In vivo gibberellin gradients visualised in elongating tissues with Gibberellin Perception Sensor 1

Annalisa Rizza [1], Ankit Walia [1], Viviane Lanquar [2], Wolf B Frommer [2,3]*, Alexander M Jones [1,2]*

[1] Sainsbury Laboratory, Cambridge University, Cambridge, UK

[2] Carnegie Institution for Science, Department of Plant Biology, Stanford, CA, USA

[3] New address: Institute for Molecular Physiology, Heinrich Heine Universität, Düsseldorf, and Max Planck Institute for Plant Breeding Research, Cologne, Germany

*Corresponding authors: alexander.jones@slcu.cam.ac.uk, frommew@hhu.de

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Abstract

The phytohormone gibberellin (GA) is a key regulator of plant growth and development. Although the upstream regulation and downstream responses to GA vary across cells and tissues, developmental stages, and environmental conditions, the spatiotemporal distribution of gibberellin *in vivo* remains unclear. Using a combinatorial screen in yeast, we engineered an optogenetic biosensor, Gibberellin Perception Sensor 1 (GPS1) that

25 senses nanomolar levels of bioactive gibberellins. *Arabidopsis thaliana* plants expressing a
26 nuclear localised GPS1 report on gibberellins at the cellular level. GA gradients were
27 correlated with gradients of cell length in rapidly elongating roots and dark-grown
28 hypocotyls. In roots, accumulation of exogenously applied GA also correlated with cell
29 length, intimating that a root GA gradient can be established independently of GA
30 biosynthesis. In hypocotyls, GA levels were reduced in a *phytochrome interacting factor*
31 (*pif*) quadruple mutant in the dark and increased in a phytochrome double mutant in the
32 light, indicating that PIFs elevate GA in the dark and that phytochrome inhibition of PIFs
33 could lower GA in the light. As GA signalling directs hypocotyl elongation largely through
34 promoting PIF activity, PIF promotion of GA accumulation represents a positive feedback
35 loop within the molecular framework driving rapid hypocotyl growth.

36 **Introduction**

37 Gibberellins (GA) are plant hormones that promote organ growth in a variety of
38 developmental contexts and mutants defective in GA biosynthesis are characterized by
39 reduced elongation of roots, stems, and floral organs [1]. Although GA was first
40 discovered as a plant growth regulator nearly a century ago (reviewed here [2]),
41 knowledge of GA distribution at the cellular level remains limited by an inability to
42 quantify GA *in vivo*. Reporter constructs driven by the promoters of GA biosynthetic
43 enzymes have revealed dynamic expression maps over development [3-5], suggesting that
44 spatiotemporal distribution of GA is tightly regulated. However, inference of GA

45 distribution from enzyme expression profiles is complicated when GA isozymes exhibit
46 contrasting expression patterns, for example during hypocotyl de-etiolation where
47 expression of *AtGA3ox1* decreases over 6-fold while *AtGA3ox2* increases 16-fold [6].
48 Furthermore, GA distributions are also regulated by catabolism, conjugation, and, because
49 GAs are mobile signals, transport steps [1]. Although overall GA levels can be quantified
50 directly with biochemical methods, such as gas chromatography/mass spectrometry
51 (GC/MS) [7], these approaches average over many cells and do not enable dynamic
52 analyses.

53 In primary root tips, where GA deficiency reduces cell elongation and cell proliferation
54 rate leading to shorter roots, the biological function of GA in the Arabidopsis root has
55 received considerable attention [8-13]. Ubeda-Tomás et al. (2008) found that blocking GA
56 signalling specifically in the endodermis is sufficient to disrupt GA-directed cell elongation
57 in the *Arabidopsis* root, indicating that the endodermis is a key site of GA action in root
58 elongation [11]. This hypothesis is supported by Shani et al. (2013), who observed
59 accumulation of exogenous fluorescein-GA_3 and fluorescein-GA_4 over time in the vacuoles
60 of endodermal cells of the elongation zone [13]. However, a multi-scale model of the
61 relationship between GA distribution and root cell growth proposed by Band et al. (2012)
62 posits that cellular GA levels decrease – owing to cytosolic dilution - as cells progress
63 through the root elongation zone [9]. Despite such investigations using cell-specific

64 genetic intervention into GA signalling [11], fluorescein labelled GAs [13], and multi-scale
65 modelling of GA levels [9], the distribution of GA in roots remains unclear.

66 In several tissues, GA levels are altered in response to environmental stimuli, and this
67 redistribution of GA is critical for key developmental transitions such as germination,
68 photomorphogenesis, and the transition to flowering [1]. For example, in Arabidopsis
69 seeds, light stimuli trigger activation of the phytochrome photoreceptors (PHYs), leading
70 to elevated GA levels, which promote germination [14, 15]. Interestingly, in hypocotyls the
71 opposite is true, as light signalling lowers GA levels and the cessation of the rapid
72 elongation observed in darkness [16-18]. In seeds, phytochromes raise GA levels in part
73 through relieving PHYTOCHROME INTERACTING FACTOR (PIF) mediated inhibition of GA
74 accumulation [19]. In the case of the hypocotyl, it remains unclear how light signalling
75 lowers GA levels or how elevated GA levels are promoted by darkness. Also, the
76 distribution of GA in hypocotyls is yet unknown.

77 Here we used an optogenetic biosensing approach, based on a Förster Resonance Energy
78 Transfer (FRET) biosensor for GA for high-resolution quantification of spatiotemporal GA
79 distribution. Small molecule FRET biosensors that directly interact with and report on
80 ligand concentration have been used in living plants to generate high-resolution
81 measurements of calcium [20, 21], sugars [22, 23], and the plant hormone abscisic acid
82 [24, 25]. In these synthetic fusion proteins, binding of the ligand to a sensory domain
83 induces structural rearrangements that alter the amount of energy transfer between a

84 donor fluorescent protein moiety and an acceptor fluorescent protein moiety. Sensor
85 output can be tracked by exciting the donor and measuring the ratio of acceptor emission
86 over donor emission; this emission ratio is independent of biosensor expression levels and
87 is proportional to ligand concentration [26]. Compared with GC/MS, optogenetic
88 biosensors that directly report on small molecule concentrations are minimally invasive,
89 provide high-resolution, and require only light inputs once the biosensor is expressed *in*
90 *vivo* [26].

91 To develop and optimize a FRET biosensor for GA, we used plant hormone receptors as
92 sensory domains. GIBBERELLIN INSENSITIVE DWARF 1 (GID1) proteins are soluble
93 receptors that interact with GA in an internal binding pocket [27]. GA binding promotes
94 GID1 interaction with members of the DELLA family of growth regulators. The GID1-GA
95 complex binds the N-terminal DELLA domain, which leads to the degradation of the
96 DELLA protein after ubiquitination of the C-terminal GRAS domain [28]. We endeavoured
97 to co-opt the Arabidopsis GA perception machinery into a conformationally dynamic GA
98 binding domain within a FRET biosensor by fusing GID1 variants to DELLA N-termini. Such
99 a fusion would effectively convert GA-dependent *inter*molecular interactions into GA-
100 dependent *intra*molecular structural rearrangements.

101 We screened potential biosensor constructs expressed in yeast [24] for fluorescence
102 emission ratio changes in response to GA addition. A systematic screen of GA sensory
103 domains, donor and acceptor fluorescent protein variants, and variant linkers fusing the

component domains led to the identification of Gibberellin Perception Sensor 1 (GPS1). GPS1 functions as a biosensor for GA that responds with an increase in emission ratio to nanomolar concentrations of bioactive GAs (e.g. K_d = 24 nM for GA_4).

The GPS1 biosensor expressed in Arabidopsis and targeted to cell nuclei (nuclear localization signal GPS1, nlsGPS1) reports GA distribution *in vivo* in multiple tissues and growth conditions. Here we report GA gradients along the growth axis of *Arabidopsis* roots and hypocotyls as well as elevated GA in stamen filaments. We also report that exogenously applied GA_4 accumulates preferentially in the root elongation zone intimating that GA transport and catabolic activities can generate GA gradients independently of GA biosynthesis. We furthermore observed reduced GA levels in dark grown hypocotyls of a *pif* quadruple mutant (*pif1, pif3, pif4, pif5* (*pif q* [26, 27])) and increased GA levels in light grown hypocotyls of a phytochrome double mutant (*phyA, phyB* [29]), implicating that PIFs promote GA accumulation in the dark and that phytochrome mediated degradation of PIFs could lead to lowering of GA levels in the light.

Results

Combinatorial screen for optimized FRET-based GA biosensors

Engineering FRET biosensors is a semi-empirical process that often requires the screening of ligand binding domains fused to pairs of FRET donor/acceptor variants to identify fusion proteins that exhibit ligand-induced changes in FRET (Figure 1a, [26]). Here we

used a combinatorial FRET biosensor cloning approach in which a series of Gateway Entry clones coding for potential GA binding domains were recombined with a set of previously designed Gateway destination vectors (pDR-FLIPs) which carry fluorescent protein gene variants capable of FRET [24].

A matrix of 24 potential GA binding domain constructs were generated as Gateway Entry clones. GA binding domains were derived from either AtGID1A, B, or C linked via a flexible 12-amino-acid linker (L12) to either the N- or C-terminus of two different truncations of the DELLA proteins AtRGA or AtGAI. These 24 candidates were recombined with a Gateway Destination vector (pDR-FLIP39 [24]) that encodes enhanced dimerization (ed) variants of Aphrodite (edAFP) as FRET acceptor and edeCFP as FRET donor. We screened fusion proteins expressed in protease-deficient yeast [30] for GA-dependent alterations in the fluorescence emission curves after FRET donor excitation at 430 nM (donor excitation (Dx); Figure S1a). For two of the 24 fusion proteins, GA_3 triggered an increase in emission at 535 nm (donor excitation acceptor emission, DxAm) and a concomitant decrease in emission at 485 nm (donor excitation donor emission, DxDm), characteristic of a FRET biosensor with a positive ratio change (ΔDxAm/DxDm). We next sought to optimize the ratio change and fluorescent proteins of one of these fusion proteins by combinatorially screening a series of variants of the L12 linker and a series of FRET pair variants (Figure S1b). This screen resulted in an optimized biosensor, named GPS1, which consisted of an N-terminal edAFP linked via the translated attB1 to a 74-amino-acid truncation of AtGAI

144 linked via L12 to AtGID1C linked via the translated attB2 site to edCerulean (Figure 1b).
145 Based on the crystal structure of AtGID1A in complex with AtGAI and our observed
146 DxAm/DxDm values for GPS1 (hereafter referred to as GPS1 emission ratio) with and
147 without GA_3 (Figure 1c), one hypothesis is that GPS1 switches from a moderate FRET to
148 high FRET average state upon binding to GA.

149 **Generation of the non-responsive control GPS1-NR**

150 Non-responsive variants of GPS1, as important controls for interpretation of GPS1-derived
151 *in vivo* data, were generated via mutation of AtGID1C residues involved in GA binding and
152 AtGAI residues involved in interaction with GID1 proteins. GPS1-S114A/F115A carries two
153 alanine substitutions in the GA binding pocket of AtGID1C based on corresponding
154 mutations in the rice GID1 protein that had been shown to disrupt GA binding [27].
155 GPS1–S114A/F115A shows no detectable response to GA *in vitro* (Figure 1e). In GPS1-
156 E51K/E54R, the substitution of two positively charged residues for negatively charged
157 residues in AtGAI likely disrupts two salt-bridges that function in the interaction with
158 AtGID1 [31]. By combining the AtGID1C-S114A/F115A mutant with the AtGAI-E51K/E54R
159 mutant, we generated a mutant biosensor (termed GPS1-non-responsive, GPS1-NR) that is
160 apparently non-responsive to GA and, importantly, whose component GAI domain is
161 predicted to be deficient in *in planta* interactions with endogenous GAI binding factors
162 including GIDs (Figure 1e).

163 **Characterization of GPS1**

To determine the dynamic range of GA detection by GPS1, we measured the K_d of metal-affinity purified GPS1 *in vitro* by tracking dose-dependent changes in GPS1 emission ratio for several bioactive, precursor, and catabolized GAs (Figure 2a and b). It had been shown that the K_d of AtGID1C for 16,17-dihydro-GA_4 is 1.9 μM and that the K_d decreases to 46 nM in the presence of AtGAI [32], indicating that AtGAI increases AtGID1C affinity for GA 50-fold. The K_d of GPS1, which contains full-length AtGID1C and the DELLA domain of AtGAI, was 24 nM for GA_4 (Figure 1B). This affinity is comparable with the AtGID1C affinity for GA_4 in the presence of AtGAI [32]. The K_d of GPS1 for GA_1 was 110 nM and for GA_3 was 240 nM. Overall, GPS1 was responsive to several GAs (Figure 2a and b), with lower affinities relative to GA_4, consistent with the properties of unfused GID and GAI [32]. We examined whether GA binding by GPS1 is reversible by treating GPS1 with different GAs, performing two 20 minute steps of buffer-exchange chromatography, and then treating with GA a second time. GPS1 was partially reversible for GA_1 and GA_3 but not for GA_4 (Figure 2c). Because the GPS1 response to GA_4 remains even after removal of GA_4 from solution, GPS1 emission ratio change is likely unable to report rapid GA depletion *in vivo*.

Expression of GPS1 *in planta*

To assess GA distribution *in planta,* we generated stable transgenic *Arabidopsis* lines expressing a nuclear targeted variant of GPS1 (nlsGPS1) under the control of a promoter fragment previously shown to direct a broad expression pattern (p16, [33]). Targeting the biosensor to the nucleus allowed for analysis of GA accumulation in the compartment

most relevant to endogenous GA perception and facilitates comparison of GPS1 emission ratios between neighbouring cells. The use of the p16 promoter led to successful expression of GPS1 transgenes in both roots and shoots of wild-type Col-0 *Arabidopsis*. Importantly, GPS1 expression did not result in detectable phenotypic changes in GA-dependent hypocotyl or root growth (Figure S2a and b). We further analysed whether the presence of GPS1 might interfere with GA-dependent seedling growth by comparing nlsGPS1 or GPS1 with wild-type Col-0 in response to treatment with the GA biosynthesis inhibitor paclobutrazol (PBZ) alone or in combination with GA_4 (Figure 2d and Figure S2c). All lines exhibited an expected reduction in hypocotyl elongation in presence of PBZ that was gradually reversed by increasing concentrations of GA_4. However, nlsGPS1 and GPS1 seedlings were hyposensitive to PBZ treatment (Figure 2d and Figure S2c), a phenotype that might be due to increased GA sensitivity resulting from overexpression of the full length AtGID1C moiety of the GPS1 biosensor. Similar hormone hypersensitivity had been observed for Arabidopsis lines expressing ABACUS1 biosensors [24]. The increased GA sensitivity resulting from expression of the GPS1 biosensor, although not apparent under normal growth conditions, must be considered when interpreting results from all analyses. Thus, further engineering is required to create GA biosensors with reduced interactions with endogenous machinery as was accomplished in the second generation of cameleon calcium sensors [34].

Live-cell imaging of GPS1 response to endogenous GAs in roots

To explore whether GPS1 is suitable for measuring GA distribution in plants, we investigated nlsGPS1 emission ratios in roots of *Arabidopsis* seedlings grown on agar plates and exposed to long-day cycling (16 h light/ 8 h dark) for three days (hereafter referred to as 'light-grown', Figure 3, Figure S3). GA signalling in roots is required for meristem size and cell elongation [11, 12]. In roots, we observed an apparent gradient of GA with low nlsGPS1 emission ratios in the apical cell division zone transitioning to increasing nlsGPS1 emission ratios in the elongation zone (Figure 3a). Although local variation was observed, mean nlsGPS1 emission ratios increased significantly along the root growth axis (Figure 3b). Interestingly, the increase in nlsGPS1 emission ratios in the elongation zone was observed in epidermal and cortical cells and was not limited to the endodermis (Figure 3c and d), which is thought to be a main site of action for GA signalling in root elongation [11]. To evaluate whether nlsGPS1 emission ratios report actual GA distribution, we analysed emission ratios in seedlings expressing nlsGPS1-NR that do not respond to GA. These plants showed low emission ratios in all root zones with a small but significant increase from the division zone to the elongation zone (Figure 3a and b). However, the difference in emission ratios between the division zone and the late elongation zone is small (mean difference = 0.13) when compared with nlsGPS1 (mean difference = 0.89), implying that the gradient in nlsGPS1 emission ratios along the root growth axis is not artefactual (Figure 3b). We also observed greatly reduced nlsGPS1 emission ratios in roots of a *ga3ox1, ga3ox2* double mutant (Figure 3a and b), as one

might expect for a mutant with greatly reduced GA levels [4]. GA signalling results in proteasome mediated degradation of DELLAs [35]. We assessed the distribution of the DELLA protein AtRGA through imaging Arabidopsis lines expressing a pRGA::GFP-RGA translational fusion ([35], Figure S4) and observed pRGA::GFP-RGA protein distribution to be anti-correlated with nlsGPS1 emission ratios in roots (Figure S4a). Taken together, the distribution of pRGA::GFP-RGA fluorescence and nlsGPS1 emission ratios indicate that GA levels correlate with cell length in Arabidopsis roots (Figure 3).

Live-cell imaging of GPS1 response to exogenous GAs in roots

Plants translocate GAs [36] from source to sink tissues during normal development to trigger responses such as stem growth and flowering [37-40]. Indicative of GA's role as a mobile signal, a variety of plant tissues have been shown to accumulate and respond to exogenously supplied GAs [38]. Treatments of Arabidopsis roots with fluorescein-GA_3 and fluorescein-GA_4 resulted in dye accumulation first in all cells of the elongation zone before subsequent concentration in vacuoles of the endodermis in the elongation zone [13]. However, it remains unclear which cells and cell types are competent in accumulating unmodified GAs. We analysed nlsGPS1 emission ratios in roots of *Arabidopsis* seedlings before and 20 minutes after treatment with exogenous GA_4, GA_1, and GA_3 at concentrations ~40-, 90- and 40-fold higher than the respective GPS1 K_d (Figure 4a – d, Figure S5a). Treatment of Arabidopsis roots with exogenously applied GA_1 or GA_3 did not increase nlsGPS1 emission ratios, whereas exogenous GA_4 led to increased nlsGPS1

emission ratios specifically in the elongation zone. Thus, the mechanisms governing accumulation of exogenously applied GAs can effectively discriminate between GAs, and preferentially accumulate GA_4 – the dominant bioactive GA in *Arabidopsis* - over GA_3 or GA_1. Similar to endogenous GA, accumulation of exogenous GA_4 was not limited to the endodermis, though it should be noted that following an initial accumulation of exogenous GA_4, nlsGPS1 would not report on depletion of GA from epidermal and cortical cells nor on accumulation of GA in vacuoles.

The accumulation of exogenously applied GAs as detected by nlsGPS1 in the nuclei of root cells results from an ensemble of import activity less depletion activities such as catabolism, compartmentation, and export. To quantify this ensemble activity with high spatiotemporal resolution, we performed time-course experiments of *Arabidopsis* roots growing in the RootChip16, a microfluidic device that allows imaging of roots growing in controlled perfusion chambers [23, 24]. After twenty minutes of perfusion with GA_4, GA accumulation closely mirrored the gradient of endogenous GA prior to treatment (Figure 4e and f, Movie 1). The differential accumulation of exogenous GA across the root could result from differential depletion activity, as had been shown for ABA depletion activities in different zones of Arabidopsis roots [24]. Direct analysis of GA depletion rates was not possible due to the low apparent reversibility of GPS1 binding to GA (Figure 1c). To determine if biosensor turnover could nevertheless lead to a lowering of nlsGPS1 emission ratios to baseline levels, we analysed roots after a 20 minute treatment with 100 nM GA_4

264 and found that nlsGPS1 emission ratios in GA responsive cells remained high for at least
265 two hours and were not highly responsive to a subsequent 20 minute treatment with 100
266 nM GA_4 (Movie 1, Figure S5). Consistent with the observation that nlsGPS1 responses are
267 stable *in vivo*, we did not observe rapid turnover of nlsGPS1 protein *in vivo*, as would
268 have been indicated by loss of YFP fluorescence intensity, after treatment with the protein
269 synthesis inhibitor cycloheximide for three hours (Figure S6).
270 An alternative hypothesis is that differential import activity, rather than differential
271 catabolism, results in an accumulation gradient of exogenous GA_4 (Figure 4e and f). As
272 GAs are mobile, differential import could also contribute to gradients of endogenous GA
273 (Figures 3 and 4). Band et al. (2012) concluded from analyses of *AtGA2ox* expression
274 patterns and root growth phenotypes of a *ga2ox* quintuple mutant that GA catabolism
275 was likely low in both division and elongation zones [9]. Further investigations are needed
276 to clarify how spatial regulation of GA enzymes and transporters set cellular GA
277 distribution such that GA levels are correlated with cell length in Arabidopsis roots.

278 **Live-cell imaging of GPS1 in stamen filaments**

279 Stamen filaments undergo rapid elongation in order to deposit the pollen from the anther
280 to the stigma [41]. We observed high nlsGPS1 emission ratios compared with nlsGPS1-NR
281 in elongating filaments, indicating that GA levels increase during filament growth (Figure
282 S7). Our results are consistent with the strong expression of *AtGA3ox* biosynthetic
283 enzymes in stamen filaments [3].

Live-cell imaging of GPS1 in light- and dark-grown hypocotyls

Environmental stimuli are known to regulate GA levels in several tissues, for example in basal hypocotyls where light signalling lowers GA leading to slower elongation growth [16-18]. In nuclei of light-grown hypocotyl cells, we observed low nlsGPS1 emission ratios near the shoot apical meristem and variable nlsGPS1 emission ratios in the faster growing central region of the hypocotyl (Figure 5, Figure S8). As in roots, both wild-type seedlings expressing nlsGPS1-NR and *ga3ox1, ga3ox2* double mutant seedlings expressing nlsGPS1 exhibited reduced emission ratios (Figure 5a and b), suggesting that the nlsGPS1 emission ratios observed in light-grown, wild-type hypocotyls are indicative of GA distribution. We investigated the relationship between the GA distribution and hypocotyl cell size by co-imaging nlsGPS1 emission ratios with cell margins stained with propidium iodide (Figure 6a, Figure S9a). Unlike in rapidly elongating root tips, where larger cells exhibit higher nlsGPS1 emission ratios, we did not detect a correlation between cell size and nlsGPS1 emission ratios in light-grown hypocotyls (r= -0.09, Figure 6a and b). Compared to light-grown hypocotyls, dark-grown hypocotyls exhibited dramatic, GA-dependent cell elongation that can result in a 10-fold increase in overall hypocotyl length [42, 43]. The rapidly elongating region of dark-grown hypocotyls exhibited larger cells, higher nlsGPS1 emission ratios, and a positive correlation between the two (r= 0.64, Figure 6a and b). Overall, in dark-grown hypocotyls we observed a gradient of nlsGPS1 emission ratios indicative of high GA levels in longer cells transitioning to lower GA levels in the smaller

304 cells of the apical hook (Figure 6e-f, Figure S10). The emission ratio gradient was not
305 observed in dark-grown hypocotyls of seedlings expressing nlsGPS1-NR or dark-grown
306 hypocotyls of the *ga3ox1, ga3ox2* mutant expressing nlsGPS1 (Figure 6e-f), suggesting
307 that the gradient of nlsGPS1 emission ratios observed in dark-grown, wild-type hypocotyls
308 is indicative of an actual GA gradient. We also observed pRGA::GFP-RGA protein
309 distribution to be anti-correlated with nlsGPS1 emission ratios in the dark-grown
310 hypocotyl, further supporting the conclusion that nlsGPS1 emission ratios are indicative of
311 GA levels in dark-grown hypocotyls (Figure S4). Combined with the observation of GA
312 accumulation in the root elongation zone (Figure 3) and stamen filaments (Figure S7),
313 these results indicate that high GA levels correlate with rapid cell elongation in plant
314 development.

315 Rapid cell elongation in dark-grown hypocotyls depends on both PIF proteins to promote
316 growth [44-46] and elevated GA levels that lead to degradation of growth-repressing
317 DELLA proteins [1]. PIF and GA do not function independently in the hypocotyl as DELLA
318 proteins repress PIF function, and thus elevated GA contributes to PIF-mediated hypocotyl
319 elongation in the dark [47, 48]. There is also evidence that light signalling through
320 phytochromes can lower GA levels [17, 18], thus reducing hypocotyl elongation by
321 repressing PIF activity both directly, via PIF degradation, and indirectly, via promotion of
322 DELLA accumulation. Compared with wild-type, nlsGPS1 emission ratios were modestly
323 increased in light-grown hypocotyls of a *phyA, phyB* mutant (Figure 6c-d). Because much

of phytochrome signalling functions through repression of PIFs, we hypothesized that PIF proteins may regulate the GA gradient in dark-grown hypocotyls, and that the loss of PIFs will lead to reduced GA levels in the light. Correspondingly, nlsGPS1 emission ratios of *pifq* mutant hypocotyls were reduced compared with wild-type hypocotyls grown in the dark (Figure 6e-f), consistent with PIF proteins being in part responsible for GA elevation in the dark. In the light, where the *pifq* mutant displays no hypocotyl elongation phenotype, we did not observe substantive differences in hypocotyl nlsGPS1 emission ratios between *pifq* and wild-type (Figure S11). The finding that PIF proteins elevate GA in hypocotyls in the dark is supported by RNA-seq experiments indicating that PIFs positively regulate expression of the GA biosynthetic enzyme *AtGA20ox3* in dark-grown seedlings [49]. Interestingly, the elevation of nlsGPS1 emission ratios below the apical hook of etiolated hypocotyls is reduced in the *pifq* mutant, indicating a potential role for the PIFs in controlling cellular GA distribution in hypocotyls (Figure 6e-f). As GA accumulation promotes PIF activity through stimulating degradation of the PIF-repressing DELLA proteins, our findings support a hypocotyl growth model with a PIF positive feedback loop through GA signalling.

Discussion

Plant hormones are mobile, small signaling molecule that play central roles in plant development and environmental responses. The goal of a systems level understanding of the upstream regulation and downstream consequences of hormone accumulations and

depletions demands a more accurate and higher resolution quantification of dynamic hormone patterning. Unlike other quantification methods, fluorescent biosensors that directly report on plant hormones are minimally invasive, high-resolution, and require only light inputs once the biosensor is expressed from the host cell genome [26]. Compared with transcriptional reporters and signalling sensors, hormone quantification using direct biosensors is also less susceptible to non-linear signalling responses, variations in signalling components independent of the hormone, and crosstalk from other signalling pathways [50]. Here we report the engineering and analysis of the first such biosensor for GA, a key signalling molecule that affects plant morphogenesis.

Engineering of a biosensor for GA

The GA biosensor GPS1 was developed and optimized using a combinatorial FRET biosensor engineering platform developed previously [24]. GPS1 reports GA levels via a ratiometric, fluorescent output (Figure 1). GPS1 is a single synthetic polypeptide composed of an output domain based on improved variants of cyan fluorescent protein and yellow fluorescent protein fused with a sensory domain based on the GID1C – GAI receptor complex from *Arabidopsis* (Figure 1b). GPS1 directly interacts with GA_4 with high affinity (*in vitro* apparent K_d for GA_4 = 24 nM) and other bioactive GAs with lower affinity (Figure 2a). Although GA_4 is considered to be the dominant bioactive GA in *Arabidopsis*, GPS1 could also report on other GAs *in vivo* if present at appreciable concentrations (e.g. around the K_d of 110 nM for GA_1). In the future, GPS biosensors could potentially be re-

364 engineered to have altered selectivity and affinity for the various GAs. The high-affinity
365 association of GPS1 with GA_4 is apparently not rapidly reversible (Figure 2c), and thus
366 GPS1 emission ratios likely report the highest recent GA concentration reached in the
367 solution or cellular compartment harbouring the biosensor. Thus, GPS1 will probably not
368 report decreasing GA levels. Nevertheless, the ability to deploy the GPS1 fusion protein in
369 living cells permits minimally invasive GA biosensing with unprecedented spatiotemporal
370 resolution.

371 **GA gradients *in vivo***

372 The GPS1 biosensor was targeted to the nucleus (nlsGPS1) in transgenic Arabidopsis
373 plants to investigate the GA distribution in nuclei elongating root and shoot tissues and
374 the quantitative relationship between GA accumulation and cell elongation. It has been
375 proposed that GA levels in root tip cells are inversely correlated with cell length, i.e. to be
376 high in the root division zone and to be depleted due to cytosolic dilution as cells
377 elongate [9]. Rather than an inverse relationship, we found GA – as indicated by GPS1
378 emission ratios – to be positively correlated with cell length (Figures 3 and 4). In another
379 tissue where pronounced cell elongation requires GA, the dark-grown hypocotyl, we again
380 observed GA to be correlated with cell length (Figures 6). These correlations are
381 compatible with a hypothesis that GA induces cell growth locally in a dose-dependent
382 manner. Thus, the patterning of GA enzymatic and transport activities that set GA levels
383 would influence the patterning of cell elongation. We show that the pattern of

accumulation of exogenous GA_4 in roots closely mirrors the distribution of endogenous GA, indicating that differential accumulation of GA from other cells or tissues could generate a GA gradient, and thus a gradient of cell length, in the root elongation zone (Figure 4). Understanding the signalling pathways that regulate these activities, whether via patterning GA enzyme activity, transport activity, or both, is key to understanding how GA distribution links environmental stimuli and endogenous factors to their downstream effects on plant growth and development.

Factors affecting GA gradients

Because light signalling is known to impact expression of GA enzymes in monocots and dicots, including Arabidopsis [16-18], we investigated the regulation of the GA distribution by light. In the light, activation of phytochromes, such as PhyA and PhyB, contributes to a reduction in hypocotyl cell length compared to hypocotyls grown in the dark [29]. Compared with wild-type, light-grown hypocotyls of a *phyA, phyB* mutant [29] exhibited elevated GA, confirming a role for light signalling through phytochromes in lowering GA levels alongside hypocotyl cell length (Figure 6). However, it remained unknown what factors promote GA elevation in dark-grown hypocotyls and how phytochrome signalling lowers GA levels in the light. We provide genetic evidence that the PIFs fulfil, at least in part, both roles in affecting GA levels (Figure 6). As GA signalling influences hypocotyl elongation largely through promoting PIF activity [47, 48], PIF promotion of GA accumulation represents a positive feedback loop within the molecular framework driving

404 rapid hypocotyl growth. Furthermore, positive regulation of GA by PIFs in the hypocotyl
405 stands in marked contrast to repression of GA by PIF1 activity in seeds [19], and
406 represents a new link between light signalling and hormone distribution that underpins
407 cellular growth and developmental plasticity. However, because we observed only a
408 modest increase in GA levels in *phyA, phyB* mutant hypocotyls in the light and partial
409 reduction in GA levels in *pifq* mutant hypocotyls in the dark (Figure 6), it is likely that
410 other phytochromes and other light signalling components beyond the phytochromes and
411 the PIFs regulate GA in hypocotyls. Indeed, during photomorphogenesis, both
412 phytochromes and cryptochromes affect GA biosynthetic and catabolic enzymes [18].

413 Because myriad other signals have been shown to regulate GA levels [1], and because
414 local GA elevation may define regions of rapid cell elongation (Figures 3 and 6), it is likely
415 that a finely tuned ensemble of GA enzymatic and transport steps in concert with their
416 regulators together determine GA distribution dynamics *in vivo*. A precise understanding
417 of how GA distribution is controlled and how it, in turn, contributes to patterning plant
418 cell growth warrants further investigation. Such an understanding could lead to strategic
419 interventions into GA distributions in crop plants for specific improvements in
420 germination, flowering-time, plant architecture, organ size, and environmental responses.

421

Materials and Methods

Gateway entry clone and destination vector libraries

Potential GA binding domains to be incorporated in a screen for FRET GA biosensors were designed as combinations of an AtGID1 GA receptor protein linked to a truncation of a DELLA protein and cloned as a Gateway Entry Clone library. The specific components were full-length AtGID1A (AT3G05120), AtGID1B (AT3G63010), and AtGID1C (AT5G27320), truncated AtGAI D28 – D101 and G26 – L91 (At1g14920), truncated AtRGA D44 – N117 and N41 – N108 (At2g01570), and five internal linkers described previously (L12, L52, L65, L71, L118 [24]). Potential GA binding domains were amplified in two PCR steps. In the first step, N-terminal domain sequences were amplified with 5′ primers containing the attB1 site and 3′ primers containing a 30-base pair sequence for overlapping PCR. C-terminal domain sequences were amplified with 5′ primers containing the same 30-base pair sequence for overlapping PCR and 3′ primers containing the attB2 site. In the second step, two products from the first reactions were amplified together in an overlap extension PCR reaction, and the resulting product was recombined into pDONR221 in a BP reaction resulting in a potential GA binding domain Entry Clone. The 30-base pair overlap sequence codes for a flexible 12-amino acid linker (L12) and contains *Asc*I and *Fse*I restriction sites for subsequent insertion of additional linker sequences. Primers for amplification are listed in Supplemental Table 1. In the second round of screening, the selected GA binding domain Entry Clones were digested with *Asc*I and *Fse*I (NEB) for

insertion of one of four additional linker sequences as described previously [24]. The pDR FLIP Destination vectors for expression of biosensors in yeast were described previously [24] and are available from AddGene (https://www.addgene.org/Wolf_Frommer/).

Generation of GPS1-NR mutants

The GA binding domain Entry Clone for GPS1 was altered using QuikChange (Agilent) site-directed mutagenesis according to manufacturer's instructions to generate the GPS1-NR mutations. Primers for site-directed mutagenesis of GPS1 to create GPS1-NR are included in Supplemental Table 1.

Expression of sensors in protease deficient yeast

pDR FLIP Destination vectors were recombined in Gateway LR reactions with GA binding domain Entry Clones resulting in yeast expression plasmids coding for various biosensor designs. *Saccharomyces cerevisiae* strain BJ5465 (ATCC 208289 [*MATa ura3-52 trp1 leu2-Δ1 his3-Δ200 pep4::HIS3 prb1-Δ1.6R can1 GAL*] [30]) was transformed with yeast expression plasmids in 96-well format using a lithium acetate transformation protocol [51]. Transformed yeast were plated on synthetic complete (SC) medium with 0.8 % agar (BD) supplemented with 240 mg/L leucine, and 20 mg/L tryptophan (SC agar +Leu,Trp) to select for complementation of uracil auxotrophy by the URA3 marker of the pDR FLIP-based expression clones. Transformants were grown in 2 x 1-ml cultures in SC medium +Leu,Trp in 96-well culture blocks (Greiner) for preliminary fluorescence analysis of sensor

461 expression and for high-throughput screening of sensors in cleared cell lysates. For metal-
462 affinity chromatography purification of sensors, yeast strains expressing sensors were
463 grown in 30-ml cultures in SC medium +Leu,Trp in 50-ml culture tubes.

464 **Fluorescence analysis of cell lysates**

465 Yeast cell cultures (OD_{600} ~1.0) were centrifuged at 5000 g for 7 minutes, washed once in
466 1 mL PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4),
467 transferred to a 96-well culture block (in the case of 30 mL cultures), and centrifuged a
468 second time at 5000 g for 7 minutes. The cell pellets were then frozen at -80 ºC. Frozen
469 cell pellets were resuspended in 1 ml PBS buffer. A 50 µl aliquot was transferred to a clear
470 bottom microtiter plate (Greiner) for analysis of whole cell fluorescence. Cells were then
471 centrifuged at 5000 g for 7 minutes before 700 µl of chilled glass bead slurry [PBS buffer,
472 0.1% Triton X-100 and 50% (v/v) Zirconia/Silica beads (0.5 mm, BioSpec]] were added to
473 each cell pellet. The culture block was then sealed with aluminium foil tape (3M 439 Silver,
474 3-1/4 in width) and vortexed until pellets were resuspended. The block was then loaded
475 into a Retsch MM300 mixer mill, which was run for 15 minutes at a frequency setting of
476 20. Cell lysate was centrifuged at 5000 g for 10 min at 4 ºC, after which 200 µl of
477 supernatant was transferred to a microtiter plate and centrifuged again (5000 g for 5 min)
478 to remove remaining cell debris. The resulting supernatant was then diluted in PBS buffer
479 for use in fluorescence analysis in clear bottom microtiter plates using a fluorometer
480 (Tecan). Samples were diluted in 20 mM MOPS pH 7.4 in order to obtain fluorescence

emission at 480 nm and 530 nm between 5000 and 50000 relative fluorescence units (RFU) with an excitation wavelength of 428 nm and a gain between 70 and 100. For all imaging, bandwidth was set to 12 nm, number of flashes was 10, and integration time was 40 µs. Fluorescence readings were acquired for donor fluorophore (excitation 428 nm, emission 485 nm, abbreviated DxDm), acceptor fluorophore (excitation 500 nm, eYFP emission 535 nm, abbreviated AxAm) and for energy transfer from donor to acceptor (excitation 428 nm, emission 530 nm, abbreviated DxAm). Additionally, a fluorescence emission scan reading from 470 to 550 nm (step size 5 nm) with an excitation wavelength of 428 nm was acquired. Cell lysates from BJ5465 yeast containing an empty vector were used as a negative control for background subtraction.

To analyze sensor response to GA, 100 µl of each sensor sample were combined with 50 µl of 10 µM GA_3 in water (diluted from 10 mM GA_3 in 100% ethanol stock solution) or a mock treatment lacking GA. Replicates were averaged, and a ratio of DxAm/DxDm was calculated as an approximation of FRET ratio. GA-dependent ratio change was calculated as the DxAm/DxDm with GA over DxAm/DxDm mock.

Fluorescence analysis of purified sensors

For purification of the biosensors by metal affinity chromatography, yeast lysates were diluted 1:2 in 20 mM TRIS-HCl, 5 mM imidazole, pH 7.4 and then filtered through a 0.45 µm PES filter and bound to Poly-Prep chromatography columns (Bio-Rad) containing His-bind resin (Novagen). Columns were then washed twice with 20 mM Tris-HCl, 5 mM

501 imidazole, pH 7.4 and eluted in 20 mM TRIS-HCl, 200 mM imidazole, pH 7.4. Alternatively,
502 lysates were diluted 1:2 in 50 mM MOPS, 10 mM imidazole, pH 7.4 and then filtered
503 through a 0.45 µm PES filter and bound to Poly-Prep chromatography columns (Bio-Rad)
504 containing His-Pur Cobalt resin (Pierce). Columns were then washed twice with 50 mM
505 MOPS, 10 mM imidazole, pH 7.4 and eluted in 50 mM MOPS, 150 mM imidazole, pH 7.4.
506 In all cases, samples were diluted in 50 mM MOPS pH 7.4 and fluorescence was analyzed
507 on a Safire fluorometer (Tecan) as described for cleared cell lysates or a SpectraMax
508 fluorometer (Molecular Biosciences). Determination of the apparent K_d of GPS1 for
509 various GAs was performed as described previously [52]. All chemicals were from Sigma
510 with the exception of the GAs, which were from OlChemIm. Data are reported as means
511 and standard deviations of 3-4 replicates, and each experiment was performed at least
512 three times with similar results. The reversibility of binding of GAs by GPS1 protein was
513 tested using Zeba™ Spin Desalting Columns, 0.5 ml, 40K MWCO (Thermo Scientific). The
514 purified GPS1 protein was pre-treated with 0 µM GA, 0.1 µM GA_4, 1 µM GA_3, or 1 µM GA_1.
515 After two 20 minute buffer exchange steps with 50 mM MOPS pH 7.4 using Zeba Spin
516 columns according to manufacturer's instructions, the eluted GPS1 in 50 mM MOPS pH
517 7.4 was treated 0 µM GA and 0.1 µM GA_4 and fluorescence was analyzed on a SpectraMax
518 fluorometer. The donor fluorophore was excited at a wavelength of 430nm and the
519 fluorescence emission scan from 460 to 560 nm (step size 5 nm) was acquired. The

emission ratio was subsequently calculated dividing the mean value across the 530-535 nm range by that across the 480-485 nm range.

Expression of GPS1 *in planta*

The p16 promoter [33] from the AT3G60245 gene encoding a 16S ribosomal subunit was used to drive the nuclear-localized GPS1 fusion biosensor in plants. The following construct was inserted into the multiple cloning site of the p16-Kan vector [33]: 5′ – a sequence coding for the SV40 derived nuclear localization signal LQPKKKRKVGG [53]; a sequence coding for the enhanced dimer variant of Aphrodite with a nine amino-acid C-terminal truncation (edAFPt9); a Gateway cassette including attR1, Chloramphenicol resistance gene, ccdB terminator gene, and attR2; a sequence coding for the enhanced dimer variant of Cerulean (edCer); and a sequence coding for the cMyc epitope tag - 3′. The resulting Gateway Destination vector (p16-FLIPnls43) was then recombined in Gateway LR reactions with GA binding domain Entry Clones resulting in GPS1 and GPS1 – NR expression clones. The map and sequence of p16-nlsGPS1 and p16-nlsGPS1-NR are included in Supplemental File 1.

Transgenic plant lines expressing GPS1

Transgenic plant lines were generated using the *Agrobacterium* floral dip method as described previously [22]. Transformants were selected on agar plates containing ½ × MS medium with Kanamycin. Fluorescence expression of transformants on agar plates was

analyzed using a FluorChem Q imager (Alpha Innotech) with CY2 excitation and emission and the following settings: 12 second exposure time, normal speed, ultra-resolution and level 2 noise reduction. All GPS1 plant lines are described in Supplemental Table 1 including GPS1 lines in the following mutant backgrounds sourced from ABRC (CS6224 - *phyA, phyB*, CS66049 – *pif1, pif3, pif4, pif5*, CS6944 – *ga3ox1, ga3ox2*). pRGA::GFP-RGA in Col0 (NASC ID – N16360) is described here [35].

Phenotypic characterization of plant lines expressing GPS1

For hypocotyl and root length assays, plant lines were grown vertically on ½ × MS agar medium [½ × MS salts (PhytoTechnology Lab), 0.8 % Agar (BD), 0.05% (w/v) MES pH 5.7]. Plates were stratified for 2 days at 4 ºC, after which they were exposed to 30 h of light and then transferred to a dark growth chamber (24 h dark, 22 ºC, 70% RH). For experiments, 10 mM GA_4 (Olchemim) and 10 mM Paclobutazol (PBZ, Sigma-Aldrich) stock solutions were prepared in 70% ethanol. Germinated seeds were transferred to ½ × MS agar medium supplied with PBZ (0 µM, 1µM and 10µM) and GA_4 (0 µM, 0.01µM and 10µM). Plates were imaged after 5 days, and hypocotyl and root lengths were measured using FIJI (http://fiji.sc/). Each experiment included ~15 seedlings per genotype and was repeated three independent times with similar results. Homozygous biosensor expressing lines were compared with isogenic wild-type lines segregated from heterozygous T1 parents.

Seedling Growth, RootChip16 Device Preparation, and Root Perfusion

559 The RootChip16 device was used as described in detail previously [23, 54]. Briefly, the
560 device was sterilized by UV light exposure and immersed in liquid growth media [¼ × MS
561 salts, 0.025% (w/v) MES pH 5.7 supplemented with B vitamins at final concentrations of
562 niacin 2.3 µM, thiamine 1.5 µM, pyridoxin 1.2 µM and myo-inositol 0.3 µM (Sigma)], for
563 removal of air in the root observation chambers by application of suction at the solution
564 outlets using a microliter pipette. Arabidopsis seedlings had been germinated on 5-mm
565 long portion of 10 µl pipette tips filled with solidified growth medium [½ × MS salts, 1%
566 Agar, 0.05% (w/v) MES pH 5.7 supplemented with B vitamins at final concentrations of
567 niacin 4.6 µM, thiamine 3.0 µM, pyridoxin 2.4 µM and myo-inositol 0.6 µM). At 5 days, the
568 seedlings were placed onto the chip by plugging the tips into the root inlets. The chip
569 was incubated for 24 h under standard long-day growth conditions (16 h light/8 h dark
570 cycling, 22 ºC, 70% RH) to allow roots to grow into the observation chambers. Before
571 imaging, the RootChip16 was inserted from below into the central aperture of the carrier
572 and fixed with tape and pressure lines, for control over micromechanical valves, and
573 connected to solution lines. To actuate the push-up valves on the chip, a closing pressure
574 of 15 p.s.i (1.03 bar) was applied to the water-filled control lines. Solution vials were
575 pressurized with 5-10 p.s.i. (0.34 bar) to drive perfusion solutions through the flow lines of
576 the device.

577 ***In planta* analysis of GPS1 using the RootChip16**

The RootChip16 was constantly perfused with ¼ × MS medium (¼ × MS salts, 0.025% MES pH 5.7). For experiments, 10 mM GA_4 stock solutions were prepared in 100% ethanol and diluted in ¼ × MS medium. Vials with solutions were connected to root chip flow lines and pressurized. Perfusion of solutions was controlled by the RootChip16 control valves [23, 54] connected to a valve controller controlled by a LabView (National Instruments) program.

Fluorescence microscopy

Arabidopsis plants were grown vertically on ½ × MS agar medium (½ × MS salts, 0.8 % Agar, 0.05% MES pH 5.7) plates. Plates were stratified for 2 days at +4 °C in the dark prior to being placed in a growth chamber with long-day growth conditions (16 h light/8 h dark cycling, temperature cycling 22 °C day/18 °C night, 70% RH) or darkness (accomplished by wrapping agar plates in foil) for the indicated number of days post sowing. Dark-grown seedlings were additionally exposed to 3 – 6 hours of light before wrapping in foil. *ga3ox1, ga3ox2* mutant seedlings were additionally treated with 1 µM GA_4 during stratification followed by washing with water and plating. All light-grown seedling experiments were initiated between 4 – 10 hours after the onset of the light period. Seedlings were placed in solution containing ¼ × MS medium (¼ × MS salts, 0.025% MES pH 5.7) and prepared for imaging on either glass slides or the RootChip16. For GA and cycloheximide treatments on glass slides, seedlings were placed on glass slides with 50 µL of solution and surrounded with a rectangle of vacuum grease and

598 covered with a square cover slip equal in height and half the width of the vacuum grease
599 rectangle. The GA treatment solution could then be exchanged beneath the coverslip by
600 addition to the left and removal from the right side of the coverslip. Images were
601 acquired before and 20 minutes after exchange of the solution around the seedlings.

602 Confocal images were acquired on a Leica SP8-FLIMan using a 20x dry 0.70 HC PLAN APO
603 objective. To excite CFP and YFP, 448 nm and 514 nm lasers were used, respectively. The
604 552 nm laser line was used to excite propidium iodide (PI). The 448 nm laser line set to
605 10% for GFP excitation. For root imaging with PI (10 mg/L working solution), a 25x 0.95
606 NA water objective was used. Fluorescence emission was detected by HyD SMD detectors,
607 set to detect 460 to 500 nm for CFP, 525 to 560 nm for YFP, 590 to 635 nm for propidium
608 iodide and 500 to 535nm for GFP. The laser power was set between 0.5-2% with detector
609 gain set to 110 to image CFP or YFP. A line average of four was used for the majority of
610 experiments, except for imaging nlsGPS1-NR, where a line average of 8 was used to
611 improve the signal-to-noise ratio. The z-stacks were acquired with a step-size of 1-2 μm
612 depending upon the experiment.

613 RootChip imaging was performed using a 20x 0.70 HC PL APO objective on an inverted
614 spinning disk confocal; 442 nm and 514 nm lasers were used for excitation of CFP and
615 YFP, respectively. Emission filters were 458-482 nm for CFP and 520-550 nm for YFP.
616 Fluorescence emission was detected by Andor iXon camera. A typical acquisition used an
617 intensification setting of 300, a gain setting of 2, a 400 ms exposure for DxDm and DxAm

618 or 100 ms exposure for AxAm, a Z-stack of three 5 µm steps, and imaging intervals of 5
619 minutes.

620 **Image Processing and Analysis**

621 For confocal images acquired on glass slides, image processing and analysis were
622 performed by using IMARIS 8.3.1 (Bitplane). Surfaces were segmented based on YFP
623 channel using the 'surfaces wizard' and the following settings: background subtraction
624 (local contrast) set to 3 µm, thresholding set as default. For the cycloheximide assay and
625 pRGA::GFP-RGA distribution, surfaces were false-colored according to the mean of the
626 masked YFP or GFP channel, respectively. The ratio channel (DxAm/DxDm) was calculated
627 by using the IMARIS Xtension 'XT *Mean Intensity Ratio*', which computes a ratio between
628 existing mean intensity statistics in IMARIS using Matlab. The extension computes and
629 stores the ratio of the intensity means of individual surfaces in the provided channels. For
630 Root-Chip experiments, the ratio channel (DxAm/DxDm) was calculated by using the
631 Matlab extension '*Channel Arithmetics*'.

632 For confocal images acquired using the RootChip16, image processing and analysis were
633 performed using deprecated 3-D stitching (FIJI Macro by Stephan Preibisch, http://fly.mpi-
634 cbg.de/~preibisch/contact.html) of the 4 root positions followed by 4-D analysis using
635 IMARIS 8.3.1 (BitPlane).

In this work, we presented data using beeswarm and box plot of raw data. In the beeswarm and box plot graphs, the central rectangle spans the first quartile to the third quartile, while the line inside the rectangle shows the median. The whiskers denote 1.5 interquartile ranges from the box and outlying values plotted beyond the whiskers. All the statistical analyses were performed using OriginPro (http://www.originlab.com/Origin).

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contributions

AR contributed experimentation, analysis of results, and writing of the manuscript; AW contributed experimentation and analysis of results; VL contributed experimentation and analysis of results; WBF contributed project design, project supervision, and revision of the manuscript; AMJ contributed project design, project supervision, experimentation, analysis of results, and writing of the manuscript.

Corresponding authors

AMJ: alexander.jones@slcu.cam.ac.uk, WBF: frommew@hhu.de

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657

658 **Figure legends**

659 **Figure 1**. Engineering and GA response of GPS1 and GPS1-NR. (a) Representation of
660 variant domains included in potential FRET biosensors for GA: GA binding domain variants
661 (GA binding domain I and II connected through linker variants) were fused via attB1 and
662 attB2 linkers to fluorescent protein FRET pairs (variant donor and acceptor fluorescent
663 proteins). (b) Structural model of GPS1 bound to GA_4. The GID1C protein (green), GAI
664 truncation (purple), and GA_4 (magenta) representations are from a published structure of
665 an AtGID1A, AtGAI truncation, GA_4 ternary complex (PDB 2ZSI [31]). The edAphrodite
666 (yellow) representation is from a published structure of Venus (PDB 1MYW, [55]) and the
667 edCerulean representation is from a published structure of Cerulean (PDB 2WSO, [56]).
668 The representations of three linkers (translated attB1, L12 and translated attB2) were built
669 in PyMol software. (c) Fluorescence emission curve of purified GPS1 protein with and
670 without GA_4 treatment. (d) The GPS1 residues of the GAI and GID1 domains mutagenized
671 to make GPS1-NR construct are highlighted in red and blue, respectively. (E) Fluorescence
672 emission ratio of purified GPS1, GPS1-S114A/F115A, GPS1-E51K/E54R, and GPS1-NR
673 proteins with and without GA_4 treatment. (c and e) (Student's t-test **** p-value < 0.0001,
674 *** p-value< 0.001). Means and standard deviations of three replicates are presented.
675 Complete experiments were repeated at least three times with similar results.

676

Figure 2. Fluorescence emission ratio response of purified GPS1 to GAs. (a) GPS1 fluorescence response to increasing concentrations of bioactive GAs (GA_4, GA_1, or GA_3). (b) Percent maximal ratio change of GPS1 in response to 200 nM GAs. (c) GPS1 emission ratio response to a second GA treatment (0.1 μM GA_4) after a prior GA treatment and 2x buffer exchange. Means and standard deviations of three replicates are presented. Complete experiments were repeated at least three times with similar results. (d) Hypocotyl length of two nlsGPS1 lines were compared with wild-type Col0 siblings (isogenic) with and without paclobutrazol (PBZ, 1 μM and 10 μM) and GA_4 (0.01 μM and 10 μM) treatments. Student's t-test, **** p-value < 0.0001, *** p-value < 0.001. At 10 μM PBZ and 0 μM GA_4: solid lines Cohen's d = 5.92, dashed lines Cohen's d = 6.04. At 10 μM PBZ and 0 μM GA_4 : solid lines Cohen's d = 6.50, dashed lines Cohen's d = 7.31. Means and standard deviations of three replicates are presented. Complete experiments were repeated at least three times with similar results.

Figure 3. Emission ratio of nlsGPS1 in root tips. (a) 3D images of nlsGPS1 or nlsGPS1-NR emission ratios of roots 3 days post sowing in Wild-type Col0 or *ga3ox1, ga3ox2* mutant backgrounds. Two independent lines of each genotype are also presented in Figure S3. (b) Beeswarm and box plot of nlsGPS1 or nlsGPS1-NR emission ratios for nuclei of late division zone (DZ, 100 - 200 μm from quiescent center) early elongation zone (eEZ, 200 - 400 μm from quiescent center) and late elongation zone (lEZ, 400 - 800 μm from

697 quiescent center). Zones indicated graphically in the example image at right (n > 68 nuclei
698 from 3 independent seedlings for each genotype and root zone). For Col0 nlsGPS1, eEZ vs
699 DZ Kruskal Wallis test **** p-value< 0.0001, Cohen's *d* = 1.83 and lEZ vs eEZ Kruskal
700 Wallis test **** p-value< 0.0001, Cohen's *d* = 1.16. For Col0 nlsGPS1-NR, eEZ vs DZ
701 Kruskal Wallis test **** p-value< 0.0001, Cohen's *d* = 0.60 and lEZ vs eEZ (Mann-Whitney
702 U test test p-value = 0.41, Cohen's *d* = 0.13. For nlsGPS1 *ga3ox1, ga3ox2*, eEZ vs DZ DZ
703 Kruskal Wallis test **** p-value< 0.0001, Cohen's *d* = 0.73 and lEZ vs eEZ Kruskal Wallis
704 test **** p-value< 0.0001, Cohen's *d* = 0.84. (c) Longitudinal slice of nlsGPS1 in a Col0
705 root tip co-imaged with propidium iodide (PI) staining of cell outlines. (d) Orthogonal
706 projection of the longitudinal slice from (c). Complete experiments were repeated at least
707 three times with similar results.
708
709 **Figure 4.** Emission ratio of nlsGPS1 in roots before and after treatment with GAs. (a and c)
710 3D images of nlsGPS1 roots before and after GA_4, GA_3 and GA_1 treatment. (b and d)
711 Beeswarm and box plot of nlsGPS1 emission ratios for nuclei of late elongation zone, 400
712 - 600 μm from quiescent center (region demarcated with frame in (a and c), n > 24
713 nuclei). nlsGPS1 emission ratio was statistically different before and after GA_4 (Student's *t*-
714 test, **** p-value < 0.0001, Cohen's *d* = 3.38). (e) Still frames from Movie 1 at zero and
715 twenty minutes post-GA_4 treatment. Seedlings were grown in the RootChip16. (f) nlsGPS1
716 emission ratios for individual nuclei before (gray) and after (blue) GA_4 treatment in

717 relation to distance from the root tip (µm from bottom). Complete experiments were
718 repeated at least three times with similar results.
719
720 **Figure 5.** Emission ratio of nlsGPS1 in light-grown hypocotyls. (a) 3D images of nlsGPS1
721 or nlsGPS1-NR emission ratios in hypocotyls 3 days post sowing (upper panels). Enlarged
722 view of central region of hypocotyls, 250 - 450 µm from shoot apical meristem (lower
723 panels, corresponds to frames in upper panels). Wild-type Col0 was compared with
724 *ga3ox1, ga3ox2* mutant. Two independent lines of each genotype are also presented in
725 Figure S8. (b) Beeswarm and box plot of nlsGPS1 emission ratios for nuclei of central
726 region (n > 261 nuclei from 3 independent seedlings for each genotype). nlsGPS1
727 emission ratios were statistically different in *ga3ox1, ga3ox2* compared to Col0 as were
728 nlsGPS1–NR emission ratios compared to nlsGPS1 (Kruskal-Wallis test **** p-value<
729 0.0001, for nlsGPS1 vs nlsGPS1-NR Cohen's d = 3.83, for nlsGPS1 vs *ga3ox1, ga3ox2*
730 mutant Cohen's d = 4.41). Complete experiments were repeated at least three times with
731 similar results.
732
733 **Figure 6.** Relationship between cell size and nlsGPS1 emission ratio. (a) nlsGPS1 emission
734 ratios and propidium iodide-labelled (PI) cell outlines in light and dark grown Col0
735 hypocotyl epidermal cells. Scale bar = 30 µm. Complete experiments were repeated at
736 least three times with similar results. (b) Scatter plot showing nlsGPS1 emission ratio and

737 2D cell section area for individual epidermal cells. For light-grown hypocotyls, correlation
738 coefficient r = -0.09; n = 214 cells from 6 hypocotyls. For dark-grown hypocotyls,
739 correlation coefficient r = 0.64; n = 106 cells from 6 hypocotyls. (c) 3D images of nlsGPS1
740 or nlsGPS1-NR emission ratios of light-grown hypocotyls 3 days post sowing. Wild-type
741 Ler was compared with *phyA, phyB* mutant. Two independent lines of each genotype are
742 also presented in Figure S10. (d) Beeswarm and box plot of nlsGPS1 emission ratios for
743 nuclei of central region of hypocotyls, 250 - 450 µm from shoot apical meristem (n > 90
744 nuclei from 3 independent seedlings for each genotype); frames demarcating central
745 region are shown. nlsGPS1 emission ratios were statistically different in *phyA, phyB*
746 compared to Ler (Mann-Whitney U test **** p-value = 0.0001, Cohen's *d* = 0.42). (e) 3D
747 images of dark-grown hypocotyls 3 days post sowing of nlsGPS1 or nlsGPS1-NR. Wild-
748 type Col0 was compared with *ga3ox1, ga3ox2* and *pifq* mutants. Two independent lines of
749 each genotype are also presented in Figure S10. (f) Beeswarm and box plot of nlsGPS1
750 emission ratios for nuclei of central region of hypocotyls, 400 – 600 µm from shoot apical
751 meristem (n >100 nuclei from 3 independent seedlings for each genotype); example
752 frames demarcating central region are shown (e). nlsGPS1 emission ratios were statistically
753 different in *ga3ox1, ga3ox2* and *pifq* compared to Col0 as were nlsGPS1–NR emission
754 ratios compared to nlsGPS1 (Kruskal-Wallis test **** p-value< 0.0001, for nlsGPS1 vs *pifq*
755 mutant Cohen's *d* = 1.06, for nlsGPS1 vs nlsGPS1 Cohen's *d* = 4.48, for nlsGPS1 vs

ga3ox1, ga3ox2 mutant Cohen's *d* = 4.40). Complete experiments were repeated at least three times with similar results.

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