

# METHODS IN MOLECULAR BIOLOGY

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**University of Hertfordshire**

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# **Autophagy**

## **Methods and Protocols**

Edited by

**Nicholas Ktistakis and Oliver Florey**

*Signalling Programme, Babraham Institute, Cambridge, UK*

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*Editors*

Nicholas Ktistakis  
Signalling Programme  
Babraham Institute  
Cambridge, UK

Oliver Florey  
Signalling Programme  
Babraham Institute  
Cambridge, UK

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## Preface

The process of self-eating as a way to survive periods of starvation has been known for over 150 years, and this response was from the beginning designated as “autophagy.” Following early progress in establishing the morphological and some biochemical parameters of the autophagic response in the 1950s and 1960s, the field of autophagy was transformed when Yoshinori Ohsumi and others applied a genetic approach to isolate a near complete set of autophagy genes in yeast, which allowed the characterization of equivalent genes and processes in other organisms including mammals.

We now know that autophagy is a highly conserved catabolic and quality control pathway across all eukaryotes. Its dysregulation can underpin disease and developmental problems, frequently in ways not immediately predictable. Despite tremendous progress in researching the autophagic response in a variety of experimental settings, much remains to be discovered, from the basic mechanisms to the physiological functions.

This volume hopes to be a comprehensive and extensive set of protocols for the study of autophagy *in vitro* and *in vivo*, primarily in mammals but also in various model organisms including yeast, *Drosophila*, and *C. elegans*. Protocols are also presented for the study of autophagy-related processes outside of the canonical autophagy pathways.

We first present 11 chapters describing protocols for studying autophagy *in vitro*, i.e., using pure or semi-pure components; these studies have contributed a great deal toward understanding autophagy in molecular terms, and it is likely that they will continue to do so in the future. The next 8 chapters describe protocols for the study of autophagy (canonical and noncanonical) by imaging methods, either using light microscopy or EM. Being able to follow the formation of autophagosomes and to study their unique architecture has traditionally been a productive area of research, and it is likely that novel insights from such studies will continue to be derived in the future. In the last few years, much effort has been devoted to the establishment of assays and screening platforms for large-scale discovery efforts to identify genes and chemicals affecting autophagy; here we have included 10 chapters describing a set of up-to-date protocols for such studies. The next 9 chapters deal with the study of autophagy not in tissue culture but in whole organisms. We believe that work along those lines is likely to come to dominate the autophagy field in the future, and it is therefore very useful to have a set of protocols mapping out these useful approaches. The final 10 chapters describe assays and techniques for studying selective autophagy, i.e., the induction of autophagy for the elimination of specific cargoes. Although autophagy is sometimes solely considered a starvation response, it can be argued that its function as a specific eliminator of unwanted substances is a major physiological function and therefore likely to continue to be studied intensely.

We wish to thank all authors who took time off from their own work in order to provide the protocols included in this book.

Cambridge, UK

Nicholas Ktistakis  
Oliver Florey

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## Contributors

- HAGAI ABELIOVICH • *Biochemistry, Food Science and Nutrition, Hebrew University of Jerusalem, Rehovot, Israel*
- CHRISTINE ABERT • *Department of Biochemistry and Cell Biology, Max F. Perutz Laboratories (MFPL), Vienna Biocenter (VBC), University of Vienna, Vienna, Austria*
- JAIME AGUDO-CANALEJO • *Rudolf Peierls Centre for Theoretical Physics, University of Oxford, Oxford, UK; Department of Chemistry, The Pennsylvania State University, University Park, PA, USA*
- SONJA AITS • *Cell Death and Lysosomes Group, Experimental Neuroinflammation Laboratory, Department of Experimental Medical Science, Lund University, Lund, Sweden; Peter MacCallum Cancer Centre, Melbourne, Australia*
- ALICIA ALONSO • *Instituto Biofisika (CSIC, UPV/EHU), Bilbao, Spain; Departamento de Bioquímica y Biología Molecular, Universidad del País Vasco, Bilbao, Spain*
- ZURIÑE ANTÓN • *Instituto Biofisika (CSIC, UPV/EHU), Bilbao, Spain; Departamento de Bioquímica y Biología Molecular, Universidad del País Vasco, Bilbao, Spain*
- RITSUKO ARAI • *Department of Anatomy and Histology, Fukushima Medical University, School of Medicine, Fukushima, Japan*
- MEENAKSHI BANERJEE • *Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY, USA*
- DARIO BARBONE • *Zuckerberg San Francisco General Hospital and Trauma Center, University of California San Francisco, San Francisco, CA, USA*
- NASTASSIA BAYLAC • *Molecular and Integrative Biosciences Research Program, University of Helsinki, Helsinki, Finland*
- PETRA BELI • *Institute of Molecular Biology (IMB), Mainz, Germany*
- ÅSA BIRNA BIRGISDOTTIR • *Molecular Cancer Research Group, Department of Medical Biology, University of Tromsø – The Arctic University of Norway, Tromsø, Norway*
- KEITH B. BOYLE • *Division of Protein and Nucleic Acid Chemistry, MRC Laboratory of Molecular Biology, Cambridge, UK*
- RAPHAEL BUENO • *Division of Thoracic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA*
- MAEVE A. CALDWELL • *Trinity College Institute for Neuroscience, Trinity College, Dublin, Ireland*
- YANG CHEN • *Center for Precision Medicine Multi-Omics Research, Peking University, Health Science Center, Beijing, China*
- XIGUI CHEN • *Department of Neuropathology, Medical Research Institute and Center for Brain Integration Research, Tokyo Medical and Dental University, Tokyo, Japan*
- MIRUNA CHIPARA • *Institute of Cancer and Genomic Sciences, Institute of Biomedical Research, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK*
- PATRICE CODOGNO • *Institut Necker-Enfants Malades (INEM), INSERM U1151-CNRS UMR 8253, Université Paris Descartes-Sorbonne Paris Cité, Paris, France*

- V. COURTNEY BROADDUS • *Zuckerberg San Francisco General Hospital and Trauma Center, University of California San Francisco, San Francisco, CA, USA*
- LUCY CROMPTON • *Cell Biology Laboratories, School of Biochemistry, University of Bristol, Bristol, UK*
- A. M. CUERVO • *Department of Developmental and Molecular Biology, Bronx, NY, USA; Institute for Aging Studies, Albert Einstein College of Medicine, Bronx, NY, USA*
- JÖRN DENGJEL • *Department of Biology, University of Fribourg, Fribourg, Switzerland; Department of Dermatology, Medical Center—University of Freiburg, Freiburg, Germany*
- BRITTA DIEDRICH • *Agilent Technologies, Waldbronn, Germany*
- IVAN DIKIC • *Institute of Biochemistry II, Goethe University School of Medicine, Frankfurt, Germany; Buchmann Institute for Molecular Life Sciences, Goethe University, Frankfurt, Germany*
- AUDREY DONG • *Department of Neuroscience, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA*
- NICOLAS DUPONT • *Institut Necker-Enfants Malades (INEM), INSERM U1151-CNRS UMR 8253, Université Paris Descartes-Sorbonne Paris Cité, Paris, France*
- NIKOLAI ENGEDAL • *Centre for Molecular Medicine Norway (NCMM), Nordic EMBL Partnership, University of Oslo, Oslo, Norway*
- EEVA-LIISA ESKELINEN • *Molecular and Integrative Biosciences Research Program, University of Helsinki, Helsinki, Finland*
- KATHERINE FLETCHER • *Signalling Programme, Babraham Institute, Cambridge, UK*
- OLIVER FLOREY • *Signalling Programme, Babraham Institute, Cambridge, UK*
- CARLO FOLLO • *Zuckerberg San Francisco General Hospital and Trauma Center, University of California San Francisco, San Francisco, CA, USA*
- IAN G. GANLEY • *MRC Protein Phosphorylation and Ubiquitylation Unit, School of Life Sciences, University of Dundee, Dundee, UK*
- LIANG GE • *State Key Laboratory of Membrane Biology, Beijing, China; Tsinghua-Peking Center for Life Sciences, Beijing, China; School of Life Sciences, Tsinghua University, Beijing, China*
- A. GIATROMANOLAKI • *Department of Pathology, Democritus University of Thrace, and University General Hospital of Alexandroupolis, Alexandroupolis, Greece*
- DANIEL GRASSO • *Pathophysiology Department, Institute of Biochemistry and Molecular Medicine (CONICET), School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina*
- SIGURDUR GUDMUNDSSON • *Molecular and Integrative Biosciences Research Program, University of Helsinki, Helsinki, Finland*
- AUDREY GUESDON • *Structural and Computational Biology Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany*
- JENS C. HAMANN • *Meyer Cancer Center, Weill Cornell Medicine, New York, NY, USA; Cell Biology Program, Sloan Kettering Institute for Cancer Research, New York, NY, USA; Louis V. Gerstner, Jr. Graduate School of Biomedical Sciences, Memorial Sloan Kettering Cancer Center, New York, NY, USA*
- MAHO HAMASAKI • *Andor Technology Ltd., Newark, DE, USA*
- SIVAN HENIS-KORENBLIT • *The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel*
- JAVIER H. HERVÁS • *Instituto Biofisika (CSIC, UPV/EHU), Bilbao, Spain; Departamento de Bioquímica y Biología Molecular, Universidad del País Vasco, Bilbao, Spain*

- ERIKA L. F. HOLZBAUR • *Department of Physiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA*
- MICHAEL HOWELL • *Tumour Cell Death Laboratory, Cancer Research UK Beatson Institute, Glasgow, UK; High Throughput Screening, The Francis Crick Institute, London, UK*
- CHENG-WEI HSIEH • *Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan*
- YUNJIE HUANG • *Division of Pulmonary Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA*
- NAZMUL HUDA • *Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA*
- ANNE-CLAIRE JACOMIN • *School of Life Sciences, University of Warwick, Coventry, UK*
- ELISE JACQUIN • *Signalling Programme, Babraham Institute, Cambridge, UK; INSERM, U1231, Université de Bourgogne Franche Comté, Dijon, France*
- MING JIANG • *Tumour Cell Death Laboratory, Cancer Research UK Beatson Institute, Glasgow, UK*
- NATALIA JIMENEZ-MORENO • *Cell Biology Laboratories, School of Biochemistry, University of Bristol, Bristol, UK*
- TERJE JOHANSEN • *Molecular Cancer Research Group, Department of Medical Biology, University of Tromsø – The Arctic University of Norway, Tromsø, Norway*
- SMITA JOSHI • *Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY, USA*
- GÁBOR JUHÁSZ • *Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest, Hungary; Institute of Genetics, Biological Research Centre, Szeged, Hungary*
- THOMAS JURETSCHKE • *Institute of Molecular Biology (IMB), Mainz, Germany*
- Y. R. JUSTE • *Department of Developmental and Molecular Biology, Bronx, NY, USA; Institute for Aging Studies, Albert Einstein College of Medicine, Bronx, NY, USA*
- STÉPHANIE KAESER-PEBERNARD • *Department of Biology, University of Fribourg, Fribourg, Switzerland*
- JENNY KAHLHOFER • *Division of Cell Biology, Biocenter, Medical University of Innsbruck, Innsbruck, Austria*
- D. KALAMIDA • *Department of Radiotherapy/Oncology, Democritus University of Thrace, and University General Hospital of Alexandroupolis, Alexandroupolis, Greece*
- KATRI KALLIO • *Molecular and Integrative Biosciences Research Program, University of Helsinki, Helsinki, Finland*
- ELEFTHERIOS KARANASIOS • *Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London, Maurice Wohl Clinical Neuroscience Institute, London, UK*
- BILON KHAMBU • *Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA*
- SUNG EUN KIM • *Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA, USA; Cell Biology Program, Sloan Kettering Institute for Cancer Research, New York, NY, USA*
- ROLAND L. KNORR • *Department of Theory and Bio-Systems, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany; Department of Biochemistry and Molecular Biology, Graduate School and Faculty of Medicine, The University of Tokyo, Tokyo, Japan*
- PANAGIOTA KOLITSIDA • *Biochemistry, Food Science and Nutrition, Hebrew University of Jerusalem, Rehovot, Israel*

- MASAAKI KOMATSU • *Department of Physiology, Juntendo University Graduate School of Medicine, Tokyo, Japan*
- KANO H KONDO • *Department of Neuropathology, Medical Research Institute and Center for Brain Integration Research, Tokyo Medical and Dental University, Tokyo, Japan*
- M. I. KOUKOURAKIS • *Department of Radiotherapy/Oncology, Democritus University of Thrace, and University General Hospital of Alexandroupolis, Alexandroupolis, Greece*
- TANJA KUHM • *Structural and Computational Biology Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany*
- VINEET VINAY KULKARNI • *Department of Neuroscience, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA*
- OUIN KUNITAKI • *Department of Genetics, Osaka University Graduate School of Medicine, Osaka, Japan*
- JON D. LANE • *Cell Biology Laboratories, School of Biochemistry, University of Bristol, Bristol, UK*
- CÉLINE LARGEAU • *Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Sud, Université Paris-Saclay, Gif-sur-Yvette cedex, France*
- MARIA M. LAZARI • *Institut Necker-Enfants Malades (INEM), INSERM U1151-CNRS UMR 8253, Université Paris Descartes-Sorbonne Paris Cité, Paris, France*
- RENAUD LEGOUIS • *Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Sud, Université Paris-Saclay, Gif-sur-Yvette cedex, France*
- LAURE-ANNE LIGEON • *Viral Immunobiology, Institute of Experimental Immunology, University of Zürich, Zürich, Switzerland*
- EIRINI LIONAKI • *Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Crete, Greece*
- MONICA LOI • *Viral Immunobiology, Institute of Experimental Immunology, University of Zürich, Zürich, Switzerland*
- JACLYN S. LONG • *High Throughput Screening, The Francis Crick Institute, London, UK*
- MORTEN LUHR • *Centre for Molecular Medicine Norway (NCMM), Nordic EMBL Partnership, University of Oslo, Oslo, Norway*
- CHRISTIAN MÜNZ • *Viral Immunobiology, Institute of Experimental Immunology, University of Zürich, Zürich, Switzerland*
- SANDRA MADAY • *Department of Neuroscience, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA*
- SASCHA MARTENS • *Department of Biochemistry and Cell Biology, Max F. Perutz Laboratories (MFPL), Vienna Biocenter (VBC), University of Vienna, Vienna, Austria*
- PETER MAYRHOFER • *Unit Membrane Biochemistry and Transport, Institut Pasteur, Paris, France*
- THOMAS G. MCWILLIAMS • *MRC Protein Phosphorylation and Ubiquitylation Unit, School of Life Sciences, University of Dundee, Dundee, UK; Translational Stem Cell Biology Research Program, Research Programs Unit, Faculty of Medicine, University of Helsinki, Helsinki, Finland*
- ALICIA MELÉNDEZ • *Department of Biology, Queens College, The City University of New York, Flushing, NY, USA; Biology and Biochemistry PhD Programs, The Graduate Center of the City University of New York, New York, NY, USA*
- ZOLTAN METLAGEL • *Thermo Fisher Scientific, Hillsboro, OR, USA*
- ANDREW S. MOORE • *Department of Physiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA*

- AMELIE J. MUELLER • *Interfaculty Institute of Cell Biology, Eberhard Karls University Tuebingen, Tübingen, Germany; International Max Planck Research School “From Molecules to Organisms”, Tübingen, Germany*
- MARIA NEW • *Molecular Cell Biology of Autophagy Laboratory, The Francis Crick Institute, London, UK*
- IOANNIS P. NEZIS • *School of Life Sciences, University of Warwick, Coventry, UK*
- PAUL NISTOR • *School of Clinical Medicine, University of Bristol, Bristol, UK*
- NOBUO N. NODA • *Laboratory of Structural Biology, Institute of Microbial Chemistry, Tokyo, Japan*
- KAZUTO OHASHI • *Institute for Molecular and Cellular Regulation, Gunma University, Gunma, Japan*
- HITOSHI OKAZAWA • *Department of Neuropathology, Medical Research Institute and Center for Brain Integration Research, Tokyo Medical and Dental University, Tokyo, Japan*
- IDIL ORHON • *Department of Cell Biology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands*
- CHINATSU OTOMO • *Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA, USA*
- TAKANORI OTOMO • *Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA, USA*
- MADHU M. OUSEPH • *Department of Pathology and Laboratory Medicine, Brown University, Providence, RI, USA*
- MICHAEL OVERHOLTZER • *Cell Biology Program, Sloan Kettering Institute for Cancer Research, New York, NY, USA*
- KONSTANTINOS PALIKARAS • *Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Crete, Greece*
- JANA PETRI • *Department of Animal Behavior, Freie Universität Berlin, Berlin, Germany*
- DUŠAN PETROVIĆ • *Department of Chemistry, BMC, Uppsala University, Uppsala, Sweden; Institute of Complex Systems ICS-6 (Structural Biochemistry), Forschungszentrum Jülich, Jülich, Germany*
- IRINA POKROVSKAYA • *Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR, USA*
- TASSULA PROIKAS-CEZANNE • *Interfaculty Institute of Cell Biology, Eberhard Karls University Tuebingen, Tübingen, Germany; International Max Planck Research School “From Molecules to Organisms”, Tübingen, Germany*
- FELIX RANDOW • *Division of Protein and Nucleic Acid Chemistry, MRC Laboratory of Molecular Biology, Cambridge, UK; Department of Medicine, Addenbrooke’s Hospital, University of Cambridge, Cambridge, UK*
- MADS SKYTTE RASMUSSEN • *Molecular Cancer Research Group, Department of Medical Biology, University of Tromsø – The Arctic University of Norway, Tromsø, Norway*
- WILLIAM G. RICHARDS • *Division of Thoracic Surgery, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA*
- ALEJANDRO ROPOLO • *Pathophysiology Department, Institute of Biochemistry and Molecular Medicine (CONICET), School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina*
- TATIANA R. ROSENSTOCK • *Department of Physiological Science, Santa Casa de São Paulo School of Medical Science, São Paulo, Brazil*
- KEVIN M. RYAN • *Tumour Cell Death Laboratory, Cancer Research UK Beatson Institute, Glasgow, UK; High Throughput Screening, The Francis Crick Institute, London, UK*

- CARSTEN SACHSE • *Structural and Computational Biology Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany; Ernst-Ruska Centre for Microscopy and Spectroscopy with Electrons (ER-C-3/Structural Biology), Forschungszentrum Jülich, Jülich, Germany*
- JUN-ICHI SAKAMAKI • *High Throughput Screening, The Francis Crick Institute, London, UK*
- SOVAN SARKAR • *Institute of Cancer and Genomic Sciences, Institute of Biomedical Research, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK*
- REBECCA SAUNDERS • *Tumour Cell Death Laboratory, Cancer Research UK Beatson Institute, Glasgow, UK*
- MELANIE SCHWARTEN • *Institute of Complex Systems ICS-6 (Structural Biochemistry), Forschungszentrum Jülich, Jülich, Germany*
- PER O. SEGLEN • *Centre for Molecular Medicine Norway (NCMM), Nordic EMBL Partnership, University of Oslo, Oslo, Norway*
- ELENA SERANOVA • *Institute of Cancer and Genomic Sciences, Institute of Biomedical Research, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK*
- PETROS STATHAKOS • *Cell Biology Laboratories, School of Biochemistry, University of Bristol, Bristol, UK*
- BRIAN STORRIE • *Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR, USA*
- BIRGIT STRODEL • *Institute of Complex Systems ICS-6 (Structural Biochemistry), Forschungszentrum Jülich, Jülich, Germany; Institute of Theoretical and Computational Chemistry, Heinrich Heine University Düsseldorf, Düsseldorf, Germany*
- QIAN PETER SU • *Institute for Biomedical Materials & Devices (IBMD), Faculty of Science, University of Technology Sydney, New South Wales, Australia*
- PAULA SZALAI • *Centre for Molecular Medicine Norway (NCMM), Nordic EMBL Partnership, University of Oslo, Oslo, Norway*
- GYÓZÓ SZENCI • *Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest, Hungary*
- SAROLTA TÓTH • *Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest, Hungary*
- SZABOLCS TAKÁTS • *Hungarian Academy of Sciences, Premium Postdoctorate Research Program, Budapest, Hungary; Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest, Hungary*
- ABUL K. TARAFDER • *Structural and Computational Biology Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany*
- NEKTARIOS TAVERNARAKIS • *Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Crete, Greece; Faculty of Medicine, Department of Basic Sciences, University of Crete, Crete, Greece*
- SHARON A. TOOZE • *Molecular Cell Biology of Autophagy Laboratory, The Francis Crick Institute, London, UK*
- PANAGIOTIS TSAPRAS • *School of Life Sciences, University of Warwick, Coventry, UK*
- HIROYUKI UEDA • *Department of Genetics, Osaka University Graduate School of Medicine, Osaka, Japan; Department of Intracellular Membrane Dynamics, Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan*
- TAKASHI UENO • *Laboratory of Proteomics and Biomolecular Science, Research Support Center, Juntendo University Graduate School of Medicine, Tokyo, Japan*

- MARIA I. VACCARO • *Pathophysiology Department, Institute of Biochemistry and Molecular Medicine (CONICET), School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina*
- TIM VAN ACKER • *Molecular Cell Biology of Autophagy Laboratory, The Francis Crick Institute, London, UK*
- SATOSHI WAGURI • *Department of Anatomy and Histology, Fukushima Medical University, School of Medicine, Fukushima, Japan*
- QING JUN WANG • *Department of Ophthalmology and Visual Sciences, University of Kentucky, Lexington, KY, USA*
- CARL WARD • *Institute of Cancer and Genomic Sciences, Institute of Biomedical Research, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK*
- YASUNORI WATANABE • *Department of Bioscience, Graduate School of Agriculture, Ehime University, Matsuyama, Japan; Laboratory of Structural Biology, Institute of Microbial Chemistry, Tokyo, Japan*
- OLIVER H. WEIERGRÄBER • *Institute of Complex Systems ICS-6 (Structural Biochemistry), Forschungszentrum Jülich, Jülich, Germany*
- SIDNEY W. WHITEHEART • *Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY, USA*
- DIETER WILLBOLD • *Institute of Complex Systems ICS-6 (Structural Biochemistry), Forschungszentrum Jülich, Jülich, Germany; Institut für Physikalische Biologie, Heinrich Heine University Düsseldorf, Düsseldorf, Germany*
- MARTINA WIRTH • *Molecular Cell Biology of Autophagy Laboratory, The Francis Crick Institute, London, UK*
- THOMAS WOLLERT • *Unit Membrane Biochemistry and Transport, Institut Pasteur, Paris, France*
- AKINORI YAMASAKI • *Laboratory of Structural Biology, Institute of Microbial Chemistry, Tokyo, Japan*
- SHENGMIN YAN • *Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA*
- WEI YUAN YANG • *Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan; Institute of Biochemical Sciences, College of Life Sciences, National Taiwan University, Taipei, Taiwan*
- XIAO-MING YIN • *Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA*
- LI YU • *State Key Laboratory of Membrane Biology, Biomedical Pioneering Innovation Center (BIOPIC), School of Life Sciences, Peking University, Beijing, China*
- MIN ZHANG • *State Key Laboratory of Membrane Biology, Beijing, China; Tsinghua-Peking Center for Life Sciences, Beijing, China; School of Life Sciences, Tsinghua University, Beijing, China*
- JINCHAO ZHANG • *Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY, USA*
- HUI ZOU • *Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA; College of Veterinary Medicine, Yangzhou University, Yangzhou, People's Republic of China*