SUPPLEMENTAL MATERIAL

Nussbaum et al., https://doi.org/10.1084/jem.20162031

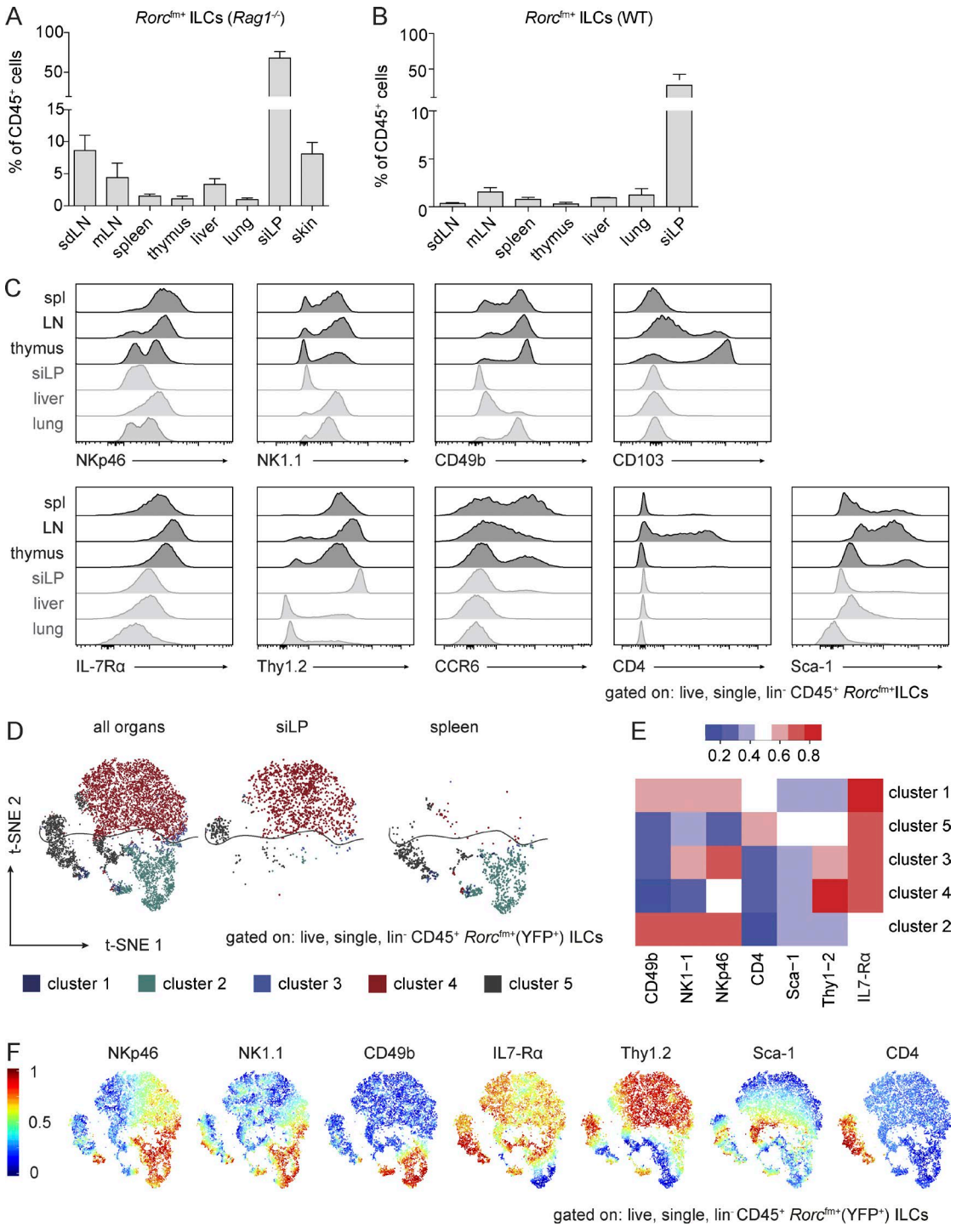


Figure S1. ***Rorc*$^{fm+}$ ILCs from various organs possess different expression pattern.** (A) Frequencies of *Rorc*$^{fm+}$ ILCs within the CD45 compartment of various organs of *Rorc*^{fm}-*Rag1*$^{-/-}$ mice. Graphs represent pooled data from two independent experiments, $n \geq 4$ each (means ± SEM). (B) Frequencies of *Rorc*$^{fm+}$ ILCs of within the CD45 compartment of various organs of *Rorc*^{fm} (WT) mice. Graphs represent pooled data from two independent experiments, $n \geq 4$ each (means ± SEM). (C) Histograms of lymphoid (dark) splenic (spl), LN, thymic, and nonlymphoid (light) siLP, liver, or lung *Rorc*$^{fm+}$ ILCs. Representative histograms of two independent experiments, $n \geq 5$ each. (D) Dimensionality reduction using t-SNE. Data from *Rorc*$^{fm+}$ ILCs of spleen and siLP (of *Rorc*^{fm} [WT]) mice; gated on live, single lin$^-$CD45$^+$*Rorc*$^{fm+}$ ILCs, which were transformed and plotted in two t-SNE dimensions using R software. Clustering was performed using the flowSOM algorithm ($k = 5$). Depicted are the combined spleen and siLP data sets (left), the siLP dataset only (middle), and spleen dataset only (right). (E) ILC3- and NK cell–associated markers plotted in a heat map across flowSOM clusters from D. (F) Expression pattern of ILC3- and NK cell–associated markers depicted in the two t-SNE dimensions.

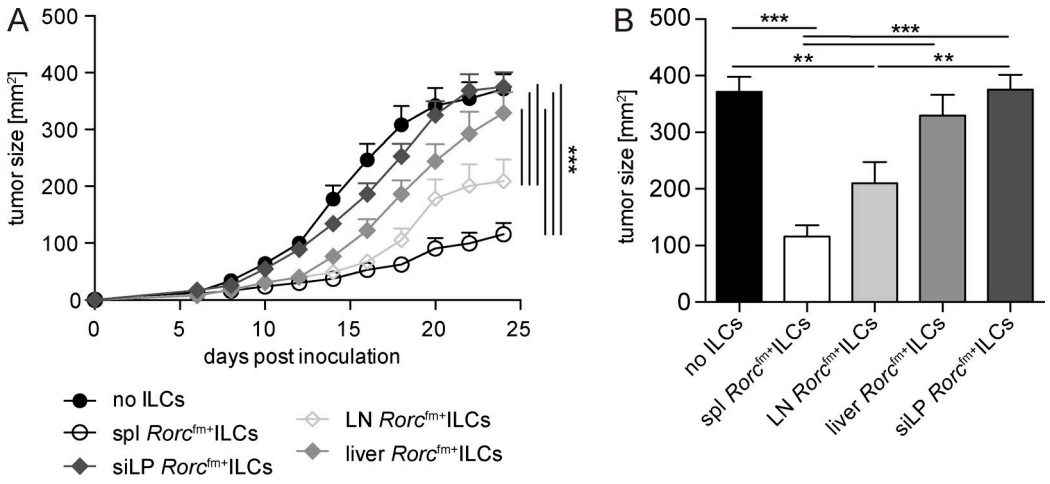


Figure S2. **Lymphoid *Rorc*$^{fm+}$ ILCs suppress tumor growth, whereas nonlymphoid *Rorc*$^{fm+}$ ILCs fail to do so.** *Il12rb2*$^{-/-}$ mice were s.c. challenged with B16–IL-12 coinjected with splenic (spl)-, LN-, siLP-, or liver-derived *Rorc*$^{fm+}$ ILCs or no ILCs. (A) Tumor growth of B16–IL-12 tumor cells coinjected with splenic (spl) *Rorc*$^{fm+}$ ILCs (open circles), siLP-derived *Rorc*$^{fm+}$ ILCs (dark gray squares), LN-derived *Rorc*$^{fm+}$ ILCs (gray open squares), hepatic (liver) *Rorc*$^{fm+}$ ILCs (light gray squares), or the absence of ILCs (closed circles) over time. For comparison of the tumor growth curve two-way ANOVA with Tukey's multiple comparisons test was used. ***, $P < 0.001$. (B) Quantification of tumor burden 24 d after tumor inoculation. Graph represents pooled data from three independent experiments, $n \geq 5$ each (means ± SEM). One-way ANOVA with Tukey's multiple comparisons test was performed. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

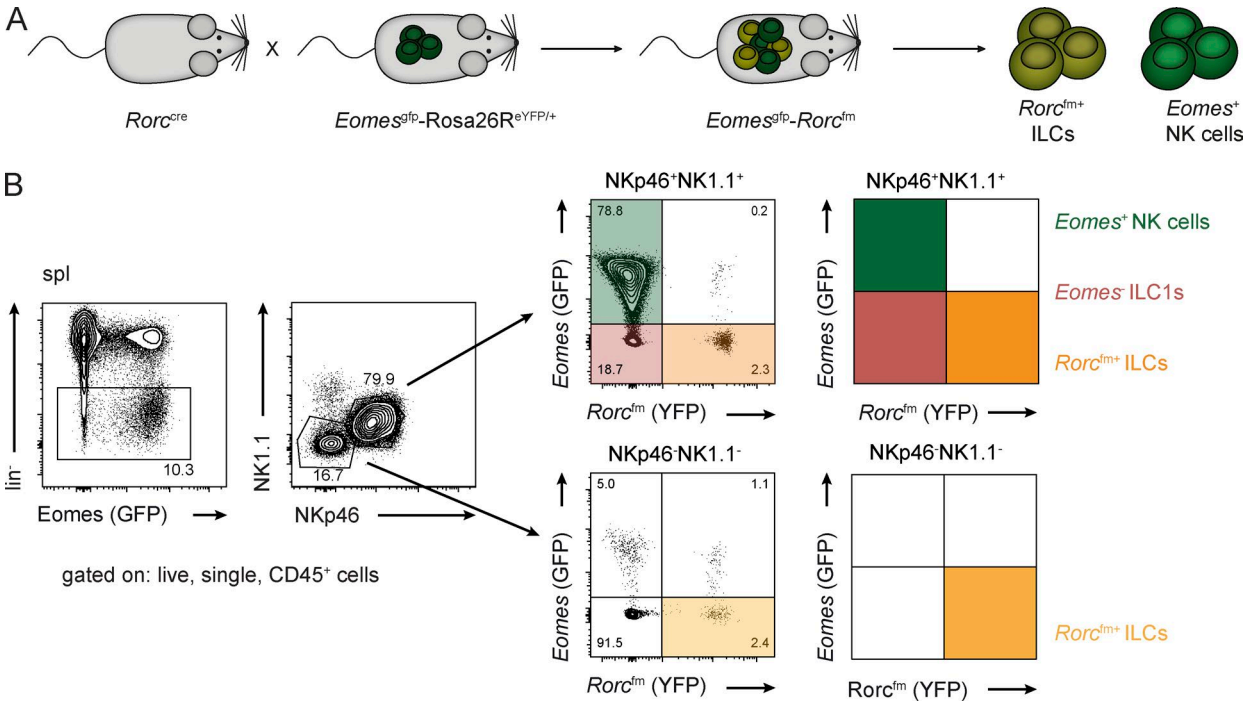
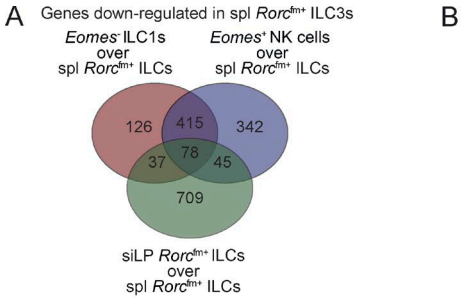
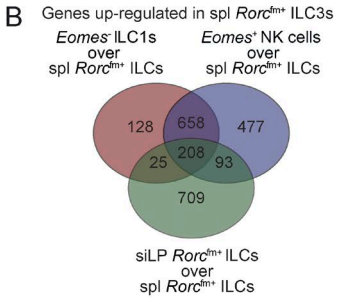


Figure S3. ***Rorc*-fate map crossed to *Eomes*-reporter mice allow identification of type 1 and type 3 ILC subsets.** (A) Schematic representation of the *Rorc*^{cre} mice crossed to the *Eomes*^{GFP}-Rosa26R$^{eYFP/+}$ mice, labeling all cells expressing RORγt with YFP and all cells expressing *Eomes* with GFP. (B) Gating strategy to identify ILC3s derived from the *Rorc* lineage (*Rorc*$^{fm+}$ ILCs), *Eomes*-expressing NK cells, and *Rorc*$^{fm-}$*Eomes*$^{-}$ ILC1s in the spleen (spl). For exclusion of the adaptive immune cells and myeloid cells lin^{-} (CD3^{-}CD5^{-}CD11c^{-}CD19^{-}GR-1^{-}), live, single CD45^{+} cells were gated. ILC1s, NK cells, and NCR^{+} ILC3s were identified within the NCR^{+} (NK1.1^{+}NKp46^{+}) cell population, whereas NCR^{-} ILC3s were identified in the NCR^{-} population. NCR^{+} and NCR^{-}ILC3 are summarized as *Rorc*$^{fm+}$ ILCs.





C Genes down-regulated by spl *Rorc*$^{fm+}$ ILCs

Eomes$^-$ ILC1s over spl *Rorc*$^{fm+}$ ILCs	*Eomes*$^+$ NK cells over spl *Rorc*$^{fm+}$ ILCs	siLP *Rorc*$^{fm+}$ ILCs over spl *Rorc*$^{fm+}$ ILCs
Htra1	*Lamb1*	*Igsf5*
Podxl	*Mcam*	*Podxl*
Entpd1	*Adrb1*	*Frmd5*
Lamb1	*Ndrg1*	*Lamb1*
Sorcs2	*Trdc*	*Slc16a6*
Adrb1	*Sgk1*	*Ndrg1*
Mcam	*Ubxn11*	*Htra1*
Trbj1-3	*Sept4*	*Kcnk5*
Igsf5	*Gzmb*	*Mcam*
Trbd1	*Trbj1-3*	*Trbj1-3*
Trdc	*Zeb2*	*Ust*
Sgk1	*Podxl*	*Gzmb*
Cdk14	*Ust*	*Sorcs2*
Gzmb	*Sorcs2*	*Fhl2*
Spc25	*Kcnk5*	*Smim3*
Trbj1-1	*Shcbp1*	*Ephx1*
Ubxn11	*Trbd1*	*Cdk14*
Sept4	*Prdm1*	*Cd3g*
Gimap7	*Entpd1*	*Mical2*
Depdc1a	*Spc25*	*Fam46a*
Prdm1	*Frmd5*	*Prdm1*
Mtfr2	*Mtfr2*	*Zfp609*
Trdv4	*Cdk14*	*Mxd1*
Shcbp1	*Ppm1j*	*Mmd*
Zeb2	*Kifc1*	*Ets2*
Fads1	*Depdc1a*	*Selenbp1*
Ephx1	*Asap2*	*Osbpl3*
Frmd5	*Trbj1-4*	*Serpinb6b*
Ppm1j	*Htra1*	*Adrb1*
Serpinb9	*Igsf5*	*Ubxn11*

D Genes up-regulated by spl *Rorc*$^{fm+}$ ILCs

Eomes$^-$ ILC1s over spl *Rorc*$^{fm+}$ ILCs	*Eomes*$^+$ NK cells over spl *Rorc*$^{fm+}$ ILCs	siLP *Rorc*$^{fm+}$ ILCs over spl *Rorc*$^{fm+}$ ILCs
Arhgef26	*Mapk10*	*Cnga3*
Lingo4	*Lingo4*	*Scn3b*
Ccr6	*Ccr6*	*Fut7*
S1pr3	*Cntn1*	*Aldh3a1*
Mapk10	*Arhgef26*	*Popdc3*
Scn3b	*Cd4*	*Fam26e*
Cd4	*Fam26e*	*Olig2*
Dsg2	*Dsg2*	*Fstl4*
Fam26e	*Arnt2*	*Arnt2*
Erbb2	*Cxcr5*	*Arhgef26*
Cnga3	*Aatk*	*Marveld3*
Piwil4	*Scn3b*	*Wisp2*
Col18a1	*S1pr3*	*Ramp1*
Moxd1	*Col18a1*	*Gpr15*
Dscaml1	*Cnga3*	*Mapk8ip1*
Wbscr17	*Erbb2*	*Syt6*
Nfe2l3	*Aldh3a1*	*Trgj2*
Adamtsl4	*Dscaml1*	*Tmem51*
Lrrc3	*Wbscr17*	*Cd4*
Adm	*Ldhb*	*Agpat4*
Cdh2	*St6galnac2*	*Zc3h12d*
Sorcs1	*Gucy1a3*	*Bmp2*
Popdc3	*Moxd1*	*Mical3*
Plekhh1	*Tox2*	*Tktl1*
St6galnac2	*Fstl4*	*Nptxr*
Cxcr5	*Nfe2l3*	*Plxnb2*
Cntn1	*Cdh2*	*Spink2*
Olig2	*Tcrg-C2*	*Nfe2l3*
Ip6k3	*Azin2*	*Lingo2*
Tox2	*She*	*Rarg*

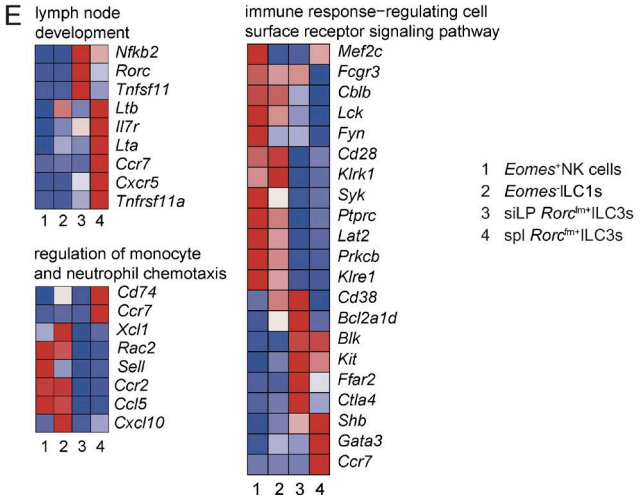


Figure S4. **NGS reveals differentially expressed genes by splenic *Rorc*$^{fm+}$ ILCs compared with other ILC subsets.** (A and B) Pairwise comparison of the four experimental groups depicted in a Venn diagram. 78 genes are significantly down-regulated, and 208 are up-regulated when comparing all conditions (*Eomes*$^+$ NK cells, *Eomes*$^-$ ILC1s, and siLP *Rorc*$^{fm+}$ ILCs) to splenic (spl) *Rorc*$^{fm+}$ ILCs (altered to a minimum significance threshold of $P < 0.01$ and fold change >1 or -1). (C) Top 30 list of genes down-regulated by splenic (spl) *Rorc*$^{fm+}$ *ILCs* compared with *Eomes*$^+$ NK cells, *Eomes*$^-$ ILC1s, and siLP *Rorc*$^{fm+}$ ILCs. (D) Top 30 list of genes up-regulated by splenic (spl) *Rorc*$^{fm+}$ *ILCs* compared with *Eomes*$^+$ NK cells, *Eomes*$^-$ ILC1s, and siLP *Rorc*$^{fm+}$ ILCs. (E) Heat maps of differentially expressed genes clustered to the indicated category. Heat maps show representative data of one sample per group.

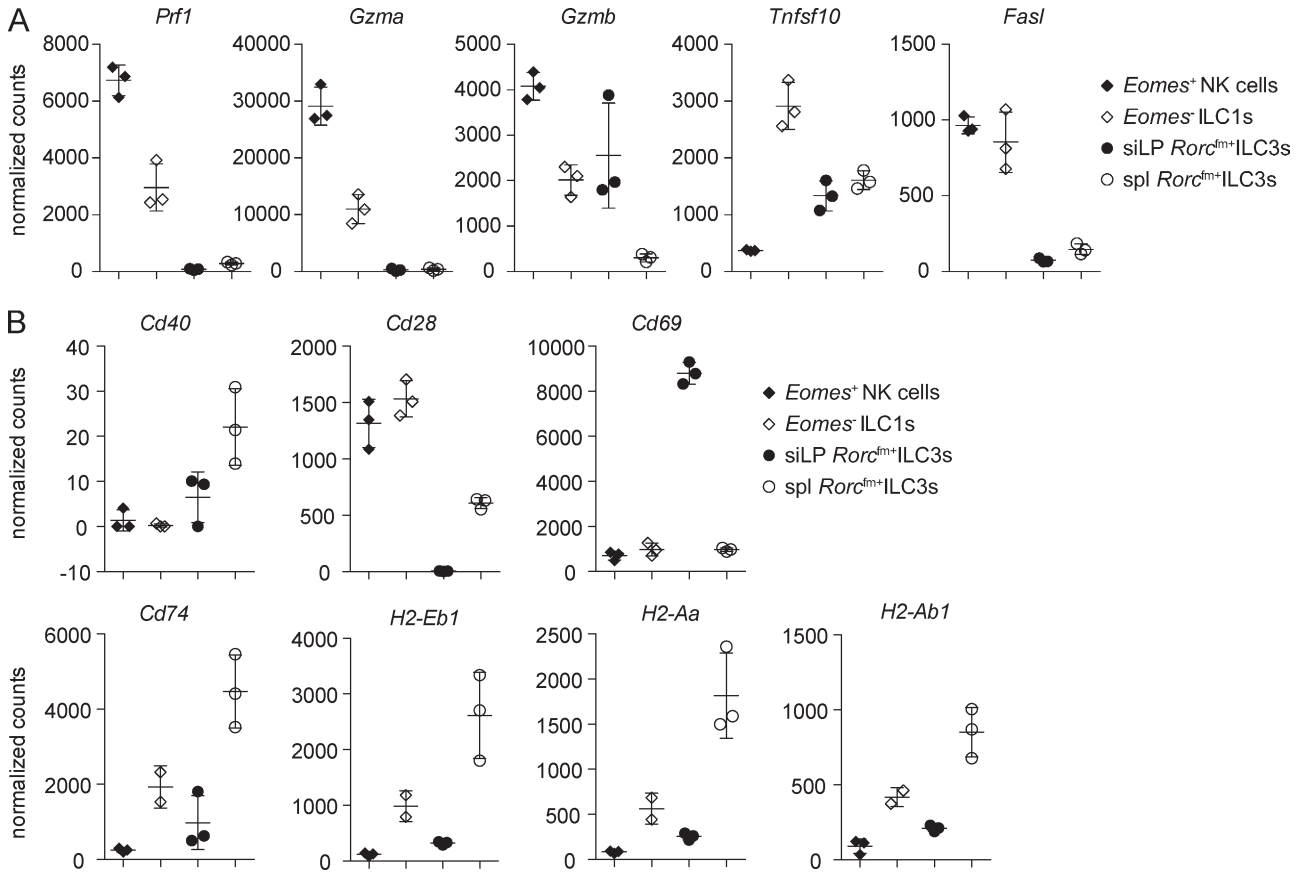


Figure S5. **Splenic *Rorc*$^{fm+}$ ILCs only express low amounts of cytotoxic molecules and an activated phenotype.** (A) Expression pattern of cytotoxic molecules by the different splenic and siLP *Rorc*$^{fm+}$ ILC data from NGS (means ± SD; Fig. 6). (B) Expression pattern of activation markers by the different splenic and siLP *Rorc*$^{fm+}$ ILCs; data from NGS (means ± SD; Fig. 6).