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Supporting Information for Proteomics

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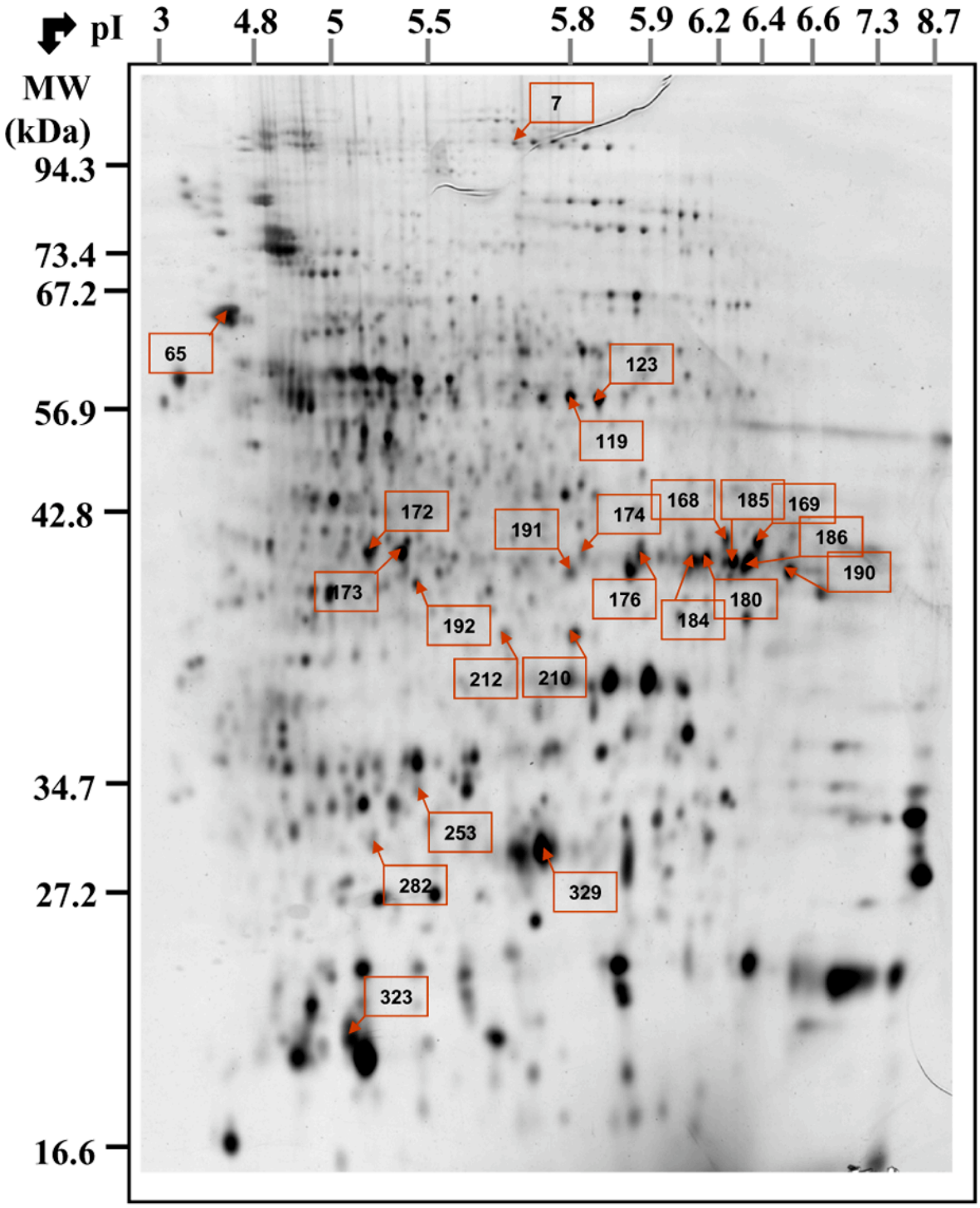
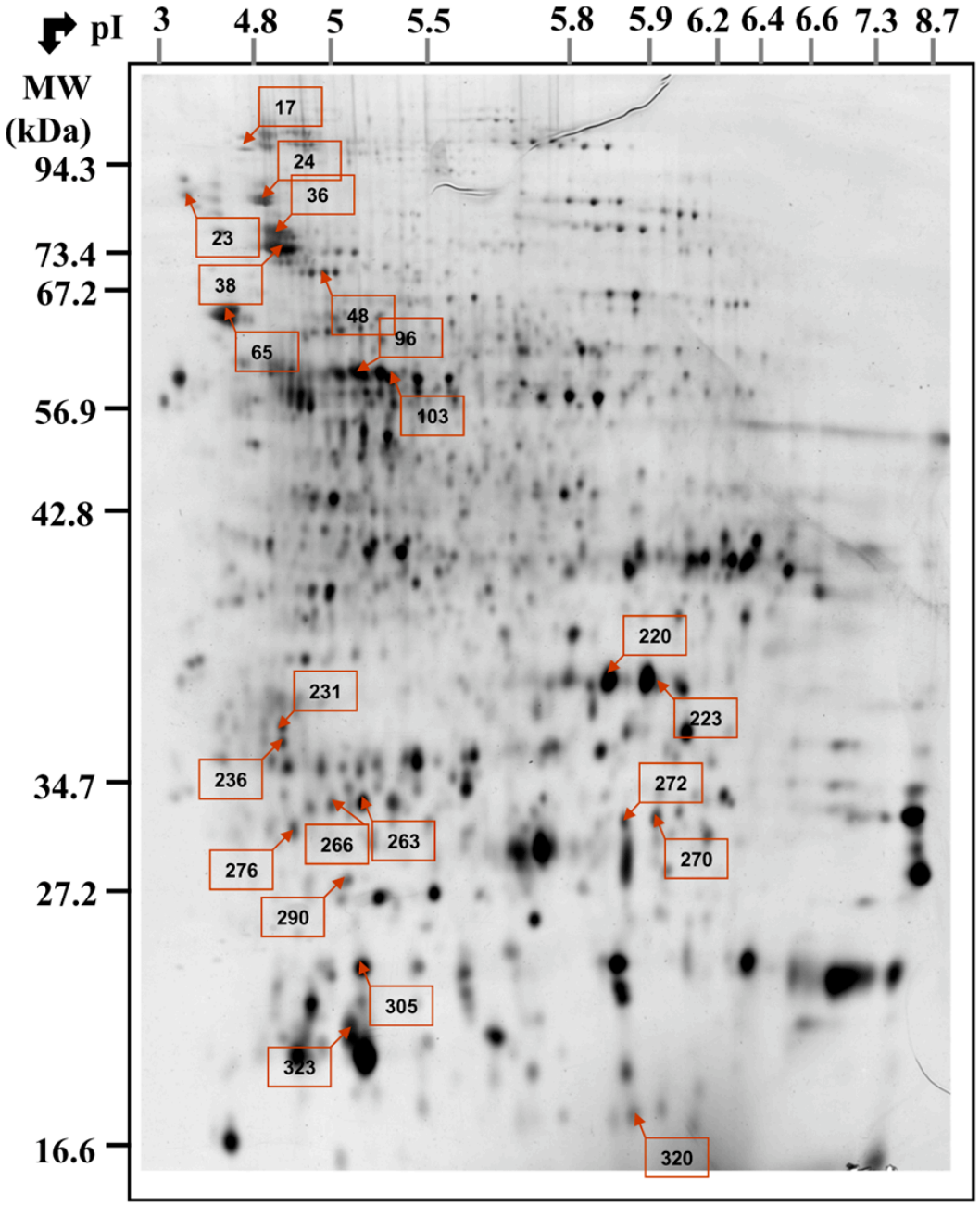
Proteomics reveal tissue-specific features of the cress (*Lepidium sativum* L.) endosperm cap proteome and its hormone-induced changes during seed germination

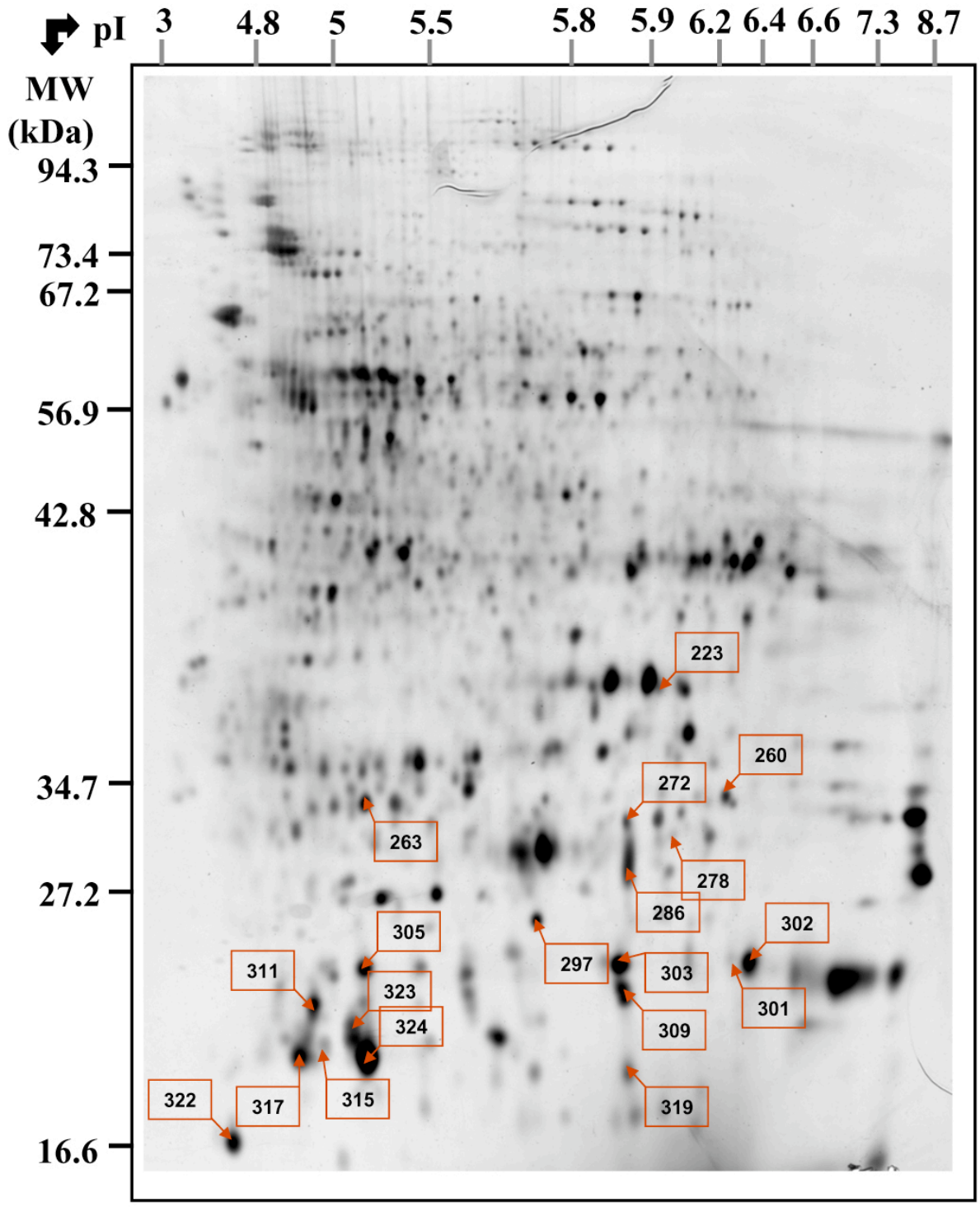
Supplemental Figure S1. Position of the protein spots listed in Supplemental Table S2 on a silver nitrate stained 2D gel. For spot characteristics, refer to Supplemental Table S1.



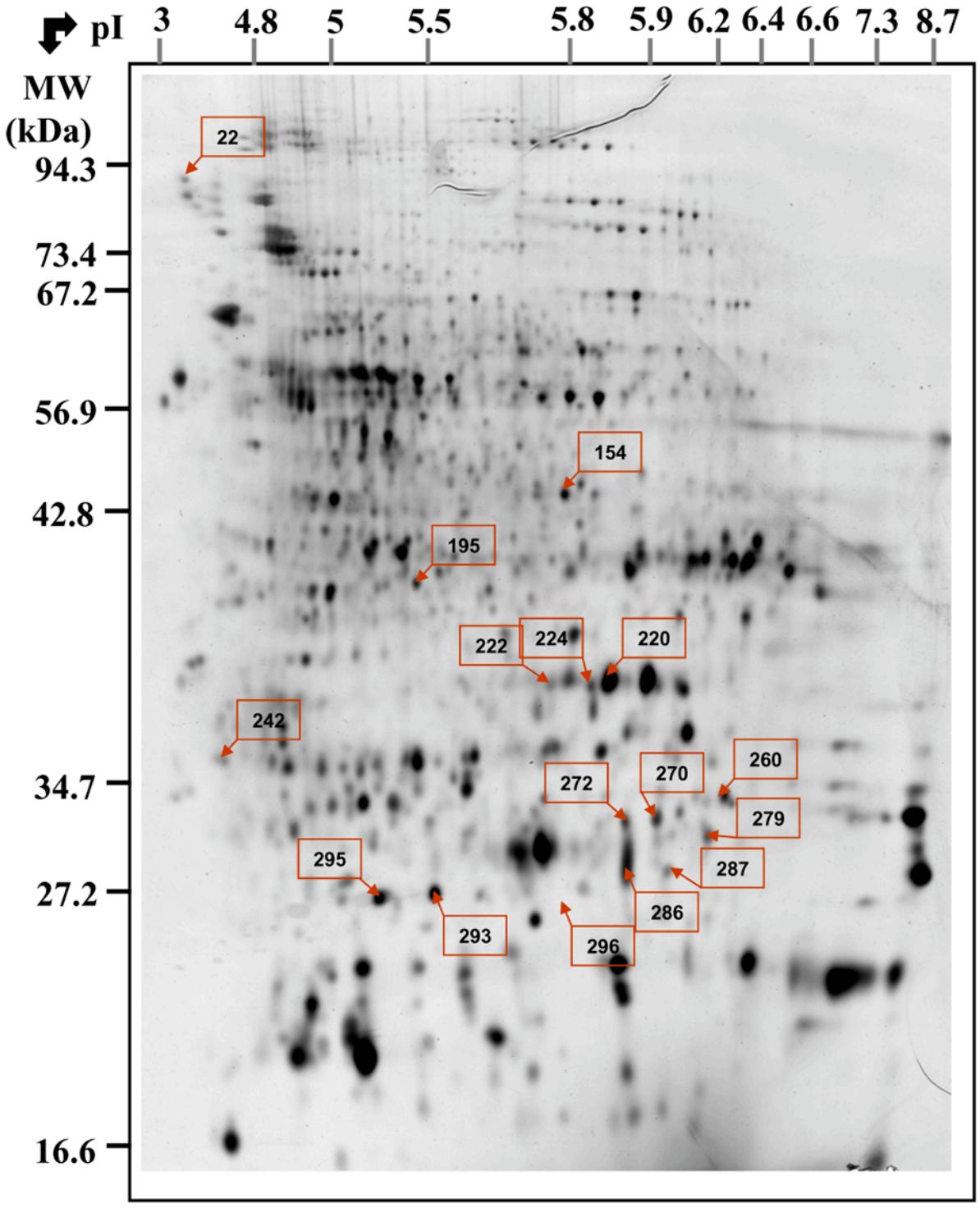
Supplemental Figure S2.1. Position of the protein spots listed in Supplemental Table S3 on a silver nitrate stained 2D gel. Numbers of storage proteins can be found in Supplemental Figure S2.2. For spot characteristics, refer to Supplemental Table S1.



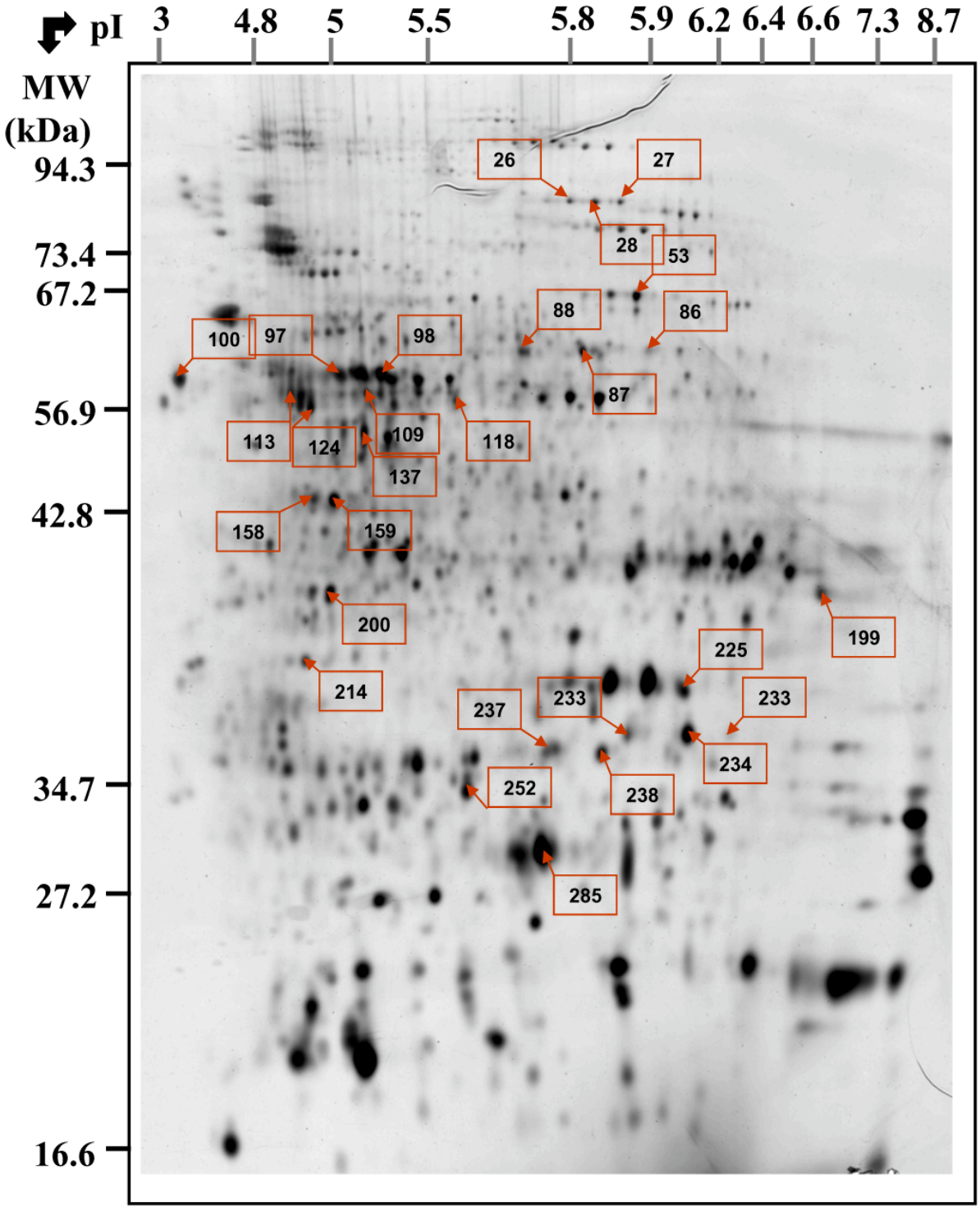
Supplemental Figure S2.2. Position of the protein spots referring to storage proteins listed in Supplemental Table S3 on a silver nitrate stained 2D gel. For spot characteristics, refer to Supplemental Table S1.



Supplemental Figure S3. Position of the protein spots listed in Supplemental Table S4 on a silver nitrate stained 2D gel. For spot characteristics, refer to Supplemental Table S1.



Supplemental Figure S4. Position of the protein spots not listed in Supplemental Tables S2-4 on a silver nitrate stained 2D gel. For spot characteristics, refer to Supplemental Table S1.



Supplemental Tables S1-S4

Legend for Supplemental Table S1 (provided as Excel file)

Supplemental Table S1. List of all identified proteins from endosperm caps of germinating cress seeds. Proteins were analyzed by two-dimensional electrophoresis and identified by LC/MS-MS.

N° = spot label on the reference maps presented in Supplemental Figures S1-S4;

8 h CON (av volume) = average normalized spot volume, calculated from three analyzed gels with extracts from biological replicates of 1000-2000 cress endosperm caps from seeds imbibed for 8 h;

18 h CON (av volume) = average normalized spot volume, calculated from three analyzed gels with extracts from biological replicates of 1000-2000 INTACT cress endosperm caps from seeds imbibed for 18 h;

18 h CON ruptured (av volume) = average normalized spot volume, calculated from three analyzed gels with extracts from biological replicates of 1000-2000 RUPTURED cress endosperm caps from seeds imbibed for 18 h;

18 h ABA (av volume); 96 h ABA (av volume) = average normalized spot volume, calculated from three analyzed gels with extracts from biological replicates of 1000-2000 cress endosperm caps from seeds imbibed for 18 h and 96 h, respectively, in the presence of 10 µM ABA;

SD = standard deviation;

fold change = calculated from the averaged means of normalized spot volumes of three 2D-gels loaded with biological replicates. Red numbers indicate statistically significant differences at $p < 0.05$ in a t-test;

Number of id per spot, number of proteins identified in the corresponding spot; quantitation of spot volumes is only presented for spots containing a single protein;

Cluster, cluster 1, proteins whose accumulation level remained constant; cluster 2, proteins whose accumulation level varied after endosperm rupture; clusters 3 and 4, proteins whose accumulation level varied prior to endosperm rupture in water (cluster 3) or ABA (cluster 4);

Exp MM (Da) = experimental molecular mass;

Exp pI = experimental isoelectric point;

Theo MM (Da) = theoretical molecular mass;

Theo pI = theoretical isoelectric point;

Organism = organism in which the protein has been identified;

No. NCBI accession = accession number in NCBI database;

Real Mascot matched queries = number of unique peptides identified with Mascot software;

Peptides = list of peptides identified;

Mascot score = identification score obtained with Mascot software;

Mascot coverage % = percentage of sequence coverage obtained with identified peptides with Mascot software for the orthologous protein;

Function category and Function description = functional categories defined according to the ontological classification of Bevan et al. [Bevan et al. (1998) Nature 391:485-488].

Supplemental Table S2. Identified proteins from the cap of germinating cress seeds belonging to functional category 02–energy. Only spots in which just one protein was identified are listed. "Fold change" is calculated from the averaged means of normalized spot volumes of three 2D-gels loaded with biological replicates. Bold numbers indicate statistically significant differences at $p<0.05$ in a t-test. For detailed information on spot characteristics, refer to Supplemental Table S1 and Supplemental Fig. S1.

	fold change					
spot #	8 to 18	18 to 18 ruptured	8 to 18 ABA	18 ABA to 96 ABA	protein name	functional category
168	**2.97**	**0.63**	0.53	**3.92**	fructose-bisphosphate aldolase, putative	02.01 glycolysis
169	**2.47**	**0.42**	0.58	**2.17**	fructose-bisphosphate aldolase, cytoplasmic isozyme	02.01 glycolysis
174	1.12	**0.52**	**2.22**	**3.91**	fructose-bisphosphate aldolase	02.01 glycolysis
176	**2.65**	0.78	**3.31**	1.30	fructose-bisphosphate aldolase, putative	02.01 glycolysis
253	0.66	1.06	0.60	0.74	triosephosphate isomerase, cytosolic	02.01 glycolysis
180	0.68	1.07	1.22	**1.84**	putative glyceraldehyde-3-phosphate dehydrogenase	02.01 glycolysis
185	0.75	1.11	0.78	0.55	glyceraldehyde-3-phosphate dehydrogenase	02.01 glycolysis
186	**2.01**	1.10	1.36	1.16	glyceraldehyde-3-phosphate dehydrogenase	02.01 glycolysis

190	**2.29**	**0.48**	1.41	**1.81**	glyceraldehyde-3-phosphate dehydrogenase	02.01 glycolysis
173	1.13	**0.41**	1.40	1.24	PGK (phosphoglycerate kinase)	02.01 glycolysis
192	**3.17**	**0.18**	2.92	1.07	PGK (phosphoglycerate kinase)	02.01 glycolysis
7	1.26	**0.50**	1.09	1.57	cytoplasmic aconitate hydratase	02.02 gluconeogenesis
191	0.87	0.66	0.68	**3.52**	malate dehydrogenase, cytosolic, putative	02.02 gluconeogenesis
210	0.72	1.20	1.20	0.90	malate dehydrogenase	02.10 TCA
212	0.82	1.00	0.92	1.03	malate dehydrogenase (NAD), mitochondrial	02.10 TCA

Supplemental Table S3. Identified proteins from the endosperm cap of germinating cress seeds belonging to functional category 06–protein folding and stability. Only spots in which just one protein was identified are listed. "Fold change" is calculated from the averaged means of normalized spot volumes of three 2D-gels loaded with biological replicates. Bold numbers indicate statistically significant differences at $p<0.05$ in a t-test. For detailed information on spot characteristics, refer to Supplemental Table SI and Supplemental Fig. S2.

	fold change					
spot #	8 to 18	18 to 18 ruptured	8 to 18 ABA	18 ABA to 96 ABA	protein name	functional category
17	**2.01**	**0.13**	**1.51**	0.73	SHD (SHEPHERD); HSP 90-like	06.01 folding and stability
23	**2.44**	1.05	1.88	1.29	protein disulfide isomerase precursor-like	06.01 folding and stability
24	0.89	**0.37**	1.30	**0.57**	heat shock cognate protein 80	06.01 folding and stability
276	**1.71**	**1.75**	1.07	0.94	chaperonin 10	06. folding and stability
290	**0.47**	**2.53**	0.92	1.05	chaperonin 10	06.01 folding and stability
236	**2.82**	0.77	1.31	**1.52**	aspartyl protease family protein	06.13 proteolysis
266	0.99	0.76	1.46	**3.63**	aspartyl protease family protein	06.13 proteolysis
223	**1.88**	1.30	1.13	1.10	26S proteasome regulatory subunit, putative	06.13 proteolysis
278	**3.06**	**4.60**	1.22	1.50	Cruciferin CRU1 precursor (11S globulin)	06. storage protein
301	0.85	**2.16**	0.67	**0.22**	CRU3 (CRUCIFERIN 3)	06.20 storage protein
302	0.82	**2.32**	**0.23**	2.44	CRU3 (CRUCIFERIN 3)	06.20 storage protein
317	1.37	**0.49**	1.07	**0.62**	CRA1 (CRUCIFERIN A)	06.20 storage protein
297	**0.33**	**0.57**	**0.12**	0.91	seed storage protein beta-chain 6 (fragment)	06.20 storage protein

Supplemental Table S4. Identified proteins from the endosperm cap of germinating cress seeds belonging to functional category 11–disease and defense. Only spots in which just one protein was identified are listed. "Fold change" is calculated from the averaged means of normalized spot volumes of three 2D-gels loaded with biological replicates. Bold numbers indicate statistically significant differences at $p<0.05$ in a t-test. For detailed information on spot characteristics, refer to Supplemental Table SI and Fig. S3.

	fold change					
spot #	8 to 18	18 to 18 ruptured	8 to 18 ABA	18 ABA to 96 ABA	protein name	functional category
154	**0.59**	0.83	**0.53**	**0.20**	12-oxophytodienoate reductase	11.02 defense regulated
222	**4.58**	**0.51**	0.87	0.51	thiocyanate forming protein	11.02 defense regulated
224	**1.77**	1.09	**0.43**	1.40	thiocyanate forming protein	11.02 defense regulated
242	1.01	**0.50**	0.89	**3.11**	GF14 psi chain	11.05 stress response
279	0.72	**1.84**	0.88	1.02	ATPER1 (1-cysteine peroxiredoxin 1)	11.05 stress response
195	**5.82**	**0.68**	**3.04**	**2.16**	aldo/keto reductase family protein	11.06 detoxification
287	**3.57**	**1.31**	2.25	**1.83**	glutathione S-transferase	11.06 detoxification
293	**0.63**	0.83	1.34	**1.75**	glutathione transferase	11.06 detoxification
295	1.00	0.86	1.10	**0.62**	glutathione transferase	11.06 detoxification
296	0.87	**3.24**	0.92	0.90	glutathione transferase	11.06 detoxification