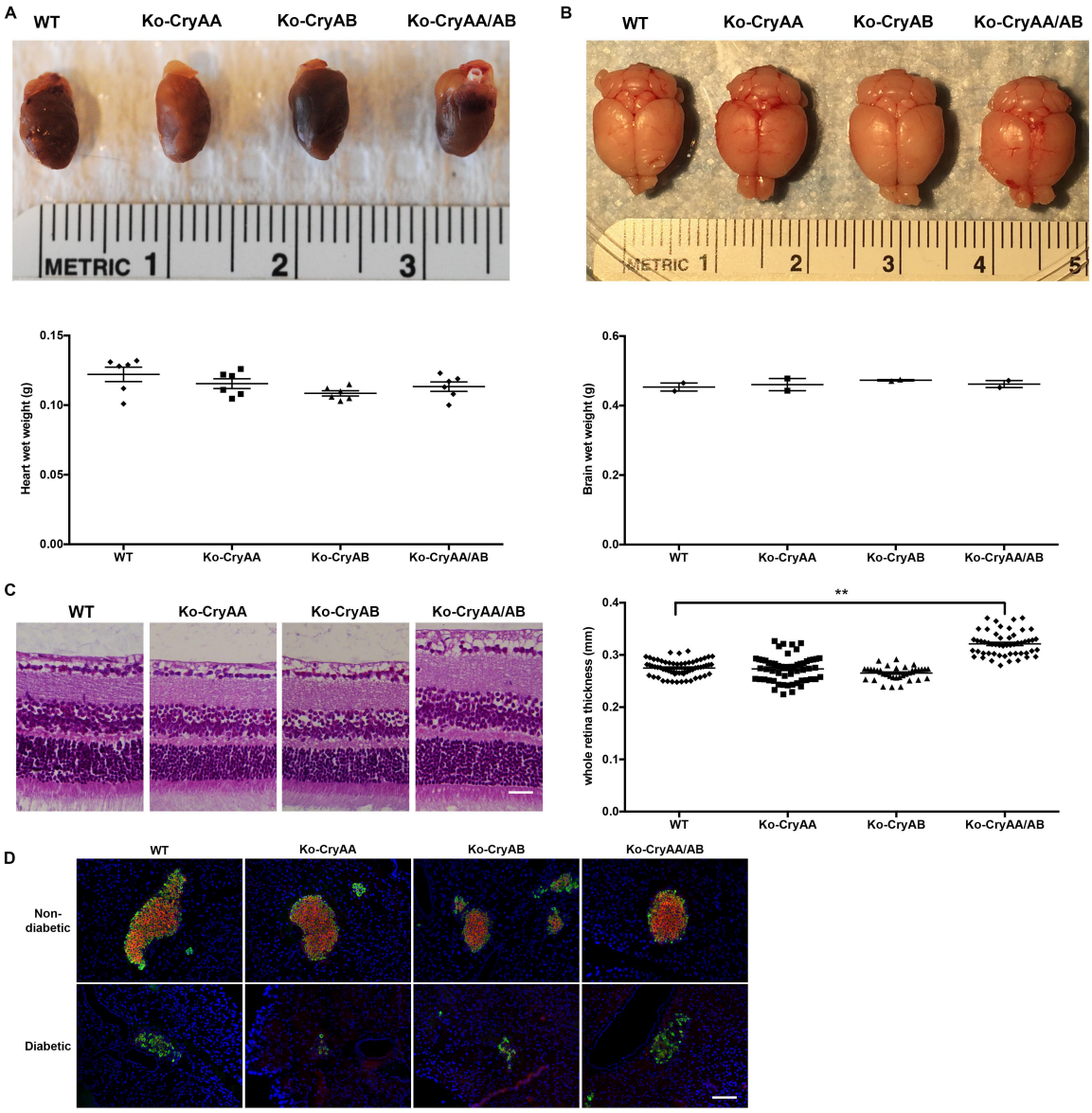
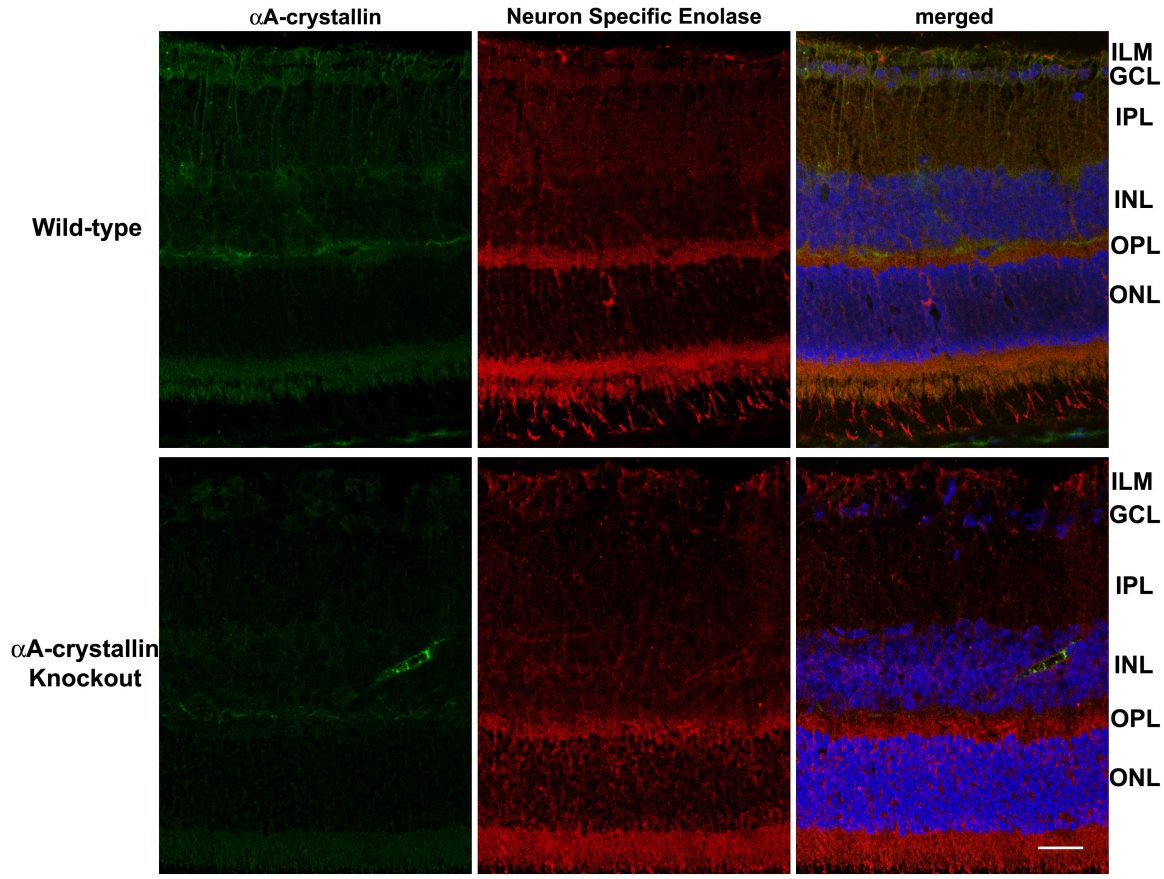
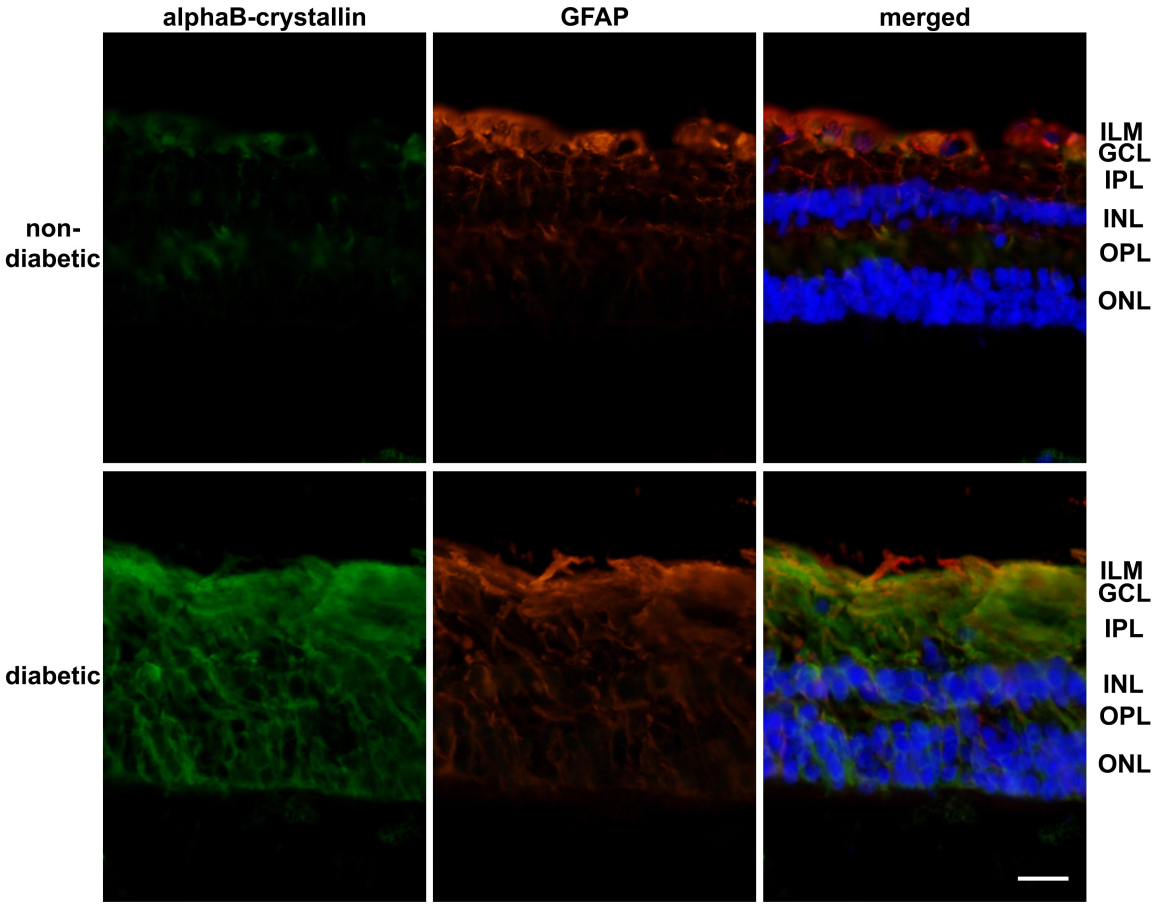


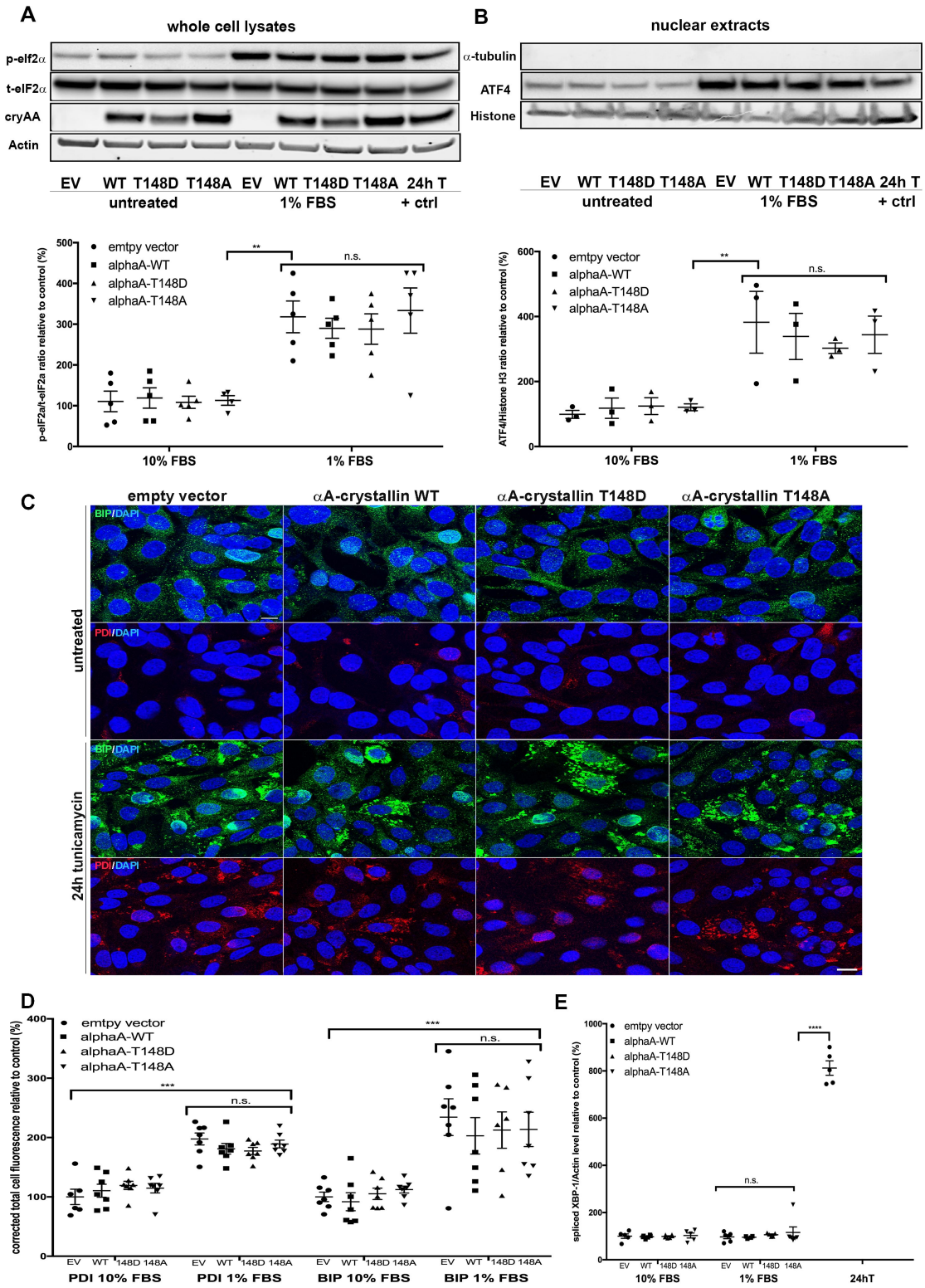
Supplemental Figure 1: α-crystallin knockout (KO) mice do not display any systemic alterations. Magnified images and weights of heart (**A**) and brain (**B**) of WT, αA-crystallin KO (Ko-CryAA), αB-crystallin KO (Ko-CryAB) and double-KO (Ko-CryAA/AB) non diabetic mice (n= 6) confirm similar weights and sizes of the organs between the different genotypes. Hematoxylin and Eosin (H&E) staining of retinal cross sections was used to assess the general anatomy and retinal thickness (**C,** n=6) and revealed no anatomical differences, but an increase in retinal thickness exclusively in the double KO mice (*p≤0.05). Immunhistochemical staining for glut2 (51) and insulin (green) were performed in non diabetic (upper panel) and streptozotocin-induced diabetic (lower panel) mice to assess islets numbers and sizes as a function of genotypes. Representative images are shown in (**D**). Nuclei were counterstained with Hoechst (blue). (scale bar = 20μm for **C** and 50μm for **D**)



Supplemental Figure 2: Cell-specificity of the rexpression of αA-crystallin in the retina using αA-crystallin KO mice. Representative images of immunohistochemical staining for αA-crystallin (green) and neuron specific enolase (NSE) on retinal cross-sections from αA-crystallin knockout mice compared to wild-type mice. Nuclei were counterstained with Hoechst (blue). (scale bar = 20μm)



Supplemental Figure 3: Cell specific upregulation of αB-crystallin in diabetic patients with diabetic retinopathy. Representative images of immunohistochemical staining for αB-crystallin (green) and glial-fibrillary acidic protein (GFAP) on retinal cross-sections from a non-diabetic human donor and a donor with diabetic retinopathy (bottom). Nuclei were counterstained with Hoechst (blue). (scale bar = 20μm)



Supplemental Figure 4: αA-crystallins does not influence the endoplasmic reticulum (ER) stress response in Müller glial cells subjected to metabolic stress conditions. (a-e) Müller glial cells were transfected with vectors overexpressing either wild-type (αA-WT), the phosphomimetic (αA-T148D) or the non-phosphorylatable mutant (αA-T148A) of αA-crystallin on threonine 148 or the empty vector (EV) control. The cells were then subjected to serum starvation (1% FBS) or tunicamycin (0.5µg/ml, positive

control) and the ER stress response was assessed by analysis of eif2α phosphorylation (**a**), ATF4 nuclear translocation (**b**), BIP and PDI induction (**c-d**) and XBP-1 splicing (**e**). Representative images of the immunoblots and the corresponding graphic representation of the quantifications are shown for p-eIF2α, t-eIf2α, Actin, αA-crystallin (cryAA) (**a**) and ATF4, Histone H3 and α-tubulin (**b**). Representative images of the immunofluorescent staining obtained for BIP (green), PDI (51) and DAPI (blue) (**c)** and the corresponding graphic representation of the relative quantification (**d**) are provided (scale bar=7.5µm). Graphic representation of the quantitative real time PCR for spliced XBP-1 (**e**). Gene expression was normalized to the Actin encoding gene *Actb*. Significantly different from untreated EV transfected cells (**p≤0.01, ***p≤0.001, ****p≤0.0001). Significantly different from serum deprived EV transfected cells ($^{\#\#\#\#}$p≤0.0001). Each endpoint was measured on a minimum of 3 technical replicates in at least 3 independent experiments.

Supplementary Table 1. MRM Transitions for LC-MS/MS Measurement of Peptide Abundances

Peptide	**Sequence**	**Precursor *m/z***	**Transitions (fragment ion, *m/z*)**
αA 145-156	VQSGLDAGHSER	628.30^{++}	y5, 585.27 y6, 656.31 y7, 771.34 y9, 941.44 y10, 1028.48
αA 145-156 phos.	VQ**pS**GLDAGHSER	668.29^{++}	y5, 585.27 y6, 656.31 y7, 771.34 y9, 941.44 y10, 1010.47[a]

[a] y10 ion monitored as $[m-98]^{+}$ ion

Supplementary Table 2. Overview over the quantitative real time PCR probes

rat genes info

Gene Symbol	**Gene name**	**Rat ref seq accession**	**Applied Biosystems Assay #**
XBP-1 (S)	X-box binding protein 1	NM_000394.3	Rn03464499_m1
XBP-1 (U)	X-box binding protein 1	NM_001004210.2	Rn03464498_s1

β-Actin was used as the housekeeping gene (Applied Biosystems #4352341E)

human genes info

Gene Symbol	**Gene name**	**Rat ref seq accession**	**Applied Biosystems Assay #**
CRYAA	crystallin alpha A	NM_001271731.1	Hs00166138_m1
CRYAB	crystallin alpha B	NM_001289807.1	Hs00157107_m1

β-Actin was used as the housekeeping gene (Applied Biosystems #4325788)