ROCK2, but not ROCK1 interacts with phosphorylated STAT3 and co-occupies TH17/TFH gene promoters in TH17-activated human T cells

Wei Chen[1], Melanie S. Nyuydzefe[1], Jonathan M. Weiss[1], Jingya Zhang[1], Samuel D. Waksal[1, 2,] and Alexandra Zanin-Zhorov[1]*

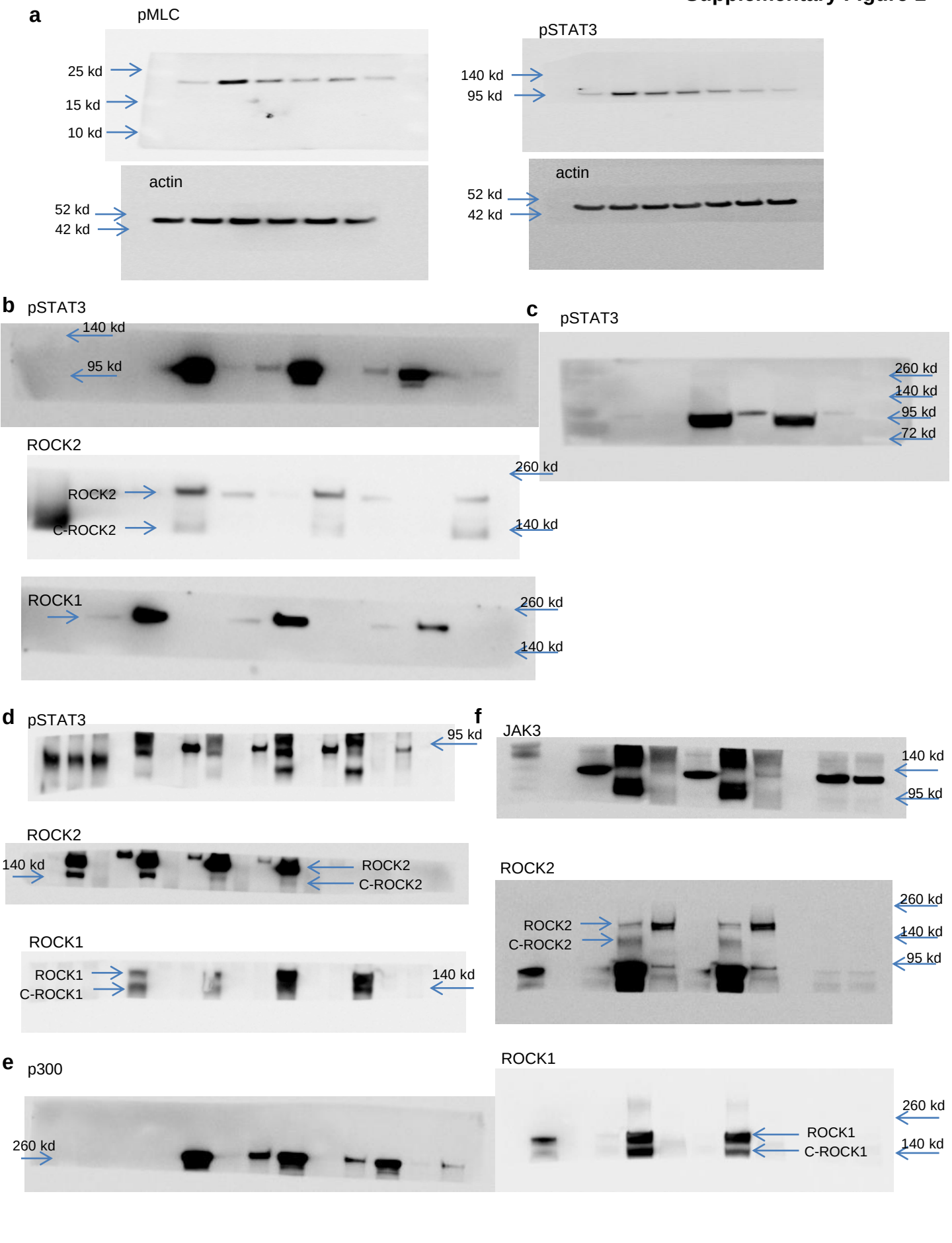
[1]Kadmon Corporation, LLC, New York, NY 10016

[2]Current Weill Cornell Medicine, New York, NY 10021

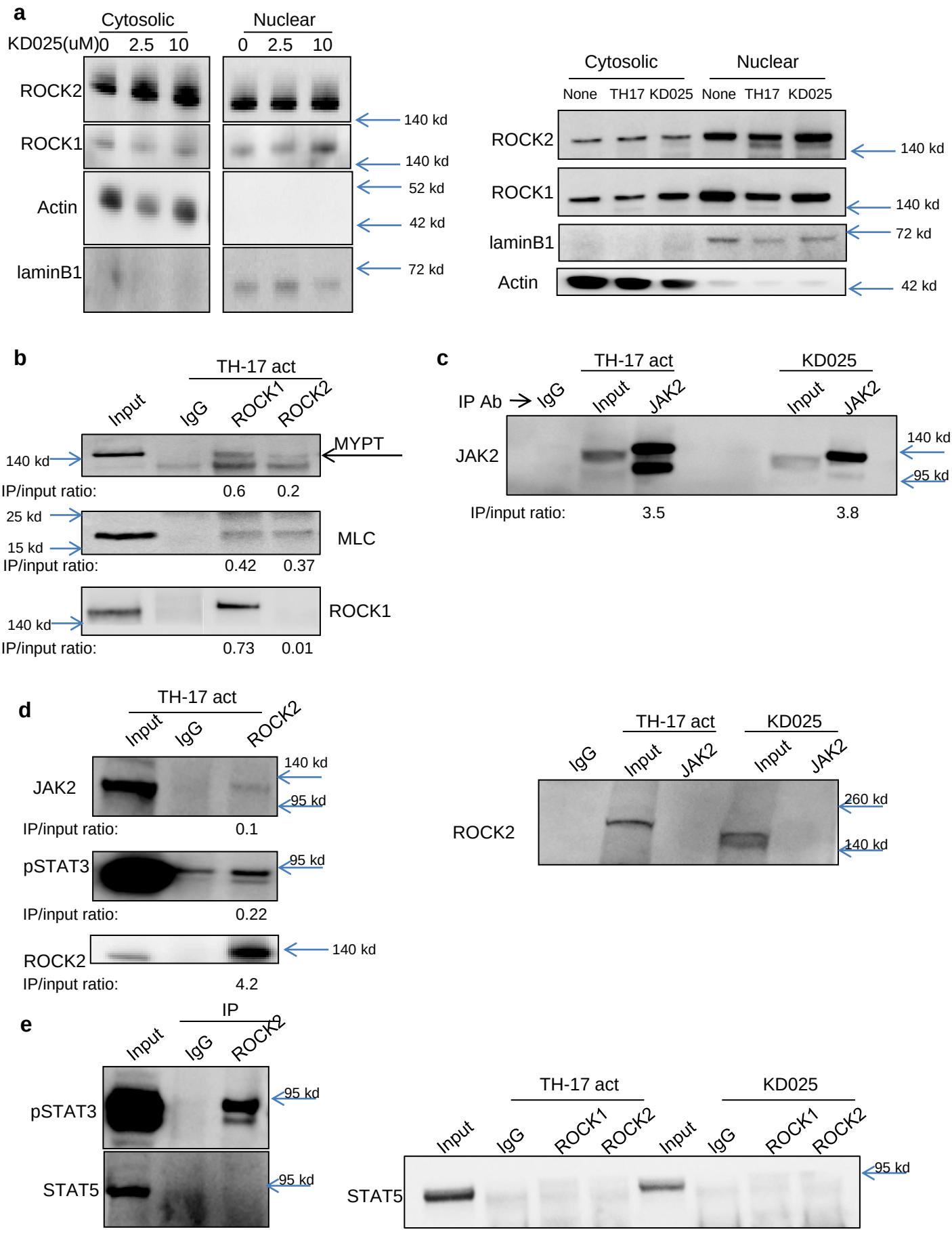
*Correspondence: Alexandra.Zanin-Zhorov@kadmon.com; Kadmon Corporation, LLC, 450 East 29th street, New York, NY 10016. Tel: 212-308-6000

Running title: ROCK2 interacts with STAT3 in human T cells

Supplementary Figure 1

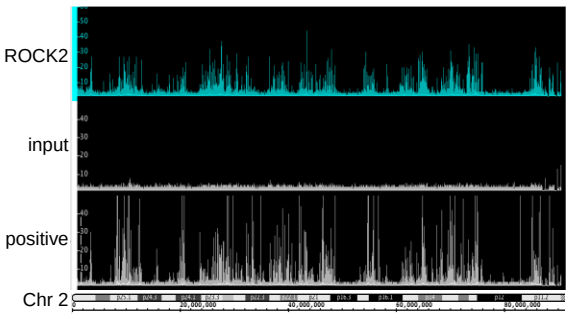


Supplementary Figure 2



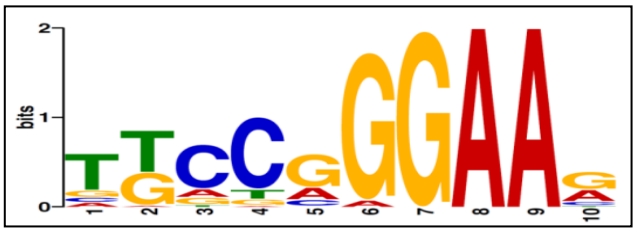
Supplementary Figure 3

a

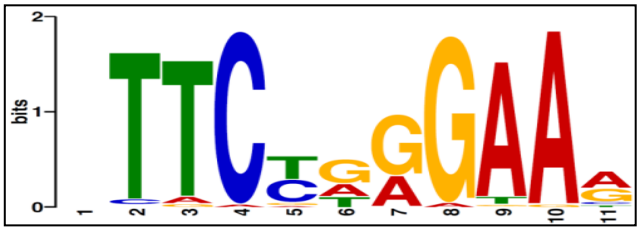


b

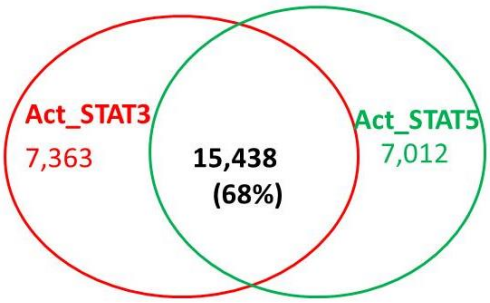
STAT3

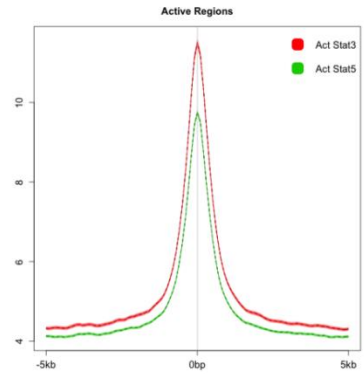


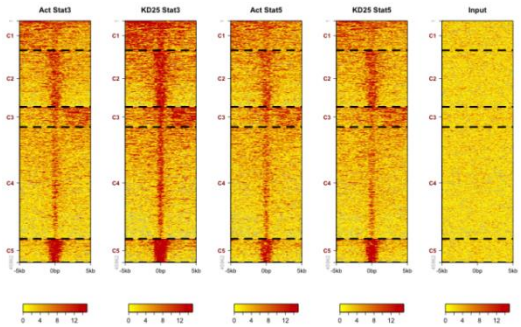
STAT5



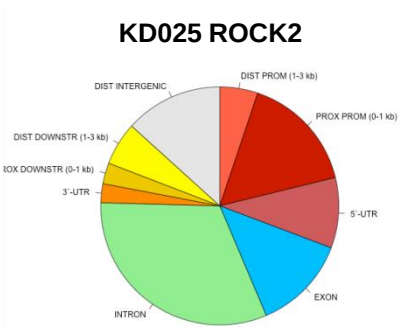
c







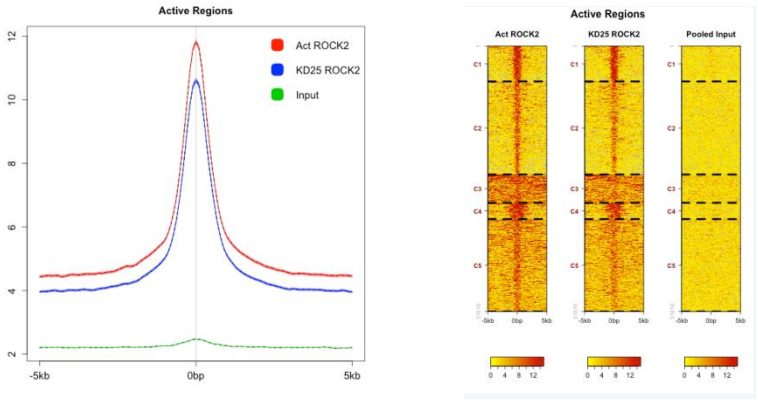
d



Genomic regions	Control	Act ROCK2	KD025 ROCK2
Dist prom (1-3 kb)	1.94%	5.65%	5.75%
Prox prom (0-1 kb)	1.03%	14.85%	17.81%
5'-UTR	0.25%	8.47%	10.65%
Exon	1.61%	13.1%	14.34%
Intron	40.6%	38.2%	35.42%
3'-UTR	1.19%	3.06%	2.86%
Prox downstr (0-1 kb)	0.99%	3.2%	3.17%
Dist downstr (1-3 kb)	1.96%	6.51%	6.53%
Dist intergenic	51.27%	16.78%	14.79%

Supplementary Figure 4

a



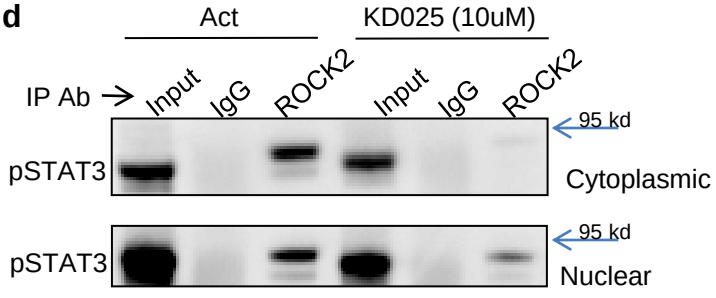
b

	Act ROCK2_increased	KD025 ROCK2_increased
Number of peaks	348	75
Number of peak with value >50	0	10

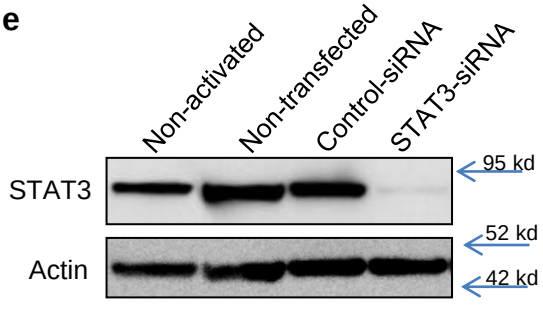
c

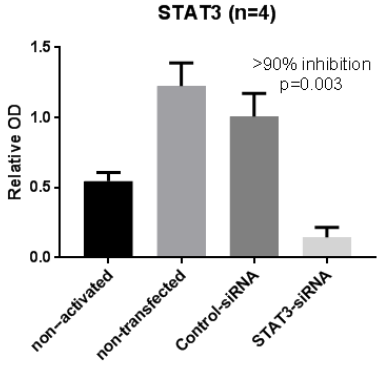
	Genes bound by ROCK2		Genes bound by STAT3	
Gene List	Dist to Start	ROCK2 Peak Value	Dist to Start	Stat3 Peak Value
BCL6	957	41	775	106
PRDM1	-60	16	514	22
PRDM1	1,478	16	11,989	87
PRDM1	12,236	15	14,674	9
IRF4	1,026	77	662	79
RORC	14,749	24	6968, 15653, -13565	139
CXCR5, BCL9L	713, 26359	18	-403	31
ICOS	-1,727	15	69	23
PDCD1, C2orf85	-10883, 55	23	23	-400
BATF	-321	22	870	113
MAF	-89	39	-179	54
STAT3	134	59	219	270
TXLNA	663	35	780	163
TOB1	-2462, -22	37	-2459, -25	50

d



e





f

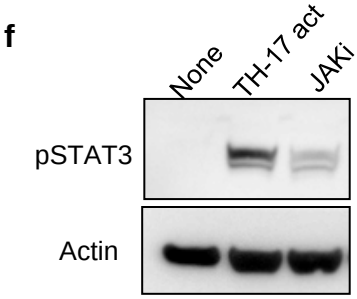


Figure 1. Full-length gels for Figure 1

Figure 2. Both ROCK1 and ROCK2 exist in the cytoplasmic as well as the nuclear fraction of human CD4$^+$ T cells. Human peripheral blood CD4$^+$ T cells were stimulated under Th17-skewing conditions for 2 hours, then nuclear and cytoplasmic fractions were purified and subjected to western blot analyses. (a) Actin and LaminB1 served as quality controls for cytoplasmic and nuclear fractions respectively. (b) Both anti-ROCK1 and anti-ROCK2 antibodies can co-precipitates MYPT and MLC. (c) Anti-JAK2 antibody specifically pulls down JAK2. pSTAT3 (d), but not STAT5 (e), co-immunoprecipitated with ROCK2. (d) Weak JAK2 being detected by anti-ROCK2 co-immunoprecipitation, but no detectable ROCK2 by anti-JAK2 co-immunoprecipitation (d). Numbers indicate ratio between the precipitated protein and the input.

Figure 3. ROCK2, STAT3 and STAT5 are co-localized on chromatin in human T cells. (a) A screen shot of ROCK2 peaks, together with input and a positive antibody at Chromosome 2 from ROCK2 antibody ChIP-seq validation assay. (b) STAT3 and STAT5 occupancy on an average gene peak at transcription start region; STAT3 and STAT5 binding motif. (c) Venn diagram with numbers of genomic sites bound by STAT3 and STAT5. (d) Pie chart of ROCK2 active peaks distribution in KD025-treated cells over control.

Figure 4. STAT3 mediates ROCK2 recruitment to the chromatin. (a) ROCK2 binding peaks at transcription start site regardless KD025 treatment. (b) Number of peaks changed after KD025 treatment shows KD025 does not alter genome wide ROCK2 binding profile. (c) List of TH17/TFH cell related genes with high ROCK2 and STAT3 peaks analyzed by ChIP-seq. (d) ROCK2 interacts with pSTAT3 in human CD4$^+$ T cells stimulated by TH17 skewing conditions for 48 hours. (e) STAT3 siRNA robustly down-regulates the expression of STAT3, a representative Western blot and quantification from four independent experiments is shown. (f) Phosphorylation of STAT3 is decreased by treatment of cells with a JAK inhibitor, Baricitinib.