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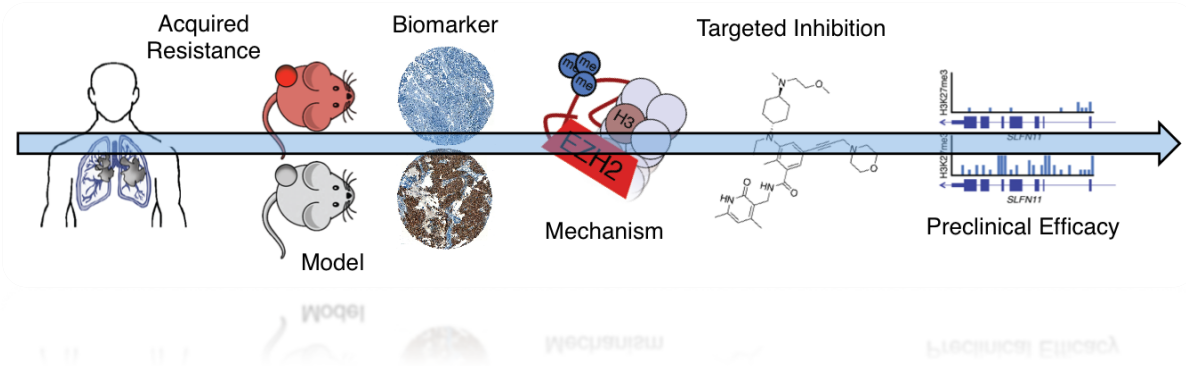
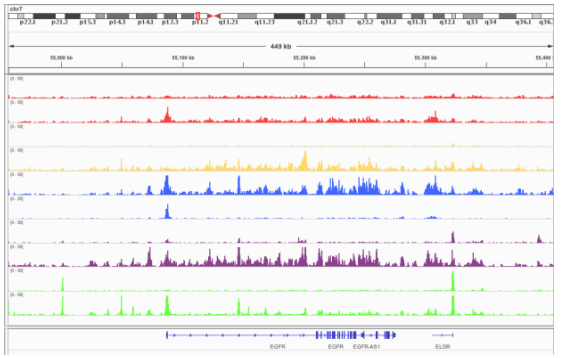
Acquired chemoresistance and EZH2



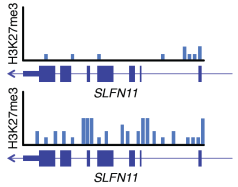


March 16th, 2018

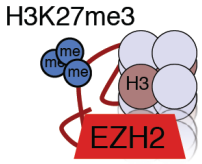
Eric E. Gardner, PharmD, PhD
Postdoctoral Associate in Medicine – Varmus Lab (WCMC)

SCLC2018 NCI meeting

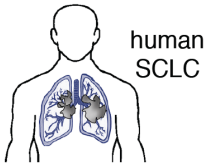
- Modeling
  - Epi/Genetics of acquired resistance to chemotherapy
  - Identifying recurrent changes in chemosensitive relapse



- *SLFN11* as an EZH2-regulated gene in SCLC
  - SLFN11 expression correlates with clinical response
  - EZH2 down-regulates SLFN11 following DNA damage



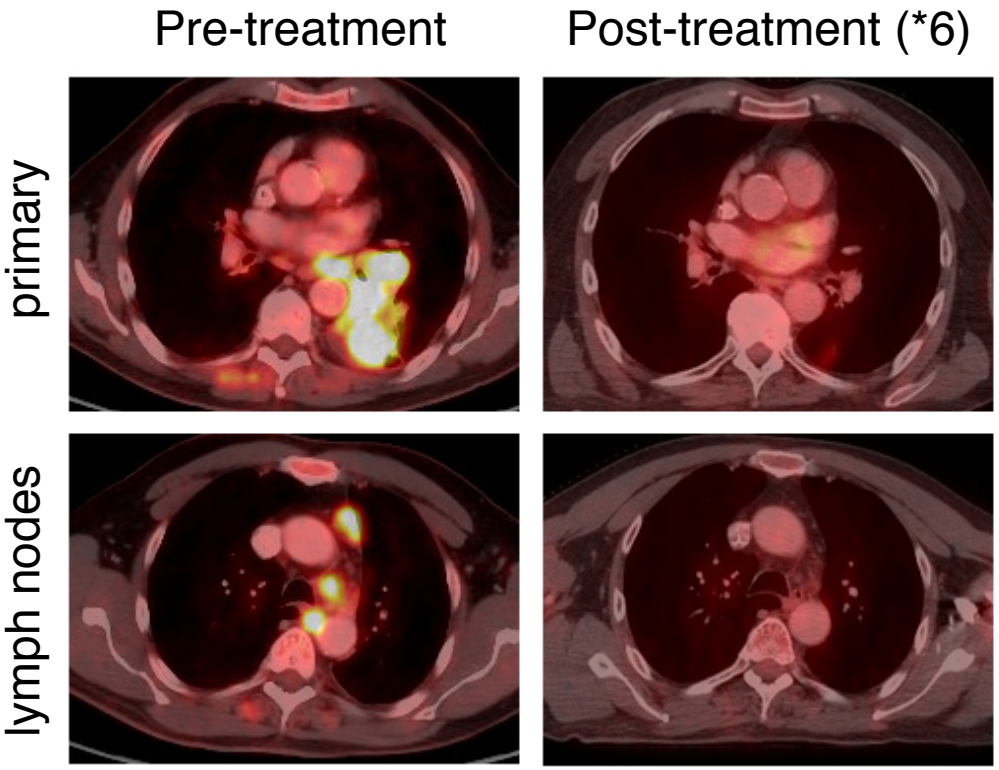
- EZH2 inhibition as a therapeutic strategy
  - Rescue of SLFN11 expression & gene body methylation
  - Potent activity with SOC in multiple models



- What happens to chromatin w/ EZH2i
  - What is permitting *SLFN11* re-expression?
  - How does chromatin in the SLFN neighborhood change w/ EZH2i?

Why model acquired resistance in PDXs?

FDG-PET scans of MSK-LX40 patient

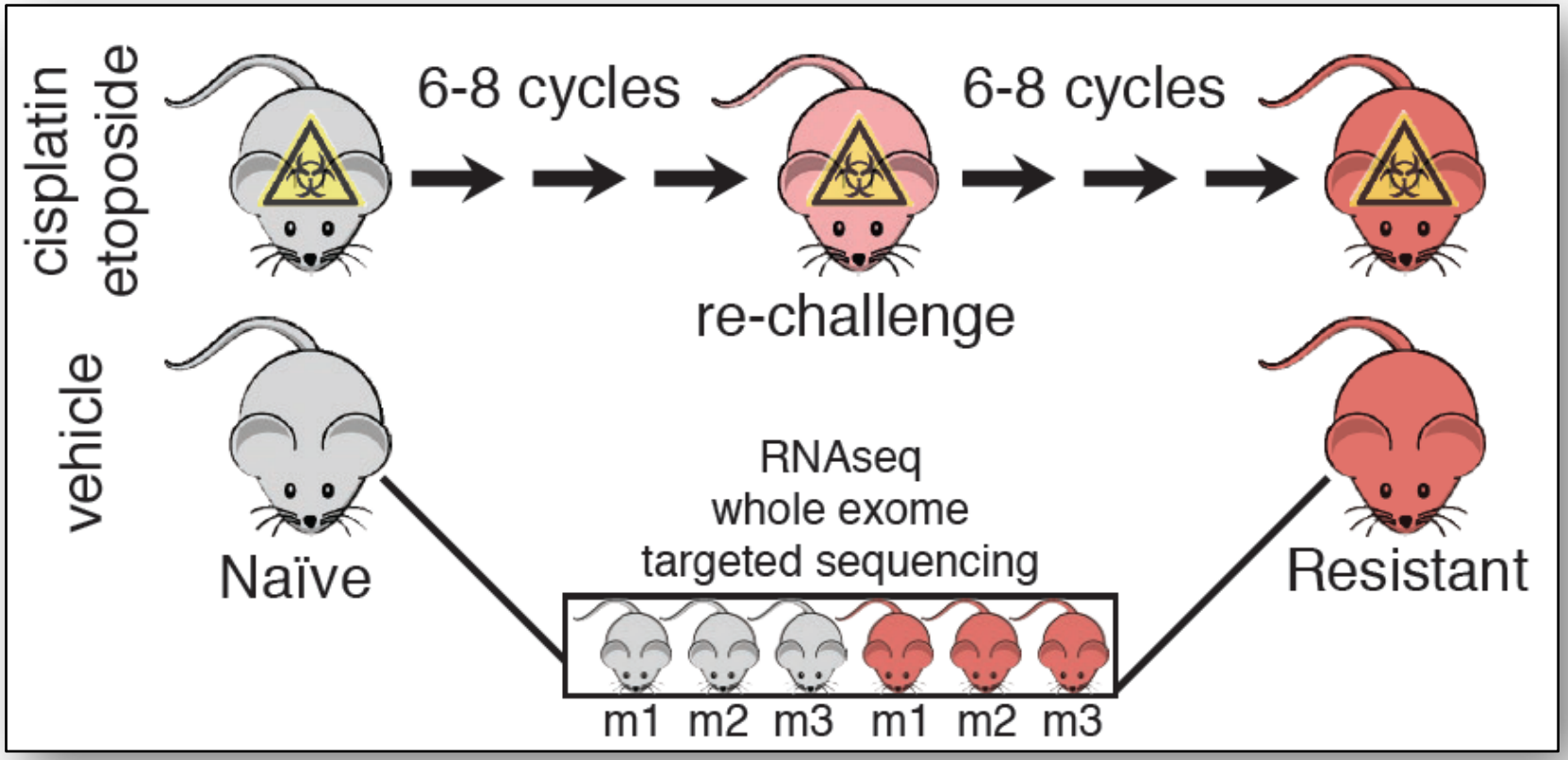


Ideal Setting…

- ✧ repeat biopsies
- ✧ multi-site sampling
- ✧ compare genomics
- ✧ basket trial inclusion
- ✧ guide decisions at relapse

- Repeat biopsies may not be feasible/possible
- No definitive biomarkers of response/resistance
- >90% of patients w/ ES-SCLC relapse

Treat & Transplant Approach to Generate Resistance



- Characterize paired models by **RNA-seq**, **WES** and targeted (**IMPACT**) sequencing
- Emphasis placed on chemonaive models that showed initial response to C/E

Gardner EE *et al*. *Cancer Cell* **2017**.

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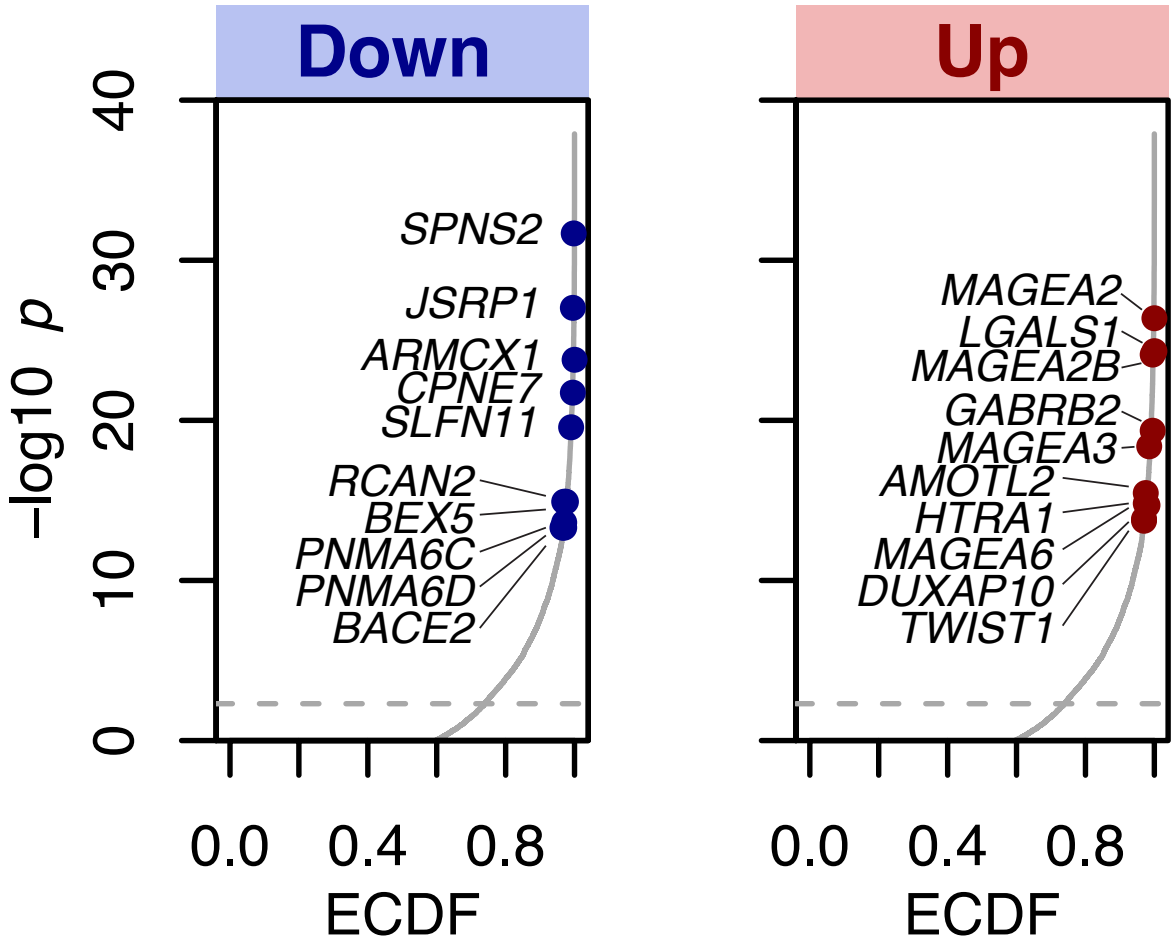
Pooled Differential Changes by RNA-sequencing



- Recurrent defined as occurrence in at least **3 of 10** models
- Up-regulation of *TWIST1* and down-regulation of *SLFN11* were **mutually-exclusive**

Gardner EE *et al*. *Cancer Cell* **2017**.

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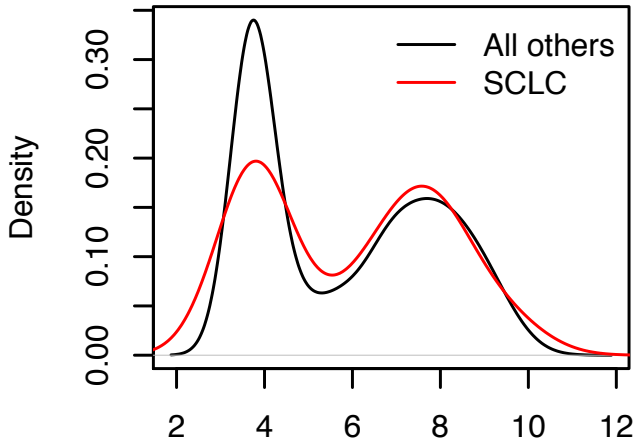
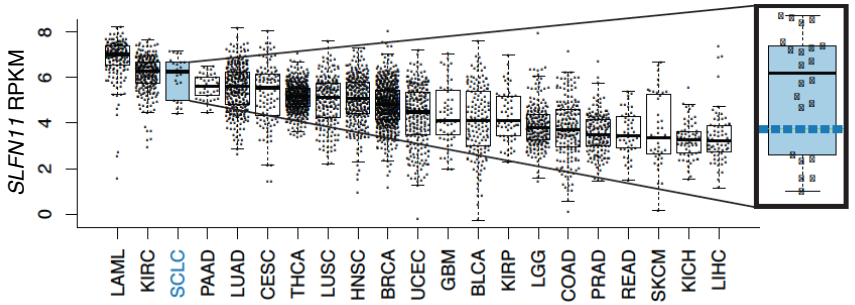
SLFN11 is high in primary SCLC & lower post-treatment

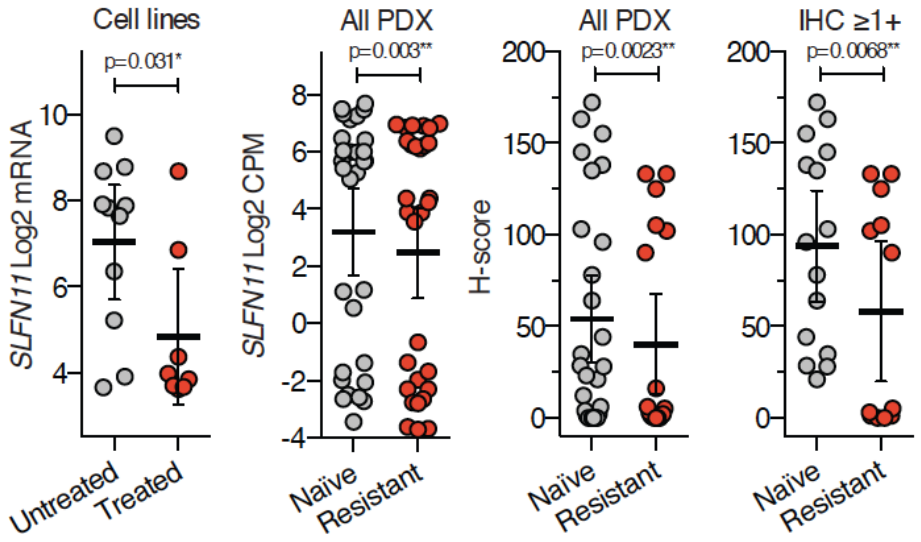
SLFN11 – SCLC cell lines (CCLE)

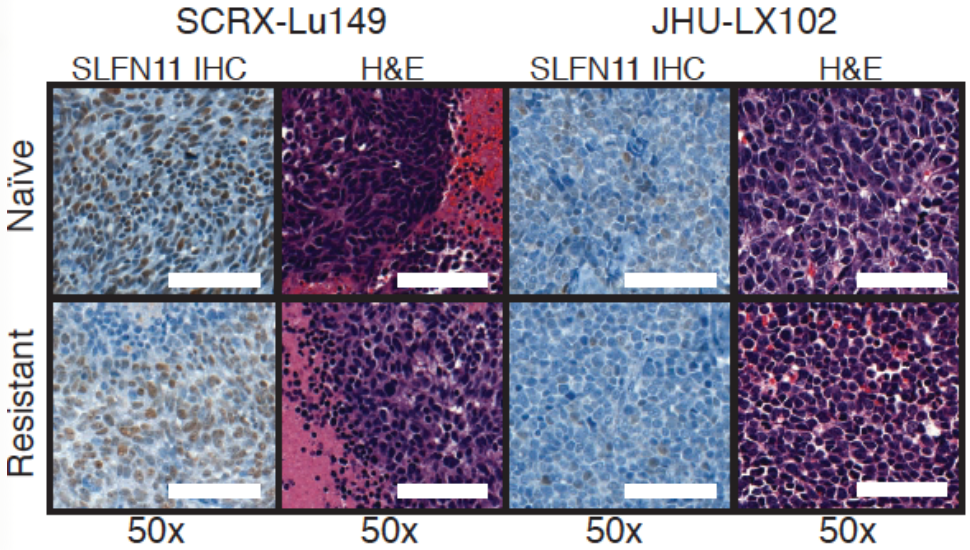


SLFN11 – primary SCLC (TCGA)



SLFN11 is significantly **decreased** in previously-treated SCLC models





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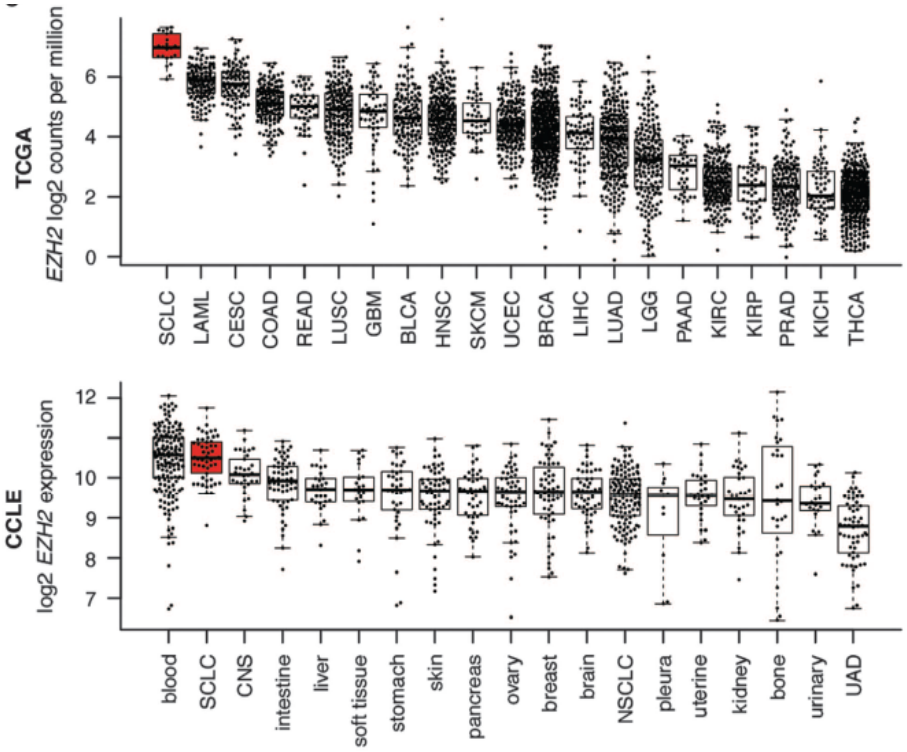
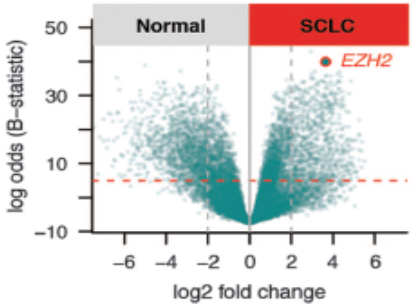
EZH2 in SCLC – *RB1* loss and E2Fs

- EZH2 inhibitors have activity in SCLC
- SWI/SNF mutations ***may*** create synthetic lethality
- Ras/MAPK activation confers resistance (rare in SCLC)

EZH2 is highest in SCLC (TCGA)



EZH2 is higher in tumor vs. adjacent lung



20% of SCLC have mutated SWI/SNF

George JS, *et al. Nature* **2015;**524:47-53 / Poirier JT, *et al. Oncogene* **2015**;34:5896-78. / Campbell JE, *et al. ACS Med. Chem. Lett.* **2015**;6:491-5. / Byers LA, *et al. Cancer Discovery* **2015**;9:798-811. / Fillmore CM, *et al. Nature* **2015**;520:239-41. / Kim KH, et al. Nature Medicine **2015**;12:1491-6.

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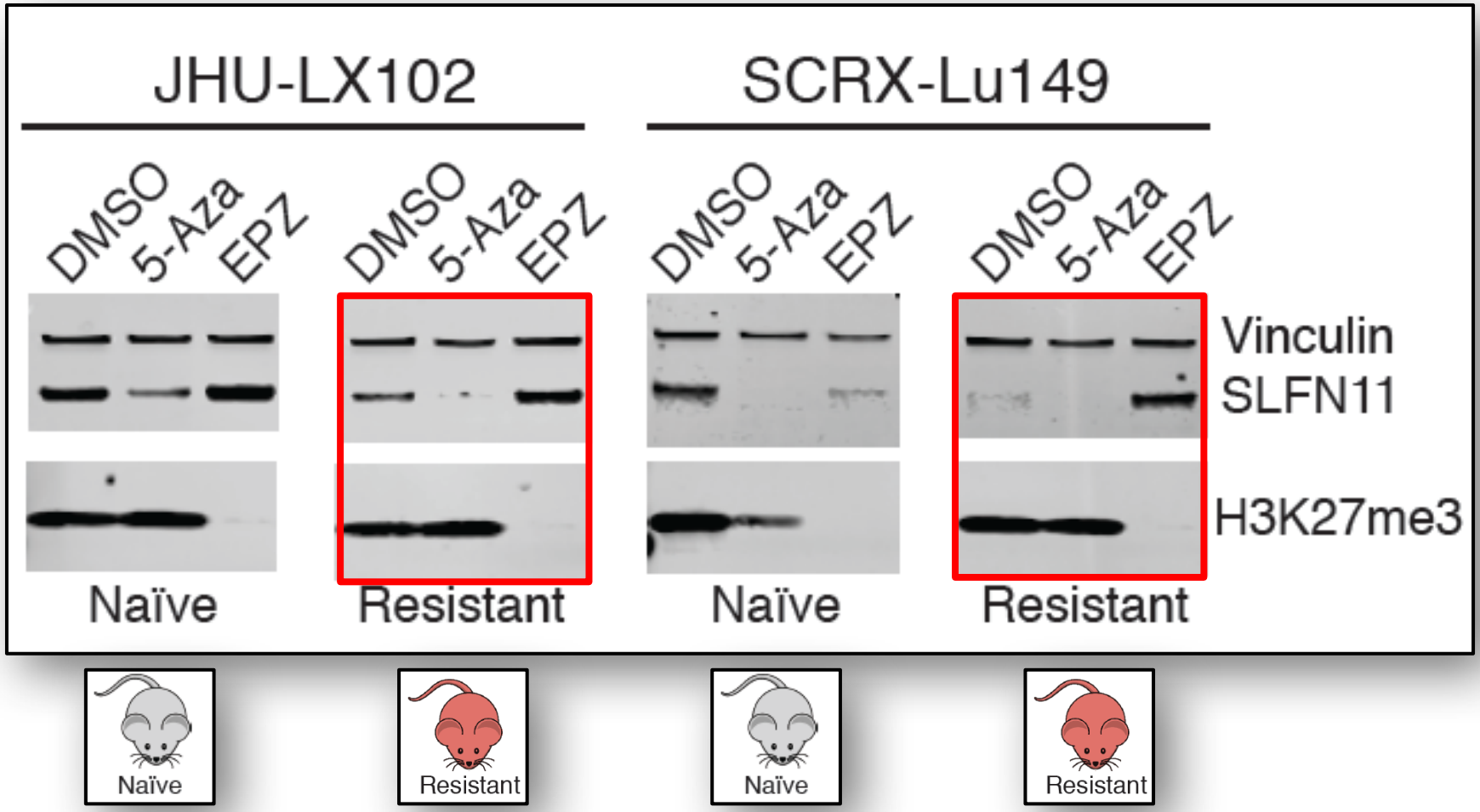
Chemical Inhibition of EZH2 can restore SLFN11



- Broad DNA methylation inhibitors do not appear to rescue SLFN11 in SCLC
- Histone modifications may be at the core of *SLFN11* silencing

Gardner EE *et al. Cancer Cell* **2017**.

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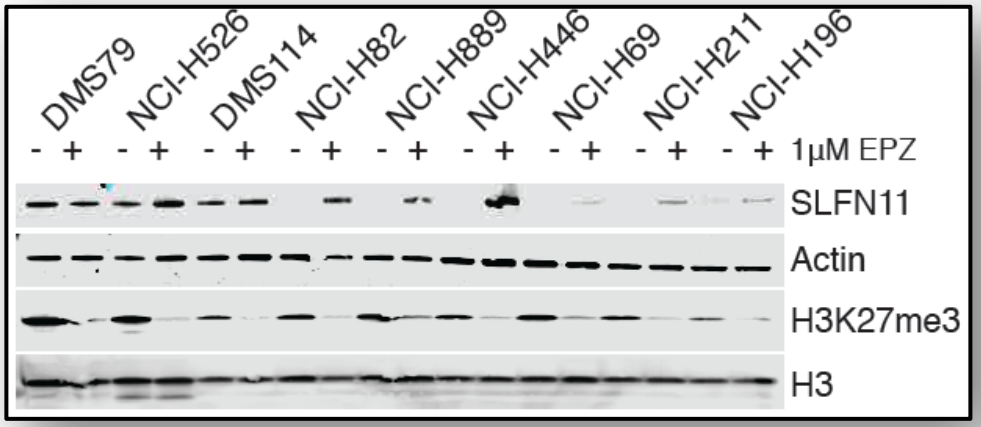
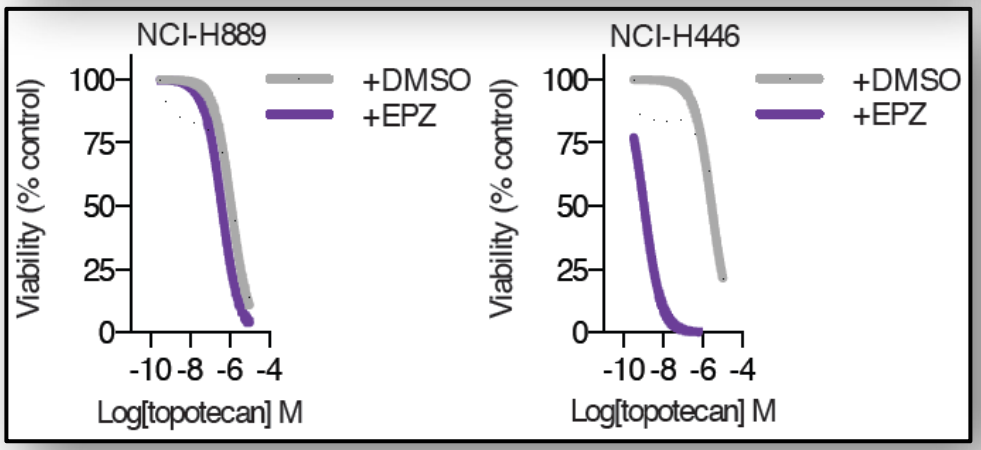
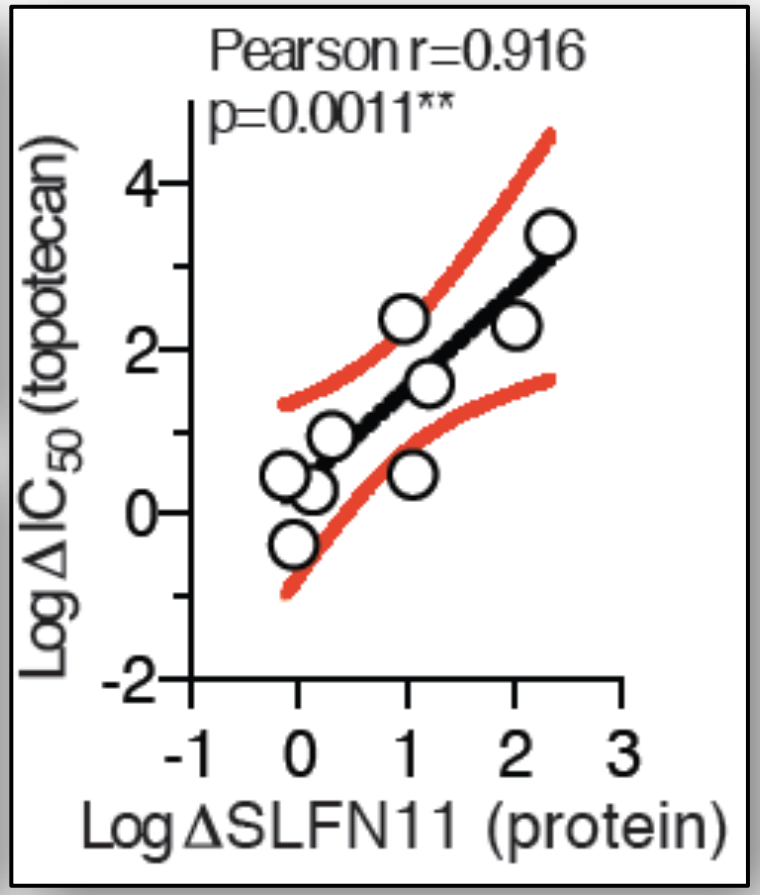
Hypothesis: *If SLFN11 can be re-expressed, then we can chemosensitize to DNA damage*







The greater the re-expression w/ EZH2 inhibition, the greater the chemosensitization

Gardner EE *et al. Cancer Cell* **2017**.

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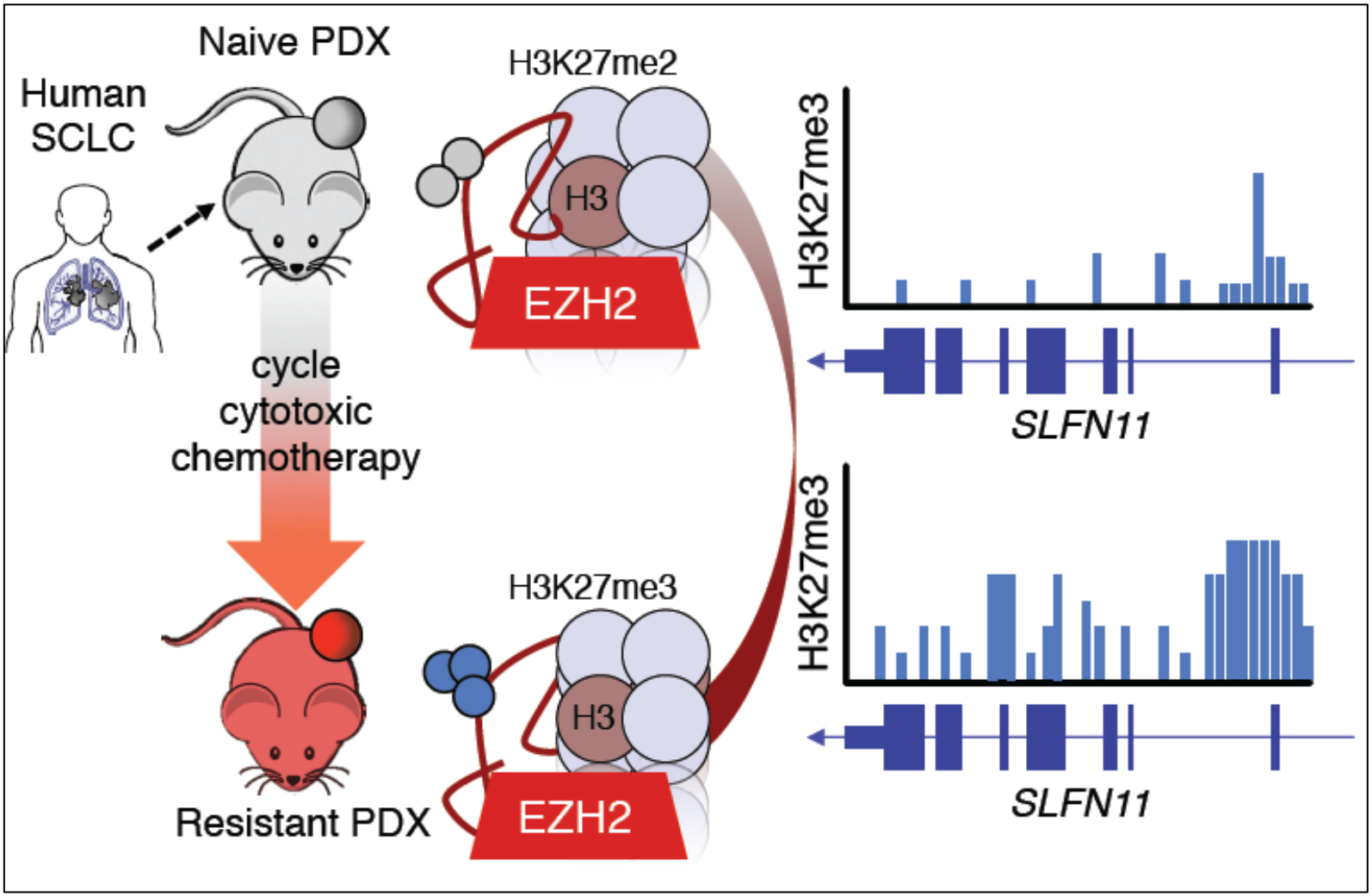
We ***hypothesize*** that EZH2 is responsible for global silencing events in SCLC that permit resistance to chemotherapy and chemical inhibition of EZH2 can partially reverse and/or prevent this resistance *in vivo*



Gardner EE *et al. Cancer Cell* **2017**.

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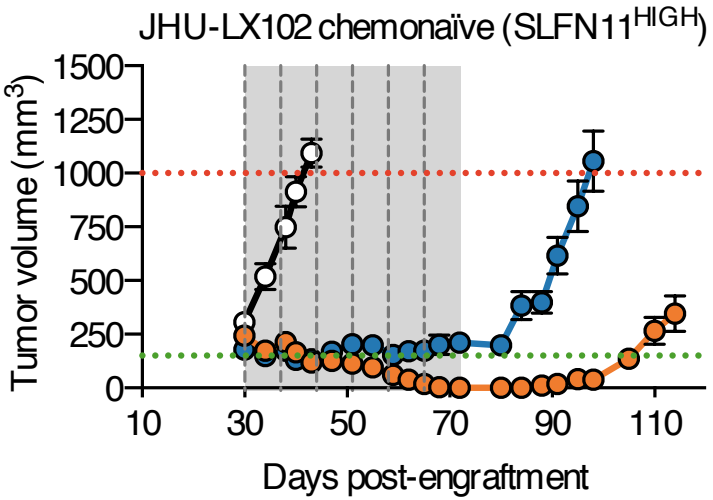
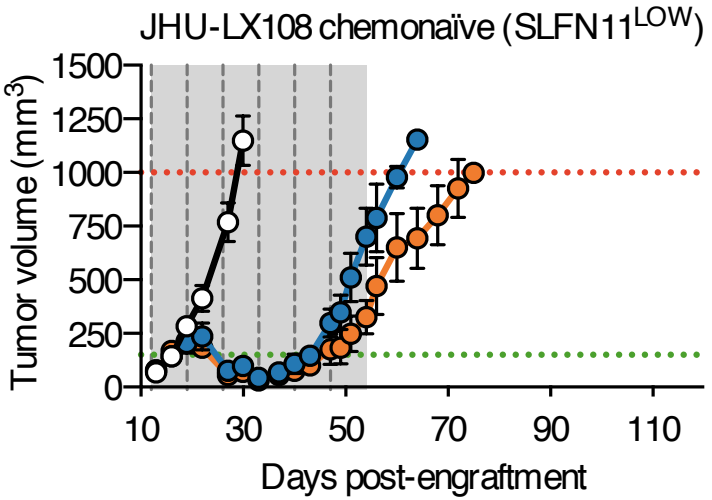
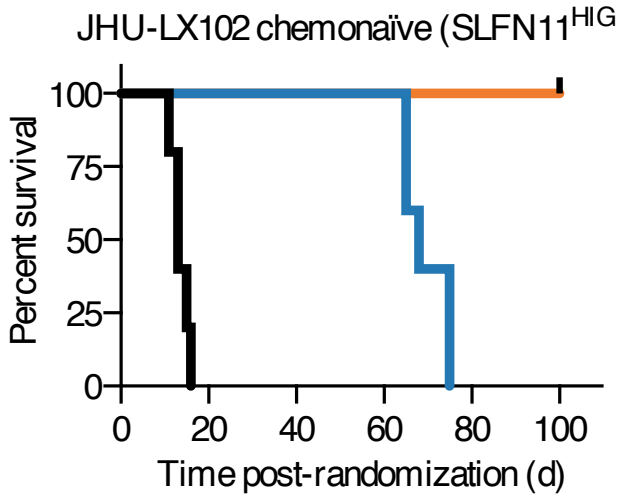
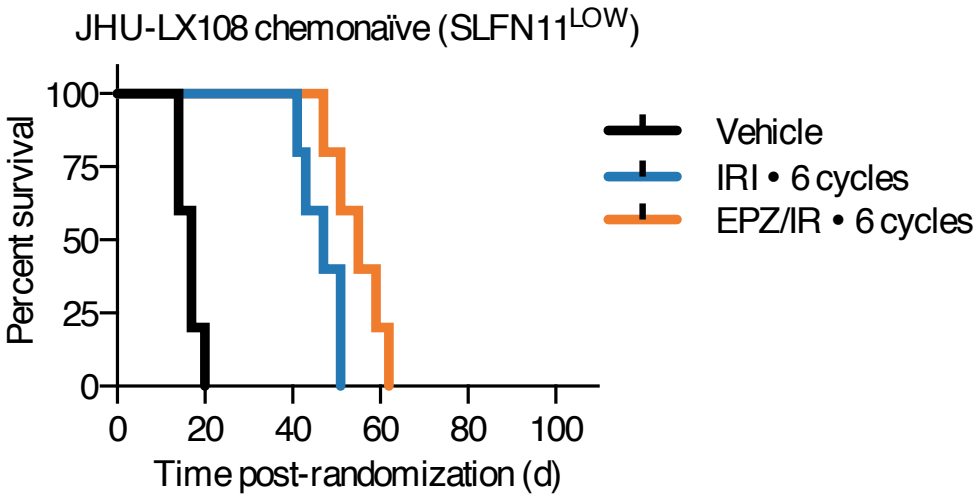
Efficacy in SLFN11^{HIGH} / SLFN11^{LOW} PDX models









Ideal scenario – combining a topoisomerase 1 poison w/ an EZH2 inhibitor in SLFN11^{HIGH}, chemosensitive relapse (**MYC status appears to trump SLFN11**)

Gardner EE *et al. Cancer Cell* **2017**.

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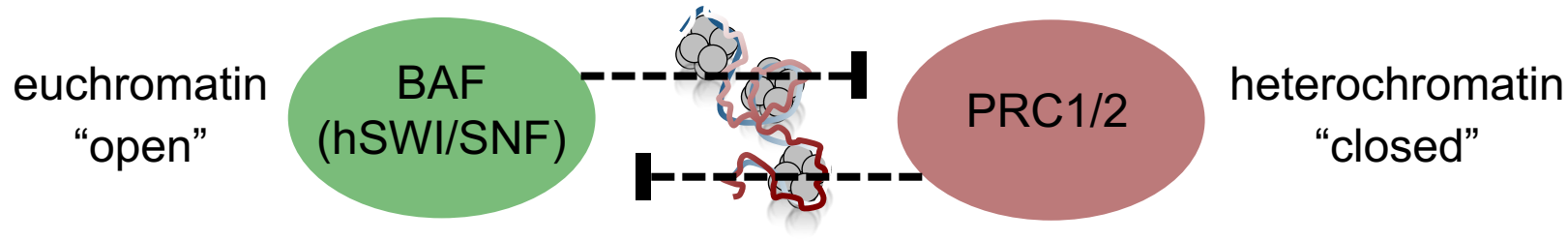
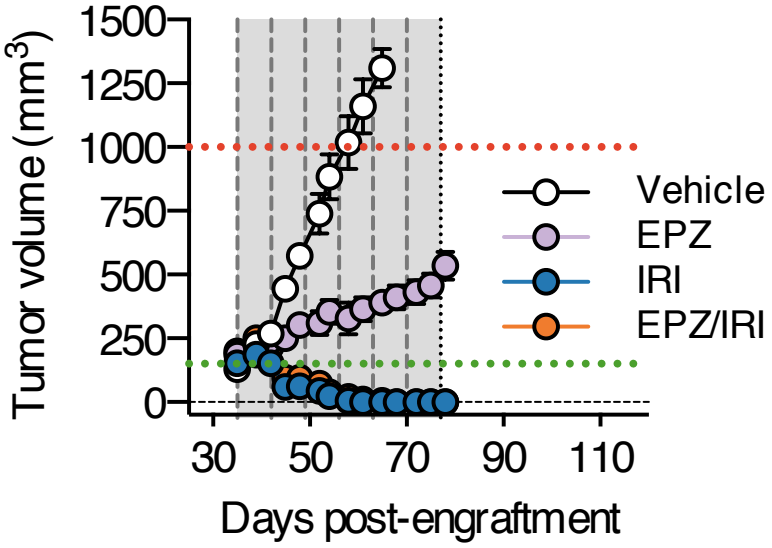
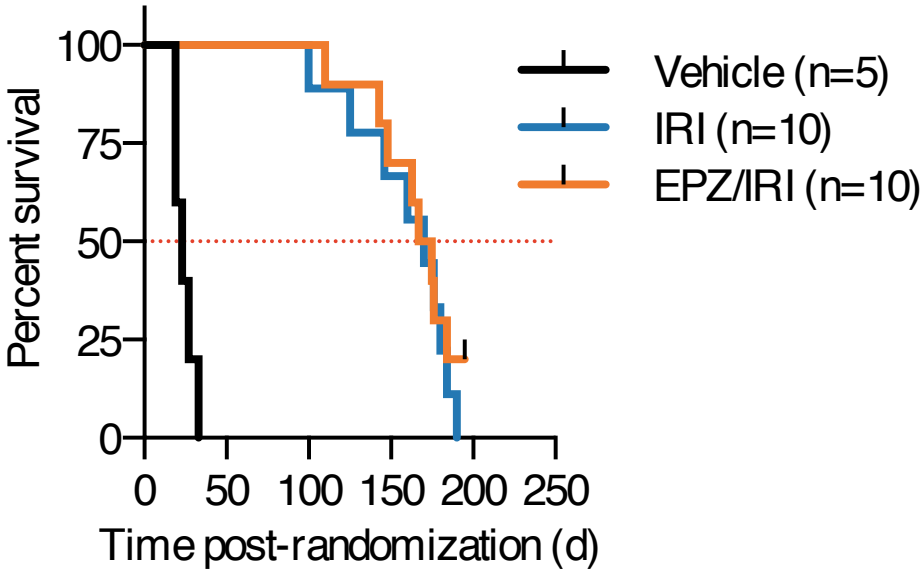
~15-20% of SCLC cases have inactivating BAF mutations



MSK-LX40 (SLFN11^{HIGH}) *SMARCD1-mut*





- BAF^{MUT} SCLC may benefit from EZH2i **maintenance** post-chemotherapy
- Combination efficacy (EPZ/IRI) must be administered **together, chronically**

Gardner EE *et al. unpublished*

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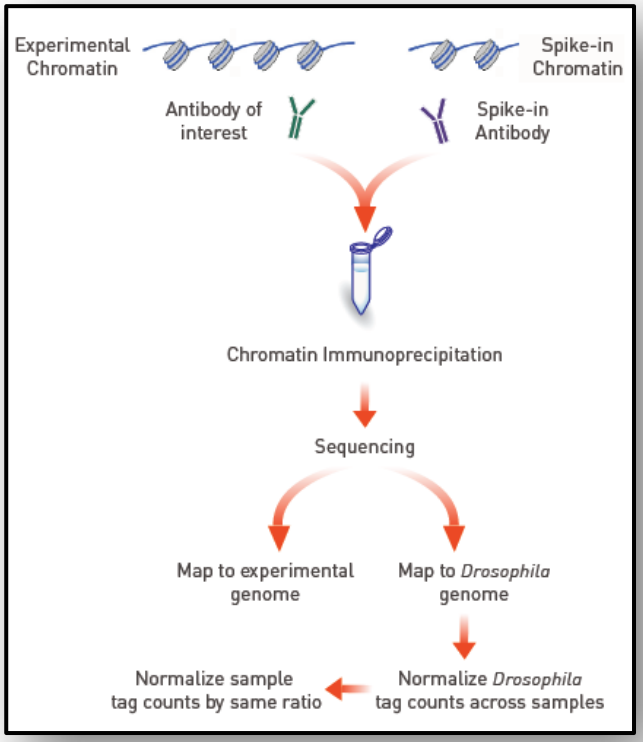
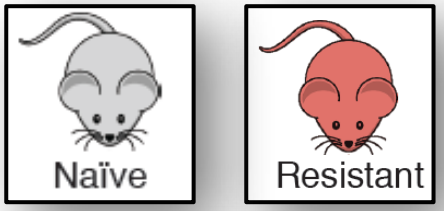
ChIP-sequencing suggest global rescue of *TSS* silencing

Normalize Scaling with exogenous chromatin source (Drosophila S2 spike-in)

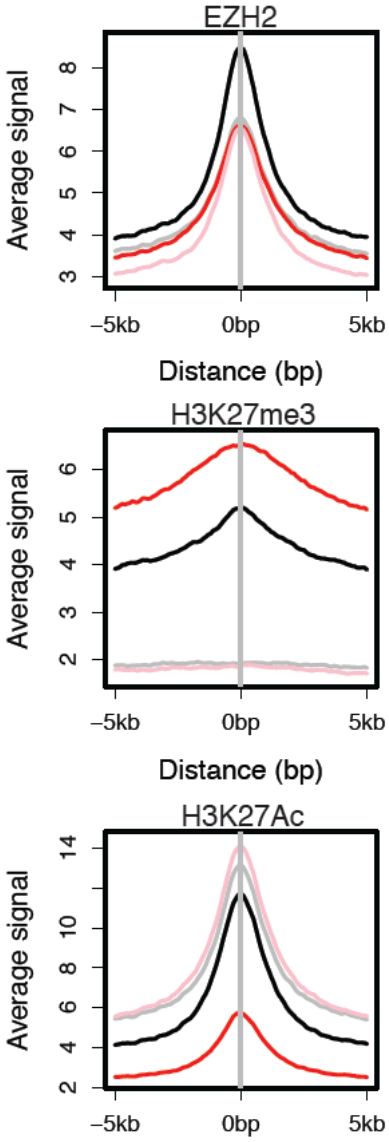


± EZH2 inhibition



Chemonaïve
Chemonaïve + EPZ
Chemoresistant
Chemoresistant + EPZ

H3K27me3 – **repressed**
H3K27Ac – **active**



- H3K27me3 ↑ and K27Ac ↓ near TSS in resistant disease
- EZH2 chemical inhibition reverses both of these changes

Gardner EE *et al. Cancer Cell* **2017**.

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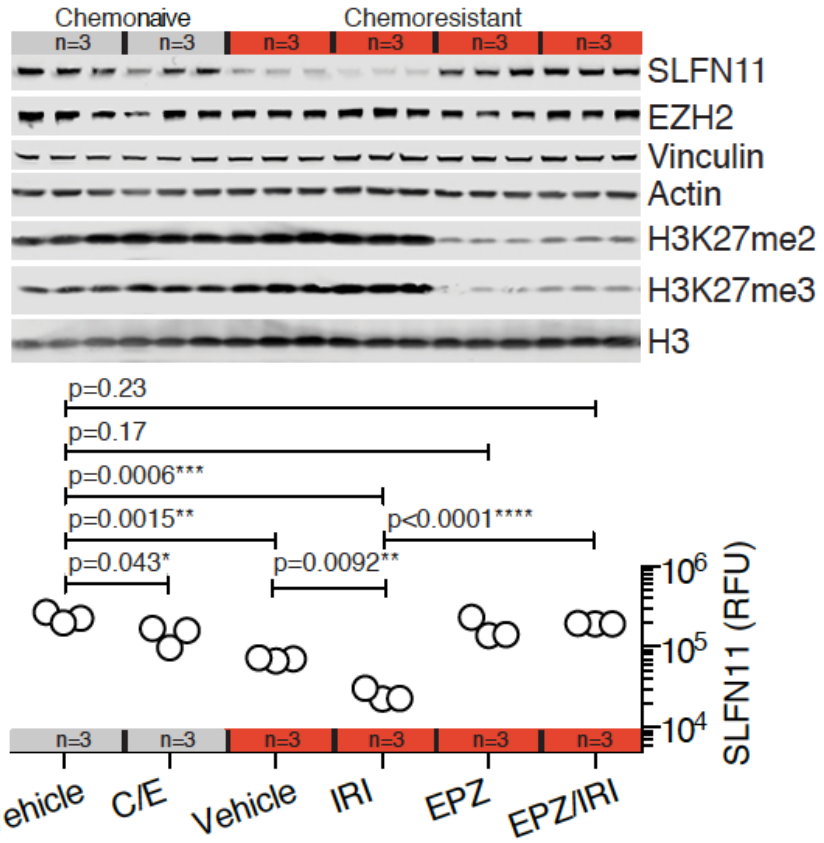
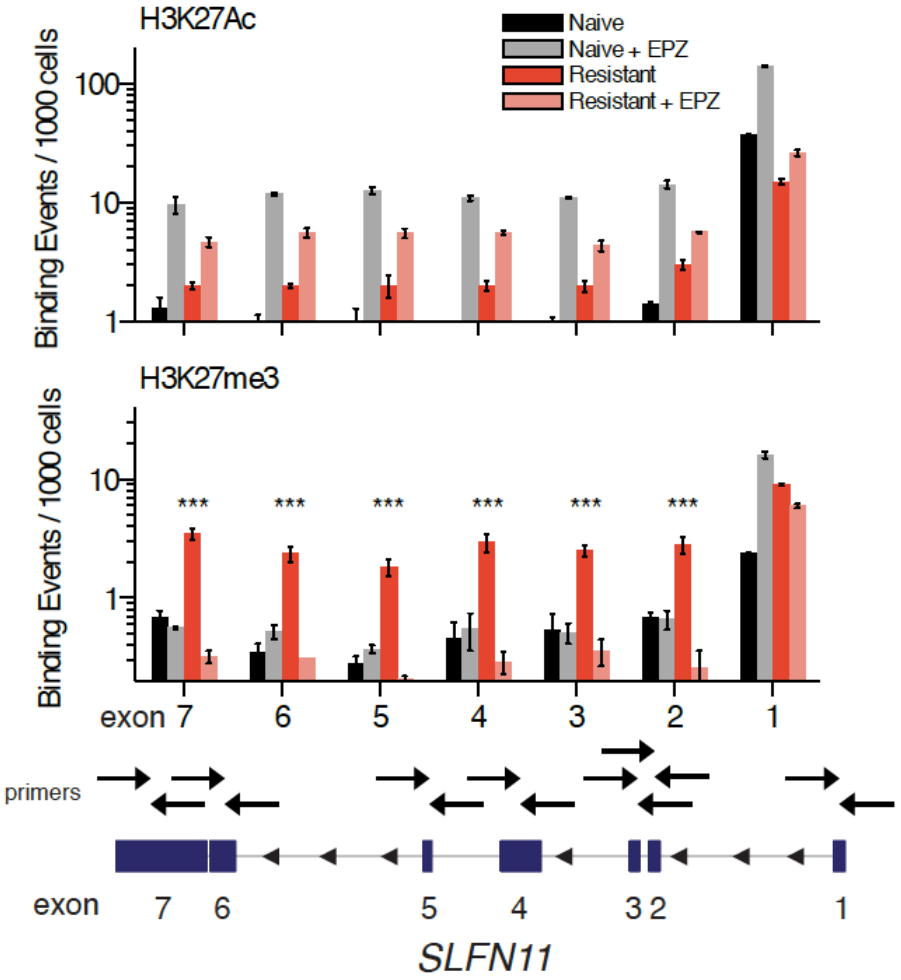
Chemical EZH2 inhibition sustains SLFN11 expression during cycles of DNA damage *in vivo*

SLFN11 expression in tumors



H3K27me3 / Ac ChIP-qPCR



SLFN11 expression is **maintained *in vivo*** during cycles of chemotherapy with EZH2 inhibition

Gardner EE *et al. Cancer Cell* **2017**.

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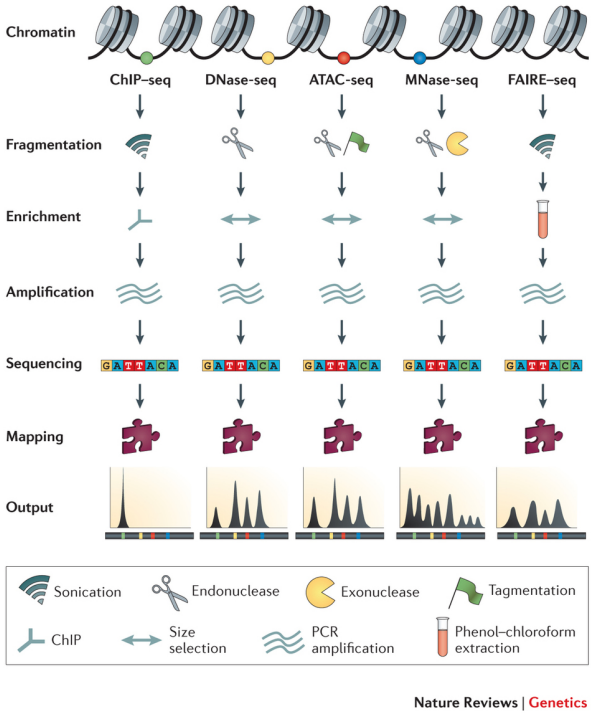
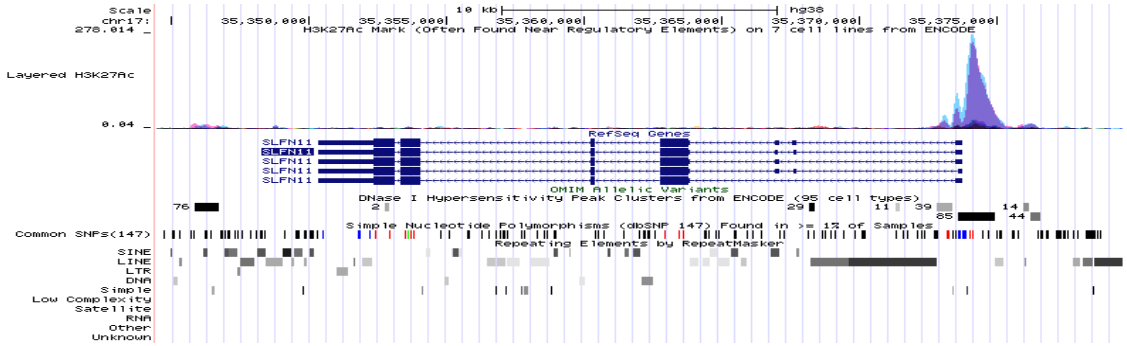
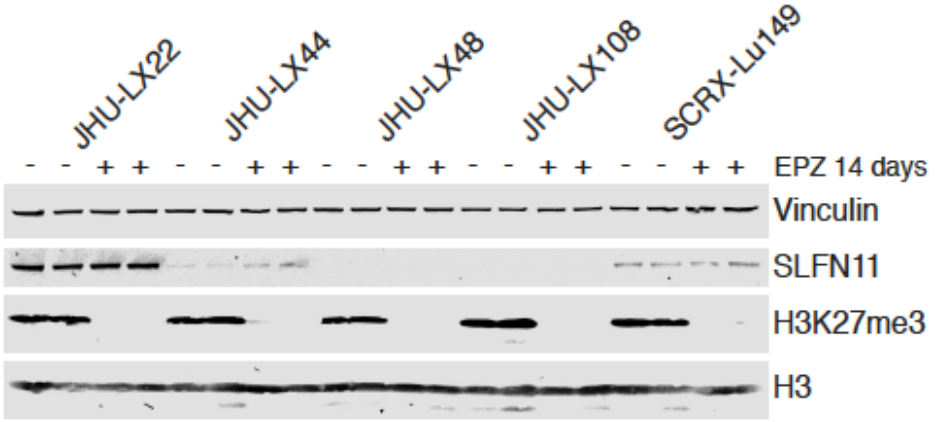
What regulates the *SLFN11* locus in human SCLC?





Not all SCLC can "re-express" SLFN11 under EZH2i



What approaches can we readily apply?

RNA-seq EZH2i time course for re-expression
ATAC-seq +/- EZH2i
ChIP-seq +/- EZH2i – define bivalency, enhancers, etc.
Proteomic Trapping – HALO-dCas9 purifications
CRISPR positive selection screens

A bunch of NSG experiments is not mechanism nor is it direct evidence…

Meyer CA, XS Liu. *Nature Reviews Genetics* **2015**
Gardner EE *et al. Cancer Cell* **2017**
Gardner EE et al. *unpublished*

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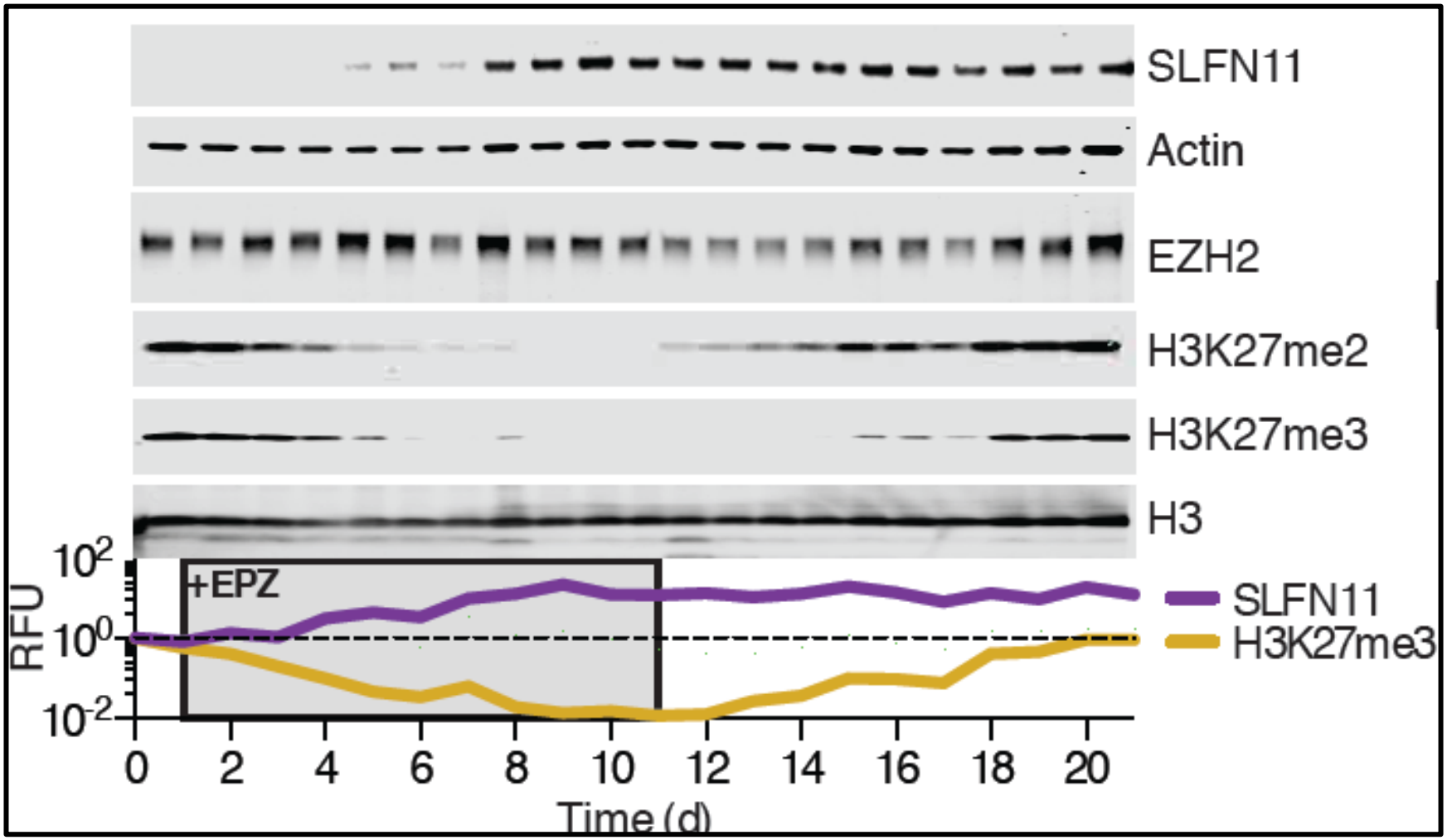
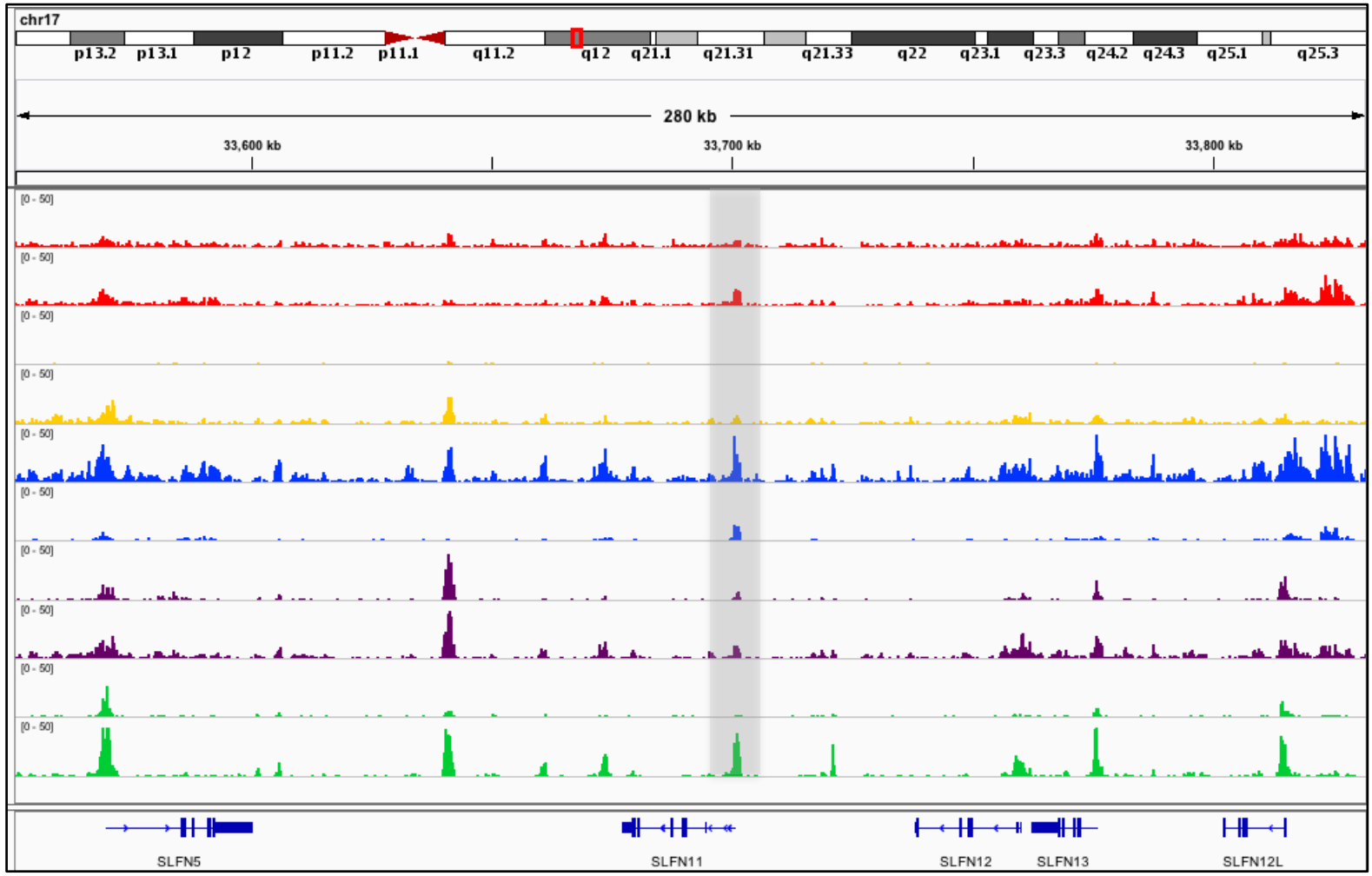
EZH2i takes time to rescue SLFN11 expression

Western Blot sampling of daily treatment of NCI-H82 cells with 1uM EZP/d for 10-ON / 10-OFF days



SLFN11 is not clearly a bivalent PRC2 target in SCLC

ChIP-sequencing of the *SLFN* "neighborhood" after one week of EZH2 chemical inhibition



Gardner EE *et al. unpublished*

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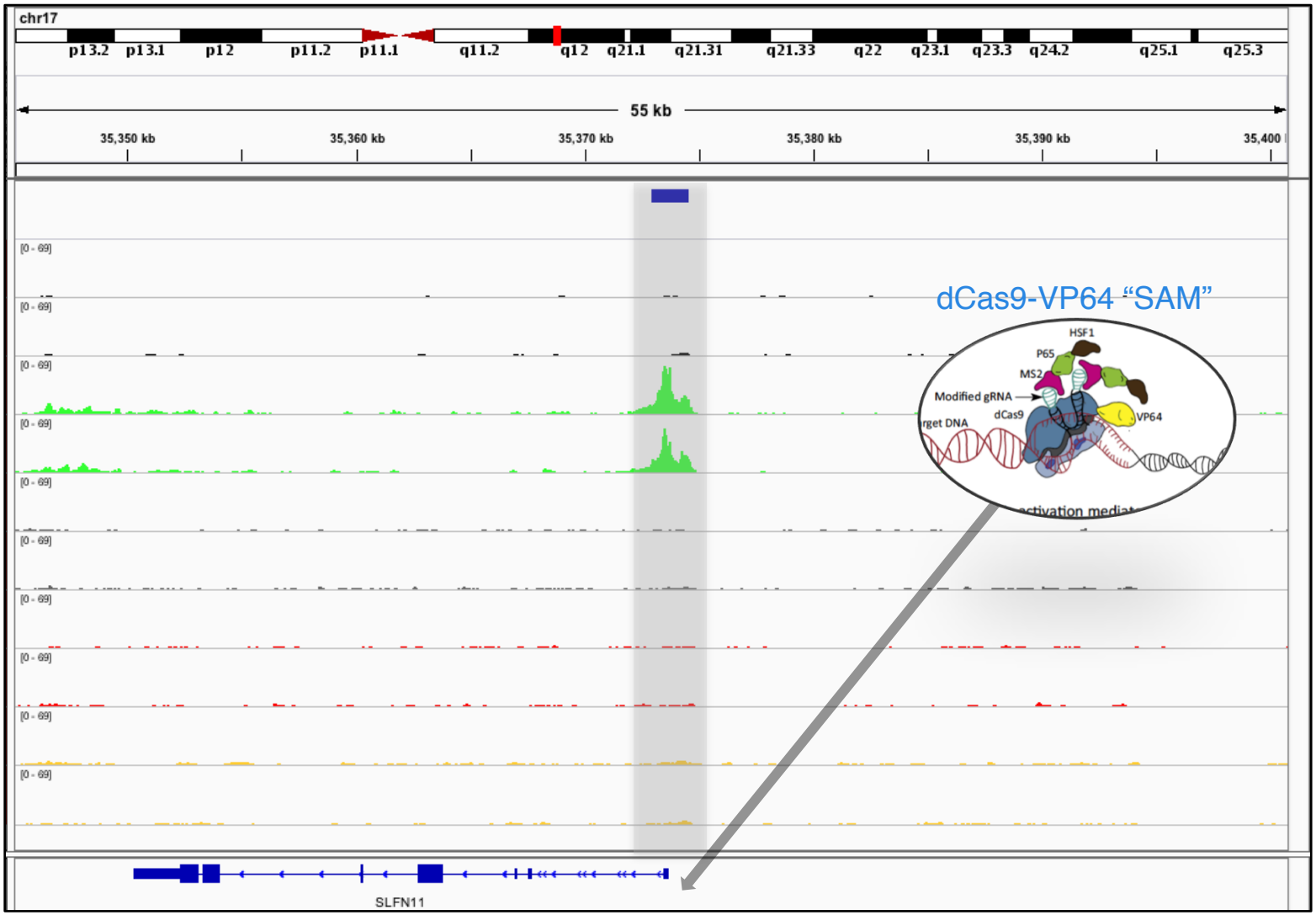
EZH2i does not open local *SLFN11* chromatin

ATAC-sequencing to assess local and global changes in chromatin accessibility to Tn5 transposase



Joung J, Konermann S, *et al. Nature Protocols* **2017**
Gardner EE *et al. unpublished*

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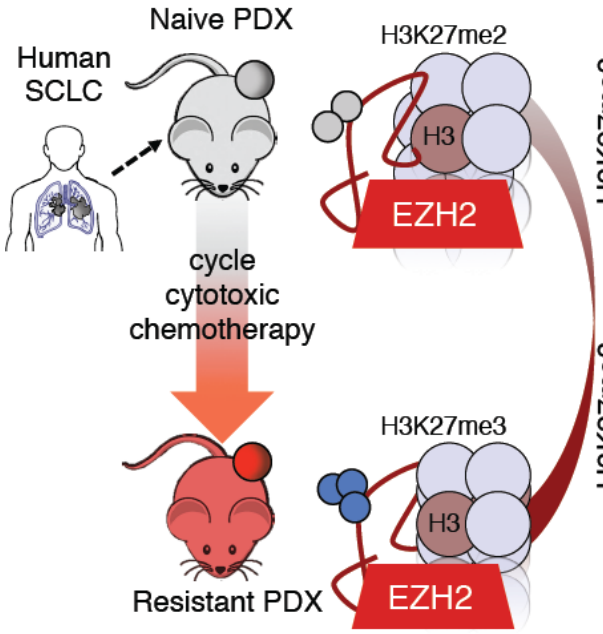
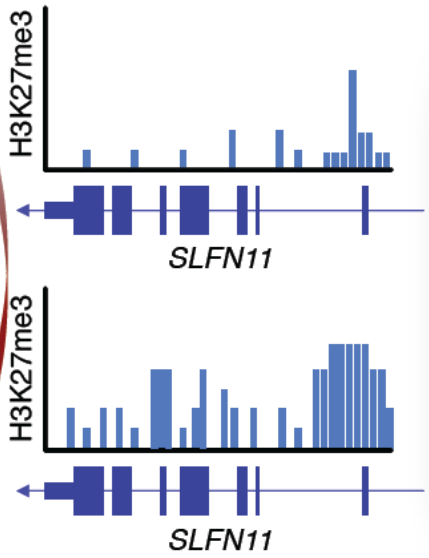
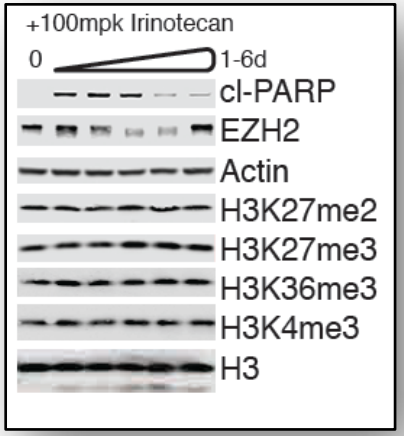
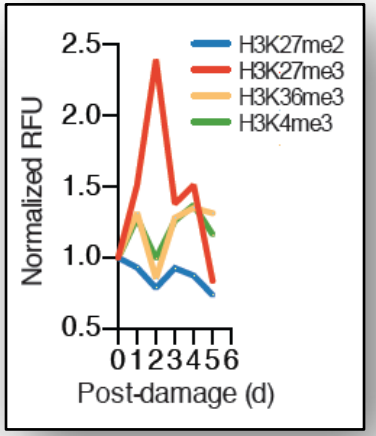
Summary of EZH2's role in remodeling the SCLC epigenome…

- EZH2 promotes global silencing in SCLC during acquired resistance to SOC
- Chemical (catalytic) EZH2 inhibition can rescue/prevent some of these…
- *SLFN11* locus is not a PRC2 bivalent region, nor opened (ATAC) with EZH2i
- Rescue of *SLFN11* may be regulated outside of local chromatin remodeling









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  - Valentina Schneeberger
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  - Inna Khodos (AACF)
  - Ralph Garippa (RNAi)
  - Mesruh Turkel (MCCF)
  - Integrated Genomics Operation
- Pathology
  - Patrice Desmeules
  - Natasha Rekhtman
- Epidemiology and Biostatistics
  - Andy Ni

- Vanderbilt
  - Pierre Massion
- Case Western
  - Afshin Dowlati
- Stanford
  - Julien Sage
  - Thuyen Nguyen
- Epizyme
  - Scott Ribich
  - John Campbell

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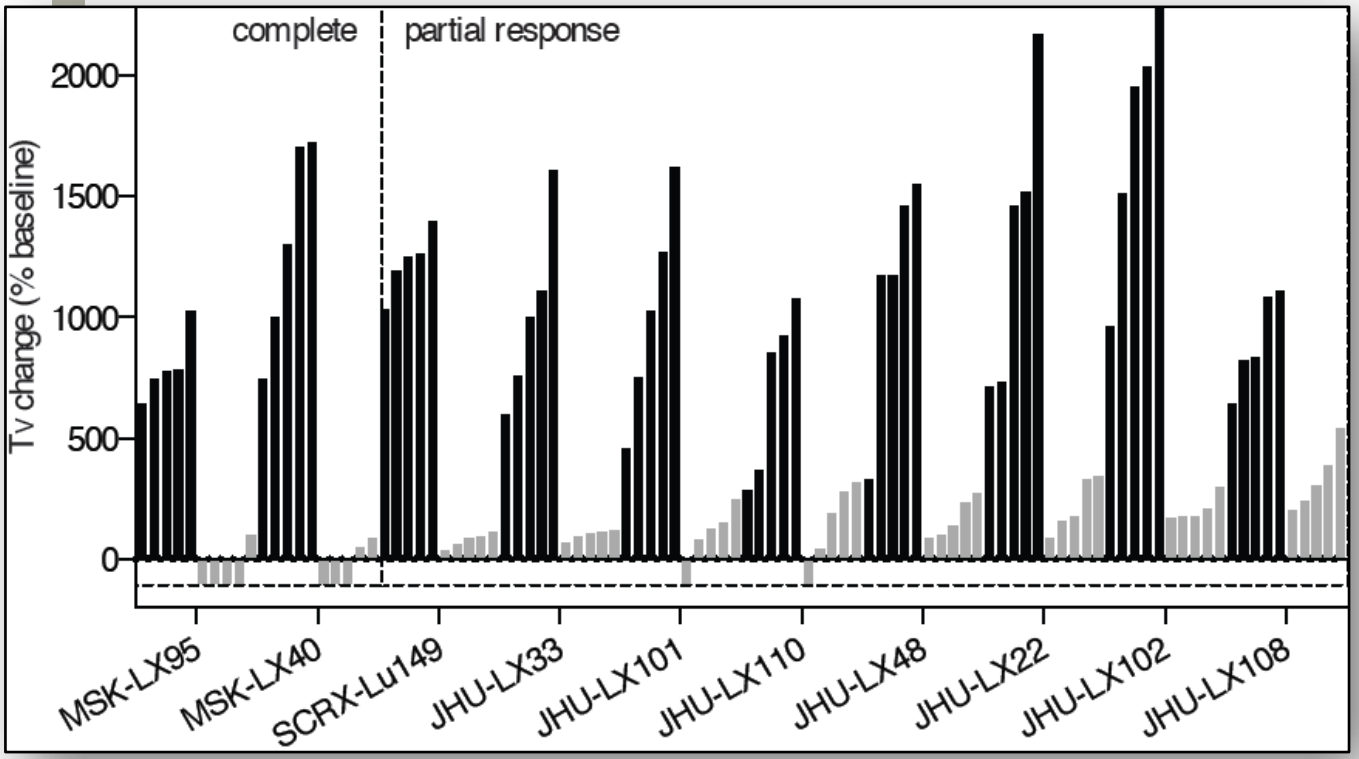
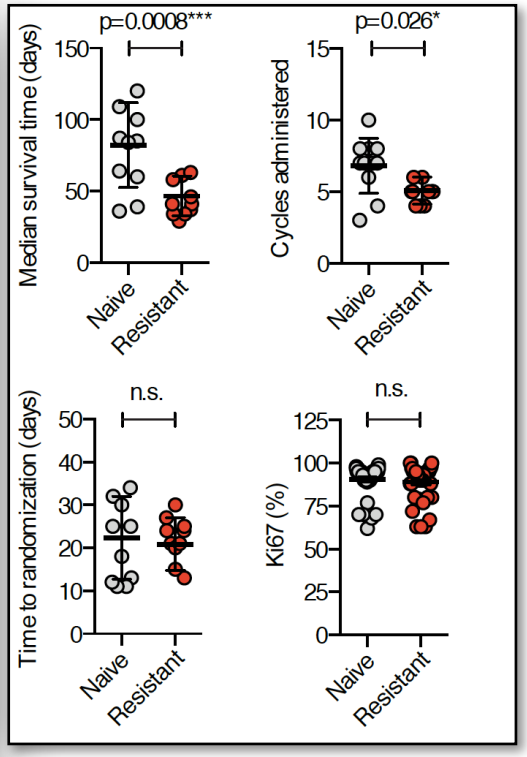
Credentialing responsiveness of PDX models





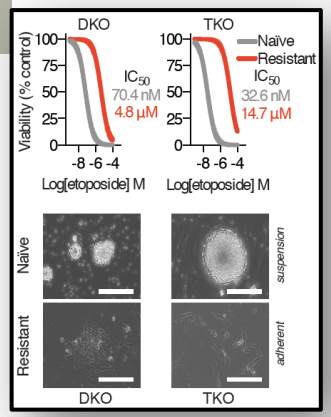
Focused analyses on **chemoresponsive** PDXs where we observed some measurable response to C/E

Gardner EE *et al. Cancer Cell* **2017**.

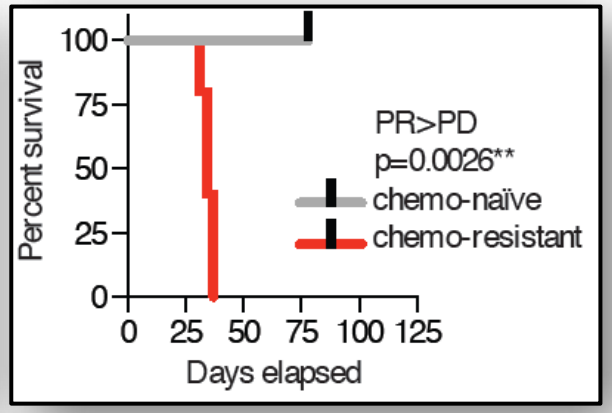
Memorial Sloan Kettering Cancer Center

Targeting *TWIST1* **does not** affect resistance in Hu/Mu-SCLC

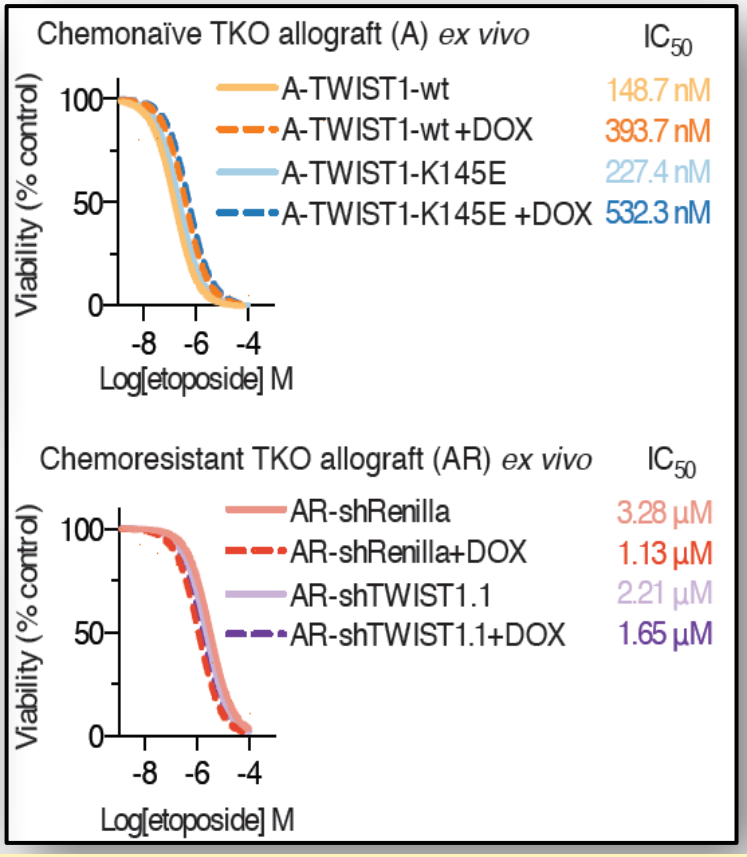
mSCLC cell lines



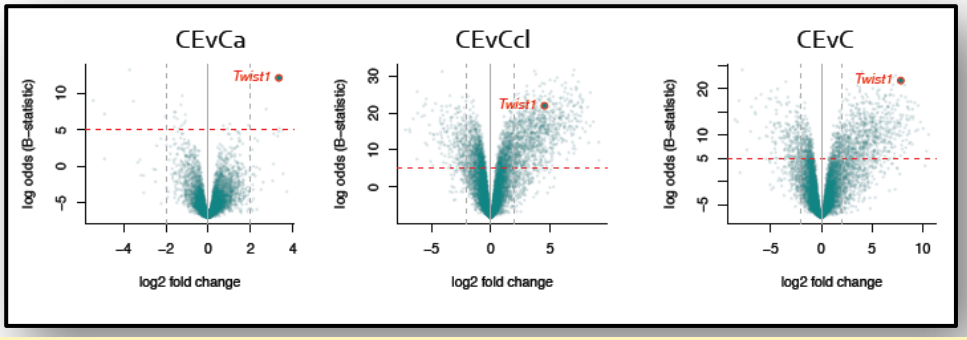
mSCLC allograft from GEMM



Conditional suppression / expression



TWIST1 is up-regulated across mSCLC systems



- *TWIST1* up-regulation may be associated w/, but not a determinant of resistance

Gardner EE *et al.*, *Cancer Cell* **2017**.

TWIST1^{HIGH} resistant TKO-A cells are less aggressive *in vivo*