Supporting Information

Temporal and spatial pattern of zinc and iron accumulation during barley (*Hordeum vulgare*) grain development

Detterbeck, A, Pongrac, P, Persson. DP, Vogel-Mikuš, K, Mitja, K, Vavpetic, P, Pelicon, P, Arčon, I, Husted, S, Schjoerring, JK, Clemens, S

The following Supporting Information is available for this article:

Fig. S1 Barley grains and positions within the grain chosen for mapping

Fig. S2 Sulphur (S) concentration in grain of two contrasting barley lines at four developmental stages extracted from µ-PIXE maps.

Fig. S3 Phosphorus (P) concentration in grain of two contrasting barley lines at different developmental stages extracted from µ-PIXE maps.

Fig. S4 Co-localization of phosphorus (P) and sulfur (S) with zinc (Zn).

Fig. S5 Co-localization of phosphorus (P) and sulfur (S) with iron (Fe).

Fig. S6 Positions of line scans obtained from Line scan positions of µ-PIXE distribution maps.

Fig. S7 Image of representative zinc (66Zn), iron (56Fe), sulphur (34S) and phosphorus (31P) distribution in and around the embryo in barley grains 27 days after pollination, analyzed by LA-ICP-MS.

Table S1 Signal intensities of LA-ICP-MS line scans in a low (LAL) and high (HAL) Zinc (Zn) accumulating line.

Fig. S1 Barley grains and positions within the grain chosen for mapping. (a) **Barley grains were harvested 7, 15, 27 days after pollination (dap) and at maturity**. Positions of µPIXE and LA-ICP-MS measurements are indicated with bars and analyzed area in (b). For µ-PIXE, the grain was sectioned transversely at the position of the embryo and for LA-ICP-MS the grain was cut both longitudinally (d) and transversely in the middle of the grain (e). µ-PIXE analysis was performed on grains 7, 15, 27 dap and maturity (c) and for LA-ICP-MS at 27 dap (d) and maturity (e). dem: differentiating embryo, end: endosperm, em: embryo, h: husk; cr: crease, a: aleurone.

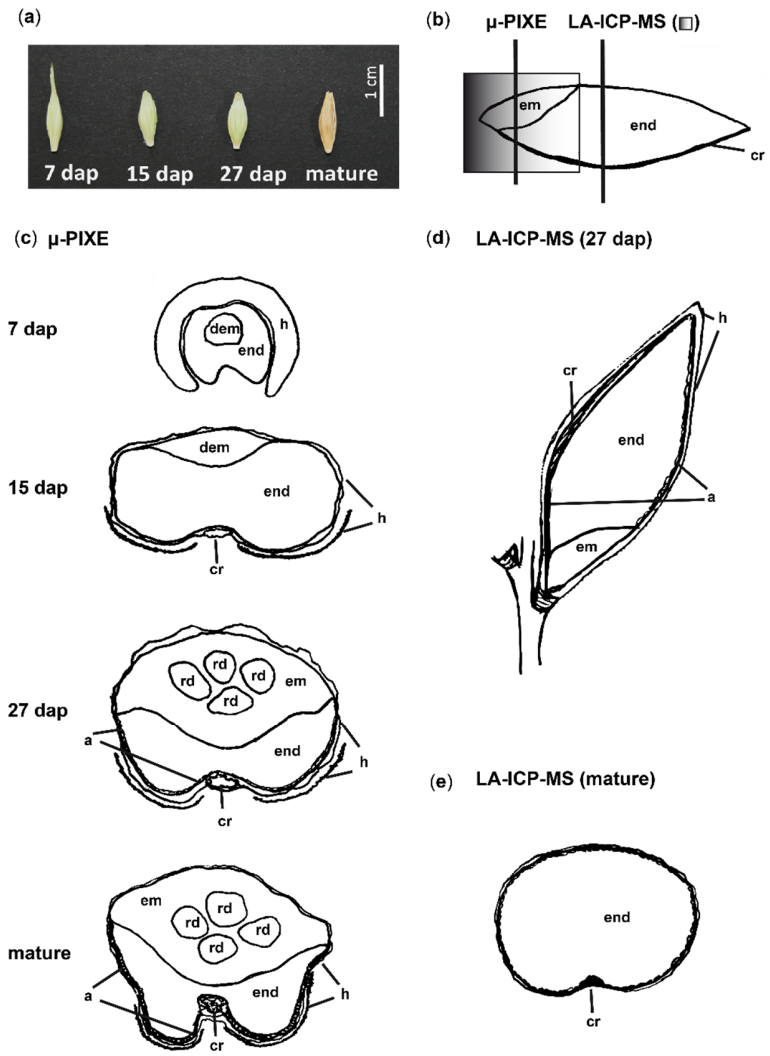

Fig. S2 Sulphur (S) concentration in grain of two contrasting barley lines at four developmental stages extracted from µ-PIXE maps. Sulphur concentrations averaged over µ-PIXE maps of three sections per line in the whole section (a), endosperm (b), embryo (c), aleurone (d), husk (e) and crease (f). At 7 dap aleurone and crease were not yet differentiated (indicated n.d.). Values are means + standard deviation. Statistical differences in grain tissue concentrations between the lines at different developmental stages were calculated using ANOVA/Sidak Holm post hoc test and are displayed as letters ($P \leq 0.05$) for comparing developmental differences within each line (LAL small letters, HAL: capital letters) and asterisks for comparison of the two different lines within one developmental stage (*:$P \leq 0.05$).

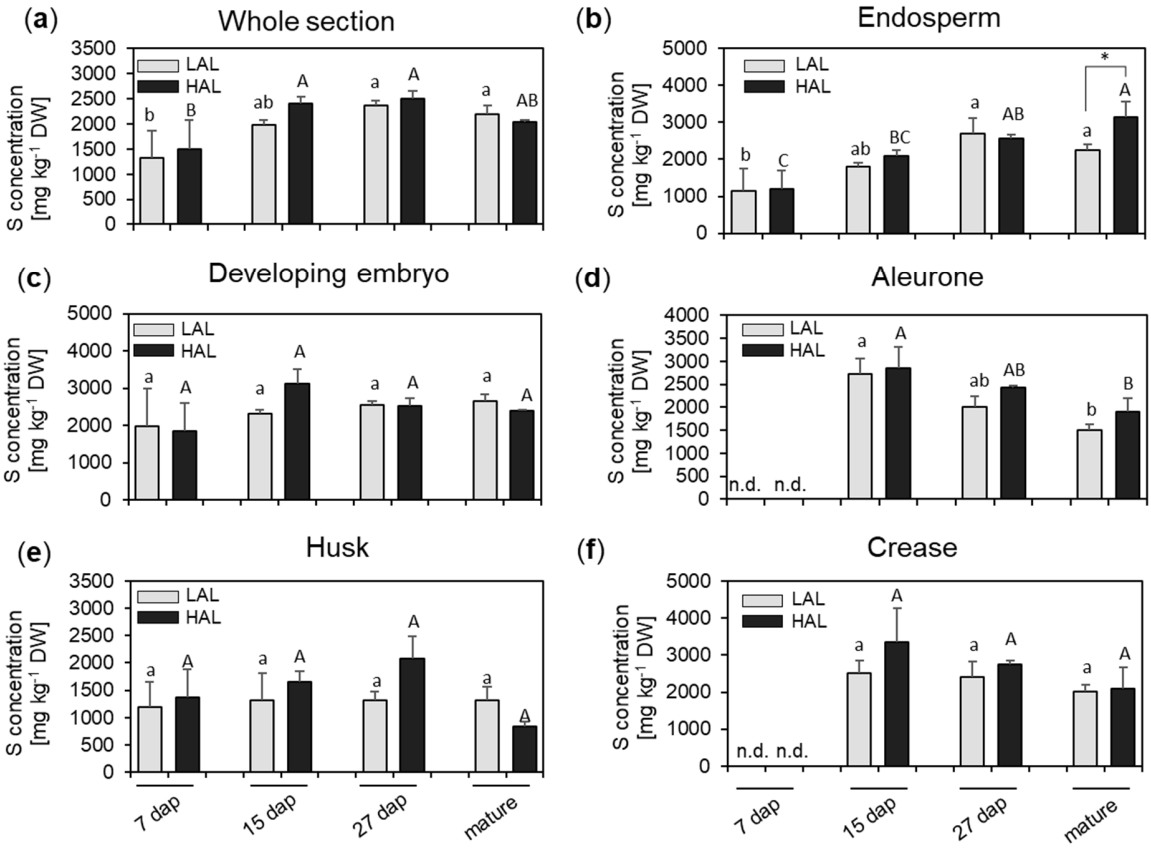

Fig. S3 Phosphorus (P) concentration in grain of two contrasting barley lines at different developmental stages extracted from µ-PIXE maps. Phosphorus concentrations averaged over µ-PIXE maps of three sections per line in the whole section (a), endosperm (b), embryo (c), aleurone (d), husk (e) and crease (f). At 7 dap aleurone and crease were not yet differentiated (indicated n.d.). Values are means + standard deviation. Statistical differences in grain tissue concentrations between the lines at different developmental stages were calculated using ANOVA/Sidak Holm post hoc test and are displayed as letters ($P \leq 0.05$) for comparing developmental differences within each line (LAL small letters, HAL: capital letters) and asterisks for comparison of the two different lines within one developmental stage (*:$P \leq 0.05$).

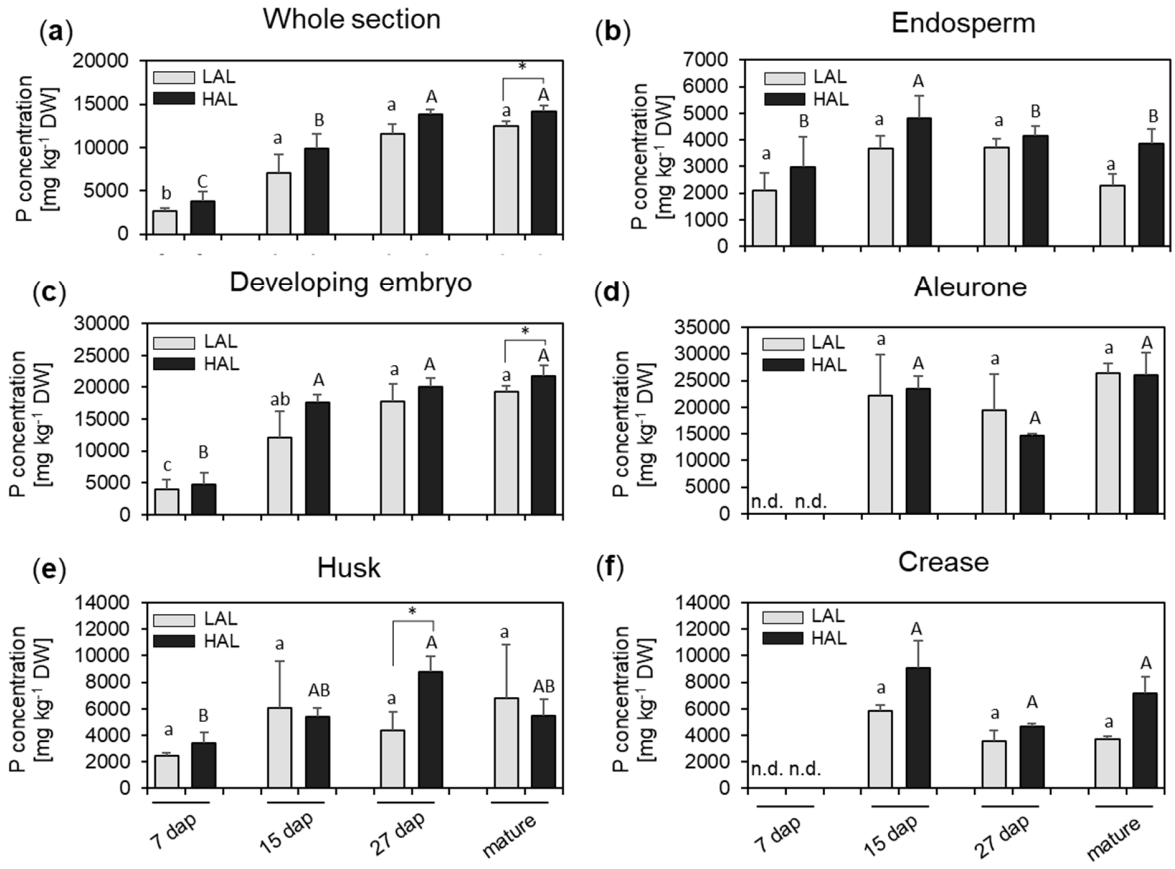

Fig. S4 Co-localization of phosphorus (P) and sulfur (S) with zinc (Zn). µ-PIXE co- localization maps for Zn (shown in green) with P (red) and S (blue) in barley grain sections of the low (LAL) Zn accumulating line and the high (HAL) Zn accumulating line at different developmental stages (7, 15 and 27 days after pollination (dap) and at maturity. Scale bars indicate 62.5 µm (7 dap) and 500 µm (15, 27 dap and mature).

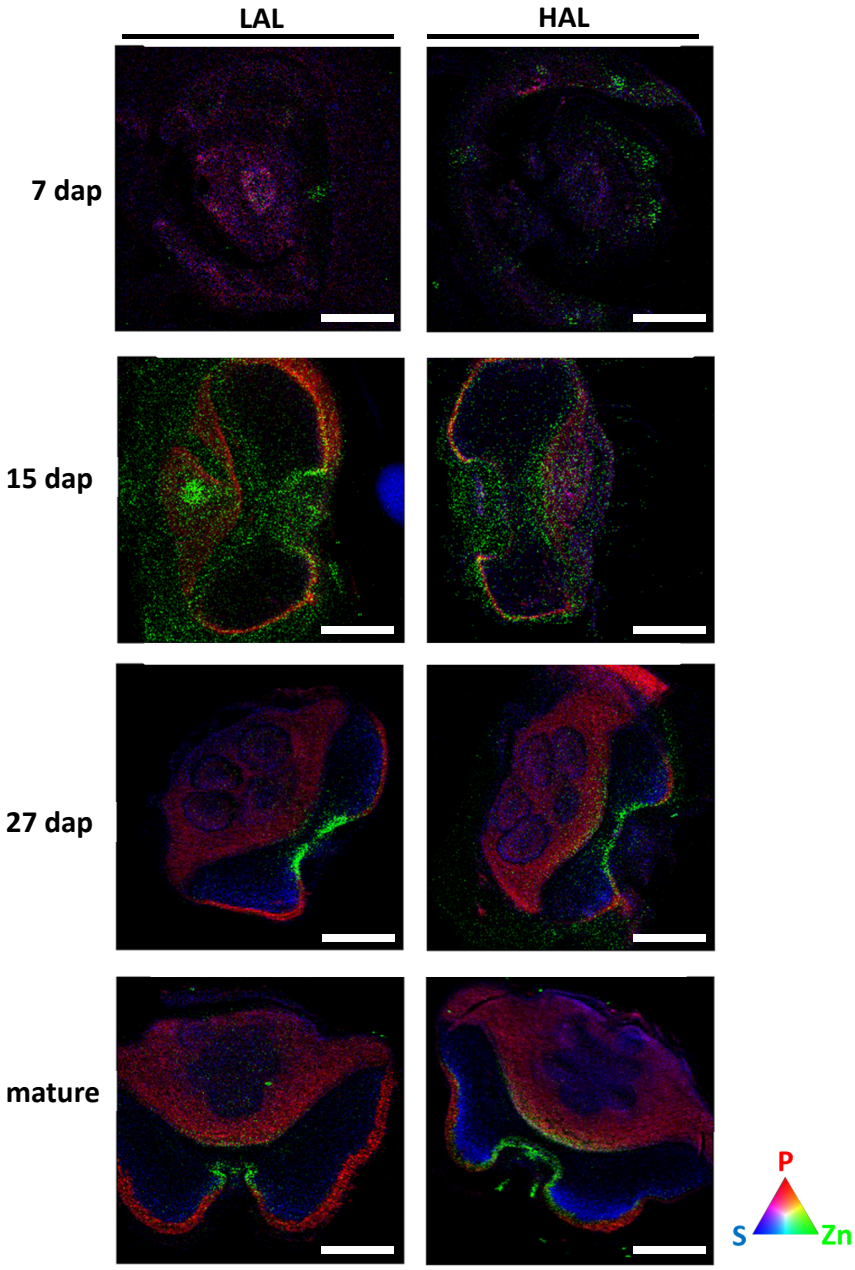

Fig. S5 Co-localization of phosphorus (P) and sulfur (S) with iron (Fe). µ-PIXE co- localization maps for Fe (shown in green) with P (red) and S (blue) in barley grain sections of the low Zn accumulating line (LAL) and the high Zn accumulating line (HAL) at different developmental stages (7, 15 and 27 days after pollination (dap) and at maturity. Scale bars indicate 62.5 µm (7 dap) and 500 µm (15, 27 dap and mature).

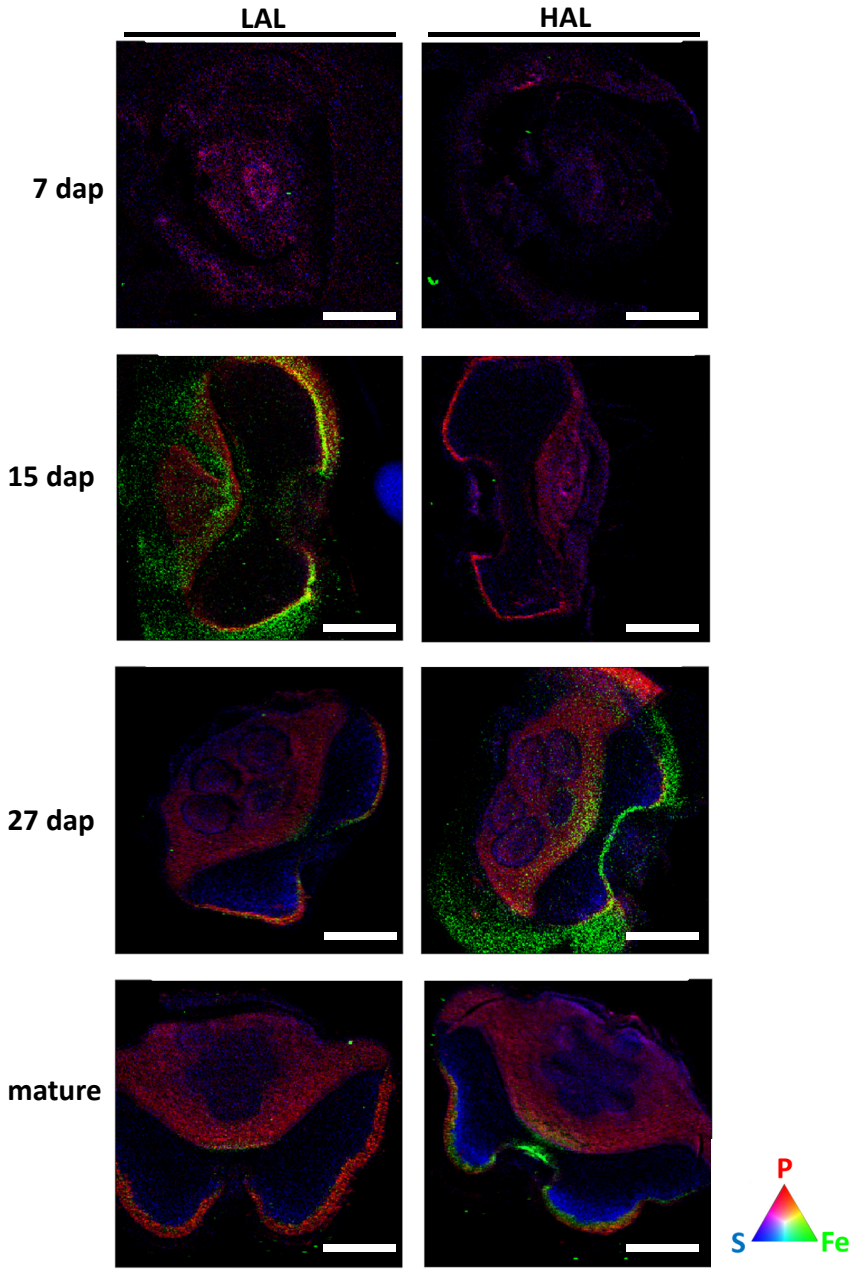

Fig. S6 Positions of line scans obtained from Line scan positions of µ-PIXE distribution maps.

Line scans were extracted from three µPIXE maps of a low (LAL) and high (HAL) Zn accumulating line at maturity as indicated by a black box using PyMca version 5.0. Line scans were transversed the aleurone and the endosperm tissue.

LAL | **HAL**

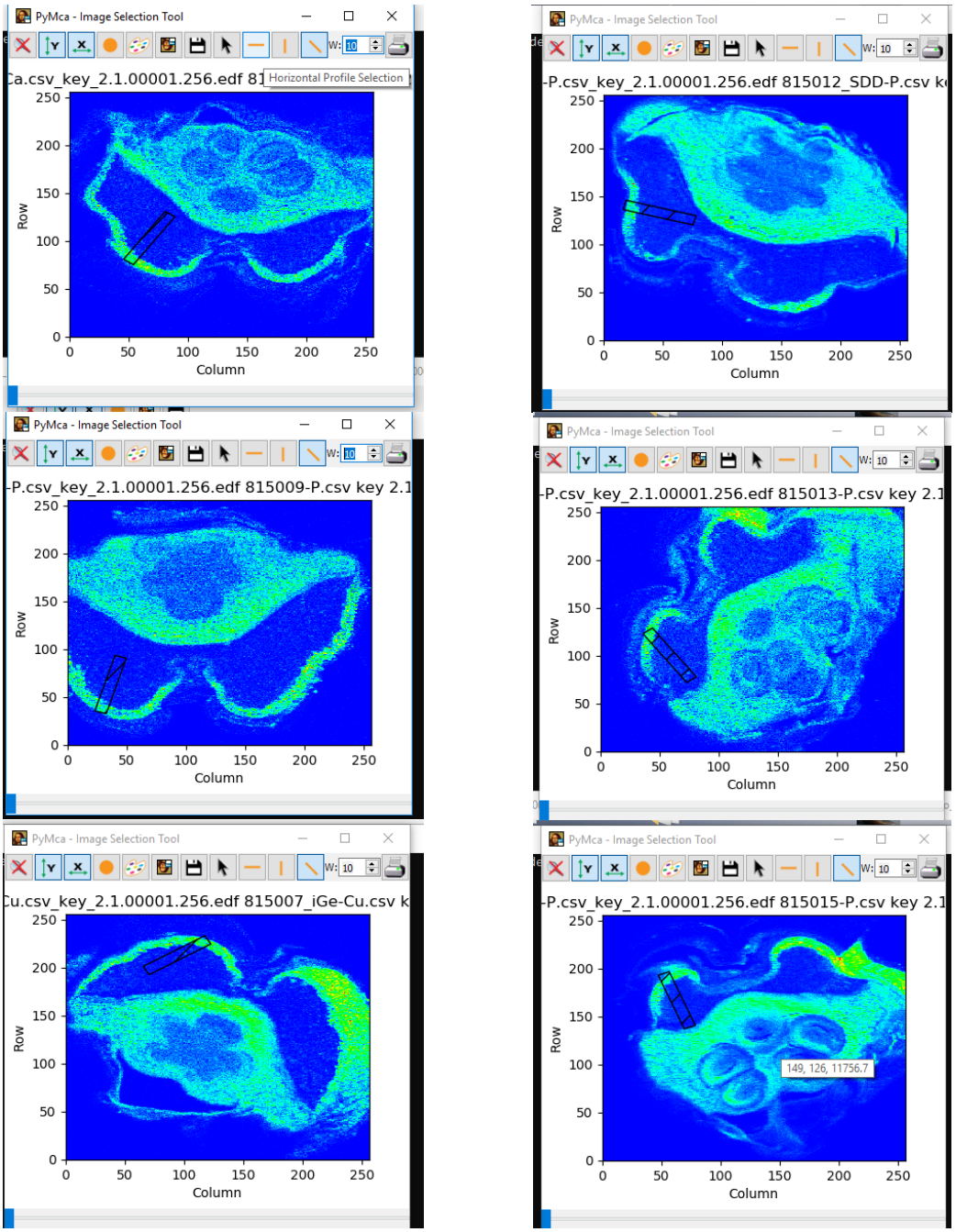

Fig. S7 Image of representative zinc (66Zn), iron (56Fe), sulphur (34S) and phosphorus (31P) distribution in and around the embryo in barley grains 27 days after pollination, analyzed by LA-ICP-MS. All signals were normalized to endogenous carbon, measured as 13C. Images were made at transversely cut grains including endosperm (end), aleurone (a), crease (cr) and husk (h) as indicated. The size bar represent 1 mm.

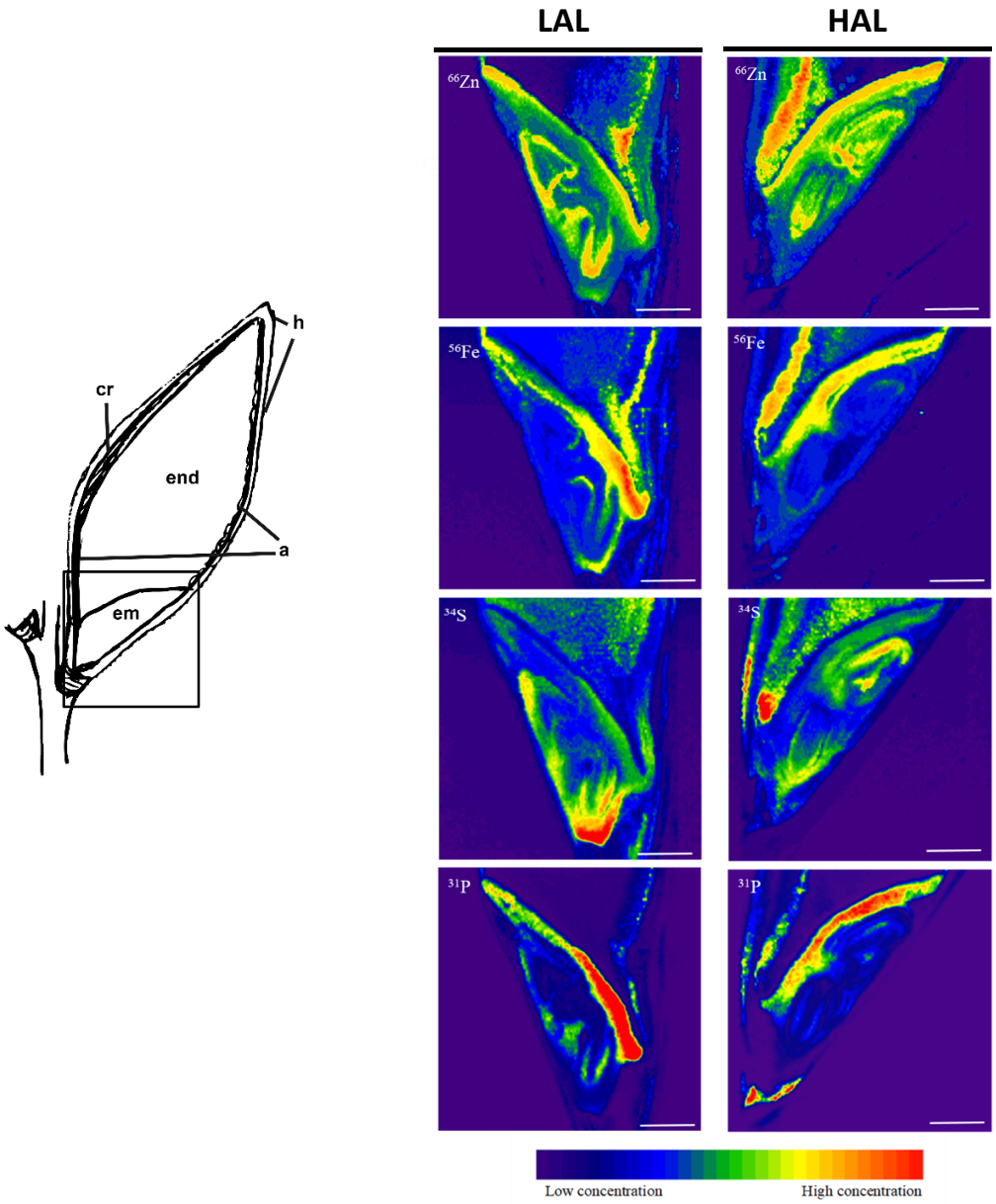

Table S1 Signal intensities of LA-ICP-MS line scans in a low (LAL) and high (HAL) Zinc (Zn) accumulating line. Average phosphours (P), sulphur (S), iron (Fe) and zinc (Zn) signals from LA-ICP-MS line-scans in aleurone (measured from crease and ventral side of the grain) and endosperm tissues (*n*=3). Significant differences are indicated with Y ($P \leq 0.05$).

	P signal			S signal			Fe signal			Zn signal		
	LAL	HAL	*P* value	LAL	HAL	*P* value	LAL	HAL	*P* value	LAL	HAL	*P* value
Aleurone crease side	**14192**	10354	N	**4184**	3507	N	**8812**	4829	Y	**2385**	1459	Y
Endosperm	1774	**2314**	Y	**4404**	4014	Y	747	925	N	381	**868**	Y
Aleurone ventral side	**12184**	9849	Y	**4057**	3416	N	**6597**	5205	N	1666	1440	N