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Supplementary Materials for

Nuclear WRAP53 promotes neuronal survival and functional recovery after stroke

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Other Supplementary Material for this manuscript includes the following:

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Supplementary Figure 1 (Fig. S1)

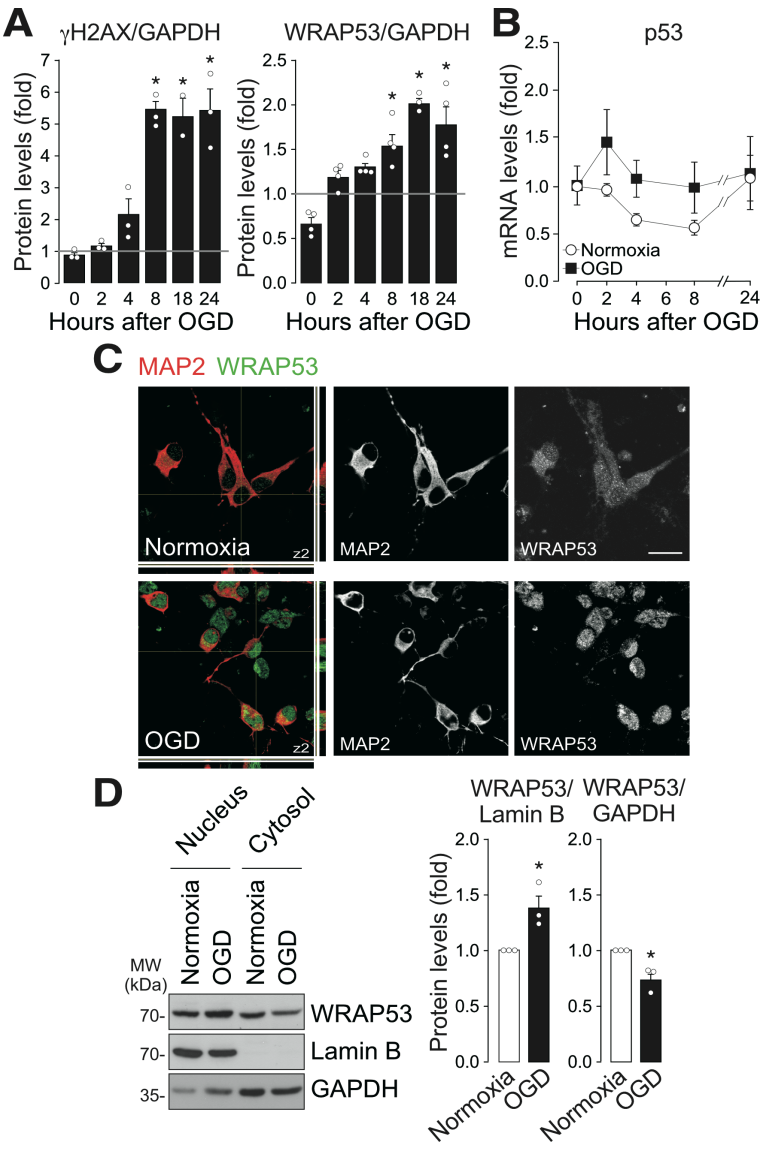

Fig. S1. Oxygen and glucose deprivation (OGD) induces WRAP53 upregulation and nuclear translocation in neurons. A. Results of the protein abundance quantification of proteins shown in Fig. 1C. OGD increased γH2AX and WRAP53 protein levels in neurons. The error bars are standard error deviation from 3-4 different neuronal cultures (*p<0.05 *versus* Normoxia; one-way ANOVA followed by the Bonferroni *post hoc* test). **B.** p53 mRNA remained unchanged after OGD. The error bars are standard error deviation from 3 different neuronal cultures (*p<0.05 *versus* Normoxia; two-way ANOVA followed by the Bonferroni

post hoc test). **C.** Orthogonal view showing WRAP53 translocation into the nucleus at 8 hours after OGD (Scale bar = 25 μm). **D.** Western blot analysis confirming WRAP53 accumulation in neuronal nuclei at 8 hours after OGD. Quantifications of the nuclear and cytosolic WRAP53 abundance. The error bars are standard error deviation from 3 different neuronal cultures (*$p<0.05$ *versus* Normoxia; Student's *t* test).

Supplementary Figure 2 (Fig. S2)

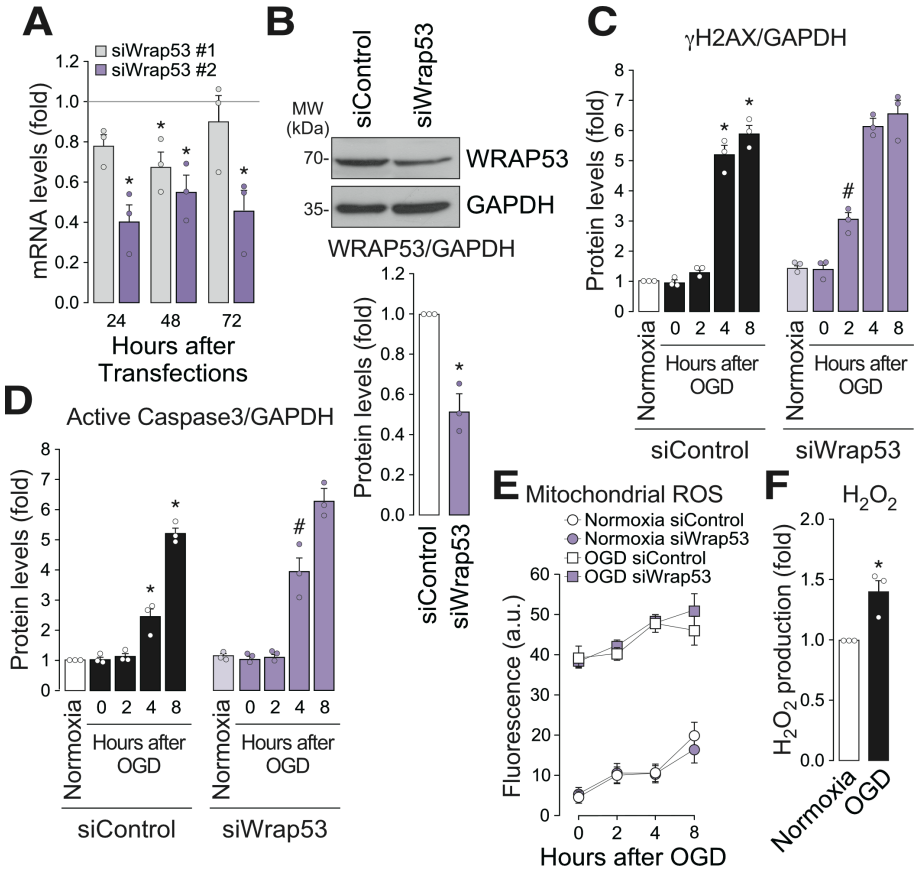

Fig. S2. WRAP53 loss renders neurons vulnerable to DNA damage and apoptosis after oxygen and glucose deprivation (OGD). A, B. WRAP53 knockdown by siRNA (siWrap53#1, and siWrap53#2 or siWrap53) transfection for 2 days triggers γH2AX and active Caspase-3 accumulation in neurons. The error bars are standard error deviation from 3 different neuronal cultures (*p<0.05 *versus* siControl; Students's *t* test). **C, D.** Results of the protein abundance quantification of proteins shown in Fig. 2A. The error bars are standard error deviation from 3 different neuronal cultures (*p<0.05 *versus* Normoxia; #p<0.05 *versus* siControl OGD; two-way ANOVA followed by the Bonferroni *post hoc* test). **E, F.** OGD-induced mitochondrial ROS generation and hydrogen peroxide (H_2O_2) production. The error bars are standard error deviation from 3 different neuronal cultures (*p<0.05 *versus* Normoxia; Student's *t* test).

Supplementary Figure 3 (Fig. S3)

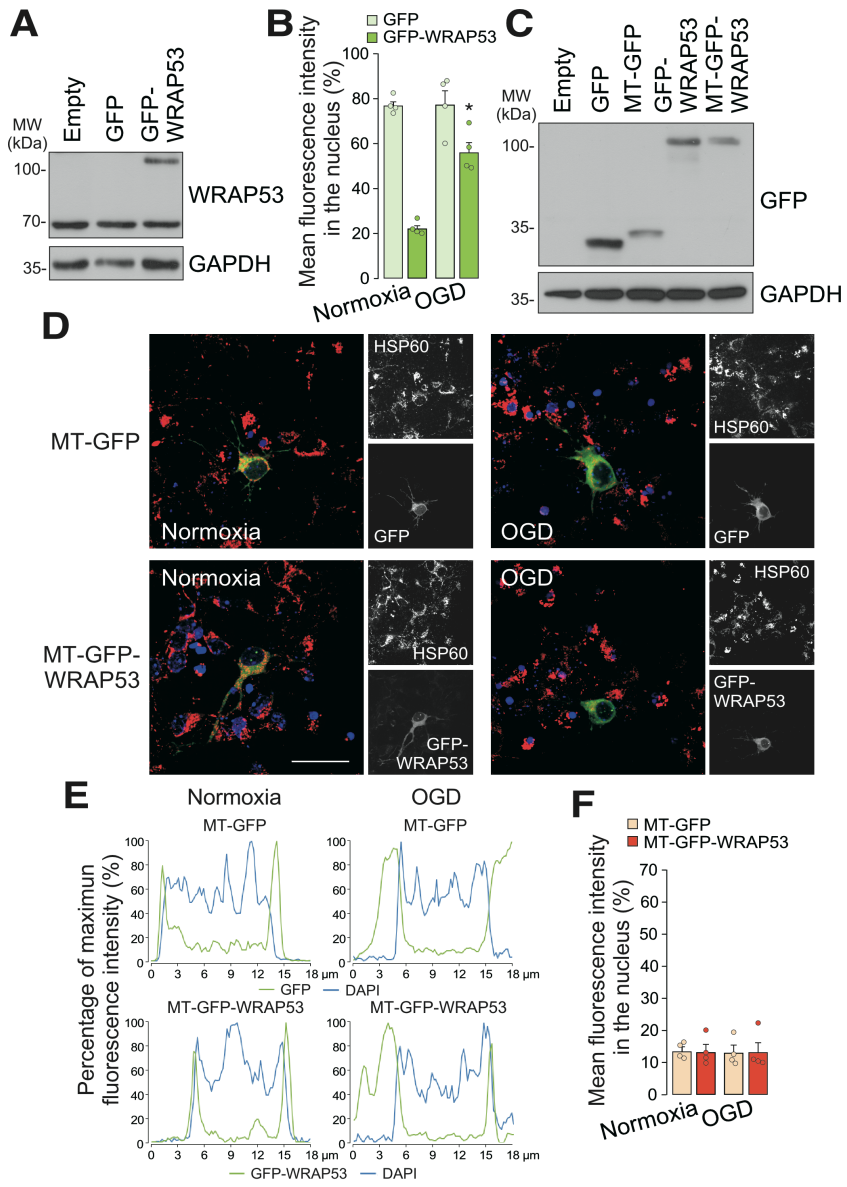

Fig. S3. WRAP53 recruitment in the cytosol prevents neuroprotection. **A, B.** Neurons were transfected with plasmids encoding GFP and GFP-WRAP53. **A.** Western blot image showing ectopic expression of GFP-WRAP53. **B.** Results of the mean fluorescence intensity of GFP and GFP-WRAP53 within the nucleus. The error bars are standard error deviation from 4 different neuronal cultures (10-15 GFP^+ neurons per culture. *$p<0.05$ *versus* Normoxia; two-way ANOVA followed by the Bonferroni *post hoc* test). **C-F.** Neurons were transfected with plasmids encoding GFP (MT-GFP) and GFP-WRAP53 (MT-GFP-WRAP53) fused to a

mitochondrial-targeting (MT) sequence of human ornithine transcarbamylase for 24 hours and were subjected to oxygen and glucose deprivation (OGD). **C.** Western blot image showing ectopic expression of GFP. **D.** Representative image of cortical neurons stained with GFP and HSP60 (mitochondrial marker). Scale bar = 25 μm. **E.** Representative cross-sectional intensity profiles for GFP (green) and DAPI (blue) staining of MT-GFP and MT-GFP-WRAP53-transfected neurons. **F.** Results of the mean fluorescence intensity of MT-GFP and MT-GFP-WRAP53 within the nucleus. The error bars are standard error deviation from 4 different neuronal cultures (10-15 GFP^+ neurons per culture).

Supplementary Figure 4 (Fig. S4)

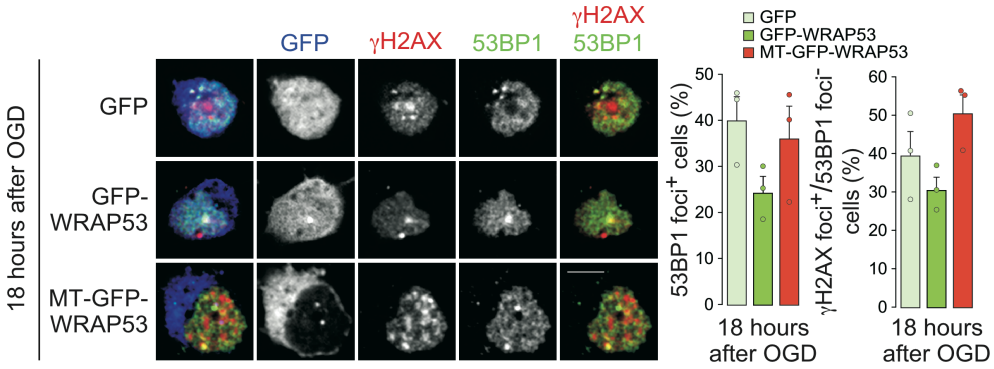

Fig. S4. Nuclear accumulation of WRAP53 promoted double-strand break (DSB) repair at 18 hours after oxygen and glucose deprivation (OGD). Neurons were transfected with plasmids encoding GFP, GFP-WRAP53 and MT-GFP-WRAP53 (MT: mitochondrial-targeting sequence) for 24 hours and were subjected to oxygen and glucose deprivation (OGD), as indicated in Fig. 1. Neurons were immunostained for GFP, γH2AX and 53BP1 at 18 hours after OGD. Representative images are shown. Scale bar = 5 μm. Quantification of 53BP1 foci positive neurons (percentage of neurons costaining γH2AX and 53BP1 foci) and percentage of γH2AX foci-positive and 53BP1 foci-negative neurons. The error bars are standard error deviation from 3 different neuronal cultures (10-12 GFP^+ neurons per culture).

Supplementary Figure 5 (Fig. S5)

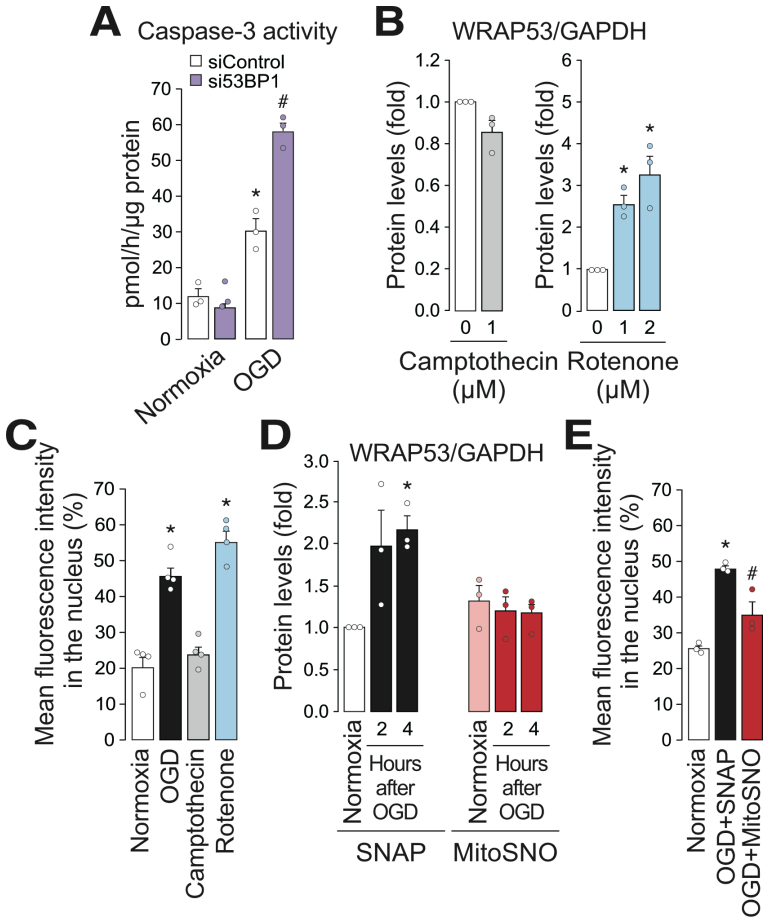

Fig. S5. Mitochondrial ROS generation promotes WRAP53 upregulation and nuclear translocation in neurons. A. Analysis of caspase-3 activity in siControl and si53BP1 transfected neurons performed at 8 hours after oxygen and glucose deprivation (OGD).The error bars are standard error deviation from 3 different neuronal cultures (*p<0.05 *versus* Normoxia; #p<0.05 *versus* OGD siControl; two-way ANOVA followed by the Bonferroni *post hoc* test). **B.** Results of the protein abundance quantification of proteins shown in Fig. 5A and B. The error bars are standard error deviation from 3 different neuronal cultures (*p<0.05 *versus* absence of treatment; one-way ANOVA followed by the Bonferroni *post hoc* test). **C.** Results of the mean fluorescence intensity of GFP-WRAP53 within the nucleus. The error bars are standard error deviation from 4 different neuronal cultures (10-15 GFP^+ neurons per culture. *p<0.05 *versus* Normoxia; one-way ANOVA followed by the Bonferroni *post hoc* test). **D.**

Results of the WRAP53 protein abundance quantification of proteins shown in Fig. 6B, after treatment with SNAP (*S*-nitroso-*N*-acetyl penicillamine) or the mitochondrial ROS production inhibitor MitoSNO (mitochondria-selective *S*-nitrosating agent). The error bars are standard error deviation from 3 different neuronal cultures (*p<0.05 *versus* Normoxia; two-way ANOVA followed by the Bonferroni *post hoc* test). **E.** Results of the mean fluorescence intensity of GFP-WRAP53 within the nucleus. The error bars are standard error deviation from 3 different neuronal cultures (10-12 GFP^+ neurons per culture. *p<0.05 *versus* Normoxia; #p<0.05 *versus* OGD+SNAP; one-way ANOVA followed by the Bonferroni *post hoc* test).

Supplementary Figure 6 (Fig. S6)

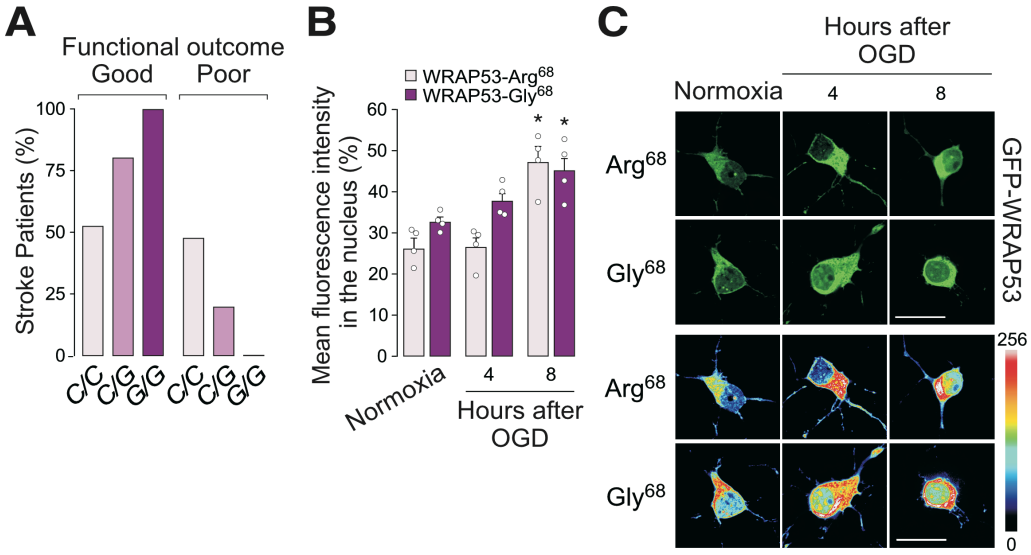

Fig S6. The *Wrap53 C>G* SNP (rs2287499) enhanced WRAP53 nuclear accumulation and functional recovery after stroke. A. Stroke patients (n = 408; rs2287499 *C/C*: 311; *C/G*: 87; *G/G*: 10) were matched by good (mRS ≤ 2) and poor (mRS > 2) functional outcome, by using the modified Ranking scale (mRS), with indicated *Wrap53* genotypes. **B.** Results of the mean fluorescence intensity of GFP-WRAP53 polymorphic variants Arg^{68} and Gly^{68} within the nucleus. The error bars are standard error deviation from 4 different neuronal cultures (10-12 GFP^{+} neurons per culture. *p<0.05 *versus* Normoxia; two-way ANOVA followed by the Bonferroni *post hoc* test). **C.** Heat maps of fluorescence intensity of GFP-WRAP53 Arg^{68} and GFP-WRAP53 Gly^{68}.

Table S1. Demographic and clinical features of stroke patients

Variables	Ischemic stroke (n=408)
Age, years	72.8 ± 12.1
Males, n (%)	242 (59.3)
TOAST:	
- Cardioembolic, n (%)	148 (36.3)
- Atherotrombotic, n (%)	38 (9.3)
- Lacunar, n (%)	46 (11.3)
- Undetermined, n (%)	167 (40.9)
- Others, n (%)	9 (2.2)
NIHSS on admission	5 [2, 11]
ASPECTS on admission	10 [8, 10]
Infarct volume at day 4-7, mL	31.1 ± 58.7
Early neurological deterioration, n (%)	43 (10.5)
mRS at 3 months	2 [1, 4]
Poor prognosis at 3 months, n (%)	161 (39.5)
Wrap53 C>G SNP (rs2287499)	
- *C/C* genotype, n (%)	311 (76.2)
- *C/G* genotype, n (%)	87 (21.3)
- *G/G* genotype, n (%)	10 (2.5)

Patients were admitted at the University Hospital of Santiago de Compostela (Galicia, Spain). Data are shown as number (%), means ± SD or medians [quartiles]. mRS: modified Rankin Scale; NIHSS: National Institute of Health Stroke Scale.

Table S2. Univariate analysis of baseline variables according to functional outcome at 3 months in stroke patients

	Stroke		
	Good outcome n=247	**Poor outcome n=161**	***p***
Age, years	70.1 ± 11.7	78.8 ± 9.9	<0.0001
Males, %	68.0	46.0	<0.0001
Hypertension, %	51.1	64.6	0.007
Diabetes, %	27.1	24.8	0.347
Smoking, %	17.4	8.1	0.005
Heavy alcohol intake, %	9.3	7.5	0.321
Hyperlipidemia, %	33.2	28.6	0.191
Coronary disease, %	13.8	14.3	0.496
Atrial fibrillation, %	14.6	24.8	0.007
SBP on admission, mm Hg	148.5 ± 25.9	155.6 ± 28.1	0.053
DBP on admission, mm Hg	81.7 ± 13.9	82.5 ± 14.7	0.708
Maximum SBP at 24 hours, mm Hg	133.1 ± 23.2	145.8 ± 25.4	<0.0001
Maximum DBP at 24 hours, mm Hg	73.8 ± 12.1	76.7 ± 13.1	0.224
Temperature on admission, ºC	36.3 ± 0.5	36.3 ± 0.5	0.296
Maximum temperature at 24 hours, ºC	36.3 ± 0.5	36.6 ± 0.5	<0.0001
Blood glucose on admission, mg/dL	133 ± 60.5	140 ± 60.6	<0.0001
Maximum blood glucose at 24 hours, mg/dL	121.1 ± 43.9	128.5 ± 56.5	0.052
Leukocytes on admission, $x10^3$/mL	8.2 ± 2.4	9.0 ± 2.8	<0.0001

Platelet number on admission, $x10^3$/mL	231.1 ± 65.8	255.3 ± 70.0	0.045
International normalized ratio on admission	1.1 ± 0.3	1.1 ± 0.2	0.003
Fibrinogen on admission, mg/dL	452.9 ± 103.8	526.9 ± 130.7	<0.0001
hs-CRP on admission, mg/dL	1.6 ± 2.4	4.6 ± 7.9	<0.0001
NIHSS on admission	3 [1, 5]	11 [6, 17]	<0.0001
Early neurological deterioration, %	3 [1, 5]	11 [6, 17]	<0.0001
Infarct Volume at day 4-7, mL	10.4 ± 16.0	58.8 ± 81.1	<0.0001
ASPECTS	10 [9, 10]	10 [7, 10]	<0.0001
TOAST			0.056
C/C genotype, %	67.6	89.4	<0.0001

Functional outcome was evaluated at 3 months using the modified Rankin Scale (mRS); mRS score >2 was considered poor prognosis. Data are percentage, means ± SD or medians [quartiles]. Percentages were compared using the χ^2 test. Student's t test was used to compare continuous variables with normal distribution between the groups. Continuous variables without normal distribution (NIHSS) were compared using the Mann-Whitney test. SBP, systolic blood pressure; DBP, diastolic blood pressure; hs-CRP, high-sensitivity C-reactive protein; NIHSS, National Institute of Health Stroke Scale.

Table S3. Multiple logistic regression analysis showing independent variables associated with poor functional outcome at 3 months (modified Rankin Scale > 2) after stroke

	Stroke		
	OR	**95% CI**	***p***
Age	1.04	1.01 - 1.07	0.003
Infarct volume	1.01	1.00 - 1.03	0.005
NIHSS on admission	1.27	1.18 - 1.37	<0.0001
Early neurological deterioration	12.84	3.84 - 42.84	<0.0001
C/C genotype	2.95	1.01 - 4.21	0.001

OR, odds ratio; NIHSS, National Institutes of Health Stroke Scale.

Table S4. Genotypes of stroke patients with poor functional outcome matched for infarct volume.

Infract volume 4th - 7th day after stroke (mL)	**N**	***C/C***	***C/G + G/G***
0 - 2	112	22.1% (17/77)	11.4% (4/35)
2 - 10	91	16.9% (11/65)	15.4% (4/26)
10-28	96	40.5% (30/74)	9.1% (2/22)*
≥ 28	100	89.7% (78/87)	53.8% (7/13)*

Patients were admitted at the University Hospital of Santiago de Compostela (Galicia, Spain). Functional outcome was evaluated at 3 months after stroke, by using the modified Rankin Scale (mRS). Percentages represent number of patients with poor functional outcome (mRS >2) in relation with total patients per genotype matched for infarct volume. N is the number of stroke patients per infarct volume range. Percentages were compared between genotype groups using the χ^2 test (*p = 0.003).