PYK2 INHIBITION OF P53 AS AN ADAPTIVE AND INTRINSIC MECHANISM FACILITATING CELL PROLIFERATION AND SURVIVAL

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Legends to Supplemental Figures

Table I. Primers used for PCR cloning, PCR mutagenesis and lentiviral shRNA knockdown.

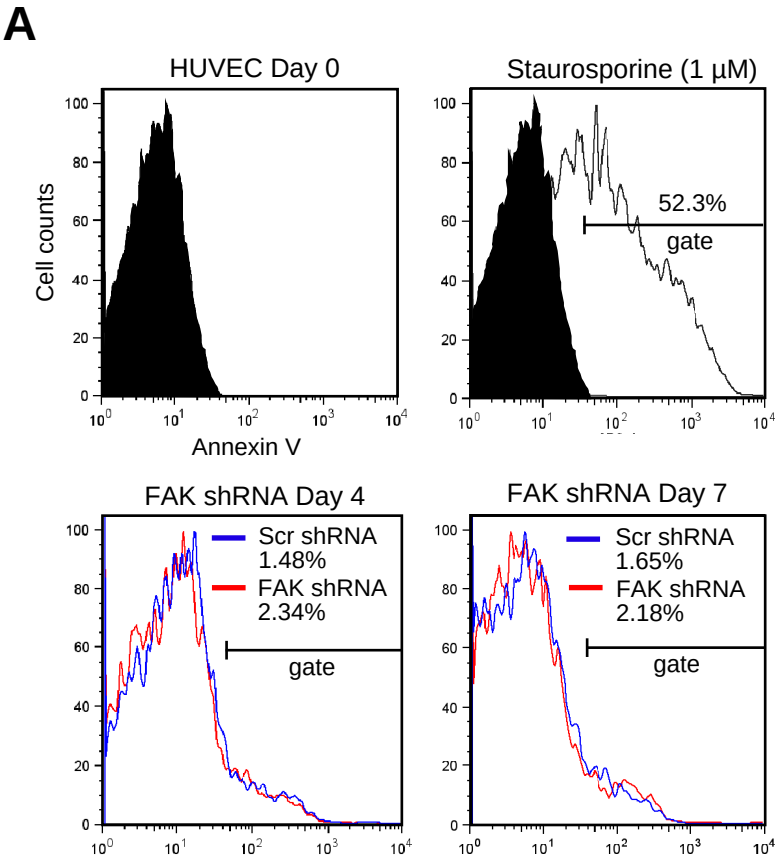
Supplemental Figure. 1. **FAK knockdown in HUVECs.** (A) Loss of FAK does not trigger apoptosis. Allophycocyanin-conjugated annexin V staining of growing (Day 0, filled) or staurosporine-treated (1 µM, 12 h) HUVECs shown as negative and positive controls, respectively. Lentiviral-mediated expression of FAK and Scr shRNA were evaluated for effects on cell apoptosis after 4 and 7 days. Knockdown of FAK (red line) did not increase annexin V staining compared to Scr control (blue line), annexin positive population was gated. The gated region was constant and was set using staurosporine treatment of HUVECs. (B) FAK, Pyk2, p53 levels after lentiviral-mediated anti-FAK or control scrambled (Scr) shRNA expression in HUVECs as determined. Actin was used as a loading control. Days 4 or 7 are time after initial infection.

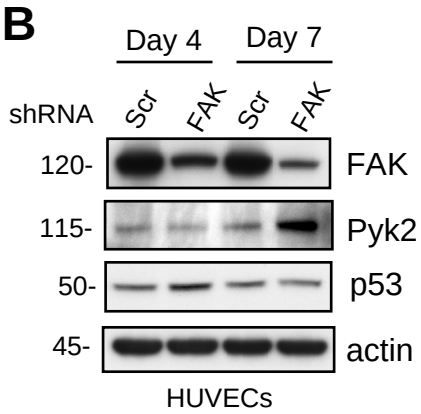
Supplemental Figure 2. **Pyk2 knockdown does not increase FAK-/-p21-/- MEF apoptosis.** APC-annexin V staining of growing (Day 0, filled) or staurosporine-stressed (1 µM, 12 h) FAK-/-p21-/- fibroblasts shown as negative and positive controls, respectively. Lentiviral-mediated expression of Scr or Pyk2 shRNA were evaluated for effects on cell apoptosis after 3 days. Pyk2 knockdown did not increase annexin V staining compared to Scr control. The gated region was constant and was set using staurosporine treatment. (B) Pyk2 shRNA-expressing FAK-/-p21-/- MEFs grow slower than scrambled shRNA control. Passage 10 FAK-/-p21-/- MEFs were transduced with different anti-mouse lentiviral Pyk2 shRNA constructs and cell growth over 4 days was compared to scrambled (Scr) control shRNA. Resulting cell counts from 3 dishes at each experimental day are mean cell number ± SD.

Supplemental Table 1. Primers used for PCR cloning, PCR mutagenesis and shRNA.

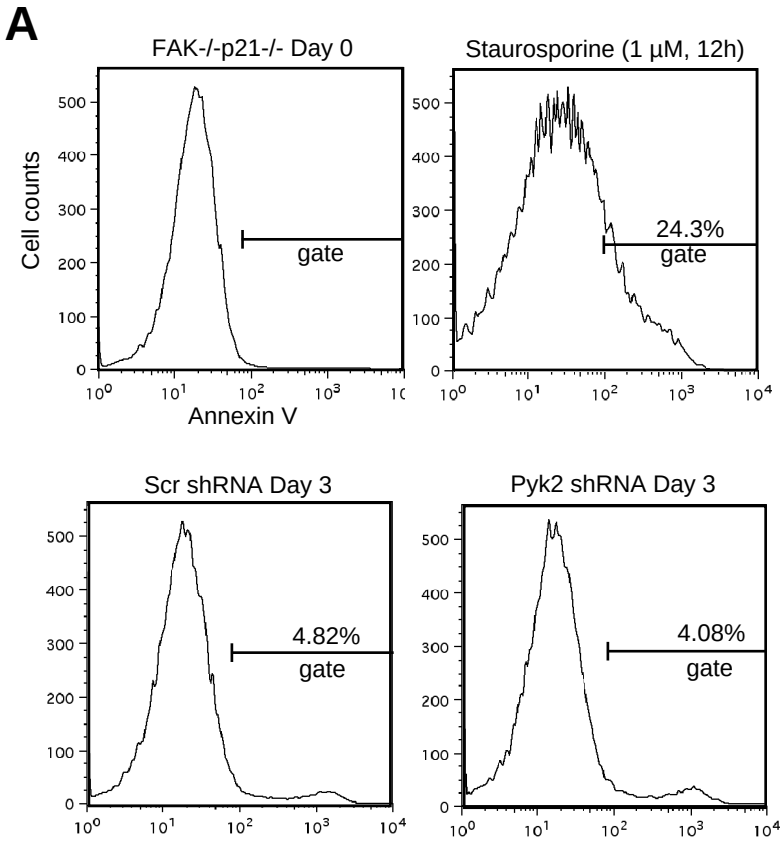
Primer	Sequence	Note
Pyk2_BglI-5'	5'-gcaggagatctcgtatcctcaaggtctgcttc-3'	Pyk2 39-367 cloning BglI underlined
Pyk2_XbaI-3'	5'-gctcgtctagacctaggatggatgatgagaga-3	Pyk2 39-367 cloning XbaI underlined
Pyk2 FERM R184T/R185T-5'	5'-cagctgggctgcctggagttaacgacgttcttcaaggatat-3'	R184T/R185Tmutagenesis Mutated sequences underlined
Pyk2 FERM R184T/R185T-3'	5'-tttaagcttttacctaggatggatgatgagagagccttg-3'	R184T/R185Tmutagenesis Mutated sequences underlined
Pyk2_FERM_HindIII-5'	5'-tttaagcttcgtatcctcaaggtctgcttctatagc-3'	Ad-Myc-Pyk2 FERM subcloning HindIII underlined
Pyk2_FERM_HindIII-3'	5'-tttaagcttttacctaggatggatgatgagagagccttg-3'	Ad-Myc-Pyk2 FERM subcloning HindIII underlined
Mouse Pyk2 shRNA-5' #1	5'-*t*GAAGTAGTTCTTAACCGCA*ttcaagaga*TGCGGTTAAGAACTA CTTC*tttttt*c-3'	shRNA cloning
Mouse Pyk2 shRNA-3' #1	5'-TCGA*gaaaaaa*GAAGTAGTTCTTAACCGCATC*tctcttgaa*TGCGG TTAAGAACTACTTC*a*-3'	5'TCGA is XhoI overhang
Mouse Pyk2 shRNA-5' #2	5'-*t*AGCCTCTGTGACACGTCTA*ttcaagaga*TAGACGTGTCACAGA GGCT*tttttt*c-3'	shRNA cloning
Mouse Pyk2 shRNA-3' #2	5'-TCGA*gaaaaaa*GAAGTAGTTCTTAACCGCATC*tctcttgaa*GTGCG GTTAAGAAGAACTA*a*-3'	5'TCGA is XhoI overhang
Mouse FAK shRNA-5' #1	5'-*t*GAATGGCAGCTGCTTATCTT*tcaagag*AAGATAAGCAGCTGCC ATTC*tttttt* c-3	shRNA cloning
Mouse FAK shRNA-3' #1	5'-TCGA*gaaaaaa*GAATGGCAGCTGCTTATCTT*ctcttga*AAGATAAG CAGCTGCCATTCa-3'	5'TCGA is XhoI overhang
Mouse FAK shRNA-5' #2	5'-*t*GAAGGGATCAGTTACCTGA*ttcaagaga*TCAGGTAACTGATCCC TTCTT*ttttc*-3'	shRNA cloning
Mouse FAK shRNA-3' #2	5'-TCGAgaaaaaaGAAGGGATCAGTTACCTGATCtcttgaaTCAGGTAA CTGATCCCTTCa-3'	5'TCGA is XhoI overhang
Human FAK shRNA-5' #1	5'-tGAACCTCGCAGTCATTTATTtcaagagAATAAATGACTGCGA GGTTCttttttc-3'	shRNA cloning
Human FAK shRNA-3' #1	5'-TCGAgaaaaaaGAACCTCGCAGTCATTTATTctcttgaAATAAATGAC TGCGAGGTTCa-3'	5'TCGA is XhoI overhang
Human FAK shRNA-5' #2	5'-tGGCATTTATATGAGTCCAGTtcaagagACTGGACTCATATAAA TGCCTttttc-3'	shRNA cloning
Human FAK shRNA-5' #2	5'-TCGAgaaaaaaGAACCTCGCAGTCATTTATTCAGTCtcttgaaCTGG ACTCATATAAATGCCa3'	5'TCGA is XhoI overhang

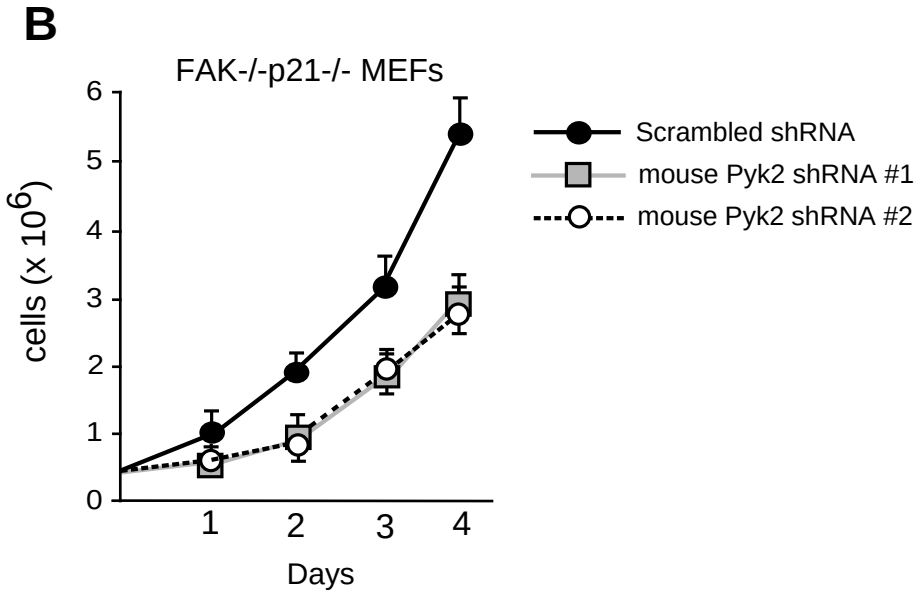
5' primer represents forward and 3' for reverse primers. Italicized characters are accessory components of shRNA.





Supplemental Figure 1





Supplemental Figure 2