TidyMass: An Object-oriented Reproducible Analysis Framework for LC-MS Data

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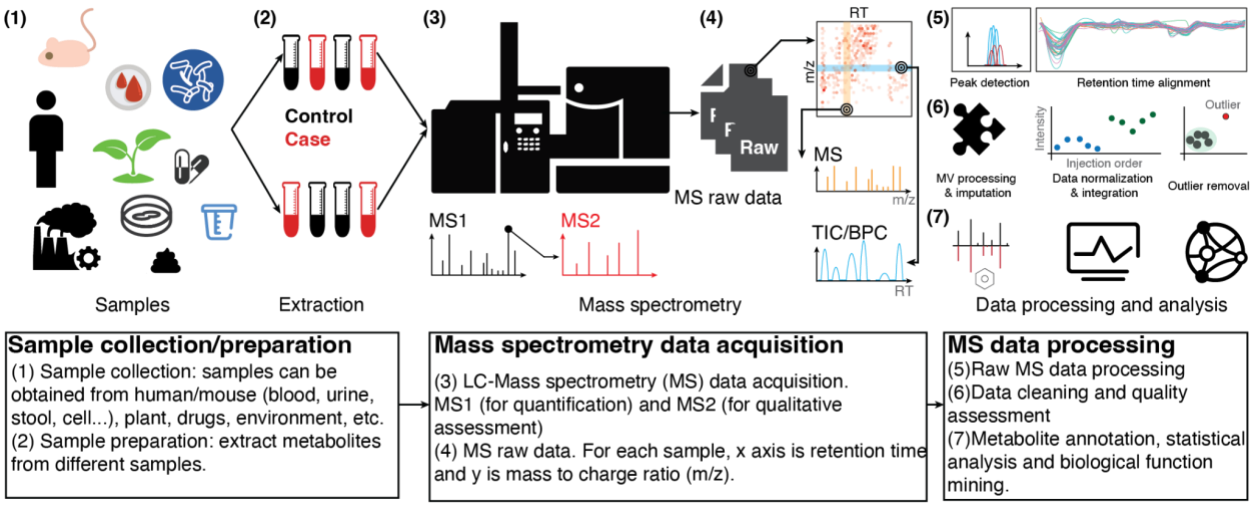
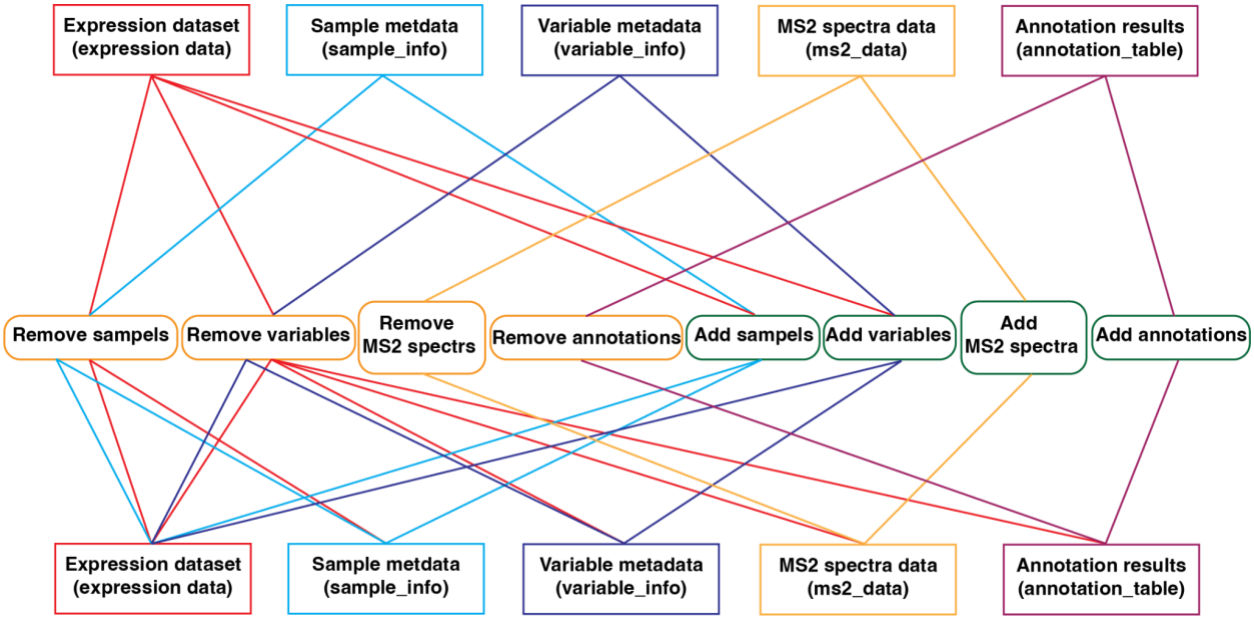
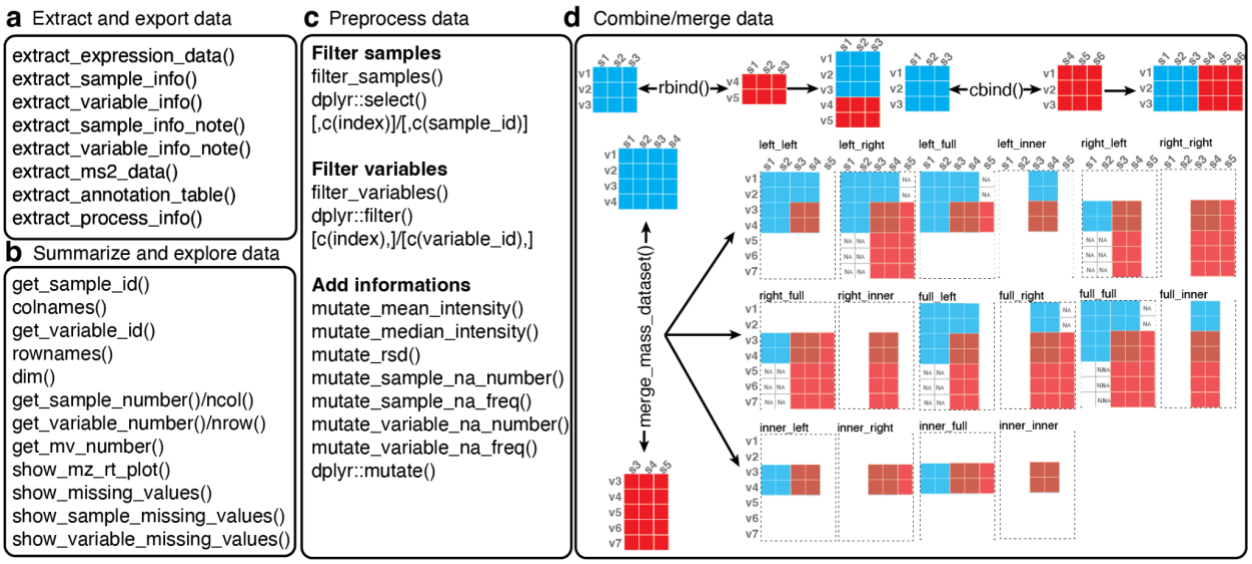


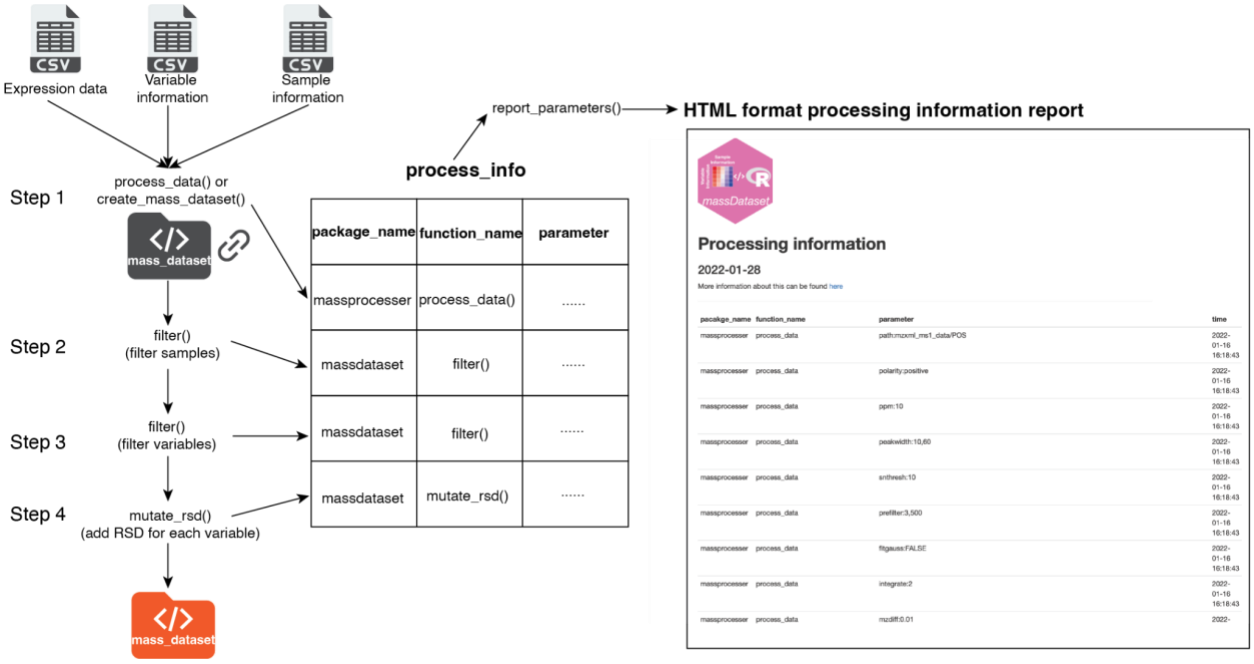
Supplementary Figure 1. Example of an untargeted high-resolution LC-MS experiment. A diagram of an experimental and analysis workflow for LC-MS-based untargeted metabolomics. LC-MS-based untargeted metabolomics involves several fundamental steps: **(1)** sample collection and preparation; **(2)** metabolite extraction; **(3)** mass spectrometry data acquisition and raw data generation; **(4)**, **(5)** raw data processing; **(6)** data cleaning; and **(7)** metabolite annotation and biological function mining. The intended role for *tidymass* is in MS data processing and analysis (steps 5-7).



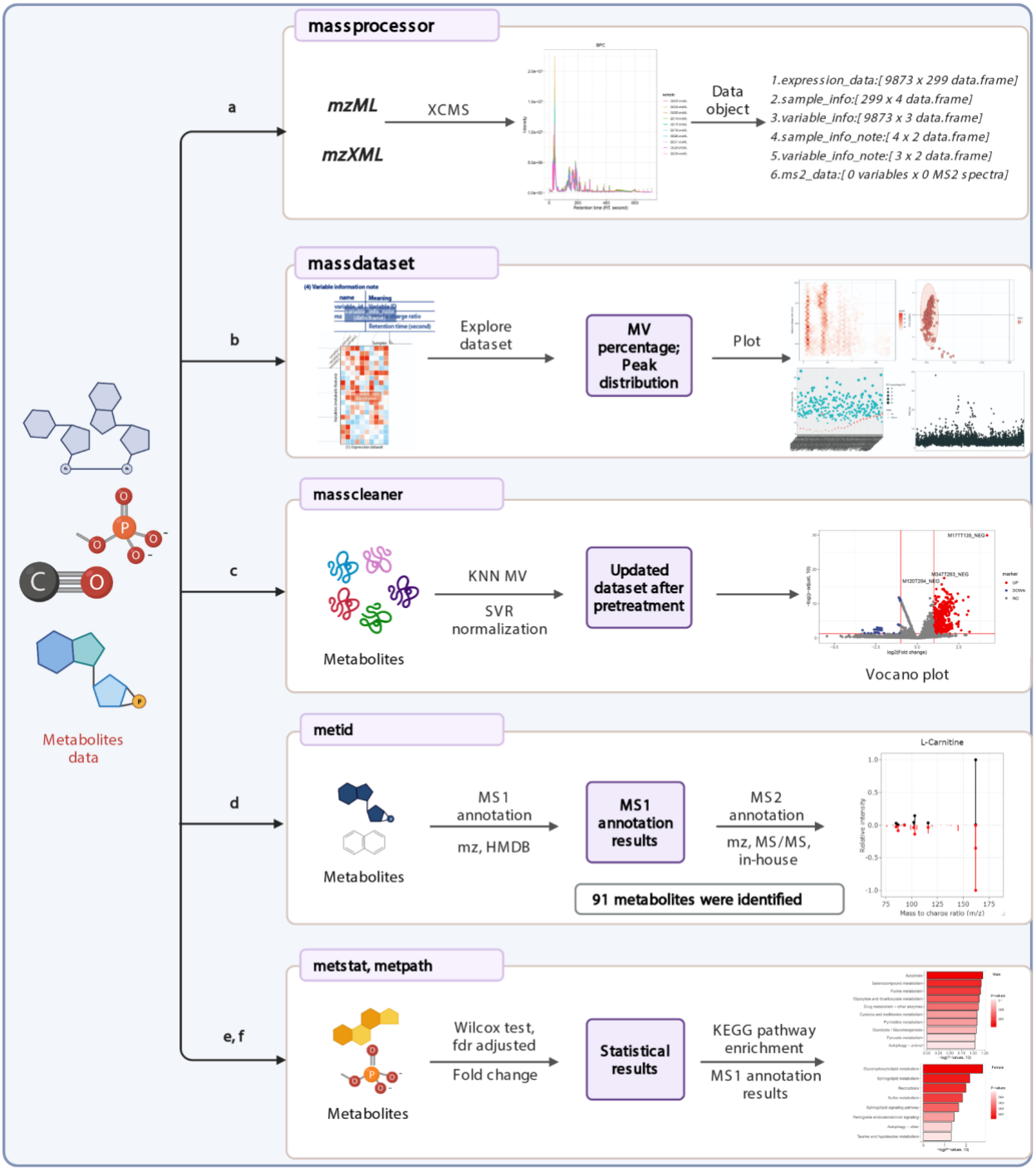
Supplementary Figure 2. Automatic synchronization of components in the "mass_dataset" class.



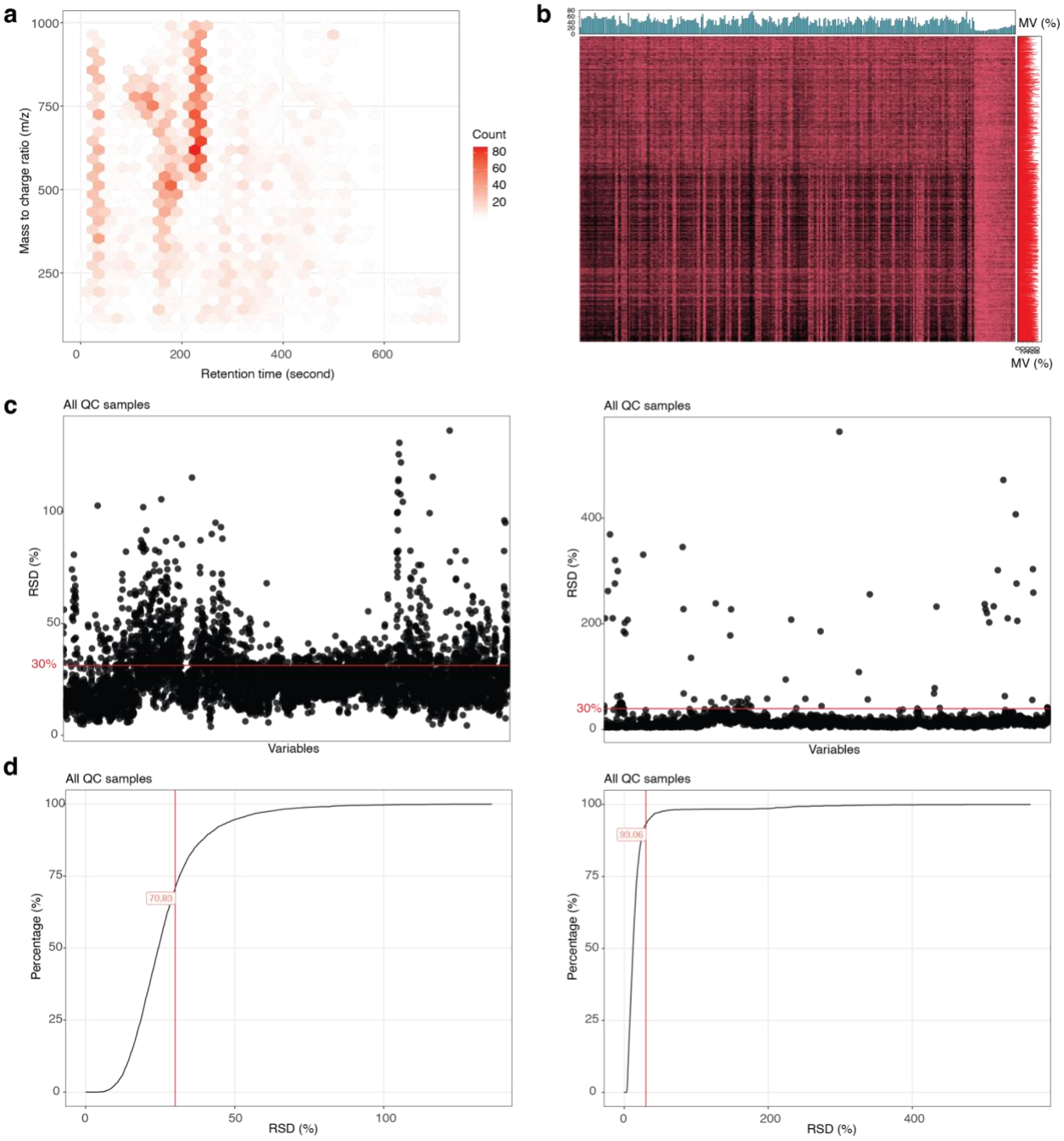
Supplementary Figure 3. Functions that support the "mass_dataset" class. (a) Functions to extract and export data. **(b)** Functions to summarize and explore data. **(c)** Functions for preprocessing data. (d) Functions to combine/merge data.



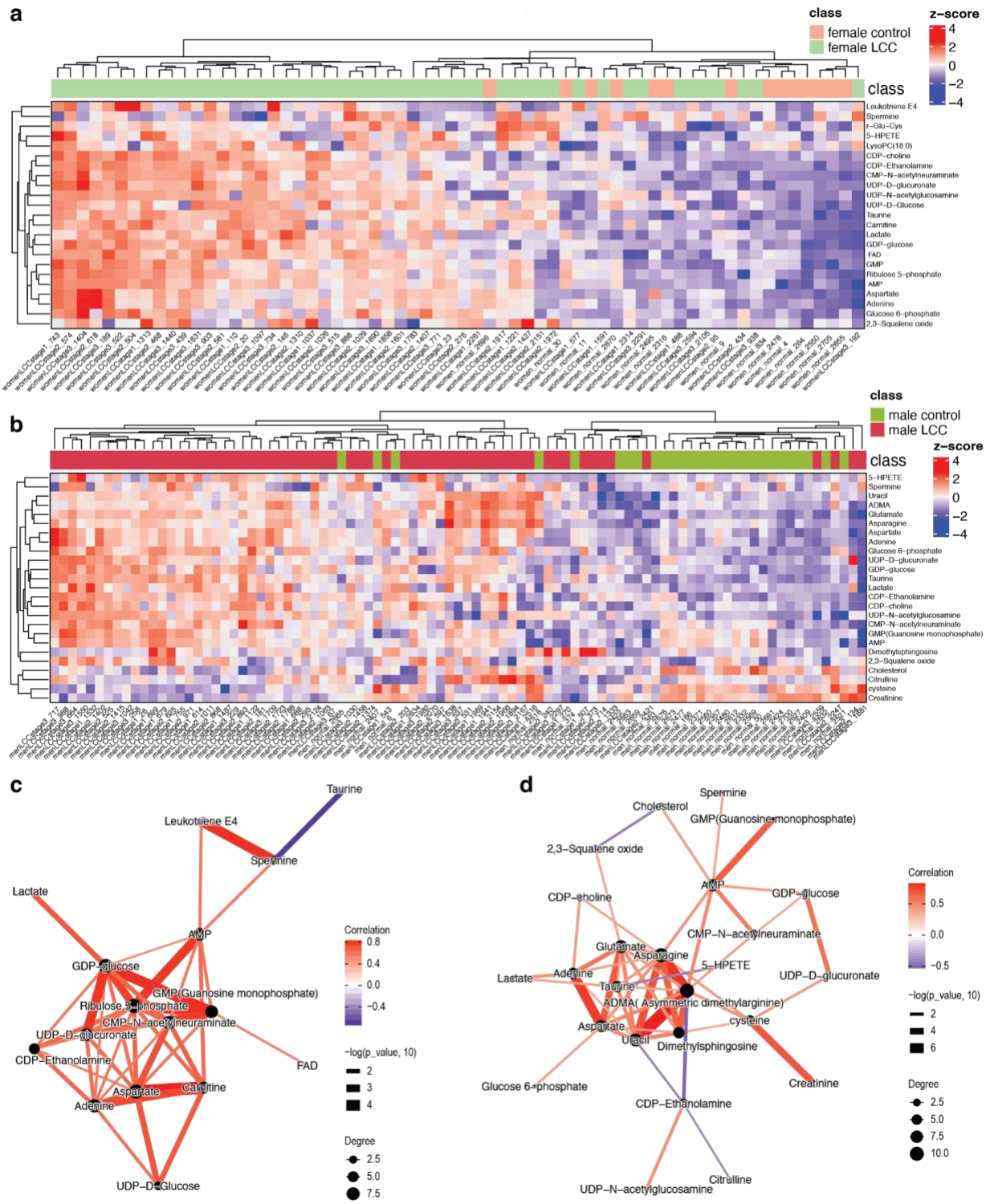
Supplementary Figure 4. Processing information in the "mass_dataset" class. Different functions from different packages can apply to the "mass_dataset" class step by step, and the parameters will be recorded in the "process_info" slot. The "report_parameters()" function from the massDataset package can be used to extract them and output them as an HTML format processing information report.



Supplementary Figure 5. Analysis workflow of the case study. **(a)**, **(b)**, **(c)**, **(d)**, **(e)**, and **(f)** correspond to the step in **Fig. 2**.



Supplementary Figure 6. Data quality assessment before and after data cleaning using tidyMass. Here the HILIC positive mode data is used as an example. **(a)** The distribution of metabolic features. **(b)** Missing distribution. The X-axis displays samples and the y-axis displays metabolic features. **(c)** RSD for all the metabolic features in QC samples before (left) and after data cleaning (right). **(d)** RSD cumulative plot before (left) and after data cleaning (right).



Supplementary Figure 7. Differential expressed metabolites (DEMs) for tumors from female and male patients with left-sided colorectal cancer (LCC). (a) Heatmap for normal colon (control) and tumor tissues from female patients using DEMs. **(b)** Heatmap for normal colon (control) and tumor tissues from male patients using DEMs. **(c)** Correlation networks for metabolites dysregulated in tumors compared to normal tissues from female **(c)** and male patients **(d)**.

Supplementary Table 1. Functions in the tidyMass project for data processing and analysis.

Function name	package	Input data format	Output	Fcuntion
docker_pull_pwiz()	massConverter	-	-	Pull pwiz docker image.
create_msconvert_parameter()	massConverter	-	msconvert_parameter	Create parameter class for masssConverter.
convert_raw_data()	massConverter	Mass spectrometry raw data	Other format data	Convert MS raw data to other format data
create_mass_dataset()	massDataset	-	"mass_dataset" class object	Create "mass_dataset" class object
extract_expression_data()	massDataset	"mass_dataset" class object	data.frame	Extract expression data
extract_sample_info()	massDataset	"mass_dataset" class object	data.frame	Extract sample information
extract_variable_info()	massDataset	"mass_dataset" class object	data.frame	Extract variable inforamtion
extract_annotation_table()	massDataset	"mass_dataset" class object	data.frame	Extract annotation table
extract_variable_info_note()	massDataset	"mass_dataset" class object	data.frame	Extract variable information metadata
extract_sample_info_note()	massDataset	"mass_dataset" class object	data.frame	Extract sample information metadata
extract_process_info()	massDataset	"mass_dataset" class object	"tidymass_parameter" class	Extract processing information
extract_ms2_data()	massDataset	"mass_dataset" class object	"ms2_data" class	Extarc MS^2 data
mz_rt_match()	massTools	"mass_dataset" class object	data.frame	Match two "mass_dataset" class according to mz and rt
fliter_samples()	massDataset	"mass_dataset" class object	"mass_dataset" class object	Filter samples
filter_variabels()	massDataset	"mass_dataset" class object	"mass_dataset" class object	Filter variables

filter()	dplyr	“mass_dataset” class object	“mass_dataset” class object	Filter rows from components in “mass_dataset” class
mutate()	dplyr	“mass_dataset” class object	“mass_dataset” class object	Mutate new columns to components in “mass_dataset” class
select()	dplyr	“mass_dataset” class object	“mass_dataset” class object	Select columns from components in “mass_dataset” class
mutate_ms2()	massDataset	“mass_dataset” class object	“mass_dataset” class object	Add MS^2 data to “mass_dataset” class
mutate_mean_intensity()	massDataset	“mass_dataset” class object	“mass_dataset” class object	Add mean intensity to variable information
mutate_median_intensity()	massDataset	“mass_dataset” class object	“mass_dataset” class object	Add median intensity to variabel information
mutate_rsd()	massDataset	“mass_dataset” class object	“mass_dataset” class object	Add RSD to variable information
mutate_sample_na_number()	massDataset	“mass_dataset” class object	“mass_dataset” class object	Add variable NA numbers to sample inforamtion
mutate_sample_na_freq()	massDataset	“mass_dataset” class object	“mass_dataset” class object	Add variable NA frequency to sample inforamtion
mutate_sample_na_number()	massDataset	“mass_dataset” class object	“mass_dataset” class object	Add sample NA numbers to variable inforamtion
mutate_sample_na_freq()	massDataset	“mass_dataset” class object	“mass_dataset” class object	Add sample NA frequency to variable inforamtion
report_parameters()	massDataset	“mass_dataset” class object	HTML format report	Report for processing information
show_mz_rt_plot()	massDataset	“mass_dataset” class object	ggplot2 plot class object	Metabolic feature plot
show_missing_values()	massDataset	“mass_dataset” class object	ggplot2 plot class object	Missing value distribution plot
left_join()	dplyr	“mass_dataset” class object	“mass_dataset” class object	Mutate new columns to components in “mass_dataset” class

cbind()	base	“mass_dataset” class object	“mass_dataset” class object	Bind two “mass_dataset” class objects by rows
rbind()	base	“mass_dataset” class object	“mass_dataset” class object	Bind two “mass_dataset” class objects by columns
merge_mass_dataset()	massDataset	“mass_dataset” class object	“mass_dataset” class object	Merge two “mass_dataset” class objects
process_data()	massProcesser	mzXML format data	“mass_dataset” class object	Raw data processing
extract_eic()	massProcesser	-	-	Extract EIC
detect_outlier()	massCleaner	“mass_dataset” class object	“outlier_samples” class object	Detect outlier samples
extract_outlier_table()	massCleaner	“outlier_samples” class object	data.frame	Outlier sample table
impute_mv()	massCleaner	“mass_dataset” class object	“mass_dataset” class object	Impute missing values
integrate_data()	massCleaner	“mass_dataset” class object	“mass_dataset” class object	Interate data
normalize_data()	massCleaner	“mass_dataset” class object	“mass_dataset” class object	Normalize data
optimize_loess_span()	massCleaner	“mass_dataset” class object	data.frame	Optimize the parameters for loess regression
align_batch()	massCleaner	“mass_dataset” class object	“mass_dataset” class object	Align two “mass_dataset” class objects
massqc_cumulative_rsd_plot()	massQC	“mass_dataset” class object	ggplot2 plot class object	Cummulative RSD plot
massqc_pca()	massQC	“mass_dataset” class object	ggplot2 plot class object	PCA score plot
massqc_rsd_plot()	massQC	“mass_dataset” class object	ggplot2 plot class object	RSD distribution plot
massqc_sample_box plot()	massQC	“mass_dataset” class object	ggplot2 plot class object	Sample box plot
massqc_sample_correlation()	massQC	“mass_dataset” class object	ggplot2 plot class object	Smaple correlation plot
massqc_report()	massQC	“mass_dataset” class	HTML format report	HTML format QC

		object		report
annotate_metabolites_mass_dataset()	metID	“mass_dataset” class object	“mass_dataset” class object	Annotate features in “mass_dataset” by databases
annotate_single_peak_mass_dataset()	metID	“mass_dataset” class object	“mass_dataset” class object	Annotate one feature in “mass_dataset” by databases
ms2_plot_mass_dataset()	metID	“mass_dataset” class object	ggplot2 plot class object	MS^2 matching plot
convert_dummy_variable()	massStat	vector	data.frame	Convert vector to dummy variable
convert_mass_dataset2graph()	massStat	“mass_dataset” class object	tbl_gh class	Convert “mass_dataset” class to graph object
cor_mass_dataset()	massStat	“mass_dataset” class object	data.frame	Correlation matrix
dist_mass_dataset()	massStat	“mass_dataset” class object	data.frame	Distance matrix
Heatmap()	massStat	ComplexHeatmap	ggplot2 plot class object	Heatmap plot
pls()	massStat	mixOmics	“pls” object	PLS analysis
plsda()	massStat	mixOmics	“plsda” object	PLS-DA analysis
mutate_fc()	massStat	“mass_dataset” class object	“mass_dataset” class object	Add fold changs to variable inforamtion
mutate_p_value()	massStat	“mass_dataset” class object	“mass_dataset” class object	Univariable test
run_pca()	massStat	“mass_dataset” class object	pca class object	PCA analysis
pca_score_plot()	massStat	pca class object	ggplot2 plot class object	PCA score plot
scale_data()	massStat	“mass_dataset” class object	“mass_dataset” class object	Scale data
volcano_plot()	massStat	“mass_dataset” class	ggplot2 plot class object	Volcano plot
filter_pathway()	metPath	“pathway_database” class	“pathway_database” class	Filter pathways
get_hmdb_pathway()	metPath	-	“pathway_database” class	Get or download HMDB pathway

get_kegg_pathway()	metPath	-	“pathway_database” class	Get or download KEGG pathway
enrich_kegg()	metPath	Query metabolite ID	“enrich_result” class	Pathway enrichment
enrich_hmdb()	metPath	Query metabolite ID	“enrich_result” class	Pathway enrichment
enrich_bar_plot()	metPath	“enrich_result” class	ggplot2 plot class object	Barplot to show the enriched pathways
enrich_scatter_plot()	metPath	“enrich_result” class	ggplot2 plot class object	Scatter plot to show the enriched pathways
enrich_network()	metPath	“enrich_result” class	ggplot2 plot class object	Network to show the enriched pathways

Supplementary Table 2. Parameters for conversion using massConvereter.

Argument name	Meaning	Format	Range
output_format	Output format	character	mzXML, mzXL, mz5, mgf, text, ms1, cms1, ms2, cms2
binary_encoding_precision	Binary encoding precision	character	32 or 64
zlib = TRUE	Zlib or not	logical	TURE or FALSE
write_index	Write index or not	logical	TRUE or FALSE
peak_picking_algorithm	Peak picking algorithm	character	vendor, cwt, no
vendor_mslevels	Vender MS levels	vector	1-n
cwt_mslevels	Cwt MS level	vector	1-n
cwt_min_snr	Cwt minimum signal to noise rate	numeric	0-1
cwt_min_peak_spacing	Cwt minimum peak spacing	numeric	0-1
subset_polarity	Subset polarity	character	any, positive, negative
subset_scan_number	Subset scan number	vector	0-n
subset_scan_time	Subset scan time	vector	0-n
subset_mslevels	Subset MS level	vector	1–n
zero_samples_mode	Zero sample mode	character	no, removeExtra, addMissing
zero_samples_mslevels	Zero sample MS levels	vector	1-n
zero_samples_add_missing_flanking_zero_count	Zero sample add missing flanking zero count	numeric	0-10

Supplementary Note

Colon tumor tissues and normal colon tissues were acquired from surgery and prospectively collected on 736 stage I-IV CRC patients in the period 1991–2001 at Memorial Sloan-Kettering Cancer Center (MSKCC, New York, NY, United States)[1]. Clinical data were recorded and updated retrospectively. Each tissue sample was snap-frozen in liquid nitrogen in the operating room and immediately stored in a −80 °C freezer. For this study, samples were selected that were from patients ≥ 55 years old to reduce the

confounding effects of estrogen signaling on metabolism before menopause, also patients with early-onset CRC have a different etiology and biology than later-onset CRC. All normal colon tissues were selected from stage I-IV CRC patients (n = 39), and tumor tissue samples were selected from RCCs (right-sided colon cancer) and LCCs (left-sided colon cancer) stage I-III (n = 197). Only the normal and LCC samples were used for subsequent analysis. Clinical information was provided in **Table S3**.

Supplementary Table 3. Demographics of colon cancer patients from samples used within this study. Right-sided colon cancer = RCC, Left-sided colon cancer = LCC.

	Normal (n=39)	Stage I (n=47)		Stage II (n=86)		Stage III (n=64)	
		RCC (n=22)	LCC (n=25)	RCC (n=44)	LCC (n=42)	RCC (n=32)	LCC (n=32)
Sex, n							
Male	27	10	15	23	25	15	14
Female	12	12	10	21	17	17	18
				Age, mean (sd)			
Male	69.3 (9.6)	73.9 (6.5)	69.3 (5.8)	72.9 (7.8)	72.2 (8.5)	73.5 (7.8)	63.7 (5.8)
Female	63.3 (16.1)	72.1 (6.2)	69.6 (7.6)	73.5 (9.8)	69.1 (7.8)	72.2 (6.6)	71.1 (6.0)
				Race/Ethnicity, n			
NHWs	35	20	22	40	37	26	25
Hispanic	2	2	3	2	3	4	2
AA	1	0	0	1	0	1	4
API	1	0	0	1	2	1	1

NHWs: non-Hispanic whites, AA: African-Americans, API: Asian-Pacific Islander

Supplementary Data 1. Processed data ("mass_dataset" class) from the massProcesser package.
Supplementary Data 2. Code file (Rmd format) for the case study.

References

1. Cai, Y. *et al.* Sex Differences in Colon Cancer Metabolism Reveal A Novel Subphenotype. *Sci. Rep.* **10**, 4905 (2020).