

Table S1. Metabolic pathways enriched in upregulated genes by OsSRT1 RNAi

KEGG ID	Pathway name	P-value
osa00051	Fructose and mannose metabolism	0.00515
osa00010	Glycolysis/Gluconeogenesis	0.00528
osa00480	Glutathione metabolism	0.013078
osa04145	Phagosome	0.014308
osa00305	Tyrosine metabolism	0.01998
osa00940	Phenylpropanoid biosynthesis	0.020105
osa00030	Pentose phosphate pathway	0.02298
osa00710	Carbon fixation photosynthetic organism	0.024823

Table S2. OsGAPDH1 peptides Lys modification identified by mass spectrometry.

peptide	q-value
AVG K LPDLNGKLTGMSFR	0.0016
I KS K TLLGEKPVTVFGIR	0.0000
I KIGINGFGR	0.0000
K VVISAPSK	0.0000
L KGIIGYVEEDLVSTDFVGDSR	0.0000
SAIK S ASEGKLK	0.0000
TLLL G EKPVTVFGIR	0.0000
TV D GPSS K DWR	0.0000
VIHDNFG I IEGLMTTVHAI T ATQ K TV D GPSSK	0.0086
VLPDLNG K LTGMSFR	0.0030
V V VISAPSKDAPMFVCGVNED K YTSDIDIVSNASCT	0.0000
YDTVHGQW K HSDIK	0.0000
AASFNIIP S STGAA K AVGK	0.0000
IE K AAASYDAIK	0.0000
NPDEIPWAEAGAEYVVESTGVFTD K EK	0.0000
AASYDAI K SAIK	0.0000

Table S3. Summary of glycolytic genes used in the study

Locus	Annotation	Gene name
LOC_Os01g09570	ATP-dependent 6-phosphofructokinase 6	6-PFK 6
LOC_Os02g32490	Acetyl-coa synthetase	AcCoA Syn
LOC_Os04g38530	aldose 1-epimerase, putative, expressed	Ald-ep
LOC_Os04g38540	aldose 1-epimerase, putative, expressed	Ald-ep
LOC_Os05g36270	fructose-1,6-bisphosphatase, putative, expressed	F-1,6-BP
LOC_Os06g45590	glyceraldehyde-3-phosphate dehydrogenase GAPCP1, chloroplastic	GAPCP1
LOC_Os09g30240	ATP-dependent 6-phosphofructokinase 2	6-PFK 2
LOC_Os07g26540	hexokinase, putative, expressed	HXK1
LOC_Os08g03290	glyceraldehyde-3-phosphate dehydrogenase, putative, expressed	GAPDH1

Table S4 Primers for qPCR, genotyping and amino acid mutagenesis

Pimer name	Sequence (5'-3')
LOC_Os01g09570F	TACTGCACCCCTGCTAGCTC
LOC_Os01g09570R	CTCCGTGATTCTGTAGAACG
LOC_Os02g32490F	GTTGAGCATGAAGTGAAAGG
LOC_Os02g32490R	ATGAGGCTTTCCGTAGTTC
LOC_Os04g38530F	GTGAACCACCCCAACTTC
LOC_Os04g38530R	AGAACCGAGAACTTGTACACCAT
LOC_Os04g38540F	CAAGGTGTACCAGCAGTACG
LOC_Os04g38540R	GAGAACTTGAAGGCCATGT
LOC_Os05g36270F	CTGGGGAGCTATGATGACG
LOC_Os05g36270R	TGGTGAAGCCGTACCGTGT
LOC_Os06g45590F	CGAATGATTCAATTGGTGAC
LOC_Os06g45590R	TTGTCGTACCAAGAGACGAG
LOC_Os09g30240F	ATCAGCCCGACTTTATCCG
LOC_Os09g30240R	CTGGTTCAACCCAAACCAA
LOC_Os07g26540F	AGCAGACCTACGAGAACGTCAT
LOC_Os07g26540R	CCTCCCGATCTTCTTCAGGAT
LOC_Os08g03290F	GATAACTTGTCAAGCTTGTGC
LOC_Os08g03290R	CAGCATAGACAAAGCATACCGG
GAPDHmutantF	TCAAATGCTAGCTGCACAC
GAPDHmutantR	GCTTCCCTCTGATGCAGAC
N	AATCCAGATCCCCGAATTA
qRTActin1-F	TGTATGCCAGTGGTCGTACCA
qRTActin1-R	CCAGCAAGGTCGAGACGAA
R(57/74)-F	CAATGGAGGCACAGCGACATCAAGATCAAGGACTC CAAGACTCTGCTCTGGCGAGAGGCCGGTC
R(57/74)-R	GACCGGCCTCTGCCCAAGAGCAGAGTCTGGAGT CCTTGATCTTGATGTCGCTGTGCCTCCATTG
R(120)-F	GGTGCCAAGAGGGTTTCATC
R(120)-R	GATGACAACCCCTTGGCACC
R(217)-F	GGTGCTGCCAGAGCTTGGC
R(217)-R	GCCAACAGCTTGGCAGCACC
R(261/265)-F	CTATCAGGAGTGCTATCAGGTCTGC
R(261/265)-R	GCAGACCTGATAGCACTCCTGATAG

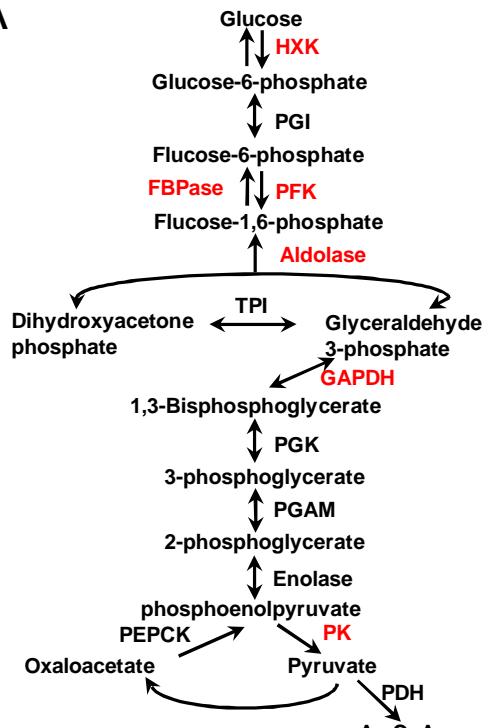
Table S5 Primers for ChIP-PCR

Pimer name	Sequence (5'-3')
LOC_Os01g09570ChIP-F	GCACACACATCTTCTCTAG
LOC_Os01g09570ChIP-R	GTGCTTCTGGTTGCGG
LOC_Os02g32490ChIP-F	CCGAATAAAATTAACTAAC
LOC_Os02g32490ChIP-R	CGCTGAATTGAAAAAAG
LOC_Os04g38530ChIP-F	GCGAGCGAATATTGCCG
LOC_Os04g38530ChIP-R	GATCCGTGCCCACGCC
LOC_Os04g38540ChIP-F	CGTTTCAGTTCTTTTTCC
LOC_Os04g38540ChIP-R	GATGACAATTGTCATTGG
LOC_Os05g36270ChIP-F	GGAAGAAGGGAGGTGATC
LOC_Os05g36270ChIP-R	CGTTGGCAAGCCGCGTCCGG
LOC_Os06g45590ChIP-F	GTAAAGAGAGAACAAAC
LOC_Os06g45590ChIP-R	TTAGATTGTTAATAACATG
LOC_Os09g30240ChIP-F	GTGGGACCAAGAGAGTCG
LOC_Os09g30240ChIP-R	GGGCTCGCGTTATGGC
LOC_Os07g26540ChIP-F	CGAGAGGTGAAACATGTGA
LOC_Os07g26540ChIP-R	CAACAGGAGACTGCTACAGG
LOC_Os08g03290CHIP-F	CGTCCTCGCATCTCCACT
LOC_Os08g03290CHIP-R	GCGAACGCCAGCAGTAA
LOC_Os01g09570CHIP-2F	TCGAAGTGAAGGAGTCCC
LOC_Os01g09570CHIP-2R	CTTCTTCTTCATCGCCCAC
LOC_Os02g32490CHIP-2F	AGCCATCGCACTCTGCA
LOC_Os02g32490CHIP-2R	TGGCGGTCGTCTTCCTC
LOC_Os04g38530CHIP-2F	GAGACGCCCTGGGAGCA
LOC_Os04g38530CHIP-2R	GCCGGATGGACGGATAT
LOC_Os04g38540CHIP-2F	CGCTGCGACTTGAGGACA
LOC_Os04g38540CHIP-2R	CCAGCCTGGTTACGGACA
LOC_Os05g36270CHIP-2F	CGCCGCTACCTCCTCAT
LOC_Os05g36270CHIP-2R	TGCTCCTTGCAGAAAACG
LOC_Os06g45590CHIP-2F	TGTTGTTCGTATGGCGATGT
LOC_Os06g45590CHIP-2R	GCGAACGAATCTCAACTGAA
LOC_Os09g30240CHIP-2F	GCCGCACATGACCAACCA
LOC_Os09g30240CHIP-2R	GATCGGGTCGGGATGG
LOC_Os07g26540ChIP-2F	GATCGCCAAGAACCTTC
LOC_Os07g26540ChIP-2R	CGTCACCACCTCTGATCT
LOC_Os08g03290CHIP-2F	GTTCGTGCTGTGCTGGGTG
LOC_Os08g03290CHIP-2R	CGATCCTCCGAAACCTGA
ChIPActin1F	TGCGTGTCCCTTCCATCT
ChIPActin1R	AAGGCGAATGAAGCGAAAAG

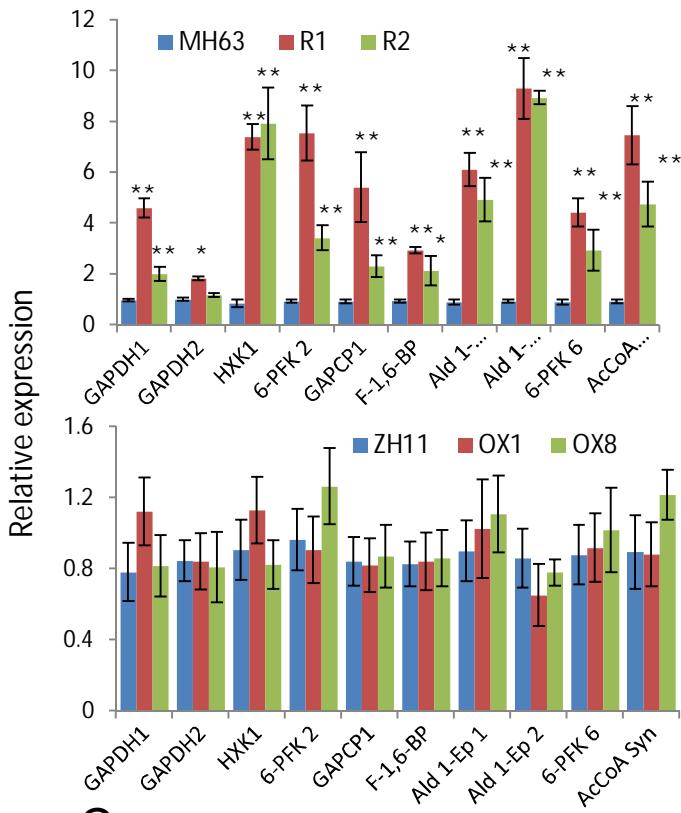
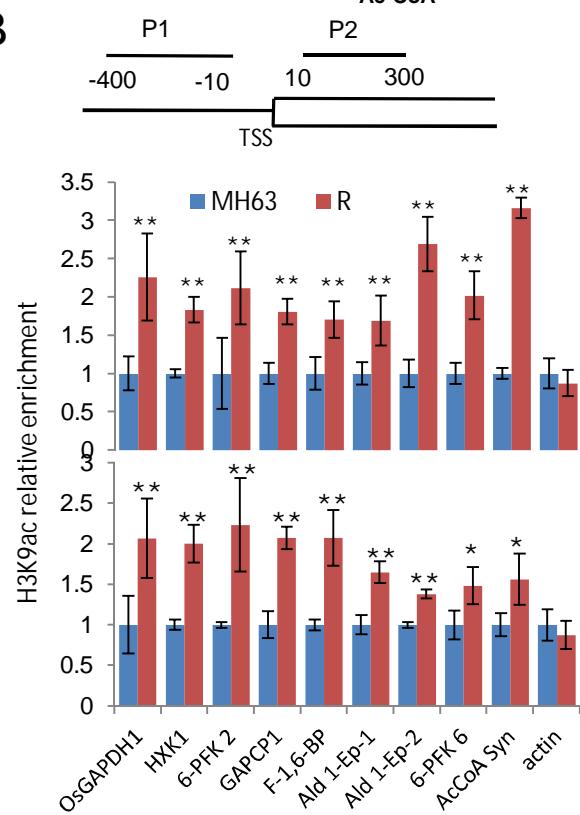
Table S5 Primers for ChIP-PCR (continue)

Pimer name	Sequence (5'-3')
LOC_Os01g09570CHIP-3F	CCGACAGCCCGCGTCGACC
LOC_Os01g09570CHIP-3R	AGGAGGGAGGGCCGCAAC
LOC_Os01g09570CHIP-4F	ATGTTTTTTGGCCTGTG
LOC_Os01g09570CHIP-4R	ACATTTGCTTAAACATTATC
LOC_Os02g32490CHIP-3F	CAGCGAGCAATCGGGTG
LOC_Os02g32490CHIP-3R	CATTACTCGTCATAAATTG
LOC_Os02g32490CHIP-4F	GGGACATGAATATCAATC
LOC_Os02g32490CHIP-4R	AACCTTAGCACATCAC
LOC_Os04g38530CHIP-3F	GACACAAAAGCCATCCAGATG
LOC_Os04g38530CHIP-3R	GATCGATGGTACATGGACGG
LOC_Os04g38530CHIP-4F	GCTCATATTTCACTGTTA
LOC_Os04g38530CHIP-4R	TGCTGTTCTACAATCCATA
LOC_Os04g38540CHIP-3F	GTGCGAGGAAGACGGTCG
LOC_Os04g38540CHIP-3R	GACGGTACCTTGGAATC
LOC_Os04g38540CHIP-4F	GTACTTGTCTGATCAAGA
LOC_Os04g38540CHIP-4R	AAAATTCCACCATTTTG
LOC_Os05g36270CHIP-3F	CGTCAACAAGGCCGGCCT
LOC_Os05g36270CHIP-3R	CATGGATTGTGTGGAGGAAC
LOC_Os05g36270CHIP-4F	CAGCCACGAGTTCGACAAG
LOC_Os05g36270CHIP-4R	ATCGATATTGAGGGAGCC
LOC_Os06g45590CHIP-3F	ACGTGACTTCTCTTTGG
LOC_Os06g45590CHIP-3R	TTATTGAGGATTGACTCCG
LOC_Os06g45590CHIP-4F	AGATTATTCGAATTCCCTG
LOC_Os06g45590CHIP-4R	CACAGAGCAAAACCACA
LOC_Os09g30240CHIP-3F	GAGAGCTCGTCGTCGGGCT
LOC_Os09g30240CHIP-3R	CGAGCGGGACGACCTCGT
LOC_Os09g30240CHIP-4F	ACTGCTGCCTCATCCCGGA
LOC_Os09g30240CHIP-4R	CTTGGGATGAGACGTTGGC
LOC_Os07g26540CHIP-3F	CTGTGCATCTTGCTACGTTG
LOC_Os07g26540CHIP-3R	GCAACGGAAAAATAATAGGG
LOC_Os07g26540CHIP-4F	TCTCCTCCCCGTGCACCAA
LOC_Os07g26540CHIP-4R	TGCAGCGCCGCCACGACGTCC
LOC_Os08g03290CHIP-3F	GTATGTGTCCTAACAGATTG
LOC_Os08g03290CHIP-3R	TGTATGACAGGTGGGATCA
LOC_Os08g03290CHIP-4F	CTACTGCATATGGCTTGA
LOC_Os08g03290CHIP-4R	TTCCATTGGCCGTGCACGGTAT

A



B



C

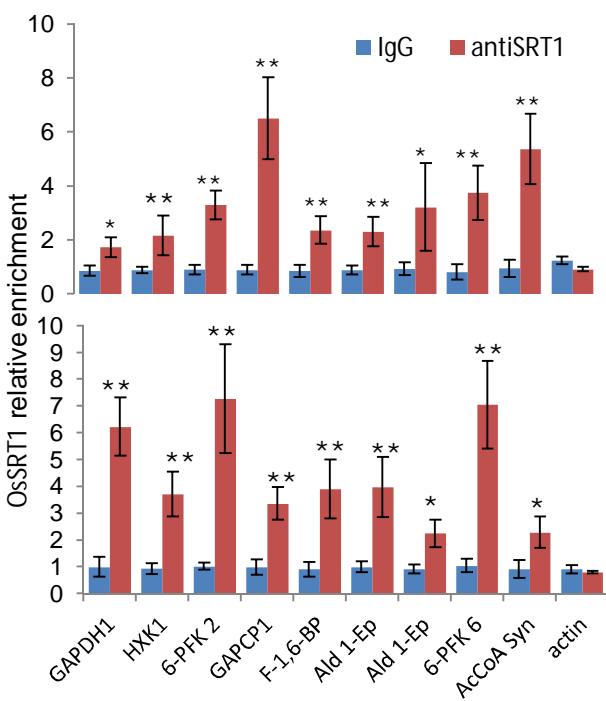


Figure S1. OsSRT1 represses glycolytic gene expression. (A) Left, analyzed genes in glycolysis pathway are indicated in red. Right, transcript levels of the genes in two *OsSRT1*RNAi lines (R1, R2) compared to wild type seedlings. (B) H3K9ac levels of glycolytic genes in wild type (MH63) and combined RNAi lines R1 and R2 (R) detected by ChIP-PCR. P1 is located between -400 to -10 relative to TSS. P2 is located between +10 to +300 relative to TSS. The exact positions of the primers are indicated in Table S2. (C) Enrichment of OsSRT1protein on glycolytic gene loci in wild type plants by ChIP-PCR using anti-OsSRT1 and IgG as controls. The used primer sets are the same as in (B). Bars represent means+/-SD of three biological replicates of the RT-PCR and ChIP-PCR experiments. Significance of differences are indicated (** p<0.01; *p<0.05).

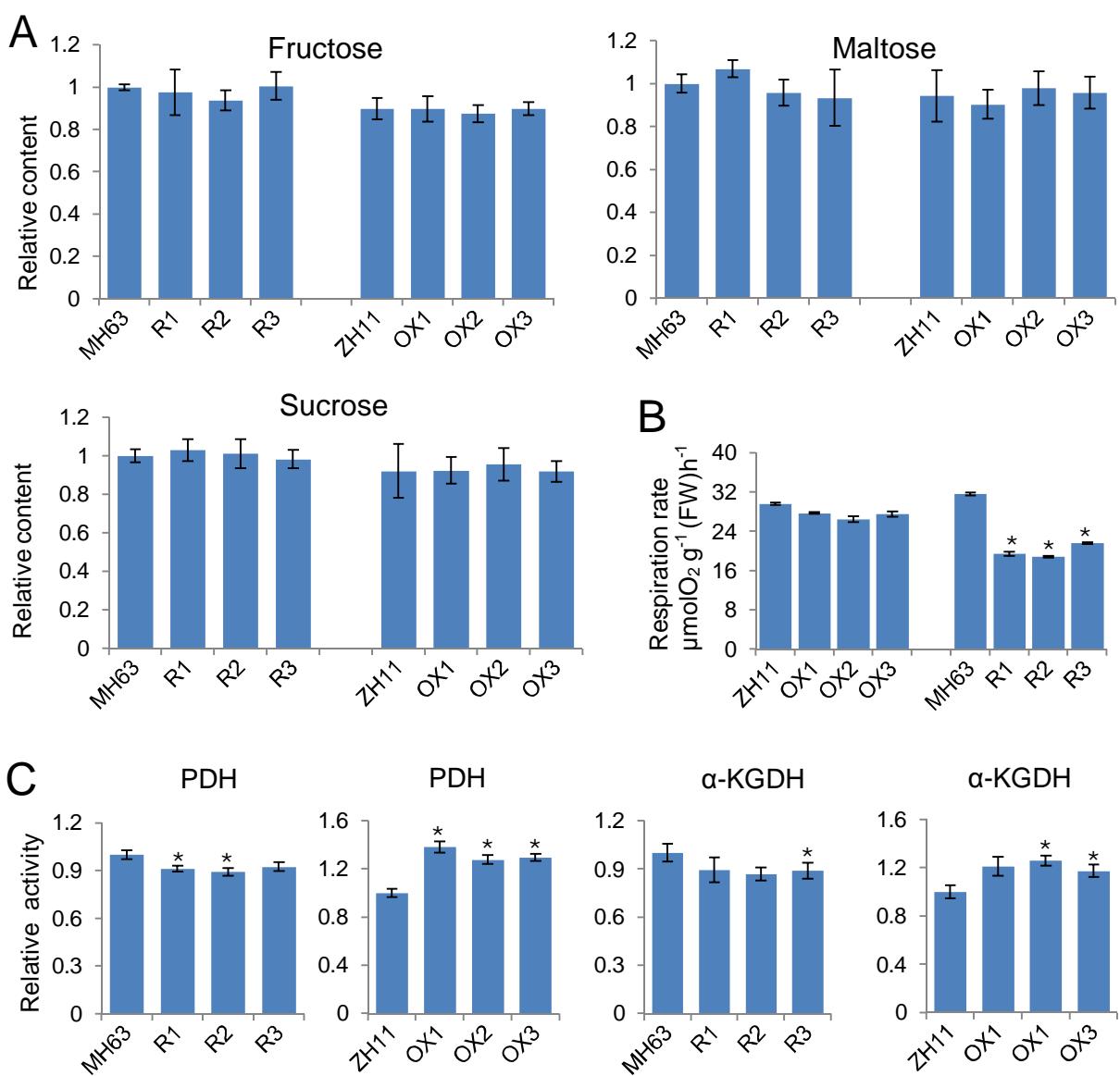


Figure S2. OsSRT1 RNAi and over-expression effects on sugar accumulation and mitochondrial respiration. (A) Sugar contents in *OsSRT1* transgenic (RNAi, over-expression) plants compared to the respective wild type (MH63 and ZH11). (B) Mitochondrial respiration rates in *OsSRT1* RNAi and over-expression leaves compared to the respective wild type, measured 30 min after light to dark transition. (C) Activities of pyruvate dehydrogenase and α -ketoglutarate dehydrogenase in *OsSRT1* transgenic plants compared to wild type. Bars= means +/- SD of 3 biological replicates (each replicates with 3 technical repetitions). Significances of differences between wild type and transgenic lines are indicated (* P value < 0.05, Chi-2 tests).

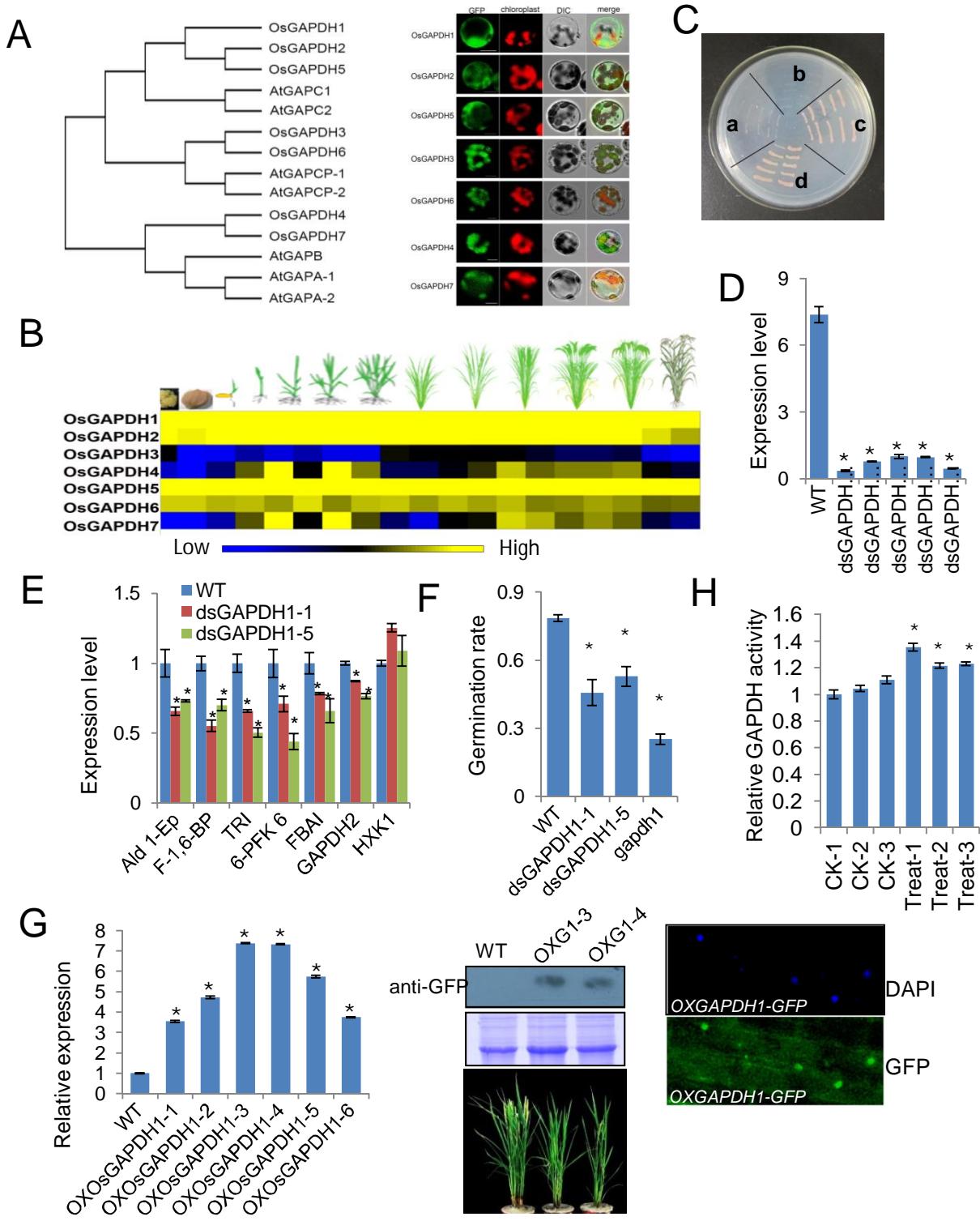


Figure S3. Rice GAPDH genes and OsGAPDH1 RNAi and over-expression lines. (A) Phylogenetic relationship of rice and Arabidopsis GAPDH gene families and the Subcellular localization of rice GAPDH-GFP proteins. (B) Expression profiles of rice GAPDH genes at different developmental stages. (C) Protein interaction between OsGAPDH1 and OsGAPDH2 in yeast two hybrid assays. (D) Transcript levels (relative to Actin1) of OsGAPDH1 in wild type and 5 RNAi (ds) lines. (E) Transcript levels of glycolytic genes in two RNAi lines compared to wild type. (F) Seed germination rates of two RNAi lines and the T-DNA mutant compared to wild type. (G) Production of OsSRT1 over-expression plants. Left, OsGAPDH1 transcript levels in 35S::OsGAPDH1-GFP transgenic lines. Middle, protein level of OsGAPDH1-GFP in two line and phenotype at mature stage. Right, detection of OsGAPDH1-GFP in root cells (upper part, DAPI; lower part, GFP fluorescence). (H) Relative GAPDH activity in 12 d seedlings treated with or without 0.1% H_2O_2 . Bars are means +/- SD of three biological replicates. Significances of difference are indicated * ($p < 0.01$).

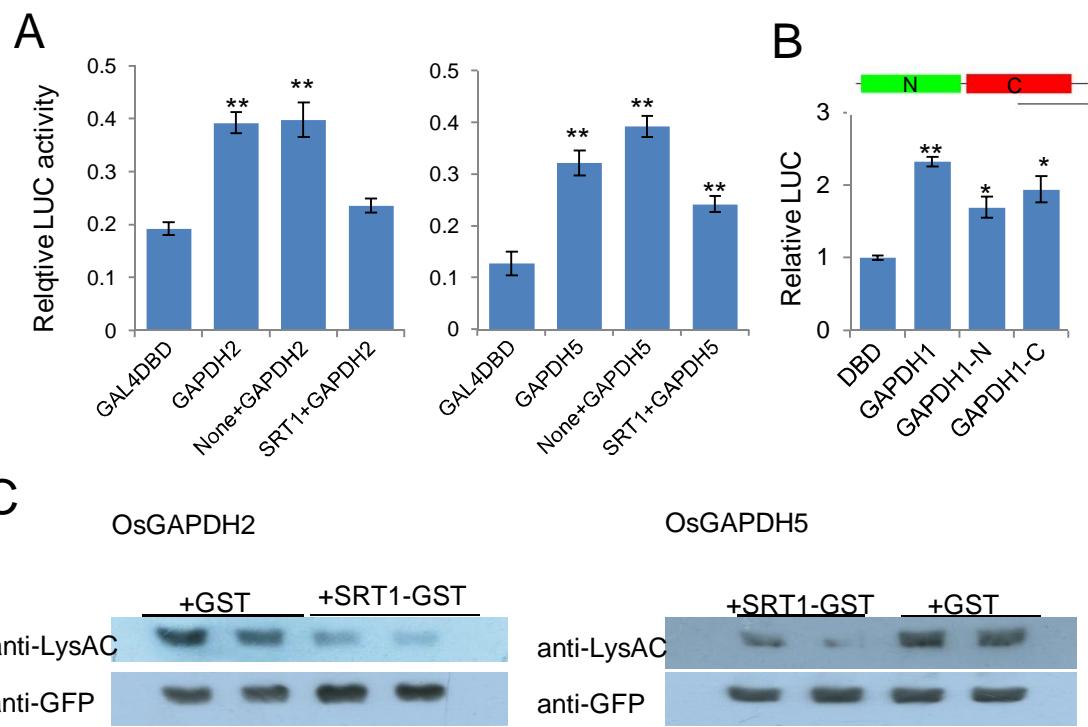


Figure S4. OsSRT1-regulated transcriptional function and lysine deacetylation of rice GAPDH proteins. (A) Transactivation of UAS reporter promoters (described in Figure 4A) by OsGAPDH2, and OsGAPDH5 fused with DBD in the presence or absence of OsSRT1 assayed in transfected tobacco protoplasts. (B) Deletion analysis of OsGAPDH1 transcription activity. Grey bar=100 amino acids. (C) E coli-produced OsSRT1-GST protein deacetylates tobacco cell -produced OsGAPDH2-GFP and OsGAPDH5-GFP in vitro. Anti-GFP was used for loading controls and anti-Lys detected lysine acetylation levels of OsGAPDH1-GFP in the absence or presence of OsSRT1-GST. Significances of differences between wild type and transgenic lines are indicated (* P value < 0.05; (** P value < 0.01).

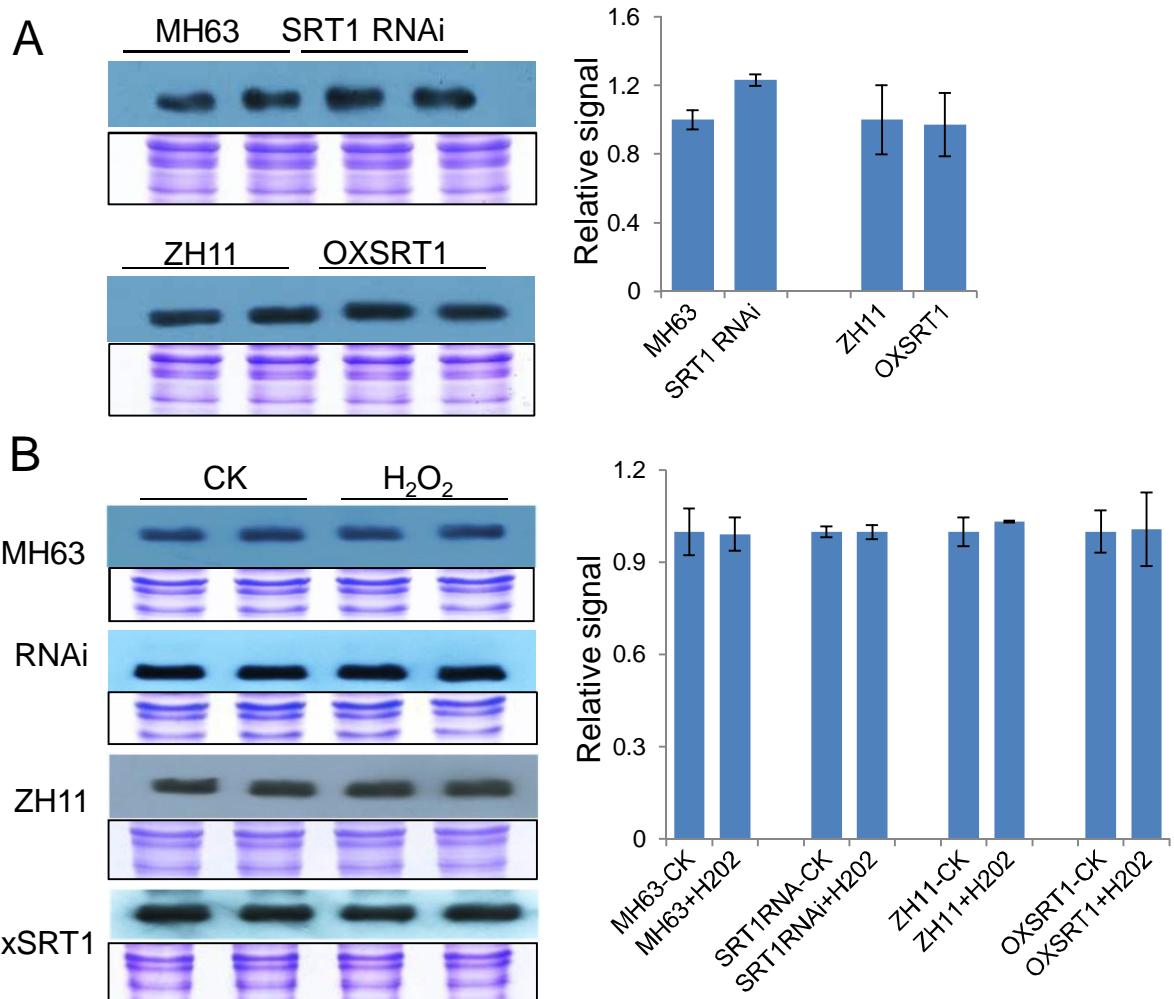
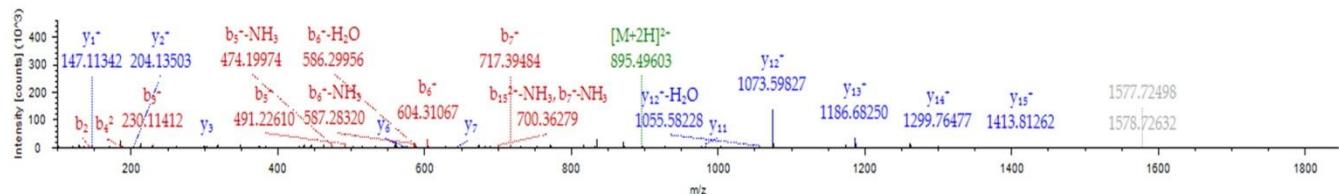


Figure S5. Relative endogenous GAPDH protein levels. GAPDH protein levels in Wild type (ZH11, MH63), SRT1 RNAi and overexpression (Ox) seedling leaves under normal growth conditions (A) and treated with or without H_2O_2 (0.1 %) during 30 min (B) were detected by immunoblots using anti-GAPDH antibody. The bands were quantified by the ImageJ program. Bars are means \pm SD from three measures of each of the two repeats.

Supplemental Data Set 1. MS spectra of 16 GAPDH1 peptides

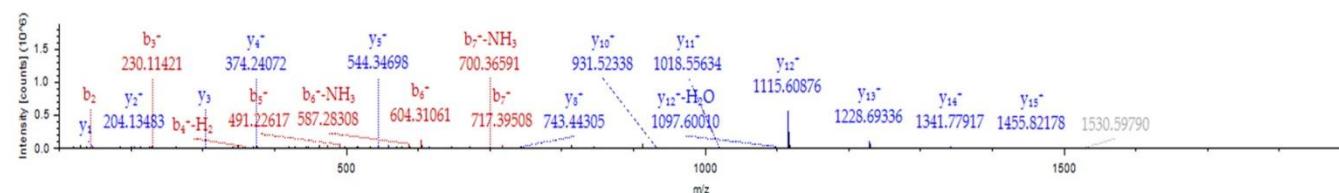
AASFNI IPSSTGAAKAVGK

$z=+2$, Mono $m/z=895.49689$ Da, $MH+=1789.98650$ Da, Match Tol.=0.02 Da



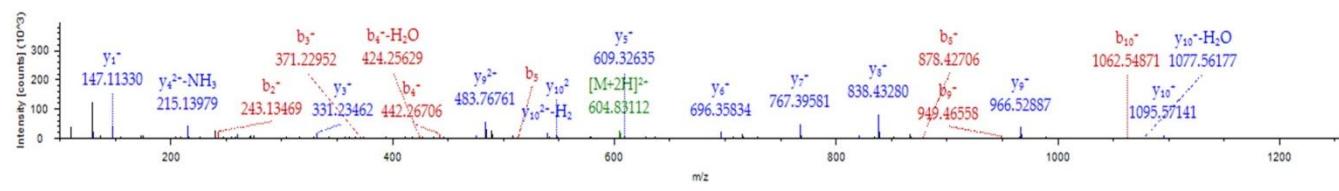
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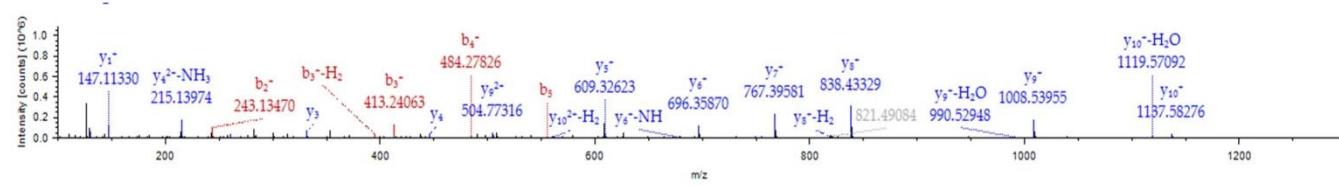
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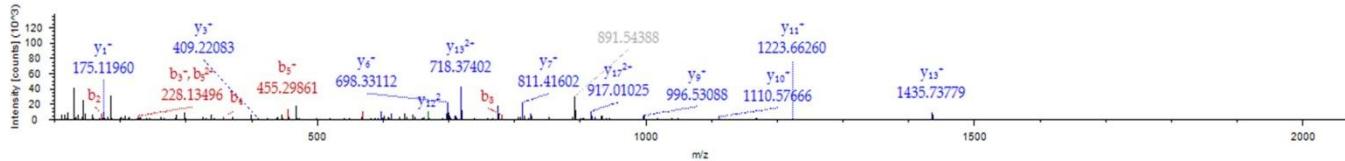
IEK (ac) AASYDAIK

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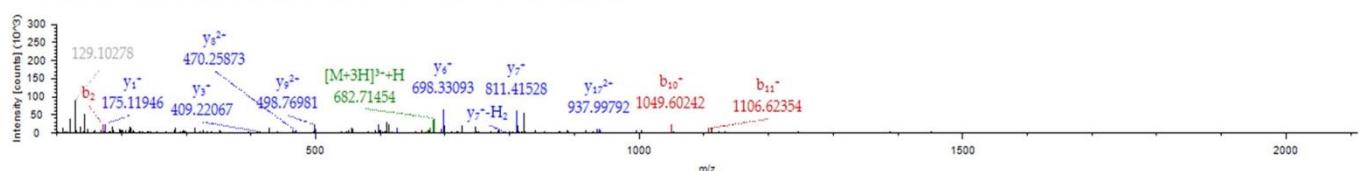
AVGKVLPDNLNGKLTGMSFR

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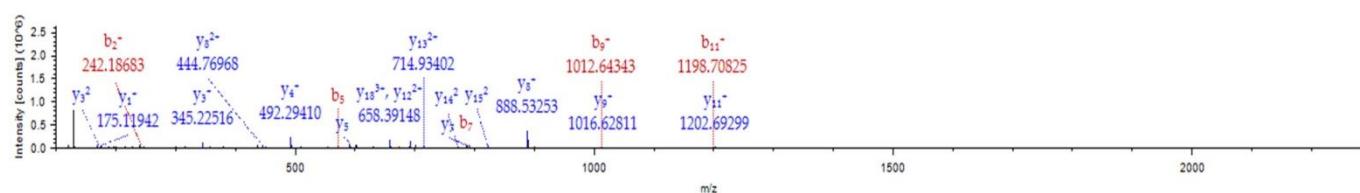
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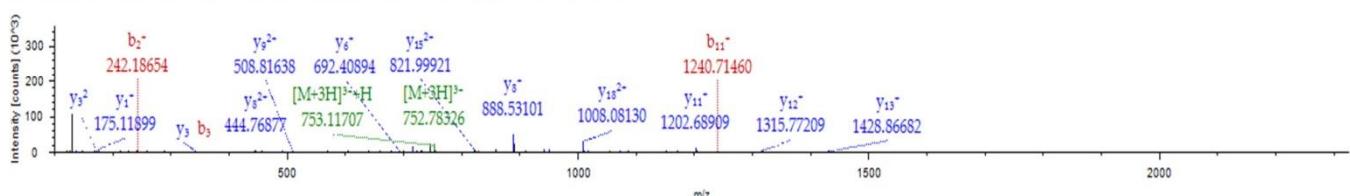
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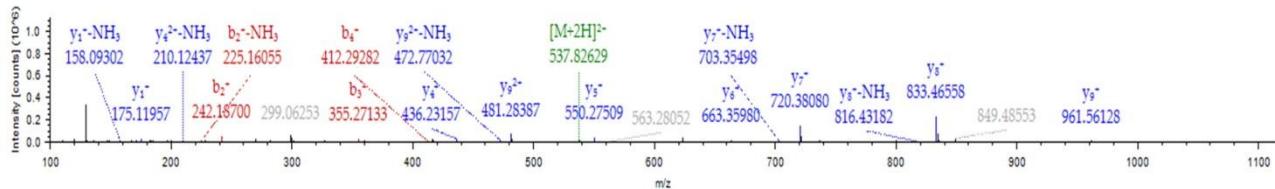
IKDSK (ac) TLLLEGEKPVTVFGIR

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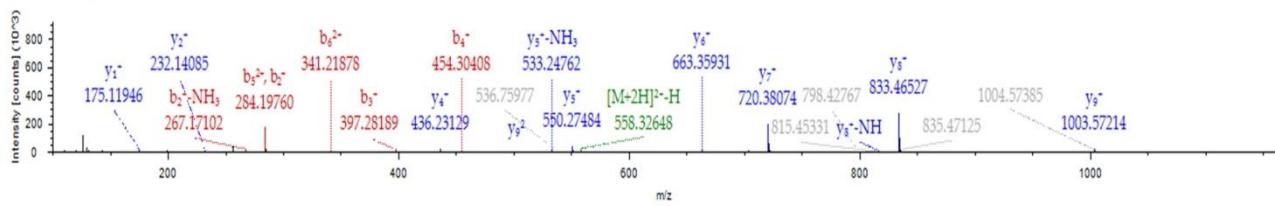
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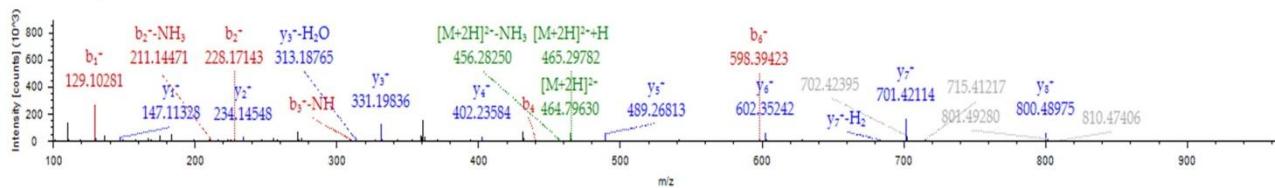
IK(ac)IGINGFGR

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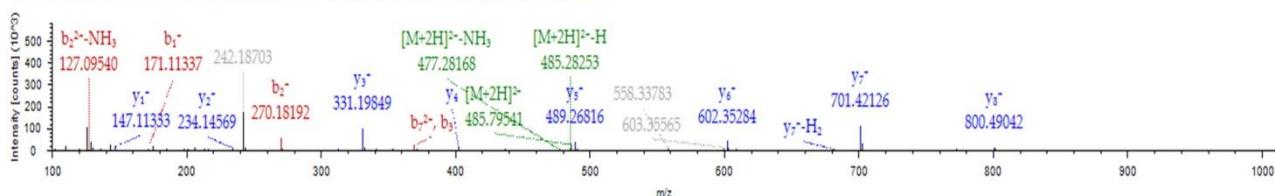
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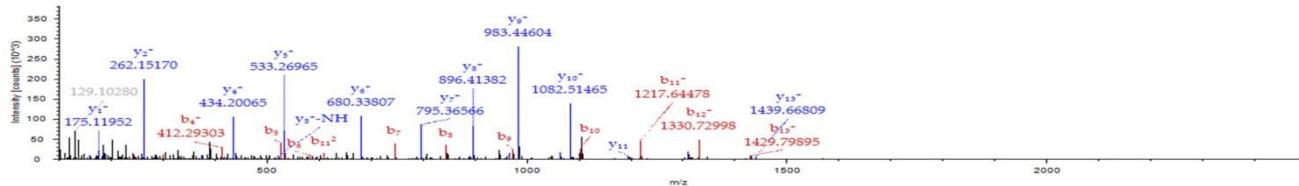
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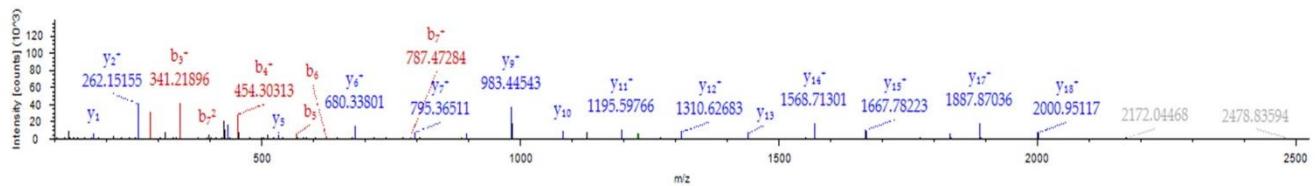
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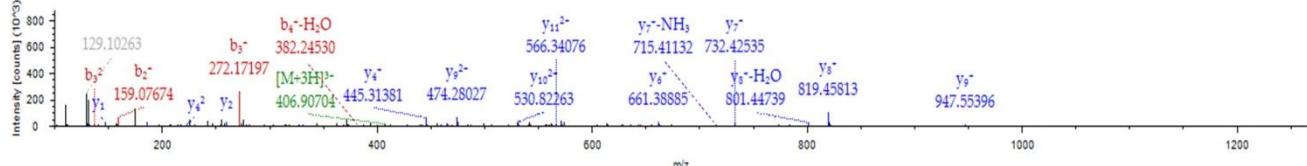
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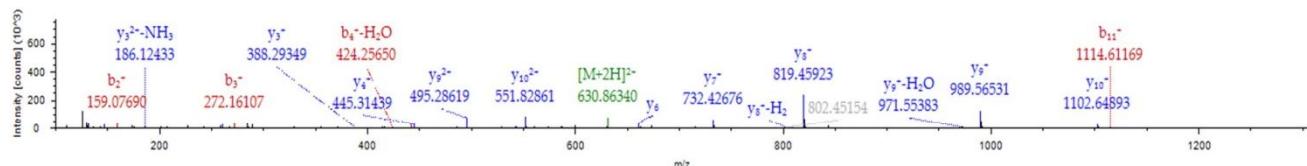
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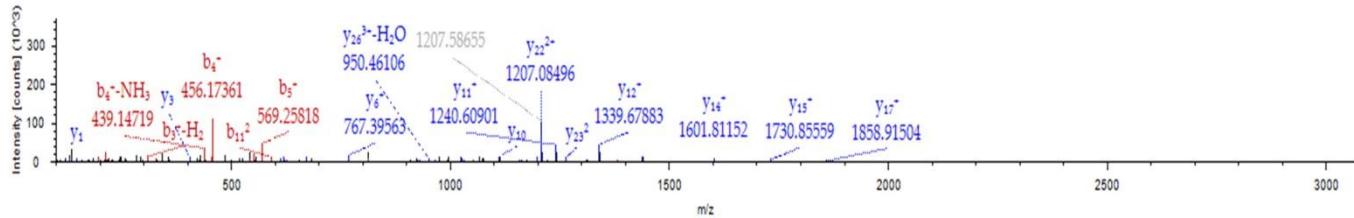
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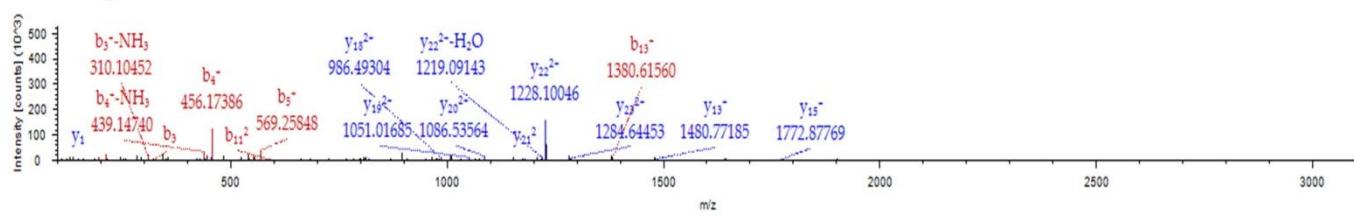
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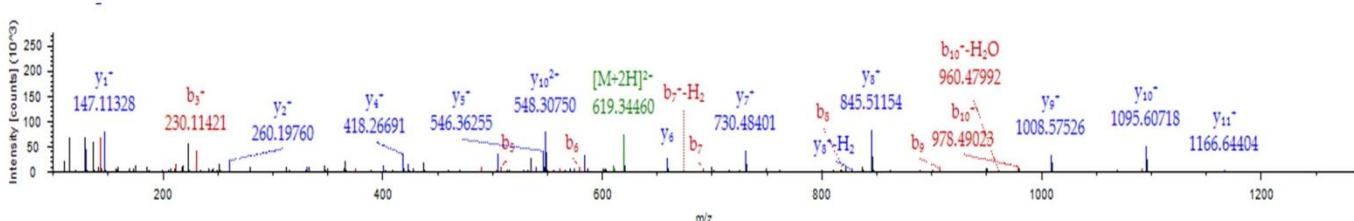
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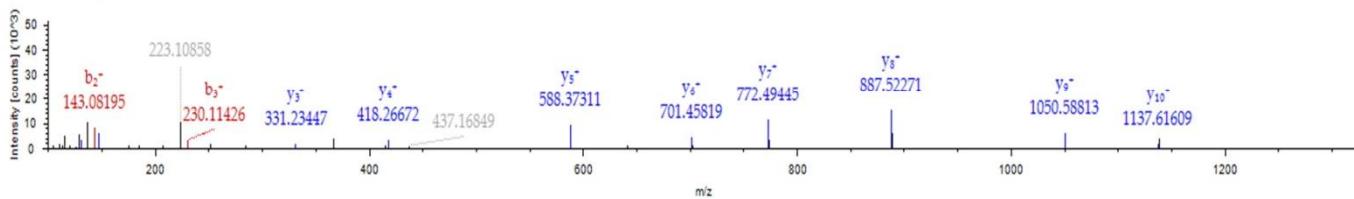
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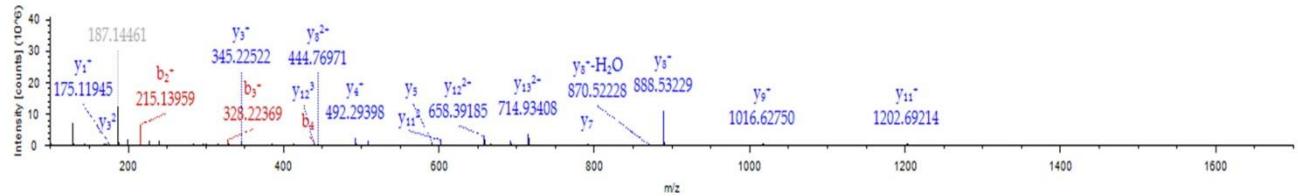
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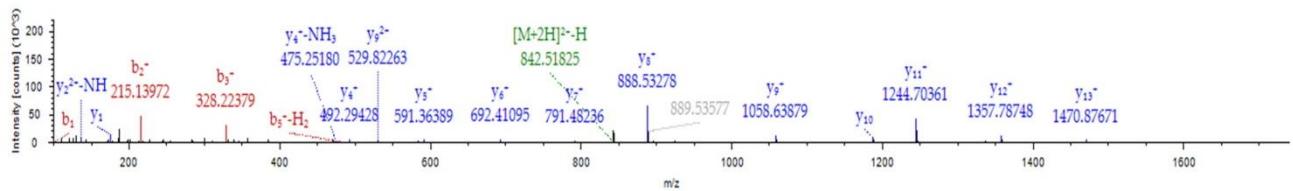
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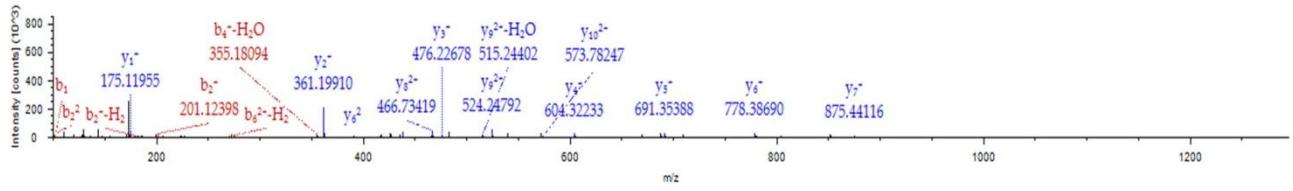
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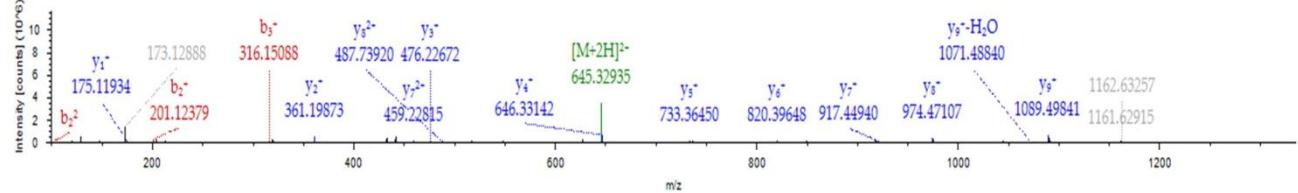
TVDGPSSKDWR

z=+3, Mono m/z=416.53976 Da, MH+=1247.60474 Da, Match Tol.=0.02 Da



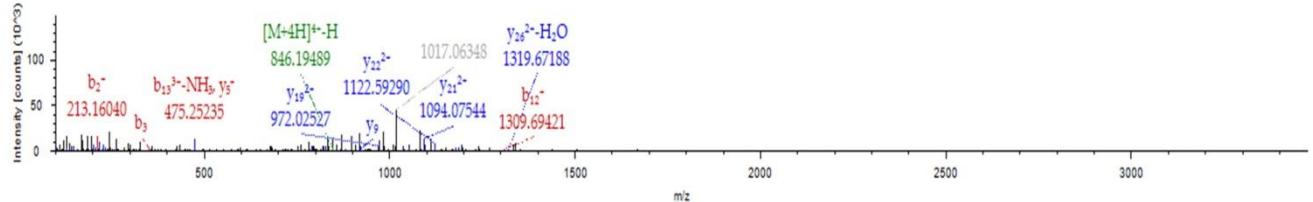
TVDGPSSK (ac) DWR

z=+2, Mono m/z=645.31152 Da, MH+=1289.61577 Da, Match Tol.=0.02 Da



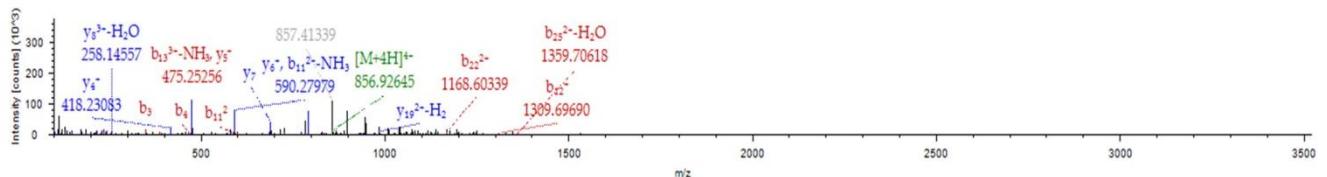
VIHDNFGIIEGLMTTVHAITATQKTVDGPSSK

$z=+4$, Mono $m/z=846.44012$ Da, $MH+=3382.73867$ Da, Match Tol.=0.02 Da



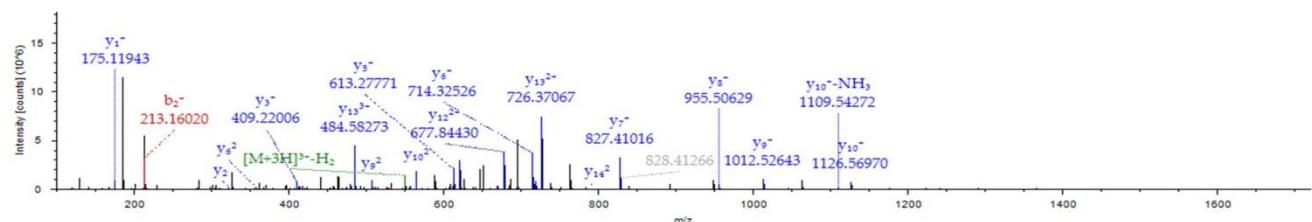
VIHDNFGIIEGLMTTVHAITATQK (ac) TVDGPSSK

$z=+4$, Mono $m/z=856.94379$ Da, $MH+=3424.75332$ Da, Match Tol.=0.02 Da



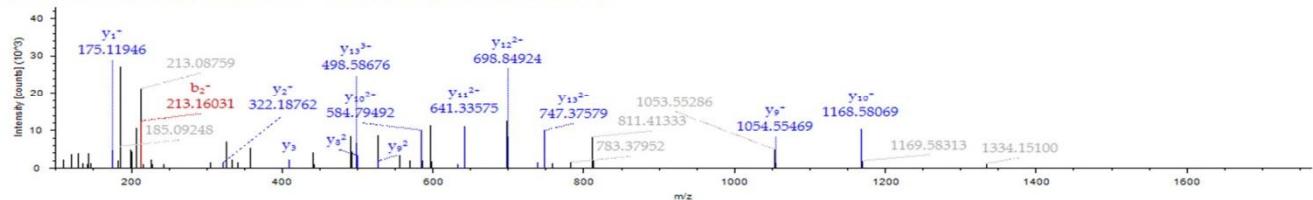
VLPDLNGKLTGMSFR

$z=+3$, Mono $m/z=555.30176$ Da, $MH+=1663.89072$ Da, Match Tol.=0.02 Da



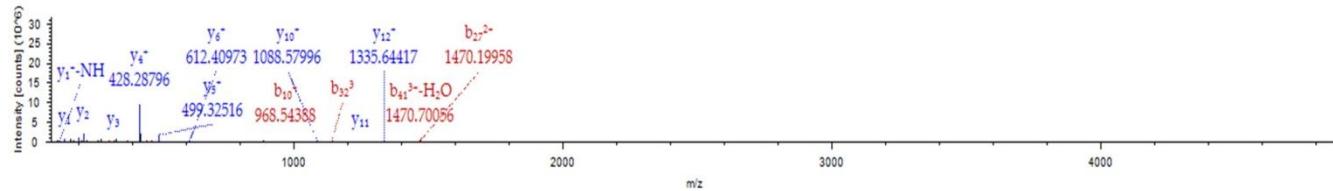
VLPDLNGK (ac) LTGMSFR

$z=+3$, Mono $m/z=569.30463$ Da, $MH+=1705.89933$ Da, Match Tol.=0.02 Da



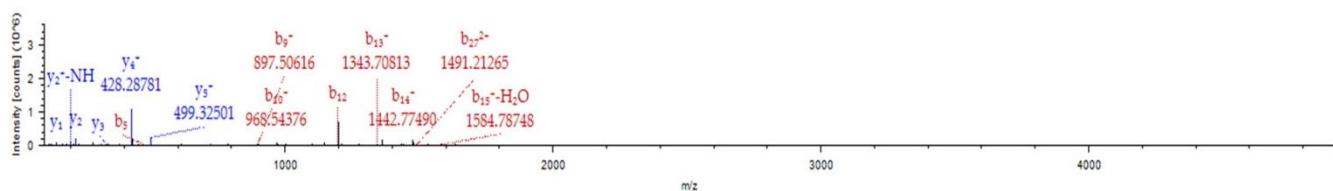
VVISAPSKDAPMFVCGVNEDKYTSDIDIVSNASCTTNCLAPLAK

$z=+4$, Mono m/z=1190.33044 Da, MH+=4758.29995 Da, Match Tol.=0.02 Da



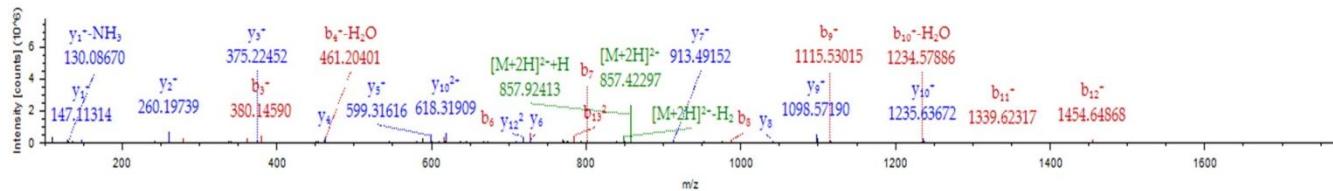
VVISAPSKDAPMFVCGVNEDK (ac) YTSDIDIVSNASCTTNCLAPLAK

$z=+4$, Mono m/z=1200.83789 Da, MH+=4800.32973 Da, Match Tol.=0.02 Da



YDTVHGQWKHS DIK

$z=+2$, Mono m/z=857.42371 Da, MH+=1713.84014 Da, Match Tol.=0.02 Da



YDTVHGQWK (ac) HSD DIK

$z=+3$, Mono m/z=585.95520 Da, MH+=1755.85105 Da, Match Tol.=0.02 Da

