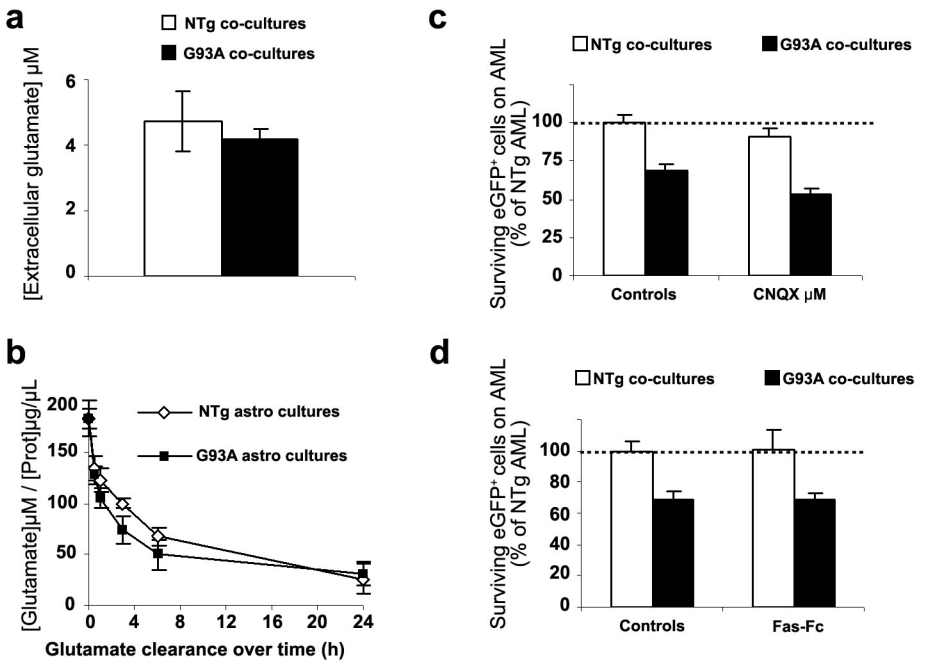
Supplementary Fig.5



e

Cytokines-Chemokines (DO ratio/positive controls)	Day 7	
	NTg astro	G93A astro
CNTF	0.62	**0.72**
MCP-1	**0.73**	0.64
MIP-3α	0.04	0.05
β-NGF	0.10	0.09
TIMP-1	0.55	**0.65**
TNF-α	0.04	0.04
VEGF	0.19	**0.27**

Supplementary Fig.5: Glutamate, Fas ligand and several major cytokines and chemokines are not involved in the toxicity of mutant SOD1 astrocytes to motor neurons. (**a**) Extracellular glutamate concentrations measurement by HPLC at 7 d post–plating in co–cultures made of ^{NTg}pMN plated on ^{NTg}AML or ^{G93A}AML did not differ (Student t–test; P=1.0). (**b**) 14 d–old confluent ^{NTg}AML or ^{G93A}AML were incubated with 200 µM of glutamate. Then, the extracellular concentration of glutamate was monitored by HPLC at 0, 0.5, 1, 3, 6 and 24 hr. No interaction was found between the timing of glutamate decrease in the medium and the genotypes of the two astrocyte layers ($F_{[2,24]}$= 0.430; P=0.826; ANOVA). (**c**) Application of 100 µM of CNQX, a potent AMPA/kainite receptor antagonist, did not influence mutant SOD1 astrocyte–mediated motor neuron death. (**d**) Incubation of the co–cultures with 1 µg/mL of Fas–Fc, the inhibitor of soluble Fas ligand, did not improve motor neuron survival on mutant astrocyte layer. (**e**) The relative contents of 19 cytokines/chemokines were screened by array in CMd from G93A and NTg astrocytes. Only, the 7 molecules with detectable quantities were reported in the table. The genotypic differences were highlighted in bold. The other 12 screened molecules with undetectable levels were: CINC–2/3, Fractalkine, GM–CSF, IL–1α/1β/4/6/10, IFN–γ, LIX, and Leptin.