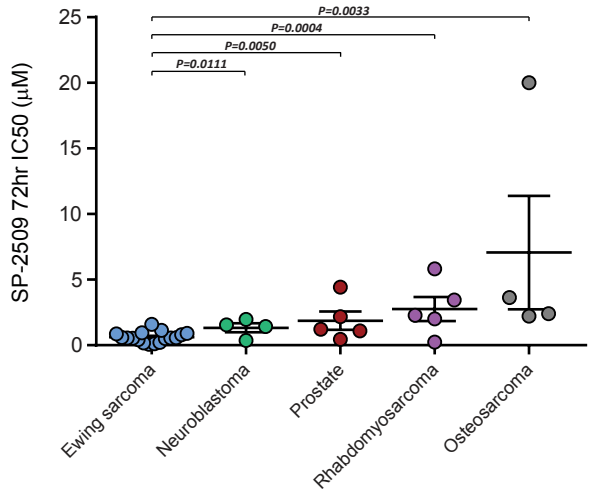
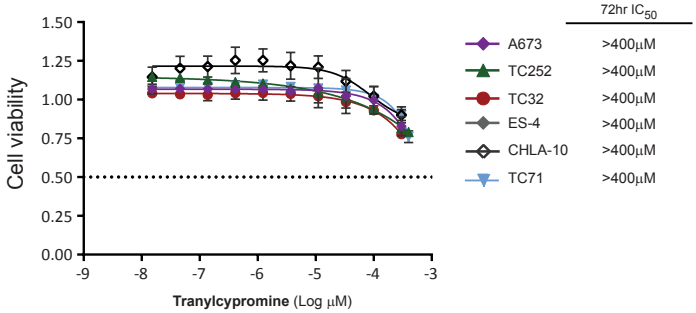
A

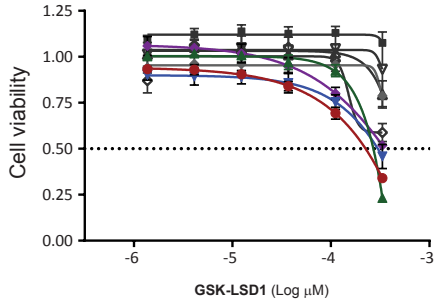


	72hr SP-2509 IC_{50}
Ewing sarcoma:	0.621 ± 0.095
Neuroblastoma:	1.323 ± 0.342
Prostate:	1.875 ± 0.698
Rhabdomyosarcoma:	2.761 ± 0.919
Osteosarcoma:	7.066 ± 4.323

B

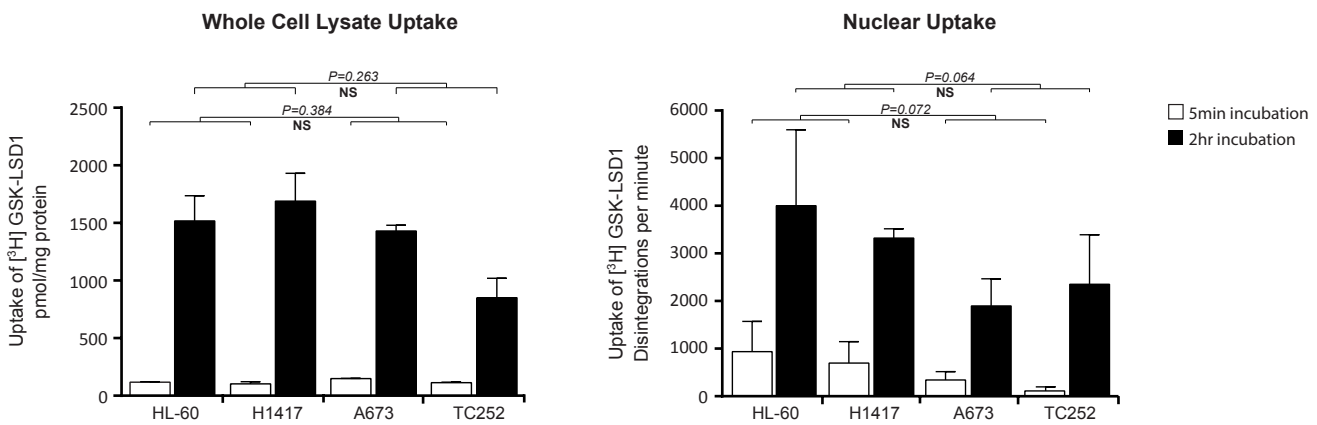


Tranylcypromine 72hr IC_{50}	
A673	>400μM
TC252	>400μM
TC32	>400μM
ES-4	>400μM
CHLA-10	>400μM
TC71	>400μM

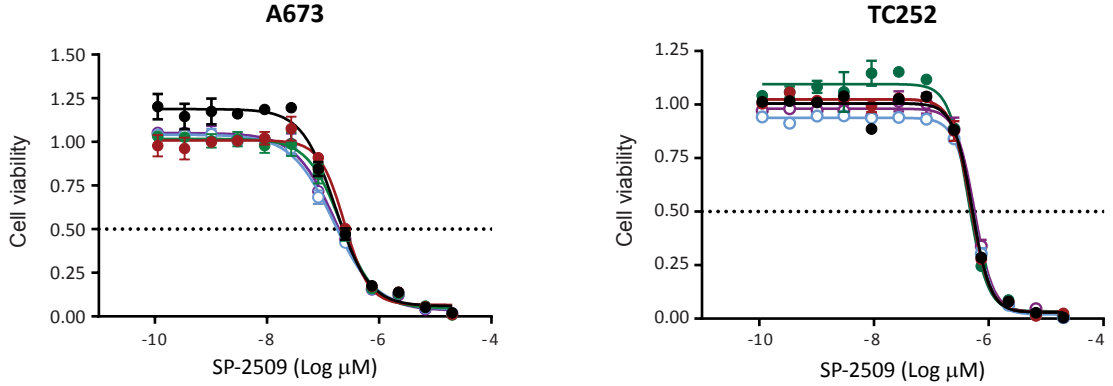


GSK-LSD1 144hr IC_{50}	
A673	346.83 ± 11.29
TC252	266.23 ± 13.00
TC32	221.68 ± 18.03
ES-4	>350μM
TTC-466	>350μM
CHLA-258	300.14 ± 43.82
EWS-502	>350μM
CHLA-10	>350μM
ES-2	>350μM

C



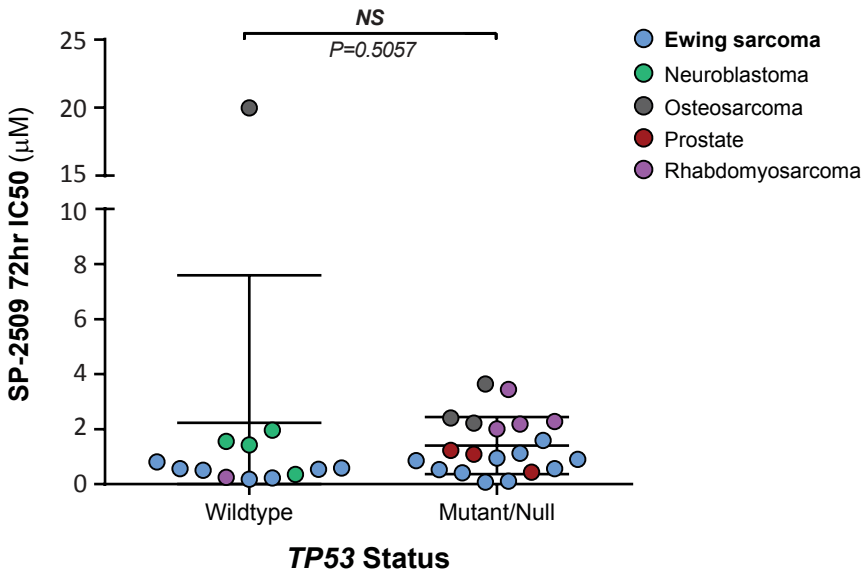
D



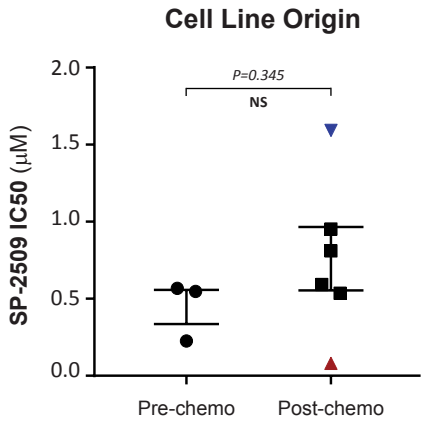
SP-2509 alone
No pre-treatment/GSK-LSD1 2μM alone
No pre-treatment/GSK-LSD1 50μM alone
2μM GSK-LSD1 pre-treatment/2μM GSK-LSD1 + SP-2509
50μM GSK-LSD1 pre-treatment/50μM GSK-LSD1 + SP-2509

	A673 72hr SP-2509 IC50	TC252 72hr SP-2509 IC50
SP-2509 alone	0.224μM	0.513μM
No pre-treatment/GSK-LSD1 2μM alone	0.174μM	0.525μM
No pre-treatment/GSK-LSD1 50μM alone	0.191μM	0.562μM
2μM GSK-LSD1 pre-treatment/2μM GSK-LSD1 + SP-2509	0.251μM	0.525μM
50μM GSK-LSD1 pre-treatment/50μM GSK-LSD1 + SP-2509	0.219μM	0.479μM

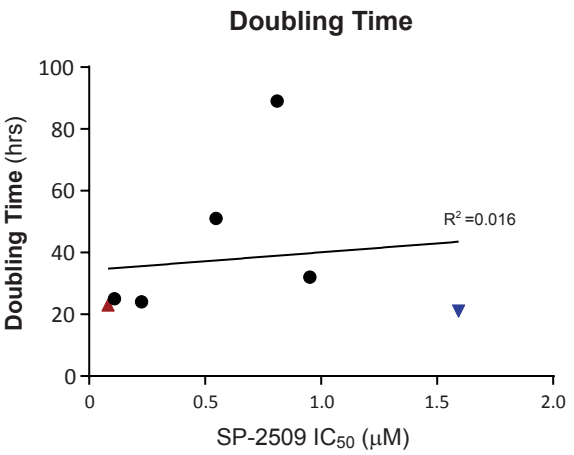
E


NS
P=0.5057
SP-2509 72hr IC50 (μM)
25
20
15
10
8
6
4
2
0
Wildtype
Mutant/Null
TP53 Status
Ewing sarcoma
Neuroblastoma
Osteosarcoma
Prostate
Rhabdomyosarcoma

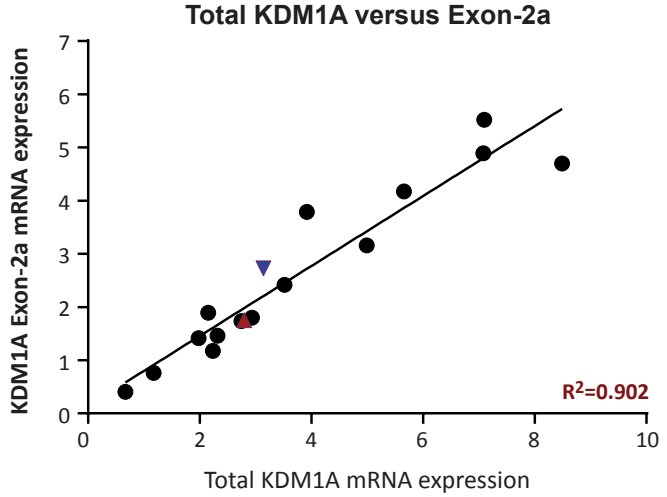
F

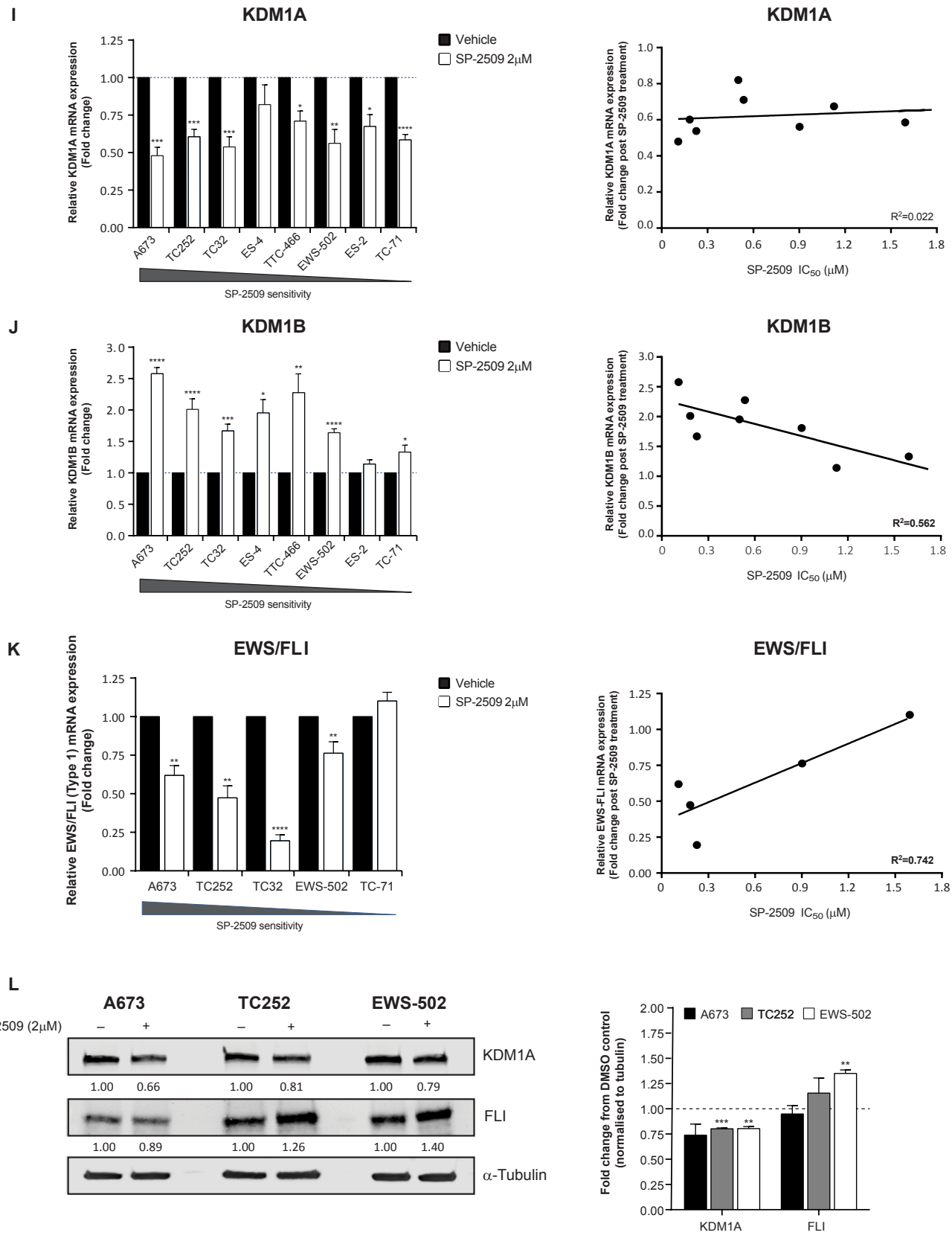

Cell Line Origin
P=0.345
NS
SP-2509 IC50 (μM)
2.0
1.5
1.0
0.5
0.0
Pre-chemo
Post-chemo

G


Doubling Time
Doubling Time (hrs)
100
80
60
40
20
0
R² =0.016
0
0.5
1.0
1.5
2.0
SP-2509 IC50 (μM)

H


Total KDM1A versus Exon-2a
KDM1A Exon-2a mRNA expression
7
6
5
4
3
2
1
0
R²=0.902
0
2
4
6
8
10
Total KDM1A mRNA expression


I
KDM1A
Relative KDM1A mRNA expression (Fold change)
Vehicle
SP-2509 2μM
A673
TC252
TC32
ES-4
TTC-466
EWS-502
ES-2
TC-71
SP-2509 sensitivity
KDM1A
Relative KDM1A mRNA expression (Fold change post SP-2509 treatment)
R²=0.022
SP-2509 IC50 (μM)
J
KDM1B
Relative KDM1B mRNA expression (Fold change)
Vehicle
SP-2509 2μM
SP-2509 sensitivity
KDM1B
Relative KDM1B mRNA expression (Fold change post SP-2509 treatment)
R²=0.562
SP-2509 IC50 (μM)
K
EWS/FLI
Relative EWS/FLI (Type 1) mRNA expression (Fold change)
Vehicle
SP-2509 2μM
SP-2509 sensitivity
EWS/FLI
Relative EWS-FLI mRNA expression (Fold change post SP-2509 treatment)
R²=0.742
SP-2509 IC50 (μM)
L
A673
TC252
EWS-502
SP-2509 (2μM)
KDM1A
FLI
α-Tubulin
1.00 0.66 1.00 0.81 1.00 0.79
1.00 0.89 1.00 1.26 1.00 1.40
Fold change from DMSO control (normalised to tubulin)
KDM1A
FLI

Supplementary Figure S3: *Cytotoxic effects of the irreversible KDM1A inhibitors GSK-LSD1 and Tranylcypromine in Ewing sarcoma cell lines*

(A) Sensitivity of Ewing (n=17), Neuroblastoma (n=4), Prostate (n=5), Rhabdomyosarcoma (n=5) and Osteosarcoma (n=4) cell lines to SP-2509. Cell viability determined through Cell Titer Glo (CTG) analysis 72hrs post treatment. Data represents mean ± SEM from three independent experiments. Neuroblastoma and Prostate IC_{50} data taken from Gupta et al., 2016 and 2018. **(B)** Ewing sarcoma cell lines were treated with escalating concentrations of either Tranylcypromine or GSK-LSD1, with cell viability determined through CTG assays 72 and 144hrs post treatment respectively. Data represents mean ± STDEV from two independent experiments (GSK-LSD1) or mean ± SEM from three independent experiments (Tranylcypromine). **(C)** Whole cell and nuclear uptake of [^{3}H] GSK-LSD1 in A673/TC252 (Ewing sarcoma), HL-60 (AML) and H1417 (SCLC) cell lines following 5min and 2hr incubation with tritiated compound. **(D)** A673 and TC252 cells were treated with either GSK-LSD1 (2μM or 50μM) or DMSO for 48hrs prior to treatment with SP-2509 alone or in combination with GSK-LSD1 for an additional 72hrs. Cell viability determined through CTG analysis. Data represents mean viability ± SEM from triplicate reactions. Lack of correlation between **(E)** *TP53* status, **(F)** cell line origin (pre or post-chemotherapy treatment), and **(G)** cell line doubling time with SP-2509 sensitivity. Doubling time taken from May et al., 2013. **(H)** Correlation between total and Exon-2a specific KDM1A mRNA expression levels in our Ewing sarcoma cell line cohort (n=17). KDM1A mRNA levels (fold change) normalized to IMR90 cells. Data represents mean ± STDEV from two independent experiments. ▲▼Denotes most and least SP-2509 sensitive cell line (SK-N-MC and TC-71) respectively. Relative mRNA expression levels of **(I)** KDM1A, **(J)** KDM1B and **(K)** EWS/FLI in Ewing sarcoma cell lines following treatment with vehicle control (DMSO) or SP-2509 (2μM) for 48hrs. Data represents mean expression ± SEM from three independent experiments. Cell lines are ranked in order of SP-2509 sensitivity (IC_{50}). Correlation between KDM1A, KDM1B and EWS/FLI (Type 1) mRNA levels post SP-2509 treatment and SP-2509 sensitivity (IC_{50}) are also shown. **(L)** Western blot analysis of KDM1A and FLI protein levels in A673, TC252 and EWS-502 cells treated as in **(I).** Quantification of protein levels (densitometry) following SP-2509 treatment (48hrs). Protein normalized to α-tubulin (loading control) and vehicle (DMSO) control. Data represents mean ± STDEV from two independent experiments. Asterisks denote statistical significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$,****$P < 0.0001$).