

SUPPLEMENTARY DATA

**Supplementary Table 1.** Baseline characteristics, according to study group.

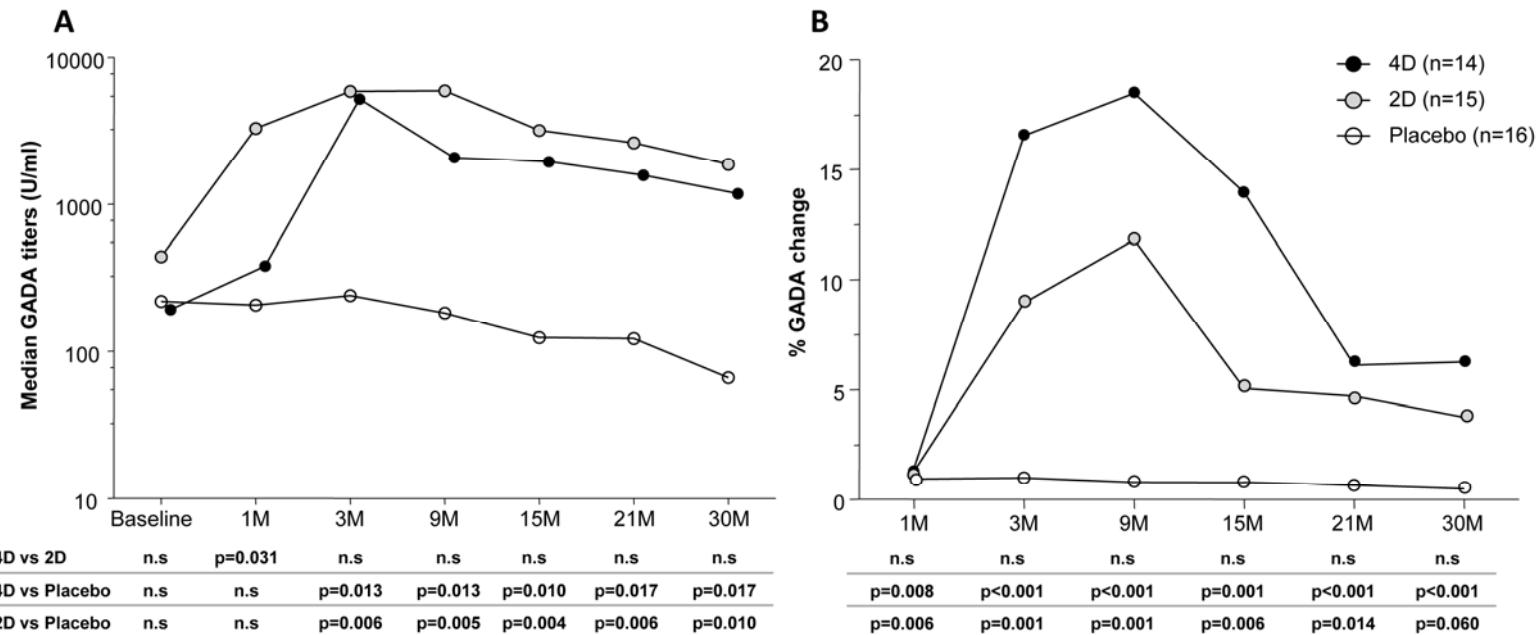
| Characteristic                         | Swedish subgroup |              |                   | Entire Swedish cohort |              |                   | Entire study cohort |               |                    |
|--|------------------|--------------|-------------------|-----------------------|--------------|-------------------|---------------------|---------------|--------------------|
|  | 4D<br>(n=14)     | 2D<br>(n=15) | placebo<br>(n=16) | 4D<br>(n=49)          | 2D<br>(n=49) | placebo<br>(n=50) | 4D<br>(n=111)       | 2D<br>(n=108) | placebo<br>(n=115) |
| <b>Age, years</b>                      | 13.2±2.4         | 13.3±2.4     | 13.4±2.7          | 13.3±2.1              | 13.3±2.2     | 13.4±2.5          | 12.9±2.4            | 12.9±2.1      | 13.3±2.3           |
| <b>Days since diagnosis</b>            | 76.4±26.1        | 67.7±22.8    | 67.6±23.5         | 74.6±20.3             | 73.3±20.0    | 71.0±20.6         | 73.6±25.0           | 76.9±23.3     | 74.2±23.4          |
| <b>Gender distribution, n (%)</b>      |                  |              |                   |                       |              |                   |                     |               |                    |
| Female                                 | 9 (64)           | 6 (40)       | 8 (50)            | 25 (51)               | 26 (53)      | 20 (40)           | 58 (52)             | 48 (44)       | 55 (48)            |
| Male                                   | 5 (36)           | 9 (60)       | 8 (50)            | 24 (49)               | 23 (47)      | 30 (60)           | 53 (48)             | 60 (56)       | 60 (52)            |
| <b>HLA classification, n (%)</b>       |                  |              |                   |                       |              |                   |                     |               |                    |
| Very high risk                         | 3 (21.3)         | 7 (47)       | 6 (38)            | 14 (29)               | 18 (37)      | 16 (32)           | 30 (28)             | 30 (28.3)     | 31 (28.2)          |
| High risk                              | 6 (43)           | 6 (40)       | 5 (31)            | 21 (44)               | 23 (47)      | 23 (46)           | 41 (38.3)           | 41 (38.7)     | 40 (36.4)          |
| Moderate risk                          | 3 (21.3)         | 2 (13)       | 4 (25)            | 8 (17)                | 4 (8)        | 8 (16)            | 24 (22.4)           | 23 (21.7)     | 28 (25.5)          |
| Low risk                               | 2 (14.3)         | 0 (0)        | 1 (6)             | 5 (10)                | 4 (8)        | 3 (6)             | 12 (11.2)           | 12 (11.3)     | 11 (10)            |
| <i>Total Observations</i>              | 14               | 15           | 16                | 48                    | 49           | 50                | 107                 | 106           | 110                |
| <b>Tanner, n (%)</b>                   |                  |              |                   |                       |              |                   |                     |               |                    |
| 1                                      | 2 (14.3)         | 4 (27)       | 0 (0)             | 6 (12)                | 10 (20)      | 5 (10)            | 15 (14)             | 22 (21)       | 12 (11)            |
| 2+3                                    | 2 (14.3)         | 2 (13)       | 8 (50)            | 17 (35)               | 12 (25)      | 16 (32)           | 43 (40)             | 30 (28)       | 37 (34)            |
| 4+5                                    | 10 (71.3)        | 9 (60)       | 8 (50)            | 26 (53)               | 27 (55)      | 29 (58)           | 49 (46)             | 54 (51)       | 59 (55)            |
| <i>Total Observations</i>              | 14               | 15           | 16                | 49                    | 49           | 50                | 107                 | 106           | 108                |
| <b>C-peptide (nmol/l)</b>              |                  |              |                   |                       |              |                   |                     |               |                    |
| Fasting C-peptide                      | 0.37±0.19        | 0.23±0.09    | 0.26±0.10         | 0.30±0.17             | 0.25±0.11    | 0.26±0.14         | 0.29±0.18           | 0.29±0.15     | 0.28±0.16          |
| Stimulated C-peptide AUC               | 0.71±0.33        | 0.61±0.23    | 0.71±0.21         | 0.71±0.35             | 0.66±0.26    | 0.64±0.24         | 0.66±0.35           | 0.68±0.31     | 0.65±0.30          |
| <b>Glycated hemoglobin (%)</b>         | 7.07±0.72        | 7.15±0.91    | 7.04±1.11         | 6.84±0.65             | 6.81±0.91    | 6.87±1.04         | 7.10±1.20           | 6.98±1.05     | 7.19±1.21          |
| <b>Glycated hemoglobin (mmol/mol)</b>  | 54               | 55           | 53                | 51                    | 51           | 52                | 54                  | 53            | 55                 |
| <b>Insulin dose (IU/Day/kg)</b>        | 0.64±0.26        | 0.71±0.27    | 0.51±0.23         | 0.59±0.29             | 0.62±0.31    | 0.55±0.23         | 0.54±0.28           | 0.60±0.35     | 0.57±0.28          |
| <b>Fasting plasma glucose (mmol/l)</b> | 6.81±1.92        | 6.32±2.15    | 5.87±1.24         | 6.28±2.16             | 5.95±1.74    | 5.69±1.19         | 6.31±2.05           | 6.28±2.05     | 6.59±2.18          |
| <b>Median GADA (Units/ml)</b>          | 193              | 440          | 219               | 204                   | 312          | 190               | 199                 | 159           | 237                |
| <b>Median IA-2A (Units/ml)</b>         | 408              | 667          | 943               | 472                   | 620          | 736               | NA                  | NA            | NA                 |

Values are mean±SD unless stated otherwise. HLA, human leukocyte antigen; 4D, 4 doses of GAD-alum; 2D, 2 doses of GAD-alum; AUC, area under the curve;

GADA, Glutamic acid decarboxylase antibodies; IA-2A, Insulinoma antigen 2 antibodies. HLA risk classification is based on HLA class II type. The Tanner puberty stage ranges from 1 to 5, with higher stages indicating more developed genitalia.

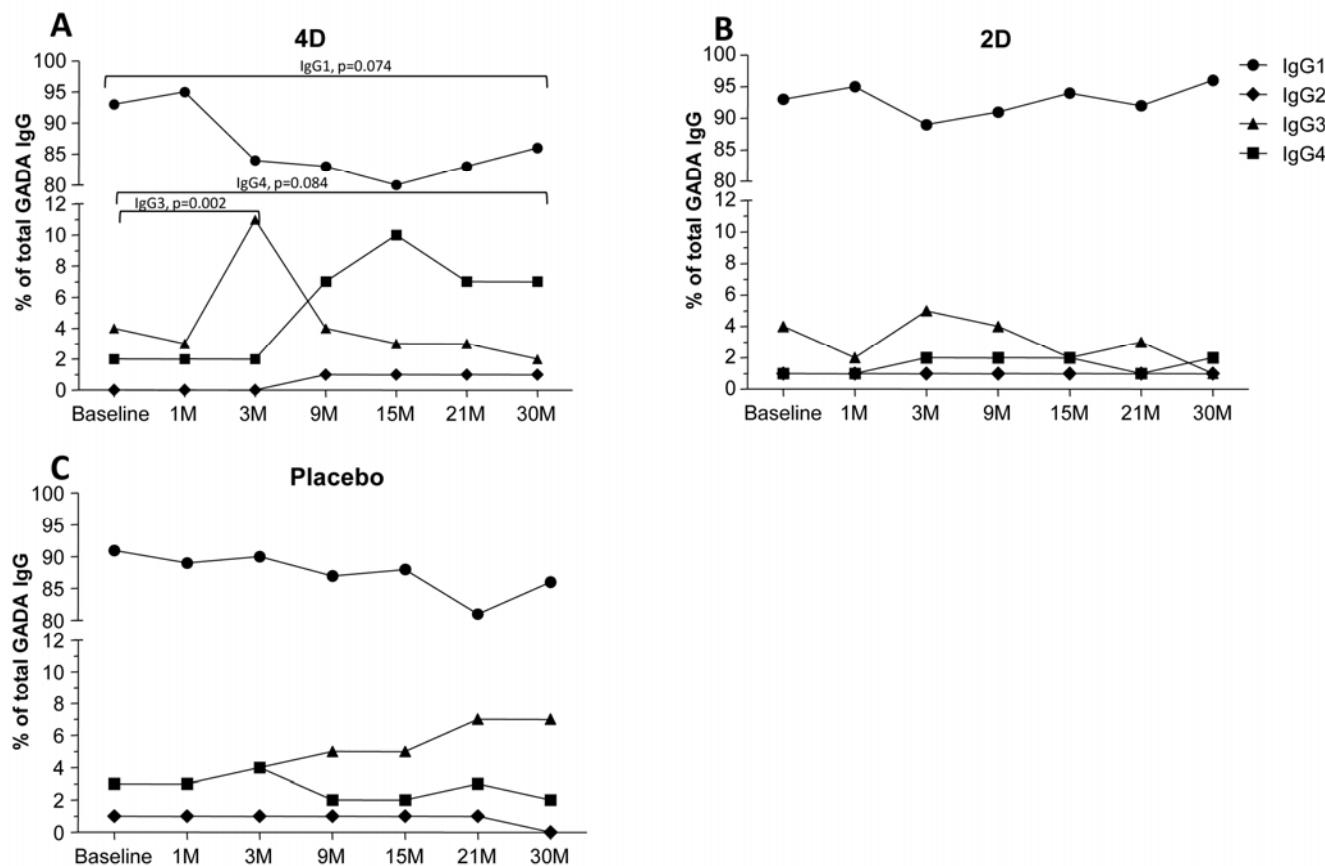
## SUPPLEMENTARY DATA

**Supplementary Figure 1.** Changes in autoantibody titers. Median GADA titers (A) and median GADA fold-change from baseline (B) in 4D (black circles), 2D (grey circles) and placebo (empty circles). Significant differences are indicated as p-values.



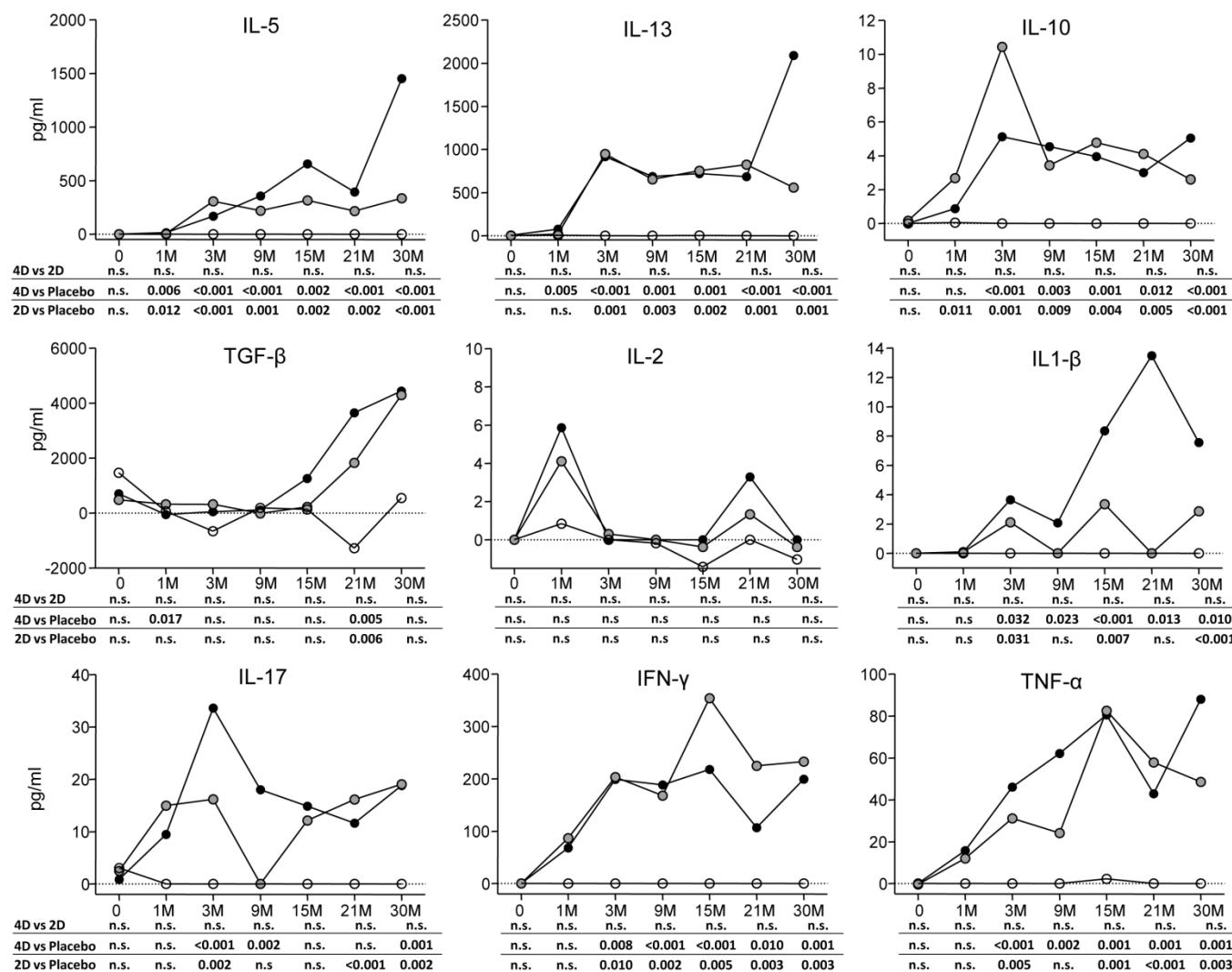
## SUPPLEMENTARY DATA

**Supplementary Figure 2.** Median GADA IgG1-4 subclass distribution. The GADA IgG subclass distribution presented as a percentage of total IgG for 4D (A), 2D, (B) and placebo (C). Results were expressed as cpm, and positivity of each sample was calculated by subtraction of the mean cpm value plus three times the standard deviation (SD) obtained for the negative control. Significant differences are indicated as p-values.



## SUPPLEMENTARY DATA

**Supplementary Figure 3.** Cytokine secretion upon in vitro PBMC stimulation. Median secretion of IL-1 $\beta$ , IL-2, IL-5, IL-10, IL-13, IFN- $\gamma$ , TNF- $\alpha$  and TGF- $\beta$  (pg/ml) in the subgroup of patients followed for 30 months, detected by Luminex in PBMC supernatants after 7 days culture with GAD<sub>65</sub>, in patients receiving 4 doses of GAD-alum (4D, black circles), 2 doses of GAD-alum (2D, gray circles) or placebo (white circles). GAD<sub>65</sub>-induced cytokine secretion is given after subtraction of spontaneous secretion. Significant differences are indicated as p-values.



## SUPPLEMENTARY DATA

**Supplementary Figure 4.** Relative contribution of each cytokine to the GAD<sub>65</sub>-induced secretion. Bar chart illustrating the relative contribution (%) of each cytokine to the GAD<sub>65</sub>-induced secretion detected by Luminex from 1 month to 30 months follow-up in patients receiving 4 doses of GAD-alum (4D), 2 doses of GAD-alum (2D) or placebo.

