Assessing the effect of two factors on a continuous response variable

y ~ a + b + a*b

Parametric test: 2-way ANOVA

→ Tipp: use anova_test() to account for repeated measures (package **rstatix** in R)

→ Links:

https://www.r-bloggers.com/2021/04/repeated-measures-of-anova-in-r-complete-tutorial/

Non-parametric test: Aligned ranks transformation ANOVA (ART ANOVA)

→ package **ARTool** in R

→ Tipp: use Error(term) to account for repeated measures

→ Links:

https://cran.r-project.org/web/packages/ARTool/readme/README.html
https://depts.washington.edu/acelab/proj/art/

Differential abundance testing

Goal: identify features that show statistically significant differences in a response variable across certain conditions
Origin: RNAseq data → contain many zeros (needs to be taken into account in each model)

Feature table

	S1	S2	S3	S4	S5	S6
Feature 1	350	4000	0	1500	100	500
Feature 2	20	10	5000	650	780	8000
Feature 3	9000	6500	800	880	400	30
Feature 4	0	0	80	3000	3500	7000
…	…	…	…	…	…	…
Feature n	7800	200	4500	60	80	20

+

Metadata table

SampleID	Treatment	Time	Sex
S1	Control	1	M
S2	Treatment	1	M
S3	Treatment	3	F
S4	Treatment	4	F
…	…	…	…
Sn	Treatment	2	M

- $Y_{Feature\ 1}$ ~ Treatment (+ …)
- Multiple testing
- **DESeq2** package in R
- ATTENTION: use non-rarefied data (internal normalization within DESeq-function)

Links:
https://bioc.ism.ac.jp/packages/2.14/bioc/vignettes/DESeq2/inst/doc/beginner.pdf
Plotting: https://github.com/kevinblighe/EnhancedVolcano