

## **Supplemental Online Data**

### **- Experimental**

The dicistronic pcDNA3 expression vectors containing the different p110<sup>PITSLRE</sup> fragments (Di-4 and Di-4 mutants) inserted between the *Renilla* (r) and firefly (f) luciferase (luc) genes were obtained by two steps of three-point ligation as follows: i) p110<sup>PITSLRE</sup> and ODC PCR fragments digested with *XbaI-NcoI* were cloned together with the *Renilla* luciferase gene obtained as a *KpnI-XbaI* fragment from pGL3-rluc (see below) in the *KpnI-NcoI* linearized vector pUC19-IRES-lacZ that was described in [Cornelis, 2000 #3]. ii) The rluc-PITSLRE and rluc-ODC inserts were then recovered as *KpnI-NcoI* fragments and cloned in the *KpnI-EcoRI* linearized pcDNA3 expression plasmid together with the firefly luciferase gene obtained as an *NcoI-EcoRI* fragment from the pBluescript-fluc plasmid described below.

The pGL3-rluc vector was made by cloning the *HindIII-XbaI* digested PCR amplification product obtained from pRL-SV40 plasmid (Promega) by using the sense primer, 5'-ATCCCAAGCTTAGCCACCATGACTTCGAAAGTTTATG-3' and the antisense primer, 5'CTAGTCTAGATATTATTCATCATTTTTGAGAACTCGCTCAACGAACG-3', in the *HindIII-XbaI* linearized pGL3-basic vector (Promega). pBluescript-fluc was made by cloning the firefly luciferase gene from the pGL3-basic vector as a *Sall-XbaI* fragment.

The sense and antisense primers used for amplification of the different PITSLRE PCR fragments were as follows: Di-4 sense, 5'-CTAGTCTAGACATCACCGAACGATGAGAGAGG-3', antisense 5'-TTCTTCATCTTCACCCATGGCTTCCTCACTTAC-3'; Di-4 mut A sense, (idem Di-4), antisense, 5'-GCTGTCGCTGATGTCCATGGCTGTAAGTCGGA-3'; Di-4 mut B sense, (idem Di-4), antisense, 5'-CATGCCATGGAGAACCTGAGCCTGATTCTGCTGACGA-3'; Di-4 mut C sense, 5'-TCCTCGTCA GCAGAATCAGGCTCAGGTTCTAGAGGAAGAAGAG-3', antisense (idem Di-4); Di-4 mut D sense, 5'GAGGAAGGGAGCACCATCTAGAGTGAATCAGAGGAGGAA-3', antisense (idem Di-4); Di-4 mut E sense, (idem Di-4), antisense 5'-CATGCCATGGTTCAGAGTTGCTGCCGGTCTCCTCCT-3'; Di-4 mut F sense, (idem Di-4), antisense 5'-CATGCCATGGTTCCTCCTCACGCAGACTGCTCTGATGCCTCCTCAG-3'; Di-2 sense, 5'-CTAGTCTAGAAAAGTGAAAACCTTAGATGAAATTC-3', antisense 5'-TGCATGCCATGGTCCTCTCTCATCGTTCGGTGATG-3'; ODC IRES sense, 5'-CTAGTCTAGAGCTGGCCTGCGGCGCCTGGGCGCTCT

GAGATTG-3; antisense, 5'-CATGCCATGGATTTCTTGATGTTTCCTATGGAA AAC-3'.

- **Supplementary table S1**

**Table S1: Efficiency of different fragments of the PITSLRE IRES to mediate internal initiation of translation.**

Absolute values of Fluc and Rluc activities from the experiment described in Figure 1C.

| <i>Dicistronic vector<sup>a</sup></i> | <i>Fluc activity (IRES-dependent translation)</i> | <i>Rluc activity (cap-dependent translation)</i> | <i>Relative IRES activity (Fluc/Rluc)</i> |
|---------------------------------------|---|--|---|
| Di-4                                  | 6983 ± 932  | 54071 ± 5774                                     | 0.1291 ± 0.0104                           |
| Di-4 mutA                             | 1352 ± 115  | 51902 ± 3704                                     | 0.0261 ± 0.0017                           |
| Di-4 mutB                             | 1539 ± 158  | 54034 ± 4269                                     | 0.0285 ± 0.0012                           |
| Di-4 mutC                             | 8951 ± 1696                                       | 50920 ± 5240                                     | 0.1748 ± 0.0159                           |
| Di-4 mutD                             | 2377 ± 257  | 51643 ± 2677                                     | 0.0459 ± 0.0026                           |
| Di-4 mutE                             | 3784 ± 281  | 58757 ± 4098                                     | 0.0646 ± 0.0063                           |
| Di-4 mutF                             | 3494 ± 334  | 51484 ± 4533                                     | 0.0679 ± 0.0030                           |
| Di-2                                  | 945 ± 118   | 47299 ± 2080                                     | 0.0120 ± 0.0022                           |

a. Cells were transfected with the indicated plasmid as described under the “Experimental” section.

- Supplementary table S2

**Table S2: Increased phosphorylation of eIF-2 $\alpha$  is permissive for PITSLRE IRES activity**

Absolute values of Fluc and Rluc activities from the experiment described in Figure 5C.

| IRES    | PKR/PKRmut(ng) | Fluc activity | Rluc activity      | Relative IRES activity (F/R) |                      |
|---------|----------------|---------------|--------------------|------------------------------|----------------------|
| PITSLRE | PKR            | 50            | 36065 $\pm$ 1245   | 1209638 $\pm$ 11756          | .02981 $\pm$ .00074  |
|         |                | 10            | 30669 $\pm$ 1546   | 1319680 $\pm$ 37983          | .02323 $\pm$ .00050  |
|         |                | 2             | 28223 $\pm$ 192    | 1576169 $\pm$ 76598          | .01793 $\pm$ .00099  |
|         |                | -             | 28007 $\pm$ 1228   | 2273318 $\pm$ 24129          | .01232 $\pm$ .00041  |
|         | PKRmut         | 50            | 49080 $\pm$ 1601   | 3728910 $\pm$ 256290         | .01320 $\pm$ .00134  |
|         |                | 10            | 47096 $\pm$ 873    | 3226873 $\pm$ 17615          | .01459 $\pm$ .00035  |
|         |                | 2             | 44067 $\pm$ 4943   | 3016542 $\pm$ 2399           | .01461 $\pm$ .00165  |
|         |                | -             | 30943 $\pm$ 309    | 2495408 $\pm$ 18796          | .01240 $\pm$ .00030  |
| ODC     | PKR            | 50            | 27837 $\pm$ 1861   | 1601659 $\pm$ 19754          | .01737 $\pm$ .00095  |
|         |                | 10            | 23965 $\pm$ 1541   | 2106625 $\pm$ 217128         | .01140 $\pm$ .00044  |
|         |                | 2             | 15039 $\pm$ 416    | 2343314 $\pm$ 152166         | .00643 $\pm$ .00024  |
|         |                | -             | 13757 $\pm$ 1631   | 3123235 $\pm$ 36856          | .00441 $\pm$ .00058  |
|         | PKRmut         | 50            | 18810 $\pm$ 547    | 4259188 $\pm$ 62737          | .00442 $\pm$ .00020  |
|         |                | 10            | 14623 $\pm$ 67     | 3357854 $\pm$ 326622         | .00438 $\pm$ .00045  |
|         |                | 2             | 15710 $\pm$ 966    | 3433670 $\pm$ 31527          | .00458 $\pm$ .00024  |
|         |                | -             | 13859 $\pm$ 969    | 2983508 $\pm$ 233824         | .00465 $\pm$ .00040  |
| HRV     | PKR            | 50            | 33837 $\pm$ 2459   | 1003753 $\pm$ 1420           | .03371 $\pm$ .00250  |
|         |                | 10            | 44925 $\pm$ 2027   | 1143182 $\pm$ 117398         | .03942 $\pm$ .00228  |
|         |                | 2             | 56437 $\pm$ 274    | 1552047 $\pm$ 59076          | .03640 $\pm$ .00156  |
|         |                | -             | 85848 $\pm$ 10293  | 2326931 $\pm$ 296648         | .03691 $\pm$ .00028  |
|         | PKRmut         | 50            | 314072 $\pm$ 18994 | 7008002 $\pm$ 389473         | .04481 $\pm$ .00022  |
|         |                | 10            | 249111 $\pm$ 12607 | 6064138 $\pm$ 23773          | .04107 $\pm$ .00050  |
|         |                | 2             | 285020 $\pm$ 4032  | 6343979 $\pm$ 45025          | .04493 $\pm$ .000317 |
|         |                | -             | 225319 $\pm$ 20597 | 5505056 $\pm$ 354740         | .04089 $\pm$ .00110  |
| EMCV    | PKR            | 50            | 8696 $\pm$ 522     | 83499 $\pm$ 3895             | .10411 $\pm$ .00134  |

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|        |    |              |                |                   |
|--------|----|--------------|----------------|-------------------|
|        | 10 | 10292 ± 2423 | 99776 ± 11608  | .102432 ± .01236  |
|        | 2  | 14761 ± 843  | 146749 ± 4107  | .10054 ± .00293   |
|        | -  | 24399 ± 5055 | 199899 ± 24353 | .12141 ± .01050   |
| PKRmut | 50 | 41950 ± 2639 | 365555 ± 10582 | .1147 ± .00390    |
|        | 10 | 31113 ± 1292 | 280971 ± 6408  | .11071 ± .00207   |
|        | 2  | 25563 ± 5544 | 249489 ± 18087 | .101924 ± .014831 |
|        | -  | 23882 ± 2249 | 208159 ± 3445  | .114835 ± .01270  |

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