Supplemental figures and figure legends

Supplementary Fig. S1

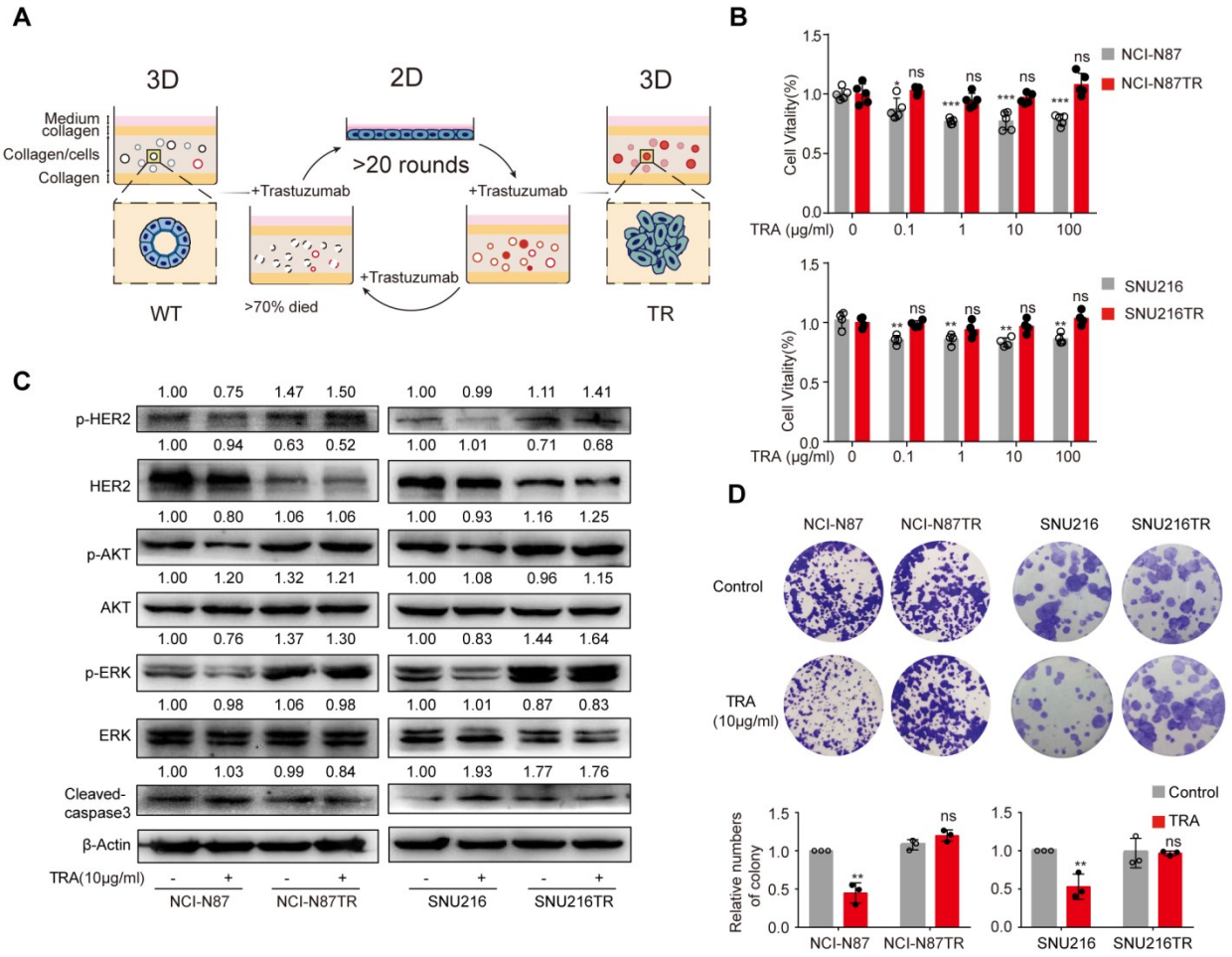

Fig. S1. Establishment and characterization of trastuzumab-resistant cell lines. (**A**) Schematic of experimental approach to establish trastuzumab-resistant (TR) cells in 3D culture system. In the presence of trastuzumab (10 μg/ml) in 3D type-1 collagen culture media, greater than 70% of colonies died. Residual colonies were isolated and iteratively passaged in 2D and 3D culture in the continued stimulation of trastuzumab over approximately 20 rounds. (**B**) MTT was used to evaluate growth inhibition of WT and TR cells treated with trastuzumab at the gradient concentrations for 4 d (n=4). (**C**) WB analysis of WT and TR cells exposed to trastuzumab (TRA, 10 μg/ml) for 48 h. β-Actin served as the loading control. (**D**) Colony-formation assay and the quantitative graph of WT and TR cells exposed to trastuzumab (TRA, 10 μg/ml) for 2 weeks. Representative images (upper) and the quantification (lower) of numbers of

colonies is are shown (n=3). Student's t-test was performed in (B) and (D). Data are presented as mean ± SD. *** $P<0.001$, ** $P<0.01$, * $P<0.05$, ns $P>0.05$.

Supplementary Fig. S2

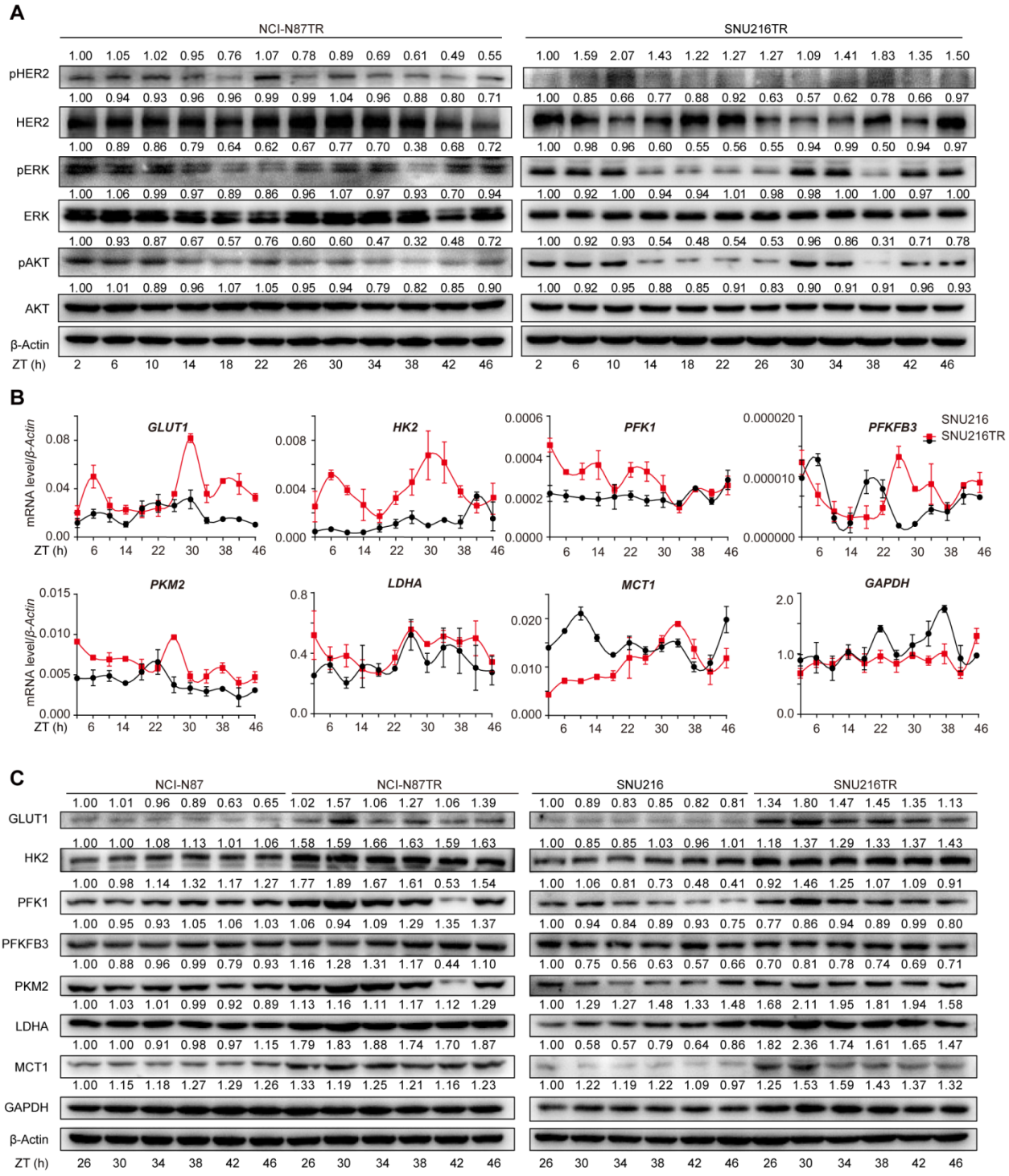

Fig. S2. The circadian rhythms of ERBB2 pathway proteins and glycolysis-related genes in trastuzumab-resistant HER2-positive gastric cancer. (**A**) WB analysis of time-series (every 4h) expression on pHER2, HER2, AKT, pAKT, ERK, pERK in synchronized TR cells. (**B**)

Quantitative PCR of glycolysis-related genes in synchronized SNU216 and SNU216TR cells at indicative ZTs (n=3). (**C**) WB analysis of glycolysis-related genes in WT and TR cells at the indicated ZT26 to ZT46. β-Actin serves as the loading control.

Supplementary Fig. S3

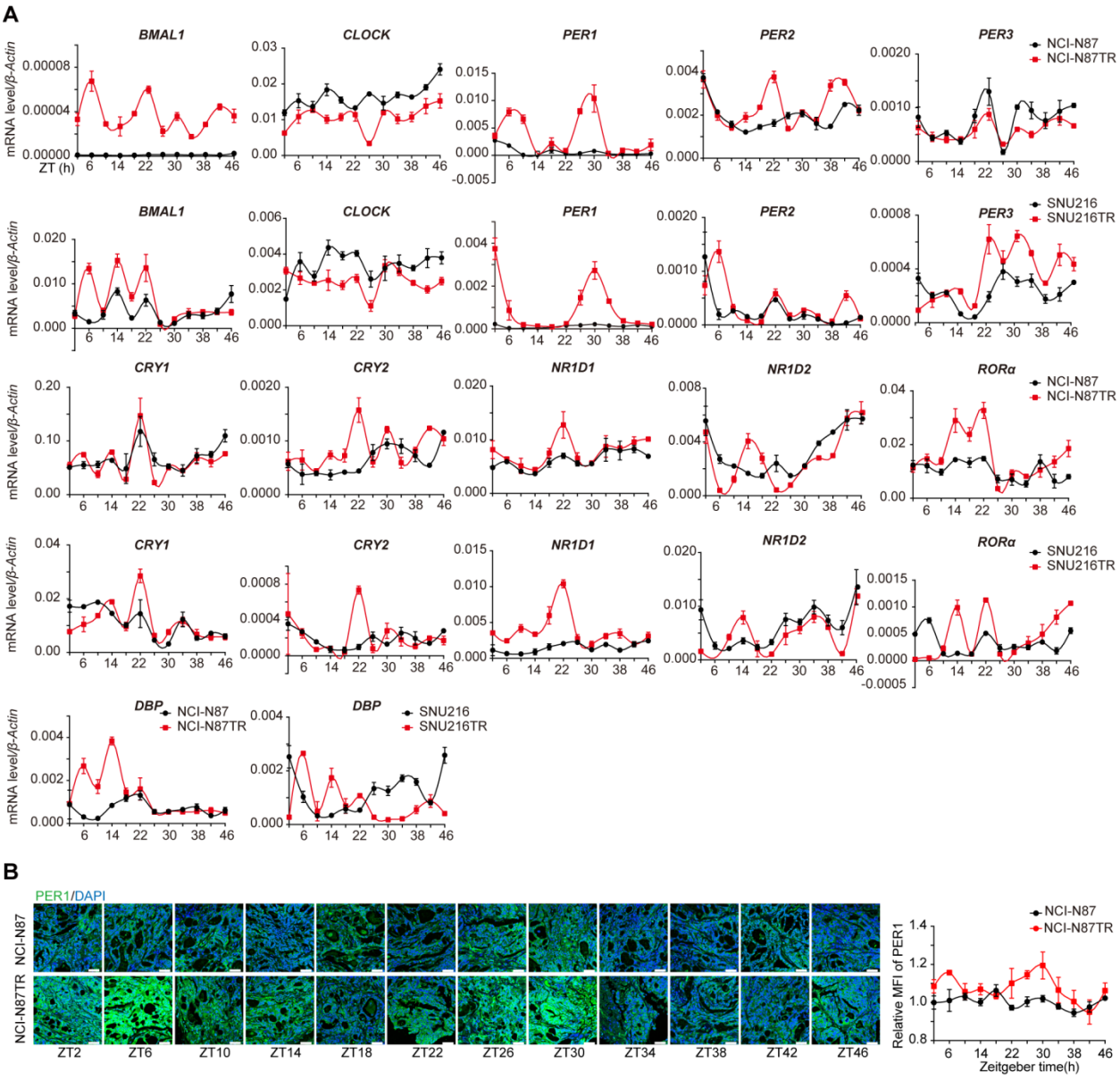

Fig. S3. Screening of core clock genes in trastuzumab-resistant cells. (**A**) Quantitative PCR of core clock genes in WT and TR cells synchronized with dexamethasone (n=3). (**B**) Immunofluorescence analysis for PER1 expression in WT and TR tumor tissues. Representative IF images (left) and the quantification (right) of mean fluorescence intensity (MFI) were shown (n=4). Scale bar: 50 μm.

Supplementary Fig. S4

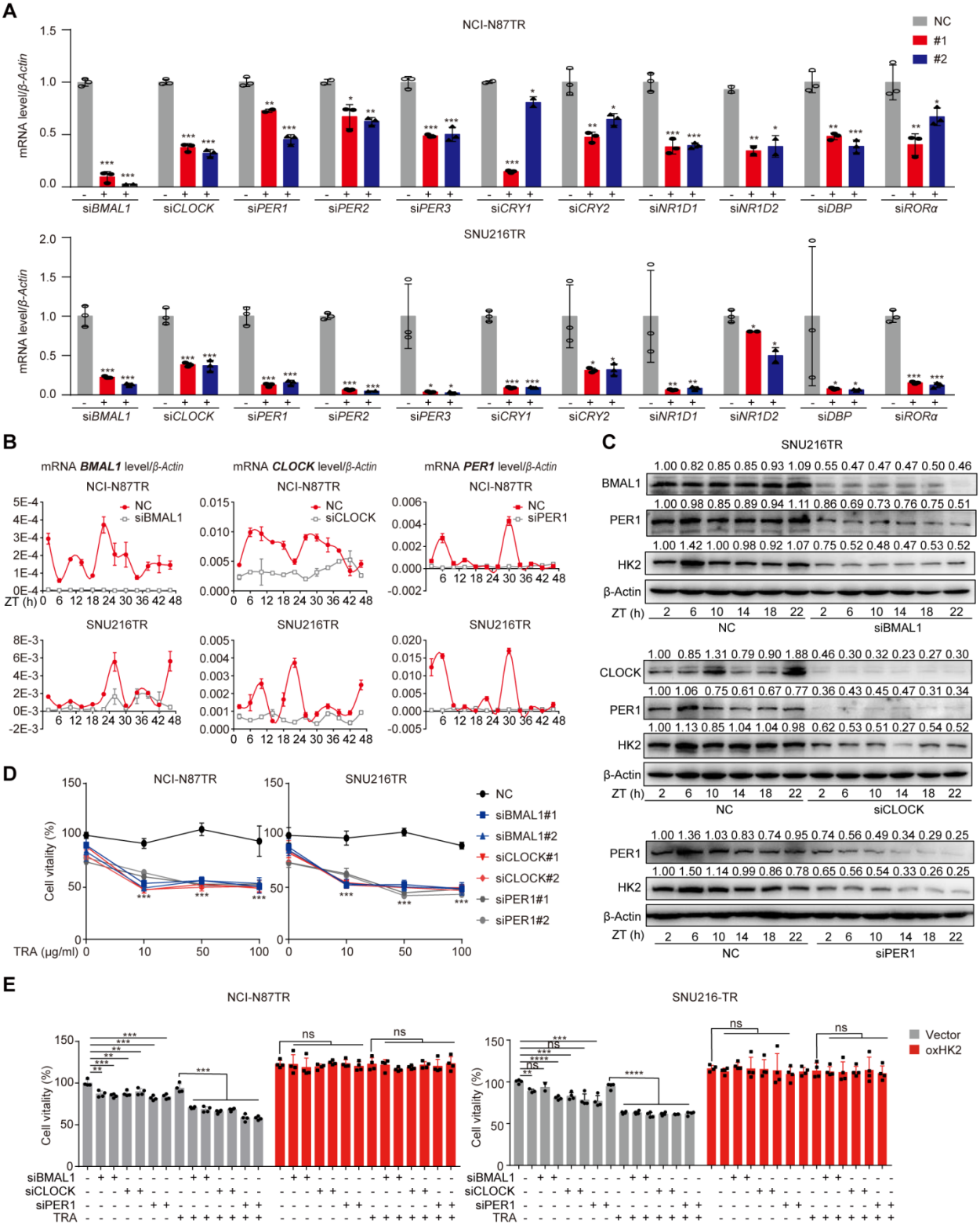

Fig. S4. BMAL1-CLOCK-PER1 axis triggered trastuzumab resistance via regulating of HK2 circadian oscillation in TR cells. (**A**) Quantitative PCR of mRNA levels for core clock

genes in TR cells transfected with or without targeting siRNA (n=3). (**B**) Quantitative PCR of mRNA levels of *BMAL1*, *CLOCK* and *PER1* in synchronized TR cells transfected with target siRNAs (n=3). (**C**) WB analysis of PER1 and HK2 proteins in synchronized SNU216TR cells infected with siRNA of *BMAL1*, *CLOCK* and *PER1*. (**D**) Growth inhibition of TR cells treated with trastuzumab at the gradient concentrations after knockdown with siRNA of *BMAL1*, *CLOCK* and *PER1* (n=4). (**E**) MTT was used to assess cell viability of TR cells treated with trastuzumab (TRA, 10 μg/ml) after transfection with siRNA of *BMAL1*, *CLOCK* and *PER1* with or without overexpressing *HK2*. Student's t-test was performed in (A), (D) and (E). Data are presented as mean ± SD. *** $P<0.001$, ** $P<0.01$, * $P<0.05$, ns $P>0.05$.

Supplementary Fig. S5

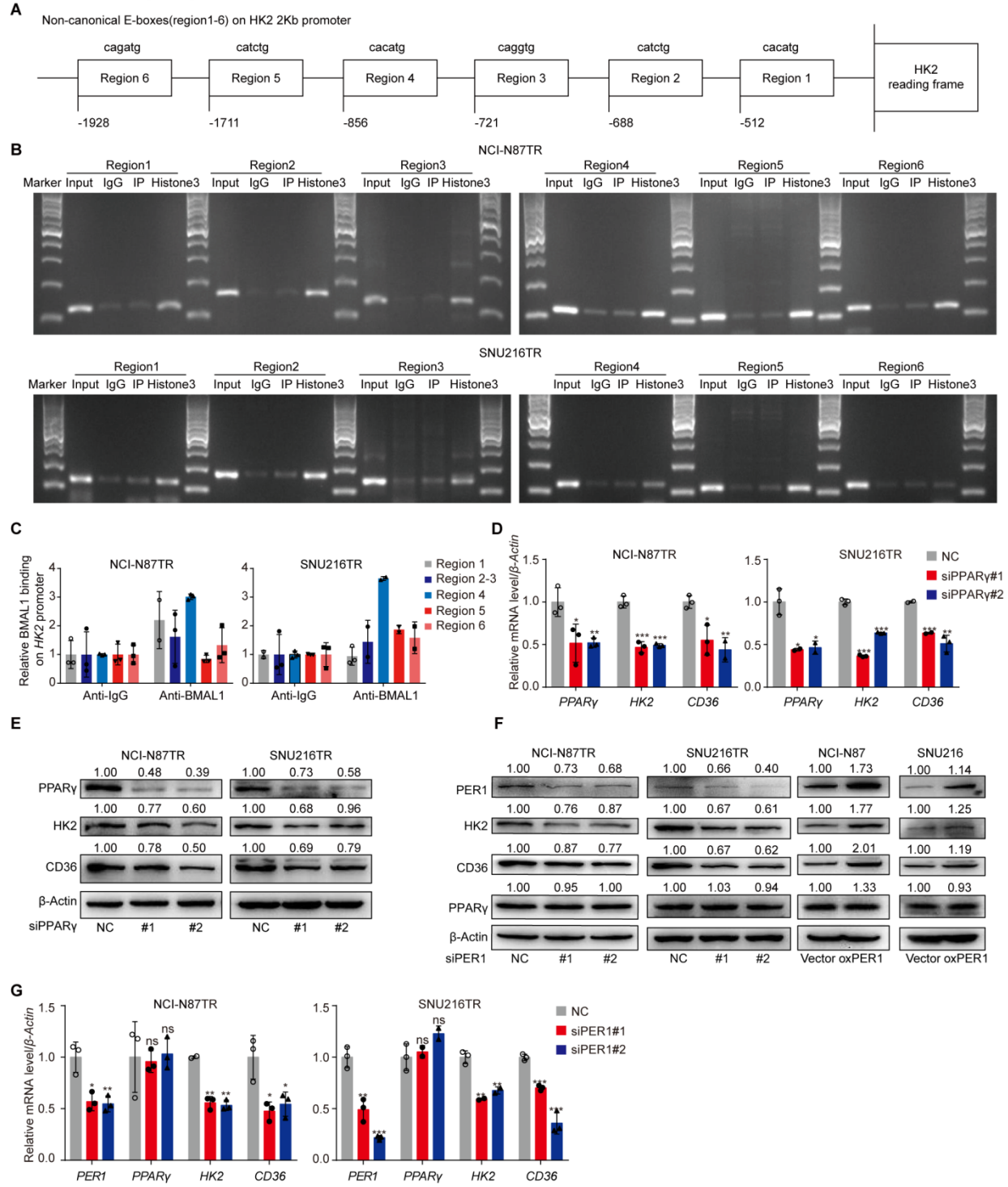

Fig. S5. PER1 interacts with PPARγ to enhance transcript regulation of HK2. (**A**) Schematic representation of non-canonical E-boxes (region1-6) on the HK2 promoter domains. (**B-C**) Gel electrophoresis (**B**) and quantitative PCR (**C**) of immunoprecipitated DNA with specific primers for the E-box locus region of HK2 by ChIP assay in TR cells. Input: DNA

fragments without any specificity antibody in cells; IP: DNA fragments precipitated by BMAL1 antibody; IgG: DNA fragments precipitated by IgG antibody served as negative control; Histone3: DNA fragments precipitated by Histone3 antibody served as positive control. (**D-E**) Quantitative PCR (**D**) and WB analysis (**E**) of PPARγ, HK2 and CD36 in TR cells infected with siRNA of PPARγ. (**F-G**) WB analysis (**F**) and quantitative PCR (**G**) in TR cells infected with siRNA or overexpression of *PER1*. Statistics were calculated with 3 samples in (C), (D) and (G). *P* value was determined by Student's t-test. Data are graphed as the mean ± SD, *** $P<0.001$, ** $P<0.01$, * $P<0.05$, ns $P>0.05$.