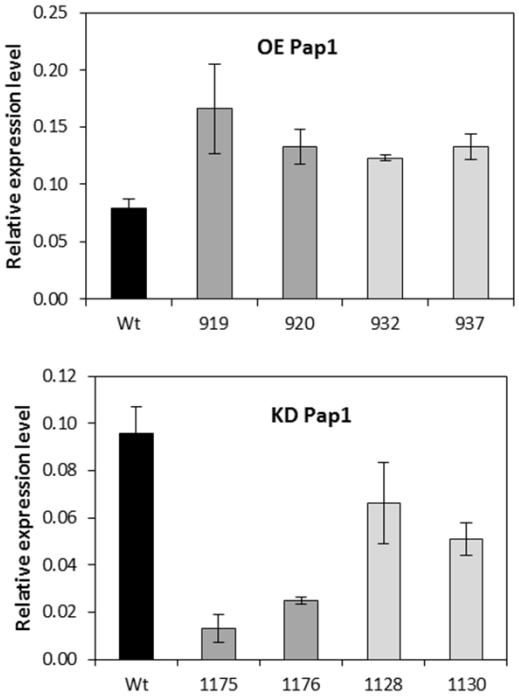
A

	Construct	Event	Number of lines	Selected lines
Over-Expressing lines (OE)	OE Pap1	E04	12	-
		E06	15	-
		E07	13	-
		E13	6	**919, 920**
		E15	20	**932, 937**
		E27	20	-
amiRNA silencing lines (KD)	KD Pap1	E22	14	-
		E23	4	**1175, 1176**
		E24	4	-
		E25	14	**1128, 1130**

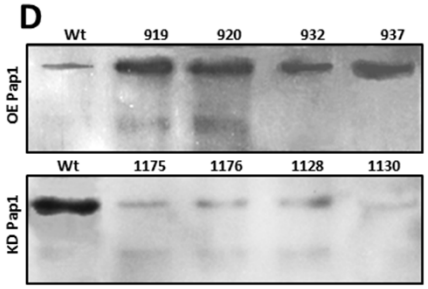
B

Lines	$2^{-\Delta\Delta Ct} \pm SE$	CN
Wt	0.93 ± 0.09	**1**
919	2.45 ± 0.25	**2**
920	1.81 ± 0.38	**2**
932	1.56 ± 0.18	**2**
937	2.06 ± 0.23	**2**
Wt	0.00 ± 0.00	**0**
1128	0.81 ± 0.15	**1**
1130	1.07 ± 0.06	**1**
1175	1.00 ± 0.08	**1**
1176	0.86 ± 0.16	**1**

C



D



Supplemental Figure S1. Selection of *HvPap-1* transgenic homozygous lines of barley generated by double haploid technology. Over-expressing (OE Pap1) and knock-down (KD Pap1) plants were selected following a double criteria, single transgene integration and high mRNA and protein content. **A**, Number of independent homozygous lines per transformation event, number of events per construct and final selected lines used for molecular characterization. **B**, Estimation of transgene copy number by RT-qPCR assays coupled to the $2^{-\Delta\Delta Ct}$ method. Values are expressed as the average ± standard error of triplicate measurements. *Hv4hppd* and *HvCycl* genes were used as references for single copy and endogenous calibrators, respectively. CN: copy number for each group. **C**, Expression levels of the *HvPap-1* gene in transgenic barley lines by RT-qPCR technology, referred as relative mRNA levels of C1A CysProt genes normalized to barley *cyclophilin* mRNA content. **D**, Expression of HvPap-1 proteins in transgenic barley lines by western-blot assays.

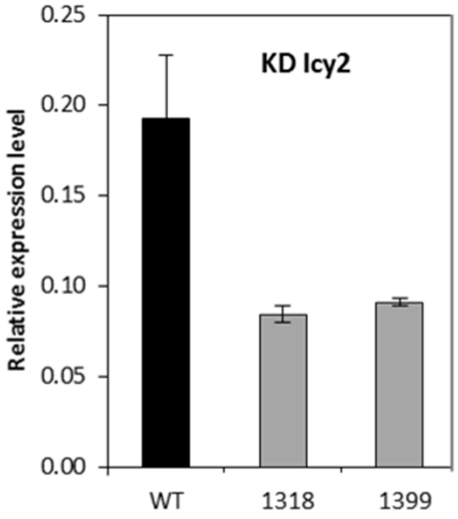
A

	Construct	Event	Number of lines	Selected lines
amiRNA silencing lines (KD)	KD Icy2	E02	11	**1318**
		E03	13	-
		E04	17	**1399**
		E05	21	-

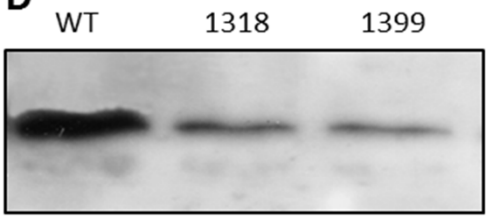
B

Lines	$2^{-\Delta\Delta Ct}$ ± SE	CN
WT	0.00 ± 0.00	**0**
1318	0.74 ± 0.08	**1**
1399	1.03 ± 0.08	**1**

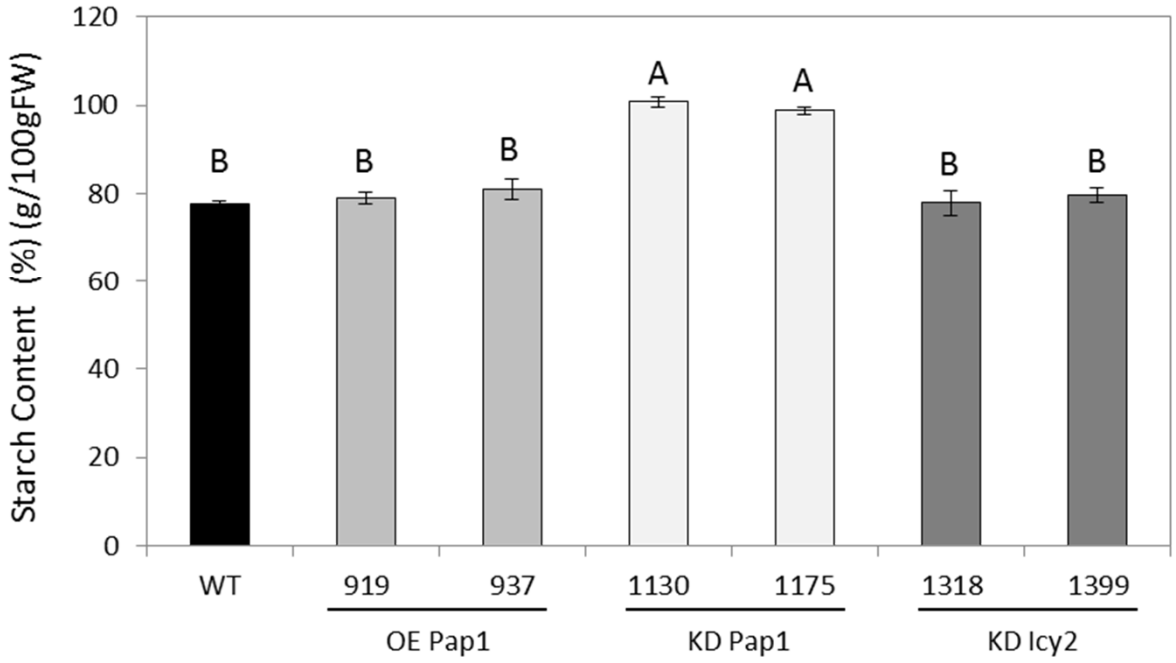
C



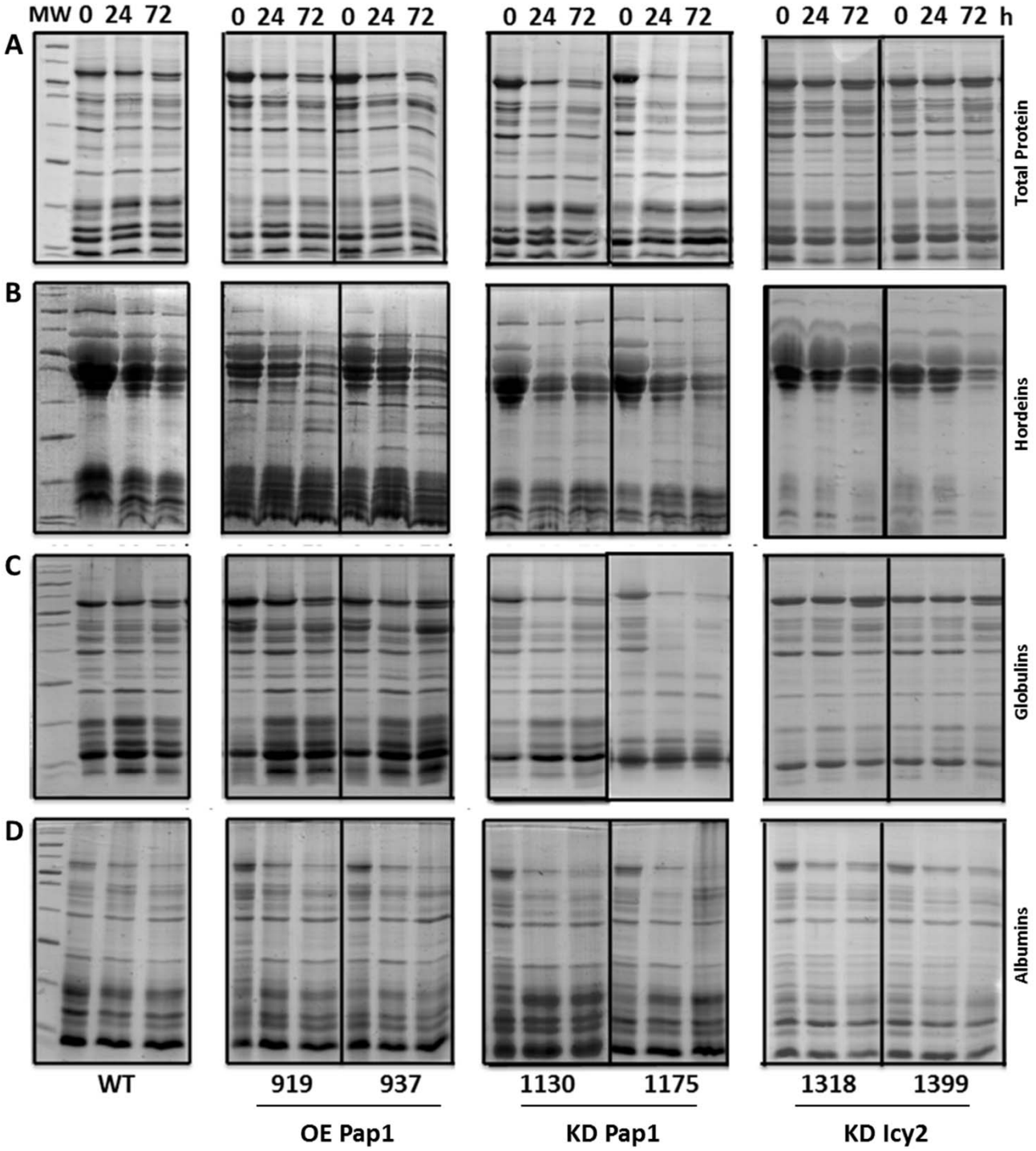
D



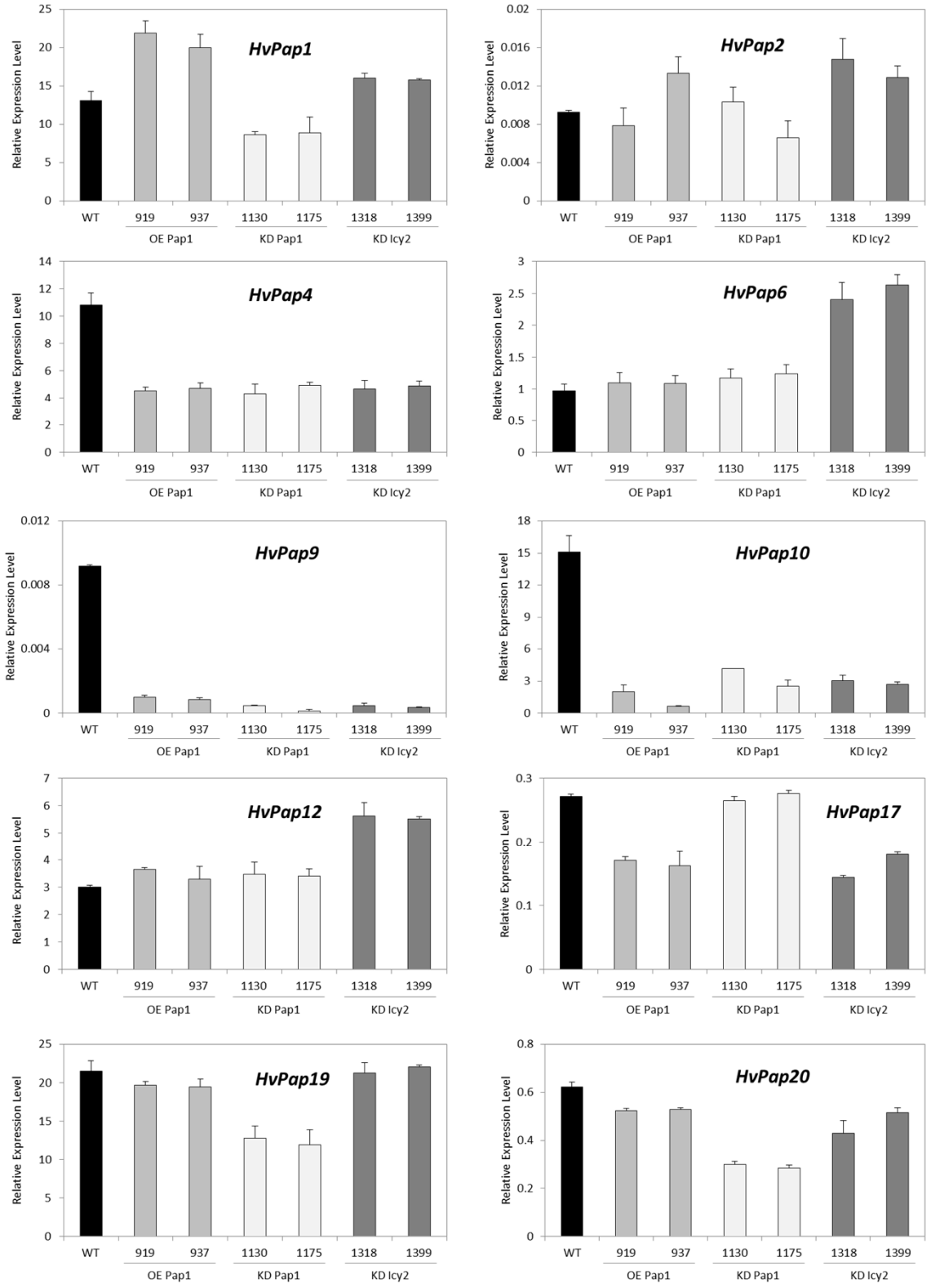
Supplemental Figure S2. Selection of *Icy-2* transgenic homozygous lines of barley generated by double haploid technology. Knock-down (KD Icy2) plants were selected following a double criteria, single transgene integration and low mRNA and protein content. A, Number of independent homozygous lines per transformation event, number of events per construct and final selected lines used for molecular characterization. B, Estimation of transgene copy number by RT-qPCR assays coupled to the $2^{-\Delta\Delta}$Ct method. Values are expressed as the average ± standard error of triplicate measurements. *Hv4hppd* and *HvCycI* genes were used as references for single copy and endogenous calibrators, respectively. CN: copy number for each group. C, Expression levels of the *HvIcy-2* gene in transgenic barley lines by RT-qPCR technology, referred as relative mRNA levels normalized to barley *cyclophilin* mRNA content. D, Expression of HvCPI-2 protein in transgenic barley lines by western-blot assays.



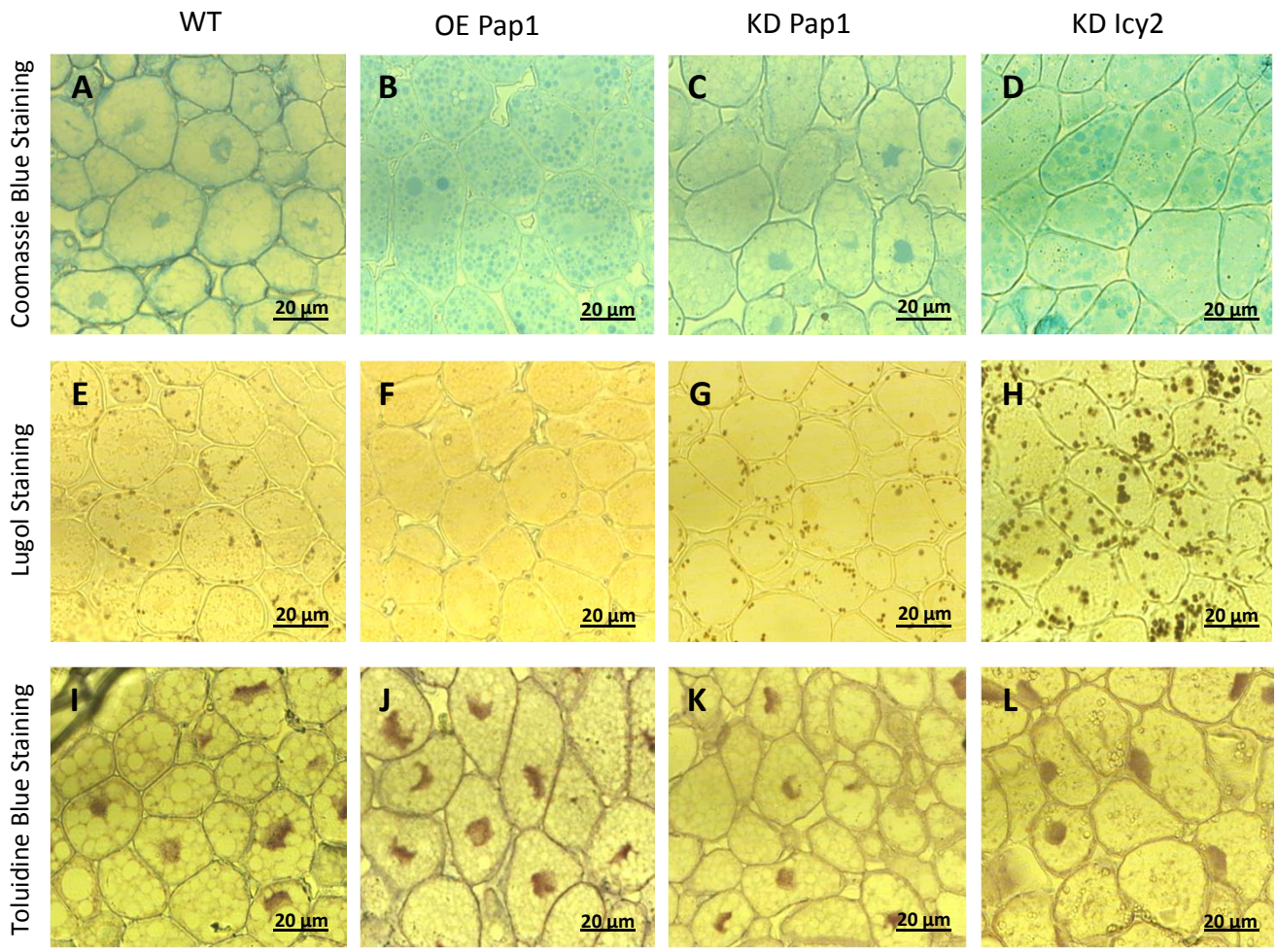
Supplemental Figure S3. Starch content of de-embryonated dry grain of OE Pap1: 919, 937 lines, KD Pap1: 1130 and 1175 lines, KD Icy2: 1318 and 1399 lines, and control (WT) plants. Data, referred as grams of transformed starch per 100 grams of fresh weight, are means ± standard error of three independent replicates. Significant differences between control wild-type and transgenic lines are indicated with capital letters ($P < 0.05$, HSD).



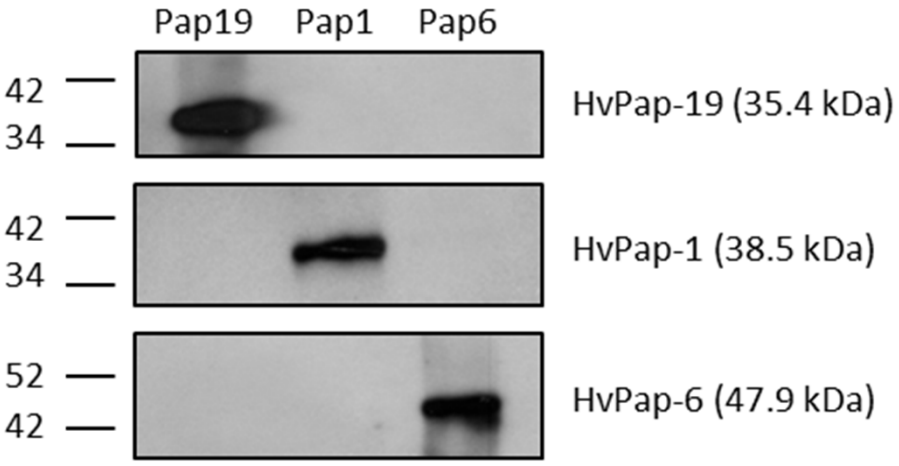
Supplemental Figure S4. Protein patterns of de-embryonated grains at different germination times (0, 24 and 72 hours after imbibition) of control wild-type and transgenic OE Pap1, KD Pap1 and KD Icy2 lines carried out by SDS-PAGE and stained with Coomassie Brilliant Blue G-250. 20 µg of each grain protein extract and of each hordein, globulin and albumin enriched fraction were used.



Supplemental Figure S5. RT-qPCR analyses of the mRNA accumulation in embryos of wild-type and transgenic lines at 24 hours after imbibition. Ten different C1A CysProt were analyzed (HvPap-1, -2, -4, -6, -9, -10, -12, -17, -19, -20). Relative expression was normalized to barley *cyclophilin* mRNA content.



Supplemental Figure S6. Structural characterization of wild-type, OE Pap1, KD Pap1 and KD Icy2 embryos at 24 hours after imbibition. A-D, Protein structures stained with Coomassie Brilliant Blue G-250. E-H, Starch accumulation stained with Lugol. I-L, Structural changes observed in cells stained with Toluidine Blue O. Images were observed on a Zeiss Axiophot microscope under bright field.



Supplemental Figure S7. Immuno-blot of recombinant barley CysProt (HvPap-19, 1, and 6) purified from *E. coli* cultures used to check peptide specificity. Only protein bands corresponding to the inactive form of each CysProt were observed. Protein detection was analysed by using specific antibodies.