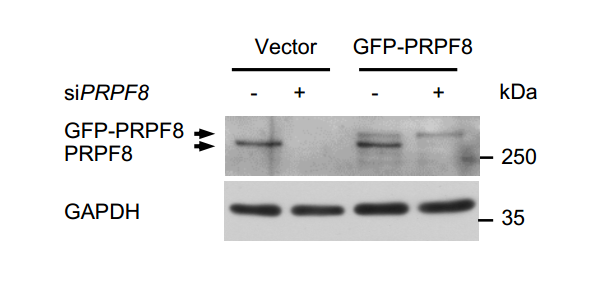
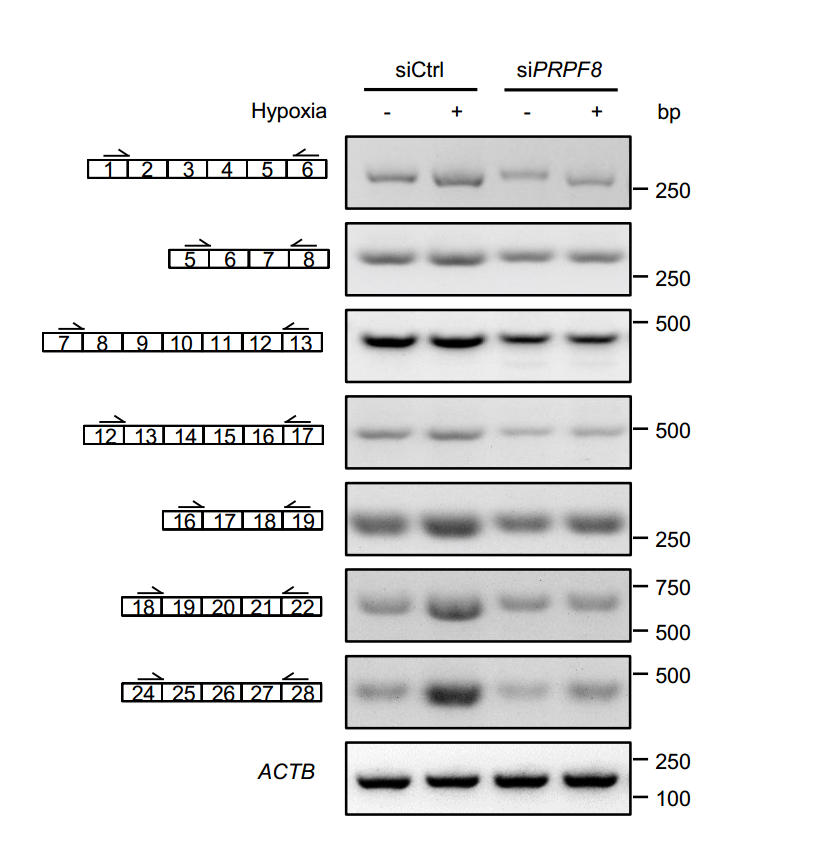


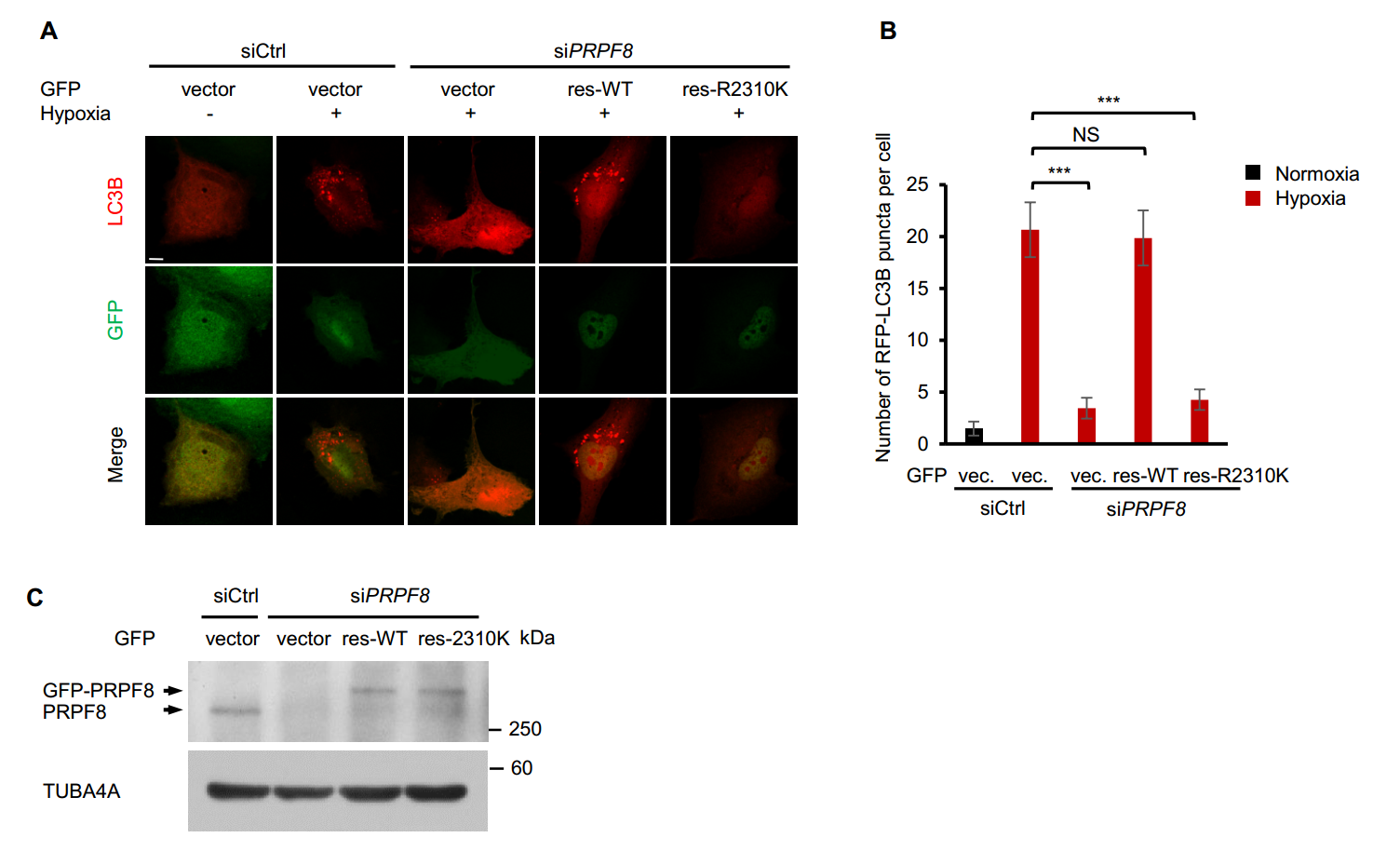
**Figure S1.** HeLa cells expressing mt-Keima can rapidly and sensitively response to pH changes. (**A**) Representative confocal images of HeLa cells expressing mt-Keima equilibrated in 1 or 10 minutes with different buffers at pH 4 and 7. (**B**) Quantification of relative mitophagy index in (Fig. 1A), n=3 independent experiments. Data are shown as the mean ± SD (One-way ANOVA, \*\*\*p < 0.001, NS, nonsignificant).(**C**) Layout of the RNAi screen conditions. HeLa cells stably expressing mt-Keima were simultaneously transfected with arrayed pools of siRNAs in 96-well plates. Following knockdown, cells were treated with hypoxia (1% O2) for 24 h to induce mitophagy. Scale bars: 10 μm.



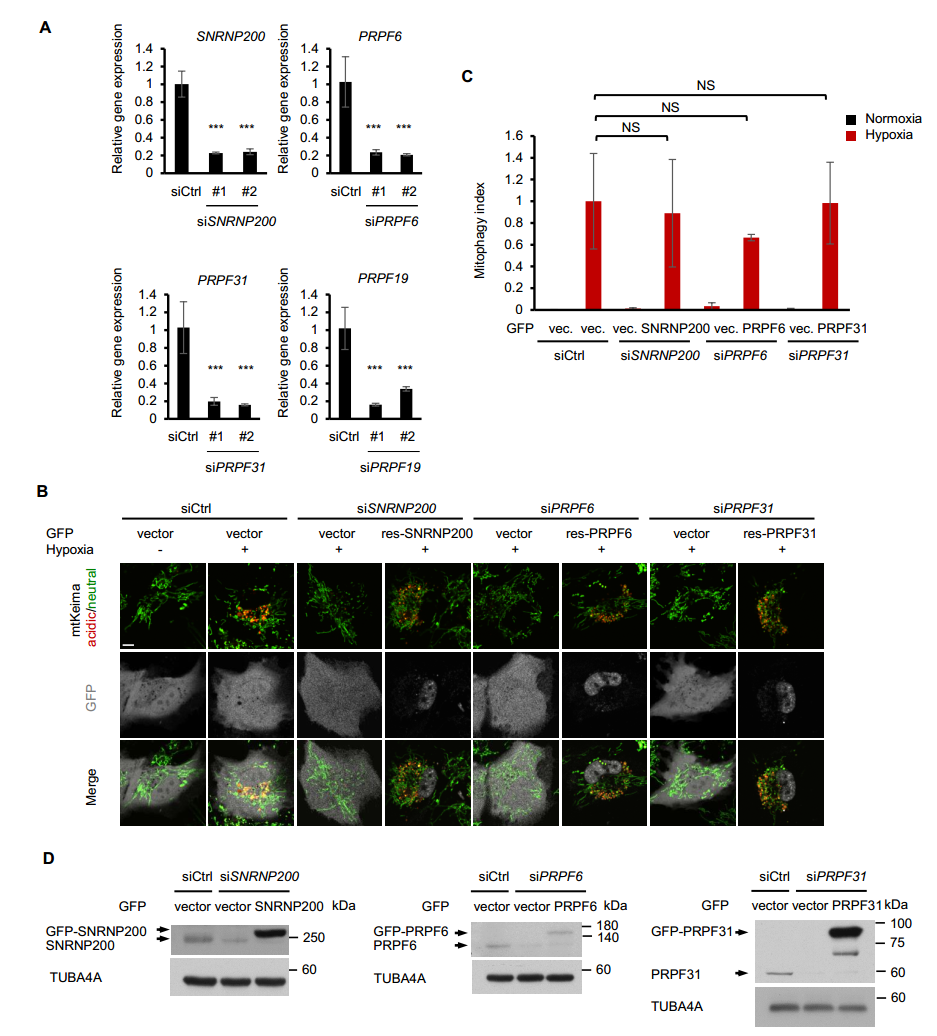
**Figure S2.** Western blot analysis of HeLa cells transfected with the indicated siRNAs and siRNA-resistant PRPF8-expressing plasmid.



**Figure S3.** Knockdown of PRPF8 does not affect other PCR products of *ULK1* mRNA. A representative gel picture shows bands corresponding to spliced products of *ULK1* mRNA in HeLa cells transfected with the indicated siRNAs during normoxia or hypoxia (1% O2, 24 h) in RT-PCR assays. Each product is confirmed by DNA sequencing. *ACTB* was used as a loading control.



**Figure S4.** APRPF8 retinitis pigmentosa disease-associated mutant has defects in mitophagy in RPE cells.(**A**) Representative confocal images of RFP-LC3 signals in RPE cells transfected with the siRNAs and plasmids indicated as shown under normoxia or hypoxia (1% O2) for 12 h. (**B**) Quantification of the number of RFP-LC3 puncta per cell in (B). n=3 independent experiments. Data are shown as the mean ± SD (One-way ANOVA, \*\*\*p < 0.001, NS, nonsignificant). Scale bars: 10 μm. (**C**) Western blot analysis of RPE cells transfected with the indicated siRNAs and siRNA-resistant PRPF8-expressing plasmids.



**Figure S5.** Retinitis pigmentosa-associated spliceosomal proteins regulate mitophagy under hypoxia in RPE cells. (**A**) Quantitative real-time PCR analysis of mRNA levels for the indicated genes in HeLa cells. Data are shown as the mean ± SD from a representative of 3 independent experiments. (**B**) mt-Keima imaging in RPE cells transfected with *SNRNP200*, *PRPF6* or *PRPF31* siRNAs and GFP-tagged siRNA-resistant plasmids after hypoxia treatment (1% O2, 24 h). GFP fluorescence is depicted in gray. (**C**) Quantification of mitophagy index of GFP-positive cells in (B). Mitophagy index in hypoxia-treated control cells was normalized to 1. n=3 independent experiments. (**D**) Western blot analysis of RPE cells transfected with the indicated siRNAs and siRNA-resistant plasmids. Data are shown as the mean ± SD (One-way ANOVA, \*\*\*p < 0.001, NS, nonsignificant). Scale bars: 10μm.

**Table S1.** siRNA duplexes used in this study.

|  |  |  |
| --- | --- | --- |
| Gene symbol | siRNA no. | Target sequences (5'-3') |
| Negative Control |  | UUCUCCGAACGUGUCACGU |
| *PRPF8* | #1 | CACGUAUCAAGAUUGGACU |
|  | #2 | GGAUUAUGAUGCGCCGAGA |
|  | #3 | CUCAUGAAACAUGAUGUUA |
| *SNRNP200* | #1 | GACUAUUUGUGCAGAGUUU |
|  | #2 | GACAUAUGUGGGUAUCACA |
| *PRPF6* | #1 | GAGAAGAUUGGGCAGCUUA |
|  | #2 | GACAGUUGUGUAGCCCACA |
| *PRPF31* | #1 | CGUAUGAGCUUCGGAGAGA |
|  | #2 | CUGAGUUCCUCAAGGUCAA |
| *Prpf8* (mouse) |  | CAGAUCAUUGUCACUCGGA |

**Table S2.** Primers for *ULK1* splicing analysis in RT-PCR assays.

|  |  |  |
| --- | --- | --- |
|  | Primer name | Target sequences (5'-3') |
| E1-E6 | Forward | TCTTCAAGGGCCGCCACC |
|  | Reverse | GTGTCCTCGCTCAGCGTGC |
| E5-E8 | Forward | GGGACCTGGCCGACTACC |
|  | Reverse | AGTGCTGGGACATGATGACC |
| E7-E13 | Forward | CTCTGCGGCTCCCCCATG |
|  | Reverse | CTGCAGCTGCTGCATCTCG |
| E12-E17 | Forward | CTCCACCTCCCACCTGGCC |
|  | Reverse | TGCCTGACCTGCGGATGG |
| E16-E19 | Forward | CCCACCCAGTTCCAAACACC |
|  | Reverse | GAGTGCTCGGGTGCAGAGG |
| E18-E22 | Forward | TGGCAGGTCCCCTCGTCC |
|  | Reverse | CCTCCAAAGCCAGCTGAGG |
| E21-E25 | Forward | GCACGGAGAGCCTGCAGGAGAAGCC |
|  | Reverse | GGTACAGCACCAGCTGTTCC |
| E24-E28 | Forward | CCTGCTGAGCCGAGAATGG |
|  | Reverse | TCTCCGCTCAATGCACAGC |

**Table S3.** Primers for quantitative PCR used in this study.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Gene name | Primer name | Target sequence (5'-3') | Gene name | Primer name | Target sequence (5'-3') |
| *PRPF8* | Forward | TGACAAAAGGGTTTACTTGGGTG | *MTOR* | Forward | ATGCTTGGAACCGGACCTG |
|  | Reverse | CTCATTGACGAAGGAAATGGCT |  | Reverse | TCTTGACTCATCTCTCGGAGTT |
| *SNRNP200* | Forward | CCAAGCTGACCGTTCTCTCAT | *ULK1* | Forward | CAGAACTACCAGCGCATTGA |
|  | Reverse | GCCTTGTCTCCCATACGGG |  | Reverse | TCCACCCAGAGACATCTTCC |
| *PRPF6* | Forward | CCGTTGGGGACCAGATGAAG | *BECN1* | Forward | TCCACAGAAAGTGCCAACAG |
|  | Reverse | GGCGTTCCATACGATATTTCTCT |  | Reverse | GACGTTGAGCTGAGTGTCCA |
| *PRPF31* | Forward | GAGATCGAAAACGAGCTGAACA | *ATG4A* | Forward | TGCTGGTTGGGGATGTATGC |
|  | Reverse | GTGACGCTGACGACCATGAT |  | Reverse | GCGTTGGTATTCTTTGGGTTGT |
| *PRPF19* | Forward | GGCACGGATGTCCAGATCTAC | *ATG5* | Forward | AAAGATGTGCTTCGAGATGTGT |
|  | Reverse | CACGCCAAGTTCATCGCTTC |  | Reverse | CACTTTGTCAGTTACCAACGTCA |
| *ATG9B* | Forward | CCCCTCATACAAGAAGCTCCC | *ATG7* | Forward | ATGATCCCTGTAACTTAGCCCA |
|  | Reverse | TGCAGGTTGAGCCTGTGTTG |  | Reverse | CACGGAAGCAAACAACTTCAAC |
| *ATG13* | Forward | AGACAGTTCGTGTTGGGACAG | *WIPI1* | Forward | AACAGGTCTATGTGCTCTCTCT |
|  | Reverse | CTCAAATTGCCTGGTAGACATGA |  | Reverse | CTCATGGGCAGCAATAGTGC |
| *ACTB* | Forward | AGAAAATCTGGCACCACACC | *ATG16L1* | Forward | AACGCTGTGCAGTTCAGTCC |
|  | Reverse | AGAGGCGTACAGGGATAGCA |  | Reverse | AGCTGCTAAGAGGTAAGATCCA |