

Supplementary Figures

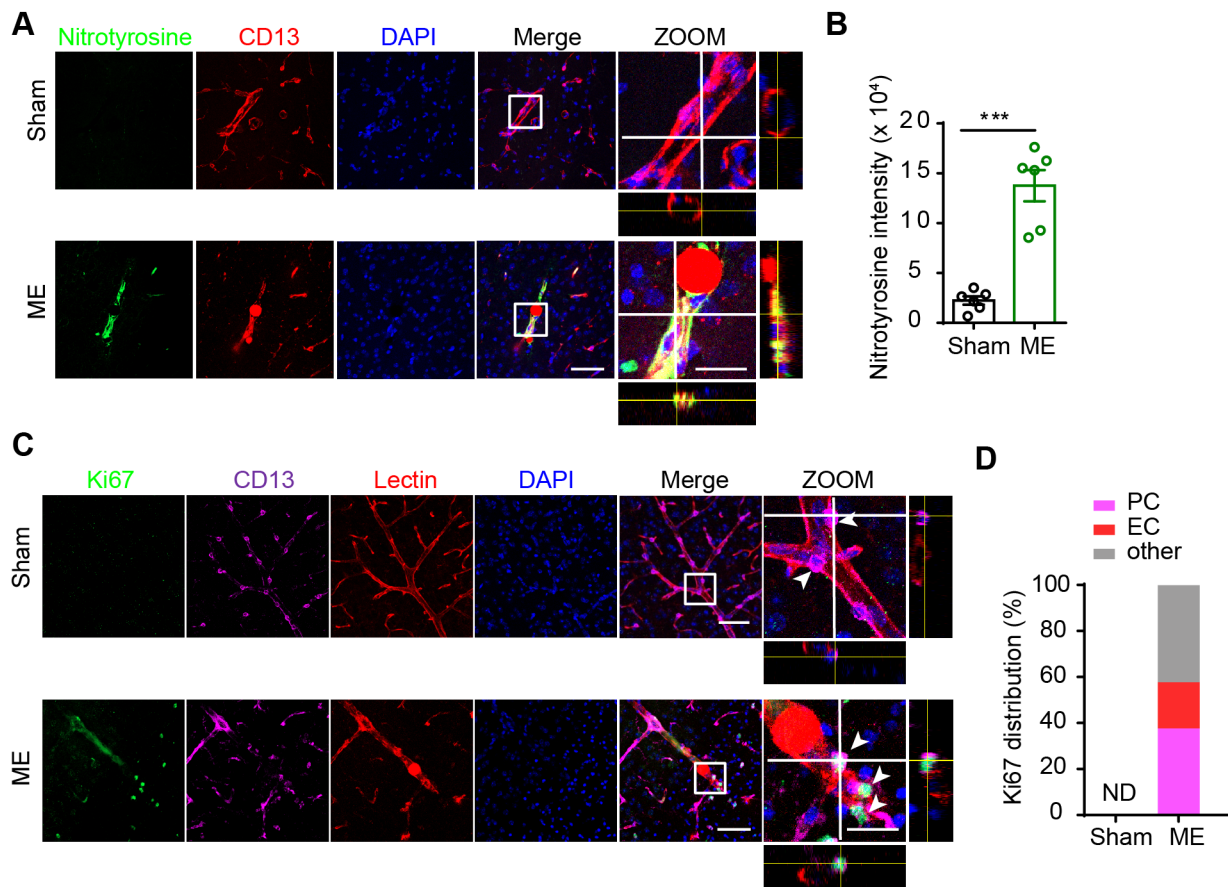


Figure S1. Nitrosative stress observed in the vessel after ME induction. (A) Maximum intensity projections of confocal images from brain sections stained for Nitrotyrosine (green), CD13 (red) and DAPI (blue, nuclei) at 72 h following ME. The images on the right are magnifications of the boxed regions in the images. Scale bar: 50 μm , magnified images, 20 μm . **(B)** Signal intensities of Nitrotyrosine staining quantified from **A** (dot plot, Sham: $n = 6$; ME: $n = 6$). **(C)** Maximum intensity projections of confocal images from brain sections stained for Ki67 (green), CD13 (magenta), Lectin (red) and DAPI (blue, nuclei) at 72 h following ME. The images on the right are magnifications of the boxed regions in the images. Scale bar: 50 μm , magnified images, 20 μm . The arrowheads indicate pericytes. **(D)** Quantification of

the percentage of Ki67 in pericytes, endothelial cells and other cells (Sham: n = 6; ME: n = 6). *** $P < 0.001$ vs. control. The data are presented as the mean \pm s.e.m. Statistical significance was determined by unpaired Student's t-test. ND, not detected.

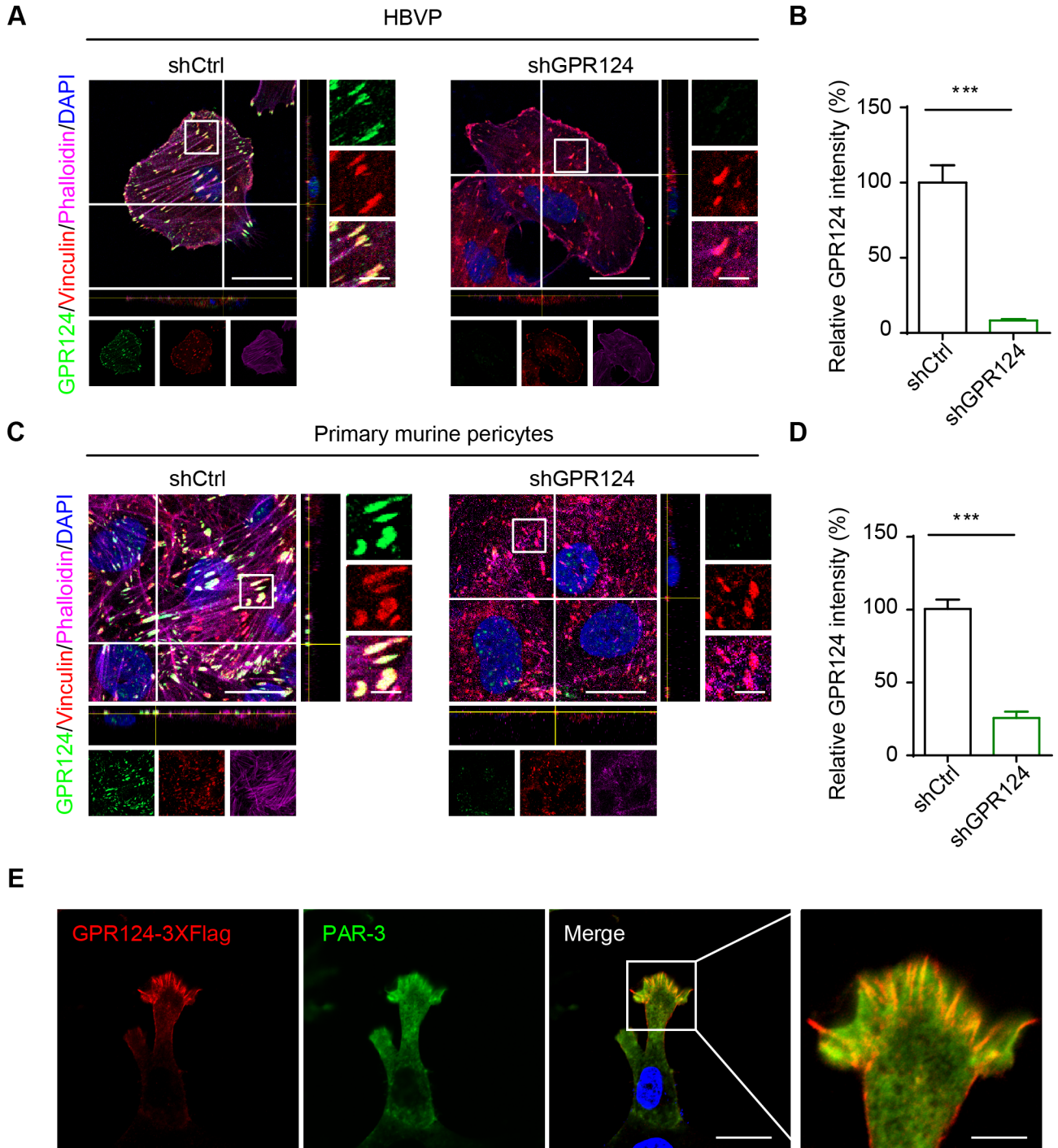


Figure S2. Representative images for knockdown and overexpression of GPR124

in pericytes. (A) Knockdown of GPR124 expression in HBVPs infected with either shCtrl or shGPR124 lentiviruses for 72 h. Cells were stained with GPR124 (red), Vinculin (green), Phalloidin (magenta) and DAPI (blue, nuclei). Scale bar: 20 μ m (right), magnified images, 5 μ m. (B) Signal intensities of GPR124 staining quantified from A (shCtrl: n = 20; shGPR124: n = 22). (C) Knockdown of GPR124 expression in primary mouse brain pericytes infected with either shCtrl or shGPR124 lentiviruses for 72 h. Cells were stained with GPR124 (red), Vinculin (green), Phalloidin (magenta) and DAPI (blue, nuclei). Scale bar: 20 μ m (right), magnified images, 5 μ m. (D) Signal intensities of GPR124 staining quantified from C (shCtrl: n = 21; shGPR124: n = 22). (E) Confocal images of Flag (red), PAR-3 (green) and DAPI (blue, nuclei) staining in the bodies of HBVPs showing the colocalization of GPR124 and polarization protein PAR-3 in the movement of the front tip of the cell. Scale bar: 20 μ m (left) and 5 μ m (right). *** $P < 0.001$ vs. control. The data are presented as the mean \pm s.e.m.

Statistical significance was determined by unpaired Student's t-test.

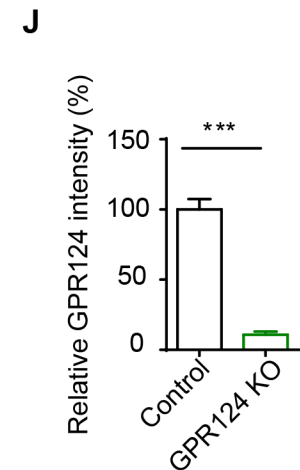
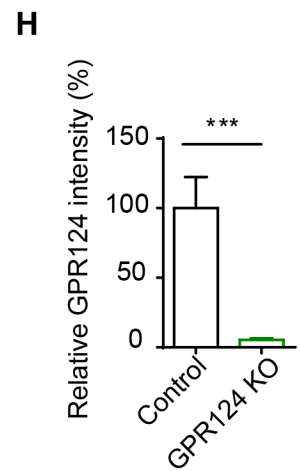
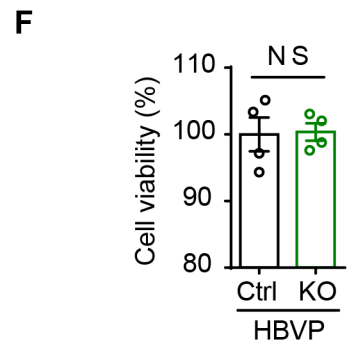
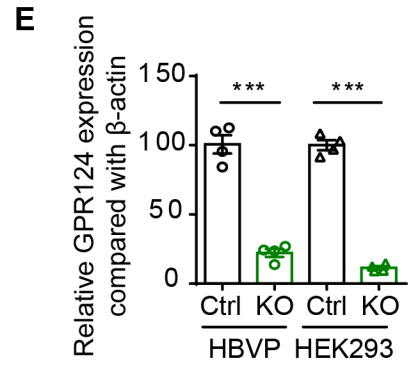
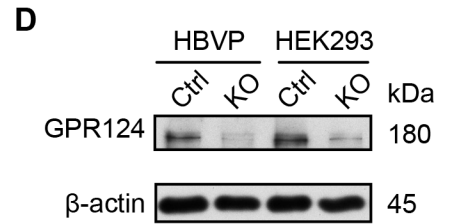
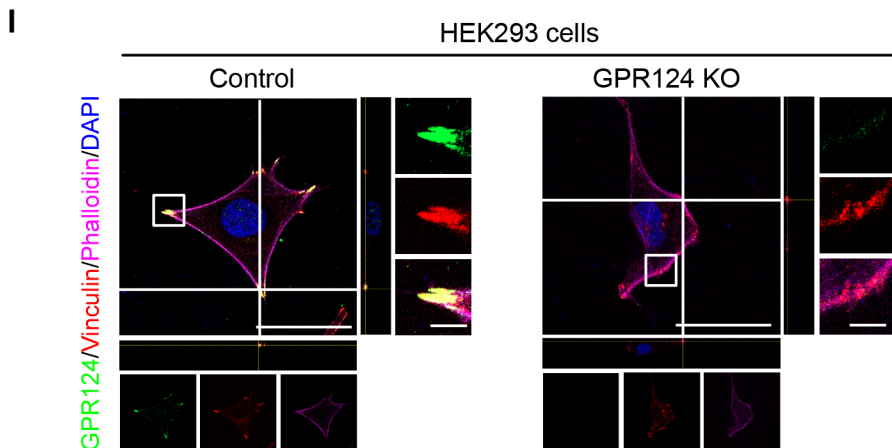
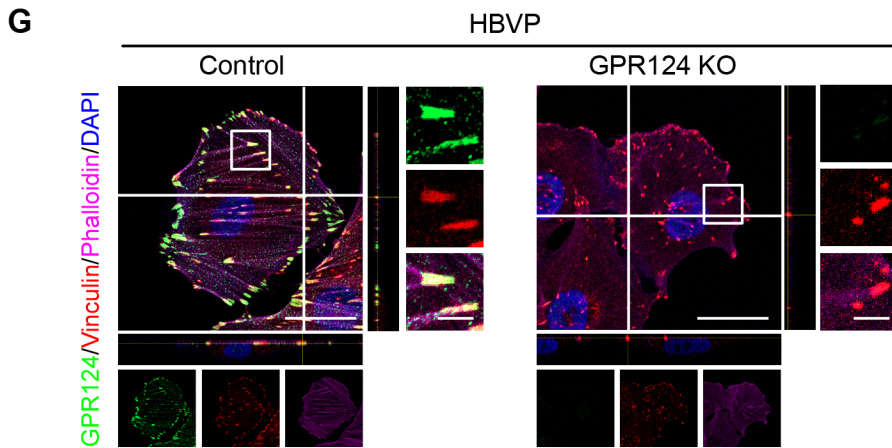
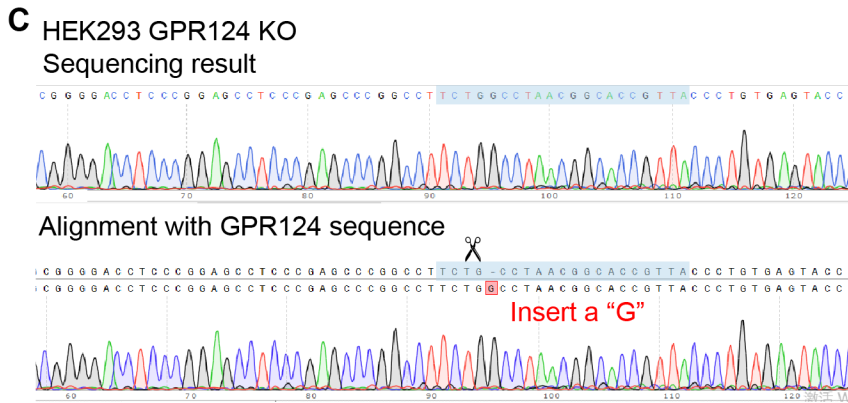
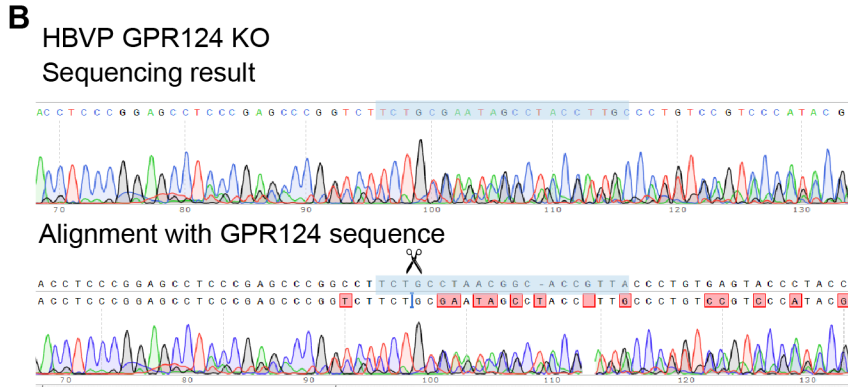


Figure S3. CRISPR-Cas9-mediated GPR124 deletion in HEK293 cells and HBVPs. (A) Schematic of the Cas9-sgRNA-targeting sites in mutant GPR124 gene. Blue lines label the sgRNA-targeting sequences. (B, C) DNA sequence of PCR products amplified from the GPR124 gene of HBVPs (B) or HEK293 cells (C) aligns to the reference sequence. A "G" was inserted in the sequence of the GPR124 gene in HEK293 cell individual clones. Insertions are indicated with red labels. Lots of overlapped peaks were present in the sequences of PCR products amplified from the GPR124 gene in HBVP multiple clones. (D) Immunoblot analysis of GPR124 proteins level in Ctrl-HBVPs, GPR124 KO-HBVPs, Ctrl-HEK293 cells and GPR124 KO-HEK293 cells to confirm depletion of the corresponding proteins. (E) Quantitative analyses of the results as shown in D and presented in the bar graph as the densitometry ratio of the control from four independent experiments. (F) Cell viability was measured by the CCK8 assay in Ctrl-HBVPs and GPR124 KO-HBVPs. The data within each group were normalized to the viability of Ctrl (n = 4). (G-J) Confocal images of GPR124 (green), Vinculin (red), Phalloidin (magenta) and DAPI (blue, nuclei) staining in Ctrl-HBVPs and GPR124 KO-HBVPs (G), Ctrl-HEK293 cells and GPR124 KO-HEK293 cells (I). Scale bar: 40 μ m, magnified images, 5 μ m. H and J showed that signal intensities of GPR124 staining quantified from G and I, respectively (H, n = 12; J, n = 14). *** $P < 0.001$ vs. control. The data are presented as the mean \pm s.e.m. Statistical significance was determined by unpaired Student's t-test. NS, not statistically significant.

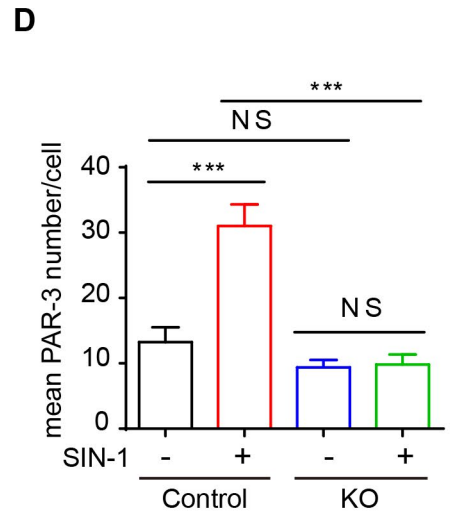
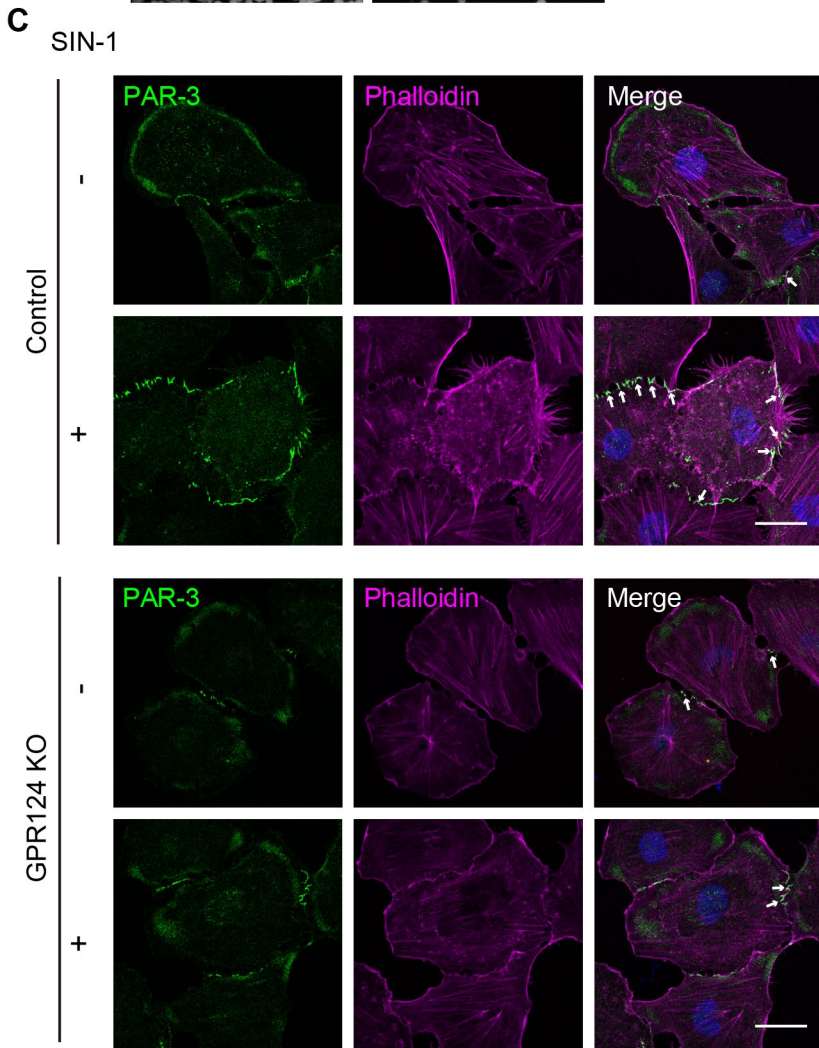
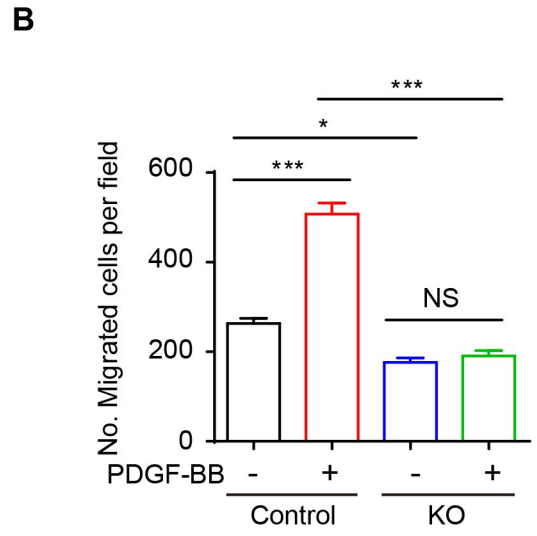
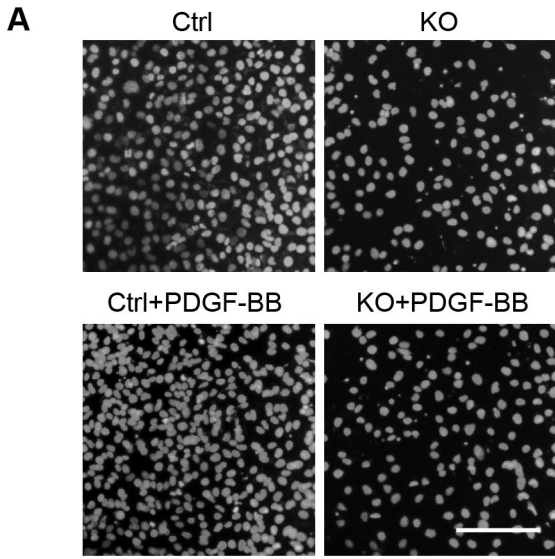


Figure S4. GPR124 regulates cell polarity in HBVPs upon nitrosative stress. (A)

Role of GPR124 on the migratory response of HBVPs to PDGF-BB was detected by a transwell invasion assay. PDGF-BB, 100 ng/mL. Scale bar: 200 μ m. **(B)**

Quantification of the number of migrated cell per field. Data are expressed as mean \pm s.e.m. from three independent experiments (n = 3). **(C)** Confocal images of PAR-3

(green), Phalloidin (magenta) and DAPI (blue, nuclei) staining in the normal HBVPs or GPR124-KO HBVPs. The cells were seeded on glass-bottom 24-well plates overnight and then stimulated with or without SIN-1 (0.5 mM, 12 h). The distribution of PAR-3 at the cell edges is indicated by arrows. Scale bar: 20 μ m. **(D)**

Quantification of PAR-3-labeled filopodia for mean filopodia number per cell (n = 20).

* $P < 0.05$ and *** $P < 0.001$ vs. control. The data are presented as the mean \pm s.e.m.

Statistical significance was determined by one-way ANOVA with Dunnett's multiple comparisons test. NS, not statistically significant.

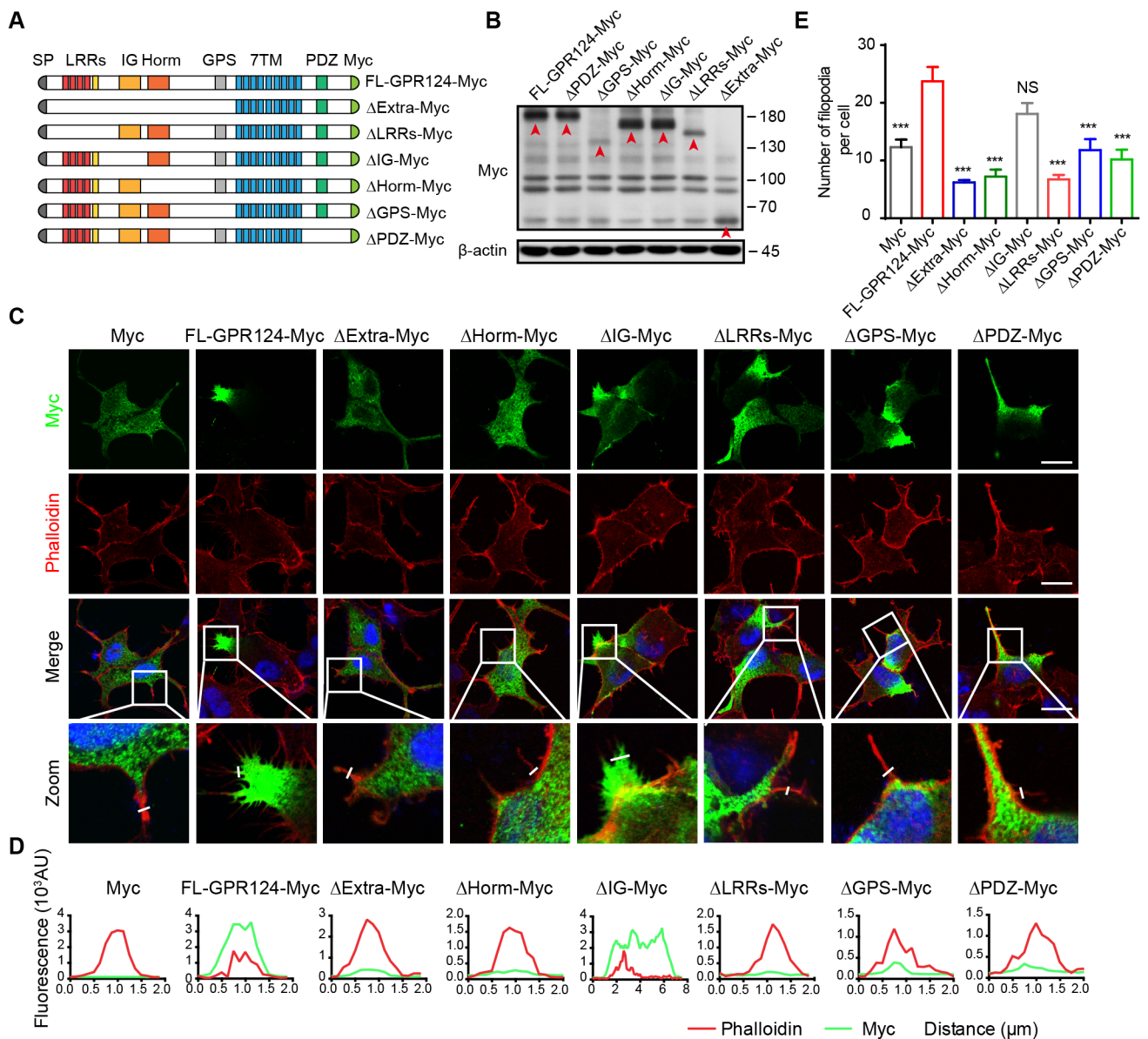


Figure S5. Essential domain of GPR124 required for SIN-1-induced migration in HEK293 cells. (A) Schematic diagram of full-length (FL) GPR124 and the N-terminal or C-terminal deletion mutants. (B) Immunoblot analysis of FL-GPR124-Myc or Myc-tagged GPR124 truncation mutants produced by transfection of HBVPs. Actin serves as a loading control. Molecular weight markers are indicated at the left. (C) Confocal images of Myc (green), phalloidin (red) and

DAPI (blue) stained HEK293 cells. GPR124-KO HEK293 cells transfected with FL-GPR124-Myc or Myc-tagged GPR124 truncation mutants as indicated. Cells were plated on coverslips 12 h before plasmid transfection and then incubated with SIN-1 (0.5 mM, 4 h). Single channels are shown in rows 1 and 2. The 4th row shows higher-magnification images of the boxed regions in the 3rd row. Scale bar: 50 μm (1st, 2nd and 3rd rows) and 10 μm (4th row). **(D)** Plots of the fluorescence intensity in arbitrary units (AU) along the solid lines indicated in the high-magnification images in **C**. **(E)** Quantification of the number of filopodia per cell (n = 10, 11, 19, 19, 12, 11, 15, 11). *** $P < 0.001$ vs. FL-GPR124-Myc. The data are presented as the mean \pm s.e.m. Statistical significance was determined by unpaired Student's t-test. NS, not statistically significant.

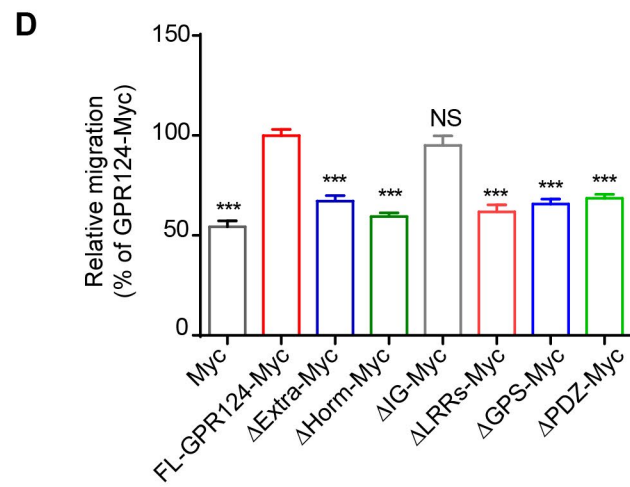
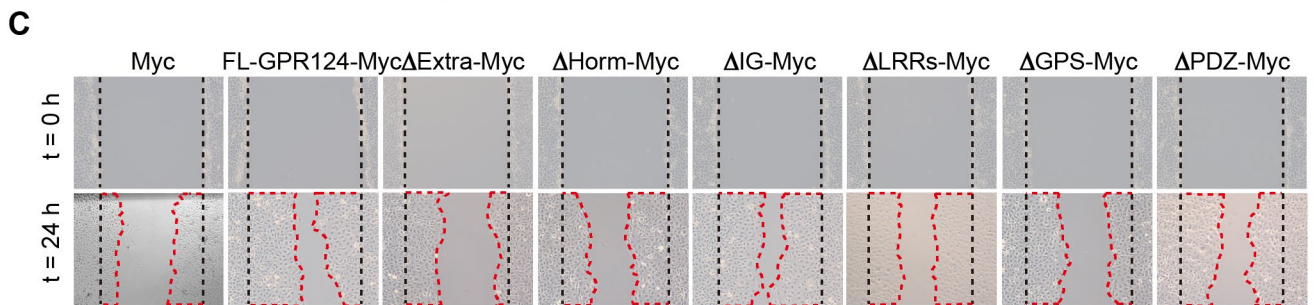
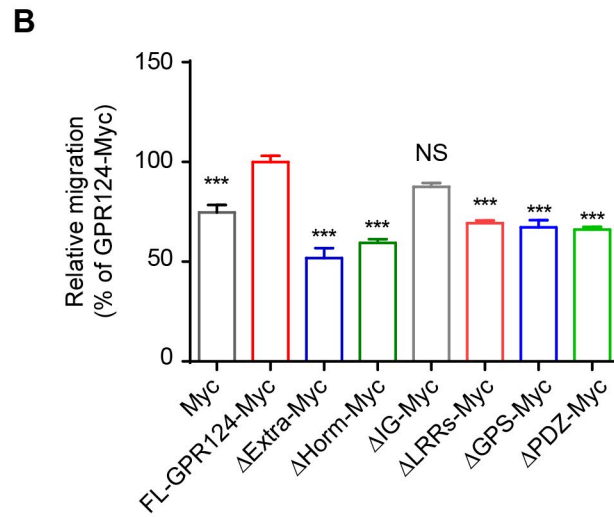
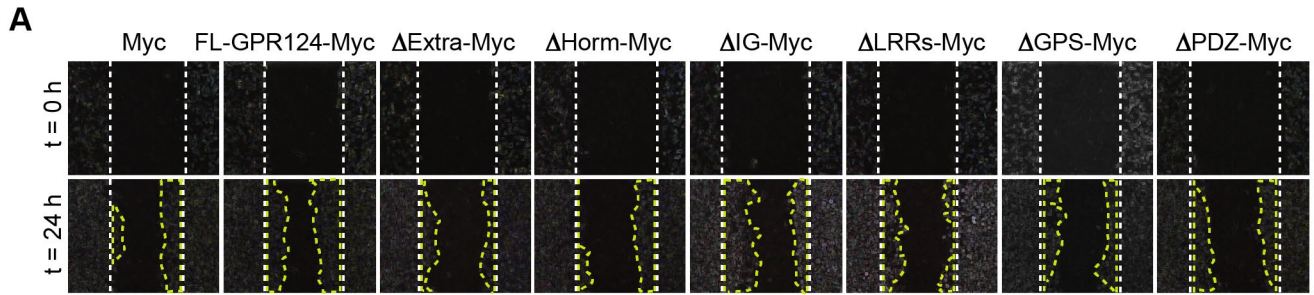


Figure S6. GPR124 regulates HBVPs migration through Horm domain. (A)

Representative images of GPR124-KO HEK293 cells transfected with FL-GPR124-Myc or Myc-tagged GPR124 truncation mutants at the 0 h and 24 h of the wound healing assay. The dashed lines (white) indicate the edges of the wound immediately after scratch. The dashed lines (yellow) outline the area of cell migration.

(B) Quantification of relative migration by control and GPR124 truncates in a wound healing assay after 24 h (normalized to FL-GPR124-Myc values; n = 4 in each group).

(C) Representative images of GPR124-KO HEK293 cells transfected with FL-GPR124-Myc or Myc-tagged GPR124 truncation mutants at the 0 h and 24 h of the wound healing assay. The dashed lines (black) indicate the edges of the wound immediately after scratch. The dashed lines (red) outline the area of cell migration. **(D)**

Quantification of relative migration by control and GPR124 truncates in a wound healing assay after 24 h (normalized to FL-GPR124-Myc values; n = 4 in each group).

*** $P < 0.001$ vs. FL-GPR124-Myc. The data are presented as the mean \pm s.e.m.

Statistical significance was determined by unpaired Student's t-test. NS, not statistically significant.

Table S1

Table. Mean, s.e.m. and statistical analyses for data presented in Figures 1-8, Related to Figures 1-8.						
FIGURE NUMBER	TEST USED	n		DESCRIPTIVE STATS (AVERAGE, VARIANCE)	P VALUE	DEGREES OF FREEDOM & F/t/z/R/ETC VALUE
Figure	WHICH TEST?	EXACT VALUE	DEFINED?	REPORTED?	EXACT VALUE	VALUE
1B	unpaired two-tailed Student's t-test	4, 4	Mice	error bars are mean +/- SEM	P = 0.0015	
1C	unpaired two-tailed Student's t-test	4, 4	Mice	error bars are mean +/- SEM	P = 0.0089	
1D	unpaired two-tailed Student's t-test	4, 4	Mice	error bars are mean +/- SEM	P = 0.5816	
1E	unpaired two-tailed Student's t-test	4, 4	Mice	error bars are mean +/- SEM	P = 0.0575	
1I	unpaired two-tailed Student's t-test	6, 6	Mice	error bars are mean +/- SEM	P = 0.0034	
4C	unpaired two-tailed Student's t-test	4, 4	experiments	error bars are mean +/- SEM	P < 0.0001	
4F	unpaired two-tailed Student's t-test	4, 4, 4	experiments	error bars are mean +/- SEM	P < 0.0001; P < 0.0001	
4G	unpaired two-tailed Student's t-test	4, 4	experiments	error bars are mean +/- SEM	P = 0.3079	
4I	unpaired two-tailed Student's t-test	4, 4	experiments	error bars are mean +/- SEM	P < 0.0001; P < 0.0001	
4M	one-way ANOVA followed by a	4, 4,4,4,4,4	experiments	error bars are mean +/- SEM	P < 0.0001	F (5, 18) = 10.39

	Dunnett's Multiple Comparisons Test					
4N	one-way ANOVA followed by a Dunnett's Multiple Comparisons Test	3, 3,3,3,3,3	experiments	error bars are mean +/- SEM	P = 0.0002	F (5, 12) = 12.18
5B	one-way ANOVA followed by a Dunnett's Multiple Comparisons Test	9, 18, 9, 9	cell	error bars are mean +/- SEM	P < 0.0001	F (3, 41) = 48.8
5C	one-way ANOVA followed by a Dunnett's Multiple Comparisons Test	11, 16, 11, 10	cell	error bars are mean +/- SEM	P < 0.0001	F (3, 44) = 17.79
5E	one-way ANOVA followed by a Dunnett's Multiple Comparisons Test	12, 12, 12, 12	cell	error bars are mean +/- SEM	P < 0.0001	F (3, 44) = 69.8
5F	one-way ANOVA followed by a Dunnett's Multiple Comparisons Test	12, 30, 12, 11	cell	error bars are mean +/- SEM	P = 0.0014	F (3, 64) = 5.804
5H	one-way ANOVA followed by a Dunnett's Multiple	18, 15, 12, 9	cell	error bars are mean +/- SEM	P = 0.0415	F (3, 50) = 2.999

	Comparisons Test					
5I	one-way ANOVA followed by a Dunnett's Multiple Comparisons Test	12, 17, 11, 19	cell	error bars are mean +/- SEM	P < 0.0001	F (3, 54) = 8.615
7I	unpaired two-tailed Student's t-test	26, 26, 26, 26, 26, 26	cell	error bars are mean +/- SEM		
				Myc vs. FL-GPR124-Myc ΔExtra-Myc vs. FL-GPR124-Myc ΔHorm-Myc vs. FL-GPR124-Myc ΔIg-Myc vs. FL-GPR124-Myc ΔLRRMyc vs. FL-GPR124-Myc ΔGPS-Myc vs. FL-GPR124-Myc ΔPDZ-Myc vs. FL-GPR124-Myc	P < 0.0001 P < 0.0001 P < 0.0001 P = 0.3027 P = 0.1451 P = 0.7321 P = 0.6139	
7J	unpaired two-tailed Student's t-test	20, 20, 20, 20, 20, 20, 20	cell	error bars are mean +/- SEM		
				Myc vs. FL-GPR124-Myc ΔExtra-Myc vs. FL-GPR124-Myc ΔHorm-Myc vs. FL-GPR124-Myc ΔIg-Myc vs. FL-GPR124-Myc ΔLRRMyc vs. FL-GPR124-Myc ΔGPS-Myc vs. FL-GPR124-Myc ΔPDZ-Myc vs. FL-GPR124-Myc	P < 0.0001 P < 0.0001 P < 0.0001 P = 0.4953 P < 0.0001 P < 0.0001 P < 0.0001	

8B	one-way ANOVA followed by a Dunnett's Multiple Comparisons Test	3, 3, 3, 3	experiments	error bars are mean +/- SEM	P = 0.0008	F (3, 8) = 17.04
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Table. Mean, s.e.m. and statistical analyses for data presented in Supplementary Figures 1-6, Related to Supplementary Figures 1-6.

FIGURE NUMBER	TEST USED	n		DESCRIPTIVE STATS (AVERAGE, VARIANCE)	P VALUE	DEGREES OF FREEDOM & F/t/z/R/ETC VALUE
Figure	WHICH TEST?	EXACT VALUE	DEFINED?	REPORTED?	EXACT VALUE	VALUE
S1B	unpaired two-tailed Student's t-test	6, 6	mice	error bars are mean +/- SEM	P < 0.0001	
S2B	unpaired two-tailed Student's t-test	20, 22	cell	error bars are mean +/- SEM	P = 0.0002	
S2D	unpaired two-tailed Student's t-test	21, 22	cell	error bars are mean +/- SEM	P < 0.0001	
S3E	unpaired two-tailed Student's t-test	4, 4, 4, 4	experiments	error bars are mean +/- SEM	P < 0.0001; P < 0.0001	
S3F	unpaired two-tailed Student's t-test	4, 4	experiments	error bars are mean +/- SEM	P = 0.9010	
S3H	unpaired two-tailed Student's t-test	12, 12	cell	error bars are mean +/- SEM	P < 0.0001	
S3J	unpaired two-tailed Student's t-test	14, 14	cell	error bars are mean +/- SEM	P < 0.0001	
S4B	one-way ANOVA followed by a	3, 3, 4, 3	experiments	error bars are mean +/- SEM	P < 0.0001	F (3, 9) = 99.45

	Dunnett's Multiple Comparisons Test					
S4D	one-way ANOVA followed by a Dunnett's Multiple Comparisons Test	20, 20, 20, 20	cell	error bars are mean +/- SEM	P < 0.0001	F (3, 76) = 50.29
S5E	unpaired two-tailed Student's t-test	10, 11, 19, 19, 12, 11, 15, 11	cell	error bars are mean +/- SEM		
				Myc vs. FL-GPR124-Myc ΔExtra-Myc vs. FL-GPR124-Myc ΔHorm-Myc vs. FL-GPR124-Myc ΔIg-Myc vs. FL-GPR124-Myc ΔLRRMyc vs. FL-GPR124-Myc ΔGPS-Myc vs. FL-GPR124-Myc ΔPDZ-Myc vs. FL-GPR124-Myc	P = 0.0009 P < 0.0001 P < 0.0001 P = 0.0828 P < 0.0001 P = 0.0007 P = 0.0002	
S6B	unpaired two-tailed Student's t-test	4, 4, 4, 4, 4, 4, 4, 4	experiments	error bars are mean +/- SEM		
				Myc vs. FL-GPR124-Myc ΔExtra-Myc vs. FL-GPR124-Myc ΔHorm-Myc vs. FL-GPR124-Myc ΔIg-Myc vs. FL-GPR124-Myc	P < 0.0001 P = 0.0002 P < 0.0001 P = 0.0001	

				FL-GPR124-Myc ΔLRRMyc vs. FL-GPR124-Myc ΔGPS-Myc vs. FL-GPR124-Myc ΔPDZ-Myc vs. FL-GPR124-Myc	0.4192 P < 0.0001 P = 0.0001 P = 0.0001	
S6D	unpaired two-tailed Student's t-test	4, 4, 4, 4, 4, 4, 4, 4	experiments	error bars are mean +/- SEM		
				Myc vs. FL-GPR124-Myc ΔExtra-Myc vs. FL-GPR124-Myc ΔHorm-Myc vs. FL-GPR124-Myc ΔIg-Myc vs. FL-GPR124-Myc ΔLRRMyc vs. FL-GPR124-Myc ΔGPS-Myc vs. FL-GPR124-Myc ΔPDZ-Myc vs. FL-GPR124-Myc	P < 0.0001 P = 0.0002 P < 0.0001 P = 0.2141 P < 0.0001 P = 0.0005 P < 0.0001	