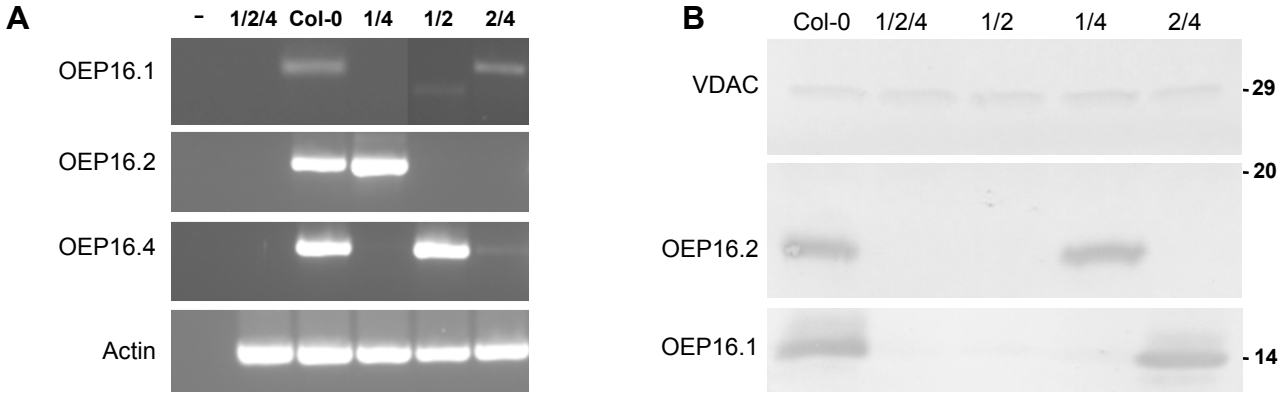
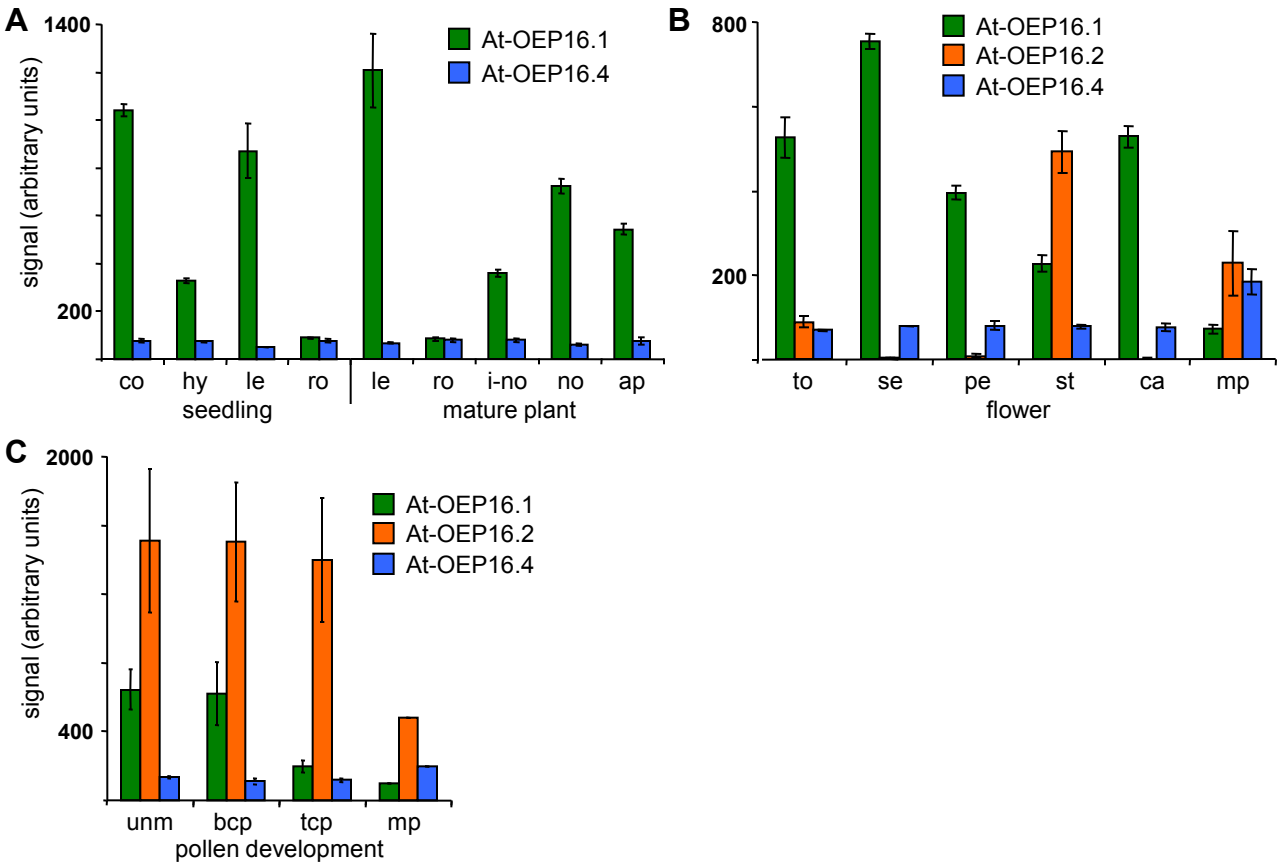
The plastid outer envelope protein OEP16 affects metabolic fluxes during ABA-controlled seed development and germination

Birgit Pudelski, Annette Schock, Stefan Hoth, Ruslana Radchuk, Hans Weber, Jörg Hofmann, Uwe Sonnewald, Jürgen Soll, Katrin Philippar

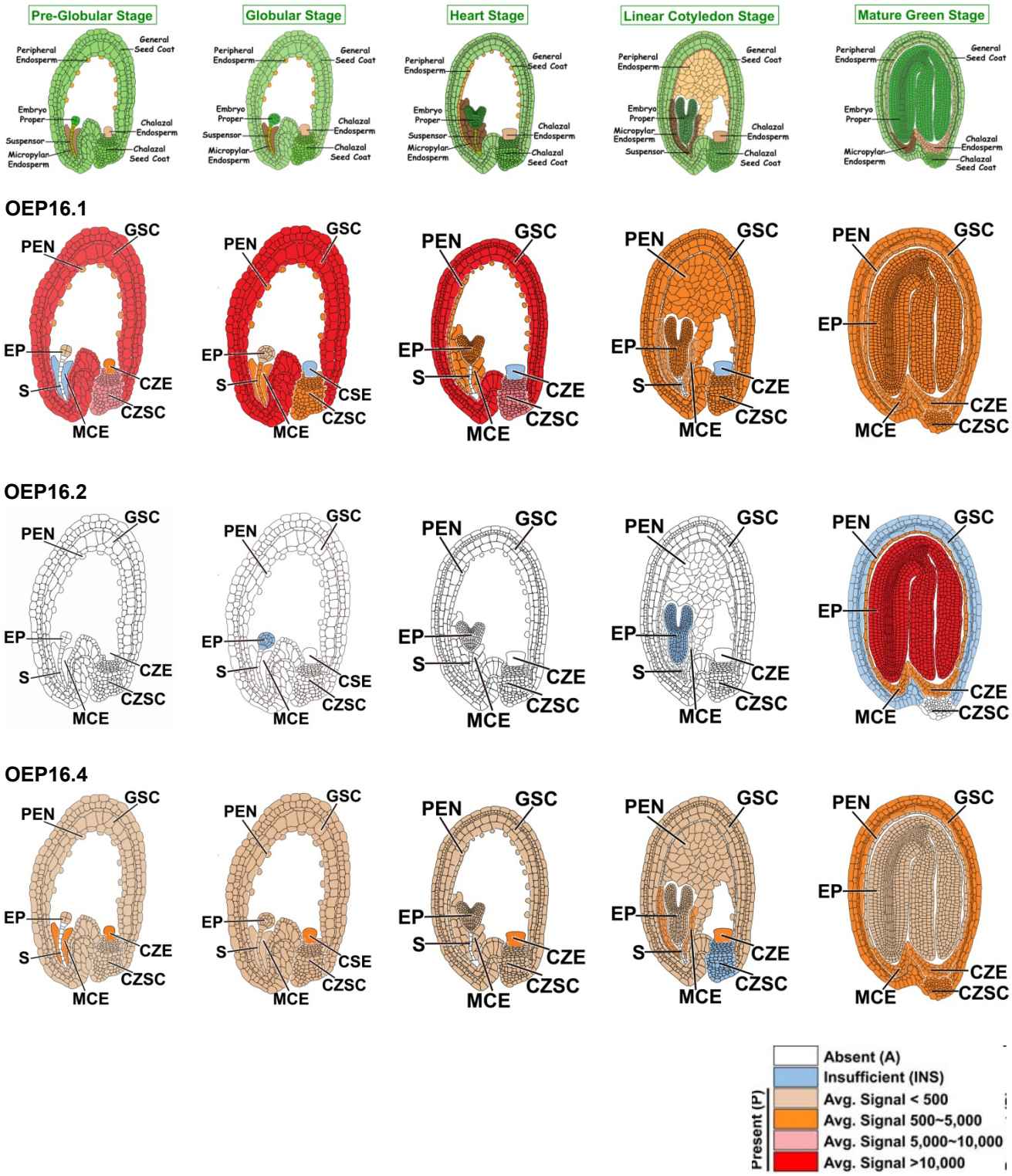
Supplementary material



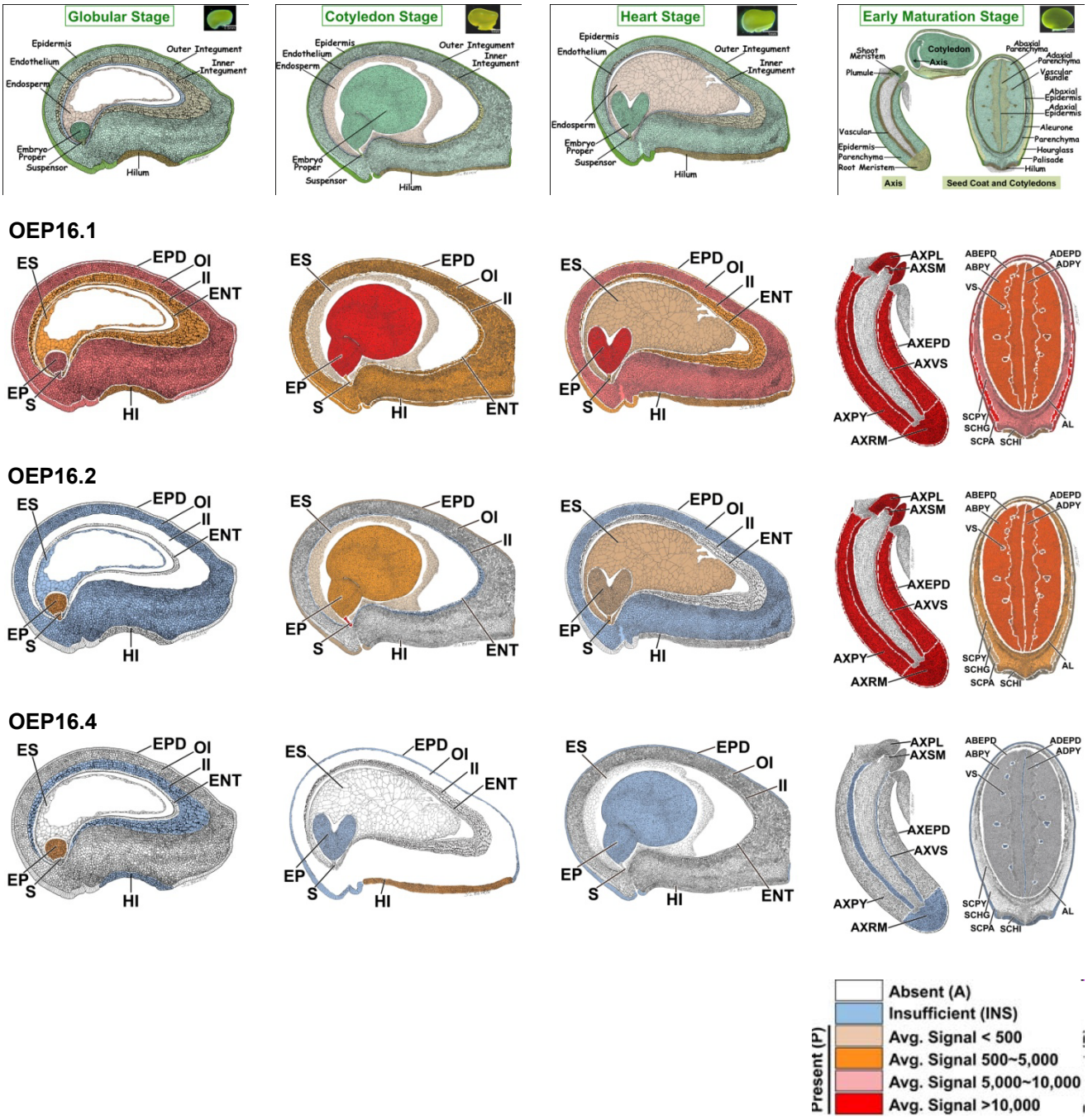
Supplementary Fig. S1. Characterisation of OEP16 mutant lines.
(A) RT-PCR on green silique/seed tissue of the homozygous *Arabidopsis* mutant lines *oep16.1/2/4*, Col-0 wild-type, *oep16.1/4*, *oep16.1/2*, and *oep16.2/4*. Fragment sizes of the specific PCR products are 331bp (OEP16.1), 401bp (OEP16.2), 339bp (OEP16.4), and 435bp (actin control). -: Negative control without cDNA. RT-PCR was performed as described in Philippar et al. (2007). For primer sequences see Supplementary Table S1.
(B) Immunoblot analysis of At-OEP16.2 and At-OEP16.2 on Col-0 wild-type and the mutant lines described in (A). 25µg of protein isolated from seeds germinated for 48h (OEP16.2, VDAC) and of 7-day-old seedlings (OEP16.1) were loaded in each lane. Antiserum against the marker protein VDAC (outer membrane of mitochondria) was used as loading control. Numbers indicate the molecular mass of proteins in kDa.



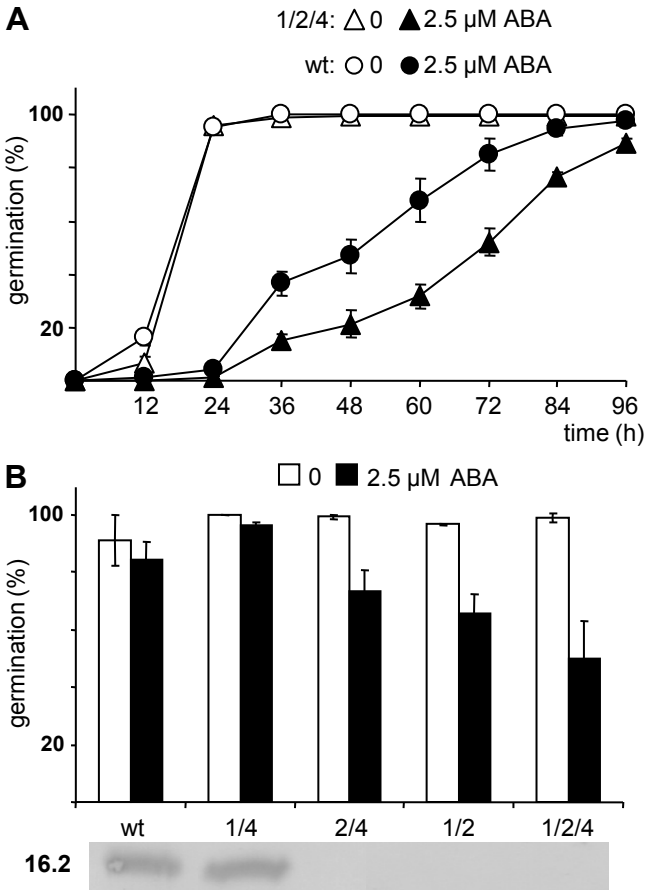
Supplementary Fig. S2. OEP16 expression profiles during *Arabidopsis* development.
Data used to create the digital Northern blots of *OEP16.1* (green), *OEP16.2* (orange) and *OEP16.4* (blue) expression were obtained from the respective experiments deposited at the NASCArrays website (http://affy.arabidopsis.info/narrays/experimentbrowse.pl). Mean signal intensities (arbitrary units ± SD) were averaged from 2 to 3 replicates.
(A) Data from several AtGenExpress developmental series (NASCARRAYS-149, 150, 151, 153). 7-day-old seedlings are separated into cotyledon (co), hypocotyl (ho), green shoots (le), and roots (ro). Mature plant tissue is dissected into 21-day-old rosettes (le), 17-day-old roots (ro) as well as stem tissue of the 2nd internode (i-no), 2nd node (no) and shoot apex/inflorescence (ap).
(B) AtGenExpress developmental series of flowers, stage 12 (NASCARRAYS-152). to: total flower tissue; se: sepals; pe: petals; st:stamen; ca: carpel; mp: mature pollen.
(C) Transcriptome analysis of pollen development (NASCARRAYS-48) as described by Honys and Twell (2003). unm: uninucleate microspores; bcp: bicellular pollen; tcp: tricellular pollen; mp: mature pollen.



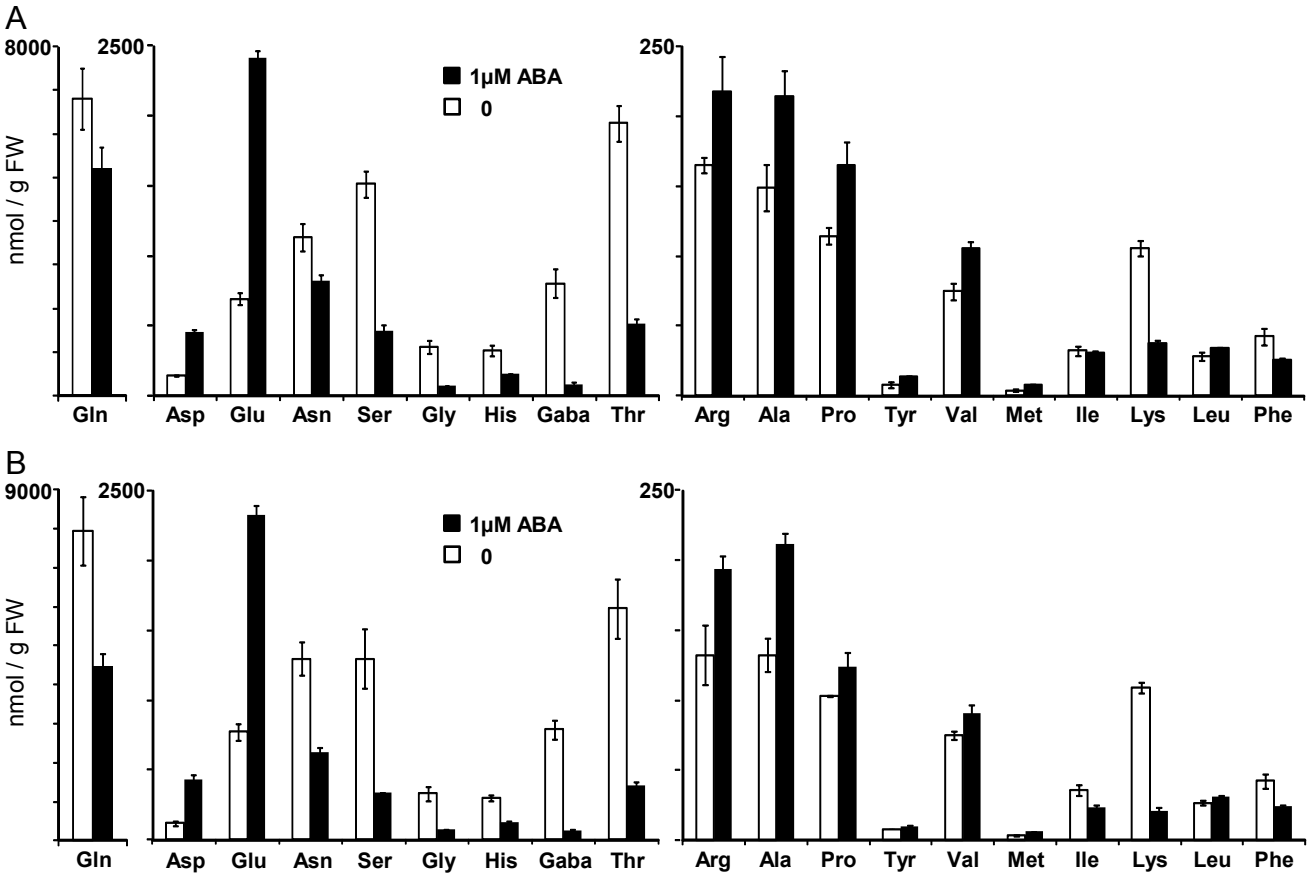
Supplementary Fig. S3. Tissue-specific expression of OEP16 genes in *Arabidopsis* seed development. Expression pattern of OEP16.1, OEP16.2 and OEP16.4 are obtained from the Harada-Goldberg Arabidopsis miccroarray data set of laser capture microdissected seeds ("Gene Networks in Seed Development" at http://estdb.biology.ucla.edu/seed/). Stages of seed development (upper panel) are: Pre-Globular Stage, Globular Stage, Heart Stage, Linear Cotyledon Stage and Maturation Green Stage. Tissue are specified as follows: CZE - Chalazal Endosperm; CZSC - Chalazal Seed Coat; EP - Embryo Proper; GSC - General Seed Coat; MCE - Micropylar Endosperm; PEN - Peripheral Endosperm; S - Suspensor; WS - Whole Seed. Colour codes for transcript density are depicted, for signal values see Supplementary Table S2.



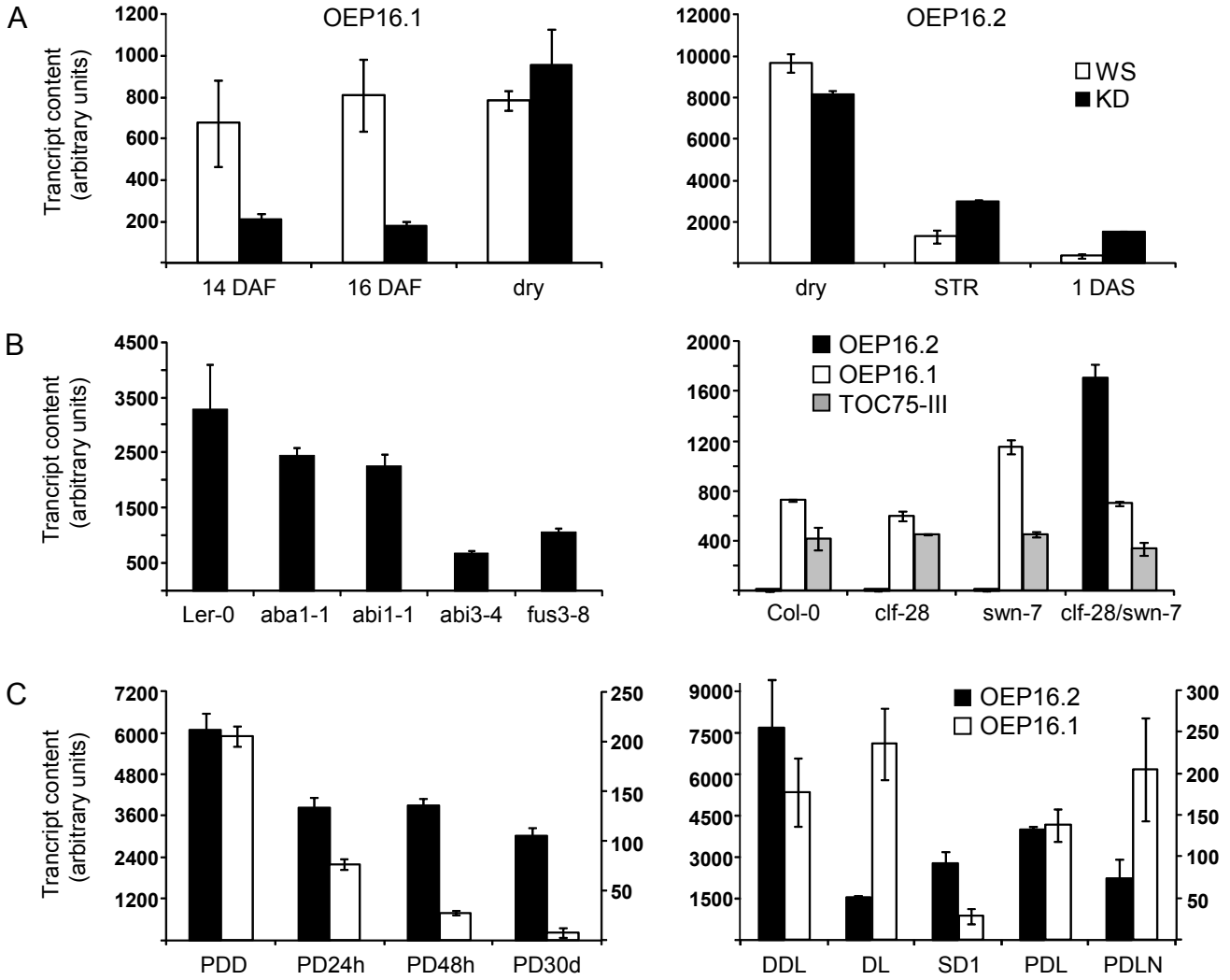
Supplementary Fig. S4. Tissue-specific expression of OEP16 isoforms in soybean seed development. Expression pattern of OEP16.1, OEP16.2 and OEP16.4 orthologs are obtained from the Harada-Goldberg soybean miccroarray data set of laser capture microdissected seeds ("Gene Networks in Seed Development" at http://estdb.biology.ucla.edu/seed/). Stages of seed development (upper panel) are: Globular Stage, Heart Stage, Cotyledon Stage, and Early Maturation Stage. Tissue are specified as follows: AB - Abaxial; AD - Adaxial; AL - Aleurone; AX - Axis; Cot - Cotyledon; EP - Embryo Proper; EPD - Epidermis; ENT - Endothelium; ES - Endosperm; HG - Hourglass; HI - Hilum; II - Inner Integument; OI - Outer Integument; PA - Palisade; PL - Plumule; PY - Parenchyma; RM - Root Meristem; S - Suspensor; SC - Seed Coat; SM - Shoot Meristem; VS - Vascular Bundle; WS - Whole Seed. Colour codes for transcript density are depicted, for signal values see Supplementary Table S3.



Supplementary Fig. S5. Hypersensitivity during germination correlates with the loss of OEP16.2.
(A) Germination rates in % of *oep16* triple mutant (*1/2/4*, triangles) and corresponding wild-type seeds (circles). Seed germination was followed for 96h in the absence and presence of 2.5 µM ABA (open and filled symbols, respectively). Data represent mean values ± SE of n=3 independent experiments.
(B) Germination of *oep16* double mutant (*1/4*, *1/2*, *2/4*), *oep16* triple mutant (*1/2/4*) and corresponding wild-type seeds after 72h incubation without (white) and with 2.5µM ABA (black). Germination rates are given in % (n=3 ± SE). Lower panel: Signals of At-OEP16.2 antiserum in 25µg protein from seeds of the respective mutant lines, germinated for 48h (compare Supplementary Fig. S1).



Supplementary Fig. S6. ABA-induced amino acid changes in *Arabidopsis* seedlings.
Amino acid concentrations (nmol/g FW, n=3-4 ± SD) in 6-day-old *Arabidopsis* Col-0 wild-type (A) and *oep16* triple mutant (B) seedlings. Plantlets were grown in the absence (white bars) and presence (black bars) of 1µM ABA. Please note that 3 different scalings were used for y-achses.



Supplementary Fig. S7. Regulation of *OEP16* expression in a seed high-lysine mutant, within the ABA-signalling chain, and during dormancy and germination.

Data used to create the digital Northern blots of *OEP16.1* and *OEP16.2* expression were obtained from the respective publications or from microarray data sets deposited at the NASCArrays website (http://affy.arabidopsis.info/narrays/experimentbrowse.pl). Mean signal intensities (arbitrary units ± SD) were averaged from 2 to 3 replicates. For detailed conditions and complete data sets compare original references.

(A) OEP16 isoforms are differentially expressed in the high-lysine mutant KD (black bars) when compared to the corresponding wild type Wassilewskija (WS, white bars). Whereas during seed development (14 and 16 days after flowering, DAF) *OEP16.1* is down-regulated in the KD mutant (Angelovici et al., 2009), *OEP16.2* transcripts in stratified (STR) and germinated seeds (1 day after stratification, DAS) are increased in KD and thus show a delay in down-regulation upon early germination (Angelovici et al., 2011).

(B) *OEP16.2* expression is under control of the ABA-signalling transcription factors ABI3 and FUS3. (Left) In *abi3* and *fus3* mutant seeds *OEP16.2* transcripts (black bars) are decreased about 3-4 fold when compared to Col-0 wild type (NASCARRAYS-61, Carrera Bergua: "Functional genomics of shoot meristem dormancy"; tissue: freshly harvested, non-dormant seeds, imbibed for 24h). (Right) Whereas RNA-levels for *OEP16.1* (white bars) and for *TOC75-III* (protein translocon at the outer plastid envelope membrane, grey bars) do not change significantly in polycomb mutant seedlings, *OEP16.2* expression is drastically induced in CURLY LEAF (clf) and SWINGER (swn) double mutants (NASCARRAYS-425, Goodrich: "Polycomb mutant seedlings"; tissue: 10-12-day-old seedlings).

(C) *OEP16.1* and *OEP16.2* RNA (white and black bars, respectively) in seeds of the deep dormant ecotype Cvi ("Cape Verde Islands", NASCARRAYS-69, Finch-Savage: "A genomic approach to understanding seed dromancy"). The left y-axis is scaled for *OEP16.2*, the right y-axis for *OEP16.1*. Treatment and dormancy stages of seeds as described in Cadman et al. (2006), Finch-Savage et al. (2007) are as follows: PDD – primary dormant, dry; PD24h, 48h, 30d – primary dormant, imbibed for 24h, 48h, and 30 days in dark; DDL – non-dormant, dry; DL – non-dormant, imbibed for 24h in dark, SD1 – secondary dormant, DL seeds imbibed for 24 days in dark; PDL – primary dormant, after-ripened for 91 days, imbibed for 24h in light; PDLN – PDL seeds imbibed in presence of 10mM KNO_3. Please note that of all dormant seeds only the PDLN seeds are able to complete germination without further treatment (light and nitrate).

Supplementary Table S1. Oligonucleotide primers used in this study

I = Inosin, M = A/C, R = A/G, Y = C/T.

Isolation of Ps-OEP16.2 cDNA:	
Ps-OEP16.2 fw_a	TGATCT[I]GG[I]CA[Y]CCTCT[I]CT[I]AA[Y][M]G
Ps-OEP16.2 fw_b	AAGCTGT[I]TCT[M]G[I]GA[R]GCTTATTT[Y]AC
Ps-OEP16.2 rev_a	TAAAT[I]CCTGCAGC[I]AC[I]CCCCATTG
Ps-OEP16.2 rev_b	AGC[I]ACTGCAGA[R]TT[Y]TTCCAATCATG
Ps-OEP16.2(RACE)fw	AGCCGTTTCTCGTGATGCTTATTTCTCAG
Ps-OEP16.2(RACE)rev	ATATGGGCTGAAGGAAGCTCGTGGA
Ps-OEP16.2(NcoI)fl_fw	ATACCATGGATGAACTTGAACACGAGC
Ps-OEP16.2(XhoI)fl_rev	GTGCTCGAGGAAAATCCCAGTGAG
RT-PCR on oep16 mutants:	
OEP16.1_fw	TGTTAGCACGCCGAAG
OEP16.1_rev	CTTACCAACCGCTGAG
OEP16.2_fw	CTTAACCGTATCGCAGA
OEP16.2_rev	AGAGCAGACTGAACCAC
OEP16.4_fw	GTCGAGTCAGTTCTCCG
OEP16.4_rev	CCTGGTGCAATTAGCC
Actin_fw	GGTGATGGTGTGTCT
Actin_rev	ACTGAGCACAATGTTAC
Promoter-GUS constructs:	
OEP16.1-GUS_fw	CCCGCAAACAATCGGGTGC
OEP16.1-GUS_rev	CTTTTCTTCTTCTTTCTTCC
OEP16.2-GUS_fw	CCTTCTCGACGGCGTGCAATG
OEP16.2-GUS_rev	TTTTTTCTTTCTTTCTTCACTTGTTGC