

Supp. Table 1: General study eligibility**Inclusion Criteria:**

- participants of both sexes between 18 and 60 years of age
- completed primary TBE immunization and at least 1 booster vaccination
- willingness to sign written informed consent form

Exclusion Criteria:

- age < 18 and > 60 years
- BMI 25-30
- previous TBE infection
- pregnancy or breast feeding
- acute infection on day of inclusion (day 0), body temperature > 37,9°C
- concomitant medications: systemic cortisone therapy, chemotherapy, immunotherapy (allergy), immunosuppressive therapy 4 weeks prior or during the study
- administration of other vaccines 4 weeks before/after day 0
- planned surgery within 2 weeks before/after TBE booster
- any contraindication to administration of FSME-Immun® vaccine according to manufacturer's instructions
- malignant diseases within 5 years prior to the study
- autoimmune diseases
- kidney insufficiency, dialysis
- drug addiction
- plasma donor
- receipt of blood transfusions or immunoglobulins within 3 months prior to study entry
- severe disease with hospitalization or surgery 3 months before or during the study
- participation in a clinical trial simultaneously to visit 1-4 with receipt of vaccination and/or investigational product within one month before booster

Obesity, sex and vaccine responsiveness

Supp. Table 2: *Changes in metabolic parameters 4 weeks after TBE booster*

Concentrations of triglycerides, total cholesterol, HDL cholesterol, glucose (all mg/dl); insulin (mIU/L), fructosamine ($\mu\text{mol/L}$) and hsCRP (mg/dl) were measured before (d0) and 4 weeks (d28) after booster vaccination. Cholesterol ratio was calculated as total cholesterol/HDL cholesterol and LDL cholesterol was calculated according to the Friedewald formula; values are indicated as geometric mean (GM) concentrations with 95% CI.

Abbreviations: tgl, triglycerides; chol, total cholesterol; LDL, LDL cholesterol; HDL, HDL cholesterol; chol ratio, cholesterol ratio; fruct, fructosamine; L 95% CI, lower 95% confidence interval; U 95% CI, upper 95% confidence interval.

			tgl	chol	LDL	HDL	chol ratio	glucose	insulin	fruct	hsCRP
female	obese	d0	116,9	197,6	119,3	53,9	3,67	94,7	16,38	232,1	0,43
		L 95% CI	94,8	181,1	102,9	47,6	3,2	87,8	12,57	221,8	0,27
		U 95% CI	144,2	215,5	138,3	61,0	4,21	102,1	21,35	242,9	0,66
	obese	d28	106,6	188,5	111,0	52,9	3,56	95,5	13,84	225,7	0,31
		L 95% CI	86,0	174,7	97,8	46,8	3,11	88,1	10,58	217,0	0,24
		U 95% CI	132,1	203,4	126,0	59,8	4,08	103,5	18,12	234,8	0,40
	controls	d0	77,3	225,0	118,5	85,7	2,61	86,6	7,66	258,7	0,04
		L 95% CI	62,0	205,5	101,5	75,3	2,26	80,3	5,88	246,9	0,02
		U 95% CI	96,3	246,4	138,4	97,6	3,02	93,4	9,98	271,0	0,06
	controls	d28	72,8	218,3	114,2	84,6	2,58	85,0	6,20	243,2	0,16
		L 95% CI	58,2	201,6	100,0	74,4	2,24	78,5	4,74	233,6	0,13
		U 95% CI	91,2	236,4	130,4	96,1	2,98	92,1	8,11	253,3	0,21
male	obese	d0	152,9	196,8	137,6	40,9	4,82	97,0	22,84	229,4	0,32
		L 95% CI	121,8	179,2	117,2	35,7	4,15	89,3	17,15	218,4	0,20
		U 95% CI	191,8	216,2	161,4	46,7	5,6	105,2	30,42	241,0	0,52
	obese	d28	164,0	190,3	126,8	40,1	4,76	104,8	23,96	230,9	0,27
		L 95% CI	130,0	175,3	110,5	35,1	4,11	96,1	17,91	221,2	0,21
		U 95% CI	206,8	206,6	145,4	45,8	5,5	114,4	32,06	241,0	0,35
	controls	d0	88,9	204,6	117,9	62,0	3,31	89,4	6,53	247,2	0,02
		L 95% CI	69,8	185,2	99,4	53,8	2,82	82,1	4,85	234,9	0,01
		U 95% CI	113,2	226,1	139,7	71,5	3,88	97,3	8,79	260,1	0,04
	controls	d28	96,1	208,6	131,9	56,2	3,72	87,2	6,70	236,2	0,15
		L 95% CI	75,0	191,2	114,0	48,8	3,19	79,7	4,95	226,0	0,11
		U 95% CI	123,0	227,7	152,7	64,7	4,35	95,5	9,06	246,9	0,20

Obesity, sex and vaccine responsiveness

Supp. Table 3: Serum hormone levels

Concentrations of the sex hormones testosterone, estrogen and progesterone (in ng/ml) and luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (in mU/ml) indicated as geometric mean (GM) concentrations with 95% CI.

Abbreviations: L 95% CI, lower 95% confidence interval; U 95% CI, upper 95% confidence interval.

	controls male	L 95% CI	U 95% CI	controls female	L 95% CI	U 95% CI
testosterone	6,05	5,16	7,10	0,13	0,09	0,19
estrogen	28,92	24,76	33,78	19,24	9,92	37,33
progesterone	0,18	0,12	0,28	0,31	0,14	0,73
FSH	5,52	4,38	6,95	17,89	7,45	42,95
LH	5,29	4,47	6,25	9,91	3,89	25,24
	obese male			obese female		
testosterone	3,42	2,87	4,07	0,22	0,17	0,29
estrogen	29,61	25,98	33,75	29,92	18,11	49,41
progesterone	0,29	0,20	0,44	0,30	0,15	0,60
FSH	4,43	3,57	5,51	16,63	9,80	28,22
LH	5,16	4,23	6,28	12,46	7,82	19,87

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Supp. Table 4: *Differences in total B cells, naïve and memory B subsets and plasma blasts according to sex*

PBMCs were stained with fluorochrome-labeled mAbs and analyzed on a BD FACS Canto II flow cytometer. Distributions of total CD19+ B cells and plasma blasts as % of total lymphocytes as well as of naïve, un-switched memory, switched memory and double-negative (DN) B subsets as % of total B cells before (d0) and one week (d7) after TBE booster vaccination are listed. Values for the entire group (all) and separately for females (F) and males (M) before (d0) and 1 week (d7) after booster are indicated.

Abbreviations: ly, lymphocytes; tot, total; unsw, unswitched; sw, switched; DN, double negative; PB, plasma blasts

		total B as % of ly		naïve as % tot B		unsw as % tot B		sw as % tot B		DN as % tot B		PB as % of ly	
		d0	d7	d0	d7	d0	d7	d0	d7	d0	d7	d0	d7
obese	all	10,58	10,56	67,13	68,01	15,16	14,18	13,86	14,11	3,84	3,71	1,69	1,89
	F	10,25	10,40	69,03	69,30	13,85	13,93	13,36	13,32	3,76	3,46	1,69	1,88
	M	10,97	10,74	64,88	66,47	16,72	14,47	14,46	15,05	3,94	4,01	1,69	1,91
controls	all	8,70	7,83	60,97	59,07	18,41	19,57	15,60	16,70	5,01	4,65	1,61	2,47
	F	8,94	7,80	62,66	60,07	15,78	17,73	15,76	17,05	5,80	5,16	1,40	2,49
	M	8,37	7,87	58,68	57,73	21,98	22,07	15,40	16,24	3,94	3,97	1,90	2,45

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Supp. Table 5: Differences in CD4 and CD8 T cell naïve and memory subsets according to sex

PBMCs were stained with fluorochrome-labeled mAbs and analyzed on a BD FACS Canto II flow cytometer. Distributions of CD3⁺/CD4⁺ helper T cells and CD3⁺/CD8⁺ cytotoxic T cells as % of total lymphocytes. T naïve, T central memory (T_{CM}), T effector memory (T_{EM}) and T effector memory RA (T_{EMRA}) subsets as % of total CD4 and total CD8 T cells respectively are listed. Values for the entire group (all) and separately for females (F) and males (M) before (d0) and 1 week (d7) after booster are indicated.

Abbreviations: ly, lymphocytes; p sex, p value in respect to sex differences.

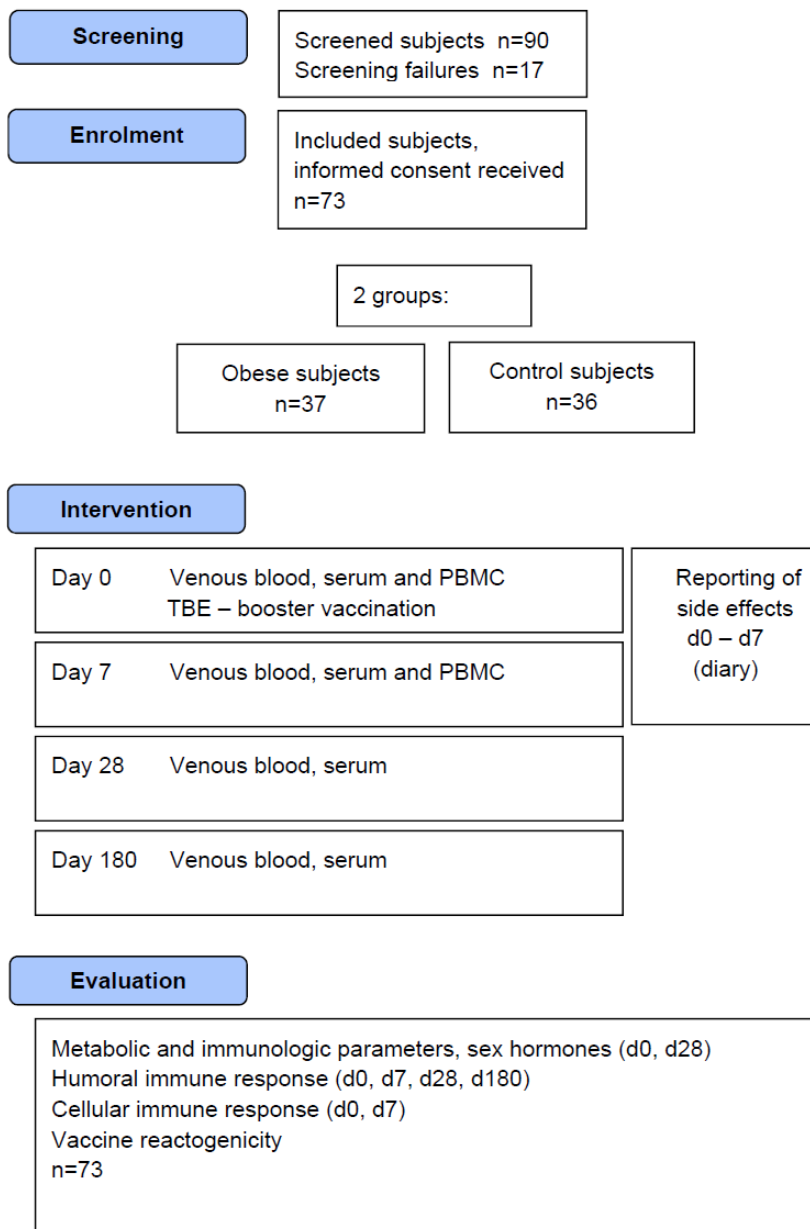
ANOVA with linear contrasts; *p≤0.05, **p≤0.01

		CD4 as % of ly		CD4 naïve as % CD4		CD4 T _{CM} as % CD4		CD4 T _{EM} as % CD4		CD4 T _{EMRA} as % CD4	
		d0	d7	d0	d7	d0	d7	d0	d7	d0	d7
obese	all	45,4	43,7	51,7	51,5	34,5	33,7	10,6	11,2	3,2	3,6
	F	48,7	46,0	54,6	54,2	30,9	30,5	10,5	10,9	4,0	4,4
	M	41,3	40,8	48,0	48,1	39,0	37,8	10,7	11,6	2,3	2,6
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controls	all	44,5	43,8	52,5	52,2	28,9	29,0	15,1	15,2	3,4	3,6
	F	46,4	46,1	55,1	55,1	27,0	27,4	14,7	14,0	3,3	3,5
	M	41,8	40,8	49,1	48,2	31,6	31,3	15,7	16,9	3,6	3,7
	p sex	**p=0.005	*p=0.01	*p=0.02	*p=0.01	*p=0.03	p=0.05	p=0.12	*p=0.04		
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		CD8 as % of ly		CD8 naïve as % CD8		CD8 T _{CM} as % CD8		CD8 T _{EM} as % CD8		CD8 T _{EMRA} as % CD8	
		d0	d7	d0	d7	d0	d7	d0	d7	d0	d7
obese	all	17,2	17,3	38,0	37,1	14,3	13,3	24,3	23,6	23,5	26,1
	F	16,3	15,9	41,3	40,2	12,8	12,4	21,7	21,6	24,3	25,9
	M	18,3	19,0	33,8	33,3	16,1	14,4	27,6	26,0	22,4	26,3
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controls	all	19,4	19,8	39,5	38,3	10,2	10,5	27,7	27,9	22,6	23,3
	F	19,0	19,7	41,5	40,2	9,2	10,4	27,1	26,0	22,2	23,4
	M	19,9	19,9	36,7	35,7	11,5	10,7	28,5	30,5	23,3	23,1
	p sex	p=0.59	p=0.45	p=0.14	p=0.17	p=0.18	p=0.64	p=0.17	p=0.10		

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Supp. Fig. 1: Study design

Flow-diagram of study design and study participants from screening to evaluation.

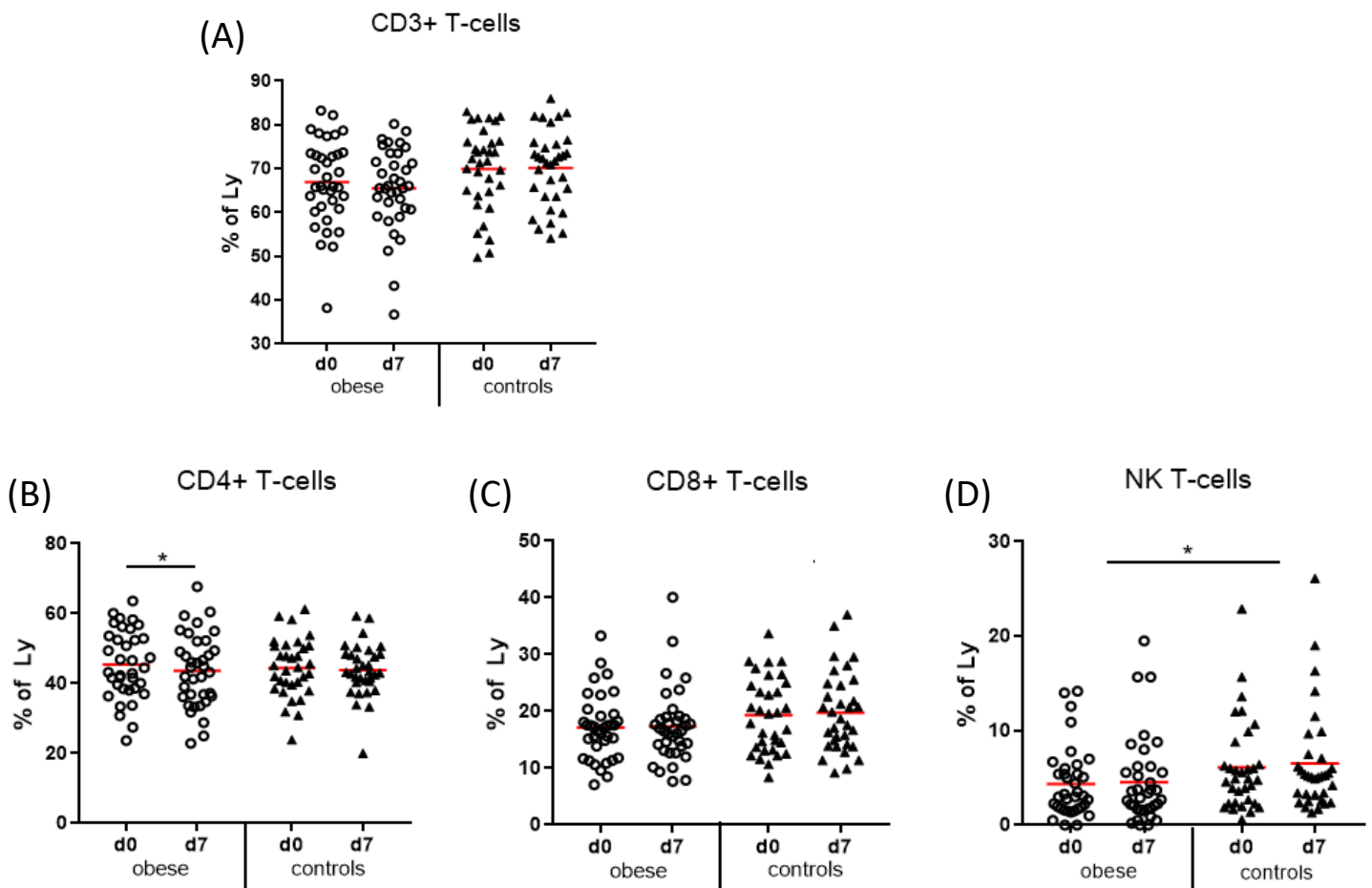


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Supp. Fig. 2: Quantification of T lymphocytes and T cell subsets

PBMC were stained with fluorochrome-labeled mAbs and analyzed on a BD FACS Canto II flow cytometer. (A) CD3+ total T-cells (B) CD3+/CD4+ T-helper cells and (C) CD3+/CD8+ cytotoxic T-cells, and D) calculated CD3+/CD4-/CD8- NK T cells before (d0) and 1 week (d7) after booster vaccination as % of lymphocytes in the two investigated groups; line is arithmetic mean.

ANOVA with linear contrasts; * $p \leq 0.05$



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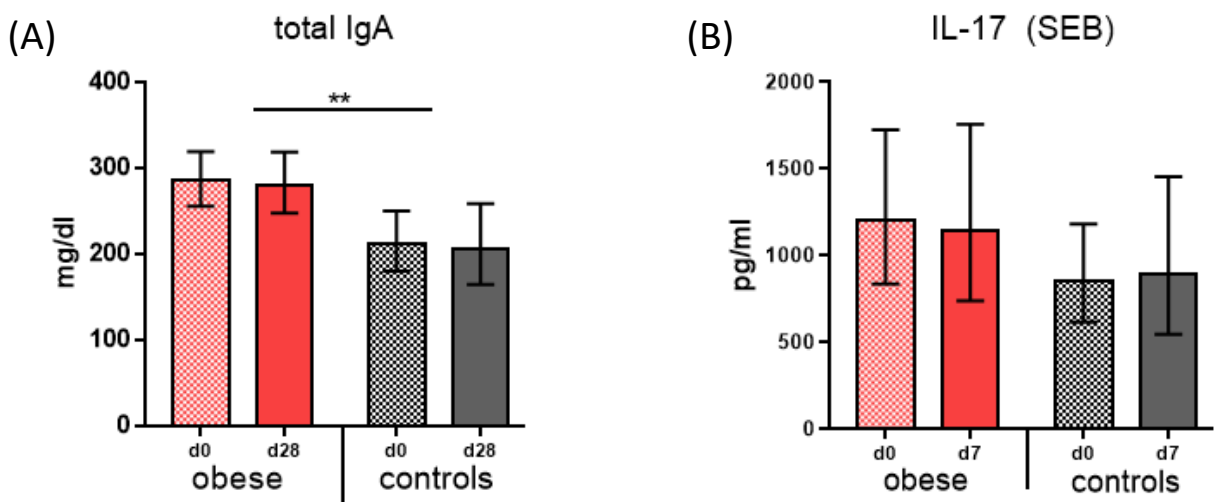
Supp. Fig. 3: Total IgA & SEB-specific IL-17 production

A) Concentrations of total serum immunoglobulin A (IgA) in obese and control cohort measured before (d0) and 4 weeks after vaccination (d28); in mg/dl as geometric mean (GM) with 95% CI.

ANOVA with linear contrasts; ** $p \leq 0.01$

B) IL-17 concentrations (pg/ml) were measured in PBMC culture supernatants with Luminex technology. PBMC obtained before (d0) and 1 week (d7) after booster were incubated with 1 $\mu\text{g/ml}$ *Staphylococcus* Enterotoxin B for 48 h. Geometric mean (GM) concentrations (pg/ml) with 95% CI.

ANOVA with linear contrasts;

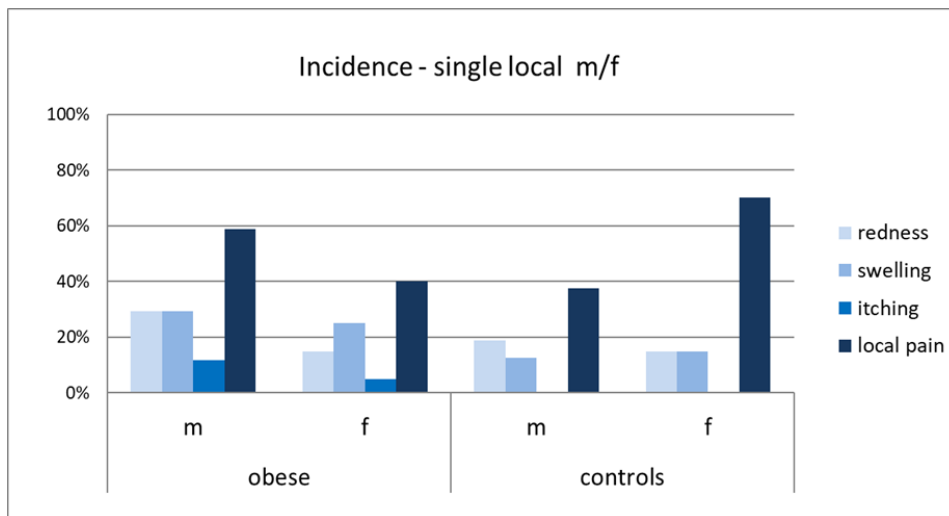


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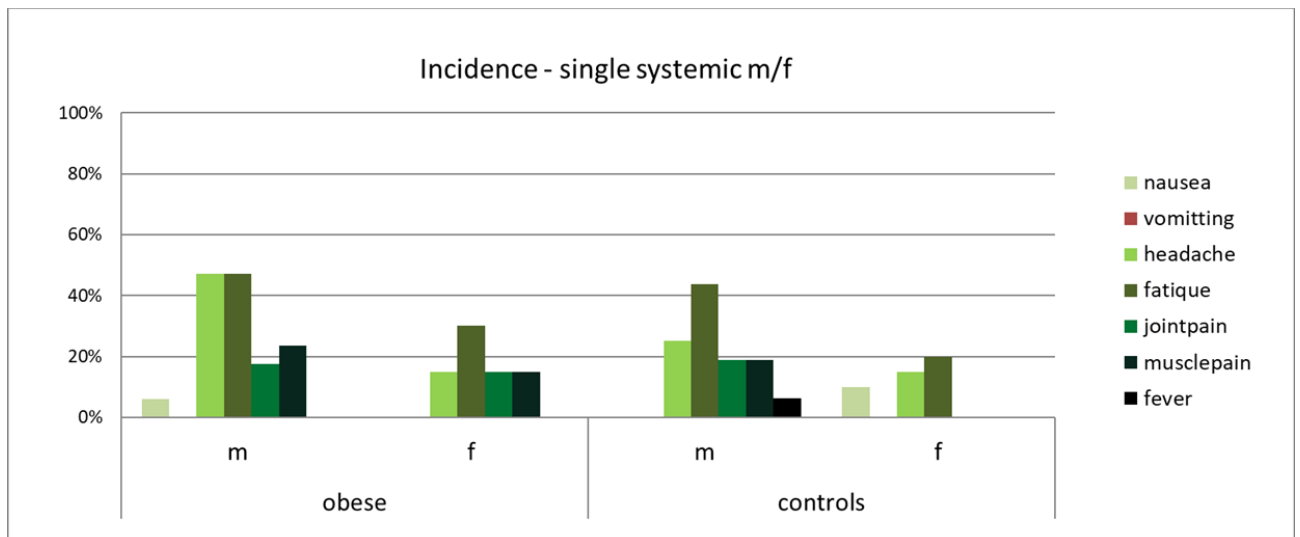
Supp. Fig. 4: Incidence of single local and systemic side effects

Incidence of (A) single local and (B) single systemic side effects according to sex

(A)



(B)

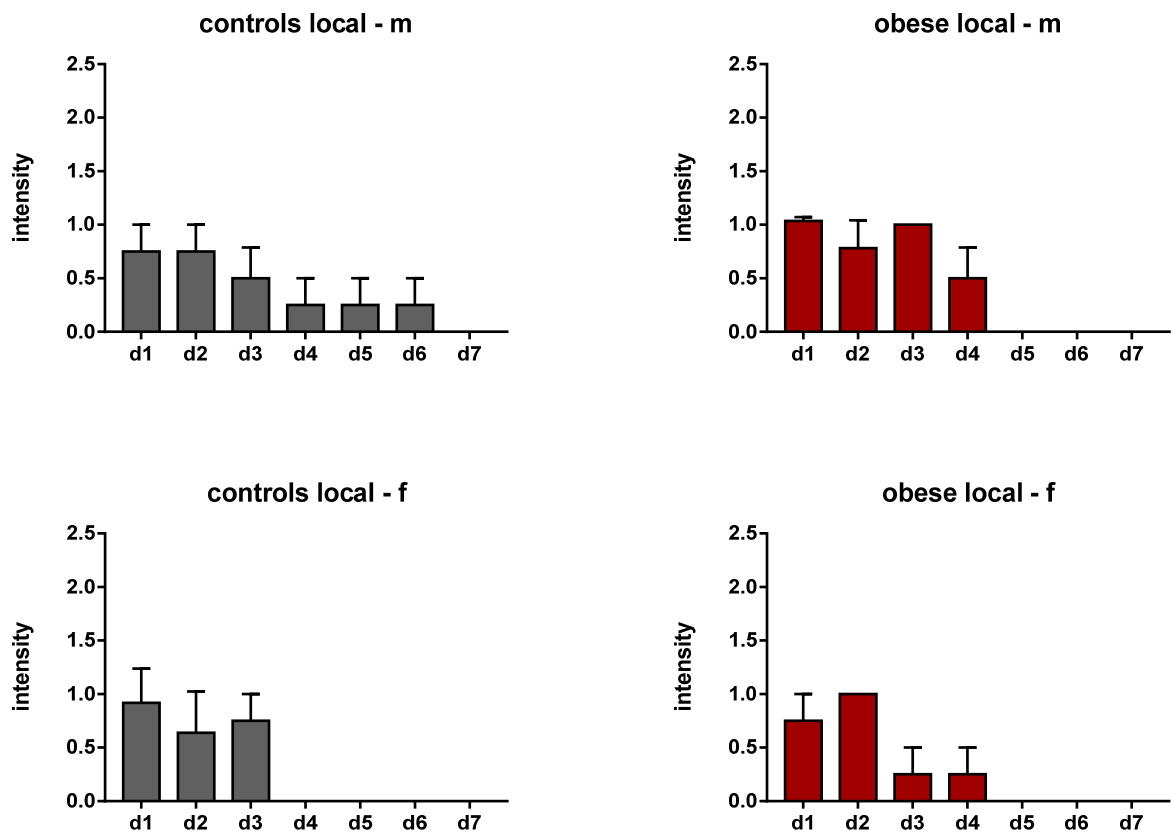


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Supp. Fig. 5: Intensity and duration of local and systemic side effects

(A) Intensity and duration of local side effects according to sex

Arithmetic means of intensities of local side effects after vaccination for males and females over a 7-day period; error bar is SEM. Local effects are pain, redness, itching and swelling at the injection site. Intensity classified as one/mild, two/intermediate, three/strong.



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(B) Intensity and duration of systemic side effects according to sex

Arithmetic means of intensities of systemic side effects after vaccination for males and females over a 7-day period; error bar is SEM. Systemic effects are nausea, vomiting, headache, fatigue, joint pain, muscle pain and fever. Intensity classified as one/mild, two/intermediate, three/strong.

