Supplemental Figure 1

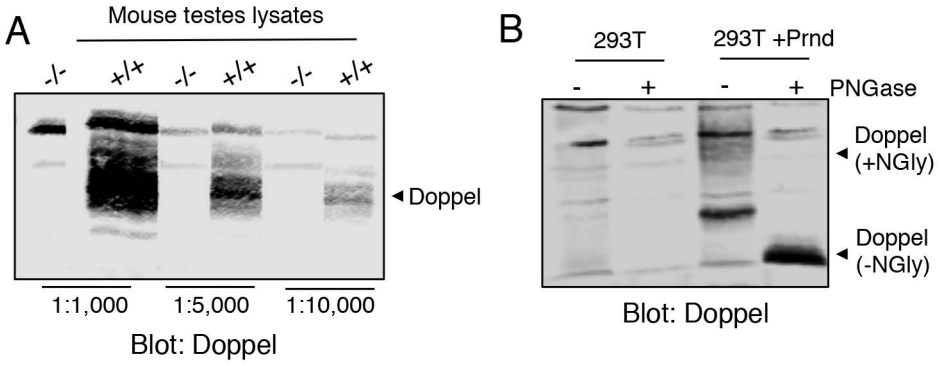



**Figure S1. Validation of an anti-Doppel rabbit polyclonal antibody. (A);** Doppel expression is confirmed in detergent-soluble lysates form wild type control (+/+) or knockout (Prnd-/-) testes by immunoblotting with anti-Doppel antisera at 1:1,000, 1:5,000 and 1,10,000 dilutions. The antibody detects a protein band in the wild type samples but not the Prnd-/- samples, which is likely due to non-specific antibody binding or expression of a related prion family member that is recognized by the anti-Doppel antibody. **(B);** Detergent-soluble lysates from HEK-293T cells infected with control pLOC lentivirus (-Prnd) or pLOC-PRND (+Prnd) expressing Doppel were treated with PNGase, revealing that N-deglycosylation of Doppel causes a shift in apparent molecular weight. The bands detected in the non-transfected lysate lanes are likely non-specific proteins bound by the anti-Doppel antibody.

Supplemental Figure 2

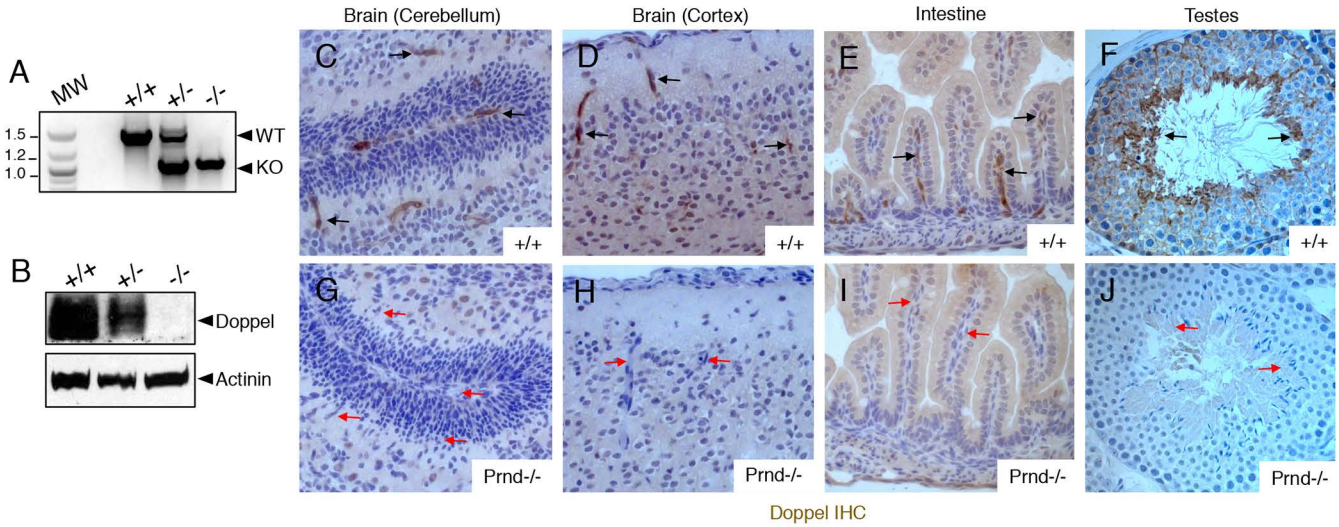
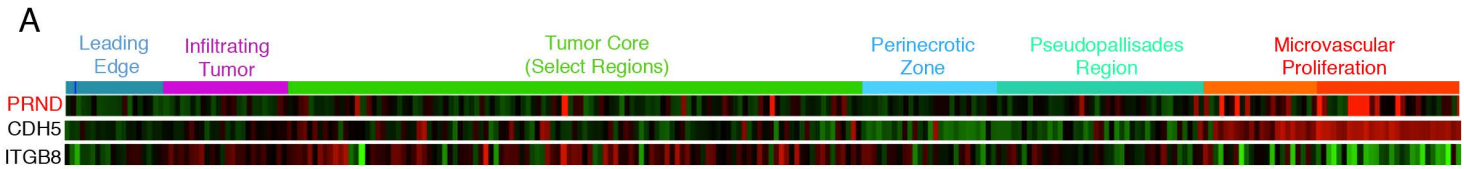



**Figure S2. Analysis of Doppel expression in wild type and Prnd-/- mice. (A);** PCR-based genotyping using genomic DNA isolated from tail samples from P30 Prnd wild type (+/+), heterozygous null (+/-) and homozygous null (-/-) mice. **(B);** Immunoblotting reveals absence of Doppel protein in testes lysates from homozygous null (Prnd-/-) mice in comparison to wild type (+/+) and heterozygous (+/-) null controls, supporting specificity of the anti-Doppel antibody. Also note the ~50% reduction of Doppel protein expression in heterozygous null (Prnd+/-) testes lysates as compared to wild type control (+/+) samples. **(C-J);** Formalin fixed and paraffin embedded sagittal brain sections from wild type (C-F) and Prnd-/- (G-J) mice were immunolabeled with anti-Doppel antibodies. Note that Doppel is expressed in vascular endothelial cells that comprise blood vessels in the cerebellum (C) and cerebral cortex (D) of the developing wild type brain. In wild type mice Doppel is also expressed in blood vessels within intestinal microvilli (E) and in Sertoli cells of testes (F). Black arrows indicate anti-Doppel immunoreactivity. Note the lack of Doppel expression in the Prnd-/- brain (G, H), intestine (I) and testes (J), demonstrating specificity for the anti-Doppel antibody.

Supplemental Figure 3

A




B

| Correlated Gene | Spearman's Correlation |
|---|---|
| FAM124B | 0.58 |
| LXN | 0.57 |
| PCDH12 | 0.57 |
| GRAP | 0.55 |
| TNFRSF4 | 0.54 |
| ACVRL1 | 0.53 |
| LRRC36 | 0.52 |
| ECSCR | 0.52 |
| CXORF36 | 0.52 |
| ESAM | 0.51 |
| DLL4 | 0.51 |
| PECAM1 | 0.51 |
| TM4SF18 | 0.51 |
| CD34 | 0.51 |
| CDH5 | 0.50 |

**Figure S3. PRND is expressed in angiogenic endothelial cells in human brain tumors. (A);** Analysis of the IVY-GAP database for human GBM reveals that PRND mRNA is expressed primarily in regions of pathological microvascular proliferation. PRND expression is similar to the bona fide vascular endothelial marker CDH5/VE-Cadherin, but unlike expression of ITGB8/β8 integrin which is present in regions of tumor cell infiltration and invasion. **(B);** Analysis of the cBioPortal human cancer database reveals that PRND mRNA is co-expressed with various well-characterized vascular endothelial cell markers, including PECAM1/CD31, CDH5/VE-Cadherin and DLL4. Bona fide vascular endothelial-expressed genes are indicated with red text. Genes co-expressed with PRND that lack published links to vascular endothelial cells are listed in black text.

Supplemental Figure 4

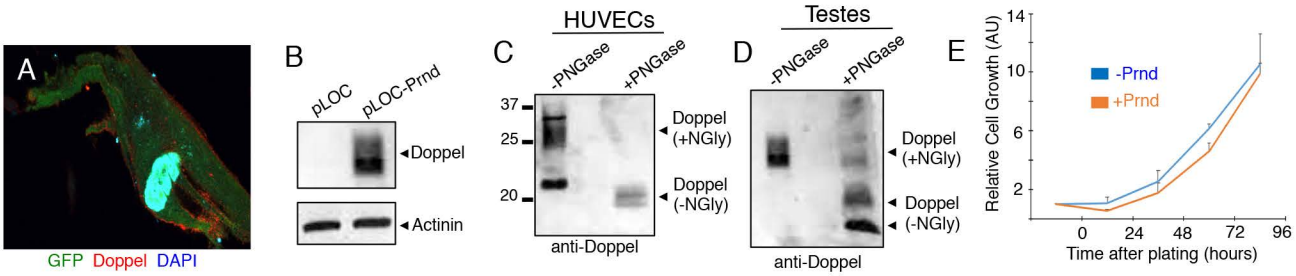



**Figure S4. Lentivirus-mediated expression of PRND/Doppel in HUVECs in vitro. (A);** HUVEC monolayers were infected with control pLOC lentivirus or pLOC lentivirus expressing PRND. Fixed and non-permeabilized cells were then analyzed for GFP (green) and Doppel (red) protein expression by double immunofluorescence. Note the Doppel protein expression in the HUVEC surface. **(B);** Validation of Doppel expression in HUVECs infected with pLOC-PRND by anti-Doppel immunoblotting of detergent-soluble lysates. **(C);** Detergent-soluble lysates from HUVECs expressing Doppel were treated with PNGase, revealing that N-deglycosylation of Doppel leads to a decreased apparent molecular weight.**(D);** As a comparative control for the PNGase treatment of HUVECs, detergent-soluble lysates from wild type mouse testes were treated with PNGase, revealing that deglycosylation induces a shift in the Doppel apparent molecular weight. **(E);** Proliferation of cultured HUVECs infected with control pLOC or pLOC-PRND were quantified every 24 hours for four days. There are no statistically significant differences in HUVEC proliferation. Similar results have been obtained for overall viability.

Supplemental Figure 5

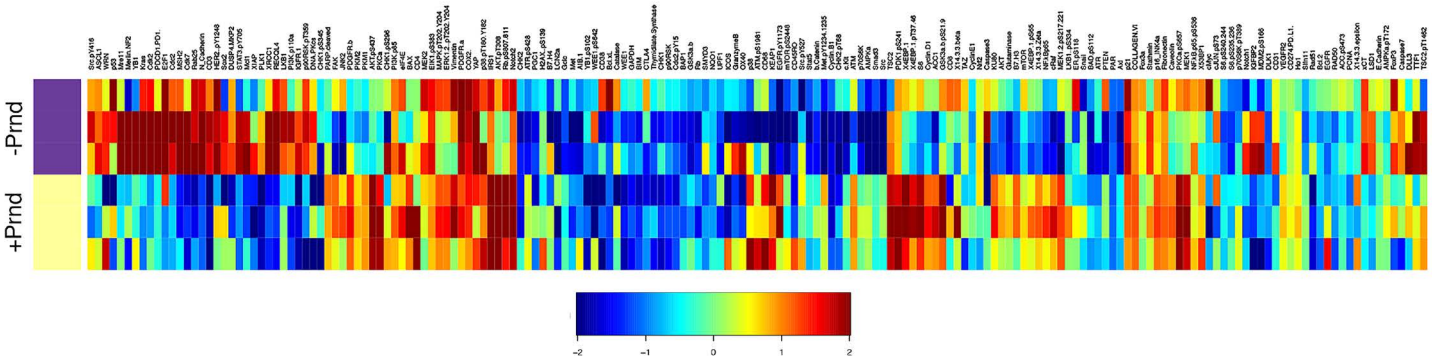



**Figure S5. Heat map showing Doppel-dependent differences in expression and/or phosphorylation of select signaling proteins based on RPPA analyses.** Detergent-soluble protein lysates rom control HBMECs (n=3) or HBMECs expressing PRND/Doppel (n=3) were analyzed by RPPA.

Supplemental Figure 6

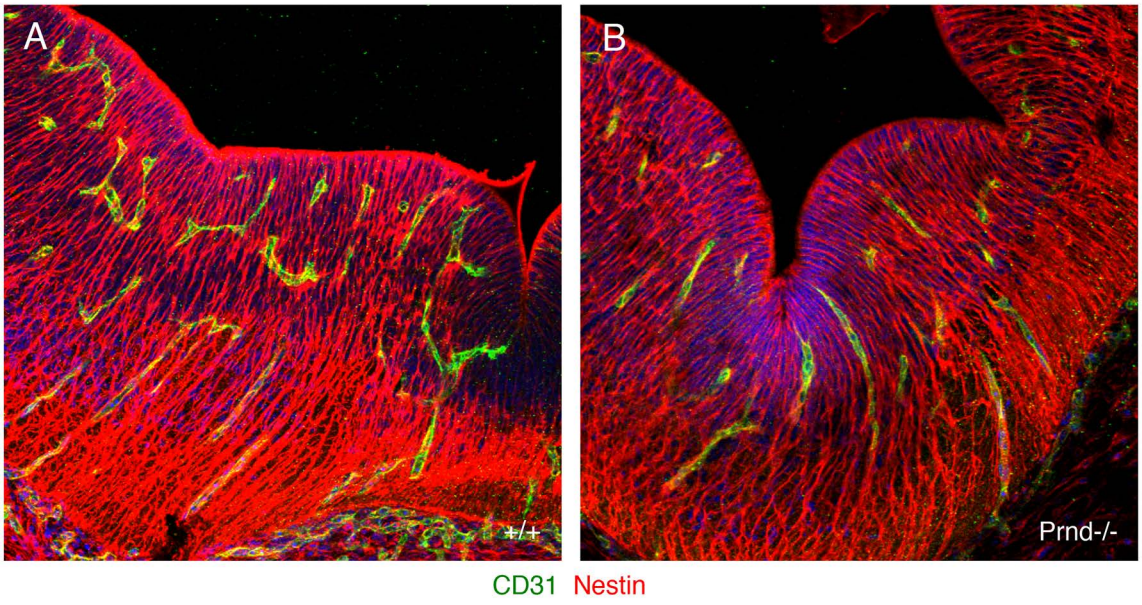



**Figure S6. Prnd-/- embryos do not display obvious defects in radial glia patterning. (A, B);** Wild type (A) and Prnd-/- (B) coronal sections through the ganglionic eminences of the E11.5 brain were immunofluorescently labeled with anti-CD31 (green) and anti-Nestin (red) antibodies to visualizes blood vessels and radial glial cells. Note the apparently normal patterning of the radial glial network in Prnd-/- brains.

Supplemental Figure 7

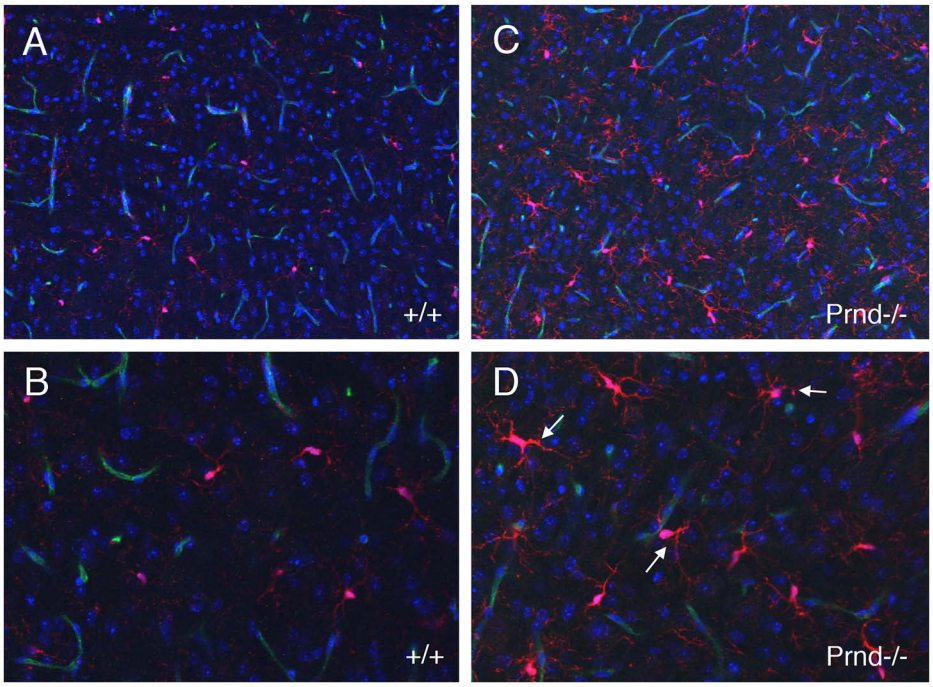



**Figure S7. Adult Prnd-/- mice display perivascular microgliosis. (A-D);**

Sagittal sections from wild type (A, B) and Prnd-/- (C, D) P30 mouse brains were generated with a vibratome and then immunofluorescently labeled with anti-CD31 and anti-Iba1 antibodies to visualize vascular endothelial cells (green) and microglia (red) respectively.

Note the increased perivascular microgliosis in Prnd-/- brain samples (white arrows in D), which is likely a secondary consequence of BBB leakage. Panels B, D are higher magnification images from panels A, C.

Supplemental Figure 8

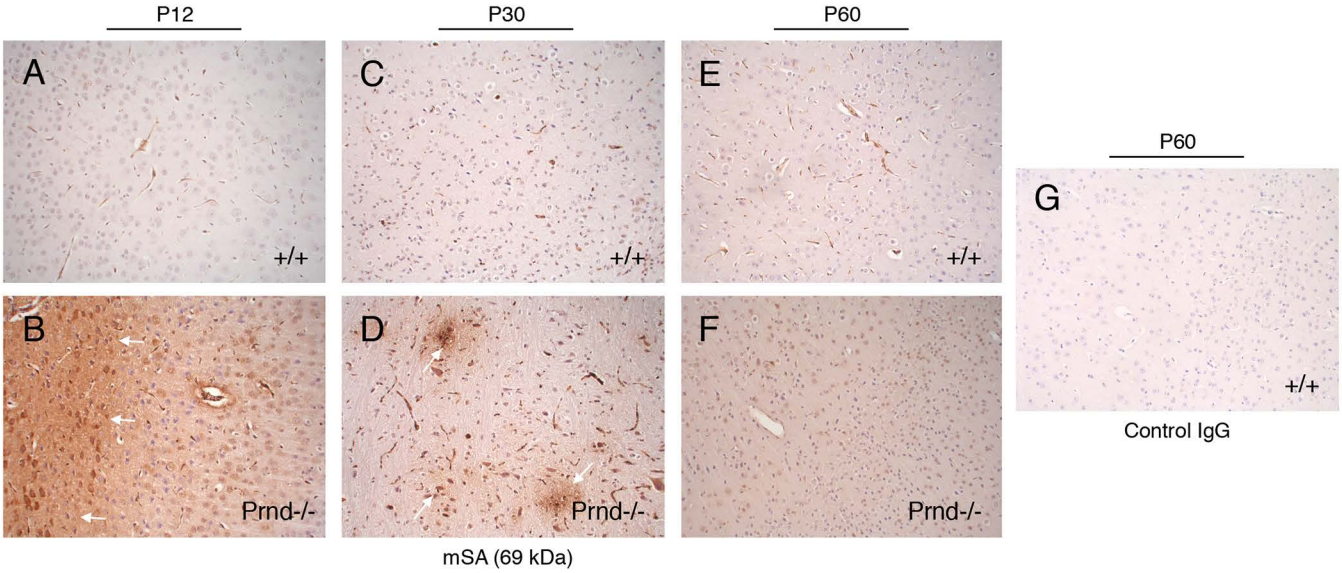



**Figure S8. Prnd-/- mice display BBB defects that diminish with age. (A-F);** Analysis of circulating mouse serum albumin (mSA), which does not normally cross the BBB, in brain sections from P12 (A, B), P30 (C, D), and P60 (E, F) wild type control (A, C, E) and Prnd-/- (B, D, F) mice. Note that P12 and P30 Prnd-/- mice display abnormal mSA extravasation into the brain parenchyma (arrows in B, D), with leakage more severe in the P12 brain. There is less pronounced mSA leakage in P60 Prnd-/- mice. **(G);** A section from a P60 wild type control mouse was stained with control rabbit IgG to show specificity for the anti-mSA antibody.

Supplemental Figure 9

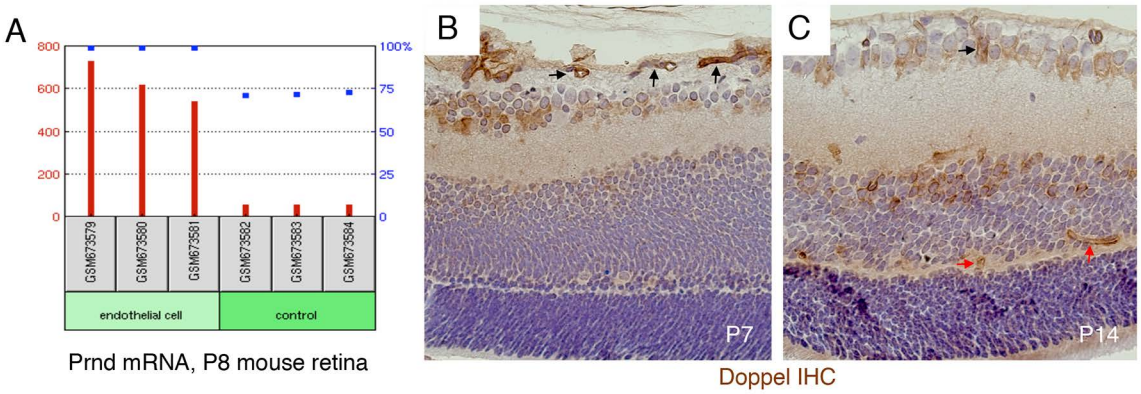
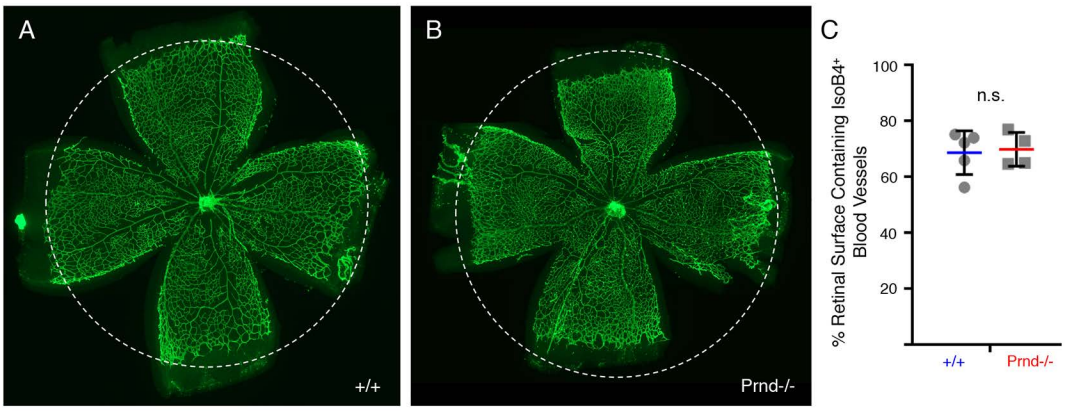



**Figure S9. Prnd/Doppel is expressed in angiogenic endothelial cells in the developing mouse retina. (A);** Prnd mRNA is enriched in vascular endothelial cells of the developing mouse retina, as revealed by analysis of the NCBI GEO database. $GFP^+$ retinal vascular endothelial cells were sorted from P8 Tie2-GFP mouse pups and gene expression was compared to $GFP^-$ retinal cells using cDNA microarray technology. **(B, C);** Formalin fixed and paraffin embedded retinal cross-sections from P7 (B) or P14 (C) neonatal mice were immunolabeled with anti-Doppel antibodies. Note that Doppel is expressed in vascular endothelial cells that comprise sprouting blood vessels of the P7 primary vascular plexus (B, black arrows) and the P14 primary (black arrows in C) secondary vascular plexi (red arrows in C).

Supplemental Figure 10




P7 Retinas

**Figure S10. Analysis of the primary vascular plexus in P7 retinas. (A, B);** Wild type (A) or Prnd-/- (B) P7 retinal flat mounts were stained with IsolectinB4-Alexa488 to fluorescently label endothelial cells in the primary vascular plexus. Note the relatively normal blood vessel coverage in the Prnd-/- retinas as compared to wild type controls. Dashed circles contain the same areas. **(C);** Quantitation of total retinal surface with blood vessel coverage in P7 wild type (n=9) and Prnd-/- (n=5) retinas. There are no statistically significant differences in between wild type and Prnd-/- samples at P7.