

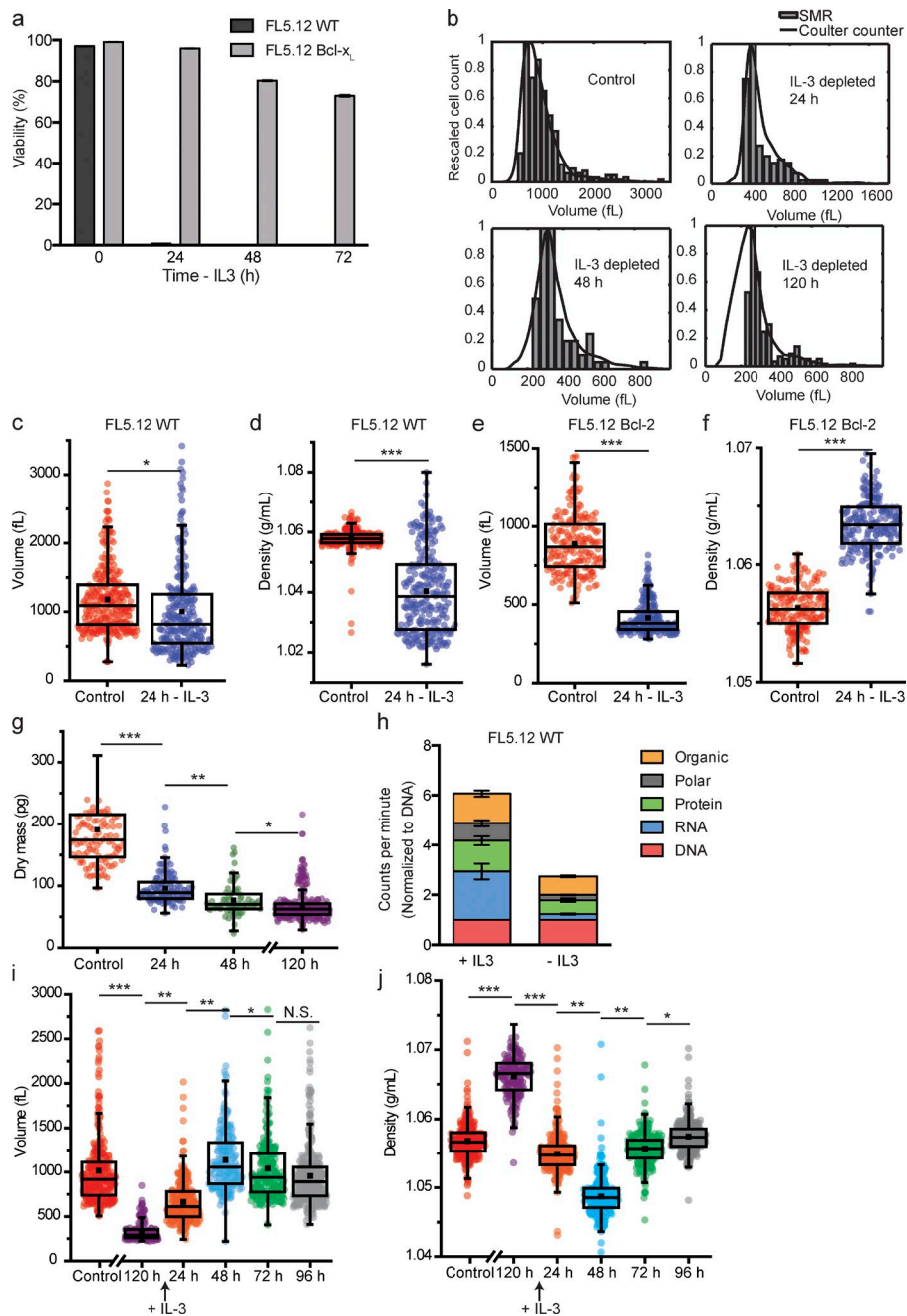
Hecht et al., <http://www.jcb.org/cgi/content/full/jcb.201506118/DC1>

Figure S1. **Biophysical responses to IL-3 deprivation in FL5.12 cell lines.** Viability of FL5.12 Bcl- x_L (light gray) and FL5.12 WT (dark gray) cells over 72 h after IL-3 depletion (a). Comparison between volume measurements obtained with the SMR (gray columns) and the Coulter counter (black curve) for IL-3-depleted FL5.12 Bcl- x_L cells (b). Volume (c) and density (d) measurements of FL5.12 WT cells after IL-3 depletion for 24 h. Volume (e) and density (f) measurements of FL5.12 Bcl-2 cells after IL-3 depletion for 24 h. Dry mass decreases in FL5.12 Bcl- x_L cells after IL-3 depletion (g). Cellular fractionation of FL5.12 WT cells after IL-3 depletion for 24 h (h). Volume (i) and density (j) changes to FL5.12 Bcl- x_L cells upon IL-3 repletion (black arrow) after 120 h of IL-3 depletion. *, $P < 9 \times 10^{-5}$; **, $P < 7.10^{-10}$; ***, $P < 3.6 \times 10^{-36}$; N.S., no significance.

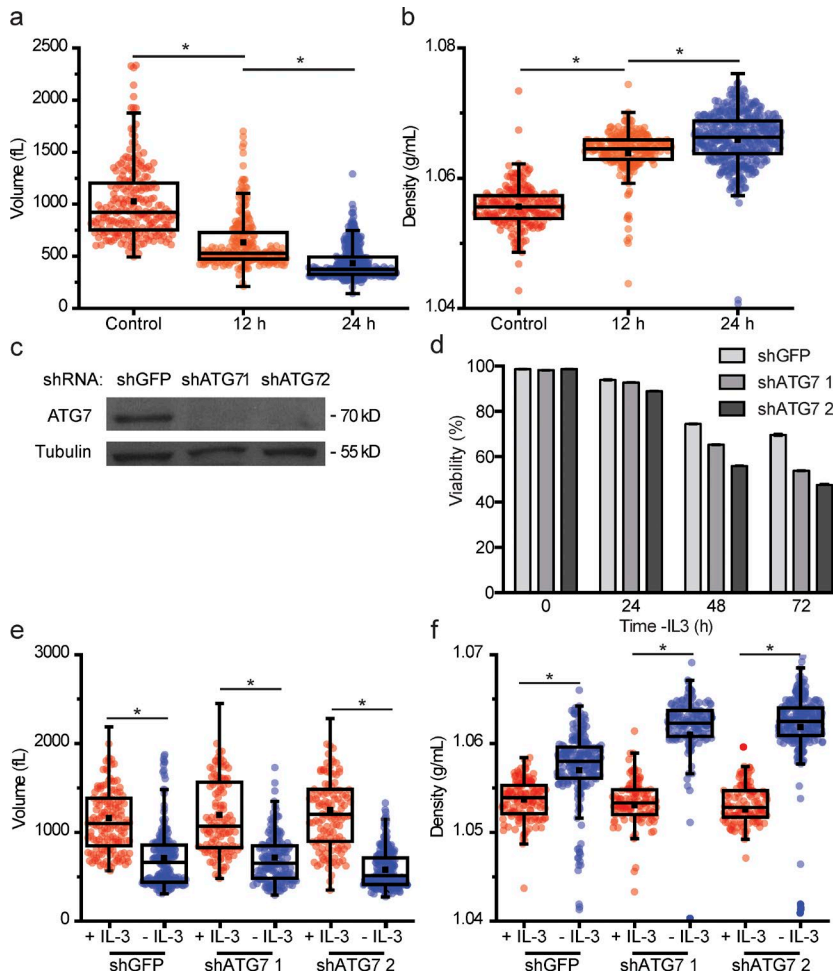


Figure S2. **Relationship between biophysical response and autophagy.** Volume (a) and density (b) changes in FL5.12 Bcl-x_i cells over 24 h after IL-3 depletion. Western blot of FL5.12 Bcl-x_i cells after shRNA knockdown of ATG7 (c). Viability of shRNA-expressing FL5.12 Bcl-x_i cells after IL-3 withdrawal over 72 h (d). Volume (e) and density (f) changes in shRNA-expressing FL5.12 Bcl-x_i cells over 24 h after IL-3 depletion. *, $P < 1.8 \times 10^{-12}$.

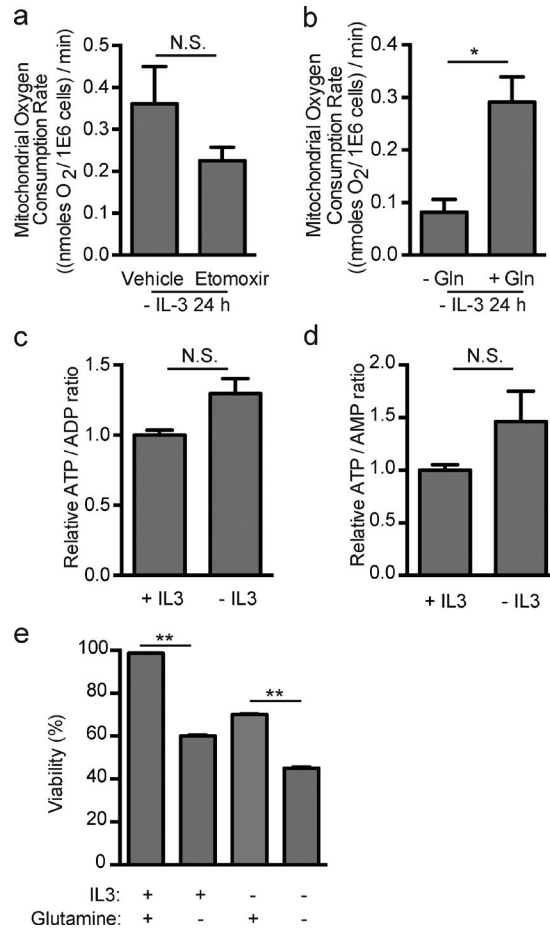


Figure S3. **Characterization of metabolic phenotypes upon IL-3 withdrawal.** Measurement of mitochondrial oxygen consumption rate in FL5.12 Bcl-x_L cells after 24 h IL-3 withdrawal and exposure to vehicle or the fatty acid oxidation inhibitor etomoxir (300 μM) (a). Measurement of mitochondrial oxygen consumption rate in FL5.12 Bcl-x_L cells after 24 h IL-3 withdrawal and 2 h glutamine depletion and subsequent glutamine (Gln) repletion (b). Liquid chromatography–mass spectrometry analysis of change in the relative ATP/ADP (c) or ATP/AMP (d) ratio in FL5.12 Bcl-x_L cells after 24-h IL-3 withdrawal. Viability of FL5.12 Bcl-x_L cells depleted of IL-3 for 24 h and subsequently incubated in glutamine-free media for an additional 48 h (e). *, P < 0.04; **, P < 2 × 10⁻⁴; N.S., no significance.

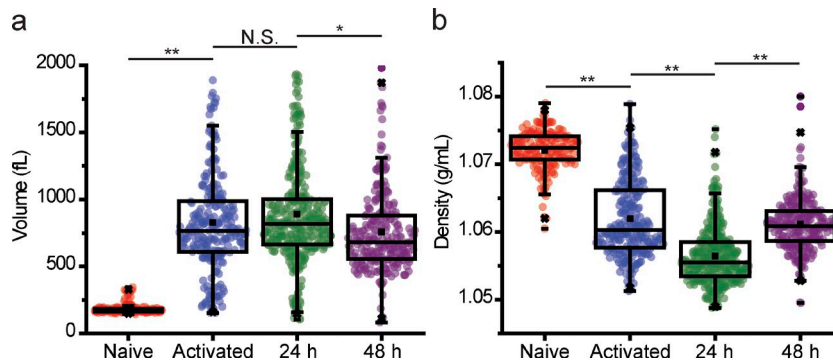


Figure S4. **Biophysical changes in continuously activated T cells.** Volume (a) and density (b) responses of CD8⁺ OT-1 cells 72 h after activation with continued exposure to IL-2 for an additional 48 h. *, P < 2.2 × 10⁻⁷; **, P < 2.9 × 10⁻³⁶; N.S., no significance.

Table S1. Doubling time of FL5.12 cells after IL-3 depletion and repletion

Condition	Time elapsed	Doubling time
	<i>h</i>	<i>h</i>
-IL3	0	11
	24	17
	48	>24
	72	>24
	96	>24
	120	>24
+IL3	24	>24
	48	>24
	72	>24
	96	11