- 1 Supplemental Figures for
- 2 LHP1-mediated epigenetic buffering of subgenome diversity and
- 3 defense responses confers genome plasticity and adaptability in
- 4 allopolyploid wheat
- 5



7 Supplemental Figure 1. Enrichment of LHP1 targeted genes in sc-triads and non-

8 sc-homoeologs with different copy number. sc-triads and non-sc-homoeologs were

9 divided in 4 groups in each subgenome: copy number = 1, $2 \le \text{copy number} \le 3, 4$

10 \leq copy number \leq 5, copy number > 5.

TaLHP1 5'										
	550	555	5 ¹³	CDS(bp)	69 ¹	60 ⁸	630		stop codon site	
WT ABD	N	N	CCCAG	AGCAAGCAGCAGC	GAGAGGT	N	Ν	mutation	WT/Mut	
А	С	G	CCCAG	A - CAAGCAGCAG	GAGAGGT	G	С	-1 bp	1194 bp/643 bp	
<i>lhp1-abd-18</i> в	G	А	CCCAG	AG <mark>A</mark> CAAGCAGCAG	GAGAGGT	С	G	+1 bp	1188 bp/597 bp	
D	G	А	CCCAGAGTCAAGCAGCAGGAGAGGT G				G	+1 bp	1194 bp/603 bp	

- 13 Supplemental Figure 2. CRISPR-editing sites in *Talhp1-abd-18* lines. The positions
- 14 in gray represent the reference SNVs differentiating homoeologs. The region from
- 15 573 to 597 is the CRISPR target site. The type of mutation is listed on the right.
- 16



Supplemental Figure 3. Mutation information of single and double mutants and comparation of H3K27me3 level in single, double and triple mutants.

- a) CRISPR-editing sites in *Talhp1-abD and Talhp1-Abd* lines. The positions in blue
 represent the reference SNVs differentiating homoeologs. The region from 573 to
 597 is the CRISPR target site. The type of mutation is listed on the right.
- b) The global H3K27me3 level in *Talhp1* triple, single and double mutants via western
 blot with specific antibody against H3K27me3.
- 26

	FUL2		FUL2	FUL2		VRN1				
	TraesCS2A02G2	61200 TraesC	S2B02G281000	TraesCS2D02G262700		TraesCS5A02G391700		TraesCS5B02G	396600 Trae	sCS5D02G401500
gene	₩-{- }		 → →- ∎	↓ → 】		€ ++			1	● · · ·)
LHP1		أستعدده والاستاد	والمراجعة ومطبق				ير بر بالأله بيره	المحيط والمتعالي والمعالي	محدر والمشغلة	[0 - 0.20]
LHP1 peak				-						
JW1 H3K27me3	- 1 - A		L	I			a della		da a	[0 - 2000]
lhp1-abd-19 H3K27me3							de a m			[0 - 2000]
lhp1-abd-18 H3K27me3										[0 - 2000]
H3K27me3 peak			B - 18 1	1.100.00		1		1 I.	B11.1	
JW1 mRNA						A		L.		[0 - 0.60]
<i>lhp1-abd-19</i> mRNA	6 . I. k		1.1.36	i				I		[0 - 0.60]
<i>lhp1-abd-18</i> mRNA			- 1 MA							[0 - 0.60]
PNAS-BGC4										
	TraesCS5D02G487900	TraesCS5D02G488300	TraesCS5D02G488400	TraesCS5D02G488500	TraesCS5D	02G488600 T	raesCS5D02G488700	TraesCS5D02G488800	TraesCS5D02G48890	0 TraesCS5D02G489000
gene					· · ·	H	• ••• •			ID 0 201
LHP1	and a start with the	<u> </u>	And the state of t	ملغ بالعالية في سينات	A Bhat.		Aba a sha	الكبد فقطعهم	i addition in a	10-0.20
LHP1 peak										[0 1800]
JW1 H3K2/me3	6				-		•			[0 - 1000]
Inp1-abd-19 H3K2/me3										[0 - 1800]
Inp1-abd-18 H3K2/me3									A	[0 - 1800]
H3K2/me3 peak			_							
JW1 mRNA										[0 - 1.00]
inp1-abd-19 mRNA			_ A A.							[0 - 1.00]
<i>lhp1-abd-18</i> mRNA							A			[0 - 1.00]

27

28 Supplemental Figure 4. Genome tracks illustrating the functional genes involved

29 in flowering and floral development (top panel), gene clusters synthesizing

- 30 defense-related metabolites (bottom panel).
- 31



33 Supplemental Figure 5. H3K27me3 preferentially targeted non 1:1 homoeologous

34 genes in polyploidy species.

35



38 Supplemental Figure 6. Changes of H3K27me3 and expression in *Talhp1-abd-18*.

- a) H3K27me3 changes in the *Talhp1-abd-18* mutant. The x-axis represents the average
 read densities in H3K27me3 target genes in the wild-type and *Talhp1-abd-18*samples, whereas the y-axis represents the log2 changes (*M* value) of H3K27me3
 in *Talhp1-abd-18*.
- b) Enrichment of homeologous groups in genes with differential H3K27me3 changes
 (*M* value) in *Talhp1-abd-18*. The color of dots represents enrichment score, with all
 high confident genes as background.
- 46 c) Enrichment of genes with up- and down-regulated expression in genes with
 47 different levels of H3K27me3 changes [represented by M = log₂(fold-change)],
 48 with all high confident genes as background.
- 49
- 50





52 Supplemental Figure 7. Scatter plot of the changes in the expression and 53 H3K27me3 of functional genes in *Talhp1-abd-18*. Different colors and shapes 54 represent different functions.

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- 56



Supplemental Figure 8. Stripe rust infection induces subgenome-diversified defense genes by removing H3K27me3 (Results of *Talhp1-abd-18*).

a) Enrichment of *Talhp1-abd*-induced genes among the genes more highly expressed

- in samples resistant to various pathogens (represented by different colors) than inthe corresponding susceptible samples.
- b) Scatter plot representing H3K27me3 changes post-inoculation and in the *Talhp1-abd-18* mutant.
- c) Enriched functional domains among the genes with H3K27me3 levels commonly
 or uniquely decreased in *Talhp1-abd-18*.
- 67



69 Supplemental Figure 9. Resistance phenotypes to stripe rust in *Talhp1-abd-18*. Left:
70 WT and *Talhp1-abd-18* plants were inoculated with *Pst* CYR32 and photographed

at 14 DPI; bar, 1 cm. Right top: H_2O_2 accumulation in WT and *Talhp1-abd-18*

72 leaves at 2 DPI revealed by DAB staining; bar, 20 μm. Right bottom: *Pst* growth

in wild-type and *Talhp1-abd-18* plants at 2 dpi (stained with wheat germ agglutinin)

- 74 conjugated to Alexa-488) ; bar, 50 μm.
- 75
- 76
- 77



- 79 Supplemental Figure 10. The global H3K27me3 level in *Talhp1* triple, single and
- 80 double mutants via western blot with specific antibody against H3K27me3 (Full
- 81 uncropped scans of gel).
- 82