## SUPPLEMENTARY METHODS

### Study population

This was a prospective, single arm, interventional study (clinicaltrial.gov: NCT03025828). All patients had histological diagnosis of primary membranous nephropathy (MN) and persistent proteinuria (>3 months) at nephrotic range (>3.5 g/24h) despite at least 3 months of angiotensin converting enzyme inhibitors or angiotensin receptor blocker therapy. The study included patients naïve to immunosuppressive treatments as well as patients who had previously received treatment with steroids, alkylating agents, calcineurin inhibitors, and/or rituximab. Patients had to be free of immunosuppression for at least 6 months. Patients with eGFR < 30 ml/min/1.73 $m^2$ (GFR was estimated based on CKD-EPI formula[S3]), kidney transplant recipients, patients with secondary causes of membranous nephropathy, and patients with uncontrolled diabetes mellitus were excluded from the study. Patients were enrolled at Mount Sinai Hospital, NY.

The study protocol was approved by the Institutional Review Board of the Mount Sinai Hospital and the study was conducted in accordance with the Declaration of Helsinki. Clinical data, including laboratory tests and renal biopsies, were collected in accordance with the center' standard clinical practice.

### Repository corticotropin injection treatment

Repository corticotropin injection (RCI, Acthar® Gel), a naturally sourced complex mixture of adrenocorticotropic hormone analogs and other pituitary peptides, whose major component is the N-25 deamidated porcine ACTH (1-39), was administered subcutaneously at the dose of 80 units for the first week and then 80 units twice weekly to complete 6 months of treatment with a cumulative dose exposure of 3,920 units.

**Patient follow-up**

After inclusion, patients underwent monthly evaluations of clinical parameters (body weight and blood pressure), biochemistry, proteinuria and eGFR up to month 3, then at month 6, 9, and 12 after treatment start. At baseline and at 3, 6, 9, and 12 months blood samples were collected for immune phenotyping.

Partial remission was defined as a urinary protein excretion (P/C) <3.0 g/g (with at least 50% reduction versus baseline) in at least two consecutive visits.

**Flow cytometry**

Peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood by Ficoll gradient and stored in liquid nitrogen for batched analysis. For multicolor flow cytometry analyses the following monoclonal antibodies were used: from Becton Dickinson (BD, Franklin Lakes, NJ), CD3-FITC, CD3-PerCP-Cy5.5, CD4-APC-Cy7, CD8-BV510, CD45RO-FITC, CD45RA-APC, CD45RA-APC-Cy7, CD71-PE, IgD-PerCP-Cy5.5, CD25-APC-Cy7, CD138-BV421, CCR4-PE, CD27-PE, CD95-BV421, CD28-BV421, TIM3-BV421, CCR6-BV421, CCR7-AF700, CXCR3-PE, IL-2-PE, IL-17-BV786, and IFN-γ-PE-Cy7; from Biolegend (San Diego, CA), CD19-BV510, CD56-FITC, CD27-APC, CD127-FITC, CD57-PerCp-Cy5.5, PD-1-APC-Cy7, CXCR5-FITC, LAG3-APC, IL-10-PerCP-Cy5.5, and TNF-α-FITC. From eBioscience (San Diego, CA), CD4-PE-Cy7, CD21-PE, and CD24-APC-Cy7 were used. From Miltenyi Biotec (Auburn, CA), CD25-APC and anti-KLRG1-PE were used. From Beckman Coulter (Indianapolis, IN), CD38-PE-Cy7 was used. Appropriate isotype controls were used for gate placement.

Data were acquired (> 1 × $10^6$ events) on a 3-laser FACSLyric flow cytometer (BD Biosciences) and analyzed with FlowJo® software.

**Imaging flow cytometry**

Imaging flow cytometry was used to measure MC1R surface expression at the single cell level. We enriched total T cells (Easysep catalog #19051, STEMCell Technologies, Vancouver, BC) from healthy volunteers and we fixed them (PFA 1.6%, 37ºC, 10 min) before staining to avoid a possible internalization of the receptor because of antibody binding. After staining with anti-MC1R-AF488 (Antibody-online; Limerick, PA) or isotype control, images of each cell were acquired at a magnification of 60× on an ImageStream flow cytometer (Amnis) and analyzed using IDEAS analysis software. Single-color controls were used for the creation of a compensation matrix that was applied to all files to correct for spectral crosstalk. Surface receptor expression was estimated by constructing a contour mask on the bright-field image and applying it to the MC1R-AF488 channel, an approach that has been successfully applied prior to quantify surface protein expression.

**$T_{REG}$ induction**

We enriched $CD4^+CD45RA^+CD45RO^-$ naïve $CD4^+$ T cells from PBMC obtained by healthy volunteers using magnetic isolation (Miltenyi Biotec) using AutoMACS (Miltenyi Biotech) (>95% purity). Isolated cells were cultured with anti-CD3 and anti-CD28 mAb (1 μg/ml each), IL-2 (100 IU/ml), TGF-β (10 ng/ml) with or without α-MSH (Tocris, Minneapolis, MN; 1 ng/ml or 10 ng/ml). FOXP3 expression was measured by flow cytometry 5 days later.

**Statistical analysis**

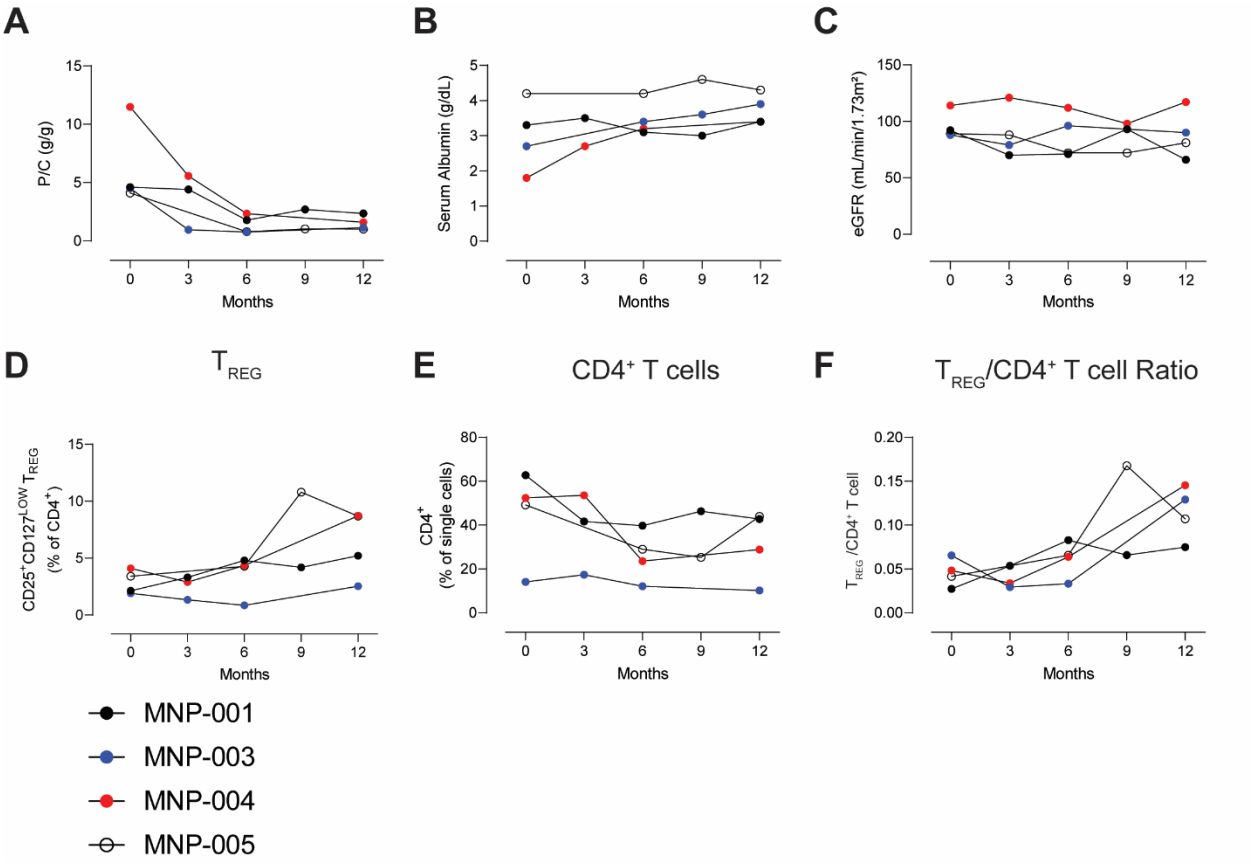
Results were presented as median and interquartile range (IQR) or mean + standard error of the mean (SEM) unless stated otherwise. Comparison of continuous variables between *in vitro* experimental groups was performed by unpaired t-test. Comparisons across serial time-points within the same group was done by ANOVA. $P < 0.05$ was considered as

statistically significant. Statistical analysis was performed using GraphpadPrism® version 8.4.2 software package (Graphpad Software Inc., San Diego, CA).

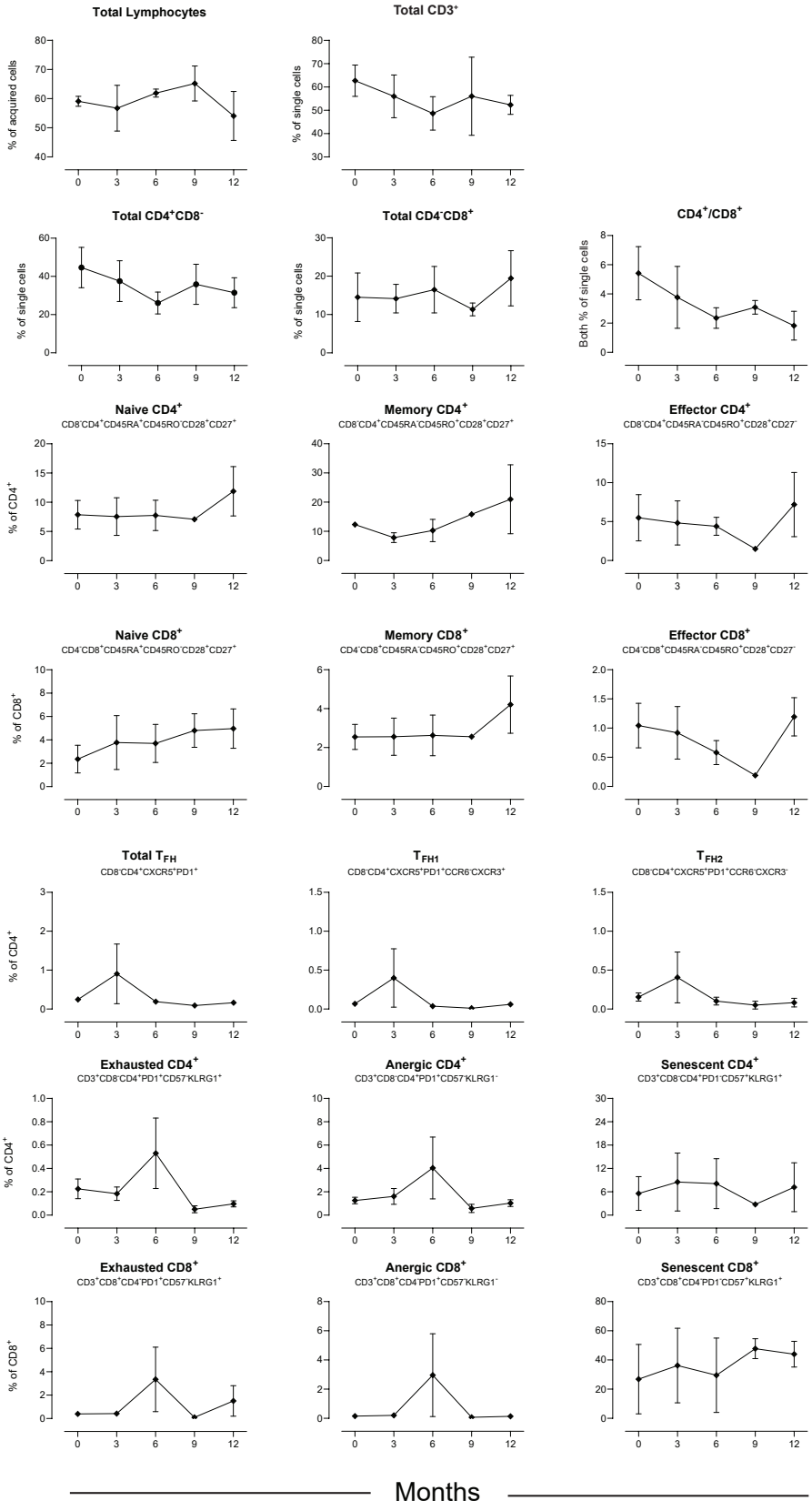
**Supplementary Table S1.** Clinical characteristics at baseline in four patients who completed treatment with Acthar® Gel.

| Patient code | Age (yrs) | Sex | Serum albumin (g/dL) | Serum creatinine (mg/dL) | Anti-$PLA_2R$ Ab (RU/mL) | P/C (g/g) | Previous treatments |
|---|---|---|---|---|---|---|---|
| MNP-001 | 52 | M | 3.3 | 0.95 | <1.8 | 4.6 | No previous treatment |
| MNP-003 | 60 | M | 2.7 | 0.93 | negative | 4.51 | Rituximab followed by cyclophosphamide |
| MNP-004 | 43 | M | 1.8 | 0.85 | negative | 11.48 | No previous treatment |
| MNP-005 | 60 | M | 4.2 | 1.13 | 5.9 | 4.1 | Cyclosporine followed by rituximab |

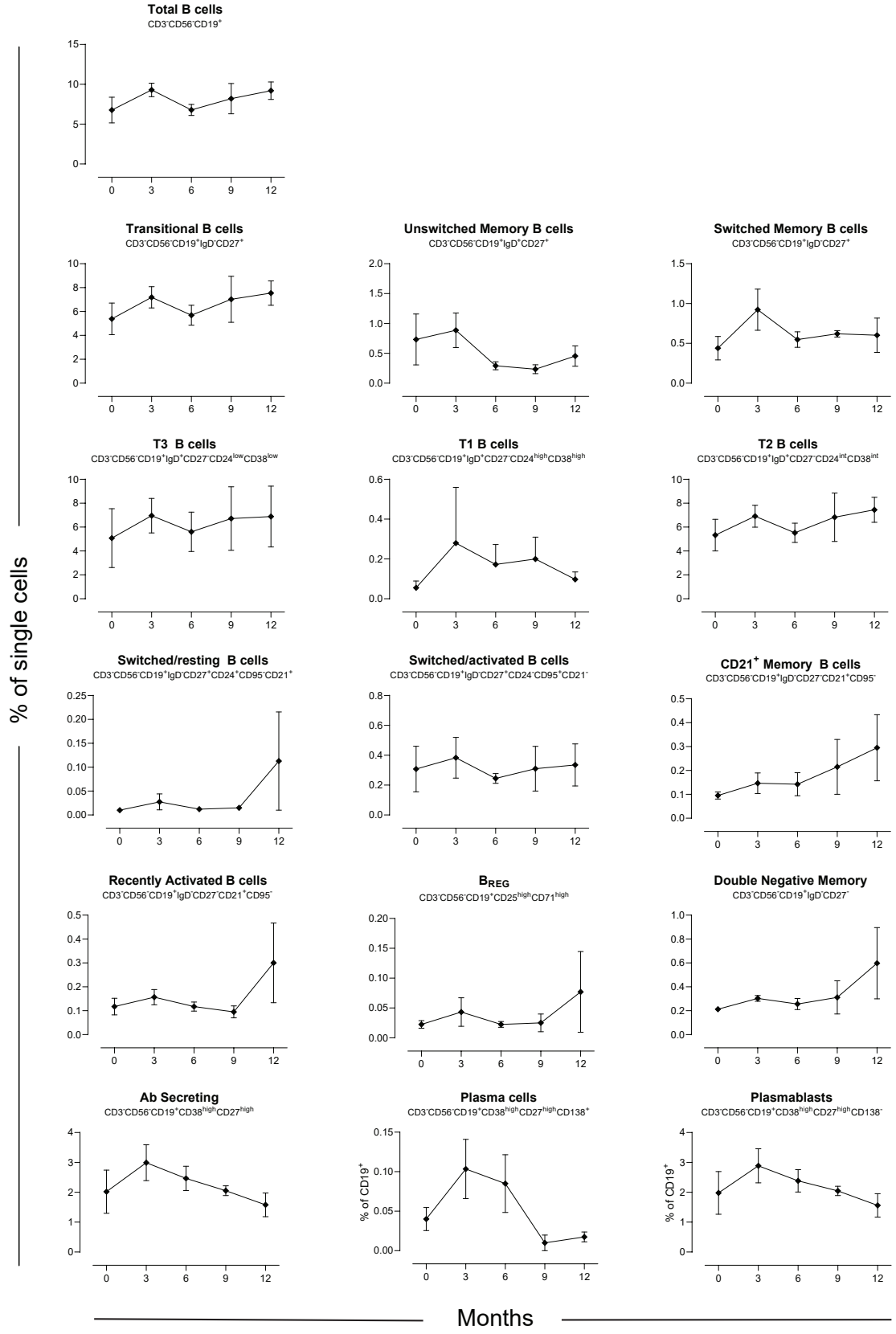
P/C: Urine protein/creatinine ratio. M: Male. Threshold for anti-$PLA_2R$ Ab positivity is 19.9 RU/mL.




**Supplementary Figure S1. A**) Urinary protein/creatinine ratio (P/C); **B**) serum albumin and **C**) eGFR. Percentages of circulating **D**) $CD25^{+}CD27^{LOW}$ $T_{REG}$, **E**) total $CD4^{+}$ T cells and **F**) $T_{REG}/CD4^{+}$ T cell ratio. Data are represented as single values for each patient, at baseline and throughout study course.




**Supplementary Figure S2**. Changes in total lymphocytes and T cell subsets. Data are represented as mean ± SEM. No subsets differed significantly between time points.




**Supplementary Figure S3**. Changes in total lymphocytes and B cell subsets. Data are represented as mean ± SEM. No subsets differed significantly between time points.

## SUPPLEMENTARY REFERENCES


S1. Xiang L, Marshall GD, Jr. Immunomodulatory effects of in vitro stress hormones on FoxP3, Th1/Th2 cytokine and costimulatory molecule mRNA expression in human peripheral blood mononuclear cells. *Neuroimmunomodulation* 2011; **18:** 1-10.

S2. Ronaldson A, Gazali AM, Zalli A*, et al.* Increased percentages of regulatory T cells are associated with inflammatory and neuroendocrine responses to acute psychological stress and poorer health status in older men and women. *Psychopharmacology (Berl)* 2016; **233:** 1661-1668.

S3. Levey AS, Stevens LA, Schmid CH*, et al.* A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; **150:** 604-612.