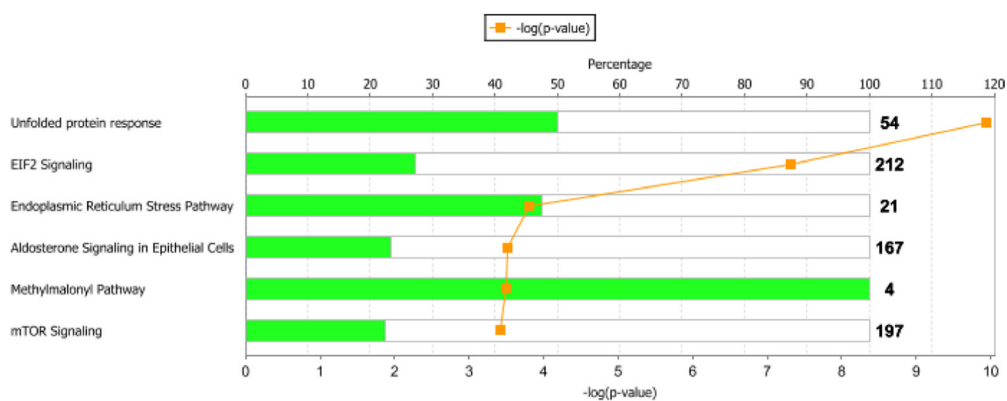


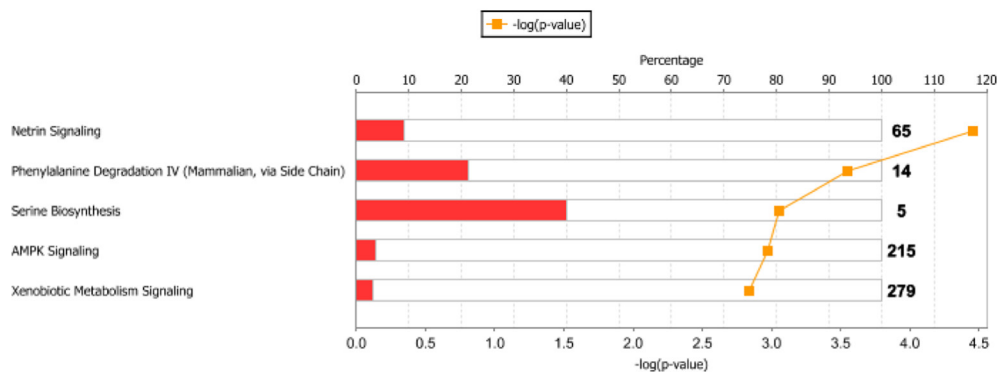
## Ewing sarcoma resistance to SP-2509 is not mediated through KDM1A/LSD1 mutation

### SUPPLEMENTARY MATERIALS

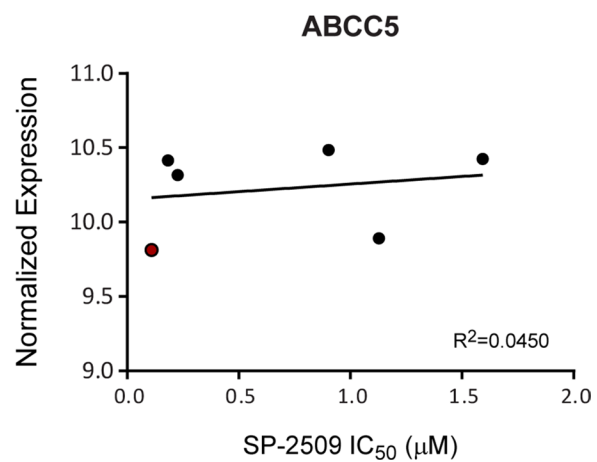
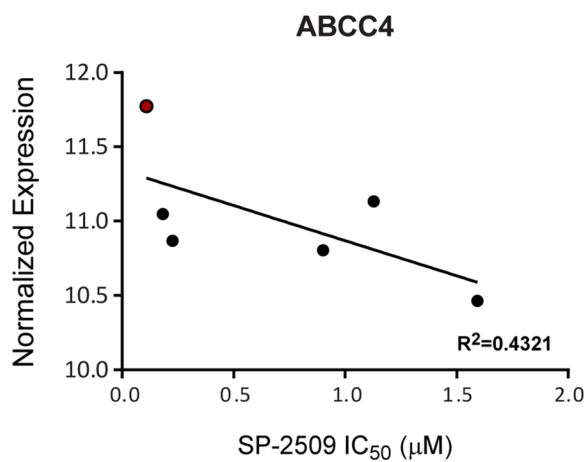
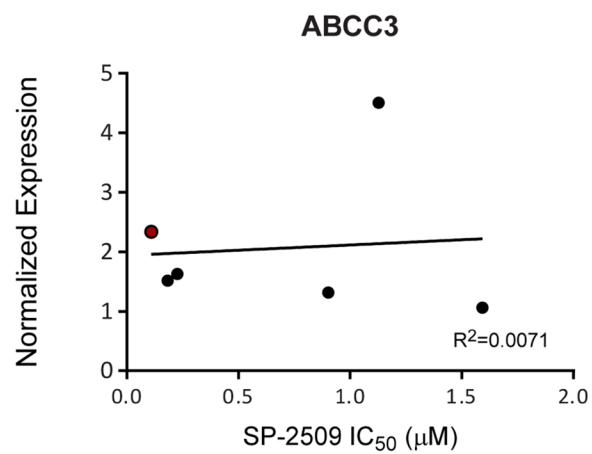
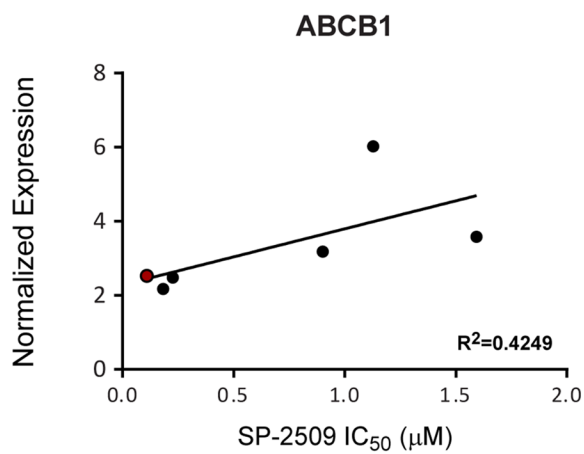
#### Up-regulated by SP-2509 in parental A673 cells



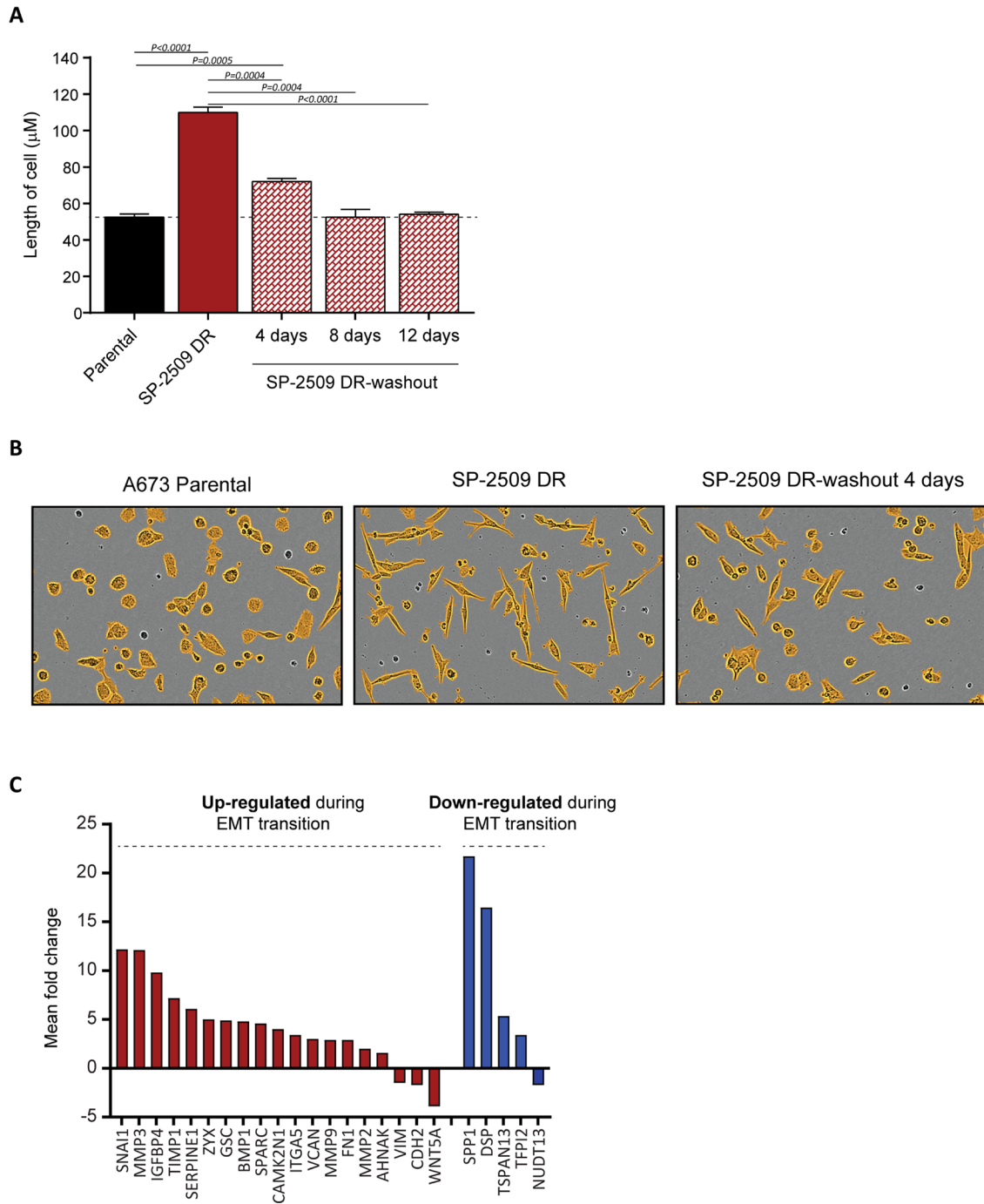
#### Up-regulated by SP-2509 in SP-2509 DR A673 cells



**Supplementary Figure 1: SP-2509 drug resistant cells do not engage the endoplasmic reticulum stress response following treatment with SP-2509.** RNA-Seq IPA of genes significantly upregulated (>1.5 fold) in parental and SP-2509 drug resistant (DR) cells following treatment with SP-2509 (2  $\mu$ M, 48 hrs). The percentage gene pathway overlap is shown. Bold numbers indicate the number of genes in that specific pathway.

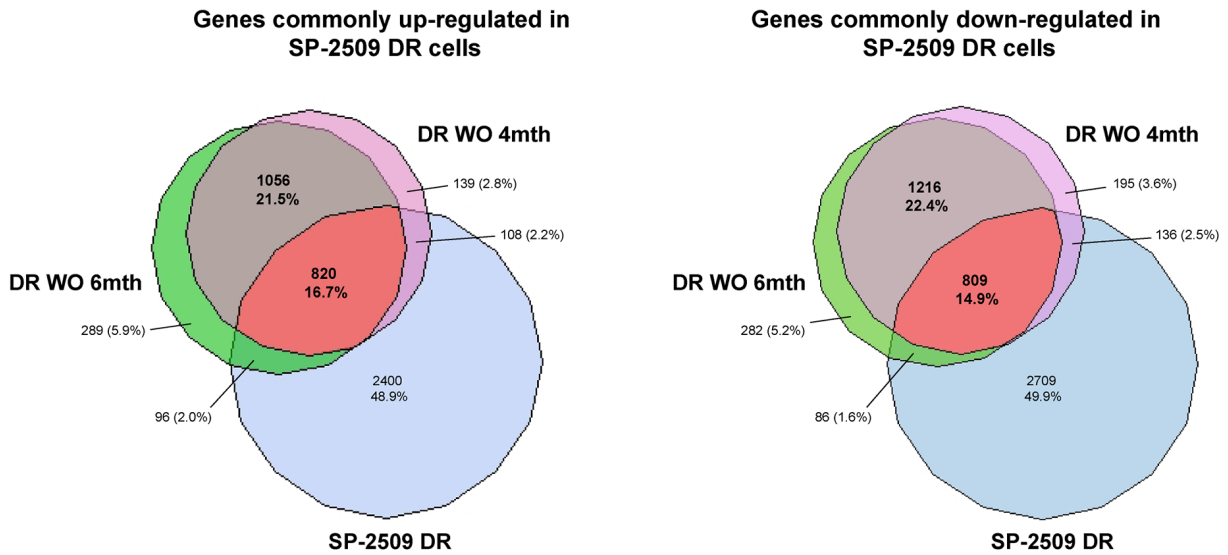


**Supplementary Figure 2: Expression of *ABCB1* and *ABCC4* correlate with sensitivity to SP-2509.** Correlation between sensitivity to SP-2509 and expression of multi-drug resistance genes. Ewing sarcoma cell line ( $n = 6$ ) SP-2509 sensitivity determined through CellTiter Glo analysis, 72 hrs post treatment. Data taken from Pishas *et al.*, 2018. Normalized expression of *ABCB1*, *ABCC3*, *ABCC4* and *ABCC5* taken from RNA-Seq analysis. ● Denotes A673 cells.



**Supplementary Figure 3: SP-2509 drug resistant cells display a reversible EMT morphology.** (A) Quantification of cell length in A673 parental, SP-2509 drug resistant (DR) and SP-2509 DR cells post washout for the indicated times. Data represents mean length  $\pm$  SEM from triplicate wells. 10 cells per well, 18 hrs post seeding were analyzed. (B) Representative phase contrast IncuCyte Zoom images of parental and SP-2509 DR cells 18 hrs post seeding. (C) RNA-Seq expression levels of EMT (epithelial to mesenchymal transition) genes significantly (>1.5 fold) altered in SP-2509 DR cells compared to parental. Data represents mean fold change from parental cells.

**A**



**B**

**Genes commonly up-regulated in SP-2509 DR cells**

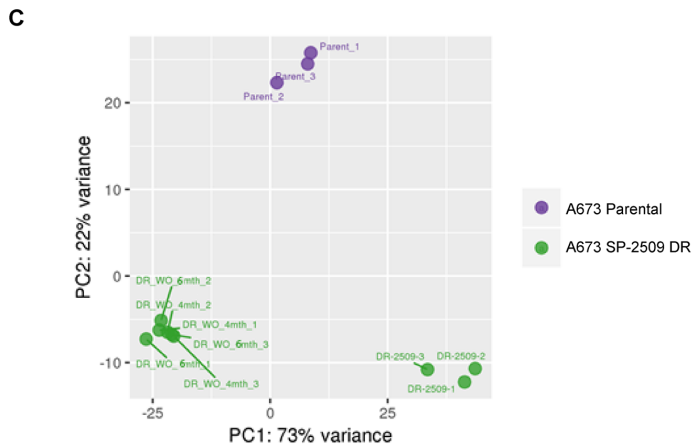
GO: Biological Process						
Rank	ID	Name	p-Value	Bonferroni	Genes from input	Genes in Annotation
1	GO:0030196	Extracellular matrix organization	1.296x10 <sup>-10</sup>	8.090x10 <sup>-7</sup>	40	354
2	GO:0043062	Extracellular structure organization	1.412x10 <sup>-10</sup>	8.815x10 <sup>-7</sup>	40	355
3	GO:0048646	Structure formation involved in morphogenesis	4.082x10 <sup>-10</sup>	2.548x10 <sup>-6</sup>	91	1299
4	GO:0001525	Angiogenesis	4.328x10 <sup>-10</sup>	2.702x10 <sup>-6</sup>	46	464

GO: Molecular Function						
Rank	ID	Name	p-Value	Bonferroni	Genes from input	Genes in Annotation
1	GO:0050840	Extracellular matrix binding	4.957x10 <sup>-7</sup>	7.524x10 <sup>-4</sup>	12	56
2	GO:0019838	Growth factor binding	3.021x10 <sup>-6</sup>	4.587x10 <sup>-3</sup>	18	142
3	GO:0005518	Collagen binding	8.107x10 <sup>-6</sup>	1.231x10 <sup>-2</sup>	12	72
4	GO:0005102	Signaling receptor binding	1.498x10 <sup>-5</sup>	2.275x10 <sup>-2</sup>	89	1601

**Genes commonly down-regulated in SP-2509 DR cells**

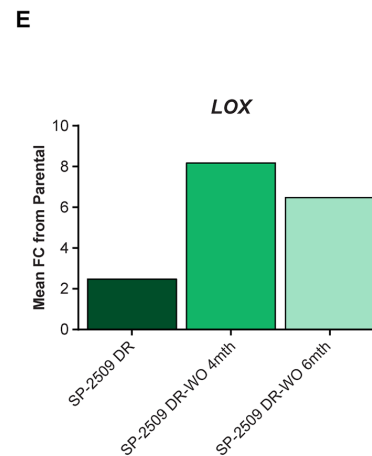
GO: Biological Process						
Rank	ID	Name	p-Value	Bonferroni	Genes from input	Genes in Annotation
1	GO:0048667	Cell morphogenesis involved in neuron differentiation	5.396x10 <sup>-8</sup>	3.148x10 <sup>-4</sup>	50	590
2	GO:0007409	Axonogenesis	5.426x10 <sup>-7</sup>	3.165x10 <sup>-3</sup>	41	475
3	GO:0048812	Neuron projection morphogenesis	9.523x10 <sup>-7</sup>	5.556x10 <sup>-3</sup>	49	630
4	GO:0000904	Cell morphogenesis involved in differentiation	9.977x10 <sup>-7</sup>	5.820x10 <sup>-3</sup>	60	840

GO: Molecular Function						
Rank	ID	Name	p-Value	Bonferroni	Genes from input	Genes in Annotation
1	GO:0000982	Transcription factor activity, RNA polymerase II proximal promoter sequence-specific DNA binding	6.278x10 <sup>-6</sup>	8.946x10 <sup>-3</sup>	32	365
2	GO:0044212	Transcription regulatory region DNA binding	7.673x10 <sup>-6</sup>	1.093x10 <sup>-2</sup>	58	860
3	GO:0000976	Transcription regulatory region sequence-specific DNA binding	8.387x10 <sup>-6</sup>	1.195x10 <sup>-2</sup>	50	704
4	GO:0001067	Regulatory region nucleic acid binding	9.441x10 <sup>-6</sup>	1.345x10 <sup>-2</sup>	58	866



**D**

NuRD/REST component	Fold change in expression from parental A673		
	SP-2509 DR	SP-2509 DR-WO 4mth	SP-2509 DR-WO 6mth
BHC80/PHF21A	-5.2	-2.2	-2.1
CHD3	-1.6		
CHD4	-1.9		
GATAD2A	-5.9		
GATAD2B			
HDAC1			
HDAC2	-5.3		
MBD2			
MBD3			
MTA1	-5.0		
MTA2	-1.6		
MTA3			
RBBP4	-2.1		
RBBP7			
RCOR1/CoREST1	-3.5	-2.3	-2
RCOR2/CoREST1	-4.1	2.1	2.5
SIN3A	-4.6		
SIN3B	-3.2		



**Supplementary Figure 4: Core SP-2509 drug resistant transcriptional profile.** (A) Venn diagram analysis of RNA-Seq genes universally up/down-regulated ( $\geq 1.5$  fold change) in SP-2509 drug resistant (DR) and SP-2509 DR-washout (WO) cells (4 and 6 months) compared to parental A673 cells. (B) ToppGene Suite analysis of GO biological and molecular function pathways associated with core SP-2509 DR genes ( $n = 820$  up-regulated and  $n = 809$  down-regulated). (C) PCA analysis of all SP-2509 DR groups. (D) Expression levels of NuRD/REST components in SP-2509 DR cells. Data expressed as normalized fold change (FC) from parental A673 cells. (E) Expression levels of *LOX* in SP-2509 DR cells. Data expressed as normalized FC from parental A673 cells.

**Supplementary Table 1: Mutations acquired during prolong passage of A673 cells.** See Supplementary\_Table\_1

**Supplementary Table 2: Mutations identified in SP-2509 DR washout cells.** See Supplementary\_Table\_2