Figure S1. Gene set variant analysis results of The Cancer Genome Atlas cohort in the hallmark gene sets. (A) Volcano plot and (B) heatmap of differentially expressed cancer hallmark gene sets between bladder cancer and adjacent non-tumor patient tissues. Adl.P.Val, adjusted P-value; FC, fold change; con, control (adjacent non-tumor samples); trea, treated (tumor samples).



Figure S2. ROC curves of patients grouped by TNM stage and association between the prognostic value of selected genes and demographic characteristics in The Cancer Genome Atlas cohort. (A) ROC curves for The Cancer Genome Atlas patients classified according to TNM stage. (B) Boxplots illustrating the relationship of ferroptosis-related genes with sex. Data were analyzed by ANOVA. No post-hoc test was used. Kaplan-Meier curves for (C) CRYAB, (D) GCLM, (E) TFRC, (F) G6PD, (G) SQLE and (H) ZEB1. ns, not significant; \*P<0.05. G6PD, glucose-6-phosphate dehydrogenase; GCL, glutamate-cysteine ligase; CRYAB, crystalline  $\alpha$ -B; TFRC, transferrin receptor; ZEB1, zinc finger E-box binding homeobox 1; SQLE, squalene epoxidase.



Figure S3. Associations between gene expression levels and immunotherapy response and the relationship of ferroptosis-related genes with age. (A) Associations between gene expression levels and immunotherapy response. Data were analyzed by ANOVA. No post-hoc test was used. (B) The relationship of ferroptosis-related genes with age. ns, not significant; \*P<0.05; \*\*P<0.01, multiple comparison vs. control groups. ROC, receiver operating characteristic; AUC, area under the ROC curve; G6PD, glucose-6-phosphate dehydrogenase; GCL, glutamate-cysteine ligase; CRYAB, crystalline  $\alpha$ -B; TFRC, transferrin receptor; ZEB1, zinc finger E-box binding homeobox 1; SQLE, squalene epoxidase; CR, complete response; PD, progressive disease; PR, partial remission; SD, stable disease.



Figure S4. Association between G6PD expression and clinical factors in The Cancer Genome Atlas cohort. Boxplots indicating the relationship between G6PD expression and (A) age, (B) sex, (C) grade, (D) T stage, (E) N stage and (F) M stage. G6PD, glucose-6-phosphate dehydrogenase.



Figure S5. Association between glucose-6-phosphate dehydrogenase expression and immune response genes in The Cancer Genome Atlas cohort. \*P<0.05; \*\*P<0.01. CTLA4, cytotoxic T-lymphocyte associated protein 4; PD-1, programmed cell death 1; PD-L2, programmed cell death 1 ligand 2.

