Reporter assays

Dual Luciferase assay

- Cells were seeded in white (clear bottom) 96-well plate and after 24 h transfected with selected plasmids.
- 24 h after transfection cells were induced with appropriate inducers.
- Before dual Luciferase assay medium was removed and cells were lysed with 25 µL of 1x Passive lysis buffer (Promega).
- Luminescence of reporter firefly luciferase was measured with Orion (Berthold Technologies) using Luciferase buffer with luciferin as a substrate. For normalization, activity of Renilla luciferase was used. Renilla luciferase was measured using Renilla buffer supplemented with coelenterazine.

Real time measurements of light inducible systems:

- 24 h after transfection the medium was exchanged for medium with the final luciferin concentration of 1 mM.
- After 30 minutes in the dark, cells were induced with with blue light (455 nm). Plates were put in Orion (Berthold Technologies) and measured continuously for appropriate amounts of time, after which the medium was removed and the cells were lysed with 25 µL of 1x Passive lysis buffer (Promega). For normalization, activity of Renilla luciferase was used. Renilla luciferase was measured using Renilla buffer supplemented with coelenterazine.

Real time measurements of Ca-sensor:

- 24 h after transfection the medium was exchanged for medium with 1 mM luciferin and 4 mM CaCl2.
- After 3 hours cells were induced with 10 µM calcium ionophore (A23187).
- Plates were put in Orion (Berthold Technologies) and measured continuously for appropriate amounts of time, after which the medium was removed and the cells were lysed with 25 µL of 1x Passive lysis buffer (Promega).
- For normalization, activity of Renilla luciferase was used. Renilla luciferase was measured using Renilla buffer supplemented with coelenterazine.

SEAP assay

- Cells were seeded in 96 well clear plate and after 24 h transfected with selected plasmids .
- 24 h after transfection cells were induced.
- Samples of medium were collected at different time points after induction.

- 25 µL of medium was transferred into clear 96 well plate and 200 uL of QUANTI Blue™ reagent was added into each well.
- Absorbance at 630 nm was measured continuously for 2-3 h at 37°C.