Supplemental Figure Legends

Figure 1. *In situ* hybridization analysis was performed to detect RIP140 mRNA in WT ovaries at different stages of follicle development. There was an increase in RIP140 expression with eCG injection. A (untreated ovaries), B (6h eCG), C (24h eCG) and D (48h eCG) show expression localised primarily in the granulosa cells of the growing secondary as well as mural (Mu) and cumulus granulosa (Cu) cells of the antral follicles. E and F show expression in the inner layers of the mural granulosa cells and cumulus cells in the unexpanded (3h hCG) and expanded COCs (10h hCG) respectively (Scale Bar: 100 µM).

Figure 2. Quantitative PCR analysis of RIP140 expression in WT COCs. COCs were obtained from 48h eCG treated animals and cultured for 4, 8 and 16h with 100 ng/ml AREG. (n=3-4, each experiment a pool of 2-3 animals). Similar levels of RIP140 expression was maintained throughout this time course.

Figure 3. COCs isolated from WT and RIP140 KO ovaries after eCG treatment, cultured for 8 and 16h in the presence of 100 ng/ml each of AREG, EREG and BTC (Scale Bar: 100 µM).

Figure 4. Cumulus Expansion Index score of the COCs in Fig. S3.

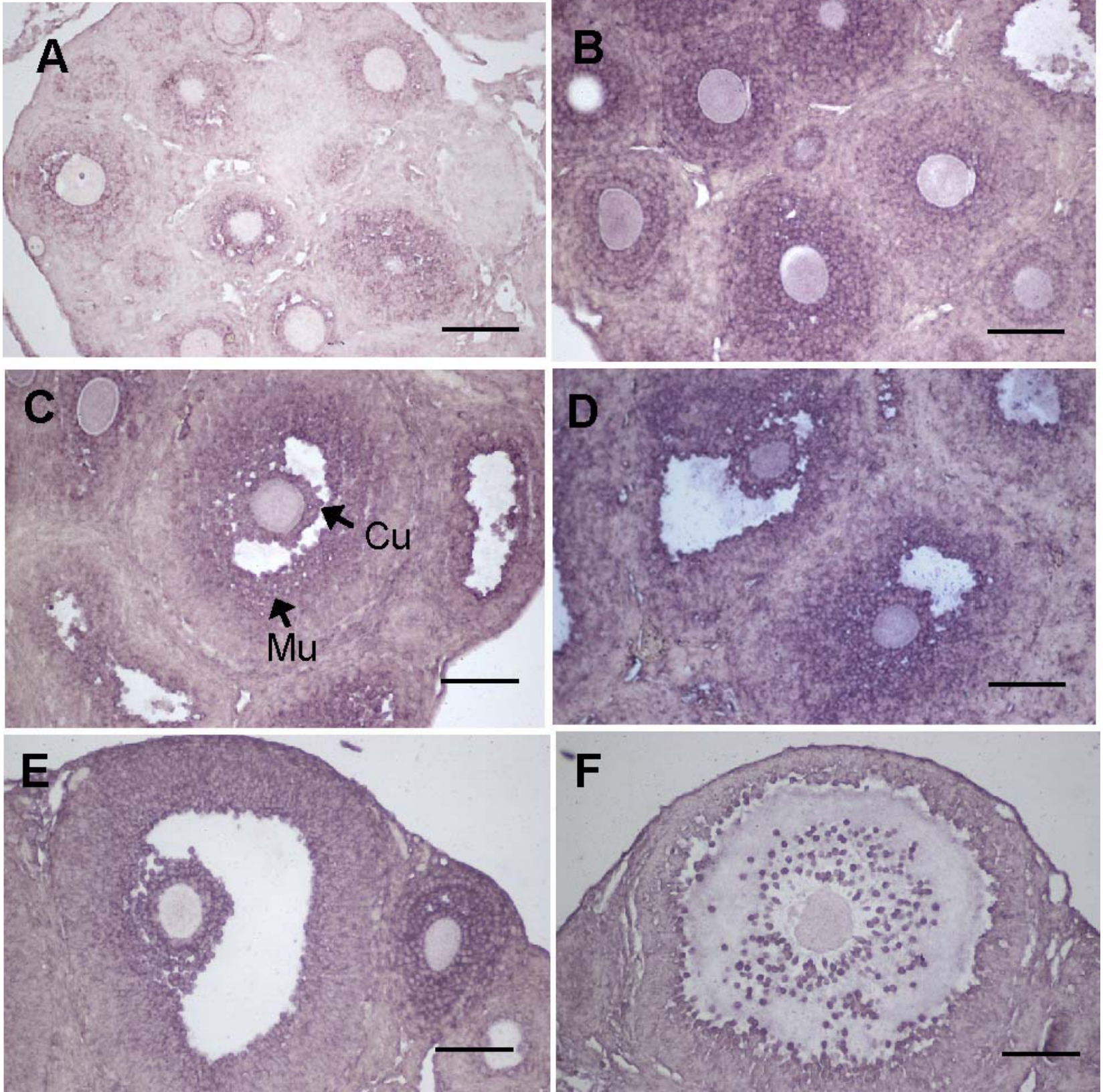
Figure 5. Quantitative PCR analysis of *Ereg* expression in cultured granulosa cells from WT and RIP140 KO ovaries cultured with, 300 ng/ml AREG, 10uM Forskolin or 500 ng/ml PGE2 for 4 hrs.

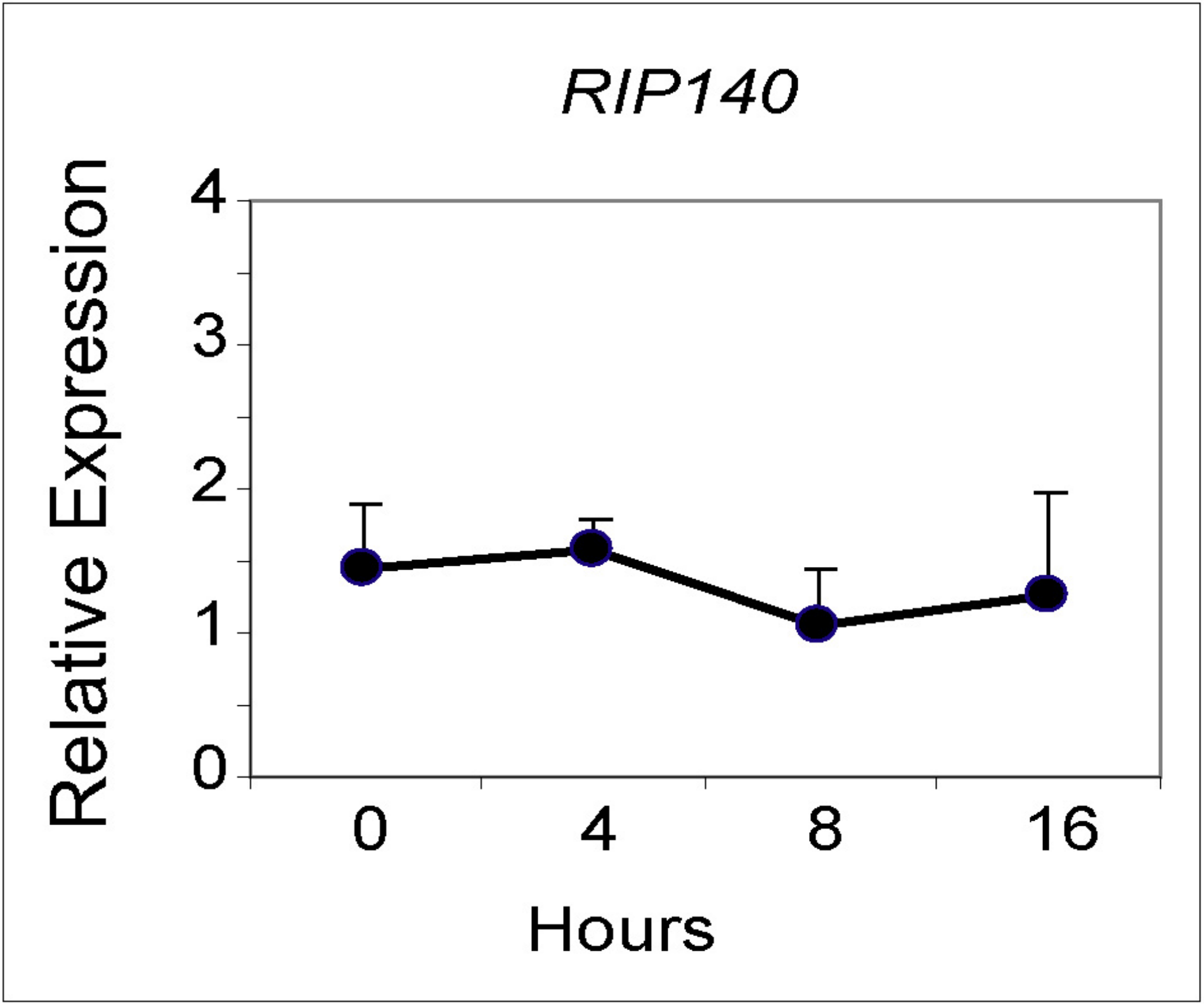
Figure 6. Quantitative PCR analysis of Areg expression in the WTF4 granulosa cells after treatment with forskolin (10uM), AREG (300 ng/ml) and PGE2 (500 ng/ml) at 0h, 1h and 4h.

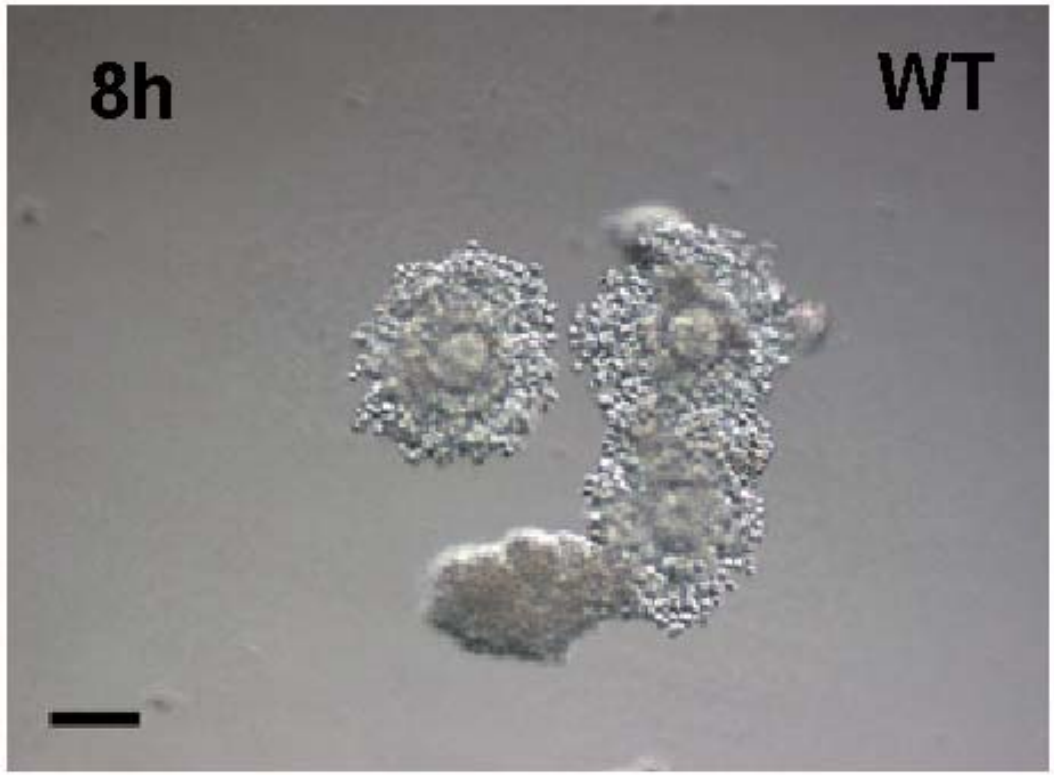
Figure 7. (A) Chromatin immunoprecipitation (ChIP assays) of WTF4 granulosa cells using antibodies specific for RIP140, p-CREB and c-Jun or nonspecific IgG in untreated or forskolin stimulated cells. Precipitated fragments were analysed by real-time PCR using an upstream region of the Areg promoter and GAPDH promoter (n=2).

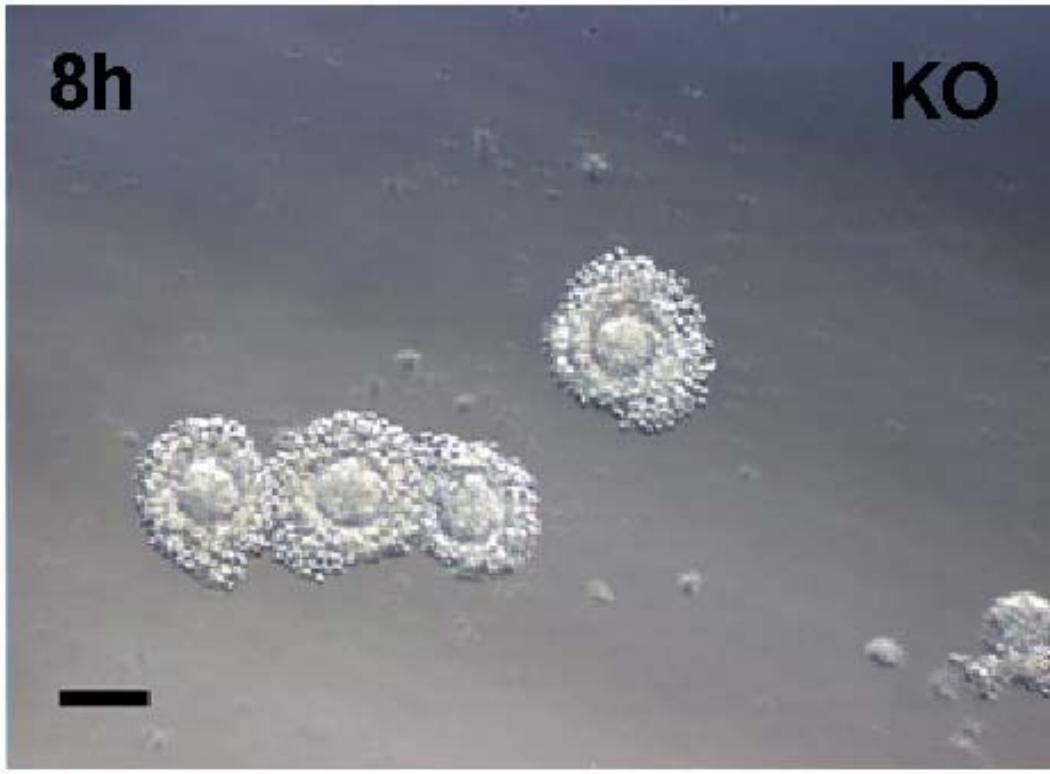
Table 1. Primers used for making *in situ* probes

Table 2. Primers used for Q-PCRs, ChIP assays, deletion constructs and siRNA.

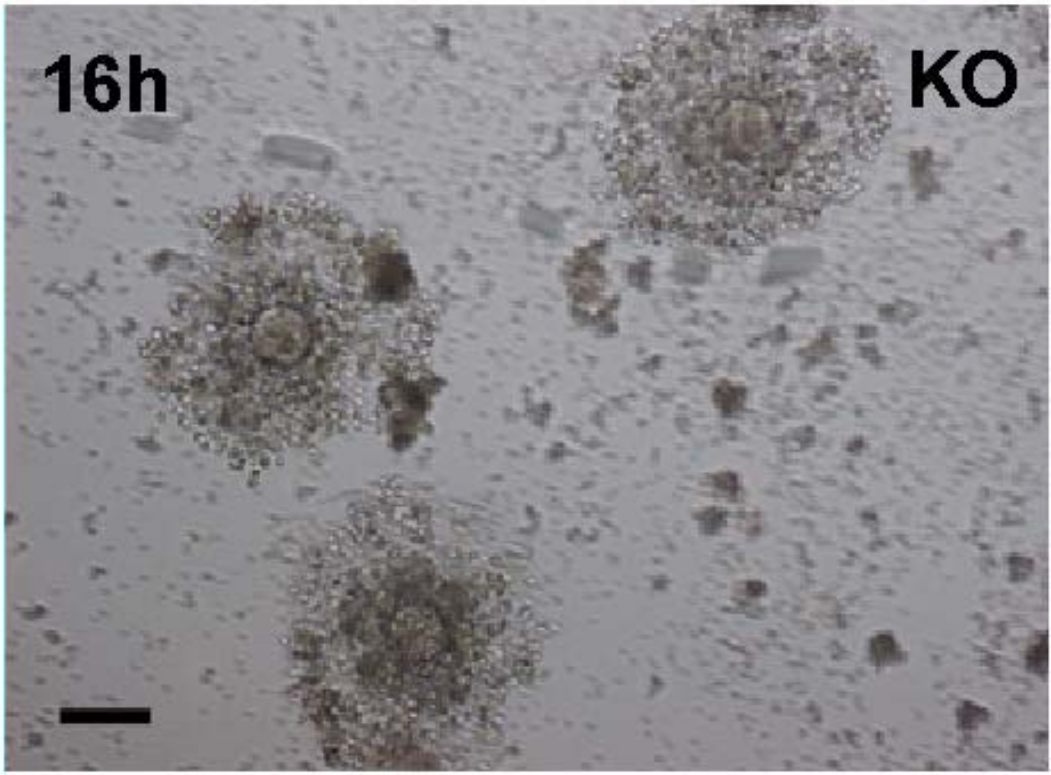

A
B
C
Cu
Mu
D
E
F

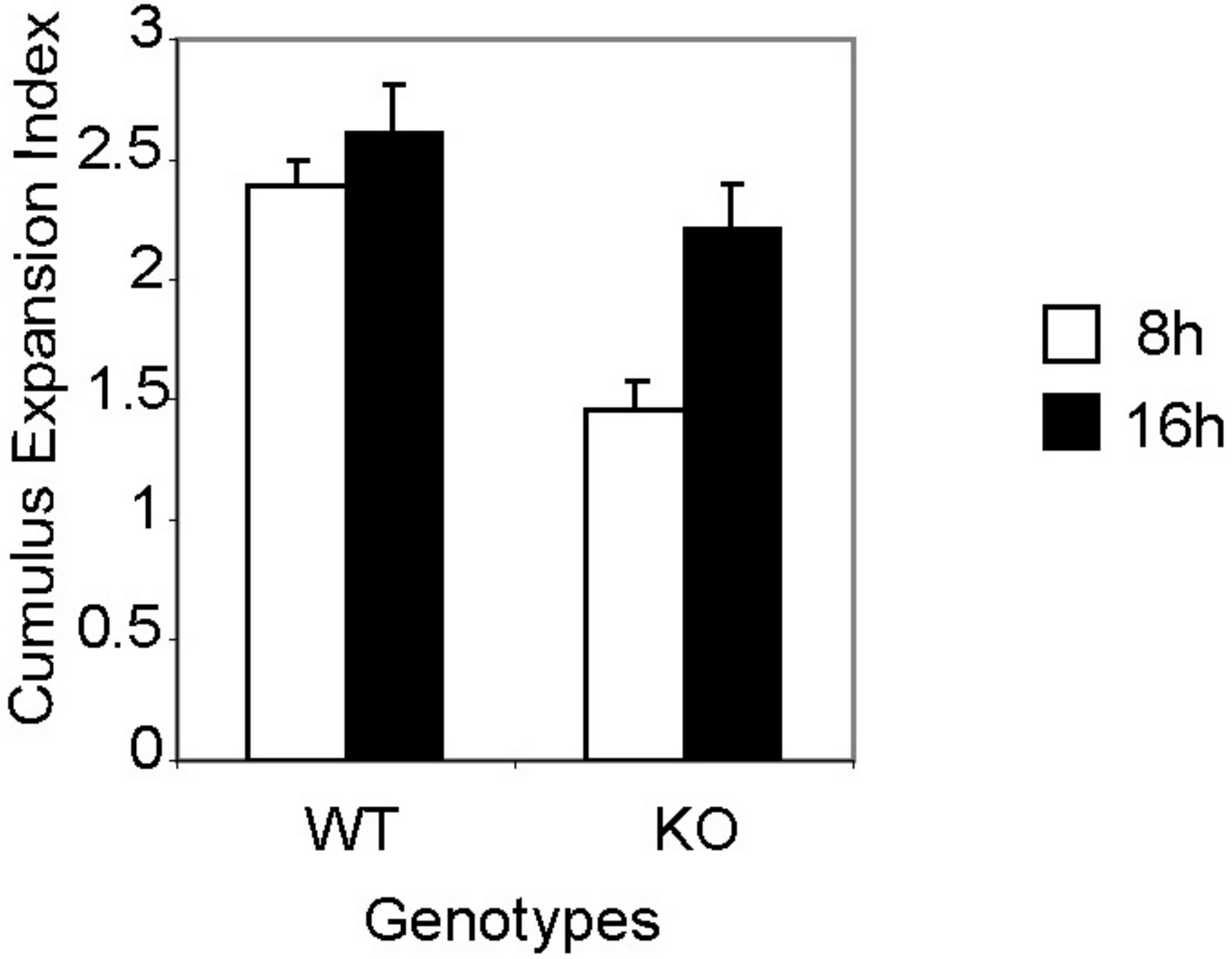

RIP140
Relative Expression
4
3
2
1
0
0
4
8
16
Hours

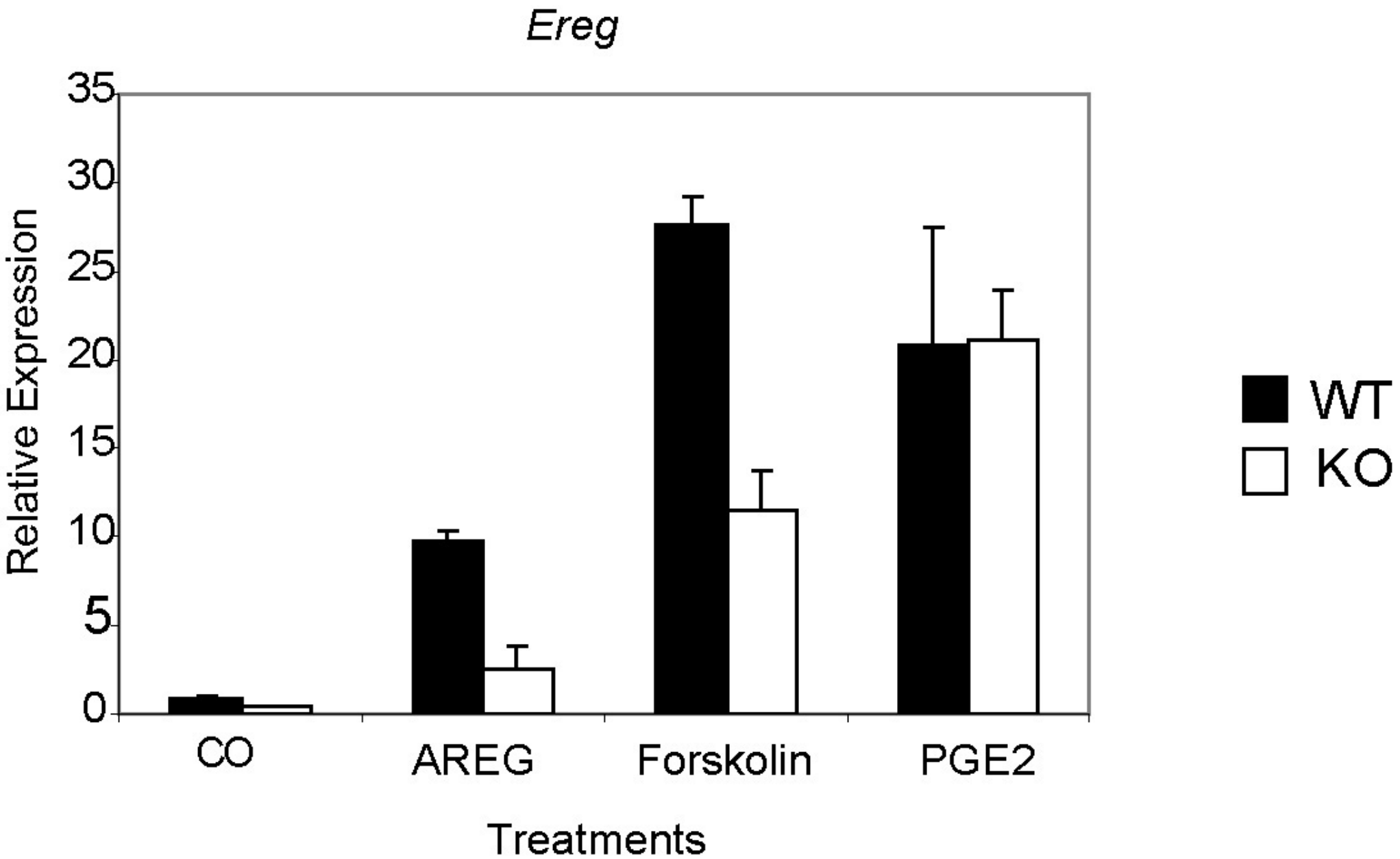

8h
WT

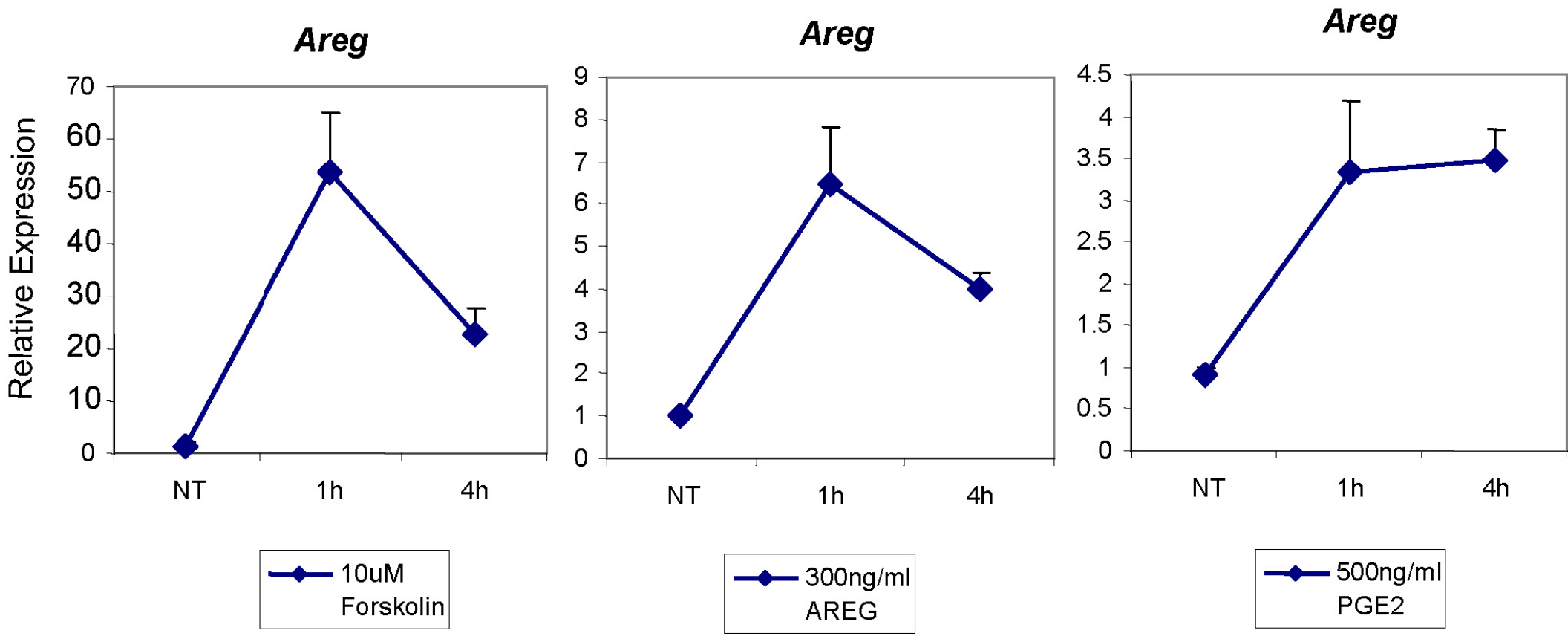

8h
KO


16h
WT


16h
KO


Cumulus Expansion Index
3
2.5
2
1.5
1
0.5
0
WT
KO
Genotypes
8h
16h


Ereg
35
30
25
20
15
10
5
0
Relative Expression
CO
AREG
Forskolin
PGE2
Treatments
WT
KO


Areg
Relative Expression
70
60
50
40
30
20
10
0
NT
1h
4h
10uM Forskolin
Areg
9
8
7
6
5
4
3
2
1
0
NT
1h
4h
300ng/ml AREG
Areg
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
NT
1h
4h
500ng/ml PGE2

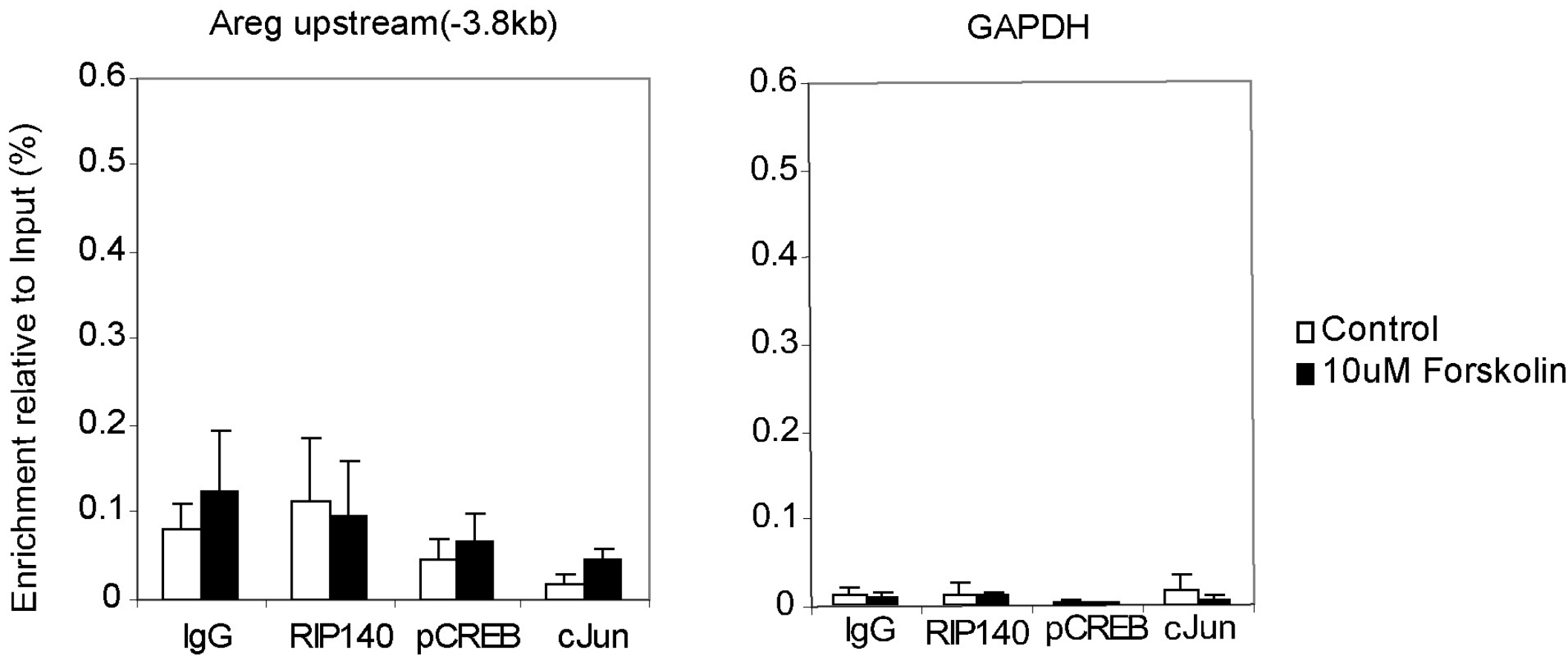

Areg upstream(-3.8kb)
GAPDH
Enrichment relative to Input (%)
0.6
0.5
0.4
0.3
0.2
0.1
0
IgG
RIP140
pCREB
cJun
Control
10uM Forskolin

Table 1: Primers Used for In Situ hybridization analysis

Primer Name	Primer Sequence
AregInSituF	TGGCAGTGAACTCTCCACAG
AregInSituR	CAATTGCATGTCACCACCTC
EregInSituF	CGCTGCTTTGTCTAGGTTCC
EregInSituR	CGCAACGTATTCTTTGCTCA
BtcInSituF	TGGTAGCAGATGGGAACACA
BtcInSituR	TGAACACCACCATGACCACT
mRIPInSituF1	AGACATTGCAGCAGAGCCCCGAAC
mRIPInSituR1	CGAGGCCGCCGCGGCTCCGGGCCGCC

Primers used for Q-PCR	
Gene	**Primer Sequences, Forward Primer (F), Reverse Primer (R) 5'-3'**
Areg	F:TCCGGCTATATTATAGATGATTCAGTCA
	R:TCTCCTTCTGTCTTGTTTTTCTTGG
Ereg	F:GCTCCCTGCCTCTTGGGT
	R:TGCCTGTAGAAGGTGGGAACC
Btc	F:CCAATGGCTCTCTTTGTGGAG
	R:TGGGTTTTCACTTTCTGTCTAGGG
Nrip1	F:CCCCAGTACCAACAGGACTACC
	R:TGAACGTGGCGGAATTTTGT
RPL7	F:AGCGAGGCTACGGCAAAA
	R:GAGACCGAGCAATCAAGGAATT
Tnfaip6	F:GGTGGTCGTCTCGCAACCTA
	R:CAAGCAGCACAGACATGGAA
Has-2	F:CAGACAGGCGGAGGACGA
	R:AGAAACCTCTCACAATGCATCTTG
Ptx-3	F:TTGCTGAGACCTCGGATGAC
	R:GCGAGTTCTCCAGCATGATGA
Primers for generating Amphiregulin deletion constructs	
Areg -935/+65F:	TAGCCCCTCACATTTGCTCT
Areg -758/+65F:	TTTCTTCTGCCCAGCAAC
Areg -574/+65F:	CACACTTATCCCCAGCAA
Areg -341/+65F:	TCAAAGTCTTCGGGCTAA
Areg -158/+65F:	AGTTTCTCCCCGCGTAAT
AregR :	TCAGGCTCAGCTGGAGATGT
Primers used in the ChIP assays	
Areg CRE (0.2kb)	F:CCGGTGGAACCAATGAGAACT
	R:TGAGCCTAAGACCAGCAGCAA
Areg Upstream(-3.8kb)	F:CACTGACAATTCTGAAGGTGCTTACT
	R:TGATGGTTACAGCAAAAGCACACT
GAPDH	F:TTCCTGAAGCCTGGAAGGAG
	R:GCCAGCCTTGGTCTACAGAG
Primers used in RNA interference assays	
siControl	5'UUCUCCGAACGUGUCACGUTT3'
	5'ACGUGACACGUUCGGAGAATT3'
siCREB	5'GGACCUUUACUGCCACAAATT3'
	5'UUUGUGGCAGUAAAGGUCCTT3'
sic-Jun	5'CAGUAACCCUAAGAUCCUATT3'
	5'UAGGAUCUUAGGGUUACUGTA3'

Table 2