SUPPLEMENTAL MATERIAL

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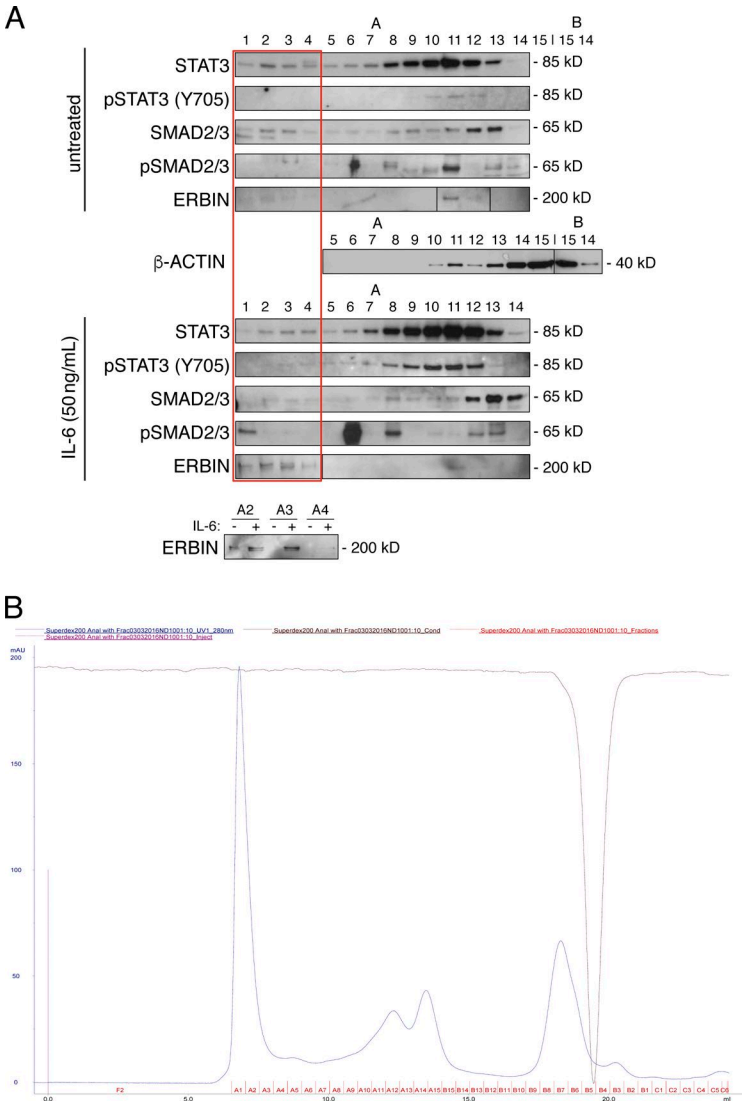

Figure S1. **A large molecular weight protein fraction contains ERBIN, STAT3, and SMAD2/3.** (A and B) Immunoblots of protein fractions obtained by gel filtration chromatography of 293T whole cell lysates from two independent experiments with or without treatment with IL-6 (A) and representative corresponding absorbance of eluent (B). Large molecular weight fractions containing ERBIN, STAT3, SMAD2/3, and, after IL-6 treatment, pSMAD2/3 are indicated by the red box. mAU, milli–arbitrary units.

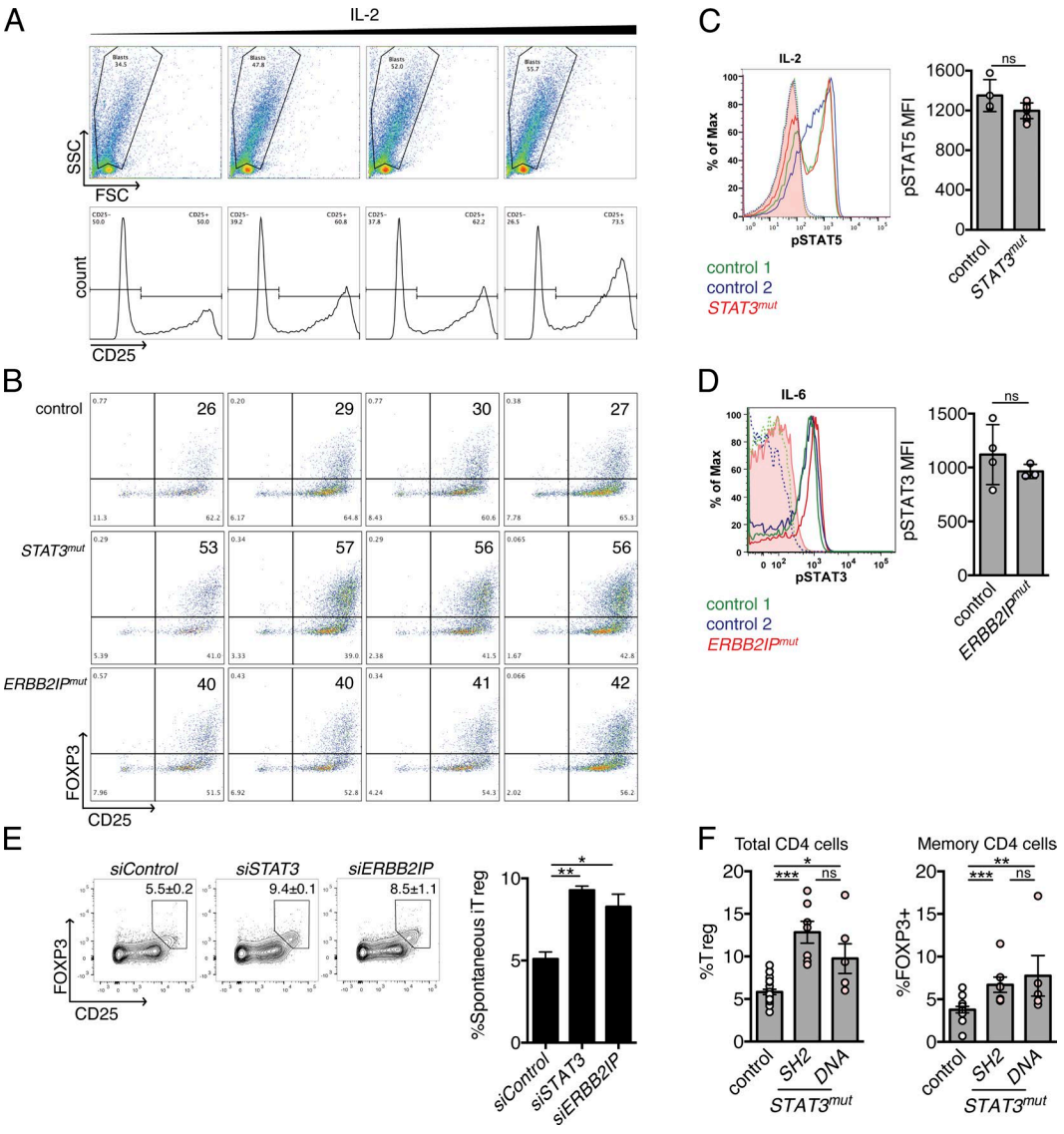

Figure S2. **Increased T reg cells in *ERBB2IP*^{mut} and *STAT3*^{mut} patients are not associated with altered IL-2 responsiveness and are independent of the mutant *STAT3* domain.** (A) Scatter plot showing blasting lymphocytes (top) and histograms (bottom) demonstrating CD25 induction in naive CD4 cells cultured under iT reg cell conditions with up to 40-fold lower IL-2 concentrations (50, 100, 250, and 500 pg/ml) increasing from left to right. FSC, forward scatter; SSC, side scatter. (B) Scatter plots showing FOXP3 and CD25 expression under the same iT reg cell conditions with reduced IL-2. iT reg cell percentage is indicated in bold. (C) Representative histogram (left) and combined data (right) showing STAT5 phosphorylation (pSTAT5; Y694) in control (n = 4) and *STAT3*^{mut} (n = 10) CD4 T cells. (D) Representative histogram (left) and combined data (right) showing STAT3 phosphorylation (pSTAT3; Y705) in control (n = 4) and *ERBB2IP*^{mut} (n = 3) CD4 T cells. (E, left) Contour plot showing FOXP3 and CD25 expression in control naive CD4$^+$ T cells after siRNA-mediated silencing of STAT3 (*siSTAT3*) or ERBIN (*siERBB2IP*), compared with scrambled siRNA control (*siControl*), showing spontaneous iT reg cells (FOXP3$^+$CD25^{bright}) after weak TCR stimulation under nonskewing conditions. (Right) Combined data. (F) FOXP3 induction in total CD4$^+$ T cells after short-term stimulation (right) and prevalence of T reg cells among memory (CD45RO$^+$) CD4 cells (left) from control (n = 20) or *STAT3*-mutant (*STAT3*^{mut}) patients with SH2-domain mutations (n = 7) or DNA-binding–domain mutations (n = 5). All data are representative or combined from at least two independent experiments and are shown as the mean ± SEM. Paired and unpaired two-tailed Student's t tests and Mann-Whitney tests were used where appropriate. *, P < 0.05; **, P < 0.005; ***, P < 0.001.

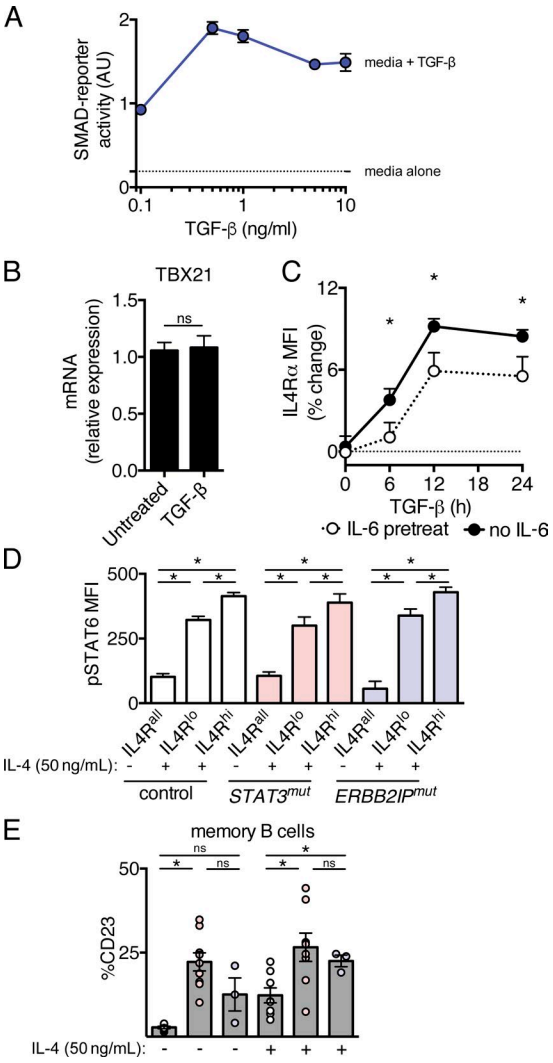

Figure S3. **TGF-β can induce functionally active IL-4Rα expression in lymphocytes to promote STAT6-specific target expression.** (A) SMAD-reporter activity induced by exogenous TGF-β (media + TGF-β) or by media alone containing 10% FCS, relative to unsupplemented media. AU, arbitrary units. (B) Relative quantitation of T-BET (*TBX21*) expression in Jurkat T cells at baseline or after treatment with 5 ng/ml TGF-β for 6 h. (C) Percent change in IL-4Rα surface expression in Jurkat T cells after treatment with 5 ng/ml TGF-β over the indicated duration, with or without pretreatment with 50 ng/ml IL-6 for 72 h. (D) Phosphorylation of STAT6 (Y641) among IL-4Rα bright (IL-4R^{hi}) and IL-4Rα dim (IL-4R^{lo}) lymphocytes from *ERBB2IP*^{mut} (n = 3), *STAT3*^{mut} (n = 5), and control (n = 4) individuals after IL-4 stimulation. (E) Percent of memory B (CD27^{+}CD19^{+}) lymphocytes from control (n = 8), *STAT3*^{mut} (n = 8), and *ERBB2IP*^{mut} (n = 3) patients expressing the STAT6-target CD23 before and after short-term in vitro exposure to 50 ng/ml IL-4. White circle, control; red circle, *STAT3*^{mut}; blue circle, *ERBB2IP*^{mut}. Data are combined from at least three independent experiments and are represented as the mean ± SEM. Unpaired two-tailed Student's *t* tests and Mann-Whitney tests were used where appropriate. *, P < 0.05.

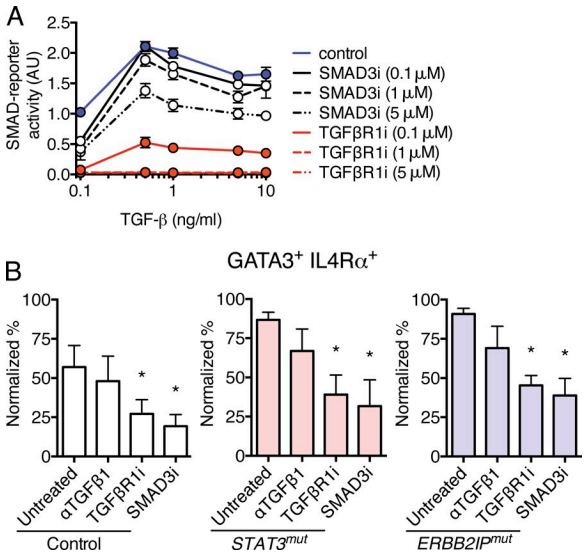

Figure S4. **Greater IL-4Rα expression and increased GATA3 induction in *STAT3*^{mut} and *ERBB2IP*^{mut} naive CD4 lymphocytes are normalized by SMAD3 inhibition.** (A) Dose response to TGF-β in the SMAD-reporter line basally and after preincubation with a selective SMAD3 inhibitor (SMAD3i) or TGF-βR1 inhibitor (TGFβR1i). AU, arbitrary units. (B) Percentage of activated (CD69$^+$) naive CD4 cells coexpressing GATA3 and IL-4Rα (GATA3$^+$IL-4Rα$^+$) after short-term culture with weak TCR stimulation under nonskewing conditions in the presence or absence of 5 µM SMAD3i, 1 µM TGFβR1i, or 20 µg/ml neutralizing TGF-β antibody (αTGFβ1). Data are combined from at least three independent experiments and are represented as the mean ± SEM. Unpaired two-tailed Student's *t* test was used. *, P < 0.05.

Table S1. **Comparison of phenotypes between *ERBB2IP*^{mut} and *STAT3*^{mut} patients**

	ERBB2IP^{mut}	*STAT3*^{mut}	Reference
Clinical phenotype			
Eczema	66% (2/3)	95%	Freeman and Holland, 2009
Scoliosis	100% (3/3)	60%	Freeman and Holland, 2009
Hyperextensibility[a]	100% (3/3)	70%	Freeman and Holland, 2009
Arterial dilation/tortuosity[b]	100% (3/3)	60%	Freeman and Holland, 2009
Eosinophilic esophagitis[c,d]	100% (3/3)	50%	Hsu et al., 1993
S. aureus susceptibility[e]	66% (2/3)	87%	Freeman and Holland, 2009
Bronchiectasis/pneumatocele	0% (0/3)	70%	Freeman and Holland, 2009
Mucocutaneous candidiasis	0% (0/3)	83%	Freeman and Holland, 2009
Food allergy	33% (1/3)	38%	Siegel et al., 2013
Immunological phenotype			
Serum IgE	normal to increased	increased	Freeman and Holland, 2009
Peripheral eosinophils	normal to increased	normal to increased	Freeman and Holland, 2009
Peripheral T reg cells	increased	increased	Freeman and Holland, 2009
Th1 cytokine production	intact	intact	Ma et al., 2015
Th2 cytokine production	increased	increased	Ma et al., 2015
IL-17A production	intact	reduced	Milner et al., 2008
IL-21 production	intact	reduced	Ma et al., 2012
T cell memory	intact	impaired	Siegel et al., 2011
B cell memory/class switch	intact	impaired	Deenick et al., 2013

[a]All *ERBB2IP*^{mut} individuals had Beighton scores >5. However, two of these individuals were ≤14 yr old at the time of this study.
[b]All *ERBB2IP*^{mut} individuals had aortic root dilation (z-score range 2.2–2.7), whereas *STAT3*^{mut} individuals have predominantly smaller arterial disease.
[c]All *ERBB2IP*^{mut} individuals had biopsy-proven eosinophilic esophagitis (>15 eosinophils/high-power field while on maximal proton pump inhibitor therapy for acid suppression).
[d]Defined as absolute eosinophil count >500 cells/µl, 1/2 normalized with treatment of eosinophilic esophagitis.
[e]Cutaneous susceptibility; all affected *ERBB2IP*^{mut} individuals also had recurrent streptococcal pharyngitis requiring tonsillectomy.

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