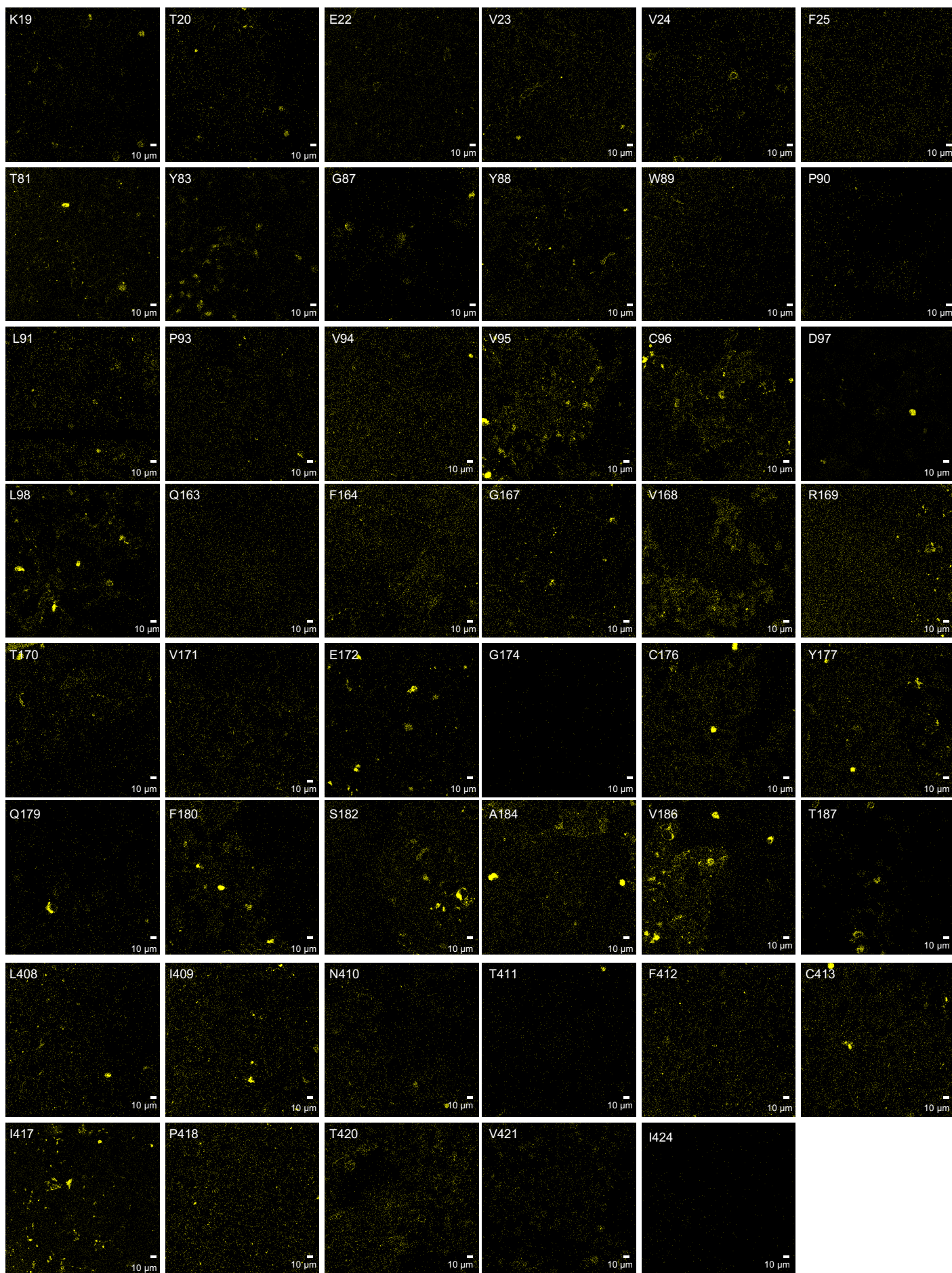
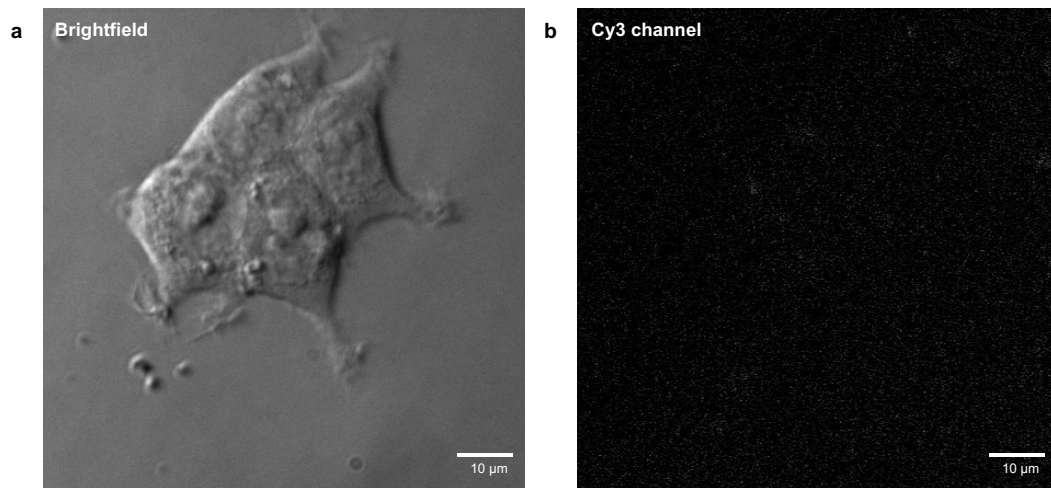

Supplementary information

Ligand-specific activation trajectories dictate GPCR signalling in cells

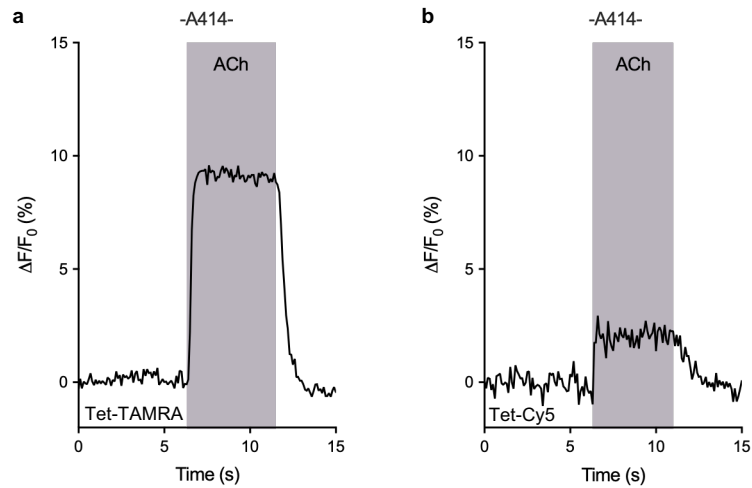
In the format provided by the
authors and unedited



Supplementary Figure 1: M₂R mutants that could not be labelled. Representative confocal images obtained from the labelling screen. The indicated M₂R mutants (SP-M₂R^{XXXTAG}) could not be labelled with Tet-Cy3 at the ncAA TCO*K incorporated at the indicated position (amino acid of the native human receptor). The data were obtained from at least 3 independent experiments.

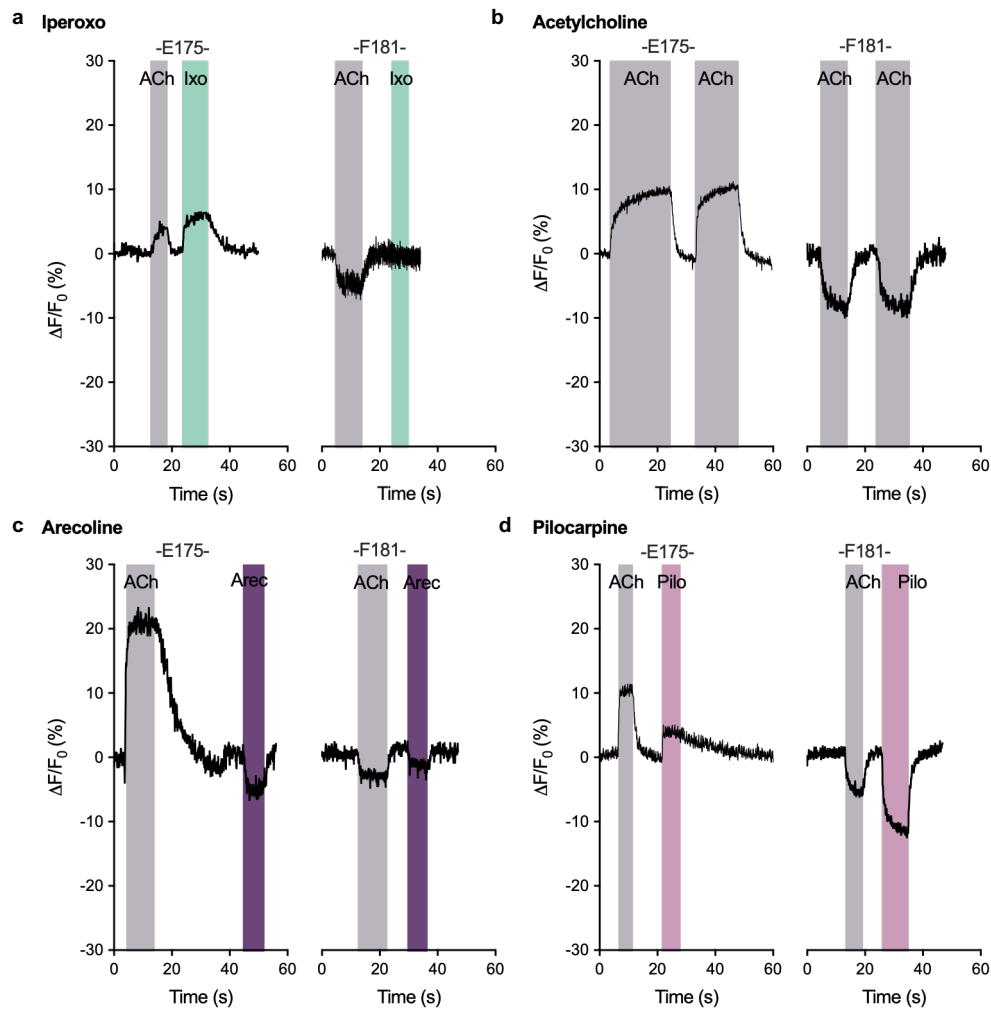


Supplementary Figure 2: Labelling of the M_2R^{414} biosensor in absence of the non-canonical amino acid TCO*K. Shown are representative epifluorescence images of HEK293T cells transfected with the cDNA of the tRNA synthetase, the tRNA cassette (*MbPylRS^{AF}/4xtRNA^{M15}*), and *SP-M₂R^{414TAG}* in absence of the ncAA TCO*K after labelling with Tet-Cy3. (a) brightfield, (b) Cy3 channel.

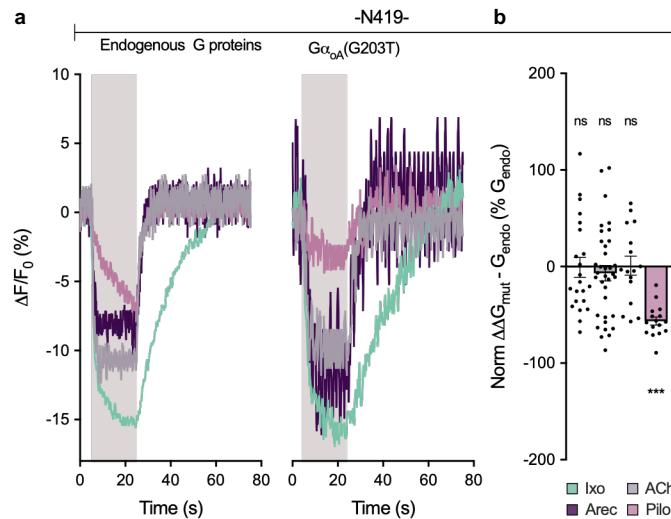


Supplementary Figure 3: Conformational changes can be monitored with fluorescent dyes of different chemistry. Representative traces of $\Delta F/F_0$ of single cells expressing the M_2R -A414 biosensor ($SP-M_2R^{A414TAG}$) and labelled with Tetrazine-TAMRA (a) or

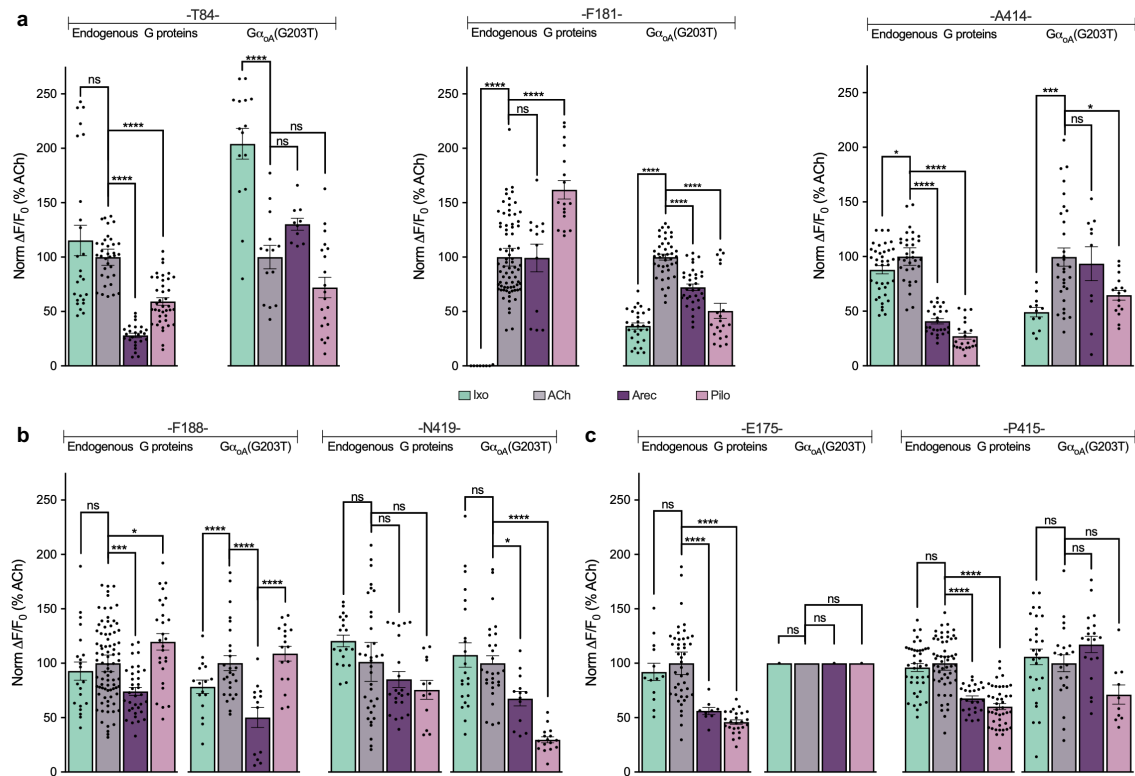
Tetrazine-Cyanine5 (b) stimulated with 1 mM ACh. Shaded areas indicate the duration of agonist superfusion, unshaded areas represent buffer application. Data obtained from 3 independent experiments. Number of cells (n): n = 31 (a), n = 27 (b).



Supplementary Figure 4: Agonist efficacies at biosensors are independent of the order of ligand addition. Representative fluorescence intensity changes ($\Delta F/F_0$) recorded in real-time from single HEK293T cells expressing indicated M_2R biosensors ($SP-M_2R^{XXXTAG}$) superfused with 1mM ACh, followed by (a) 100 μ M iperoxo (Ixo), (b) 1mM ACh, (c) 1 mM arecoline (Arec), or (d) 10 mM pilocarpine (Pilo), after washout with buffer, respectively. Application of different agonists is indicated with shaded areas in different colors. Non-shaded areas indicate buffer application. Number of individual cells (n, for Ixo, ACh, Arec, Pilo): E175 (n = 29, 54, 15, 22); F181 (n = 20, 68, 22, 14). Number of independent experiments (for Ixo, ACh, Arec, Pilo): E175 = 5, 9, 3, 3; F181 = 3, 12, 3, 3.



Supplementary Figure 5: Conformational changes at M₂R-N419 are independent of G-protein activity. (a) Representative traces of $\Delta F/F_0$ of single cells expressing the M₂R-N419 biosensor (*SP-M₂R^{N419TAG}*) at endogenous G-protein levels (left) or after G α_{oA} (G203T) overexpression (center) and stimulated with 1 mM ACh (grey), 100 μ M Ixo (green), 1 mM Arec (purple), or 10 mM Pilo (pink). Shaded areas indicate the duration of agonist superfusion, unshaded areas represent buffer application. (b) Statistical summary of the normalised changes in $\Delta F/F_0$ obtained from experiments in (a). Shown are the ligand-dependent differences in $\Delta F/F_0$ after G α_{oA} (G203T) overexpression (G_{mut}) normalised to endogenous G-protein levels (G_{endo} , set to 0%). Negative values indicate decreases in $\Delta F/F_0$ after G α_{oA} (G203T) overexpression, and positive values indicate increases in $\Delta F/F_0$ after G α_{oA} (G203T) overexpression. The bars represent means \pm sem, each data point represents a single cell. n, number of cells, number of independent experiments: n = 13, 3 (Ixo), n = 24, 4 (ACh), n = 13, 4 (Arec), n = 14, 3 (Pilo). ***p < 0.001 (0.0004), according to an unpaired two-tailed t-test. G_{endo} , endogenous G proteins; mut, mutant; ns, not significantly different.



Supplementary Figure 6: $G\alpha_{0A}(G203T)$ overexpression changes the equilibrium of receptor/G-protein complexes. Comparison of the ligand-dependent differences in $\Delta F/F_0$ after $G\alpha_{0A}(G203T)$ overexpression or at endogenous G-protein levels after activation of the indicated M_2R biosensors ($SP-M_2R^{XXXXTAG}$) with the ligands 100 μM Ixo (green), 1 mM ACh (grey), 1 mM Arec (purple), and 10 mM Pilo (pink). For each condition, the data has been normalised to the mean $\Delta F/F_0$ of ACh (set to 100%). The bars represent means \pm sem of at least 3 independent experiments, each data point represents a single cell. Number of individual cells (n, for Ixo, ACh, Arec, Pilo): n = 15, 14, 10, 20 (T84), n = 11, 10, 9, 11 (E175), n = 29, 41, 34, 20 (F181), n = 16, 26, 13, 17 (F188), n = 12, 31, 10, 16 (A414), n = 28, 21, 19, 10 (P415), n = 13, 24, 13, 14 (N419). ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05 according to an one-way analysis of variance (ANOVA multiple comparisons test) with Tukey's posthoc test. ns, not significantly different.

Supplementary Table 1 (related to Figs. 1 and 2): Mean $\Delta F/F_0$ [%] from M₂R biosensors stimulated with distinct agonists. N: total number of cells; Exp.: independent days of transfections; SEM: standard error of the mean.

T84 ^{TM2}					E175 ^{ECL2}				F181 ^{ECL2}			
Agonist	Mean	SEM	N	Exp.	Mean	SEM	N	Exp.	Mean	SEM	N	Exp.
Ixo	9.36	1.20	21	7	9.61	0.83	13	7	0.00	0.01	12	5
ACh	8.46	0.31	35	17	10.43	0.54	44	11	-5.86	0.24	76	9
Arec	-2.38	0.18	25	6	-5.86	0.35	9	4	-5.82	0.75	13	7
Pilo	5.02	0.29	39	7	4.80	0.22	25	5	-9.50	0.50	17	7

F188 ^{TM5}					A414 ^{ECL3}				P415 ^{ECL3}			
Agonist	Mean	SEM	N	Exp.	Mean	SEM	N	Exp.	Mean	SEM	N	Exp.
Ixo	-8.42	0.75	21	5	-14.4	0.61	38	12	-15.52	0.58	40	9
ACh	-11.10	0.52	96	15	-16.36	0.64	33	7	-16.15	0.52	56	9
Arec	-6.72	0.34	35	6	-6.68	0.38	26	11	-10.92	0.39	26	5
Pilo	-10.86	0.68	27	6	-4.44	0.46	22	3	-9.71	0.46	43	10

N419 ^{TM7}				
Agonist	Mean	SEM	N	Exp.
Ixo	-13.76	0.61	19	9
ACh	-11.66	1.02	34	12
Arec	-9.72	0.83	22	6
Pilo	-8.63	0.99	12	6

Supplementary Table 2 (related to Fig. 2 and Extended Data Fig. 5: Potency (pEC₅₀) and maximum efficacy (E_{max}) determined by G_{oA} TRUPATH assays of wildtype M₂R or M₂R biosensors stimulated with the indicated agonists. Each condition was performed as 3-4 independent experiments. E_{max} indicates the BRET response normalised to the maximum BRET response of ACh (% of ACh) for the respective conditions of receptor and ligand. SEM: standard error of the mean.

Wildtype M ₂ R								
Agonist	Ixo		ACh		Arec		Pilo	
	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}
Mean	10.36	100.70	7.93	100.40	6.66	116.10	2.10	85.49
SEM	0.06	0.58	0.11	0.61	0.12	3.64	0.18	0.86
T84 ^{TM2}								
Agonist	Ixo		ACh		Arec		Pilo	
	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}
Mean	10.21	106.30	8.11	102.00	6.67	114.00	6.61	71.00
SEM	0.15	1.09	0.15	0.47	0.12	1.28	0.19	1.48
E175 ^{ECL2}								
Agonist	Ixo		ACh		Arec		Pilo	
	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}
Mean	10.32	108.40	8.25	101.3	7.02	120.2	6.28	82.48
SEM	0.17	0.90	0.14	1.54	0.14	1.49	0.22	2.03
F181 ^{ECL2}								
Agonist	Ixo		ACh		Arec		Pilo	
	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}
Mean	8.36	102.40	5.81	101.60	5.10	49.97	4.97	27.92
SEM	0.10	0.74	0.08	0.70	0.12	1.09	0.01	0.58
F188 ^{TM5}								
Agonist	Ixo		ACh		Arec		Pilo	
	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}
Mean	9.58	105.80	7.02	103.80	6.06	106.00	5.60	61.62
SEM	0.03	1.47	0.02	1.63	0.02	2.00	0.08	1.82
A414 ^{ECL3}								
Agonist	Ixo		ACh		Arec		Pilo	
	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}
Mean	10.81	106.00	8.60	99.90	7.90	113.80	6.57	83.52
SEM	0.09	1.14	0.06	1.16	0.09	1.25	0.05	1.93
P415 ^{ECL3}								
Agonist	Ixo		ACh		Arec		Pilo	
	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}
Mean	10.47	105.40	8.24	101.30	7.39	115.00	6.75	82.43
SEM	0.04	1.05	0.05	1.06	0.25	1.73	0.28	1.33
N419 ^{TM7}								
Agonist	Ixo		ACh		Arec		Pilo	
	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}	pEC ₅₀	E _{max}
Mean	9.40	107.70	7.27	102.10	6.25	92.16	6.10	47.79
SEM	0.15	1.26	0.10	0.94	0.04	1.33	0.09	1.51

Supplementary Table 3 (related to Fig. 4 and Extended Data Fig. 9): Mean Tau-ON (apparent on-rates, s) values of fluorescence intensity changes from M₂R biosensors stimulated with the indicated agonists. N: total number of cells. Exp: days of independent transfections, SEM: standard error of mean, G_{endo}: endogenous G-protein level, G α _{oA}: overexpressed G α _{oA}(G203T) mutant.

Agonist	T84 ^{TM2}								E175 ^{ECL2}							
	Ixo		ACh		Arec		Pilo		Ixo		ACh		Arec		Pilo	
	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}
Mean	1.12	1.06	0.25	0.64	0.34	2.72	2.10	1.14	1.03	NA	0.50	NA	0.27	NA	0.18	NA
SEM	0.11	0.09	0.02	0.15	0.06	0.60	0.18	0.16	0.14	NA	0.02	NA	0.03	NA	0.01	NA
N	25	15	35	14	25	10	39	20	13	11	44	10	9	9	25	11
Exp	7	5	17	7	6	3	7	4	7	3	11	3	4	3	5	3

Agonist	F181 ^{ECL2}								F188 ^{TM5}							
	Ixo		ACh		Arec		Pilo		Ixo		ACh		Arec		Pilo	
	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}
Mean	NA	18.41	1.33	1.82	1.85	1.96	1.87	1.50	3.21	9.35	2.69	3.20	2.21	4.64	4.73	8.09
SEM	NA	1.48	0.04	0.04	0.16	0.13	0.10	0.32	0.26	0.45	0.11	0.12	0.14	0.45	0.34	0.54
N	12	29	76	41	13	34	17	20	21	16	96	26	35	13	27	17
Exp	5	4	9	7	7	4	7	3	5	3	15	4	6	3	6	3

Agonist	A414 ^{ECL3}								P415 ^{ECL3}							
	Ixo		ACh		Arec		Pilo		Ixo		ACh		Arec		Pilo	
	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}	G _{endo}	G α _{oA}
Mean	0.53	2.21	0.17	0.49	0.87	0.54	0.33	0.68	0.34	0.44	0.16	0.59	0.22	0.68	0.21	0.28
SEM	0.03	0.34	0.01	0.08	0.12	0.08	0.02	0.10	0.03	0.03	0.00	0.08	0.01	0.17	0.01	0.06
N	38	12	33	31	26	10	22	16	40	28	56	21	26	19	43	10
Exp	12	4	7	9	11	3	3	3	9	5	9	6	5	5	10	3

Supplementary Table 4: Mean pEC₅₀ values of the concentration-effect-curves shown in Extended Data Fig. 10. N: total number of experiments; SEM: standard error of the mean.

G α protein	Acetylcholine			Iperoxo			Arecoline			Pilocarpine		
	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
G _{i1}	7.27	0.15	4	9.30	0.10	6	6.00	0.11	4	5.18	0.13	6
G _{i2}	7.41	0.14	4	9.75	0.14	7	6.18	0.07	4	5.63	0.10	6
G _{i3}	6.55	0.16	4	8.74	0.10	6	5.68	0.10	4	5.36	0.22	5
G _{oA}	7.39	0.10	6	9.65	0.05	5	6.54	0.07	3	5.50	0.06	6
G _{oB}	7.76	0.12	5	9.83	0.10	8	6.66	0.12	3	5.82	0.10	5
G _z	6.56	0.15	5	8.90	0.15	6	5.85	0.09	4	4.77	0.28	4
G ₁₅	6.77	0.23	6	9.50	0.10	6	5.68	0.21	4	5.44	0.29	5

Supplementary Table 5 (related to Methods): Primer sequences for Gibson assembly. Primer sequences are depicted as 5'-3' reading frame. The priming region is highlighted in capital letters.

#	Sequence (5'-3')
1	ATTCTAGTTGTGGTTTGTCCAAACTCATCAA
2	ggagtttgttgagttattGCTGCCGGCGAACACCAGGCAGAAGATGTAGCTCAGGGCGATGATCGTCTTCATCAGTGTGATGGATATCTG
3	TTGATGAGTTTGGACAAACCACAAC TAGAAT
4	ataggcgctacaaggTAAGCGGCCGCTCGAGCAT
5	CAGATATCCATCACACTGATGAAGACGATCATCGCCCTGAGCTACATCTTCTGCCTGGTGTTCGCCGGCAGCaataactcaacaaactcc
6	ATGCTCGAGCGGCCGCTTAacctgtagcgctat
7	ctcctcgcccttgctcacgcctccgccaccGGTCCTTGTAGCGCCTAT
8	atggacgagctgtacaagtaaTGAGCGGCCGCTCGA
9	aggaccgglggcggaggcGTGAGCAAGGGCGAGGAG
10	tcgagcgccgctcattaCTTGTACAGCTCGTCCAT
11	TTCTGCCTGGTGTTCGCCTACCCATACGATGTTCCAGATTACGCTGGCAGCaataactcaaca
12	tgttgagttattGCTGCCAGCGTAATCTGGAACATCGTATGGGTAGGCCGAACACCAGGCAGAA
13	GACAATTGGTgctTGGCTTTGTTACATC
14	CACACAGTGTGGGG

Supplementary Table 6: Primers for site-directed mutagenesis (related to Methods). The respective M₂R residue was exchanged by an amber stop codon (TAG) using the listed forward and reverse primers. Primer sequences are depicted in the 5'-3' reading frame. The TAG is highlighted in capital letters.

M ₂ R residue	Forward (5'-3')	Reverse (5'-3')
K19	TAGacatttgaagtgggtttattgtcctggg	cttcaaatgtCTAataaggactgtgaagaccag
T20	gTAGttgaagtgggtttattgtcctgggg	cactcaaaCTActataaggactgtgaagaccag
F21	gacaTAGgaagtgggtttattgtcctggg	ccacttcCTAtgtctataaggactgtgaagac
E22	TAGgtgggtttattgtcctgggtggctgg	taaaccaccacCTAaaatgtctataaggactgtgaag
V23	gaaTAGgtgtttattgtcctgggtggctgg	taaacacCTAttcaaatgtctataaggactgtgaag
V24	gTAGttattgtcctgggtggctggatcc	ggacaataaaCTAcacttcaaatgtctataaggacttg
F25	gtgggtgTAGattgtcctgggtggctgg	gacaatCTAcaccacttcaaatgtctataaggacttg
T81	gttacTAGctctacactgtgattgggtactgg	gtagagCTAgtaacaagttcatggagaaaacacc
L82	gttacaccTAGtacactgtgattgggtactgg	gtaCTAggtgtacaagttcatggagaaaacacc
Y93	gtacaccctcTAGactgtgattgggtactg	CTAgaggggtgtacaagttcatggagaaaacac
T84	cTAGgtgattgggtactggccttggggac	aaccaatcacCTAgtagaggggtgtacaagttc
V85	ctTAGattgggtactggccttggggacc	gtaaccaatCTAagtgtagaggggtgtacaag
I86	gtgTAGgggtactggccttggggacc	ccagtaaccCTAcacagtgtagaggggtg
G87	TAGtactggccttggggacactgtggg	aaggccagtaCTAaatcacagtgtagaggggtg
Y88	ggTAgTggccttggggacactgtgg	aaaggccaCTAaccaatcacagtgtagagg
W89	cTAGccttggggacactgtggtgtg	gtcccaaaggCTAgtaaccaatcacagtg
P90	ctggTAGttggggacactgtggtgtg	ggtcccaaCTAccagtaaccaatcacagtg
L91	ggcctTAGgggacactgtggtgtg	ggtccCTAaggccagtaaccaatcac
G92	ggccttgTAGcctgtggtgtgtgac	ggCTAaaaggccagtaaccaatcacagtg
P93	ggaTAGgtggtgtgtgaccttggctag	acaccacCTAtcccaaaggccagtaacc
V94	gggacctTAGgtgtgtgaccttgg	cacacCTAaggtcccaaaggccag
V95	gTAGgtgaccttggctagccctg	aagggtcacaCTAcacaggtcccaaagg
C96	ggtgTAGgaccttggctagccctg	aagggtcCTAcaccacaggtcccaaagg
D97	ggtgtgtTAGccttggctagccctg	caaagCTAacacaccacaggtcccaaagg
L98	gtgtgacTAGtggttagccctggac	gccacCTAgtcacacaccacaggtcc
F161	ctcTAGtggcagttcattgtaggggtgag	gaactgccacCTAgagaatggctggagc
W162	ctcttcTAGcagttcattgtaggggtgag	gaactgCTAgaagagaatggctggagc
Q163	ctctctggTAGttcattgtaggggtgag	gaaCTAccagaagagaatggctggagc
F164	ctctctggcagTAGattgtaggggtgag	Actgccagaagagaatggctggagc
I165	cTAGgtaggggtgagaactgtggagg	cacccctacCTAgaactgccagaagag
V166	TAGgggggtgagaactgtggaggatgg	gttctcaccctCTAaatgaactgccagaag
G167	TAGgtgagaactgtggaggatggggag	cacagttctacCTAtacaatgaactgccag
V168	gTAGagaactgtggaggatggggag	ccacagttctCTAccctacaatgaactgc
R169	ggtgTAGactgtggaggatggggag	ccacagtCTAcaccctacaatgaactgc
T170	ggtgagaTAGgtggaggatggggag	ccacCTAtctcaccctacaatgaactgc
V171	gagaactTAGgaggatggggagtg	cctcCTAagttctcaccctacaatgaac
E172	gaactgtgTAGgatggggagtgctac	catcCTAcacagttctcaccctacaatg
D173	ctgtggagTAGggggagtgctacattc	ccCTActccacagttctcaccctac
G174	ctgtggaggatTAGgagtgctacattcag	CTAatcctccacagttctcaccctac
E175	ggatgggTAGtgctacattcagtttttcc	gcaCTAccatcctccacagttctc
C176	ggaggatggggagTAGtacattcagtttttcc	ctcccatcctccacagttctc
Y177	ggatggggagtgTAGattcagtttttcc	gcactcccatcctccacagttc
I178	tggggagtgctacTAGcagttttttccaatg	gtagcactcccatcctccacag

Supplementary Table 6: Site-directed mutagenesis primers. - Continues

# M ₂ R residue	Forward (5'-3')	Reverse (5'-3')
Q179	ggggagtgctacattTAGtttttccaatgc	tgtagcactcccatcctccacag
F180	cattcagTAGttttccaatgctgctgcacc	aaaCTActgaatgtagcactcccatcctcc
F181	TAGtccaatgctgctgcaccttggtagc	cagcattggaCTAaaactgaatgtagcactcc
S182	gtttttTAGaatgctgctgcaccttggtagc	cattCTAaaaaaactgaatgtagcactcccatcc
N183	ccTAGgctgctgcaccttggtagc	gacagcagcCTAggaaaaaactgaatgtagc
A184	TAGgctgctgcaccttggtagcgctattgc	aggtgacagcCTAattggaaaaaactgaatgtagc
A185	gctTAGGtcaccttggtagcgctattgc	aggtgacCTAagcattggaaaaaactgaatgtagc
V186	gctgctTAGaccttggtagcgctattgc	aggtCTAagcagcattggaaaaaactgaatgtagc
T187	gctgctgcTAGtttggtagcgctattgc	aCTAgacagcagcattggaaaaaactgaatgtagc
F188	gctgtcaccTAGggtacggctattgc	cCTAggtgacagcagcattggaaaaaactg
G189	gctgtcacctttTAGacggctattgcagc	Aaaaggtgacagcagcattggaaaaaactgaatg
V407	gtcatgTAGctcattaacacctttgtgcac	ttagCTAcatgacattglatggggcccaag
L498	gtcatgggtgTAGattaacacctttgtgcacc	tCTAcaccatgacattgtatggggcccaag
I409	catgggtgcTAGaacacctttgtgcacc	CTAgagcaccatgacattgtatggggcccaag
N410	TAGacctttgtgcaccttgcaccccaac	gcacaaaaggtCTAaatgagcaccatgacattg
T411	cTAGttttgtgcaccttgcaccccaac	ggtgcacaaaaCTAgttaatgagcaccatgac
F412	ccTAGtgtgcaccttgcaccccaac	ggtgcacaCTAggtttaatgagcaccatg
C413	TAGgcaccttgcaccccaacactgtg	tgcaaggtgcCTAaaaggtttaatgagcac
A414	TAGccttgcaccccaacactgtgtg	ggatgcaaggCTAacaaaaggtttaatgagc
P415	gcaTAGtgcatcccaacactgtgtg	ggatgcaCTAtgcacaaaaggtttaatgagc
C416	gcacctTAGatcccaacactgtgtg	ggggatCTAaggtgcacaaaaggtttaatg
I417	gcaccttgcTAGcccaacactgtgtg	gggCTAgcaaggtgcacaaaaggtttaatg
P418	gcaccttgcTAGaacactgtgtggac	Agatgcaaggtgcacaaaaggtttaatgag
N419	gcaccccTAGactgtgtggacaattgg	gtCTAggggatgcaaggtgcacaaaagg
T420	gcaccccaacTAGgtgtggacaattgg	TAgttgggatgcaaggtgcacaaaagg
V421	ccccaacactTAGtggaactgtgtactg	CTAagtgttgggatgcaaggtgcac
W422	ccccaacactgtgTAGacaattgtgtactg	cacagtgttgggatgcaaggtgc
T423	aacactgtgtgTAGattgttactgtgtt	Accacacagtgttgggatgcaagg
I424	cactgtgtggacaTAGggttactgtgtt	tgtccacacagtgttgggatgcaag

Supplementary Table 7: G-protein subunits for TRUPATH G-protein activation assays. (related to Methods). G β and G γ subunits used for the respective G α subunit. The combination of subunits has been previously optimized for each G α by Olsen et al⁵⁴.

G α	G γ	G β
i1	γ 9	β 3
i2	γ 8	β 3
i3	γ 9	β 3
oA	γ 8	β 3
oB	γ 8	β 3
Z	γ 1	β 3
Gust	γ 1	β 3
sS	γ 9	β 3
sL	γ 1	β 1
11	γ 13	β 3
12	γ 9	β 3
13	γ 9	β 3
15	γ 13	β 3