Dasatinib is preferentially active in the activated B-cell subtype of diffuse large B-cell lymphoma

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SUPPORTING INFORMATION FOR PUBLICATION

TABLE OF CONTENTS

Figure S-1: Schematic of proteomics workflow
Schematic representation of the MIB/MS technique. Cells are lysed, then lysates are flowed over a column of kinase inhibitor-bound beads to capture kinases. Bound kinases are then eluted, trypsinized, and identified by mass spectrometry.

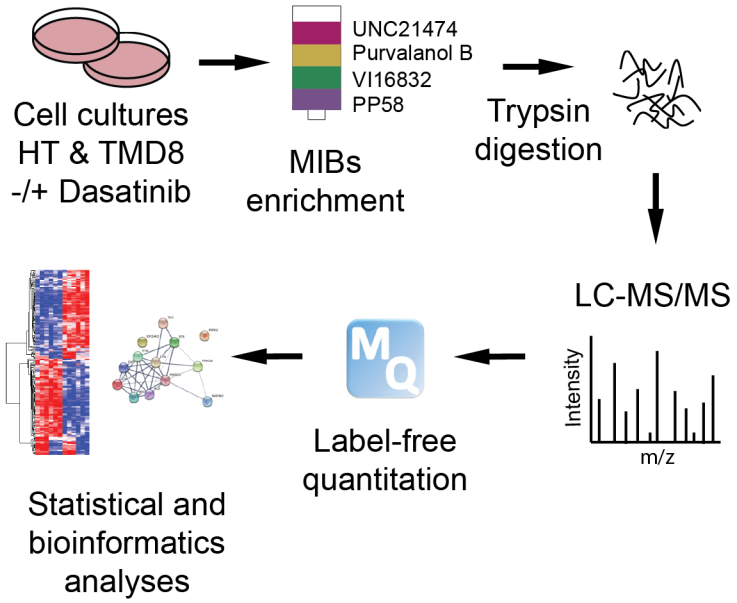


Figure S-2: Active SFKs in DLBCL cell lines
Relative expression of the individual SFK members in the panel of DLBCL cell lines, as determined by MIB/MS binding. Data are shown as normalized to the average of each SFK across all 7 cell lines. Red indicates higher MIBs binding, blue indicates less MIBs binding (relative to the average).

	HBL-1	OCI-Ly3	OCI-Ly10	TMD8	Farage	HT	Karpas 422
BLK	2.5	0.5	1.2	0.2	0.1	1.7	0.8
FGR	0.1	-	0.0	0.0	0.0	5.4	1.4
FRK	5.5	0.1	0.4	0.7	0.3	0.0	-
FYN	0.1	0.1	0.0	0.2	0.6	4.6	1.5
HCK	0.5	0.0	1.4	5.1	0.0	0.0	0.0
LCK	0.2	2.7	0.1	0.0	0.0	1.7	2.3
LYN	1.2	0.7	0.9	2.9	0.4	0.7	0.2
SRC	1.8	0.0	0.3	0.2	0.5	3.1	1.0
YES1	0.7	0.4	1.8	1.8	1.6	0.7	-

Figure S-3: MIB/MS binding after treatment with 100 nM dasatinib for 30 min

TMD8 and HT cells were treated with DMSO or 100 nM dasatinib for 30 min, and kinome changes were analyzed by MIB/MS in three independent experiments. Data are shown as ratios of kinase binding in the dasatinib-treated cells relative to the DMSO-treated cells. Ratios ≤0.5 and ≥2 denote decreased and increased MIB binding of kinases from lysates, respectively. Acronyms are the names of the various kinase families.

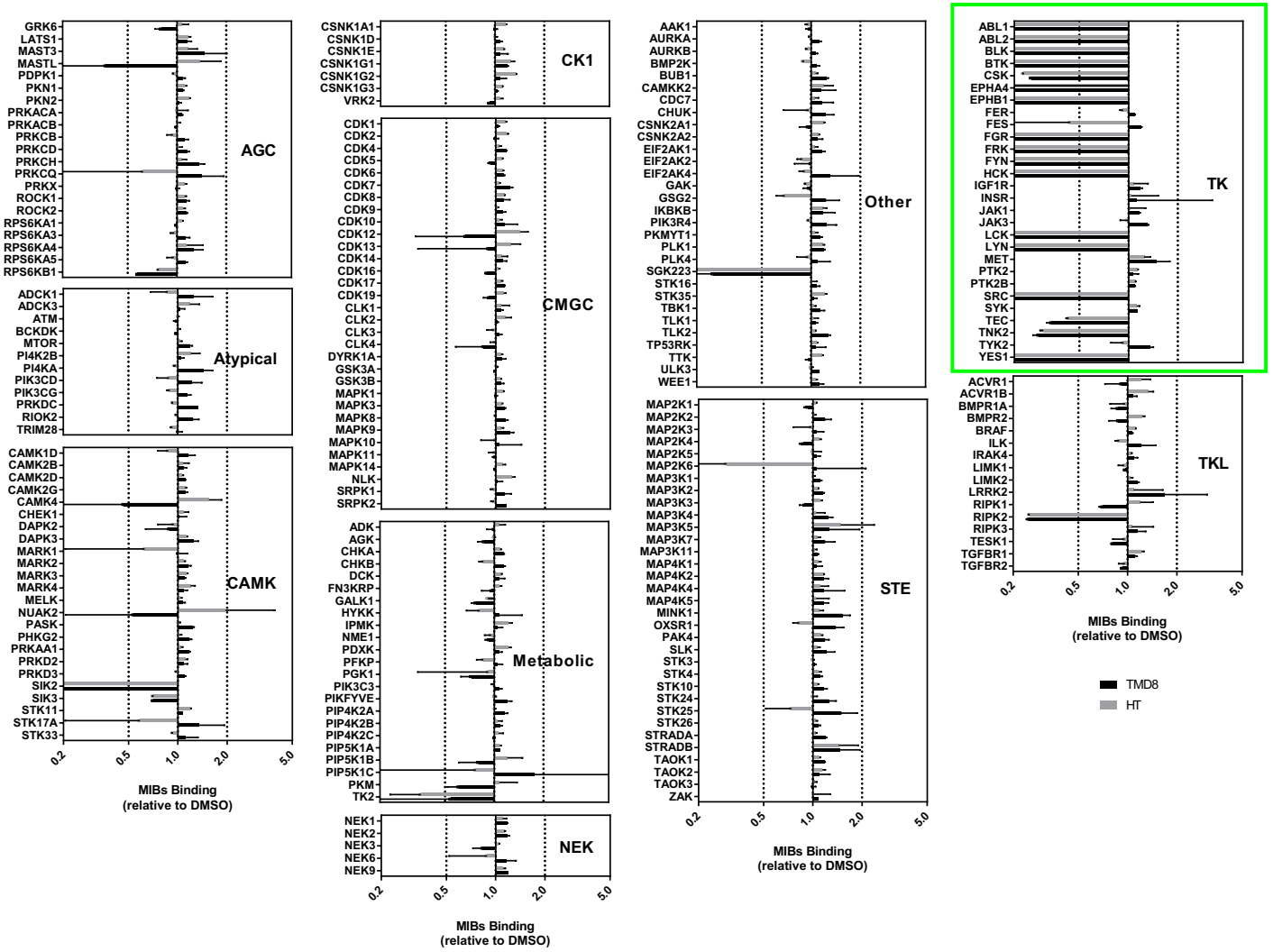


Figure S-4: SFKs are inhibited by short-term dasatinib treatment in a dose-dependent manner

(A) Western blots of SFK downstream targets after 30 min treatment with dasatinib at the indicated concentrations. Western blots shown were from the OCI-Ly3 cell line. (B) Western blot of phospho-active Lyn from cells treated with dasatinib at the indicated concentrations for 30 min. After dasatinib treatment, cells were lysed and Lyn was immunoprecipitated from whole cell lysates. Immunoprecipitations and western blots were performed using the OCI-Ly3 cell line.

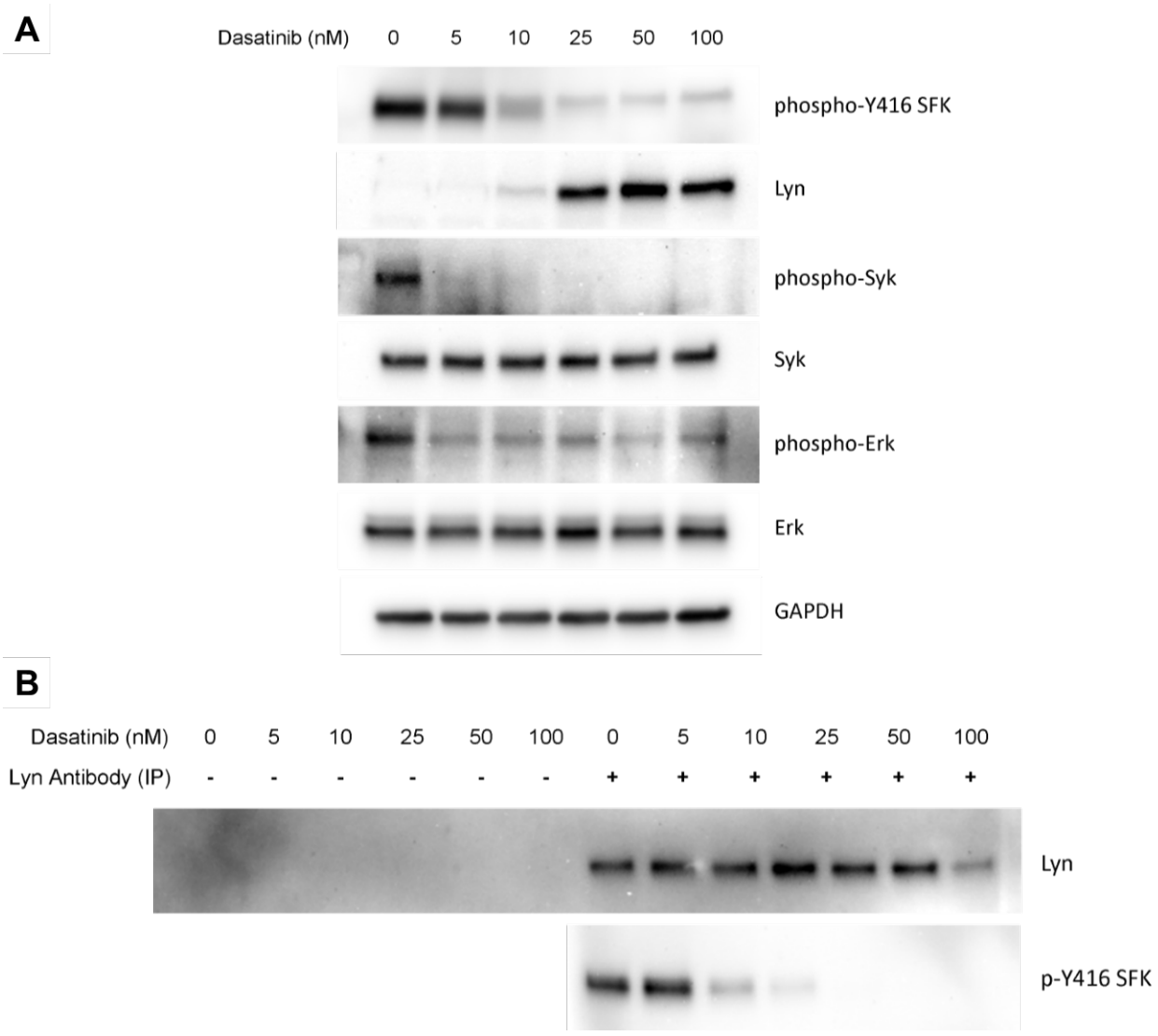


Figure S-5: MIB/MS binding after treatment with 100 nM dasatinib for 24 h

TMD8 and HT cells were treated with DMSO or 100 nM dasatinib for 24 h, and kinome changes were analyzed by MIB/MS in three independent experiments. Data are shown as ratios of kinase binding in the dasatinib-treated cells relative to the DMSO-treated cells. Ratios ≤0.5 and ≥2 denote decreased and increased MIB binding of kinases from lysates, respectively. Acronyms are the names of the various kinase families.

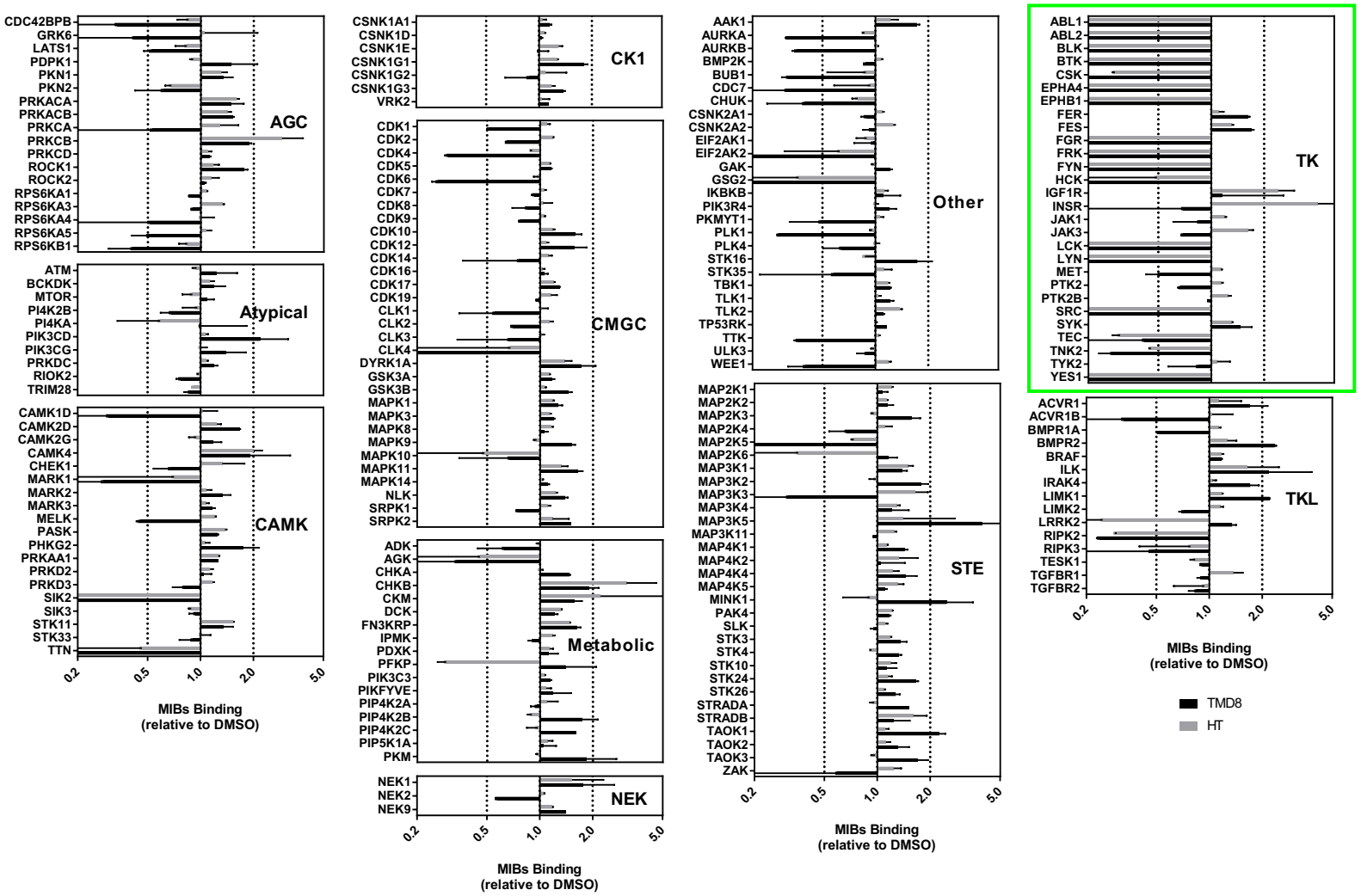


Figure S-6: Long-term dasatinib treatment has more of an impact on the kinome than short-term dasatinib treatment
Principal component analysis of kinases identified by MIB/MS after treatment with 100 nM dasatinib for (A) 30 min or (B) 24 h.

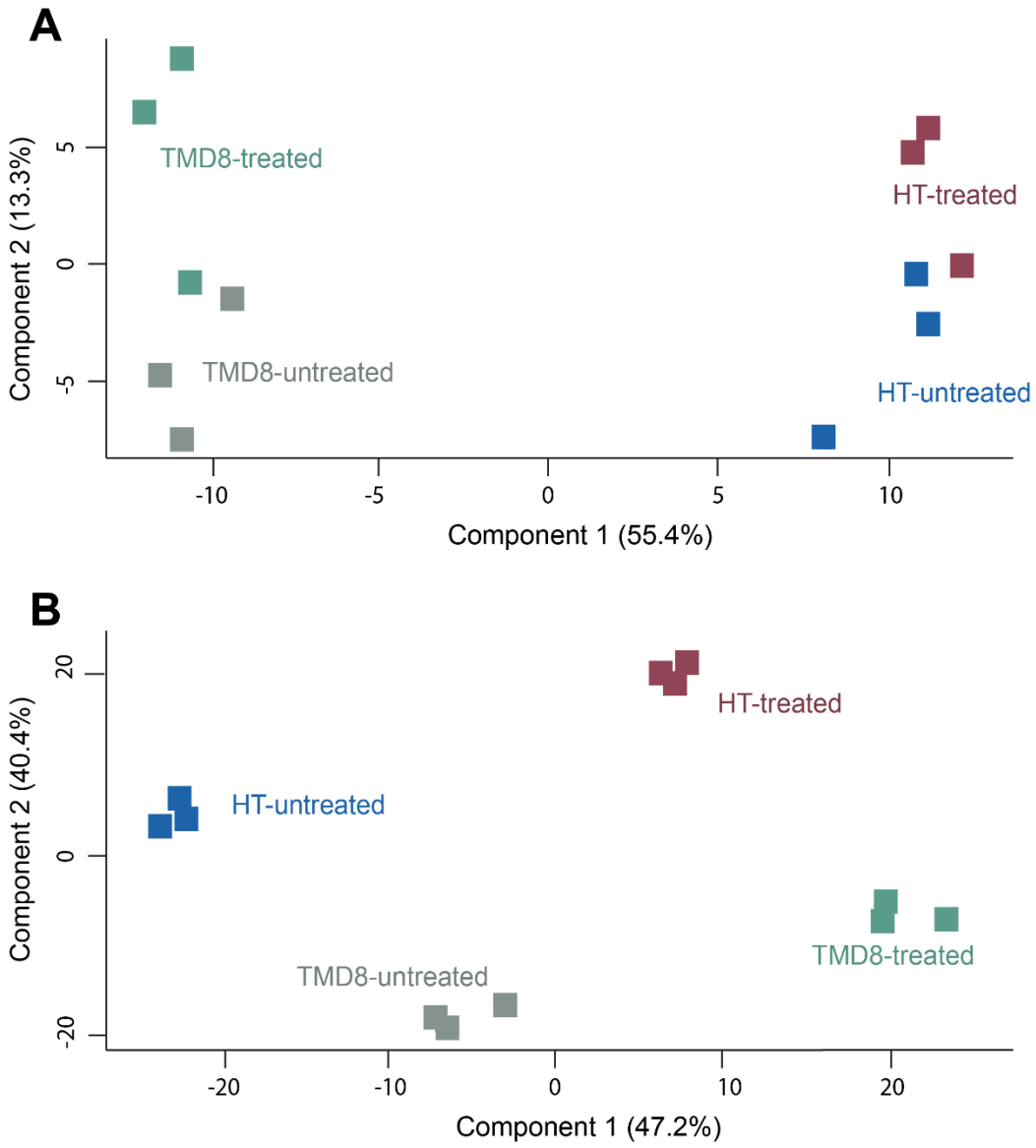


Figure S-7: Decreased expression of cyclins in the ABC DLBCL cell lines after treatment with dasatinib

(A) Western blot analysis and (B-E) corresponding quantification of several cyclins in the panel of DLBCL cell lines after treatment with 100 nM dasatinib for 24 h.

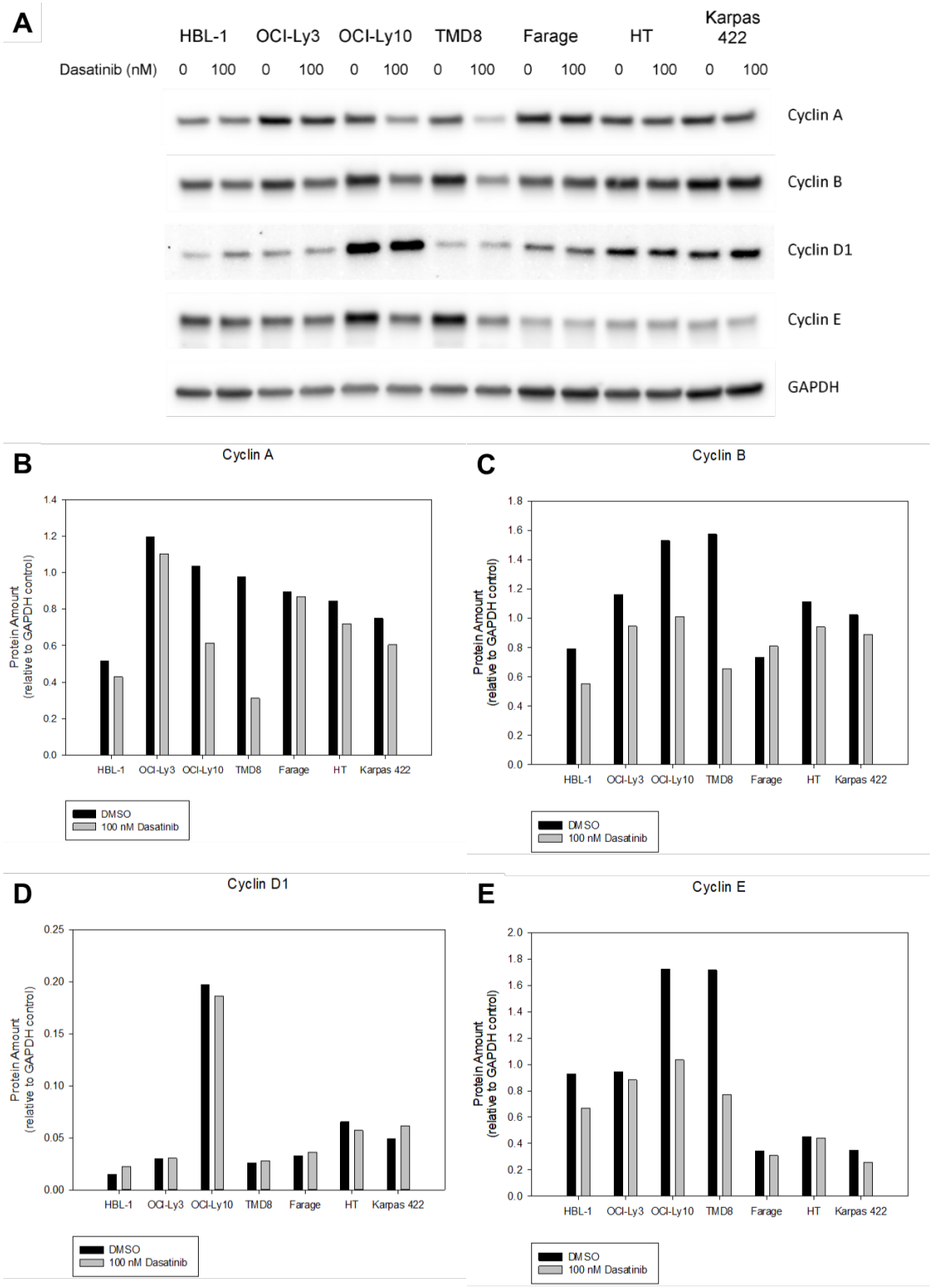
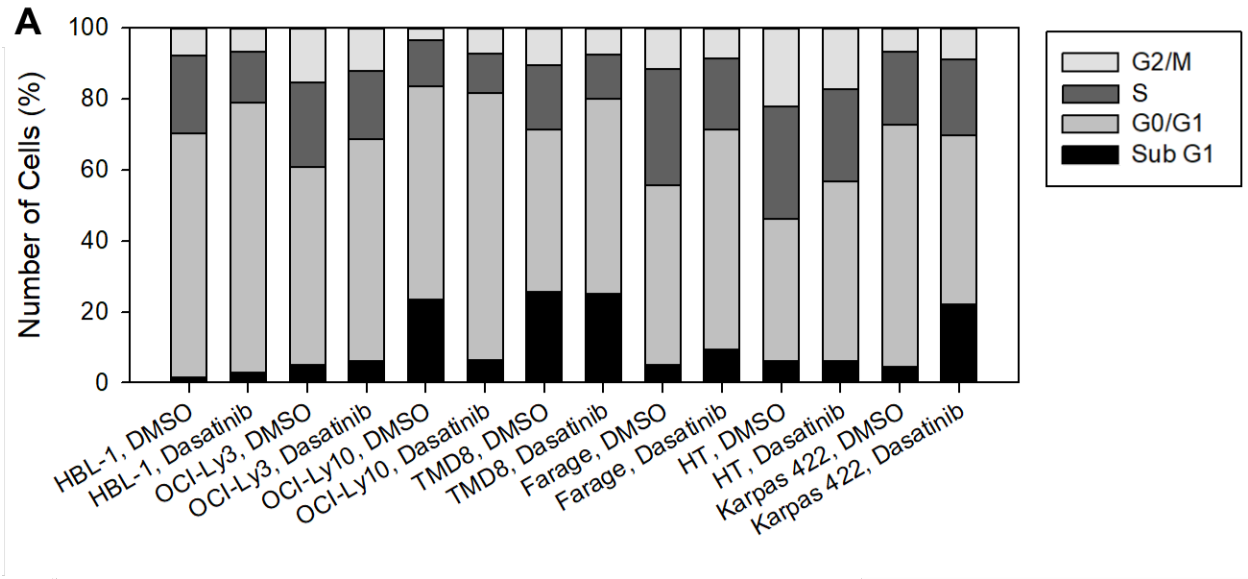
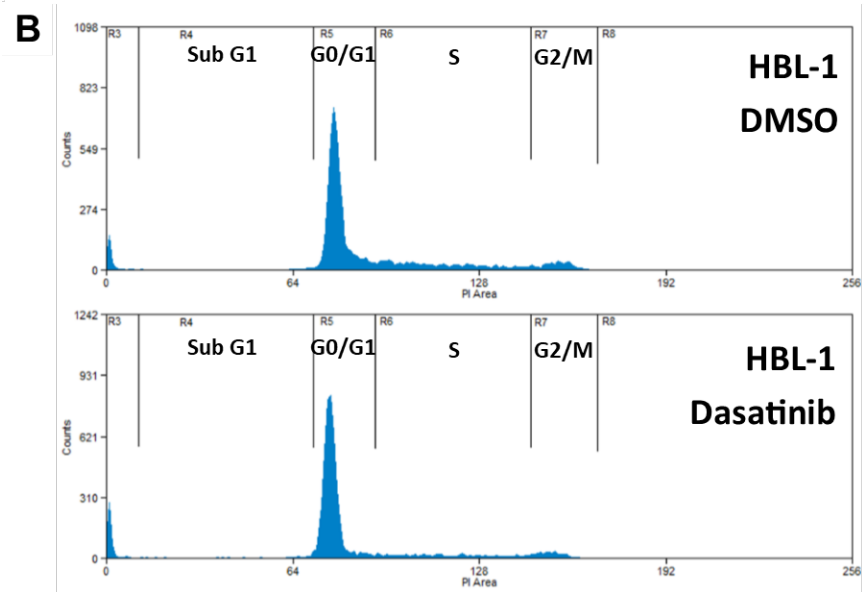
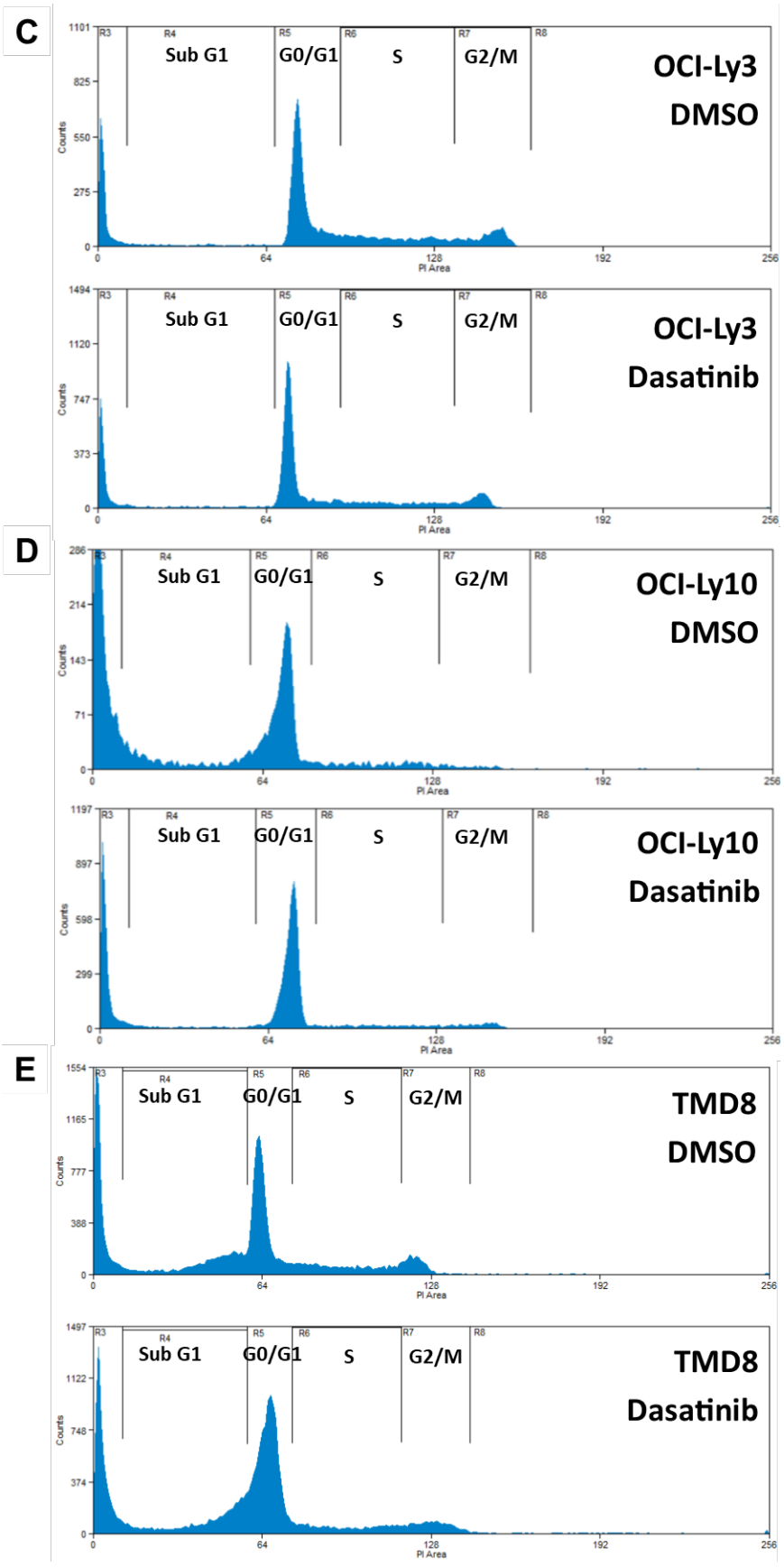


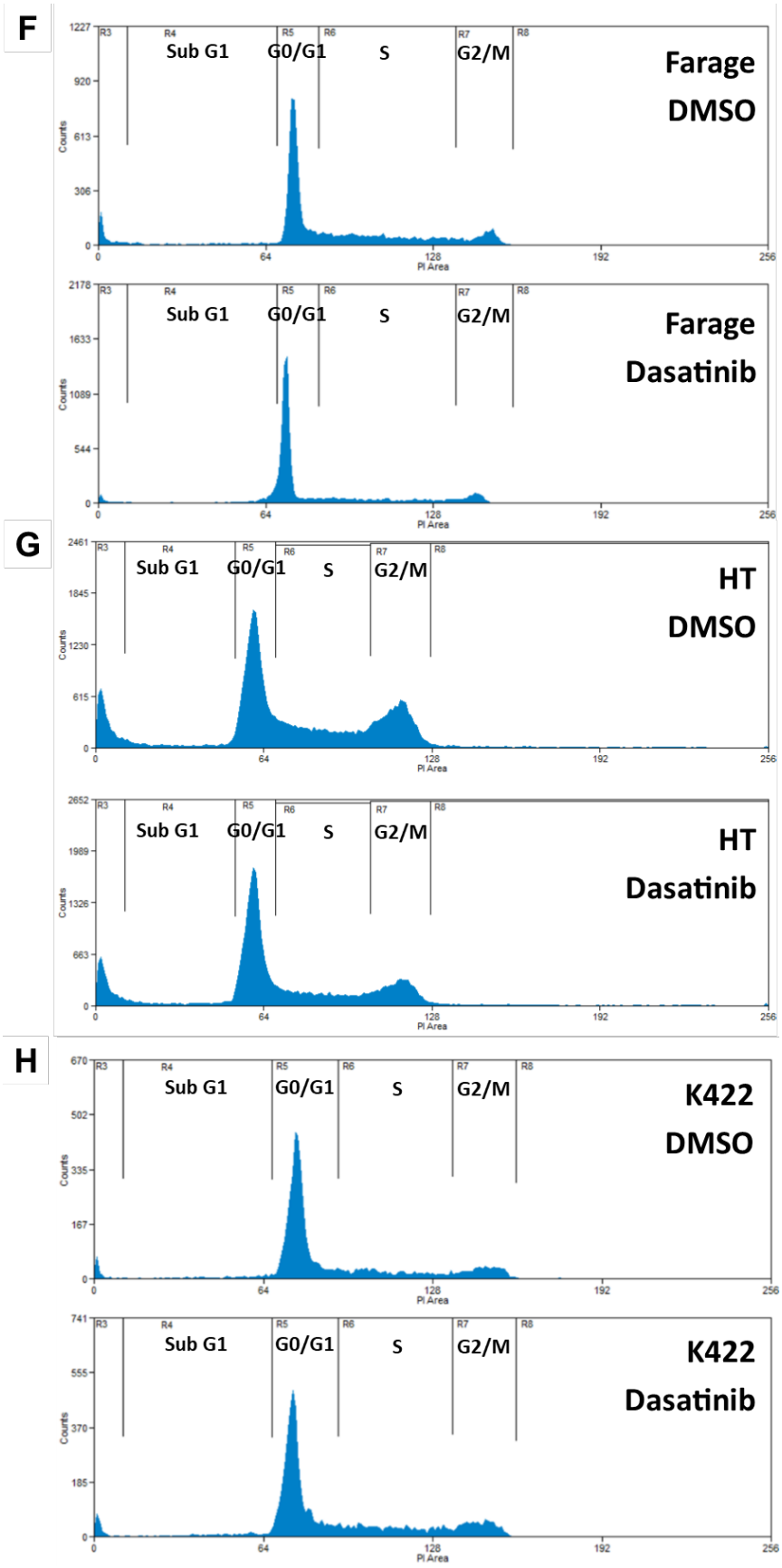
Figure S-8: Dasatinib treatment does not greatly alter cell cycle distribution after 24 h

(A) Quantification of flow cytometry analysis of DLBCL cell lines stained with propidium iodide (PI) after treatment with DMSO or 100 nM dasatinib for 24 h. (B-H) Original flow cytometry plots of PI staining.






C
Sub G1
G0/G1
S
G2/M
OCI-Ly3
DMSO
OCI-Ly3
Dasatinib
D
OCI-Ly10
DMSO
OCI-Ly10
Dasatinib
E
TMD8
DMSO
TMD8
Dasatinib
Counts
PI Area


F
Sub G1
G0/G1
S
G2/M
Farage
DMSO
Farage
Dasatinib
G
HT
DMSO
HT
Dasatinib
H
K422
DMSO
K422
Dasatinib
Counts
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