Supplementary Material

Optimised FRET pairs and quantification approaches to detect the activation of Aurora kinase A at mitosis.

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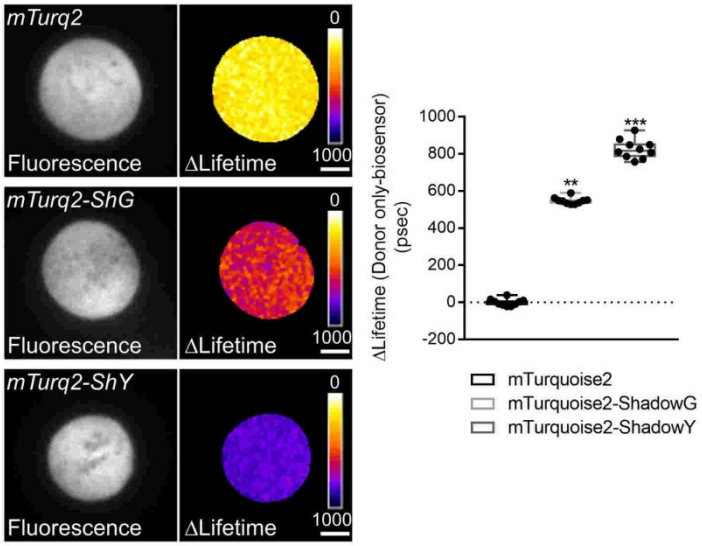


Supplementary Fig. 1. mTurquoise2-ShadowG and ShadowY are efficient donor-acceptor FRET pairs. (Left panels) Representative fluorescence (mTurquoise2 channel) and ΔLifetime (donor only-biosensor) images of U2OS cells expressing the indicated constructs and synchronised at mitosis. ShG: ShadowG; ShY: ShadowY; mTurq2: mTurquoise2. (Right panel). ΔLifetime values for individual cells represented as black dots in each boxplot. The bar in boxplots represents the median; whiskers extend from the 10th to the 90th percentiles. *n*=10 cells per condition of one representative experiment (of three). Scale bar: 10 µm. ***P*<0.01 and ****P*<0.001 against the 'mTurquoise2' condition.

A

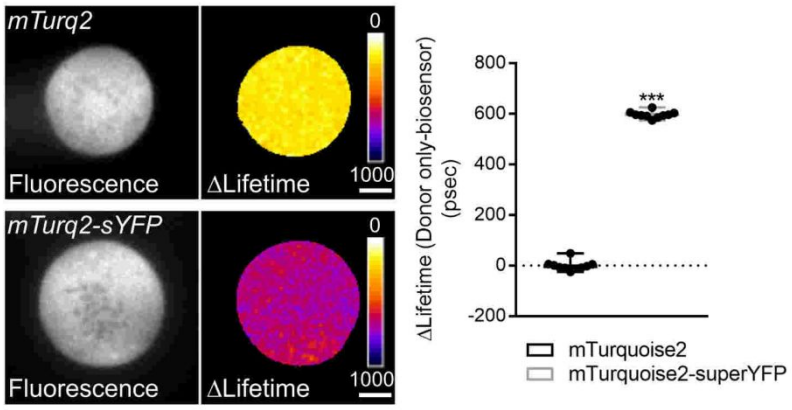
dLanYFP: (https://www.fpbase.org)

MVSK GEEDNMASLPATHELHIFGSFNGVDFDMVGRGTGNPNDGYEELNLK 50
STKGDLQFSPWILVPQIGYGFHQYLPFPDGMSPFQAAMKDGSGYQVHRTM 100
QFEDGASLTSNYRYTYEGSHIKGEFQVKGTGFPADGPVMTNSLTAADWCV 150
TKMLYPNDKTIISTFDWTYTTGNGKRYQSTARTTYTFAKPMAANILKNQP 200
MFVFRKTELKHSKTELNFKEWQKAFTDVM GMDELYK

super YFP:

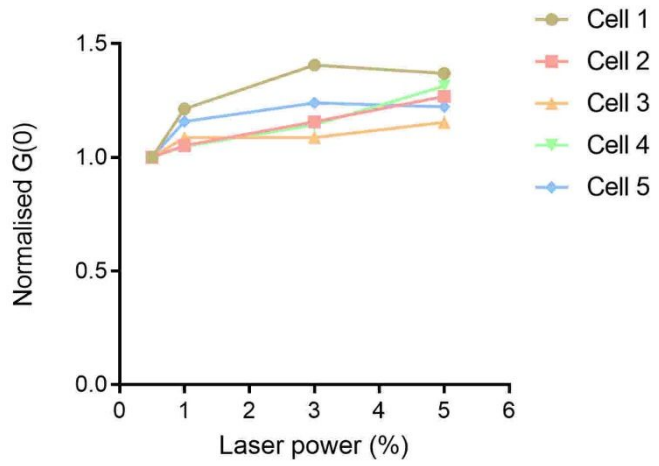
MVSK GEEDNMASLPATHELHIFGSFNGVDFDMVGRGTGNPNDGYEELNLK 50
STKGDLQFSPWILVPQIGYGFHQYLPFPDGMSPFQAAMKDGSGYQVHRTM 100
QFEDGASLTSNYRYTYEGSHIKGEFQVKGTGFPADGPVMTNSLTAADWCV 150
TKMLYPNDKTIISTFDWTYTTGNGKRYQSTARTTYTFAKPMAANILKNQP 200
MFVFRKTELKHSKTELNFKEWQKAFTDVM STGTGSTGSGSS GEEDNMASL 250
PATHELHIFGSFNGVDFDMVGRGTGNPNDGYEELNLKSTKGDLQFSPWIL 300
VPQIGYGFHQYLPFPDGMSPFQAAMKDGSGYQVHRTMQFEDGASLTSNYR 350
YTYEGSHIKGEFQVKGTGFPADGPVMTNSLTAADWCVTKMLYPNDKTIIS 400
TFDWTYTTGNGKRYQSTARTTYTFAKPMAANILKNQPMFVFRKTELKHSK 450
TELNFKEWQKAFTDVM GMDELY

B



Supplementary Fig. 2. superYFP, a dimeric version of dLanYFP, is an efficient FRET acceptor for mTurquoise2. A. Amino acidic sequence of dLanYFP as reported in www.fpbase.org, and of superYFP. superYFP consists of a dimer of dLanYFP, where the two monomers are connected by a 12-amino acidic linker (indicated in red). The linker replaces the last seven amino acids of the first dLanYFP monomer and the first four amino acids of the second one, which are indicated in black in both sequences.

B. (Left panels) Representative fluorescence (mTurquoise2 channel) and ΔLifetime (donor only-biosensor) images of U2OS cells expressing mTurquoise 2 and the mTurquoise2-superYFP tandem, and synchronised at mitosis. sYFP: superYFP; mTurq2: mTurquoise2. (Right panel). ΔLifetime values for individual cells represented as black dots in each boxplot. The bar in boxplots represents the median; whiskers extend from the 10th to the 90th percentiles. *n*=10 cells per condition of one representative experiment (of three). Scale bar: 10 μm. ***P*<0.01 and ****P*<0.001 against the 'mTurquoise2' condition.



Supplementary Fig. 3. The amplitude of superYFP is not altered by increasing the 514 nm laser power. Normalised G(0) values obtained from five independent U2OS cells expressing the superYFP-AURKA-mTurquoise2 FRET biosensor and synchronised at mitosis. Cells were illuminated with the 514 nm laser only at increasing powers, i.e. 0.5%, 1%, 3% and 5%. Measurements were taken in the cytosol; each point represents the average of three independent points per cell at 0.01 msec.