Understanding the role of sleep in innate immunity

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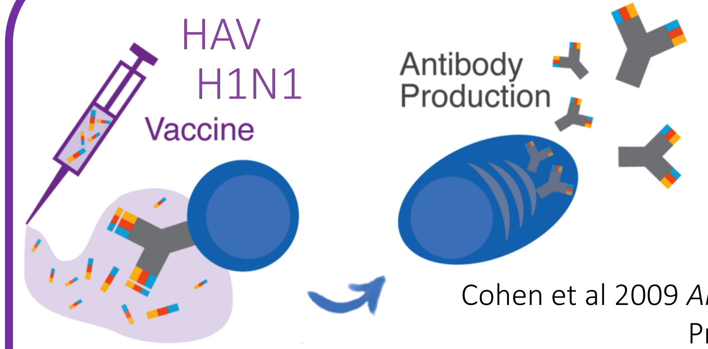
Sleep affects Immunity

< 5hr sleep

Increase Relative Risk of Infection when sleep < 5hr (RR 1.39-1.82)

Patel et al 2012 *Sleep 35*(1), 97–101
Prather et al 2016 JAMA Intern Med 176(6) 850-852



Decrease antibody titers and Th1 immune response after vaccination

Cohen et al 2009 *Archives of Internal Medicine*, *169*(1), 62–67
Prather et al 2015. *Sleep*, *38*(9), 1353–1359.
Lange et al 2003. *Psychosomatic Med* 65(5), 831-835)
Lange et al 2011. *J Immuno* 187(1), 283-290

Sleep Disruption

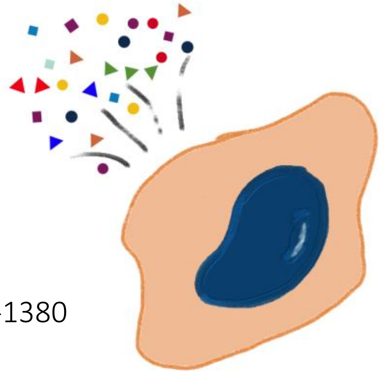
Poor Sleep

High susceptibility for common cold symptoms when inoculated with rhinovirus in nares

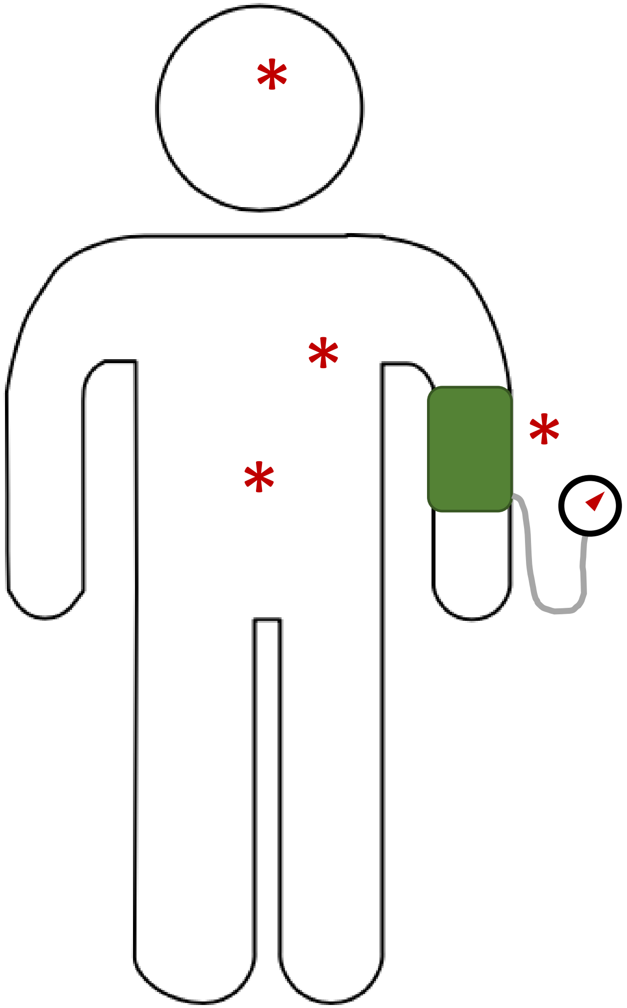
Cohen et al 2009 *Archives of Internal Medicine*, *169*(1), 62–67
Prather et al 2015. *Sleep*, *38*(9), 1353–1359.

Alters cytokines release and immune cell count and function

Besedovsky et al *Phsyiol Rev*, *99(3)*, 1325-1380



Obstructive Sleep Apnea are associated with chronic inflammatory conditions



- Stroke (Yaggi et al 2005; Redline et al, 2010; Marshall et al, 2016)
- Myocardial infarction (Hung et al, 1990)
- Coronary arterial disease (Peker et al, 2006)
- Hypertension (Peppard et al, 2000)
- Metabolic syndrome (Tasali et al 2008; Tanno et al 2014; Bakker, 2015)

How does the immune compartment knows when sleep occurred?

Sepsis: 26% mortality rate

Fleischmann, et al. (2016). Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. *American Journal of Respiratory and Critical Care Medicine*, *193*(3), 259–272
Rhee et al. (2019). Prevalence, Underlying Causes, and Preventability of Sepsis-Associated Mortality in US Acute Care Hospitals. *JAMA Network Open*, *2*(2), e187571

Sleep disruption is ubiquitous in the ICU

- Highly fragmented
- Total sleep duration reduced
- Reduced Stage 3 NREM and REM sleep
- Significant amount of sleep occurs during the day time

Elliot, et al. (2013). *Critical Care*. 17:R46
Knauert et al. (2014). *Heart Lung*. 43(5): 445-452

Simple hypothesis:

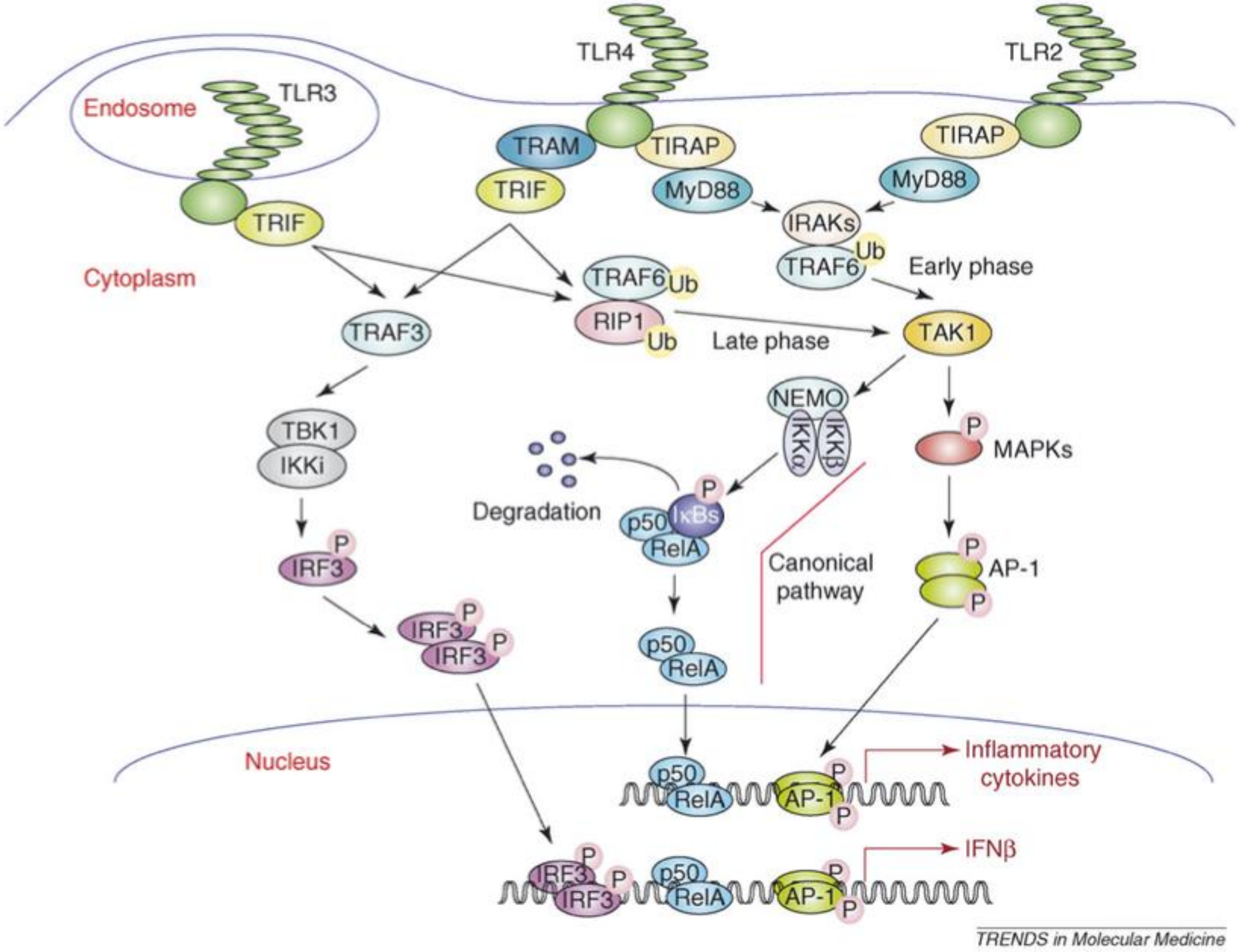
Sleep disruption worsens septic shock outcome

Sleep disruption with tactile stimulation

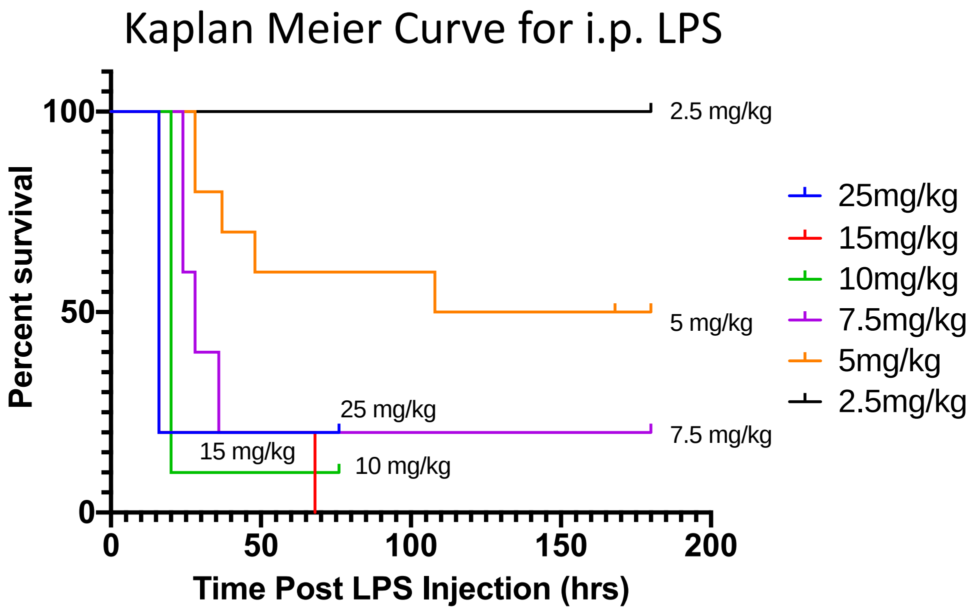
Biological phenotypes:

- Induces metabolic syndrome (Wang et al, 2013; Poroyko et al 2016)
- Predispose atherosclerosis with increase neutrophils and monocytes through Hypocretin pathway (McAlpine et al, 2019)
- Worsen lung cancer due to increase TAM infiltration (Hakim et al, 2014)

Lipopolysaccharide (LPS) mediates septic shock through Toll-like Receptor 4 (TLR4) signaling pathway



Kawai and Akira (2007). *Trends Mol Med*. 13(11) 460-469

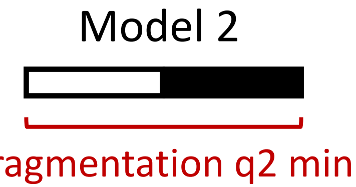


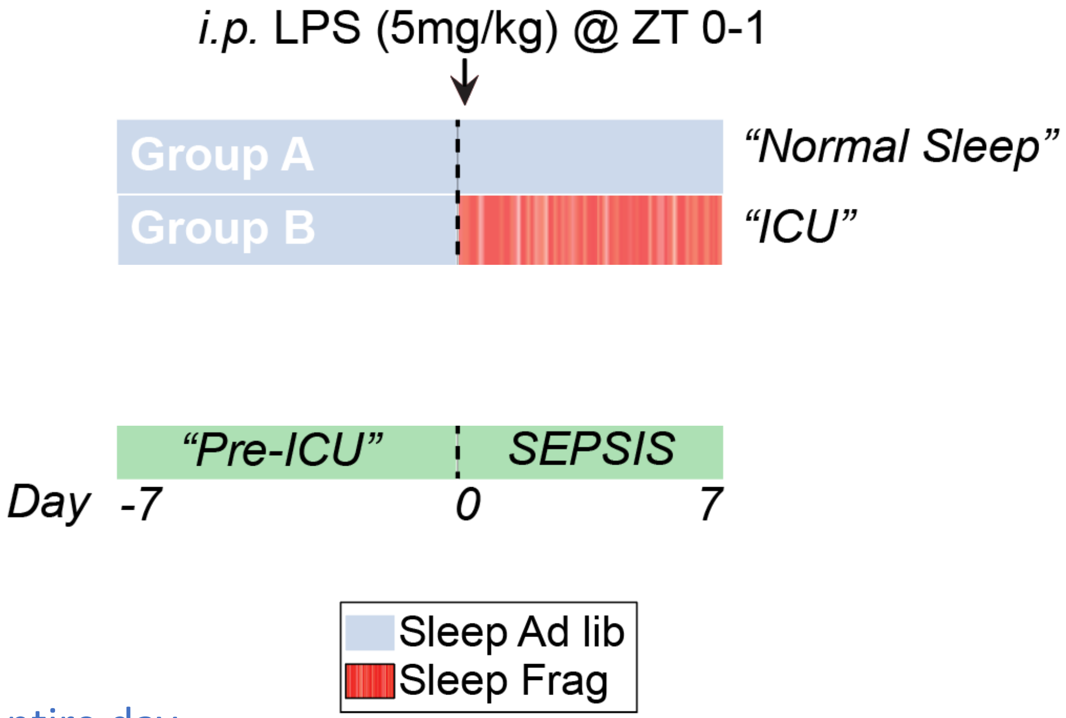
C57BL6/J males, 3-4 months, Male. LPS *i.p.* injection time ZT0-1.
n = 5 except for 10mg/kg (n = 10). With Koorosh Askari

Testing whether sleep disruption affect septic shock outcome

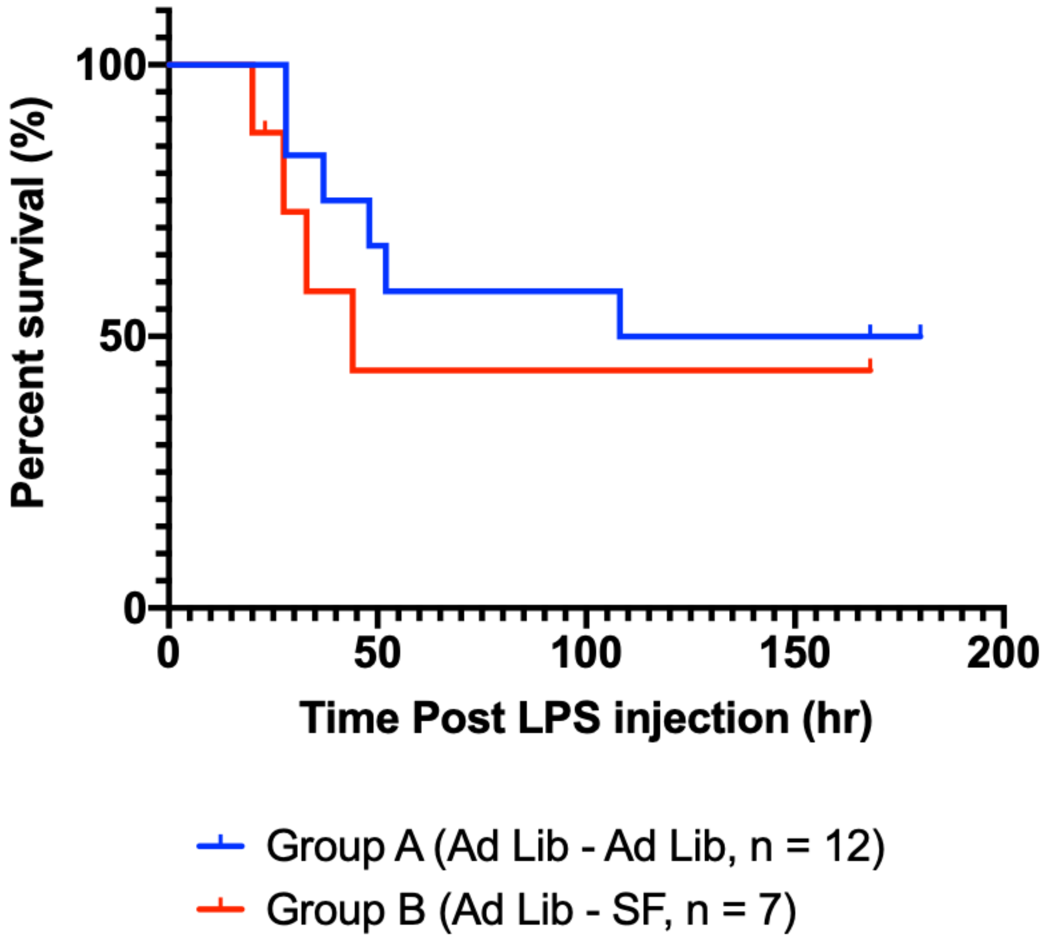
Sleep fragmentation model

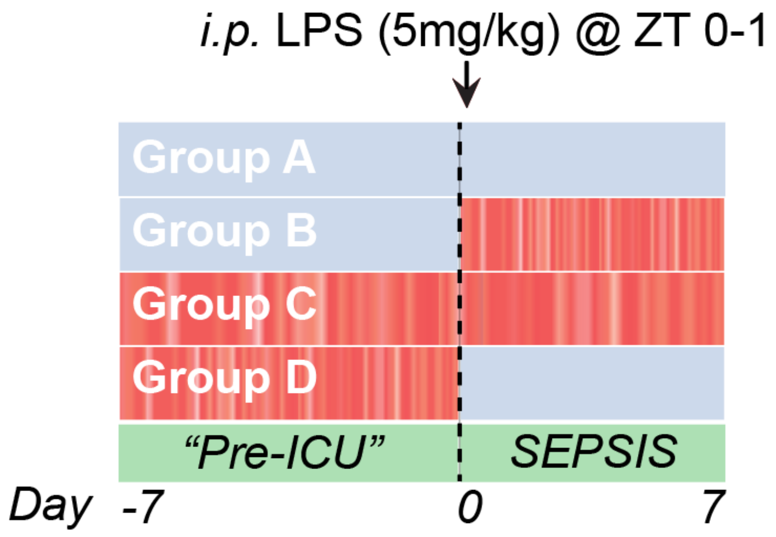
Bar moves every 2 minutes throughout the entire day





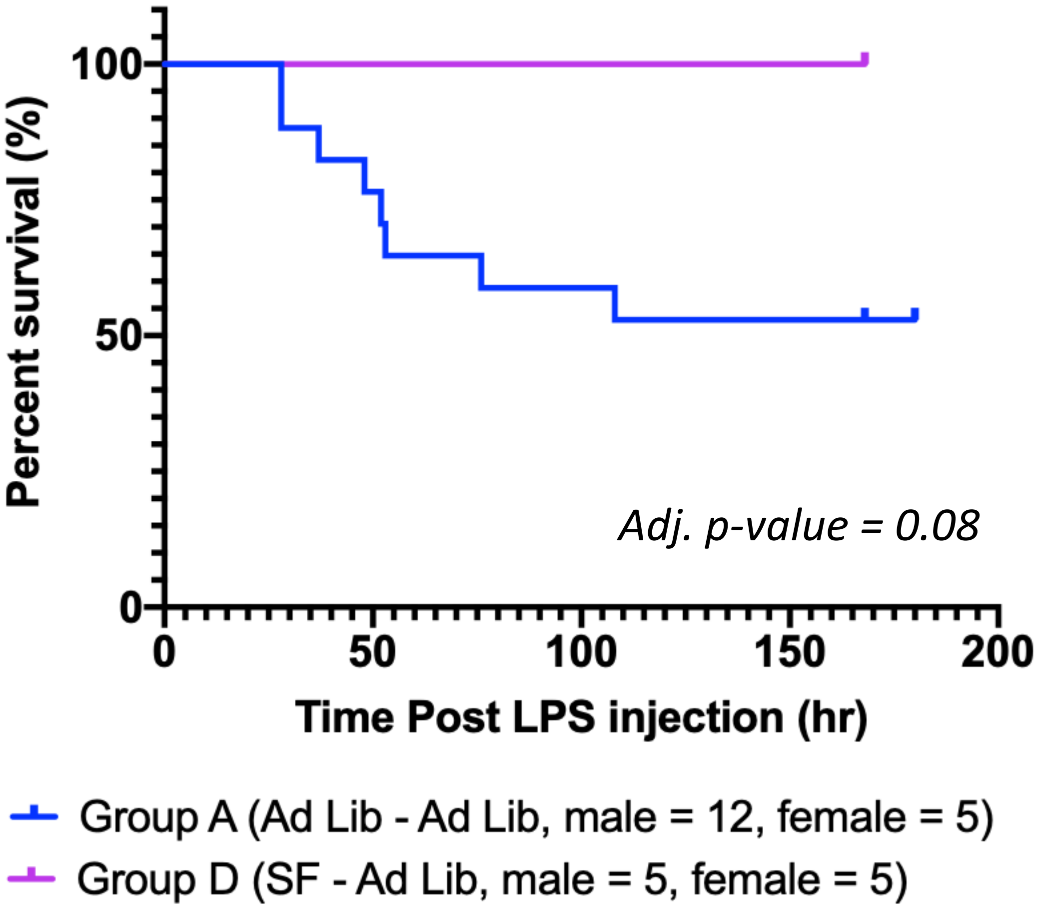
Sleep Fragmentation after septic challenge may worsen survival outcome

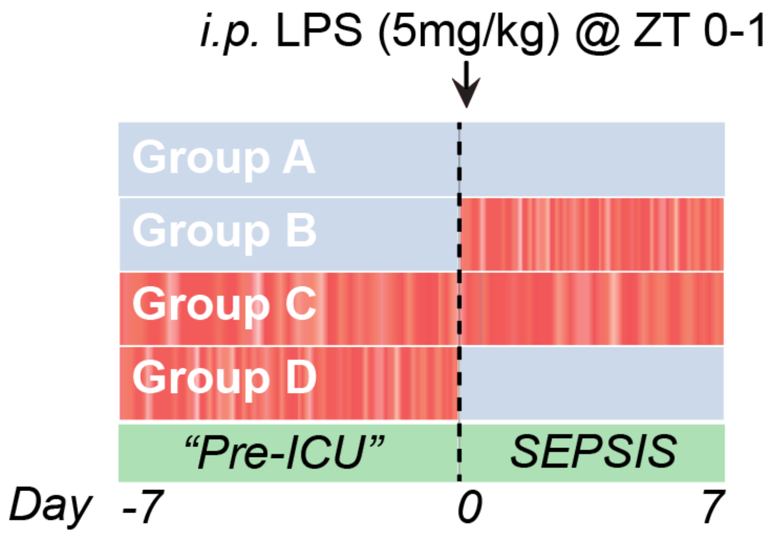




Power calculation, n = 20 per arm to observe a difference of 32-37% with type I error < 0.05.

Sleep Fragmentation prior to septic challenge may have protective effect on Septic Shock





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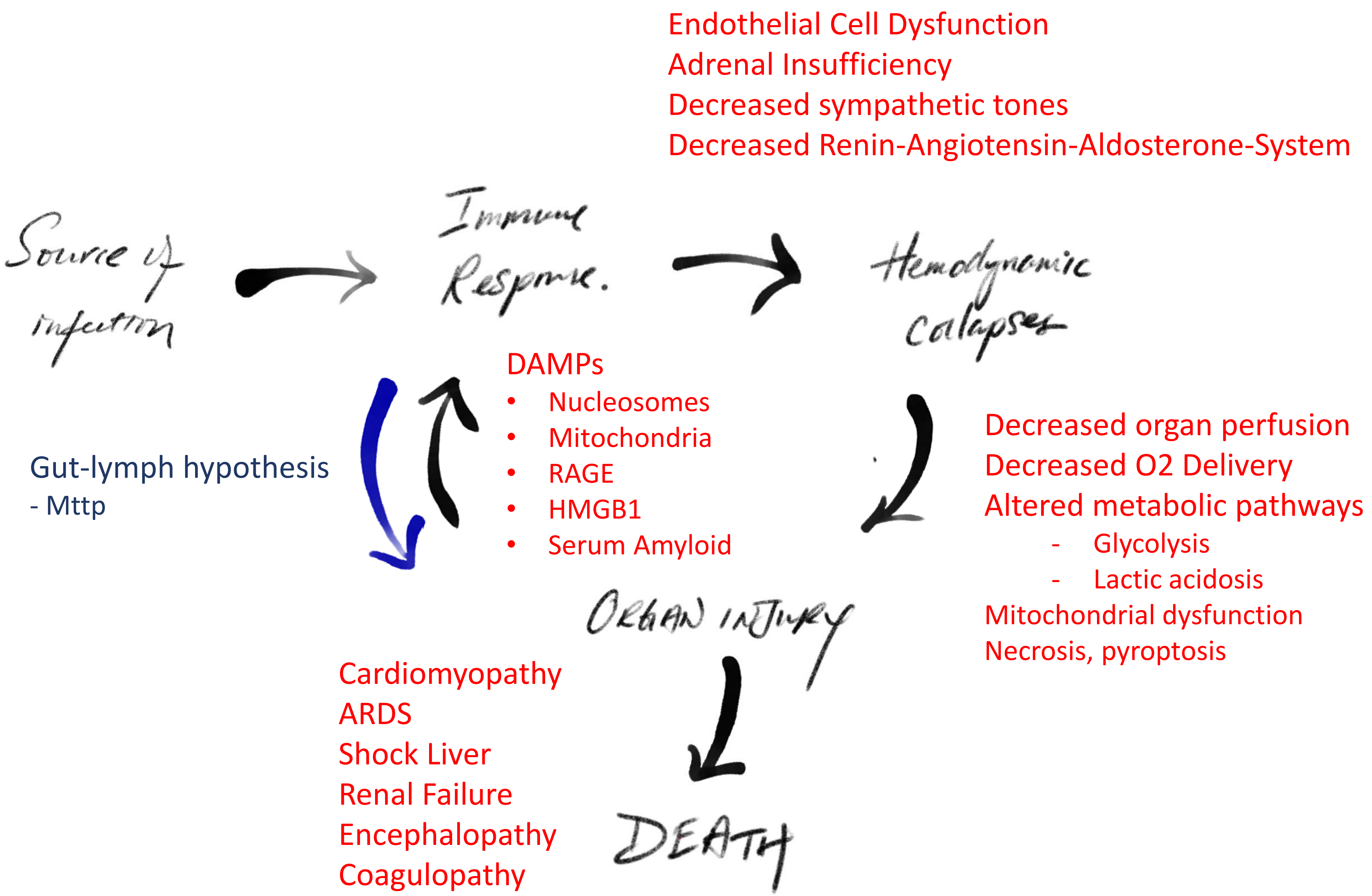
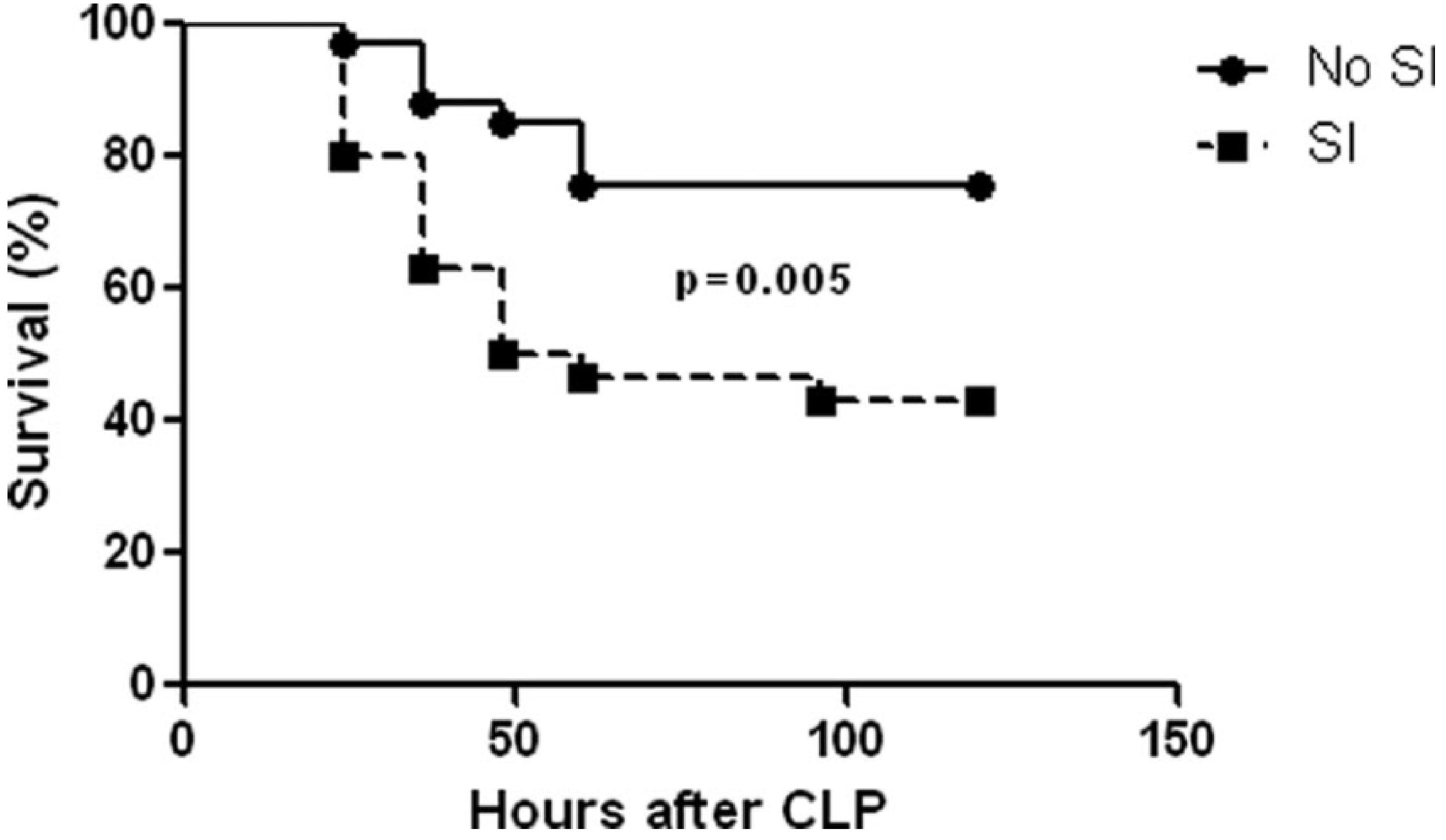

Endothelial Cell Dysfunction
Adrenal Insufficiency
Decreased sympathetic tones
Decreased Renin-Angiotensin-Aldosterone-System
Source of infection
Immune Response.
Hemodynamic calapses
DAMPs
Nucleosomes
Mitochondria
RAGE
HMGB1
Serum Amyloid
Gut-lymph hypothesis
- Mttp
Decreased organ perfusion
Decreased O2 Delivery
Altered metabolic pathways
- Glycolysis
- Lactic acidosis
Mitochondrial dysfunction
Necrosis, pyroptosis
ORGAN INJURY
Cardiomyopathy
ARDS
Shock Liver
Renal Failure
Encephalopathy
Coagulopathy
DEATH

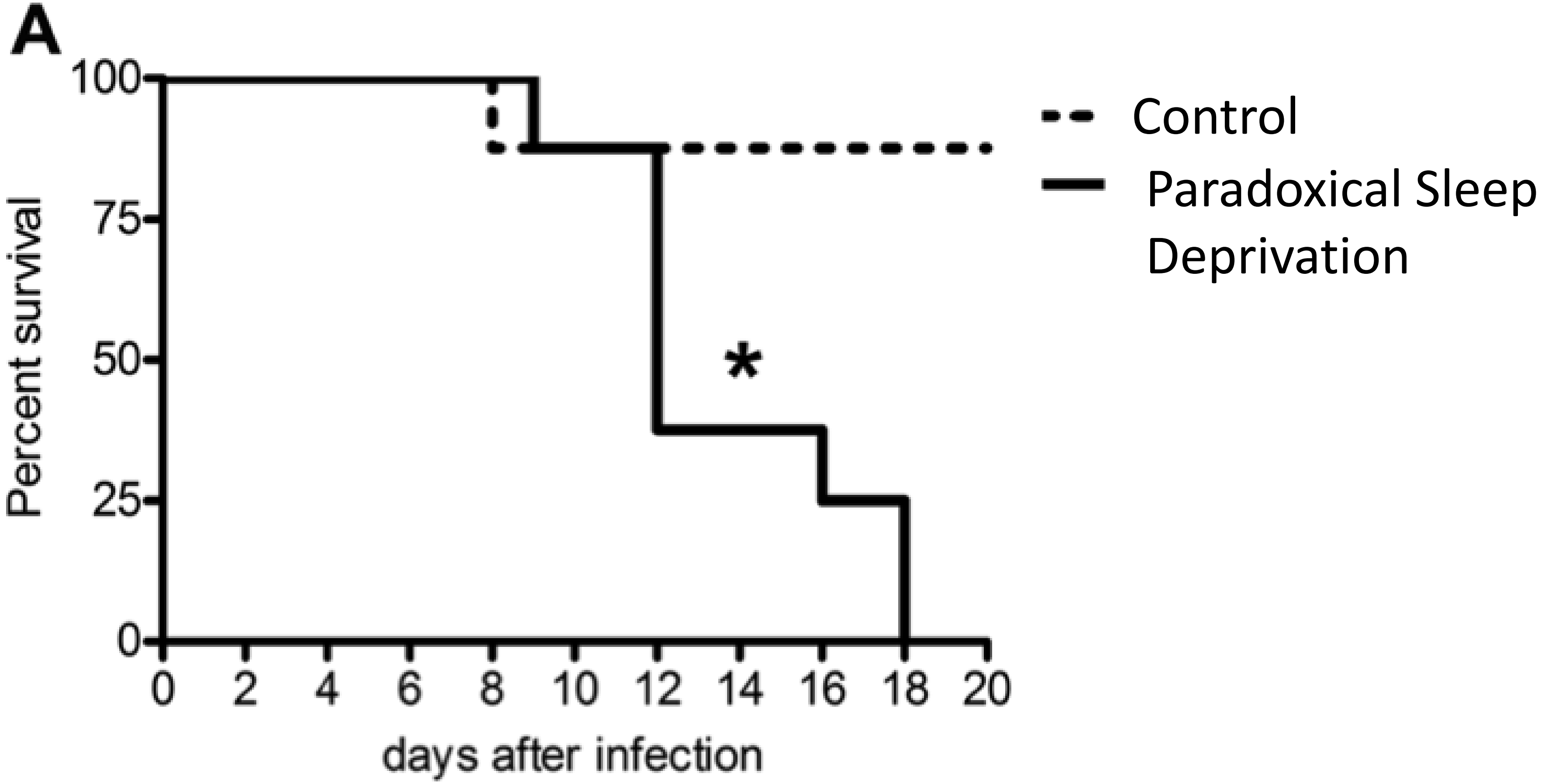
Is Sleep Fragmentation Good or Bad for Sepsis Outcome?

Sleep interruption after cecal ligation puncture results in higher mortality in mice



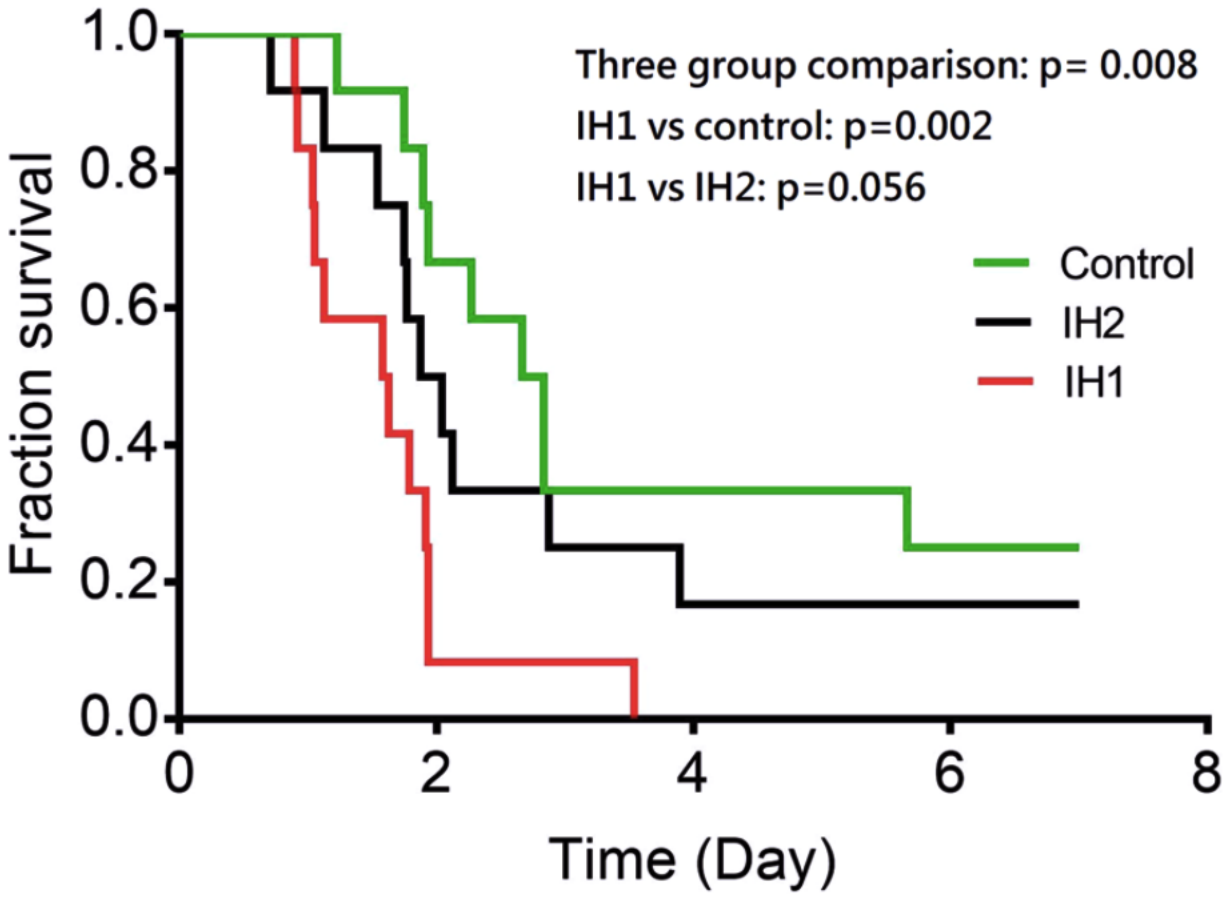
Friese, R. S., Bruns, B., & Sinton, C. M. (2009). Sleep Deprivation After Septic Insult Increases Mortality Independent of Age. *The Journal of Trauma: Injury, Infection, and Critical Care*, *66*(1), 50–54. http://doi.org/10.1097/TA.0b013e318190c3a1

REM sleep deprivation prior to inoculation of malaria results in higher mortality in mice

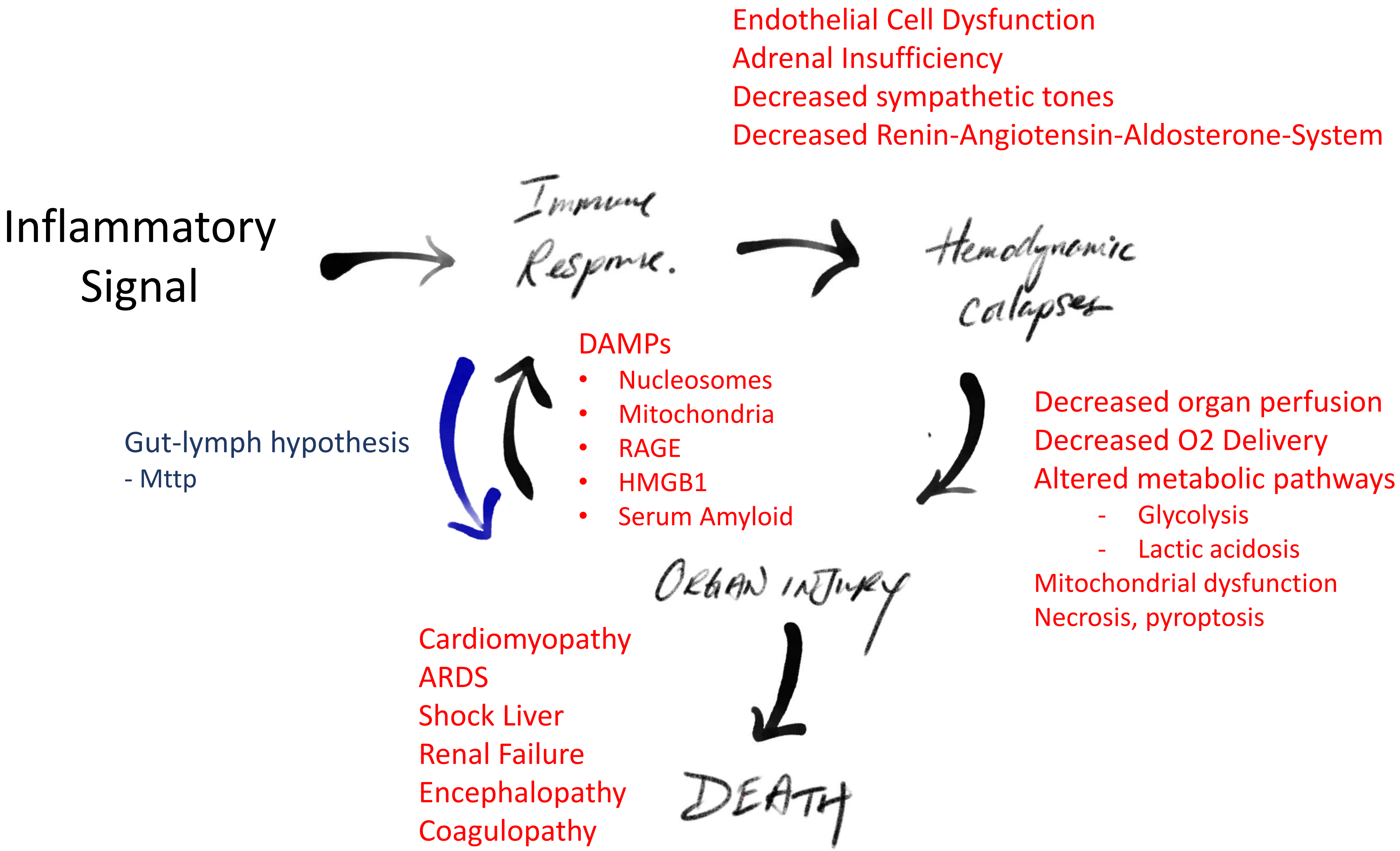


Lungato, L., Gazarini, M. L., Paredes-Gamero, E. J., Tufik, S., & D'Almeida, V. (2015). Paradoxical sleep deprivation impairs mouse survival after infection with malaria parasites. *Sleep Science*, *14*(1), 1–7. http://doi.org/10.1186/s12936-015-0690-7

Intermittent hypoxia prior to cecal ligation puncture also decreases survival of mice from bacterial peritonitis



Chou, K.-T., Cheng, S.-C., Huang, S.-F., Perng, D.-W., Chang, S.-C., Chen, Y.-M., et al. (2019). Impact of Intermittent Hypoxia on Sepsis Outcomes in a Murine Model. *Scientific Reports*, *9*(1), 1–8. http://doi.org/10.1038/s41598-019-49381-w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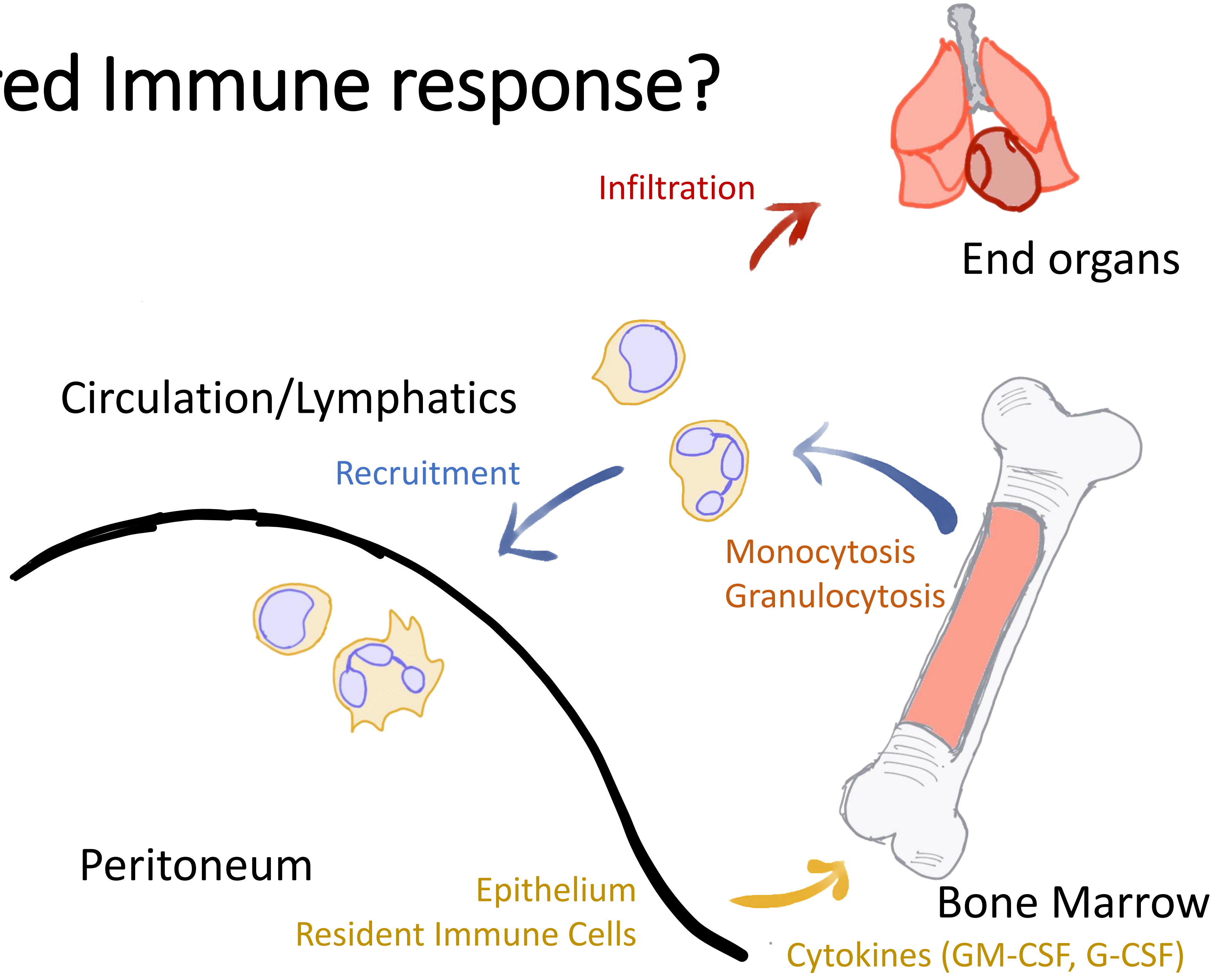
The Question: Impaired Immune response?

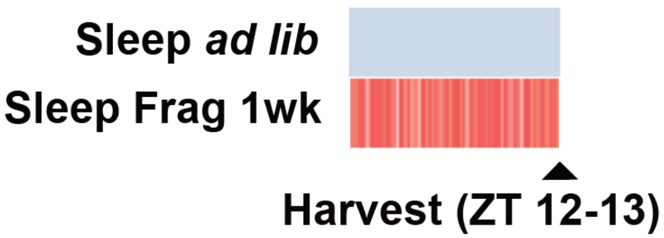
"Differentials hypotheses"

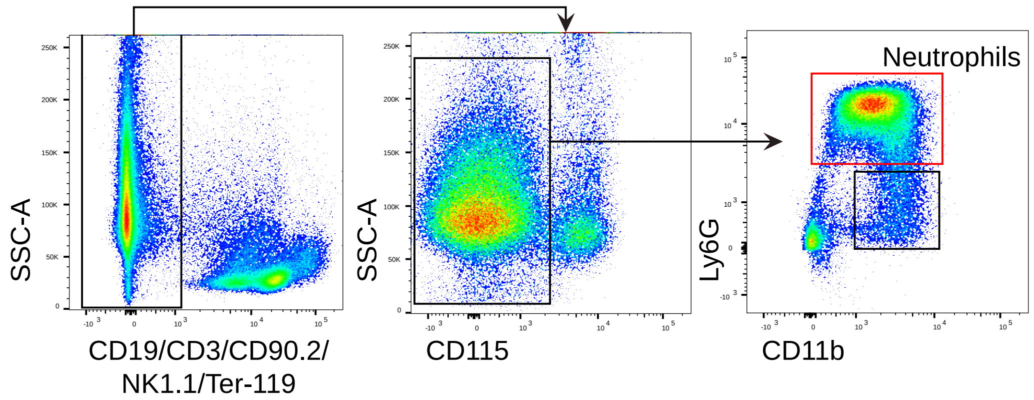
Impaired host immune responses:

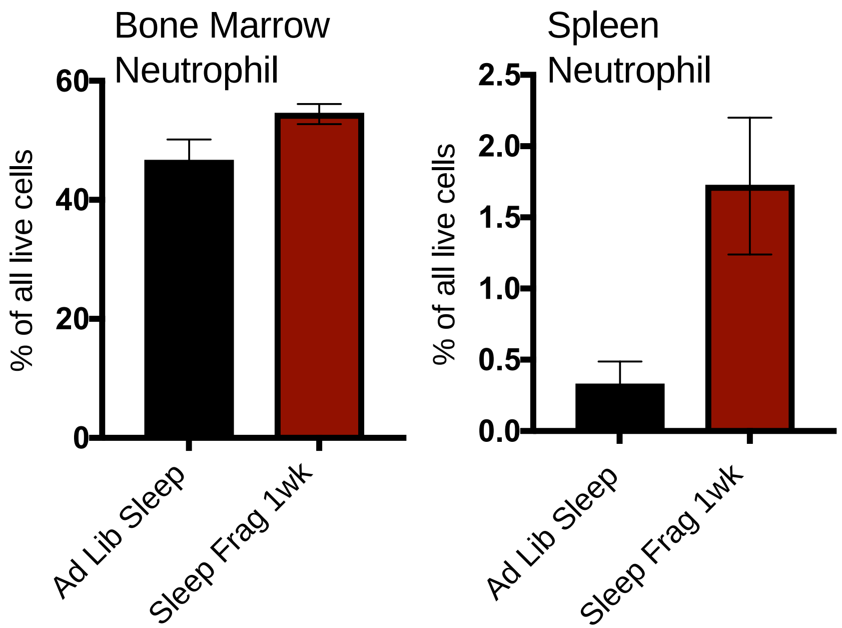
1) Defective Neutrophils recruitment (extravasation)
2) Defective granulopoiesis and monocytosis
3) Defective "aging" of neutrophils
4) Defective TLR4 signaling from resident immune cells or from peritoneum
5) Defective immune cells signaling, excessive glucocorticoid from sleep fragmentation
6) Immune tolerance



Sleep fragmentation increases number of neutrophils in bone marrow and spleen

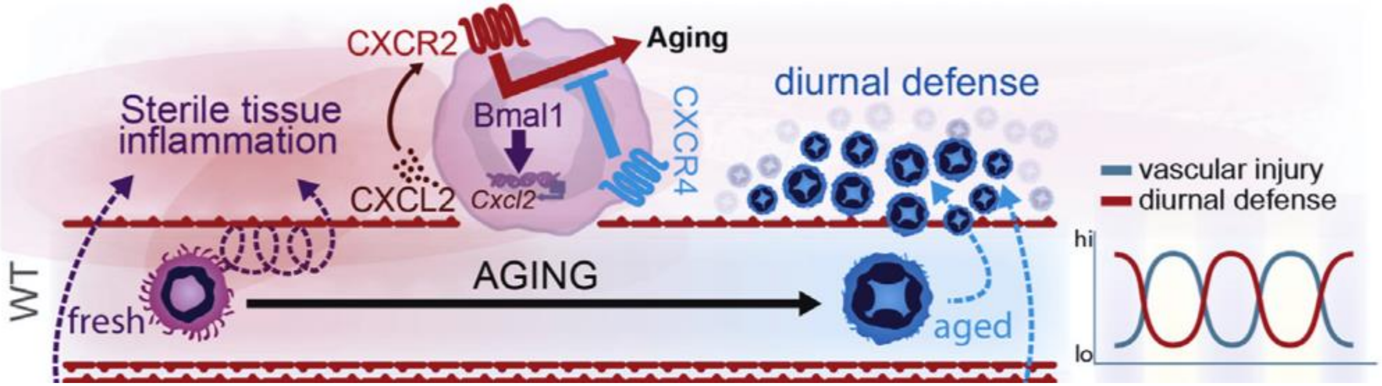






1. Neutrophils are a heterogenous population of cells
2. Cell number is only a part of the picture
3. Phenotyping with activation markers can be helpful

Specific Aim 1



FLOW MARKERS

"Fresh"	"Aged"
$Cxcr2^{Hi}$, $Cxcr4^{Lo}$, $CD62L^{Hi}$	$Cxcr2^{Lo}$, $Cxcr4^{Hi}$, $CD62L^{Lo}$

Sleep promotes maturation of $Cxcr2^{lo}$, $Cxcr4^{Hi}$, $CD62L^{lo}$ neutrophils. Sleep fragmentation reduce this population of neutrophils, resulting in reduced Neutrophil infiltration in the primary infections sites.

Adrover et al. (2019). *Immunity*, *50*(2), 390–402.e10.

The Approach

“Differentials hypotheses”

Impaired host immune responses:

1) Defective Neutrophils recruitment (extravasation)
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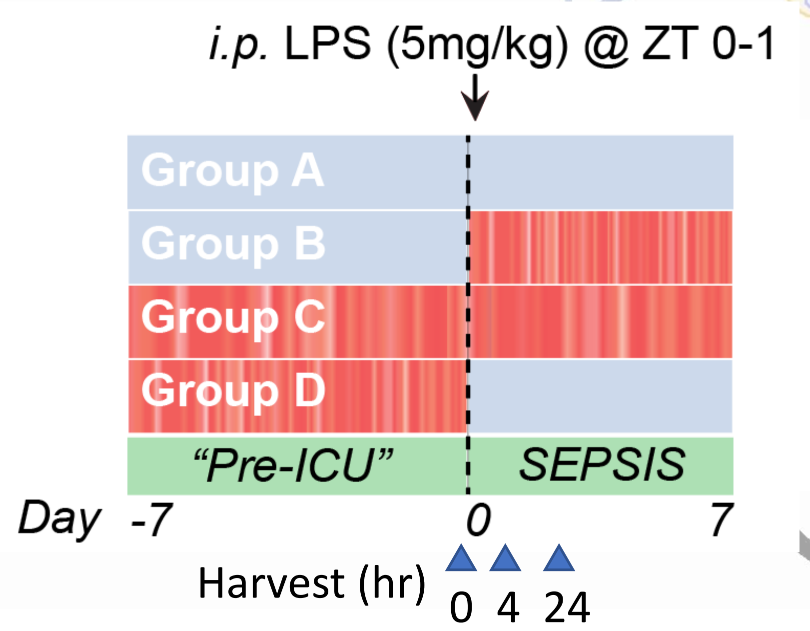
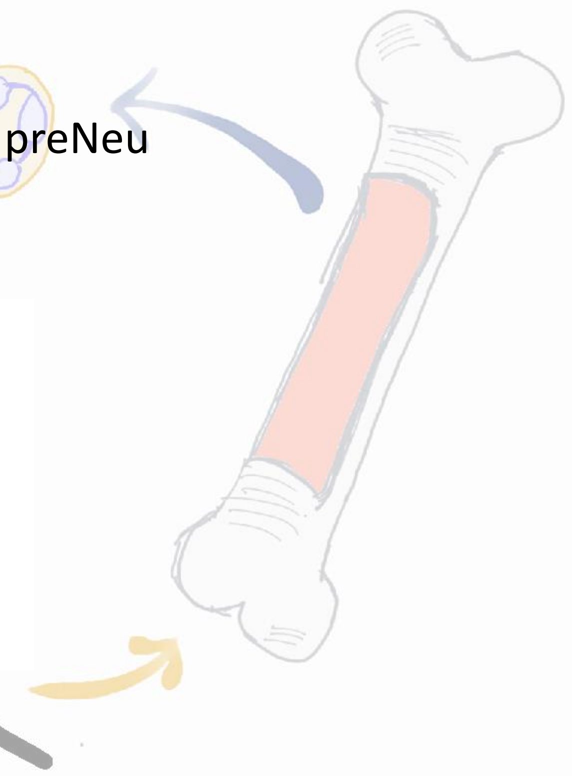
Immunophenotyping by flow cytometry

- Cell counts
  - Peritoneum
  - Circulations
  - Bone Marrow
  - End organs
- Cell types:
  - Monocytes
  - Neutrophils
    - ”fresh”: Cxcr2^{Hi}, Cxcr4^{Lo}, CD62L^{Hi}
    - “aged”: Cxcr2^{Lo}, Cxcr4^{Hi}, CD62L^{lo}
    - Bone marrow – mature, immature, preNeu

	“Fresh” Neutrophils	“Aged” Neutrophils
Peritoneum	Down	Down
Circulation	Up	Down
Bone Marrow	Up	Down
End Organs	Down	Down





Dissecting the molecular mechanisms of sleep-immune interaction

Sleep Perturbation

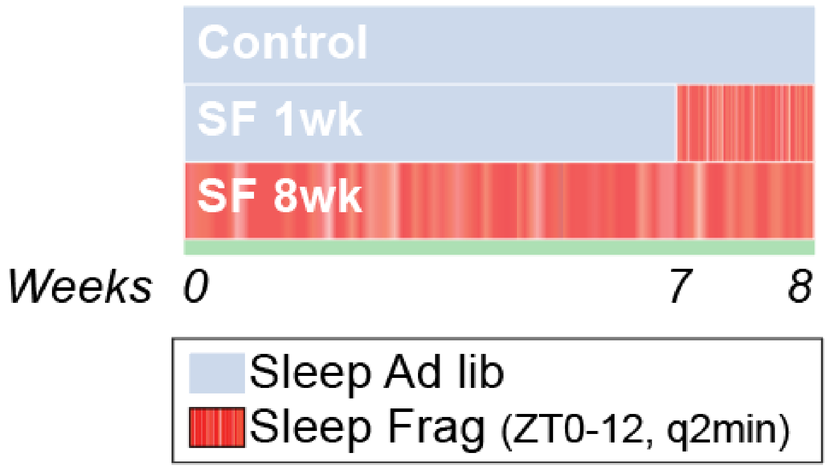
↓

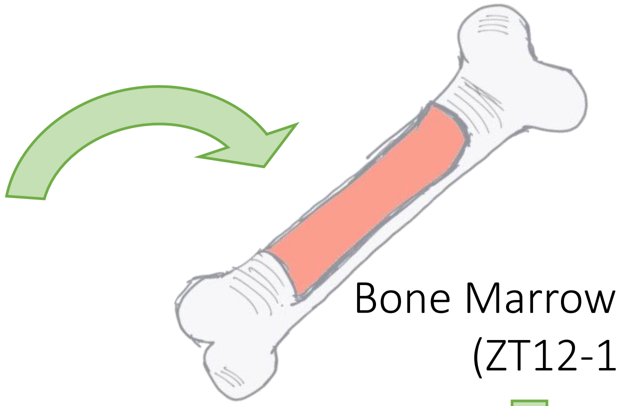
TRANSCRIPTIONAL REGULATION

↓

Immune function adaptation

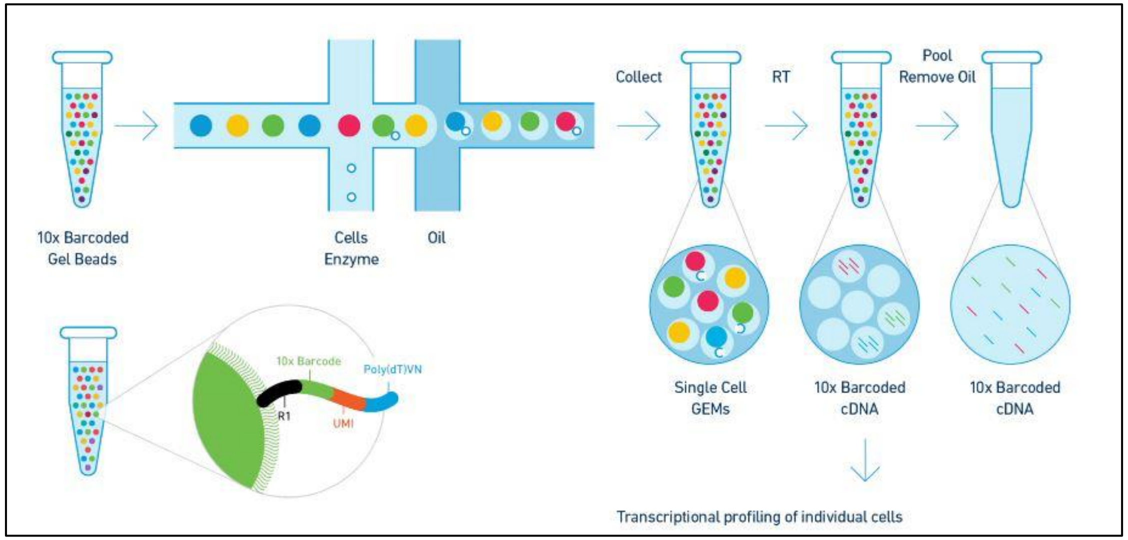
Single cell transcriptome profiling from bone marrow of sleep fragmented mice



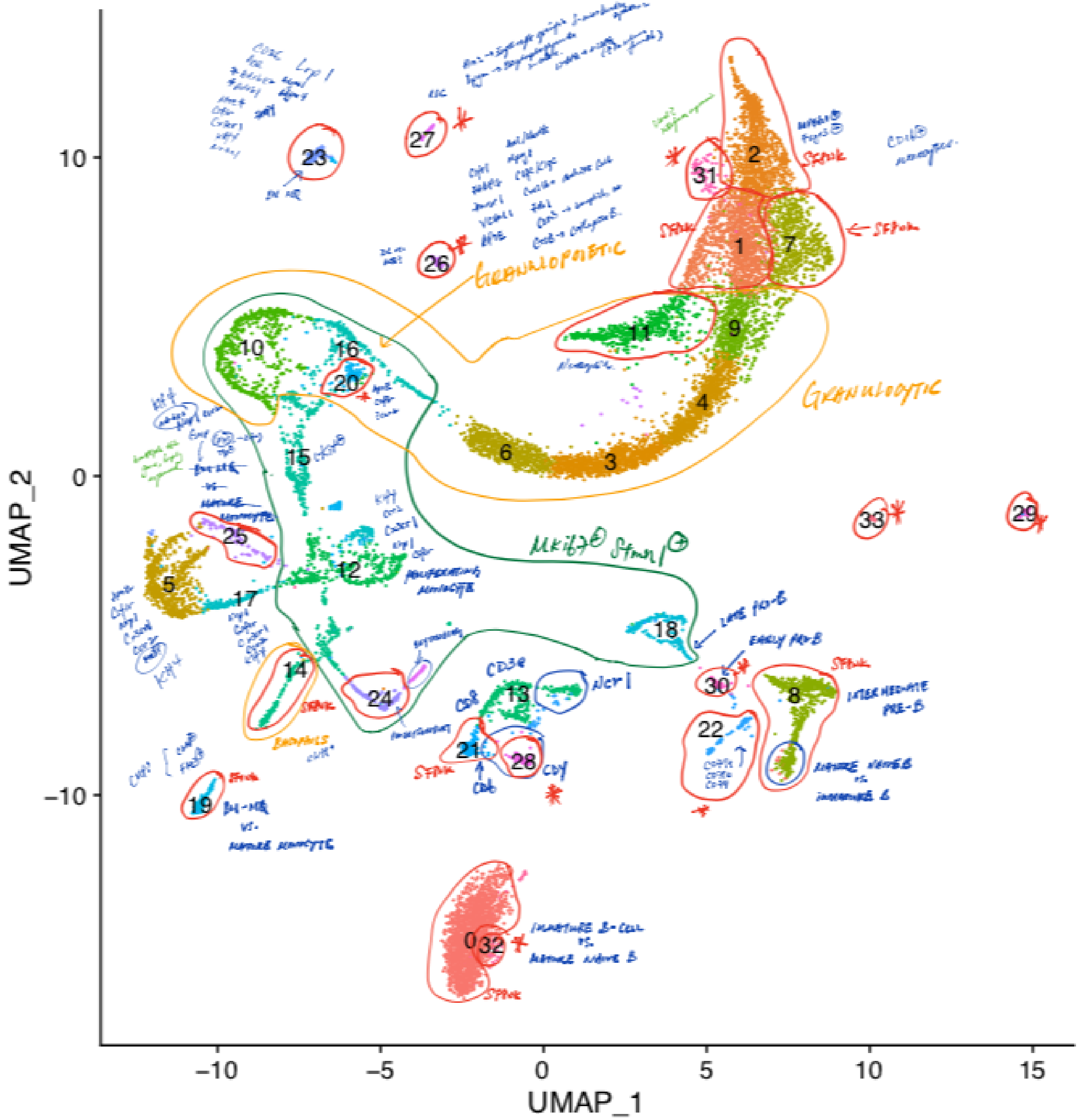


Bone Marrow Harvest
(ZT12-13)

Single Cell RNA sequencing (10X Genomics)

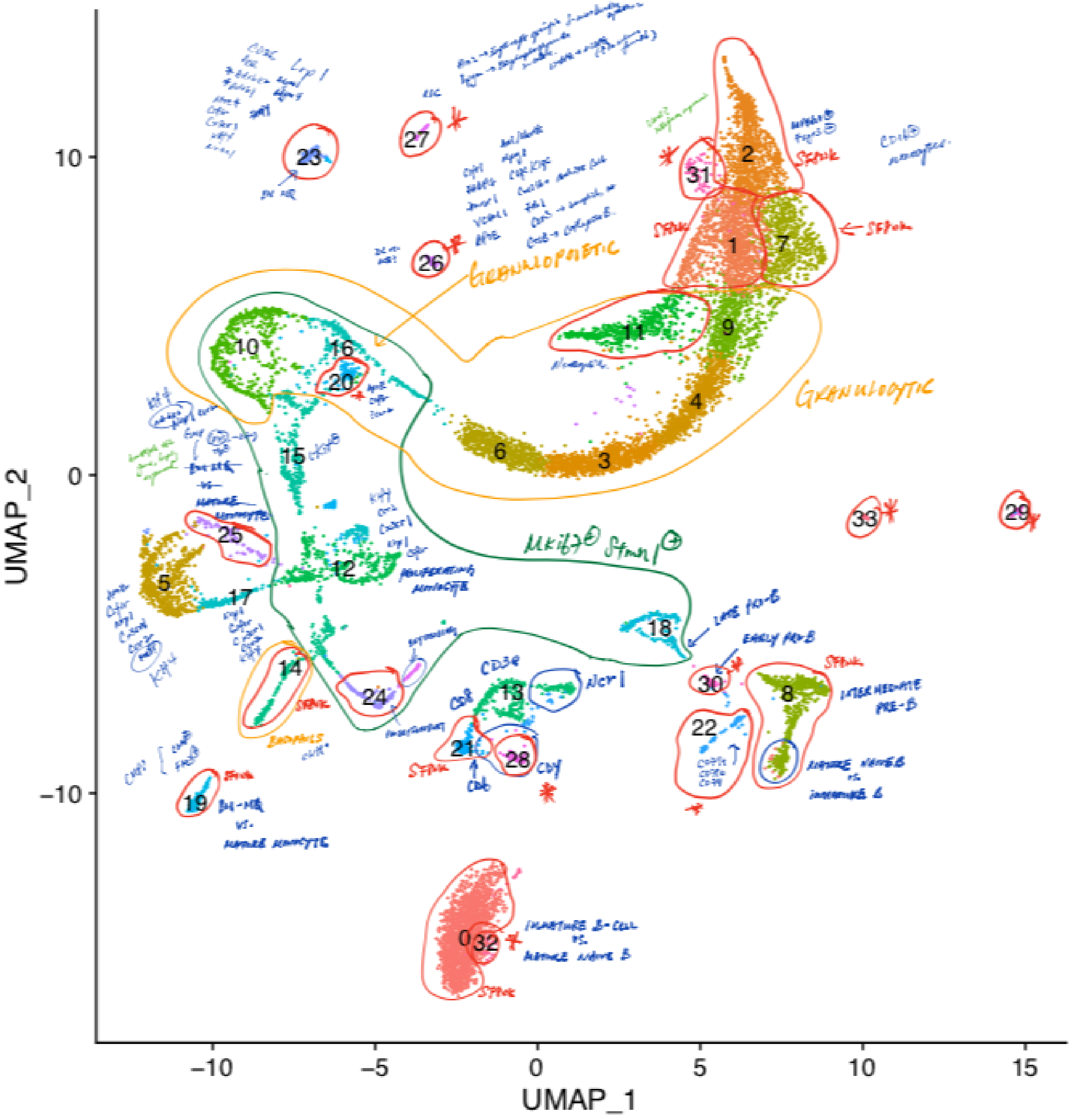


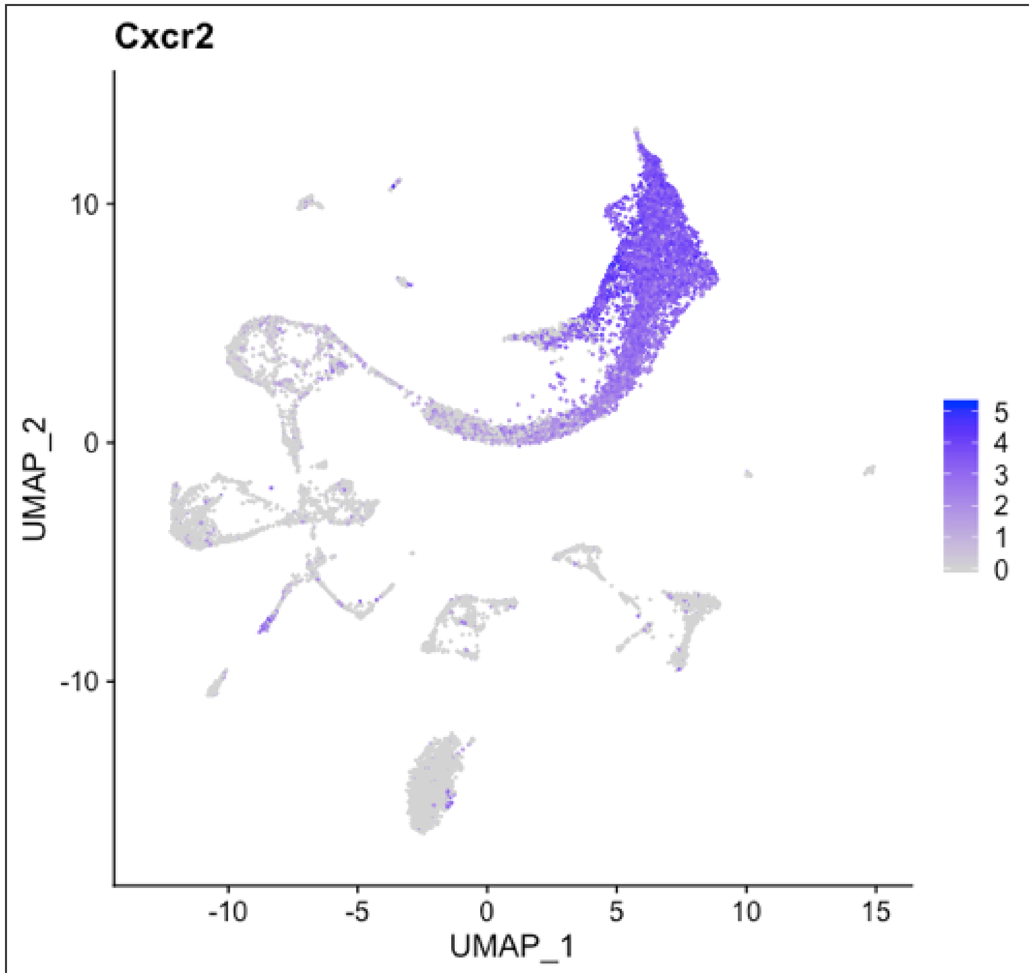
Non-linear dimension reduction for visualization



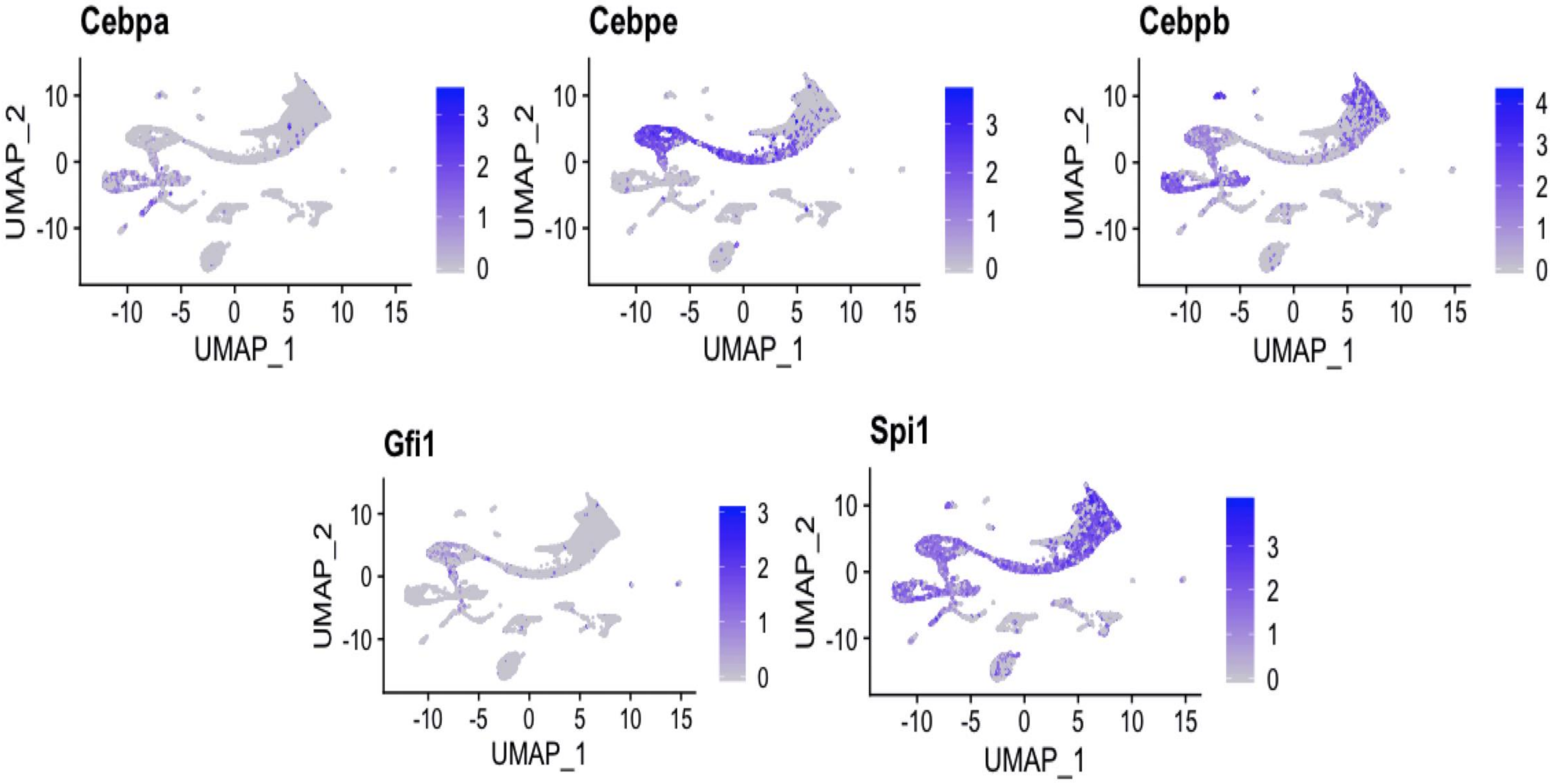
Summary	
Samples #	3
Cell #	13208
RNA features #	15381
Clusters #	34

Exp arms	# cells
Ctrl	4096
SF 1 week	4133
SF 8 weeks	4979

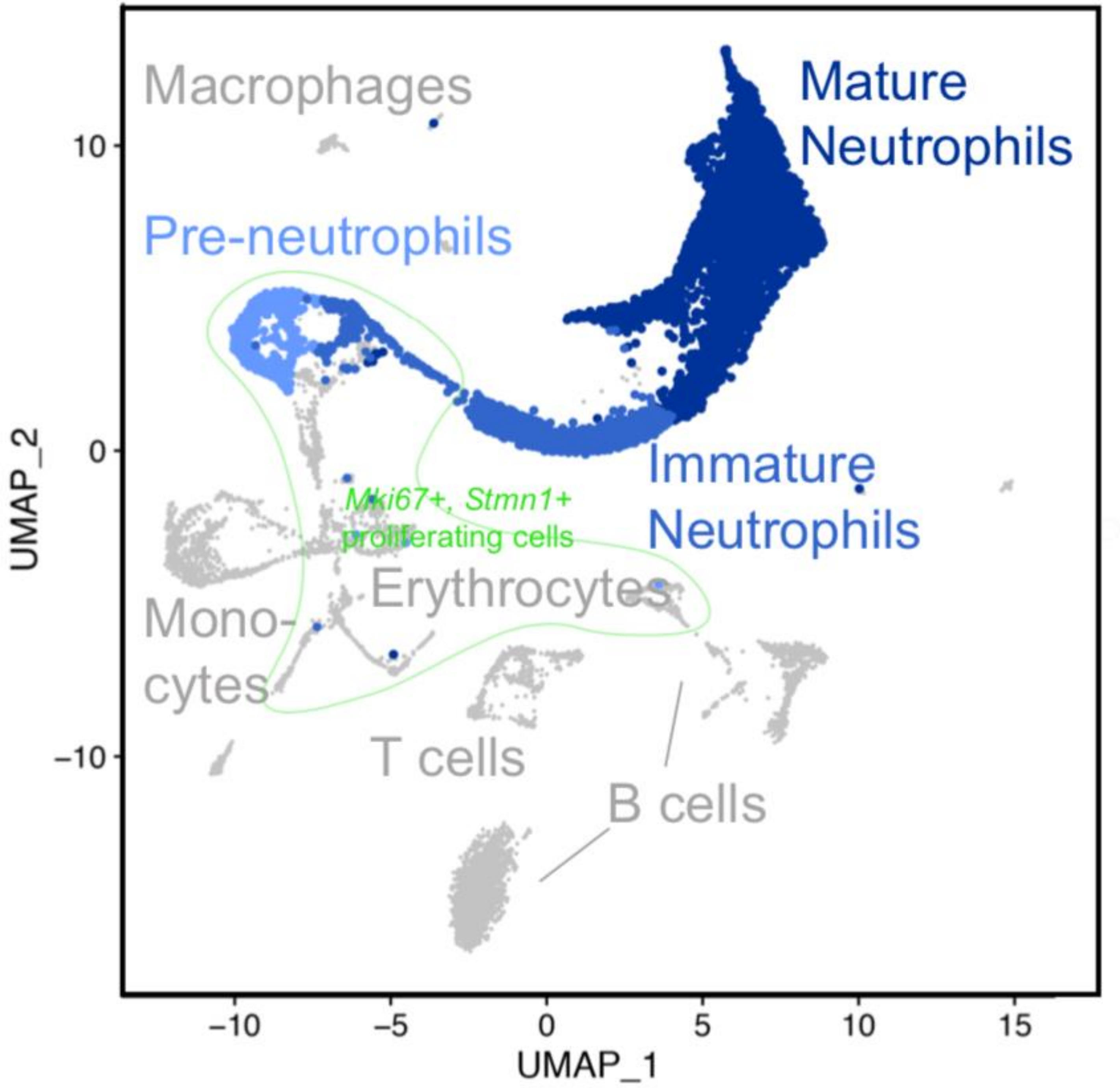

UMAP_1
UMAP_2
GRANULOPOIETIC
GRANULOCYTIC
EARLY PRE-B
INTERMEDIATE PRE-B
IMMATURE B-CELL vs. MATURE NAIVE B
CD3e
CD8
CD4
Ncr1


Cxcr2
UMAP_1
UMAP_2

Progenitor/monocytes   Immature/preNeu   Mature Neu/monocytes

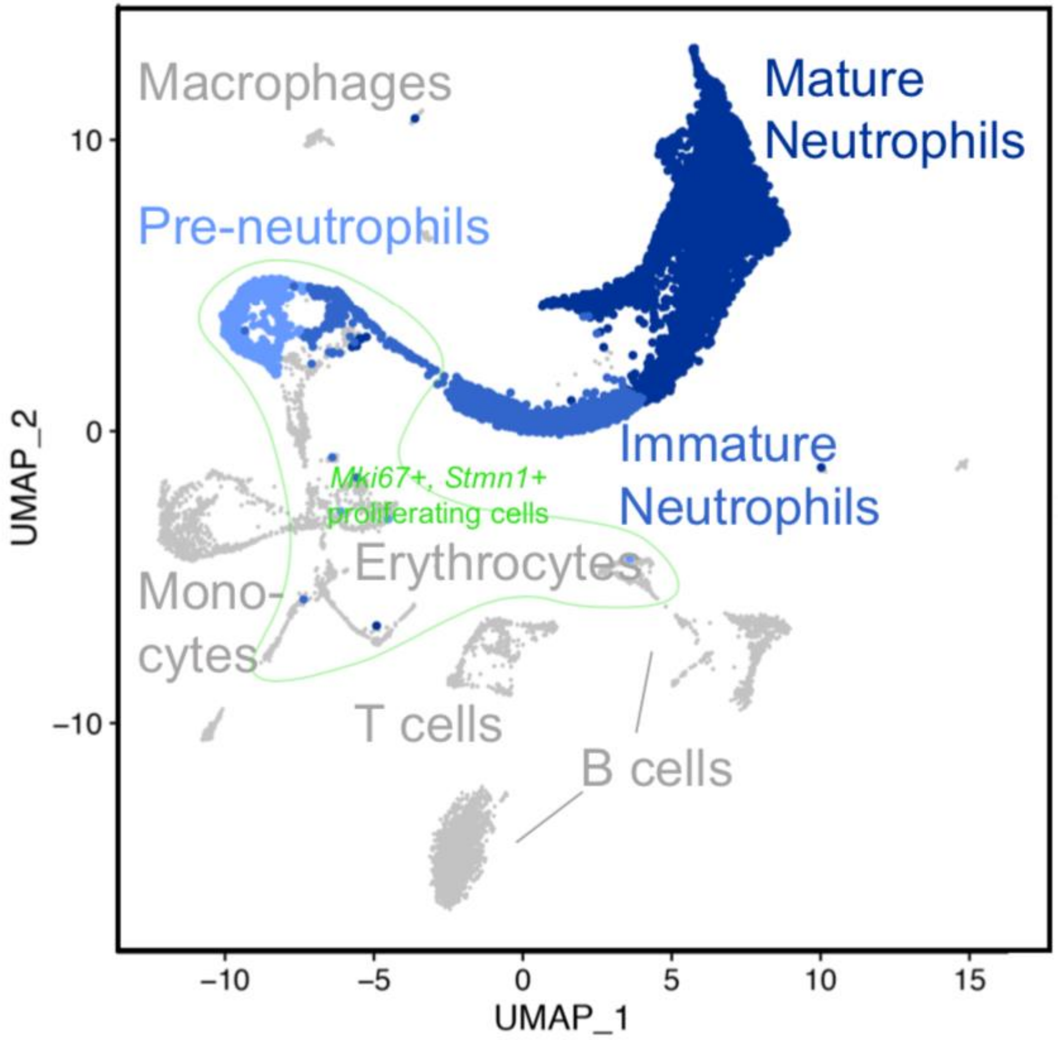


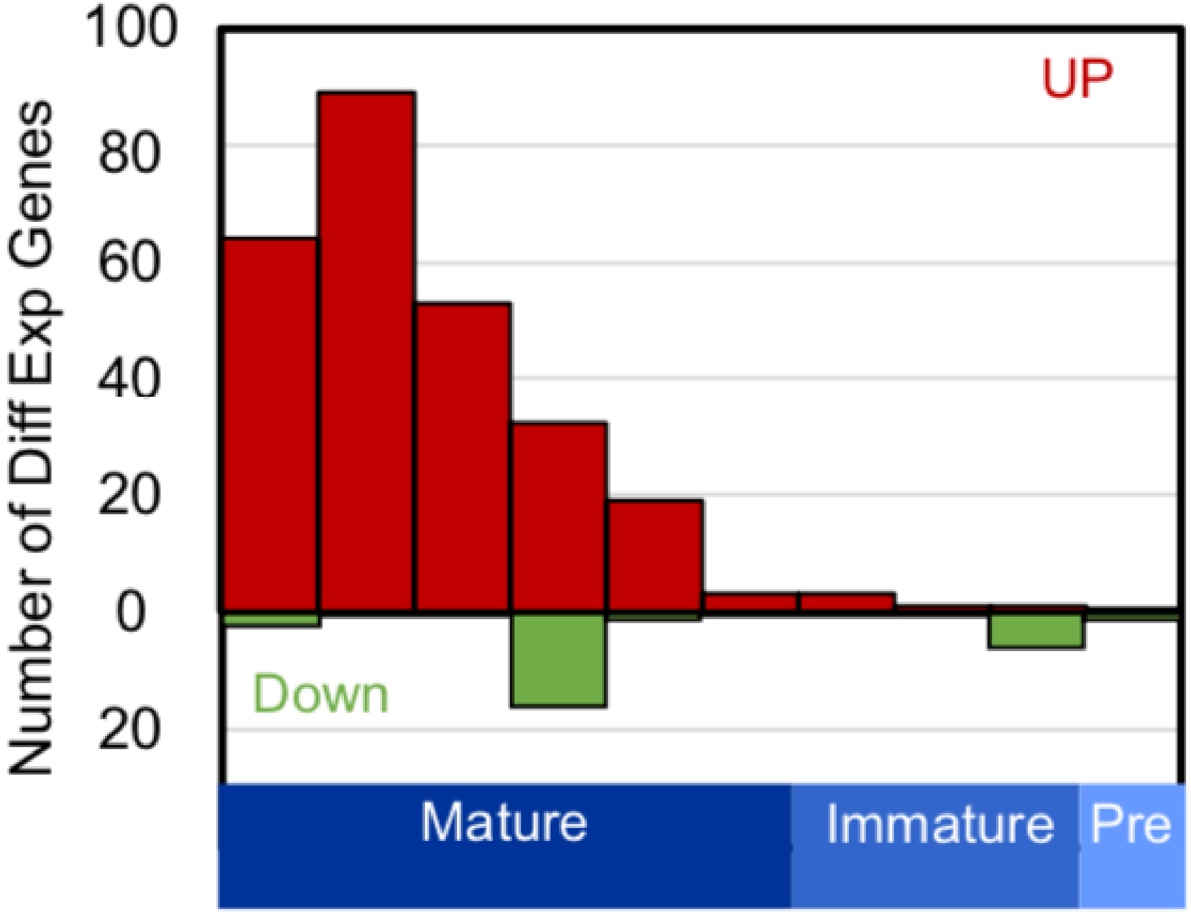
Granulocytes progenitor   Neu/Mono/B-cells



Macrophages
Mature
Neutrophils
Pre-neutrophils
Immature
Neutrophils
Mki67+, Stmn1+
proliferating cells
Erythrocytes
Mono-
cytes
T cells
B cells
UMAP_2
UMAP_1
10
0
−10
−10
−5
0
5
10
15

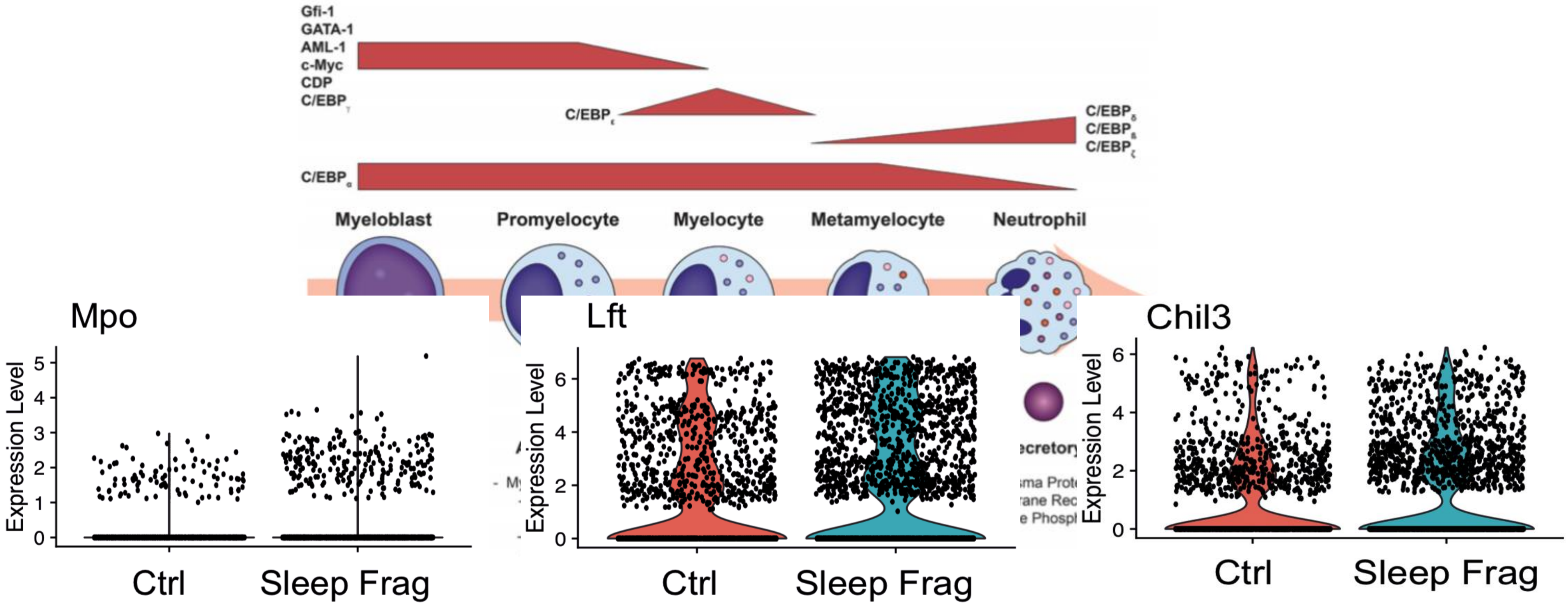
Mature Neutrophils have the highest number of differentially expressed genes after chronic sleep fragmentation





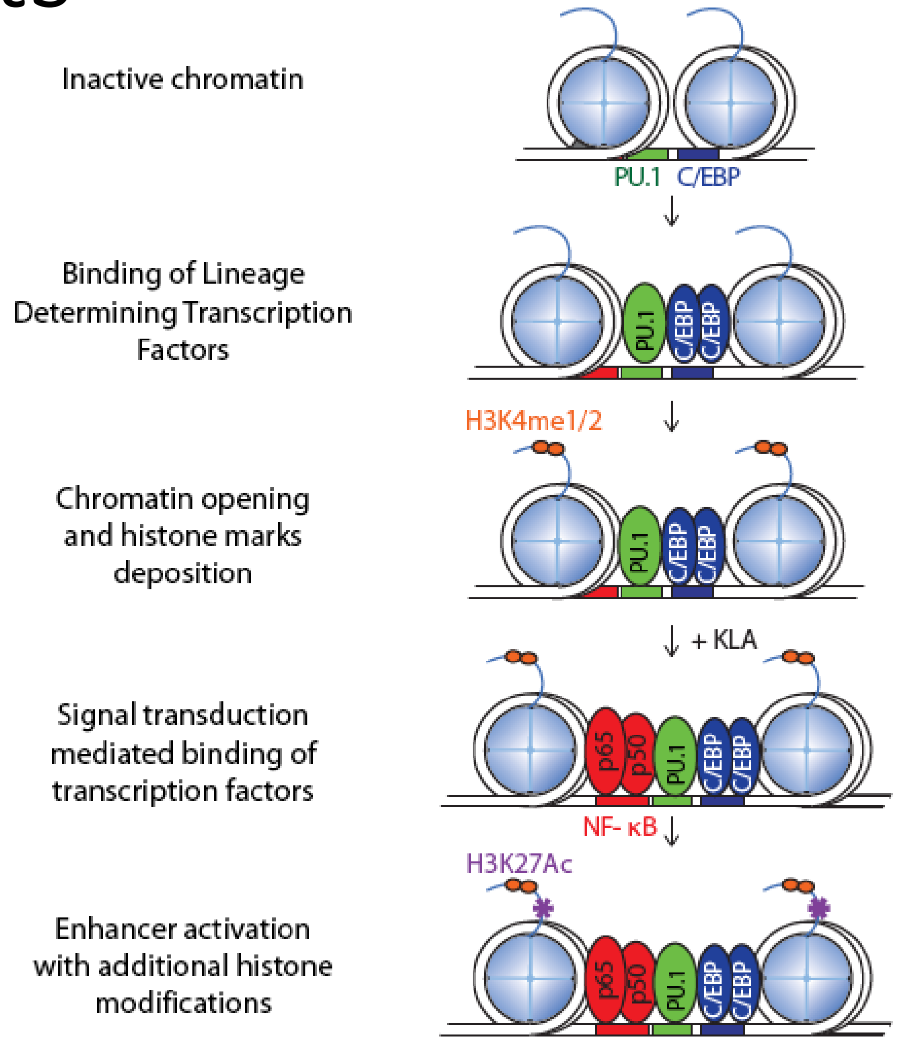
Gene Ontology from differentially expressed genes in mature neutrophils after sleep fragmentation

Description	q-value
Neutrophil degranulation	4.13E-10
Symbiont process	1.21E-05
Mitotic cell cycle process	2.03E-05
Myeloid cell differentiation	1.92E-03
Positive regulation of cytokine production	1.97E-03
Response to unfolded protein	1.97E-03



Gfi-1
GATA-1
AML-1
c-Myc
CDP
C/EBP
C/EBP
C/EBP
C/EBP
C/EBP
C/EBP
Myeloblast
Promyelocyte
Myelocyte
Metamyelocyte
Neutrophil
Mpo
Expression Level
0
1
2
3
4
5
Ctrl
Sleep Frag
Lft
Expression Level
0
2
4
6
Ctrl
Sleep Frag
Chil3
Expression Level
0
2
4
6
Ctrl
Sleep Frag

Step-wise establishment of regulatory elements



DNAse-I Hypersensitivity, FAIREseq, ATAC-seq

Lineage Determining Transcription Factor ChIPseq

Histone ChIPseq

Signal Dependent Transcription Factor ChIPseq

Active histone mark ChIPseq

The Question – what drives the changes in gene expression?

DEG in Mature Neutrophils from Sleep Fragmented Mice

↓

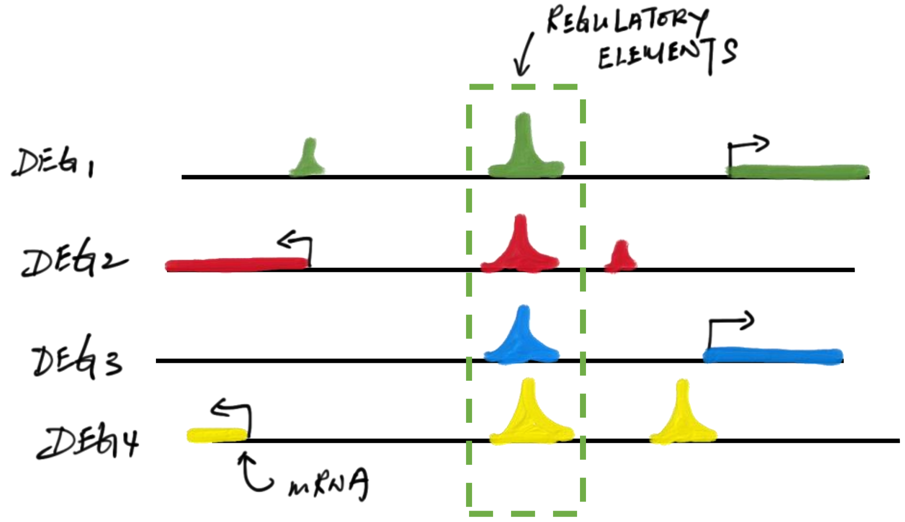
Map to nearest regulatory elements based on publicly available data set

↓

ATAC-seq from Neutrophils
Lavin et al (2014). *Cell* 159: 1312-1326

↓

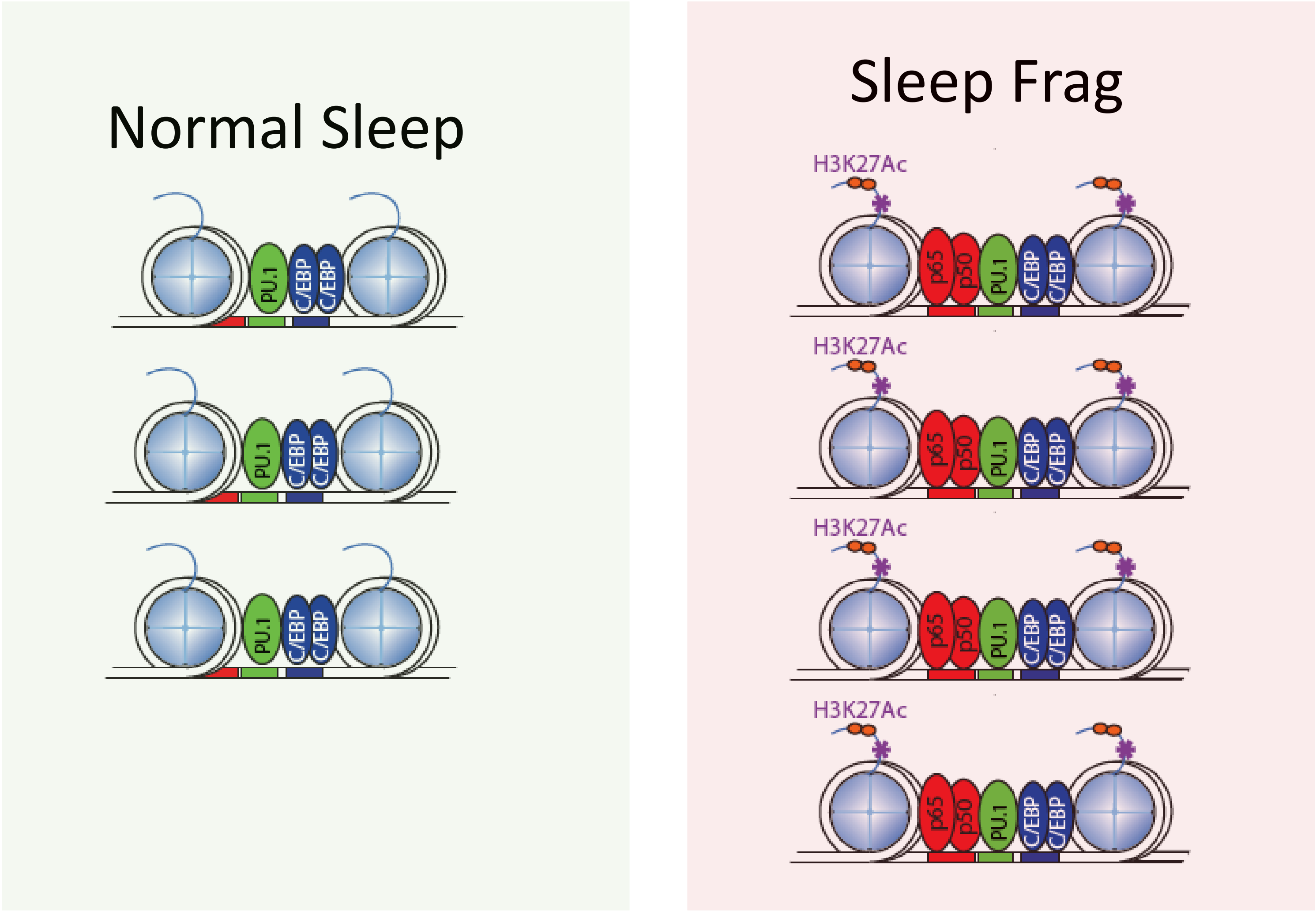
Transcription Factor Motif Analysis



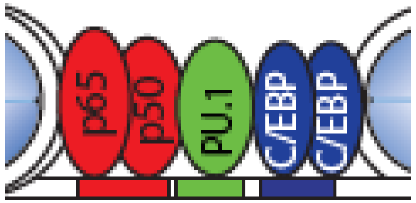
Bmal1 P-value = 1×10^{-6}

CAVEATS
Only looking at nearest opened chromatin regions
NO information about ACTIVITY of the regulatory elements
Regulatory elements with changes are NOT necessary NEAREST to changes in mRNA genes
Different experiment conditions

Specific Aim 2: Sleep fragmentation altered the cistrome and transcriptome of mature neutrophils during septic challenge by modulating the molecular clock Bmal1



Transcription Factors responsible for transcriptional regulation during sleep perturbation



The Approach

"Differentials hypotheses"

Impaired host immune responses:

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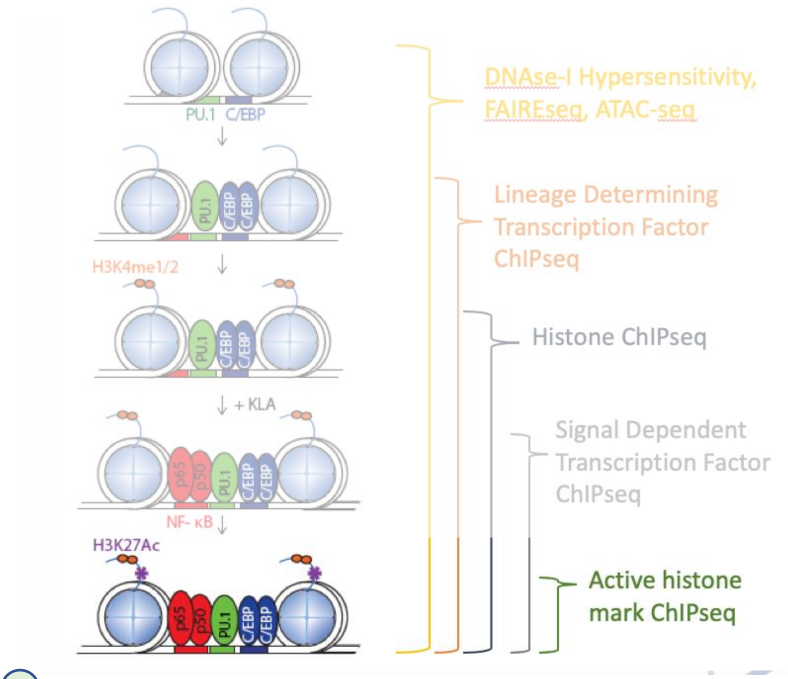

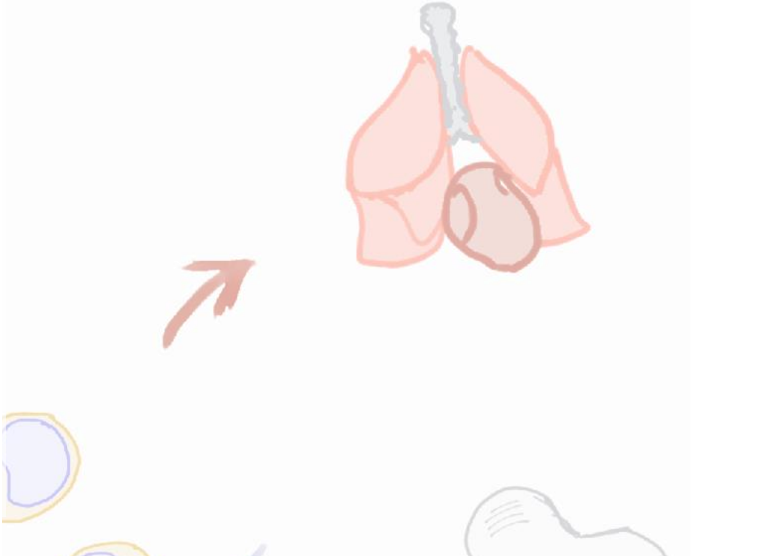

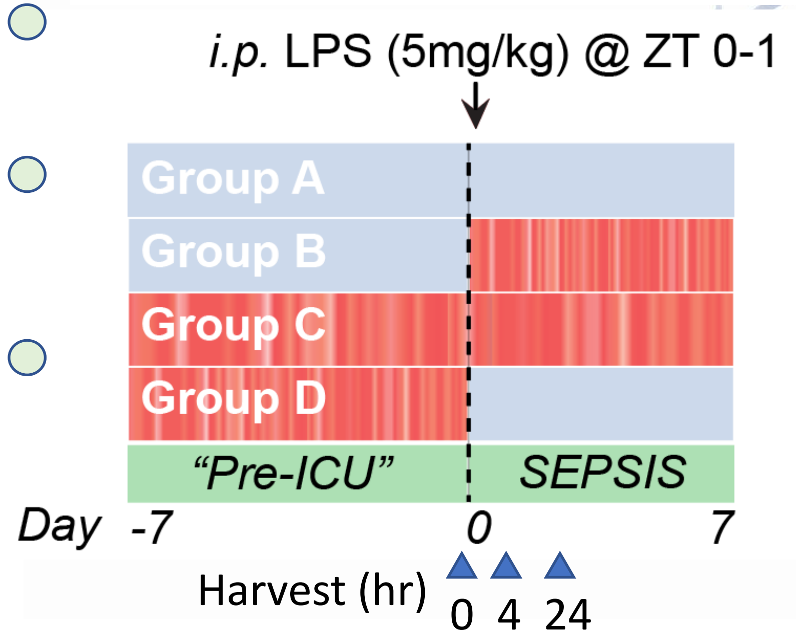

Method:
H3K27ac CUT and RUN on isolated mature Neutrophils
Determine differentially activated regulatory elements between SF versus Control
Focus on regulatory element with Bmall motifs
Complementary analysis with Total-RNAseq from mature neutrophils
Evaluate Bmall protein level and post-translational modifications
Unbiased de novo motif discovery for other transcription factors

Summary

- Sleep fragmentation prior to LPS challenge improves survival
- Sleep fragmentation increased the number of neutrophils in bone marrow and spleen
- **Specific Aim 1** examines the impact of sleep fragmentation on recruitment of neutrophils to primary site of infection
- Transcriptomes of mature neutrophils were affected after chronic sleep fragmentation
- Bmall motif enriched at nearby regulatory elements of differentially expressed genes
- **Specific Aim 2** examines the effect sleep fragmentation on the cistrome and transcriptome in mature neutrophils with a Bmall-centric perspective

Milestones (2019-2020)

Milestones (2019-2020)
Publications
Sulli, G., Lam, M. T. Y., & Panda, S. (2019). Interplay between Circadian Clock and Cancer: New Frontiers for Cancer Treatment. Trends in Cancer, 5(8), 475–494.
Boddu, S. A., Bojanowski, C. M., Lam, M. T., Advani, I. N., Scholten, E. L., Sun, X., et al. (2019). Use of E-cigarettes with Conventional Tobacco is Associated with Decreased Sleep Quality in Women. American Journal of Respiratory and Critical Care Medicine, rccm.201904–0890LE–10. http://doi.org/10.1164/rccm.201904-0890LE
Manuscripts in preparation
Chapter on Sleep and ICU with Kamdar BB, Malhotra A, and Panda S.
ATS sleep core curriculum: OSA: New Advances in PAP Therapy to Improve Management and Adherence, with Bernie Sunwoo
GRANTS (2019)
Parker B. Francis Fellowship A.P. Giannini Fellowship ALA Catalyst Award

Goals (2020-2021)
Career
Continue T32 with UC San Diego PCCSM Division, 75% research, 25% Pulmonary Clinic
Manuscripts in preparation
Chapter on Sleep and ICU with Kamdar BB, Malhotra A, and Panda S.
ATS sleep core curriculum: OSA: New Advances in PAP Therapy to Improve Management and Adherence, with Bernie Sunwoo
Sleep Fragmentation and Septic Shock (Specific AIM 1) – early 2021
Sleep Fragmentation on cistrome and transcriptome of mature neutrophils (Specific AIM 2) – mid – late 2021
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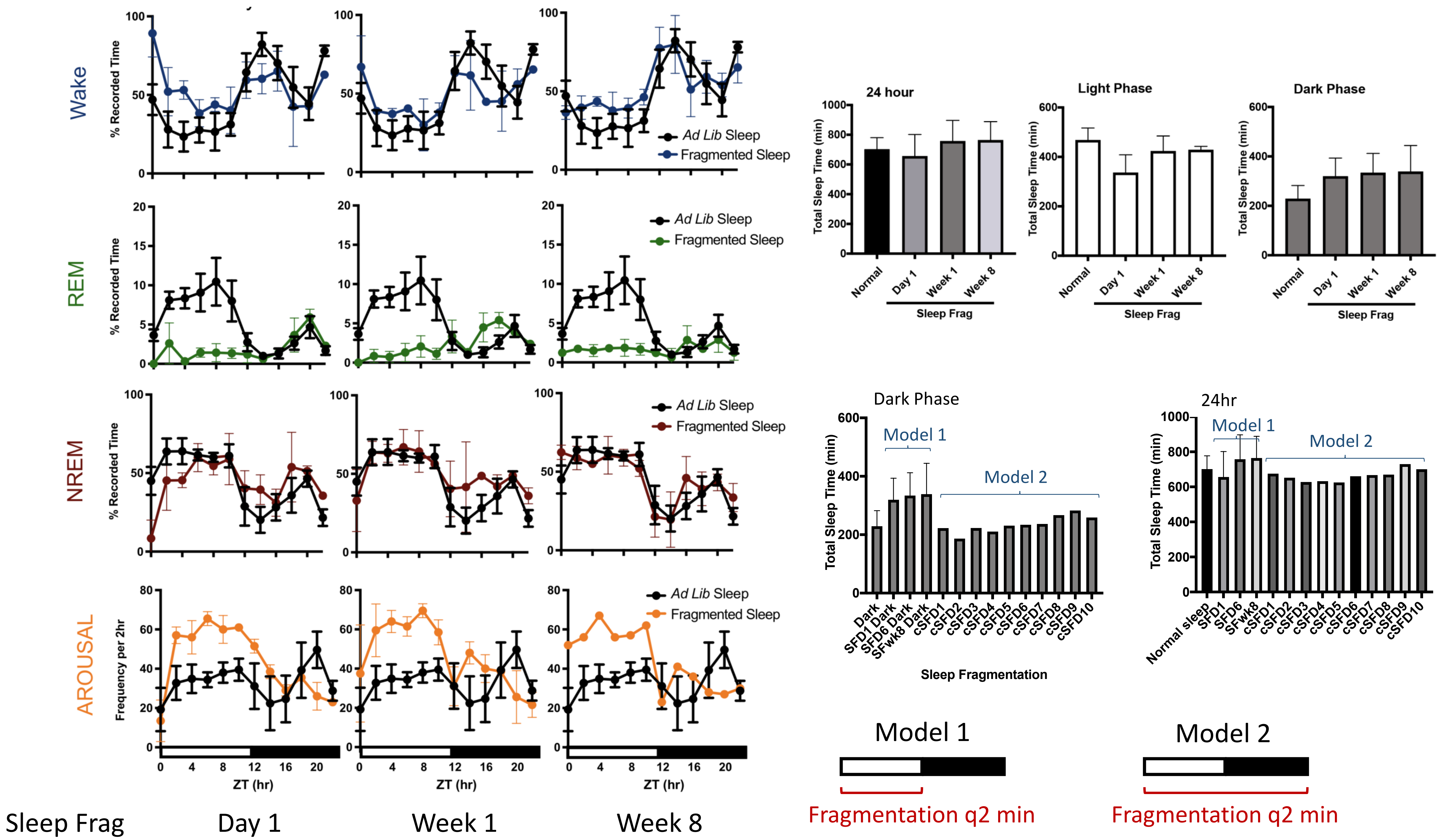
ASPIRE Fellowship

NIH T32 PCCM Fellowship



Wake
% Recorded Time
Ad Lib Sleep
Fragmented Sleep
REM
% Recorded Time
Ad Lib Sleep
Fragmented Sleep
NREM
% Recorded Time
Ad Lib Sleep
Fragmented Sleep
AROUSAL
Frequency per 2hr
Ad Lib Sleep
Fragmented Sleep
ZT (hr)
Sleep Frag
Day 1
Week 1
Week 8
24 hour
Light Phase
Dark Phase
Total Sleep Time (min)
Normal
Day 1
Week 1
Week 8
Sleep Frag
Dark Phase
Model 1
Model 2
Dark
SFD1 Dark
SFD6 Dark
SFwk8 Dark
cSFD1
cSFD2
cSFD3
cSFD4
cSFD5
cSFD6
cSFD7
cSFD8
cSFD9
cSFD10
Sleep Fragmentation
24hr
Normal sleep
SFD1
SFD6
SFwk8
Model 1
Model 2
Fragmentation q2 min
Fragmentation q2 min