#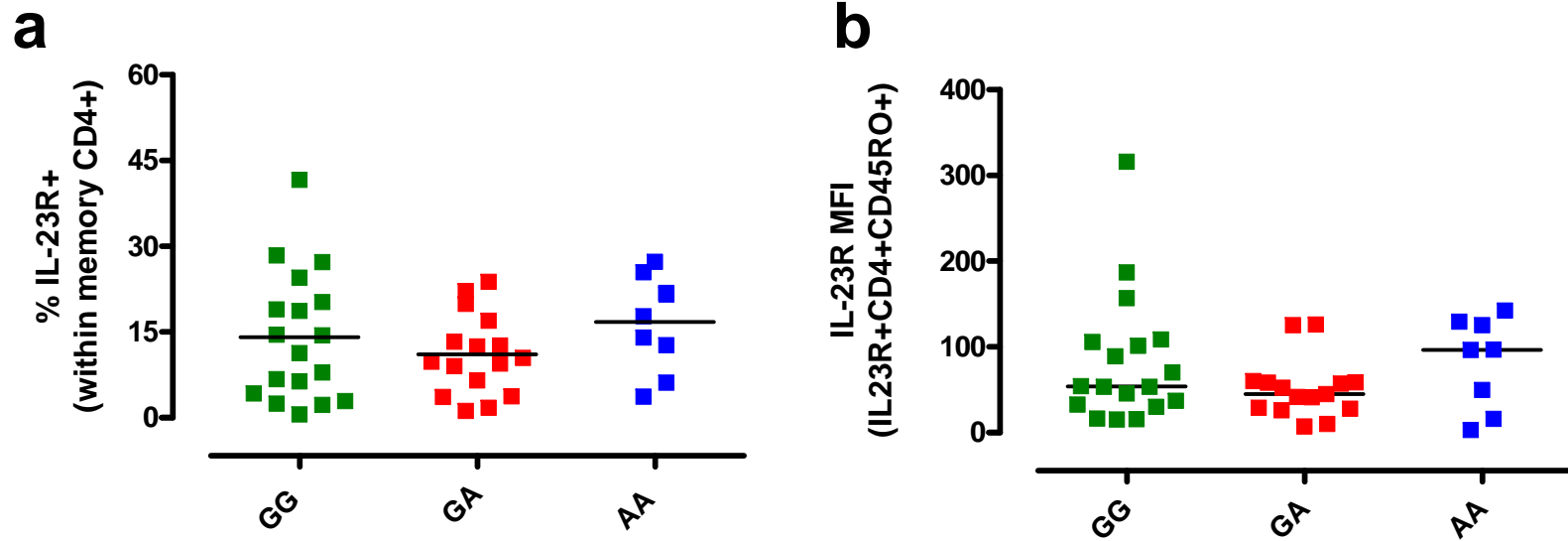 The IL23R A/Gln381 allele promotes IL-23 unresponsiveness in human memory T helper17 cells and impairs Th17 responses in psoriasis patients

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Supplemental Materials

Figure S1

IL23R A/Gln381 heterozygous and homozygous carriers have normal frequency of IL-23R+ memory T cells and IL-23R surface expression

a) Frequency of IL-23R+ cells within memory CD4+ T cells in healthy individuals homozygous for the common G allele (GG, green squares), heterozygous GA (red squares), or homozygous for the protective A allele (AA, blue squares).

b) IL23R Median Fluorescent Intensity (MFI) within IL-23R$^+$ memory T cells in GG (green), GA (red) and AA (blue) healthy individuals. Each symbol represents one individual donor. Horizontal bars represent mean (a) or median (b). One-way ANOVA, followed by Bonferroni post test (a) or Kruskal-Wallis, followed by Dunn's Multiple Comparison test (b) was performed yielding P>0.05 for all comparisons.
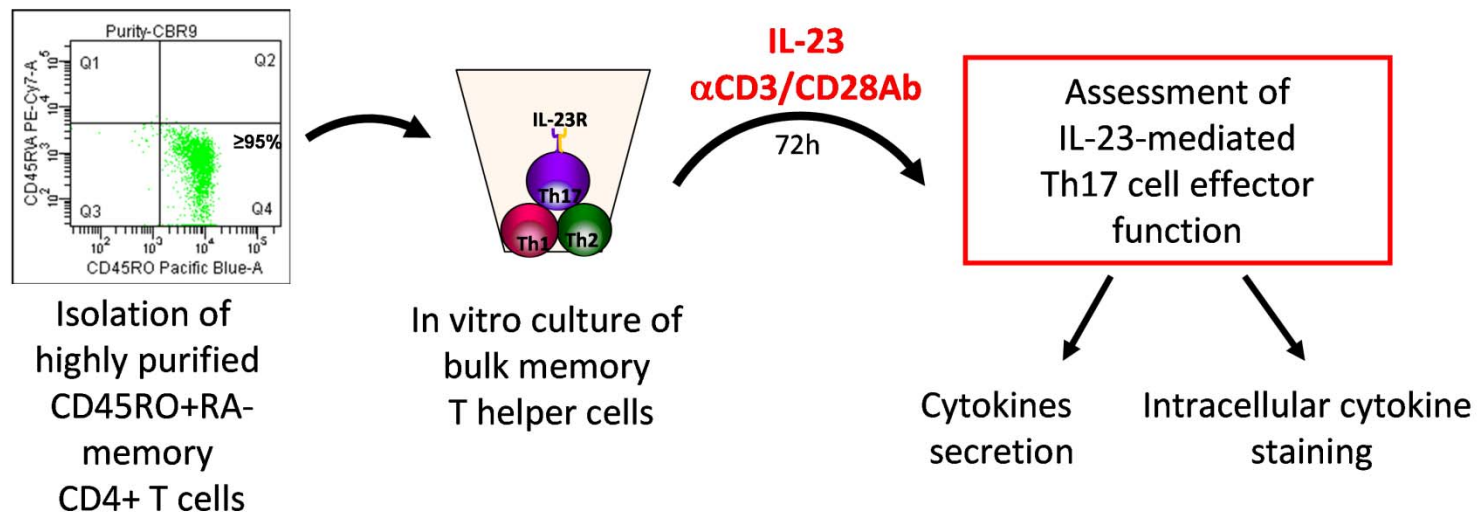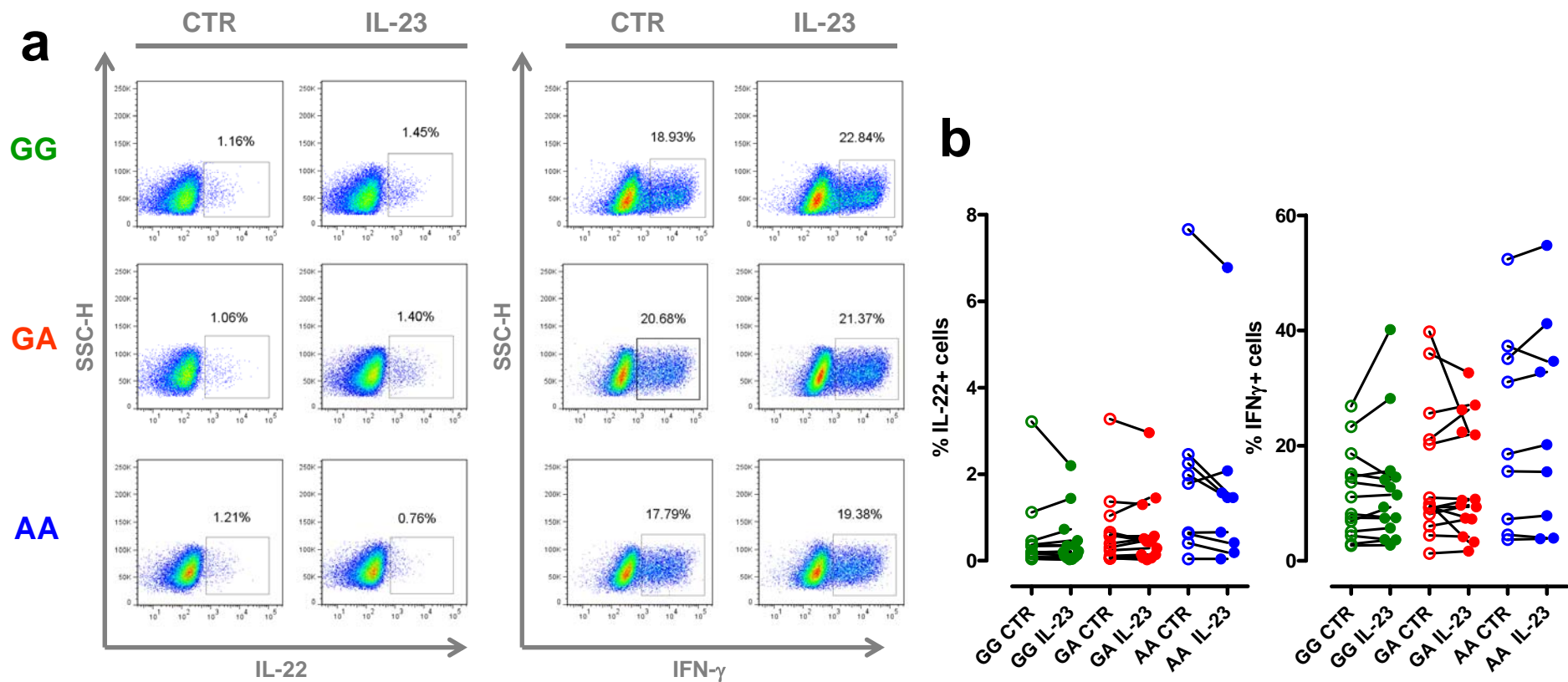
Figure S2
Memory cell experimental layout
Memory (CD4+CD45RO+RA-) T cells were obtained by incubation with Rosette Sep Human CD4+T cells enrichment cocktail followed by centrifugation through Lymphoprep and further purification by negative selection using magnetic beads and anti-CD45RA. Purity of CD45RO+CD45RA- T cells was checked by FACS and considered acceptable when over 95%. To assess IL-23-mediated Th17 cell effector function, cells were stimulated in duplicate with anti CD3/CD28 beads (1 bead/10 cells), with or without IL-23 (100 ng/ml) for 72 hours. Thereafter, cells were spun down and cell supernatants were taken to measure cytokine secretion. Cells from each condition were pulled together, fresh complete medium was added and cells were stimulated for intracellular cytokine staining with PMA (10 ng/ml) and Ionomycin (500 ng/ml) in the presence of monensin (3μM) and Brefeldin A (10 ng/ml) for 6 hours.
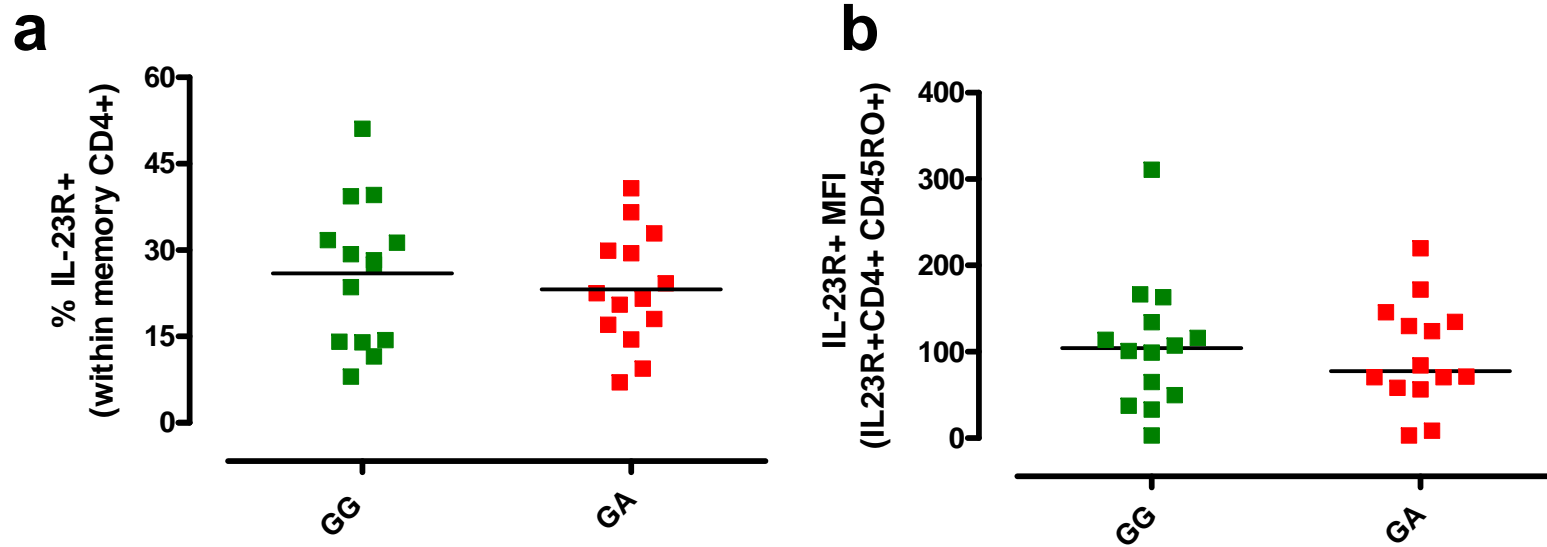
Figure S3

IL23R A/Gln381 heterozygous psoriasis patients have normal frequency of IL-23R+ memory T cells and IL-23R surface expression

a) Frequency of IL-23R+ cells within memory CD4+ T cells in psoriasis patients homozygous for the common G allele (GG, green squares) and heterozygous for the protective A allele (GA, red squares). **b**) IL-23R Median Fluorescent Intensity (MFI) within IL23R+ memory T cells in GG (green), GA (red) psoriasis patients. Each symbol represents one individual donor. Horizontal bars represent mean (a) or median (b). Unpaired t test (a) or Mann Whitney (b) was performed yielding P>0.05 for all comparisons.

Figure S4

Effect of IL23R A/Glu381 allele on IL-23-driven survival/expansion of human memory Th cells

Memory CD4+ T cells from healthy individuals homozygous for the common G allele (GG, green), heterozygous GA (red), or homozygous for the protective A allele (AA, blue), cultured with or without IL-23 for 72h, were assayed for intracellular cytokines IFN-γ and IL-22, following PMA/Ionomycin stimulation for 6 h. **a**) Representative dot plots of GG, GA and AA individual donors. **b**) Each connected symbol represents paired samples from one individual donor. Wilcoxon signed rank test was performed. P>0.05 for all paired comparisons
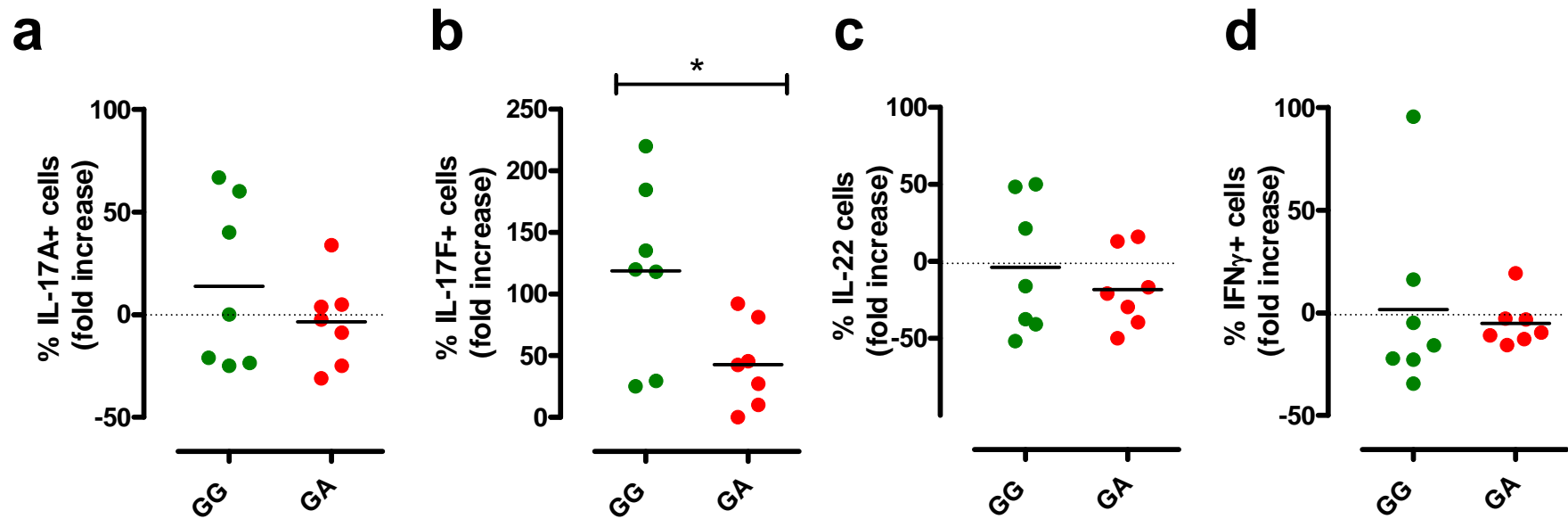
Figure S5

IL23R A/Glu381 allele impairs IL-23-driven survival/expansion of human memory T helper 17 cells from psoriatic patients

Memory CD4+ T cells from psoriasis patients homozygous for the common G allele (GG, green dots) or heterozygous for the protective A allele GA (red dots) cultured with or without IL-23 for 72h, were assayed for intracellular cytokines IL-17A (**a**), IL-17F (**b**), IL-22 (**c**) and IFN-γ (**d**), following PMA/Ionomycin stimulation for 6 h. Data are shown as fold increase in frequency of cytokines producing cells in the presence of IL-23. Each dots represents an individual donor. Mann Whitney test was performed, *P <0.05. P>0.05 for all other comparisons.

Donors	Genotype		
	GG	**GA**	**AA**
Healthy volunteers	18[1]	18[2]	9
mean age (range) *yrs*	42 (24-66)	42 (26-67)	47 (26-46)
males	8	8	6
females	10	10	3
Psoriatic patients	40	18	0
mean age (range) *yrs*	44 (25-76)	43 (30-62)	-
males	30	14	-
females	10	4	-
			-
Psoriatic Arthritis[3]	6	5	

Table S1
Demographics of healthy donors and psoriasis patients used in functional experiments
[1] of which 3 used in Di Meglio et al., PLoS ONE 2011
[2] of which 4 used in Di Meglio et al., PLoS ONE 2011
[3] Info available only for 35 GG and 16 GA

Genotype	ID	Gender	Age *yrs*	Treatment
GG	P1	M	62	etanercept
	P2	M	54	methotrexate + etanercept
	P3	M	50	topical
	P4	F	35	topical
	P5	M	40	topical
	P6	M	55	etanercept
	P7	F	43	fumaric acid ester
	P8	M	29	retinoid
	P9	M	32	cyclosporine
	P10	M	28	adalimumab
	P11	M	66	methotrexate
	P12	M	39	cyclosporine
	P13	F	65	retinoid
	P14	M	36	phototherapy
GA	P15	M	58	etanercept
	P16	M	61	methotrexate + etanercept
	P17	M	56	topical
	P18	F	30	topical
	P19	M	44	topical
	P20	M	48	etanercept
	P21	F	54	fumaric acid ester
	P22	F	34	retinoid
	P23	M	35	cyclosporine
	P24	M	29	etanercept
	P25	M	63	methotrexate
	P26	M	44	cyclosporine
	P27	M	54	retinoid
	P28	M	35	phototherapy

Table S2

Demographics of psoriasis patients used in PBMCs experiments (Fig.4c)

Genotype	ID	Gender	Age *yrs*	Treatment
GG	P9	M	32	cyclosporine
	P29	F	43	methotrexate
	P30	M	42	methotrexate
	P31	M	55	methotrexate
	P32	F	57	infliximab
	P33	M	54	etanercept
	P34	M	50	methotrexate + adalimumab
GA	P15	M	58	etanercept
	P16	M	61	methotrexate + etanercept
	P22[1]	F	37	methotrexate
	P23	M	35	cyclosporine
	P25	M	63	methotrexate
	P35	M	35	methotrexate
	P36	F	37	adalimumab

Table S3

Demographics of psoriasis patients used in memory T cells experiments (Fig. 5a)

[1] Different collection as compared to table S2

Genotype	ID	Gender	Age	Treatment
GG	P2	M	54	methotrexate + etanercept
	P10[1]	M	27	etanercept
	P30[2]	M	39	methotrexate
	P31[2]	M	51	topical
	P34	M	50	methotrexate + adalimumab
	P37	M	30	topical
	P38	M	32	none
	P39	M	37	none
	P40	M	42	none
	P41	M	37	none
	P42	F	72	methotrexate
	P43	M	43	adalimumab
	P44	M	42	none
	P45	M	74	methotrexate
	P46	F	30	topical
	P47	F	76	none
	P48	F	25	none
	P49	M	36	none
	P50	M	40	topical
	P51	M	39	topical
	P52	F	29	topical
	P53	M	41	topical
	P54	M	29	none
	P55	M	49	topical
	P56	M	NA	none
GA	P16	M	61	methotrexate + etanercept
	P20	M	48	etanercept
	P21[1]	F	56	adalimumab
	P25[1,2]	M	60	topical
	P26	M	44	cyclosporine
	P57	M	34	topical
	P58	M	34	topical

Table S4

Demographics of psoriasis patients used in skin cytokines mRNA correlation analysis (Fig. 5b)

[1] Different collection as compared to Table S2   NA= Not Available

[2] Different collection as compared to Table S3

Antigen	Fluorochrome	Company
CD3	PE-Cy5	BioLegend (San Diego, CA)
CD4	Alexa Fluor 700	BioLegend (San Diego, CA)
CD45RO	Pacific Blue	BioLegend (San Diego, CA)
IFN-g	PE-Cy7	BioLegend (San Diego, CA)
IFN-g	Alexa Fluor 700	BioLegend (San Diego, CA)
IL-17A	FITC	eBioscence (San Diego, CA)
IL-17F	PerCP e-Fluor 710	eBioscence (San Diego, CA)
IL-22	Alexa 647	eBioscence (San Diego, CA)
IL-23R	biotynilated	R&D Systems (Minneapolis, MN)
Secondary reagent	Fluorochrome	Company
Streptavidin	APC-Cy7	BD Biosciences (San Jose, CA)

Table S5
Antibodies used in multiparameter flow cytometry experiments