Red Blood Cell Metabolism in Down Syndrome:

hints on metabolic derangements in aging, inflammation and cognitive impairment

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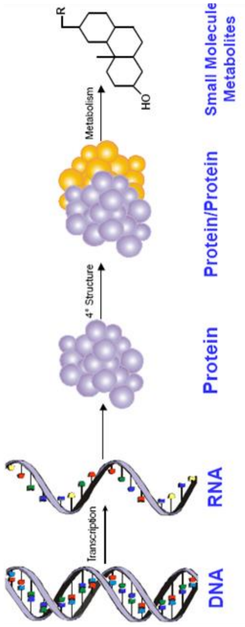
METABOLOME: complex and close to the phenotype

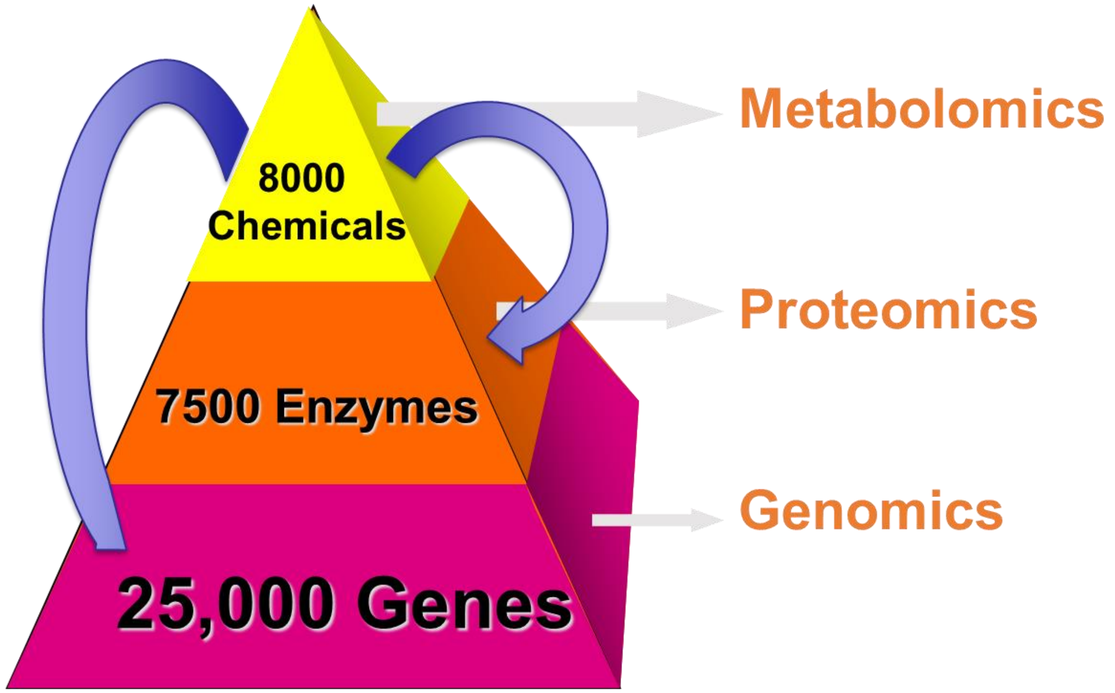
Metabolome = total metabolite pool

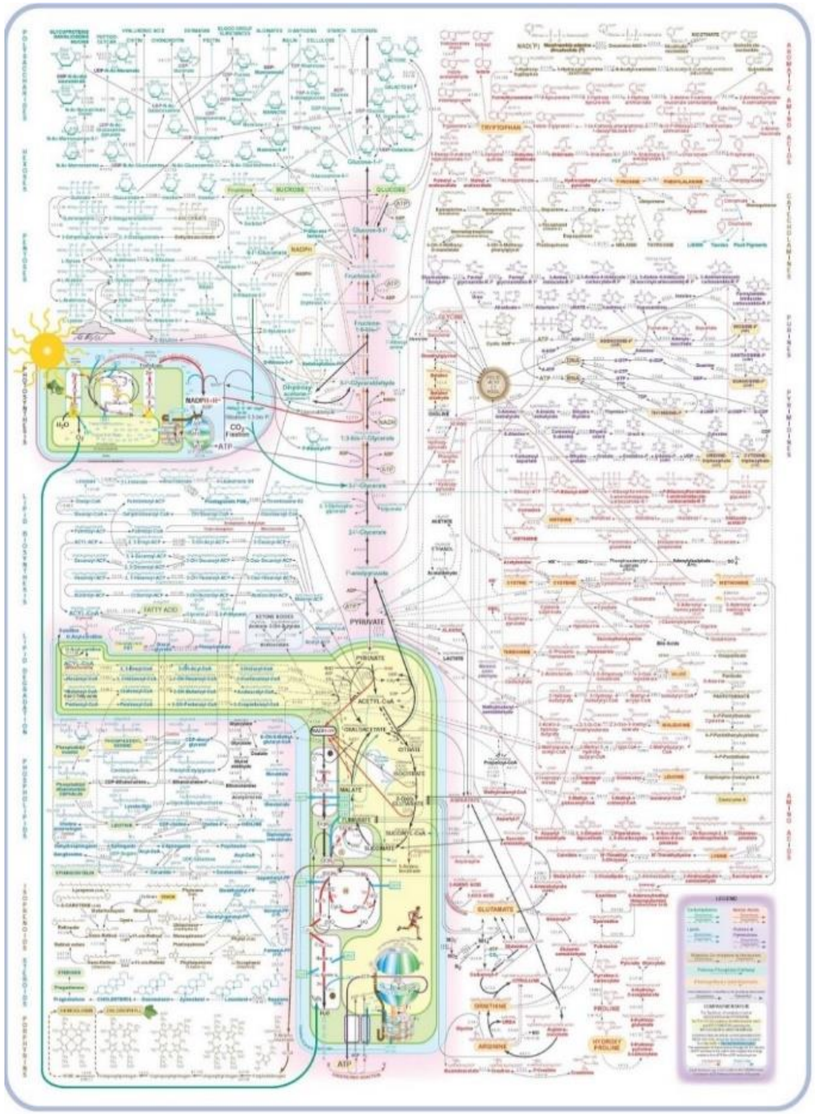
All low molecular weight (< 1500 Da)

Sugars, Nucleosides, Organic acids, Ketones, Aldehydes, Amines, Amino acids, Small peptides, Lipids, Steroids, Terpenes, Alkaloids
Drugs (xenobiotics)

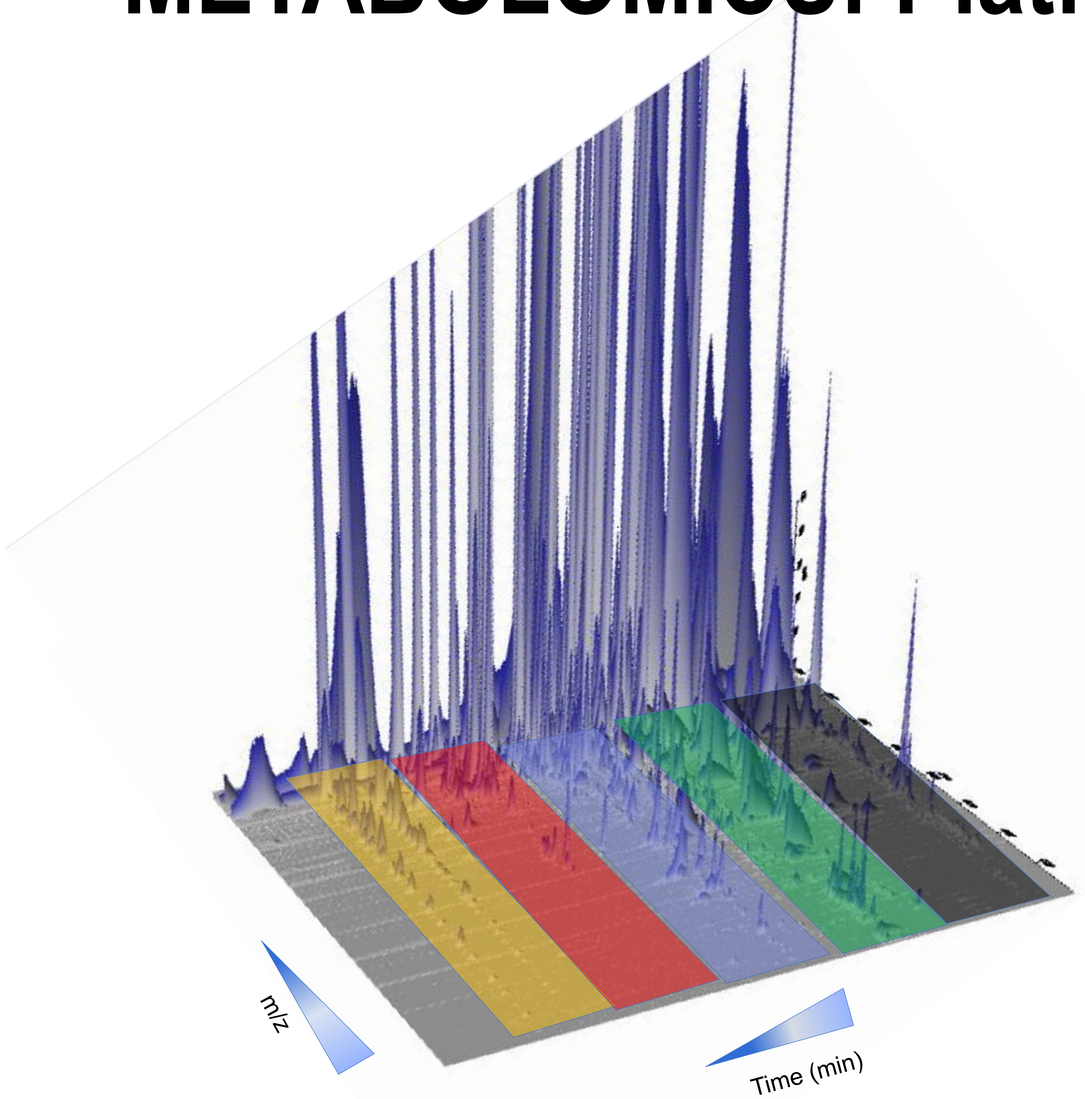
- >95% of all diagnostic clinical assays test for small molecules
- 89% of all known drugs are small molecules
- 50% of all drugs are derived from pre-existing metabolites
- 30% of identified genetic disorders involve metabolic disease
- Small molecules are cofactors and signaling molecules

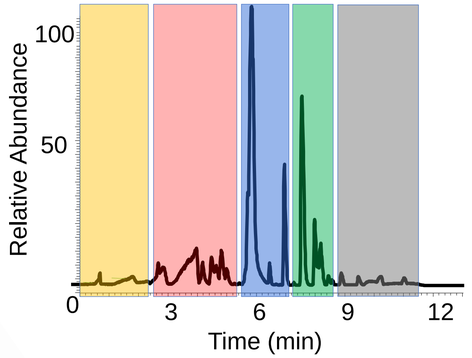






METABOLOMICS: Platform at UC Denver



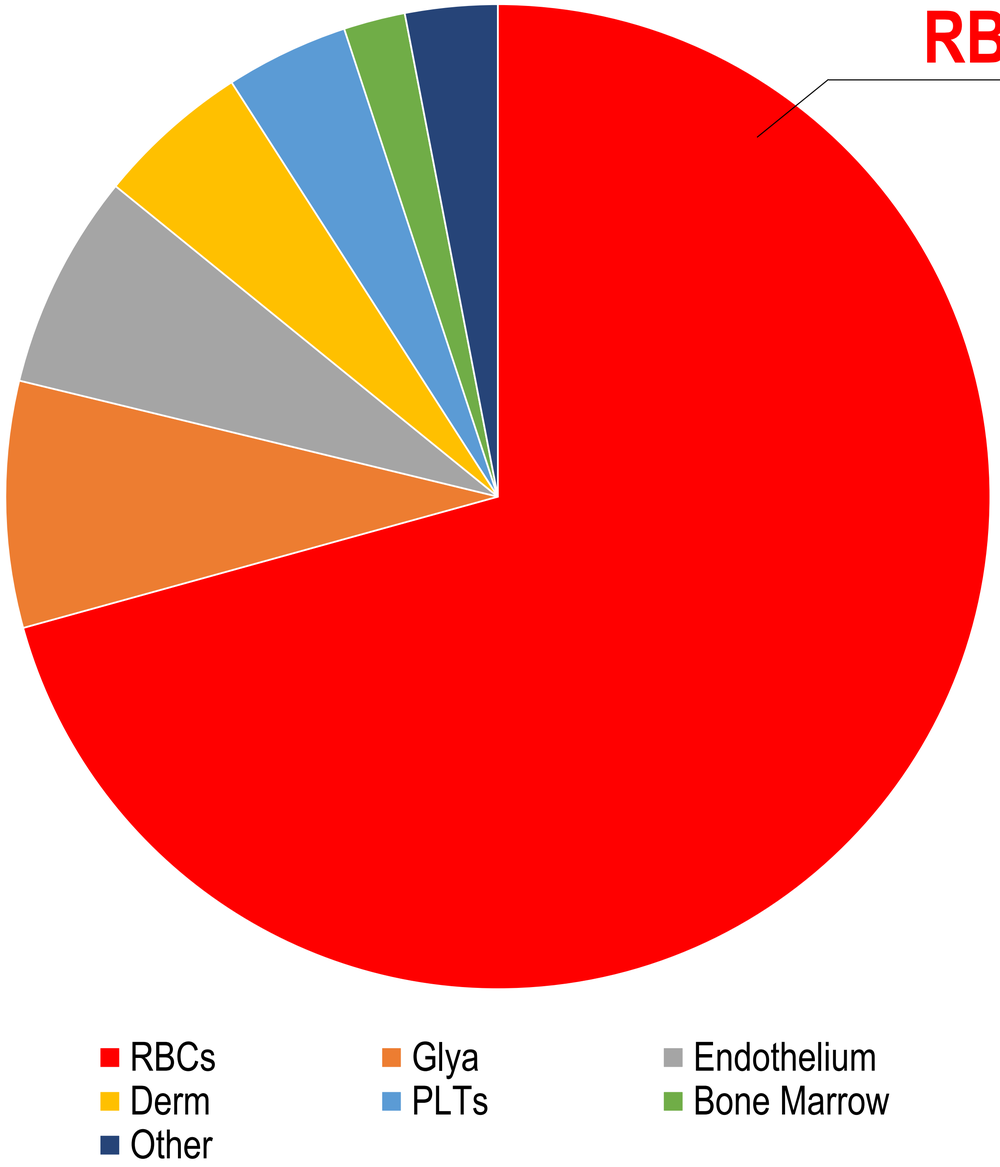


- Polyamines and basic AAs
- Sugars and Carboxylic acids
- Aromatic AAs and Small Peptides
- Phosphate and Nucleosides
- Fatty acids

>17,000 features
~800 in house standard library
~100 in house heavy internal std.

Nemkov et al. Rapid Comm Mass Spec 2017; D'Alessandro et al. Transfusion 2016; Technology Networks - 2017

Why study RBCs? Most abundant host cell in human body



- **Lifespan of ~120 days**
- **~0.2 Trillion RBCs made everyday**
- **Significant daily energy expenditure in embryonic and adult life**

SIMPLE

- Lose nuclei and organelles during maturation;
- No ribosomes: devoid of *de novo* protein synthesis capacity;
- 98% of cytosol is hemoglobin!

YET COMPLEX

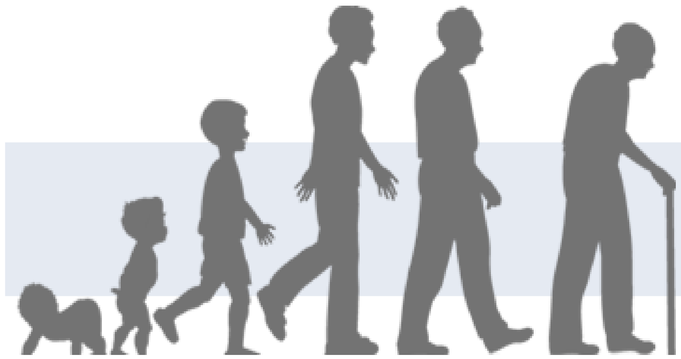
- They still preserve the proteasome;
- The 2% of the proteome that is not Hb includes more than 2900 proteins (as of now) – including **hundreds of transporters**

D'Alessandro and Zolla. Exp Rev Prot 2017

Metabolic Derangements of Down Syndrome

- Trisomy 21 (T21) = etiological factor of Down Syndrome
- T21 is a model of accelerated aging
  - Premature skin wrinkling
  - Hypothyroidism
  - Declining immune function
  - Alzheimer’s disease/cognitive impairment
- T21 is a model of chronic inflammation
  - Autoimmune disorders
  - Leukemias
  - Celiac disease
  - Autoimmune chronic hepatitis

F, D21	M, D21	F, T21	M, T21
n = 44	n = 23	n = 17	n = 13



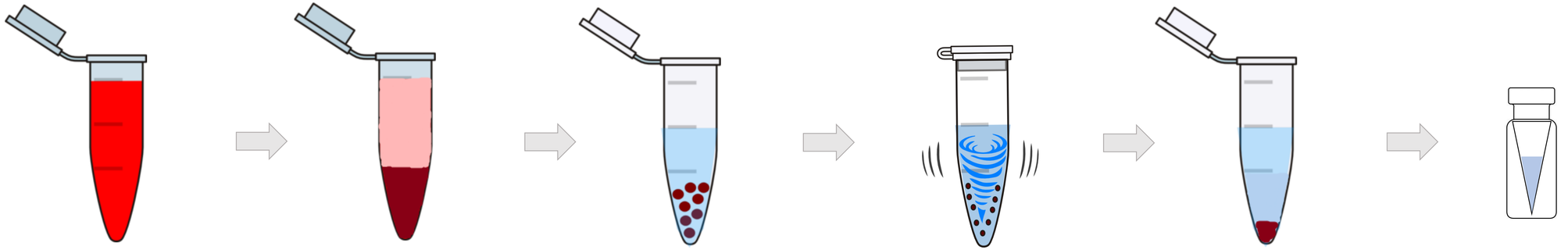
Age

Gender

Trisomy 21

RBCs

Metabolomics



Whole blood collected from donors

RBCs sorted through centrifugation

Extracted 1:10 in lysis buffer w/ heavy stds

Vortexed for 30 min @ 4°C

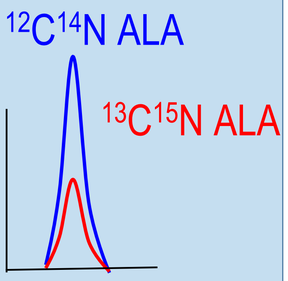
RBCs separated by centrifugation

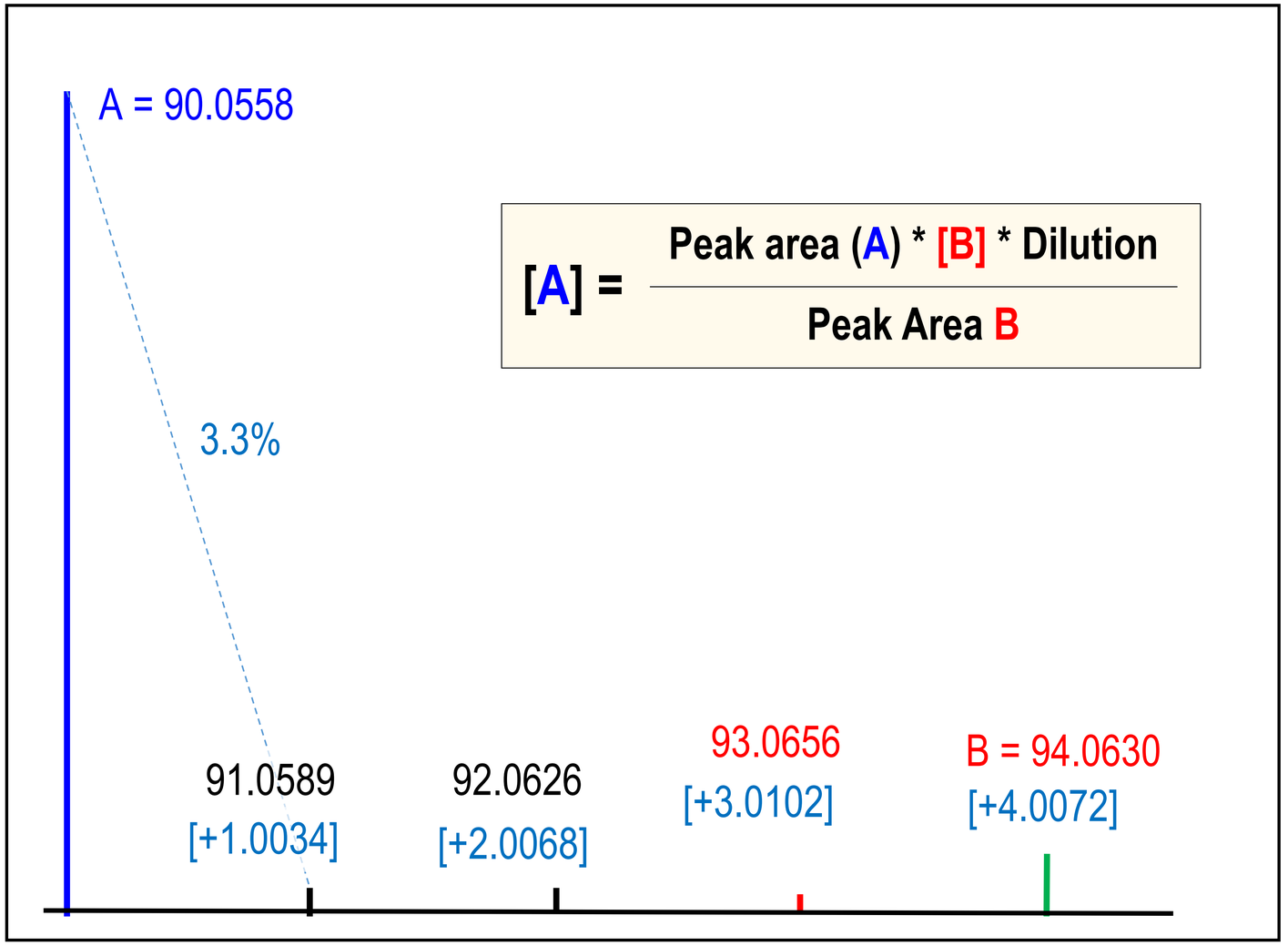
Extraction sol'n analyzed via UHPLC-MS

Culp-Hill et al. *Under review*

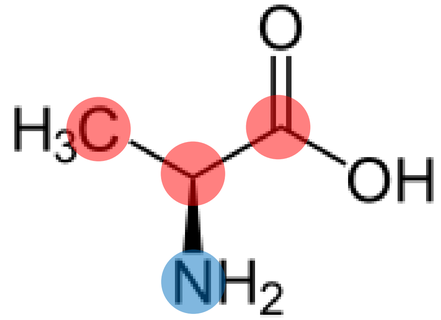
Scheme for Absolute Quant

Heavy
ALA standard





$$[A] = \frac{\text{Peak area (A)} * [B] * \text{Dilution}}{\text{Peak Area B}}$$



Mass of ^{13}C = 1.0034 m/z

Mass of ^{15}N = 0.997 m/z

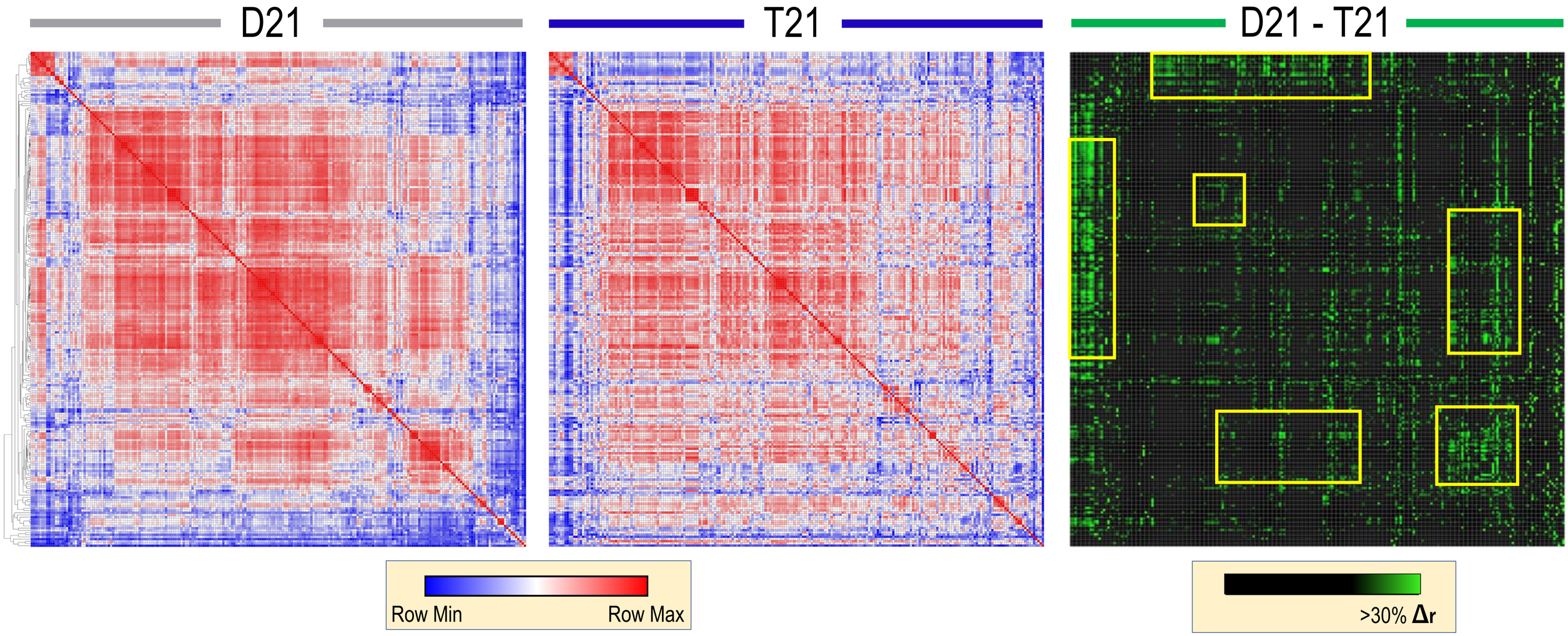
Isotopic standards used

Amino Acids	2.5	uM	Alanine
	2.5	uM	Arginine
	2.5	uM	Aspartate
	1.25	uM	Cystine
	2.5	uM	Glutamate
	2.5	uM	Histidine
	2.5	uM	Isoleucine
	2.5	uM	Leucine
	2.5	uM	Lysine
	2.5	uM	Methionine
	2.5	uM	Phenylalanine
	2.5	uM	Proline
	2.5	uM	Serine
	2.5	uM	Threonine
	2.5	uM	Tyrosine
	2.5	uM	Valine
	2.5	uM	Glycine

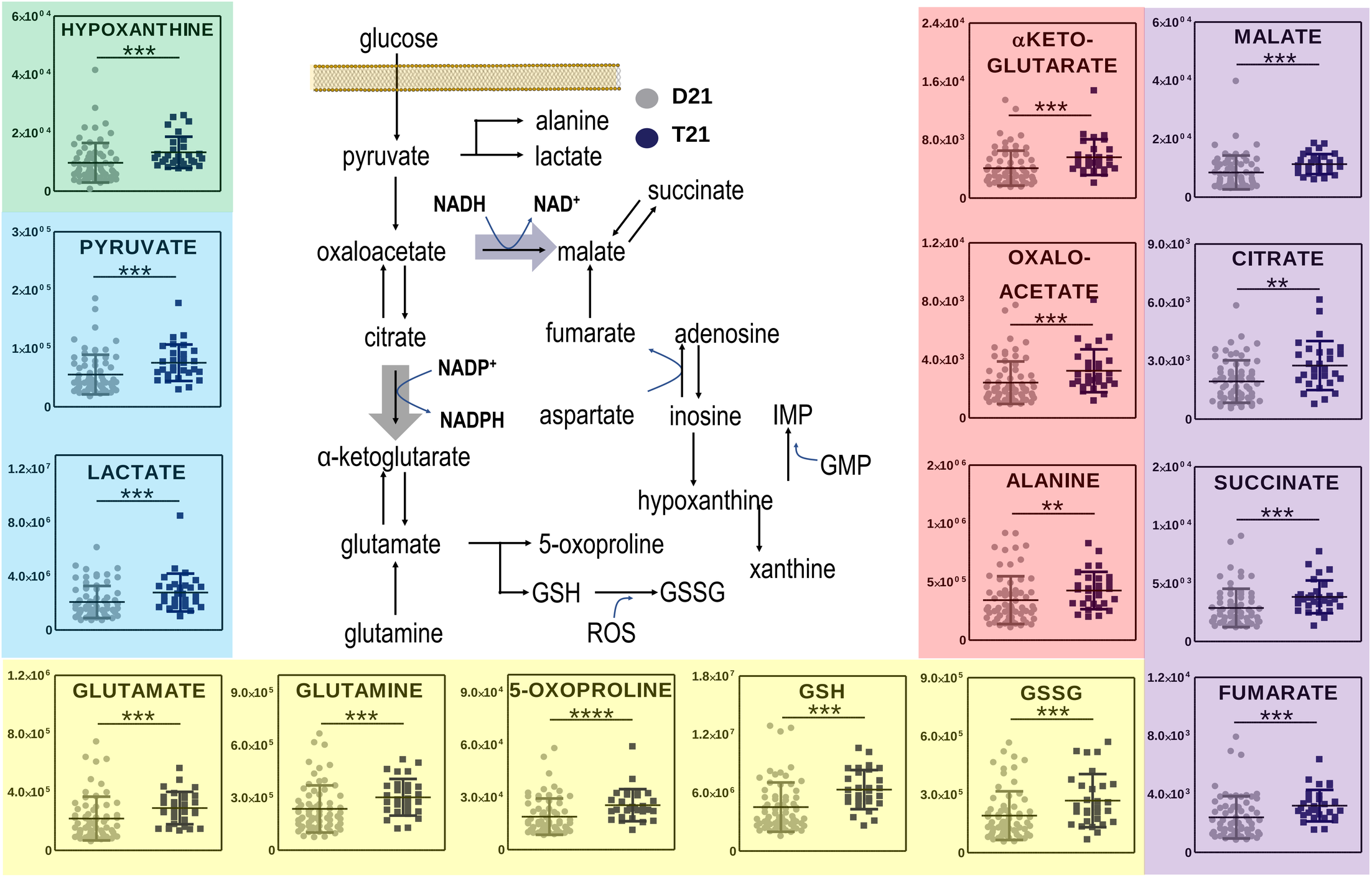
Acyl-Carnitines	729.6	nM	Carnitine
	182.4	nM	Acetyl-carnitine
	38.4	nM	Propionyl-carnitine
	38.4	nM	Butyryl-carnitine
	38.4	nM	Isovaleryl-carnitine
	38.4	nM	Octanoyl-carnitine
	38.4	nM	Myristoyl-carnitine
	76.8	nM	Palmitoyl-carnitine
Bile Acids	25	nM	Taurodeoxycholate /Taurochendeoxycholate
	25	nM	Tauroursodeoxycholate
	25	nM	Taurocholate
	25	nM	Taurolithocholate
	25	nM	Cholate
	25	nM	Deoxycholate
	25	nM	Glycocholate
	25	nM	Glycochenodeoxycholate

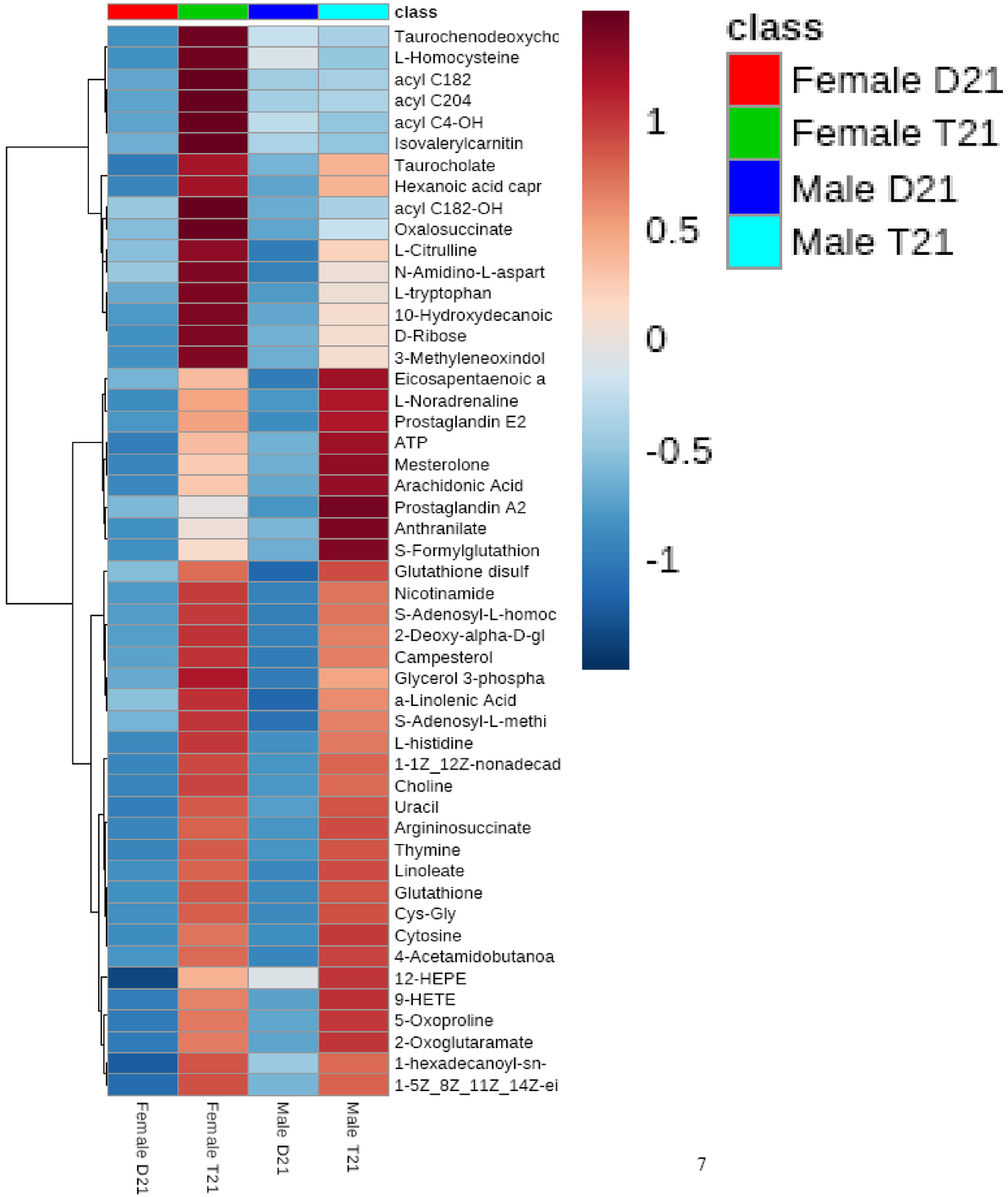
1	uM	Citrate
1	uM	Fumarate
1	uM	Succinate
1	uM	α-Ketoglutarate
10	uM	Glucose
1	uM	Pyruvate
40	uM	Lactate
0.5	uM	Glutathione
2.5	uM	Adenosine
1	uM	Urate
1	uM	Palmitate

Correlation ≠ causation, but reveals disruption



Culp-Hill et al. *Under review*

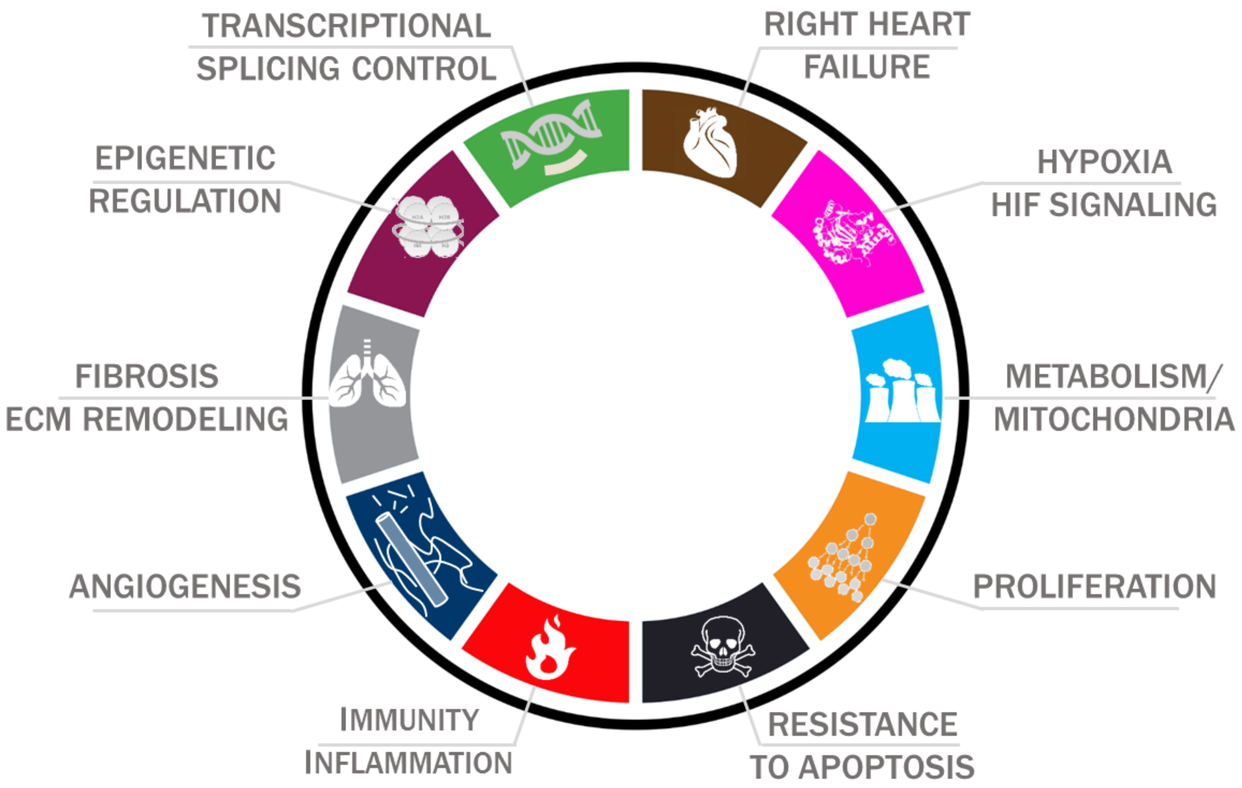

HYPOXANTHINE
***
PYRUVATE
***
LACTATE
***
glucose
D21
T21
alanine
lactate
pyruvate
NADH
NAD+
succinate
oxaloacetate
malate
citrate
fumarate
adenosine
NADP+
NADPH
aspartate
inosine
IMP
GMP
α-ketoglutarate
hypoxanthine
glutamate
5-oxoproline
xanthine
GSH
GSSG
glutamine
ROS
αKETO-GLUTARATE
***
MALATE
***
OXALO-ACETATE
***
CITRATE
**
ALANINE
**
SUCCINATE
***
GLUTAMATE
***
GLUTAMINE
***
5-OXOPROLINE
****
GSH
***
GSSG
***
FUMARATE
***
Purine catabolism
Glycolysis
Glutaminolysis, GSH homeostasis
Transamination products
TCA Cycle



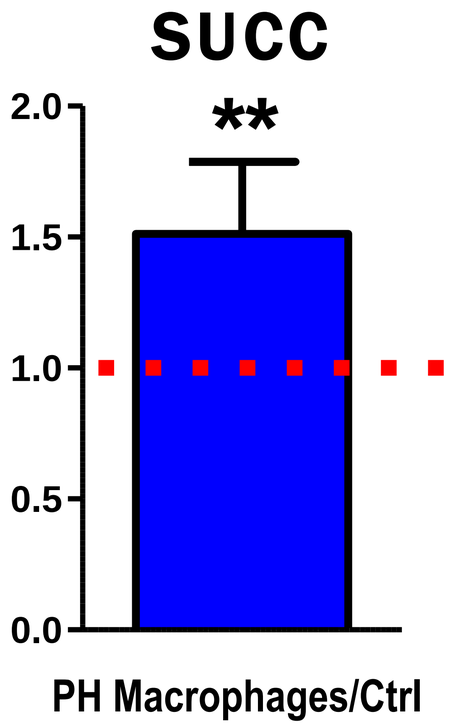
Heat map – top 50 significant metabolites by ANOVA in age-matched D21 v. T21 donors

Culp-Hill et al. *Under review*

Metabolic reprogramming (involving succinate) is a hallmark of Pulmonary Hypertension – comorbidity in T21

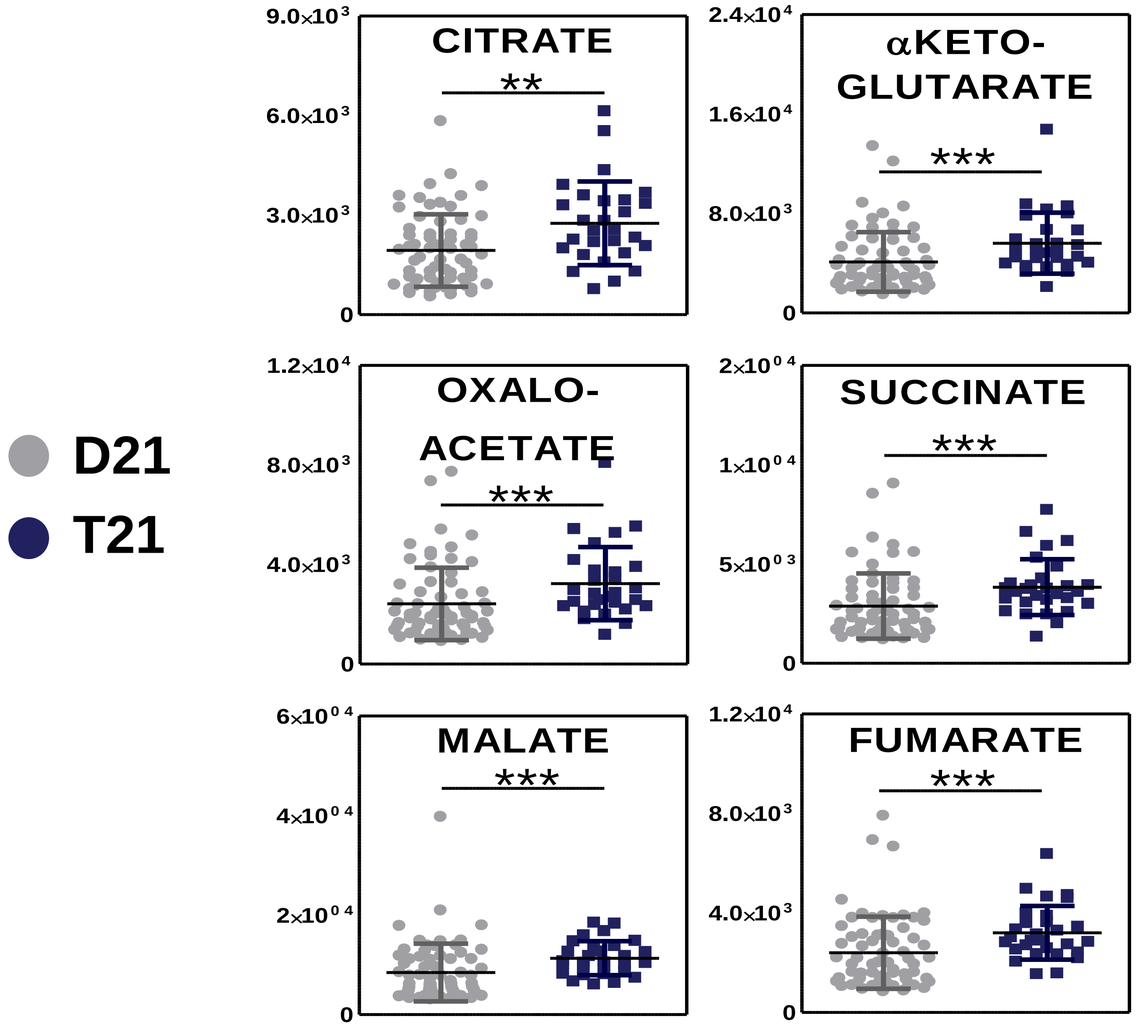


Hallmarks of Pulmonary Hypertension



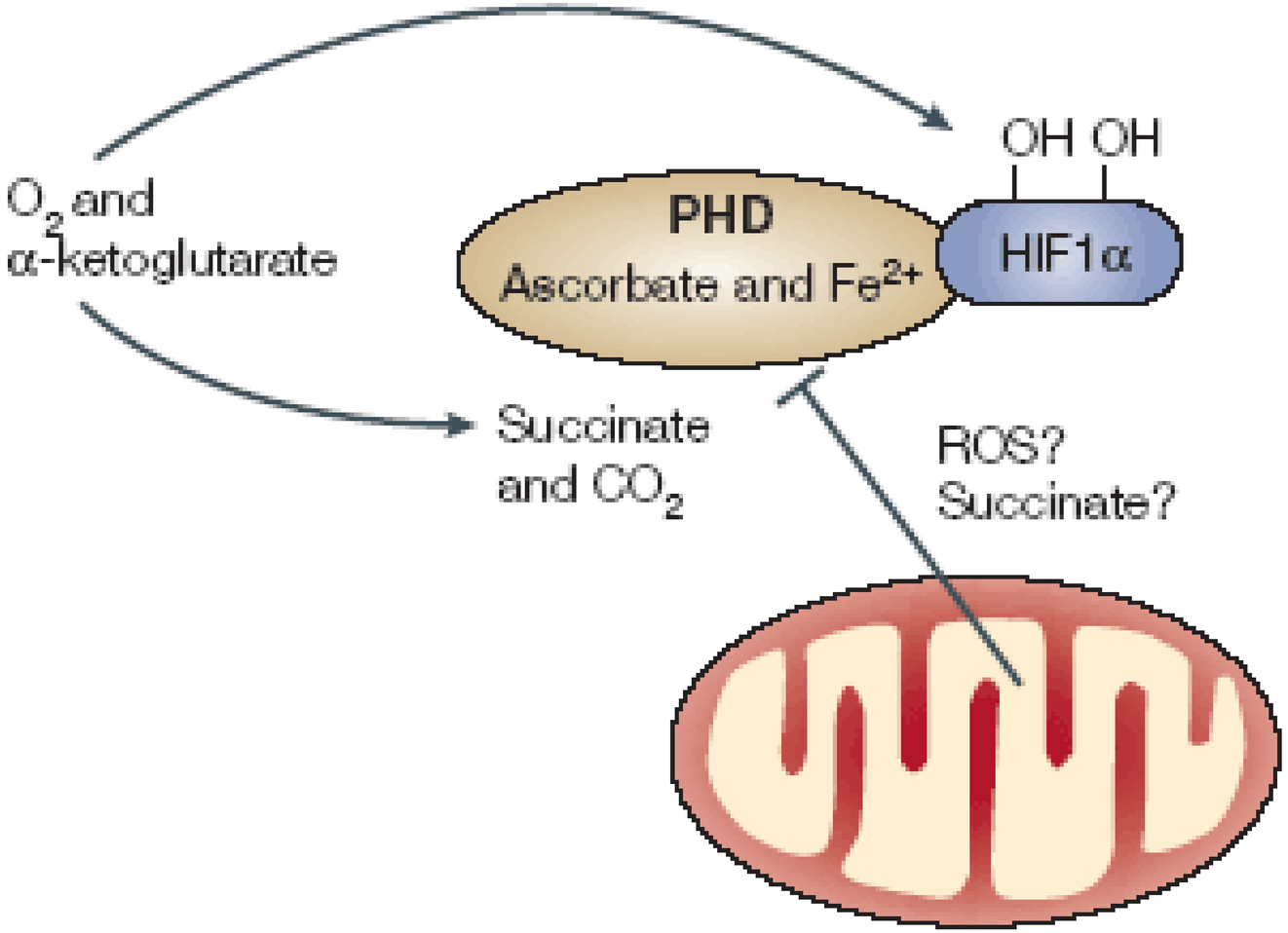
D'Alessandro et al. Antiox Redox Signal 2017; El Kasmi et al. in preparation

T21 is characterized by increased plasma and RBC carboxylic acids, especially succinate



Culp-Hill et al. *Under review*

Increased plasma and RBC succinate: a pro-inflammatory marker associated with hypoxia



Semenza et al.

Succinate: a marker of hypoxia and inflammation

Review

CellPress

Succinate: a metabolic signal in inflammation

Evanna Mills and Luke A.J. O'Neill

LETTER

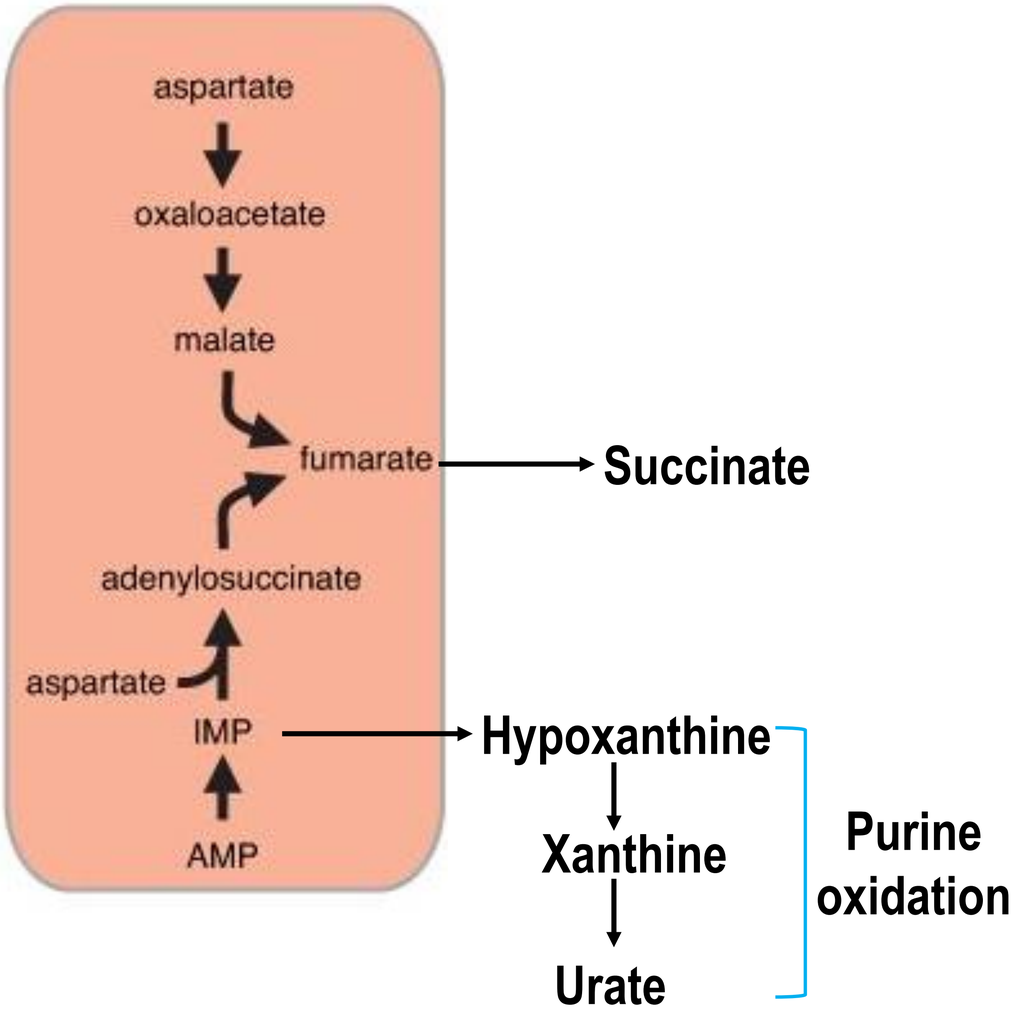
doi:10.1038/nature11986

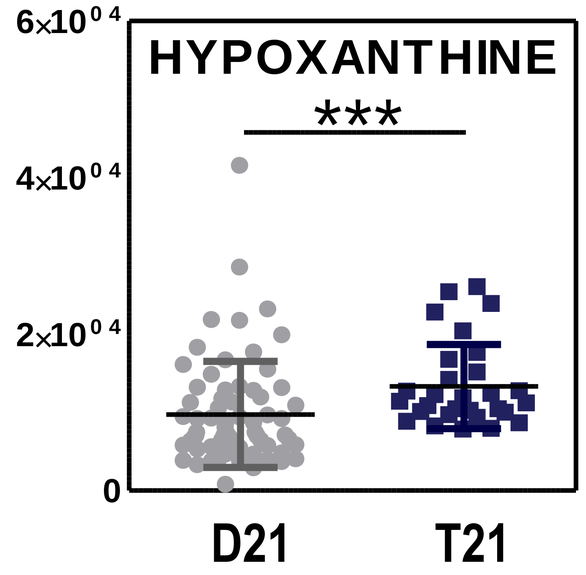
Succinate is an inflammatory signal that induces IL-1β through HIF-1α

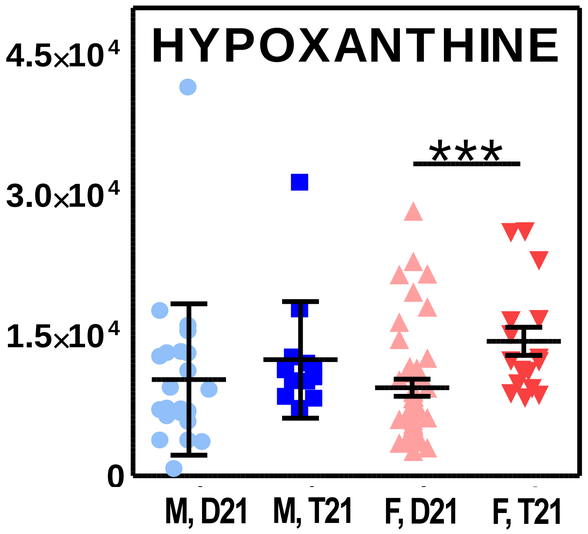
Succinate –[PHD –[HIF-1α → IL-1β → Inflammation

Tannahill et al. Nature 2014; Mill and O'Neill. Cell 2016

…and is coupled to purine oxidation



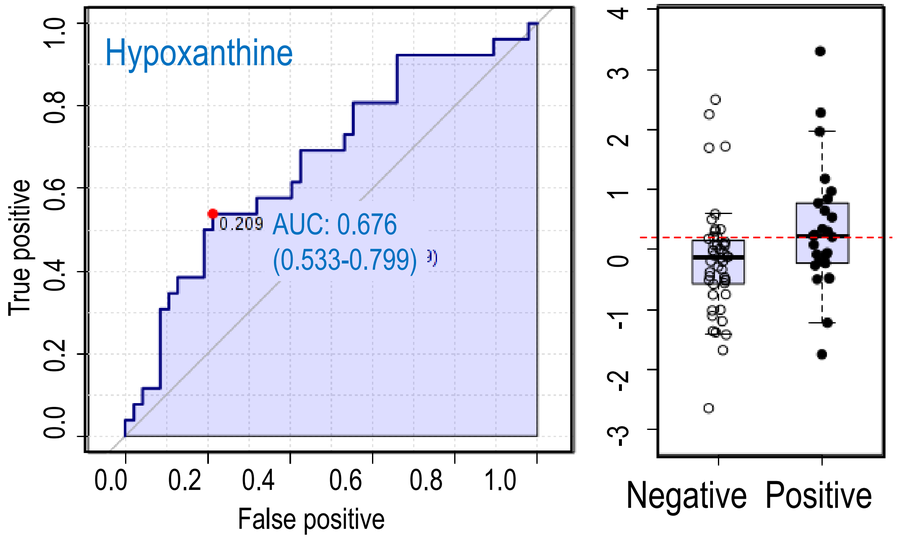




D21 Male
T21 Male
D21 Female
T21 Female

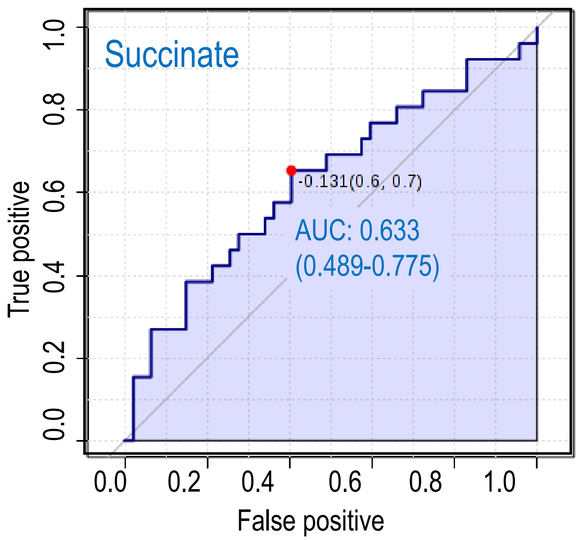
Culp-Hill et al. *Under review*

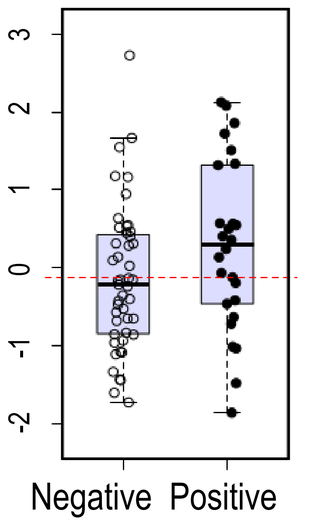
Succinate and purine oxidation are higher in adults and children with T21 and predict cognitive impairment in D21 and T21



TOTAL POPULATION (D21+T21)

(AD, ASD, Depression)



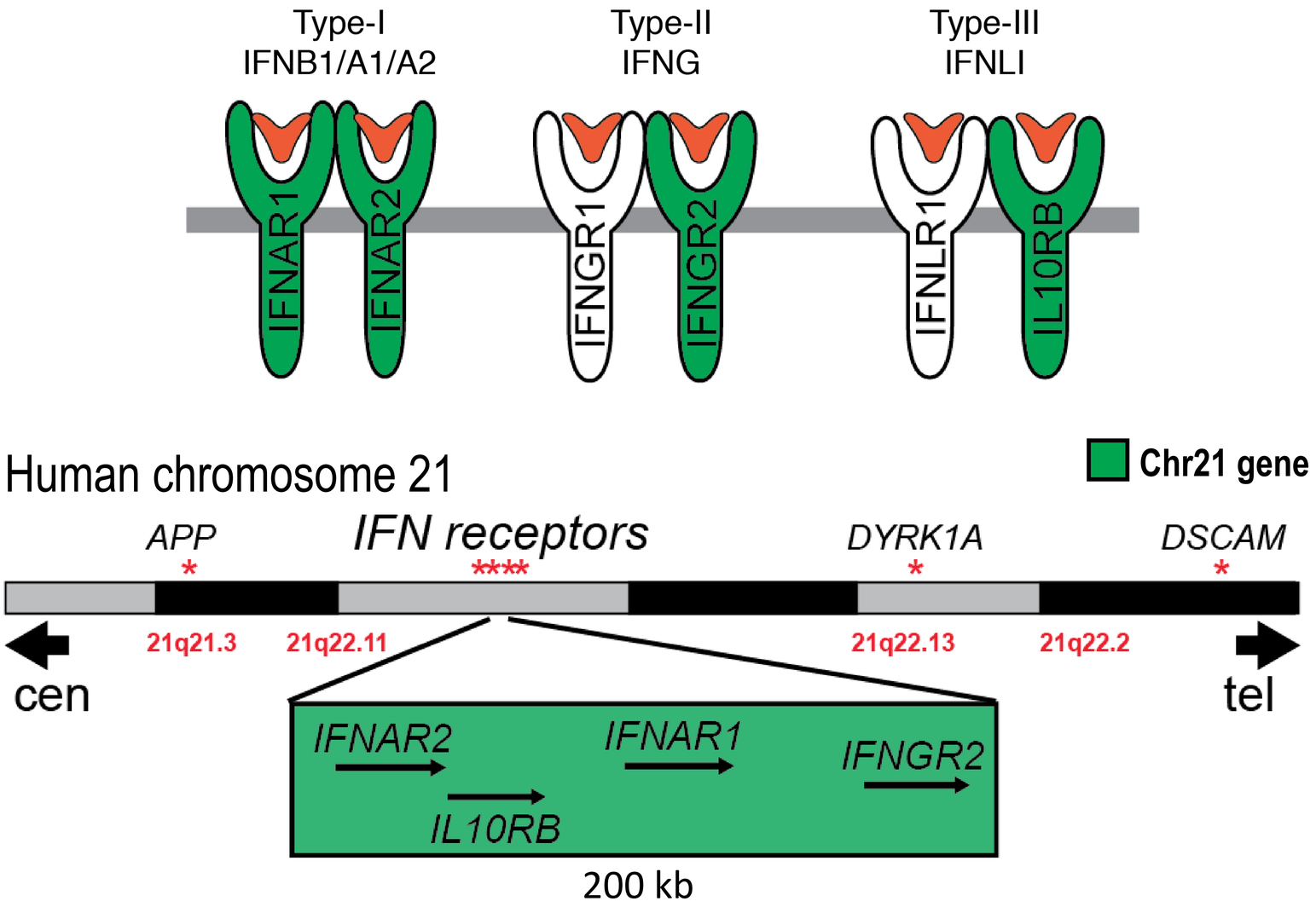


TOTAL POPULATION (D21+T21)

(AD, ASD, Depression)

Culp-Hill et al. *Under review*

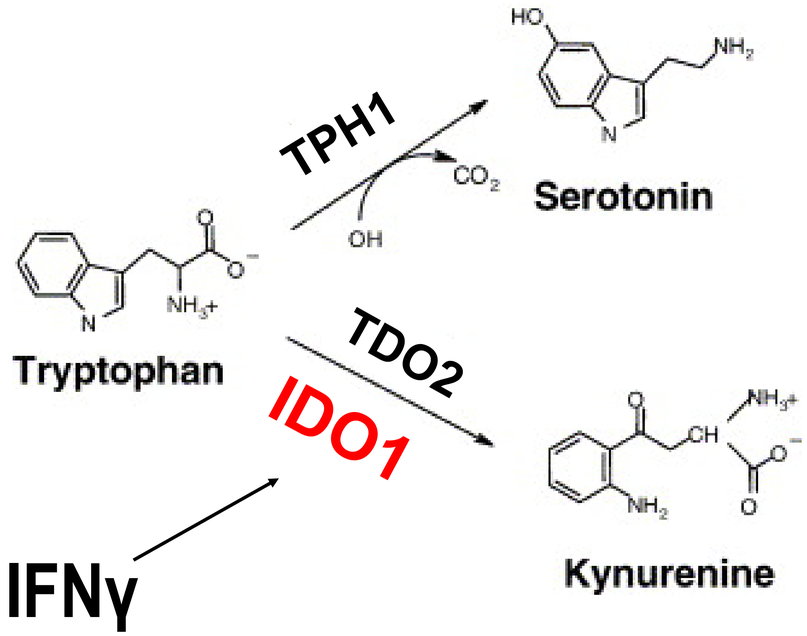
4 of the 6 IFN receptors are encoded on chr21

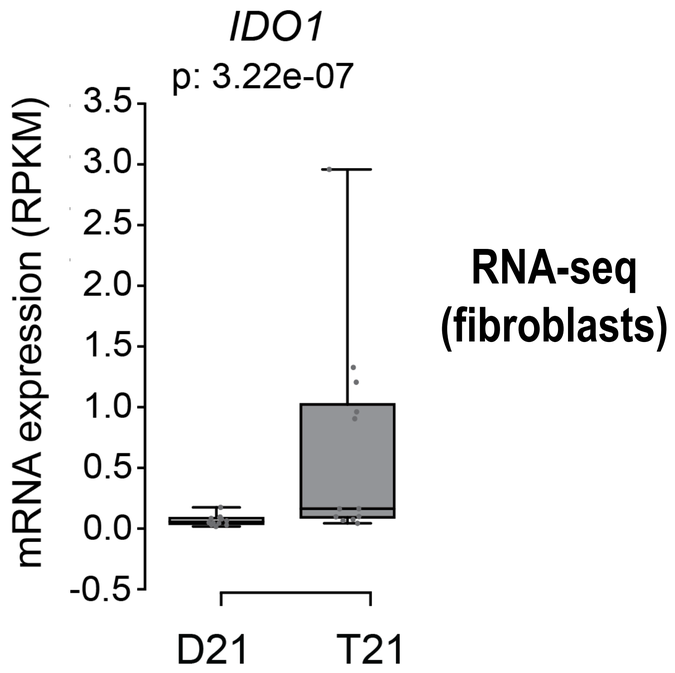


Courtesy of Dr. Kelly Sullivan – Sullivan et al. Elife 2016

Plasma metabolomics reveals strong dysregulation of tryptophan metabolism

IDO1, the key enzyme required to shunt tryptophan into
the kynurenine > quinolinic acid pathway,
is a well known Interferon Stimulated Gene!

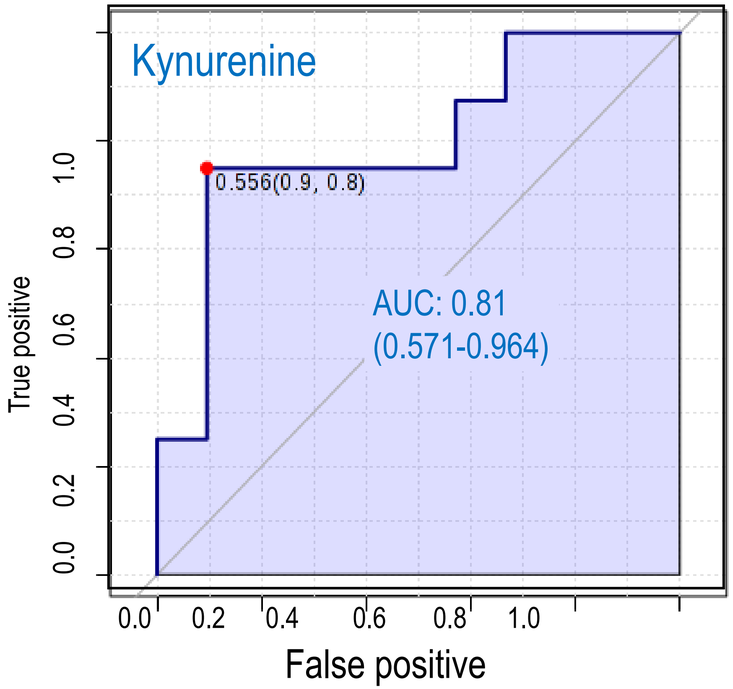




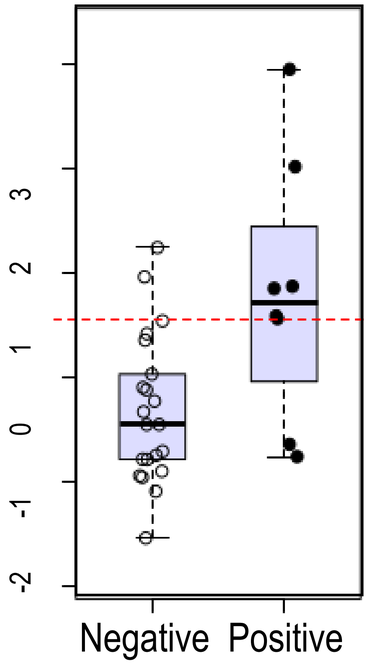
IDO1 is expressed by a variety of cells and is induced preferentially by IFN-γ
IDO1 expression is much increased in cells from people with Down syndrome

Courtesy of Dr. Kelly Sullivan – Sullivan et al. Elife 2016

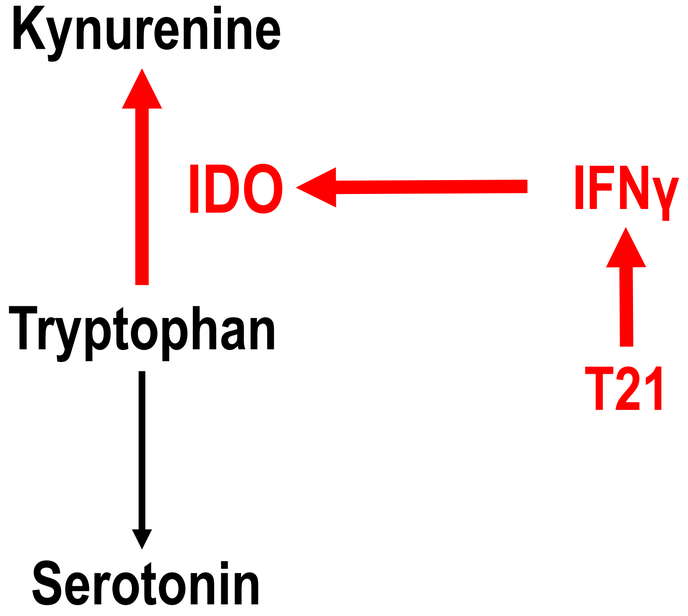
Tryptophan metabolism and kynurenine predict cognitive impairment in Down Syndrome



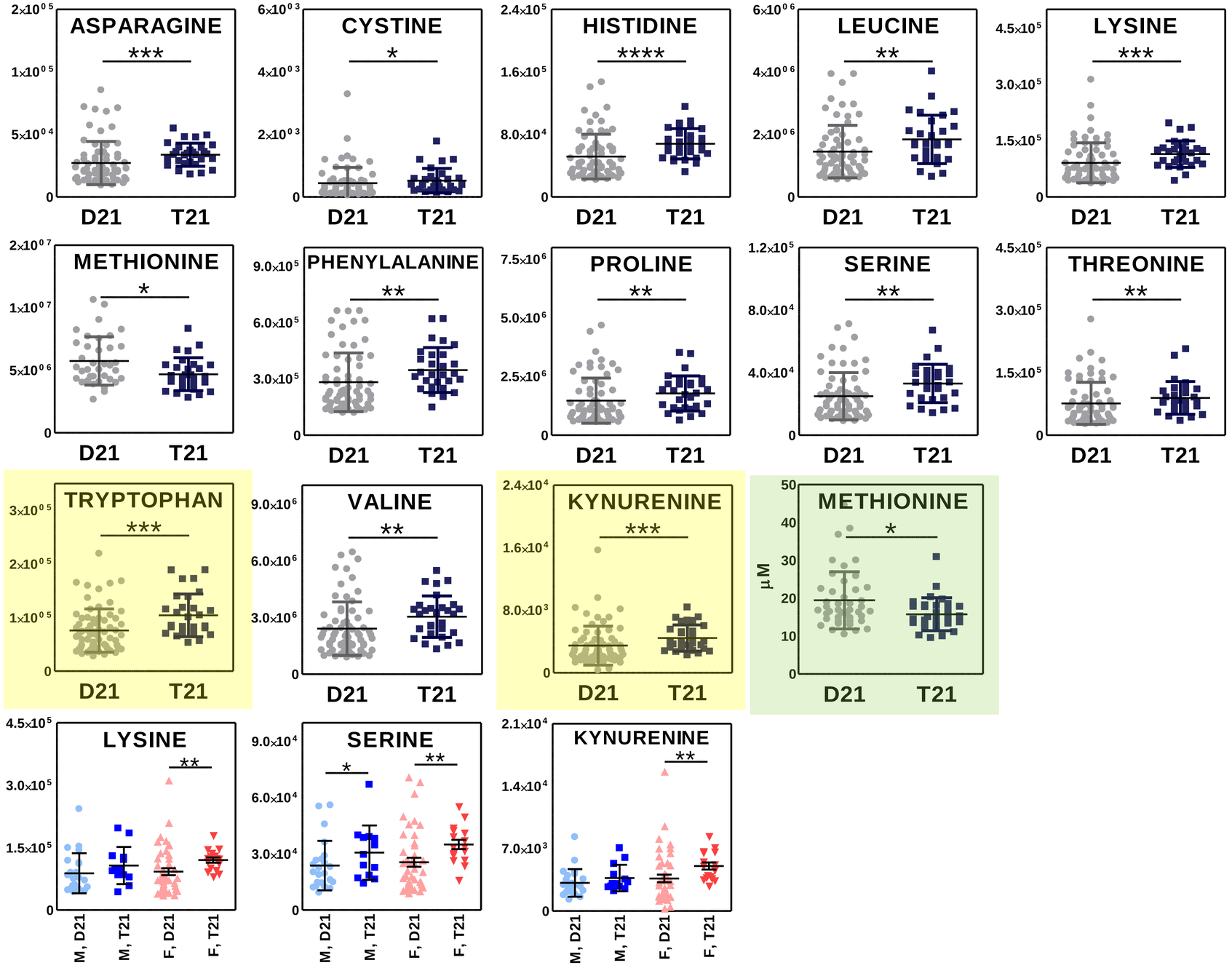
T21 ONLY



(AD, ASD, Depression)



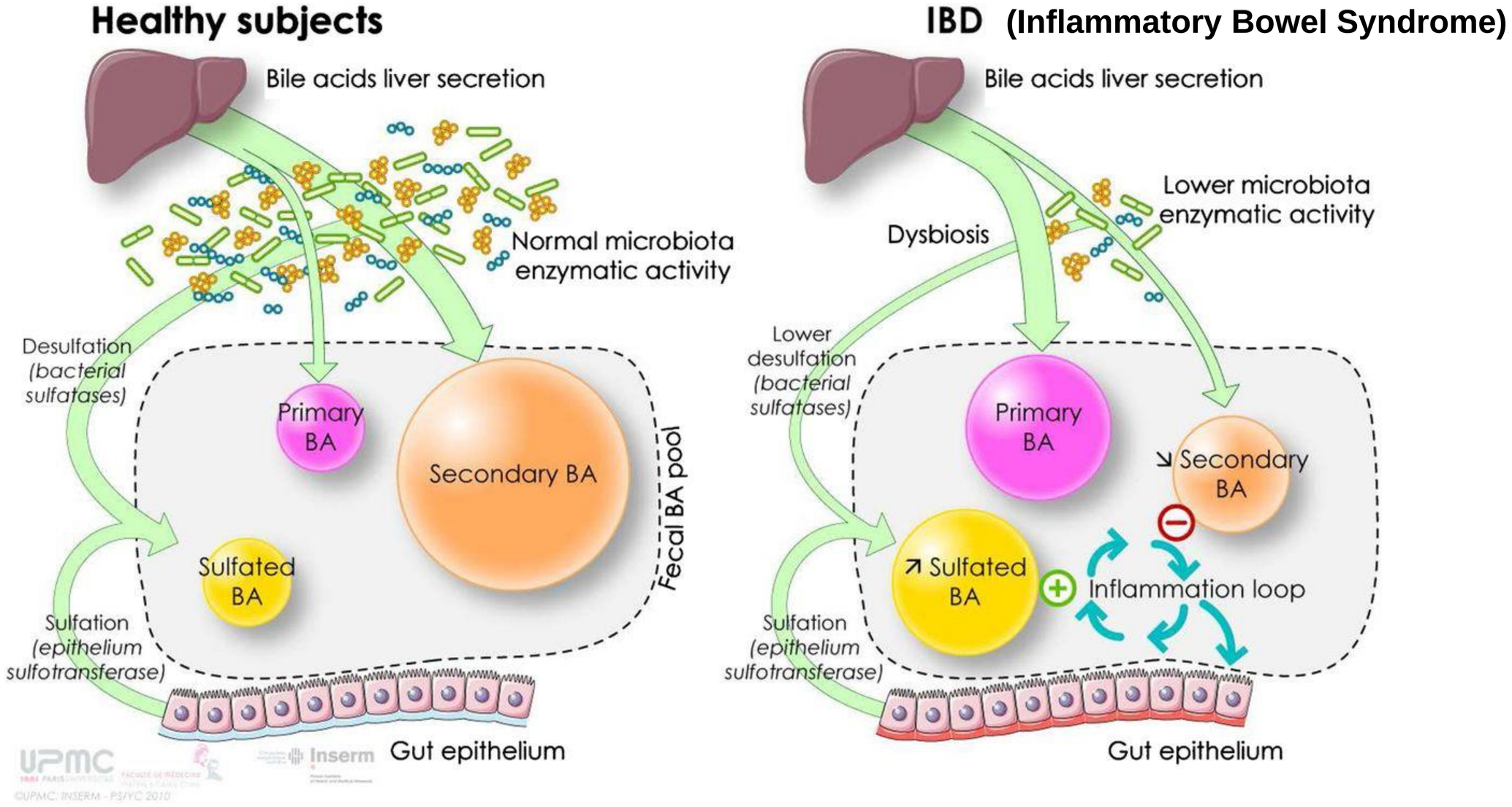
Culp-Hill et al. *Under review;* Stockwell et al. *in preparation*



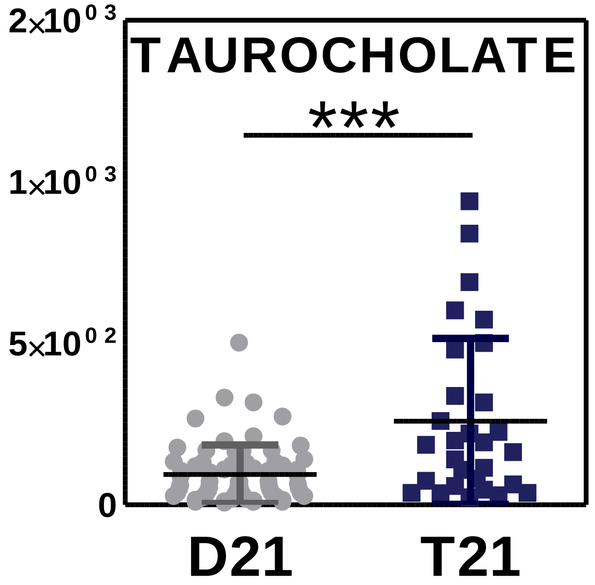
Increased free amino acids: a marker of inflammation-induced proteolysis

Culp-Hill et al. *Under review*

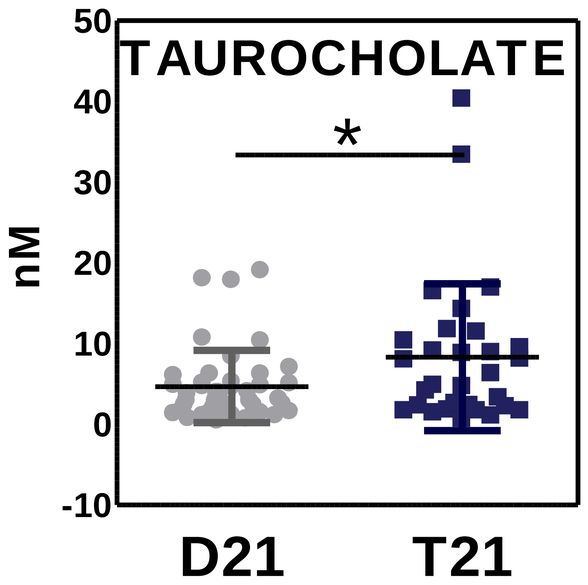
Bile acids are deconjugated by the gut microbiome: link to the GI autoimmune comorbidities in T21



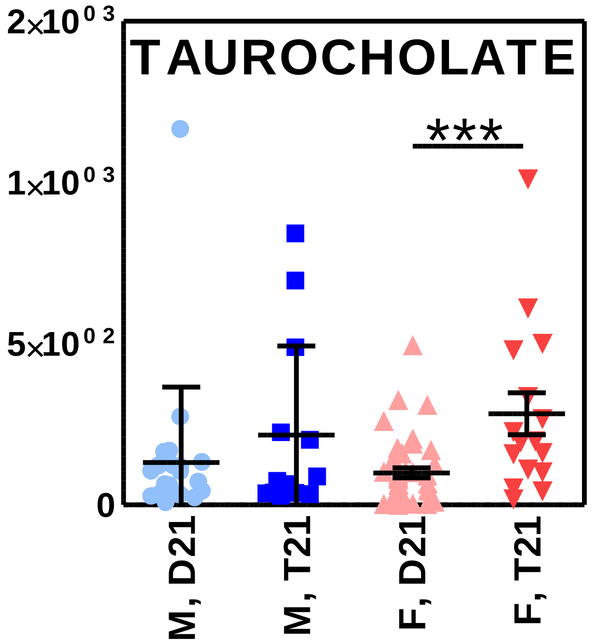
Conjugated bile acids accumulate in RBCs from adults and children with T21, especially in women



Relative quant



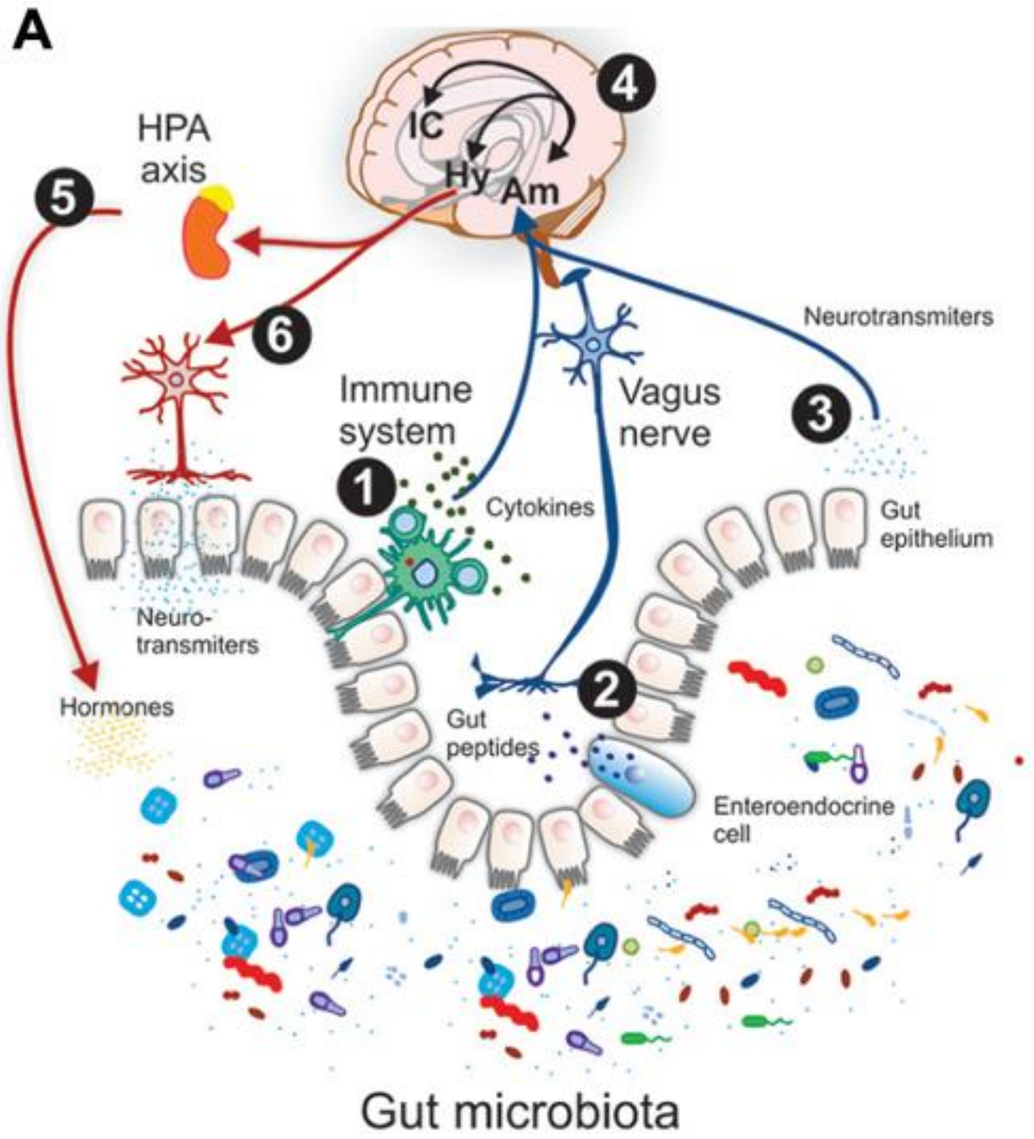
Absolute quant

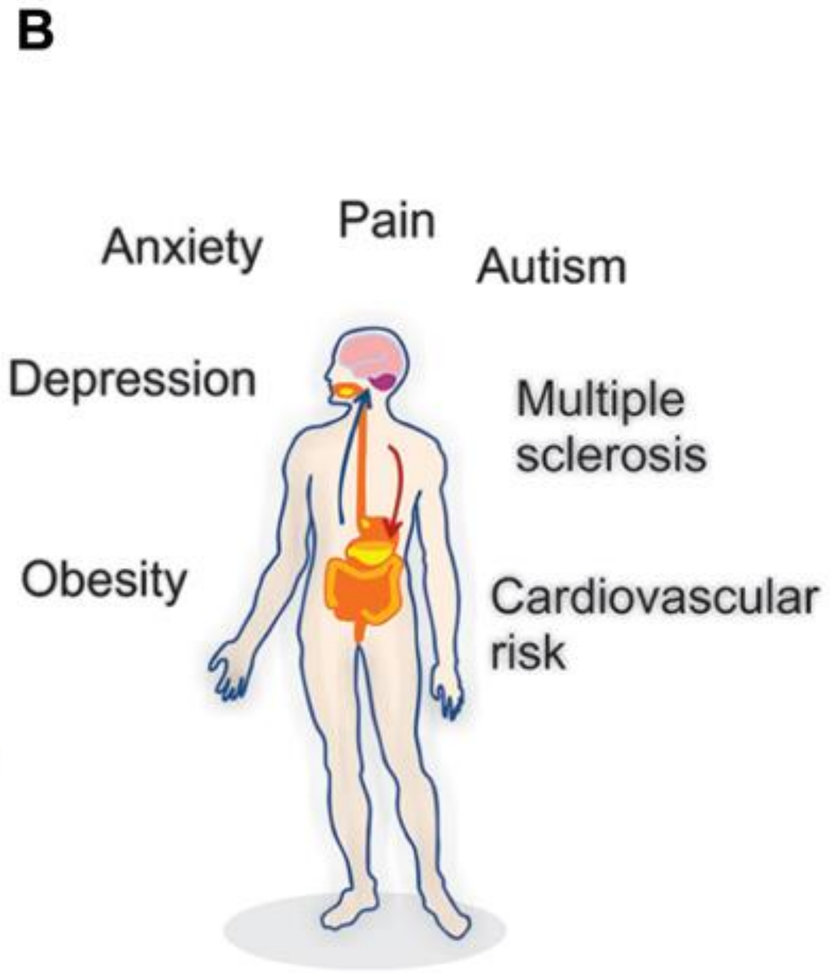


Gender-bias

Culp-Hill et al. *Under review*

Microbiome dysbiosis and autism





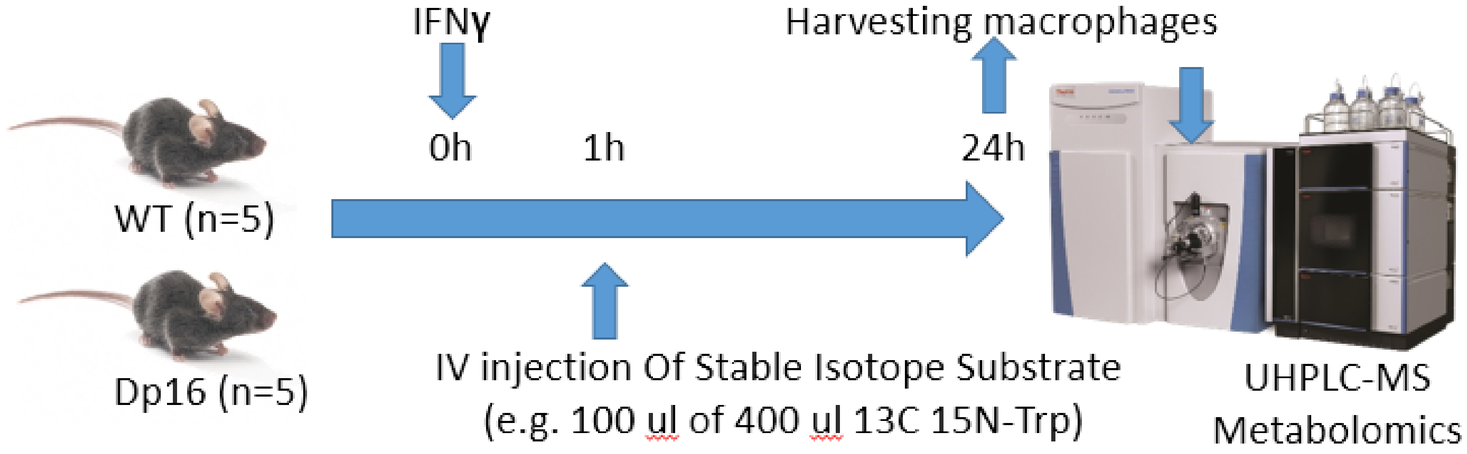
Mulle et al. Curr Psychiatric Rep 2013

Take home messages

- Succinate, hypoxanthine: hypoxia, pulmonary hypertension and inflammation
- Tryptophan metabolism: inflammaging and cognitive impairment
- Bile acids: (gut) inflammation, microbiome dysbiosis and autism spectrum disorders

Future Directions

- Use of dp10, dp16, dp17 mouse models for recapitulation of human findings
- Metabolic tracing experiments in vitro and in vivo with stable isotope tracers (Espinoza lab)
- More isotopic standards for absolute quantification (Kyn, Trp)
- Obtain larger cohort for validation of metabolic markers of comorbidities
- Metabolic interventions with drugs and diet in animal models
- Bioinformatics (Costello lab)

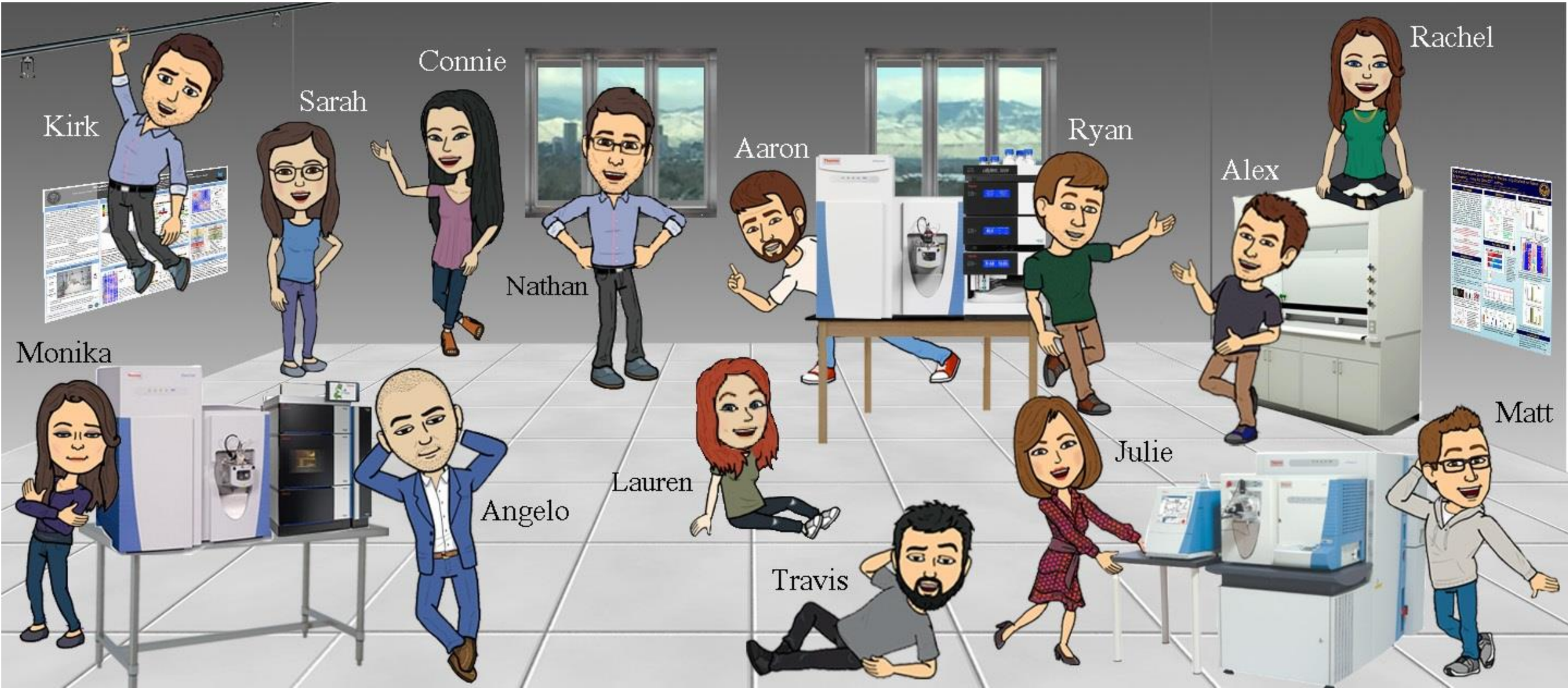


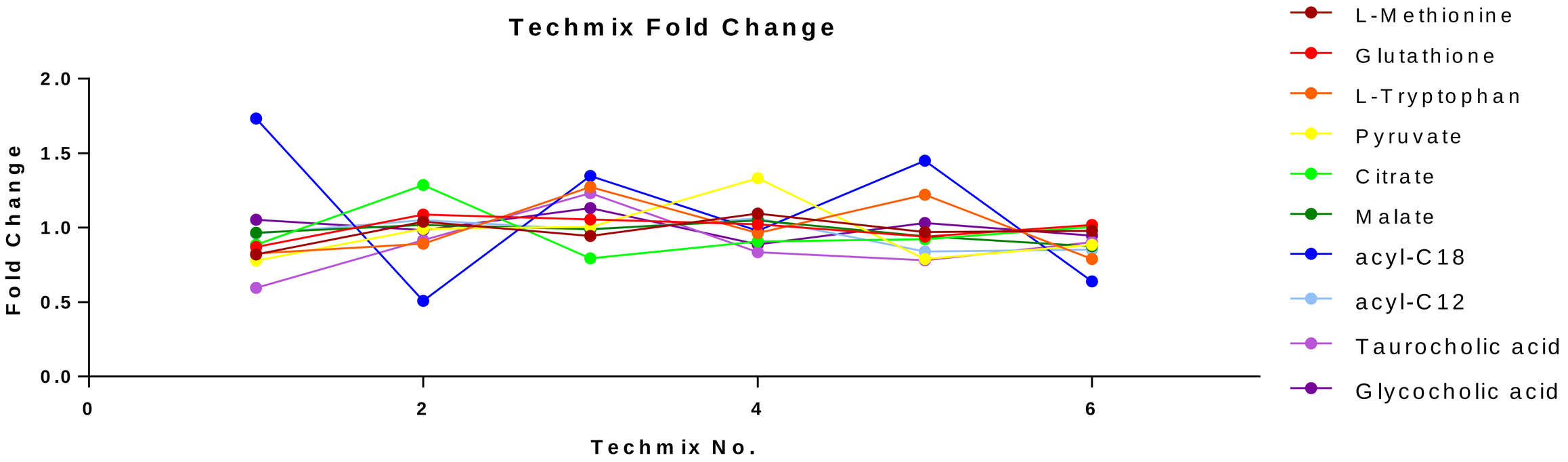
LINDA CRNIC INSTITUTE FOR DOWN SYNDROME™

Joaquin Espinoza
Tom Blumenthal
Kelly Sullivan

Angela Rachubinski
Keith Smith
Rani Powers

James Costello
Ken MacLean




Techmix Fold Change
Fold Change
2.0
1.5
1.0
0.5
0.0
0
2
4
6
Techmix No.
L-Methionine
Glutathione
L-Tryptophan
Pyruvate
Citrate
Malate
acyl-C18
acyl-C12
Taurocholic acid
Glycocholic acid