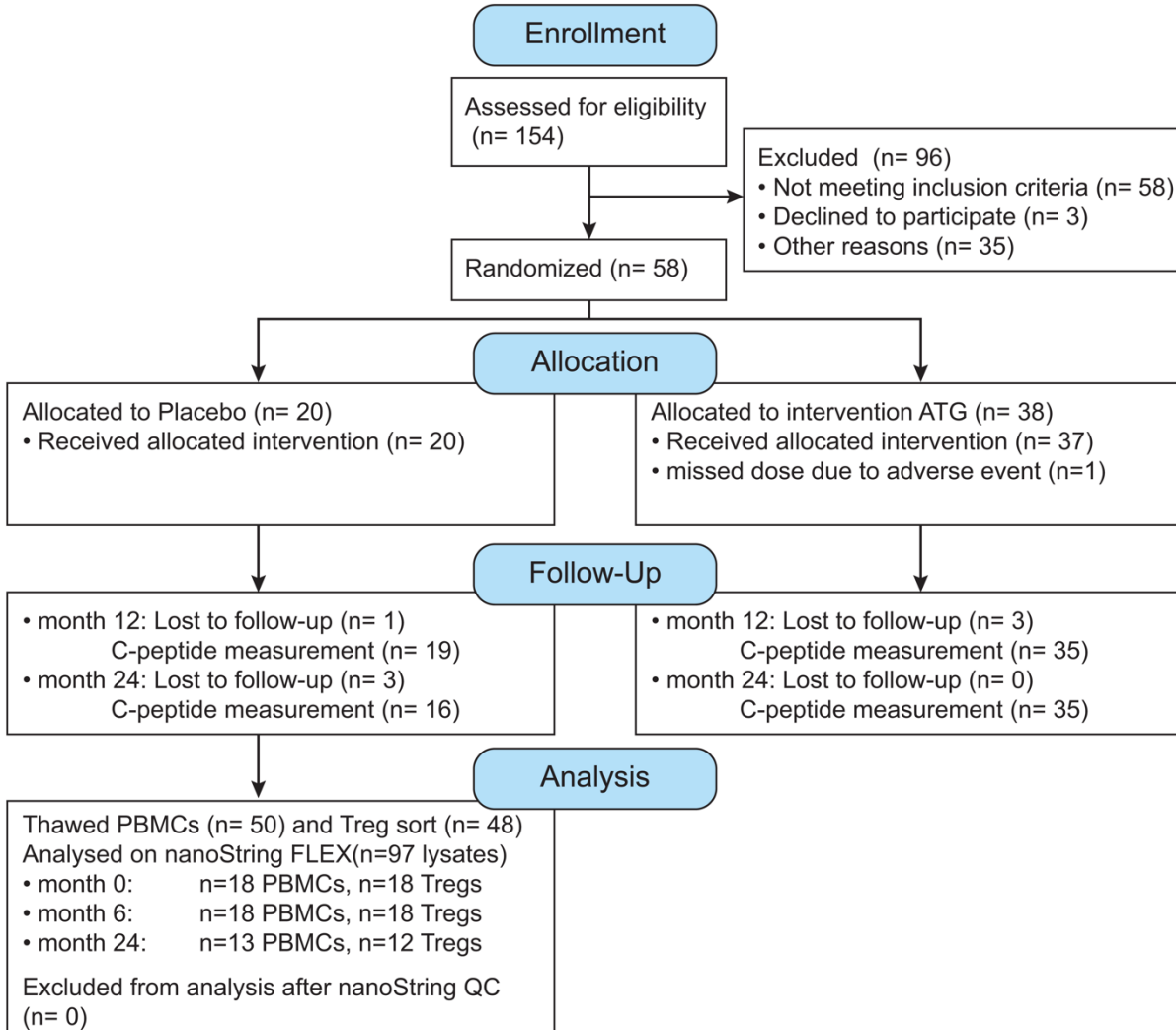**SUPPLEMENTAL MATERIAL**

Figure S1

CONSORT 2010 Flow Diagram – START trial, Treg signature analysis

Enrollment

Assessed for eligibility (n= 154)

Excluded (n= 96)
• Not meeting inclusion criteria (n= 58)
• Declined to participate (n= 3)
• Other reasons (n= 35)

Randomized (n= 58)

Allocation

Allocated to Placebo (n= 20)
• Received allocated intervention (n= 20)

Allocated to intervention ATG (n= 38)
• Received allocated intervention (n= 37)
• missed dose due to adverse event (n=1)

Follow-Up

• month 12: Lost to follow-up (n= 1)
        C-peptide measurement (n= 19)
• month 24: Lost to follow-up (n= 3)
        C-peptide measurement (n= 16)

• month 12: Lost to follow-up (n= 3)
        C-peptide measurement (n= 35)
• month 24: Lost to follow-up (n= 0)
        C-peptide measurement (n= 35)

Analysis

Thawed PBMCs (n= 50) and Treg sort (n= 48)
Analysed on nanoString FLEX(n=97 lysates)
• month 0:   n=18 PBMCs, n=18 Tregs
• month 6:   n=18 PBMCs, n=18 Tregs
• month 24:   n=13 PBMCs, n=12 Tregs

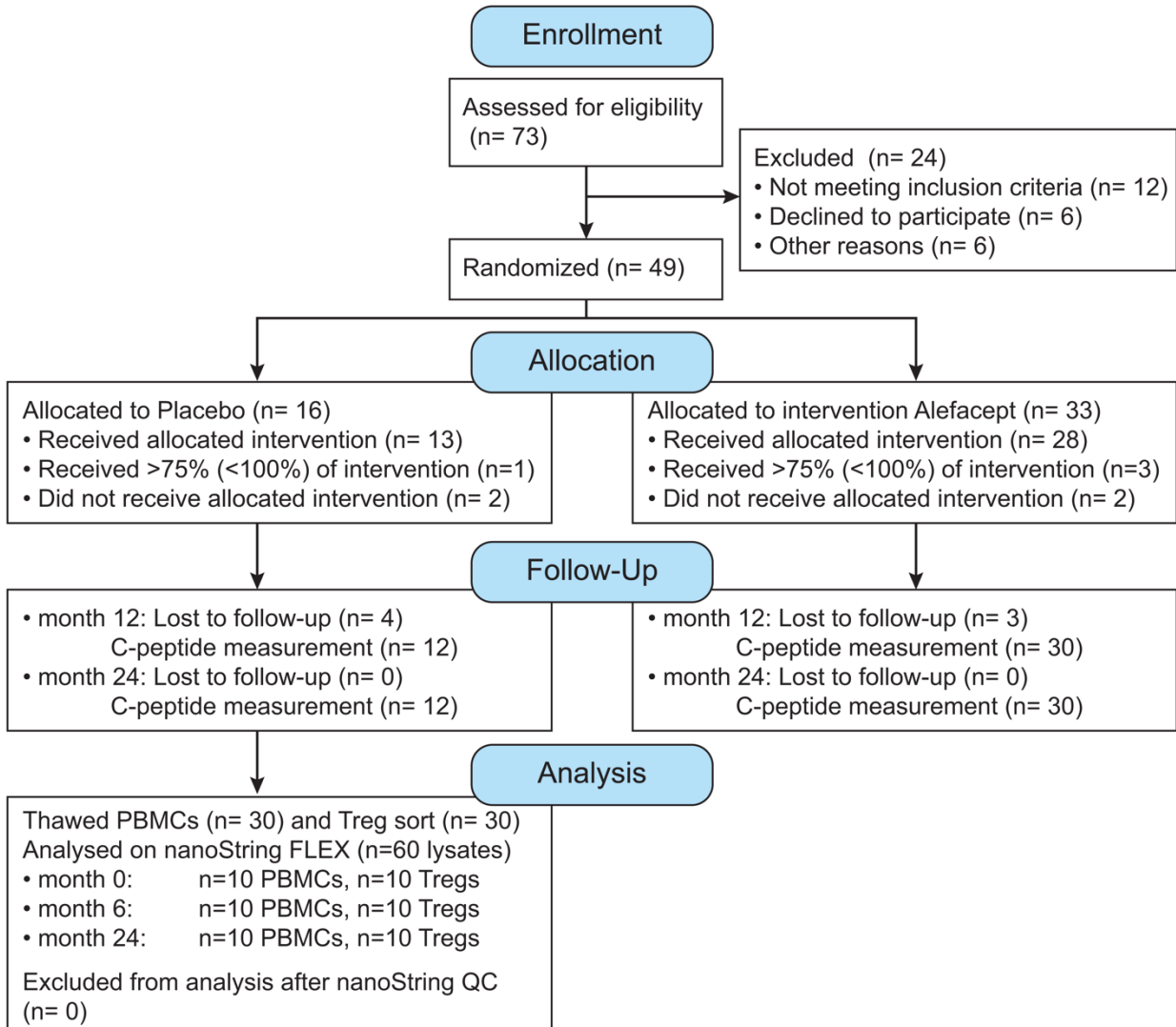Excluded from analysis after nanoString QC (n= 0)

Enrollment to follow-up details adopted from: Gitelman, S. E., et al. (2016). "Antithymocyte globulin therapy for patients with recent-onset type 1 diabetes: 2 year results of a randomised trial." Diabetologia 59(6): 1153-1161.

Supplemental Figure S1: CONSORT diagram showing allocation and disposition of study subjects in the START trial and origin of samples for Treg gene signature analysis.

Figure S2

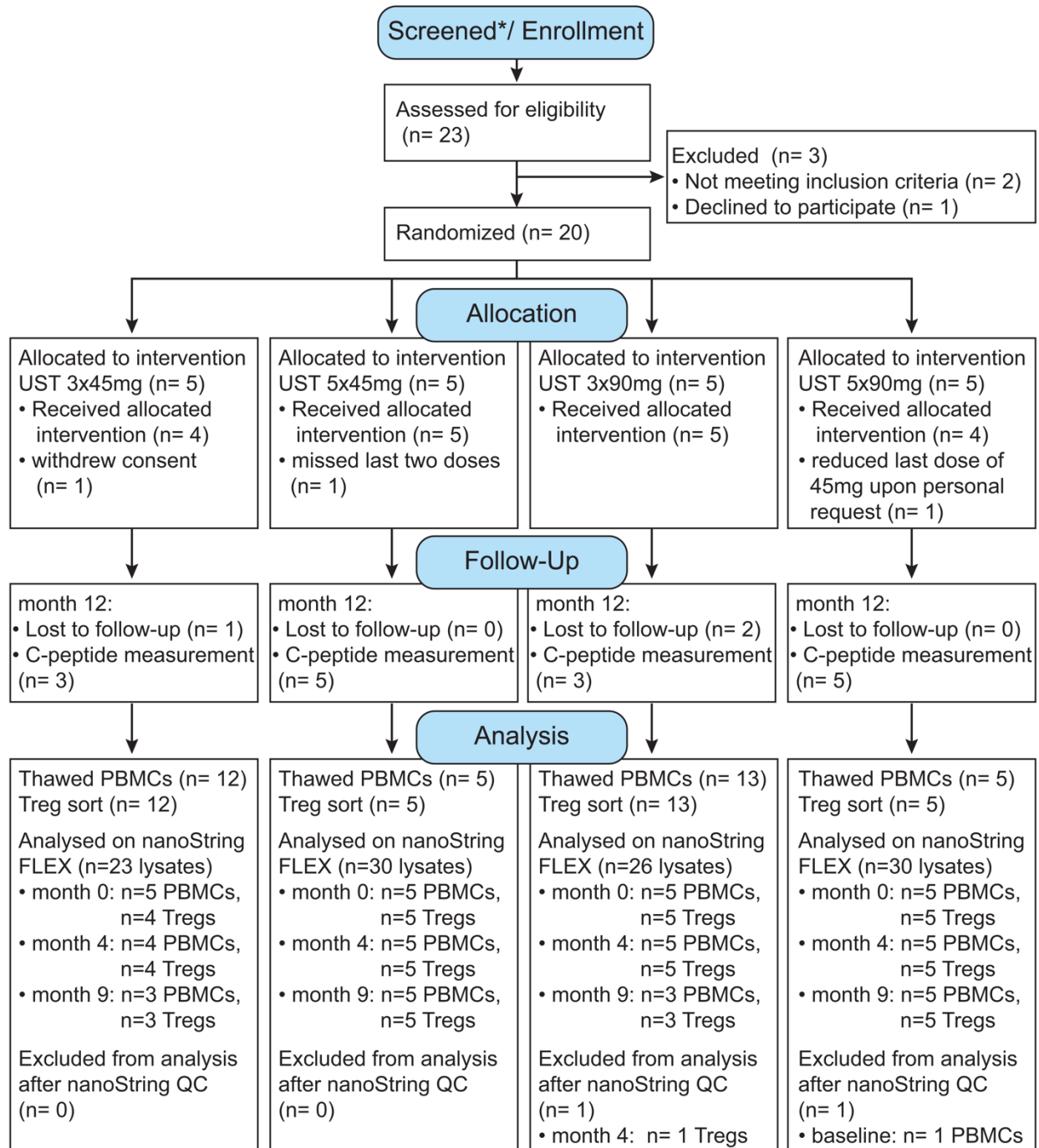CONSORT 2010 Flow Diagram – T1DAL trial, Treg signature analysis

Enrollment to follow-up details adopted from: Rigby, M. R., et al. (2015). "Alefacept provides sustained clinical and immunological effects in new-onset type 1 diabetes patients." J Clin Invest 125(8): 3285-3296

Supplemental Figure S2: CONSORT diagram showing allocation and disposition of study subjects in the T1DAL trial and origin of samples for Treg gene signature analysis.

Figure S3
CONSORT 2010 Flow Diagram – UST1D trial, Treg signature analysis

Supplemental Figure S3: CONSORT diagram showing allocation and disposition of study subjects in the UST1D trial and usage in Treg gene signature analysis. * only subjects that met inclusion criteria were approached and screened.

Supplemental Table T1: Subject characteristics for samples run on the nCounter FLEX and reported in Figures 1-5.

	new onset T1D vs healthy controls		cross sectional T1D vs healthy controls	
	new T1D	HC	T1D	HC
number	20	10	43	38
Sex: % male (number males)	40 (8)	40 (4)	44.2 (19)	42.1 (16)
average age at sample collection [years] (min-max)	24.3 (18-35)	26.3 (20-36)	28.5 (5-59)	30.9 (6.1-59)
average age at diagnosis [years] (min-max)	24.1 (17.7-34.9)	n/a	17.7 (3-35)	n/a
average time since diagnosis [months] (min-max)	2.1 (0.4-3.2)	n/a	129.1 (6-354)	n/a
average random c-peptide [ng/mL] (min-max)	n/a	n/a	0.34 (<0.05-3.75)	n/a
average HbA1c [%] (min-max)	8.6 (5.8-12.2)	n/a	7.5 ** (5.2-13.4)	n/a
average IFCC HbA1c [mmol/mol] (min-max)	70 (40-110)	n/a	58 ** (33-123)	n/a

** n=33 data points available

Supplemental Table T2: genotype for subjects' samples used in Figures 2 and 4

SNP (disease associated allele)	genotype	cross sectional T1D vs healthy controls	
		T1D	HC
Ptpn2 rs478582 (T)	(n genotype available)	47	30
	T/T	26	19
	T/C	18	10
Ptpn2 rs1893217 (C)	(n genotype available)	34	24
	C/C	7	1
	T/C	11	7
Ptpn22 rs2476601(T)	(n genotype available)	47	30
	T/T	6	1
	T/C	6	4
Cd25 rs2104286 (A)	(n genotype available)	47	29
	A/A	31	16
	G/A	12	10
Cd25 rs11594656 (T)	(n genotype available)	47	29
	T/T	29	17
	A/T	18	10
Cd25 rs12722489 (G)	(n genotype available)	34	24
	G/G	25	17
	A/G	9	6
Cd25 rs41295061 (C)	(n genotype available)	35	23
	C/C	31	17
	A/C	4	5
Cd25 rs12722495 (A)	(n genotype available)	46	19
	A/A	42	16
	G/A	4	3

Supplemental Table T3: Subject characteristics for samples run on the nCounter SPRINT and reported in Figures 6-7

	T2D, T1D, HC		
	T2D	T1D	HC
number	25	8	9
Sex: % male (number males)	36.0 (9)	37.5 (3)	22.2 (2)
average age at sample collection [years] (min-max)	39.4 (17-59)	44.4 (20-59)	44.2 (20-59)
average age at diagnosis [years] (min-max)	n/a	25.9 (5-33)	n/a
average time since diagnosis [months] (min-max)	38.7 (1-154.8)	222.5 (76-327)	n/a
average random c-peptide [ng/mL] (min-max)	n/a	0.13 (<0.05-0.34)	n/a
average HbA1c [%] (min-max)	7.3* (5.5-9.9)	7.6** (6.2-8.6)	n/a
average IFCC HbA1c [mmol/mol] (min-max)	56* (37-85)	60** (44-70)	n/a

* n=20 data points available; ** n=7 data points available

Supplemental Table T4: Subject characteristics for samples used in Figure 8

	longitudinal placebo arm ITN trials		
c-peptide decline	slow	moderate	rapid
number	9	8	7
Sex: % male (number males)	44.4 (4)	75.0 (6)	71.4 (5)
average age at sample collection [years] (min-max)	20.8 (12-33)	22.6 (14-28)	20.0 (14-34)
average age at diagnosis [years] (min-max)	n/a	n/a	n/a
average time since diagnosis [months] (min-max)	<3.3	<3.3	<3.3
average HbA1c [%] (min-max)	7.9 (5.6-15.2)	7.5 (5.5-11.0)	7.1 (4.9-10.3)
average IFCC HbA1c [mmol/mol] (min-max)	63 (38-143)	58 (37-97)	54 (30-89)
average MMTT 2h AUC at baseline (min-max)	1.03 (0.19-2.58)	0.49 (0.01-0.78)	0.83 (0.53-1.14)
average MMTT 2h AUC at M12/24 (min-max)	1.01 (0.24-2.78)	0.27 (0.03-0.54)	0.20 (0.09-0.32)

Supplemental Table T5: Subject characteristics for samples used in Figure 9.

c-peptide decline	longitudinal ustekinumab trial		
	slow	rapid	uncertain
number	11	5	4
Sex: % male (number males)	54.5 (6)	40.0 (2)	22.2 (2)
average age at sample collection [years] (min-max)	25.4 (19-35)	22.0 (18-27)	24.3 (18-31)
average age at diagnosis [years] (min-max)	25.2 (18.8-34.9)	21.8 (17.9-26.8)	24.0 (17.7-30.8)
average time since diagnosis [months] (min-max)	2.0 (0.4-2.9)	1.9 (1.3-2.3)	2.7 (1.7-3.3)
average HbA1c [%] (min-max)	8.6 (5.8-12.2)	8.3 (6.8-9.1)	9.3 (7.1-11.0)
average IFCC HbA1c [mmol/mol] (min-max)	70 (40-110)	67 (51-76)	78 (54-97)
average MMTT 2h AUC at baseline (min-max)	0.62 (0.25-1.40)	0.66 (0.39-0.83)	0.50 (0.29-0.80)
average MMTT 2h AUC at M12/24 (min-max)	0.56 (0.21-1.23)	0.17 (0.005-0.29)	n/a

Supplemental Table T6: antibody information for Treg sorting from PBMCs of cross-sectional T1D, matched controls and longitudinal samples from the placebo arms of the T1DAL and START trials

target	clone	Fluorochrome/dye	company
CD3	UCHT1	AlexaFluor700	Thermo Fisher
CD4	RPA-T4, OKT4	PE	Biolegend, Thermo Fisher
CD14	61D3	APC	Thermo Fisher
CD19	HIB19	PerCP Cy5.5, PerCP	Thermo Fisher
CD20	2H7	FITC	Thermo Fisher
CD25	CD25-4E3	Pe-eFluor610	Thermo Fisher
CD127	A019D5	BV421	Biolegend
CD8*	RPA-T8	BV650	Biolegend
Fixable viability dye	n/a	eFluor506	Thermo Fisher

*used in a subset of sorts

Supplemental Table T7: antibody information for Treg sorting from CD4 T cell enriched cells of new onset adult T1D, matched controls, T2D and longitudinal samples from the Ustekinumab trial

target	clone	Fluorochrome/dye	company
CD4	SK3	BV510	Biolegend
CD25	2A3	BB515	BD biosciences
CD127	eBioRDR5	eFluor450	Thermo Fisher
Fixable viability dye	n/a	eFluor780	Thermo Fisher