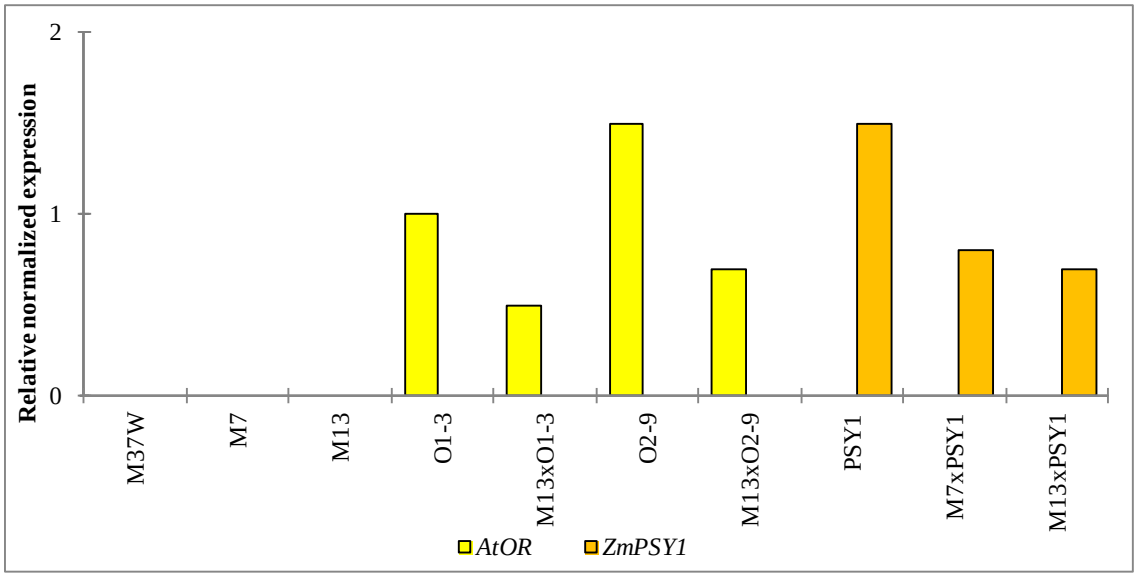

Figure S1. Transgene expression normalized against actin in wild-type (M37W) and transgenic lines presented as means of three replicates plus standard errors.

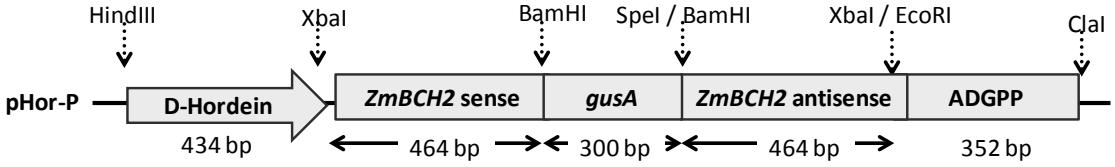

Figure S2 Schematic representation of pHorP-RNAi-ZmBCH2.

Table S1. Carotenoid content and composition of wild-type (M37W), M7, M13, B73, C17, NC356, O1-3, O2-9 and PSY1 parents and the corresponding hybrids at 30 DAP (µg/g DW±SE). The percentage of individual carotenoids in the endosperm is shown in brackets. Abbreviations: anthera, antheraxanthin; zea, zeaxanthin; lut, lutein; β-crypto, β-cryptoxanthin; α-crypto, α-cryptoxanthin; β-caro, β-carotene; phyto, phytoene; total caro, total carotenoids.

Plant line	anthera µg/g DW (%)	zea µg/g DW (%)	lut µg/g DW (%)	β-crypto µg/g DW (%)	α-crypto µg/g DW (%)	β-caro µg/g DW (%)	phyto µg/g DW (%)	Total caro µg/g DW
WT	0.0±0.00 (0)	2.0±0.10 (59)	1.1±0.00 (32)	0.0±0.00 (0)	0.0±0.00 (0)	0.0±0.00 (0)	0.3±0.00 (9)	3.4
M7	0.0±0.00 (0)	2.8±0.27 (60)	1.9±0.19 (40)	0.0±0.00 (0)	0.0±0.00 (0)	0.0±0.00 (0)	0.0±0.00 (0)	4.7
M13	0.3±0.02 (6)	2.5±0.19 (57)	1.7±0.14 (37)	0.0±0.0 (0)	0.0±0.00 (0)	0.0±0.00 (0)	0.0±0.00 (0)	4.5
B73	1.5±0.09 (5)	6.1±0.38 (19)	18.6±0.93 (57)	2.8±0.23 (8)	3.6±0.18 (11)	0.0±0.00 (0)	0.0±0.00 (0)	32.6
M7x B73	1.2±0.04 (3)	7.4±0.23 (17)	18.1±0.18 (43)	3.3±0.24 (8)	3.5±0.20 (8)	7.1±0.45 (17)	1.8±0.12 (4)	42.4
M13x B73	0.9±0.05 (3)	4.6±0.06 (13)	18.5±0.04 (54)	1.8±0.24 (5)	1.8±0.05 (5)	5.8±0.21 (17)	0.7±0.02 (2)	34.1
C17	0.0±0.00	6.2±0.20	12.0±0.30	0.6±0.03	0.0±0.00	11.4±0.90	8.7±0.02	38.9

	(0)	(16)	(31)	(1)	(0)	(29)	(22)	
M7x C17	0.8±0.01 (2)	7.3±0.20 (19)	9.0±0.21 (24)	2.6±0.15 (7)	1.0±0.01 (3)	12.6±0.17 (33)	4.8±0.13 (13)	38.1
M13x C17	0.4±0.01 (1)	11.2±0.22 (25)	8.5±0.18 (19)	2.8±0.03 (6)	1.2±0.01 (3)	14.9±0.51 (34)	5.4±0.11 (12)	44.4
NC356	3.0±0.19 (4)	45.5±0.78 (62)	10.8±0.17 (15)	5.0±0.25 (7)	3.7±0.16 (5)	5.9±0.41 (8)	0.0±0.00 (0)	73.9
M7x NC356	3.0±0.04 (3)	23.7±0.04 (23)	22.7±0.5 (22)	10.5±0.19 (10)	11.9±0.28 (12)	26.0±1.00 (25)	4.9±0.15 (5)	102.7
M13x NC356	2.2±0.00 (3)	13.2±0.17 (19)	20.2±0.10 (29)	3.0±0.19 (4)	5.2±0.16 (7)	25.3±1.40 (36)	0.4±0.00 (1)	69.5
O1-3	0.2±0.02 (2)	6.5±0.38 (61)	2.6±0.08 (25)	1.3±0.01 (12)	0.0±0.00 (0)	0.0±0.00 (0)	0.0±0.00 (0)	10.6
M13x O1-3	0.5±0.03 (7)	3.4±0.36 (45)	1.1±0.1 (14)	0.9±0.05 (12)	0.5±0.02 (6)	1.2±0.03 (15)	0.0±0.00 (0)	7.6
O2-9	0.0±0.00 (0)	9.3±2.02 (62)	3.9±0.35 (26)	1.9±0.40 (13)	0.0±0.00 (0)	0.0±0.00 (0)	0.0±0.00 (0)	15.1
M13x OR2-9	0.8±0.06 (7)	6.5±0.04 (53)	2.2±0.08 (18)	1.2±0.01 (9)	0.6±0.05 (5)	1.8±0.04 (15)	0.0±0.00 (0)	13.1
PSY1	0.0±0.00 (0)	25.5±2.14 (47)	8.5±0.35 (16)	6.6±0.29 (12)	0.0±0.00 (0)	8.7±0.75 (16)	5.0±0.18 (9)	54.3
M7x PSY1	4.5±0.05 (4)	23.8±0.19 (22)	12.3±0.17 (12)	10.5±0.25 (10)	9.3±0.09 (9)	29.9±0.49 (28)	15.7±0.10 (15)	106.0
M13x PSY1	4.2±0.3 (5)	19.2±0.25 (23)	7.3±0.15 (9)	5.9±0.12 (7)	4.6±0.03 (5)	20.7±0.09 (25)	23.0±0.18 (27)	84.9

Table S2 Maize lines with specific carotenoid profiles used to evaluate the effect of *BCH* gene silencing by RNAi.

Line	Genotype	Source	Carotenoid profile	References
B73	Inbred	USDA	High lutein Very low β/ε ratio	(Harjes et al., 2008) (Vallabhaneni and Wurtzel, 2009)
C17	Inbred	USDA	High β-carotene High β/ε ratio	(Yan et al., 2010)
NC356	Inbred	USDA	High zeaxanthin Very high β/ε ratio	(Yan et al., 2010)
PSY1	Transgenic *ZmPSY1*	Applied plant biotechnology, Universitat de Lleida, Spain	High zeaxanthin and β-carotene High β/ε ratio	(Berman et al., 2017)
O1-3	Transgenic *AtOR*	Applied plant biotechnology, Universitat de Lleida, Spain	High zeaxanthin Medium β/ε ratio	(Berman et al., 2017)
O2-9	Transgenic AtOR	Applied plant biotechnology, Universitat de Lleida, Spain	High zeaxanthin Medium β/ε ratio	(Berman et al., 2017)

USDA: United States Department of Agriculture; CSIC: Consejo Superior de Investigaciones Científicas

References

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Table S3. Primer sequences used for qRT-PCR.

Gene	Forward	Reverse
ZmACTIN	5′-CGATTGAGCATGGCATTGT-3′	5′-CCCACTAGCGTACAACGAA-3′
ZmBCH1	5′-CCACGACCAGAACCTCCAGA-3′	5′-CATGGCACCAGACATCTCCA-3′
ZmBCH2	5′-GCTTGTTAGCAGTCCGGT-3′	5′-GAAAGGAGGATGGCGATAGAT-3′
ZmCYP97A	5′-CTGGAGCGTCTGAAAGTCA-3′	5′-GGACCAAATCCAAACGAGAT-3′
ZmCYP97B	5′-CTGAGGAGAAGGACTTGA-3′	5′-TCCACTGGTCTGTCTGCGAT-3′
ZmCYP97C	5′-GTTGACATTGGATGTGATTGG-3′	5′-AACCAACCTTCCAGTATGGC-3′
ZmPSY1	5′-CATCTTCAAAGGGGTCGTCA-3′	5′-CAGGATCTGCCTGTACAACA-3′
AtOR	5′- TTCTCTATCACCGCCCAAAAC-3′	5′- GCCATAGCCATTCCTGTGC-3′