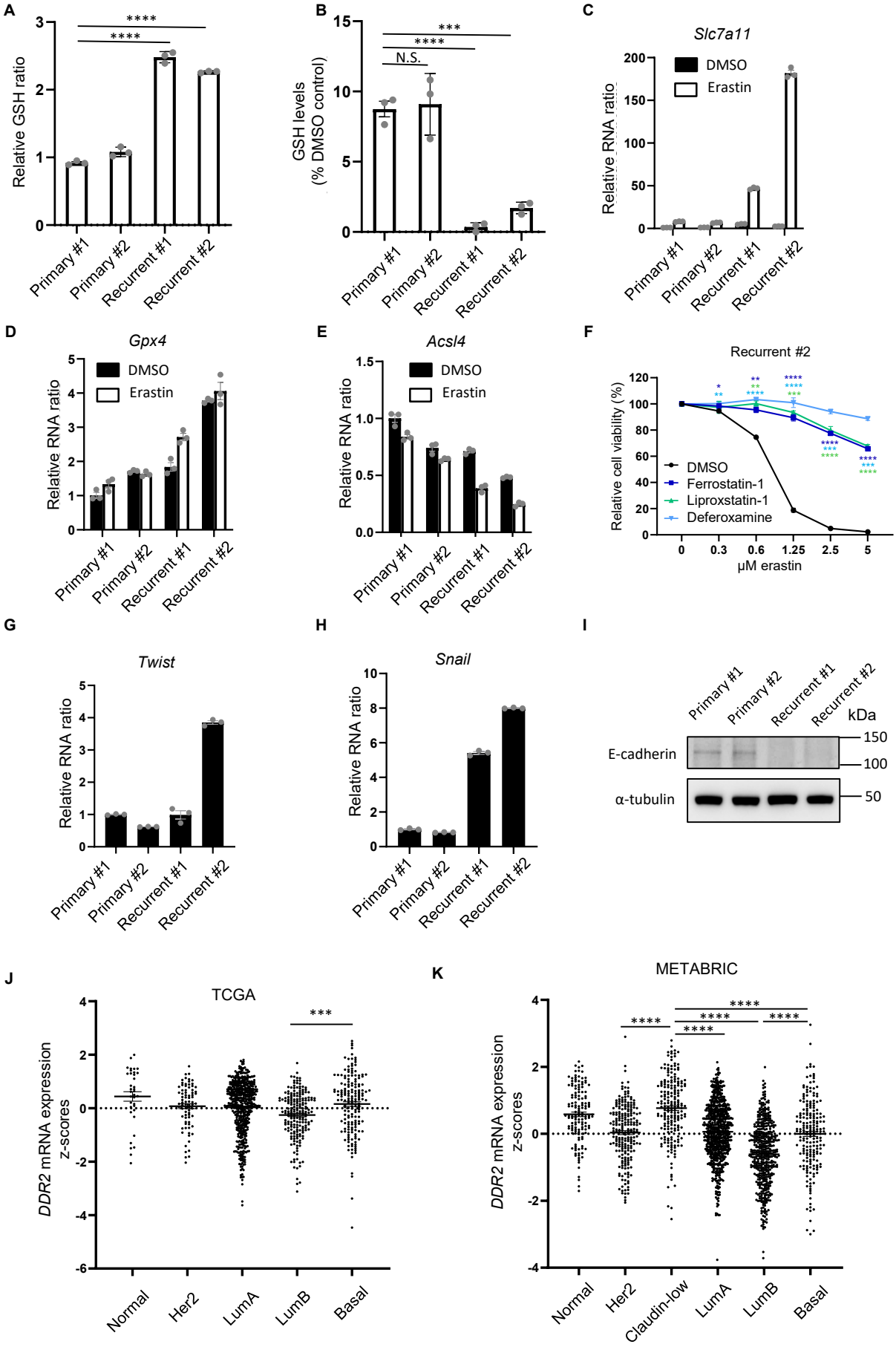
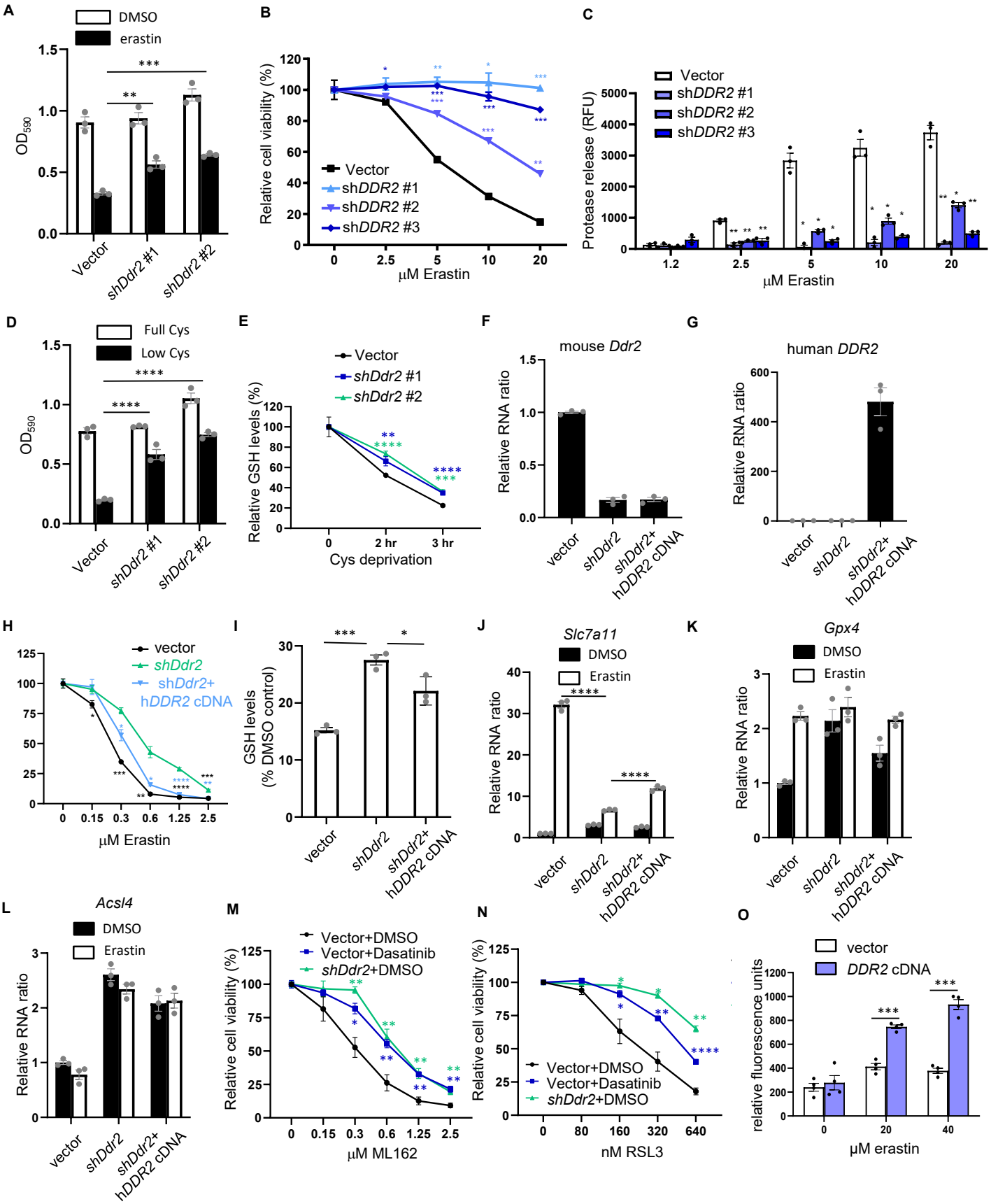

A
Relative GSH ratio
****
****
Primary #1
Primary #2
Recurrent #1
Recurrent #2
B
GSH levels (% DMSO control)
***
****
N.S.
C
Slc7a11
Relative RNA ratio
DMSO
Erastin
D
Gpx4
E
Acsl4
F
Recurrent #2
Relative cell viability (%)
DMSO
Ferrostatin-1
Liproxstatin-1
Deferoxamine
μM erastin
G
Twist
H
Snail
I
kDa
E-cadherin
α-tubulin
150
100
50
J
TCGA
DDR2 mRNA expression z-scores
Normal
Her2
LumA
LumB
Basal
K
METABRIC
Claudin-low

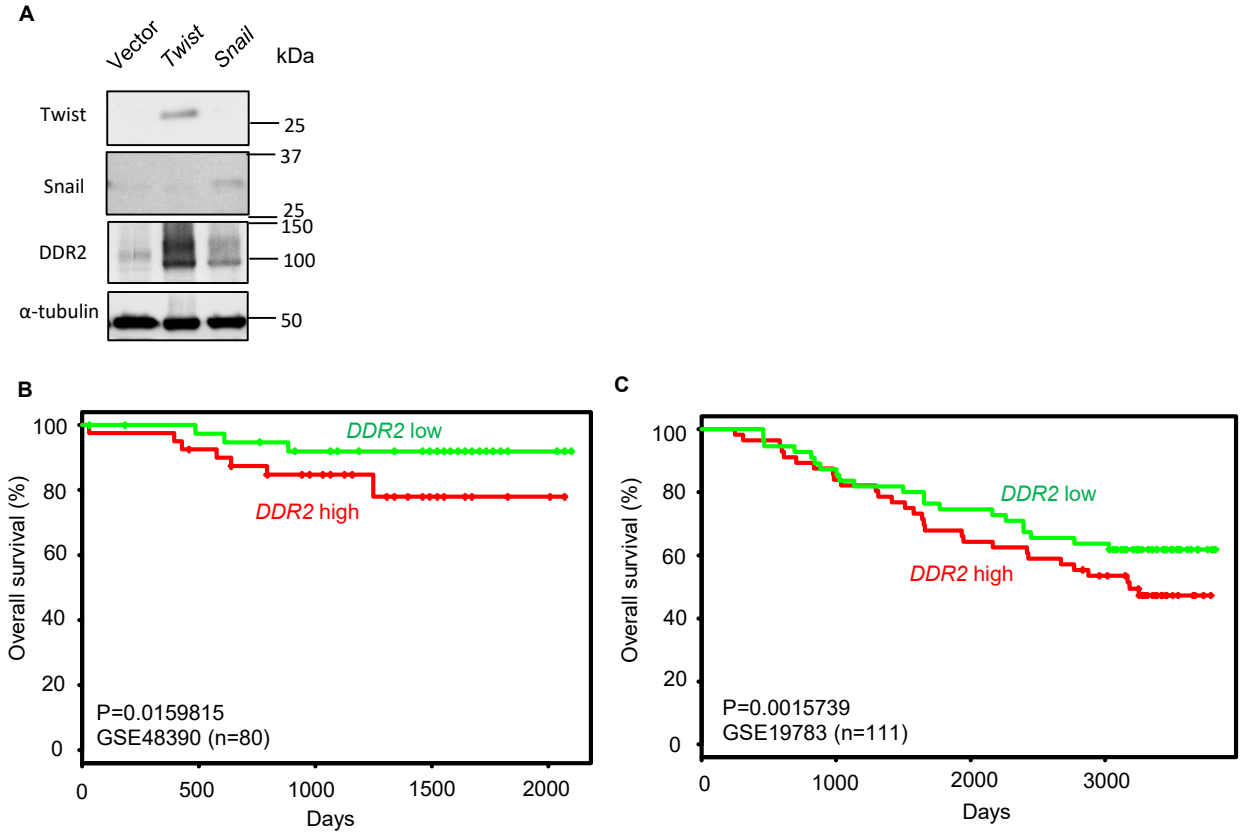
Supplementary Fig. 1

(**A**) Recurrent tumor cell lines showed a higher basal level of GSH. The GSH levels of the same number of primary and recurrent tumor cell lines were measured by GSH/GSSG-Glo assay. (**B**) GSH was depleted by erastin in recurrent tumor cells. The relative levels of GSH of primary and recurrent tumor cell lines treated with erastin (0.5 μM) for 15 hours were measured by GSH/GSSG-Glo assay. (**C-E**) Primary and recurrent tumor cell lines treated with/without erastin (0.5 μM) for 15 hours were measured by RT-PCR for the mRNA expression of *Slc7a11* (**C**), *Gpx4* (**D**), and *Acsl4* (**E**). (**F**) The erastin-induced death of an independent recurrent tumor cell was also rescued by ferroptosis inhibitors (ferrostatin-1, 10 μM; liproxstatin-1, 2 μM) and iron chelator (deferoxamine, 100 μM) as determined by Celltiter Glo assay after 19 hours of incubation. n=3 biological replicates. (**G-H**) The mRNA expression of *Twist* (**G**) and *Snail* (**H**) in primary and recurrent tumor cells were quantified by RT-PCR. (**I**) E-cadherin protein expression in primary and recurrent tumor cell lines was compared by Western blots. (**J, K**) *DDR2* mRNA expression in the different intrinsic subtypes of breast cancer in TCGA dataset (**J**) and METABRIC dataset (**K**). (**F**) Two-way ANOVA, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 Dunnett's multiple comparisons. (**A, B, J, K**) One-way ANOVA, ***p<0.001, ****p<0.0001. Bars show standard error of the mean.


A
DMSO
erastin
OD590
Vector
shDdr2 #1
shDdr2 #2
B
Relative cell viability (%)
Vector
shDDR2 #1
shDDR2 #2
shDDR2 #3
μM Erastin
C
Protease release (RFU)
Vector
shDDR2 #1
shDDR2 #2
shDDR2 #3
μM Erastin
D
Full Cys
Low Cys
OD590
E
Relative GSH levels (%)
Vector
shDdr2 #1
shDdr2 #2
Cys deprivation
F
mouse Ddr2
Relative RNA ratio
G
human DDR2
Relative RNA ratio
H
vector
shDdr2
shDdr2+ hDDR2 cDNA
μM Erastin
I
GSH levels (% DMSO control)
J
Slc7a11
DMSO
Erastin
Relative RNA ratio
K
Gpx4
DMSO
Erastin
Relative RNA ratio
L
Acsl4
DMSO
Erastin
Relative RNA ratio
M
Vector+DMSO
Vector+Dasatinib
shDdr2+DMSO
Relative cell viability (%)
μM ML162
N
Vector+DMSO
Vector+Dasatinib
shDdr2+DMSO
Relative cell viability (%)
nM RSL3
O
vector
DDR2 cDNA
relative fluorescence units
μM erastin

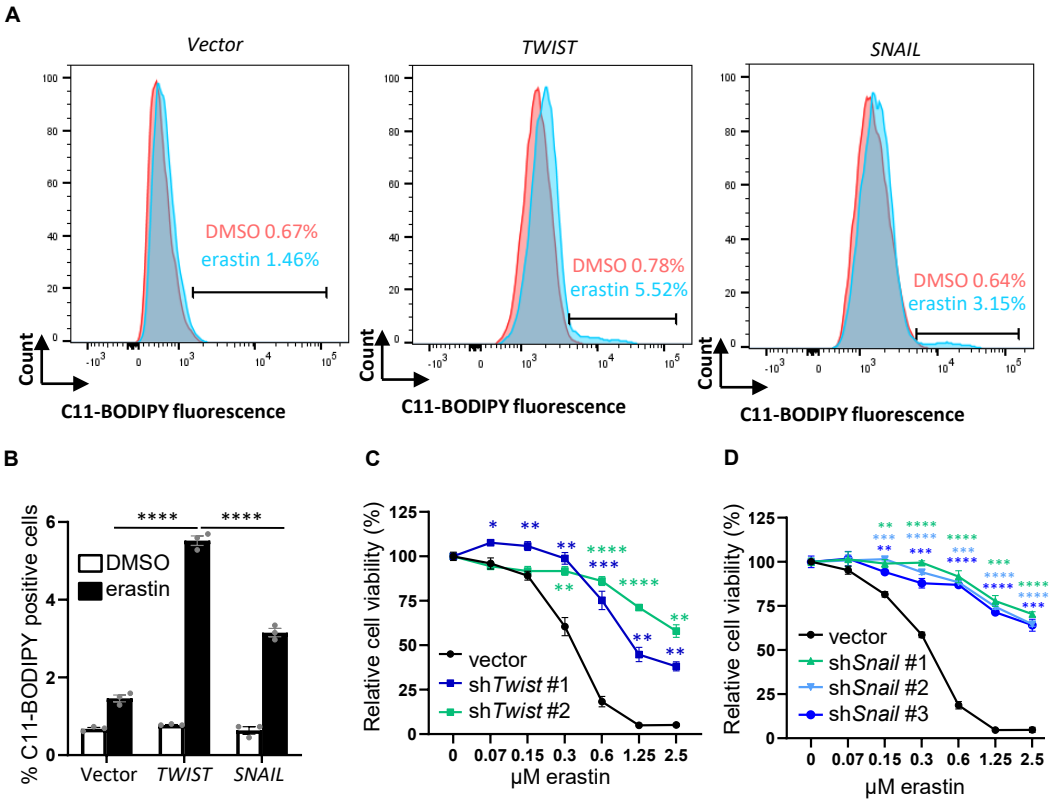
Supplementary Fig. 2

(**A**) Quantification of crystal violet staining in Fig. 2B. n=3 biological replicates. (**B-C**) MDA-MB-231 cells transduced with control or *DDR2* shRNAs were treated with an increasing dose of erastin for 18 hours and the cell viability was determined by CellTiter Glo assay (**B**) or protease release upon death by CellTox Glo assay (**C**). (**D**) Quantification of crystal violet staining in Fig. 2G. n=3 biological replicates. (**E**) Mouse recurrent tumor cells transduced with *Ddr2* shRNAs were incubated in cystine-free media for 2-3 hours. The relative levels of GSH levels, when compared to full media, were shown. (**F**) Validation of mouse *Ddr2* knockdown by mouse *Ddr2*-targeting shRNA in mouse recurrent tumor cells using RT-PCR. (**G**) Validation of increased human *DDR2* (h*DDR2*) mRNA expression upon h*DDR2* cDNA transduction in mouse recurrent tumor cells using RT-PCR. (**H-L**) Overexpression of mouse shRNA-resistant h*DDR2* cDNA in mouse recurrent tumor cells transduced with mouse *Ddr2* shRNA was analyzed for cell viability by CellTiter Glo assay after 19 hours of erastin (**H**), relative GSH levels under erastin (0.125 μM, 15 hours)(**I**) and mRNA expression of *Slc7a11* (**J**), *Gpx4* (**K**) and *Acsl4* (**L**) (0.5 μM, 15 hours). (**M, N**) Both genetic or pharmacological suppression (dasatinib, 1μM) of DDR2 conferred resistance of mouse recurrent tumor cells to ML162 (**M**) and RSL3 (**N**) for 18 hours. (**O**) Primary tumor cells transduced with DDR2 cDNA showed increased cytotoxicity as quantified by CellTox Green assay after 24 hours of incubation. (**B, E, H, M, N**) n=3 biological replicates. (**A, B, C, D, E, H, M, N, O**) Two-way ANOVA, (**I**) One-way ANOVA, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, Dunnett's multiple comparisons. Bars show S.E.M..



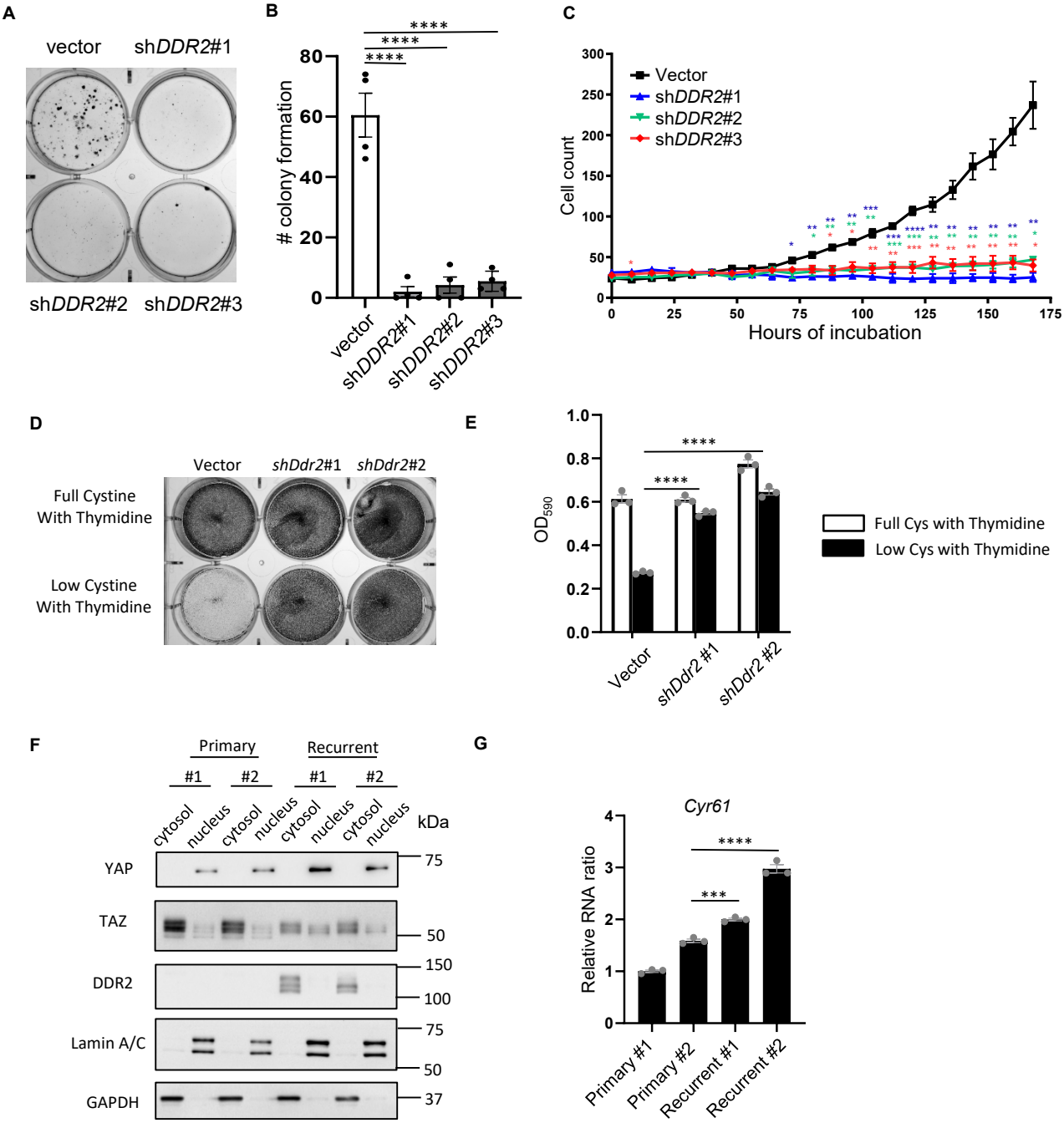
Supplementary Fig. 3

(**A**) *Twist* and *Snail* increased DDR2 protein expression. Primary tumor cells transduced with *Twist* or *Snail* cDNA showed an increase in DDR2 protein expression as determined by Western blots. (**B-C**) The breast cancer patients from Huang dataset (GSE48390) (**B**) and Enerly dataset (GSE19783) (**C**) were bifurcated into high and low *DDR2* expression groups by the median of *DDR2* expression. The differences in the overall survival were compared by Kaplan-Meyer analysis in the percentage of overall survival.



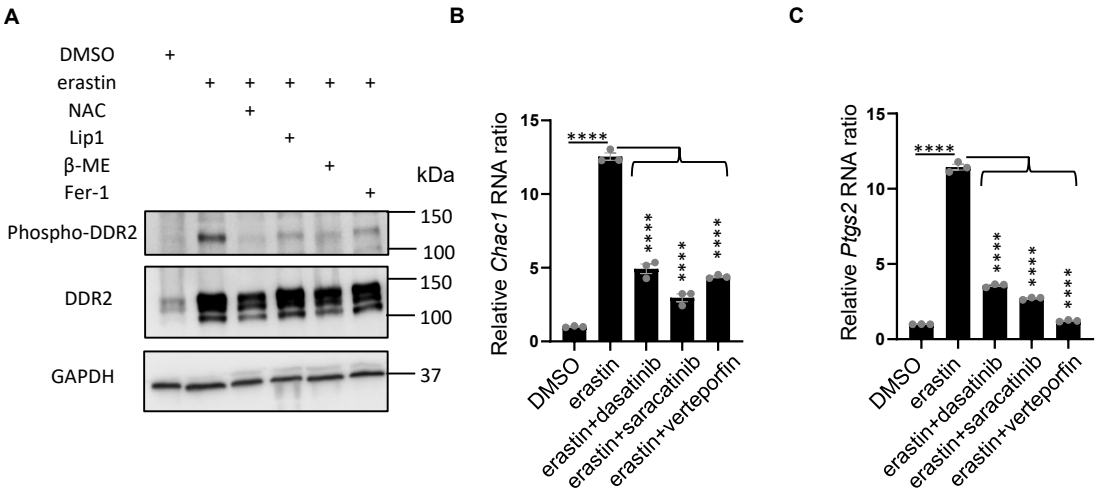
Supplementary Fig. 4

(**A-B**) T47D cells overexpressing *TWIST* or *SNAIL* showed increased lipid peroxidation after 18 hours of erastin (10 μM) treatment as determined by C11-BODIPY staining (**A**) and percentage of positive cells (**B**). (**C-D**) *Twist* or *Snail* knockdown protected ferroptosis. Recurrent tumor cells transduced with control, *Twist* shRNAs (**C**) or *Snail* shRNAs (**D**) were treated with erastin for 19 hours and the viability was determined by CellTiter Glo. n=3 biological replicates.


A
vector
shDDR2#1
shDDR2#2
shDDR2#3
B
colony formation
****
****
****
vector
shDDR2#1
shDDR2#2
shDDR2#3
C
Cell count
Hours of incubation
Vector
shDDR2#1
shDDR2#2
shDDR2#3
D
Vector
shDdr2#1
shDdr2#2
Full Cystine
With Thymidine
Low Cystine
With Thymidine
E
OD590
****
****
Vector
shDdr2 #1
shDdr2 #2
Full Cys with Thymidine
Low Cys with Thymidine
F
Primary
Recurrent
#1
#2
#1
#2
cytosol
nucleus
kDa
YAP
TAZ
DDR2
Lamin A/C
GAPDH
75
50
150
100
75
50
37
G
Cyr61
Relative RNA ratio
****
***
Primary #1
Primary #2
Recurrent #1
Recurrent #2

Supplementary Fig. 5

(**A-B**) The clonogenic assay showed a reduced amount of colony formation under the *DDR2* knockdown. MDA-MB-231 cells transduced with control or three *DDR2* shRNAs were plated on 6 well plates (250 cells/well) and incubated for 10 days. The cells were then fixed by paraformaldehyde and stained with crystal violet for direct counting of colony numbers as quantified in (**B**). (**C**) MDA-MB-231 cells with *DDR2* knockdown showed a reduction in cell proliferation under image-based monitoring of cell numbers over the indicated number of hours. MDA-MB-231 cells stably expressing histone H2B-mCherry were transduced by control or *DDR2* shRNAs and plated in a 96-well plate. The image of each well was taken every 8 hours using Incucyte S3 for the quantification of cell numbers. (**D, E**) Thymidine did not abolish the protective effects of *Ddr2* knockdown under cystine deprivation. Recurrent tumor cells transduced with control or *Ddr2* shRNAs were treated with cystine deprivation (2.5 μM) and thymidine (2 mM) and the cell numbers were determined by crystal violet staining after 16 hours of incubation (**D**) and quantification of crystal violet staining (**E**). (**F**) Nuclear/cytosol fractionation showed more nuclear YAP/TAZ accumulation in recurrent tumor cells than primary tumor cells. GAPDH: cytosolic marker; Lamin A/C: nuclear marker. (**G**) RT-PCR showed higher *Cyr61* mRNA expression in recurrent tumor cells. (**B**) One-way ANOVA, ****$p < 0.0001$ Tukey's multiple comparisons. (**C, E**) Two-way ANOVA, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ Dunnett's multiple comparisons. Bars show S.E.M..



Supplementary Fig. 6

(**A**) Antioxidants and ferroptosis inhibitors abolished erastin-induced DDR2 phosphorylation at Y740. Recurrent tumor cells under erastin treatment (0.5 μM) were co-treated with DMSO, N-acetyl cysteine (NAC, 5 mM), liproxstatin-1 (Lip-1, 2 μM), 2-Mercaptoethanol (β-ME, 50 μM) or ferrostatin-1 (Fer-1, 10 μM) for 16 hours and stained with phospho-DDR2(Y740) antibody for DDR2 phosphorylation and activation. (**B-C**) Pharmacological inhibition of DDR2, Src, and YAP suppressed erastin-induced induction of ferroptosis markers. Recurrent tumor cells under erastin treatment (0.5 μM, 16 hours) were co-treated with DMSO, dasatinib (1 μM), saracatinib (1 μM), or verteporfin (2 μM) for *Chac1*(**B**) and *Ptgs2* (**C**) mRNA expression was measured by RT-PCR.