Supplementary Figures

Heinz et al., Supplementary Figure S1

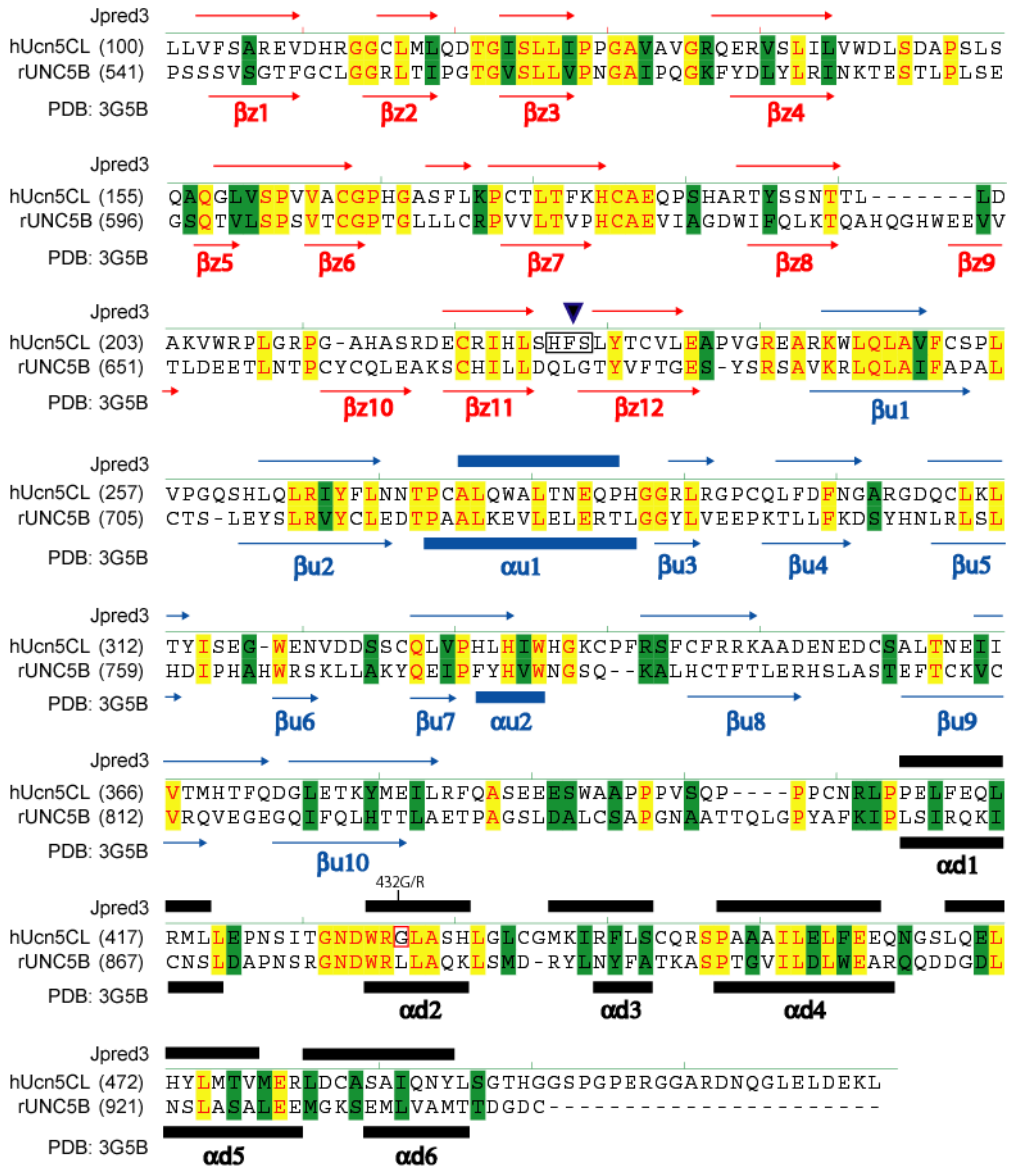

Figure S1 - Domain organization of Unc5CL. (A) ClustalW pairwise sequence alignment of human Unc5CL protein (aa 100-518) with rat Unc5B intracellular domain (aa 541-945). Yellow shading: identical amino acids, green shading: similar amino acids. Arrows and bars represent alpha-helices (α) and beta-sheets (β), respectively. The features above the alignment are derived from secondary structure prediction of human Unc5CL using Jpred3, the features below were annotated corresponding to the crystal structure of rat Unc5B ICD (PDB: 3G5B). Red (z): ZU5 domain, Blue (u): UPA domain, Black (d): DD. Black rectangle: putative autoproteolytic HFS cleavage site, triangle: specific site of cleavage. Red rectangle: aa affected by SNP rs742493 (432G/R).

Heinz et al., Supplementary Figure S2

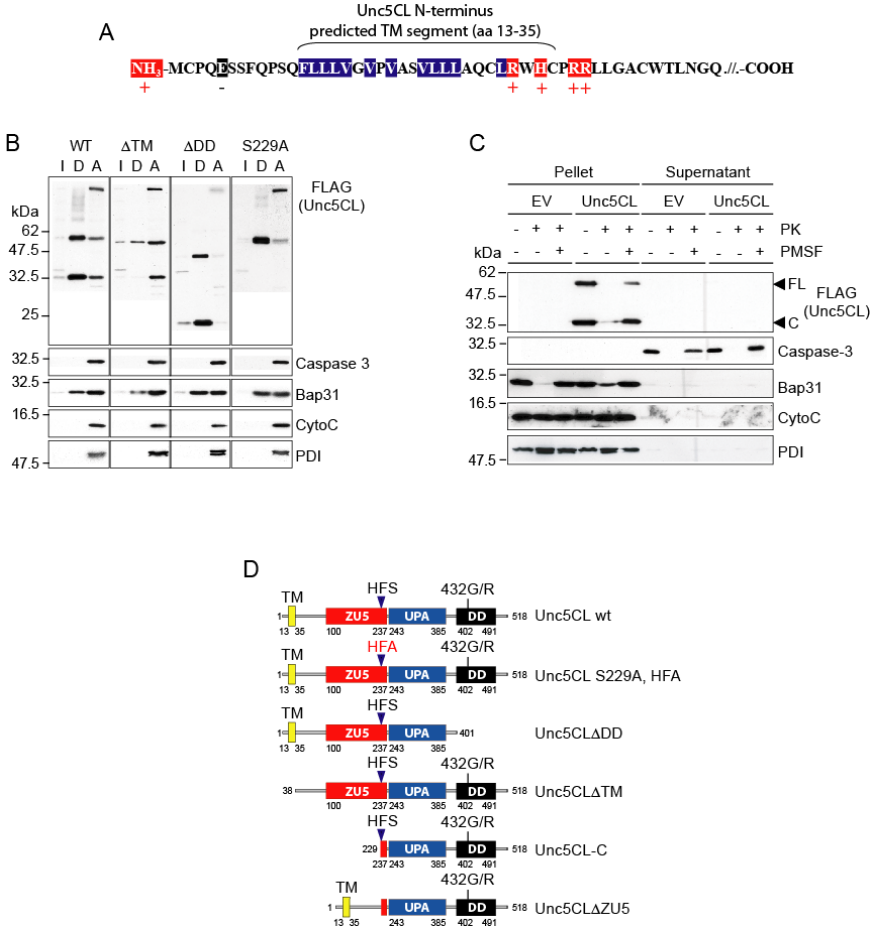

Figure S2 - Membrane association and topology of Unc5CL (A) Amino acid sequence of the Unc5CL N-terminus (aa 1-49). Red shading: positive charge, black shading: negative charge, blue shading: hydrophobic aa in the predicted transmembrane domain. (B) Proteins from HEK293T cells stably transduced with the indicated C-terminally FLAG-tagged Unc5CL MSCV constructs were subjected to TX-114 phase separation. Proteins were analyzed by western blot using the indicated antibodies. CytoC: 14 kDa Cytochrome C. I: detergent insoluble proteins, D: detergent phase, amphiphilic integral membrane proteins, A: aqueous phase, hydrophilic proteins. (C) Digitonin permeabilization and PK protection. HEK293T cells stably transduced with an empty MSCV vector (EV) or C-terminally FLAG-tagged Unc5CL were permeabilized for 10´ at 4°C with digitonin buffer containing 0.2 μg/ml digitonin (added directly before use). Samples were divided into 3 and left either untreated or were treated with 40 μg/ml PK ± 1 mM PMSF for 10´ at 4°C as indicated in the figure. Digestion was stopped by addition of 1 mM PMSF to all samples. Lysates were centrifuged at 13.000 rpm for 10´ at 4°C in a microcentrifuge to obtain a pellet and supernatant fraction and analyzed by western blot using the indicated antibodies. (D) Schematic representation of Unc5CL expression constructs used in this study.

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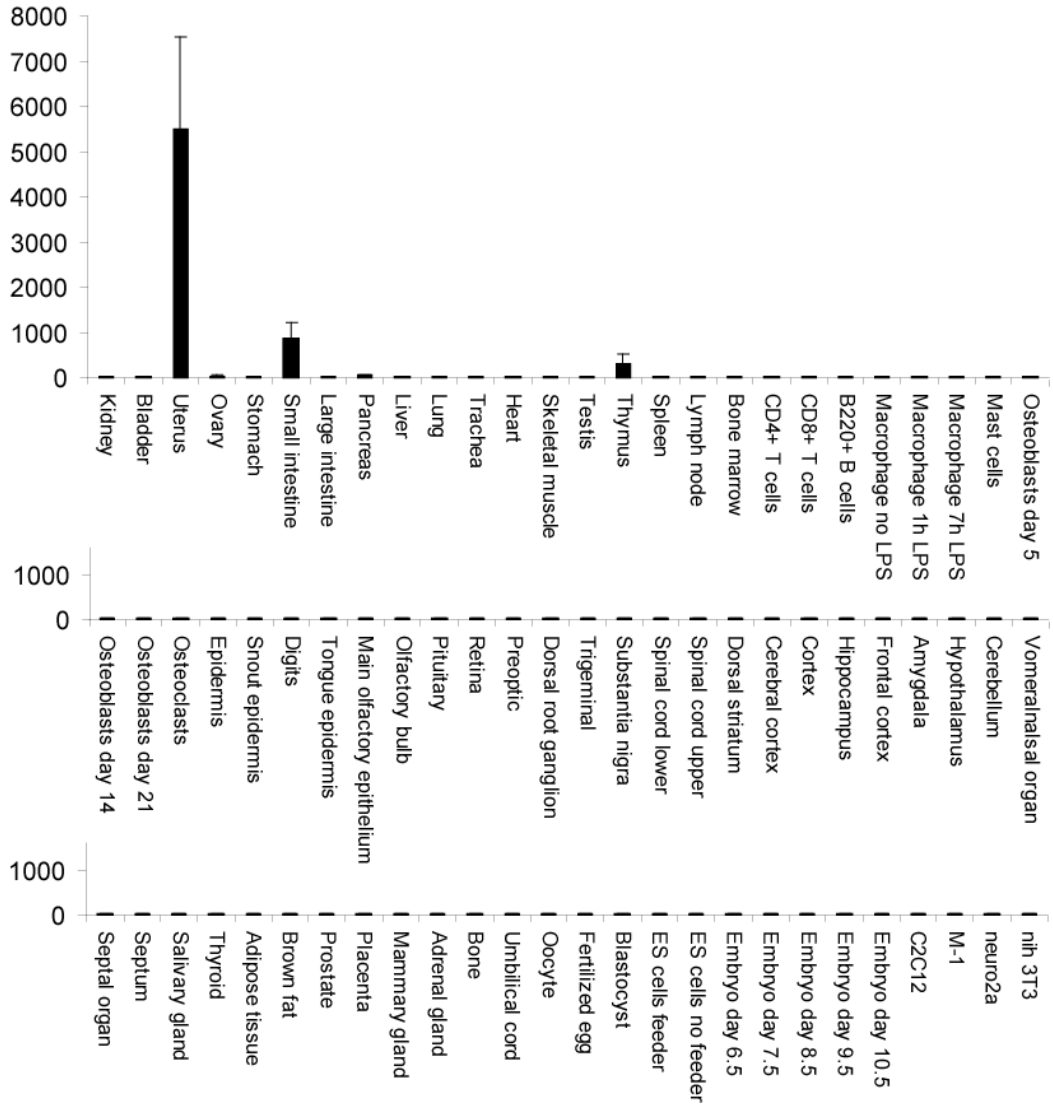

Figure S3 - Unc5CL mRNA tissue distribution. Unc5CL expression data were retrieved from the GNF1M murine tissue distribution atlas.

Heinz et al., Supplementary Figure S4

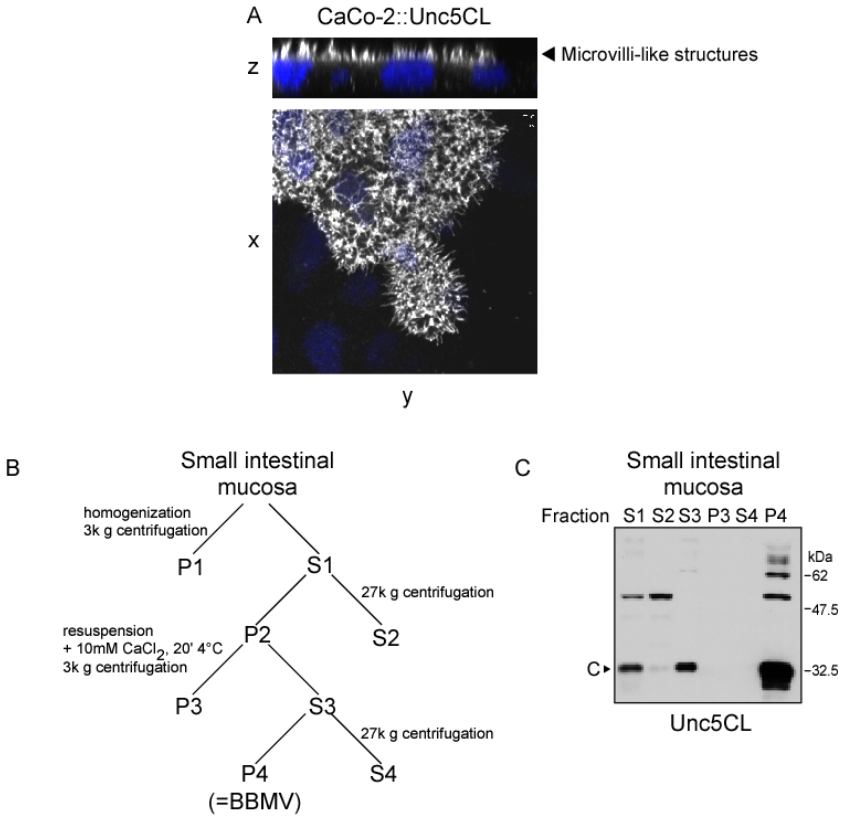

Figure S4 - Unc5CL is localized in intestinal microvilli. (A) Confocal microscopy of overexpressed C-terminally FLAG-tagged Unc5CL. 3-day post-confluent stable CaCo-2 cells were stained with FLAG-specific antibodies (white) and nuclear Hoechst stain (blue) and were analyzed by confocal microscopy. (B) Flow diagram of BBMV preparation. S: supernantant, P: pellet (C) Unc5CL-specific western blot of small intestinal mucosal fractions as described in (B).

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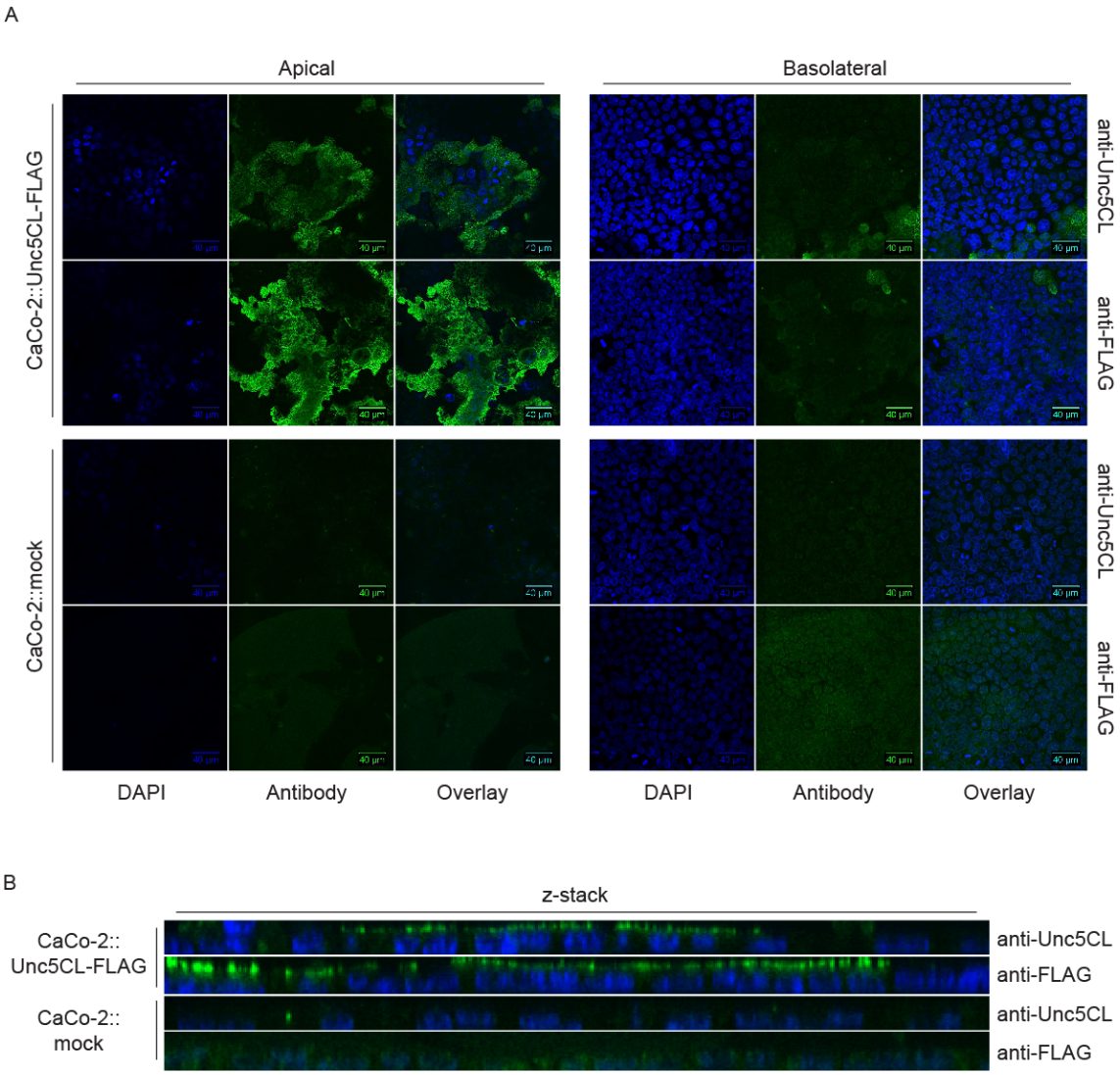

Figure S5 – Control immunofluorescent staining of Unc5CL following acetone fixation. (A) Apical and basolateral confocal sections of CaCo-2 cells stably expressing FLAG-tagged Unc5CL or mock transduced cells stained for nuclear DAPI and the indicated antibodies. Blue: nuclear DAPI staining, Green: Unc5CL. (B) z-stack reconstruction of the samples shown in (A).

Heinz et al., Supplementary Figure S6

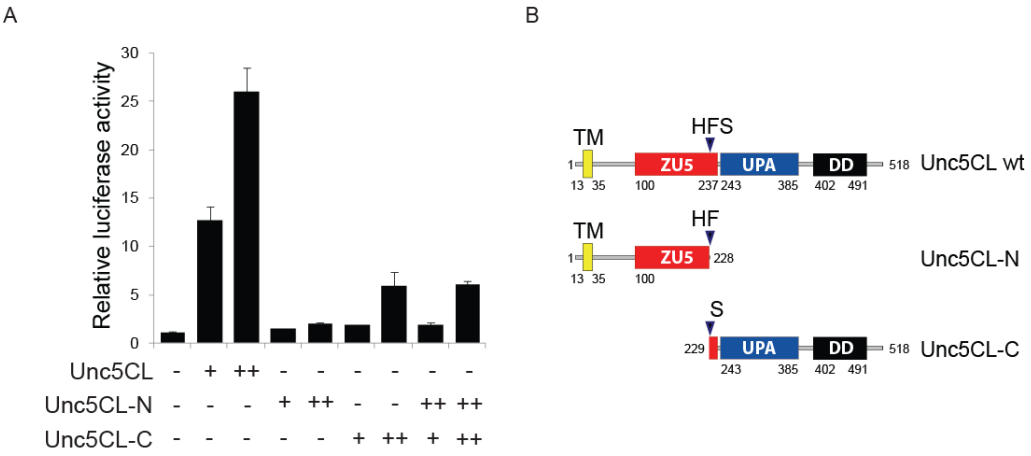

Figure S6 – Contribution of N- and C-terminal Unc5CL fragments to NF-κB activation. (A) HEK293T cells were transfected with NF-κB luciferase reporter gene plasmids together with an empty vector (EV) and increasing or single doses of the indicated expression constructs and were analyzed for NF-κB dependent luciferase activity 24h later. Data represent the mean values ± s.d. of technical triplicates; results are representative of three independent experiments. (B) Schematic representation of the expression constructs used in (A).

Heinz et al., Supplementary Figure S7

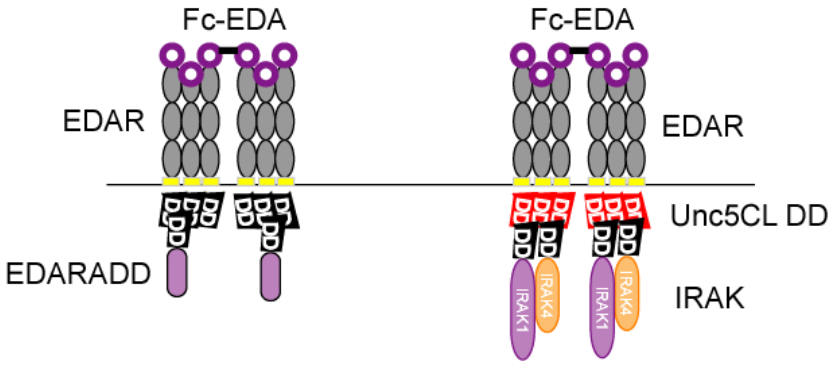

Figure S7 - Schematic representation of EDAR and EDAR-Unc5CL chimeric receptor. While oligomerization of EDAR by hexameric Fc-EDA leads to the recruitment and activation of the adapter protein EDARADD (left panel) a chimeric receptor in which the DD of EDAR is exchanged by the DD of Unc5CL (shown in red) is able to recruit and activate IRAK kinases (right panel).