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NFκB pathway dysregulation due to reduced RelB expression leads to severe autoimmune disorders and declining immunity

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SUPPLEMENTARY RESULTS

Altered transcriptional responses to LIGHT

To determine what functional significance induced P346L RelB protein may possess, we examined transcription responses to Lymphotoxin-β-receptor stimulation. The LIGHT ligand has been shown to induce subsets of transcripts that are solely dependent upon either RelA or RelB, allowing us to specifically evaluate RelB-mediated NFκB dependent gene induction and determine whether RelA dependent transcription normalised upon P364L RelB upregulation. Control, RelB null and P364L RelB expressing patient fibroblasts were pre-treated with LPS to up-regulate RelB protein expression, and then stimulated with LIGHT. RNA was isolated and analyzed by GeneChip PrimeView Human Gene Expression microarray (Applied Biosystems).

Control fibroblasts robustly up-regulating message levels of 366 genes by a factor of two-fold or greater in response to LIGHT. Of these, RelB null fibroblasts failed to up-regulate 47 transcripts, or 14% of the total (Fig E2 and E4.A, Online Repository). Thus, the LIGHT response appeared heavily tilted towards RelA-mediated gene transcription. P364L RelB fibroblasts did not demonstrate the loss of any gene transcription not also lost in RelB null cells, and 26 of the 47 genes failing to be induced in RelB null cells now demonstrating partial to normal responses, with 21 still absent (Fig E2 and E4.B, Online Repository). A clear example of differential responses were seen with TRAF family members. TRAF3 underwent normal induction in null and P364L cells, while TRAF1 failed to be induced in either (Fig E4.C, Online Repository). However, TRAF2 induction, absent in null cells, was restored in the presence of P364L RelB. Thus, when transiently upregulated, the P364L RelB protein appears to restore some alternative pathway responsiveness, which likely contributes to slowing the decline in patient immune function compared to complete RelB deficiency. However, the P364L protein appeared insufficient to fully restore responses.

P364L RelB expression and the complete absence of RelB both had a significant impact upon RelA-mediated gene expression in LIGHT-stimulated cells. While 366 genes up-regulated transcription by a factor of two-fold or greater in control cells, 535 and 479 genes went above this threshold in RelB null and P364L-expressing cells, respectively (Fig E4.D, Online Repository) - a 30-50% increase. This included not only transcripts undergoing a greater degree of up-regulation in null and P364L fibroblasts, or null cells alone, but also genes that were not LIGHT-induced in controls (Fig E4.E, Online Repository). As hypothesized to occur in poly(I:C)-stimulated cells, increased and novel transcription presumably occurs due to a loss of RelB-mediated negative regulation of RelA. Despite prior LPS-induced upregulation of the P364L RelB protein to near normal levels, patient fibroblasts still showed increased transcription responses to LIGHT, although less than in RelB null cells, suggesting once again that the P364L RelB protein was unable to fully maintain normal regulatory functions.

Surprisingly, the abnormal upregulation of a small subset of genes was seen to occur to a significantly higher level in the presence of P364L RelB than in completely RelB deficient cells. Notably, this included multiple pro-inflammatory cytokines and chemokines (e.g. CXCL8, CXCL5, CCL5, CXCL6), (Fig E4.F, Online Repository). This strongly suggested that the regulation of a subset of RelA-dependent transcription was specifically compromised by the presence of the mutated P364L RelB protein, arguing it could interfere with down-regulation or feedback inhibition of certain active promoter sites.

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Figure E1

Flow Cytometry Antibodies

BD Biosciences	catalogue number
CD3	560835 555342
CD4	557852 562970 566318
CD8	561453
CD45RO	555493
CD45RA	560674
CCR7	562555
CD95	564596
CD28	559770
CD57	560844
CD279	563076
CD38	555462
CD19	563038
CD27	562513
IgD	561314
CD24	564521
CD21	563474
IgM	563113
CD25	564467
CD69	557745
CD127	560551
CD194	562579
Il-2	563946
IFN-g	554701 564791

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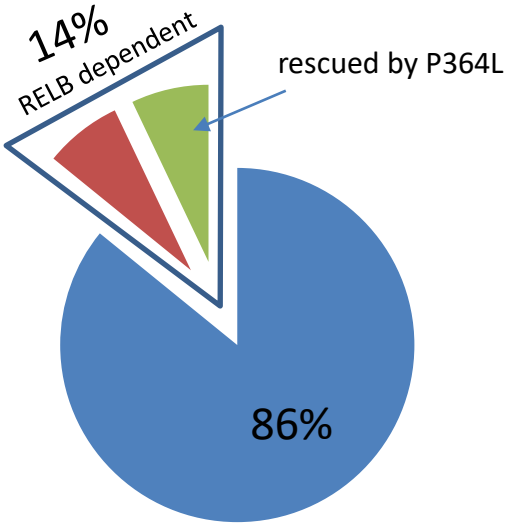
Figure E2



LIGHT-induced transcription
366 genes >2 fold

	NO INDUCTION IN RELB NULL		
P364L	**loss**	**partial**	**recovered**
	ADTRP	CCDC28B	C10orf10
	ANKRD1	CD70	APOL2
	APOL6	ELOVL2	EPHB2
	BHLHE40	FGD6	GATA6
	BTBD11	GADD45A	NES
	BTN2A2	IL15	SAMD9L
	CMAHP	LAMC2	SLFN11
	DGKI	LIMD2	TNFSF4
	EGLN3	NEDD9	TRAF2
	EGR2	PLK2	WWC1
	EHD1	TIMP1	XAF1
	KCNN4	TRAF1	
	PLXNA1	ZNF267	
	RASGRP1	DDX58	
	RGS20	DTX4	
	RNF19B		
	SCUBE3		
	SEMA7A		
	SLFN5		
	TACSTD2		
	TM4SF1		

LIGHT-induced transcription of 47 genes was lost in RelB null cells compared to control. Of these, approximately 50% were rescued in P364L expressing fibroblasts, either partially or fully. Unique loss of ERRFI1 induction was observed in P364L cells.

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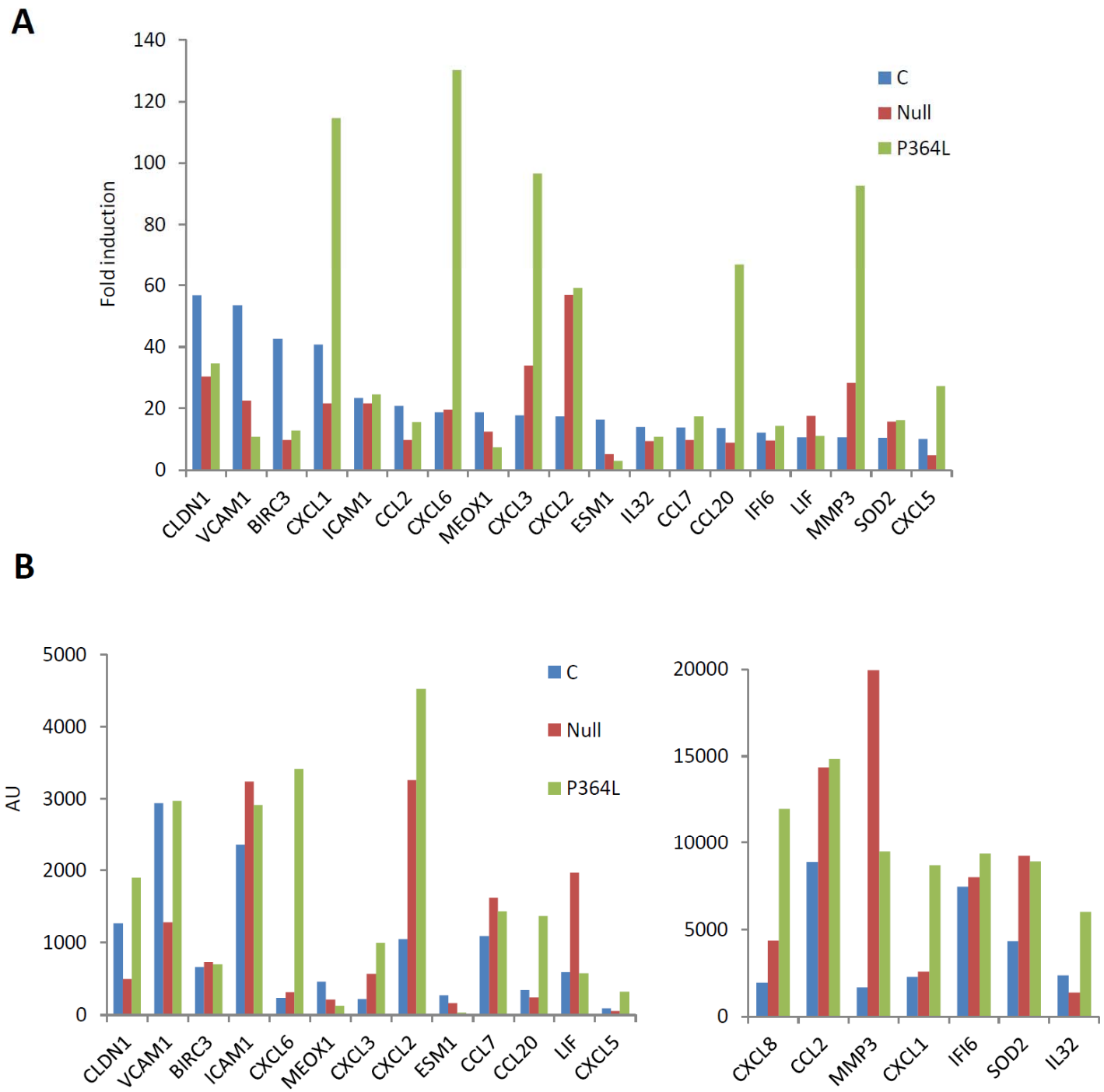
Figure E3



(A) Most of the twenty most strongly induced genes found in normal control fibroblasts were also induced strongly by LIGHT in RelB deficient cells, both null and P362L RelB. P364L RelB cells displayed significant hyper-induction of a number of these genes. **(B)** Post stimulation level of gene message (left and right panels).

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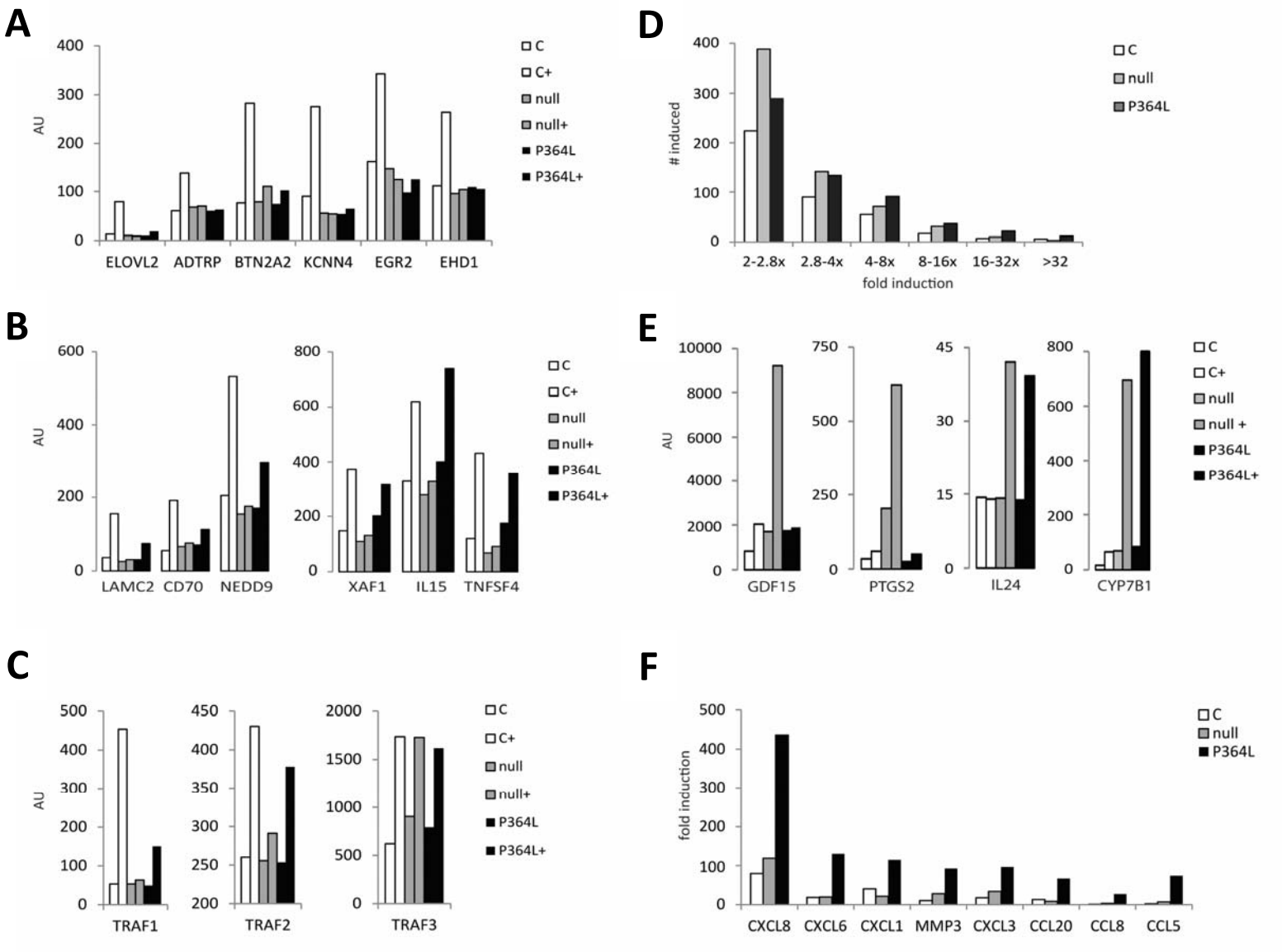
Figure E4



Aberrant transcriptional responses to LIGHT. (A) Examples of loss of LIGHT-induced transcription in P364L RelB and null RelB fibroblasts. Cells were stimulated with 200ng/ml LIGHT for 8 hours and RNA analysed by GeneChip PrimeView Human Gene Expression microarray. AU: arbitrary units. Complete list of loss in Online Repository - Fig E2. **(B)** Examples of partial and complete responses in P364L RelB cells of genes failing to undergo transcriptional upregulation in RelB null cells (see also Online Repository - Fig E2). **(C)** Loss of TRAF1 and TRAF2 transcript induction in RelB null fibroblasts, but only TRAF1 in P364L cells, with normal TRAF3 transcription in both. **(D)** Number of genes with LIGHT-induced transcription in control and patient fibroblasts. **(E)** Examples of novel LIGHT-induced transcription in null RelB only (left two panels) or null and P364L RelB (right panels). **(F)** Examples of hyper-induced cytokine and chemokine gene transcription in P364L RelB cells compared to normal and null RelB.