Supplementary Information (SI) for Chemical Communications.

Supplementary Materials for:

Development of MhOR1 as a Chemogenetic Tool for Odorant-Mediated Regulation of Insulin Release

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1.Materials and methods

Plasmid Construction

The MhOR1 was synthesized (Beijing Tsingke Biotech Co., Ltd. China). mCherry-P2A fragement was amplified by primers. According to the instruction of Gibson Assembly kit (TransGen Biotech., China), a 15-25 bp homologous fragments were introduced in both terminals of mCherry-P2A and MhOR1 and subsequently cloned into pLenti-MCS-SV40-Puro empty vector that linearized by XbaI and SpeI restriction enzymes (New England Biolabs, Inc., USA).

For the optimization of MhOR1, different signal peptides were added into the indicated terminal of MhOR1 through primers. The “ERS”, “TS” referred to as ER signal peptides FCYENEV, golgi transport signals KSRITSEGEYIPLDQIDINV, respectively. Opsin (abbreviated as “O”) tag was MNGTEGPNFYVPFSNKTG. Lucy (abbreviated as “L”) tag was MRPQILLLALLTLGLA. The peptide “QVAPAGKPIPNPLLGLDSTVNKFSL”[1] represented the human Rhodopsin ^{344}QVAPA^{348} peptide (abbreviated as “R” tag), a V5 tag (GKPIPNPLLGLDST) and the human HCN1 peptide ^{106}VNKFSL^{111} (abbreviated as “E”). OR47a was synthesized (Beijing Tsingke Biotech Co., Ltd. China) and ORCO was amplified from pDmelOR (#126476, Addgene, USA). These two fragments were linked with IRES sequence. The above plasmids were assembled with the corresponding purified PCR fragments via Gibson Assembly kit. The sequence and other constructions used in this study are listed in Supplementary tables below and all plasmids used in this study were sequenced for validation.

Cell culture and transfection

Cancer cell lines COS-7, 293T, HeLa, and RIN-5F used in our research were purchased from ATCC. COS-7 and 293T were routinely grown in Dulbecco’s Modified Essential Media (DMEM, Hyclone), while RIN-5F cells were routinely grown in RPMI 1640. The complete medium was supplemented with 10% fetal bovine serum (PAN, PAN-Biotech, Germany) and 100 U/mL penicillin/streptomycin (Beyotime Biotechnology, China). The cells were cultured at 37 °C in a humidified atmosphere with 5% CO_2. For transient over-expression assay, the cells were plated on confocal dishes. About 1-2 μg indicated plasmid were used according to the instruction of lipofectamine 3000 (Thermo Fisher Scientific, USA). For the establishment of stable cell lines, plasmids of interest along with helper plasmids (pMD2.G and psPAX2) were transfected into 293T cells. Following a 24-hour incubation period, the culture medium was replaced with fresh medium. The transfected cells were then cultured for an additional 24 hours, after which the culture medium was harvested and centrifuged at 2,000 rpm/min for 5 minutes. The resulting supernatant was filtered and utilized for incubating wild-type cells with polybrene (1 μg/mL). Positive cell selection was carried out using puromycin (1 μg/mL).

Calcium influx detection

For calcium imaging, cells were plated on the confocal dish and then were co-transfected with indicated plasmid. The transfected cells were incubated in D-HANK's buffer and the basal state of cellular calcium was captured through a AX confocal laser scanning microscope (Nikon, Japan) equipped with a 60×/1.49 numerical aperture (NA) oil immersion objective lens. 500 μL buffer containing octanol or octanol processed with mechanochemical technique was carefully added into the dish and subsequently calcium dynamic was captured. In the detection of intracellular calcium levels using flow cytometry (Beckman Coulter Cytoflex S, Beckman, USA), the instrument parameters and gate settings were established by comparing non-transfected control cells with transfected cells. Before detection, odorant molecules were added to the flow tube, mixed thoroughly, and then the samples were analyzed. For measuring calcium signals with patch-clamp, cells were seeded on round cover glass one day ahead. Borosilicate pipettes were pulled by a horizontal puller (PC-1000, Narishige, USA) to a resistance of 4-6 MΩ on the day of the experiment. The culture medium was replaced with the buffer (135 mM K-Gluconate, 10 mM HEPES, 4 mM KCl, 4 mM $CaCl_2$, 1 mM EGTA, 0.3 mM Na-GTP, 4 mM Mg-ATP, 10 mM Na_2-phosphocreatine). Using a 20× objective (LUMPLFL40XW/IR, NA 0.8, Olympus) to locate a single cell. Calcium dynamics were recorded by the amplifier (PC-505B, Warner, USA) at room temperature with consistent buffer perfusion. Pipette pressure during patch clamp steps was digitally controlled. For the insulin vesicle release assay, cells were incubate in KRBH buffer. Adjusting the angle of the incident light and entering the total internal reflection range. The first image was captured with a TIRF microscope (Olympus). Octanol (500 μM) was then added to stimulate the O.MhOR1-expressed RIN-5F cells and final images were captured after 5 min.

Improvement of octanol solubility by mechanochemical technique

The mechanochemical processing of octanol was conducted using a ball mill (Wiggens ML007, Germany). Briefly, octanol and either disodium glycyrrhizinate or HP-β-CD were combined in the drum (300 mL volume) at the indicated ratio (*w*:*w*). Additionally, eight grinding media steel balls (10 mm in diameter) were introduced into the drum. The ball mill operated under the following conditions: a rotational speed of 60 rpm/min and a processing duration of 4 hours.

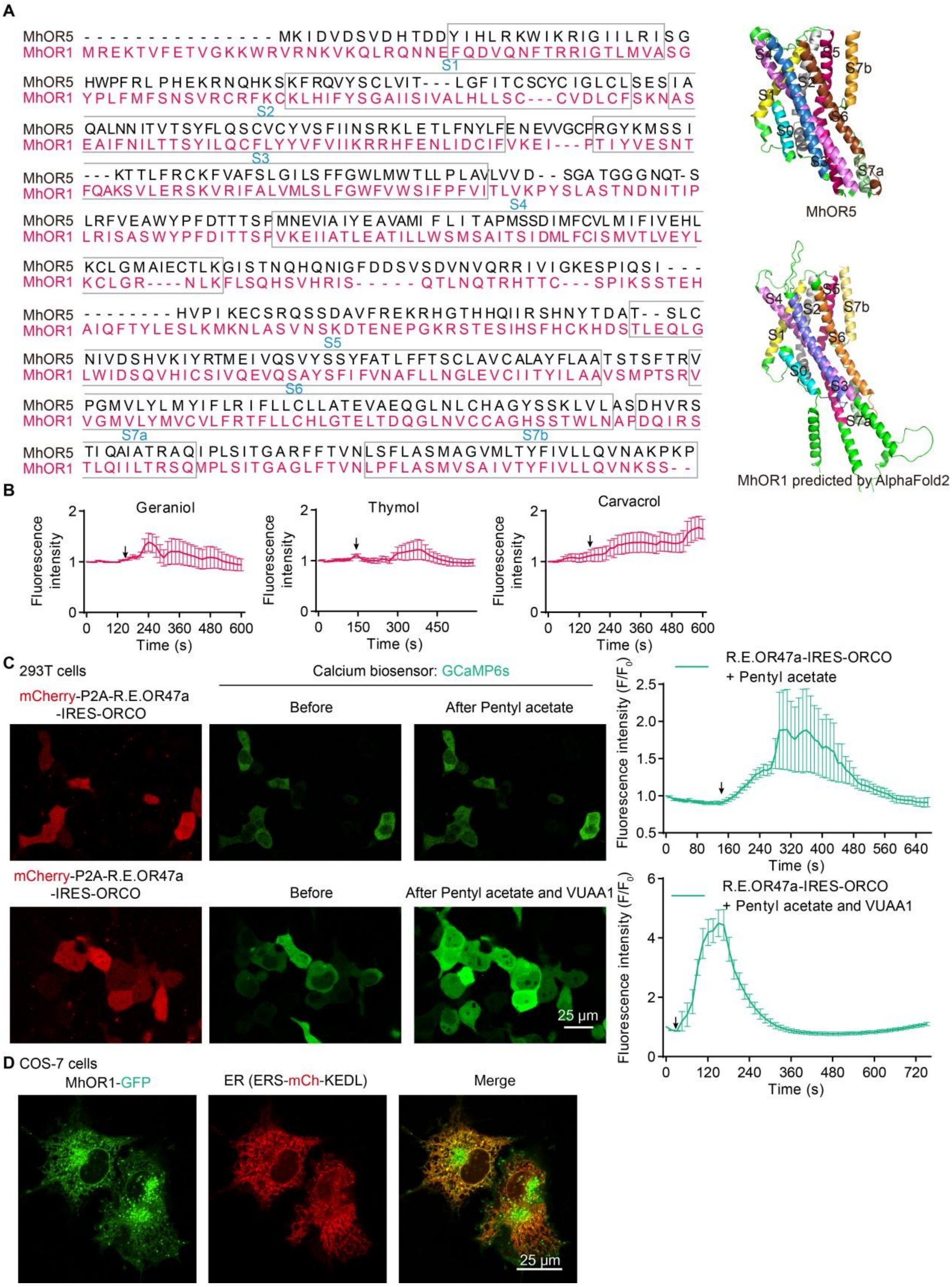
Detecting insulin levels in the culture medium

RIN-5F cells stably expressing O.MhOR1 were seeded in 12-well plates. Once the cells reached 80% confluence, 1 mL of D-Hank's buffer containing 250 μM octanol was added to each well for a 5-minute stimulation. After stimulation, 1 mL of fresh culture medium was added, and insulin was collected. Four hours later, the medium was recovered, and 1 mL of D-Hank's buffer containing 250 μM octanol was added for another 5-minute stimulation. Then the previously collected medium was reintroduced, and this step was totally repeated four times. The final collected medium was centrifuged at 3000 rpm/min for 5 minutes, and insulin levels were measured using an insulin kit (HB127-Ra, Hengyuan, China) according to the manufacturer's instructions. 0.2 mL of lysis buffer was added to each well to extract proteins, and the protein concentration was used to normalize the cell number.

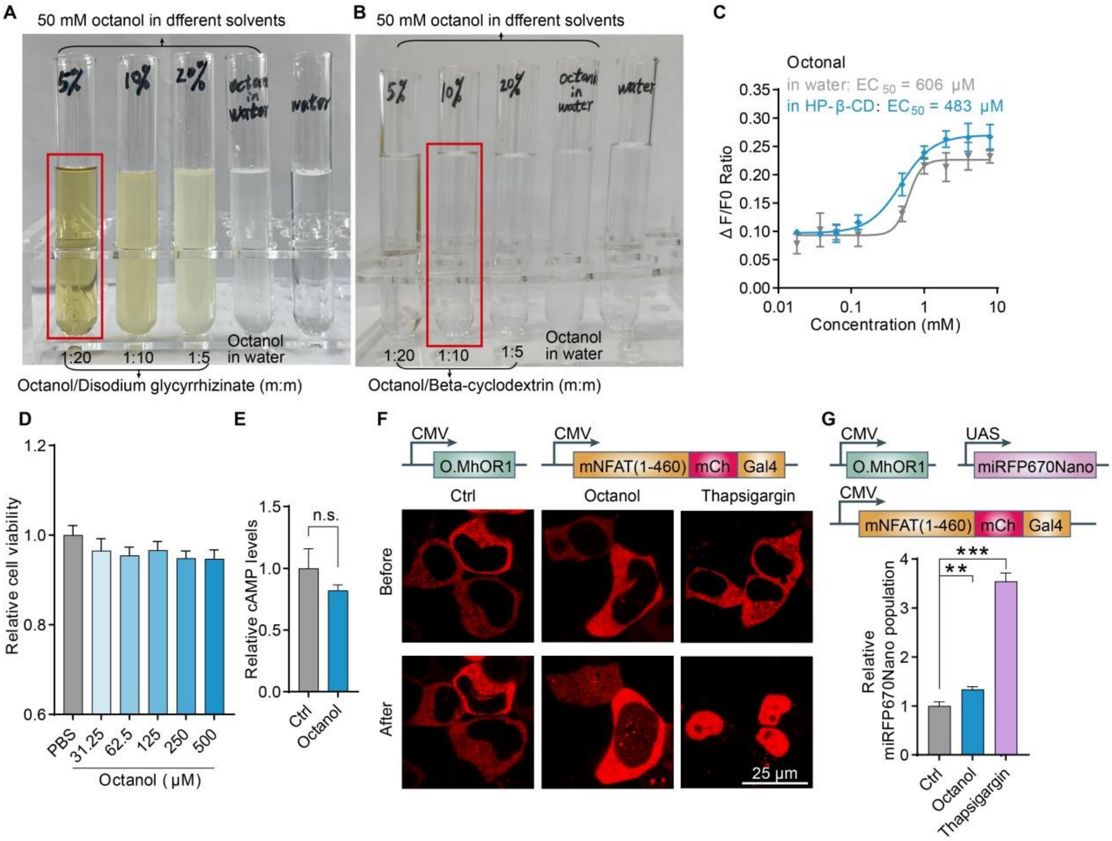
Statistical analysis

The fluorescence intensity of the images was analyzed by FiJi Image J software (Version 1.53t). Two experimental groups were analyzed by Unpaired Student's *t*-test (two-tailed). The *p* values less than 0.05 were considered as significant, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Quantitative data are presented as mean ± SEM. Details of the *n* number can be found in the appropriate figure legend. GraphPad Prism were used for data analysis and plot.

2. Supplementary figures (1-2)



Supplementary figure 1 the olfactory receptor mediated calcium influx in mammalian cells. **A**, the structural information of MhOR5 and its alignment with MhOR1, the secondary structure of MhOR1 was predicted by AlphaFold 2. **B**, quantitative analysis of geraniol-, thymol, and carvacrol (2 mM)-induced calcium flux in MhOR1-expressed cells (n ≥ 17 cells). **C**, the characteristic of OR47a-mediated calcium influx in mammalian cells (n ≥ 10 cells), the final concentration of VUAA1 and pentyl acetate were 100 μM. **D**, cellular localization of MhOR1 in COS-7 cells.



Supplementary figure 2 enhancement of the administration of hydrophobic ordorant ligands with the mechanochemical technique. Octanol was mixed with the pharmaceutical adjuvants disodium glycyrrhizinate (**A**) and HP-β-CD (**B**) in indicated ratio (*w* : *w*) and subsequently was dissolved in water at a 50-mM final concentration. **C**, the EC_{50} of octanol in induction of calcium influx in stably O.MhOR-expressed 293T cells, the fluorescence intensity was measured by fluorescence microplate (n = 4). **D**, changes in cell viability after treatments of different doses of octanol for four hours (n = 6). **E**, the intracellular cAMP levels in cells expressing O.MhOR1 after treatment with or without octanol (500 μM) for four hours (n ≥ 3) . **F**, the hybrid NFAT entered into the nucleus after octanol stimulation for one hour in 293T cells with co-expression of NFAT-mCh-Gal4 and O.MhOR1, thapsigargin (2 μM) as a positive control. **G**, MhOR1-mediated gene expression by octanol (500 μM), the cells were first incubated with solvent, octanol, thapsigargin and cell population of miRFP670 was analyzed with flow cytometer (n = 6).

3. sequence of plasmids used in this study.

Plasmid	Sequence
pLenti-mCh -P2A-MhOR1	mCh:red P2A:pink MhOR1:magenta MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGP LPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDS SLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKD GGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDE LYKATNFSLLKQAGDVEENPGPMREKTVFETVGKKWRVRNKVKQLRQNNEFQDVQNF TRRIGTLMVASGYPLFMFSNSVRCRFKCKLHIFYSGAIISIVALHLLSCCVDLCFSKNASEAI FNILTTSYILQCFLYYVFVIIKRRHFENLIDCIFVKEIPTIYVESNTFQAKSVLERSKVRIFALV MLSLFGWFVWSIFPFVITLVKPYSLASTNDNITIPLRISASWYPFDITTSPVKEIIATLEATILL WSMSAITSIDMLFCISMVTLVEYLKCLGRNLKFLSQHSVHRISQTLNQTRHTTCSPIKSST EHAIQFTYLESLKMKNLASVNSKDTENEPGKRSTESIHSFHCKHDSTLEQLGLWIDSQVHI CSIVQEVQSAYSFIFVNAFLLNGLEVCIITYILAAVSMPTSRVVGMVLYMVCVLFRTFLLCH LGTELTDQGLNVCCAGHSSTWLNAPDQIRSTLQIILTRSQMPLSITGAGLFTVNLPFLAS MVSAIVTYFIVLLQVNKSS
pLenti- MhOR1(O.MhO R1,L.MhOR1 or L.O.MhOR1)- 2×Flag	MhOR1:magenta linker:gray 2×Flag:red opsin tag:orange lucy:pink MREKTVFETVGKKWRVRNKVKQLRQNNEFQDVQNFTRRIGTLMVASGYPLFMFSNSV RCRFKCKLHIFYSGAIISIVALHLLSCCVDLCFSKNASEAIFNILTTSYILQCFLYYVFVIIKRRH FENLIDCIFVKEIPTIYVESNTFQAKSVLERSKVRIFALVMLSLFGWFVWSIFPFVITLVKPYS LASTNDNITIPLRISASWYPFDITTSPVKEIIATLEATILLWSMSAITSIDMLFCISMVTLVEY LKCLGRNLKFLSQHSVHRISQTLNQTRHTTCSPIKSSTEHAIQFTYLESLKMKNLASVNSK DTENEPGKRSTESIHSFHCKHDSTLEQLGLWIDSQVHICSIVQEVQSAYSFIFVNAFLLNG LEVCIITYILAAVSMPTSRVVGMVLYMVCVLFRTFLLCHLGTELTDQGLNVCCAGHSSTW LNAPDQIRSTLQIILTRSQMPLSITGAGLFTVNLPFLASMVSAIVTYFIVLLQVNKSSGSDY KDDDDKDYKDDDDK **O.MhOR1:** MNGTEGPNFYVPFSNKTGVVRSMREKTVFETVGKKWRVRNKVKQLRQNNEFQDVQN FTRRIGTLMVASGYPLFMFSNSVRCRFKCKLHIFYSGAIISIVALHLLSCCVDLCFSKNASEA IFNILTTSYILQCFLYYVFVIIKRRHFENLIDCIFVKEIPTIYVESNTFQAKSVLERSKVRIFALV MLSLFGWFVWSIFPFVITLVKPYSLASTNDNITIPLRISASWYPFDITTSPVKEIIATLEATILL WSMSAITSIDMLFCISMVTLVEYLKCLGRNLKFLSQHSVHRISQTLNQTRHTTCSPIKSST EHAIQFTYLESLKMKNLASVNSKDTENEPGKRSTESIHSFHCKHDSTLEQLGLWIDSQVHI CSIVQEVQSAYSFIFVNAFLLNGLEVCIITYILAAVSMPTSRVVGMVLYMVCVLFRTFLLCH LGTELTDQGLNVCCAGHSSTWLNAPDQIRSTLQIILTRSQMPLSITGAGLFTVNLPFLAS MVSAIVTYFIVLLQVNKSSGSDYKDDDDKDYKDDDDK **L.MhOR1:** MRPQILLLALLTLGLAMREKTVFETVGKKWRVRNKVKQLRQNNEFQDVQNFTRRIGTL MVASGYPLFMFSNSVRCRFKCKLHIFYSGAIISIVALHLLSCCVDLCFSKNASEAIFNILTTS YILQCFLYYVFVIIKRRHFENLIDCIFVKEIPTIYVESNTFQAKSVLERSKVRIFALVMLSLFG WFVWSIFPFVITLVKPYSLASTNDNITIPLRISASWYPFDITTSPVKEIIATLEATILLWSMSA ITSIDMLFCISMVTLVEYLKCLGRNLKFLSQHSVHRISQTLNQTRHTTCSPIKSSTEHAIQFT YLESLKMKNLASVNSKDTENEPGKRSTESIHSFHCKHDSTLEQLGLWIDSQVHICSIVQEV QSAYSFIFVNAFLLNGLEVCIITYILAAVSMPTSRVVGMVLYMVCVLFRTFLLCHLGTELTD

	QGLNVCCAGHSSTWLNAPDQIRSTLQIILTRSQMPLSITGAGLFTVNLPFLASMVSAIVTYFIVLLQVNKSSGSDYKDDDDKDYKDDDDK **L.O.MhOR1:**MRPQILLLALLTLGLAGSNGTEGPNFYVPFSNKTGVVRSMREKTVFETVGKKWRVRNKVKQLRQNNEFQDVQNFTRRIGTLMVASGYPLFMFSNSVRCRFKCKLHIFYSGAIISIVALHLLSCCVDLCFSKNASEAIFNILTTSYILQCFLYYVFVIIKRRHFENLIDCIFVKEIPTIYVESNTFQAKSVLERSKVRIFALVMLSLFGWFVWSIFPFVITLVKPYSLASTNDNITIPLRISASWYPFDITTSPVKEIIATLEATILLWSMSAITSIDMLFCISMVTLVEYLKCLGRNLKFLSQHSVHRISQTLNQTRHTTCSPIKSSTEHAIQFTYLESLKMKNLASVNSKDTENEPGKRSTESIHSFHCKHDSTLEQLGLWIDSQVHICSIVQEVQSAYSFIFVNAFLLNGLEVCIITYILAAVSMPTSRVVGMVLYMVCVLFRTFLLCHLGTELTDQGLNVCCAGHSSTWLNAPDQIRSTLQIILTRSQMPLSITGAGLFTVNLPFLASMVSAIVTYFIVLLQVNKSSGSDYKDDDDKDYKDDDDK
pLenti-MhOR1-2×Flag-ERS	MhOR1:magenta linker:gray 2×Flag:red ERS:blue MREKTVFETVGKKWRVRNKVKQLRQNNEFQDVQNFTRRIGTLMVASGYPLFMFSNSVRCRFKCKLHIFYSGAIISIVALHLLSCCVDLCFSKNASEAIFNILTTSYILQCFLYYVFVIIKRRHFENLIDCIFVKEIPTIYVESNTFQAKSVLERSKVRIFALVMLSLFGWFVWSIFPFVITLVKPYSLASTNDNITIPLRISASWYPFDITTSPVKEIIATLEATILLWSMSAITSIDMLFCISMVTLVEYLKCLGRNLKFLSQHSVHRISQTLNQTRHTTCSPIKSSTEHAIQFTYLESLKMKNLASVNSKDTENEPGKRSTESIHSFHCKHDSTLEQLGLWIDSQVHICSIVQEVQSAYSFIFVNAFLLNGLEVCIITYILAAVSMPTSRVVGMVLYMVCVLFRTFLLCHLGTELTDQGLNVCCAGHSSTWLNAPDQIRSTLQIILTRSQMPLSITGAGLFTVNLPFLASMVSAIVTYFIVLLQVNKSSGSDYKDDDDKDYKDDDDKGSFCYENEV This construction was amplified for cloning other constructions like O.MhOR1-2×Flag-ERS, L.MhOR1-2×Flag-ERS, L.O.MhOR1-2×Flag-ERS.
pLenti-MhOR1-TS-2×Flag-ERS	MhOR1:magenta linker:gray 2×Flag:red TS:green ERS:blue MREKTVFETVGKKWRVRNKVKQLRQNNEFQDVQNFTRRIGTLMVASGYPLFMFSNSVRCRFKCKLHIFYSGAIISIVALHLLSCCVDLCFSKNASEAIFNILTTSYILQCFLYYVFVIIKRRHFENLIDCIFVKEIPTIYVESNTFQAKSVLERSKVRIFALVMLSLFGWFVWSIFPFVITLVKPYSLASTNDNITIPLRISASWYPFDITTSPVKEIIATLEATILLWSMSAITSIDMLFCISMVTLVEYLKCLGRNLKFLSQHSVHRISQTLNQTRHTTCSPIKSSTEHAIQFTYLESLKMKNLASVNSKDTENEPGKRSTESIHSFHCKHDSTLEQLGLWIDSQVHICSIVQEVQSAYSFIFVNAFLLNGLEVCIITYILAAVSMPTSRVVGMVLYMVCVLFRTFLLCHLGTELTDQGLNVCCAGHSSTWLNAPDQIRSTLQIILTRSQMPLSITGAGLFTVNLPFLASMVSAIVTYFIVLLQVNKSSGSKSRITSEGEYIPLDQIDINVDYKDDDDKDYKDDDDKGSFCYENEV This construction was amplified for cloning other constructions like O.MhOR1-TS-2×Flag-ERS, L.MhOR1-TS-2×Flag-ERS, L.O.MhOR1-TS-2×Flag-ERS.
pLenti-L.R.E.MhOR1-TS-Flag-ERS	MhOR1:magenta linker:gray 2×Flag:red TS:green ERS:blue lucy:pink **R**.V5.**E** tag:orange MRPQILLLALLTLGLA**DQVAPA**GKPIPNPLLGLDST**VNKFSL**MREKTVFETVGKKWRVRNKVKQLRQNNEFQDVQNFTRRIGTLMVASGYPLFMFSNSVRCRFKCKLHIFYSGAIISIVALHLLSCCVDLCFSKNASEAIFNILTTSYILQCFLYYVFVIIKRRHFENLIDCIFVKEIPTIYVESNTFQAKSVLERSKVRIFALVMLSLFGWFVWSIFPFVITLVKPYSLASTNDNITIPLRISASWYPFDITTSPVKEIIATLEATILLWSMSAITSIDMLFCISMVTLVEYLKCLGRNLKFLSQHSV

	HRISQTLNQTRHTTCSPIKSSTEHAIQFTYLESLKMKNLASVNSKDTENEPGKRSTESIHSF HCKHDSTLEQLGLWIDSQVHICSIVQEVQSAYSFIFVNAFLLNGLEVCIITYILAAVSMPTS RVVGMVLYMVCVLFRTFLLCHLGTELTDQGLNVCCAGHSSTWLNAPDQIRSTLQIILTRS QMPLSITGAGLFTVNLPFLASMVSAIVTYFIVLLQVNKSSGSKSRITSEGEYIPLDQIDINV DYKDDDDKDYKDDDDKGSFCYENEV
pLenti-mCh -P2A-O.MhOR1	mCh:red linker:gray P2A:pink MhOR1:magenta opsin tag:orange MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGP LPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDS SLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKD GGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDE LYKATNFSLLKQAGDVEENPGPMNGTEGPNFYVPFSNKTGVVRSMREKTVFETVGKK WRVRNKVKQLRQNNEFQDVQNFTRRIGTLMVASGYPLFMFSNSVRCRFKCKLHIFYSG AIISIVALHLLSCCVDLCFSKNASEAIFNILTTSYILQCFLYYVFVIIKRRHFENLIDCIFVKEIPTI YVESNTFQAKSVLERSKVRIFALVMLSLFGWFVWSIFPFVITLVKPYSLASTNDNITIPLRIS ASWYPFDITTSPVKEIIATLEATILLWSMSAITSIDMLFCISMVTLVEYLKCLGRNLKFLSQH SVHRISQTLNQTRHTTCSPIKSSTEHAIQFTYLESLKMKNLASVNSKDTENEPGKRSTESIH SFHCKHDSTLEQLGLWIDSQVHICSIVQEVQSAYSFIFVNAFLLNGLEVCIITYILAAVSMP TSRVVGMVLYMVCVLFRTFLLCHLGTELTDQGLNVCCAGHSSTWLNAPDQIRSTLQIILT RSQMPLSITGAGLFTVNLPFLASMVSAIVTYFIVLLQVNKSS
pLenti-mCherry -P2A-OR47a-IRES-ORCO	mCh:red P2A:pink OR47a:magenta MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGP LPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDS SLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKD GGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDE LYKATNFSLLKQAGDVEENPGPDQVAPAGKPIPNPLLGLDSTVNKFSLVNKFSLDSFLQV QKSTIALLGFDLFSENREMWKRPYRAMNVFSIAAIFPFILAAVLHNWKNVLLLADAMVA LLITILGLFKFSMILYLRRDFKRLIDKFRLLMSNEAEQGEEYAEILNAANKQDQRMCTLFRT CFLLAWALNSVLPLVRMGLSYWLAGHAEPELPFPCLFPWNIHIIRNYVLSFIWSAFASTG VVLPAVSLDTIFCSFTSNLCAFFKIAQYKVVRFKGGSLKESQATLNKVFALYQTSLDMCND LNQCYQPIICAQFFISSLQLCMLGYLFSITFAQTEGVYYASFIATIIIQAYIYCYCGENLKTES ASFEWAIYDSPWHESLGAGGASTSICRSLLISMMRAHRGFRITGYFFEANMEAFSSIVRT AMSYITMLRSFS **ORCO**: MASTTSMQPSKYTGLVADLMPNIRAMKYSGLFMHNFTGGSAFMKKVYSSVHLVFLLM QFTFILVNMALNAEEVNELSGNTITTLFFTHCITKFIYLAVNQKNFYRTLNIWNQVNTHPL FAESDARYHSIALAKMRKLFFLVMLTTVASATAWTTITFFGDSVKMVVDHETNSSIPVEI PRLPIKSFYPWNASHGMFYMISFAFQIYYVLFSMIHSNLCDVMFCSWLIFACEQLQHLK GIMKPLMELSASLDTYRPNSAALFRSLSANSKSELIHNEEKDPGTDMDMSGIYSSKADW GAQFRAPSTLQSFGGNGGGGNGLVNGANPNGLTKKQEMMVRSAIKYWVERHKHVV RLVAAIGDTYGAALLLHMLTSTIKLTLLAYQATKINGVNVYAFTVVGYLGYALAQVSIKVT RQV
pLenti-MhOR1-GFP	MhOR1:magenta linker:gray GFP:green MREKTVFETVGKKWRVRNKVKQLRQNNEFQDVQNFTRRIGTLMVASGYPLFMFSNSV

	RCRFKCKLHIFYSGAIISIVALHLLSCCVDLCFSKNASEAIFNILTTSYILQCFLYYVFVIIKRRH FENLIDCIFVKEIPTIYVESNTFQAKSVLERSKVRIFALVMLSLFGWFVWSIFPFVITLVKPYS LASTNDNITIPLRISASWYPFDITTSPVKEIIATLEATILLWSMSAITSIDMLFCISMVTLVEY LKCLGRNLKFLSQHSVHRISQTLNQTRHTTCSPIKSSTEHAIQFTYLESLKMKNLASVNSK DTENEPGKRSTESIHSFHCKHDSTLEQLGLWIDSQVHICSIVQEVQSAYSFIFVNAFLLNG LEVCIITYILAAVSMPTSRVVGMVLYMVCVLFRTFLLCHLGTELTDQGLNVCCAGHSSTW LNAPDQIRSTLQIILTRSQMPLSITGAGLFTVNLPFLASMVSAIVTYFIVLLQVNKSSSRMV SKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLV TTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTL VNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLA DHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK
pLenti-ERS-mCh-KEDL	ER signal peptide:blue mCh:red linker:gray KEDL:orange MLLSVPLLLGLLGLAVALEMVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEG RPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWE RVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERM YPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTI VEQYERAEGRHSTGGMDELYKGSKEDL
pCMV-mNFAT-mCh-Gal4	Mouse NFAT (4-460):orange mCh:red Gal4:blue linker:gray MIFYPYDVPDYAGYPYDVPDYAGSYPYDVPDYAAQCGRSSPEPQPDPDGGDGPGHEP GGSPQDELDFSILFDYDYLNPIEEEPIAHKAISSPSGLAYPDDVLDYGLKPCNPLASPSGEP PGRFGEPDSIGFQNFLSPVKPAGASGPSPRIEITPSHELMQAGGALRGRDAGLSPEQPAL ALAGVAASPRFTLPVPGYEGYREPLCLSPASSGSSASFISDTFSPYTSPCVSPNNAGPDDL CPQFQNIPAHYSPRTSPIMSPRTSLAEDSCLGRHSPVPRPASRSSSPGAKRRHSCAEALV APLPAASPQRSRSPSPQPSPHVAPQDDSIPAGYPPTAGSAVLMDALNTLATDSPCGIPS KIWKTSPDPTPVSTAPSKAGLARHIYPTVEFLGPCEQEERRNSAPESILLVPPTWPKQLVP AIPICSIPVTASLPPLEWPLSNQSGSYELRIEVQPKPHHRAHYETEGSRGAVKAPTGGHPV VQLHGYMENKPLGLQIDPPVATGSMVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFE IEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFP EGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWE ASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSH NEDYTIVEQYERAEGRHSTGGMDELYKGSKLLSSIEQACDICRLKKLKCSKEKPKCAKCLK NNWECRYSPKTKRSPLTRAHLTEVESRLERLEQLFLLIFPREDLDMILKMDSLQDIKALLT GLFVQDNVNKDAVTDRLASVETDMPLTLRQHRISATSSSEESSNKGQRQLTVSANFNQ SGNIADSSLSFTFTNSSNGPNLITTQTNSQALSQPIASSNVHDNFMNNEITASKIDDGNN SKPLSPGWTDQTAYNAFGITTGMFNTTTMDDVYNYLFDDEDTPPNPKKE

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