New Sequencing technologies help revealing unexpected mutations in Autosomal Dominant Hypercholesterolemia

Sandy Elbitar [1,2,3], Delia Susan-Resiga[4], Youmna Ghaleb [1,2,3], Petra El Khoury [1,2], Gina Peloso[5], Nathan Stitziel[6], Jean-Pierre Rabès [1,7], Valérie Carreau[8], Josée Hamelin[4], Ali Ben- Djoudi-Ouadda[4], Eric Bruckert[8], Catherine Boileau[1,3,9], Nabil G. Seidah[4], Mathilde Varret [1,3], Marianne Abifadel* [1,2]

Figure 2A

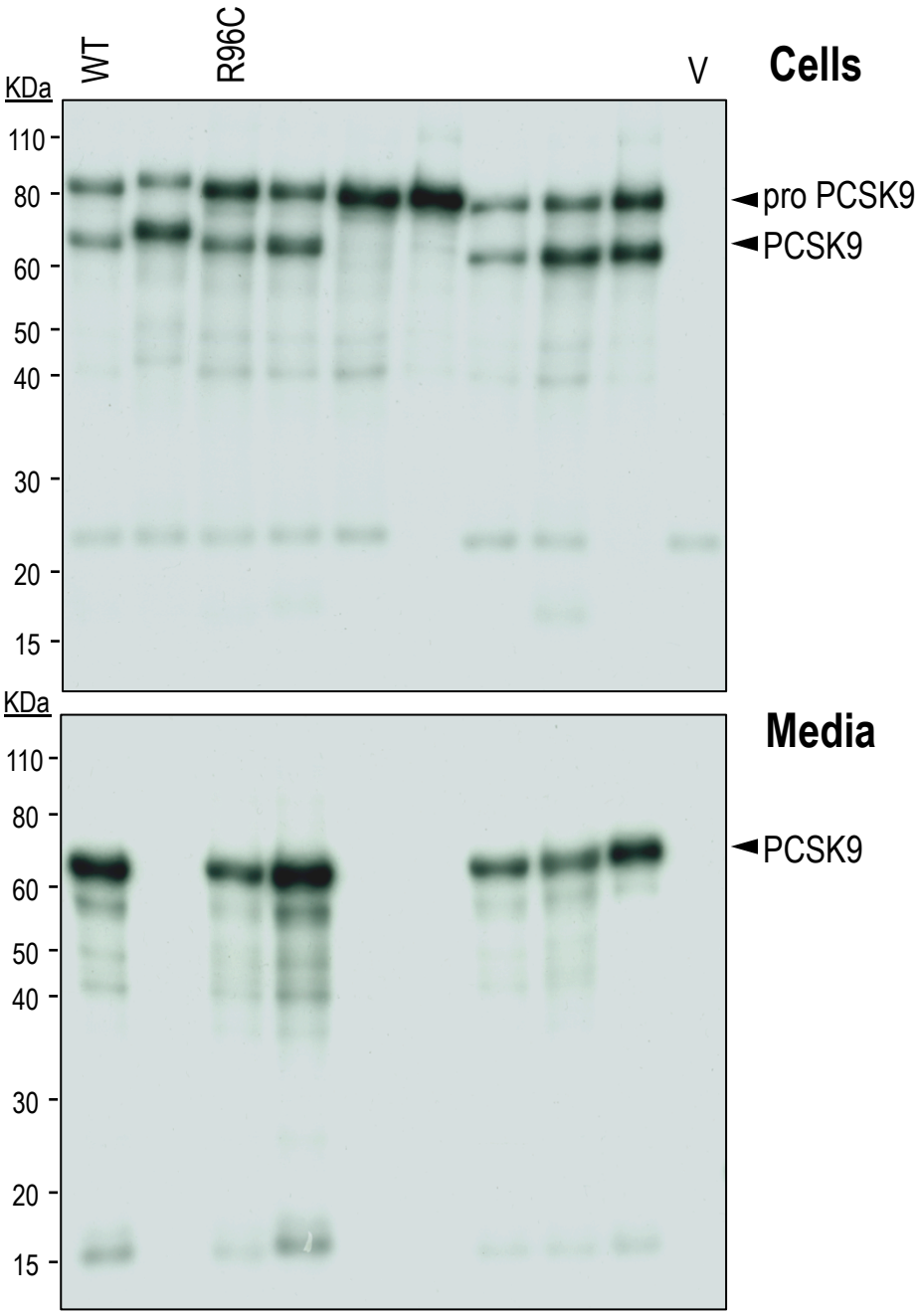

WT
R96C
V
Cells
KDa
110
80
60
50
40
30
20
15
pro PCSK9
PCSK9
KDa
Media
110
80
60
50
40
30
20
15
PCSK9

Figure 2B

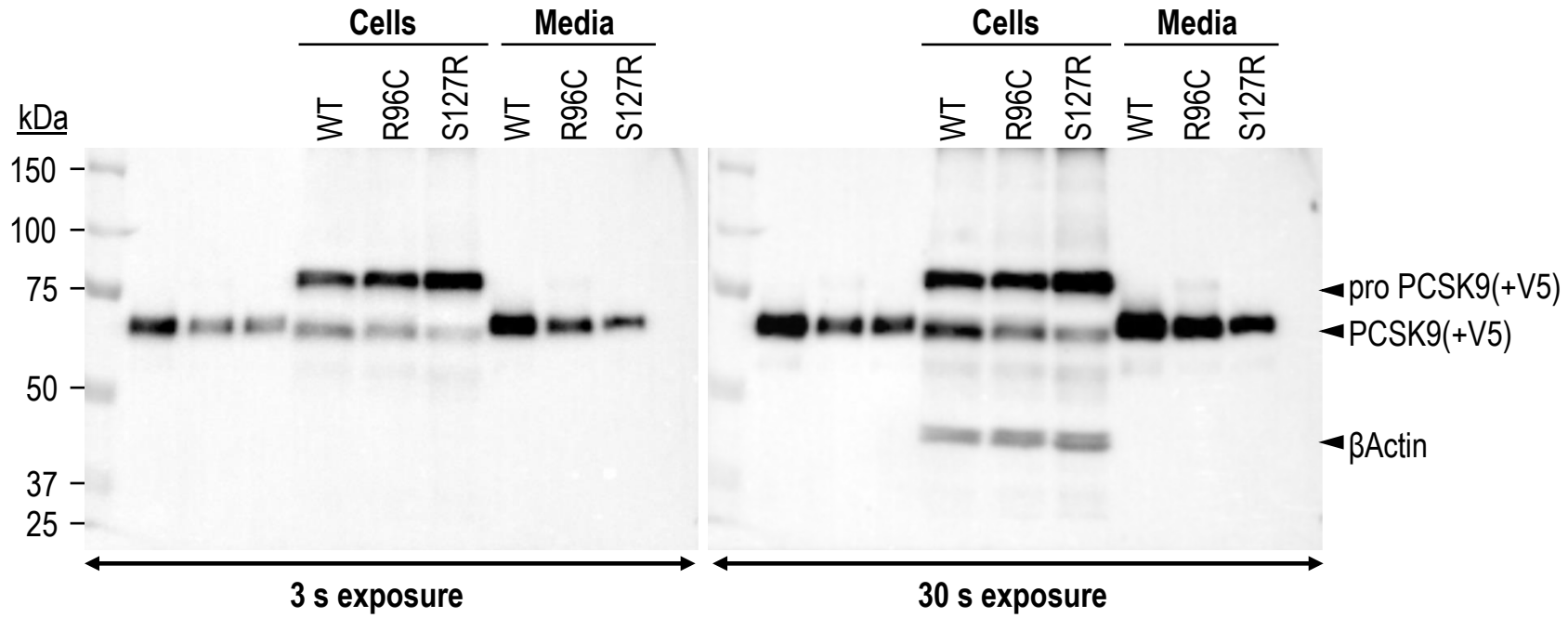
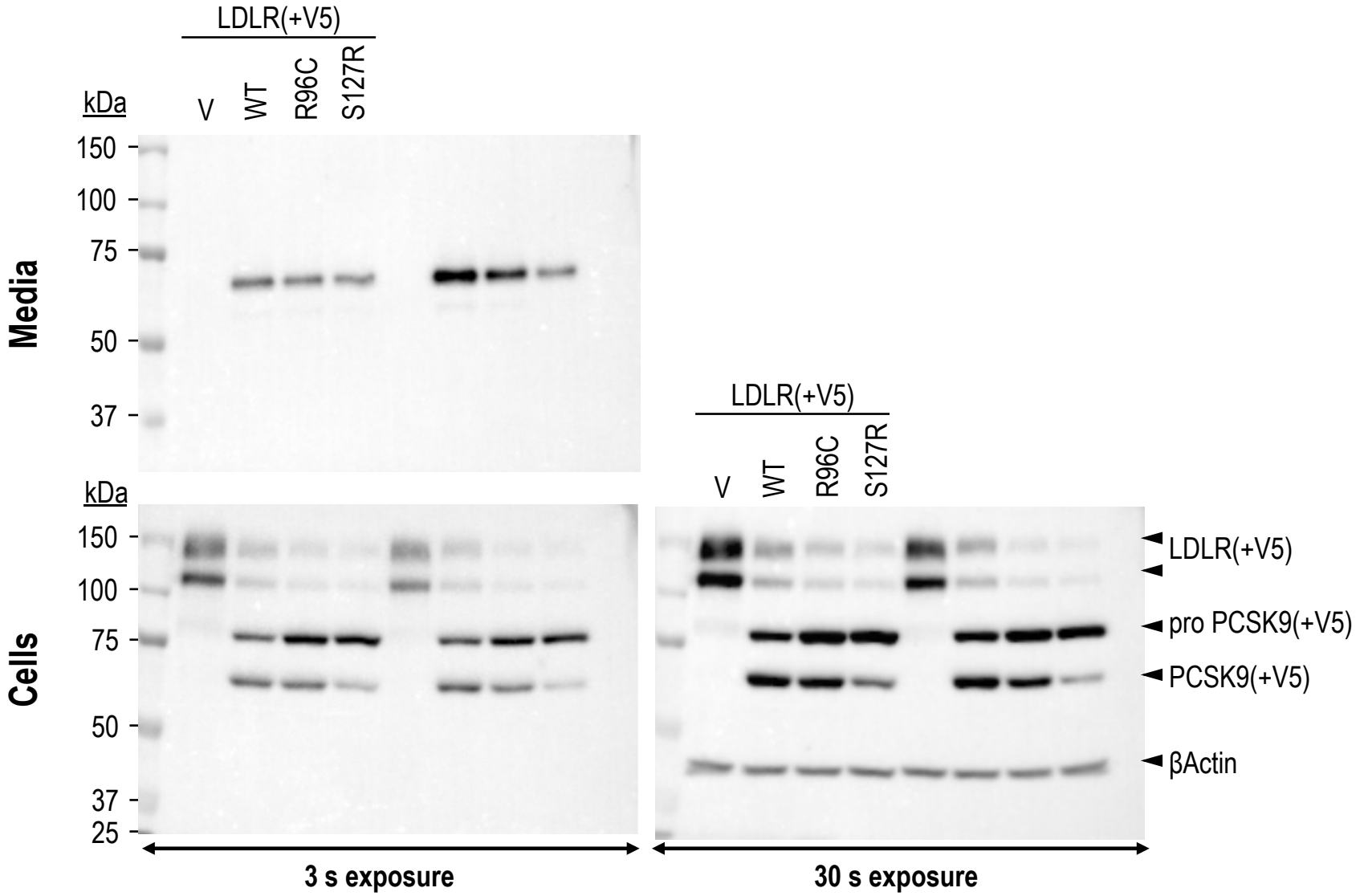

Cells
Media
WT
R96C
S127R
WT
R96C
S127R
kDa
150
100
75
50
37
25
pro PCSK9(+V5)
PCSK9(+V5)
βActin
3 s exposure
30 s exposure

Figure 2D


LDLR(+V5)
V
WT
R96C
S127R
kDa
150
100
75
50
37
Media
Cells
25
LDLR(+V5)
pro PCSK9(+V5)
PCSK9(+V5)
βActin
3 s exposure
30 s exposure

Supplementary Figure 2: Full-length blots of Figures 2A, 2B and 2D. Only the lanes selected in Figure 2 are labeled. 2A represents the biosynthetic analysis: 48h post-transfection cells were pulsed-labeled with [^{35}S]Met/Cys for 3h, followed by anti-V5 immunoprecipitation, SDS-PAGE and autoradiography. 2B represents WB analysis using anti V5-HRP and anti-β-Actin. The bands corresponding to proPCSK9 and PCSK9 in cells and media were quantified and their values normalized to β-Actin. In 2D, transfected HEK293 cells were analyzed by WB using anti V5-HRP after that V5-tagged LDLR was co-transfected with V5-tagged PCSK9-WT, PCSK9-R96C, PCSK9-S127R or empty vector as control and their values normalized to β-Actin.