

Supplemental information belonging to the manuscript:

β -Hexosaminidases along the secretory pathway of *Nicotiana benthamiana* have distinct specificities towards engineered helminth N-glycans on recombinant glycoproteins

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Table S1. Primer overview

This table gives an overview of all the primers used in this study to perform 3' RACE for HEXO2 orthologs and amplification of full-length HEXO open reading frames. Restriction sites used for subcloning are underlined. Capital letters refer to gene specific sequences.

| Primer name | Sequence (5' – 3') | Characteristics (underlined) |
|----------------|-------------------------------------------------|-------------------------------------|
| HEXO1A-fw | cct <u>catgagc</u> ATGTCCTCAAATCCCAATGTC | BspHI site for subcloning into pHYG |
| HEXO1A-rv | <u>gggtaca</u> TTATTGTTTCATAGCATGATCCTGG | BsrGI site for subcloning into pHYG |
| HEXO2A-fw | cct <u>catgagc</u> ATGGCTTACTTCATTTCAATTTCTCTCC | BspHI site for subcloning into pHYG |
| HEXO2A-rv | <u>gggtacc</u> TCAGCTGGTAAATGGATGAACTGTG | KpnI site for subcloning into pHYG |
| HEXO2B-fw | cct <u>catgagc</u> ATGGCTTACTTCAACTTCAAAG | BspHI site for subcloning into pHYG |
| HEXO2B-rv | <u>gggtacc</u> TCAGCTGGTAAATGGATGAAC | KpnI site for subcloning into pHYG |
| HEXO3A-fw | cct <u>catgagc</u> ATGGGGAAGTTAGGATTCCG | BspHI site for subcloning into pHYG |
| HEXO3B-fw | <u>ccccATGGG</u> GGAAGTTAGGAGTGCA | NcoI site for subcloning into pHYG |
| HEXO3-rv | <u>gggtacc</u> TTATTGCTGATAGCAAGAACCTG | KpnI site for subcloning into pHYG |
| Oligo(dT)-AP | <u>ggccacgcgtcgactagtac</u> TTTTTTTTTTTTTTTTTT | Adaptor sequence for 3' RACE |
| Adaptor primer | ggccacgcgtcgactagtac | Reverse primer for 3' RACE of HEXO2 |
| HEXO2-RACE-fw | TCCTTCGGAGCCGGAGCTC | Forward primer for 3' RACE of HEXO2 |

Table S2. Overview of β -Hexosaminidases

This table gives an overview of HEXO sequences from *Arabidopsis thaliana* and *Nicotiana benthamiana* reported in literature and from this study. For each HEXO variant we provide the accession number, homology between the two plant species, information on whether the gene encodes a functional protein, size of the encoded protein, presence of a signal peptide for secretion (SP) and/or transmembrane domain (TMD), the cellular localisation, and literature citations. Sequence homology between Arabidopsis and Nicotiana orthologs was determined on amino acid level by using Clustal Omega alignments. The signal peptide was predicted by the SignalP server (v5) and the transmembrane domain was predicted by the TMHMM server v2.0. Y: yes; N: no.

| | Accession | Protein coding / Active | Homology with Arabidopsis (%) | Protein Length (aa) | SP (aa) | TMD (aa) | Localisation | Citation |
|------------------------------|------------------------------------------------------|-------------------------|-------------------------------|---------------------|---------|----------|---------------------------|---------------------|
| <i>A. thaliana</i> | | | | | | | | |
| HEXO1 | At3g55260 | Y / Y | - | 541 | 1-20 | | vacuole | 8, 14 |
| HEXO2 | At1g05590 | Y / Y | - | 580 | 1-28 | 7-29 | plasma membrane, apoplast | 8, 14 |
| HEXO3 | At1g65590 | Y / Y | - | 535 | 1-24 | 7-26 | plasma membrane, apoplast | 8, 14 |
| <i>N. benthamiana</i> | | | | | | | | |
| HEXO1A | Niben101Scf03794g01004.1 | Y / Y | 71.51 | 541 | 1-24 | - | vacuole | 15, this manuscript |
| HEXO1B | Niben101Scf10015g07014.1 | N | - | - | - | - | - | this manuscript |
| HEXO2A | Niben101Scf09360g01009 | Y / N | 59.54 | 548 | - | 21-43 | plasma membrane, apoplast | this manuscript |
| HEXO2B | Niben101Scf15928g00003.1 Niben101Scf01151g00003.1 | Y / Y | 63.53 | 602 | - | 28-50 | - | this manuscript |
| HEXO3A | Niben101Scf02017g00001 | Y / Y | 68.30 | 530 | 1-24 | - | apoplast | 15, this manuscript |
| HEXO3B | Niben101Scf02405g02003 | Y / N | 69.13 | 530 | 1-24 | - | - | this manuscript |

| | | |
|--------------------|--------------------------------------------------------------------------------|-----|
| AtHEXO1 | MSTN-----LLRLILFLI---TSLITSSL-STPSPADSPPYLWPLPAEFSFGNETLSV | 49 |
| HEXO1A | MSSNSQCLLKTFLFLLFI FPLVNARSIKSTLHKTELEDESPLYLWPLPSQFTFGNDTLTV | 60 |
| 101-03794 | MSSNSQCLLKTFLFLLFI FPLVNARSIKSTLHKTELEDESPLYLWPLPSQFTFGNDTLTV | 60 |
| Ctg15557/101-10015 | MSSNSQFLKLTLSFLLLIPLVNSRSTKSTLHKTELEDESPLYLWPLPSQFTFANDTLTV | 60 |
| | **:* * *::: : * .:* * . : * *****:*.*:*.**:* | |
| AtHEXO1 | DPTVTLIVAGNGGSLIIRAADFDRYMGII FKHASGRGSLLSRIRFLKMVEYDITSLKIVV | 109 |
| HEXO1A | DPNLSIVFTGNGGGSVIVKEAFERYKKIIFKHGSKSGDF-----FDVTQLTVIV | 109 |
| 101-03794 | DPNLSIVFTGNGGGSVIVKEAFERYKKIIFKHGSKSGDF-----FDVTQLTVIV | 109 |
| Ctg15557/101-10015 | DPNLSIVFTGNGGESVIVKQAFERYKKIIFKHGSKSGEF-----FDVTQLTVIV | 109 |
| | **.::::.*:*.**: *:* * *****.* *.: :*:*.*:.* | |
| AtHEXO1 | HSDSEELQLGVDESITLVMVSKKNEQSI VGAATIEANTVYGALRGLETFSQLCAFYDITKS | 169 |
| HEXO1A | HSDNDELQLGVDESISLLVTKSNEHSI IGEVSI EANSIYGALRGLETMSQLCIFDYGVKT | 169 |
| 101-03794 | HSDNDELQLGVDESISLLVTKSNEHSI IGEVSI EANSIYGALRGLETMSQLCIFDYGVKT | 169 |
| Ctg15557/101-10015 | HSDNDELQLGVDESISLLVTKSNKHSI IGEVSI EANSIYGALRGLETMSQLCIFDYGVKT | 169 |
| | **.:*****:*.*:*.**: * .:*****:*****:**** * * .: | |
| AtHEXO1 | VQIYKAPWYIQDKPRFGYRGLLIDTSRHYLPIDVIKQI IESMSFAKLNVLHWHIVDEQSF | 229 |
| HEXO1A | VQIHKAPWFIQDKPRFAYRGLLLDTSRHYLPIEIIKQI IESMSYAKLNVLHWHIIDESF | 229 |
| 101-03794 | VQIHKAPWFIQDKPRFAYRGLLLDTSRHYLPIEIIKQI IESMSYAKLNVLHWHIIDESF | 229 |
| Ctg15557/101-10015 | VQIRKAPWFIQDKPRFAYRGLLLDTSRHYLPIEIIKQI IESMSYAKLNVLHWHIIDESF | 229 |
| | ** * * * : * * * * . * * * * : * * * * * : * * * * * : * * * * * : * * * * * | |
| AtHEXO1 | PLETPTYPNLWKGAYSRWERYTVEDASEIVRFKMRGINVMAEVDVPGHAESWGTGYPDL | 289 |
| HEXO1A | PLEVPSYPNLWKGYSYTKWERYTVEDAYEIVDFAKLRGINVMAEVDVPGHAESWGAGYPDL | 289 |
| 101-03794 | PLEVPSYPNLWKGYSYTKWERYTVEDAYEIVDFAKLRGINVMAEVDVPGHAESWGAGYPDL | 289 |
| Ctg15557/101-10015 | PLEVPSYPNLWKGYSYTKWERYTVEDAYEIVDFAKMRGINVMAEVDVPGHAESWGAGYPDL | 289 |
| | **.*:*****:*.*:***** * * * * :*****:*****:*****:***** | |
| AtHEXO1 | WPSLSCREPLDVTKNFTFDVISGILADMRKIFPFELFHLGGDEVNTDCWKNTHVKEWLQ | 349 |
| HEXO1A | WPSPSCKEPLDVSKNYTFDVIAGILADMRKIFPFKLFHLGGDEVNTTCWTTTPHLKQWLQ | 349 |
| 101-03794 | WPSPSCKEPLDVSKNYTFDVIAGILADMRKIFPFKLFHLGGDEVNTTCWTTTPHLKQWLQ | 349 |
| Ctg15557/101-10015 | WPSPSCKEPLDVSKNYTFDVIAGILADMRKIFPFELFHLGGDECC-----SPHVSVCRLQ | 344 |
| | ** * * * : * * * * * : * * * * * : * * * * * : * * * * * . * * * * | |
| AtHEXO1 | GRNFTTKDAYKYFVLRQQIAISKNWTPVNWEETFSSFGK--DLDPRTVIQNLVSDICQ | 407 |
| HEXO1A | DHNMTSKDAYEYFVLRQAEIAISHNWTVPVNWEETFNTFPS--KLNPTQVVHNWLRGGVCQ | 407 |
| 101-03794 | DHNMTSKDAYEYFVLRQAEIAISHNWTVPVNWEETFNTFPS--KLNPTQVVHNWLRGGVCQ | 407 |
| Ctg15557/101-10015 | DHNMTSKDAYEYFVLRQAEIAISHNWTVPVWLRGGVCQEAQVAKGFRCIYSNQLRGGVCQ | 404 |
| | .*:*.**:*****:*****:***** . . : * .:.* | |
| AtHEXO1 | KAVAKGFRCIFSNQGYWYLDHLDVWPWEVYNTPEPLNGIEDPSLQKLVIGGEVCMWGETAD | 467 |
| HEXO1A | AAVAKGFRCIYSNQGFWYLDHLDVWPWDKVVYYTEPLEGIKSISEQKLLLGGEACMWGETAD | 467 |
| 101-03794 | AAVAKGFRCIYSNQGFWYLDHLDVWPWDKVVYYTEPLEGIKSISEQKLLLGGEACMWGETAD | 467 |
| Ctg15557/101-10015 | AAVAKGFRCIYSNQGFWYLDHLDVWPWDKVVYYTEPLEGIKSISEQKLLLGGEACMWGETAD | 464 |
| | *****:*****:*****:*. * * * * : * * . * * * * : * * * * * . * * * * * | |
| AtHEXO1 | TSVVLQTIWPRAAAAERMWSTREAVSKGNITLTALPRLHYFRCLLNRRGVPAAAPVDNFY | 527 |
| HEXO1A | ASDVQQTWPRAAAVAERLWSDKEATSTNTTSSALQRLEYFRCLLTRRGVPAAPVTNFY | 527 |
| 101-03794 | ASDVQQTWPRAAAVAERLWSDKEATSTNTTSSALQRLEYFRCLLTRRGVPAAPVTNFY | 527 |
| Ctg15557/101-10015 | ASDVQQTWPRAAAVAERLWSDKEATSTNTTSSALQRLEYFRCLLTRRGVPTAPVTNFY | 524 |
| | :* * * * * * * * . * * * * : * * . * * : * * * * . * * * * * . * * * * * * * * | |
| AtHEXO1 | ARRPPLGPGSCYAQ | 541 |
| HEXO1A | ARRPPLGPGSCYEQ | 541 |
| 101-03794 | ARRPPLGPGSCYEQ | 541 |
| Ctg15557/101-10015 | ARRPPLGPGSCYEQ | 538 |
| | ***** * * | |

Figure S1. Amino acid sequence alignment (Clustal Omega) of *A. thaliana* HEXO1, predicted open reading frames (ORFs) of HEXO1 orthologs from the Sol Genomics Network draft genome for *N. benthamiana* (v1.0.1) and retrieved HEXO1 ORFs from Shin and co-workers (Shin et al., 2017). For one predicted ORF the sequences of Niben101Ctg15557g00001.1 (highlighted in orange) and Niben101Scf10015g07014.1 were combined.

| | | |
|-----------------|---------------------------------------------------------------------------|-----|
| AtHEXO2 | -----MLTLSKF-HVILIPILFFITLLSPLFSIALPINIWP | 35 |
| 101-09360 | --MAY----FISFLSSKQHHKMRGEKTFFSFLPLLLISLL--FFIFISHTTATNYPINIWP | 53 |
| NbHEXO2A | --MAY----FISFLSSKQHHKMRGEKTFFSFLPLLLISLL--FFIFISHTTATNYPINIWP | 53 |
| 101-15928/01151 | --MAYFNFKVILFLSSKQHHKMRGEKTFFSFLPLFLVSL--FFIFISQTTATNYPINWVP | 57 |
| NbHEXO2B | MSMAYFNFKVILFLSSKQHHKMRGEKTFFSFLPLFLVSL--FFILISQTTATNYPINWVP | 59 |
| | * : . * : : * : * : : * : : * : : * : : * : : * | |
| AtHEXO2 | KPRFLSWPQHKAIALSPNFTILAPEHQYLSASVTRYHNLIRSENYSPPLISYVVKLMKRYT | 95 |
| 101-09360 | KPTTFNWPNPKSIIFLSPNFTISHPPHRYLTPAVYRYLHLILSEHHRPIITPAFNLTSSSTP | 113 |
| NbHEXO2A | KPTTFNWPNPKSIIFLSPNFTISHPPHRYLTPAVYRYLHLILSEHHRPIITPAFNLTSSSTP | 113 |
| 101-15928/01151 | KPTTFNWPNPKSIIFLSPNFTISHPPHRYLTPAVYRYLHLILSEHHRPIITPAINLTSSYKP | 117 |
| NbHEXO2B | KPTTFNWPNPKSIIFLSPNFTISHPPHRYLTPAVYRYLHLILSEHHRPIITPAINLTSSYKP | 119 |
| | ** . : . * : * * : * * * : * * : * : * : * : * : * : * : . : * . | |
| AtHEXO2 | LRNLVVTVDLFDLPLHGHVDESXYKLSIPI--GSFSAHLLAHSWAMRGLETFSQMIWGT | 154 |
| 101-09360 | LRSLIISISDVTSPPLAHGVNESYALSTPSDGSPSAYITAETVWGAMRGLETFSQVLYGK | 173 |
| NbHEXO2A | LRSLIISISDVTSPPLAHGVNESYALSTPSDGSPSAYITAETVWGAMRGLETFSQVLYGK | 173 |
| 101-15928/01151 | LHSLIISISDVTSPPLAHGINESYALSTPSDGSPSAYITAETVWGAMRGLETFSQVLYGNP | 177 |
| NbHEXO2B | LHSLIISISDVTSPPLAHGINESYALSTPSDGSPSAYITAETVWGAMRGLETFSQVLYGNP | 179 |
| | * : . : : : : * : . * : : * : * * : * * : * : * : * : . : * . : * . : * . | |
| AtHEXO2 | PDLCLPVGIIYIQDSPLFGHRGVLLDTSRNYGVDDIMRTIKAMSANKLNVFHHITDSQS | 214 |
| 101-09360 | TR--VAAGVYIHDLPFAHRGVMLDTSRNFYGVDDLLRLIKAMSMNKLNVFHHITDSHS | 231 |
| NbHEXO2A | TR--VAAGVYIHDLPFAHRGVMLDTSRNFYGVDDLLRLIKAMSMNKLNVFHHITDSHS | 231 |
| 101-15928/01151 | TR--VAAGVYIRDLPFAHRGVMLDTSRNFYGVDDLLRLIKAMSMNKLNVFHHITDSHS | 235 |
| NbHEXO2B | TR--VAAGVYIRDLPFAHRGVMLDTSRNFYGVDDLLRLIKAMSMNKLNVFHHITDSHS | 237 |
| | : . * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | |
| AtHEXO2 | FPLVIPSEPELAAKGLSPDMVYTPEDVSKIIVQYGFEGHVRVLPEDIDPGHTGSWEAYP | 274 |
| 101-09360 | FPLVIPSEPELAGKAYGNEMMYSPADVQKIVEFGLEHGVRVLPEDIDPAHTGSWAEAYP | 291 |
| NbHEXO2A | FPLVIPSEPELAGKAYGNEMMYSPADVQKIVEFGLEHGVRVLPEDIDPAHTGSWAEAYP | 291 |
| 101-15928/01151 | FPLVIPSEPELAGKAYGNEMMYSPADVQKIVEFGLEHGVRVLPEDIDP----- | 284 |
| NbHEXO2B | FPLVIPSEPELAGKAYGNEMMYSPADVQKIVEFGLEHGVRVLPEDIDPAHTGSWAEAYP | 297 |
| | *** : *** : * . * : * : * : * : * : * : * : * : * : * : * : * : * : * | |
| AtHEXO2 | EIVTCANMFWWPAGKSWEEERLASEPGTGQNLNPLSPKTYEVVKNVIQDIVNQPFESFFHGG | 334 |
| 101-09360 | EIITCANMFWWPAASS--PALAAEPGTGQNLNPLSPKTYEVAKNVIIRDITIAMFPDLLFHGG | 349 |
| NbHEXO2A | EIITCANMFWWPAASS--PALAAEPGTGQNLNPLSPKTYEVAKNVIIRDITIAMFPDLLFHGG | 349 |
| 101-15928/01151 | -----MFWWPAASS--PALAAEPGTGQNLNPLSPKTYEVAKNVIIRDITIAMFPDLLFHGG | 335 |
| NbHEXO2B | EIITCANMFWWPAASS--PALAAEPGTGQNLNPLSPKTYEVAKNVIIRDITIAMFPDLLFHGG | 355 |
| | ***** . * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | |
| AtHEXO2 | GDEVIPGCWKTDPAINSFLSSGGLTSQLEKYINSTLPIYIVSQNRTVVYVWEDVLLDAQIK | 394 |
| 101-09360 | ADEINSACWNTDPSIQKFVTSNGTSLQLEMFVNNTLPEIISLNRVTVYVWEDVILSANVK | 409 |
| NbHEXO2A | ADEINSACWNTDPSIQKFVTSNGTSLQLEMFVNNTLPEIISLNRVTVYVWEDVILSANVK | 409 |
| 101-15928/01151 | ADEINSACWNTDPSIQKFVASNGTSLQLEIFINNTLPEIISLNRVTVYVWEDVILSANVK | 395 |
| NbHEXO2B | ADEINSACWNTDPSIQKFVASNGTSLQLEIFINNTLPEIISLNRVTVYVWEDVILSANVK | 415 |
| | . * : . * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | |
| AtHEXO2 | ADPSVLPKEHTILQTNNGPENTKRIVAAGYRVIVSSSEFYLLDCGHGGFLGNDSIYDQK | 454 |
| 101-09360 | VNPSLLSPQNVIMQTNNGPNTKRLVTSYGRYIVSSADYYLLDCGHGSGFVGNDSRYEQP | 469 |
| NbHEXO2A | VNPSLLSPQNVIMQTNNGPNTKRLVTSYGRYIVSSADYYLLDCGHGSGFVGNDSRYEQP | 469 |
| 101-15928/01151 | VNPSLLSPENVIMQTNNGPNTKRLVTSYGRYIVSSADYYLLDCGHGSGFVGNDSRYDQP | 455 |
| NbHEXO2B | VNPSLLSPENVIMQTNNGPNTKRLVTSYGRYIVSSADYYLLDCGHGSGFVGNDSRHDQP | 475 |
| | . : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | |
| AtHEXO2 | ---ESGGGWCAPFKTWQSIYNYDIADGLLNEEERKLVLGGEVALWSEQADSTVLDLRSRL | 510 |
| 101-09360 | PGLTDQNGGSGWCGPFTWETIYNYDITYGLTD--EEAKLVIGGEVALCPNKLI-----QLL | 523 |
| NbHEXO2A | PGLTDQNGGSGWCGPFTWETIYNYDITYGLTD--EEAKLVIGGEVALCPNKLI-----QLL | 523 |
| 101-15928/01151 | PGLTDQNGGSGWCGPFTWETIYNYDITYGLTD--EEAQLVIGGEVAL----- | 500 |
| NbHEXO2B | PGLTDQNGGSGWCGPFTWETIYNYDITYGLTD--EEAQLVIGGEVALWSEQADSTVMDLRSRI | 534 |
| | . : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | |
| AtHEXO2 | WPRASALAESLWGSNRDER-GVKRCGEAVDRLNLWRYRMVARGIGAEPDPLWCLKNPDM | 569 |
| 101-09360 | WIQESGQEHQQWQKHCQGQGIAMKQE----- | 548 |
| NbHEXO2A | WIQESGQEHQQWQKHCQGQGIAMKQE----- | 548 |
| 101-15928/01151 | ----- | 500 |
| NbHEXO2B | WPRGSAMAETLWGSNRDET-GMKRYAEATDRLNEWRYRMVARGIGAEPDPLWCVKNPDM | 593 |
| | ----- | |
| AtHEXO2 | CNTVHGALQDQ | 580 |
| 101-09360 | ----- | 548 |
| NbHEXO2A | ----- | 548 |
| 101-15928/01151 | ----- | 500 |
| NbHEXO2B | CNTVHFFTS-- | 602 |

Figure S2. Amino acid sequence alignment (Clustal Omega) of *A. thaliana* HEXO2, predicted open reading frames (ORFs) of HEXO2 orthologs from the Sol Genomics Network draft genome for *N. benthamiana* (v1.0.1) and retrieved HEXO2 ORFs in this study. For one predicted ORF we combined the sequences of Niben101Scf15928g00003.1 and Niben101Scf01151g00003.1.

| | | |
|-----------|---------------------------------------------------------------|-----|
| AtHEXO3 | MRGSGAKIAGVLPFLMFLIAGTISAFEDIERLRIWPLPAQ-----SGDFKLVTEG | 50 |
| 101-02405 | -----MNLIVISADANIKNLRIWMPPLSVSYGHRNLQLSNDFVLKTEG | 43 |
| NbHEXO3B | MGKLGQVS---ILFAVIVQILVISADANIKNLRIWMPPLSVSYGHRNLQLSNDFVLKTEG | 57 |
| 101-02017 | MGKLGFRS---ILFAAIVQILVVSADANIKNLRIWMPPLSVSYGHRNLLSNDFVLKTEG | 57 |
| NbHEXO3A | MGKLGFRS---ILFAAIVQILVVSADANIKNLRIWMPPLSVSYGHRNLLSNDFVLKTEG | 57 |
| | . :.* * :*:.*:***:* . *.* * ** | |
| AtHEXO3 | SKYGDASGILKEGFDRLGVVRLSHVISGDRNSSGTGGSALLQGLHVI ISSSTDELEYGA | 110 |
| 101-02405 | SKYPDASGILKEGFSRLLDVVKAAHVVDAN--FSYAGPSVPLKGIQVVVLSPSDELQYGV | 101 |
| NbHEXO3B | SKYPDASGILKEGFSRLLDVVKAAHVVDAN--FSYAGPSVPLKGIQVVVLSPSDELQYGV | 115 |
| 101-02017 | SKYPDTSGLKEGFSRLLDVVVKAHVVDAN--FSYAGPSVPLKGIQVVVLSPNDELQYGV | 115 |
| NbHEXO3A | SKYPDTSGLKEGFSRLLDVVVKAHVVDAN--FSYAGPSVPLKGIQVVVLSPNDELQYGV | 115 |
| | *** *:*****.*:*.** :**:. : * : * * :*:.*:***: * .***:***. | |
| AtHEXO3 | DESYKLVVPSPEKPSYAQLEAKSVYGALHGLQTFSQLCHFNLKKKVIIEILMTPWNIIDQP | 170 |
| 101-02405 | DESYNLTIPAHGNPAYAHLTAKTVYGALHGLQTFSQLCHFNFTRTIEVHQVPWSIVDRP | 161 |
| NbHEXO3B | DESYNLTIPAHGNPAYAHLTAKTVYGALHGLQTFSQLCHFNFTRTIEVHQVPWSIVDRP | 175 |
| 101-02017 | DESYNLTIPAHGNPAYAYLTAKTVHGHGLQTFSQLCHFNFTRTIEVHQVPWTIVDRP | 175 |
| NbHEXO3A | DESYNLTIPAHGNPAYAYLTAKTVHGHGLQTFSQLCHFNFTRTIEVHQVPWTIVDRP | 175 |
| | ****:*. :* :* * * :*:*****:*****:.. :* .**.*:*** | |
| AtHEXO3 | RFSYRGLLIDTSRHYLPLPVILKVIDSMYAKNLVHWHIVDTQSFPLEIPSPKLNWGA | 230 |
| 101-02405 | RFSYRGLLIDTSRHYLPPFVILKVIDSMYAKNLVHWHIVDTQSFPLEIPSPRLWGA | 221 |
| NbHEXO3B | RFSYRGLLIDTSRHYLPPFVILKVIDSMYAKNLVHWHIVDTQSFPLEIPSPKLNWGA | 235 |
| 101-02017 | RFSYRGLLIDTSRHYLPLPVILKVIDSMYAKNLVHWHIVDTQSFPLEIPSPKLNWGA | 235 |
| NbHEXO3A | RFSYRGLLIDTSRHYLPLPVILKVIDSMYAKNLVHWHIVDTQSFPLEIPSPKLNWGA | 235 |
| | *****:*** :****:*****:*****:.. :* .**.*:*** | |
| AtHEXO3 | YSSSQRYTFEDAAEIV---NYARRRGIHVLAEIDVPGHALSWGKYPALWPSKNCQEPL | 286 |
| 101-02405 | YSSSERYTVVDAAEIVRQSCRYAGRRGINVLAELDVPGHQSWGTGYPPLWPSKDCQQPL | 281 |
| NbHEXO3B | YSSSERYTVVDAAEIV---RYAGRRGINVLAELDVPGHQSWGTGYPPLWPSKDCQQPL | 291 |
| 101-02017 | YSSSERYTVTDAAEIVRQSCRYAGRRGINVLAELDVPGHQSWGNGYPSLWPSKDCQQPL | 295 |
| NbHEXO3A | YSSSERYTVTDAAEIV---RYAGRRGINVLAELDVPGHQSWGNGYPSLWPSKDCQQPL | 291 |
| | ****:***. ***** .** *****:***** ***** **.*.***:*****:*** | |
| AtHEXO3 | DVSSDFTFKVIDGILSDFSKIFKRFVHLGGDEVNTTCSAT----- | 328 |
| 101-02405 | DISNDFTFKLDIGILSDFSKIFKRFVHLGGDEVDTIVIPGDKNTGRCVCHYMNSSKLQF | 341 |
| NbHEXO3B | DISNDFTFKLDIGILSDFSKIFKRFVHLGGDEVDTSCWTLT----- | 333 |
| 101-02017 | DISNDFTFKVIDGILSDFSKIFKRFVHLGGDEVDTSKLTCSDNCPDVLVVSIFLDCDILLF | 355 |
| NbHEXO3A | DISNDFTFKVIDGILSDFSKIFKRFVHLGGDEVDTSCWTL----- | 332 |
| | *:*.*****:*****:*****:*****:* | |
| AtHEXO3 | ----PRIAQWLKHKRMEKEAYQYFVLAQKIALSHGYEINWEETFNFGSKLNRKTVV | 384 |
| 101-02405 | RSSHFLGMLKQKLNLTAYEYFVLAQKIALSHGYEINWEETFNFGKLIPTIV | 401 |
| NbHEXO3B | ----PRIRKWLKQKLNLTAYEYFVLAQKIALSHGYEINWEETFNFGKLIPTIV | 389 |
| 101-02017 | PW-TKTLVDGLRQKLNLTAYEYFVLAQKIALSHGYEINWEETFNFGKLSRNSIV | 414 |
| NbHEXO3A | ---TPRIRKWLQKLNLTAYEYFVLAQKIALSHGYEINWEETFNFGKLSRNSIV | 389 |
| | : * : : : : . ** :*****:***** ***** **.* ** : : * | |
| AtHEXO3 | HNW-----LNTGLVENVTASGLRCI | 404 |
| 101-02405 | HNWCVLL-----LGGGVAQQVTAAGLRCI | 425 |
| NbHEXO3B | HNW-----LGGGVAQQVTAAGLRCI | 409 |
| 101-02017 | HNWCVLLSCCISWKLHISSYLFSLRFLHFLYSLPIKVIKVRKLGGAQQVTAAGLRCI | 474 |
| NbHEXO3A | HNW-----LGGGVAQQVTAAGLRCI | 409 |
| | *** * . * : : : * : * : * : * | |
| AtHEXO3 | VSNQEFWYLDHIDAPWQGFYANEPFQNIITDKKQQLVSLGGEVCMWGEHIDASDIEQTIWP | 464 |
| 101-02405 | VSNQDKWYLDHIDTTWQDFYSNEPLTNIRNPKQQLVIGGEVCMWGEHIDGSIETTIWP | 485 |
| NbHEXO3B | VSNQDKWYLDHIDTTWQDFYSNEPLTNIRNPKQQLVIGGEVCMWGEHIDGSIETTIWP | 469 |
| 101-02017 | VSNQDKWYLDHIDTTWQDFYSNEPLTNITNPKQQLVIGGEVCMWGEHIDGSIETTIWP | 534 |
| NbHEXO3A | VSNQDKWYLDHIDTTWQDFYSNEPLTNITNPKQQLVIGGEVCMWGEHIDGSIETTIWP | 469 |
| | ****: *****: **.*:***: * : * * * :*****:*****.*:*** ** | |
| AtHEXO3 | RAAAAERLWTPYAKLAKNPNNVTRRLAHFRCLLNQRGVAAAPLVGGGRVVPFEPGSCLA | 524 |
| 101-02405 | RAAAAERLWTAYDNLAKNPSQVTRRLAHFRCLLNQRGVASVPLTGGGRAAPEDPADVTR | 545 |
| NbHEXO3B | RAAAAERLWTAYDNLAKNPSQVTRRLAHFRCLLNQRGVASVPLTGGGRAAPEDPGSCYQ | 529 |
| 101-02017 | RAAAAERLWTAYDNLAKNPSQVTRRLAHFRCLLNRRGVASGPLTGGGRAAPEDPGSCYQ | 594 |
| NbHEXO3A | RAAAAERLWTAYDNLAKNPSQVTRRLAHFRCLLNRRGVASGPLTGGGRAAPEDPGSCYQ | 529 |
| | ***** * :****.*:*****:*****: **.*.***.* : * . | |

Figure S3. Amino acid sequence alignment (Clustal Omega) of *A. thaliana* HEXO3, predicted open reading frames (ORFs) of HEXO3 orthologs from the Sol Genomics Network draft genome for *N. benthamiana* (v1.0.1) and retrieved HEXO3 ORFs from Shin and co-workers (Shin et al., 2017) and this study.

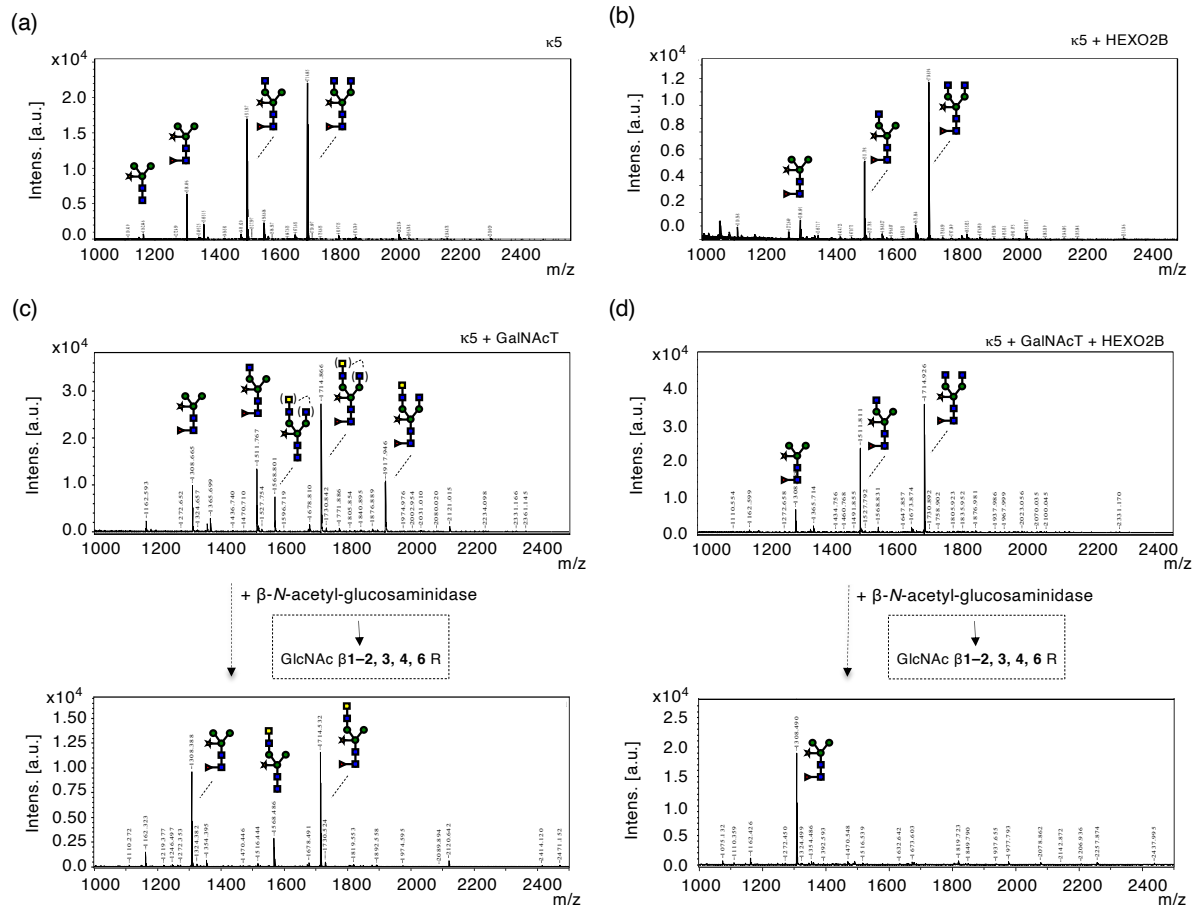


Figure S4. HEXO2B substrate specificity. Kappa-5 was co-expressed with p19 and combinations of HEXO2B and/or CeGalNAcT in wild-type plants. Kappa-5 was purified from the apoplast fluid (at 6 dpi) and the N-glycan composition of kappa-5 was analyzed MALDI-TOF-MS. MS profiles are given for N-glycans of wild-type kappa-5 (a), kappa-5 with HEXO2B (b), kappa-5 with CeGalNAcT (c) and kappa-5 with CeGalNAcT and HEXO2B (d). To distinguish terminal GlcNAc and GalNAc residues we treated the N-glycans with glucosaminidase for which the substrate specificity is indicated in the dashed box. When a MS peak represents multiple N-glycan structures of identical mass, the possible positions of these sugar residues on the N-glycan are indicated between brackets.

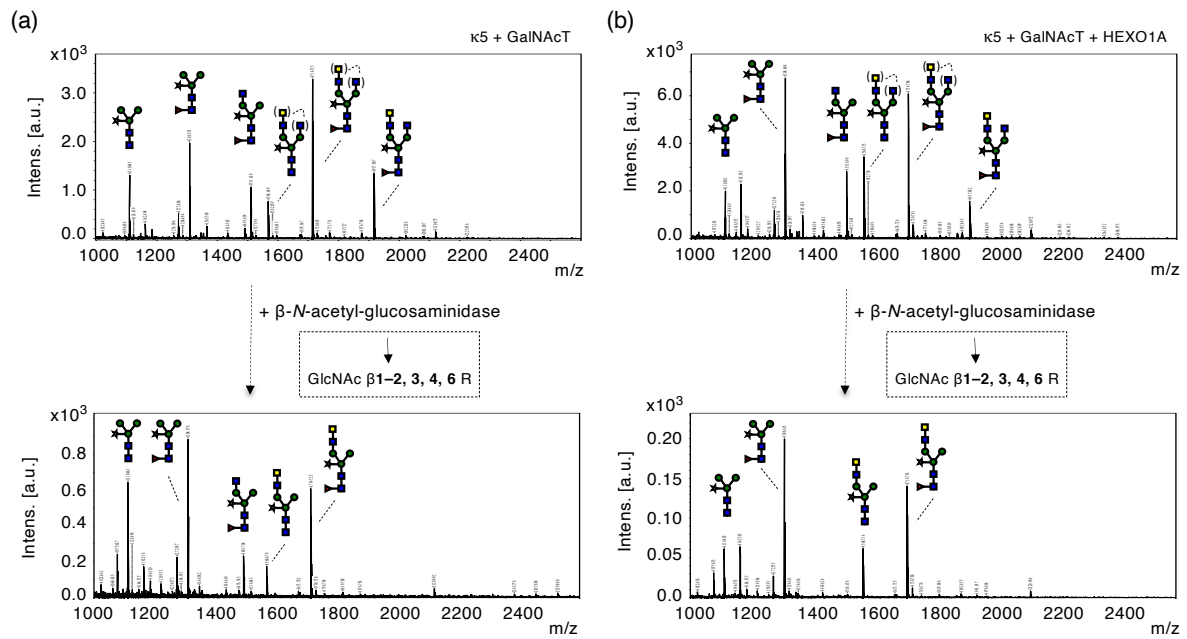


Figure S5. HEXO1A substrate specificity. Kappa-5 was co-expressed with p19, CeGalNAcT and with/without HEXO1A in wild-type plants. Kappa-5 was purified from the apoplast fluid (at 6 dpi) and the N-glycan composition of kappa-5 was analyzed MALDI-TOF-MS. MS profiles are given for N-glycans of kappa-5 with CeGalNAcT (a) and kappa-5 with CeGalNAcT and HEXO1A (b). To distinguish terminal GlcNAc and GalNAc residues we treated the N-glycans with glucosaminidase for which the substrate specificity is indicated in the dashed box. When a MS peak represents multiple N-glycan structures of identical mass, the possible positions of these sugar residues on the N-glycan are indicated between brackets.

CLUSTAL O(1.2.4) multiple sequence alignment

| | | |
|---------|---------------------------------------------------------------------|------|
| HEX02A | -----ATGGCTTACTT-----CATTTCTTCCTCAAAACACACAC | 42 |
| HEX02B | ATGAGATGGCTTACTTCACTCAAAATGATTTATTTCTTCCTCAAAACACACAC | 60 |
| hairpin | ----- | 0 |
| HEX02A | AAAATGAGAGAGAGAAAATTTTTCCTTCCTCCACTACTCTTATCTCTGTTA | 102 |
| HEX02B | AAAATGAGAGAGAGAAAATTTTTCCTTCCTCCACTACTCTTATCTCTGTTA | 120 |
| hairpin | ----- | 0 |
| HEX02A | TTCTTCATCTTCATTTCTCACACACAGCTACAAATFACCAATCAATATCTGGCCCAAG | 162 |
| HEX02B | TTCTTCATCTTCATTTCTCACACACAGCTACAAATFACCAATCAATATCTGGCCCAAG | 180 |
| hairpin | ----- | 0 |
| HEX02A | CCCAACACATTCATTTGGCCCAACCAAAATCCATCTTCCTCCCAAACTTCAACATC | 222 |
| HEX02B | CCCAACACATTCATTTGGCCCAACCAAAATCCATCTTCCTCCCAAACTTCAACATC | 240 |
| hairpin | -----CCATCTCTCTCTCCAACTTCAACATC ***** | 29 |
| HEX02A | TCCACCCACCCACCGTTATCTCACTCCCGGGTTACCGTTATCTCCACTATCTC | 282 |
| HEX02B | TCCACCCACCCACCGTTATCTCACTCCCGGGTTACCGTTATCTCCACTATCTC | 300 |
| hairpin | TCCACCCACCCACCGTTATCTCACTCCCGGGTTACCGTTATCTCCACTATCTC ***** | 89 |
| HEX02A | TCCGAGCACCCCGGATCATCACTCCGGCTTTTAACTGACGTATCCACGGCGTTA | 342 |
| HEX02B | TCCGAGCACCCCGGATCATCACTCCGGCTTTTAACTGACGTATCCACGGCGTTA | 360 |
| hairpin | TCCGAGCACCCCGGATCATCACTCCGGCTTTTAACTGACGTATCCACGGCGTTA ***** | 149 |
| HEX02A | CGTAGCCTCATCATCTTCTCCGACGCTCACTCACCCTTCTCAGGAGTCAACGAA | 402 |
| HEX02B | CATAGCCTCATCATCTTCTCCGACGCTCACTCACCCTTCTCAGGAGTCAACGAA | 420 |
| hairpin | CATAGCCTCATCATCTTCTCCGACGCTCACTCACCCTTCTCAGGAGTCAACGAA ***** | 209 |
| HEX02A | TCCTATGCTCTTCCACTCCTCCGACGGCTCCCTCCGCCTATATCACCAGGAACT | 462 |
| HEX02B | TCCTATGCTCTTCCACTCCTCCGACGGTCCCTCCGCCTATATCACCAGGAACT | 480 |
| hairpin | TCCTATGCTCTTCCACTCCTCCGACGGTCCCTCCGCCTATATCACCAGGAACT ***** | 220 |
| HEX02A | GTATGGGAGCAATGCGAGGCTCTGAGACTTTCTGCAACTCTGTACGGAAACCTACC | 522 |
| HEX02B | GTATGGGAGCAATGCGAGGCTCTGAGACTTTCTGCAACTCTGTACGGAAACCTACC | 540 |
| hairpin | GTATGGGAGCAATGCGAGGCTCTGAGACTTTCTGCAACTCTGTACGGAAACCTACC ***** | 220 |
| HEX02A | AGAGTCGCCCGCGGCTGTACATACAGATCTGCCGATTTTCGCCACGAGGTGTATG | 582 |
| HEX02B | AGAGTCGCCCGCGGCTGTACATACAGATCTGCCGATTTTCGCCACGAGGTGTATG | 600 |
| hairpin | AGAGTCGCCCGCGGCTGTACATACAGATCTGCCGATTTTCGCCACGAGGTGTATG ***** | 220 |
| HEX02A | CTGGACTTCGAGAACTTTTACGGCGTGTATGTTGTTAGGCTTATTAAAGCTATG | 642 |
| HEX02B | CTGGACTTCGAGAACTTTTACGGCGTGTATGTTGTTAGGCTTATTAAAGCTATG | 660 |
| hairpin | CTGGACTTCGAGAACTTTTACGGCGTGTATGTTGTTAGGCTTATTAAAGCTATG ***** | 220 |
| HEX02A | AGTATGAACAAGTTGAATGTTTCCACTGGCACATACTGATTCACATTCGTTCCGGCT | 702 |
| HEX02B | AGTATGAACAAGTTGAATGTTTCCACTGGCACATACTGATTCACATTCGTTCCGGCT | 720 |
| hairpin | AGTATGAACAAGTTGAATGTTTCCACTGGCACATACTGATTCACATTCGTTCCGGCT ***** | 220 |
| HEX02A | GTGATTCCTCGGAGCGGAGCTCGCCGAAAAGGAGCATAGGCAATGAGATGATGTAC | 762 |
| HEX02B | GTGATTCCTCGGAGCGGAGCTCGCCGAAAAGGAGCATAGGCAATGAGATGATGTAC | 780 |
| hairpin | GTGATTCCTCGGAGCGGAGCTCGCCGAAAAGGAGCATAGGCAATGAGATGATGTAC ***** | 220 |
| HEX02A | TCCCGCGGAGCTGCGAGAATCTGGAAATTTGACTGGAAACAGGATAGAGTTTTA | 822 |
| HEX02B | TCCCGCGGAGCTGCGAGAATCTGGAAATTTGACTGGAAACAGGATAGAGTTTTA | 840 |
| hairpin | TCCCGCGGAGCTGCGAGAATCTGGAAATTTGACTGGAAACAGGATAGAGTTTTA ***** | 220 |
| HEX02A | CCTGAAATGACATGCTGACATACAGGATCATGGGCTGAGCTTACCTGAGATATC | 882 |
| HEX02B | CCTGAAATGACATGCTGACATACAGGATCATGGGCTGAGCTTACCTGAGATATC | 900 |
| hairpin | CCTGAAATGACATGCTGACATACAGGATCATGGGCTGAGCTTACCTGAGATATC ***** | 220 |
| HEX02A | ACTTGTGCAAAATGTTCTGGTGGCTGCTGCAAGTAGTCCAGCTCTTGCAGCTGAACCA | 942 |
| HEX02B | ACTTGTGCAAAATGTTCTGGTGGCTGCTGCAAGTAGTCCAGCTCTTGCAGCTGAACCA | 960 |
| hairpin | ACTTGTGCAAAATGTTCTGGTGGCTGCTGCAAGTAGTCCAGCTCTTGCAGCTGAACCA ***** | 220 |
| HEX02A | GGTACTGGTCACTGAACCCCTTGGAGTCCCAAGACTATGAAGTAGCCAAGATGTATC | 1002 |
| HEX02B | GGTACTGGTCACTGAACCCCTTGGAGTCCCAAGACTATGAAGTAGCCAAGATGTATC | 1020 |
| hairpin | GGTACTGGTCACTGAACCCCTTGGAGTCCCAAGACTATGAAGTAGCCAAGATGTATC ***** | 220 |
| HEX02A | CGCATACTATGCCATGTTCCAGATTTGCTCTTTTATGGGGGAGAGATGATCAAT | 1062 |
| HEX02B | CGCATACTATGCCATGTTCCAGATTTGCTCTTTTATGGGGGAGAGATGATCAAT | 1080 |
| hairpin | CGCATACTATGCCATGTTCCAGATTTGCTCTTTTATGGGGGAGAGATGATCAAT ***** | 220 |
| HEX02A | TCAGCTTGTGGAATCTGATCCATCAATCAAAAATTTGTCTAGCAATGGAAGCTCTC | 1122 |
| HEX02B | TCAGCTTGTGGAATCTGATCCATCAATCAAAAATTTGTCTAGCAATGGAAGCTCTC | 1140 |
| hairpin | TCAGCTTGTGGAATCTGATCCATCAATCAAAAATTTGTCTAGCAATGGAAGCTCTC ***** | 220 |
| HEX02A | AGTCAGCTACTGAAAATGTTTGTCAATAAATTTACCTGAAATCTCTCACTCAACCGT | 1182 |
| HEX02B | AGTCAGCTACTGAAAATGTTTGTCAATAAATTTACCTGAAATCTCTCACTCAACCGT | 1200 |
| hairpin | AGTCAGCTACTGAAAATGTTTGTCAATAAATTTACCTGAAATCTCTCACTCAACCGT ***** | 220 |
| HEX02A | ACGGTGTCTACTGGGAGATGTTATATGAGTCTAATGTAAGTGAATCCATCTCTG | 1242 |
| HEX02B | ACGGTGTCTACTGGGAGATGTTATATGAGTCTAATGTAAGTGAATCCATCTCTG | 1260 |
| hairpin | ACGGTGTCTACTGGGAGATGTTATATGAGTCTAATGTAAGTGAATCCATCTCTG ***** | 220 |
| HEX02A | CTTTCTCCAGAAATGTTATGCAAACTTGGAAATAGGCAAACTAATAAGCGG | 1302 |
| HEX02B | CTTTCTCCAGAAATGTTATGCAAACTTGGAAATAGGCAAACTAATAAGCGG | 1320 |
| hairpin | CTTTCTCCAGAAATGTTATGCAAACTTGGAAATAGGCAAACTAATAAGCGG ***** | 220 |
| HEX02A | CTTGTCACTCTGGCTACCGTCTATTGTCTACTGAGATTAATTACTTGGATTGT | 1362 |
| HEX02B | CTTGTCACTCTGGCTACCGTCTATTGTCTACTGAGATTAATTACTTGGATTGT | 1380 |
| hairpin | CTTGTCACTCTGGCTACCGTCTATTGTCTACTGAGATTAATTACTTGGATTGT ***** | 220 |
| HEX02A | GGTCATGGGAGCTTCTGTTGAAATGACAGCCGCTATGAGCAACACAGTACTGACCAA | 1422 |
| HEX02B | GGTCATGGGAGCTTCTGTTGAAATGACAGCCGCTATGAGCAACACAGTACTGACCAA | 1440 |
| hairpin | GGTCATGGGAGCTTCTGTTGAAATGACAGCCGCTATGAGCAACACAGTACTGACCAA ***** | 220 |
| HEX02A | GGCAATGGTGGATCTGCTGGGACCTTCAAGACTGGGAAACCATATACAATATGAT | 1482 |
| HEX02B | GGCAATGGTGGATCTGCTGGGACCTTCAAGACTGGGAAACCATATACAATATGAT | 1500 |
| hairpin | GGCAATGGTGGATCTGCTGGGACCTTCAAGACTGGGAAACCATATACAATATGAT ***** | 220 |
| HEX02A | ATAACTATGGCTGACTGATGAGGAGCTAAATGGTAATGGAGGGGAATGACATTA | 1542 |
| HEX02B | ATAACTATGGCTGACTGATGAGGAGCTAAATGGTAATGGAGGGGAATGACATTA | 1560 |
| hairpin | ATAACTATGGCTGACTGATGAGGAGCTAAATGGTAATGGAGGGGAATGACATTA ***** | 220 |
| HEX02A | TG-TCCGAAACAAGCTGATCAACTGTTATGATTAAGAACTTGGCCAGAGCATAGCA | 1601 |
| HEX02B | TG-TCCGAAACAAGCTGATCAACTGTTATGATTAAGAACTTGGCCAGAGCATAGCA | 1620 |
| hairpin | TG-TCCGAAACAAGCTGATCAACTGTTATGATTAAGAACTTGGCCAGAGCATAGCA ***** | 220 |
| HEX02A | ATGGCAGAAACATTTGGTCAAGGAAATCCGATGAACAGGAATGAAGAGATATCCAGAG | 1647 |
| HEX02B | ATGGCAGAAACATTTGGTCAAGGAAATCCGATGAACAGGAATGAAGAGATATCCAGAG | 1680 |
| hairpin | ATGGCAGAAACATTTGGTCAAGGAAATCCGATGAACAGGAATGAAGAGATATCCAGAG ***** | 220 |
| HEX02A | ----- | 1647 |
| HEX02B | GCTACTGATCAGTGAATGAATGGAGTACAGAAATGGTTCAGGGGAATAGGTGCTGAA | 1740 |
| hairpin | ----- | 220 |
| HEX02A | ----- | 1647 |
| HEX02B | CGAATCAGCCTCTGTGGTGTGTCAAAACCCAGGACTGTATACACAGTTCATCAATTT | 1800 |
| hairpin | ----- | 220 |
| HEX02A | ----- | 1647 |
| HEX02B | ACCAGCTGA | 1809 |
| hairpin | ----- | 220 |

Figure S6. Nucleotide sequence alignment (Clustal Omega) of HEX02A, HEX02B and the target region of the $\Delta 2$ hairpin construct.

CLUSTAL O(1.2.4) multiple sequence alignment

| | | |
|---------|----------------------------------------------------------------------|------|
| HEX03B | ATGGGGAAGTTAGGAGTGCAGACATATTTATTTGCAGTATCGTTCAGATTCTAGTATA | 60 |
| HEX03A | ATGGGGAAGTTAGGAGTCCGGAGCATATTTATTTGCAGTATCGTTCAGATTCTAGTATA | 60 |
| hairpin | ----- | 0 |
| HEX03B | TCAGCAGATGCTAATATTAAGAATTGAGAATATGCCGATGCCACTATCGGTAGCTAT | 120 |
| HEX03A | TCAGCAGATGCTAATATTAAGAATTGAGAATATGCCGATGCCACTATCGGTAGCTAT | 120 |
| hairpin | ----- | 0 |
| HEX03B | GGACATAGGAATCTTCAACTTAGTAATGATTTTGTGCTCAAGACTGAAGGCAGCAAGTAC | 180 |
| HEX03A | GGACATAGGACTCTTCTACTTAGTAATGATTTTGTGCTCAAGACTGAAGGCAGCAAGTAC | 180 |
| hairpin | ----- | 0 |
| HEX03B | CCTGATGCCTCGGAATTCFAAGGAAGTTTTTCAAGGCTACTTGAATGCTTAAGGCA | 240 |
| HEX03A | CCTGATACCTCGGAATTCFAAGGAAGTTTTTCAAGGCTACTTGAATGCTTAAGGCA | 240 |
| hairpin | ----- | 0 |
| HEX03B | GCTCATGTGTGATGCAACTTCTCTATGCGCGCCCTCTCCAGTCTTAAGGGAATT | 300 |
| HEX03A | GCTCATGTGTGATGCAACTTCTCTATGCGCGCCCTCTCCAGTCTTAAGGGAATT | 300 |
| hairpin | ----- | 0 |
| HEX03B | CAAGTGGTGTCTTCTCCAAGTATGAGCTACAATATGGTGTGATGAGTCTCAAC | 360 |
| HEX03A | CAAGTGGTGTCTTCTCCAAGTATGAGCTACAATATGGTGTGATGAGTCTCAAC | 360 |
| hairpin | ----- | 0 |
| HEX03B | TTGACCATACCAGCACATGAAACCAGCCTATGCATATTTGACGGCAAAAACAGTTTAT | 420 |
| HEX03A | TTGACCATACCAGCACATGAAACCAGCCTATGCATATTTGACGGCAAAAACAGTTTAT | 420 |
| hairpin | -----GCAAAAACAGTTTAT ***** ** | 15 |
| HEX03B | GGGGCTTTGCATGGTTTGCAGACATTTAGTCAAGTATGCCATTTTAACTTTACACCAGA | 480 |
| HEX03A | GGGGCTTTGCATGGTTTGCAGACATTTAGTCAAGTATGCCATTTTAACTTTACACCAGA | 480 |
| hairpin | GGGGCTTTGCATGGTTTGCAGACATTTAGTCAAGTATGCCATTTTAACTTTACACCAGA ***** | 75 |
| HEX03B | ACAATGAAGTTCATCAAGTCCATGGACATAGTGTGATGACCAAGATTCCTTTATCGA | 540 |
| HEX03A | ACAATGAAGTTCATCAAGTCCATGGACATAGTGTGATGACCAAGATTCCTTTATCGA | 540 |
| hairpin | ACAATGAAGTTCATCAAGTCCATGGACATAGTGTGATGACCAAGATTCCTTTATCGA ***** | 135 |
| HEX03B | GGGCTTTAATGATACTTCCCGTCACTACTGCCATTTCTGTGATATGAAGGTATT | 600 |
| HEX03A | GGGCTTTAATGATACTTCCCGTCACTACTGCCATTTCTGTGATATGAAGGTATT | 600 |
| hairpin | GGGCTTTAATGATACTTCCCGTCACTACTGCCATTTCTGTGATATGAAGGTATT ***** | 195 |
| HEX03B | GATTCGATGGCTTATGCAAACTGAATGTGCTGCACCTGGCATTGTAGATACACAATCA | 660 |
| HEX03A | GATTCGATGGCTTATGCAAACTGAATGTGCTGCACCTGGCATTGTAGATACACAATCA | 660 |
| hairpin | GATTCGATGGCTTATGCAAACTGAATGTGCTGCACCTGGCATTGTAGATACACAATCA ***** | 220 |
| HEX03B | TTCCCACTTGAGATACCTTCAATTTCCGAGACTGGAATGGTCTTACTCAAGTTCAGAG | 720 |
| HEX03A | TTCCCACTTGAGATACCTTCAATTTCCGAGACTGGAATGGTCTTACTCAAGTTCAGAG | 720 |
| hairpin | TTCCCACTTGAGATACCTTCAATTTCCGAGACTGGAATGGTCTTACTCAAGTTCAGAG | 220 |
| HEX03B | CGGTACACTGTAGTTGATGCTGCTGAGATTGTAAGATATGCAGGAAGACGTGGAATCAAT | 780 |
| HEX03A | CGGTACACTGTAGTTGATGCTGCTGAGATTGTAAGATATGCAGGAAGACGTGGAATCAAT | 780 |
| hairpin | CGGTACACTGTAGTTGATGCTGCTGAGATTGTAAGATATGCAGGAAGACGTGGAATCAAT | 220 |
| HEX03B | GTATTAGCTGAATPAGACGTTCCAGGACATGCTCAATCTGGGGTACTGGTTATCCTTCT | 840 |
| HEX03A | GTATTAGCTGAATPAGACGTTCCAGGACATGCTCAATCTGGGGTACTGGTTATCCTTCT | 840 |
| hairpin | GTATTAGCTGAATPAGACGTTCCAGGACATGCTCAATCTGGGGTACTGGTTATCCTTCT | 220 |
| HEX03B | TTATGGCCCTCAAAGATTGTCACAGCCACTTGACATAAGCAATGATTTCACTTTCAA | 900 |
| HEX03A | TTATGGCCCTCAAAGATTGTCACAGCCACTTGACATAAGCAATGATTTCACTTTCAA | 900 |
| hairpin | TTATGGCCCTCAAAGATTGTCACAGCCACTTGACATAAGCAATGATTTCACTTTCAA | 220 |
| HEX03B | CTCATAGACGGAATTCCTCAGATTTTAGCAAGATATCAAAACAGATTGTTCACTTA | 960 |
| HEX03A | CTCATAGACGGAATTCCTCAGATTTTAGCAAGATATCAAAACAGATTGTTCACTTA | 960 |
| hairpin | CTCATAGACGGAATTCCTCAGATTTTAGCAAGATATCAAAACAGATTGTTCACTTA | 220 |
| HEX03B | GGAGGTGATGAAGTGGATACAAGTTGCTGGACGTTAAGTCTCGTATAAGAAAGTGGTTG | 1020 |
| HEX03A | GGAGGTGATGAAGTGGATACAAGTTGCTGGACGTTAAGTCTCGTATAAGAAAGTGGTTG | 1020 |
| hairpin | GGAGGTGATGAAGTGGATACAAGTTGCTGGACGTTAAGTCTCGTATAAGAAAGTGGTTG | 220 |
| HEX03B | AAGCAAAACAAGTTGAATGGAACCACTGCTTATGAGTACTTGTCTTGAGAGCACAGAAG | 1080 |
| HEX03A | AAGCAAAACAAGTTGAATGGAACCACTGCTTATGAGTACTTGTCTTGAGAGCACAGAAG | 1080 |
| hairpin | AAGCAAAACAAGTTGAATGGAACCACTGCTTATGAGTACTTGTCTTGAGAGCACAGAAG | 220 |
| HEX03B | ATAGCTTTATCTCATGGATATGAAATATAAAGTGGGAAGACGTTCAATAAATTGGGA | 1140 |
| HEX03A | ATAGCTTTATCTCATGGATATGAAATATAAAGTGGGAAGACGTTCAATAAATTGGGA | 1140 |
| hairpin | ATAGCTTTATCTCATGGATATGAAATATAAAGTGGGAAGACGTTCAATAAATTGGGA | 220 |
| HEX03B | AATAAGTTGATCCAAACACTATAGTTCAACAATGGCTCGGGGGCGGTGTTCTCAGCAA | 1200 |
| HEX03A | AATAAGTTGATCCAAACACTATAGTTCAACAATGGCTCGGGGGCGGTGTTCTCAGCAA | 1200 |
| hairpin | AATAAGTTGATCCAAACACTATAGTTCAACAATGGCTCGGGGGCGGTGTTCTCAGCAA | 220 |
| HEX03B | GTAAGTGCAGCTGGATTGCGGTGCATTTGTAAGCAACAGGACAAAGTGGTATTTGGACCAT | 1260 |
| HEX03A | GTAAGTGCAGCTGGATTGCGGTGCATTTGTAAGCAACAGGACAAAGTGGTATTTGGACCAT | 1260 |
| hairpin | GTAAGTGCAGCTGGATTGCGGTGCATTTGTAAGCAACAGGACAAAGTGGTATTTGGACCAT | 220 |
| HEX03B | ATAGATACCACATGGCAGGATTTCTATTCAAATGAGCCACTAATAATATFAGAAATCCC | 1320 |
| HEX03A | ATAGATACCACATGGCAGGATTTCTATTCAAATGAGCCACTAATAATATFAGAAATCCC | 1320 |
| hairpin | ATAGATACCACATGGCAGGATTTCTATTCAAATGAGCCACTAATAATATFAGAAATCCC | 220 |
| HEX03B | AAGCAACAACGGCTGGTTATTGGGGGTGAGTATGATGTTGGGTGAGCATATTGATGGA | 1380 |
| HEX03A | AAGCAACAACGGCTGGTTATTGGGGGTGAGTATGATGTTGGGTGAGCATATTGATGGA | 1380 |
| hairpin | AAGCAACAACGGCTGGTTATTGGGGGTGAGTATGATGTTGGGTGAGCATATTGATGGA | 220 |
| HEX03B | TCAACATCGAAACACCATATGGCCCGTGCAGCAGCAGCTGCAGAGAGGCTATGGACT | 1440 |
| HEX03A | TCAACATCGAAACACCATATGGCCCGTGCAGCAGCAGCTGCAGAGAGGCTATGGACT | 1440 |
| hairpin | TCAACATCGAAACACCATATGGCCCGTGCAGCAGCAGCTGCAGAGAGGCTATGGACT | 220 |
| HEX03B | GCTTATGACAACCTTCCCAAGAATCCAAGTCAAGTACTCCAGGCTGGCTCATTTTAGG | 1500 |
| HEX03A | GCTTATGACAACCTTCCCAAGAATCCAAGTCAAGTACTCCAGGCTGGCTCATTTTAGG | 1500 |
| hairpin | GCTTATGACAACCTTCCCAAGAATCCAAGTCAAGTACTCCAGGCTGGCTCATTTTAGG | 220 |
| HEX03B | TGCCATTGAAATCAAGAGGAGTGGCTTCTGTTCCATTAAGTGGAGCGGGCGAGCTGCA | 1560 |
| HEX03A | TGCCATTGAAATCAAGAGGAGTGGCTTCTGTTCCATTAAGTGGAGCGGGCGAGCTGCA | 1560 |
| hairpin | TGCCATTGAAATCAAGAGGAGTGGCTTCTGTTCCATTAAGTGGAGCGGGCGAGCTGCA | 220 |
| HEX03B | CCAGAAGATCCAGGTTCTTCTATCAGCAATAG | 1593 |
| HEX03A | CCAGAAGATCCAGGTTCTTCTATCAGCAATAG | 1593 |
| hairpin | CCAGAAGATCCAGGTTCTTCTATCAGCAATAG | 220 |

Figure S7. Nucleotide sequence alignment (Clustal Omega) of HEXO3A, HEXO3B and the target region of the $\Delta 3$ hairpin construct.