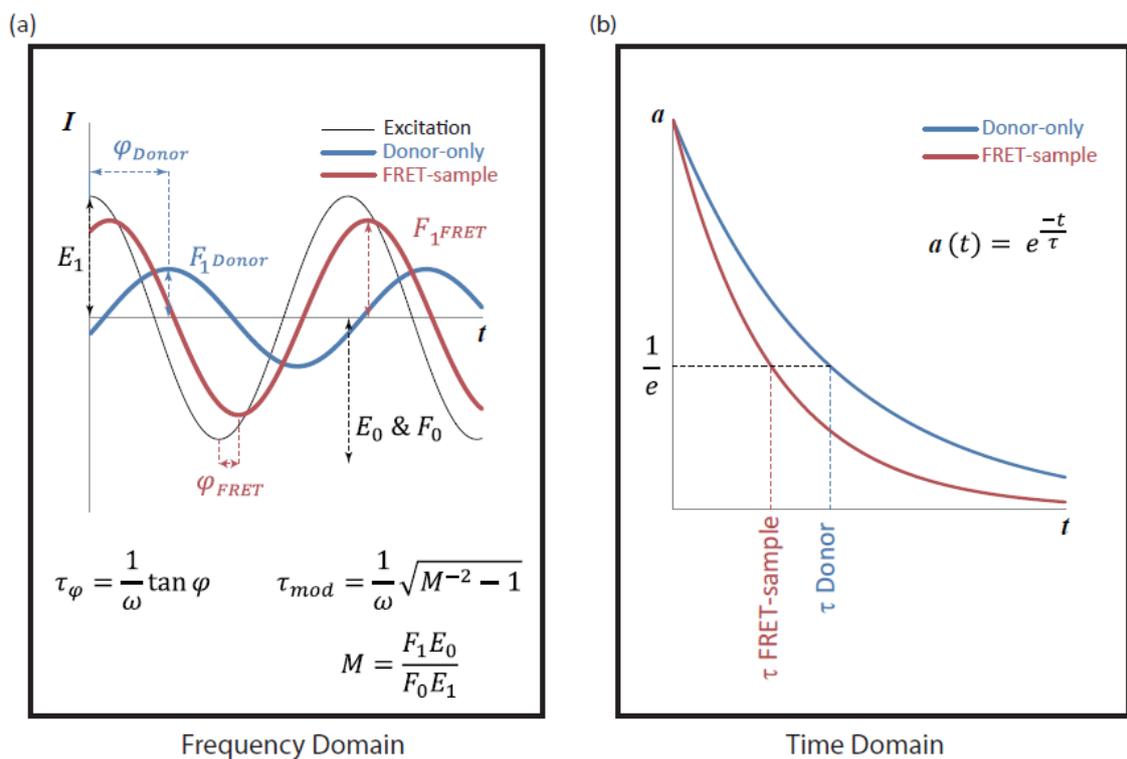


**Supplementary Figure 1: Diagram of *Arabidopsis thaliana* root meristem.**

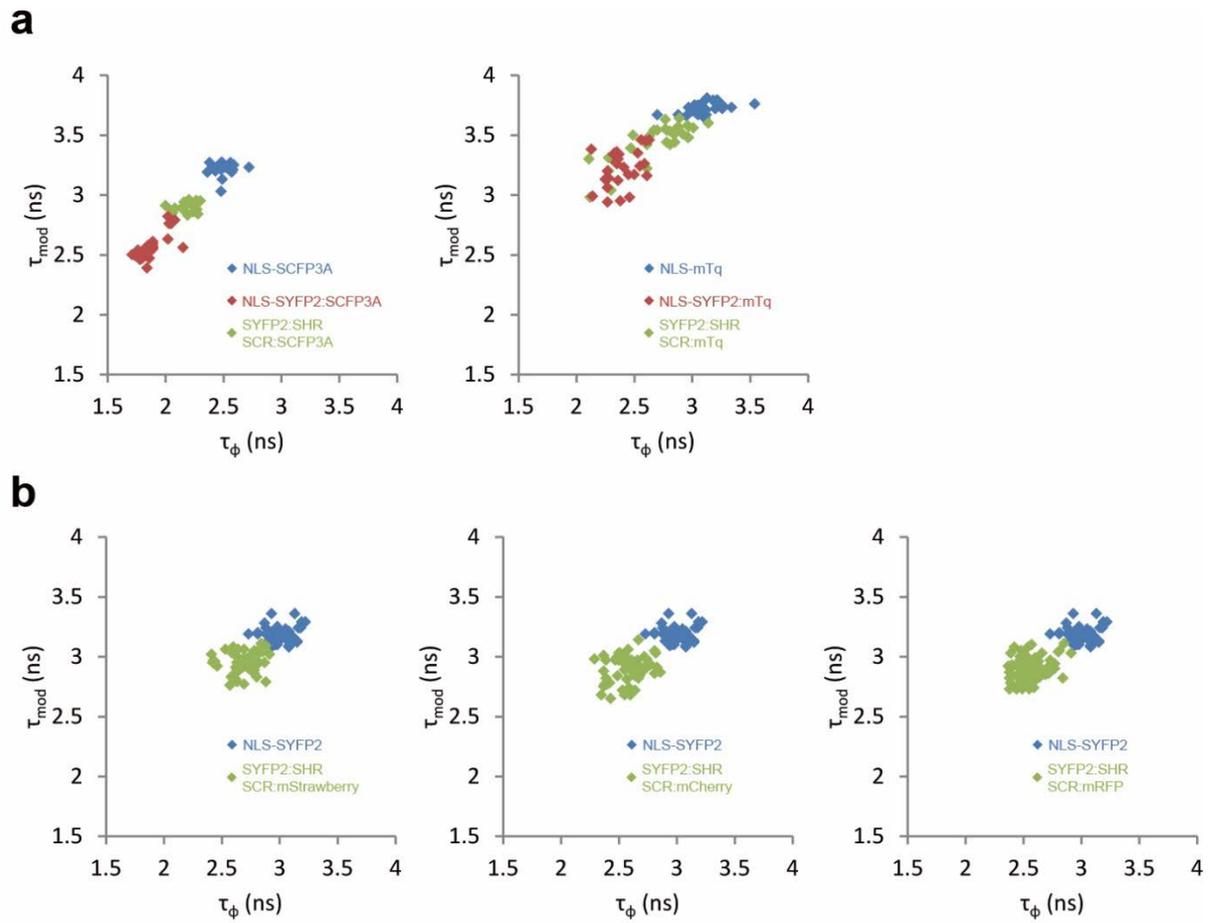
*Arabidopsis thaliana* root meristem marked with different cell types. QC, quiescent center; CEI, cortex-endodermal initial. Right half panel summarizes SHR and SCR localization.



### Supplementary Figure 2

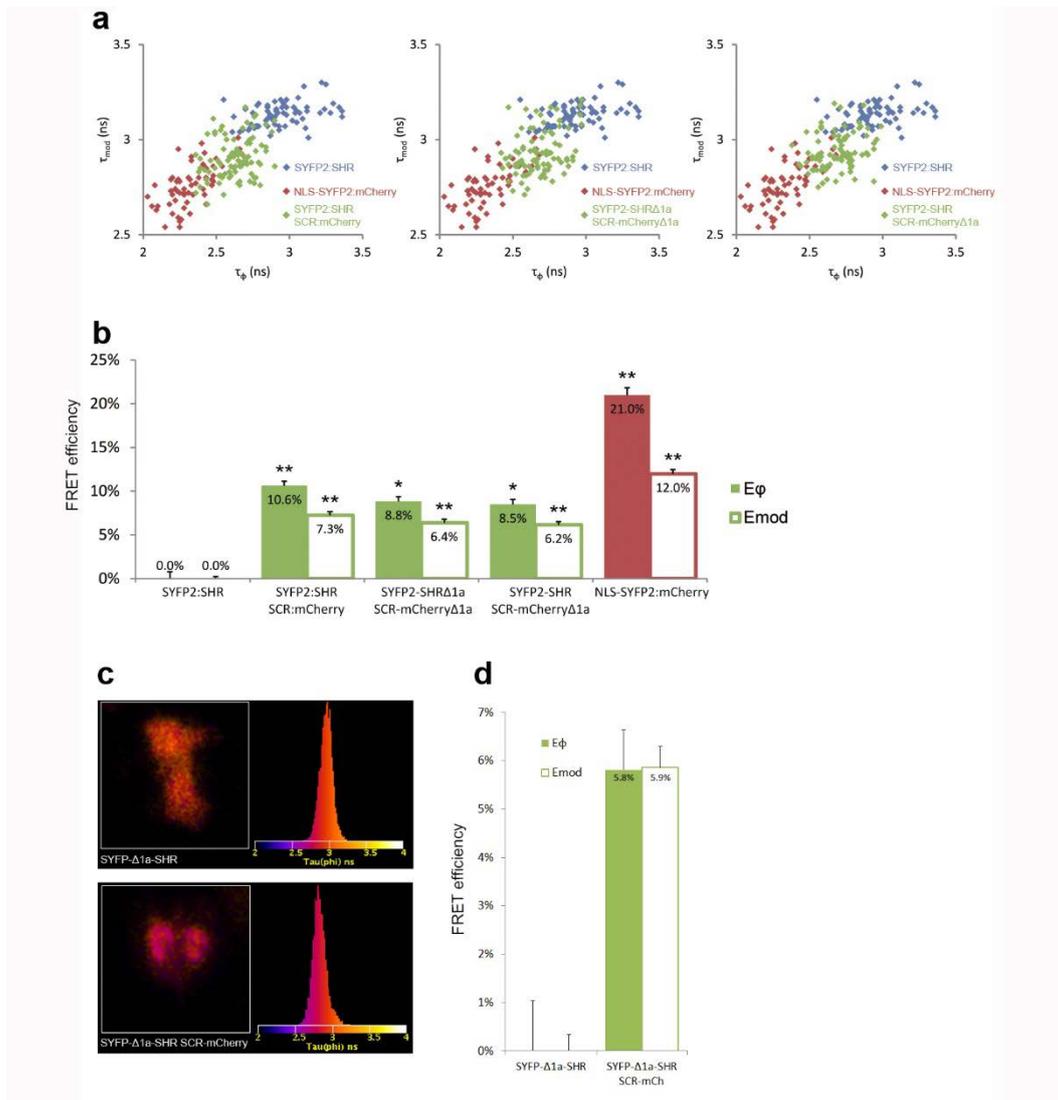
Schematic representations of the time domain and frequency domain for fluorescence lifetimes determination.

(a) Frequency-domain FLIM resolves lifetime from the phase shift and modulation of the fluorescence emission.  $I$ , fluorescence intensity (co-aligned);  $\varphi$ , phase shift;  $\omega$ , laser angular frequency,  $M$ , modulation ratio;  $E$ , Excitation;  $F$ , Emission. In the frequency domain the life times are represented by two values, ( $\tau_{\varphi}$ ) derived from the phase angle ( $\varphi_{\omega}$ ) and ( $\tau_{mod}$ ) determined from the modulation. The  $\tau_{\varphi}$  and  $\tau_{mod}$  values are usually strongly correlated and each value represents a different weighted average of the decay times displayed by the sample. (b) Time-domain FLIM relies on single-photon counting, molecules are excited by a very short pulse. The fluorescence life time lifetime is based on the exponential decay of fluorescence signal measured by Time-Correlated Single Photon Counting (TCSPC).  $a$ , normalized photon count;  $t$ , time;  $\tau$ , lifetime;



**Supplementary Figure 3: Optimization of tags-of-choice for FRET-FLIM between SHR and SCR.**

(a) Scatterplots showing distribution of phase lifetime  $\tau_\phi$  against modulation lifetime  $\tau_{mod}$  from protoplast measurements between SYFP2:SHR and SCR:SCFP3A or SCR:mTq. (b) Scatterplots of fluorescence lifetime measurements between SYFP2:SHR and SCR:mStrawberry, SCR:mCherry or SCR:mRFP in protoplasts.



**Supplementary Figure 4: Optimization of linker composition for FRET-FLIM between SHR and SCR.**

(a) Scatterplot of fluorescence lifetime measurements between SHR and SCR with different linker composition in protoplasts. SYFP2-SHRΔ1a has DKVA linker, SCR-mCherryΔ1a has NKVA linker, while SYFP2-SHR has no linker between SYFP2 and SHR. (b) Bar chart of FRET efficiency  $E$  derived from (a), with error bars of standard error of mean. \*,  $10^{-20} < p < 10^{-2}$ ; \*\*,  $p < 10^{-20}$ . (c) Fluorescence lifetime heatmaps and histograms of SYFP-Δ1a-SHR in donor-only or FRET sample HeLa cells. (d) Bar chart showing FRET efficiency  $E$  derived from  $\tau_\phi$  and  $\tau_{mod}$  in (c), error bars represent standard errors within one set of experiment,  $n > 10$ .