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**FBXW7 interaction with Cyclin E1 - cellular assay**

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**Methods**

HEK293T cells were plated in 96-well plates (2 x 104/well) and 4 h later transfected with 0.03 µg/well C-terminally HT-tagged FBXW7 (WT or R465E, R505E mutant) or HT and 0.001 µg/well C-terminally NL-tagged cyclin E1 using Xtreme gene HP transfection reagent (Roche), following manufacturer’s instructions. Next day media was replaced with 40 µl of DMEM/F12 (no phenol red) +/- HaloTag® NanoBRET™ 618 Ligand (1 µl/ml, Promega) and 4 h later 10 µl of NanoBRET™ Nano-Glo Substrate (10 µl/ml in DMEMF12 no phenol red, Promega) was added, and the signal was read. Donor emission at 450 nm (filter: 450 nm/BP 80nm) and acceptor emission at 618 nm (filter: 610nm/LP) was measured within 10 minutes of substrate addition using CLARIOstar microplate reader (Mandel). Mean corrected NanoBRET ratios were determined by subtracting the mean of 618/460 signal from cells without NanoBRET™ 618 Ligand x 1000 from the mean of 618/460 signal from cells with NanoBRET™ 618 Ligand x 1000.

**Constructs:**

Cyclin E1 was cloned into pNLF1-C vector and FBXW7 was cloned into pHTC – HaloTag vector (Promega). R465E/R505E double mutant was madeusing Q5® Site-Directed Mutagenesis kit (NEB), following the manufacturer’s instructions.

**FBXW7(Q969H0)**

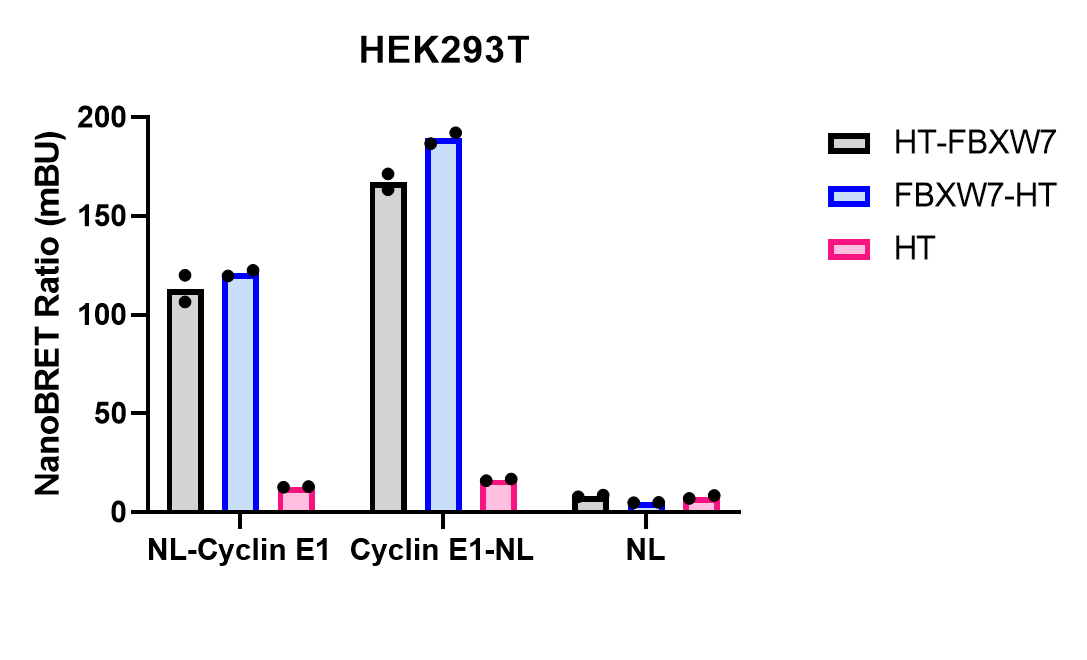
MNQELLSVGSKRRRTGGSLRGNPSSSQVDEEQMNRVVEEEQQQQLRQQEEEHTARNGEVVGVEPRPGGQNDSQQGQLEENNNRFISVDEDSSGNQEEQEEDEEHAGEQDEEDEEEEEMDQESDDFDQSDDSSREDEHTHTNSVTNSSSIVDLPVHQLSSPFYTKTTKMKRKLDHGSEVRSFSLGKKPCKVSEYTSTTGLVPCSATPTTFGDLRAANGQGQQRRRITSVQPPTGLQEWLKMFQSWSGPEKLLALDELIDSCEPTQVKHMMQVIEPQFQRDFISLLPKELALYVLSFLEPKDLLQAAQTCRYWRILAEDNLLWREKCKEEGIDEPLHIKRRKVIKPGFIHSPWKSAYIRQHRIDTNWRRGELKSPKVLKGHDDHVITCLQFCGNRIVSGSDDNTLKVWSAVTGKCLRTLVGHTGGVWSSQMRDNIIISGSTDRTLKVWNAETGECIHTLYGHTSTVRCMHLHEKRVVSGSRDATLRVWDIETGQCLHVLMGHVAAVRCVQYDGRRVVSGAYDFMVKVWDPETETCLHTLQGHTNRVYSLQFDGIHVVSGSLDTSIRVWDVETGNCIHTLTGHQSLTSGMELKDNILVSGNADSTVKIWDIKTGQCLQTLQGPNKHQSAVTCLQFNKNFVITSSDDGTVKLWDLKTGEFIRNLVTLESGGSGGVVWRIRASNTKLVCAVGSRNGTEETKLLVLDFDVDMK

**FBXW7 (R465E, R505E)**

MNQELLSVGSKRRRTGGSLRGNPSSSQVDEEQMNRVVEEEQQQQLRQQEEEHTARNGEVVGVEPRPGGQNDSQQGQLEENNNRFISVDEDSSGNQEEQEEDEEHAGEQDEEDEEEEEMDQESDDFDQSDDSSREDEHTHTNSVTNSSSIVDLPVHQLSSPFYTKTTKMKRKLDHGSEVRSFSLGKKPCKVSEYTSTTGLVPCSATPTTFGDLRAANGQGQQRRRITSVQPPTGLQEWLKMFQSWSGPEKLLALDELIDSCEPTQVKHMMQVIEPQFQRDFISLLPKELALYVLSFLEPKDLLQAAQTCRYWRILAEDNLLWREKCKEEGIDEPLHIKRRKVIKPGFIHSPWKSAYIRQHRIDTNWRRGELKSPKVLKGHDDHVITCLQFCGNRIVSGSDDNTLKVWSAVTGKCLRTLVGHTGGVWSSQMRDNIIISGSTDRTLKVWNAETGECIHTLYGHTSTVECMHLHEKRVVSGSRDATLRVWDIETGQCLHVLMGHVAAVECVQYDGRRVVSGAYDFMVKVWDPETETCLHTLQGHTNRVYSLQFDGIHVVSGSLDTSIRVWDVETGNCIHTLTGHQSLTSGMELKDNILVSGNADSTVKIWDIKTGQCLQTLQGPNKHQSAVTCLQFNKNFVITSSDDGTVKLWDLKTGEFIRNLVTLESGGSGGVVWRIRASNTKLVCAVGSRNGTEETKLLVLDFDVDMK

**Cyclin E1 (P24864)**

MPRERRERDAKERDTMKEDGGAEFSARSRKRKANVTVFLQDPDEEMAKIDRTARDQCGSQPWDNNAVCADPCSLIPTPDKEDDDRVYPNSTCKPRIIAPSRGSPLPVLSWANREEVWKIMLNKEKTYLRDQHFLEQHPLLQPKMRAILLDWLMEVCEVYKLHRETFYLAQDFFDRYMATQENVVKTLLQLIGISSLFIAAKLEEIYPPKLHQFAYVTDGACSGDEILTMELMIMKALKWRLSPLTIVSWLNVYMQVAYLNDLHEVLLPQYPQQIFIQIAELLDLCVLDVDCLEFPYGILAASALYHFSSSELMQKVSGYQWCDIENCVKWMVPFAMVIRETGSSKLKHFRGVADEDAHNIQTHRDSLDLLDKARAKKAMLSEQNRASPLPSGLLTPPQSGKKQSSGPEMA



**Fig.1. FBXW7 interaction with cyclin E1 - NanoBRET assay.** HEK293T cells were co-transfected with C- or N-terminally NanoLuc (NL)-tagged cyclin E1 or NL alone and C- or N-terminally Halo-tagged (HT) FBXW7 or HT alone for 24 h. The interaction was measured using NanoBRET assay. The results are MEAN of 2 technical replicates.



**Fig.2. R465E, R505E double mutation decreases the interaction of FBXW7 with cyclin E1.** HEK293T cells were co-transfected with C-terminally Halo-tagged (HT) FBXW7 (WT or R465E/R505E or HT and C-terminally NanoLuc (NL)-tagged cyclin E1 for 24 h. The interaction was measured using NanoBRET assay. The results are MEAN of 2 technical replicates.