Gordon Andrews (designer)
Gazelle chair (c. 1950) designed, 1957 manufactured
plywood, aluminium, wool
74.0 x 48.0 x 55.0 cm
Museum of Applied Arts and Sciences, Sydney
Purchased, 1989 (89/499)

ECOSCOPE
routing the microcosmos

Visual design principles for scientific data

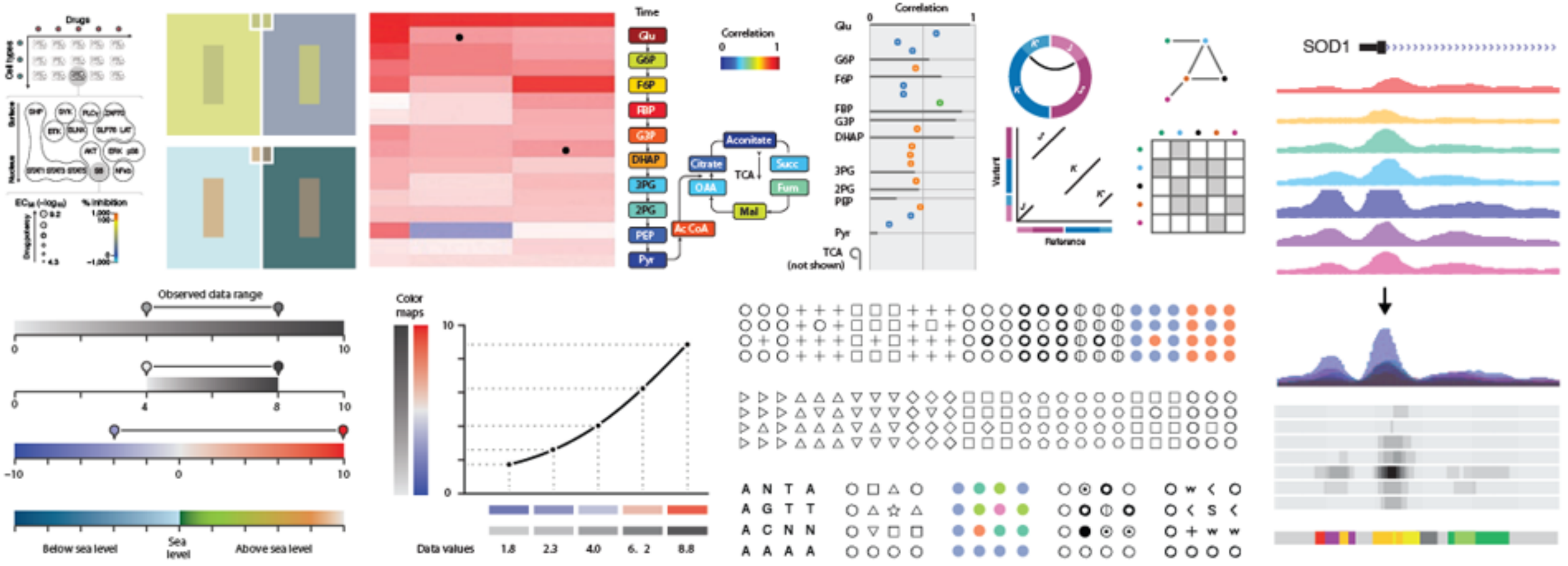
Martin Krzywinski
http://mkweb.bcgsc.ca

7 March 2016
12:30-1:30pm, LSC 3, Life Science Institute
2350 Health Sciences Mall, UBC

nature methods

POINTS OF VIEW

Wong · Krzywinski · Gehlenborg · Nielsen · Soresh · Kjaegaard · Savig · Cairo



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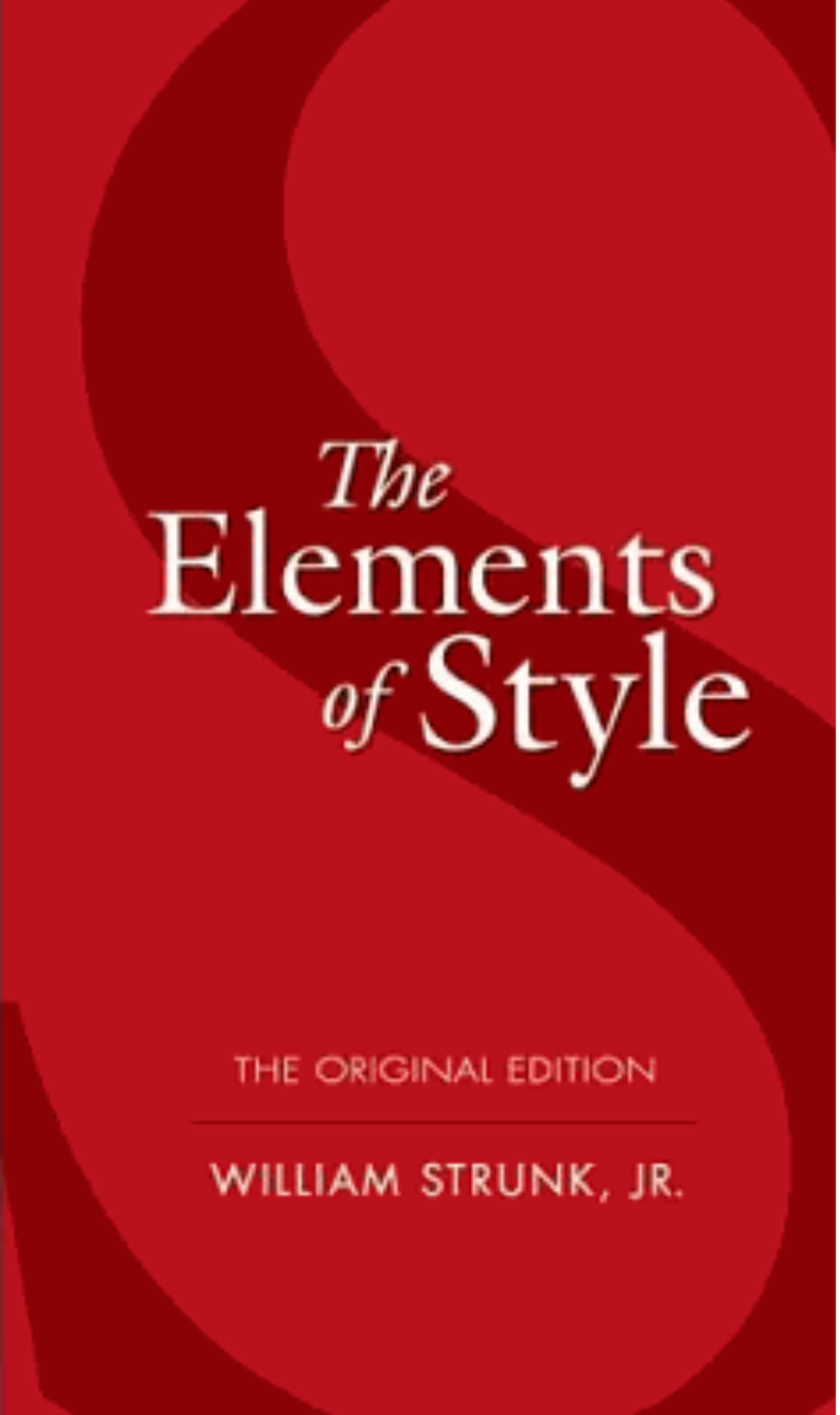

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POINTS OF VIEW

Elements of visual style

Translate the principles of effective writing to the process of figure design.

We all use words to communicate information—our ability to do so is extremely sophisticated. We have large vocabularies, understand a variety of written styles and effortlessly parse errors in real time. But when we need to present complex information visually, we may find ourselves 'at a loss for words', graphically speaking.

We can rationalize figure creation by applying principles of effective written communication. By leveraging our training and experience with words, we can turn graphical improvisation into a structured and reproducible process in which we assess and optimize each part of a figure just as we would each paragraph, sentence and word in a manuscript. Let's look at how Strunk and White's classic but stern *The Elements of Style*[1] can be applied to figures. (I encourage you to revisit your own favorite writing resources in the context of visual representation.)

Figure 1 | A flood of identical symbols triggers semantic satiation, a phenomenon in which overwhelming repetition results in loss of meaning. As an accurate but visually unparsable representation of a breakpoint graph[5], the figure breaks Strunk and White's rule "Do not explain too much."[1]

A popular example of disregarding Strunk and White's dictum "Do not take shortcuts at the expense of clarity"[1] is the syntactically correct but incomprehensible sentence "Buffalo buffalo Buffalo buffalo buffalo buffalo Buffalo buffalo"[2]. Unfortunately, visual analogs of this construct appear all too frequently in the literature. If we cannot parse this eight-word sentence, how can we cope with the complexity of **Figure 1**?

Strunk and White also ask us to avoid overwriting because "rich, ornate prose is hard to digest, generally unwholesome, and

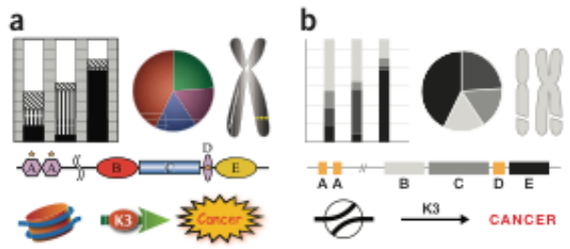

Figure 2 | Use the simplest visual representation[6] for objects and "omit needless words"[1]. (**a**) Visually garnished elements shout at the reader, who is at a loss to determine what is important. If you wouldn't write it this way, don't draw it either. (**b**) Simple shapes provide an elegant presentation. Complex shapes may carry unintended meaning (such as unduplicated versus duplicated chromosomes). In schematics, reserve the use of color for emphasis, where possible.

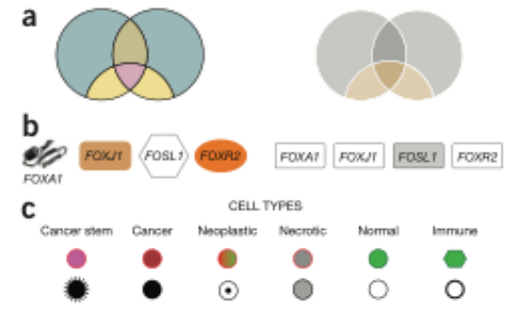

Figure 3 | Objects that interact or share common meaning should be formatted in a similar way that appeals to intuition. (**a**) Venn diagram colors should be selected to naturally communicate overlap. This can be automated by using blend modes in applications such as Illustrator or Inkscape. (**b**) Entity similarities in pathway diagrams are hard to identify when diverse icons are used. When only tone varies, *FOSL1* immediately stands out from the *FOX* gene family. (**c**) Symbols in a series should reflect the concept of progression as naturally as possible. For example, immune cells aren't actually a different shape, and it is not intuitive that pink cells should give rise to red cells.

sometimes nauseating"[1]. The visual equivalent is "chartjunk," a term coined by Tufte[3]. Examples are shimmering textures, gradients and a proliferation of shapes (**Fig. 2**), which all make interpreting the data more difficult, act as exclamation marks that make selective emphasis impossible, and "can never rescue a thin data set"[3]. If you cannot easily emphasize an element in your figure, chances are that it is overstated.

To reinforce the content and function of related ideas, use the visual equivalent of parallel construction and "express coordinate ideas in similar form"[1]. Choose shapes and colors that intuitively embody overlap, category hierarchy and importance (**Fig. 3**).

Keep in mind the needs and experience of your audience and "place yourself in the background"[1]: do not rely solely on your personal aesthetic (for example, black text overlaid on your favorite color may lack sufficient contrast to be legible). Instead, strive for simplicity and clarity. "Use definite, specific, concrete language"[1]. Be legible without shouting. Concise, but not opaque.

In his play *Horace*, Corneille wrote, "Un premier mouvement ne fut jamais un crime" ("A first impulse was never a crime")[4]. But in the process of making figures, it can be. Avoid the temptation of going with your first idea. Instead, use it as the starting point and then refine and clarify your message. A good figure, like good writing, doesn't simply happen—it is crafted. "Revise and rewrite"[1] becomes "revise and redraw."

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

Martin Krzywinski

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2. Pinker, S. *The Language Instinct* (W. Morrow, New York, 1994).
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4. Corneille, P. *Horace* (http://openlibrary.org/books/OL6939036M/Corneille's_Horace/) line 1648 (Heath, 1904).
5. Alekseyev, M.A. & Pevzner, P.A. *Genome Res.* **19**, 943–957 (2009).
6. Wong, B. *Nat. Methods* **8**, 611 (2011).

Martin Krzywinski is a staff scientist at Canada's Michael Smith Genome Sciences Centre.

top-down

bottom-up

redundancy

consistency

conciseness

clarity

focus & emphasis

salience & relevance

accuracy & detail

INFORMS

SATISFIES

data encoding

symbols

color

typeface

arrows

line weight

alignment

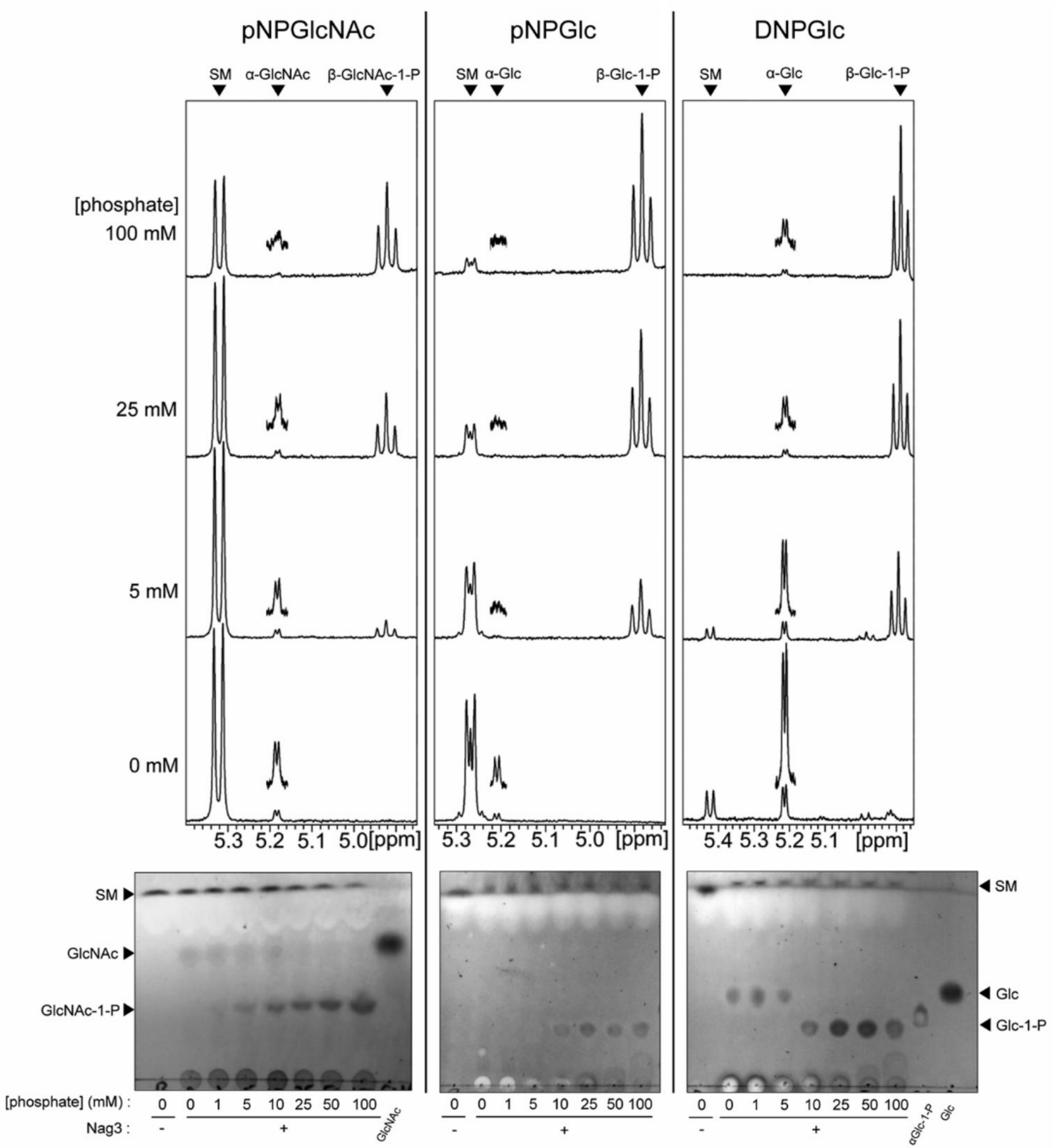
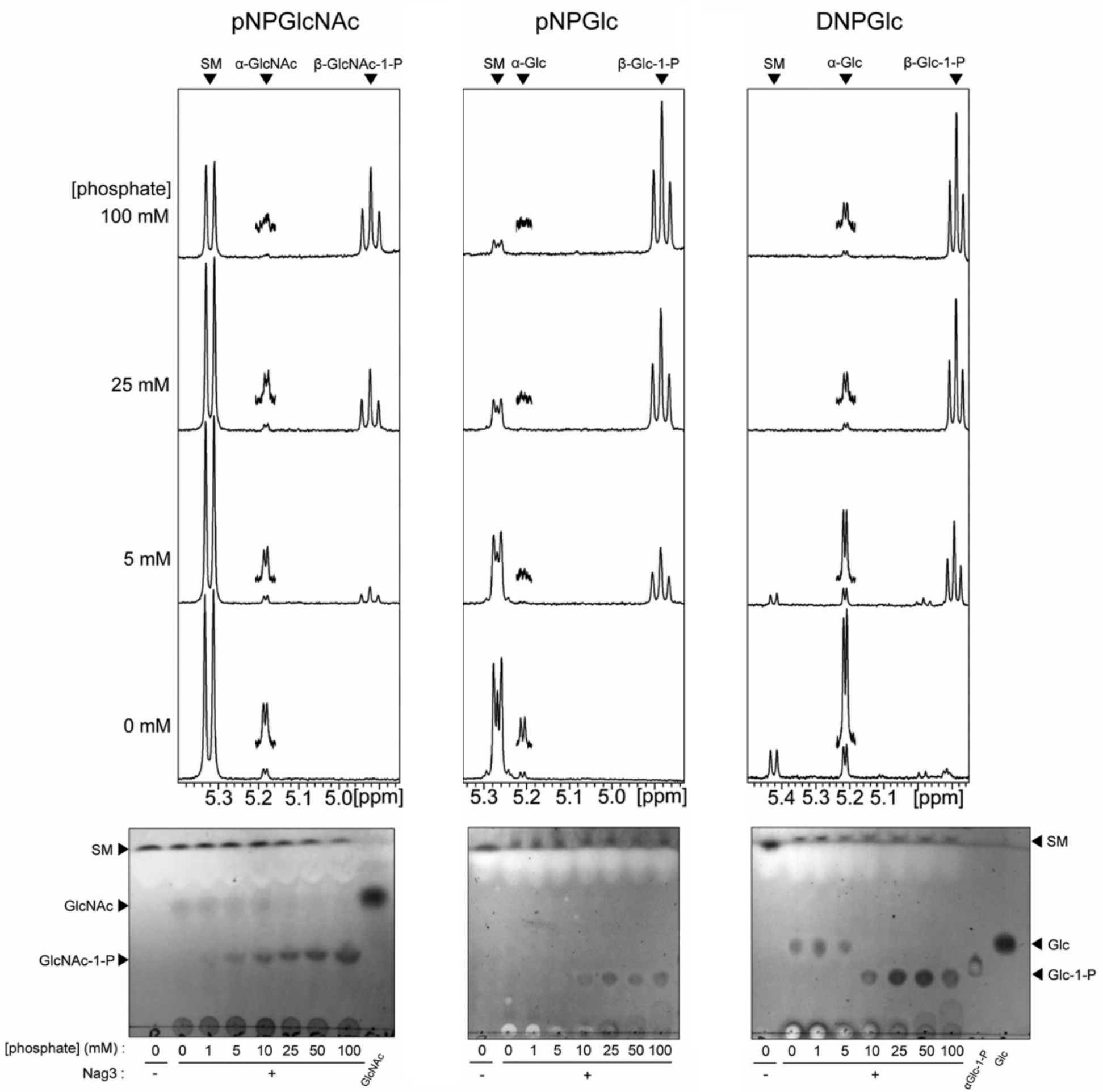


FIGURE 2. *Top panel,* ^{1}H NMR spectra for Nag3 and 10 mM pNPGlcNAc, pNPGlc, and DNPGlc with 0, 5, 25, and 100 mM phosphate. Insets show $\times 4$ amplification of the α-Glc/α-GlcNAc peak regions. Unfortunately the β-GlcNAc and β-Glc anomeric proton falls right under the large peaks from residual HOD, thus only the peaks from α-anomers are shown. *Bottom panel,* TLC analysis of Nag3 hydrolysis and phosphorolysis of 10 mM pNPGlcNAc, pNPGlc, and DNPGlc with 0, 1, 5, 10, 25, 50, and 100 mM phosphate. The aryl glycoside starting materials (*SM*) are labeled.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pNPGlc
DNPGlc
SM
α-GlcNAc
β-GlcNAc-1-P
SM
α-Glc
β-Glc-1-P
SM
α-Glc
β-Glc-1-P
[phosphate]
100 mM
25 mM
5 mM
0 mM
5.3 5.2 5.1 5.0[ppm]
5.3 5.2 5.1 5.0 [ppm]
5.4 5.3 5.2 5.1 [ppm]
SM
GlcNAc
GlcNAc-1-P
SM
Glc
Glc-1-P
[phosphate] (mM) :
0 0 1 5 10 25 50 100
Nag3 :
- +
GlcNAc
0 0 1 5 10 25 50 100
- +
0 0 1 5 10 25 50 100
- +
αGlc-1-P
Glc

pNPGlcNAc

pNPGlc

DNPGlc

SM α-GlcNAc β-GlcNAc-1-P

SM α-Glc β-Glc-1-P

SM α-Glc β-Glc-1-P

[phosphate]
100 mM

25 mM

5 mM

0 mM

5.3 5.2 5.1 5.0[ppm]

5.3 5.2 5.1 5.0 [ppm]

5.4 5.3 5.2 5.1 [ppm]

SM

GlcNAc

GlcNAc-1-P

[phosphate] (mM) : 0 0 1 5 10 25 50 100

Nag3 : - +

GlcNAc

0 0 1 5 10 25 50 100

- +

SM

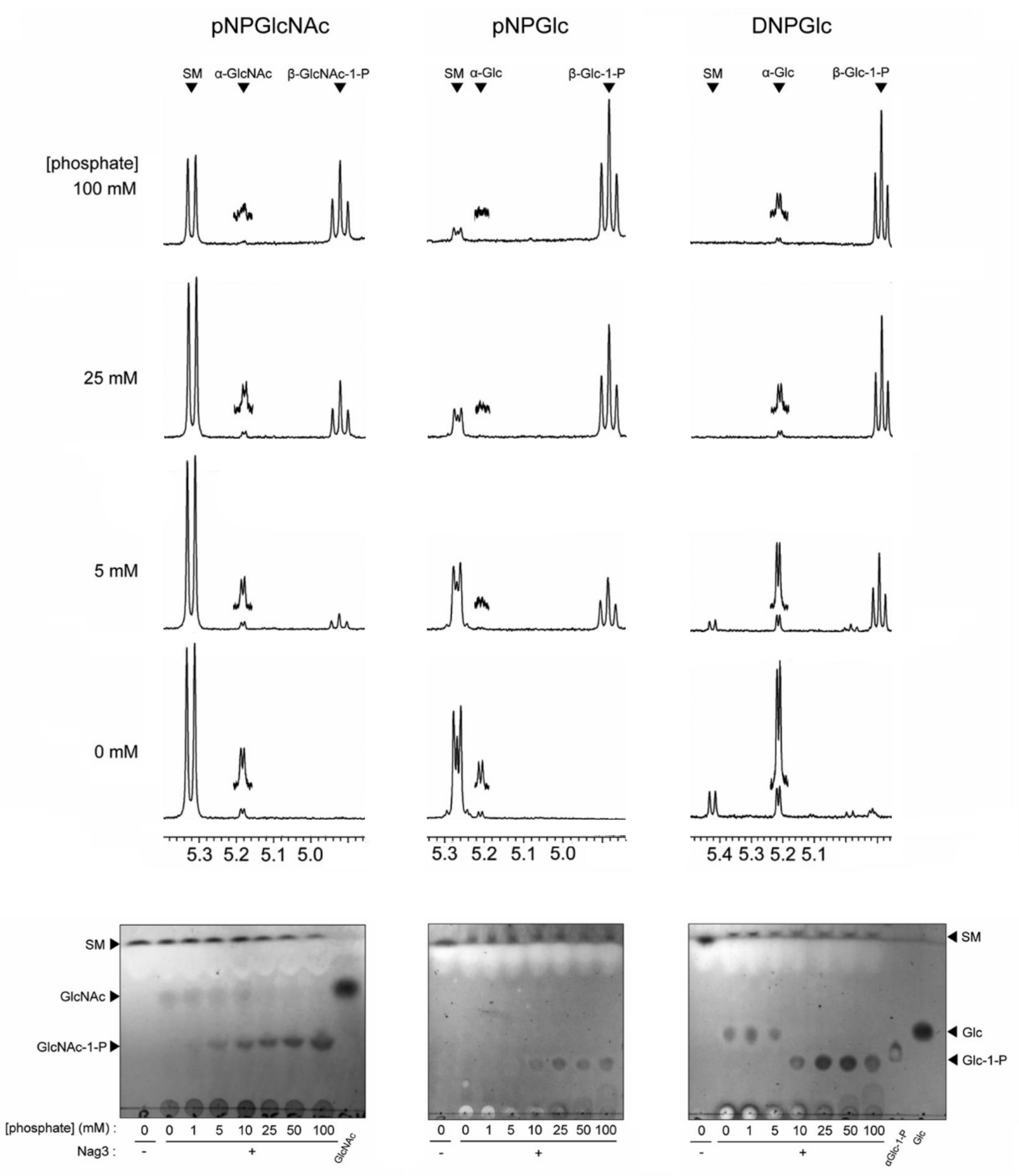
Glc

Glc-1-P

0 0 1 5 10 25 50 100

- +

αGlc-1-P Glc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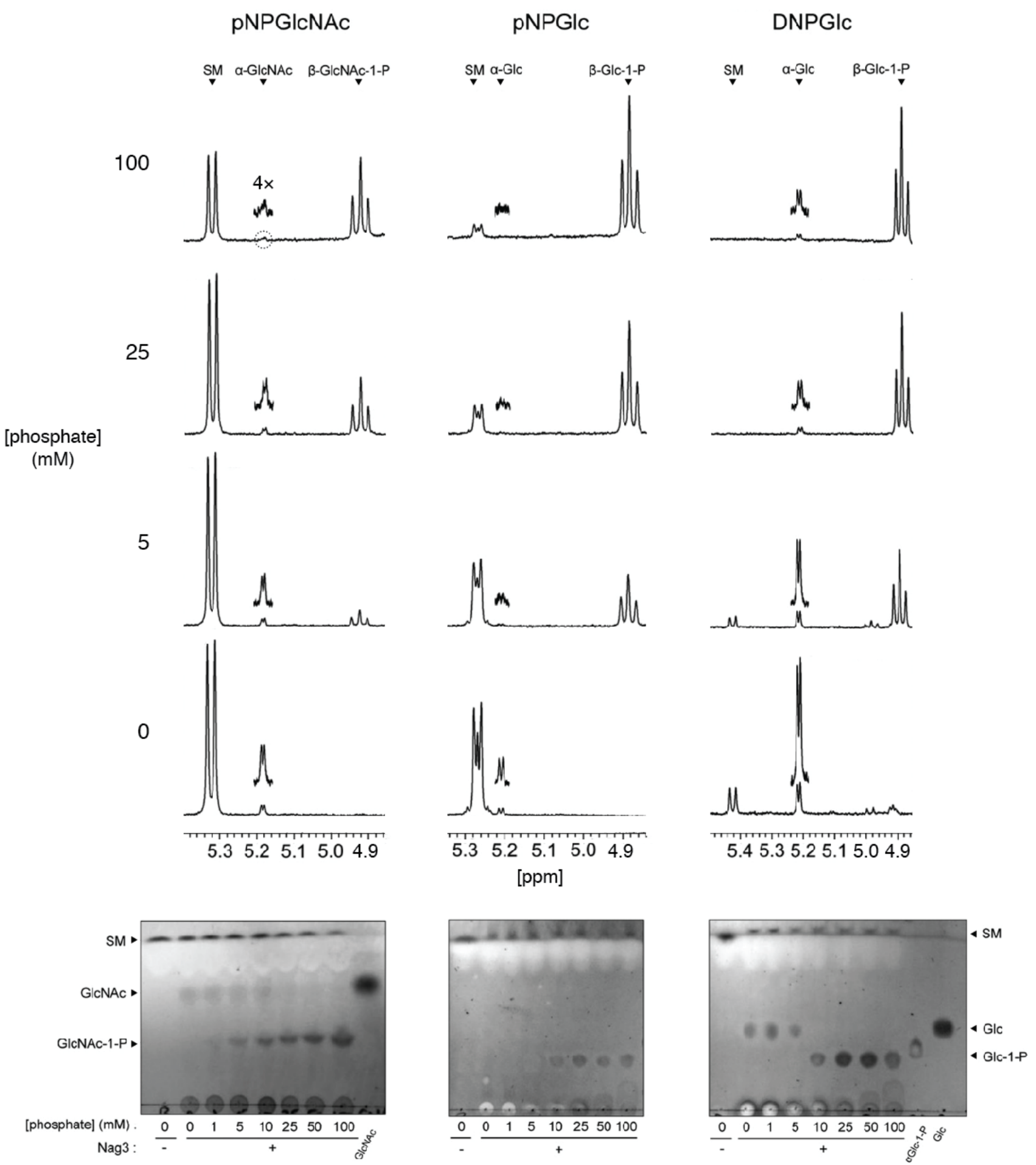
pNPGlc
DNPGlc
SM
α-GlcNAc
β-GlcNAc-1-P
α-Glc
β-Glc-1-P
[phosphate]
100 mM
25 mM
5 mM
0 mM
5.3 5.2 5.1 5.0
5.4 5.3 5.2 5.1
GlcNAc
GlcNAc-1-P
Glc
Glc-1-P
[phosphate] (mM) :
Nag3 :
0 0 1 5 10 25 50 100
-
+
aGlc-1-P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pNPGlc
DNPGlc
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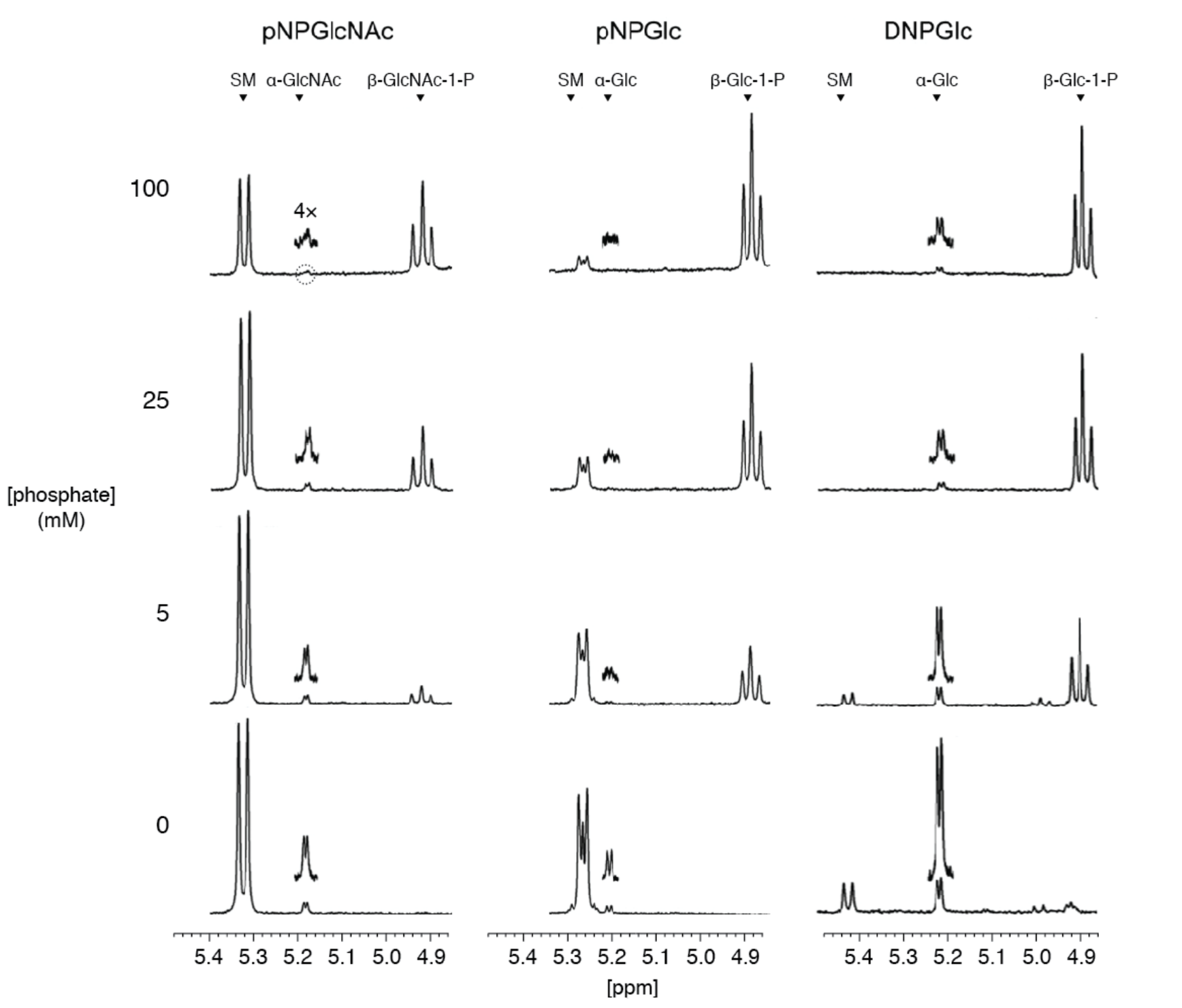
β-GlcNAc-1-P
SM
α-Glc
β-Glc-1-P
SM
α-Glc
β-Glc-1-P
100
4×
25
5
0
[phosphate]
(mM)
5.3 5.2 5.1 5.0 4.9
5.3 5.2 5.1 5.0 4.9
5.4 5.3 5.2 5.1 5.0 4.9
[ppm]
SM
GlcNAc
GlcNAc-1-P
[phosphate] (mM)
0 0 1 5 10 25 50 100
Nag3 : - +
GlcNAc
0 0 1 5 10 25 50 100
- +
0 0 1 5 10 25 50 100
- +
αGlc-1-P
Glc
SM
Glc
Glc-1-P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pNPGlc
DNPGlc
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β-GlcNAc-1-P
SM
α-Glc
β-Glc-1-P
SM
α-Glc
β-Glc-1-P
100
4×
25
5
0
[phosphate]
(mM)
5.4 5.3 5.2 5.1 5.0 4.9
[ppm]
SM
GlcNAc
GlcNAc-1-P
[phosphate] (mM) 0 0 1 5 10 25 50 100
Nag3 : - +
GlcNAc
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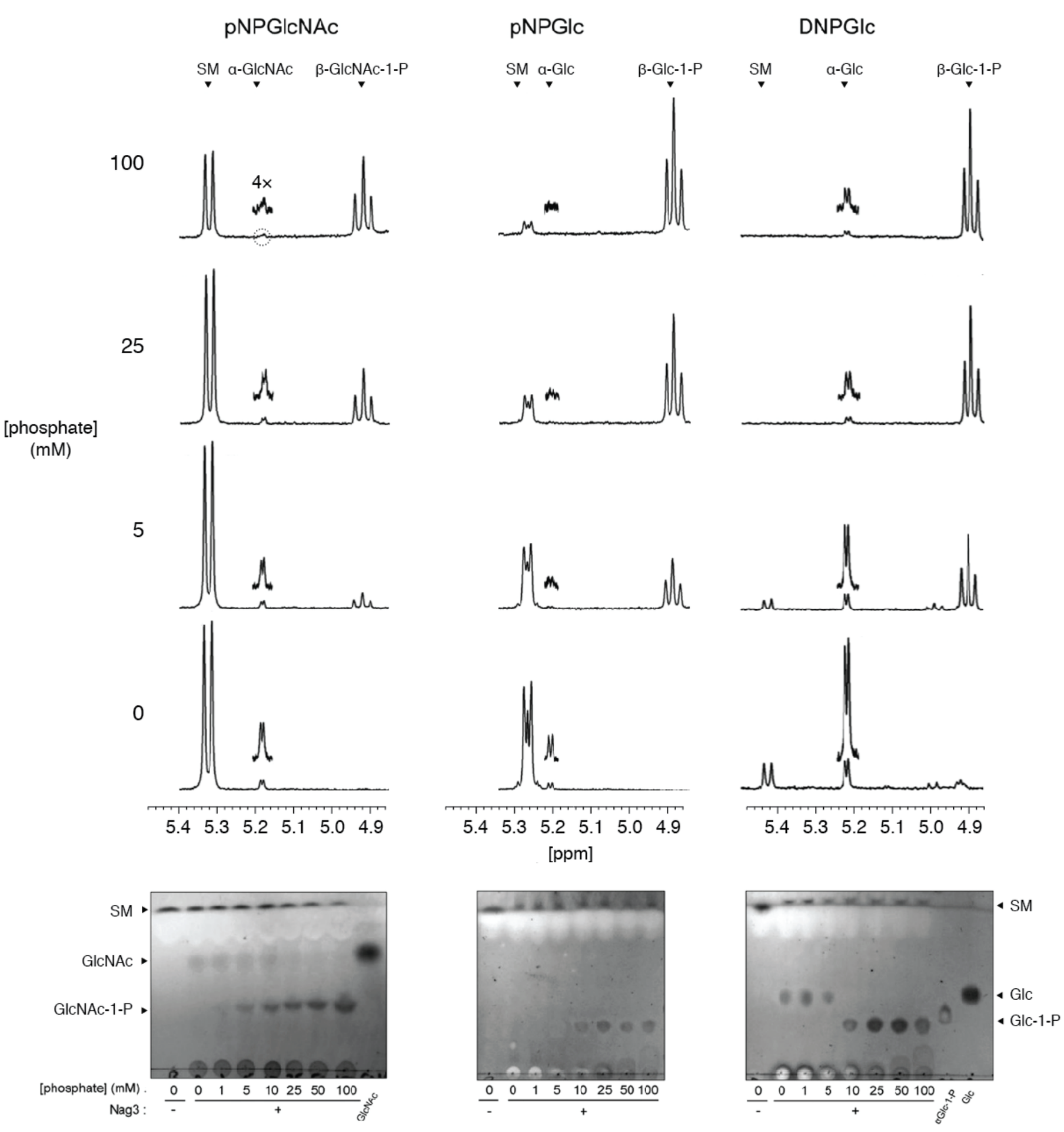
- +
0 0 1 5 10 25 50 100
- +
αGlc-1-P
Glc
SM
Glc
Glc-1-P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pNPGlc
DNPGlc
SM
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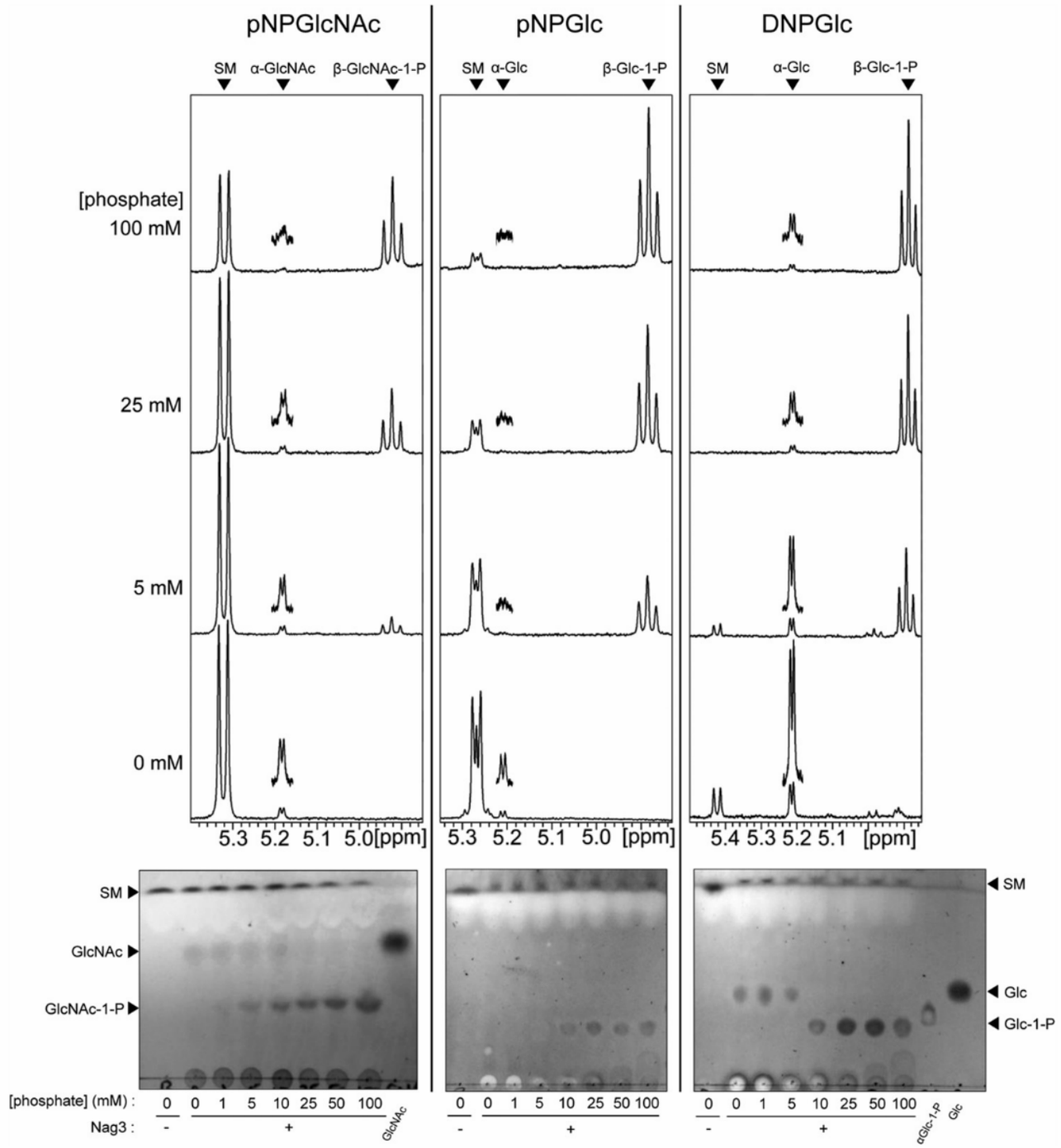
β-GlcNAc-1-P
SM
α-Glc
β-Glc-1-P
SM
α-Glc
β-Glc-1-P
100
4×
25
[phosphate]
(mM)
5
0
5.4 5.3 5.2 5.1 5.0 4.9
[ppm]
SM
GlcNAc
GlcNAc-1-P
[phosphate] (mM)
0 0 1 5 10 25 50 100
Nag3 :
- +
GlcNAc
SM
Glc
Glc-1-P
αGlc-1-P
Glc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pNPGlc
DNPGlc
SM
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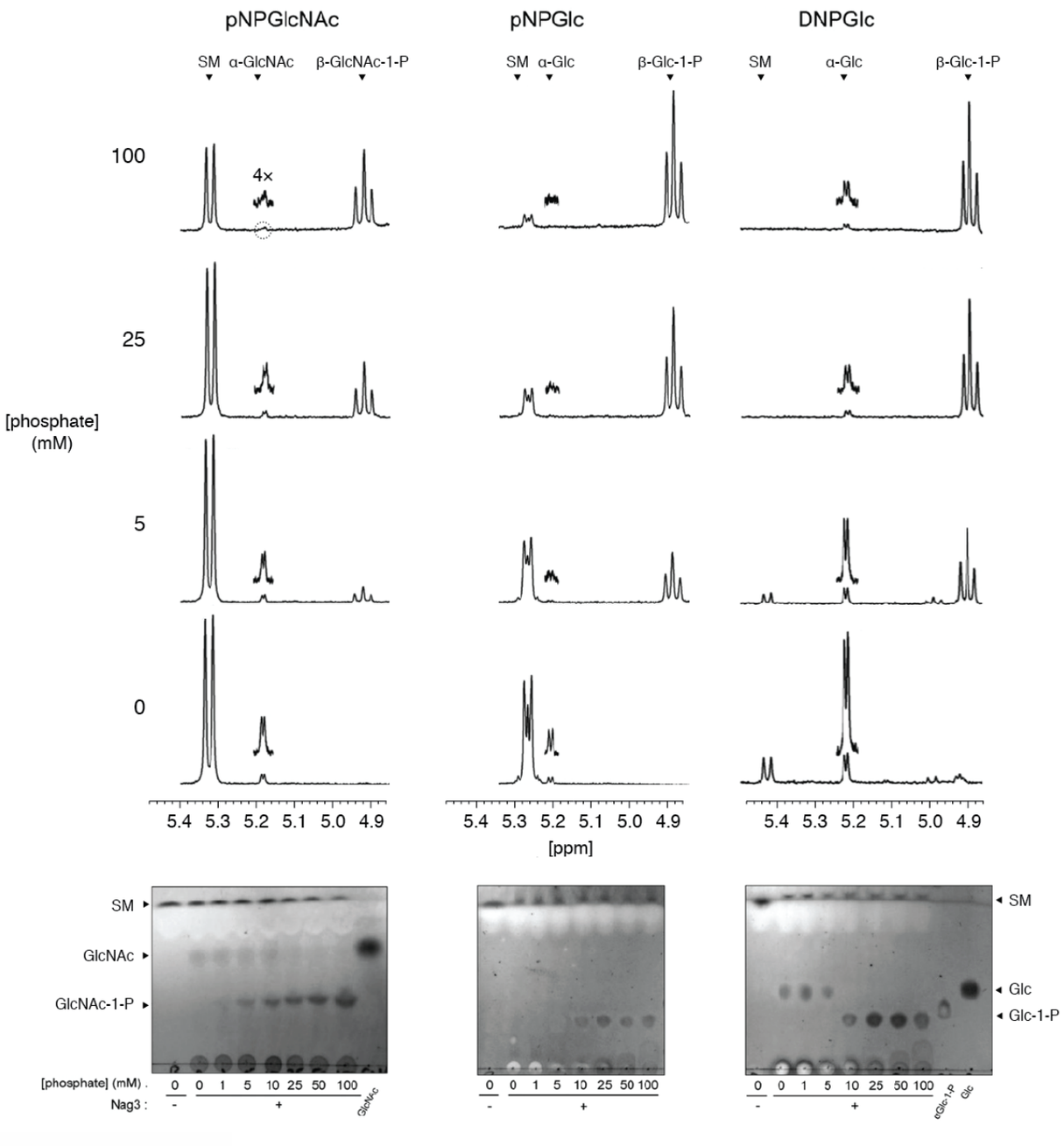
β-GlcNAc-1-P
α-Glc
β-Glc-1-P
[phosphate]
100 mM
25 mM
5 mM
0 mM
5.3 5.2 5.1 5.0[ppm]
5.3 5.2 5.1 5.0 [ppm]
5.4 5.3 5.2 5.1 [ppm]
SM
GlcNAc
GlcNAc-1-P
Glc
Glc-1-P
[phosphate] (mM) : 0 0 1 5 10 25 50 100
Nag3 : - +
GlcNAc
αGlc-1-P
Glc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pNPGlc
DNPGlc
SM
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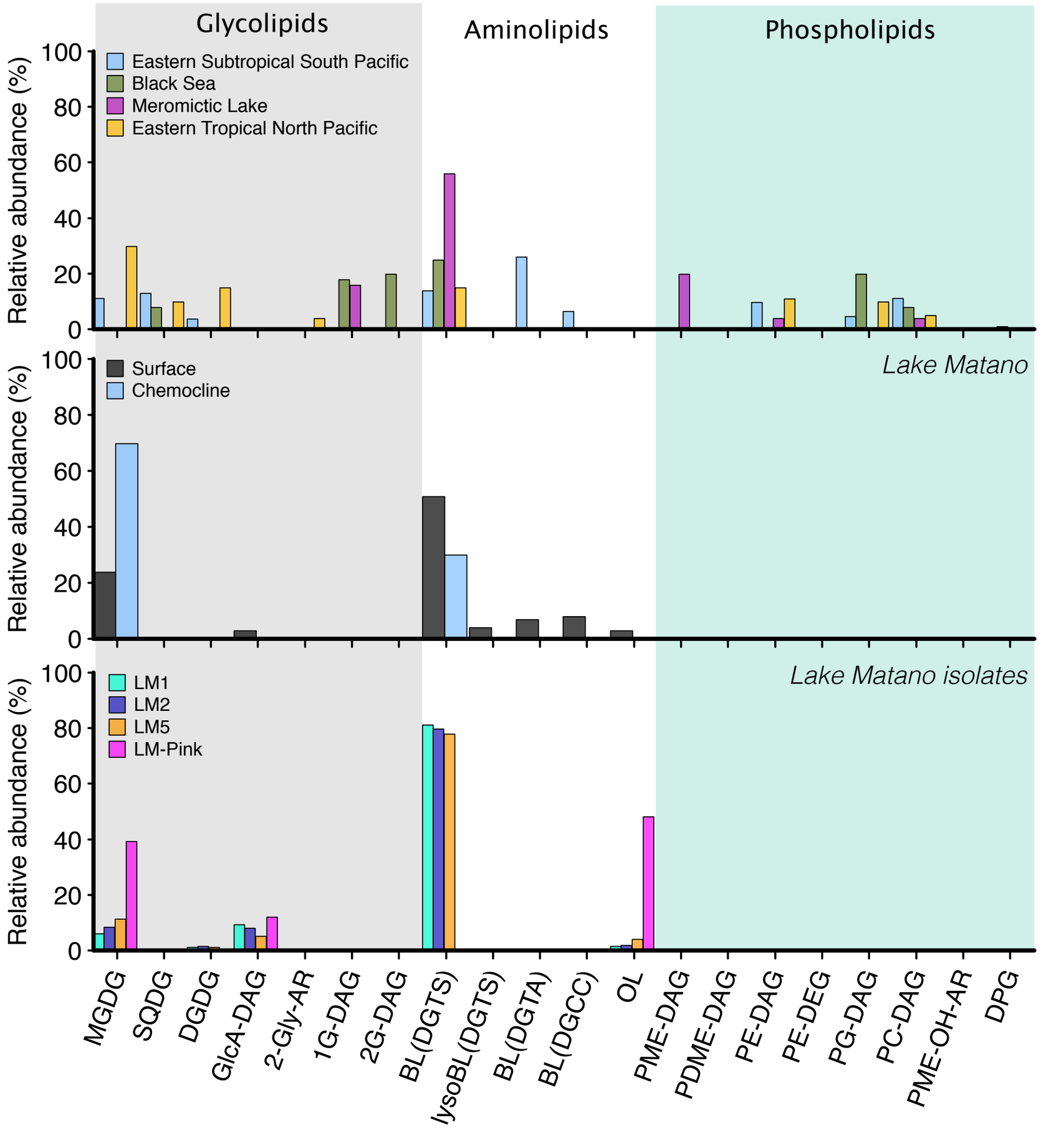
β-GlcNAc-1-P
α-Glc
β-Glc-1-P
4×
100
25
5
0
[phosphate]
(mM)
5.4 5.3 5.2 5.1 5.0 4.9
[ppm]
SM
GlcNAc
GlcNAc-1-P
Glc
Glc-1-P
[phosphate] (mM) : 0 0 1 5 10 25 50 100
Nag3 : - +
GlcNAc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Aminolipids
Phospholipids
Eastern Subtropical South Pacific
Black Sea
Meromictic Lake
Eastern Tropical North Pacific
Relative abundance (%)
100
80
60
40
20
0
Lake Matano
Surface
Chemocline
Relative abundance (%)
Lake Matano isolates
LM1
LM2
LM5
LM-Pink
MGDG
SQDG
DGDG
GlcA-DAG
2-Gly-AR
1G-DAG
2G-DAG
BL(DGTS)
lysoBL(DGTS)
BL(DGTA)
BL(DGCC)
OL
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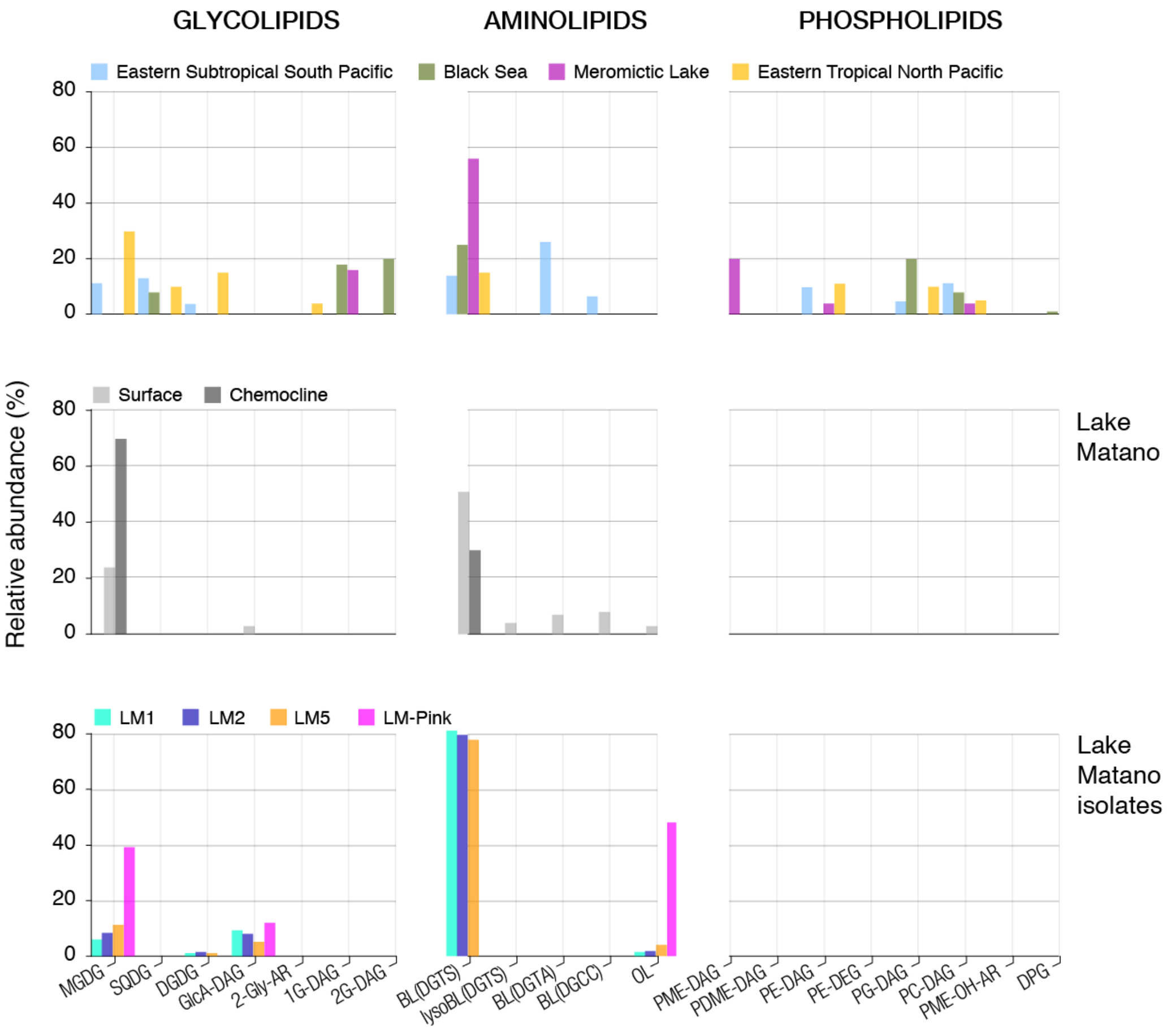
DPG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PHOSPHOLIPIDS
Eastern Subtropical South Pacific
Black Sea
Meromictic Lake
Eastern Tropical North Pacific
Surface
Chemocline
Relative abundance (%)
0
20
40
60
80
LM1
LM2
LM5
LM-Pink
MGDG
SQDG
DGDG
GlcA-DAG
2-Gly-AR
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BL(DGTA)
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OL
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PC-DAG
PME-OH-AR
DPG
Lake Matano
Lake Matano isolates

GLYCOLIPIDS

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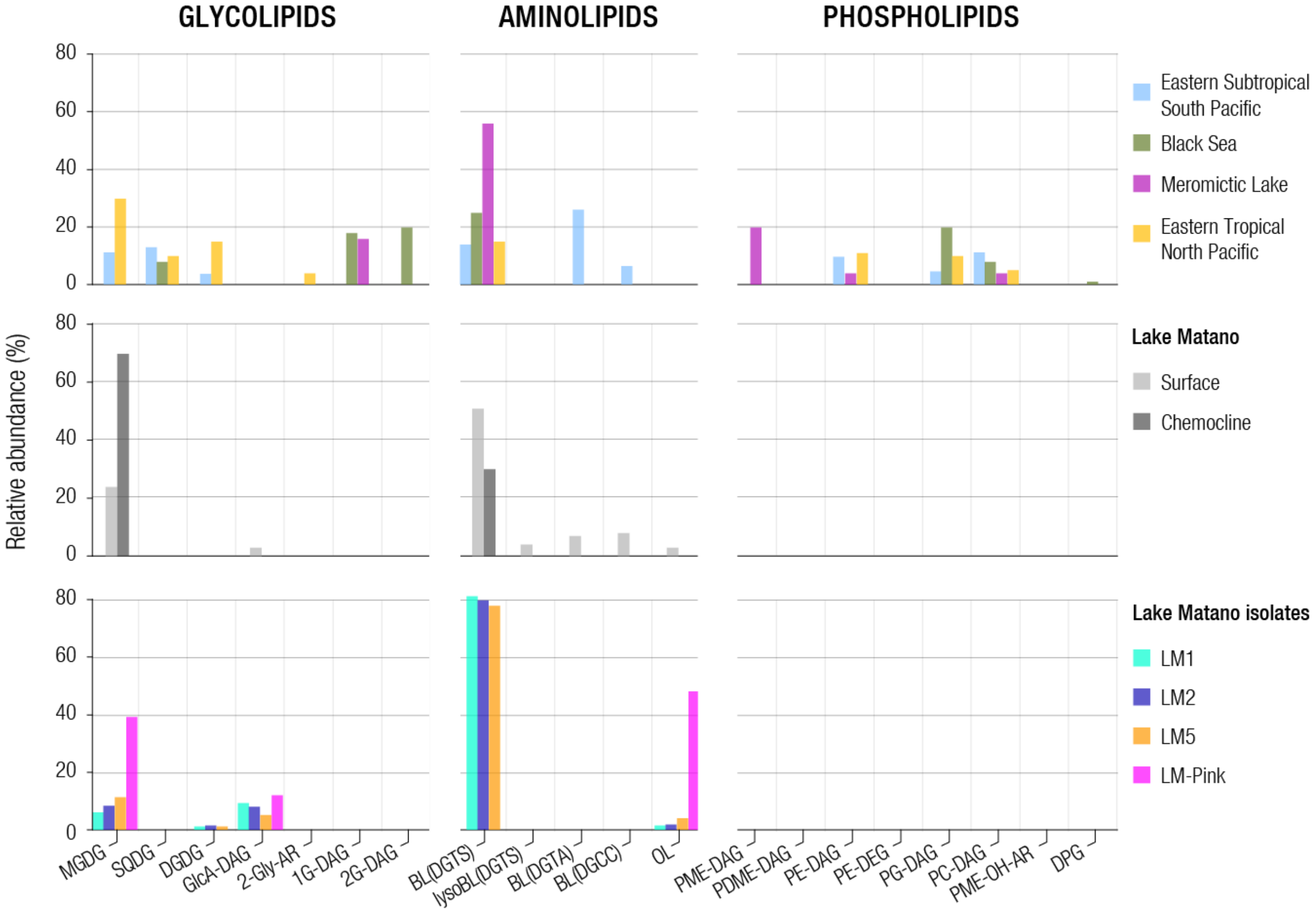
PME-OH-AR

DPG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PHOSPHOLIPIDS
Relative abundance (%)
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40
60
80
Eastern Subtropical South Pacific
Black Sea
Meromictic Lake
Eastern Tropical North Pacific
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BL(DGTA)
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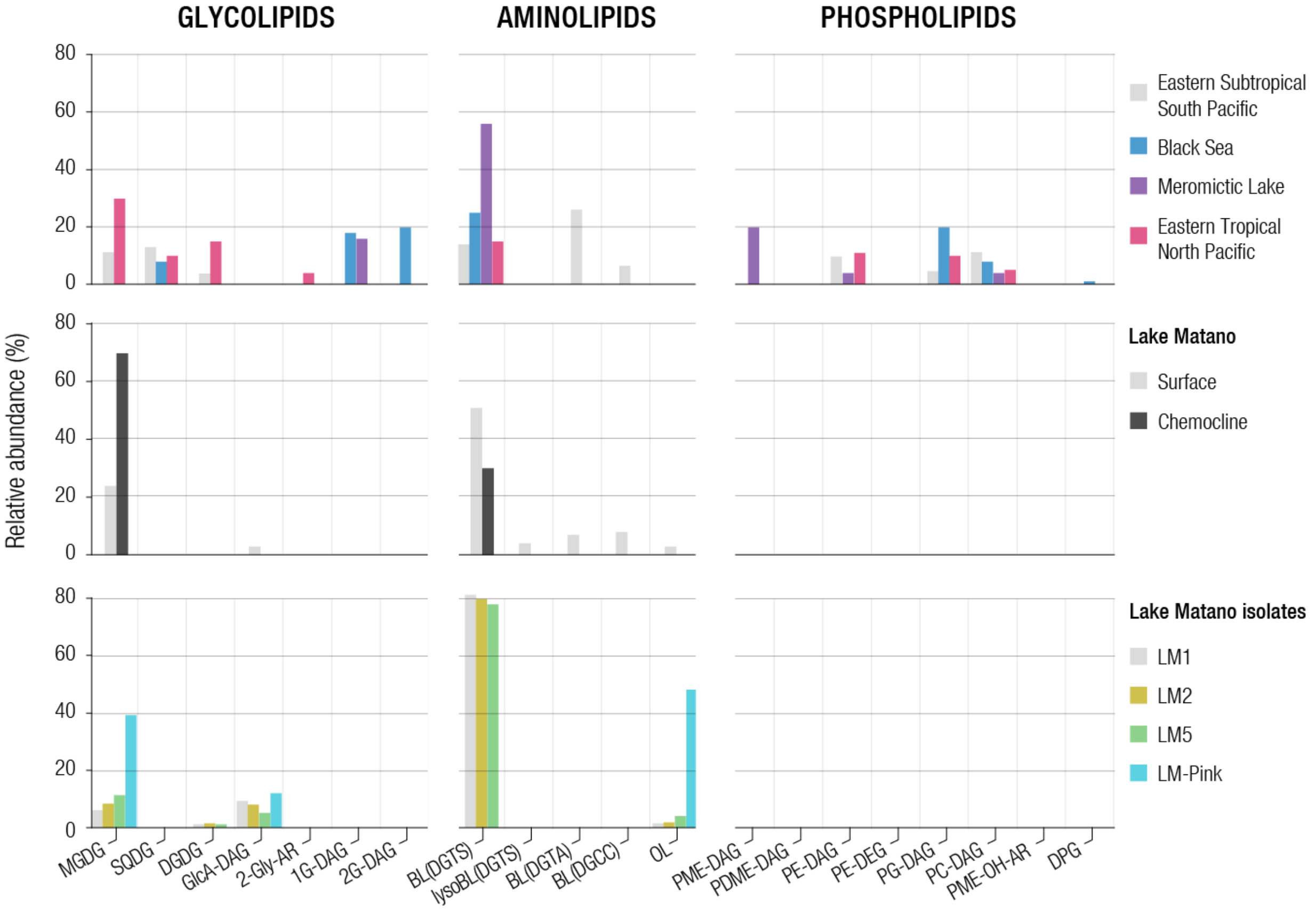
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Eastern Subtropical South Pacific
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GlcA-DAG
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lysoBL(DGTS)
BL(DGTA)
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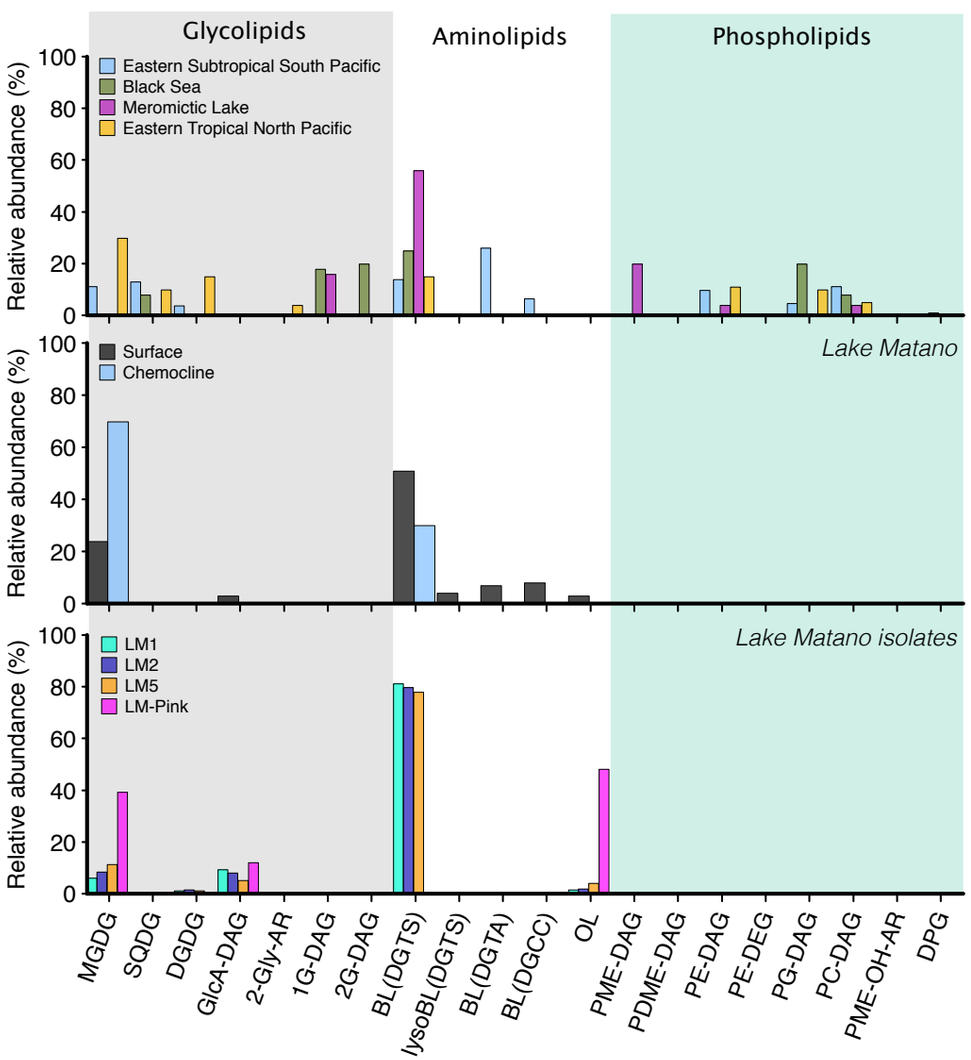
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Aminolipids
Phospholipids
Eastern Subtropical South Pacific
Black Sea
Meromictic Lake
Eastern Tropical North Pacific
Relative abundance (%)
Surface
Chemocline
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LM1
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MGDG
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lysoBL(DGTS)
BL(DGTA)
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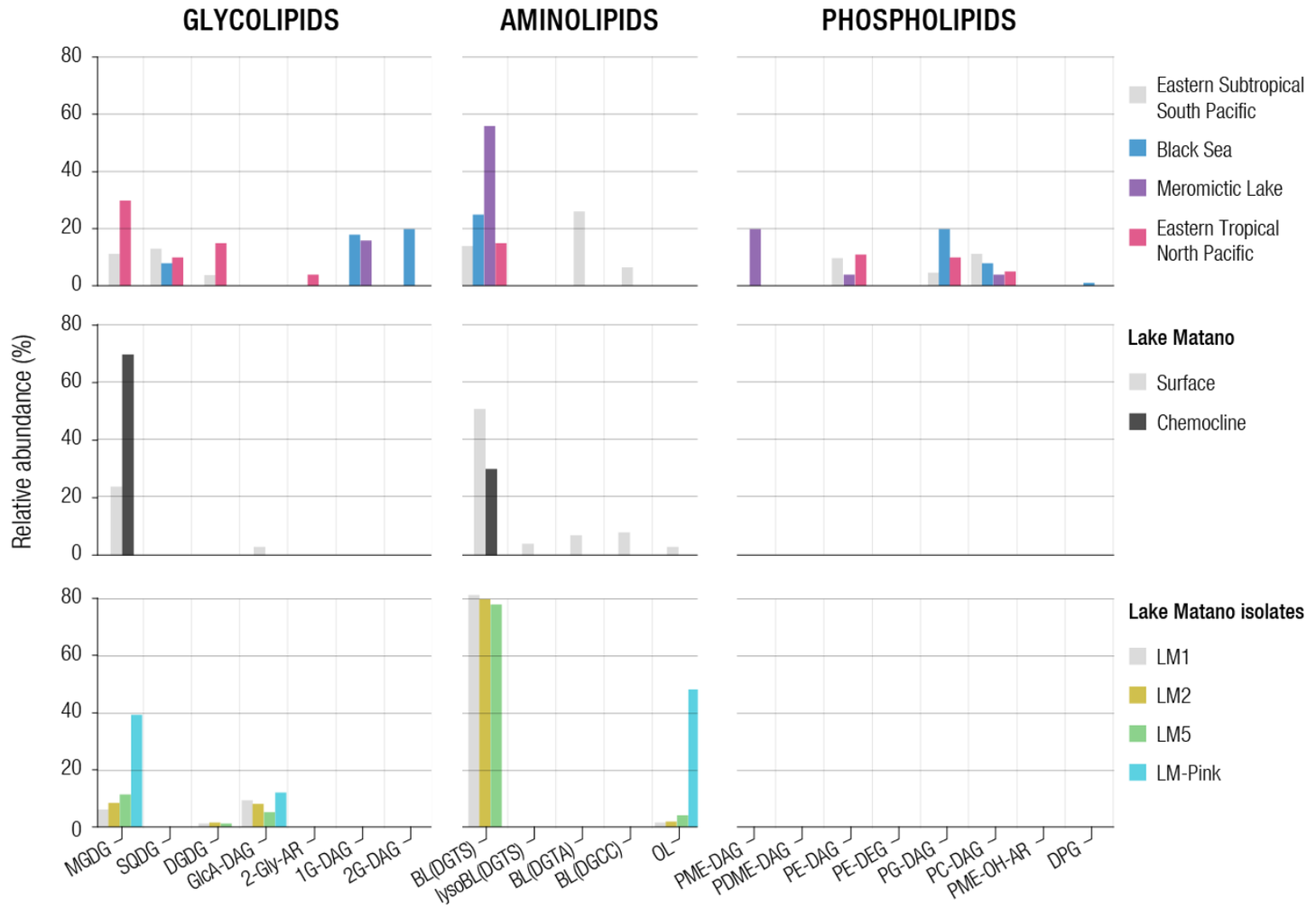
GLYCOLIPIDS
AMINOLIPIDS
PHOSPHOLIPIDS
Relative abundance (%)
Eastern Subtropical South Pacific
Black Sea
Meromictic Lake
Eastern Tropical North Pacific
Lake Matano
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1G-DAG
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BL(DGTS)
lysoBL(DGTS)
BL(DGTA)
BL(DGCC)
OL
PME-DAG
PDME-DAG
PE-DAG
PE-DEG
PG-DAG
PC-DAG
PME-OH-AR
DPG

Table 4: Composition of most prevalent 2-, 3- and 4-drug combinations and count of participants reporting each

	2-Drug Combos						3-Drug Combos							4-Drug Combos							
Cannabis	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X		Cannabis
Ecstasy / MDMA		X				X	X	X	X	X				X	X	X	X	X		X	Ecstasy / MDMA
Alcohol	X					X	X				X	X		X	X	X	X		X	X	Alcohol
Ketamine				X				X			X				X			X			Ketamine
LSD / Acid			X						X				X					X			LSD / Acid
Cocaine (powder)																X			X	X	Cocaine (powder)
Mushrooms					X					X		X	X	X					X	X	Mushrooms
2C-x Family																	X				2C-x Family
Count of Reports	12	6	4	3	2	2	5	5	2	2	2	2	2	6	3	2	2	2	2	2	Count of Reports

Table 4. Composition of the most prevalent 2-, 3- and 4-drug combinations, the count of participants reporting each and the number of times the use of a drug has been reported.

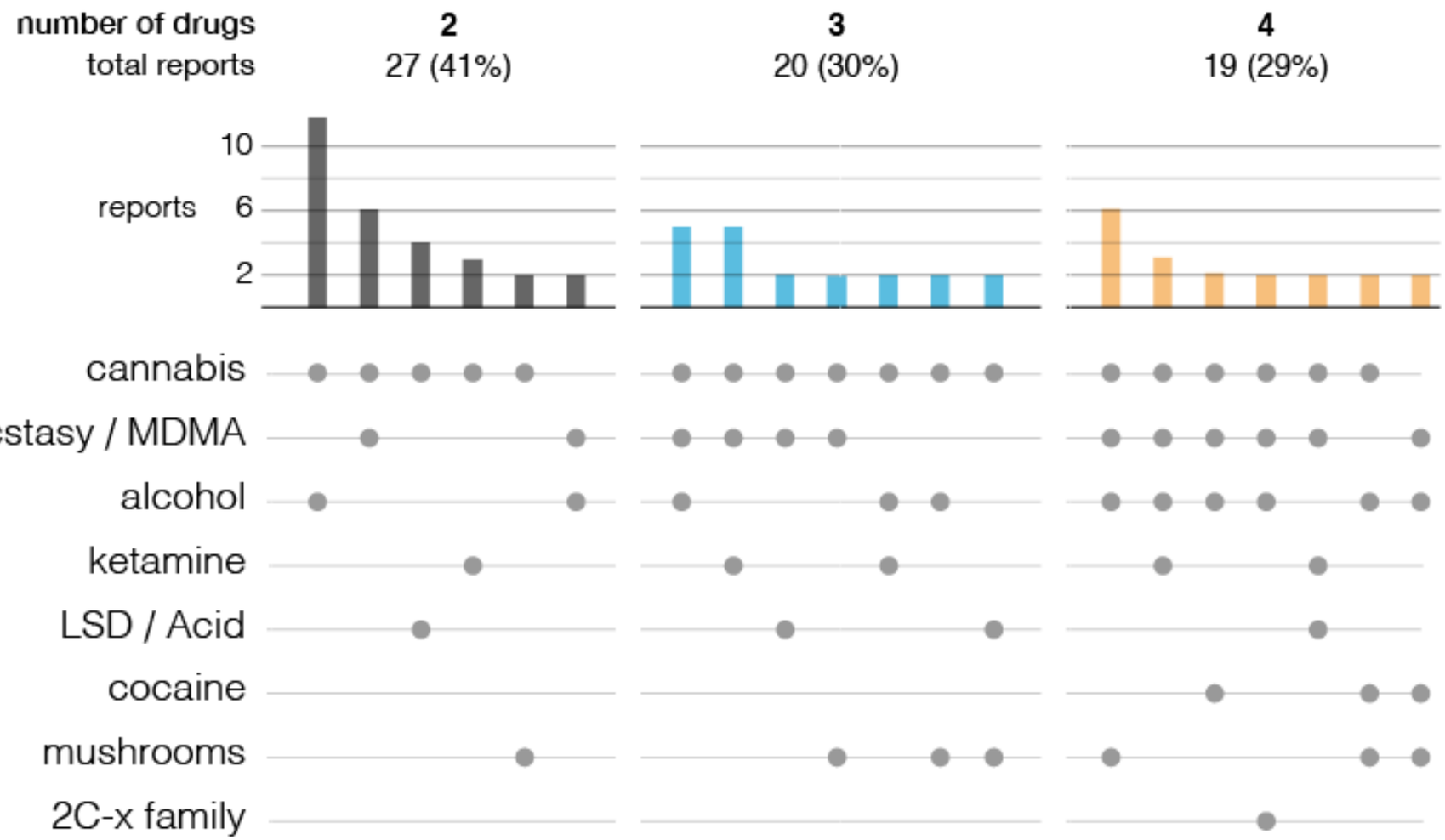


Table 4. Composition of the most prevalent 2-, 3- and 4-drug combinations, the count of participants reporting each and the number of times the use of a drug has been reported.

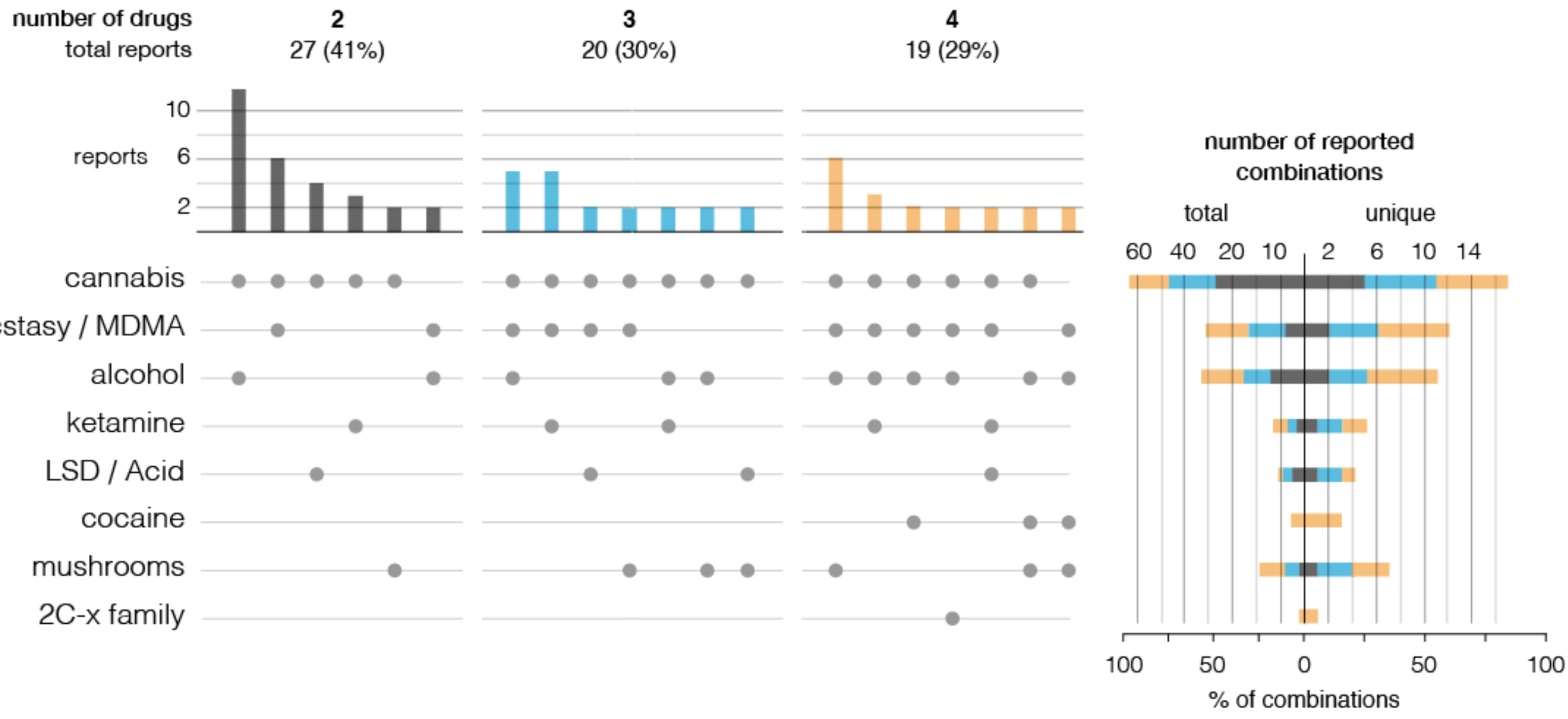
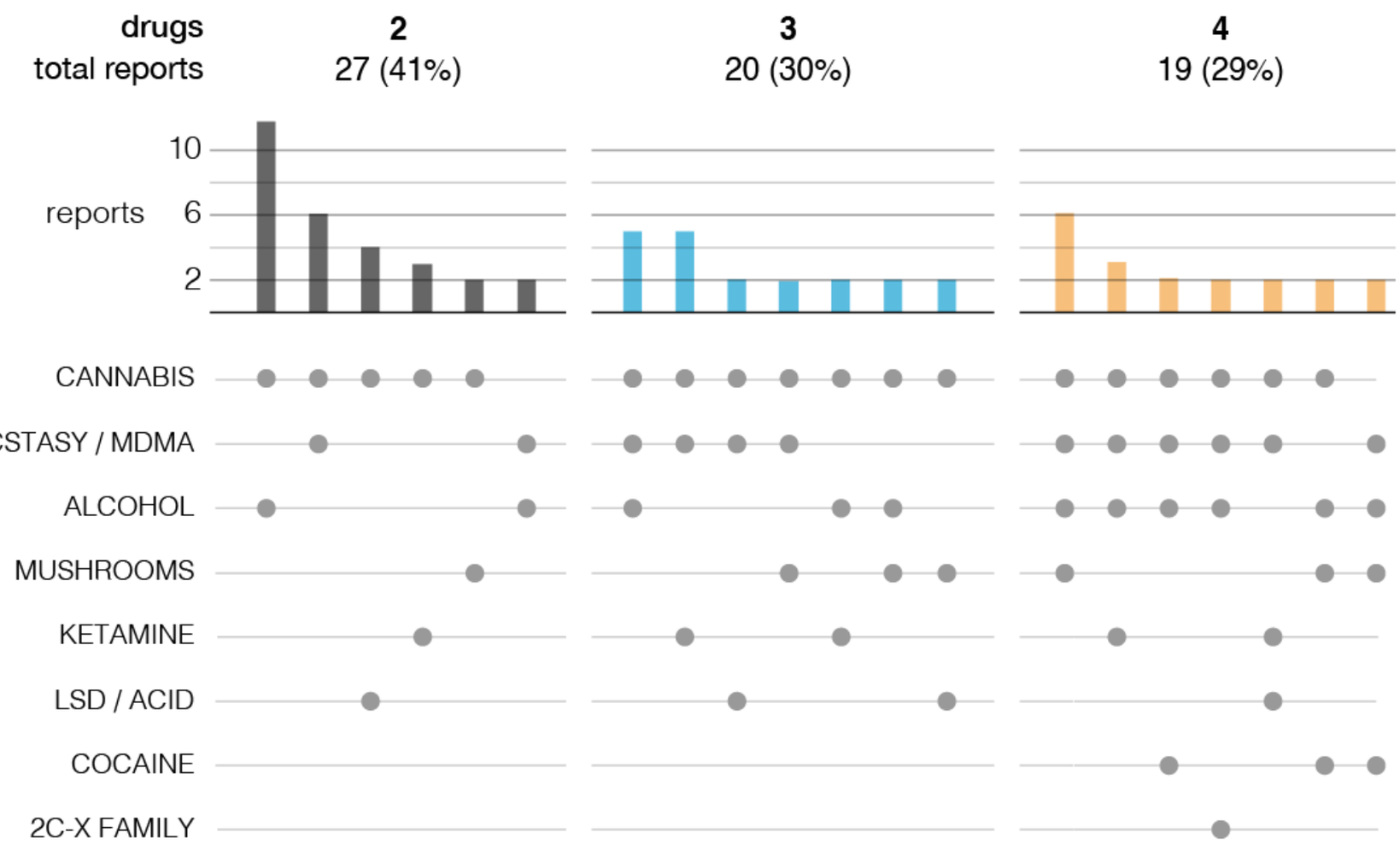


Table 4. Composition of the most prevalent 2-, 3- and 4-drug combinations, the count of participants reporting each and the number of times the use of a drug has been reported.



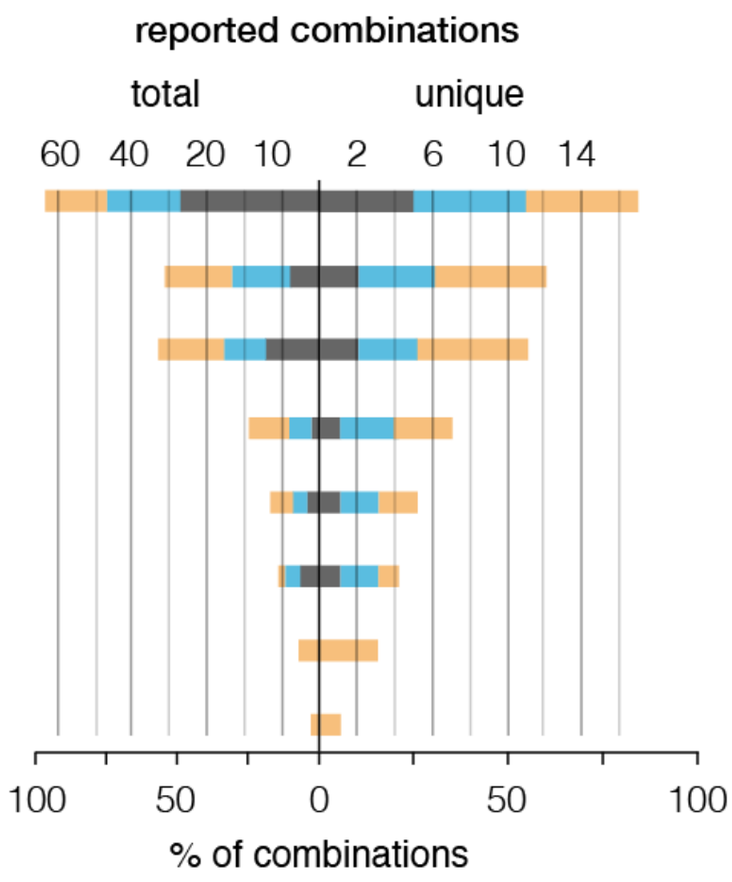
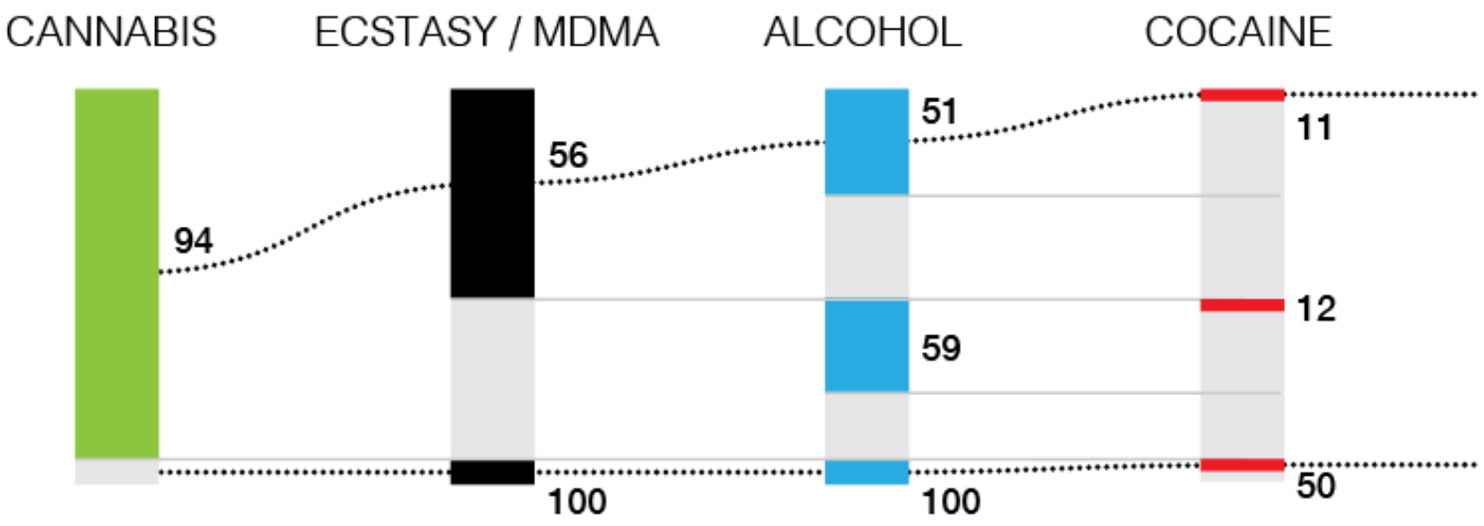


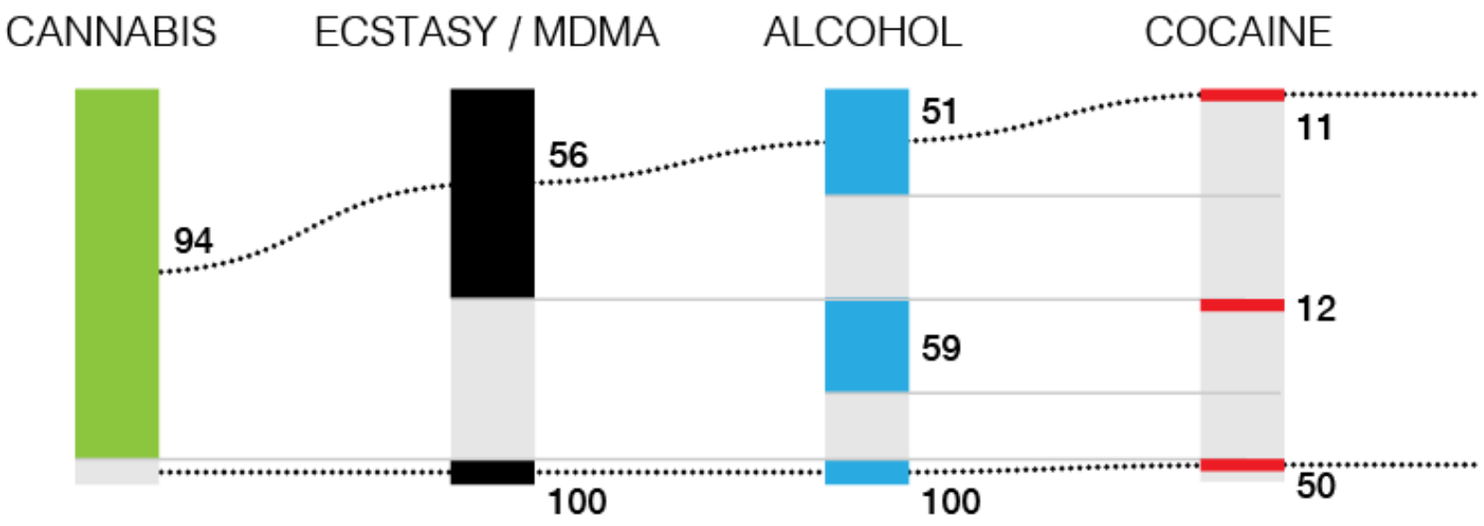
Table 5. Want to lower your risk of cocaine use? Smoke pot.



94% of respondents did pot.
Of those, 56% did ecstasy.
Of those, 51% did alcohol.
Of those, 11% did cocaine.

6% of respondents did not do pot.
Of those, 50% did a combination of ecstasy, alcohol and cocaine.

Table 5. Want to lower your risk of cocaine use? Smoke pot.



94% of respondents did pot.
Of those, 56% did ecstasy.
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Of those, 11% did cocaine.

6% of respondents did not do pot.
Of those, 50% did a combination of ecstasy, alcohol and cocaine.

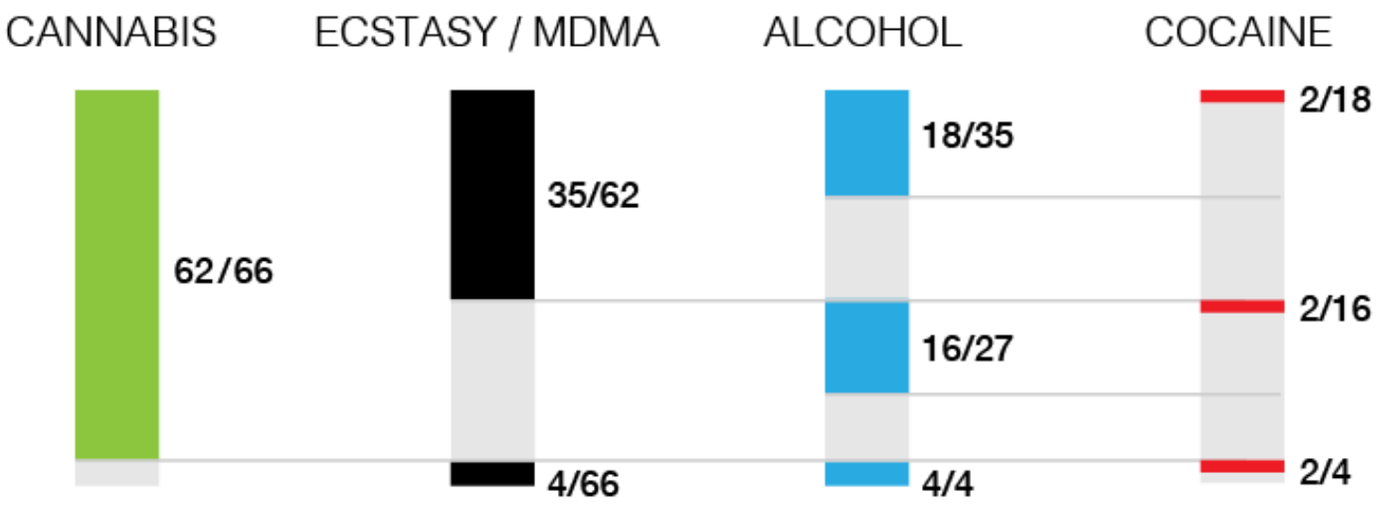
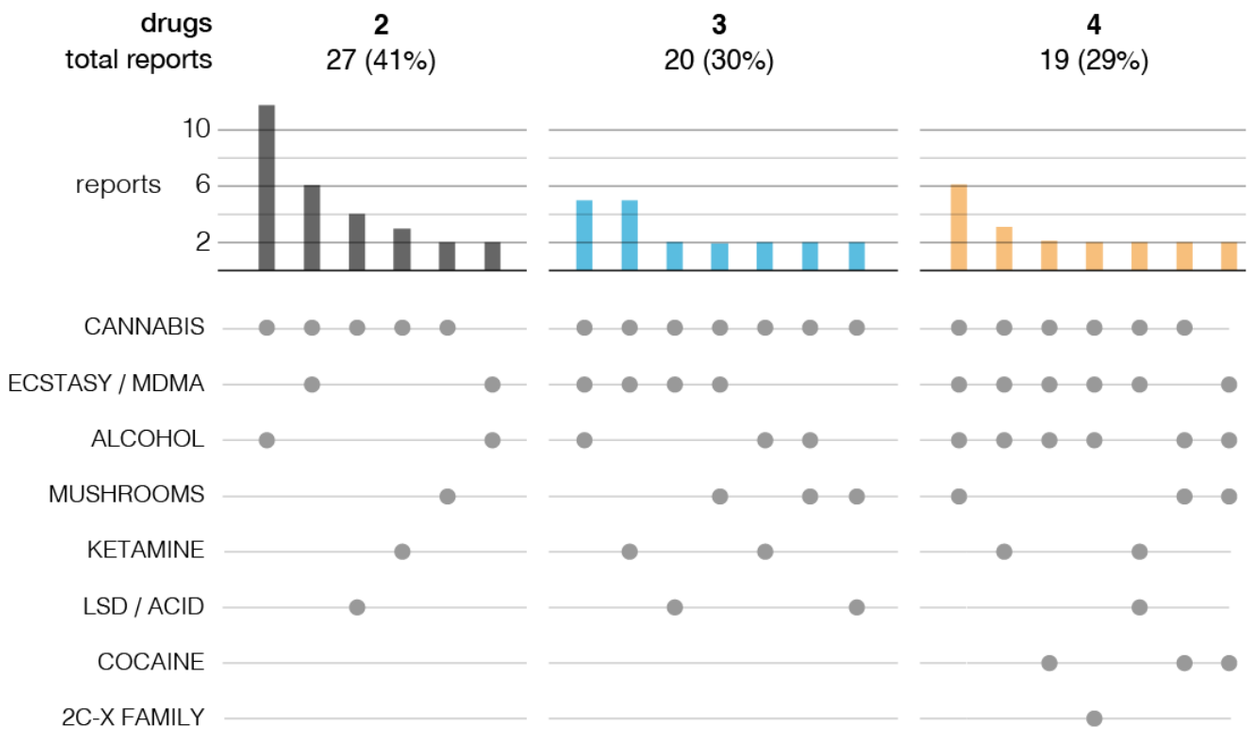
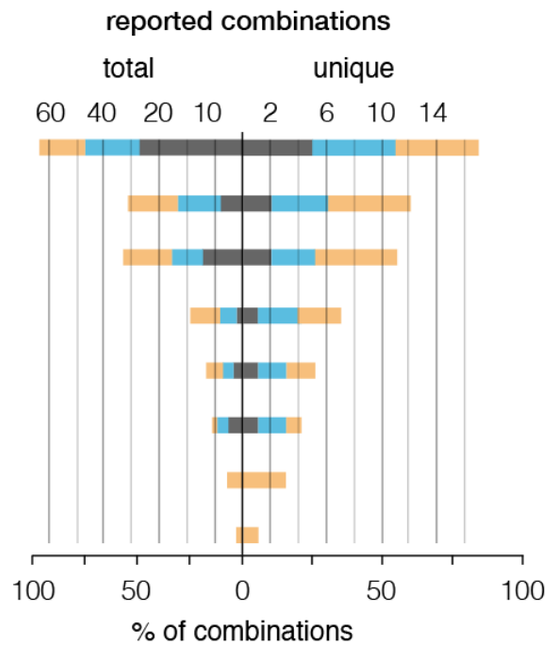


Table 4: Composition of most prevalent 2-, 3- and 4-drug combinations and count of participants reporting each

	2-Drug Combos						3-Drug Combos							4-Drug Combos							
Cannabis	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X		Cannabis
Ecstasy / MDMA		X				X	X	X	X	X				X	X	X	X	X		X	Ecstasy / MDMA
Alcohol	X					X	X				X	X		X	X	X	X		X	X	Alcohol
Ketamine				X				X			X				X			X			Ketamine
LSD / Acid			X						X				X					X			LSD / Acid
Cocaine (powder)																X			X	X	Cocaine (powder)
Mushrooms					X					X		X	X	X					X	X	Mushrooms
2C-x Family																	X				2C-x Family
Count of Reports	12	6	4	3	2	2	5	5	2	2	2	2	2	6	3	2	2	2	2	2	Count of Reports

Table 4. Composition of the most prevalent 2-, 3- and 4-drug combinations, the count of participants reporting each and the number of times the use of a drug has been reported.





important! important! important!
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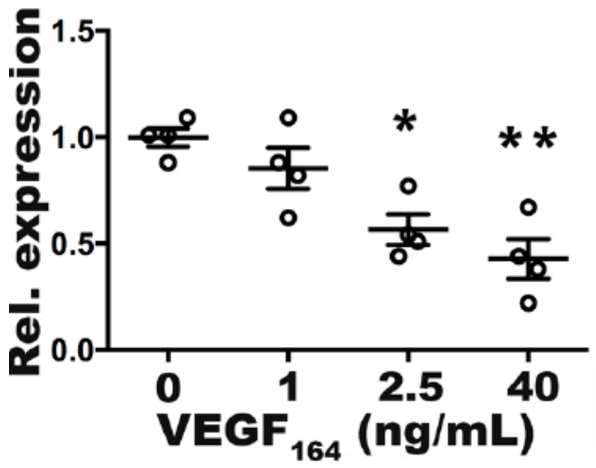
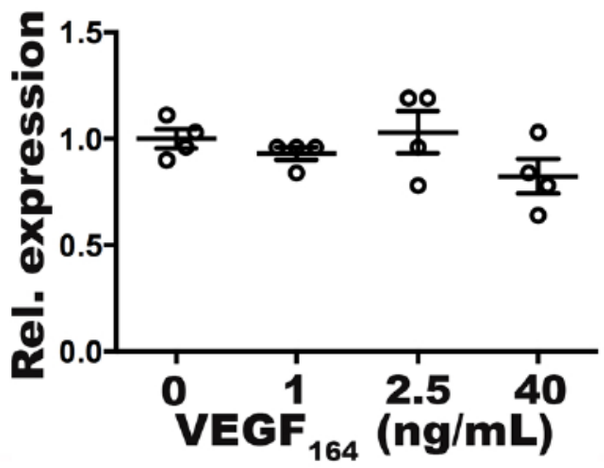
important! important! important!
important! important! important!
important! important! important!

look here

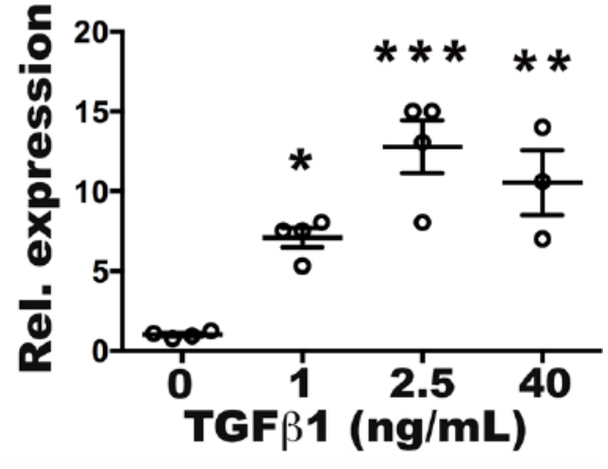
A


Sema3A
Rel. expression
1.5
1.0
0.5
0.0
*
**
0
1
2.5
40
VEGF164 (ng/mL)

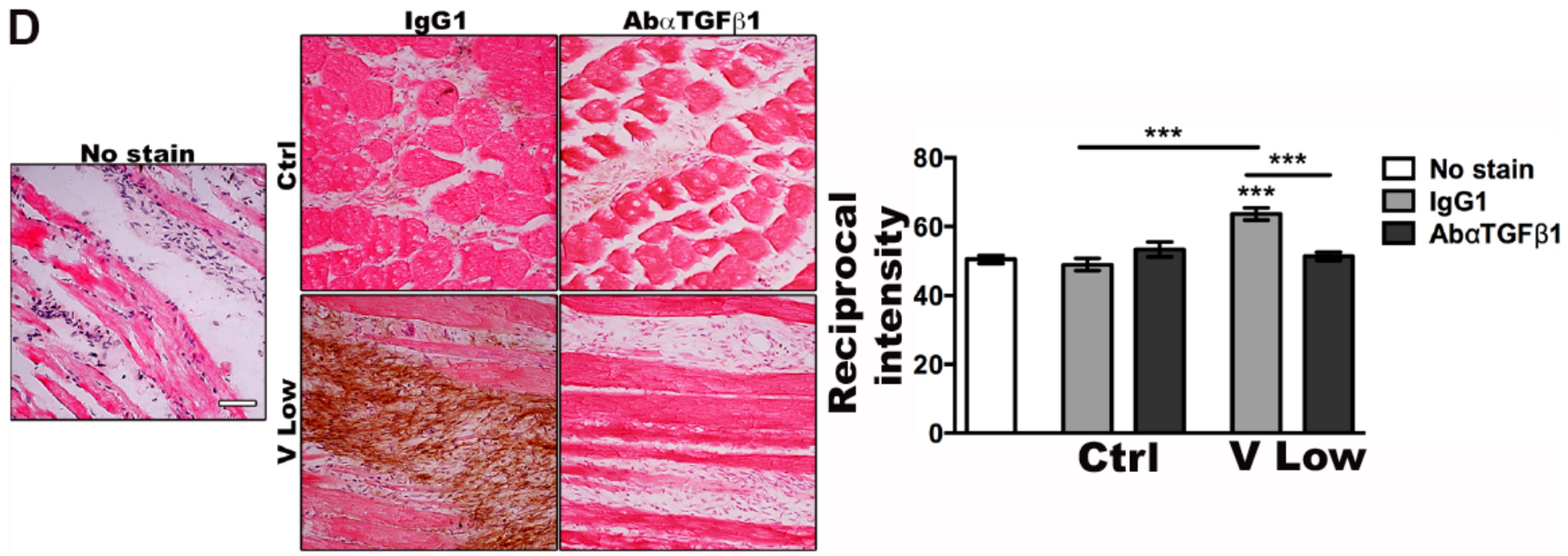
B


TGF-β1
Rel. expression
1.5
1.0
0.5
0.0
0
1
2.5
40
VEGF164 (ng/mL)

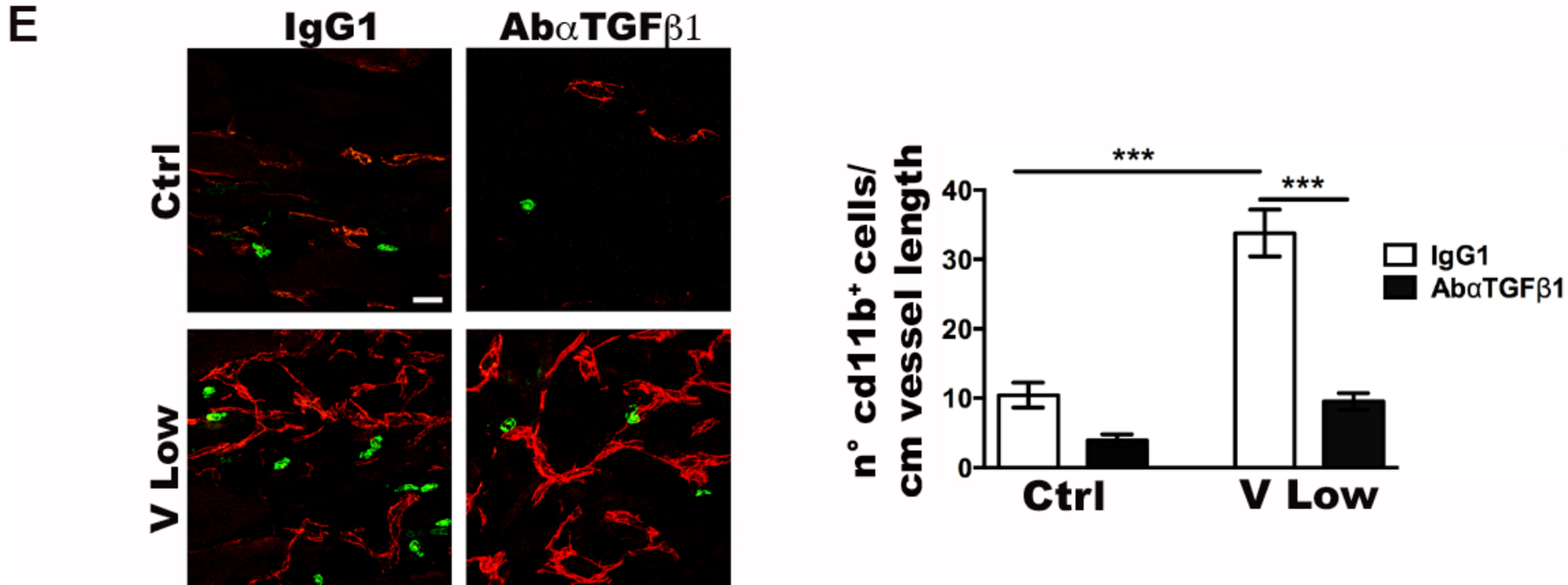
C

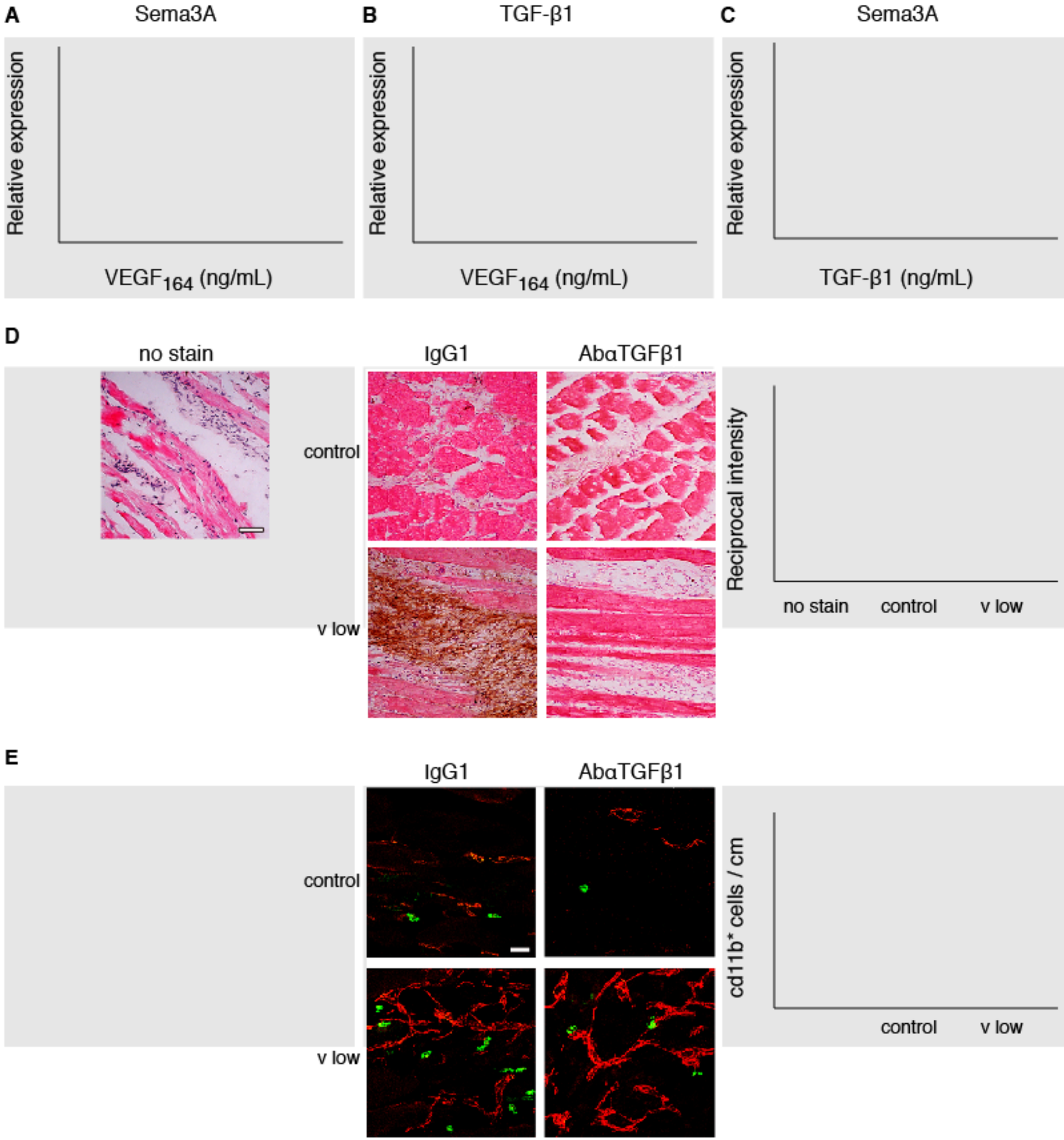

Sema3A
Rel. expression
20
15
10
5
0
*
***
**
0
1
2.5
40
TGFβ1 (ng/mL)

D


IgG1
AbαTGFβ1
No stain
Ctrl
V Low
Reciprocal intensity
80
60
40
20
0
***
***
***
No stain
IgG1
AbαTGFβ1
Ctrl
V Low

E


IgG1
AbαTGFβ1
Ctrl
V Low
n° cd11b+ cells/ cm vessel length
40
30
20
10
0
***
***
IgG1
AbαTGFβ1
Ctrl
V Low


A
Sema3A
Relative expression
VEGF164 (ng/mL)
B
TGF-β1
Relative expression
VEGF164 (ng/mL)
C
Sema3A
Relative expression
TGF-β1 (ng/mL)
D
no stain
IgG1
AbαTGFβ1
control
v low
Reciprocal intensity
no stain
control
v low
E
IgG1
AbαTGFβ1
control
v low
cd11b* cells / cm
control
v low

A

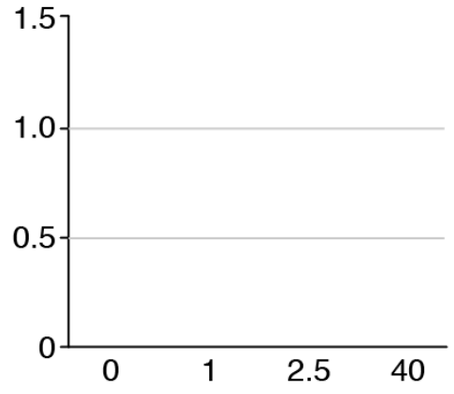
B

C

D

E

A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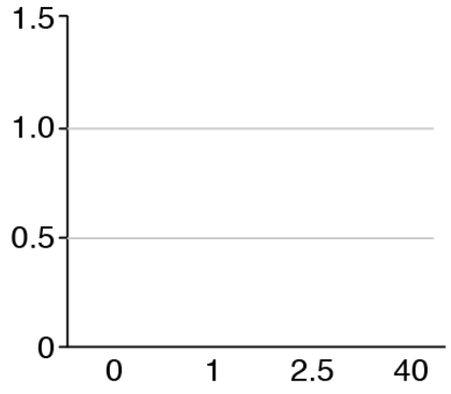
1.0
0.5
0
0
1
2.5
40

B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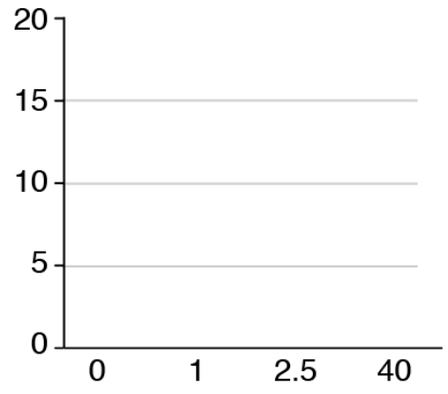
1.0
0.5
0
0
1
2.5
40

C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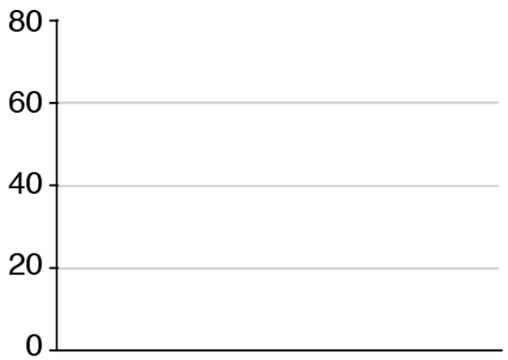
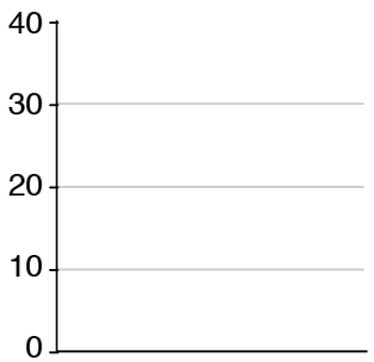
15
10
5
0
0
1
2.5
40

D


80
60
40
20
0

E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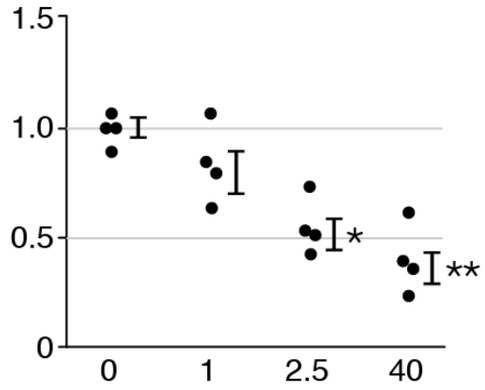
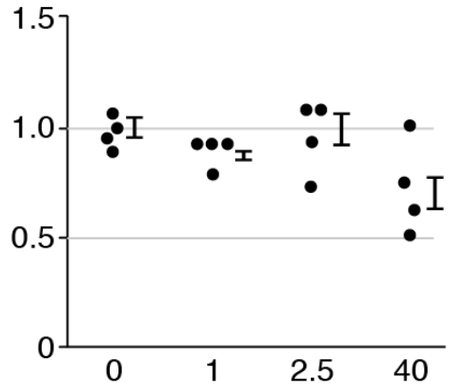
30
20
10
0

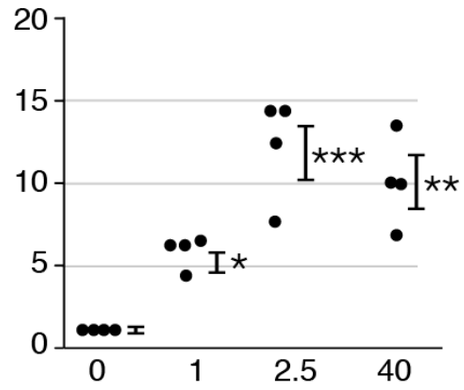
A


1.5
1.0
0.5
0
0
1
2.5
40
*
**

B


1.5
1.0
0.5
0
0
1
2.5
40

C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15
10
5
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2.5
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*
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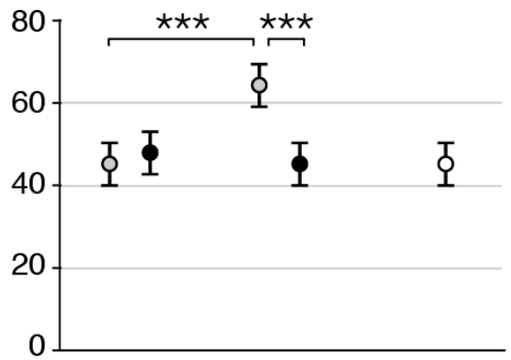
**

D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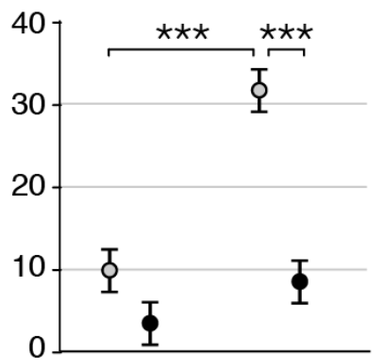
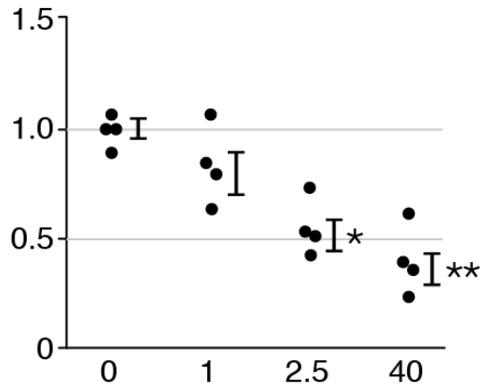
E


40
30
20
10
0
***
***

A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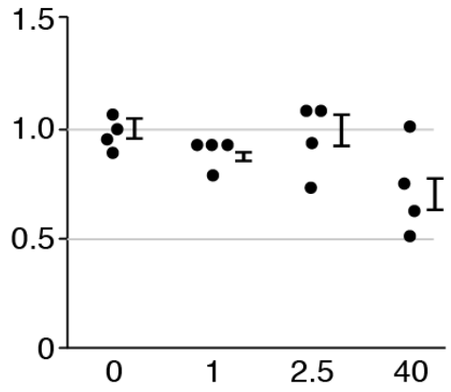
1.0
0.5
0
0
1
2.5
40
*
**

B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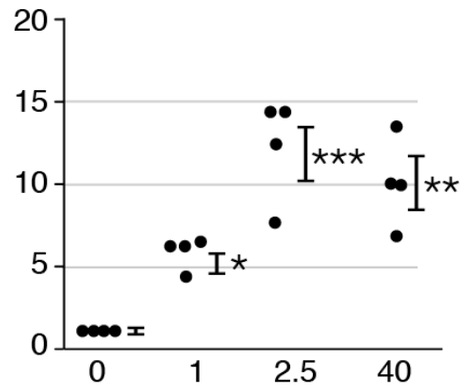
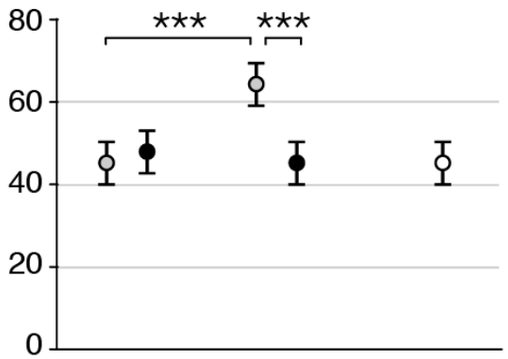
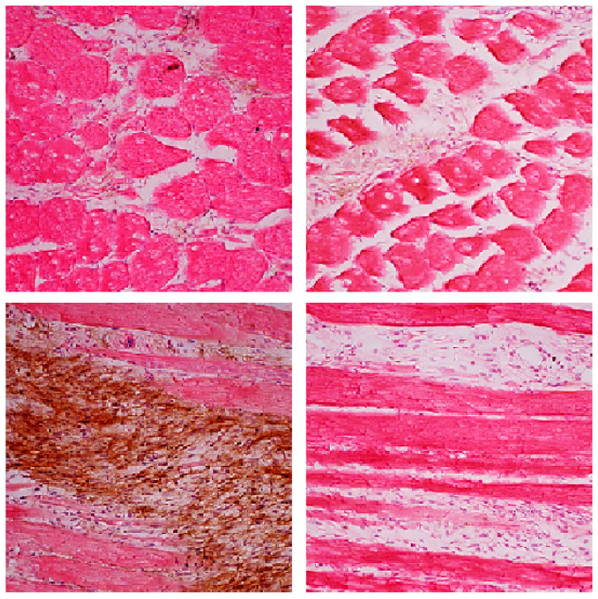
1.0
0.5
0
0
1
2.5
40

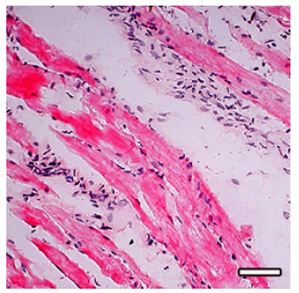
C


20
15
10
5
0
0
1
2.5
40
***
**
*

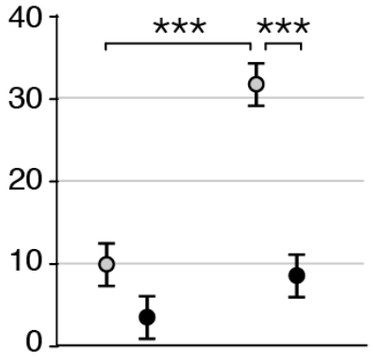
D

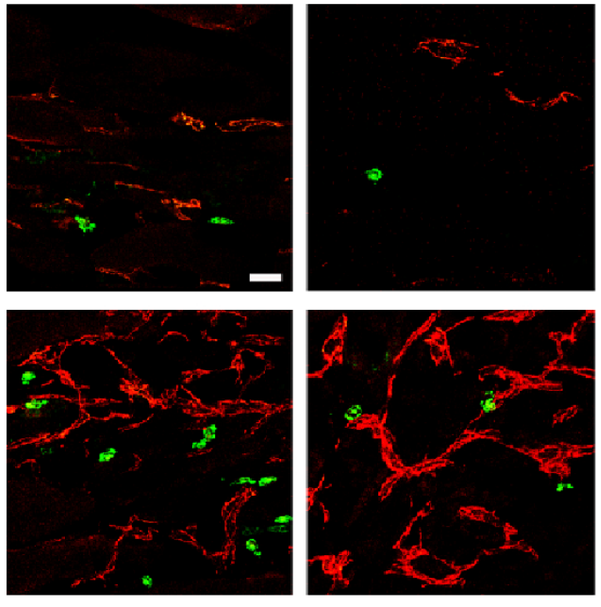

80
60
40
20
0
***
***

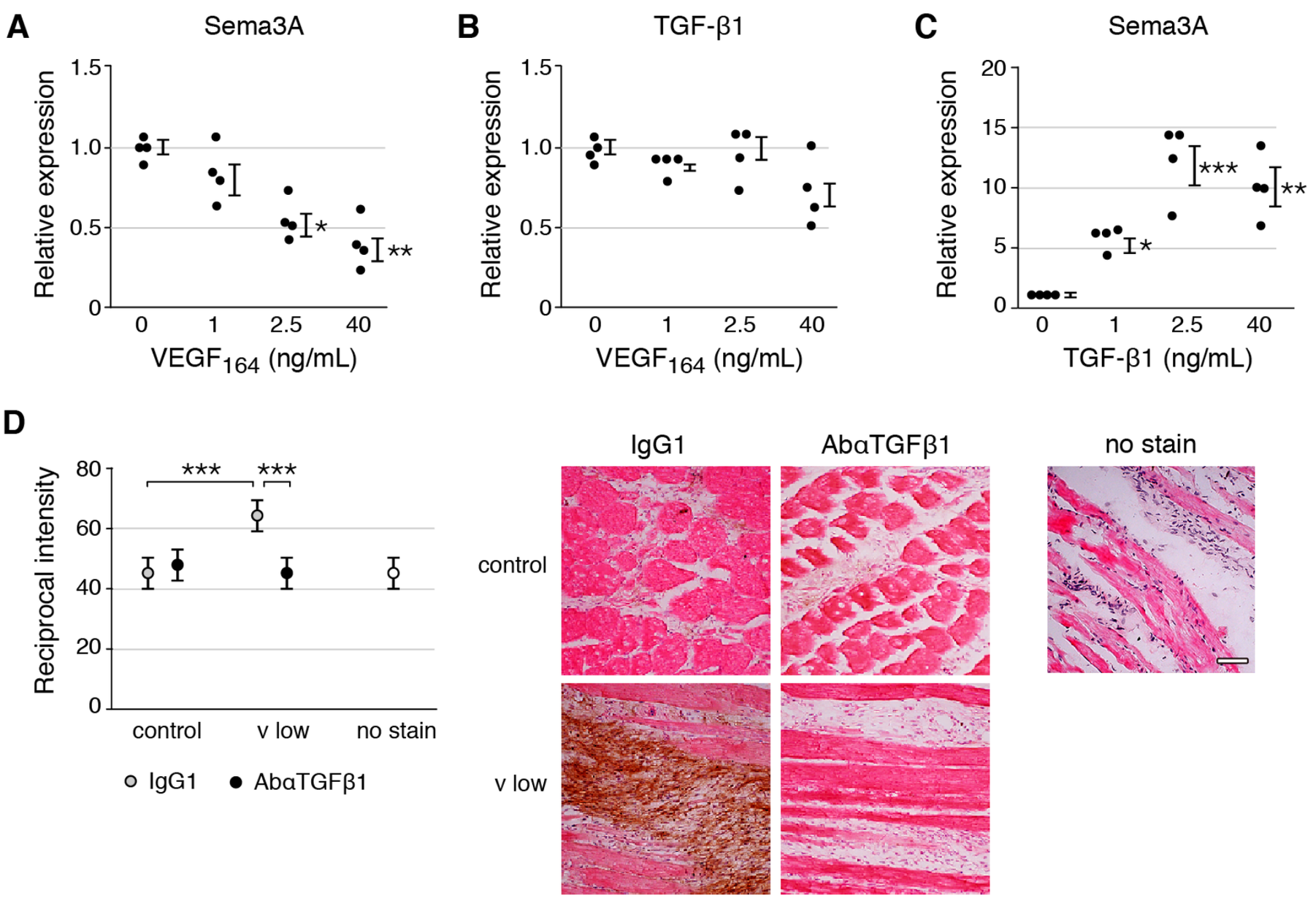




E


40
30
20
10
0
***
***




A
Sema3A
Relative expression
1.5
1.0
0.5
0
0
1
2.5
40
VEGF164 (ng/mL)
*
**
B
TGF-β1
Relative expression
1.5
1.0
0.5
0
0
1
2.5
40
VEGF164 (ng/mL)
C
Sema3A
Relative expression
20
15
10
5
0
0
1
2.5
40
TGF-β1 (ng/mL)
***
**
*
D
Reciprocal intensity
80
60
40
20
0
***
***
control
v low
no stain
IgG1
AbαTGFβ1
IgG1
AbαTGFβ1
no stain
control
v low
E
cd11b* cells / cm
40
30
20
10
0
***
***
control
v low
IgG1
AbαTGFβ1
IgG1
AbαTGFβ1
control
v low

Sema3A

Relative expression

$VEGF_{164}$ (ng/mL)

TGF-β1

Relative expression

$VEGF_{164}$ (ng/mL)

Sema3A

Relative expression

TGF-β1 (ng/mL)

Reciprocal intensity

control

control

v low

no stain

IgG1

AbαTGFβ1

v low

IgG1

AbαTGFβ1

cd11b* cells / cm

control

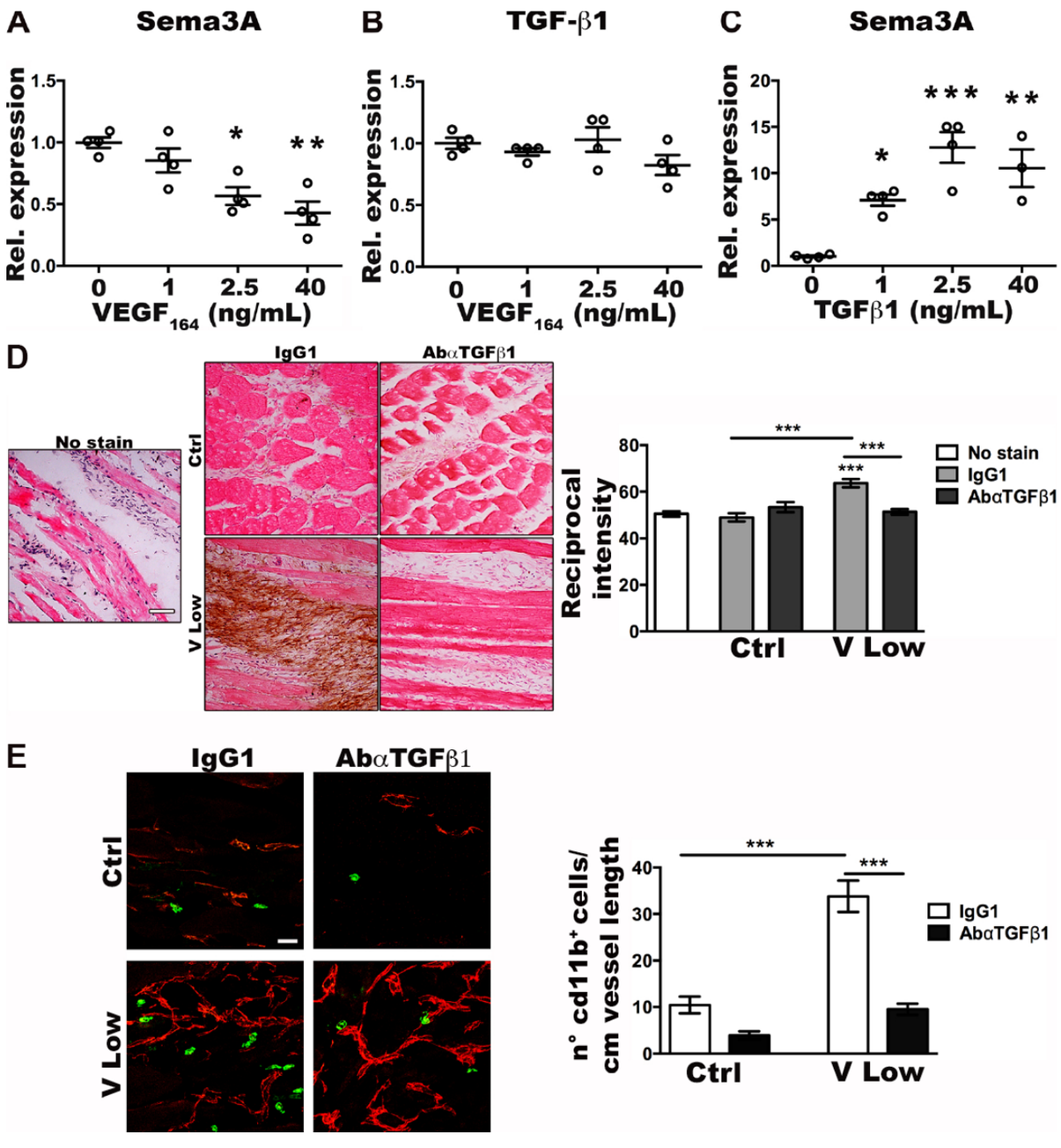
control

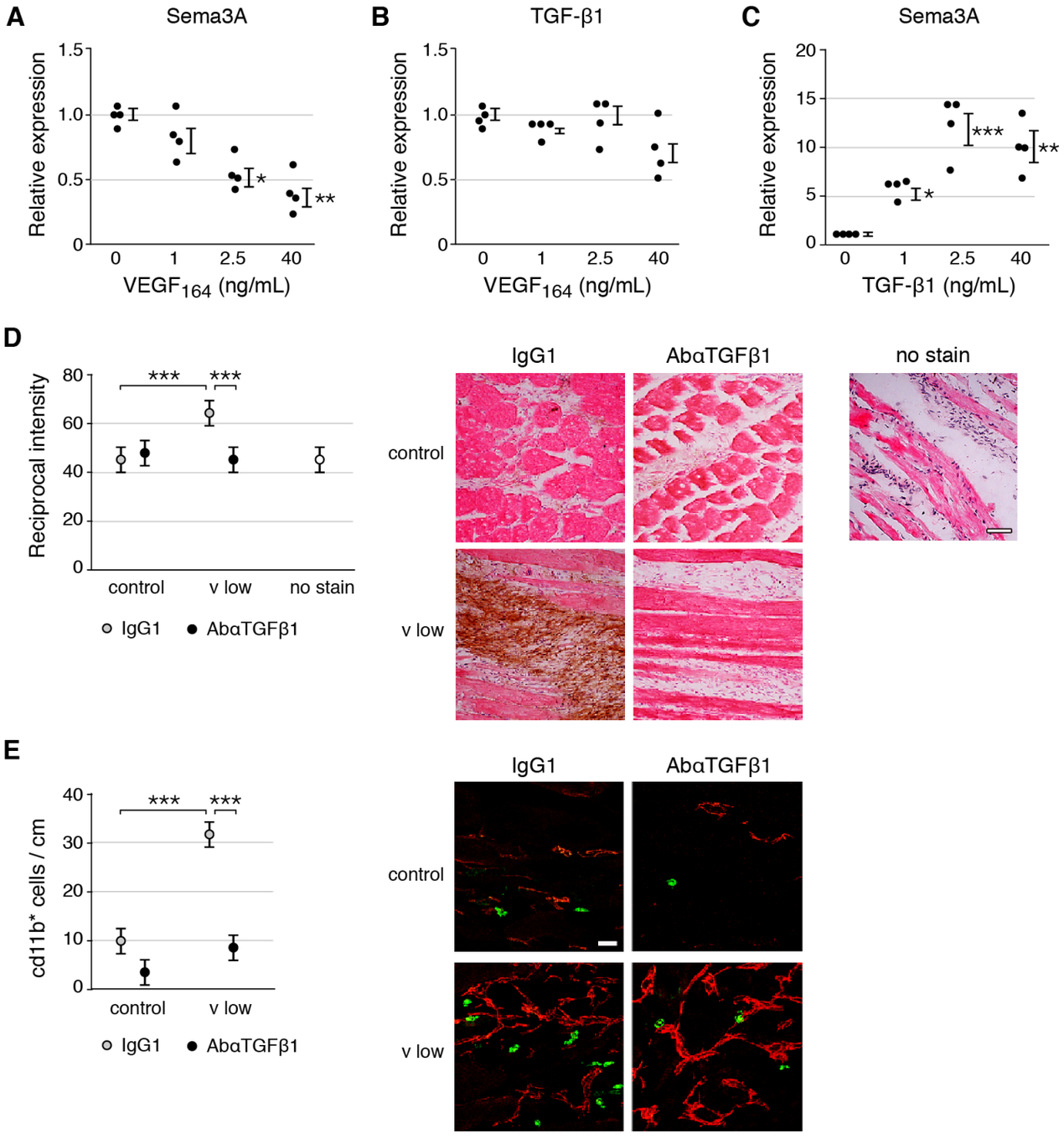
v low

IgG1

AbαTGFβ1

v low


A
Sema3A
Rel. expression
VEGF164 (ng/mL)
B
TGF-β1
C
Sema3A
TGFβ1 (ng/mL)
D
IgG1
AbαTGFβ1
No stain
Ctrl
V Low
Reciprocal intensity
No stain
IgG1
AbαTGFβ1
E
n° cd11b+cells/ cm vessel length


A
Sema3A
Relative expression
VEGF164 (ng/mL)
B
TGF-β1
C
Sema3A
TGF-β1 (ng/mL)
D
Reciprocal intensity
control
v low
no stain
IgG1
AbαTGFβ1
E
cd11b* cells / cm

POINTS OF SIGNIFICANCE

Visualizing samples with box plots

Use box plots to illustrate the spread and differences of samples.

Visualization methods enhance our understanding of sample data and help us make comparisons across samples. Box plots are a simple but powerful graphing tool that can be used in place of histograms to address both goals. Whereas histograms require a sample size of at least 30 to be useful, box plots require a sample size of only 5, provide more detail in the tails of the distribution and are more readily compared across three or more samples. Several enhancements to the basic box plot can render it even more informative.

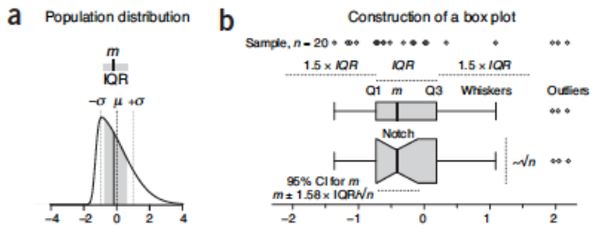


Figure 1 | The construction of a box plot. (**a**) The median ($m = -0.19$, solid vertical line) and interquartile range (IQR = 1.38, gray shading) are ideal for characterizing asymmetric or irregularly shaped distributions. A skewed normal distribution is shown with mean $\mu = 0$ (dark dotted line) and s.d. $\sigma = 1$ (light dotted lines). (**b**) Box plots for an $n = 20$ sample from **a**. The box bounds the IQR divided by the median, and Tukey-style whiskers extend to a maximum of 1.5 × IQR beyond the box. The box width may be scaled by $\sqrt{n}$, and a notch may be added approximating a 95% confidence interval (CI) for the median. Open circles are sample data points. Dotted lines indicate the lengths or widths of annotated features.

Box plots characterize a sample using the 25th, 50th and 75th percentiles—also known as the lower quartile (Q1), median (m or Q2) and upper quartile (Q3)—and the interquartile range (IQR = Q3 – Q1), which covers the central 50% of the data. Quartiles are insensitive to outliers and preserve information about the center and spread. Consequently, they are preferred over the mean and s.d. for population distributions that are asymmetric or irregularly shaped and for samples with extreme outliers. In such cases these measures may be difficult to intuitively interpret: the mean may be far from the bulk of the data, and conventional rules for interpreting the s.d. will likely not apply.

The core element that gives the box plot its name is a box whose length is the IQR and whose width is arbitrary (**Fig. 1**). A line inside the box shows the median, which is not necessarily central. The plot may be oriented vertically or horizontally—we use here (with one exception) horizontal boxes to maintain consistent orientation with corresponding sample distributions. Whiskers are conventionally extended to the most extreme data point that is no more than 1.5 × IQR from the edge of the box (Tukey style) or all the way to minimum and maximum of the data values (Spear style). The use

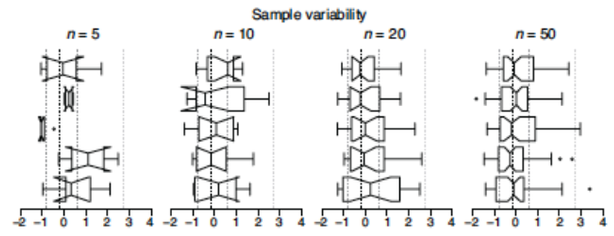


Figure 2 | Box plots reflect sample variability and should be avoided for very small samples ($n < 5$), with notches shown only when they appear within the IQR. Tukey-style box plots for five samples with sample size $n = 5$, 10, 20 and 50 drawn from the distribution in **Figure 1a** are shown; notch width is as in **Figure 1b**. Vertical dotted lines show Q1 (−0.78), median (−0.19), Q3 (0.60) and Q3 + 1.5 × IQR (2.67) values for the distribution.

of quartiles for box plots is a well-established convention: boxes or whiskers should never be used to show the mean, s.d. or s.e.m. As with the division of the box by the median, the whiskers are not necessarily symmetrical (**Fig. 1b**). The 1.5 multiplier corresponds to approximately $\pm 2.7\sigma$ (where σ is s.d.) and 99.3% coverage of the data for a normal distribution. Outliers beyond the whiskers may be individually plotted. Box plot construction requires a sample of at least $n = 5$ (preferably larger), although some software does not check for this. For $n < 5$ we recommend showing the individual data points.

Sample size differences can be assessed by scaling the box plot width in proportion to $\sqrt{n}$ (**Fig. 1b**), the factor by which the precision of the sample's estimate of population statistics improves as sample size is increased.

To assist in judging differences between sample medians, a notch (**Fig. 1b**) can be used to show the 95% confidence interval (CI) for the median, given by $m \pm 1.58 \times \text{IQR}/\sqrt{n}$ (ref. 1). This is an approximation based on the normal distribution and is accurate in large samples for other distributions. If you suspect the population distribution is not close to normal and your sample size is small, avoid interpreting the interval analytically in the way we have described for CI error bars[2]. In general, when notches do not overlap, the medians can be judged to differ significantly, but overlap does not rule out a significant difference. For small samples the notch may span a larger interval than the box (**Fig. 2**).

The exact position of box boundaries will be software dependent. First, there is no universally agreed-upon method to calculate quartile values, which may be based on simple averaging or linear interpolation. Second, some applications, such as R, use hinges instead of quartiles for box boundaries. The lower and upper hinges are the median of the

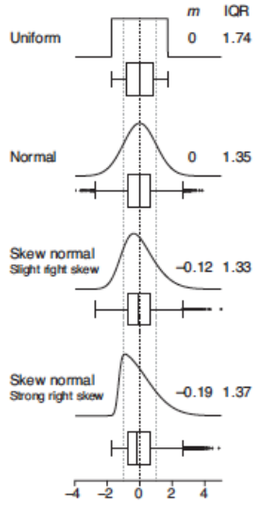


Figure 3 | Quartiles are more intuitive than the mean and s.d. for samples from skewed distributions. Four distributions with the same mean ($\mu = 0$, dark dotted line) and s.d. ($\sigma = 1$, light dotted lines) but significantly different medians (m) and IQRs are shown with corresponding Tukey-style box plots for $n = 10{,}000$ samples.

POINTS OF SIGNIFICANCE

Error bars

The meaning of error bars is often misinterpreted, as is the statistical significance of their overlap.

Last month in Points of Significance, we showed how samples are used to estimate population statistics. We emphasized that, because of chance, our estimates had an uncertainty. This month we focus on how uncertainty is represented in scientific publications and reveal several ways in which it is frequently misinterpreted.

The uncertainty in estimates is customarily represented using error bars. Although most researchers have seen and used error bars, misconceptions persist about how error bars relate to statistical significance. When asked to estimate the required separation between two points with error bars for a difference at significance $P = 0.05$, only 22% of respondents were within a factor of 2 (ref. 1). In light of the fact that error bars are meant to help us assess the significance of the difference between two values, this observation is disheartening and worrisome.

Here we illustrate error bar differences with examples based on a simplified situation in which the values are means of independent (unrelated) samples of the same size and drawn from normal populations with the same spread. We calculate the significance of the difference in the sample means using the two-sample t-test and report it as the familiar P value. Although reporting the exact P value is preferred, conventionally, significance is often assessed at a $P = 0.05$ threshold. We will discuss P values and the t-test in more detail in a subsequent column.

The importance of distinguishing the error bar type is illustrated in **Figure 1**, in which the three common types of error bars—standard deviation (s.d.), standard error of the mean (s.e.m.) and confidence interval (CI)—show the spread in values of two samples of size $n = 10$ together with the P value of the difference in sample means. In **Figure 1a**, we simulated the samples so that each error bar type has the same length, chosen to make them exactly abut. Although these three data pairs and their error bars are visually identical, each represents a different data scenario with a different P value. In **Figure 1b**, we fixed the P value to $P = 0.05$ and show the length of each type of bar for this level of significance. In this latter scenario, each of the three pairs of points represents the same pair of samples, but the bars have different lengths because they indicate different statistical properties of the same data. And because each bar is a different length, you are likely to interpret each one quite differently. In general, a gap between bars

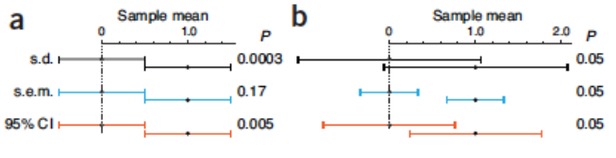


Figure 1 | Error bar width and interpretation of spacing depends on the error bar type. (**a**,**b**) Example graphs are based on sample means of 0 and 1 ($n = 10$). (**a**) When bars are scaled to the same size and abut, P values span a wide range. When s.e.m. bars touch, P is large ($P = 0.17$). (**b**) Bar size and relative position vary greatly at the conventional P value significance cutoff of 0.05, at which bars may overlap or have a gap.

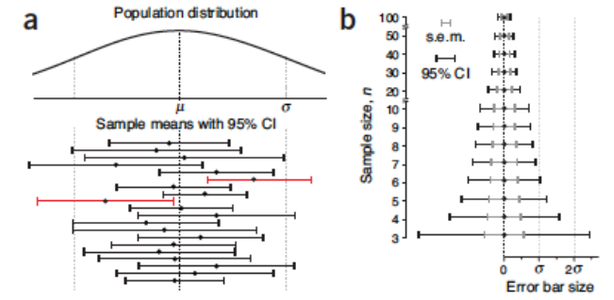


Figure 2 | The size and position of confidence intervals depend on the sample. On average, CI% of intervals are expected to span the mean—about 19 in 20 times for 95% CI. (**a**) Means and 95% CIs of 20 samples ($n = 10$) drawn from a normal population with mean μ and s.d. σ. By chance, two of the intervals (red) do not capture the mean. (**b**) Relationship between s.e.m. and 95% CI error bars with increasing n.

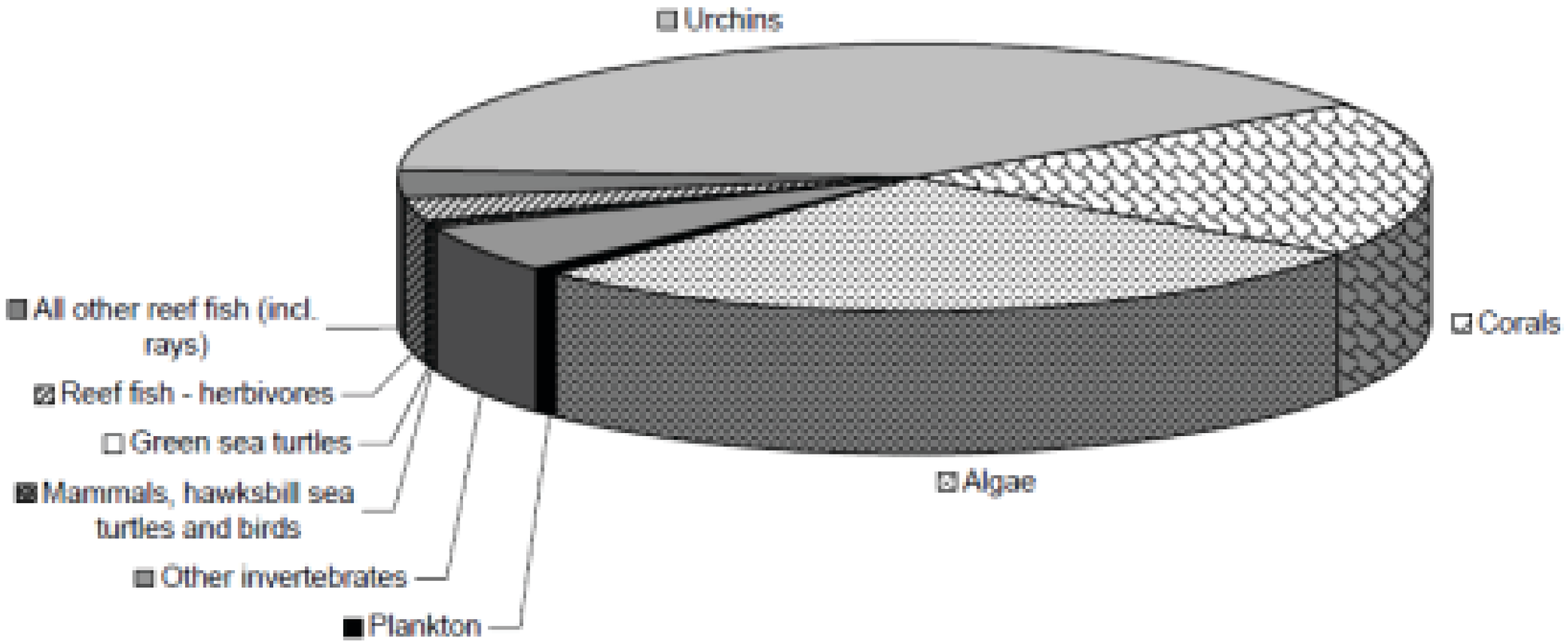
does not ensure significance, nor does overlap rule it out—it depends on the type of bar. Chances are you were surprised to learn this unintuitive result.

The first step in avoiding misinterpretation is to be clear about which measure of uncertainty is being represented by the error bar. In 2012, error bars appeared in *Nature Methods* in about two-thirds of the figure panels in which they could be expected (scatter and bar plots). The type of error bars was nearly evenly split between s.d. and s.e.m. bars (45% versus 49%, respectively). In 5% of cases the error bar type was not specified in the legend. Only one figure[2] used bars based on the 95% CI. CIs are a more intuitive measure of uncertainty and are popular in the medical literature.

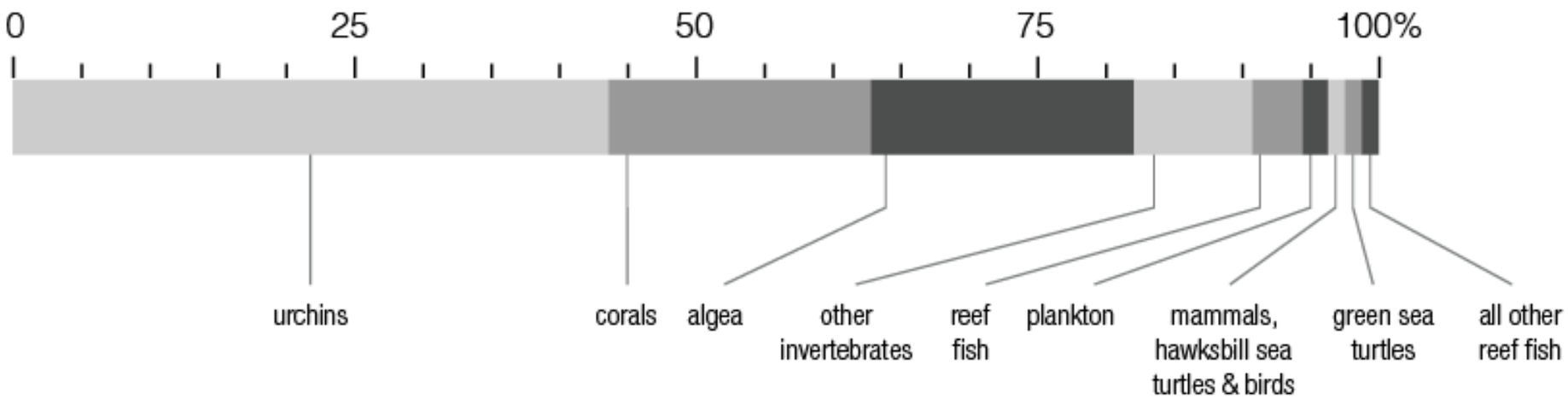
Error bars based on s.d. inform us about the spread of the population and are therefore useful as predictors of the range of new samples. They can also be used to draw attention to very large or small population spreads. Because s.d. bars only indirectly support visual assessment of differences in values, if you use them, be ready to help your reader understand that the s.d. bars reflect the variation of the data and not the error in your measurement. What should a reader conclude from the very large and overlapping s.d. error bars for $P = 0.05$ in **Figure 1b**? That although the means differ, and this can be detected with a sufficiently large sample size, there is considerable overlap in the data from the two populations.

Unlike s.d. bars, error bars based on the s.e.m. reflect the uncertainty in the mean and its dependency on the sample size, n (s.e.m. = s.d./$\sqrt{n}$). Intuitively, s.e.m. bars shrink as we perform more measurements. Unfortunately, the commonly held view that "if the s.e.m. bars do not overlap, the difference between the values is statistically significant" is incorrect. For example, when $n = 10$ and s.e.m. bars just touch, $P = 0.17$ (**Fig. 1a**). Conversely, to reach $P = 0.05$, s.e.m. bars for these data need to be about 0.86 arm lengths apart (**Fig. 1b**). We cannot overstate the importance of recognizing the difference between s.d. and s.e.m.

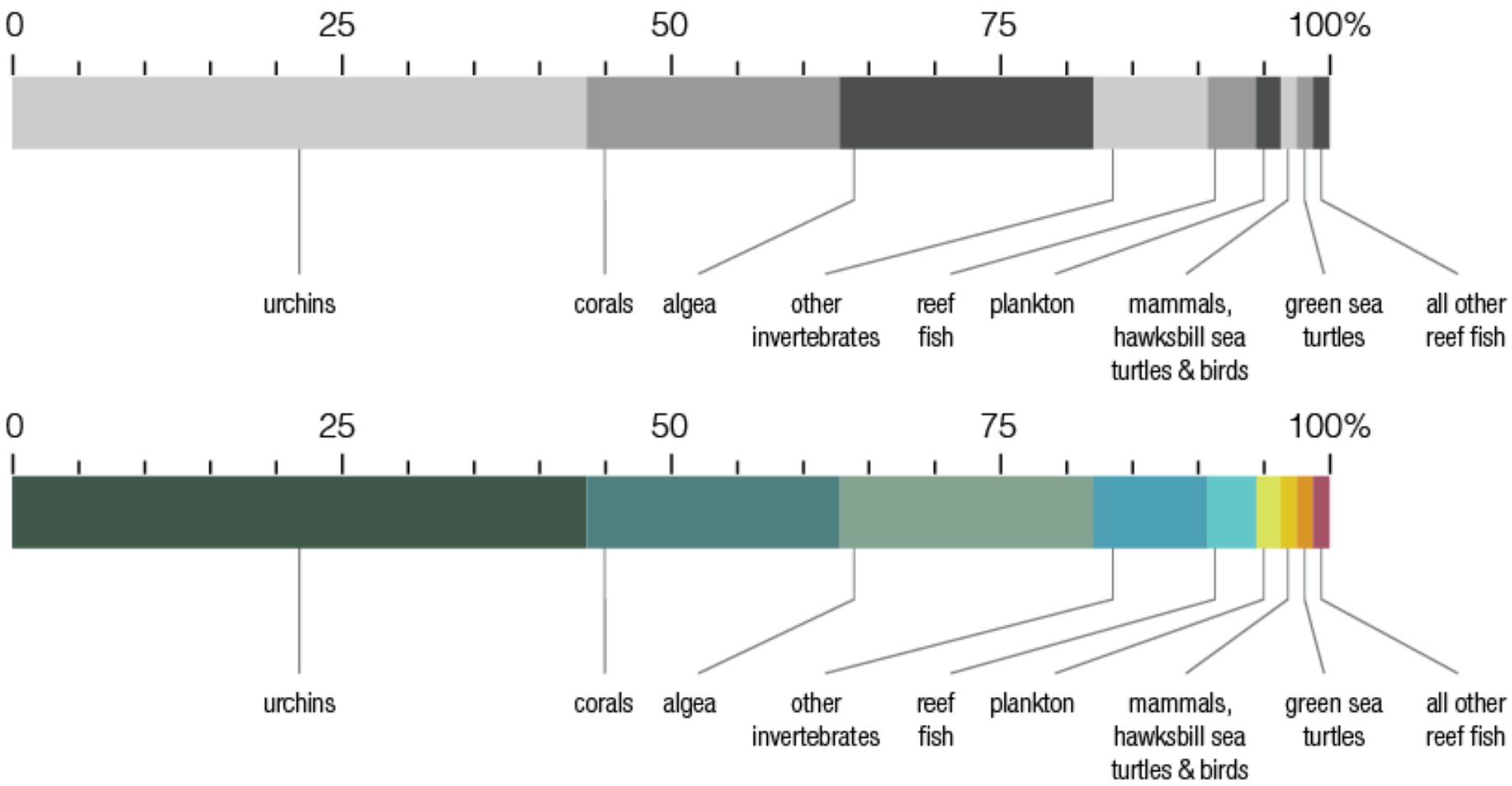
The third type of error bar you are likely to encounter is that based on the CI. This is an interval estimate that indicates the reliability of a measurement[3]. When scaled to a specific confidence level (CI%)—the 95% CI being common—the bar captures the population mean CI% of the time (**Fig. 2a**). The size of the s.e.m. is compared to the 95% CI in **Figure 2b**. The two are related by the t-statistic, and in large samples the s.e.m. bar can be interpreted as a CI with a confidence level of 67%. The size of the CI depends on n; two useful approximations for the CI are 95% CI ≈ 4 × s.e.m ($n = 3$) and 95% CI ≈ 2 × s.e.m. ($n > 15$).

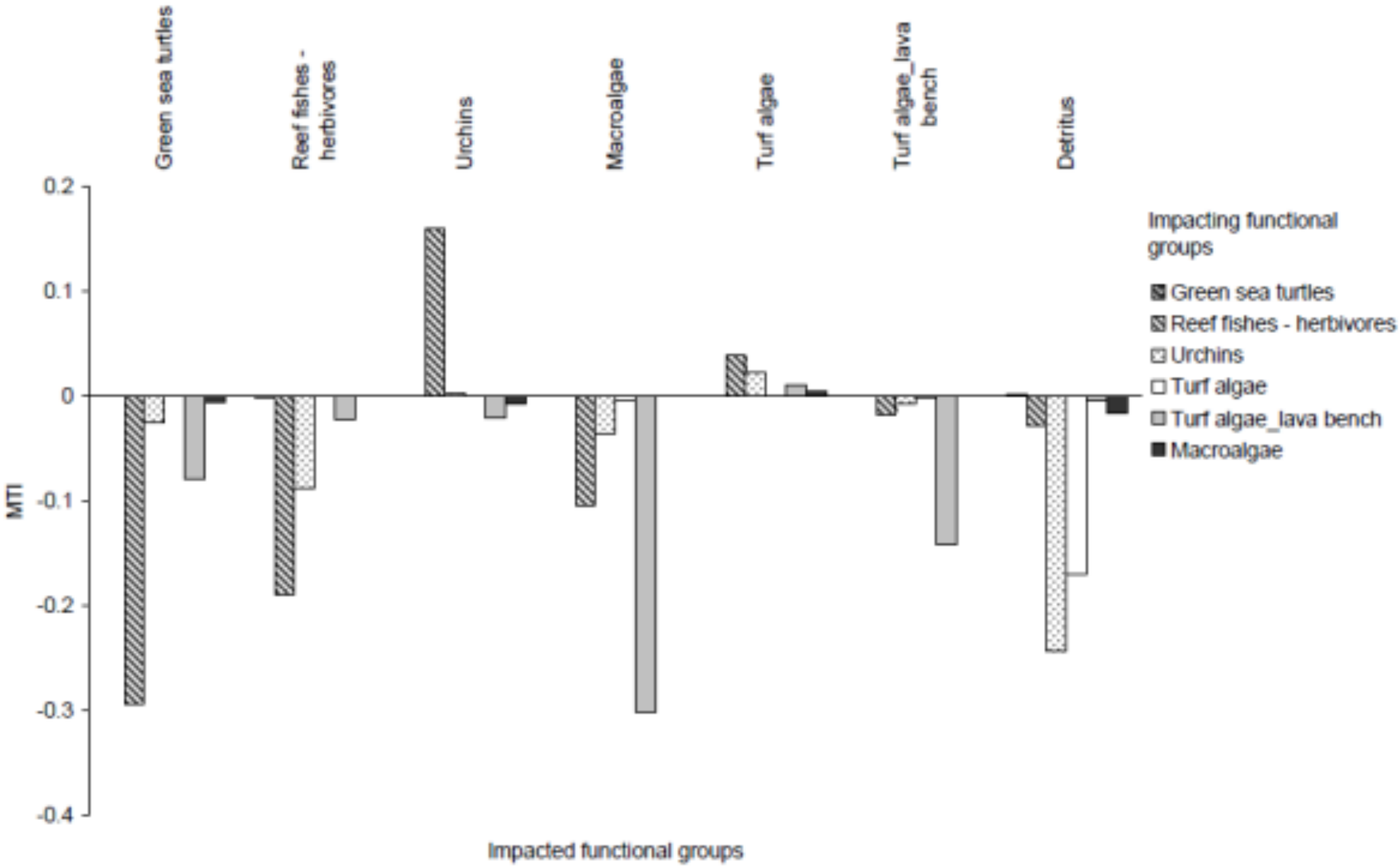

Urchins
All other reef fish (incl. rays)
Reef fish - herbivores
Green sea turtles
Mammals, hawksbill sea turtles and birds
Other invertebrates
Plankton
Corals
Algae

Proportions (%), in terms of biomass (t/km^2) of different groups within the Kaloko National Marine Park ecosystem



Proportions (%), in terms of biomass (t/km^2) of different groups within the Kaloko National Marine Park ecosystem




Green sea turtles
Reef fishes - herbivores
Urchins
Macroalgae
Turf algae
Turf algae_lava bench
Detritus
0.2
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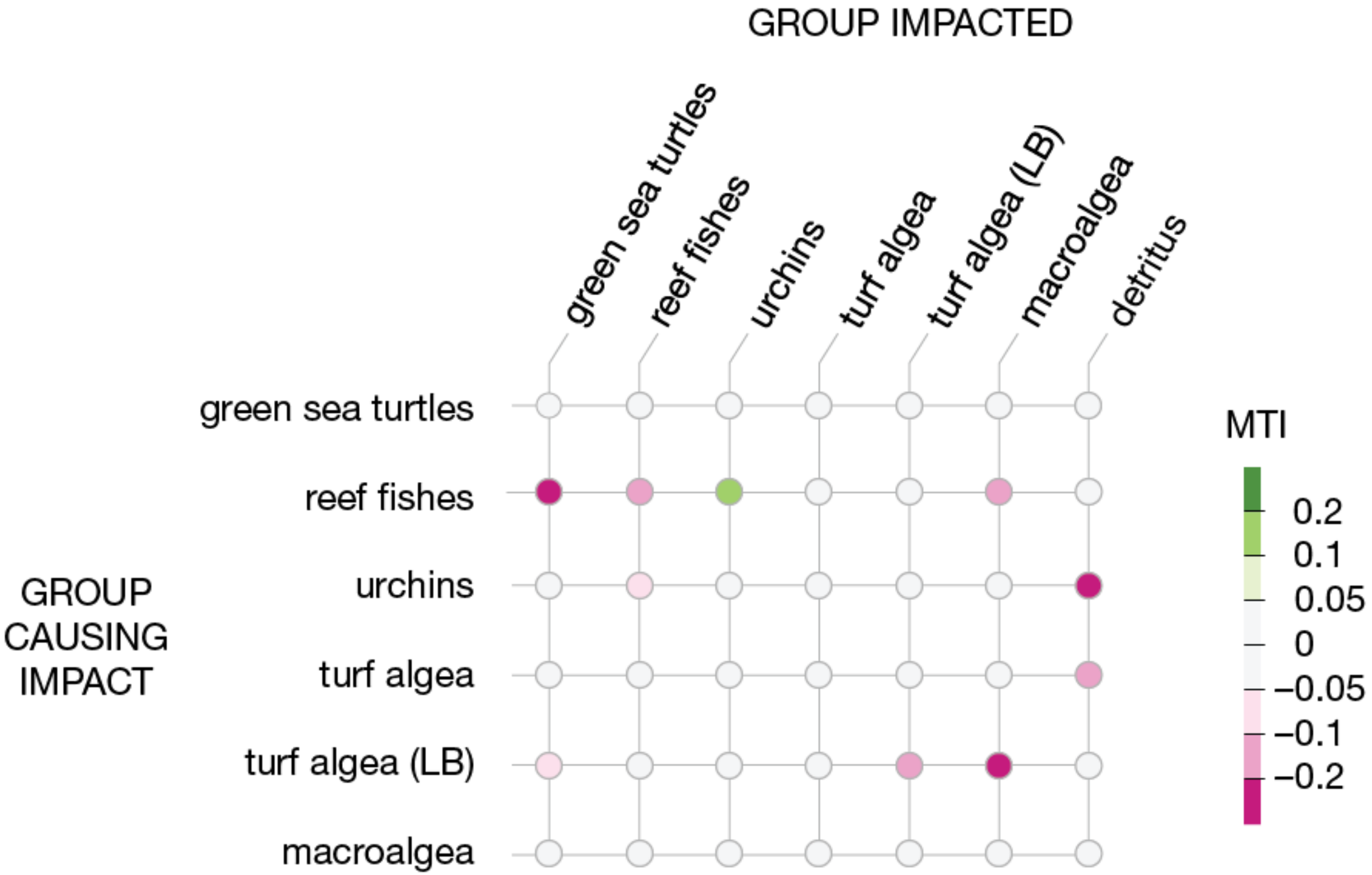
-0.2
-0.3
-0.4
MTI
Impacting functional groups
Green sea turtles
Reef fishes - herbivores
Urchins
Turf algae
Turf algae_lava bench
Macroalgae
Impacted functional groups


GROUP IMPACTED
green sea turtles
reef fishes
urchins
turf algea
turf algea (LB)
macroalgea
detritus
GROUP CAUSING IMPACT
green sea turtles
reef fishes
urchins
turf algea
turf algea (LB)
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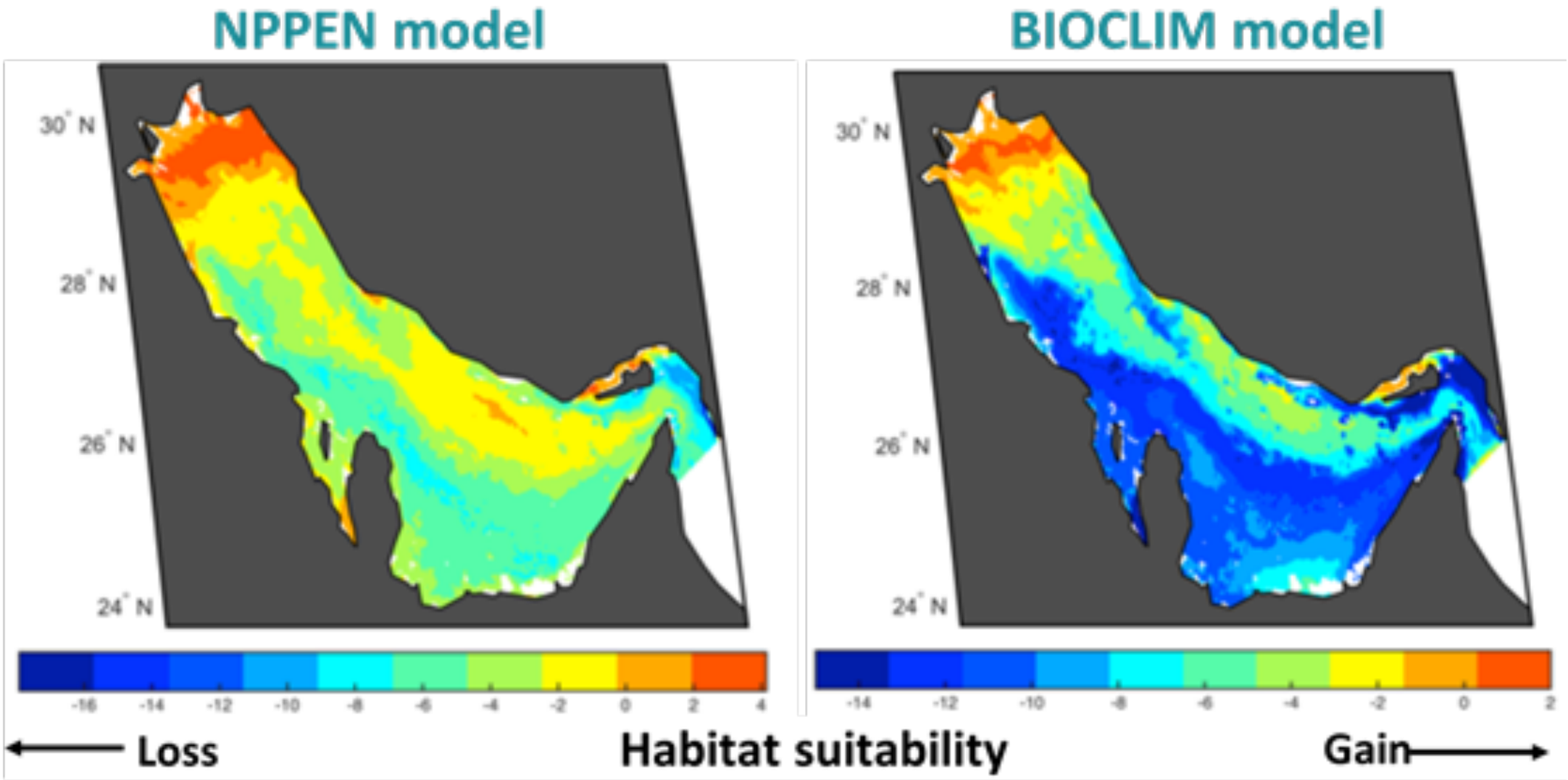
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BIOCLIM model
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28° N
26° N
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-14 -12 -10 -8 -6 -4 -2 0 2
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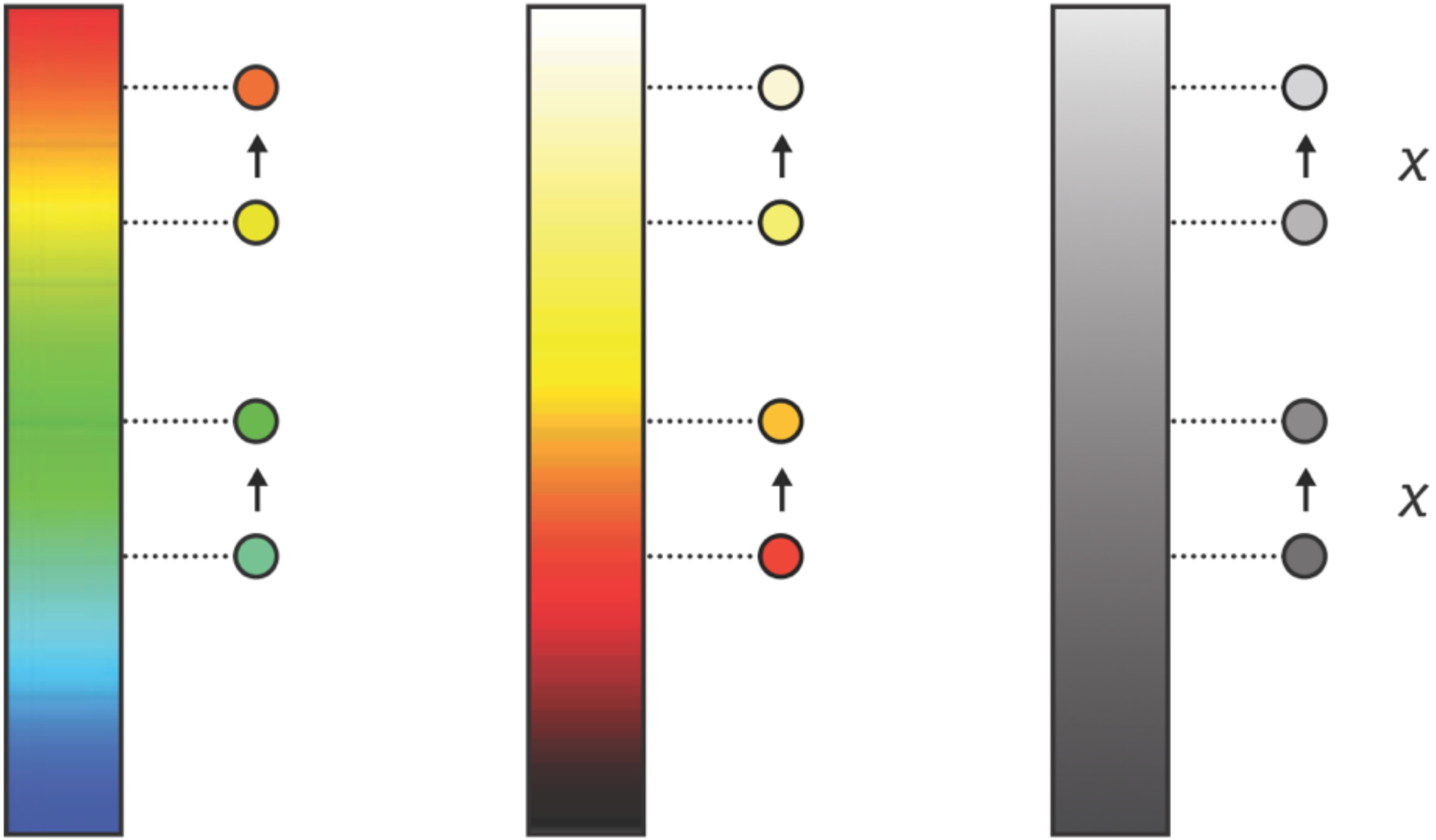
Habitat suitability
Gain



Wong, B. (2011) Points of View: Points of Review II. Nat Meth 8:189.

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REFERENCE

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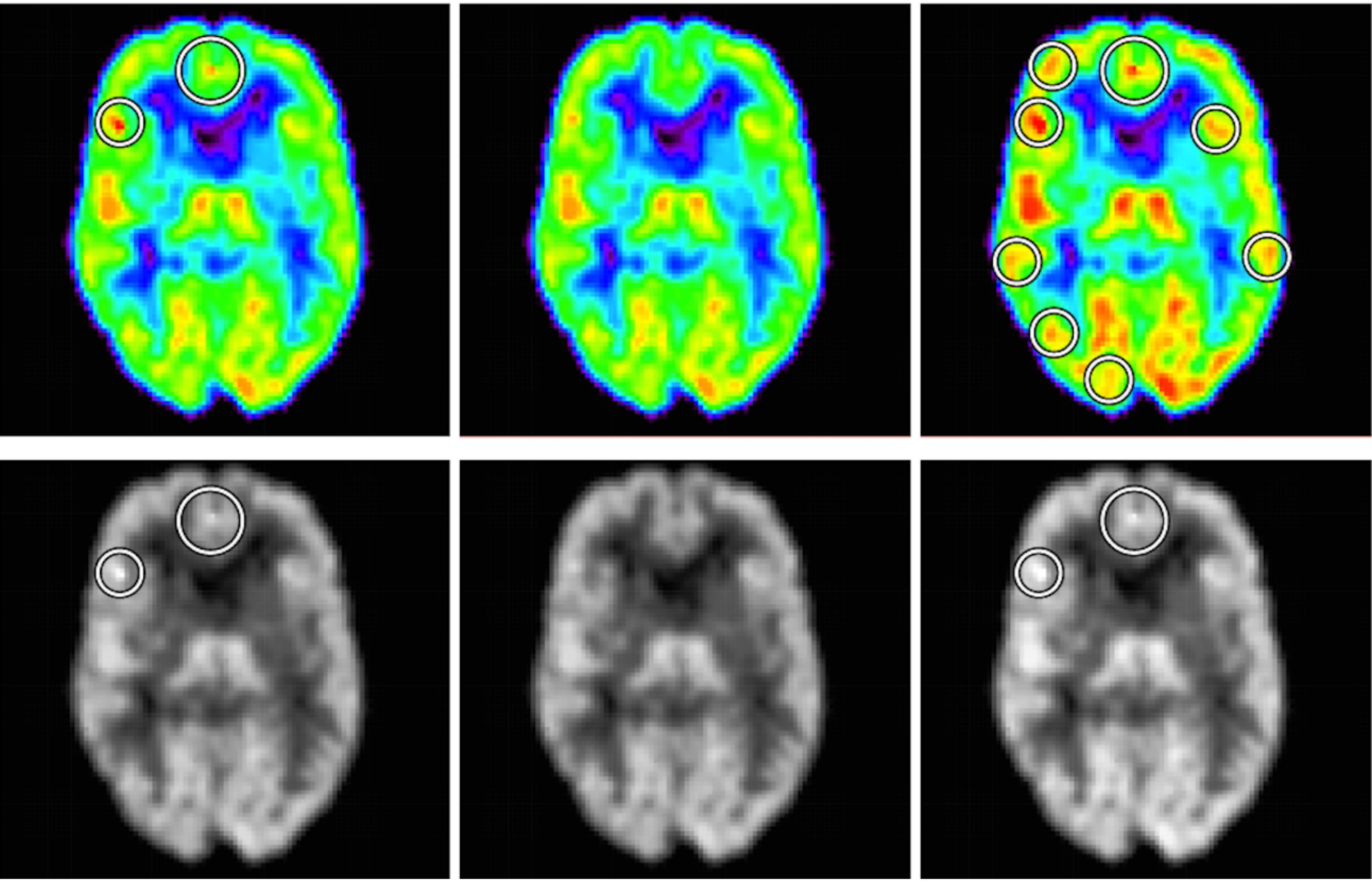

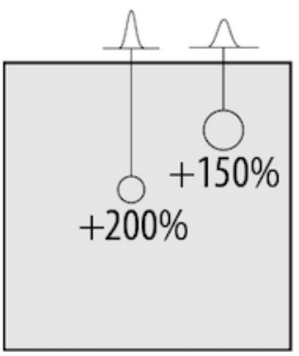
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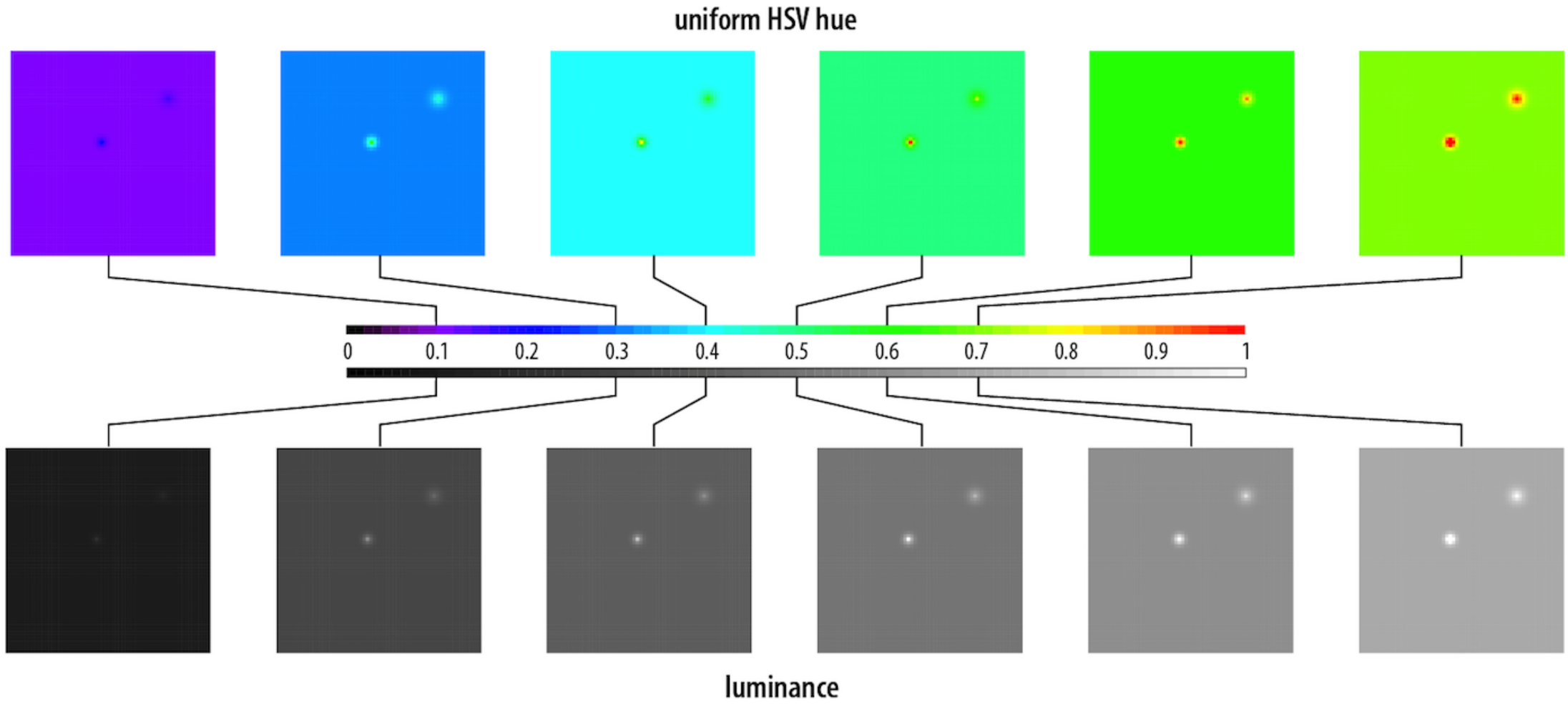
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BREWER PALETTES

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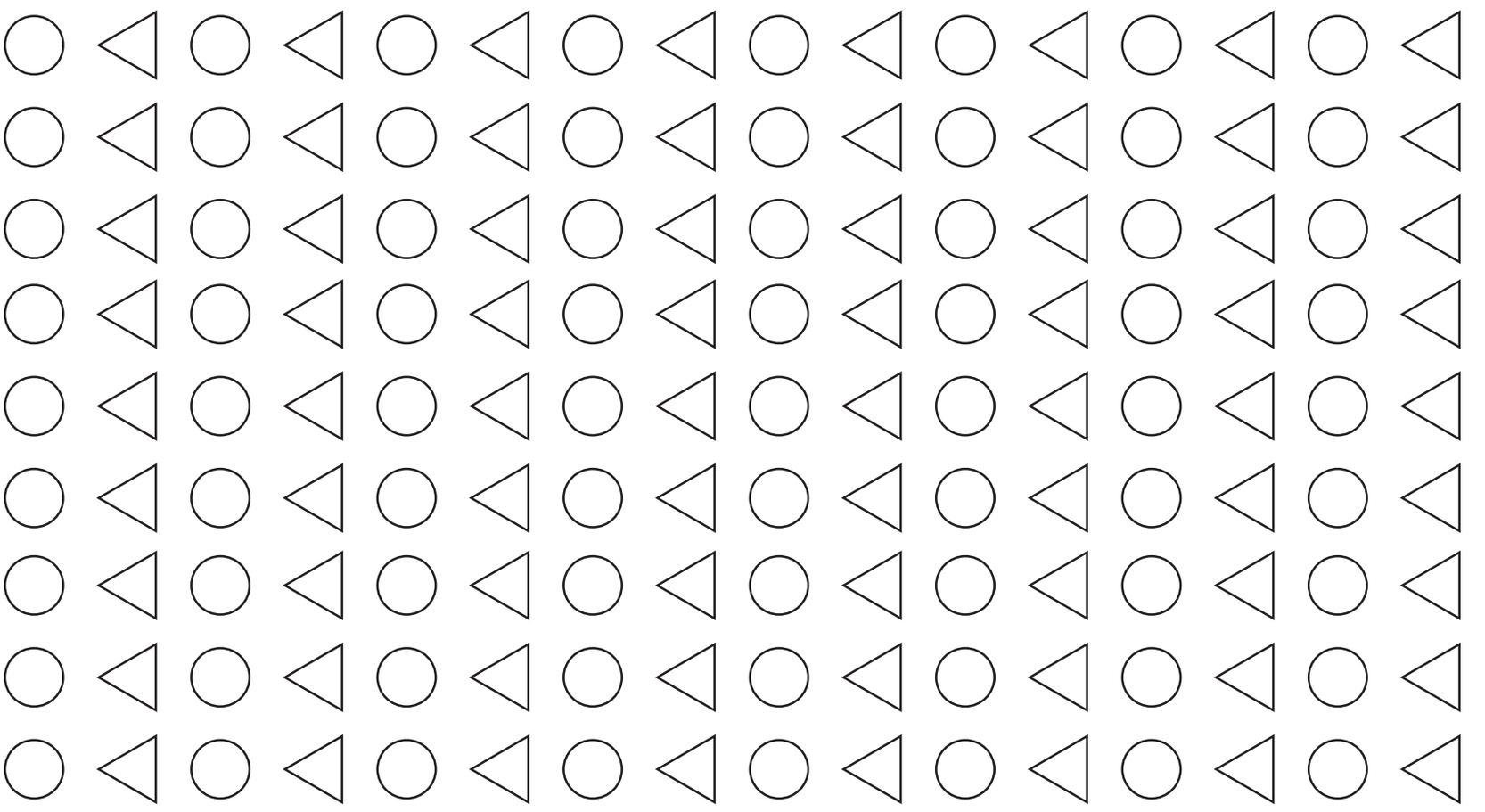
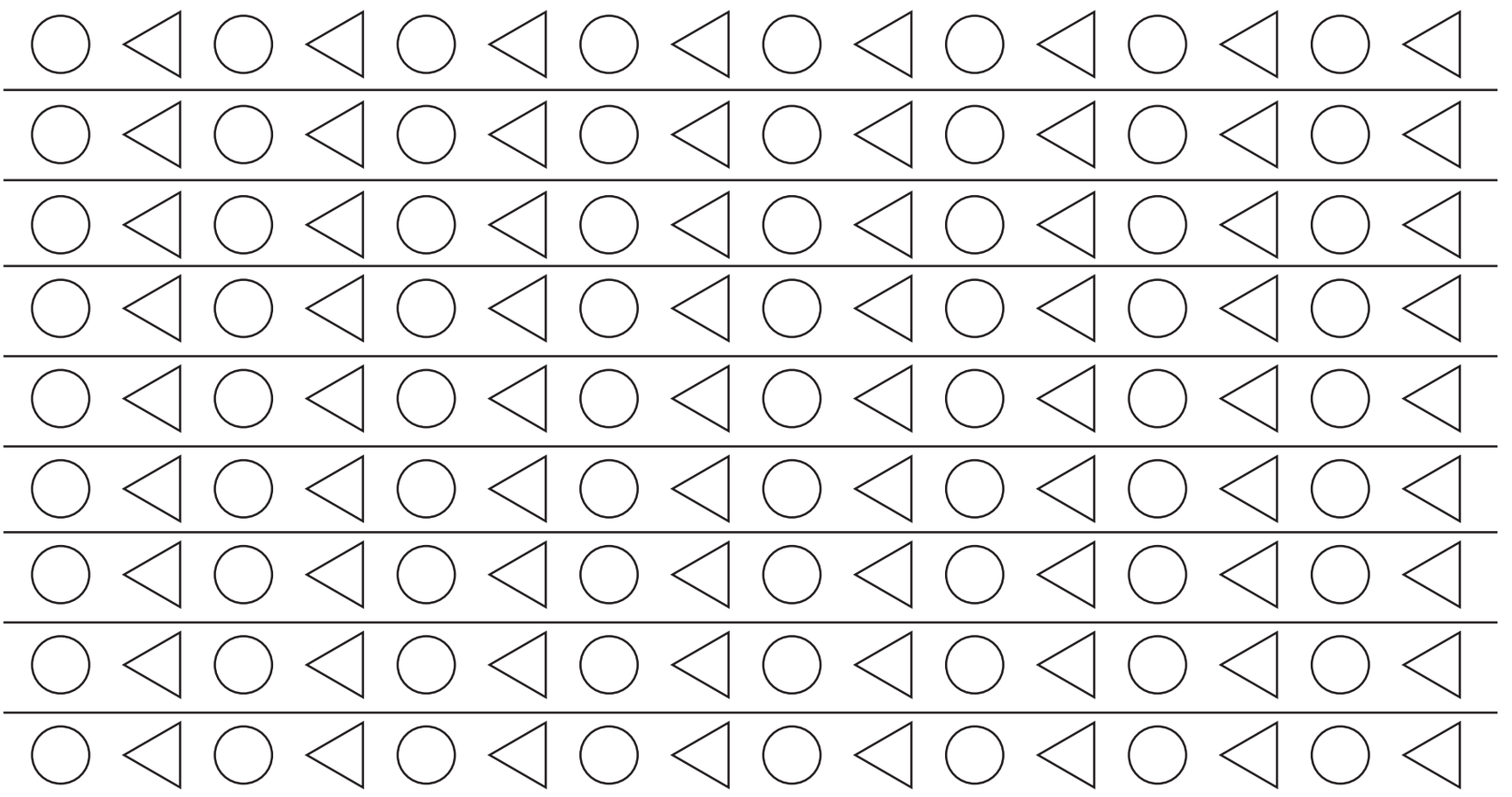
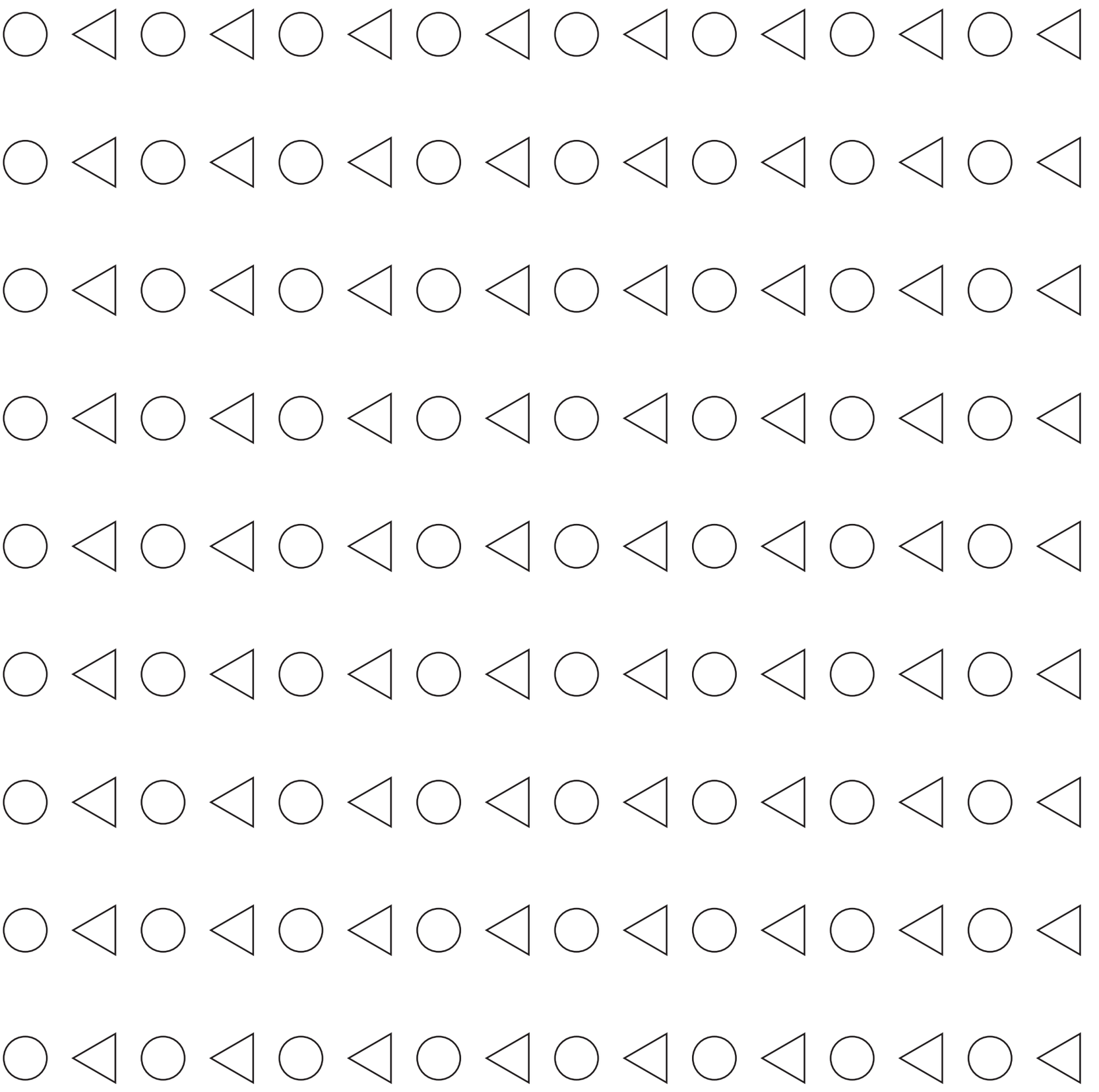
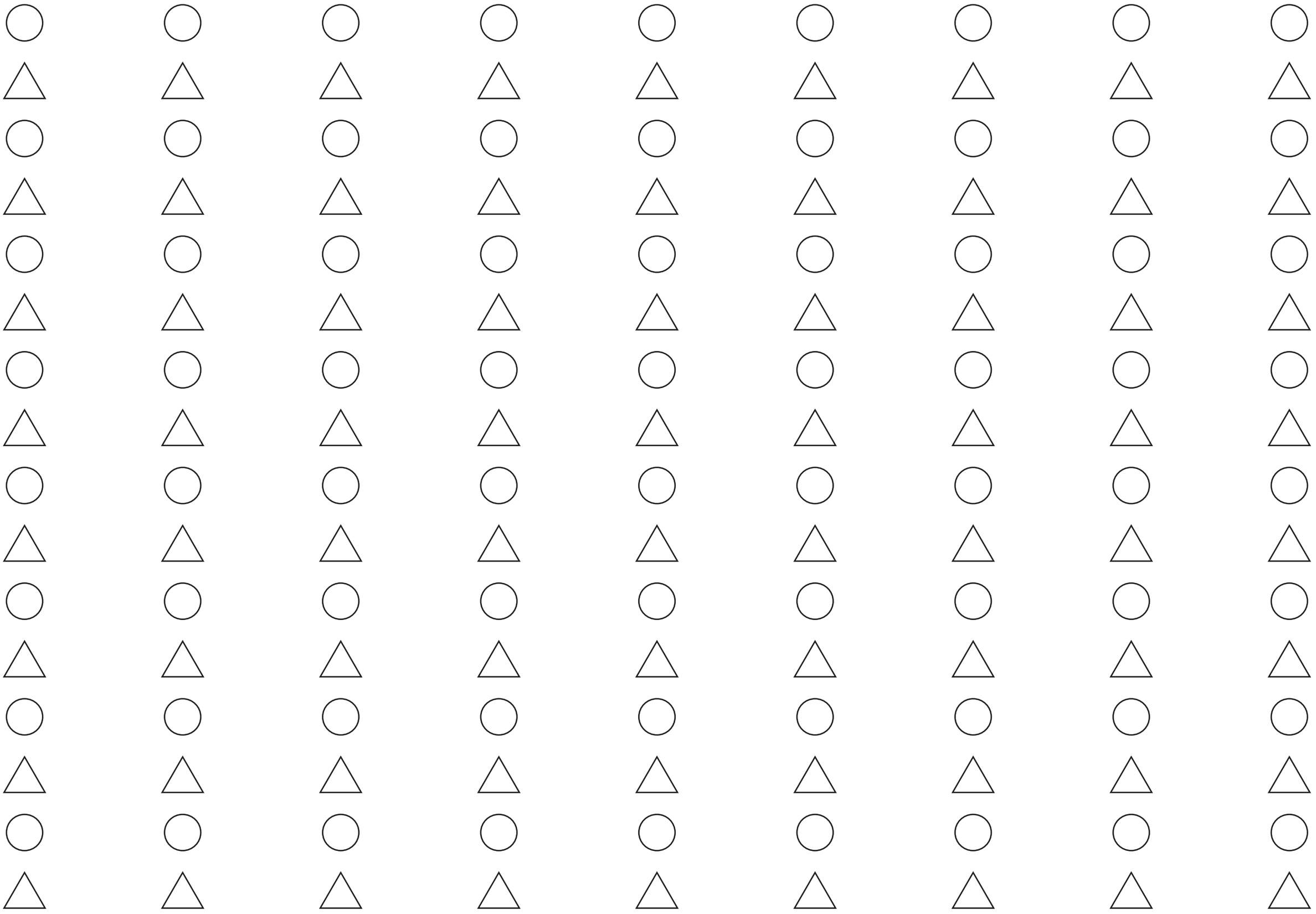
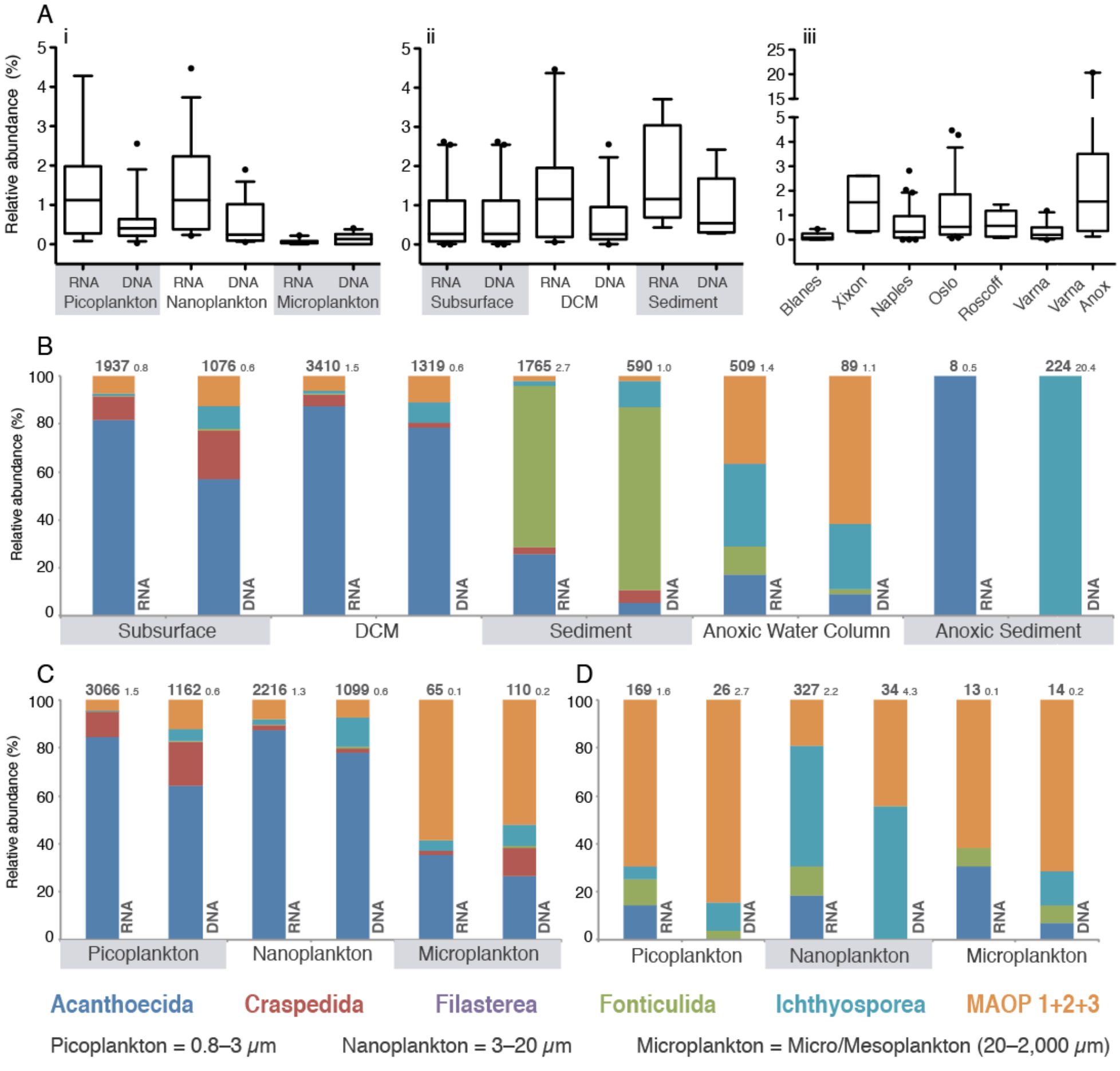


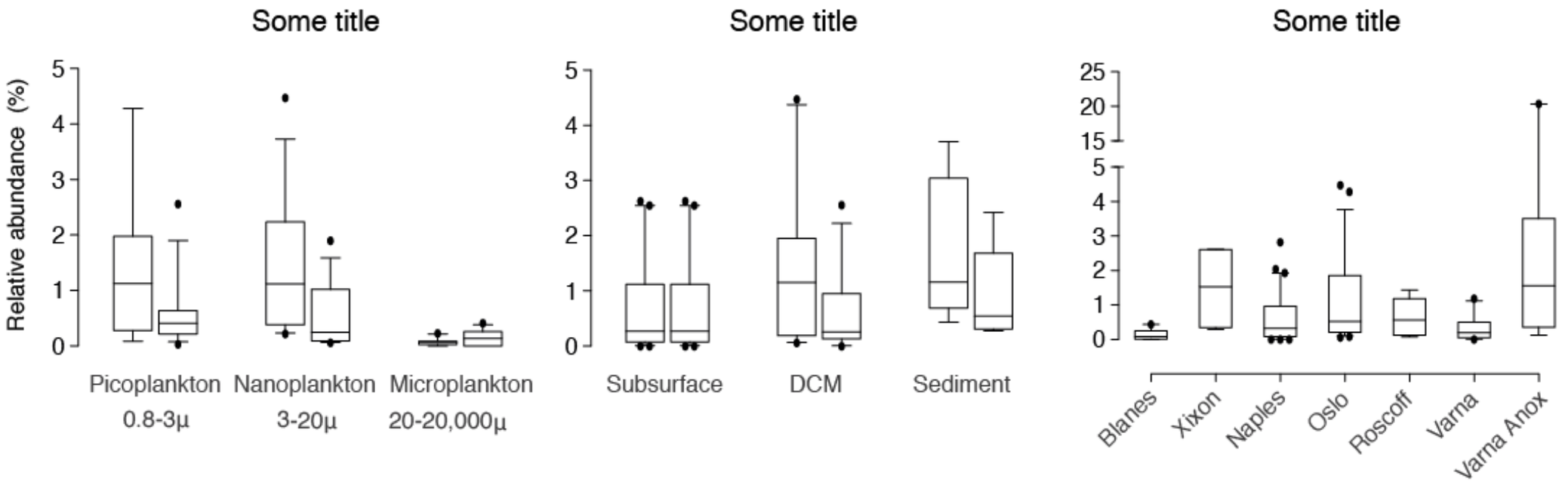







A
i
ii
iii
Relative abundance (%)
RNA
DNA
Picoplankton
Nanoplankton
Microplankton
Subsurface
DCM
Sediment
Blanes
Xixon
Naples
Oslo
Roscoff
Varna
Varna
Anox
B
1937 0.8
1076 0.6
3410 1.5
1319 0.6
1765 2.7
590 1.0
509 1.4
89 1.1
8 0.5
224 20.4
Anoxic Water Column
Anoxic Sediment
C
D
3066 1.5
1162 0.6
2216 1.3
1099 0.6
65 0.1
110 0.2
169 1.6
26 2.7
327 2.2
34 4.3
13 0.1
14 0.2
Acanthoecida
Craspedida
Filasterea
Fonticulida
Ichthyosporea
MAOP 1+2+3
Picoplankton = 0.8–3 μm
Nanoplankton = 3–20 μm
Microplankton = Micro/Mesoplankton (20–2,000 μm)

A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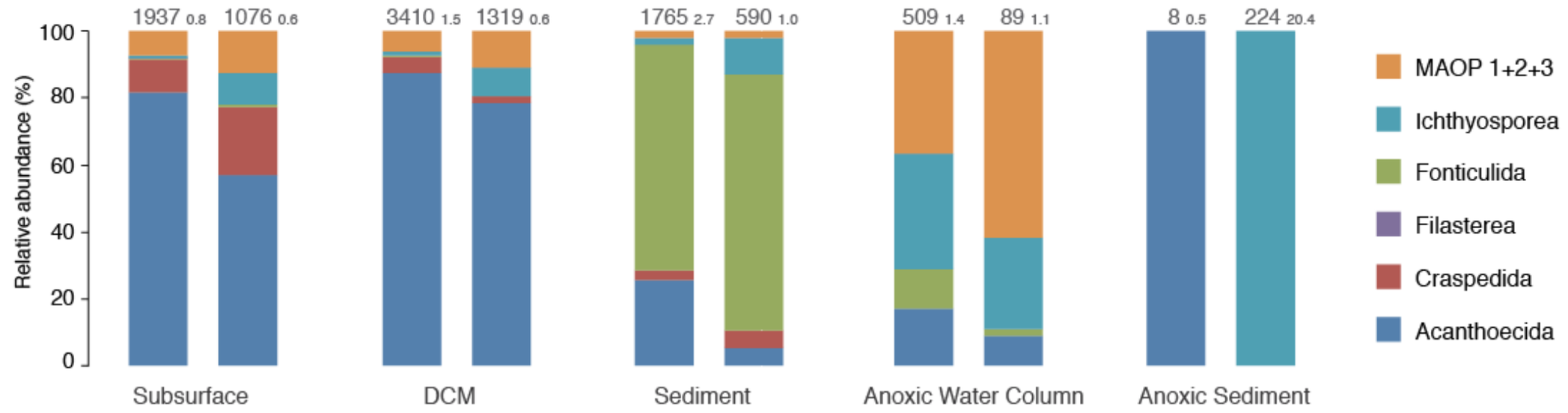
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Relative abundance (%)
Subsurface
DCM
Sediment
Anoxic Water Column
Anoxic Sediment
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Fonticulida
Filasterea
Craspedida
Acanthoecida

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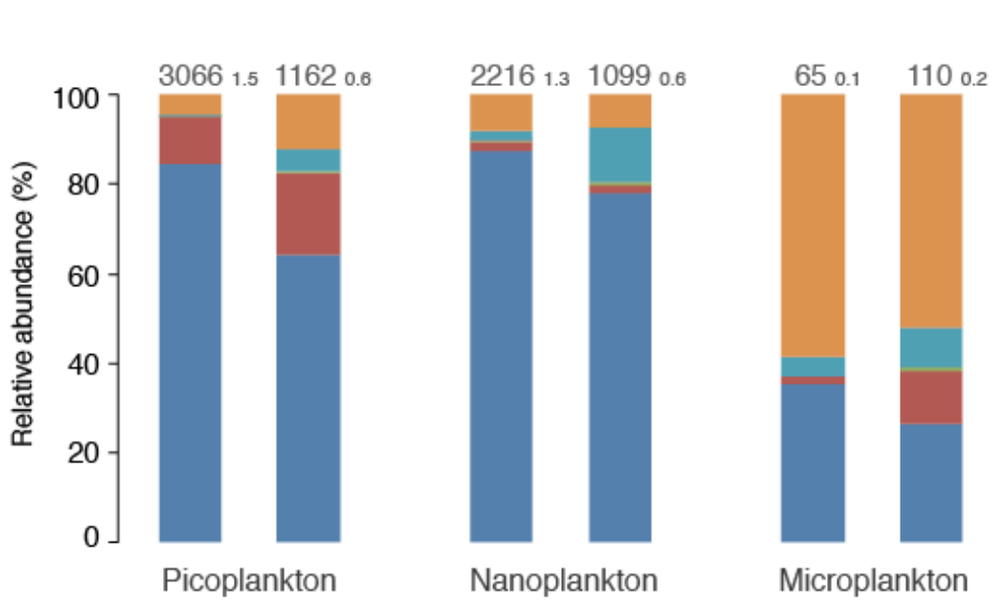
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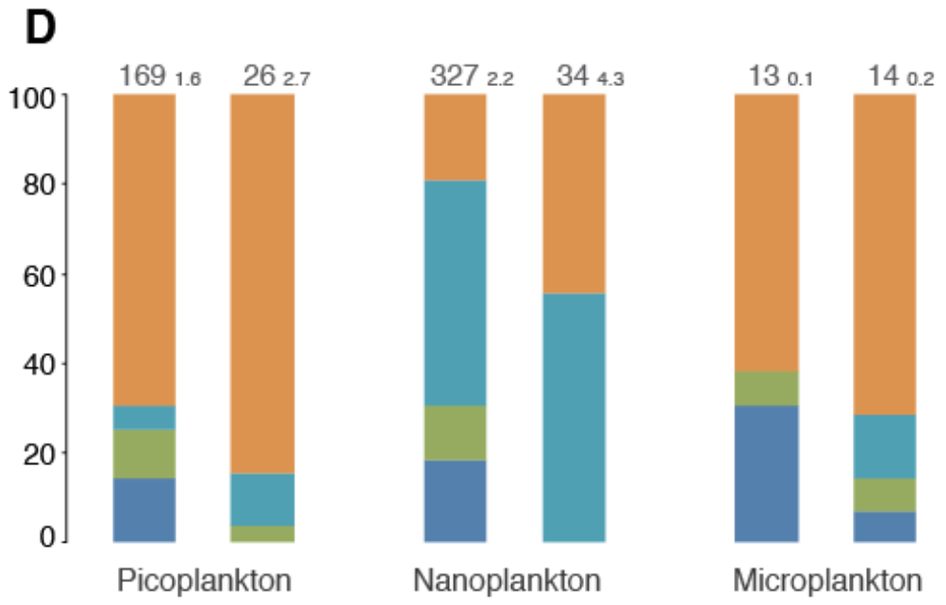
Relative abundance (%)
Picoplankton
Nanoplankton
Microplankton

D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14 0.2
Picoplankton
Nanoplankton
Microplankton

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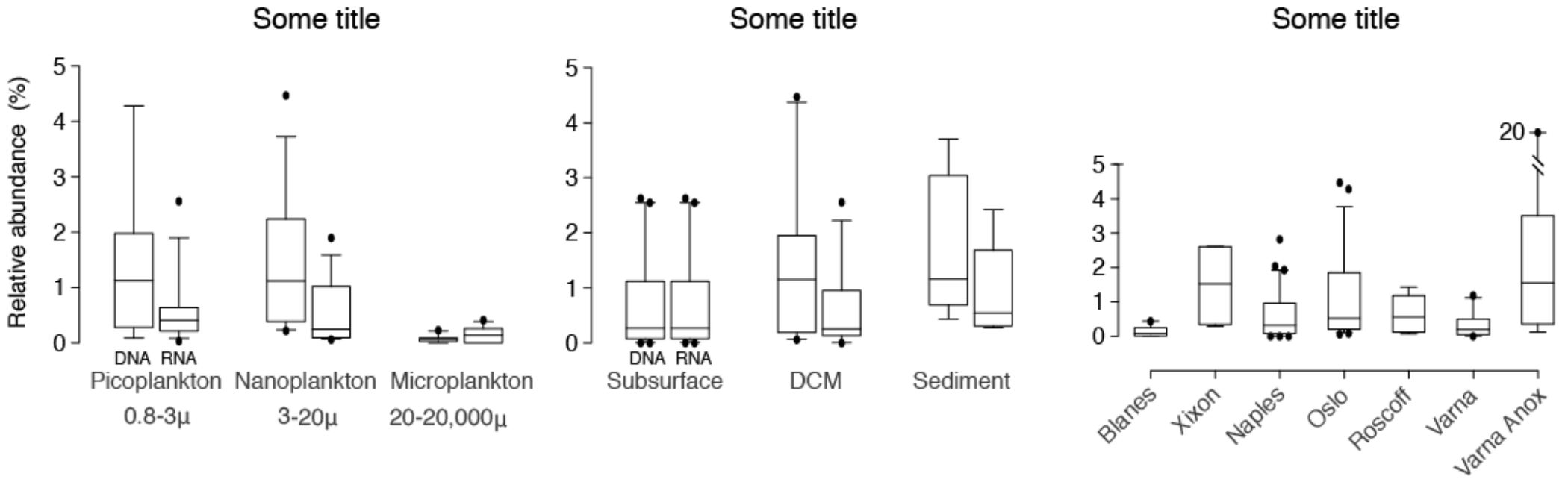
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Microplankton
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Naples
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Roscoff
Varna
Varna Anox

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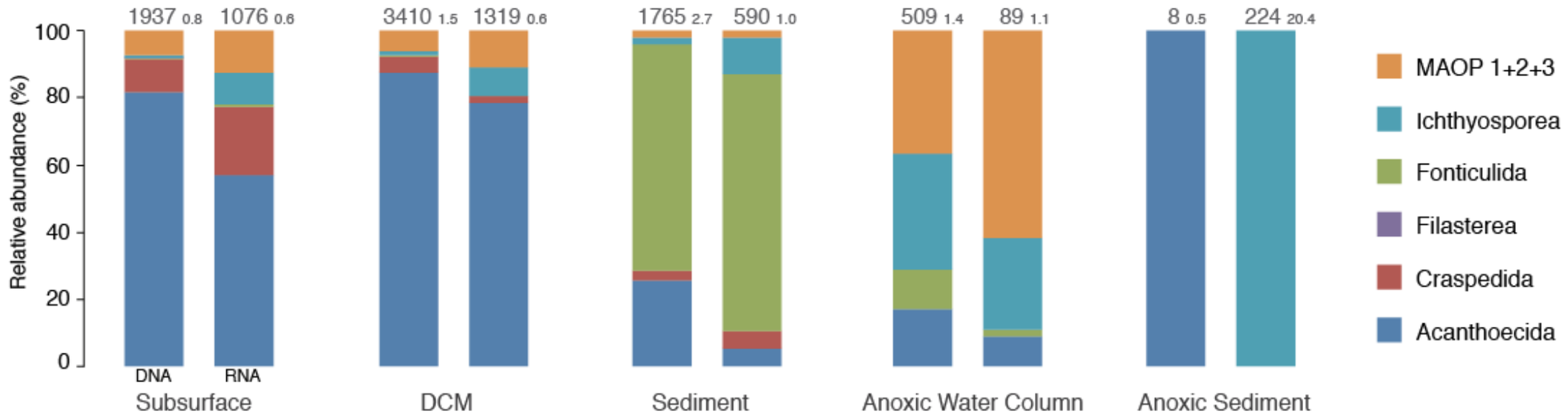
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89 1.1
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224 20.4
Relative abundance (%)
DNA RNA
Subsurface
DCM
Sediment
Anoxic Water Column
Anoxic Sediment
MAOP 1+2+3
Ichthyosporea
Fonticulida
Filasterea
Craspedida
Acanthoecida

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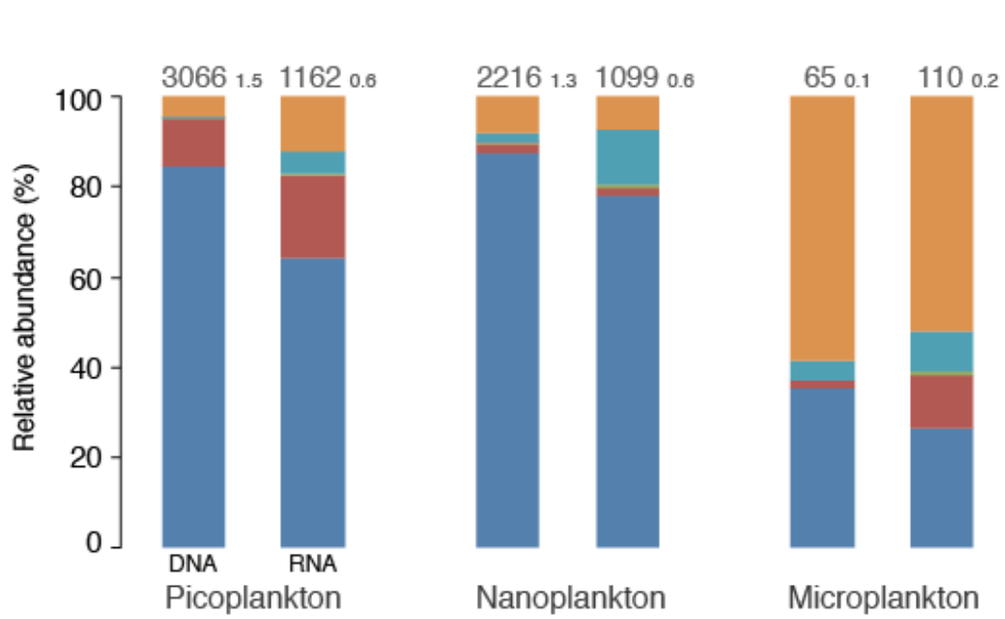
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Relative abundance (%)
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Nanoplankton
Microplankton

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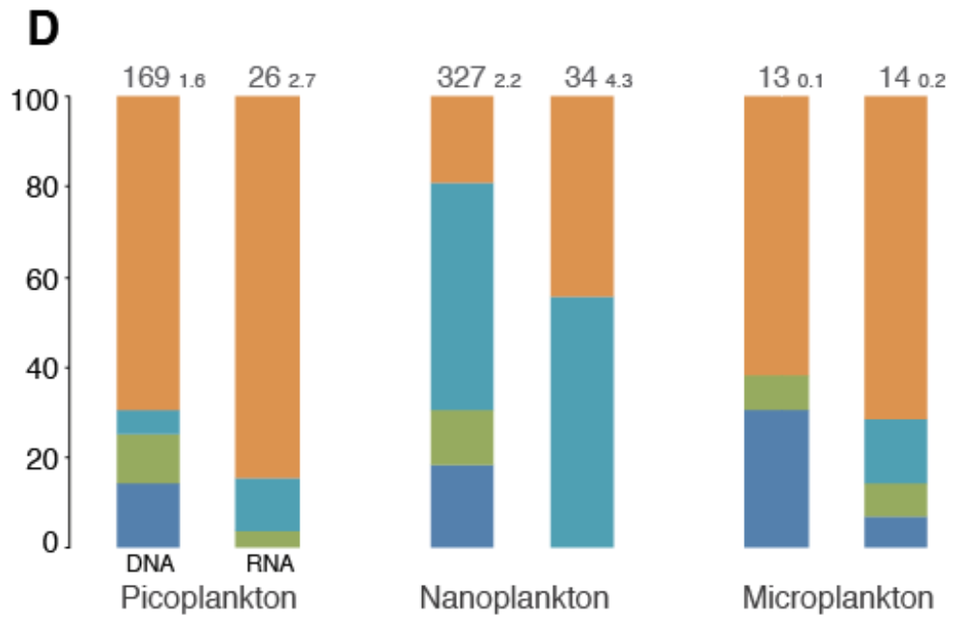
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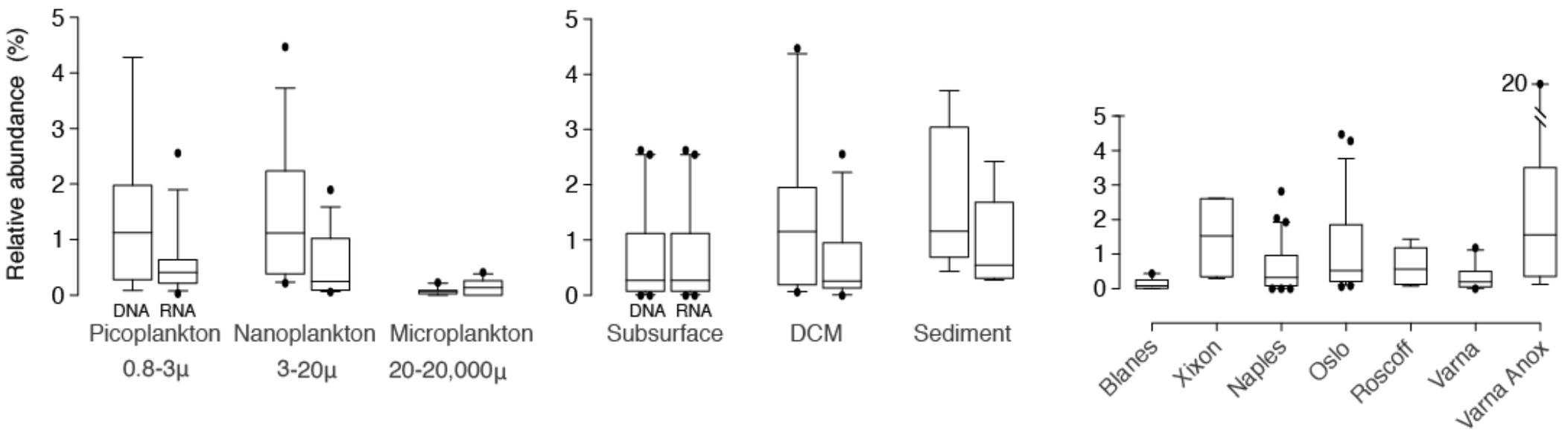
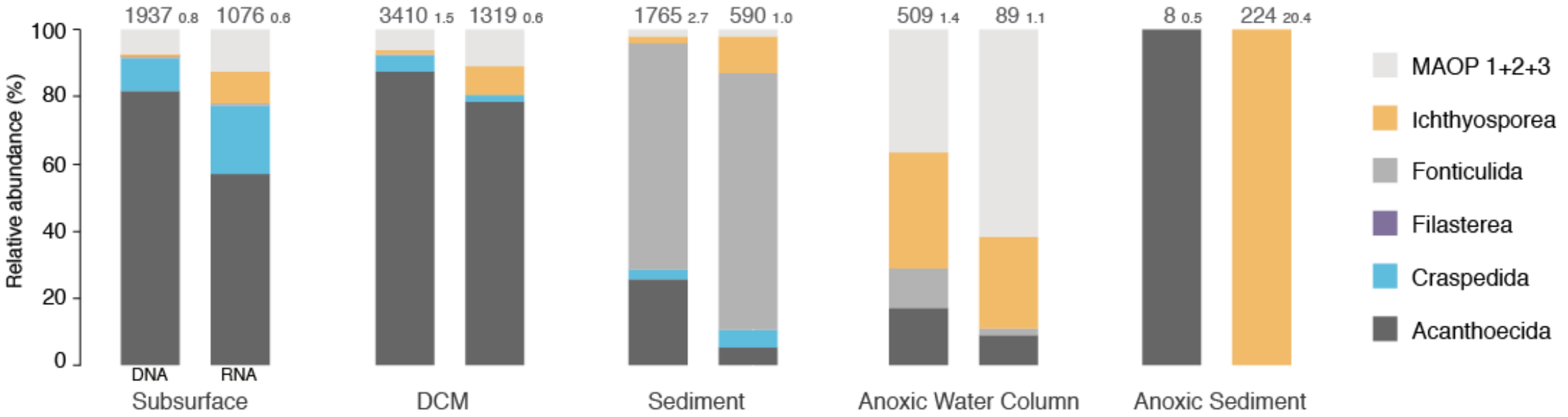
Nanoplankton
Microplankton

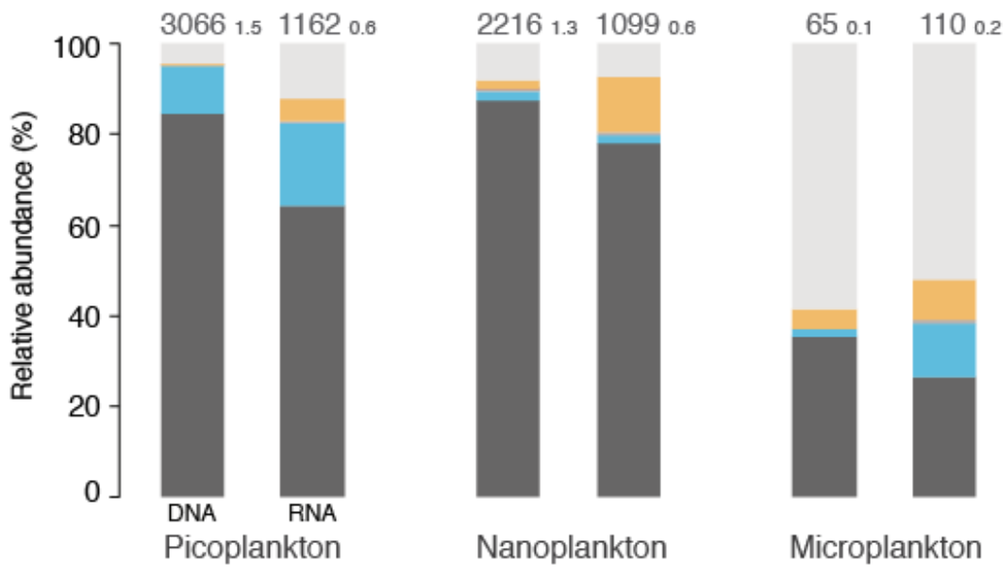
A


Some title
Some title
Some title
Relative abundance (%)
DNA RNA
Picoplankton
0.8-3µ
Nanoplankton
3-20µ
Microplankton
20-20,000µ
Subsurface
DCM
Sediment
20
Blanes
Xixon
Naples
Oslo
Roscoff
Varna
Varna Anox

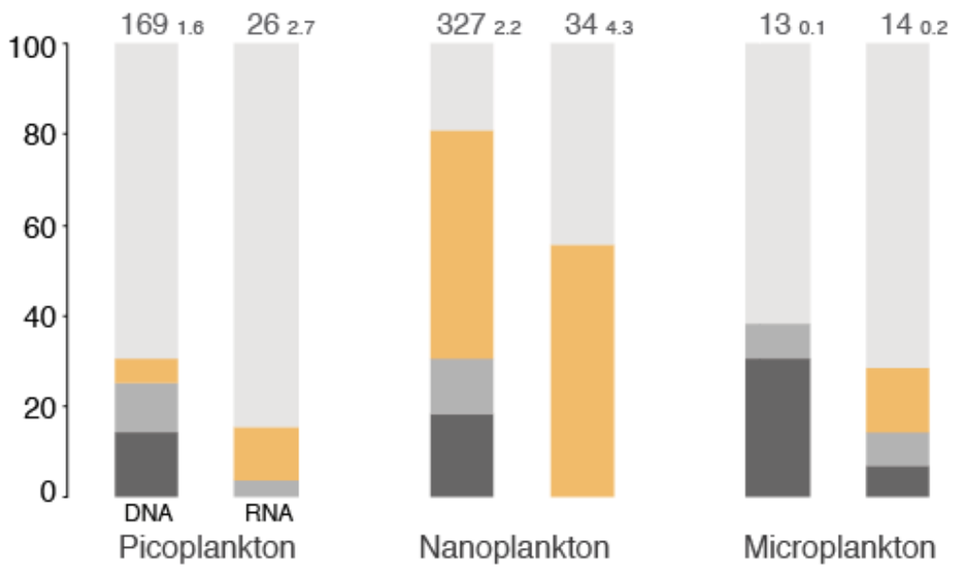
B

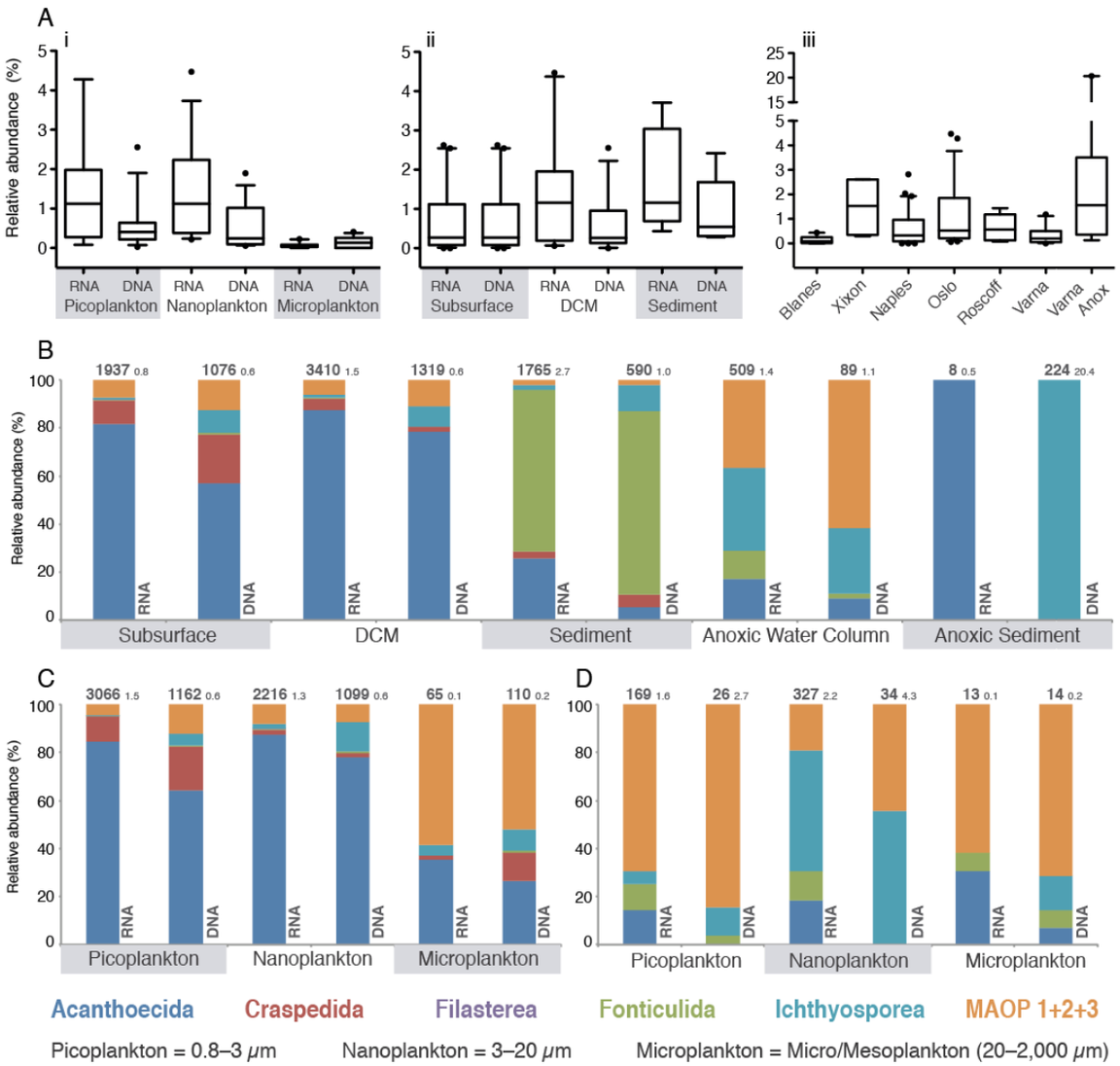

1937 0.8
1076 0.6
3410 1.5
1319 0.6
1765 2.7
590 1.0
509 1.4
89 1.1
8 0.5
224 20.4
Relative abundance (%)
DNA RNA
Subsurface
DCM
Sediment
Anoxic Water Column
Anoxic Sediment
MAOP 1+2+3
Ichthyosporea
Fonticulida
Filasterea
Craspedida
Acanthoecida

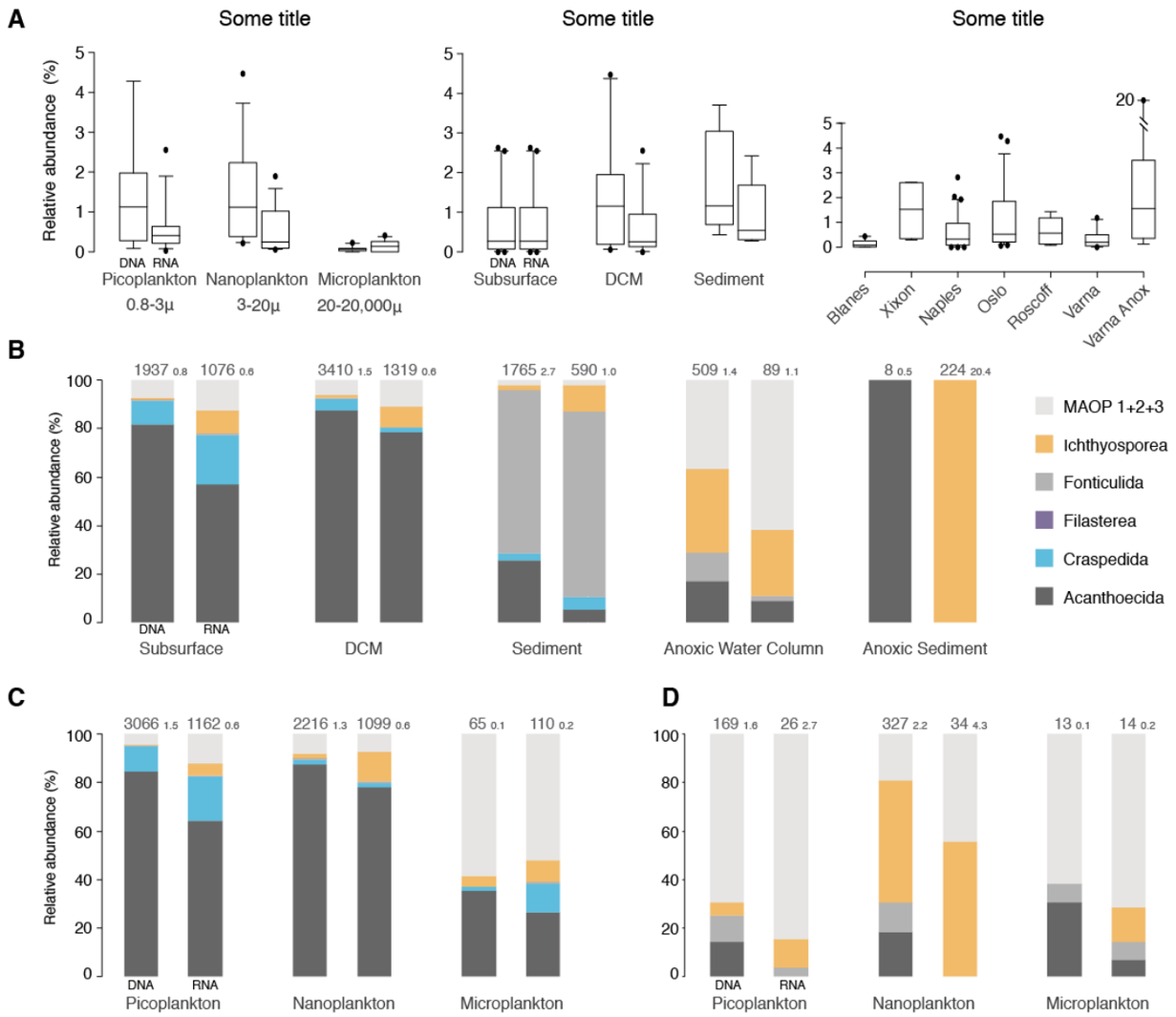
C

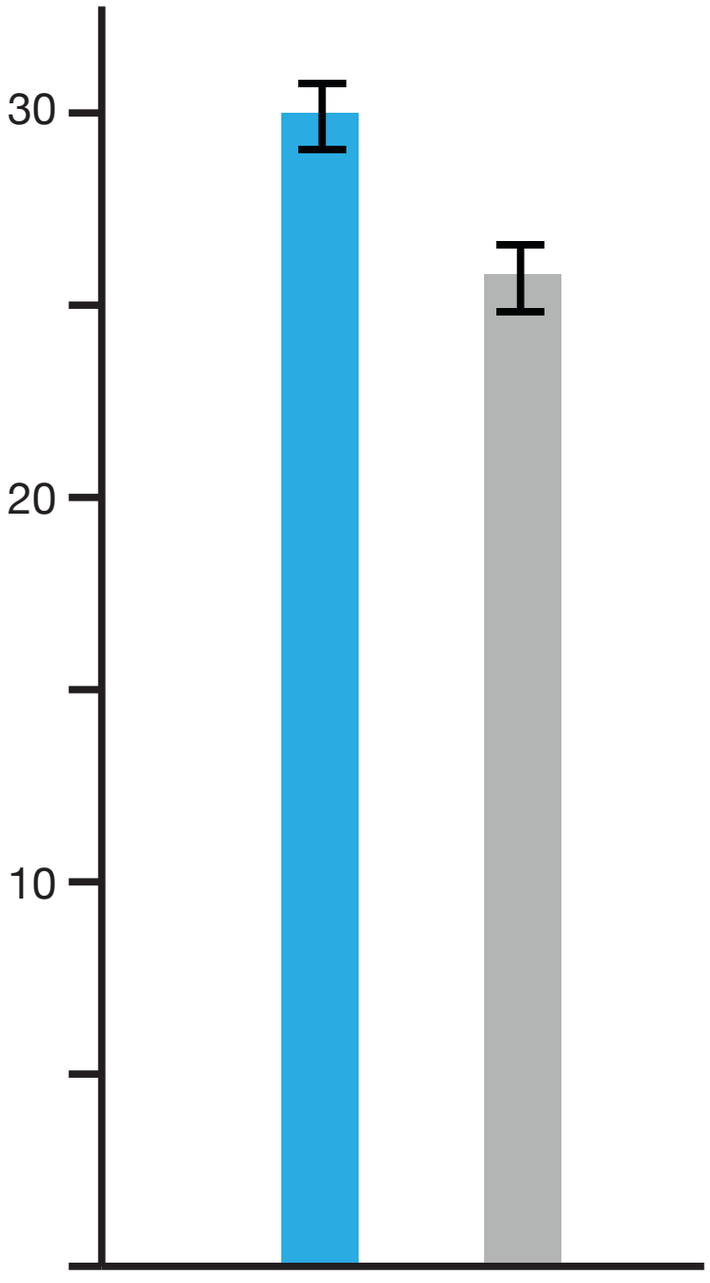

3066 1.5
1162 0.6
2216 1.3
1099 0.6
65 0.1
110 0.2
Relative abundance (%)
DNA RNA
Picoplankton
Nanoplankton
Microplankton

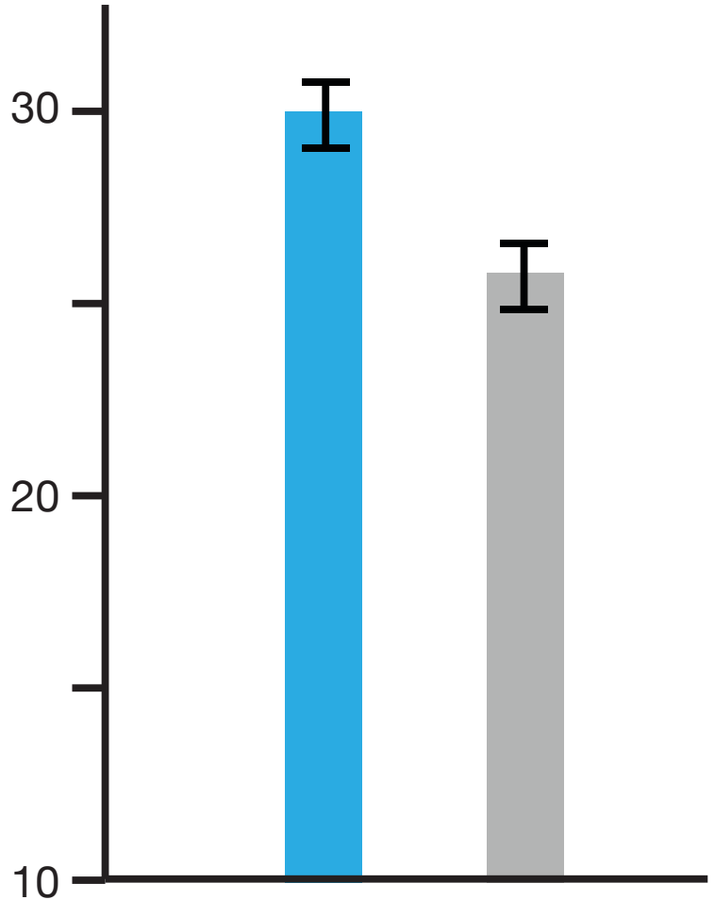
D

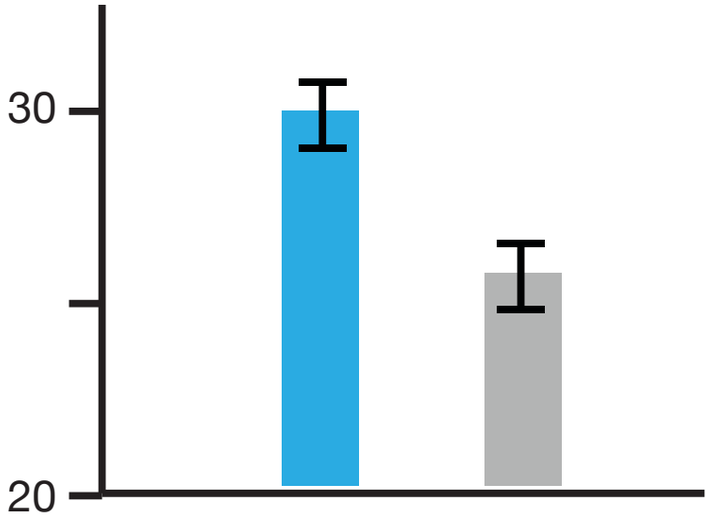

169 1.6
26 2.7
327 2.2
34 4.3
13 0.1
14 0.2
DNA RNA
Picoplankton
Nanoplankton
Microplankton

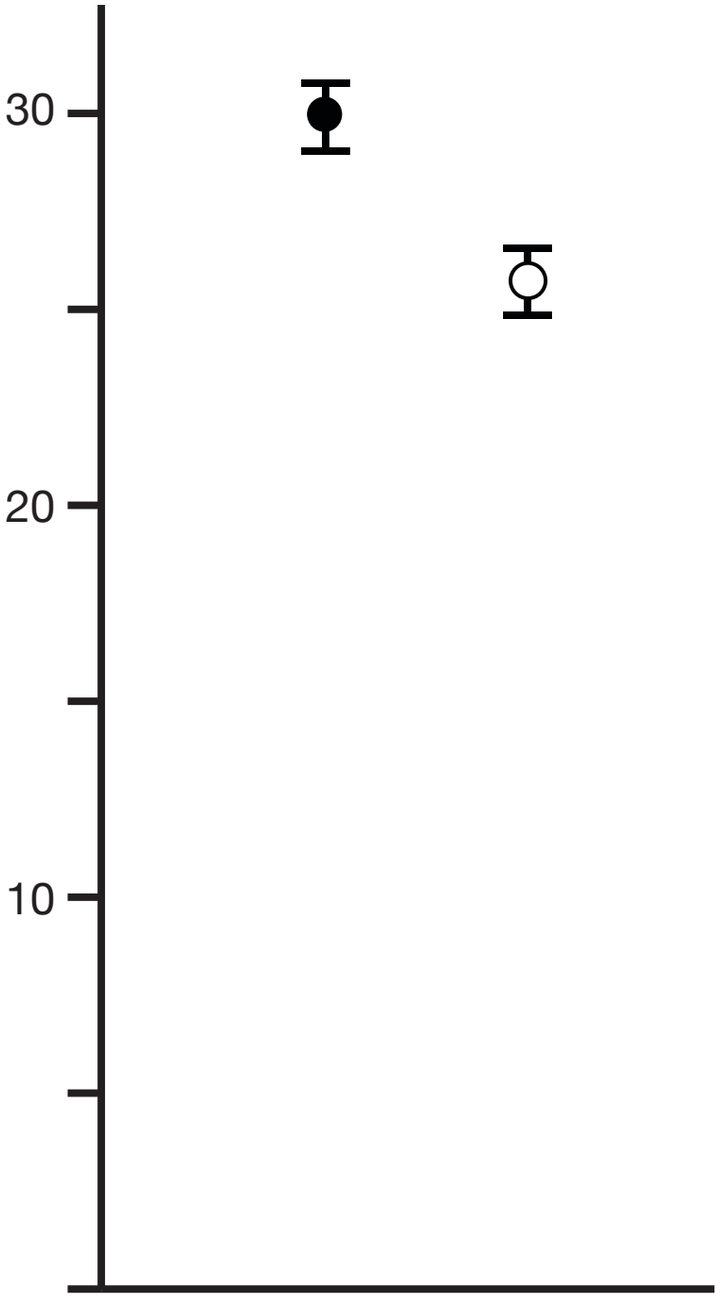

A
i
ii
iii
Relative abundance (%)
RNA DNA RNA DNA RNA DNA
Picoplankton Nanoplankton Microplankton
RNA DNA RNA DNA RNA DNA
Subsurface DCM Sediment
Blanes Xixon Naples Oslo Roscoff Varna Varna Anox
B
1937 0.8
1076 0.6
3410 1.5
1319 0.6
1765 2.7
590 1.0
509 1.4
89 1.1
8 0.5
224 20.4
Relative abundance (%)
RNA DNA
Subsurface DCM Sediment Anoxic Water Column Anoxic Sediment
C
3066 1.5
1162 0.6
2216 1.3
1099 0.6
65 0.1
110 0.2
Picoplankton Nanoplankton Microplankton
D
169 1.6
26 2.7
327 2.2
34 4.3
13 0.1
14 0.2
Picoplankton Nanoplankton Microplankton
Acanthoecida Craspedida Filasterea Fonticulida Ichthyosporea MAOP 1+2+3
Picoplankton = 0.8–3 μm Nanoplankton = 3–20 μm Microplankton = Micro/Mesoplankton (20–2,000 μm)

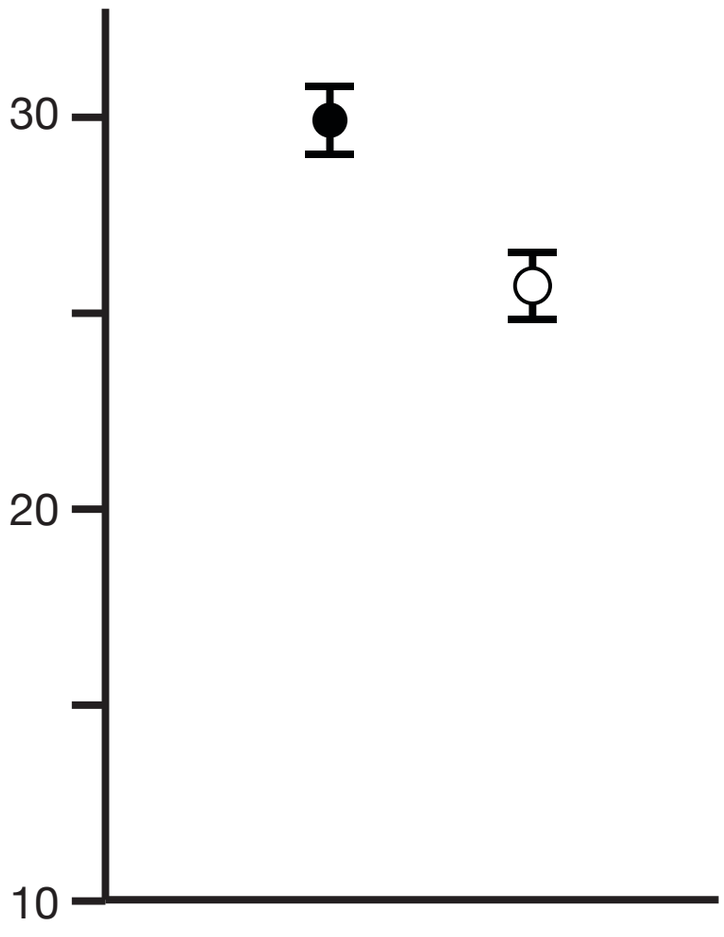

A
Some title
Some title
Some title
Relative abundance (%)
DNA RNA
Picoplankton Nanoplankton Microplankton
0.8-3μ 3-20μ 20-20,000μ
DNA RNA
Subsurface DCM Sediment
Blanes Xixon Naples Oslo Roscoff Varna Varna Anox
B
1937 0.8
1076 0.6
3410 1.5
1319 0.6
1765 2.7
590 1.0
509 1.4
89 1.1
8 0.5
224 20.4
Relative abundance (%)
DNA RNA
Subsurface DCM Sediment Anoxic Water Column Anoxic Sediment
MAOP 1+2+3
Ichthyosporea
Fonticulida
Filasterea
Craspedida
Acanthoecida
C
3066 1.5
1162 0.6
2216 1.3
1099 0.6
65 0.1
110 0.2
Picoplankton Nanoplankton Microplankton
D
169 1.6
26 2.7
327 2.2
34 4.3
13 0.1
14 0.2
Picoplankton Nanoplankton Microplankton

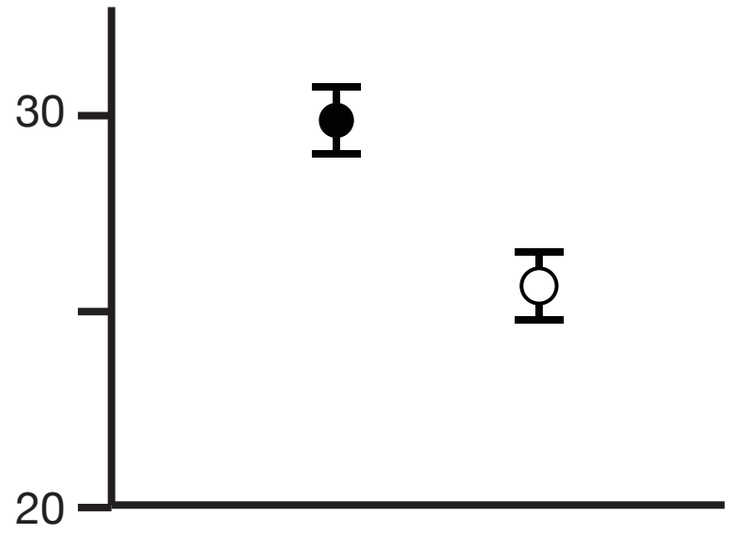

30
20
10

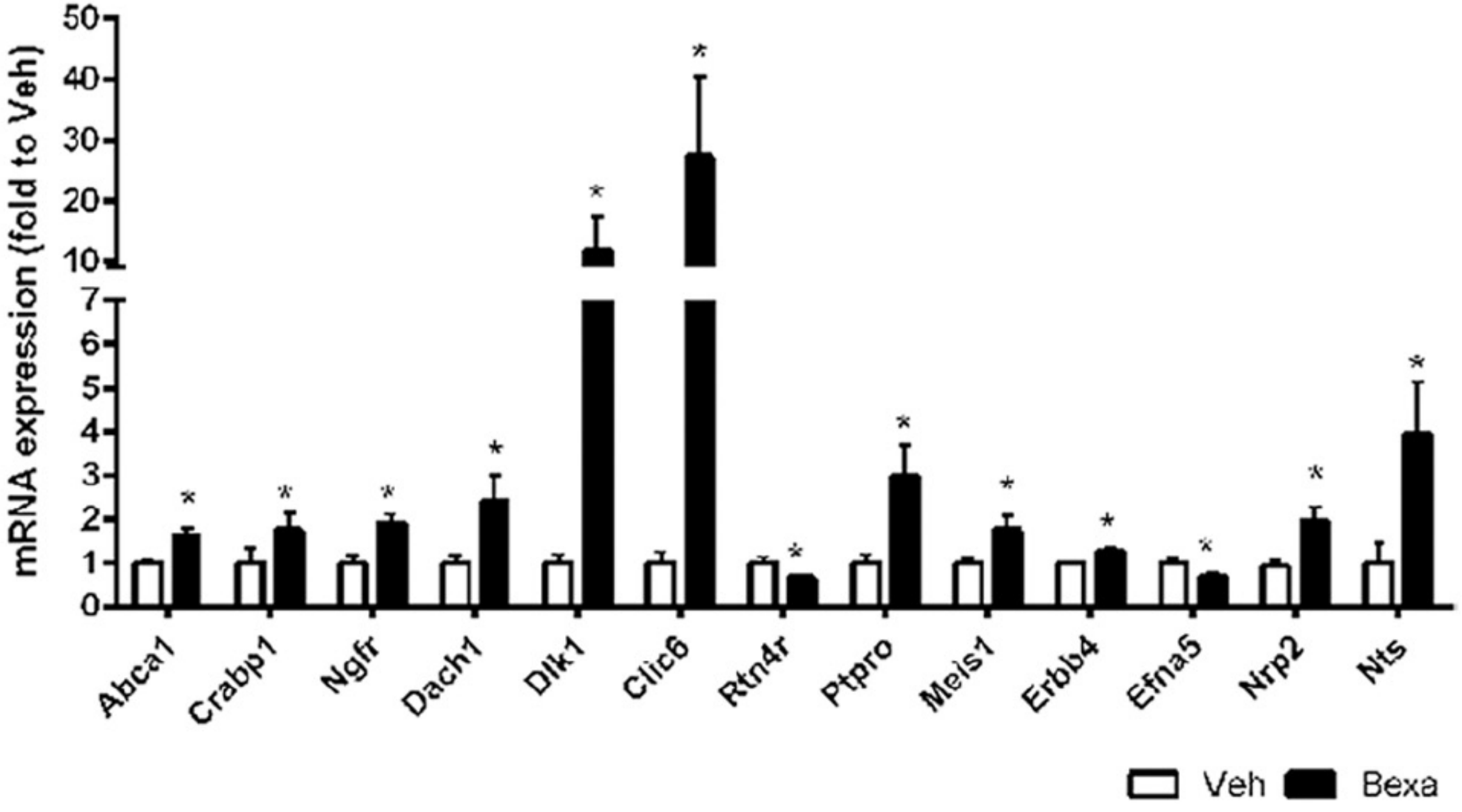

30
20
10

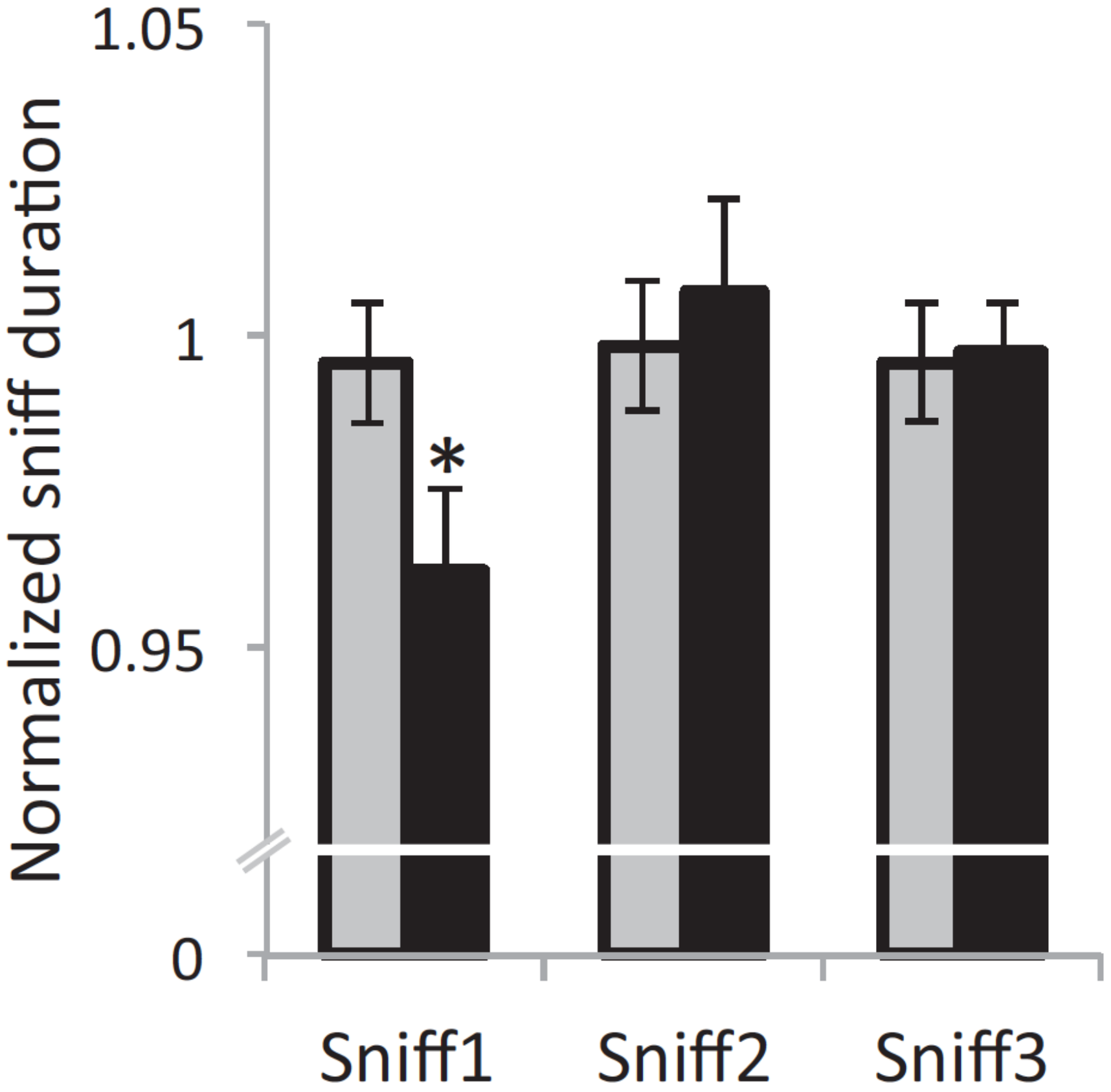

30
20

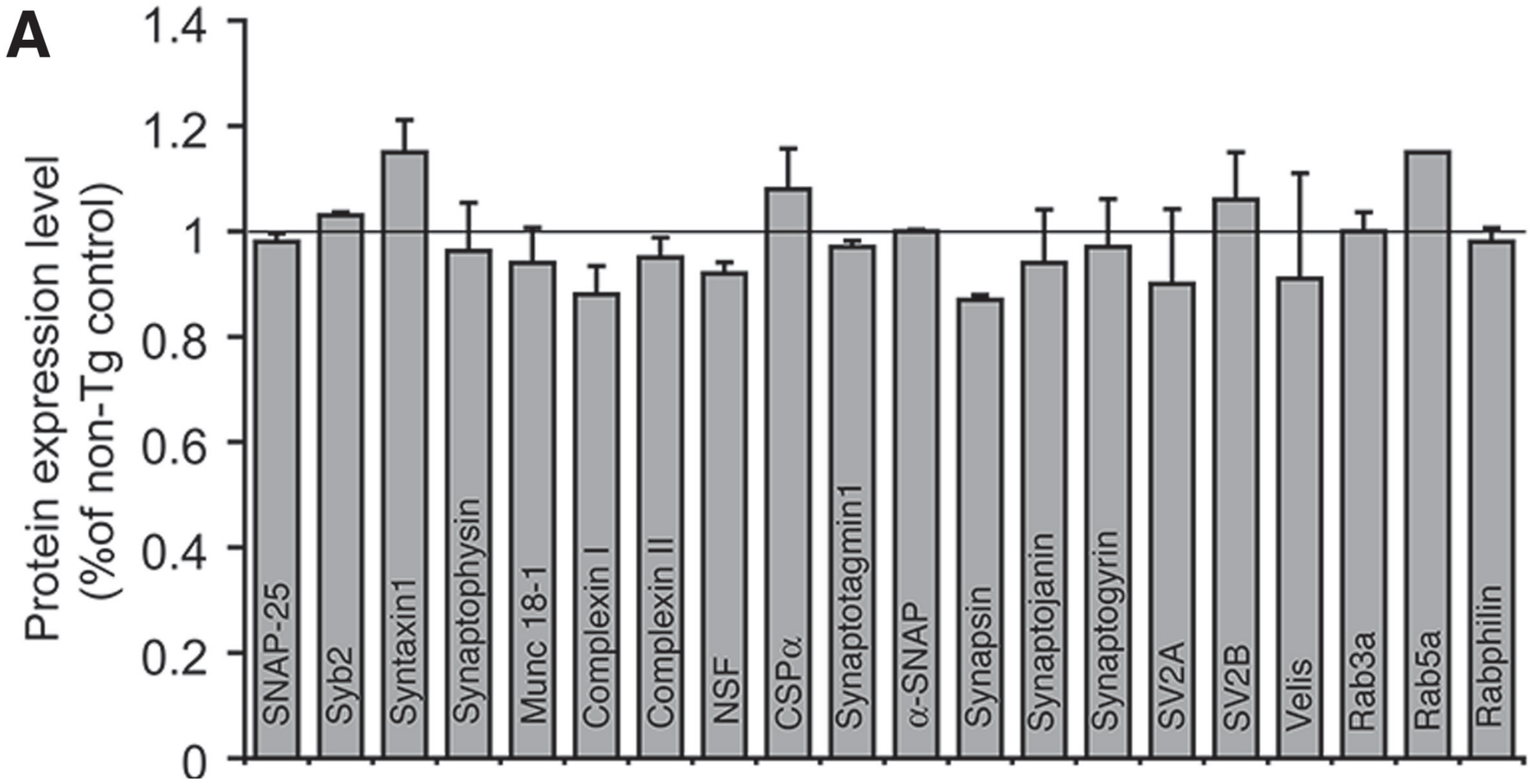

30
20
10


30
20
10

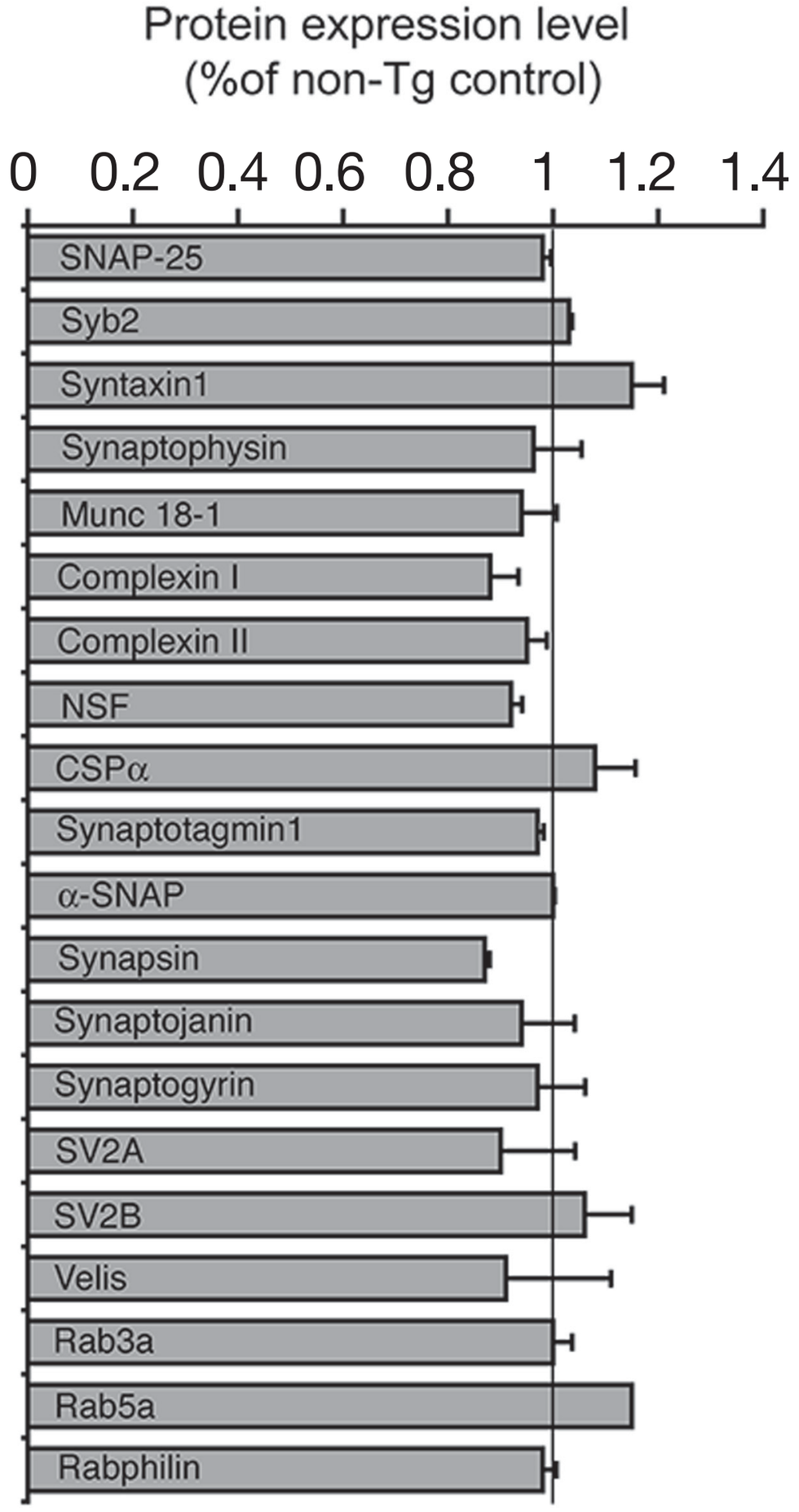

30
20

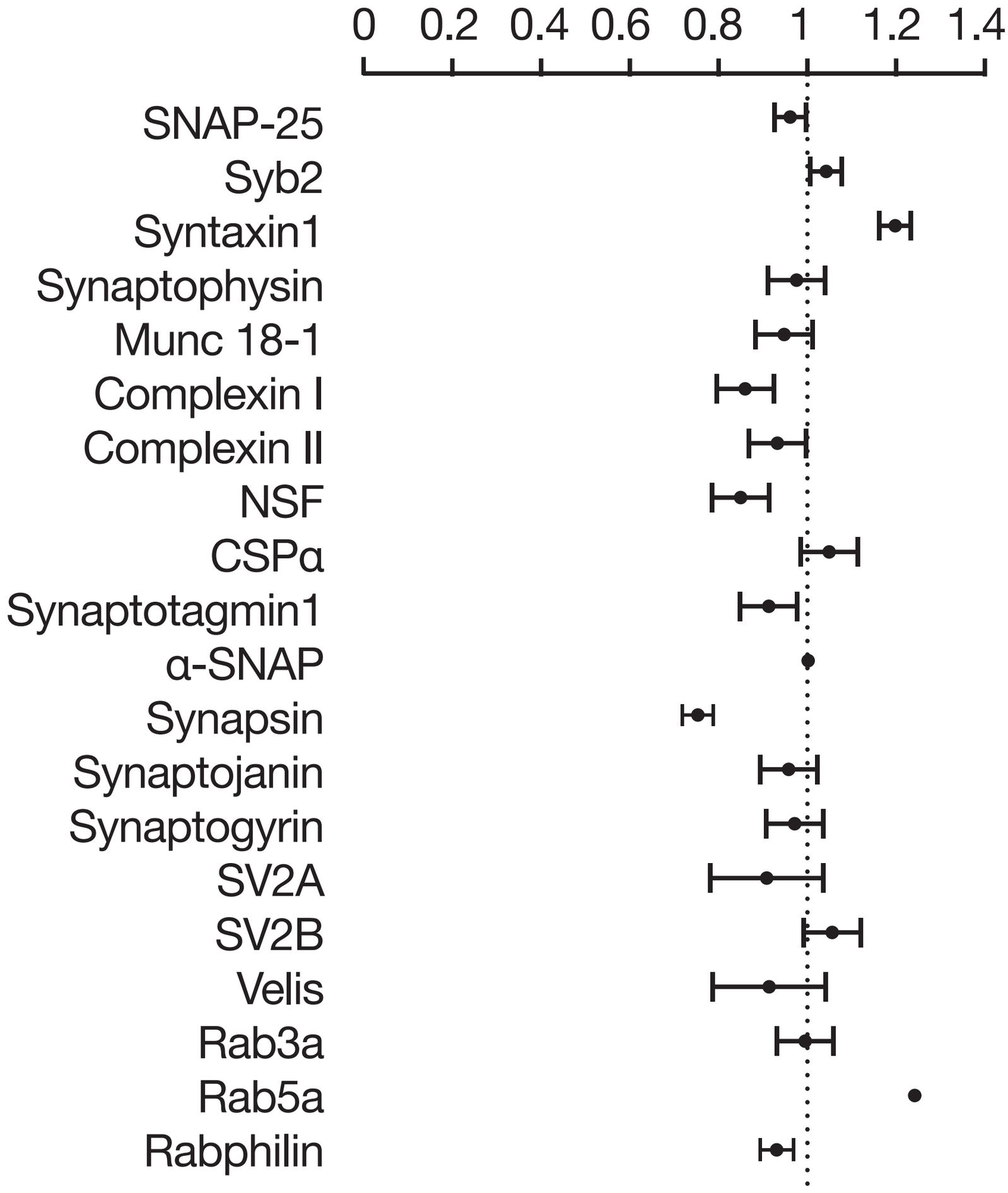

mRNA expression (fold to Veh)
50
40
30
20
10
7
6
5
4
3
2
1
0
Abca1
Crabp1
Ngfr
Dach1
Dlk1
Clic6
Rtn4r
Ptpro
Meis1
Erbb4
Efna5
Nrp2
Nts
Veh
Bexa

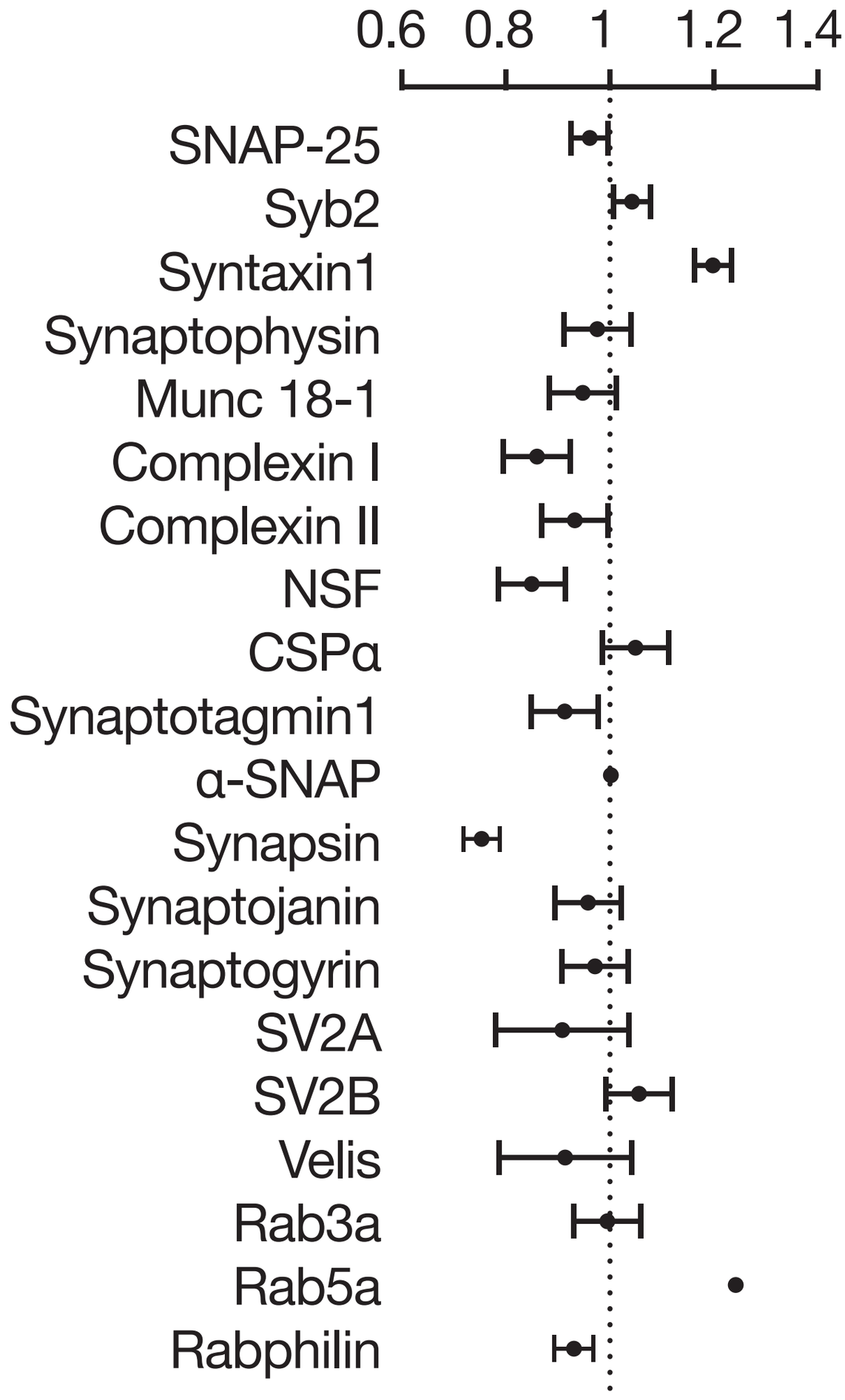

1.05
1
0.95
0
Normalized sniff duration
*
Sniff1
Sniff2
Sniff3


A
Protein expression level
(%of non-Tg control)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
SNAP-25
Syb2
Syntaxin1
Synaptophysin
Munc 18-1
Complexin I
Complexin II
NSF
CSPα
Synaptotagmin1
α-SNAP
Synapsin
Synaptojanin
Synaptogyrin
SV2A
SV2B
Velis
Rab3a
Rab5a
Rabphilin

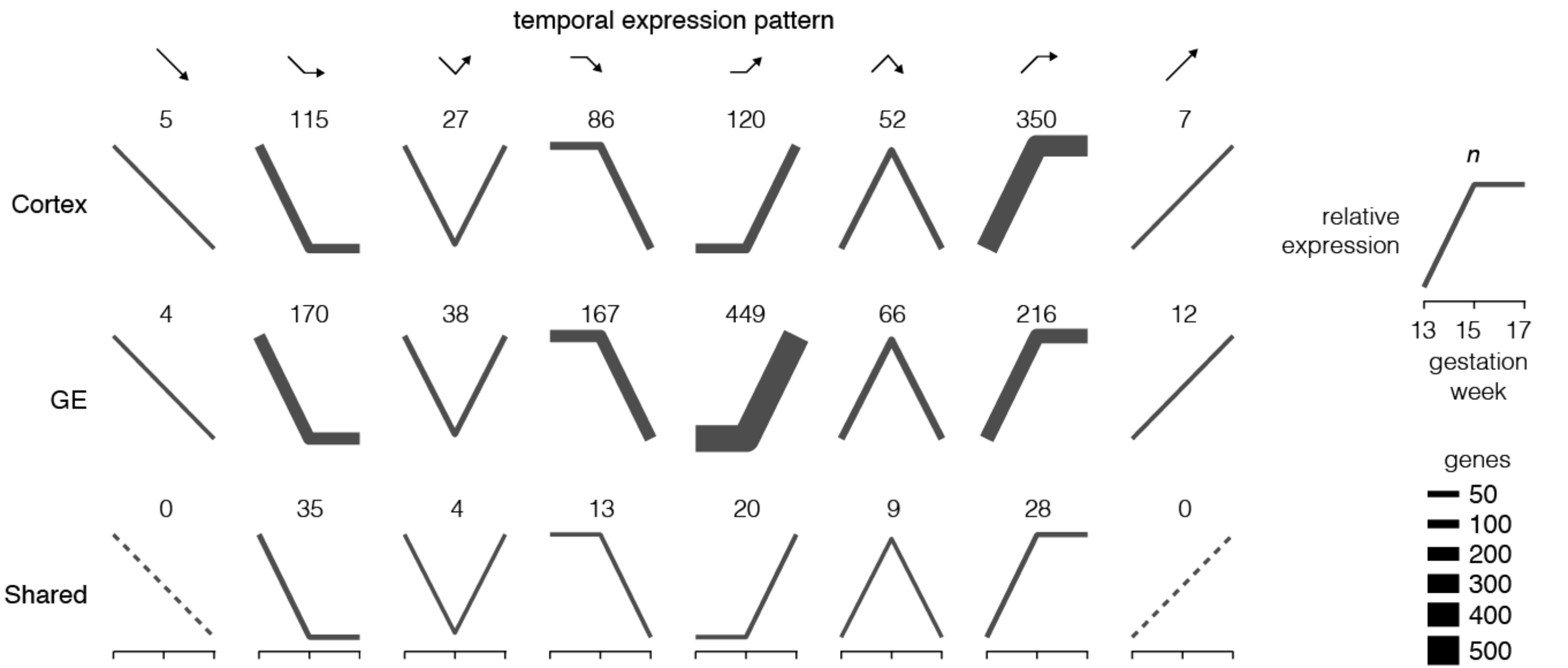
A

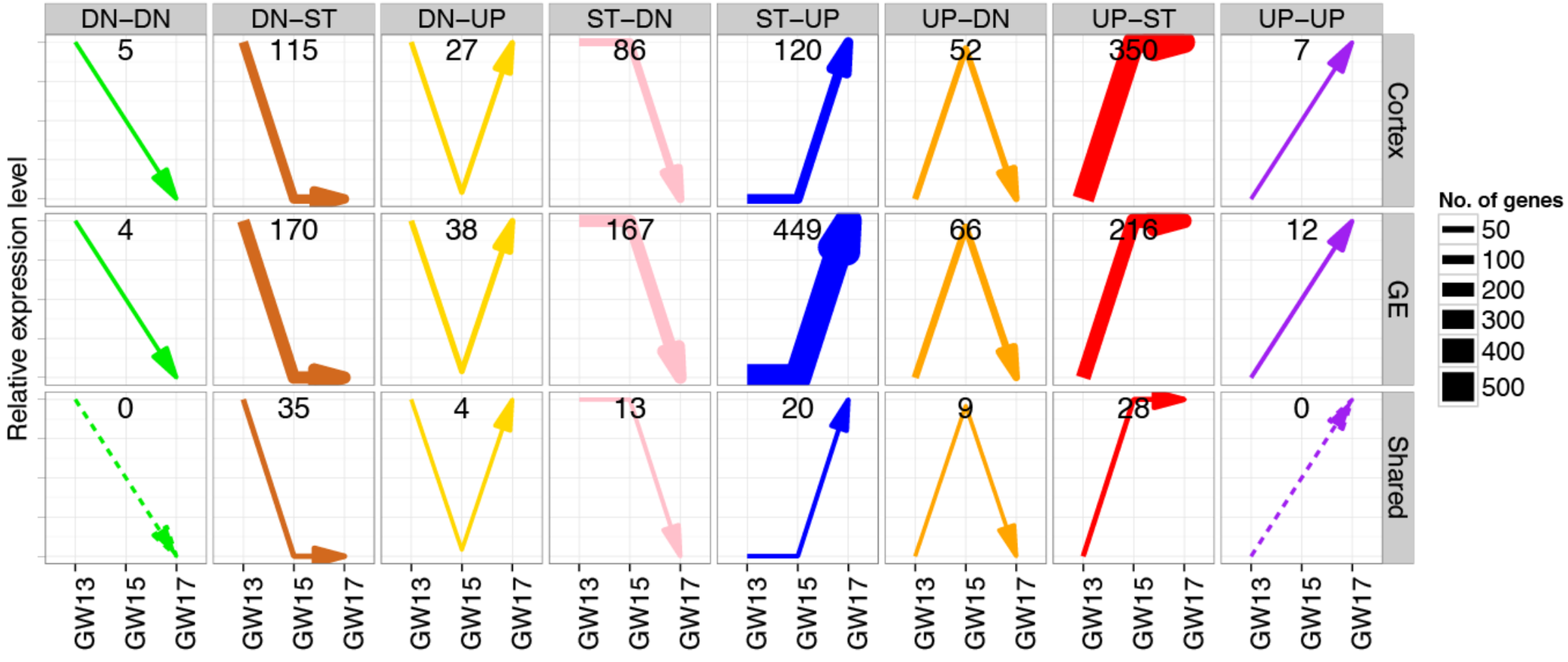

Protein expression level
(%of non-Tg control)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
SNAP-25
Syb2
Syntaxin1
Synaptophysin
Munc 18-1
Complexin I
Complexin II
NSF
CSPα
Synaptotagmin1
α-SNAP
Synapsin
Synaptojanin
Synaptogyrin
SV2A
SV2B
Velis
Rab3a
Rab5a
Rabphilin


0
0.2
0.4
0.6
0.8
1
1.2
1.4
SNAP-25
Syb2
Syntaxin1
Synaptophysin
Munc 18-1
Complexin I
Complexin II
NSF
CSPα
Synaptotagmin1
α-SNAP
Synapsin
Synaptojanin
Synaptogyrin
SV2A
SV2B
Velis
Rab3a
Rab5a
Rabphilin


0.6
0.8
1
1.2
1.4
SNAP-25
Syb2
Syntaxin1
Synaptophysin
Munc 18-1
Complexin I
Complexin II
NSF
CSPα
Synaptotagmin1
α-SNAP
Synapsin
Synaptojanin
Synaptogyrin
SV2A
SV2B
Velis
Rab3a
Rab5a
Rabphilin

DN–DN
DN–ST
DN–UP
ST–DN
ST–UP
UP–DN
UP–ST
UP–UP
Cortex
GE
Shared
5
115
27
86
120
52
350
7
4
170
38
167
449
66
216
12
0
35
4
13
20
9
28
0
Relative expression level
GW13
GW15
GW17
No. of genes
50
100
200
300
400
500

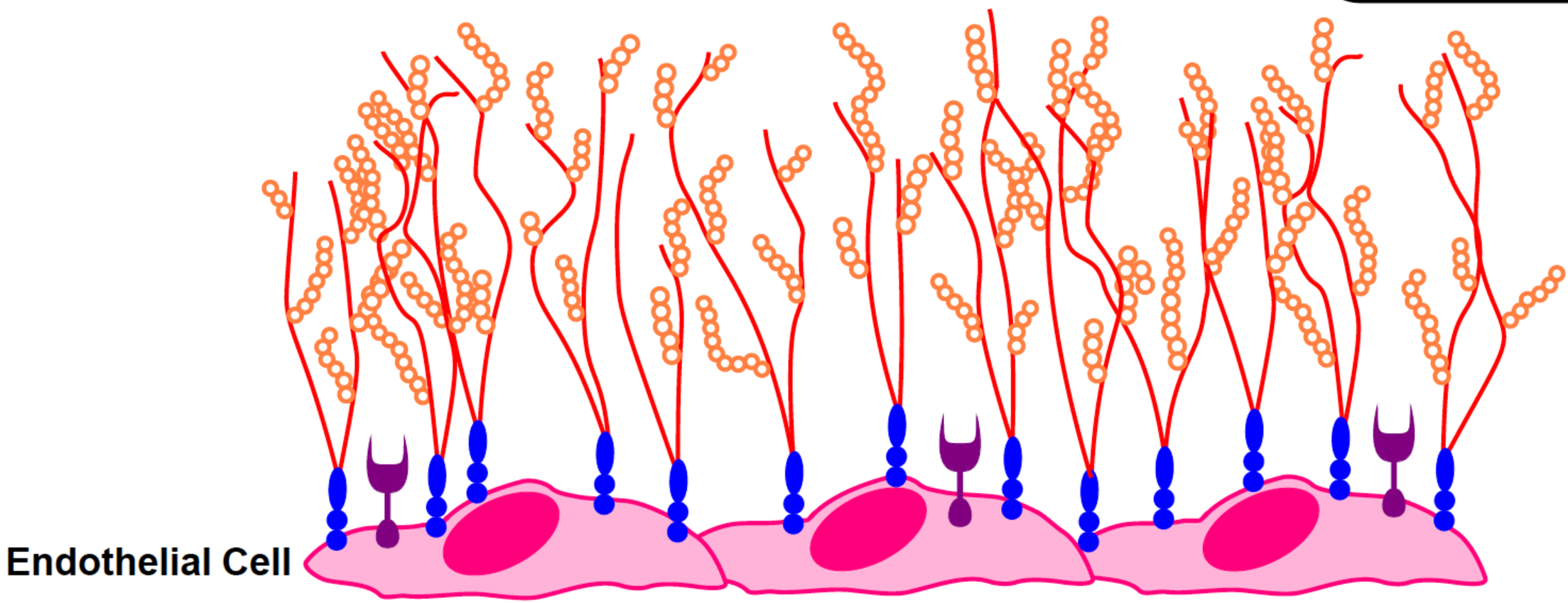

temporal expression pattern
5
115
27
86
120
52
350
7
Cortex
4
170
38
167
449
66
216
12
GE
0
35
4
13
20
9
28
0
Shared
relative expression
n
13
15
17
gestation week
genes
50
100
200
300
400
500


DN−DN
DN−ST
DN−UP
ST−DN
ST−UP
UP−DN
UP−ST
UP−UP
Cortex
GE
Shared
5
115
27
86
120
52
350
7
4
170
38
167
449
66
216
12
0
35
4
13
20
9
28
0
Relative expression level
GW13
GW15
GW17
No. of genes
50
100
200
300
400
500
temporal expression pattern
Cortex
GE
Shared
relative expression
n
13 15 17
gestation week
genes
50
100
200
300
400
500

The Role of the Glycocalyx

○ = Carbohydrate Chain

= Leukocyte Adhesion Receptor (ICAM-1)



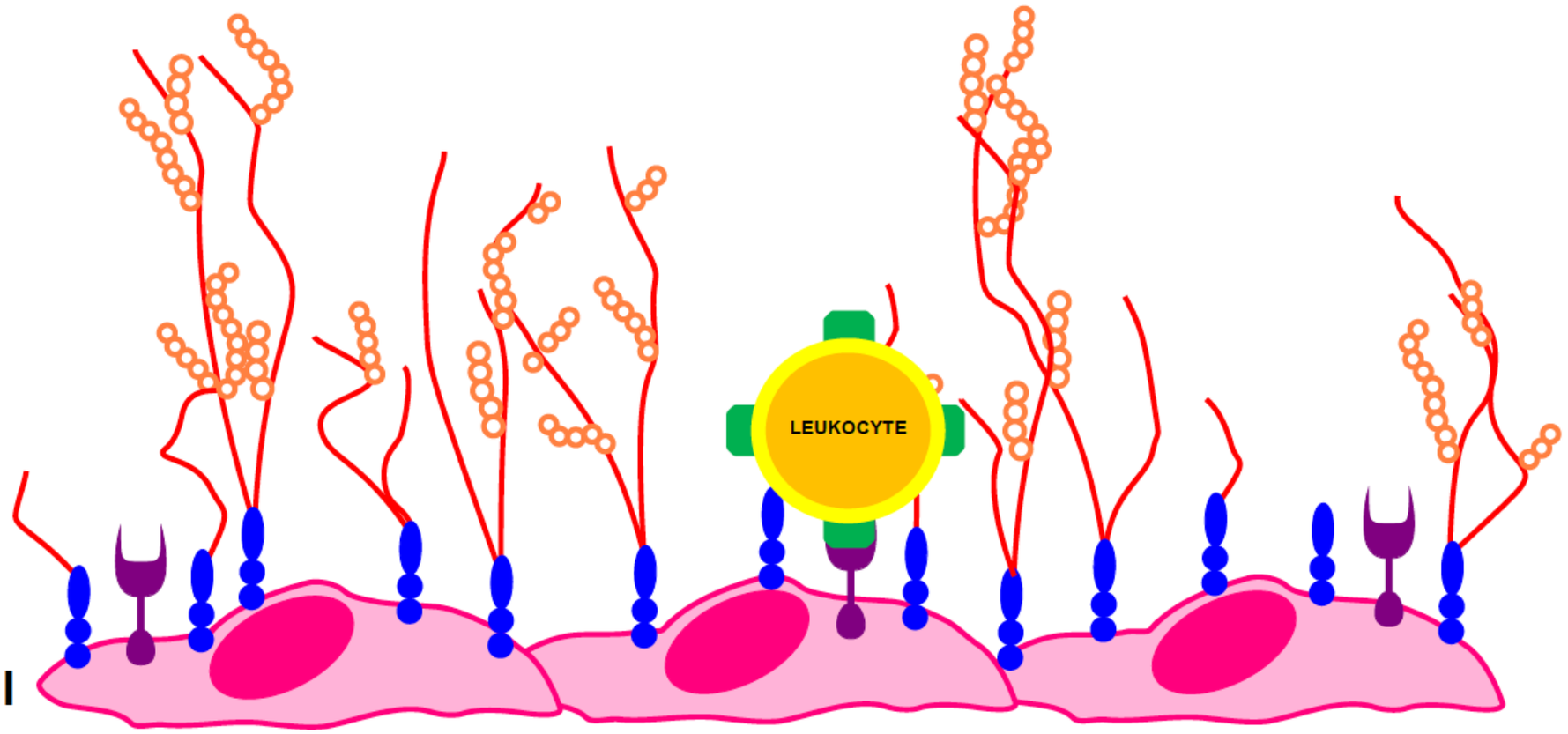
*structures not to scale

Glycocalyx is a protective ‘shield’ to tissue

The Role of the Glycocalyx

○ = Carbohydrate Chain

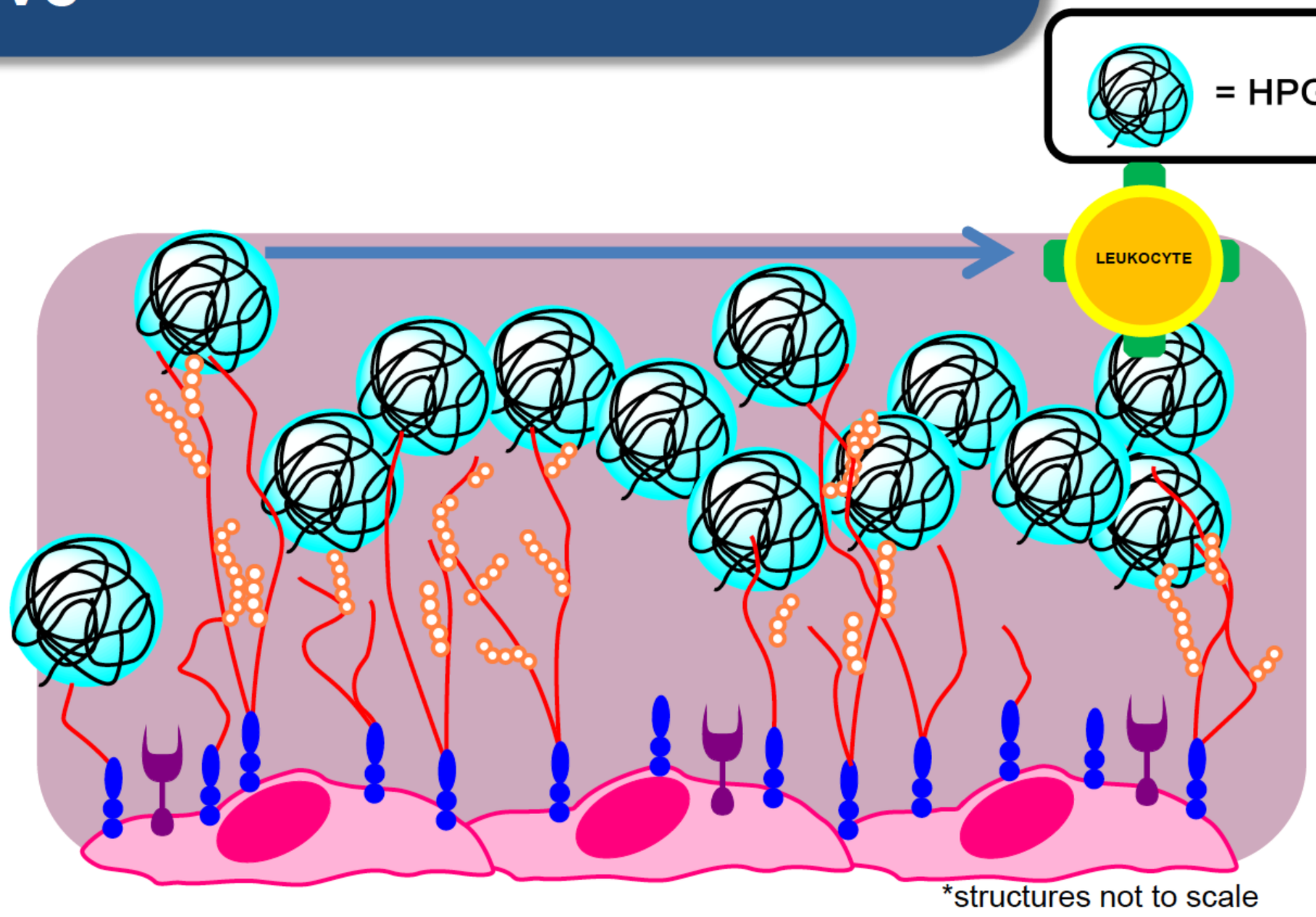
= Leukocyte Adhesion Receptor (ICAM-1)



*structures not to scale

Uncover surface proteins that allow for leukocyte adhesion followed by inflammation and graft death

Objective



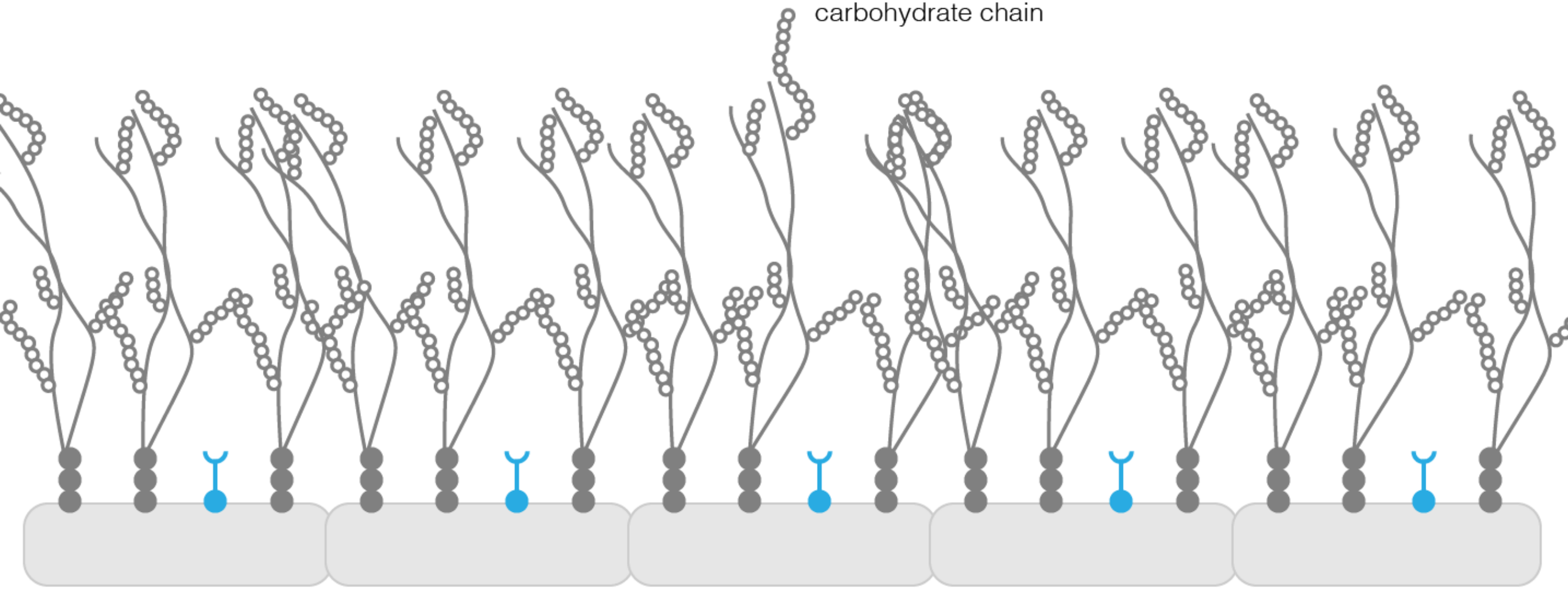
*structures not to scale

1) Incubation in Cold Storage Solution containing Polymer

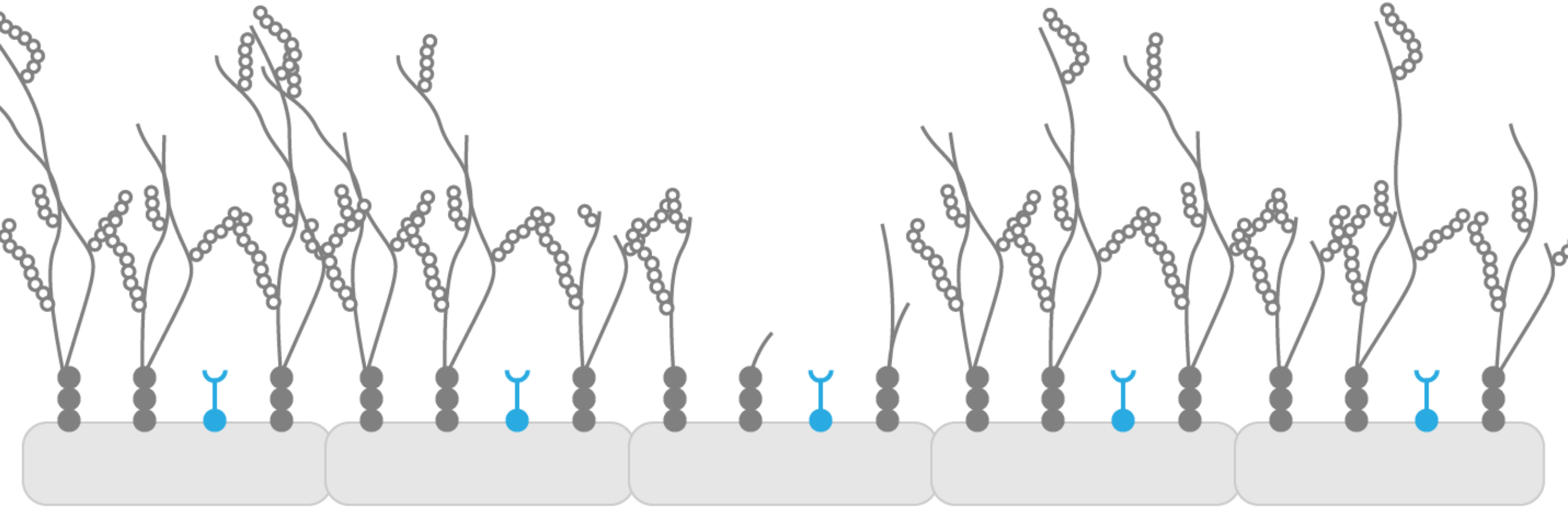
2) Covalent Attachment to Damaged Glycocalyx

3) Protective Effect Remains Upon Reintroduction of Blood (and Immune Cells)

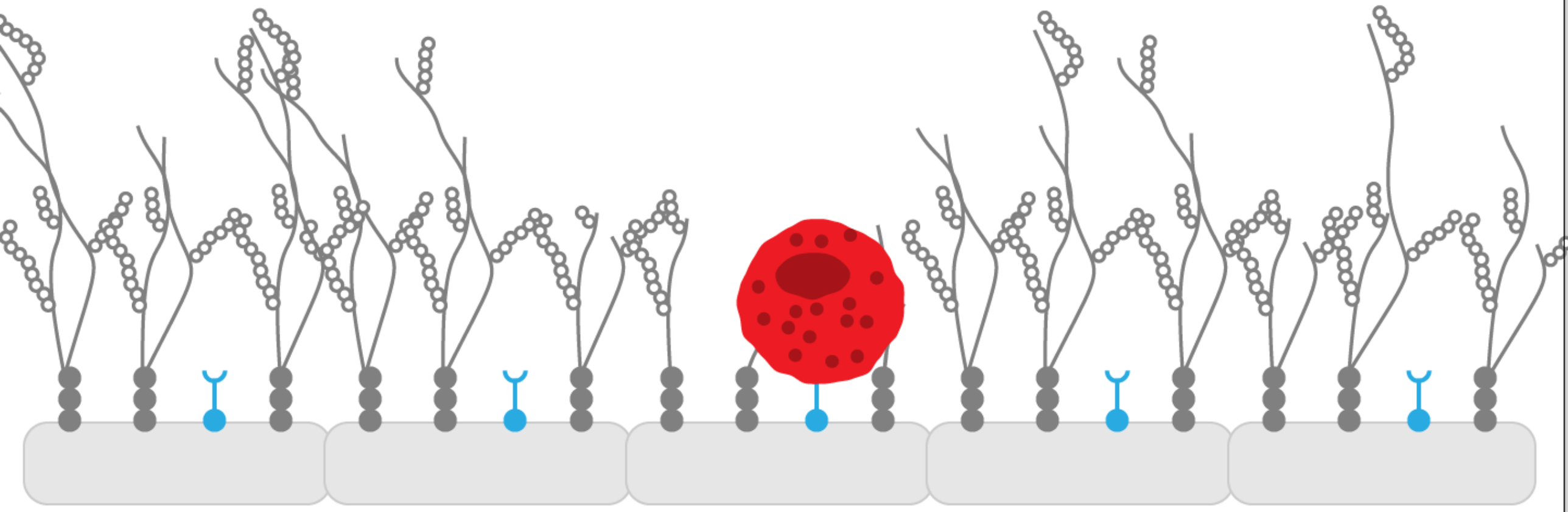
Glycocalyx is a protective shield to tissue.



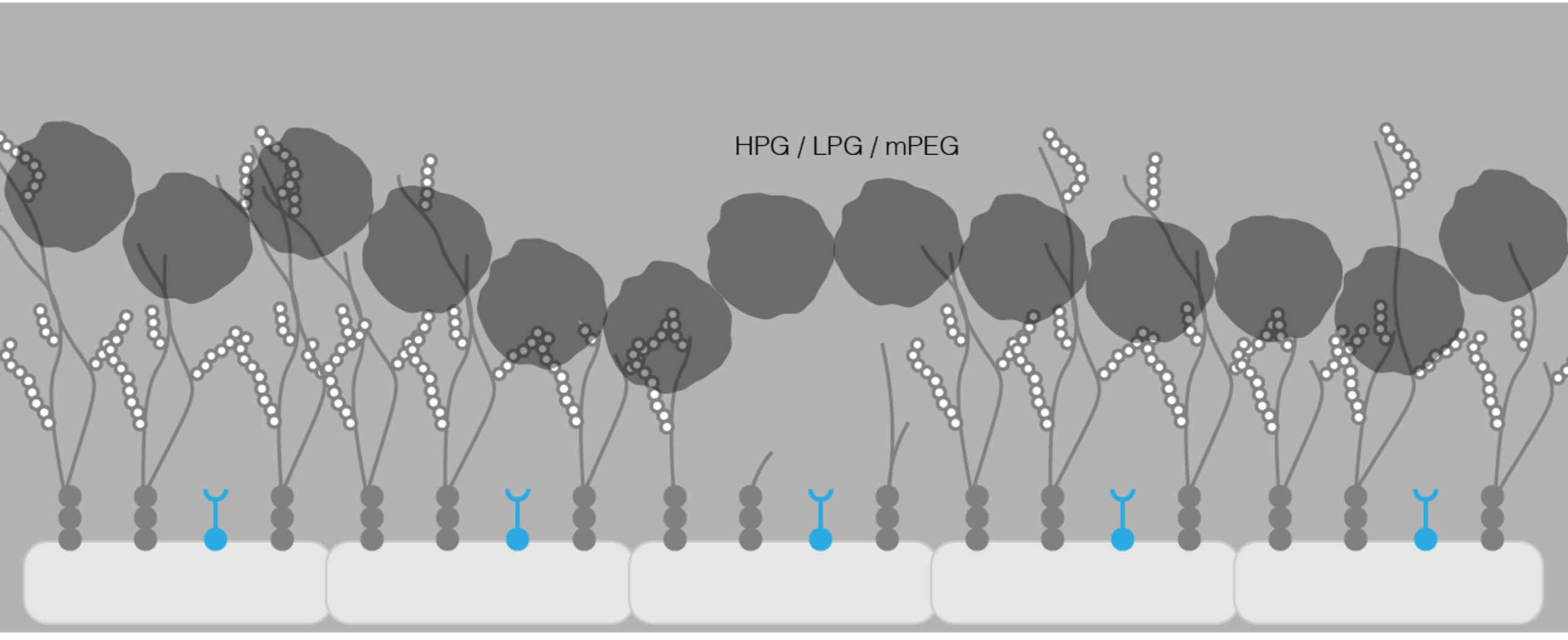
Reactive oxygen species and cold shock cause breakdown of the glycocalyx.



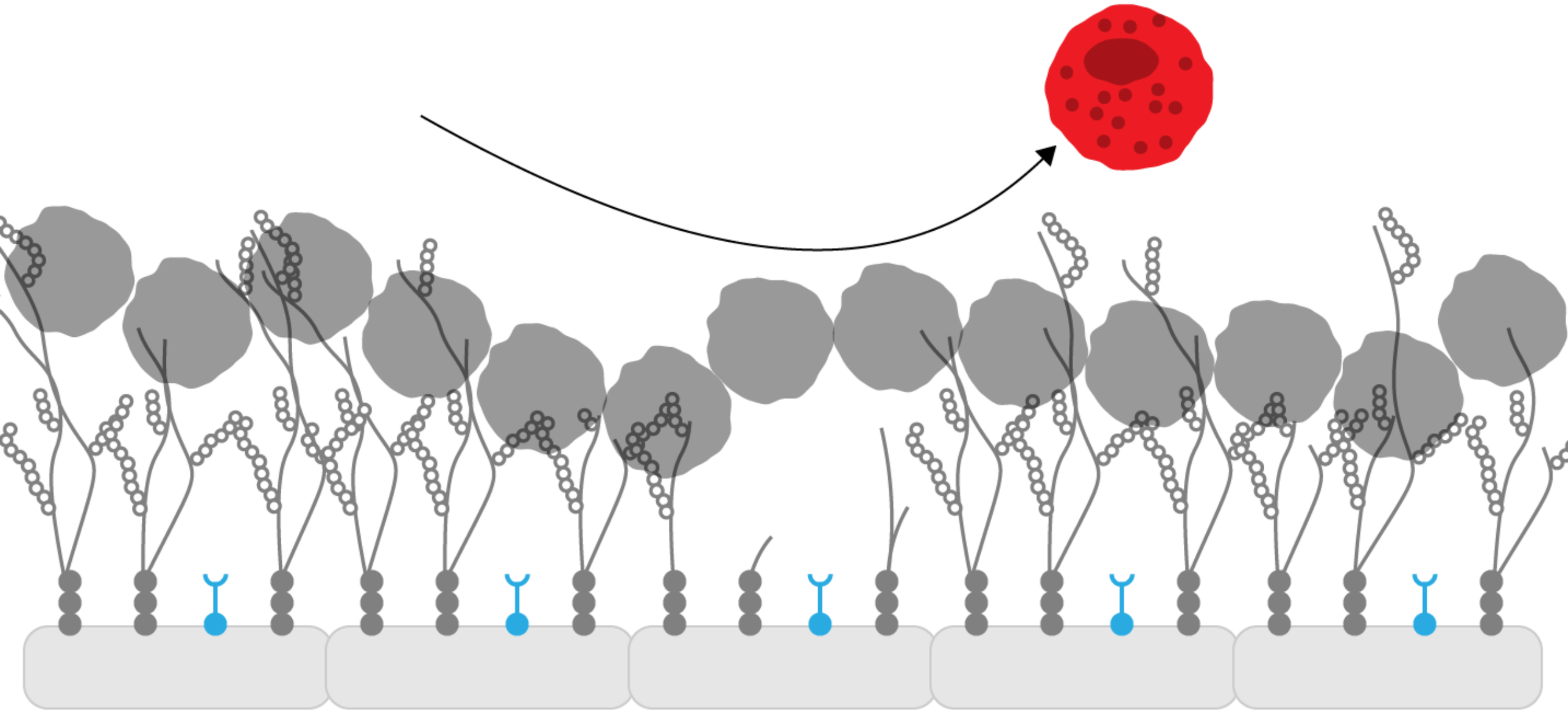
Leukocytes adhere to exposed surface proteins and cause inflammation and graft death.



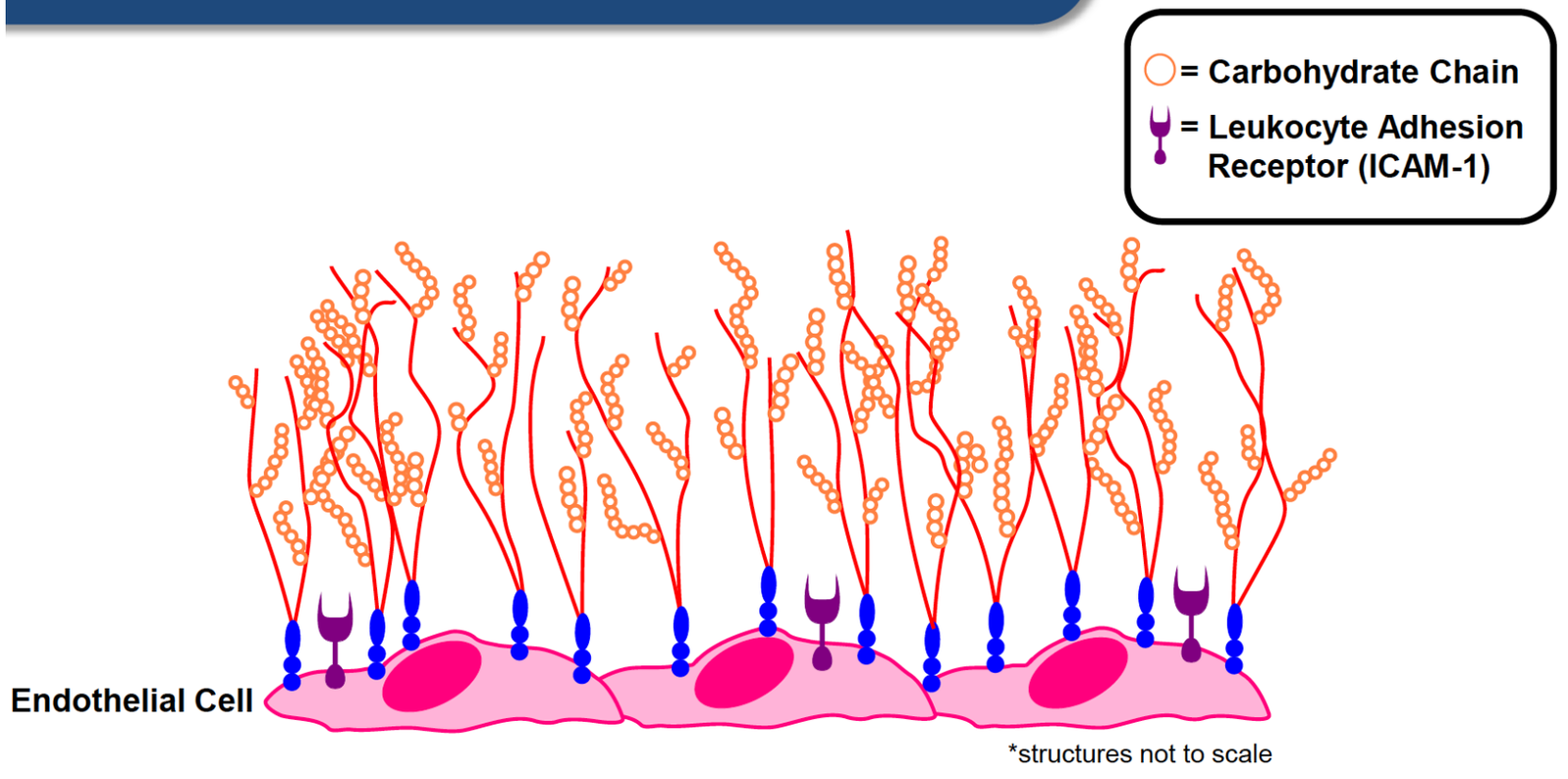
Polymer attaches covalently to damaged glycocalyx during incubation in cold storage.



Protective effect remains even when
blood and immune cells are reintroduced.

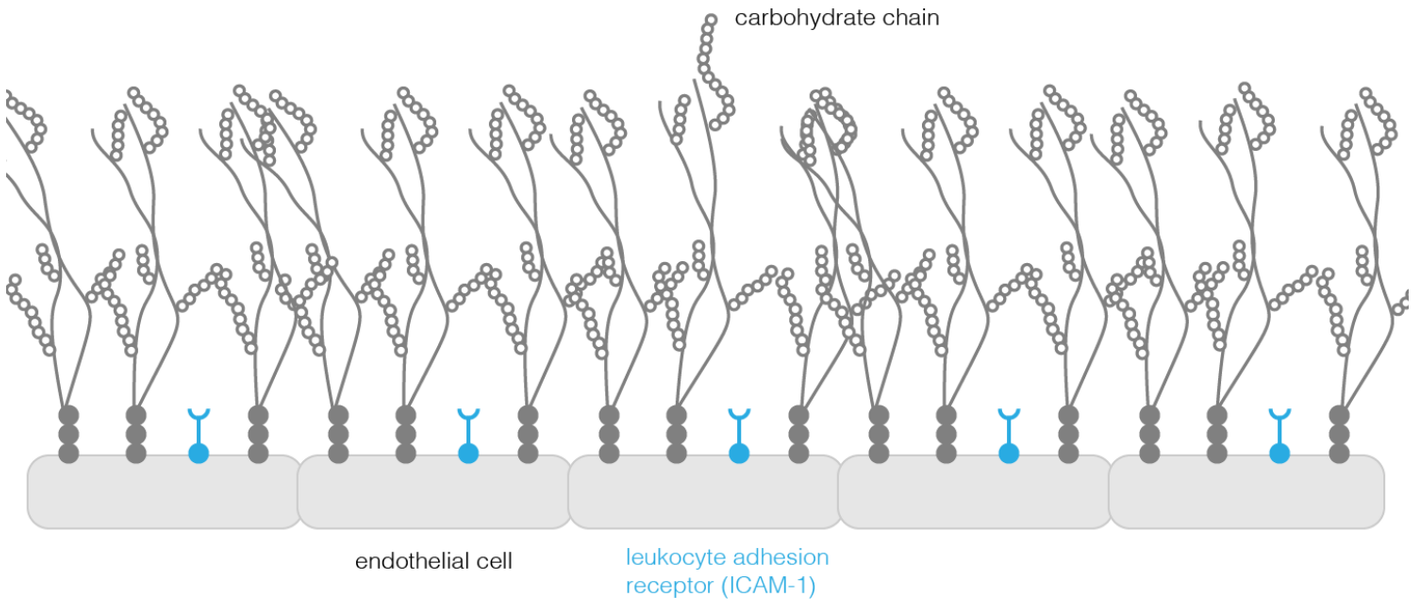


The Role of the Glycocalyx



Glycocalyx is a protective ‘shield’ to tissue

Glycocalyx is a protective shield to tissue.



Gordon Andrews (designer)
Gazelle chair (c. 1950) designed, 1957 manufactured
plywood, aluminium, wool
74.0 x 48.0 x 55.0 cm
Museum of Applied Arts and Sciences, Sydney
Purchased, 1989 (89/499)