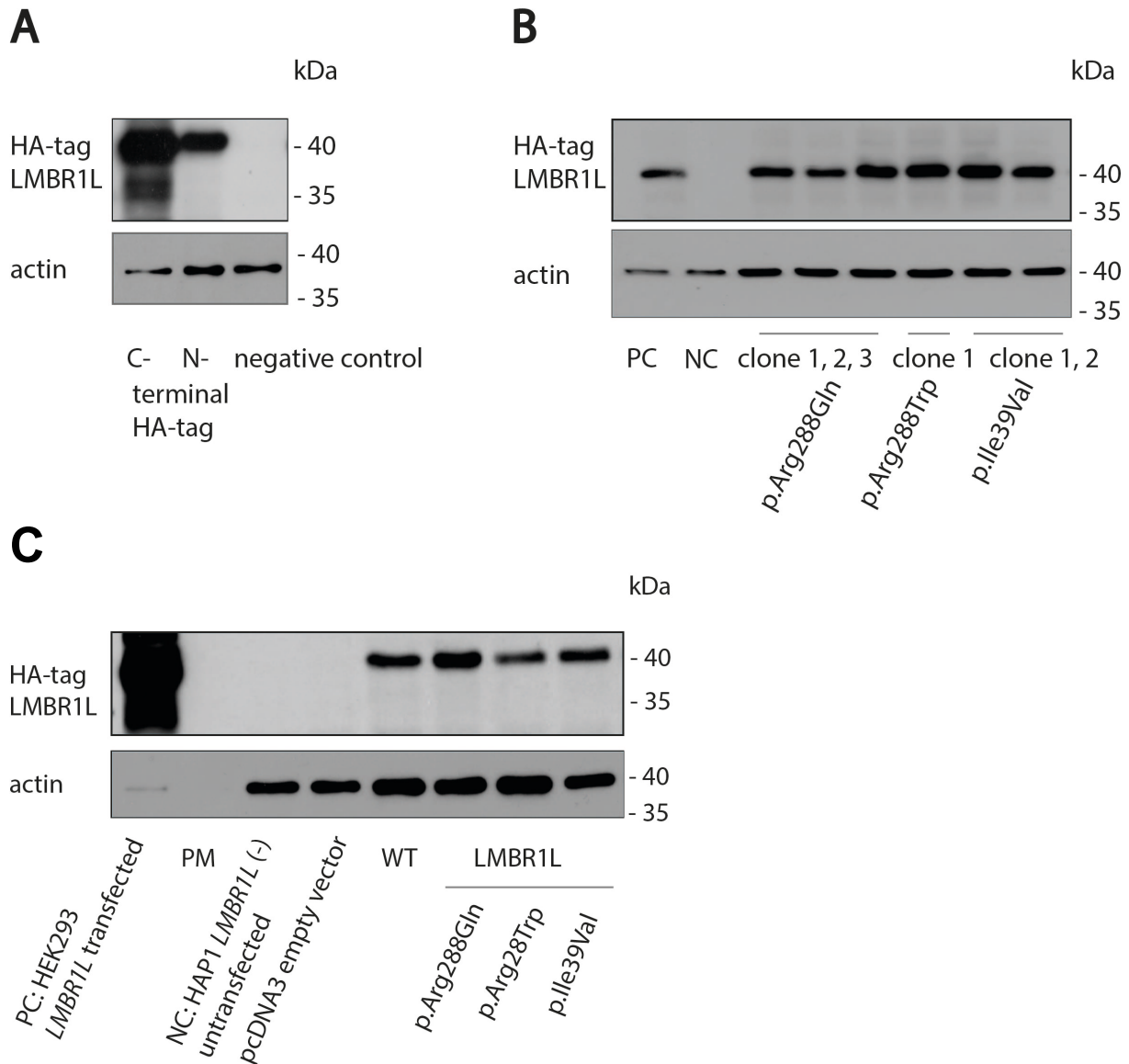


Supplemental Figure S1

Cloning and transfection of N- and C-terminally HA-tagged *LMBR1L* variants



A: N- or C-terminally HA-tagged *LMBR1L* cDNA was cloned into pcDNA3-vector and transiently transfected into HEK293 cells. Western blotting of total cellular protein with an antibody directed against the HA-tag revealed a major band above 40 kDa. The C-terminally HA-tagged *LMBR1L* expressed more strongly. Non-transfected cells did not show a band (NC negative control). β -actin served as reference.

B: Site-directed mutagenesis was performed on pcDNA3 vector containing C-terminally HA-tagged *LMBR1L*. Stable transfection of *LMBR1L*-knockout HAP1 cells with HA-*LMBR1L* variants. 1-3 stable clones were analyzed by western blotting with an anti-HA antibody. NC negative control (empty vector transfected), PC positive control (transiently transfected HEK293 cells). β -actin served as reference.

C: Characterization of *LMBR1L*-knockout HAP1 cells stably transfected with HA-*LMBR1L* variants that were used for surface biotinylation assays in Figure 5. Western blot using anti-HA antibody. PC positive control (transiently transfected HEK293 cells); PM protein marker lane; NC negative control (untransfected); NC (empty vector transfected); WT wild-type *LMBR1L* sequence; p.Arg288Gln, p.Arg288Trp, p.Ile39Val *LMBR1L* variants.

Supplemental Figure S2:

LMBR1L-deficient HAP1 cells

Haploid HAP1 cells have been obtained from Horizon/PerkinElmer (catalog number HZGHC007405c011) that have been engineered by a CRISPR-mediated 7 base-pair deletion to be deficient in *LMBR1L*.

Documentation available at https://horizondiscovery.com/-/media/Files/Horizon/resources/Data%20Sheets/5/HZGHC007405c011_DataSheet.pdf retrieved on October 7, 2021.

We have verified the presence of the mutation (in red) by reverse transcription of mRNA isolated from HZGHC007405c011 HAP1 cells, followed by PCR amplification of the *LMBR1L* cDNA.

The PCR product was subjected to Sanger sequencing and compared with the reference sequence NM_018113.3:

26IE70 – Primer: 5' UTR fwd

```
Query 1 ATGGAAGCACCTGACTACGAAGTGCTATCCGTGCGAGAACAGCTATTCCACGAGAGGATC 60
      |||
Sbjct 94 ATGGAAGCACCTGACTACGAAGTGCTATCCGTGCGAGAACAGCTATTCCACGAGAGGATC 153

Query 61 CGCGAGTGTATTATATCAAACTTCTGTTTGCAAACTGTACATCCTCTGCCACATCTTC 120
      |||
Sbjct 154 CGCGAGTGTATTATATCAAACTTCTGTTTGCAAACTGTACATCCTCTGCCACATCTTC 213

Query 121 CTGACCCGCTTCAAGAAGCCTGCTGAGTTCACCACAGTGGATGATGAAGATGCCACCGTC 180
      |||
Sbjct 214 CTGACCCGCTTCAAGAAGCCTGCTGAGTTCACCACAGTGGATGATGAAGATGCCACCGTC 273

Query 181 AACAAAGATTGCGCTCGAGCTGTGCACCTTTACCCTGGCAATTGCCCTGGGTGCTGTCTTG 240
      |||
Sbjct 274 AACAAAGATTGCGCTCGAGCTGTGCACCTTTACCCTGGCAATTGCCCTGGGTGCTGTCTTG 333

Query 241 CTCCTGCCCTTCTCCATCATCAGCAATGAGGTGCTGCTCTCCCTGCCTCGGAACACTACTAC 300
      |||
Sbjct 334 CTCCTGCCCTTCTCCATCATCAGCAATGAGGTGCTGCT-----CCTCGGAACACTACTAC 386

Query 301 ATCCAGTGGCTCAACGGCTCCCTCATCCATGGCCTCTGGAACCTGTTTTTCTCTTCTCC 360
      |||
Sbjct 387 ATCCAGTGGCTCAACGGCTCCCTCATCCATGGCCTCTGGAACCTGTTTTTCTCTTCTCC 446

Query 361 AACCTGTCCCTCATCTTCTCATGCCCTTTGCATATTTCTTCACTGAGTCTGAGGGCTTT 420
      |||
Sbjct 447 AACCTGTCCCTCATCTTCTCATGCCCTTTGCATATTTCTTCACTGAGTCTGAGGGCTTT 506

Query 421 GCTGGCTCCAGAAAGGGTGTCTGGGCCGGGTCTATGAGACAGTGGTGATGTTGATGCTC 480
      |||
Sbjct 507 GCTGGCTCCAGAAAGGGTGTCTGGGCCGGGTCTATGAGACAGTGGTGATGTTGATGCTC 566

Query 481 CTCACTCTGCTGGTGCTAGGTATGGTGTGGGTGGCATCAGCCATTGTGGACAAGAACAAG 540
      |||
Sbjct 567 CTCACTCTGCTGGTGCTAGGTATGGTGTGGGTGGCATCAGCCATTGTGGACAAGAACAAG 626

Query 541 GCCAACAGAGAGTCACTCTATGACTTTTGGGAGTACTATCTCCCCTACCTCTACTCATGC 600
      |||
Sbjct 627 GCCAACAGAGAGTCACTCTATGACTTTTGGGAGTACTATCTCCCCTACCTCTACTCATGC 686

Query 601 ATCTCCTTCCTTGGGGTTCTGCTGCTCCTGGTGTGTACTCCACTGGGTCTCGCCCGCATG 660
      |||
Sbjct 687 ATCTCCTTCCTTGGGGTTCTGCTGCTCCTGGTGTGTACTCCACTGGGTCTCGCCCGCATG 746

Query 661 TTCTCCGTCACCTGGGAAGCTGCTAGTCAAGCCCCGGCTGCTGGAAGACCTGGAGGAGCAG 720
      |||
Sbjct 747 TTCTCCGTCACCTGGGAAGCTGCTAGTCAAGCCCCGGCTGCTGGAAGACCTGGAGGAGCAG 806
```

Query 721 CTGTACTGCTCAGCCTTTGAGGAGGCAGCCCTGACCCGCAGGATCTGTAATCCTACTTCC 780
 |||
 Sbjct 807 CTGTACTGCTCAGCCTTTGAGGAGGCAGCCCTGACCCGCAGGATCTGTAATCCTACTTCC 866

Query 781 TGCTGGCTGCCTTTAGACATGGAGCTGCTACACAGACAGGTCTGGCTCTGCAGACACAG 840
 |||
 Sbjct 867 TGCTGGCTGCCTTTAGACATGGAGCTGCTACACAGACAGGTCTGGCTCTGCAGACACAG 926

Query 841 AGGGTCCTGCTGGAGAAGAGGCGGAAGGCTTCAGCCTGGCAACGGAACCTGGGCTACCCC 900
 |||
 Sbjct 927 AGGGTCCTGCTGGAGAAGAGGCGGAAGGCTTCAGCCTGGCAACGGAACCTGGGCTACCCC 986

Query 901 CTGGCTATGCTGTGCTTGCTGGTGCTGACGGGCCTGTCTGTGCTCATTGTGGCCATCCAC 960
 |||
 Sbjct 987 CTGGCTATGCTGTGCTTGCTGGTGCTGACGGGCCTGTCTGTGCTCATTGTGGCCATCCAC 1046

Supplemental Table 1: Primer sequences for Sanger sequencing

Primer name	Sequence
gp.Arg288Gln Trp Fw	5' GGCTGCCTTTAGACATGGAGCTGCTAC 3'
gp.Arg288Gln Trp Rv	5' CAGGGCAAACCTGCACAACCTCCTCACTG 3'
gp.Ile39Val Fw	5' CTTTGCCCATTTACCAACCAGAG 3'
gp.Ile39Val Rv	5' GAGAGGAGATGACACAGAGCCTG 3'
p.Ser1707Phe Fw	5' CCTGTACTGGGGCCTCTGGAAGGACAC 3'
p.Ser1707Phe Rv	5' GGACACAGCACATGTTCAAGTCGCAATTGG 3'
p.Thr2086Ser Fw	5' ATGCCGCCGAATCCTCAAATGGCTGTAG 3'
p.Thr2086Ser Rv	5' GAAAAATCTGGGGACGAGGTCTGGAGC 3'
p.Ile3389Val Fw	5' GCTCTCTTTCTTTTCTGAATGCAG 3'
p.Ile3389Val Rv	5' GTTGCACACTGAGCTGACTACC 3'
p.Glu3763Val Fw	5' GAGAGATCAGATCACCTTTGGAC 3'
p.Glu3763Val Rv	5' CCTAAGACCCAACACCAAATCAG 3'