Table S2

Table S2. LC-MS/MS parameters for DDA analyses (for generation of spectral libraries and quantification by MS1 feature detection (MaxLFQ)).

	LHCSR3 phosphosite mutants				High light kinetics				
	Trypsin digested samples		LysC digested samples		200 µmol photons m^{-2} s^{-1}		500 µmol photons m^{-2} s^{-1}		
	non-enriched	enriched	non-enriche	enriched	non-enriched	enriched	non-enriched	enriched	
Eluent compositions	Peptide trapping: 0.05% trifluoroacetic acid (TFA) in ultrapure water (A1), 0.05% TFA in 80% acetonitrile (B1) Peptide separation: 0.1% formic acid (FA) in ultrapure water (A2), 0.1% FA in 80% acetonitrile (B2)								LC parameters
Trap Column	C18 PepMap 100, 300 µM x 5 mm, 5 µm particle size, 100 Å pore size; Thermo Scientific								
Peptide trapping (eluents A1+B1)	2.5% B1 at 10 µl/min for 10 min				2.5% B1 at 10 µl/min for 5 min				
Flow rate	300 nl/min								
Gradient for peptide separation	2.5-30% B2 over 240 min, 30-50% B2 over 60 min, 50%-99% B2 over 5 min 99% B2 over 15 min				2.5-18% B2 over 105 min, 18%-32% B2 over 55 min, 32%-99% B2 over 5 min, 99% B over 20 min		2.5-18% B2 over 65 min, 18%-35% B2 over 40 min, 35%-99% B2 over 5 min, 99% B2 over 20 min		
Separation column	Acclaim PepMap C18, 75 µm x 50 cm, 2 µm particle size, 100 Å pore size; Thermo Scientific								
Resolution at *m/z* 200 (FWHM)	70,000								MS1 settings
Chromatographic peak width	30 s				20 s	30 s	20 s	30s	
AGC target	1e6								
Maximum injection time	50 ms	100 ms	50 ms		30 ms	50 ms	50 ms	100 ms	
Scan range	375-1600 *m/z*				350-1600 *m/z*		350-1500 *m/z*		
TopN	12								MS2 settings
Resolution at *m/z* 200 (FWHM)	17,500								
Isolation window	1.5 *m/z*								
AGC target	5e4		1e5		5e4				
Maximum injection time	55 ms	125 ms	55 ms		55 ms	110 ms	55 ms	125 ms	
Normalized collision energy (NCE)	27								
Minimum AGC target	2e2	1.25e3	5e2		5.5e2	1e3	5.5e2	1.25e3	
Intensity threshold	3.6e3	1e4	9.1e3		1e4	9.1e3	1e4		
Charge exclusion	unassigned,1, >7				unassigned,1, >4		unassigned,1, >5		
Dynamic exclusion	60 s				auto		auto	30 s	