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Supplementary Data

RHBDF2 Mutations Are Associated with Tylosis, a Familial Esophageal Cancer Syndrome

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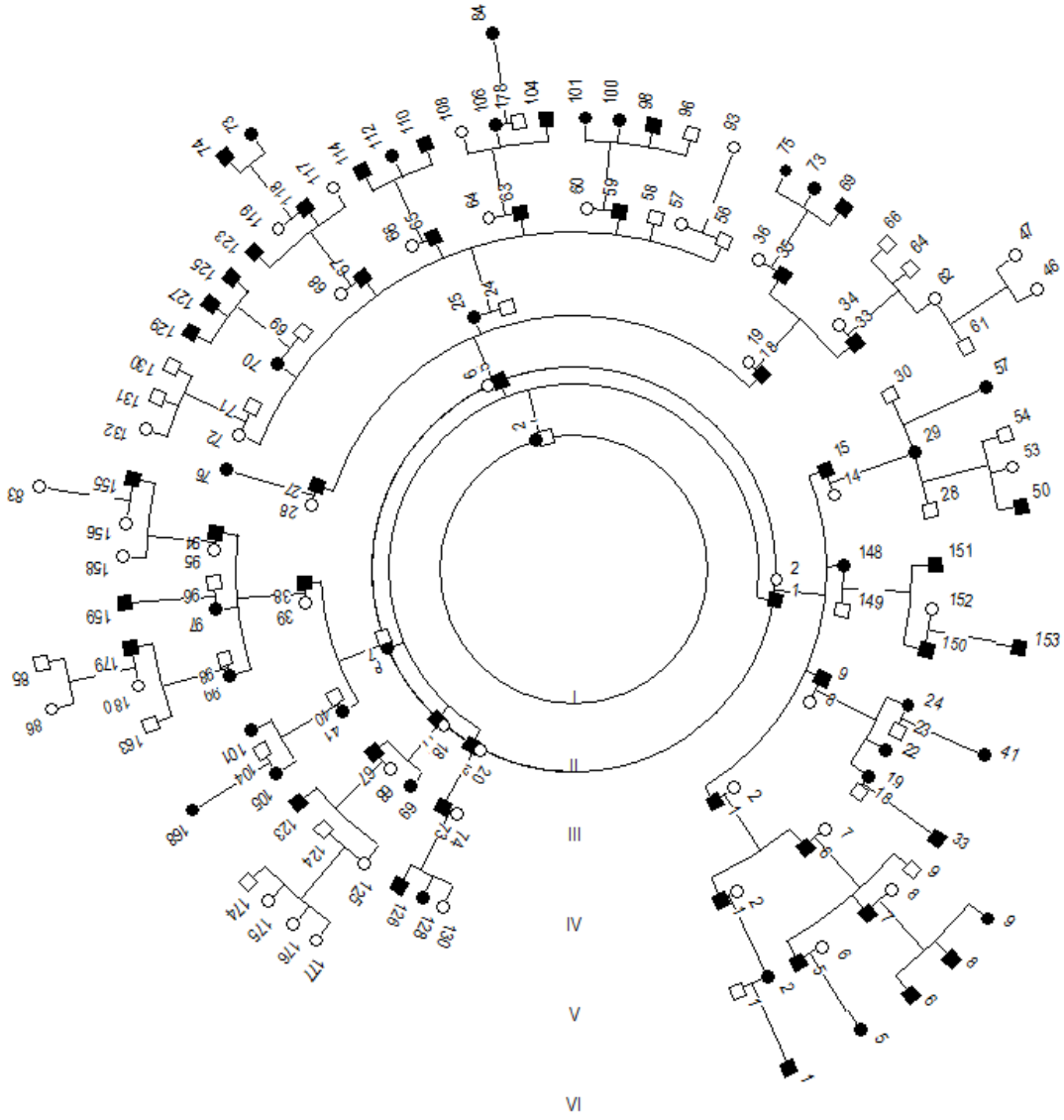

Figure S1. A Subset of the UK Tylosis Family

Schematic showing a subset of the extensive UK Tylosis family that are in current contact with the study. Closed symbols indicate those affected with Tylosis.

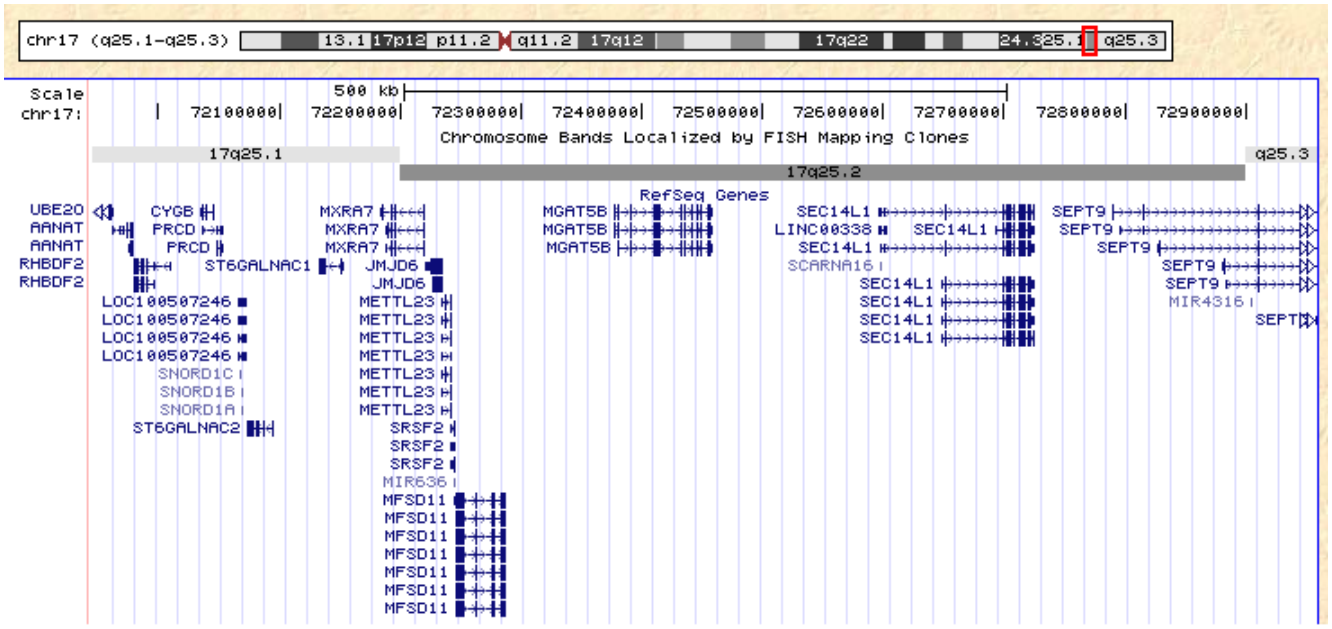

Figure S2. Extended Tylosis Minimal Region

Screen capture from UCSC browser showing the revised Tylosis minimal region on chr17q25.1-q25.3. Probes to all exons, and some non-coding sequence, within this region were included on a capture array and the captured DNA was sequenced, by next-generation sequencing, in one affected individual from the UK family with tylosis.

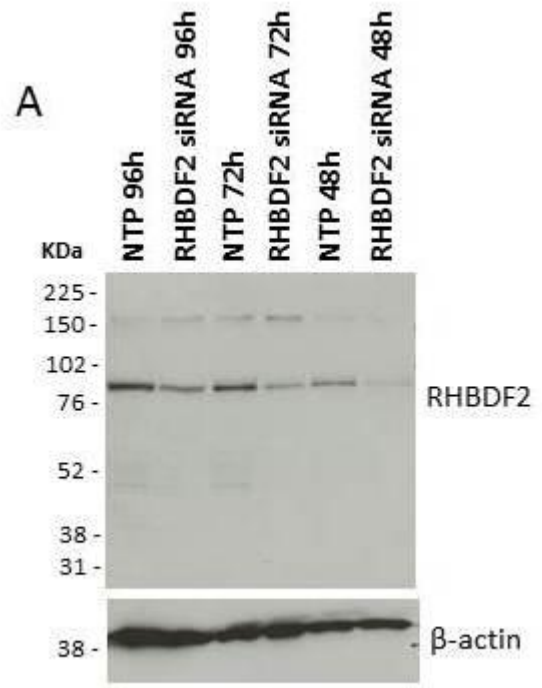

Figure S3. Immunoblot Illustrating α-RHBDF2 Antibody Specificity

Immunoblot of lysates collected from Neb1 keratinocyte cells transfected with RHBDF2 siRNA, or a non-targetting pool (NTP) of siRNA after 48 h, 72 h and 96 h and probed using the rabbit polyclonal anti-RHBDF2 antibody (Sigma, HPA018080). Anti-β-actin is used to demonstrate equal loading.

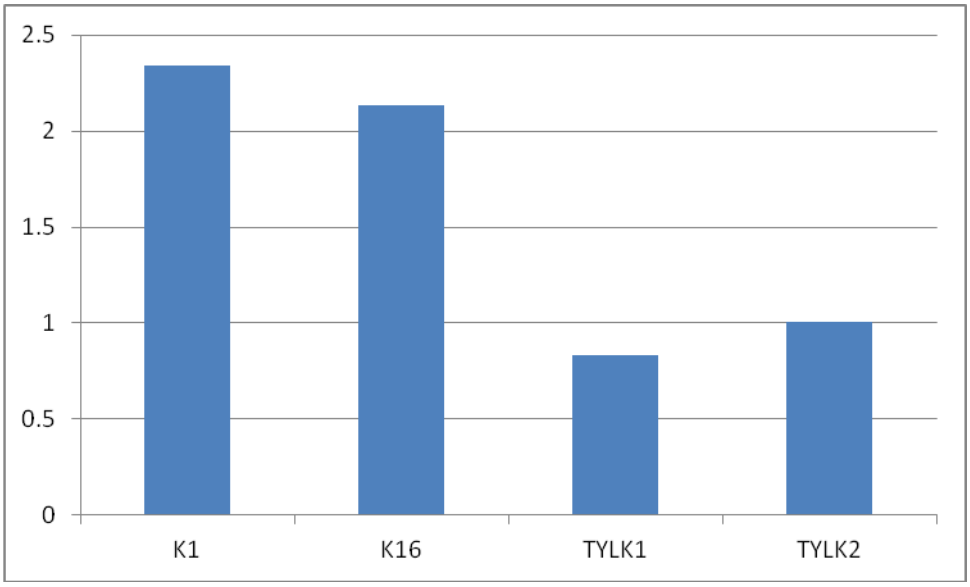

Figure S4. Quantification of Total EGFR Levels Relative to Anti-β-actin (from data in Fig. 3A)

Quantification of Western blots of lysates from control cells, K1 and K16, and tylotic cells, TYLK1 and TYLK2, blotted with anti-EGFR (total), showing a reduction in the levels of total EGFR in the lysates from tylotic cells. Anti-β-actin was used to demonstrate equal loading. Cells were grown in the absence of exogenous EGF.

Table S1. Primers Used for Pyrosequencing Assay

Forward primer (biotin-labelled)	Reverse primer	Pyrosequencing primer
GGACCCTAATGGCTCTGCTT	ATGTCCATCTCCTCCGGGT	GCCAGCGGATCCACA

The PCR primers and a pyrosequencing primer (all shown 5'-3') were designed to cover the c.557T>C mutation site, in order to screen other members of the UK and US families for the presence of this mutation.