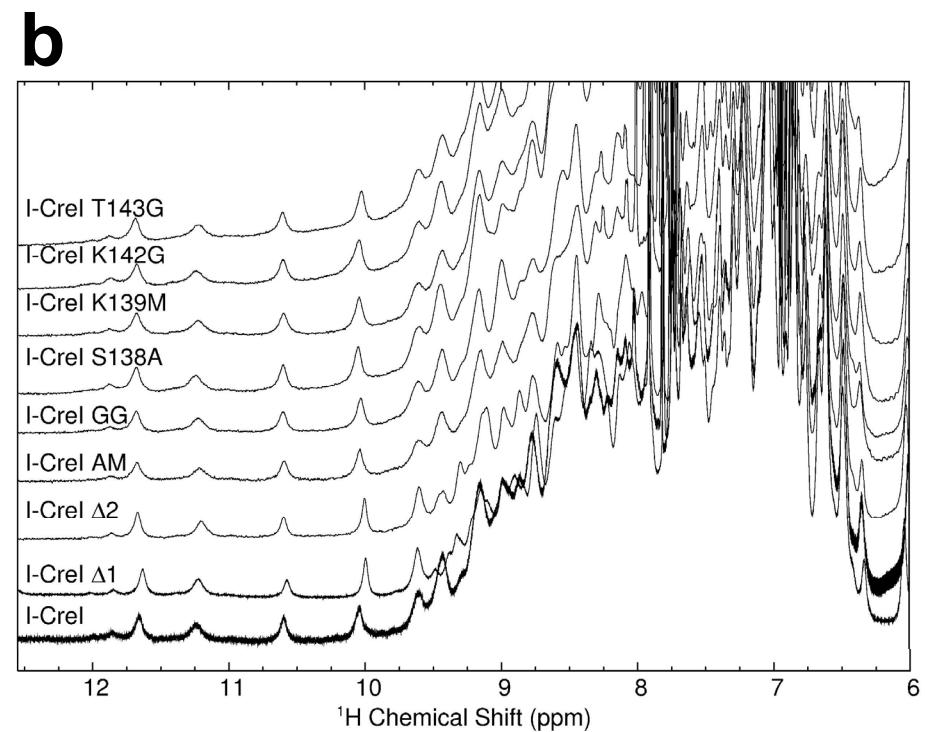
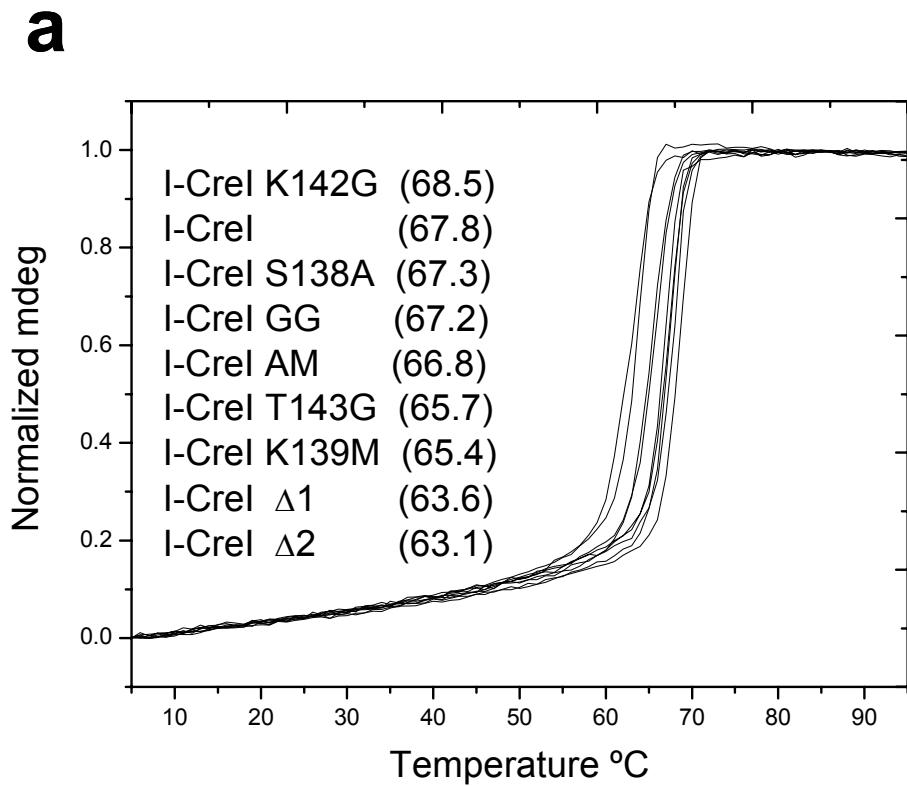
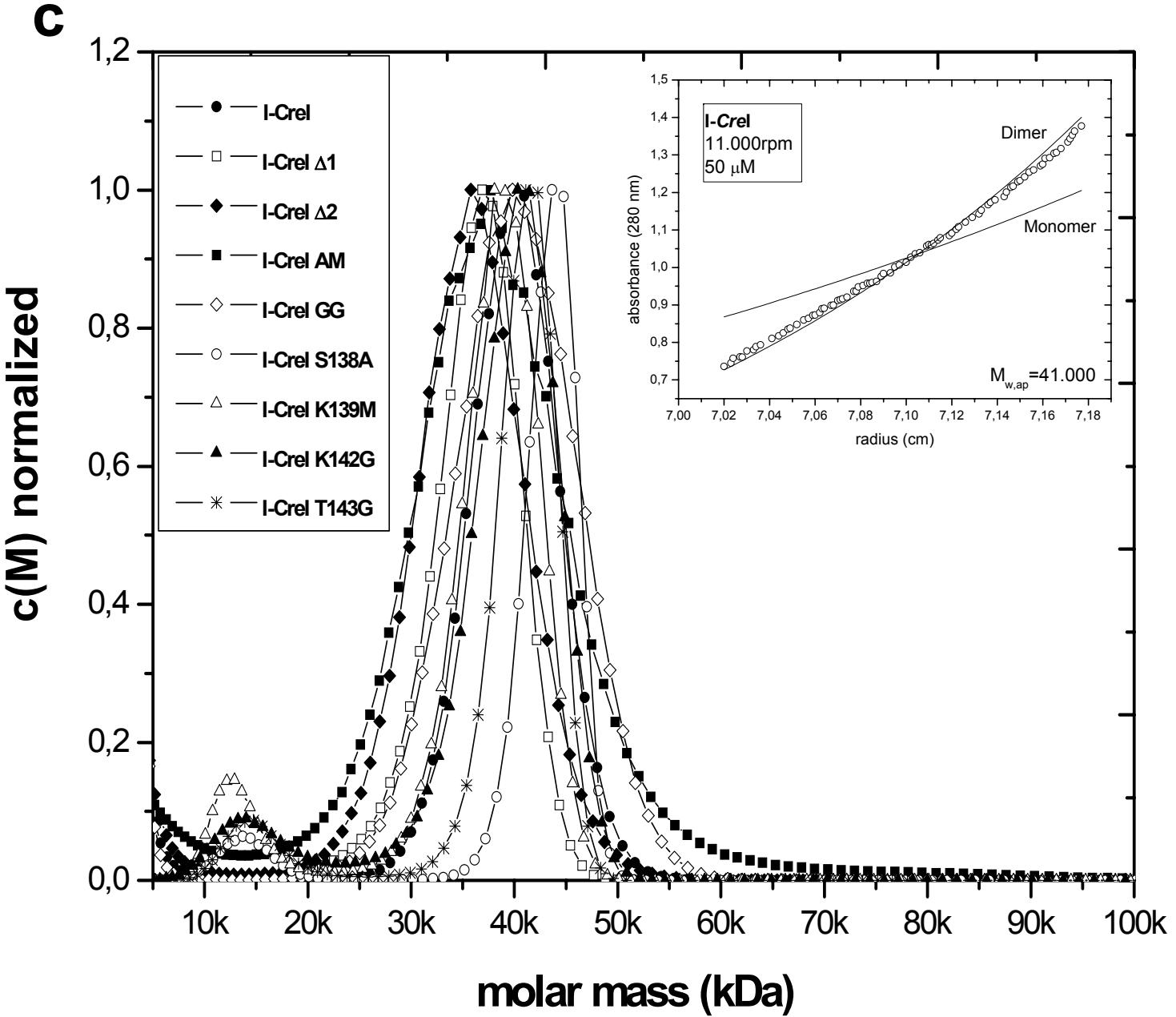


## Supporting material figure 1.-



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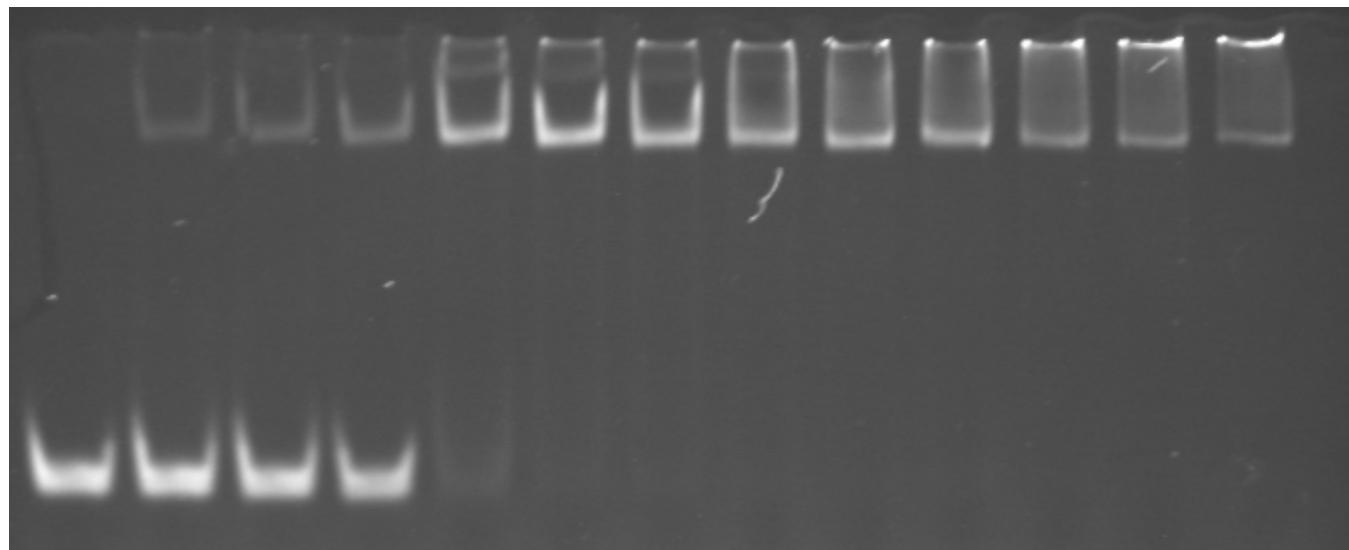
**Supporting material figure 1.-** Biophysical characterization of the I-Crel C-terminal region mutants. **a)** Circular dichroism thermal denaturation. **b)** One dimensional  $^1\text{H}$  NMR spectra. **c)** Analytical ultracentrifugation measurements. Sedimentation velocity distribution of the I-Crel proteins (1mg/ml in PBS buffer) at 42,000 rpm and 20°C. Inset, sedimentation equilibrium gradient of I-Crel proteins (4mg/ml in PBS buffer) at 11,000 rpm and 20°C. Open circles represent the experimental data, the two solid lines represent the theoretical gradients of a I-Crel monomer (20,045 Da) and dimer (41,000 Da).

## **Gels for the dissociation constants ( $K_D$ )**

**Ca<sup>2+</sup>**

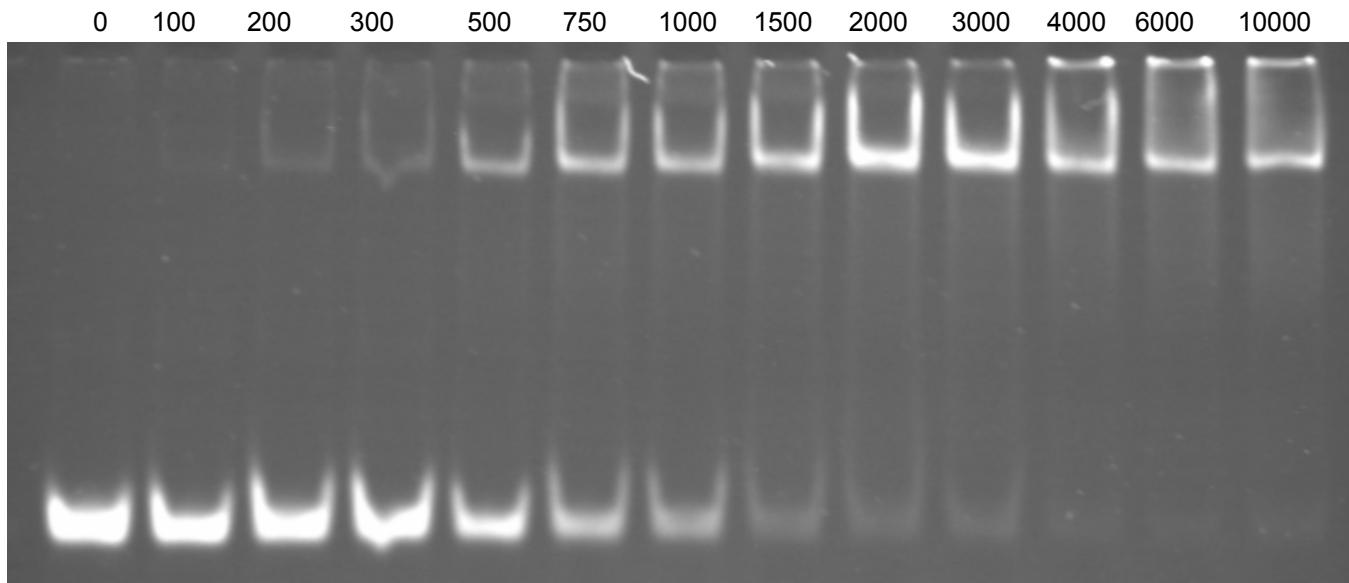
**I-Crel wt (nM)**

0    100    200    300    500    750    1000    1500    2000    3000    4000    6000    10000



**Ca<sup>2+</sup>**

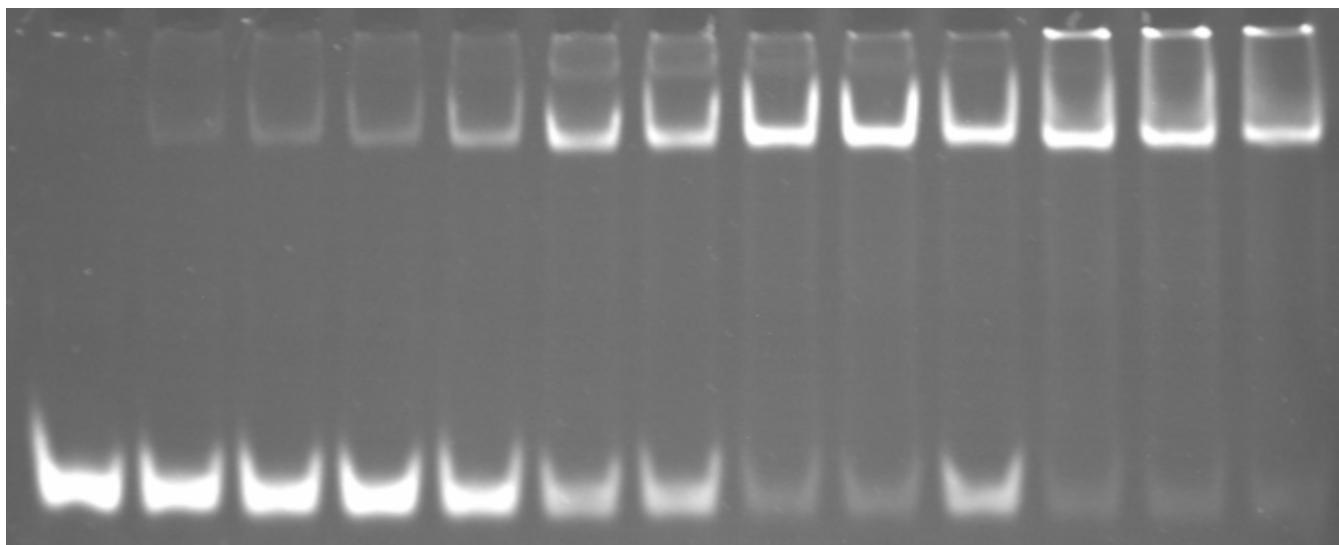
**I-Crel T143G (nM)**



**Ca<sup>2+</sup>**

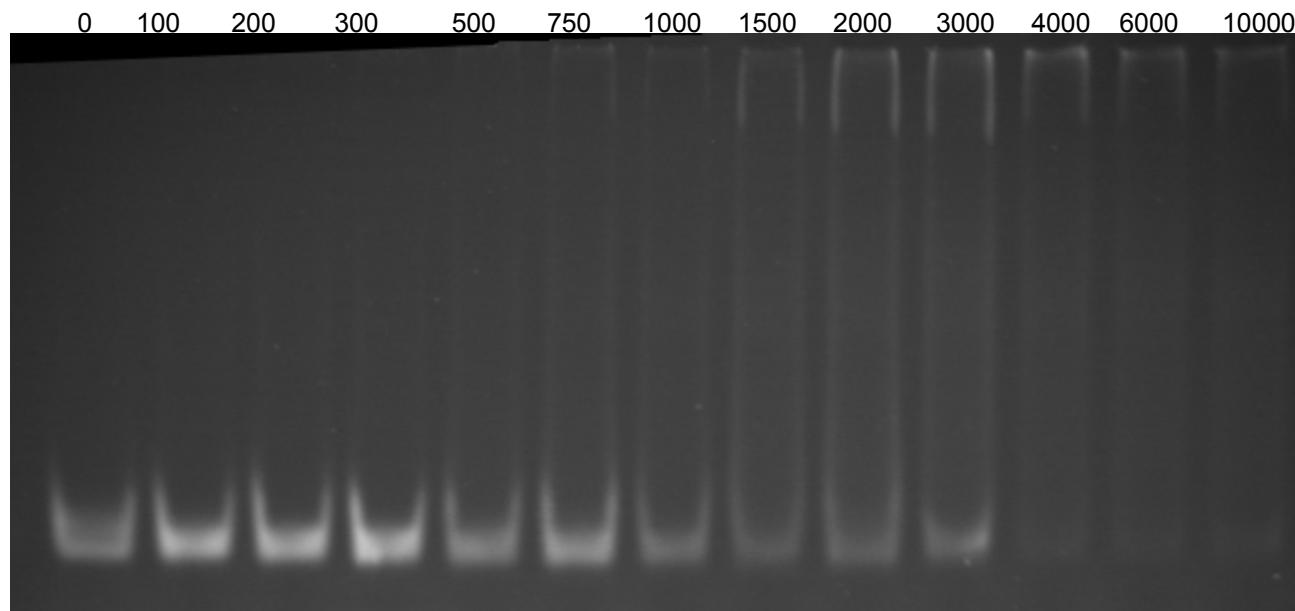
**I-Crel K142G (nM)**

0    100    200    300    500    750    1000    1500    2000    3000    4000    6000    10000



**Ca<sup>2+</sup>**

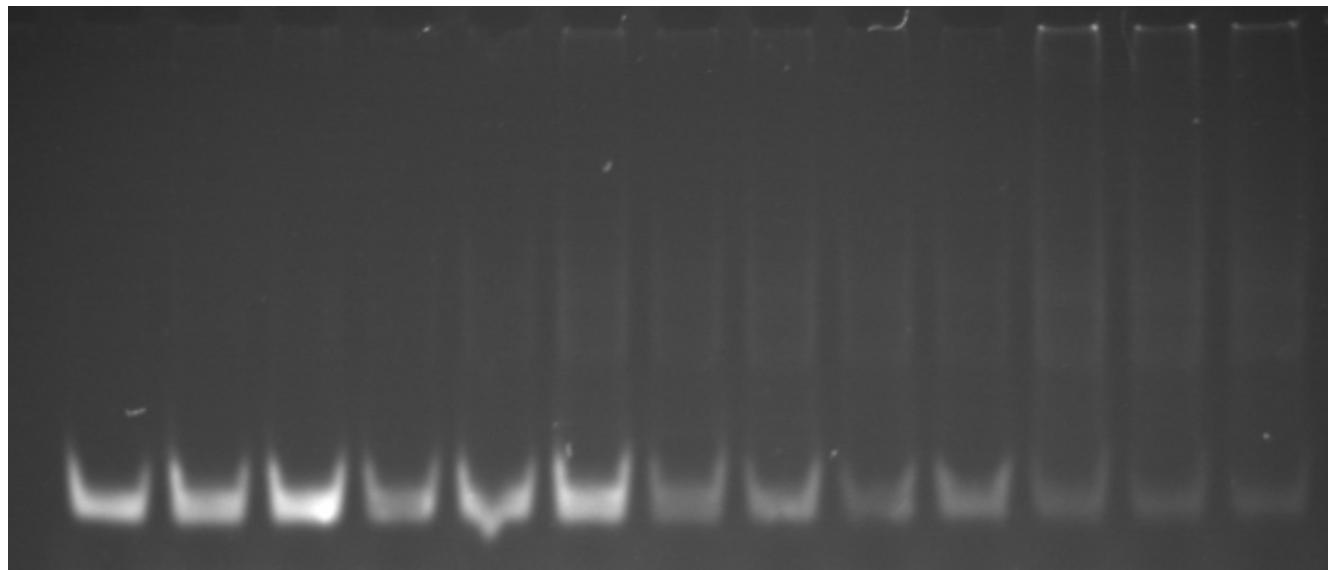
**I-Crel S138A (nM)**



**Ca<sup>2+</sup>**

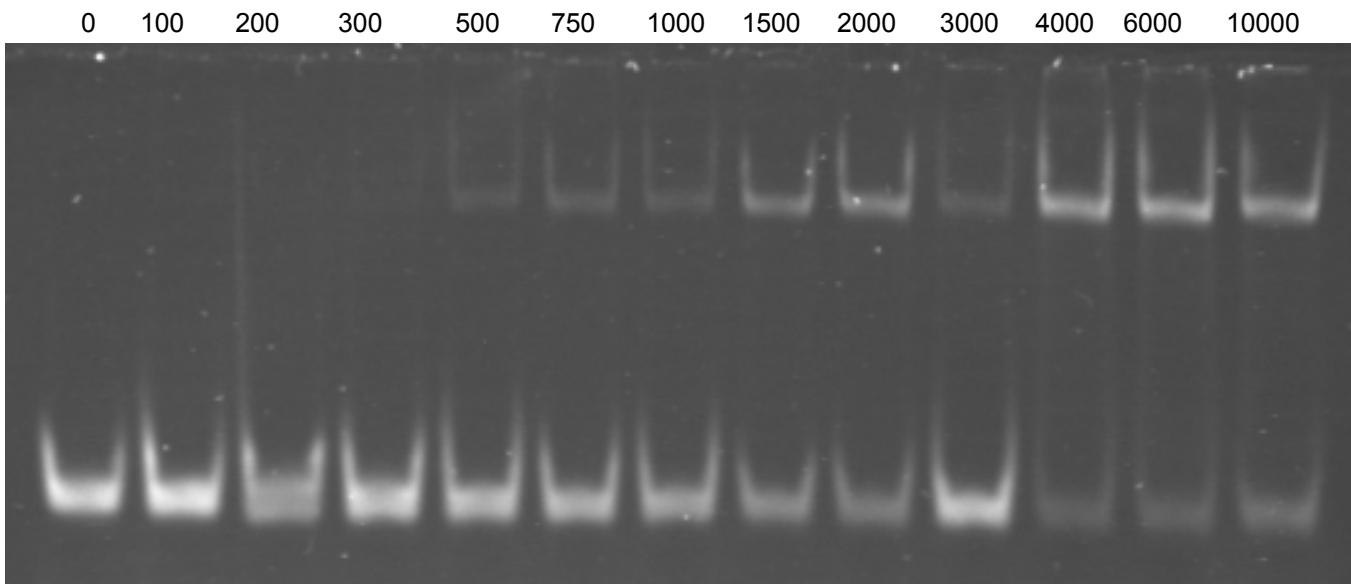
**I-Crel K139M (nM)**

0    100    200    300    500    750    1000    1500    2000    3000    4000    6000    10000



**Ca<sup>2+</sup>**

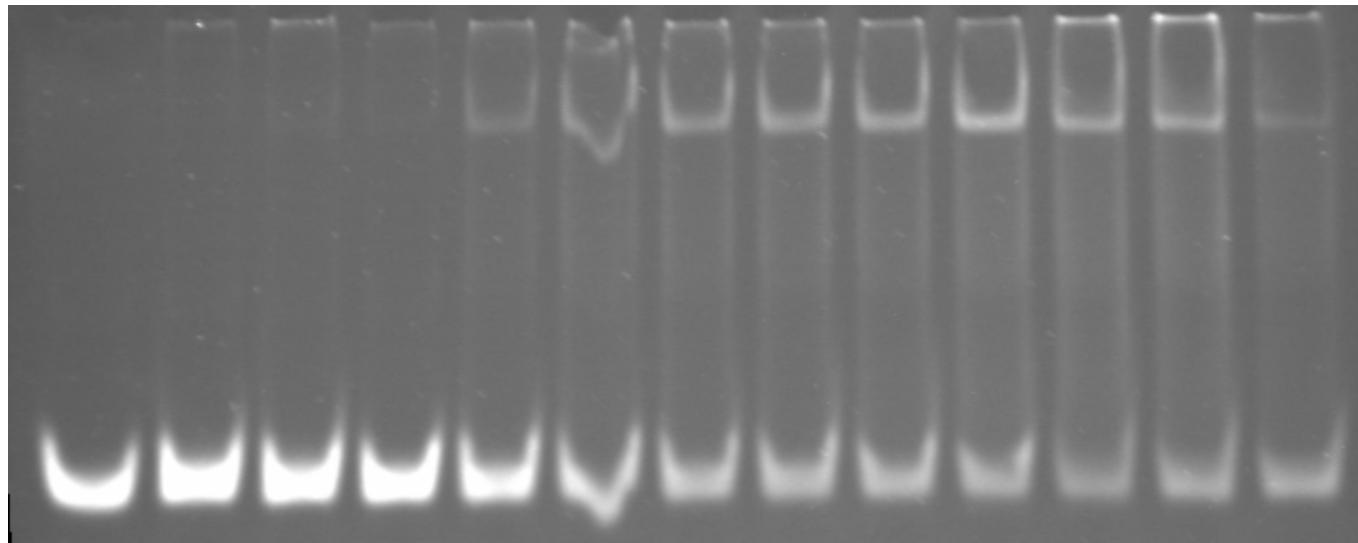
**I-Crel Δ2 (nM)**



**Ca<sup>2+</sup>**

**I-Crel GG (nM)**

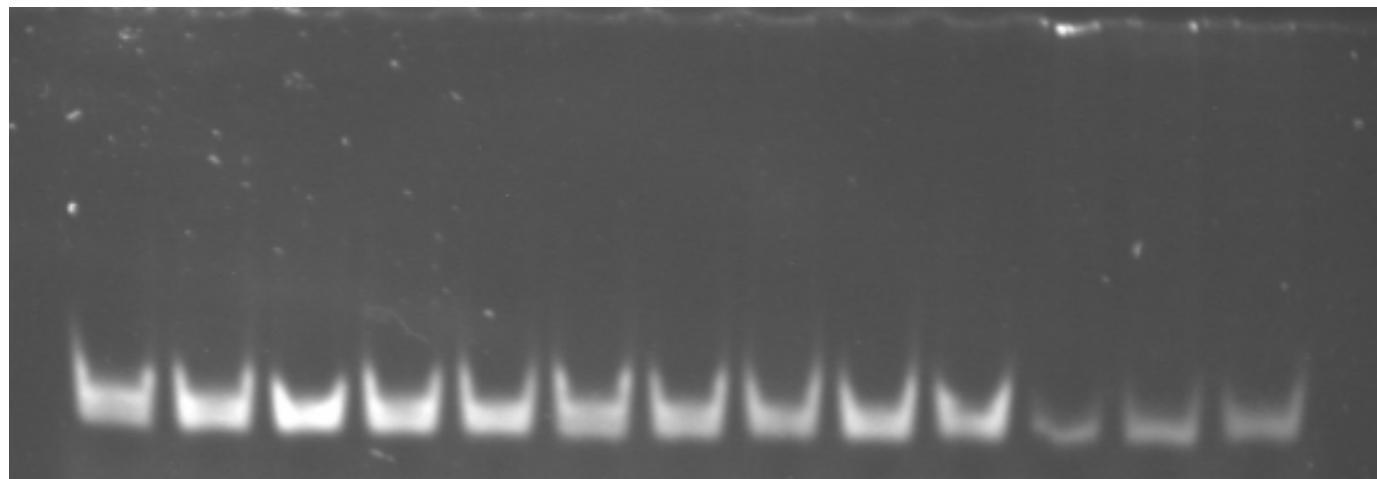
0    100    200    300    500    750    1000    1500    2000    3000    4000    6000    10000



**Ca<sup>2+</sup>**

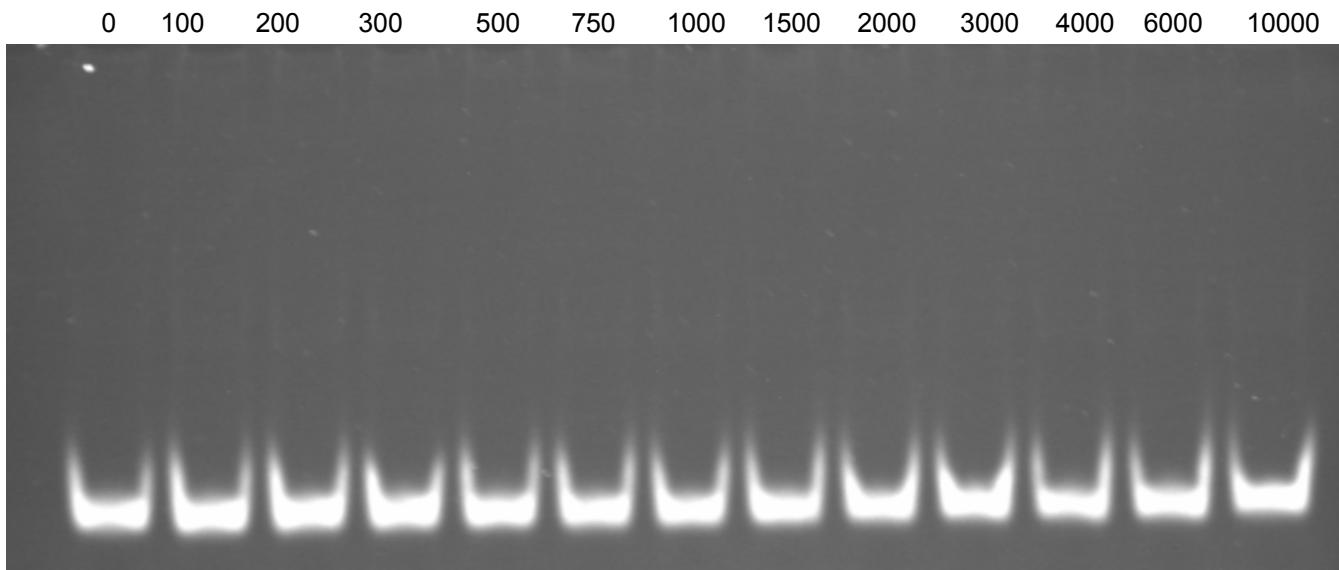
**I-Crel AM (nM)**

0    100    200    300    500    750    1000    1500    2000    3000    4000    6000    10000



**Ca<sup>2+</sup>**

**I-Crel Δ1 (nM)**

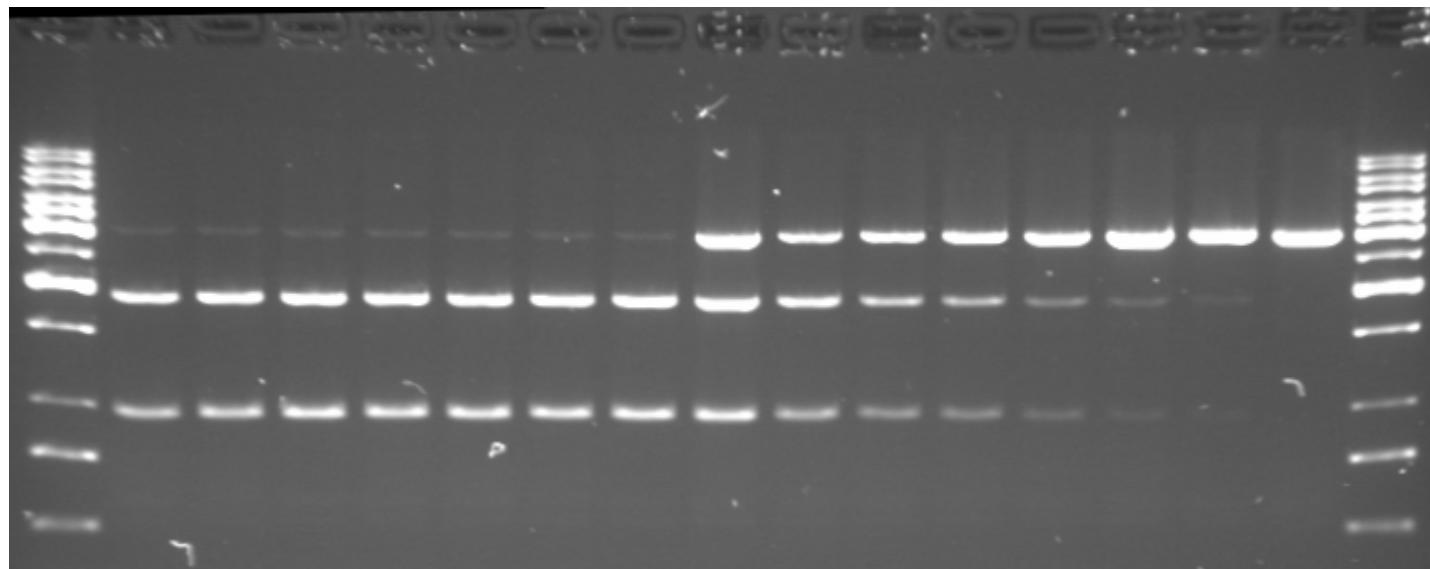


## **Gels for the cleavage C<sub>50</sub>**

**Mg<sup>2+</sup>**

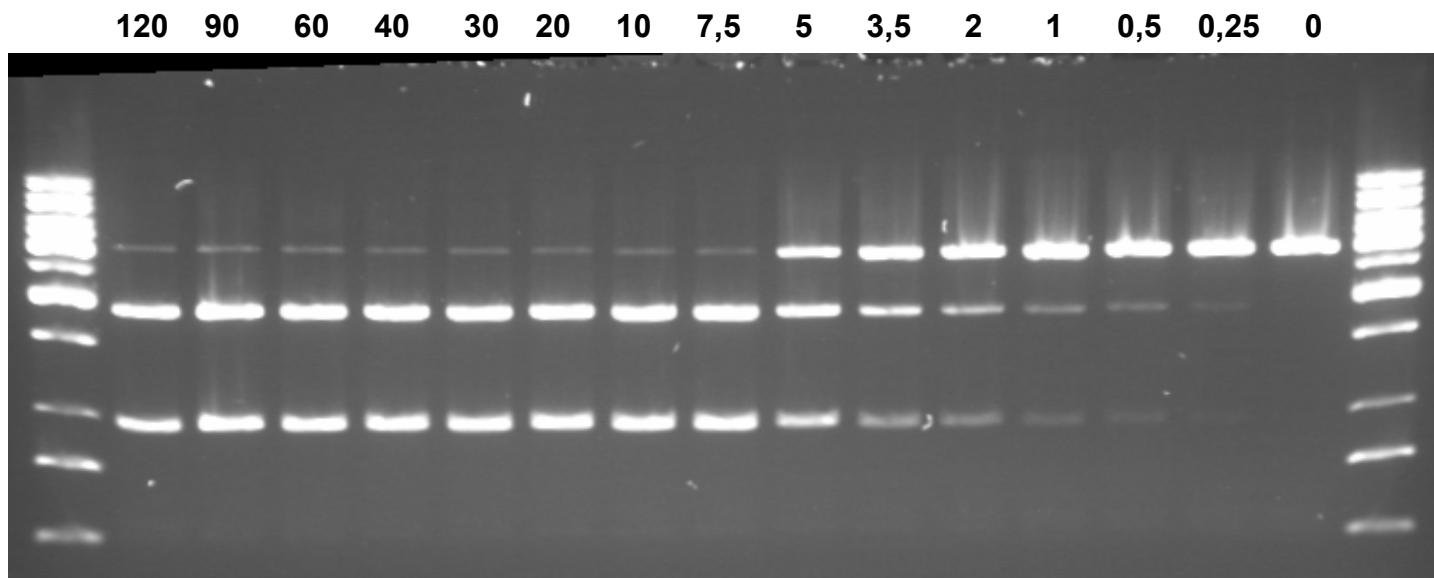
**I-Crel wt (ng)**

120 90 60 40 30 20 10 7,5 5 3,5 2 1 0,5 0,25 0



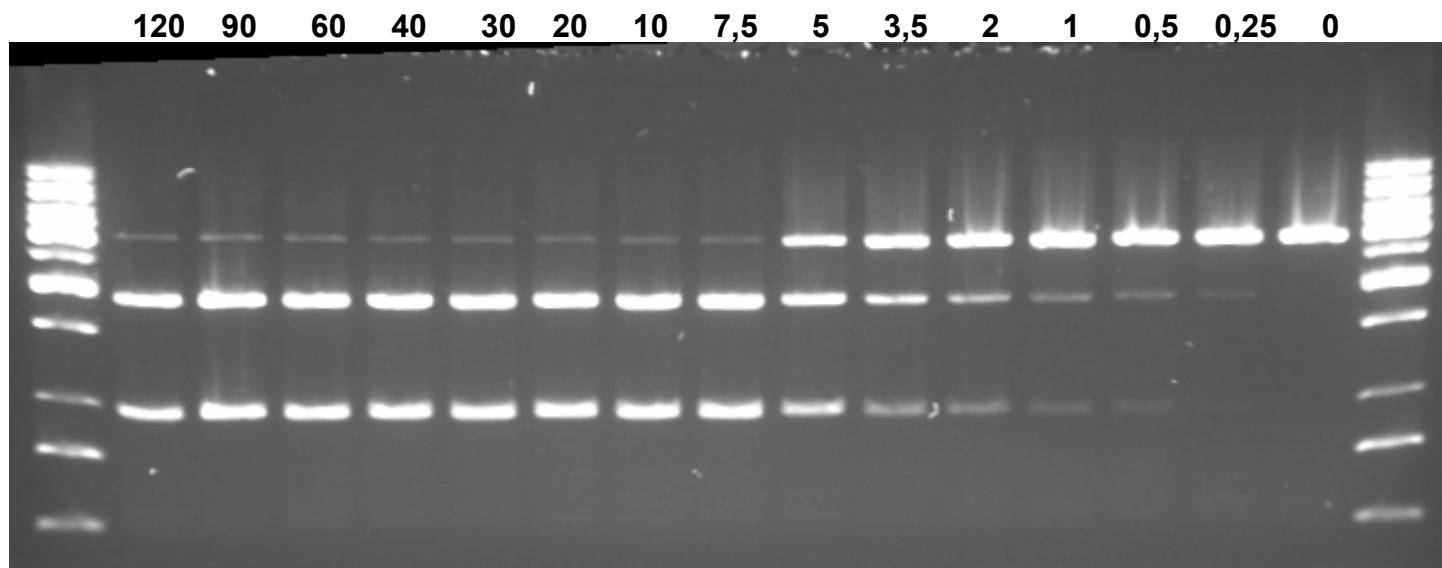
**Mg<sup>2+</sup>**

**I-Crel T143G (ng)**



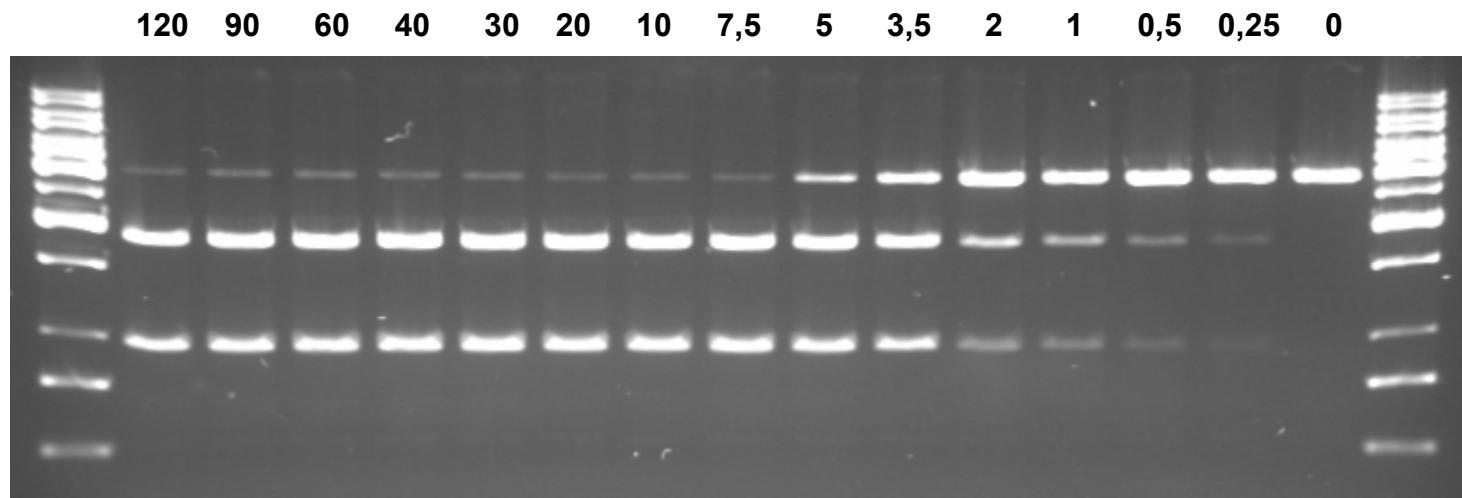
**Mg<sup>2+</sup>**

**I-CreI K142G (ng)**



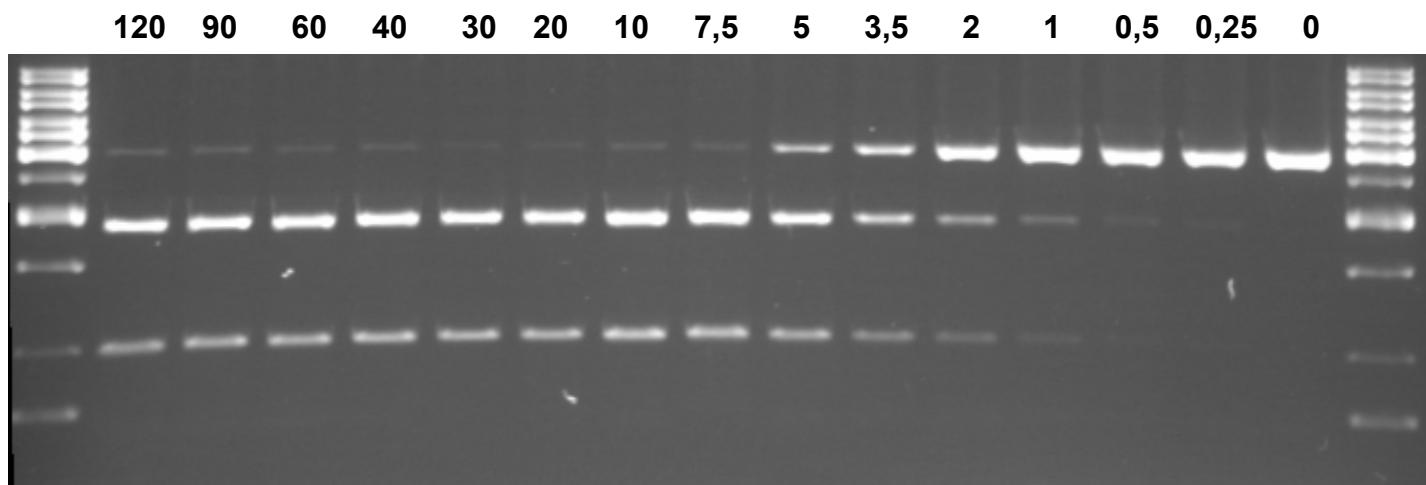
**Mg<sup>2+</sup>**

**I-CreI K139M (ng)**



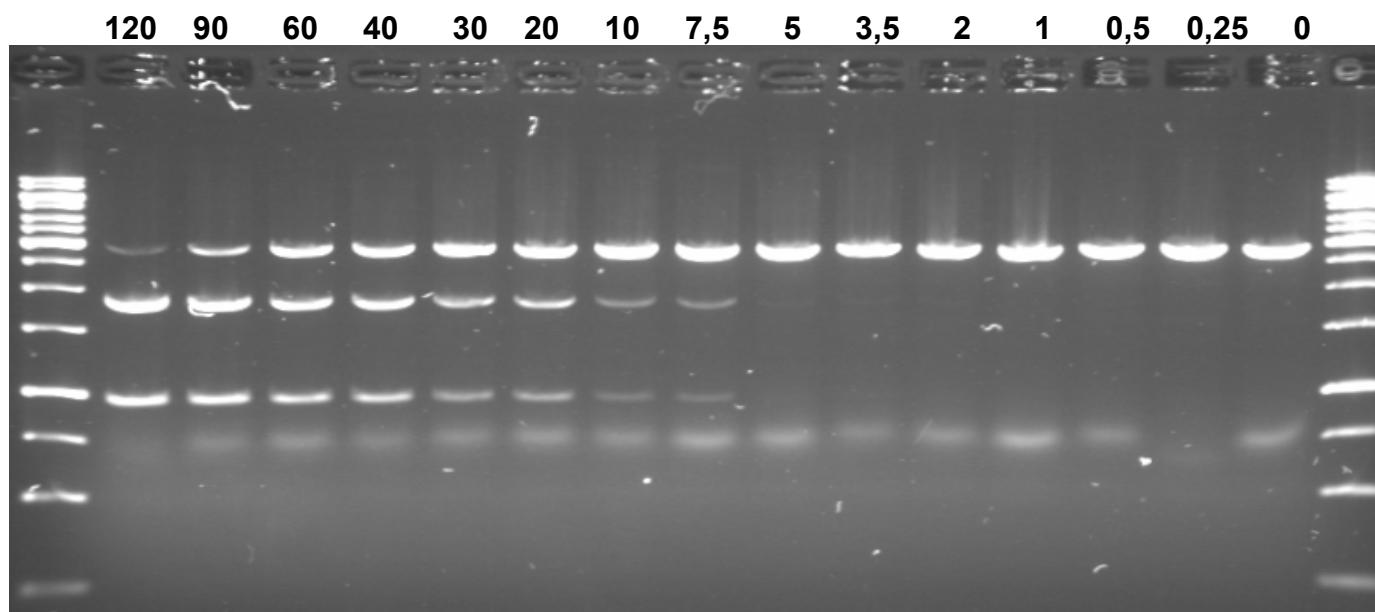
**Mg<sup>2+</sup>**

**I-Crel S138A (ng)**



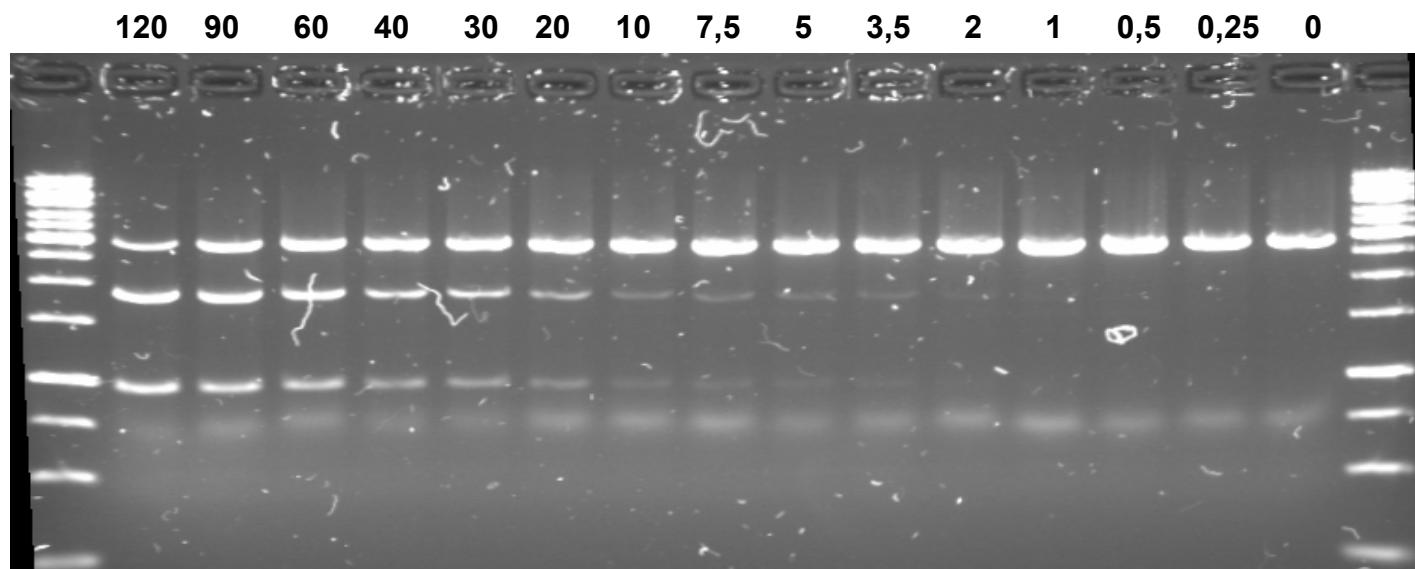
**Mg<sup>2+</sup>**

**I-Crel Δ2 (ng)**



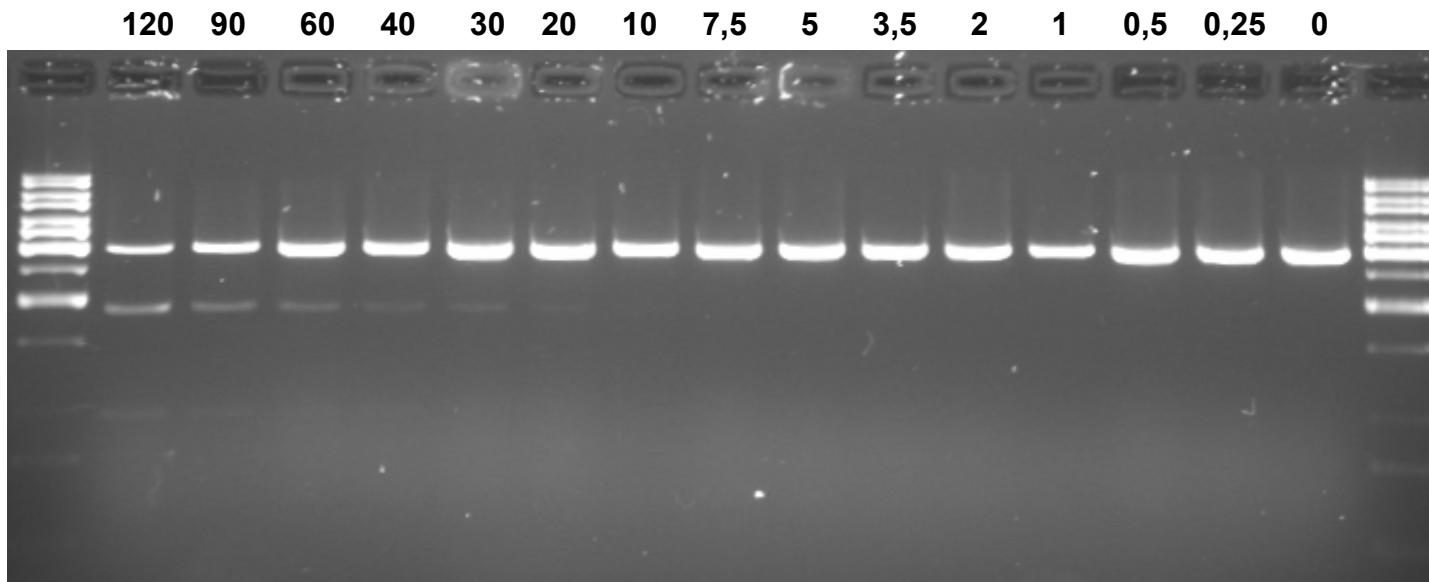
**Mg<sup>2+</sup>**

**I-Crel AM (ng)**



**Mg<sup>2+</sup>**

**I-Crel GG (ng)**



**Mg<sup>2+</sup>**

**I-Crel Δ1 (ng)**

