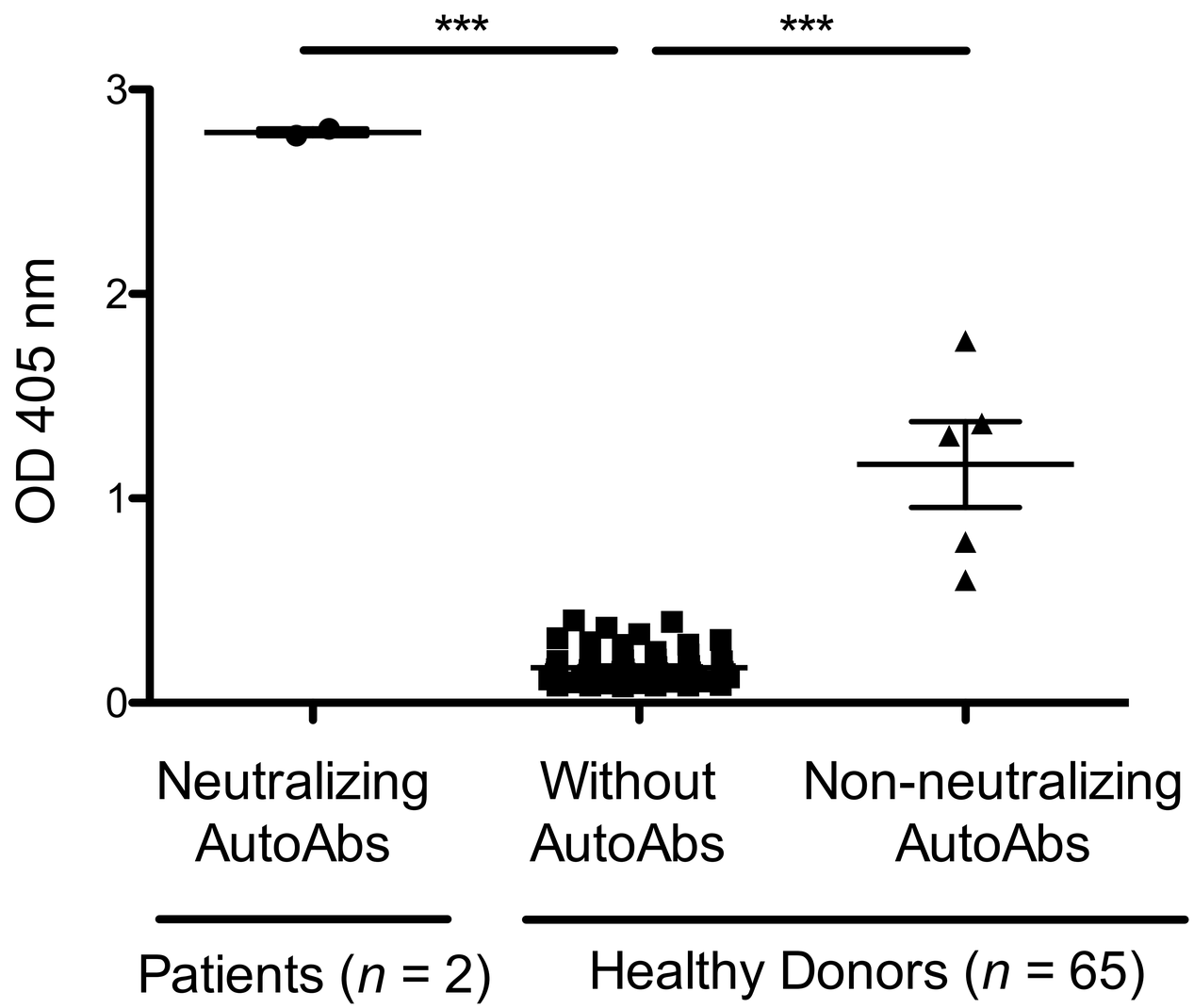
Supplementary Figure 1

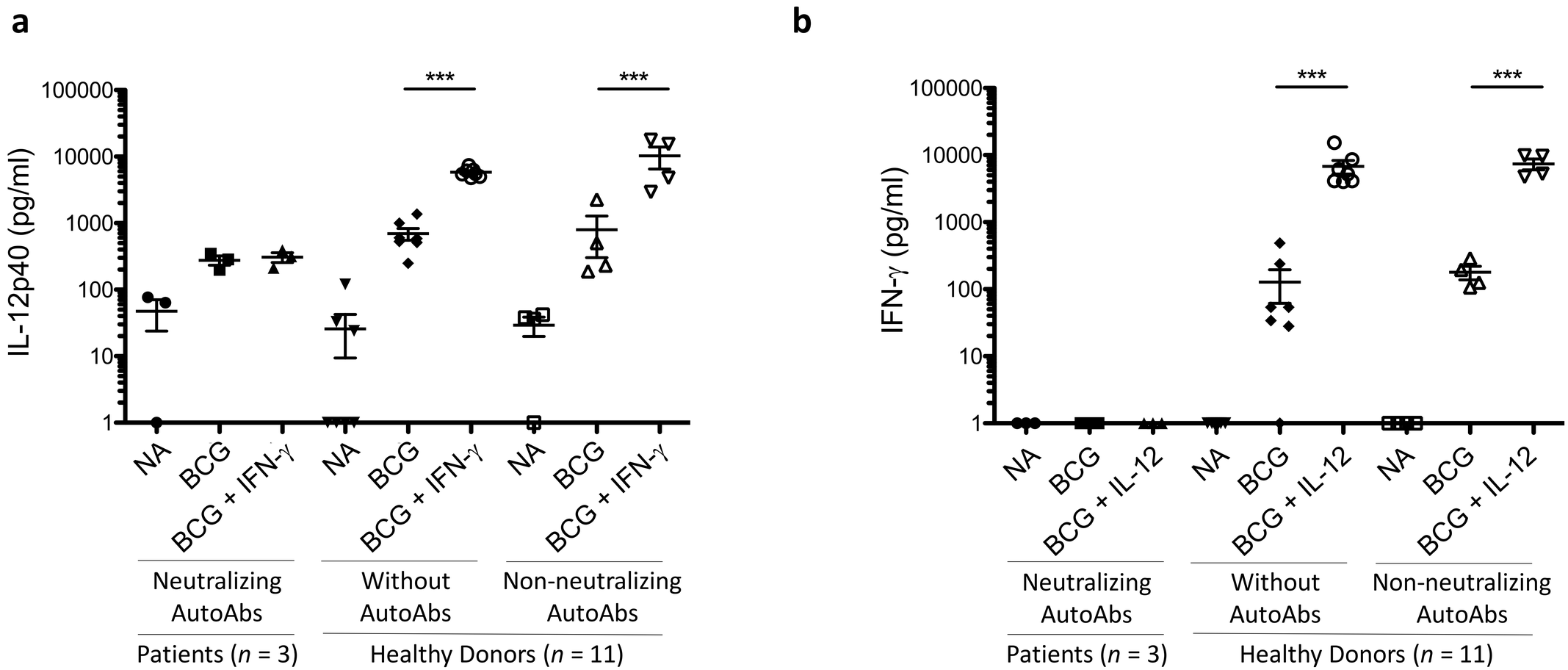


Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 1. Direct IFN-γ ELISA used to detect AutoAbs against IFN-γ. The data are expressed as the mean optical density (OD) at 405 nm; plasma samples from IFN-γ AutoAbs patients (n = 2), and healthy controls (n = 65) were used. Individual data points are shown, together with the mean and s.e.m. of each group in a representative experiment. ***P < 0.001 in one-way ANOVA with a post-hoc Tukey's test.

Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 2

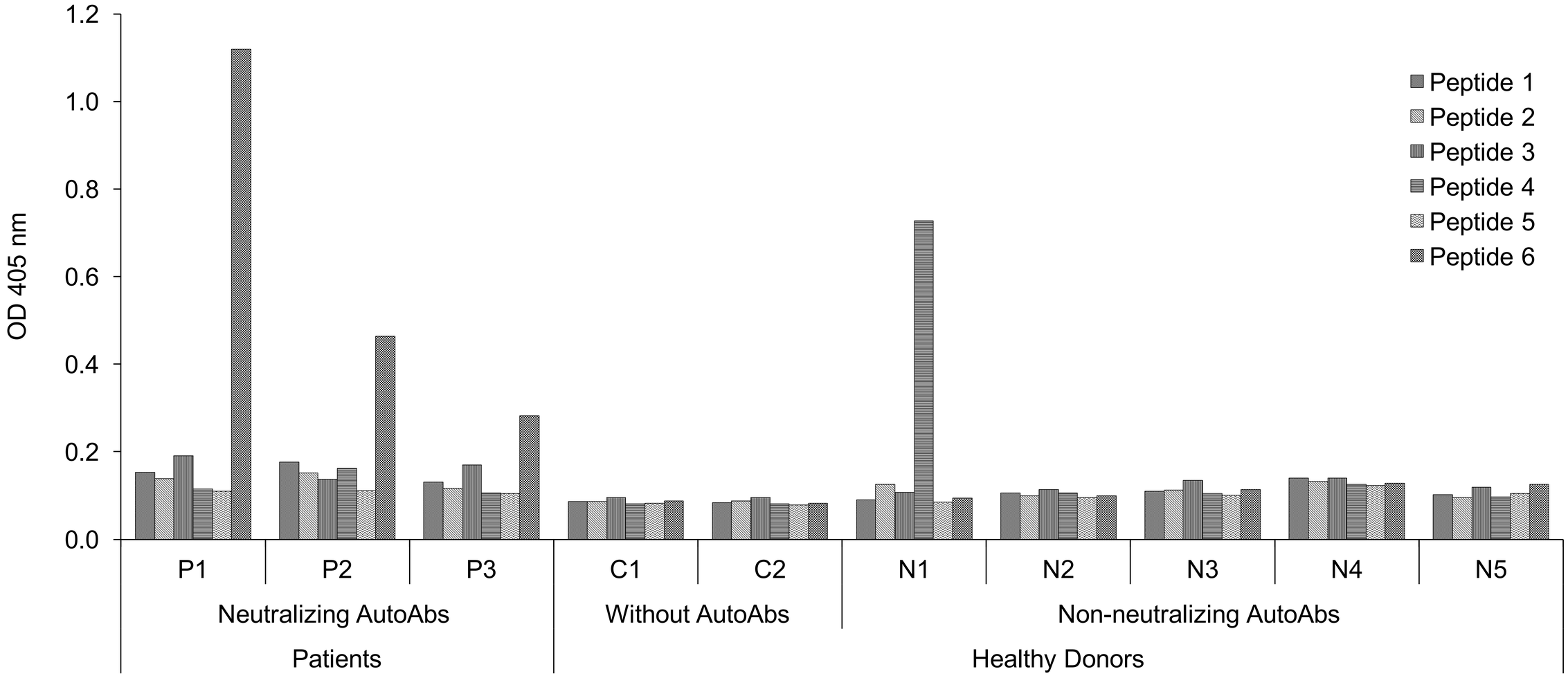


Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 2. Non-neutralizing AutoAbs against IFN-γ did not affect the ability of IFN-γ to induce IL-12 production. (**a**) IL-12p40 production levels were determined by ELISA in the absence of activation, or after activation with BCG or BCG plus IFN-γ, in whole blood from individuals from three groups: IFN-γ AutoAbs patients (n = 3), healthy controls without AutoAbs (n = 7), and healthy controls with non-neutralizing IFN-γ AutoAbs (n = 4). (b) IFN-γ production levels were determined by ELISA in the absence of activation or after activation with BCG or BCG plus IL-12 in whole blood from individuals from three groups: IFN-γ AutoAbs patients (n = 3), healthy controls without AutoAbs (n = 7), and healthy controls with non-neutralizing IFN-γ AutoAbs (n = 4). Individual data points are shown, together with mean and s.e.m. values for each group in a representative experiment. ***$P < 0.001$ in one-way ANOVA with a post-hoc Tukey's test.

Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 3

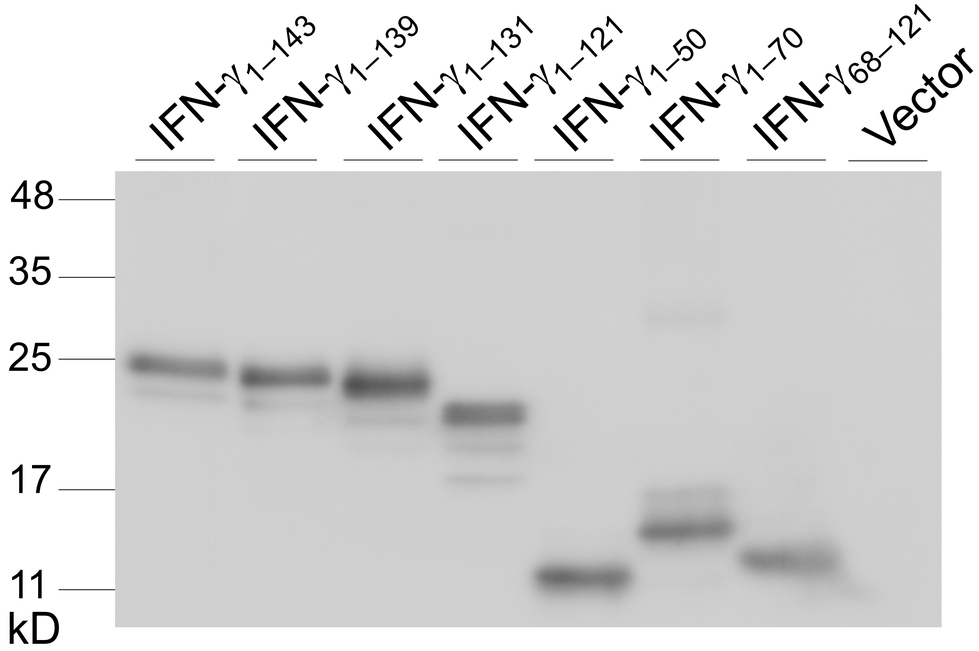


Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 3. Epitope mapping to determine the affinity, in plasma, of synthetic peptide binding to IFN-γ. Epitope mapping to assess the binding affinity of the synthetic peptide for IFN-γ, presented as the mean optical density (OD) at 405 nm; we used plasma samples from IFN-γ AutoAbs patients (*n* = 3, P1–3), healthy controls without AutoAbs (*n* = 2, C1 and C2), and healthy controls with non-neutralizing IFN-γ AutoAbs (*n* = 5, N1–5)

Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 4

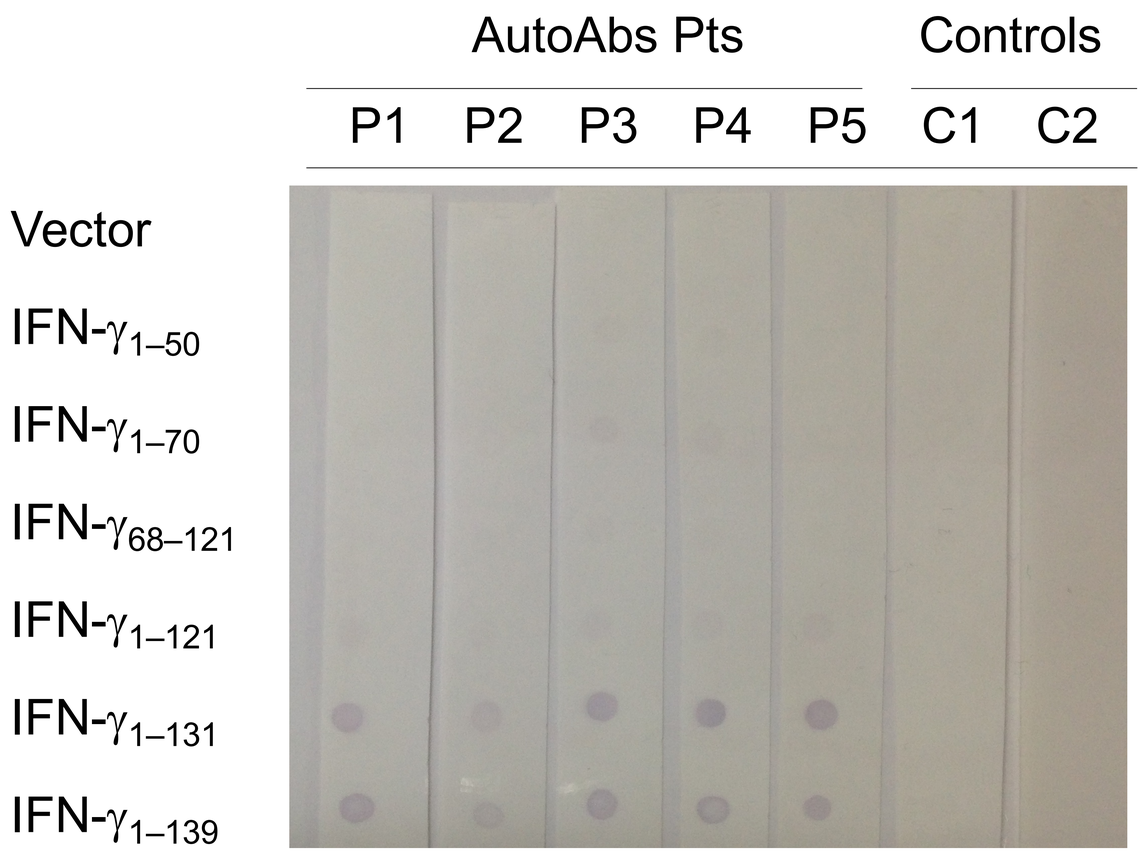


Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 4. Expression of different forms of recombinant IFN-γ. Western blot analysis showed the expression of different forms of recombinant IFN-γ detected with a V5-tagged antibody.

Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 5

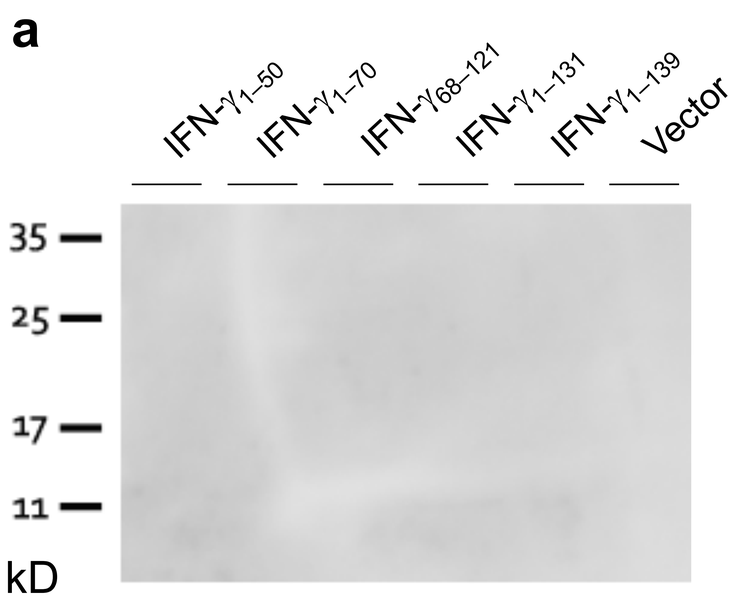


Nature Medicine: doi:10.1038/nm.4158

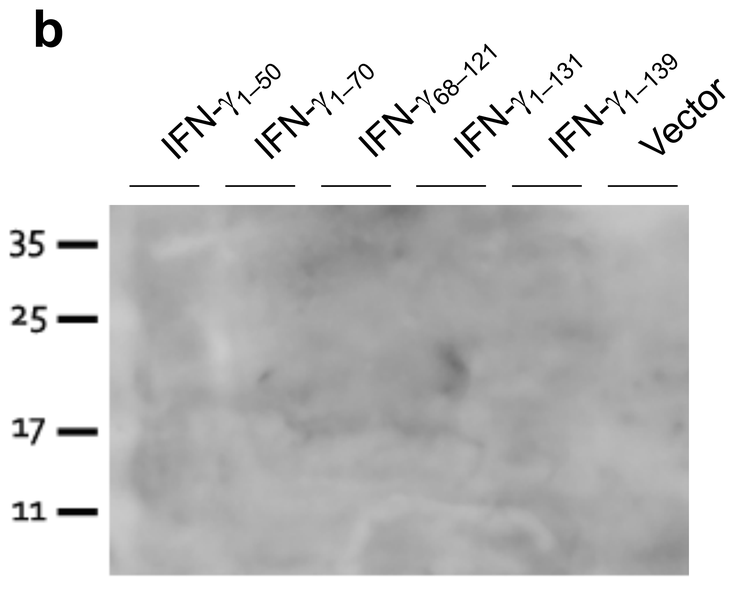
Supplementary Figure 5. Dot blot to assess the affinity of binding, in plasma, of the recombinant proteins to IFN-γ. Plasma samples from randomly selected IFN-γ AutoAbs patients (n = 5, P1–5) and healthy controls (n = 2, C1 and C2) were probed for the control vector and IFN-$\gamma_{1–50}$, IFN-$\gamma_{1–70}$, IFN-$\gamma_{68–121}$, IFN-$\gamma_{1–121}$, IFN-$\gamma_{1–131}$, and IFN-$\gamma_{1–139}$ recombinant proteins.

Nature Medicine: doi:10.1038/nm.4158

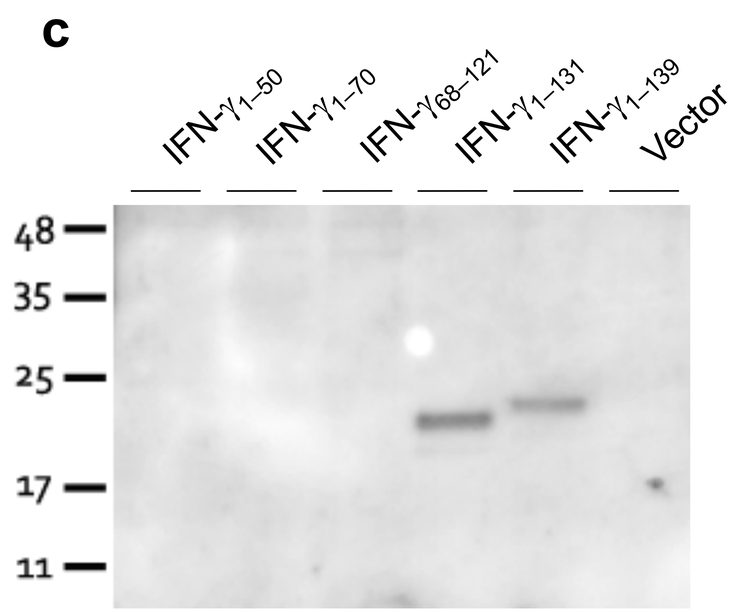
Supplementary Figure 6



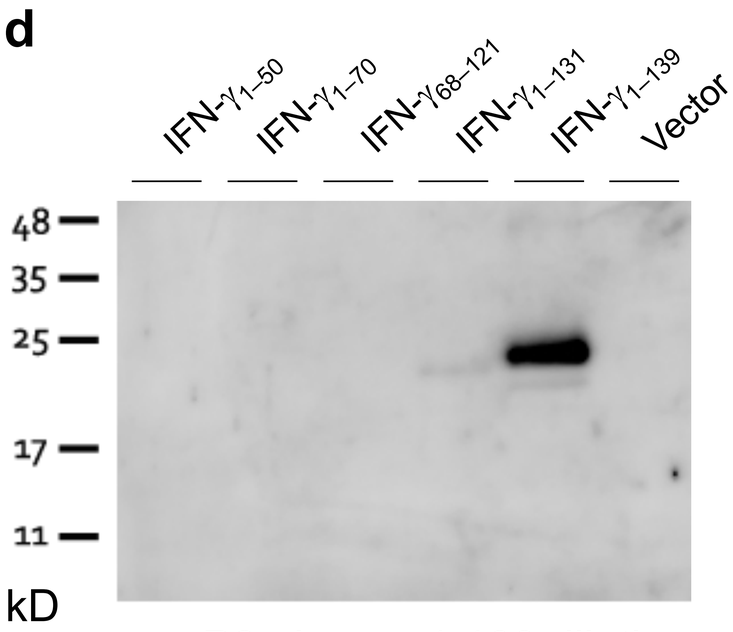
C1 plasma 1:100 dilution



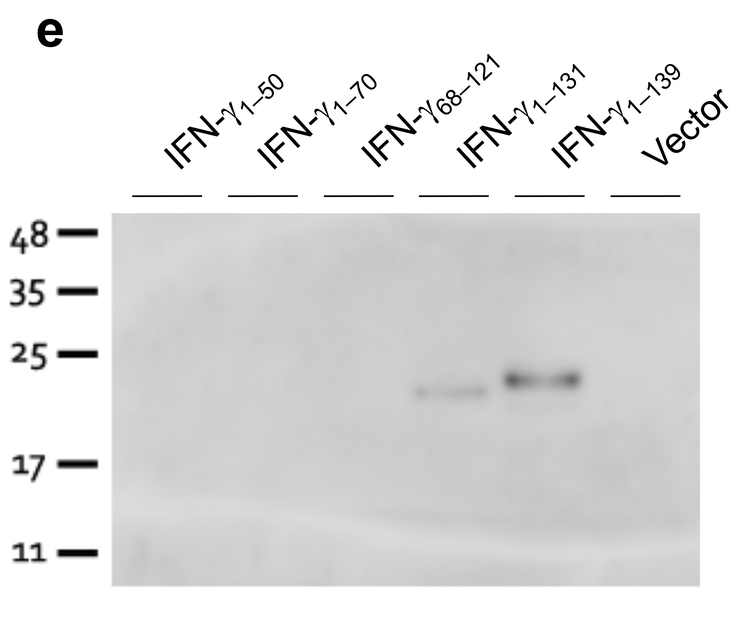
C2 plasma 1:100 dilution



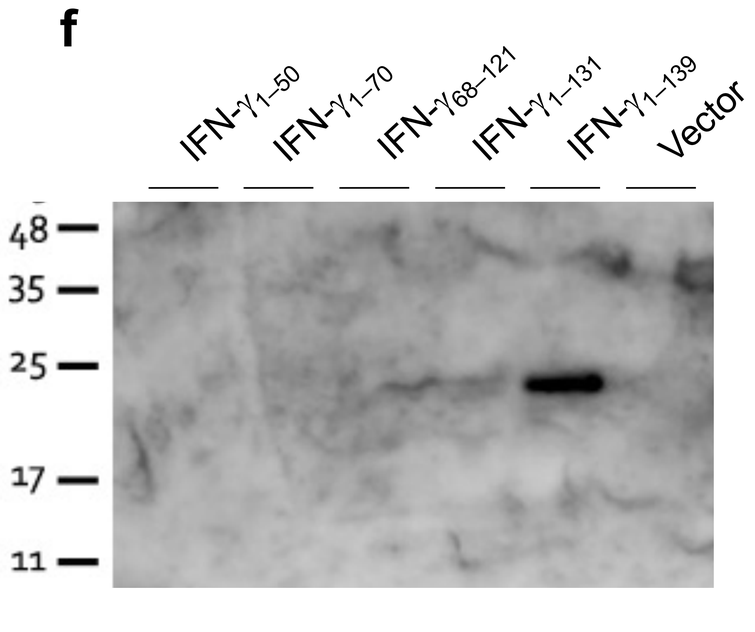
P1 plasma 1:100 dilution



P2 plasma 1:100 dilution



P3 plasma 1:100 dilution



P4 plasma 1:100 dilution

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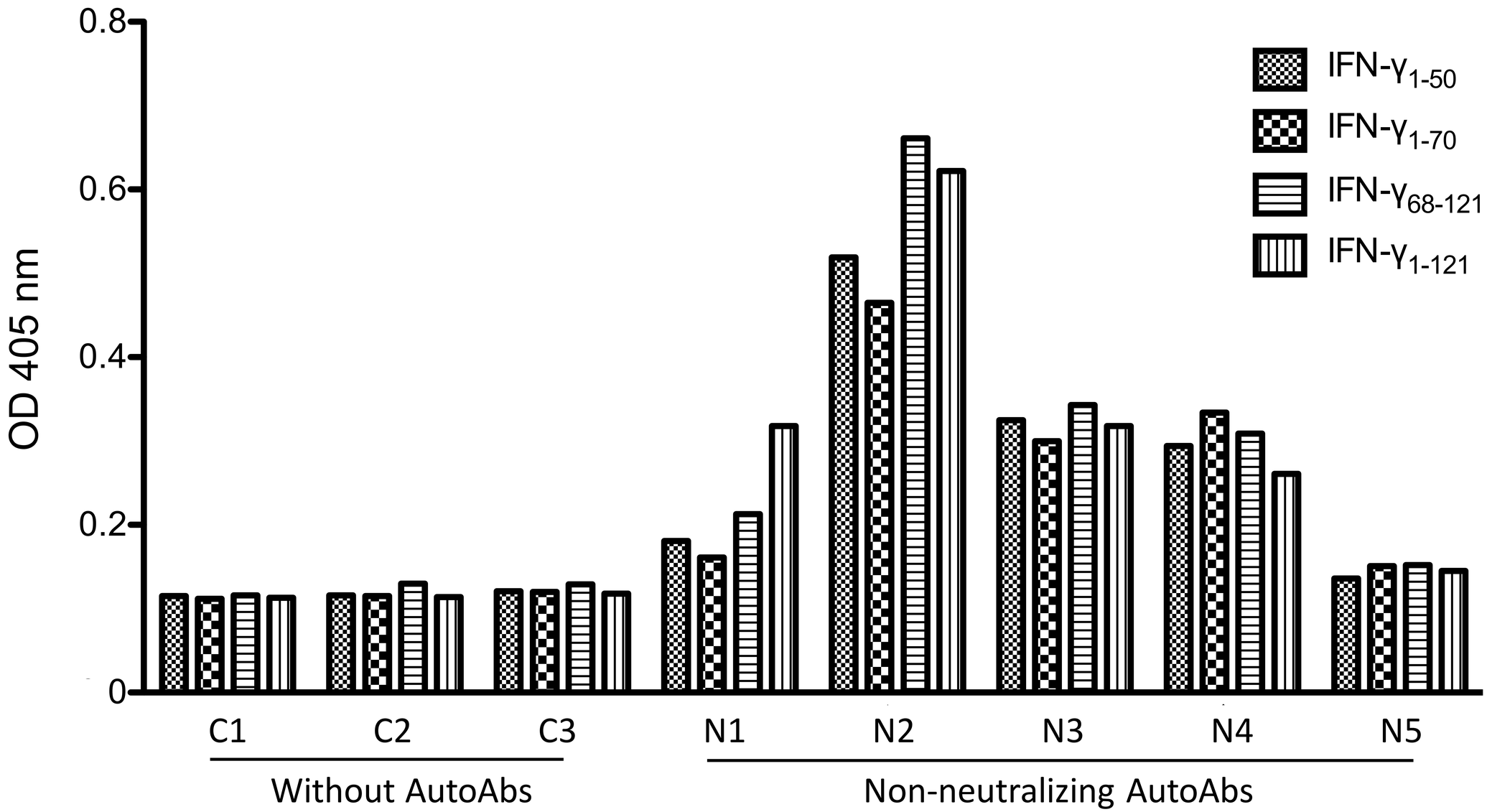
Supplementary Figure 6. IFN-$\gamma_{1–139}$ and IFN-$\gamma_{1–131}$ were recognized by anti-IFN-γ AutoAbs, whereas other truncated IFN-γ proteins were not. Western blot showing the ability of plasma from randomly selected Controls (*n* = 2, C1 and C2) (**a** and **b**) and IFN-γ AutoAbs Pts (*n* = 4, P1–4) (**c**–**f**) to bind various truncated forms of IFN-γ. Results from a representative experiment are shown. Similar results were obtained in at least three replicated experiments.

Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 7

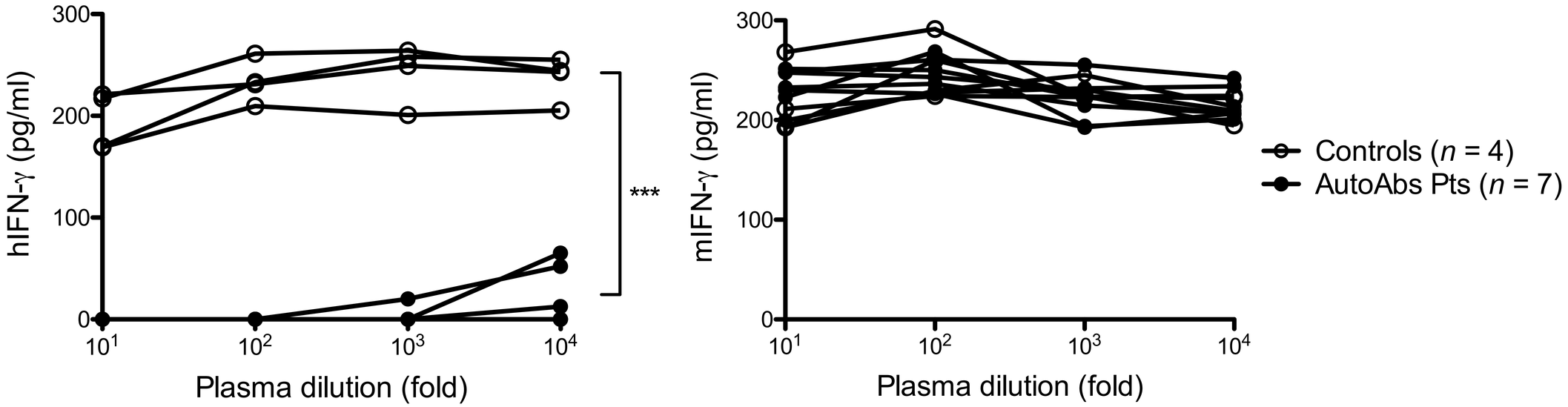


Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 7. Non-neutralizing AutoAbs recognized IFN-$\gamma_{1–50}$, IFN-$\gamma_{1–70}$, IFN-$\gamma_{68–121}$ and IFN-$\gamma_{1–121}$. Protein mapping was used to assess the affinity of binding of the recombinant proteins to IFN-γ in plasma, presented as the mean optical density (OD) at 405 nm; the plasma samples used were from healthy controls without AutoAbs (*n* = 3, C1–3), and healthy controls with non-neutralizing IFN-γ AutoAbs (*n* = 5, N1–5).

Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 8

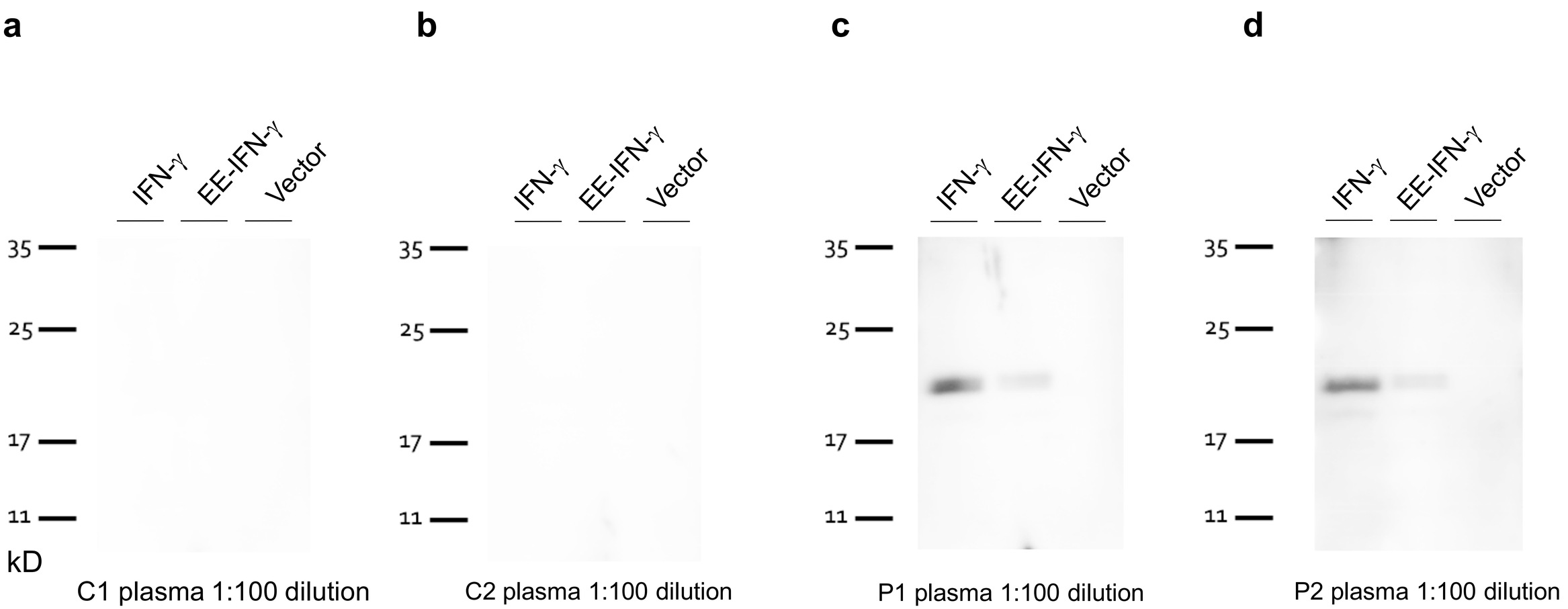


Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 8. Plasma samples from patients with anti-IFN-γ AutoAbs were unable to bind mouse IFN-γ. Seven plasma samples from randomly selected patients were serially diluted and incubated with 200 pg/mL human IFN-γ (hIFN-γ) or murine IFN-γ (mIFN-γ). Plasma from healthy donors did not block the detection of hIFN-γ or mIFN-γ. However, plasma from patients with AutoAbs against IFN-γ inhibited the detection of hIFN-γ at dilutions of up to $1/10^4$, but did not block the detection of mIFN-γ. $***P < 0.001$ at plasma dilutions of $1{:}10^{-1}$ to $1{:}10^{-4}$ in one-way ANOVA with a post-hoc Tukey's test.

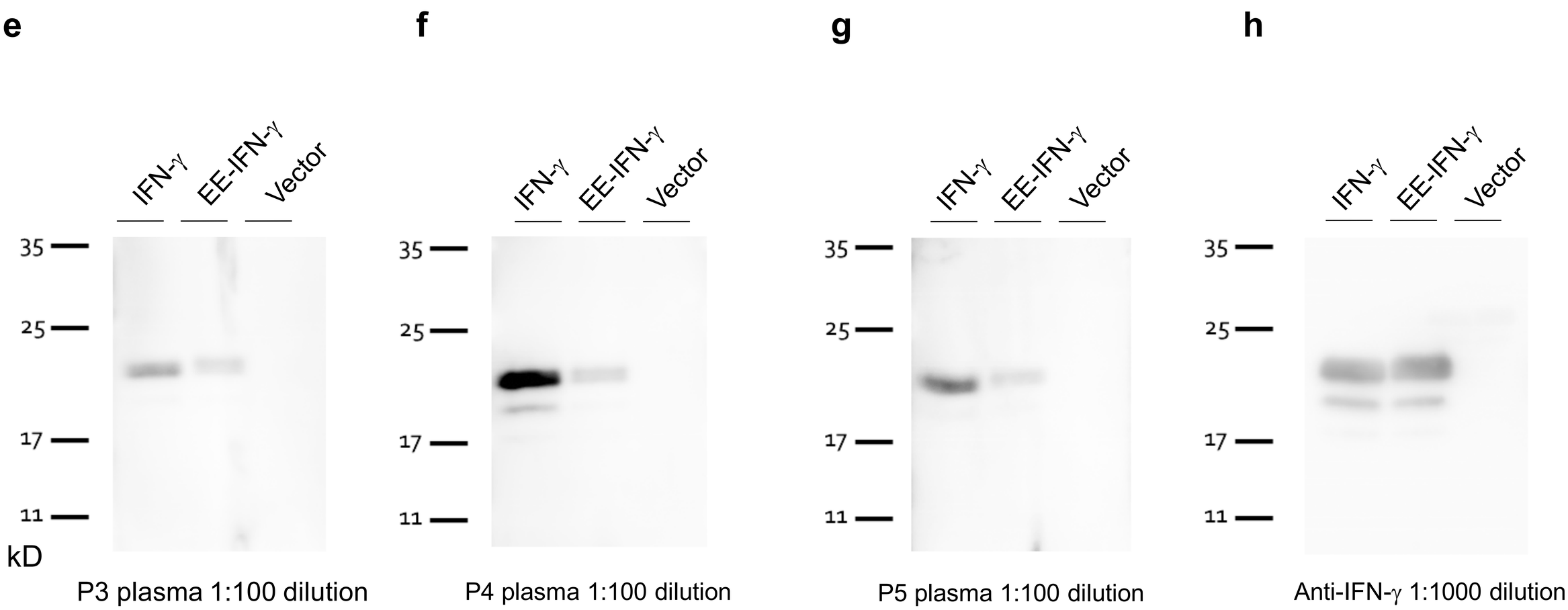
Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 9



Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 9



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Supplementary Figure 9. Western blotting revealed differences in the binding strength of anti-IFN-γ AutoAbs for EE-IFN-γ and IFN-$\gamma_{1–131}$. Western blot showing the binding of plasma from randomly selected healthy controls (*n* = 2, C1 and C2) (**a** and **b**) and anti-IFN-γ AutoAbs patients (*n* = 5, P1–5) (**c**–**g**) to IFN-$\gamma_{1–131}$ and EE-IFN-γ. IFN-$\gamma_{1–131}$ and EE-IFN-γ loading control were detected with anti-IFN-γ antibody (**h**). Similar results were obtained in at least three replicated experiments.

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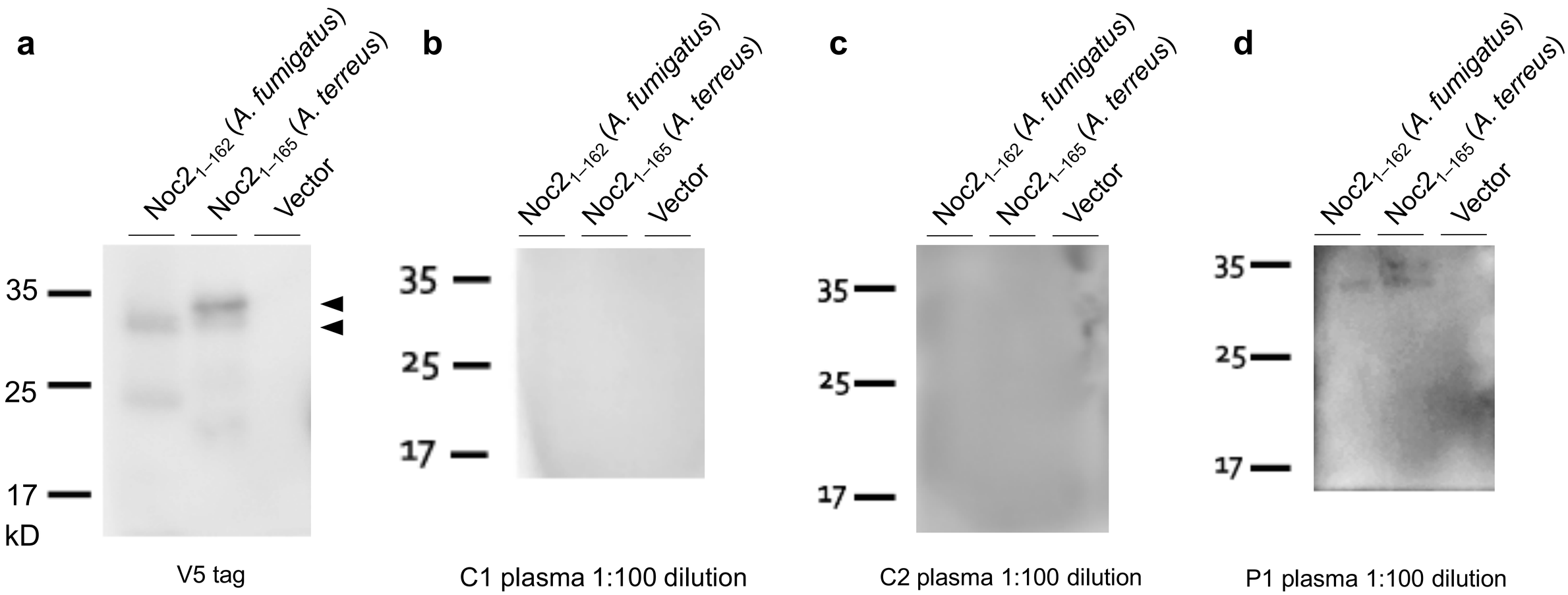
Supplementary Figure 10



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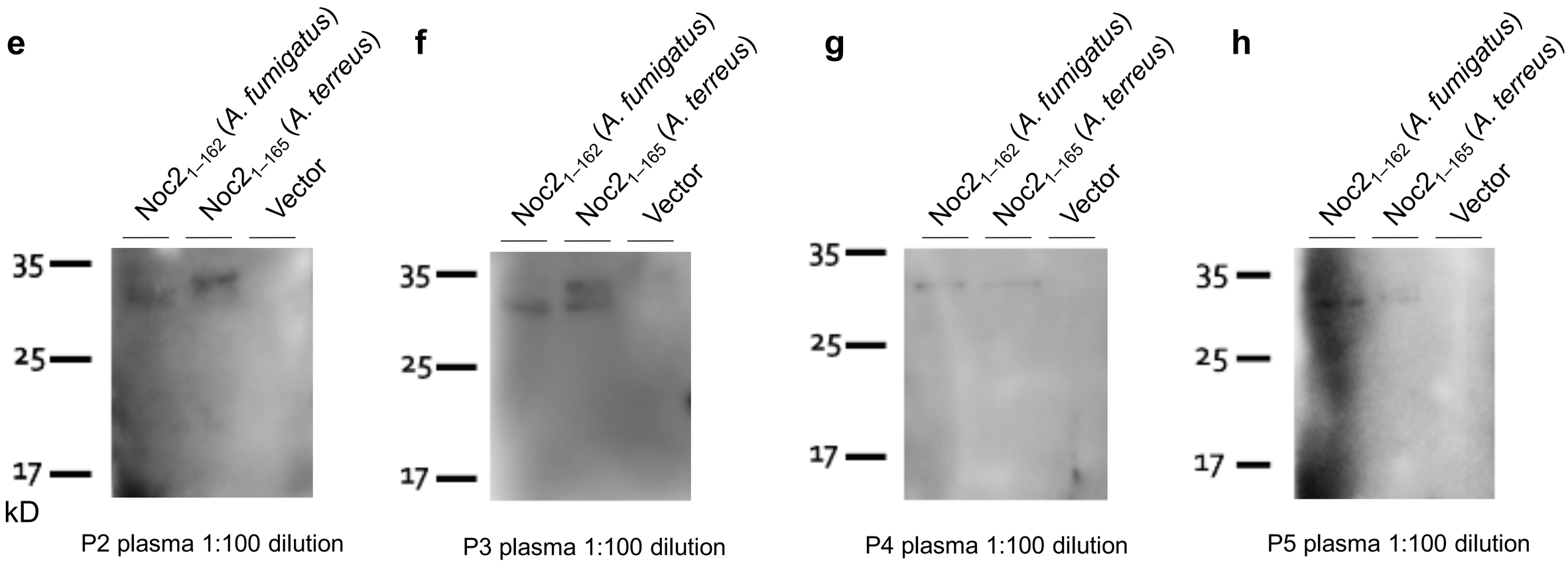
Supplementary Figure 10



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Supplementary Figure 10. AutoAbs against IFN-γ bound to *Aspergillus* Noc2. Western blot analysis of the different forms of *Aspergillus fumigatus* (amino acids 1–162) and *Aspergillus terreus* (amino acids 1–165) recombinant Noc2 protein detected with a V5-tagged antibody (**a**). Western blot showing the ability of plasma from randomly selected Controls (*n* = 2, C1 and C2) (**b** and **c**) and IFN-γ AutoAbs Pts (*n* = 5, P1–5) (**d**–**h**) to bind various truncated forms of Noc2. Results from a representative experiment are shown. Similar results were obtained in at least three replicated experiments.

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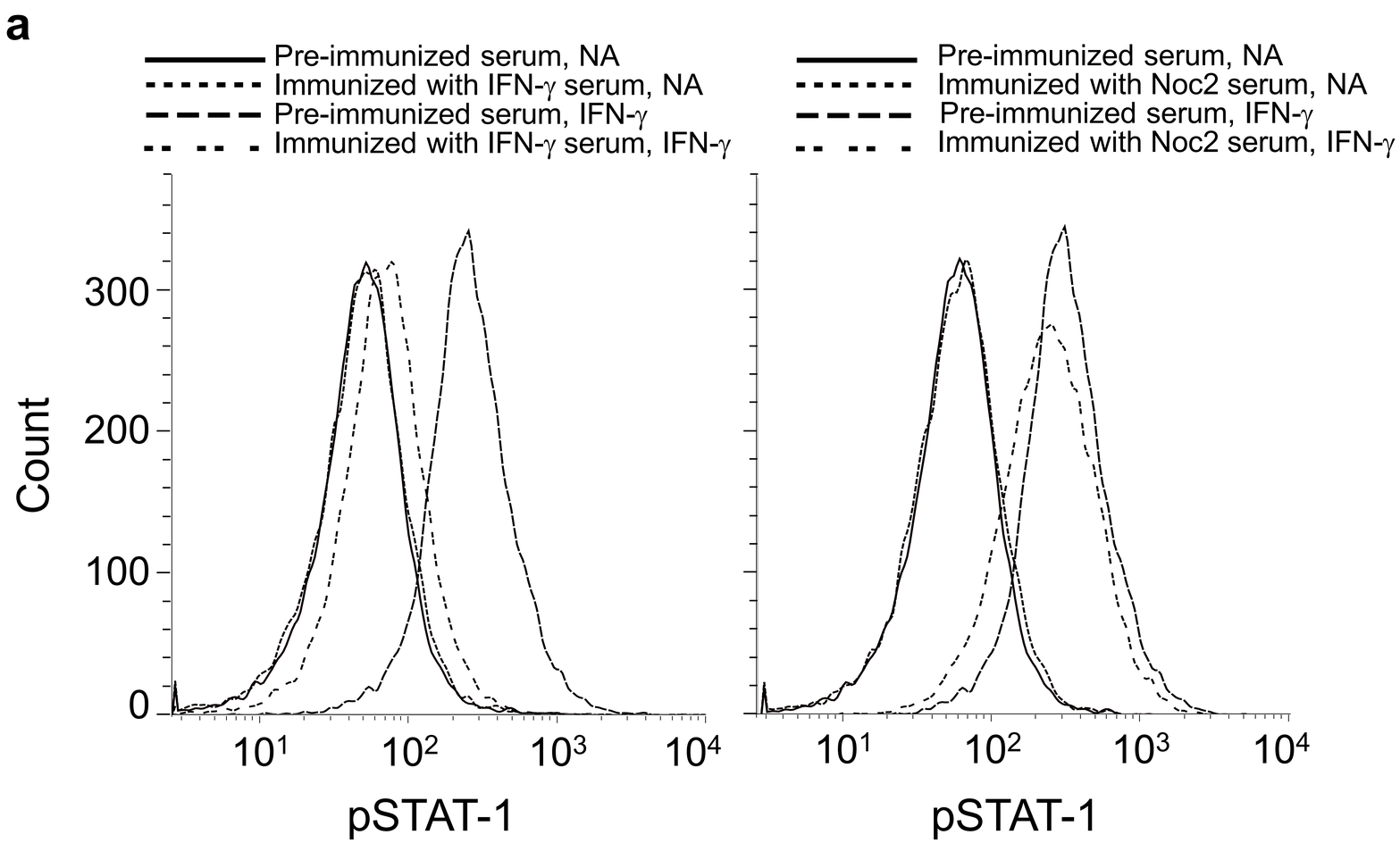
Supplementary Figure 11



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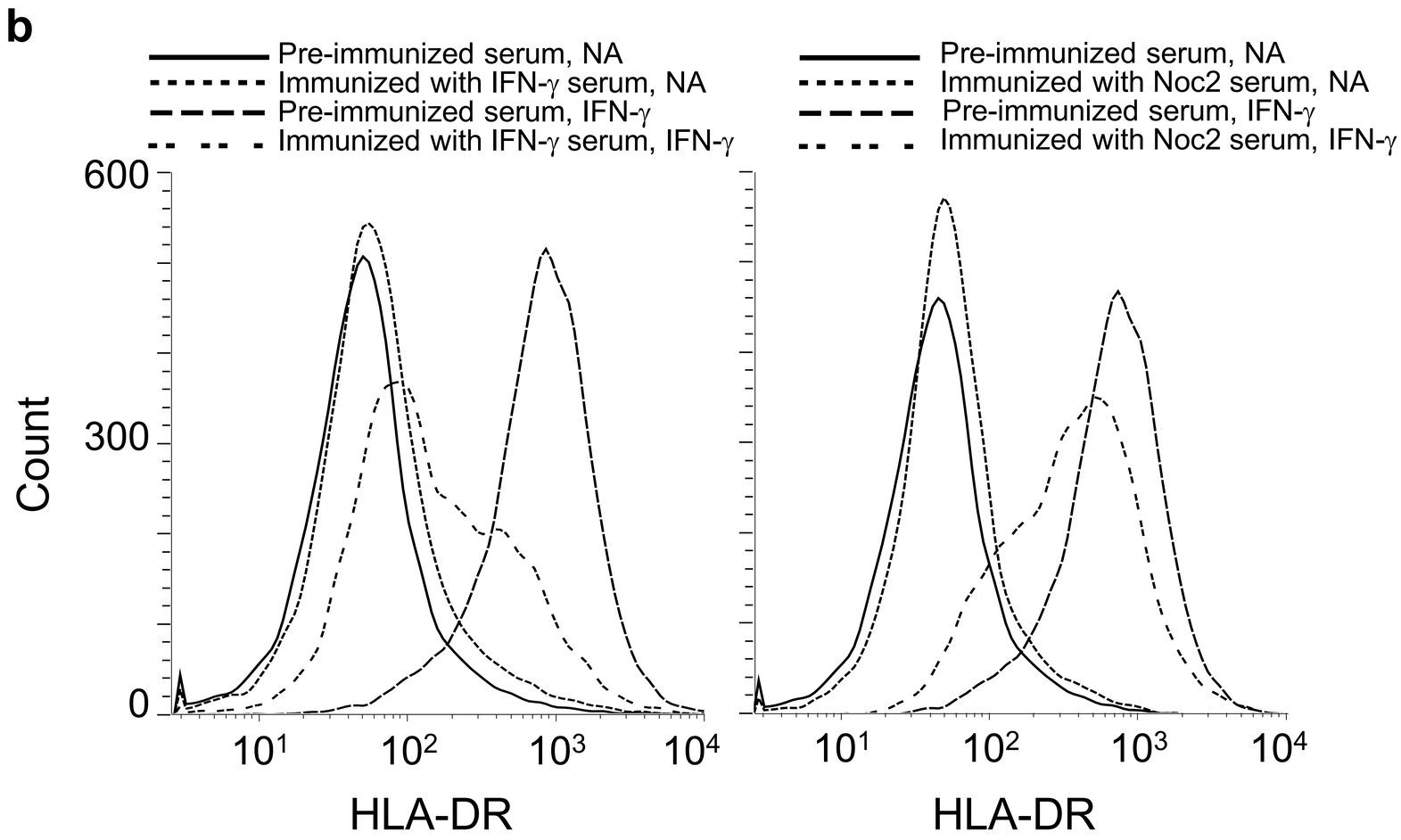
Supplementary Figure 11



Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 11. Purified IgG from rats immunized with Noc2 or IFN-γ blocked the IFN-γ-induced upregulation of p-STAT1 and HLA-DR. THP1 cells were incubated with IgG from non-immunized rats (n = 6), Noc2 peptide-immunized rats (n = 6) or IFN-γ peptide 6-immunized rats (n = 6) and treated with recombinant IFN-γ. P-STAT1 levels were measured by flow cytometry with the p-STAT1 monoclonal antibody; representative histograms (**a**) are shown. HLA-DR expression was measured by flow cytometry with an HLA-DR monoclonal antibody; representative histograms (**b**) are shown.

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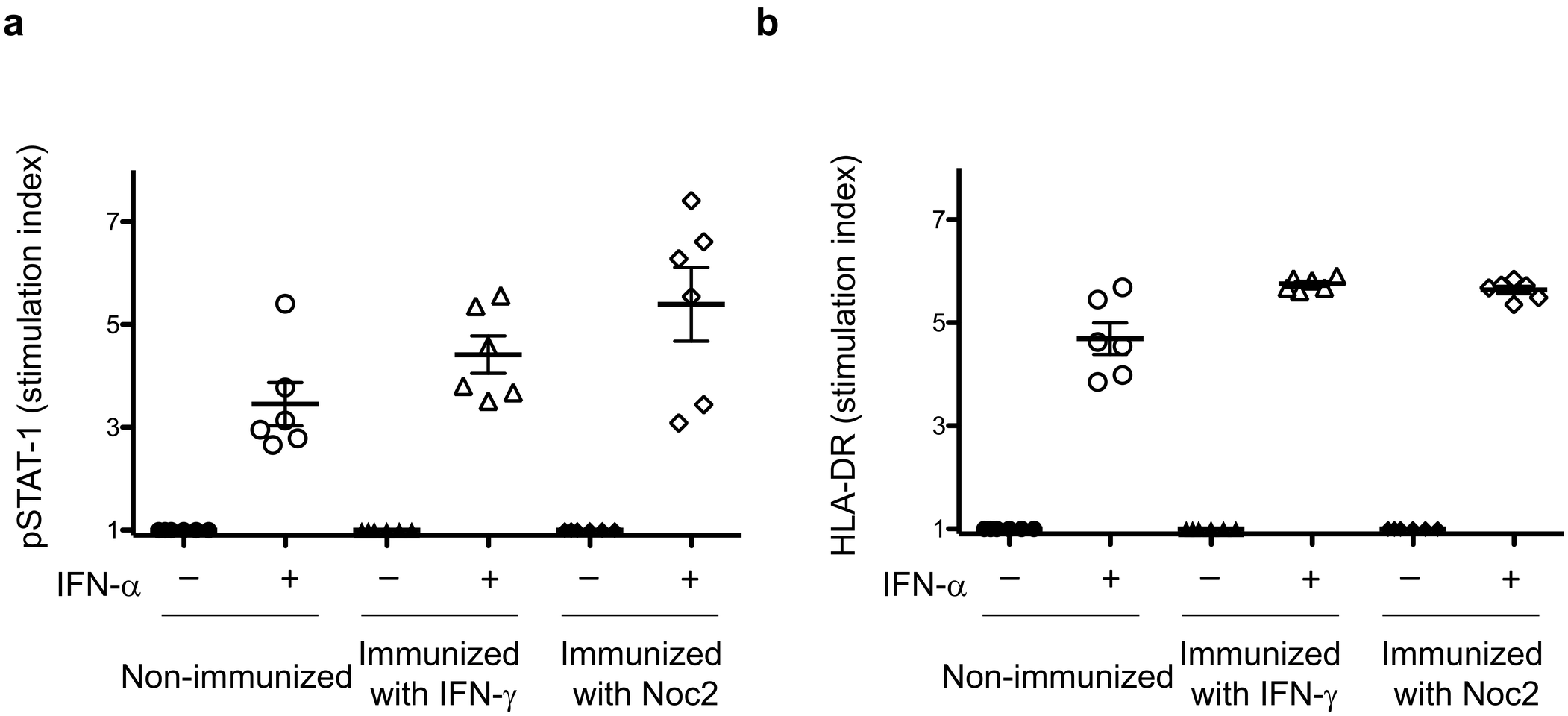
Supplementary Figure 12



Nature Medicine: doi:10.1038/nm.4158

Supplementary Figure 12. Purified IgG from rats immunized with Noc2 or IFN-γ did not block the IFN-α-induced upregulation of p-STAT1 and HLA-DR. THP1 cells were incubated with IgG from non-immunized rats (n = 6), Noc2 peptide-immunized rats (n = 6) or IFN-γ peptide 6-immunized rats (n = 6) and treated with recombinant IFN-α. p-STAT1 levels were measured by flow cytometry with the p-STAT1 monoclonal antibody, and the individual stimulation index (stimulated/unstimulated median fluorescence intensity ratio) is shown, together with the mean and s.e.m. (**a**). HLA-DR expression was measured by flow cytometry with an HLA-DR monoclonal antibody; the individual stimulation index (stimulated/unstimulated median fluorescence intensity ratio) is shown, together with the mean and s.e.m. (**b**).

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Supplementary Table 1

	AutoAbs recognization						EE-IFN-γ functional assay							Patient	
	Mapping			Competition		Affinity	Patients' Plasma		Autologous plasma			Whole Blood		HLA typing	
P	IFN-γ Peptide	IFN-γ Protein	Noc2 peptide	IFN-γ	Noc2	EE-IFN-γ	p-STAT1	IL12p40	p-STAT1	IL12p40	HLA-DR	p-STAT1	IL12p40	DRB1	DQB1
P1	+++	+++	+++	+++	+++	+++	++	+++	+	+++	+++	+	++	4:05	4:01
P2	++	+++	+++	+++	+++	+++	+	+	+	++	++	++	++	16:02	5:02
P3	+	+++	n.d.	+++	++	+++	+++	+++	+	n.d.	−	n.d.	−	16:02	5:02
P4	+++	+++	+++	+++	++	n.d.	+++	+++	+	++	n.d.	++	+++	4:05	4:01
P5	++	+++	+++	+++	n.d.	n.d.	+	+	++	+	+	++	+	n.d.	n.d.
P6	++	+++	n.d.	n.d.	++	n.d.	+++	+++	++	+++	n.d.	+	++	16:02	5:02
P7	−	+++	n.d.	n.d.	++	n.d.	+++	+++	n.d.	+	n.d.	++	n.d.	16:02	5:02
P8	n.d.	+++	n.d.	n.d.	++	n.d.	+	+	n.d.	+++	++	n.d.	+++	16:02	5:02
P9	+	+++	−	n.d.	n.d.	n.d.	++	+++	+	+++	+	+	+	n.d.	n.d.
P10	++	+++	n.d.	n.d.	n.d.	n.d.	++	++	++	n.d.	n.d.	+	+++	16:02	5:02
P11	n.d.	+++	n.d.	n.d.	n.d.	n.d.	++	+	+	+	+	n.d.	+	16:02	5:02
P12	+	+++	−	n.d.	n.d.	+++	+++	+	n.d.	n.d.	n.d.	+	n.d.	16:02	5:02
P13	+	+++	−	n.d.	n.d.	n.d.	n.d.	++	n.d.	++	+++	n.d.	++	n.d.	n.d.
P14	+	+++	−	n.d.	n.d.	n.d.	+	n.d.	n.d.	+++	n.d.	++	+	n.d.	n.d.
P15	−	+++	n.d.	+++	n.d.	n.d.	n.d.	+++	n.d.	++	n.d.	n.d.	n.d.	16:02	5:02
P16	+++	n.d.	+++	+++	n.d.	n.d.	n.d.	n.d.	n.d.	+	n.d.	n.d.	n.d.	16:02	5:02
P17	n.d.	+++	n.d.	n.d.	n.d.	n.d.	+++	++	n.d.	++	n.d.	n.d.	n.d.	16:02	5:02
P18	−	n.d.	n.d.	n.d.	n.d.	n.d.	+	n.d.	+	n.d.	n.d.	n.d.	n.d.	16:02	5:02
P19	n.d.	n.d.	n.d.	+++	n.d.	+++	n.d.	+	n.d.	n.d.	n.d.	n.d.	n.d.	4:05	4:01

Supplementary Table 1. Summary of patients and the experiments performed. This table presents a list of patients and the experiments performed. n.d., not done. In mapping assays: (+++) OD values > 0.8; (++) OD values > 0.3; (+) OD values > 0.15; (-) OD values < 0.15. In competition assays: (+++) *P* values < 0.001; (++) *P* values < 0.01 at 240 mg/ml. In affinity assays: (+++) decrease in OD values > 30%. In EE-IFN-γ functional assays: (+++) stimulation index > 2 or fold-increase > 10; (++) stimulation index > 1.5 or fold-increase > 5; (+) stimulation index > 1 or fold-increase > 1; (-) stimulation index < 1 or fold-increase < 1.

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