

**Fig. S1.** Screening of plant materials. HI activity in crude extract prepared from each plant material was determined by standard assay. Data are means  $\pm$  SE (*n*=3). ND, not detected.



**Fig. S2.** Detection of HI activity by HPLC. Typical chromatograms of DNP-derivatized samples prepared from reaction mixtures before and after reaction are shown. Arrows indicate peaks of which retention time were identical to those of authentic compound-DNPs, respectively. Substrates are (*Z*)-3-hexenal (*A*), (*E*)-2-hexenal (*B*), (*E*)-2-nonenal (*C*), and (*Z*,*Z*)-3,6-nonadienal (*D*). (*E*)-2-Hexenal was not isomerized (*B*).



Fig. S2. (continued)



**Fig. S3.** pH dependence of activities of native CaHI (A) and rCaHI (B). Enzyme activity was determined by standard assay in 50 mM Na-acetate buffer (pH 3.0-7.0) or 50 mM Tris-HCl buffer (pH 8.0-10.0). Data of native CaHI and rCaHI are from single assay and triplicate assays (means  $\pm$  SE), respectively. Maximum activity was set to 100%.



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Α

MDLILASKKADKTIVEVEGVGGYYTWSRKFPVLSQKKLAAGLLVLQPRGF 1 ALPHYADSSKIAYVIEGECIAGLISPEDSKEEVIKIQKGDSVPVPIGATS WWYNGGDTRLSIIFLGESGEYTPGEFCYFFLTGAAGILNGFSNELLAQTF HMTKTESEKLKKDQSSLNIIIKISEGIKTPDPCNSGIHKLVFNLDGAKPS VEMKNGGVLTSVSVRDLPLLGDIGLSANRVVLEGGGMLGPLFTADSSVHL 2 SYVTKGSGRVVIVGLFGKVVLDTKVDEGDLFFVPKFFPFVVEADEGGIEF FSVKTSSKQIYGALSGGPKSVFVAESPSILEASLNMTPDFTKSFKSKIAK GAVIAPP

**Fig. S4.** Determination of internal amino acid sequence of CaHI. (*A*) Purified CaHI was cleaved by BrCN and then the resultant peptide fragments were separated with Tricine-SDS-PAGE. Peptides blotted onto PVDF membrane were stained by CBB R-250. Arrows indicate peptides of which sequences could be determined. (*B*) Determined internal amino acid sequences are underlined on the whole amino acid sequence of CaHI.



- 5. 2nd wash steps
  - 6. Final elution

5 10 15 20 25 30 0 **Retention time (min)** 

Fig. S5. Production of recombinant HI by heterogeneous expression in E. coli. (A) Purification of recombinant CaHI (rCaHI). Proteins in each fraction were electrophoresed by SDS-PAGE. Purified rCaHI is indicated by an arrowhead (60 kD = 35 kD (CaHI) + 25 kD (Tag)). (B) Activity of the purified rCaHI was confirmed by the production of (E)-2hexenal. (C) By same strategy, activities of recombinant HIs from tomato (rSlHI1) and potato (rStHI1 and 2) were confirmed, on the contrary, recombinant HI-like protein from tomato (rSlHI-like1) did not show activity.



Fig. S5. (continued)



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		10	20	30	40	50	60 •	70	80
[	StHI1	- MDLILSSKK	ADKTIVEVEG	VGG - YYTWSS	SQFPVLSQKK	IAGGLLVLQP	RGFALPHYAD	SSKIGYVCEG	- ECIAGLISP
	StHI2	- MDLILSSKK	ADKTIVEVEG	VGG - YYTWSS	TQFPVLSQKK	IAAGLLVLQH	RGFALPHYAD	SSKIAYVCEG	- ECIAGLTSP
		- MDLILSSKK	ADKTIVEVEG	VGG - YYTWSS	SQFPVLSQKQ	TAAGLELLQP	RGLALPHYAD	SSKIAYVCEC	- ECIAGLISP
	GmHI2	- MELDLTPKT	AEALFEG	DGGGYYTWSS	SQVPLLAKNN	VGAGRLVLQP	RGFALPHYAD	SSKIGYVIQG	TDGVVGMVLP
	MsHI	- MELDLTPKT	AQP LLEG	DGGGYYIWLS	SQVPVLAKTN	VGAGQLVLQP	RGFALPHYAD	SNKVGYVIEG	TDGVVGMVLP
HI -	MtHI	- MELDLTPKA	AQP LFEG	DGGCYYIWLS	SQVPVLAKTN	VGAAHLVLHP	GGLALPHYGD	CSKVGYVVEG	TNGVVGMILP
•••	OsHI1     OsHI2	MAATDMS	PKAGKPLVEN	DAGSYLAWSG	KDQPAVAGEK			SGKEGYVLGG	SAVVGVLPAG
	OsHI3	MAAPDMS	PKAGKPLVQN	DAGSYLAWSG	KDQPTLAGEK	LGCGLLVLKP	LGFALPHYAD	SGKFGYVLGG	SAVVGVLPVG
	CsHI1	- MELNLKPMD	P S N F F T G	EGGSFHKWFP	SDFPIISQTK	VGAGRLLLHP	RGFAVPHNSD	SSKVGYVLQG	- SGVAGIIFP
	CsHI2	MEEQNLKAMN	PRKHFEG	VGGSYNKWYP	SDYPLLAQSK	VGAGMLLLHP	RGFAILHYSD	ASKVGYVLRG	NNGVTGFIFP
	CSHI3 CaHI	- MDL LLASKK	ADKTIVEVEG	VGG-VYTWSR	- KEPVISOKK		RGFAUPHYSD	SSKLAYVIEG	-ECLAGILSP
Ĺ	CaHI-like	SKK	ADKTIVEIEG	VGG - YYTWSS	THFPLLSQKK	LAAGLLVLHP	RVFSQPYYSV	SSKLAYVCEG	- ECIVGLITP
	AtHI-like1	- MELDLSPRL	P K K V Y G G	DGGSYFAWCP	EELPMLRDGN	IGASKLALEK	YGLALPRYSD	SPKVAYVLQG	- AGTAGIVLP
HI-like -	<ul> <li>SIHI-like</li> <li>Stull like</li> </ul>	- MDLILASKK	ADKIIMEVEG	VGG - YYGWSS	SQFPLLSQKK	LAAGLELLQP	HAFVQPYYSV	SSKIAYVCQG	- ECIVGLISS
	<ul> <li>CsHI-like</li> </ul>	- MEIDLTPQL	PKKIYGS	DGGSYYAWSP	KELPMLREGN	IGASKLALEK	NGFALPRYSD	SAKVAYVLQG	- NGVAGIILP
l	GmHI-like2	- MEIDLSPQL	AKKVYES	NGGSYHAWSP	SELPMLHEGN	IGAAKLALQK	NGFALPQYSD	SSKVAYVLQG	- SGVAGIVLP
							v	_	
	_	90	100	110	120	130	140	150	160
ſ	StHI1	EDSKEEVIKI	QKGDALPITV	GTVSWWYNAG	D-TKLKIIFL	GESSEDYTPG	EFCYFFLTSA	AGILNGFSNE	LIAKTFHMTQ
	StHI2	EDSKEEVVKI	QKGDTLPVTV	GTVSWWYNAG	D-TKLTIIFL	GESSEDYTPG	EYCYFFLTSA	AGMLNGFSNE	LIAKCFHMNK
	<ul> <li>SIHI1</li> <li>SIHI2</li> </ul>	EDSKEEVVKI		GTVSWWYNAG	D-IKLIIIFL D-SKITIIFL	GESSKDYTPG	EYCYFFLISA	AGILNGEPNE	VIAKSEHMINI
	GmHI2	NTKEEVVLKL	KKGDVIPVPI	GAVSWWFNDG	D-SDLIIAFL	GETSKALVPG	QFTYFFLTGA	LGLVGGFSNE	LTSKVYGLDN
	MsHI	STGKEVVLKL	KKGDVIPVPI	GGVSWWFNDG	E-SDLNIIFL	GETSIAHVPG	EFTYFFLSGV	QGLLSSFSSE	LISKVYNFNK
HI -	MtHI	STGKEVVLKL	KQGDIVPVPI	GAVSWWFNDG	D-SDFKIIYL	GETSNALVPG	EFTYFILGGV	LGLLGSFSSE	LISKVYNFNK
	OsHI2		EAGDVIAMRA	GEVTWWYNDA		GDTARAASPG	DISYEVLAGE	MGVLGGLDAG	LLATASGLTS
	OsHI3	VDARERVVRL	EAADVIAMRA	GEVTWC		P G	DFSYFILAGP	MSVLGGLDAG	LLATASGLTS
	CsHI1	CKSEEAAVRL	KKGDVIPVPE	GVTSWWFNDG	D - SDFEVLLV	G D T R N A L I P G	DITYVVFAGP	LGVLQGFSSD	YIEKVYDLTE
	CsHI2	NTSNEEVIKL	KKGDLIPVPT	GVTSWWYNDG	D-SDLEIAFL	GETKYAHVPG	DISYYLLSGP	QGILQGFSQD	YVAKTENLNE
	<ul> <li>CaHI</li> </ul>	EDSKEEVIKI	QKGDSVPVPI	GATSWWYNGG	D-TRLSIIFL	GESGE - YTPG	EFCYFFLTGA	AGILNGFSNE	LLAQTEHMIK
ſ	CaHI-like	EDSKEKVIKI	EKGDALPINL	KAISWWYNGG	D-AKLKIIFL	GEYDDENTPG	TPCYFFLTGV	AGILKGFSNE	LIAKSFHMTK
111 121-4	AtHI-like1	E - KEEKVIAI	KKGDSIALPF	GVVTWWFNNE	D-TELVVLFL	GETHKGHKAG	QFTDFYLTGS	NGIFTGFSTE	FVGRAWDLDE
HI-IIKe ⊣	SIHI-IIKe StHI-Iike	EDSKEEVIKL	QKGDILPLIM	KEVSWWYNDG	E-SKLETTFL	- EYDDEYTPG	EYCGECLAGE	IGTENGESNE	ELAKSEHMIK
	<ul> <li>CsHI-like</li> </ul>	E-SEEKVIAI	KKGDAIALPF	GVVTWWFNKE	A - TDLVVLFL	GDTSKAHKSG	EFTDFFLTGA	NGIFTGFSTE	FVGRAWDMDE
l	- GmHI-like2	E - SEEKVLAI	KKGDALALPF	GVITWWYNKE	D - TELVVLFL	GDTSKAHKTG	EFTDFYLTGS	NGIFTGFSTE	F V G R AWD L E E
	_	170	180	190	200	210		230	240
	StHI1	TESEKLVKDQ	SGLNIIIKVN	EGIQIPNGSS	S - AKRKFVYN	LDGAKPC-VE	VKNGGHLSSL	SIKNIPLLGE	IGLSANRVVL
	StHI2	TESEKLMKDQ	SSENLLIKIN	ESIQMPNSSN	S - AKRKLVYN	LDGAKPC-VE	VKNGGHLSSV	SGKNIALLGE	VGLSANRGVL
	<ul> <li>SIHI1</li> <li>SIHI2</li> </ul>	TESEKLMKDQ	SSENILIKVN	EGIPIPNPSN	S - AKRKLVYS	LYDAKPC-VD	VKNGGVLSSV	SGKNIALLGE	IGLSANRLVL
	GmHI2	DEVEKLTKSQ	TGV-LIIKLD	KSQPMPKPQ-	MNMTKKLVYN	IDAARPENVV	E - NAGLVKTL	TEKDFPFIGD	VGLSVMRVKL
	MsHI	DEVTKLTQSQ	KGV-VIIKLE	KGQPMPKPQ-	LDLTKDFVYD	IDAKKPDIKA	Q-NVGLVTTL	TDKDFPFIKD	VGLSVIRVKL
HI -	MtHI	DEVIKLIQSQ	TGV-TIIKLE	KGQPMPKPQ-	MDLTKDLVYD		K-NVGLVTSL		VGLSVIRVKL
	OsHI2	PEQAATAFRS	QPAVLLTRLS	RKLQDVRPR-	EHDRHGIVVN	AARMPADSST	GGAAAGTKIV	TAAHLPVLGQ	LGFSVGLTPL
	OsHI3	PEQAATAFRS	QPAALLTRLS	RKLHGVRPR-	EHDRHGIVVN	AARVPPDS-T	G G K T V	TAAHLPALAQ	LGLSVGLALL
	CsHI1	KEREVLLKSQ	PNG-LIFKLK	DDQTLPEP	- DCHSDLVFN	IYHTAPDAVV	K-GGGSVTVL	TEEKFPFIGK	SGLTAVLEKL
	CsHI2 CsHI3	FETNTELKSQ	PNV-LIFTVQ	PSOSIPKP	- HKYSKI VYN		KVGDAAVTMV	TESTEPEIGO	TGLAVVVERL
l	• CaHI	TESEKLKKDQ	SSLNIIIKIS	EGIKTPDPCN	S-GIHKLVFN	LDGAKPS-VE	MKNGGLLTSV	SVRDLPLLGD	IGLSANRVVL
ſ	CaHI-like	TEFEKIIKDQ	SSSNVLIKIK	EGTKMPDPCN	NDVKHKLAFN	LDSAKPC-VE	VKNGGTLSVV	TCRNLLLGD	VGLSANRVVL
	<ul> <li>AtHI-like1</li> <li>SIHI-like</li> </ul>	PEIEKIIKEE	IGN-GIVKVD	ASLKMPEPK-	KGDRKGFVLN	CLEAPLD-VD			
ni-like -	StHI-like	IESEKLIKDQ	SRTNLLIKIN	EGTKMPHPCN	K - VKHKLVFN	LDSAKPC-VE	VKNGGVLSAV	TCRNLPLLGD	VGLSANRVVL
	CsHI-like	ASVKSLVKNQ	TGT-GIVKLK	EGTKMPEPK-	KEHRNGMALN	CEEAPLD-VD	VKNGGRVVVL	NTKNLPLVGE	VGLGADLVRL
L	<ul> <li>GmHI-like2</li> </ul>	KDVKTLVGKQ	SGN-GIVKLE	GNINLPEPK-	EEHRKGMALN	CEEAPLD-VD	IKNGGRVVVL	NTKNLPLVGE	VGLGADLVRL

**Fig.S7.** Alignment of HI and HI-like proteins. Positions of catalytic HKY are boxed. Red and blue dots indicate that their recombinant proteins showed activities and not, respectively.

		250	260	270	280	290	300	310	320
	_	I T							
	StHI1	EC-GAVLGPI	YTPDSFIHLS	Y I T K G S G R V V	I V G L F G K - V V	LDTKVEEGDL	FYVPKFFPFV	VEAD - EGGIE	FFSMKTSSKE
	StHI2	EG-GAVLGPI	FTADSSIHLS	Y I T K G S G R V V	I V G L F G K - V V	LDTKVEEGEL	FFVPKFFPFV	VEAD - EGGIE	FFSLKTSSKQ
	SIHI1	EP-GAVLGPI	FTADSSIHLS	Y I T K G S G R V V	I V G L F G K - V V	LDAKVEEGEL	FFVPKFFPFV	VEAD - EGGIE	FFSLKTSSKQ
	SIHI2	ER-GAVLGPI	FTADSSIHLS	Y I T K G S G R V V	IVGLSGK-VV	LDTKVEEGQL	FFVPKFFPFV	VEAD - EGGIE	LFSLKTSSKQ
	GmHI2	EP-GAIKAPS	YPTNPTVQLI	YIARGSGKIE	IVDFSGK-SV	LNTQVEAGHL	LVVPQFFVLA	EIAG-EEGIE	SYSIVITTKP
	MsHI	EP - NAIKAPS	NLITPAIQLI	YIARGSGKIE	IVGLNGK-RV	LDAQVKAGHL	IVVPQFFVVA	KVAG-EDGME	SYSIVTTTKP
ш .	MtHI	EP - NAIKAPS	NLITPGIQLI	YIARGSGKIE	IVGINGK-RV	LDSQVKPGHL	IVVPKFFVIA	QIAG-EEGME	SYSIVTTTKP
	OsHI1	DAGAAVRGPW	VLRDAAAQAV	Y V A R G S G R V Q	VAGAGGASTL	LDAEVAAGSL	LVVPRYGVSL	AAADDAGGME	LVSLIKSPRP
	OsHI2	DAGAAVRGPW	VLRDAAAQAV	Y V A R G S G R V Q	VAGAGGASTL	LDAEAAAGSL	LVVPRYAVAL	VGVD - AGGME	LVSLIKSPRP
	OsHI3	DAGAAVRGPW	VLRDAAAQAV	Y V A R G S G R V Q	VASAGGASTL	LDAEVAAGSL	LVVPRYAVAL	VAADDAGGME	LVSLIKSSRP
	CsHI1	EA-NAVRSPV	YVADPSVQLI	Y	IAETFMR-YQ	IDAEVKAGQL	VLVPKYFAVG	KMAG-EEGLE	CFTIITTTQ-
	CsHI2	GP - NVVRSPV	LLVSPADQLI	Y V A <b>R G</b> S G T V Q	IVGLSSS-SK	IELHVESGQL	IFVPKYFAAG	KIAA-EQGME	FFSILTAKLG
	CsHI3	DA - NAIRSPV	YIAEPSDQLI	Y V T K G S G K I Q	VVGFSSK	FDADVKTGQL	ILVPRYFAVG	KIAG-EEGLE	CISMIVATHP
	CaHI	EG-GGMLGPL	FTADSSVHLS	YVTKGSGRVV	I V G L F G K - V V	LDTKVDEGDL	FFVPKFFPFV	VEAD-EGGIE	FFSVKTSSKQ
	CaHI-like	EG-GALFGPI	FTADSSVQLS	Y V T K G S G R V Q	IVGPFGK-VV	LDTKVEEGEL	FFVPKFFPFV	VEAD-EGGME	FFSVITSSKQ
	AtHI-like1	DG-HSMCSPG	FSCDSALQVT	Y I V G G S G R V Q	IVGADGK-RV	LETHVKAGVL	FIVPRFFVVS	KIAD-SDGLS	WFSIVTTPDP
HI_lika	SIHI-like	EG-GALFGPV	FTADLSVQLS	Y V T K G S G R V Q	IVGPLRK-VV	LDTKVKEGEL	FFVPKFFPFV	VEAD - EGGME	FFSVITSSKQ
	StHI-like	ES-GALFGPI	FTADLSVQLS	Y V T K G S G R V Q	IVGPLRK-VV	LDTKVEEGGL	FFVPKFFPFV	VEAD-EGGME	FFSVITSSKQ
	CsHI-like	DG-SAMCSPG	FSCDSALQVT	YIVKGSGRAE	VVGVDGK-KV	LETRVKAGNL	FIVPRFFVVS	KIGD-PEGME	WFSIISTPNP
	GmHI-like2	DG-KAMCSPG	FSCDSAFQVT	Y I V R G S G R A Q	VVGADGR-RV	LETTVKAGNL	FIVPRFFVVS	KIAD-SDGLE	WFSIITTPNP
		330	340	350	360	370			
		330	340 · · · ·   · · · ·	350	360 	370			
	• StHI1	330 VYGELSGGKK	340    SVWEAASPSI	350 I LEASLNMTPD	380    LTKSFKSKIA	370 II KGAVIAPPSN	V		
	• StHI1 StHI2	330 VYGELSGGKK TYGELSSGKK	340  S VWE A A S P S I S I WE A A S P S I	350 LEASLNMTPD LEASLNMTPD	380 LTKSFKSKIA LTKSFKSKIA	370 KGAVIAPPSN KGAVIAPPST	V I		
	StHI1     StHI2     SIHI1	330 VYGELSGGKK TYGELSSGKK TYRELSGGKK	340 SVWEAASPSI SIWEAASPSI SIWEAASPSI SIWEAASPSI	350 LEASLNMTPD LEASLNMTPD LEASLNRTPD	380 LTKSFKSKIA LIKSFKSKIT LTKSFKAKIA	370 KGAVIAPPSN KGAVIAPPST KGAVISPPSN	V I G		
	StHI1     StHI2     SIHI1     SIHI1     SIHI2	330 VYGELSGGKK TYGELSSGKK TYRELSGGKK TYGELSGGKK	340 SVWE AASPS I SIWE AASPS I SIWE AASPS I SIWE AASPS I SIWE AASPS I	350 LEASLNMTPD LEASLNMTPD LEASLNRTPD LEASLNRTPD LEASLNMTPD	380 LTKSFKSKIA LIKSFKSKIT LTKSFKAKIA LTKSFKSKIA	370 KGAVIAPPSN KGAVIAPPST KGAVISPPSN KGSVIAPPST	V I G A		
	StHI1     StHI2     SIHI1     SIHI2     GmHI2	330 VYGELSGGKK TYGELSSGKK TYRELSGGKK TYGELSGGKK LFEELAG-RR	340 SVWE A ASPS I SIWE A ASPS I	350 LEASLNMTPD LEASLNMTPD LEASLNRTPD LEASLNMTPD QQVSLNVDSD	380 LTKSFKSKIA LIKSFKSKIT LTKSFKAKIA LTKSFKSKIA FQKFFISKIK	370 KGAVIAPPSN KGAVIAPPST KGAVISPPSN KGSVIAPPST ESTNLIPPTV	V I G A		
	StH11     StH12     SIH12     SIH12     GmH12     MSH1	330 VYGELSGGKK TYGELSSGKK TYRELSGGKK TYGELSGGKK LFEELAG-RR LFEELAG-ET	340 SVWE AASPS I SIWE AASPS I SIWE AASPS I SIWE AASPS I SIWE AASPS I SIWN AFSPTL SVWG ALSPTV	300 LEASLNMTPD LEASLNMTPD LEASLNRTPD LEASLNMTPD QQVSLNVDSD QQVSFNVDSE	380 LTKSFKSKIA LIKSFKSKIT LTKSFKAKIA LTKSFKSKIA FQKFFISKIK FQELFISKTT	370 KGAVIAPPSN KGAVIAPPST KGAVISPPSN KGSVIAPPST ESTNLIPPTV ETTSLILPTI	V I G A -		
HI	StHI1     StHI2     SIHI1     SIHI2     GmHI2     GmHI2     MsHI     MtHI	330 VYGELSGGKK TYGELSGGKK TYRELSGGKK TYGELSGGKK LFEELAG-RR LFEELAG-DT LFEELAG-DT	340 SVWE AASPS I SIWE AASPS I SIWE AASPS I SIWE AASPS I SIWE AASPS I SIWN AFSPT L SVWG ALSPT V SVWG ALSPT V	350 LEASLNMTPD LEASLNMTPD LEASLNMTPD QUVSLNVDSD QQVSFNVDSE QQVSFNVDSE	300 LTKSFKSKIA LIKSFKSKIA LTKSFKSKIA LTKSFKSKIA FQKFFISKIK FQELFISKT FQNLFISKST	370 KGAVIAPPSN KGAVIAPPSN KGAVISPPSN KGSVIAPPST ESTNLIPPTV ETTSLIPTI	V G A -		
HI	StHi1     StHi2     SiHi1     SiHi2     GmHi2     MsHi     MtHi     OsHi1	330 VYGELSGGKK TYGELSGGKK TYRELSGGKK TYGELSGGKK LFEELAG-RR LFEELAG-T LFEELAG-T ATEHFTG-KG	340 SVWE AASPS I SIWE AASPS I SIWE AASPS I SIWE AASPS I SIWE AASPS I SIWE AASPS I SVWG ALSPT V SVWG ALSPT V SVWG ALSPT V	380 LEASLNMTPD LEASLNMTPD LEASLNMTPD LEASLNMTPD QQVSLNVDSD QQVSFNVDSE QQVSFNVDSE VQAALNVSPE	300 LTKSFKSKIA LIKSFKSKIA LTKSFKSKIA LTKSFKSKIA FQELFISKIK FQELFISKT FQLFISKST FVEQLRTKY-	370 KGAVIAPPSN KGAVISPPSN KGSVISPPSN KGSVIAPPST ESTNLIPPTV ETTSLILPTI KTTNLILPTI	V I G A - -		
HI -	StHI1     StHI2     SIHI1     SIHI2     GmHI2     MsHI     MtHI     OsHI1     OsHI2	330 TYGELSGGKK TYGELSGGKK TYGELSGGKK TYGELSGGKK LFEELAG-RT LFEELAG-DT ATEHFG-KG AMKQFTG-KG	340 SVWE AASPSI SIWE AASPSI SIWE AASPSI SIWE AASPSI SIWE ASPSI SVWG ALSPTV SVWG ALSPTV SVWG ALSPTV SVIGGLTAEI	350 LEASLNMTPD LEASLNMTPD LEASLNMTPD QQVSLNVDSD QQVSFNVDSE QQVSFNVDSE VQAALNVSPE VQAALNVSPE	380 LTKSFKSKIA LIKSFKSKIA LTKSFKSKIA FQKFFISKIK FQELFISKIK FQELFISKT FVEQLRMTK- LVEQLRMTK-	370 KGAVIAPPSN KGAVIAPPSN KGAVISPPSN KGSVIAPPST ESTNLIPPTV ETTSLILPTI KTTNLILPTI	V I G A - -		
HI -	StHi1     StHi2     SiHi2     SiHi2     GmHi2     MsHi     MtHi     OsHi1     OsHi2     OsHi3	330 TYGELSGGKK TYGELSGGKK TYGELSGGKK LFEELAG-RR LFEELAG-RT LFEELAG-DT ATEHFTG-KG AMKAFTG-KG AMEHFTG-KG	340 SVWE AASPS I SIWE AASPS I SIWE AASPS I SIWE AASPS I SIWE AASPS I SIWN AFSPTL SVWG ALSPTV SVWG ALSPTV SVVG GLTAE I SVIGG LTPE I	350 LEASLNMTPD LEASLNMTPD LEASLNMTPD QQVSLNVDSD QQVSFNVDSE QQVSFNVDSE VQAALNVSPE VQAALNVSPE VQAALNVSPE	300 LTKSFKSKIA LIKSFKSKIA LTKSFKSKIA FQKFFISKIK FQLFISKIK FQLLFISKT FVEQLRTKY- LVEQLRMTK-	370 KGAVIAPPSN KGAVISPPSN KGAVISPPSN KGSVIAPPST ESTNLIPPTV ETTSLILPTI KTTNLILPTI	V G A - - -		
HI -	StHi1     StHi2     SiHi1     SiHi2     GmHi2     GmHi2     MsHi     MtHi     OsHi1     OsHi3     CsHi1	330 VYGELSGGKK TYGELSGGKK TYRELSGGKK TYGELSGGKK LFEELAG-RR LFEELAG-R LFEELAG-DT ATEHFTG-KG AMKQFTG-KG	340 SVWE AASPS I SIWE AASPS I SIWE AASPS I SIWE AASPS I SIWE AASPS I SIWN AFSPTL SVWG ALSPTV SVWG ALSPTV SVWG ALSPTV SVIGG LTAE I SVIGG LTPE I SVIGG LTPE I	380 LEASLNMTPD LEASLNMTPD LEASLNMTPD LEASLNMTPD QQVSLNVDSD QQVSFNVDSE QQVSFNVDSE VQAALNVSPE VQAALNVSPE VQAALNVSPE	300 LTKSFKSKIA LIKSFKSKIA LTKSFKSKIA LTKSFKSKIA FQELFISKT FQELFISKT FQLLFISKST FVEQLRTKY- LVEQLRTK- LVEQLRTK-	370 KGAVIAPPSN KGAVIAPPST KGAVISPPSN KGSVIAPPST ESTNLIPPTV ETTSLILPTI KTTNLILPTI	V I G A - - - -		
HI -	StHI1     StHI2     SIHI1     SIHI2     GmHI2     MsHI     MtHI     OsHI1     OsHI3     CsHI1     CsHI2	330 TYGELSGGKK TYGELSGGKK TYGELSGGKK TYGELSGGKK LFEELAG-RT LFEELAG-BT ATEHFTG-KG AMKQFTG-KG AMKQFTG-KG	340 SVWE AASPSI SIWE AASPSI SIWE AASPSI SIWE AASPSI SIWE ASPSI SVWG ALSPTV SVWG ALSPTV SVWG ALSPTV SVIGGLTAEI SVIGGLTPEI SVIGGLTPEI SVME ALSAEV	350 LEASLNMTPD LEASLNMTPD LEASLNMTPD QQVSLNVDSD QQVSFNVDSE QQVSFNVDSE VQAALNVSPE VQAALNVSPE VQAALNVSPE	380 LTKSFKSKIA LIKSFKSKIA LTKSFKSKIA FQKFFISKIK FQELFISKIK FQELFISKT FVEQLRTKY- LVEQLRTKY- LVEQLRTK- FEKVLRSNTT	370 KGAVIAPPSN KGAVIAPPSN KGAVISPPSN KGSVIAPPST ESTNLIPPTV ETTSLILPTI KTTNLILPTI	V I G A - - - -		
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**Fig. S8.** HI homologs of alfalfa (A), cucumber (B), and rice (C) showed HI activity. Catalytic pocket of each HI by homology modeling is shown in lower panel. Catalytic HKY locate in the same pocket. PDB ID of template protein, identity between CaHI and template protein, and QMEAN score are shown in parentheses.



**Fig. S9.** Production of point-muted rCaHI by heterogeneous expression in *E. coli*. (*A*) Purified rCaHI recovered in soluble fractions were electrophoresed by SDS-PAGE. Purified rCaHI is indicated by an arrowhead (60 kD = 35 kD (CaHI) + 25 kD (Tag)). (*B*) Activity of the purified rCaHI was determined by the production of (*E*)-2-hexenal.