

**Supplementary Files** (Zurli et al. CD2 signaling in cytotoxic T cells regulates AMPK-dependent lytic granule polarization and killing of B cells)

Supplemental Tables 1-7, legends.

Supplemental Figures 1-8.

## Supplementary Tables

### Table S1

Comparative label-free quantitative proteomics analysis of B cell surface proteome ( $\log_2$  fold change  $\geq 1.0$ ; false discovery rate (FDR)  $\leq 0.09$ ). Statistical analysis was performed with Perseus software. Related to Figure 1.

### Table S2

Quantification of phosphopeptides in human freshly isolated CTLs, related to Figure 3.

### Table S3

Quantification of phosphopeptides present at statistically different levels in CD2-stimulated CTLs compared to isotype-stimulated CTLs ( $\log_2$  fold change  $\geq 1.0$ ; false discovery rate [FDR]  $\leq 0.01$ ). Statistical analysis was performed with Perseus software. Related to Figure 3.

### Table S4

Cluster enrichment analysis of proteins containing CD2-regulated phosphopeptides (Fisher's exact test, FDR  $\leq 0.01$ ). Analysis was performed in Cytoscape. The list was filtered to remove redundant terms, redundancy cut-off 0.2. Related to Figure 4.

### Table S5

NetworkKIN/NetPhorest Predicted Kinases of the CD2-Regulated Phosphosites. Related to Figure 5.

### Table S6

CD2-regulated lysosome-associated proteins in human freshly isolated CTLs. The list was generated through the analysis in Cytoscape.

### Table S7

Comparison of the CD2-regulated and published TCR- and CD28-regulated phosphoproteomes (ref. Mayya V, et al. *Sci Signal* 2009; Tian R, et al. *Proc Natl Acad Sci* 2015). Analysis was performed in Cytoscape.

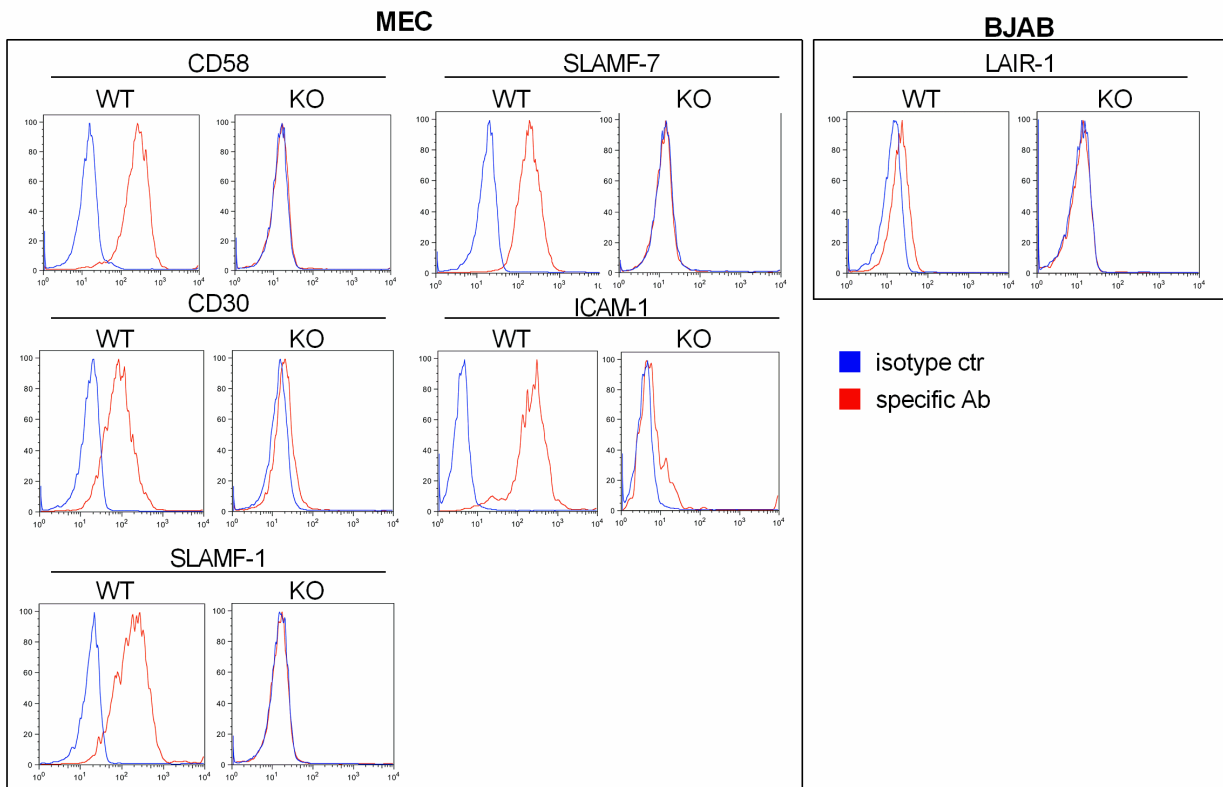
## Supplementary Figures

### Figure S1

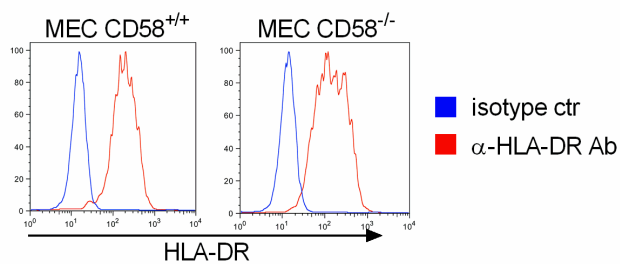
(A) Flow cytometry analysis of indicated surface receptors on CRISPR/Cas9-modified MEC and BJAB cells.

(B) Flow cytometry analysis showing similar levels of HLA-DR (major histocompatibility class II molecules to which SAg binds) on CD58<sup>+/+</sup> and CD58<sup>-/-</sup> MEC cells.

A



B



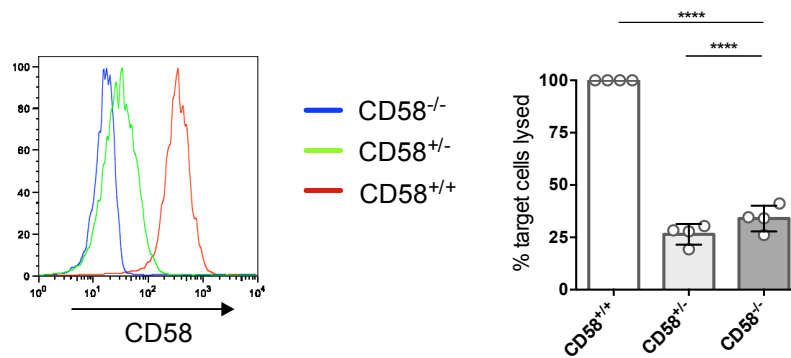
**Figure S2. Influence of CD58 levels on target cells on CTL killing and degranulation**

(A) Left panel: CD58 levels on wild-type MEC cells transfected with empty CRISPR/Cas9-modifying vector (CD58<sup>+/+</sup>), and on CRISPR/Cas9-modified MEC cells with heterozygous (CD58<sup>+/-</sup>) and homozygous (CD58<sup>-/-</sup>) CD58 knockout.

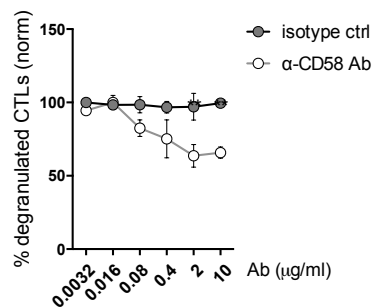
Right panel: Flow cytometry assay of cytotoxic killing with freshly isolated CTLs and indicated SAg-loaded MEC cells.

(B) Analysis of degranulation in CTLs incubated with MEC targets in the presence of either isotype or CD58-specific blocking antibody (n=3 experiments). Normalization was performed setting the values for the treatment with 0.0032 µg/mL isotype antibody as 100% (degranulation ranged 9.6-12.1% of CTLs).

A



B



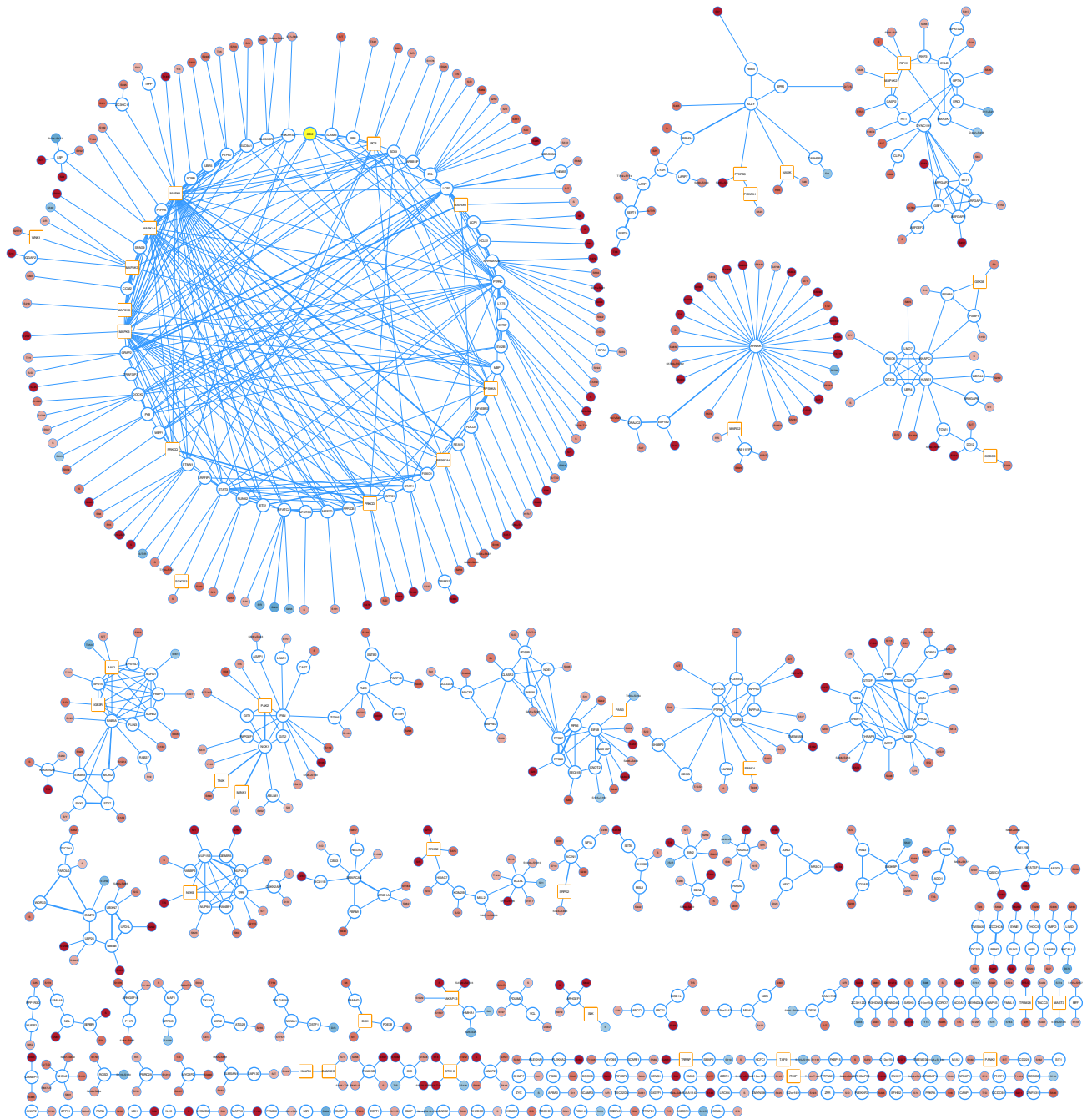
**Figure S3**

Pearson's correlation analysis of CD2-regulated phosphopeptide fold-change quantification among the three donors.

	<u><math>\alpha</math>-CD2 Ab</u>				<u>isotype Ab</u>			
Donor	1	2	3		1	2	3	
	1	0.964	0.929	1	1	0.92	0.904	1
		1	0.929	2		1	0.972	2
			1	3			1	3

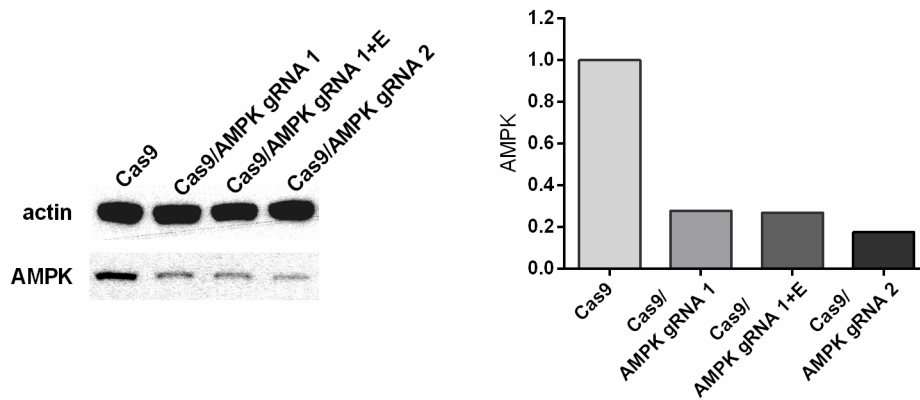
### Figure S4. CD2-regulated phospho-proteome

An integrative map of 373 CD2-regulated phosphoproteins generated using the STRING (confidence threshold 0.4) and ClusterMaker (confidence threshold 0.641) applications in Cytoscape. Nodes corresponding to kinases are square-shaped.



**Figure S5. Validation of AMPK-targeting guide RNAs in in vitro expanded CTLs (CTL blasts)**

Immunoblot analysis of AMPK expression in CRISPR/Cas9-modified CTL blasts at 72 hours post-nucleofection. Cells were nucleofected with Cas9-ribonucleoprotein complexes and several combination of AMPK-targeting guide RNAs. gRNA 1 stands for the combination of GAAGATCGGCCACTACATTC and GGCTGTGCGCCATCTTTCTCC guides; gRNA 1+E is the same combination of gRNAs with the addition of a carrier DNA Alt-R Cas9 Electroporation Enhancer (Integrated DNA Technologies); gRNA 2 is the combination of AMPK-targeting gRNAs (AAGATCGGCCACTACATTCT, ATTCGGAGCCTTGATGTGGT) used in the main manuscript.

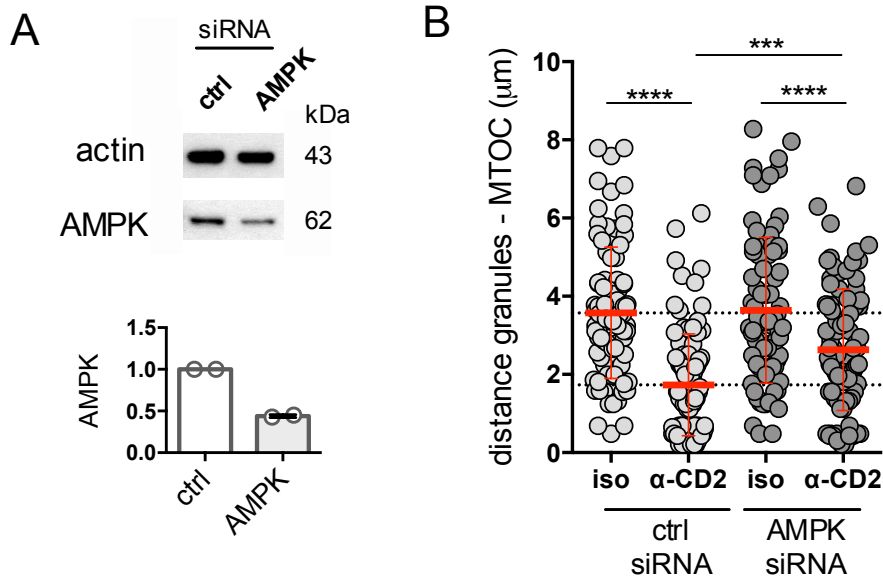


**Figure S6. AMPK knockdown by small interfering RNAs (siRNAs) significantly impaired CD2-dependent granule polarization**

(A) Immunoblot analysis of CTLs electroporated with control siRNA or AMPK-specific siRNA at 40 hours post-electroporation.

(B) Quantitative immunofluorescence analysis of granule polarization in siRNA-electroporated CTLs (n=2 experiments).

Shown are mean values  $\pm$  SD; ns, not significant; two-way ANOVA test \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

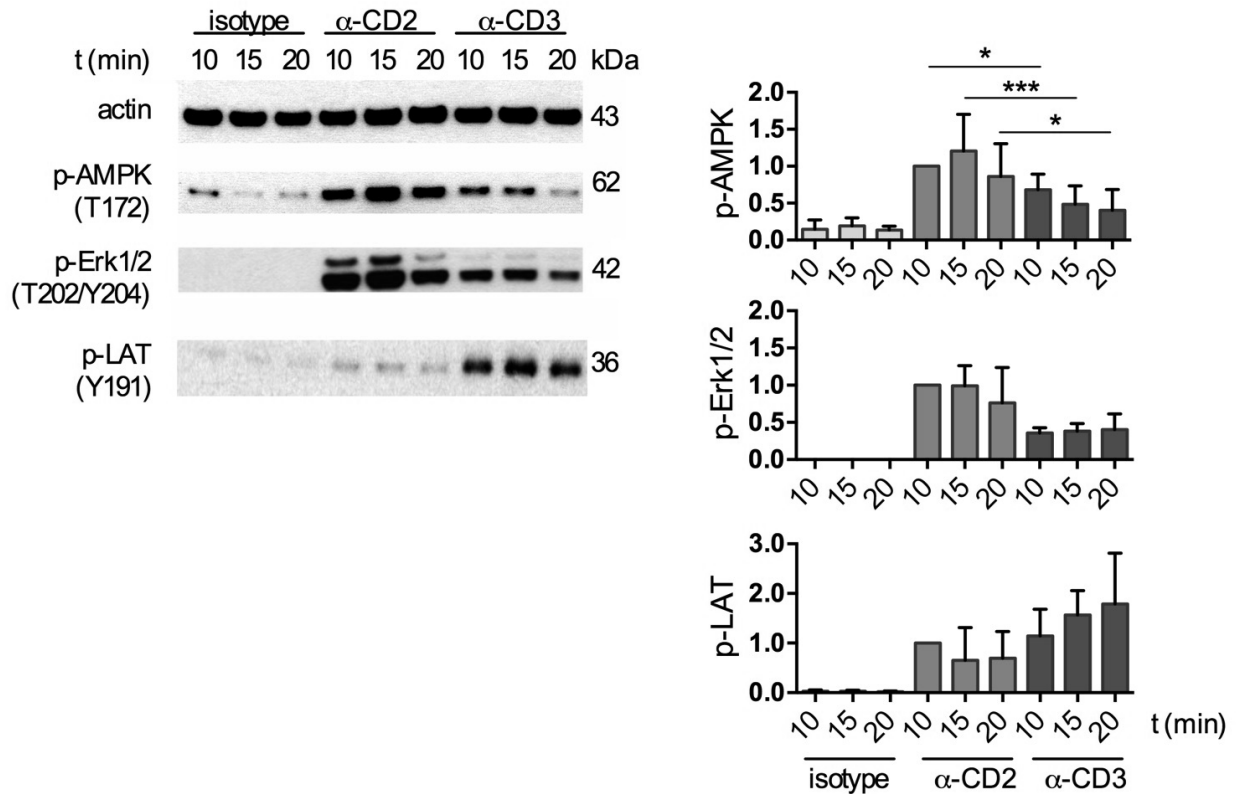




**Figure S7 In freshly isolated CTLs CD2 stimulation triggers enhanced AMPK activation in comparison to the TCR stimulation.**

Freshly isolated CTLs were stimulated with either CD2-activatory antibodies, the TCR-activatory antibody UCHT1, or isotype antibody control (all 10 µg/mL). Left panel: immunoblot analysis of AMPK activation in isotype-, CD2- and CD3-stimulated CTLs (n=4-5). pErk and p-LAT immunoblotting was performed as control blots of the signaling activation. Right panel: immunoblot quantification.

Shown are mean values ± SD; one-way ANOVA test \* p<0.05, \*\*\*p<0.001.



### Figure S8

Representative quantitative analysis of lytic granule polarization to the MTOC in CTLs treated with vehicle (complete medium), 3 mM metformin, or CD2-specific antibodies (n=2 experiments). Each dot represents individual granule. Shown are mean values  $\pm$  SD; two-way ANOVA test \*\*\*\*p<0.0001.

