SUPPLEMENTARY MATERIAL

Supplementary Figure 1. Total cysteine protease activity in (**A**) wild-type and *Lmna*$^{-/-}$ MEFs, (**B**) wild-type MEFs depleted of A-type lamins, (**C**) *Lmna*$^{-/-}$ MEFs depleted of CTSL and (**D**) wild-type MEFs overexpressing CTSL. In "bee swarm" plots horizontal bar indicates the average value.

Supplementary Figure 2. Representative images of total RNA after Northern blotting from: (lanes 1 and 2) wild-type cells retrovirally transduced with shRNA control or shRNA specific for depletion of A-type lamins; and (lanes 3 and 4) *Lmna*$^{-/-}$ cells retrovirally transduced with shRNA control or shRNA specific for depletion of CTSL. A CTSL probe was used in the hybridization. Hybridization with a probe specific for 28S RNA was carried out for normalization.

Supplementary figure 3. (**A**) Representative images of individual DNA comets from wild-type and *Lmna*$^{-/-}$ MEFs lentivirally transduced with either control or CTSL shRNAs. Note how the length of the comet decreases faster over time in wild-type and CTSL depleted *Lmna-/-* cells, indicating proficient repair of DNA DSBs. (**B**) Western blots showing acute depletion of 53BP1 in wild-type and *Lmna*$^{-/-}$ MEFs lentivirally transduced with either control or CTSL shRNAs. (**C**) Representative images of individual DNA comets from CTSL-depleted *Lmna*$^{-/-}$ MEFs lentivirally transduced with either control or 53BP1 shRNAs. Note how the length of the comet decreases faster over time in CTSL-depleted *Lmna*$^{-/-}$ cells, but not in 53BP1-depleted cells. (**D**) Representative images of individual DNA comets from cells retrovirally transduced with CTSL or an empty vector control.

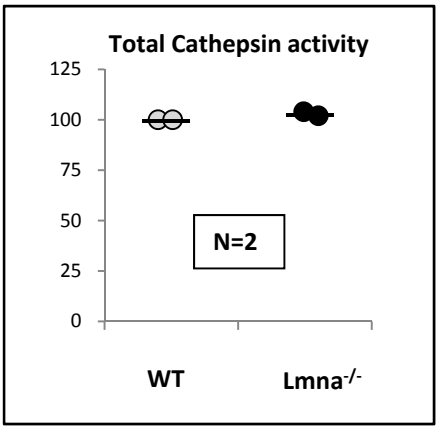
Supplementary Figure 4. Subcellular fractionation of wild-type MEFs lentivirally transduced with shLmna or shControl, followed by western blots to monitor the levels of 53BP1, Lamin A and CTSL in the nucleus and the cytoplasm. β-tubulin was used as marker of cytoplasmic

fraction and LAP2α as marker of nuclear fraction († non-specific band detected with LAP2α antibody). Graph shows the quantitation of two independent experiments. Note how loss of A-type lamins leads to accumulation of CTSL in the nucleus and an increase of 53BP1 in the cytoplasm. In “bee swarm” plots horizontal bar indicates the average value.

Supplementary Figure 5. Representative images of immunofluorescence studies with 53BP1 antibody performed in wild-type MEFs lentivirally transduced with shLmna or shControl, followed by incubation with vitamin D or vehicle. Note the decrease in the intensity of 53BP1 labeling in the nucleus upon loss of A-type lamins, which is rescued by treatment with vitamin D. Labeling of cells depleted of 53BP1 was used as a negative control.

Supplementary figure 6. (**A**) Representative images of individual DNA comets from wild-type and *Lmna*$^{-/-}$ MEFs treated with either vitamin D or vehicle. Note how the length of the comet decreases faster over time in *Lmna*$^{-/-}$ cells treated with vitamin D. (**B**) Representative images of individual DNA comets from control or 53BP1-depleted *Lmna*$^{-/-}$ MEFs treated with either vitamin D or vehicle. Note how the length of the comet decreases faster in vitamin D-treated *Lmna*$^{-/-}$ cells than in 53BP1-depleted cells. (**C**) Representative images of individual DNA comets from control and CTSL overexpressing wild-type MEFs treated with either vitamin D or vehicle. Note how vitamin D treatment restores DNA repair in CTSL overexpressing cells.

A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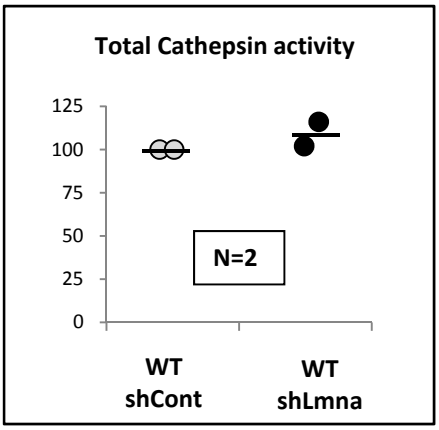
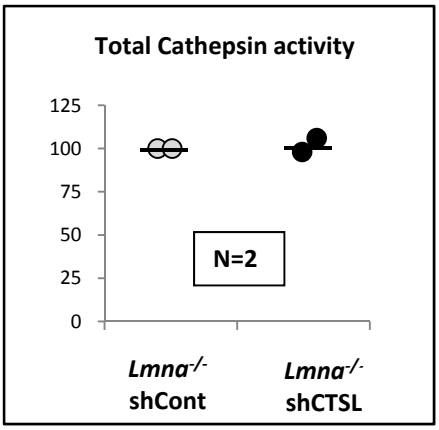
125
100
75
50
25
0
N=2
WT
Lmna-/-

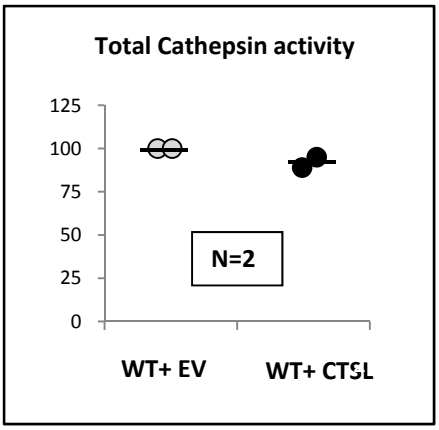
B


Total Cathepsin activity
125
100
75
50
25
0
N=2
WT shCont
WT shLmna

C


Total Cathepsin activity
125
100
75
50
25
0
N=2
Lmna-/- shCont
Lmna-/- shCTSL

D


Total Cathepsin activity
125
100
75
50
25
0
N=2
WT+ EV
WT+ CTSL

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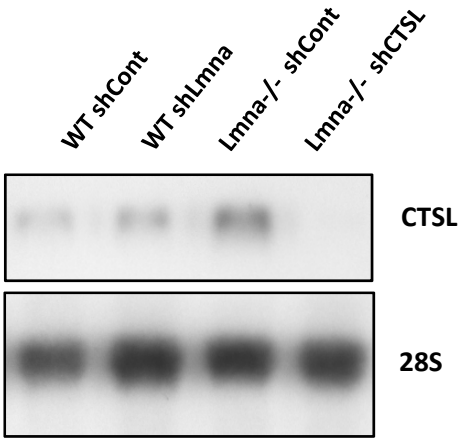
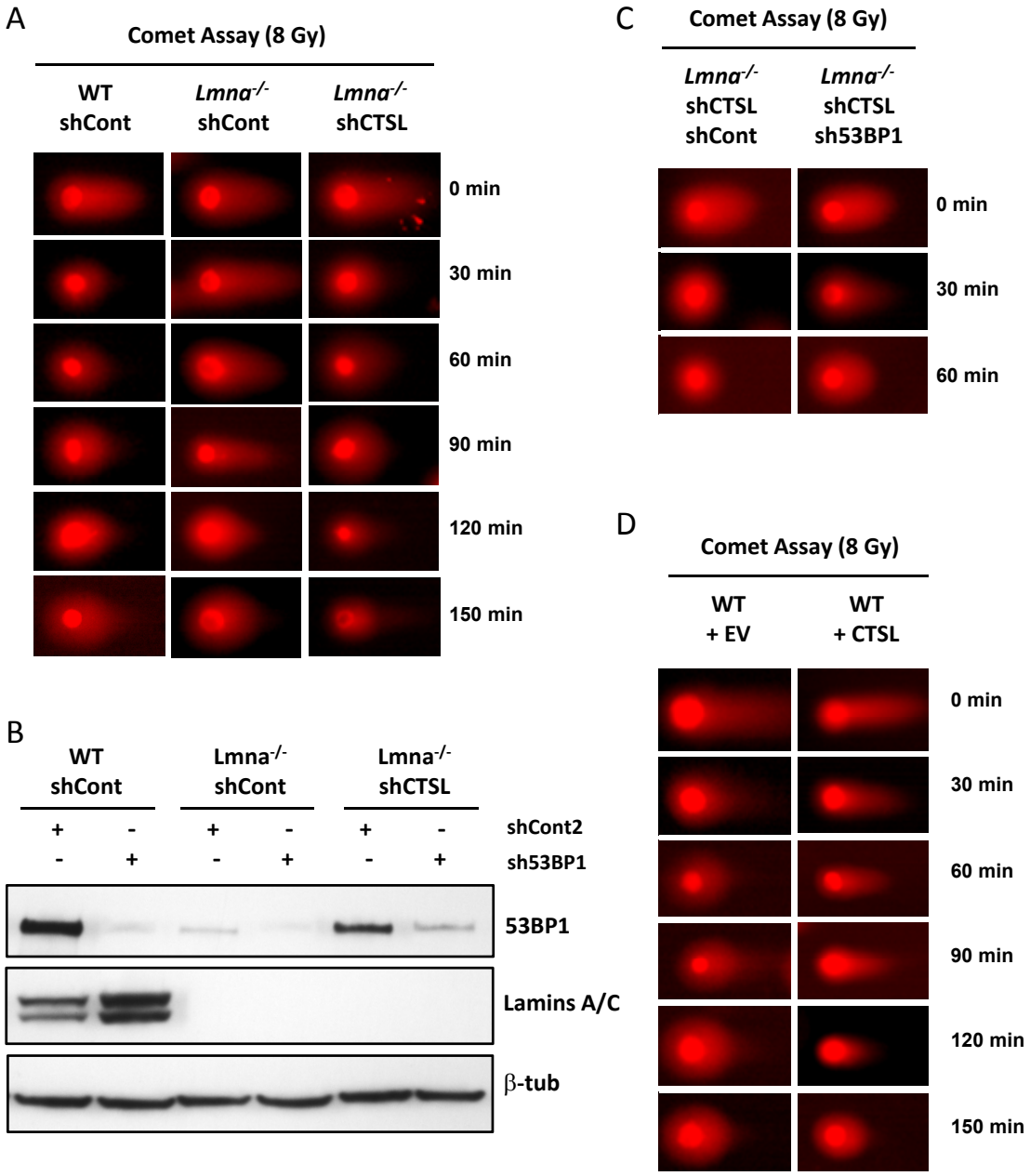
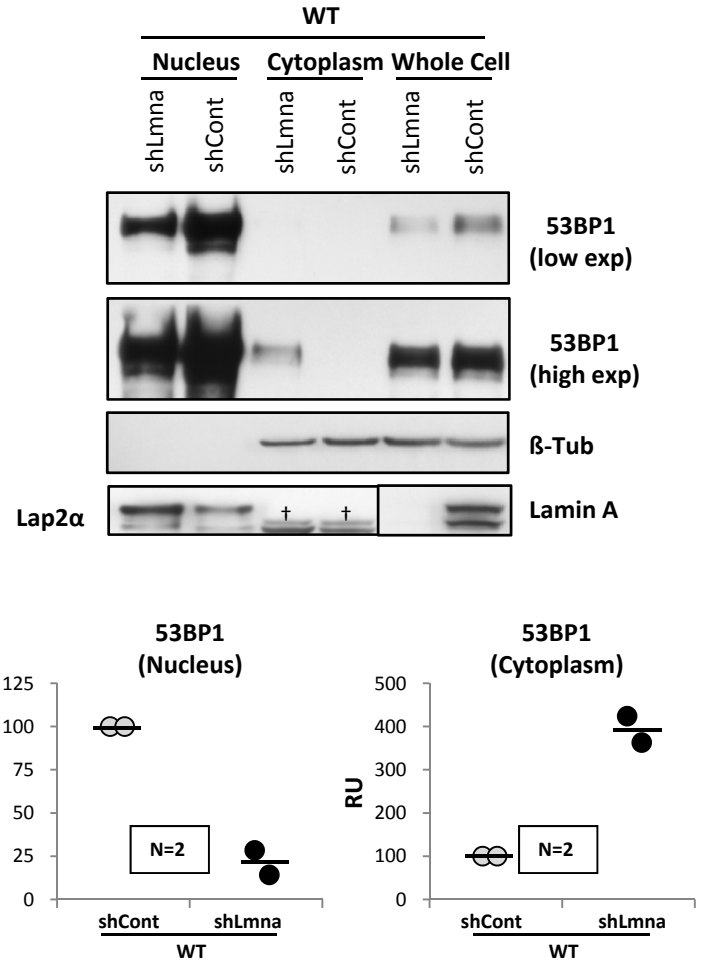
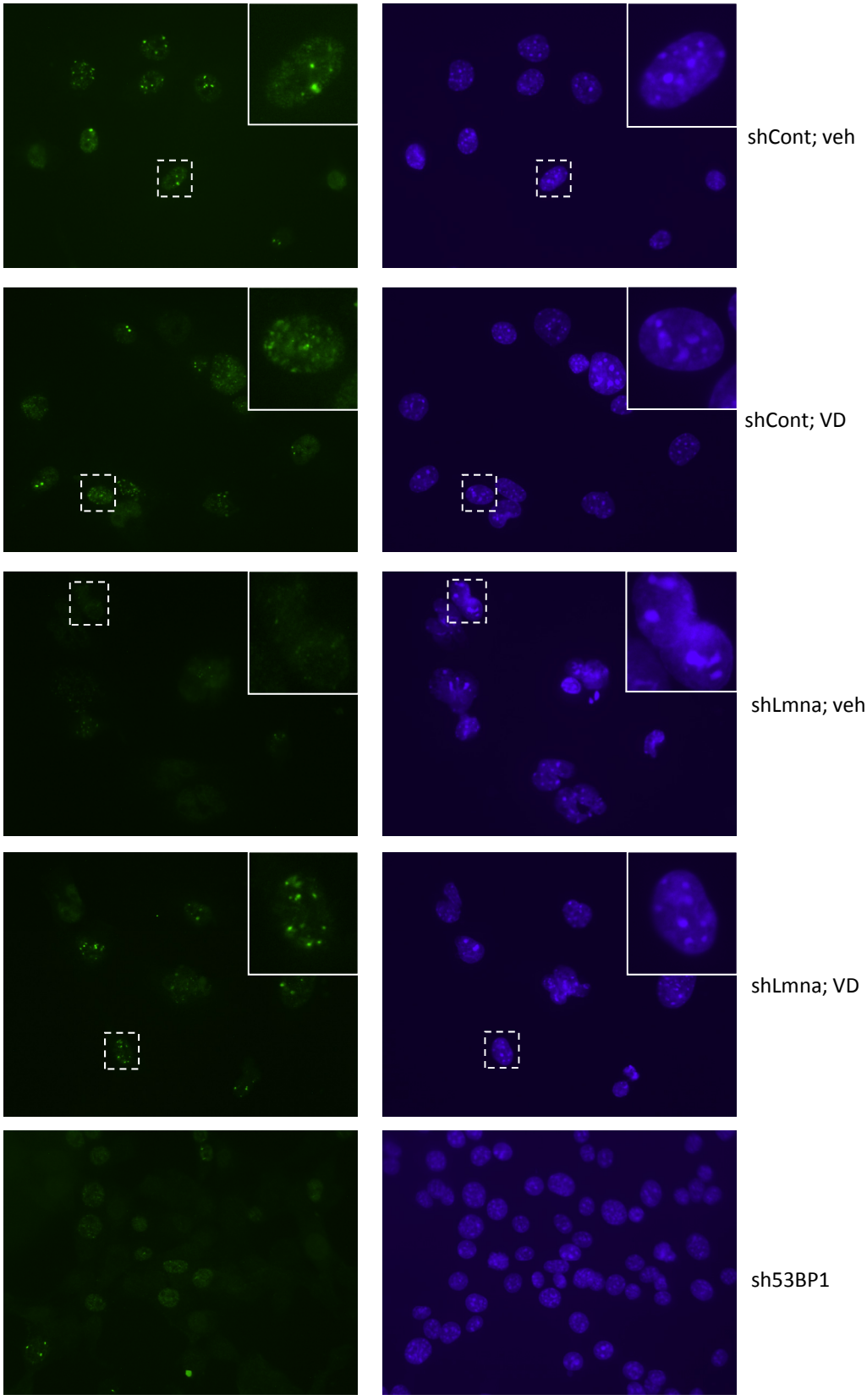
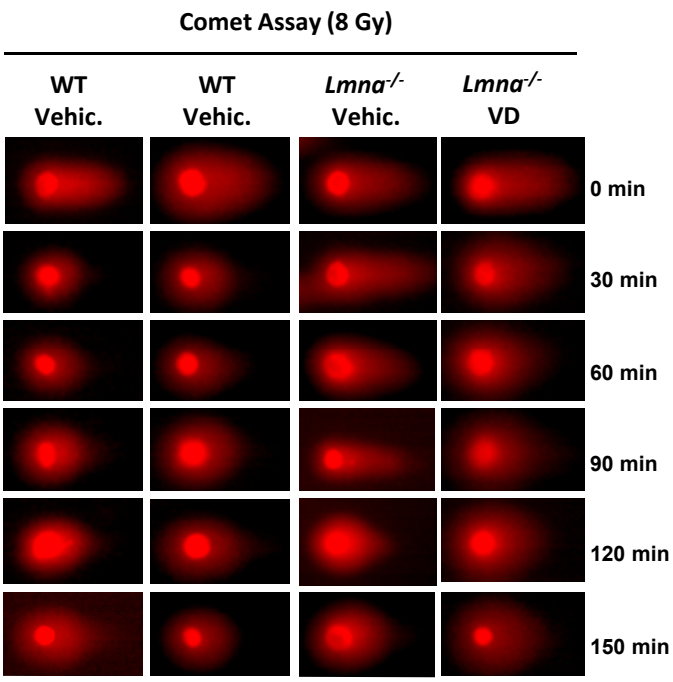



A
Comet Assay (8 Gy)
WT shCont
Lmna-/- shCont
Lmna-/- shCTSL
0 min
30 min
60 min
90 min
120 min
150 min
B
WT shCont
Lmna-/- shCont
Lmna-/- shCTSL
shCont2
sh53BP1
53BP1
Lamins A/C
β-tub
C
Comet Assay (8 Gy)
Lmna-/- shCTSL shCont
Lmna-/- shCTSL sh53BP1
0 min
30 min
60 min
D
Comet Assay (8 Gy)
WT + EV
WT + CTSL
0 min
30 min
60 min
90 min
120 min
150 min

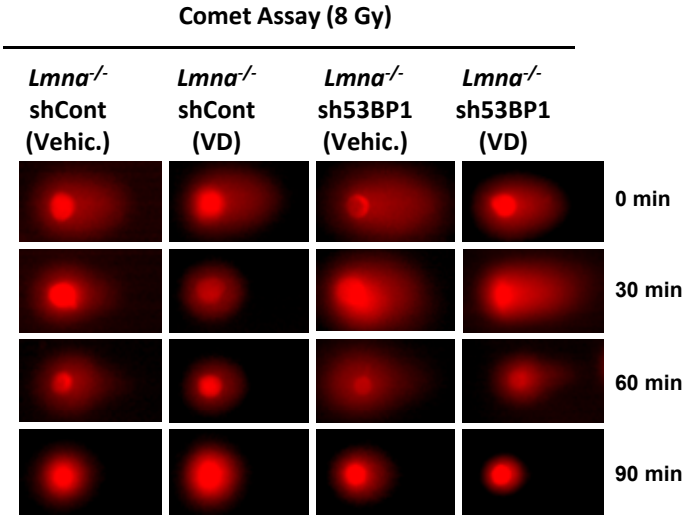

WT
Nucleus
Cytoplasm
Whole Cell
shLmna
shCont
shLmna
shCont
shLmna
shCont
53BP1
(low exp)
53BP1
(high exp)
ß-Tub
Lap2α
Lamin A
53BP1
(Nucleus)
RU
125
100
75
50
25
0
N=2
shCont
shLmna
WT
53BP1
(Cytoplasm)
RU
500
400
300
200
100
0
N=2
shCont
shLmna
WT


shCont; veh
shCont; VD
shLmna; veh
shLmna; VD
sh53BP1

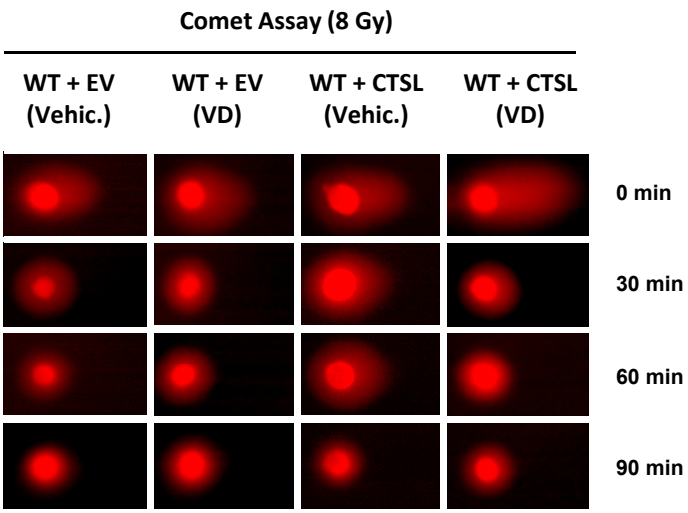
A


Comet Assay (8 Gy)
WT Vehic.
WT Vehic.
Lmna-/- Vehic.
Lmna-/- VD
0 min
30 min
60 min
90 min
120 min
150 min

B


Comet Assay (8 Gy)
Lmna-/- shCont (Vehic.)
Lmna-/- shCont (VD)
Lmna-/- sh53BP1 (Vehic.)
Lmna-/- sh53BP1 (VD)
0 min
30 min
60 min
90 min

C


Comet Assay (8 Gy)
WT + EV (Vehic.)
WT + EV (VD)
WT + CTSL (Vehic.)
WT + CTSL (VD)
0 min
30 min
60 min
90 min