Supplementary Figures

Table of Contents

Supplementary Table Legends

Supplementary Table 1: Complete list of reagents, antibodies, and equipment including manufacturer, cat#, and RRID

Supplementary Table 2: qPCR primers used in this study

Supplementary Table 3: RNAseq normalized counts of genes in Hallmark EMT gene set for T1 cells treated with siNC, siERRα, or siNC+imatinib (n=3/group)

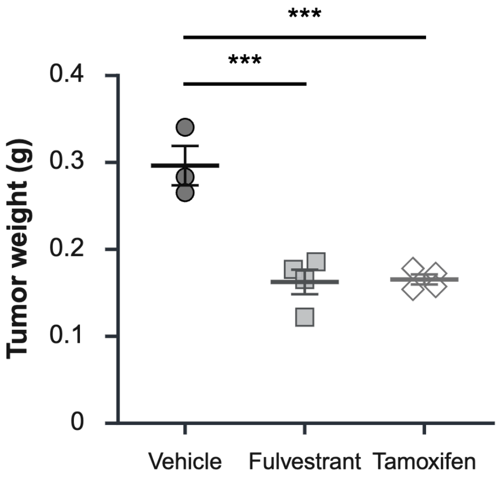

Figure S1. **ERα inhibition by tamoxifen and fulvestrant in *Kit*$^{\Delta V558/+}$ mice**. *Kit*$^{\Delta V558/+}$ mice were treated with vehicle (n=3), fulvestrant (n=4), or tamoxifen (n=4) for 3 weeks. Medians are indicated. Statistical significance assessed with One-way ANOVA with Dunnett multiple comparisons test. ***p<0.001.

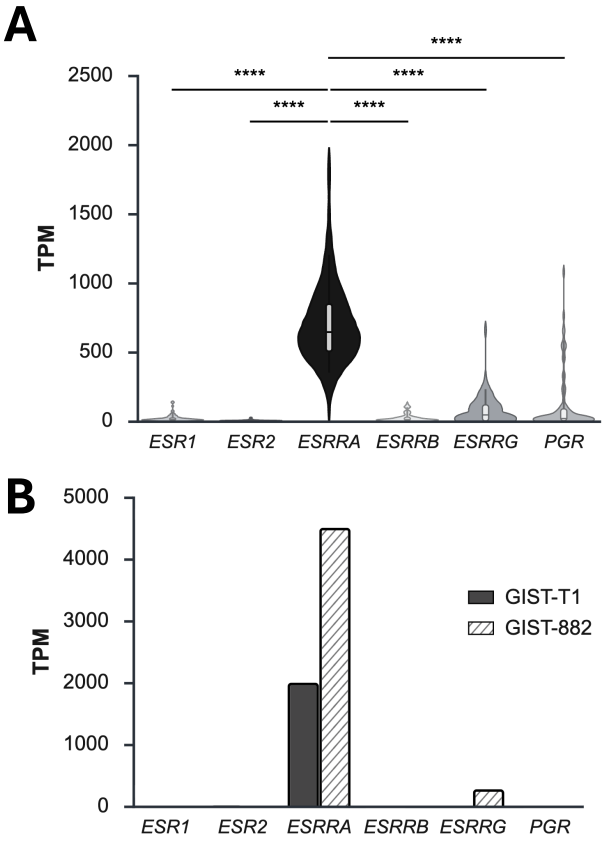

Figure S2. Nuclear receptor expression in GIST cell lines and tumors. mRNA expression of estrogen receptors-α and β (*ESR1, ESR2*), estrogen-related receptors-α,-β,and -γ (*ESRRA, ESRRB, ESRRG*), progesterone receptor (*PGR*) using **A)** our previously published RNA-seq expression data of 75 primary and metastatic human GIST tumor specimens (27) and **B)** GIST-T1 and GIST-882 mRNA expression profiles from a publicly available dataset (GSE143547). Data presented are in transcripts per million (TPM) and scaled by count. Statistical significance assessed Kruskal-Wallis test with Dunn's multiple comparisons test. ****p<0.0001.

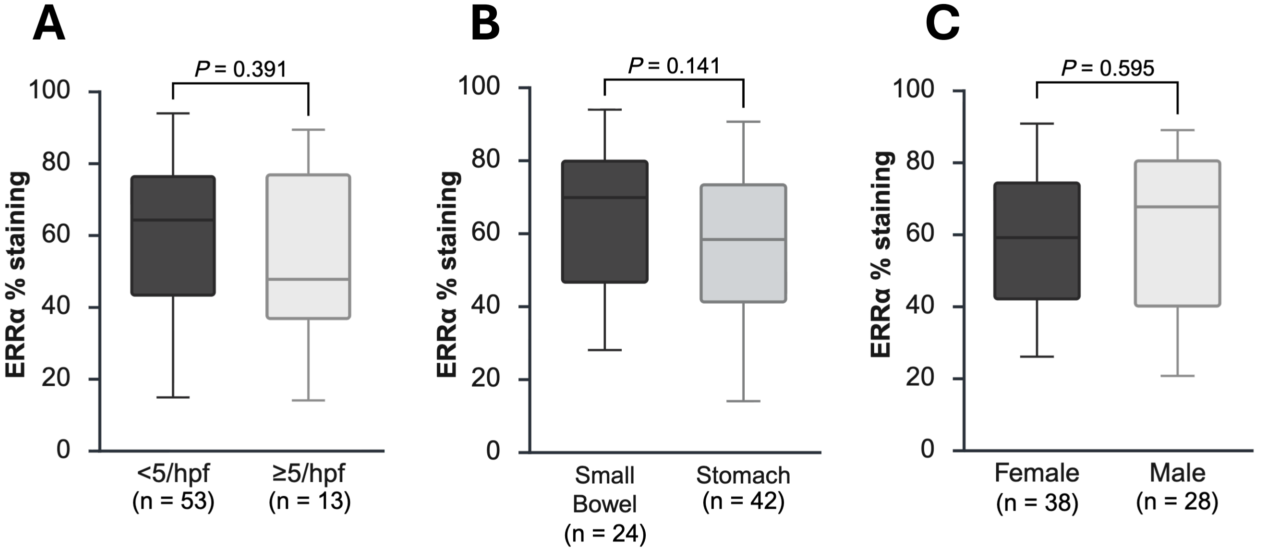

Figure S3. **ERRα expression in human GIST by tissue microarray (TMA).** Immunohistochemical ERRα percent staining from a human GIST TMA grouped by **A)** tumor mitotic rate **B)** location and **C)** gender. Data represent mean ± SEM. Statistical significance assessed with Student's t-test.

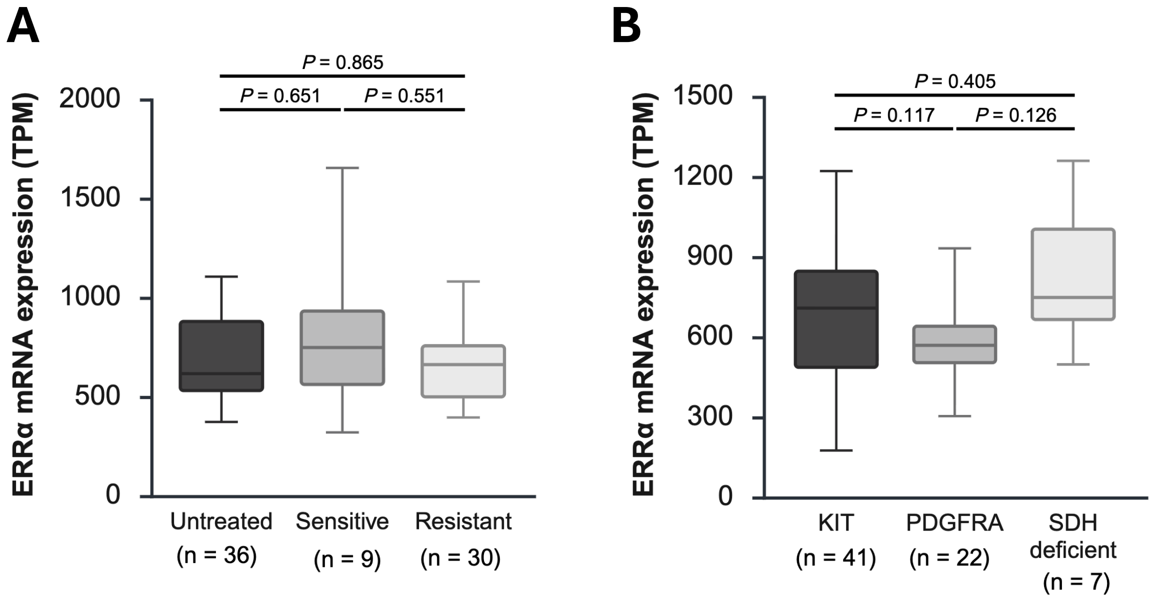

Figure S4. **ERRα expression by GIST tumor bulk RNA-seq.** ERRα expression of 75 human GIST tumors from a previously published RNA-seq dataset (27) comparing **A)** treatment status and **B)** mutation status. Untreated means never treated with imatinib, sensitive means the tumor had responded to imatinib at the time of surgery, and resistant means that the tumor was progressing at the time of surgery. Data represent mean ± SEM. Statistical significance assessed with Kruskal-Wallis test with Dunn's multiple comparisons test (A) and Welch's one-way ANOVA with Games-Howell multiple comparisons test (B).

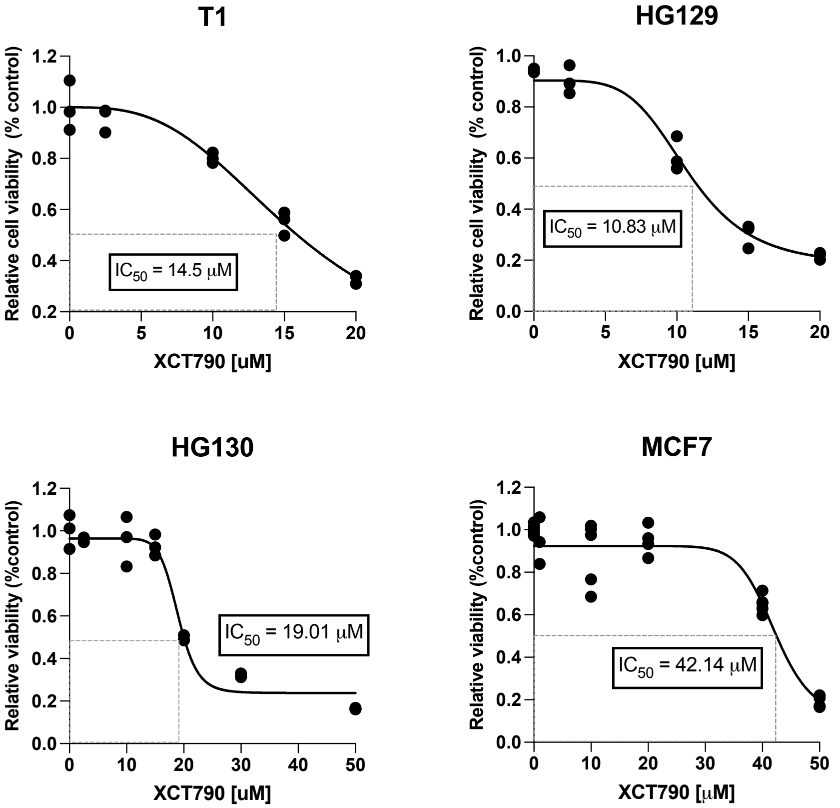

Figure S5. **Dose response and IC_{50} of GIST cell lines treated with XCT790.** Relative cell viability in human GIST cell lines (T1, HG129, HG130) and MCF7 breast cancer cell line treated with XCT790. IC_{50} was estimated using non-linear regression models to fit the curves.

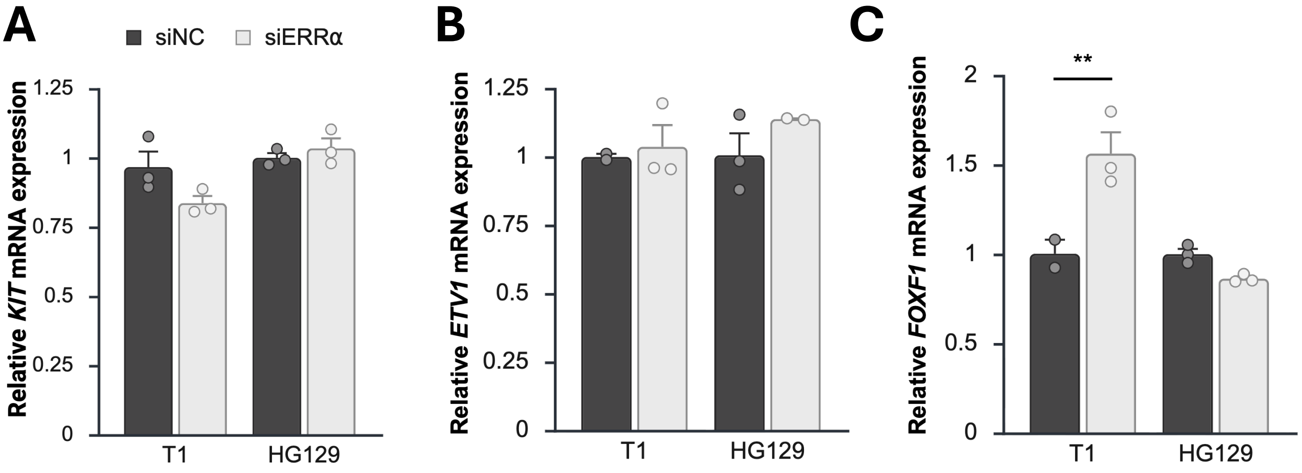

Figure S6. *KIT, ETV1,* and *FOXF1* expression after ERRα knockdown. Relative mRNA expression after ERRα knockdown (siERRα) compared to control (siNC) (n=3/group) in T1 and HG129 cells at 48h. Data represent mean ± SEM. Statistical significance assessed with two-way ANOVA with Bonferroni multiple comparisons test. **p<0.01.

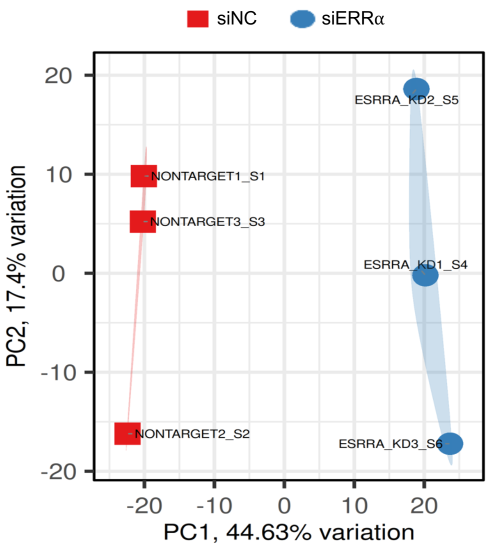

Figure S7. PCA analysis of RNA-seq samples after ERRα knockdown vs. non-target control. PCA plot of T1 cells treated with siRNA-mediated knockdown of ERRα (siERRα) or non-target control (siNC) (n=3 replicates/group).

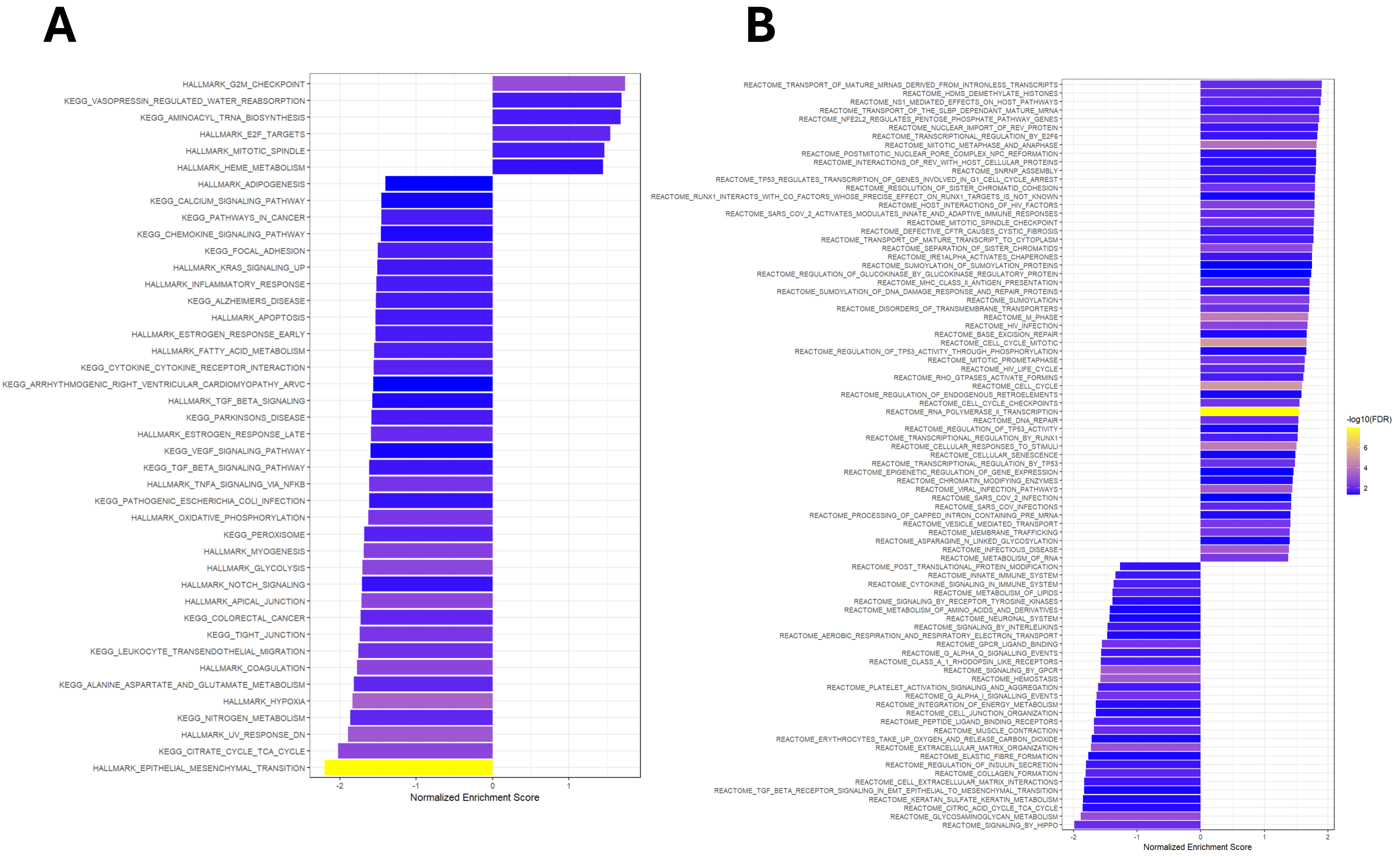

Figure S8. Complete GSEA analysis of RNA-seq data ERRα knockdown. The complete list of significantly enriched **A)** KEGG/HALLMARK and **B)** REACTOME pathways from GSEA analysis with an FDR<0.5.

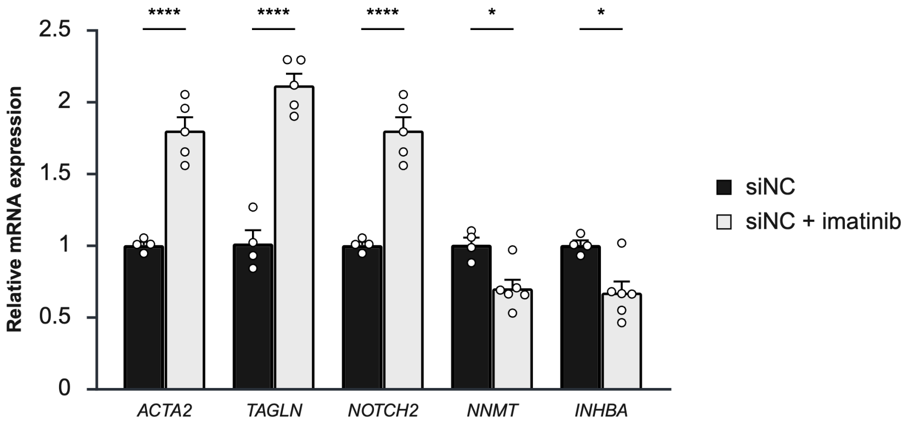

Figure S9. qPCR validation of RNA-seq data with EMT genes after imatinib treatment vs. non-target control. Quantitative PCR was performed in triplicate to assess mRNA expression of various EMT DEGs in T1 cells treated with non-target siRNA (n=5) ± imatinib (n=4) at 48h. Mean ± SEM. *p<0.05, **p<0.01, ****p<0.0001.