iScience, Volume *24*

Supplemental information

Dissecting VEGF-induced acute versus chronic vascular hyperpermeability: Essential roles of dimethylarginine dimethylaminohydrolase-1

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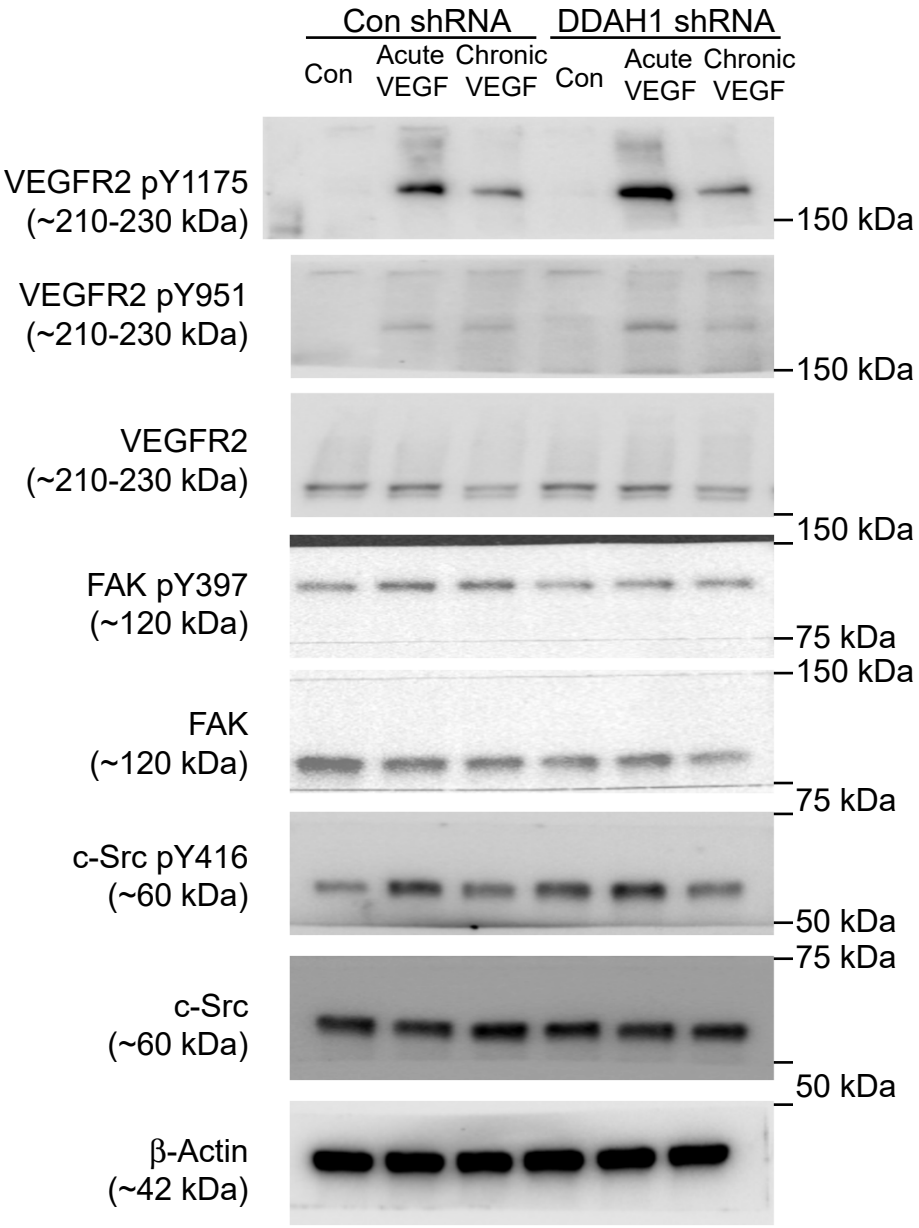

Figure S1. Related to Fig.1A: Full blots of Fig.1A.

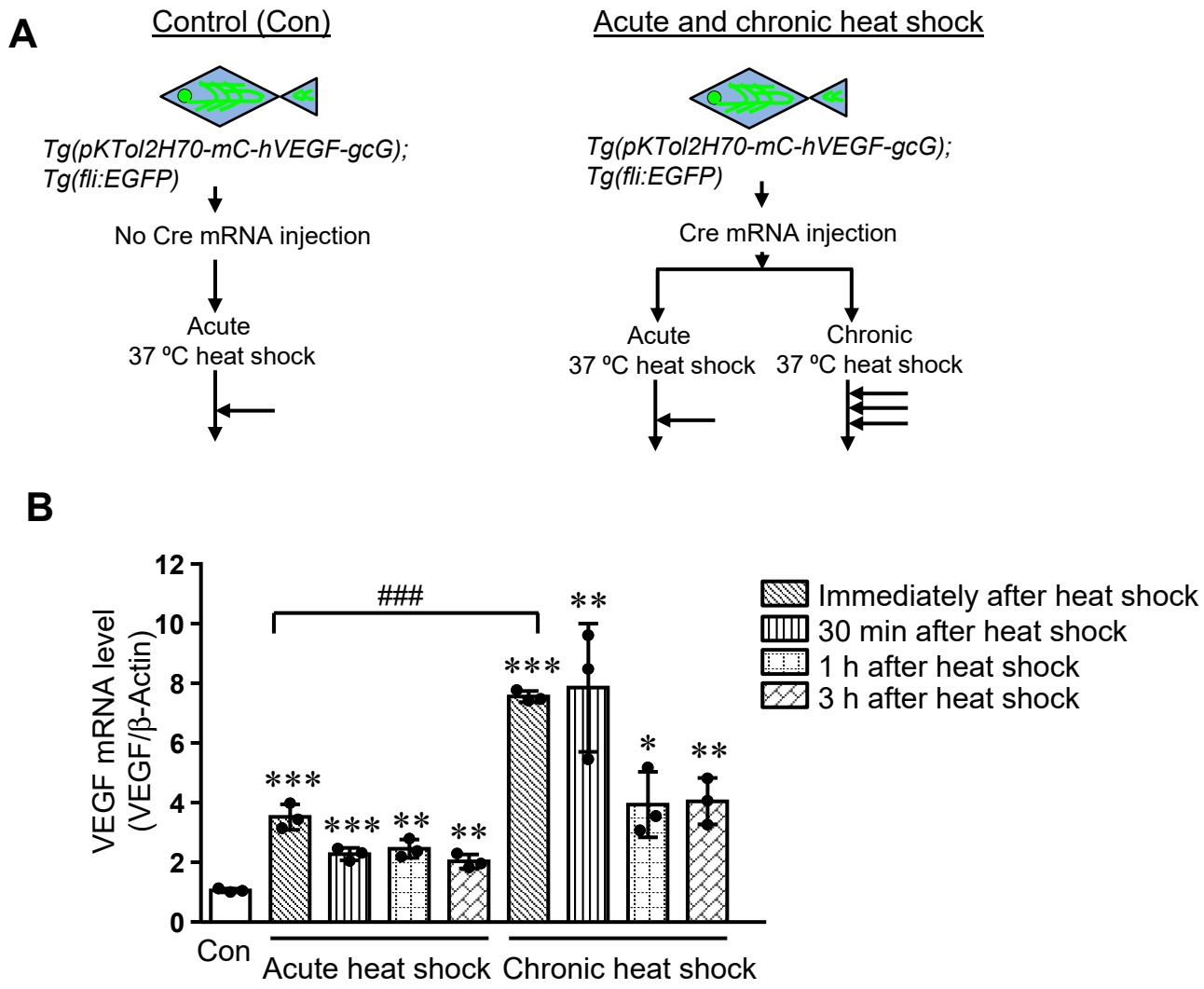

Figure S2. Related to Figure 2: Heat shock-induced VEGF mRNA expression in zebrafish. A-B. Double transgenic zebrafish *Tg(pKTol2H70-mC-hVEGF-gcG);Tg(fli:EGFP)* which received Cre mRNA injection at 1 day post fertilization (dpf) were subjected to acute and chronic heat shock at 37°C at 3 dpf. Total mRNA was collected immediately after the heat shock, 30 min, 1 hour, and 3 hours after heat shock. The double transgenic zebrafish which did not receive Cre mRNA injection and subjected to acute heat shock, then mRNA was collected immediately after heat shock and used as control (con). *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$, compared with control group.

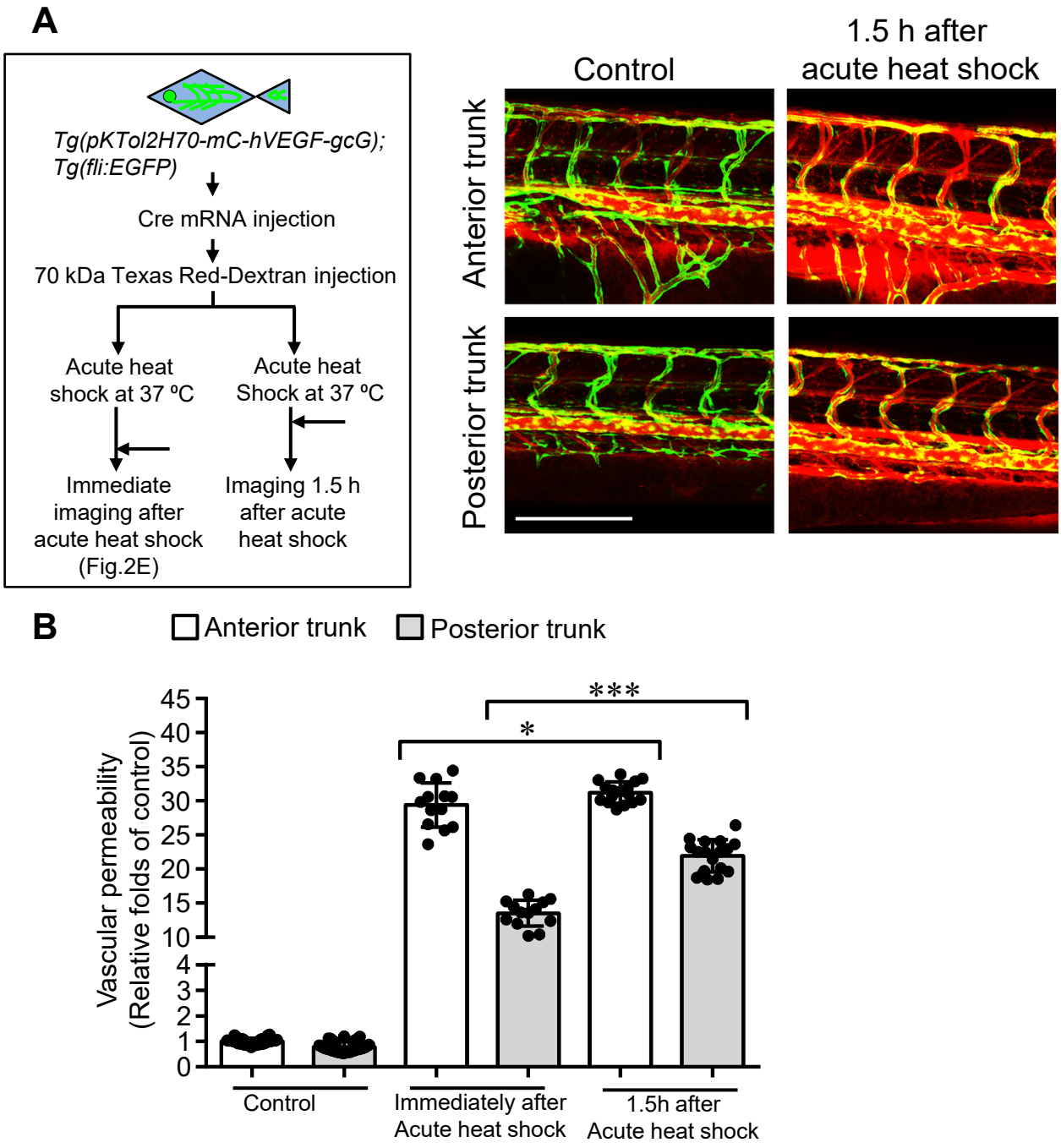

Figure S3. Related to Fig.2: Time-dependent response of acute heat shock-induced vascular permeability. A. VEGF-induced vascular hyperpermeability in zebrafish was examined at base line (no exposure to heat shock) and upon acute (37°C for 30 minutes) exposure to heat shock as demonstrated. Images were acquired immediately and 1.5 h after heat shock, respectively, using a Zeiss LSM 880 confocal microscope with standard FITC and dsRed filter sets. **B**. The vascular permeability of anterior and posterior regions of trunks was quantified and compared. *, $p<0.05$, ***, $p<0.001$. Zebrafish numbers in each group are presented in the scatter blot. Scale bar: 200 μm.

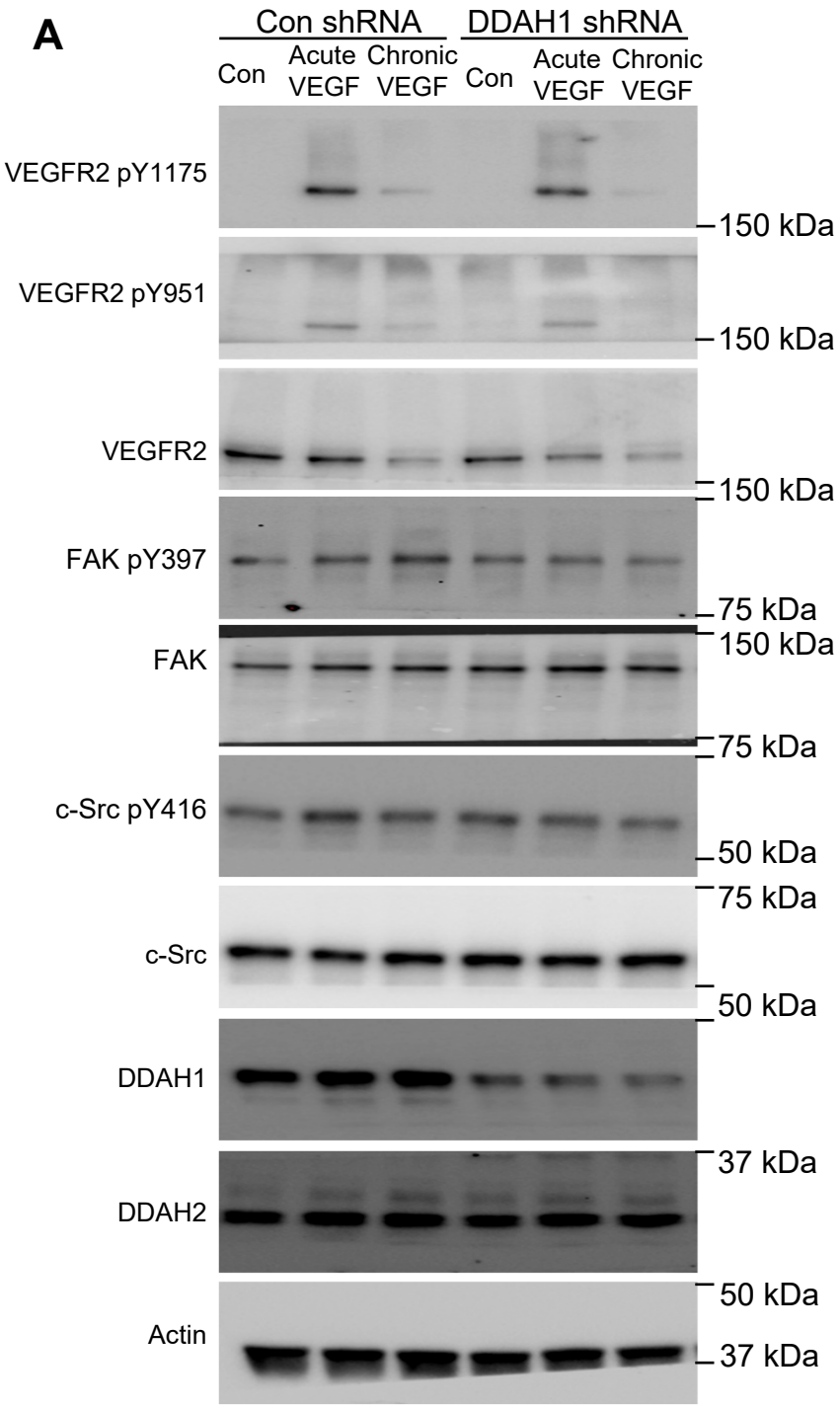

Figure S4. Related to Fig.3A: Full blots of Fig.3A.

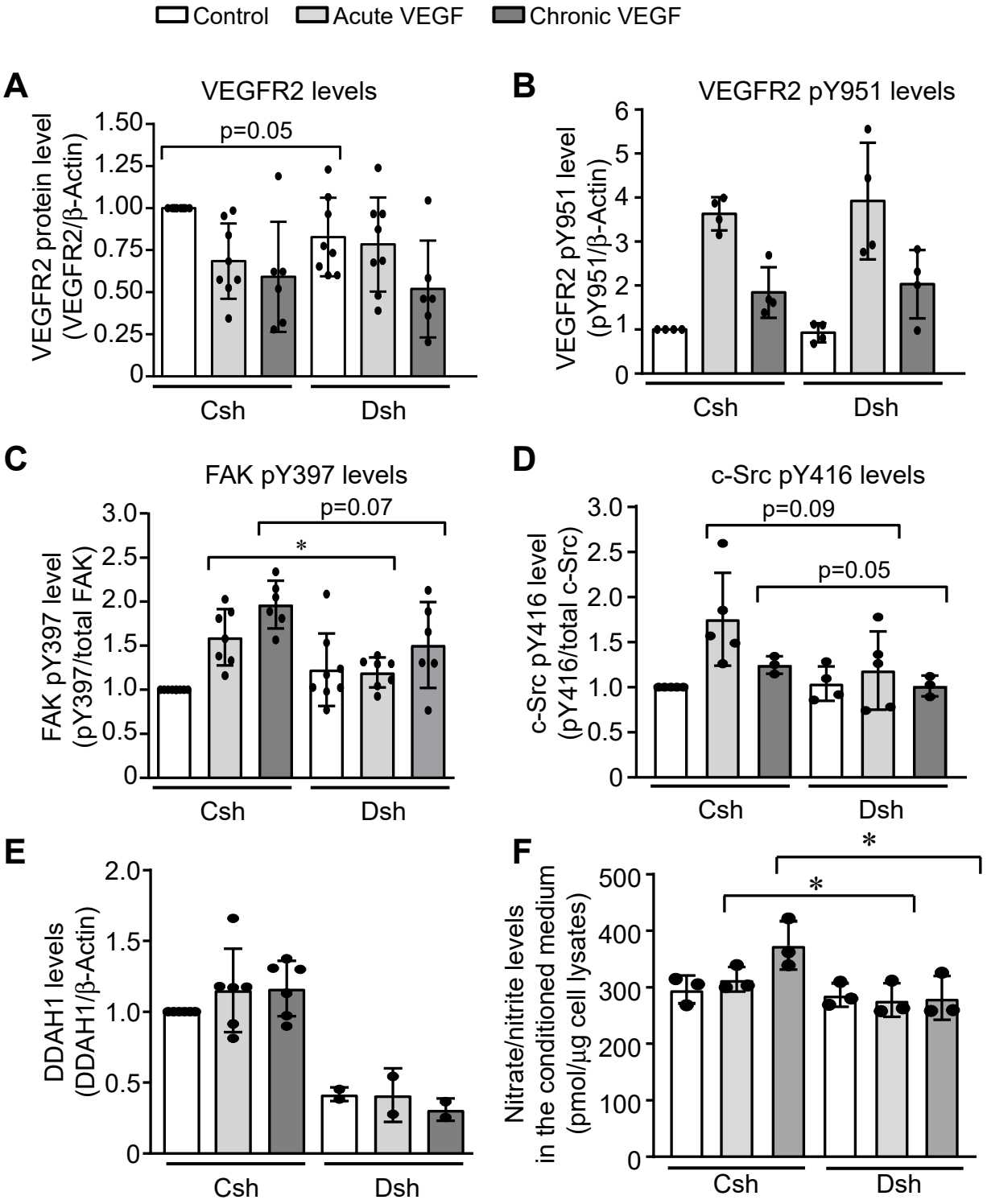

Figure S5. Related to Fig.3: Signaling pathways and nitric oxide levels in DDAH1 knockdown cells. Western blots in Fig.3A were quantified and compared. Relative levels of total VEGFR2 (A), VEGFR2 pY951 (B), FAK Y397 (C), c-Src Y416 (D) and DDAH1 (E) were compared. Conditioned medium of control and DDAH1 knockdown endothelial cells were collected and subjected to analysis of nitrate/nitrite levels. *, $p<0.05$.

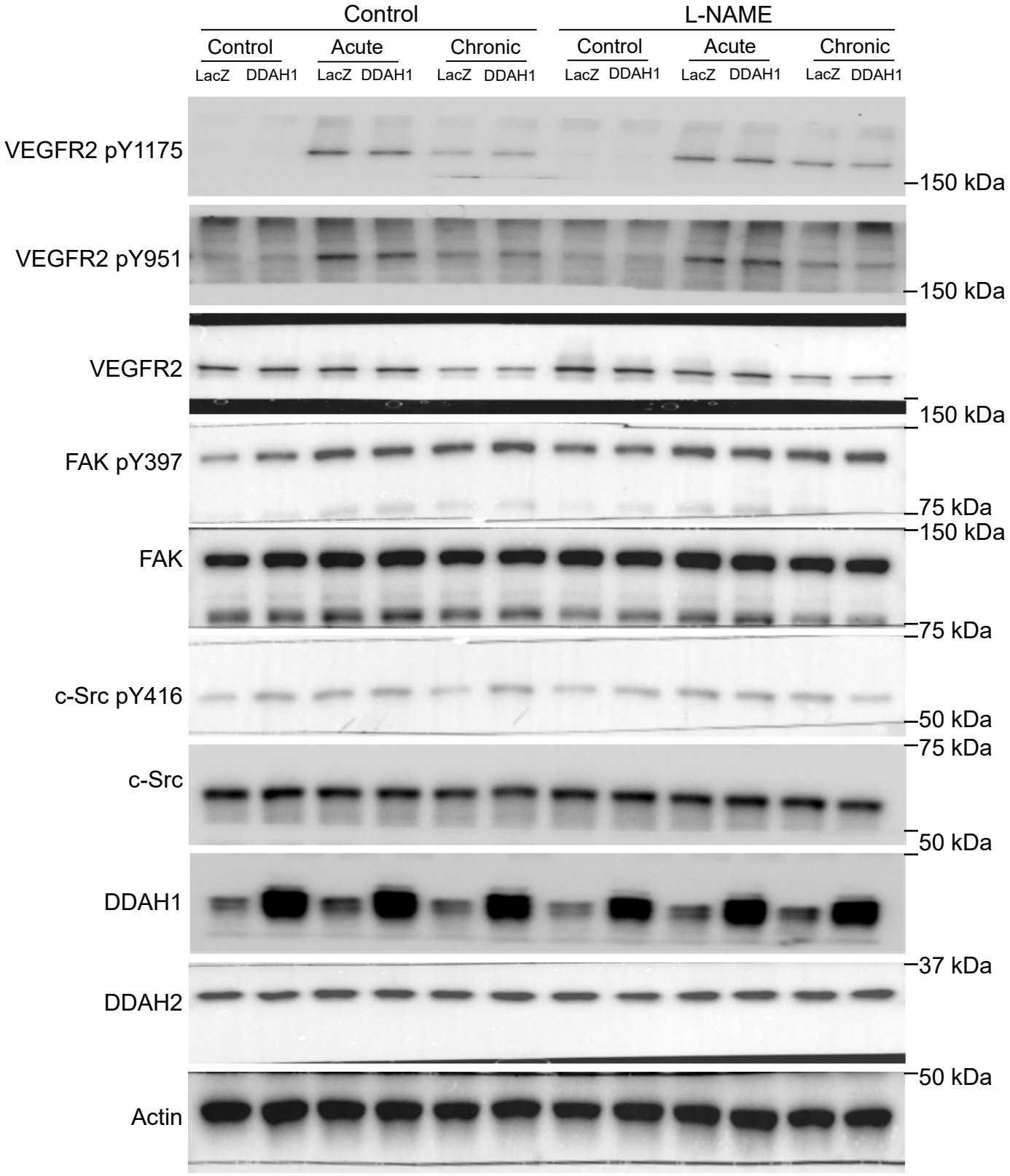

Figure S6. Related to Fig.3C: Full blots of Fig.3C.

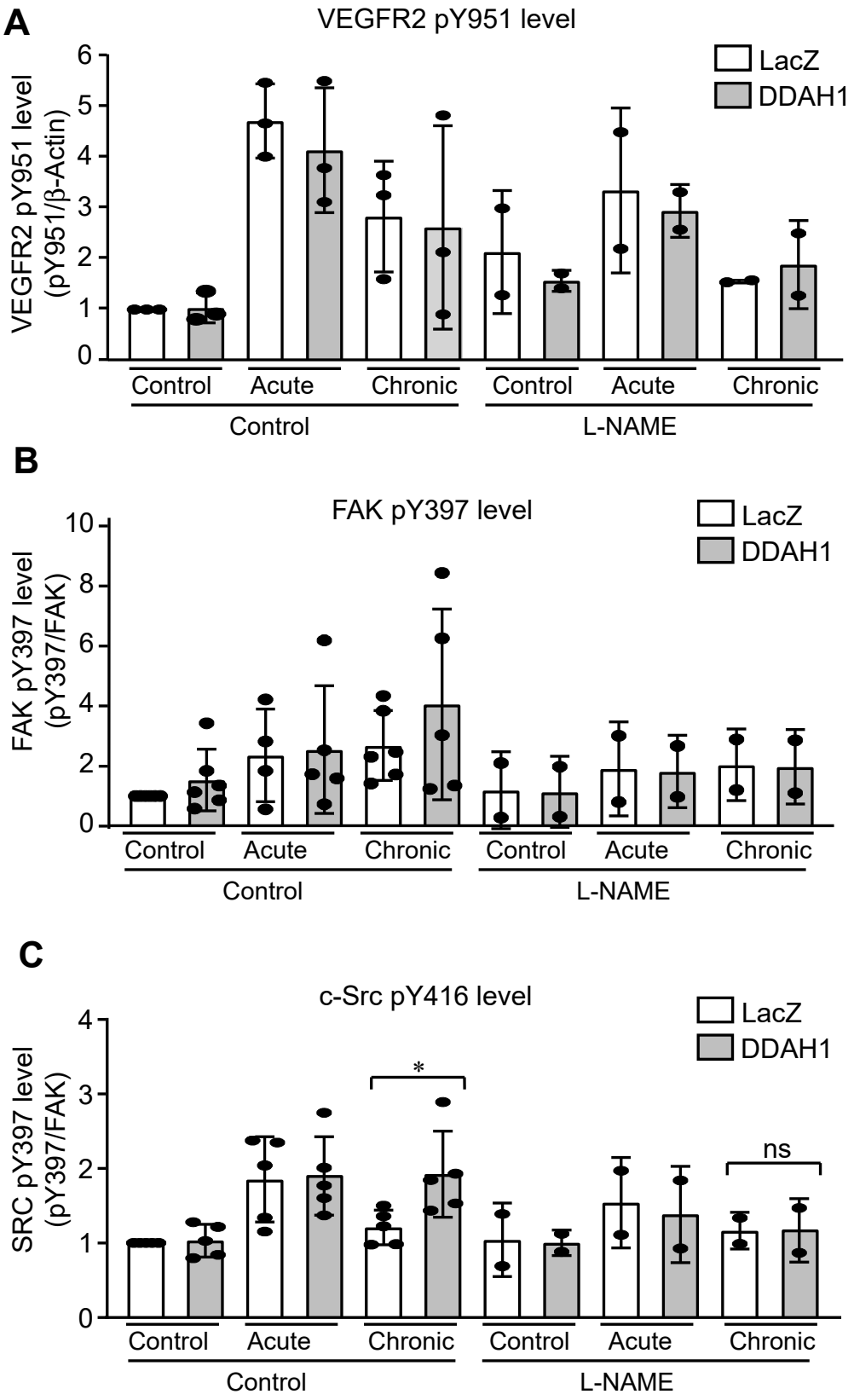

Figure S7. Related to Fig.3: Signaling pathways in DDAH1 overexpressing cells. Western blots in Fig.3C were quantified and compared. Relative levels of VEGFR2 pY951 (A), FAK Y397 (B), c-Src Y416 (C) were compared. *, $p<0.05$. ns. No significance.

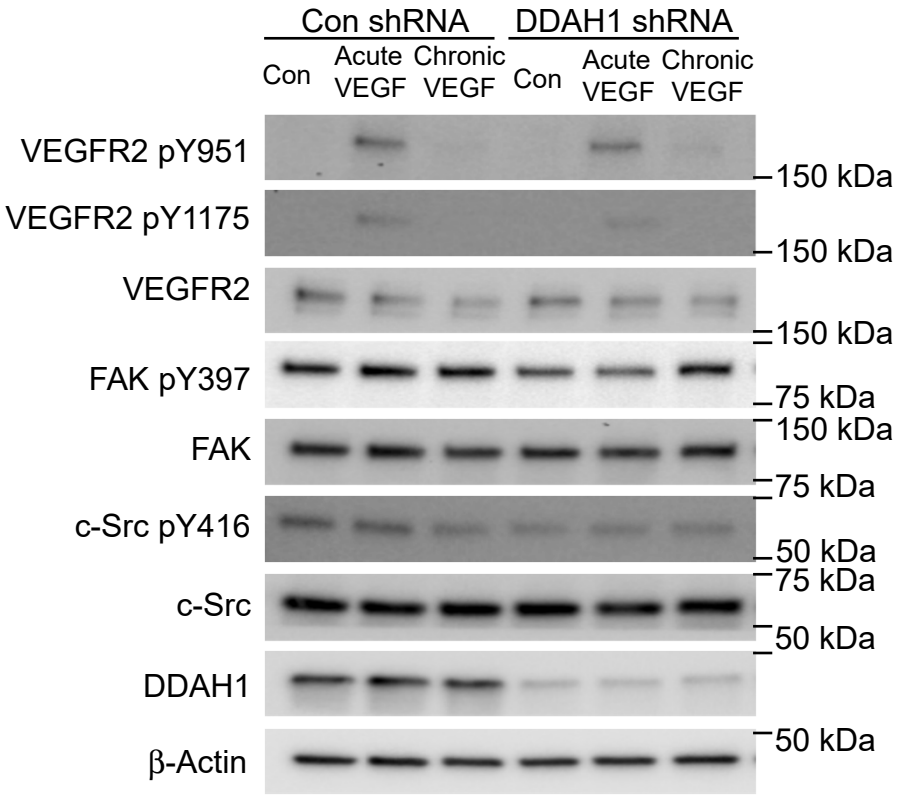

Figure S8. Related to Fig.3. Effect of DDAH1 knockdown on VEGF-induced signaling pathways in retinal endothelial cells. HRMVECs were infected with lentivirus expressing of control shRNA and DDAH1 shRNA, cultured in low-serum medium for 4 h and then exposed to stimuli of acute (single stimulus of VEGF at 10 ng/mL) and chronic VEGF (three stimuli of VEGF at 10 ng/mL with 30 min intervals), respectively. Protein were collected 10 minutes after VEGF stimulation and analyzed with western blotting.

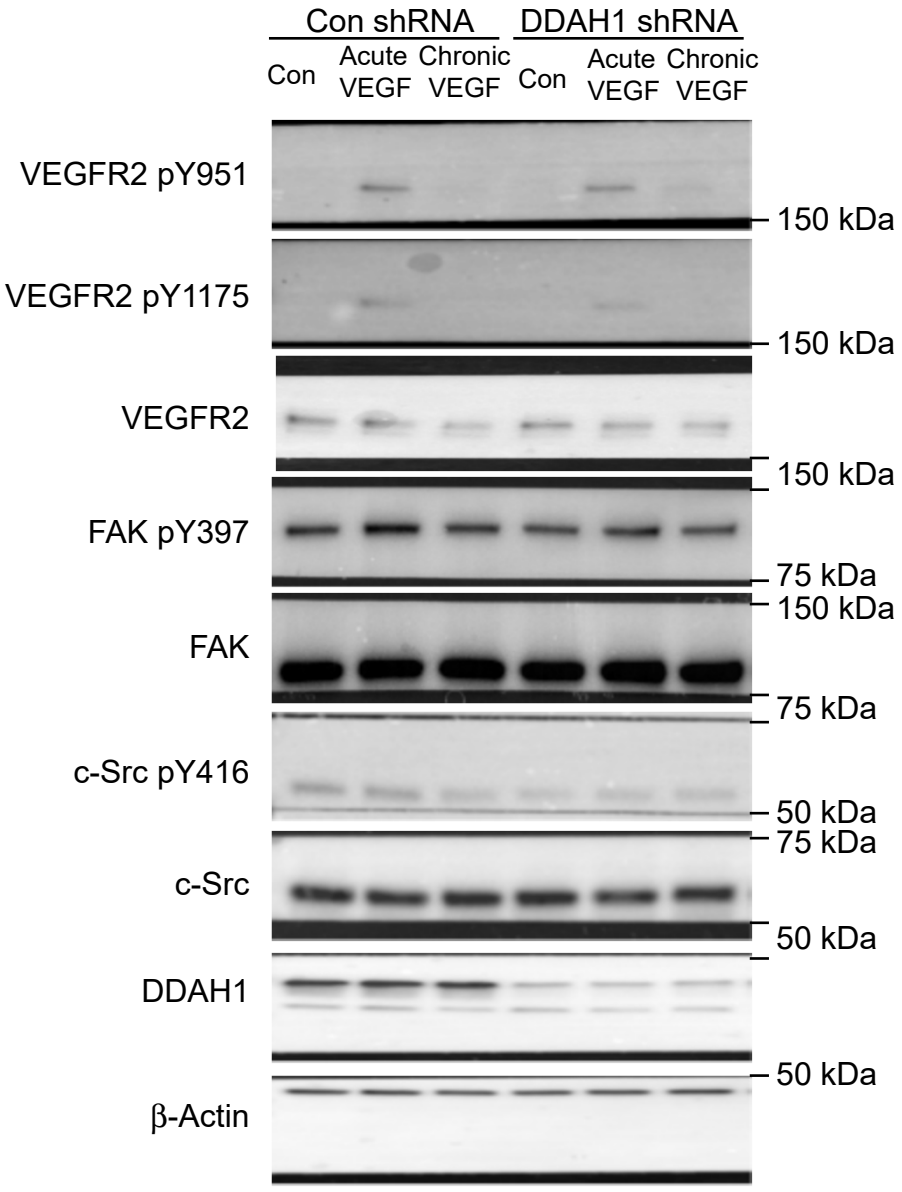

Figure S9. Related to Fig.3. Full blots of Fig.S8.