Table 1. GFP^2 and Rluc fusion proteins

Combination	C-terminal GFP^2 fusion protein	N-terminal GFP^2 fusion protein	C-terminal Rluc fusion protein	N-terminal Rluc fusion protein
1	Protein A		Protein B	
2	Protein A			Protein B
3		Protein A	Protein B	
4		Protein A		Protein B

Table 2. BRET^2 transfection matrix

		Rluc-Protein B			
		0μg	1μg	3μg	10μg
GFP^2-Protein A	0μg	Condition 1	Condition 2	Condition 7	Condition 12
	3μg	-	Condition 3	Condition 8	Condition 13
	10μg	-	Condition 4	Condition 9	Condition 14
	20μg	-	Condition 5	Condition 10	Condition 15
	40μg	-	Condition 6	Condition 11	Condition 16

Table 3. Cell number titration in Optiplate

	50,000 cells			100,000 cells			200,000 cells		
Condition 1 Non-treated									
Condition 1 + Treatment									
Condition 2 Non-treated									
Condition 2 + Treatment									
Condition 3 Non-treated									
Condition 3 + Treatment									

Calculation #1:

$$\frac{\text{GFP}^2 \text{ emission (515nm)} - \text{GFP}^2 \text{ emission of non-transfected cells (515nm)}}{\text{Rluc emission (410 nm)} - \text{Rluc emission of non-transfected cells (410 nm)}}$$
$$=$$
$$\text{Corrected BRET}^2 \text{ Ratio}$$

Calculation #2:

$$\text{Corrected BRET}^2 \text{ Ratio} - \text{Correction Factor (Cf)}$$

Where Cf =
$$\frac{\text{GFP}^2 \text{ emission (515nm)} - \text{GFP}^2 \text{ emission of non-transfected cells (515nm)}}{(\text{Rluc emission (410nm)} - \text{Rluc emission of non-transfected cells (410)}}$$

for the Rluc vector expressed with the empty GFP^2 vector