Metabolic Consequences of Hyperhomocysteinemia

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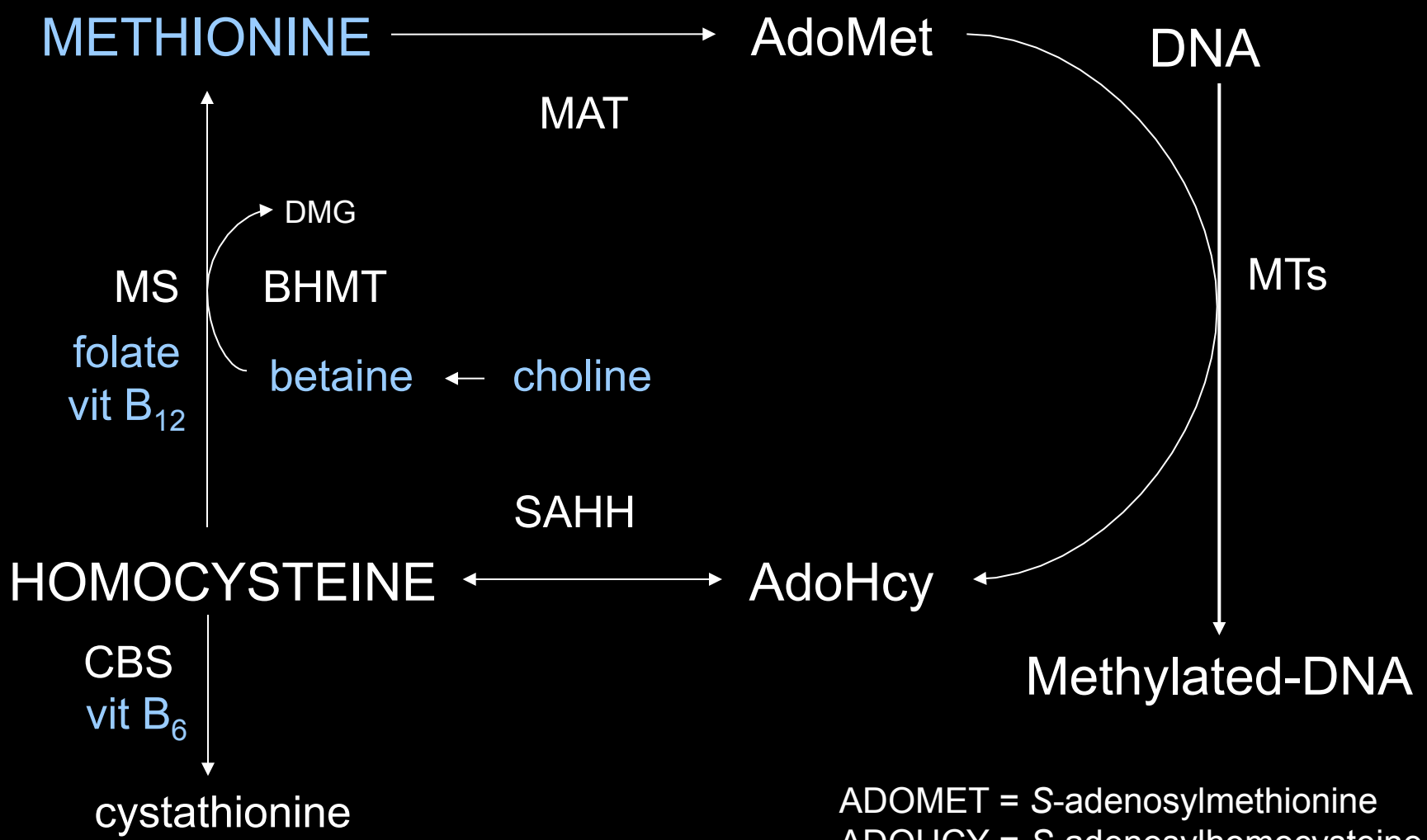
Metabolic Consequences of HHcy

- Endothelial dysfunction (impaired vasodilitation) is a common feature of hyperhomocysteinemia
- What are the mechanisms?
- Homocysteine is metabolically linked to:
  - DNA methylation
  - Lipid metabolism

Where to start…

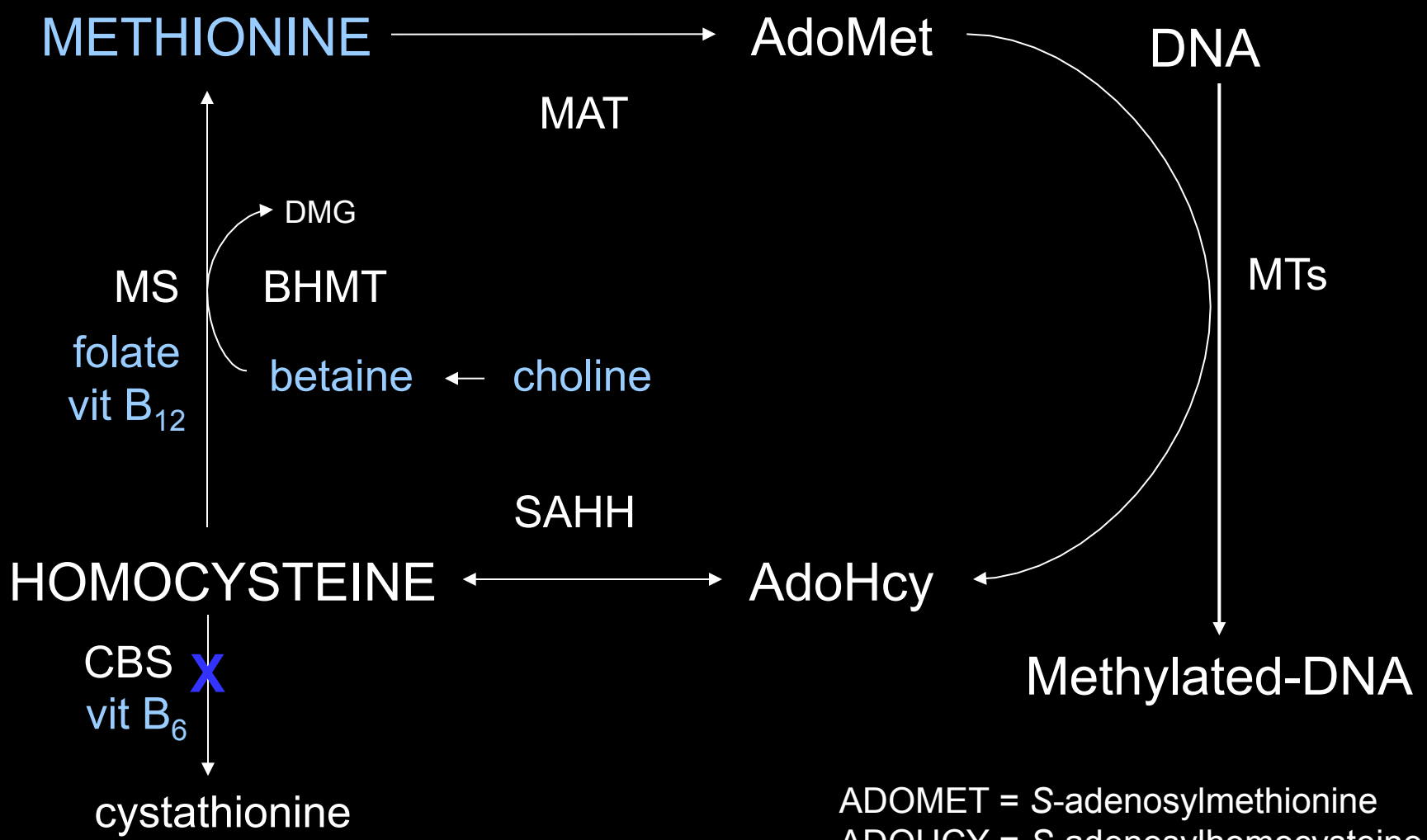
- DNA methylation (CpG methylation) regulates gene expression
- Allele-silencing of genomically imprinted genes
  - *H19*/*Igf2*
- Silencing of gene expression
  - *Fads2* (lipid metabolism)
  - *Nr3c1*

Homocysteine & DNA Methylation



ADOMET = *S*-adenosylmethionine
ADOHCY = *S*-adenosylhomocysteine

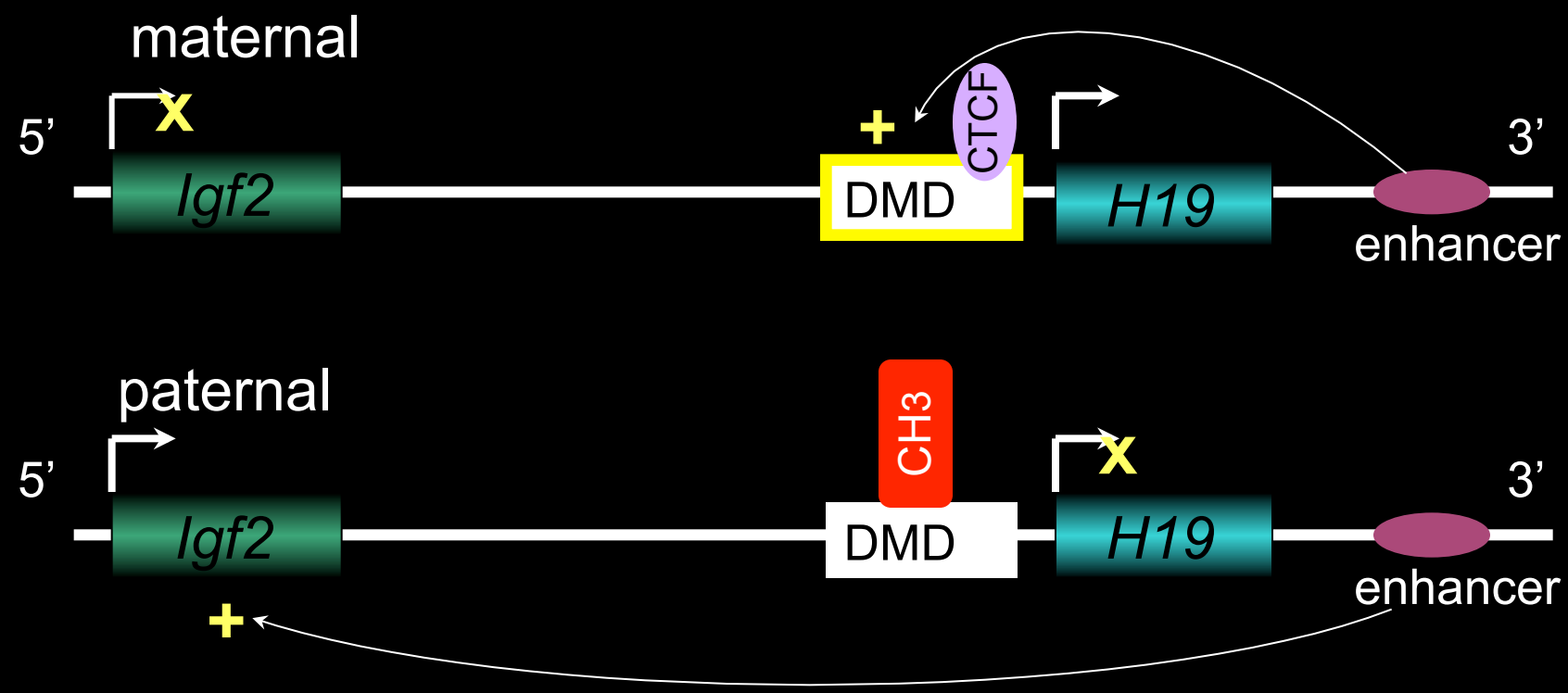
Homocysteine & DNA Methylation



ADOMET = *S*-adenosylmethionine
ADOHCY = *S*-adenosylhomocysteine

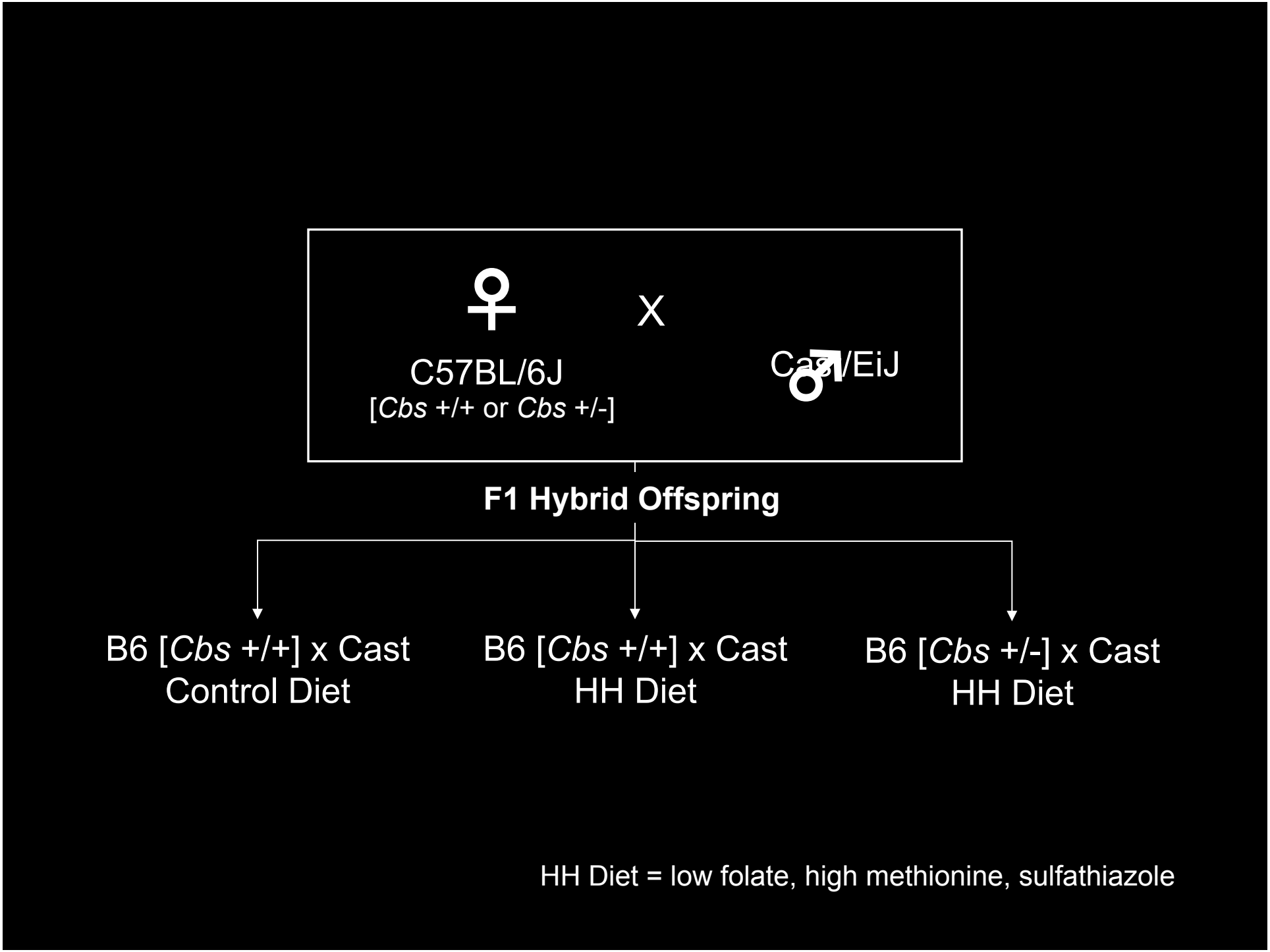
Genomically Imprinted *H19/Igf2*

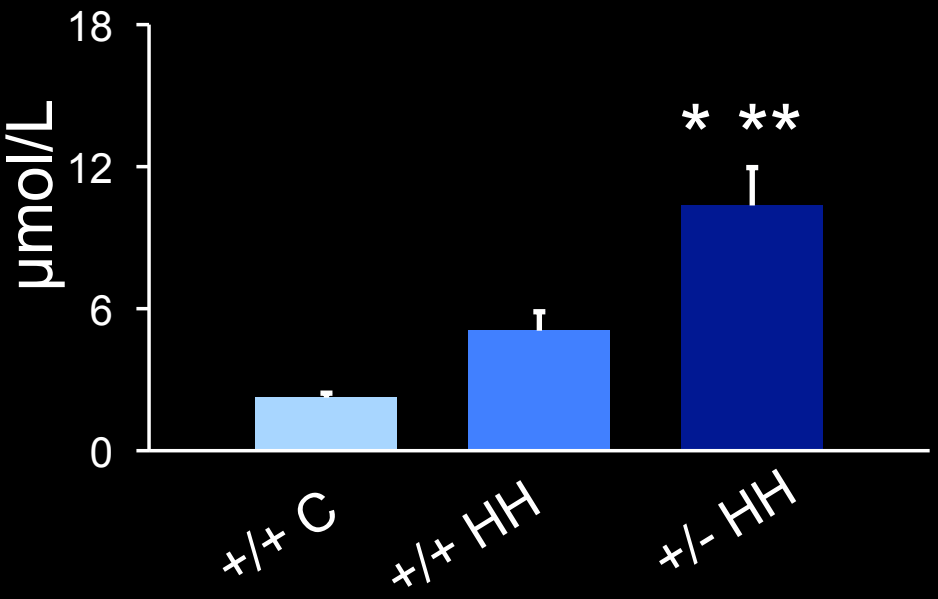
- *H19*/*Igf2* are well characterized reciprocally imprinted genes
- Biallelic expression of *H19* has been reported in human subjects with renal disease and HHcy
- Reversed by folate supplementation
  (Ingrosso et al *Lancet* 2003; 361:1693-1699)
- *H19* is a good 'proof-of-concept' target to investigate the relevance of changes in AdoMet and AdoHcy to changes in DNA methylation and tissue-specific differences

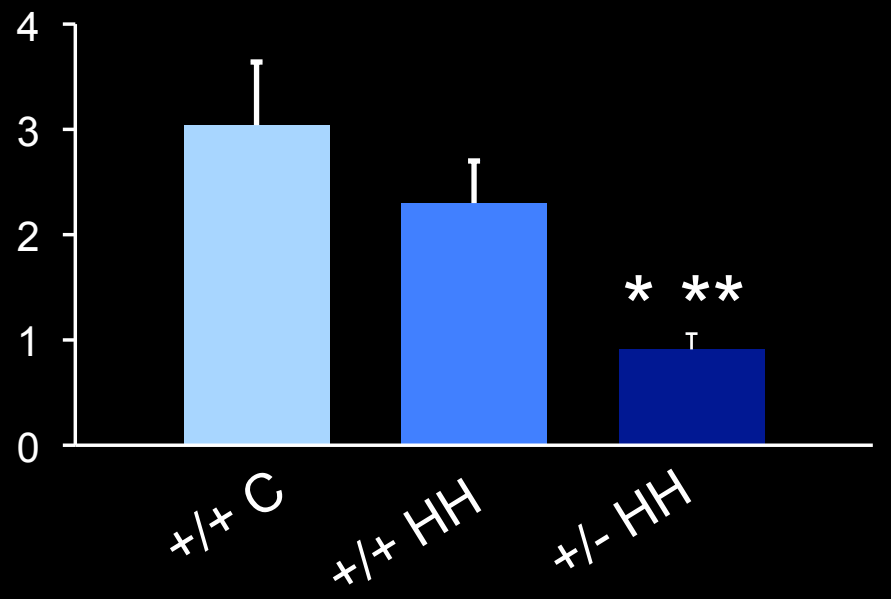


- *H19* is expressed from the maternal allele, *Igf2* expressed from the paternal allele
- Expression is regulated by the methylation status of a differentially methylated domain (DMD)

(reviewed by Ideraabdullah & Bartolomei *Mut Res*, 2008, 647:77-85)

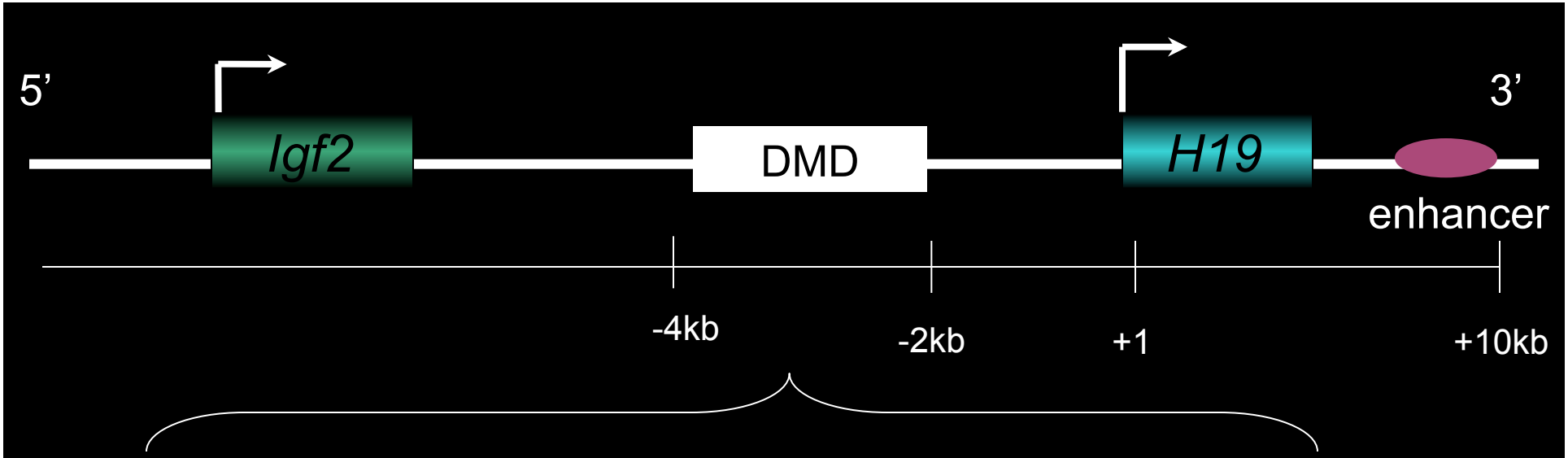

♀
X
C57BL/6J
[*Cbs* +/+ or *Cbs* +/-]
Cast/EiJ
♂
F1 Hybrid Offspring
B6 [*Cbs* +/+] x Cast
Control Diet
B6 [*Cbs* +/+] x Cast
HH Diet
B6 [*Cbs* +/-] x Cast
HH Diet
HH Diet = low folate, high methionine, sulfathiazole





* $P < 0.01$, ** $P < 0.05$

Reduced liver methylation capacity with HHcy.

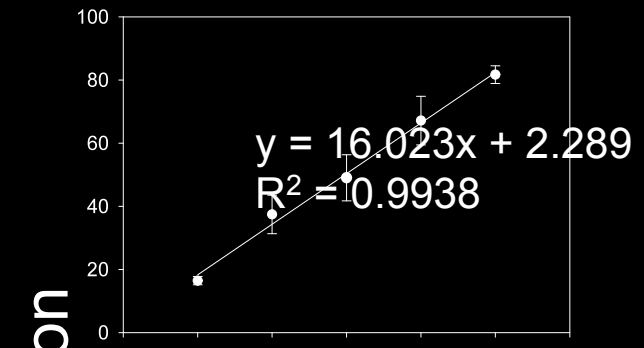



```
                                        1
-4455 gactcccaaa tcaacaagGt cggcttactc tctgcaaaga

                                     2     3  4
-4415 atcctttgtg tgtaaagacc agggttgccg cacggcggca

                  5          6
-4375 gtgaagtctc gtacatcgca gtcctaaaa
```

G (C57BL/6J) allele → A (Cast allele) at nucleotide -4437

Identified a strain-specific variant that was used for allele-specific methylation.

Methylation Assay Reliability

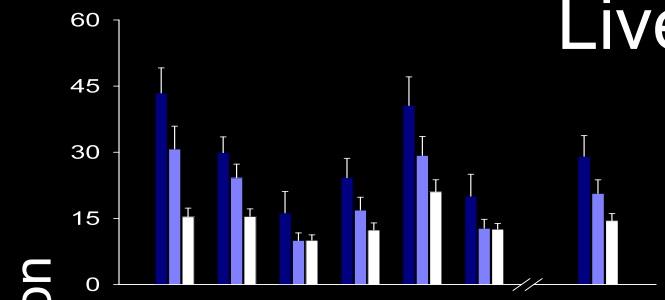


% Methylation

100/0 75/25 50/50 25/75 0/100

% Maternal Allele / % Paternal Allele

Liver Maternal Allele



* $P < 0.05$, ** $P < 0.05$

% methylation

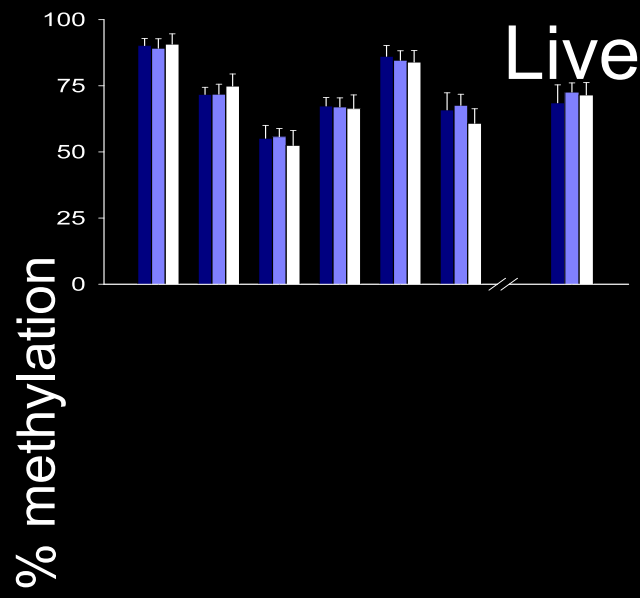
* ** * ** * * *

1 2 3 4 5 6 mean

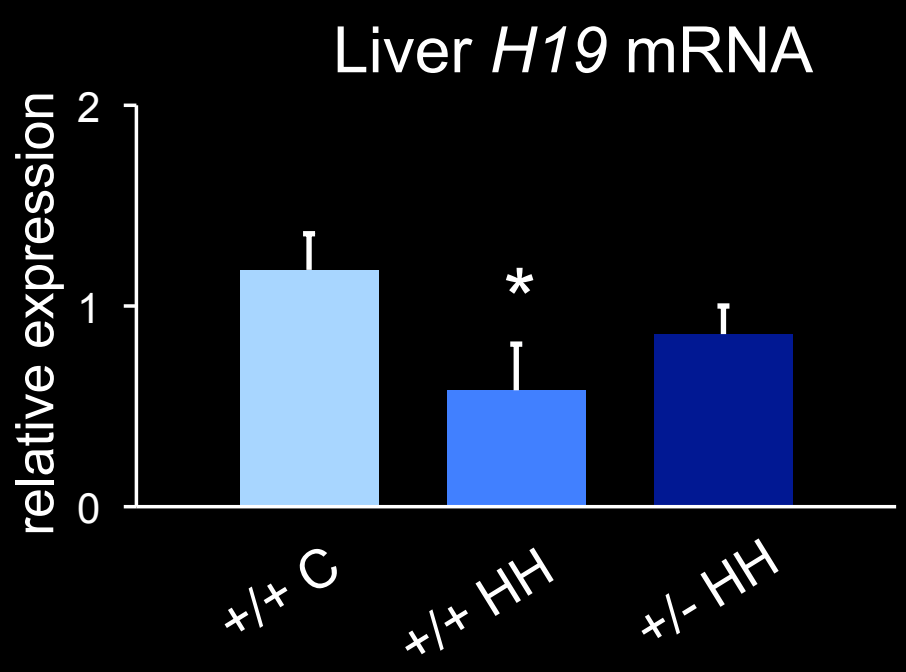
CpG Site

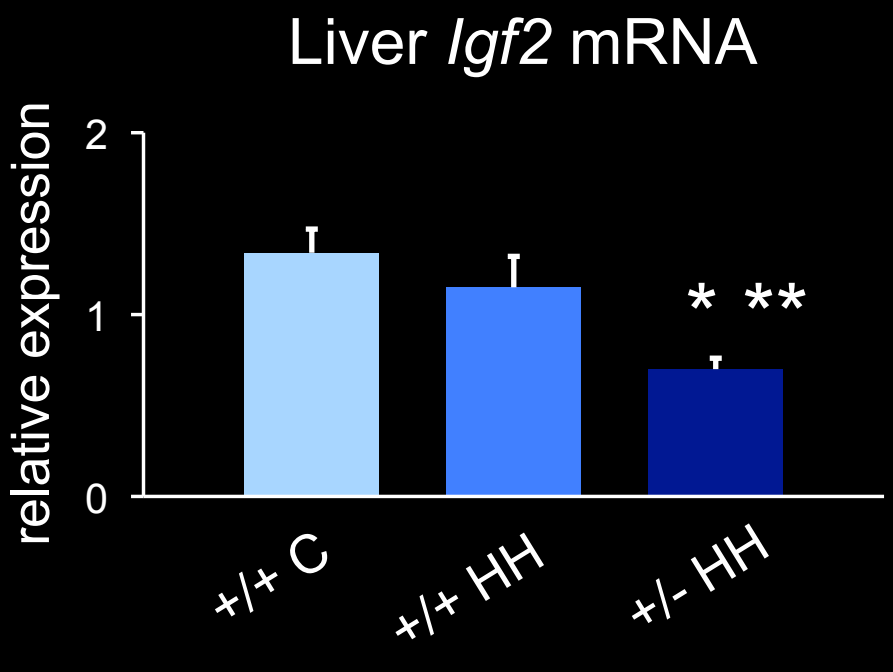
Decreased methylation of the maternal *H19* allele in liver from mice with HHcy.

Liver Paternal Allele



No effect of HHcy on paternal *H19* allele methylation in liver.





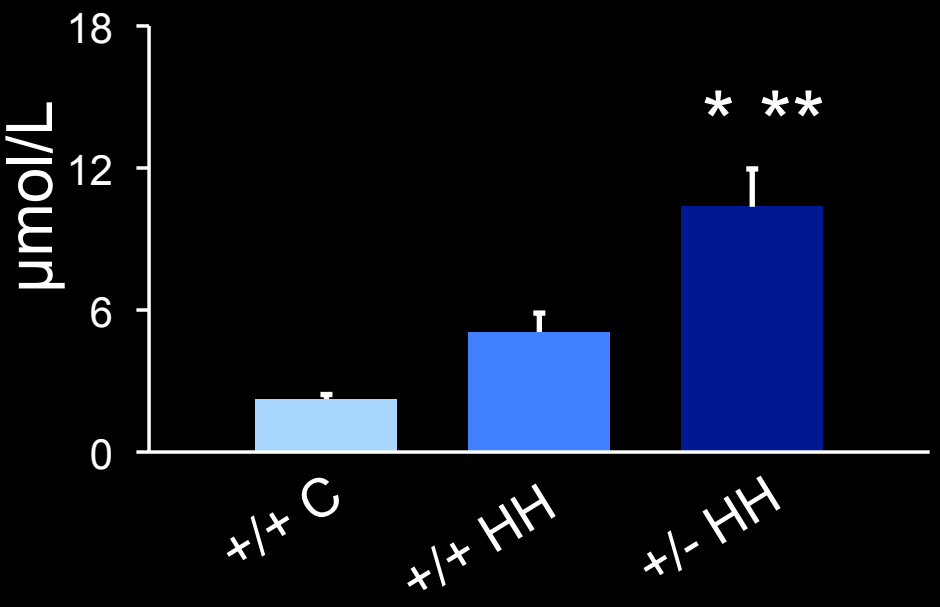
monoallelic expression

monoallelic expression

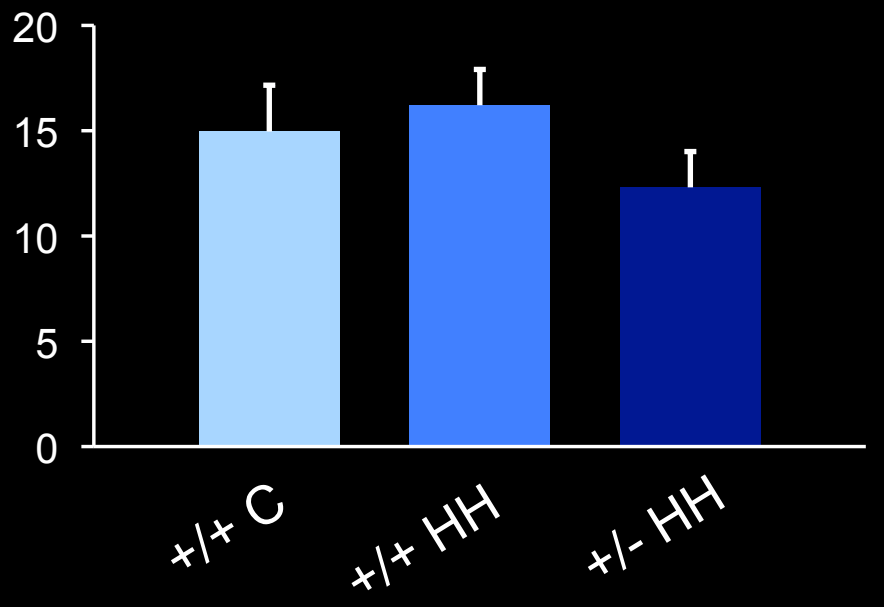
* ** $P < 0.05$

Decreased levels of *Igf2* mRNA with decreased *H19* maternal allele methylation in liver from mice with HHcy.

Plasma Total Homocysteine

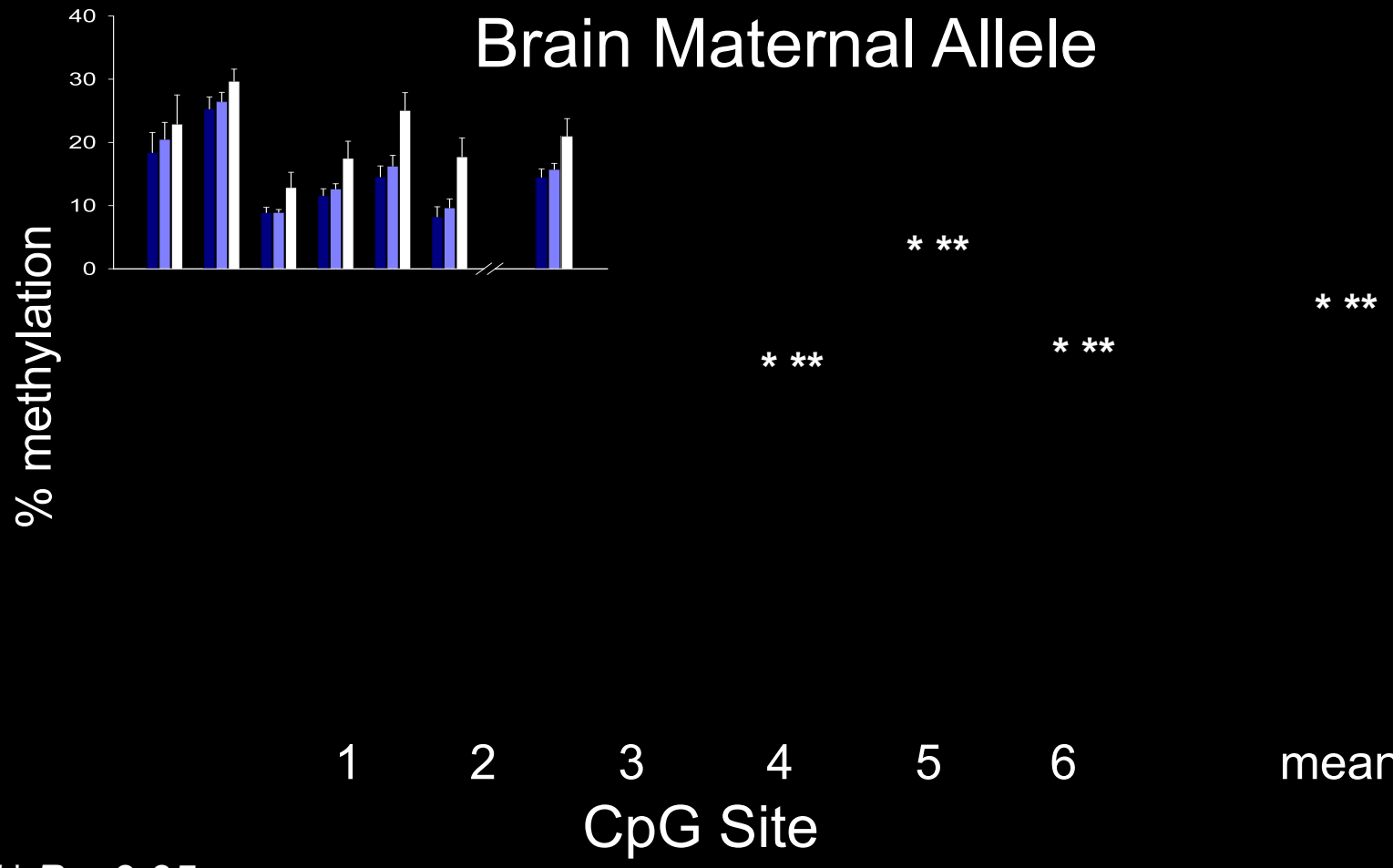


Brain AdoMet/AdoHcy



* ** $P < 0.05$

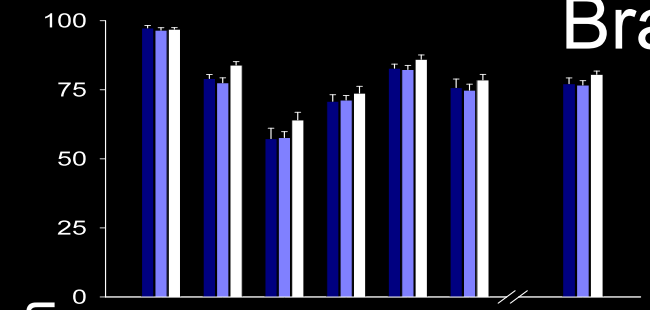
No effect of HHcy on brain methylation capacity.



* ** $P < 0.05$

Increased methylation of the maternal *H19* allele in brain from mice with HHcy (despite no change in methylation capacity).

Brain Paternal Allele

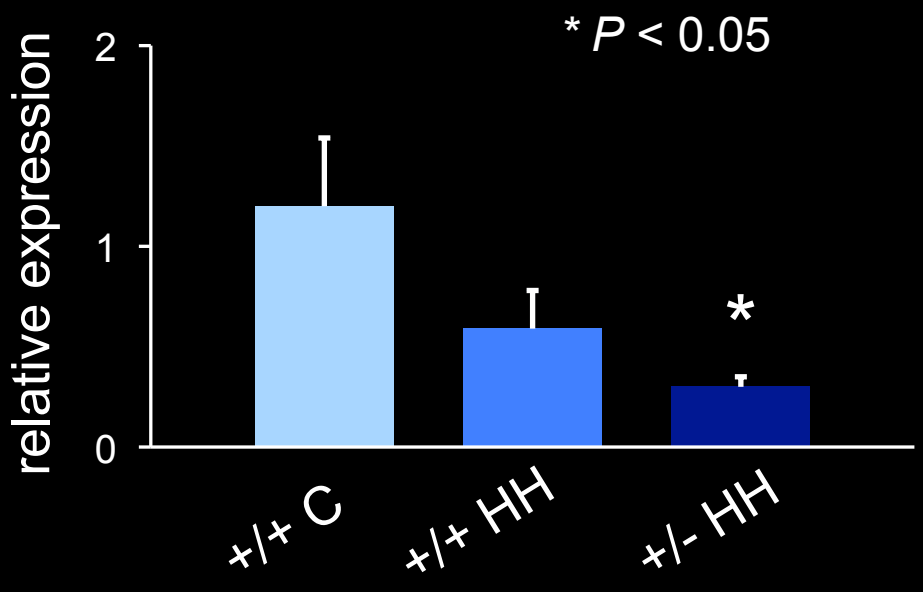


% methylation

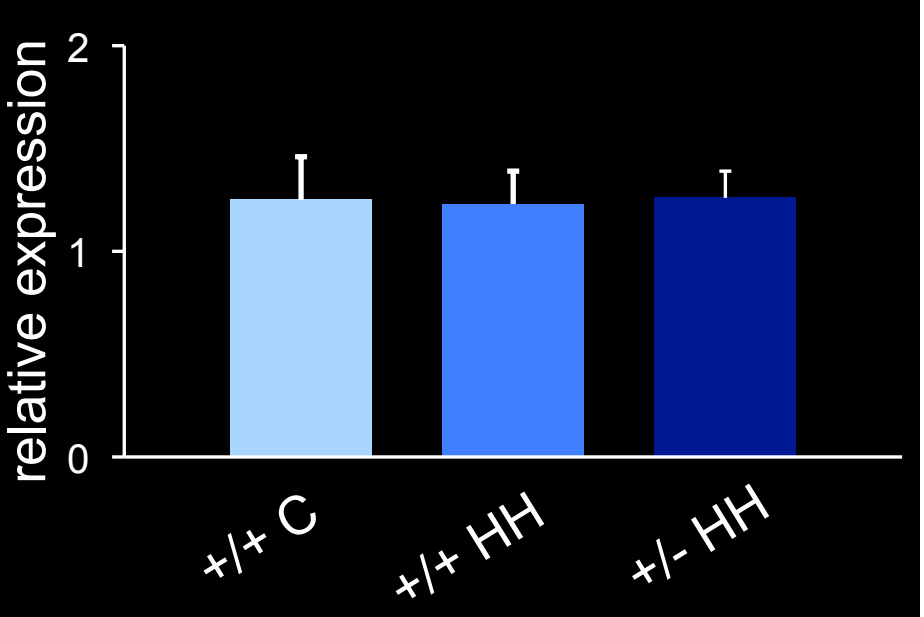
1 2 3 4 5 6 mean

CpG Site

No effect of HHcy on paternal *H19* allele methylation in brain.



monoallelic expression



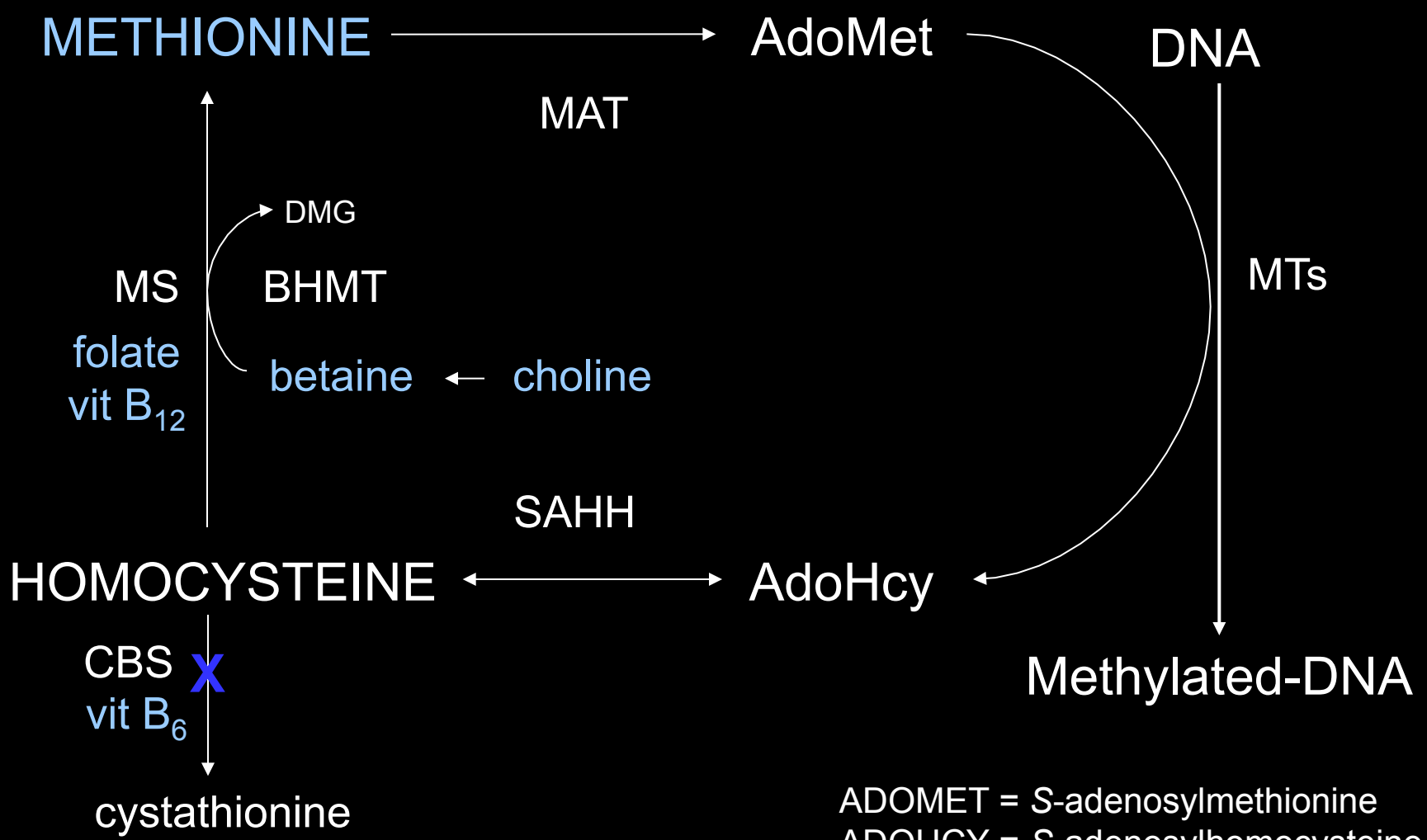
biallelic expression

Decreased levels of *H19* mRNA with increased methylation of the *H19* maternal allele in brain from mice with HHcy.

Summary: *H19*/*Igf2* & HHcy

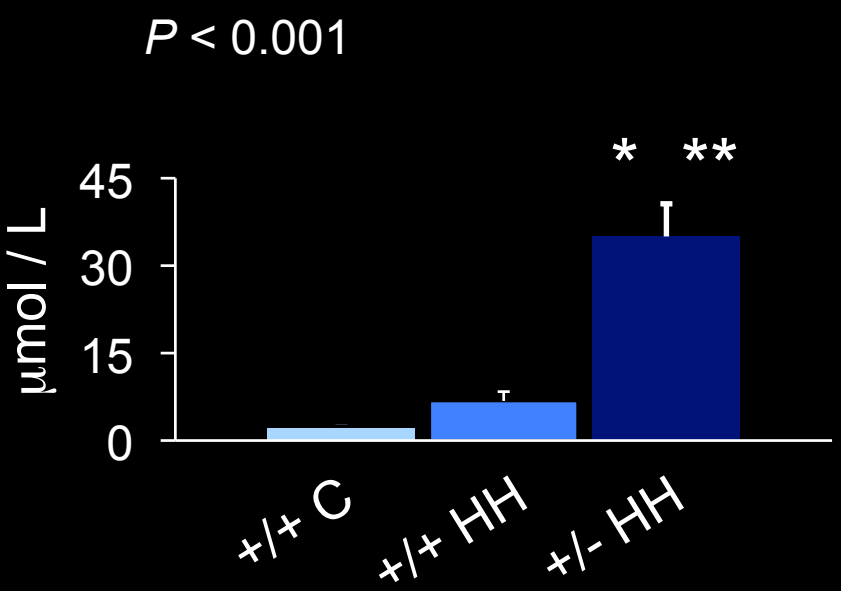
- HHcy is associated with gene-specific changes in DNA methylation
- Changes in methylation are tissue specific
- Changes in DNA methylation can occur in tissues despite no changes in methylation capacity (AdoMet/AdoHcy)

Homocysteine & DNA Methylation

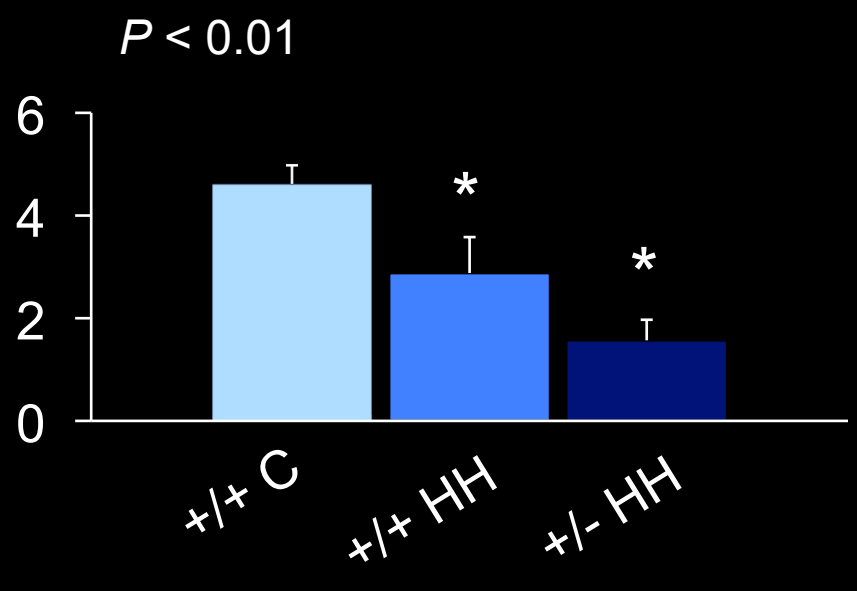


ADOMET = *S*-adenosylmethionine
ADOHCY = *S*-adenosylhomocysteine

Plasma Homocysteine

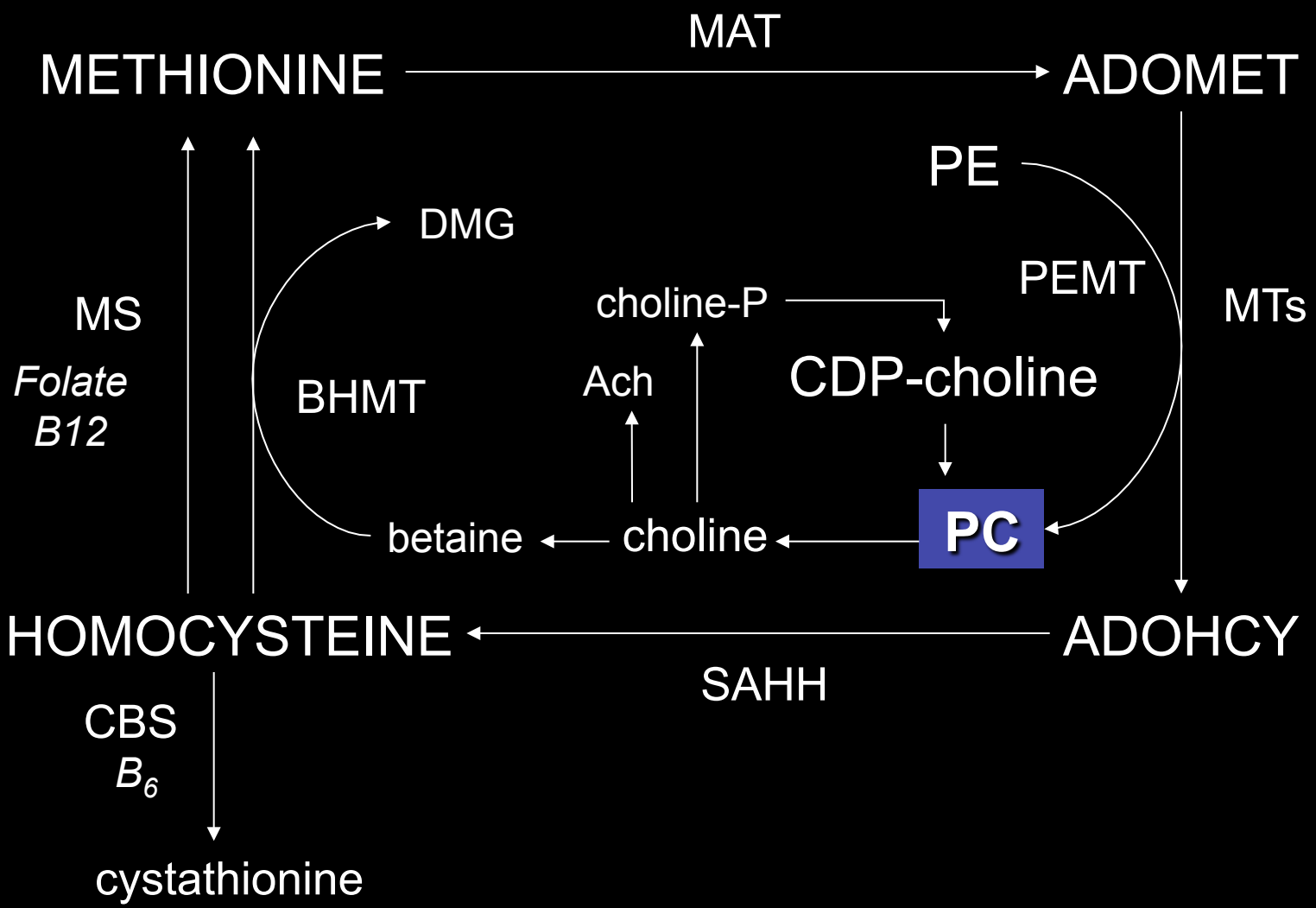


Liver AdoMet/AdoHcy

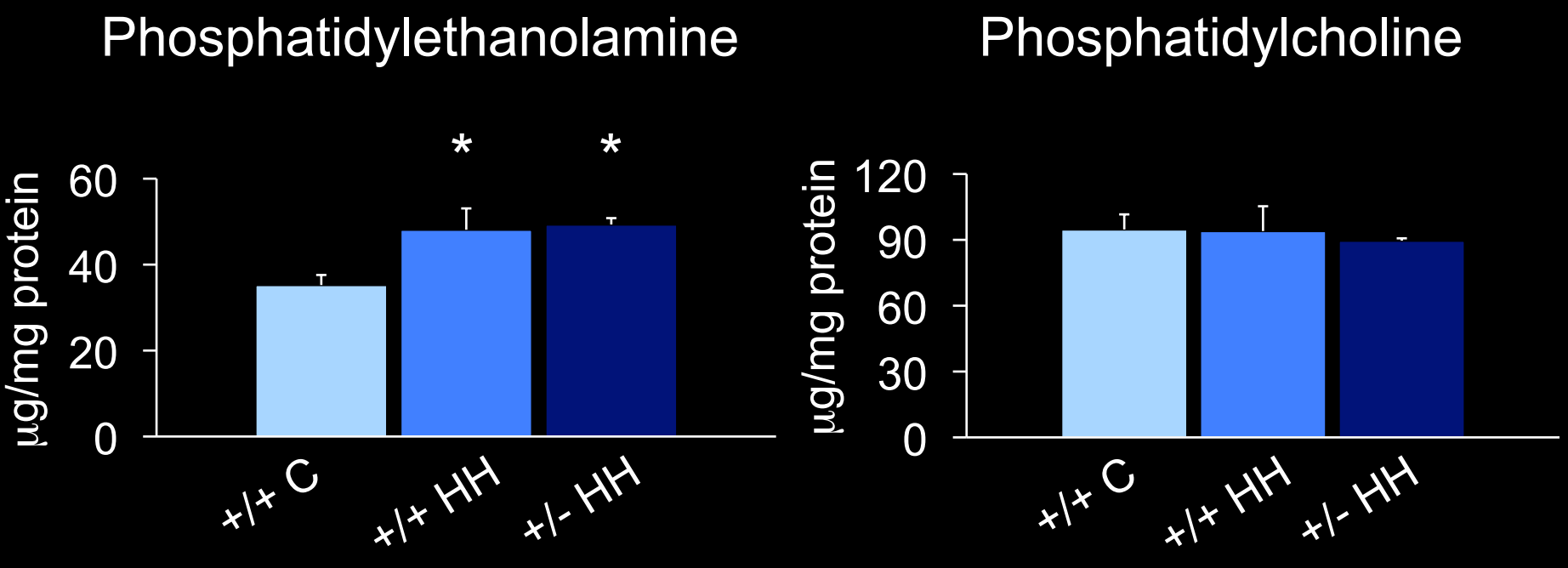


Reduced liver methylation capacity with HHcy.

Intersection of Lipids & Methyl Metabolism



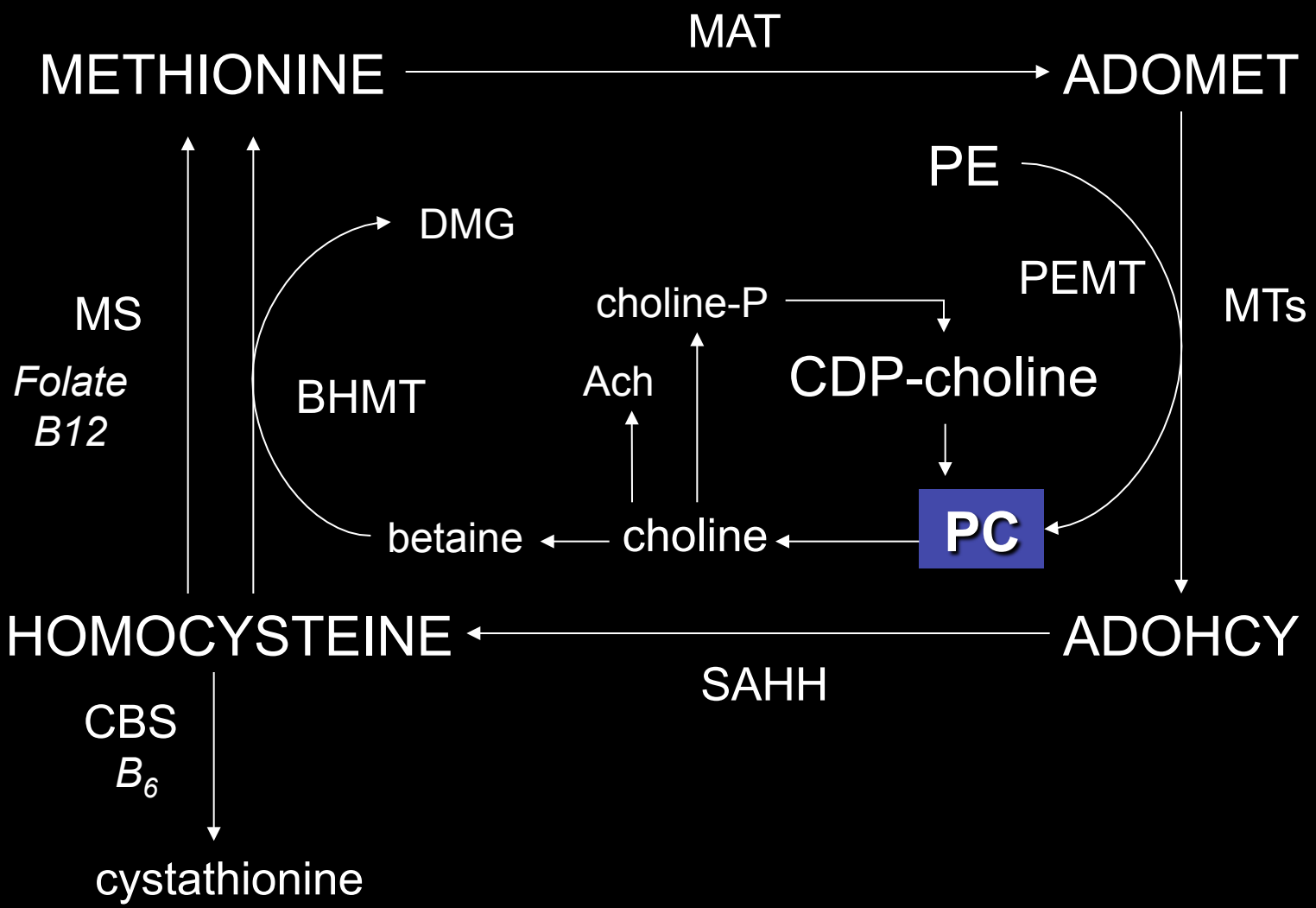
liver



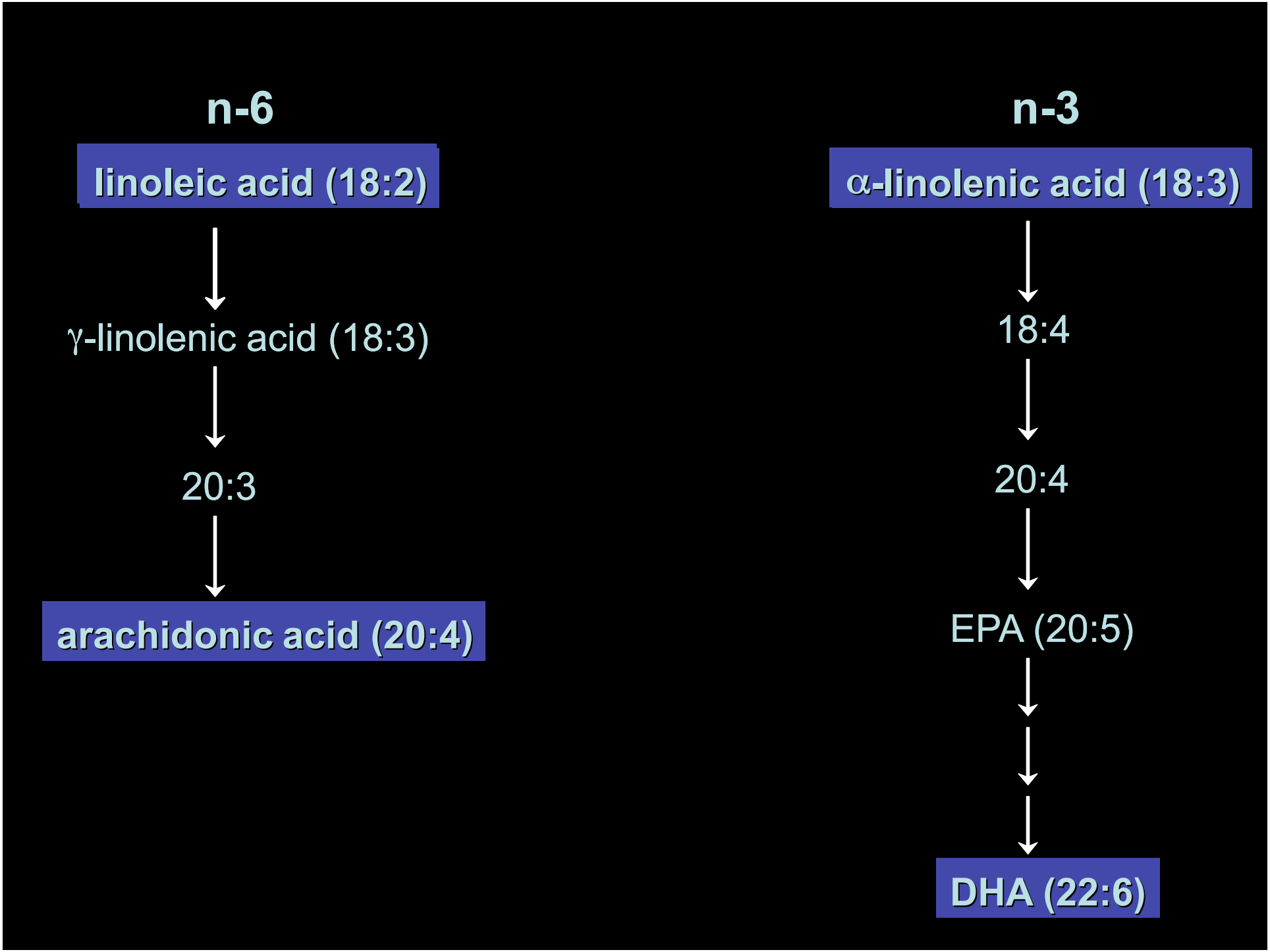
* $P < 0.01$

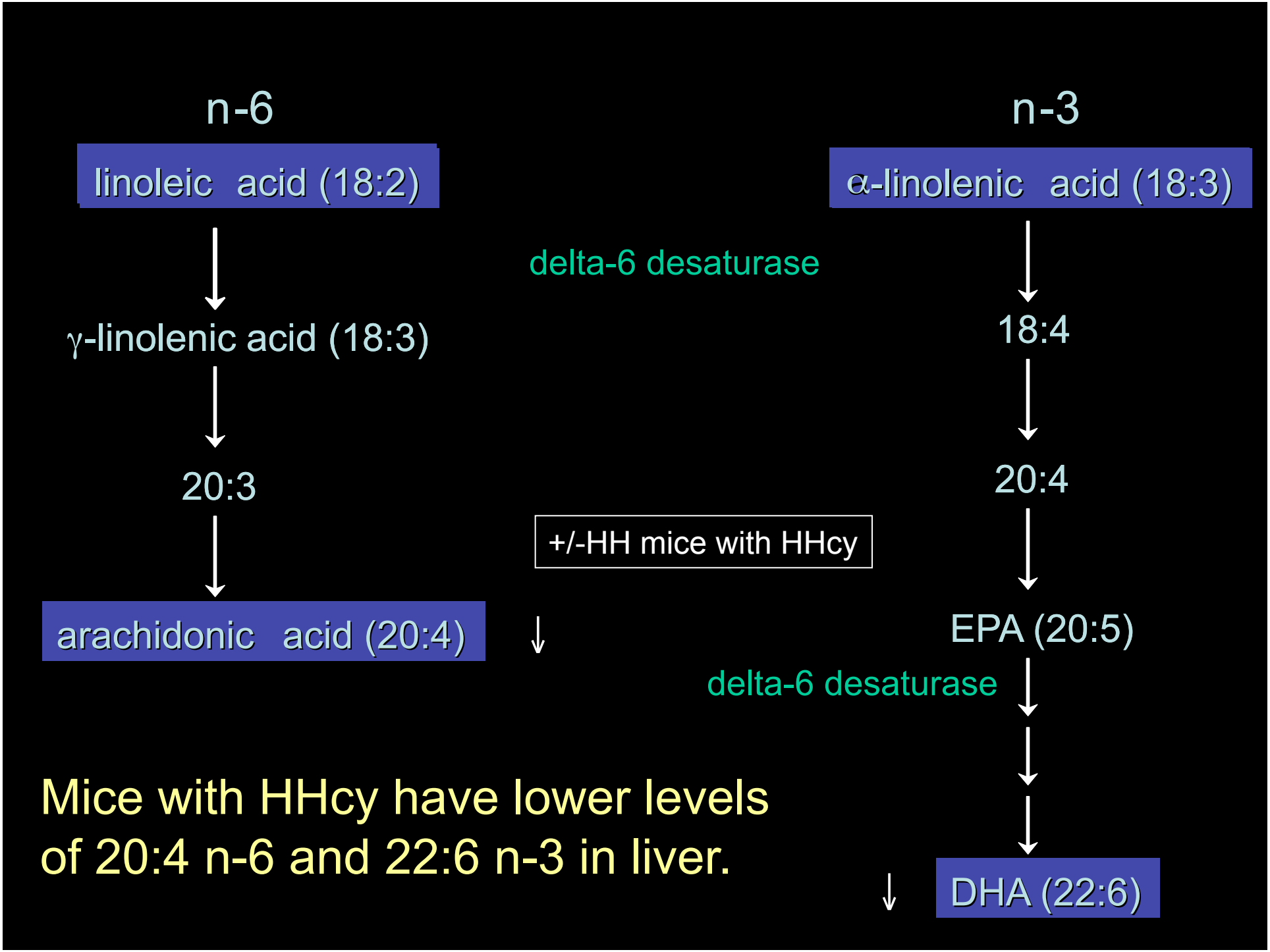
Increased liver phosphatidylethanolamine in HHcy.

Intersection of Lipids & Methyl Metabolism

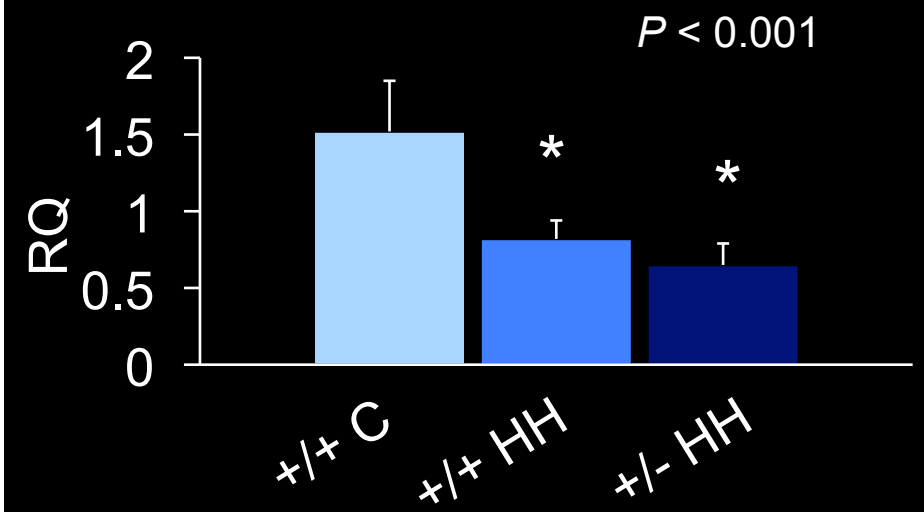


liver

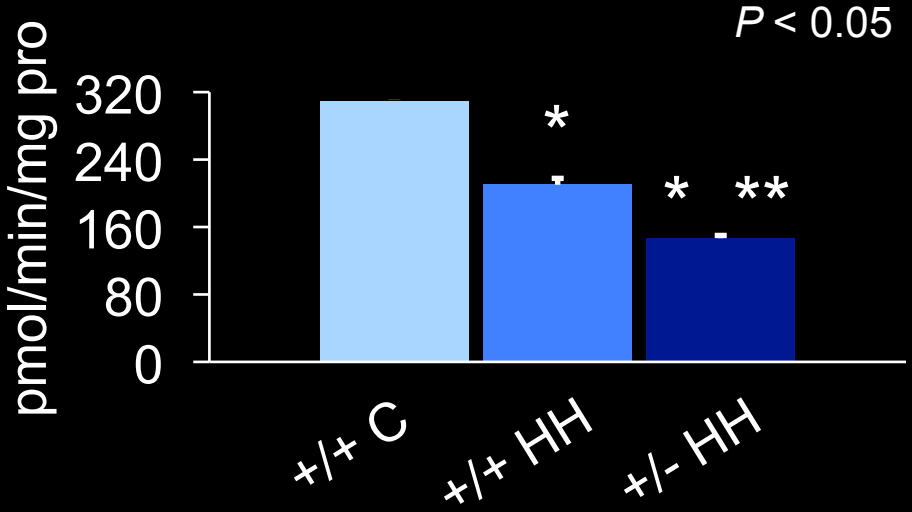

n-6
linoleic acid (18:2)
γ-linolenic acid (18:3)
20:3
arachidonic acid (20:4)
n-3
α-linolenic acid (18:3)
18:4
20:4
EPA (20:5)
DHA (22:6)


n-6
linoleic acid (18:2)
γ-linolenic acid (18:3)
20:3
arachidonic acid (20:4)
delta-6 desaturase
+/-HH mice with HHcy
n-3
α-linolenic acid (18:3)
18:4
20:4
EPA (20:5)
delta-6 desaturase
DHA (22:6)
Mice with HHcy have lower levels of 20:4 n-6 and 22:6 n-3 in liver.

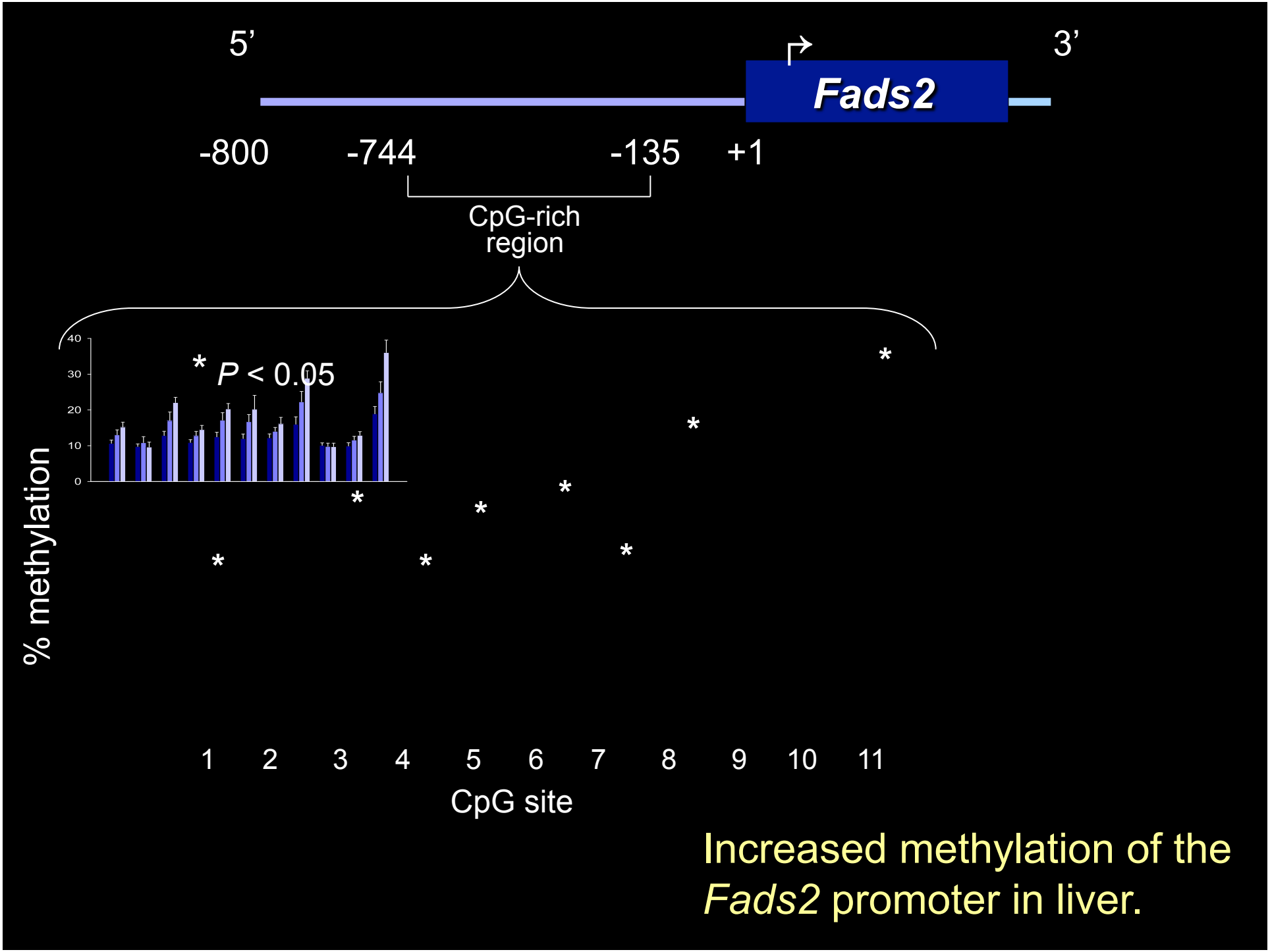
Fads2 mRNA



Delta-6 Desaturase Activity

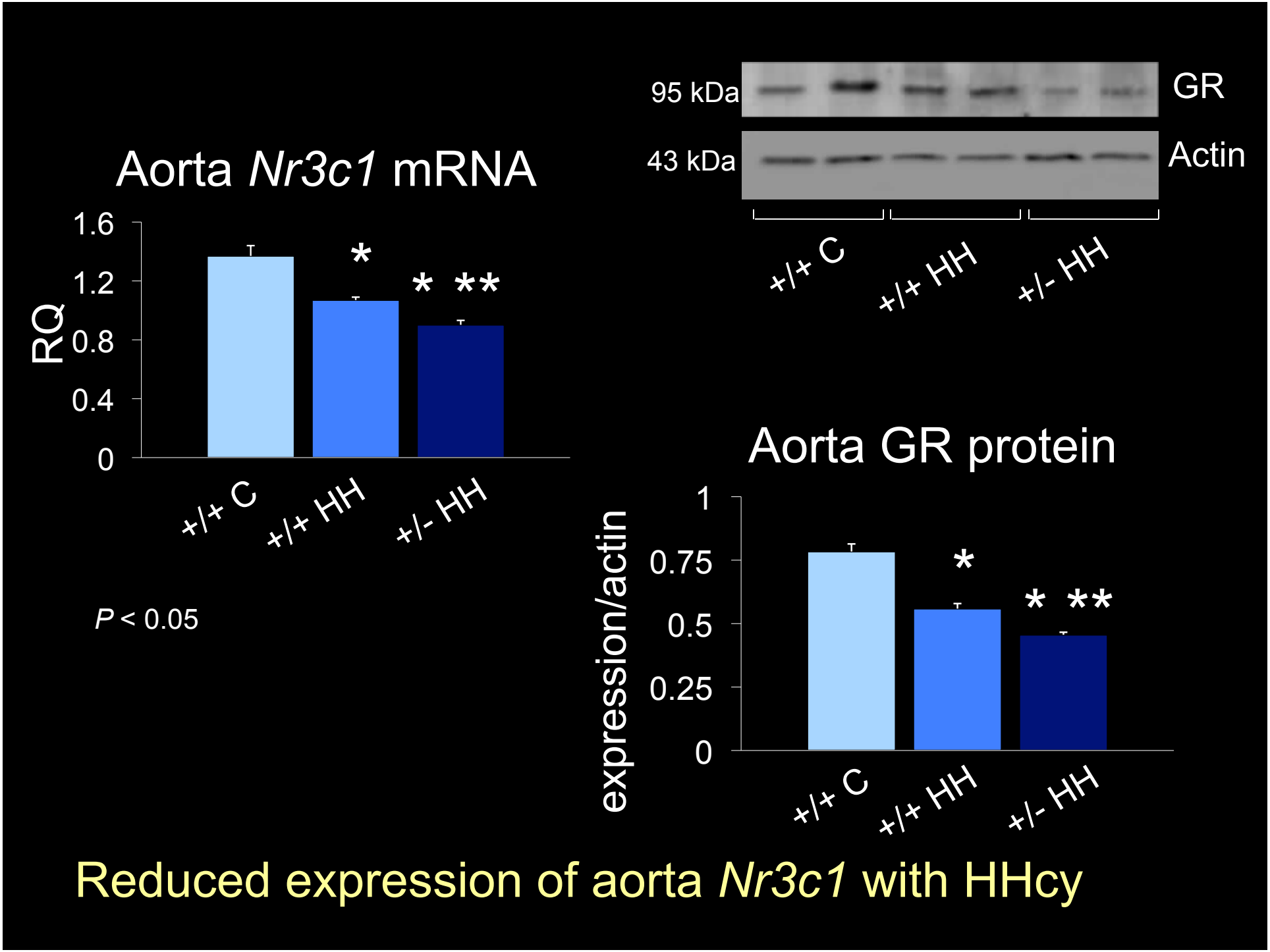


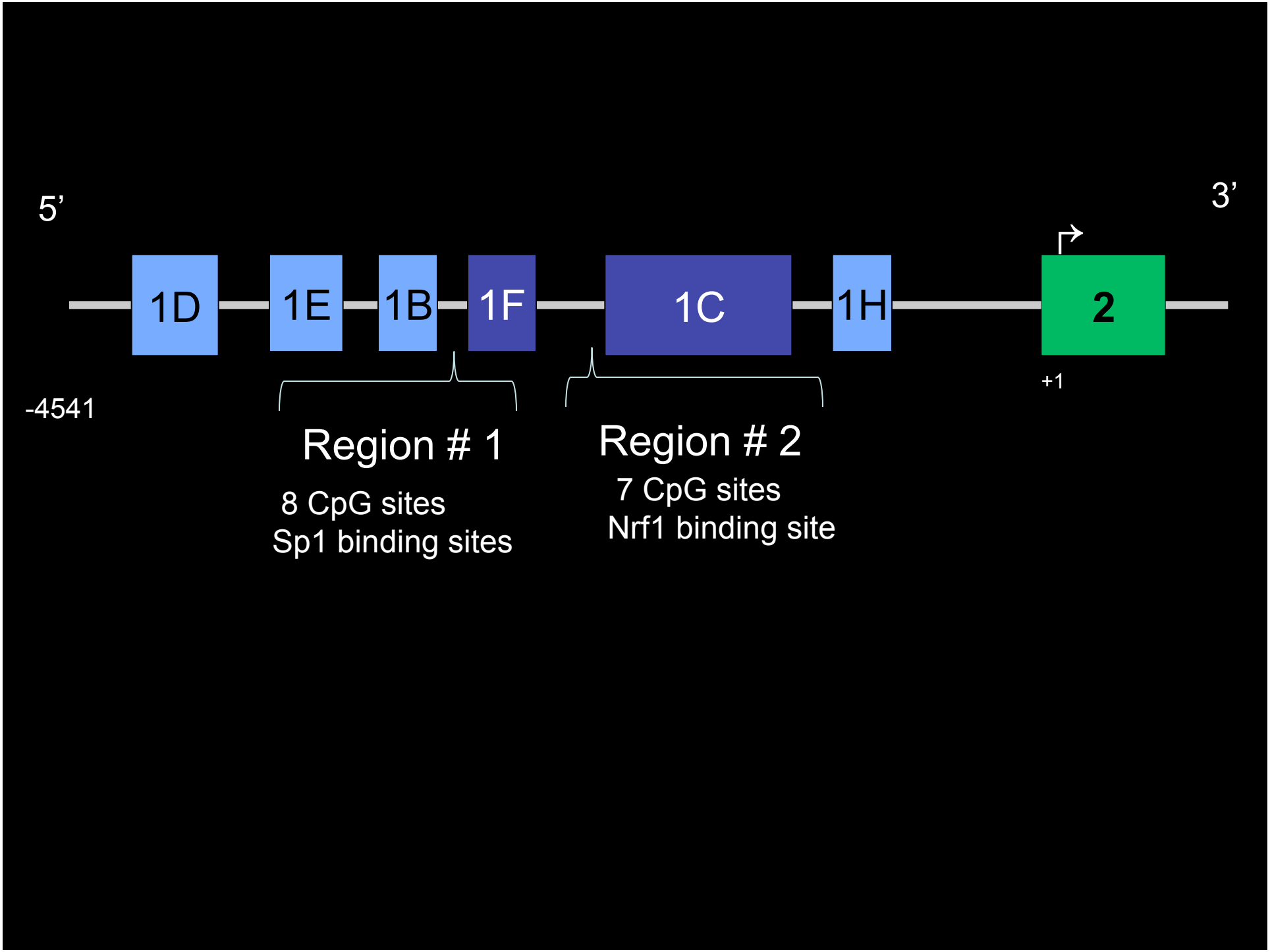
Reduced expression of liver *Fads2* with HHcy.

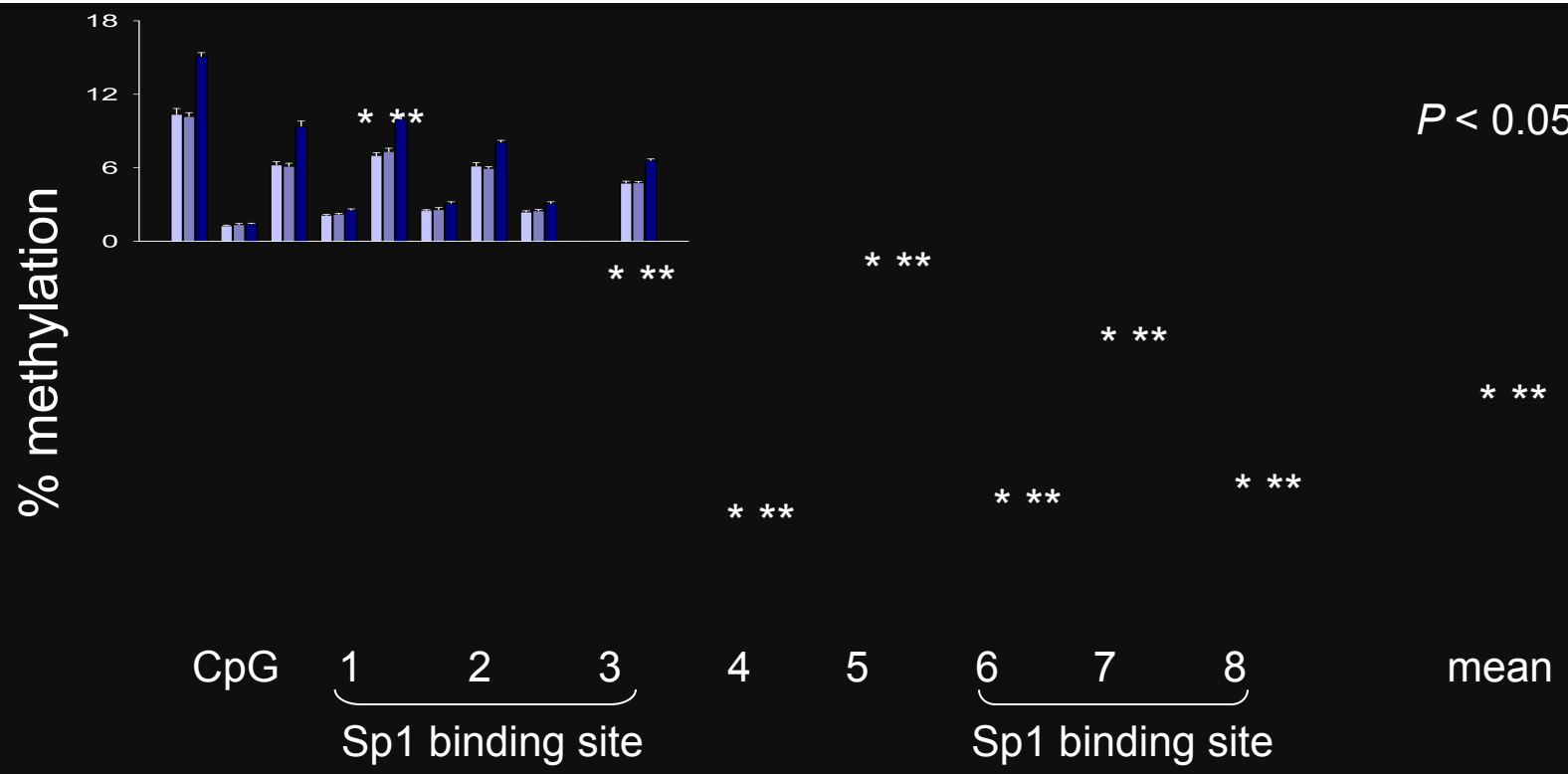

5’
3’
Fads2
-800
-744
-135
+1
CpG-rich region
40
30
20
10
0
* P < 0.05
% methylation
1
2
3
4
5
6
7
8
9
10
11
CpG site
Increased methylation of the *Fads2* promoter in liver.

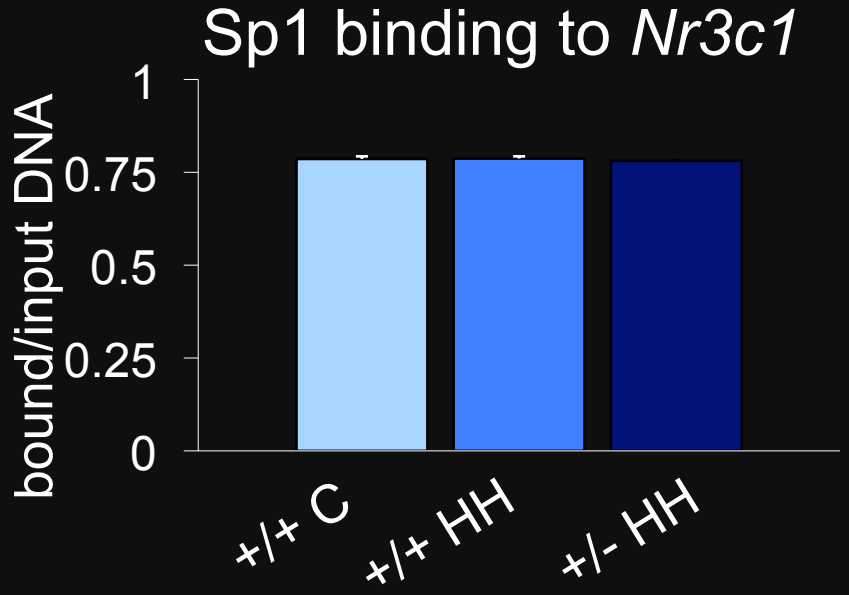
HHcy & DNA Methylation

- Mice with HHcy have methylation silencing of *Fads2* expression and changes in long chain fatty acid composition in liver
- Vascular tissue?
- Targeted *Nr3c1* (encodes GR)
- Expression regulated by methylation (hippocampus)
- Glucocorticoids may have cardioprotective effects

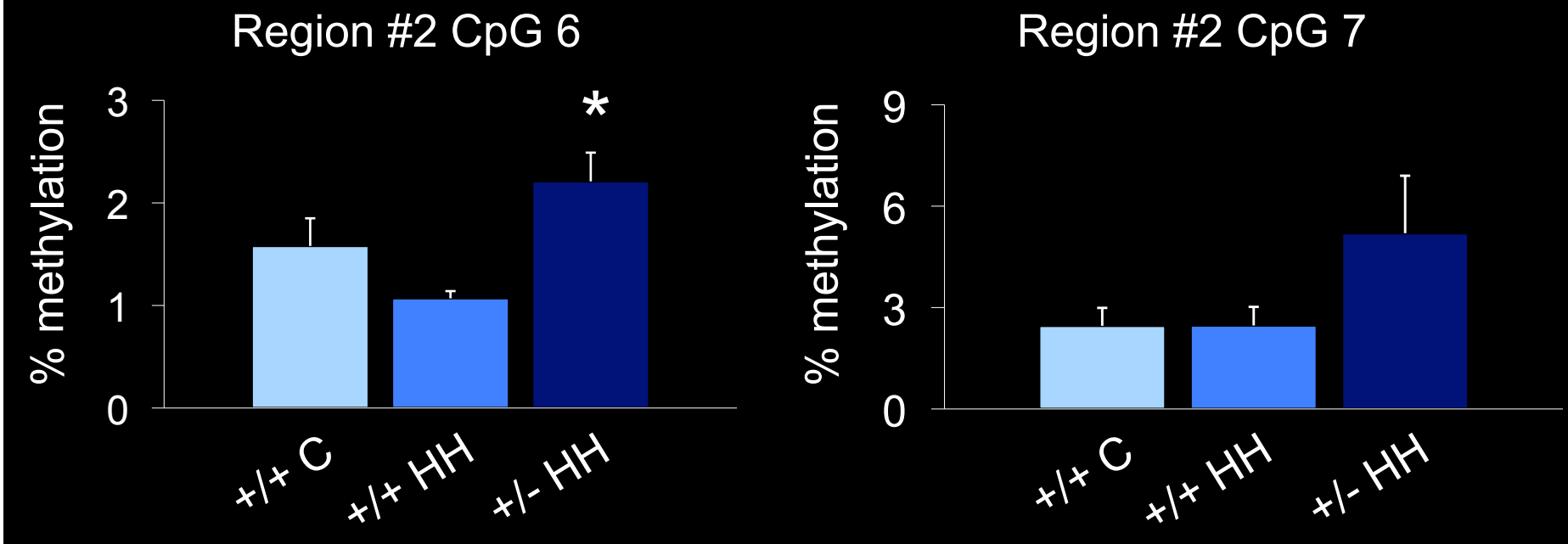

Aorta *Nr3c1* mRNA
RQ
1.6
1.2
0.8
0.4
0
*
* **
+/+ C
+/+ HH
+/- HH
P < 0.05
95 kDa
GR
43 kDa
Actin
+/+ C
+/+ HH
+/- HH
Aorta GR protein
expression/actin
1
0.75
0.5
0.25
0
*
* **
+/+ C
+/+ HH
+/- HH
Reduced expression of aorta *Nr3c1* with HHcy


5’
3’
1D
1E
1B
1F
1C
1H
2
+1
-4541
Region # 1
8 CpG sites
Sp1 binding sites
Region # 2
7 CpG sites
Nrf1 binding site



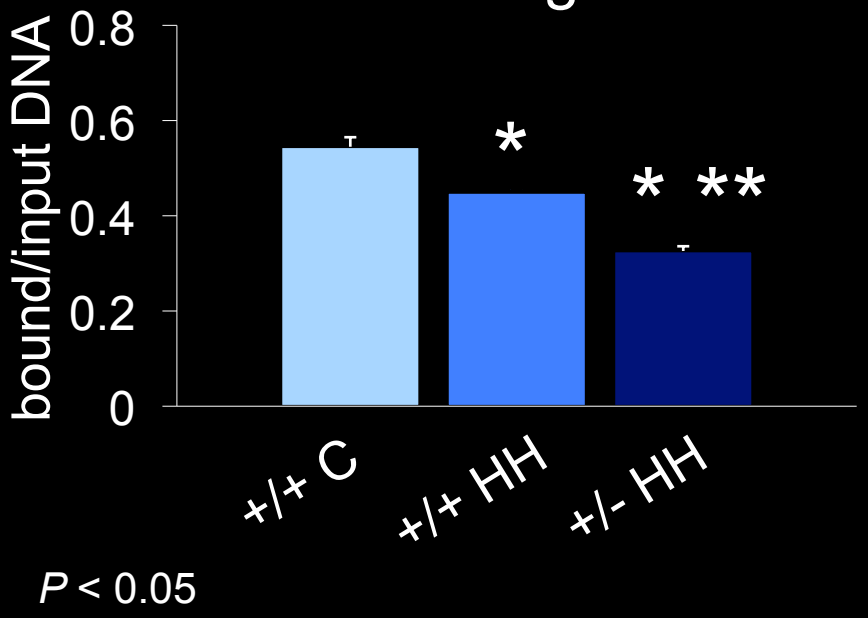


Increased methylation of Sp1 binding sites but no change in Sp1 binding to *Nr3c1* in aorta.

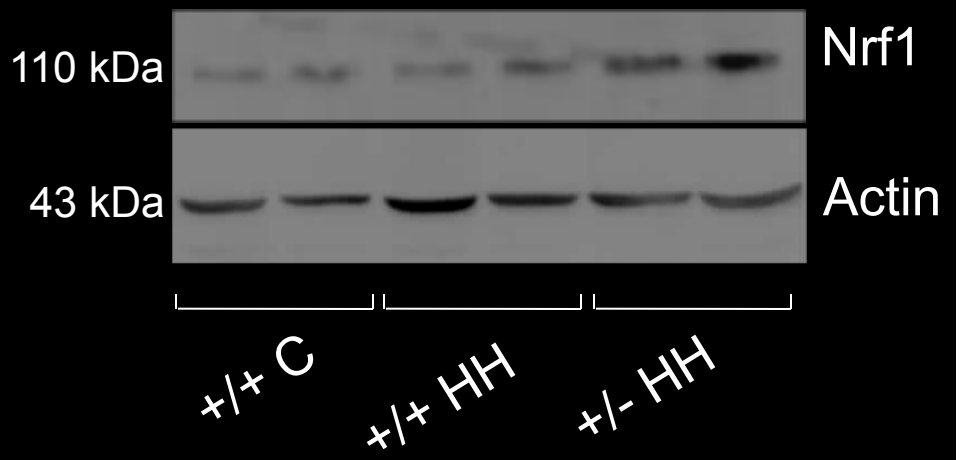


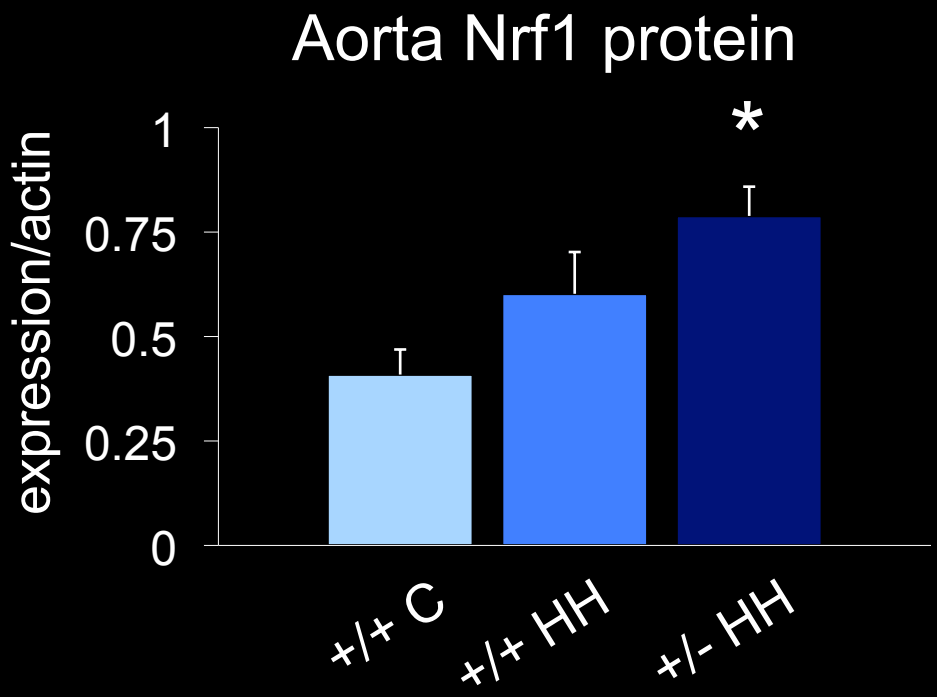
$P < 0.05$

Increased methylation of the Nrf1 binding site in *Nr3c1* in aorta.



$P < 0.05$





Increased methylation of the Nrf1 binding site associated with decreased binding of Nrf1 to *Nr3c1* in aorta.

Overall Summary & Conclusions

- Regulation of gene expression by DNA methylation may play a role in the pathology of HHcy
- Effect of HHcy on DNA methylation is tissue-specific and gene-specific
- AdoMet/AdoHcy is not always a good biomarker of changes in DNA methylation
- Alterations in liver fatty acid metabolism observed in mice with HHcy may be involved in the pathology of HHcy

Acknowledgements

Devlin Lab

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