

## Supplementary file

### Figure legends

#### **Figure S1: Increased percentages of NK and B cells in PBMCs obtained from ALS patients.**

PBMCs of healthy individuals and ALS patients were isolated from peripheral blood as described in Materials and Methods section. PBMCs ( $2 \times 10^5$  cells) were analyzed using flow cytometer to determine the percentages of CD14+, CD16+CD56+, CD19+, CD3+CD4+, CD3+CD8+, and CD3+CD56+ subsets within CD45+ immune cells (**a** (n=25), **b** (n=37))

#### **Figure S2: Increased secretion levels of IFN- $\gamma$ in PBMCs obtained from ALS patients.**

PBMCs of healthy individuals and ALS patients were isolated from peripheral blood as described in Materials and Methods. PBMCs were treated with a combination of IL-2 (1000 U/ml) and anti-PD1 (20  $\mu$ g/ml), 18-20 hours of treatments, the number of cells secreting IFN- $\gamma$  were determined as spot counts using ELISpot assay (**a** (n=11), **b** (n=15))

#### **Figure S3: Number of NK cells and IFN- $\gamma$ secretion in OC-expanded NK cells of ALS patient and healthy individual.**

OCs and OC-expanded NK cells were generated as described in the Materials and Methods section. OC-expanded NK cells ( $2 \times 10^5$  cells) from healthy individual and ALS patient were analyzed using flow cytometer to determine the percentages of CD16+ cells on days 6, 9, 12, and 27 of culture (**a**). OCs and OC-expanded NK cells were generated as described in the Materials and Methods section. The supernatants were harvested on days 6, 9, 12, 15, 19 and 22 from OC-expanded patient and healthy twin NK cell cultures to determine IFN- $\gamma$  secretion using single ELISA (**b**).

#### **Figure S4. Autologous and allogeneic monocytes from ALS patients and those of healthy individuals increase NK cell-mediated cytotoxicity and secretion of IFN- $\gamma$ .**

NK cells and monocytes from ALS patients and healthy individuals were isolated from PBMCs as described in Material and Methods section. NK cells were treated with a combination of IL-2 (1000 U/ml) and anti-CD16 mAbs (3 µg/ml). A crisscross NK cells and monocyte co-cultures were performed with 3 sets of NK cells with allogeneic and autologous monocytes. NK cell-mediated cytotoxicity were measured 18 hours after co-culture using standard 4-hour <sup>51</sup>Cr release assay against OSCSCs. The lytic units (LU) 30/10<sup>6</sup> cells were determined using inverse number of NK cells needed to lyse 30% of target cells OSCSCs ×100 (a). NK and monocyte co-cultures were performed as described in Fig. S4a. After 18 hours of co-culture, supernatants were harvested and used in ELISA to measure IFN-γ secretion (b).

**Figure S5: Different levels of secreted factors by CD8+ T cells of ALS patients.**

CD8+ T cells of healthy individuals and ALS patients were isolated from PBMCs as described in Materials and Methods. CD8+ T cells (1×10<sup>6</sup> cells/ml) were treated with IL-2 (100 U/ml) and anti-PD1 (20 µg/ml) for 18 hours before, the supernatants were harvested to determine the levels of IFN-γ using single ELISA (a (n=5), b (n=7)). CD8+ T cells were treated with IL-2 (100 U/ml) and anti-CD3/28 antibody (25 µl/ml) for 18 hours before, the supernatants were harvested to determine levels of secreted factors using multiplex assay (c, d, e).

**Figure S6. Increased inflammatory cytokines in peripheral blood-derived serum of ALS patients in comparison to those from healthy individuals.**

Sera were obtained from the peripheral blood of healthy individuals and ALS patients, and analyzed for the levels of cytokines, chemokines, and growth factors using a multiplex array kit (a (N=5-17), b (n=7)).

**Figure S7: CD8+ T cells' supernatant of ALS patients induced higher cell death and differentiation in OSCSCs.**

CD8+ T cells of healthy individuals and ALS patients were isolated from PBMCs as described in Materials and Methods. CD8+T cells ( $1 \times 10^6$  cells/ml) were treated with a combination of IL-2 (100 U/ml) and anti-CD3/28 antibody (25  $\mu$ l/ml) before the supernatants were harvested to determine IFN- $\gamma$  secretion using single ELISA. Supernatant containing equal amounts of IFN- $\gamma$  from the healthy individuals and ALS patients' CD8+ T cells were added to OSCSCs for 4 days. On day 4, live and dead tumor cells were manually counted after they were stained with trypan blue (a-b), and the surface expression levels of CD44, and CD54 on OSCSCs were determined using flow cytometry (c). IgG2 isotype was used as control.

**Figure S8: Percentages of Treg at different time-points during CD4+ T cells differentiation in healthy individuals and ALS patients.**

CD4+ T cells of healthy individuals and ALS patients were isolated from PBMCs as described in Materials and Methods. For Treg differentiation, CD4+ T cells ( $1 \times 10^6$  cell/ml) were cultured with ImmunoCult-XF T cell expansion medium supplemented with Immunocult human CD3/CD28 T cell activator (25  $\mu$ l/ml). On days 10, 18, and 20, the surface expression levels of Foxp3 on CD4+CD25+ cells was analyzed using flow cytometric analysis. IgG2 isotype was used as control.

**Figure S9. Weekly NAC injections in ALS patients did not affect the secretion levels of GMCSF in peripheral blood of ALS patient.**

Sera were obtained from the peripheral blood of healthy individuals and ALS patients (before and after NAC injection in patient), and analyzed for the levels of GMCSF using a multiplex array kit (n=8).

Table S1: Patient information

Patient Initials	Age	Gender	Confirmed ALS diagnosis
W.O	57	M	Yes
R.S	62	M	Yes
J.C	60	M	Yes
S.G	54	F	Yes
J.C	62	M	Yes

Figure S1

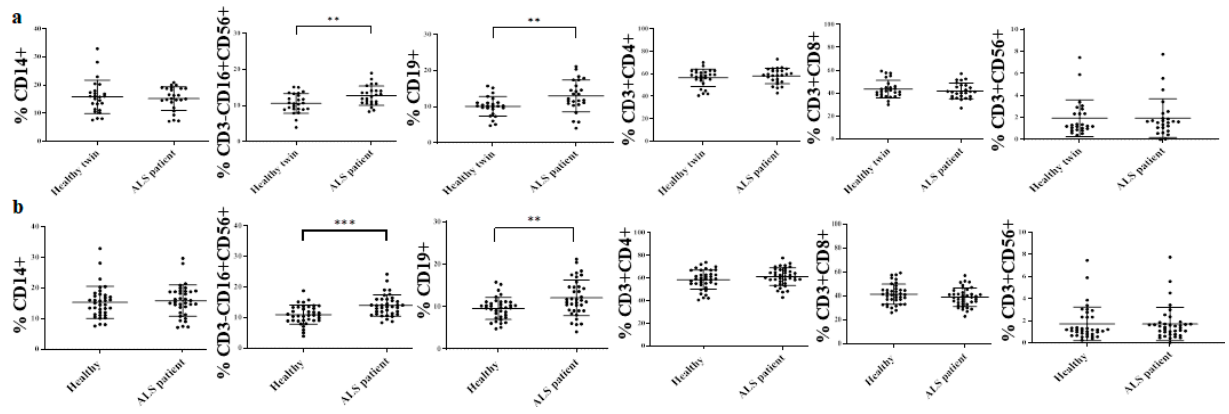
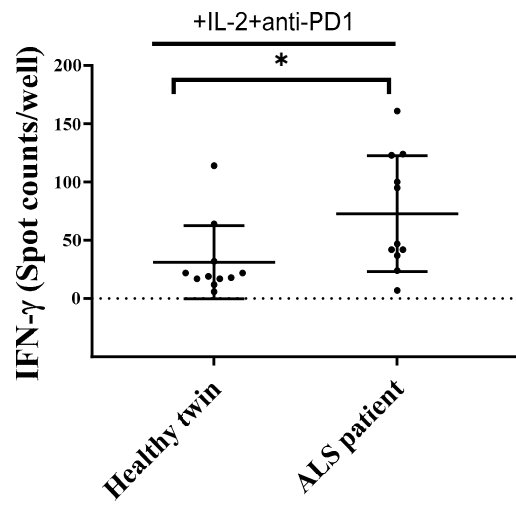


Figure S2

a



b

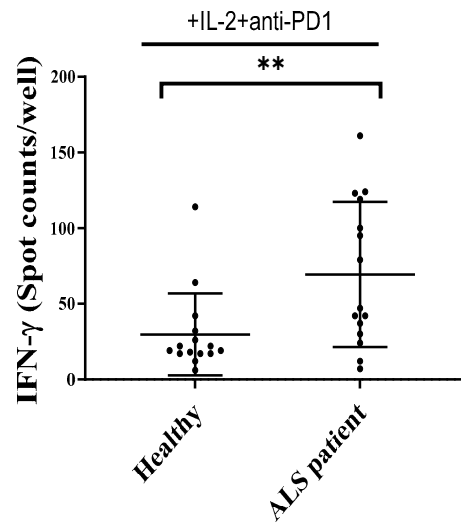


Figure S3

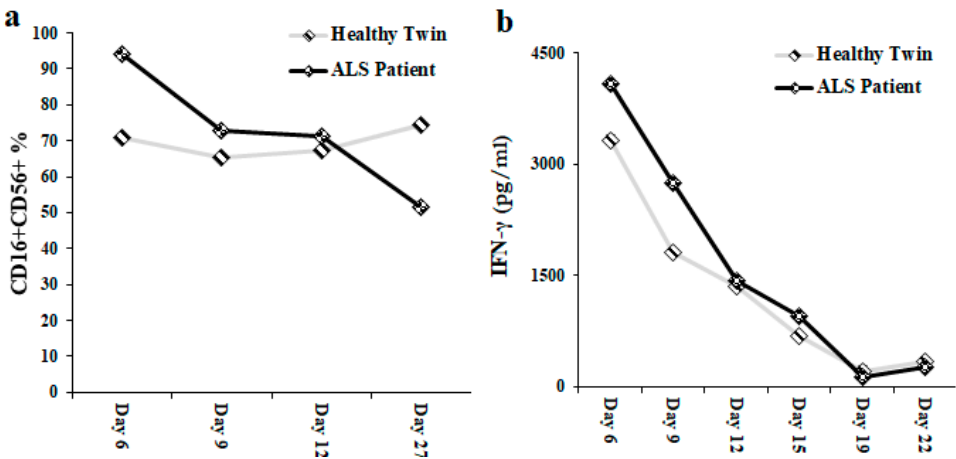


Figure S4

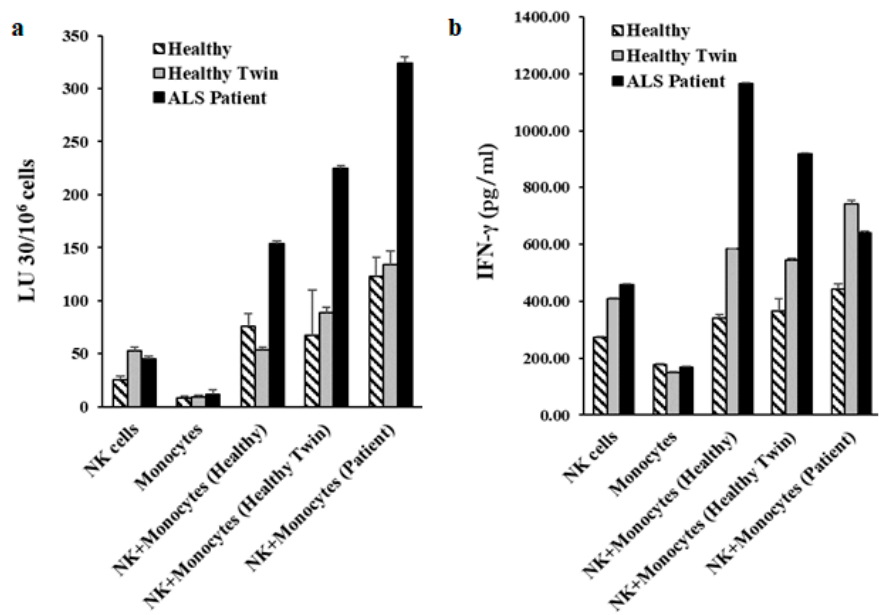
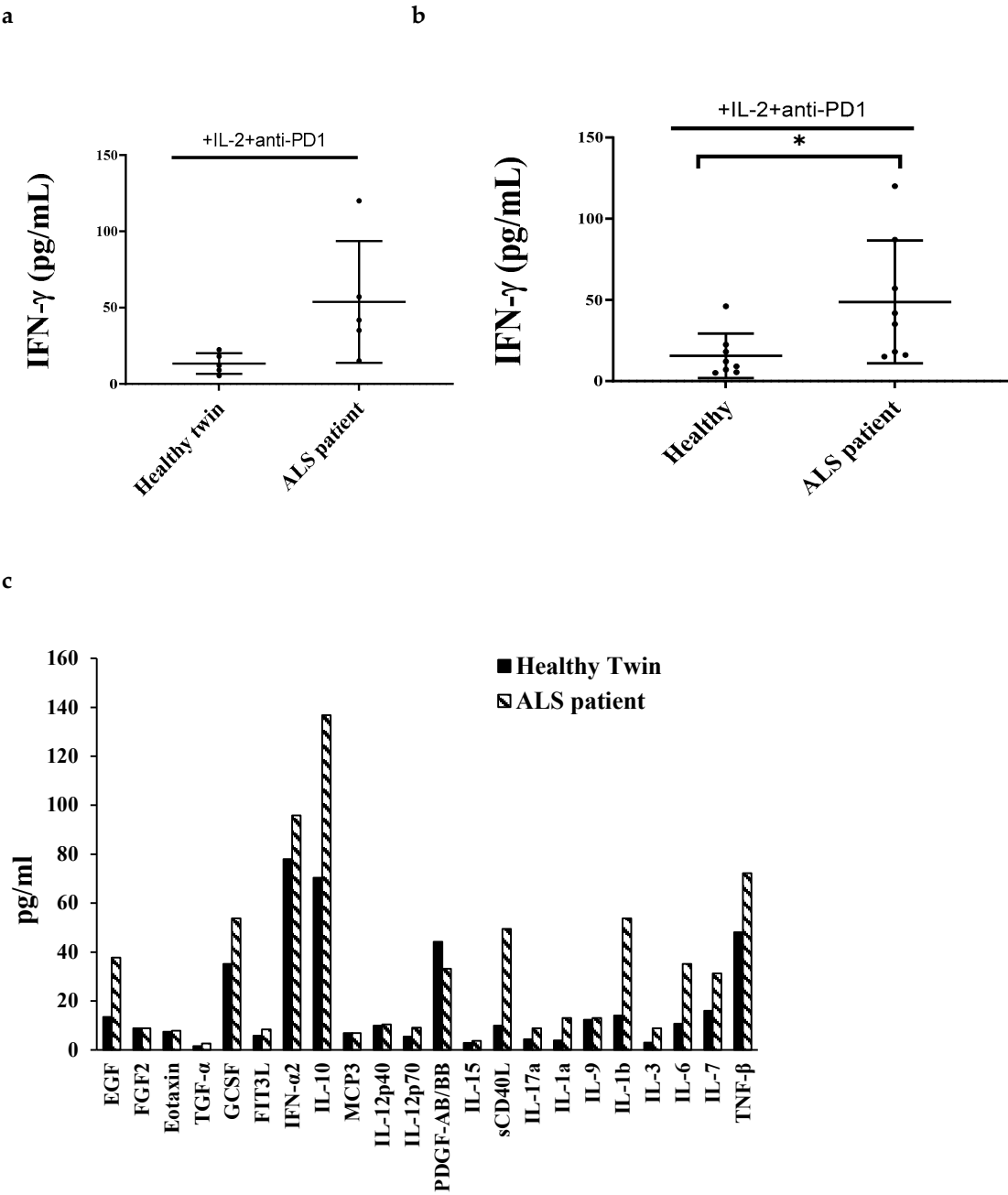
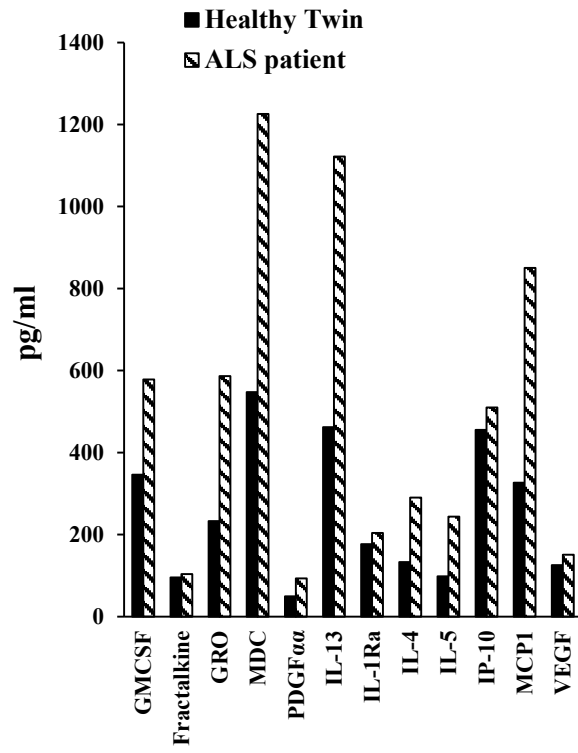




Figure S5



d



e

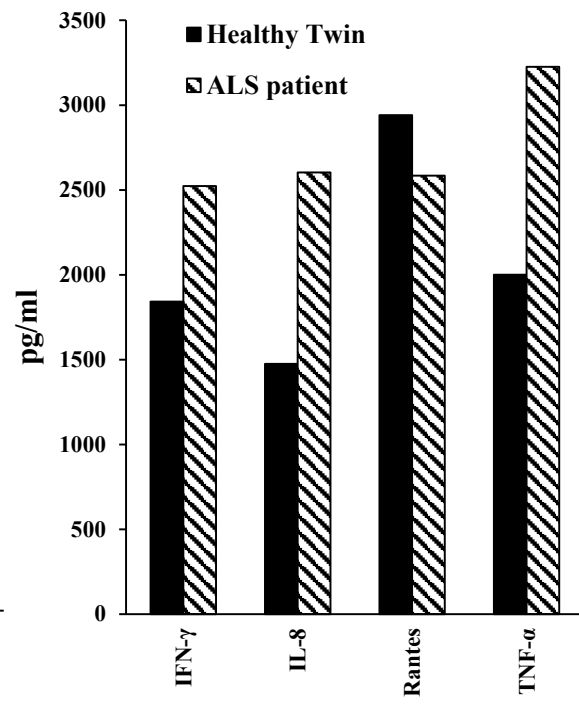


Figure S6

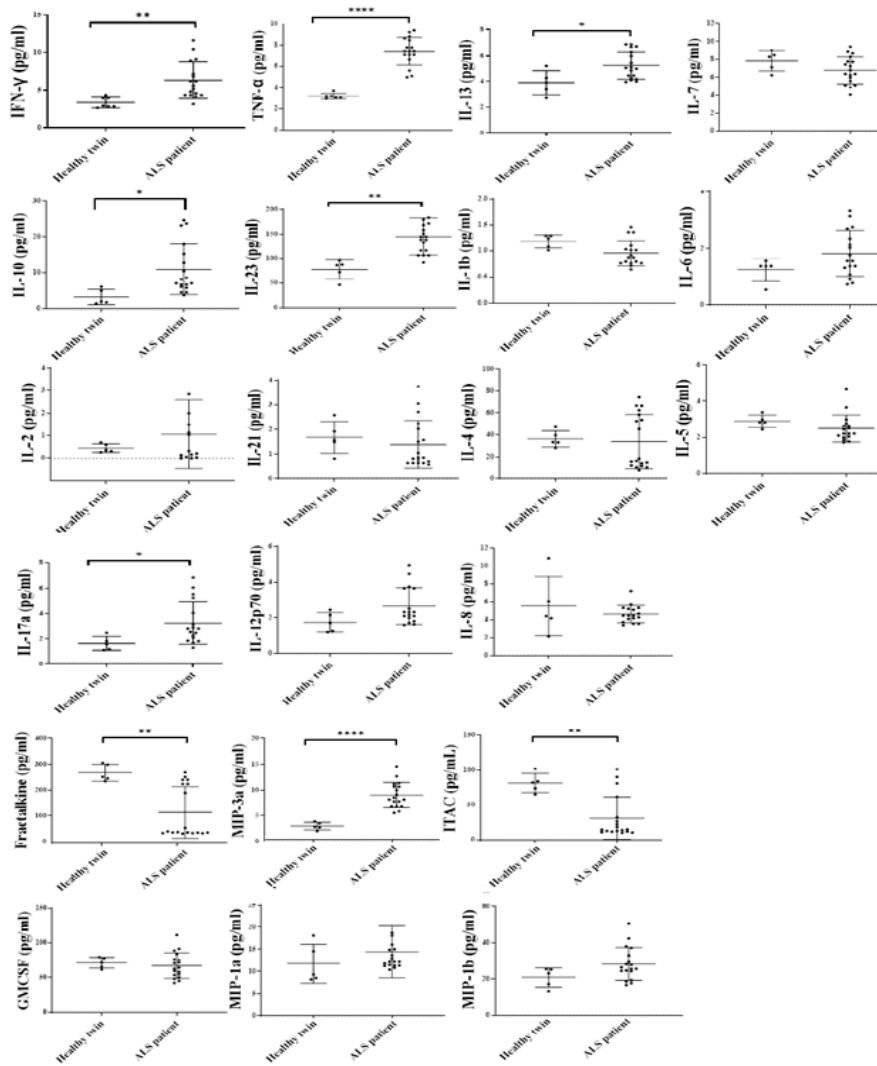
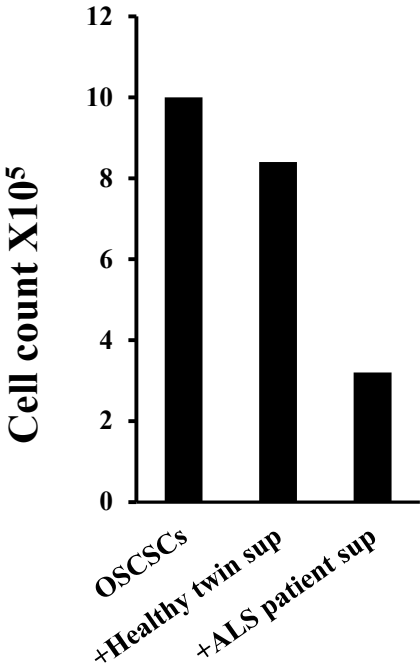
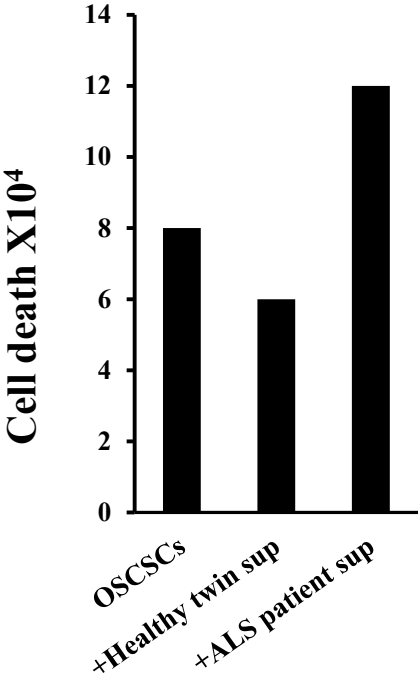


Figure S7

a



b



c

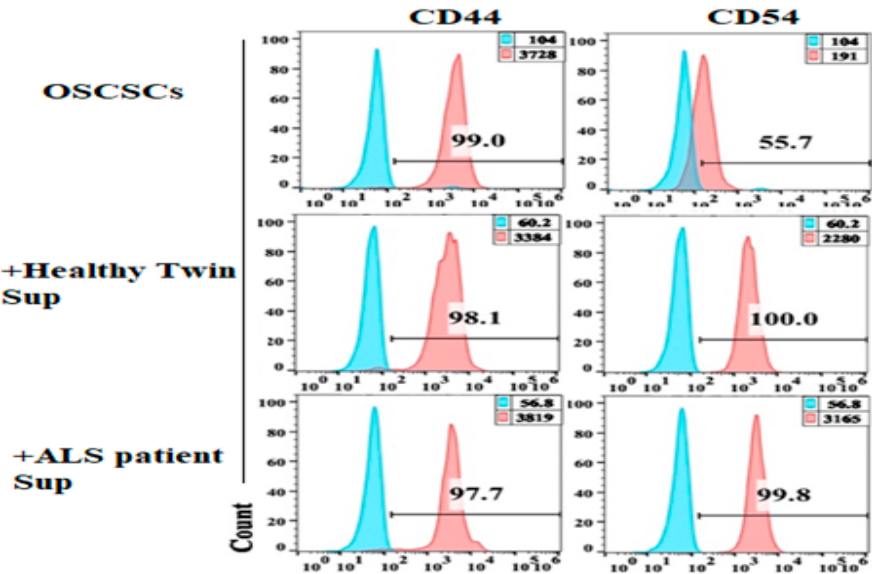


Figure S8

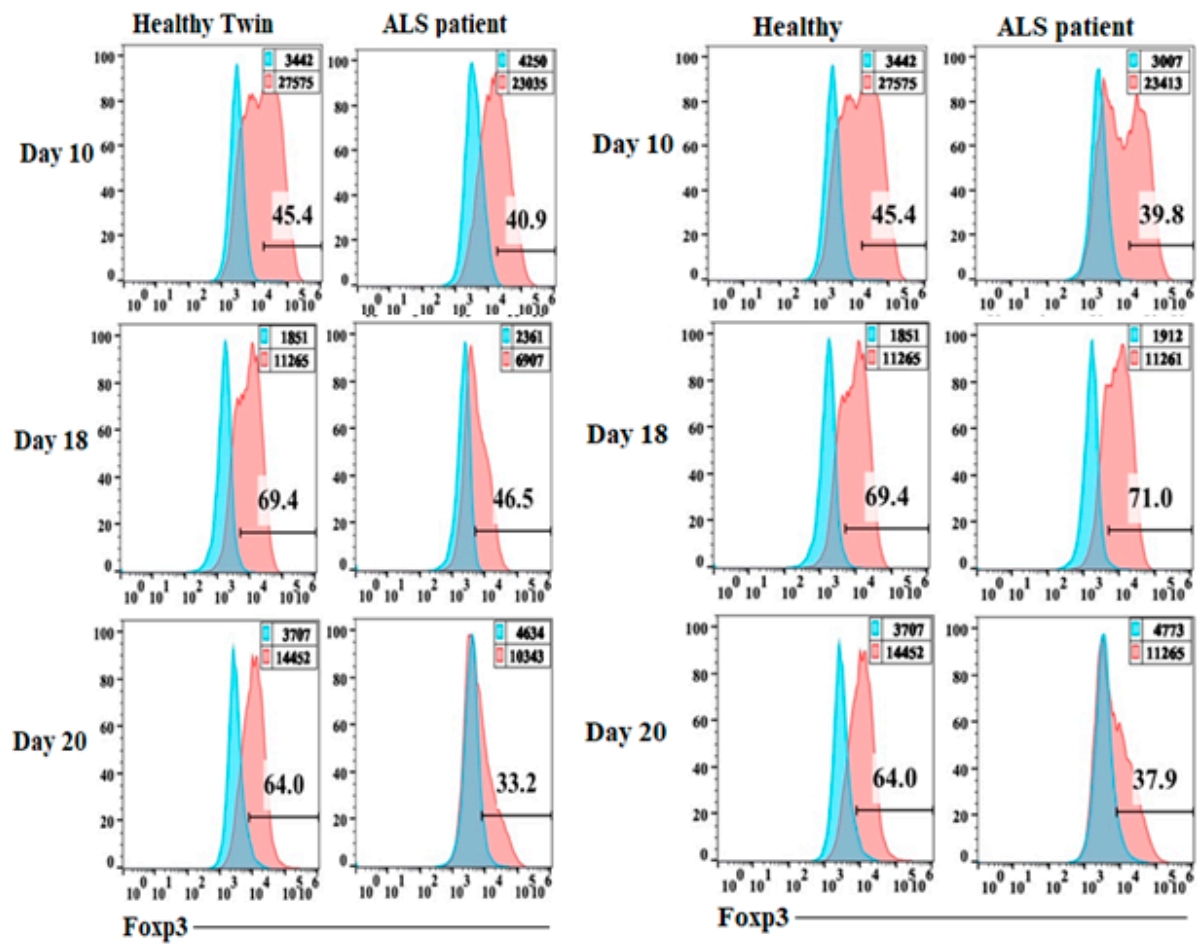


Figure S9

