# ETV1 is a lineage survival factor that cooperates with KIT in gastrointestinal stromal tumours

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## ABSTRACT:

**Introduction:** ETV1 is an ETS family transcription factors involved in recurrent genomic alterations (translocation and amplification) in prostate cancer, Ewing sarcoma, and melanoma. Here, we uncover an oncogenic role of ETV1 in gastrointestinal stromal tumours (GIST) pathogenesis.

**Results:** We show that ETV1 is universally highly expressed in GISTs and is required for growth of imatinib-sensitive and resistant GIST cell lines. Unlike other ETS dependent tumors where aberrant expression is mediated by genomic alterations, ETV1 is physiologically highly expressed in the subtypes of interstitial cells of Cajal (ICCs) that are the precursors of GISTs, and is required for their development. In addition, transcriptome profiling and global analyses of ETV1-binding sites suggest that ETV1 is a master regulator of an ICC-GIST-specific transcription network mainly through enhancer binding. The ETV1 transcriptional program is further regulated by activated KIT, which prolongs ETV1 protein stability and cooperates with ETV1 to promote tumorigenesis.

**Conclusion:** ETV1 is a lineage-specific transcription factor of the ICC/GIST lineage and cooperates with activated KIT in oncogenic transformation of GIST.

## INTRODUCTION:

GIST is the most common human sarcoma and is primarily defined by activating mutations in the *KIT* or *PDGFRA* receptor tyrosine kinases. *KIT* is highly expressed in the interstitial cells of Cajal (ICCs)—the presumed cell of origin for GIST—as well as in hematopoietic stem cells, melanocytes, mast cells and germ cells. Yet, families harbouring germline activating *KIT* mutations and mice with knock-in *Kit* mutations almost exclusively develop ICC hyperplasia and GIST, suggesting that the cellular context is important for *KIT* to mediated oncogenesis.

## RESULTS:

### *ETV1 is universally highly expressed and required for tumour growth and survival in GIST*

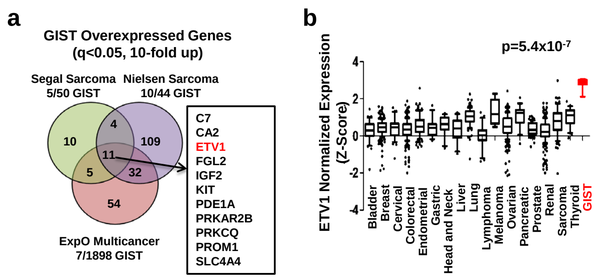


**Figure 1. a**, Venn diagram of GIST-signature genes from three publically available datasets. **b**, Expression of *ETV1* in multiple tumour types from the ExpO dataset. Box, 25-75 percentile; error bar, 10-90 percentile; dots, outliers.

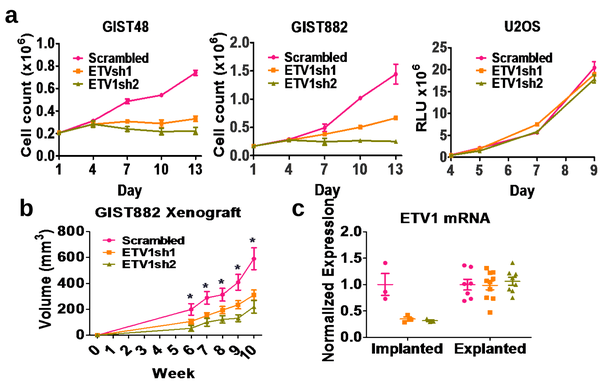


**Figure 2. ETV1 is required for growth and survival in GIST. a,** Growth curves of imatinib sensitive GIST882, imatinib resistant GIST48 and U2OS osteosarcoma cells after shRNA-mediated *ETV1* suppression compared to control. Mean±SEM, n=3. **b,** Tumour volume over time in SCID mice implanted with GIST882 cells after shRNA-mediated *ETV1* suppression compared to scrambled shRNA controls. Mean±SEM, * *p<0.05*; n=7, 10, 8 for scrambled, ETV1sh1, and ETV1sh2 respectively. **c**, *ETV1* mRNA levels of preimplanted GIST882 cells and explanted xenografts at week 10. Mean±SD.

### *ETV1 is required for the development of the ICC lineage*

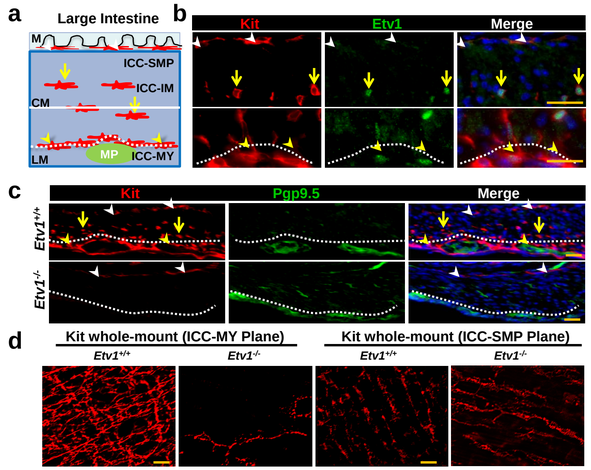


**Figure 3. a**, In the large intestine, there are three ICC subtypes that all express Kit. Schematic showing localization of ICC-MY (yellow arrowheads), ICC-IM (yellow arrows) and ICC-SMP (white arrowheads) in the large intestine. M: mucosa, CM: circular muscle, LM: longitudinal muscle. Only ICC-MY and ICC-IM, but not ICC-SMP are susceptible to mutant-Kit mediated transformation in GIST mouse models. **b**, Co-immunofluorescence (divided into two microscopy fields) of Kit (red), Etv1 (green) and DAPI (blue) shows that ICC-MY and ICC-IM, but not ICC-SMP express Etv1. **c**, Co-immunofluorescence of Kit (red), Pgp9.5 (green), and DAPI (blue) of the large intestine of *Etv1*$^{+/+}$ and *Etv1*$^{-/-}$ mice showing selective loss of ICC-MY and ICC-IM in *Etv1*$^{-/-}$ mice. **d,** Representative deconvoluted whole-mount Kit-immunofluorescence images of the large intestine of *Etv1*$^{+/+}$ and *Etv1*$^{-/-}$ mice. A single microscopy field focused to the ICC-MY and ICC-SMP planes are shown. The entire stack is not shown. Scale bar, 20 μm.

### *ETV1 is a master regulator of the ICC-GIST lineage*

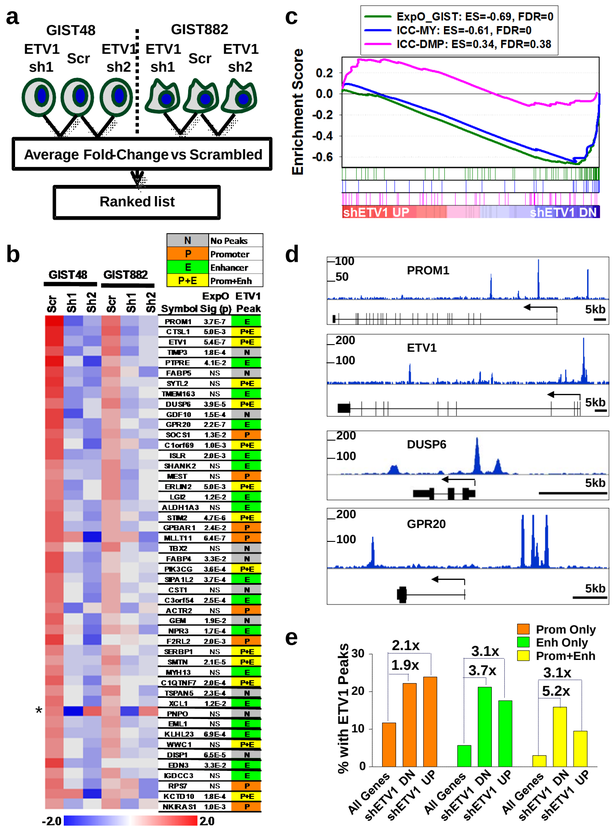


**Figure 4. a**, Ranked list of ETV1 regulated genes was generated based on the average fold-change by the two *ETV1* hairpins in two GIST cell lines. **b,** Table of top 48 genes downregulated by ETV1 knockdown. *indicates PNPO, likely off-target of hairpin 1. *Column 3* indicates the T-test p-value of expression of gene in GIST vs. other tumors showing that 32/48 genes are GIST specific. *Column 4* shows whether the gene has ETV1 enhancer, promoter, or both binding sites by ChIP-Seq. **c,** GSEA plots of the shETV1 ranked list using three gene sets: GIST signature genes from ExpO dataset, ICC-MY and ICC-DMP signature genes in mouse small intestine, showing that GIST and ICC-MY specific genes are highly enriched among genes suppressed by ETV1 knockdown. ES, enrichment score; FDR, false discovery rate. **d**, Representative ChIP-Seq reads in top ETV1 target genes. **e**, Plot of percent of all genes, genes averagely downregulated 1.4-fold by shETV1 (n=410), and genes averagely upregulated 1.4-fold by shETV1 (n=380) with promoter only, enhancer only and both promoter and enhancer ETV1 binding. Fold enrichment over all genes is shown above the plots. ETV1 regulated genes are more likely bound by ETV1, especially at enhancer regions.

### *KIT signalling synergizes with ETV1 in GIST tumorigenesis by stabilization of ETV1 protein*

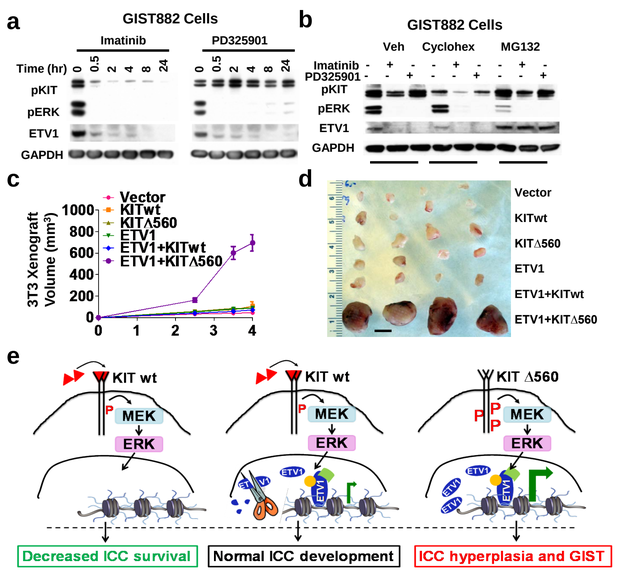


**Figure 5. a**, Immunoblots of GIST882 cells treated with the KIT inhibitor- imatinib (1 μM) or the MEK inhibitor-PD325901 (100 nM) for the indicated time points, showing rapid loss of ETV1 protein by KIT or MEK inhibition. **b,** Immunoblots of GIST882 cells treated for 2 hours with imatinib or PD325901 in combination with cyclohexamide (10 μg/ml) or MG132 (10 μM), showing that loss of ETV1 is due to proteosomal degradation. **c,** Growth of xenografts of engineered NIH3T3 cells stable expressing the indicated genes (n=12, Mean ±SEM). **d**, Photograph of 4 representative explanted xenografts at 4 weeks after implanting. Scale bar 1 cm. **e**, Model of the role of ETV1 in ICC maintenance and GIST oncogenesis. Normal level of KIT activation by KIT ligand (red triangle) stabilizes ETV1 transcription factor through the MAPK pathway, and results in physiological ETV1 transcriptional output critical for ICC development (middle). In the absence of ETV1, there is decreased ICC development, which phenocopies genetic loss of KIT signalling (left). Activating mutation of *KIT* (e.g. *KITΔ560*) leads to constitutive activation of the KIT-MAPK signalling pathway, increased ETV1 stabilization and augmented ETV1 transcriptional output that promotes tumorigenesis (right).

## CONCLUSIONS:

1. **ETV1 is highly expressed in the GIST/ICC lineage and is required for survival of normal ICC cells and of GIST tumors.**
2. **ETV1 is a master regulator of an GIST/ICC lineage specific transcriptional program and directly binds to regulatory regions of these genes.**
3. **ETV1 is stabilized by KIT/MAPK signaling.**
4. **ETV1 cooperates with mutant KIT in oncogenesis, suggesting that endogenous expression of ETV1 in ICC-MY and ICC-IM provides the context of KIT mediated oncogenesis. Other KIT expressing tissues (ICC-SMP, melanocytes, hematopoetic stem cells, etc) without ETV1 expression are resistant to mutant KIT mediated oncogenesis in human and mice with germline KIT mutations.**