Targeting Trends

Reporting the latest news in Molecular Surgery
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Highlight

Impaired reach-to-grasp responses in mice depleted of striatal cholinergic interneurons

by Nilupaer Abudukeyoumu, Marianela Garcia-Munoz, Yoko Nakano, Gordon W. Arbuthnott.
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Cholinergic interneurons (ChIs) are sparsely distributed within the striatum, a nucleus that plays important roles in voluntary motor control, associated learning, procedural memory, execution of movement, action selection, and planning.[1]

ChIs comprise 1-3% of all striatal neurons, are the main source of striatal acetylcholine, and have long been associated with deficits in Parkinson's disease. A striatal imbalance between dopamine and acetylcholine has been suggested as one of the causes of parkinsonism.[2]

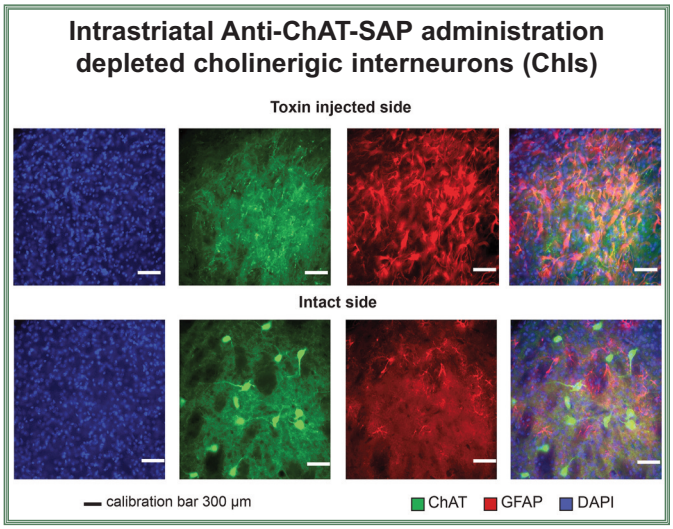


Fig. 1

To selectively deplete ChIs in the dorsolateral striatum of 21-day-old male mice (C57BL/6J), we used the saporin immunotoxin that targets choline acetyltransferase (**Anti-ChAT-SAP**, Cat. #IT-42). Experimental animals received a stereotaxic unilateral infusion of the targeted toxin (0.3µl/3min) and sham controls received the same volume of sterile saline (sham control). Our histological analysis encompassed Weeks 2-6 postsurgery performed at 2-week intervals (Fig. 1). The loss of cells reached a stable ~70% level by 4 to 6 weeks with the additional surprising finding that axon terminals stained with a vesicular acetylcholine transporter antibody were more numerous two weeks after the injection, returning to control levels by six weeks.

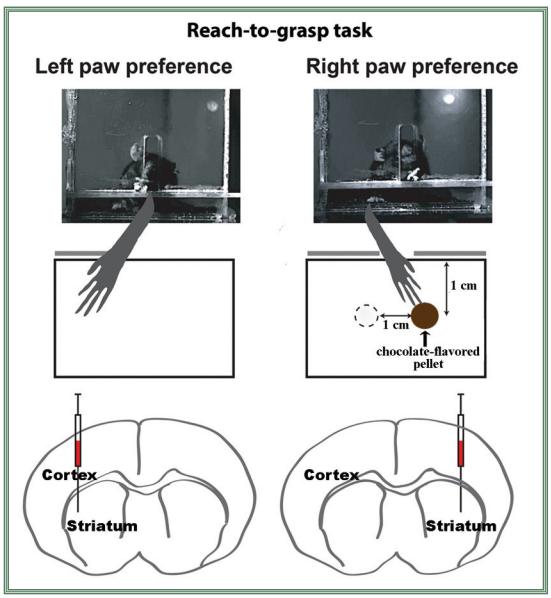


Fig. 2. Damage to ChIs impaired the use of the paw preference observed before **Anti-ChAT-SAP** intrastriatal adminisration.

From a functional point of view, it will be important to find out if despite the cell loss, axon terminals sprout to invade the Anti-ChAT-SAP injected area from ~30% surviving ChIs or from ChIs in the surrounding tissue. To begin the study of dorsolateral striatal function following Anti-ChAT-SAP-induced ChI loss, we followed the same procedures as before[3] and observed the animal's perfomance in a reach-to-grasp task (Fig. 2). Mice were divided in two control groups (intact and sham) and one experimental Anti-ChAT-SAP-injected group. Training started one week postsurgery during the animal's active circadian cycle and following 12 hours of food deprivation.

(continued on page 6)

The 2018 Poster of the Year Award Goes To . . .

Nilupaer Abudukeyoumu! Her poster was presented at the 2018 Society for Neuroscience meeting that was held in San Diego, CA:

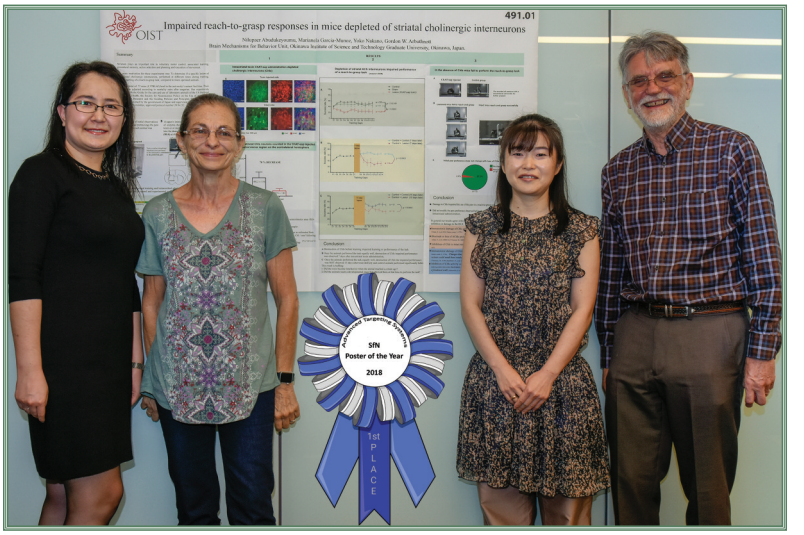
Impaired reach-to-grasp responses in mice depleted of striatal cholinergic interneurons

Authors on the winning poster are pictured at right L. to R.: Nilupaer Abudukeyoumu**,** Marianela Garcia-Munoz, Yoko Nakano, and Gordon W. Arbuthnott.

Nilupaer Abudukeyoumu is a PhD student in Dr. Arbuthnott's laboratory in the Brain Mechanism for Behavior Unit, Okinawa Institute of Science and Technology Graduate University, Japan.

See Cover article for more details about their exciting work.

Congratulations!

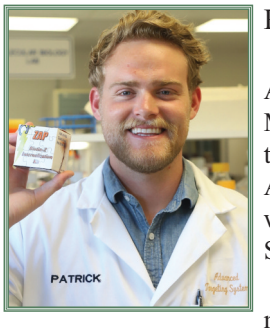


Product Managers

LEONARDO ANCHETA

Leonardo has worked as a scientist at Advanced Targeting Systems for over 15 years and was recently promoted to Vice President. His expertise in conjugations, flow cytometry, and research assay development are a major reason for the company's success.

Leonardo is also Scientific Director of ATS's partner organization, CytoLogistics. He oversees all the contract laboratory services (e.g. compound screening, cell-based assays, custom conjugations). Streptavidin-ZAP and many other secondary conjugates are under his watchful eye and he is ready to discuss your custom conjugation needs.



PATRICK SHRAMM

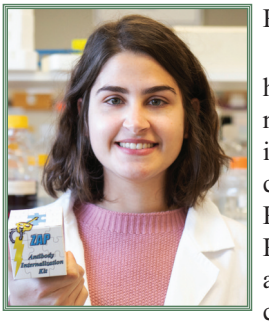
Patrick just completed his 7th year at ATS. He is a Product Manager for the Melanopsin product line and several of our targeted toxins. Patrick also developed the ATS pHast line of products and is currently working on the latest addition: Streptavidin-pHast (Strep-pHast).

Patrick's molecular biology expertise makes him a valuable contributor to new product development. His scientific research on a CRISPR line of targeting tools keeps him busy in the laboratory when he's not answering customer questions or working on product conjugations.

MIGUEL GALVAN

Miguel is a Product Manager in his second year at ATS. He is responsible for the production and handling of one of our most important products: Saporin. His skills in the laboratory and attention to detail make him a valuable asset to the team. He continues to be involved in Research and Development and is currently in the final stages of a new product line of transfection kits that will greatly improve efficiency and success in cell transfections.

Miguel's other products include the recombinant IB4 products, the AB-T conjugated antibodies, and the essential line of Saporin Control Conjugates.



RASCHEL BOUAJRAM

Raschel, our newest Product Manager, has been on the ATS team for over a year now. She has great expertise in the important assays needed to provide quality control for our conjugates and antibodies. Raschel produced one of our new products: Recombinant MOA (Marasmius oreades agglutinin). Unavailable from any other commercial source, her success in expressing and purifying the material was much needed. She is also responsible for the Saporin conjugate version: MOA-SAP. This targeted toxin recognizes blood group B antigens and has a high affinity to alpha gal, a carbohydrate found on B blood type cells. Raschel is also responsible for the Angiotensin antibodies and is currently working on a mouse-specific version.

Targeting Topics: 2018 Scientific References

TARGETED TOXINS

Targeting agent attached to Saporin

IT-01 192-IgG-SAP

Neuropharmacology of attention.
Burk JA, *et al.* Eur J Pharmacol 835: 162-168, 2018

Effect of Placenta-Derived Mesenchymal Stem Cells in a Dementia Rat Model via Microglial Mediation: a Comparison between Stem Cell Transplant Methods.
Cho JS, *et al.* Yonsei Med J 59: (3):406-415, 2018.

Intracerebroventricular Administration of 192IgG-Saporin Alters Expression of Microglia-Associated Genes in the Dorsal But Not Ventral Hippocampus.
Dobryakova YV, *et al.* Front Mol Neurosci 10: (Article 429), 2018.

Lacrimal Gland Denervation Alters Tear Protein Composition and Impairs Ipsilateral Eye Closures and Corneal Nociception.
Hegarty DM, *et al.* Invest Ophthalmol Vis Sci 59: (12):5217-5224, 2018.

Neuronal Activity-Dependent Control of Postnatal Neurogenesis and Gliogenesis.
Káradóttir RT, *et al.* Annu Rev Neurosci 41: 139-161, 2018.

Impact of Chronic Stress on the Spatial Learning and GR-PKAc-NF-κB Signaling in the Hippocampus and Cortex in Rats Following Cholinergic Depletion.
Lee S-Y, *et al.* Mol Neurobiol 55: (5):3976-3989, 2018.

Cholinergic basal forebrain structures are not essential for mediation of the arousing action of glutamate.
Lelkes Z, *et al.* J Sleep Res 27: (4):e12605, 2-18.

Effects of Cholinergic Lesions and Cholinesterase Inhibitors on Aromatase and Estrogen Receptor Expression in Different Regions of the Rat Brain.
Li J, *et al.* Neurosci 384: 203-213, 20183.

Cholinergic modulation of frontoparietal cortical network dynamics supporting supramodal attention.
Ljubojevic V, *et al.* J Neurosci 38: (16):3988-4005, 2-18.

The hot 'n' cold of cue-induced drug relapse.
Pitchers KK, *et al.* Learn Mem 25: (9):474-480, 2018.

The role of the supramammillary area of the hypothalamus in cognitive functions.
Shim HS, *et al.* Anim Cells Sys 22: (1):37-44.

Disruption of medial septum and diagonal bands of Broca cholinergic projections to the ventral hippocampus disrupt auditory fear memory.
Staib JM, *et al.* Neurobiol Learning Memory 152: 71-79, 2018.

Ontogenetic and Phylogenetic Approaches for Studying the Mechanisms of Cognitive Dysfunctions.
Zhuravin IA, *et al.* Evolutionary Physiology and Biochemistry, 2018.

Cholinergic modulation of spatial learning, memory and navigation.
Solari N, *et al.* Eur J Neurosci 48: (5):2199-2230, 2018.

IT-03 Anti-DBH-SAP

Mo1548 - How Does the Extrinsic Nervous System (ENS) Connect with the Intrinsic Nervous System (INS) of the Esophagus and Stomach?
Jiang Y, *et al.* Gastroenterology 154: (6, Supplement 1):S-748-S-749, 2018.

Modelling the dopamine and noradrenergic cell loss that occurs in Parkinson's disease and the impact on hippocampal neurogenesis.
Ermine CM, *et al.* Hippocampus 28: (5):327-337, 2018.

Noradrenergic Hypothesis Linking Neurodegeneration-Based Cognitive Decline and Astroglia.
Leanza G, *et al.* Front Mol Neurosci 11: 254, 2018.

Brainstem catecholaminergic neurones and breathing control during postnatal development in male and female rats.
Patrone LGA, *et al.* J Physiol 596: (15):3299-3325, 2018.

Essential role of hippocampal noradrenaline in the regulation of spatial working memory and TDP−43 tissue pathology.
Pintus R, *et al.* J Comp Neurol 526: (7):1131-1147, 2018.

Why I can’t say “no” to hindbrain catecholamine neurons.
Ritter S. Appetite 126: 210, 2018.

Mo1545 - Vagal Nerve Modulates the Effects of Esophageal Acid on the Periaqueductal Gray Functional Connectivity in a Rat Model.
Sanvanson P, *et al.* Gastroenterology 154: (6, Supplement 1):S-747-S-748, 2018.

Mo1547 - Enriched Environment Housing Alleviates Post-Colitis Pain, Visceral Hypersensitivity and Mood Disorders in Rats.
Winston J. Gastroenterology 154: (6, Supplement 1):S-748, 2018.

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Targeting Topics: 2018 Scientific References

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Mo1546 - Neonatal Colon Inflammation-Induced Increases in Pituitary Adenylate Cyclase Activating Peptide Expression in the Parabrachial Nucleus Contributes to Reduced Meal Consumption, to Increased Meal-Induced Aversive and Anxiety-Like Behaviors in Adult Rats.

Winston J. Gastroenterology 154: (6, Supplement 1):S-748, 2018.

Catecholaminergic Projections into an Interconnected Forebrain Network Control the Sensitivity of Male Rats to Diet-Induced Obesity.

Lee SJ, *et al.* Am J Physiol Regul Integr Comp Physiol 314: (6):R811-R823, 2018.

Involvement of median preoptic nucleus and medullary noradrenergic neurons in cardiovascular and sympathetic responses of hemorrhagic rats.

Naves LM, *et al.* Sci Rep 8: (1):11276, 2018.

IT-06 Mac-1-SAP (mouse)

The Critical Role of IL-10 in the Antineuroinflammatory and Antioxidative Effects of Rheum tanguticum on Activated Microglia.

Meng J, *et al.* Oxid Med Cell Longev (Article ID 1083596):12, 2018.

Targeting macrophage and microglia activation with colony stimulating factor 1 receptor inhibitor is an effective strategy to treat injury-triggered neuropathic pain.

Lee S, *et al.* Mol Pain 14: 1-12, 2018.

Summary: Depletion of spinal microglia with Mac-1-SAP was able to prevent and reverse neuropathic pain behavior.

Involvement of lysophosphatidic acid-induced astrocyte activation underlying the maintenance of partial sciatic nerve injury-induced neuropathic pain.

Ueda H, *et al.* Pain 159: (11):2170-2178, 2018.

Reduced Microglial Activity and Enhanced Glutamate Transmission in the Basolateral Amygdala in Early CNS Autoimmunity.

Acharjee S, *et al.* J Neurosci 38: (42):9019-9033, 2018.

IT-10 IB4-SAP

Fentanyl Induces Rapid Onset Hyperalgesic Priming: Type I at Peripheral and Type II at Central Nociceptor Terminals.

Araldi D, *et al.* J Neurosci 38: (9):2226-2245, 2018.

The Combinational Use of CRISPR/Cas9 and Targeted Toxin Technology Enables Efficient Isolation of Bi-Allelic Knockout Non-Human Mammalian Clones.

Watanabe S, *et al.* Int J Mol Sci 19: (4):E1075, 2018.

Summary: Results indicate that a combination of the CRISPR/Cas9 system and targeted toxin technology using IB4-SAP allows efficient enrichment of genome-edited clones, particularly bi-allelic KO clones.
Dose: Cells were trypsinized 3 days after transfection and approximately 80% were incubated for 30 min at 37°C in a solution (25 mcL) containing 0.5–1.0 mcg IB4-SAP.

CD44 Signaling Mediates High Molecular Weight Hyaluronan-Induced Antihyperalgesia.

Ferrari LF, *et al.* J Neurosci 38: (2):308-321, 2018.

IT-11 SSP-SAP

Role of GPCR (mu-opioid)-receptor tyrosine kinase (epidermal growth factor) crosstalk in opioid-induced hyperalgesic priming (type II).

Araldi D, *et al.* Pain Epub 16 Feb, 2018

Breathing regulation and blood gas homeostasis after near complete lesions of the retrotrapezoid nucleus in adult rats.

Souza G, *et al.* J Physiol 596: (13):2521-2545, 2018.

Modulation of chronic post-thoracotomy pain by NK-1 neurons in the rostral ventromedial medulla is not paralleled by changes spinal MAPKinase activation.

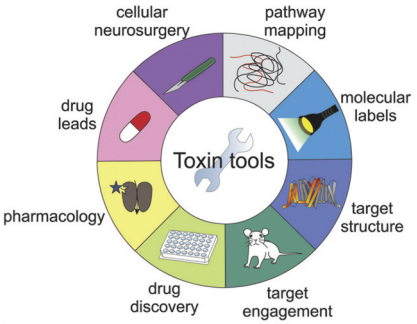
Strichartz G, *et al.* J Pain 19: (3):S14, 2018.

The Transient Intermediate Plexiform Layer, a Plexiform Layer-like Structure Temporarily Existing in the Inner Nuclear Layer in Developing Rat Retina.

Park HW, *et al.* Exp Neurobiol 27: (1):28-33, 2018.

Toxins in Neurobiology: New tools from old molecules.

Vetter I. Neurosci Lett, 679 1-3. 2018/05/09, 2018.



Vetter I. Neurosci Lett, 679 1-3. 2018/05/09, 2018.

IT-12 Dermorphin (MOR)-SAP

Selective ablation of striatal striosomes produces the deregulation of dopamine nigrostriatal pathway.

Shumilov K, *et al.* PLOS ONE 13: (8):e0203135, 2018.

IT-14 CTB-SAP

Neuroprotective effects on the morphology of somatic motoneurons following the death of neighboring motoneurons: A role for microglia?

Chew C, *et al.* Dev Neurobiol Epub 14 Nov, 2018.

Phrenic long-term facilitation following intrapleural CTB-SAP-induced respiratory motor neuron death.

Nichols NL, *et al.* Respir Physiol Neurobiol 256: 43-49, 2018.

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Targeting Topics: 2018 Scientific References

Targeted Ablation of Distal Cerebrospinal Fluid-Contacting Nucleus Alleviates Renal Fibrosis in Chronic Kidney Disease.

Qiu M, *et al.* Front Physiol 21 Nov, 2018.

The Establishment of a CSF-Contacting Nucleus "Knockout" Model Animal.

Song S-Y, *et al.* Front Neuroanat. 12: 22-32, 2018.

Targeted ablation of cardiac sympathetic neurons improves ventricular electrical remodelling in a canine model of chronic myocardial infarction.

Xiong L, *et al.* Europace 20: (12):2036-2044, 2018.

Objective: To evaluate the cardiac electrophysiologic effects of targeted ablation of cardiac sympathetic neurons (TACSN) in a canine model of chronic myocardial infarction (MI).
Summary: Targeted ablation of cardiac sympathetic neuron attenuates sympathetic remodelling and improves ventricular electrical remodelling in the chronic phase of MI. These data suggest that TACSN may be a novel approach to treating ventricular arrhythmias.
Dose: 20 µl of CTB-SAP (1.2 mg/ml) was mixed with 4 µl of 3% Evans blue dye to make it visible (CTB-SAP is colorless), ensuring localization within the ganglia. The CTB-SAP/Evans blue dye solution was slowly and intermittently injected into the left stellate ganglia using a glass micropipette.

Targeted ablation of cardiac sympathetic neurons attenuates adverse post-infarction remodeling and left ventricle dysfunction.

Xiong L, *et al.* Exp Physiol 103: (9):1221-1229, 2018.

IT-16 mu p75-SAP

Real−time electrochemical monitoring of choline during systemic inflammation in the freely−moving mouse.

Doyle S, *et al.* Monitoring Molecules in Neuroscience, Conference, 2018.

Removal of p75 Neurotrophin Receptor Expression from Cholinergic Basal Forebrain Neurons Reduces Amyloid-β Plaque Deposition and Cognitive Impairment in Aged APP/PS1 Mice.

Qian L, *et al.* Mol Neurobiol Epub 29 Oct 2018.

Objective: To investigate the contribution of CBF neuronal p75NTR to the progression of Alzheimer's Disease.
Summary: Data indicate that a direct interaction between CBF-expressed p75NTR and Aβ does not contribute significantly to the regulation of Aβ load.
Dose: To lesion CBF neurons, a single infusion of mu p75-SAP or control Rabbit IgG-SAP (0.4 mg/ml) was stereotaxically-injected into the basal forebrain.

Acute Down-regulation of BDNF Signaling Does Not Replicate Exacerbated Amyloid-β Levels and Cognitive Impairment Induced by Cholinergic Basal Forebrain Lesion.

Turnbull MT, *et al.* Front Mol Neurosci 11: 51, 2018.

Cholinergic modulation targeting medial prefrontal cortex leads to behavior deficit in interval timing task.

Zhang Q, *et al.* (P5.195) Neurology 90: (15 Supplement), 2018.

IT-20 Orexin-SAP

Depletion of Hypocretin/Orexin Neurons Increases Cell Proliferation in the Adult Subventricular Zone.

Arias-Carrion O, *et al.* CNS Neurol Disord Drug Targets 17: (2):106-112, 2018.

Orexinergic neurons are involved in the chemosensory control of breathing during the dark phase in a Parkinson's disease model.

Oliveira LM, *et al.* Exp Neurobiol 309: 107-118, 2018.

IT-23 Anti-SERT-SAP

Serotonin-specific lesions of the dorsal raphe disrupt maternal aggression and caregiving in postpartum rats.

Holschbach MA, *et al.* Behav Brain Res 348: 53-64, 2018.

Raphe Pallidus is Not Important to Central Chemoreception in a Rat Model of Parkinson's Disease.

Oliveira LM, *et al.* Neuroscience 369: 350-362, 2018.

IT-25 Anti-DAT-SAP

Macrophage migration inhibitory factor mediates peripheral nerve injury-induced hypersensitivity by curbing dopaminergic descending inhibition.

Wang X, *et al.* Exper Mol Med 50: (2):e445, 2018.

IT-31 CCK-SAP

A Neural Circuit for Gut-Induced Reward.

Han W, *et al.* Cell 175: (3):665-678, 2018.

IT-33 Mac-1-SAP (rat)

Early CALP2 expression and microglial activation are potential inducers of spinal IL-6 upregulation and bilateral pain following motor nerve injury.

Chen SX, *et al.* J Neurochem 145: (2):154-169, 2018.

Microglial pannexin-1 channel activation is a spinal determinant of joint pain.

Mousseau M, *et al.* Sci Adv 4: (8):1-12, 2018.

IT-40 Bombesin-SAP

Tac1-Expressing Neurons in the Periaqueductal Gray Facilitate the Itch-Scratching Cycle via Descending Regulation.

Gao Z-R, *et al.* Neuron 101: (1):45-59, 2018.

Spinal Mechanisms of Itch Transmission.

Barry DM, *et al.* Neurosci Bull 34: (1):156-164, 2018.

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Impaired reach-to-grasp responses in mice depleted of striatal cholinergic interneurons

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Once the animals passed the initial acquisition phase, the successful performance in the reach-to-grasp task -- expressed as mean ± SD percentage is shown in Fig. 3.

Controls: 51.11 ± 3.83; n = 25 [intact], 48.79 ± 4.6; n = 9 [sham]

Treated: 26.28 ± 3.74; n = 13

The significantly-impaired performance of the experimental group compared to controls was present even when the animals were pretrained. The loss of ChIs impairs the performance of striatally-mediated motor tasks, which suggests that cholinergic synaptic function is more important than non-synaptic communication in this situation. A non-synaptic cholinergic tone may be important for setting functional striatal states in other circumstances,[4] however, these specific lesions of ChI cells suggest that performance of a learned forelimb task requires that the cholinergic synaptic circuits of the striatum are intact.

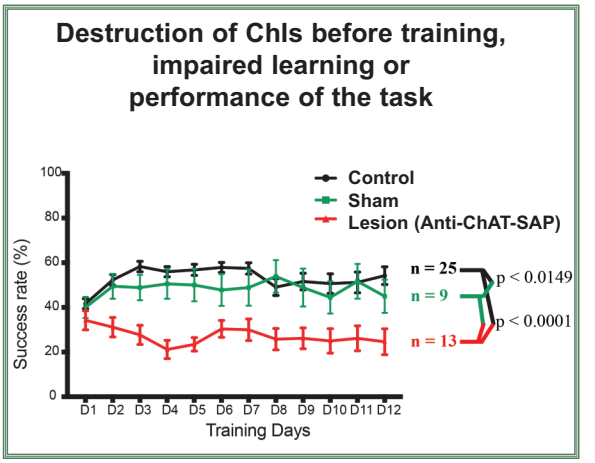

Fig. 3. Effect of intrastriatal administration of Anti-ChAT-SAP or sterile saline (300 nl) into the dorsolateral-sensimotor-striatum at different times during task training.

References

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2. Aosaki T, Miura M, Suzuki T, Nishimura K, & Masuda M. Acetylcholine-dopamine balance hypothesis in the striatum: an update. (2010). *Geriatr Gerontol Int.*, 10 Suppl 1 S148-157. 2010/07/16.

3. Lopez-Huerta VG, Nakano Y, Bausenwein J, Jaidar O, Lazarus M, Cherassse Y, Garcia-Munoz M, & Arbuthnott G. The neostriatum: two entities, one structure? (2016). *Brain Struct Funct*, 221 (3):1737-1749. 2015/02/06. PMC4819794.

4. Pittman-Polletta BR, Quach A, Mohammed AI, Romano M, Kondabolu K, Kopell NJ, Han X, & McCarthy MM. Striatal cholinergic receptor activation causes a rapid, selective and state-dependent rise in cortico-striatal beta activity. (2018). *Eur J Neurosci.* 48 (8):2857-2868.

Other References Using Anti-ChAT-SAP

Liu A, Aoki S, Wickens J. (2017) A Streamlined Method for the Preparation of Gelatin Embedded Brains and Simplified Organization of Sections for Serial Reconstructions. *BioProtoc*. 7(22). DOI: 10.21769/2610.

Xiao H, Li M, Cai J, Li N, Zhou M, Wen P, Xie Z, Wang Q, Chang J, Zhang W. (2017) Selective Cholinergic Depletion of Pedunculopontine Tegmental Nucleus Aggravates Freezing of Gait in Parkinsonian Rats. *Neurosci Lett* 659:92-98. PMID: 28803956

Abudukeyoumu N, Garcia-Munoz M, Jaidar OP, Arbuthnott G (2016) Striatal cholinergic interneurons: their depletion and its progression. *Soc Neurosci Meeting Abstract* 245.09

Aoki S, Liu AW, Zucca A, Zucca S, Wickens JR. (2015) Role of striatal cholinergic interneurons in set-shifting in the rat. *J Neurosci* 35(25):9424-9431.

Aoki S, Wickens JR. (2015) Anti-ChAT-SAP elucidates a causal role in behavioral flexibility. *Targeting Trends* 16(4).

Kucinski A. (2015) Impairments in gait, posture and complex movement control in rats modeling the multi-system, cholinergic-dopaminergic losses in Parkinson's Disease. *Targeting Trends* 16(1).

Xu M, Kobets A, Du JC, Lennington J, Li L, Banasr M, Duman RS, Vaccarino FM, DiLeone RJ, Pittenger C. (2015) Targeted ablation of cholinergic interneurons in the dorsolateral striatum produces behavioral manifestations of Tourette syndrome. *Proc Natl Acad Sci USA* 112(3):893-898.

LaPlante F. (2013) Role of cholinergic neurons in the nucleus accumbens and their involvement in schizophrenic pathology. *Targeting Trends* 14(1).

LaPlante F, Dufresne MM, Ouboudinar J, Ochoa-Sanchez R, Sullivan RM. (2013) Reduction in cholinergic interneuron density in the nucleus accumbens attenuates local extracellular dopamine release in response to stress or amphetamine. *Synapse* 67(1):21-29.

LaPlante F, Zhang ZW, Huppe-Gourgues F, Dufresne MM, Vaucher E, Sullivan RM. (2012) Cholinergic depletion in nucleus accumbens impairs mesocortical dopamine activation and cognitive function in rats. *Neuropharmacology* 63(6):1075-1084.

LaPlante F, Lappi DA, Sullivan RM (2011) Cholinergic depletion in the nucleus accumbens: Effects on amphetamine response and sensorimotor gating. *Prog Neuropsychopharmacol Biol Psychiatry* 35(2):501-509.


Kermit enjoys a winter's nap!

Targeting Topics: 2018 Scientific References

(continued from page 5)

IT-44 Melanopsin-SAP

DSCAM promotes self-avoidance in the developing mouse retina by masking the functions of cadherin superfamily members.

Garrett AM, *et al.* Proc Natl Acad Sci 115: (43):E10216-E10224, 2018.

Summary: Focus on DSCAM (Down syndrome cell adhesion molecule 1) self-avoidance function in the mouse retina. DSCAM and members of the cadherin superfamily have also emerged as key contributors to a variety of neurodevelopmental disorders, including autism, schizophrenia, bipolar disease, Down syndrome and intellectual disability.

Immunotoxin-Induced Ablation of the Intrinsically Photosensitive Retinal Ganglion Cells in Rhesus Monkeys.

Ostrin LA, *et al.* Front Neurol 9: 1000, 2018.

Immunolesion of melanopsin neurons causes gonadal regression in Pekin drakes (Anas platyrhynchos domesticus).

Potter H, *et al.* Gen Comp Endocrinol 256: 16-22, 2018.

IT-50 Anti-CD103-SAP

Indoleamine 2,3-dioxygenase-dependent expansion of T-regulatory cells maintains mucosal healing in ulcerative colitis.

Acovic A, *et al.* Therap Adv Gastroenterol 11: 1-22, 2018.

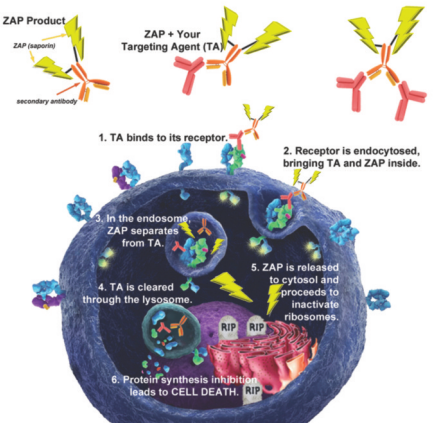
IT-69 Nppb-SAP

Circuit dissection of the role of somatostatin in itch and pain.

Huang J, *et al.* Nature Neurosci 21: (5):707-716, 2018.

SECONDARY CONJUGATES

Use your primary targeting agent



IT-04 Mab-ZAP

An Agonistic Antibody to EPHA2 Exhibits Antitumor Effects on Human Melanoma Cells.

Sakamoto A, *et al.* Anticancer Res 38: (6):3273-3282, 2018.

Objective: Investigate the therapeutic potential of antibody to EPHA2 against melanoma *in vitro*.
Summary: Observations indicate a promising role for EPHA2 as a target in antibody treatments for melanoma, and demonstrate the potential therapeutic effects of an agonistic antibody to EPHA2.
Dose: A375 cells were plated into a flat-bottom, 96-well plate (2,000 cells per well) and incubated for 4 days at 37˚C. Cell suspension included different concentrations of Mab-ZAP, along with either anti-EPHA2 mAb (SHM16, SHM17, or SHM20 at 2 µg/ml final concentration), or a control IgG1 mAb (2 µg/ml final concentration).

Targeting of embryonic annexin A2 expressed on ovarian and breast cancer by the novel monoclonal antibody 2448.

Cua S, *et al.* Oncotarget 9: (17):13206-13221, 2018.

IT-22 Hum-ZAP

Conservation of oncofetal antigens on human embryonic stem cells enables discovery of monoclonal antibodies against cancer.

Tan HL, *et al.* Sci Rep 8: (1):11608, 2018.

IT-27 Streptavidin-ZAP

Sequential Prodrug Strategy to Target and Eliminate ACPA-Selective Autoreactive B cells.

Lelieveldt LPWM, *et al.* Mol Pharm Epub 26 Oct, 2018.

Human anti-NKp46 antibody for studies of NKp46-dependent NK cell function and its applications for type 1 diabetes and cancer research.

Berhani O, *et al.* Eur J Immunol Epub 7 Dec, 2018.

Objective: To investigate human NKp46 activity and its critical role in Natural Killer (NK) cell biology.
Summary: A unique anti-human NKp46 monoclocal antibody was developed and conjugated to Saporin. Targeted toxin inhibits growth of NKp46-positive cells; thus, exemplifying the potential as an immunotherapeutic drug to treat NKp46-dependent diseases, such as, type I diabetes and NK and T cell related malignancies.
Dose: Conjugation of the antibodies to Saporin, treatment of cells, and cell viability assay Biotin-Z Kit instructions.

Enhanced targeting of triple-negative breast carcinoma and malignant melanoma by photochemical internalization of CSPG4-targeting immunotoxins.

Eng MS, *et al.* Photochem Photobiol Sci 17: (5):539-551, 2018.

Antibody Drug Conjugates Targeted to CD45 or CD117 Enable Allogeneic Hematopoietic Stem Cell Transplantation in Animal Models.

Palchaudhuri R, *et al.* ASH Annual Meeting, 2018.

Receptor-mediated Delivery: New Targets
See Back Cover for details!

(continued on page 8)

(continued from page 7)

IT-27 Streptavidin-ZAP

Synergistic Cytotoxic Effect on Gastric Combine Phage Antibody Display Library Selection on Patient Tissue Specimens with Laser Capture Microdissection to Identify Novel Human Antibodies Targeting Clinically Relevant Tumor Antigens.

Su Y, *et al.* Methods Mol Biol 1701: 331-347, 2018.

Cancer Cells of an Immunotoxin Cocktail in Which Antibodies Recognize Different Epitopes on CDH17.

Kusano-Arai O, *et al.* Monoclon Antib Immunodiagn Immunother 37: (1):1-11, 2018.

Characterization of the first fully human anti-TEM1 scFv in models of solid tumor imaging and immunotoxin-based therapy.

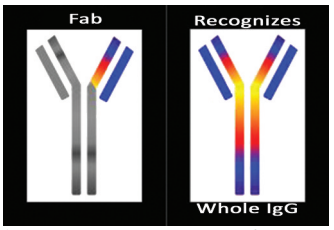
Yuan X, *et al.* Cancer Immunol Immunother 67: (2):329-339, 2018.

Development and evaluation of T-Zap: a novel antibody-drug conjugate for the treatment of Her2 positive breast cancer.

Hoffmann RM, *et al.* AACR Ann Mtg 2018. 78: (13 Suppl):Abstract LB-001, 2018.

Interaction between the retrotrapezoid nucleus and the parafacial respiratory group to regulate active expiration and sympathetic activity in rats.

Zoccal DB, *et al.* Am J Physiol Lung Cell Mol Physiol Epub 7 Nov, 2018.



Fab-ZAP products are made with monovalent (Fab) antibodies that recognize the whole IgG, without bivalent capping.

IT-51 Fab-ZAP human

Engineering elastic properties into an anti-TNFα monoclonal antibody.

Sadhukhan R, *et al.* Cogent Biol 4: (1):1-19, 2018.

IT-65 FabFc-ZAP human

Adalimumab:TNF complexes are cleared more efficiently by human osteoclasts than those with etanercept through FCG-receptor binding and internalization.

Harvey BP, *et al.* Ann Rheum Dis 77: (Suppl 2):SAT0058 893, 2018.

SELECTED ANTIBODIES

AB-02 Anti-CRH

Immunohistochemical detection of prolactin-releasing peptide2 in the brain of the inshore hagfish Eptatretus burger.

Amano M, *et al.* Gen Comp Endocrinol, 2018.

In vitro examination of microglia-neuron crosstalk with BV2 cells, and primary cultures of glia and hypothalamic neurons.

Tao X, *et al.* Heliyon 4: (8):e00730-e00730, 2018.

Histological and morphofunctional parameters of the hypothalamic-pituitary-adrenal system are sensitive to daidzein treatment in the adult rat.

Trifunovic S, *et al.* Acta Histochem 120: (2):129-135, 2018.

AB-213 Anti-6His

Isolation of blood-brain barrier-crossing antibodies from a phage display library by competitive elution and their ability to penetrate the central nervous system.

Thom G, *et al.* mAbs 10: (2):304-314, 2018.

AB-N01 Anti-mu p75

Non-canonical Ret signaling augments p75-mediated cell death in developing sympathetic neurons.

Donnelly CR, *et al.* J Cell Biol 217: (9):327, 2018.

Nicotinamide Mononucleotide Adenylyltransferase 2 maintains neuronal structural integrity through the maintenance of golgi structure.

Pottorf T, *et al.* Neurochem Int 121: 86-97, 2018.

Enteric Neurodegeneration is Mediated Through Independent Neuritic and Somal Mechanisms in Rotenone and MPP+ Toxicity.

Virga DM, *et al.* Neurochem Res 43: (12):2288-2303, 2018.

Myoepithelial Cells of Submucosal Glands Can Function as Reserve Stem Cells to Regenerate Airways after Injury.

Tata A, *et al.* Cell Stem Cell 22: (5):668-683, 2018.

AB-N01AP Anti-mu p75

affinity-purified

Nitration and Glycation Turn Mature NGF into a Toxic Factor for Motor Neurons: A Role for p75NTR and RAGE Signaling in ALS.

Kim MJ, *et al.* Antioxid Redox Signal 28: (18):1587-1602, 2018.

Cell-Specific Transcriptome Analysis Shows That Adult Pillar and Deiters' Cells Express Genes Encoding Machinery for Specializations of Cochlear Hair Cells.

Liu H, et al. Front Mol Neurosci 11: 356-356, 2018.

Regenerative effects of human embryonic stem cell-derived neural crest cells for treatment of peripheral nerve injury.

Jones I, et al. J Tissue Eng Regen Med 1-11, 2018.

(continued on page 10)

Targeting Tools: Top Twenty (Five Each in Four Categories)

Top Five Targeted Toxins

#1. CTB-SAP (Cholera Toxin B-Saporin) (Cat. #IT-14)
targets cells expressing GM1 receptor

#2. Anti-DBH-SAP (Cat. #IT-03)
targets cells expressing rat dopamine beta-hydroxylase (DBH)

#3. 192-IgG-SAP (192-Saporin) (Cat. #IT-01)
targets cells expressing rat p75^{NTR}

#4. Anti-ChAT-SAP (Cat. #IT-42)
targets cells expressing choline acetyltransferase

#5. Nppb-SAP (Neuropeptide natriuretic polypeptide B) (Cat. #IT-06)
targets cells expressing Nppb or BNP receptor

Top Five Antibodies

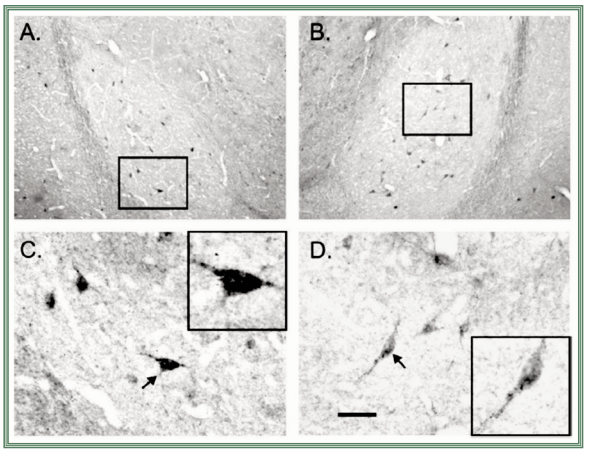
#1. Angiotensin II receptor (AT-1r) Rabbit Polyclonal, affinity-purified (Cat. #AB-N27AP)

#2. Trans-hydroxyproline Rabbit Polyclonal (Cat. #AB-T044)

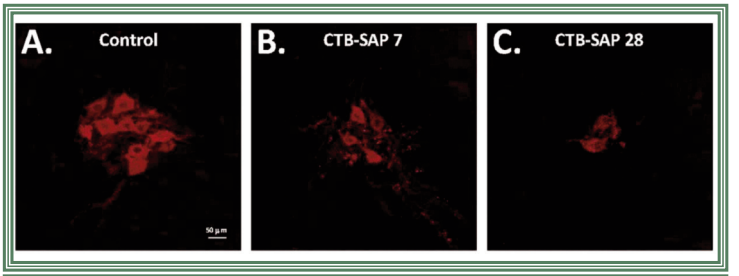
#3. Melanopsin Rabbit Polyclonal (Cat. #AB-N38)

#4. NGFr (mu p75) Rabbit Polyclonal, affinity-purified (Cat. #AB-N01AP)

#5. Angiotensin II receptor (AT-2r) Rabbit Polyclonal, affinity-purified (Cat. #AB-N28AP)



AB-N27AP (Angiotensin IIR - AT1R. Photomicrographs in A,B) and C,D) represent low and high magnification, respectively; images of IHC localization of AT1R in left and right basolateral amygdala of an adult male rat. Black box inserts in A and B are high magnification photomicrographs shown in C and D, respectively. Black box inserts in C and D are higher magnification images of neurons in C and D that are indicated by arrows. Scale bars: 200 µm, A,B; 50 µm, C,D.
Johnson et al. Prog Neuro-Psychopharmacol Biol Psychiatry, 44:248, 2013.



Phrenic motor neuron counts in controls and CTB-SAP treated rats after 7 and 28 days. A-C. Representative CTB-stained sections from C4 spinal ventral horn from a control rat (A) Control, (B) 7-day, (C) 28-day. Phrenic motor neuron survival is significantly decreased in CTB-SAP treated rats versus control rats (*; $p < 0.05$), but was not different when comparing 7 and 28 day CTB-SAP treated rats ($p > 0.05$). Means ± 1 SEM. Scale bar at 20X = 50 µm.
Nichols et al. Respir Physiol Neurobiol 256:43, 2018.

Top Five ZAP Products

#1. Streptavidin-ZAP (Cat. #IT-27)
Uses your biotinylated material in order to evaluate the ability of the reagent to internalize upon binding to its receptor

#2. FAB-ZAP human (Cat. #IT-51)
Uses your primary human monoclonal antibody

#3. FAB-ZAP mouse (Cat. #IT-48)
Uses your primary mouse monoclonal antibody

#4. Mab-ZAP (Cat. #IT-04)
Uses your primary mouse monoclonal antibody

#5. Fab-ZAP rat (Cat. #IT-55)
Uses your primary rat monoclonal IgG antibody

Top Five in Number of Publications

#1. 192-IgG-SAP (192-Saporin) (Cat. #IT-01)
targets cells expressing rat p75^{NTR}

#2. Anti-DBH-SAP (Cat. #IT-03)
targets cells expressing rat dopamine beta-hydroxylase (DBH)

#3. Streptavidin-ZAP (Cat. #IT-27)
Uses your biotinylated material in order to evaluate the ability of the reagent to internalize upon binding to its receptor

#4. NGFr (mu p75) Rabbit Polyclonal, *affinity-purified (Cat. #AB-N01AP)*

#5. Melanopsin Rabbit Polyclonal (Cat. #AB-N38)

(continued from page 8)

AB-N01AP Anti-mu p75
affinity-purified

Differentiation of adipose-derived stem cells into Schwann cell-like cells through intermittent induction: potential advantage of cellular transient memory function.

Sun X, et al. Stem Cell Res Ther 9: (1):133, 2018.

Low Osteogenic Yield in Human Pluripotent Stem Cells Associates with Differential Neural Crest Promoter Methylation.

Sparks NRL, et al. Stem Cells 36: (3):349-362, 2018

Neurotrophically Induced Mesenchymal Progenitor Cells Derived from Induced Pluripotent Stem Cells Enhance Neuritogenesis via Neurotrophin and Cytokine Production.

Brick RM, et al. Stem Cells Transl Med 7: (1):45-58, 2018.

The soluble form of LOTUS inhibits Nogo receptor-mediated signaling by interfering with the interaction between Nogo receptor type 1 and p75 neurotrophin receptor.

Kawakami Y, et al. J Neurosci 38: (10):2589-2604, 2018.

AB-N07 Anti-p75 ME20.4

Induced pluripotent stem cells with NOTCH1 gene mutation show impaired differentiation into smooth muscle and endothelial cells: Implications for bicuspid aortic valve-related aortopathy.

Jiao J, et al. J Thorac Cardiovasc Surg 156: (2):515-522, 2018.

Directed differentiation of periocular mesenchyme from human embryonic stem cells.

Lovatt M, et al. Differentiation 99: 62-69, 2018.

Selective Laminin-Directed Differentiation of Human Induced Pluripotent Stem Cells into Distinct Ocular Lineages.

Shibata S, et al. Cell Rep 25: (6):1668-1679, 2018.

Extracellular Matrix from Periodontal Ligament Cells Could Induce the Differentiation of Induced Pluripotent Stem Cells to Periodontal Ligament Stem Cell-Like Cells.

Hamano S, et al. Stem Cells Dev 27: (2):100-111, 2018.

AB-N27AP Angiotensin IIR
(AT1R)

Eriobotrya japonica ameliorates cardiac hypertrophy in H9c2 cardiomyoblast and in spontaneously hypertensive rats.

Chiang JT, et al. Environ Toxicol 33: (11):1113-1122, 2018.

Selective abdominal venous congestion induces adverse renal and hepatic morphological and functional alterations despite a preserved cardiac function.

Cops J, et al. Sci Rep 8: (1):17757, 2018.

Nanoparticle-mediated delivery of Tanshinone IIA reduces adverse cardiac remodeling following myocardial infarctions in a mice model: role of NF-kappaB pathway.

Mao S, et al. Artif Cells Nanomed Biotechnol 1-10, 2018.

Developmental and degenerative cardiac defects in the Taiwanese mouse model of severe spinal muscular atrophy.

Maxwell GK, et al. J Anat 232: (6):965-978, 2018.

Erythropoietin alleviates post-resuscitation myocardial dysfunction in rats potentially through increasing the expression of angiotensin II receptor type 2 in myocardial tissues.

Zhou H, et al. Mol Med Rep 17: (4):5184-5192, 2018.

The Effect and Mechanism of Chinese Herbal Formula Sini Tang in Heart Failure after Myocardial Infarction in Rats.

Zhu Y, et al. Evid Based Complement Alternat Med 5629342, 2018.

AB-N28AP Angiotensin IIR
(AT2R)

Effect of Chronic Intermittent Hypoxia on Angiotensin II Receptors in the Central Nervous System.

Morgan BJ, et al. Clin Exp Hypertens 41: (2):1-7, 2018.

Expression of the intrarenal angiotensin receptor and the role of renin-angiotensin system inhibitors in IgA nephropathy.

Zhang Z, et al. Mol Cell Biochem Epub 29 Aug, 2018.

AT2R Activation Prevents Microglia Pro-inflammatory Activation in a NOX-Dependent Manner: Inhibition of PKC Activation and p47(phox) Phosphorylation by PP2A.

Bhat SA, et al. Epub 3 Aug, 2018.

The angiotensin II type 2 receptors protect renal tubule mitochondria in early stages of diabetes mellitus.

Micakovic T, et al. Kidney Int 94: (5):937-950, 2018.

AB-N32 Anti-mGluR2

Myocyte Enhancer Factor 2c Regulates Dendritic Complexity and Connectivity of Cerebellar Purkinje Cells.

Kamath P, et al. Mol Neurobiol Epub 1 Oct, 2018.

AB-N33AP Anti-NK-1R

Allopregnanolone suppresses mechanical allodynia and internalization of neurokinin-1 receptors at the spinal dorsal horn in a rat postoperative pain model.

Fujita M, et al. Korean J Pain 31: (1):10-15, 2018.

AB-N38 Anti-Melanopsin

Intersectional Strategies for Targeting Amacrine and Ganglion Cell Types in the Mouse Retina.

Jo A, et al. Front Neural Circuits 12: 66, 2018.

(continued on page 12)

Combinatorial Effects of Alpha- and Gamma-Protocadherins on Neuronal Survival and Dendritic Self-Avoidance.
Ing-Esteves S, et al. J Neurosci 38: (11):2713-2729, 2018.

The M6 cell: A small-field bistratified photosensitive retinal ganglion cell.
Quattrochi LE, et al. J Comp Neurol 527: (1):297-311, 2018.

Synaptic circuits for irradiance coding by intrinsically photosensitive retinal ganglion cells.
Sabbah S, et al. bioRxiv, 2018.

The M5 Cell: A Color-Opponent Intrinsically Photosensitive Retinal Ganglion Cell.
Stabio ME, et al. Neuron 97: (1):150-163, 2018.

Retina-specific loss of lkbkap/Elp1 causes mitochondrial dysfunction that leads to selective retinal ganglion cell degeneration in a mouse model of familial dysautonomia.
Ueki Y, et al. Dis Model Mech 11: (7), 2018.

Differential roles for cryptochromes in the mammalian retinal clock.
Wong JCY, et al. FASEB J 32: (8):4302-4314, 2018.

AB-N39 Anti-Melanopsin
affinity-purified

Assembly of functionally antagonistic visual circuits for controlling pupil dynamics.
Dhande O, et al. Cell Rep Epub 19 Dec, 2018.

Melanopsin expression in the cornea.
Delwig A, et al. Vis Neurosci 35: (E004), 2018.

Melanopsin retinal ganglion cells are not labeled in Thy-1YFP-16 transgenic mice.
Grillo SL, et al. Neuroreport 29: (2):118-122, 2018.

AB-N40 Anti-SERT
Serotonin (5HT) Transporter

Single Quantum Dot Tracking Reveals Serotonin Transporter Diffusion Dynamics are Correlated with Cholesterol-Sensitive Threonine 276 Phosphorylation Status in Primary Midbrain Neurons.
Bailey DM, et al. ACS Chem Neurosci 9: (11):2534-2541, 2018.

AB-N43 Anti-p75 (192 IgG)

Adult skin-derived precursor Schwann cell grafts form growths in the injured spinal cord of Fischer rats.
May Z, et al. Biomed Mater 13: (3):034101, 2018.

CUSTOM SERVICES

Conjugations

Leveraging Siglec-8 endocytic mechanisms to kill human eosinophils and malignant mast cells.
O'Sullivan JA, et al. J Allergy Clin Immunol 141: (5):1774-1785, 2018.

Summary: Therapeutic payloads can be targeted selectively to eosinophils and malignant mast cells by exploiting this Siglec-8 endocytic pathway.
Dose: Eosinophil cell death was assessed with 2C4 mAb or isotype control (both at 2.5 µg/mL).

Targeting of embryonic annexin A2 expressed on ovarian and breast cancer by the novel monoclonal antibody 2448.
Cua S, et al. Oncotarget, 9 (17):13206-13221, 2018.

Summary: The novel IgG1, 2448, was shown to target a unique glycosylated surface epitope on ANXA2. As a possible therapeutic candidate for ovarian and breast cancer, 2448 demonstrated anti-tumor activity.
Dose: A Custom ADC was created by direct conjugation of Saporin to ch2448. As a control, an isotype chimeric IgG was also conjugated to saporin (IgG-SAP). Compared to using Secondary Saporin conjugates, ch2448-SAP induced an increase of 20–30% cytotoxicity.

ASSAYS FOR INTERNALIZATION

pHast Conjugates

The fastest tools for quantitative testing of your primary antibody's specificity, binding, and internalization, providing results in 1 day.

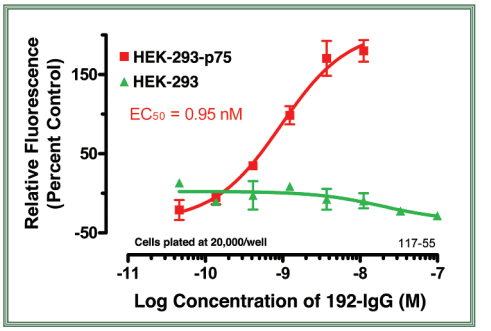
Fab-pHast products bind to your primary antibody via a secondary antibody cross-linked to a pH-dependent fluorescent reporter. This fluorescent reporter increases intensity as the pH of its surroundings becomes more acidic, as evident when exposed to the environment inside a cell.

PH-01 Fab-pHast human

SLC46A3 as a Potential Predictive Biomarker for Antibody-Drug Conjugates Bearing Noncleavable Linked Maytansinoid and Pyrrolobenzodiazepine Warheads.
Kinneer K, *et al*. Clin Cancer Res, 24 (24):6570-6582, 2018.

Objective: To develop biomarkers to uncover the underlying mechanism of resistance by certain cell lines for ADCs.
Summary: Loss of SLC46A3 expression was found to be a mechanism of innate and acquired resistance to ADCs bearing DM1 and SG3376.
Dose: For Lysosomal trafficking, ADCs were labeled with Fab-pHast human. Cells were incubated with 3 mg/mL of labeled ADCs at 37°C for desired time points and fluorescence quantified by flow cytometry.

pHast Ab Internalization Assay



Parental HEK-293 cells, and HEK-293 cells transfected with the p75 receptor, were plated in a 96-well plate overnight. Titrated 192-IgG antibody (Cat. #AB-N43) was incubated at RT with 50 nM of Fab-pHast Mouse (Cat. PH-02) for 20 min prior to addition to cells. Plates were incubated overnight to allow maximum internalization, but a few hours is sufficient for detection.

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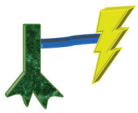
Choose an ANTIBODY§ specific to your cell type.



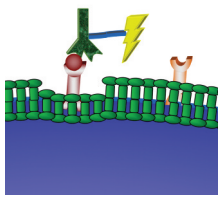
SAPORIN is a potent cytotoxin. Safe in the lab. Lethal in the cell.

ATS binds SAPORIN with your ANTIBODY to make a powerful targeting agent.

§ or anything recognized on the cell surface and internalized.

The targeting agent is administered to the cells (*in vivo* or *in vitro*).

The antibody seeks out its target receptor on the cell surface.



Cells that do not have the receptor will not be affected.

The conjugate is internalized and SAPORIN breaks away from the antibody.

SAPORIN inactivates the ribosomes.

The result is **CELL DEATH**.

Advanced Targeting Systems and its Contract Service partner, **CytoLogistics** announce a

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Selected researchers will receive support for smaller scale or pilot research projects ($1,000 - $25,000) with the targeting tools and contract services needed for the identification and characterization of new cell-surface markers for specific delivery of a payload.

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- Successful candidates will be notified of award within 30 days of receipt of application.
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