

TABLE OF CONTENT

AGRADECIMIENTOS	3
ABSTRACT	8
RESUMEN	10
RESUM	13
LIST OF FIGURES	18
LIST OF TABLES	19
ABBREVIATIONS	21
GENERAL INTRODUCTION	25
1. POLYQ EXPANSION DISEASES	26
1.1 Molecular and pathological features of polyQ expansion diseases	26
1.2 Huntington's disease	30
1.2.1 Clinical, molecular, and therapeutic overview	30
1.2.2 Systemic manifestations and huntingtin function	31
1.2.3 Genetic modifiers and variability in disease onset	32
2. <i>CAENORHABDITIS ELEGANS</i> AS A MODEL ORGANISM IN BIOMEDICAL RESEARCH	33
2.1 <i>C. elegans</i> as a model for polyQ diseases and HD	34
3. MODULATORS OF POLYGLUTAMINE AGGREGATION IN <i>C. ELEGANS</i>	35
3.1 AMPK as a Therapeutic Target in HD	36
3.2 Sirtuins	38
3.2.1 Sirtuins in <i>C. elegans</i>	40
3.3 Autophagy	42
3.4 Regulation of intracellular calcium by phospholipases C	44
3.4.1 PLC families and structural domains	45
3.4.2 PLC isoforms in <i>C. elegans</i>	46
3.4.3 PLC signalling in neurodegeneration	47
4. THE EXCRETORY SYSTEM AND EPITHELIAL POLARITY IN <i>C. ELEGANS</i>	48
4.1 Excretory cell polarity and relevance to human disease	50
4.2 Conserved regulators of apical-basal polarity	51
OBJECTIVES	53
CHAPTER I	55
ABSTRACT	56
INTRODUCTION	57
MATERIALS AND METHODS	58
RESULTS	69
DISCUSSION	89
SUPPLEMENTARY MATERIAL	93
CHAPTER II	112
ABSTRACT	112
INTRODUCTION	113
MATERIALS AND METHODS	115
RESULTS	119
DISCUSSION	132
SUPPLEMENTARY MATERIAL	134
CHAPTER III	141
ABSTRACT	142
INTRODUCTION	143

TABLE OF CONTENT

MATERIALS AND METHODS.....	145
RESULTS	148
DISCUSSION	155
SUPPLEMENTARY MATERIAL.....	158
GENERAL DISCUSSION	164
CONCLUSIONS.....	179
APPENDIX	182
BIBLIOGRAPHY.....	185

LIST OF FIGURES

GENERAL INTRODUCTION	25
Figure 1. The process of aggregation of proteins with polyQs.	26
Figure 2. Negative correlation between CAG repeats length and the age at which motor symptoms manifest in HD.	33
Figure 3. SIRT4-ANT2 regulation of AMPK activity	40
Figure 4. Overview of macroautophagy.....	43
Figure 5. IP ₃ /calcium signalling pathway.....	45
Figure 6. Schematic representation of the PLC ϵ structure.	47
Figure 7. Scheme of the excretory system in <i>C. elegans</i>	49
CHAPTER I	55
Figure 1. <i>sir-2.3/SIRT4</i> loss-of-function has a neuroprotective role in polyQ toxicity.	71
Figure 2. Silencing SIRT4 ameliorates the accumulation of mutant HTT aggregates.	74
Figure 3. The protective role on polyQ toxicity mediated by <i>sir-2.3</i> depletion is AMPK-dependent.	77
Figure 4. Transcriptomic analysis reveals altered protein homeostasis pathways in <i>sir-2.3</i> mutants.	81
Figure 5. <i>sir-2.3</i> depletion induces an increase in autophagy.....	83
Figure 6. The protective role against polyQ toxicity mediated by <i>sir-2.3</i> depletion is <i>daf-16</i> partially dependent.	86
Figure 7. Mild ATP synthase inhibition mimics <i>sir-2.3</i> ablation effect in mHTT-expressing worms.	88
Figure S1. <i>sir-2.3</i> ablation improves protein homeostasis.....	93
Figure S2. <i>sir-2.3</i> modulates neuronal polyQ aggregation via tissue-intrinsic and extrinsic mechanisms.	94
Figure S3. <i>sir-2.2</i> and <i>sir-2.3</i> are expressed from independent promoters.	95
Figure S4. <i>sir-2.2</i> modulates <i>sir-2.3</i> -dependent regulation of polyQ aggregation and neuronal function.	96
Figure S5. Structural comparison of sirtuins SIRT-4 from <i>Xenopus tropicalis</i> and SIR-2.2 and SIR-2.3.	97
Figure S6. KEGG and Go terms analysis from the transcriptome of <i>sir-2.3</i> vs wild type animals.	99
Figure S7. <i>sir-2.3</i> mutants exhibit enhanced autophagic flux revealed by chloroquine treatment.....	100
Figure S8. Mild ATP synthase inhibition mimics <i>sir-2.3</i> ablation effects in polyQ-expressing worms.	102
Figure S9. α -ketoglutarate levels may be higher in <i>sir-2.3</i> mutants.	103
Figure S10. ATP synthase inhibitors do not further improve neuronal function in <i>sir-2.3</i> mutants.	104
Figure S11. Tomatidine and bedaquiline improve neuronal function independently of the metabolism of the <i>E. coli</i> strain OP50.....	105
CHAPTER II	112
Figure 1. Loss of phospholipase C function enhances polyQ aggregation and impairs motor performance in <i>C. elegans</i>	120
Figure 2. PLC-1 modulates polyQ toxicity in <i>C. elegans</i> through the IP ₃ receptor ITR-1.....	124
Figure 3. IP ₃ sequestration by sponge constructs enhances polyQ aggregation in muscle cells	126
Figure 4. <i>plc-1</i> depletion reduces autophagic flux.....	129
Figure 5. <i>plc-1(vlt28)</i> enhances α -synuclein aggregation.	131
Figure S1. Some representative confocal images of the expression pattern of the PLC enzymes.....	134
CHAPTER III	141
Figure 1. Schematic representation of the <i>C. elegans</i> excretory system.	147
Figure 2. Excretory system defects in <i>plc-1</i> mutants.	149
Figure 3. Genetic interaction between <i>plc-1</i> and <i>fln-2</i> in excretory system morphology.	151
Figure 4. PLC-1 and FLN-2 colocalize in the apical domain of the excretory cell.	153
Figure 5. Co-localization of PLC-1 and FLN-2 with core polarity proteins in the excretory system.	154
Figure S1. Apical localization of FLN-2 in the excretory canal.	158
Figure S2. Apical localization of PLC-1 in the excretory canal.....	159
GENERAL DISCUSSION	164
Figure 1. Model of action proposed.	171

LIST OF TABLES

GENERAL INTRODUCTION	25
Table 1. Characteristics of polyQ expansion diseases.....	29
CHAPTER I	55
Table S1. <i>C. elegans</i> strains used in this work.....	106
Table S2. Primers used for genotyping worm strains.	108
Table S3. Primers used to produce pEntry vectors for further cloning using Gateway technology.	109
Table S4. Sequence of the crRNAs and ssODNs used for CRISPR knock-in experiments.	109
Table S5. Primers used for qPCR.	110
CHAPTER II	112
Table 1. Expression patterns of the different PLCs	128
Table S1. Strains used in this work.	136
Table S2. Primers used for genotyping worm strains.	138
Table S3. Sequence of the crRNAs and ssODNs used for CRISPR experiments.....	139
Table S4. Injection mix to generate deletions by CRISPR/Cas9.....	139
Table S5. Injection mix to generate point mutations by CRISPR/Cas9	139
CHAPTER III	141
Table 1. <i>fln-2</i> genetically interacts with <i>plc-1</i> in the regulation of the excretory system of <i>C. elegans</i>	150
Table S1. Strains used in this work.	160
Table S2. Primers used for genotyping worm strains.	161
Table S3. Sequence of the crRNAs and ssODNs used for CRISPR experiments.....	162
Table S4. Injection mix to generate deletions by CRISPR/Cas9.....	162
Table S5. Injection mix to generate point mutations by CRISPR/Cas9	162