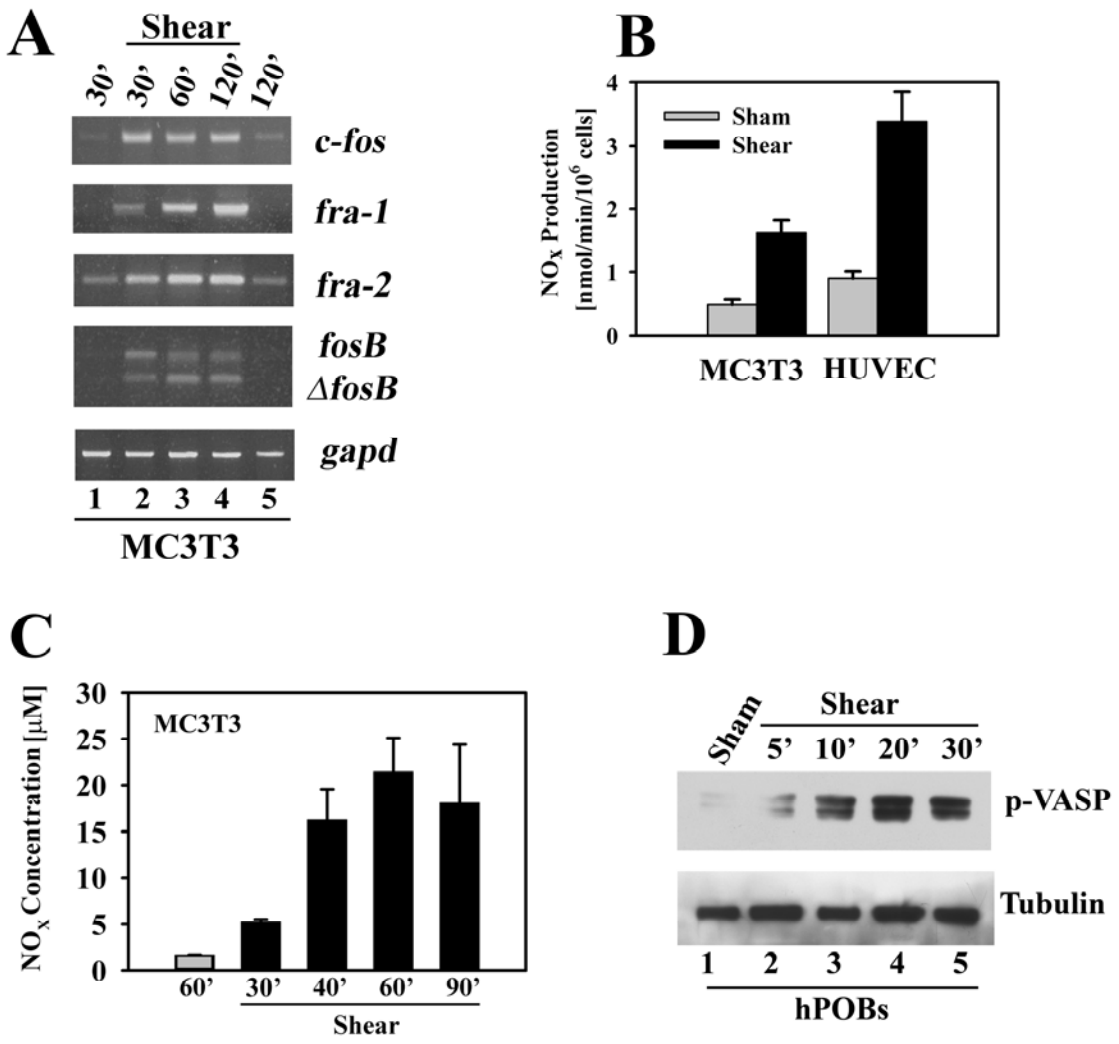
Supplemental Table I: PCR Primers Used for RT-PCR

Gene	PCR Primers		Product Size (bp)
	Sense (5'-3')	Anti-Sense (5'-3')	
***gapd* (murine)**	TGTCTTCACCACCATGGAGAACG	GTGGATGCAGGGATGATGTTCTG	333
***c-fos* (murine)**	CGCAGAGCATCGGCAGAAGG	TCTTGCAGGCAGGTCGGTGG	258
***fra-1* (murine)**	TGCGAGCAGATCAGCCCAGAG	CTGGAGAAAGGGAGATGCAAGG	347
***fra-2* (murine)**	GGAGGAGAAGCGTGCAATCC	GGGGCTGATTTTGCACACG	217
***fosB* (murine)**	AAAAGGCAGAGCTGGAGTCGG	TGTACGAAGGGCTAACAACGG	324
***pkg I* (murine)**	GTCACTAGGGATTCTGATGTATGA	AGAATTTCCAAAGAAGATTGCAAA	129
***pkg II* (murine)**	GTGACACAGCGCGGTTGTT	TGGGAATGGAAAAGGACAAC	235
***gapd* (human)**	AACGGATTTGGTCGTATTGGG	TGGAAGATGGTGATGGGATTTC	209
***c-fos* (human)**	GTGCCAACTTCATTCCCACG	GAGATAACTGTTCCACCTTGCCC	231
***fra-1* (human)**	TGTGAACAGATCAGCCCGGAG	CTGGGGAAAGGGAGATACAAGG	331
***fra-2* (human)**	ATTATCCCGGGAACTTTGACAC	ATGTGTCCAGGGACAGAGGCC	274
***fosB* (human)**	AAAAAGCAGAGCTGGAGTCGG	TGTACGAAGGGTTAACAACGG	323

Suppl. Fig. 1



Supplemental Fig. 1:

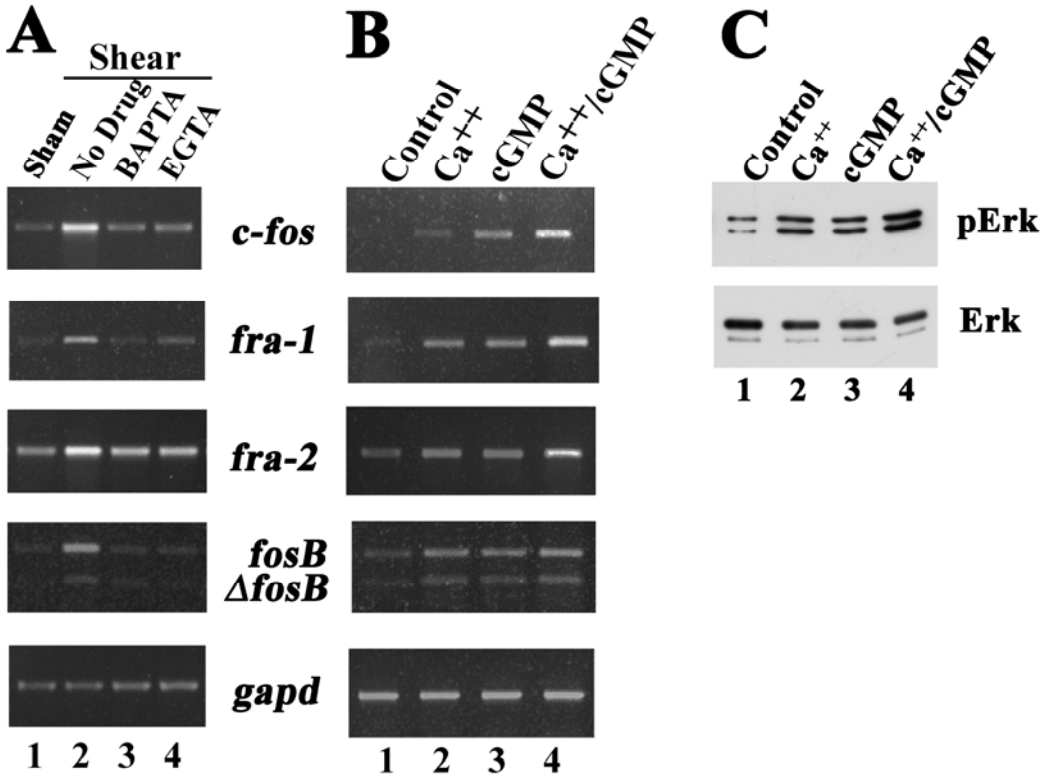
A. Serum-deprived MC3T3 cells were incubated in a parallel plate flow chamber for the indicated times; cells were exposed to laminar flow for the first 20 min (lanes 2-4), or kept under static conditions (lanes 1 and 5). At the indicated times, *c-fos, fra-1, fra-2, fosB/ΔfosB,* or *gapd* mRNA levels were determined by semi-quantitative RT-PCR as described in Experimental Procedures.

B. In parallel experiments, MC3T3 cells and HUVEC cells were kept either under static conditions (grey bars) or were exposed to laminar flow (black bars) for 20 min. After that, cells were kept in the chamber for three additional minutes, and nitrate plus nitrite (NO_x) concentrations were measured in the media collected from the chamber. NO_x production was calculated as nmol/min/10^6 cells over the three min interval.

C. MC3T3 cells were kept under static conditions for 60 min (grey bar), or were exposed to laminar flow (black bars) for 20 min; media was harvested from the flow chamber at the indicated times after the onset of flow to measure NO_x concentrations (expressed in μM).

D. hPOBs were kept under static conditions (lane 1) or were exposed to laminar flow for up to 20 min (lanes 2-4); cells in lane 5 were exposed to 20 min of flow and harvested 10 min later. Cell lysates were analyzed by SDS-PAGE/Western blotting using antibodies specific for VASP phosphorylated on Ser^{259} (upper panel), or for α-tubulin (lower panel).

Suppl. Fig. 2



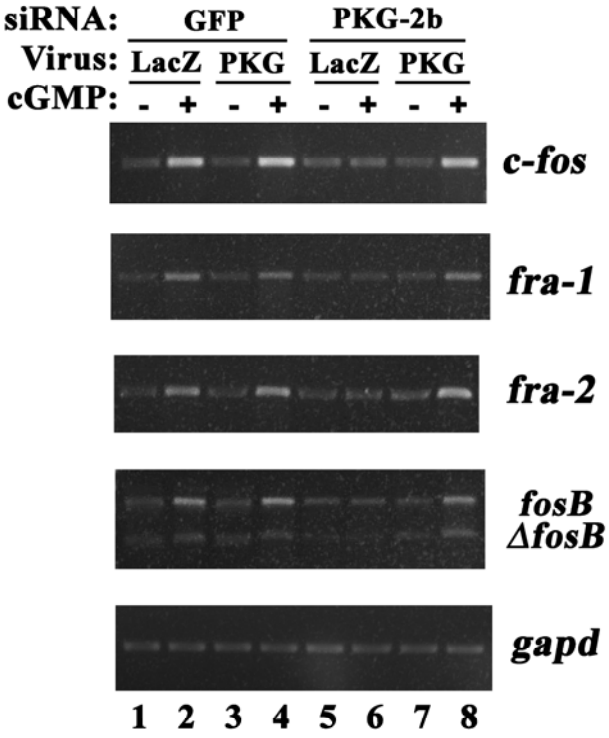
Supplemental Fig. 2:

A. MC3T3 cells were preincubated for 1 h in the presence of media alone (lanes 1 and 2), 10 μM BAPTA-AM (lane 3), or 5 mM EGTA (lane 4). After that, cells were either kept under static conditions (lane 1), or were exposed to laminar flow for 20 min (lanes 2-4), and 10 min later, *c-fos, fra-1, fra-2, fosB/ΔfosB* and *gapd* mRNA levels were determined by semi-quantitative RT-PCR as described in Experimental Procedures.

B. Cells were treated for 30 min with the calcium ionophore A23187 (0.3 μM, lanes 2 and 4) and/or 8-pCPT-cGMP (50 μM, lanes 3 and 4), and *c-fos, fra-1, fra-2, fosB/ΔfosB* and *gapd* mRNA levels were determined as in panel A.

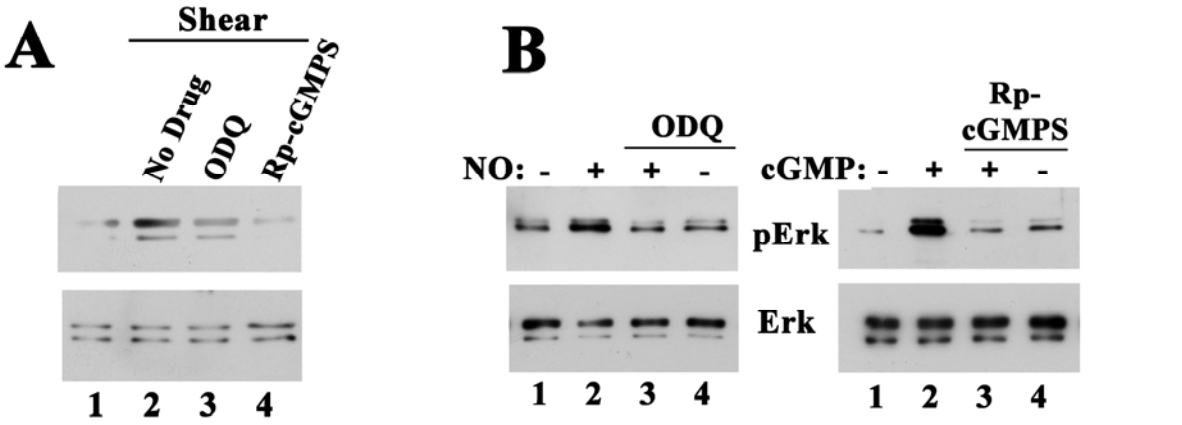
C. Cells were incubated for 20 min with the calcium ionophore A23187 (0.3 μM, lanes 2 and 4) and/or 8-pCPT-cGMP (50 μM, lanes 3 and 4), and cell lysates were analyzed by Western blotting using a phospho-Erk1/2-specific antibody (upper panel); duplicate blots were probed with an antibody recognizing Erk1/2 irrespective of their phosphorylation state (lower panel).

Suppl. Fig. 3



Supplemental Fig. 3:
MC3T3 cells were transfected with either GFP siRNA (lanes 1-4) or the mouse PKG II-specific siRNA PKG-2b (lanes 5-8); 8 h later, cells were infected with control virus encoding β-galactosidase (LacZ, lanes 1, 2, 5, and 6) or virus encoding siRNA-resistant rat PKG II (lanes 3, 4, 7, and 8). Forty-eight hours later, cells were treated with 8-pCPT-cGMP for 1 h, and *fos* family gene expression was determined by semiquantitative RT-PCR as described in Experimental Procedures.

Suppl. Fig. 4



Supplemental Fig. 4:

A. Serum-deprived hPOBs were incubated for 1 h without drug, with 10 μM ODQ, or Rp-8-pCPT-PET-cGMPS as indicated, prior to a 10 min exposure to laminar flow (lanes 2-4); cells in lane 1 were kept under static conditions (sham-treated). Erk phosphorylation was assessed as described in Figs.7-10 and Supplemental Fig. 2C, using an antibody specific for Tyr-phosphorylated Erk1 ($pTyr^{204}$, clone E-4).

B. Serum-deprived hPOBs were incubated for 1 h with ODQ or Rp-8-pCPT-PET-cGMPS as indicated on top, prior to receiving 10 μM PAPA-NONOate (NO, left panels) or 50 μM 8-pCPT-cGMP (cGMP, right panels) for 10 min as indicated; cells in lane 1 were mock-treated. Erk phosphorylation was assessed using an antibody specific for dually-phosphorylated Erk1 ($pThr^{202}/pTyr^{204}$).