

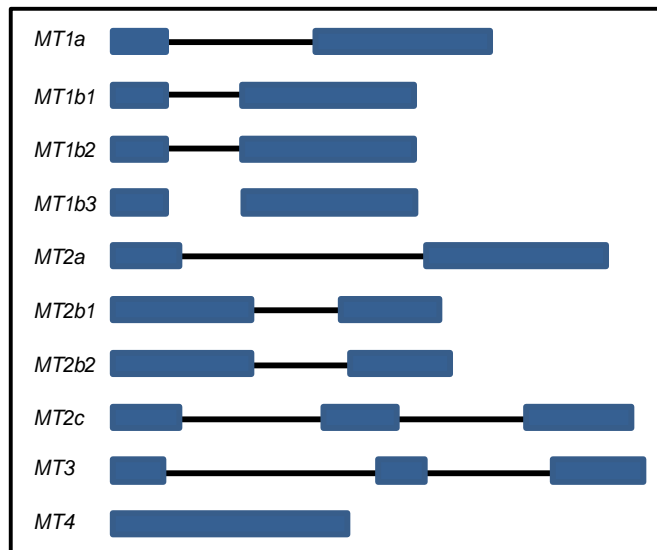
Supplemental Table S1. Identity scores (%) among MT1 and MT2 protein subgroups in barley.

	MT1a	MT1b1	MT1b2	MT1b3
MT1a	100	75.6	75.1	75.6
MT1b1	75.6	100	91.1	91.1
MT1b2	75.1	91.1	100	95.5
MT1b3	75.6	91.1	95.5	100
	MT2a	MT2b1	MT2b2	MT2c
MT2a	100	65.4	65.1	66.2
MT2b1	65.4	100	94	79.6
MT2b2	65.1	94	100	78.4
MT2c	66.2	79.6	78.4	100

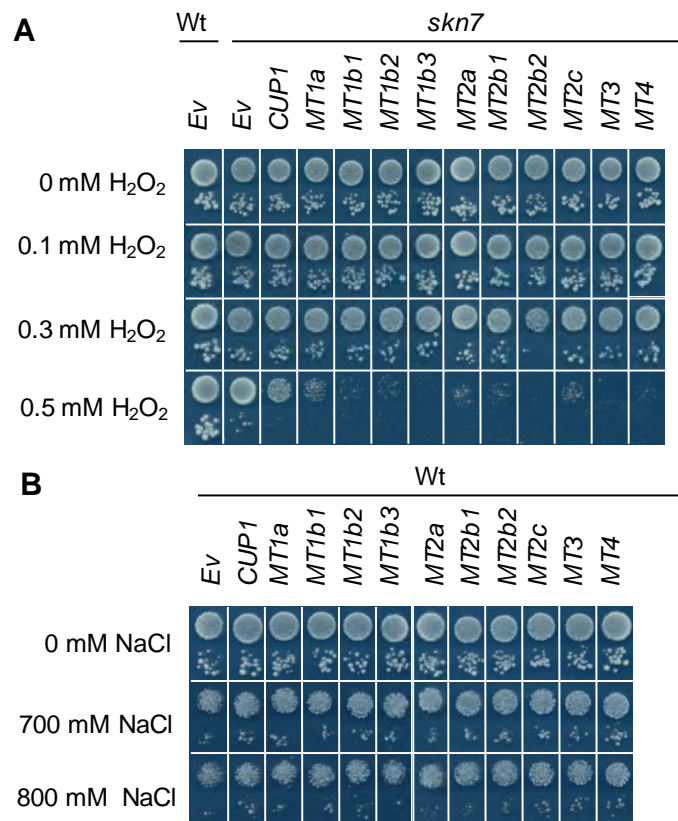
Supplemental Table S2. Primers used in this study

Gene/cDNA	Primer ¹	Sequence 5'-3'
Cloning		
<i>MT1a</i>	1a	GGCTTAAU TTCGGGTT CACCAGATCT
	1b	GGTTTAAUCAGATCATCAGACGCTCG
<i>MT1b1</i>	2a	GGCTTAAUGGCAACACAACAGAACCA
	2b	GGTTTAAUCACAAGCGCATCGACTGC
<i>MT1b2/MT1b3</i>	3a	GGCTTAAUAGCAGCACAGGTTACAGC
	3b	GGTTTAAUCACCACAATCGCATAACACC
<i>MT2a</i>	4a	GGCTTAAUATAACCAACTCGAGGGAG
	4b	GGTTTAAUCCGTCTCCATGTGTTGAT
<i>MT2b1</i>	5a	GGCTTAAUCAGGAAGCTCCTAGCCAA
	5b	GGTTTAAUATGCACGCACGGTGCATG
<i>MT2b2</i>	5a	as above
	5d	GGTTTAAUATGCAGGCAGGTGCTTACA
<i>MT2c</i>	6a	GGCTTAAUTCGCCACCGCCACCAAG
	6b	GGTTTAAUCGTGCTCGCTCTGCCGG
<i>MT3</i>	7a	GGCTTAAUGAGGCACTTCTCCGATCA
	7b	GGTTTAAUGACACGGGTTACTAGTACGGG
<i>MT4</i>	8a	GGCTTAAUACGCCTGAGAAGATCGAG
	8b	GGTTTAAUGGCTCAGCGGCACTTATT
<i>CUP1</i>	9a	GGCTTAAUACTCCTTGTCTTGTATCA
	9b	GGTTTAAUTTATTCTTGGGGCGACAT
Subcloning into p462		
<i>MT1a</i>	10a	GCAGATCTATGTCTTGCAATTGTGGATC
	10b	CTCGAGTTAACAGTTACAGGGGTTG
<i>MT1b1/MT1b2</i>	11a	AGATCTATGTCTTGACAGCTGTGGA
	11b	CTCGAGTTAGCAGTTACAAGGGTTG
<i>MT1b3</i>	11c	AGATCTATGTCTTGACAGTTGTGGATC
	11b	as above
<i>MT2a</i>	12a	AGATCTATGTCTGCTGCGGAGGC
	12b	CTCGAGTCACTTGACAGGTGCACGG
<i>MT2b1/MT2b2</i>	13a	CGGATCCATGTCTTGCTGCGGAGGA
	13b	CTCGAGCTAGCAACTGCAGCAGGAGC
<i>MT2c</i>	14a	GGATCCATGTCTTGCTGCGGCGGC
	14b	GCTCGAGTCACTTGCAACTGCAGCAGG
<i>MT3</i>	15a	AGATCTATGGCTGACAAGTGC GGCAAC
	15b	CTCGAGTCAGTGTCCGCAGGTGCA
<i>MT4</i>	16a	CAGATCTATGGGCTGCGACGACAAGTGCG
	16b	CTCGAGTCAGGCGGTCGAGCCGCA
<i>CUP1</i>	17a	CGGATCCATGTTACAGCAATTGATTAAC
	17b	CTCGAGTCATTTCCCAGAGCAGCA
Subcloning into pMal-c2		
<i>MT3</i>	15c	GAAGGATTTCAATGGCTGACAAGTGC GGCAAC
	15b	as above
<i>MT4</i>	16c	GAAGGATTTCAATGGGCTGCGACGACAAGTGCG
	16b	as above
RT-qPCR		
<i>MT3</i>	18a	GACAAG ACC CAG TGC GTG AA
	18b	GGTGTCAACCATGACGATGC
<i>MT4</i>	19a	GCGTGTGTGAAAGTGTGTGTATA
	19b	CACTGATGCGAGTAGGTGGTAGG
<i>GAPDH</i>	20a	GCTCAAGGGTATCATGGGTTACG
	20b	GCAATTCAGCCTTAGCATCAAAG
<i>LTP2</i>	21a	ACCCAACTACGGGCACT
	21b	AGTGCGGTACGGGTATGC
<i>ITR1</i>	22a	CAGTTTCGGGGATTTCGTG
	22b	GACTGACCACGTAGGTGCG
<i>HOR2</i>	23a	CCCAAC AGT CGG TCC AAG
	23b	CGACTTGCTCCTCCTGCA

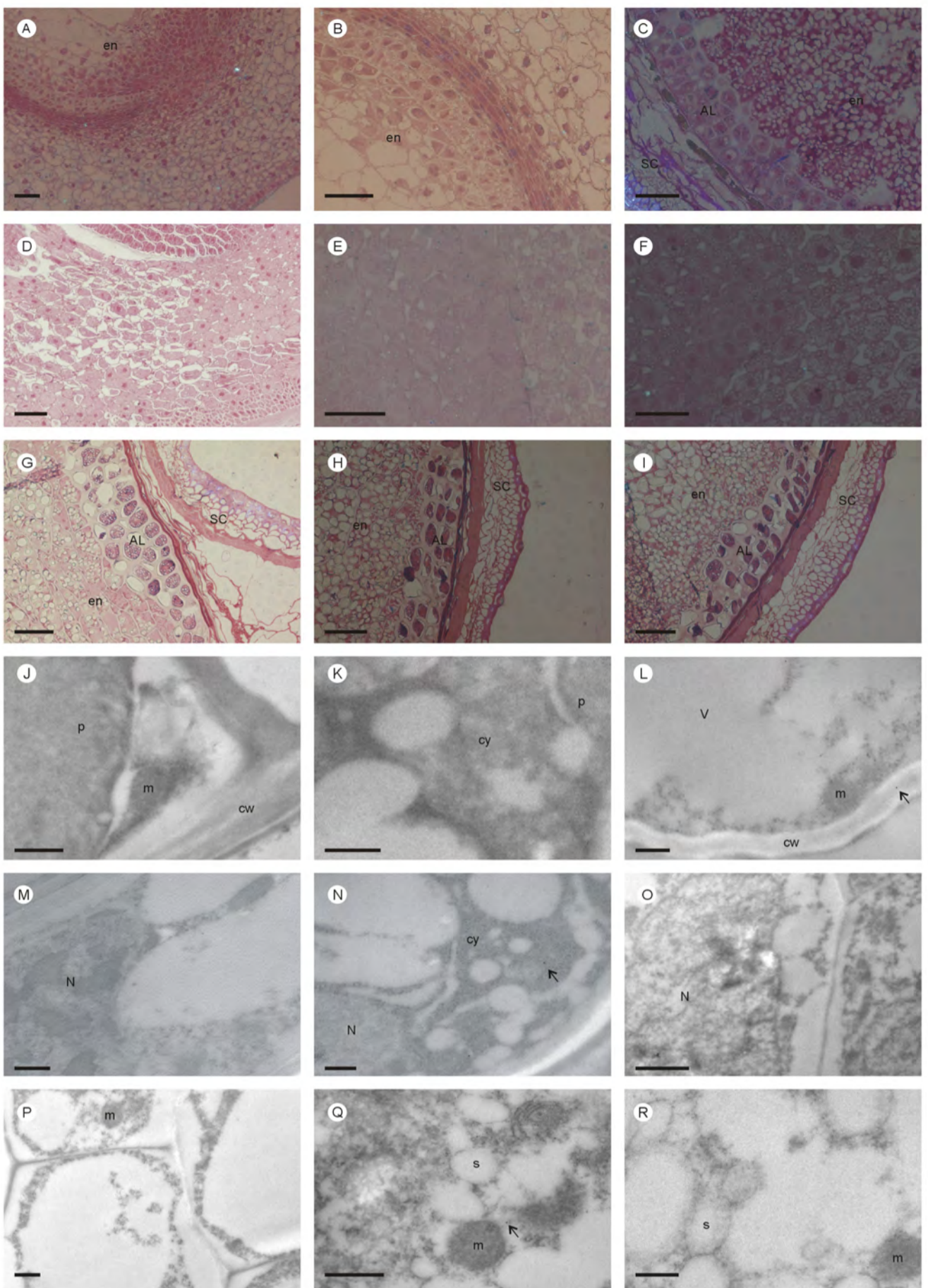
¹All a and c primers are forward orientated whereas b and d represent reverse primers. Modifications with 2'-deoxyuridine are indicated with a U.



Supplemental Figure S1. Exon-intron gene organization of barley MT genes. Exons and introns are indicated with blue boxes and lines, respectively. *MT2b1* and *MT2b2* introns are not identical but share intron positions. Genomic DNA encoding *MT1b3* was not detected.



Supplemental Figure S3. Effects of barley MT expression on H₂O₂ tolerance in *skn7* and salt tolerance in wild type yeast. Expression of *MT1a*, *MT1b1*, *MT1b2*, *MT1b3*, *MT2a*, *MT2b1*, *MT2b2*, *MT2c*, *MT3* and *MT4* in *skn7* (A) and in wild type (B). In both (A) and (B) cells were spotted in two concentrations (OD₆₀₀ = 0.05 and OD₆₀₀ = 0.0005). *skn7* or wild type were transformed with vector p426 or CUP1 as negative and positive controls, respectively.



Supplemental Figure S4. (A-I) Controls of developing grain transversal sections stained by fuchsin and examined under bright field epipolarized light (A) Control section of a 7 dap grain lacking the incubation with the anti-MT antibody. (B) Control sections of 7 dap grain (B) and 14 dap grain (C) treated with MT4 pre-immune serum. Control sections of 21 dap embryo lacking the incubation with the anti-MT antibody (D), treated with MT3 (E) or MT4 (F) pre-immune serum. Control sections of mature grain lacking the incubation with the anti-MT antibody (G), treated with MT3 (H) or MT4 (I) pre-immune serum. (J-R) Control sections of developing grain transversal sections observed with a transmission electron microscope. View of pericarp cells (J, K) and of endosperm cells (L) of a 7 dap grain sections lacking the incubation with the anti-MT antibody (J) or treated with MT4 (K, L) pre-immune serum. View of embryo cells of a 14 dap grain (M-N) and of a 21 dap grain (O) sections lacking the incubation with the anti-MT antibody (M) or treated with MT3 (N, O). View of embryo cells (P) and of the aleurone layer (Q-R) of 21 dap grain section treated with MT3 pre-immune serum (P, Q) or lacking the incubation with the anti-MT antibody (R). Arrows indicate the weak presence of gold particles. Abbreviations; AL, aleurone cell layer; cw, cell wall; cy, cytosol; en, endosperm; m, mitochondria; N, nucleus; p, plastid; s, spherosome; SC, seed coat. v, vacuole. Bars = 100 μm (A-I) and = 0.5 μm (J-R).