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Supplemental Material

Supplemental Figure 1. Comparison of the 5' leader sequences of IFRD1 TR1 and TR2
The 5' leader sequences of the two IFRD1 transcripts are shown. TR2 contains a 260 nt region that is not found in TR1. TR1 contains a unique 46 nt region corresponding to the gap in the TR2 sequence. The remaining 186 nts are identical. The TR1 5' leader encodes a 156 nt ORF. The start and stop codons are outlined and the ORF is underlined. The AUG start codon of the major ORF is also outlined.

Supplemental Figure 2. Tm sensitivity is not influenced by mRNA levels
HeLa tet-off cells were transfected with either 5'TR1-KC (5μg/dish) or 5'TR2-KC (0.2 μg/dish) in order to obtain comparable levels of KC mRNA and 3 hr after transfection were separated into 5 individual Petri dishes and cultured overnight. Cultures were either untreated or treated with vehicle or Tm for the indicated times prior to analysis of KC and GAPDH (not shown) mRNA by northern hybridization and secreted KC protein by Elisa.

Supplemental Figure 3. IFRD1 TR1 5' leader sequence selectively reduces protein production from the major open reading frame
HeLa tet-off cells were co-transfected with either 5'TR1-KC (5 μg/dish) or 5'TR2-KC (0.2 μg/dish) along with a plasmid encoding firefly luciferase (also in pTRE2) and 3 hr after transfection were separated into 3 individual Petri dishes and cultured overnight. One dish was used to prepare total RNA at t = 0 (**a**) while a second dish was washed and incubation continued with fresh medium for 3 hrs. The supernatant was harvested for determination of KC protein by Elisa while the cells were used to prepare total RNA at t = 3 hrs (**b**). KC and GAPDH mRNA levels were determined by northern hybridization. The 3rd dish was used to prepare whole cell extract for determination of luciferase activity to normalize for transfection efficiency. Results are presented as mean +/- 1 S.D. from three separate experiments.

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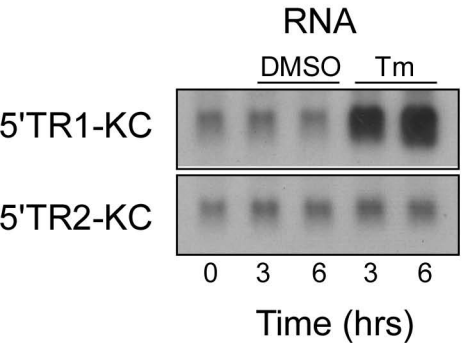
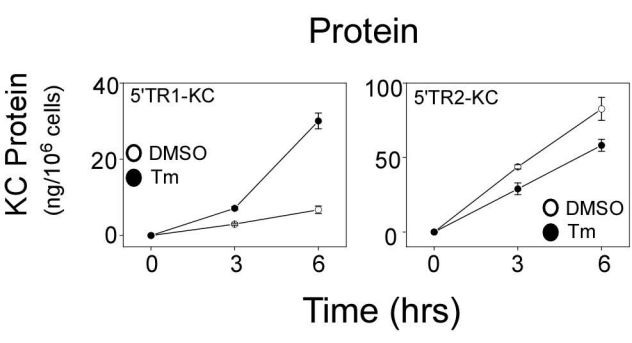
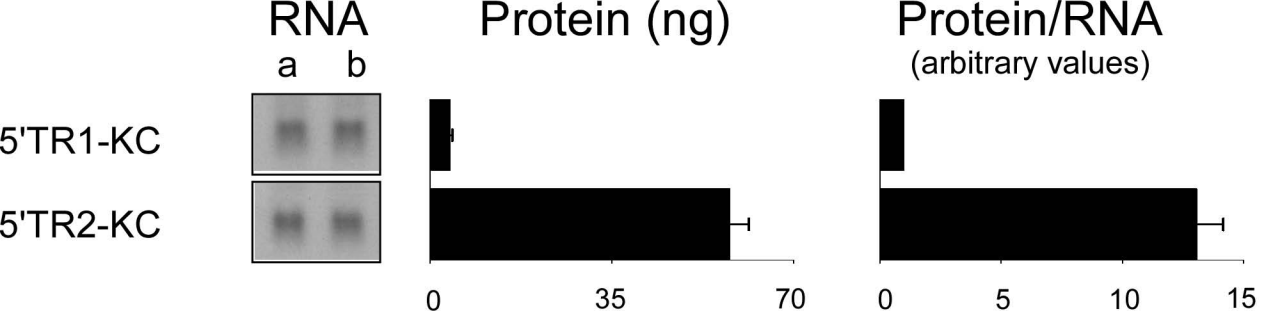
Zhao supplemental fig 1

Zhao supplemental fig 2






RNA
a
b
Protein (ng)
Protein/RNA
(arbitrary values)
5'TR1-KC
5'TR2-KC
0
35
70
0
5
10
15