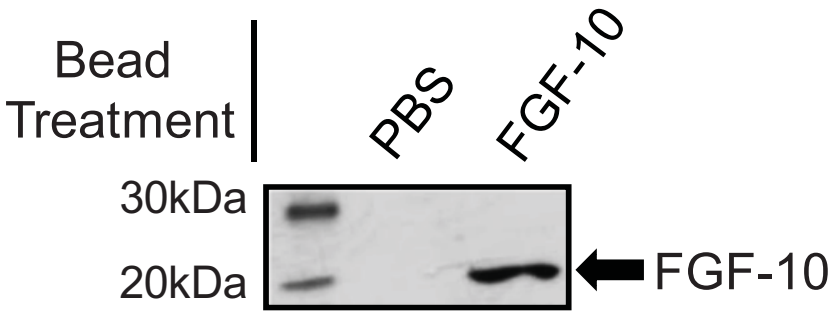
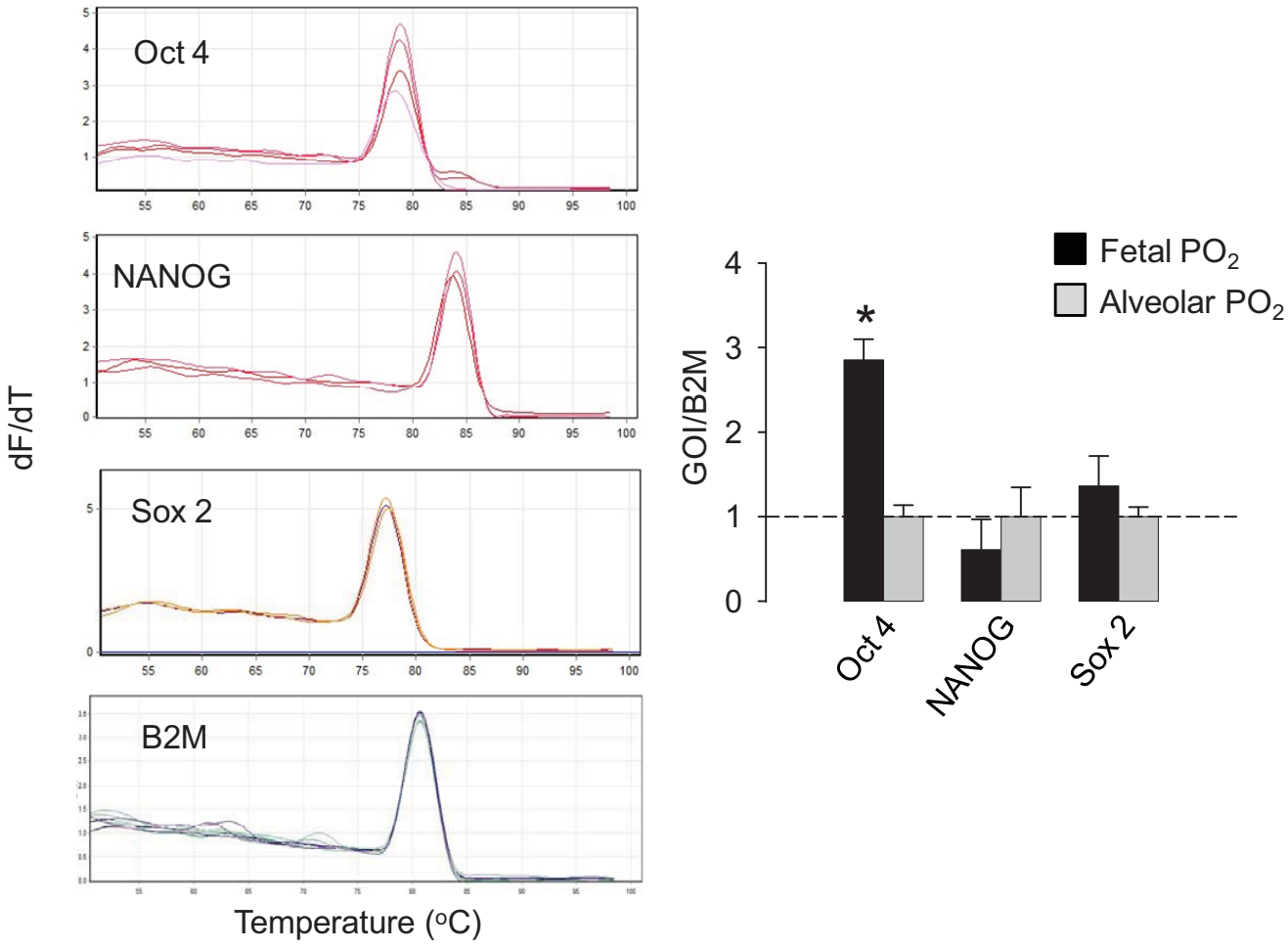
On-Line Supplementary Figure S1.

Confirmation of FGF-10 binding to heparin-coated acrylic beads. Beads were soaked for a minimum of 3h in 1μg.ml^{-1} FGF-10 in PBS with agitation at 4^{o}C. Un-bound FGF-10 was then washed off with three rinses in PBS before grafting of beads into the peripheral mesenchyme of E12 mouse lung explants. Successful coating was confirmed by boiling a sample of control (PBS washed) and FGF-10-coated beads in Laemmi sample buffer and a western blot was performed to detect FGF-10.



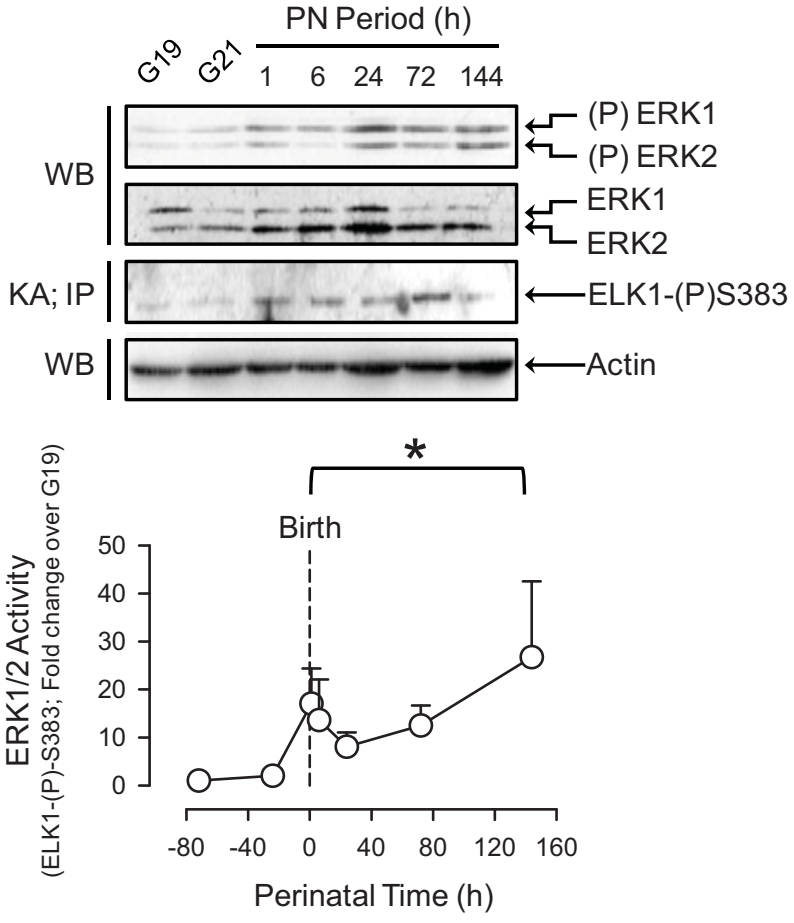
On-Line Supplementary Figure S2.

HELMF cells express pleuripotency markers Oct4, Sox2 and Nanog. HELMF cells were cultured in 6-well plastic culture dishes at fetal (23mmHg) or alveolar (100mmHg) PO_2 for 16 in SFM. Total RNA was isolated using Promega's SV RNA isolation kit and 1μg RNA was reverse transcribed into DNA using Maloney Monkey Leukemia Virus (MMLV) reverse transcriptase. Abundance of Oct4, Nanog and Sox2 were normalised against β2-microglobulin (B2M) expression using Qiagen's Quantitect Primers for each target gene using a Rotorgene Q H2M qPCR machine. Results were calculated using the Pfaffl Method as Expression = $(E_{GOI})^{Ct\ GOI}/(E_{B2M})^{Ct\ B2M}$ where GOI= gene of interest, E is the efficiency of the reaction, Ct is the measured critical threshold value at fetal or alveolar PO_2. E values were: Oct4 (0.86), Sox2 (0.74), Nanog (1.54) and B2M (0.98) and all four genes produced a single peak with melt analysis (shown to left of figure). The histogram shows that Oct4, Nanog and Sox2 are expressed in HELMF and that Oct4 expression is significantly induced by culture of HELMF at fetal PO_2. Results are expressed as the fold difference of each gene at fetal compared to alveolar PO_2 and are mean ± standard error from 4 independent RNA preparations. *$P<0.05$ relative to alveolar PO_2, paired Students t-test.


Oct 4
NANOG
Sox 2
B2M
dF/dT
Temperature (°C)
Fetal PO_2
Alveolar PO_2
GOI/B2M
*
Oct 4
NANOG
Sox 2

On-Line Supplementary Figure S3.

ERK1/2 activity is augmented from birth in the perinatal rat lung. Rat lungs were obtained from fetuses and neonates at the times shown and disrupted in kinase lysis buffer by 3 x 30s passes of an Ultra Tarrax homogeniser. The cleared protein lysate was used to determine perinatal ERK1/2 (T202/Y204) phosphorylation by western blotting (WB) and kinase activity by immunoprecipitation (KA;IP) of recombinant ELK-1 followed by WB using an ELK-(P)S383 specific antibody. Blots are representative of 4 lungs sampled from independent litters at each time point. Compiled ELK-1 S383 phosphorylation data is shown graphically and reveals a statistically significant increase in ERK1/2 activity over the early postnatal period (*P<0.05; n=4). Data is expressed as fold relative to the level of phosphorylation detected at G19 (-72h).

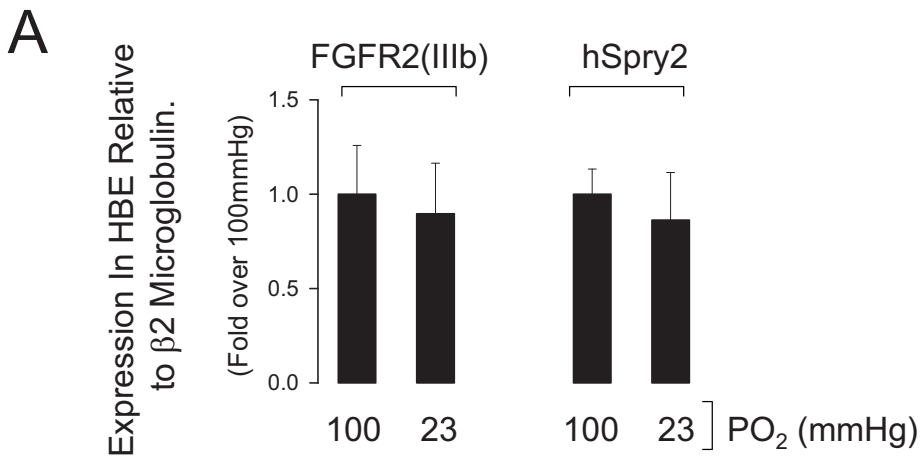


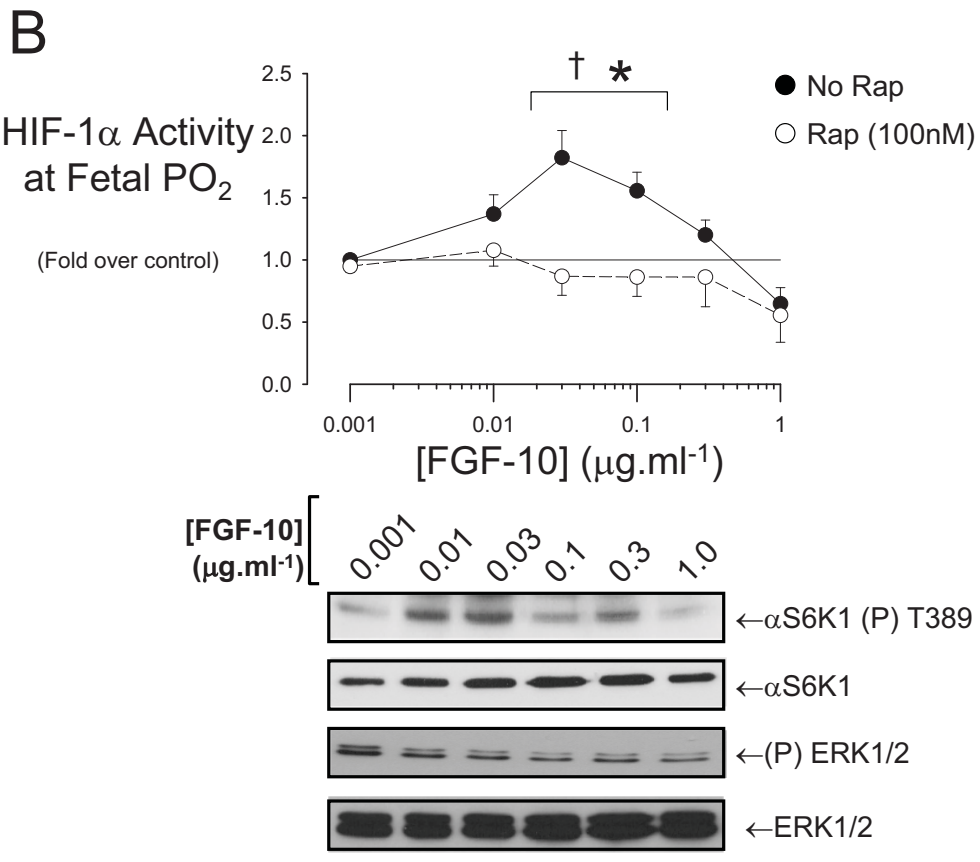
On-Line Supplementary Figure S4.

FGF-10 modulates HIF-1α activity via mTORC1 at Fetal PO_2 in HBE.

A. HBE express the high-affinity FGF-10 receptor, FGFR2(IIIb) and hSpry2 independently of oxygen tensions. Quantitative PCR was performed for these genes in HBE cultured for 24h at alveolar (100mmHg) or fetal (23mmHg) PO_2. n=4. To determine if HBE express FGFR2b (Genbank Accession Number NM-022972.2), qPCR, primers were designed to yield a 130bp product spanning the Ava1 restriction site unique to the IIIb splice variant. The primers were: Forward: 5' CTG CAA GGT TTA CAG TGA TGC CC 3'; Reverse: 5'GGA ACT ATT TAT CCC GAG TGC TTG 3'. Sequencing was performed on a larger, 616bp product spanning the same sequence (Forward: 5' CTG CAA GGT TTA CAG TGA ATG CCC 3'; Reverse: 5' CAG TGT CAG TTA TCT CTT GGA AAC 3'). Human Sprouty 2 (hSpry2) expression was determined using Qiagen's Quantitect Primer Assay for this gene and all results were standardised to β2 microglobulin (B2M) (B2M Quantitect Primer Assay) using the Pfaffl Method as in figure S1.

B. HBE transfected with a HIF reporter gene were cultured for 2h at fetal PO_2 (23mmHg) and then exposed for a further 2h to increasing concentrations of FGF-10 in SFM in the presence or absence of rapamycin (100nM). Values are mean±SEM, n=5 . *P<0.05 relative to respective rapamycin treatment; †P<0.05 relative to control, n=5. Blots below show induction of mTORC1 activity in the absence of ERK1/2 phosphorylation.


A
Expression In HBE Relative to β2 Microglobulin.
(Fold over 100mmHg)
FGFR2(IIIb)
hSpry2
1.5
1.0
0.5
0.0
100
23
100
23
PO2 (mmHg)

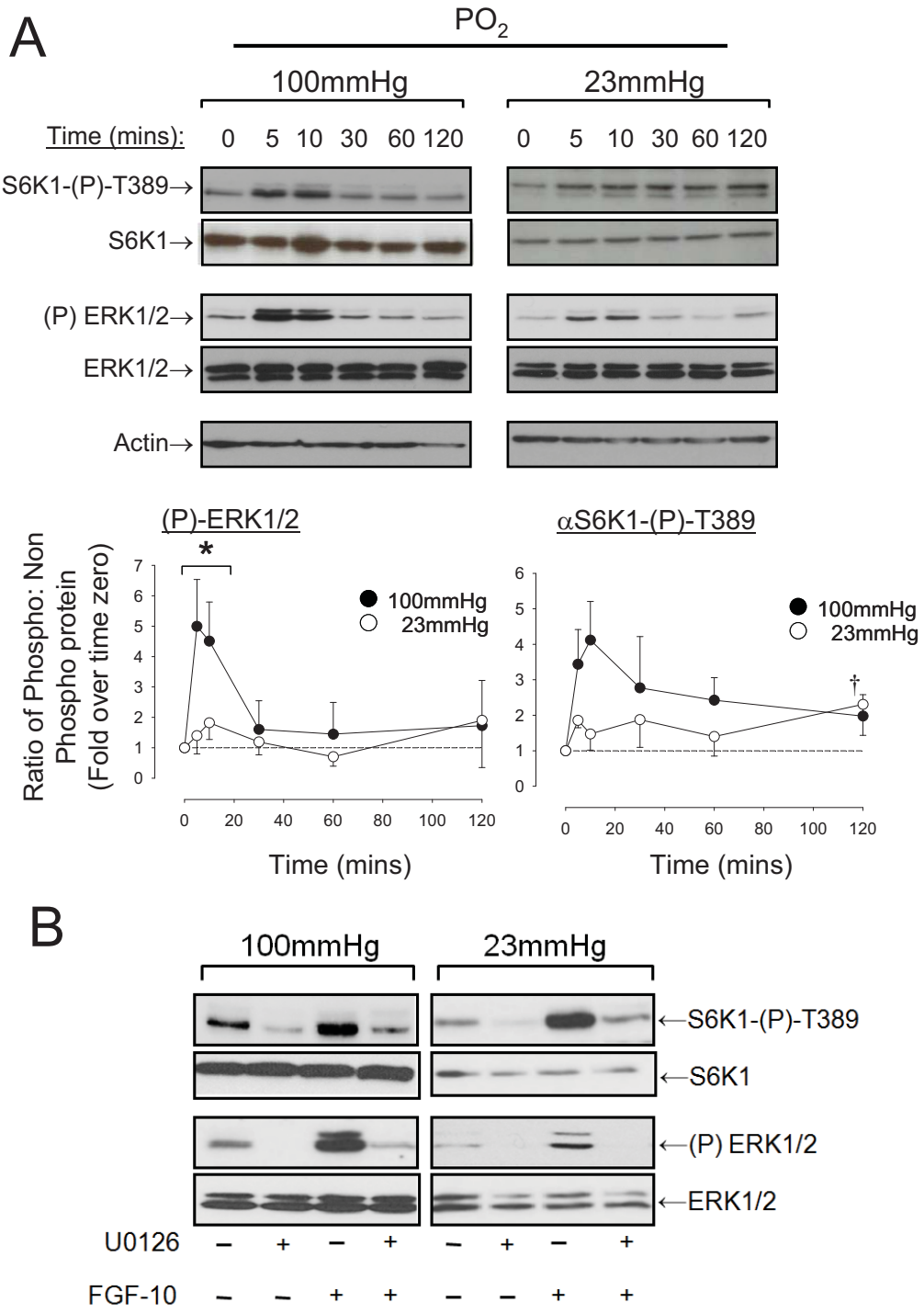

B
HIF-1α Activity at Fetal PO2
(Fold over control)
† *
No Rap
Rap (100nM)
2.5
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
[FGF-10] (μg.ml-1)
[FGF-10] (μg.ml-1)
0.001
0.01
0.03
0.1
0.3
1.0
←αS6K1 (P) T389
←αS6K1
←(P) ERK1/2
←ERK1/2

On-Line Supplementary Figure S5.

FGF-10 signalling in HBE.

A. Representative western blots showing rapid and transient induction of mTOR (S6K1 (T389) phosphorylation) and ERK1/2 activity by 0.1μg.ml^{-1} recombinant human FGF-10 at alveolar (100mmHg) or fetal (23mmHg) PO_2 in HBE maintained in SFM. Histogram presents densitometry data from 4 independent experiments and reveals parallel regulation of mTOR and ERK1/2 by FGF-10 at alveolar PO_2 but protracted mTOR activation despite temporal ERK1/2 inactivation at fetal PO_2. *P<0.01 relative to 0 mins; † P<0.05 relative to 0 mins. Values are mean±SEM, n=4.

B**.** The MEK1 inhibitor, U0126, attenuates FGF-10-evoked ERK1/2 mTOR activity at alveolar and fetal PO_2. Cells were incubated in FGF-10 (100ng.ml^{-1}) for 10mins prior to lysis. Blots are representative of 4 independent experiments.


A
PO2
100mmHg
23mmHg
Time (mins): 0 5 10 30 60 120 0 5 10 30 60 120
S6K1-(P)-T389→
S6K1→
(P) ERK1/2→
ERK1/2→
Actin→
(P)-ERK1/2
αS6K1-(P)-T389
Ratio of Phospho: Non Phospho protein (Fold over time zero)
100mmHg
23mmHg
Time (mins)
B
100mmHg
23mmHg
←S6K1-(P)-T389
←S6K1
←(P) ERK1/2
←ERK1/2
U0126 – + – + – + – +
FGF-10 – – + + – – + +