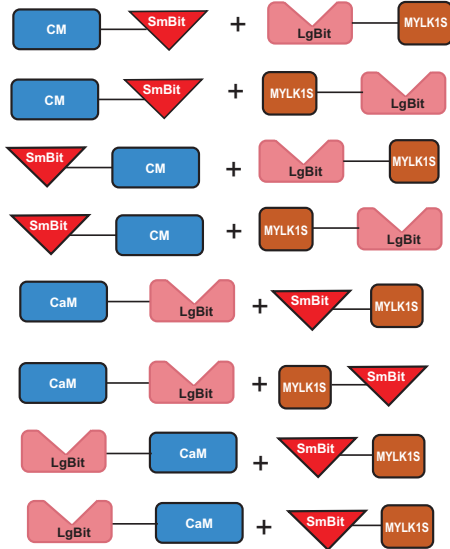
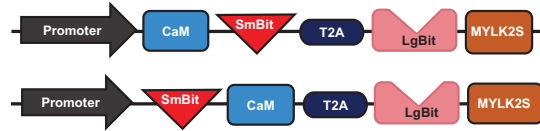




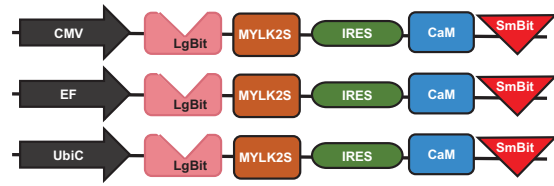
8 combinations of NanoBIT constructs



T2A constructs



IRES constructs



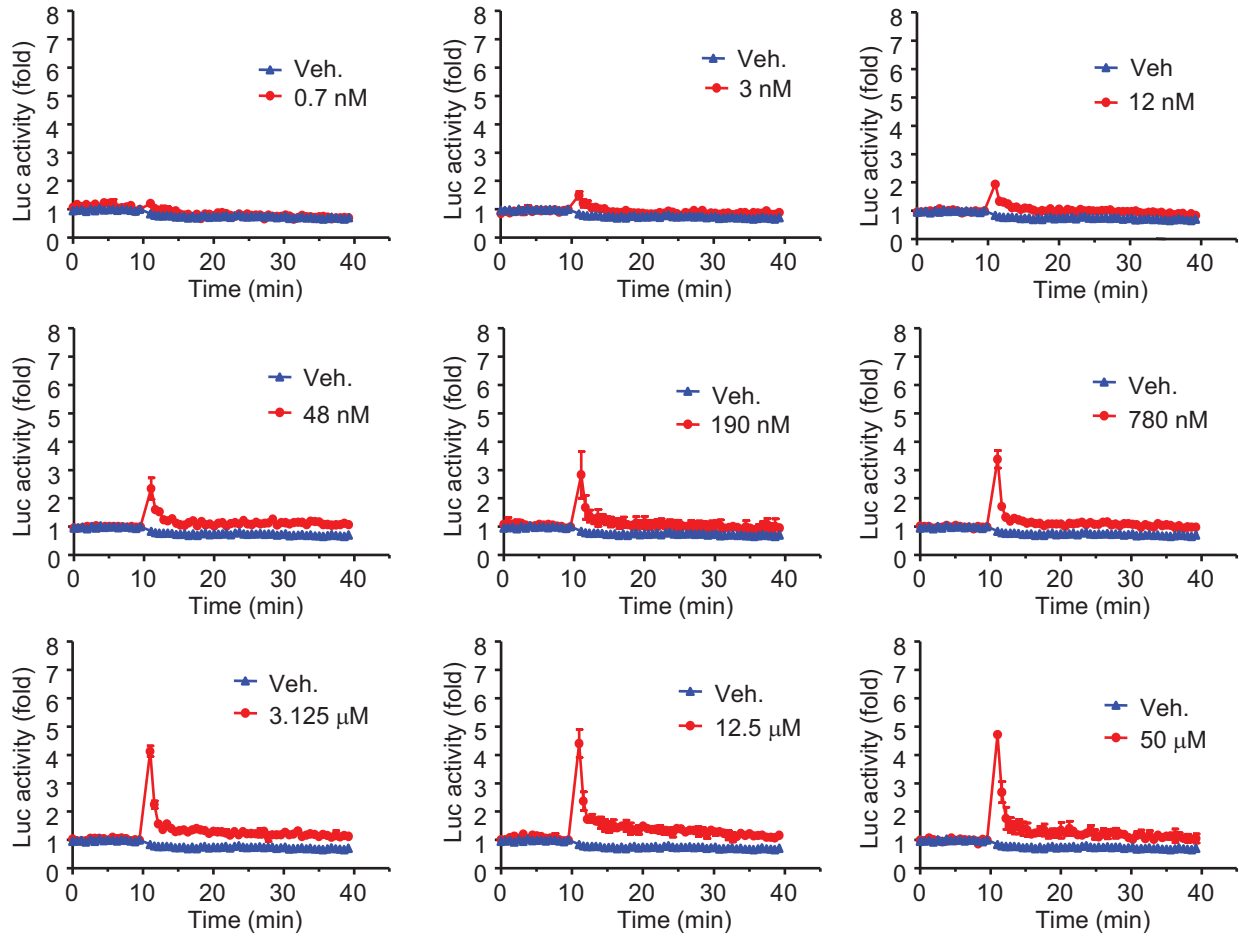
3 vector system



2 vector system

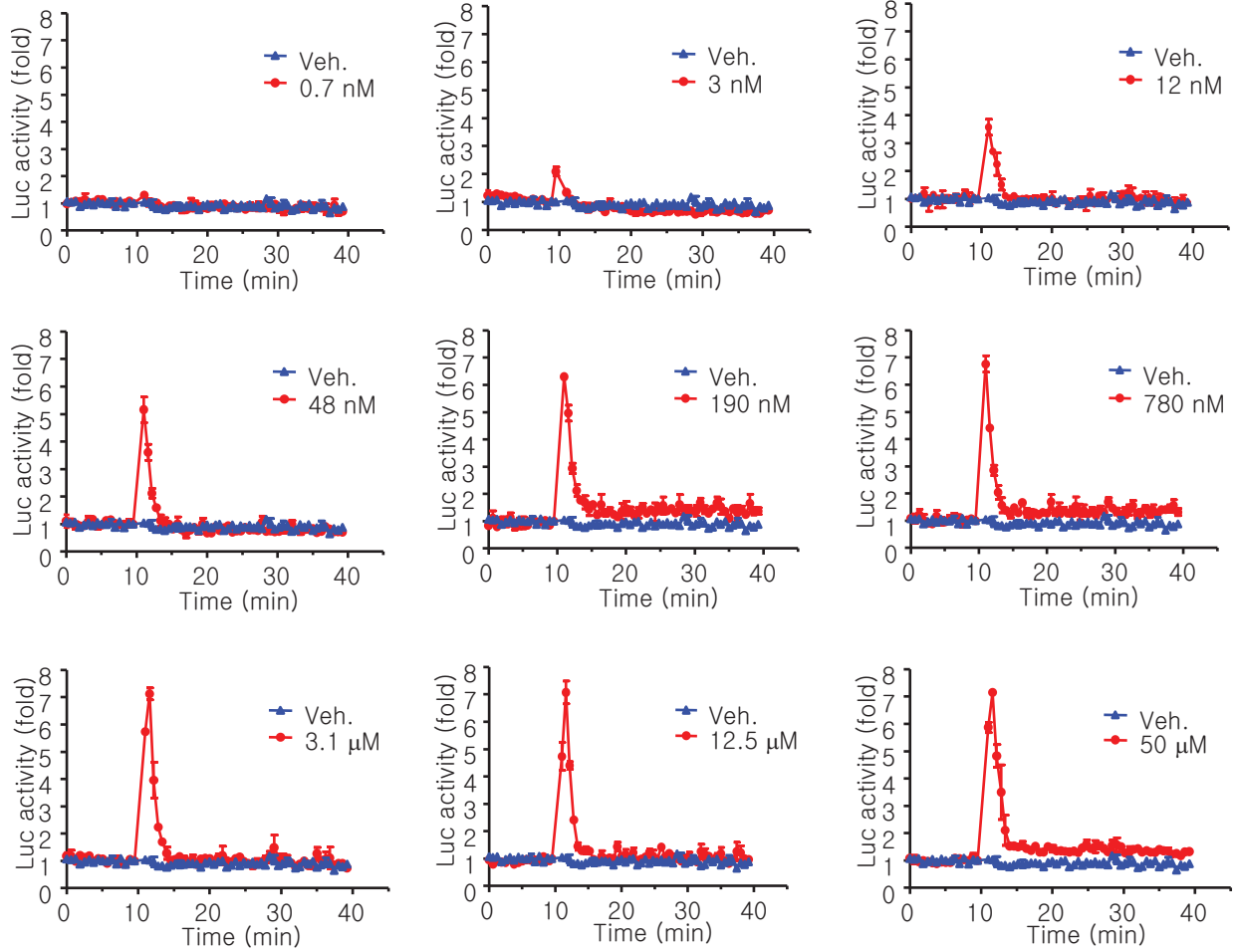
Supplementary Fig. S1. Schematics of the constructs and their combinations.

5-HT_{2A}R: CM-SmBiT/LgBiT-MYLK1S (5-HT)



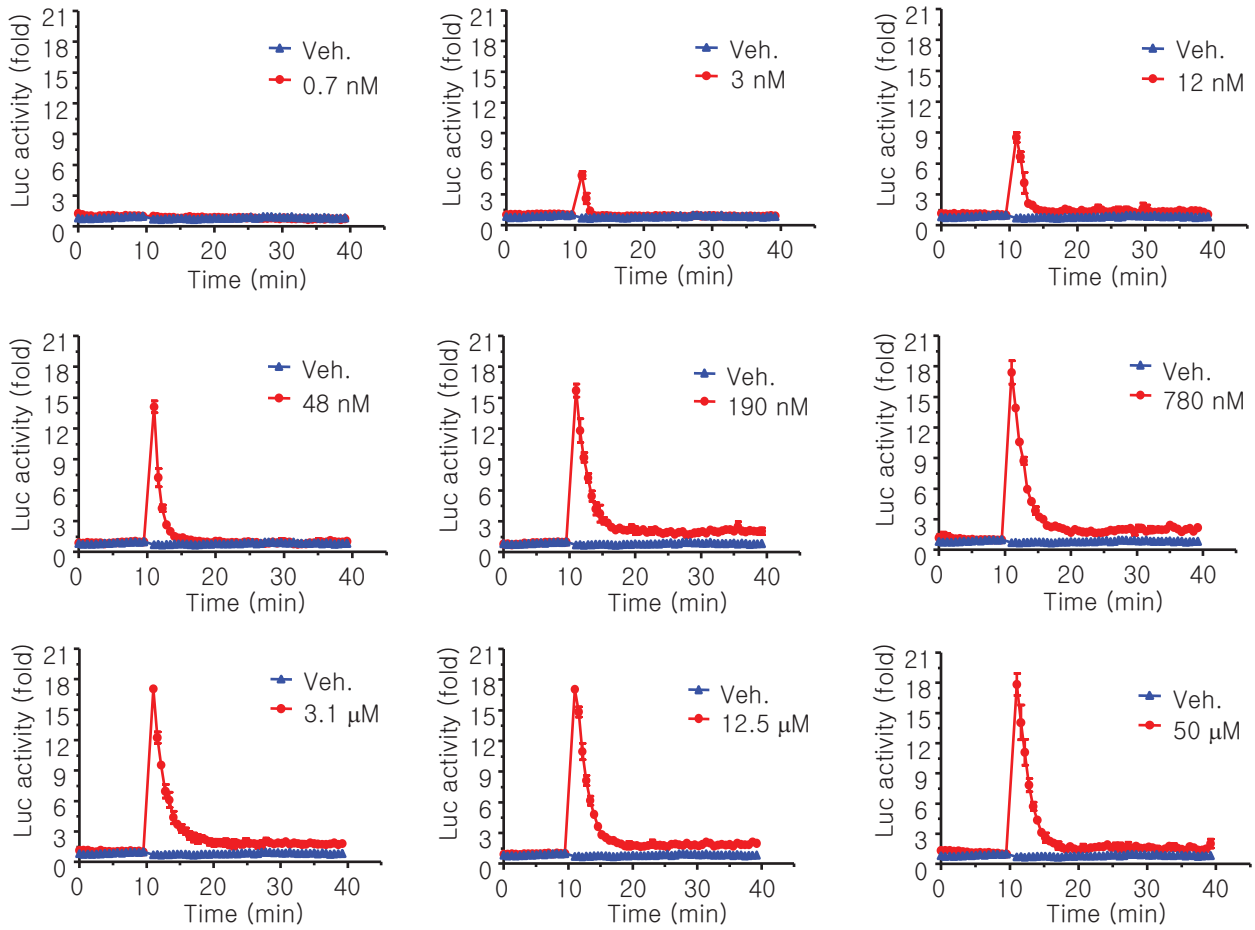
Supplementary Fig. S2. Dose dependency of the luciferase activities by 5-HT in cells expressing calmodulin-SmBiT and LgBiT-MYLK1S, and CMV-driven 5-HT_{2A}R.

5-HT2AR: CM-SmBiT/LgBiT-MYLK2S (5-HT)



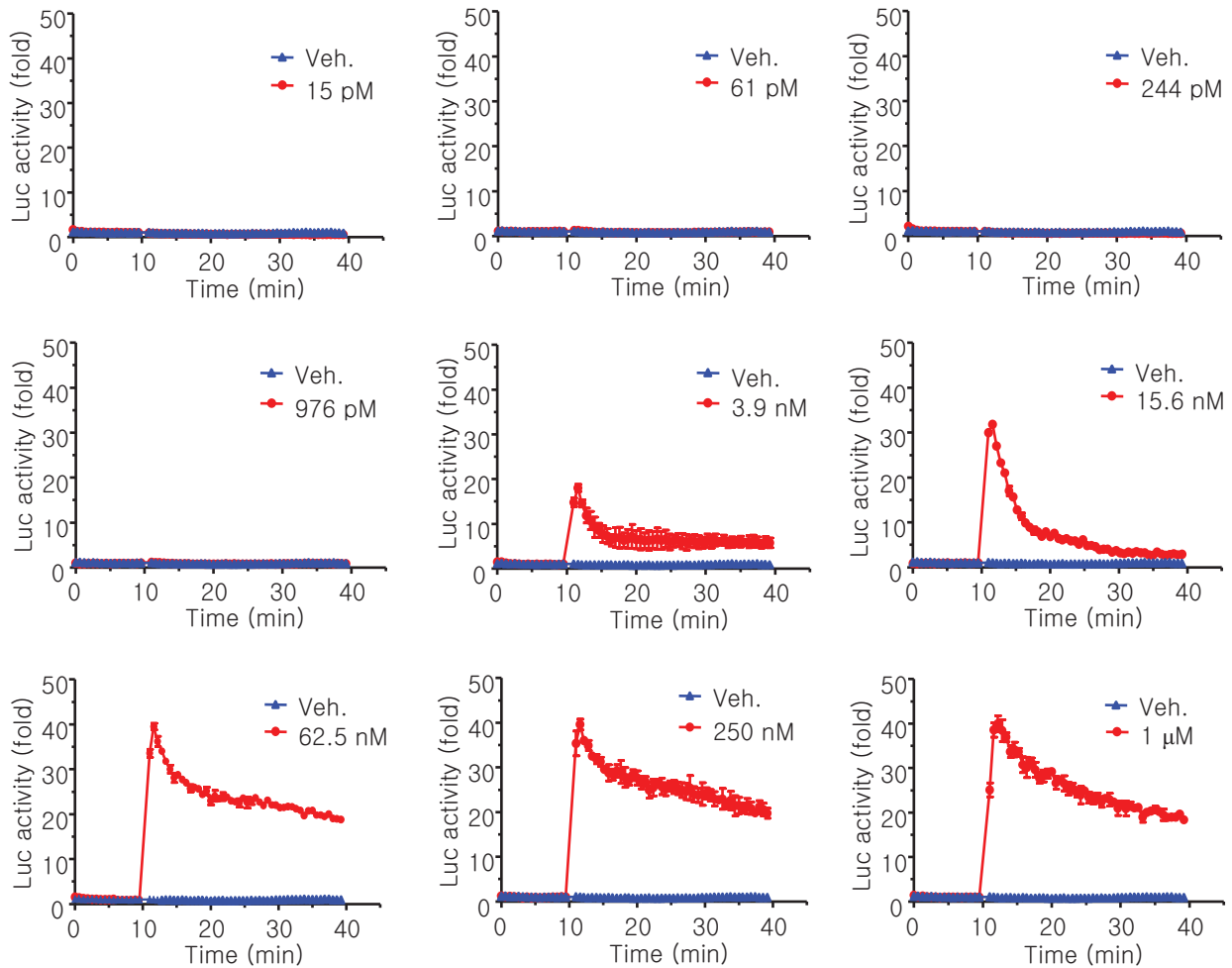
Supplementary Fig. S3. Dose dependency of the luciferase activities by 5-HT in cells expressing calmodulin-SmBiT and LgBiT-MYLK2S, and CMV-driven 5-HT2AR.

5-HT_{2A}R: CM-SmBiT/LgBiT-MYLK2S (5-HT)



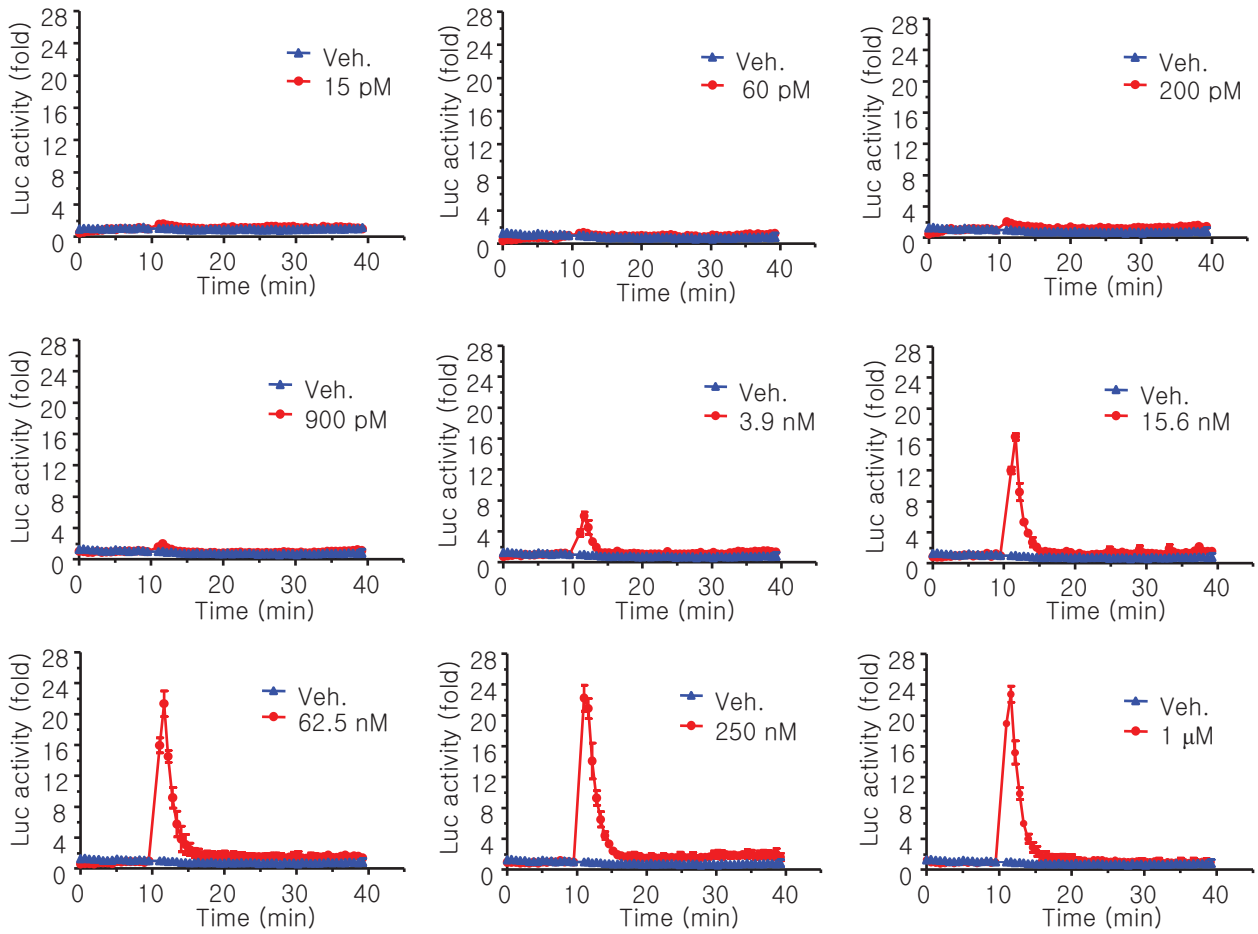
Supplementary Fig. S4. Dose dependency of the luciferase activities by 5-HT in cells expressing calmodulin-SmBiT and LgBiT-MYLK2S, and UbiC-driven 5-HT_{2A}R.

TACR1: CM-SmBiT/LgBiT-MYLK2S (Substance P)



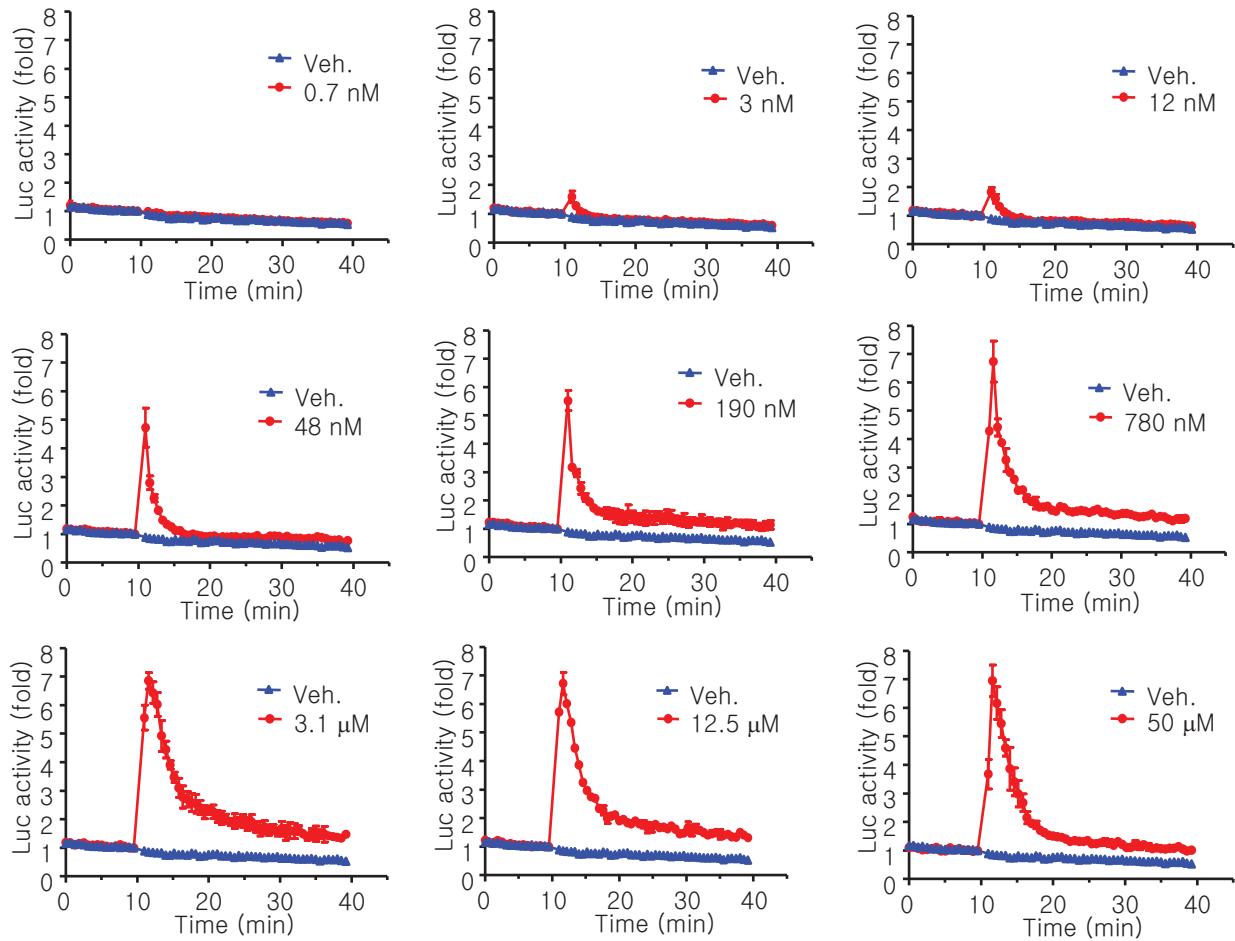
Supplementary Fig. S5. Dose dependency of the luciferase activities by substance P in cells expressing calmodulin-SmBiT and LgBiT-MYLK2S, and UbiC-driven TACR1.

AGTR1: CM-SmBiT/LgBiT-MYLK2S (Ang II)



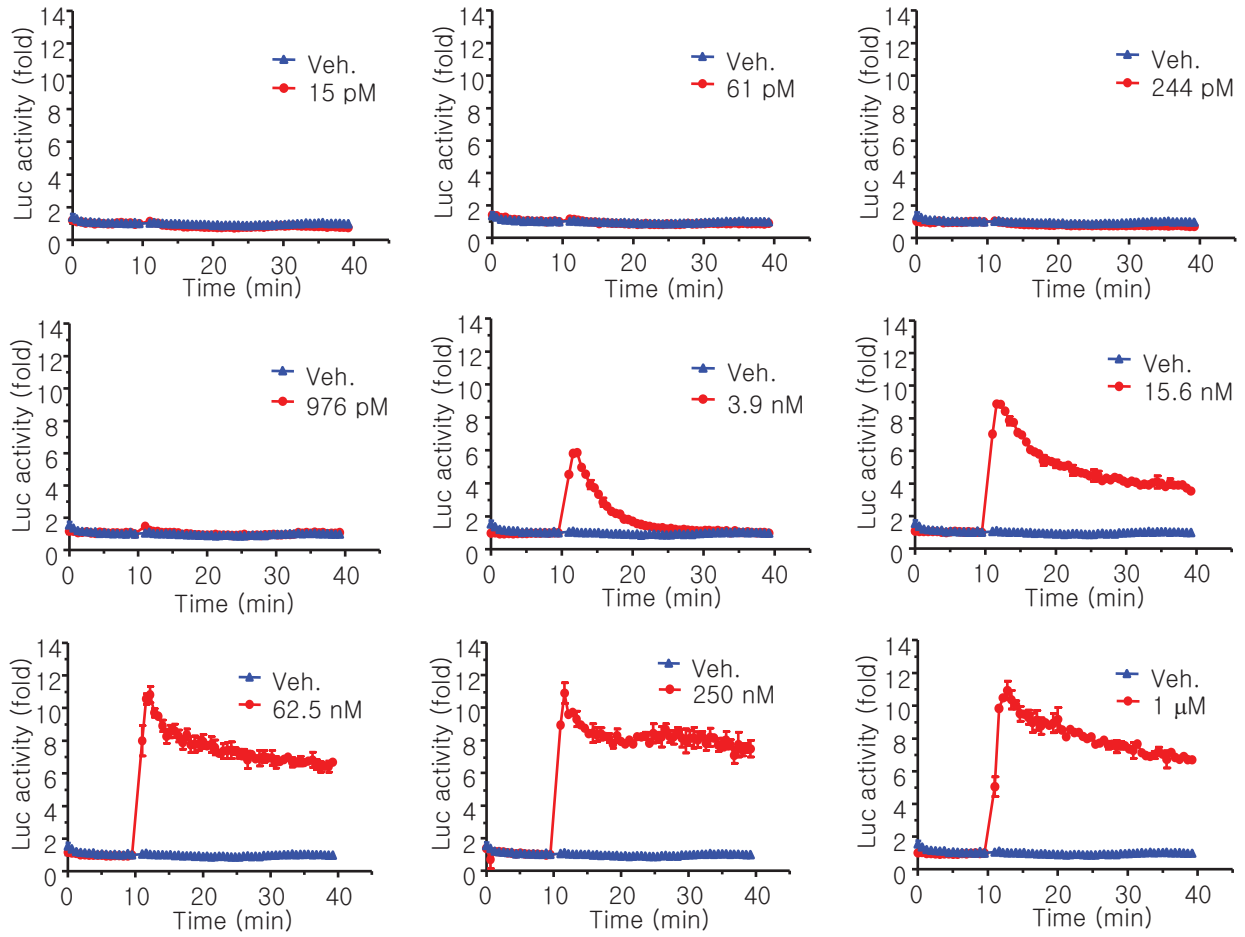
Supplementary Fig. S6. Dose dependency of the luciferase activities by angiotensin II in cells expressing calmodulin-SmBiT and LgBiT-MYLK2S, and UbiC-driven AGTR1.

5-HT_{2A}R: EF/LgBiT-MYLK2S-IRES-CM-SmBiT (5-HT)



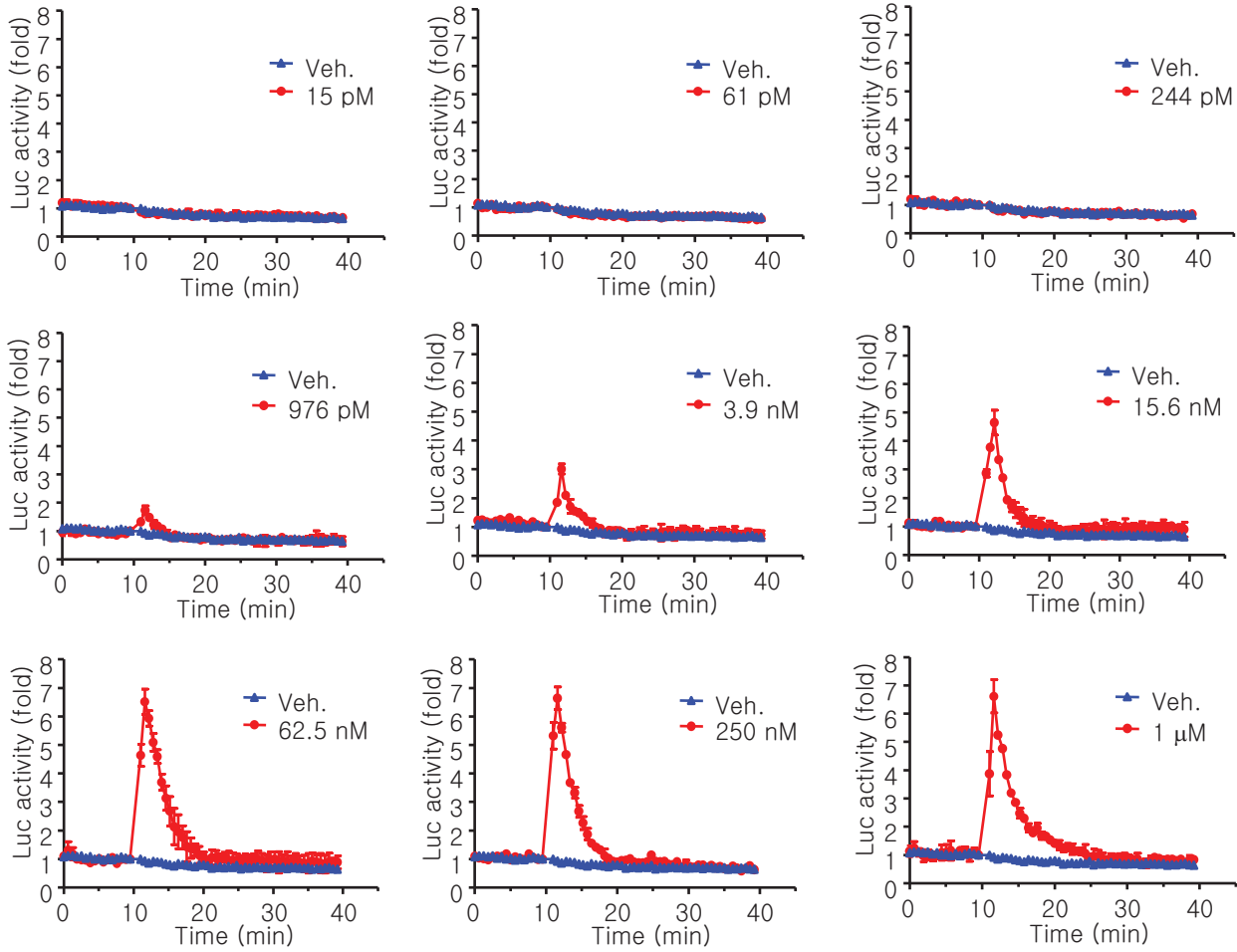
Supplementary Fig. S7. Dose dependency of the luciferase activities by 5-HT in cells expressing EF-driven calmodulin-SmBiT-IRES-LgBiT-MYLK2S, and UbiC-driven 5-HT_{2A}R.

TACR1: EF/LgBiT-MYLK2S-IRES-CM-SmBiT (Substance P)



Supplementary Fig. S8. Dose dependency of the luciferase activities by substance P in cells expressing EF-driven calmodulin-SmBiT-IRES-LgBiT-MYLK2S, and UbiC-driven TACR1.

AGTR1: EF/LgBiT-MYLK2S-IRES-CM-SmBiT (Ang II)



Supplementary Fig. S9. Dose dependency of the luciferase activities by Angiotensin II in cells expressing EF-driven calmodulin-SmBiT-IRES-LgBiT-MYLK2S, and UbiC-driven AGTR1.

Supplementary Table S1. Representative luciferase activities (RLU) from all constructs

	Constructs	Basal	Highest	Fold-increase	Note
Fig. 1	SmBiT-CM/LgBiT-MYLK1S	12128	40497	3.34	HEK293T cells pNB vectors (HSV-tk) CM: calmodulin
	SmBiT-CM/MYLK1S-LgBiT	45427	70603	1.55	
	LgBiT-CM/MYLK1-SmBiT	7099	7217	1.02	
	LgBiT-CM/SmBiT-MYLK1S	342	345	1.01	
	CM-SmBiT/LgBiT-MYLK1S	12663	33933	2.68	
	CM-SmBiT/MYLK1S-LgBiT	3104	6110	1.97	
	CM-LgBiT/SmBiT-MYLK1S	345	345	1.00	
Fig. 2	CM-LgBiT/MYLK1S-SmBiT	187	219	1.17	HEK293 cells Promoter : UbiC CM: calmodulin
	SmBiT-CM/LgBiT-MYLK1S	1879	5880	3.13	
	SmBiT-CM/MYLK1S-LgBiT	2046	1863	0.91	
	CM-SmBiT/LgBiT-MYLK1S	2813	11070	3.94	
	CM-SmBiT/MYLK1S-LgBiT	534	517	0.97	
	SmBiT-CM/LgBiT-MYLK2S	423	2002	4.73	
	SmBiT-CM/MYLK2S-LgBiT	283	476	1.68	
	CM-SmBiT/LgBiT-MYLK2S	2081	17767	8.54	
	CM-SmBiT/MYLK2S-LgBiT	1150	5568	4.84	
	SmBiT-CM/LgBiT-CAMK2S	12645	19841	1.57	
	SmBiT-CM/CAMK2S-LgBiT	8292	8617	1.04	
Fig. 3	CM-SmBiT/LgBiT-CAMK2S	4363	14423	3.31	HEK293 cells Receptor: CMV CM, MYLK2S: UbiC
	CM-SmBiT/CAMK2S-LgBiT	1358	2836	2.09	
	5-HT2A/CM-SmBiT/LgBiT-MYLK2S	3444	19743	5.73	
	AGTR1/CM-SmBiT/LgBiT-MYLK2S	2776	33372	12.02	
	TACR1/CM-SmBiT/LgBiT-MYLK2S	5020	76072	15.15	
	ADRA1A/CM-SmBiT/LgBiT-MYLK2S	3873	28002	7.23	
Fig. 4	ADRB1/CM-SmBiT/LgBiT-MYLK2S	3155	4680	1.48	Receptor: CMV Receptor: UbiC Receptor: HSV-tk
	EGFR/CM-SmBiT/LgBiT-MYLK2S	1102	8179	7.42	
	5-HT2A/CM-SmBiT/LgBiT-MYLK2S	138678	661939	4.77	
		7490	186395	24.89	
		10957	198359	18.10	
	TACR1/CM-SmBiT/LgBiT-MYLK2S	7917	101422	12.81	
		3440	114899	33.40	
		5228	46022	8.80	
	AGTR1/CM-SmBiT/LgBiT-MYLK2S	7032	38679	5.50	
3763		54342	14.44		
5475		47693	8.71		
Fig. 5	SmBiT-CM-T2A-LgBiT-MYLK2S	45817	55709	1.22	UbiC promoter
		CM-SmBiT-T2A-LgBiT-MYLK2S	760273	1093633	
	UbiC/LgBiT-MYLK2S-IRES-CM-SmBiT	7607	22226	2.92	pCubi3.1-TACR1
		7495	30177	4.03	pCubi3.1-ADRA1A
		10527	19630	1.86	pCubi3.1-AGTR1
	EF/LgBiT-MYLK2S-IRES-CM-SmBiT	28637	171290	5.98	pCubi3.1-TACR1
		31267	207573	6.64	pCubi3.1-ADRA1A
		12577	47307	3.76	pCubi3.1-AGTR1
	CMV/LgBiT-MYLK2S-IRES-CM-SmBiT	322603	1228985	3.81	pCubi3.1-TACR1
		413580	1599206	3.87	pCubi3.1-ADRA1A
343816		671899	1.95	pCubi3.1-AGTR1	
Fig. 6	5-HT2A:EF/LgBiT-MYLK2S-IRES-CM-SmBiT	99197	393611	3.97	Receptor: CMV
		4624	33414	7.23	Receptor: UbiC
		12918	33287	2.58	Receptor: HSV-tk
	TACR1:EF/LgBiT-MYLK2S-IRES-CM-SmBiT	34601	153666	4.44	Receptor: CMV
		11645	114485	9.83	Receptor: UbiC
		13205	55106	4.17	Receptor: HSV-tk
	AGTR1:EF/LgBiT-MYLK2S-IRES-CM-SmBiT	11244	57681	5.13	Receptor: CMV
		9737	72657	7.46	Receptor: UbiC
		13279	48527	3.65	Receptor: HSV-tk