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# Supplementary Materials for

## StrigoQuant: A genetically encoded biosensor for quantifying strigolactone activity and specificity

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**This PDF file includes:**

## Supplementary Results

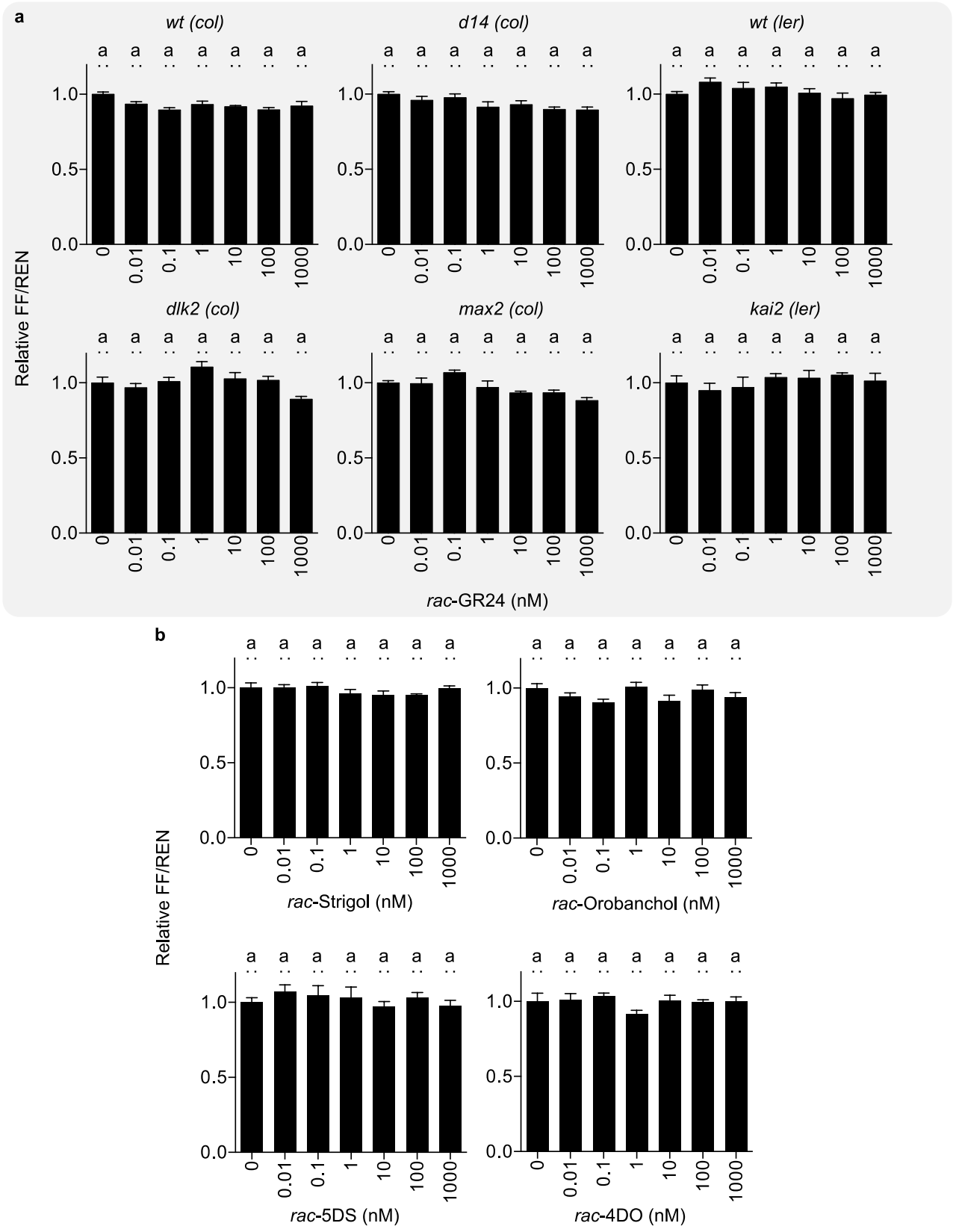


**fig. S1. CtrlQuant activity upon incubation with racemic SLs in WT and mutant *Arabidopsis* protoplasts.** (**A**) Characterization of the CtrlQuant sensor construct in protoplasts isolated from WT and mutant *Arabidopsis* backgrounds upon addition of increasing concentrations of *rac*-GR24. Protoplasts were isolated from WT (Columbia, *col-0*, ecotype), *Atd14* (*col*), *max2* (*col*), *dlk2* (*col*), WT (Landsberg erecta, *ler*,

ecotype), and *kai2* (*ler*) seedlings and transformed with CtrlQuant. 24 hours after transformation, protoplasts were supplemented with increasing concentrations of a *rac*-GR24 serial dilution for 2 hours prior to luciferase activity determination. (**B**) Activity of CtrlQuant upon incubation with strigol- and orobanchol-like SLs. WT *Arabidopsis* protoplasts transformed with CtrlQuant were incubated with racemic mixtures of strigol-like (*rac*-strigol, *rac*-5DS) and orobanchol-like (*rac*-orobanchol and *rac*-4DO) SLs for 2 hours prior to luminescence activity determination. The data shown corresponds to one representative experiment out of four (**A**) and three (**B**) replicated experiments. Results are averaged FF/REN ratios, normalized to the sample without addition of any inducer substrate for each genotype. Error bars represent standard error of the mean (SEM), n = 6. Statistical significance between the tested concentrations within a genotype is indicated with lower case letters above each bar, where "a" significantly differs from "b," "b" from "c," and so on. One-way ANOVA, $P < 0.01$.

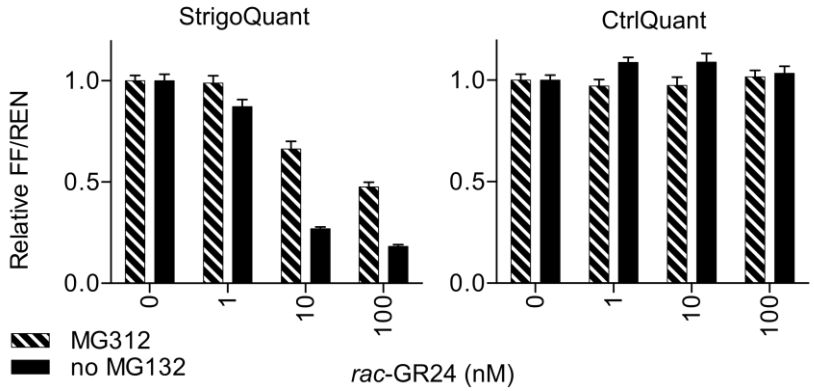


**fig. S2. SL-dependent degradation of the SMXL6-FF sensor component is mediated by the 26*S* proteasome.** WT *Arabidopsis* protoplasts were transformed with StrigoQuant (left panel) and CtrlQuant (right panel) and treated with 40 µM of the proteasomal inhibitor MG132, 22 hours after transformation. 2 hours prior to supplementation with increasing concentrations of *rac*-GR24. After additional 2 hours, luciferase activity was determined. The data shown corresponds to one representative experiment out of five replicated experiments. Results are averaged FF/REN ratios, normalized to the sample without addition of any inducer substrate for each genotype. Error bars represent standard error of the mean (SEM), n = 6.

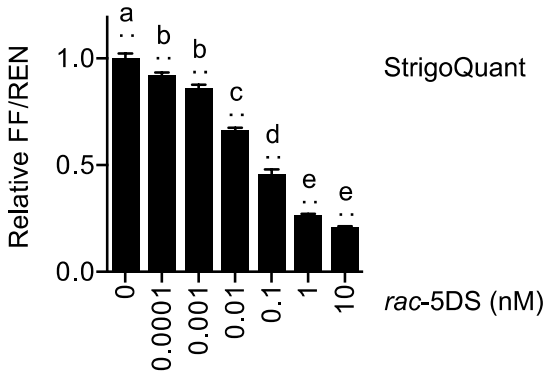


**fig. S3. Specificity and sensitivity of StrigoQuant to 5DS.** WT *Arabidopsis* protoplasts were transformed with StrigoQuant and 24 hours later treated with increasing concentrations of a *rac*-5DS serial dilution for 2 hours prior to luciferase activity determination. Results are averaged FF/REN ratios, normalized to the sample without addition of any inducer substrate. The data shown corresponds to one representative experiment out of four replicated experiments. Error bars represent standard error of the mean (SEM), n = 6. Statistical significance between the tested concentrations within a genotype is indicated with lower case letters above each bar, where “a” significantly differs from “b,” “b” from “c,” and so on. One-way ANOVA, $P < 0.01$.

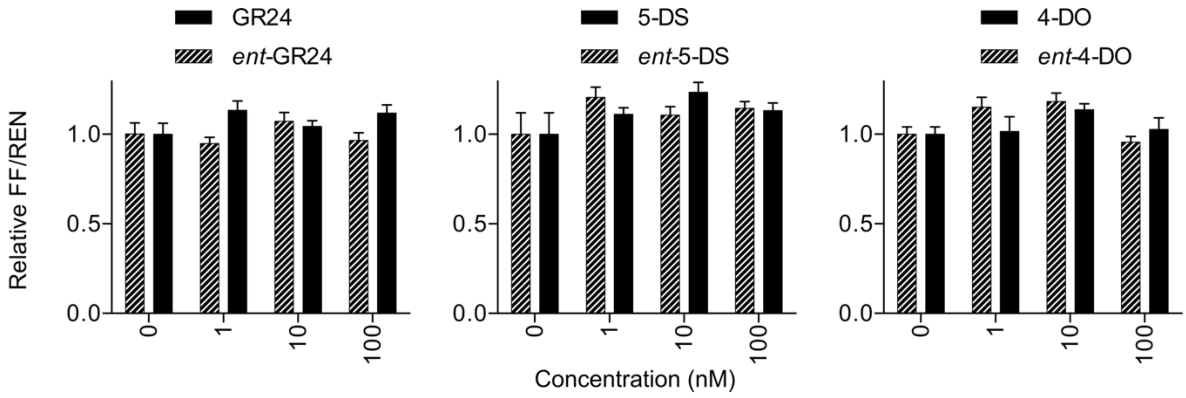


**fig. S4. Stereoselectivity analysis of SL species with CtrlQuant.** WT protoplasts transformed with CtrlQuant and incubated with separated enantiomers GR24 and *ent*-GR24, 5DS and *ent*-5DS, 4DO and *ent*-4DO for 2 hours prior to luminescence activity determination. Dose-response curves for substrates with the 2´*R* (+) D-ring configuration are shown in dashed lines, 2´*S* (-) configured substrates in solid black. The data shown corresponds to one representative experiment out of three replicated experiments. Results are averaged FF/REN ratios, normalized to the sample without addition of inducer for each substrate tested. Error bars represent standard error of the mean (SEM), n = 6.

**table S1. Amino acid sequences of the components of the StrigoQuant and CtrlQuant sensors.**

| | | |
|---|---|---|
| Renilla luciferase | MTSKVYDPEQRKRMITGPQWWARCKQMNVLDSFINYYDSEKHAENAVIFLHGNAASSY<br>LWRHVVPHIEPVARCIIPDLIGMGKSGKSGNGSYRLLDHYKYLTAWFELLNLPKKIIFVG<br>HDWGACLAFHYSYEHQDKIKAIVHAESVVDVIESWDEWPDIEEDIALIKSEEGEKMVLE<br>NNFFVETMLPSKIMRKLEPEEFAAYLEPFKEKGEVRRPTLSWPREIPLVKGGKPDVVQIV<br>RNYNAYLRASDDLPKMFIESDPGFFSNAIVEGAKKFPNTEFVKVKGLHFSQEDAPDEMG<br>KYIKSFVERVLKNEQ | |
| 2A peptide | PVKQLLNFDLLKLAGDVESNPGP | |
| Sensor module | StrigoQuant | MPTPVTTARECLTEEAARALDDAVVVARRRSHAQTTSLHAVSALLAMPSSI<br>LREVCVSRAARSVPYSSRLQFRALELCVGVSLDRLPSSKSPATEEDPPVSNSL<br>MAAIKRSQANQRRHPESYHLQQIHASNNGGGGCQTTVLKVELKYFILSILDD<br>PIVNRVFGEAGFRSSEIKLDVLHPPVTQLSSRFSRGRCPPLFLCNLPNSDPNRE<br>FPFSGSSGFDENSRRIGEVLGRKDKKNPLLIGNCANEALKTFTDSINSGKLGF<br>LQMDISGLSLISIEKEISEILADGSKNEEEIRMKVDDLGRTVEQSGSKSGIVLN<br>LGELKVLTSEANAALEILVSKLSDLLKHESKQLSFIGCVSSNETYTKLIDRFPT<br>IEKDWDLHVLPITASTKPSTQGVYPKSSLMGSFVPFGGFFSSTSNFRVPLSST<br>VNQTLSRCHLCNEKYLQEVAAVLKAGSSLSLADKCSEKLAPWLRAIETKED<br>KGITGSSKALDDANTSASQTAALQKKWDNICQSIHHTPAFPKLGFQSVSPQF<br>PVQTEKSVRTPTSYLETPKLLNPPISKPKPMEDLTASVTNRTVSLPLSCVTTD<br>FGLGVIYASKNQESKTTREKPMLVTLNSSLEHTYQKDFKSLREILSRKVAWQ<br>TEAVNAISQIICGCKTDSTRRNQASGIWLALLGPDKVGKKKVAMTLSEVFFG<br>GKVNYICVDFGAEHCSLDDKFRGKTVVDYVTGELSRKPHSVVLLENVEKAE<br>FPDQMRLSEAVSTGKIRDLHGRVISMKNVIVVVTSGIAKDNATDHVIKPVKF<br>PEEQVLSARSWKLQIKLGDATKFGVNKRKYELETAQRAVKVQRSYLDLNLP<br>VNETEFSPDHEAEDRDAWFDEFIEKVDGKVTFKPVDFDELAKNIQEKIGSHF<br>ERCFGSETHLELDKEVILQILAASWSSLSSGEEEGRTIVDQWMQTVLARSFA<br>EAKQKYGSNPMLGVKLVASSSGLASGVELPAKVDVIW |
| | CtrlQuant | GAGAGAGAGAGAGA |
| Firefly luciferase | MEDAKNIKKGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEM<br>SVRLAEAMKRYGLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPANDIYNERELLNSM<br>GISQPTVVFVSKKGLQKILNVQKKLPIIQKIIIMDSKTDYQGFQSMYTFVTSHLPPGFNEYD<br>FVPESFDRDKTIALIMNSSGSTGLPKGVALPHRTACVRFSHARDPIFGNQIIPDTAILSVVPF<br>HHGFGMFTTLGYLICGFRVVLMYRFEEELFLRSLQDYKIQSALLVPTLFSFFAKSTLIDKY<br>DLSNLHEIASGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILITPEGDDKPGAVGKV<br>VPFFEAKVVDLDTGKTLGVNQRGELCVRGPMIMSGYVNNPEATNALIDKDGWLHSGDI<br>AYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIFDAGVAGLPDDDAGELPAA<br>VVVLEHGKTMTEKEIVDYVASQVTTAKKL<br>RGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGKIAV | |

**table S2. Oligonucleotides used for the cloning of the sensor constructs.**

| Oligo | Sequence (5´-> 3´) |
|---|---|
| oHB556 | ATTTCATTTGGAGAGAACACGGGGACTCTAGCGCTACCGGTCGCCACCATGACTTCGAAAGTTTATGATCCAGAACAAAGG |
| oHB557 | CGCCTCGAGATCAGTTATCTAGATCCGGTGGATCCAAGCTTTTACAAGTCTTCTTCTGAGATTAATTTTTGTTCCAC |
| oHB562 | CAAGCATTCTCTCGCCGTAGTCACCGGCGTCGGCATGCGCGCGGGTCCAGG |
| oHB565 | GGAGTAGAATTGCCGGCGAAGGTGGATGTGATATGGGCTAGCATGGAAGACGCCAAAAAC |
| oHB576 | ATTTCATTTGGAGAGAACACGGGGACTCTAGCGCTACCGGTCGCCACCATGCTGCGATCGACGCATCC |
| oHB577 | CGCCTCGAGATCAGTTATCTAGATCCGGTGGATCCAAGCTTCTAGGCGTAGTCGGGCACGTCGTAGGGGTAGTACCGGGCGAGAGCGC |
| oSW237 | GTTCATTTCATTTGGAGAGAACACGGGGACTCTAGCGCTACCGGTTGGCTAGGTAAGCTTGGTAC |
| oSW238 | CGAGCCCGGGGAATTCGGCCGCTGCCGCAGCGGCAGCGGCCGCTTACAAGTCTTCTTCTGAG |