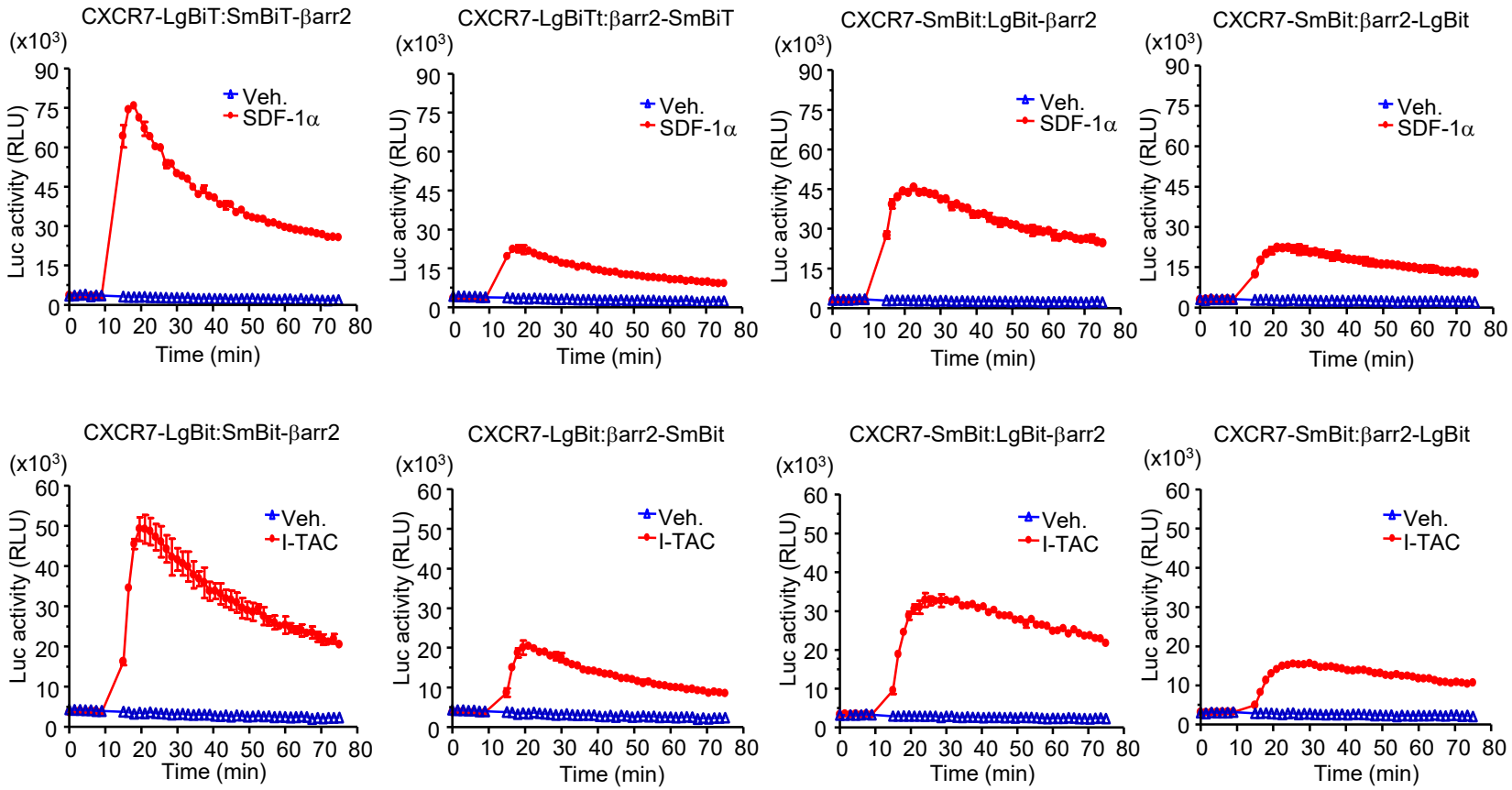

Fig. S1 Ligand-stimulated real-time luciferase activities in HEK293 cells expressing different combination of NanoBit constructs of CXCR7 and β-arrestin2

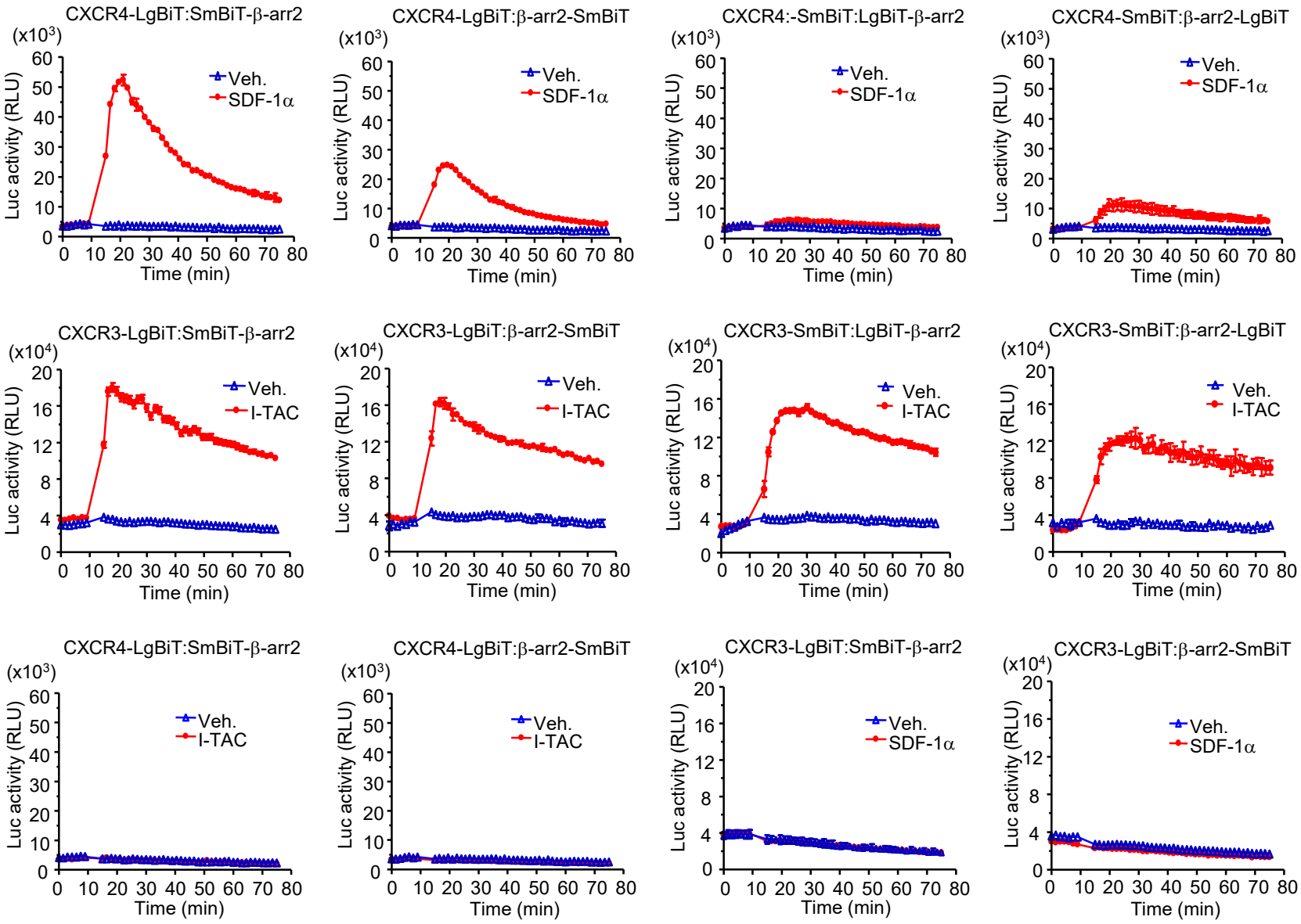

Fig S2 Ligand-stimulated real-time luciferase activities in HEK293 cells expressing different combination of NanoBit constructs of β-arrestin2 with CXCR4 or CXCR3

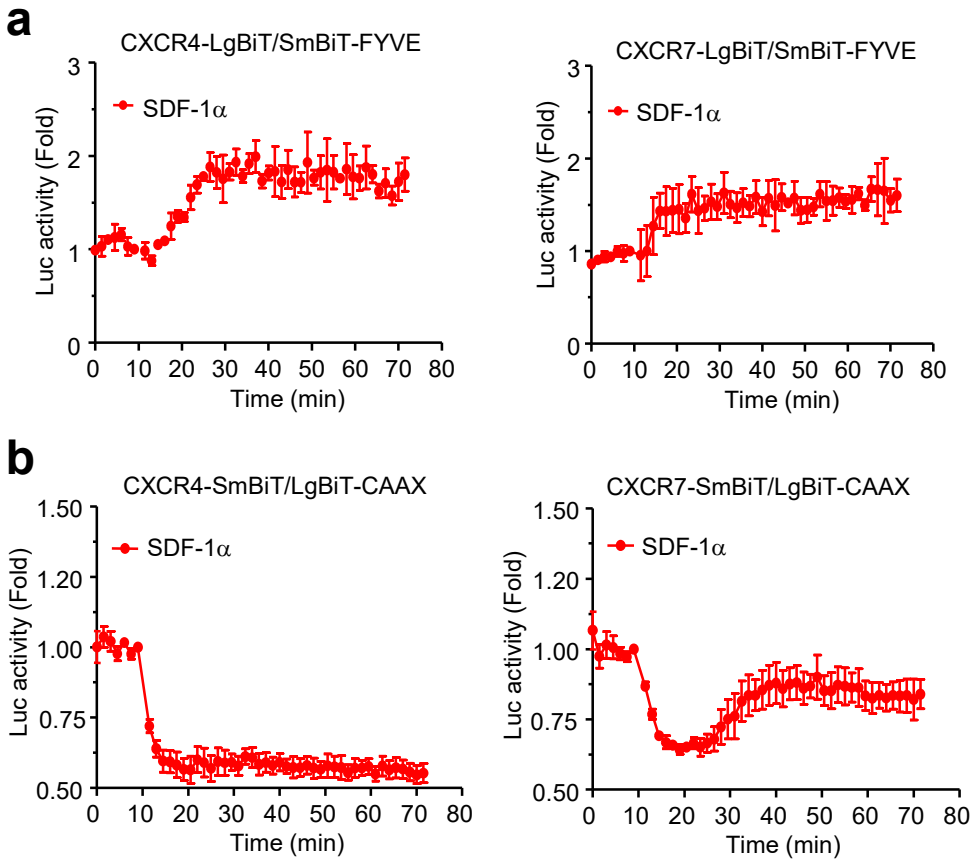

Fig S3 Receptor internalization assay using NanoBit constructs. (a) Cells expressing receptor-LgBiT and SmBiT-FYVE domain of EEA1 were treated with SDF-1α and the luciferase activities were measured in real time. (b) Cells expressing receptor-SmBiT and LgBiT-CAAX sequence were used the NanoBit assay.

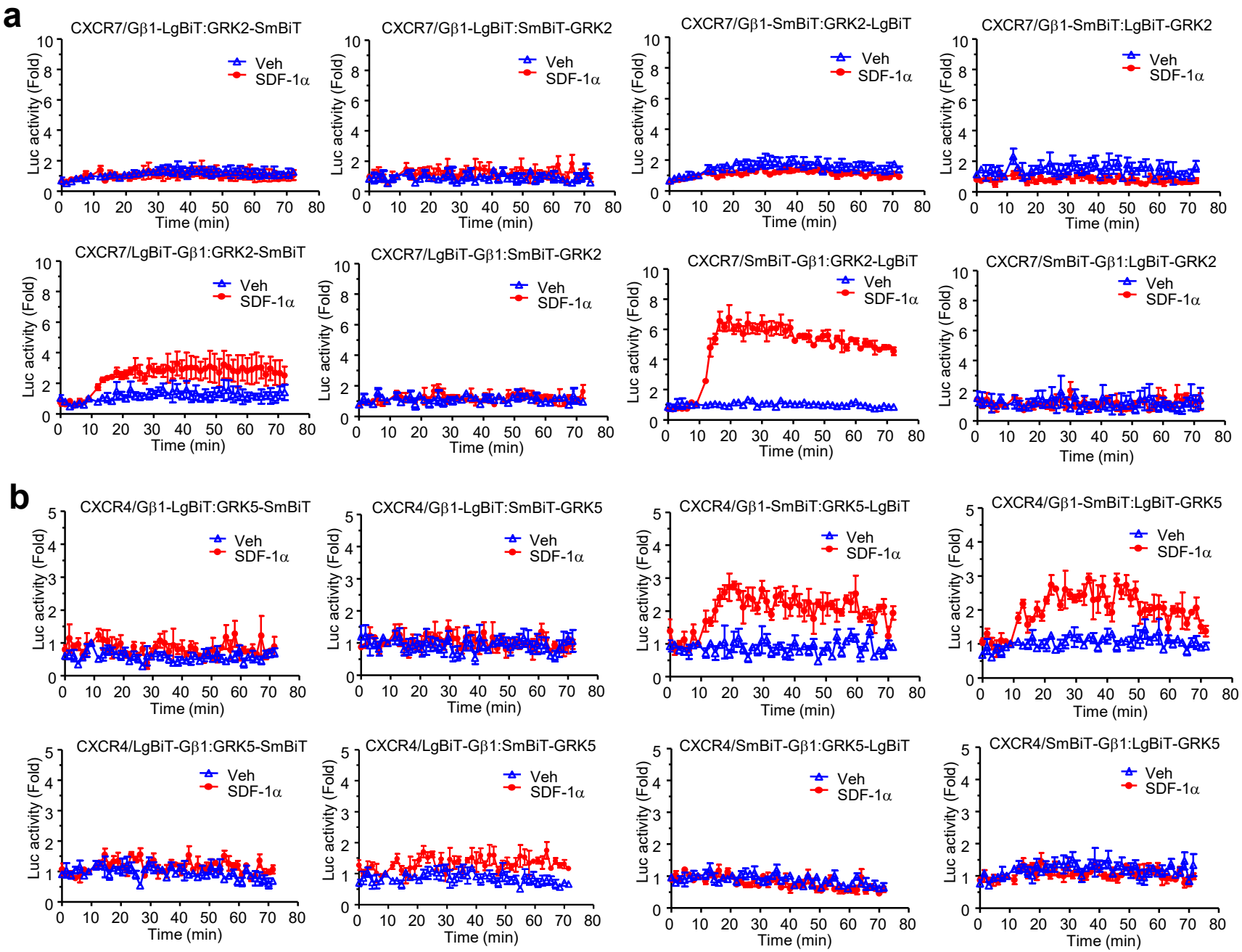

Fig S4 Optimization of NanoBit construct combinations of Gβ1 and GRKs. a and b showed luciferase activities in cells expressing different combinations of NanoBiT constructs

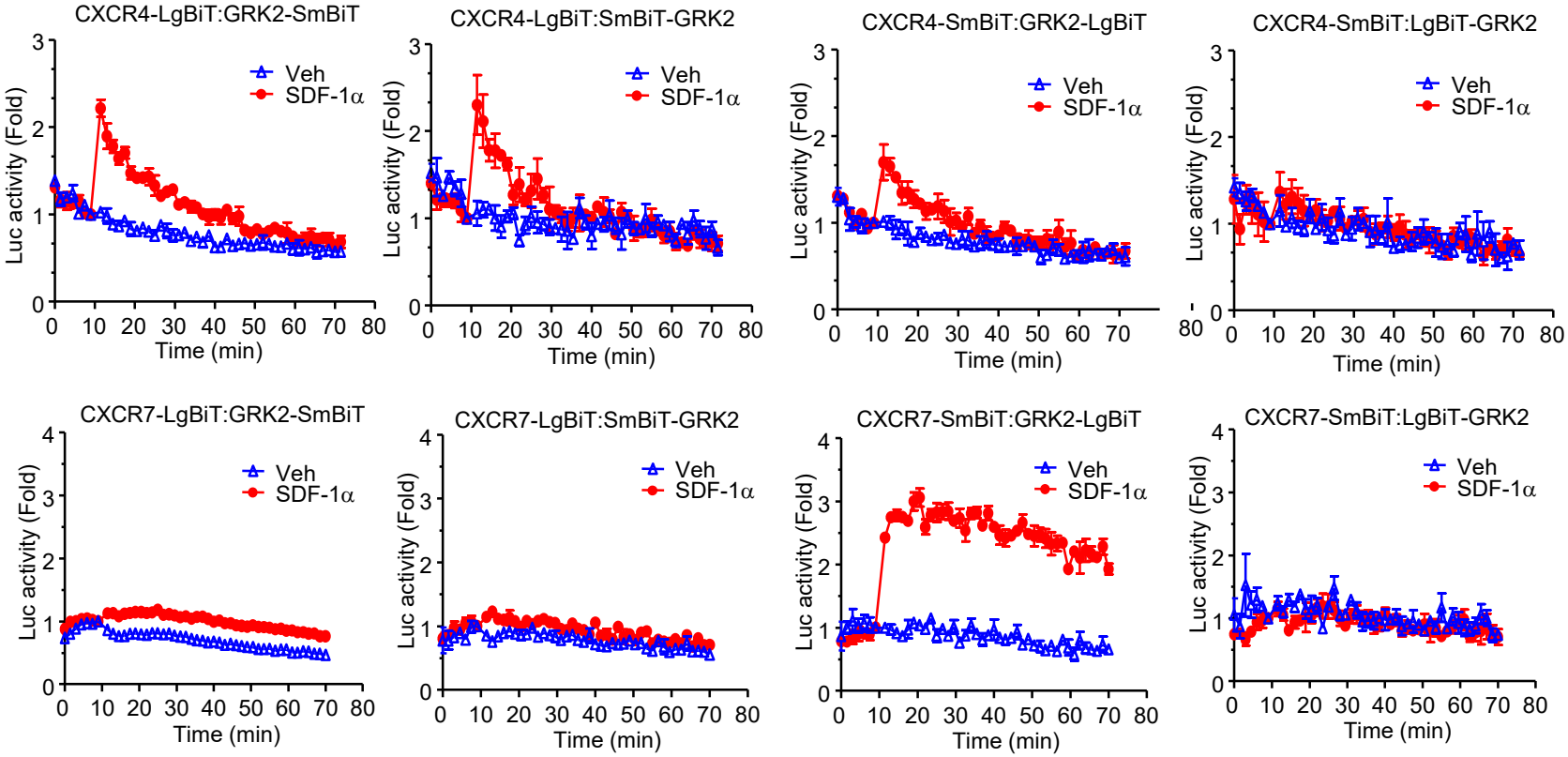

Fig S5 Optimization of NanoBit construct combinations of receptor and GRK2.

a

HEK293/CXCR4-KO

```
ATATACACTTCAGATAACTACACCGAGGAAATGGGCTCAGGGGACTATGACTCCATGAAGGAACCC
ATATACACTTCAGATAACTACACCGAGGAAATGGG-----GGGACTATGACTCCATGAAGGAACCC
                                 65nt del  GGACTATGACTCCATGAAGGAACCC
```

HEK293/CXCR7-KO

```
CTTCGACTACTCAGAGCCAGGGAACTTCTCGGACATCAGCTGGCCATGCAACAGCAGCGACTGCATC
CTTCGACTACTCAGAGCCAGGGAACTTCTCGGACATCAG----CCATGCAACAGCAGCGACTGCATC
CTTCGACTACTCAGAGCCAGGGAACTTCTCGGACA-------GCCATGCAACAGCAGCGACTGCATC
```

HeLa/CXCR4-KO

```
ATATACACTTCAGATAACTACACCGAGGAAATGGGCTCAGGGGACTATGACTCCATGAAGGAACCC
ATATACACTTCAGATAACTACACCGAGGAAATGGGC--AGGGGACTATGACTCCATGAAGGAACCC
ATATACACTTCAGATAACTACACCGAGGAAATGGG-------GACTATGACTCCATGAAGGAACCC
```

HeLa/CXCR7-KO

```
CTTCGACTACTCAGAGCCAGGGAACTTCTCGGACATCAGCTGGCCATGCAACAGCAGCGACTGCATC
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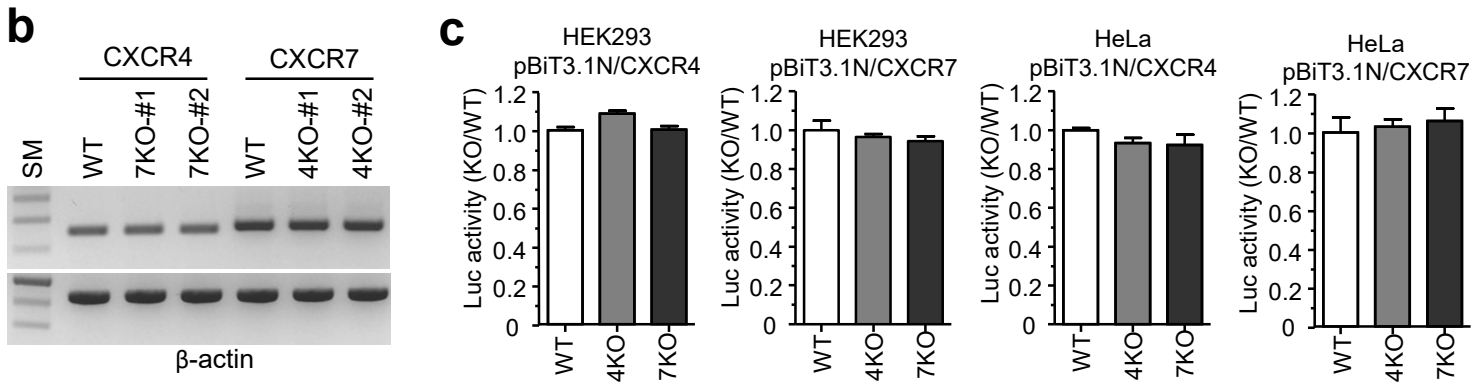

Fig S6 Generation of cells lacking receptors using CRISPR system. (a) Genomic DNA PCR products from cells established with CRISPR-Cas9 were cloned and sequenced. Red colors designates guide RNA target regions. (b) RT-PCR products of either CXCR4 or CXCR7 were compared in wild type and receptor KO HeLa cell clones. β-actin products were used as a control. (c) Membrane expression of exogenous receptors were not affected by deletion of CXCR4 or CXCR7. HiBiT constructs of the receptors were expressed in wild type and receptor KO of HEK293 and HeLa cells, and the cells were applied to HiBiT assay.