Supporting information for

Improved Protein-protein Interaction Assay FlimPIA by the Entrapment of Luciferase Conformation

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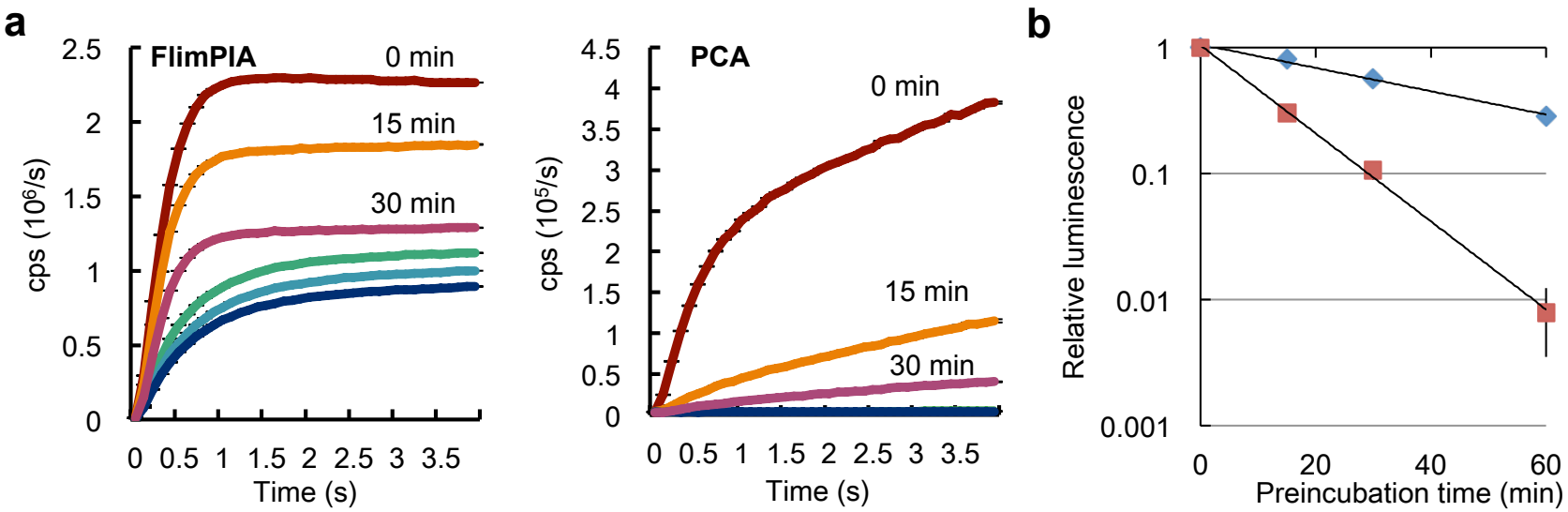

Figure S-1. Comparison of thermostability. (a) Probes (50 nM each) were preincubated at 37°C for 0-30 min with or without equimolar rapamycin. The luminescent intensity was measured for 4 s after adding substrates (LH_2 and ATP). Left: FlimPIA, Right: Fluc-based PCA. Red: incubation for 0 min with rapamycin, Orange: 15 min with rapamycin, pink: 30 min with rapamycin, green: 0 min without rapamycin, light blue: 15 min without rapamycin, dark blue: 30 min without rapamycin. (n=3) (b) Inactivation time course. Relative luminescent intensities at 4 s after reaction start were normalized at the value obtained with 0 min pre-incubation (n=3).

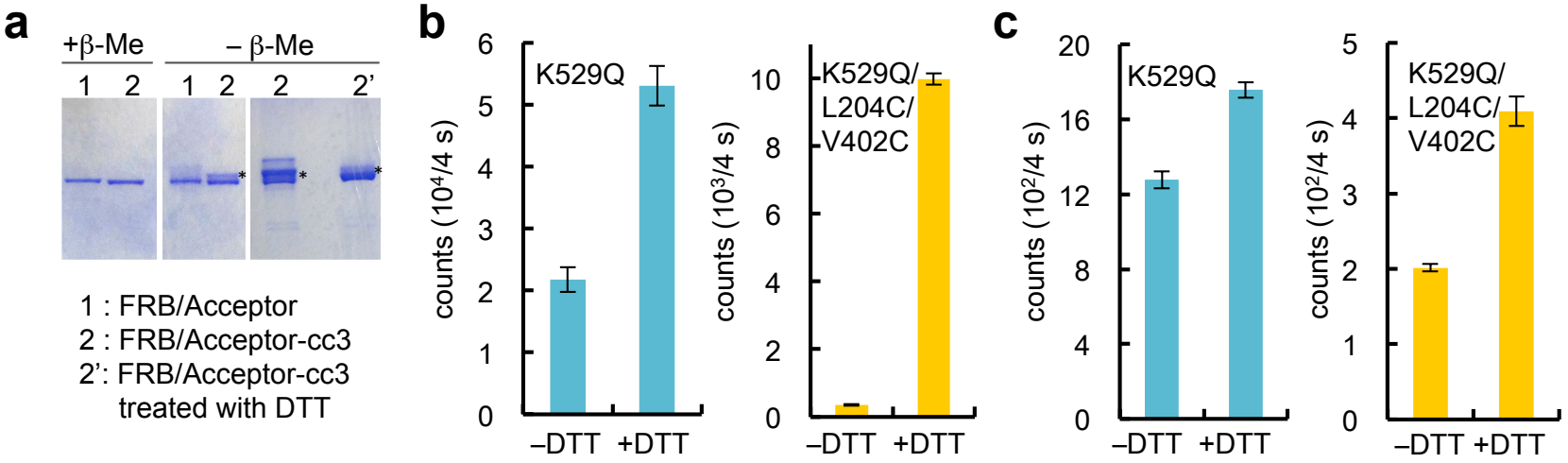

Figure S-2. Reduction of adenylation activity of Acceptor by disulfide bonding. (a) SDS-PAGE of the FRB/Acceptors. Band shifts of Acceptor-cc3 was observed only in the non-reducing condition. (b and c) Luminescent activity of the conventional Acceptor (left) and Acceptor-cc3 (right). Reactions with LH_2 and ATP (b) and those with LH_2-AMP (c) are shown (n=3).

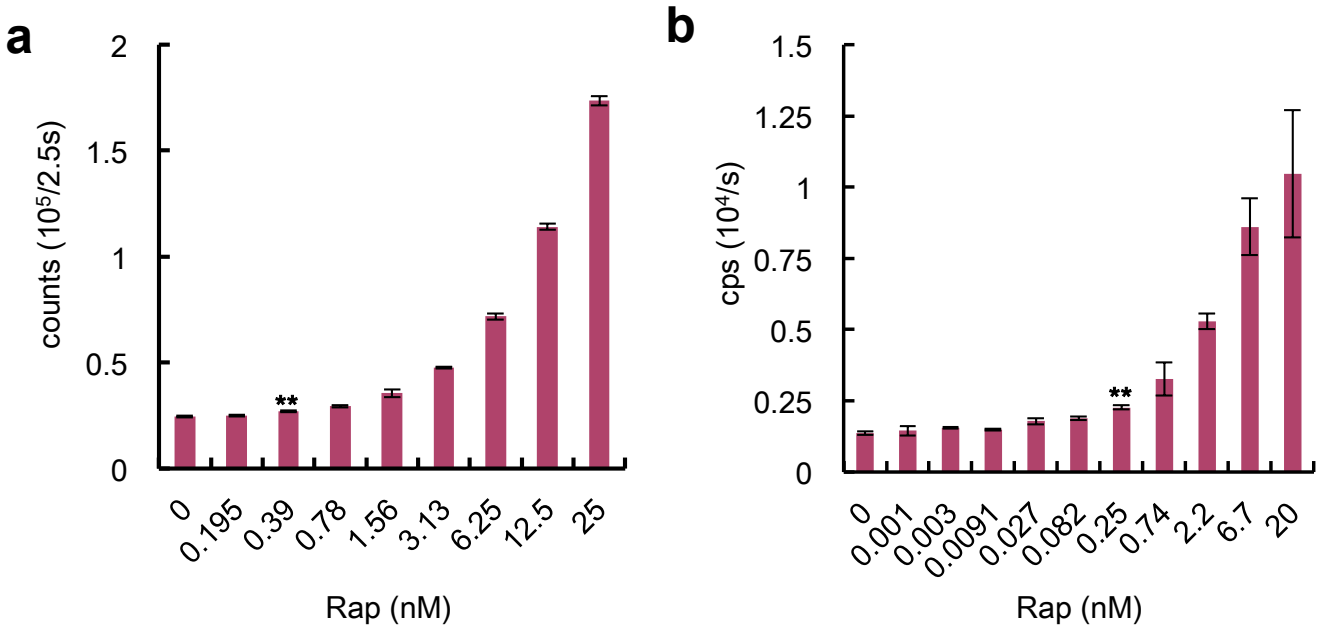

Figure S-3. Sensitivity of the conventional FlimPIA and Fluc PCA. (a) Sensitivity of the conventional FlimPIA. FKBP/Donor and FRB/Acceptor (50 nM each) were added with indicated concentration of rapamycin (n=3). (b) Sensitivity of Fluc PCA using purified probes. 50 nM each of FKBP/C and FRB-N was added with indicated concentrations of rapamycin (n=3). **Limit of detection

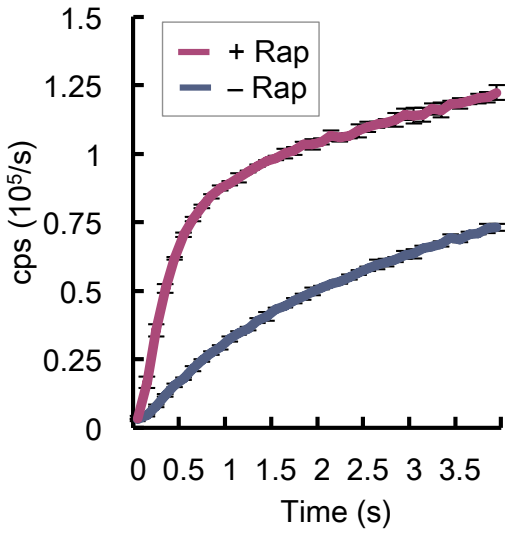

Figure S-4. FlimPIA detecting FKBP12-FRB interaction over long distance with a thermostabilized Donor. The improved FlimPIA was performed with a probe pair FKBP/Fn7-8/Donor and FRB/Acceptor-cc3 (50 nM each), where the Donor introduced with a thermostabilization mutation A296C/A326C was used. Average ± 1 SD of three measurements are shown.