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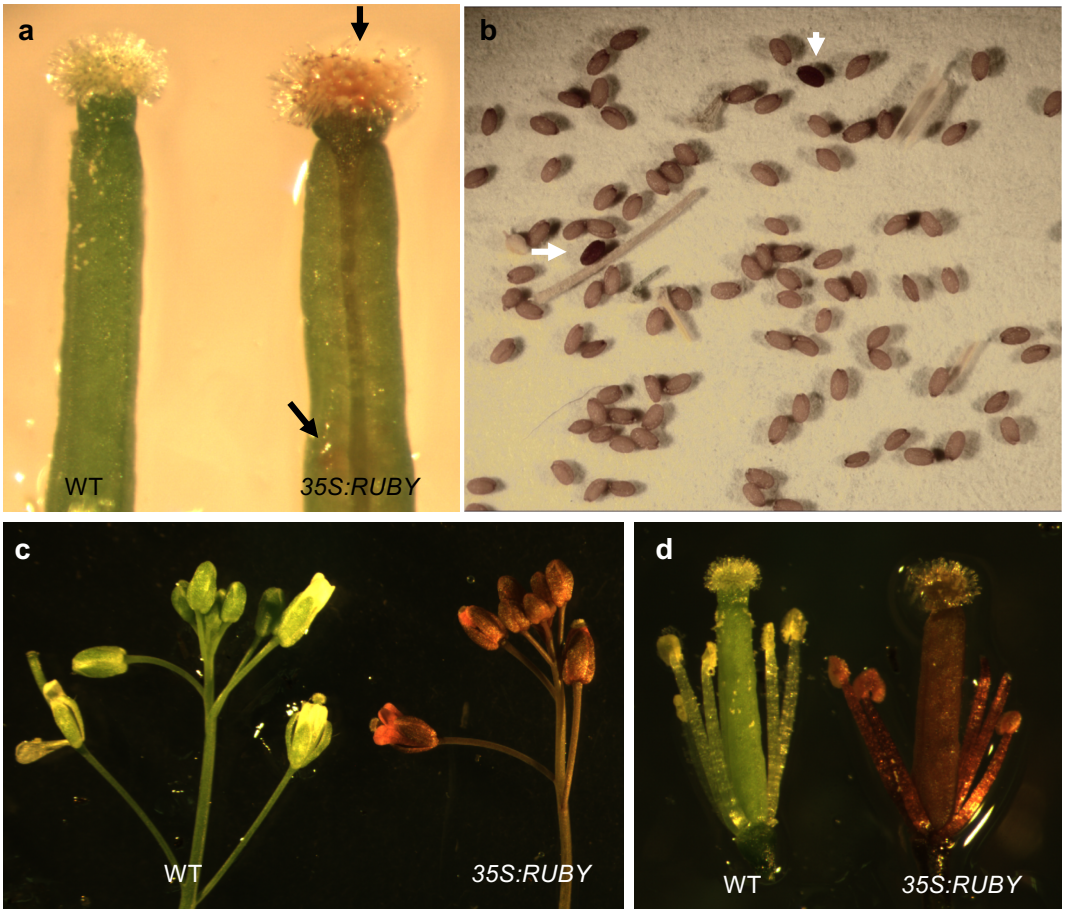
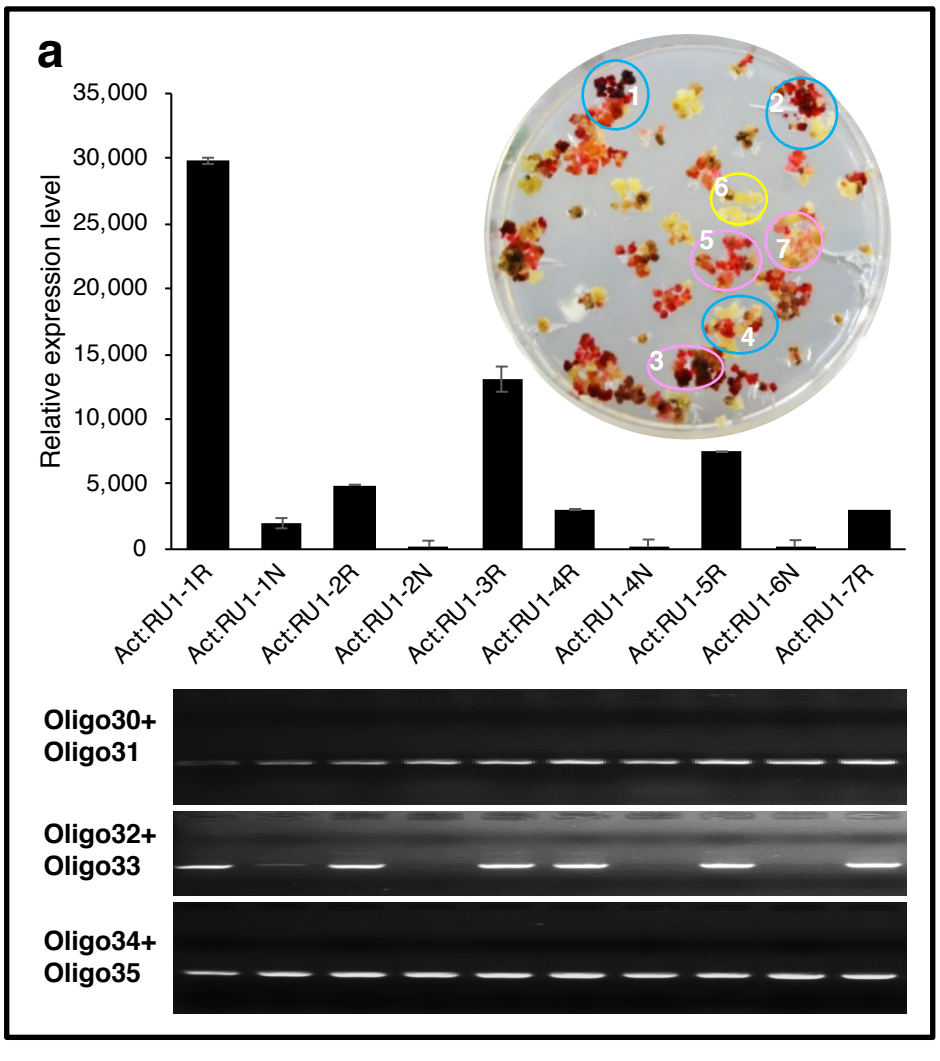
CAGCATATAACTACTGTATGTGCTATGGTATGGACTATGGAATATGATTAAAGATAAG

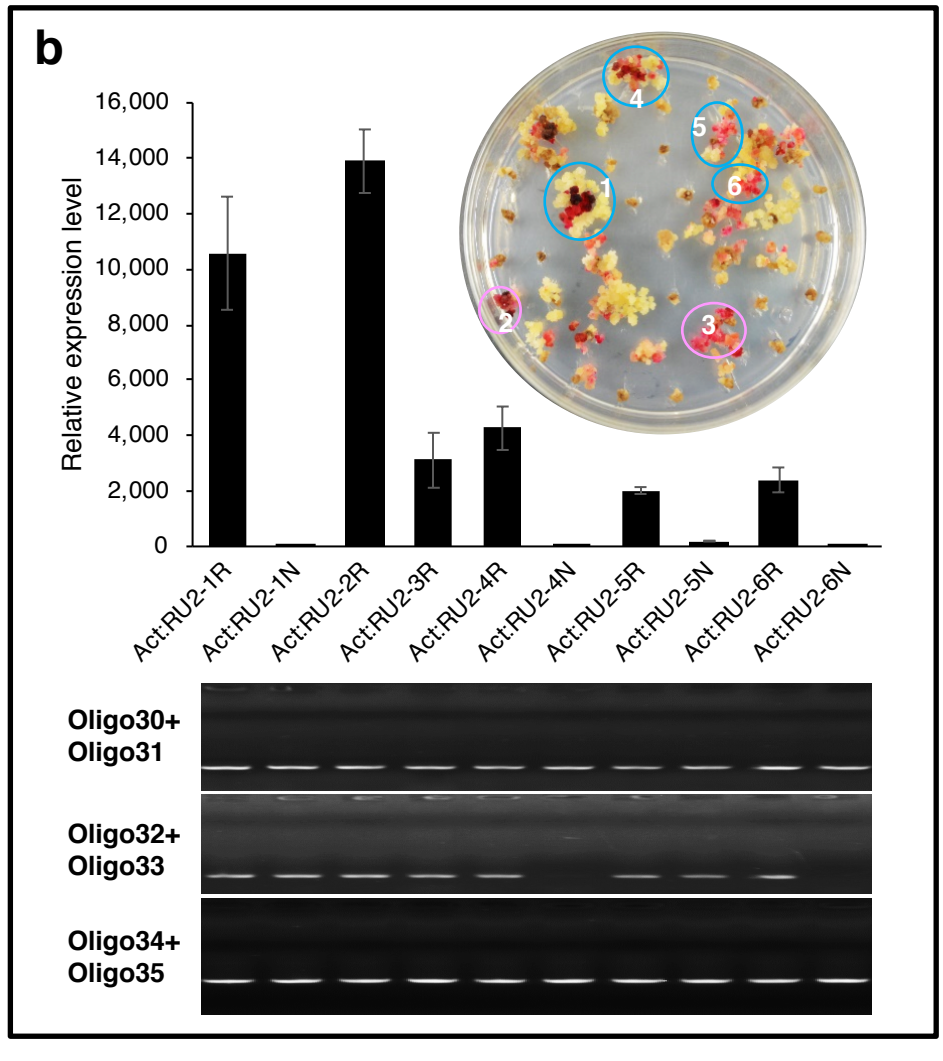
Supplemental Fig. 1. The complete sequence of *RUBY* reporter. The betalain biosynthetic genes *CYP76AD1* (highlighted in light blue), *DODA* (yellow), and *Glucosyltransferase* (gray), were arranged as a single open reading frame. The three genes were linked by sequences (red) that encode 2A peptides. Note that the two 2A sequences differed in DNA sequences, but both encoded the same peptide, which had a sequence of GSGATNFSLLKQAGDVEENPGP. The RUBY ORF was followed by the Arabidopsis *HSP18.2* terminator. For rice plasmids, we used F2A peptide (QLLNFDLLKLAGDVESNPGP). The corresponding DNA sequence is: CAATTGTTGAATTTTGATTTGTTGAAGTTGGCTGGAGATGTTGAATCTAATCCTGGAC CT

Supplemental Fig. 2. Transient expression of *RUBY* in tobacco leaves. Agrobacteria that contain the expression cassette of *RUBY* under the control of *CaMV 35S* promoter were infiltrated into tobacco leaves. The picture was taken 72 hours after infiltration.



Supplemental Fig. 3. Expression of *RUBY* driven by the *CaMV 35S* promoter in Arabidopsis. **a**) Two days after floral dipping, red betalain was visible in some flowers (arrow) (right), suggesting that the betalain enzymes were produced transiently. **b***)* Transgenic seeds (arrow) were easily distinguishable from non-transgenic seeds among the seeds harvested from plants underwent floral dipping. **c**)T1 plants of *35S:RUBY* (right) had red flowers and red stem . **d)** Betalain was synthesized in all floral organs in *35S:RUBY* plants.





Supplemental Fig. 4. Correlation of *RUBY* expression and the brightness of betalain color. Independent hygromycin-resistant calli tissue blocks were used for quantitative analysis of the expression level of *RUBY* from two independent *OsACTIN1: RUBY* transformation experiments **(a)** and **(b)**. *Act: RU1* and *Act: RU2* refer to plate 1 and plate 2 of two independent *OsACTIN1: RUBY* transformation events. The calli samples used for amplifying the DNA fragments of *RUBY* and *HptII,* and analyzing the *RUBY* expression level were numbered and labeled by circles with different colors. The circles in pink and yellow represent only red (R) and yellow (N) calli, respectively. The blue circle refers to both the red (R) and yellow (N) calli in same tissue block were analyzed separately. “R” in Act:RU1-1R and other terms refer to red tissue while “N” in the terms refer to negative of RUBY phenotypes. Oligo30/Oligo31, Oligo32/Oligo33 and Oligo34/Oligo35 refer the primer pairs used for detecting the presence the rice genome DNA, *RUBY* DNA and selection marker gene *HptII*, respectively. The primers were described in Supplemental Table 1.

Supplemental Fig. 5. RUBY is useful for monitoring transgenes in intact rice plants.
The expression of *DR5:RUBY* in Xiaowei^{NIP} background was obvious in one month old plant grown in the field. WT refers to Xiaowei^{NIP} plant.

Primer name	Sequence	Annotation
Oligo1	CACTGATAGTTTAAACTAGTATGGATCATGCGACCCTCGC	*RUBY* construction
Oligo2	CACCTGCCTGCTTAAGGAGGCTAAAATTGGTAGCTCCGCTACCGTAGCGCGGAATCGGGA	*RUBY* construction
Oligo3	CCTTAAGCAGGCAGGTGATGTAGAAGAGAACCCCGGGCCTATGAAGATGATGAACGGCGA	*RUBY* construction
Oligo4	CTCCTGCTTGCTTGAGCAGGCTAAAGTTGGTTGCTCCGGATCCGGCGGAGGTGAACTTGT	*RUBY* construction
Oligo5	GCTCAAGCAAGCAGGAGATGTTGAGGAAAATCCTGGCCCCATGACCGCCATCAAGATGAA	*RUBY* construction
Oligo6	GCTAGCTTACTCAGTTAGGTCTTATCTTTAATCATATTCC	*RUBY* construction
Oligo7	CTGTCAAACACTGATAGTTTTGAGACTTTTCAACAAAGGG	*35S:RUBY* construction
Oligo8	CGCATGATCCATACTAGTTTTCAGCGTGTCCTCTCCAAAT	*35S:RUBY* construction
Oligo9	CTGTCAAACACTGATAGTTTCTGCAGTGCAGCGTGACCCG	*ZmUbq:RUBY* construction
Oligo10	CGCATGATCCATACTAGTTTCTGCAGAAGTAACACCAAAC	*ZmUbq:RUBY* construction
Oligo11	CTGTCAAACACTGATAGTTTATGTCCAACATGCATGCG	*AtYUC4:RUBY* construction
Oligo12	CGCATGATCCATACTAGTTTGTCGACTAATAAAAGCGAAAGAGAG	*AtYUC4:RUBY* construction
Oligo13	CTGTCAAACACTGATAGTTTTAAGCTGGCACAACTATATT	*AtAT2S3:RUBY* construction
Oligo14	CGCATGATCCATACTAGTTTTGTTGGTACCGTTTTGCTAT	*AtAT2S3:RUBY* construction
Oligo15	TAAGGGACTGACCACCCGGGGATCCGGTATCGATAAGCTTGCAGCC	*pDR5:eGFP* construction
Oligo16	CAGCGCTGAAGCTTGGCTGCAGTGTAATTGTAAATAGTAATTGTAATG	*pDR5:eGFP* construction
Oligo17	CACTGCAGCCAAGCTTCAGCGCTGTAGATCTCCATGGATCATGCGACCCTCG	*pDR5:RUBY* construction
Oligo18	CATCTCCAGCCAACTTCAACAAATCAAAATTCAACAATTGGTAGCGCGGAATCGGGATG	*pDR5:RUBY* construction
Oligo19	GTTGAAGTTGGCTGGAGATGTTGAATCTAATCCTGGACCTAAGATGATGAACGGCGAGG	*pDR5:RUBY* construction
Oligo20	CGATCGGGGAAATTCGAGCTGGTCACCGGCGGAGGTGAACTTGTAGG	*pDR5:RUBY* construction
Oligo21	GTTGAAGTTGGCTGGAGATGTTGAATCTAATCCTGGACCTACCGCCATCAAGATGAACA	*pDR5:RUBY* construction
Oligo22	TCTTCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCTACCGCCATCAAGATGAACA	*pDR5:RUBY* construction
Oligo23	ACGATCGGGGAAATTCGAGCTGGTCACCTCACTATCACTGGAGGCTTG	*pDR5:RUBY* construction
Oligo24	CCTACAAGTTCACCTCCGCCGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAG	*pDR5:RUBY* construction
Oligo25	CTACAAGTTCACCTCCGCCCAATTGTTGAATTTTGATTTGTTGAAGTTGGCTGGAGATG	*pDR5:RUBY* construction
Oligo26	AACAGCATCCTTGAGTCTCTTCG	qPCR primer of *RUBY*
Oligo27	TTCTCTTTGGAGATCTCGCCTTC	qPCR primer of *RUBY*
Oligo28	AACCAGCTGAGGCCCAAGA	qPCR primer of endogenous reference gene
Oligo29	ACGATTGATTTAACCAGTCCATGA	qPCR primer of endogenous reference gene
Oligo30	CTCAACCCCAAGGCTAACAG	detection the presence of rice genome DNA
Oligo31	ACCTCAGGGCATCGGAAC	detection the presence of rice genome DNA
Oligo32	AGAAGATTTTCTCCAAGAAGCCGA	detection the presence of *RUBY* DNA
Oligo33	ATAATGAGCACGCCATCATTCTTG	detection the presence of *RUBY* DNA
Oligo34	ACGGTGTCGTCCATCACAGTTTGCC	detection the presence of *HptII* gene
Oligo35	GGAGAGGACACGCTGAAATCACCA	detection the presence of *HptII* gene

Supplemental Table I: Primers used in this study.