



Figure S2: T cells express an extranuclear isoform of RARa (Related to Figure 2)



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Exon4 ATTTGCCTGTCGGGGCCTCCCGAGAAGGAAGTGAG Exon5

ATTTGCCTGTCGGGGGCCTCCCGAGAAGGAAGTGAGGTGAAAACTGGGGGCA GGGCAGTTGTGTGTGGGCATGAGCAGCAGATTGCAGCAG Exon6

CCGCAGGAGTCTTTCACAGCCTGGCATAACACATGCTGTTGGATGCTTGAG CACAGGAACCGTGTATATCCAGCACTCAACTGG

Exon7 ATTIGCCTGTCGGGGCCTCCCGAGAAGGAAGTGAGGTGAAAACTGGGGCA GGCAGTTGTGTGAGCATGAGCAGCAGCAGTGCAGCAGGTCAGTCCTTTG GACCCCTGGGGGTTGTAGGCATGGACAACGTCCTTTGCACGCGGGGTTGAAGAA GGGAACATGGGTTGAAGCAACGTTGGACAGCCCGGCTGATGTCCAGGCC AAGTAGAAGCCAGGAAAGTCCTGCCAAGGCCATTGGCGGAGTTCCCTGGT GTGTTTGCAG

Exon8

ATTTGCCTGTCGGGGgCCTCCCCGAGAAGGAAGTGAGGTGAAAACTGGGGC AGGGCAGTTGTGTGTAGGCATGAGCAGCAGATTGCAGCAGGTCAGTCCTTT GGACCCCTGGGGGTTGTAGGCATGGAAAAGTCCTTTTCCATGGGTTAAAGA AGGGAACATGGGTTaCAGCAACGTTGGACAGCCCGGCTGATGTCCAGGCC CAAGTAGAAGCCAGGAAAGTCCTGCCAAGGCCATTGGCGGAGTTCCCTG

Exon8.1 ATTTGCCTGTCGGGGCCTCCCGAGAAGGAAGTGAGGTGAAAACTGGGGC AGGGCAGTTGTGTGTGGGCATGAGCAGCAGATTGCAGCAGGTCAGTCCTT TGGACCCCTGGGGGTTGTAGGCATGGAAAAGTCCTTTTCCATGGGTTAAAG AAGGAACATGGGTTACASCAACGTTGGACAGCCCGGCTGATGTCCAGGC CCAAGTAGAACCCGGAAAGTCCTGCCAAGGCCCGGCTGATGTCCCAGGC GTGTGTTTGCAGGTAGGAGGGTGGTAGTATGCAACCAGGCTCCTGTGTCCC CAAGTGTCCTAACCTCTCTGCCTGGGCTTTGCAGCCGCAGGAGTCTTTCAC AGCCTGGCATAACACATGCTGTTGGATGCTTGAGCACAGGAACCGTGTATAT CCAGCACTCAACTGG





D		10	20	30	40	50	60	70	80	90
hRARα1 mRARα1	1 MASNSSSC MASNSSSC	PTPGGGHLNG PTPGGGHLNG	YPVPPYAFFFI YPVPPYAFFFI	PMLGGLSPPO	GALTTLQHQLI GALTSLQHQLI	PVSGYSTPSP PVSGYSTPSP	ATIETQSSSS: ATIETQSSSS:	EEIVPSPPSP EEIVPSPPSP	PPLPRIYKPC PPLPRIYKPC	FVCQDKSSGYHY 100 FVCQDKSSGYHY
		110	120	130	140	150	160	170	180	190
hRARα1 : mRARα1 mRARα3	01 GVSACEGC GVSACEGC	KGFFRRSIQKI KGFFRRSIQKI DNA bi	MVYTCHRDKN MVYTCHRDKN MVYTCHRDKN	ICIINKVTRNE ICIINKVTRNE ICIINKVTRNE ain	RCQYCRLQKCI RCQYCRLQKCI RCQYCRLQKCI	EVGMSKESV DVGMSKESV DVGMSKESV	RNDRNKKKE RNDRNKKKKE RNDRNKKKE	VPKPECSESY APKPECSESY APKPECSESY	TLTPEVGELI TLTPEVGELI TLTPEVGELI	EKVRKAHQETFP 200 EKVRKAHQETFP EKVRKAHQETFP 80
		210	220	230	240	250	260	270	280	290
hRARα1 mRARα1 mRARα3	201 ALCQLGKY 81 ALCQLGKY	TTNNSSEQRV TTNNSSEQRV TTNNSSEQRV	SLDIDLWDKFS SLDIDLWDKFS SLDIDLWDKFS	ELSTKCIIK ELSTKCIIK ELSTKCIIK	IVEFAKQLPGI IVEFAKQLPGI IVEFAKQLPGI	FTTLTIADQI FTTLTIADQI FTTLTIADQI	TLLKAACLDI TLLKAACLDI TLLKAACLDI	LILRICTRYT LILRICTRYT LILRICTRYT	PEQDTMTFSD PEQDTMTFSD PEQDTMTFSD	GLTLNRTOMHNA 300 GLTLNRTOMHNA GLTLNRTOMHNA 180
Ligand binding domain										
		310	320	330	340	350	360	370	380	390
hRARα1 30 mRARα1 mRARα3 ¹⁸	301 GFGPLTDL GFGPLTDL 181 GFGPLTDL	VFAFANQLLP VFAFANQLLP VFAFANQLLP	LEMDDAETGLI LEMDDAETGLI LEMDDAETGLI	SAICLICGDE	RQDLEQPDRVI RQDLEQPDKVI RQDLEQPDKVI	OMLQEPLLEA OMLQEPLLEA OMLQEPLLEA	LKVYVRKRRP LKVYVRKRRP LKVYVRKRRP	SRPHMFPKML SRPHMFPKML SRPHMFPKML	MKITDLRSIS MKITDLRSIS MKITDLRSIS	AKGAERVITLKM 400 AKGAERVITLKM 280
		410	420	430	440 GAAS	450	460			
hRARa1 mRARa1 mRARa3	01 EIPGSMPPLIQEMLENSEGLDTLSGQFGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG									
					F-doma	in				

Figure S3: Extranuclear RAR α controls TCR signal transduction (Related to Figure 3)





Figure S4: RA counteracts extranuclear RARα signaling in T cells (Related to Figure 5)

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Figure S5: Extranuclear RAR α signaling controls TCR-induced proliferation *in vivo* (Related to Figure 6)



Figure S6: Extranuclear RAR α signaling controls effector differentiation *in vivo* (Related to Figure 7)



SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1: RA enhances Treg cell conversion independently of nuclear RARα (Related to Figure 1)

(**A**) Gating strategy for analysis of FOXP3 expression among WT CD4 T cells stimulated for 96hrs with α -CD3/CD28 with or without TGF- β or RA. (**B**) Cell surface expression for CCR9 on WT, DNRAR α or nRAR α -deficient naïve spleen CD4 T cells stimulated for 16hrs *in vitro* with 1.0 µg/ml of plate-bound α -CD3 and 0.5 µg/ml of α -CD28, then cells were washed and cultured 48 more hours (64hrs in total) in cRPMI supplemented with 25 ng/ml of IL-2 with or without RA (10nM). Representative histograms of 2 independent experiments are shown. Statistical analyses of frequency of CCR9⁺ T cells on the right was calculated with the two-tailed Student t-test. Mean +/- SEM is depicted.

FIGURE S2: T cells express an extranuclear isoform of RARα (Related to Figure 2)

(A) Heatmap for junction-use in spleen and DP thymocytes calculated and drawn by SGseq package on R. (B) PCR analysis performed on cDNA from DP thymocytes using forward primers F1 and F2 in combination with reverse R primers as shown. F1+R9 and F2+R9 detect *Rara1* and *Rara2*, respectively. (C) DNA sequences of new exons as indicated. Shared sequence between exons 6 and 8.1 is in red. (D) Amino acid sequences of human and mouse RAR α 1 and mouse RAR α 3 are aligned. Lighter colored amino acids indicate mismatches between human and mouse. The first methionine used by mRAR α 3 is in red in the sequence of hRAR α 1 and mRAR α 1. The amino acid sequences shared by hRAR α 1 or mRAR α 1 and mRAR α 3 are in bold. Sequence encoding the DNA Binding Domain (brown), the Ligand Binding Domain (green) and the F Domain (blue) are indicated. The numbers indicate amino acid number. Serine (S445) in the F Domain phosphorylated upon TCR stimulation is marked.

FIGURE S3: Extranuclear RAR α controls TCR signal transduction (Related to Figure 3)

(**A**) Primary spleen T cells were treated with DMSO, a pan-RAR antagonist LE540 (iRARs), an RAR α -specific antagonist, Ro 41-5253 (iRAR α) or an RAR γ antagonist, MM11253 (iRAR γ) and activated with anti-CD3/CD28 in the presence of (10nM) RA for 72hrs. CCR9 expression was then assessed. Dot plots are from a representative experiment. Graphs represent the means +/- SEM of CCR9⁺ T cells from 3 independent experiments. (**B**) Primary spleen T cells unstimulated or activated for 24hrs with anti-CD3/CD28 alone or with γ -secretase inhibitor (GSI) were analyzed with western blot for cleaved NOTCH1-intracellular domain with the quantification of its optical density relative to control HSP90. Representative western blot from 2 independent experiments. (**C**) Primary spleen T cells treated with DMSO or iRARs and activated with anti-CD3/CD28 for 4hrs were analyzed for the expression of c-MYC protein. Dot plots are from a representative experiments.

FIGURE S4: RA counteracts extranuclear RAR α signaling in T cells (Related to Figure 5)

(A) WT or CRABP2-deficient spleen T cells were activated with α -CD3/CD28 in the presence of RA (10nM) for 72hrs. CCR9 expression was then assessed. Dot plots show

one representative experiment out of three independent experiments. **(B)** Thymocytes isolated from 5 (10week-old and gender matched) WT and CRABP2-deficient mice were analyzed for phenotype, frequency and absolute number. Data are represented as the means +/- SEM.

FIGURE S5: Extranuclear RAR α signaling controls TCR-induced proliferation *in vivo* (Related to Figure 6)

Tracking of endogenous OVA-reactive CD8 T cells in the spleen of WT CD45.1⁺CD45.2⁺ recipient mice with Vitamin A or Vitamin A-free, infected i.v. with ActA⁻ Lm-OVA and assessed 7 days later. Graphs show the percentage and absolute number of OVA-reactive CD8 T cells among the total CD8 T cells.

FIGURE S6: Extranuclear RAR α signaling controls effector differentiation *in vivo*

(Related to Figure 7)

EAE was induced in WT and *Crabp2* conditional deletion mutant mice and cells from spinal cords were analyzed by FACs at day25 of EAE induction. (**A**) Clinical scores were assessed daily. (**B**) Gating strategy for live TCR β and CD45 positive cells with a representative dot plot for FOXP3 and ROR_Yt expressions. (**C**) Quantifications of FOXP3⁺ROR_Yt⁻ Treg and FOXP3⁺ROR_Yt⁺ cell frequencies and FOXP3 mean fluorescence intensity (MFI) among CD4 T cells. (**D**) Frequency of IFN_Y⁺ and ROR_Yt⁺ (upper) or IL17A⁺(lower) cells are shown in representative dot plots. Data are representative of 2 independent EAE experiments. Statistical significance calculated with two-tailed Student t-test. Shown mean +/- SD.