

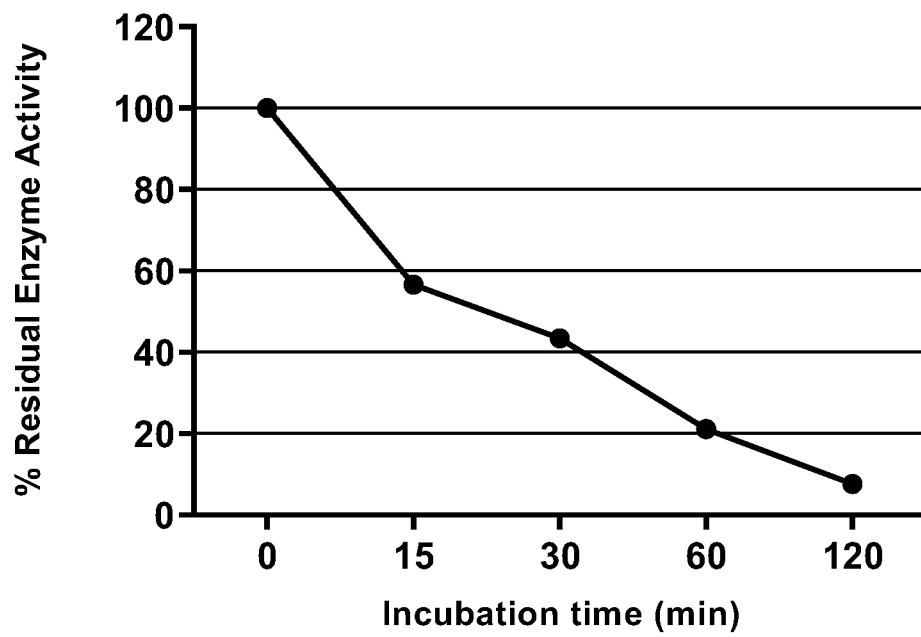
Figure 1

Figure 2

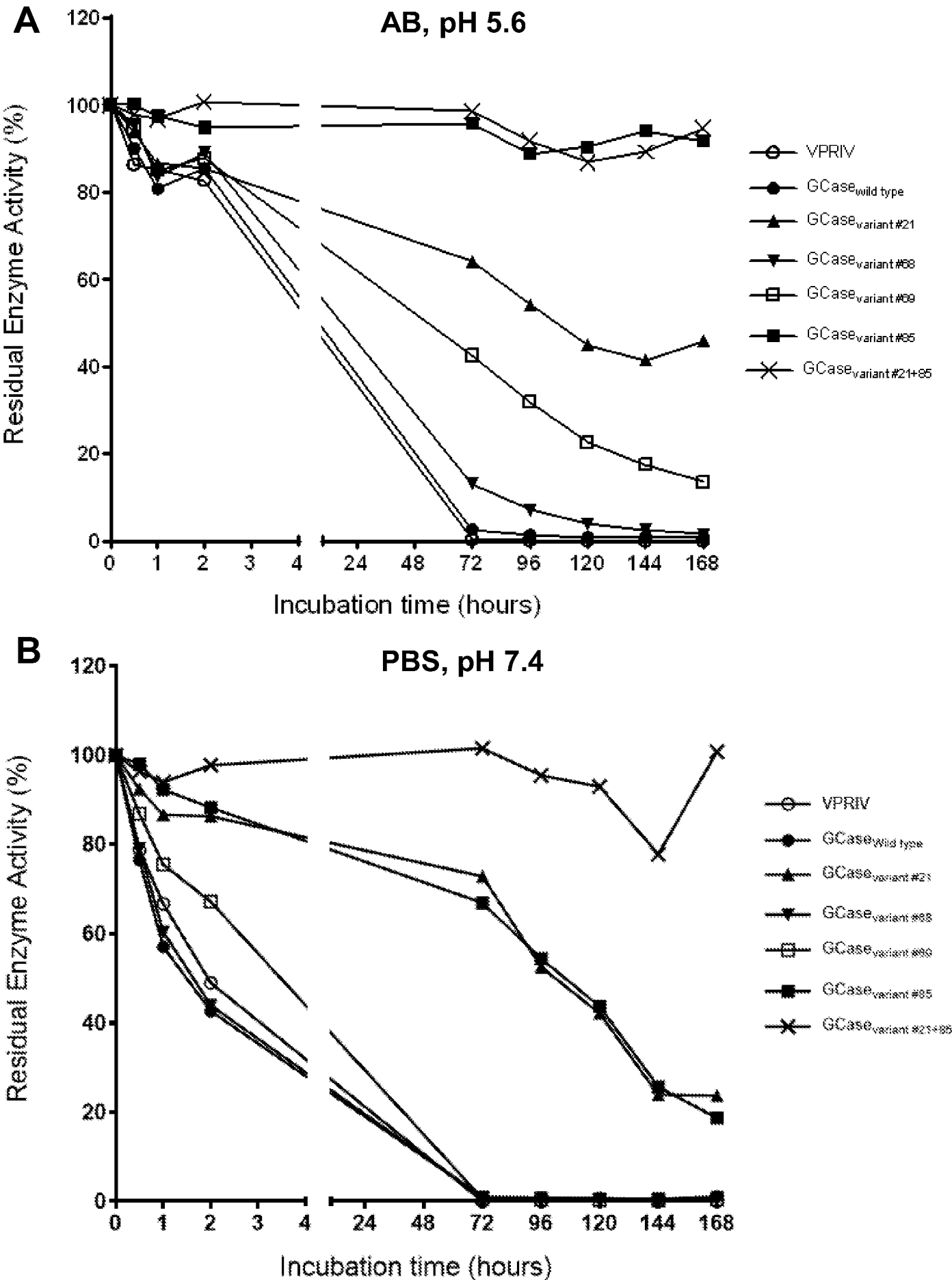


Figure 3

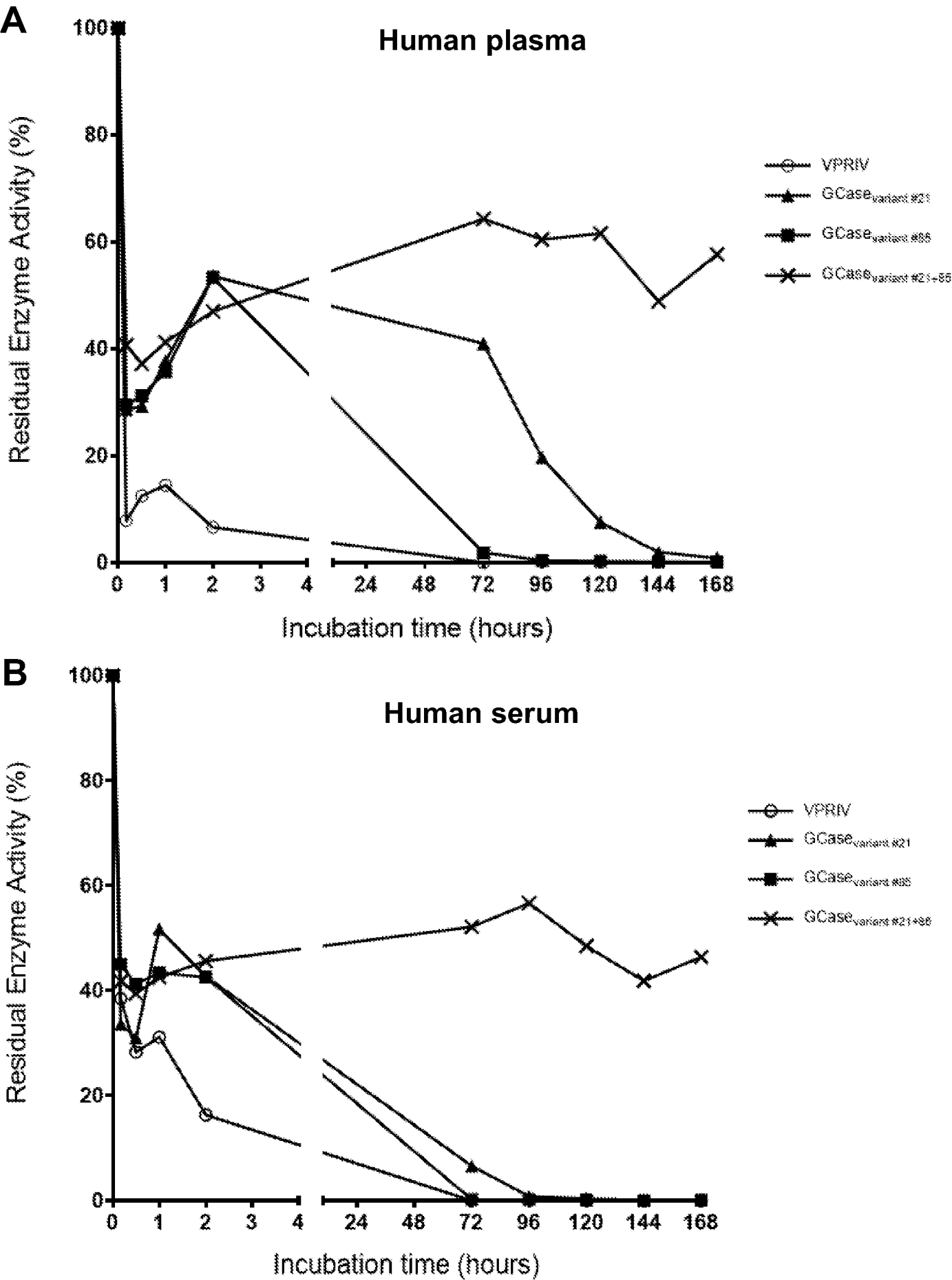
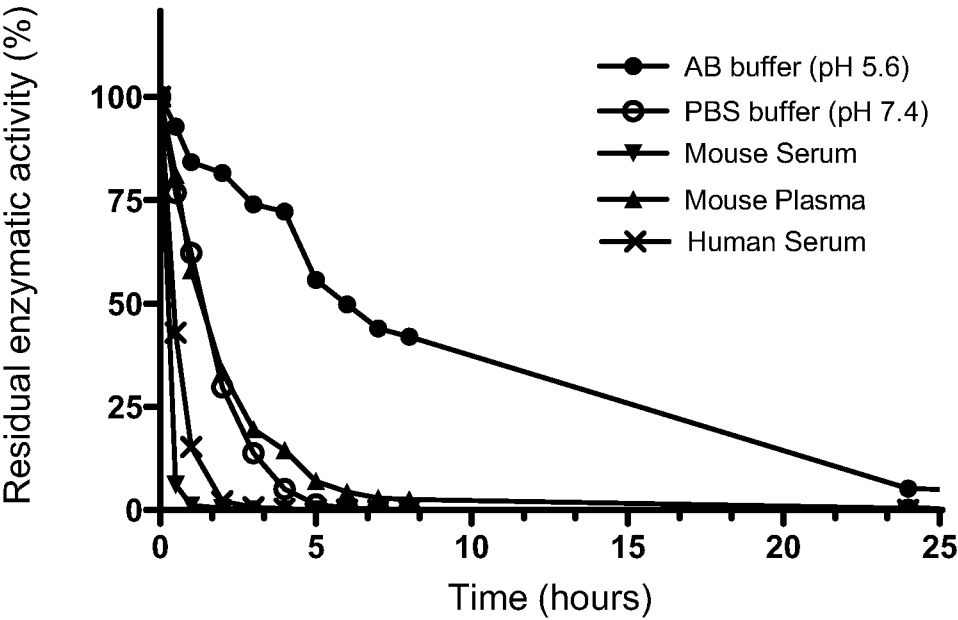


Figure 4

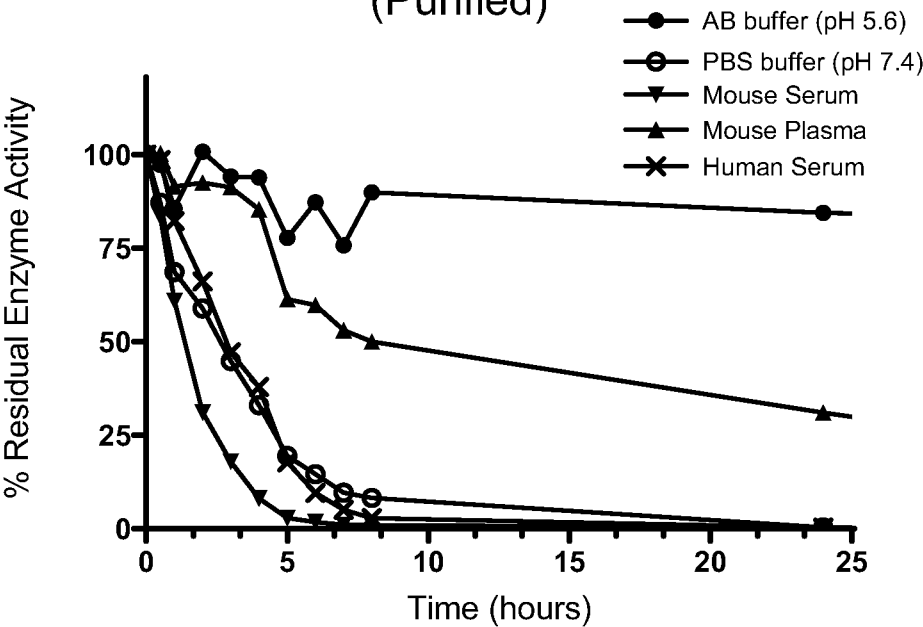
A

Velaglucerase alfa



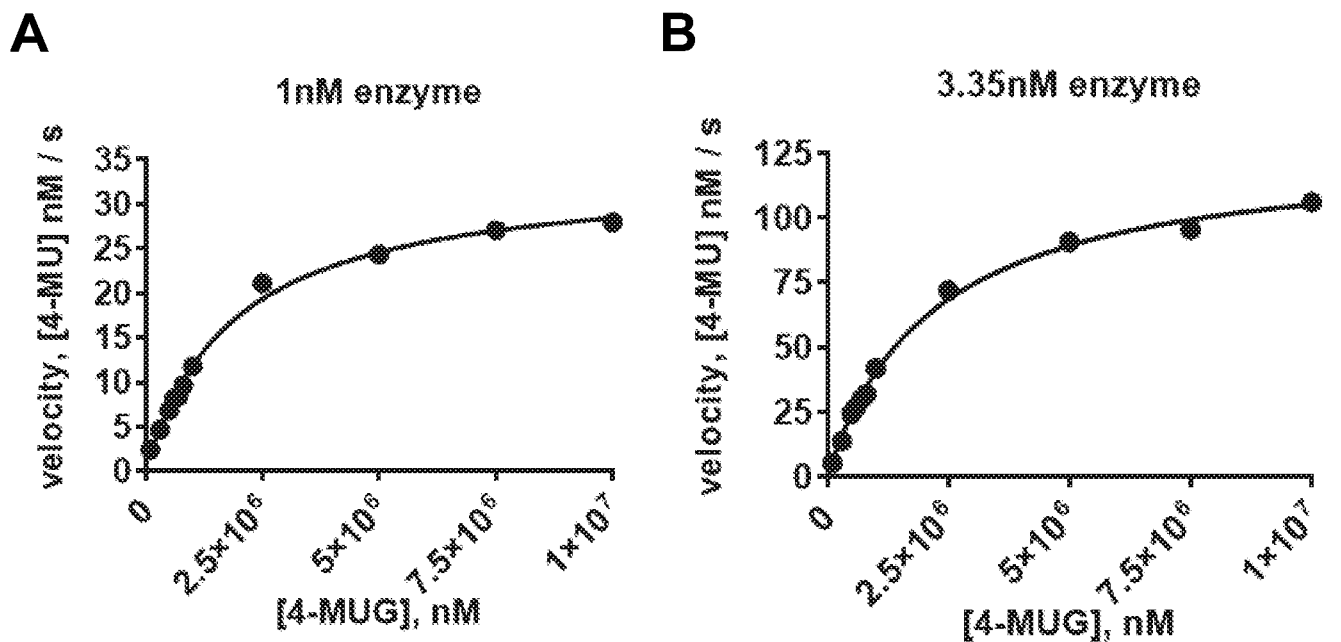
B

GCase_{variant #85}
(Purified)



C

Product	Lysosomal pH	Half-life (minutes)		
		Physiological pH	Mouse serum	Human serum
VPRIV®	388.0	70.1	7.4	24.0
GCase _{variant 85}	≥8,640	141.0	74.0	143.0

Figure 5**C**

Enzyme	Substrate	Kcat (S ⁻¹)	Km (mM)	Reference
GCCase variant #85	4-MUG	36.0 ± 3.2	2.0 ± 0.2	Based on the data shown in Figures 5 A and B
Imiglucerase	4-MUG	N/A	1.8	Liou et al., JBC, 2006 ¹
GCCase – wild type	4-MUG	N/A	1.9	Liou et al., JBC, 2006 ¹
Velaglucerase alpha	4-MUG	N/A	1.6 ± 0.2	Xu et al, PLOsone, 2010 ²
Imiglucerase	4-MUG	N/A	1.7 ± 0.3	Xu et al, PLOsone, 2010 ²

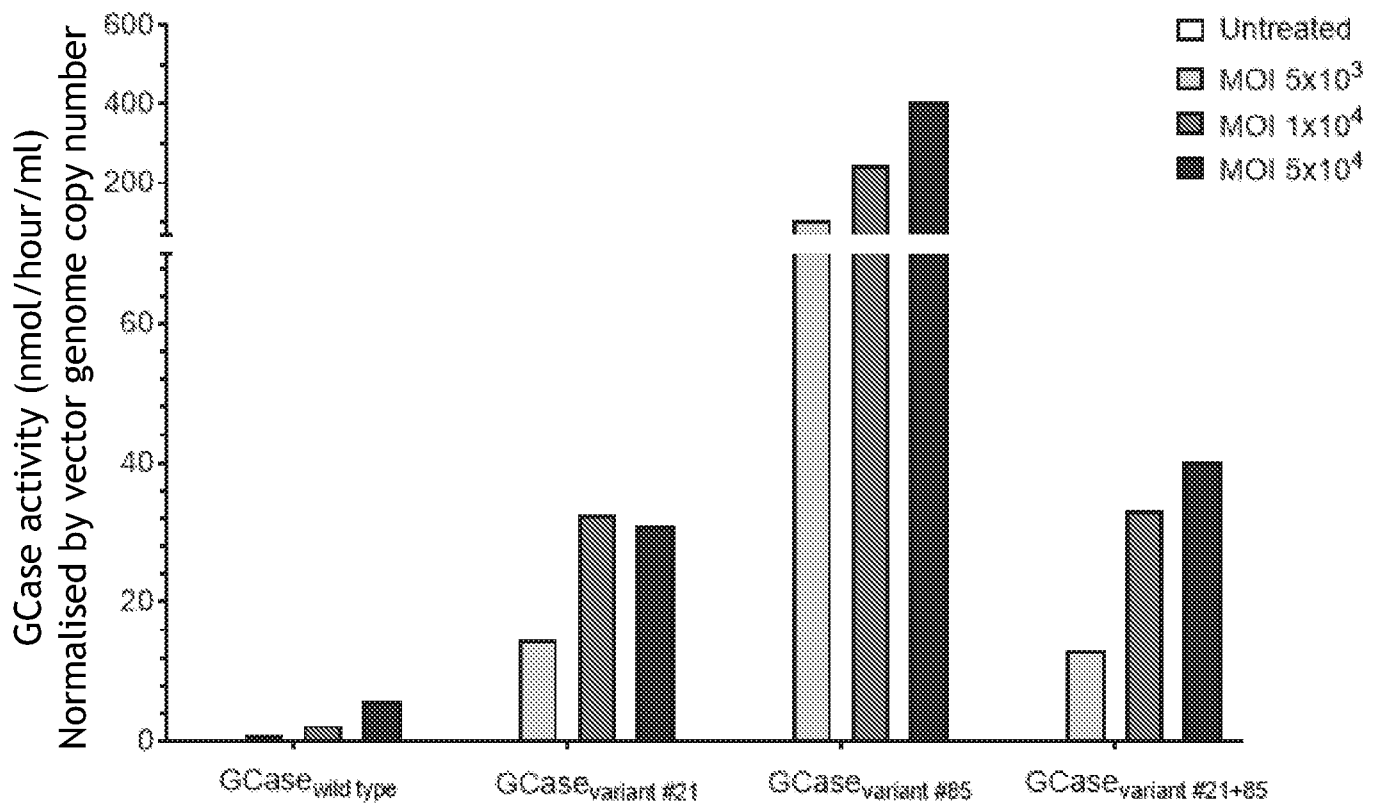
¹ Analyses of variant acid beta-glucosidases: effects of Gaucher disease mutations. Liou B1, Kazimierczuk A, Zhang M, Scott CR, Hegde RS, Grabowski GA. J Biol Chem. 2006 Feb 17;281(7):4242-53.

² Comparative Therapeutic Effects of Velaglucerase Alfa and Imiglucerase in a Gaucher Disease Mouse Model. You-Hai Xu, Ying Sun, Sonya Barnes, Gregory A. Grabowsk. PLOS One 2010. <https://doi.org/10.1371/journal.pone.0010750>

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Figure 6

MHC Class	Peptide binding category	GCaseWT	Gcase variant #85
HLA I	Strong	19	16
	Intermediate	36	38
HLA II	Strong	36	32
	Intermediate	100	99

Figure 7

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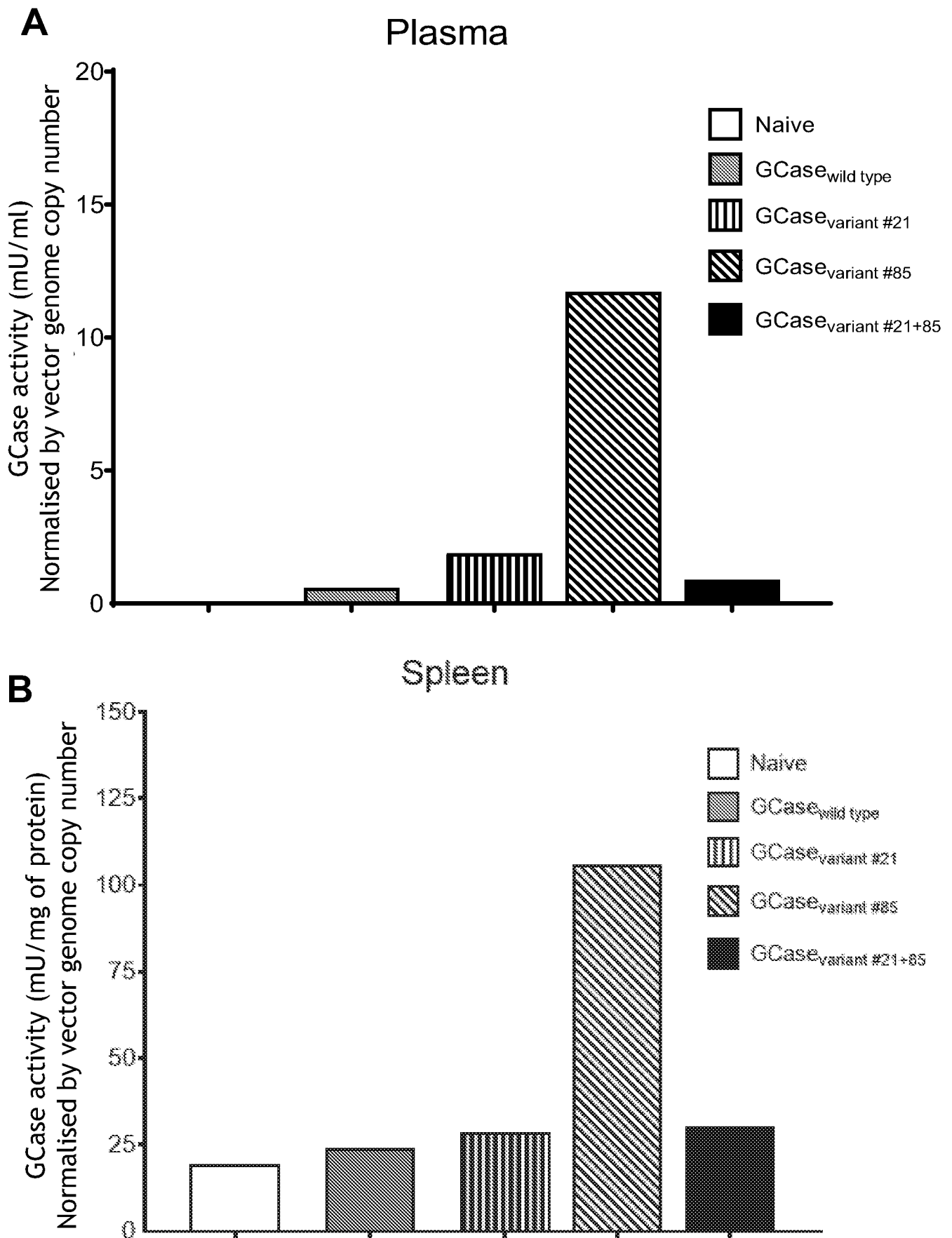
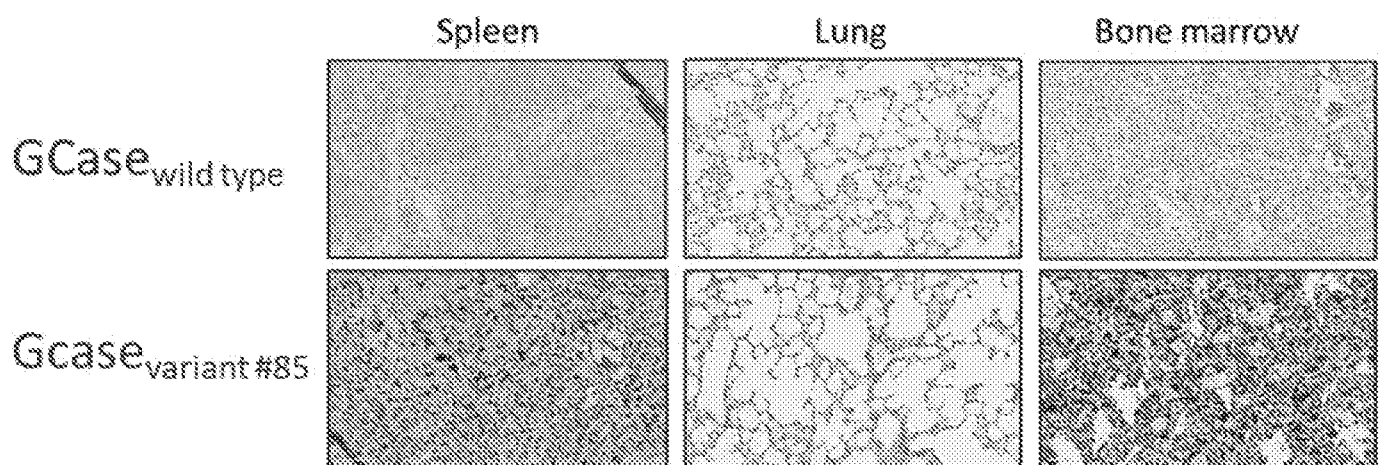
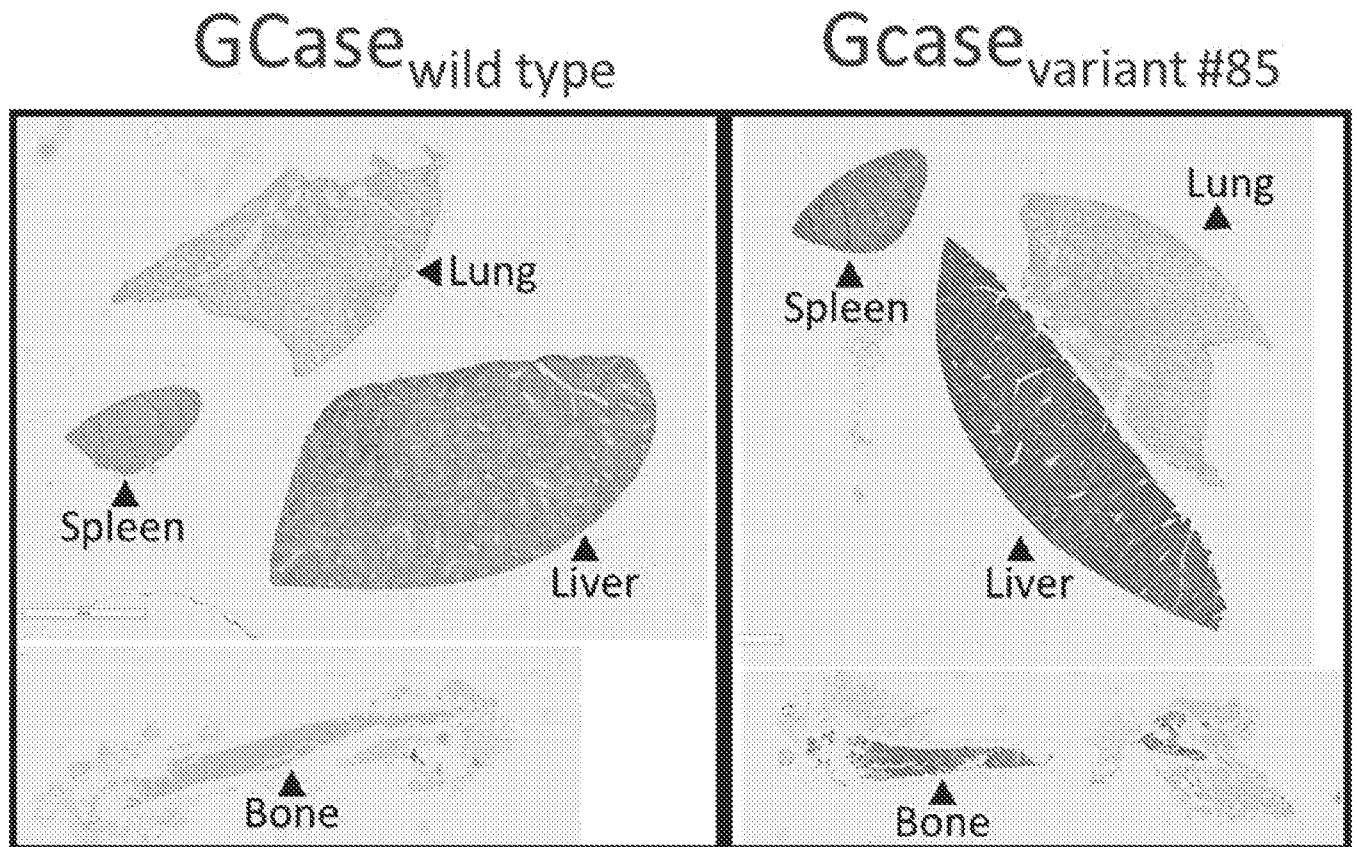
Figure 8

Figure 8 (cont)**C**

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Figure 9

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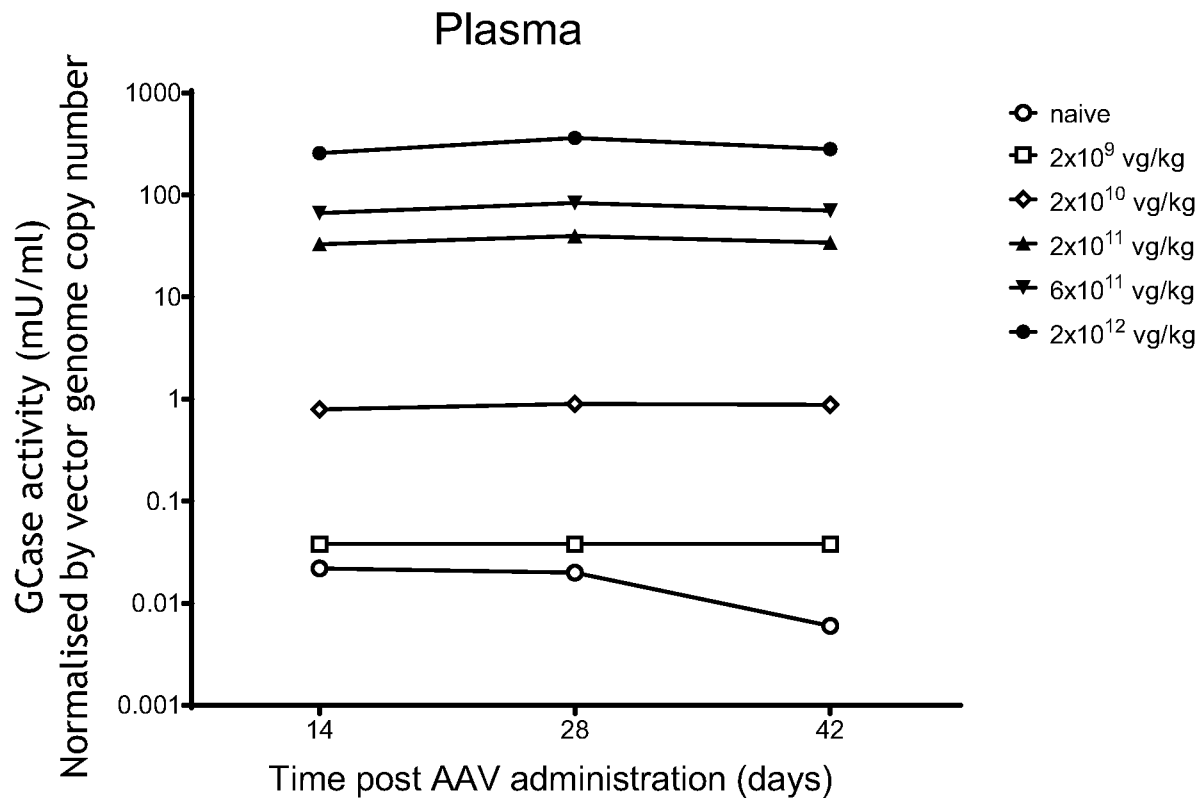
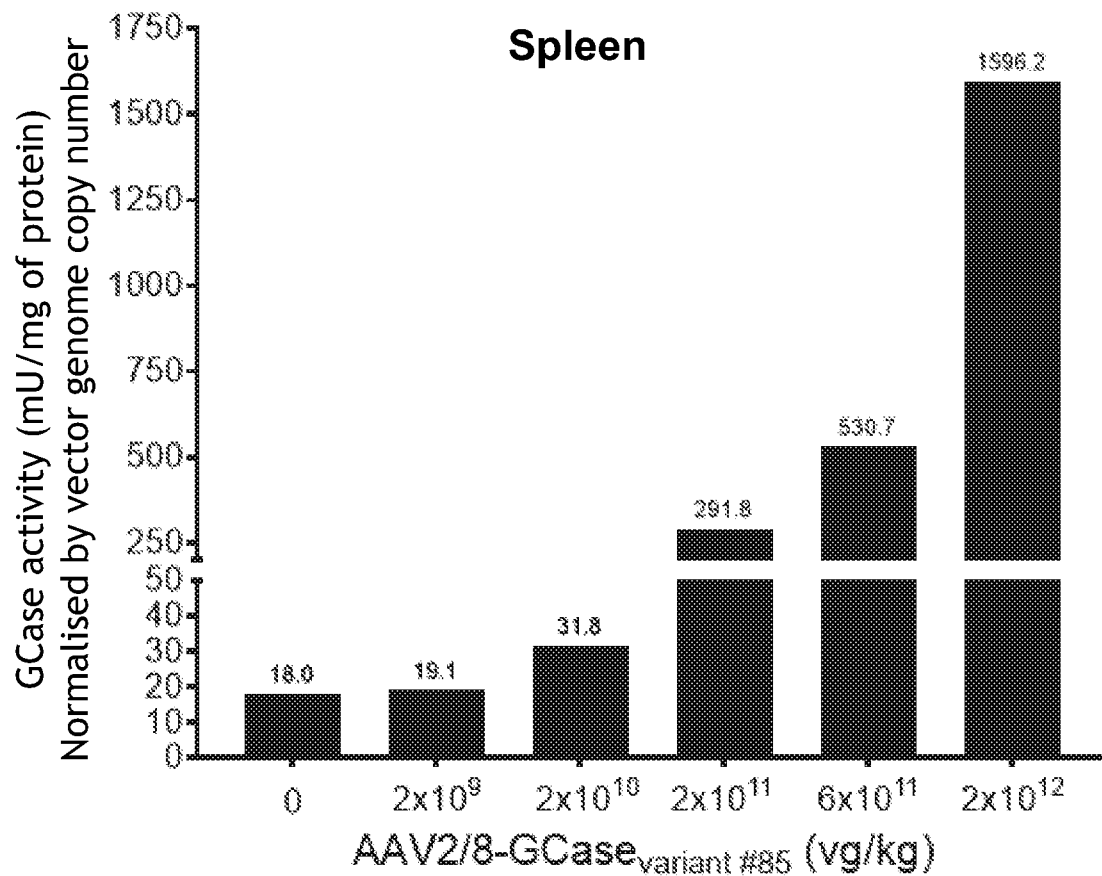
Figure 10**A****B**

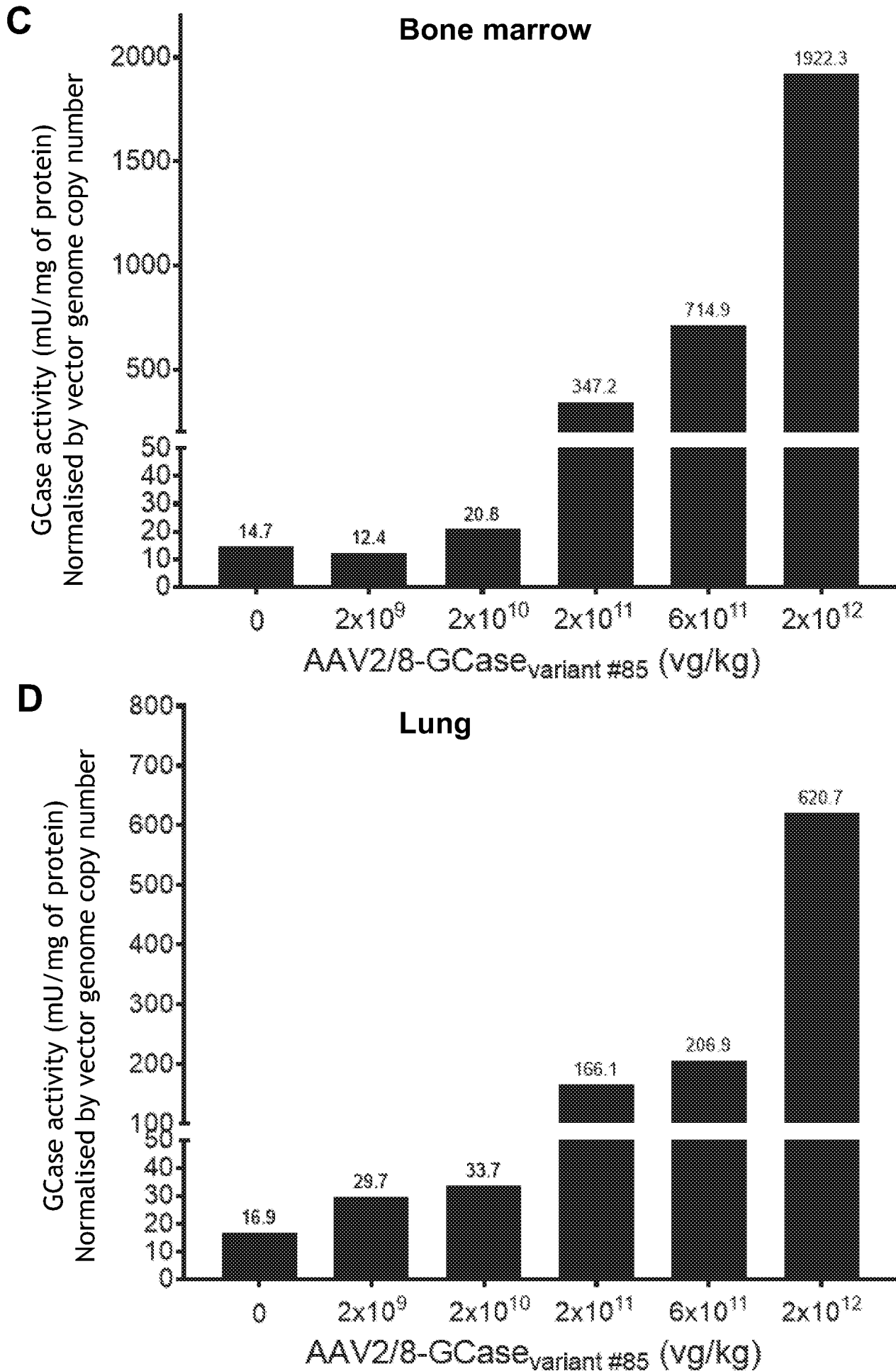
Figure 10 (cont)

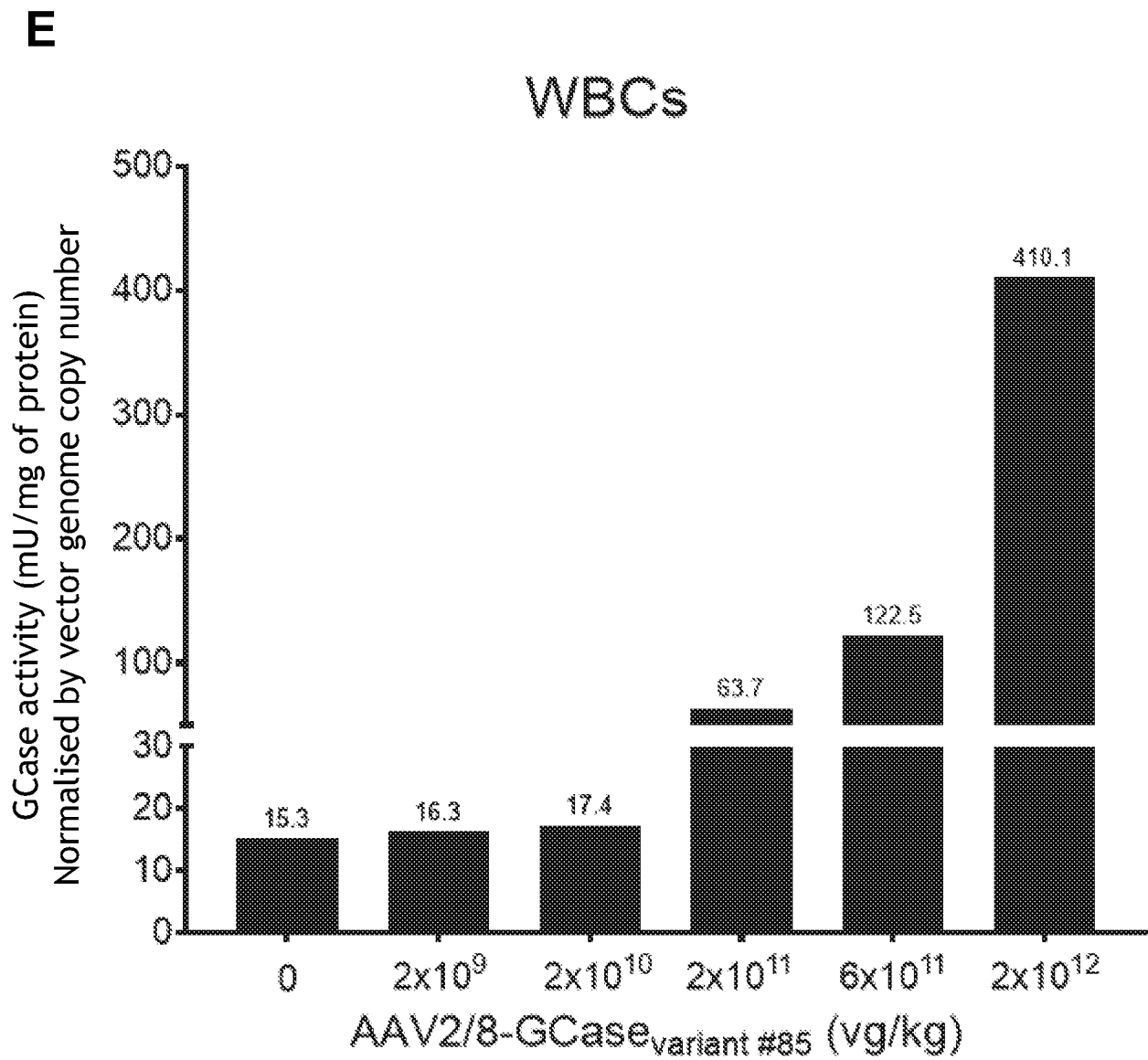
Figure 10 (cont)

Figure 11

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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SEQ ID NO: 28

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Figure 11 (cont)

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Figure 11 (cont)

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SEQ ID NO: 35

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cattgcaagcagcaaacagcaaacacacagccctccctgcctgctgaccttggagctggggcagaggtcag
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tgtttgctcctccgataactggggtgaccttgggttaattaccagcagcctcccccggttggccctctggatc
cactgcttaataacggacgaggacagggccctgtctcctcagcttcaggcaccaccactgacctgggacagt
gaatgatccccctgatctgcggcc

SEQ ID NO: 36

MAADGYLPDWLEDNLSEGIREWWALKPGVPQPKANQQHQDNRRGLVLPGYKYLPGNG
LDKGEPVNEADAAALEHDKAYDQQLKAGDNPYLKYNHADADEFQERLQEDTSFGGNLGRA
VFQAKKRILEPLGLVEEAAKTAPGKKRPVDQSPQEPDSSSGVGKSGKQPARKRLNFGQTGD
SESVPDPQPLGEPPAAPTSLGSNTMASGGGAPMADNNEGADGVGNSSGNWHCDSQWL
DRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSRWD
QRLINNNWGFPRPKLSFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSA
HQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYTFED
VPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQGTTSGTTNQSRLIFSQAGPQSMSLQARN
WLPGPCYRQQRLSKTANDNNNSNFPWTAASKYHLNGRDSLVPNGPAMASHKDDEEKFFP
MHGNLIFGKEGTTASNAELDNVMITDEEEIRTTNPVATEQYGTVANNLQSSNTAPTTRTVN
DQGALPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPMLGGFGLKHPPPQIMIKNTPVPA
NPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYSVNVDFTVDTN
GVYSEPRPIGTRYLTRNL

SEQ ID NO: 37

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGN
LDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADADEFQERLQEDTSFGGNLGRA
VFQAKKRVLEPLGLVEEGA KTAPGKKRPVDQSPQEPDSSSGVGKSGKQPARKRLNFGQTG
DSESVPDPQPLGEPPAAPTSLGSNTMASGGGAPMADNNEGADGVGNSSGNWHCDSQWL
GDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSRWD
WQRLINNNWGFPRPKLSFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSA
AHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYTFE
DVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQGTTSGTTNQSRLIFSQAGPQSMSLQARN
WLPGPCYRQQRLSKTANDNNNSNFPWTAASKYHLNGRDSLVPNGPAMASHKDDEEKFFP
MHGNLIFGKEGTTASNAELDNVMITDEEEIRTTNPVATEQYGTVANNLQSSNTAPTTRTVN
DQGALPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPMLGGFGLKHPPPQIMIKNTPVPA

Figure 11 (cont)

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NPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTN
GVYSEPRPIGTRYLTRNL

SEQ ID NO: 38

MAADGYLPDWLEDNLSEGIREWWALQPGAPKPKANQQHQDNARGLVLPGYKYLGPNG
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VFQAKKRILLEPLGLVEEAAKTAPGKKRPVDQSPQEPDSSSGVGKSGKQPARKRLNFGQTG
DSESVDPDPQLGEPPAAPTSLGSNTMASGGGAPMADNNEGADGVGNSSGNWHCDSQWL
GDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYDFNRFHCHFSPRD
WQRLINNNWGFPRPKLSFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGS
AHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYTFE
DVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQGTTSGTTNQSRLFSQAGPQSMSLQARN
WLPGPCYRQQRLSKTANDNNNSNFPWTAASKYHLNGRDSLVPNGPAMASHKDDEEKFFP
MHGNLIFGKEGTTASNAELDNVMITDEEEIRTTPVATEQYGTVANNLQSSNTAPTTRTVN
DQGALPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQIMIKNTPVPA
NPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTN
GVYSEPRPIGTRYLTRPL

SEQ ID NO: 39

MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPNG
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VFQAKKRILLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQAQAKRRLNFGQTGD
TESVPDPQPIGEPPAAPSGVGSALTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGD
RVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYDFNRFHCHFSPRD
WQRLINNNWGFPRKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLG
SAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFE
NVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIP
GPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSG
SLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGVATNHQSAQAQAQTGWVQN
QGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADP
PTAFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEG
VYSEPRPIGTRYLTRNL

SEQ ID NO: 40

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ctacagctcgggtggtgtgtgtctgcaatgccacatactgtgactcctttgacccccgaccttcctgcccttg
gtaccttcagccgctatgagagtacacgcagtgggcgacggatggagctgagtatggggcccatccaggcta
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ggggccatgacagatgctgctgctctcaacatccttgccctgtcaccctgccccaaatttgctacttaa
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Figure 11 (cont)

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cacctacacctatgcagacacccctgatgatttccagttgcacaacttcagcctcccagaggaagataccaa
gctcaagatacccttgattcaccgagccctgcagttggcccagcgtcccgtttcactccttgccagcccctgg
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catctaccaccagacctgggcccagatactttgtgaagttcctggatgcctatgctgagcacaagttacagttc
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tccgcctactcatgctggatgaccaacgcttgctgctgccccactgggcaaaggtggtactgacagaccag
aagcagctaaatatgttcatggcattgctgtacattggtacctggactttctggctccagccaaagccaccct
aggggagacacaccgcctgttccccaacaccatgctctttgcctcagaggcctgtgtgggctccaagttctgg
gagcagagtgtgaggctaggctcctgggatcgaggatgcagtacagccacagcatcatcacgaacctcctg
taccatgtggtcggctggaccgactggaaccttgccctgaaccccgaaggaggaccaattgggtgcgtaac
ttgtcgacagtcccatcattgtagacatcaccaaggacacgttttacaacagcccatgttctaccaccttg
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acgcagtggcactgatgcatcccgatggctctgctgttggtcgtgctaaaccgctcctctaaggatgtgcct
cttaccatcaaggatcctgctgtgggcttctggagacaatctcacctggctactccattcacacctacctgt
ggcgtcgccagtga

SEQ ID NO: 41

MEFSSPSREECPKPLSRVSIMAGSLTGLLLLQAVSWASGARPCIPKSFGYSSVVCVCNATYCD
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MTDAAALNILALSPPAQNLLLKSYFSEEGIGYNIIRVPMASCDIFSIRTYTYADTPDDFQLHNFS
LPEEDTKLKIPLIHRALQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT
WARYFVKFLDAYAEHKLQFWAVTAQNEPSAGLLSGYPFQCLGFTPEHQ RDFIARDLGPTLA
NSTHHNVRLMLDDQRLLLPHWAKVVLTDPEAAKYVHGIAVHWYLD FLAPAKATLGETHR
LFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYSHSIITNLLYHVVGWTDWNLALNPE
GGPNWVRNFVDSPPIVDITKDTFYKQPMFYHLGHFSKFIPEG SQRVGLVASQKNDLDAVAL
MHPDGS AVVVVLNRSSKDVPLTIKDPAVGFLETISPGYSIHTYLWRRQ

SEQ ID NO: 42

MEFSSPSREECPKPLSRVSIMAGSLTGLLLLQAVSWASGARPCIPKSFGYSSVVCVCNATYCD
SFDPPTFPALGTFSRYESTRSGRRMELSMGPIQANHTGTGLLLTLQPEQKFQKVKGF GGA
MTDAAALNILALSPPAQNLLLKSYFSEEGIGYNIIRVPMASCDIFSIRTYTYADTPDDFQLHNFS
LPEEDTKLKIPLIHRALQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT
WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQ RDFIARDLGPTLA
NSTHHNVRLMLDDQRLLLPHWAKVVLTDPEAAKYVHGIAVHCYLD FLAPAKATLGETHRL
FPNTMLFASECCVGSKFWEQSVRLGSWDRGMQYSHSIITNLLYHVVGWTDWNLALNPEG
GPNWVRNFVDSPPIVDITKDTFYKQPMFYHLGHFSKFIPEG SQRVGLVASQKNDLDAVALMH
PDGS AVVVVLNRSSKDVPLTIKDPAVGFLETISPGYSIHTYLWRRQ

SEQ ID NO: 43

MEFSSPSREECPKPLSRVSIMAGSLTGLLLLQAVSWASGARPCIPKSFGYSSVVCVCNATYCD
SFDPPTFPALGTFSRYESTRSGRRMELSMGPIQANHTGTGLLLTLQPEQKFQKVKGF GGA

Figure 11 (cont)

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MTDAAALNILALSPPAQNLLLKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFS
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WARYFVKFLDAYAEHKLQFWAVTAQNEPSAGLLSGYPFQCLGFTPEHQRFIARDLGPTLA
NSTHHNVRLMLDDQRLLLPHWAKVVLTDPEAAKYVHGIAPHVHCYLDLAPAKATLGETHRL
FPNTMLFASECCVGSKFWEQSVRLGSWDRGMQYSHSIITNLLYHVVGWTDWNLALNPEG
GPNWVRNFVDSPPIVDITKDTFYKQPMFYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMH
PDGSVVVVLNRSSKDVPLTIKDPVGVLETISPGYSIHTYLWRRQ

SEQ ID NO: 44

ARPCIPKSFGYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELSMGPIQANHTG
TGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLLLKSYFSEEGIGYNIIRVPM
SCDFSIRTYTYADTPDDFQLHNFSLPEEDTKLKIPLIHRALQLAQRPVSLASPWTSPWLK
TNGAVNGKGSLLKGQPGDIYHQTWARYFVKFLDAYAEHKLQFWAVTAQNEPSAGLLSGYPF
QCLGFTPEHQRFIARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDPEAAKYVH
GIAPHVWYLDLAPAKATLGETHRLFPNTMLFASECCVGSKFWEQSVRLGSWDRGMQYSHS
IITNLLYHVVGWTDWNLALNPEGGPNWVRNFVDSPPIVDITKDTFYKQPMFYHLGHFSKFI
PEGSQRVGLVASQKNDLDAVALMHPDGSVVVVLNRSSKDVPLTIKDPVGVLETISPGYSIH
TYLWRRQ

SEQ ID NO: 45

ARPCIPKSFGYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELSMGPIQANHTG
TGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLLLKSYFSEEGIGYNIIRVPM
SCDFSIRTYTYADTPDDFQLHNFSLPEEDTKLKIPLIHRALQLAQRPVSLASPWTSPWLK
TNGAVNGKGSLLKGQPGDIYHQTWARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPF
QCLGFTPEHQRFIARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDPEAAKYVH
GIAPHVHCYLDLAPAKATLGETHRLFPNTMLFASECCVGSKFWEQSVRLGSWDRGMQYSHS
ITNLLYHVVGWTDWNLALNPEGGPNWVRNFVDSPPIVDITKDTFYKQPMFYHLGHFSKFI
EGSQRVGLVASQKNDLDAVALMHPDGSVVVVLNRSSKDVPLTIKDPVGVLETISPGYSIHT
YLWRRQ

SEQ ID NO: 46

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SCDFSIRTYTYADTPDDFQLHNFSLPEEDTKLKIPLIHRALQLAQRPVSLASPWTSPWLK
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QCLGFTPEHQRFIARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDPEAAKYVH
GIAPHVHCYLDLAPAKATLGETHRLFPNTMLFASECCVGSKFWEQSVRLGSWDRGMQYSHS
ITNLLYHVVGWTDWNLALNPEGGPNWVRNFVDSPPIVDITKDTFYKQPMFYHLGHFSKFI
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YLWRRQ

Figure 11 (cont)

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SEQ ID NO: 47

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gcaccttcagcagatatgagagcaccaggtctgggaggaggatggagctgagcatggggcccatccaggct
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tgatgctgtggctctgatgcacctgatggctctgctgtggtggtggtgctgaacaggagctctaaggatgtg
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ctgtggaggaggcagtga

SEQ ID NO: 48

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Figure 11 (cont)

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SEQ ID NO: 49

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ggaggaggcagtga

SEQ ID NO: 50

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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Figure 11 (cont)

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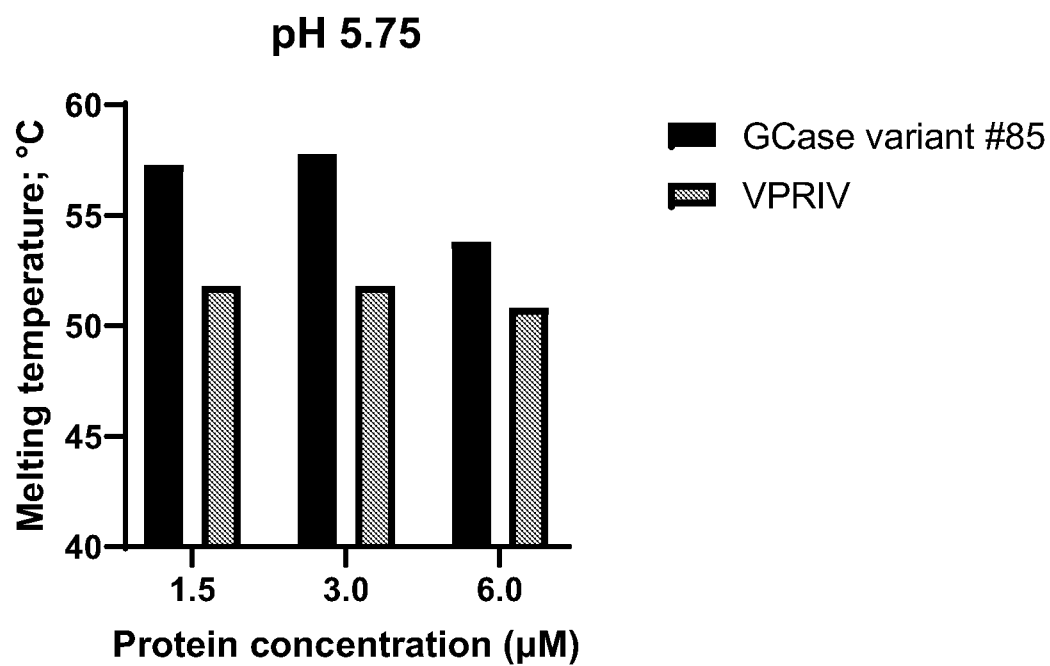
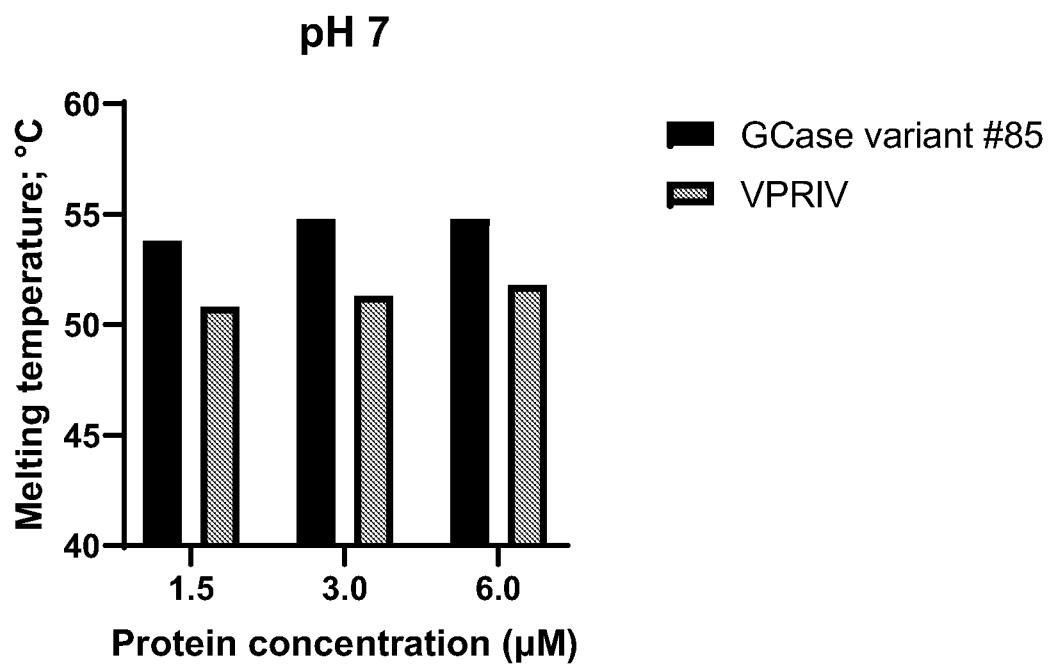
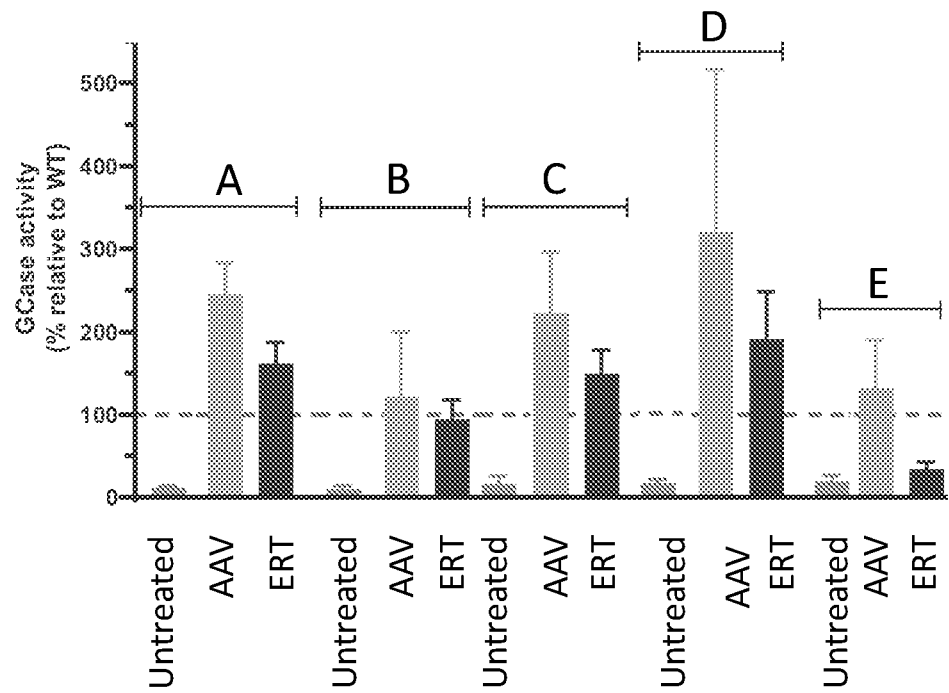
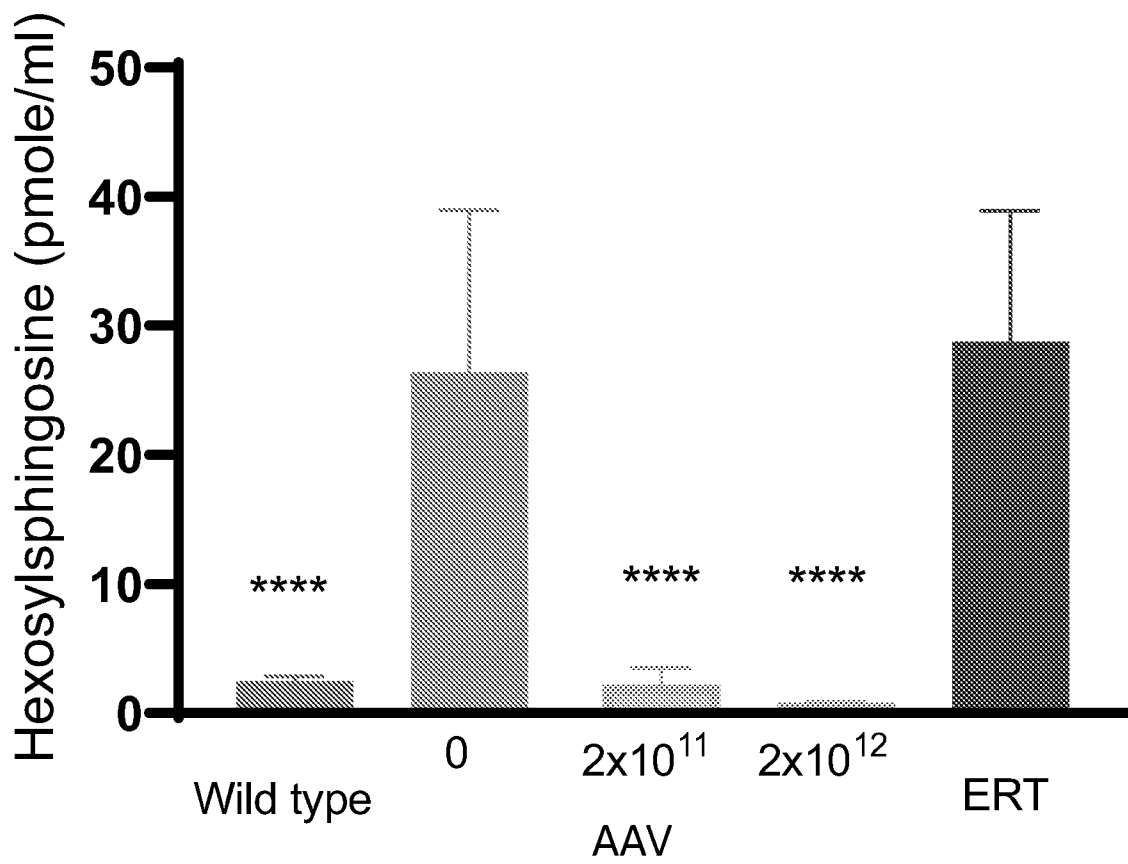
Figure 12**A****B**

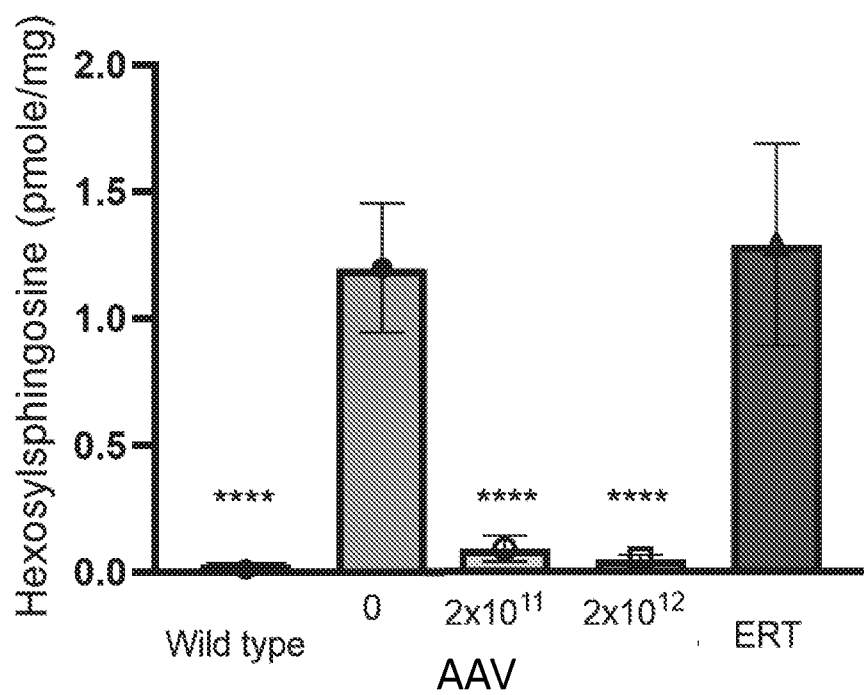
Figure 13



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Figure 14

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Figure 15A

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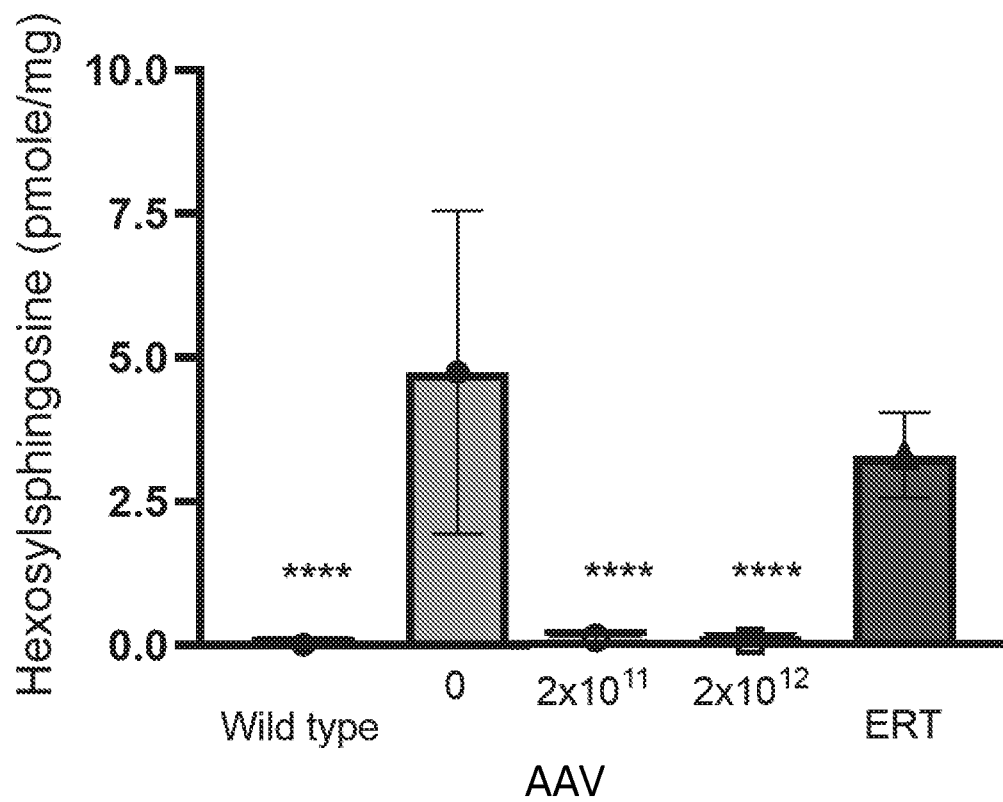
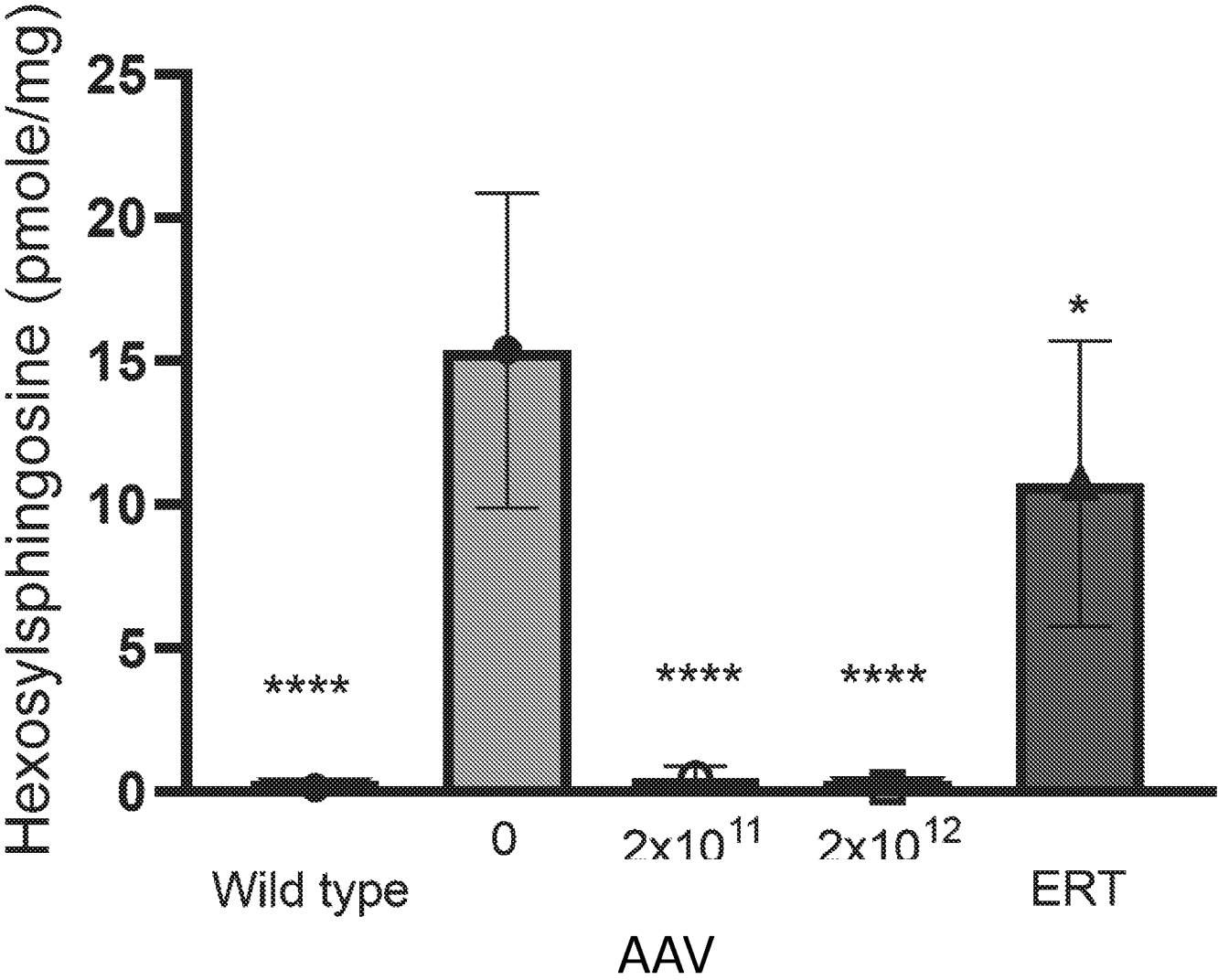
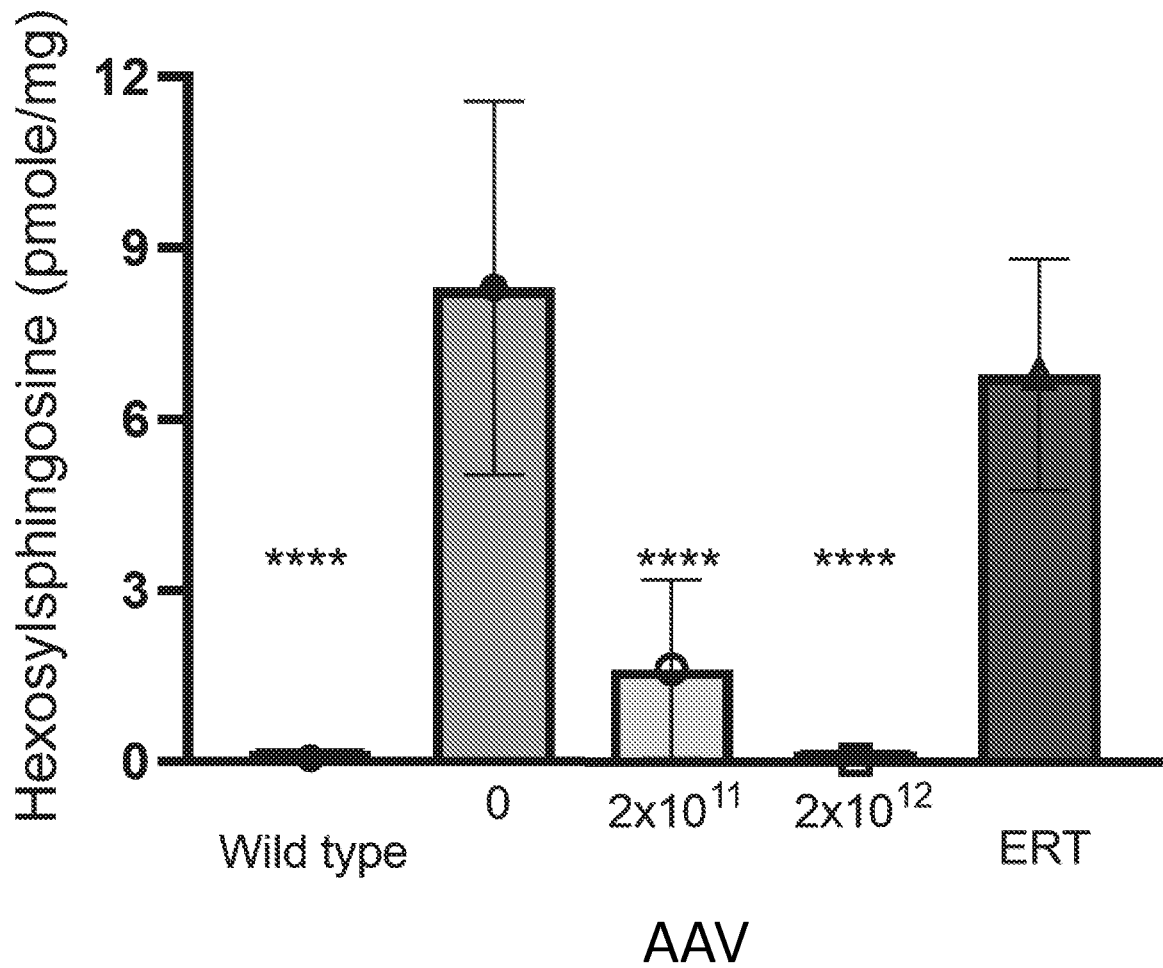
Figure 15B

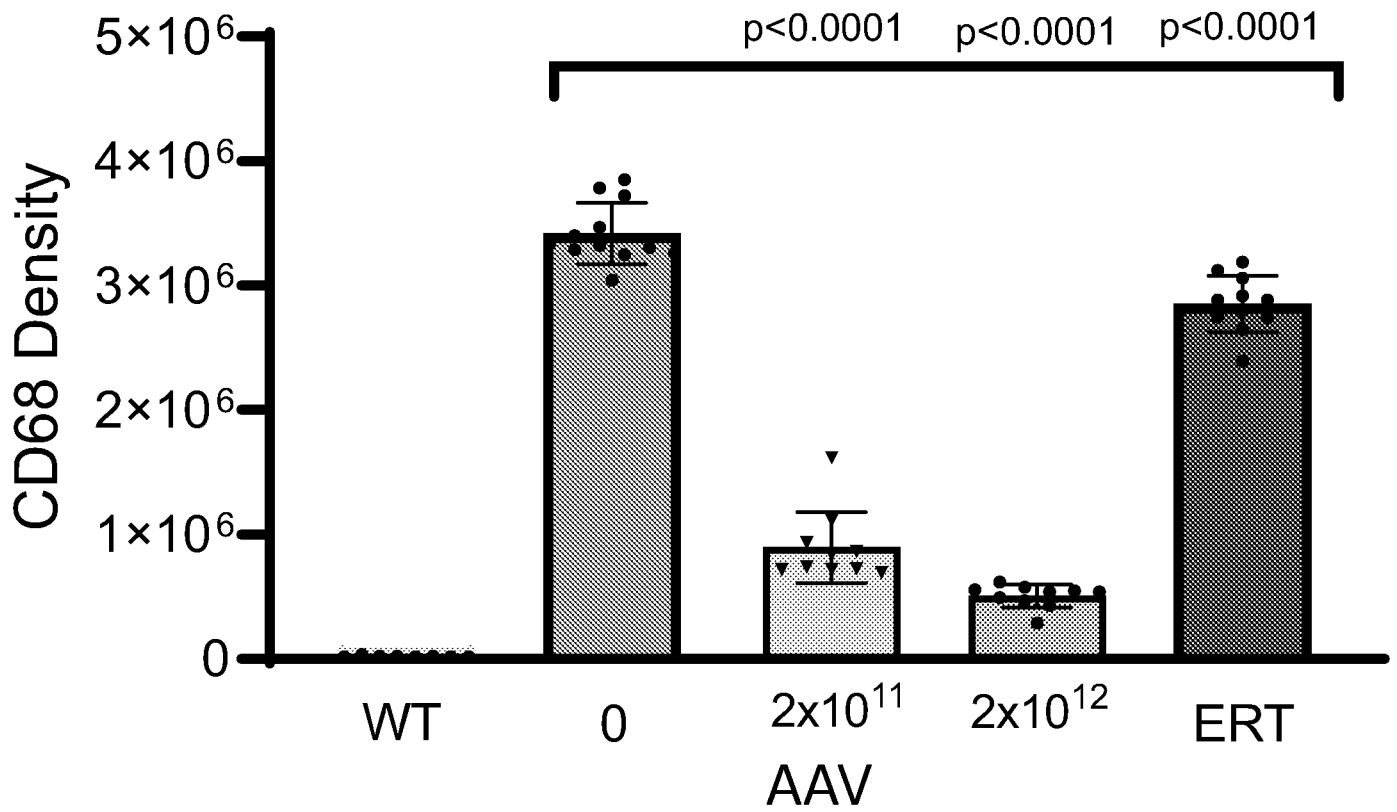
Figure 15C



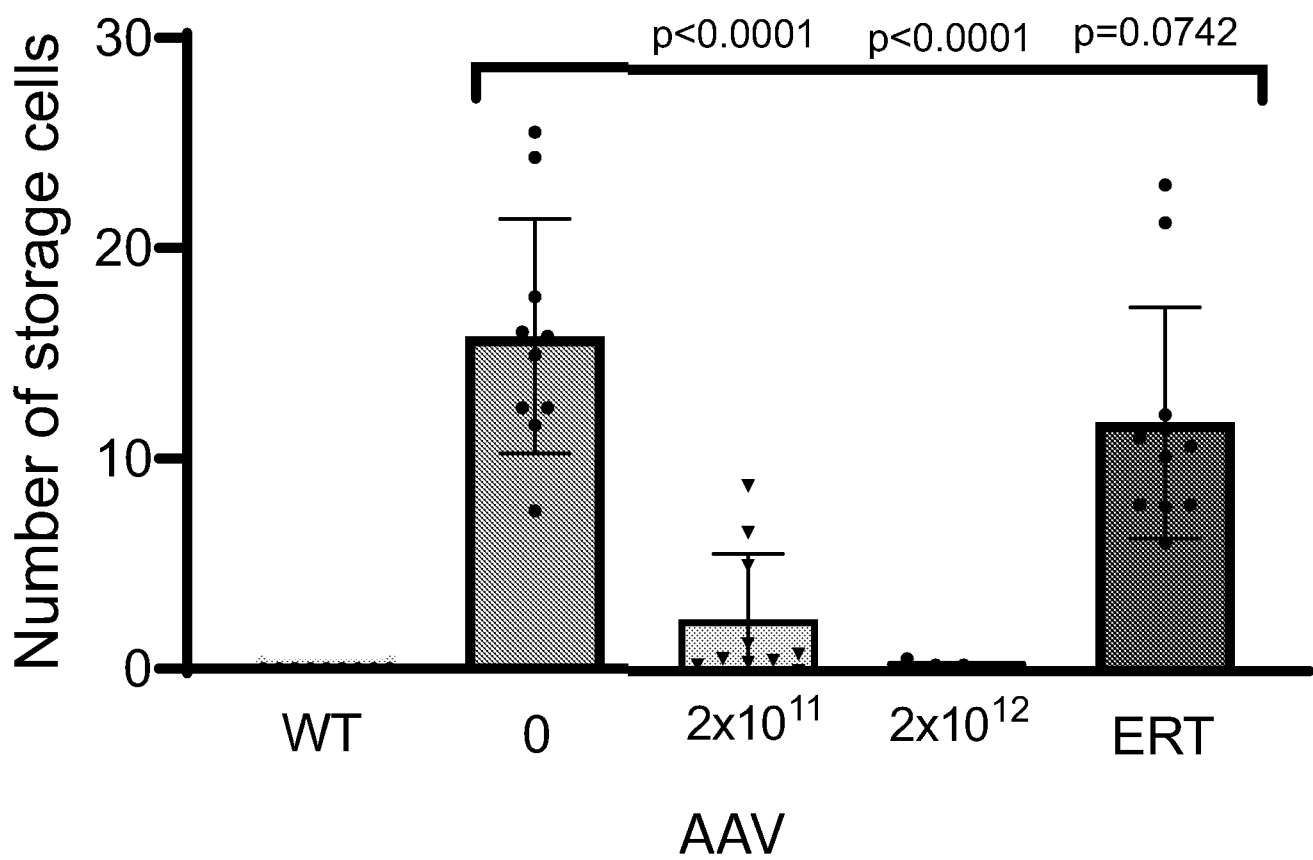
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Figure 15D

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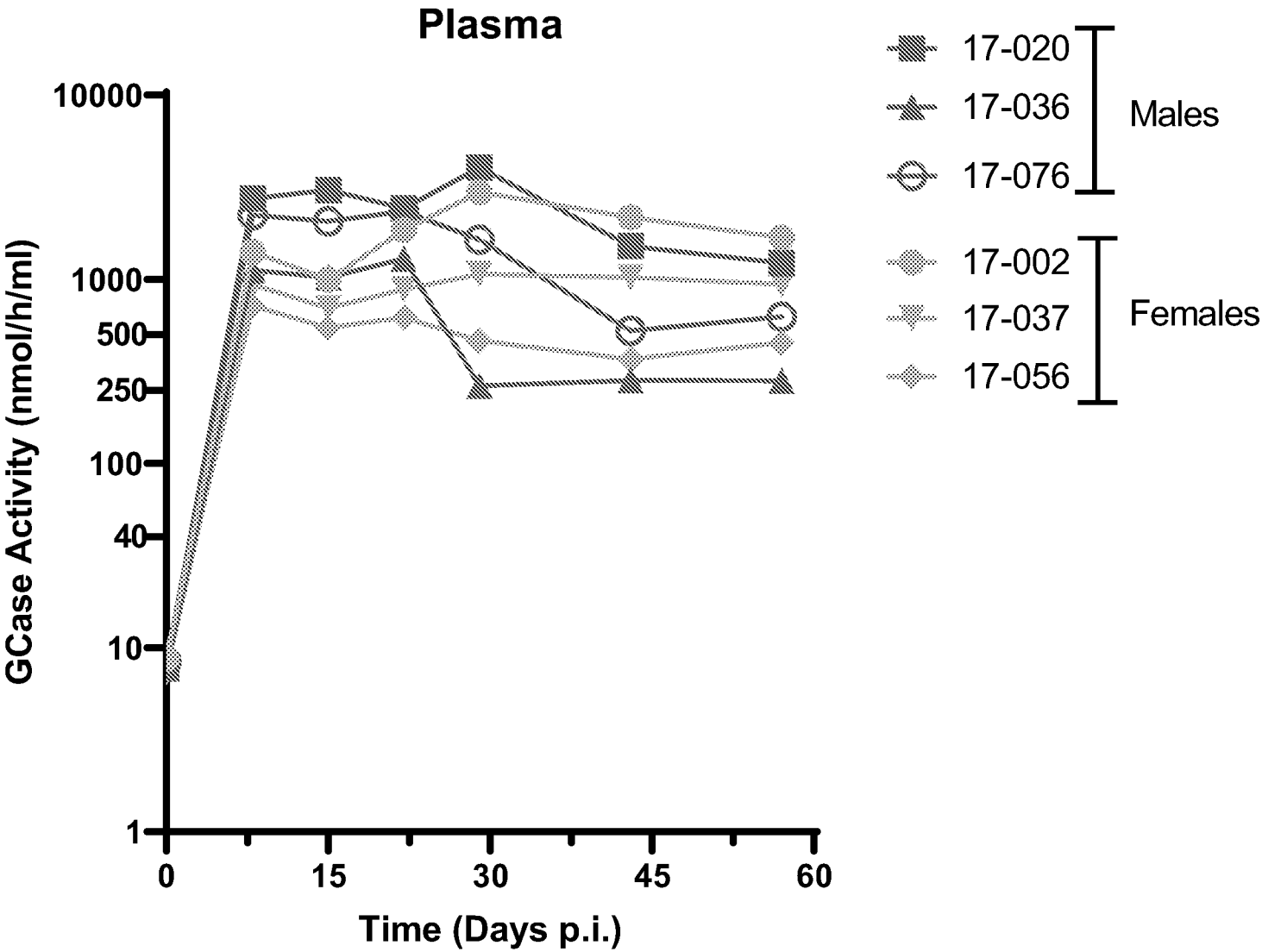
Figure 16A

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Figure 16B

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Figure 17A



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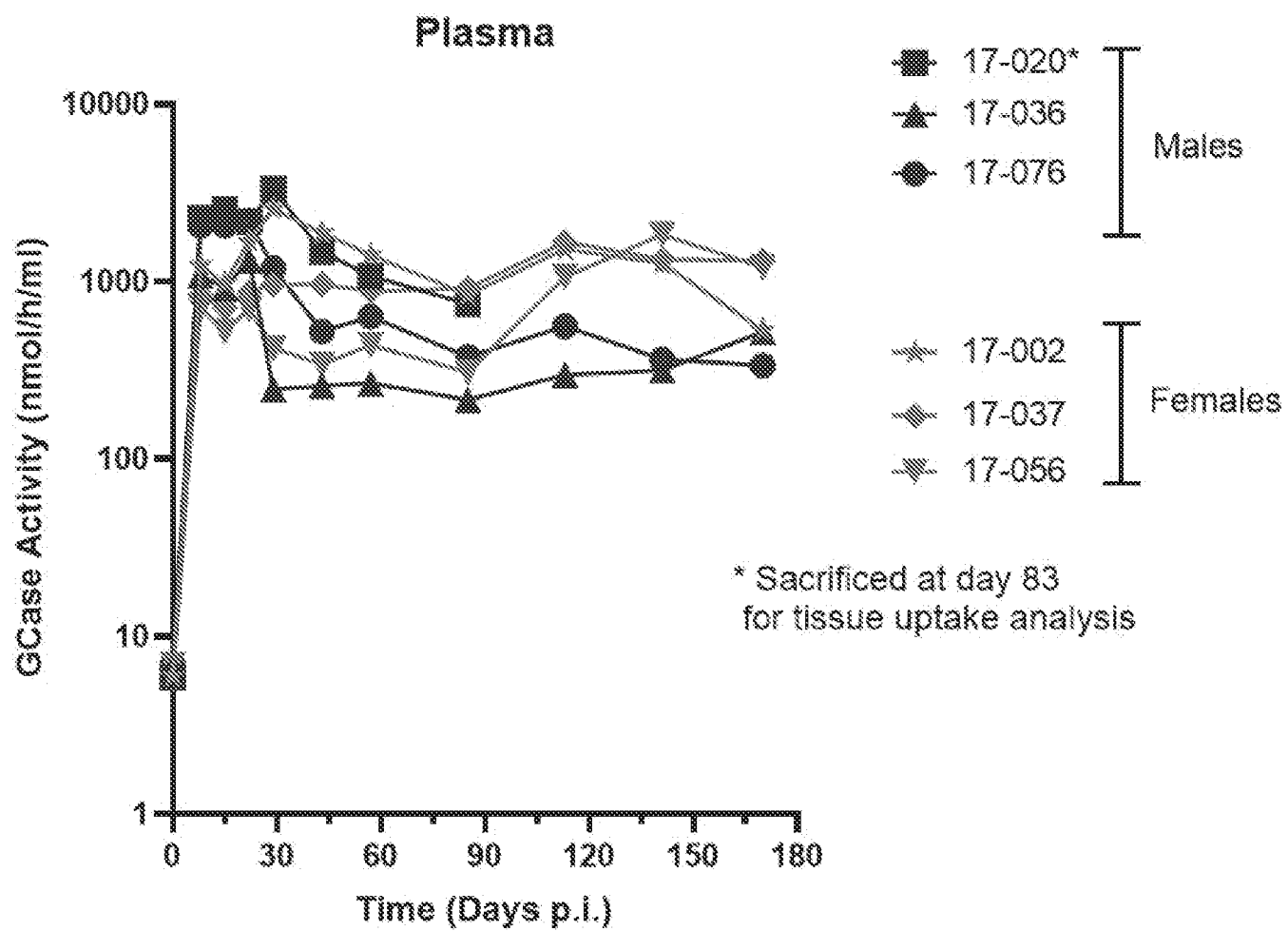
Figure 17B

Figure 18

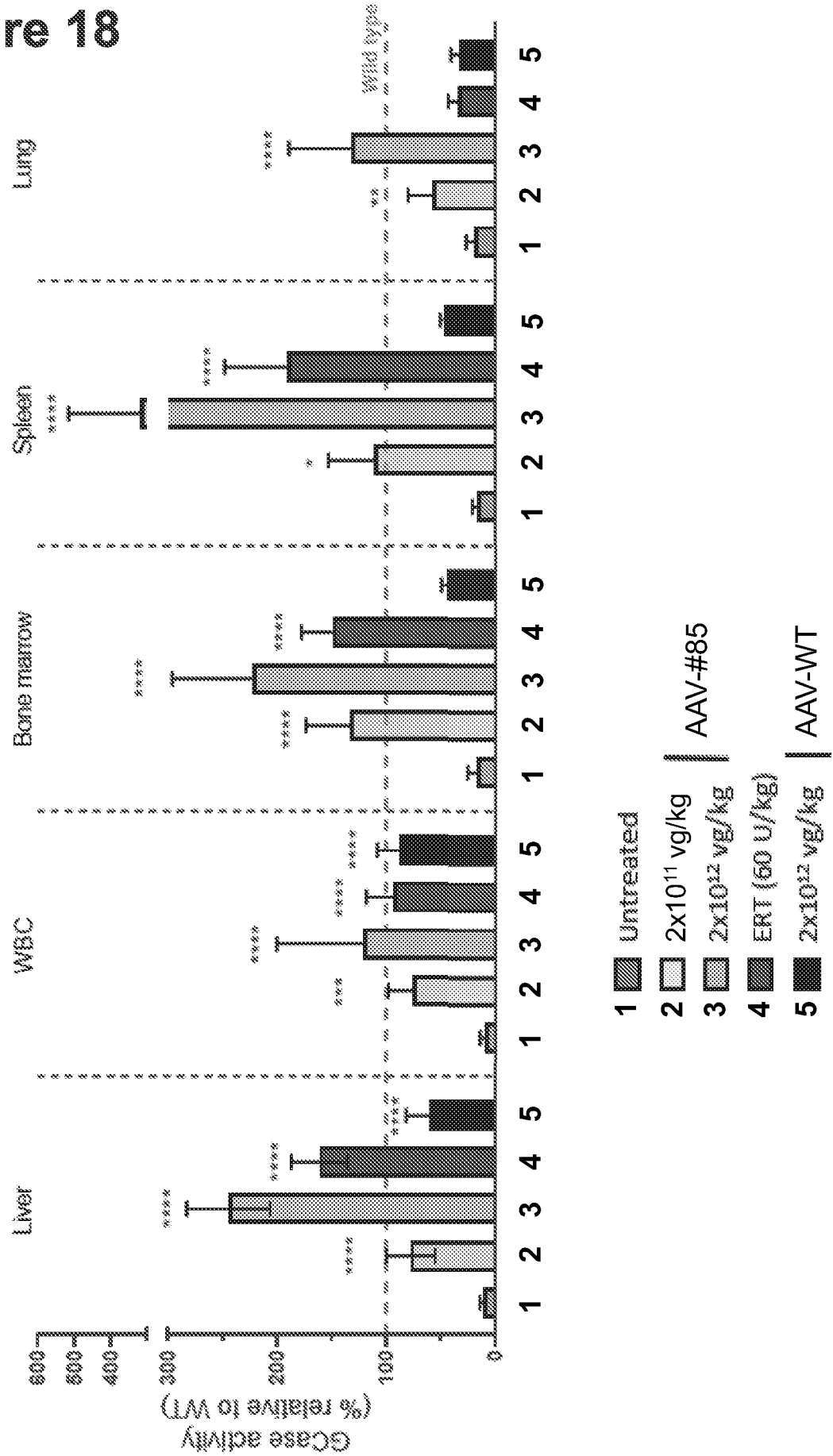
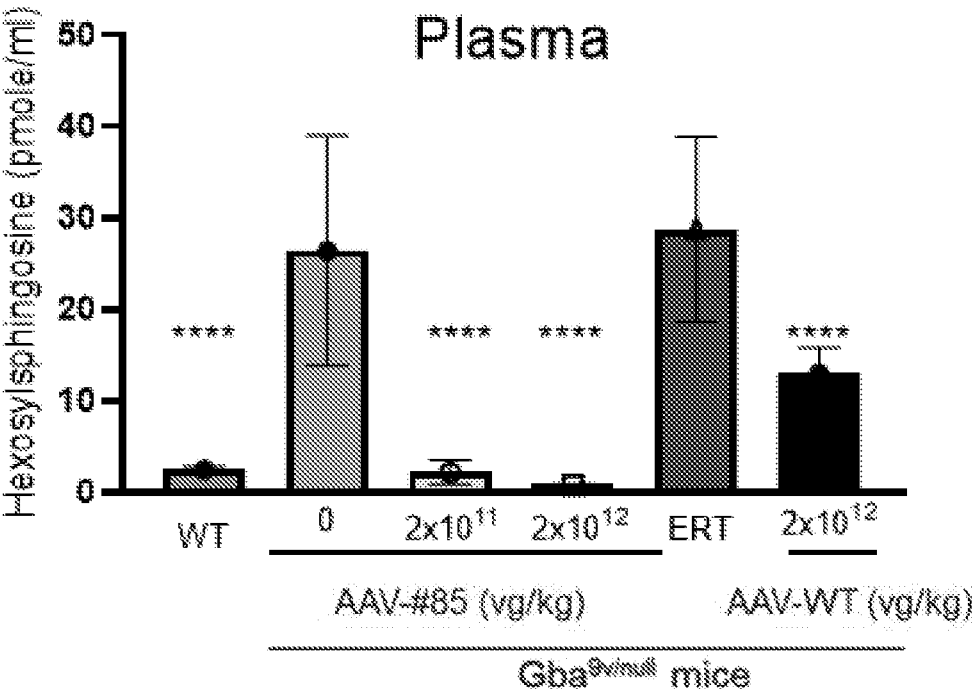


Figure 19

A



B

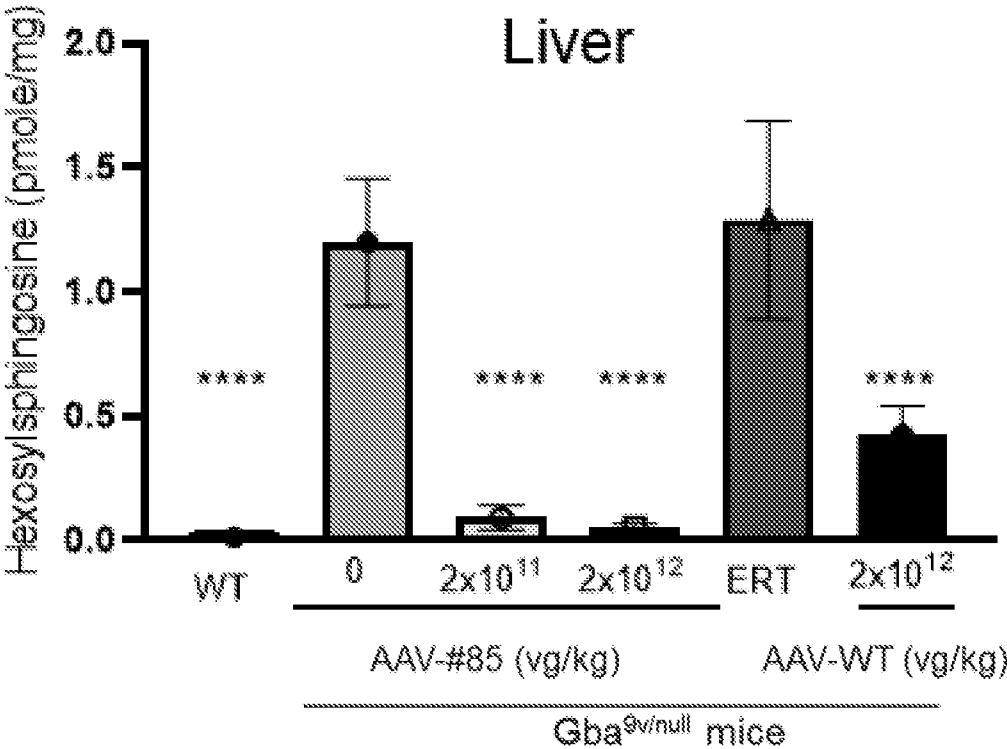
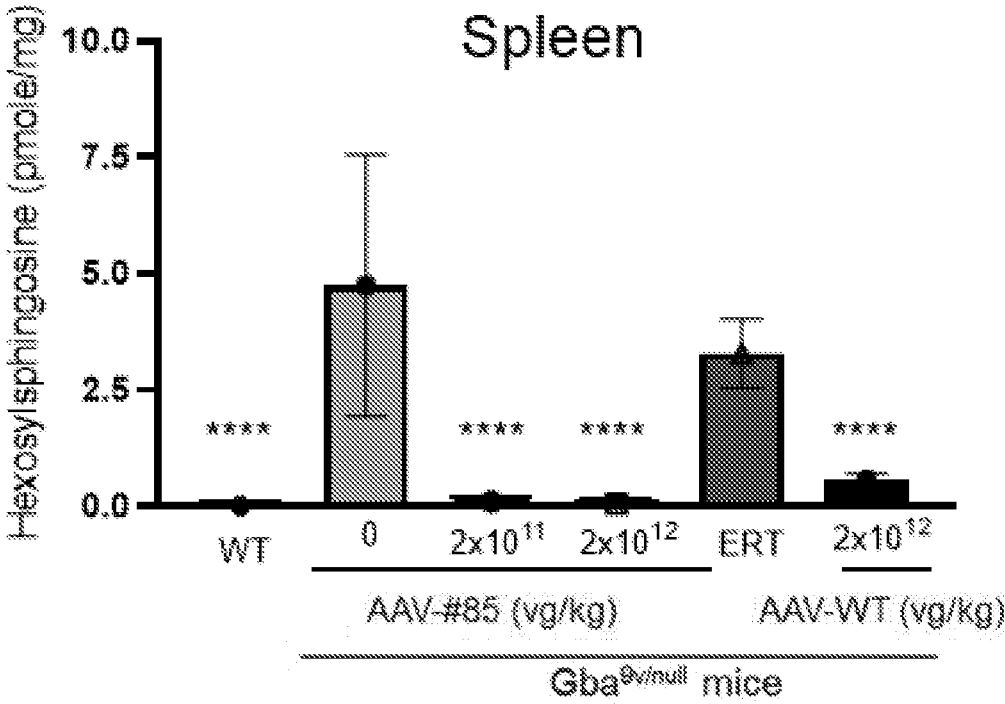


Figure 19 (cont)

C



D

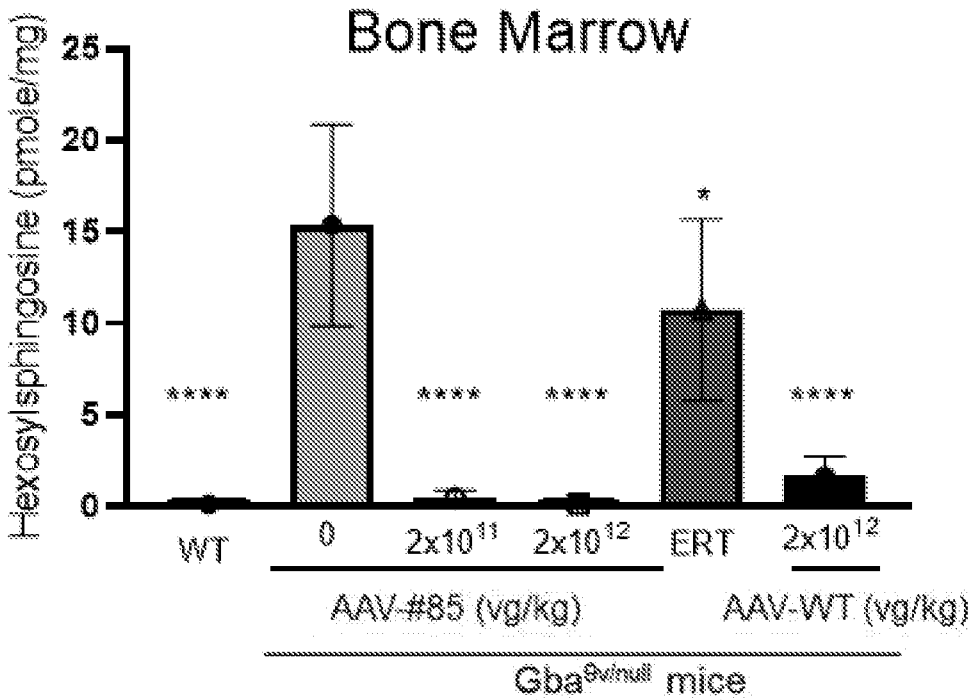


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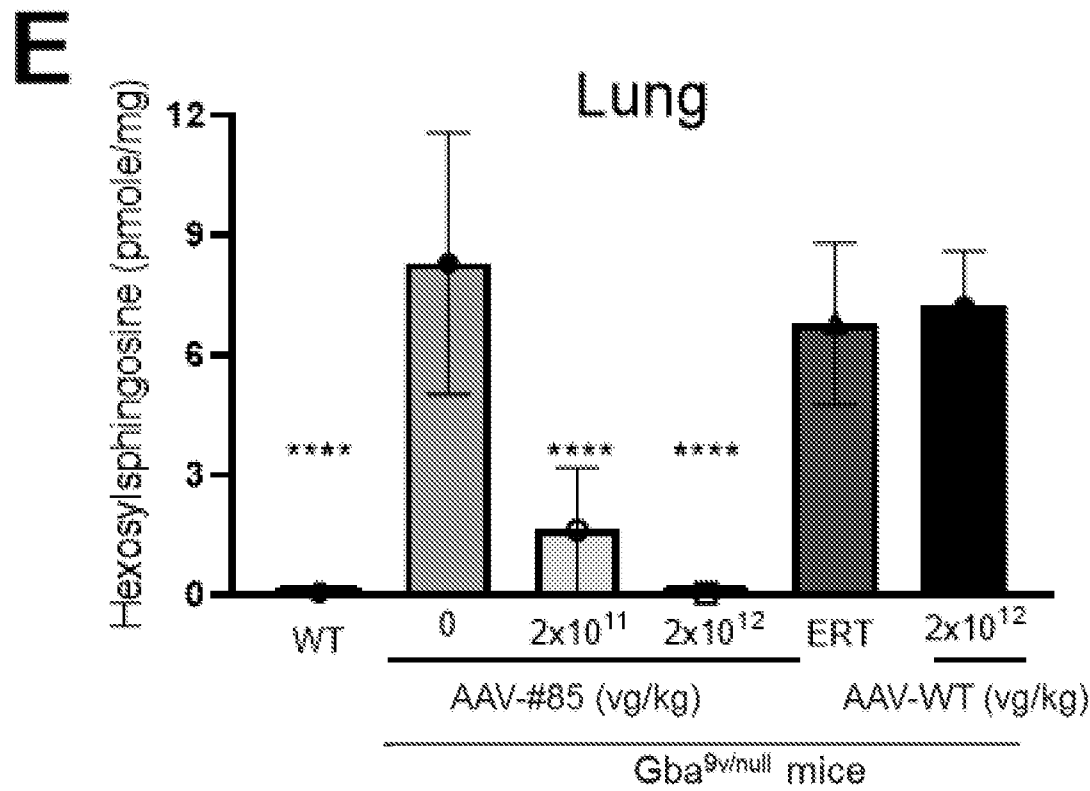


Figure 20

