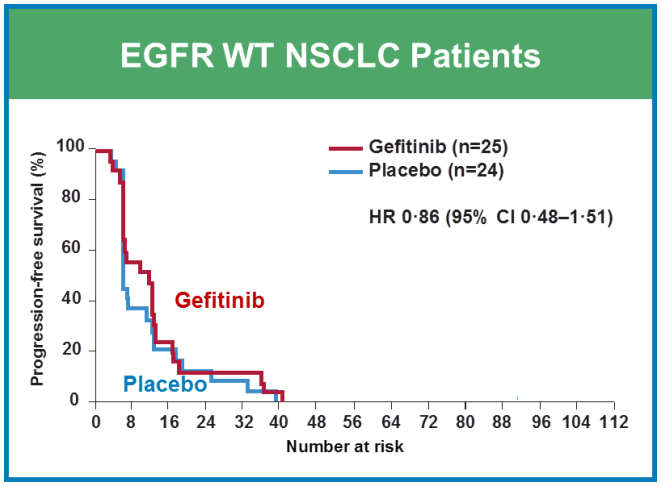
Metabolic collateral vulnerabilities of MTAP-deleted cancers as therapeutic opportunities

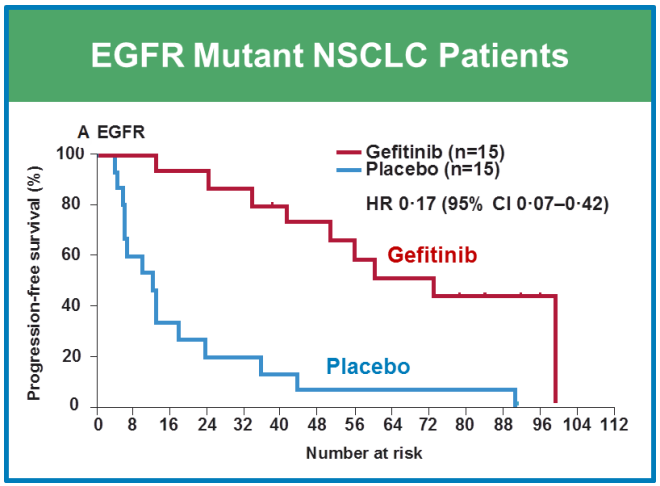
Keystone on Tumor Metabolism 2017

5 – 9 March 2017 | Whistler, Canada

The Challenge: Identifying Precision Medicine Approaches in Cancer Metabolism

Directly drugging ‘driver mutations’ has yielded transformative medicines



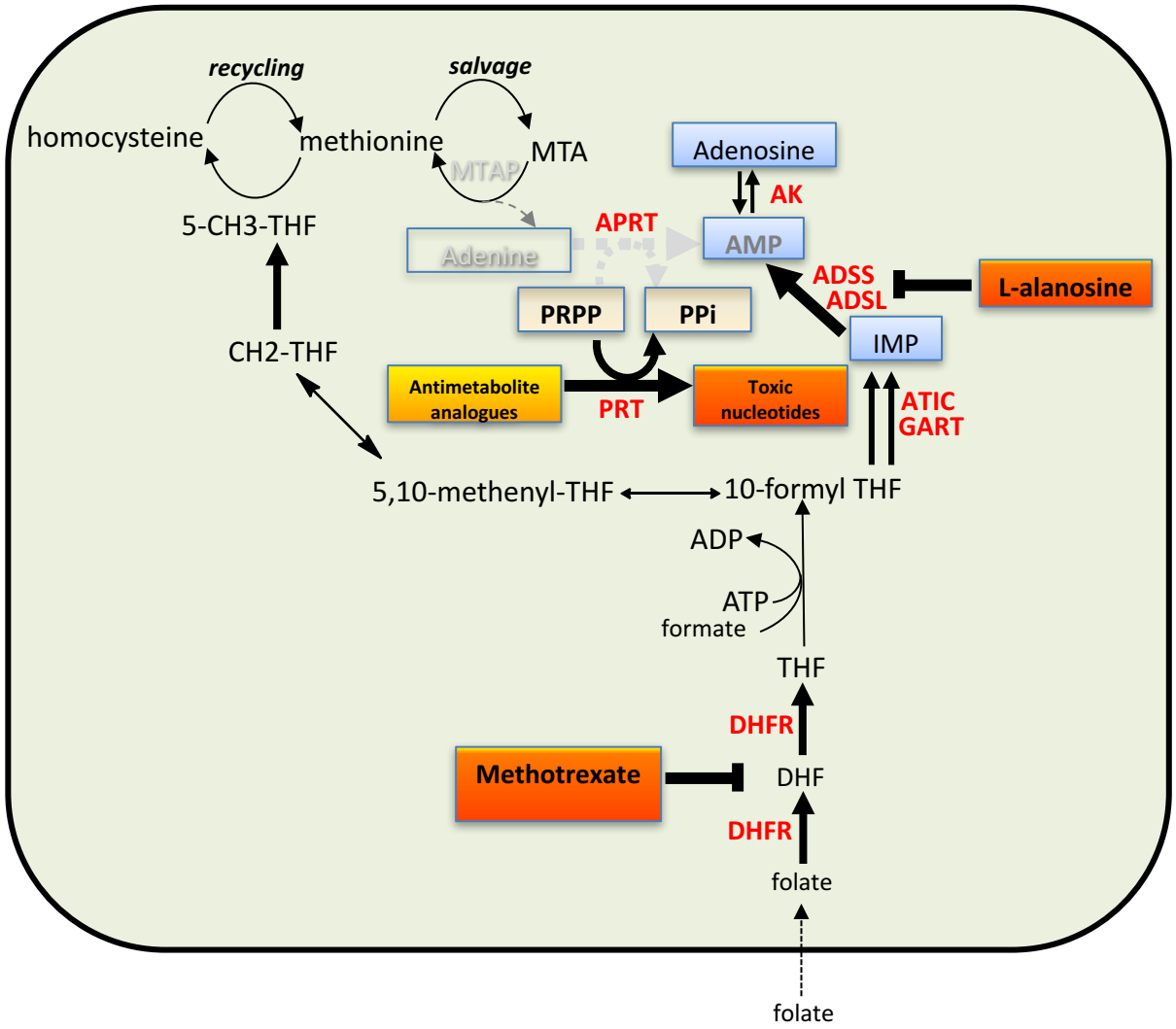


Zhang, et al, Lancet Oncology, 2012

but…DNA sequencing has identified only 1 classic, gain-of-function metabolic ‘driver’ mutation out of 2000+ metabolic genes

IDH1/2

History of targeting MTAP-null cancers: purine biosynthesis story



- MTAP-null cancer cell lines are not selectively sensitive to purine biosynthesis inhibition
- MTA selectively rescues MTAP wt cell lines from purine biosynthesis inhibition *in vitro*
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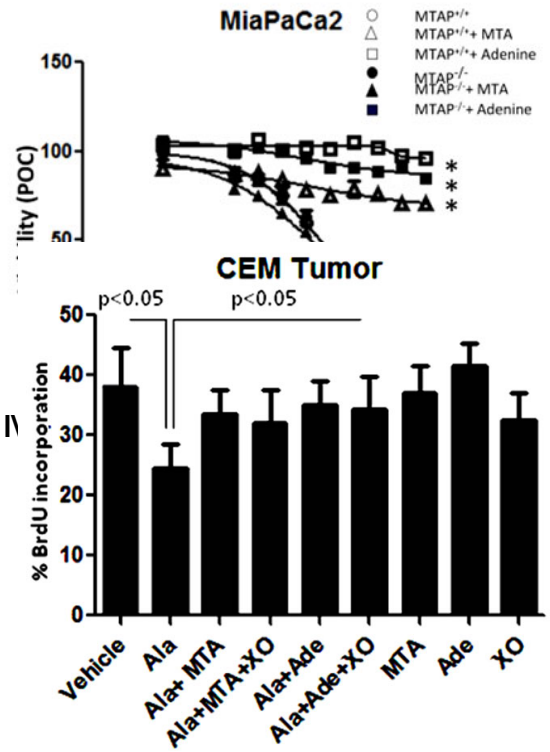

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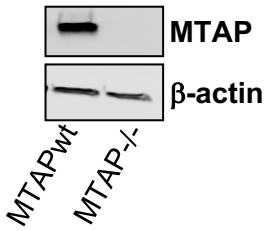
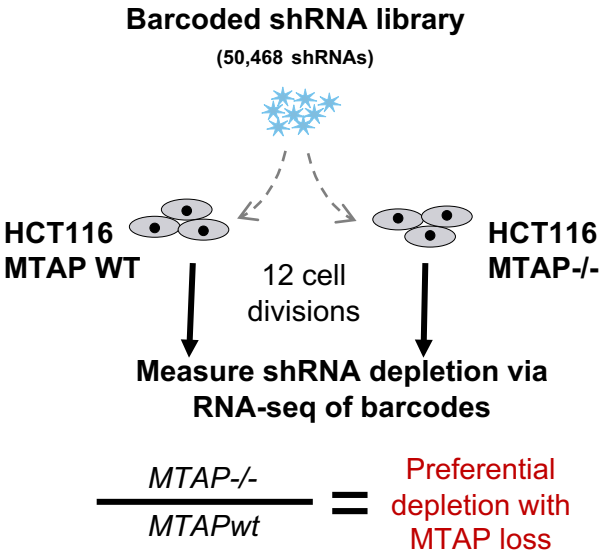
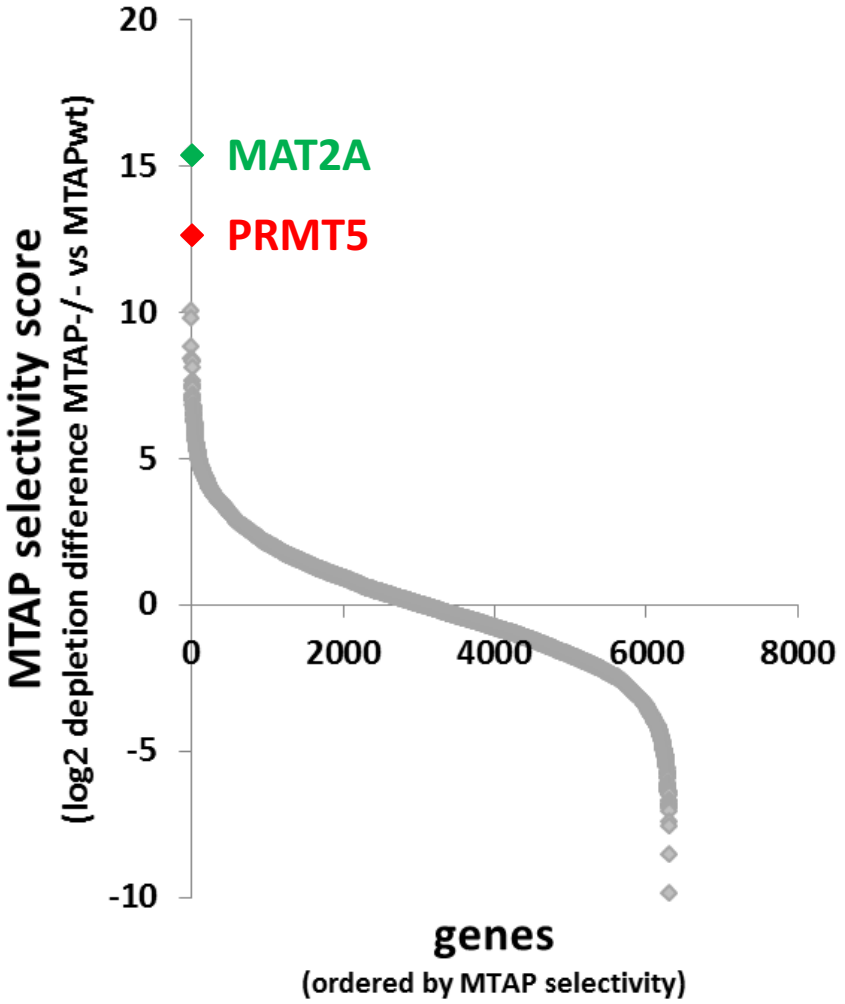
Astrid Ruefli-Brasse et al., J. of Cancer Therapy 2011
Hedy Lee Kindler et al., Invest. New Drugs 2009

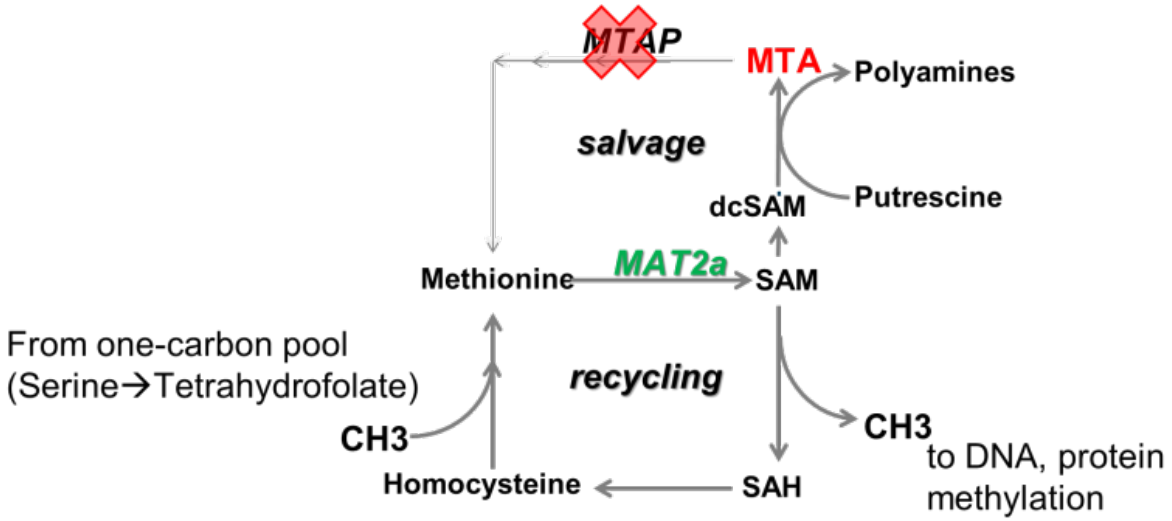
shRNA Screening Identifies Candidate MTAP Synthetic Lethal Targets



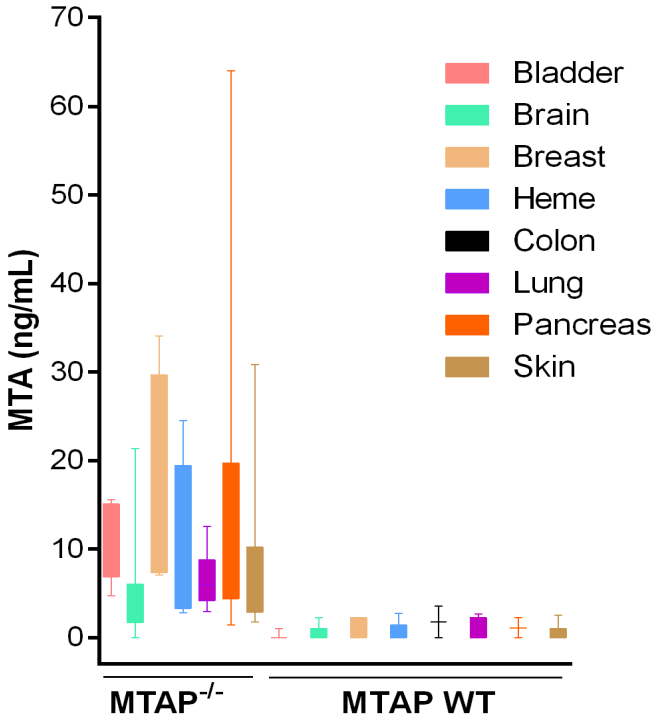




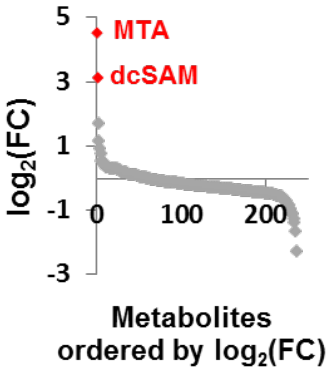
Metabolomics Reveals Substantial Accumulation of MTAP Substrate MTA in MTAP-null Cells

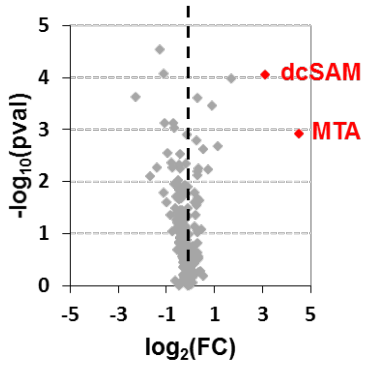


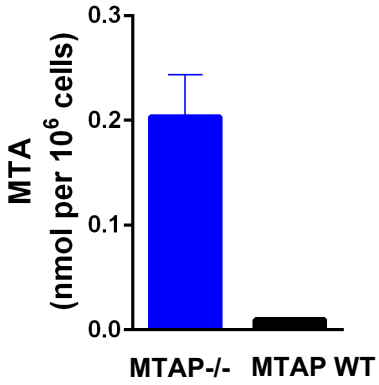
Media MTA profiling in broad cell line panel (n=249)



Metabolomics in HCT116 MTAP$^{-/-}$ and HCT116 MTAP wt cells

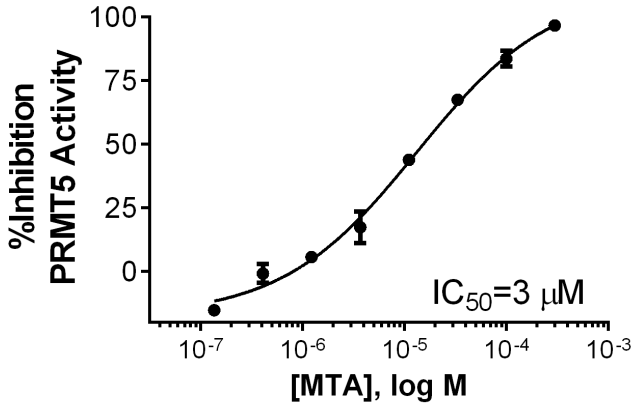


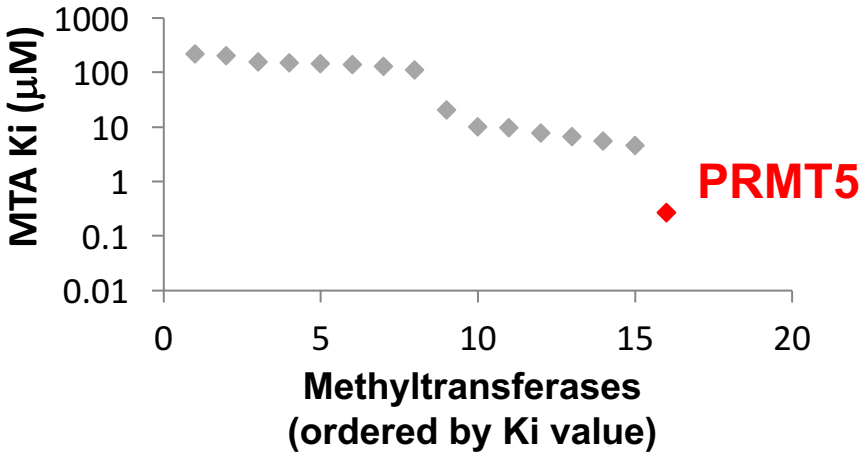




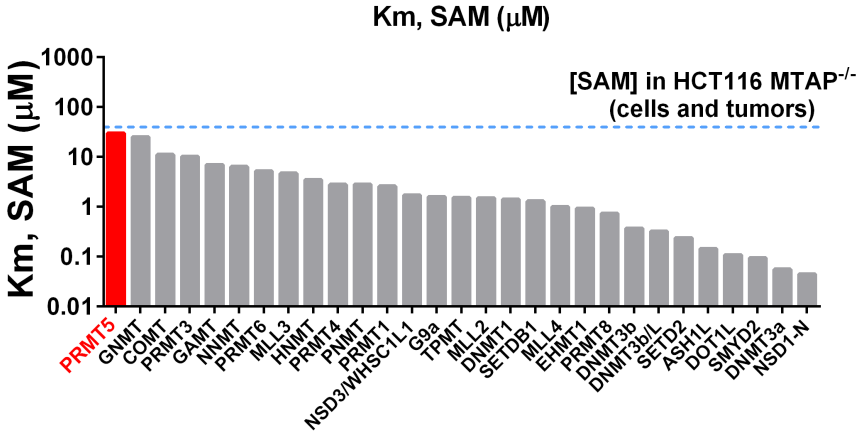
PRMT5 biochemical features make it sensitive to double hit of MTA accumulation and SAM reduction

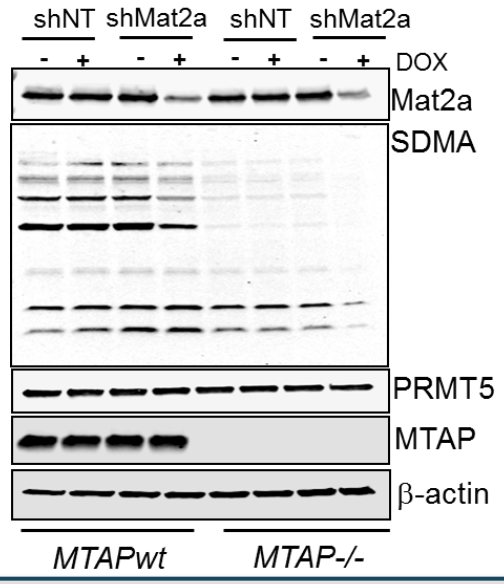
PRMT5 is >20 fold more sensitive to MTA than any other methyltransferase tested in *in vitro* assay



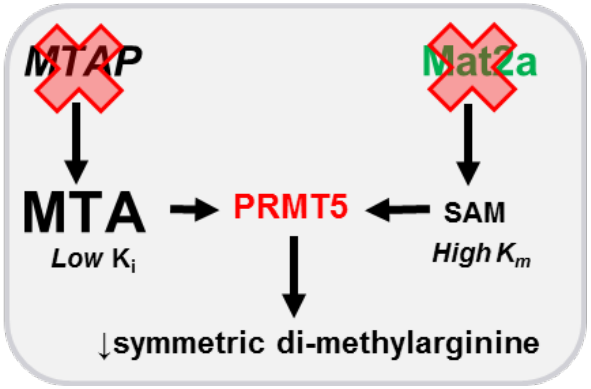


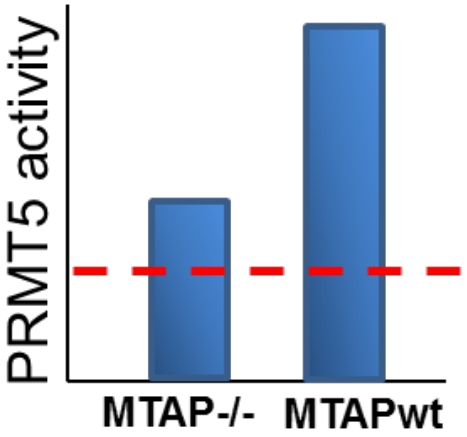
Biochemical profiling of methyltransferases indicates PRMT5 Km is poised near physiologic [SAM]



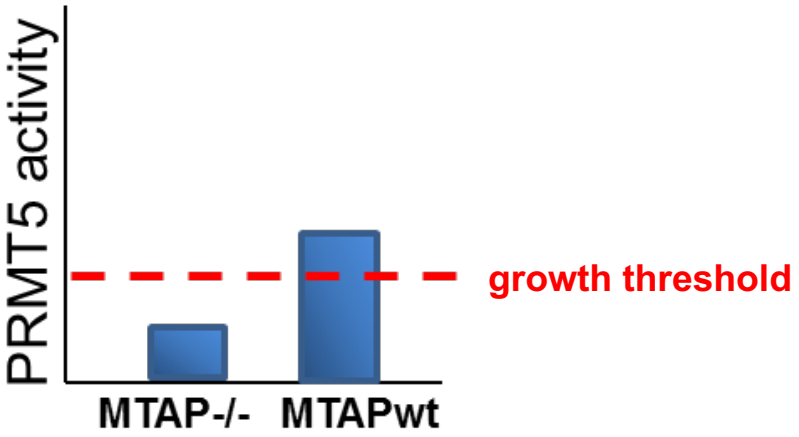


Fortuitous Biochemical Features of PRMT5 can Explain the Vulnerability of PRMT5 and MAT2A in MTAP-deleted Cancers



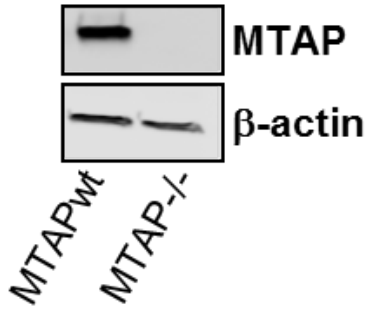


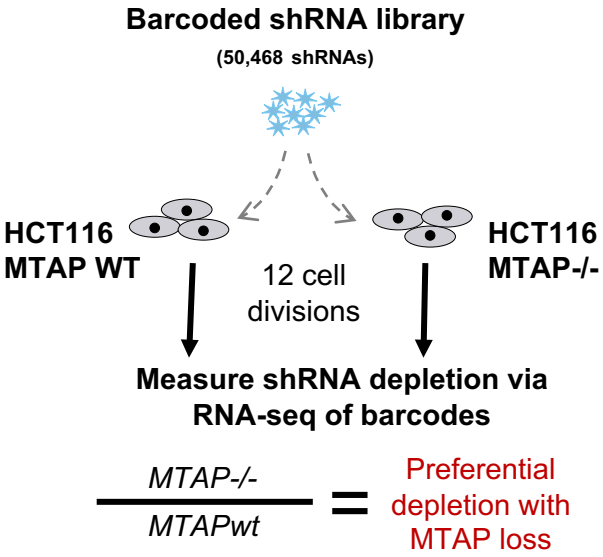
PRMT5 is inhibited by MTA at concentrations that arise in MTAP-null cancers

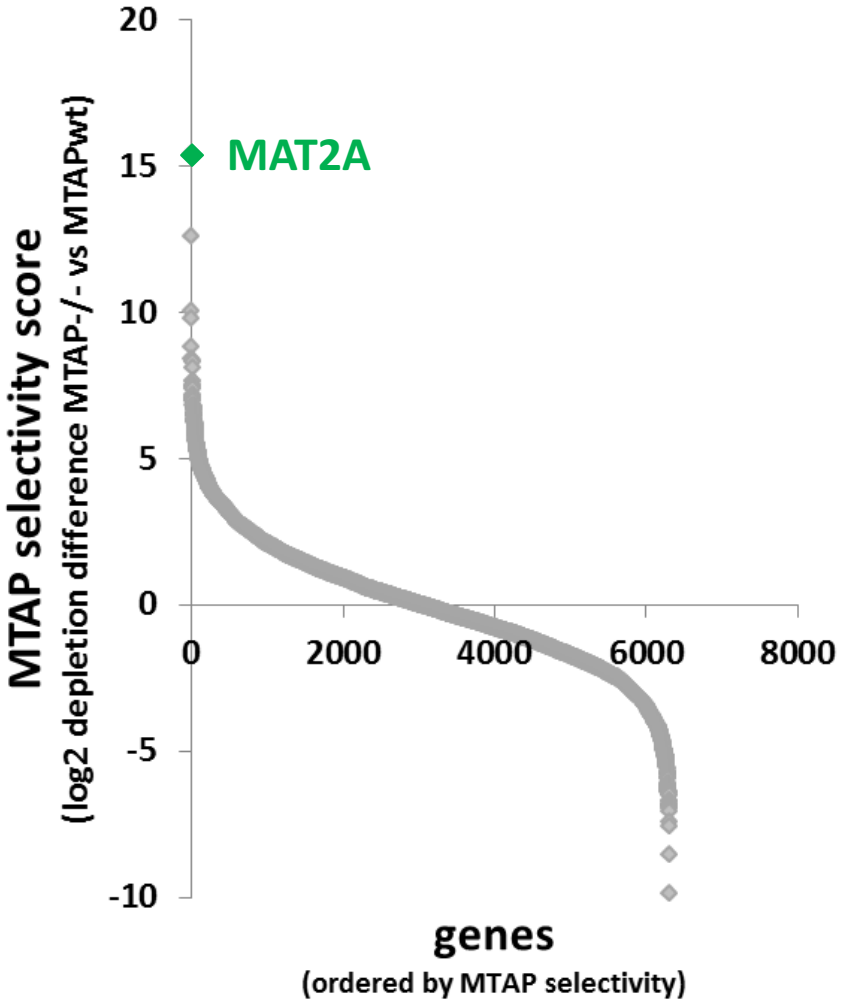


Low affinity of PRMT5 for SAM leads to further reduction in activity upon MAT2A ablation

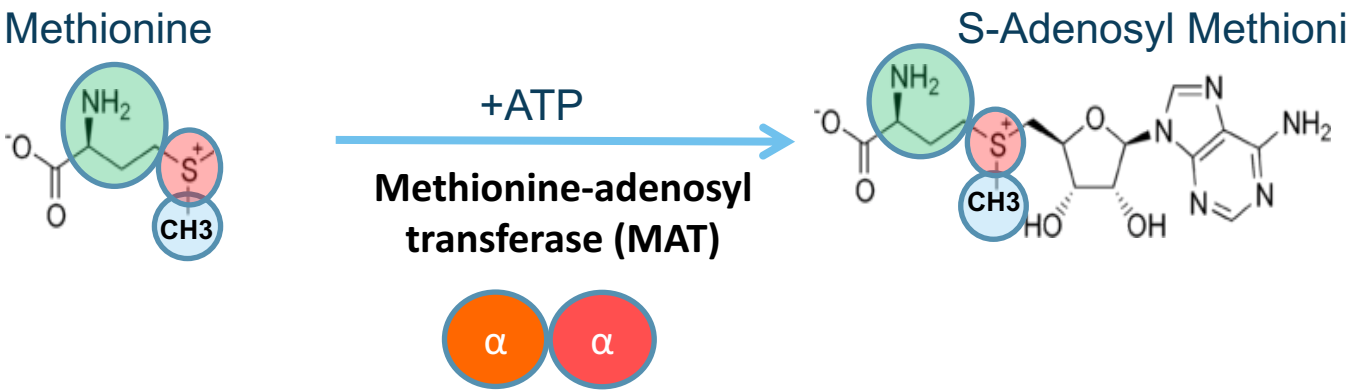
shRNA Screening Identifies Candidate MTAP Synthetic Lethal Targets



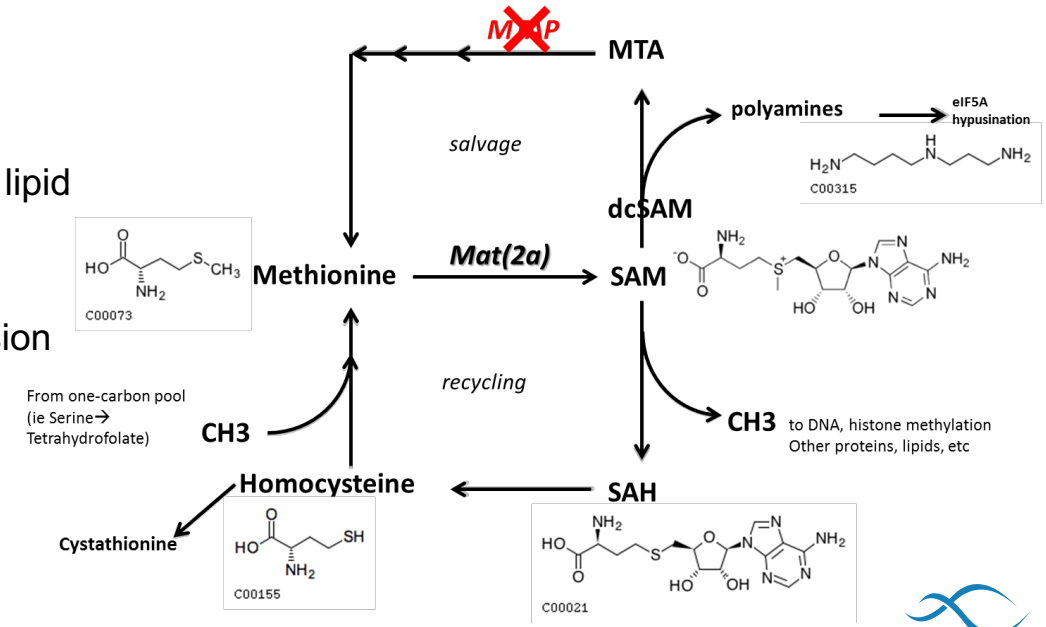




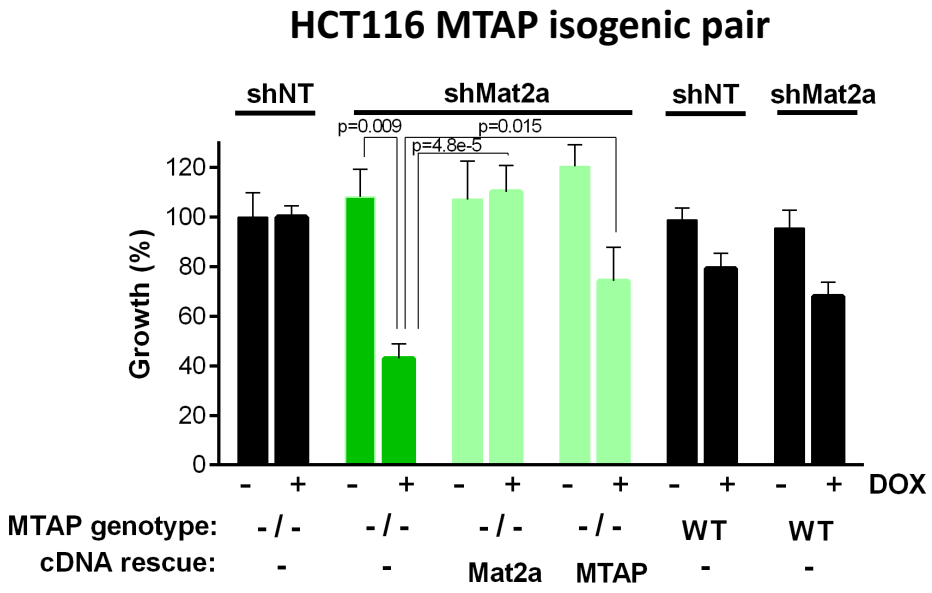
MAT2A: Methionine Adenosyltransferase 2A

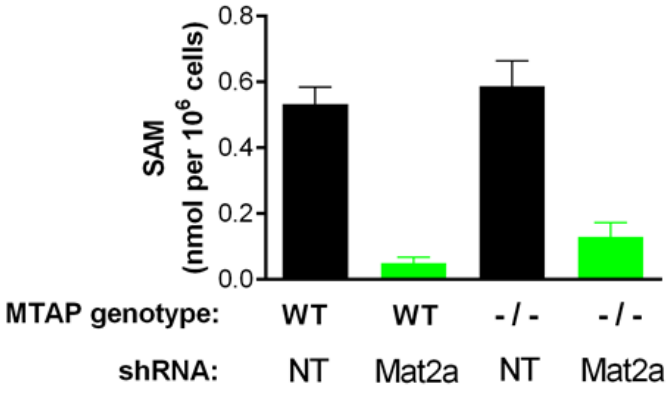


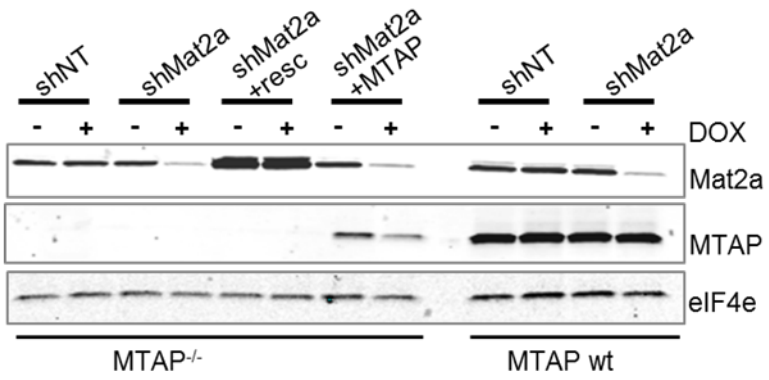
- MAT2A is the key enzyme that produces SAM in cancer & normal cells
- SAM (S-adenosyl methionine) is a 'hub' metabolite utilized in a number of pathways. Fates include:
  - **methyl group**
    - methylation of histone, DNA, protein, lipid
  - **aminopropyl group**
    - polyamines → regulate gene expression
  - **sulfur**
    - glutathione → protect vs ROS

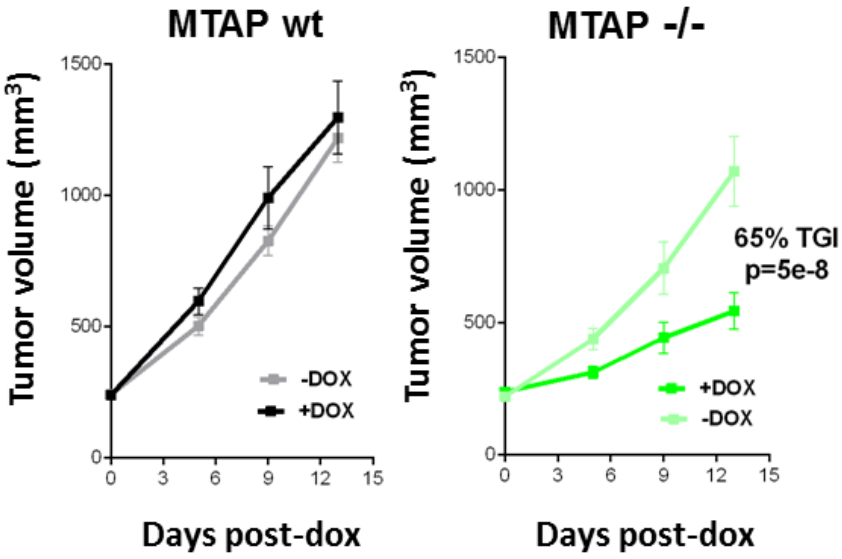


Genetic tools validate MAT2A as a Selective Vulnerability in MTAP-null Cancers



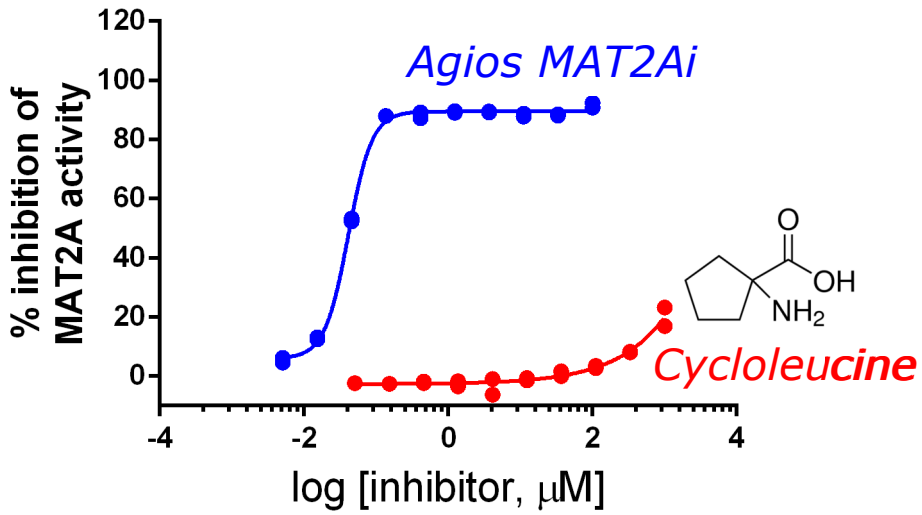




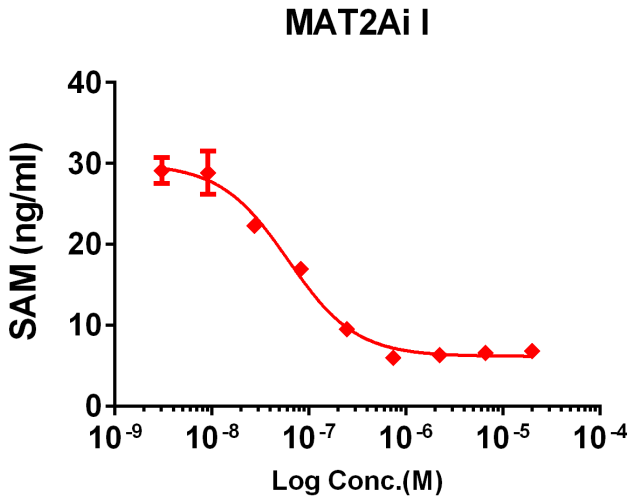


In vitro and cellular activity of first-in-class MAT2A small molecule inhibitor

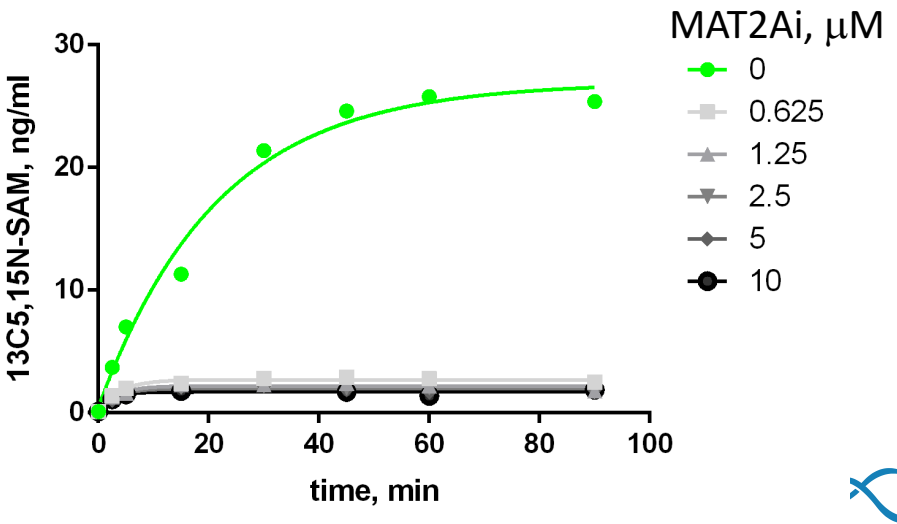
In vitro biochemical assay



SAM levels in HCT116 cells

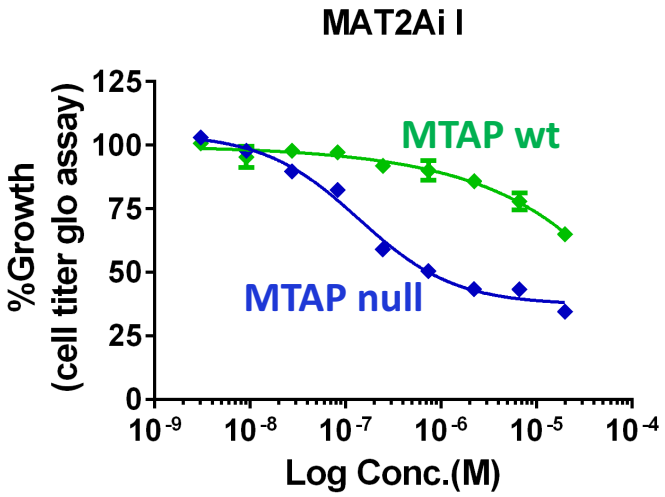


SAM production rate in HCT116 cells

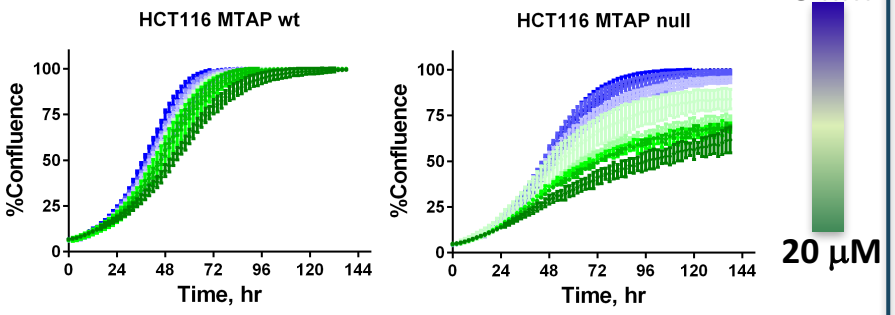


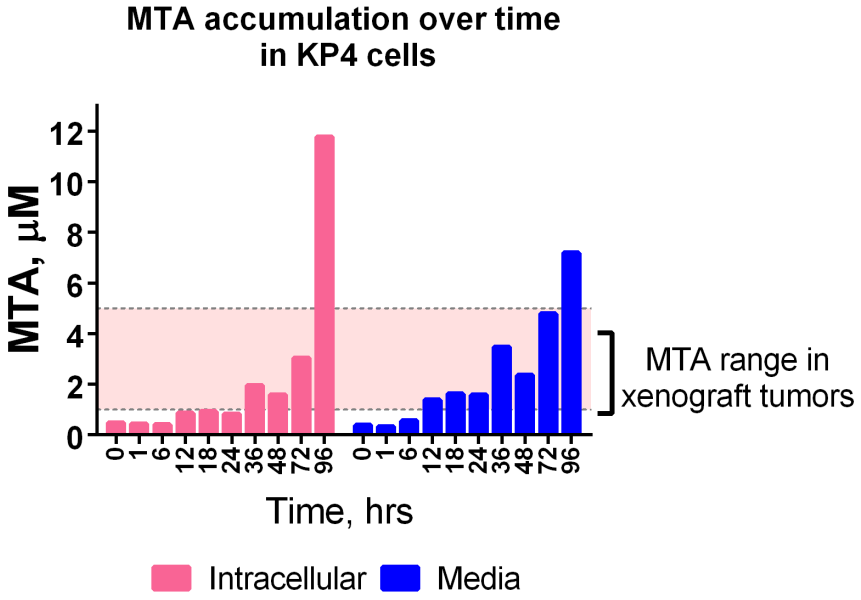
MAT2A Inhibitors Selectively Block Growth of MTAP-null cancer cells *in vitro*

4 day proliferation assay



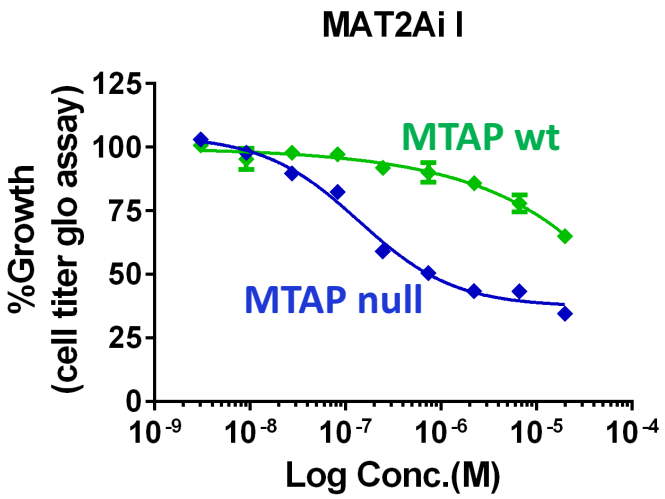
IncuCyte Continuous growth assay



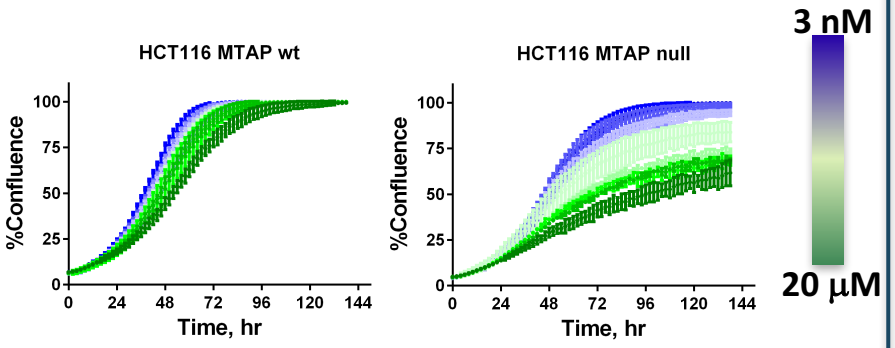


MAT2A Inhibitors Selectively Block Growth of MTAP-null cancer cells *in vitro*

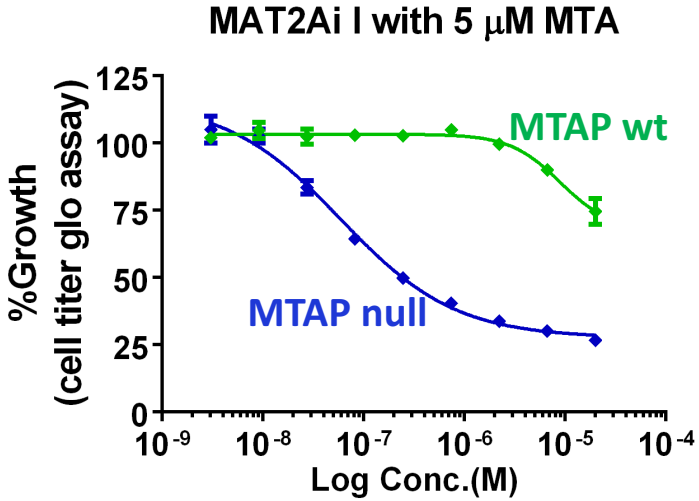
4 day proliferation assay



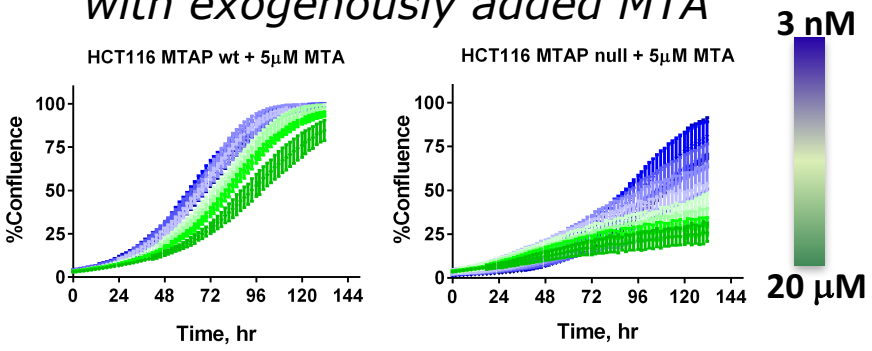
IncuCyte Continuous growth assay



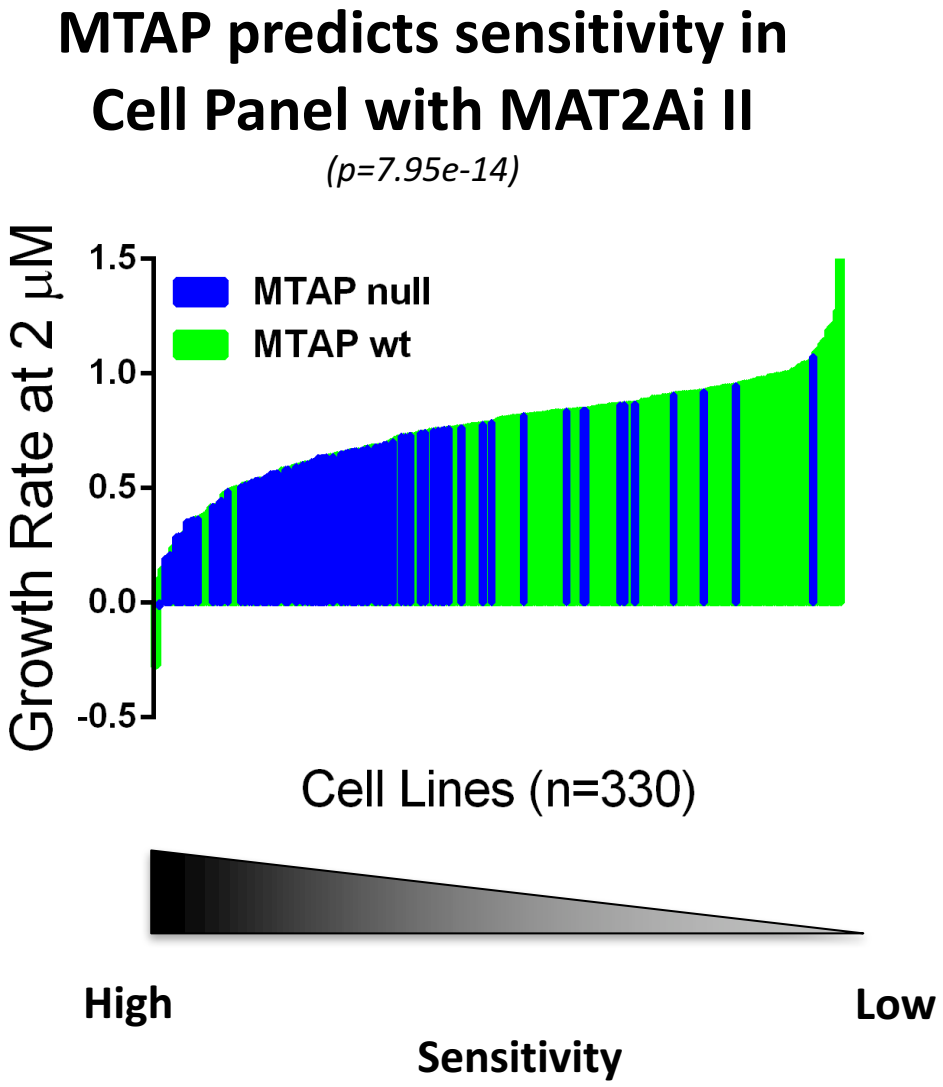
4 day proliferation assay



IncuCyte Continuous growth assay with exogenously added MTA

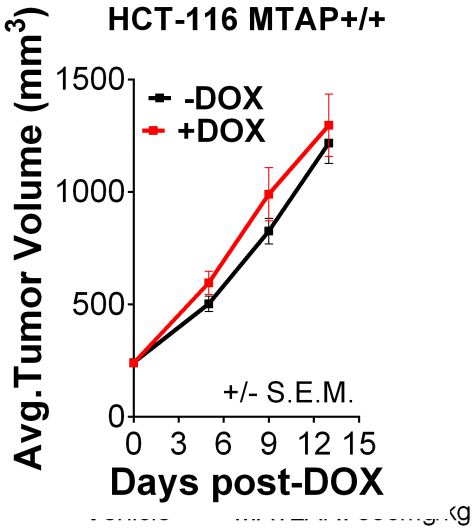


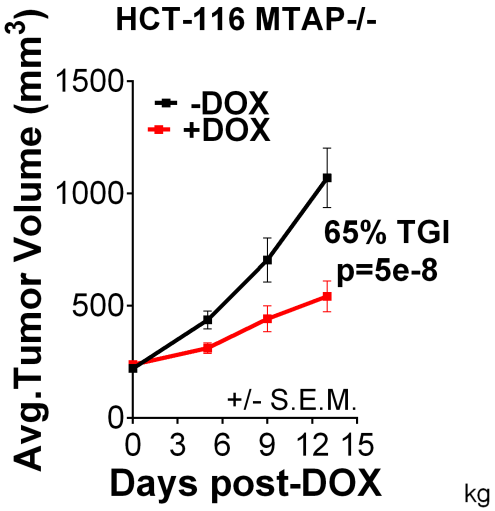
MAT2A Inhibitors Selectively Block Growth of MTAP-null cancer cells *in vitro*

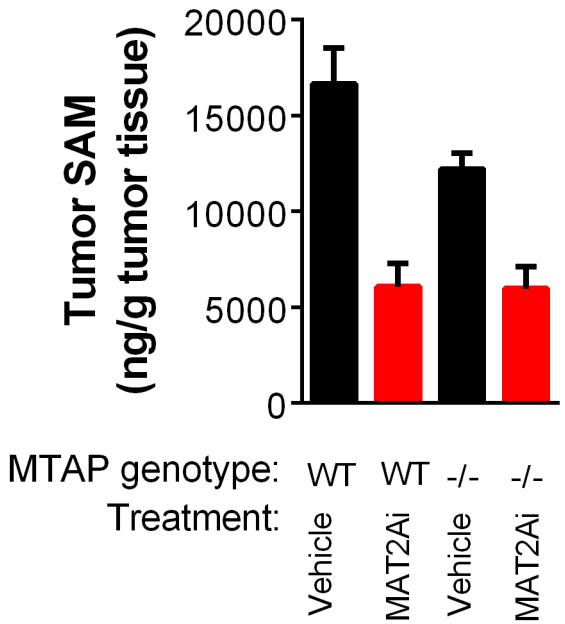


Genetic and Pharmacologic targeting of MAT2A Selectively Blocks Growth of MTAP-null cancer cells *in vivo*

] Impact in vivo with MAT2Ai ols

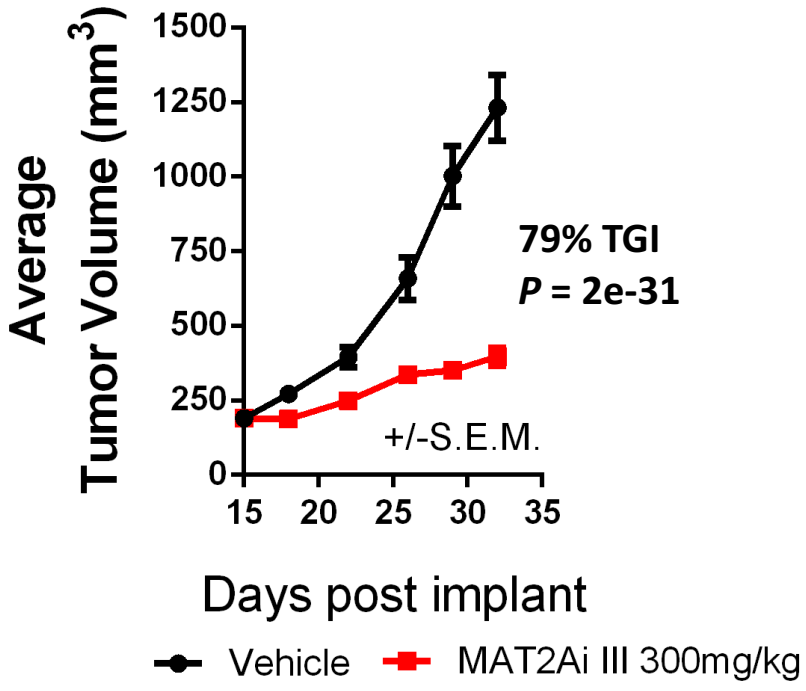




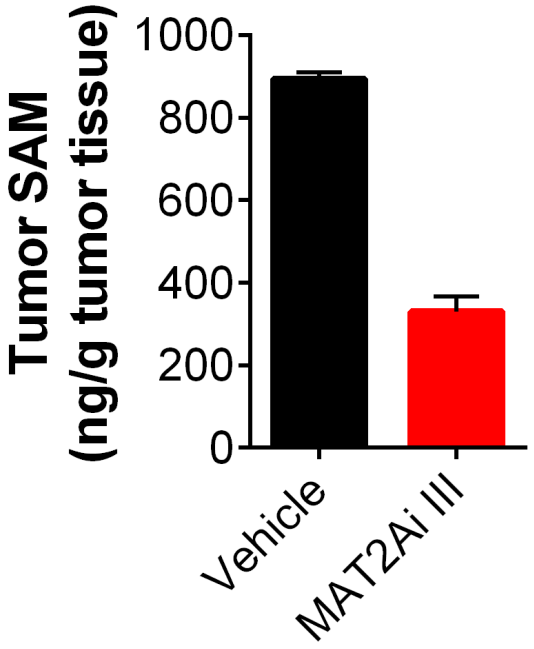


- Similar SAM decrease is observed in HCT116-MTAP-/- and HCT116-MTAP+/+ tumors

Treatment with MAT2Ai reduces growth of naturally MTAP-null KP4 pancreatic cancer cell line xenografts



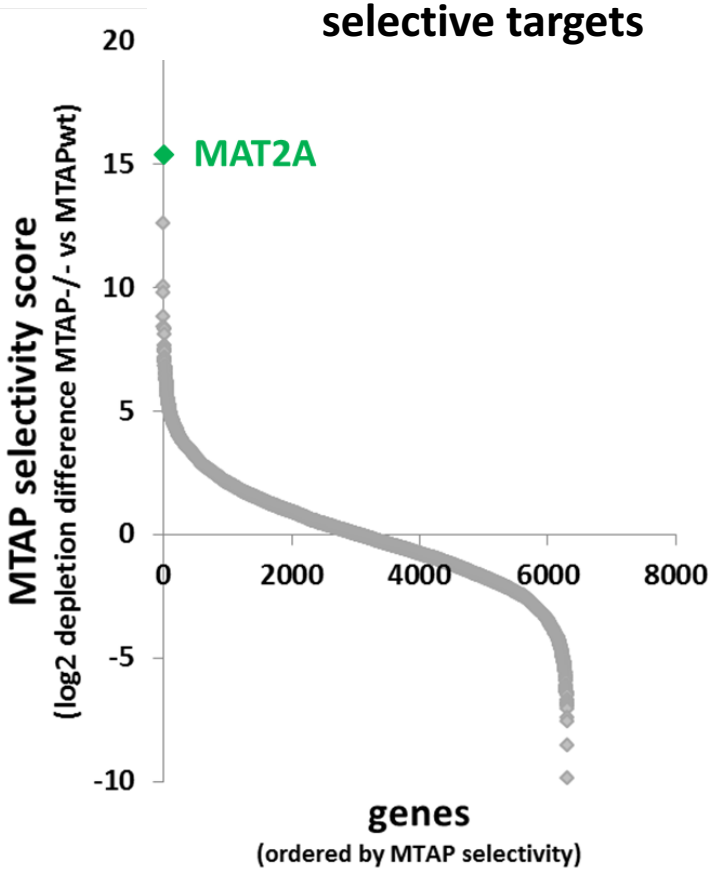
Tumor SAM reduction after MAT2Ai III Treatment (300 mg/kg)



MAT2Ai had significant anti-tumor activity in the KP4 Sub-q model

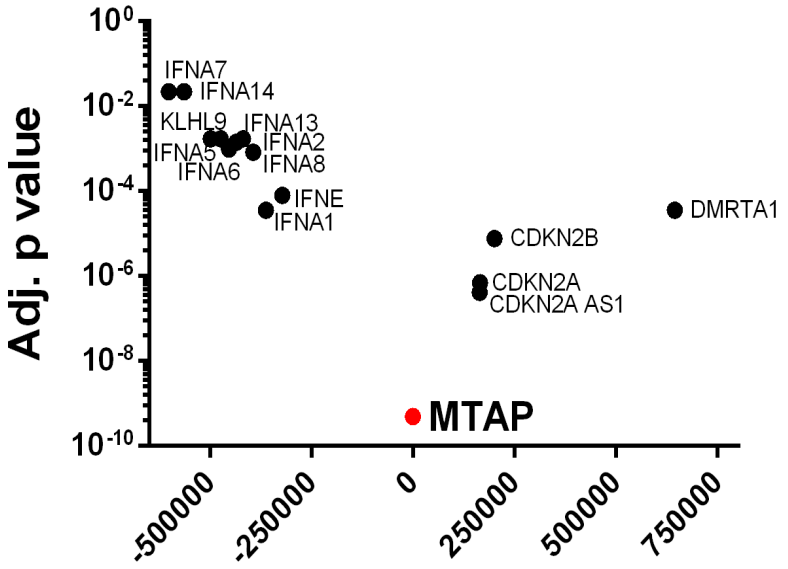
Strong reciprocal connection/synthetic lethality between MAT2A and MTAP

shRNA screen for MTAP-selective targets



MAT2A is the top hit in an MTAP-selective shRNA screen

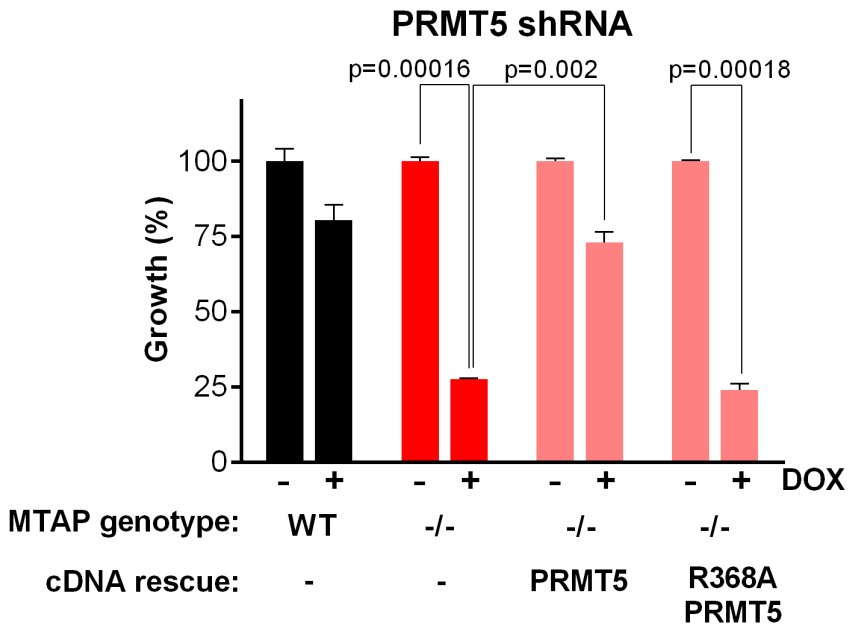
Correlation of MAT2Ai GI50 with Copy Number

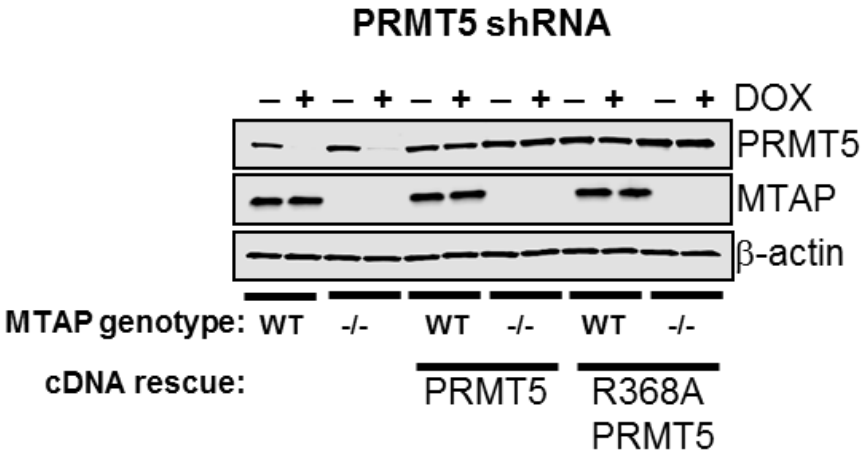


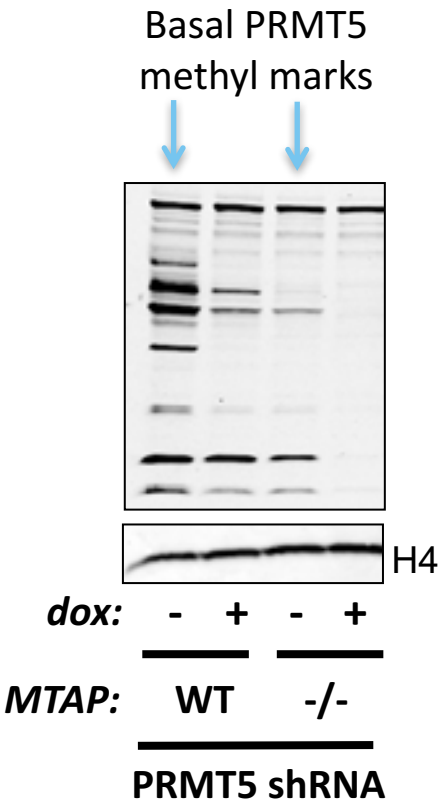
Meanwhile, MTAP deletion is the genetic feature that best predicts sensitivity to MAT2A inhibitor

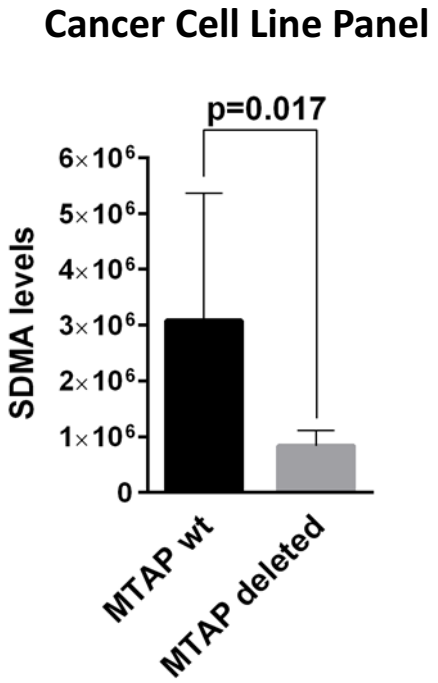
PRMT5 is also a Selective Vulnerability in MTAP-null Cancers

HCT116 MTAP isogenic pair







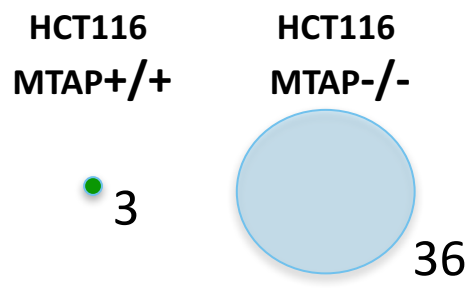


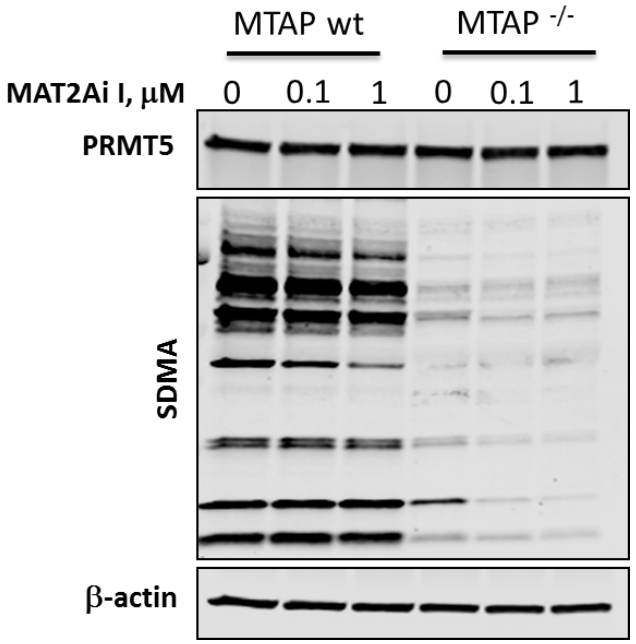
Methylation Proteomics Corroborates Role for PRMT5 as a Key Downstream Mediator of MAT2Ai in MTAP-deleted Cells

HCT116 MTAP$^{-/-}$ and HCT116 MTAP wt cells → **3-day treatment with MAT2Ai or DMSO** → **IP with PTM-specific antibodies** → **LC-MS/MS**

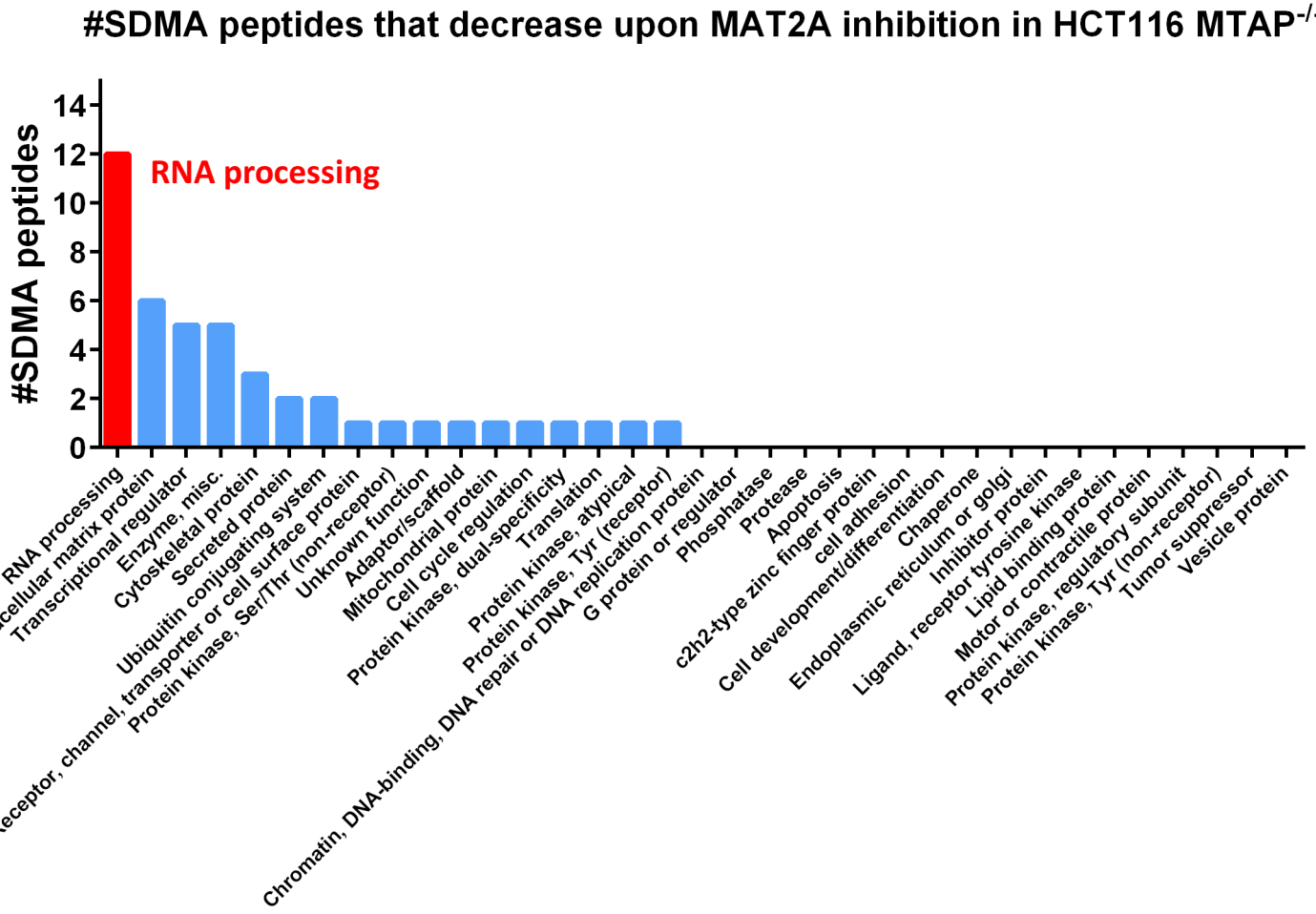
- **Mono-Methyl-Arginine**
- **Asymmetrical di-methyl Arginine**
- **Symmetrical di-methyl Arginine**
- **Pan-methyl Lysine**

 # PRMT5 SDMA peptides reduced >4-fold upon MAT2Ai II treatment:



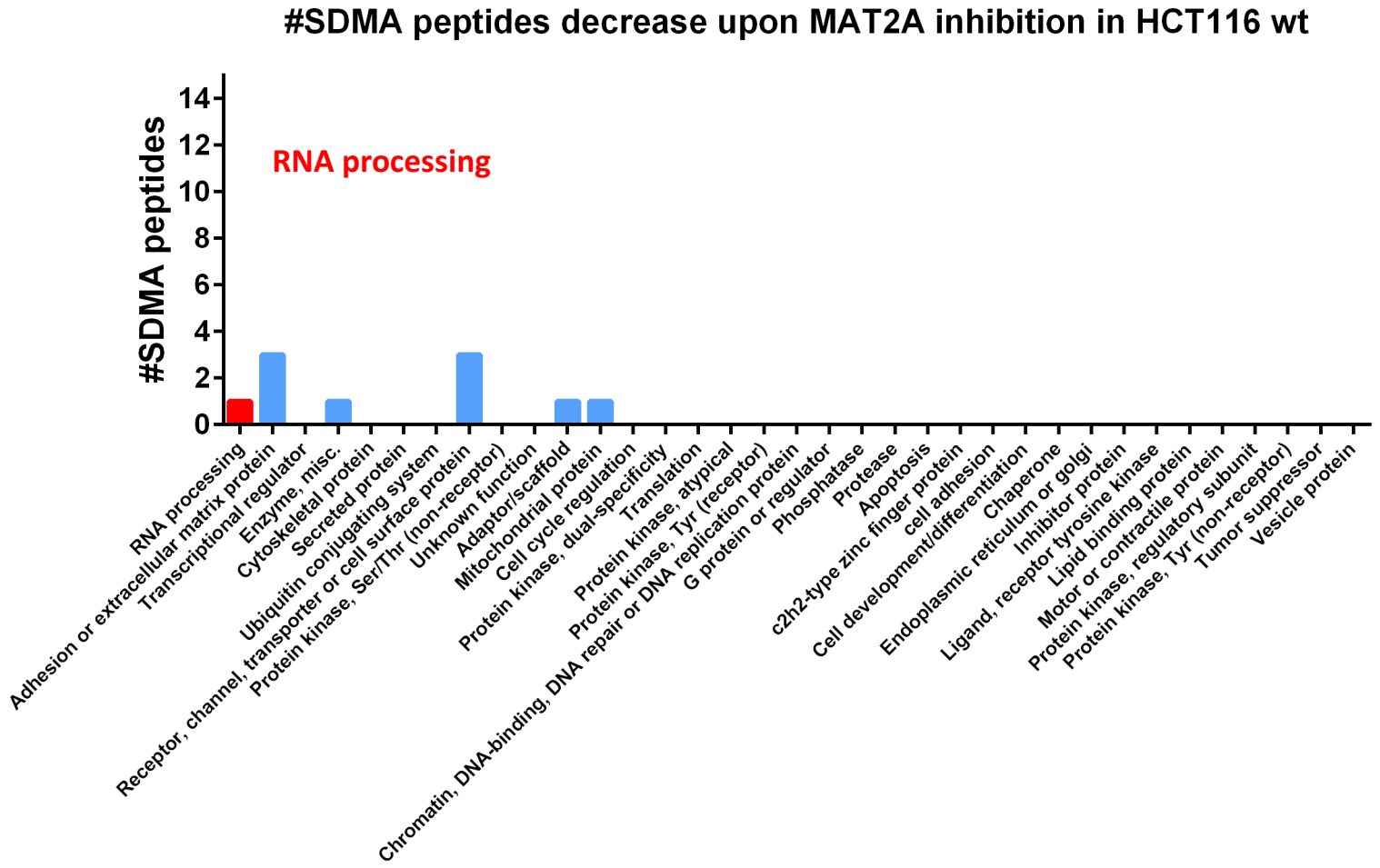


Methylation Proteomics Indicates MAT2A Inhibition Reduces Methylation of RNA Processing Machinery in MTAP-null Cells



Methylation proteomics identifies loss of methylation of RNA processing machinery upon MAT2A inhibitor treatment

Methylation Proteomics Indicates MAT2A Inhibition Reduces Methylation of RNA Processing Machinery in MTAP-wt Cells

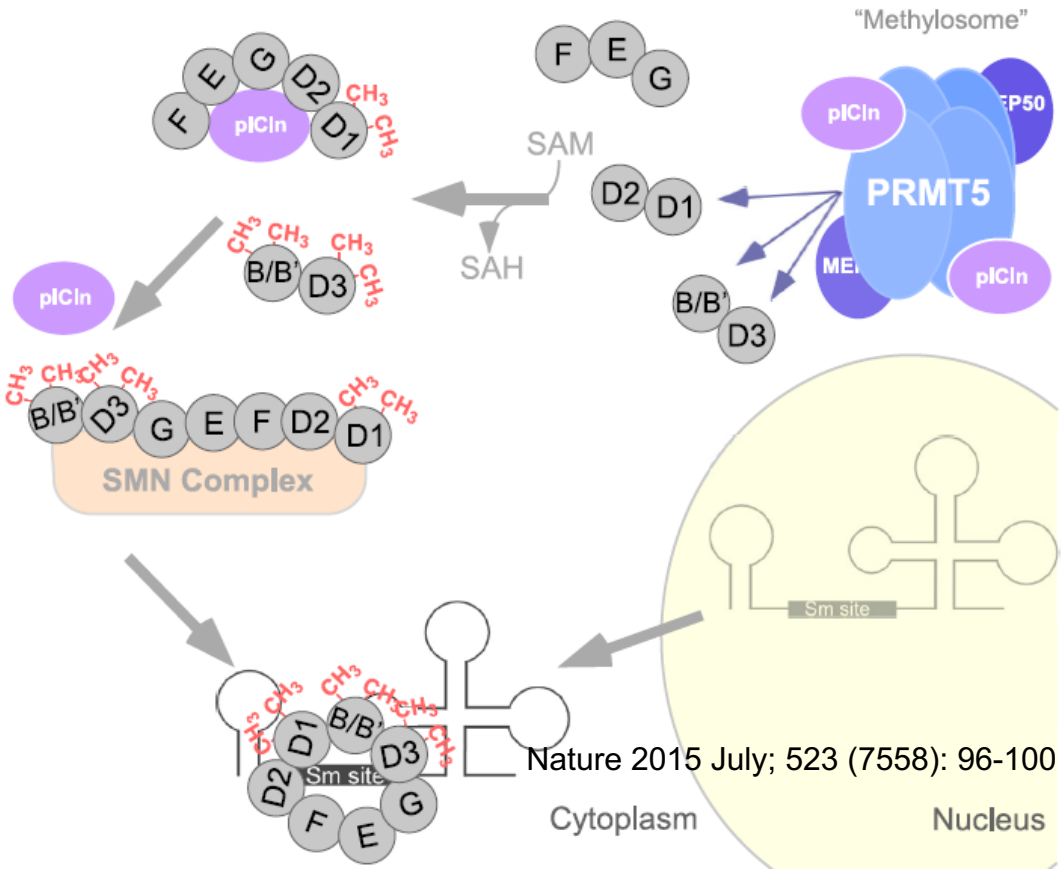


Methylation proteomics identifies loss of methylation of RNA processing machinery upon MAT2A inhibitor treatment

Symmetric Arginine Methylation of Spliceosome components by PRMT5 is Important for Spliceosome Maturation

Published substrates include:

- SmD1,SmD3,SmB/B' (*Brahms, RNA 2001 and Friesen Mol Cell 2001)*
  - Methylation is required for interaction w/ SMN
- PRMT5 KO mouse NPCs have splicing defects *(Bezzi, Genes Dev 2013)*



Nature 2015 July; 523 (7558): 96-100

Cell Mol Life Sci. 2015 Jun; 72(11): 2041–2059

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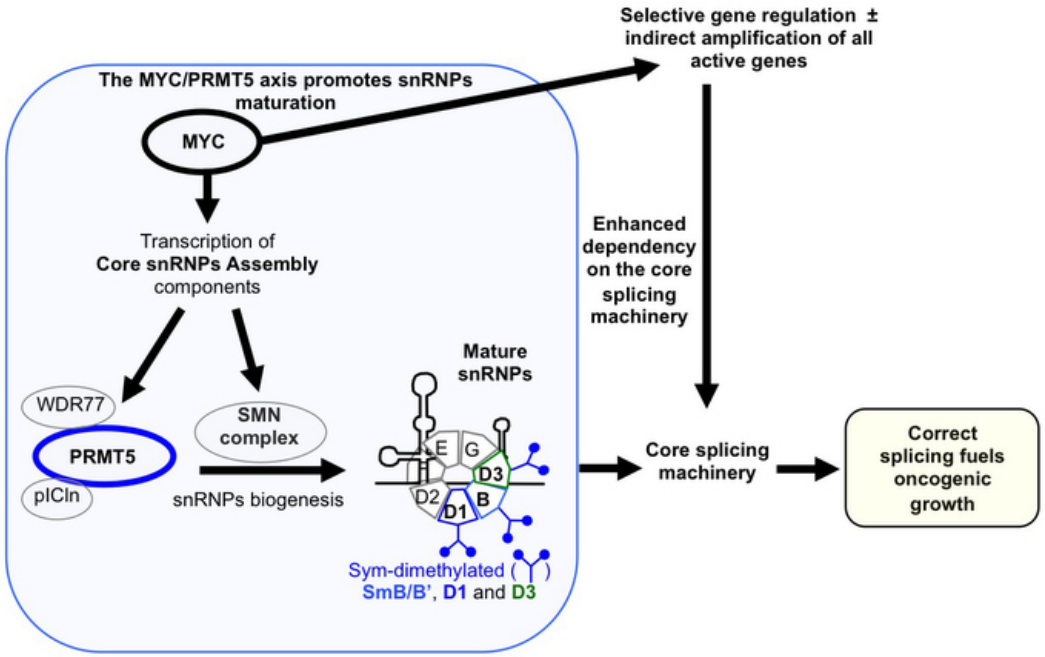
Splicing regulation is an essential step in lymphomagenesis:

- MYC directly upregulates the core snRNP assembly genes, including PRMT5
- PRMT5 is overexpressed in non-Hodgkin lymphoma (NHL) cell lines and clinical samples *(Chang J Biol Chem 2013)*
- PRMT5 is required for proliferation of B lymphoma cell lines (*Chan-Penebre NCB 2015)*



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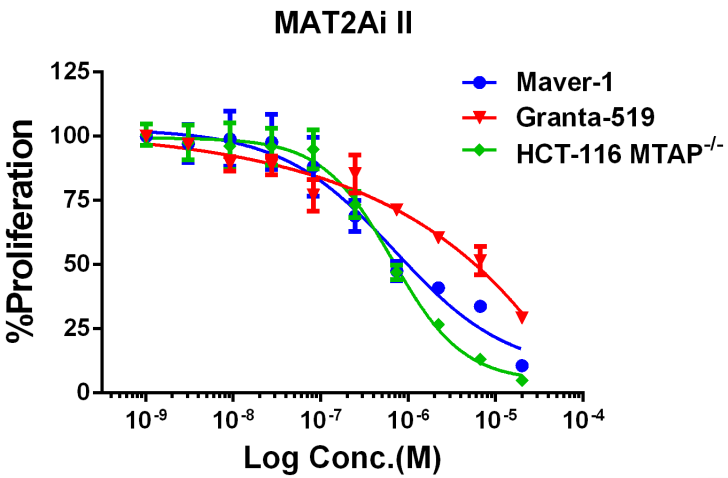
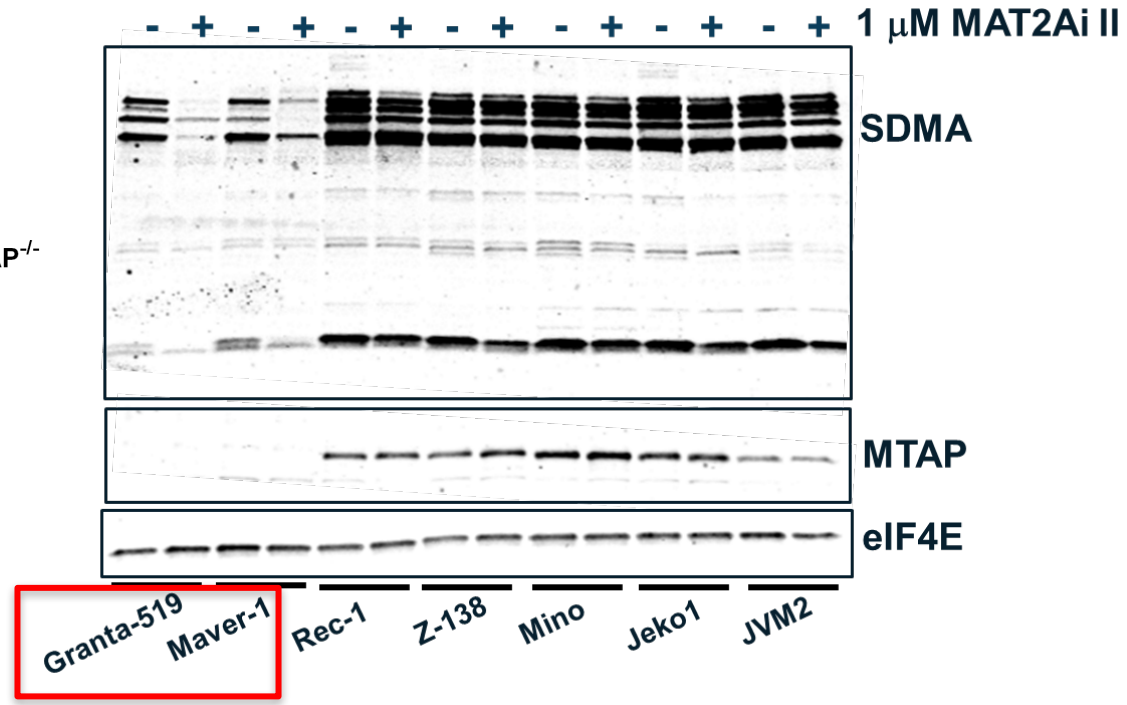
NHL B lymphoma MTAP-null models show reduced growth and downstream impact on PRMT5 SDMA marks upon treatment with MAT2Ai

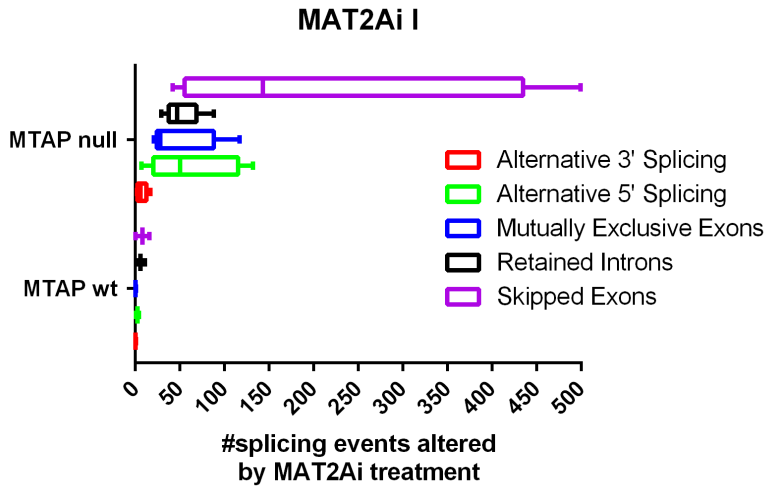


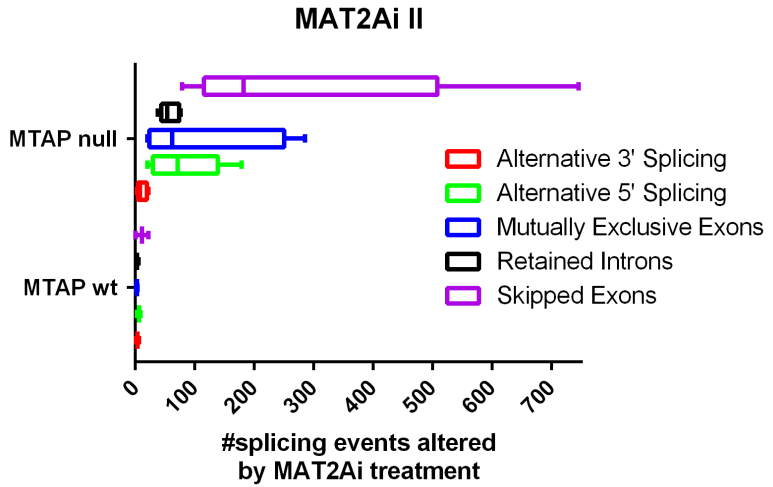


MAT2Ai Treatment Perturbs Splicing Selectively in MTAP-null NHL B lymphoma Cell Lines

Tophat2 (mapping reads to transcriptome) → **rMATS: alt splicing (FDR < 0.05)**

MTAP Status	Cell Line	Treatment	Alternative 3' Splicing	Alternative 5' Splicing	Mutually Exclusive Exons	Retained Introns	Skipped Exons
NULL	DOHH2	MAT2Ai I					
		MAT2Ai II					
	Granta-519	MAT2Ai I					
		MAT2Ai II					
	Maver1	MAT2Ai I					
		MAT2Ai II					
	OCI-LY19	MAT2Ai I					
		MAT2Ai II					
	OCI-LY3	MAT2Ai I					
		MAT2Ai II					
WT	Jeko	MAT2Ai I					
		MAT2Ai II					
	Mino	MAT2Ai I					
		MAT2Ai II					





MAT2Ai Treatment Perturbs Splicing Selectively in MTAP-null Cell Lines

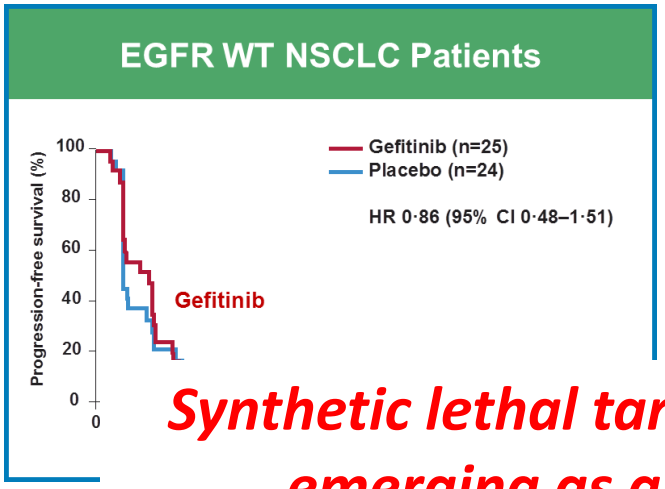
Summary

- MTAP is frequently deleted in a variety of cancer indications (~15% of all human cancer)
  - MTAP deletion leads to its substrate MTA accumulation in MTAP null tumors
  - PRMT5 unique biochemical features make it sensitive to double hit of MTA accumulation and SAM reduction (downstream from MAT2A)
- **Agios discovered first-in-class small molecule inhibitors of MAT2A**
- MAT2A pharmacologic targeting substantially reduces SAM levels and SAM *de novo* synthesis in cells
- **Pharmacologic inhibition of MAT2A recapitulates findings with MAT2A-targeting genetic tools** and selectively attenuates growth of $MTAP^{-/-}$ but not MTAP wt cancer cells *in vitro* and *in vivo*
  - Inhibition of MAT2A selectively attenuates growth of $MTAP^{-/-}$ HCT116 cells and naturally MTAP-deleted cancer cell lines (n=330 cell lines)
  - Inhibition of MAT2A significantly attenuates growth of MTAP-deficient HCT116 and KP4 cells *in vivo*
- **MAT2A inhibition and MTAP loss exhibit strong reciprocal connection** mediated at least in part via impact on PRMT5 activity and downstream function (symmetric Arg di-methylation of RNA processing machinery and splicing)

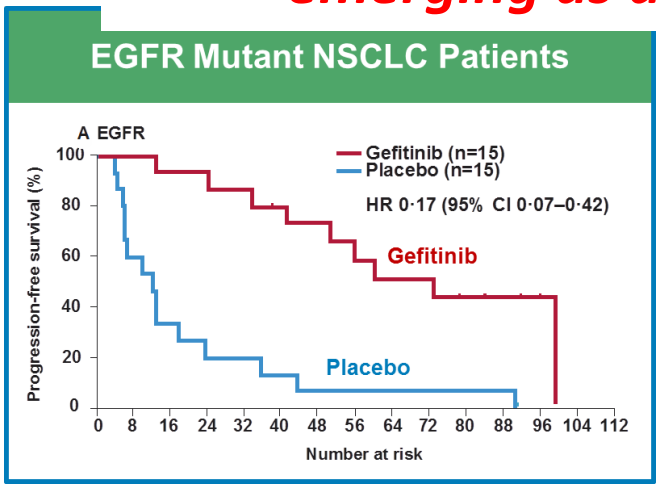
The Challenge: Identifying Precision Medicine Approaches in Cancer Metabolism

Directly drugging 'driver mutations' has yielded transformative medicines

but…DNA sequencing has only ID'd 1 classic, gain-of-fuction metabolic 'driver' mutation out of 2000+ metabolic genes



Synthetic lethal targeting of collateral vulnerabilities emerging as a key solution to this challenge



Zhang, et al, Lancet Oncology, 2012

IDH1/2

Acknowledgements

Agios 2016 Founders Day Retreat

- Agios Pharmaceuticals team
- Cell Signaling Technology proteomics core