



Whole Body Integrative Approach and CLARITY-BPA Consortium Studies

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CLARITY-BPA Consortium Studies

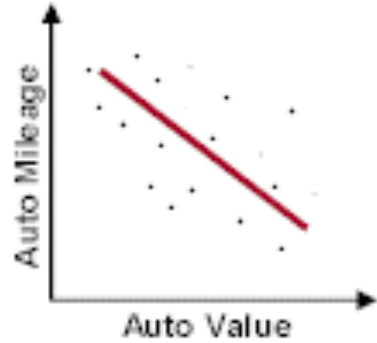
- This Consortium project involved 13 independent investigators and many of them received tissues from the same animals.
- Independent investigators have published their own data that clearly shows that even low dose exposure to bisphenol A (BPA) leads to harmful effects in the organ/system studied.
- One of the key goals though established at the outset of the studies was to integrate results across studies.
- However, the FDA and NTP has yet to do such analyses.
- In our current publication (Heindel et al., *Repro Toxicol*, 2020), we report on a method, mixOmics analyses, that integrates datasets across laboratories for three ages (juvenile, young adult, and older adult) and three doses of BPA (2.5 mg/kg, 250 mg/kg, and 2500 mg/kg body weight).

Types of Correlation Analyses

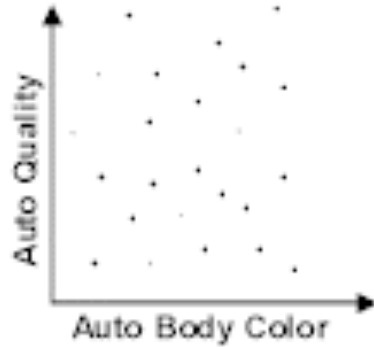
Correlation

Relationship Between Two Quantities
Such That When One Changes, the Other Does

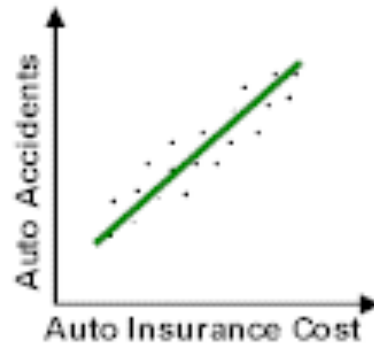
Negative



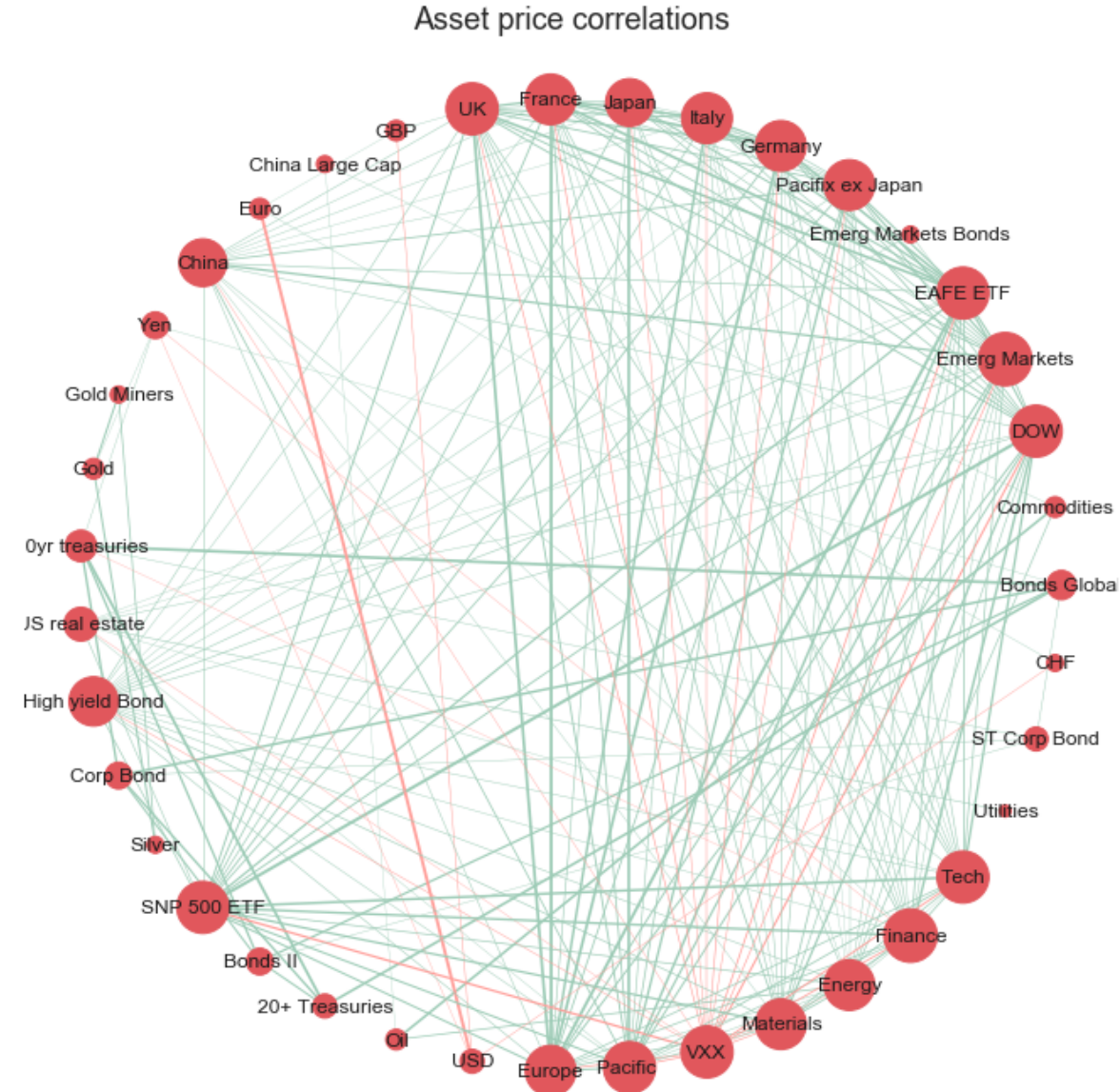
Zero



Positive



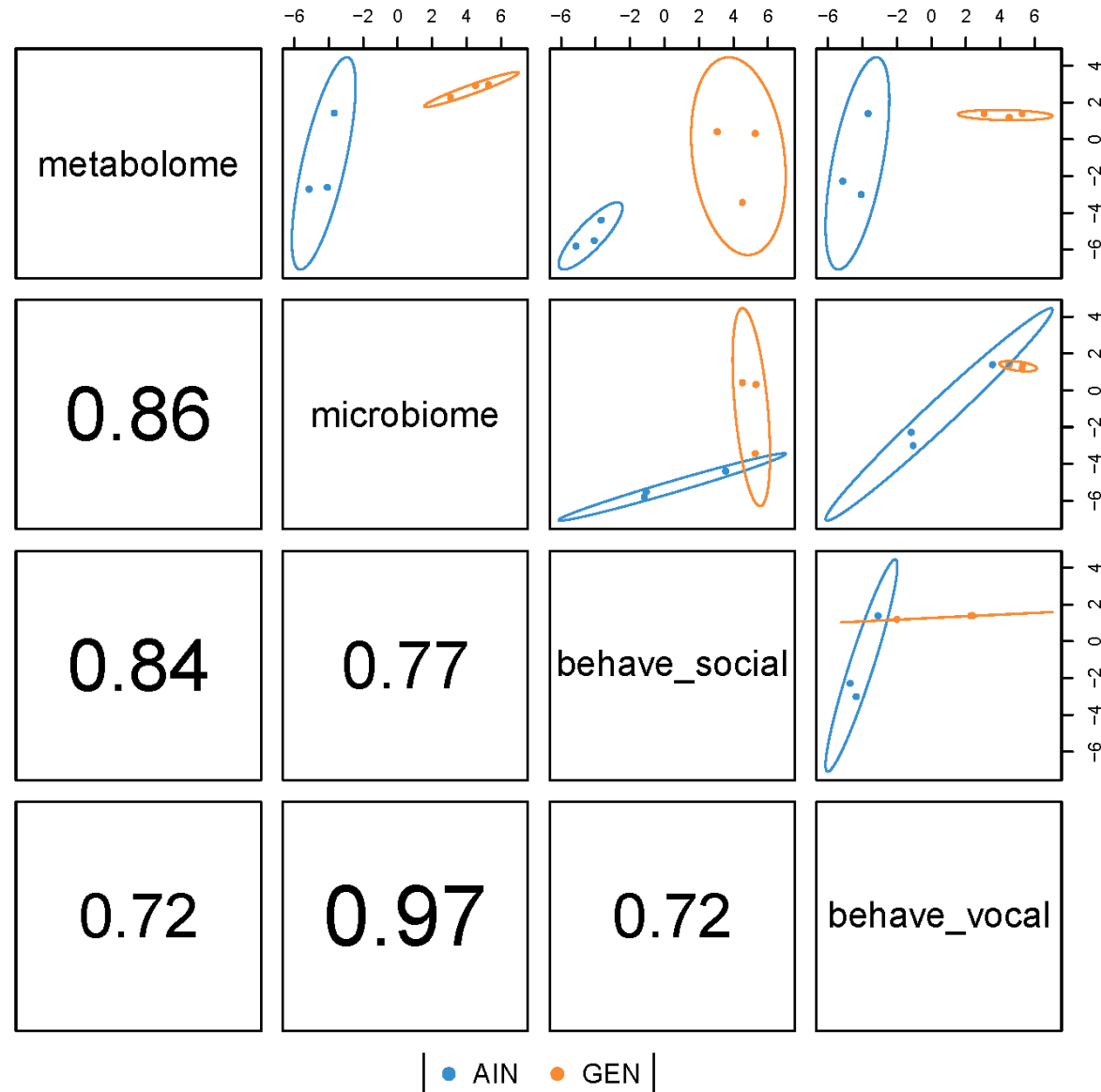
Correlating More than Two Factors



MixOmics Analysis

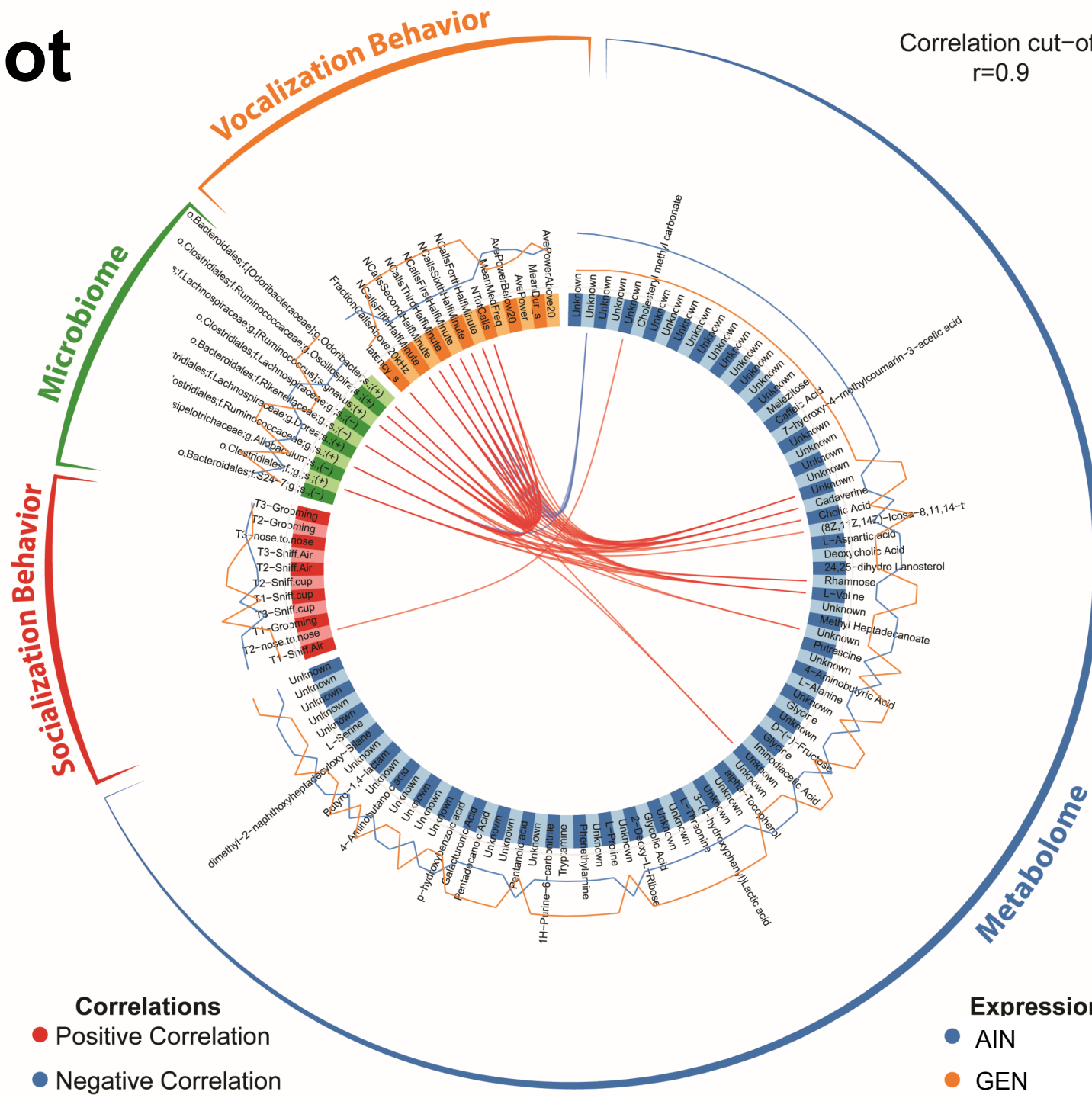
- “We introduce ***mixOmics***. By adopting a systems biology approach, the toolkit provides a wide range of methods that ***statistically integrate several data sets***. This method allows for simultaneous correlation analyses to permit “***data integration across independent studies***.” Rohart et al., *Plos Comp Biol* 2017 13(11):e1005752.
- It is thus designed to compare heterogenous and large datasets.
- As mentioned above, it was also designed with the idea of comparing datasets across independent studies.
- We have used it to integrate 'omics and phenotypic data (*Marshall et al. J Endocrinol* 2019; *Mao et al., PNAS* 2020; *Butler et al., J Neuroendocrinol* 2020; *Kaur et al., Sci Reports* 2020).
- To our knowledge, though, this is the **first usage of this program to integrate datasets from multiple independent laboratories.**

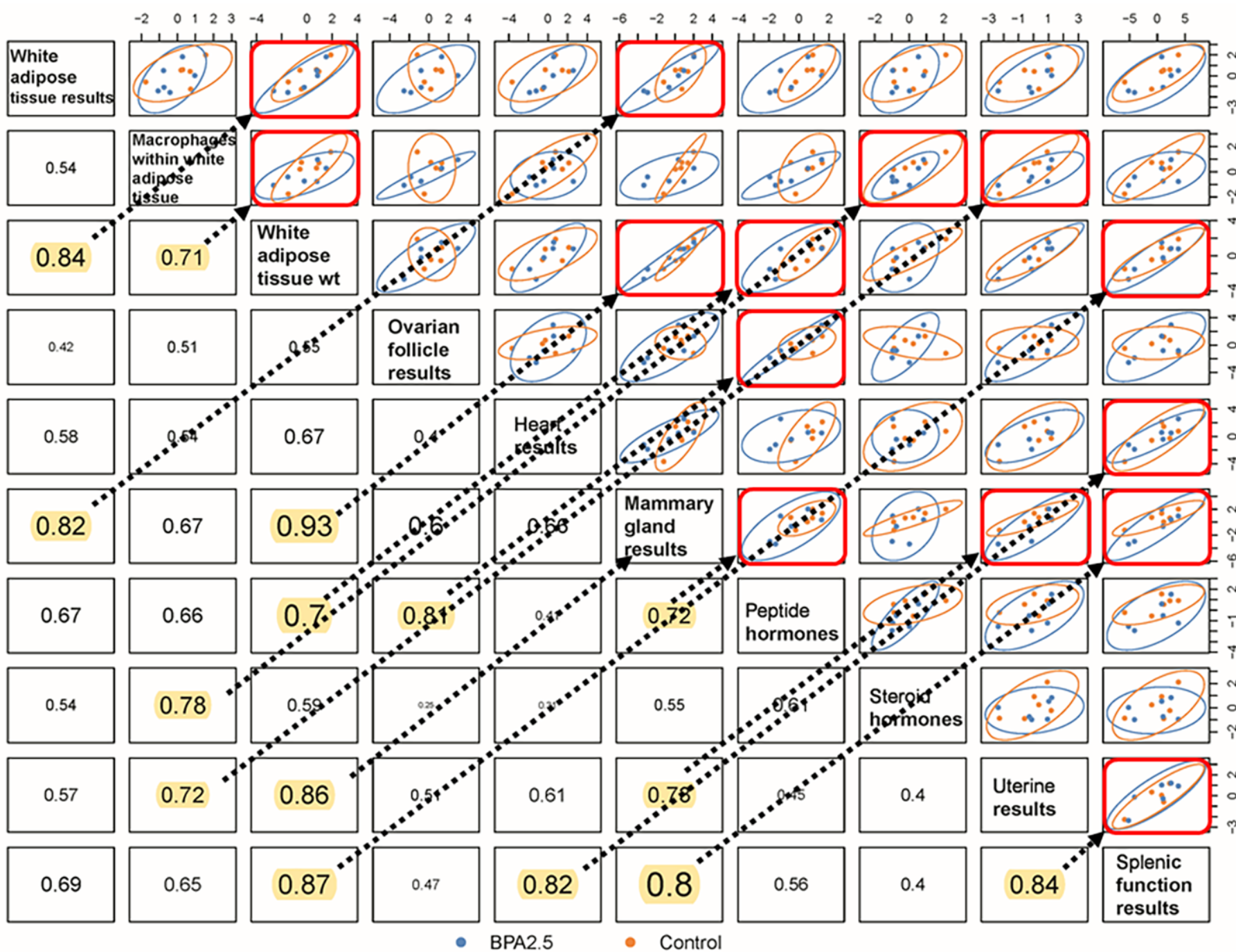
Diablo "R" Plot



Circos Plot

Correlation cut-off
r=0.9



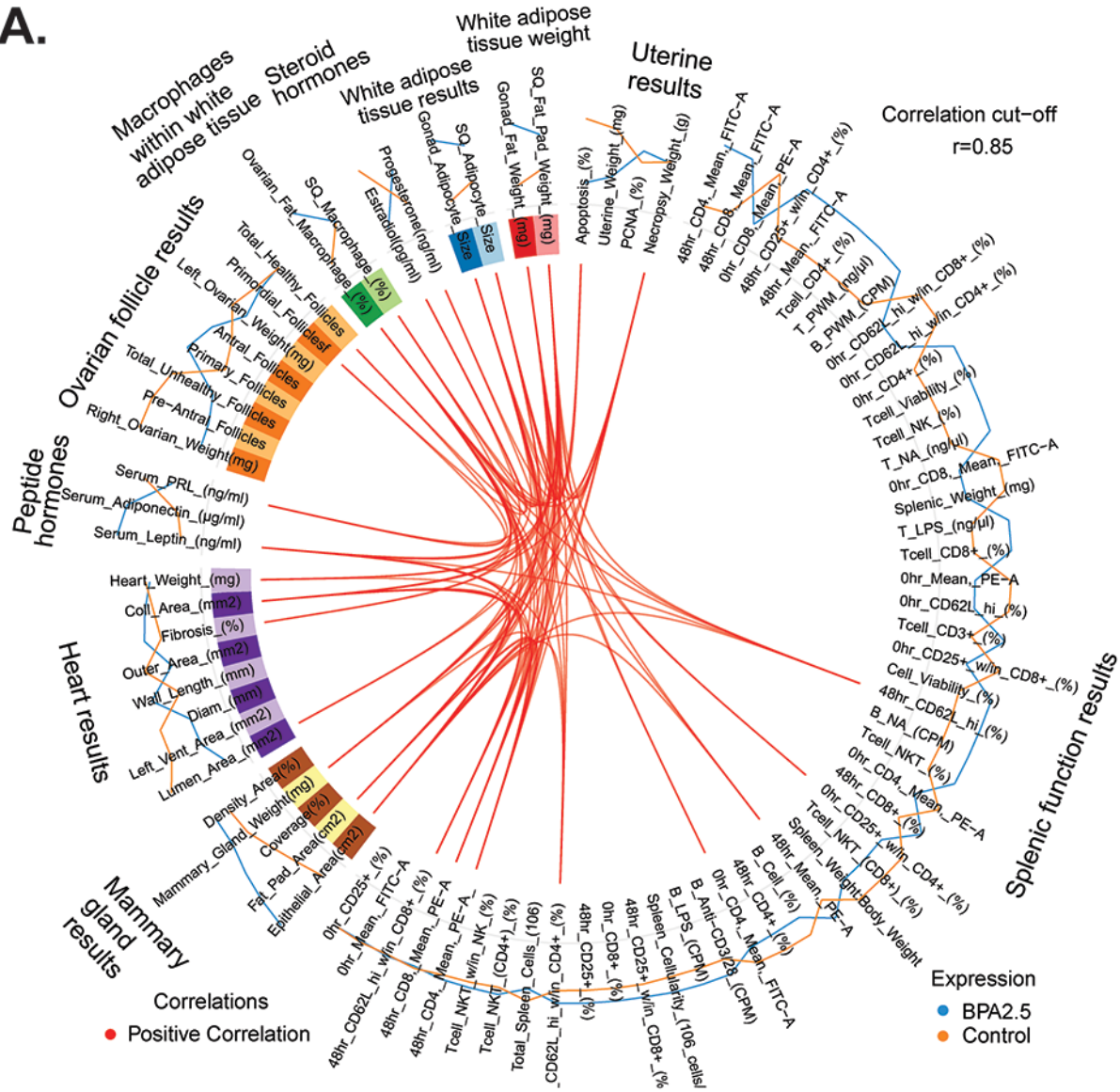


R Plot
 6-month-old female data
 for low-dose BPA
 exposure (2.5 µg/kg/day)
 and controls

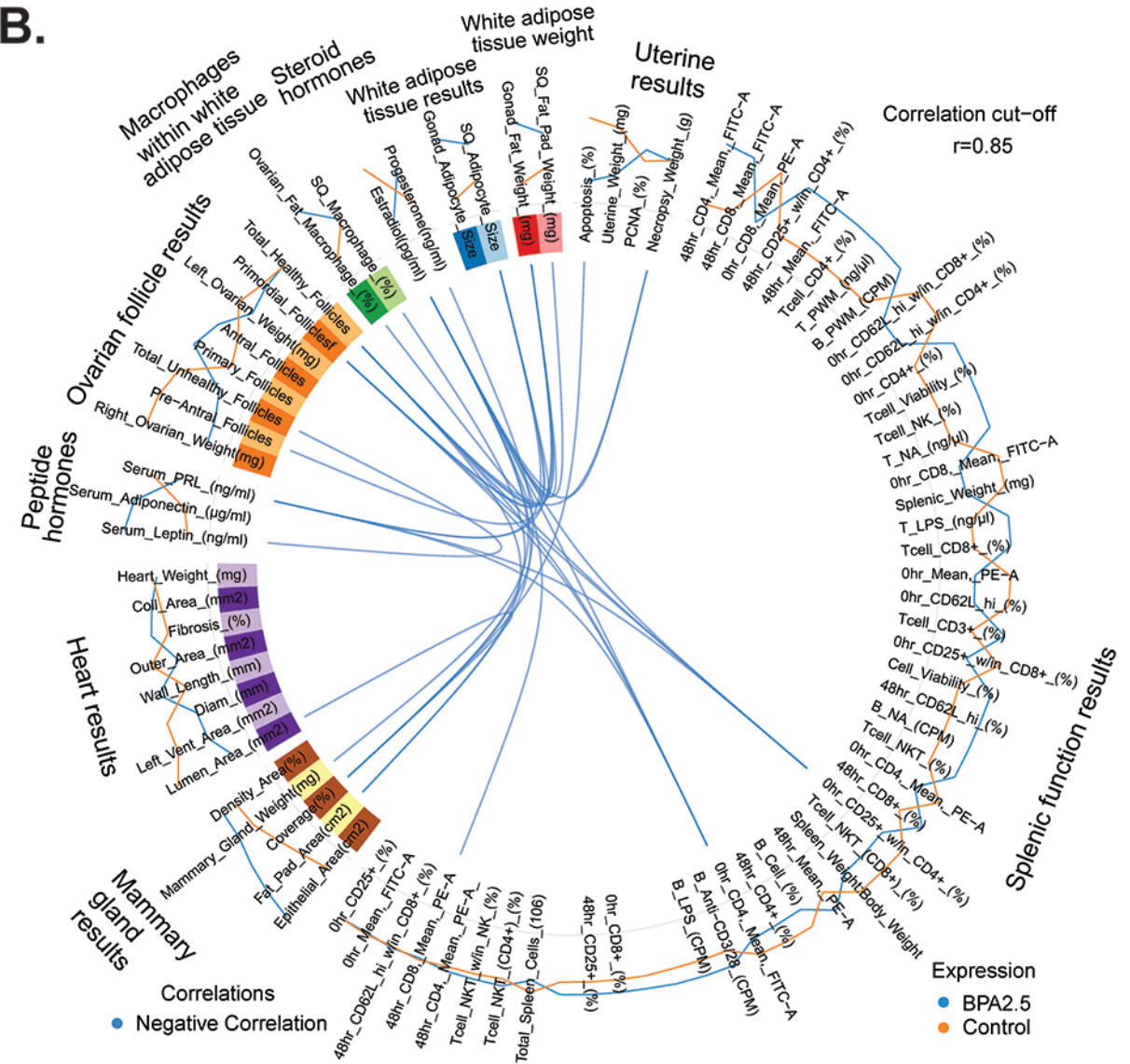
Circos Plot:

6-month-old female data for low-dose BPA exposure (2.5 µg/kg/day) and controls

A.



B.

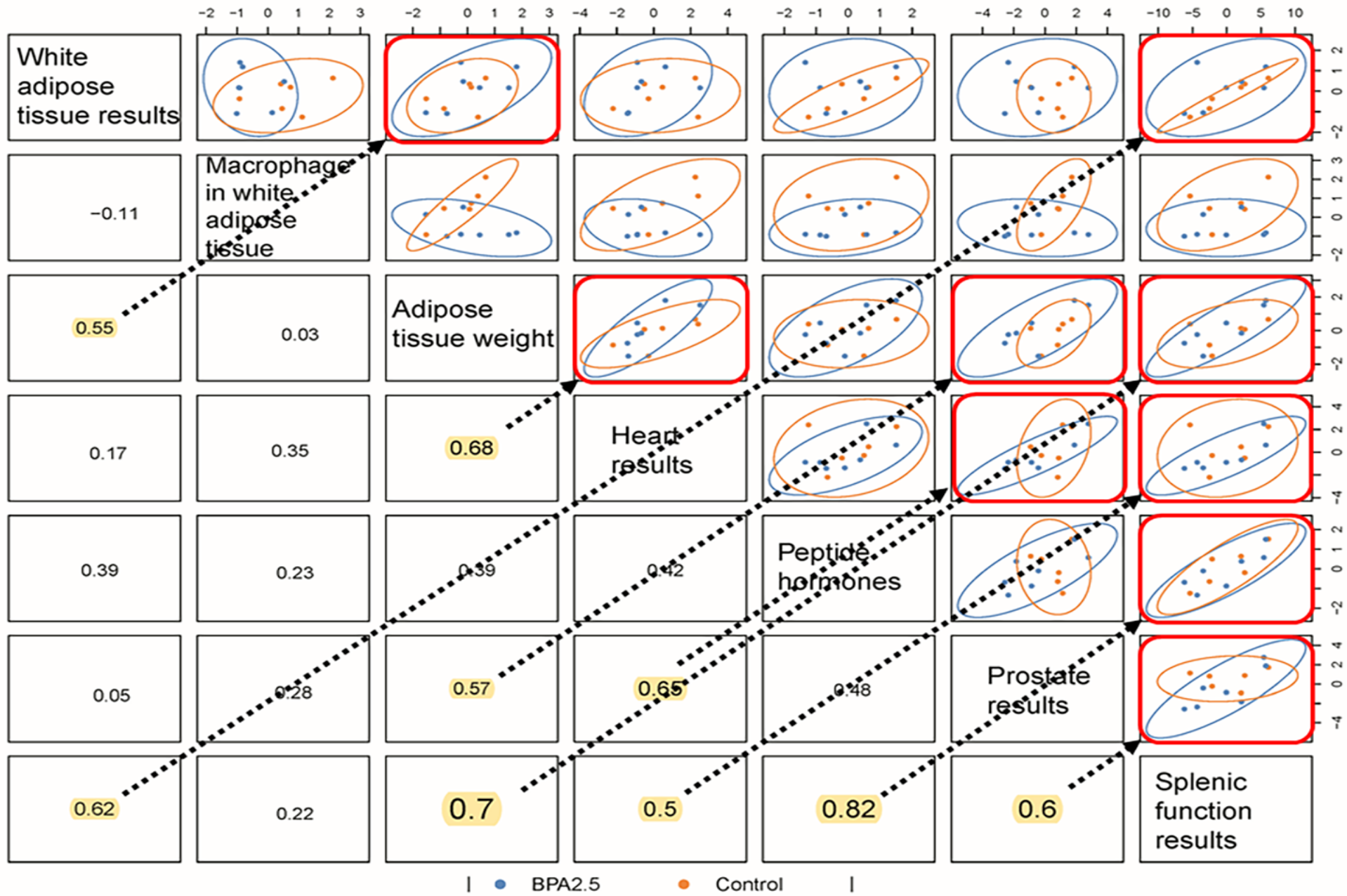


MixOmics data integration comparison at 2.5, 250 and 2500 µg/kg/day of BPA at PND180 in females

		Correlation coefficient		
Phenotype A	Phenotype B	BPA2.5	BPA250	BPA2500
White adipose tissue results	Macrophages within white adipose tissue	0.54	ND	0.03
	White adipose tissue weight	0.84	0.75	0.75
	Heart results	0.58	0.6	0.65
	Mammary gland results	0.82	0.51	0.64
	Peptide hormones	0.67	0.54	0.61
	Steroid hormones	0.54	0.13	0.3
	Uterine results	0.57	0.37	0.3
	Splenic function results	0.69	0.51	0.53
Macrophages within white adipose tissue	White adipose tissue weight	0.71	0.77	0.35
	Ovarian follicle results	0.51	0.25	0.29
	Heart results	0.54	0.7	0.23
	Mammary gland results	0.67	0.87	0.22
	Peptide hormones	0.66	0.74	0.34
	Steroid hormones	0.78	0.52	0.17
	Uterine results	0.72	-0.06	0.06
	Splenic function results	0.65	0.37	0.53
White adipose tissue weight	Ovarian follicle results	0.55	0.42	0.62
	Heart results	0.67	0.67	0.55
	Mammary gland results	0.93	0.88	0.78
	Peptide hormones	0.7	0.86	0.87
	Steroid hormones	0.59	0.5	0.68
	Uterine results	0.86	0.29	0.24
	Splenic function results	0.87	0.61	0.78

MixOmics data integration comparison at 2.5, 250 and 2500 µg/kg/day of BPA at PND180 in females

		Correlation coefficient		
Phenotype A	Phenotype B	BPA2.5	BPA250	BPA2500
Ovarian follicle results	Heart results	0.4	0.22	0.52
	Mammary gland results	0.6	0.31	0.89
	Peptide hormones	0.81	0.58	0.64
	Steroid hormones	0.25	0.37	0.75
	Uterine results	0.51	0.6	0.68
	Splenic function results	0.47	0.66	0.62
Heart results	Mammary gland results	0.66	0.59	0.64
	Peptide hormones	0.41	0.77	0.58
	Uterine results	0.61	0.32	0.26
	Splenic function results	0.82	0.61	0.69
	Mammary gland results	0.72	0.8	0.74
Mammary gland results	Steroid hormones	0.55	0.52	0.69
	Uterine results	0.73	0.05	0.66
	Splenic function results	0.8	0.43	0.66
	Peptide hormones	0.61	0.48	0.55
Peptide hormones	Steroid hormones	0.61	0.48	0.55
	Splenic function results	0.56	0.78	0.82

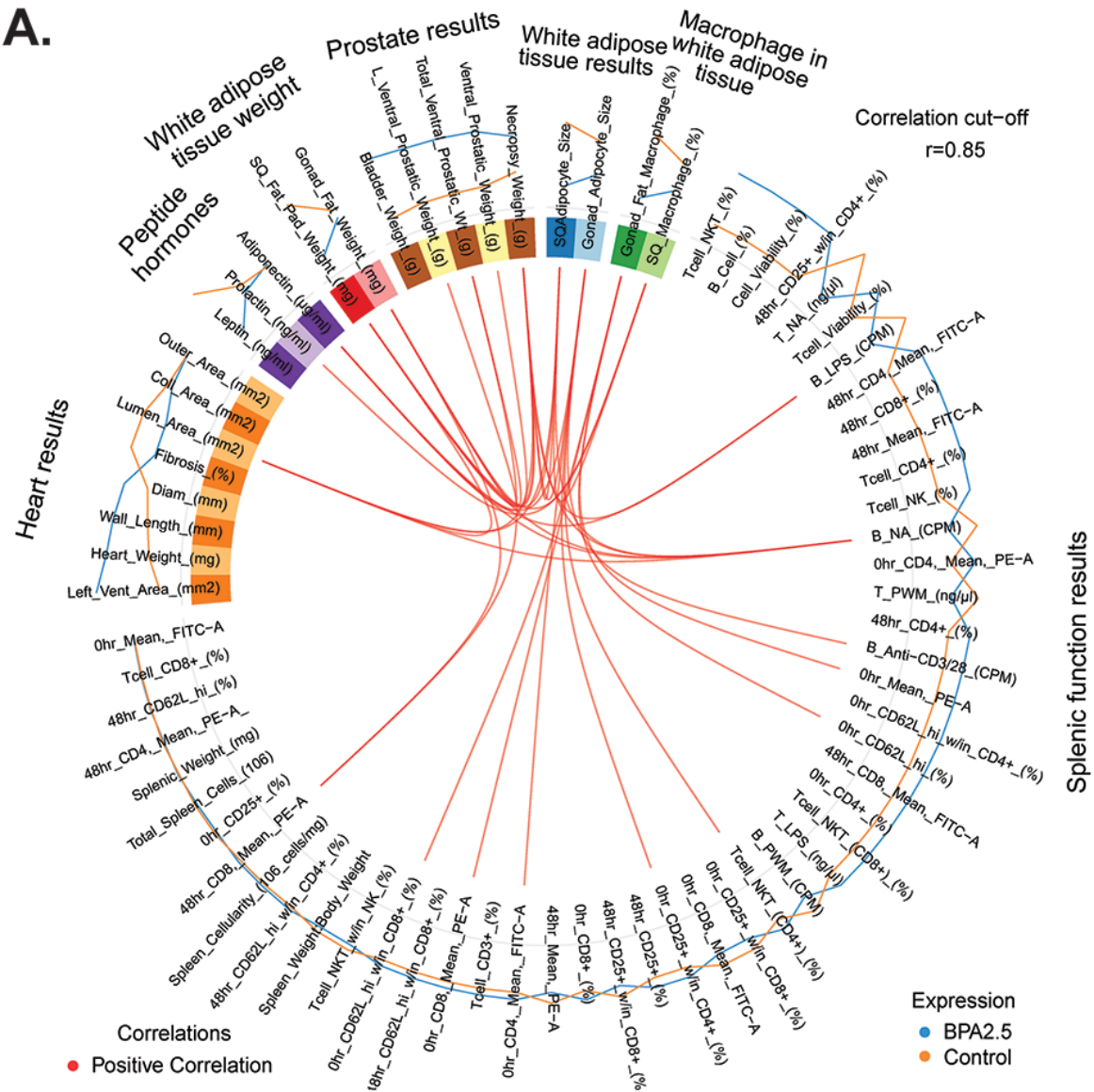


R plot correlations across 6-month-old male data for low-dose BPA exposure (2.5 µg/kg/day) and controls.

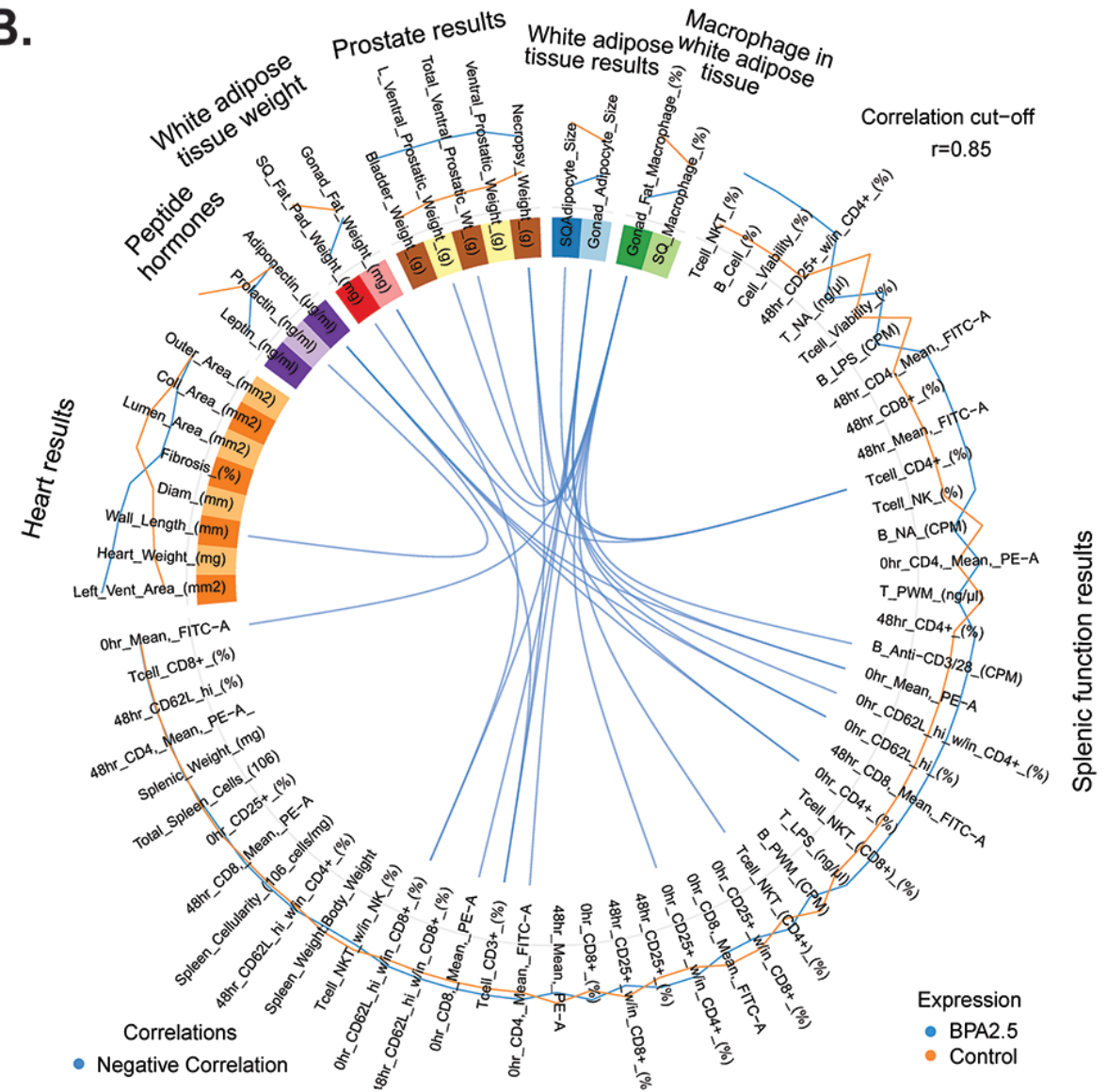
Circos Plot:

6-month-old male data for low-dose BPA exposure (2.5 µg/kg/day) and controls

A.



B.

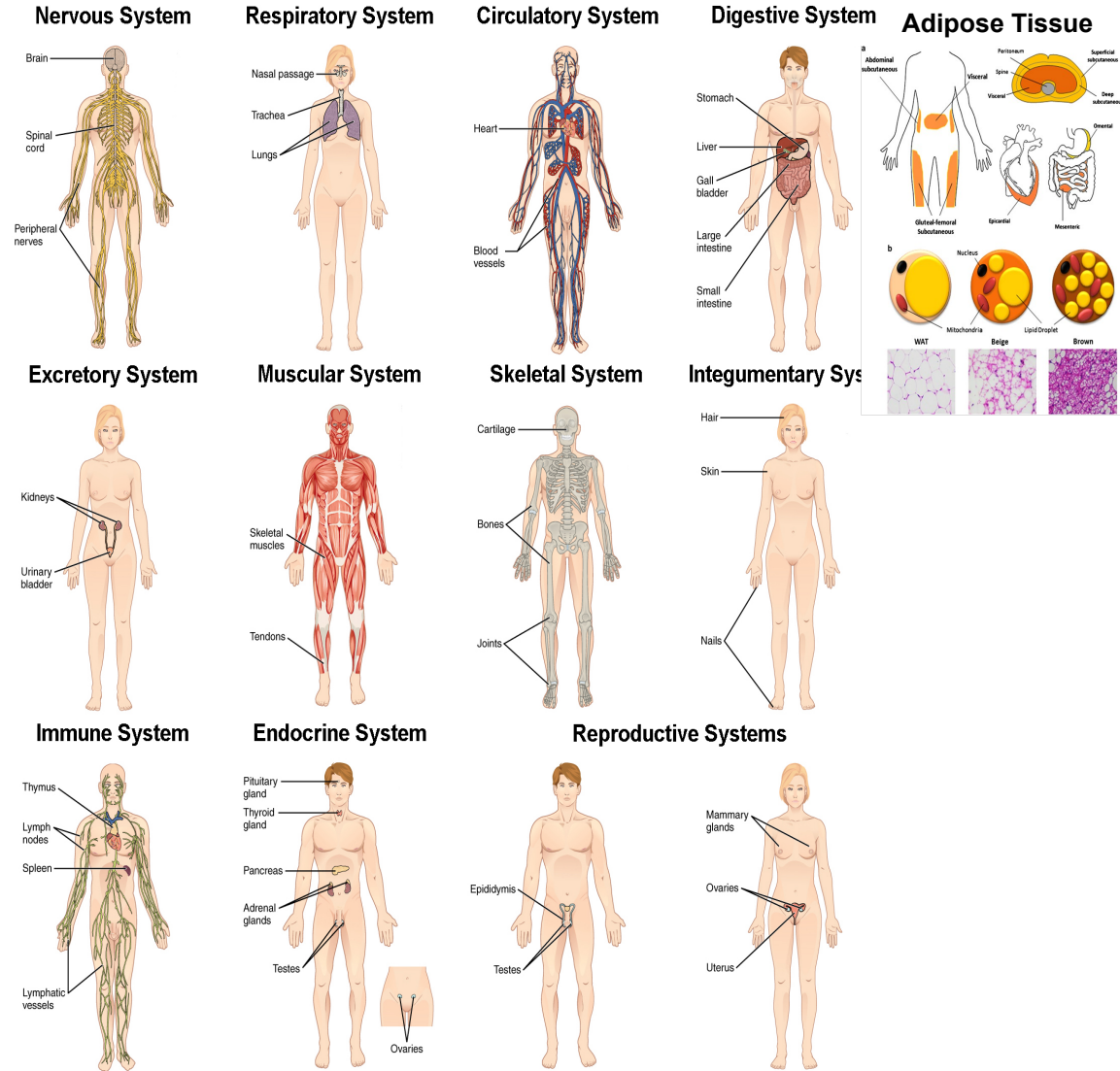


MixOmics data integration comparison at 2.5, 250 and 2500 µg/kg/day of BPA at PND180 in males.

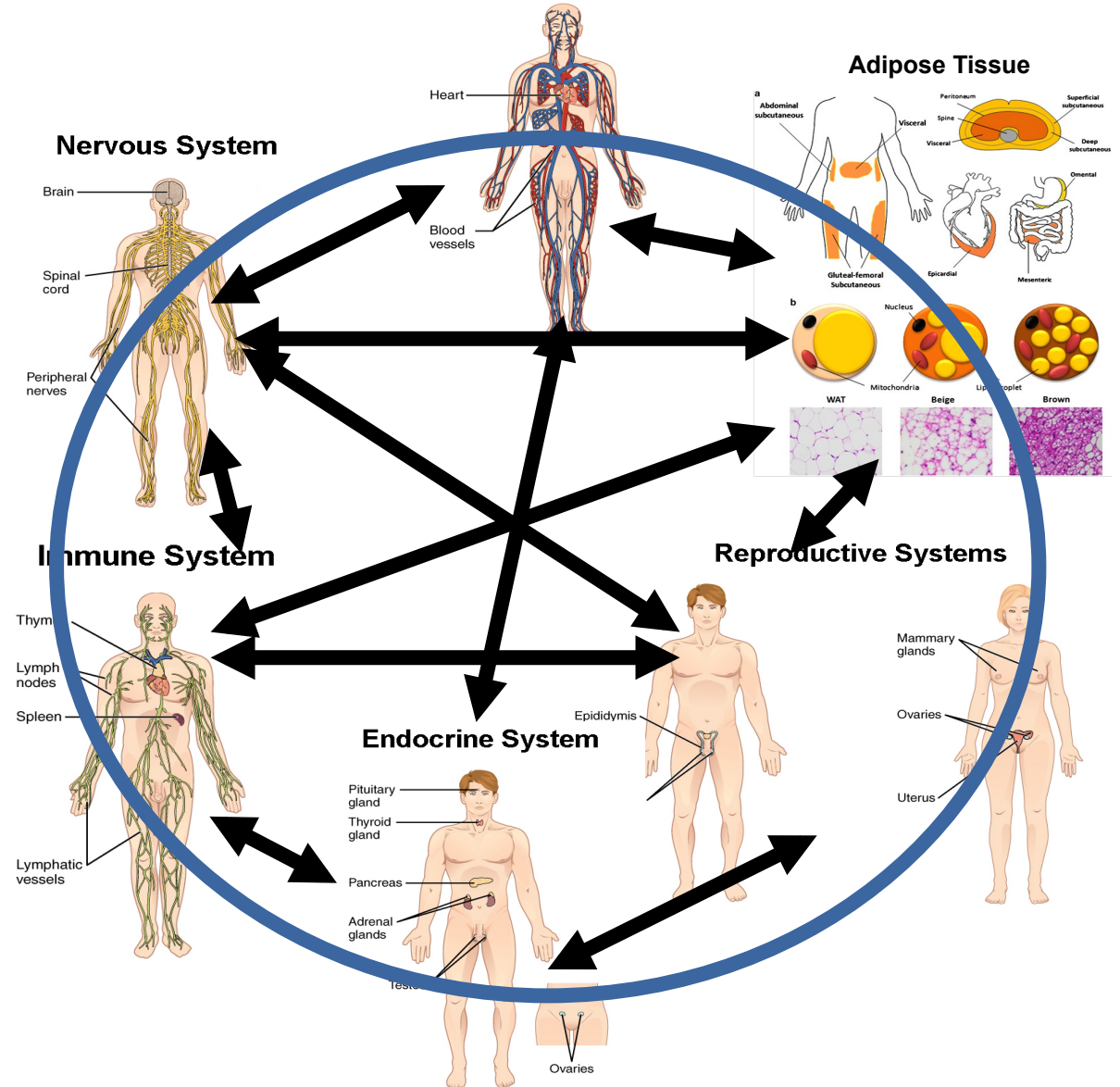
		Correlation coefficient		
Phenotype A	Phenotype B	BPA2.5	BPA250	BPA2500
White adipose tissue results	Adipose tissue weight	0.55	0.71	0.74
	Heart results	0.17	0.4	0.57
	Splenic function results	0.62	0.67	0.72
Macrophage in white adipose tissue	Prostate results	0.28	0.58	ND
Adipose tissue weight	Heart results	0.68	0.73	0.66
	Peptide hormones	0.39	0.72	0.45
	Prostate results	0.57	0.85	ND
Heart results	Splenic function results	0.7	0.81	0.56
	Peptide hormones	0.42	0.66	0.27
	Prostate results	0.65	0.69	ND
Peptide hormones	Splenic function results	0.5	0.72	0.69
	Splenic function results	0.82	0.66	0.76
Prostate results	Splenic function results	0.6	0.7	ND

Rethinking How We View Organ Systems and Effects of BPA on the Human Body

Old Way



New Way Circulatory System



Conclusions

- To our knowledge, the current work represents the **first study** to use this informatics approach to **integrate data across multiple independent investigator labs**.
- By using a whole body integrative approach, it reveals that even **low doses of BPA currently considered safe by the FDA can simultaneously lead to effects in multiple organs** in both males and females and at all ages studied.
- The most surprising correlations were those associated with BPA-exposure, the immune system, and other systems in both females and males.
- The main limitation of the mixOmics approach is that it can only establish correlation. However, follow-up studies can then be designed to determine which BPA-induced changes may be precipitating factors and lead to systemic consequences.