SUPPLEMENTARY MATERIAL

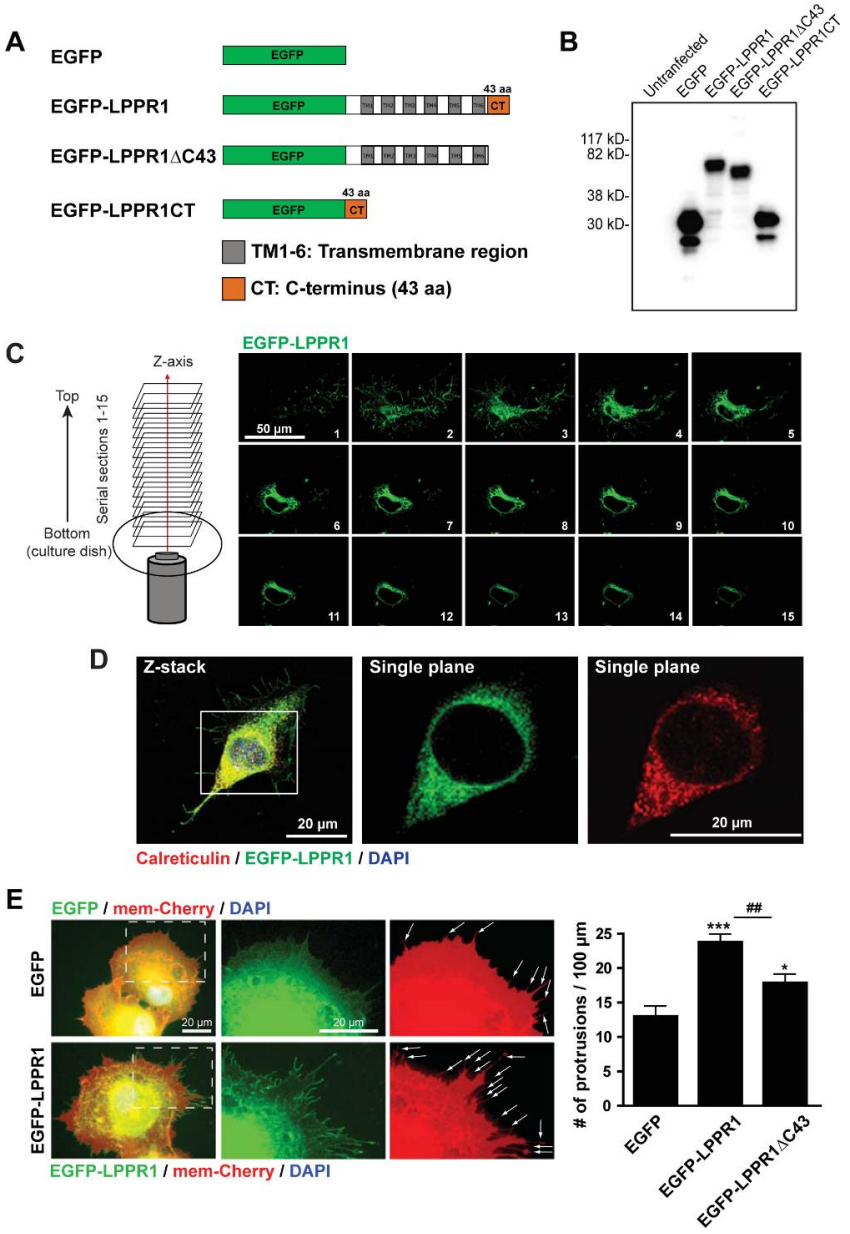

Fig. S1. LPPR1 overexpression and its effect on the formation of membrane protrusions. (A) Schematic representation of EGFPLPPR1, LPPR1ΔC43 and EGFP-LPPR1CT constructs; (B) Western blot analysis with anti-GFP antibody confirmed the correct expression of LPPR1 constructs; (C) Representative serial z-stack images from bottom to the top of a Neuro-2A cell expressing EGFP-LPPR1. (D) Staining of the EGFP-LPPR1 transfected cells with ER marker calreticulin (red). (E) Representative images and quantification of membrane protrusions in Cos-7 cells transfected with EGFP, EGFP-LPPR1 or EGFP-LPPR1ΔC43 together with a construct expressing a membrane-targeting mCherry (mem-Cherry) for quantification the number of membrane protrusions (arrows). * $p<0.05$, *** $p<0.001$, compared to EGFP; ## $p<0.01$, compared to EGFP-LPPR1ΔC43 (Oneway ANOVA and Tukey's multiple comparison test). Scale bar: 50 µm in C; 20 µm in D and E.

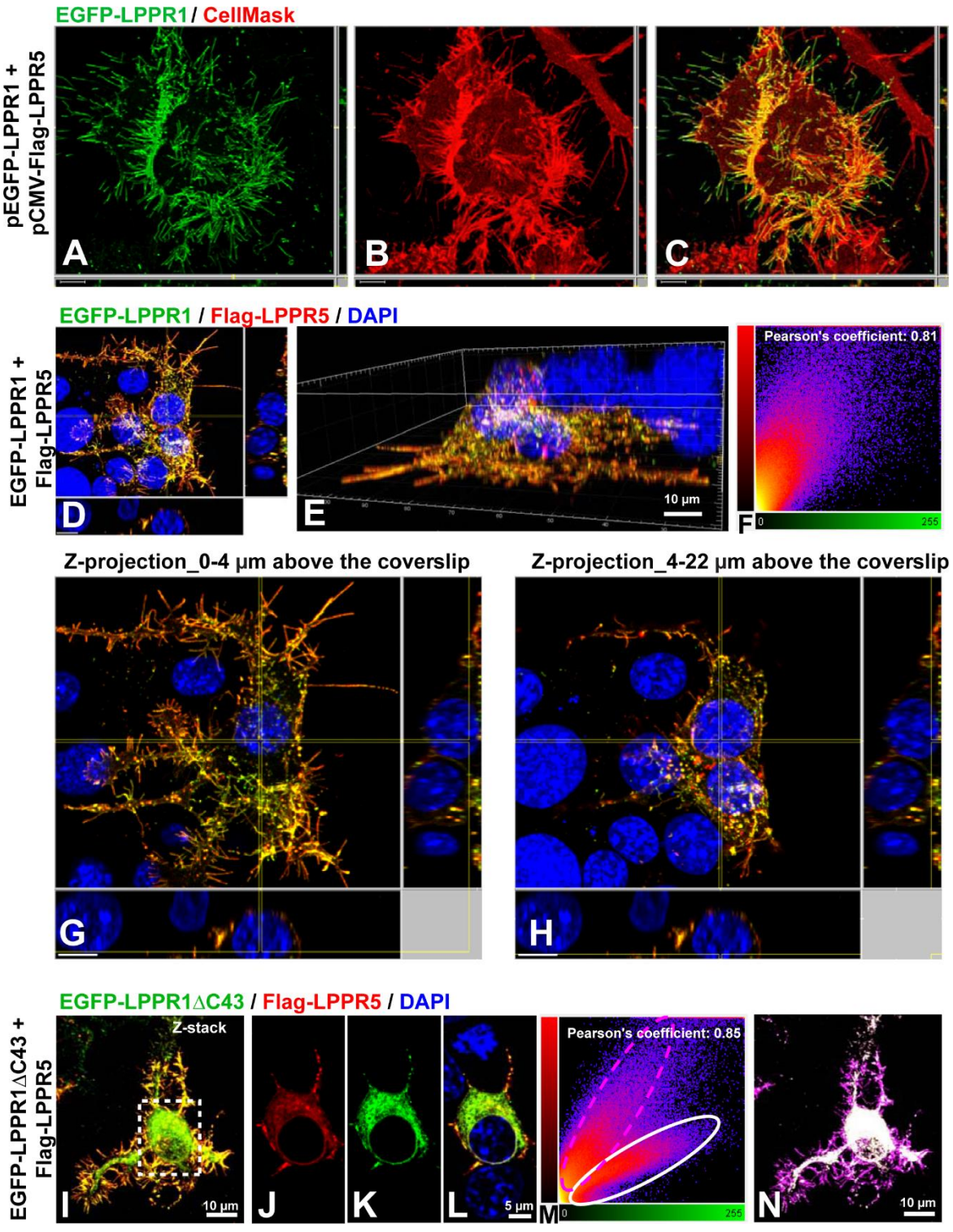
EGFP-LPPR1 / CellMask
pEGFP-LPPR1 + pCMV-Flag-LPPR5
A
B
C
EGFP-LPPR1 / Flag-LPPR5 / DAPI
EGFP-LPPR1 + Flag-LPPR5
D
E
10 µm
Pearson's coefficient: 0.81
F
0
255
Z-projection_0-4 µm above the coverslip
Z-projection_4-22 µm above the coverslip
G
H
EGFP-LPPR1ΔC43 / Flag-LPPR5 / DAPI
EGFP-LPPR1ΔC43 + Flag-LPPR5
Z-stack
I
10 µm
J
K
L
5 µm
Pearson's coefficient: 0.85
M
0
255
N
10 µm

Fig. S2. Colocalization of LPPR1 with LPPR5 at plasma membrane protrusions in Neuro2A cells. (A-C) Plasma membrane staining with CellMask followed by live cell imaging, showing localization of LPPR1 towards the plasma membrane protrusions when co-expressed with LPPR5; (D and E) Top view and side view of a 3D confocal image of cells cotransfected with EGFP-LPPR1 and Flag-LPPR5, showing the colocalization of LPPR1 with LPPR5 predominantly on plasma membrane protrusions; (F) 2D fluorogram showing colocalization of EGFP-LPPR1 and Flag-LPPR5 as distribution of pairs of pixel intensities (with greater diagonal alignment correlating to higher colocalization). The Pearson's colocalization coefficient (1, perfect correlation; 0, no correlation; –1, perfect inverse correlation) in the colocalized volume between EGFP-LPPR1 and Flag-LPPR5 was also calculated using Imaris software. (G and H) Z-projection view of serial images collected from the first 4 μm and 4-22 μm above the coverslip, respectively, showing majority LPPR-induced protrusions are located at the cell bottom. (I) A z-stack image of Neuro-2A cells cotransfected with EGFP-LPPR1ΔC43 and Flag-LPPR5, showing the colocalization of EGFP-LPPR1ΔC43 with Plag-LPPR5, notably on many plasma membrane protrusions. (J-L) Single plane images of the boxed cell body area in I, which showed some Flag-LPPR5 protein was also localized intracellularly together with EGFP-LPPR1ΔC43. (M) Colocalization analysis using Imaris showing two clusters of colocalization between EGFPLPPR1ΔC43 and Flag-LPPR5 in the 2D fluorogram. (N) Pixels within the dashed magenta oval of the fluorogram are represented as magenta, while pixels within the solid white oval are colored white, demonstrating that the clusters represent intracellular localization (white) and membrane localization (magenta).

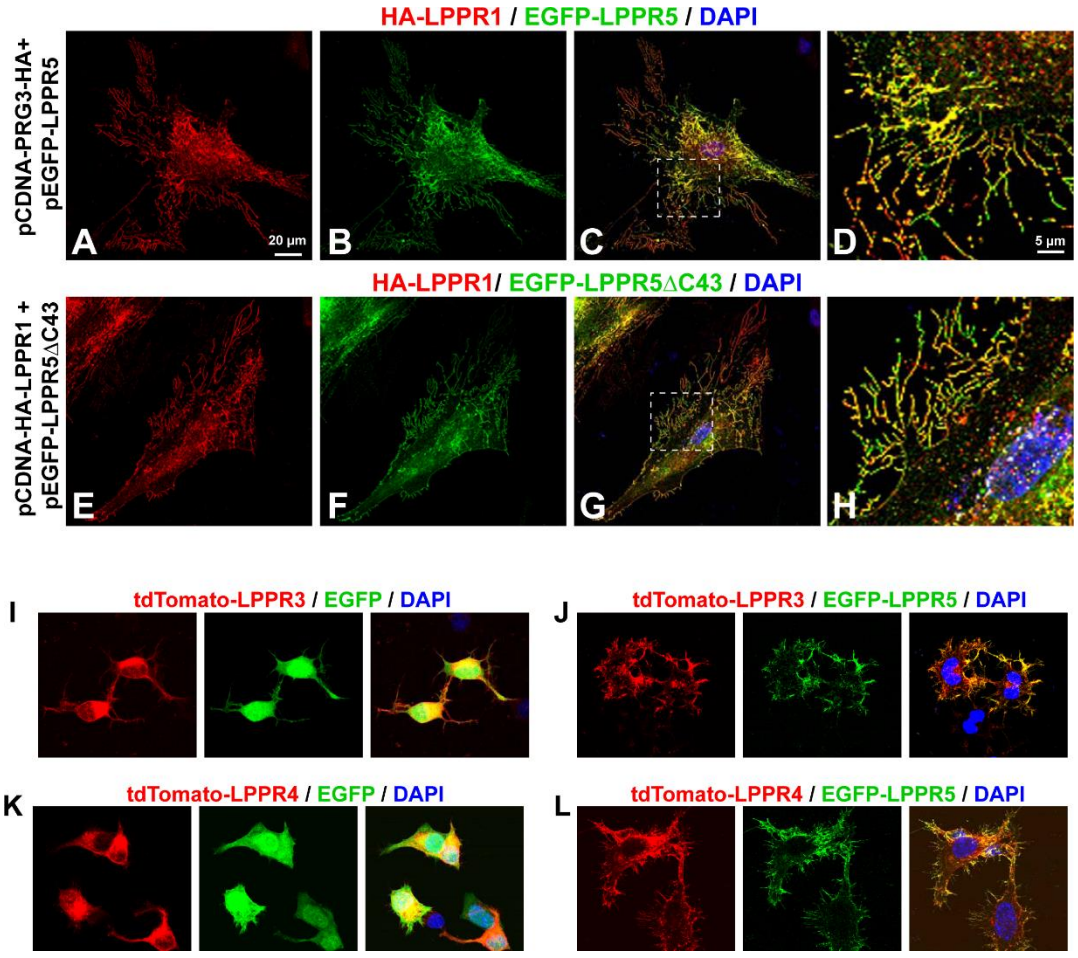

Fig. S3. Co-localization of LPPR family members at plasma membrane protrusions. (A-H) Representative images of human fibroblasts co-expressing LPPR1-HA and EGFPLPPR5 (A-D), or LPPR1-HA and EGFP-LPPR5ΔC39 (E-H) showing LPPR1 and LPPR5 were co-localized at plasma membrane protrusions, and deletion of the C-terminal portion of LPPR5 had little effect on their distribution as well as co-localization. D and H are higher magnification images of the boxed areas in C and G, respectively. Scale bar: 20 μm in A-C and E-G; 5 μm in D and H. (I-L) LPPR5 facilitated the localization of LPPR3 and LPPR4 to the plasma membrane. Neuro2A cells were co-transfected with tdTomato-LPPR3 and EGFP (I), tdTomato-LPPR3 and EGFP-LPPR5 (J), tdTomato-LPPR4 and EGFP (K) or tdTomato-LPPR4 and EGFP-LPPR5 (L). LPPR3 or LPPR4 was predominantly intracellular when co-expressed with EGFP control (I and K). However, when co-expressed with EGFP-LPPR5, a large port of LPPR3 or LPPR4 was then co-localized with LPPR5 on the plasma membrane protrusions (J and L).

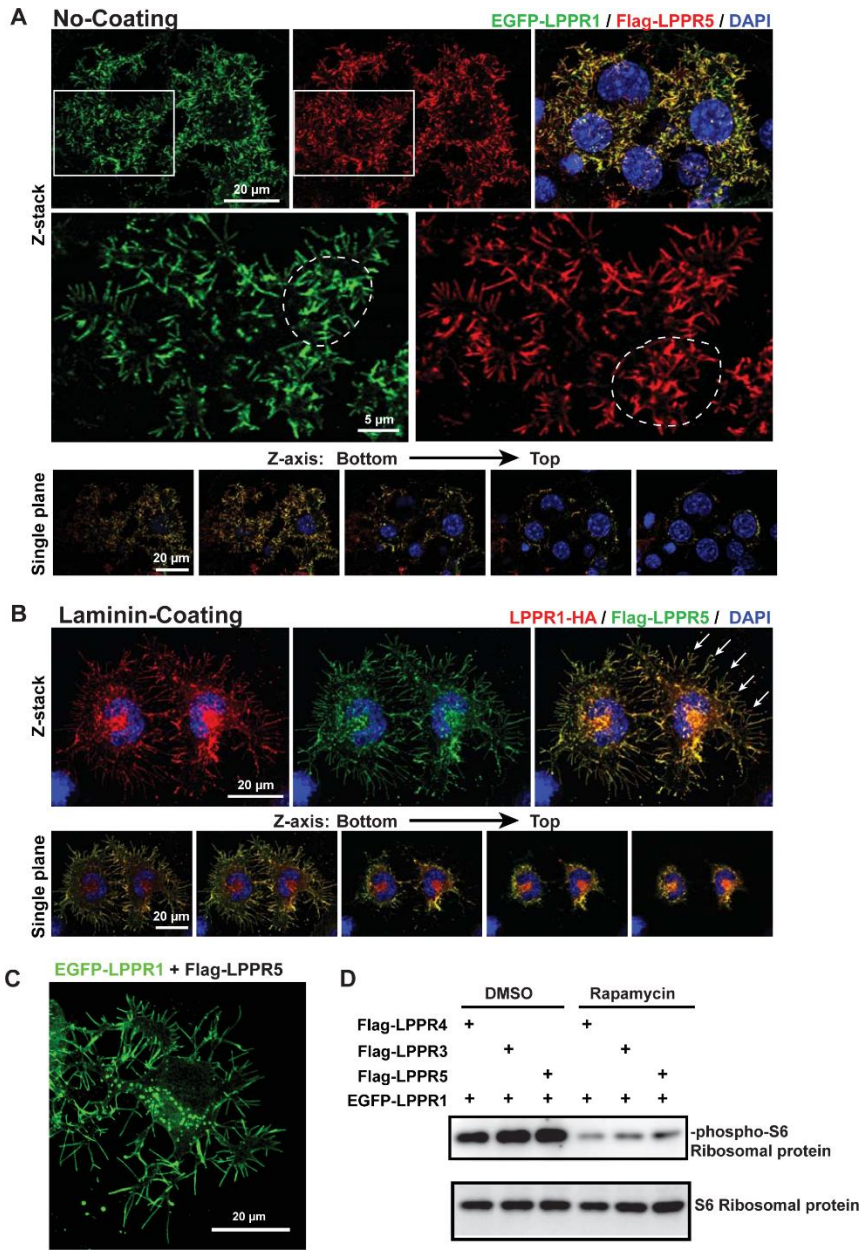

Fig. S4. Characterization of the LPPR-induced membrane protrusions. (A and B) Laminin-coating changed the orientation of LPPR-induced membrane protrusions. Z-stack and corresponding single plane image serial (from bottom to top) of EGFP-LPPR1 and Flag-LPPR5 co-transfected Neuro2A cells growing on uncoated surface (A), or on Laminin-coated surface (B). When cells were plated on uncoated surface, lots of protrusions are generated from the ventral surface and extend downward towards the culture surface in random orientation. While cells were on laminin-coated surface, their protrusions mostly extended outward towards cell periphery (arrows in B). (C and D) Blocking mTOR pathway with rapamycin showed little effect on the protrusion formation induced by LPPRs. Representative of EGFP-LPPR1 and Flag-LPPR5 cotransfected Neuro2A cells treated with 100 nM rapamycin (C). Western blot result showing the phosphorylation of S6 ribosomal protein was blocked by rapamycin (D).

Table S1

Click here to Download Table S1

Table S2

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Table S3

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