Supplementary Figures

Avenin-like protein A (F2EGD5):

MKTMLILALIAFAATSAVAQLDTTC**SQGYGQCQQQPQQQMNTCAAFLQQCSRTPYVQSQMWQASGCQLMRQQCCQPLAQIS**
EQARCQAVCSMAQVIMRQQQGQSFTQPQQQQSQSFGQPQQQVPVEVMRMVLQTLPSMCSVNIPQYCTTTPCSTITPTIYSI
PMAATCAGGVC

B1-hordein (I6SJ22):

MKTFLIFALLAIAATSTIA**QQQPLPQQPIPQKPQPYPQQPFPPQQPFPQQPVPQQPQPYPQQPQQPFPPQQPFPQQPPFGL**
QQPFPQQPPFGLQQRILSQQQPCTPQQTPLPQGQLYQTLLQLQIPYVHPSILQQLNPCKVFLQQQCSPVRMPQLIARLQML
QLSSCHVLQQQCCQQLPQISEQFRHEAIRAIVYSIFLQEQPQQSVQGVSQTQQQLQQEKVGQCSFQQPQPQQLGQPQQVPQ
SVFLQPHQIAQLEATTSIALRTLPRMCNVNVPLYDIMPPDFWH

B3-hordein (I6SJ26)

MKTFLIFALLAIVATSTIA**QQQPYPQQPQPFPQQPIPQQPQPFPQQPQPYPQQPQPF**PQQPI**PQQPQPYPQQPQPF**PLQPFPSQQPFPQQPPFWQQQPVLSQQQPC**TPQQTPLPQGQQDQMLVQVQIPFVHPSILQQLNPCKV**
FLQQQCSPVAMSQRIARSQMLQQSSCYVLQQQCCQQLPQIPEQFRHEAVRAIVY**SIVLQEQPQQLVQGVSQPQQQSQLHQV**
GQCSFQQPQPQQGQQQQVPQSVFLQPLQLAQLEATASIALRTLPMMCSVNVPFYRILPFGIDTRVGV

B3-hordein (I6TRT5):

MKTFLIFALLAIVATSTIA**QQQPYPQQPQPFPQQPQPYPQQPQPFPQQPIPQQPQPYPQQPQPF**PLQPFPSQQPFPQQPPF
WQQQPVLSQQQPC**TQEQTPLLQEQQDQMLLQVQIPFVHPSILQQLNPCKVFLQQQCSPVAMSQRIARSQMLQQSSCHVLQQ**
QCCQQLPQIPEQLRHEAVR**AIVYSIVLREQSLQLVQGVSQPQQQSQQQQVGQCSFQQPQPQQGQQQQVPQSVLLQPHQIAQ**
LEATTSIALRTLPTMCSVNVPLYRIVPLAIDTRVGV

C-hordein (Q41210):

MKTFLTFVLLAMVMSIVTTA**RQLNPSSQEL**QSPQQSY**LQQPYPQNPY**LPQQPFPVQQPFHTPQQY**FPYLPEELSPQY**QIPT
PL**QPQQPFPQQPQQPLPRPQQPFPW**QPQQPFPQPQQPIPYQPQQPFNQQPQQIIS**QPQQPFPQQPQQPFPQPQQPFPWQP**
QQPFPQPQQPFPLQPQQPFPWQPQQPFPQPQQPIAHQPQQPFSFSQQPQQPFPLQPQQPFPQQPQQPFPQQPQQIIFQQPQ
QSYPVQPQQPFPQPQPVPQQRPQQASPL**QPQQPFPQGSEQIIPQQPQQPFPLQP** HQPYTQQTIWSMV

C-hordein (Q40053):

MKTFLTFVLLAMVMSIVTTA**RQLNPSSQEL**QSPQQSY**LQQPYPQNPY**LPQKPFPVQQPFHTPQQY**FPYLPEELFPQYQIPT**
PLQPQQPFPQQPQQPLPRPQQPFPWQPQQPFPQPQEPIPQQPQQPFPQQPQQPFPQQPQQIIFQQPQQSYPVQPQQPFPQP
QPVPQQRPQQASPLQPQPQQASPL**QPQQPFPQGSEQIIPQQPFPLQPQPFPQQPQQPLPQPQQPF**RQQAEL**IIPQQPQQPL**
PLQPHQPYTQQTIWSMV

D-hordein (I6TRS8):

MAKRLVLFVAVIVALVALTTA**EREINGNNIFLDSRSRQLQCERELQESSLEACRRVVDQQLVGQLPWSTGLQMQCCQQLRD**
VSPECRPVALSQVVRQYEQQTEVPSKGGSFYPGGTAPPLQQGGWWGTSVKWYYPDQTSSQQSWQGQQGYHQSVTSSQQPGQ
GQQGSYPGSTFPQQPGQGQQPGQRQPWSYPSATFPQQPGQGQGQQGYYPGATSLLQPGQGQQGPYQSATSPQQPGQGQGQQ
ETYPIA**TSPHQPGQW**QQPGQGQQGYYPSVTSPQQSGQGQQGYPSTTSPQQSGQGQQLGQGQQPGQGQQGYPSATFPQQPGQ
WQQGSYPSTTSPQQSGQGQQGYNPSGTSTQQPGQVQQ**LGQGQQGYYPIATSPQQPGQGQQLGQGQQPGHGQQLVQGQQQGQ**
GQQGHYPSMTSPHQTGQGQKGYYPSAISPQQSGQGQQGYQPSGASSQGSVQGACQHSTSSPQQQAQGCQASSPKQGLGSLY
YPSGAYTQQKPGQGYNPGGTSPLHQQGGGFGGGLTTEQPQGGKQPFHCQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQT

TVSPHQGQQTTVSPHPGQQTTVSPHQGQQTTVSPHPGQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQTT
VSPHQGQQPGEQPCGFPGQQTTVSLHHGQQSNELYYGSPYHVSVEQPSASLKVAKAQQLAAQLPAMCRLEGGGGLLASQ

Gamma-1-hordein (I6TMV6):

MKILIILTILAMATTFATSEMQVNPSVQVQPTQQQPYPESQQPFISQSPQQFPLPQQPFPQQPQQPFPQSQQQCLQQPQHQ
FPQPTQQFPQRPLLPFTHPFLPFPDQLLPQPPHQSFPQPPQSYPQPPLQPFPQPPQQKYPEQPQQPFPWQQPTIQLYLQQQ
LNSCKEFLLQQCRPVSLLSYLWSKILQQSSCRVMQQQCCLQLAQIPEQYKCTAIDSIVHAIFMQQGQRQGVQIVQQQPQPQ
QVGQCVLVQGQGVAQPQQLAQMEAIRTLVLQSVPSMCNFNVPPNCSTIKAPFVGVVTGVGGQ

Gamma-1-hordein (M0XYT2):

MKILIILIILAMATSFATSEMQVNPSVQVQPTQQQTHPESQQPFIHHSQQQFPQPQQSFPQQPQQPFPQSQQPCLQQPQHQ
FPQPSQPFPRQPLQPFPRPFLPFPEQPLPQPPQESFPQPPQSYPQPPLQPFPQPPQESFPQPPQSYPQPLLQPFPQPPQQQ
YPEQPQQPFPRPPQEQFPNQPQQPFPWQQPSIQLYLQQQLNPCKEFLLQQCRPVSLVSYLWSKIVQQSNCQVMQEQCCLQL
AQIPEQYKCTTIDSIVHAIFMQQGQRQGVQIVQQQPQPQEVGQCVLVQGRDIVQPQQLAQMEAIRSLVLQSVPAMCNFNVP
PNCSTMRAPFFSLVNAGML

Gamma-3-hordein (I6TEV2):

MKIFLLFSLLGVATAITTTTMQFNPSGLELERPQQLFPQWQPLPQQPPFLQQEPEQPYPQQQPLPQQQPFPQQPQLPHQHQ
FPQQLPQQQFPQQMPLQPQQQPQFPQQKPFGQYQQPLTQQPYPQQQPLAQQQPSIEEQHQLNLCKEFLLQQCTLDEKVPLL
QSVISFLRPHISQQNSCQLKRQQCCQQLANINEQSRCPAIQTIVHAIVMQQQQQQVQQQVDHGFVQSQLQQLGQGMPIQLQ
QQPGQAFVLPQQQAQFKVVGSLVIQTLPMLCNVHVPPYCSPFGSMATGSGGQ

Figure S1. Amino acid sequence coverage of representative hordeins from each class as analysed using liquid chromatography mass spectrometry. The discovery data presented here was derived from a previous study of barley cv Sloop (Colgrave *et al*, 2016, Anal. Chem., 88, 9127). Legend: amino-acids in green were confidently identified (≥95%), while amino acids in yellow were identified with 50-95% confidence and those in red with <50% confidence. Residues in grey were not detected. There was no evidence of signal peptides (predicted by SignalP server, underlined).

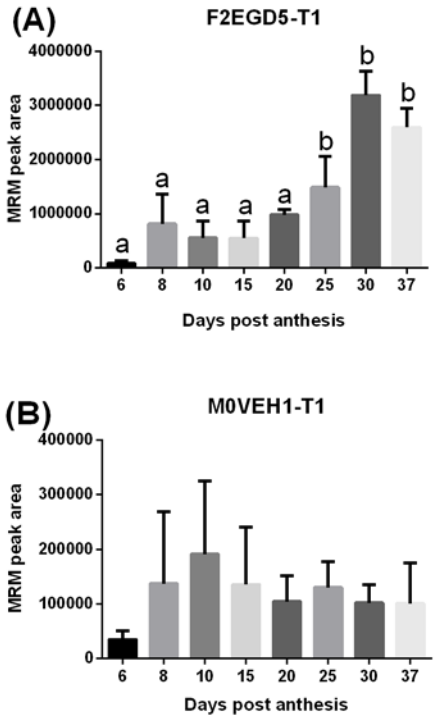

Figure S2. The effect of maturity (days post anthesis, DPA) on the accumulation of the indicated avenin-like protein (ALP) specific peptides following trypsin cleavage. The mean multiple reaction monitoring (MRM) peak area ± SE (n=3) from 5 µg extracted protein is shown for the indicated peptide. Within A, points with different letters were significantly different at $p<0.05$ by one-way ANOVA and Tukey's test. No points were significantly different in B.

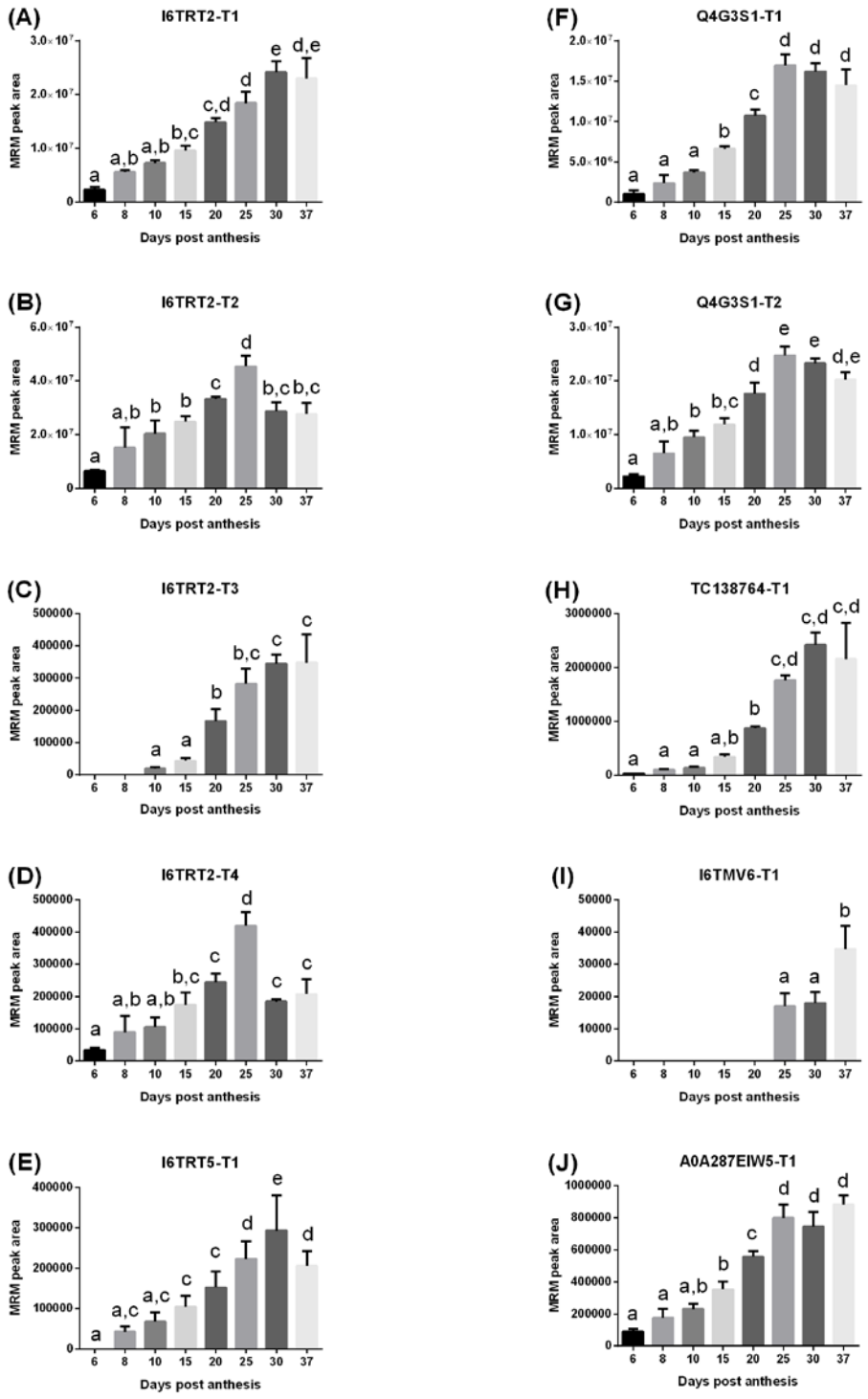

Figure S3. The effect of maturity (days post anthesis, DPA) on the accumulation of the indicated B1-hordein specific peptides following trypsin cleavage. The mean multiple reaction monitoring (MRM) peak area ± SE (n=3) from 5 µg extracted protein is shown for the indicated peptide. Within each graph, points with different letters were significantly different at $p<0.05$ by one-way ANOVA and Tukey's test.

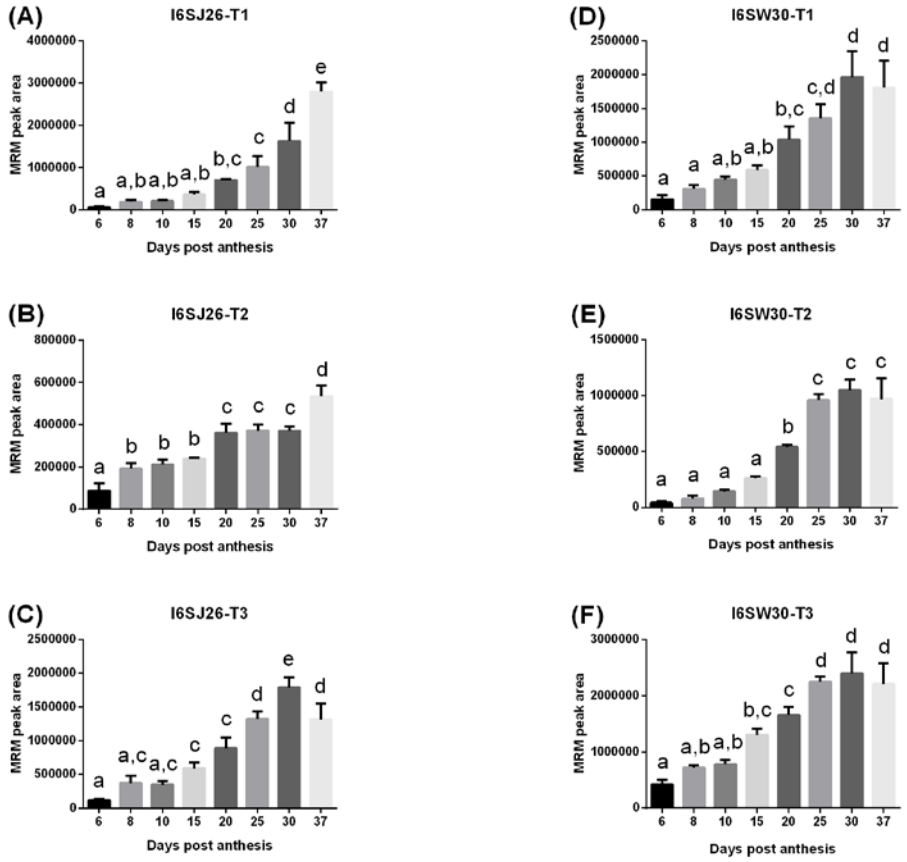

Figure S4. The effect of maturity (days post anthesis, DPA) on the accumulation of B3-hordein specific peptides following trypsin cleavage. The mean multiple reaction monitoring (MRM) peak area ± SE (n=3) from 5 µg extracted protein is shown for the indicated peptide. Within each graph, points with different letters were significantly different at $p<0.05$ by one-way ANOVA and Tukey's test.

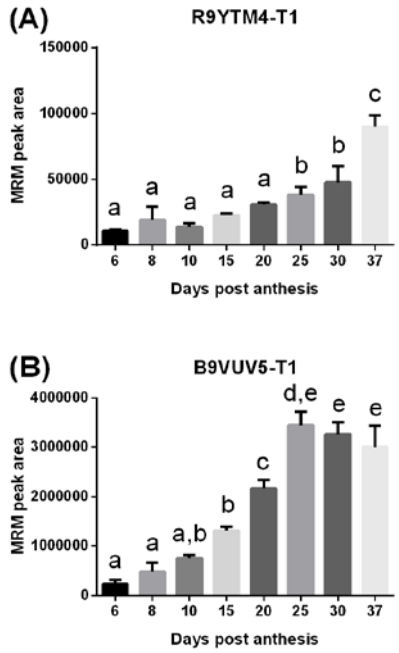

Figure S5. The effect of maturity (days post anthesis, DPA) on the accumulation of the indicated B-hordein (identified by mapping peptides to low molecular weight glutenin subunits, LMW-GS) specific peptides following trypsin cleavage. The mean multiple reaction monitoring (MRM) peak area ± SE (n=3) from 5 µg extracted protein is shown for the indicated peptide. Within each graph, points with different letters were significantly different at $p<0.05$ by one-way ANOVA and Tukey's test.

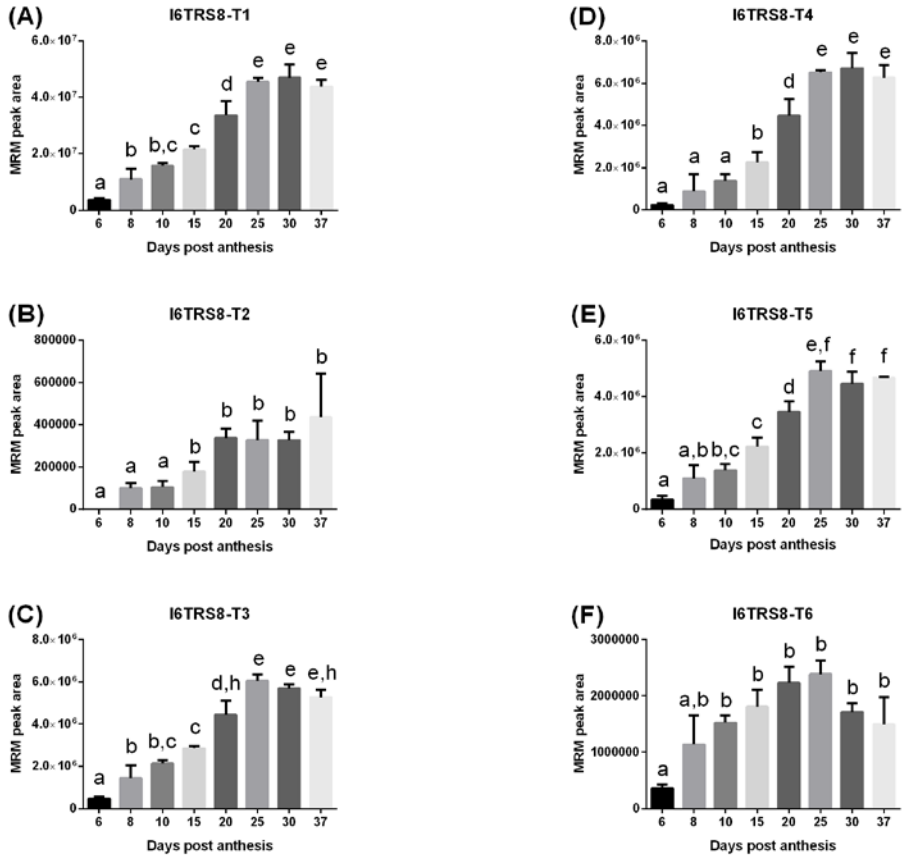

Figure S6. The effect of maturity (days post anthesis, DPA) on the accumulation of the indicated D-hordein specific peptides following trypsin cleavage. The mean multiple reaction monitoring (MRM) peak area ± SE (n=3) from 5 µg extracted protein is shown for the indicated peptide. Within each graph, points with different letters were significantly different at $p<0.05$ by one-way ANOVA and Tukey's test.

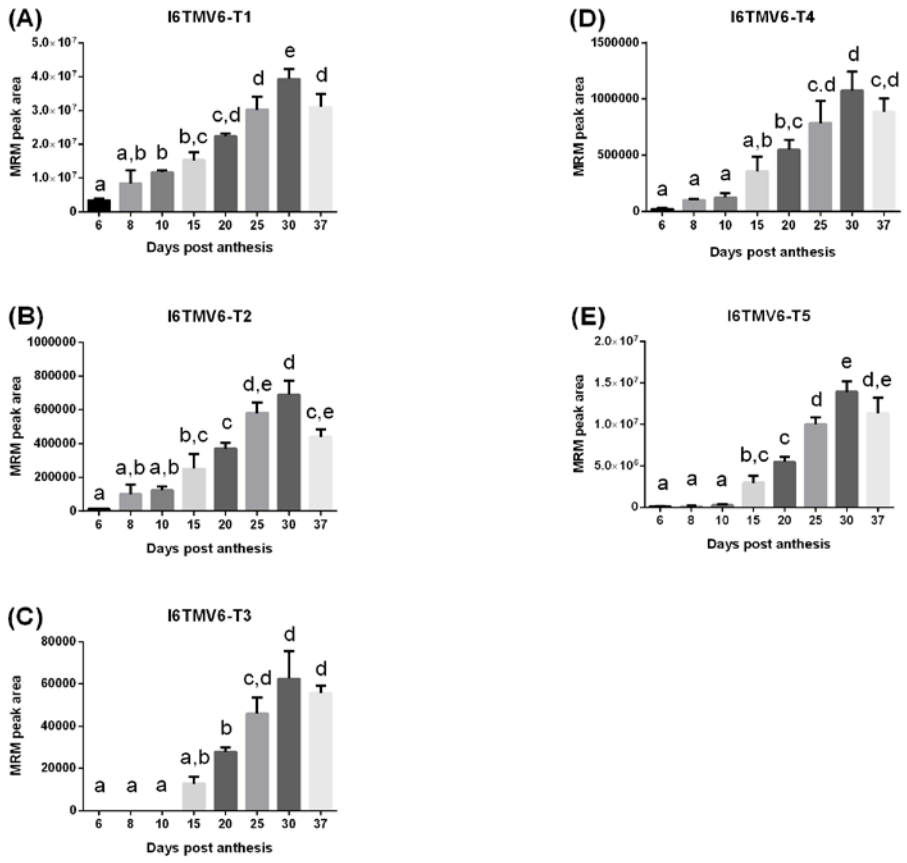

Figure S7. The effect of maturity (days post anthesis, DPA) on the accumulation of the indicated gamma-1-hordein specific peptides following trypsin cleavage. The mean multiple reaction monitoring (MRM) peak area ± SE (n=3) from 5 µg extracted protein is shown for the indicated peptide. Within each graph, points with different letters were significantly different at $p<0.05$ by one-way ANOVA and Tukey's test.

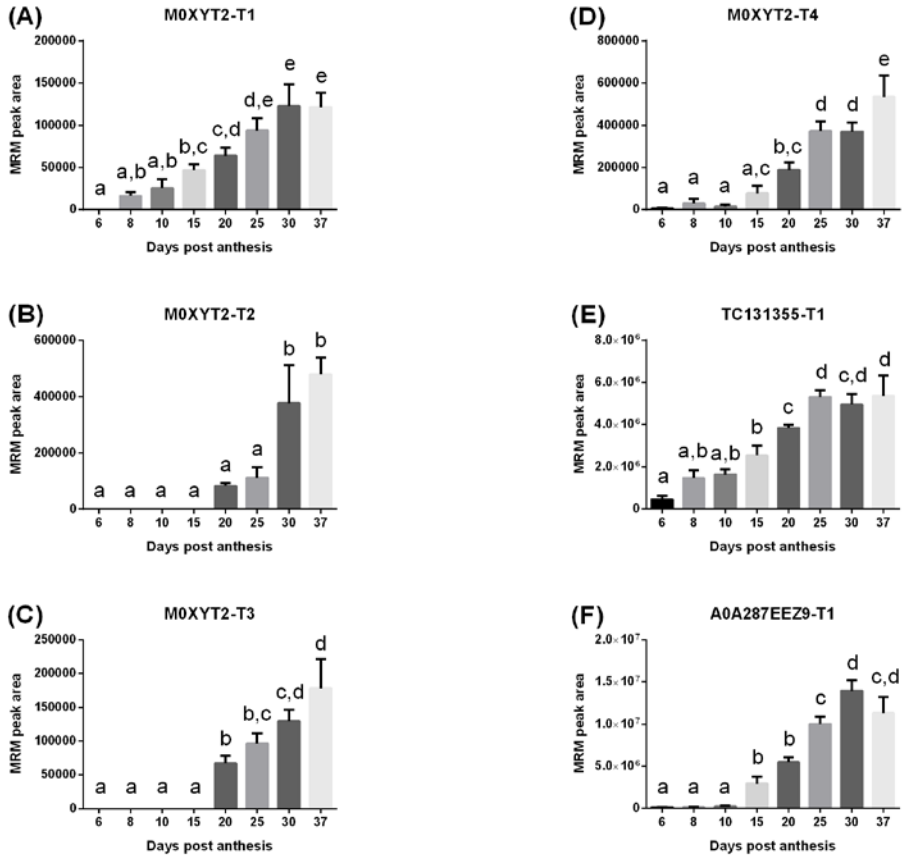

Figure S8. The effect of maturity (days post anthesis, DPA) on the accumulation of the indicated gamma-1-hordein specific peptides following trypsin cleavage. The mean multiple reaction monitoring (MRM) peak area ± SE (n=3) from 5 µg extracted protein is shown for the indicated peptide. Within each graph, points with different letters were significantly different at $p<0.05$ by one-way ANOVA and Tukey's test.

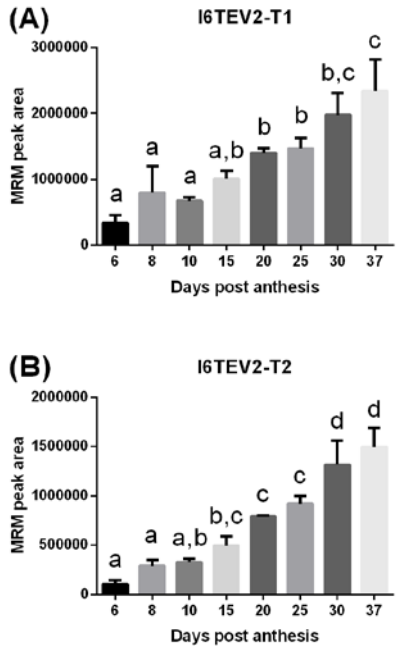

Figure S9. The effect of maturity (days post anthesis, DPA) on the accumulation of the indicated gamma-3-hordein specific peptides following trypsin cleavage. The mean multiple reaction monitoring (MRM) peak area ± SE (n=3) from 5 µg extracted protein is shown for the indicated peptide. Within each graph, points with different letters were significantly different at $p<0.05$ by one-way ANOVA and Tukey's test.

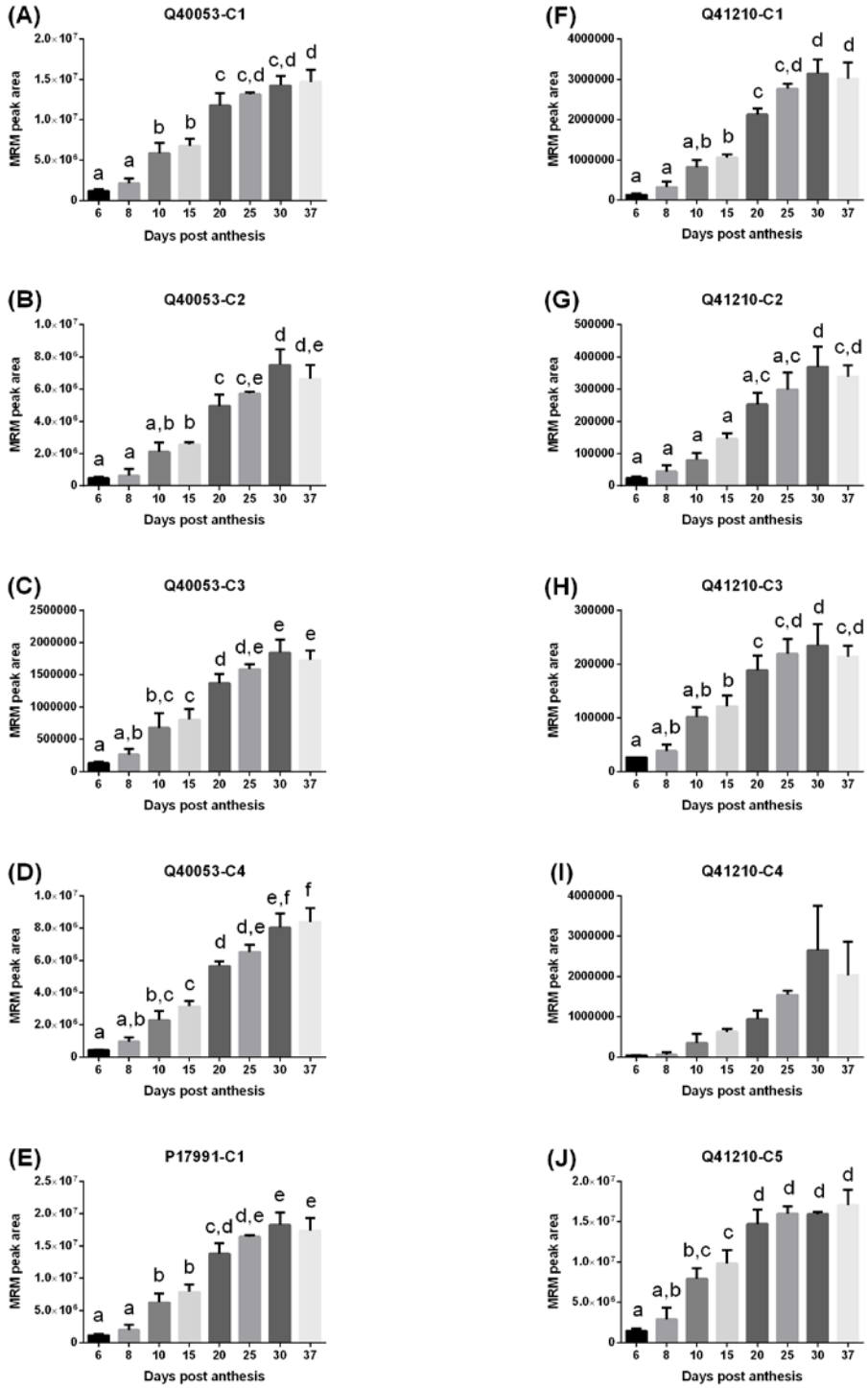

Figure S10. The effect of maturity (days post anthesis, DPA) on the accumulation of the indicated C-hordein specific peptides following chymotrypsin cleavage. The mean multiple reaction monitoring (MRM) peak area ± SE (n=3) from 5 µg extracted protein is shown for the indicated peptide. Within each graph, points with different letters were significantly different at $p<0.05$ by one-way ANOVA and Tukey's test.