Supplementary Information

Systems biology of ferroptosis: a modeling approach

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S1 Initial conditions for the mathematical model

Initial conditions were set such that levels of input parameters (ACSL4, SCD1, ferroportin, TFRC, and p53) were set to low (level = 0), with the exception of ACSL4, which was set to high (level = 2). Since ACSL4 controls the generation of PUFA-CoA moieties for PUFAs, a lower level of it was found by simulation to not generate a maximal ferroptotic response under any conditions of the other input variables, therefore we consider the baseline cell to have maximal availability of PUFA-CoA. Other values of ACSL4 with respect to the input parameters are explored in Section 2.2.2 of the main text. The remainder of the initial conditions are in Table S1. The initial system was considered to have moderate MUFA, PUFA, ALOX15, GPX4, SLC7A11, and LPCAT3 levels, low ROS, PUFA incorporation into the membrane (LH-P), and peroxidated phospholipids (LOOH, $LO^{\bullet}$). In order to test system dependence on the initial conditions, we randomly varied initial MUFA, PUFA, LH-P, LIP, ALOX15, SLCA7A11, and LPCAT3 between all levels (0,1, or 2), and GPX4 between moderate and high levels (1 or 2). We maintained ROS, LOOH, and $LO^{\bullet}$ at low levels during these simulations because we assumed that the baseline cell is not under oxidative stress before the start of the simulation. We also maintained the values of the input parameters as discussed above, since altering those does change baseline cellular behavior and response to ferroptosis. We ran 1000 simulations using random initial conditions for treated and untreated simulated cells, and found that steady states to be identical to the steady states obtained with initial conditions described in Table S1. For this set of simulations, SFA and AA availability for downstream reactions were considered to be non-rate-limiting – and thus were maintained at high levels for the course of the simulations.

S2 Correlation analysis for individual input variables to ferroptotic response

In order to ascertain whether varying one input variable can serve to predict the ferroptotic response irrespective of the values of the other input variables, we set each input variable to low, medium, and high levels, and varied all other input variables while recording the steady state output of $LO^{\bullet}$, a peroxide radical that is indicative of ferroptosis. We performed the simulations in erastin – and + conditions (Figure S1). Since TFRC and Fptn effect only levels of the labile iron pool (LIP), which is upstream of $LO^{\bullet}$, we combined the impact of these two input variables in the variable $TFRC^{*}/Fptn^{*} = (TFRC+1)/(Fptn+1)$.

We observed several trends: when ACSL4 is low or SCD1 is high, $LO^{\bullet}$ is not produced with or without erastin, and when $TFRC^{*}$/$Fptn^{*}$ is low, $LO^{\bullet}$ is not produced without erastin. Otherwise, the production of $LO^{\bullet}$ varies, indicating that ascertaining the values of the other input variables is critical for predicting the ferroptotic response.

Table S1: Ferroptosis model initial conditions (I.C.s). Input parameters are in bold (values of input parameters do not change during the course of the simulation). *If treatment with erastin or RSL3 is simulated, the initial condition is set to 2 for the respective treatment.

Variable	**I.C.**	**Variable**	**I.C.**
MUFA	1	ROS	0
SFA	2	ALOX15	1
PUFA	1	GPX4	1
LH-P	1	erastin*	0
SCD	1	RSL3*	0
TFRC	1	**p53**	1
Fptn	1	SLC7A11	1
LIP	1	LPCAT3	1
LOOH	0	**ACSL4**	2
$LO^{\bullet}$	0		

S3 Systems biology of ferroptosis: intermediate response

When we consider the intermediate ferroptotic response to a systems-level simulation of all possible input variables (Section 2.2.2), which accounts for ~22.22% of the cases, we observe that in order for ferroptosis to increase upon GPX4 treatment, we must still have SLC7A11 and GPX4 to be high and p53 to be low/intermediate (except for special cases, discussed in Figure 7), whereas now ACSL4 and SCD1 can take on high/intermediate and low/intermediate values, respectively (Figure S2). As in the high ferroptosis case (Figure 5), most cases of maximal p53 result in a non-responsive cell due to relatively high levels of ferroptosis without erastin that cannot be further increased with a ferroptotic agent.

S4 ACSL4 and SCD1 are up-regulated in transformed ovarian cancer stem cells.

To assess the baseline level of ACSL4 and SCD1 in FT-i cells, we compared their expression levels to those of FT and FT-i cells (Figure S3). As can be observed, FT-t cells display significantly increased expression of both genes as compared to FT cells. Therefore, we model FT-t cells as having ACSL4 and SCD1 at high level before knockdown.

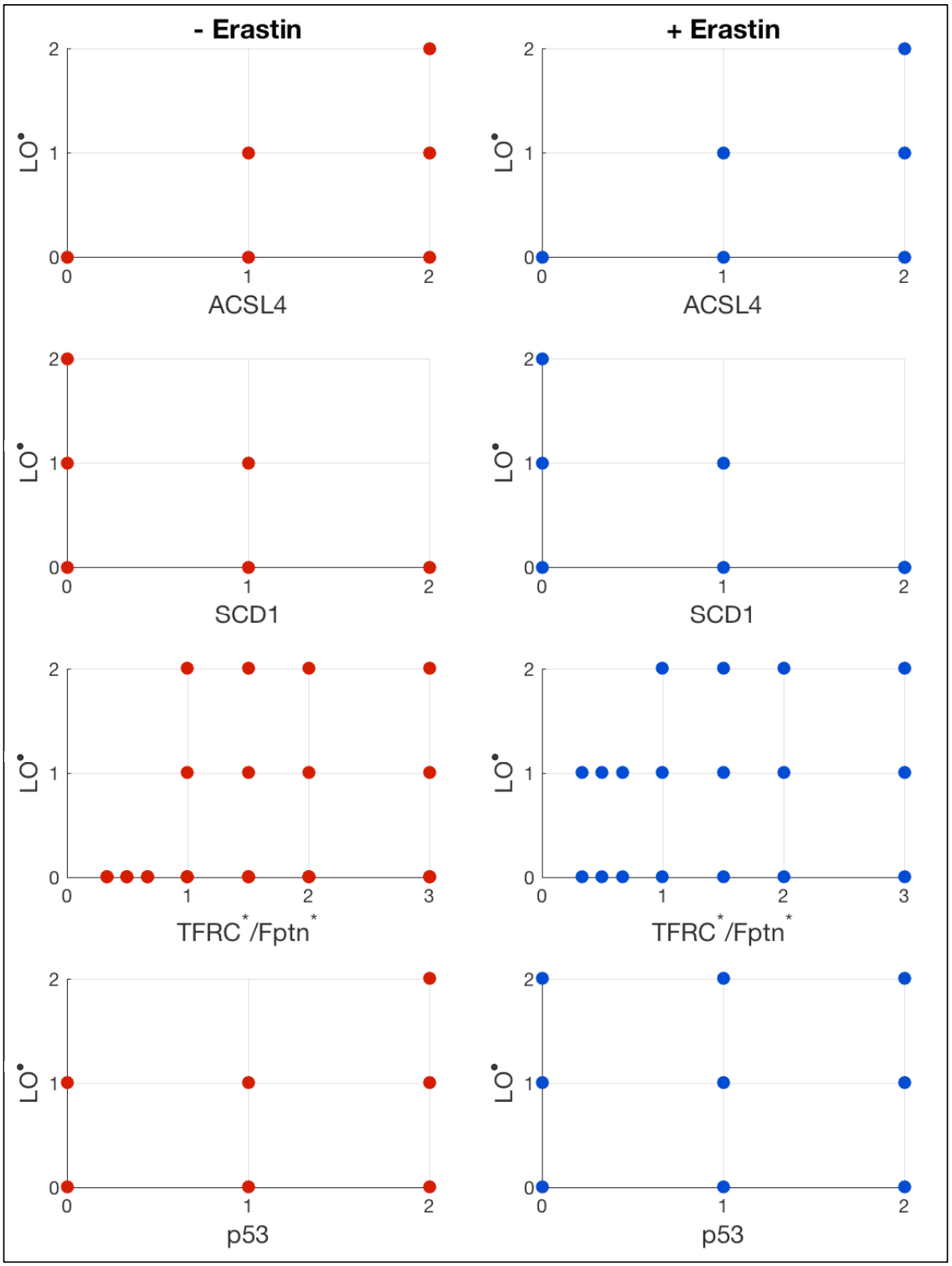

Figure S1: Correlation of input variable (ACSL4, SCD1, TFRC, Fptn, p53) levels with production of $LO^{\bullet}$, a lipid radical that precedes ferroptosis, in erastin – (red) and + (blue) conditions. Since TFRC and Fptn impact only levels of LIP, which is upstream of $LO^{\bullet}$, via their combined activity, their impact is modeled together using the equation $TFRC^{*}/Fptn^{*}$ = (TFRC+1)/(Fptn+1).

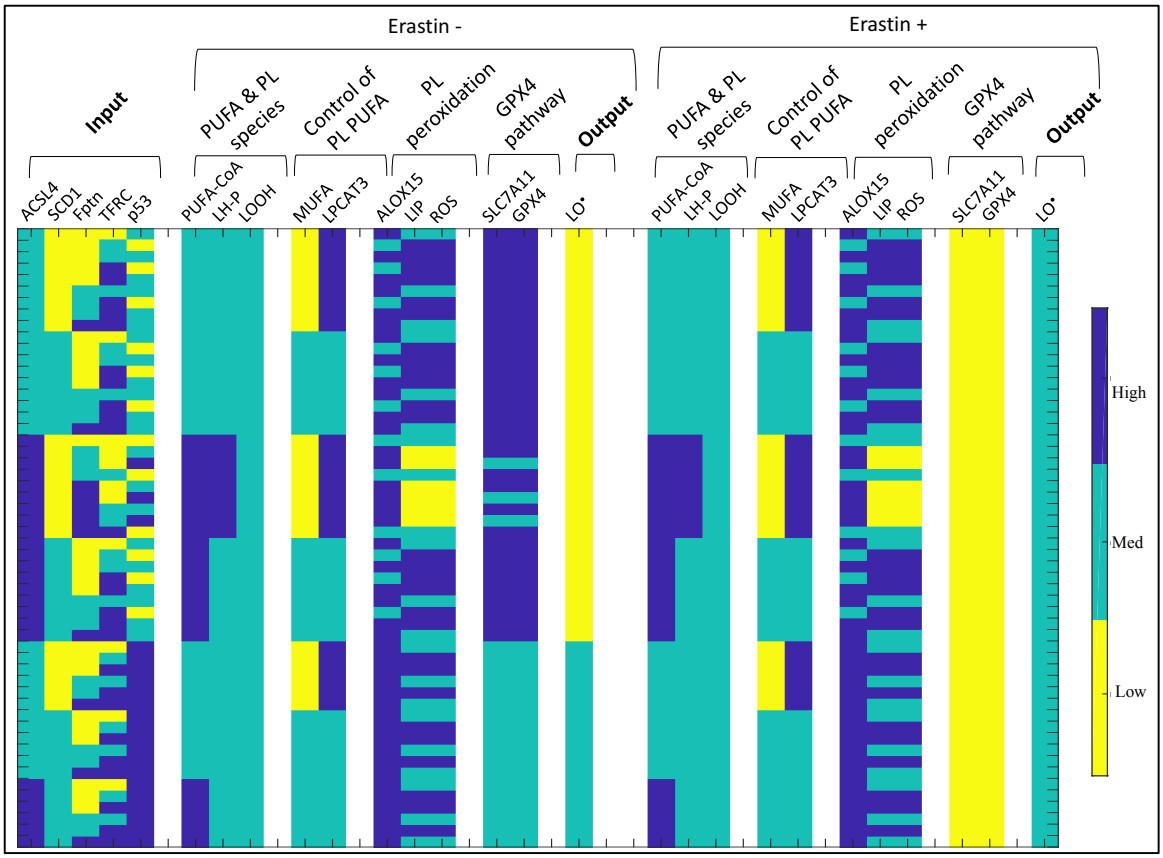

Figure S2: Ferroptosis model output with all combinations of input conditions, sorted by ferroptotic response, focus on intermediate ferroptosis after erastin treatment.

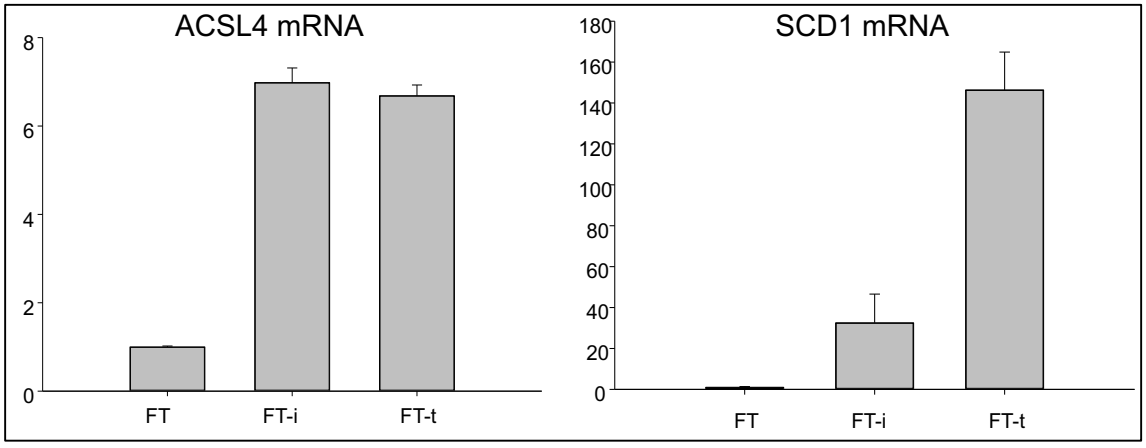

Figure S3: ACSL4 and SCD1 mRNA in normal fallopian stem (FT), immortalized fallopian tube cells (FT-i) and transformed fallopian tube stem cells (FT-t) were analyzed using RT-q-PCR. Experiments are representative of two to three independent experiments. Graphs are means and standard deviation of 3 replicates in one representative experiment.