**Supplementary Materials 2**

**Bicistronic expression of SplitΔR:**

**Fig. S1.** (A) Relative expression of CFTR half-molecules, expressed as the ratio of front : back after 24, 48 or 72 h induction with Pon. A (10 µM). The density of each band was normalized to that of wild-type (WT) CFTR before calculating the ratios. Values represent means ± s.e. for n = 3 - 6 Western blots at each time point. *** indicates p<0.022. Density of GST-R (B) and RDpNUT (C) bands in precipitates obtained using antibody against the front (black bars) or back half (white bars) of the SplitDRpIND after treatments as indicated. In (B), * indicates a significant difference in binding to the front (p = 0.005) and back half (p = 0.042) when compared with unphosphorylated control samples. (#) indicates the density measured after co-immunoprecipitation of the R domain with the front half differs significantly from that obtained with the back half (p = 0.019; n=4). Results for (C) are as follows:

<table>
<thead>
<tr>
<th>Exptl. Condition</th>
<th>IP of R domain with front half</th>
<th>IP of R domain with back half</th>
<th>Front vs Back</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean x 10^3</td>
<td>s.d. x 10^3</td>
<td>P vs control</td>
</tr>
<tr>
<td>H7</td>
<td>1.1</td>
<td>0.42</td>
<td>0.001</td>
</tr>
<tr>
<td>Control</td>
<td>3.2</td>
<td>0.23</td>
<td>-</td>
</tr>
<tr>
<td>cAMP</td>
<td>6.9</td>
<td>1.26</td>
<td>0.008</td>
</tr>
</tbody>
</table>
**Supplementary Fig. S1**

**A**
- Graph showing the ratio of expression (Front half / Back half) over different induction times (24 hrs, 48 hrs, 72 hrs).
- Three bars for each induction time,
  - White bar: SplitΔR_{pIND}
  - Black bar: SplitΔR_{pIND} + RD_{pNUT}***

**B**
- Bar chart showing GST-R density levels.
  - IP: Front half
  - IP: Back half
  - Conditions: Unphospho, + PKA

**C**
- Bar chart showing RD_{pNUT} density levels.
  - IP: Front half
  - IP: Back half
  - Conditions: H7, control, cpt-cAMP + IBMX

**Legend:**
- *: Significant difference
- #: Significant difference

### Notes
- Graph A highlights the induction of expression ratios over time, with a significant increase at 72 hrs.
- Graph B and C display phosphorylation differences between front and back half samples under various conditions.