Supplementary Table: Timeless methods for biological discovery applied to *S. rosetta*

Technology	Method	Gene Delivery	*S. rosetta*	In other Choanoflagellates
Cell Biology				
Light Microscopy				
•Vital Dyes and Stains	Cross-reactive antibodies and dyes	N	Cytoskeleton Staining: anti-Tubulin Antibody & Phalloidin for F-Actin Stain (Sebé-Pedrós et al., 2013)	Collar Complex Architecture (Karpov and Leadbeater, 1998) Tubulin and F-actin (King et al., 2009)
	Custom Antibodies	N	Rosetteless (Levin et al., 2014) Homer (Burkhardt et al., 2014) Synaptobrevin (Göhde et al., 2021)	Cadherin (Abedin and King, 2008) Myc (Young et al., 2011)
•Live cell imaging	Time-lapse	N	Rosette Development (Fairclough et al., 2010; Larson et al., 2020) Cell differentiation (Dayel et al., 2011) Phagocytosis (Dayel and King, 2014) Aerotaxis (Kirkegaard et al., 2016) pH-taxis (Miño et al., 2017) Fluid Dynamics (Roper et al., 2013)	Collective contractility (Brunet et al., 2019)
	Genetically encoded fluorescent markers	Y	Amoeboid Motility (Brunet et al., 2021) Glycosyl Transferase (Wetzel et al., 2018) Septin Localization (Booth et al., 2018)	STING (Woznica et al., 2021)
Electron Microscopy				
•Transmission EM	Heavy Metal Staining	N	Intercellular Bridges in Colonial Cells (Fairclough et al., 2013)	General Cell Biology (Leadbeater, 1983, 2015) Collar Architecture (Karpov and Leadbeater, 1998) Flagellar Vane (Leadbeater, 2006)
	3D Reconstruction	N	Spatial Cell Differentiation (Laundon et al., 2019; Naumann and Burkhardt, 2019)	
•Scanning EM	Heavy Metal Staining	N	Phagocytosis and Collar Links (Dayel and King, 2014)	Loricate Structure (Frösler and Leadbeater, 2009)

Biochemistry				
Pharmacological Inhibitors	Highly conserved gene inhibitors	N	Aphidicolin (Fairclough et al., 2010) Nocodazole (Anderson et al., 2016) Tyrosine Kinases (King et al., 2003) Actin-based Motility Inhibitors: Blebbistatin/Latrunculin B/CK666 (Brunet et al., 2021)	Phosphodiesterase Inhibitors: Caffeine/IBMX (Brunet et al., 2019) Cell Permeable Nucleotides (Brunet et al., 2019; Woznica et al., 2021) Germanium (Marron et al., 2016) Cytoskeletal Inhibitors for Loricate patterning (Frösler and Leadbeater, 2009)
Biochemical Resconstitution	Chemical Ecology/Classical Biochemistry	N	Rosette Inducing Lipids (Alegado et al., 2012; Beemelmanns et al., 2014; Woznica et al., 2016) EroS (Woznica et al., 2017) Ciliary Proteome (Sigg et al., 2017)	
	Recombinant Protein Activity	N	Homer (Burkhardt et al., 2014) GKPID (Anderson et al., 2016) CamKII (Bhattacharyya et al., 2016) Cbl (Amacher et al., 2018)	Myc (Young et al., 2011) Src (Manning et al., 2008)
Genetics				
Genomics	Whole Genome Sequence	N	*S. rosetta* (Fairclough et al., 2013)	*M. brevicollis* (King et al., 2008) Environmental sample (López-Escardó et al., 2019)
	Transcriptomics	N	Differential gene expression (Fairclough et al., 2013)	Comparative Transcriptomes (Brunet et al., 2019; Richter et al., 2018)
Forward Genetics	Mutagenesis	N	Sex and recombination (Levin and King, 2013)	
	Genetic Mapping	N	Rosetteless (Levin et al., 2014) Glycosyl Transferase (Wetzel et al., 2018)	
Reverse Genetics	Genome Editing	Y	Point Mutation and Gene Knockout (Booth and King, 2020)	Gene Knockout (Woznica et al., 2021)
	Transgenesis	Y	Complementation (Wetzel et al., 2018) Reporter gene expression and localization (Booth et al., 2018)	STING Localization (Woznica et al., 2021)

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