Supplemental Data. Penfield and Hall (2009). A role for multiple circadian clock genes in the response to signals that break seed dormancy in Arabidopsis.



Supplemental Figure 1. A, B. The germination after 7 days of freshly harvested seeds in response to cold stratification. (A) *lhy* and *cca1* single mutants, compared to the *lhy cca1* double mutant, and (B) *LHY* and *CCA1* overexpressing seeds. (C) The germination of freshly harvested WS, *lhy cca1* and *gi-11* seeds at four constant temperatures. The germination of all genotypes is poor at harvest indicating strong dormancy. Data represents the mean and standard error of 5 independent seed batches. Asterisks indicate significant differences from wild type (P<0.05).



Supplemental Figure 2. The germination frequency of freshly harvested (Fr) and 3 month after-ripened (St) wild type seeds from 12 to 27°C after 7 days. Data represents the mean and standard error of 5 independent seed batches. The genetic basis of these differences is largely unknown.



Supplemental Figure 3. The expression of genes encoding Arabidopsis circadian clock components under diurnal light/ dark cycles in imbibed 3 month after-ripened seeds in WS (blue) *gi-11* (green) and *lhy cca1* (pink) during the first 48 hours of imbibition. Data represents the mean and standard error of two biological replicates.



Supplemental Figure 4. Real time RT-PCR to show the expression of *GA3OX1* after the release of freshly harvested wild type WS and the cold stratification non-responsive *gi-11* mutant seeds into ambient temperatures from 3 days cold stratification at 4°C. Data points represent the mean and standard deviation of two biological replicates.

AGI	GENE	EXPERIMENT								PDD	NDD
NUMBER	NAME	PDD1	PDD2	PDD3	PDD4	NDD1	NDD2	NDD3	NDD4	MEAN	MEAN
At5g61380	TOC1	271.8	237.53	256.73	238.04	194.29	258.81	223.2	126.6	251.03	200.73
At1g01060	LHY	19.38*	13.99*	5.88*	12.79*	2.51*	37.11*	4.52*	8.23*	13.01	13.09
At2g46830	CCA1	2.16*	13.82*	2.93*	8.93*	4.77*	10.2*	1.19*	14.71*	6.96	7.72
At5g02810	PRR7	158.01	182.34	164.75	201.55	103.57	107.41	99.2	66.11	176.66	94.07
At1g22770	GI	235.7	281.96	273.2	211.73	166.59	212.9	156.26	117.6	250.65	163.34
At2g46790	PRR9	124.61	126.02	112.18	141.14	86.68	131.81	80.46	120.35	125.99	104.83
At3g46640	LUX	217.56	235.72	236.56	225.65	337.25	274.71	315.37	507.5	228.87	358.71
AT5G6010	PRR3	283.29	282.88	230.71	267.61	439.60	446.69	302.10	441.19	266.12	407.40
AT5G24470	PRR5	8.99*	17.19*	20.66*	5.18*	1.5*	14.02*	7.14*	2.07*	13.0	6.18
AT2G25930	ELF3	192.51	198.08	182.75	191.15	153.95	239.43	178.58	105.14	267.48	258.40
AT2G40080	ELF4	1397.0	1319.7	1108.5	1404.6	1312.4	697.28	1417.3	1617.4	1307.5	1261.0
AT2G21660	CCR2	179.94	168.17	177.82	167.58	480.17	278.61	525.71	556.82	173.28	460.32
At1g20620	CAT3	2756.7	2510.5	2824.3	1763.4	3932.9	5255.9	4437.4	4056.5	2713.7	4420.6
At1g29920	CAB2	2.18*	1.53*	5.04*	0.48*	10.07*	1.53*	11.54*	2.13*	2.31	6.31

* indicates an absent call by MAS5.

Supplemental Table 1. Microarray data showing expression of Arabidopsis circadian clock genes from freshly harvested dry seeds, and dry after-ripened dry seeds (PDD; primary dormant, fresh from the mother plant growing in the greenhouse in light/dark cycles; or NDD; seeds stored dry in the dark at 20°C for 1 year). Data taken from Finch-savage et al. (2007). Note that the morning-expressed (red) genes are called absent in both treatments.

Supplemental Reference:

Finch-Savage, W.E., Cadman, C.S., Toorop, P.E., Lynn, J.R., Hilhorst, H.W. (2007) Seed dormancy release in Arabidopsis Cvi by dry after-ripening, low temperature, nitrate and light shows common quantitative patterns of gene expression directed by environmentally specific sensing *Plant J.* **51**, 60-78.