Figure 1

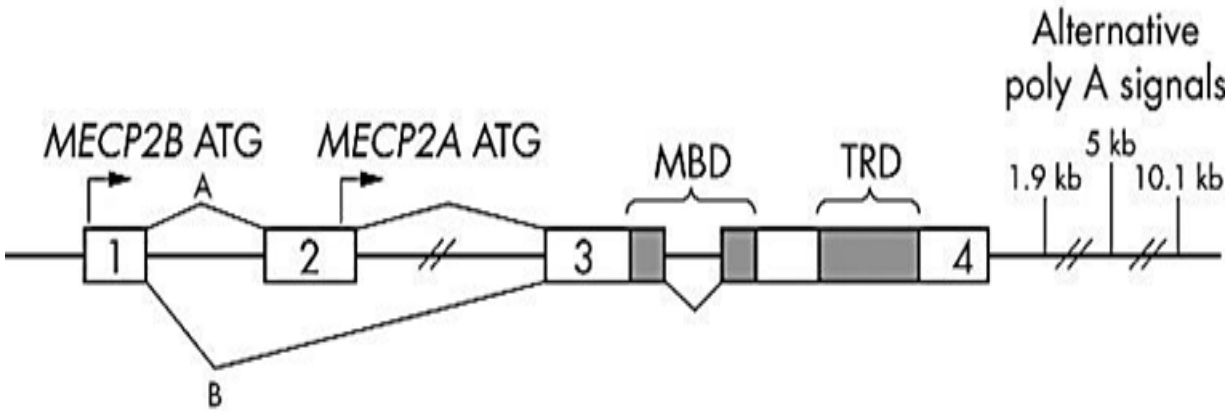

Alternative
poly A signals
MECP2B ATG
MECP2A ATG
MBD
TRD
5 kb
1.9 kb
10.1 kb
A
1
2
3
4
B

Figure 2

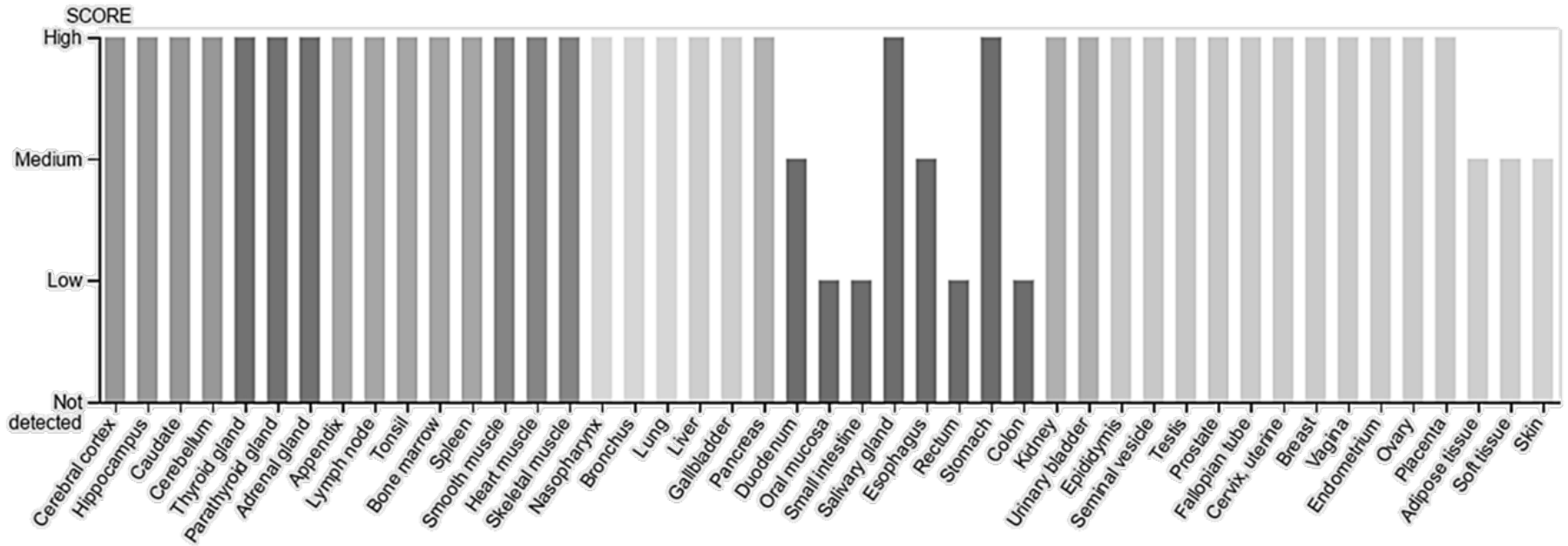

SCORE
High
Medium
Low
Not detected
Cerebral cortex
Hippocampus
Caudate
Cerebellum
Thyroid gland
Parathyroid gland
Adrenal gland
Appendix
Lymph node
Tonsil
Bone marrow
Spleen
Smooth muscle
Heart muscle
Skeletal muscle
Nasopharynx
Bronchus
Lung
Liver
Gallbladder
Pancreas
Duodenum
Oral mucosa
Small intestine
Salivary gland
Esophagus
Rectum
Stomach
Colon
Kidney
Urinary bladder
Epididymis
Seminal vesicle
Testis
Prostate
Fallopian tube
Cervix, uterine
Breast
Vagina
Endometrium
Ovary
Placenta
Adipose tissue
Soft tissue
Skin

Figure 3

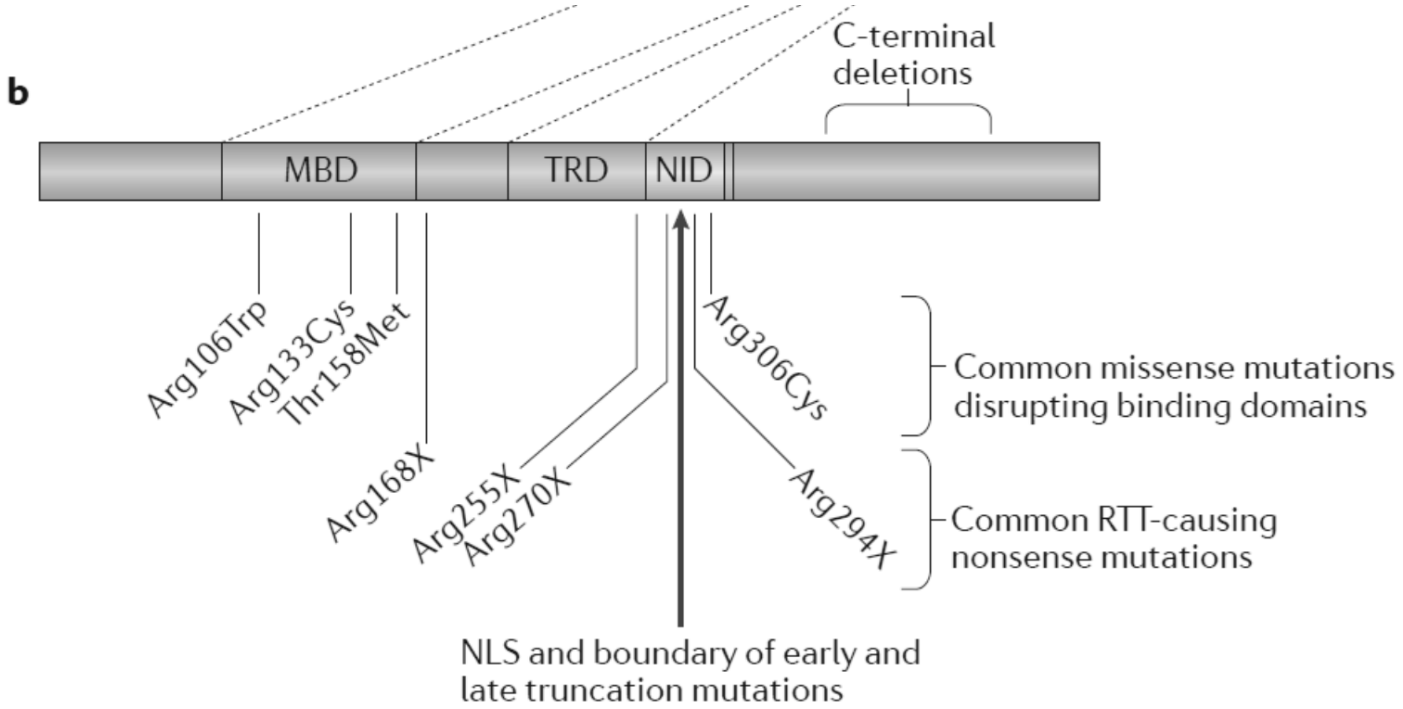

b
C-terminal deletions
MBD
TRD
NID
Arg106Trp
Arg133Cys
Thr158Met
Arg168X
Arg255X
Arg270X
Arg306Cys
Arg294X
Common missense mutations disrupting binding domains
Common RTT-causing nonsense mutations
NLS and boundary of early and late truncation mutations

Figure 4

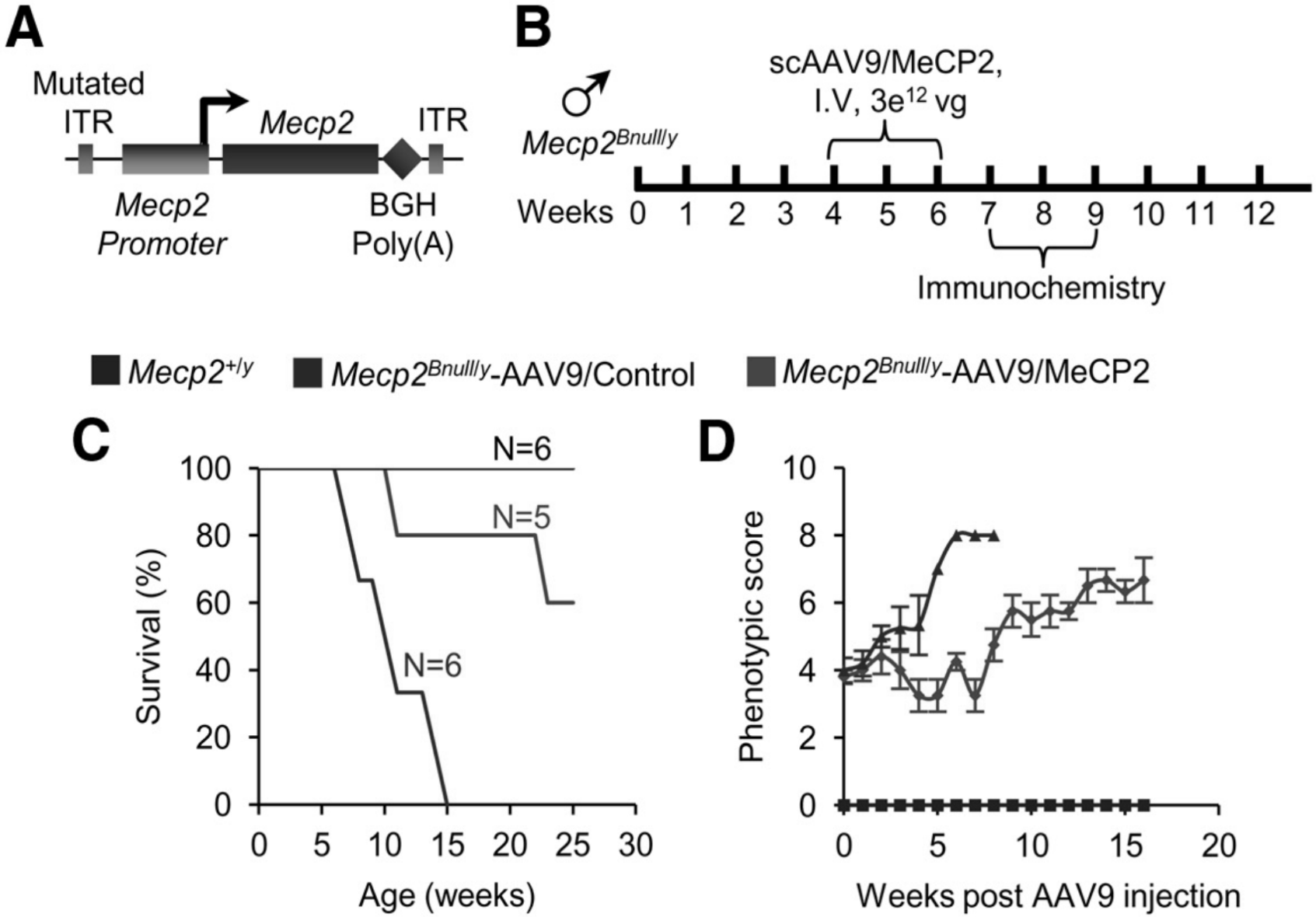

A
Mutated
ITR
Mecp2
ITR
Mecp2
Promoter
BGH
Poly(A)
B
scAAV9/MeCP2,
I.V, 3e12 vg
Mecp2Bnull/y
Weeks 0 1 2 3 4 5 6 7 8 9 10 11 12
Immunochemistry
Mecp2+/y
Mecp2Bnull/y-AAV9/Control
Mecp2Bnull/y-AAV9/MeCP2
C
N=6
N=5
N=6
Survival (%)
100
80
60
40
20
0
0 5 10 15 20 25 30
Age (weeks)
D
Phenotypic score
10
8
6
4
2
0
0 5 10 15 20
Weeks post AAV9 injection

Figure 5

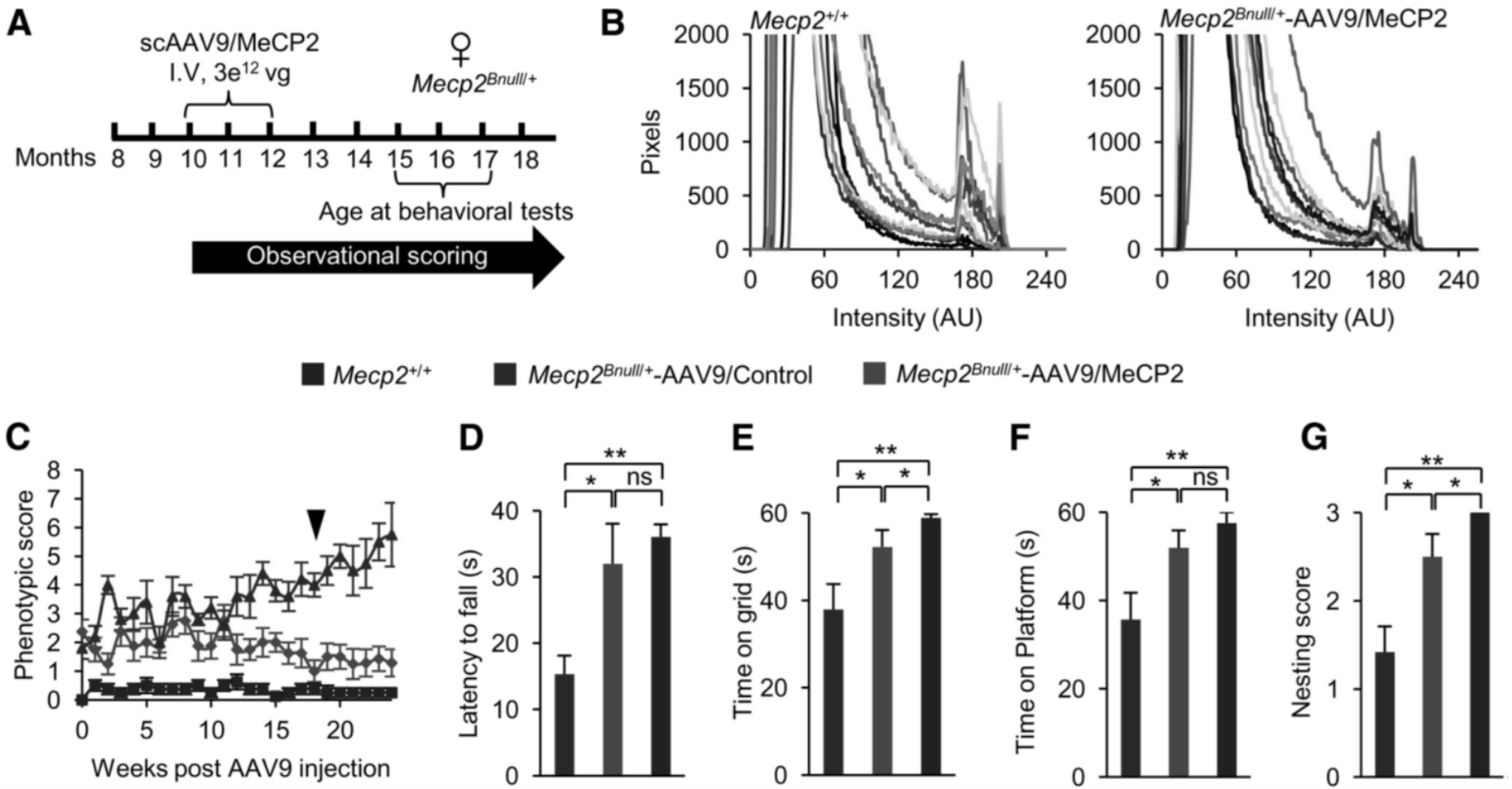

A
scAAV9/MeCP2
I.V, 3e12 vg
♀
Mecp2Bnull/+
Months 8 9 10 11 12 13 14 15 16 17 18
Age at behavioral tests
Observational scoring
B
Mecp2+/+
Mecp2Bnull/+-AAV9/MeCP2
Pixels
Intensity (AU)
Mecp2+/+
Mecp2Bnull/+-AAV9/Control
Mecp2Bnull/+-AAV9/MeCP2
C
Phenotypic score
Weeks post AAV9 injection
D
Latency to fall (s)
E
Time on grid (s)
F
Time on Platform (s)
G
Nesting score
ns

Figure 6

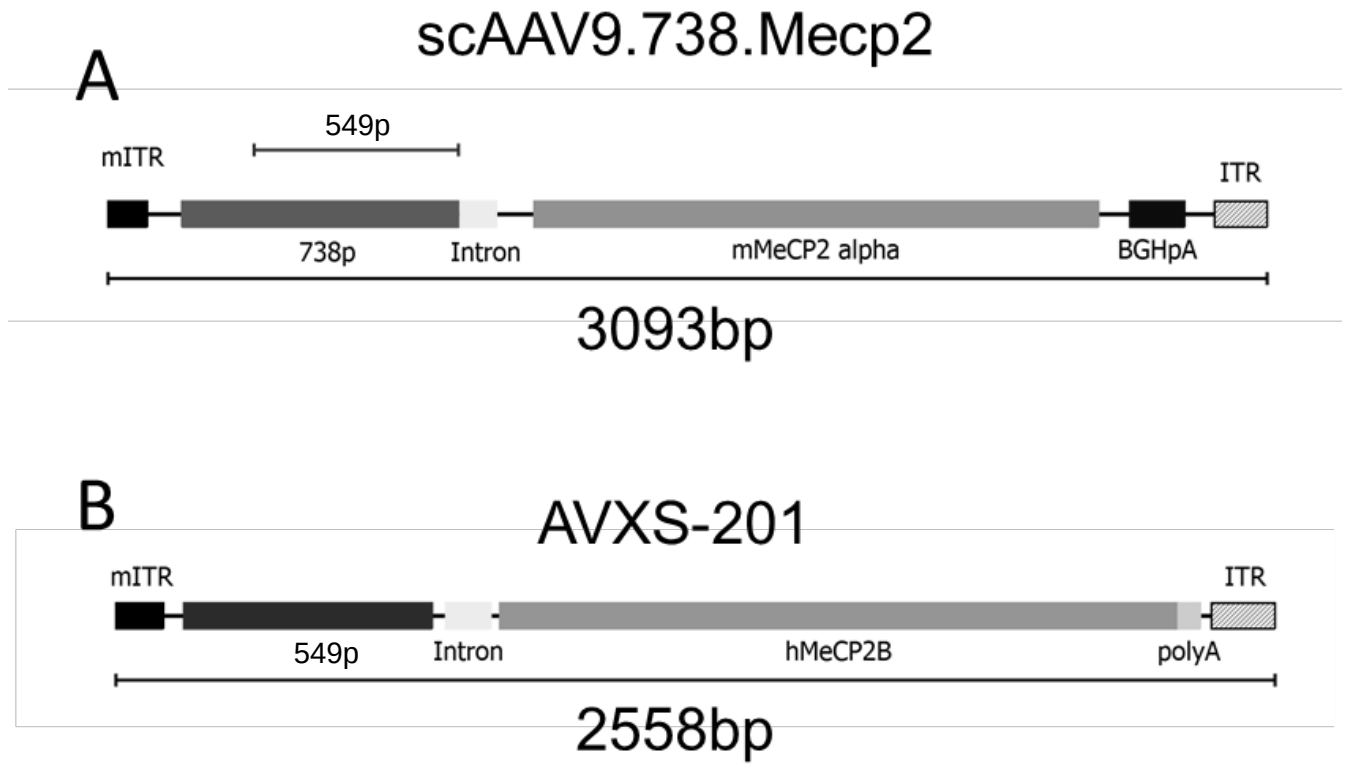
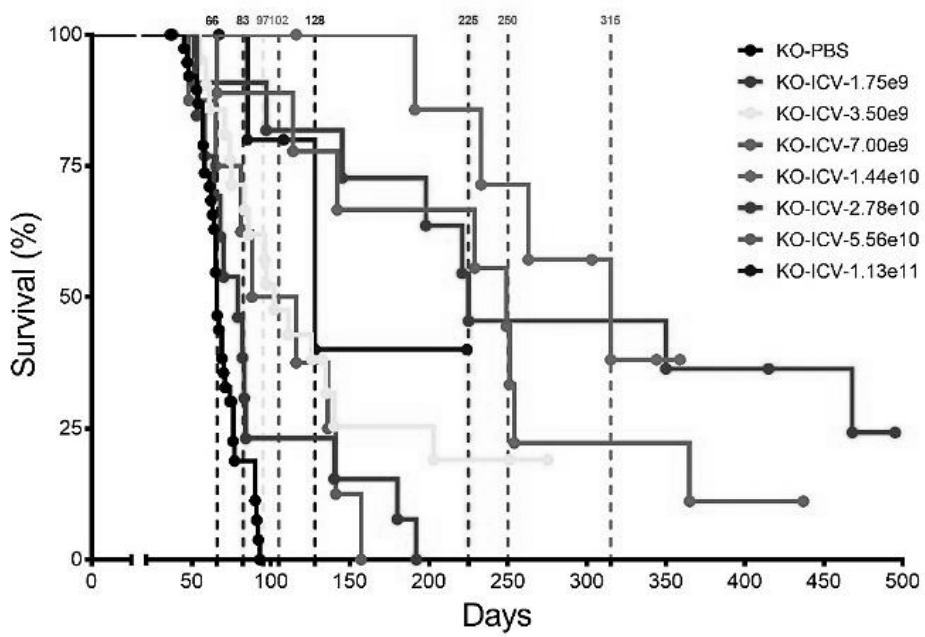

A
scAAV9.738.Mecp2
549p
mITR
ITR
738p
Intron
mMeCP2 alpha
BGHpA
3093bp
B
AVXS-201
mITR
ITR
549p
Intron
hMeCP2B
polyA
2558bp

Figure 7

A


66
83
97102
128
225
250
315
100
75
50
25
0
Survival (%)
0
50
100
150
200
250
300
350
400
450
500
Days
KO-PBS
KO-ICV-1.75e9
KO-ICV-3.50e9
KO-ICV-7.00e9
KO-ICV-1.44e10
KO-ICV-2.78e10
KO-ICV-5.56e10
KO-ICV-1.13e11

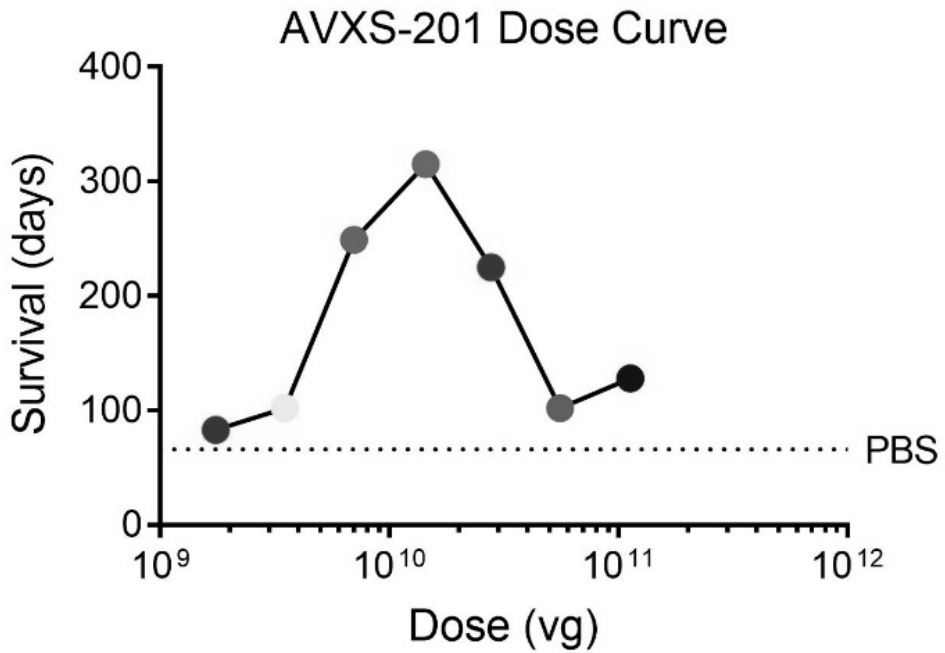

AVXS-201 Dose Curve
400
300
200
100
0
Survival (days)
PBS
10^9
10^10
10^11
10^12
Dose (vg)

Figure 8

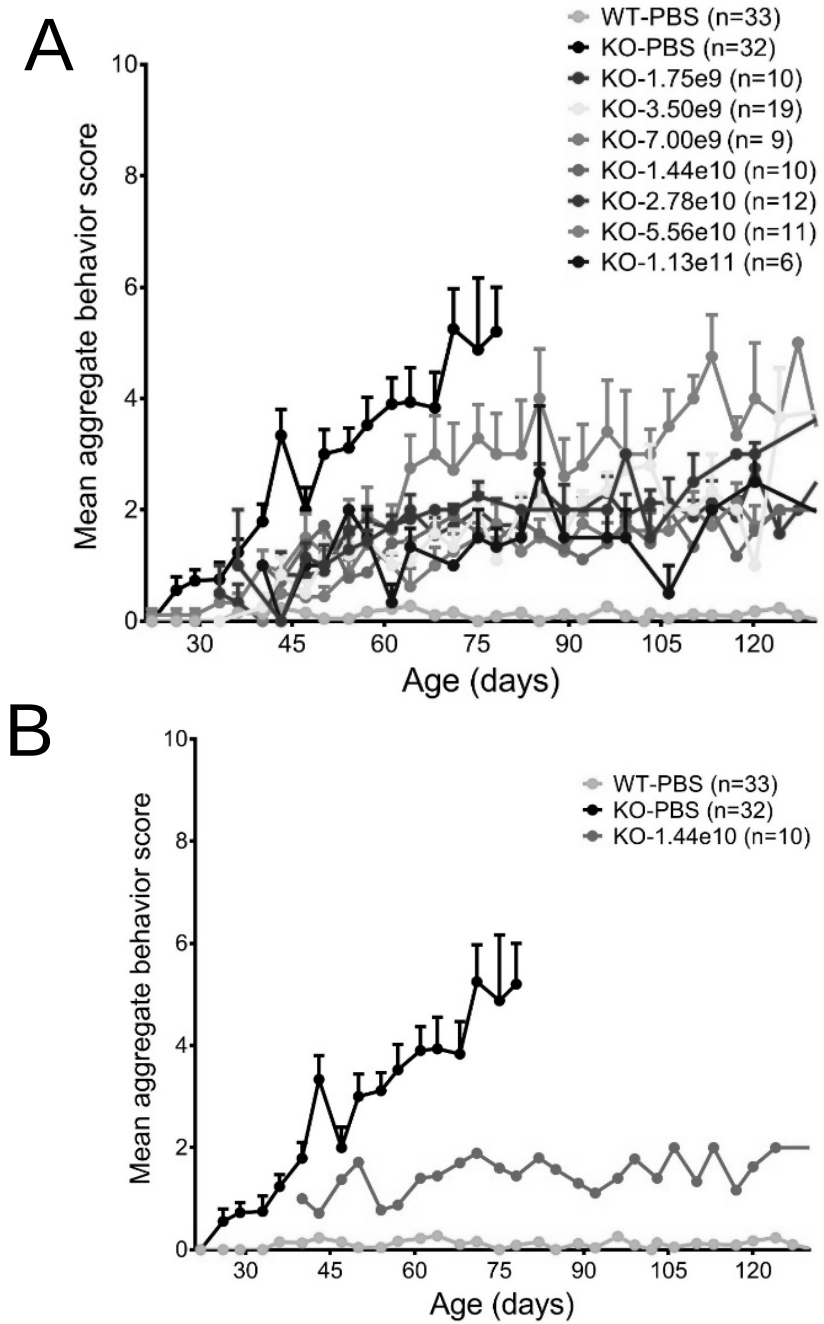

A
WT-PBS (n=33)
KO-PBS (n=32)
KO-1.75e9 (n=10)
KO-3.50e9 (n=19)
KO-7.00e9 (n= 9)
KO-1.44e10 (n=10)
KO-2.78e10 (n=12)
KO-5.56e10 (n=11)
KO-1.13e11 (n=6)
Mean aggregate behavior score
0
2
4
6
8
10
30
45
60
75
90
105
120
Age (days)
B
WT-PBS (n=33)
KO-PBS (n=32)
KO-1.44e10 (n=10)
Mean aggregate behavior score
0
2
4
6
8
10
30
45
60
75
90
105
120
Age (days)

Figure 9

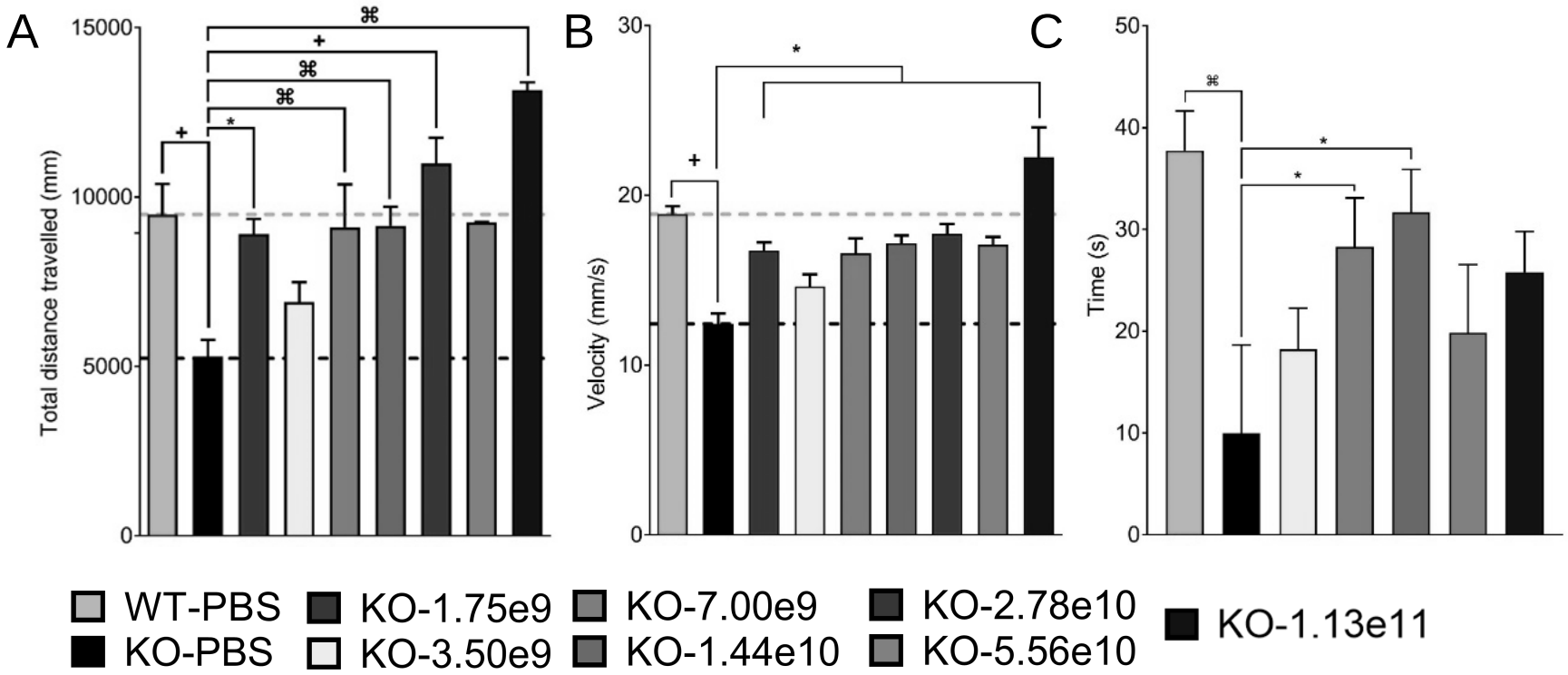

A
B
C
Total distance travelled (mm)
15000
10000
5000
0
Velocity (mm/s)
30
20
10
0
Time (s)
50
40
30
20
10
0
WT-PBS
KO-PBS
KO-1.75e9
KO-3.50e9
KO-7.00e9
KO-1.44e10
KO-2.78e10
KO-5.56e10
KO-1.13e11

Figure 10

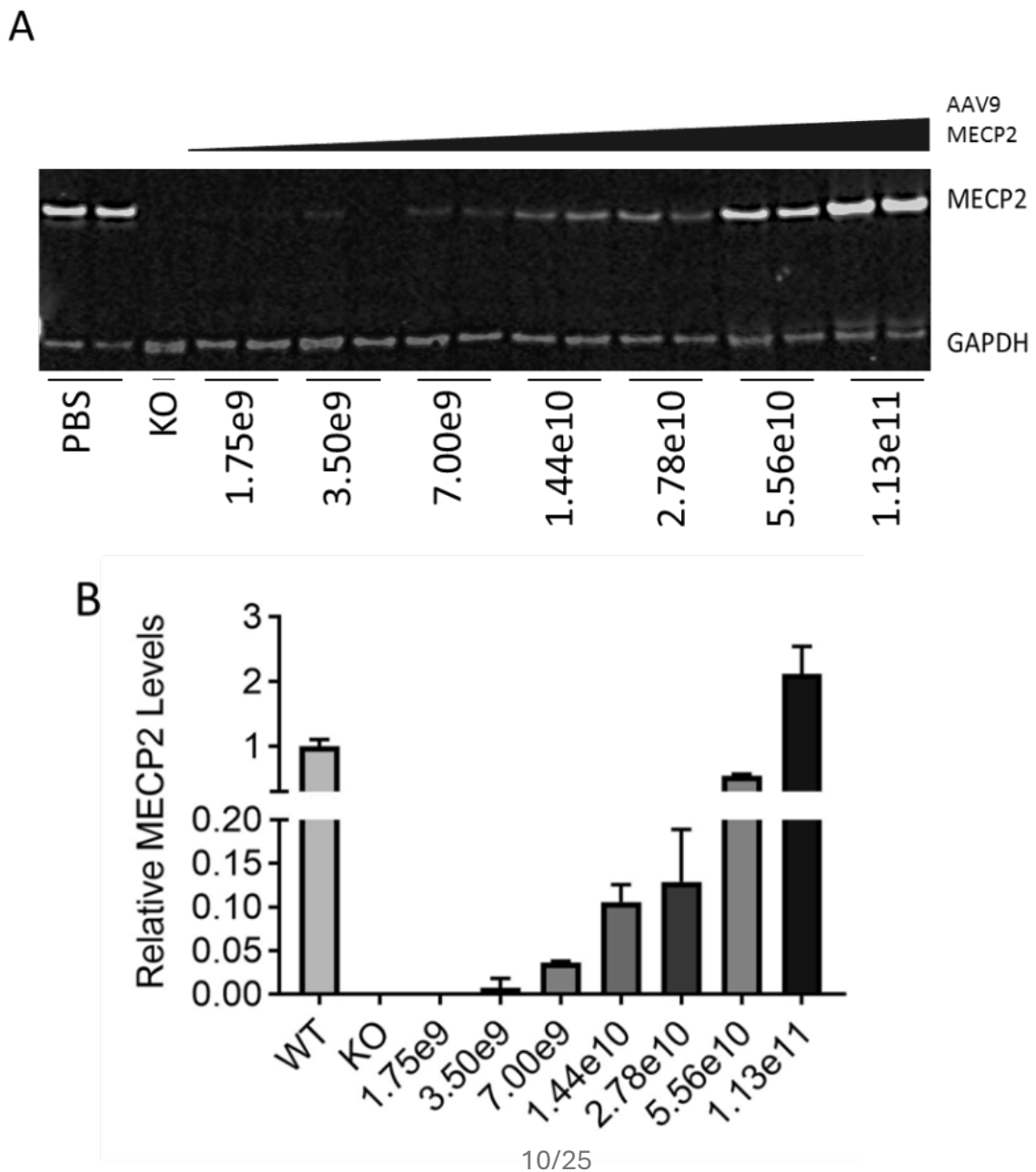

A
AAV9
MECP2
MECP2
GAPDH
PBS
KO
1.75e9
3.50e9
7.00e9
1.44e10
2.78e10
5.56e10
1.13e11
B
Relative MECP2 Levels
3
2
1
0.20
0.15
0.10
0.05
0.00
WT
KO
1.75e9
3.50e9
7.00e9
1.44e10
2.78e10
5.56e10
1.13e11

Figure 11

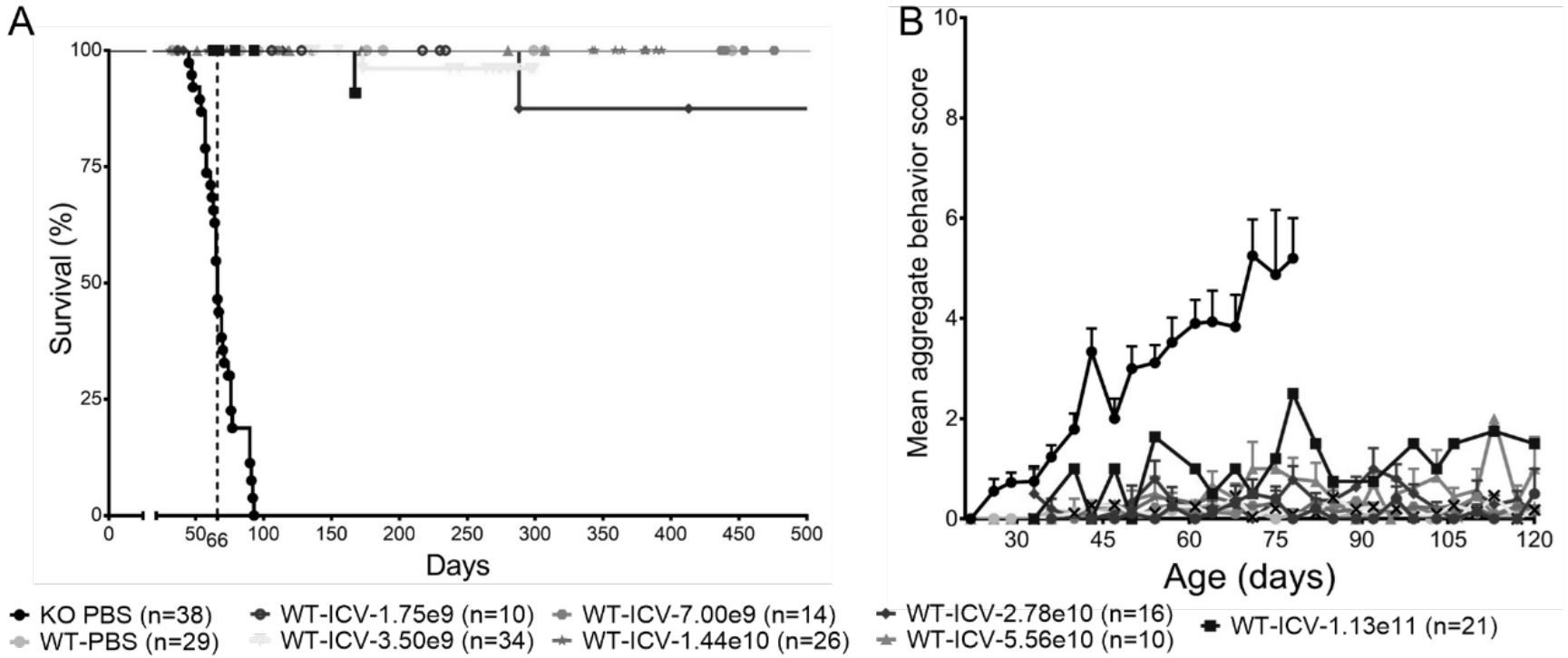

A
Survival (%)
Days
B
Mean aggregate behavior score
Age (days)
KO PBS (n=38)
WT-PBS (n=29)
WT-ICV-1.75e9 (n=10)
WT-ICV-3.50e9 (n=34)
WT-ICV-7.00e9 (n=14)
WT-ICV-1.44e10 (n=26)
WT-ICV-2.78e10 (n=16)
WT-ICV-5.56e10 (n=10)
WT-ICV-1.13e11 (n=21)

Figure 12

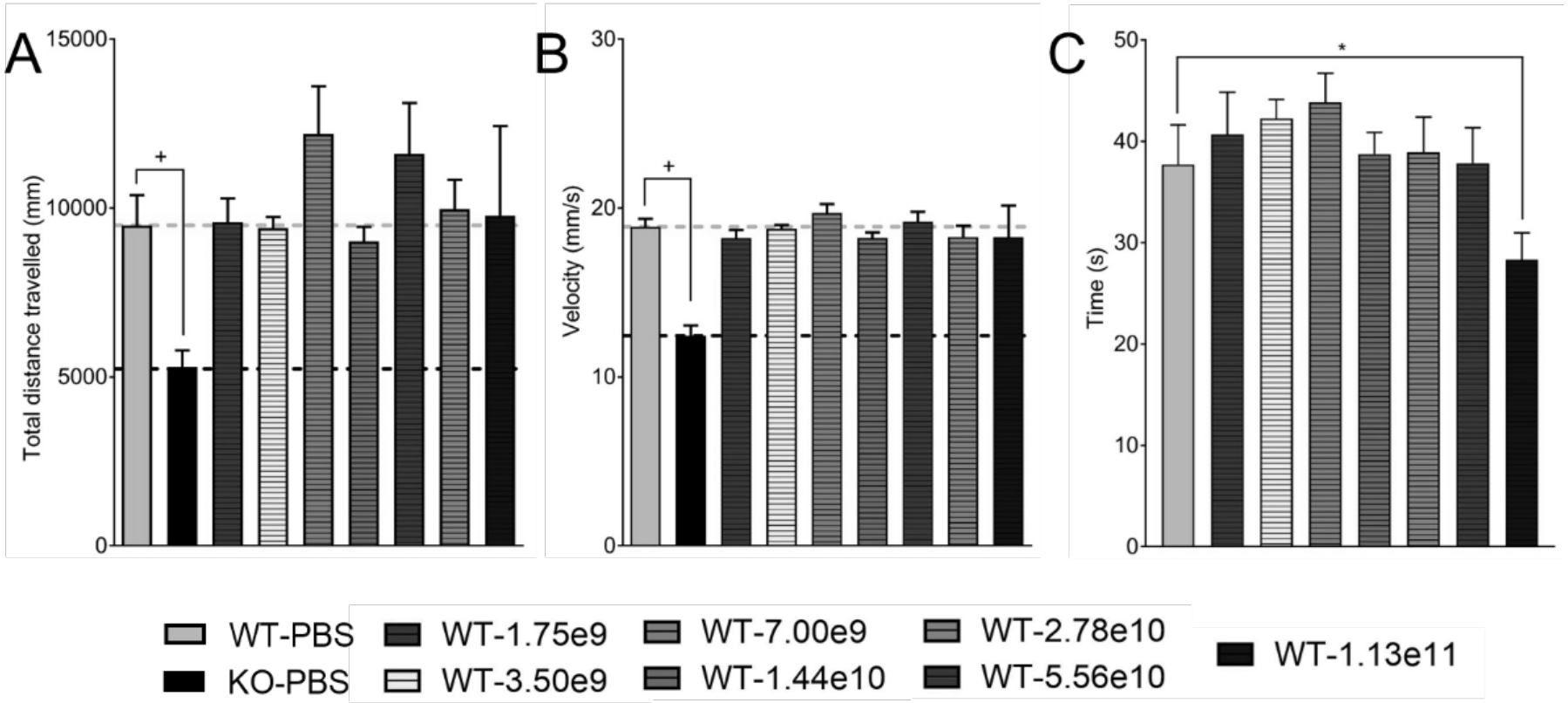
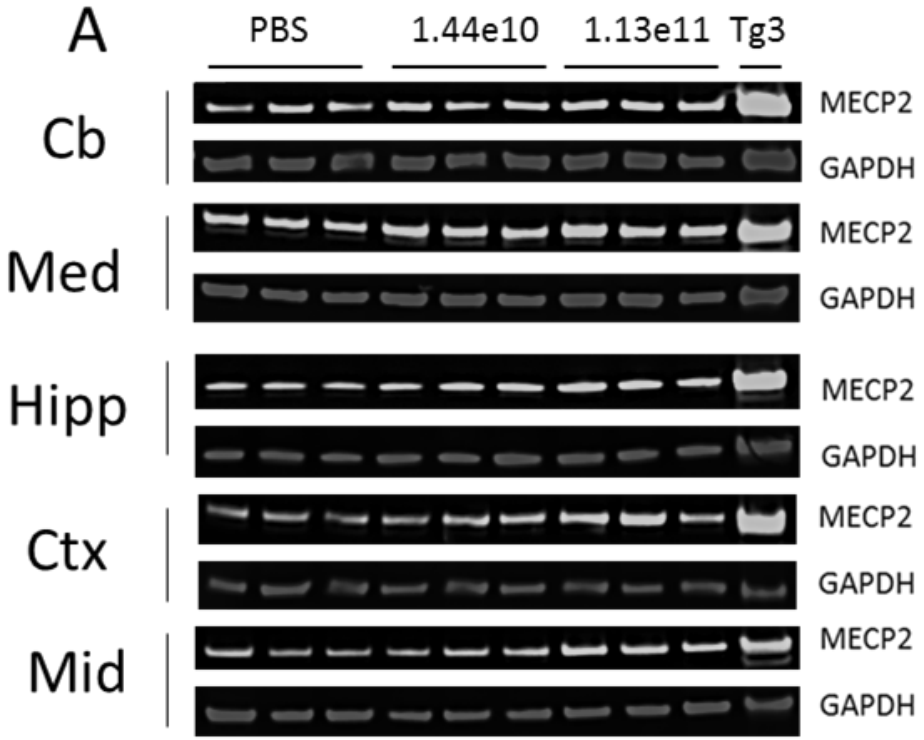

A
Total distance travelled (mm)
15000
10000
5000
0
+
B
Velocity (mm/s)
30
20
10
0
+
C
Time (s)
50
40
30
20
10
0
*
WT-PBS
KO-PBS
WT-1.75e9
WT-3.50e9
WT-7.00e9
WT-1.44e10
WT-2.78e10
WT-5.56e10
WT-1.13e11

Figure 13


A
PBS
1.44e10
1.13e11
Tg3
Cb
Med
Hipp
Ctx
Mid
MECP2
GAPDH

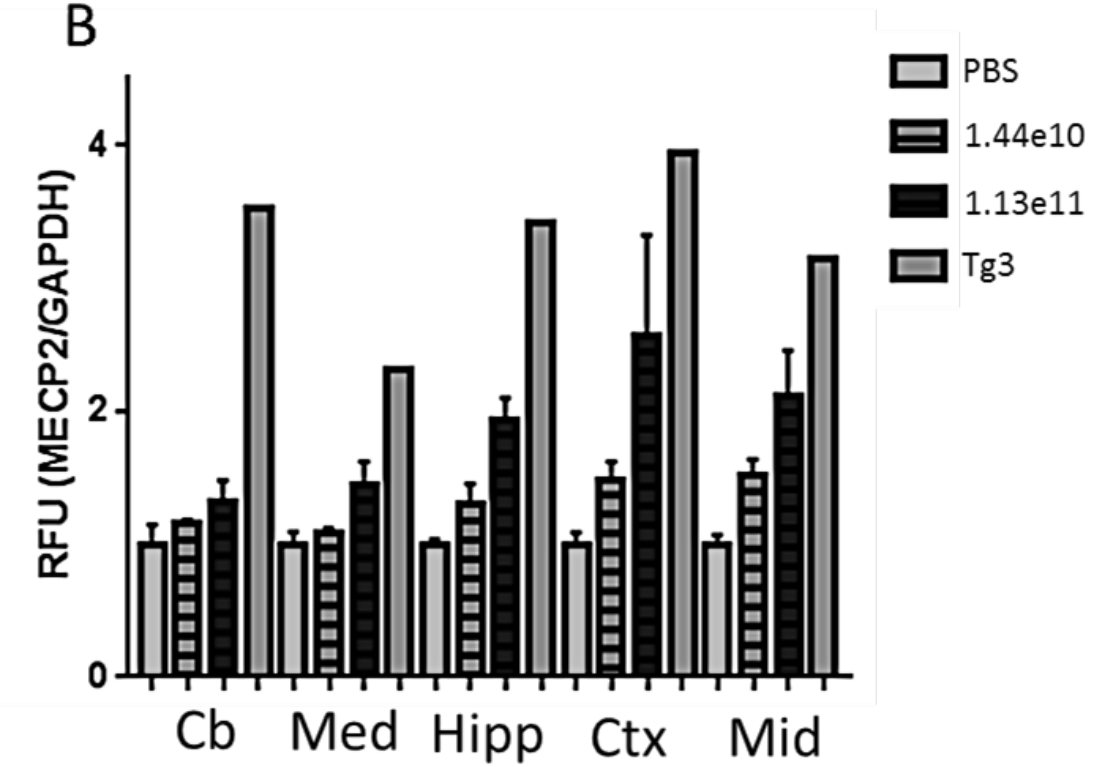

B
RFU (MECP2/GAPDH)
0
2
4
Cb
Med
Hipp
Ctx
Mid
PBS
1.44e10
1.13e11
Tg3

Figure 14

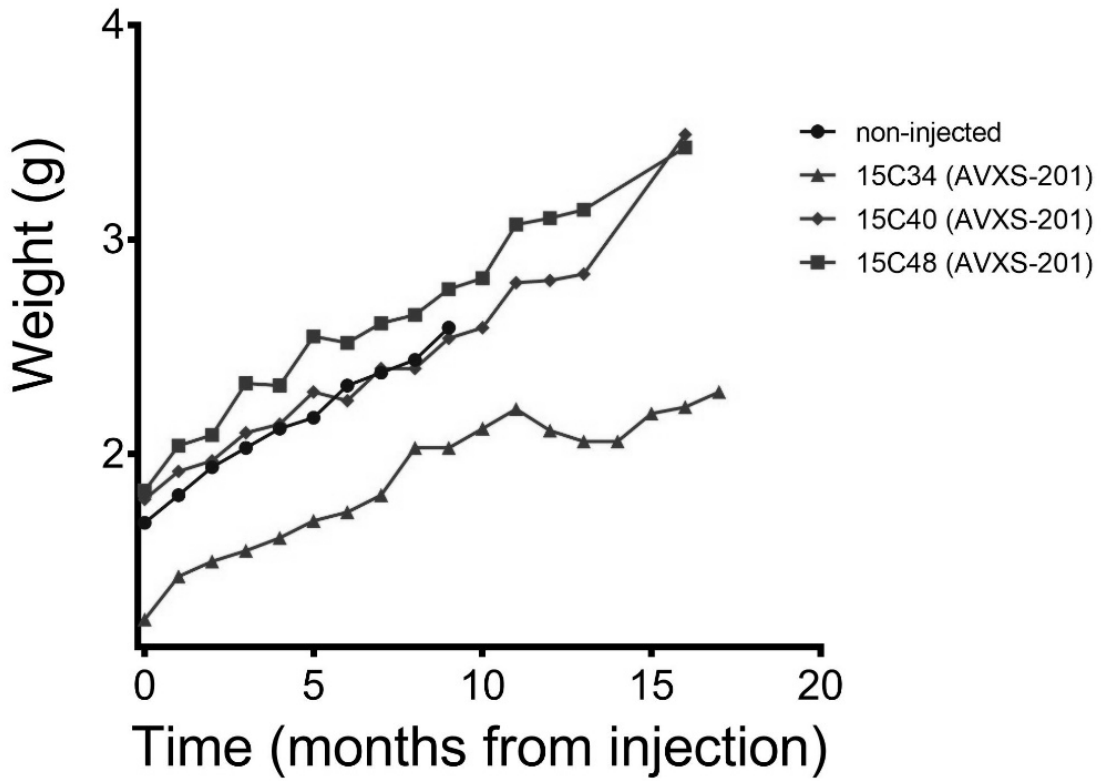

Weight (g)
4
3
2
0
5
10
15
20
Time (months from injection)
non-injected
15C34 (AVXS-201)
15C40 (AVXS-201)
15C48 (AVXS-201)

Figure 15

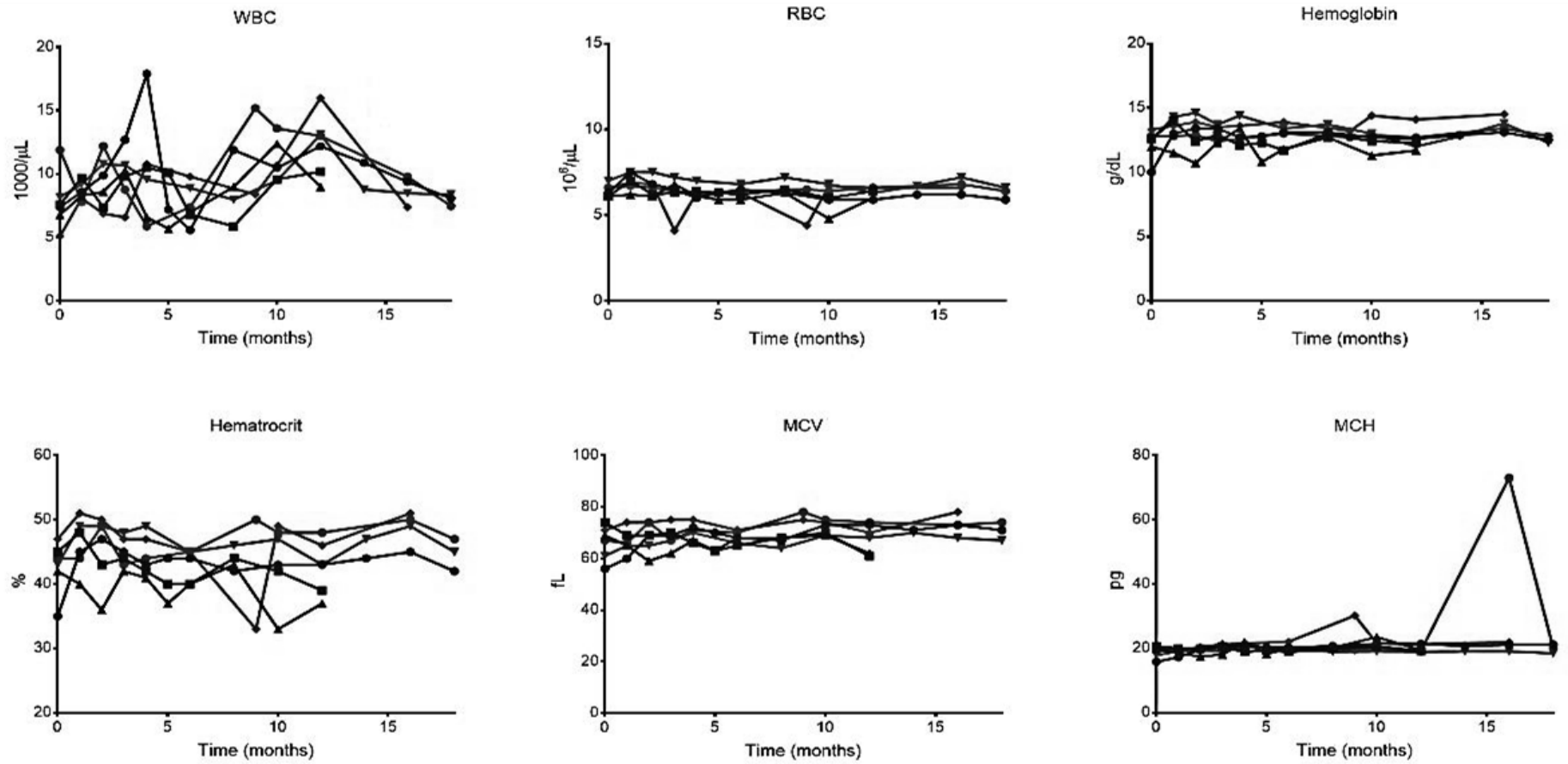

WBC
1000/µL
Time (months)
RBC
10^6/µL
Time (months)
Hemoglobin
g/dL
Time (months)
Hematrocrit
%
Time (months)
MCV
fL
Time (months)
MCH
pg
Time (months)
PBS 113
PBS 126
PBS 130
15C34 (AVXS-201)
15C40 (AVXS-201)
15C48 (AVXS-201)

Figure 15 cont.

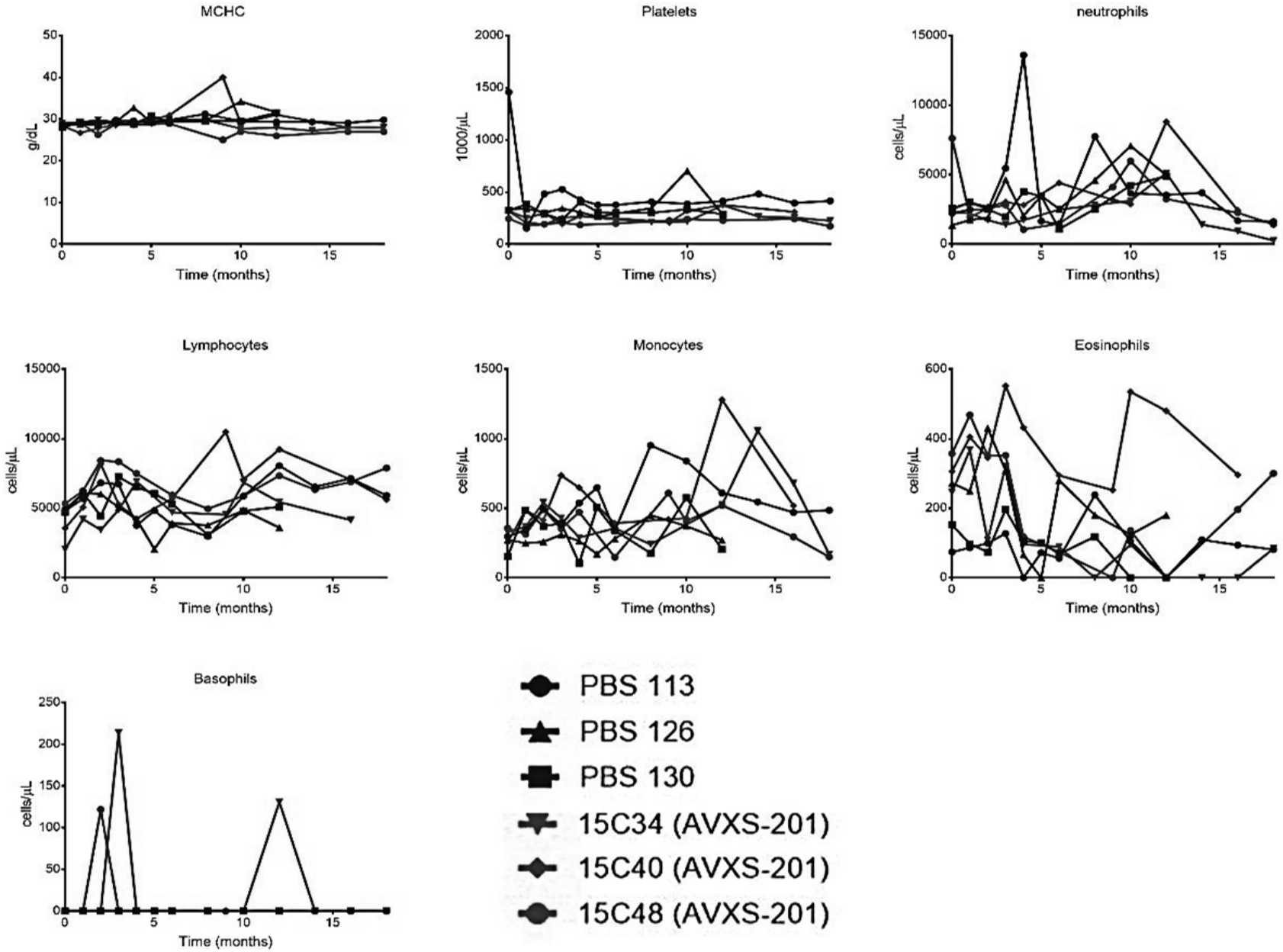


Figure 16

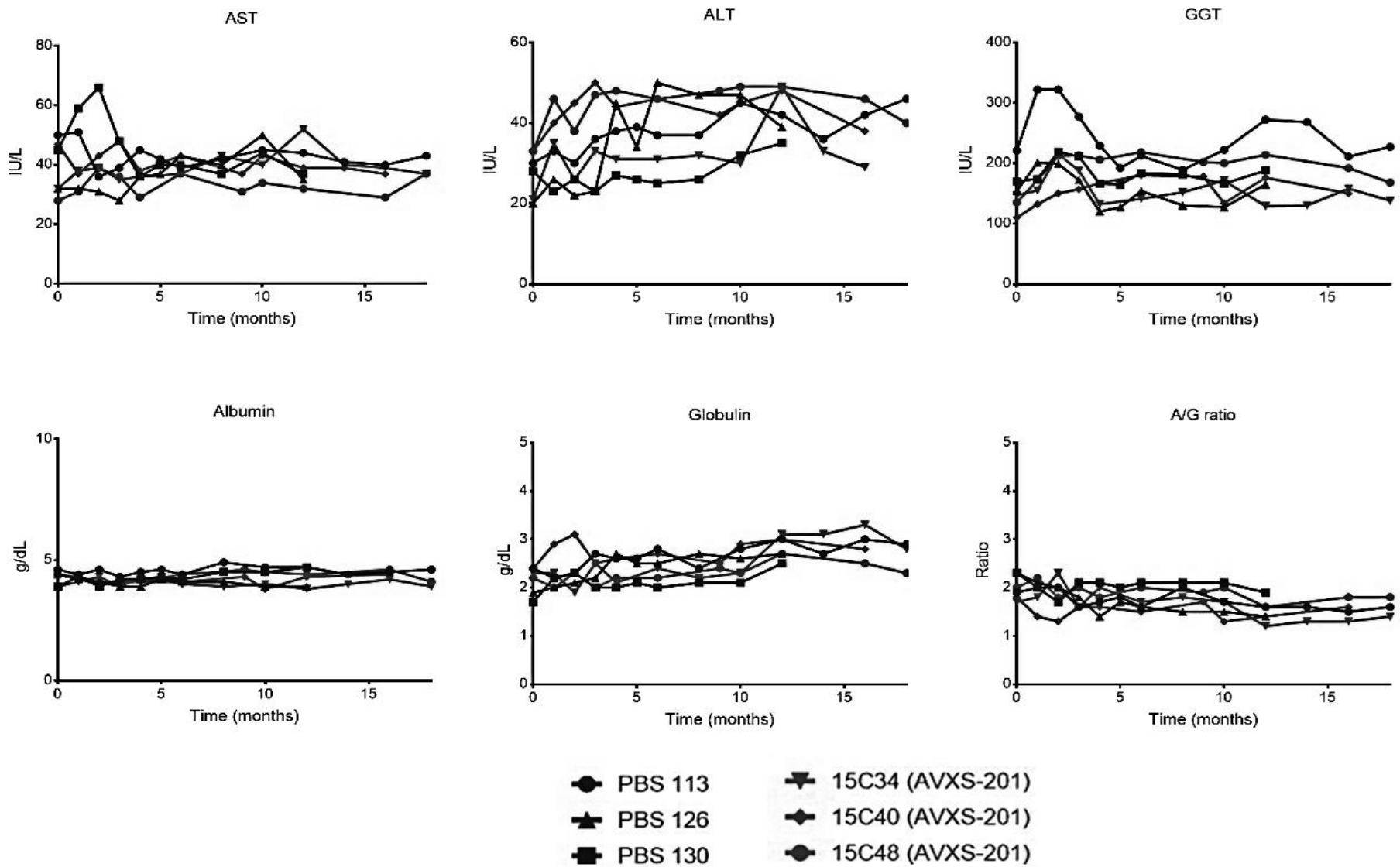

AST
IU/L
Time (months)
ALT
IU/L
Time (months)
GGT
IU/L
Time (months)
Albumin
g/dL
Time (months)
Globulin
g/dL
Time (months)
A/G ratio
Ratio
Time (months)
PBS 113
PBS 126
PBS 130
15C34 (AVXS-201)
15C40 (AVXS-201)
15C48 (AVXS-201)

Figure 16 cont.

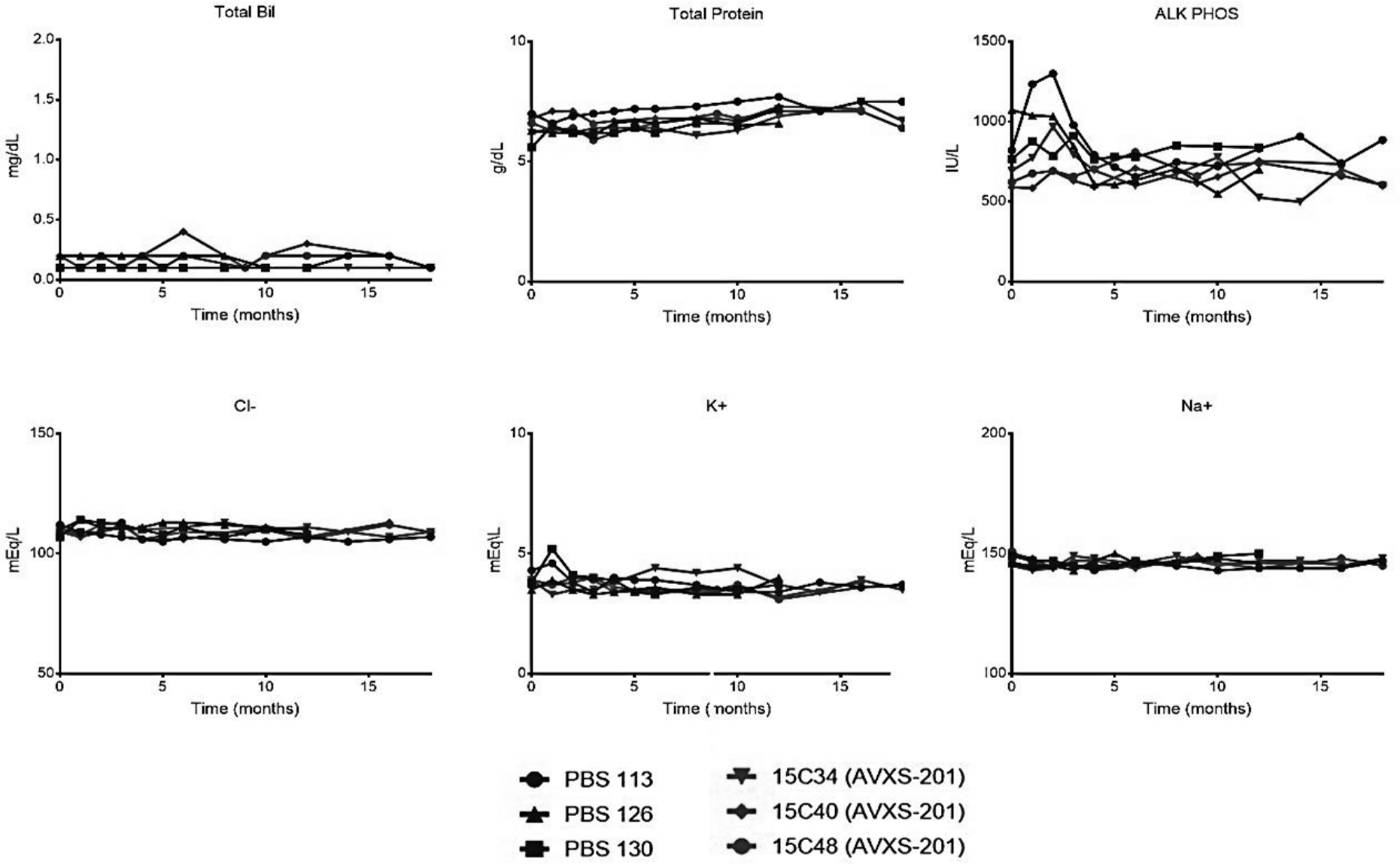


Figure 17

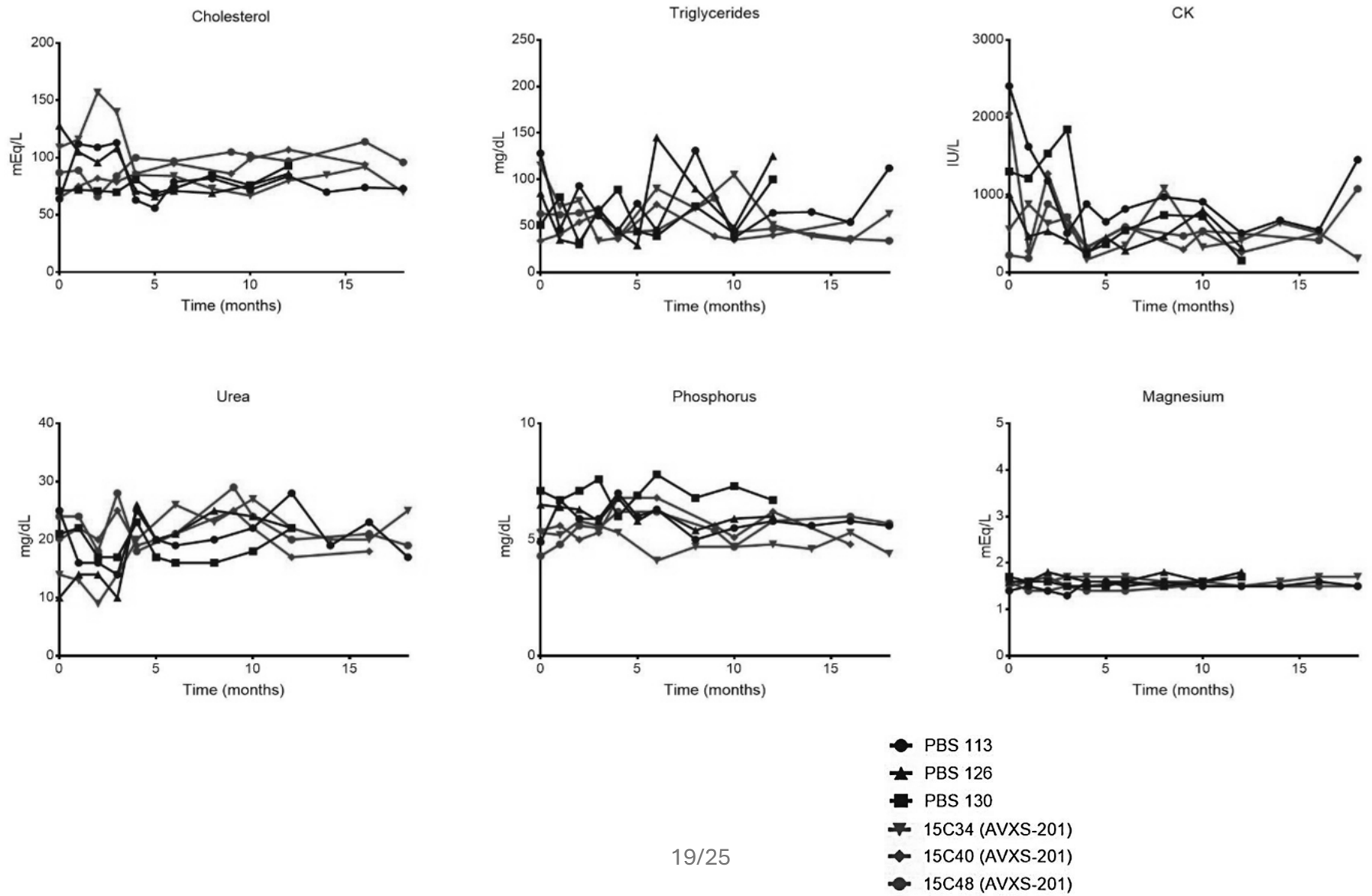

Cholesterol
mEq/L
Time (months)
Triglycerides
mg/dL
Time (months)
CK
IU/L
Time (months)
Urea
mg/dL
Time (months)
Phosphorus
mg/dL
Time (months)
Magnesium
mEq/L
Time (months)
PBS 113
PBS 126
PBS 130
15C34 (AVXS-201)
15C40 (AVXS-201)
15C48 (AVXS-201)

Figure 17 cont.

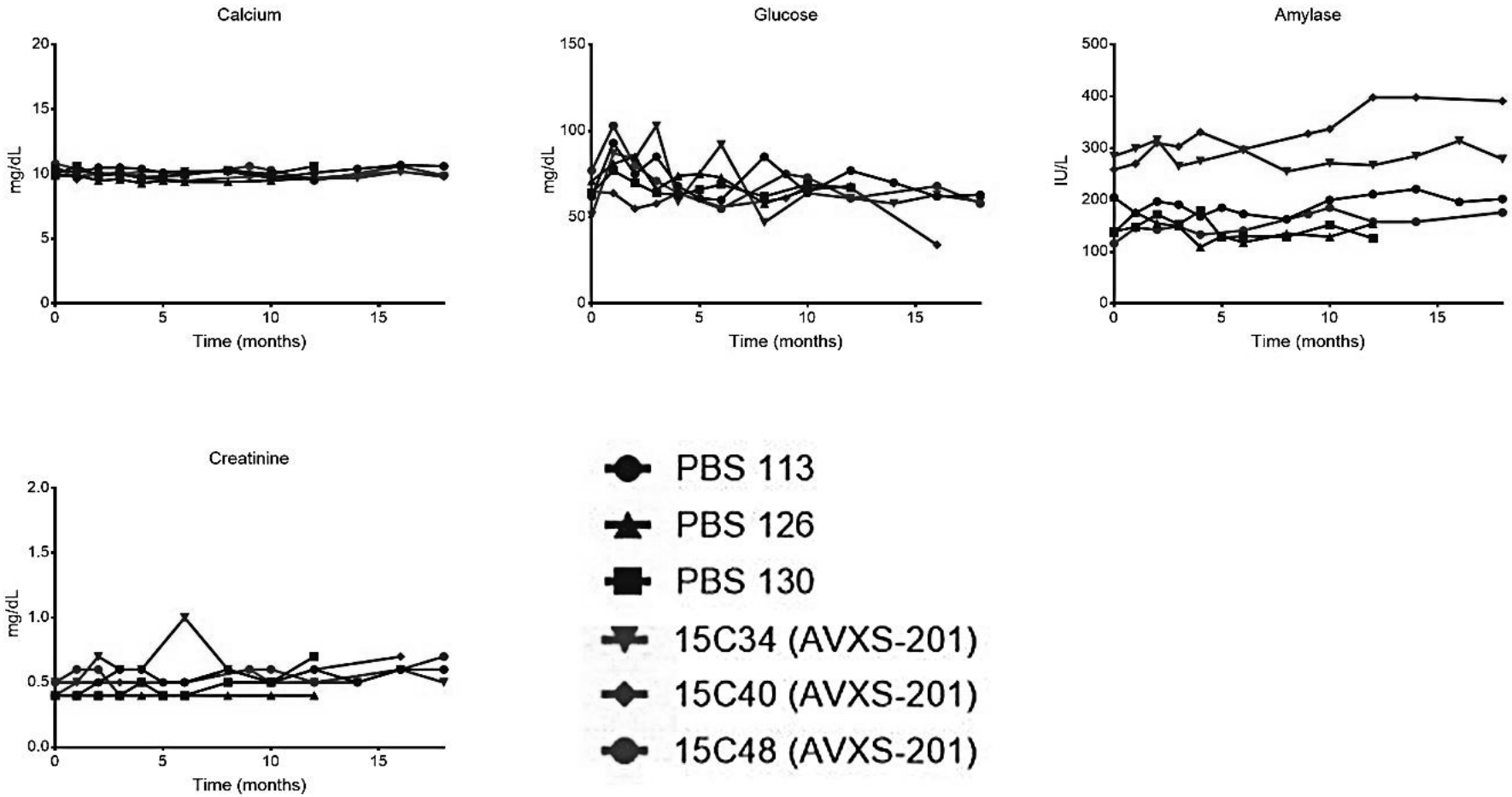


Figure 18

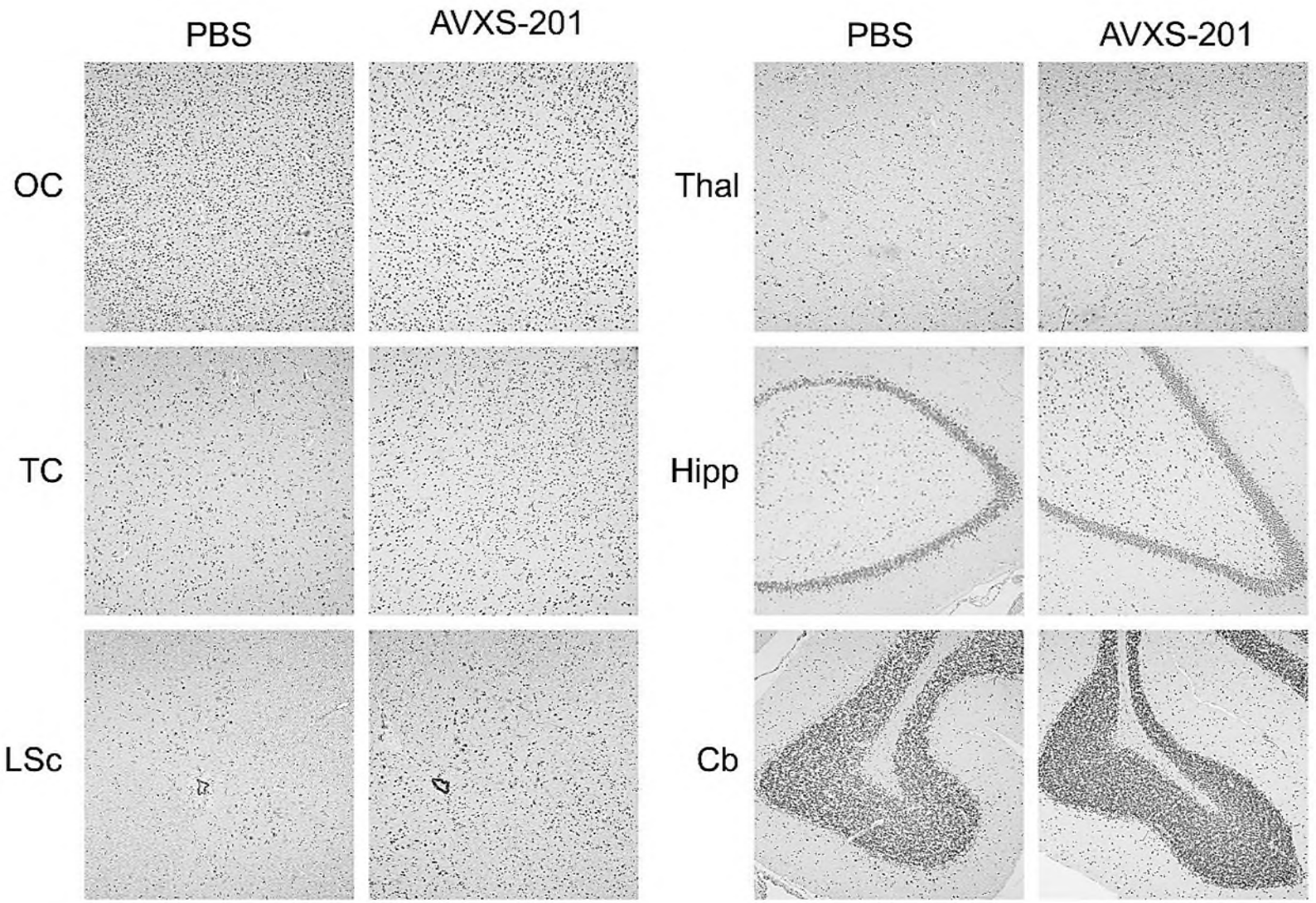


Figure 19

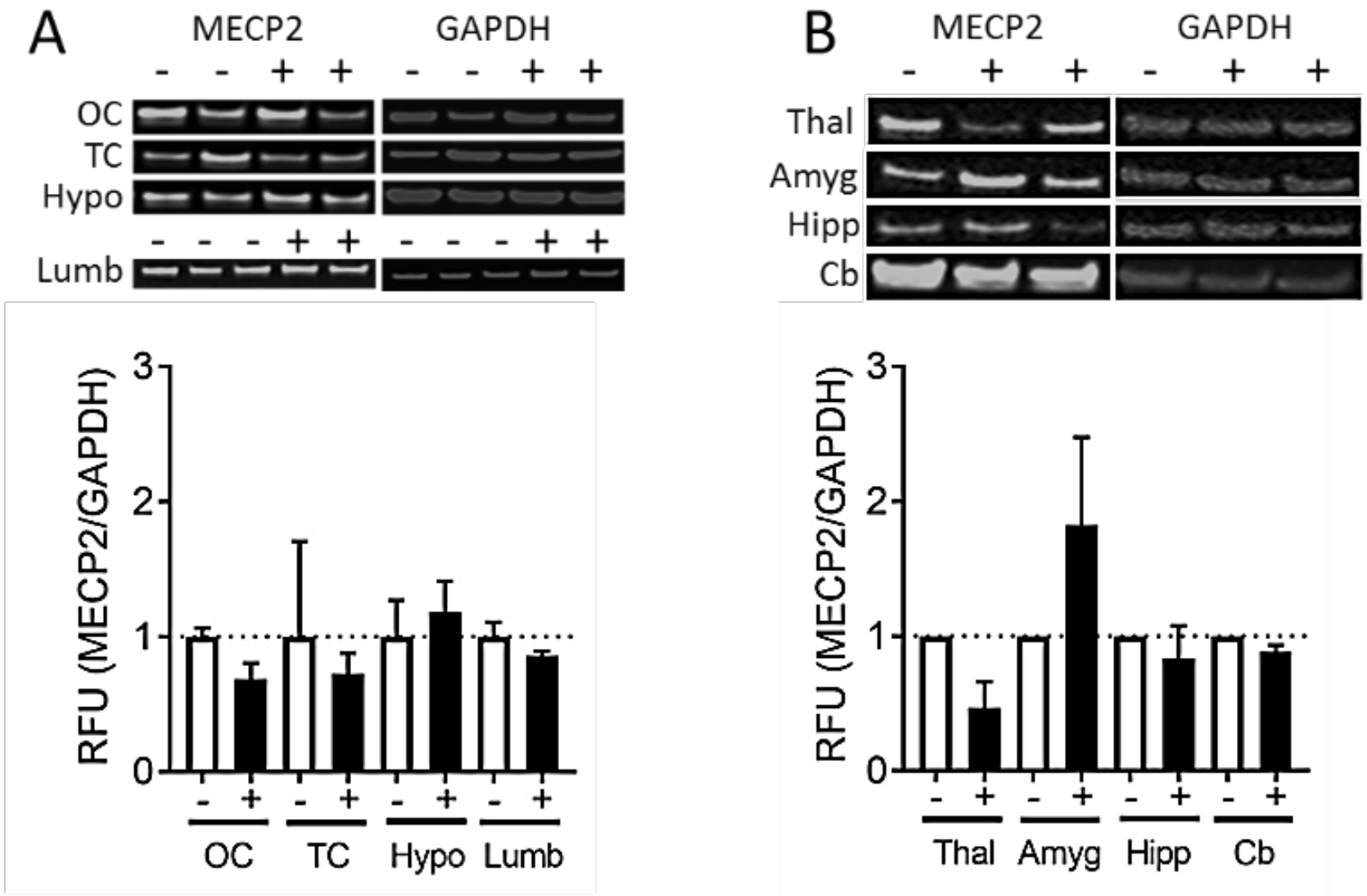

A
MECP2
GAPDH
- - + + - - + +
OC
TC
Hypo
- - - + + - - - + +
Lumb
RFU (MECP2/GAPDH)
0
1
2
3
- + - + - + - +
OC
TC
Hypo
Lumb
B
MECP2
GAPDH
- + + - + +
Thal
Amyg
Hipp
Cb
RFU (MECP2/GAPDH)
0
1
2
3
- + - + - + - +
Thal
Amyg
Hipp
Cb

Figure 20

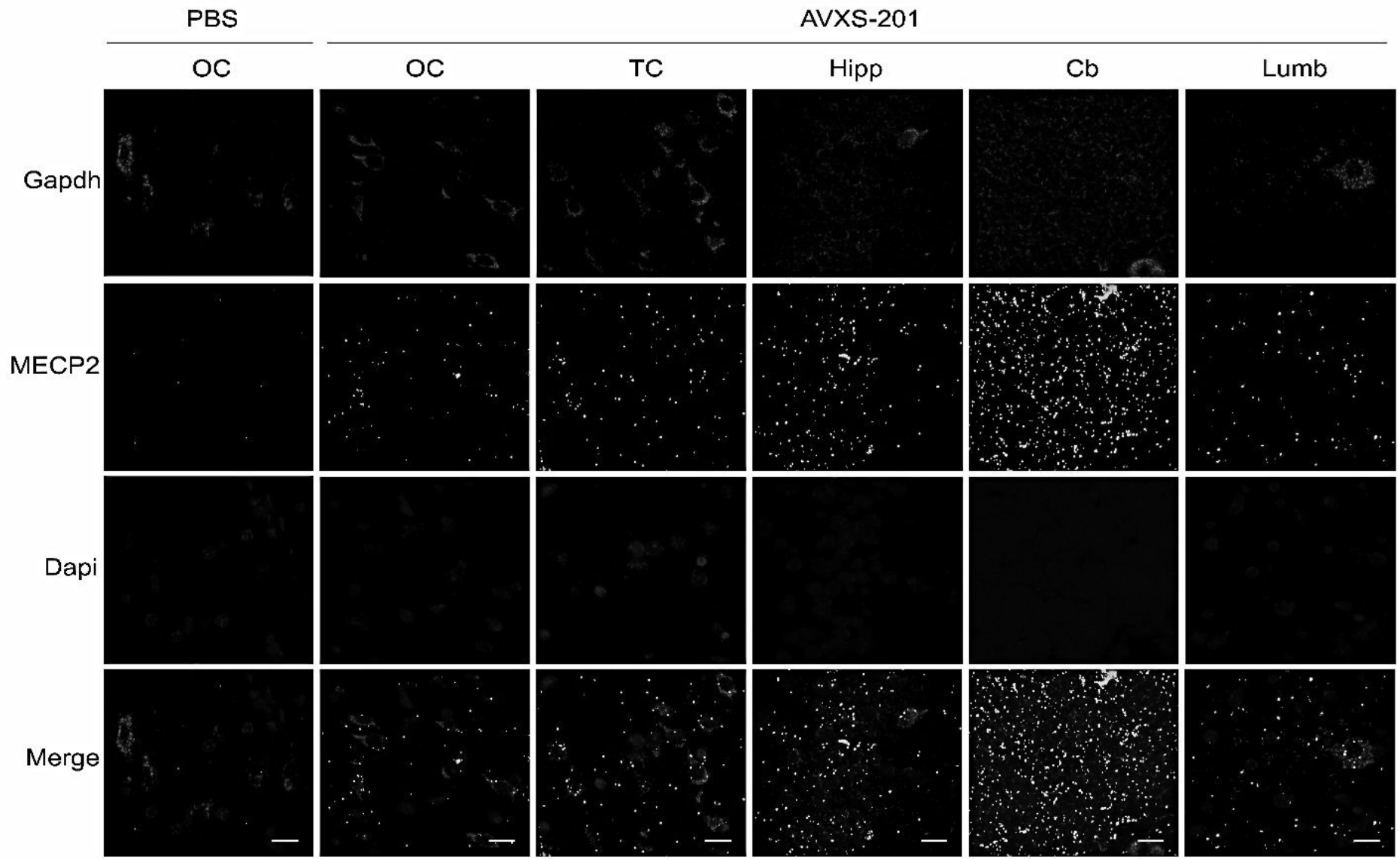
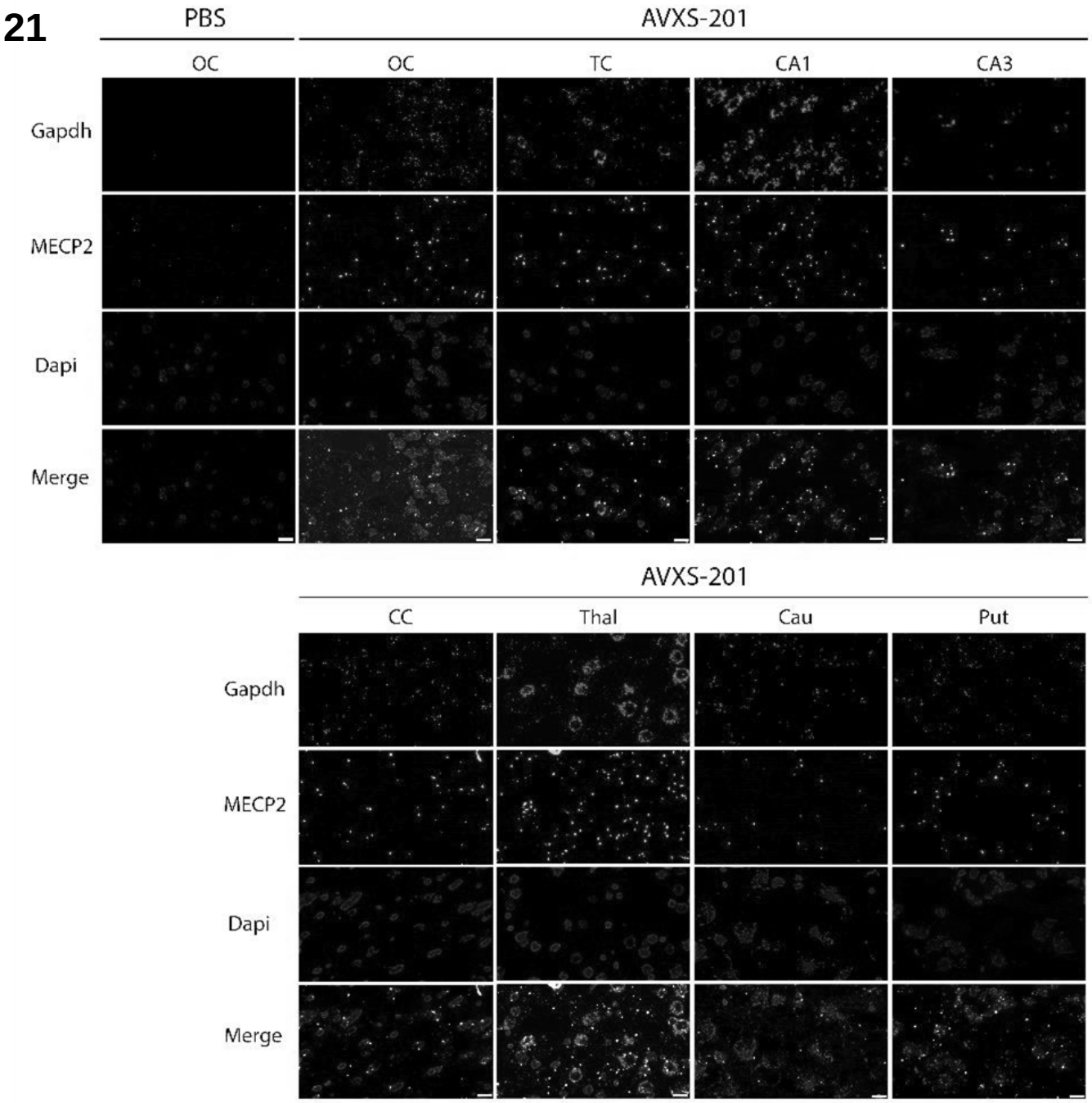


Figure 21


PBS
AVXS-201
OC
OC
TC
CA1
CA3
Gapdh
MECP2
Dapi
Merge
AVXS-201
CC
Thal
Cau
Put
Gapdh
MECP2
Dapi
Merge

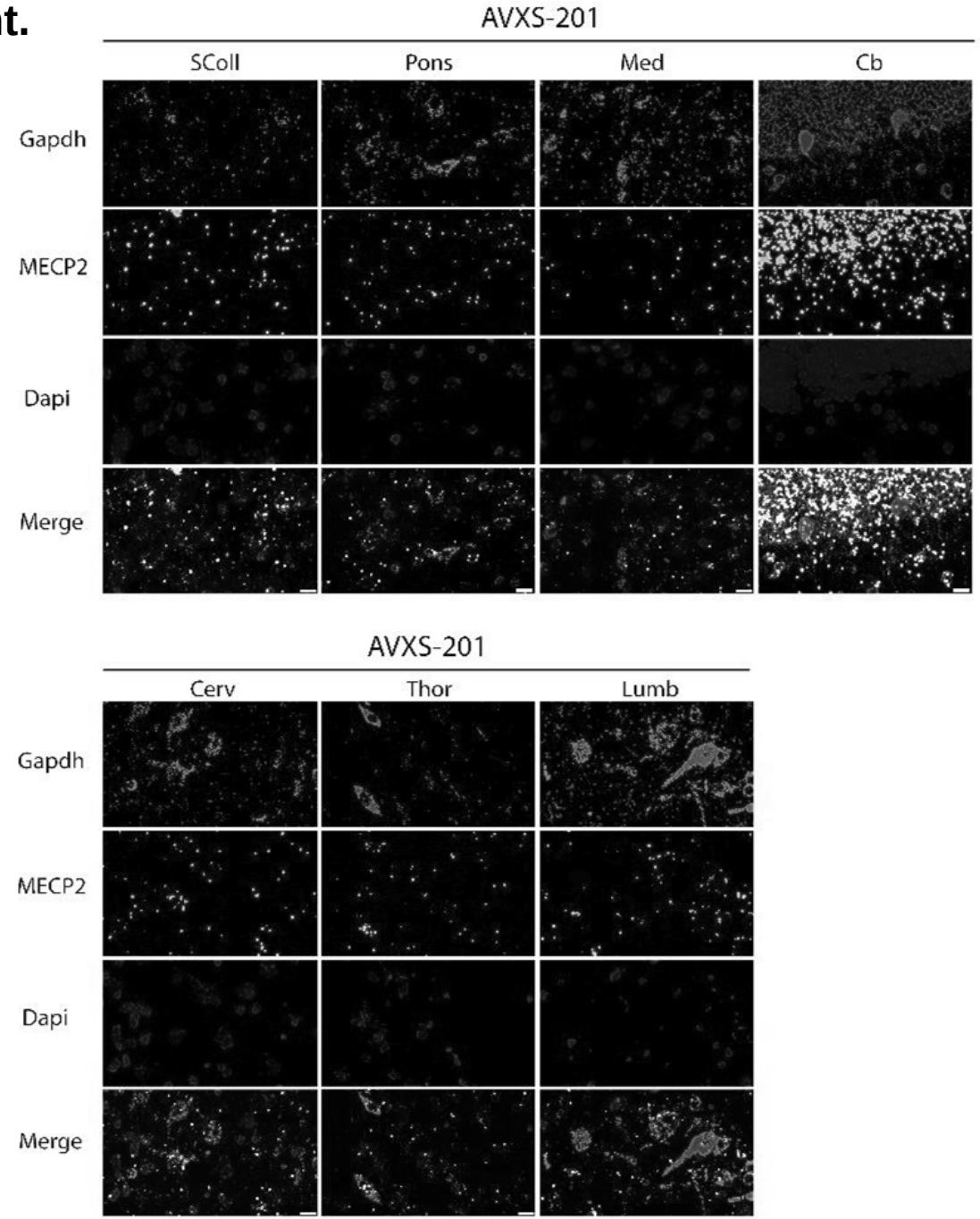



Figure 21 cont.