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Supplemental Material to:

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Impaired autophagy and delayed autophagic clearance of transforming growth factor β-induced protein (TGFBI) in granular corneal dystrophy type 2

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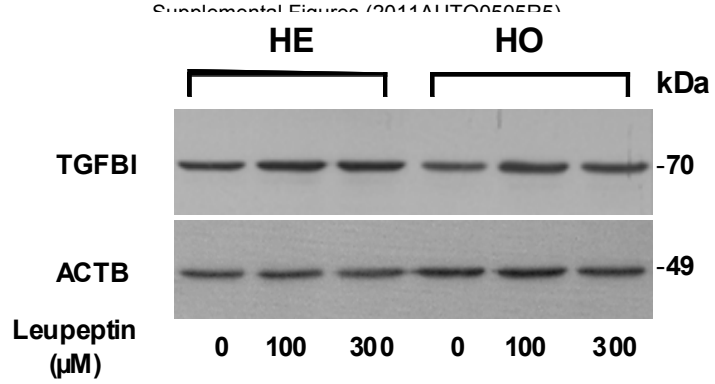
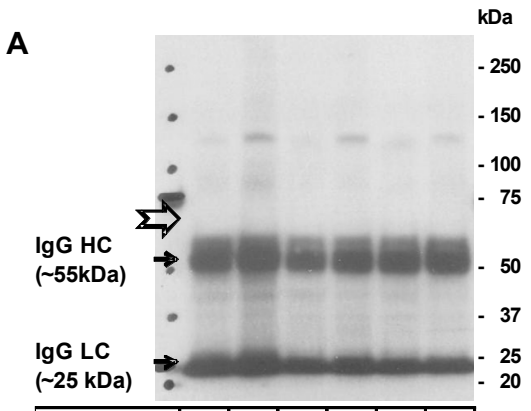
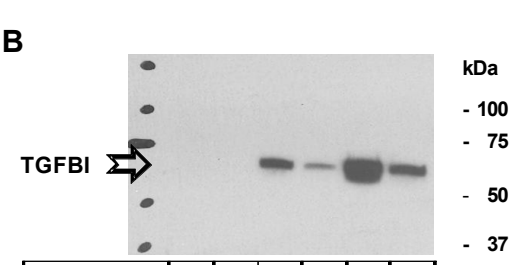


Figure S1. TGFBI was degraded by Autophagy. Primary HE and HO corneal fibroblasts were incubated with different doses of leupeptin (100 and 300 μg/ml), and TGFBI protein levels were determined by immunoblot analysis with an anti-TGFBI antibody.

FIGURE S2



MG132	-	+	-	+	-	+
Cell Types	WT		WT		HO	
IP with antibodies	Normal mice IgG		Anti -TGFBI			
WB with antibodies	Anti -Ubiquitin					



MG132	-	+	-	+	-	+
Cell Types	WT		WT		HO	
IP with antibodies	Normal mice IgG		Anti -TGFBI			
WB with antibodies	Anti -TGFBIp					

Figure S2. TGFBI was not ubiquitinated in WT and GCD2 Corneal Fibroblasts. WT and GCG2 corneal fibroblasts treated with or without MG132 for 12 h. Cells were lysed and incubated with anti-TGFBI or normal mouse IgG (2 µl of antibodies for 500 µl of cells homogenized in RIPA buffer). Immunoprecipitated proteins were analyzed on 10% SDS PAGE gels, western blotted then probed with either anti-ubiquitin antibodies (1: 1000 dilution) (A) purchased from LifeSensor (Malvern, VU101) and used at a 1/1000 dilution or anti-TGFBI antibodies (B).

FIGURE S3

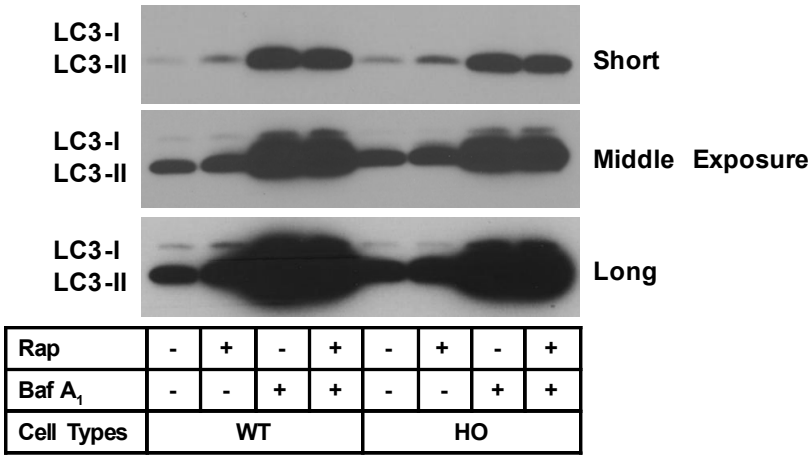


Figure S3. WT and GCD2 HO corneal fibroblasts were treated with Rap (100 nM) and Baf A_1 (0.1 µM) individually and in combination for 14 h. Cells were lysed and immunoblotted with anti-LC3 antibodies. Short (first panel), middle (second panel), and long (third panel) exposure times were applied in order to better visualize the differences in LC3-I and LC3-II levels between the samples.

FIGURE S4

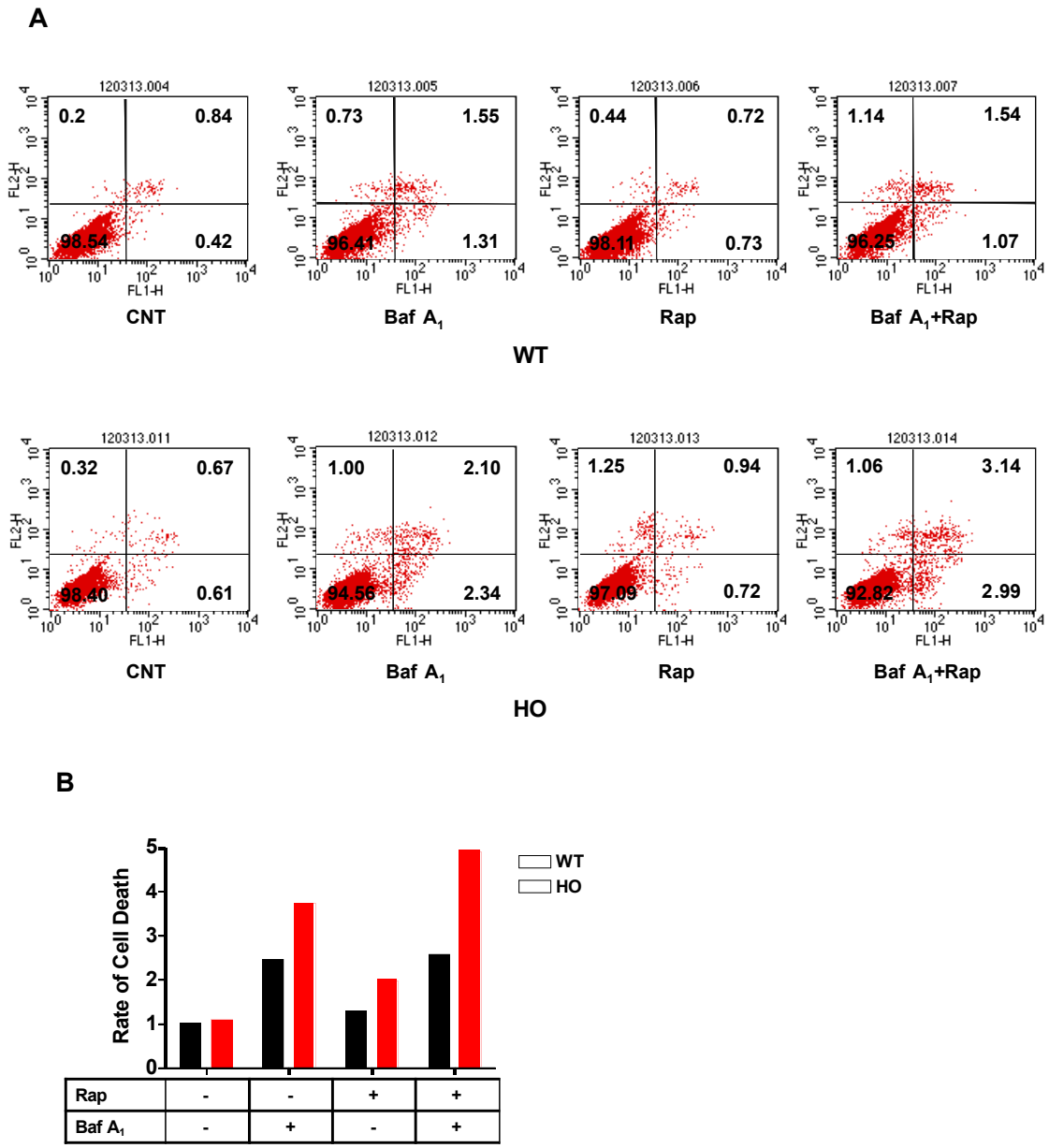


Figure S4. GCD HO corneal fibroblasts were most susceptible to autophagy inhibition-induced cell death. (**A**) Cell death was quantified using AnnexinV-FITC and PI staining in FACS. Percentages indicate percentage of cells in the respective quadrants. Corneal fibroblasts cells were divided into four groups: control (no treatment), Baf A_1 (treated

with 0.1 μM of Baf A_1), Rap (treated with 100 nM of Rap), and Baf A_1 plus Rap (treated with both 0.1 μM of Baf A_1 and 100 nM of Rap) for 24 h. The cell viability was measured by FACS analysis. The gate setting divides the cells in four populations: upper left (UL), necrotic cells; upper right (UR), late apoptotic cells; Lower left (LL), viable cells; lower right (LR) early apoptotic cells. Cells in the UL, UR and LR regions combined together give the proportion with cell death. (**B**) Cells in the UL, UR and LR regions represent the rate of cell death.