

Title: Toll-like Interleukin 1 receptor regulator (TILRR) protein, a major modulator of inflammation, is expressed in normal human and macaque tissues and PBMCs

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Running Title: TILRR protein expression in tissues and PBMCs

†In Memoriam

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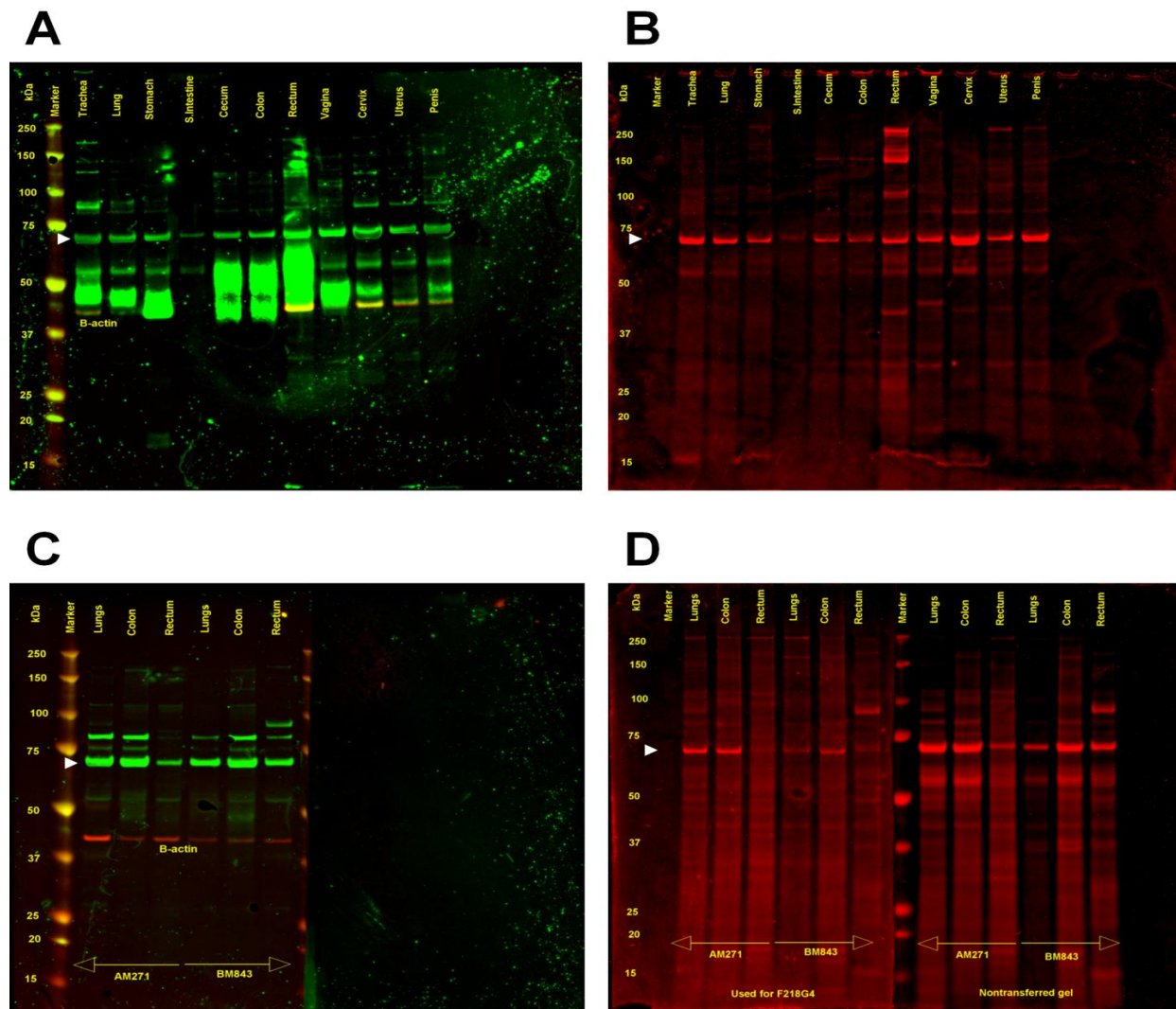


Figure S1: Full-length un-cropped membranes and gels run for normal human and Cynomolgus monkey tissues. A-B) Western blot analysis (A) and Coomassie blue staining (B) of normal human tissue lysates; **C-D)** Western blot analysis (C) and Coomassie blue staining (D) of monkey tissue cell lysates. TILRR protein (70 kDa, green color) (A and C) was probed by primary mouse anti-TILRR F218G4 mAb followed by secondary goat anti-mouse IRDye 800CW antibody (1:10000, LI-COR). β -actin protein (42 kDa, red color) (A and C) was probed by primary rabbit anti- β -actin antibody (1:1000, LI-COR) followed by secondary goat anti-rabbit IRDye 680RD antibody (1:10000, LI-COR). Coomassie blue staining of gels (B and D), showing untransferred proteins after iBlot transfer in the case of human tissue lysates (B) and Cynomolgus monkey tissue cell lysates (D). Western blot and Coomassie blue staining images were acquired by Odyssey CLx imaging system (LI-COR, USA) with auto channels (both 700 and 800), 42 μ m resolution, high image quality, 0 mm focal offset for membranes, and 0.5 focal offsets for the gels. A white-colored arrowhead indicates the 70 kDa-sized protein band. Other observed bands (<70 kDa or >70 kDa) in the blot could be the additional variants of FREM1 (yet uncharacterized). Tissues' names are mentioned on the top of the membranes and gels. AM271 and BM843 are the monkeys' identification numbers. S. intestine, small intestine (ileum); kDa, kiloDalton.

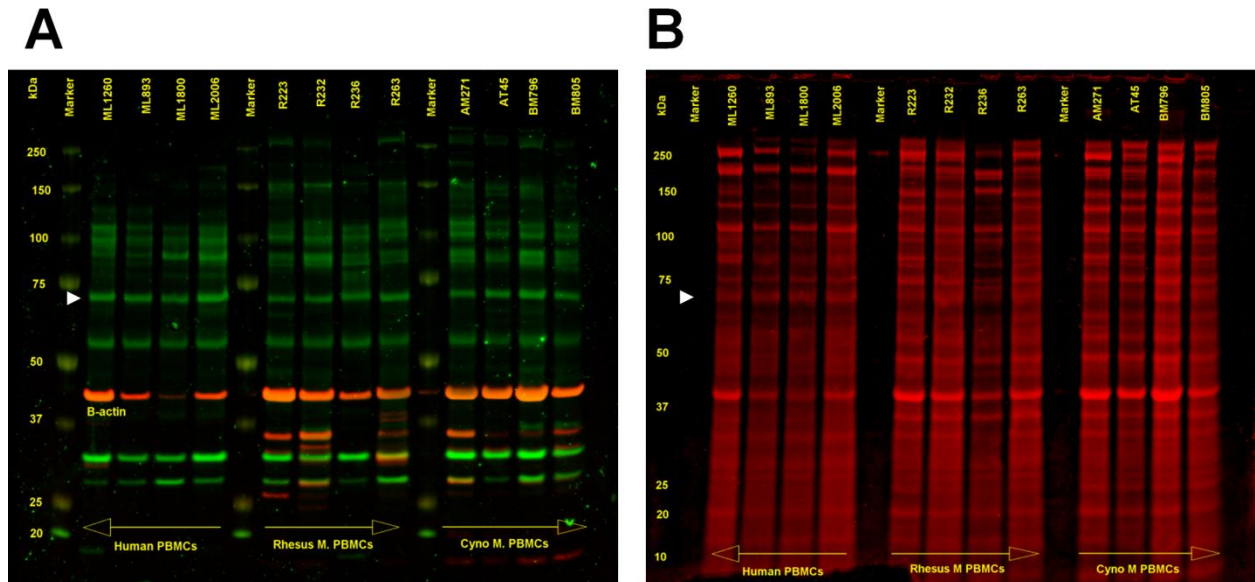


Figure S2: Full-length un-cropped membrane and gel run for normal human and macaques PBMCs. **A)** Western blot analysis of TILRR protein in PBMC lysates of humans, rhesus monkeys, and cynomolgus monkeys. TILRR protein (70 kDa, green color) was probed by primary mouse anti-TILRR F218G4 mAb followed by secondary goat anti-mouse IRDye 800CW antibody (1:10000, LI-COR). β -actin protein (42 kDa, red color) was probed by primary rabbit anti- β -actin (1:1000, LI-COR) followed by secondary goat anti-rabbit IRDye 680RD antibody (1:10000, LI-COR). **B)** Coomassie blue staining of gel, showing untransferred proteins after iBlot transfer of PBMC lysates in humans, rhesus monkeys, and cynomolgus monkeys. Western blot and Coomassie blue staining images were acquired by Odyssey CLx imaging system (LI-COR, USA) with auto channels (both 700 and 800), 42 μ m resolution, high image quality, 0 mm focal offset for membrane, and 0.5 focal offsets for the gel. A white-colored arrowhead indicates the 70 kDa-sized protein band. Other observed bands (<70 kDa or >70 kDa) in the blot could be the additional variants of FREM1 (yet uncharacterized). Patients' and animals' identification numbers are mentioned on the top of the membrane and gel. kDa, kiloDalton; PBMCs, peripheral blood mononuclear cells, Rhesus M, rhesus monkey; Cyno M, cynomolgus monkey.

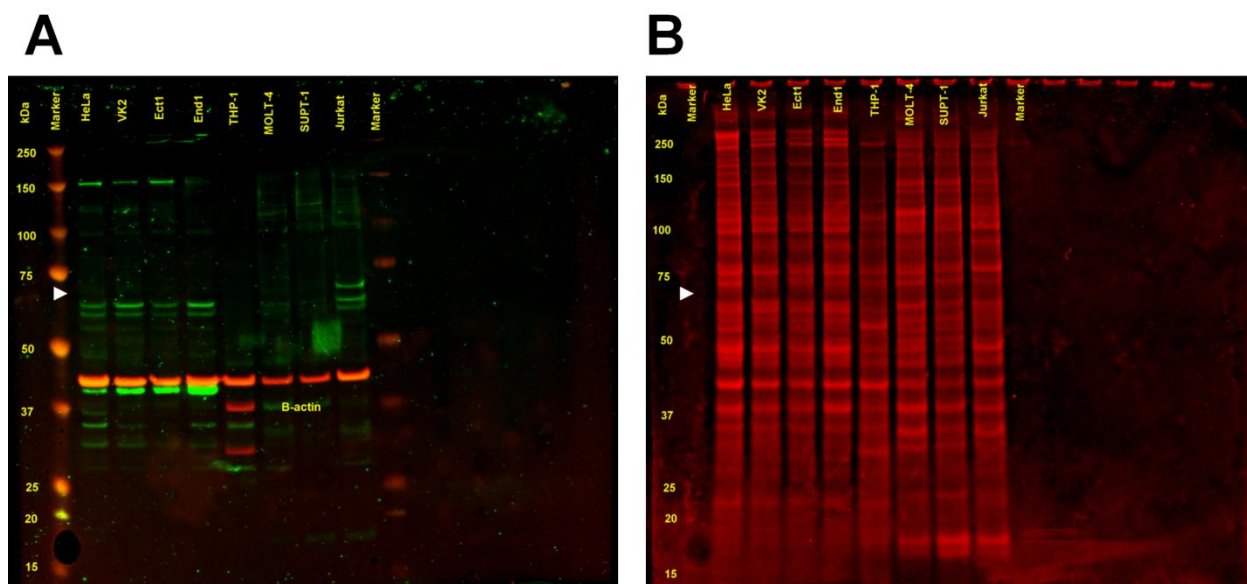


Figure S3: Full-length un-cropped membrane and gel run for human cell lines. **A)** Western blot analysis of TILRR protein in human cell line lysates. TILRR protein (70 kDa, green color) was probed by primary mouse anti-TILRR F218G4 mAb followed by secondary goat anti-mouse IRDye 800CW antibody (1:10000, LI-COR). β -actin protein (42 kDa, red color) was probed by primary rabbit anti- β -actin antibody (1:1000, LI-COR) followed by secondary goat anti-rabbit IRDye 680RD antibody (1:10000, LI-COR). **B)** Coomassie blue staining of gel, showing untransferred proteins after iBlot transfer of human cell line lysates. Western blot and Coomassie blue staining images were acquired by Odyssey CLx imaging system (LI-COR, USA) with auto channels (both 700 and 800), 42 μ m resolution, high image quality, 0 mm focal offset for membrane, and 0.5 focal offsets for the gel. A white-colored arrowhead indicates the 70 kDa-sized protein band. Other observed bands (<70 kDa or >70 kDa) in the blot could be the additional variants of FREM1 (yet uncharacterized). Cell line names are mentioned on the top of the membrane and gel. kDa, kiloDalton.

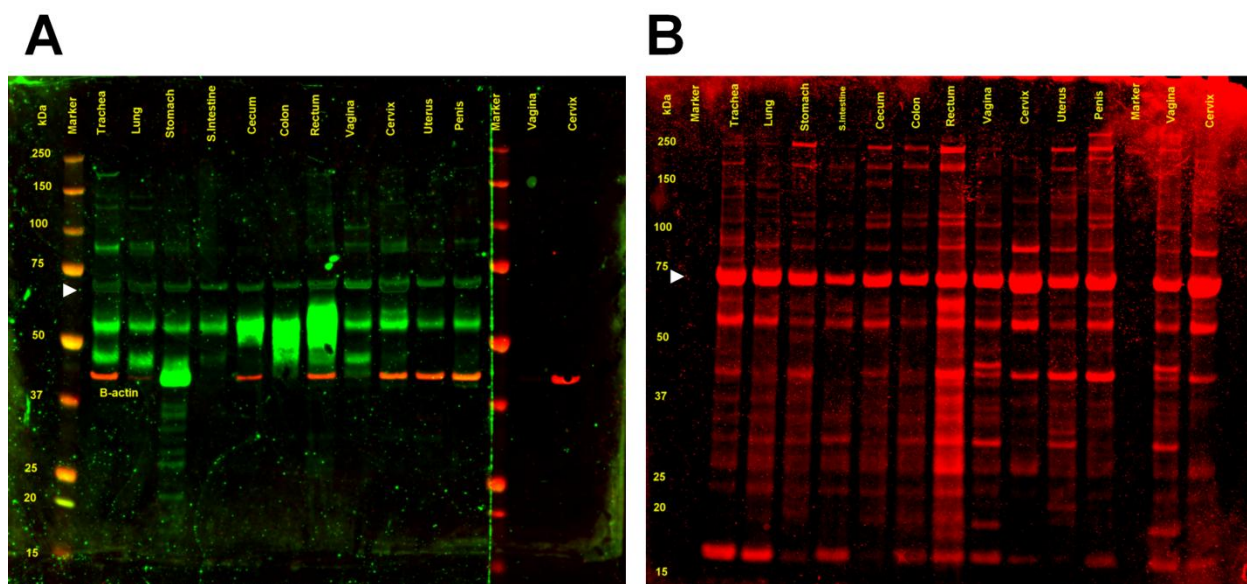


Figure S4: Full-length un-cropped membrane and gel run to validate anti-TILRR mAb. A) Western blot analysis of TILRR protein in human tissue lysates. TILRR protein (70 kDa, green color) was probed by either primary mouse anti-TILRR F218G4 mAb or primary mouse IgG1 mAb (isotype control) (abcam, Canada) followed by secondary goat anti-mouse IRDye 800CW antibody (1:10000, LI-COR). β -actin protein (42 kDa, red color) was probed by primary rabbit anti- β -actin antibody (1:1000, LI-COR) followed by secondary goat anti-rabbit IRDye 680RD antibody (1:10000, LI-COR). **B)** Coomassie blue staining of gel, showing untransferred proteins after iBlot transfer of human tissue lysates. Western blot and Coomassie blue staining images were acquired by Odyssey CLx imaging system (LI-COR, USA) with auto channels (both 700 and 800), 42 μ m resolution, high image quality, 0 mm focal offset for membrane, and 0.5 focal offsets for the gel. A white-colored arrowhead indicates the 70 kDa-sized protein band. Other observed bands (<70 kDa or >70 kDa) in the blot could be the additional variants of FREM1 (yet uncharacterized). Tissue names are mentioned on the top of the membrane and gel. S. intestine, small intestine (ileum); kDa, kiloDalton.