

1 **PARK7/DJ-1 promotes pyruvate dehydrogenase activity and maintains Treg** 2 **homeostasis**

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38 Abstract

39 Pyruvate dehydrogenase (PDH) is the gatekeeper enzyme into the tricarboxylic acid (TCA) cycle. Here we show that
40 PARK7/DJ-1, a key familial Parkinson's disease (PD) gene, is a pacemaker controlling PDH activity in CD4 regulatory
41 T cells (Tregs). DJ-1 bound to PDH-E1 beta (PDHB), inhibiting the phosphorylation of PDH-E1 alpha (PDHA), thus
42 promoting PDH activity and oxidative phosphorylation (OXPHOS). *Dj-1* depletion impaired Treg proliferation and
43 cellularity maintenance in older mice, increasing the severity during the remission phase of experimental autoimmune
44 encephalomyelitis (EAE). The compromised proliferation and differentiation of Tregs in *Dj-1* knockout mice were caused
45 via regulating PDH activity. These findings provide novel insight into the already complicated regulatory machinery of
46 the PDH complex and demonstrate that the DJ-1-PDHB axis represents a potent target to maintain Treg homeostasis,
47 which is dysregulated in many complex diseases.

49 Introduction

50 Mitochondria are the organelle providing cellular bioenergy through tricarboxylic acid (TCA), oxidative phosphorylation
51 (OXPHOS) and fatty acid oxidation. Compelling evidence shows that mitochondrial dysfunction contributes to several
52 major neurological diseases such as Parkinson's disease (PD)^{1,2} and Alzheimer's disease³, but also to diseases of the
53 gastrointestinal tract⁴, diabetes⁵ and cancer⁶. In PD patients, a particular deficiency in mitochondrial complex I activity
54 or general mitochondrial functions has been described^{7,8}. The activity and maintenance of the mitochondrial complex I
55 is regulated by DJ-1/PARK7, a key risk gene for early-onset familial PD⁹⁻¹¹, via binding to its subunits¹². As a
56 consequence, murine or human cells that are devoid of DJ-1/PARK7 expression showed mitochondrial defects affecting
57 either mitochondrial respiration or integrity¹³⁻¹⁵. Our current understanding of the functions of DJ-1 in mitochondria is
58 essentially based on immortalized neurectodermal cancer cell lines and on primary or derived neurons^{16,17}. However, due
59 to the existence of cell type- and tissue-specific protein interactions and differences in mitochondrial organelle number
60¹⁸, these studies do not allow to extrapolate whether DJ-1 is also involved in the regulation of mitochondrial functions in
61 non-neuronal cells such as in cells of the immune system, where mitochondrial dysfunction has been linked to the
62 autoimmune diseases multiple sclerosis and rheumatoid arthritis^{19,20}. Recently, significant progress has already been
63 made in understanding the role of *Dj-1* in innate immune cells, e.g., macrophages, where *Dj-1* interacts with p47phox, a
64 subunit of the NADPH oxidase^{21,22}. As DJ-1 protects dopaminergic neurons from oxidative stress-induced cell death, the
65 influence of DJ-1 on brain resident immune cells has also been investigated²³. Deletion of *Dj-1* indeed enhances activation
66 of astrocytes and microglia, possibly through a STAT signaling pathway²⁴.

67 A potential contribution of the immune system to the pathogenesis of PD has been postulated for some time^{25,26}. As there
68 is a complete penetrance of early-onset PD among individuals with homozygous DJ-1 loss-of-function mutations, we
69 hypothesized that assessing the functions of DJ-1 in immune cells could provide valuable information on the impact of
70 the molecular function of DJ-1 in cells that have a potential relevance for PD pathogenesis beyond neurons. So far, it still
71 remains elusive whether and how DJ-1 is involved in the regulation of mitochondrial energetic metabolism on a molecular
72 level and whether this could be potentially important for the physiological role of adaptive immune cells such as subsets
73 of CD4⁺ T cells that have been associated with the pathogenesis of PD²⁷. While DJ-1 was already linked with the
74 differentiation of inducible regulatory T cells (iTregs), no influence of DJ-1 on natural Tregs (nTregs) could be observed
75²⁸. Tregs are critical immune cells maintaining immune homeostasis and self-tolerance in both physiological and
76 pathological conditions, such as in autoimmune diseases²⁹⁻³³. Previous studies on DJ-1 function in Tregs were done by
77 using young adult mice only²⁸. Aging, however, is the most important risk factor for PD³⁴⁻³⁶. Furthermore, a growing
78 body of evidence showed a possible association between PD and autoimmunity^{37,38}. Thus, we hypothesized that the
79 effects of DJ-1 on nTregs might become only evident during the aging process.

80 We undertook this study to examine the effects of DJ-1, a major genetic risk factor for early onset PD, on the CD4⁺ T
81 cell function. Our data provide thorough evidence that DJ-1 is crucial in maintaining the cellularity of nTregs only in
82 older adult mice, while no effect was seen in young adult mice. The mechanistic studies illustrate that DJ-1 interacts with
83 pyruvate dehydrogenase (lipamide) beta (PDHB), a subunit of the PDH super-complex, preferentially in CD4⁺CD25⁺

84 Tregs, but not in CD4⁺CD25⁻ T cells. Furthermore, our results show that DJ-1 serves as a pacemaker governing the
85 activity of PDH, the central metabolic enzyme linking the glycolytic pathway and the TCA cycle^{39,40}, preferentially in
86 Tregs. Thus, by using both, *Dj-1* knockout (KO) mice and primary human T cells, we describe a previously unrecognized
87 molecular mechanism through which DJ-1 critically regulates bioenergetic functions in mitochondria via the metabolic
88 gatekeeper enzyme PDH. *Dj-1* depletion impaired Treg proliferation and maintenance of Treg cellularity in older mice,
89 thereby increasing the severity of induced autoimmunity during the remission phase of experimental autoimmune
90 encephalomyelitis (EAE). Thus, our data provide evidence that DJ-1 is an important component acting with PDH in Treg
91 homeostasis, which contributes to an immune phenotype that becomes only apparent in adult mice at older age and that
92 can be phenocopied through PDH inhibition in Tregs.

93 Results

94 Identification of *DJ-1* as a potential key gene in Tregs

95 Starting from our published dataset of high-time-resolution (HTR) time-series transcriptome data during the first 6 h of
96 T cell receptor (TCR) stimulation⁴¹, we found that *DJ-1* was the most highly expressed among 20 reported PD-related
97 genes⁴² in both unstimulated and stimulated human Tregs and Teffs (**Fig. 1a**). The immunoblot analysis from independent
98 healthy donors confirmed the protein expression of *DJ-1* in both Tregs and Teffs (**Fig. 1b**, for Treg characterization refer
99 to **Fig. EV1a-c**). Furthermore, by utilizing the so-called ‘queen-bee-surrounding principle’ network analysis strategy,
100 derived from the ‘guilt-by-association’ concept⁴³⁻⁴⁵, we analyzed the first-degree neighborhoods in the *DJ-1* subnetwork.
101 However, the first-degree neighbors of *DJ-1* were not significantly enriched in a collection of 400 genes potentially
102 related to T-cell function⁴¹. Since the functions of the second-degree neighbors in the network might also be indicative
103 of the roles of the given node^{43,44}, we further extended to scrutinize the subnetwork composed of second-order neighbors
104 of *DJ-1*. Interestingly, *DJ-1* was then highly connected with well-known key players in Treg functions (**Fig. 1c**, $P=4.3E-$
105 12 , cumulative binomial distribution), such as *FOXP3*, *CTLA4*, *ICOS*, *GATA3*, and *CD44*³⁰. None of the other known
106 PD genes were highly expressed in Tregs and Teffs and significantly surrounded by potential T-cell-related genes in the
107 Treg-specific network (**Table EV1**). Together, this network analysis results predict that DJ-1 might play a role in Tregs
108 that warrants further investigation of its potential impact on Treg function and cellularity.

109 *Dj-1* depletion impairs Treg cellularity maintenance in aged mice

110 To test whether DJ-1 maintains Treg cellularity as suggested above, we analyzed the Treg compartment in *Dj-1*^{-/-} knockout
111 (KO) mice as developed elsewhere⁴⁶. Since DJ-1 is one of the key PD familial genes and aging is the major risk factor
112 of PD^{35,36}, we investigated both young adult and older mice. For both young adult mice (short as ‘young mice’, 8–12
113 wks) and older adult mice (short as ‘old mice’, ~45 wks), the frequency of total CD4⁺ T cells in spleen and peripheral
114 lymph nodes was not significantly changed between *Dj-1* KO mice and *Dj-1*^{+/+} littermate control mice (WT, **Fig. 2a**). The
115 frequency of total Tregs (CD4⁺FOXP3⁺ T cells, Tregs) among total CD4⁺ T cells was also not different between young
116 *Dj-1* KO mice and WT mice. Consistent with other reports⁴⁷, the frequency of Tregs among total CD4⁺ cells was increased
117 with age in WT mice (**Fig. 2b**). Interestingly, the frequency of Tregs was significantly decreased in spleen and peripheral
118 lymph nodes of old *Dj-1* KO mice compared with that of age- and gender-matched WT mice (**Fig. 2b, c**). The percentages
119 of CD4⁺CD25⁺ T cells among total CD4⁺ T cells showed similar changes as that of CD4⁺FOXP3⁺ T cells (**Fig. 2d, e**).
120 The total absolute number of splenic Tregs was also significantly reduced in old *Dj-1* KO mice compared with WT mice
121 (**Fig. 2f**). The ratio between CD4⁺FOXP3⁻ Tconv and Tregs was increased in old but not young *Dj-1* KO mice (**Fig. 2g**).
122 As Helios is a marker distinguishing natural Tregs (nTregs) from induced Tregs (iTregs), we also analyzed expression of
123 Helios. Interestingly, the percentages of Helios positive cells among CD4⁺FOXP3⁺ T cells also declined in old but not
124 young *Dj-1* KO mice vs. WT mice (**Fig. 2h, i**). Consistent with reduced Tregs, the frequency of splenic IL-10-producing
125 CD4⁺ cells following *in vitro* PMA/ionomycin stimulation was significantly decreased in old *DJ-1* KO mice vs. WT
126 littermates (**Fig. 2j**). Furthermore, the age-dependent effect of the *Dj-1* loss on Treg cellularity was not simply caused by
127 the potential differential expression of *Dj-1* between young and old WT mice (**Fig. 2k**). Despite the reduced Treg
128 cellularity, old *Dj-1* KO mice up to the tested age did not develop spontaneous autoimmune phenotypes, possibly due to
129 the compensation by imbalanced Tconv cell subsets (unpublished own data).

130 Functional Tregs are critical for the active phases (e.g., remission) but not the onset of experimental autoimmune
131 encephalomyelitis (EAE)⁴⁸, a murine model of the human autoimmune disease multiple sclerosis. We therefore evaluated
132 the *in vivo* effects of reduced Treg accumulation in *Dj-1 KO* mice by using the myelin oligodendrocyte glycoprotein
133 (MOG35-55)-induced EAE model⁴⁹. In agreement with not seeing a significant difference in the Treg frequency between
134 young *Dj-1 KO* mice and their WT littermates, no obvious difference in the clinical scores of different disease phases was
135 observed (**Fig. 2I**). Interestingly, old *Dj-1 KO* mice relative to their age- and gender-matched WT controls exhibited
136 deteriorated EAE symptoms during the Treg-dependent disease remission phase (**Fig. 2m**). These data suggested an
137 impairment of total *in vivo* immunosuppression capability in *Dj-1 KO* mice attributable to a decreased number of Tregs.

138 The decreased Treg accumulation in aged mice could be caused by an impairment either in Treg development or Treg
139 homeostatic proliferation. To test these two possibilities, we first assessed the thymic Treg compartment in 4-wk-old mice
140 as thymocytes peak in mice aged at 4-6 weeks and gradually evolve afterwards⁵⁰. In 4-wk-old mice, we did not observe
141 any difference between *Dj-1 KO* mice and WT mice in total thymocytes, single-positive CD4 T cells (CD4 SP), single-
142 positive CD8 T cells (CD8 SP) or the percentages of total FOXP3⁺ Tregs among CD4 SP (**Fig. 3a-d**). We also did not
143 find any difference in the four subsets determined by the combination of positive or negative expression of CD25 and
144 FOXP3 (**Fig. 3e**). Neither Treg markers, nor proliferation or functional markers, such as Helios, Ki67 or PD-1, CTLA4
145 and GITR exhibited any difference between 4-wk-old *Dj-1 KO* and WT mice (**Fig. 3f-h**). These data suggest that *Dj-1*
146 depletion does not influence Treg development in juvenile mice before thymic involution. As decreased Treg
147 accumulation only appeared in aged *Dj-1 KO* mice, we also analyzed the cellular composition of thymocytes in old mice
148 (~45 wks). As thymic atrophy already started in 45-wk-old mice, the total thymocytes were dramatically reduced in old
149 WT mice relative to 4-wk-old mice (**Fig. 3a**). However, the number of total thymocytes was not significantly affected
150 when comparing old *Dj-1 KO* mice with WT mice at the tested age (**Fig. 3a**). No significant difference was observed
151 between old KO and WT mice with respect to the frequency of CD4 SP, CD8 SP and the frequency of total Tregs among
152 CD4 SP (**Fig. 3b-d**). Interestingly, *Dj-1* ablation selectively decreased the frequency of Helios-expressing cells among
153 FOXP3⁺CD4⁺ T cells (**Fig. 3g**) and modestly but significantly reduced homeostatic proliferation of FOXP3⁺CD4⁺ T cells
154 (**Fig. 3h, i**). Therefore, *Dj-1* depletion did not affect Treg development, neither in 4-wk-old nor in aged mice, indicating
155 that the observations in peripheral lymphoid tissues of aged mice might be caused by deficiency in Treg homeostatic
156 proliferation.

157 ***Dj-1* depletion impairs Treg proliferation and activation in aged mice, but not Treg suppressor function**

158 The finding of a decreased frequency of Tregs in old *Dj-1 KO* mice prompted us to seek for the underlying molecular
159 pathways mediated by DJ-1. We performed transcriptomic analysis of sorted *Dj-1 KO* and WT Tregs from spleen of old
160 mice. As expected, the upregulated Treg-signature genes were expressed much higher than Tconv-signature genes⁵¹,
161 indicating a highly-purified cell sorting (**Fig. 4a**). Consistent with the view of DJ-1 as a transcriptional coactivator⁵², *Dj-1*
162 deficient Tregs exhibited much larger fractions of downregulated genes compared with those of upregulated genes (**Fig.**
163 **4b**). In old *Dj-1 KO* Tregs, in line with reduced Ki67 staining (**Fig. 3h, i**), the genes involved in cell cycle and proliferation
164 were significantly enriched among the downregulated list (**Fig. 4c, d**). Moreover, the loss of *Dj-1* in Tregs of old mice
165 reduced the transcription of many key Treg genes, such as *Tnfrsf1b*, *Tnfrsf8* and *Itgae*⁵¹, as well as many of the MHC II-
166 related genes, which are involved in the early-contact-dependent suppression⁵³ (**Fig. 4c**). Many of these MHC II-related
167 genes were also observed in the human Treg-specific correlation network surrounding *DJ-1* (**Fig. 1c**). The MHC II genes
168 are reported to be expressed in activated T cells of many species, but are not expressed in activated murine T cells⁵⁴,
169 possibly because this aspect has never been analyzed in depth in aged mice. The observation of decreased MHC II genes
170 is in agreement with the decreased homeostatic expression of activation markers in Tregs (**Fig. 4e, f**), such as GITR and
171 ICOS, in old *Dj-1 KO* mice vs. WT mice. This unbiased transcriptome analysis demonstrates that *Dj-1* deficiency leads
172 to a downregulation of pathways mediating Treg homeostatic proliferation and activation, which accumulatively
173 contributes to the reduced Treg compartment observed in old *Dj-1 KO* mice. In conclusion, DJ-1 is critical for maintaining
174 Treg cellularity via regulating their activation and proliferation, which becomes evident only in aged mice.

175 As outlined earlier by the computational correlation network analysis, we also tested whether *Dj-1* plays a critical role in
176 controlling Treg suppressor function (TSF). When we analyzed TSF from young mice *in vitro* using Tregs, CD4 T
177 conventional cells (Tconv) and feeder cells in co-culture suppressive assays, no significant difference was observed

178 between young *Dj-1 KO* and WT mice (**Fig. EV2a**), consistent with a previous study²⁸ in which the authors also
179 investigated the suppressor function of DJ-1-KO Treg, but only focused on young mice. Interestingly in old *Dj-1 KO*
180 mice relative to WT mice, *in vitro* TSF was also not compromised (e.g., at the ratio of 1:1, **Fig. EV2b**), but even slightly
181 augmented with a higher ratio between Tconv and Tregs (e.g., at the ratio of 4:1, **Fig. EV2b**). To further characterize the
182 Tregs, we investigated the protein expression of known key Treg effector molecules, such as FOXP3, CTLA4, and IL2RA
183 (CD25) in Tregs of old DJ-1 mice. Although the fraction of CTLA4 positive cells among old *Dj-1 KO* Tregs was not
184 different compared with that among WT Tregs (**Fig. EV2c**), the expression levels of CTLA4 among CTLA4 positive
185 cells were modestly, but significantly increased in spleen (**Fig. EV2c**). Furthermore, FOXP3 and CD25 expression levels
186 were also modestly increased in spleen of old *Dj-1-KO* individual Tregs (**Fig. EV2d, e**). Therefore, our data demonstrate
187 that DJ-1 is critical for maintaining Treg cellularity, but dispensable for Treg suppressor function.

188 **DJ-1 promotes PDH activity preferentially in Tregs**

189 To further identify the detailed underlying molecular mechanisms through which DJ-1 maintains Treg cellularity, we next
190 examined the binding partners of DJ-1 in resting and activated human primary nTregs by coimmunoprecipitation (IP)-
191 mass spectrometry (MS) analysis. We identified around 20 novel potential binding partners of DJ-1 in human nTregs by
192 employing a combination of five strict filtering criteria (**Fig. 5a, Table EV2**, refer to Methods). Interestingly, five proteins
193 regulating RNA splicing, namely, HNRNP3, PRPF8, KHSRP, SKIV2L2 and SART1, bound to DJ-1 preferentially in
194 the stimulated Tregs and Teffs compared with those in the resting cells ($P=2.8E-4$, **Fig. 5a, Table EV2**). This is consistent
195 with the dynamic binding behavior of DJ-1 to RNAs¹¹, which binds to RNAs in a resting state and then dissociates
196 following activation-driven oxidative stress as shown in a neuroblastoma cell line. Our results also confirmed 11 known
197 DJ-1 binding partners identified previously from other cell types (**Table EV3**, refer to Methods), demonstrating the
198 reliability of our technique.

199 To prioritize and test the most promising binding candidates, we ranked the top 20 novel binding partners based on their
200 preferential binding to DJ-1 relative to the IgG control (**Fig. 5a**). Unexpectedly, the top-ranked candidate was PDHB
201 (pyruvate dehydrogenase [lipoamide] beta). Furthermore, PDHB also occupied the highest fold change in binding affinity
202 between unstimulated and stimulated Treg samples in the MS analysis (**Table EV2**). Following the DJ-1 Co-IP-MS
203 identification of PDHB (**Fig. 5a, b**), we confirmed that DJ-1 bound to PDHB by coIP-immunoblotting analysis and
204 observed that the DJ-1 binding was more predominant in Tregs than in Teffs (**Fig. 5c**). Moreover, using ImageStream
205 analysis, we quantitatively demonstrated that DJ-1 preferentially colocalized with PDHB in Tregs rather than in Teffs,
206 with or without anti-CD3/-CD28/IL-2 stimulatory beads or antibodies (**Fig. 5d, e**). Coincidentally, an available murine
207 microarray database, analyzing the mRNA coexpression in different integrated datasets in a cell-type-specific manner,
208 demonstrated that *Dj-1* and *Pdhb* are significantly correlated in murine Tregs (**Fig. EV3a**), but not in other CD4 T-cell
209 subsets, such as effector memory CD4 T (CD4 Tem) cells, Th1 or Th2 cells⁵⁵ (**Fig. EV3b**). This finding is not only in
210 line with the reported role of Dj-1 in glucose homeostasis of pancreatic islets⁵⁶, but also suggests a novel molecular
211 pathway that connects Treg function or maintenance with metabolism. Taken together, our data from cobinding,
212 colocalization and coexpression strongly support that DJ-1 binds to PDHB preferentially in Tregs relative to Teffs.

213 To further investigate whether the physical binding between DJ-1 and PDHB might result in any functional consequences,
214 we checked PDH enzymatic activity. Interestingly, knocking down *DJ-1* significantly reduced PDH enzymatic activity
215 in human Tregs (**Fig. 5g**), while adding recombinant human DJ-1 protein to the T-cell lysates promoted PDH activity in
216 a high-dose-dependent manner (**Fig. 5h**). The requirement for a high-dose of recombinant DJ-1 protein to further enhance
217 PDH activity is most likely due to the high endogenous expression of DJ-1. Since the PDH enzyme is mainly activated
218 through site-specific dephosphorylation of three sites (Ser232, Ser293 and Ser300) on the E1 alpha subunit (PDH-E1A,
219 also known as PDHA)⁴⁰, we measured the site-specific phosphorylation in human Tregs. Consistent with the impact on
220 PDH enzymatic activity, knocking down *DJ-1* in Tregs significantly increased the phosphorylation levels of Ser293 but
221 not another tested site (Ser300) (**Fig. 5i**). In line with the weaker but detectable binding between DJ-1 and PDHB in Teffs
222 relative to Tregs (**Fig. 6c**), knocking down *DJ-1* in Teffs modestly dampened PDH activity (**Fig. 5j**) and enhanced Ser293
223 phosphorylation on PDHA (**Fig. 5k**). Notably, a stronger effect of DJ-1 on the phosphorylation of Ser293 was observed
224 in the unstimulated Tregs and Teffs (**Fig. 5i, k**) compared to the stimulated counterparts. This finding is in line with the
225 preferential binding in unstimulated vs. stimulated T cells as determined by MS analysis (**Table EV2**). A stronger effect

226 on PDH activity in resting T cells is also in agreement with the fact that following stimulation, T cells switch their
227 energetic model from mainly OXPHOS to glycolysis⁵⁷, a situation in which T cells rely less on PDH activity. Taken
228 together, these findings support the notion that binding of DJ-1 to PDHB promotes PDH enzymatic activity by inhibiting
229 Ser293 phosphorylation levels of PDHA in Tregs.

230 Reduced PDH activity following DJ-1 knockdown in Tregs could be also directly regulated by the expression levels of
231 PDH kinases (*Pdks*) and phosphatases (*Pdps*)⁵⁸ as DJ-1 is also known as a transcription co-activator. We therefore
232 analyzed those genes using the Treg transcriptomic data sorted from old *Dj-1 KO* and WT mice, but none of the *Pdp*
233 genes showed significant difference at the transcriptional level (**Fig. EV4a**). Interestingly, the kinase gene *Pdk1* was
234 upregulated while the other two kinase genes *Pdk2* and *Pdk3* were downregulated in old *Dj-1 KO* mice, three of which
235 might compensate each other and contribute to the overall PDH activity in Tregs (**Fig. EV4a**). The change in PDH activity
236 was not simply rendered by a change in mRNA of the genes encoding the PDH supercomplex⁵⁹ as no change was
237 observed for any of these genes (**Fig. EV4b**). Thus, DJ-1 regulates the PDH activity in Tregs, not only via a physical
238 interaction with PDHB, but also possibly in parallel via transcriptional regulation of PDH kinase genes. Nevertheless, to
239 distinguish the contribution of each mechanism to PDH activity requires further investigation.

240 Since PDH is the critical TCA-cycle-entry enzyme and crucial for ATP generation, a partial knockdown of *DJ-1* in Tregs
241 indeed decreased both the ATP/ADP and ATP/AMP ratios, with or without TCR stimulation (**Fig. 5l, m**). For stimulated
242 Tregs, DJ-1 downregulation reduced both ratios (**Fig. 5l, m**). Therefore, these data demonstrate that DJ-1 also regulates
243 ATP production in Tregs.

244 Having identified DJ-1 as a binding partner of PDHB, which consequently affects PDH activity, the critical gatekeeper
245 enzyme entering the TCA cycle, we sought to further evaluate whether DJ-1 has any effect on OXPHOS of Tregs, as
246 Tregs critically depend on OXPHOS⁶⁰. Particularly, we measured the mitochondrial reserve or spare respiratory
247 capability (SRC) and maximum respiration. These measurements are used as a proxy for the extra mitochondrial capacity
248 available to produce energy through OXPHOS under activation or cellular stress conditions, which is important for the
249 long-term survival of the cell⁶¹. Due to the loss of *Dj-1* and its binding with PDHB, an impaired SRC was indeed
250 observed in Tregs of old *DJ-1 KO* mice (**Fig. 6a**), resulting in a lower bioenergetic production and consequently reduced
251 proliferation (**Fig. 3h, i**). Interestingly, the baseline OXPHOS was only reduced in *Dj-1 KO* Tregs from old but not young
252 mice (**Fig. 6a, b**), which might subsequently impair Treg homeostatic proliferation (**Fig. 3h, i**). However, the maximum
253 respiration was already decreased in young *Dj-1 KO* Tregs (**Fig. 6b**), indicating that under stressful cellular or activation
254 conditions, Treg survival or proliferation might be affected. The aging progression itself could be considered as a chronic
255 inflammatory/activation process or model and could be the reason why we observed an impaired accumulation of Treg
256 cellularity in aged, but not young *Dj-1 KO* mice. We did not observe any significant difference in the baseline OXPHOS
257 and SRC of Tconv, neither in young nor old *Dj-1 KO* and WT mice (**Fig. 6c, d**). This is not only consistent with the
258 current notion that Tregs rely on OXPHOS much more than do Tconv cells^{60,62}, but also is reflected by our observation
259 that Tconv cellularity was not affected in both young and old *Dj-1 KO* mice (**Fig. 2a**). In short, DJ-1 binds to PDHB
260 preferentially in Tregs and, as a functional consequence, regulates Treg OXPHOS, which becomes more obvious in aged
261 mice.

262 **Dj-1 regulates Treg maintenance via PDH**

263 Having observed that DJ-1 regulates PDH activity via interacting with PDHB in nTregs and that DJ-1 depletion impairs
264 nTreg proliferation and cellularity in aged mice, we further asked whether there is any causal link from the detected DJ-
265 1-PDHB interaction to the observed Treg phenotypes. We therefore inhibited PDH activity to measure any potential effect
266 on the proliferation and cell survival of WT nTregs sorted from young mice. Although we observed nTreg phenotypes in
267 aged mice, we speculated the effects of PDH inhibition should already be visible in young mice as a specific PDH inhibitor
268 should have a strong inhibition on PDH activity while DJ-1 only partially inhibits PDH activity. Compared with the
269 vehicle treatment, the usage of a PDH inhibitor, CPI-613 (EC50 concentration around 200 uM in many tested tumor cell
270 lines⁶³), essentially impaired proliferation, as assessed by a cell tracking dye (CellTracer Violet, CTV) and slightly
271 compromised cell survival of nTregs following anti-CD3/-CD28/IL2 stimulation for 3 days (**Fig. 7a**). But the in vitro
272 Treg lineage stability during the stimulation period of 3 days was not impaired by PDH inhibition (**Fig. 7a**). Since DJ-1

273 deficiency impaired SRC/maximum respiration in Tregs (**Fig. 6b**), we also inhibited PDH to evaluate whether SRC is
274 affected in Tregs to further confirm whether DJ-1 regulates SRC via regulating PDH. As demonstrated, inhibiting PDH
275 decreased SRC and maximum respiration in Tregs (**Fig. 6b**), indicating that DJ-1 regulates SRC via PDH.

276 In line with the work done by others²⁸, *Dj-1* depletion also impaired differentiation and proliferation of iTregs (**Fig. 7b**,
277 **c**). We therefore assessed whether these effects occur via PDH. Notably, inhibiting PDH activity in naïve T cells with
278 CPI-613 significantly weakened the differentiation toward iTreg in a dose-dependent manner (**Fig. 7d**). Inhibiting PDH
279 activity also impaired proliferation and cell survival although the administration dose of CPI-613 (ranging from 50 μ M
280 to 100 μ M) was lower than the EC50 concentration (**Fig. 7e, f**). The effect on proliferation was already noticeable from
281 the CPI-613 treatment with a concentration as low as 50 μ M (**Fig. 7e**). A reduction in iTreg survival was also observed
282 following *Dj-1* depletion²⁸. Since PDH activity is inhibited by PDH kinases, we assumed that inhibition of PDH kinases
283 should generate opposite effects to PDH blockade. As speculated, treating naïve T cells with 10 mM of DCA
284 (dichloroacetate, a PDH kinase inhibitor) relative to the control, we indeed observed an enhanced differentiation of iTregs
285 (**Fig. 7g**), in line with the observations made elsewhere⁶⁴. In short, PDH inhibition phenocopied *Dj-1* depletion in nTregs
286 and iTregs, indicating that DJ-1 maintains total Treg cellularity via regulating PDH activity in consideration of our
287 observation that DJ-1 regulates PDH activity.

288 Discussion

289 Here we uncovered a novel critical link between DJ-1 and PDHB that affects T cell immunity with a preferential impact
290 on Tregs relative to the other CD4+ T cells (i.e., Tconv). We found that *Dj-1* depletion impaired Treg proliferation and
291 the maintenance of Treg cellularity in older adult mice only. Another key observation of our study was that the apparent
292 dysregulation in nTreg homeostasis of aged mice had a functional consequence by aggravating the symptom score during
293 the remission phase of the induced autoimmunity disease model EAE. Pyruvate dehydrogenase (PDH) is a multiunit
294 gatekeeper enzyme controlling the entry of glucose-derived pyruvate into the tricarboxylic acid cycle (TCA), a vital
295 pathway providing cellular energy. Mitochondrial dysfunction in general^{2,65}, defects in PDH enzyme subunits⁶⁶,
296 deficiency of critical cofactors of the PDH enzyme (e.g., thiamine⁶⁷) or deleterious mutations in pyruvate transporters^{68,69}
297 contribute not only to a variety of metabolic diseases⁵, but also to several major neurological diseases, such as PD.
298 At the same time, particular homozygous mutations in *DJ-1* cause 100% penetration of early-onset familial PD, which so
299 far has been attributed mainly to the loss of anti-oxidative function in a cell-autonomous manner in neurons⁹⁻¹¹. Moreover,
300 *Dj-1* has been shown to regulate mitochondrial respiratory functions and mitochondrial integrity^{12,13,70}. The effects of
301 DJ-1 on mitochondrial functions might be attributable to cell type- and tissue microenvironment-specific regulations that
302 eventually contribute to a complex disease phenotype. Although the previous functional and genetic studies described
303 above have already suggested a functional convergence of DJ-1 and the PDH subunits leading to similarities in the
304 phenotypic outcome, it remained elusive until now whether and how these two key cellular regulators interact. In line
305 with the previously reported independent observations on DJ-1 and PDH functions already indicating convergent
306 phenotypes, our unbiased proteomic data together with both, in vitro and in vivo analyses on the molecular and cellular
307 level have revealed a physical and functional link between DJ-1 and PDH, which has a key impact on mitochondrial
308 bioenergetic functions in nTregs, a crucial subset of CD4 T cells. Furthermore, we also showed that PDH inhibition
309 phenocopied the effects of DJ-1 deletion in Tregs. Thus, our findings add several new aspects to the understanding of the
310 DJ-1/PARK7-based monogenic defect, which leads to early onset PD with full penetrance: i) the finding of significant
311 immune dysregulation affecting T cell immunity beyond the cell-autonomous dysregulation already described in neurons;
312 ii) the obvious age-dependency of the observed immune phenotypes; and iii) the layer of critical interaction of major
313 intracellular metabolic pathways at the crossroads of mitochondrial functions and the TCA cycle.

314 While loss-of-function defects or inhibition of DJ-1 and PDH lead to partially overlapping and convergent cellular and
315 disease phenotypes, no direct interaction of these two key cellular regulators has been described so far. Our results
316 indicated that DJ-1 regulates PDH activity and OXPHOS in Tregs in a novel and unexpected way. Through mass
317 spectrometry analysis followed by anti-DJ-1 co-immunoprecipitation in unstimulated and stimulated human Tregs and
318 Tconv, we detected that PDHB was the most prominent protein with binding preference for DJ-1. Furthermore, we found

319 a much higher abundance of PDHB protein bound to DJ-1 in unstimulated cells than stimulated ones. The binding between
320 DJ-1 and PDHB indeed regulated the enzymatic activity of PDH via modulating phosphorylation levels of PDHA mainly
321 in Tregs and in resting Tconv. Due to the preferential interaction between DJ-1 and PDHB in Tregs, the loss of DJ-1
322 reduced OXPHOS capacity in Tregs as a functional consequence, especially in aged mice. These findings are in line with
323 previously reported data where it has been shown that Tregs rely more on OXPHOS than Tconv^{62,71}. Furthermore, the
324 biased effect on PDH activity in unstimulated T cells can further be explained by the fact that T cells switch their metabolic
325 pathway mode from OXPHOS to glycolysis upon activation⁵⁷. Thus, our study has identified a novel and hitherto
326 unknown protein-protein interaction between DJ-1 and PDH that connects two major cellular pathways, mitochondrial
327 functions with DJ-1 as a crucial player and the TCA cycle with PDH as a key enzyme.

328 An appropriate homeostatic regulation of Treg activity and function is crucial for a balanced immune response during the
329 physiological aging process³⁰. Our study demonstrated that DJ-1 depletion impairs nTreg proliferation and cellularity in
330 older adult mice only. Since OXPHOS is critical for the development and long-term survival of Tregs⁷¹, it is plausible
331 that the compromised spared respiratory capability, which we have demonstrated in aged mice devoid of *Dj-1*, contributed
332 to the impaired Treg accumulation. The spared or maximum respiration is often not necessary under homeostatic and
333 disease-free conditions, but is required under cellular stress or activation conditions such as in low-grade inflammation
334 in the elderly, a state that is also known as ‘inflammaging’⁷². In line with this notion, age-dependent mitochondrial
335 dysfunction has been observed in skeletal muscle of *Dj-1*-depleted mice¹³. We further elucidated the underlying
336 mechanisms and found that the impairment of nTreg proliferation and cellularity occurred via PDHB, as blockade of the
337 PDH enzymatic activity phenocopied the effects of *Dj-1* deletion on nTreg cellularity and OXPHOS capacity. The
338 functional consequences of PDH inhibition through *Dj-1* deletion on Treg cellularity appear to be compensated by other
339 pathways during young age⁷³. However, these effects on Treg proliferation and cellularity cannot be balanced anymore
340 in old *Dj-1*-deficient mice beyond a critical age and tipping-point⁷⁴. In our study we have applied an inhibitor of PDH
341 that phenocopied the effects of *Dj-1* depletion in purified nTregs. In line with our findings, the administration of the same
342 PDH inhibitor in preclinical trials induced systemic inflammatory responses as the primary and only side effect, which is
343 indicative of systemic immune dysregulation⁷⁵. Thus, our data provide strong evidence that *Dj-1* deletion compromises
344 nTreg cellularity and accumulation in aged mice only, via a previously unrecognized interaction of DJ-1 with PDHB.

345 The depletion of *Dj-1* has previously already been linked to an impaired differentiation of iTregs from naïve CD4 T cells
346 in young adult mice²⁸. Our results confirm that *Dj-1* depletion impairs iTreg differentiation. However, the study by Singh
347 and coworkers²⁸ provided no clear-cut underlying molecular mechanism of how iTreg differentiation is impaired in *Dj-1*
348 *KO* mice. Our results showed that inhibiting PDH also impaired differentiation, proliferation and survival of iTregs in
349 an identical manner as it was observed with *Dj-1* deletion. Since OXPHOS is required for activation of naïve T cells⁷⁶,
350 it is not surprising that PDH blockade impaired the downstream TCA-driven OXPHOS and consequently compromised
351 differentiation of naïve T cells towards iTregs. Interestingly, depletion of *Dj-1* did not impede Treg suppressor function,
352 which is indicative that OXPHOS might be more vital to the long-term survival rather than the suppressive function of
353 Tregs. Together, these results demonstrate that DJ-1 regulates overall Treg cellularity via modulating PDH enzyme
354 activity.

355 Since Tregs are critical during the stable and remission phases of various autoimmune diseases⁴⁸, we hypothesized that
356 reduced Treg cellularity, as observed in our study after *Dj-1* depletion, should worsen disease symptoms. As a critical
357 role of Tregs has especially been demonstrated in EAE⁷⁷, a murine model for the human autoimmune disease multiple
358 sclerosis, we decided to analyze the role of DJ-1 further in this particular autoimmune disease model. Indeed, our data
359 showed that depletion of *Dj-1* deteriorated symptoms during the remission-phase of EAE in old mice as compared to age-
360 and gender-matched WT mice. As no difference in Treg frequency was observed between young *Dj-1 KO* and WT mice,
361 we also did not observe a difference in the EAE scores between the corresponding young adult groups. Therefore, *Dj-1*
362 depletion impaired Treg cellularity and consequently Treg pathophysiological functions in aged mice. We thus concluded
363 that *Dj-1* depletion enhances autoimmune responses in a well-characterized disease model in an age-dependent way.

364 Although PDH enzyme activity is tightly regulated by several PDH kinases and phosphatases, our findings on DJ-1
365 promoting PDH activity add a new dimension to the regulation of the PDH complex, thus enabling a better understanding

366 of the highly organized PDH supercomplex system ⁷⁸. Although we cannot exclude potential effects of other cell types
367 on the degree of accumulation of total Tregs in mice with a constitutional deletion of *Dj-1* used in this study, our work
368 underscores that the DJ-1-PDHB interaction could be used as a potential pharmaceutical target to interfere with various
369 complex diseases involving dysregulation of Treg frequency in the aged population. Uncovering this unexpected critical
370 physical and functional interaction of the familial PD gene DJ-1 on PDH activity in Tregs suggests that other genes
371 involved in neurodegenerative diseases and regulating mitochondrial respiratory functions might be among potential
372 candidates that steer the activity of PDH or other TCA enzymes. In line with our discoveries, another PD familial gene,
373 PARK2/PARKIN, regulates the important glycolysis rate-limiting enzyme pyruvate kinase M2 in tumor cell lines ⁷⁹.
374 However, pyruvate kinase M2 is not expressed in human CD4 T cells. Our data demonstrate that DJ-1 counteracts the
375 role of the PDH kinases, e.g., PDK1 on PDH ⁶⁴, mainly in the specific CD4 T-cell subset of Tregs. Future experiments
376 are needed to determine whether the observed DJ-1-PDHB interaction also holds true in dopaminergic neurons and
377 accordingly contributes to the death of such neurons, which essentially causes the motor dysfunction of PD ¹⁶.

378

379 **Materials and Methods**

380 **Human T cell-related experiments.**

381 **Human T cell isolation.**

382 The buffy coats or leukopaks from healthy donors used for primary T cell culture and analysis were provided by the
383 Luxembourg Red Cross. Human primary natural Tregs and Teffs (CD4 effector T cells) were derived respectively from
384 sorted CD4⁺CD25^{high}CD127^{low} and CD4⁺CD25⁻ T cells of peripheral blood mononuclear cells (PBMC) on BD
385 FACSAriaTM III cell sorter. The PBMCs were isolated by using Ficol-Paque plus (17-1440-03, GE Healthcare) or
386 Lymphoprep (07801, StemCell) followed by magnetic separation with anti-human CD4 microbeads (130-045-101,
387 Miltenyi Biotec), as described by the manufacturers, before being stained with mouse monoclonal [RPA-T4] anti-human
388 CD4 FITC (555346, BD Biosciences) (dilution 1:20), mouse monoclonal [M-A251] anti-human CD25 APC (555434,
389 BD Biosciences) (dilution 1:20) and mouse monoclonal [HIL-7R-M21] anti-human CD127 V450 (560823, BD
390 Biosciences) (dilution 1:20) for sorting with BD FACSAriaTM III.

391 **Human T cell culturing.**

392 We followed the same protocol as described in our previous work ⁴¹. Sorted T cells were cultured in IMDM (21980-032,
393 Thermo Fisher Scientific) complete medium supplemented with 10% heat-inactivated (56°C, 45 min) Gibco® fetal
394 bovine serum (FBS) (10500-064, Thermo Fisher Scientific), 1x Penicillin+Streptomycin (15070-063, Thermo Fisher
395 Scientific), 1x MEM non-essential amino acids (M7145, Sigma-Aldrich) and 50 µM beta-mercaptoethanol (M7522,
396 Sigma-Aldrich). All the human T cells were cultured in 37°C 7.5% CO₂ incubators, unless specified. 100 U/ml
397 recombinant human IL2 (known as Proleukin® in medication) (PZN 2238131, Novartis) was added daily to Treg (but
398 not Teff) cell culture medium and the same amount of IL2 was added to Tregs unless otherwise stated. Every seven days,
399 all T-cells were restimulated with irradiated Epstein-Barr virus (EBV preparation from VR-1492, ATCC)-transformed
400 B-cells ⁸⁰ (EBV-B cells), at a 1:1 ratio of T and EBV-B cells, to expand and maintain the T-cells. The EBV-B cells were
401 irradiated in an RS2000 X-Ray Biological Irradiator (Rad Source Technologies) for 30 min at a rate of 2.80 Gy/min. The
402 T-cells were regularly characterized by co-staining CD4, CD25, FOXP3 and Helios protein levels by flow cytometry
403 (**Fig. EV1a, b**). When the primary Tregs were older than 6 weeks or the expression level of FOXP3 or Helios or the
404 cellular viability was apparently decreased, the cells were discarded and new T cells were isolated from different healthy
405 donors. The antibodies used for cell quality assessment were: mouse monoclonal [RPA-T4] anti-human CD4 BUV395
406 (564724, BD Biosciences) (dilution 1:100), mouse monoclonal [M-A251] anti-human CD25 FITC (555431, BD
407 Biosciences) (dilution 1:100), mouse monoclonal [22F6] anti-human Helios Pacific blue (13721, BioLegend) (dilution
408 1:100), mouse monoclonal [206D] anti-human FOXP3 Alexa Fluor 647 (320119, BioLegend) (dilution 1:10). The
409 LIVE/DEAD® Fixable Near-IR Dead Cell Stain (L10119, Thermo Fisher Scientific) (dilution 1:500) was used to
410 distinguish living cells from dead cells. In certain cases, we directly compared the markers of our isolated human Tregs

411 with TregThu, a golden standard isolated from our previous work⁸⁰. The procedure for the staining of extracellular and
412 intracellular markers is described below.

413 **Gene knockdown in human T cells.**

414 Targeted gene expression was knocked-down by using P3 Primary Cell 4D-Nucleofector X Kit L (V4XP-3024, Lonza)
415 with 100 µl P3 primary cell solution and 100 pmol of the corresponding si_RNAs (resuspended in 10 µl RNase-free
416 H₂O): si_Non-Specific (si_NS or si_CTL) (sc-37007, Santa Cruz) and si_PARK7/_DJ-1 (SI00301091, *AATG GAGG*
417 *TCAT TACA CCTA C*, Qiagen). Amaxa 4D-Nucleofector™ X System (Lonza) was used for the experiments with the
418 manufacturer's recommended program for stimulated human primary T cells. Following the siRNA transfection
419 procedure, T cells were transferred into 12-well plates with pre-warmed complete medium supplemented with 100 U/ml
420 IL-2 for Tregs and kept at 37°C for 1 day. They were then stimulated with Dynabeads Human T-Activator CD3/CD28
421 (11131D, Thermo Fisher Scientific) (ratio of cells and beads: 1:1) or with ImmunoCult Human T Cell Activator (10991,
422 STEMCELL Technologies) (25 µl/ml) in 24-well plates for 1 or 2 days depending on the corresponding experiments,
423 with or without additional recombinant IL-2 for Tregs and Teffs, respectively.

424 **RNA extraction and cDNA synthesis.**

425 RNA was extracted as previously described⁴¹. RNA samples for standard and quantitative PCR were prepared by using
426 the RNeasy Mini Kit (74106, Qiagen) starting with lysing the cells with RLT buffer supplemented with 1% beta-
427 mercaptoethanol (63689, Sigma-Aldrich), following the manufacturer's instructions and including the digestion of
428 genomic DNA with DNase I (79254, Qiagen). The RNA concentration was measured with a NanoDrop 2000c
429 Spectrophotometer (Thermo Fisher Scientific) followed by a quality check of the RNA integrity number (RIN). For RIN
430 assessment, the Agilent RNA 6000 Nano kit (5067-1511, Agilent) was used together with the Agilent 2100 Bioanalyzer
431 Automated Analysis System (Agilent) according to the manufacturer's protocol.

432 A maximum of 500 ng RNA was used for cDNA synthesis. A master mix for the first step was prepared with 0.5 µl of 50
433 µM Oligo(dT)₂₀ primers (18418020, Thermo Fisher Scientific), 0.5 µl of 0.09 units/µl Random Primers (48190-011,
434 Thermo Fisher Scientific), 1 µl of 10 mM dNTP mix (R0192, Thermo Fisher Scientific) and RNase-free water made up
435 to a final volume of 13 µl in 0.2 ml PCR Tube Strips (732-0098, Eppendorf). The tubes were transferred into a Professional
436 Standard Gradient 96 Thermocycler (Biometra) for 5 min at 65°C and 2 min at 4°C. After the first step, the reaction was
437 supplemented with 40 units RNaseOUT™ Recombinant Ribonuclease Inhibitor (10777019, Thermo Fisher Scientific),
438 200 units SuperScript™ III Reverse Transcriptase (18080-044, Thermo Fisher Scientific) and dithiothreitol (DTT)
439 (70726, Thermo Fisher Scientific) to give a final concentration of 5 mM in a total reaction volume of 20 µl. The PCR
440 tubes were returned to the thermocycler at 50°C for 60 min, 70°C for 15 min and 4°C until further usage.

441 **Standard polymerase chain reaction (ST-PCR) and realtime quantitative PCR (qPCR).**

442 The DreamTaq Green PCR Master Mix (K1081, Thermo Fisher Scientific) was used as a base for the ST-PCR. Forward
443 and reverse primers together with cDNA and RNase-free water were added. PCR was performed in a Professional
444 Standard Gradient 96 Thermocycler (Biometra). Following the amplification, the samples and MassRuler DNA Ladder
445 Mix (SM0403, Thermo Fisher Scientific) were loaded onto 2% agarose (A9539, Sigma-Aldrich) gel with ethidium
446 bromide (E1510, Sigma-Aldrich) or SYBR Safe (S33102, Thermo Fisher Scientific, dilution 1:10 000) and the gel was
447 run for 1 hr at 120 V in TAE buffer. Images of the bands were taken with the G:Box gel doc system (Syngene).

448 Real-time quantitative PCR (RT-PCR/qPCR) was performed by using LightCycler 480 SYBR Green I Master Mix
449 (04707516001, Roche), supplemented with cDNA and primers in a reaction volume of 10 µl. LightCycler 480 Multi-well
450 White Plates (04729749 001, Roche) with 384 wells and LC 480 Sealing Foil (04729757001, Roche) were used in these
451 experiments. The reaction was undertaken on a LightCycler 480 (384) platform (LightCycler 480 (384), Roche). The
452 results were analysed with LightCycler 480 SW 1.5 software. The annealing temperature of 55°C was set for the various
453 genes unless stated.
454 The primers used for human qPCR were: PARK7 (QT00055811, Qiagen), RPS9 (QT00233989, Qiagen) or GAPDH
455 (QT00079247, Qiagen) as reference genes.

456 **Human suppression assay.**

457 Human Teff cells were stained with 1 μ M CFSE (carboxyfluorescein succinimidyl ester, C34554, Thermo Fisher
458 Scientific) dye in PBS for 2min 45sec in the dark at room temperature. The reaction was stopped with cold FBS (2min,
459 dark, 4°C) before the cells were washed and recultured in complete IMDM medium. Tregs were transfected with
460 scrambled control or gene-specific siRNA for 1 day before being cocultured with Teffs and EBV-B cells. Serial dilution
461 of Tregs was performed in a 96-well plate with a starting cell number of 6E4/well. 6E4 CFSE-stained Teff cells were
462 distributed in each well with various amounts of Treg cells and stimulated with 6E4 irradiated EBV B-cells (irradiation
463 conditions were the same as for the cell culture). As positive or negative proliferation controls, the same number of CFSE-
464 stained Teff cells were cultured without Tregs, with and without irradiated EBV B-cells. The cell mixtures were cultured
465 at 37°C for 5 days and FACS analysis was carried out to determine the proliferation capacity of Teff cells by additionally
466 staining with CD4 APC ab and the LIVE/DEAD® Fixable Near-IR Dead Cell Stain reagent.

467 **Co-immunoprecipitation (Co-IP).**

468 The same number (around 1E7 T cells) of Tregs and Teffs were either left unstimulated or were stimulated for 5 hrs with
469 Dynabeads Human T-Activator CD3/CD28 for cell expansion and activation (11131D, Thermo Fisher Scientific) (ratio
470 of 1:1). After 5 hrs, cell pellets were collected, lysed in homogenization buffer with 0.2% Triton X100, 100 mM NaCl, 2
471 mM EDTA and 50 mM Tris (pH=8.0). Lysates were pre-cleared with 10 μ l washed Dynabeads Protein G (10003D,
472 Thermo Fisher Scientific) in 10 μ l lysis buffer for 1 hr at room temperature and the beads were then removed. The total
473 proteins in each sample were quantified by standard Bradford assay (5000006, Bio-Rad) in a microplate Cytation 5 cell
474 imaging multi-mode reader (1509096, BioTek Instruments GmbH). The lysates were diluted to a 3 μ g/ μ l protein
475 concentration with protease inhibitor cocktail (PIC) at a dilution of 1:100 to give the final volume. The samples were then
476 evenly distributed into two parts. One part of the sample was immune-precipitated with rabbit monoclonal [EP2815Y]
477 anti-human PARK7/DJ-1 antibody (ab76008, Abcam) and another part was immune-precipitated with normal rabbit IgG
478 (sc-2027, Santa Cruz) as a negative control, for 1 hr at room temperature (for both target and control antibodies, the
479 dilution was 1:10) before the addition of Dynabeads (20 μ l beads for 7 μ l of antibody) overnight at 4°C with gentle
480 rocking. The beads were washed 3x in lysis buffer and sequentially 2x in lysis buffer without detergent. The DJ-1 ab pull-
481 out complexes were then eluted from the beads by using 0.1% RapiGest (186001861, Waters) in 50 mM ammonium
482 bicarbonate (A6141, Sigma-Aldrich) solution with 5 mM DTT (43819, Sigma-Aldrich) at 60°C for 1 hr, dried in a
483 SpeedVac Concentrator at 4°C (Thermo Fisher Scientific) and resuspended in a small volume of lysis buffer (for Western
484 blot) or in ultra-pure H₂O with 1% acetonitrile (AE70.2, Carl-Roth) (for mass spectrometry analysis).

485 **Western Blotting (WB).**

486 Protein samples were mixed 1:3 with 4x Laemmli sample buffer (NP0007, Life Technologies) containing 50 mM
487 dithiothreitol (DTT), heated for 5min at 95°C, cooled on ice and loaded on gradient PAGER™ Gold Precast Gels (58505,
488 Lonza). ProSieve™ EX Running (200307, Westburg) and ProSieve™ EX Transfer (200309, Westburg) buffers were
489 used in the assay according to manufacturer's recommendations and both PageRuler™ Prestained Protein Ladder (26616,
490 Thermo Fisher Scientific) and Precision Plus Protein™ All Blue Prestained Protein Standards (Bio-rad, 1610373) were
491 used to determine the size of the protein bands. Proteins were transferred to a methanol (34860, Sigma-Aldrich)-activated
492 (5min, room temperature) Immun-Blot PVDF membrane (162-0177, Bio-Rad) and then blocked with 5% milk (70166,
493 Sigma-Aldrich) in PBS (14190169, Thermo Fisher Scientific) with 0.2% Tween-20 (P7949, Sigma-Aldrich) for 1 hr at
494 room temperature with gentle shaking. All primary antibodies were diluted in the blocking solution and incubated
495 overnight at 4°C. The membrane was washed (3x10min) with PBS-Tween-20 (0.2%) before and after incubation with
496 secondary antibodies. Chemiluminescence-based Amersham ECL Prime Western Blotting Detection Reagent (RPN2232,
497 VWR/GE-Healthcare) was employed to detect the bands, which were visualized in the Westburg Odyssey FC Imaging
498 System (Li-COR). If needed, the membrane was stripped with Restore Western Blot Stripping Buffer (21059, Thermo
499 Fisher Scientific) for 30min at room temperature, washed, blocked and blotted again. The main antibodies used for
500 Western blotting were: rabbit monoclonal [EP2815Y] anti-human PARK7/DJ-1 antibody (ab76008, Abcam) (dilution
501 1:2000), rabbit Anti-Pyruvate Dehydrogenase E1-alpha subunit [EPR11098] (ab168379, Abcam) (dilution 1:3000), rabbit
502 anti-human Pyruvate Dehydrogenase E1 beta subunit (PA5-31519, Thermo Fisher Scientific) (dilution 1:1000), rabbit
503 anti-human Pyruvate Dehydrogenase E1-alpha subunit (pSer293) (ab92696, Abcam)(dilution 1:300), rabbit anti-human
504 phospho PHE1-A type I (pSer300) (ABS194, Merck Millipore)(dilution 1:1000), rabbit monoclonal [6C5] anti-human
505 GAPDH (sc-32233, Santa Cruz) (dilution 1:1000).

506 A goat anti-rabbit IgG (H+L)-HRP conjugated antibody (172-1019, Bio-Rad) (dilution 1:5000) was used as the secondary
507 antibody.

508 For the assessment of the phosphorylation of PDHE1A, DJ-1/PARK7 were knocked-down in 5E6 Treg cells or in 5E6
509 Teff cells, according to the manufacturer's protocol for 24 hrs (P3 Primary Cell 4D-Nucleofector® X Kit, Lonza); see
510 the section 'Gene Knockdown'. The siRNA transfected cells were then stimulated with ImmunoCult Human CD3/CD28
511 T Cell Activator (25 µl/ml) (Stemcell Technologies, France) for 5, 24 or 48 hrs. Tregs were supplemented with IL-2 (100
512 U/ml) daily for the duration of the experiment. Proteins were quantified, separated, transferred, blocked and stained by
513 means of the same approaches as those described above. Detection was carried out on an ECL Chemocam Imager
514 (INTAS). For antibody information, please refer the paragraph above. If needed, the contrast and brightness of the whole
515 image was adjusted by using the *ImageJ* (<https://imagej.nih.gov/ij/index.html>) free software.

516 **Mass spectrometry (MS).**

517 The quality of the samples prepared for MS analysis was checked by Western Blot by loading a small amount of the re-
518 suspended IP elution products onto acrylamide gel. Only if the bait protein (PARK7/DJ-1) was detected on the blotted
519 membrane, was the IP considered successful and only these samples were analysed by mass spectrometry in the
520 proteomics facility (Institute for Systems Biology, Seattle). Eluted proteins were reduced with 5 mM dithiothreitol (DTT),
521 alkylated with 25 mM iodoacetamide and digested with Lys-C for 3 hrs at 37°C (11420429001, Promega) (dilution 1:200)
522 followed by an overnight digestion with Trypsin (V5113, Promega) (dilution 1:25) at 37°C. Peptides were acidified with
523 formic acid (33015, Sigma-Aldrich) to stop digestion and remove RapiGest. Peptides were purified by using C18
524 reversed-phase chromatography followed by hydrophilic interaction chromatography (HILIC, Nest Group). We separated
525 purified peptides by online nanoscale HPLC (Agilent) with a C18 reversed-phase column packed 14 cm (ReproSil-Pur
526 C18-AQ 3 µm; Dr. Maisch Products) over an increasing gradient of 3-35% Buffer B (100% acetonitrile, 0.1% formic
527 acid) for 90 min at a flow rate of 300 nl/min. Eluted peptides were analyzed with an Orbitrap Elite mass spectrometer
528 (Thermo Fisher Scientific) operated in a data-dependent mode. The top 15 most intense peptides per MS1 survey scan
529 were selected for further MS2 fragmentation by collision-induced dissociation (CID). We performed MS1 survey scans
530 in the Orbitrap at a resolution of 240,000 at m/z 400 with charge state rejection enabled, whereas CID MS2 was done in
531 the dual linear ion trap with a minimum signal of 1000. We set dynamic exclusion to 30 sec. We employed Maxquant
532 (v1.5.2.8) to analyze raw output data files⁸¹. Peptides were compared with the UniProt human database (02-2015 release).
533 We imposed a strict 1% FDR cut-off to a reverse sequence database. The MaxLFQ algorithm was used for label-free
534 quantification⁸¹. Microsoft Excel and Perseus (v1.5.0.15)⁸² were utilized for data processing. We removed contaminants,
535 decoys and single peptide identifications for further analysis. Zero values in the remaining data were substituted by
536 imputation. The results were pre-processed and certain criteria were used to validate previously known PARK7/DJ-1
537 binding partners and to predict and validate the as yet unknown ones. We selected potential novel binding partners by
538 using a combination of the following criteria: (1) the average mRNA expression levels of the corresponding transcripts
539 in Tregs over the first 6 hrs following TCR stimulation should be higher than 250 (the average expression level of FOXP3
540 in human natural Tregs from the time-series transcriptomic datasets is around 250); (2) the LFQ value of MS-identified
541 proteins bound to DJ-1 antibody should be at least 2.5-fold higher than those bound to the IgG control in human primary
542 T cells; (3) the fold changes in the LFQ value of the bound proteins between stimulated and unstimulated T cells for all
543 the three analysed conditions (Teffs, Tregs with or without IL-2 addition) should be equal to or higher than 2; (4) a similar
544 change trend (increase or decrease) in binding affinity between stimulated and unstimulated samples should be consistent
545 for all the three analysed conditions; (5) the LFQ value of the bound proteins in the eluates of Tregs, with or without IL2
546 addition, should be higher than the 25th percentile of all the detected binding proteins in the given unstimulated or
547 stimulated samples.

548 The known DJ-1 binding partners in human cells were downloaded from the NCBI database. Independent of the mRNA
549 expression levels, the binding partners were considered confirmed when the following two criteria were met: (1) the
550 eluted protein LFQ value was at least 2-fold higher than that in the corresponding IgG controls of either human T cells or
551 the Jurkat cell line (ACC282, DSMZ GmbH); (2) the fold enrichment (stimulated vs. unstimulated) from at least 2 out of
552 the 4 measured conditions (Teffs, Jurkat cells, Tregs with or without IL2 addition) was similar.

553 **Flow cytometry (FACS) and ImageStream for human cells.**

554 Once the cells had been collected and the medium removed, the extracellular proteins of the cells were stained in FACS
555 buffer (PBS (14190169, Thermo Fisher Scientific) containing 2% heat-inactivated FBS (10500-064, Thermo Fisher
556 Scientific) with the cell surface antibodies, the concentration of which was first optimized, for 30 min at 4°C in the dark.
557 Intracellular staining was performed by using the FOXP3 staining kit (421403, BioLegend) following the manufacturer's
558 instructions. Stained cells were analysed with BD LSRFortessa™. We excluded dead cells from the further analysis by
559 using LIVE/DEAD® Fixable Near-IR Dead Cell Stain (L10119, Thermo Fisher Scientific) (dilution 1:500).
560 Various combinations of the following antibodies were used for FACS analysis: mouse monoclonal [RPA-T4] anti-human
561 CD4 APC (561840, BD Biosciences) (dilution 1:200), mouse monoclonal [RPA-T4] anti-human CD4 FITC (555346, BD
562 Biosciences) (dilution 1:200), mouse monoclonal [G44-26] anti-human CD4 V450 (561292, BD Biosciences) (dilution
563 1:200), mouse monoclonal [RPA-T4] anti-human CD4 BUV395 (564724, BD Biosciences) (dilution 1:200), mouse
564 monoclonal [L200] anti-human CD4 PE-Cy7 (560644, BD Biosciences) (dilution 1:200), mouse monoclonal [M-A251]
565 anti-human CD25 APC (555434, BD Biosciences) (dilution 1:200), mouse monoclonal [M-A251] anti-human CD25
566 FITC (555431, BD Biosciences) (dilution 1:200), mouse monoclonal [M-A251] anti-human CD25 V450 (560356, BD
567 Biosciences) (dilution 1:200), mouse monoclonal [HIL-7R-M21] anti-human CD127 V450 (560823, BD Biosciences)
568 (dilution 1:200), mouse monoclonal [50G10] anti-human GARP (221 011, Synaptic Systems GmbH) (dilution 1:100),
569 mouse monoclonal [B56] anti-human Ki-67 V450 (561281, BD Biosciences) (dilution 1:100), mouse monoclonal [206D]
570 anti-human FOXP3 Alexa Fluor 647 (320119, BioLegend) (dilution 1:10), mouse monoclonal IgG1 (50-167-013,
571 BioLegend) (dilution 1:10), mouse monoclonal [22F6] anti-human Helios Pacific blue (137210, BioLegend) (dilution
572 1:100), CellTrace CFSE Cell Proliferation Kit (C34554, Thermo Fisher Scientific) (dilution 1:5000). For staining GARP
573 expression, anti-mouse IgG3 (biotin) (553401, BD) and Streptavidin-Phycoerythrin (PE, 349023, BD Biosciences) were
574 used as secondary antibodies. The FCS files from human-related experiments were analysed by FlowJo 7.6.5 or FlowJo
575 v10 (Tree Star).

576
577 For ImageStream experiments, Tregs and Teffs were stimulated and stained as described above, but with staining panels
578 other than those for the FACS analysis. We used the following staining panels: LIVE/DEAD® Fixable Near-IR Dead
579 Cell Stain (L10119, Thermo fisher Scientific) (dilution 1:500), mouse monoclonal [RPA-T4] anti-human CD4 V450
580 (560346, BD Biosciences) (dilution 1:100), rabbit monoclonal [EP2815Y] anti-human PARK7/DJ-1 Alexa Fluor 488
581 (ab203989, Abcam) (dilution 1:50), rabbit monoclonal [EPR11097(B)] anti-human PDHB Alexa Fluor 594 (ab211838,
582 Abcam) (dilution 1:100), including strict negative controls (rabbit monoclonal [EPR25A] IgG Alexa Fluor 488
583 (ab199091, Abcam) (dilution 1:50) and rabbit monoclonal [EPR25A] IgG Alexa Fluor 594 (ab208568, Abcam) (dilution
584 1:100). The samples were acquired on an ImageStream®^X Mark II Imaging Flow Cytometer (Amnis, EMD Millipore) at
585 60x magnification. The results were analysed by using IDEAS 6.2 software (Amnis) and the Similarity score between
586 PDHB and DJ-1 was determined by means of a similarity dilate algorithm often used for the nuclear translocation
587 quantification of a transcription factor. We utilized the PDHB-positive area as the targeted location and then calculated
588 to what degree DJ-1 was translocated to the PDHB-positive areas. Only cells gated as living focused singlets were
589 included in the analysis.

590 **PDH enzymatic assay.**

591 Various genes were knocked-down for the enzymatic assay, as described in the previous sections. The same number (1E6)
592 of Treg and/or Teff cells were either left unstimulated or stimulated for 5 hrs with Dynabeads Human T-Activator anti-
593 CD3/CD28 (11131D, Thermo Fisher Scientific; the ratio between beads and T cells is 1:1) with (Tregs) or without IL-2
594 (Teffs). Cell pellets were then collected and either lysed in lysis buffer or stored at -80°C until further analysis. Protein
595 concentrations were determined by using the Bradford assay and the same amount of protein was used for each sample
596 within one experiment. PDH activity was determined by means of the colorimetric PDH Activity Assay Kit (MAK183,
597 Sigma-Aldrich) following the manufacturer's recommendations in Nunc MicroWell 96-well microplates (167008,
598 Thermo Fisher Scientific) in an Infinite 200 Pro plate reader (Tecan) measuring the absorbance at 450 nm every 10-30
599 seconds over a time course of 1-5 hrs. Various amounts of recombinant DJ-1 protein (ab51198, Abcam) were used (0-20
600 µg) to test the effect of recombinant human DJ-1 on PDH activity.

601 **Nucleotide analysis.**

602 The gene knockdown protocol described above was used to prepare knockdown conditions in Treg cells, namely control
603 si_NS (sc-37007, Santa Cruz) and si_PARK7/DJ-1 (SI00301091, Qiagen) and two knockdown conditions in Teff cells,

604 namely si_NS (sc-37007, Santa Cruz) and si_DJ-1 (SI00301091, Qiagen). On the following day, half of the samples were
605 stimulated with Dynabeads Human T-Activator CD3/CD28 for cell activation (11131D, Thermo Fisher Scientific) for 1
606 day with IL2 (Tregs) or without IL-2 (Teffs) in 48-well plates in a total volume of 1ml complete IMDM medium+10%
607 FBS. An aliquot of 0.5 ml cell suspension was transferred to a 2 ml Eppendorf tube containing 1.5 ml of 60% methanol,
608 0.85 % AMBIC, pH=7.4 at -60°C. The tubes were gently shaken before the cells were separated by centrifugation (3 min,
609 500 G, -10°C). The supernatant was discarded and the cell pellet was stored at -80°C until intracellular metabolite
610 extraction. Intracellular metabolites were extracted by using 50% Methanol TE buffer, pH=7.0 at -20°C. Chloroform at -
611 20°C was used to improve the cell lysis. Cell lysates were taken from the freezer, resuspended in extraction fluid and
612 chloroform and incubated on a shaking device at -20°C for 2 hrs. Next, cell debris were separated by centrifugation (10
613 min, 10 000 rpm, -10°C). The extract was filtered (0.22 µm CA) and stored at -80°C until analysis. The volume of
614 extraction fluid and chloroform was adjusted to the cell number in the pellet (0.5 ml per 3E6 cells). Extracted nucleotides
615 were separated on a SeQuant® ZIC®-HILIC 3.5µm, 100 Å 150 x 2.1 mm column (Merck Millipore) at 25 °C and 0.400
616 mL/min and analyzed on a Dionex UltiMate 3000 UHPLC Systems coupled to a Q Exactive™ Hybrid Quadrupole-
617 Orbitrap™ Mass Spectrometer (Thermo Fisher Scientific). The separation of the nucleotides was carried out using a
618 binary gradient with buffer A: water and B: 90 % acetonitrile, both containing 20 mM ammonium acetate with pH 7.5.
619 The liquid chromatography started with a 10 min linear gradient of 90 – 50 % of B, followed by a second linear gradient of
620 50 – 20 % B for 1 min. The washing step was completed within 1 min by increasing buffer B to 90 %, followed by 8 min
621 of column equilibration with initial condition. Full MS scans were acquired at a target value of 3e6 ions with resolution
622 $R = 70,000$ over 3 mass ranges of 345.5 – 346.5; 425.5 – 426.5; 505.5 – 506.5 m/z.

623 Mouse-related experiments.

624 B6.129P2-Park7^{Gt}(XE726)^{Byg}/Mmucd mice were developed and characterized as described elsewhere ⁴⁶. The *Dj-1*^{-/-} (*KO*),
625 *Dj-1*^{+/-}, *Dj-1*^{+/+} (WT) mice used in our experiments were gender- and age-matched siblings generated from heterozygous
626 *Dj-1*^{+/-} breeding pairs. All mice were maintained in our SPF animal facility and all animal experimental procedures were
627 performed following the approval of the Animal Welfare Society (AWS) of University of Luxembourg and Luxembourg
628 Institute of Health.

629 FACS for murine T cells.

630 The same number of cells from spleen or peripheral lymph nodes were first incubated with anti-mouse CD16/CD32 Fc
631 blocker (553141, BD Biosciences) and then stained with various surface and intracellular antibodies. Cell numbers were
632 determined by CASY (Innovatis AG). Cell surface markers were stained by rat anti-mouse CD4-BUV496 (564667, BD
633 Biosciences) (dilution 1:200), rat anti-mouse CD25-PE-Cy7 (25-0251-82, eBioscience) (dilution 1:200), rat anti-mouse
634 CD8-BUV805 (564920, BD Biosciences) (dilution 1:200), hamster Anti-Mouse PD-1-BV711 (744547, BD Biosciences)
635 (dilution 1:100), rat anti-mouse GITR-BUV395 (740322, BD Biosciences) (dilution 1:100) and rat anti-mouse ICOS-
636 FITC (11-9942-82, eBioscience) (dilution 1:100). Of note, all these antibodies might not be necessarily used in one
637 staining panel due to the spectrum compatibility. For cytokine staining, cells were fixed and permeabilized with
638 Cytofix/Cytoperm buffer (554714, BD Biosciences). Anti-IL-10 (505028, BioLegend) antibody was diluted in
639 Perm/Wash buffer (554714, BD Biosciences). Intracellular staining for Foxp3 (17-5773-82, eBioscience) (dilution
640 1:100), CTLA4 (12-1522-83, eBioscience) (dilution 1:100), Helios (137220, BioLegend) (dilution 1:100) and Ki67 (48-
641 5698-82, eBioscience) (dilution 1:100) was performed by using the Foxp3 staining kit (00-5523-00, eBioscience). Rat
642 anti-mouse Ki67-BV605 (652413, BioLegend) (dilution 1:100) was used in the thymic analysis. Samples were measured
643 on a BD LSRFortessa™ and data were analysed with FlowJo (v10, Tree Star).

644 Murine Treg suppression assay.

645 Spleens and lymph nodes were minced through a 70 µm-pore strainer. Centrifuge the cell suspension at 350g for 5 min
646 at 4 °C. Discard the supernatant and add 3ml of 1x Red blood lysis buffer for spleen per mouse and incubate at room
647 temperature for 7 min. Add 10 ml of FACS buffer and centrifuge the cells and discard the supernatant. Using CD90.2
648 microbeads to isolate the CD90.2+ pan T cells. Stain the cells with antibodies of anti-mouse CD4-FITC (11-0042-82,
649 eBioscience) (dilution 1:200) and anti-mouse CD25-PECY7 (557192, BD Bioscience) (dilution 1:200) at 4 °C for 20
650 mins plus LIVE/DEAD® Fixable Near-IR (L10119, Thermo Fisher Scientific) (dilution 1:500) for dead cell Staining.
651 After staining, sort Tregs (CD4⁺CD25^{high}) and conventional CD4⁺CD25⁻ T cells (Tconv) by BD FACSAria™ III sorter.

652 Tconv (1E5) from WT C57BL/6 mice were first labelled with 1 μ M CFSE (C34554, Life Technology). Tregs were sorted
653 from *Dj-1^{-/-}* mice or wild-type (WT) littermates. Splenocytes depleted of T cells by using CD90.2 beads (130-049-101,
654 Miltenyi Biotec) were irradiated with a total dose of 30 Gy by RS2000 (Rad Source Technologies) and used as feeder
655 cells (2E5). Tconv cells were co-cultured with irradiated feeder cells and Treg cells from various genotypes in the
656 presence of 1 μ g/ml soluble anti-CD3 antibody (no azide/low endotoxin, 554829, BD Biosciences). The mice T-cell
657 culture media is the complete RPMI media, which included RPMI 1640 medium (21870084, Thermo Fisher Scientific)
658 supplemented with 10% heat-inactivated fetal bovine serum (FBS, 10500-064, Thermo Fisher Scientific), 50 U/ml
659 penicillin, 50 μ g streptomycin (15070-063, Thermo Fisher Scientific), 1 mM sodium pyruvate (11360070, Thermo Fisher
660 Scientific), 2 mM GlutaMAX (35050061, Thermo Fisher Scientific), 0.1 mM non-essential amino acids (M7145, Sigma
661 Aldrich), 50 μ M beta-mercaptoethanol (M7522, Sigma Aldrich) and 10 mM HEPES (15630080, Thermo Fisher
662 Scientific). All the mice cells were cultured in 37°C 5% CO₂ incubators, unless specified. Gated CD4⁺ viable single
663 CFSE-labelled cells were measured three days later in a BD LSRFortessa™. The viability of cells was assessed by using
664 the LIVE/DEAD® Fixable Near-IR Dead Cell Stain kit (L10119, Thermo Fisher Scientific) (dilution 1:500).

665 **EAE model.**

666 The EAE model was performed as previously described⁴⁹. In brief, each mouse was injected subcutaneously (s.c.) with
667 115 μ g MOG35-55 peptide (Washington Biotech) emulsified in CFA (263810, Difco) plus an intraperitoneal (i.p.)
668 injection of 300 ng pertussis toxin (NC9675592, List Biological) on days 0 and 2. Clinical signs were assessed daily as
669 described⁸³.

670 **Intracellular cytokines measurement.**

671 For intracellular cytokine measurement, cells (2E5) from spleen and draining lymph nodes were re-stimulated by 50
672 ng/ml PMA (Phorbol 12-myristate 13-acetate, P8139, Sigma-Aldrich) and 750 ng/ml ionomycin (I0634, Sigma-Aldrich)
673 in the presence of Golgiplug (555029, BD Biosciences) and Golgistop (554724, BD Biosciences) for 5 hrs in 96-well
674 plates. Following cell surface staining, cells were fixed and permeabilized with Cytotfix/Cytoperm buffer (554714, BD
675 Biosciences).

676 **qPCR of sorted murine Tregs.**

677 Total CD25^{high}CD4⁺ Tregs were sorted from spleen of young adult (8-12 wks) and very old (~75 wks) WT *Dj-1^{+/+}* mice.
678 RNA was extracted by using the RNeasy Mini Spin Kit (74104, Qiagen) including column-genome DNA removal steps.
679 cDNA was synthesized by means of the SuperScript III Reverse Transcriptase Kit (18080-044, Invitrogen). qPCR was
680 performed with the LightCycler 480 SYBR Green I Master Mix (4707516001, Roche Applied Science) and on the
681 LightCycler 480 (384-well) real-time PCR platform (Roche) as previously described⁴¹. The housekeeping gene for
682 detecting *Dj-1* mRNA expression (QT00167013, Qiagen) in total Tregs was *Rps13* (QT00170702, Qiagen).

683 **Microarray analysis.**

684 RNA samples of CD25^{high}CD4⁺ T cells (Tregs) sorted from around 45-week-old *Dj-1^{-/-}* mice and *Dj-1^{+/+}* littermates, were
685 analysed with the Affymetrix mouse Gene 2.0 ST Array at EMBL Genomics core facilities (Heidelberg). Samples were
686 first checked with an Agilent Bioanalyzer 2100 by using the RNA 6000 Pico-kit (50671513, Agilent) and only RNA
687 samples with RIN higher than 8.5 were further processed for microarray measurement. The expression signal at the exon
688 level was summarized by the Affymetrix PLIER algorithm⁸⁴ with DABG and PM-GCBG options by means of the sketch-
689 quantile normalization approach (Affymetrix Expression Console v1.4). The corresponding probesets were considered
690 differentially expressed if they passed the following combinatory filters⁸⁵: (a) whether the change folds were ≥ 2
691 between the means of DJ-1 KO and WT Tregs or Teffs; (b) whether the P-value, resulting from a two-tailed Student t-
692 test, was ≤ 0.05 ; (c) whether the cross-hyb type of the probeset was equal to 1; (d) whether the probeset with the highest
693 expression level was higher than 100 (with the median value of ~90 for each our sample); (e) for a given group (e.g. WT)
694 with the higher mean intensity value of the probeset, whether the probeset in all the replicates of the given group was
695 detected as 'present' according to the default setting of the Affymetrix Expression Console. DAVID v6.7 was used to
696 perform functional enrichment analysis for the lists of differentially up- or down-regulated genes⁸⁶. To simplify the
697 analysis of the DAVID enrichment results, we mainly selected representative GO-terms and KEGG pathways with a P-
698 value < 0.05 and fold enrichment ≥ 1.5 from each enrichment cluster. Heatmaps were generated using the R Heatmap.2
699 (gplots package) with scaled row values (with mean 0 and standard deviation 1). The microarray data were deposited into

700 the GEO series database ([GSE115269](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE115269)).

701 **Seahorse metabolic assay.**

702 CD4⁺CD25^{high} Tregs and CD4⁺CD25⁻ Tconv cells from the spleen of old *Dj-1* (~45 wks) KO and WT mice were sorted
703 on BD FACSAria™ III. 4E5 freshly isolated cells were allowed to rest for 3 hrs in the complete RPMI media before
704 being plated in wells by using Cell-TAK (354240, Corning). Tregs were pooled together from different mice of the same
705 group to reach 4E5/well due to a limited number of Tregs in each individual mouse. The oxygen consumption rate (OCR)
706 was measured in XF base medium (102353-100, Agilent Technologies, from the Seahorse XF Cell Mito Stress Test Kit),
707 containing 1 mM pyruvate (S8636, Sigma-Aldrich), 2 mM L-glutamine (G8540, Sigma-Aldrich) and 25 mM glucose
708 (G8769, Sigma-Aldrich), under basal conditions and in response to 1 μM Oligomycin (103015-100, Agilent
709 Technologies), 1.5 μM FCCP (carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone) (103015-100, Agilent
710 Technologies) and 1 μM Rotenone and 1 μM Antimycin A (103015-100, Agilent Technologies) by the Seahorse XF96
711 analyser (Agilent). The results were analysed by Wave 2.6.0 (Agilent Technologies).

712 **Treg cell proliferation and iTreg differentiation assay.**

713 CD4 T cells were first enriched by anti-mice CD4 (L3T4) microbeads (130-117-043, Miltenyi Biotec) from spleen and
714 peripheral LNs following manufacturer's instructions. Stain the CD4 T cells with antibodies of anti-mouse CD4-FITC
715 (11-0042-82, eBioscience) (dilution 1:200), anti-mouse CD25-PECY7 (557192, BD Bioscience) (dilution 1:200), anti-
716 mouse CD44-PE (560569, BD Bioscience) (dilution 1:200) and anti-CD62L-PerCP-CY5.5 (560513, BD Bioscience)
717 (dilution 1:200) plus LIVE/DEAD® Fixable Near-IR (L10119, Thermo Fisher Scientific) (dilution 1:500) for dead cell
718 Staining. After staining, sort the naïve CD4 T cells (CD4⁺CD25⁻CD62L^{high}CD44^{low}) on BD FACSAria™ III sorter.

719 Naïve CD4 T cells were isolated from spleen and peripheral LNs of *Dj-1*^{+/+} WT B6 mice aged 8-12 weeks old. Add 1 μl
720 of 5 mM CellTrace™ Violet dye (CTV, C34557, Thermo Fisher Scientific) into 1 ml of PBS and use it to suspend naïve
721 CD4 T cells. Incubate the cell mixture at 37°C for 7 min and then wash the cells with complete medium for three times.
722 1.5E5 naïve CD4 T cells were seeded in one well of 48-w plate with flat bottom coated 2 μg/ml of anti-mouse CD3
723 (553057, BD Bioscience) and 2 μg/ml of anti-mouse CD28 (553294, BD Bioscience) antibodies. Add 2ng/ml human
724 TGF-β (100-21, Peprotech) and 10U/ml mouse IL-2 (212-12, Peprotech) for iTreg differentiation. Cells were treated with
725 PDH inhibitor (CPI-613) (SML0404, Sigma Aldrich) with different concentrations or the vehicle (DMSO, D2650, Sigma
726 Aldrich) or dichloroacetate sodium (DCA, 347795, Sigma Aldrich). Cells were incubated in the incubator for 3 days. On
727 day 2, exchange half of the medium with cytokines and the inhibitor CPI-613 or DCA. On day3, harvest the cells and
728 perform the staining for FACS analysis.

729 For nTregs proliferation, CD4⁺CD25^{high} nTregs were isolated from the spleen and peripheral LNs of WT B6 mice aged
730 8-12 weeks old and labelled by CellTrace™ Violet dye. 1E5 nTregs were seeded into 96-well round bottom plate coated
731 with 1 μg/ml of anti-CD3 and 1 μg/ml of anti-CD28 antibodies. 50 U/ml of IL2 was added for nTreg proliferation. On
732 day3, harvest the cells and perform the staining for FACS analysis.

733 **Statistical analysis.**

734 P values were calculated with non-paired two-tailed Student t test (Graphpad prism or Excel) as specified in Figure legend.
735 The EAE clinical scores between WT and KO groups were compared by paired two-tailed Student t test (Graphpad). All
736 error bars represent the standard deviation. The P-values associated with Pearson correlation analysis were from a two-
737 tailed test generated by GraphPad Prism.

738 **Data availability.**

739 The microarray data used in this study have been deposited in the Gene Expression Omnibus (GEO) under the access
740 number of [GSE115269](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE115269). To review, go to <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE115269> Enter the
741 token **qlqbmoqmpryvxp** into the box.

742 The proteomic mass spectrometry data [[doi:10.25345/C5437F](https://doi.org/10.25345/C5437F)] of the Co-IP analysis has been deposited in
743 ProteomeXchange via MassIVE ⁸⁷ under the identifier PXD016610. To review, go to
744 <https://massive.ucsd.edu/ProteoSAFe/static/massive.jsp> (login in the top right of the webpage) User:
745 **MSV000084659_reviewer**; Password: **ISB.FengHe.2019**

746

747 Expanded view figures and tables

748 Expanded view (EV) figures and tables are attached in the end of the manuscript and will be available online.

749 Author contributions

750 E.D. and N.Z. designed and performed major parts of the healthy human- and mouse-related experiments, respectively.
751 N.Z. analyzed mouse-related data. C.C. performed healthy human-, patient- and mouse-related experiments. N.P. and
752 C.L.L. performed and analyzed metabolome-related experiments. D.G.F. and S.D. performed parts of primary human T-
753 cell related experiments. G.G.G. and J.C.S contributed to the Seahorse assays. M.G. and J.R. performed and analyzed
754 proteomic experiments. H.K., M.G. and D.B. performed EAE-related experiments. C.G. supervised the flow-cytometry
755 related experiments. D.C. and C.L. supervised mouse-related experiments. D.M.V.W. performed part of EAE
756 experiments. W.W., R.K., R.B. and M.O. provided insights and supervised parts of the experiments. R.B. and M.O.
757 revised the manuscript. F.H. conceived and directed the project and wrote the manuscript.

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769 Competing financial interests

770 The authors declare no competing financial interests.

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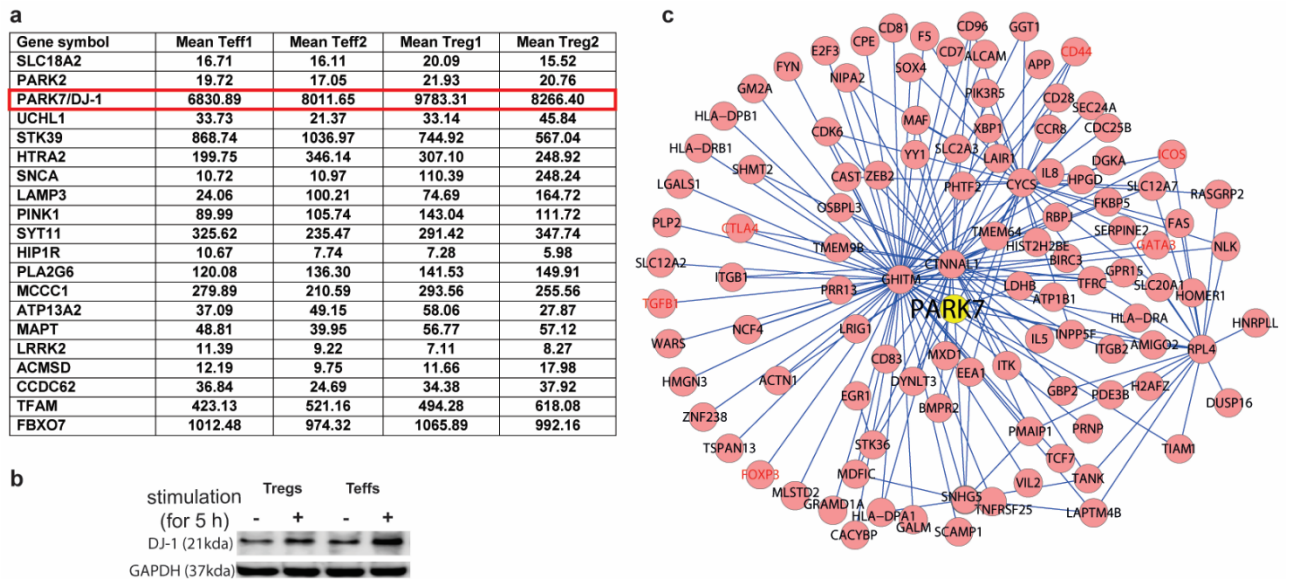
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Figures



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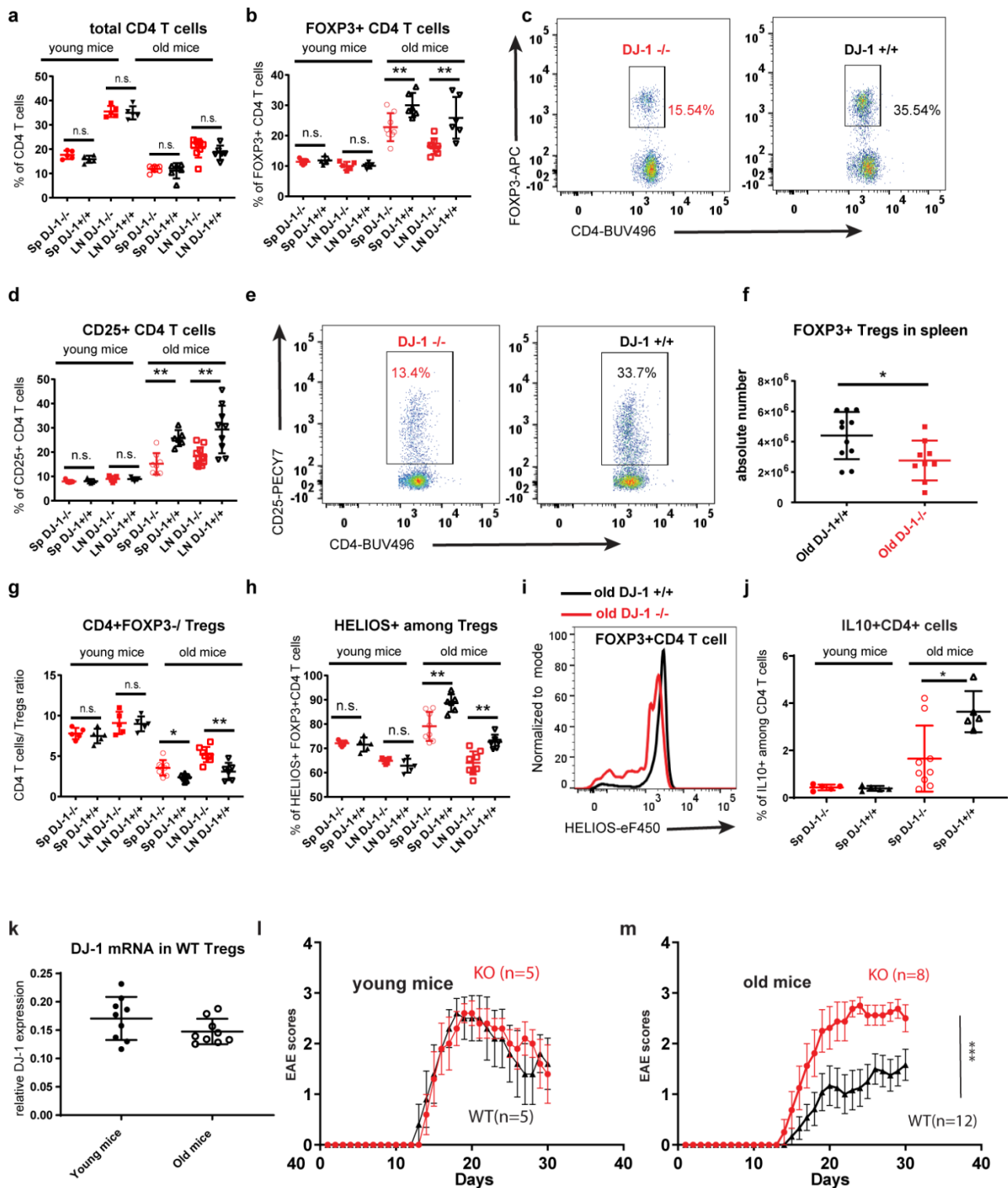
Figure 1. DJ-1 is highly expressed in human Tregs and connected with well-known Treg genes in Treg specific-correlation network.

975 **a**, mean transcription expression of the known PD genes measured in the first 6 h of anti-CD3/-CD28/IL2
 976 stimulation assessed by our published high-time-resolution (HTR) time-series transcriptome (with 19 time
 977 points) ⁴¹.

978 Treg1 and Treg2 (or Teff1 and Teff2) represent two independently repeated HTR time-series experiments for
 979 Tregs (or Teffs). DJ-1 is highlighted by red rectangle.

980 **b**, DJ-1 protein expression in human primary Tregs and Teffs with or without anti-CD3/-CD28/IL-2 stimulation
 981 assessed by Western Blotting.

982 **c**, The PARK7/DJ-1 subnetwork extracted from the constructed human Treg-specific correlation network. Each
 983 oval represents one gene. A selection of known key Treg genes were highlighted in red. Each line between
 984 DJ-1 and the other genes represents a correlation-based functional linkage and the other genes represents a
 985 correlation-based functional linkage.



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Figure 2. *Dj-1* depletion reduces Treg accumulation in old mice.

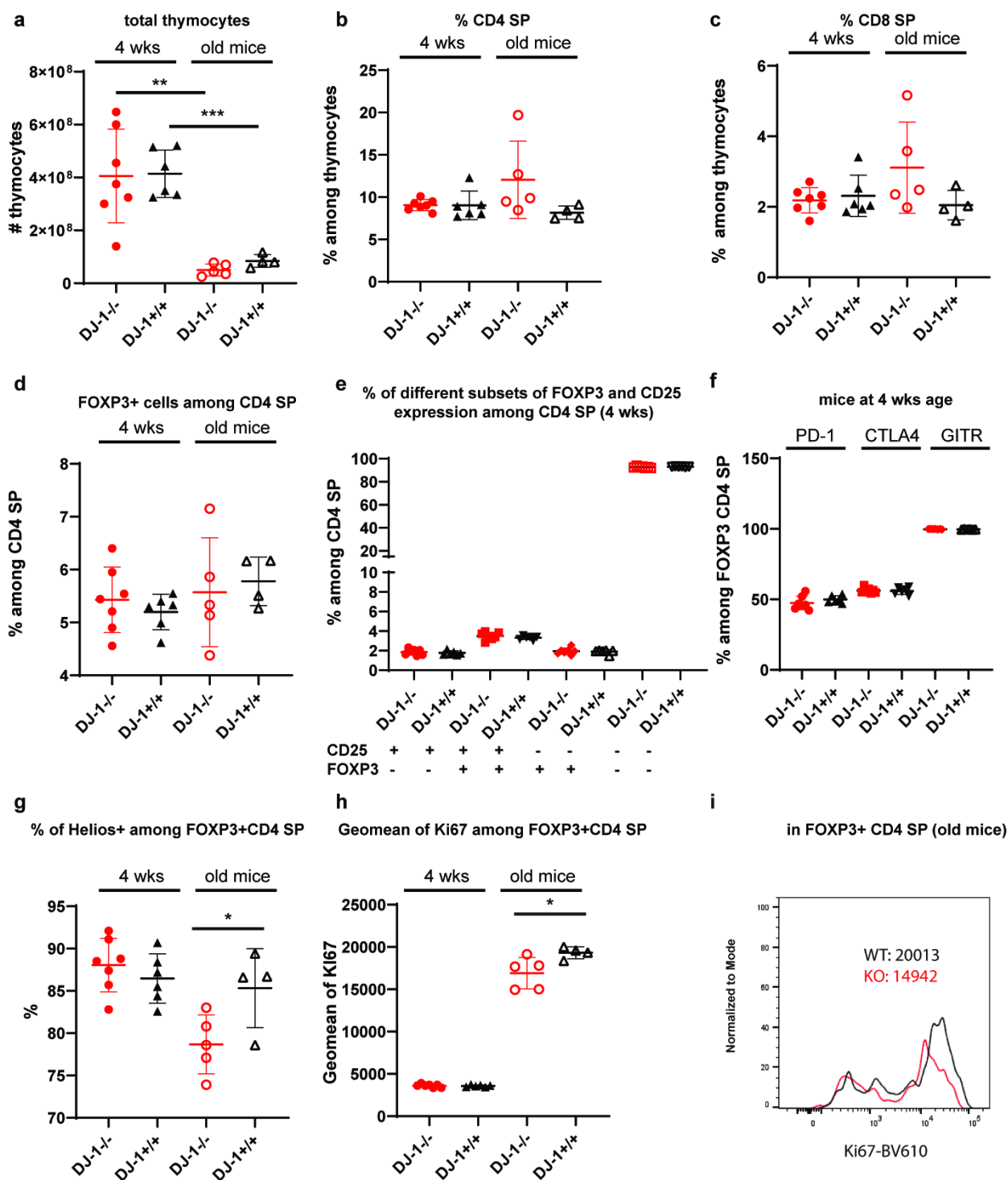
989 **a**, Percentages of CD4⁺ T cells from spleen (Sp) or peripheral lymph nodes (LN) (young KO, n=5; young WT, n=5; old KO, n=8; old WT, n=6; for old mice, data pooled from 2 independent experiments). Each symbol represents one mouse unless otherwise mentioned.

992 **b**, Percentages of FOXP3⁺ subsets among CD4 T cells.

993 **c**, Representative flow-cytometry plots of FOXP3⁺ cells among total CD4 T cells in spleen of old WT or DJ-1 KO mice.

994

995 **d**, Percentages of CD25+ subsets among CD4 T cells (young mice number as in **a**, but for old mice: spleen
996 WT, n=6;spleen KO, n=8;LN WT, n=9; LN KO, n=11, data pooled from 2 independent experiments).
997 **e**, Representative flow-cytometry plots of CD25+ cells among total CD4 T cells in spleen of old WT or DJ-1
998 KO mice.
999 **f**, Number of FOXP3⁺CD4⁺ T cells in spleen of indicated genotype mice (estimated from total splenocytes, WT,
1000 n=9; KO, n=11, pooled data).
1001 **g**, The relative ratios between non-Treg cells and FOXP3⁺CD4⁺ Tregs.
1002 **h**, Percentages of Helios⁺ subsets among FOXP3⁺CD4⁺ Tregs.
1003 **i**, Representative histogram overlay of Helios expression among total FOXP3⁺CD4⁺ Tregs between old WT or
1004 DJ-1 KO mice.
1005 **j**, Expression of IL-10 in CD4 T cells after in-vitro stimulation using PMA/ionomycin for 5 h.
1006 **k**, qPCR quantification of Dj-1 expression in sorted Tregs from young or old WT mice (n=3/group and 3
1007 technical replicates/mouse). Results represent at least four (**a-j**) and two (**k,l,m**) independent experiments.
1008 Data are mean± s.d. The P-values are determined by a two-tailed Student's t-test. ns or unlabelled, not
1009 significant, *P<=0.05, **P<=0.01 and ***P<=0.001.
1010 **l, m**, EAE clinical scores on the indicated days following immunization with MOG₃₅₋₅₅ in young (**l**) or old mice
1011 (**m**). The number of age- and gender-matched mice per group are indicated in the subfigures. Data are
1012 representative for young mice and a summary of 2 independent experiments for old mice groups. Young mice
1013 (~12 wks, left) and old mice (~45 wks, right).



1014

1015 **Figure 3. *Dj-1* ablation does not affect thymic Treg development independent of age.**

1016 **a**, Total thymocytes of 4-wk-old or old *Dj-1* KO and WT mice (young KO, n=7; young WT, n=6; old KO, n=5; old
1017 WT, n=4).

1018 **b, c**, Percentages of CD4 single positive (SP) cells (**b**) and CD8 SP (**c**) among thymocytes.

1019 **d**, Percentages of FOXP3⁺ among CD4SP cells.

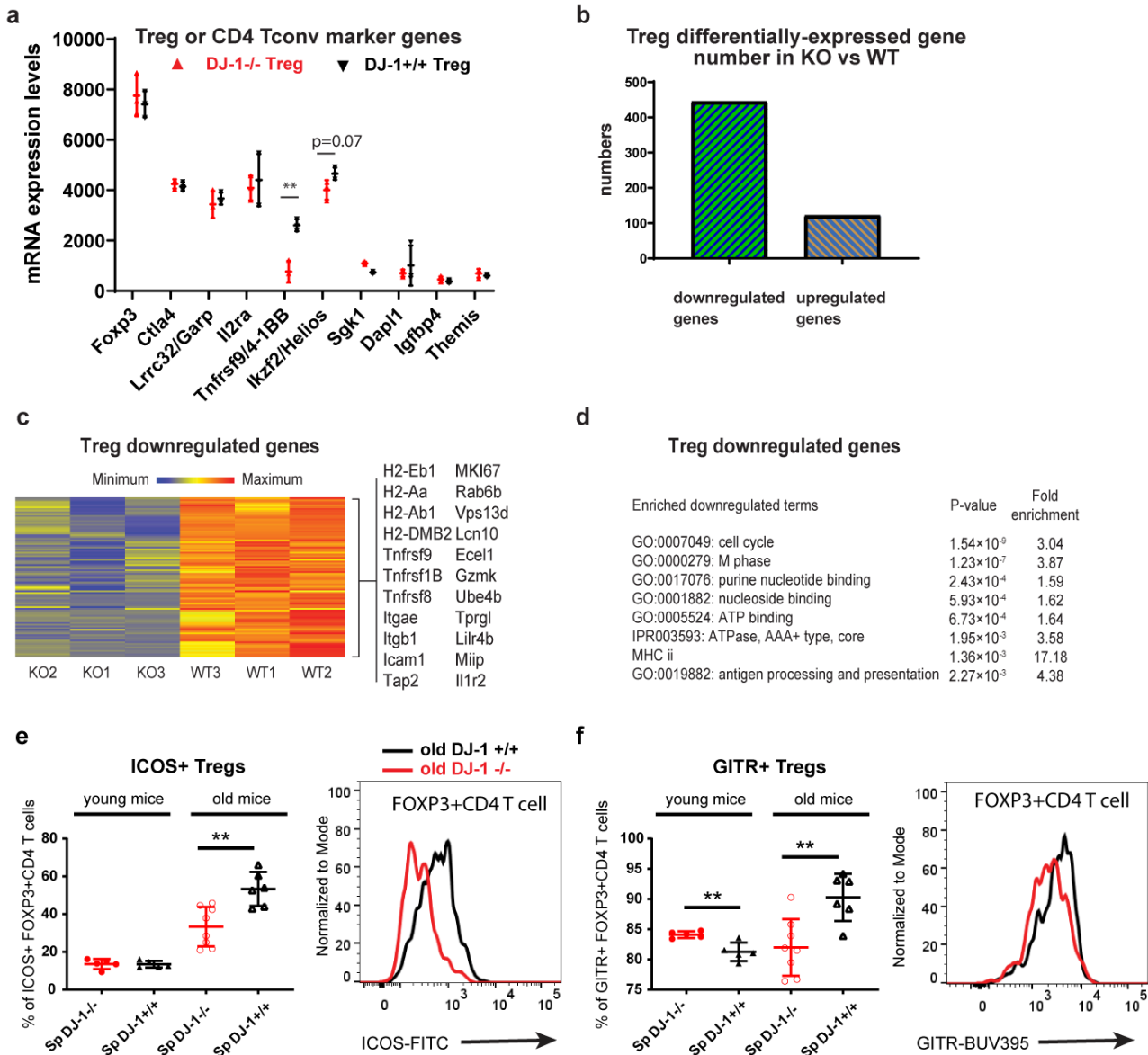
1020 **e**, Percentages of PD-1, CTLA4 and GITR among FOXP3⁺CD4SP cells in 4-wk-old mice

1021 **f**, Percentages of the four subsets defined by the presence or absence of FOXP3 and CD25 among CD4SP
 1022 cells in 4-wk-old mice.

1023 **g**, Percentages of Helios⁺ among FOXP3⁺CD4SP cells.

1024 **h**, Geometric mean of Ki67 among FOXP3⁺CD4SP cells.

1025 **i**, Representative histogram overlay of Ki67 expression among total FOXP3⁺CD4⁺SP cells between old WT or
 1026 *Dj-1* KO mice. Results represent three (**a-i**) independent experiments. Data are mean± s.d. The P-values are
 1027 determined by a two-tailed Student's *t*-test. ns or unlabeled, not significant, *P<=0.05, **P<=0.01 and
 1028 ***P<=0.001.



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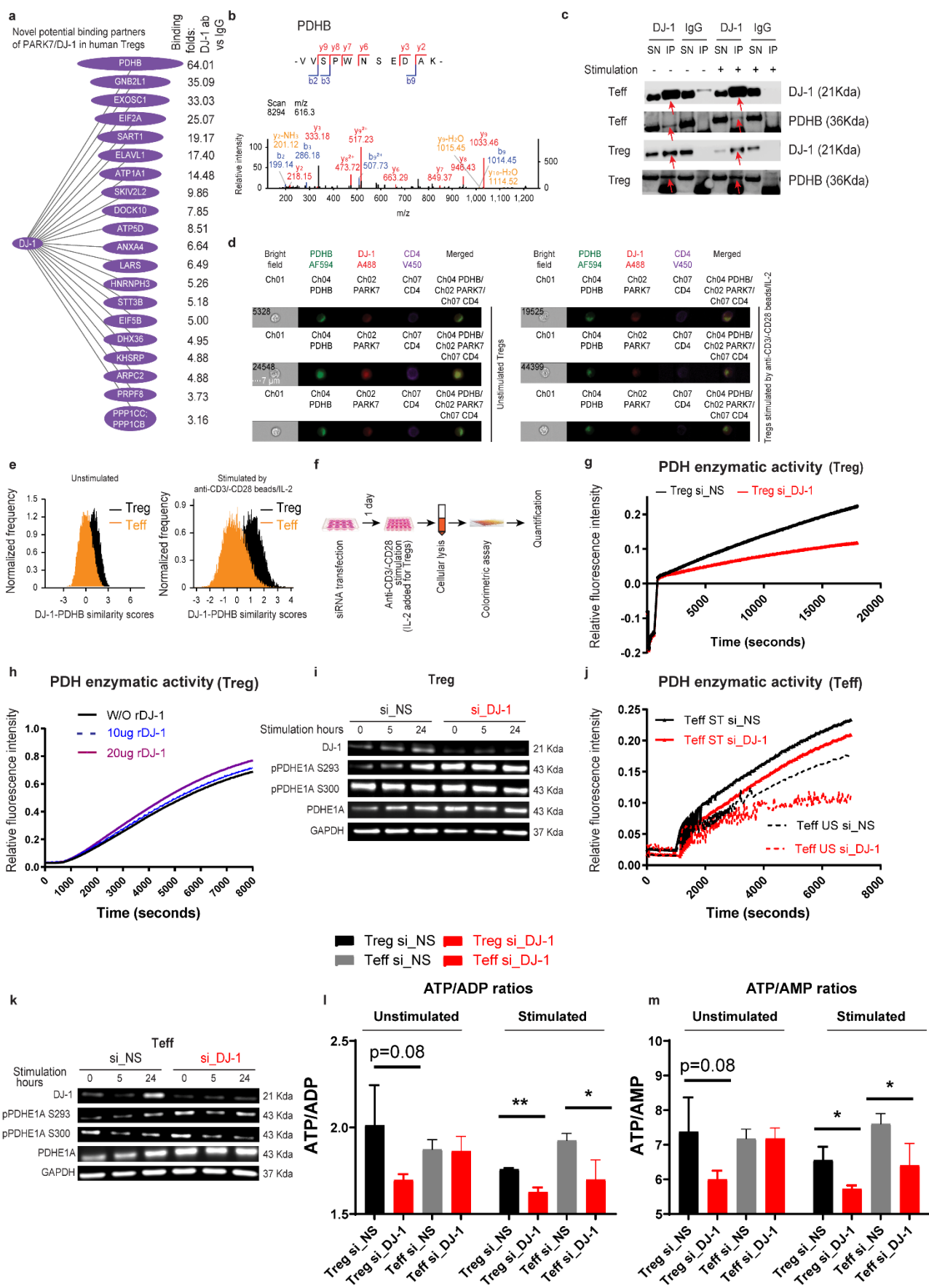
1030 **Figure 4. *Dj-1* depletion impairs Treg proliferation and activation pathways in old mice**

1031 **a**, mRNA expression of upregulated Treg-signature and Tconv-signature genes in Tregs freshly isolated from
 1032 old *Dj-1* KO and WT littermates.

1033 **b**, The number of differentially-expressed up- and down-regulated genes in Tregs.

1034 **c**, Heatmap showing relative abundance of selected differentially downregulated genes in Tregs from old *Dj-1*
 1035 KO mice vs. the age-matched WT littermates. n=3 mice/group

1036 **d**, The selected significantly enriched GO terms among downregulated genes in Tregs from old *Dj-1* KO mice
1037 vs. the age-matched WT littermates.
1038 **e, f**, Percentages of ICOS+ (**e**) or GITR+ (**f**) subsets among FOXP3+CD4+ T cells (left) and representative
1039 histogram overlay of ICOS expression among total FOXP3+CD4+ Tregs between old WT or *Dj-1* KO mice
1040 (right) (young KO, n=5; young WT, n=5; old KO, n=8; old WT, n=6). Results are the summary of the
1041 microarray datasets. Data are mean± s.d. The P-values are determined by a two-tailed Student's *t*-test. ns or
1042 unlabelled, not significant, *P<=0.05, **P<=0.01 and ***P<=0.001.

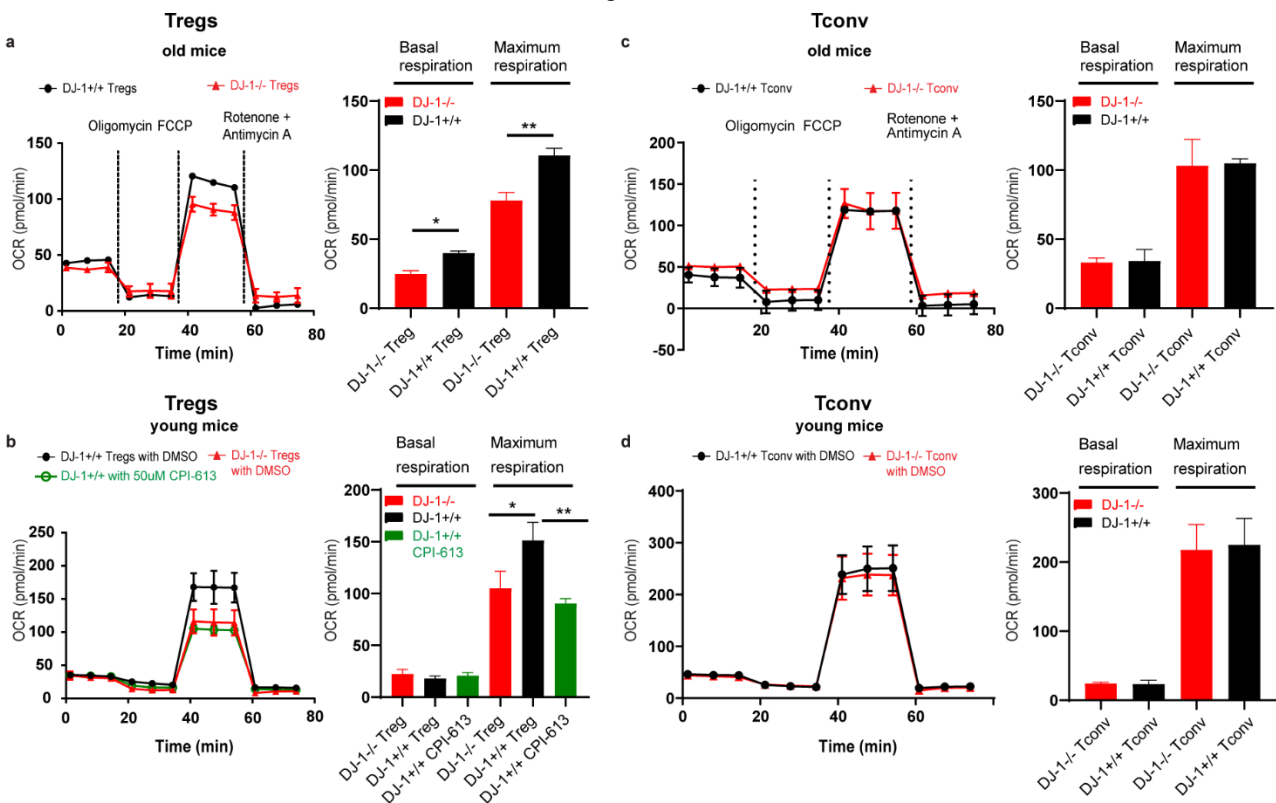


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Figure 5. DJ-1 promotes PDH activity selectively in Tregs.

1045 **a**, Potential novel binding partners of DJ-1 as identified by the co-IP-MS analysis. The binding enrichment of
 1046 the corresponding protein to anti-DJ-1 ab relative to IgG control in human primary Tregs is presented on the
 1047 plot. The width of the oval is relatively correlated to the binding specificity.
 1048 **b**, Representative MS/MS spectrum for a peptide used to identify PDHB. Observed b- and y- fragment ions
 1049 are labeled within the spectrum, and summarized in the peptide sequence shown above.
 1050 **c**, Validation of DJ-1 bound to PDHB preferentially in human Tregs relative to Teffs by Co-IP-immunoblotting
 1051 (Co-IP-IB) analysis. The red arrow indicates DJ-1 or PDHB band from anti-DJ-1 IP elution. Co-IPs were
 1052 performed with anti-DJ-1 or IgG in the lysates of primary human Tregs/Teffs with or without anti-CD3/-CD28/IL-
 1053 2 stimulation for 5 hours. To independently validate the cobinding discovery from the MS analysis, the cells
 1054 used in the immunoprecipitation-immunoblotting and colocalization analysis were isolated from different
 1055 healthy donors. SN, supernatant; IP, eluted complexes pulled out by anti-DJ-1 or IgG control ab.
 1056 **d**, Representative single cell images using Imagestream. While scale bar, 7 μ m; original magnification x60.
 1057 **e**, DJ1/PARK7 and PDHB are preferentially co-localized in Tregs relative to Teffs. The similarity scores are
 1058 trans-location similarity scores showing to which degree DJ-1/PARK7 is translocated to the specific area,
 1059 where PDHB is located.
 1060 **f**, Schematic on how the enzymatic assay of PDH was performed.
 1061 **g**, Representative data of PDH enzymatic activity of stimulated human Tregs treated with specific siRNA
 1062 against DJ-1 or non-specific scrambled siRNA.
 1063 **h**, PDH enzymatic activities of stimulated human Tregs without (W/O) or with various amount of addition of
 1064 recombinant human DJ-1 protein.
 1065 **i, k**, Site-specific phosphorylation and expression of PDHA in Tregs (**i**) or Teffs (**k**) transfected with or without
 1066 DJ-1 knockdown followed by different stimulation periods.
 1067 **j**, PDH enzymatic activities of unstimulated (US) or stimulated (ST) Teffs with or without *DJ-1* knockdown.
 1068 **l, m**, Ratios between intracellular ATP and ADP (**l**) or between intracellular ATP and AMP (**m**) in Tregs or
 1069 Teffs with or without *DJ-1* knockdown, and with or without stimulation. Results represent four (**c, g**), three (**d,**
 1070 **e, i, j, k, l, m**) and one (**a, b**) independent experiments. Data are mean \pm s.d. The P-values are determined by
 1071 a two-tailed Student's *t*-test. ns or unlabelled, not significant, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

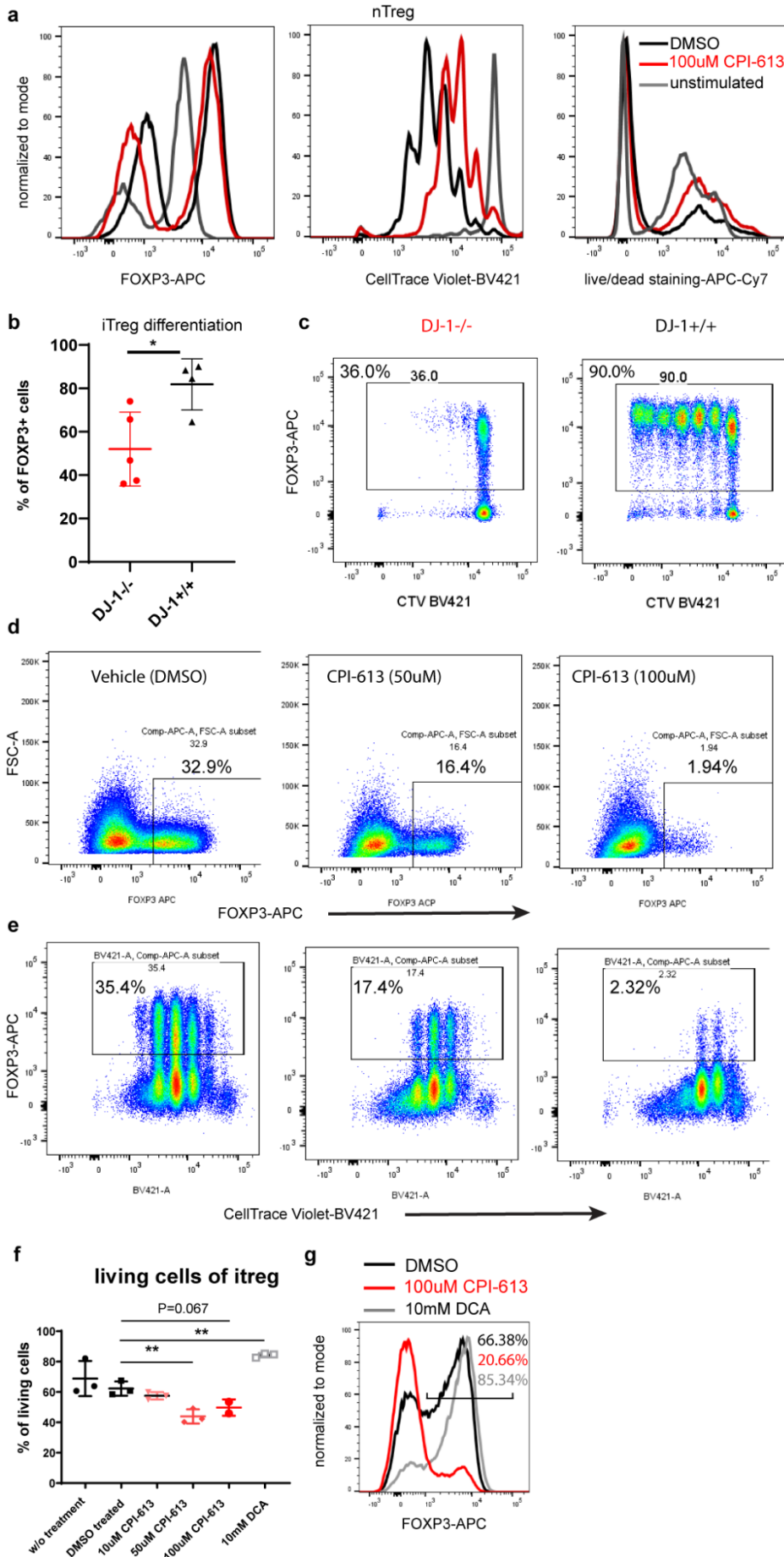


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Figure 6. *Dj-1* depletion impairs OXPHOS selectively in Tregs.

1074 **a, b, c, d**, Oxygen consumption rate (OCR) of freshly isolated Tregs (**a**, old mice; **b**, young mice) and CD4
1075 conventional cells (Tconv) (**c**, old mice; **d**, young mice) from *Dj-1 KO* and WT mice. left of each panel,
1076 representation plots following Mito stress test. right of each panel, quantitation of basal and maximum
1077 respiration under Mito stress test conditions. Results represent three (**a-d**) independent experiments. Data are
1078 mean± s.d. The P-values are determined by a two-tailed Student's *t*-test. ns or unlabelled, not significant,
1079 *P<=0.05, **P<=0.01 and ***P<=0.001.



1081

Figure 7. *Dj-1* depletion inhibits nTreg proliferation and iTreg differentiation via PDH

1082

a, Representative histogram overlay of FOXP3 expression, cell proliferation (as labelled by celltrace violet [CTV]) or cell survival as measured by live/dead staining among CD4⁺ cells differentiated from Th0 cells under the iTreg differentiation condition stimulated for 3 days treated with PDH inhibitor (CPI-613) or control vehicle or without stimulation.

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b, Percentages of FOXP3 expression among DJ-1 KO or WT CD4⁺ cells differentiated from Th0 cells under the iTreg differentiation condition stimulated for 3 days.

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c, Representative flow-cytometry plots of FOXP3 and CTV staining among DJ-1 KO or WT CD4 cells differentiated from Th0 cells under the iTreg differentiation stimulated condition for 3 days.

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d, Representative flow-cytometry plots of FOXP3 expression among total WT CD4 T cells differentiated from Th0 cells treated with different doses of CPI-613 or control vehicle.

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e, Representative flow-cytoemtry plots of FOXP3 and CTV staining among total WT CD4 T cells differentiated from Th0 cells treated with different doses of CPI-613 or control vehicle.

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f, Percentage of living cells under the iTreg differentiation condition for 3 days with different doses of CPI-613 or DCA or control vehicle.

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g, Representative histogram overlay of FOXP3 expression differentiated from Th0 cells treated with CPI-613 or DCA or control vehicle. The percentages of FOXP3 expressing cells among gated living CD4 T cells were marked.

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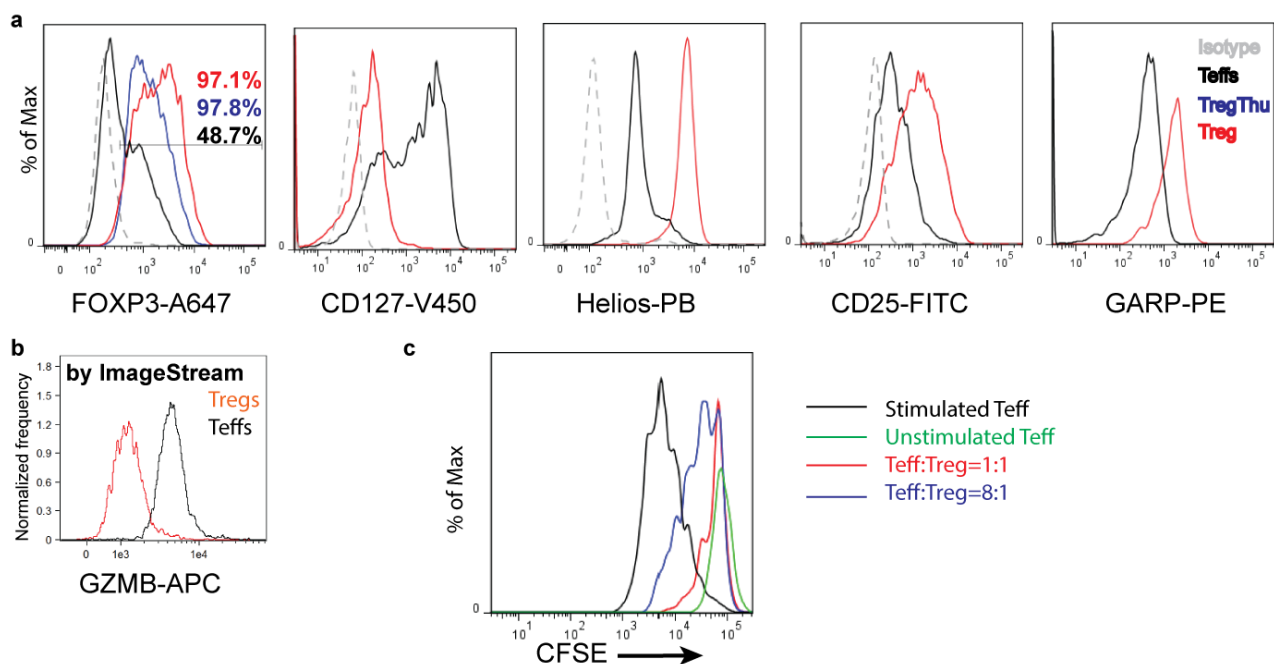
Results represent four (**a**, **d**, **e**), three (**f**, **g**) and two (**b**, **c**) independent experiments. Data are mean± s.d. The P-values are determined by a two-tailed Student's *t*-test. ns or unlabeled, not significant, *P<=0.05, **P<=0.01 and ***P<=0.001.

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Expanded view (EV) figures



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Figure EV1. Charaterization of highly purified human nTregs.

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a, Characterization of highly purified human nTregs (CD4⁺CD25^{high}CD127^{low}). Representative flow-cytometry plots of FOXP3, CD127, Helios, and CD25 on human Tregs relative Teffs (the markers were not stained in the panel). 'TregThu' is a golden standard of isolated human Tregs (refer to Methods and the previous work⁸⁰). The expression of *LRRC32* (GARP) was measured after 3-day stimulation by irradiated EBV-transformed B cells.

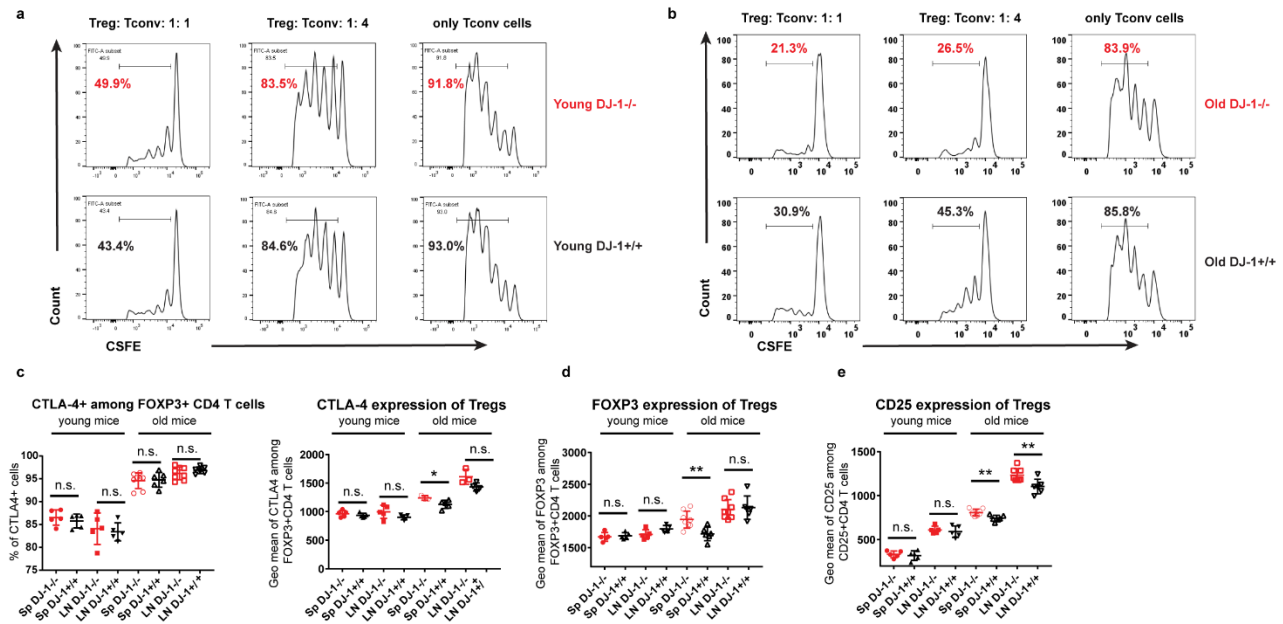
1108

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b, Representative ImageStream plot of GZMB expression in unstimulated Tregs or Teffs⁸⁸.

1111 **c**, Representative *In-vitro* proliferation assay of human Teffs suppressed by human Tregs at different ratios, in
 1112 coculture with irradiated EBV-B cells for 5 days. Results represent 7-10 (**a-c**) independent experiments (from
 1113 different healthy donors).



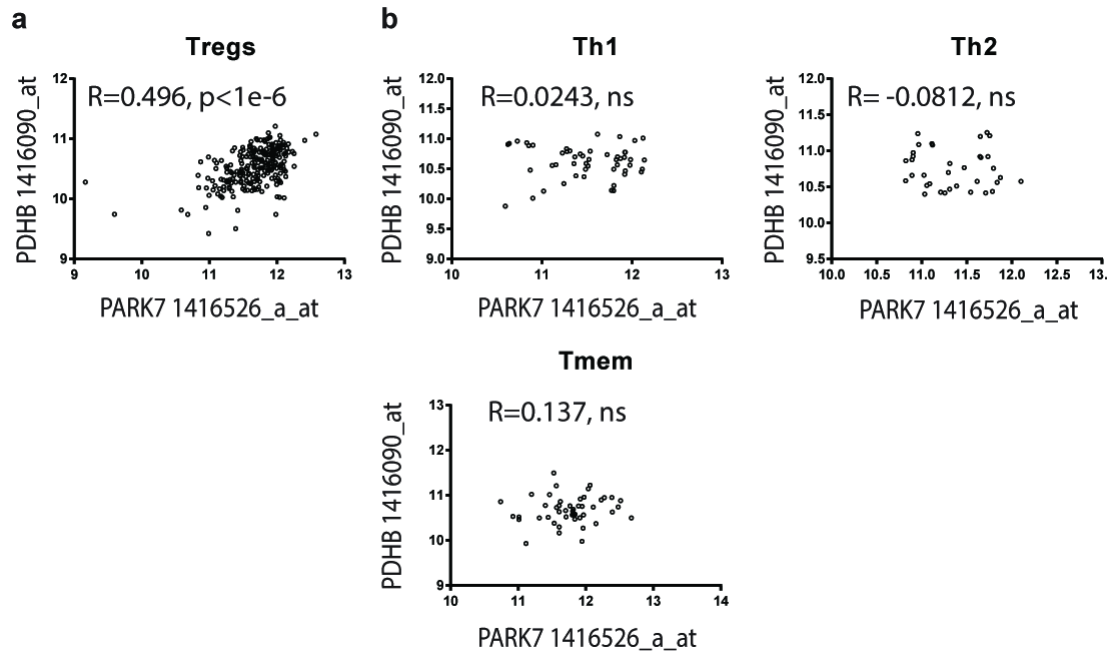
1114

1115 **Figure EV2. *Dj-1* depletion did not compromise individual Treg suppressor function (TSF).**

1116 **a, b**, *In-vitro* suppression assay of *Dj-1^{-/-}* or *Dj-1^{+/+}* Tregs isolated from young (**a**) or old (**b**) mice in coculture
 1117 with Teff cells and irradiated feeder cells in the presence of anti-CD3 for 3.5 days. Percentage of dividing cells
 1118 from the total population (the total population indicates the gated DAPI-negative and CD4⁺, CFSE stained Ths)
 1119 is presented on the plots.

1120 **c**, Frequency of CTLA4-expressing cells among CD4⁺FOXP3⁺ Treg cells (left) and Geometric mean of CTLA4
 1121 among CD4⁺FOXP3⁺ Tregs from spleen (Sp) and lymph nodes (LN) from *Dj-1^{-/-}* and *Dj-1^{+/+}* WT littermates.

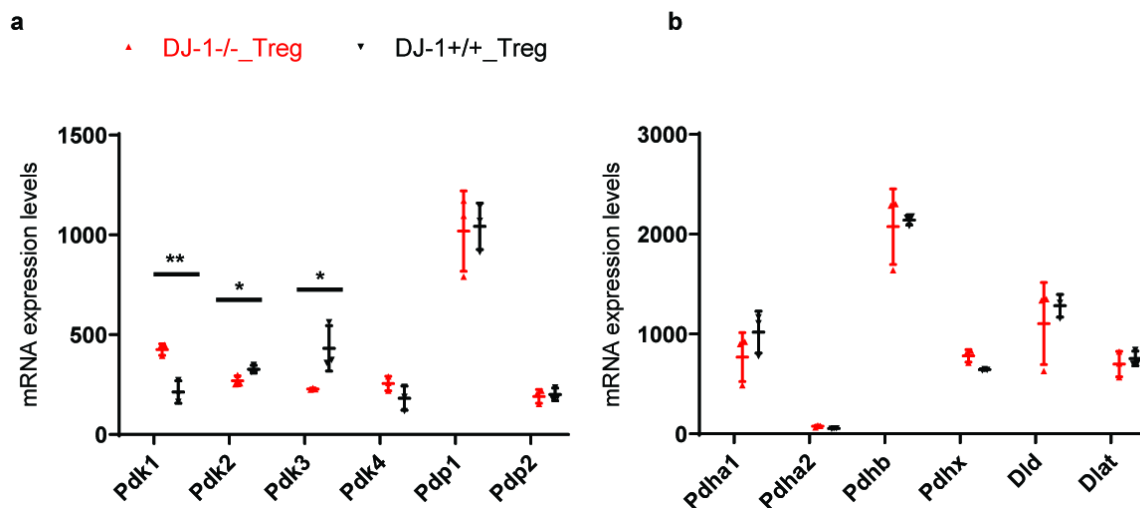
1122 **d, e**, Geometric mean of FOXP3 and CD25 expression among CD4⁺FOXP3⁺ Tregs. Young/old mouse results
 1123 are marked in each panel (young KO, n=5; young WT, n=5; old KO, n=8; old WT, n=6; for results of old mice
 1124 except for CTLA4 Geomean measurement (old KO, n=3; old WT, n=5 sp; old WT, n=4 LN; old KO, n=3 LN; of
 1125 note, for comparability, the CTLA4 Geomean results were not pooled from different batches), data pooled from
 1126 2 independent experiments). Results represent three (**a, b**) and four (**c, d, e**) independent experiments. Data
 1127 are mean ± s.d. The P-values are determined by a two-tailed Student's *t*-test. ns, not significant, *P<=0.05,
 1128 **P<=0.01 and ***P<=0.001.



1129

1130 **Figure EV3. Treg-specific correlation between *Dj-1* and *Pdhb*.**

1131 **a**, mRNA expression of *Park7/Dj-1* is highly correlated with *Pdhb* only in murine Tregs but not in other tested
 1132 CD4 T cell subsets. The number of microarray samples used for correlation analysis is 240, 51, 35 and 47
 1133 from Tregs, Th1, Th2 and Tmem (memory CD4 T cells) respectively. The R value corresponds to Pearson
 1134 correlation coefficient and the associated P-value is from a two-tailed test (Prism). Ns, non-significant and
 1135 otherwise, the P-value is provided for the corresponding cell type. For the details, refer to the database (from
 1136 *Sakaguchi's lab*): [https://sysimm.ifrec.osaka-u.ac.jp/immuno-](https://sysimm.ifrec.osaka-u.ac.jp/immuno-navigator/?o=10090&probe1=1416526_a_at&probe2=1416090_at&cell=5)
 1137 [navigator/?o=10090&probe1=1416526_a_at&probe2=1416090_at&cell=5](https://sysimm.ifrec.osaka-u.ac.jp/immuno-navigator/?o=10090&probe1=1416526_a_at&probe2=1416090_at&cell=5).



1138

1139 **Figure EV4. Expression analysis of PDH complex encoding and regulatory machinery genes in Tregs of**
 1140 **old mice.**

1141 **a**, mRNA expression of PDH kinases (*Pdks*) and pyruvate dehydrogenase phosphatases (*Pdps*) in Tregs
 1142 freshly isolated from old *Dj-1* KO and WT littermates.

1143 **b**, mRNA expression of several PDH complex encoding genes in Tregs freshly isolated from old *Dj-1* KO and
 1144 WT littermates. n=3 per group of mice. Results are the summary of the microarray datasets. Data are mean±
 1145 s.d. The P-values are determined by a two-tailed Student's *t*-test. ns or unlabelled, not significant, *P<=0.05,
 1146 **P<=0.01 and ***P<=0.001.

1147 Expanded View (EV) Tables

1148 **Table EV1.** The known PD-related genes measured in published human Treg time-series
1149 microarray datasets ⁴¹.

1150

Gene symbol ^a	Mean Teff_rep1 ^b	Mean Teff_rep2	Mean Treg_rep1	Mean Treg_rep2	Number of first-neighbor genes among 400 Treg potential genes ^c	Number of first-neighbor genes in Treg correlation network ^c	P-value for the first-neighbor Treg correlation network (cumulative binomial distribution test)
SLC18A2	16.71	16.11	20.09	15.52			
PARK2	19.72	17.05	21.93	20.76			
PARK7/DJ-1	6830.89	8011.65	9783.31	8266.40	0	7	2.56E-01
UCHL1	33.73	21.37	33.14	45.84			
STK39	868.74	1036.97	744.92	567.04	13	281	2.78E-01
HTRA2/PAKR13	199.75	346.14	307.10	248.92	47	1039	2.40E-01
SNCA	10.72	10.97	110.39	248.24	1	10	6.19E-02
LAMP3/CD208	24.06	100.21	74.69	164.72	0	13	4.23E-01
PINK1	89.99	105.74	143.04	111.72	1	31	3.70E-01
SYT11	325.62	235.47	291.42	347.74	1	9	5.09E-02
HIP1R	10.67	7.74	7.28	5.98			
PLA2G6	120.08	136.30	141.53	149.91	0	32	7.42E-01
MCCC1	279.89	210.59	293.56	255.56	0	0	
ATP13A2/PARK9	37.09	49.15	58.06	27.87			
MAPT	48.81	39.95	56.77	57.12	0	0	
LRRK2/PARK8	11.39	9.22	7.11	8.27			
ACMSD	12.19	9.75	11.66	17.98			
CCDC62	36.84	24.69	34.38	37.92			
TFAM	423.13	521.16	494.28	618.08	39	872	2.78E-01
FBXO7/PARK15	1012.48	974.32	1065.89	992.16	68	1728	6.40E-01

1151

1152 Notes:

1153 **a**, the list was collected based on literature, such as the work ⁴² and others. PARK3 and PARK10 were not included due
1154 to the probeset absence in the original time-series microarray datasets ⁴¹.

1155 **b**, The mean value indicates the average mRNA expression value through all the measured time points of the given gene
1156 for a given probeset.

1157 **c**, the blank value indicates that no linkage for the given gene was found in the Treg-specific correlation network.

1158

1159 **Table EV2.** The Co-IP-MS identified potential binding partners of DJ-1 in human primary
1160 Tregs/Tefts.

1161

Gene symbol	Mean Teff_rep1	Mean Teff_rep2	Mean Treg_rep1	Mean Treg_rep2	Fold change (Tregs sti vs. unsti) ^b	Fold change (Tregs sti vs unsti (without IL-2))	Fold change (Teffs sti vs. unsti)	Fold change (DJ-1 ab vs IgG in Teffs)	Binding preference ^c
PDHB	1304 .83	1248. 61	1650.1 3	1331.09	198.13	2.43	70.53	64.01	unsti
GNB2L1	8549 .45	14203 .38	11974. 55	9578.58	36.47	295.49	16.63	35.09	sti
EXOSC1	425. 11	452.6 9	736.40	555.02	5.11	2.17	66.07	33.03	sti
EIF2A	3478 .47	3370. 57	2809.8 5	2844.83	3.33	22.16	9.86	25.07	sti
SART1	253. 46	335.8 2	279.24	265.06	13.87	63.89	29.02	19.17	sti
ELAVL1	788. 85	1908. 74	1253.6 9	999.38	19.02	117.64	46.47	17.40	sti
ATP1A1	489. 10	1548. 97	891.64	615.27	7.43	21.02	4.06	14.48	unsti
SKIV2L2	639. 83	703.7 1	717.41	794.02	28.65	34.13	89.77	9.86	sti
DOCK10	1883 .18	1345. 03	1584.8 8	1580.57	2.11	3.79	2.89	8.51	sti
ATP5D	273. 57	687.9 8	469.23	330.71	76.51	99.86	3.05	7.85	sti
ANXA4	359. 40	660.3 6	1084.7 9	955.29	7.58	14.57	2.09	6.64	sti
LARS	597. 75	744.0 1	857.39	705.88	3.88	6.06	63.76	6.49	unsti
HNRNPH 3/HNRPH 3	2211 .08	1599. 24	1270.3 1	1961.07	4.63	20.33	2.61	5.26	sti
STT3B	4887 .15	4743. 66	4601.9 4	5918.17	6.32	5.45	6.73	5.18	unsti
EIF5B	2652 .07	2294. 71	1998.9 2	3190.99	5.80	3.11	11.02	5.00	sti
DHX36	2543 .54	1342. 67	1245.6 6	1596.94	2.26	14.27	4.74	4.95	unsti
KHSRP	294. 26	557.8 5	439.18	506.72	4.22	17.58	17.45	4.88	sti
ARPC2	1139 0.36	10741 .65	10140. 17	8212.70	5.37	82.96	17.28	4.88	unsti
PRPF8	765. 71	895.5 1	784.17	603.34	4.65	9.36	11.26	3.73	sti
PPP1CC; PPP1CB ^a	6423 .858	5781. 311	6891.0 76	8885.82 1	2.80669 1	2.65	17.76	3.16	sti

1162

1163

Notes:

1164 **a**, the mean mRNA expression value in Tregs/Teffs was taken from PPP1CB. The mean expression intensity values
 1165 through the first 6hr period following the TCR stimulation were taken from the previous time-series microarray dataset
 1166 ⁴¹.
 1167 **b**, the short term “unsti” and “sti” represents the word “unstimulated” and “stimulated”, respectively
 1168 **c**, for those bound preferentially in unstimulated T cells, the reciprocal fold changes are shown (i.e., 1/x).
 1169

1170 **Table EV3. List of confirmed known DJ-1-binding partners (the list of known DJ-1 binding**
 1171 **partners were downloaded from NCBI or pubmed literatures).**

Gene symbol	Mean Teff_rep1	Mean Teff_rep2	Mean Treg_rep1	Mean Treg_rep2	Fold change (Treg sti vs. unsti)	Fold change (Treg sti vs unsti (without IL2))	Fold change (Teff sti vs. unsti)	Fold change (Jurkat cells sti vs. unsti)	Fold change (DJ-1 ab vs IgG in unsti Teff)	Fold change (DJ-1 ab vs IgG in unsti Jurkat)
HDAC1	981.49	2009.77	2142.04	1322.45	192.53	1.11	1.36	0.78	2.34	12.02
NPM1	4093.49	4232.00	5464.82	5882.71	35.58	1.55	0.16	1.60	17.62	131.70
PARK7	6830.89	8011.65	9783.31	8266.40	0.71	0.95	2.25	1.18	2.66	56.98
TALDO1	1291.26	2576.75	2603.60	1181.23	0.04	2.70	2.70	0.06	4.56	3.54
PRDX2	1220.91	3986.84	2964.41	1512.84	0.01	0.13	1.40	0.98	1.78	3.69
CHD4	780.61	622.41	673.84	560.56	28.62	49.22	1.37	0.83	13.32	31.95
RBBP4	893.34	1434.22	838.35	788.77	0.54	1.05	2.44	0.92	4.22	21.06
MTA1	334.51	518.85	388.81	324.99	3.32	39.47	0.72	0.76	38.24	165.48
GATAD2 B	256.19	183.64	203.31	257.81	0.62	1.13	0.95	0.75	10.00	26.41
MTA2	8.15	7.00	9.91	4.45	1.59	0.92	1.42	0.82	4.92	28.43
STAT1	2473.879	3330.837	5127.192	6578.421	10.57	0.053	0.86	1.49	42.29	Binding to igG in Jurkat

1172
 1173 Notes: The list included the genes independent of their mean mRNA expression levels. Here only the reported binding
 1174 partners of DJ-1 confirmed by our MS analysis were included. The Jurkat cells were stimulated by PMA (10 ng/ml) and
 1175 ionomycin (1 µg/ml) for 4 h. The human primary T cells were stimulated as described in the Materials and Methods.