SUPPLEMENTAL DATA

siRNA validation of PTGIR and COX-2 inhibition

Human pulmonary artery smooth muscle cells (Lonza, UK), passage 6,were grown to 50% confluence on a 24 well plate in smooth muscle basal media (SMBM) (Lonza, UK) at 37^0C, 5% CO_2 with 100% humidity. On the day of siRNA transfection the culture volume was reduced to 100µl per well and the transfection mix of 6.6µl of HiPerFect (Qiagen, Hilden, Germany) and 10nM siRNA (Qiagen, Hilden, Germany) in 100µl of DMEM (serum free, phenol red free)(Sigma Aldrich, Poole , UK) was incubated for 30 minutes at room temperature prior to transfection. 3 hrs after transfection the culture volume was increased to a total of 500ul with complete SMBM. Transfections were incubated for 72hrs prior to assay .SMBM media was replaced with serum free media 24hrs before assay. Transfections were conducted in triplicate for each time point. Endothelin-1 (Calbiochem, Nottingham, UK) was added for 0hrs and 2hrs at a final concentration of $1x10^{-8}$M. Time points were terminated with a single wash of phosphate buffered saline, total RNA extracted and RT-QPCR performed for each target gene as detailed in the Materials and Methods section. All measurements represent the mean +/- SEM of 3 independent experiments.

siRNA for PTGIR (IP-receptor)

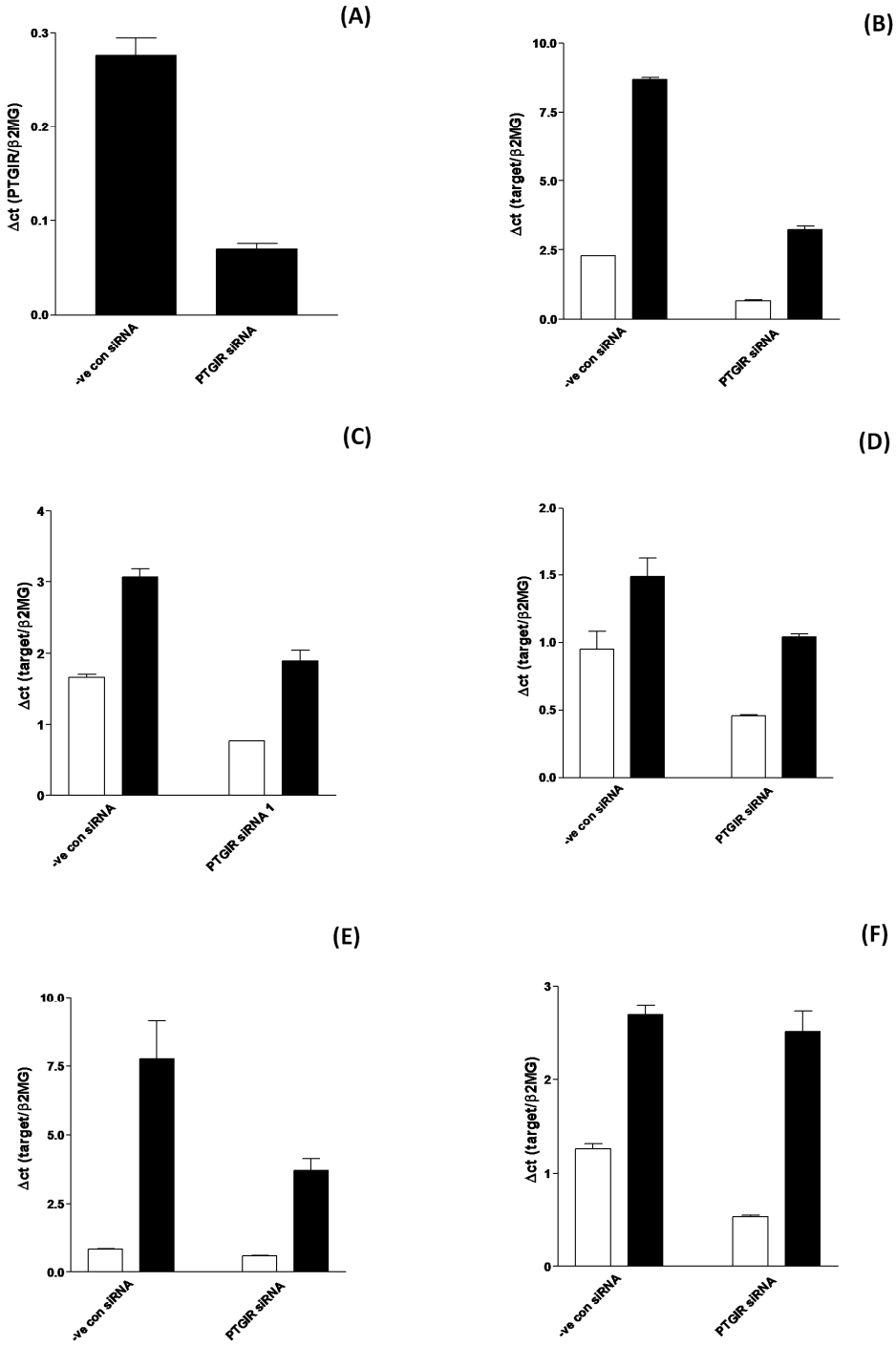
All siRNA were purchased from Qiagen (Hilden, Germany) in the Flexitube, 1nM, format. siRNA were reconstituted according to the manufacturers protocol.

Target gene	Assay name	product name	Catalogue number
IP receptor	PTGIR siRNA 1	Hs_PTGIR_1 HP	SI00019250
COX-2	COX2 siRNA	Hs_PTGS2_1 HP	SI00019334

Supplemental Figure legends/results;

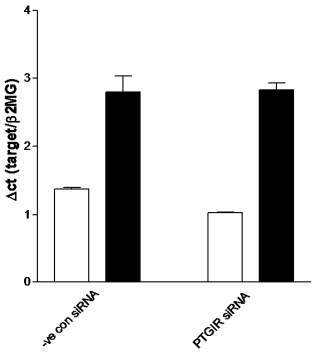
IP-receptor siRNA (PTGIR1) reduced ET-1 induced gene expression for IP dependent genes but not for ET-1 dependent genes in response to ET-1. PTGIR siRNA reduce the accumulation of IP-receptor (PTGIR) after 72 hrs in HPASMC (Supplemental Figure 1 A). PTGIR siRNA reduces the accumulation of amphiregulin (Supplemental Figure 1 B), inhibin-beta-A (Supplemental Figure 1 C), epiregulin (Supplemental Figure 1 D) and follistatin (Supplemental Figure 1 E) post ET-1 treatment of HPASMC for 2 hours. PTGIR siRNA transfection for 72hr does not reduce the accumulation of COX-2 (Supplemental Figure 1 F), HBEGF (Supplemental Figure 1 G), CYR61 (Supplemental Figure 1 H) mRNA, in response to ET-1 for 2 hrs.

Supplemental data figure 1

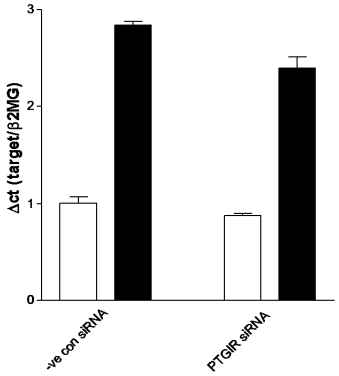


Supplemental data figure 1

(G)



(H)



SUPPLEMENTARY DATA;

Table 1

INHIBITOR/ ANTAGONIST	TARGET	Target agonist or function	REFERENCE
BQ123	ET_a ,G-protein coupled receptor	ET-1	(1)
BQ788	ET_b ,G-protein coupled receptor	ET-1	(2)
Bosentan	ET_a and ET_b G- protein coupled receptort	ET-1	(3)
NS398	Cyclooxygenase -2	Eicsoaniod synthesis	(4)
Indomethacin	Cyclooxygenase -1 and 2	Eicsoaniod synthesis	(5)
cPLA2-I	Phospholipase A_2 (inhibitor)	Arachidonate release from phospholipids	(6)
RO3244794	IP, G-Protein coupled receptor	PGI_2	(7)
AH6809	EP_1 and EP_2 , G-protein coupled receptor	PGE_2	(8)
L161982	EP_4 , G-protein coupled receptor	PGE_2	(9)
EGTA	Calcium /magnesium (non cell permanent chelator)		
BAPTA-AM	Intracellular calcium (chelator)		(10)
Nicardipine	L-type voltage-gated calcium channels	Voltage gated calcium cell entry	(11)
U73122	Phospholipase -C	Cleavage of phospholipids to DAG/IP3	(12)
ω-agatoxin-IVA	P and N-type voltage operated calcium channel inhibitor (at micromolar levels)	Voltage gated calcium cell entry	(13)
ω-conotoxin-MVIIC	N/P/Q type voltage operated calcium channel inhibitor.	Voltage gated calcium cell entry	(14)
ω-conotoxin-GVIA	N-type voltage operated calcium channel inhibitor	Voltage gated calcium cell entry	(15)
Mibefradil	L-type and T-type voltage gated calcium channel inhibitor	Voltage gated calcium cell entry	(16)

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