

***Lipids peroxidation of the HCT116 WT and GPX4 KO clones,
24 h after erastin [10 μ M] exposition***

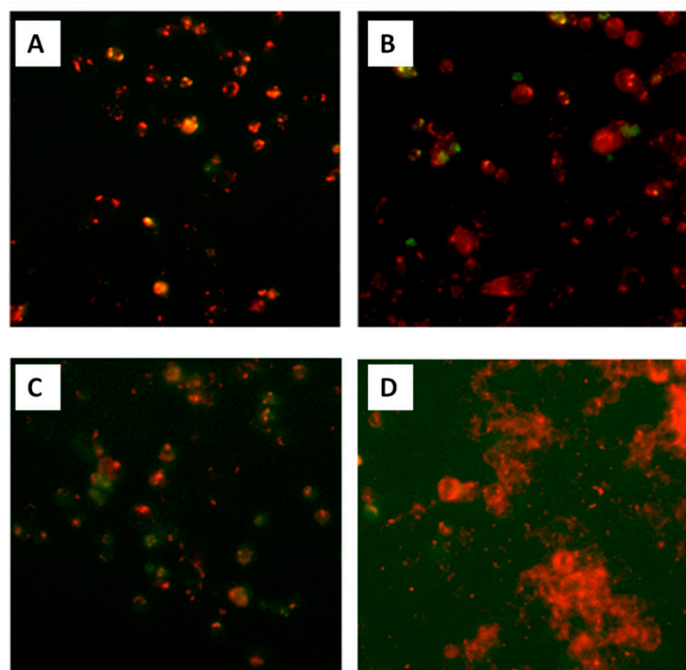


Figure S2. Imaging of lipid peroxidation from merged microscopic channels, red (reduced lipids) and green (oxidized lipids) from untreated controls: HCT116 WT (A) and GPX4 KO (C); and cells treated for 24 h with 10 μ M of erastin: HCT116 WT (B) and GPX4 KO (D).

RNA correlation between genes

The analysis of RNA level correlation between genes was conducted based on data contained in the FerrDb database. FerrDb is a database encompassing information regarding regulators and markers of ferroptosis, as well as associations between ferroptosis and diseases. Annotations are generated from currently available articles on ferroptosis in the PubMed publication database. The analysis was carried out using built-in tools within the database (Utilities tab, see **Figure S4**). After selecting "correlation analysis," a new window appears where the user can choose from which database to pull data for analysis, the study acronym (in our case, it was TCGA-COAD, where COAD stands for Colon Adenocarcinoma), the number of genes to be analysed, and their respective names (**Figure S5**).

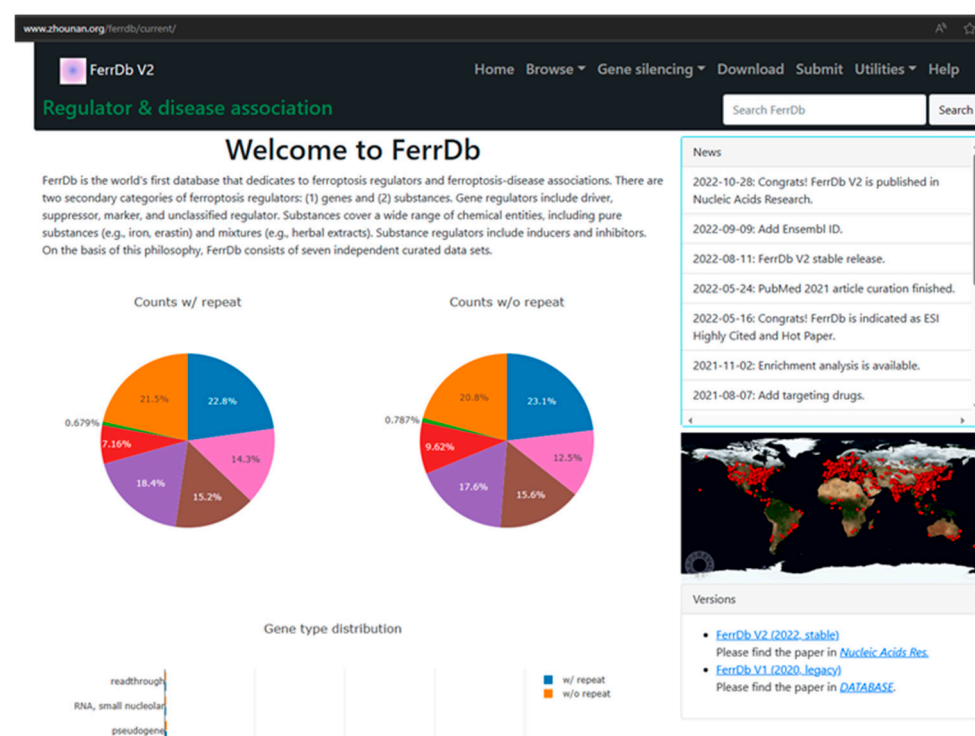


Figure S3. Database Homepage.

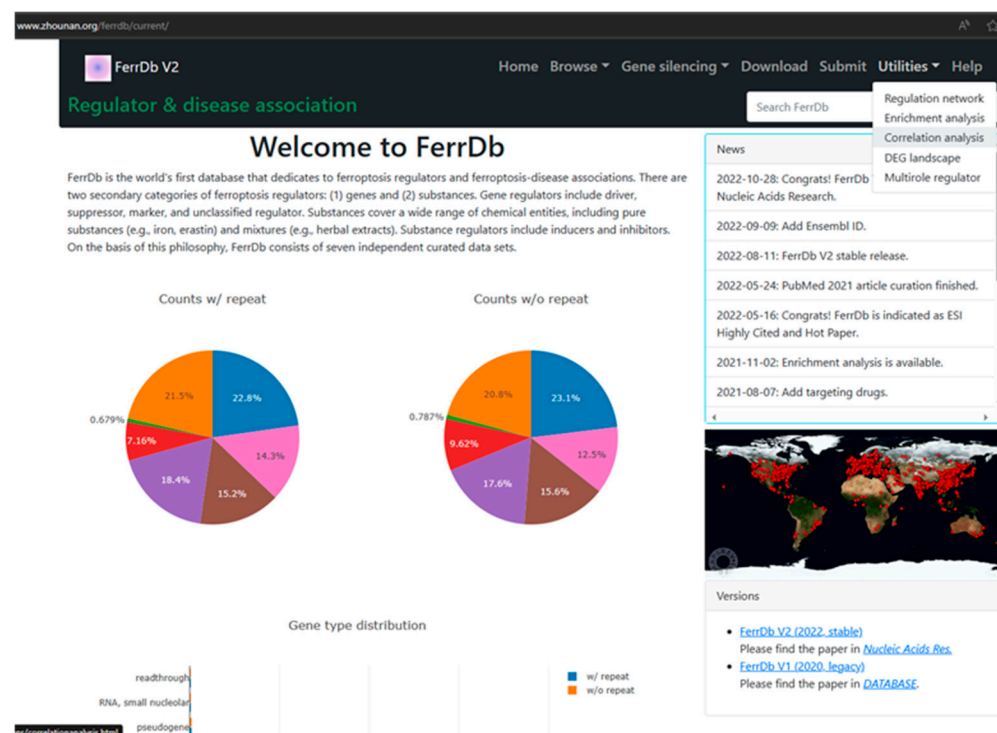


Figure S4. Utilities tab on FerrDb homepage.

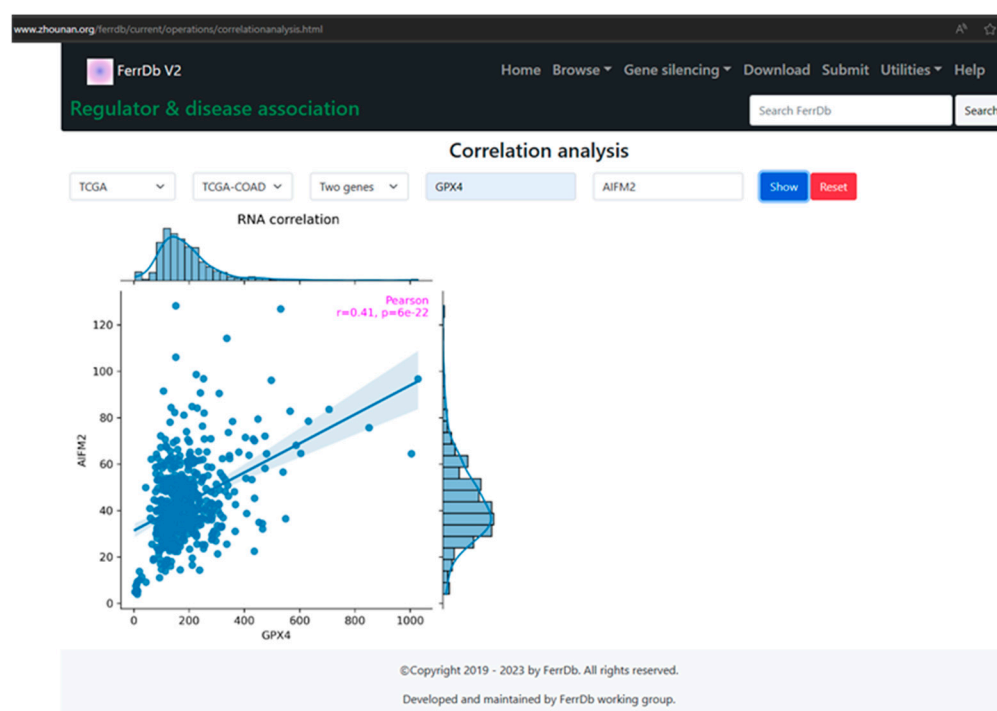


Figure S5. Example of RNA levels correlation analysis for GPX4 and AIFM2(FSP1).

Western Blot original film presentation

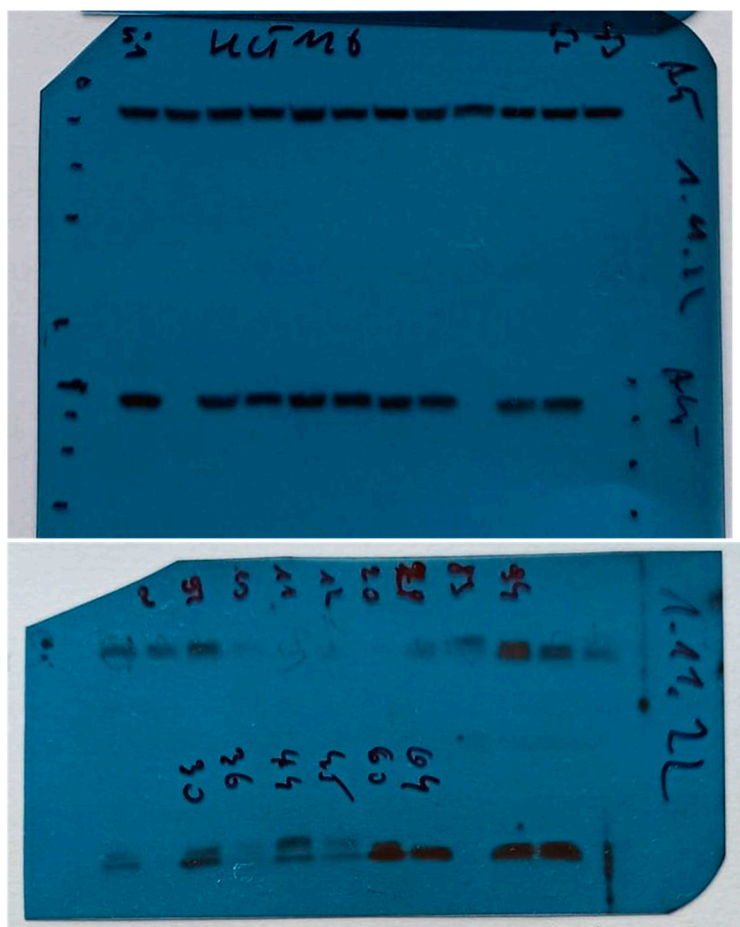


Figure S6. Original Western Blot film presentation.

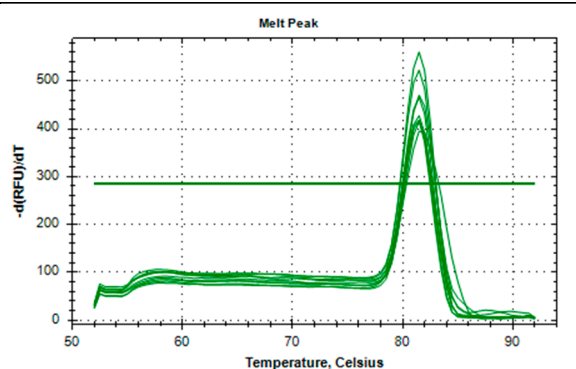
Table S1. PCR gene expression analysis – statistical significance.

HCT 116 cell line	WT		GPX4 KO		GPX4 POSITIVE CONTROL	
ACSL4	CTR	ERASTIN 10 µM	CTR	ERASTIN 10 µM	CTR	ERASTIN 10 µM
average	1	0,5407526	1	1,7398498	1	0,6803623
SD	0	0,0466356	0	0,030602	0	0,0469131
test t		0,0339632		0,012811		0,0028193
HCT 116 cell line	WT		GPX4 KO		GPX4 POSITIVE CONTROL	
TFRC	CTR	ERASTIN 10 µM	CTR	ERASTIN 10 µM	CTR	ERASTIN 10 µM
average	1	0,3810812	1	1,1543234	1	0,4895249
SD	0	0,0913219	0	0,1320607	0	0,0921362
test t		0,0132124		0,0103635		0,0272745
HCT 116 cell line	WT		GPX4 KO		GPX4 POSITIVE CONTROL	
GPX4	CTR	ERASTIN 10 µM	CTR	ERASTIN 10 µM	CTR	ERASTIN 10 µM
average	1	0,9695858			1	0,4671758
SD	0	0,166051			0	0,1254838
test t		0,7169949				0,10449
HCT 116 cell line	WT		GPX4 KO		GPX4 POSITIVE CONTROL	
FSP1	CTR	ERASTIN 10 µM	CTR	ERASTIN 10 µM	CTR	ERASTIN 10 µM
average	1	0,7888401	1	1,8501584	-	n.a.
SD	0	0,1496217	0	0,2000384	-	n.a.
test t		0,2123869		0,0342602	-	n.a.
HCT 116 cell line	WT		GPX4 KO		GPX4 POSITIVE CONTROL	
PRDX1	CTR	ERASTIN 10 µM	CTR	ERASTIN 10 µM	CTR	ERASTIN 10 µM
average	1	0,6641726	1	1,4216603	1	0,4841059
SD	0	0,0259535	0	0,0430206	0	0,0484576
test t		0,0019889		0,0034447		0,0029458
HCT 116 cell line	WT		GPX4 KO		GPX4 POSITIVE CONTROL	
TRX	CTR	ERASTIN 10 µM	CTR	ERASTIN 10 µM	CTR	ERASTIN 10 µM
average	1	0,7776127	1	1,105816	1	0,5081168
SD	0	0,0718326	0	0,0166117	0	0,02237
test t		0,0336824		0,0081025		0,0006896
HCT 116 cell line	WT		GPX4 KO		GPX4 POSITIVE CONTROL	
PROM1	CTR	ERASTIN 10 µM	CTR	ERASTIN 10 µM	CTR	ERASTIN 10 µM
average	1	0,6999002	1	1,4000544	1	0,5877065
SD	0	0,0915564	0	0,1446081	0	0,0666175
test t		0,1327422		0,0399374		0,0766253

The results presented in the tables are the means from 3 biological experiments, the standard deviation and the T-test for non-treated controls; results below the threshold of $p < 0.05$ are considered statistically significant. n.a. – not analysed.

Table S2. PROM2 RT-qPCR gene expression analysis – the positive control of the resistance to the erastin-induced ferroptosis cell line.

Sample/Ct value	PROM2	RPL41
WT control	36,94	24,55
Erastin 5 μ M	35,29	23,96
Erastin 10 μ M	35,04	24,01
GPX4 KO control	36,79	24,39
Erastin 5 μ M	36,05	24,18
Erastin 10 μ M	38,49	23,75
GPX4 positive control, control	36,52	22,84
Erastin 5 μ M	38,61	23,39
Erastin 10 μ M	39,24	23,22



Ct – threshold cell cycle of the RT-qPCR reaction (on the left) and the melting temperature of the positive product for PROM2 expression (on the right).