated with β-GPA ester and/or phosphocreatine. (H) In vitro MtCK1 enzyme activity of recombinant MtCK1 protein with creatine and cyclocreatine. The enzymatic activity was determined by the rate at which creatine or cyclocreatine were converted to their phosphorylated forms by MtCK1. (I) Illustration of the inhibition model of MtCK1 by cyclocreatine. Cr; creatine, PCr; phosphocreatine, cyclo-Cr; cyclocreatine, p-cyclo-Cr; phospho-cyclocreatine. (J) Effect of CCr on viability of trastuzumab-resistant HER2+ PDX organoids. All results represent experiments of three independent replicates with Error bars ± standard deviation of 3 independent measurements. P values were determined by a two-tailed Student's t test. ***$P<0.001$.

***Figure S6***

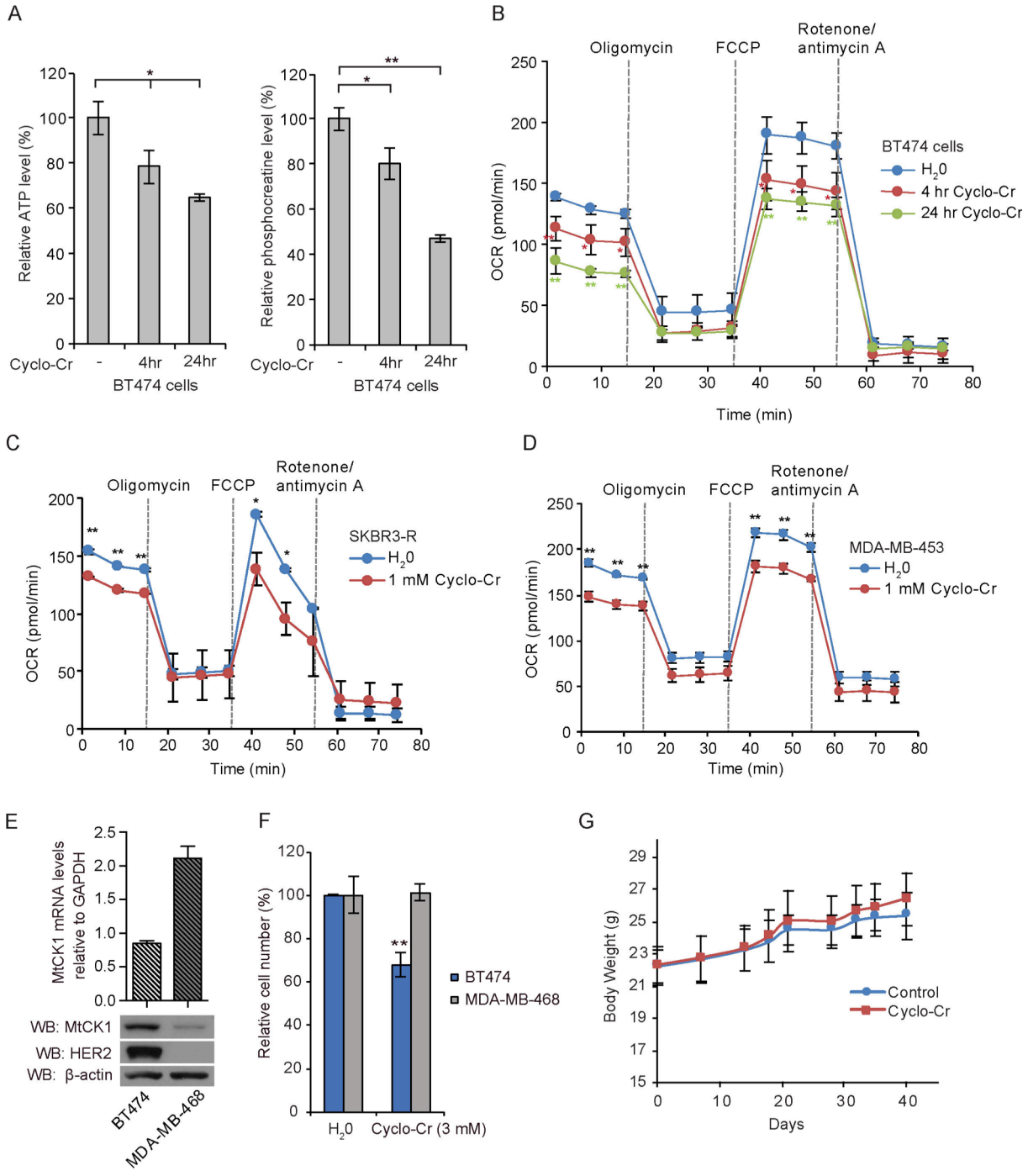
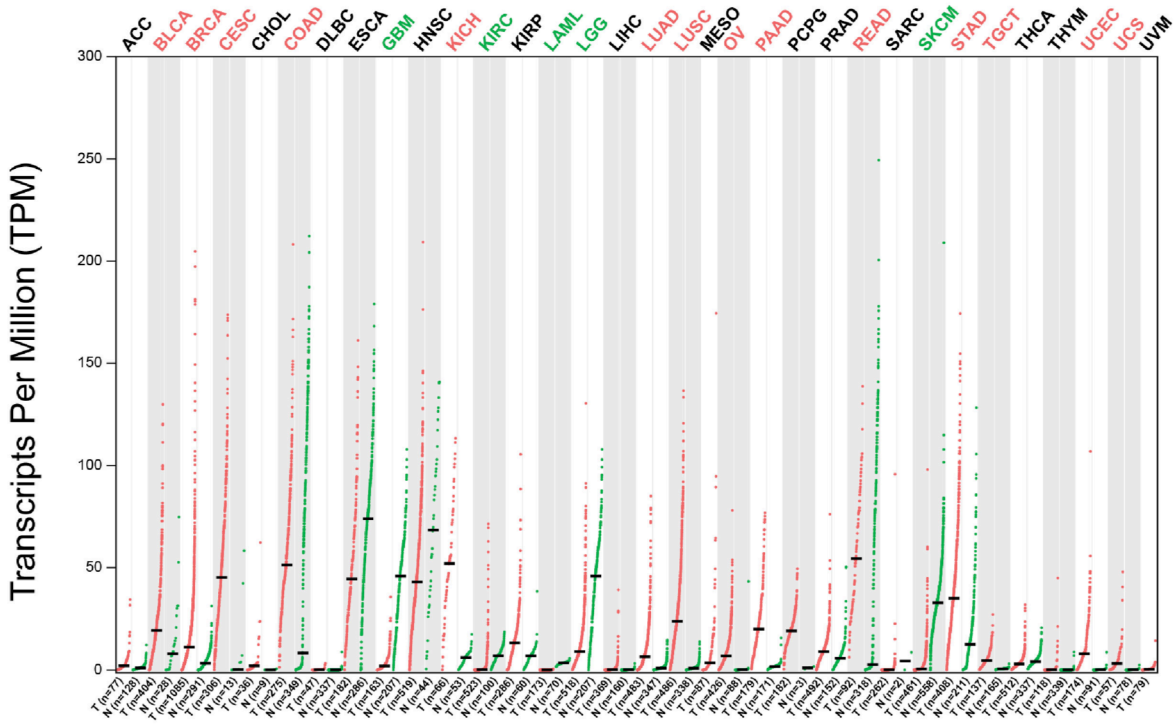

A
Relative ATP level (%)
Cyclo-Cr
-
4hr
24hr
BT474 cells
Relative phosphocreatine level (%)
B
Oligomycin
FCCP
Rotenone/
antimycin A
OCR (pmol/min)
Time (min)
BT474 cells
H20
4 hr Cyclo-Cr
24 hr Cyclo-Cr
C
SKBR3-R
1 mM Cyclo-Cr
D
MDA-MB-453
E
MtCK1 mRNA levels
relative to GAPDH
WB: MtCK1
WB: HER2
WB: β-actin
BT474
MDA-MB-468
F
Relative cell number (%)
Cyclo-Cr (3 mM)
G
Body Weight (g)
Days
Control
Cyclo-Cr

**Figure S6. Cyclocreatine specifically inhibits phosphocreatine energy shuttle and mitochondrial function in trastuzumab –sensitive and –resistant HER2$^+$ breast cancer cells, Related to Figure 6**

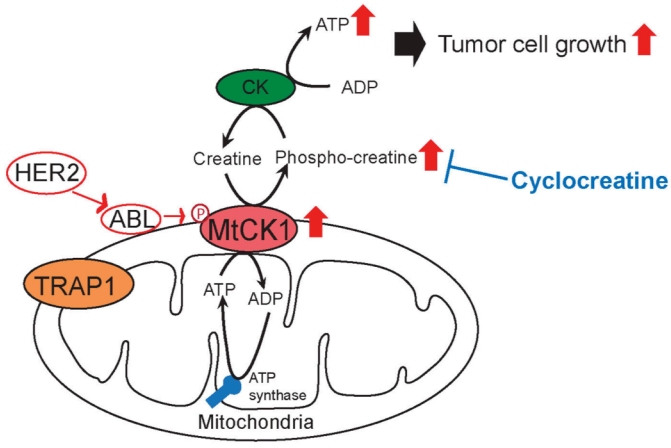
(A) Steady state ATP levels (*left*) and PCr levels (*right*) in BT474 cells treated with Cyclo-Cr for 4 h and 24 h. Bar graphs were generated from the whole cell metabolomics analysis data shown in Supplemental Table S1. (B) Mitochondrial OCR of BT474 cells treated with Cyclo-Cr (5 mM) for 4 h and 24 h under basal conditions and in response to oligomycin (1μM), FCCP (1μM) and rotenone/antimycin A (1μM). (C & D) Mitochondrial OCR of SKBR3-R (C) and MDA-MB-453 (D) treated with 1mM Cyclo-Cr under basal conditions and in response to oligomycin (1μM), FCCP (1μM) and rotenone/antimycin A (1μM). (E) *Top:* qPCR results of MtCK1 mRNA in BT474 and MDA-MB-468 cells. *Bottom:* Anti-MtCK1, anti-HER2, and anti-β-actin immunoblots of lysates from BT474 and MDA-MB-468 cells. (F) Cell proliferation assay results of BT474 and MDA-MB-468 cells with or without cyclocreatine (CCr) treatment. (G) Body weights were compared between mice given drinking water with 0.3% cyclocreatine and control mice with regular drinking water for 40 days. All results represent experiments of three independent replicates with Error bars ± standard deviation of 3 independent measurements. P values were determined by a two-tailed Student's t test. *P<0.05, **P<0.01.

***Figure S7***

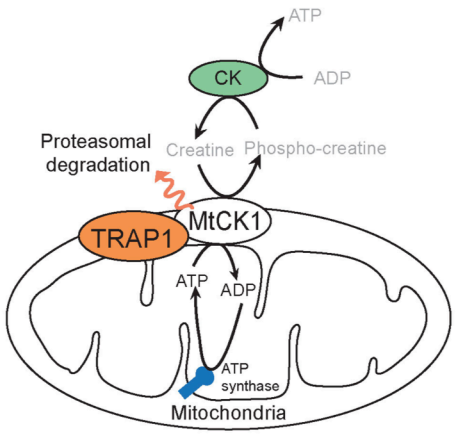
A




B




HER2 signaling "ON"




HER2 signaling "OFF"

**Figure S7. MtCK1 as a metabolic target in cancer, Related to Figure 7**

(A) *CKMT1A* expression profile from 33 different types of cancers and corresponding normal control tissues. The expression status of *CKMT1A* in the TCGA and GTEx datasets from total 33 different types of cancers along with corresponding normal tissues were examined by a web-based tool GEPIA (Gene Expression Profiling Interactive Analysis: http://gepia.cancer-pku.cn/). Each dot represents one sample (dot in red: tumor, dot in green: normal tissue). Abbreviations: ACC, Adrenocortical carcinoma; BLCA, Bladder Urothelial Carcinoma; BRCA, Breast invasive carcinoma; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, Cholangio carcinoma; COAD, Colon adenocarcinoma; DLBC, Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA, Esophageal carcinoma; GBM, Glioblastoma multiforme; HNSC, Head and Neck squamous cell carcinoma; KICH, Kidney Chromophobe; KIRC, Kidney renal clear cell carcinoma; KIRP, Kidney renal papillary cell carcinoma; LAML, Acute Myeloid Leukemia; LGG, Brain Lower Grade Glioma; LIHC, Liver hepatocellular carcinoma; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; MESO, Mesothelioma; OV, Ovarian serous cystadenocarcinoma; PAAD, Pancreatic adenocarcinoma; PCPG, Pheochromocytoma and Paraganglioma; PRAD, Prostate adenocarcinoma; READ, Rectum adenocarcinoma; SARC, Sarcoma; SKCM, Skin Cutaneous Melanoma; STAD, Stomach adenocarcinoma; TGCT, Testicular Germ Cell Tumors; THCA, Thyroid carcinoma; THYM, Thymoma; UCEC, Uterine Corpus Endometrial Carcinoma; UCS, Uterine Carcinosarcoma; UVM, Uveal Melanoma. (B) Proposed model: MtCK1 as a metabolic target in $HER2^{+}$ breast cancer.

## SUPPLEMENTAL TABLES

**Table S1. Quantification of 116 metabolites from BT474 cells, Related to Figure 1**

**Table S2. Heat map of BT474 cell metabolites, Related to Figure 1**

**Table S3. 1 to 29 of the total 29 entries identified as proteasome subunit, Related to Figure 3**

| Uniport ID | Gene Symbol | Y153F/ WT | WT | Y153F | Gene Name |
|---|---|---|---|---|---|
| P25789 | PSMA4 | 19.42 | 1.2 | 23.3 | proteasome (prosome, macropain) subunit, alpha type, 4 |
| P28070 | PSMB4 | 10.58 | 2.4 | 25.4 | proteasome (prosome, macropain) subunit, beta type, 4 |
| O00232 | PSMD12 | 7.80 | 1 | 7.8 | proteasome (prosome, macropain) 26S subunit, non-ATPase, 12 |
| P35998 | PSMC2 | 7.10 | 1 | 7.1 | proteasome (prosome, macropain) 26S subunit, ATPase, 2 |
| O14818 | PSMA7 | 5.45 | 2 | 10.9 | proteasome (prosome, macropain) subunit, alpha type, 7 |
| P28074 | PSMB5 | 4.33 | 1.2 | 5.2 | proteasome (prosome, macropain) subunit, beta type, 5 |
| P25788 | PSMA3 | 4.13 | 3 | 12.4 | proteasome (prosome, macropain) subunit, alpha type, 3 |
| P49720 | PSMB3 | 3.47 | 1.5 | 5.2 | proteasome (prosome, macropain) subunit, beta type, 3 |
| Q13200 | PSMD2 | 3.00 | 1 | 3 | proteasome (prosome, macropain) 26S |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | subunit, non-ATPase, 2 |
| Q8TAA3 | PSMA8 | 2.90 | 2.1 | 6.1 | proteasome (prosome, macropain) subunit, alpha type, 8 |
| P20618 | PSMB1 | 2.69 | 1.3 | 3.5 | proteasome (prosome, macropain) subunit, beta type, 1 |
| P17980 | PSMC3 | 2.60 | 1 | 2.6 | proteasome (prosome, macropain) 26S subunit, ATPase, 3 |
| P25787 | PSMA2 | 2.44 | 1.6 | 3.9 | proteasome (prosome, macropain) subunit, alpha type, 2 |
| P62191 | PSMC1 | 1.96 | 5.5 | 10.8 | proteasome (prosome, macropain) 26S subunit, ATPase, 1 |
| P43686 | PSMC4 | 1.90 | 1 | 1.9 | proteasome (prosome, macropain) 26S subunit, ATPase, 4 |
| P60900 | PSMA6 | 1.40 | 25.6 | 35.9 | proteasome (prosome, macropain) subunit, alpha type, 6 |
| O43242 | PSMD3 | 1.04 | 22.7 | 23.6 | proteasome (prosome, macropain) 26S subunit, non-ATPase, 3 |
| O00487 | PSMD14 | 1.03 | 3.1 | 3.2 | proteasome (prosome, macropain) 26S subunit, non-ATPase, 14 |
| P28072 | PSMB6 | 0.97 | 3 | 2.9 | proteasome (prosome, macropain) subunit, beta type, 6 |
| P28066 | PSMA5 | 0.56 | 3.6 | 2 | proteasome (prosome, macropain) subunit, alpha type, 5 |
| P25786 | PSMA1 | ∞ | 0 | 10.3 | proteasome (prosome, macropain) subunit, alpha type, 1 |
| P62333 | PSMC6 | ∞ | 0 | 2.8 | proteasome (prosome, macropain) 26S subunit, ATPase, 6 |
| Q9UNM6 | PSMD13 | ∞ | 0 | 1 | proteasome (prosome, macropain) 26S subunit, non-ATPase, 13 |
| Q99460 | PSMD1 | ∞ | 0 | 1 | proteasome (prosome, macropain) 26S subunit, non-ATPase, 1 |
| P49721 | PSMB2 | ∞ | 0 | 1 | proteasome (prosome, macropain) subunit, beta type, 2 |
| Q16401 | PSMD5 | ∞ | 0 | 1 | proteasome (prosome, macropain) 26S subunit, non-ATPase, 5 |
| O00231 | PSMD11 | ∞ | 0 | 1 | proteasome (prosome, macropain) 26S subunit, non-ATPase, 11 |
| P55036 | PSMD4 | 0 | 1 | 0 | proteasome (prosome, macropain) 26S subunit, non-ATPase, 4 |
| Q99436 | PSMB7 | 0 | 1 | 0 | proteasome (prosome, macropain) subunit, beta type, 7 |

**Table S4 Complete blood profiles of nude mice treated with either control or 0.3% cyclocreatine for 40 days, Related to Figure 7**

| | Units | Reference Range | Control ($H_2O$) | Treatment (CCr) |
|---|---|---|---|---|
| WBC | $10^9$/L | 2.6-10.1 | 2.08 ± 0.60 | 4.62 ± 1.26 |
| LYM | $10^9$/L | 1.3-8.4 | 1.19 ± 0.72 | 3.36 ± 1.04 |
| MON | $10^9$/L | 0-0.3 | 0.14 ± 0.04 | 0.22 ± 0.09 |
| NEU | $10^9$/L | 0.5-3.8 | 0.76 ± 0.18 | 1.04 ± 0.31 |
| LYM% | % | 0-99.9 | 51.20 ± 27.08 | 72.43 ± 6.12 |
| MON% | % | 0-99.9 | 6.83 ± 1.17 | 4.83 ± 1.27 |
| NEU% | % | 0-99.9 | 42.00 ± 26.82 | 22.73 ± 4.91 |
| | | | | |
| RBC | $10^{12}$/L | 6.5-10.1 | 7.70 ± 0.87 | 7.36 ± 1.05 |
| HGB | g/dL | 10-16.1 | 13.45 ± 1.64 | 13.10 ± 2.42 |
| HCT | % | 32.8-48 | 43.29 ± 6.40 | 42.09 ± 6.87 |
| MCV | fl | 42.3-55.9 | 56.25 ± 4.92 | 57.00 ± 2.65 |
| MCH | pg | 13.7-18.1 | 17.48 ± 0.26 | 17.70 ± 0.78 |
| MCHC | g/dL | 29.5-35.1 | 31.20 ± 2.17 | 31.10 ± 1.91 |
| RDWc | % | 0-99.9 | 15.95 ± 0.45 | 15.83 ± 0.87 |
| RDWs | fl | 0-99.9 | 35.05 ± 1.8 | 35.93 ± 2.71 |
| | | | | |
| PLT | $10^9$/L | 200-1540 | 52.25 ± 30.21 | 96.00 ± 40.73 |
| MPV | fl | 0-99.9 | 8.15 ± 1.24 | 8.37 ± 1.36 |
| PCT | % | 0-99.9 | 0.05 ± 0.02 | 0.08 ± 0.03 |
| PDWc | % | 0-99.9 | 35.70 ± 4.17 | 36.27 ± 3.67 |
| PDWs | fl | 0-99.9 | 11.03 ± 3.6 | 11.67 ± 3.38 |

**Table S5 (related to Figure 7). Complete blood profiles of NOD scid mice treated with either control or 0.3% cyclocreatine for 21 days, Related to Figure 7**

| | Units | Reference Range | Control ($H_2O$) | Treatment (CCr) |
|---|---|---|---|---|
| WBC | $10^9$/L | 2.6-10.1 | 2.12 ± 1.99 | 6.81 ± 5.69 |
| LYM | $10^9$/L | 1.3-8.4 | 0.82 ± 0.99 | 0.75 ± 0.73 |
| MON | $10^9$/L | 0-0.3 | 0.05 ± 0.05 | 0.50 ± 0.38 |
| NEU | $10^9$/L | 0.5-3.8 | 1.25 ± 1.02 | 5.56 ± 4.60 |
| LYM% | % | 0-99.9 | 31.73 ± 12.37 | 8.40 ± 4.95 |
| MON% | % | 0-99.9 | 3.97 ± 5.57 | 7.83 ± 1.16 |
| NEU% | % | 0-99.9 | 64.30 ± 13.22 | 83.77 ± 4.19 |
| | | | | |
| RBC | $10^{12}$/L | 6.5-10.1 | 6.39 ± 3.33 | 9.66 ± 1.14 |
| HGB | g/dL | 10-16.1 | 10.17 ± 4.09 | 13.30 ± 1.65 |
| HCT | % | 32.8-48 | 29.95 ± 15.70 | 44.46 ± 4.92 |
| MCV | fl | 42.3-55.9 | 46.67 ± 0.58 | 46.00 ± 1 |
| MCH | pg | 13.7-18.1 | 17.23 ± 3.79 | 13.80 ± 0.26 |
| MCHC | g/dL | 29.5-35.1 | 36.83 ± 8.11 | 29.93 ± 1.01 |
| RDWc | % | 0-99.9 | 17.33 ± 0.91 | 17.53 ± 0.57 |
| RDWs | fl | 0-99.9 | 32.30 ± 1.61 | 32.00 ± 0.80 |
| | | | | |
| PLT | $10^9$/L | 200-1540 | 61.00 ± 47.76 | 658.33 ± 485.99 |
| MPV | fl | 0-99.9 | 7.83 ± 0.49 | 6.73 ± 0.15 |
| PCT | % | 0-99.9 | 0.05 ± 0.04 | 0.44 ± 0.32 |
| PDWc | % | 0-99.9 | 36.17 ± 1.70 | 31.83 ± 2.31 |
| PDWs | fl | 0-99.9 | 10.93 ± 1.50 | 9.87 ± 4.79 |