

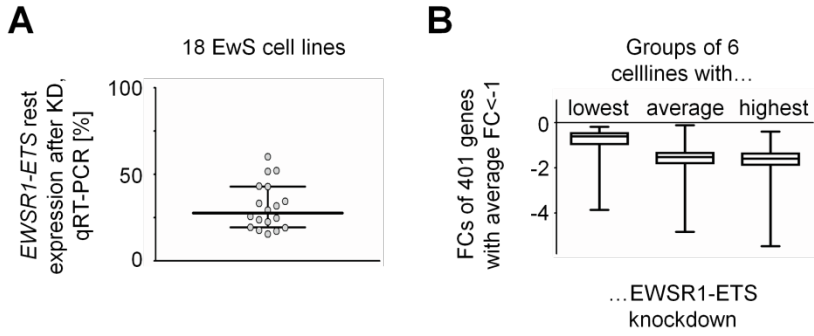
**Supplemental information**

**Systematic multi-omics cell line profiling**

**uncovers principles of Ewing sarcoma**

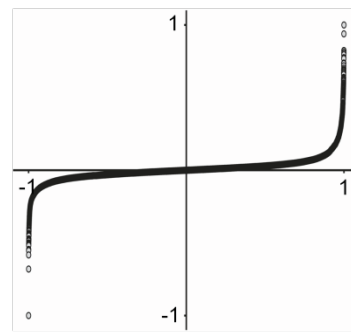
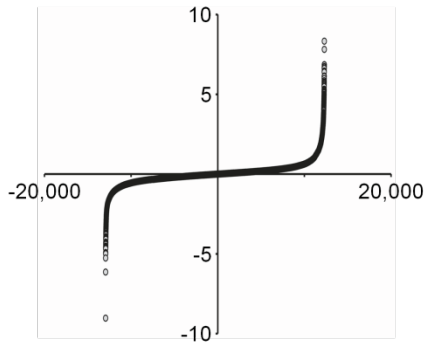
**fusion oncogene-mediated gene regulation**

**Martin F. Orth, Didier Surdez, Tobias Faehling, Anna C. Ehlers, Aruna Marchetto, Sandrine Grossetête, Richard Volckmann, Danny A. Zwijnenburg, Julia S. Gerke, Sakina Zaidi, Javier Alonso, Ana Sastre, Sylvain Baulande, Martin Sill, Florencia Cidre-Aranaz, Shunya Ohmura, Thomas Kirchner, Stefanie M. Hauck, Eva Reischl, Melissa Gymrek, Stefan M. Pfister, Konstantin Strauch, Jan Koster, Olivier Delattre, and Thomas G.P. Grünewald**

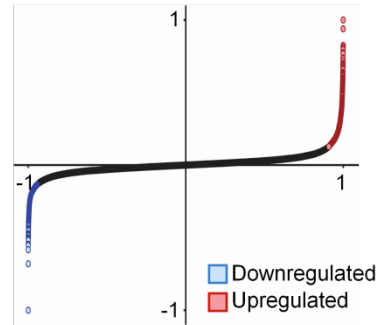
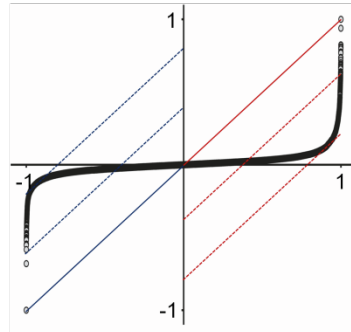


**C** Definition of differentially expressed genes with a semi-ranked approach:

1. List all genes on x-axis sorted by log<sub>2</sub> FC (EE-low vs. -high, y-axis)
2. Normalize both axes to (-)1

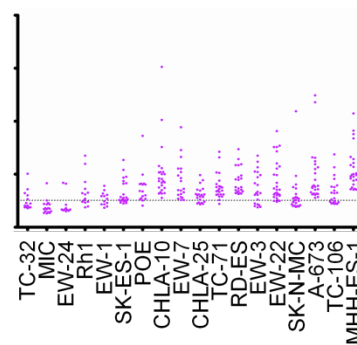
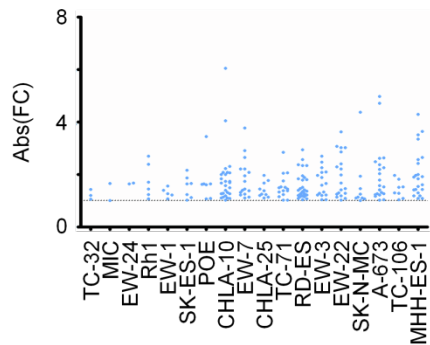


3. Slide connection line between 0|0 and most extreme values along the y-axis searching for the single intersecting point (slope is 1)
4. Designate all genes from the ones with slope 1 to the extremes as regulated



**D** Applying  $\text{abs}(\text{FC}) > 1$  and  $P < 0.01$  as cut-off

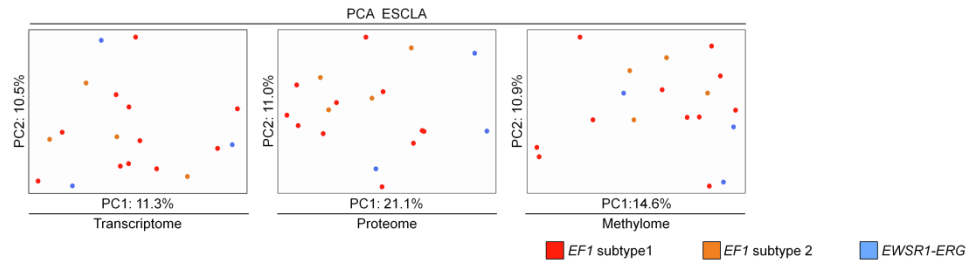
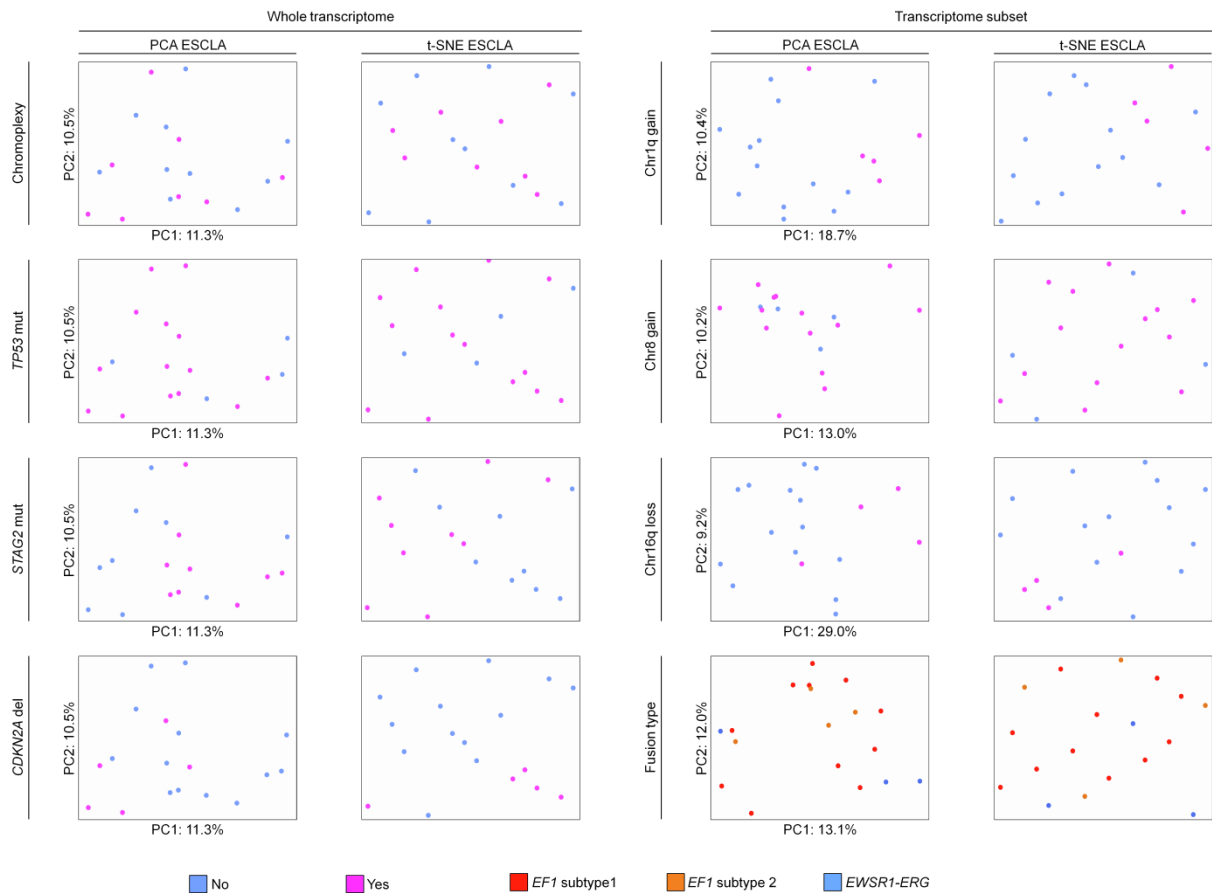
Applying approach depicted in C, cell line specific cut-off



■ ■ Genes identified as regulated by the respective approach from 252 exemplarily and randomly picked genes represented in the transcriptome data

**Supplementary Figure S1, related to STAR Methods - DNA microarray gene expression analyses.**  
**Designation of regulated genes**

**A)** qRT-PCR after EWSR1-ETS knockdown induction in the 18 EwS models of the ESCLA revealed variable rest expression levels. Median is indicated as horizontal bar and the interquartile range as whiskers. **B)** Comparing cell lines with lowest, average and highest knockdown efficiencies (groups of 6 cell lines) revealed highly significant ( $P < 0.0001$ ) association of EWSR1-ETS knockdown level with the FC of the 401 genes with strongest average expression reduction upon EWSR1-ETS knockdown ( $FC < -1$ ). Box plot indicates median and interquartile range of fold changes, whiskers indicate minimum and maximum fold changes. **C)** Workflow for the designation of regulated genes applied per cell line at the transcriptome and proteome levels of the ESCLA to avoid arbitrary cut-offs and  $P$ -value based approaches, and account for differential EWSR1-ETS knockdown efficiencies. **D)** Comparison of genes identified as regulated by a conventional approach with arbitrary cut-off (left) and the here applied approach (right); for better visibility, only 1% of genes represented in the transcriptome data ( $n=252$ ) were investigated and those designated as regulated in the respective cell line indicated as colored dot. KD: knockdown; EE-low/-high: EWSR1-ETS-low/-high; FC: fold change.

**A****B**

### Supplementary Figure S2, related to Figure 3. Dimension reduction of the ESCLA stratified by various genomic aberrations

**A)** PCA of the transcriptome, proteome and methylome of the ESCLA, each dot represents the mean level assessed per cell line in EWSR1-ETS-high condition, different fusion types are color coded. **B)** Left: PCA and t-SNE plots for the whole transcriptome of the ESCLA, each dot represents one cell line in EWSR1-ETS-high condition stratified by the feature indicated on the left site. Right: PCA and t-SNE plots based on a subset of the ESCLA transcriptome data. For chromosomal aberrations only genes localized on the respective chromosome were included in the analysis. For the stratification by fusion type, only those genes were included that were in at least 50% of cell lines at least moderately regulated by EWSR1-ETS. EF1: EWSR1-FLI1; PC: principal component; mut: mutation; del: deletion.

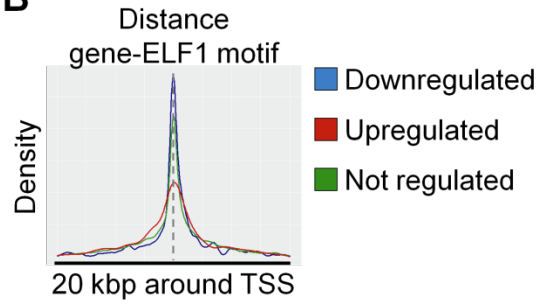
**A**

*De novo* called motif in  
EWSR1-ERG binding sites

Score:  
0.79

bg/target:  
0.9/22.9%

EWSR1-FLI1 (ref. motif)

**B**

**Supplementary Figure S3, related to Figure 4. Motif analyses for EWSR1-ERG bindings sites and ELF1**

**A)** *De novo* called motif in EWSR1-ERG binding sites with highest enrichment in the target sites versus background (bg) and the aligned reference binding motif for EWSR1-FLI1 (score for match 0.79). Only those EWSR1-ERG binding sites were analyzed, which were shared across all EWSR1-ERG positive cell lines in the ChIP data of the ESCLA. **B)** Density plot of the distance between the TSS of genes down-, up- and not regulated upon EWSR1-ETS knockdown and the closest ELF1 binding motif. Regulation was observed in at least 33% of all cell lines in one direction, never in the opposite direction for down- and up-regulated genes; not regulated genes were never found to be regulated in any of the 18 ESCLA cell lines. The genes are color-coded.