Supplementary document.

Phosphorylation of CRN2 by CK2 regulates F-actin and Arp2/3 interaction and inhibits cell migration.

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This document contains the following supplementary information:

Supplementary figure legends

Figure S1

Three-dimensional model of human CRN2 displaying structural features of functional relevance.

The model was elaborated by sequence threading through the known structure of CRN4 (synonym: coronin 1A [1]). N-terminal signature motif (green); WD40 domains (alternating light/dark brown); C-terminal segment (blue) with an α-helical portion (red) with the phospho-S463 site confined to the CRN2 subfamily. Amino acids highlighted in magenta denote sites predicted by SDPfox and show evidence of a conservation pattern able to distinguish individual coronin subfamilies; an example is labeled “SDP” (see also Fig. 1A, asterisks). "KGD", a motif universally conserved in coronin proteins (aa485-487 in CRN2).

Figure S2

Low resolution solution structure of the synthetic S463-phosphorylated coiled coil peptide of CRN2 (aa435-474).

Shape restoration using small-angle X-ray scattering data (protein concentration ρ^* = 18 mg ml^{-1}) revealed a cylindrical elongated particle (shown in grey) with the approximate dimensions of 55 Å (height) x 15 Å (diameter). The shape can be fitted with the predicted model of the peptide (see Fig. 1B) adopting the fold of a three-stranded coiled coil (shown in colored stick representation). The goodness of fit of experimental and theoretical scattering curves was χ = 5.5, as calculated with CRYSOL [2].

Figure S3

Quantitative illustration of the lamellipodial patterns shown in Fig. 8. Groups of bars are given for CRN2 S463D as well as CRN2 wild-type and S463A. For each group the first two bars illustrate the texture and width of the CRN2 distribution, the third and fourth bars illustrate the texture and width of the F-actin distribution, and the last bars indicate the width of the Arp2/3 distribution in the front of lamellipodia. While S463D CRN2 expressing cells predominantly

showed spiky and irregular distributions of CRN2, actin and Arp2/3, the cells expressing S463A CRN2 mainly showed smooth and regular patterns. Individual lamellipodia of cells expressing wild-type CRN2 showed patterns either similar to the findings for S463D or S463A CRN2. Rough, spiky pattern; irregular, varying width of distribution.

Figure S4

Subcellular localization of CRN2 constructs in primary human macrophages.
Fluorescence micrographs of macrophages expressing wild-type **(A)**, S463D **(B)**, or S463A **(C)** CRN2 fused to GFP, together with mRFP-Lifeact, labeling F-actin-rich podosomes. Note co-localization of all three constructs with F-actin in the podosome core structure.

Figure S5

siRNA-induced knock-down of CRN2 in primary human macrophages.
A, western blots of whole cell lysates of macrophages treated with CRN2-specific siRNA for 3 or 4 days, as indicated, compared to non-treated cells and cells transfected with control siRNA specific for firefly luciferase. Upper blot developed with anti-CRN2 antibody, lower blot developed with anti-β-actin antibody.
B, fluorescence micrographs of macrophages transfected with control siRNA (upper row) or CRN2-specific siRNA (lower row) and stained for CRN2 (left column) and F-actin (middle column), with merged images shown in right column.

Figure S6

Statistical evaluation of cells showing localization of CRN2 constructs at podosomes (wild-type, S463D, S463A) during over-expression (left bars; “non-treated cells”) or after podosome disruption by 30 min treatment with the Src family kinase inhibitor PP2 and subsequent podosome re-formation after 45 min of wash out (right bars, “re-formed podosomes”). The total number of cells expressing GFP-CRN2 proteins were counted and correlated with the number of cells showing a clear co-localization of GFP-CRN2 constructs

with mRFP-Lifeact at podosomes. Note that CRN2 S463A targeting to re-assembled podosomes was impaired. For each value, 3x30 cells were counted. Values are given as mean percentage ± SD of total counts.

Figure S7

FRAP analysis of CRN2 dynamics in living macrophages.

GFP-fused CRN2 wild-type **(A)**, S463D **(B)**, or S463A **(C)** was transiently expressed in primary human macrophages. FRAP experiments were performed to determine protein half-life times in F-actin-rich podosome cores. Normalized fluorescence recovery curves were corrected for acquisition photobleaching and further processed by bi-exponential regression analyses, which allowed the calculation of half-life times. Each curve represents 15 measurements from podosomes of at least 3 different cells. Values are given as mean percentages ± SD. Note especially elevated half-life time of the S463D variant (6.56 s) compared to wild-type (2.27 s).

Supplementary tables

Table S1

Parameters derived from small-angle X-ray scattering of synthetic pS463 CRN2 coiled coil peptide.

ρ* (mg ml^{-1})	18	9	4.5
Guinier fit			
R_g (Å)	20	23	26
M (g/mol)	15270	16212	14555
N	3.3	3.5	3.1
P(r)			
R_{max} (Å)	57	53	53
R_g (Å)	20	20	20
M (g/mol)	15242	15405	13127
N	3.3	3.3	2.8
Atomic model			
R_g (Å)[a]	15		
hydrodynamic R_g (Å)[b]	16		

The parameters obtained at three different concentrations are consistent and therefore indicate the presence of a true trimer rather than aggregation effects. [a]determined with MOLEMAN2 [3]; [b]calculated with HYDROPRO [4].

Supplementary References

1. Appleton, B. A., Wu, P. & Wiesmann, C. The crystal structure of murine coronin-1: a regulator of actin cytoskeletal dynamics in lymphocytes. *Structure* **14**, 87-96 (2006).

2. Svergun, D., Barberato, C. & Koch, M. CRYSOL - a program to evaluate X-ray solution scattering of biological macromolecules from atomic coordinates. *Journal of Applied Crystallography* **28**, 768-773 (1995).

3. Kleywegt, G., Zou, J., Kjeldgaard, M. & Jones, T. in *International Tables for Crystallography* Vol. F. Crystallography of Biological Macromolecules (eds M. Rossmann & E. Arnold) 353-356, 366-367 (Kluwer Academic Publishers, 2001).

4. Garcia De La Torre, J., Huertas, M. L. & Carrasco, B. Calculation of hydrodynamic properties of globular proteins from their atomic-level structure. *Biophys J* **78**, 719-730 (2000).

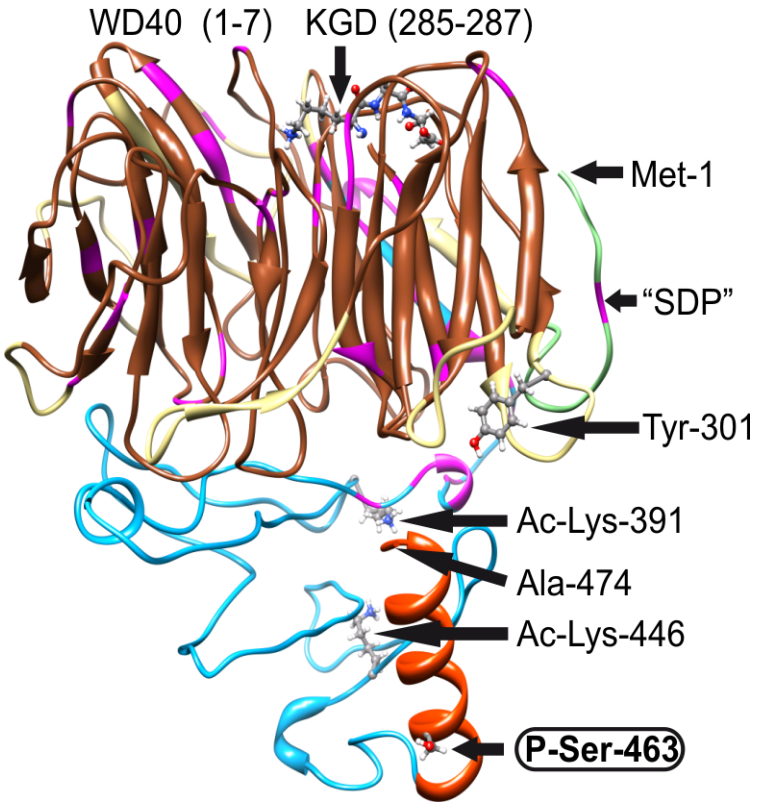
WD40 (1-7)
KGD (285-287)
Met-1
"SDP"
Tyr-301
Ac-Lys-391
Ala-474
Ac-Lys-446
P-Ser-463

Figure S1

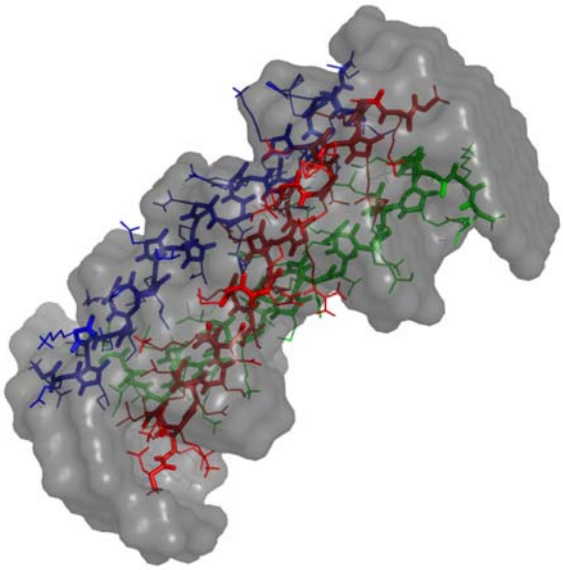

Figure S2

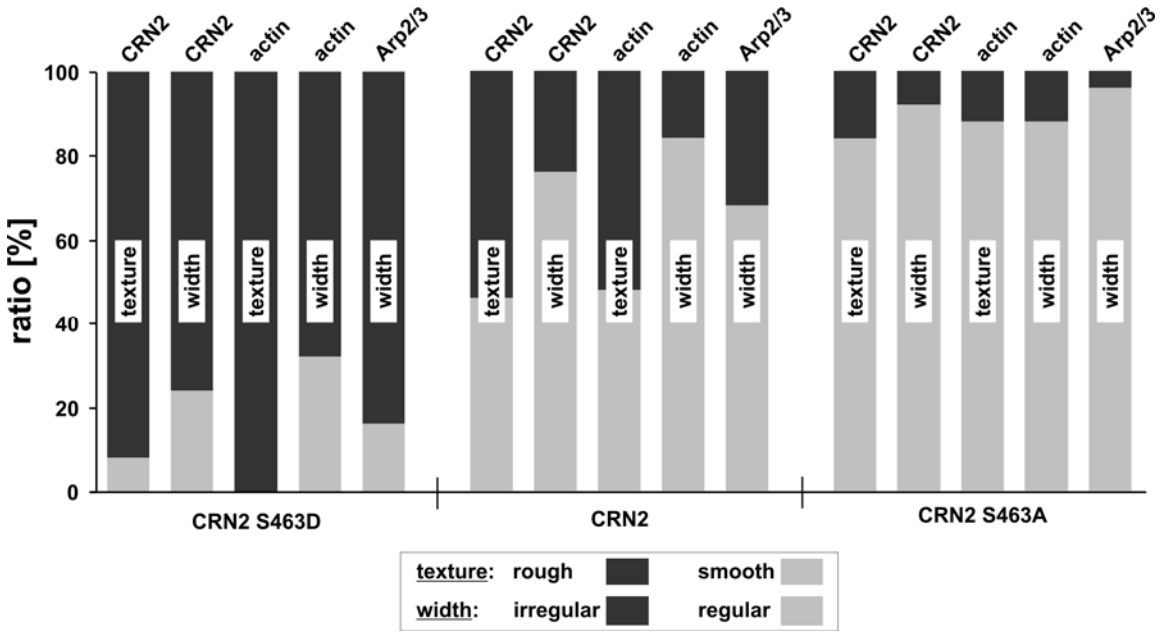
CRN2
CRN2
actin
actin
Arp2/3
CRN2
CRN2
actin
actin
Arp2/3
CRN2
CRN2
actin
actin
Arp2/3
ratio [%]
100
80
60
40
20
0
texture
width
CRN2 S463D
CRN2
CRN2 S463A
texture: rough
smooth
width: irregular
regular

Figure S3

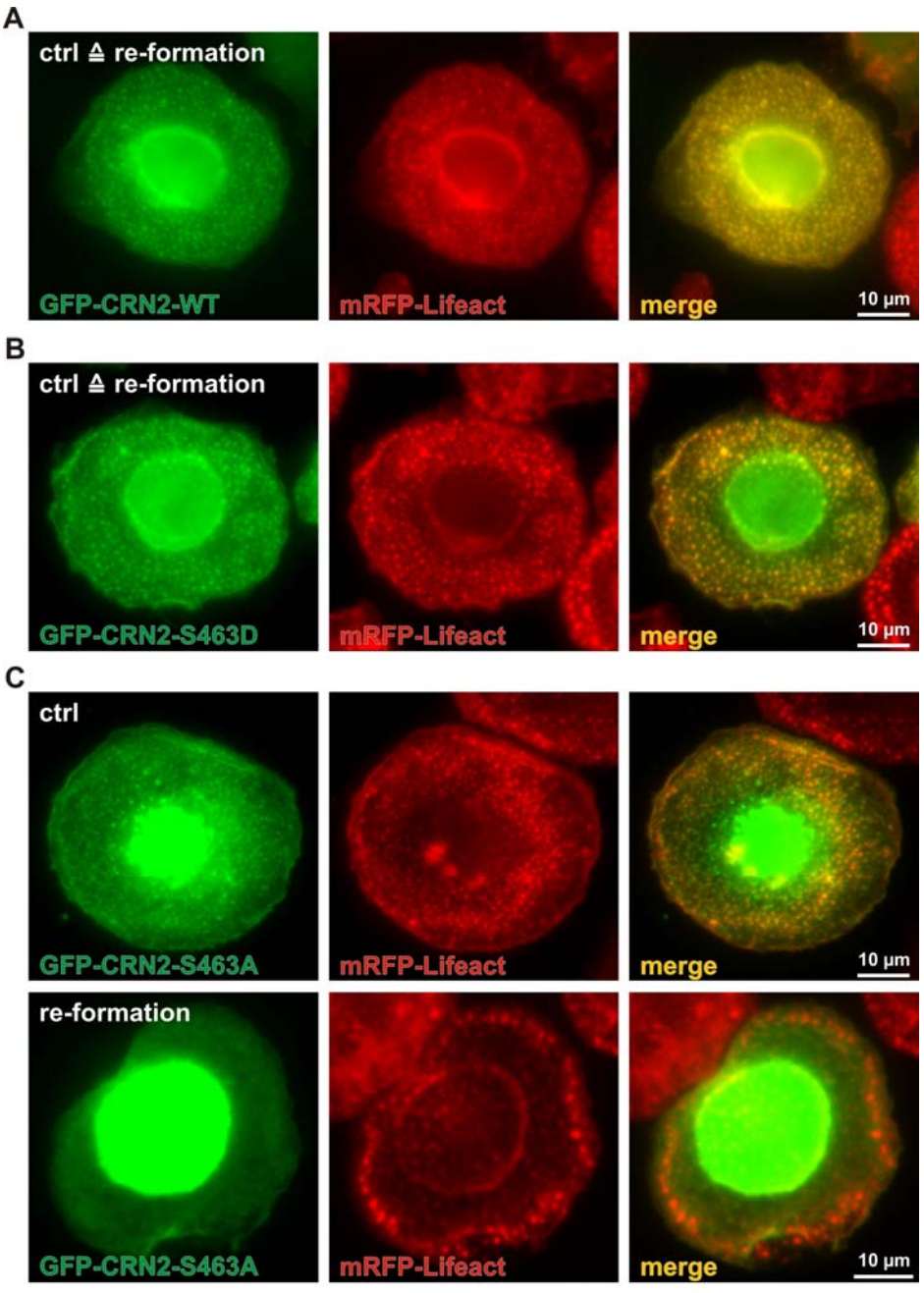
A
ctrl ≙ re-formation
GFP-CRN2-WT
mRFP-Lifeact
merge
10 µm
B
ctrl ≙ re-formation
GFP-CRN2-S463D
mRFP-Lifeact
merge
10 µm
C
ctrl
GFP-CRN2-S463A
mRFP-Lifeact
merge
10 µm
re-formation
GFP-CRN2-S463A
mRFP-Lifeact
merge
10 µm

Figure S4

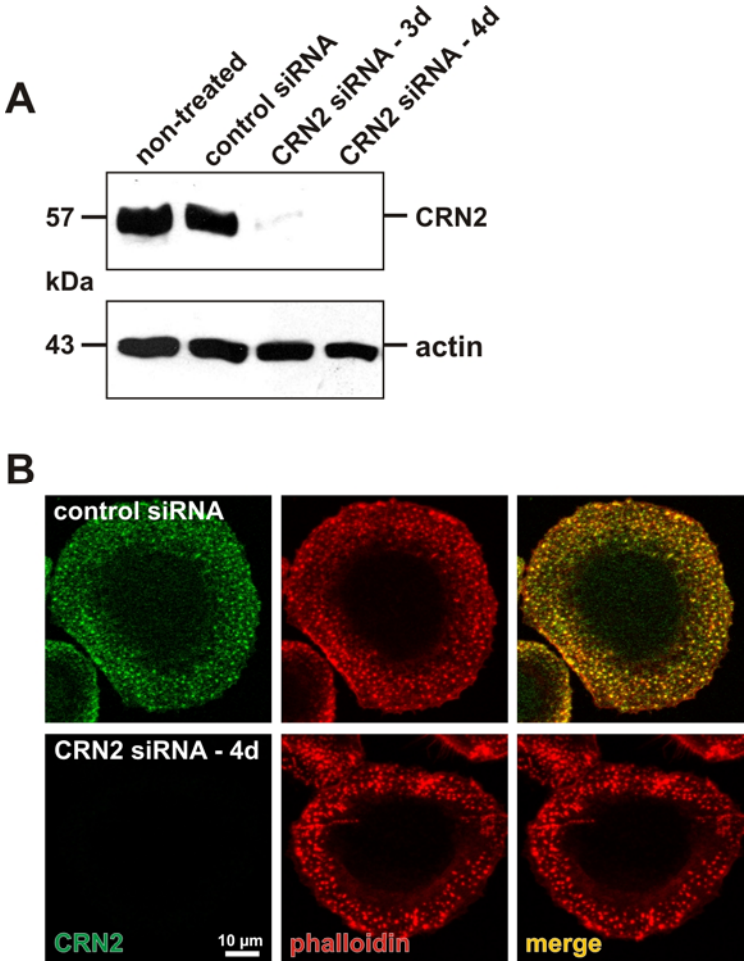
A
non-treated
control siRNA
CRN2 siRNA - 3d
CRN2 siRNA - 4d
57
kDa
CRN2
43
actin
B
control siRNA
CRN2 siRNA - 4d
CRN2
10 µm
phalloidin
merge

Figure S5

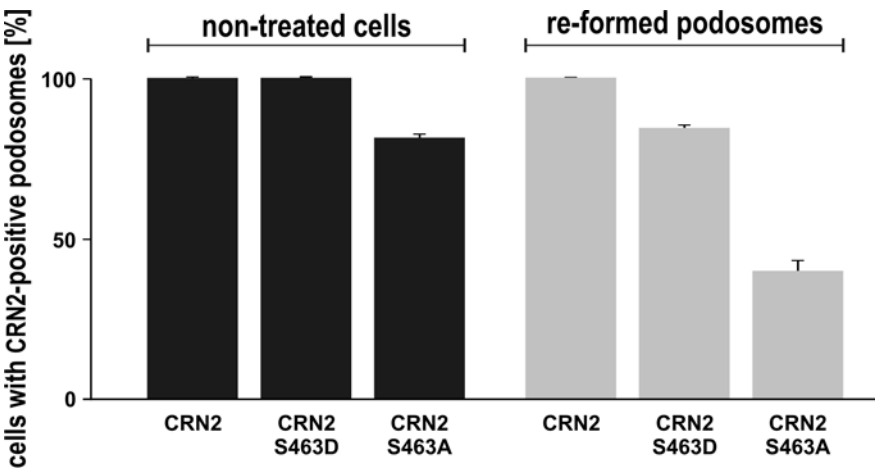
non-treated cells
re-formed podosomes
cells with CRN2-positive podosomes [%]
100
50
0
CRN2
CRN2 S463D
CRN2 S463A
CRN2
CRN2 S463D
CRN2 S463A

Figure S6

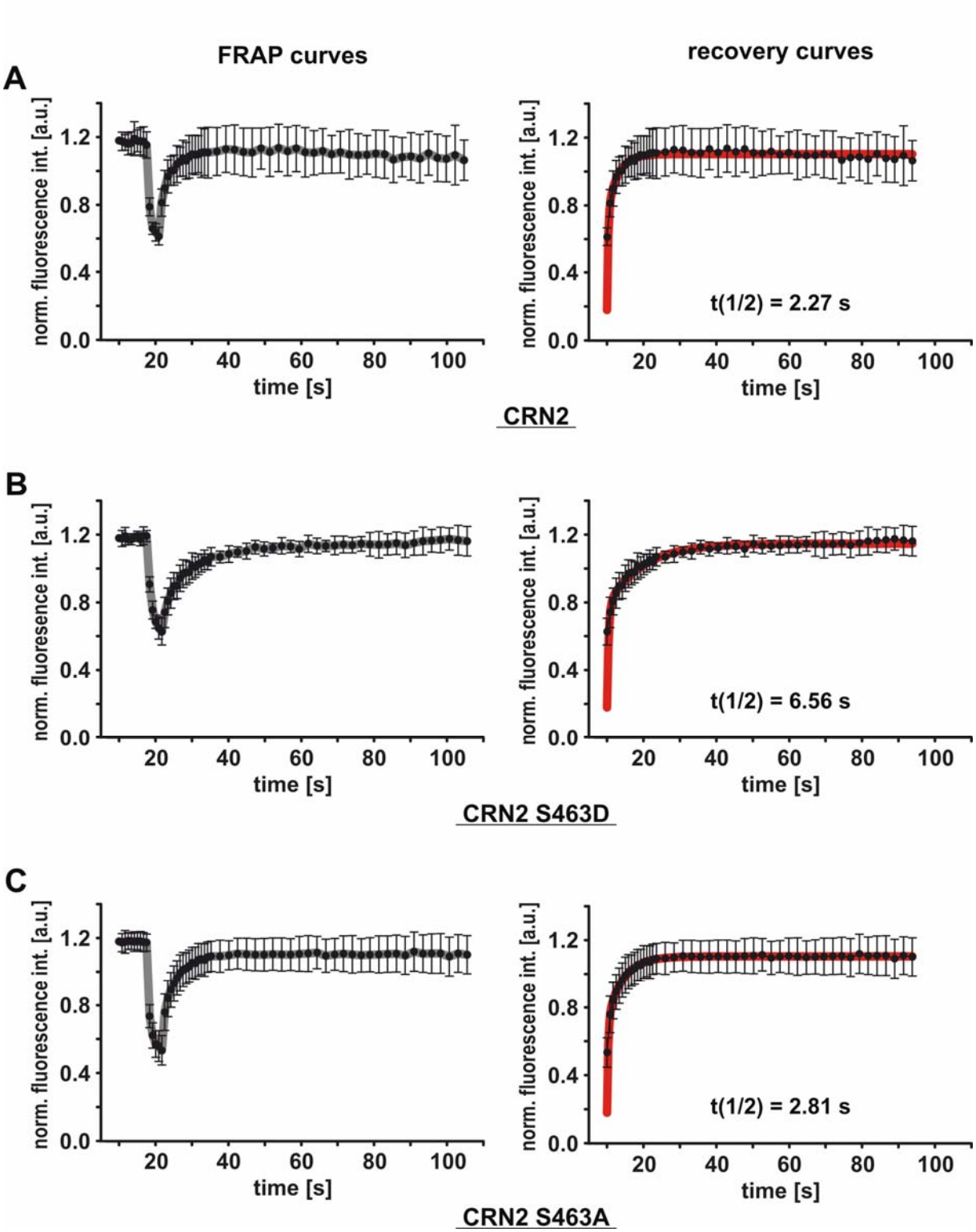
A
FRAP curves
recovery curves
norm. fluorescence int. [a.u.]
time [s]
t(1/2) = 2.27 s
CRN2
B
t(1/2) = 6.56 s
CRN2 S463D
C
t(1/2) = 2.81 s
CRN2 S463A

Figure S7