Signatures in Blood
Senescence, Exosomes and Aging Markers

S.K. Patel, C.D. King, N. Basisty, J. Rose, S. Shah, M. Chamoli, R. Beres, E. Baker, Judith Campisi, Birgit Schilling

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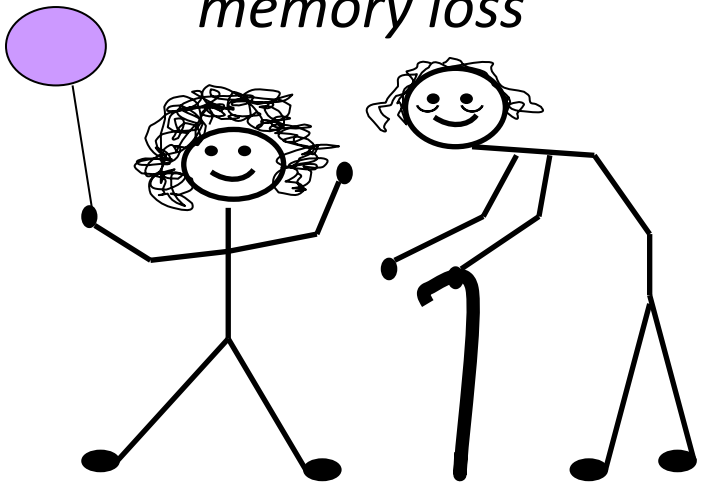
RCCN

Live better longer

Aging = susceptibility to (chronic) disease

not a coincidence! caused by basic aging process(es)

Neurodegeneration,
memory loss



Osteoporosis

Macular degeneration,
hearing loss

Heart disease

Vascular disease

Sarcopenia,
frailty

Diabetes,
metabolic syndrome

Decreased
lung, kidney, etc function

CANCER

Cellular senescence: a candidate basic aging process

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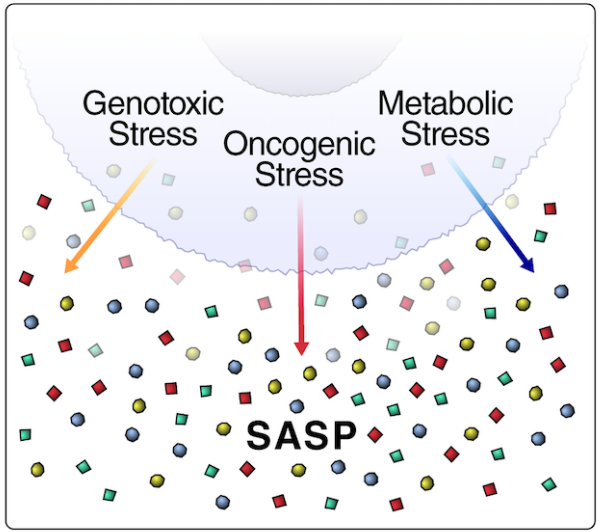
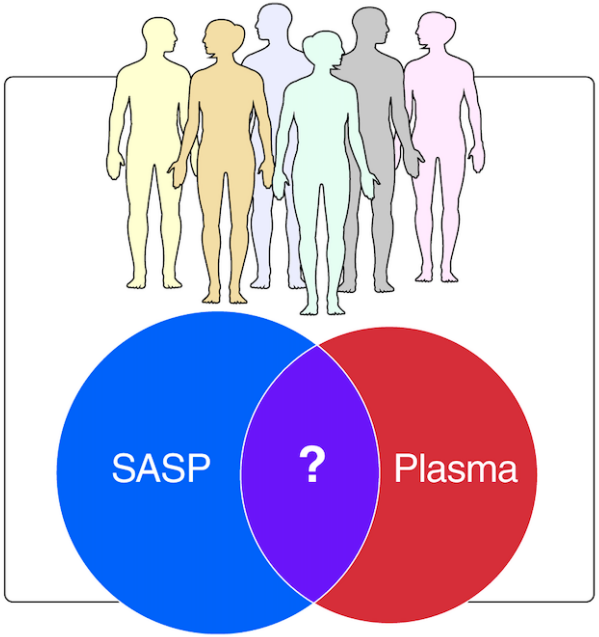
Senescence-derived Biomarkers of Aging



Phase 1: **Discovery**

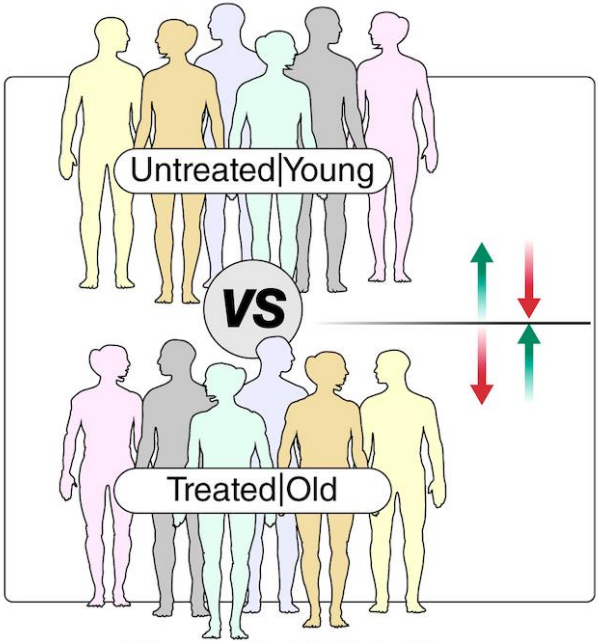
Which proteins are secreted?

Cell Culture Experiments



Phase 2: **Verification**

Which SASP proteins can be detected in plasma?



Phase 3: **Validation**

Are biomarkers elevated with age/disease?
Are biomarkers reduced with treatments?

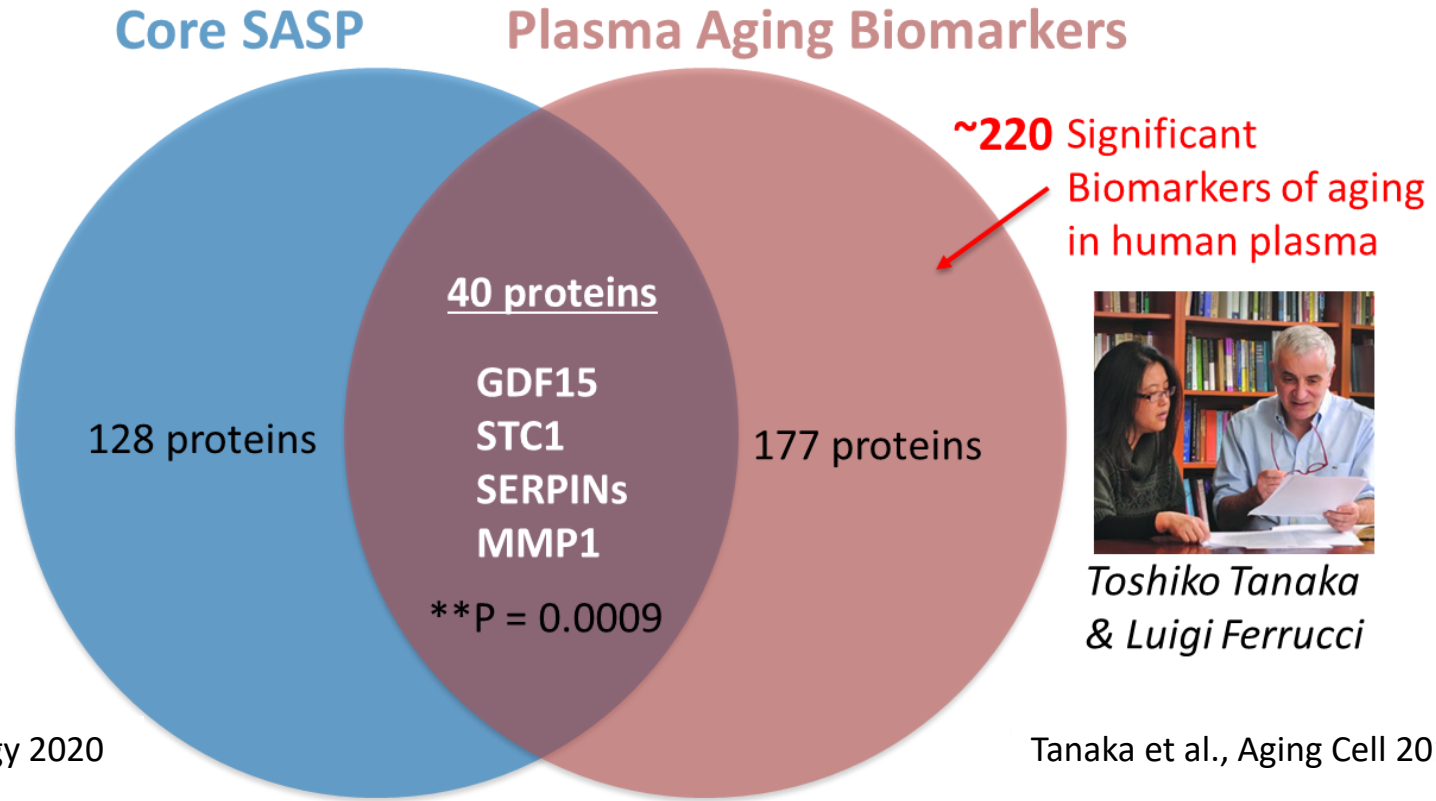
Basisty et al. Plos Biology, 2020

*SASP: **S**enescence-**A**ssociated **S**ecretory **P**henotype*

Human Plasma Aging Biomarkers are Enriched with SASP

Nathan Basisty & Birgit Schilling

Basisty et al., Plos Biology 2020



Toshiko Tanaka & Luigi Ferrucci

Tanaka et al., Aging Cell 2018

(healthy aging cohort)
now InChianti cohort

*SASP: **S**enescence-**A**ssociated **S**ecretory **P**henotype*

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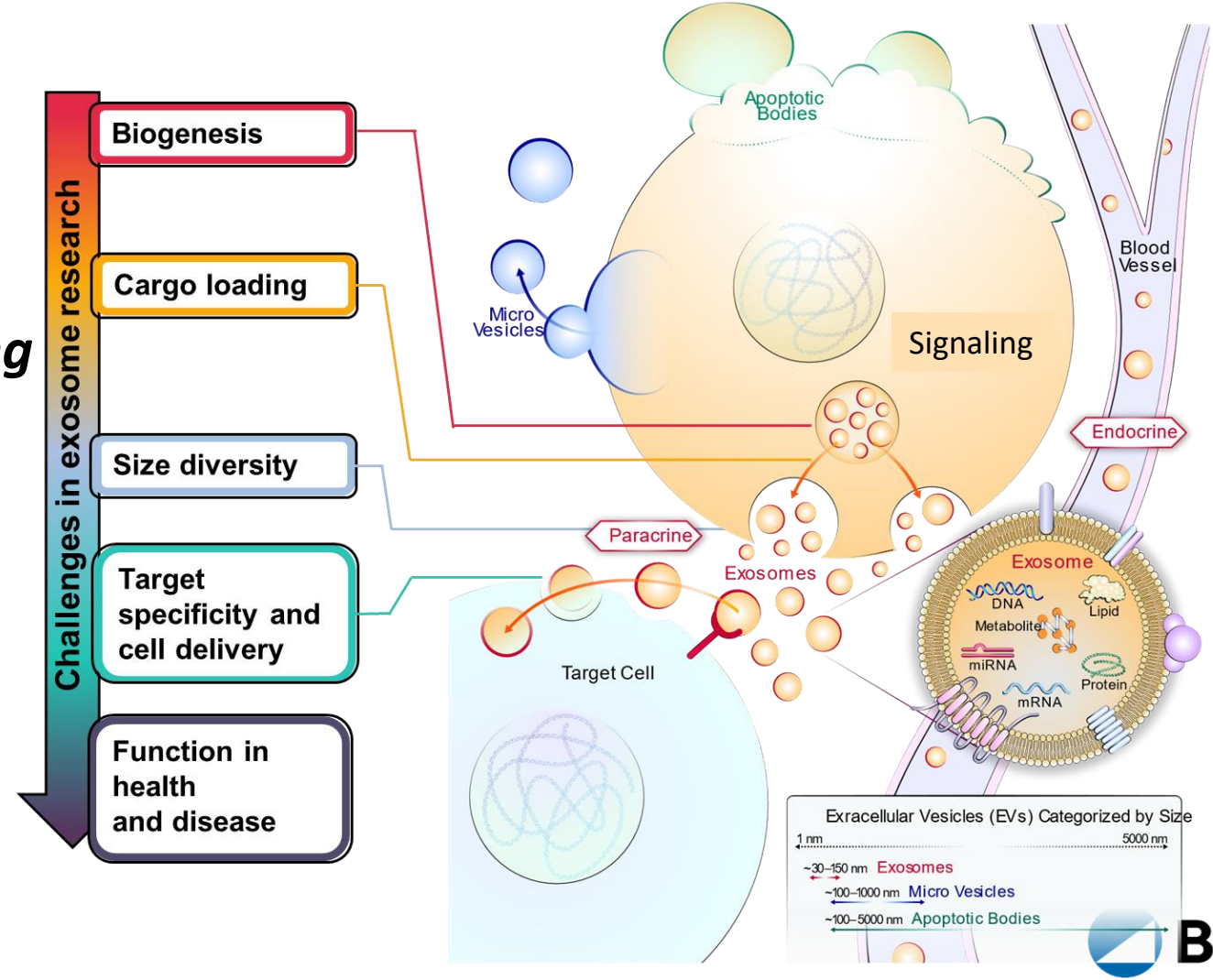
Exosome Characteristics and Challenges in Aging Research



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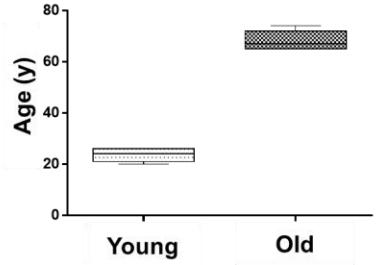
Exploring Exosomes in Plasma with DIA-MS Proteomics

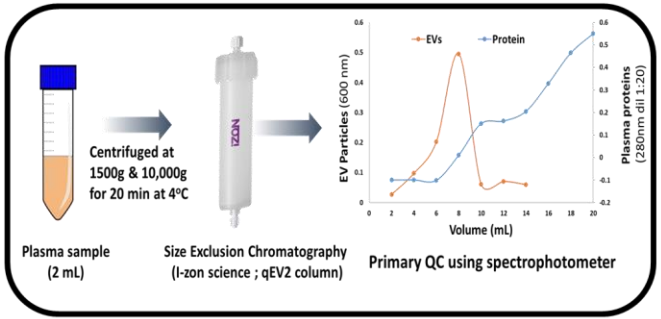
(a) Sample selection

PILOT Study

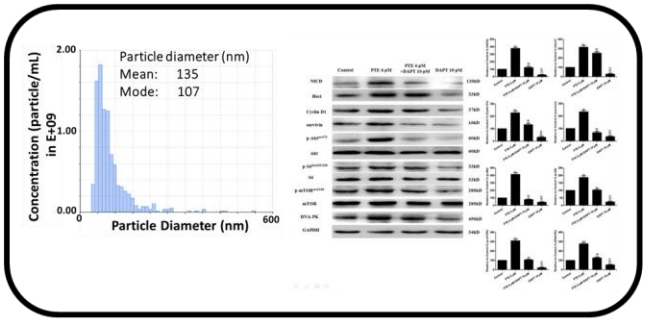


Age (n=5)

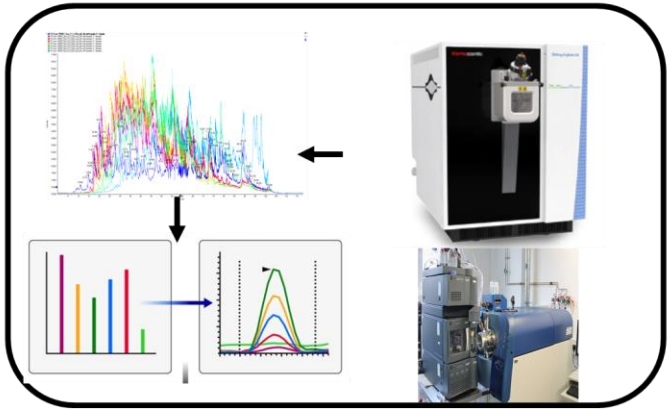
(b) Optimization of exosome extraction



(c) Characterization of exosomes

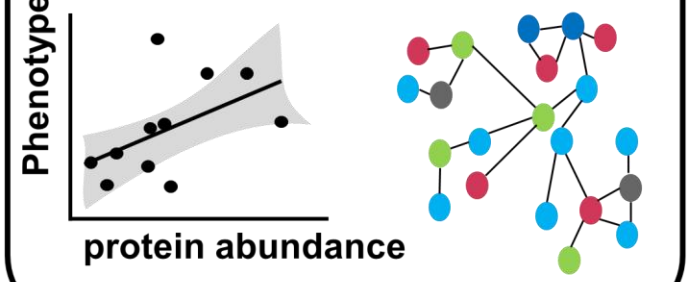


(d) DIA/DDA proteomics analysis

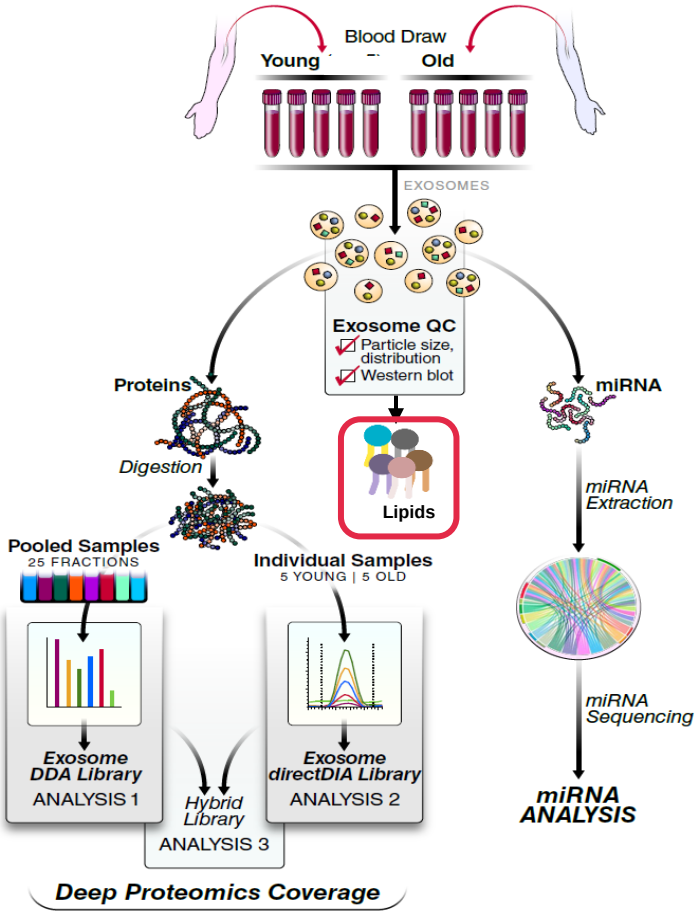


(e) Data analysis

(I) Correlation (II) Network analysis



Plasma Exosome Workflow for Multi-Omics Study



Exosome Purification from Plasma

- Proteomics
- Micro RNA
- Lipidomics (with Erin Baker, NCS)

received more plasma samples
In preparation

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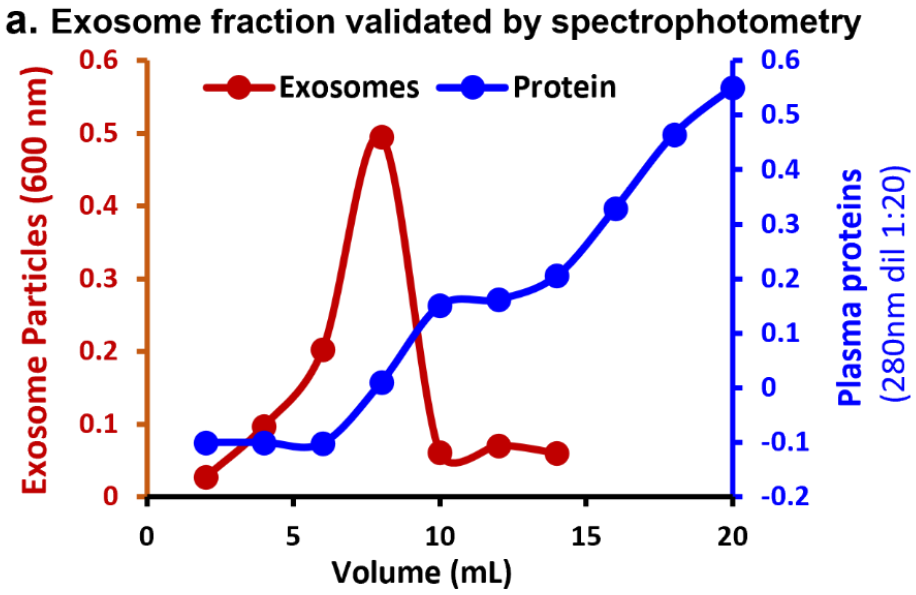
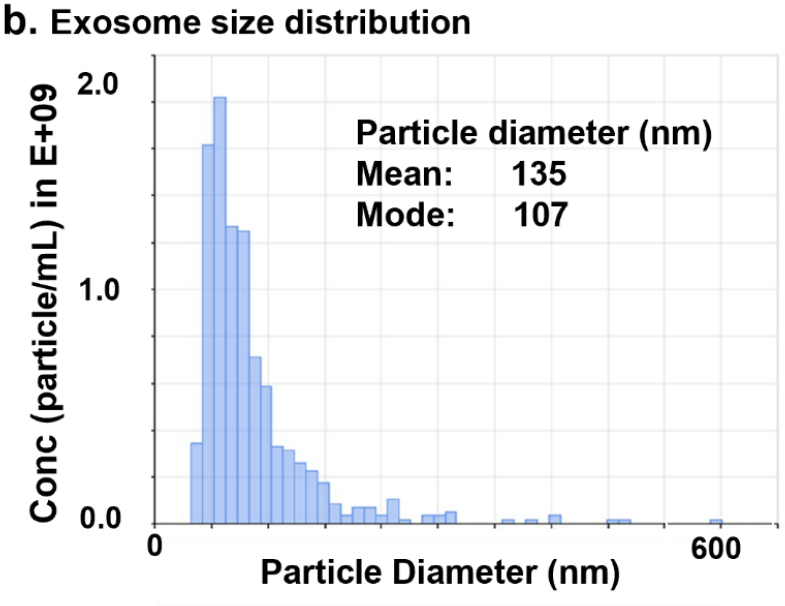
Plasma Exosome Workflow for Multi-Omics Study

Size Exclusion Chromatography and Ultrafiltration





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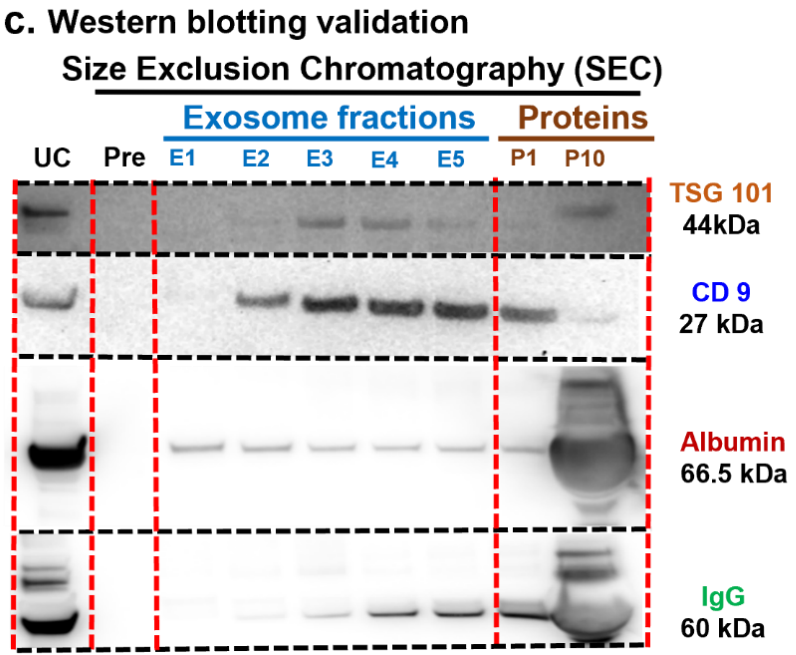
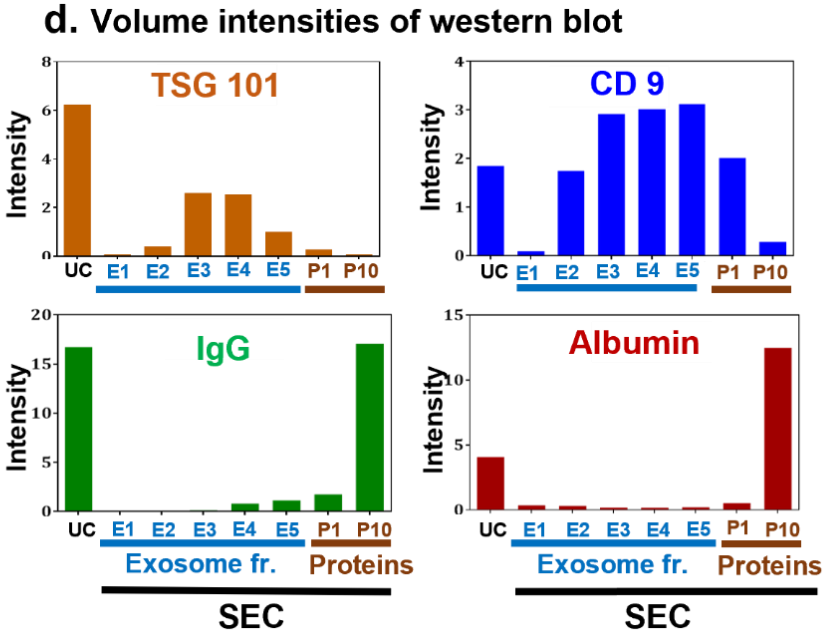
Plasma Exosome Workflow for Multi-Omics Study

Exosome Purification Quality Control





Generated Plasma Exosome Deep Spectral Libraries

Sample Details	Database	# precursors	# peptides	# proteins	# protein groups
(25 DDA)	Fractionation dataset (DDA)	43,201	26,655	5,186	2,323
(15 DIA)	directDIA dataset (DIA)	23,444	13,091	1,909	906
(25 DDA+15 DIA)	Combined dataset (DDA+directDIA)	43,492	26,745	5,221	2,341

Cohort Analysis (young and old)

Plasma Exosome DIA Quantitative analysis (young & old)

Database used	# Total Protein Groups identified & quantified in exosomes	# Proteins with exosome (GO) annotation
Combined dataset (DDA+DIA)	1,379	839
Fractionation dataset (DDA)	1,341	804

204 proteins are significantly changing !!

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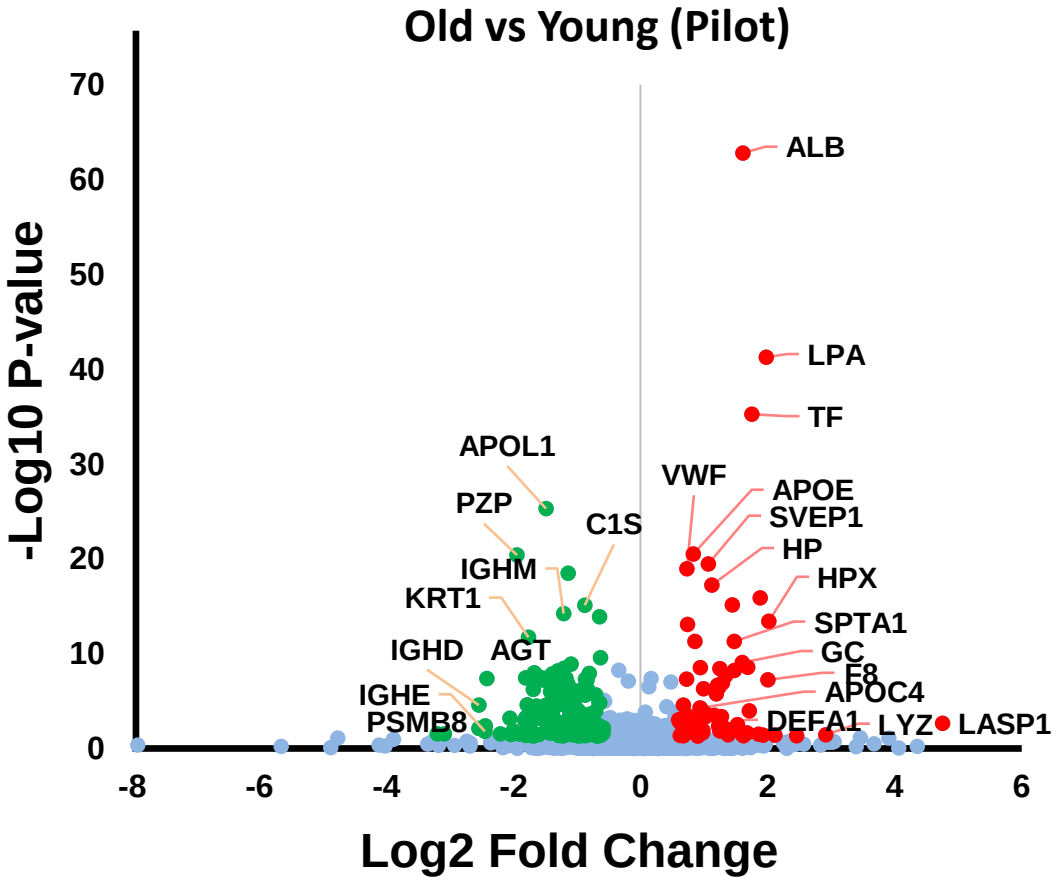
Exosome Proteins changing during Aging (Pilot Study)



ALB	Serum albumin
LPA	Apolipoprotein(a)
TF	Serotransferrin
VWF	von Willebrand factor
APOE	Apolipoprotein E
HP	Haptoglobin
PZP	Pregnancy zone protein
IGHM	Ig mu chain C region
AGT	Angiotensinogen
LYZ	Lysozyme C
LASP1	LIM and SH3 domain protein 1

~ 200 of 1,300 proteins are changing

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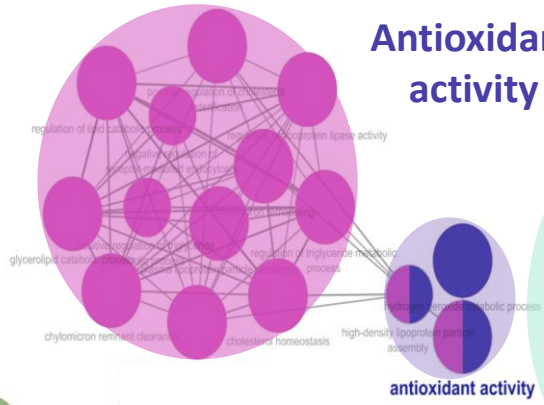
Pathway and Network Analysis Plasma Exosomes

old vs young

(i) Up-regulated

Plasma lipoprotein particle remodeling

Antioxidant activity



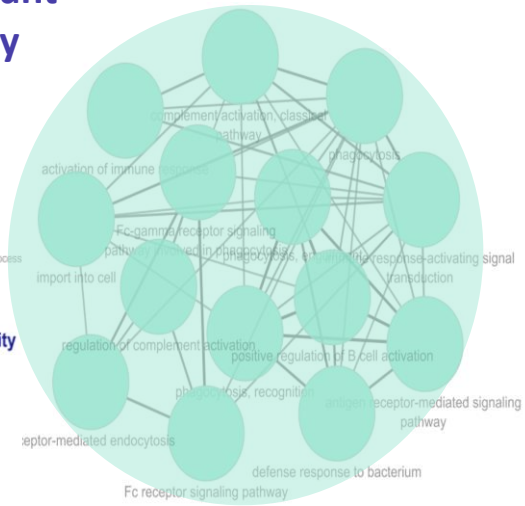
Acute inflammatory response

Regulation of humoral immune response

Blood coagulation intrinsic pathway

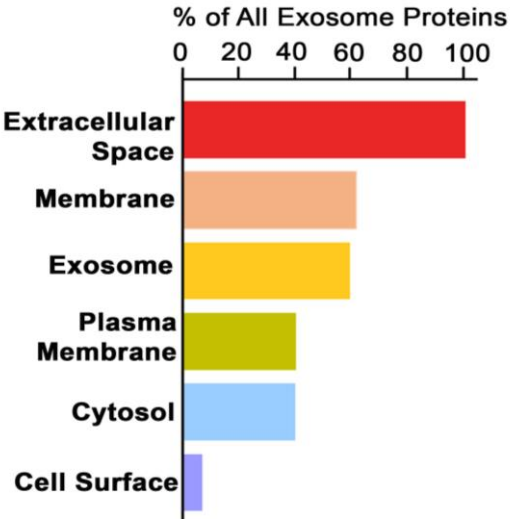
(ii) Down-regulated

Defense response



antibacterial humoral response

intermediate filament cytoskeleton organization



Proteins include:
(≥2 peptide, Q value <0.01)
(P value <0.05, FC= 1.5)

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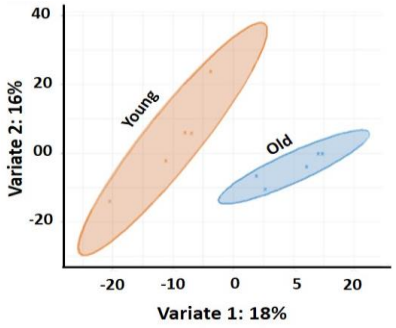
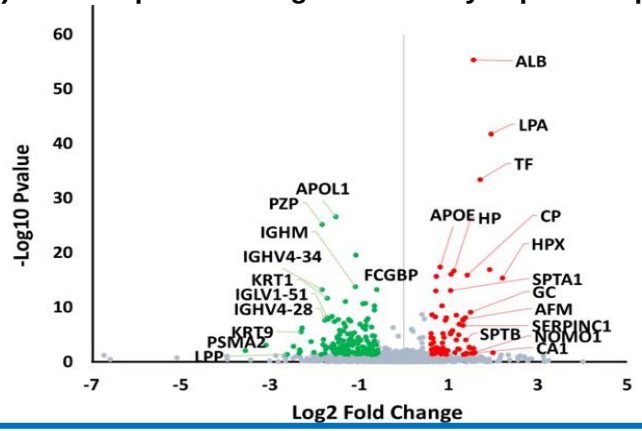
Exosome miRNA and proteins classify old and young plasma

Proteomics analysis

(i) PLS-DA analysis classify old and young

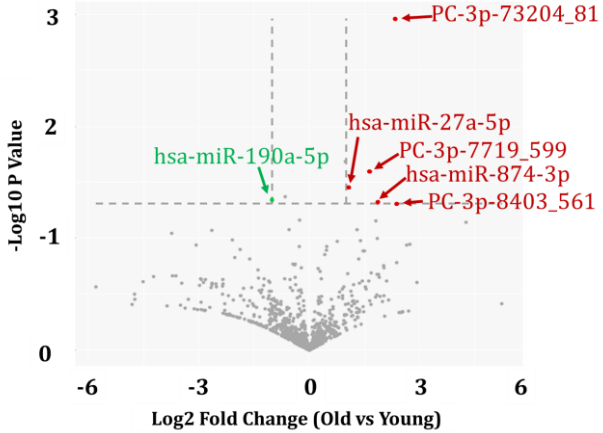


(ii) Volcano plot showing differentially expressed proteins

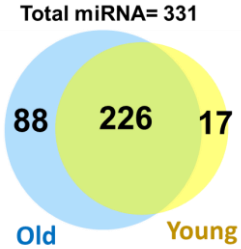


miRNA analysis

(i) Volcano plot showing differentially expressed miRNA



(ii) Venn diagram showing common and unique miRNA



hsa-miR-199b-5p
hsa-miR-409-3p

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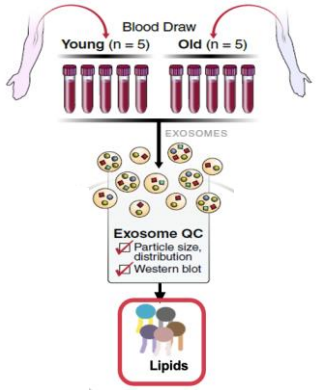
Plasma Exosomes – Lipidomics with ion mobility LC-MS/MS



Lipid Preparation from plasma exosomes

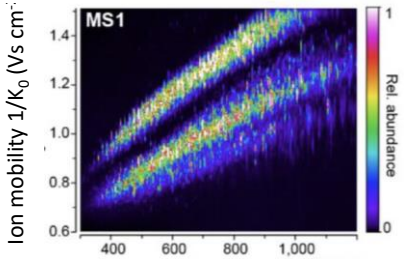
- Ultra filtration and size exclusion chromatography
- Antibody Enrichment

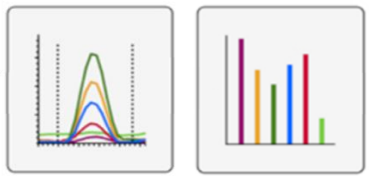
BAKER Lab
NCSU

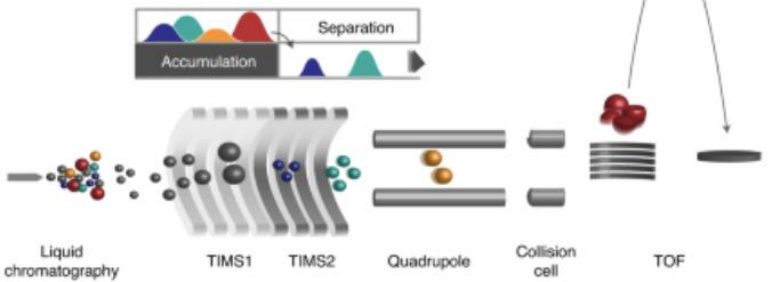
LC/MS-MS
Acquisitions on
Agilent system

DDA

Ion mobility
MS







Skyline

Bioinformatics

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Method Development for Plasma Exosome Lipidomics

Young (22-26 yr) → **Plasma** n=20

Old (65-74 yr) → **Plasma** n=20

centrifuge

1

Size Exclusion Chromatography (SEC) → Ultrafilter (UF) → Exosomes

Lipid extraction

Modified Folch extraction method Reconstituted in $CHCl_3$:MeOH (1:19)

lipids → UHPLC (Agilent 1290 Infinity II) → Drift time → IMS → Mass spectrometer

(Agilent 6560 IMQTOF MS)
Data acquisition: both positive and negative mode; mass range 50-1700, CID

Lipids associated with lipid bodies, macrovesicles, and other EVs identified

Macrovesicle (100-1000 nm)

Exosome (50-150 nm)

Apoptotic EVs (100-5000 nm)

VLDL, HDL & Chylomicrons (100-5000 nm)

2

Antibody-based approach

Exosome Isolation Kit CD63, human

CD 9, CD63, CD81

Antibody based capturing of exosomes → Exosomes

Lipid extraction

Modified Folch extraction method Reconstituted in $CHCl_3$:MeOH (1:19)

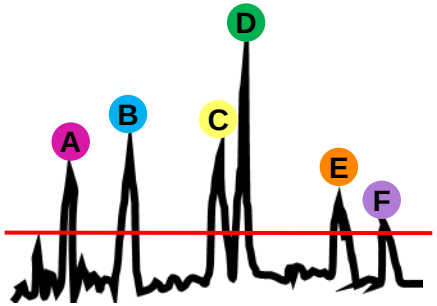
lipids → UHPLC (Agilent 1290 Infinity II) → Drift time → IMS → Mass spectrometer

Agilent 6560 IMQ TOF MS

Successfully identified exosome lipids

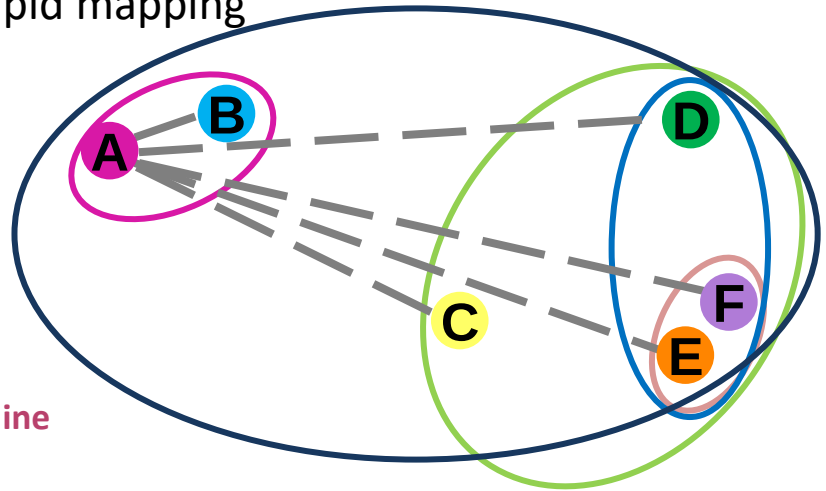
Data acquisition:
both positive and negative mode;
mass range 50-1700, CID

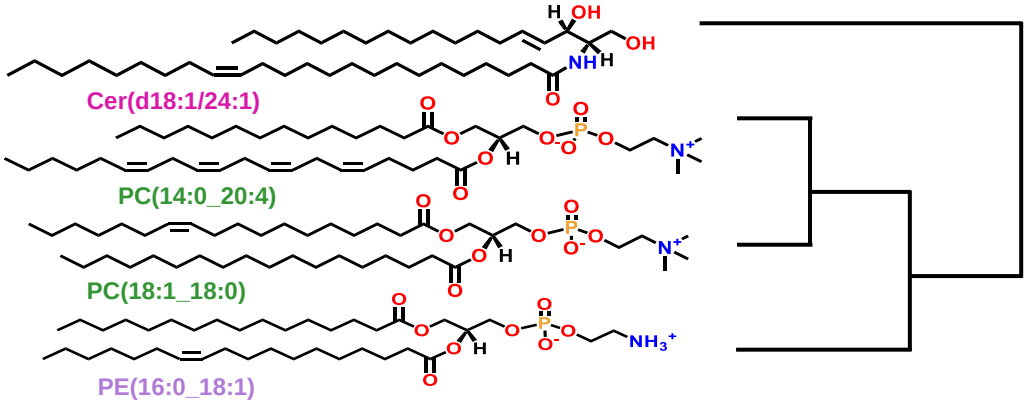
Plasma exosome lipid mapping using Ion Mobility LC MS/MS

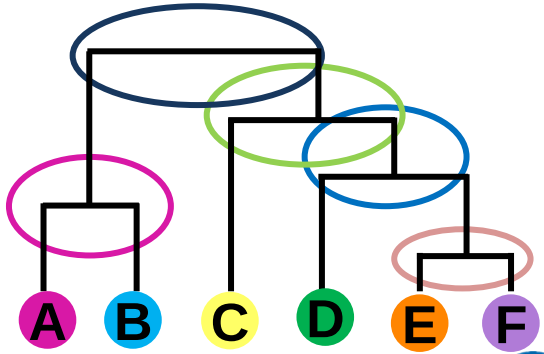


DG = Diglycerides
TG = Triglycerides
PE = Phosphatidylethanolamine
PC = Phosphatidylcholine
CE = Cholesteryl Esters
SM = Sphingomyelin
Cer = Ceramide
AC = Acetylcholines
LPC = Lysophosphatidylcholine
LPE = Lysophosphatidylethanolamine

lipid mapping







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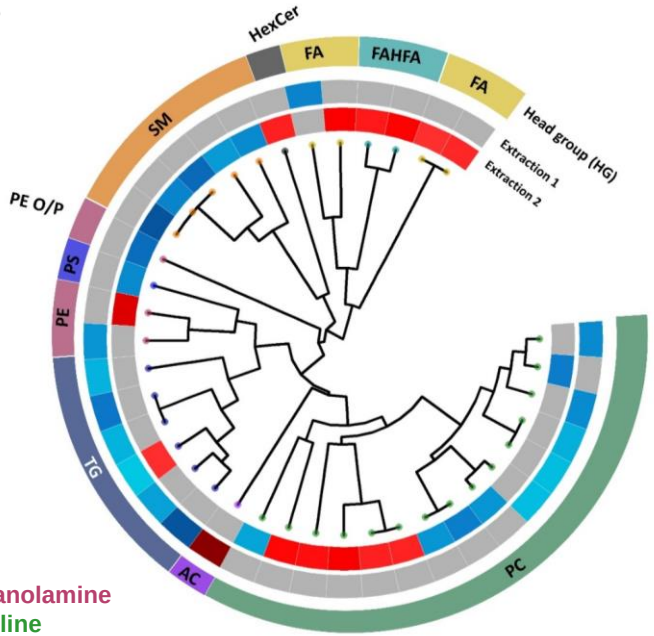
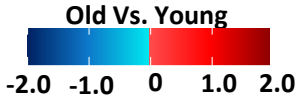
Exosome lipids classify old and young plasma

A. Significantly altered plasma exosome lipid profile for two different methods

Lipid Bodies

Exosomes





DG=Diglycerides
TG=Triglycerides
PE=Phosphatidylethanolamine
PC=Phosphatidylcholine
CE=Cholesteryl Esters
SM=Sphingomyelin
Cer=Ceramide
AC= Acetylcholine
LPC=Lysophosphatidylcholine
LPE=Lysophosphatidylethanolamine

B. Heat map showing lipid distribution in old plasma exosomes compared to young (significant)

Name	P-value	Old vs Young
AC(18:2)	2.14E-04	2.4
PE(18:0_18:3)b	1.93E-02	1.6
LPC(20:3/0:0)	4.75E-02	1.3
FA 19:3	4.17E-03	1.2
FAHFA(14:0/O-16:2)	5.79E-03	1.2
PC(O-18:0/22:5)	7.42E-03	1.1
PC(P-18:0/16:0)	2.48E-02	0.9
FAHFA(16:0/O-18:2)	3.07E-04	0.8
LPC(0:0/18:1)	1.15E-02	0.7
HexCer(d18:1/24:0)	3.46E-02	0.7
LPC(18:1/0:0)	3.11E-02	0.6
FA 12:0	2.68E-02	0.6
FA 14:0	3.29E-03	0.4
PC(16:0_20:4);PC(18:1_18:3)	4.53E-02	-0.9
PE(18:1_20:4)	4.18E-02	-0.9
PC(39:4)	2.35E-02	-1
SM(d42:5)b	3.70E-02	-1
PC(19:0_20:4)+	2.92E-02	-1
PS(18:1_22:0)	2.45E-02	-1
SM(d17:1/24:0);SM(d18:1/23:0)	4.54E-02	-1.1
FA 22:4	2.22E-02	-1.1
PC(18:0_20:1)	5.34E-03	-1.1
PC(15:0_20:4);PC(17:2_18:2)	4.97E-02	-1.2
TG(18:0_18:0_18:1)	4.60E-02	-1.2
PE(P-18:0/22:5)b	1.84E-02	-1.4
SM(d18:1/24:0)+	3.80E-02	-1.4
TG(58:8)a	2.46E-02	-1.7
SM(d18:1/20:0)	4.41E-02	-1.7

low high

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Characterization of Senescent Endothelial Cells *in cell culture*

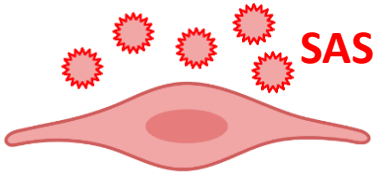
Schilling Lab
(Buck Institute)

Campisi Lab
(Buck Institute)

Francesco Neri

Normal
Endothelial Cell

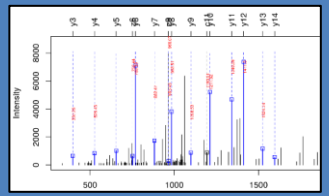
VS



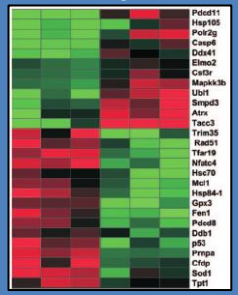
Senescent
Endothelial Cell

Deep RNA-sequencing

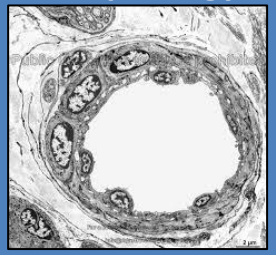
Proteomics



Transcriptomics



Morphology



SASP, splice variants

- Senescence Biomarkers
- Therapeutic Targets (Senolytics)

Francesco Neri

Ferrucci Lab
(NIA)

Lisa Hartnell (NIA)

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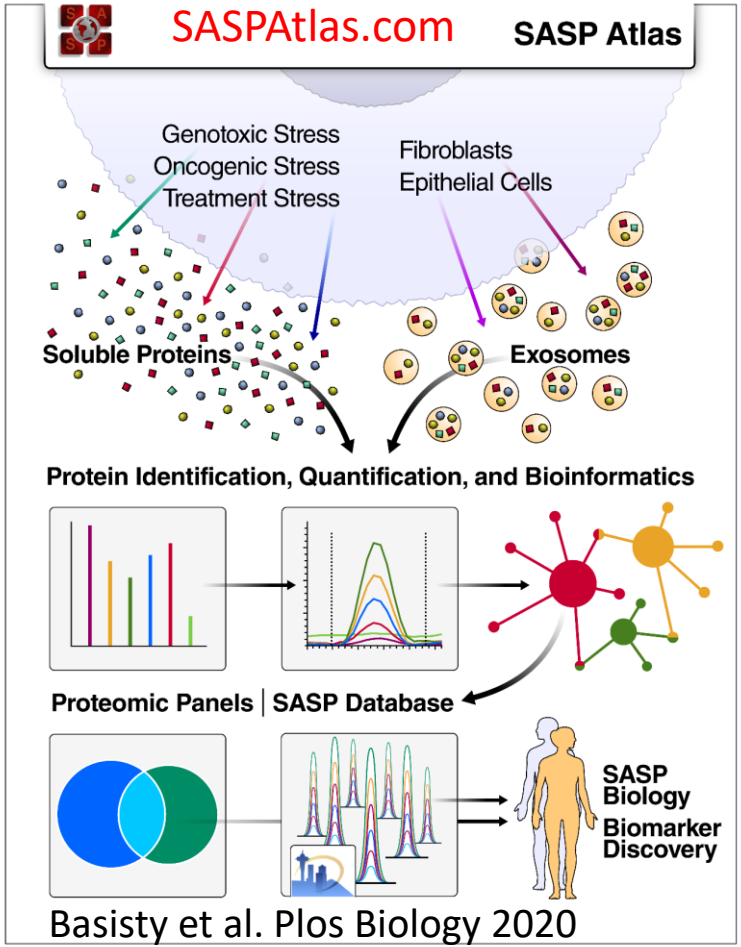
Heterogeneity of Senescence - Biomarkers



Basisty et al. Plos Biology 2020

Aging Atlas

Geographical Atlas

- **DIA workflows** – deep libraries and **directDIA**
- Soluble SASP vs released <u>exosomes</u>
- Dynamic changes over time of SASP/exosomes
- Splice Variants during senescence? Proteogenomics, **PTMs (site localization)**
- Blood signatures and **multi omics**

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Schilling Lab

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Postdoc Positions open

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