The glycolytic enzyme PFKFB3 is involved in estrogen-mediated angiogenesis via GPER1

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Supplemental data

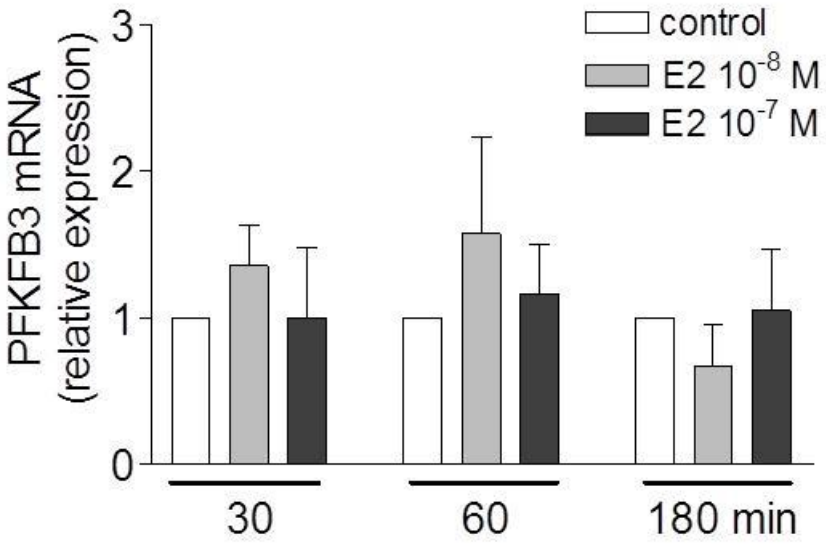
Methods

Chemicals. ICI 182,780 and the selective PFKFB3 inhibitor PFK15 were purchased from Tocris Bioscience, Bristol, UK.

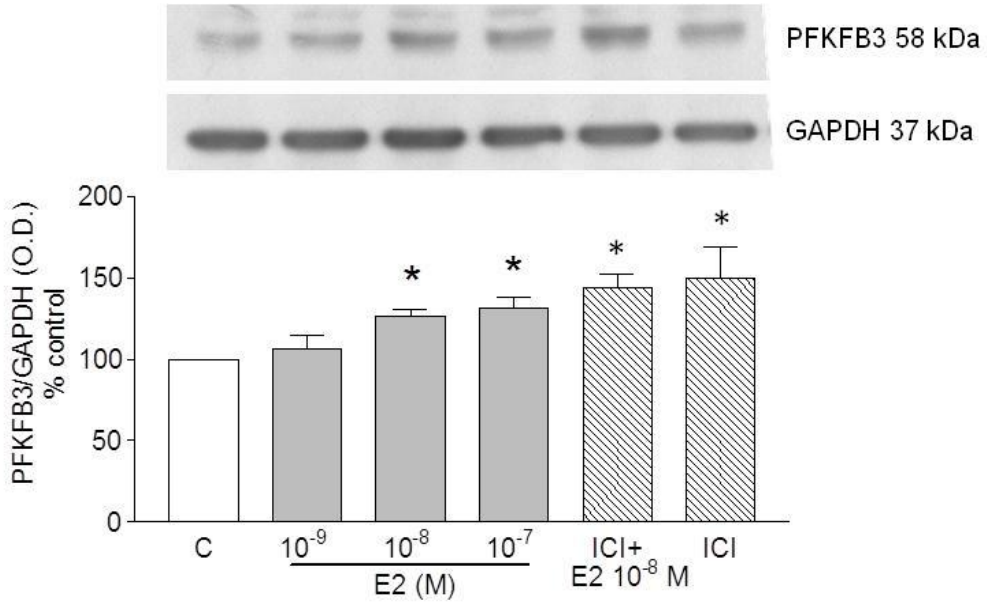
Real-time PCR. HUVECs ($3x10^5$/well) were grown in 35-mm dishes and stimulated with E2 (10^{-8} – 10^{-7} M) for 0.5 to 3 h in phenol red-free complete M199 medium with 5% FBS. mRNA levels were measured by Q-PCR using the primer pair GCGTCCCCACAAAAGTGTTC (forward) and CCGGACTTTCATGGCTTCCT (reverse), and normalized to 18S. Data are expressed as mean ± S.E.M. of 3 independent experiments.

Results

Supplemental Figure 1. 17β-Estradiol treatment did not affect *PFKFB3* mRNA levels in HUVECs.

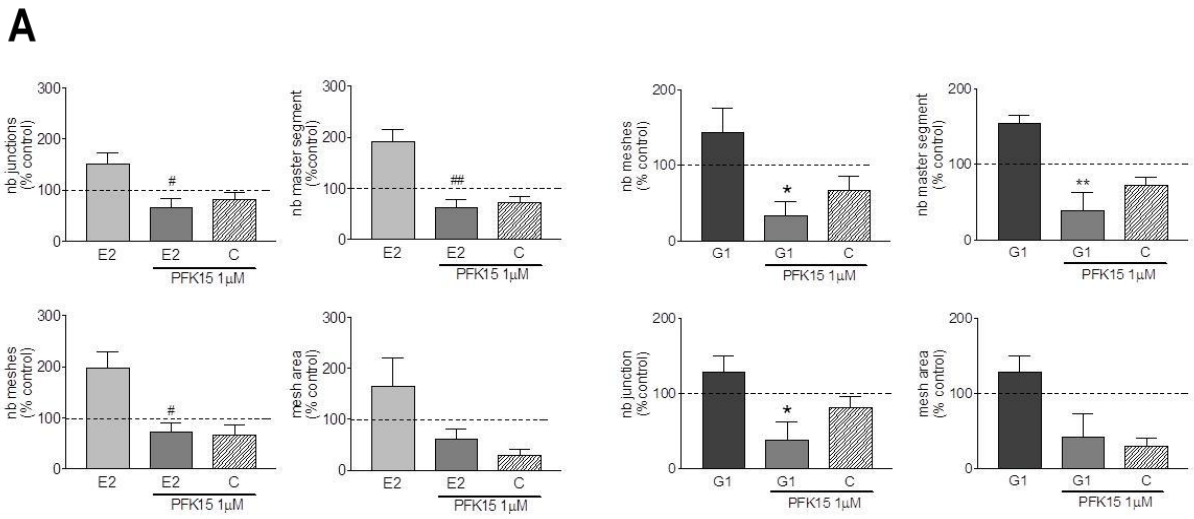


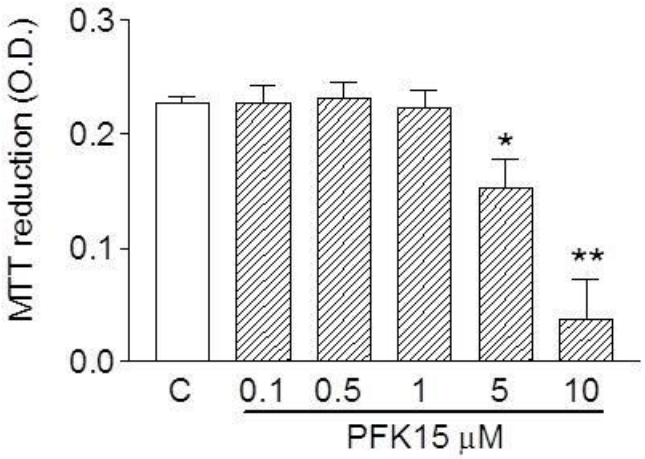
Supplemental Figure 2. ICI 182,780 failed to abolish the effect of E2 on PFKFB3 protein amount in HUVECs.



HUVECs ($3x10^5$/well) were grown in 35-mm dishes and, after reaching confluence, stimulated with E2 (10^{-9} – 10^{-7} M) for 3 h in phenol red-free complete M199 medium with 5% FBS. The ER antagonist, ICI182,780 (10^{-6} M), was added 30 min before the experiment and was present during the experiment. Cell lysates were subjected to Western blotting as described in Methods. *Upper part*: representative experiment showing the expression of PFKFB3; GADPH expression was used as loading control. *Lower part*: densitometric analysis, normalized to GADPH levels, expressed as % of control (mean ± S.E.M. from 4 independent experiments). * $P < 0.05$ *vs*. control, One-way ANOVA, Dunnett's post-hoc test).

Supplemental Figure 3. The PFKFB3 inhibitor 1-(4-pyridinyl)-3-(2-quinolinyl)-2-propen-1-one (PFK15; Tocris Bioscience) reduced the formation of tube-like structures induced by ER ligands (panel A) without affecting cell viability (panel B).





A) HUVECs ($7x10^3$ cells/well) were seeded onto Matrigel-coated 48-well plates in phenol red-free M199 medium with 5% FBS containing E_2 or G-1 (both 10^{-7} M) in the presence or absence of PFK15 (1 µM). Parameters of capillary tube formation after 4 h incubation were measured using Angiogenesis Analyser (ImageJ). Data are expressed as mean ± S.E.M. of 3 independent experiments. One-way ANOVA, Bonferroni's post-hoc test, # $P < 0.05$ *vs*. E_2; ## $P < 0.01$ *vs*. E_2; * $P < 0.05$ *vs*. G1; ** $P < 0.01$ *vs*. G1.

B) HUVECs (10^4 cells/well) were plated in 96-well plates and incubated in phenol red-free M199 medium with 5% FBS in the presence of PFK15 (0.1 – 10 µM). Cell viability was measured by MTT assay as described in Methods. Data are the mean ± S.E.M. of 3 independent experiments performed in quadruplicate. One-way ANOVA, Dunnett's post-hoc test, * $P < 0.05$ *vs*. Control; ** $P < 0.01$ *vs*. Control.