A Crucial Role of CXCL14 for Promoting Regulatory T Cells Activation in Stroke

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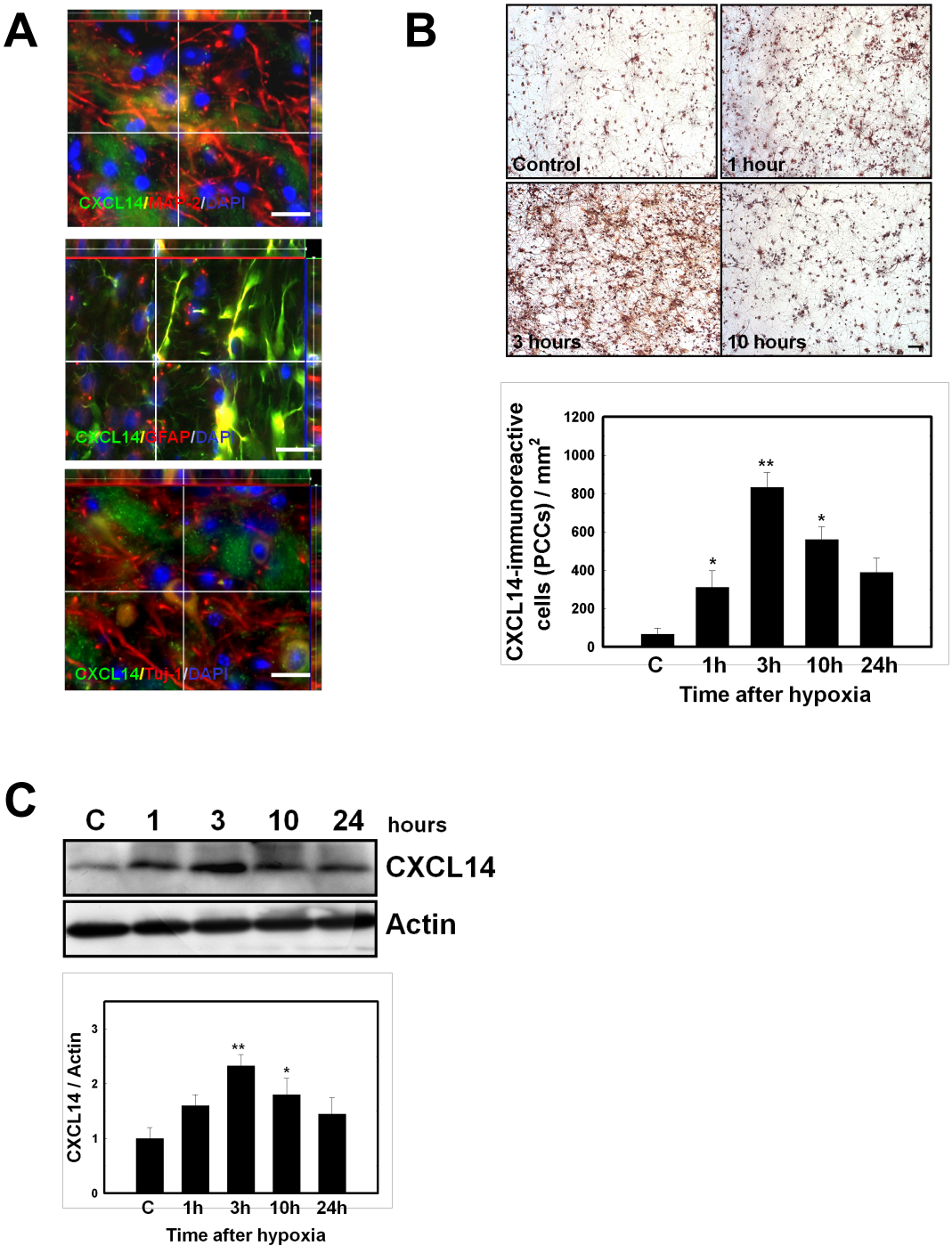
Supplementary Table S1

Demographic and immunostaining data of brain samples of each patient

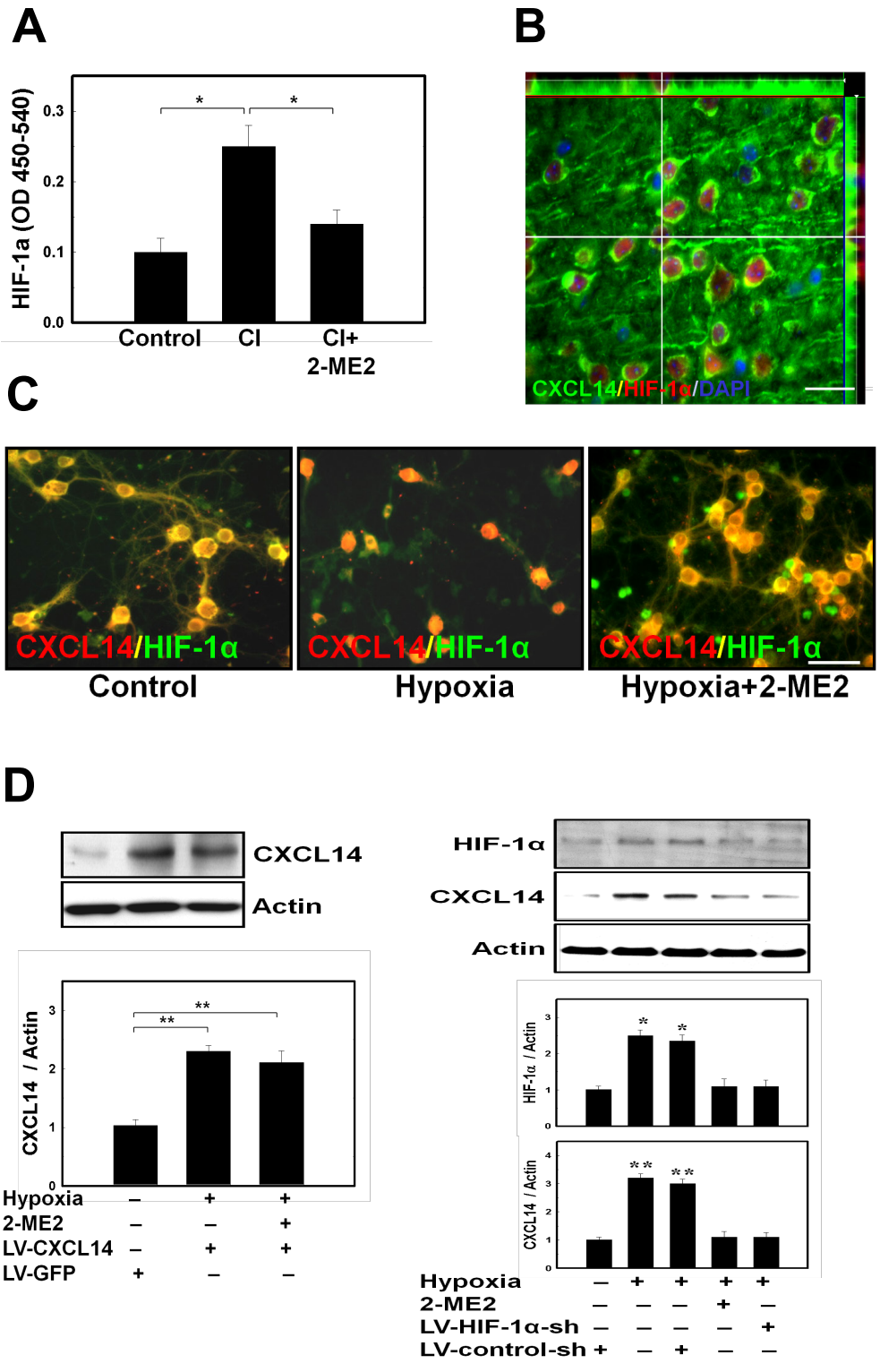
		Gender (female:F, male:M)	Age	Cause of death	CXCL14 Immunoreactivity (No. of $CXCL14^+$ cells)
Experimental group	1	M	61	fatal cerebral infarction （1day after onset）	3766/mm^2
	2	F	59	fatal cerebral infarction （1day after onset）	3154/ mm^2
	3	M	57	fatal cerebral infarction （3days after onset）	4011/ mm^2
	4	M	54	fatal cerebral infarction （3days after onset）	3416/ mm^2
Control group	1	F	61	Glioblastoma Multiforme with brainstem failure	99/ mm^2
	2	M	63	Glioblastoma Multiforme with brainstem failure	73/ mm^2
	3	M	60	Glioblastoma Multiforme with brainstem failure	44/ mm^2
	4	F	57	Glioblastoma Multiforme with brainstem failure	57/ mm^2

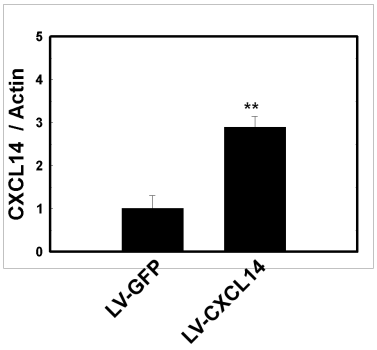
Supplementary Figures



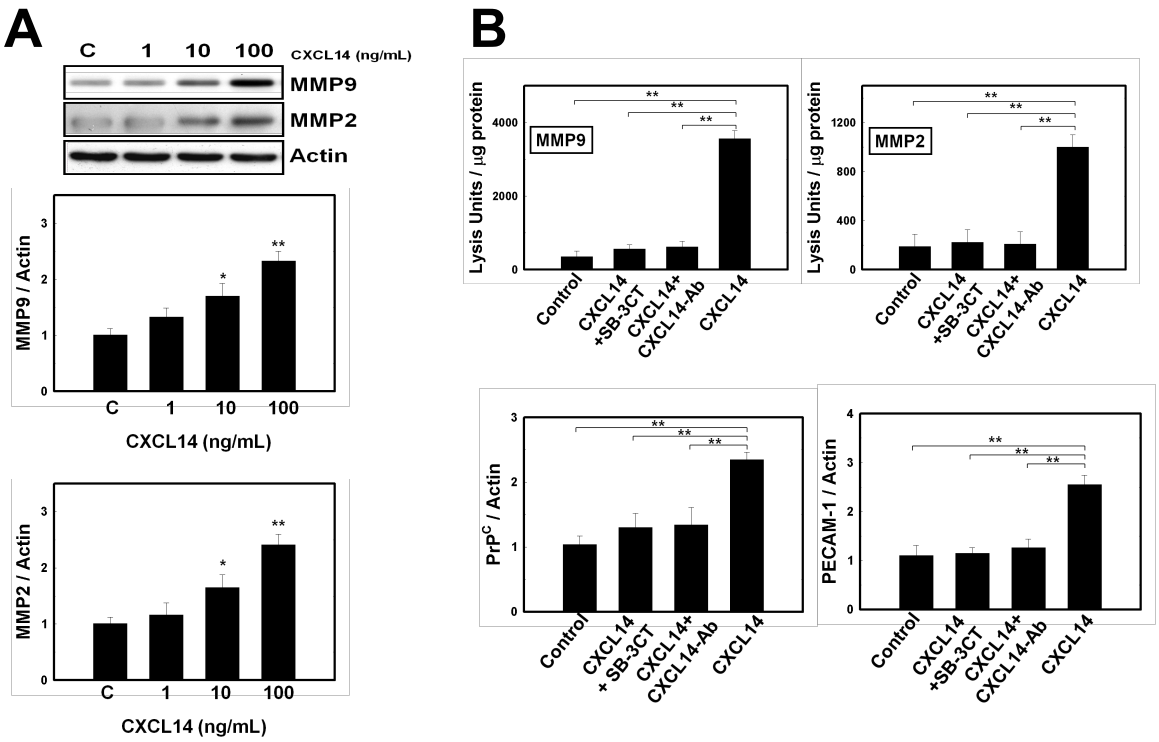
Supplementary Figure S1. HIF-1α/Ischemia Induced Upregulation of CXCL14 Expression in human Brain and Rat Brains as well as in Primary Cortical Cultures (PCCs). (**A**) In a double immunofluorescence study of rat brain (3D images), cells that expressed CXCL14 in the penumbra colocalized with the specific markers MAP-2, Tuj-1, and GFAP. (**B**) Hypoxia in PCCs increased $CXCL14^{+}$ cell density in a time-dependent manner compared with the control. (**C**) The protein

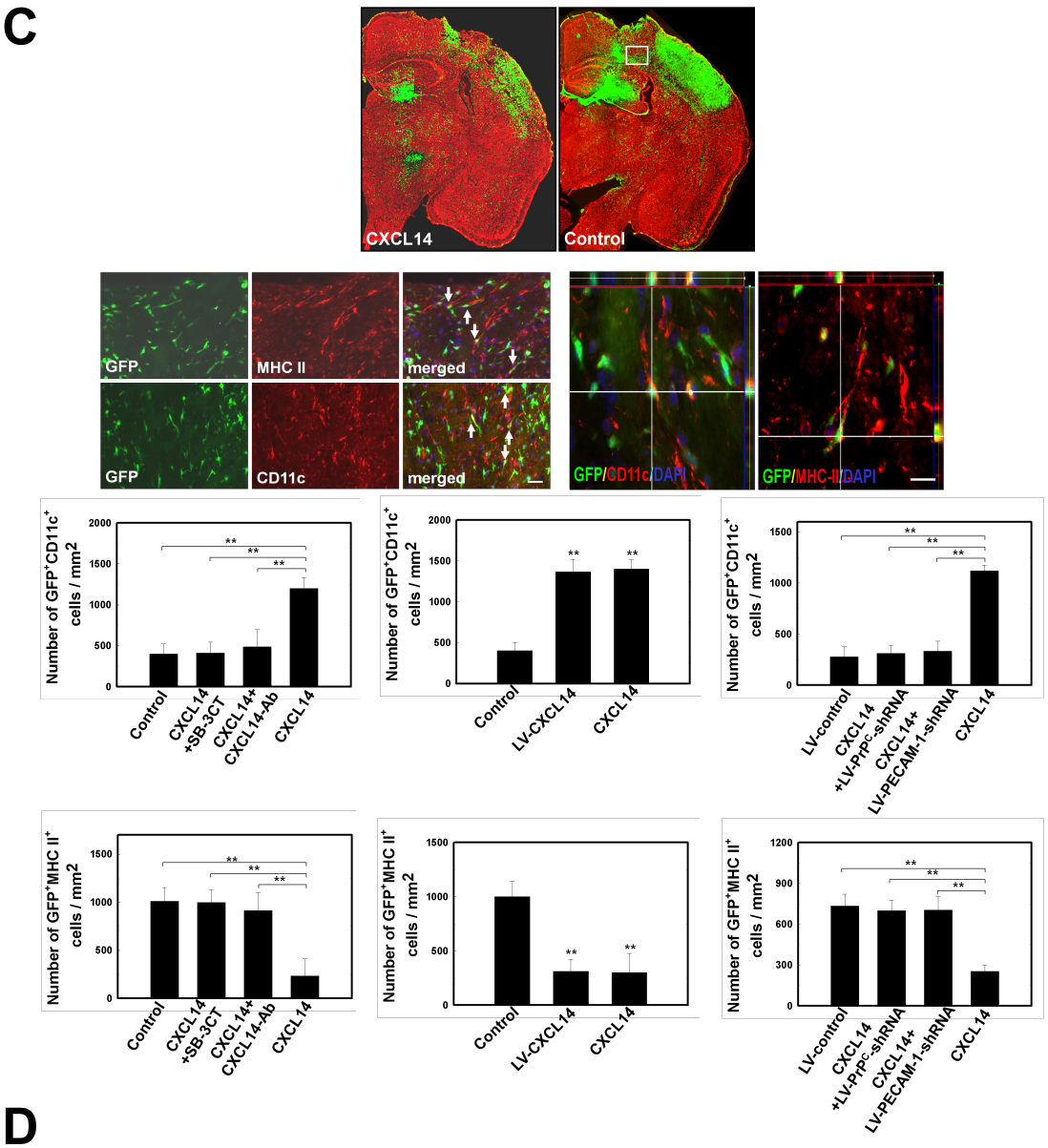
expression of CXCL14 in PCCs after hypoxia treatment was greater than in the control, and the increase was time-dependent. n = 8 per group. The mean ± SEM is shown. The mean ± SEM is shown. *$P < 0.05$ and **$P < 0.01$ vs. control. Bar = 50 μm.





Supplementary Figure S2. Upregulation of CXCL14 After Cerebral Ischemia (CI) and Hypoxia Through Induction of HIF-1α *In Vivo* and *In Vitro*. (**A**) Nuclear HIF-1α activity increased in rat brains after cerebral ischemia, which was blocked by 2-ME2 (100 mg/kg). (**B**) $CXCL14^+$ cells co-expressed with the $HIF\text{-}1\alpha^+$ cells in the ischemic penumbral area. (**C**) HIF-1α colocalized with CXCL14 and was translocated mainly into the nuclei or to perinuclear areas (arrow) of PCCs after hypoxia treatment. Pretreating PCCs with 2-ME2 (10 μM) resulted in the HIF-1α protein being distributed to the cytosol and neurite (arrow-head), as well as the nucleus after hypoxia treatment. (**D**) 2-ME2 inhibited HIF-1α-induced CXCL14 expression specifically, but not in lentiviral (LV-CXCL14) infection-induced CXCL14 expression (left panel). Both HIF-1α and CXCL14 expression were downregulated by the administration of 2-ME2 and LV-HIF-1α-shRNA (right panel). LV-CXCL14 transduction induced significant increased expression of CXCL14 in PCC (lower panel). The mean ± SEM is shown. **P* < 0.05 and ***P* < 0.01 vs. control. Bar = 50 μm.




C
CXCL14
Control
GFP
MHC II
merged
GFP
CD11c
merged
GFP/CD11c/DAPI
GFP/MHC-II/DAPI
Number of GFP+CD11c+ cells / mm2
Control
CXCL14
+SB-3CT
CXCL14+
CXCL14-Ab
CXCL14
Number of GFP+CD11c+ cells / mm2
Control
LV-CXCL14
CXCL14
Number of GFP+CD11c+ cells / mm2
LV-control
CXCL14
+LV-PrPC-shRNA
CXCL14+
LV-PECAM-1-shRNA
CXCL14
Number of GFP+MHC II+ cells / mm2
Control
CXCL14
+SB-3CT
CXCL14+
CXCL14-Ab
CXCL14
Number of GFP+MHC II+ cells / mm2
Control
LV-CXCL14
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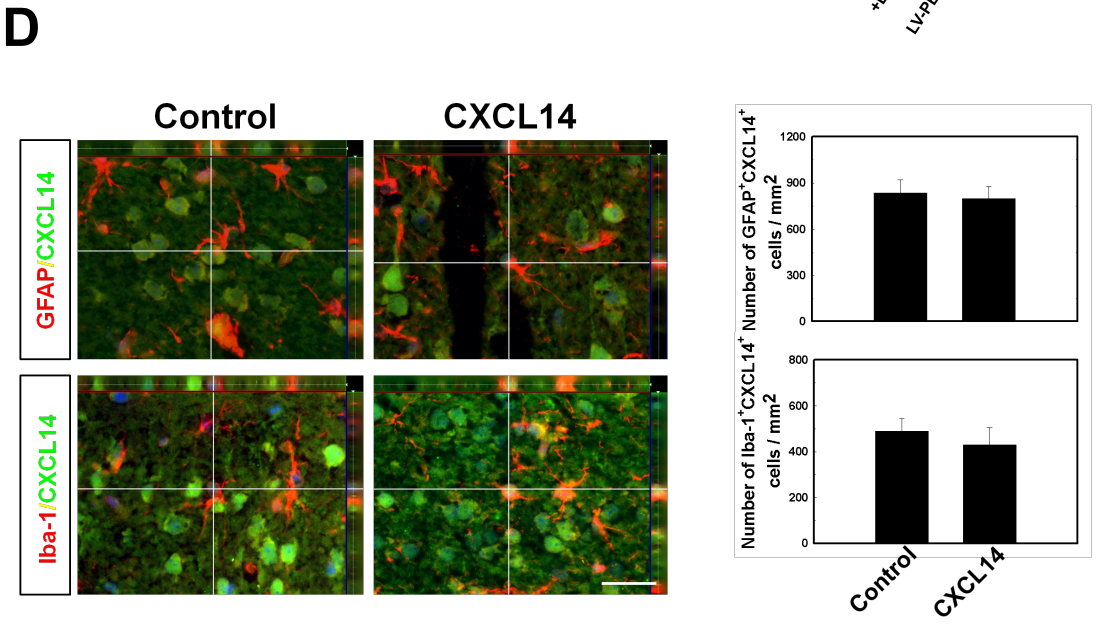
CXCL14+
LV-PECAM-1-shRNA
CXCL14


D
Control
CXCL14
GFAP/CXCL14
Iba-1/CXCL14
Number of GFAP+CXCL14+ cells / mm2
Number of Iba-1+CXCL14+ cells / mm2
Control
CXCL14

Supplementary Figure S3. CXCL14 Stimulated the Recruitment of iDC and Activation of Tregs. (**A**) Western blot showed that CXCL14 treatment upregulated the protein expression of MMP9 and MMP2. (**B**) The activity of MMP9 and MMP2, as well as the protein expression of PrP^C and PECAM-1, were increased by intravenous injection of CXCL14 (100 ng/kg) into the ischemic brain. (**C**) In the representative GFP-chimeric mice, 3 days following cerebral ischemia (green color, GFP; red color, propidium iodide stain) (right upper panel), a larger amount of GFP^+CD11c^+ iDC cells were found distributed over the right striatum, hippocampus, and the penumbral area in recombinant CXCL14 or LV-CXCL14-treated mice than in control mice (middle panel). This phenomenon was inhibited by the administration of the MMP inhibitor (SB-3CT) and CXCL14-Ab, or stereotaxic injection of LV-PrP^C-shRNA or LV-PECAM-1-shRNA. However, recruitment of $GFP^+MHC\text{-}II^+$ cells into the ischemic brain was reduced by recombinant CXCL14 or LV-CXCL14 injection. (**D**) CXCL14 treatment did not induce significant change numbers of $GFAP^+CXCL14^+$ or Iba-$1^+CXCL14^+$ cells. The mean ± SEM is shown. $^*P < 0.05$ and $^{**}P < 0.01$ vs. control. Bar = 50 μm.