cells

MDPI

Supplemental Material

Molecular regulation of the RhoGAP GRAF3 and its capacity to limit blood pressure in vivo

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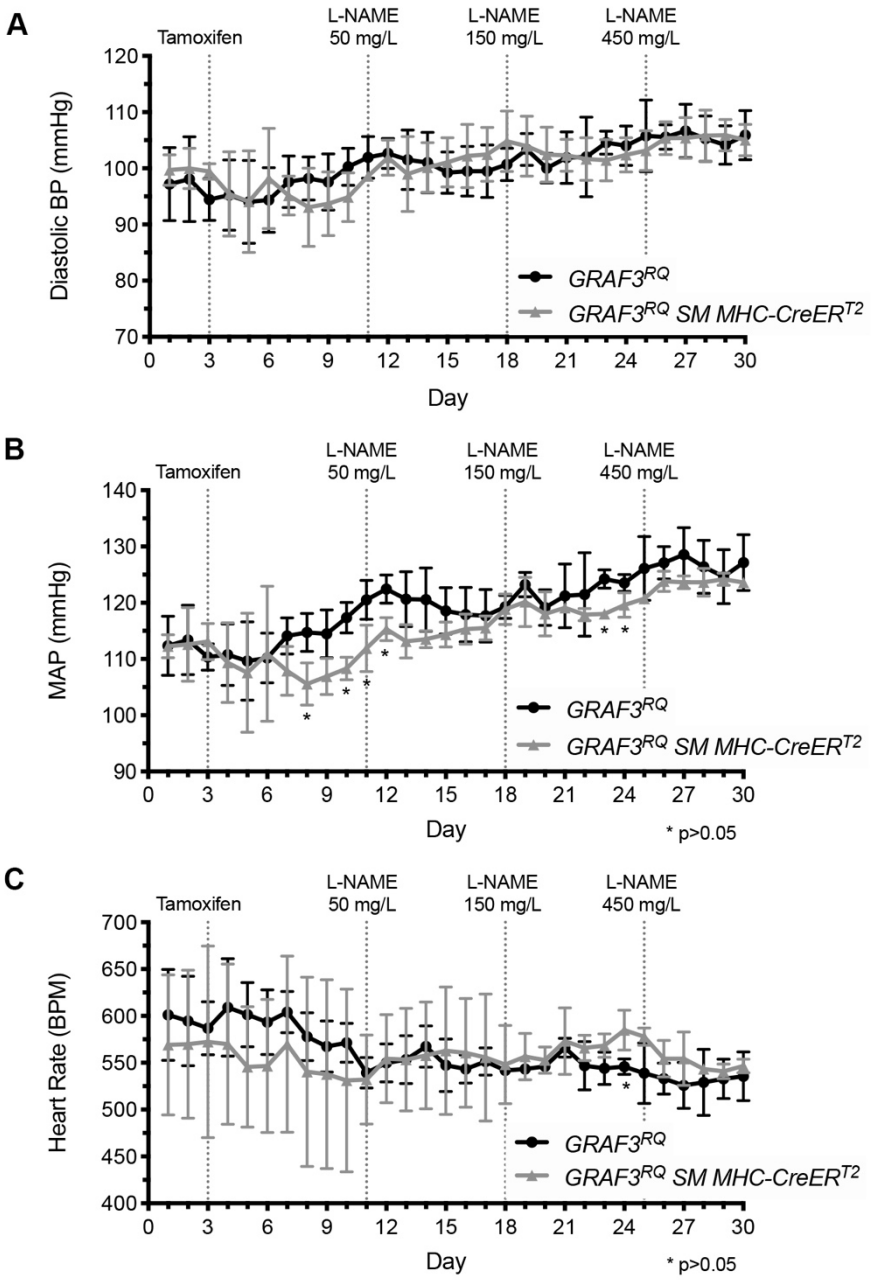

Figure S1. SMC-specific GRAF3^{RQ} expression had insignificant impact on diastolic BP, MAP, or HR. Average 24hr **(a)** diastolic BP **(b)** MAP, and **(c)** HR measured via radio-telemetry, of unrestrained, conscious *GRAF3^{RQ}* and *GRAF3^{RQ} SM MHC-CreER^{T2}* mice after tamoxifen treatment (100mg/kg for 3 consecutive days) and increasing L-NAME doses (50mg/L, 150mg/L, or 450mg/L) given for a week (each) in drinking water. Data are expressed as mean ± SD; n=4 for *GRAF3^{RQ}* mice and n=3 for *GRAF3^{RQ} SM MHC-CreER^{T2}*.*p>0.05 vs.GRAF3^{RQ} (Student's t-test).

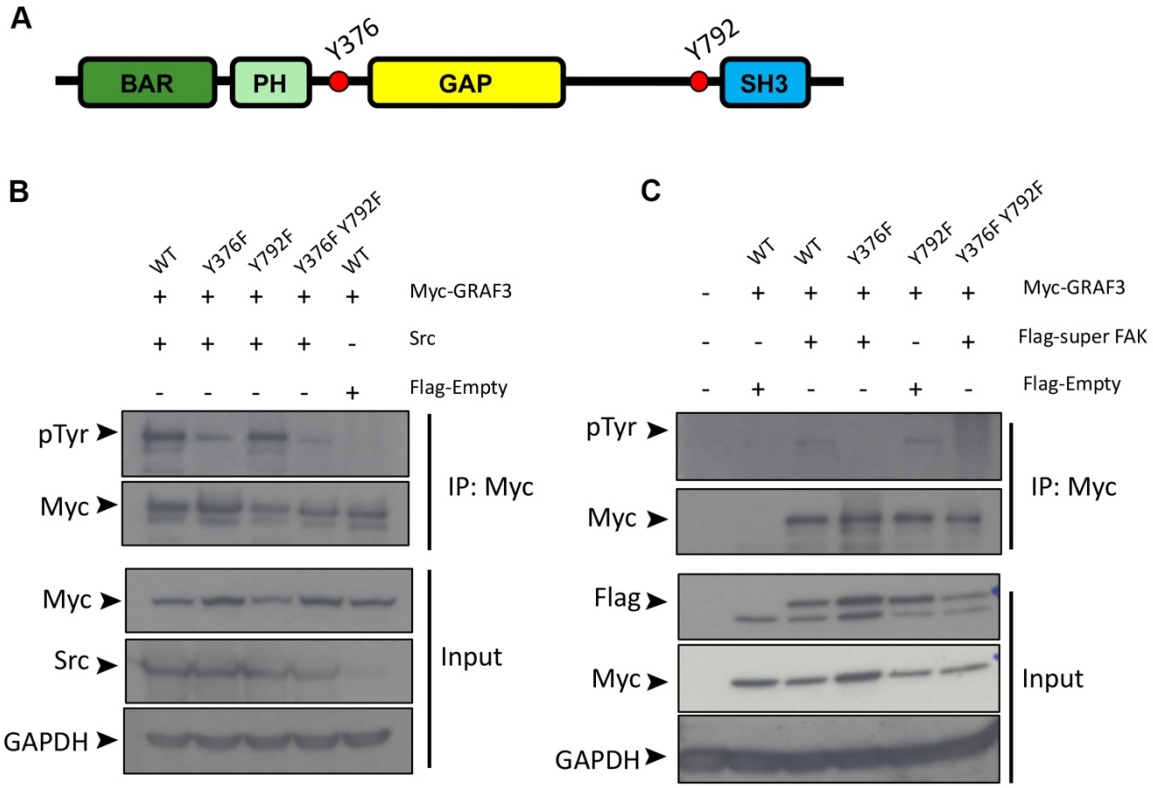

Figure S2. Y792 is not a major Src or FAK phosphorylation site. **(a)** Schematic of GRAF3 domain structure with indicated location of Y376 and Y792. Cos cells were transfected with the indicated GRAF3 variant and either **(b)** ^{529F}Src or **(c)** superFAK. Myc-tagged GRAF3 was immunoprecipitated from cell lysate and probed for tyrosine phosphorylation.

Video S1. Predicted structure of the open (active) GRAF3 BAR-PH-GAP dimer. Color scheme follows: BAR 1 (dark purple), PH 1 (light purple), BAR 2 (dark green), PH 2 (light green), GAP 1 and 2 (yellow), arginine fingers (active site) (dark blue), RhoA docking sites (pink), C-terminus of PH domain (red), N-terminus of GAP domain (orange); residues in teal aids in visualizing rotation. Model was created using Pymol and the solved, similar structures of Appl1 (BAR-PH) and GRAF1 (GAP).