ODE REPRESENTATIONS

Model 1

\[
\begin{align*}
\frac{d[EWS_R]}{dt} &= -\bar{m}_1[EWS_R] - d_1[EWS_R] + a_1, \quad (1.1) \\
\frac{d[EWS_p]}{dt} &= t_1[EWS_R] - d_2[EWS_p], \quad (1.2) \\
\frac{d[E2F3_R]}{dt} &= [E2F3_d^{\text{total}}] \frac{k_1 c_2[EWS_p] + k_2 c_1[E2F3_p]}{c_2[EWS_p] + c_1[E2F3_p] + c_1 c_2} - d_3[E2F3_R], \quad (1.3) \\
\frac{d[E2F3_p]}{dt} &= t_2[E2F3_R] - d_4[E2F3_p], \quad (1.4) \\
\frac{d[ATAD2_R]}{dt} &= [ATAD2_d^{\text{total}}] \frac{k_3 c_4[EWS_p] + k_4 c_3[E2F3_p]}{c_4[EWS_p] + c_3[E2F3_p] + c_3 c_4} - d_5[ATAD2_R]. \quad (1.5)
\end{align*}
\]

Model 3

\[
\begin{align*}
\frac{d[EWS_R]}{dt} &= -\bar{m}_1[EWS_R] - d_1[EWS_R] + a_1, \quad (3.1) \\
\frac{d[EWS_p]}{dt} &= t_1[EWS_R] - d_2[EWS_p], \quad (3.2) \\
\frac{d[E2F3_R]}{dt} &= [E2F3_d^{\text{total}}] \frac{k_1 c_2[EWS_p] + k_2 c_1[E2F3_p]}{c_2[EWS_p] + c_1[E2F3_p] + c_1 c_2} - d_3[E2F3_R], \quad (3.3) \\
\frac{d[E2F3_p]}{dt} &= t_2[E2F3_R] - d_4[E2F3_p], \quad (3.4) \\
\frac{d[ATAD2_R]}{dt} &= \frac{k_3[EWS_p]}{[EWS_p] + c_3} - d_5[ATAD2_R]. \quad (3.5)
\end{align*}
\]

Model 4

\[
\begin{align*}
\frac{d[EWS_R]}{dt} &= -\bar{m}_1[EWS_R] - d_1[EWS_R] + a_1, \quad (4.1) \\
\frac{d[EWS_p]}{dt} &= t_1[EWS_R] - d_2[EWS_p], \quad (4.2) \\
\frac{d[E2F3_R]}{dt} &= [E2F3_d^{\text{total}}] \frac{k_1 c_2[EWS_p] + k_2 c_1[E2F3_p]}{c_2[EWS_p] + c_1[E2F3_p] + c_1 c_2} - d_3[E2F3_R], \quad (4.3) \\
\frac{d[E2F3_p]}{dt} &= t_2[E2F3_R] - d_4[E2F3_p], \quad (4.4) \\
\frac{d[ATAD2_R]}{dt} &= \frac{k_4[E2F3_p]}{[E2F3_p] + c_4} - d_5[ATAD2_R]. \quad (4.5)
\end{align*}
\]
MCMC integration for marginal likelihoods

We here give the details for implementing the population MCMC sampler (1) with a simplified Manifold Metropolis-Adjusted Langevin Algorithm [simplified MMALA see (2)] used in this study. To simplify the presentation we derive all the expressions using a single variance parameter \( v \) for both RNA and protein data. Given that observations are obtained independently at each time point for each measured level, the expressions for two different variance parameters can be obtained by using the expressions below separately for each type of data (RNA and Protein) and then summing them (or multiplying them in the case of the likelihood function).

It is assumed that observations are obtained independently at each time point for each measured level. Consequently, the likelihood becomes the product of Gaussians

\[
\ell(Y|X(\theta), v) = \prod_{i=1}^{n_t} \prod_{j=1}^{n_s} \mathbb{I}_{ij} N(y_{ij}|x_{ij}(\theta), v), (S7).
\]

The \( n_t \cdot n_s \) indicators \( \mathbb{I}_{ij} \) account for missing data. If level \( j \) has been measured at time point \( t_i \), then \( \mathbb{I}_{ij} = 1 \). On the other hand, if an experimental observation has not been made at time point \( t_i \) for species \( j \), then \( \mathbb{I}_{ij} = 0 \). It follows from (6) that the log-likelihood \( \mathcal{L}(Y|X(\theta), v) \) is given by

\[
\mathcal{L}(Y|X(\theta), v) = -\frac{1}{2v} su\left[ (Y - X(\theta))^2 \right] - \frac{\log(2\pi v)}{2} su(1), (S8)
\]

where \((.) \circ (.)\) and \((.)^2\) represent the Hadamard product and Hadamard power respectively (3), \( su(.) \) refers to the sum of all elements of a matrix and \( \mathbb{I} \) is the \( n_t \cdot n_s \) indicator matrix whose \((i,j)\)-th element \( \mathbb{I}_{ij} \) informs whether the \( j \)-th level has been measured at time point \( t_i \). (4). It then follows that the log-likelihood’s gradient equals

\[
\frac{\partial \mathcal{L}(Y|X(\theta), v)}{\partial \theta_k} = \frac{1}{v} su\left[ (Y - X(\theta)) \circ \frac{\partial X(\theta)}{\partial \theta_k} \right], (S9)
\]

\[
\frac{\partial \mathcal{L}(Y|X(\theta), v)}{\partial v} = \frac{1}{2v^2} su\left[ (Y - X(\theta))^2 \right] - \frac{1}{2v} su(1), (S10)
\]

and the Fisher information \( FI(\varphi) = FI(\theta, v) \) based on log-likelihood (S8) is

\[
FI(\theta_m, \theta_k) = \frac{1}{v} su\left[ \mathbb{I} \circ \frac{\partial X(\theta)}{\partial \theta_m} \circ \frac{\partial X(\theta)}{\partial \theta_k} \right], (S11)
\]

\[
FI(v, v) = \frac{1}{2v^2} su(1), (S12)
\]

\[
FI(v, \theta_m) = FI(\theta_m, v) = 0. (S13)
\]

The derivatives of the ODE system \( \frac{dX(\theta)}{d\theta_m} \) are calculated automatically by symbolic differentiation and are then exported as a C function to be used in forward sensitivity analysis in the Sundials ODE solver package (5).

All parameters \( \varphi = (\theta, v) \) are transformed to logarithmic scale such that \( \tilde{\varphi} = \log_{10}(\varphi) \) and an independent Normal prior with 0 mean and 1 standard deviation is assumed for each of the transformed parameters \( \tilde{\varphi} \). Notice that due to the log scaling this prior gives significant mass to a large range of parameters \( \varphi \). The posterior distribution of the parameters is then
\[ p(\tilde{\theta}, \tilde{v}|Y) \propto \mathcal{L}(Y|X(\tilde{\theta}), \tilde{v}) p(\tilde{\theta}) p(\tilde{v}). \]  

A population MCMC sampler (1) is used for sampling from 50 power posteriors (6) with a simplified Manifold Metropolis-Adjusted Langevin Algorithm [simplified MMALA see (2)]. The inverse temperature parameter of the power posteriors is denoted by \( \tau \) and a fixed temperature schedule of type \( \tau_i = a_i^4 \) is set, where \( a_i \) are 50 equally spaced points in the interval \([0,1]\). Each power posterior is then defined as

\[ p_{\tau_i}(\tilde{\theta}, \tilde{v}|Y) \propto \mathcal{L}(Y|X(\tilde{\theta}), \tilde{v})^{\tau_i} p(\tilde{\theta}) p(\tilde{v}). \]  

50 Markov chains are formed, each targeting one power posterior. Each Markov chain is updated independently and in parallel using simplified MMALA. After each chain is updated, i.e. one sample is drawn, 50 neighbouring pairs of chains are selected at random to propose an exchange of their states. Each exchange between chains \( i, j \) is accepted with probability

\[ \min \left\{ 1, \frac{\mathcal{L}(Y|\tilde{\phi}_j)^{\tau_i} \mathcal{L}(Y|\tilde{\phi}_i)^{\tau_j}}{\mathcal{L}(Y|\tilde{\phi}_i)^{\tau_i} \mathcal{L}(Y|\tilde{\phi}_j)^{\tau_j}} \right\}, \]  

where \( \tilde{\phi}_i, \tilde{\phi}_j \) are the states of chains \( i, j \) respectively.

A Markov chain using the simplified MMALA algorithm is updated as follows. First a new sample \( \tilde{\phi}^* \) is proposed from a proposal distribution of the form

\[ \tilde{\phi}^* \sim N \left( \mu(\tilde{\phi}_j, s), \frac{s}{\tau_j^2} FI^{-1}(\tilde{\phi}_j) \right), \]  

where \( \tilde{\phi}_j \) is the current state of chain \( j \), \( s \) is an adjustable parameter, \( FI(\tilde{\phi}_j) \) is the Fisher Information of the model in equation (7) and

\[ \mu(\tilde{\phi}_j, s) = \tilde{\phi}_j + \frac{s}{2\tau_j} FI^{-1}(\tilde{\phi}_j) \nabla_{\tilde{\phi}_j} \log \mathcal{L}(Y|\tilde{\phi}). \]  

Then the new sample \( \tilde{\phi}^* \) is accepted with probability

\[ \min \left\{ 1, \frac{\mathcal{L}(Y|\tilde{\phi}^*)^{\tau_i} p(\tilde{\phi}_j) N(\tilde{\phi}_j|\mu(\tilde{\phi}^*, s), sFI^{-1}(\tilde{\phi}^*))}{\mathcal{L}(Y|\tilde{\phi}_j)^{\tau_i} p(\tilde{\phi}_j) N(\tilde{\phi}_j|\mu(\tilde{\phi}_j, s), sFI^{-1}(\tilde{\phi}_j))} \right\}. \]  

The gradient of the log-likelihood and the Fisher information, which are needed for computing the proposal distribution of simplified MMALA, are given by (9)-(13).

To calculate marginal likelihoods, a trapezoidal rule is used for integrating over the inverse temperature variables (6), which gives the following result:

\[ \log p(Y) \approx \frac{1}{2} \sum_{j=1}^{49} (\tau_{j+1} - \tau_j) \left( E_{\tilde{\phi}|X,\tau_{j+1}}[\log \mathcal{L}(Y|\tilde{\phi})] - E_{\tilde{\phi}|X,\tau_j}[\log \mathcal{L}(Y|\tilde{\phi})] \right). \]  

\( E_{\tilde{\phi}|X,\tau_j}[\log \mathcal{L}(Y|\tilde{\phi})] \) denotes the expectation of the log-likelihood with respect to the \( j \)-th power posterior. Given \( m \) samples \( \tilde{\phi}_j^{(i)} \) from \( j \)-th power posterior, the expectation can be approximated as

\[ E_{\tilde{\phi}|X,\tau_j}[\log \mathcal{L}(Y|\tilde{\phi})] \approx \frac{1}{m} \sum_{j=1}^{m} \log \mathcal{L}(Y|\tilde{\phi}_j^{(i)}). \]
**SUPPORTING METHODS:**

**Alternative ChIP-seq peak calling:**
The ChIP-seq peak-calling was repeated with MACS2: The MACS2 program `callpeak` was run for bam files of ChIP-sample vs. Input with default parameters and FDR < 0.05, ext-size=250). Overlapping peaks were subsequently combined into one peak region. With these settings we found 11845 peaks for EWS-FLI1, 5709 peaks for E2F3 and 3236 peaks for E2F4 (Figure S7). The percentages of overlapping peaks (corresponding to the numbers in the first paragraph of the results section, pg.9) were: E2F4-E2F3: 64.9%, E2F4-EWS-FLI1: 41.4, triple-overlap: 35%. Therefore, although the numbers of peaks varied between peak callers, the overall conclusion did not change depending on the peak-calling strategy: There is a highly significant overlap of EWS-FLI1 and E2F3/4 peaks.

**Data Access:**
Sequence data generated for this study have been submitted to the NCBI Sequence Read Archive (SRA; [http://www.ncbi.nlm.nih.gov/sra](http://www.ncbi.nlm.nih.gov/sra)) under accession number SRA096176.

**TABLE AND FIGURE LEGENDS**

TableS1: Parameters and description for model 1
TableS2: Parameters and description for model 3
TableS3: Parameters and description for model 4

Figure S1. A-C: **Coverage plots of EWS-FLI1, E2F3 and E2F4 ChIP-seq reads** for A) E2F3, B) ATAD2, C) RAD51. The plots show screen shots from the Integrated Genome Viewer ([http://www.broadinstitute.org/igv/](http://www.broadinstitute.org/igv/)). Bam files of alignments from ChIPseq experiments were directly loaded into IGV.

Figure S2. **Cell cycle analysis.** A) A673 cells were either left untreated, treated with doxycycline for 18h, 24h, 36h or 48h, treated with thymidine for 16h, released from treatment for 8h and treated again for 16h with thymidine or transfected with p57. B) Immunoblotting of untreated, thymidine treated, control empty vector transfected and p57 transfected A673 cells with EWS-FLI1 and b-actin antibodies. Treatment and transfection do not change EWS-FLI1 protein expression. C) Fold changes in reporter activity of E2F3 and RAD51 promoters in A673 cells under double thymidine block induced cell cycle arrest versus untreated control conditions.

Figure S3. **Lack of co-immunoprecipitation of EWS-FLI1 with E2F/pocket protein complexes.** IPs in the presence (+) and absence (-) of EWS-FLI1 were performed using antibodies for pRB A), E2F3 B), and p130 C) and FLI1 D). Immunoblots probing potential interaction partners were performed using anti-FLI1 and E2F3 antibodies after IP for pRB (A), anti-FLI1 after IP of E2F3 (B), anti-FLI1 and anti-E2F4 antibodies after IP of p130 (C), and antibodies to pRB, E2F3 and E2F4 after IP of EWS-FLI1 (D). Complex formation was
found only between pRB and E2F3 and between p130 and E2F4, while none of the E2F and pocket proteins was detectable in complex with EWS-FLI1.

Figure S4. **Protein and RNA expression after EWS-FLI1 knockdown.** A673 cells carrying a doxycyclin inducible EWS-FLI1 shRNA were either left untreated (0) or treated with doxycycline for 10, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45 and 48h doxycycline to induce knockdown of EWS-FLI1. Protein levels of EWS-FLI1 and E2F3 were monitored by fluorescent immunoblotting (A) and were quantified using the LICOR Odyssey® Infrared Imaging System (B). RNA expression levels were analysed by quantitative RT-PCR using primers amplifying EWS-FLI1, E2F3, ATAD2 and RAD51 and normalized to b-actin expression (C).

Figure S5. **Posterior response for model 2 and data for the ATAD2 target gene.** The plot shows solutions of the ODE systems for model 2 for 1,000 samples of the posterior distribution (blue lines) and the observed experimental data (red circles). The mean response is also shown by averaging the 1,000 simulations (green solid line).

Figure S6. **Posterior response for model 2 and data for the RAD51 target gene.** The plot shows solutions of the ODE systems for model 2 for 1,000 samples of the posterior distribution (blue lines) and the observed experimental data (red circles). The mean response is also shown by averaging the 1,000 simulations (green solid line).

Figure S7. **Venn Diagram of overlapping peaks between EWS-FLI1, E2F3 and E2F4 ChIP-seq.** For this analysis peaks were called by MACS2 (see supporting methods).

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A) EWS-FLI1
E2F3
E2F4
RefSeq Genes

B) EWS-FLI1
E2F3
E2F4
RefSeq Genes

C) EWS-FLI1
E2F3
E2F4
RefSeq Genes
A) 

EWS-FL1
E2F3
b-actin

h Dox treatment
0 10 15 18 21 24 24 30 33 36 42 45 48

B) 

EWS-FL1
E2F3

ATAD2
RAD51

Relative protein expression
0 0.5 1.0 1.5

h of Doxycycline treatment
0 10 15 20 24 27 30 33 36 42 45 48

C) 

EWS-FL1

E2F3

Relative expression
0 0.5 1.0 1.5

h of Doxycycline treatment
0 10 15 20 24 27 30 33 36 42 45 48

E2F3

Relative expression
0 0.5 1.0 1.5

h of Doxycycline treatment
0 10 15 20 24 27 30 33 36 42 45 48
Table S1: Parameters and description for model 1

| Parameter | Description |
|-----------|-------------|
| $a_1$     | EWS-FLI1 transcription rate times EWS-FLI1 DNA concentration |
| $m_1$     | shRNA mediated degradation rate constant of EWS mRNA |
| $d_1$     | EWS-FLI1 mRNA degradation rate constant |
| $t_1$     | EWS-FLI1 translation rate constant |
| $d_2$     | EWS-FLI1 protein degradation rate constant |
| $k_1$     | EWS-FLI1 mediated E2F3 transcription rate constant |
| $c_1$     | EWS-FLI1 E2F3 disassociation ratio |
| $k_2$     | E2F3 self-regulation transcription rate constant |
| $c_2$     | E2F3 protein E2F3 DNA disassociation ratio |
| $d_3$     | E2F3 mRNA degradation rate constant |
| $t_2$     | E2F3 translation rate constant |
| $d_4$     | E2F3 protein degradation rate constant |
| $k_3$     | EWS-FLI1 mediated ATAD2 transcription rate constant |
| $c_3$     | EWS-FLI1 protein ATAD2 disassociation ratio |
| $k_4$     | E2F3 mediated ATAD2 transcription rate constant |
| $c_4$     | E2F3 protein ATAD2 disassociation ratio |
| $d_5$     | ATAD2 mRNA degradation rate constant |
Table S2: Parameters and description for model 3

| Parameter | Description |
|-----------|-------------|
| \(a_1\)  | EWS-FLI1 transcription rate times EWS-FLI1 DNA concentration |
| \(m_1\)  | shRNA mediated degradation rate constant of EWS mRNA |
| \(d_1\)  | EWS-FLI1 mRNA degradation rate constant |
| \(t_1\)  | EWS-FLI1 translation rate constant |
| \(d_2\)  | EWS-FLI1 protein degradation rate constant |
| \(k_1\)  | EWS-FLI1 mediated E2F3 transcription rate constant |
| \(c_1\)  | EWS-FLI1 E2F3 disassociation ratio |
| \(k_2\)  | E2F3 self-regulation transcription rate constant |
| \(c_2\)  | E2F3 protein E2F3 DNA disassociation ratio |
| \(d_3\)  | E2F3 mRNA degradation rate constant |
| \(t_2\)  | E2F3 translation rate constant |
| \(d_4\)  | E2F3 protein degradation rate constant |
| \(k_3\)  | EWS-FLI1 mediated ATAD2 transcription rate constant |
| \(c_3\)  | EWS-FLI1 protein ATAD2 disassociation ratio |
| \(d_5\)  | ATAD2 mRNA degradation rate constant |
Table S3: Parameters and description for model 4

| Parameter | Description |
|-----------|-------------|
| $a_1$     | EWS-FLI1 transcription rate times EWS-FLI1 DNA concentration |
| $m_1$     | shRNA mediated degradation rate constant of EWS mRNA |
| $d_1$     | EWS-FLI1 mRNA degradation rate constant |
| $t_1$     | EWS-FLI1 translation rate constant |
| $d_2$     | EWS-FLI1 protein degradation rate constant |
| $k_1$     | EWS-FLI1 mediated E2F3 transcription rate constant |
| $c_1$     | EWS-FLI1 E2F3 disassociation ratio |
| $k_2$     | E2F3 self-regulation transcription rate constant |
| $c_2$     | E2F3 protein E2F3 DNA disassociation ratio |
| $d_3$     | E2F3 mRNA degradation rate constant |
| $t_2$     | E2F3 translation rate constant |
| $d_4$     | E2F3 protein degradation rate constant |
| $k_4$     | E2F3 mediated ATAD2 transcription rate constant |
| $c_4$     | E2F3 protein ATAD2 disassociation ratio |
| $d_5$     | ATAD2 mRNA degradation rate constant |