Expression of Melatonin and Dopamine D_3 Receptor Heteromers in Eye Ciliary Body Epithelial Cells and Negative Correlation with Ocular Hypertension

Irene Reyes Resina
Facultad de Farmacia y CA
Universitat de Barcelona
0902 2023

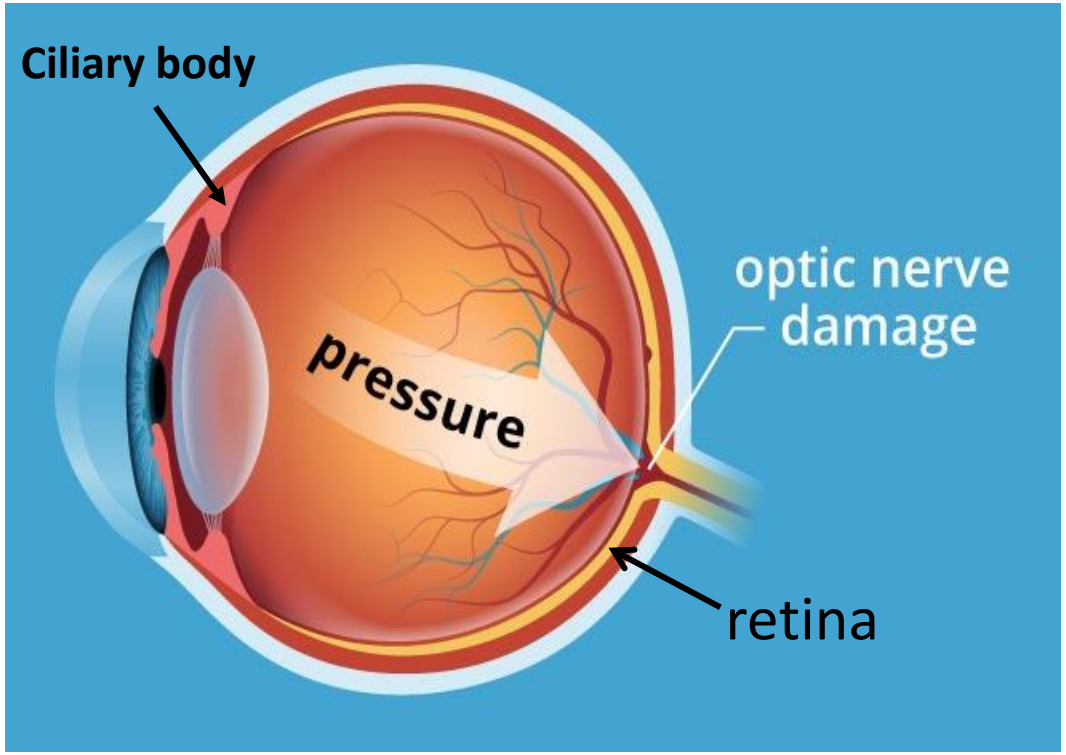
UNIVERSITAT DE BARCELONA

GLAUCOMA

Second leading cause of blindness worldwide

Intra-ocular pressure increase → Progressive death of retinal ganglion cells → Vision loss

> 21 mmHg

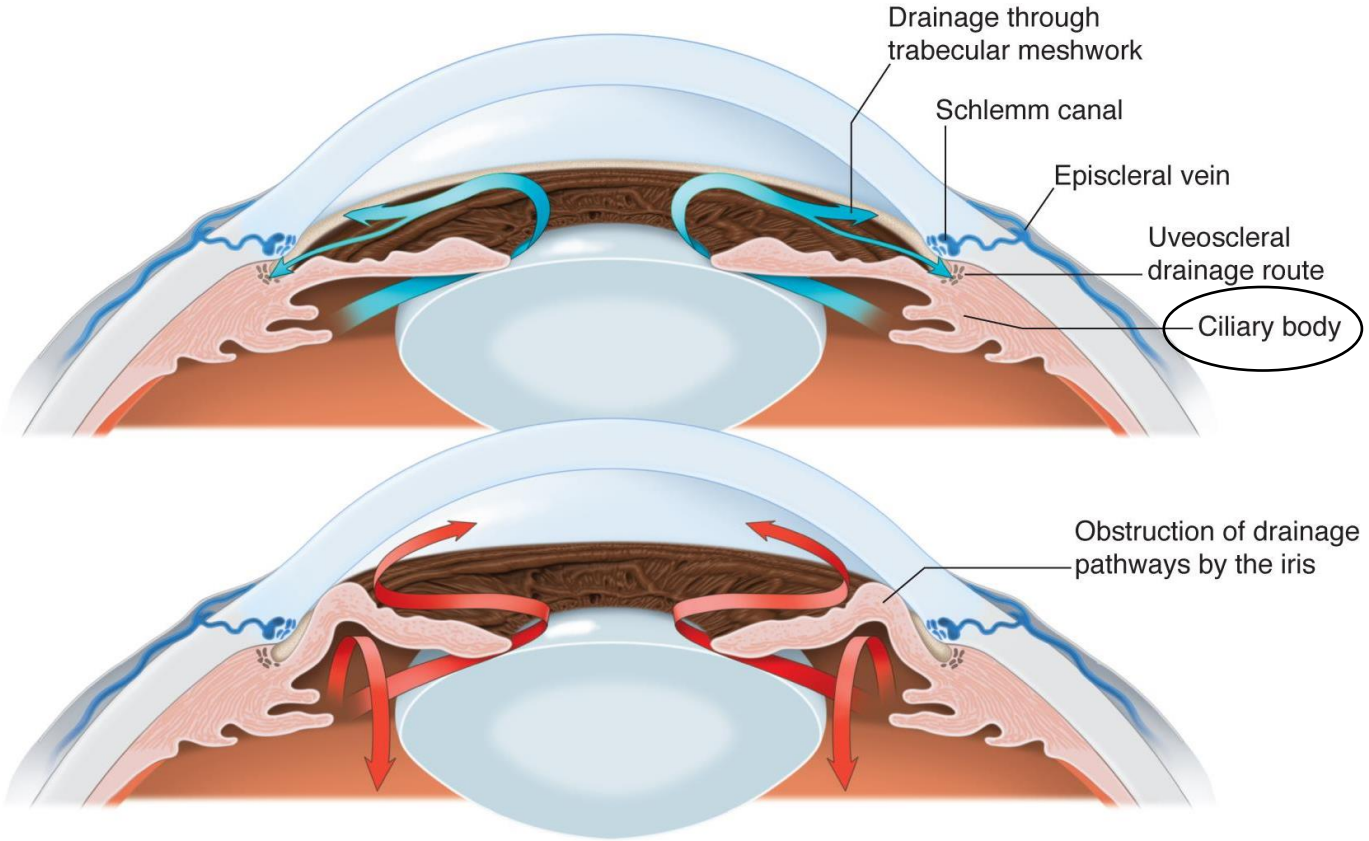


Casson et al, Clin. Experiment. Ophthalmol. 2012

Aqueous humour dynamycs

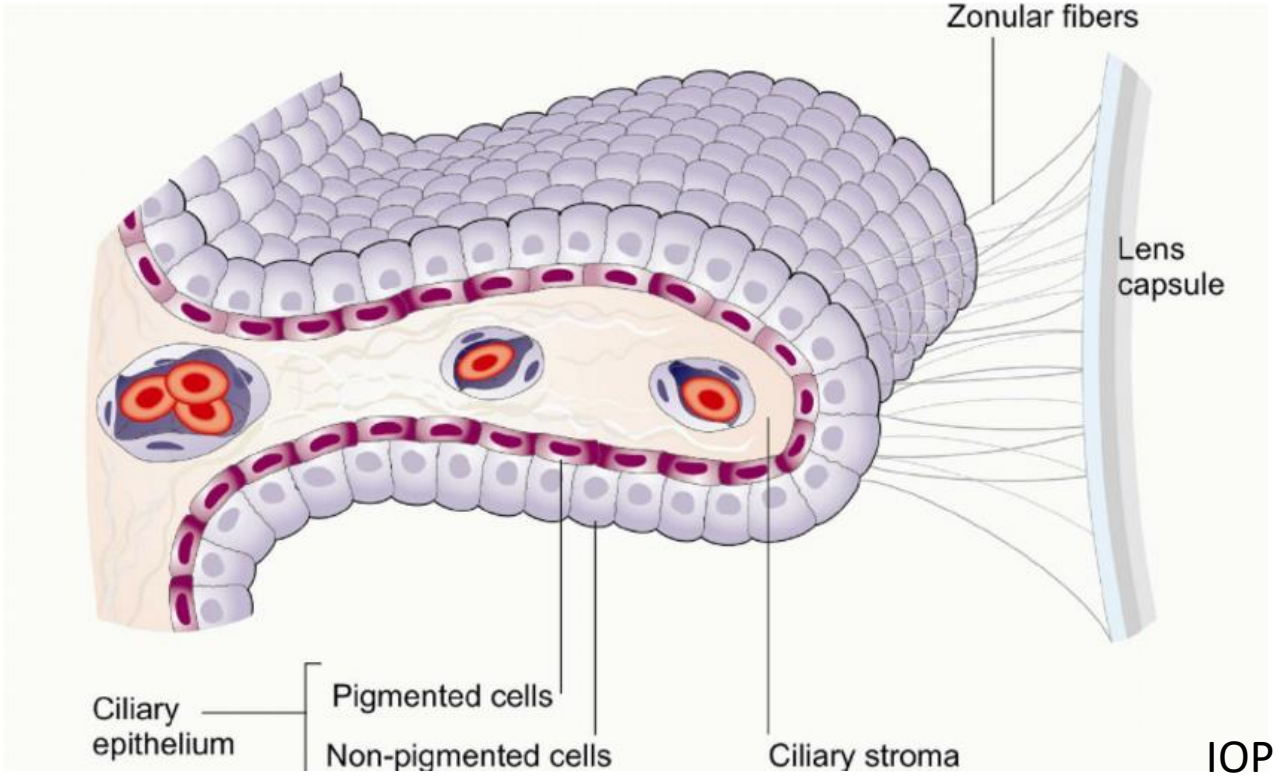
production vs drainage

Aq. humour accumulation → Intra-ocular pressure increase



Civan et al, Exp. Eye Res. 2004
Delamere et al, Adv. Organ. Biol. 2005

Ciliary body → Ciliary Processes: aqueous humor production

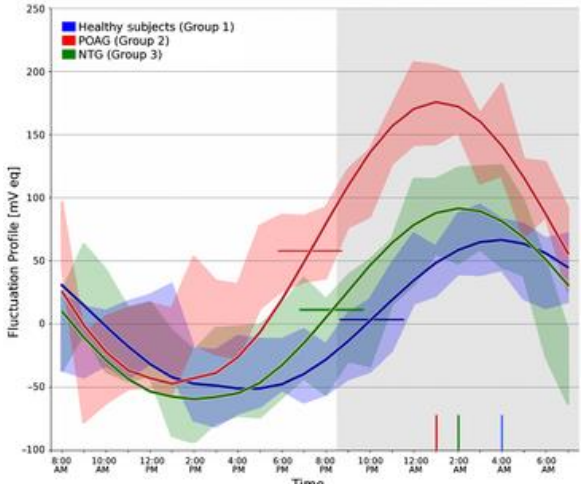


Several factors contribute to the homeostasis of IOP:

- the episcleral vein pressure
- the ratio between production and drainage of aqueous humor
- the influence of hormones
- the innervation by cranial nerves V and VII
- the circadian rhythm

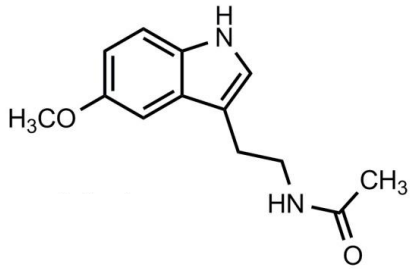
IOP varies throughout the day:
-maximal at the early morning
-minimal levels during the night

Variations are greater in the glaucomatous eye

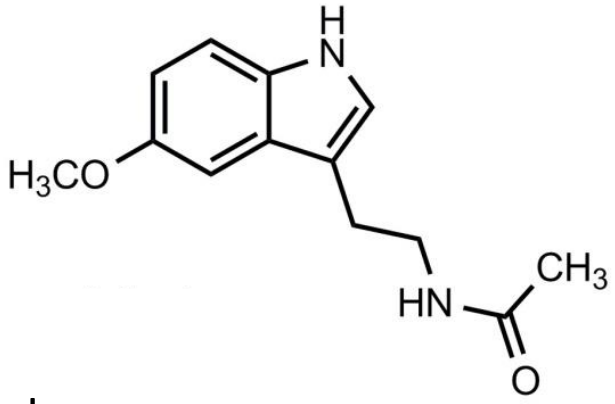


Agnifili L, et al; Acta Ophthalmol. 2015

Melatonin



Melatonin



-Neurohormone

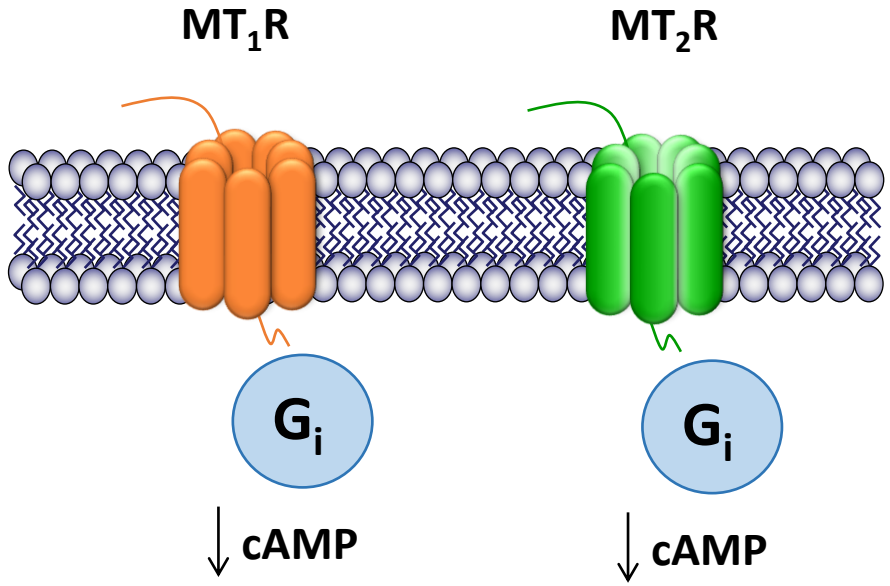
-Control of circadian rythms

-Synthesized in the pineal gland but also in eye structures (retina, ciliary body…)

Reppert et al, Neuron 1994, Reppert et al, Proc. Natl. Acad. Sci. USA 1995
Xue et al, Eur. Rev. Med. Pharmacol. Sci. 2017
Huete-Toral et al, J. Pharmacol. Exp. Ther. 2015
Cecon et al, Br. J. Pharmacol. 2017

Melatonin receptors

Family A GPCRs

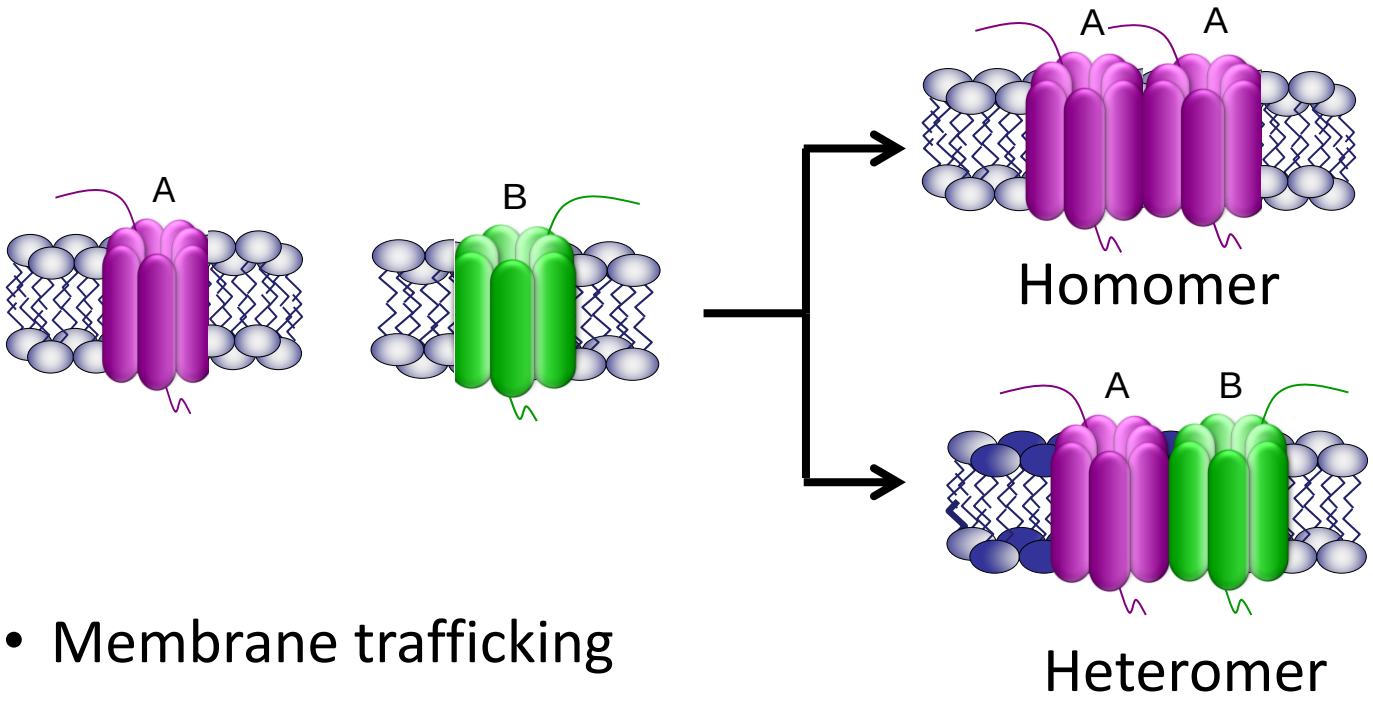


-expressed in retina, cornea, non-pigmented epithelium of ciliary body

-regulation of circadian rhythms and neuroendocrine processes in the retina and in ciliary body

-Coupling to other G proteins has been described (also increases in cAMP!)

GPCR oligomerization



- Membrane trafficking
- Pharmacology
- Signal trasnduction
- Allosteric modulation between subunits

Borroto-Escuela et al, 2014: GPCR-HetNet

MT_1R-MT2R → regulation of photoreceptor function

Baba et al, Science Signalling, 2013

MT_1R-GPR50 → Gi-coupling altered

MT_2R-GPR50

Levoye A. et al, EMBO J. 2006

MT_2R-$5HT_{2c}$ → biased agonism for agomelatine

Kamal et al, J. Biol. Chem. 2015

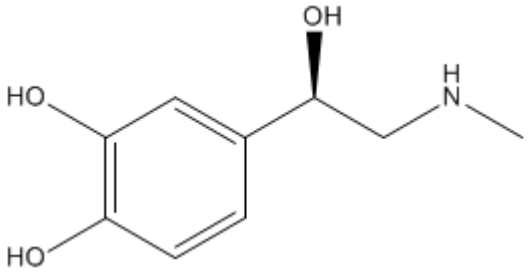
MT_1R-α_1R → heteromerization impedes coupling to cognate G proteins

MT_2R- α_1R →

Alexander et al, Br. J. Pharmacol. 2017
Alkozi et al, Br J Pharmacol. 2020

Dopamine

Present in brain but also in eye structures



Dopamine and melatonin

-Dopamine regulates melatonin synthesis in the eye

-Melatonin injection suppresses the release of dopamine

-Synthesis and liberation are under circadian control:

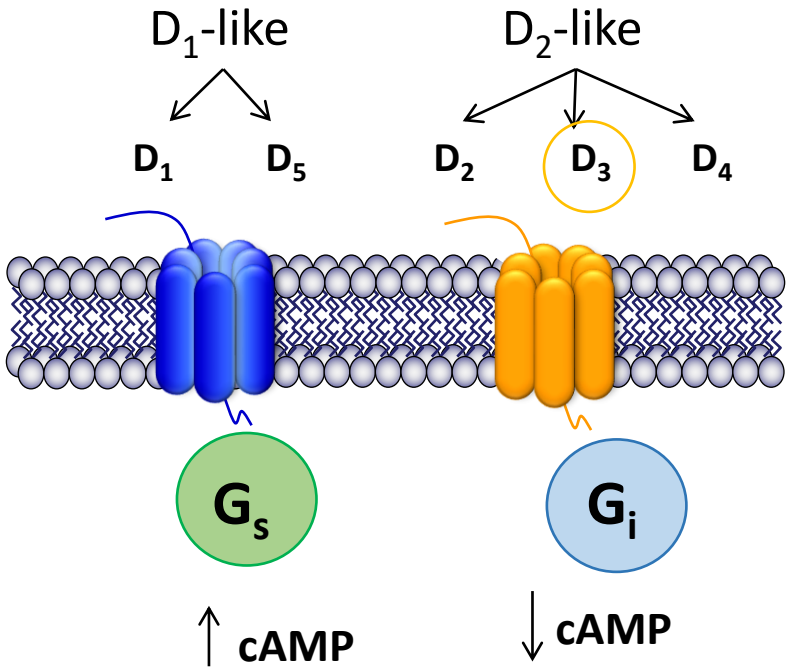
- Day:
  ↑ dopamine ↓ melatonin
- Night:
  ↓ dopamine ↑ melatonin

Melatonin ⟺ **Dopamine**

Adachi et al, Brain Res. 1998, 1999
Pescosolido et al, Biomed. Res. Int. 2013
Chu et al, J. Ocul. Pharmacol. Ther. 2004, Chu et al, J. Pharmacol. Exp. Ther. 2000

Dopamine receptors

Family A of GPCRs



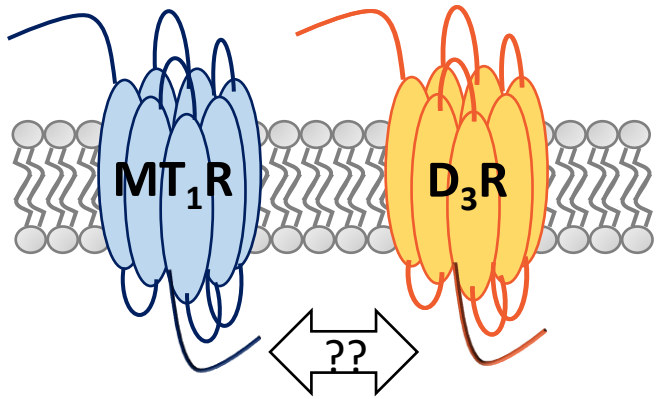
-Movement, cognition, emotions, vision, memory, reward pathway

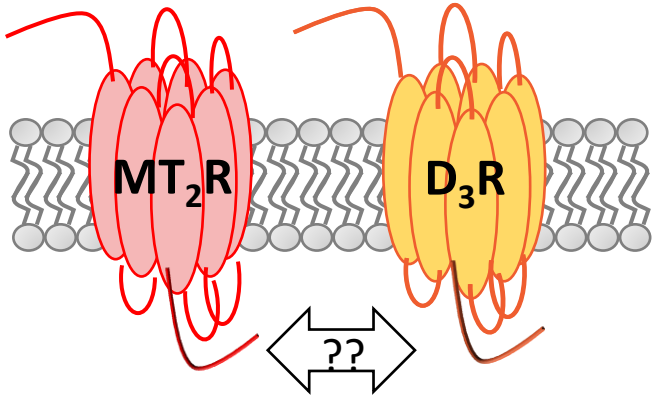
-D3R activation leads to IOP reduction

-D_3R is expressed in ciliary body epithelial cells

HYPOHESIS

Dopamine D3 and melatonin receptors might form heteroreceptor complexes whose function impact on eye physiology



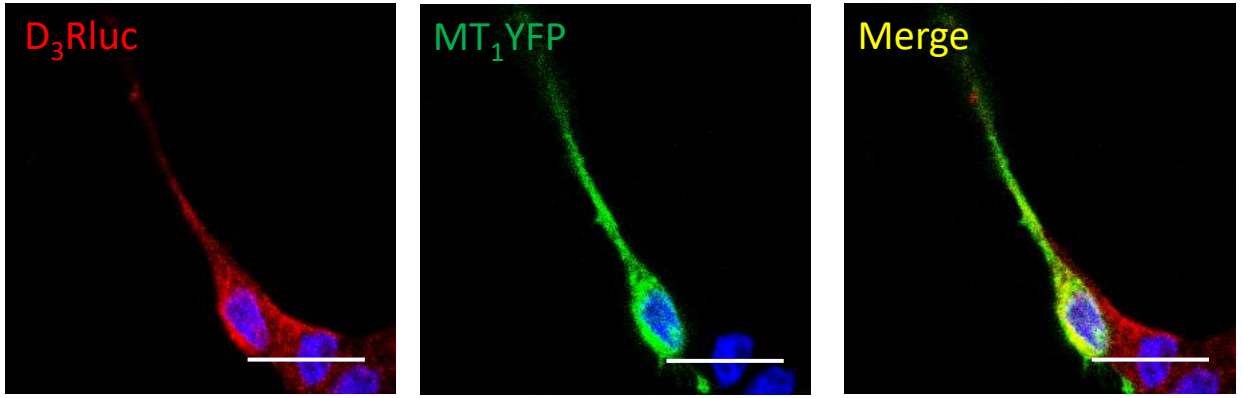


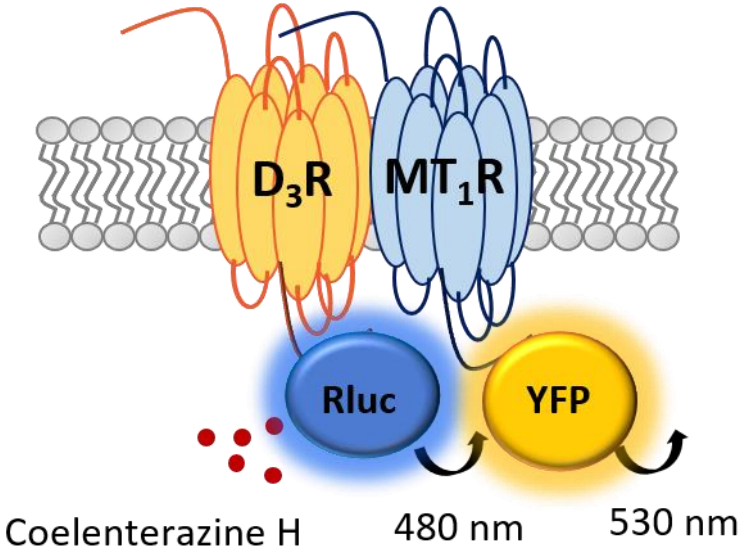
AIM

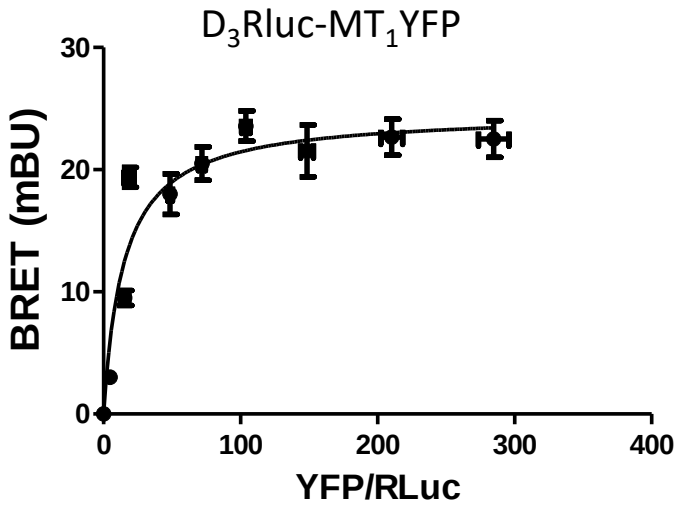
To address the potential interaction between dopamine D3 and melatonin receptors, along with the functional consequences of these interactions.

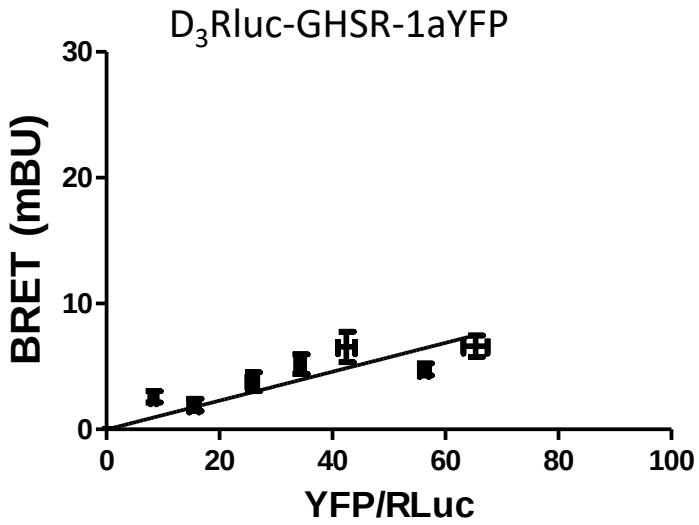
Dopamine D3 Receptors Interact with Melatonin MT1 Receptors in HEK-293T Cells

HEK-D_3R Rluc-MT_1R YFP

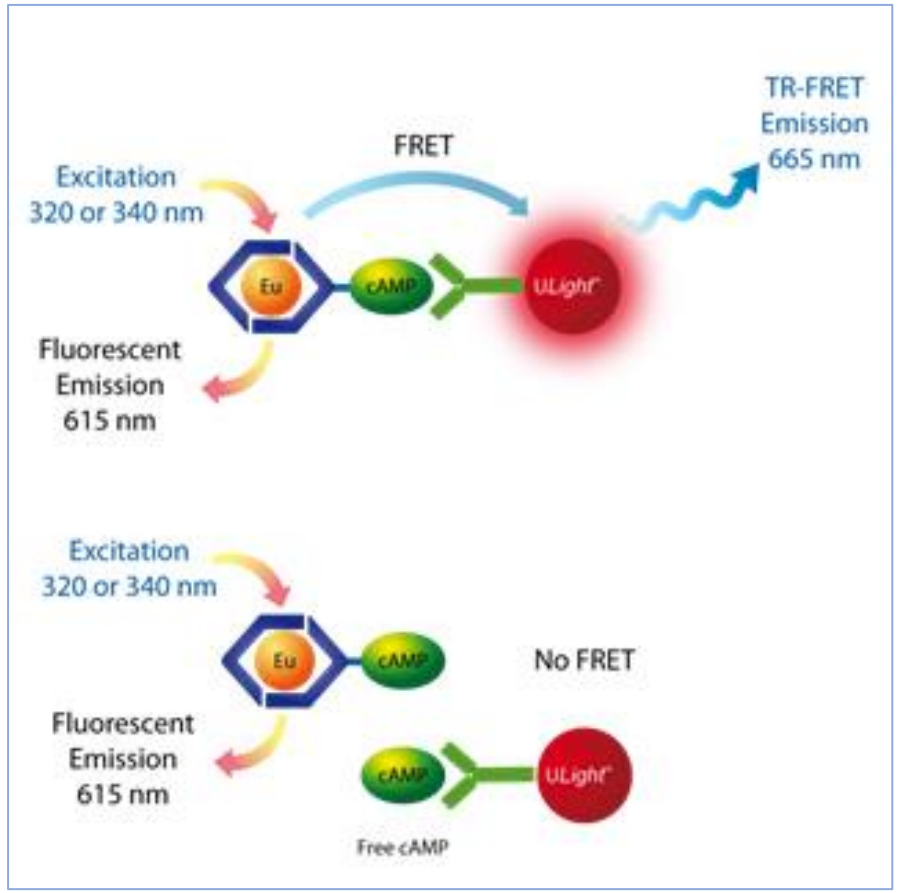




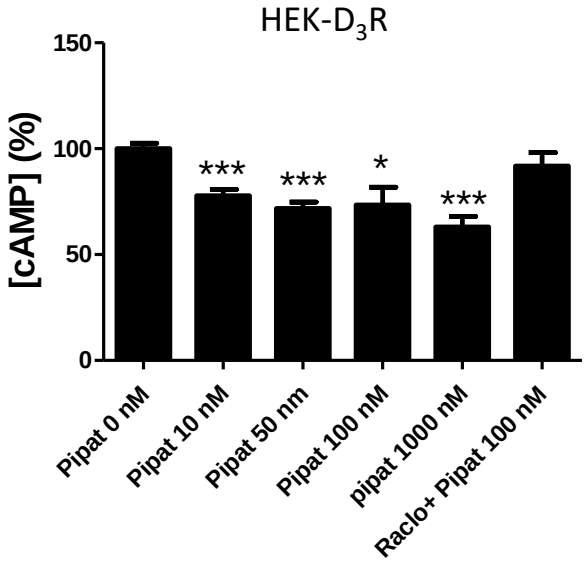


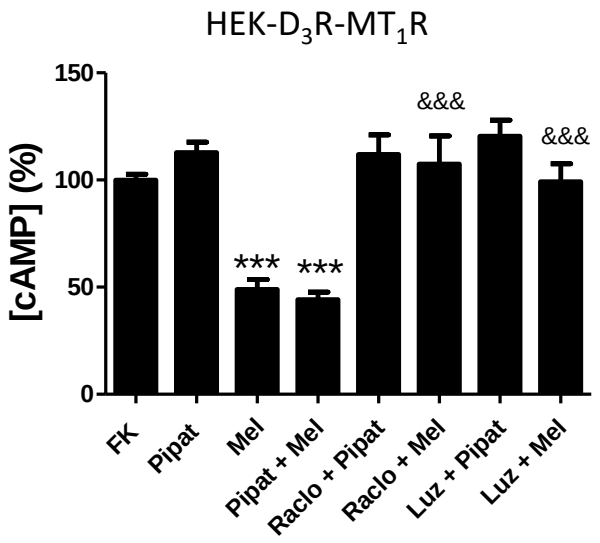


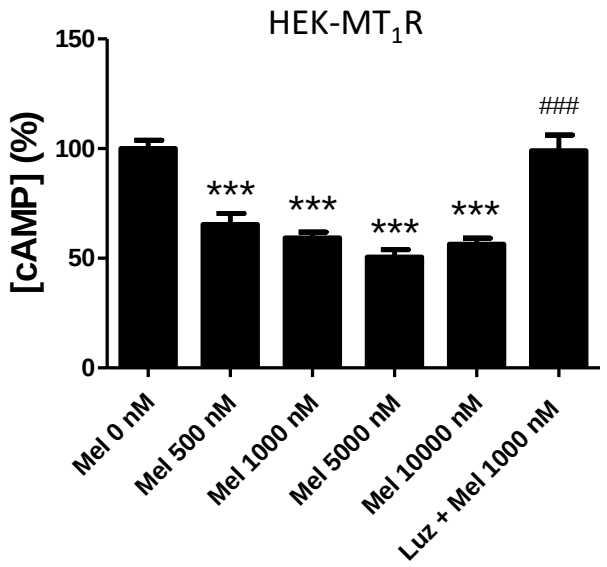
Functional Characterization of the D3-MT1 Heteroreceptor Complexes in HEK-293T Cells



- Receptor activation → low cAMP → high FRET
- Receptor inactivation → high cAMP → low FRET





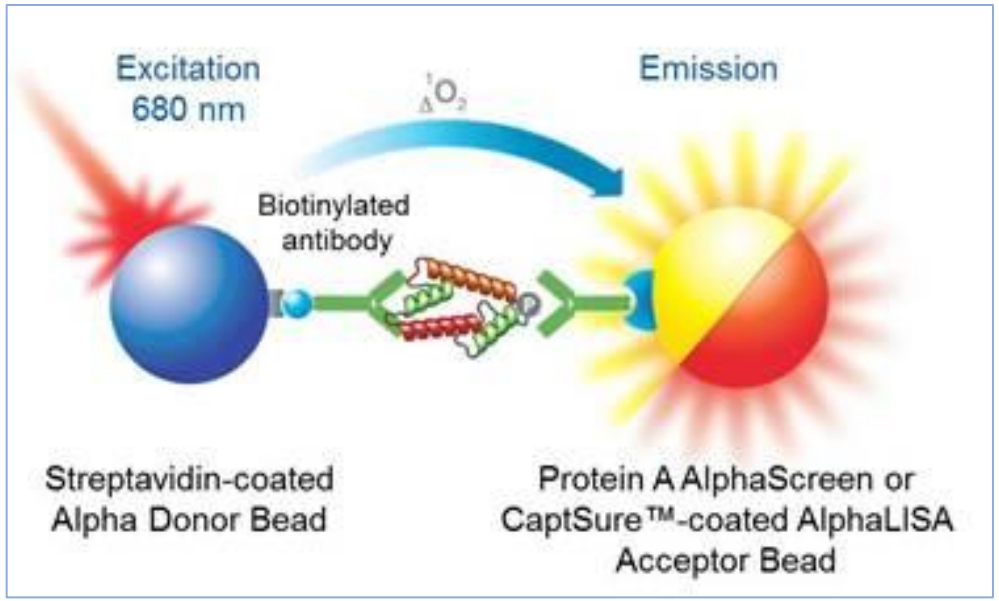


D_3R agonist: 100 nM 7-OH-PIPAT
D_3R antagonist: 1 µM raclopride
MT_1R agonist: 1 µM melatonin
MT_1R antagonist: 1 µM luzindole

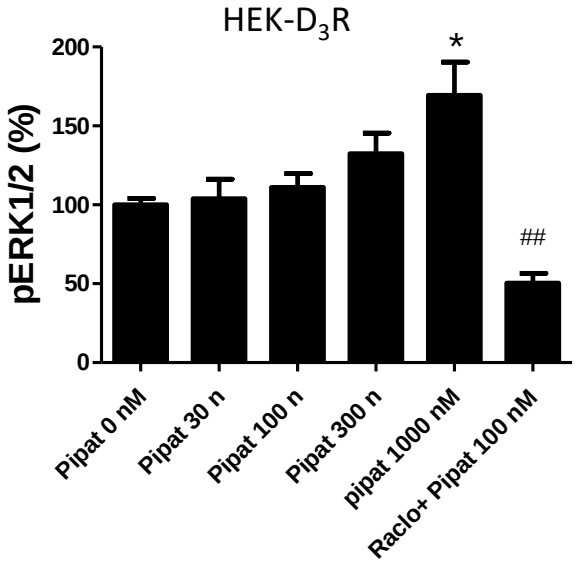
0.5 µM forskolin

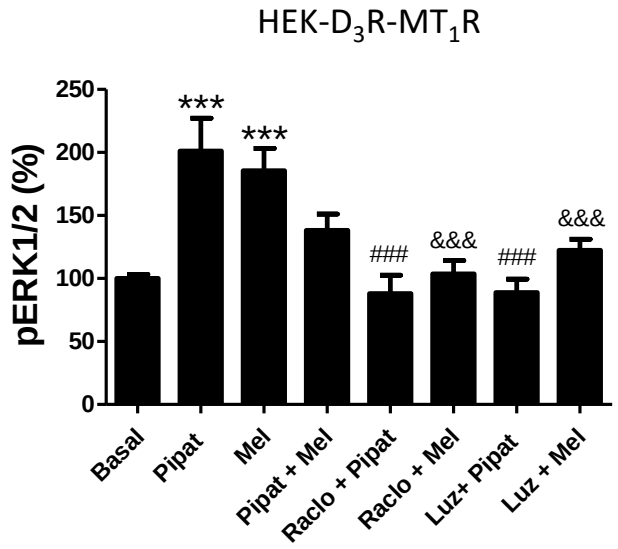
* vs forskolin
& vs melatonin

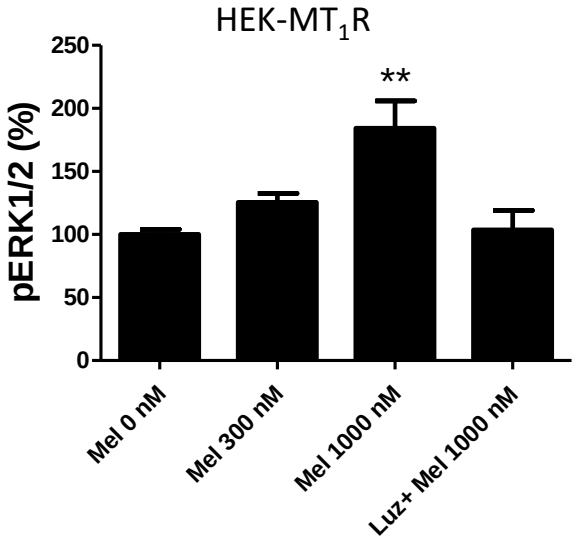
Functional Characterization of the D3-MT1 Heteroreceptor Complexes in HEK-293T Cells



- Receptor activation → high pERK → high FRET
- Receptor inactivation → low pERK → low FRET



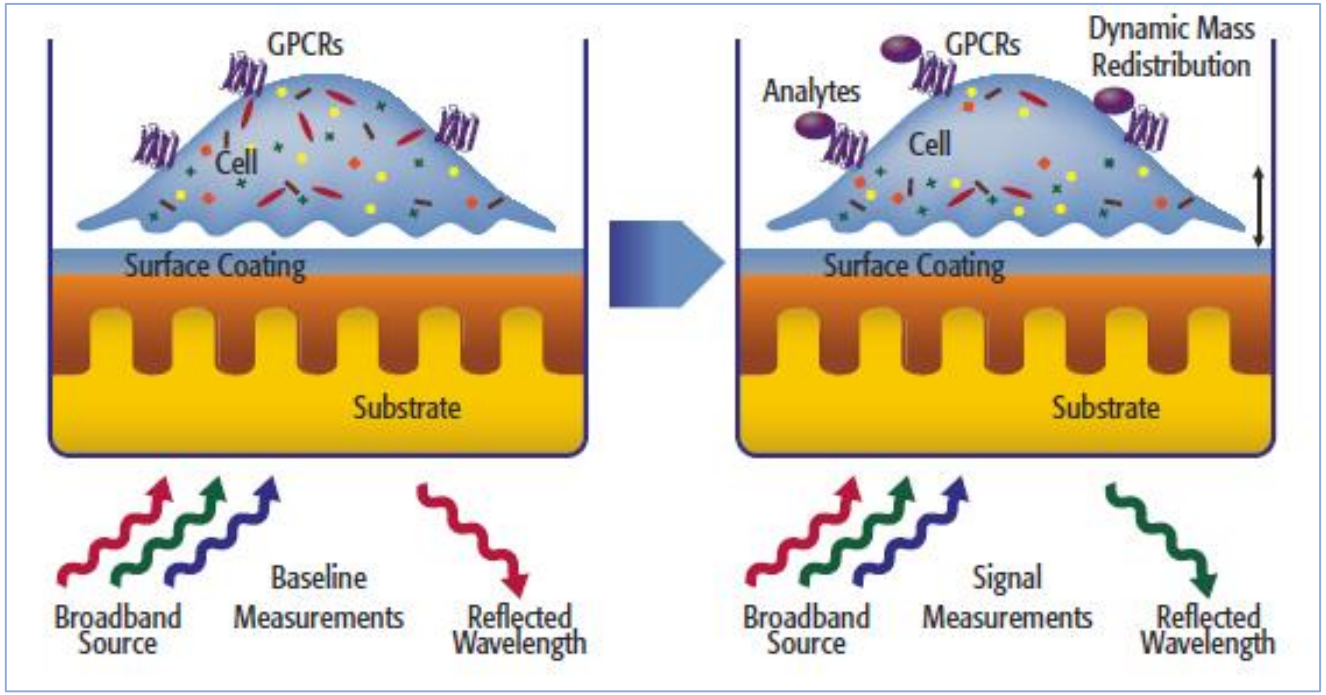




D_3R agonist: 100 nM 7-OH-PIPAT
D_3R antagonist: 1 µM raclopride
MT_1R agonist: 1 µM melatonin
MT_1R antagonist: 1 µM luzindole

* vs basal
& vs melatonin
# vs Pipat

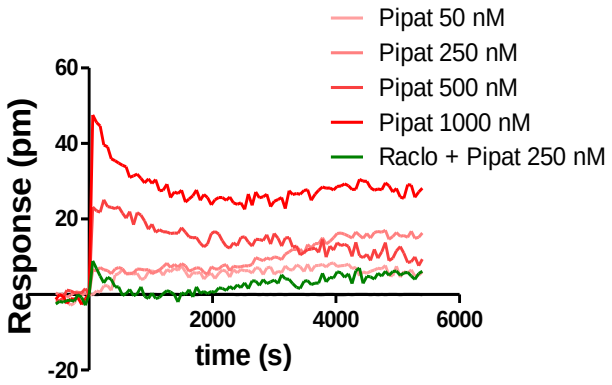
Functional Characterization of the D3-MT1 Heteroreceptor Complexes in HEK-293T Cells



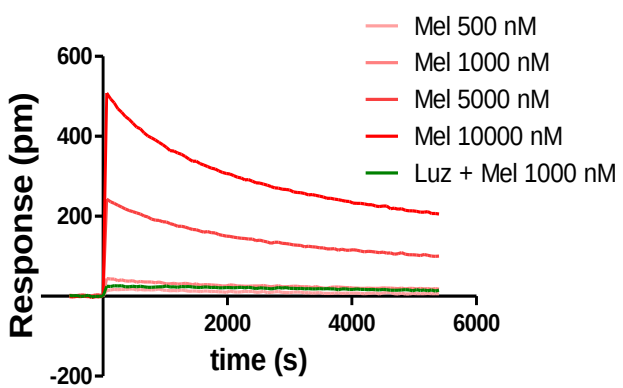
Dynamic Mass Redistribution (DMR) response

D_3R agonist: 100 nM 7-OH-PIPAT
D_3R antagonist: 1 µM raclopride
MT_1R agonist: 1 µM melatonin
MT_1R antagonist: 1 µM luzindole

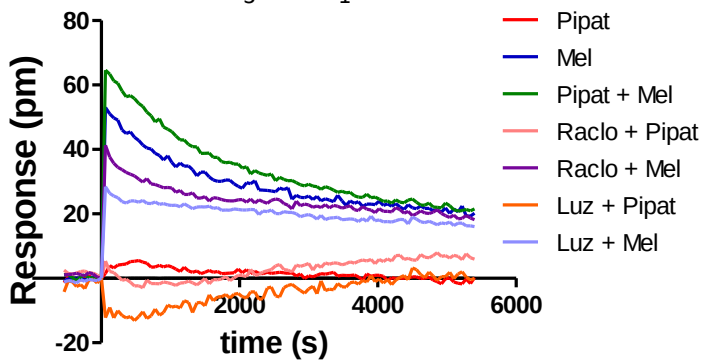
HEK-D_3R



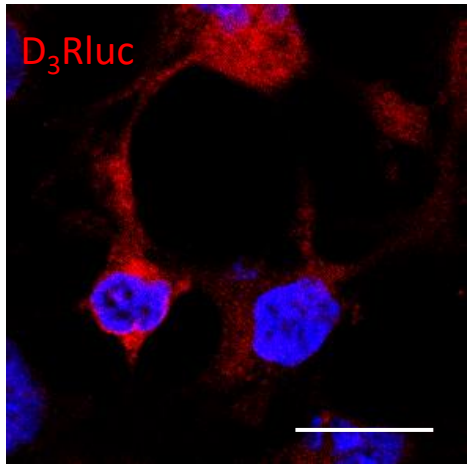
HEK-MT_1R

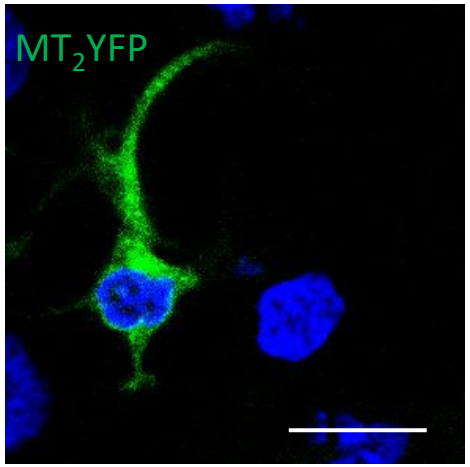


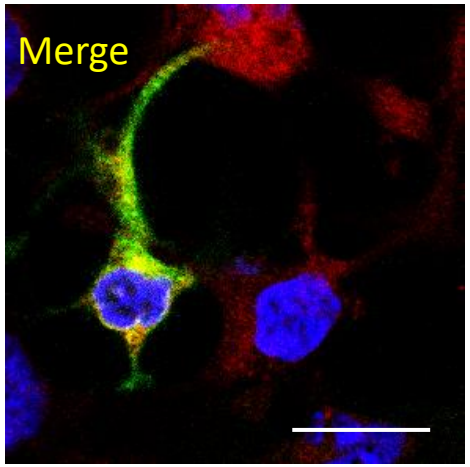
HEK-D_3R-MT_1R

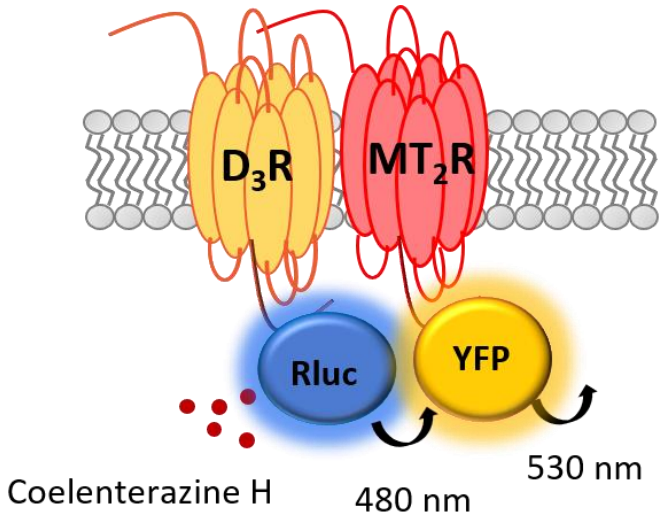


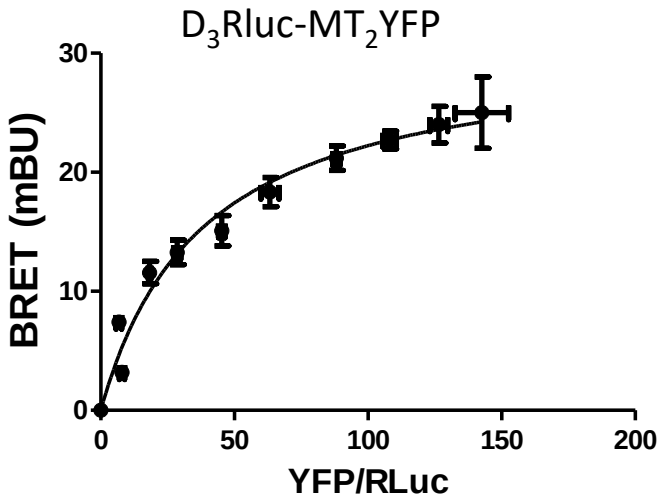
Dopamine D3 Receptors Interact with Melatonin MT2 Receptors in HEK-293T Cells









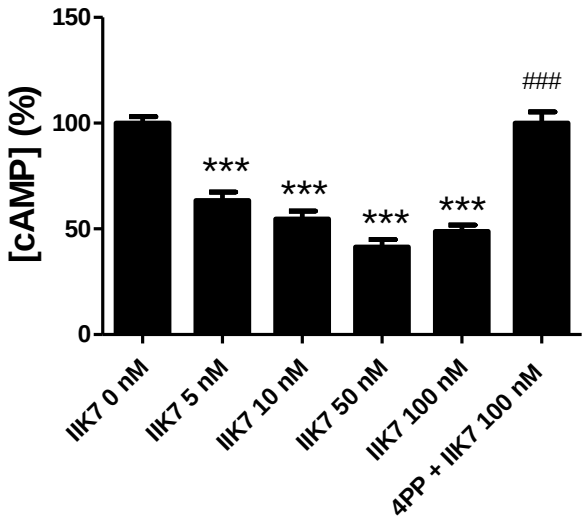


Functional Characterization of the D3-MT2 Heteroreceptor Complexes in HEK-293T Cells

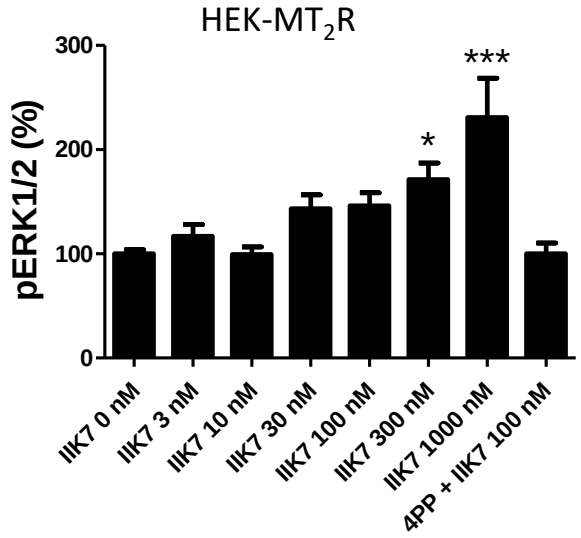
D_3R agonist: 100 nM 7-OH-PIPAT
D_3R antagonist: 1 µM raclopride
MT_2R agonist: 300 nM IIK7
MT_2R antagonist: 1 µM 4P-PDOT (4PP)

* vs FK/basal
& vs IIK7
# vs Pipat

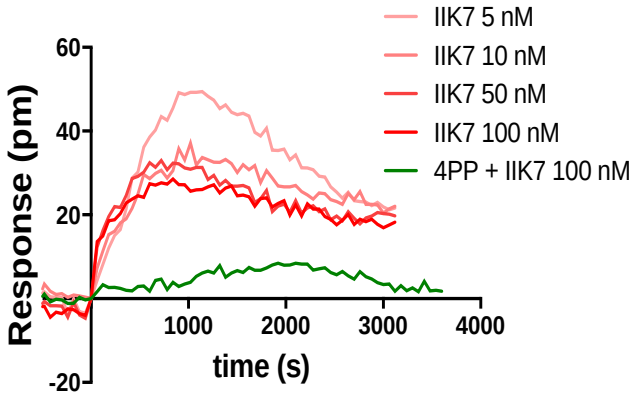
HEK-MT_2R



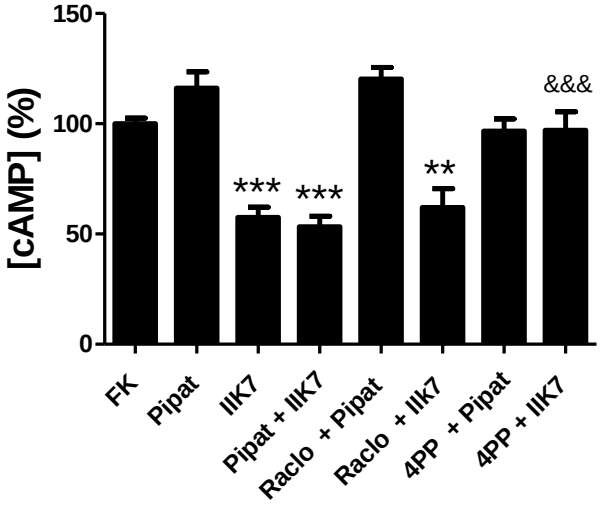
HEK-MT_2R



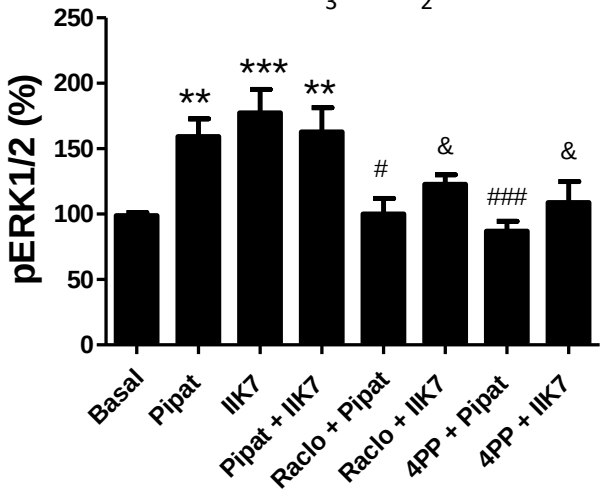
HEK-MT_2R



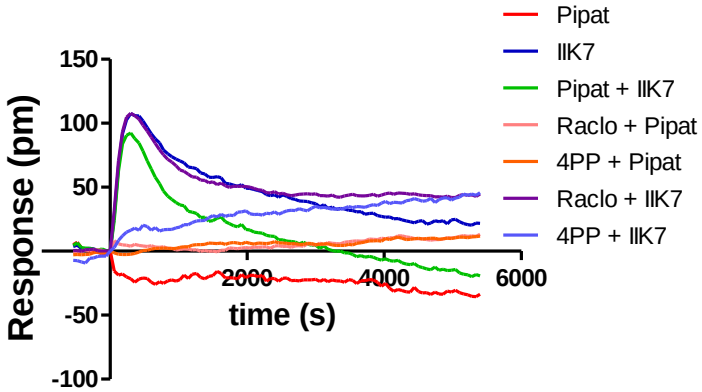
HEK-D_3R-MT_2R



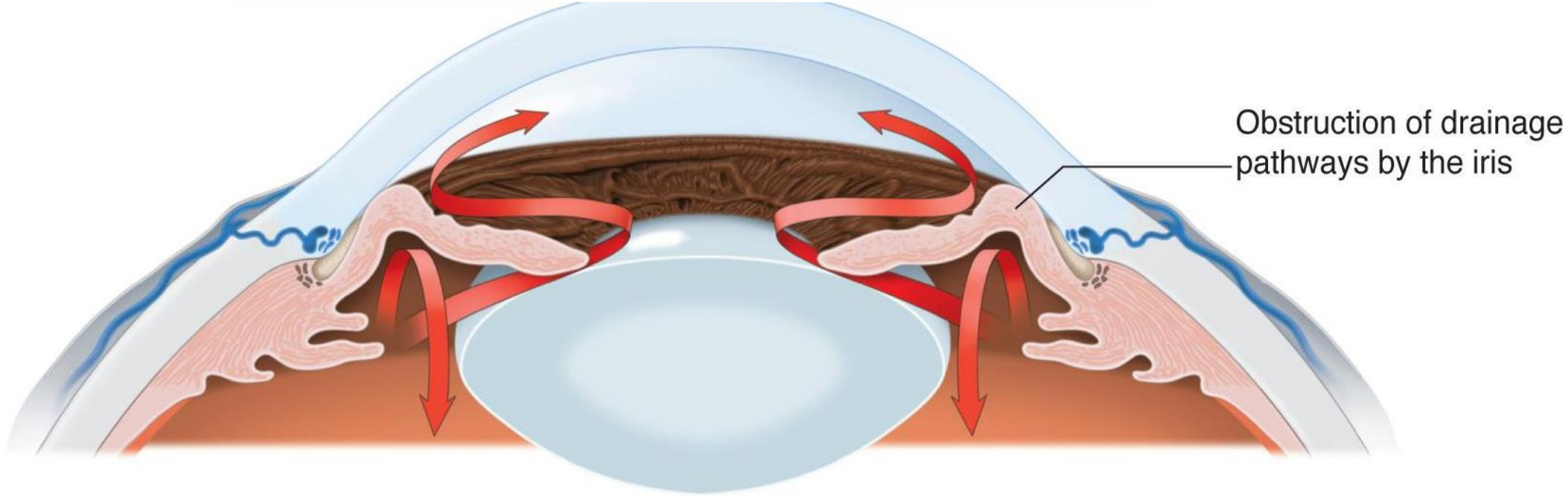
HEK-D_3R-MT_2R



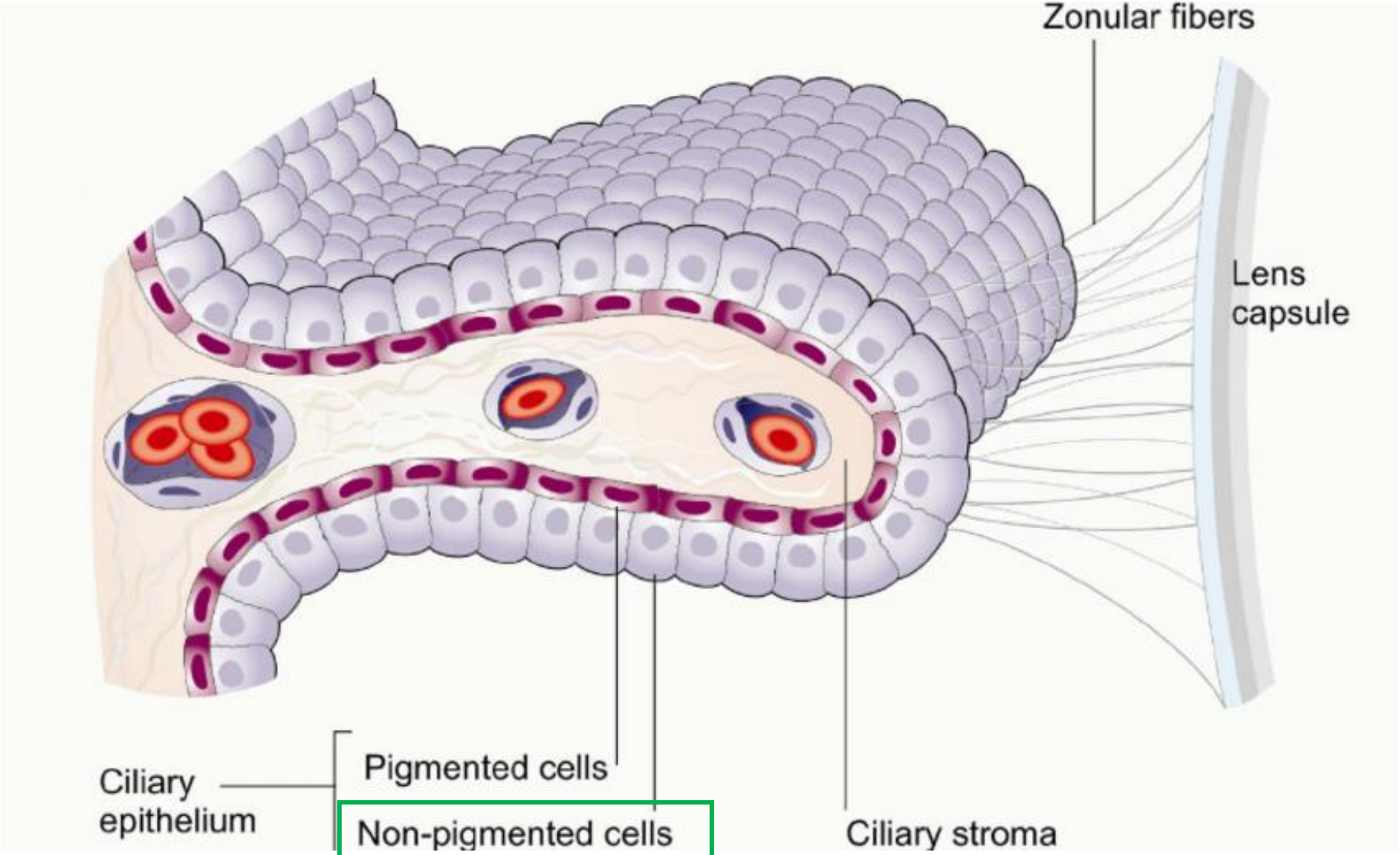
HEK-D_3R-MT_2R



CILIARY BODY

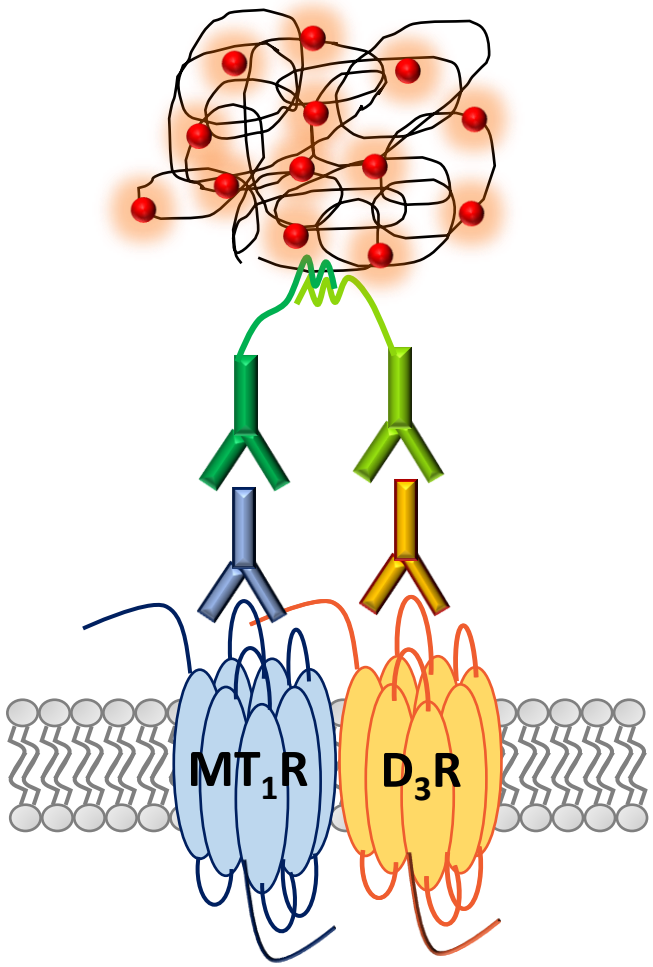


59HCE cell line: Non-Pigmented Ciliary Body Epithelial Cells



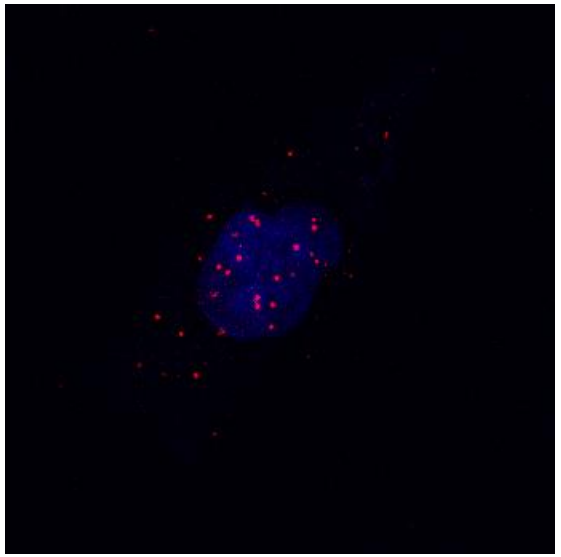
D_3-MT_1 and D_3-MT_2 Heteroreceptor Complexes in Human Non-Pigmented Ciliary Body Epithelial Cells

PROXIMITY LIGATION ASSAY (PLA)

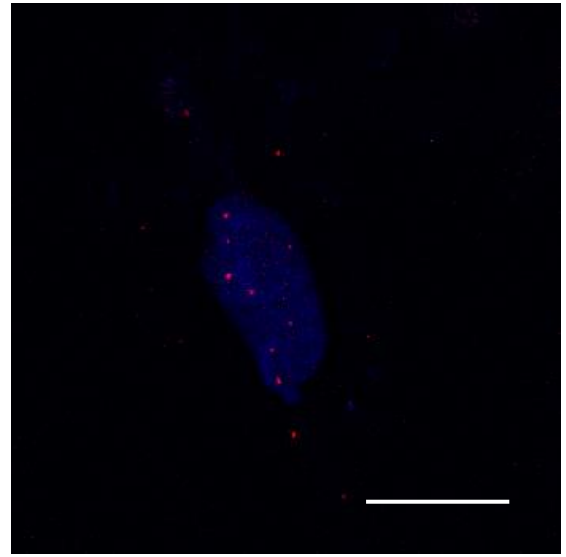


59HCE cells

D_3R-MT_1R

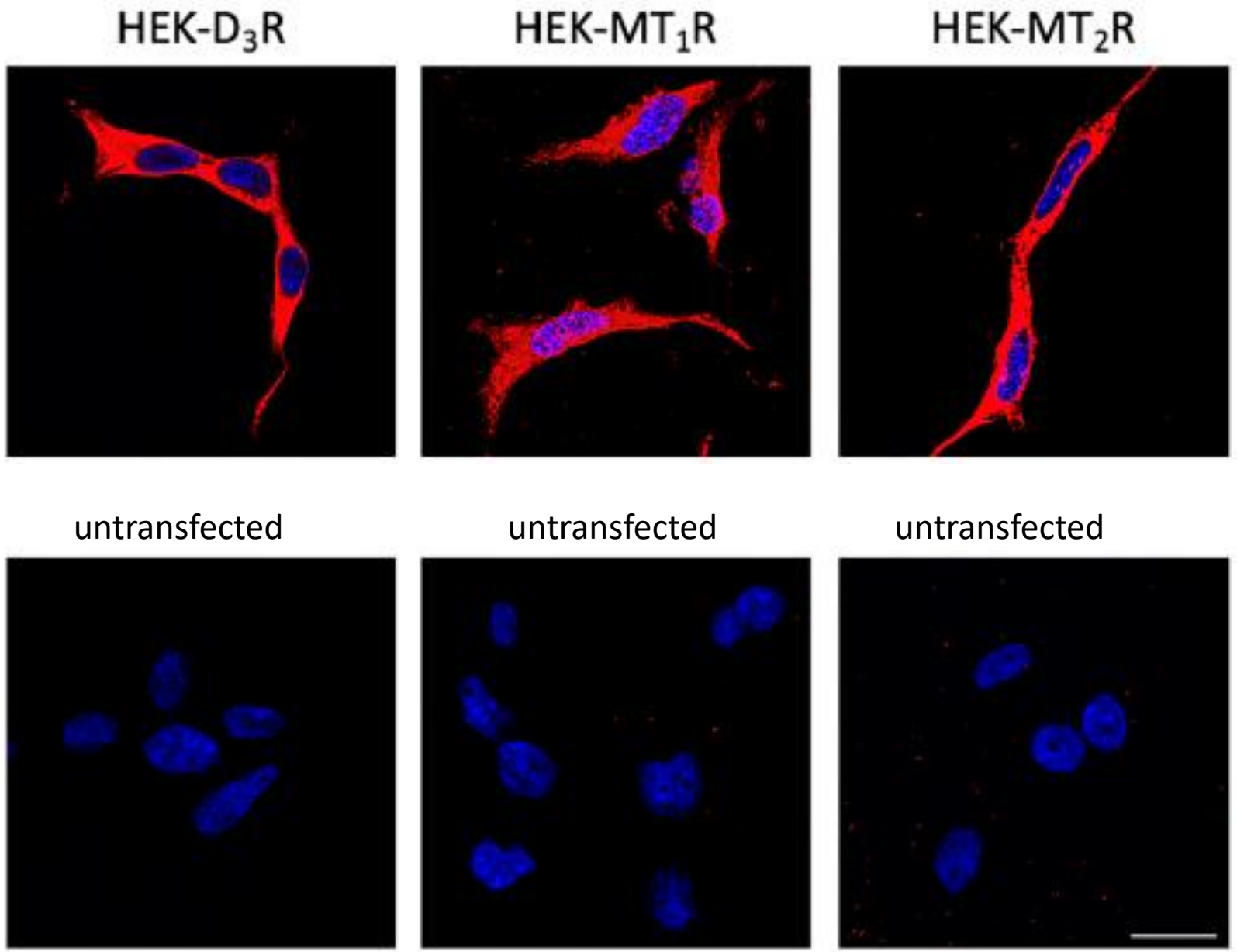


D_3R-MT_2R



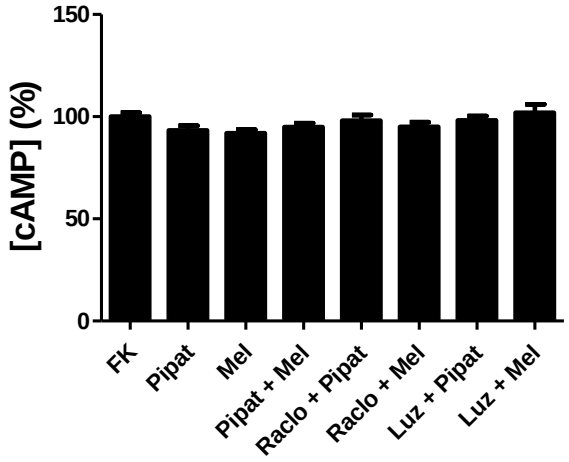
Specificity of antibodies against D3, MT1, and MT2 receptors

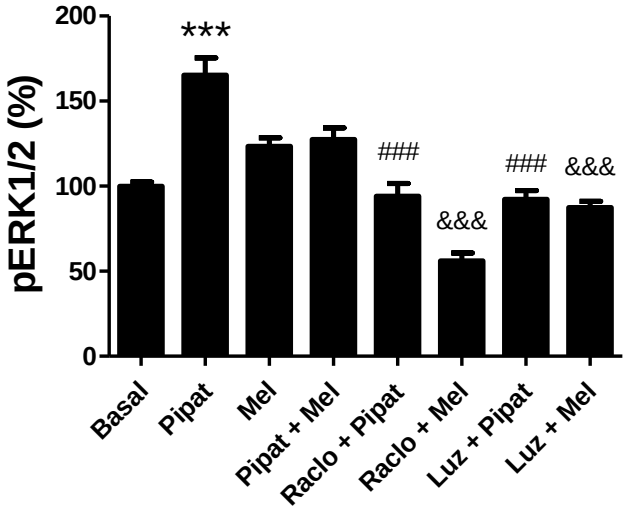
HEK293T cells

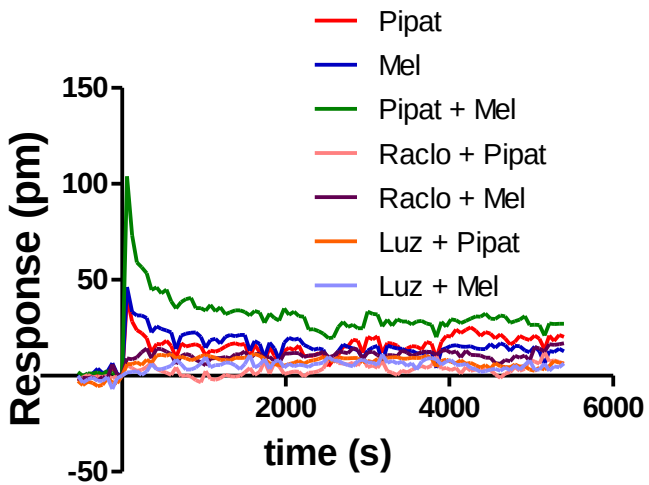


Effect of dopamine and melatonin receptor agonists in human 59HCE cells

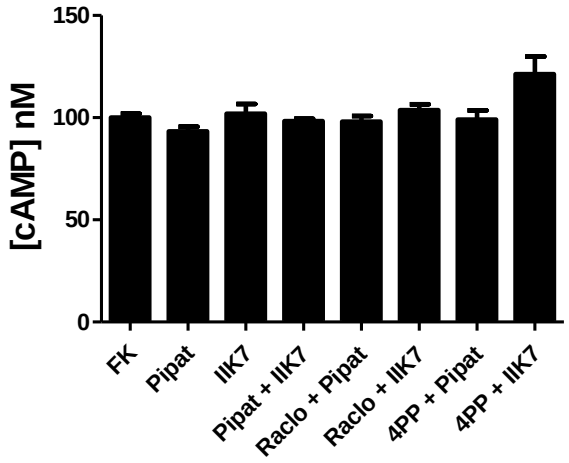
D_3R-MT_1R

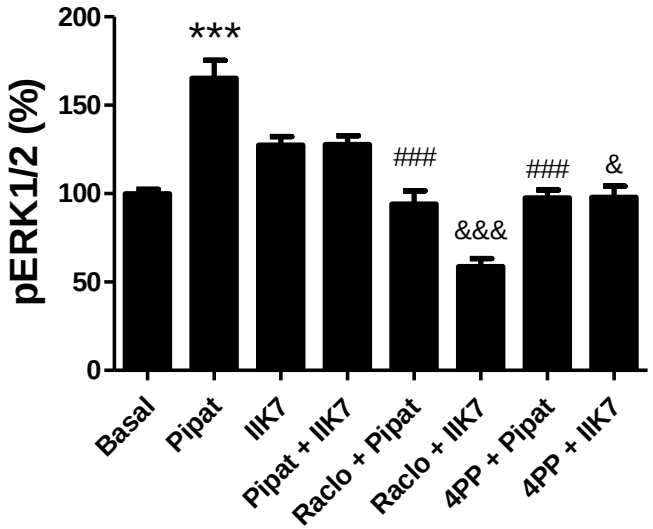


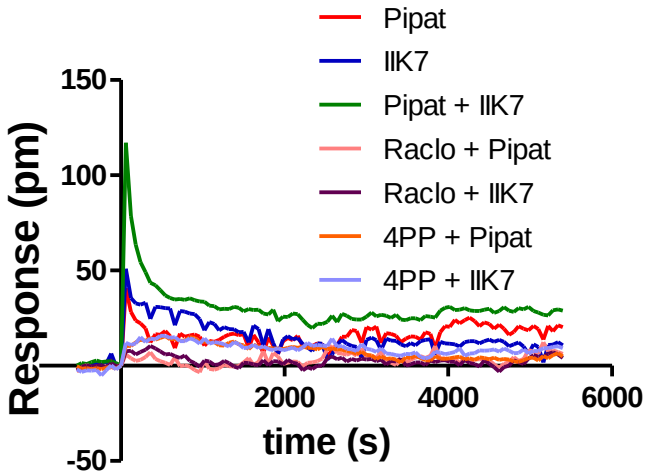




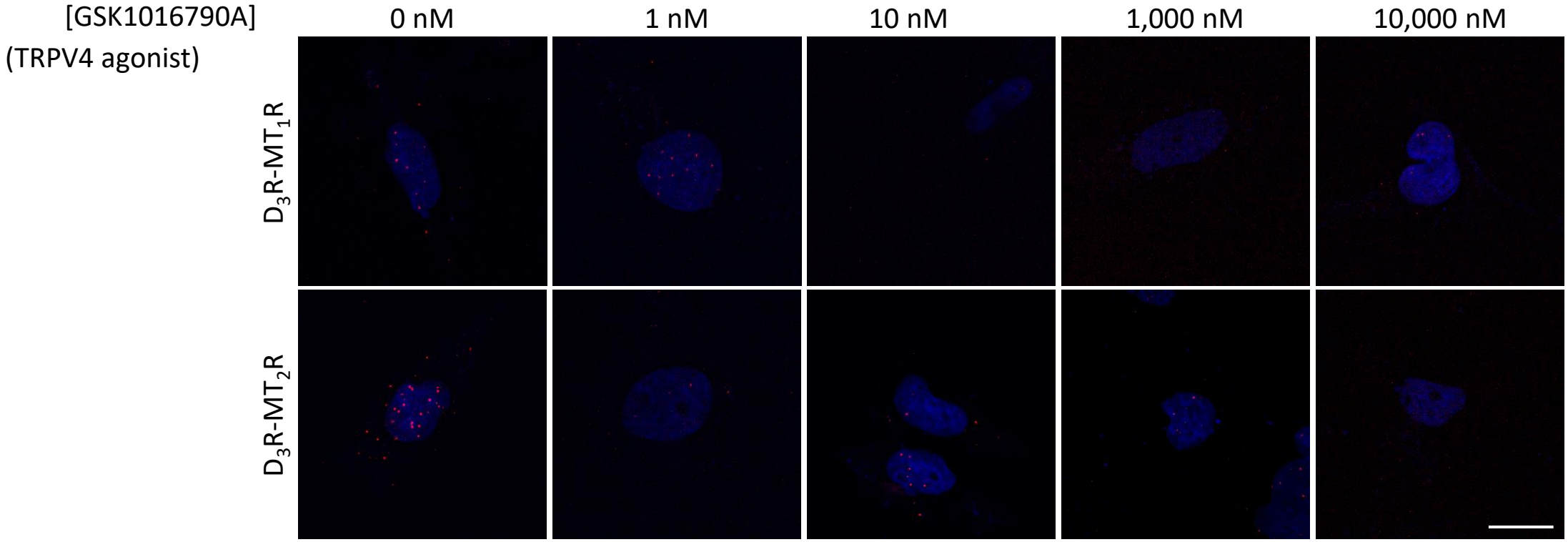
D_3R-MT_2R

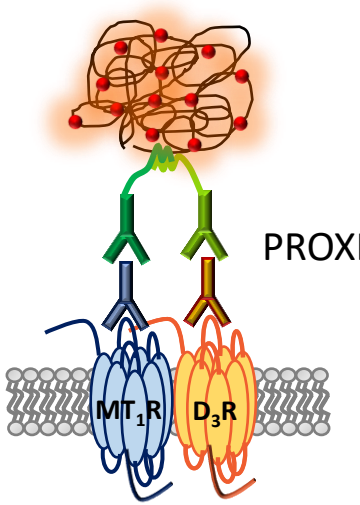




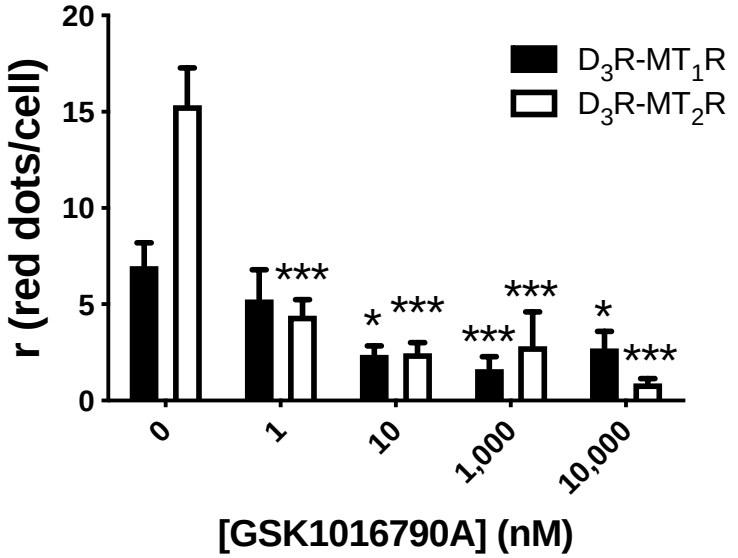


D_3-MT_1 and D_3-MT_2 Heteroreceptor Complexes in Human Non-Pigmented Ciliary Body Epithelial Cells



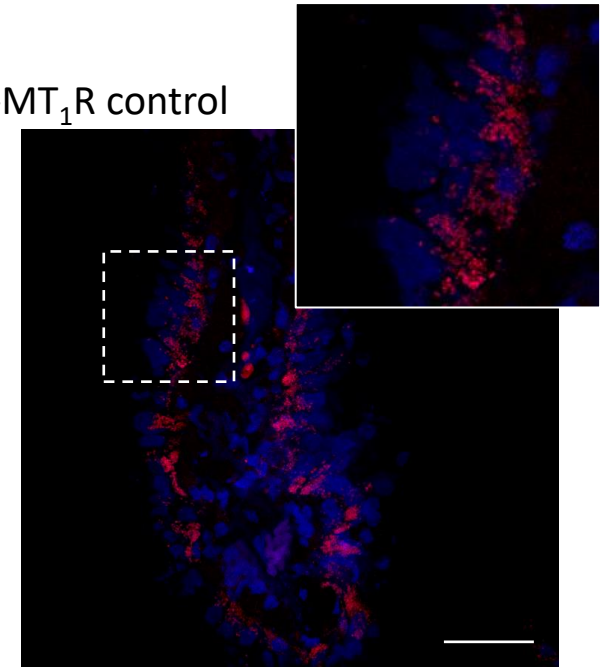


PROXIMITY LIGATION ASSAY (PLA)

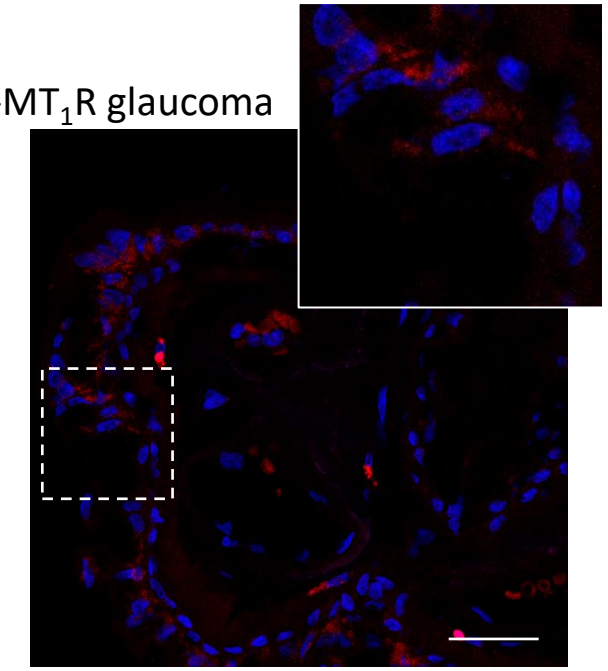


Differential expression of D_3-MT_1 and D_3-MT_2 heteroreceptor complexes in the glaucomatous eye

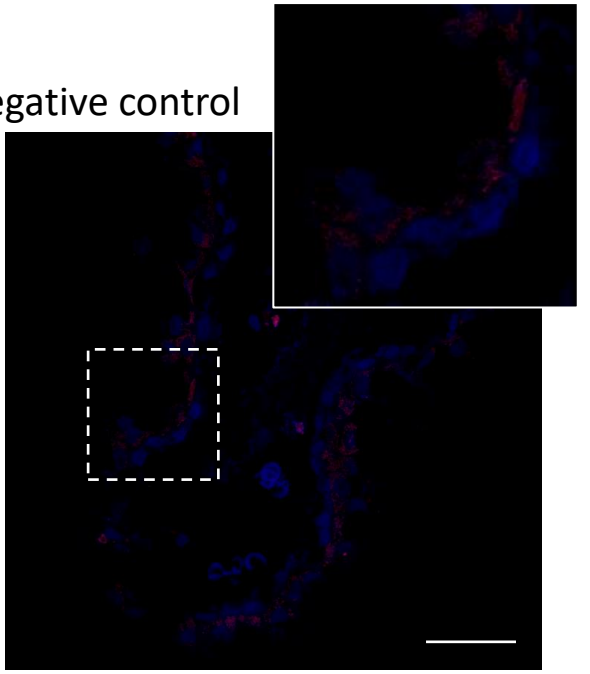
D_3R-MT_1R control



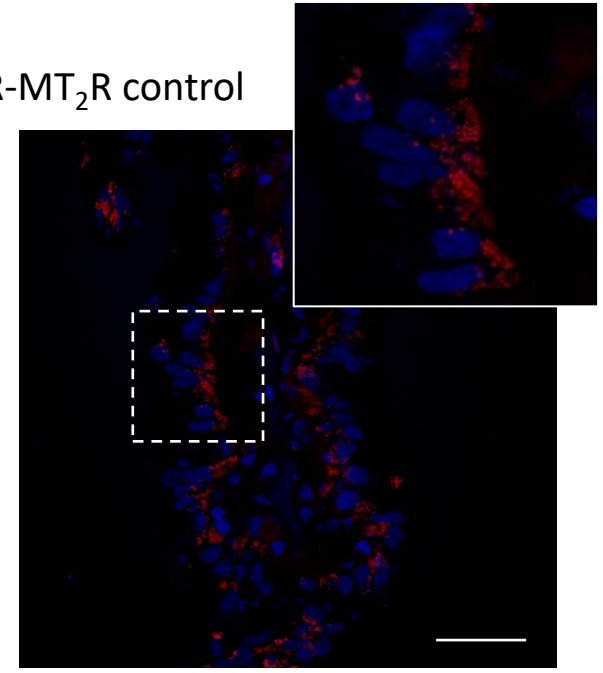
D_3R-MT_1R glaucoma



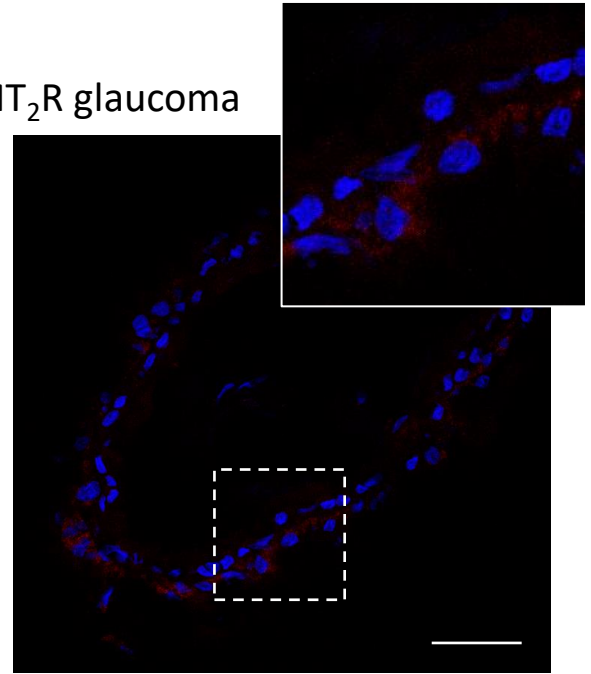
Negative control

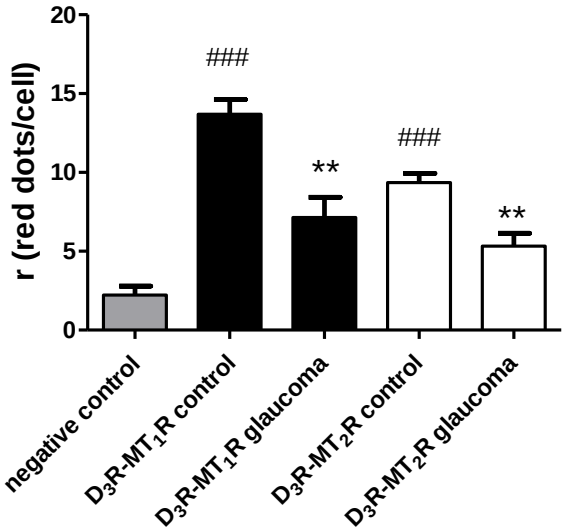


D_3R-MT_2R control



D_3R-MT_2R glaucoma





CONCLUSIONS

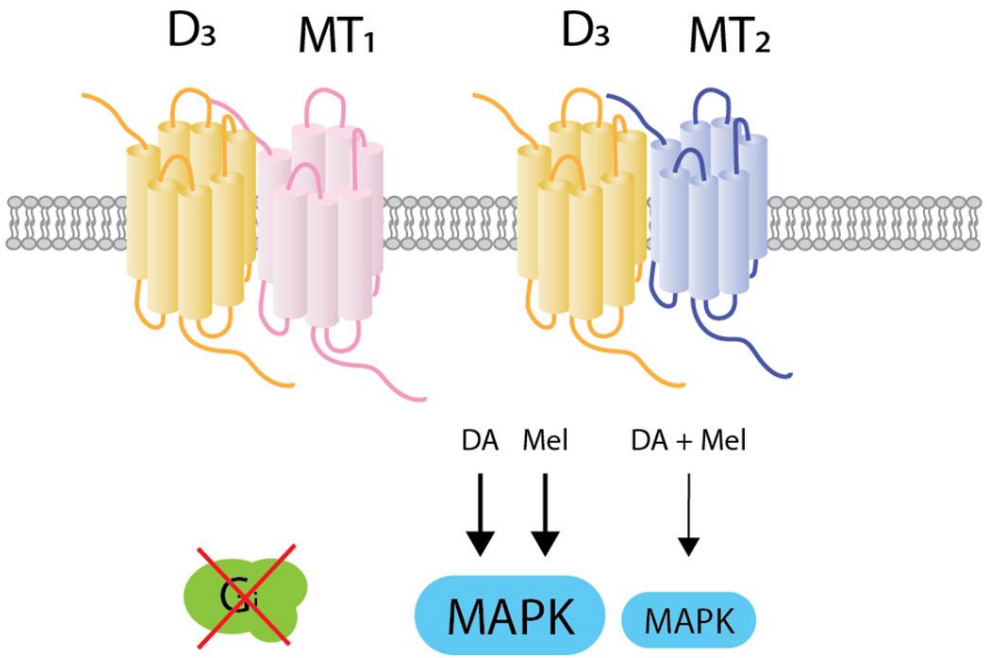
-D_3 receptors form heteroreceptor complexes with MT_1 and MT_2R receptors in transfected HEK-293T cells.

-The print of D_3R-MT_1R and D_3R-MT_2R heteroreceptor complexes consists of an abolishment of D_3R-mediated Gi signalling in the presence of MTRs, and a negative cross-talk and bidirectional cross-antagonism in MAPK signalling.

- D_3R-MT_1R and D_3R-MT_2R heteroreceptor complexes were detected in a human non-pigmented ciliary epithelial cell line and in human ciliary body samples.

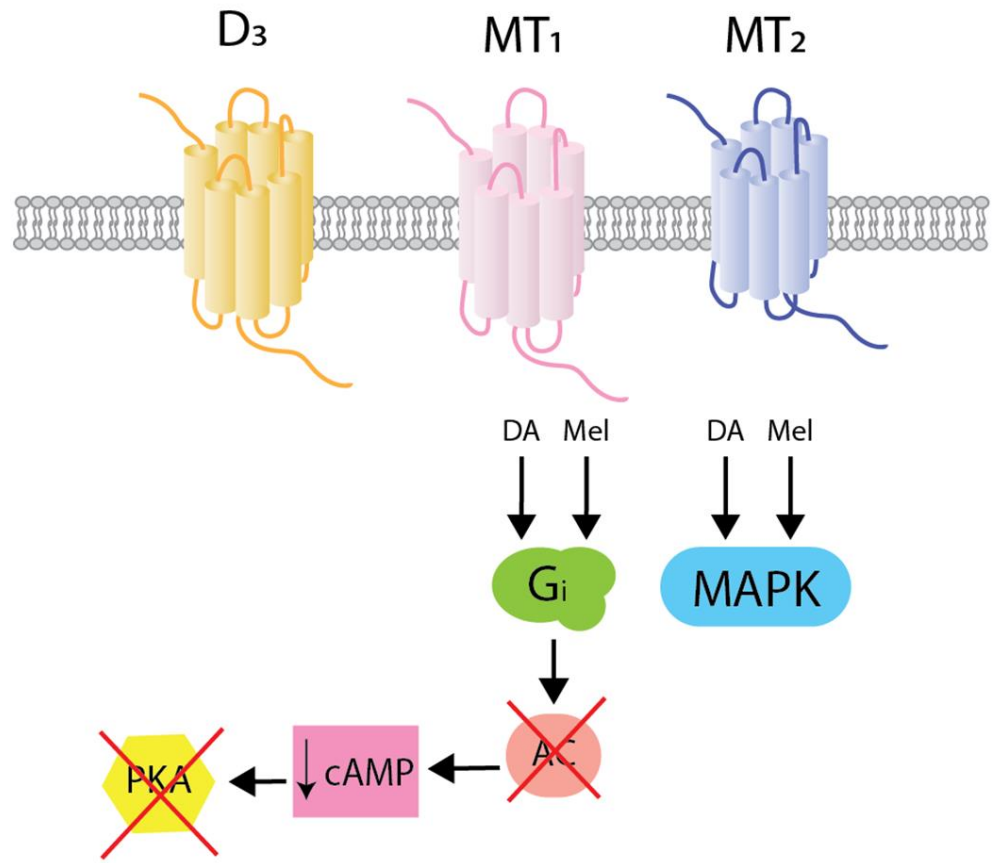
-The expression of D_3R-MT_1R and D_3R-MT_2R heteroreceptor complexes decreases in a ciliary body-based cell model of elevated IOP and in samples from human hypertensive eyes (vs normotensive), indicating a negative correlation between ocular hypertension and heteromer expression.

Healthy ciliary body cells



Balanced ion fluxes:
normotensive IOP

Glaucomatous ciliary body cells



Unbalanced ion fluxes:
hypertensive IOP

cells

MDPI

Article

Expression of Melatonin and Dopamine D_3 Receptor Heteromers in Eye Ciliary Body Epithelial Cells and Negative Correlation with Ocular Hypertension

Irene Reyes-Resina [1,2,3,*], Hanan Awad Alkozi [4], Anna del Ser-Badia [3,5], Juan Sánchez-Naves [6], Jaume Lillo [1,3], Jasmina Jiménez [3], Jesús Pintor [4], Gemma Navarro [3,7,*] and Rafael Franco [3,8,*]

1 Department of Biochemistry and Molecular Biomedicine, School of Biology, Universitat de Barcelona, 08028 Barcelona, Spain; lillojaume@gmail.com
2 Neuroplasticity Research Group, Leibniz Institute for Neurobiology, 39118 Magdeburg, Germany
3 Centro de Investigación en Red, Enfermedades Neurodegenerativas, CiberNed, Instituto de Salud Carlos III, 28029 Madrid, Spain; delserbadia@gmail.com (A.d.S.-B.); jasminajc@gmail.com (J.J.)
4 Department of Biochemistry and Molecular Biology, Faculty of Optics and Optometry, University Complutense of Madrid, 28037 Madrid, Spain; hanan-q1@live.com (H.A.A.); jpintor@ucm.es (J.P.)
5 Department de Bioquímica i Biologia Molecular, Institut de Neurociències, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain
6 Department of Ophthalmology, Balearic Islands Institute of Ophthalmology, 07013 Palma de Mallorca, Mallorca, Spain; juansanchez.naves@gmail.com
7 Department of Biochemistry and Physiology, School of Pharmacy and Food Sciences, Universitat de Barcelona, 08027 Barcelona, Spain
8 School of Chemistry, Universitat de Barcelona, 08028 Barcelona, Spain
* Correspondence: ire-reyes@hotmail.com (I.R.-R.); g.navarro@ub.edu (G.N.); rfranco123@gmail.com or rfranco@ub.edu (R.F.); Tel.: +34-934021208 (I.R.-R. & G.N.)

UNIVERSITAT DE BARCELONA

ciberned
Centro Investigación Biomédica en Red Enfermedades Neurodegenerativas

Rafael Franco, Grupo de Neurobiología Molecular, Depto. Bioquímica y Biomedicina Molecular, Facultad de Biología, UB

Gemma Navarro, Grupo de Neurofarmacología Molecular, Depto. Bioquímica y Fisiología, Facultad de Farmacia y CA, UB

Hanan Awad Alkozi

Jesús Pintor

Department of Biochemistry and Molecular Biology, Faculty of Optics and Optometry, University Complutense of Madrid

Juan Sánchez-Naves. Department of Ophthalmology, Balearic Islands Institute of Ophthalmology, Palma de Mallorca