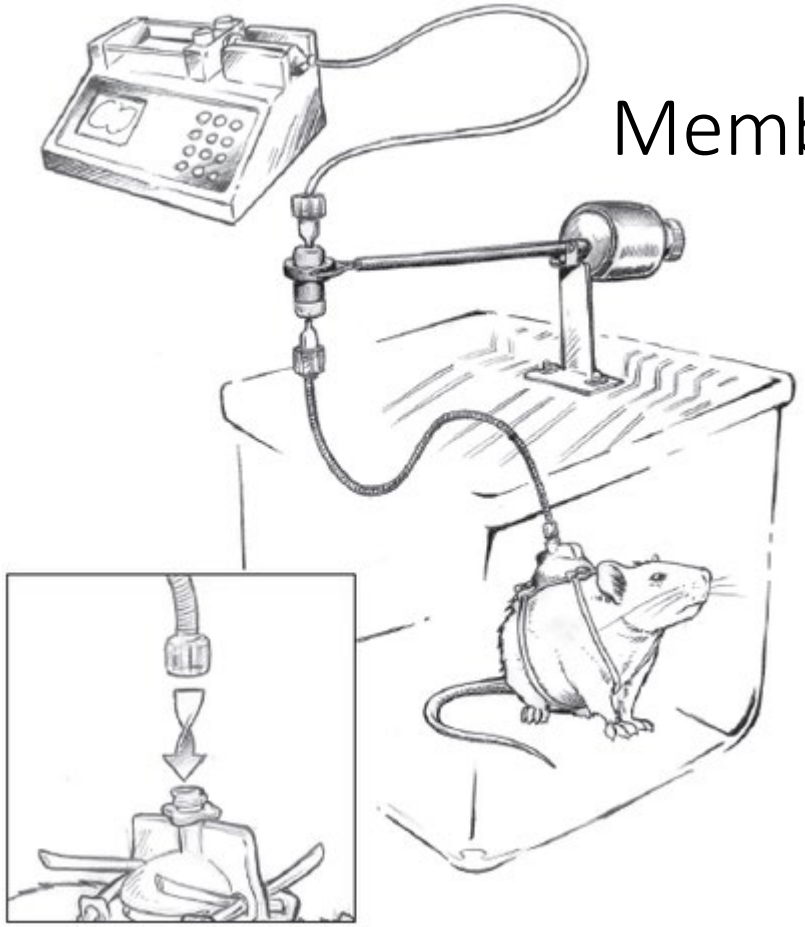
How to conduct In-vivo Metabolomics:

Rationale, Techniques, and Concerns



Membranes, Organelles, and Metabolism Meeting

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5/01/2020

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Why *In-vivo?*

1 - *In-vitro* is not the reality for Cells

Media was not designed to recapitulate nutrient conditions *in-vivo*, it was designed to keep cell cultures alive.

Example: Glucose

RPMI 1640 = 11mM

DMEM = 5-25mM

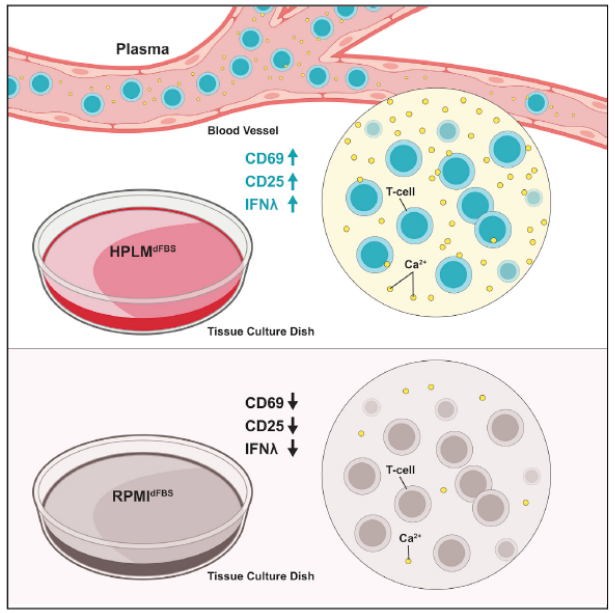
In Blood: 4-5.4mM (prediabetic = 10mM)

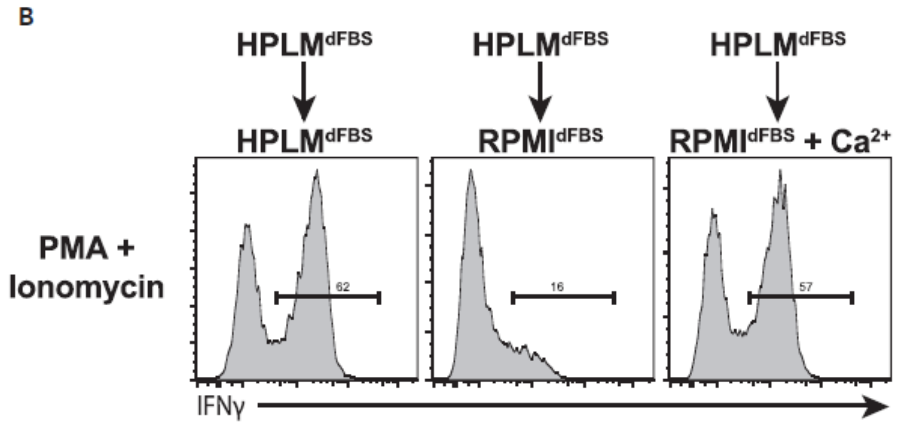
Another example: Amino acids

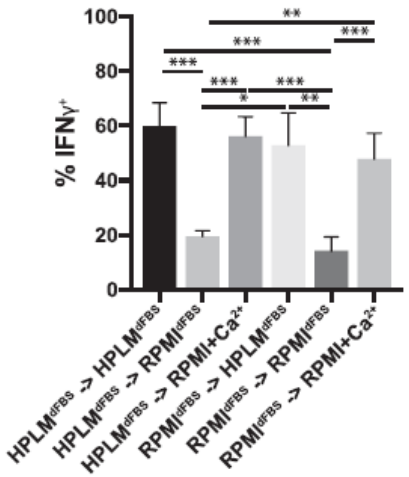
	Mean ± SD (µmol/L)	Median	RPMI
L-Alanine	319.4±71.7	315.3	0
L-Arginine	81.4±19.3	80.7	1149
L-Asparagine	48.4±9.29	47.5	378
L-Aspartic acid	6.26±2.34	6.10	150
L-Citrulline	29.8±7.91	28.8	0
L-Glutamine	657.9±106.2	652.9	2000
L-Glutamic acid	46.2±21.4	42.2	136
Glycine	255.4±65.9	244.6	133
L-Histidine	89.3±10.8	89.4	96
L-Isoleucine	77.5±15.4	76.5	381
L-Leucine	150.1±27.7	147.6	382
L-Lysine	197.4±30.7	195.9	219
L-Methionine	25.4±5.05	24.9	101
L-Ornithine	69.0±18.0	66.1	0
L-Phenylalanine	60.0±7.75	59.4	91
L-Proline	188.3±55.0	179.4	174
L-Serine	115.1±25.0	112.6	286
L-Threonine	127.9±28.2	124.5	168
L-Tryptophan	63.0±10.7	62.7	25
L-Tyrosine	62.9±12.2	61.4	11
L-Valine	243.6±45.2	241.1	171

Adapted from: Trabado S. et al, PLOSone, 2017

T-cells function is dramatically influenced by media composition



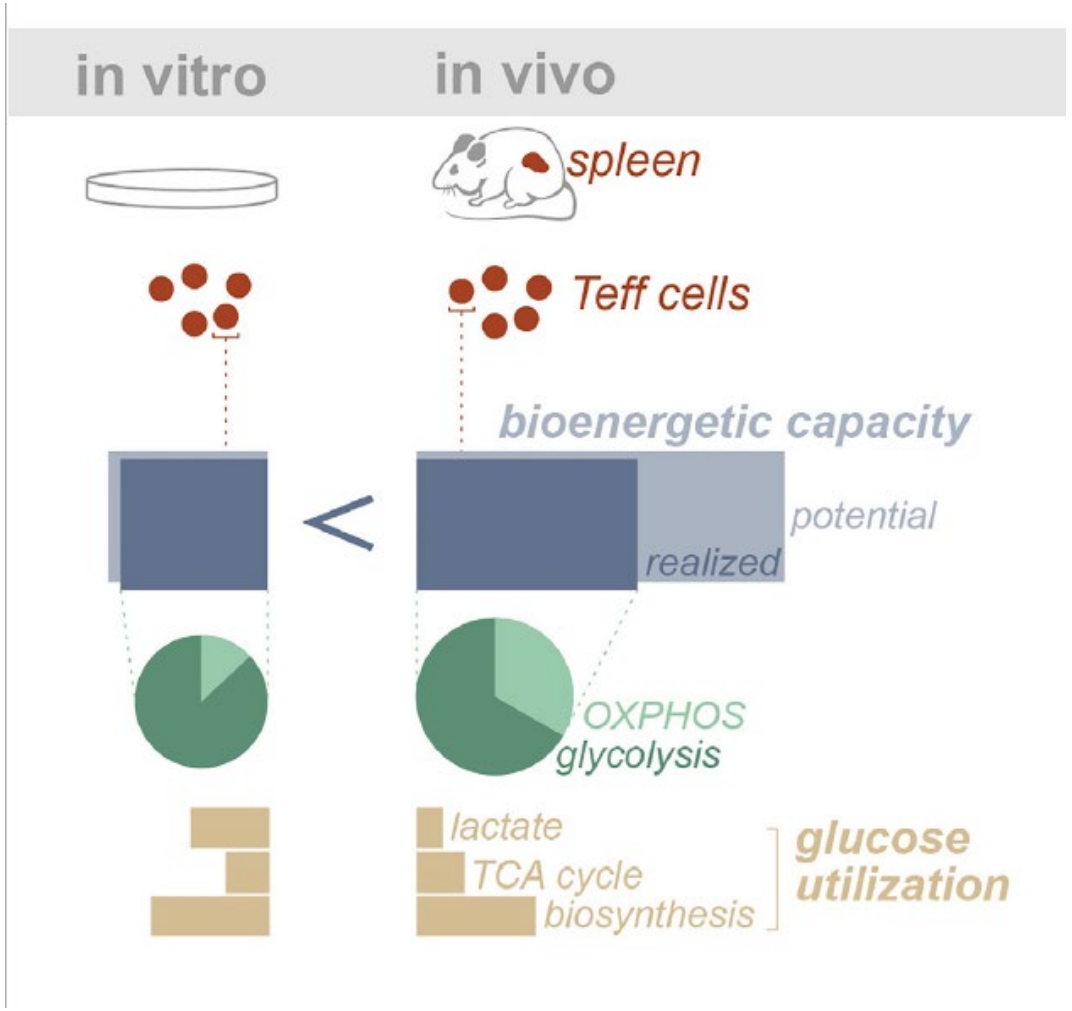


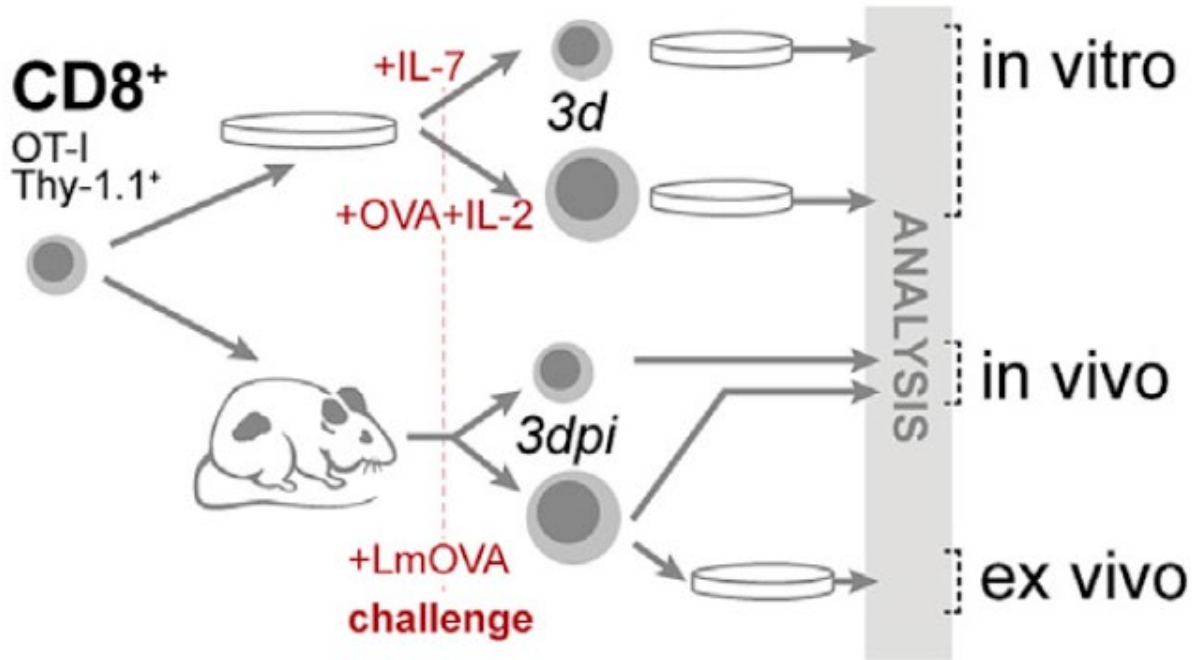


	Concentration (µM)		
	RPMI	HPLM-MIN	HPLM
Glucose	11111	5000	5000
Proteinogenic Amino acids			
Alanine	0	430	430
Arginine	1149	110	110
Asparagine	378	50	50
Aspartate	150	20	20
Cysteine	0	40	40
Cystine	208	100	100
Glutamate	136	80	80
Glutamine	2055	550	550
Glycine	133	300	300
Histidine	97	110	110
Hydroxyproline	153	0	0
Isoleucine	382	70	70
Leucine	382	160	160
Lysine	219	200	200
Methionine	101	30	30
Phenylalanine	91	80	80
Proline	174	200	200
Serine	286	150	150
Threonine	168	140	140
Tryptophan	25	60	60
Tyrosine	11	80	80
Valine	171	220	220
Ions			
Na^+	138525	132271	132271
K^+	5233	4442	4442
Ca^{2+}	424	2390	2390
Mg^{2+}	407	830	830
NH^{4+}	0	40	40
Cl^-	108781	116196	116196
HCO^{3-}	23809	24000	24000
PO_4^{3-}	5634	966	966
SO_4^{2-}	407	350	350
NO^{3-}	848	80	80

Leney-Greene MA, et al, iScience 2020

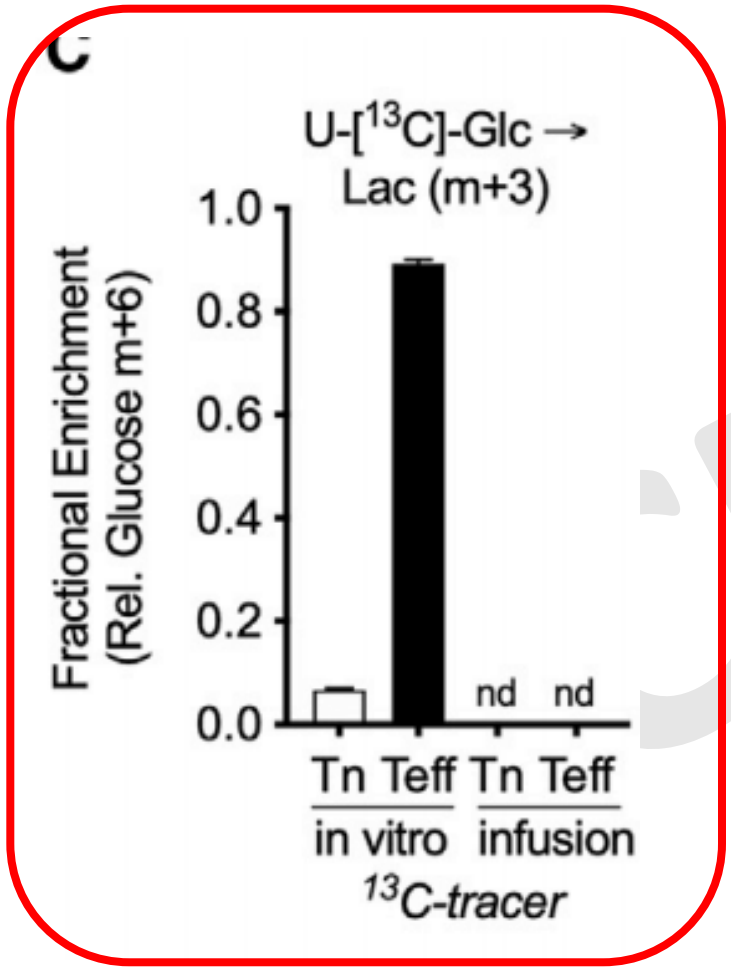
T-cell activation is dramatically different in-vivo

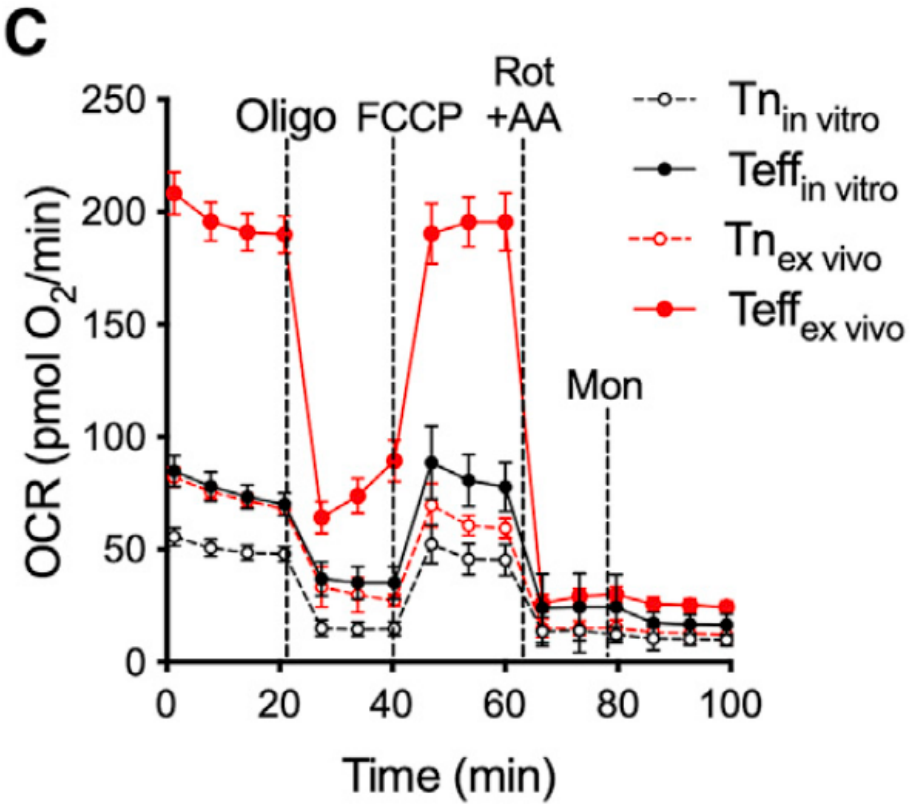




Ma, et al, Immunity, 2019

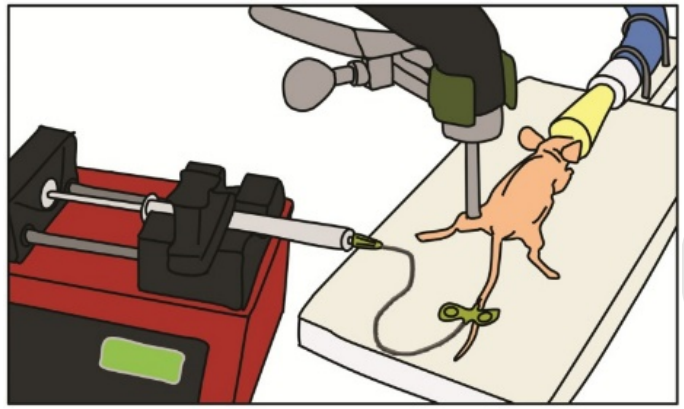
T-cell metabolism is dramatically different in-vivo



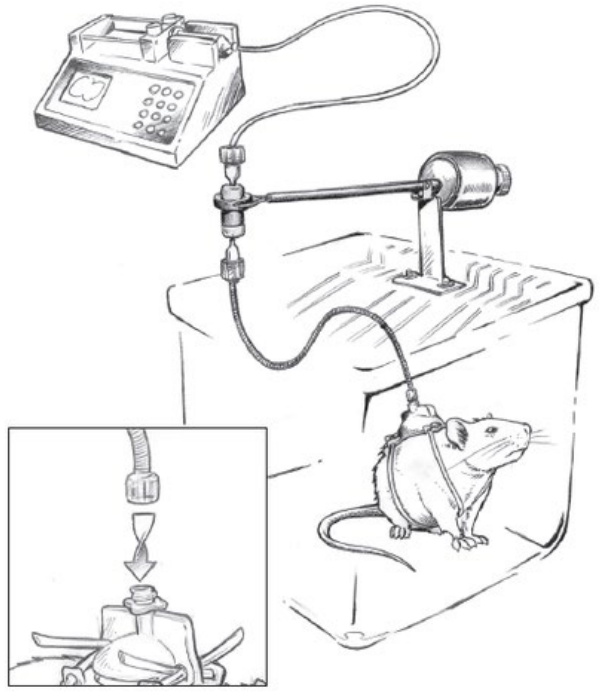


How does one do in-vivo metabolomics?

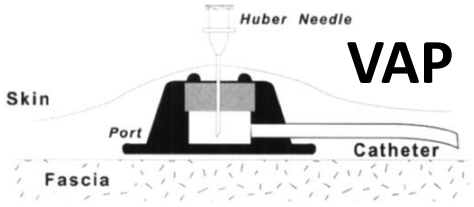
The main component: Infusion method/apparatus



Tail-Vein Infusion



DO NOT DO THIS!



Tethered Infusion

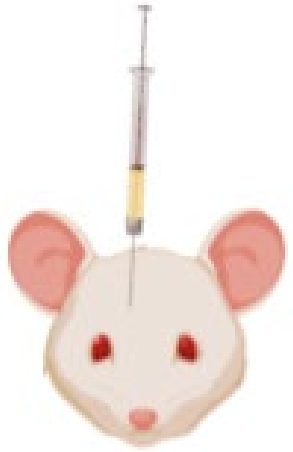
Considerations regarding infusion

- Overloading of metabolite can perturb systemic metabolism
  - Think about glucose (again):
    - If you infuse too much, you will change insulin levels…

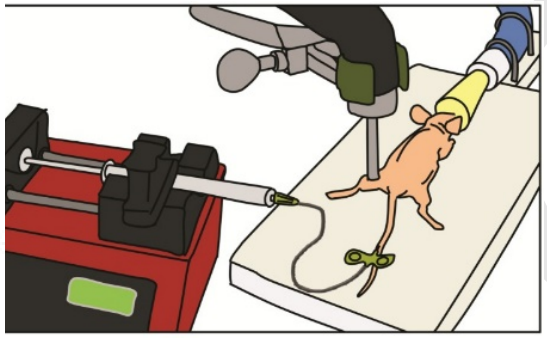
- However, The biggest issue is time to isolation!
  - Remember = Glycoysis (seconds) / OXPHOS (Minutes)

Jang. et al. *Cell*, 2018

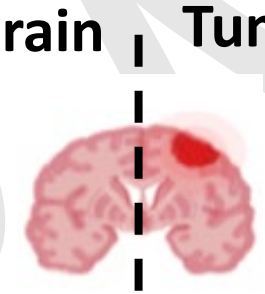
Establishment of methodology: *In-Vivo* flux analysis for murine glioblastoma

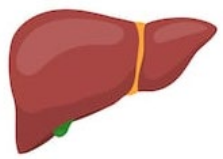


14 Days



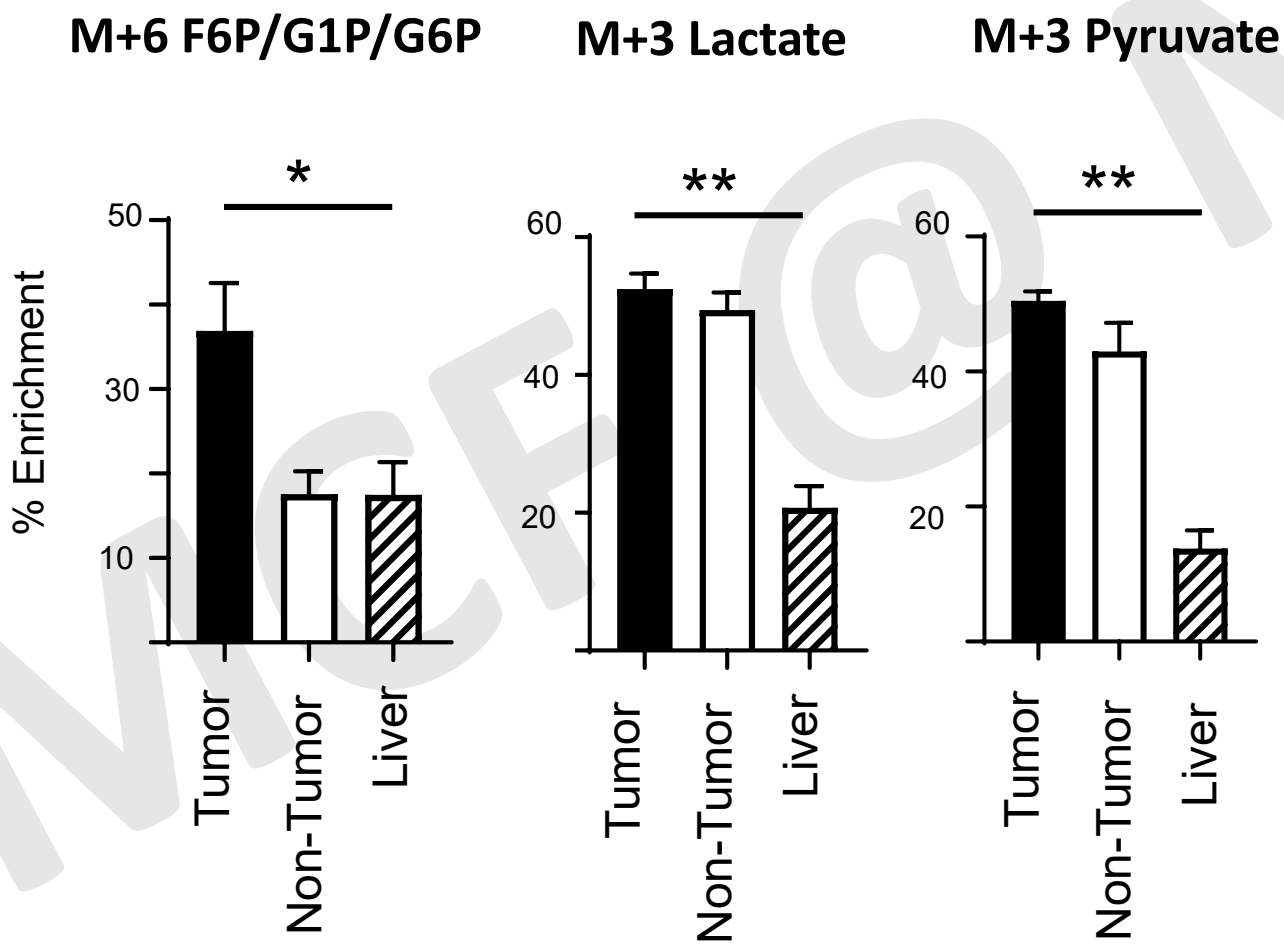
C^{13} flux in-vivo Glucose (3ul/min 100mg/ml)



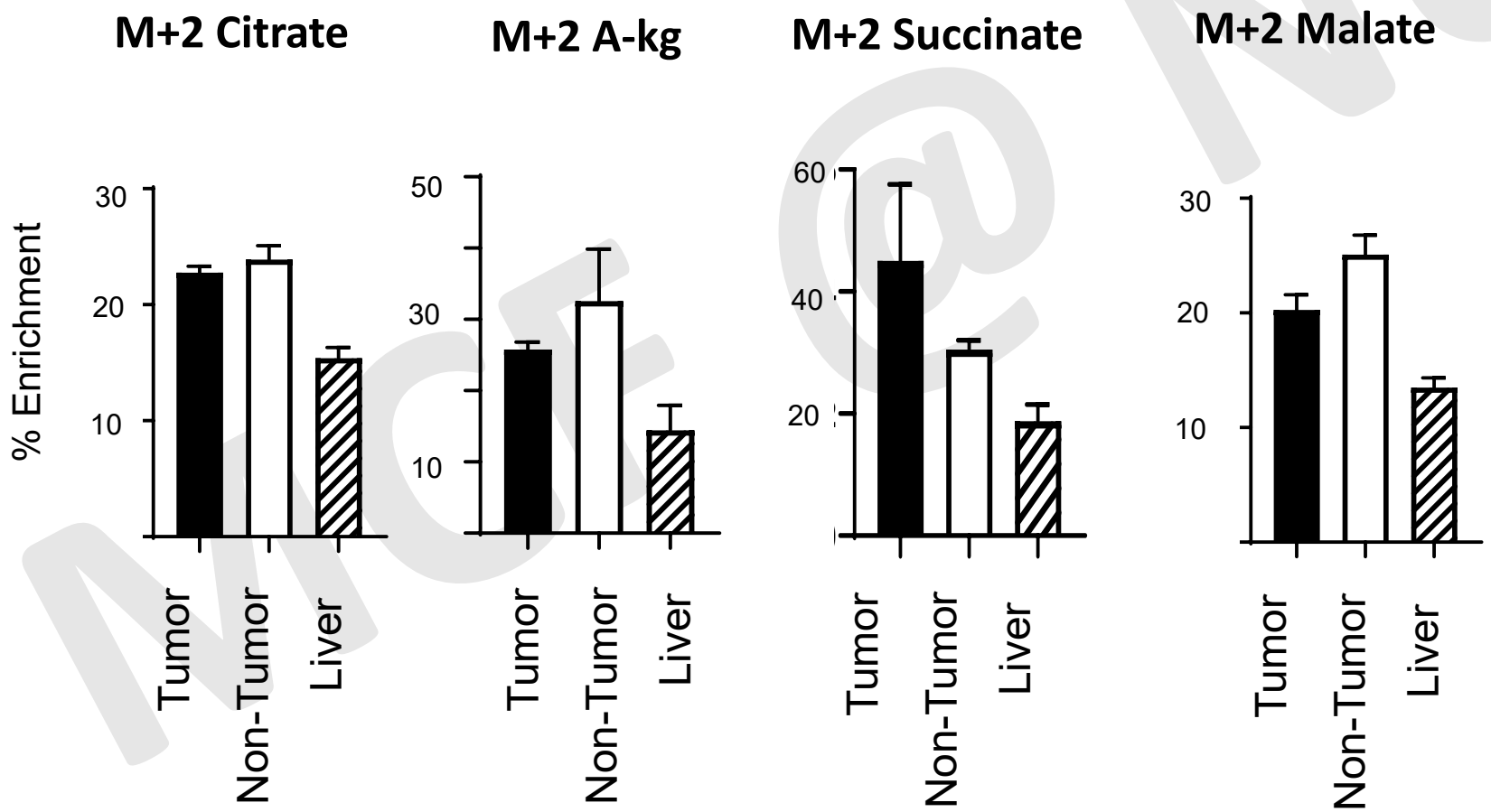


Liver

Glycolytic rate of CNS higher than liver



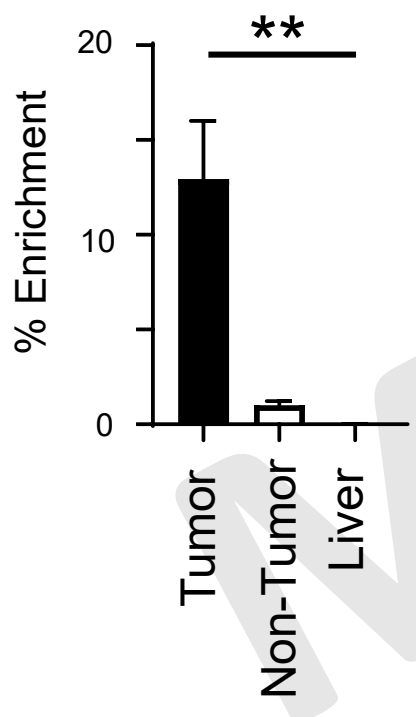
Glucose -> TCA cycle minimally changed amongst CNS tissues



There are tumor specific changes in glucose metabolism…

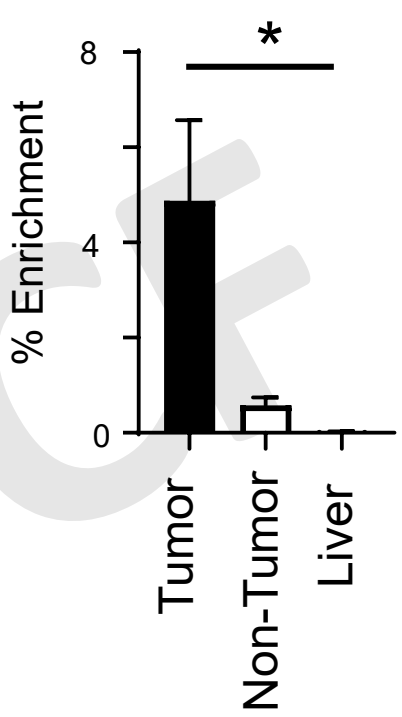
Purine

M+5 IMP

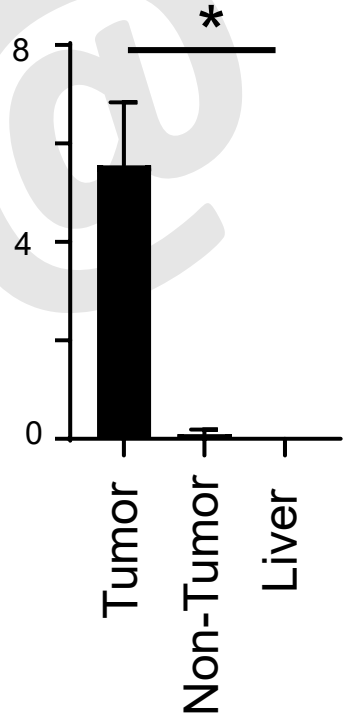


Pyrimidine

M+5 UMP

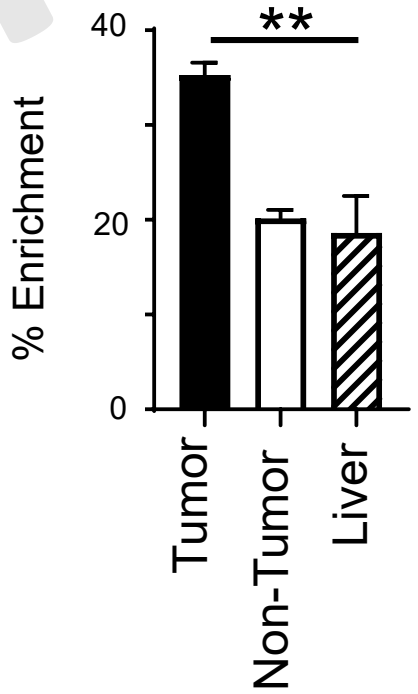


M+5 UTP



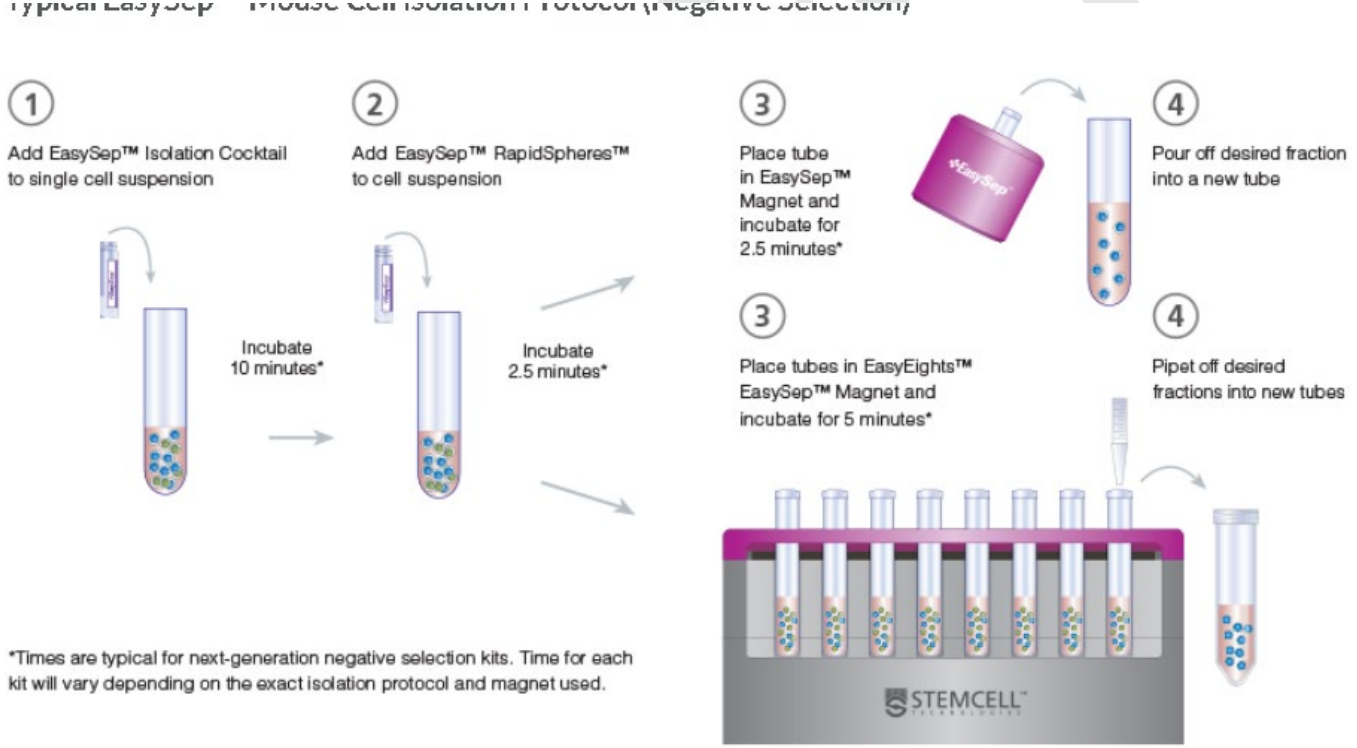
Cahill Cycle?

M+3 Alanine



What if you want to look at a specific cellular subset?

If your cell of interest is easy to isolate, then you can use magnetic bead isolation after flux



What if you want to look at a specific cellular subset?

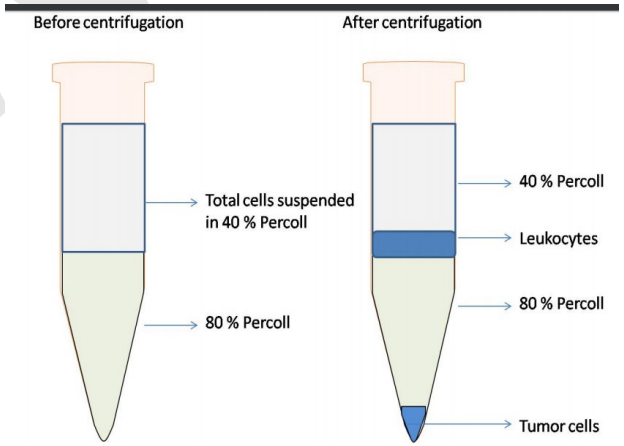
If your cell of interest is difficult to isolate….

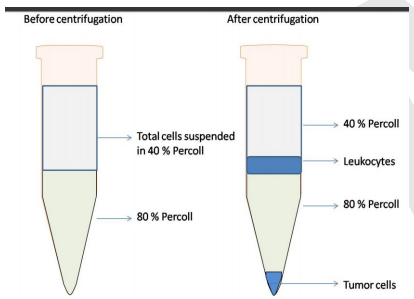
Due to cell number needed for LC-MS, 5-10 mice needed per condition!

Dissection

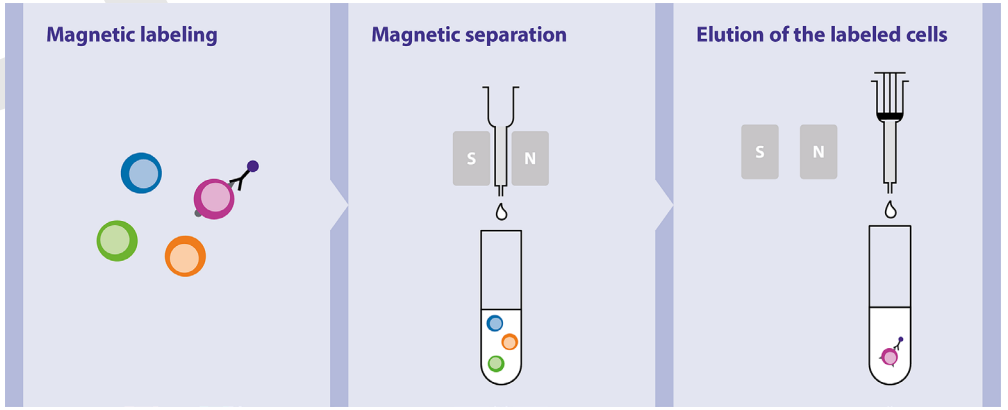
Homogenization

Density gradient centrifugation



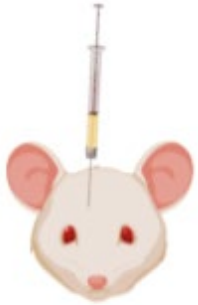


Lymphocyte layer isolation



= 6-7 hours

For challenging cell populations, Ex-vivo flux can be performed



Dissection

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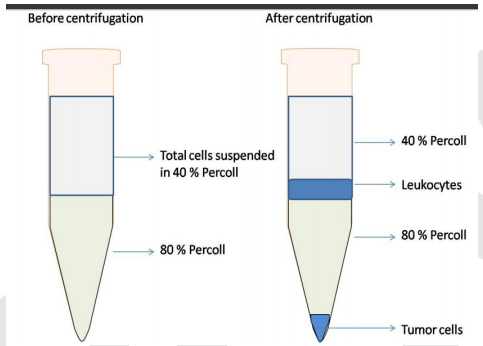
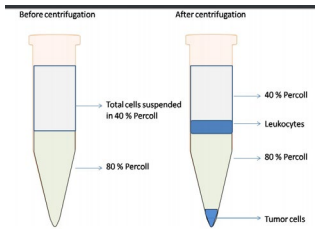
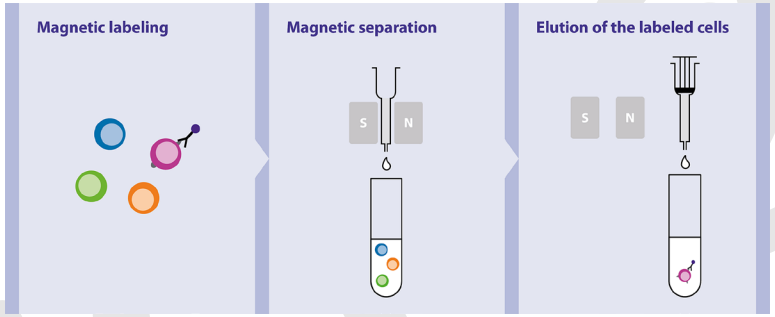


T-cell



Lymphocyte layer isolation



MDSC

T-cell

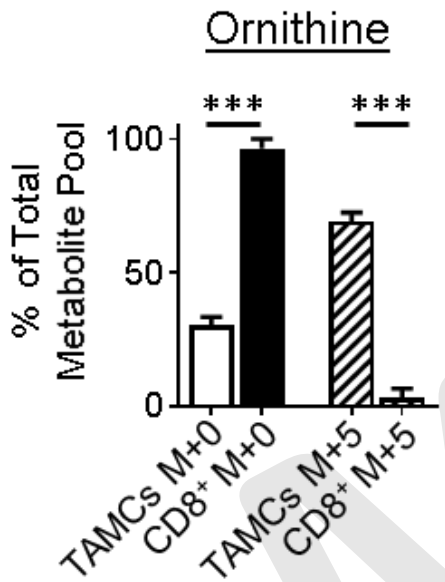
MDSC

Culture with labeled metabolite

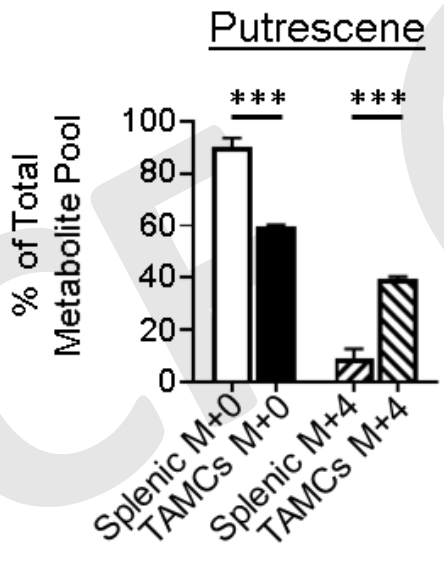
LC-MS

For challenging cell populations, Ex-vivo flux can be performed

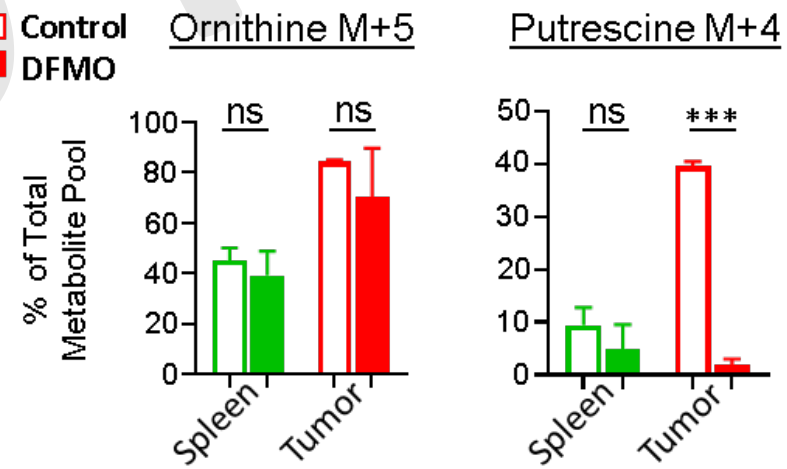
Cell Type



Location



Treatment Effects



Conclusions

1. *In-vitro* flux analysis does not recapitulate in-vivo metabolism, but is easy
   - Addition of missing factors, or utilization of specialized media can correct
2. In-vivo flux is the most scientifically accurate method for measuring metabolism, but is technically challenging
3. Ex-vivo flux is a balance between accuracy and feasibility for metabolic measurement