

## Supplementary materials

Add and Go: FRET Acceptor for Live-Cell Measurements Modulated by Externally Provided Ligand

Alexey S. Gavrikov<sup>1</sup>, Nina G. Bozhanova<sup>1</sup>, Mikhail S. Baranov<sup>1,2</sup>, Alexander S. Mishin<sup>1\*</sup>

<sup>1</sup> Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho-Maklay st. 16/10, Moscow, 117997, Russia

<sup>2</sup> Pirogov Russian National Research Medical University, Ostrovitianov 1, Moscow, 117997, Russia

\*correspondence: [mishin@ibch.ru](mailto:mishin@ibch.ru)

### 1. Supplementary materials and methods

#### 1.1 Constructs sequences

>H2B-mNeonGreen-DiB1

MPEPAKSAPAPKKGSKKAVTKAQKKGGKKRKRSRKESYSIYVYKVLKQVHPDTGISS  
KAMGIMNSFVNDIFERIAGEASRLAHYNKRSTITSREIQTAVRLLLPGELAKHAVSEGT  
KAITKYTSKDPVPVATMVSKGEEDNMASLPATHELHIFGSINGVDFDMVGQGTGNPN  
DGYEELNLKSTKGDQLQFSPWILVPHIGYGFHQYLPYPDGMSPFQAAMVDGSGYQVH  
RTMQFEDGASLTVNYRYTYEGSHIKGEAQVKGTGFPADGPVMTNSLTAADWCRSKK  
TYPNDKTIISTFKWSYTTGNGKRYRSTARTTYTFAKPMAANYLKNQPMYVFRKTELK  
HSKTELNFKEWQKAFTDVMGMDELYKGDPPVATMASSPTPPRGVTVVNNFDCKRYL  
GTWYEIARFDHRFERGLEKVTATYSLRDDGGLNVINKGYNPDRGMWQQSEGKAYFT  
GAPTRAALKVSFFGPFYGGYNVIALDREYRHALVCGPDRDYLWINSRTPPTISDEVKE  
MLAVATREGFDVSKFIWVQQPGSGS

>Vimentin-DiB1

MSTRSVSSSSYRRMFGGPGTASRPSSSSRSYVTTSTRTYSLGSALRPSTSRSLYASSPGG  
VYATRSSAVRLRSSVPGVRLLQDSVDFSLADAINTEFKNTRTNEKVELQELNDRFANY  
IDKVRFLQKQNKILLAELEQLKGQGSRLGDLYEEEMRELRRQVDQLTNDKARVEVE  
RDNLAEDIMRLREKLQEEMLQREEAENTLQSFQDQVDNASLARLDLERKVESLQEEI  
AFLKKLHEEEIQELQAQIQEQHVQIDVDVSKPDLTAALRDVRQQYESVAAKNLQEA  
EWYKSKFADLSEAANRNNDALRQAKQESTEYRRQVQSLTCEVDALKGTNESLERQ  
MREMEENFAVEAANYQDTIGRLQDEIQNMKEEMARHLREYQDLLNVKMALDIEIAT  
YRKLLEGEESRISLPLPNFSSLNLRETNLDSLPLVDTHSKRTLTIKTIVETRDGQVINETS  
QHHDDELDGPPVATGMASSTPPRGVTVVNNFDCKRYLGTWYEIARFDHRFERGLE  
KVTATYSLRDDGGLNVINKGYNPDRGMWQQSEGKAYFTGAPTRAALKVSFFGPFYGG  
YNVIALDREYRHALVCGPDRDYLWINSRTPPTISDEVKEMLAVATREGFDVSKFIWV  
QQPGSGS

>Tension sensor

MPVFHTRTIESILEPVAQQISHLVIMHEEGEVDGKAIPDLTAPVSAVQAAVSNLVRVGK  
ETVQTTEDQILKRDMPPAFIKVENACTKLVRAAQMLQADPYSVPARDYLIDGSRGILS  
GTSDLLLTDEAEVRKIIRVCKGILEYLTVAEUVETMEDLVITYTKNLGPGMTKMAKM  
IDERQQELTHQEHRVMLVNSMNTVKELLPVLISAMKIFVTTKNTKSQGIEEALKNRNF  
TVEKMSAEINEIIRVLQLTSWDEDAWASKDTEAMKRALALIDSKMNQAKGWLDPN  
APPGDAGEQAIRQILDEAGKAGELCAGKERREILGTCKTLGQMTDQLADLRARGQG  
ATPMAMQKAQQVSQGLDLLTAKVENAARKLEAMTNSKQAIKKIDAAQNLWADPN  
GGSEGEHIRGIMSEARKVAELCEEPKERDDILRSLGEISALTAKLSDLRRHGKGDSPE  
ARALAKQIATSLQNLQSKTNRAVANTRPVKAAVHLEGKIEQAQRWIDNPTVDDRGV  
GQAAIRGLVAEGRRLANVMMGPYRQDLLAKCDRVDQLAAQLADLAARGESESPQA  
RAIAAQLQDSLKDLKARMQEAMTQEVSDVFSDTTTPIKLLAVAATAPSDTPNREEVFE  
ERAAFNENHAARLGATAEKAAAVGTANKTTVEGIQATVKSARELTPQVVSAARILLR  
NPGNQAAAYEHFETMKNQWIDNVEKMTGLVDEAIDTKSLLDASEEAIKKDLKCKVA  
MANMQPQMLVAGATSIARRANRILLVAKREVENSEDPKFREAVKAAASDELSTISPM  
VMDAKAVAGNISDPGLQKSFLDSGYRILGAVAKVREAFQPQEPDFPPPPPDLEHLHLT  
DELAPPKPPLPEGEVPPPPRPPPEEKDEVEMVSKGEEDNMASLPATHELHIFGSINGVD  
FDMVGQGTGNPNDGYEELNLKSTKGDLQFSPWILVPHIGYGFHQYLPYPDGMSPFQ  
AAMVDGSGYQVHRTMQFEDGASLTVNYRYTYEGSHIKGEAQVKGTGFPADGPVMT  
NSLTAADWCRSKKTYPNDKTIISTFKWSYTTGNGKRYRSTARTTYTFAKPMAANYLK  
NQPMYVFRKTELKHSKTELNFKEWQKAFTDVMGMDELYKGPGGAGPGGAGPGGA  
GPGGAGPGGAGPGGAGPGGAGPGGAGPGGAMASSPTPPRGVTVVNNFDCKRYLGTWYEIA  
RFDHRFERGLEKVTATYSLRDDGGLNVINKGYNPDRGMWQQSEGKAYFTGAPTRAA  
LKVSFFGPFYGGYNVIALDREYRHALVCGPDRDYLWINSRTPTISDEVKQEMLAVATR  
EGFDVSKFIWVQQPGSAAAGEFPEQKAGEAINQPMMAARQLHDEARKWSSKGND  
IIAAAKRMALLMAEMSRLVRGGSGNKRALIQCADIASDEVTRLAKEVAKQCTD  
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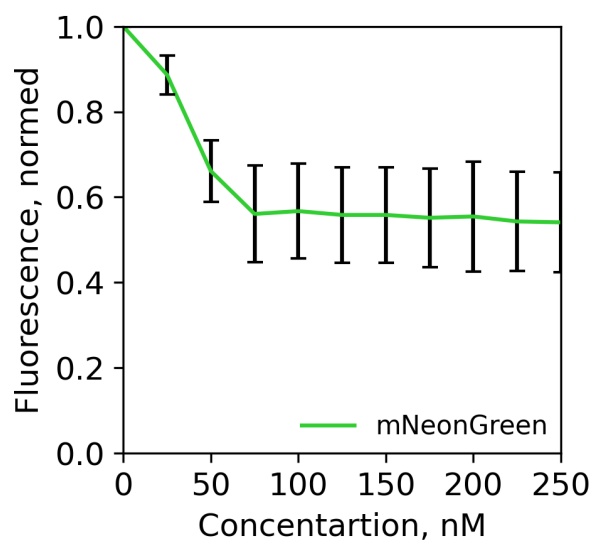
>YAP-mNeonGreen

MDPGQQPPPPQAPQGGQPPSQQPQGGQPPSGPGQPAPAATQAAPQAPPAGHQIVHV  
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AGALTPQHVRHSSPASLQLGAVSPGTLTPTGVVSGPAATPTAQHLRQSSFEIPDDVPL  
PAGWEMAKTSSGQRYFLNHIDQTTTWQDPRKAMLSQMNVTAPTSPPVQQNMMNSA  
SGPLPDGWEQAMTQDGEIYYINHKNKTTSWLDPRLDPRFAMNQRISSAPVKQPPPL  
APQSPQGGVMGGSNSNQQQQMRLQQLQMEKERLRLKQQELLRQAMRNINPSTANS  
PKCQELALRSQPLTLEQDGGTQNPVSSPGMSQELRTMTNSSDPFLNSGTYHSRDEST  
DSGLSMSSYSVPRTPDDFLNSVDEMDTGDITNQSTLPSQQNRFPDYLEAIPGTNVDLG  
TLEGDGMNIEGEELMPSLQEALSSDILNDMESVLAATKLDKESFLTWLDPPVATMVS  
KGEEDNMASLPATHELHIFGSINGVD FDMVGQGTGNPNDGYEELNLKSTKGDLQFSP  
WILVPHIGYGFHQYLPYPDGMSPFQAAMVDGSGYQVHRTMQFEDGASLTVNYRYTY  
EGSHIKGEAQVKGTGFPADGPVMTNSLTAADWCRSKKTYPNDKTIISTFKWSYTTGN  
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MGMDELYKG

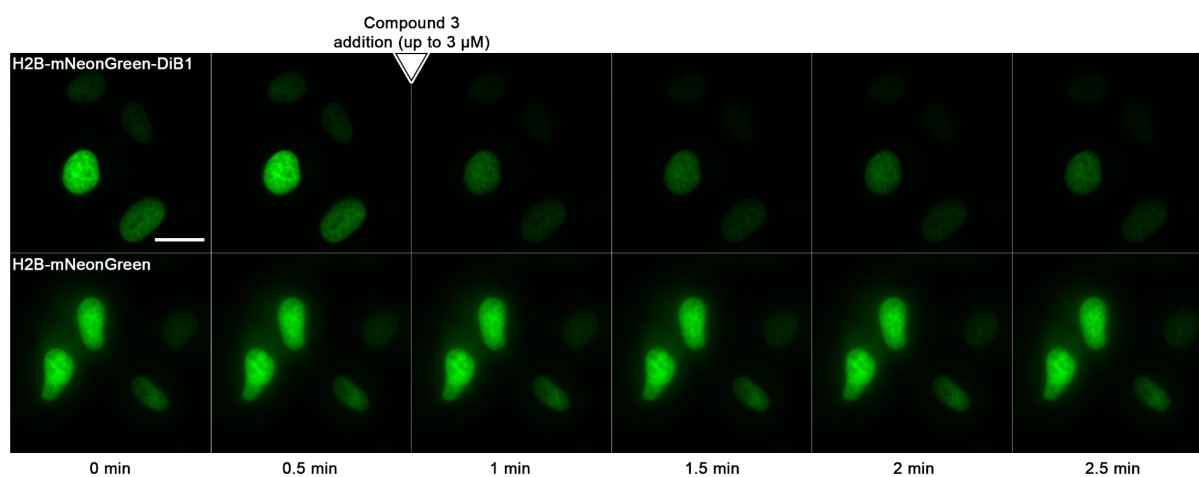
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MERASLIQKAKLAEQAERYEDMAAFMKGAVEKGEELSCEERNLLSVAYKNVVGQ  
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DAESRVFYLMKMGDYYRYLAEVATGDDKKRIIDSARSAYQEAMDISKKEMPPTNPIR  
LGLALNFSVFHYEIANSPEEAISLAKTTFDEAMADLHTLSEDSYKDSTLIMQLLRDNL  
TLWTADNAGEEGGEAPQEPQSDPPVATMASSPTPPRGVTVVNNFDCKRYLGTWYEIA  
RFDHRFERGLEKVTATYSLRDDGGLNVINKGYNPDRGMWQQSEGKAYFTGAPTRAA  
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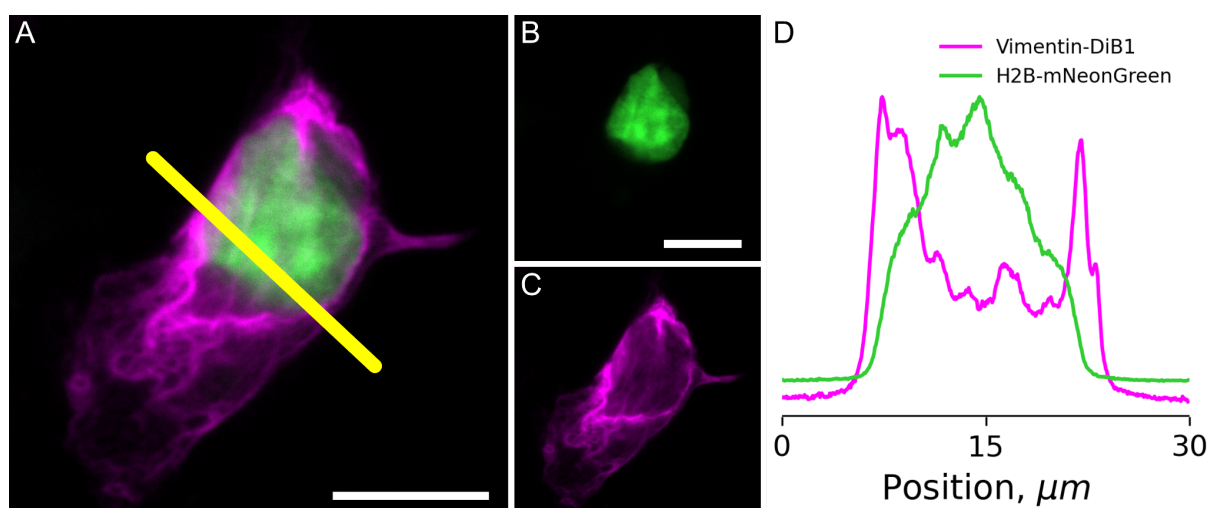
## **2. Supplementary figures and movies**



**Figure S1. Live-cell titration of modulated FRET acceptor.** Fluorescence intensity of mNeonGreen in live HeLa cells transiently transfected with H2B-mNeonGreen-DiB1 in the presence of different concentrations of compound **3**; error bars represent standard deviation (technical replicates = 17).

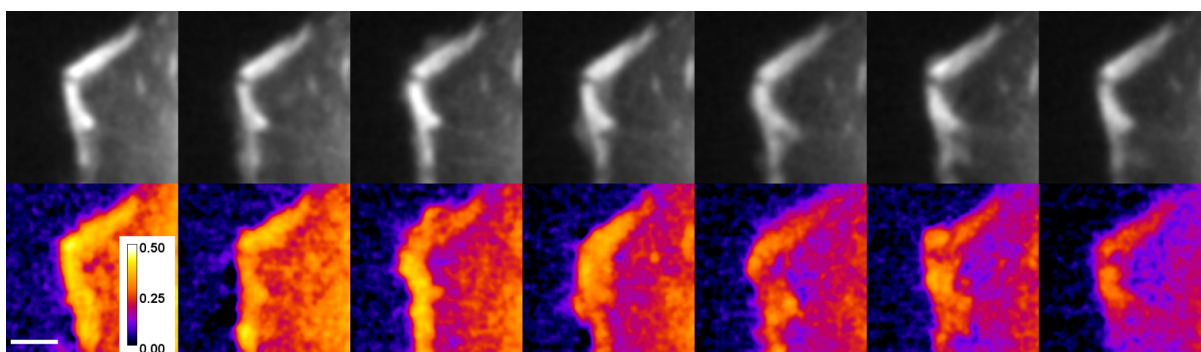


**Figure S2. Live-cell image sequence for FRET acceptor activation.** Widefield fluorescence imaging of live HeLa Kyoto cells transiently transfected with H2B-mNeonGreen (lower row) and H2B-mNeonGreen-DiB1 (upper row) constructs before and after compound **3** addition up to 3  $\mu$ M concentration; Scale bar is 20  $\mu$ m.



**Figure S3. Imaging of non-interacting proteins labeled with modulated FRET pair.**

Live-cell imaging of DiB1 and mNeonGreen on non-interacting proteins. Widefield fluorescence imaging of live HeLa Kyoto cells transiently cotransfected with H2B-mNeonGreen and vimentin-DiB1 in the presence of 100 nM of compound **3**. (A) Composed image of labeled nucleus and vimentin filaments. (B, C) Separate channels of panel (A). (D) Fluorescence intensity profile (indicated by yellow line on panel (A)); Scale bars are 20  $\mu m$ .



**Figure S4. Tension sensor with single-channel FRET detection.** Widefield fluorescence imaging of dynamic tension changes in focal contacts of live HeLa Kyoto cells transiently transfected with tension sensor construct. Frame interval – 6 minute. A cyclic FRET acceptor activation (staining with compound **3**, followed by washing with HHBS buffer) was performed on every time point. Upper row – widefield images before staining, lower row – FRET efficiency images; pseudocolor indicates FRET efficiency after the addition of 3  $\mu$ M compound **3**; Scale bar is 3  $\mu$ m.

**Supplementary Movie S1. Cyclic FRET activation.** Repeated cycles of FRET activation in live HeLa cells transiently transfected with H2B-mNeonGreen-DiB1 construct. Green lines represent average intensities of mNeonGreen (donor) fluorescence in labeled nuclei. Acceptor activation was achieved by the addition of HHBS buffer supplied with 3  $\mu$ M of compound **3**. Washing was performed with HHBS buffer; Scale bar is 10  $\mu$ m. Video plays at 30 fps.

**Supplementary Movie S2. Tension sensor with single-channel FRET detection.** Widefield fluorescence imaging of dynamic tension changes in focal contacts of live HeLa Kyoto cells transiently transfected with tension sensor construct. Frame interval - 6 minutes. A cyclic FRET acceptor activation (staining with compound **3**, followed by washing with HHBS buffer) was performed on every time point. Upper image - widefield microscopy, lower image - FRET efficiency image; pseudocolor indicates FRET efficiency after the addition of 3  $\mu$ M compound **3**; Scale bar is 3  $\mu$ m. Video plays at 2 fps.