A
Exon
1 2 3 4 5 6 7 8 9 10 11 12
M1
M2
Alternative splicing
5'
3'
PKM1 mRNA
PKM2 mRNA
B
CAG
muPKM1 cDNA
IRES
dGFP
muPKM2 cDNA
mCherry
U6
Sense
loop
Antisense
Candidate short hairpins
C
muPKM1-IRES-dGFP
muPKM2-IRES-dGFP
dGFP
mCherry
No sh
PKM1+2 sh
PKM2 sh
D
UT
No sh
PKM1+2sh
sh# 24
sh# 38
sh# 39
sh# 41
sh# 61
Scrambled
anti-PKM1
anti-GAPDH
anti-mCherry
CAG-FLAG-muPKM1
+
CAG-mCherry
Transfection
anti-PKM2
anti-GAPDH
anti-mCherry
CAG-FLAG-muPKM2
+
CAG-mCherry
Transfection
E
PKM2 sh:
-
+
-
+
anti-FLAG
anti-GAPDH
FLAG-muPKM1
FLAG-muPKM2
F
human
mus
huPKM2-IRES-dGFP
huPKM2-IRES-dGFP + PKM2sh
G
PKM1+2sh
PKM1+2sh + huPKM2
ONL
4/6
2/6
IS+OS (microns)
NS
****
CAG-mGFP
PKM1+2sh
CAG-huPKM2
H
Rho-cre> PKM2fl/fl
IS
PKM1
ONL
PKM1 DAPI
I
PKM2 sh + CAG-mGFP
mGFP
PKM1
ONL
DAPI
mGFP PKM1
DAPI
J
CAG-FLAG-muPKM1
CAG-FLAG-muPKM2
Untransfected
anti-FLAG
anti-GAPDH