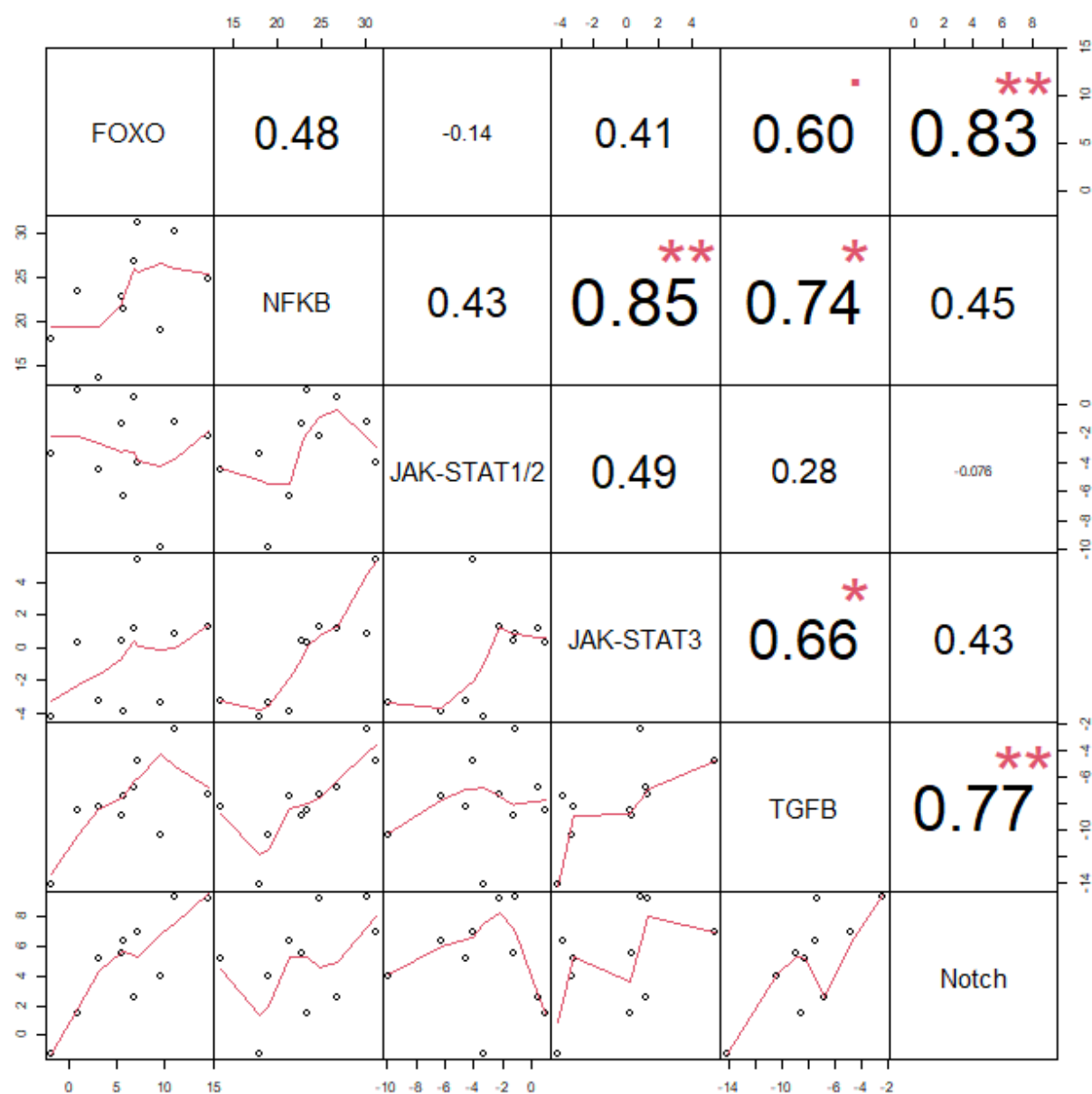


SUPPLEMENTARY INFORMATION

sample category	annotation per sample	FOXO	NFκB	JAK-STAT1/2	JAK-STAT3	TGFβ	Notch	ER	WNT
CD4+ T cells from peripheral blood	donor 1	4.5	4.5	-8.3	-5.7	-13.9	-8.9	-18.7	-19.1
	donor 2	1.3	1.4	-7.6	-10.2	-16.8	-9.6	-19.1	-21.0
	donor 3	-3.2	-7.1	-7.6	-8.4	-20.3	-8.9	-18.9	-24.5
	donor 4	-2.1	-5.7	-6.1	-9.8	-19.0	-9.3	-18.9	-21.4
	patient 1	8.5	17.4	-7.4	-2.4	-5.0	1.6	-16.0	-20.0
	patient 2	-1.6	-1.5	-7.7	-7.3	-14.5	-5.7	-18.6	-16.7
	patient 3	5.9	6.3	-8.7	-5.5	-14.7	-8.7	-18.8	-22.8
	patient 4	-3.8	-5.1	-5.6	-6.2	-19.6	-6.1	-18.6	-21.1
	patient 5	2.4	8.4	-8.7	-4.3	-11.7	-4.2	-18.2	-15.9
	patient 6	1.7	5.9	-7.2	-6.3	-13.2	-8.4	-18.8	-21.2
	patient 7	6.9	3.2	-8.3	-5.8	-14.3	-7.3	-18.9	-19.2
	patient 8	1.3	5.2	-9.3	-6.7	-14.9	-6.8	-18.7	-17.0
	patient 9	-2.5	-1.3	-4.2	-8.1	-18.4	-8.0	-19.9	-22.2
	patient 10	-2.2	-3.6	-6.2	-7.3	-18.8	-8.4	-19.3	-21.8
CD4+ T cells from axillary lymph nodes	patient 1	-1.4	13.1	-9.0	-6.7	-12.3	-2.2	-18.8	-22.7
	patient 2	2.5	11.0	-8.3	-2.9	-11.5	0.8	-17.5	-20.8
	patient 3	5.5	17.8	-8.6	-4.5	-12.2	0.3	-18.2	-20.5
	patient 4	0.8	10.1	-8.2	-4.8	-11.7	-3.0	-18.2	-21.7
	patient 5	4.2	7.4	-6.6	-7.4	-13.2	-5.4	-18.7	-20.7
	patient 6	-3.5	-2.1	-10.5	-4.6	-15.6	-8.3	-19.9	-23.4
	patient 7	2.1	12.4	-9.0	-3.1	-10.4	-3.3	-18.2	-20.3
	patient 8	0.6	7.2	-9.4	-4.8	-14.6	-5.1	-18.7	-26.9
	patient 9	-4.0	5.6	-5.7	-5.2	-17.2	-8.6	-19.1	-25.0
	patient 10	2.3	2.9	-8.1	-3.0	-14.3	-4.0	-19.1	-21.9
CD4+ T cells from breast tumor	patient 1	5.6	21.5	-6.3	-3.9	-7.5	6.3	-18.5	-21.5
	patient 2	6.9	26.8	0.5	1.2	-6.8	2.5	-15.9	-17.5
	patient 3	10.9	30.3	-1.2	0.9	-2.4	9.3	-16.9	-15.3
	patient 4	0.9	23.5	0.9	0.3	-8.5	1.5	-15.7	-19.9
	patient 5	7.2	31.3	-4.0	5.4	-4.8	7.0	-6.7	-14.2
	patient 6	9.6	19.0	-9.8	-3.3	-10.4	4.0	-19.3	-16.6
	patient 7	5.4	22.8	-1.3	0.4	-9.0	5.6	-18.3	-13.5
	patient 8	3.2	13.6	-4.5	-3.2	-8.3	5.2	-17.5	-25.4
	patient 9	-1.9	18.1	-3.4	-4.2	-14.2	-1.3	-17.6	-25.0
	patient 10	14.5	24.8	-2.2	1.3	-7.4	9.3	-18.2	-19.1

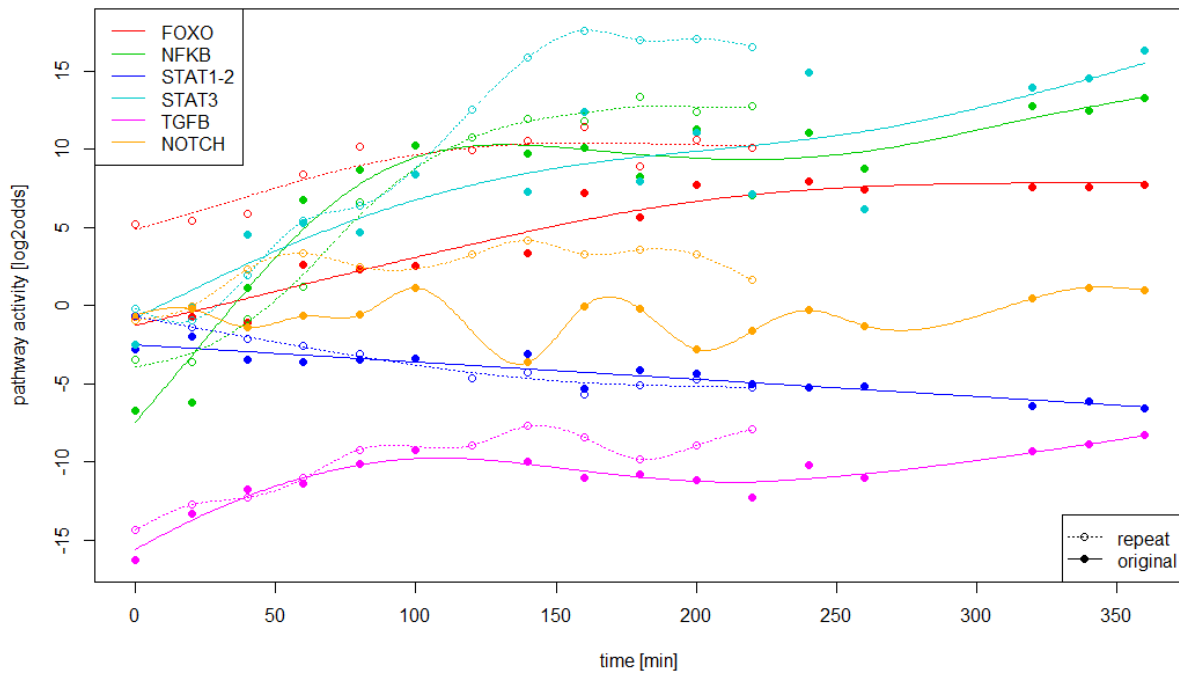
Supplementary Figure S1. Signaling pathway activities per patient from dataset GSE36765 [19].



Supplementary Figure S2. NFkB pathway activity is highly correlated with JAK-STAT3 and TGFB pathway activity.

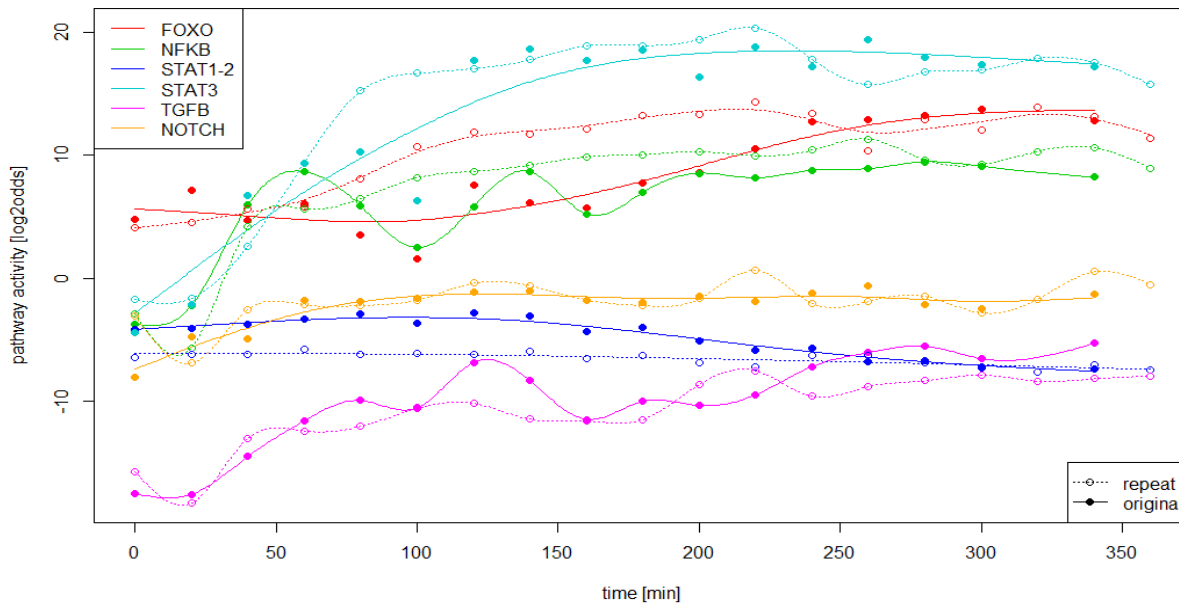
Correlation matrix between signaling pathway activities among BrCa TIL samples from dataset GSE36765 [19]. Bottom graphs show bivariate scatterplots with a fitted line and on top are the corresponding Pearson correlation coefficients, including stars for statistically significant correlations.

Treg cells



A

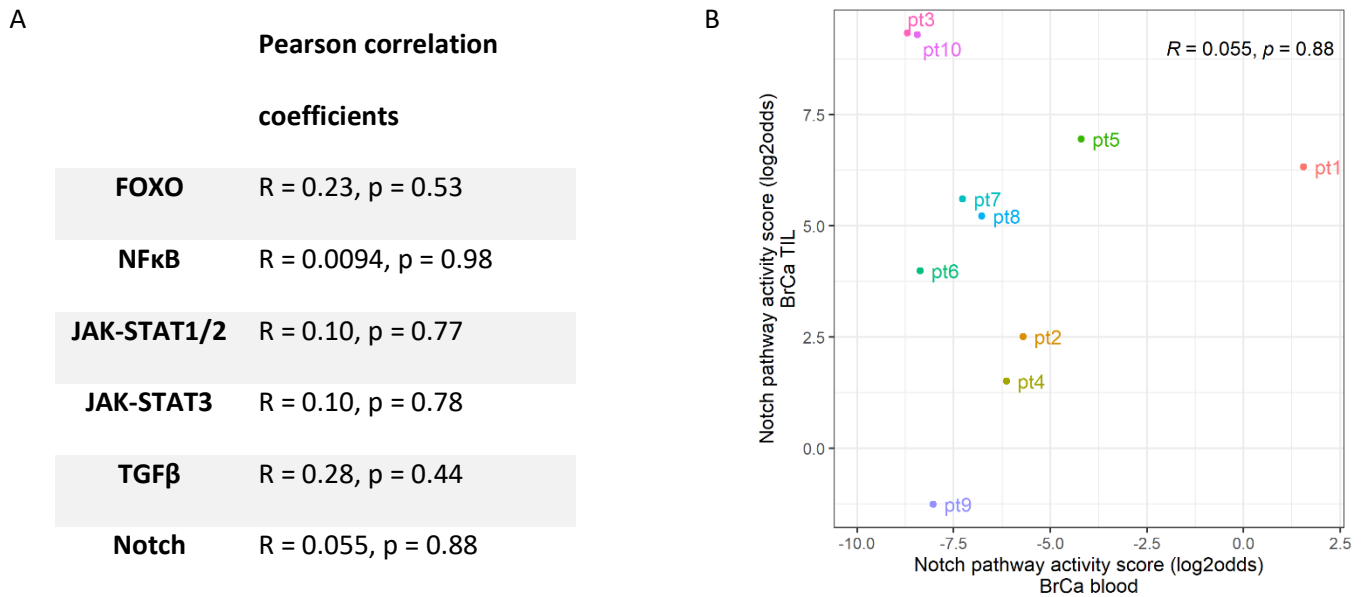
Teff cells



B

array	title	QC.passed	QC.man. passed	t	log2dds NOTCH	log2dds JAK-STAT3	log2dds NFkB	log2dds JAK- STAT1/2	log2dds TGFB	QC.man.comments
GSM285027	Human_Treg_T0min	TRUE	TRUE	0	-0.6	-2.5	-6.7	-6.3	-16.3	
GSM723815	Human_Treg_repeated_T0min	FALSE	TRUE	0	-0.9	-0.2	-3.4	-4.0	-14.3	samprep probably not performed
GSM285028	Human_Treg_T20min	FALSE	TRUE	20	-0.2	-0.1	-6.2	-5.9	-13.3	hybspike probably not performed
GSM723816	Human_Treg_repeated_T20min	TRUE	TRUE	20	0.0	-1.0	-3.6	-4.9	-12.7	
GSM285029	Human_Treg_T40min	FALSE	TRUE	40	-1.4	4.5	1.1	-6.6	-11.7	samprep and hybspike probably not performed
GSM723817	Human_Treg_repeated_T40min	TRUE	TRUE	40	2.3	1.9	-0.9	-5.4	-12.3	
GSM285030	Human_Treg_repeated_T60min	FALSE	TRUE	60	-0.7	5.3	6.8	-5.7	-11.4	hybspike probably not performed
GSM723818	Human_Treg_repeated_T60min	TRUE	TRUE	60	3.3	5.4	1.2	-5.7	-11.0	
GSM285031	Human_Treg_T80min	FALSE	TRUE	80	-0.6	4.7	8.6	-5.3	-10.1	gapdh a bit higher but .mid value OK (1.00)
GSM723819	Human_Treg_repeated_T80min	TRUE	TRUE	80	2.5	6.4	6.6	-5.7	-9.2	
GSM285032	Human_Treg_T100min	FALSE	TRUE	100	1.1	8.4	10.2	-5.6	-9.3	samprep probably not performed; hybspike OK
GSM723820	Human_Treg_repeated_T100min	FALSE	FALSE	100						SET TO FALSE! bactin (4.00), gapdh (2.60) and gapdh.mid (1.74) too high
GSM723821	Human_Treg_repeated_T120min	TRUE	TRUE	120	3.2	12.5	10.8	-5.4	-8.9	
GSM285033	Human_Treg_T140min	FALSE	TRUE	140	-3.6	7.3	9.7	-4.8	-10.0	gapdh a bit higher but .mid value OK (1.05)
GSM723822	Human_Treg_repeated_T140min	TRUE	TRUE	140	4.2	15.8	11.9	-6.3	-7.7	
GSM285034	Human_Treg_repeated_T160min	FALSE	TRUE	160	-0.1	12.4	10.1	-6.9	-11.0	samprep and hybspike probably not performed
GSM723823	Human_Treg_repeated_T160min	FALSE	TRUE	160	3.3	17.6	11.8	-6.8	-8.4	samprep probably not performed
GSM285035	Human_Treg_T180min	FALSE	TRUE	180	-0.2	7.9	8.2	-5.1	-10.8	hybspike probably not performed
GSM723824	Human_Treg_repeated_T180min	TRUE	TRUE	180	3.6	17.0	13.3	-6.6	-9.8	
GSM285036	Human_Treg_T200min	FALSE	TRUE	200	-2.8	11.1	11.3	-6.2	-11.1	hybspike probably not performed
GSM723825	Human_Treg_repeated_T200min	TRUE	TRUE	200	3.3	17.1	12.4	-6.1	-8.9	
GSM285037	Human_Treg_T220min	FALSE	TRUE	220	-1.6	7.1	7.1	-5.5	-12.3	samprep probably not performed
GSM723826	Human_Treg_repeated_T220min	TRUE	TRUE	220	1.6	16.6	12.7	-6.1	-7.9	
GSM285038	Human_Treg_T240min	FALSE	TRUE	240	-0.3	14.9	11.0	-7.1	-10.2	samprep probably not performed
GSM723827	Human_Treg_repeated_T240min	FALSE	FALSE	240						SET TO FALSE! bactin (5.18), gapdh (2.73) and gapdh.mid (1.83) too high
GSM285039	Human_Treg_T260min	FALSE	TRUE	260	-1.3	6.1	8.7	-6.2	-11.0	samprep and hybspike probably not performed
GSM723828	Human_Treg_repeated_T260min	FALSE	FALSE	260						bactin (5.46), gapdh (3.09) and gapdh.mid (2.04) too high
GSM285040	Human_Treg_T280min	FALSE	FALSE	280						bactin.mid too high (3.27)
GSM723829	Human_Treg_repeated_T280min	FALSE	FALSE	280						bactin (5.09), gapdh (2.73) and gapdh.mid (1.76) too high
GSM285041	Human_Treg_T300min	FALSE	FALSE	300						bactin (4.89) and bactin.mid (3.28) too high; failure of 6 ref genes
GSM723830	Human_Treg_repeated_T300min	FALSE	FALSE	300						SET TO FALSE! bactin (5.13), gapdh (2.51) and gapdh.mid (1.63) too high
GSM285042	Human_Treg_T320min	TRUE	TRUE	320	0.4	14.0	12.8	-7.2	-9.3	
GSM723831	Human_Treg_repeated_T320min	FALSE	FALSE	320						bactin (5.11), gapdh (2.96) and gapdh.mid (2.18) too high
GSM285043	Human_Treg_T340min	FALSE	TRUE	340	1.2	14.5	12.5	-7.3	-8.9	samprep and hybspike probably not performed
GSM723832	Human_Treg_repeated_T340min	FALSE	FALSE	340						bactin (4.98), gapdh (2.46) and gapdh.mid (1.54) too high
GSM285044	Human_Treg_T360min	FALSE	TRUE	360	1.0	16.3	13.2	-8.3	-8.3	samprep probably not performed
GSM723833	Human_Treg_repeated_T360min	FALSE	FALSE	360						SET TO FALSE! bactin (4.98), gapdh (2.49) and gapdh.mid (1.59) too high

Supplementary Figure S3. GSE11292. Pathway analysis scores for resting and activated Treg (A) and T effector (B) cells. C: QC analysis of individual sample data. Treg and T effector cells were activated in the same manner with anti-CD3/CD28 and IL2. Samples were taken every 20 minutes. N=2 time experiments (defined as "original" and "repeated") [24]. Sample data that failed our QC as described in Methods were not included in the pathway analysis. Pathway activity scores are on log2odds scale.



Supplementary Figure S4. Correlation between signaling pathway activities in CD4 T+ cells from blood and from TIL from BrCa patients (dataset GSE36765, [19]). A. Pearson correlation coefficients; B. Correlation plot for the Notch pathway.

Supplementary Table S1. Threshold values above which signaling pathway activity may be considered abnormally high. Thresholds are calculated based on activity in CD4+ T cells in blood from the four healthy donors in the GSE36765 dataset [19] and are defined as the mean pathway activity score \pm two standard deviations.

	lower threshold	upper threshold	# patients exceeding the upper threshold
FOXO	-6.9	7.2	1 (pt 1)
NFκB	-12.8	9.4	1 (pt 1)
JAK-STAT1/2	-9.2	-5.5	1 (pt 9)
JAK-STAT3	-12.6	-4.4	2 (pt 1, 5)
TGFβ	-23.1	-11.9	2 (pt 1, 5)
Notch	-9.8	-8.5	9 (pt 1-2, 4-10)