

Table S3

Table S3. LC-MS/MS parameters for PRM analyses. *, Single Ion Monitoring (SIM) data was acquired in parallel to PRM data, but was excluded from further analyses.

| | LHCSR3 phosphosite mutants* | | High light kinetics | |
|---|---|--|--|--|
| | Trypsin digested samples | LysC digested samples | 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ | 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ |
| 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) | | | |
| Trap Column | C18 PepMap 100, 300 μm x 5 mm, 5 μm particle size, 100 \AA pore size; Thermo Scientific | | | |
| Peptide trapping (eluent A1+B1) | 2.5% B1 at 10 $\mu\text{l/min}$ for 10 min | | 2.5% B1 at 10 $\mu\text{l/min}$ for 5 min | |
| Separation Column | Acclaim PepMap C18, 75 μm x 50 cm, 2 μm particle size, 100 \AA pore size; Thermo Scientific | Acclaim PepMap C18, 75 μm x 15 cm, 2 μm particle size, 100 \AA pore size; Thermo Scientific | Acclaim PepMap C18, 75 μm x 50 cm, 2 μm particle size, 100 \AA pore size; Thermo Scientific | Acclaim PepMap C18, 75 μm x 50 cm, 2 μm particle size, 100 \AA pore size; Thermo Scientific |
| Gradient for peptide separation (eluent A2+B2) | 2.5-30% B2 over 240 min, 30-50% B2 over 60 min, 50%-99% B2 over 5 min 99% B2 over 15 min | 2.5-15% B2 over 47 min, 15%-34% B2 over 33 min, 34%-99% B2 over 5 min, 99% B over 5 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 | 2.5-18% B2 over 65 min, 18%-35% B2 over 40 min, 35%-99% B2 over 5 min, 99% B2 over 20 min |
| Flow rate | 300 nl/min | | | |
| Chromatographic peak width | 15 s | | | |
| Resolution (FWHM) at m/z 200 | 70,000 | 35,000 | 35,000 | |
| AGC target | 1e5 | | 5e4 | |
| Maximum injection time | 480 ms | 240 ms | 240 ms | |
| Isolation window | 2 m/z | 3 m/z | 2 m/z | |
| Isolation offset | 0 m/z | 1 m/z | 0.5 m/z | |
| Normalized collision energy (NCE) | 27 | | | |

LC parameters

MS settings (PRM)