

Supporting information

Functional monoclonal antibody acts as a biased agonist by inducing internalization of metabotropic glutamate receptor 7

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This SI includes:

Table S1

Fig. S1

Fig. S2

Table S1. Selectivity of MAB1/28 towards mGlu receptors. Potencies of MAB1/28 and MAB1/28-derived Fab1 fragments on human mGlu₁, mGlu₂, mGlu₅, and mGlu₈ measured in Ca²⁺ mobilization assays configured for the detection of agonism or antagonism of test compounds. The concentration for the antibody and Fab1 fragments refers to the protein concentration, not to the concentration of binding sites. The mean results of several experiments performed in duplicate (reference agonists/antagonists) or as single experiments (MAB1/28/Fab1) ± SDM are reported. (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740): mGlu₂/mGlu₃ agonist. 7-(hydroxyimino)cyclopropan[b]chromen-1a-carboxylic acid ethyl ester (CPCCOEt): mGlu₁ NAM. 2-methyl-6-(phenylethyl)-pyridine (MPEP): mGlu₅ NAM. (*RS*)- α -methylserine-*o*-phosphate (MSOP): group III mGlu antagonist.

	mGlu1			mGlu2			mGlu5			mGlu8		
	mean	StDev	N	mean	StDev	N	mean	StDev	N	mean	StDev	N
Agonism (EC₅₀, nM)												
mAB 1-28	>1000		2	>1000		2	>1000		2	463.73	5.26	2
Fab (mAB 1-28)										645.75	91.41	2
L-glutamate	9500.99	2727.30	12	2225.35	531.41	12	1791.26	491.18	12			
quisqualate	492.40	123.30	4				38.58	10.74	4			
LY354740				32.34	5.09	4						
L-AP4										207.82	44.01	12
Antagonism (IC₅₀, nM)												
mAB 1-28	>1000		2	>1000		2	>1000		2	>1000		2
Fab (mAB 1-28)										>1000		2
CPCCOEt	970.34	404.58	2									
LY341495				3.60	1.32	2						
MPEP							6.45	0.97	2			
MSOP										6556.97	4916.45	2

Fig. S1. Selectivity of MAB1/28 towards mGlu receptors. MAB1/28 displayed no effect as agonist or antagonist in the HEK-293 cell lines stably expressing hmGlu_{1a} (A & B), hmGlu₂ (+ Gα16) (C & D), hmGlu₅ (E & F), and hmGlu₈ (+ Gα15) (G & H) using functional Ca²⁺ mobilization assay. The activity of reference agonists and antagonists is shown for comparison. At least three independent experiments were performed in duplicate. Error bars represent ± SEM.

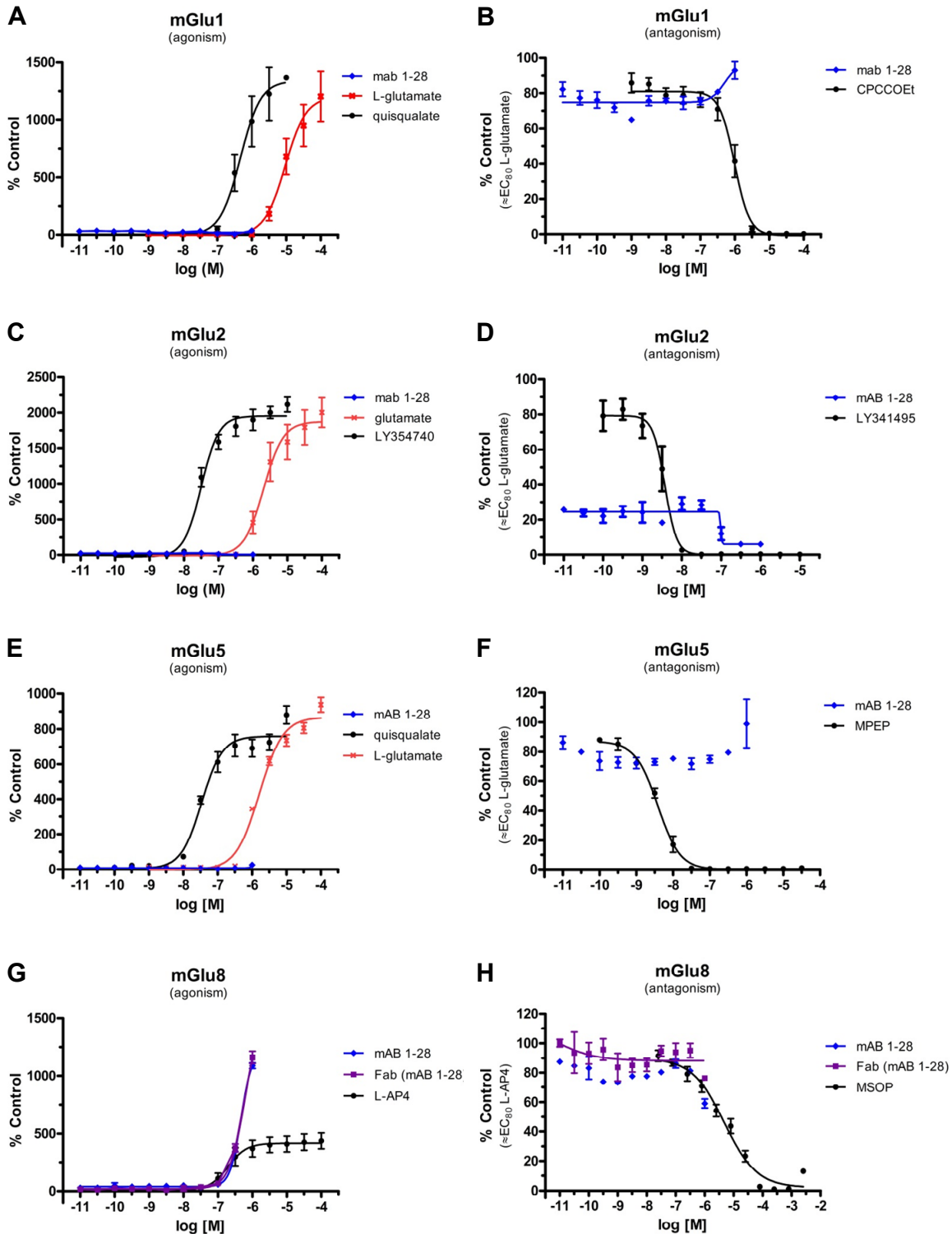


Fig. S2. Time-lapse sequence of internalising mGlu7 receptors in intact cells. CHO cells expressing mGlu7 were incubated with directly fluorescein labeled MAB1/28 on ice for 1 h and then returned to conditioned media for 6, 10, 18, 33 and 53 min to allow internalization. Confocal images suggest newly formed spot-like structures to be intracellular and forming in a time-dependent manner.

