Supporting Information

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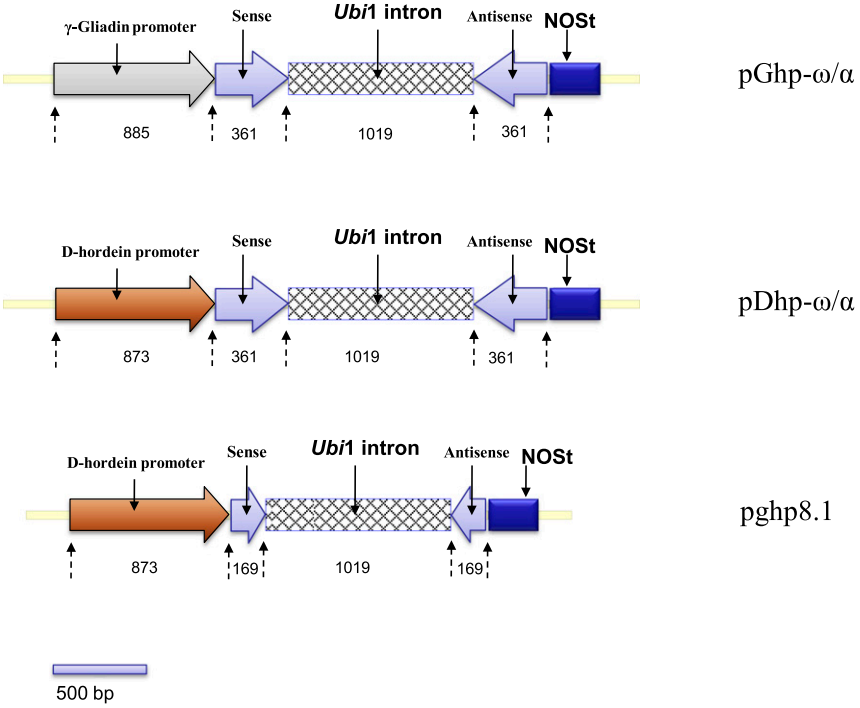

Fig. S1. Constructs for RNAi-targeting gliadin genes. The vectors contain a γ-gliadin (pGhp-ω/α) or D-hordein promoter (pDhp-ω/α and pghp8.1), the *nos* terminator sequence (NOSt), and the triggered sequences in sense and antisense orientations separated by the *Ubiquitin* 1 intron (*Ubi*1 intron). Vectors pGhp-ω/α and pDhp-ω/α include an inverted repeat (IR) chimeric 361-bp fragment from α- and ω-gliadins. Vector pghp8.1 includes a 169-bp IR fragment from γ-gliadin. Numbers indicate length in base pairs.

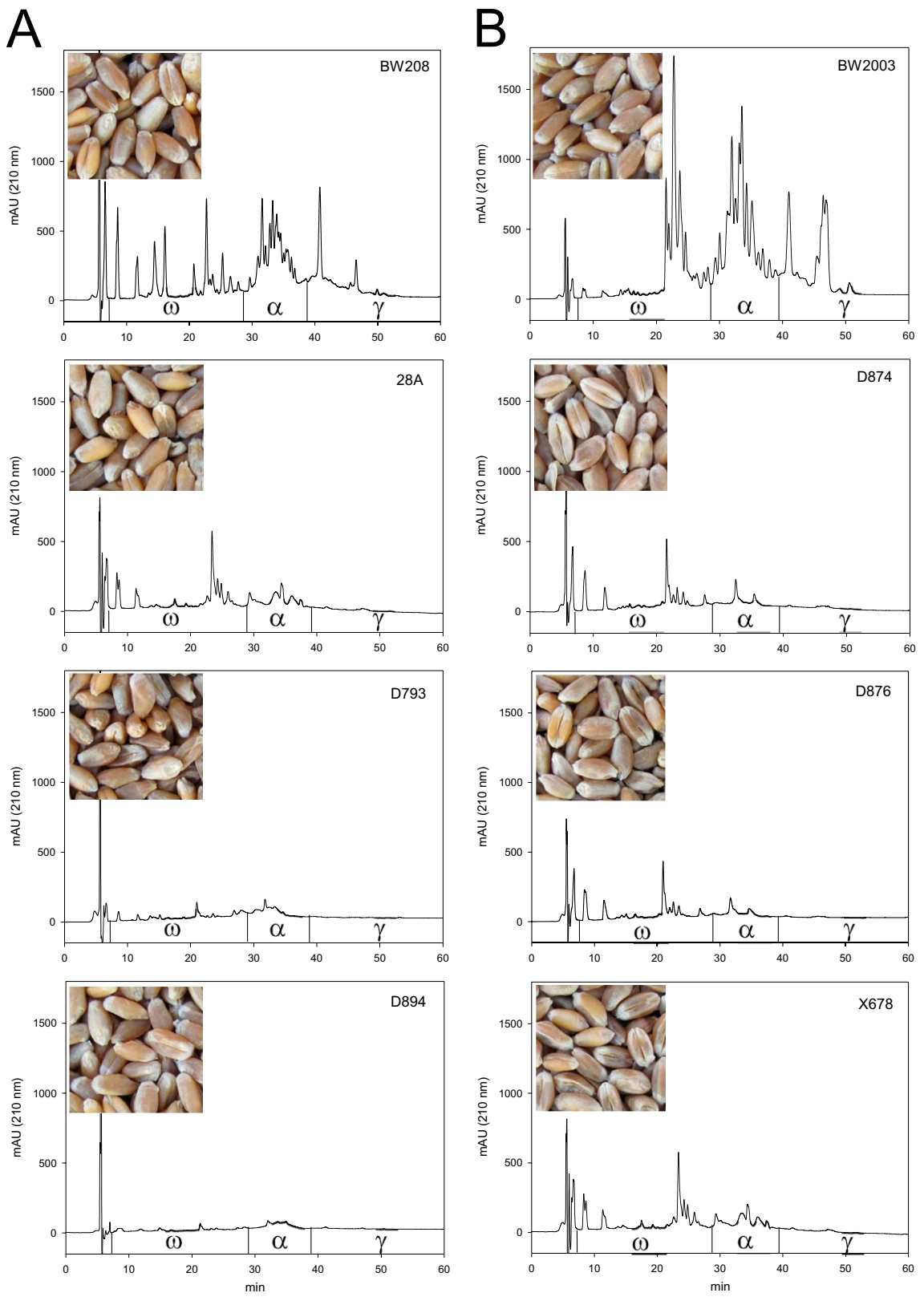

Fig. S2. RP-HPLC chromatograms of gliadin extracts from wild-type and transgenic wheat lines. (*A*) BW208 wild-type and BW208 transgenic lines. (*B*) BW2003 wild-type and BW2003 transgenic lines. ω, ω-gliadins; α, α-gliadins; γ, γ-gliadins. mAU (210 nm), milliunits of absorbance at 210 nm; min, retention time in minutes.

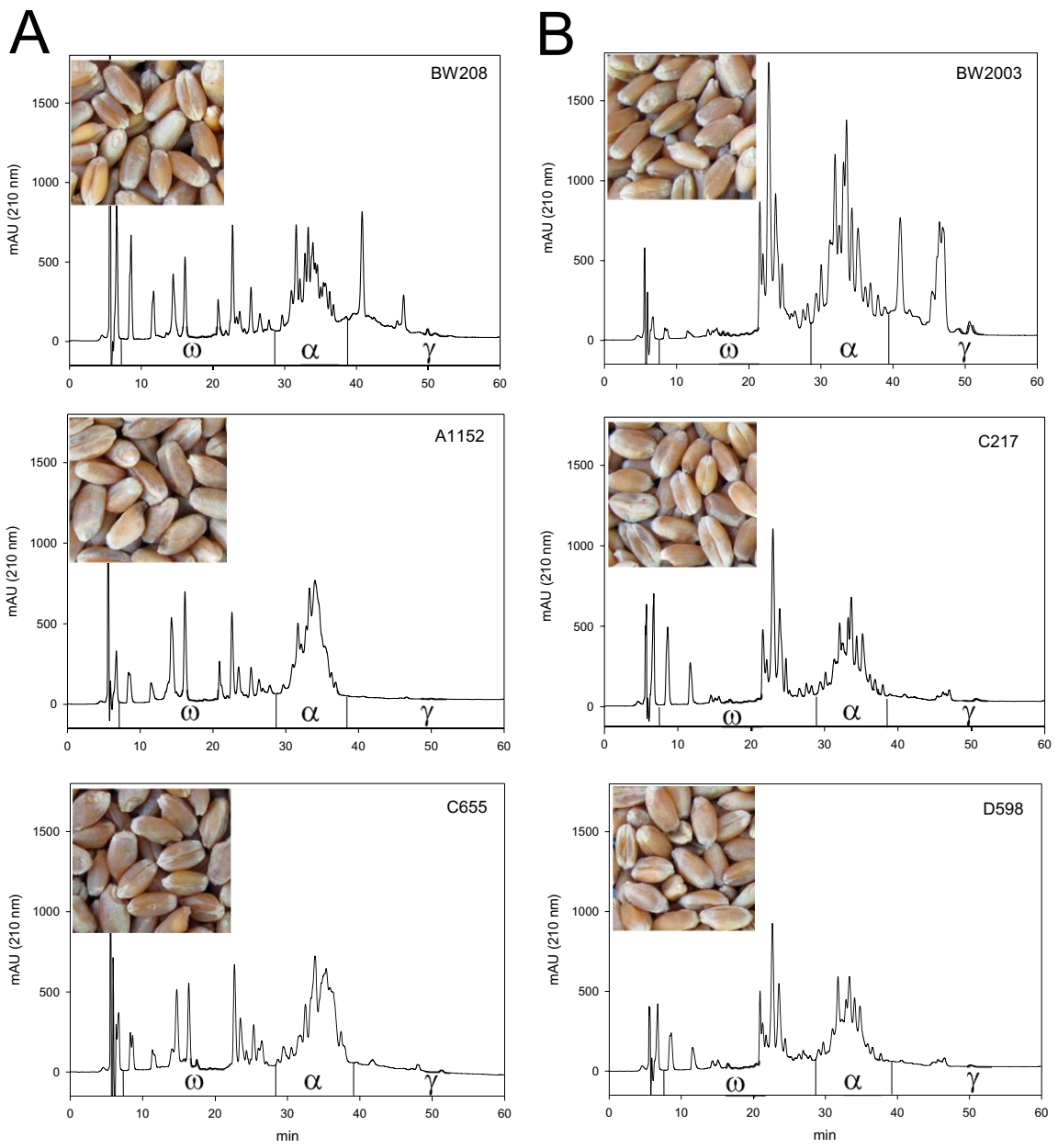

Fig. S3. RP-HPLC chromatograms of gliadin extracts from wild-type and transgenic wheat lines transformed with pghp8.1 construct. (*A*) BW208 wild-type and BW208 transgenic lines. (*B*) BW2003 wild-type and BW2003 transgenic lines. Names and symbols are as described in Fig. S2.

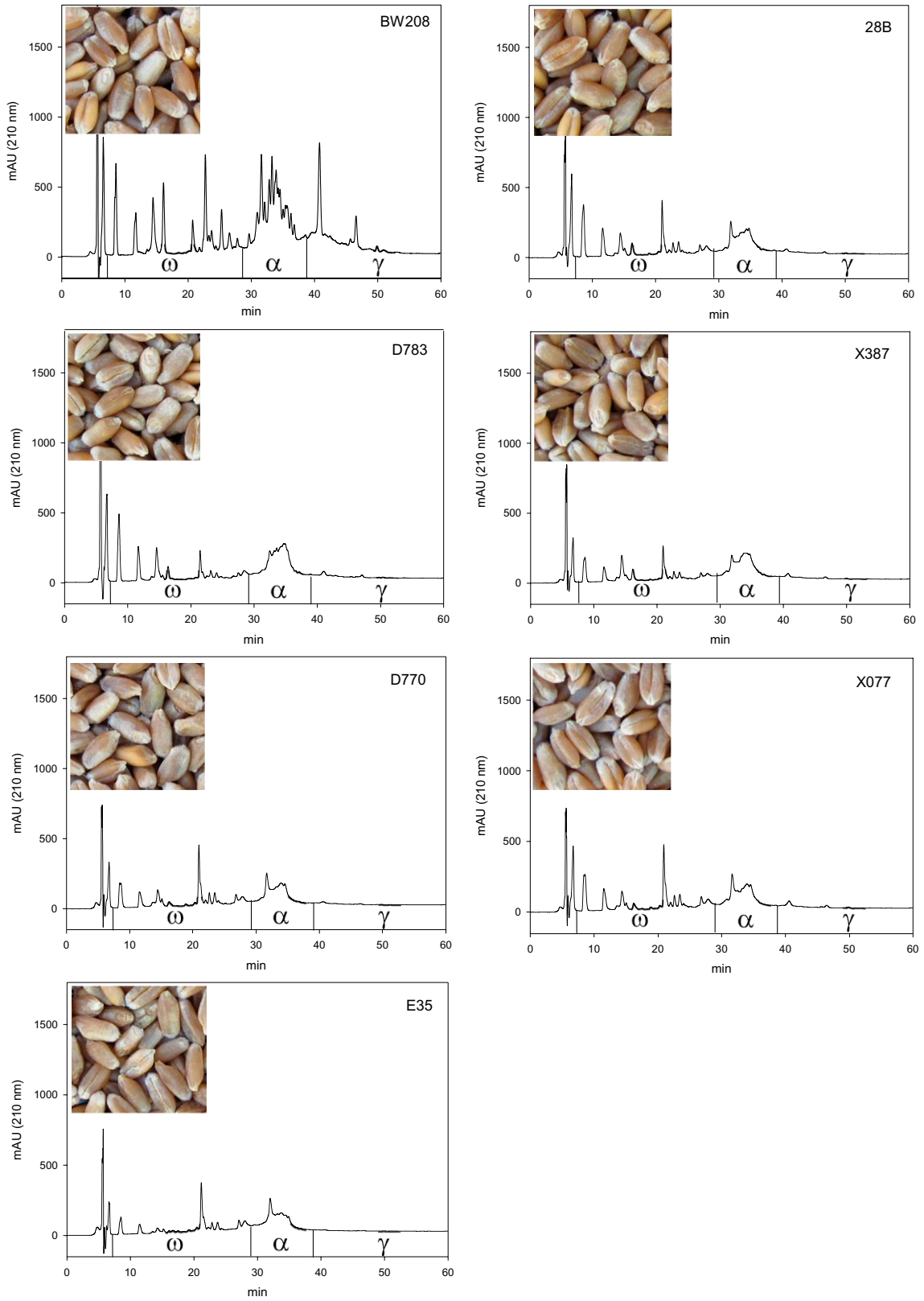

Fig. S4. RP-HPLC chromatograms of gliadin extracts from wild-type and transgenic wheat lines. BW208 wild type and BW208 transformed with vectors pDhp-ω/α, pGhp-ω/α, and pghp8.1+pDhp-ω/α. Names and symbols are as described in Fig. S2.

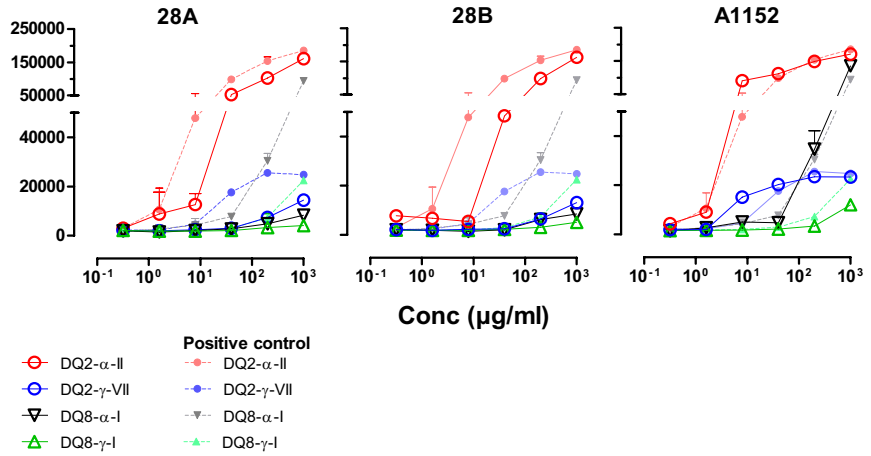

Fig. S5. Proliferative responses of gliadin-specific T-cell clones stimulated with total gluten extracts from transgenic wheat lines derived from the BW208 line. These lines gave significantly reduced T-cell proliferation compared with the wild-type line. Symbols are as described in Fig. 2.

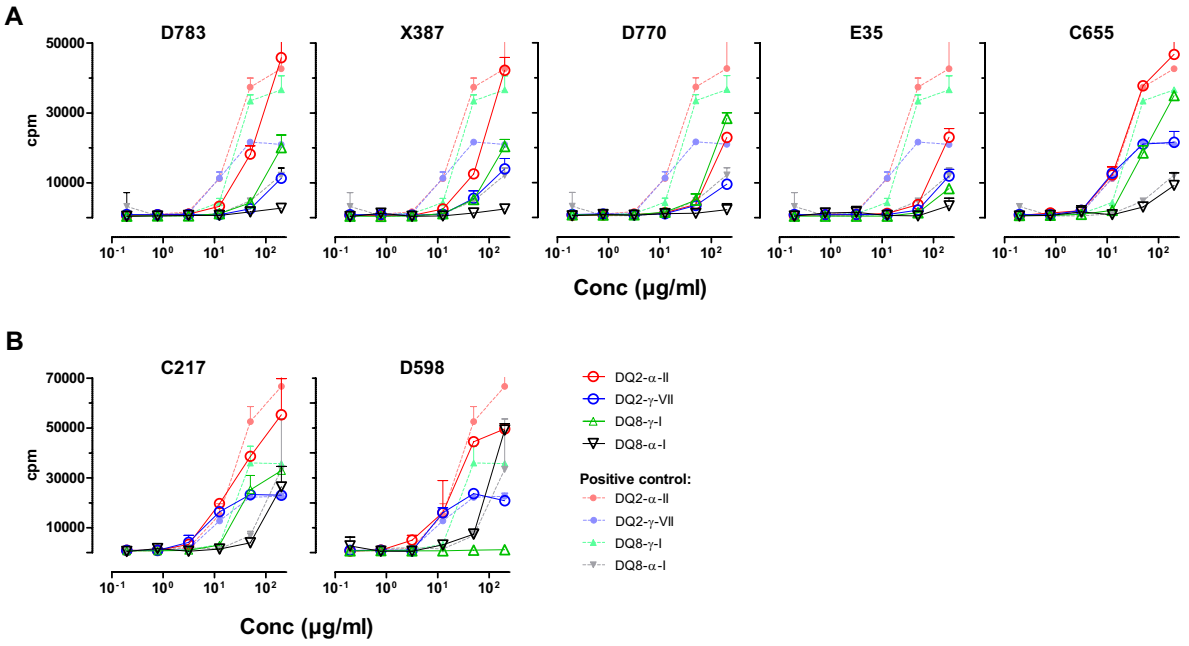

Fig. S6. Proliferative responses of gliadin-specific T-cell clones stimulated with total gluten extracts from transgenic wheat lines. These lines gave no or very little reduction in the T-cell response compared with the wild-type lines. Transgenic lines derived from line BW208 are depicted in *A*, whereas transgenic lines derived from BW2003 are depicted in *B*. Symbols are as described in Fig. 2.

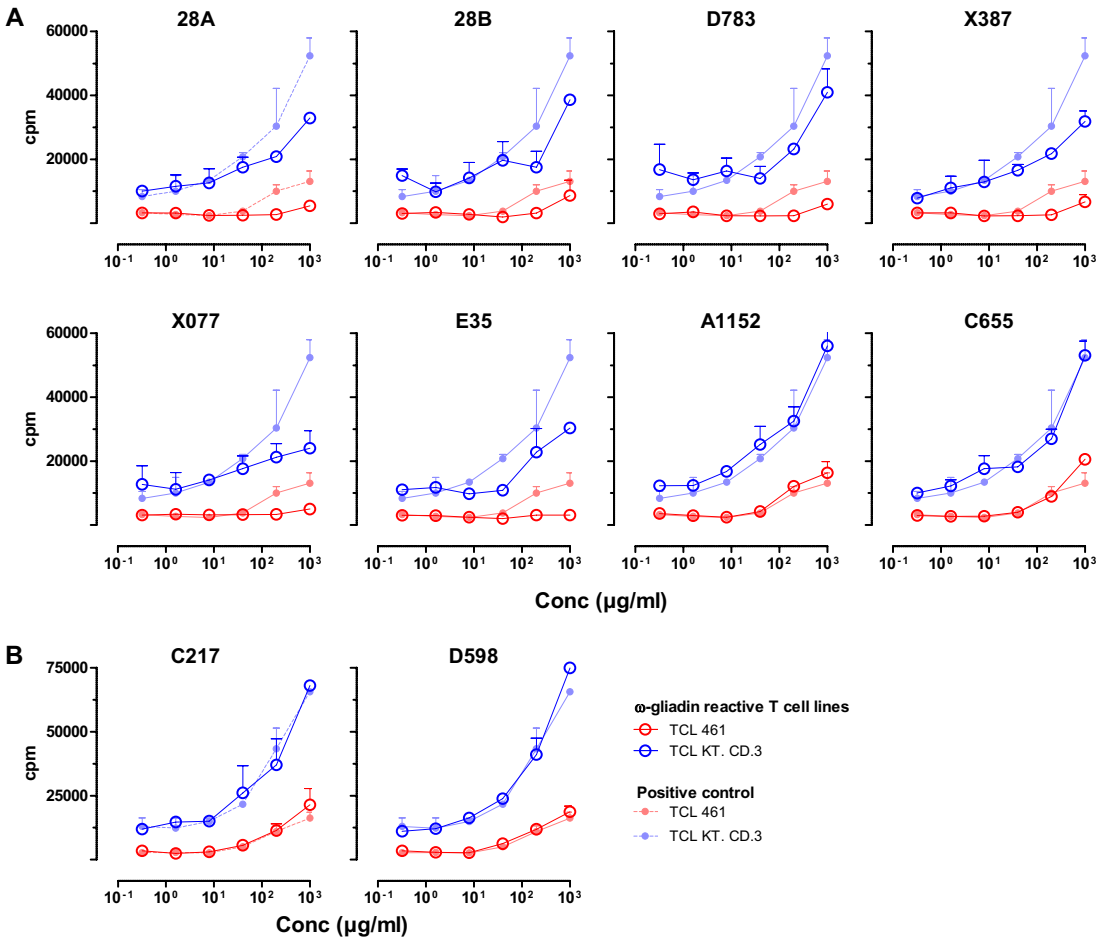

Fig. S7. Proliferative responses of ω-gliadin reactive T-cell lines stimulated with total gluten extracts from transgenic wheat lines. Transgenic lines derived from line BW208 are depicted in *A*, whereas transgenic lines derived from BW2003 are depicted in *B*. Symbols are as described in Fig. 3.

Table S1. ***Triticum aestivum*** **gliadin accession number from the nucleotide GenBank nonredundant (nr) database (http://www.ncbi.nlm.nih.gov/Genbank/)**

α-	γ-	ω-
AJ130948	AF120267	AF280605
AJ133602	AF144104	AF280606
AJ133603	AF234642	AB059812
AJ133604	AF234643	AY591334
AJ133605	AF234644	AJ937839
AJ133606	AF234645	AB181300
AJ133607	AF234646	AB181301
AJ133608	AF234647	DQ287981
AJ133609	AF234648	DQ307378
AJ133610	AF234649	EF116277
AJ133611	AF234650	EF116278
AJ133612	AF234651	FJ598069
AY293730	AF419255	FJ598070
AY496057	AJ389666	FJ598071
D84341	AJ389667	FJ598072
DQ234066	AJ389668	FJ598073
DQ234067	AJ389669	FJ598074
DQ246446	AJ389670	GQ423428
DQ246447	AJ389671	GQ423429
DQ246448	AJ389672	GQ423430
DQ246449	AJ389673	GQ423431
DQ417343	AJ389674	GQ423432
DQ417344	AJ389675	
DQ417345	AJ389676	
EF165551	AJ389689	
EF165552	AJ389690	
EF165553	AJ389691	
EF561274	AJ389692	
EF561275	AJ389693	
EF561276	AJ389694	
EF561277	AJ389695	
EF561278	AJ389696	
EF561279	AJ389697	
EF561280	AJ389698	
EF561281	AJ416336	
EF561282	AJ416337	
EF561283	AJ416338	
EF561284	AJ416339	
EF561285	AY338386	
EF561286	AY338387	
EF561287	AY338388	
EF561288	AY338389	
EF569971	AY338390	
EF569972	DQ432029	
EF569973	DQ432030	
EF569974	EF151018	
EF569975	EF426565	
EF569976	FJ006589	
EF569977	FJ006590	
EF569978	FJ006591	
EF569979	FJ006592	
EF569980	FJ006593	
EF569981	FJ006594	
EF569982	FJ006595	
EF569983	FJ006596	
EF569984	FJ006597	
EF569985	FJ006598	
EF569986	FJ006599	
EU018268	FJ006600	
EU018269	FJ006601	
EU018270	FJ006602	

Table S1. Cont.

α-	γ-	ω-
EU018271	FJ006603	
EU018272	FJ006604	
EU018273	FJ006605	
EU018274	FJ006606	
EU018275	FJ006607	
EU018276	FJ006608	
EU018277	FJ006609	
EU018278	FJ006610	
EU018279	FJ006611	
EU018280	FJ006612	
EU018281	FJ006613	
EU018282	FJ006614	
EU018283	FJ006615	
EU018284	FJ006616	
EU018285	FJ006617	
EU018286	FJ006618	
EU018287	FJ006619	
EU018288	FJ006620	
EU018289	FJ006621	
EU018290	FJ006622	
EU018291	FJ006623	
EU018292	FJ006678	
EU018293	FJ006679	
EU018294	FJ006680	
EU018295	FJ006681	
EU018296	FJ006682	
EU018297	FJ006683	
EU018298	FJ040741	
EU018299	FJ040742	
EU680852	FJ040743	
EU680853	FJ040744	
EU680854	FJ040745	
EU680855	FJ040746	
FJ478466	FJ040747	
FJ478467	FJ040748	
FJ478468	FJ040749	
FJ595934	FJ040750	
FJ595935	FJ040751	
GQ891681	FJ040752	
GQ891682	FJ040753	
GQ891683	FJ040754	
GQ891684	FJ040755	
GQ891685	FJ040756	
GQ891686	FJ231103	
GQ891687	FJ595936	
U08287	FJ595937	
U50984	GQ857626	
U51302	GQ871769	
U51303	GQ871770	
U51304	GQ871771	
U51305	GQ871772	
U51306	GQ871773	
U51307	GQ871774	
U51308	GQ871775	
U51309	GQ871776	
U51310	GQ871777	
X54517	M11077	
X54688	M11335	
X54689	M11336	
	M13712	
	M13713	
	M16060	
	M16064	
	M36999	

Table S2. Average identity of the three RNAi fragments with α-, γ-, and ω-gliadin sequences

Construct	RNAi fragment	Average identity with gliadin groups (%)		
		α	γ	ω
pghp8.1	γ	71.9 (4.8)	80.7 (11.4)	66.6 (1.9)
pGhp-ω/α and	α	91.4 (6.1)	69.2 (2.6)	60.1 (2.7)
pDhp-ω/α	ω	79.2 (5.5)	69.3 (5.5)	75.6 (13.1)

Each RNAi fragment was compared with gliadin sequences from *T. aestivum* present in the GenBank database as follows: 120 α-gliadin sequences, 125 γ-gliadin sequences, and 22 ω-gliadin sequences. Average values are presented in percentages, and SDs are in brackets. The identity between the IR fragments and the α-, γ-, and ω- gliadin sequences was calculated using Needle and Supermatcher programs (http://emboss.sourceforge.net/).

Table S3. Primers used for the synthesis and detection of the RNAi constructs

Primer	Description	Sequence 5′ to 3′
Synthesis and detection of pGhp-ω/α and pDhp-ω/α plasmids		
alpha_hp_F	Forward primer for the synthesis of the 170-bp α-gliadin fragment	CAACAACAACTGATTCCATGC
alpha_hp_R	Reverse primer for the synthesis of the 170-bp α-gliadin fragment	AYRACATTRTGGATGGCYTG
omega_III_F	Forward primer for the synthesis of the 191-bp ω-gliadin fragment	CCTTCCTCATCTTTGTCCTCC
omega_III_R	Reverse primer for the synthesis of the 191-bp ω-gliadin fragment and detection of the ω/α hpRNA plasmids in transgenic plants	AAATGGTTGTTGCGATGGATA
omega_III_R_overlapping	Reverse primer for the synthesis of the 361-bp ω/α RNAi fragment	CAGTTGTTGTTGAAATGGTTGTTGCGATGG
alpha_F_overlapping	Forward primer for the synthesis of the 361-bp ω/α RNAi fragment	CAACAACCATTTCAACAACAACTGATTCCA
prGli_F+SphI	Forward primer for the synthesis of γ-gliadin promoter and detection of the pGhp-ω/α plasmid in transgenic plants	GAGCATGCTTCCAGAAAAAACTTTGCTAATG
prGli_R+XhoI	Reverse primer for amplification of γ-gliadin promoter	CGCTCGAGGGTGGATTTGCGTTAACTACTAC
pHorD_F+SphI	Forward primer for amplification of D-hordein promoter and detection of the pDhp-ω/α plasmid in transgenic plants	GCGCATGCCCATTAATTGAACTCATTCG
pHorD_R+XhoI	Reverse primer for amplification of D-hordein promoter	CGCTCGAGCATCCGACTGTCAATGAATT
Ubi_F+EcoRV	Forward primer for amplification of *Ubi*1 intron	TTGATATCCACCTCCGCTTCAAGGTACG
Ubi_R+EcoRV	Reverse primer for amplification of *Ubi*1 intron	GCGATATCAAGTAACACCAAACAACAGGGTGA
nost_F+EcoRI	Forward primer for amplification of *nos* terminator	TAGAATTCATCGTTCAAACATTTGGCAATA
nost_R+EcoRI	Reverse primer for amplification of *nos* terminator	GAATTCTGTTYGACAGCTTATCATCG
Detection of pghp8.1 plasmid		
prHorD*3	Forward primer for detection of pghp8.1 plasmid in transgenic plants	GGGGTACCCATTAATTGAACTCATTCGGGAAGC
SUbi_R	Reverse primer for detection of pghp8.1 plasmid in transgenic plants	GCGTACCTTGAAGCGGAGGTGGTCGACTCTAGA TTGCAACACCAATGATCTGATCG
Detection of pAHC25 plasmid		
BAR_F	Forward primer for detection of *bar* gene in transgenic plants	GTCTGCACCATCGTCAACC
BAR_R	Reverse primer for detection of *bar* gene in transgenic plants	GAAGTCCAGCTGCCAGAAAC