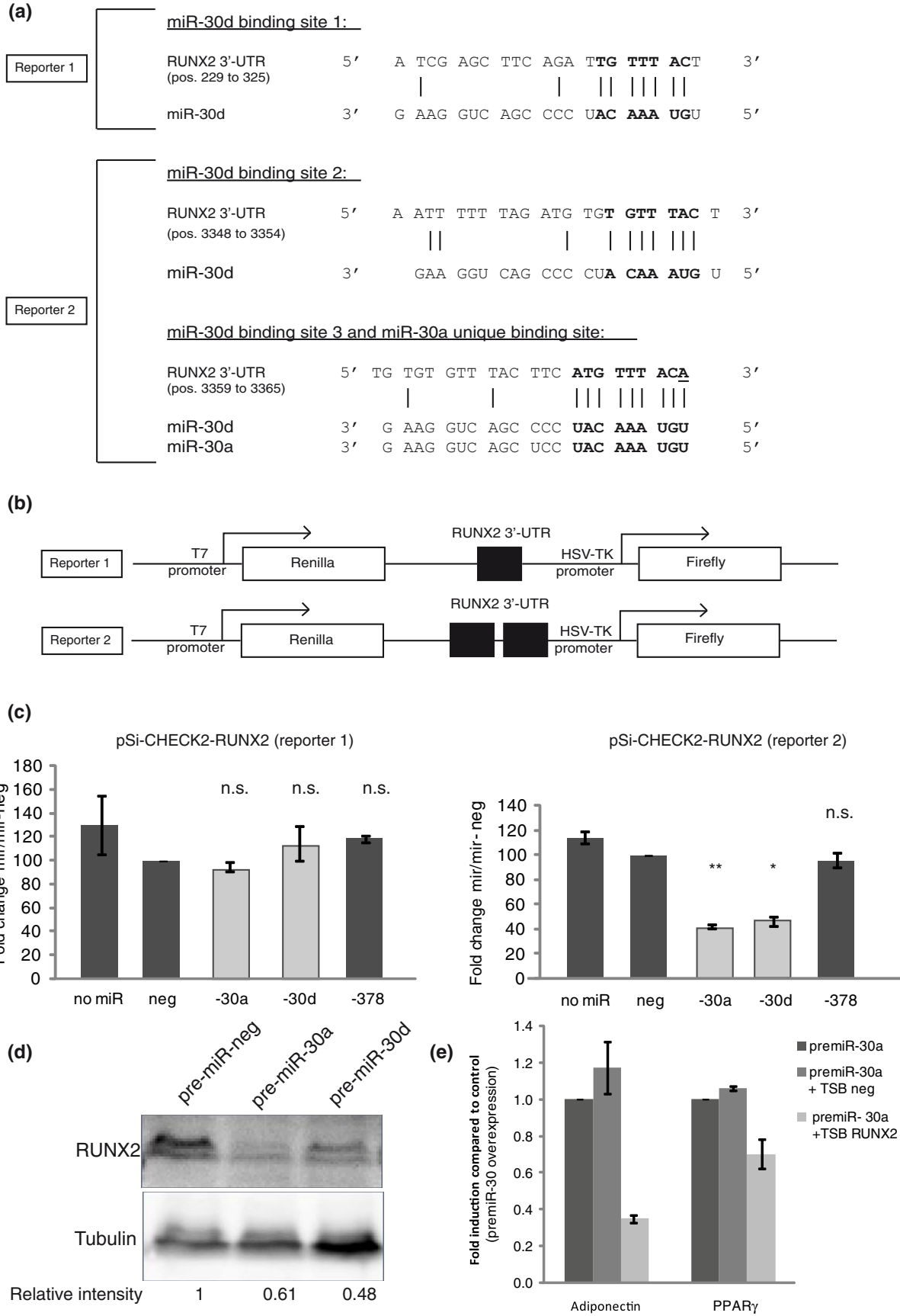
(a)
Reporter 1
miR-30d binding site 1:
RUNX2 3'-UTR (pos. 229 to 325) 5' A TCG AGC TTC AGA TTG TTT ACT 3'
miR-30d 3' G AAG GUC AGC CCC UAC AAA UGU 5'
Reporter 2
miR-30d binding site 2:
RUNX2 3'-UTR (pos. 3348 to 3354) 5' A ATT TTT TAG ATG TGT GTT TAC T 3'
miR-30d 3' GAA GGU CAG CCC CUA CAA AUG U 5'
miR-30d binding site 3 and miR-30a unique binding site:
RUNX2 3'-UTR (pos. 3359 to 3365) 5' TG TGT GTT TAC TTC ATG TTT ACA 3'
miR-30d 3' G AAG GUC AGC CCC UAC AAA UGU 5'
miR-30a 3' G AAG GUC AGC UCC UAC AAA UGU 5'
(b)
Reporter 1
T7 promoter
Renilla
RUNX2 3'-UTR
HSV-TK promoter
Firefly
Reporter 2
T7 promoter
Renilla
RUNX2 3'-UTR
HSV-TK promoter
Firefly
(c)
pSi-CHECK2-RUNX2 (reporter 1)
Fold change mir/mir-neg
180
160
140
120
100
80
60
40
20
0
n.s.
n.s.
n.s.
no miR
neg
-30a
-30d
-378
pSi-CHECK2-RUNX2 (reporter 2)
Fold change mir/mir - neg
140
120
100
80
60
40
20
0
**
*
n.s.
no miR
neg
-30a
-30d
-378
(d)
pre-miR-neg
pre-miR-30a
pre-miR-30d
RUNX2
Tubulin
Relative intensity 1 0.61 0.48
(e)
Fold induction compared to control (premiR-30 overexpression)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
premiR-30a
premiR-30a + TSB neg
premiR- 30a +TSB RUNX2
Adiponectin
PPARγ