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Supplemental Data

Mutations in *C8orf37*, Encoding a Ciliary Protein, are Associated with Autosomal-Recessive Retinal Dystrophies with Early Macular Involvement

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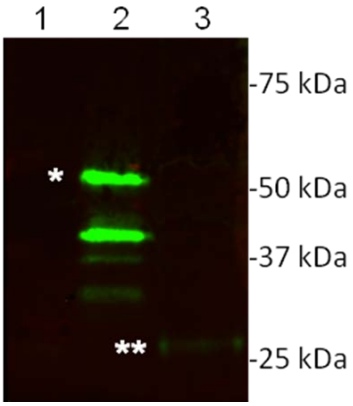

Figure S1. Western blot of bovine retinal extract and hTERT RPE1 cell extracts using anti-C8orf37

hTERT RPE1 cell extract (lane 1), hTERT RPE1 cells transfected with fused mRFP-C8orf37 (lane 2), and bovine retinal extracts (lane 3) were subjected to immunoblot analysis using anti-C8orf37 (rabbit polyclonal, Sigma-Aldrich, MO, USA). *C8orf37* cDNA constructs were generated by PCR, using IMAGE clones of *C8orf37* (clone 5520656), and were cloned in pDEST733 from the Gateway cloning system (Invitrogen), resulting in N-terminally fused mRFP-C8orf37. hTERT RPE1 cells were transfected with pDEST733-C8orf37 by using Effectene (Qiagen) according to the manufacturer's instructions and lysed as describe previously.[1] Bovine retinal extracts were prepared as describe previously.[2] The immunoblot revealed a predicted band of ~23.4 kDa (**) for bovine c8orf37 (lane 3) and a predicted band of ~53.4 kDa (*) for the fused RFP-C8orf37 protein. Some additional bands are present in the immunoblot (lane 2), which likely are due to the degradation of the fused RFP-C8orf37 protein. Endogenous C8orf37 is not visible in hTERT RPE1 extracts (lane 1).

References:

1. Boldt, K., Mans D.A., Won, J., van Reeuwijk, J., Vogt, A., Kinkl, N., Letteboer, S.J., Hicks, W.L., Hurd, R.E., Naggert, J.K, et al. (2011).Disruption of intraflagellar protein transport in photoreceptor cilia causes Leber congenital amaurosis in humans and mice. J. Clin. Invest. *121*, 2169-2180.

2. van Wijk, E., Kersten, F.F.M., Kartono, A., Mans, D.A., Brandwijk, K., Letteboer, S.J., Peters, T.A., Märker, T., Yan, X., Cremers, C.W.R.J. et al. (2009) Usher syndrome and Leber congenital amaurosis are molecularly linked via a novel isoform of the centrosomal ninein-like protein. Hum. Mol. Genet. *18*, 51-64.