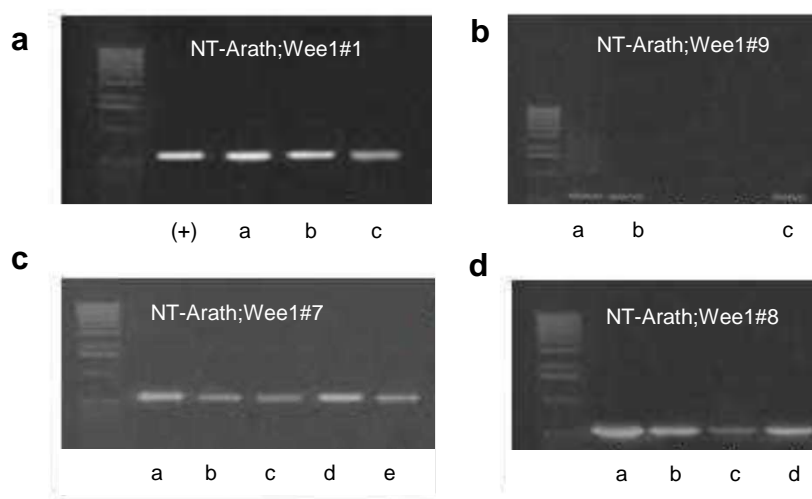


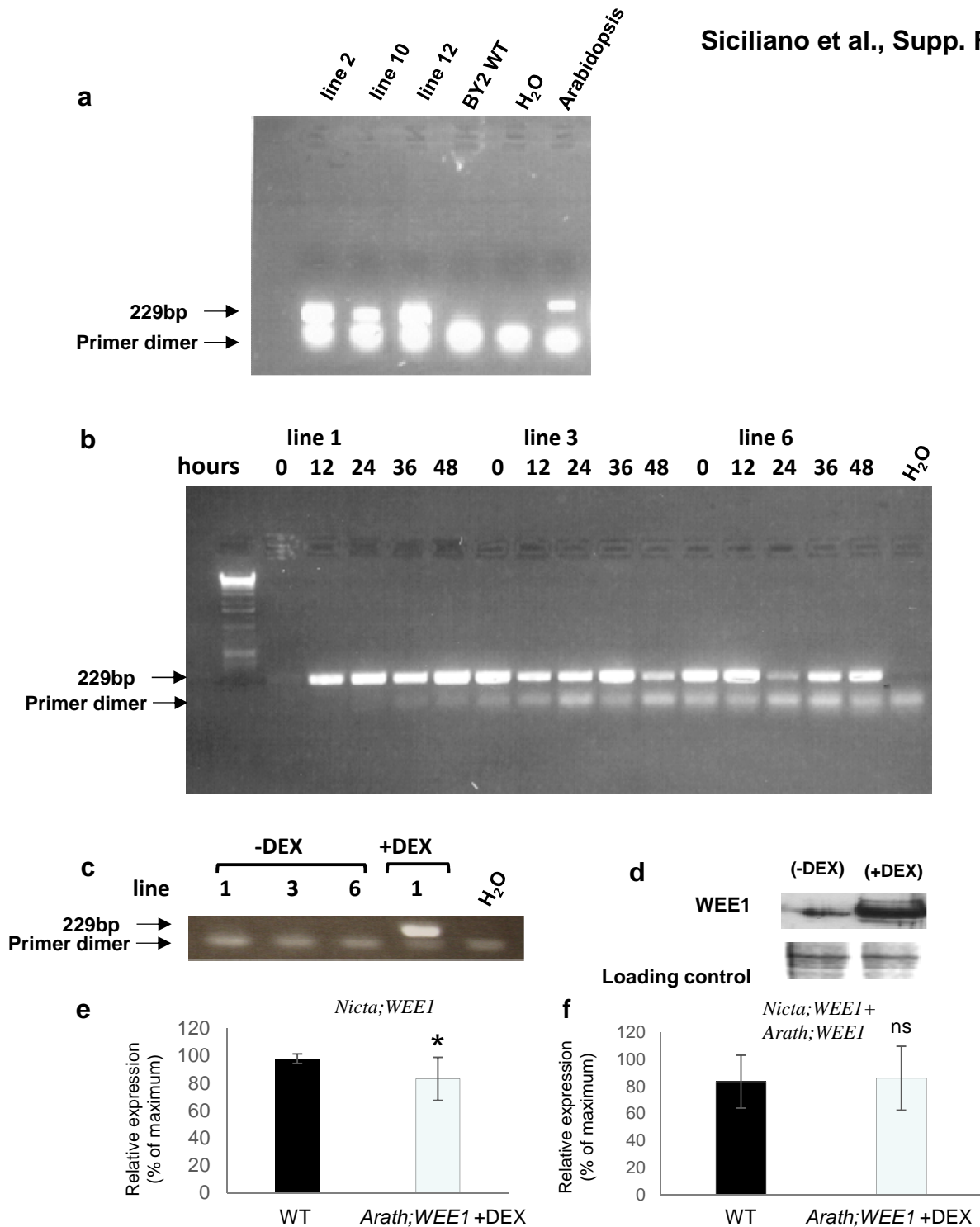
Expression of Arabidopsis *WEE1* in tobacco, induces unexpected morphological and developmental changes.

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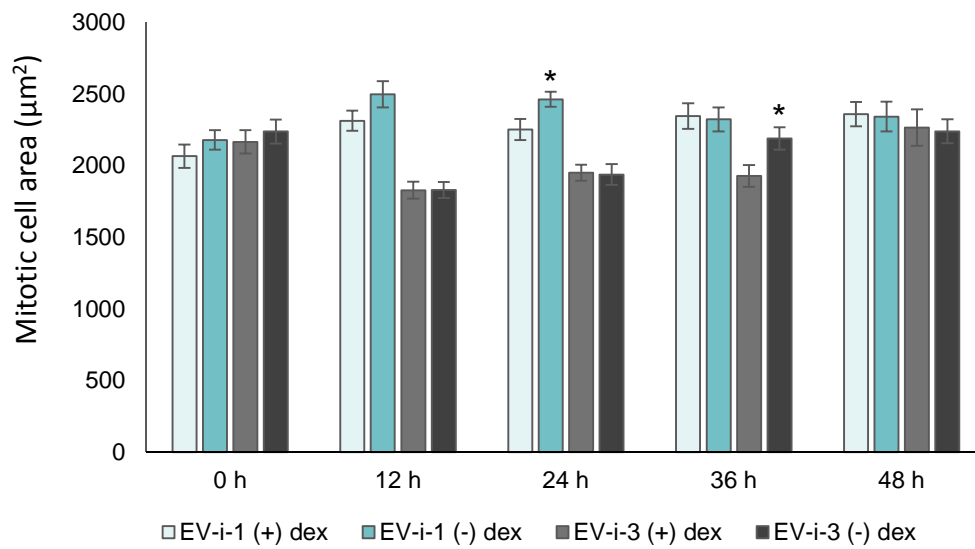
Siciliano et al., Supp. Fig. 1



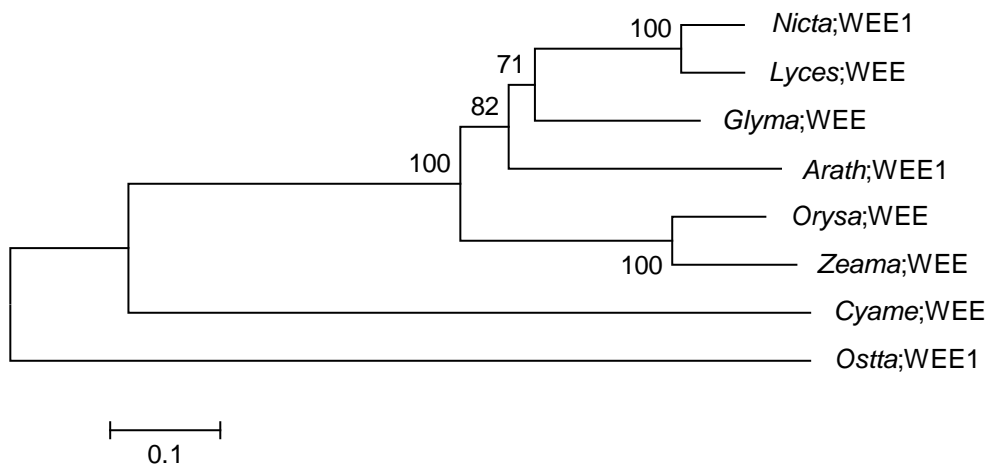
Supplementary Fig. 1: RT-PCR to confirm expression *Arath;Wee1* in transgenic tobacco plants in the T2 generation (a) NT-Arath;Wee1#1 plants (+) = positive control (cDNA from Arabidopsis), a – c are individual plants (b) NT-Arath;Wee1#9 plants: a – c are individual plants (c) NT-Arath;Wee1#7 a – (d) NT-Arath;Wee1#8: a – d are individual plants



Supplementary Figure 2: Expression of WEE1 in unsynchronised tobacco BY2 cells (a) RT-PCR expression of *Arath;WEE1* in three independent BY-2 cell lines constitutively expressing *Arath;WEE1*: 2, 10 and 12 (b) RT-PCR profile of *Arath;WEE1* expression induced by dexamethasone (DEX) at intervals after induction in three independent lines: i-WEE1-1, 3 and 6 (c) comparison of induced (+DEX) and uninduced (-DEX) *Arath;WEE1* expression in *Arath;WEE1* inducible line i-WEE1-1 (d) WEE1 protein in BY2 cells expressing inducible *Arath;WEE1* ± induction with DEX. (e) Expression of *Nicta;WEE1* and (f) Expression of *Nicta;WEE1* + *Arath;WEE1* in BY2 cell line expressing inducible *Arath;WEE1* induced with DEX, and WT BY2 cells; star indicates significant difference. (P < 0.05) based on a Student's t test, ns= non-significant.



Supplementary Fig. 3: Effect of DEX on empty vector lines Mitotic cell area (μm^2) of two independent lines carrying the pTA7002 vector (empty vector, EV-i-1 and EV-i-3) +/- addition of dexamethasone ($1 \mu\text{M}$) in the first 48 hours following induction.



Supplementary Figure 4: phylogenetic tree showing relationship between five plant WEE1 protein sequences using MEGA3.0 (Kumar et al., 2004). The tree was constructed using the Neighbour-joining algorithm, bootstrap values are indicated at branches. *Zeama*;WEE1, *Zea Mays*, (AAD52983); *Arath*;WEE1, *Arabidopsis thaliana* (CAD28679) *Glyma*;WEE1, *Glycine max* (AAS13373), *Orysa*;WEE1, *Oryza sativa* (XP_464040); *Lyces*;WEE1, *Lycopersicon esculentum* (AM180939); *Cyame*;WEE1, *Cyanidioschyzon merolae* (CMT590C); *Ostta*;WEE1, *Ostreococcys tauri* (AY675101); *Nicta*;WEE1, *Nicotiana tabacum* (accession nos: AJ866274, AJ866275, AJ866276, and AJ866277)

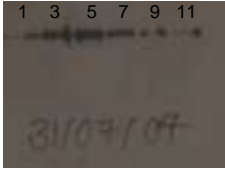
<i>Nicta</i> ;WEE1	M KRRTLN R ST P RR R NNK S NT K RM N K G SL F T 30
<i>Arath</i> ;WEE1	M FEK N G- R TL L AK R KT Q GT I K T R A SK K IR K 29
<i>Nicta</i> ;WEE1	V GF S K V SL P PL P NO Q LO S S F ST L PL S N-- 58
<i>Arath</i> ;WEE1	M E G T L ER H SL L Q F GL S K I S F EN R PS S N V A 59
<i>Nicta</i> ;WEE1	P SR F OK L LD S EV L PP A Q S N F PS I LE S NT D A 88
<i>Arath</i> ;WEE1	S SA F Q G LL D SD----- S SEL R N Q LG S - A D S 83
<i>Nicta</i> ;WEE1	D A A D G DN K D F IL S Q D FF C TP D YL T PD A PA I 118
<i>Arath</i> ;WEE1	D AN C GE- K D F IL S Q D FF C TP D YL T PD N Q N L 112
<i>Nicta</i> ;WEE1	C N G LD G N K DD C MP C PK S PE K LO T V A R K R O R 148
<i>Arath</i> ;WEE1	M SG L DI S K D - H SP C PR S P V KL N T V K S K R CR 141
<i>Nicta</i> ;WEE1	L AV K S A T S LS S DF P G Q Q L AD I PE D AF G SD 178
<i>Arath</i> ;WEE1	Q ES F T G N H S N ST W SS K HR V DE Q EN D DI D TD 171
<i>Nicta</i> ;WEE1	E TK S E K IT E SE K GH S - Y VS Q S A T A L R Y R VM 207
<i>Arath</i> ;WEE1	E VM G DK L Q A N Q T E RT G V S Q A A V AL R CR A M 201
<i>Nicta</i> ;WEE1	P PP C IR N PY L RD A SE I D V DP F GN R RS K CA G 237
<i>Arath</i> ;WEE1	P PP C L K NP Y V L N Q SE T AT D PF G H O RS K CA S 231
<i>Nicta</i> ;WEE1	F NP V IF G ND G LS R Y R S D F H E I E Q IC T GN F S 267
<i>Arath</i> ;WEE1	F LP V ST S GD G LS R Y L T D F H E I R Q IG A GH F S 261
	II
<i>Nicta</i> ;WEE1	R V F K V L K R I D G C M Y A V K H S T K Q L H Q D T DR R 297
<i>Arath</i> ;WEE1	R V F K V L K R M D G CL Y AV K H S TR K L Y LD S ER R 291
	III IV
<i>Nicta</i> ;WEE1	K AL M EV Q AL A AL G PH E N V V G Y S SW F ENE H 327
<i>Arath</i> ;WEE1	K AM M EV Q AL A AL G PH E N I V G Y S SW F ENE Q 321
	V
<i>Nicta</i> ;WEE1	L Y I Q E ML C D H SL S N-- K K Y S K LS S EV A V L E 355
<i>Arath</i> ;WEE1	L Y I Q E EL C D H SL S AL P K K S L K V S E RE I LV 351
	Via Vib
<i>Nicta</i> ;WEE1	A MY Q V A K A L Q Y T H Q R G V A HL D V K PD N I Y V K 385
<i>Arath</i> ;WEE1	I M H Q I A K A L H F V H E K G I A H LD V K P D N I Y I K 381
	VII VIII
<i>Nicta</i> ;WEE1	S GV Y KL G DF G CA T L L DK S Q P TE E GD A RY M P 415
<i>Arath</i> ;WEE1	N GV C KL G DF G CA T RL D K S LP V E E GD A RY M P 411
	IX
<i>Nicta</i> ;WEE1	Q E I LN E Y D H L DK V D F SL G AA I Y E L I R G S 445
<i>Arath</i> ;WEE1	Q E I LN E D Y E H L D K V D I F S LG V T V Y E L I K G S 441
	X
<i>Nicta</i> ;WEE1	P LP E SG P H F LN L REG L PL L PG H SL Q F Q N L 475
<i>Arath</i> ;WEE1	P L T ES R N Q SL N I K E G KL PL LP G H S L Q L Q OL 471
	XI
<i>Nicta</i> ;WEE1	L K V MM D EP T RR P SA K DL V DN P IF E R C ORN 505
<i>Arath</i> ;WEE1	L K T MM D RD P K R RP S ARE L L D HP M FD R IR G - 500
<i>Nicta</i> ;WEE1	ANK 508
<i>Arath</i> ;WEE1	--- 500

Supplementary Figure 5:

Alignment of *Nicta*;WEE1 to other plant WEE1 genes showing conserved (black) and similar (grey) residues. Roman numerals indicate kinase subdomains in the catalytic domain. Closed circles indicate residues conserved in all the ORFs, inverted closed triangles indicate the conserved 'EGD' motif. Asterisks indicate two serine residues in *Arath*;WEE1 which conform to the RXXS consensus for phosphorylation sites and which are in similar positions to those in animal and yeast WEE1 proteins known to be involved in 14-3-3 regulation.

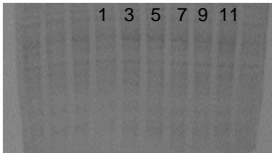
Full blot image for Figure 6 (b)

-DEX

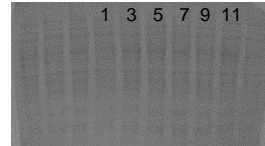


Full gel images for Figure 6 (b)

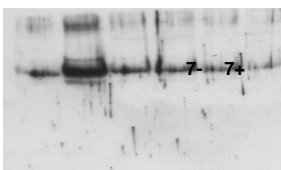
-DEX



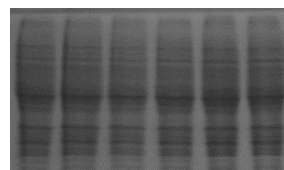
+DEX



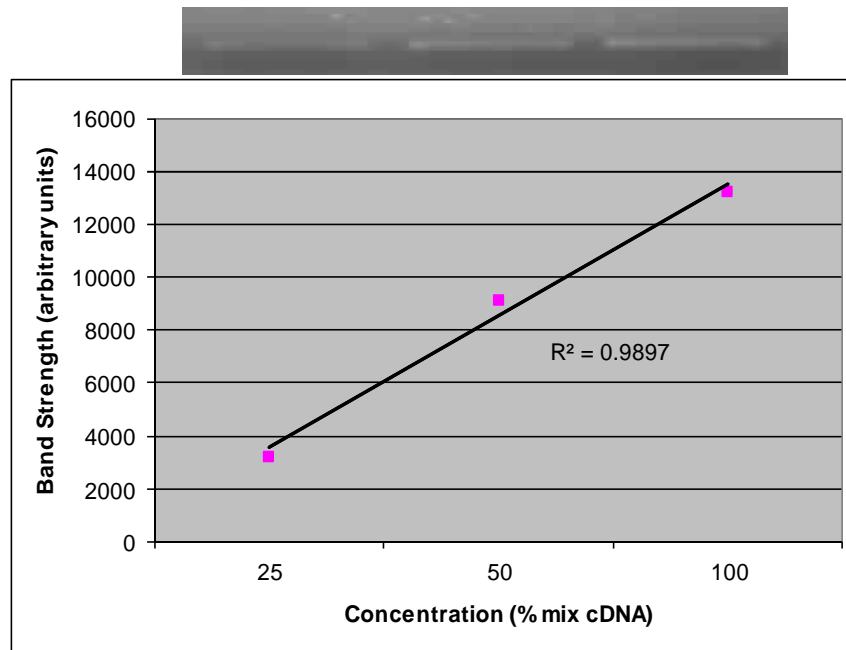
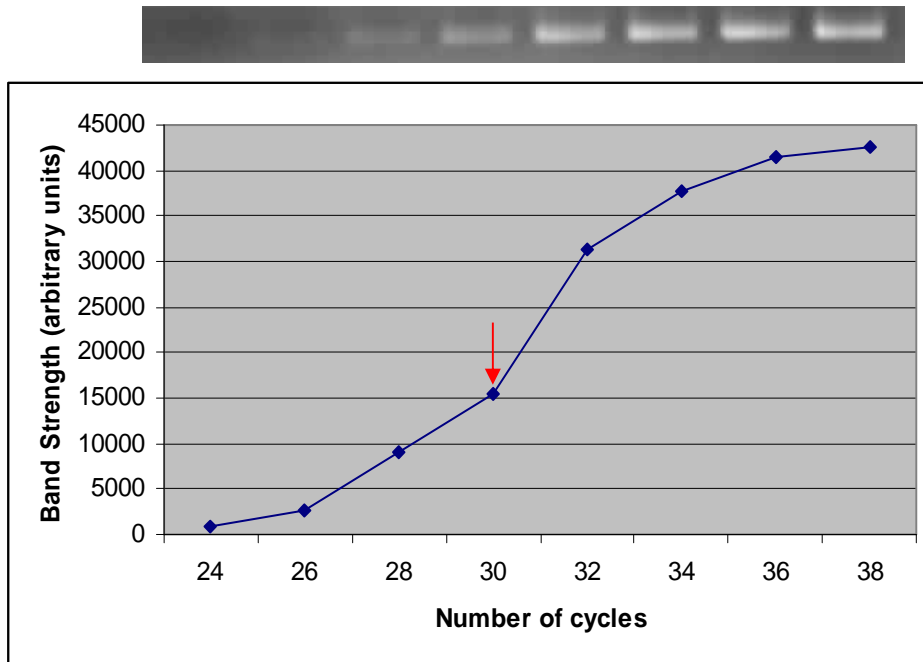
Full blot image for Supp. Fig 2(d)



Full gel image for Supp. Fig 2(d)

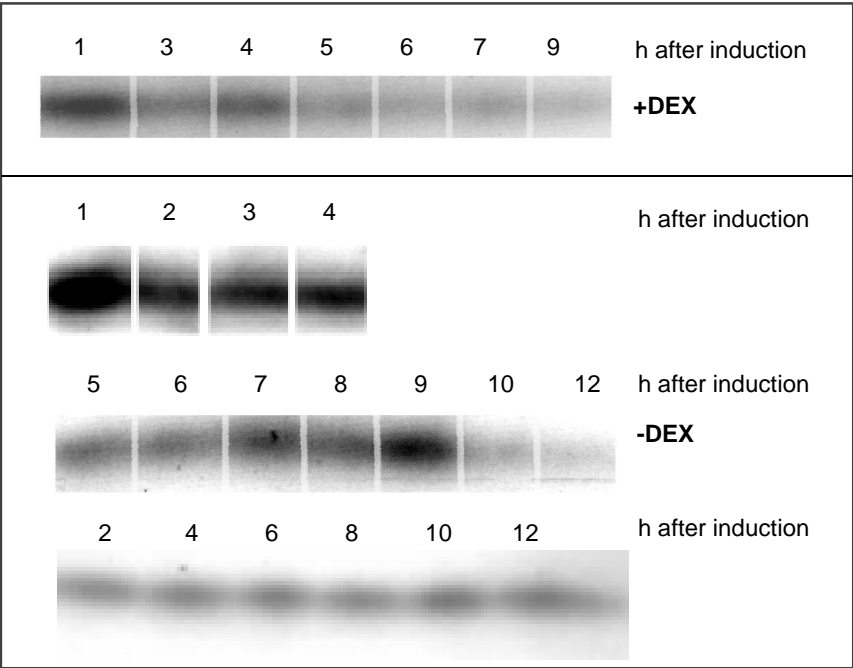


Supplementary Figure 6: Full gel images for blots and gels shown as sections in Figures and Supp Figures for the reviewers.

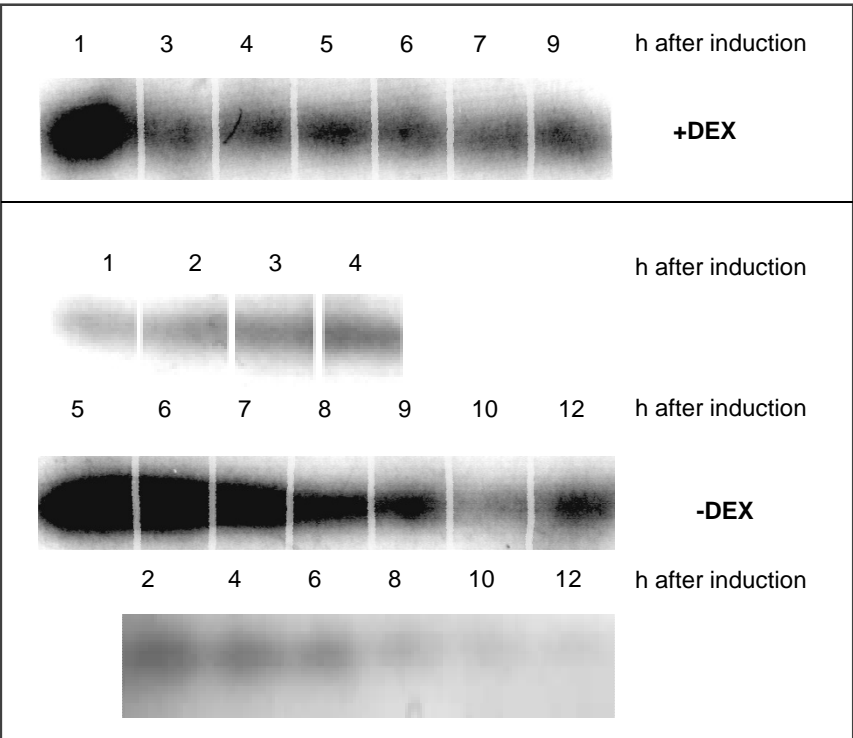


Supplementary Figure 7: Basis for semi-quantitative analysis of real-time PCR (a) Example of estimation of optimum cycle number to use for semi-quantitative RT-PCR for histone H4 from WEE1-i-1 cDNA. Arrow indicates cycle number chosen for further experiments, (b) Calibration plots using dilutions of WEE1-i-1 mix cDNA sample and histone H4 primers at an optimum number of cycles.

CDKA



CDKB



Supplementary Figure 8. Nicta;CDKB1 kinase activity is altered when *Arath;WEE1* is expressed in BY-2 cells CDKA and CDKB H1 kinase activities in i-WEE1-1, ± DEX. Immunoprecipitates from replicate experiments were quantified and shown as a % of maximum in Figure 7.

Note that early and later time points for the -DEX experiments were derived from separate gels and therefore absolute band intensity will vary amongst the gels. Hence they were normalised to % of maximum for deriving quantitative data.